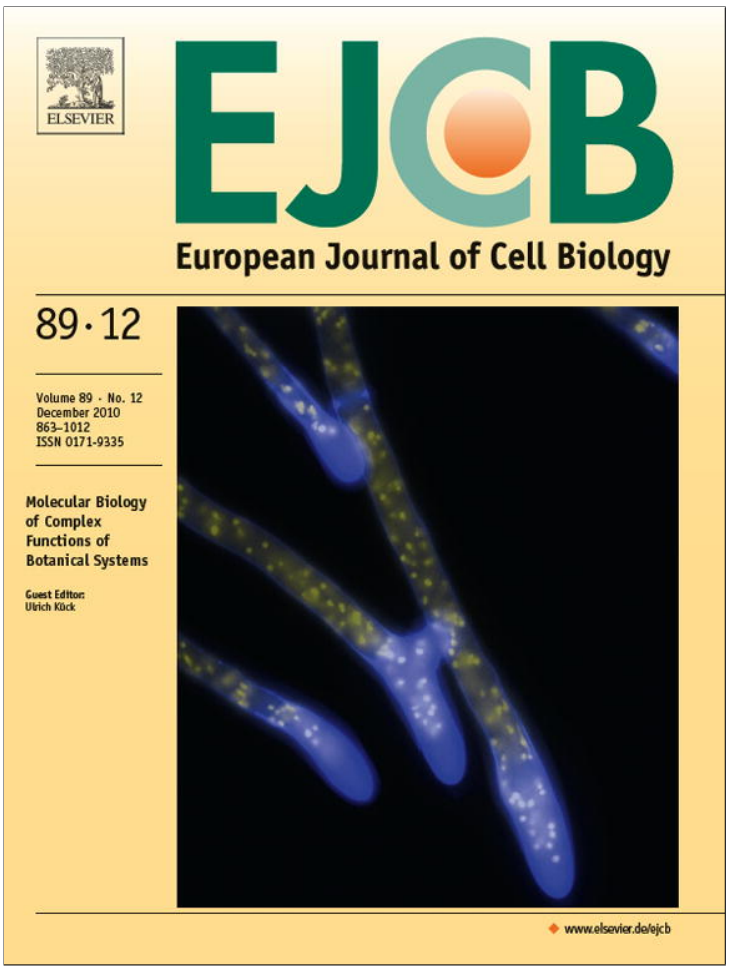

European Journal of Cell Biology 89 (2010) 974–982

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Minireview

Dynamics of the cyanobacterial photosynthetic network: Communication and modification of membrane protein complexes

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ARTICLE INFO

Keywords:
Thermosynechococcus elongatus
Cytochrome b_6f complex
Photosystem 2
Regulation
Assembly
Repair

ABSTRACT

Cyanobacterial photosystem 2 and cytochrome b_6f complexes have been structurally resolved up to the molecular level while the adjustment of their function in response to environmental and intracellular parameters is based on various modifications of these complexes which have not yet been resolved in detail. This minireview summarizes recent results on two central modifications for each complex: (a) for the cytochrome b_6f complex the implication of PetP, a new subunit, and of three copies of PetC, the Rieske protein, for the fine-tuning of the photosynthetic electron transport is evaluated; (b) for photosystem 2, the heterogeneity of the D1 subunit and the role of subunit Psb27 is discussed in relation to stress response and the biogenesis/repair cycle. The presented "dynamic" models for both complexes should illustrate the need to complement structural by more extensive functional models which consider the flexibility of individual complexes in the physiological context – beyond structure.

Introduction

All major membrane protein complexes of the cyanobacterial electron transport (ET) network (Fig. 1), i.e. photosystem 2 (PS2), cytochrome b_6f complex (Cyt b_6f) and photosystem 1 (PS1), have been structurally well characterized (Baniulis et al., 2009; Ferreira et al., 2004; Guskov et al., 2009; Jordan et al., 2001; Kamiya and Shen, 2003; Kurisu et al., 2003; Loll et al., 2005; Stroebel et al., 2003; Yamashita et al., 2007). However, substantial information on the dynamics and on modifications of these complexes in their native environment, i.e. the thylakoid membrane (TM), is still missing. This includes the regulation of their biogenesis and degradation as well as the "communication" (crosstalk) of these complexes within their membrane or between membranes, i.e. the cytoplasmic membrane (CM) and the thylakoid membrane.

After all, the available 3D structures of the isolated complexes represent only a "snapshot", i.e. one dominating complex which has been isolated and purified under certain conditions from the (native) membrane, while several other, less prominent modifications of these complexes co-existing at the same time in the membrane, are missing: due to lower abundance and/or instability they may be more difficult to find, isolate and characterize. Also, components which are attached to these complexes which may represent important intermediates in biogenesis, degradation or adaptation to environmental conditions may have been lost in the course of (harsh) purification procedures which are required for crystallization purposes. For this reason it may be worthwhile to focus on such complexes which may also be important for the communication within the components of the ET chain by providing a quick response towards internal and external changes.

Starting from a gallery of co-existing intermediate complexes, which may be found adjacent to routine preparation procedures, their quantity may be increased by choosing either milder isolation procedures or by exposing the cells to various stress conditions. Structural and functional characterization of these new complexes may then be combined with the creation of deletion mutants, heterologous overexpression, and also with pulse labeling studies to find out about the sequence of events in correlation with the appearance and disappearance of these components.

In this contribution we focus on heterogeneity of PS2- and Cyt b_6f complexes in cyanobacteria, namely in the mesophile *Synechocystis* sp. PCC 6803 (*S.*6803) and the thermophile *Thermosynechococcus elongatus* (*T. elongatus*) which are both well characterized in respect of their genomic sequence and the structure of their intrinsic photosynthetic membrane proteins, respectively.

New structure–function relationships of cyanobacterial Cyt b_6f (beyond the crystal structure)

3D structures of the Cyt b_6f complex from the green algae *Chlamydomonas reinhardtii* and two cyanobacteria, *Mastigocladus*

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doi:10.1016/j.ejcb.2010.08.008

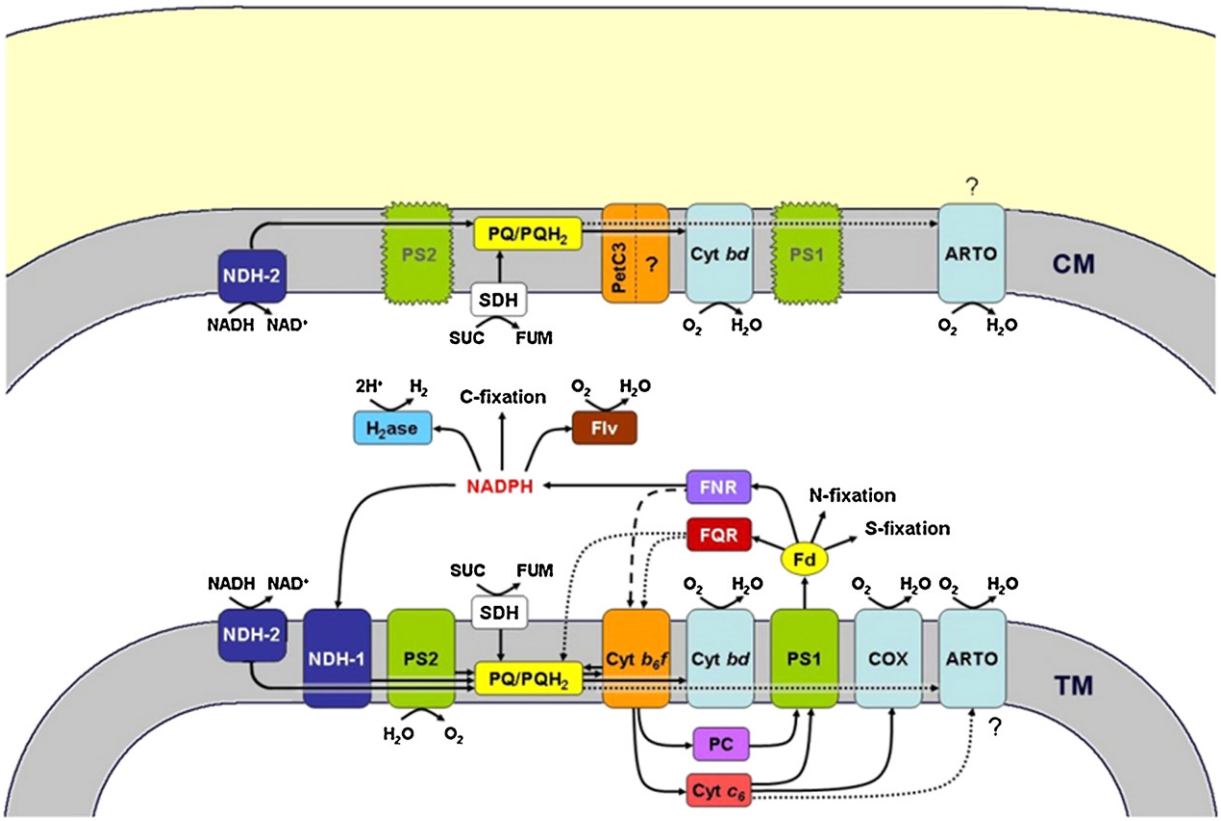

Fig. 1. Major components and routes of the cyanobacterial ET network in the CM and TM of *Synechocystis* sp. PCC 6803. ET processes in the CM (upper part) must be limited due to incomplete or missing components. Question marks and dotted lines indicate that localization and substrate of the alternative terminal oxidase (ARTO) as well as the ET route of plastoquinol (PQH_2) reduction *via* ferredoxin:plastoquinone (Fd:PQ) oxidoreductase (FQR) are still to be identified. The dashed line shows the proposed direct Cyt b_6f-reduction *via* Fd/FNR. COX, cytochrome *c* oxidase; Cyt *bd*, cytochrome *bd* oxidase; H_2ase, hydrogenase; Flv, flavoprotein-1 and -3 (Helman et al., 2003), Allakhverdieva et al., manuscript in preparation; FNR, Fd:$NADP^+$ oxidoreductase; NDH-1 and -2, type I and II NADPH dehydrogenase, respectively; PC, plastocyanin; SDH, succinate dehydrogenase. For further details and references, see Bernát and Rögner (in press).

laminosus and *Nostoc* sp. PCC 7120, are available at 3.1 and 3.0 Å resolution, respectively (Baniulis et al., 2009; Kurisu et al., 2003; Stroebel et al., 2003; Yamashita et al., 2007). They all show a functional dimer of two monomers linked by domain swapping. Each monomer consists of four large (17.5–32 kDa) subunits [Cyt *f* (PetA), Cyt b_6 (PetB), the Rieske 2Fe–2S protein (PetC) and subunit IV (PetD)] and four small subunits (3.3–4.1 kDa; PetG, -L, -M and -N) at unit stoichiometry. While most of these subunits are encoded by single genes, the Rieske protein is encoded by a gene family in most cyanobacteria (Schneider et al., 2002, 2004a,b); for a review see (Schneider and Schmidt, 2005); besides, two putative b_6 polypeptides are reported in *Gloeobacter violaceus* (Dreher et al., 2010; Nakamura et al., 2003). Beyond the eight Cyt b_6f subunits which can be identified in all X-ray structures, there is evidence for additional protein components which may interact transiently with the Cyt b_6f complex: in higher plants, Cyt b_6f has been co-isolated with ferredoxin:$NADP^+$ oxidoreductase (FNR) (Zhang et al., 2001), and the functional coupling of a small phosphoprotein, PetO (Hamel et al., 2000) to Cyt b_6f has also been reported. In cyanobacteria, PetP has been proposed as a new cyanobacterial Cyt b_6f subunit (Gendrullis et al., 2008; Volkmer et al., 2007) which might be analogous to PetO. However, the role of PetC (and PetB) heterogeneity and the function of the weakly bound PetP in cyanobacteria are still unknown.

According to mutagenesis studies, the minimal functional Cyt b_6f should consist of six essential subunits: Cyt *f*, Cyt b_6, the Rieske 2Fe-2S protein, subunit IV, PetG, and PetN. It has been shown that deletion of PetP or two *bona fide* small subunits, PetL and PetM – although they have an impact on the ET properties – keep the Cyt b_6f complex functional (Schneider et al., 2001, 2007a,b; Volkmer et al., 2007), while deletion of any other subunit is lethal. Decreasing b_6f activity or a normal b_6f activity with inability to effectively oxidize an over-reduced PQ-pool seems to have a high impact on the activation/activity and abundance of the Cyt *bd* oxidase: this enzyme can partly take over the role of Cyt b_6f in PQH_2-reoxidation as could be shown by deletion of the subunits PetM and PetC1 from Cyt b_6f (Schneider et al., 2001, 2004a,b; Tsunoyama et al., 2009) and by stress conditions such as high light (HL) or glucose treatment (Berry et al., 2002; Gendrullis et al., 2008), respectively. In this section we shortly summarize the most prominent results on the new PetP subunit (in both *T. elongatus* and *S.*6803) and on the multiple Rieske proteins (in *S.*6803).

PetP as a new subunit of the meso- & thermophilic cytochrome b_6f complex

Highly purified Cyt b_6f complexes from *S.*6803 and *T. elongatus* contain a potential new Cyt b_6f subunit with a molecular mass of 7.2 and 7.1 kDa, respectively, coined PetP, i.e. the 9th Cyt b_6f subunit (Gendrullis et al., 2008; Volkmer et al., 2007). This polypeptide is highly conserved in cyanobacteria and encoded by the open reading frames (ORFs) *ssr2998* and *tsr0524* in *S.*6803 and *T. elongatus*, respectively (Volkmer et al., 2007). Sequence analysis predicted PetP as a cytoplasm-localized soluble protein. The absence of this subunit in all up to now published cyanobacterial structures suggests, that PetP is loosely/temporarily associated with the complex, similar to PetO of the plastid Cyt b_6f complex (Hamel et al., 2000). However, in contrast to PetO, PetP is apparently not a phosphopro-

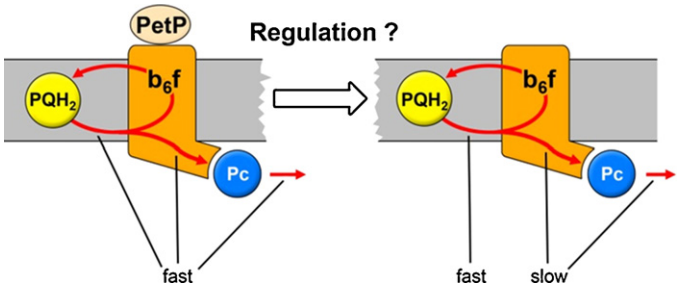

Fig. 2. Cartoon illustrating the impact of PetP on the Cyt b_6f-mediated electron transport. For further details, see text.

tein and not (directly) involved in the regulation of state transitions. *petP* deletion strains from both cyanobacteria perform twofold slower growth rates, especially under HL, in comparison with the corresponding wild types (WT). Also, flash-induced absorption change measurements show that the re-reduction kinetics of both Cyt f^+ and $P700^+$ is slowed down by a factor of 1.7 in the absence of PetP (Gendrullis et al., 2008; Volkmer et al., 2007) whereas the PQ-pool is highly reduced. In combination, both effects clearly indicate an impaired ET through Cyt b_6f, suggesting either a structural or a regulatory role for PetP, which seems also indispensible for achieving/maintaining the maximal ET rate through the complex although it is not an essential subunit (Fig. 2). As important prerequisite for further studies, the heterologous overexpression of PetP in *E. coli* was reported (Gendrullis et al., 2008; Rexroth et al., unpublished).

Multiple Rieske proteins in Synechocystis – impact on physiology?

Among the eight *bona fide* Cyt b_6f proteins, only the Rieske protein is encoded by a *petC* family (i.e. multiple genes) in most cyanobacterial genomes. In contrast to *T. elongatus* and *G. violaceus* which contain – as all eukaryotes – only one *petC* gene, *Nostoc punctiforme*, *Synechococcus* sp. PCC 7002 and *Synechocystis* sp. PCC 6803 show three Rieske isoforms (*petC1-3*) (for a review see Schneider and Schmidt, 2005; for a sequence alignment of the encoded proteins see Fig. 3). Some other cyanobacteria like *Nostoc* sp. PCC 7120 even contain an additional fourth Rieske protein, PetC4, which has a high sequence similarity to PetC1 and PetC2 and apparently plays a role in nitrogen fixation and/or heterocyst formation; it is also proposed to be a redox sensor (Schneider and Schmidt, 2005). For a more detailed characterization, PetC1, PetC2 and PetC3 from *S.*6803 have been heterologously overexpressed in *E. coli* (Schneider et al., 2000, 2002).

Different from *Synechococcus* sp. PCC 7002 (Yan and Cramer, 2003), *petC1* of *S.*6803 can be deleted. Altogether, five different Δ*petC* mutants have been generated in this strain of which only the double deletion mutant Δ*petC1*/Δ*petC2* was lethal (Schneider et al., 2004a,b). This indicates that either PetC1 or PetC2 is essential and that they can partly replace each other (Fig. 4) – in agreement with their high sequence similarity.

An in depth *in vivo* analysis of the various *petC* gene mutants indicated that PetC1, which is phylogenetically close to the group of chloroplast Rieske proteins (Schneider et al., 2002), is the major Rieske protein in *S.*6803. Its deletion results in an eightfold lower electron transport rate through the Cyt b_6f complex – in spite of similar redox potentials of PetC1 and PetC2. This may be due to a lower expression of the Cyt *f* encoding *petA* gene which is organized in the *petCA* operon (Kallas et al., 1988) as is apparent from the low amount of functional Cyt b_6f complexes (PetC2-Cyt b_6f) in this strain (Schultze et al., 2009; Tsunoyama et al., 2009). In consequence, the PQH_2-oxidation is impaired, which in turn results in an inability to perform state transitions, an increased sensitivity towards high light stress, a decreased PS2/PS1 ratio and an enhanced Cyd *bd* activity (Fig. 4; Schneider et al., 2004a,b; Tsunoyama et al., 2009).

While PetC2 shows a low abundance in WT *S.*6803 under normal conditions, a significantly increased transcript accumulation was observed under HL or in the absence of PetC1 (see above). In combination with the exceptional high growth rate of the Δ*petC2* strains under HL conditions, this suggests a role of PetC2 in the long-term light adaptation (Fig. 4; Tsunoyama et al., 2009).

Due to its unique feature, PetC3 can neither substitute PetC1 nor PetC2. Molecular reasons are: (a) its N-terminal end lacks more than ten residues in comparison with PetC1/PetC2, (b) the sequence of its putative transmembrane helix is poorly conserved and (c) the connected hinge region is absent (Fig. 3; Schneider et al., 2002). Also, its midpoint redox potential of 135 mV (*vs.* 300–320 mV for PetC1/PetC2) excludes oxidation of PQH_2, i.e. a role in the linear ET chain (Schneider et al., 2002, 2004a,b). In contrast to PetC1 and PetC2, which are exclusively located in the TM, PetC3 is exclusively located in the CM with all other major Cyt b_6f subunits missing (Aldridge et al., 2008; Schultze et al., 2009). Interestingly, sequence analysis predicts PetC3 to be a lipoprotein (D. Schneider and Y. Tsunoyama, pers. communication). In summary, these properties strongly indicate that PetC3 is not a component of the "classical" Cyt b_6f complex which apparently is missing completely in the CM (see Fig. 1). Nevertheless, current data indicate a regulatory role of PetC3 for the photosynthetic ET and for long-term light adaptation, for instance by activation of the cyclic ET around PS1 under HL conditions (Fig. 4; Tsunoyama et al., 2009).

Heterogeneity of photosystem 2 complexes in the native thylakoid membrane

Photosystem 2 (PS2) is a large multisubunit membrane protein complex located in the thylakoid membranes of cyanobacteria, algae and vascular plants. It catalyzes one of the most important reactions in nature: the light driven oxidation of water. Oxygen is released as a by-product in this reaction, leading to an aerobic atmosphere which was the prerequisite for the development of all animal life on our planet. This reaction is catalyzed by a Mn_4Ca cluster which is located near the luminal side of the complex shielded by the extrinsic proteins PsbO, PsbV and PsbU. Structural studies have provided a detailed static view of PS2 (Ferreira et al., 2004; Guskov et al., 2009; Kamiya and Shen, 2003; Loll et al., 2005). The monomeric complex is assembled of at least 20 protein subunits, of which the two core center proteins D1 and D2 each contain five transmembrane helices and bind most of the redox centers of the intrinsic electron transfer chain, i.e. six chlorophyll *a* (Chl *a*) molecules, two pheophytins, two quinones and a non-heme iron. A third quinone (Q_C) was identified recently next to Q_B (Guskov et al., 2009). Most of the chlorophyll *a* molecules of the complex are bound by the intrinsic antenna proteins CP43 and CP47, which forms six transmembrane helices each. In comparison with other membrane protein complexes, the large number of small subunits, often containing only one transmembrane helix is remarkable, although their function is largely unknown (Shi and Schroder, 2004).

Despite the availability of detailed structural information, only little is known about the dynamic aspects of the PS2 life cycle. The electron transfer reactions in PS2 require a precise positioning of the redox cofactors for proper function and the assembly of the complex has to occur in a highly coordinated way. Moreover, PS2 is continuously attacked by light which leads to a permanent cycle of damage and specific repair. The repair cycle of PS2 requires its partial disassembly to enable degradation and exchange of the damaged D1 protein and insertion of a new D1 copy. During the biosynthesis cycle, D1 is processed at its C-terminus prior to assembly of the donor side, resulting finally in a fully func-

Fig. 3. Sequence alignment of the three PetC proteins encoded in the genome of *S.*6803 (redrawn after Schneider et al., 2004a,b). Conservative substitutions and identical residues are marked by color background and lines around, respectively. The iron-sulfur binding sites (Box I and II) are highlighted in yellow, while amino acids forming putative transmembrane helices are shown as boldface letters. The position of the hinge region is underlined.

tional monomeric complex which dimerizes in the last step of the cycle.

Additionally, the native PS2 complex can be modified in order to cope with stress conditions such as light stress, nutrient deficiency, etc. This has been shown, for instance, by binding of the subunit IdiA to the PS2 acceptor side under iron deficient conditions (Lax et al., 2007).

Here we will focus on transient complexes and factors which are involved in biogenesis, adaptation, maintenance, degradation and repair of PS2.

Heterogeneity of D1 proteins for physiological adaptations – dynamics of stress response

In contrast to higher plants and green algae the cyanobacterial D1 subunit of photosystem 2 is not encoded by a single *psbA* gene but a *psbA* gene family – similar to the Rieske gene family of *Synechocystis* PCC 6803. The amount of members and their gene products varies species-dependant but there are only 4 different types of D1 proteins (D1:1, D1:2, D1m and D1′) classified so far (Mulo et al., 2009).

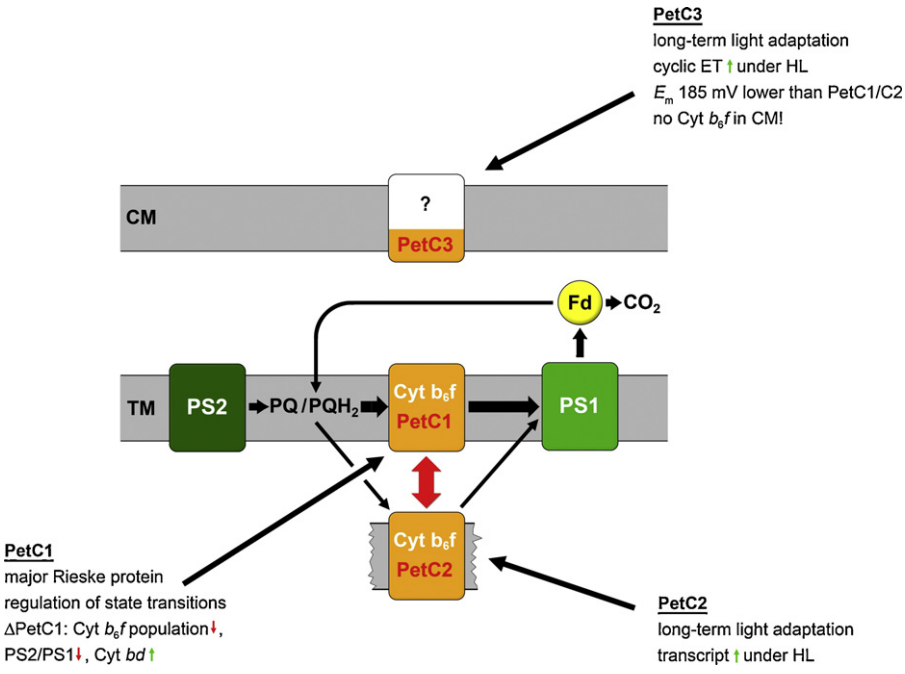

Fig. 4. Cartoon illustrating the PetC heterogeneity in *S.*6803 concerning function and localization of the three Rieske isoforms. Electron transport routes and proposed regulatory pathways are also indicated. For further details, see Tsunoyama et al., 2009, and text.

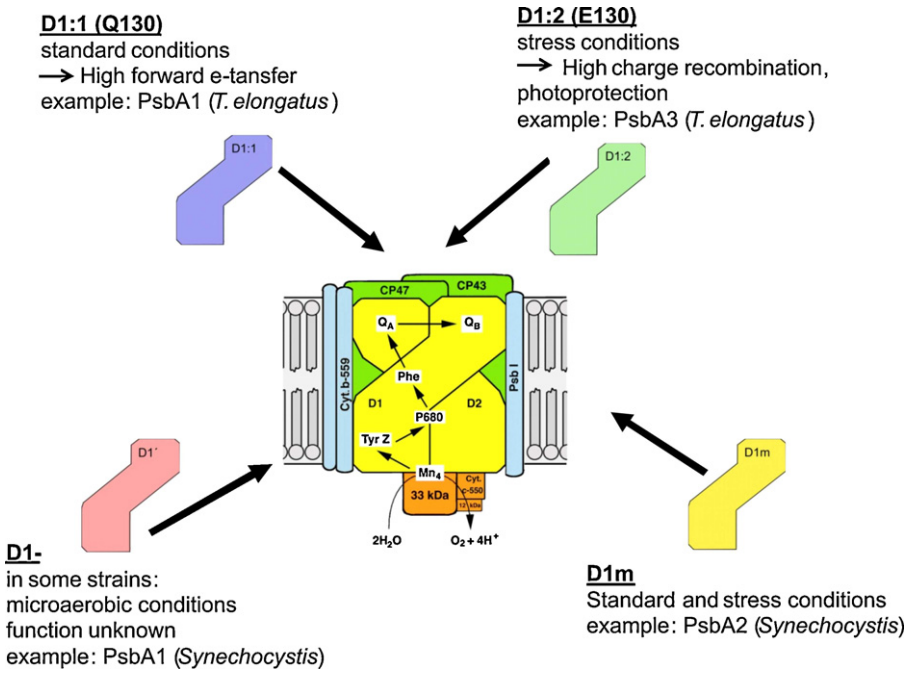

Fig. 5. Schematic model of the exchange between the four D1 isoforms as an adaptation towards stress conditions.

Under standard growth conditions D1:1 as well as D1m are expressed and represent the main D1 form (Fig. 5). In contrast, D1:2 is only expressed under stress conditions (high light, UV light, low temperature) – combined with a repression of D1:1, whereas D1m is still expressed. The D1m expression under standard and stress condition is no functional adaptation to stress but rather guarantees the maintenance of active PS2 complexes by increasing the amount of new D1 in compensation for damaged D1.

The only conserved amino acid exchange between D1:1 and D1:2 occurs at position 130, which is changed from glutamine (in D1:1) to glutamate (in D1:2). Residue Q130 forms a hydrogen bond to pheophytin and by this is at least indirectly involved in the electron transfer across D1 (Loll et al., 2008). The impact of the Q130E exchange on the electron transfer has been studied by knock-out mutants containing either D1:1 or D1:2 as well as by point mutants at this specific residue (Cser and Vass, 2007; Rappaport et al., 2002; Sander et al., 2010). In both cases D1:2 or mutants containing E130 showed a higher resistance against photoinhibition, possibly due to a higher charge recombination via alternative electron routes which lead to less photodamage.

Under those conditions, the divergent D1′ form is almost silent and has only been shown to be expressed under microaerobic conditions in some strains (Sicora et al., 2008; Summerfield et al., 2008). However, the functional role of D1′ is still elusive up to now.

The cyanobacterial D1 gene family does not always consist of all four D1 forms. *Synechocystis* PCC 6803, *Synechococcus* PCC 7942 and *T. elongatus* have *psbA* gene families with three members each but their expression pattern differs significantly. In *Synechocystis*, *psbA1* is only expressed in traces under standard growth conditions but may be expressed under microaerobic conditions (D1′ form), while *psbA2* and *psbA3* encoding for identical proteins make up the majority of the *psbA* pool both under standard and stress (high light) conditions (D1m form). There is no D1:2 form with E130 in *Synechocystis* (Sicora et al., 2006). In contrast, *Synechococcus* PCC 7942 and *T. elongatus* both have at least one D1:2 form (PsbA2 and PsbA3 in *Synechococcus* PCC 7942 and PsbA3 in *T. elongatus*) induced under stress conditions, which shows distinct functional differences compared to the D1:1 form (PsbA1 in both cases) (Campbell et al., 1998; Kulkarni and Golden, 1994). Fluorescence decay and thermoluminescence measurements indicated a higher charge recombination rate in PS2 complexes containing D1:2 (Kos et al., 2008; Sander et al., 2010). This might lead to a decreased probability of repopulation of the excited P680, which could be engaged in production of harmful reactive oxygen species (ROS). Additionally, PsbA2 of *T. elongatus* can be regarded as D1′ form, as it has been shown to be expressed under microaerobic conditions (Sicora et al., 2008), whereas no D1′ exists in *Synechococcus* 7942.

Similar to the Rieske gene family, the cyanobacterial *psbA* gene family is an excellent example for the fast and efficient adaptation of an organism to changing environmental conditions. This adaptation is at least twofold: On the one hand the amount of active PS2 complexes is maintained under stress conditions by enhancing the number of the most labile component, the D1 subunit; on the other hand its function is slightly triggered by an exchange of functionally distinct isoforms (Sander et al., 2010).

Identification of PS2-intermediate complexes with function in biogenesis, repair and degradation: The role of Psb27

Due to its transient binding mode, the 11 kDa protein Psb27 is not present in the recently published PS2 crystal structures (Ferreira et al., 2004; Guskov et al., 2009; Kamiya and Shen, 2003; Loll et al., 2005, 2008). It was first identified in a PS2 preparation from a *Synechocystis* PCC 6803 mutant strain lacking PS1 (Ikeuchi et al., 1995). Together with Psb27, another PS2 subunit, Psb28, was enriched in this preparation. Kashino et al. (2002) later confirmed the presence of Psb27 in a his-tagged PS2 preparation from the same organism, although in this case Psb27 was present in substoichiometric amounts. Originally this protein was named 11 kDa protein or PsbZ, but this caused confusion as one of the smaller PS2 subunits was also referred to as PsbZ (Swiatek et al., 2001). The new nomenclature, i.e. Psb27 (Kashino et al., 2002) is now established, but based on new results (e.g. transient binding mode, see below) another renaming may be required. Homologues of psb27 are present in all known genomes of oxygenic photosynthetic organisms with the exception of *Gloeobacter violaceus*. Interestingly, this primitive cyanobacterium is lacking a separate internal thylakoid membrane system (Rippka et al., 1974).

Spatial distribution of PS2 biogenesis and repair

Cyanobacteria, like all gram negative bacteria, contain three different membrane systems: outer-, cytoplasmic- and thylakoid membrane. Since Zak et al. reported preassembled PS2 complexes in the cytoplasmic membrane (Zak et al., 2001) there is an ongoing debate about the spatial distribution of PS2 complexes in cytoplasmic and thylakoid membrane. Additionally these authors could show that the C-terminal processing peptidase (CtpA) for the D1 subunit is located exclusively in the cytoplasmic membrane. In consequence, every newly synthesized copy of D1 has to be located – at least for this step – in the cytoplasmic membrane during biogenesis or repair, which is supported by the periplasmic location of PratA, a factor required for the efficient C-terminal processing of D1 (Klinkert et al., 2004). Additionally PratA might play a role during transfer between the two membrane systems (Schottkowski et al., 2009). On the other hand, such a model implies a tremendous traffic between cytoplasmic and thylakoid membrane which is in contrast to the missing direct connection (Liberton et al., 2006; Nevo et al., 2007) and the lacking evidence for an efficient turnover of the lipid phase (Schneider et al., 2007a,b) between the two membrane systems.

Inactivation of *ctpA* in *Synechocystis* PCC 6803 resulted in accumulation of preassembled Psb27-PS2 complexes (Roose and Pakrasi, 2004), indicating a transient interaction with PS2 during C-terminal processing of D1. However, direct evidence is still missing.

Characterization of Psb27 deletion mutants

Several deletion mutants of Psb27 have been studied in various pro- and eukaryotic organisms: out of two homologues of Psb27 in *Arabidopsis thaliana*, one (At1g03600, Psb27-H1) was found by proteomic analysis of the chloroplast lumen (Peltier et al., 2002); although it is apparently involved in recovery after photoinhibition (Chen et al., 2006), a loss of function mutant showed that it is not essential for PS2 activity. The other copy of Psb27 in this organism (At1g05835, Lpa19, Psb27-H2) is expressed only at low levels (Chen et al., 2006) but it seems to be essential for PS2 biogenesis (Wei et al., 2010). C-terminal processing of D1 is impaired in the Psb27-H2 deletion mutant which results in a strong reduction of the PS2 content. In contrast, inactivation of *psb27* in *Synechocystis* PCC 6803 showed that functional complexes could still be assembled. However, under nutrient deficient conditions which require an efficient Mn_4Ca cluster assembly for survival, Psb27 provides a selective advantage for cyanobacterial cells (Roose and Pakrasi, 2008), i.e. Psb27 seems to facilitate manganese incorporation by preventing the premature association of the lumen-exposed, extrinsic PS2 proteins. A *psb27* deletion mutant of *T. elongatus* showed impaired growth under stress conditions (Grasse et al., unpublished results). Especially low temperature stress (30 °C) in combination with high light stress (500 μE) had a large impact on the survivability of the mutant cells in comparison with wild type. Double deletion of Psb27 and PsbM, which forms – together with PsbT and PsbL – the connecting interface of the PS2 dimer, prevented PS2 assembly, whereas deletion of PsbM alone had only a slight effect (Bentley et al., 2008). In contrast, double deletion of Psb27 and PsbT does not affect assembly but impairs the recovery of PS2 after photoinhibition (Bentley et al., 2008).

Isolation and characterization of transient PS2 complexes

The thermophilic cyanobacterium *T. elongatus* is the ideal source for stable membrane protein complexes. Our strategy to purify distinct PS2 populations *via* a two step chromatographic system yielded four different PS2 complexes with different oxygen evolving activity: inactive monomer, active monomer, active dimer and less active dimer (Nowaczyk et al., 2006). The inactive monomer lacks the three extrinsic proteins PsbO, PsbU and PsbV and instead contains Psb27 bound to the luminal side. This complex contained no oxidizable Mn at the donor side of PS2 and showed an impaired forward electron transfer between Q_A and Q_B, which explains the lack of oxygen evolution (Mamedov et al., 2007). On the other hand, charge separation is not impaired (Nowaczyk et al., 2006) and neither the presence of Psb27 nor the absence of the $CaMn_4$ cluster affected the protein matrix around Y_D or the acceptor side quinones (Mamedov et al., 2007). Mass spectrometry analysis of the isolated PS2-Psb27 complex revealed a specific N-terminal lipid modification of Psb27 with diacylglycerol (with one octadecanoic and one hexadecanoic fatty acid residue) bound *via* thioether linkage to the N-terminal cystein residue. Additionally, the N-terminus is modified by a single amide-linked hexadecanoic acid residue (Nowaczyk et al., 2006) which is typical for bacterial lipoproteins in the periplasmic space of gram negative bacteria. In the case of Psb27, this lipid modification mediates a very strong interaction with PS2. In contrast to the extrinsic proteins, Psb27 cannot be removed by salt and/or detergent treatment. Moreover, in the presence of Psb27 the extrinsic PS2 subunits cannot bind to the isolated PS2-Psb27 complex (Nowaczyk et al., 2006). How Psb27 is released and replaced by PsbO during biogenesis and repair is still an open question.

The structure of Psb27 in solution – implications for transient binding to PS2

The 3D structure of Psb27 was recently solved by NMR spectroscopy (Cormann et al., 2009a,b; Mabbitt et al., 2009). The core protein is a right-handed four-helix bundle with an up-down-up-down topology and the electrostatic potential of the surface generated by the amphipathic helices shows a dipolar distribution. Size, shape and surface potential of Psb27 fit perfectly to a groove on the surface of PS2 mainly formed by the CP47 and D2 subunits as concluded from a docking model (Fig. 6) (Cormann et al., 2009a,b). This potential binding niche, which is completed by the C-terminal region of the D1 protein and a loop of the luminal domain of CP43, is occupied by the PsbO subunit in the PS2 crystal structure (Guskov et al., 2009). The proposed localization of Psb27 is in good agreement with biochemical data as Psb27 seems to prevent the binding of PsbO in order to facilitate the assembly of the Mn_4Ca Cluster (Roose and Pakrasi, 2008). Moreover, this model with the lipid modified N-terminus being located towards the monomer-membrane interface could explain how Psb27 is released from the complex: In contrast to the situation in the detergent micelle (Nowaczyk et al., 2006), Psb27 could be released much easier in the native lipid environment – at least in the monomer (Fig. 6a). In the dimer (Fig. 6b) the lipid anchor is placed between the two monomers, but it might be part of a lipid cluster at the monomer–monomer interface (Guskov et al., 2009) with contact to the lipid phase of the surrounding membrane.

The life cycle of PS2

The inactive monomeric Psb27–PS2 complex is one example for a transient intermediate in the life cycle of PS2. In order to analyze the time-dependent distribution of this and the other PS2 subcomplexes, ^{15}N pulse label experiments in combination with mass spectrometry were used (Nowaczyk et al., 2006). They could show that the monomeric Psb27–PS2 complex is an early assembly intermediate mainly in the repair cycle, but also for the biogenesis of PS2 (see Fig. 7). Therefore this subcomplex is certainly important for bridging the events of C-terminal processing of D1 on the one side and activation of water-splitting activity on the other. In extension of this model we could recently also isolate a new dimeric Psb27–PS2 complex which accumulates under specific stress conditions (for instance low temperature stress or high light stress) and seems to be involved in the replacement of damaged D1 (Grasse et al., unpublished results). Labeling studies showed that this com-

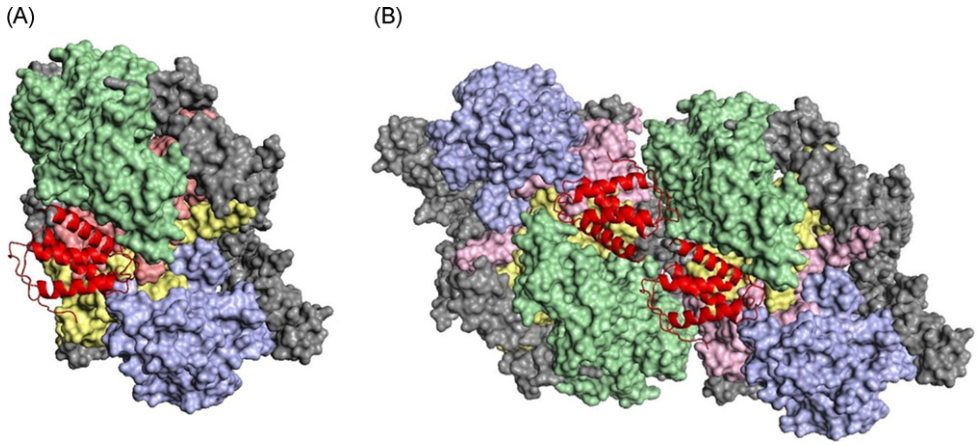

Fig. 6. Structural model of Psb27 bound to (A) monomeric and (B) dimeric PS2 based on the PS2 crystal structure (Guskov et al., 2009) and *in silico* docking simulations (Cormann et al., 2009a,b). Psb27 (red) is probably localized in a groove formed by CP47 (green) and D2 (salmon). However, the C-terminal domain of D1 (yellow) and the luminal domain of CP43 (blue) also seem to contribute to the binding of Psb27.

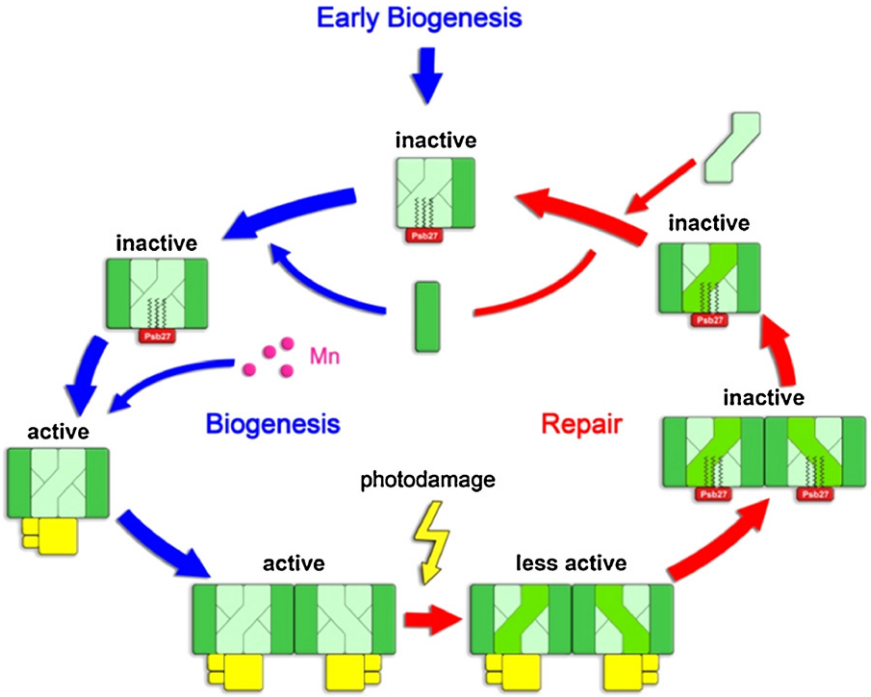

Fig. 7. Transient complexes involved in the biogenesis and repair cycle of PS2. During biogenesis (left side, blue arrows) Psb27 is attached to a PS2 precomplex which is inactive in water splitting. After incorporation of manganese and release of Psb27 the extrinsic proteins PsbO, PsbU and PsbV are attached to gain an active complex. Damage of D1 (right side, red arrows) leads to replacement of the extrinsics by Psb27 and a new D1 copy is introduced. The presence of a monomeric Psb27–PS2 complex with damaged D1 is postulated but it was not isolated yet. After exchange of D1 the complex is activated again. The steps of early biogenesis and the replacement of damaged D1 might take place at the cytoplasmic membrane but there is no direct evidence yet that Psb27 is also present in the CM.

plex appears later than the fully active dimeric complex which is in agreement with its predicted repair function. Fig. 7 summarizes all present results for the transient PS2 complexes.

Conclusion

Structure–function and life-time-analysis of intermediate membrane-bound complexes is important for understanding the dynamics of membrane-located processes and their coordination in a regulatory photosynthetic network. Future directions should also include processes occurring in the CM and explore the possibly of their communication with the TM. A prominent example here is the still essentially unknown function of the Rieske PetC3 isoform. While we were able to show, that this subunit is certainly not part of a "regular" Cyt b_6f complex in the CM, a regulatory function with impact on ET processes in the TM was strongly suggested by our results. Similarly, the *psbA*-gene family of PS2 provides modulations of PS2-internal and external ET responding to changing environmental conditions – especially light as shown here.

In addition to the analysis on the isolated protein level, these studies have to be routinely extended to the membrane level (where the monomer-/oligomer dynamics of all major membrane proteins has already been established), and finally to observations on the whole cell level, i.e. a systemic approach. With this, also the role of an increasing number of new subunits can be elucidated, which transiently interact with intrinsic membrane proteins such as Cyt b_6f and PS2 and which may have an important regulatory function as shown here for subunits PetP and Psb27 of Cyt b_6f and PS2, respectively.

Acknowledgements

We thank Ursula Altenfeld, Melanie Arendt, Claudia König and Regina Oworah-Nkruma for excellent technical assistance and the German Research Council (DFG) for continuous support of our project C1 within SFB 480.

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