Functional flexibility of photosystem I in cyanobacteria

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Cyanobacteria

The autotrophic (auto = "self" tropho = "nourishment", Greek) cyanobacteria are the largest group of phototrophic eubacteria. The cells of these bacteria are often much larger than those of other bacteria, which in the past led this group to be classified as algae rather than bacteria. In fact, cyanobacteria are still sometimes referred to as blue-green algae. Cyanobacteria share with plants the ability to carry out oxygenic photosynthesis, which is the process of using light energy to oxidize water (forming molecular oxygen) and to generate electrons (and protons) that eventually can be used to fix carbon dioxide into sugars.

The photosynthetic apparatus is localized in the thylakoids (Fig. 1). There are observations suggesting that the thylakoid membrane is contiguous with the plasma membrane (Gantt, 1994), but functional components of the photosynthetic apparatus are only found in the thylakoids (Peschek et al., 1989; Schmetterer, 1994). The thylakoids are oriented differently in different groups of cyanobacteria. Many unicellular and some filamentous cyanobacteria have multilayers of thylakoid membranes close to the periphery. Recently, complete sequencing of the genome from the ancient cyanobacterium Gloeobacter violaceus PCC 7421 was finalized. G. violaceus is a unicellular cyanobacterium with unusual characteristics (Nakamura et al., 2003). This strain lacks thylakoids and has phycobilisomes that are attached to the plasma membrane. Phylogenetic analysis using multiple criteria strongly suggests that this strain is an early branching member of the cyanobacterial lineage (Nakamura et al., 2003) and so it can be considered to be the most primitive cyanobacterium that has yet been studied.

Because cyanobacteria are so widely distributed, from the poles to the tropics and in oceans, lakes, on rocks and in soils, they make a major contribution to the evolution of the earth's atmosphere to its present state. They fix carbon dioxide from the atmosphere, and so contribute to reducing global warming, and they release oxygen back into the atmosphere.

The cyanobacteria have also been tremendously important in shaping the course of evolution and ecological change throughout earth's history. The question how old are cyanobacterial fossils is widely discussed nowadays. The commonly accepted oldest known fossils of living matter (cyanobacteria from Archaean rocks of western Australia, dated 3.5 billion years old (Schopf, 1993)), are criticized as disordered carbonaceous materials of indeterminate origin (Garcia-Ruiz et al., 2003; Brasier et al., 2004). The oldest proven fossils which are well preserved and have morphological and ecological similarity to modern cyanobacteria are the about 2.0 billion years old fossils from Belcher Islands, Canada, (Hofmann, 1976). This makes cyanobacteria the earliest known group of organisms
Figure 1. Transmission electron microscopy of *Synechocystis* sp. PCC 6803 cells.
Adapted from Electron Microscopy Facility, School of Life Science, ASU. The light reactions take place in the thylakoid membrane. In eukaryotes (plants and algae), the thylakoid membranes are located in chloroplasts and often are found in membrane stacks (grana). The chloroplast is a relatively large complex organelle with a double membrane. Cyanobacteria do not have chloroplasts and photosynthetic pigment-protein complexes are in the thylakoid membranes that are directly immersed in the cytoplasm. The thylakoid membranes are lipid bi-layers in which the proteins involved in photosynthesis (and in cyanobacteria those for respiration as well) are embedded. These membranes run in pairs, like long stretched vesicles, that sometimes seem continuous and mimic a snail shell in appearance. The thylakoid membrane vesicles create two compartments: an inner thylakoid membrane space called lumen and a compartment in which the thylakoid vesicles are embedded called stroma in chloroplasts and cytoplasm in cyanobacteria. The cytoplasm is the site of the light-independent (dark) reactions of photosynthesis (the so called Calvin cycle). The inner thylakoid membrane space, called lumen, bears the manganese cluster involved in the lysis of water by PSII, and carriers for electrons in transfer between the PQ-cytochrome $b_{6}f$ complex and PSI. A major difference between chloroplasts and cyanobacteria is the light harvesting antennae; these are all in the hydrophobic membrane in chloroplasts; but in cyanobacteria the major light harvesting antenna, called phycobilisomes, are exposed on the hydrophilic cytoplasmic side of the thylakoid membrane.

that contributed greatly to the formation of the oxygen that we breathe. Even today, cyanobacteria and oxychlorobacteria (prochlorophytes) are responsible for a large portion of the photosynthetic production in the open ocean.

Cyanobacteria can change remarkably in appearance, also depending on the environmental conditions. Until now, the largest cyanobacterial diversity has been found in freshwater, but cyanobacteria are widespread in marine environments and in damp soil as well. Many can survive in extreme environments in a wide range of temperatures, from the -4°C (Antarctic) to 72°C in hot springs, in aerobic and anaerobic habitats and from acidic (pH 3.5) to basic (pH 11) conditions. Cyanobacteria include unicellular and multicellular forms and can be divided into 5 different groups based on their morphology and genetic
properties: I) Single cells or cell aggregates; II) Pleurocapsalean: reproduce by formation of small spherical cells called baeocytes produced through multiple fission; III) Oscillatorian: filamentous cells that divide by binary fission in a single plane; IV) Nostocalean: filamentous cells that produce heterocysts; V) Branching: cells divide to form branches (Rippka et al., 1979; Waterbury and Rippka, 1989). Cyanobacteria can occur as symbionts of protozoans, diatoms, lichen-forming fungi and vascular plants; they are also known to contain toxin-producing species forming blooms that are hazardous for life. Cyanobacteria are unique in that they can perform oxygenic photosynthesis and respiration simultaneously in the same compartment and some species can fix molecular nitrogen in an oxygenic environment via temporal separation of the oxygen-producing photosynthetic process from oxygen-labile nitrogen fixation. This combination of metabolic pathways is unusual and this metabolic flexibility may be responsible for the evolutionary hardness of the cyanobacteria and their ability to adapt and survive in a broad spectrum of conditions.

All cyanobacteria contain chlorophyll \( a \) and most contain phycobilins, which are both heme derivatives. The most prevalent phycobilins are phycocyanin and phycoerythrin, which give the cells a typical blue-green to greyish-brown colour. A few genera, however, lack phycobilins and have chlorophyll \( b \) as well as \( a \), giving them a bright green colour (Prochloron, Prochlorothrix and Prochlorococcus). These were originally grouped together as the prochlorophytes or oxychlorobacteria, but since phylogenetically they are interspersed among the cyanobacteria, they appear to have developed in several different lines of cyanobacteria (Palenik and Haselkorn, 1992; La Roche et al., 1996).

It is believed that the first chloroplasts found in eukaryotes (algae and higher plants) were ancient cyanobacteria that were taken up by other cells. This endosymbiotic hypothesis is supported by various structural and genetic similarities. Primary chloroplasts are found in green plants and most green algae, where they contain chlorophyll \( b \), and among the red algae and glaucophytes, where they contain phycobilins. Other algae likely took their chloroplasts from these forms by secondary endosymbiosis or ingestion. The question of whether chloroplasts had a single phylogenetic origin or developed in multiple lines has not yet been settled.

Since this thesis deals with photosynthesis with emphasis on photosystem I (PSI) studies with cyanobacteria, use of Synechocystis sp. PCC 6803 has many advantages as an excellent experimental system. The genome of Synechocystis sp. PCC 6803 is the first completely sequenced genome of a photosynthetic organism (Kaneko et al., 1996). Since then, genomes of additional cyanobacterial species have been sequenced. Synechocystis
offers the advantages of an accessible genomic database and a relatively small genome size, which together with the possibility of integration of foreign DNA via homologous recombination (Shestakov and Khuyen, 1970) make cloning procedures for *Synechocystis* sp. PCC 6803 straightforward. Additionally, the absence of accessory membrane-bound light-harvesting complexes and the presence of a trimeric organization of PSI allow rapid purification of PSI complexes that can be used in sophisticated spectroscopic investigations of photosynthetic processes. This, together with simple nutrient requirements for growth, has rendered *Synechocystis* into a favourite model for solving the structure-function relationships and acclimation strategies of the photosynthetic machinery.

**Oxygenic photosynthesis**

Photosynthesis is one of the oldest and most fascinating biological processes on earth. Photosynthesis is the biological conversion of light energy to chemical bond energy that is stored in the form of organic carbon compounds. Photosynthesis can be written as an oxidation-reduction reaction of the general form:

\[
2 \text{H}_2\text{A} + \text{CO}_2 + \text{Light} \rightarrow (\text{CH}_2\text{O}) + \text{H}_2\text{O} + 2 \text{A}
\]

All photosynthetic bacteria except cyanobacteria and prochlorophyta are incapable to evolve oxygen. In the latter organisms compound A is, for example, an atom of sulphur and the pigments involved are bacteriochlorophylls (Blankenship *et al.*, 1995; Van Niel, 1941). Plants, green algae, cyanobacteria and prochlorophytes perform oxygenic photosynthesis in the so-called light reaction:

\[
2 \text{H}_2\text{O} + \text{CO}_2 + \text{Light} \rightarrow (\text{CH}_2\text{O}) + \text{H}_2\text{O} + \text{O}_2,
\]

where Chl *a* is the ubiquitous plant pigment chlorophyll *a*. Chlorophyll *a* catalyses a series of reactions whereby light energy is used to oxidize water:

\[
2 \text{H}_2\text{O} + \text{Light} \rightarrow 4 \text{H}^+ + 4 \text{e}^- + \text{O}_2,
\]
yielding gaseous molecular oxygen.

The oxidation of water is accompanied by a reduction reaction resulting in the formation of NADPH:

\[
2 \text{NADP}^+ + 2 \text{H}_2\text{O} \rightarrow 2 \text{NADPH} + 2 \text{H}^+ + \text{O}_2
\]

(oxidized form) (reduced form) (oxygen)

The latter reaction is linked or coupled to yet another reaction resulting in the formation of a highly energetic compound, called adenosine triphosphate (ATP). As this reaction involves the addition of a phosphate group (labeled as P) to a compound called adenosine diphosphate (ADP), during the light reaction, it is called photophosphorylation:

\[
\text{ADP} + \text{P}_i \rightarrow \text{ATP}
\]
General Introduction

The light reaction is a process by which organisms “capture and store” radiant energy as they produce oxygen gas. This energy is initially stored in the form of chemical bonds of the compounds such as NADPH and ATP and then used to reduce carbon dioxide to glucose. Reduction of carbon dioxide does not require light and this reaction is often referred to as the “dark reaction”.

\[ 6 \text{CO}_2 + 24 \text{H}^+ + 24 \text{e}^- \rightarrow C_6\text{H}_{12}\text{O}_6 + 6 \text{H}_2\text{O} \]

Electron Transfer

**Photosystem II and the cytochrome b₆f complex**

The photosynthetic machinery is embedded in the thylakoid membrane, which is contained in the chloroplast in plants and in the intracellular space (cytoplasm) in cyanobacteria. In oxygenic photosynthesis, three integral membrane protein complexes (photosystem II (PSII), the cytochrome b₆f complex and photosystem I (PSI)) are involved in electron transport and generate the transmembrane electrochemical proton gradient used for energy transduction by a fourth membrane protein complex, the ATP-synthase (Fig. 2).

Photosynthesis starts with the simultaneous excitation of special reaction centre (RC) chlorophyll a molecules in PSII and PSI. The excitation energy comes either from directly absorbed light or (most often) by energy transfer from adjacent pigment molecules in protein complexes called antennas. These “antenna” pigment molecules (chlorophylls and carotenoids) absorb light energy and then transmit it by inductive resonance from one molecule to the next, finally to the RC. Excitation is over within a few femtoseconds (10⁻¹⁵ s). [Note: a second has as many femtoseconds in it as 31 million years have seconds].

The oxygen evolution occurs in the PSII complex, which contains in its reaction centre (RC) a unique kind of chlorophyll known as P₆₈₀ (primary electron donor absorbing light at 680 nm). The excited state of P₆₈₀ (P₆₈₀⁺) is able to pass the electron to a specific acceptor molecule, phaeophytin (a chlorophyll molecule missing the central Mg²⁺), precisely positioned for rapid charge separation. P₆₈₀⁺ is an extremely strong oxidant with an oxidizing potential of more than 1 V. This high redox potential is needed to oxidize water, with help of a catalytic centre composed of four manganese atoms located at the PSII reaction centre protein designated as D1 (Debus, 1992). A special tyrosine residue (Yz) of D1 donates an electron to P₆₈₀⁺, regenerating P₆₈₀, ready to accept the next excitation (Tommos et al., 1994). The tyrosine is re-reduced by an electron from a cluster of Mn atoms bound to the luminal side of the PSII complex. The Mn cluster forms part of the “water-splitting complex” and acts as a charge accumulator - donating a series of 4 e⁻ to 4 successive PSII charge-separation cycles, and then mediating a concerted extraction of 4 e⁻ from 2 H₂O, releasing O₂ and 4H⁺
Figure 2. The integral membrane protein complexes responsible for the light reactions and ATP synthesis in oxygenic photosynthesis. Adapted from Kurisu et al. (2003). The structures are from thermophilic cyanobacterial sources: Synechococcus elongatus for the reaction centers of PSI and PSII, and Mastigocladus laminosus for the cytochrome \( b_{f} \) complex. PQ-plastoquinone, PQH\(_{2}\)-plastoquinol, PC-plastocyanin, Fd-ferredoxin, FNR-ferredoxin-NADPH\(^{-}\) reductase. Solid lines indicate electron and proton transfer, dashed line represents PSI-driven cyclic electron flow.

into the thylakoid lumen. The concerted oxidation is energetically essential and avoids production of dangerously reactive oxygen intermediates.

From phaeophytin the liberated e\(^{-}\) is passed through a quinone A (\( Q_{A} \)) to plastoquinone (PQ) at the \( Q_{B} \) site, the first mobile element of the electron transfer chain. After receiving a second electron, from the next photocycle of \( P_{680}^{+} \), PQ\(_{2}^{2-}\) takes up two protons (\( H^{+} \)) from the stromal space to form plastoquinol (PQH\(_{2}\)). PQH\(_{2}\) then diffuses out of its binding site adjacent to PSII, moving within the hydrophobic environment of the membrane plane. Electrons are passed from PQH\(_{2}\) to a membrane-bound cytochrome-containing complex, cytb\(_{f}\). Within the \( b_{f} \) complex, one electron is transferred to a high potential electron transfer chain, consisting of the Rieske iron-sulfur protein and cytochrome \( f \) on the electropositive (lumen, i.e. thylakoid interior) side of the membrane. The other electron donated by PQH\(_{2}\) to cytb\(_{f}\) is transferred to a low potential cytochrome \( b_{o} \), which equilibrates via a second high potential \( b \)-type cytochrome, situated at the cytoplasmic side of the thylakoid membrane. This electron is transferred to PQ again, in conjunction with an electron from PSII, which closes the Q and \( b \) cycles. Note that only the non-ionic forms of PQ (either PQ as such, or PQH\(_{2}\)) can pass through the hydrophobic lipid containing thylakoid membrane. The final result of this cycle, also known as the cytochrome or \( Q \) cycle (Mitchell, 1975) is the release of two protons to
the aqueous lumen phase, which creates an electrochemical proton gradient, called proton-motive force, that is used for ATP synthesis, and the transfer of one electron towards PSI via a soluble electron carrier in the lumen. The recently obtained crystal structure at 3 Å of the dimeric $b_{0f}$ complex from the thermophilic cyanobacterium *Mastigocladus laminosus* provides a high-resolution structural description of the family of cytochrome $bc$ complexes and describes the structural changes that occur in and around the central quinone exchange cavity in response to the demands of photosynthesis (Kurisu et al., 2003).

**Photosystem I**

In cyanobacteria the soluble electron carrier in the lumen may be plastocyanin or cytochrome $c_{553}$, depending on the species and on the availability of copper (plastocyanin is a copper containing enzyme). This mobile carrier serves as an electron donor to the PSI reaction centre (RC). Photons are absorbed by PSI, converting the unique P$_{700}$ chlorophylls (primary electron donor absorbing light at 700 nm) to P$_{700}^-$, which again is a strong reducing agent (-1.1 V). The oxidized form of the reaction centre chlorophyll is formed by a light-induced transfer of an electron from PSI via a series of electron carriers ($A_{0}$, a chlorophyll monomer, $A_{1}$, phylloquinone, $F_{X}$, $F_{A}$ and/or $F_{B}$, iron sulfur clusters) to ferredoxin (Fd) (Chitnis, 1996), a water-soluble mobile carrier on the cytoplasmic/stromal side of the membrane and eventually to NADP$^+$. Two ferredoxin molecules can reduce NADP$^+$ to NADPH, via a bound semi-quinone of the flavoprotein ferredoxin-NADPH reductase. NADPH is used as redox currency for many biosynthesis reactions (e.g. CO$_{2}$ fixation). Ferredoxin also participates directly in some reductive pathways, notably N$_{2}$ fixation, nitrate- and sulfate reduction.

The light energy harvested by PSI and PSII, and then processed via the electron transfer system, is creating the proton electrochemical potential across the photosynthetic membrane. Two types of reactions participate in this process: (1) the release of protons during the oxidation of water by PSII and the translocation of protons from the outer aqueous phase to the inner aqueous phase by the coupled reactions of PSII and the cytochrome $b_{0f}$ complex in reducing and oxidizing plastoquinone on opposite sides of the membrane; (2) primary charge separation at the reaction center and in the $b_{0f}$ complex drives an electron across the photosynthetic membrane, which creates an electric potential across the membrane. Together, these two forms of energy make up proton electrochemical potential across the photosynthetic membrane which is the sum of the pH difference and the electrical potential difference across the membrane (for example, during photosynthesis the outer pH is typically near 8 and the inner pH is typically near 6, and can reach even pHi 4 in high light, giving a pH difference of at least 2 across the membrane that is equivalent to 120 mV).
Photophosphorylation

The protons from the lumen are passed back to the stroma via a multi subunit protein complex known as ATP synthase, catalyzing ATP synthesis with simultaneous conversion of proton electrochemical energy into free energy of chemical bonds).

ATP synthase is a multiprotein complex with an average molecular mass of about 600 kDa. It consists of a 200 kDa CF₀(CF – coupling factor) integral membrane protein part and a 400 kDa CF₁ peripheral protein part attached to CF₀ on the stromal side. The CF₀ part spans the photosynthetic membrane and forms a proton channel through the membrane. The CF₁ part is attached to the top of the CF₀ on the outside of the membrane and is located in the aqueous phase. The crystal structure revealed that CF₁ is composed of a cylinder of 6 subunits, alternating alpha and beta subunits, which form a ring around an asymmetrical gamma subunit. Facilitated diffusion of protons causes the CF₀ particle to rotate, dragging along the gamma subunit of CF₁, while the major CF₁ subunits are fixed in place (Abrahams et al., 1994). This rotation forces conformational changes in the CF₁ particle, eventually leading to the synthesis of ATP. The CF₀F₁ particle is a reversible ATP synthase. Large enough quantities of ATP cause this particle to create a proton gradient. Under physiological conditions, the thylakoid-membrane-bound-ATP-synthase generally functions in creating ATP, while using the proton motive force from the electron transfer chain as a source of energy. The overall process of energy transduction in this fashion is termed photo-phosphorylation.

The concerted action of PSII and PSI renders NADPH and ATP according to the Z-scheme and is termed linear (or non-cyclic) oxygenic photosynthetic electron transfer (Fig. 3). [Note: the form of the diagram commonly used to illustrate reduction potential changes during the events initiated by PSI and PSII gives rise to its designation as the Z-scheme]. This reaction, also known as the “Hill reaction” (Hill, 1937), represents photosynthetic electron transfer driven by both PSII and PSI coupled to evolution of oxygen and requires an electron acceptor for PSI (ferredoxin or flavodoxin; the latter in cases of iron-limitation).

In an alternative pathway for photosynthetic energy transduction, called cyclic photophosphorylation, reduced ferredoxin does not lead to the reduction of NADP⁺. Instead, electrons are transferred back to the cyt$b_6$f complex, possibly via the plastoquinone pool. The mechanism of cyclic photophosphorylation may be closely related to the above mentioned concerted electron transfer between high potential cytochrome $b_6$, $Q_B$ of PSII and PQ. Processing might involve the plastoquinone pool or high potential cytochrome $b_6$ to act as electron acceptor, directly from ferredoxin or via intermediary components (see Chapter 3). However, cyclic photosynthetic electron flow is driven by the light energy harvested by PSI
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Figure 3. The Z-Scheme Diagram of Photosynthesis. Adapted from Veit and Govindjee (www.molecadv.com). The Z - scheme represents the movement of an electron during photosynthesis, with respect to the free energy of the electron. Electrons are removed from water and passed from molecule to molecule until they are used to reduce NADP' to from NADPH. Abbreviations used are: Mn - a manganese complex containing 4 Mn atoms, bound to the PSII reaction center; Tyr - a particular tyrosine in PSII; P 680 - for the reaction center chlorophyll in PSII; P 680* - excited P 680 carrying the energy of a photon of light; Pheo - pheophytin; Q a - a plastoquinone molecule tightly bound to PSII; Q b - another plastoquinone molecule that is loosely bound to PSII; FeS - rieske iron sulfur protein; Cyt f - cytochrome f, Cyt b 6 (L and H) for cytochrome b 6 (of low and high potential); PC - the copper protein plastocyanin; P 700 - the reaction center chlorophyll of PSI; P 700* - excited P 700 with the energy of a photon of light; A m - a special chlorophyll a molecule (primary electron acceptor of PSI); A 1 - a phylloquinione (vitamin K) molecule; F X, F A, and F B - three separate iron sulfur centers; FD - ferredoxin; FNR - ferredoxin-NADP-oxidoreductase (FNR).

only; it requires no input of free energy by PSII and thus involves no production of O 2. Cyclic electron transfer by PSI gives rise to cyclic photophosphorylation: PSI is effectively used to drive a proton pump, which is contributing to and possibly controlling maintenance of a stable phosphate potential (a way for expression of the energy status in the cell).

Another reaction that also can generate ATP at the expense of light energy is the Mehler reaction or pseudocyclic transport. This process involves electron transfer from the donor side of PSII to the reducing side of PSI, where ferredoxin can reduce oxygen to form superoxide (O 2 -) and eventually hydrogen peroxide (H 2 O 2), which is extremely dangerous
for biological membranes. Also in this way \( \text{O}_2 \) generated by the oxidation of water is reduced, ultimately leading to the production of \( \text{H}_2\text{O} \). This process is also termed the “water to water cycle” (Asada, 1999). In contrast to cyclic electron transport, oxygen reduction by Photosystem I in the Mehler reaction results in the production of toxic reactive oxygen species, which require enzymatic detoxification by peroxidases.

Cyclic electron transfer

Cyclic electron flow around PSI was first discovered based on its coupling with ATP synthesis approximately 50 years ago (cyclic phosphorylation) (Arnon et al., 1954; Arnon, 1959; Arnon and Chain, 1975), prior to the discovery of linear electron flow. It is most likely that cyclic electron flow around PSI contributes to the generation of ATP in cyanobacteria (Mi et al., 1995), the green alga *Chlamydomonas reinhardtii* (Ravenel et al., 1994; Finazzi et al., 2002), and possibly in C4 plants (Asada et al., 1993). The major difference between cyanobacteria and plants is that the cyanobacteria perform oxygenic photosynthesis and respiration simultaneously in the same compartment and that the stoichiometry of PSI over PSII is much larger than 1 (in higher plants an equal amount of PSI and PSII is the rule). One possible explanation for this unusual stoichiometry in cyanobacteria is the involvement of PSI in cyclic electron flow around this photosystem, another is the abundance of respiratory electron transfer pathways that donate electrons into the PQ pool (Matthijs and Lubberding, 1988), whereas the capacity of respiratory electron flow out of the PQ pool may be more limited (Vermaas, 2001). In cyclic electron transfer electrons flow from PSI/Fd back to PQ and cytochrome \( b_6f \), and from there to PSI again (Bendall and Manasse, 1995). This electron transport pathway contributes to a proton gradient, which can be used for ATP synthesis, but does not lead to net NADP\(^+\) reduction.

In cyanobacteria several routes operating in parallel can be involved in PSI cyclic electron flow (Fig. 4). The important one proceeds via the NDH-1 – NAD(P)H dehydrogenase complex, catalysing electron flow from NAD(P)H to plastoquinone. NAD(P)H dehydrogenase has been shown to provide the largest contribution to plastoquinone reduction in dark respiratory activity (Mi et al., 1992; Schmetterer, 1994), as well as during PSI-mediated cyclic electron transfer (Mi et al. 1994, 1995). The NDH-1 subunits are encoded by at least 10 single copy genes organized in 5 operons (\( ndhAIGE, ndhB, ndhCJK, ndhH, \) and \( ndhL \)) and two multigene families (the \( ndhF \) family with three members and the \( ndhD \) family with six members; Badger and Price, 2003). Reverse genetics approaches have revealed that the NdhF3, NdhD3, NdhF4, and NdhD4 subunits contribute mainly to the uptake of \( \text{CO}_2 \) (Klughammer et al., 1999; Ohkawa et al., 2000a, 2000b; Shibata et al., 2001),
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Figure 4. A scheme of electron transfer routes involved in linear and cyclic photosynthetic flow, respiration and CO₂ fixation pathways in thylakoid membranes of the cyanobacterium *Synechocystis* sp. PCC 6803. The plastoquinone pool and the cytochrome *b₅f* complex are is the central switching points of the photosynthetic and respiratory pathways. Plastoquinone is the acceptor of a) electrons from photosystem II, b) cyclic electron flow from photosystem I, c) respiratory dehydrogenases, and, on the other hand, the donor of electrons for photosystem I, and the terminal and alternative oxidases. The PQ pool is important for balancing photosynthetic and respiratory electron transport and for regulation of the different enzymes of which the role can be investigated by a combined strategy using inhibitors and deletion strains that lack one or more of these enzymes. The directions of electron flow are shown by arrows. The parallel operating PSI mediated quinone reductase cycles are labeled I, II, and III. I) is the Antimycin A sensitive FQR mediated pathway; II) is the rotenone sensitive NDH-1 “indirect” pathway (it relies on intermediary NAD(P)H present in cytoplasm); III) the NaCl inducible, NEM sensitive pathway that runs via FNR. Known inhibitors of a particular electron flow routes are marked by circles 1 through 9. A detailed description of the mechanism of action of the inhibitors is presented in Table 1.

whereas NdhF1, NdhD1, and NdhD2 are involved in respiration and cyclic electron transfer around PSI (Yu et al., 1993; Klughammer et al., 1999; Ohkawa et al., 2000a). Indeed, recent proteomics studies of thylakoid membrane protein complexes in *Synechocystis* revealed four distinct complexes containing NDH-1 subunits, each with a different function (Herranen et al., 2004).

A mutant of *Synechocystis* sp. PCC 6803 lacking NDH-1 activity (denoted M55, acquired by deletion of subunit NdhB of the NAD(P)H dehydrogenase complex), showed strongly reduced respiratory- and PSI cyclic electron transfer rates (Mi et al., 1992, 1995; Jeanjean et al., 1998). NAD(P)H dehydrogenase activity involved in PSI cyclic activity has also been suggested to be present in *Chlamydomonas reinhardtii*, in which several independent pathways may coexist in vivo (Ravenel et al., 1994) through which the PQ pool
<table>
<thead>
<tr>
<th>No*</th>
<th>Abbreviation</th>
<th>Compound</th>
<th>Function and mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rotenone</td>
<td>1,2,6,8α,12,12α-hexahydro-2-isopropenyl-8,9-dimethoxychromeno [3,4-b]furo[2,3-h] chromen-6-one</td>
<td>Blocks Type I NADH dehydrogenase. Acts on the ubiquinone-reducing Fe-S centers.</td>
<td>Trumpower, 1981</td>
</tr>
<tr>
<td>2</td>
<td>DCMU</td>
<td>3-[3,4-dichlorophenyl]-1,1-dimethylurea</td>
<td>Inhibits electron transfer on the acceptor side of PSII. Prevents the reduction of PQ. Binds to Q&lt;sub&gt;0&lt;/sub&gt; pocket in PSII, substitutes for PQ.</td>
<td>Arnon, 1977</td>
</tr>
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<td></td>
<td>atrazine</td>
<td>2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine</td>
<td>Inhibits electron transfer on the acceptor side of PSII. Prevents the reduction of PQ. Binds to Q&lt;sub&gt;0&lt;/sub&gt; pocket in PSII, substitutes for PQ.</td>
<td>Arnon, 1977</td>
</tr>
<tr>
<td>3</td>
<td>DBMIB</td>
<td>2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone</td>
<td>Prevents the oxidation of PQ. Binds to the Q&lt;sub&gt;0&lt;/sub&gt; site of the cytochrome b&lt;sub&gt;f&lt;/sub&gt; complex and blocks the transfer of electrons from the PQ pool to the cytochrome b&lt;sub&gt;f&lt;/sub&gt; complex. Can also bind at the Q&lt;sub&gt;0&lt;/sub&gt; site of the PS II reaction center, acting as a DCMU-type inhibitor.</td>
<td>Trebst, 1980</td>
</tr>
<tr>
<td></td>
<td>DNP-INT</td>
<td>2-iodo-6-isopropyl-3-methyl-2',4',4'-trinitrophenyl ether</td>
<td>Inhibitor of cytochrome b&lt;sub&gt;f&lt;/sub&gt; complex at the level of plastoquinol oxidation.</td>
<td>Oettmeier &lt;em&gt;et al.&lt;/em&gt;, 1982</td>
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<td></td>
<td>HQNO</td>
<td>2-n-heptyl-4-hydroxyquinolone N-oxide</td>
<td>Inhibits cytochrome b&lt;sub&gt;f&lt;/sub&gt; reoxidation. Binds to PQ docking site on the cytochrome b&lt;sub&gt;f&lt;/sub&gt; complex.</td>
<td>Sälak and Whitmarsh, 1982</td>
</tr>
<tr>
<td>4</td>
<td>DSPD</td>
<td>N,N'-disalicylidene-1,3-propanediamine</td>
<td>PSI inhibitor.</td>
<td>Håder, 1976</td>
</tr>
<tr>
<td>5</td>
<td>Antimycin A</td>
<td>N-ethylmaleimide</td>
<td>Inhibitor of cyclic electron flow around PSI by inhibition of ferredoxin-quinone reductase (FQR).</td>
<td>Tagawa &lt;em&gt;et al.&lt;/em&gt;, 1963</td>
</tr>
<tr>
<td>6</td>
<td>NEM</td>
<td>N-ethylmaleimide</td>
<td>Inhibitor of ferredoxin-NADPH reductase (FNR).</td>
<td>Fieschi &lt;em&gt;et al.&lt;/em&gt;, 1996</td>
</tr>
<tr>
<td>7</td>
<td>azide</td>
<td>N&lt;sub&gt;3&lt;/sub&gt; (used as NaN&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>Weak inhibitor of respiratory chain by binding cytochrome oxidase.</td>
<td>Ridgway, 1977</td>
</tr>
<tr>
<td></td>
<td>cyanide</td>
<td>CN&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Potent inhibitor of the respiration chain by binding cytochrome oxidase.</td>
<td>Ridgway, 1977</td>
</tr>
<tr>
<td>8</td>
<td>TFFA</td>
<td>Thienyl trifluoro acetone</td>
<td>Blocks succinate dehydrogenase.</td>
<td>Trumpower, 1981</td>
</tr>
<tr>
<td>9</td>
<td>DCCD</td>
<td>N,N'-dicyclohexylcarbodiimide</td>
<td>Blocks ATP synthase by covalent modification of a single glutamic acid residue in sub-unit III of the hydrophobic part CF&lt;sub&gt;0&lt;/sub&gt;.</td>
<td>Nelson &lt;em&gt;et al.&lt;/em&gt;, 1977</td>
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is non-photochemically reduced and subsequently reoxidized in the dark using molecular oxygen as a terminal acceptor, commonly described as chlororespiration (Bennoun, 1982; Peltier et al., 1987; Nixon, 2000). However, the prominent role of NDH-1 in electron flow from the stroma in *Synechocystis* sp. PCC 6803 has been disputed based on the *in vitro* assays in which the oxidation of NADPH and reduction of PQ appeared insufficient to cover the electron consumption in respiration, whereas added succinate would (Cooley et al., 2000) and from the fact that absence of turnover of NADPH in M55 restrains the *in vivo* function of SDH, which was proven to importantly contribute to cyclic electron flow (Cooley and Vermaas, 2001).

However, the above mentioned pathway of cyclic electron flow around PSI may be accompanied by another antimycin A-sensitive, ferredoxin-dependent pathway. The ferredoxin-quinone reductase (FQR) enzyme, connecting the stroma-exposed side of PSI and the PQ-cytochrome $b_6f$ complex, still has not yet been detected with molecular techniques in chloroplasts and cyanobacteria, but involvement of a cytochrome $b$-559 has been shown (Miyake et al., 1995). In higher plants FQR catalyses the main pathway of cyclic electron flow around PSI, rather than NDH-1. In *Arabidopsis thaliana* one of the genes required for efficient cyclic electron transfer and a potential candidate for the long sought gene encoding for ferredoxin–quinone oxidoreductase, is described by Munekage et al. (2002). The predicted protein product is termed PGR5, for proton gradient regulation. The *pgr5* mutant phenotype is one of decreased PSI cyclic electron transfer, as shown by its inability to increase the fluorescence yield when NADPH and ferredoxin are added to thylakoids in *vitro*. In wild type thylakoids, the plastoquinone pool becomes reduced and fluorescence rises because PSII has lack of acceptors to send electrons to, indicating an electron-transfer pathway from ferredoxin to plastoquinone, just as required by the cyclic chain. The PGR5 protein is membrane bound, but it does not have an extensive hydrophobic sequence, which suggests that it is not intrinsic to the thylakoid membrane. Nor does PGR5 have any obvious motif suggesting a redox-active prosthetic group. Therefore, it is unlikely that PGR5 mediates the electron transfer from ferredoxin to plastoquinone as an electron carrier and the lack of PGR5 may destabilize or inactivate a complex catalyzing ferredoxin-dependent plastoquinone reduction.

In energetically demanding conditions, such as cultivation in growth medium with added NaCl (usually 0.5 M) to induce maximum cyclic phosphorylation capacity, ferredoxin-NAD$^+$ reductase (FNR) could be involved in PSI cyclic electron transfer (van Thor et al., 2000). Inducible PSI cyclic electron flow in *Synechocystis* sp. PCC 6803 requires thylakoid-
Figure 5. Structural model of the PS I trimer from *Synechococcus elongatus* at 2.5 Å resolution. Adapted from Jordan et al. (2001). a) Top view from the stromal side of the thylakoid membrane. Different structural elements are shown in each of the three monomers (I, II and III). I, a complete set of cofactors shown with the transmembrane α-helices. II, arrangement of the transmembrane α-helices (cylinders); the subunits are labeled. Six helices in extra-membranous loop regions are drawn as spirals. III, membrane-intrinsic subunits. In addition, the transmembrane α-helices of the stromal and lumenal loop regions are shown in ribbon representation. b) Side view of the arrangement of all proteins in one monomer of PSI, including the stromal subunits PsaC, PsaD, and PsaE. The position of the monomer inside of the thylakoid membrane is indicated.

membrane-attached FNR (van Thor et al., 2000) and/or flavodoxin (Hagemann et al., 1999). It appeared that the FNR enzyme in cyanobacteria is larger than the one in chloroplasts by about 12 kDa. The N-terminal extension is essential for FNR function in PSI cyclic electron transfer (Van Thor et al., 2000). The existence of FNR in chloroplasts in a PSI cyclic route, separate from FQR, has been suggested from the distinct inhibitory effects of antimycin and the sulphydryl-alkylating agent NEM (Shahak et al., 1981; Hosler and Yocum, 1985; Garab et al., 1990; Matthijs et al., 2002). However, in chloroplasts, due to the absence of the N-terminal domain of FNR, its association with the thylakoid membrane relies on the presence of binding protein (Matthijs et al., 1986, 2002).

Several strategies can be applied to monitor cyclic electron flow. In particular, PSI-mediated cyclic electron flow in far-red light (known to excite mainly PSI chlorophylls) may be analyzed indirectly by measuring the light transmission signal at 535 nm. This so-called carotenoid band shift is only visible in non-scattering preparations of isolated thylakoids, and reflects changes in the trans-thylakoid pH gradient (Heber et al., 1992, 1995; Cornic et al., 2000). An alternative way is to measure of the re-reduction rate of the oxidized primary
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electron donor in PSI ($P_{700}^+$) after switching off the far-red light (Maxwell and Biggins, 1976; Asada et al., 1992; Schreiber and Vidaver, 1976). Additionally, study of mutants with knocked-out pathways and application of inhibitors blocking particular electron routes (Fig. 4, Tab. 1) is a powerful strategy to gain insight into molecular mechanisms of cyclic electron flow.

Photosystem I

Photosystem I is a large multi-subunit protein complex, embedded in the thylakoid membrane. It catalyses the light induced electron transfer from plastocyanin or cytochrome $c_6$ on the luminal side of the membrane (inside the thylakoids) to ferredoxin or flavodoxin on the stromal side of the membrane. Several major routes towards the investigation of the topography and function of the subunits in PSI have been explored: deletion mutants of subunits, electron microscopy (EM) investigations, site-directed mutagenesis on functionally important parts of PSI, cross-linking experiments, and X-ray crystallography.

PSI belongs to a larger group of protein-pigment complexes that are able to perform light induced charge separation: the photosynthetic reaction centres (RCs). These can be divided into two classes defined by the nature of the terminal electron acceptor. A quinone molecule is the terminal electron acceptor in type II reaction centres (quinone-type reaction centres), which include the PSII reaction centre and the reaction centres of purple bacteria. In type I reaction centres the terminal electron acceptor is a [4Fe-4S] cluster (FeS type reaction centre). Apart from its presence in chloroplasts and cyanobacteria, a type I RC is present also in green sulfur bacteria and heliobacteria. It is comprised of an interpolypeptide [4Fe-4S] cluster, $F_x$, that bridges the PsaA and PsaB subunits, and two terminal [4Fe-4S] clusters, $F_A$ and $F_B$, which are bound to the PsaC subunit. PsaC, PsaD and PsaE do not contain transmembrane $\alpha$-helices. They are located on the stromal side of PSI and are involved in the docking of ferredoxin and/or flavodoxin.

The molecular structure of the PSI core complex has recently been resolved from the cyanobacterium *Synechococcus elongatus* to a resolution of 2.5 Å (Jordan et al., 2001) and from a higher plant, *Pisum sativum var. Alaska*, to 4.4 Å resolution (Ben-Shem et al., 2003). It is remarkable that in the structure of the plant PSI reaction centre the positions of virtually all of the cyanobacterial chlorophylls were found almost precisely preserved. Since chloroplasts diverged from their cyanobacterial ancestors at least 1 billion years ago, they have undergone a separate evolution. During 1 billion years of evolutionary processes an enormous number of random mutations took place, yet both cyanobacteria and higher plants maintained the same chlorophyll arrangement. The cyanobacterial PSI is smaller in size
compared with plant PSI, having a reaction centre that resembles the one in plants but with no peripheral antenna like LHCI.

In the crystalline state as well as in vivo, PSI from Synechococcus elongatus occurs as a trimer (Jordan et al., 2001; Krauss et al., 1993) whereas plant PSI is monomeric. The trimer has a diameter of 210 Å and a maximal height of 90 Å. Each monomer (ca. 356 kDa) contains 127 cofactors (96 chlorophylls, 2 phylloquinones, 3 [4Fe-4S] clusters, 22 carotenoids, 4 lipids) and consists of 12 polypeptides, of which 9 subunits have transmembrane α-helices (Fig. 5).

The central part of one monomeric unit of the PSI trimer is formed by a heterodimer, consisting of the large membrane intrinsic subunits PsaA and PsaB with a molecular mass of 83 kDa and 11 α-helices each. The PsaA/PsaB heterodimer harbours most of the redox cofactors and a large number of antenna chlorophylls and carotenoids. A cavity at the luminal side of PsaA/PsaB, close to the symmetry axis, is the binding site for plastocyanin and cytochrome c₆ (Fromme et al., 1994; Sun et al., 1999; Sommer et al. 2002). At the cytoplasmic side, subunit PsaC (9 kDa) harbours two of the three [4Fe-4S] clusters (F₆ and F₇), and subunits PsaD and PsaE, which are both surrounding subunit PsaC, could contribute to the binding of ferredoxin or flavodoxin (Fromme et al., 1994). Different functions have been reported for PsaE: direct involvement in the anchoring of ferredoxin (Sonoike et al., 1993; Rousseau et al., 1993; Weber and Strotmann, 1993), playing a role in cyclic electron transfer (Zhao et al., 1993; van Thor et al., 1999), and crosslinking in barley with the FNR via its N-terminal extension (Andersen et al., 1992; Scheller, 1996).

Six small intrinsic membrane protein components of PSI have been identified from the gene sequence in S. elongatus (Mühlenhoff et al., 1993): the subunits PsaF (15 kDa), PsaI (4.3 kDa), PsaJ (4.4 kDa), PsaK (8.5 kDa), PsaL (16.6 kDa) and PsaM (3.4 kDa). In the 2.5 Å resolution structure (Jordan et al., 2001), a 12th subunit of PSI, PsaX, which contains one transmembrane α-helix, was identified. All of the small membrane integral subunits are located peripherally to the subunits PsaA and PsaB. The main function of the small subunits is the stabilisation of the antenna system and the quaternary structure of PSI. The small subunits can be divided into two groups according to their location in the complex: PsaL, PsaI and PsaM are located in the region where the adjacent monomers face each other in the trimeric PS I complex, whereas PsaF, PsaJ, PsaK and PsaX are located at the peripheral, detergent-exposed, surface of the photosystem (Fromme et al., 2001). The location of PsaL in the trimerisation domain was first proposed by mutagenesis studies, because no trimers can be detected in PsaL deletion mutants of cyanobacteria (Chitnis and Chitnis, 1993). The
psaF gene in *Synechocystis* encodes a mature protein of 15,705 Da that is synthesized with a 23-amino-acid extension (Hippler *et al.*, 1996). PsaF is an integral membrane protein with one transmembrane $\alpha$-helix and a substantial N-terminal domain, exposed on the luminal side of PSI. For a long time it was expected that subunit PsaF was an extrinsic subunit located at the luminal side of PSI. This assumption was mainly based on the fact that subunit PsaF in plants contains two pre-sequences, one for the import into the chloroplast and a second for import into the thylakoid lumen. It was shown that this protein is imported by the same pathway as that used for the import of plastocyanin (the soluble luminal electron carrier) (Karnauchov *et al.*, 1994). In plants electron transfer from plastocyanin to PSI is two orders of magnitude faster than in cyanobacteria. The removal of PsaF from the PSI complex of *Synechocystis elongatus* had no effect on the rate of electron transfer from cytochrome $c_6$ to $P_{700}$ (Hatanaka *et al.*, 1993). In contrast, in eukaryotic cells, PsaF function was proposed essential for plastocyanin or cytochrome $c_6$ docking on the oxidizing side of PSI, based on biochemical depletion- and chemical crosslinking experiments (Hippler *et al.*, 1998). Inactivation of the *psaF* gene from *Chlamydomonas reinhardtii* resulted in a mutant that still assembles functional PSI complex and is capable of photoautotrophic growth. However, the rate of electron transfer from plastocyanin to $P_{700}^+$ is dramatically reduced in

![Figure 6. Structural model of (a) plant monomeric PSI with its antenna complex and (b) cyanobacterial supercomplex with trimeric PSI and 18 copies of the IsiA protein. Adapted from Ben-Shem *et al.*, 2003 (a), and Bibby *et al.*, 2001 (b). View from the stromal side of the thylakoid membrane. a). Subunits F, G, H and K of the reaction centre are indicated; four Light-harvesting membrane proteins (LHCl) marked as Lhca 1-4 b). The position of subunit F on the outer edge of the monomer is shown.](image-url)
this mutant, providing evidence that PsaF plays an important role in docking plastocyanin to PSI in chloroplasts (Farah et al., 1995). Indeed the crystal structure of PSI from a higher plant, *Pisum sativum* var. *Alaska*, at 4.4 Å resolution (Ben-Shem et al., 2003) revealed the extra 18 amino acid residues in the N terminus of plant PsaF, which probably may mediate more efficient plastocyanin binding in plants compared to cyanobacteria. This controversy over the function of PsaF in plants and cyanobacteria was addressed by the peculiarities of molecular recognition between plastocyanin and PsaF (Hippler et al., 1996). Subunit PsaF could thus play a significant role in the interaction of PSI with any (or both) of its two electron donor proteins (Hervas et al., 2003) and/or in the stabilisation of the luminal surface of photosystem I (Fromme et al., 2001, Xu et al., 1994). PsaF interacts with PsaJ, a 4.4 kDa hydrophobic subunit of PSI. The N-terminus of PsaJ is located in the stroma, the C-terminus is located in the lumen. PsaJ contains one transmembrane α-helix. It binds three chlorophylls and is in hydrophobic contact with carotenoids. Analysis of a *psaJ*-deficient mutant in *Synechocystis* sp. PCC 6803 showed that PsaJ is not required for the electron transfer in PSI, but by interaction with both PsaE and PsaF, may stabilize PSI (Xu et al., 1994). PsaF does not axially coordinate chlorophylls, but forms hydrophobic interactions with several carotenoids. A possible role of the transmembrane part of PsaF could be a shielding of the carotenoids and chlorophylls from the lipid phase (Fromme et al., 2001).

**Interaction of PSI with its peripheral antenna systems**

Photosynthesis begins with the absorption of light and the transfer of its energy to reaction centres, where the energy is used in electrogenic charge separation. The major difference between PSI from cyanobacteria and that from green algae (or higher plants) originates from the peripheral light-harvesting antennae. In plants Light-harvesting Complex I (LHCI), bound to PSI, act as internal antenna. The LHCI proteins are very similar to the light-harvesting proteins LHCII of PSII. Cyanobacterial PSI complexes assemble in thylakoids as a trimer, whereas the eukaryotic PSI exists as a monomer associated with dimers of Lhca polypeptides (Scheller et al., 2001; Haldrup et al., 2001). Electron microscopy investigations identify 11-14 LHCI polypeptides attached to the monomeric eukaryotic PSI core (Germano et al., 2002; Kargul et al., 2003). The crystal structure of PSI complexes revealed the four antenna proteins assembled into two dimers (assigned to Lhca1–Lhca4 and Lhca2–Lhca3), arranged in series, creating a half moon-shaped belt that docks to the reaction center’s subunit F side (Ben-Shem et al., 2003) (Fig. 6a).

In cyanobacteria large membrane-extrinsic phycobilisomes (large supramolecular assemblies of phycobiliproteins, located at the cytoplasmic side of the thylakoid membranes)
function as peripheral antenna systems for both PSII and PSI (MacColl, 1998).

There are two basic models of energy distribution over the two photosystems (Murata, 1969). The energy spillover model is based on the permanent association of phycobilisomes with the PSII complex; thus in this model the energy transfer takes place between the chlorophyll antennae of PSII and PSI. In the mobile antenna model, it is assumed that phycobilisomes can move over the surface of the thylakoid membrane and attach to either one of the two photosystems. In this way, the energy from phycobilisomes is transferred directly to PSII (in state 1) or to PSI (in state 2). Details of the interaction site between PSI and the phycobilisomes are so far unknown; a possible role of allophycocyanin to link energetically the phycobilisomes and the photosystems has been discussed (Fromme et al., 2003). Some evidence from mutants lacking PsaF (Hippler et al., 1999) suggests that PsaF may be involved in the docking of the phycobilisomes.

Under iron-limiting conditions, a common condition in many aquatic ecosystems, the content of PSI decreases relative to that of PSII. This change in stoichiometry is usually compensated by a decreased synthesis of phycobilisomes (Sandström et al., 2002) and the accumulation of the so-called IsiA or CP43’ protein, a chlorophyll-binding protein that is structurally related to the CP43 protein of PSII (Bricker and Frankel, 2002). Recent evidence has indicated that 18 copies of the IsiA protein can encircle a trimeric PSI core complex (Fig. 6b), both in Synechocystis sp. PCC 6803 (Bibby et al., 2001; Nield et al., 2003) and in Synechococcus PCC 7942 (Boekema et al., 2001). In these supercomplexes, tight structural connection of the outer IsiA ring to the PSI trimer results in a strong energy coupling and faster transfer of excitation energy from the periphery of the complex to the PSI core (Andrizhiyevskaya et al., 2002; Melkozernov et al., 2003).

The IsiA protein shows clear sequence homology with the CP43 protein of PSII (Burnap et al., 1993) and is therefore often referred to as CP43’. CP43’ binds at least 12 chlorophyll molecules within its six-helix bundle (Zouni et al., 2001). Therefore the additional antenna size of the iron stress induced CP43’-PSI supercomplex is composed of approximately 216 chlorophyll a molecules. As the PSI trimer contains about 300 chlorophyll molecules (Schubert et al., 1997), the light-harvesting ability of the supercomplex has increased by approximately 72% (Melkozernov et al., 2003) or 100% (Andrizhiyevskaya et al., 2002) compared with that of the normal trimer (formed under Fe-replete conditions). It cannot be excluded, however, that the IsiA protein also fulfills other roles, e.g., as an excitation energy dissipator (Sandström et al., 2001, 2002) or as a chlorophyll storage protein (Burnap et al., 1993).
Inspection of the protein surface suggests that the PsaA, PsaB, PsaF, PsaK and PsaX proteins may interact with the membrane-intrinsic antenna complexes (Fromme et al., 2003). PsaF together with PsaJ may form the major interaction site with the IsiA ring. The sequence comparison between eukaryotic and prokaryotic PsaF and PsaJ shows a high degree of sequence similarity for both proteins. In plants, a direct contact of PsaF with the light harvesting systems has been suggested by experiments in which the subunit PsaF was isolated as a Chl-protein complex with LHCl proteins (Anandan et al., 1989) and from the crystal structure of the plant PSI (Ben-Shem et al., 2003).

**Scope and outline of this thesis**

The research presented in this thesis is concentrated on the molecular and physiological aspects of cyanobacterial photosynthesis. Particularly detailed studies of Photosystem I organization and light harvesting, together with elucidation of Photosystem I driven cyclic electron flow pathways, is in the focus of interest.

During adaptation to salt stress as energy demanding condition *Synechocystis* cells are known to induce their capacity for cyclic electron flow. Genes encoding potential participants in this PSI-mediated electron flow are supposed to alter their level of expression in salt stress. As the first cyanobacterium with a sequenced genome, *Synechocystis* sp. PCC 6803 is an excellent organism for studying gene expression by DNA array technology. This technique allows monitoring of transcription of all genes simultaneously, and, thus has advantages compared to more traditional molecular approaches.

In **Chapter 2** the procedures for the application of a DNA array composed of overlapping M13 clones, directly acquired from the *Synechocystis* genome-sequencing project, are described. In that Chapter we discuss the technical challenges that were met and resolved during the project. **Chapter 3** deals with investigation of patterns of gene expression in salt stress by DNA arrays. Patterns of gene expression obtained have been overlaid with those for other stresses (N/P starvation, in collaboration with V. Krasikov) to obtain insight into stress-specific responses and data are discussed according to the metabolic category to which the regulated genes belong. Among a great number of ORFs encoding hypothetical or unknown proteins that showed altered expression, several have been chosen for further mutagenesis analysis.

The scope of **Chapter 4** is investigation of the role of two ORFs ssr2016 and slr1208 by cloning and subsequent interruption of those ORFs with antibiotic resistance cassettes. Both of these ORF have an increased expression level specifically in salt stress. ssr2016
encodes a hypothetical protein highly homologous to the recently discovered PGR5 protein from *Arabidopsis thaliana* (see General Introduction) and could potentially be involved in the Antimycin A sensitive pathway of cyclic electron flow. Situated directly downstream, *slr1208* encodes a probable oxidoreductase that has been suggested to belong to the photosynthesis related genes by comparative genomic studies (Raymond *et al.*, 2002). Since the so-called indirect pathway of cyclic electron flow through the NDH-1 and/or SDH complexes takes a main part in cyanobacteria (Jeanjean *et al.*, 1998; Matthijs *et al.*, 2002; Cooley *et al.*, 2000; Cooley and Vermaas, 2001), knockout mutagenesis of *slr1208* and *ssr2016* was also performed on the M55 mutant (which lacks NdhB and is defective in the main route of cyclic electron flow) as well as on the wild type. Single, double and triple mutants were fully segregated and studied with respect to P700 RC re-reduction rates by the pulse amplitude modulation technique (PAM, Schreiber and Krieger, 1996.), via PSI-cyclic driven energy storage capacity by photoacoustic spectroscopy (PAS), in direct photophosphorylation assay, and with various inhibitors (NEM, antimycin A) of the cyclic electron transfer chain. The PSI driven cyclic electron flow pathways with components that were revealed by the DNA array experiments are discussed.

In Chapter 5 the properties of the *Synechocystis* mutant lacking the F and J subunits of PSI are examined. Whereas subunit F, located on the outer edge of Photosystem I is vitally essential in higher plants, in cyanobacteria a knockout mutant of the gene encoding this subunit did not show any significant phenotype in earlier research. Our research shows that interruption of the *psaFJ* genes leads to a relatively minor change as compared to the WT. The difference is a longer adaptation period (lag-phase) after transfer of cells in the exponential phase of growth from the standard- to a high-salt medium (0.5 M NaCl). Suppression of PSI in the *psaFJ* mutant is accompanied by impaired electron flux from the acceptor site of PSI to the PQ-cytocthrome *b,c* complex (cyclic flow), and is going hand in hand with the de-repression of the *isiAB* operon. The latter is encoding the iron-starvation inducible proteins IsiA (or CP43') and IsiB (flavodoxin). The relation between the lack of the PsaFJ subunits of PSI, the ample availability of iron in our cultures, and the induction of *isiA* is discussed. It is proposed that oxidative stress is the cause of *isiA* expression rather than iron limitation *per se*.

Earlier research has shown that induction of the IsiA protein under iron-limited conditions results in the formation of a supercomplex consisting of trimeric PSI encircled by 18 IsiA subunits.

Spectroscopic characterization of PSI-IsiA supercomplexes from cyanobacteria grown
under iron starvation suggests that the IsiA ring increases the absorption cross-section of PSI by about 70-100%. Chl a molecules bound to the low-molecular weight polypeptides of PSI (three of these chlorophylls are bound to PsaJ) have been suggested to play a role in energy transfer from the outer IsiA ring towards the reaction centre. Since isiA transcript was found in a psaFJ mutant even in iron replete conditions, we have decided to investigate the structural composition of IsiA-PSI supercomplexes from this mutant. Structural characterization, by electron microscopy and image analysis of a supramolecular complex from the psaFJ mutant consisting of PSI and a ring of 17 IsiA units, is described in Chapter 6.

The psaFJ mutant has a slightly decreased PSI content even in iron-replete medium. After prolonged growth under iron-free conditions PSI disappeared nearly completely whereas the iron-starvation inducible protein IsiA becomes the by far most abundant chlorophyll-protein complex. Chapter 7 deals with partial purification and characterization by mass spectrometry, spectroscopy, and electron microscopy of several types of IsiA aggregates that are not associated with PSI. The accumulation of IsiA in a form that does not contribute to light harvesting for PSI together with disappearance of PSI in the psaFJ mutant under prolonged iron limitation can be attributed to the combination of oxidative stress and iron limitation exerted on PSI. The structure and potential function of PSI-free IsiA aggregates are discussed.

In Chapter 8 the relation between the number of bound IsiA subunits in PSI-IsiA supercomplexes, the monomeric or trimeric aggregation state of PSI, and the degree of iron starvation is studied. PSI-IsiA complexes from the wild-type and from a psaFJ mutant at various times after inoculation of a culture in an iron-free medium were analysed by electron microscopy and image analysis of a very large set of single particle projections.

In Chapter 9 a general discussion will put the acquired observations in perspective. In particular, the implications of the functional flexibility of PSI for cyanobacterial growth under energy-limited and iron-limited conditions will be discussed. A summary with conclusions and highlights based on the interesting mix of molecular, biophysical and physiological studies done, with suggestions for further investigations, concludes this thesis.
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