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Minireview

Structure and function of photosystem I: interaction with its soluble electron carriers and external antenna systems

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Abstract Photosystem I (PS I) is a large membrane protein complex that catalyzes the first step of solar conversion, the light-induced transmembrane electron transfer, and generates reductants for CO_2 assimilation. It consists of 12 different proteins and 127 cofactors that perform light capturing and electron transfer. The function of PS I includes inter-protein electron transfer between PS I and smaller soluble electron transfer proteins. The structure of PS I is discussed with respect to the potential docking sites for the soluble electron acceptors, ferredoxin/flavodoxin, at the stromal side and the soluble electron donors, cytochrome c_6/plastocyanin, at the luminal side of the PS I complex. Furthermore, the potential interaction sites with the peripheral antenna proteins are discussed.

Key words: Photosynthesis; Photosystem I; Ferredoxin; Plastocyanin; Antenna

1. Introduction

Oxygenic photosynthesis is the major biological process in the biosphere that transforms solar energy and converts CO_2 into carbohydrates. The first, and one of the most important, steps of this process is the light-induced transmembrane charge separation, catalyzed by two large membrane protein complexes, photosystems (PS) I and II.

In PS I and II the energy of photons from sunlight is used to translocate electrons across the photosynthetic membrane. Water, oxidized by PS II to O_2 and 4 H^+, serves as the electron donor for the electron transfer chain. The electron is transferred from PS II to PS I via a pool of plastoquinones, the cytochrome (cyt) b_6f complex and the soluble electron carriers, plastocyanin or cyt c_6. PS I catalyzes the transmembrane charge separation from plastocyanin/cyt c_6, which is located at the luminal side of the membrane, to ferredoxin at the stromal side. Finally, the electron is transferred to ferredoxin-$NADP^+$ oxidoreductase, which reduces $NADP^+$ to NADPH. The electron transfer processes induce formation of a proton gradient across the membrane. The resulting electrochemical potential drives the synthesis of ATP from ADP and P_i by ATP synthase. In the subsequent dark reactions, ATP and NADPH are used for the reduction of CO_2 to carbohydrates.

In cyanobacteria, PS I assembles as a trimer with a molecular weight of more than 1 million Da. PS I is the largest and most complex membrane protein for which the structure has been determined. The structure at 2.5 Å resolution showed that each monomeric unit of PS I contains 12 proteins, 96 chlorophylls (Chl), 22 carotenoids, 3 [4Fe4S] clusters, two phylloquinone molecules and four lipids [1]. A major structural feature of the PS I complex is a fusion of the protein that binds the core antenna chlorophylls, with the protein that binds the redox cofactors of the electron transfer. The core antenna of the PS I monomer consists of a network of 90 antenna chlorophylls and 22 carotenoids. The energy is transferred through this network to the center of the complex, where an excitation trap is located [2,3]. The excitation trap or the primary electron donor is a special pair of two chlorophylls designated P700 because of its absorption maximum at 700 nm. P700 launches a transmembrane electron transfer via a chain of electron carriers with descending redox potentials. These electron carriers had been previously identified by spectroscopic investigations, and their historical names have been maintained [4]. The electron transfers stepwise from P700 to A (a chlorophyll *a* molecule), A_0 (also a chlorophyll *a* molecule), A_1 (a phylloquinone molecule) and from there to the three [4Fe4S] clusters, F_X, F_A and F_B. The distal iron–sulfur cluster F_B transfers the electron to ferredoxin, for which the binding site is located at the stromal side of PS I. Being reduced, the ferredoxin leaves the docking site to transfer the electron to the ferredoxin-$NADP^+$ reductase. Under iron deficiency flavodoxin can act as the tertiary electron acceptor instead of ferredoxin [5].

On the luminal side of the membrane the secondary electron donors bind to soluble electron carrier proteins participating in the re-reduction of $P700^+$. This process prevents a loss of energy in the reaction center via charge recombination. The docking site for the soluble electron carriers is located close to P700. In green plants reduced Cu-binding plastocyanin docks to this site and transfers the electron to $P700^+$, whereas in cyanobacteria cyt c_6 can replace plastocyanin or function as an alternative electron donor for PS I [6].

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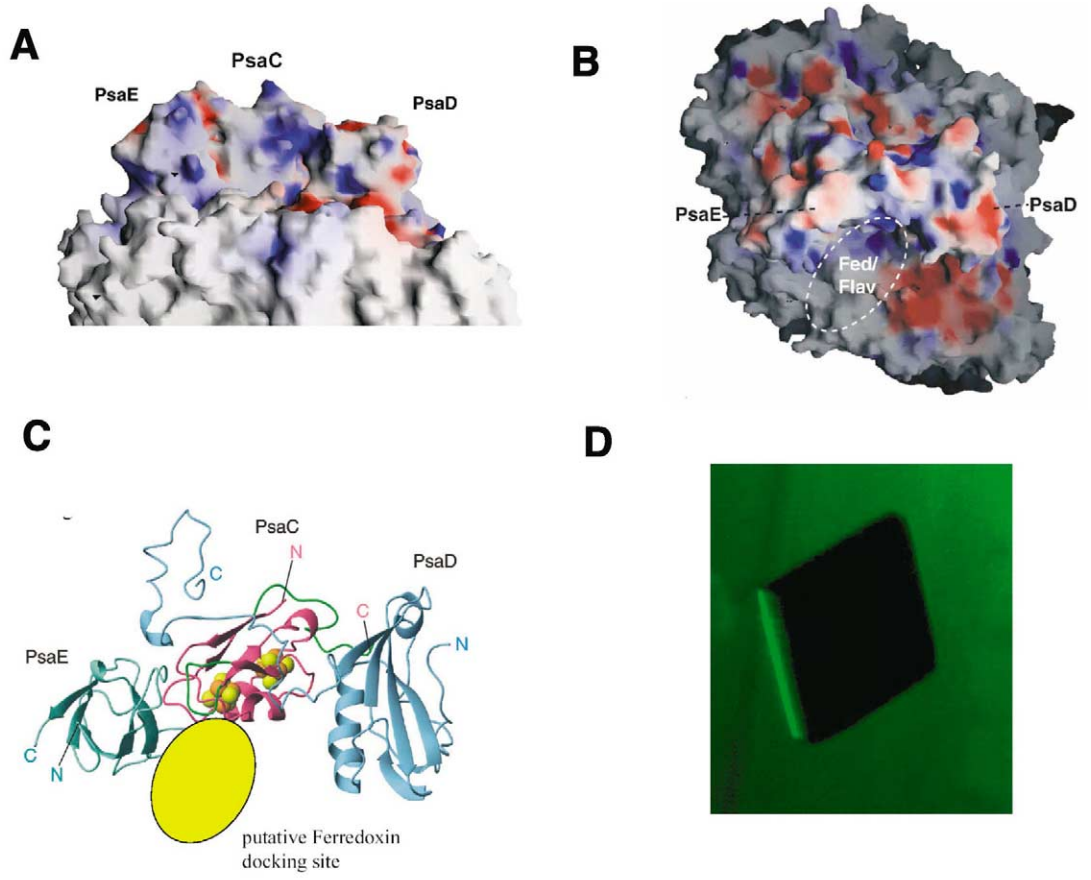

Fig. 1. The docking site of ferredoxin to PS I. A: View of the surface charges of the stromal hump of the PS I complex, formed by PsaC, PsaD and PsaE. The view direction is parallel to the membrane plane. Neutral regions are shown in gray, positive regions in blue and acidic regions are shown in red. B: View of the ferredoxin docking site from the stromal side onto the membrane plane. The color coding is the same as in A. C: Structure of subunits PsaC (pink), PsaD (blue) and PsaE (turquoise). All three subunits form the docking site for ferredoxin. D: Co-crystal of PS I with ferredoxin.

To increase the absorption cross-section for the light-harvesting process the photosynthetic core complexes (PS I and PS II) acquire additional antenna systems. In cyanobacteria, phycobilisomes act as peripheral antenna systems, whereas under iron deficiency a membrane-intrinsic ring of 18 IsiA proteins surrounding one PS I trimer can act as a peripheral antenna [7,8]. In green algae and higher plants the PS I and PS II core complexes are surrounded by integral transmembrane Chl *a*/*b* containing light-harvesting antennae [9].

This review focuses on interactions of the PS I complex with its soluble electron carriers and on potential interaction sites with the peripheral antenna systems.

2. The docking site of ferredoxin and flavodoxin

The three stromal subunits of the PS I complex, PsaC, PsaD and PsaE, participate in the docking of ferredoxin/flavodoxin on the stromal side of the membrane. Fig. 1A,B shows the distribution of surface charges on the stromal side of the PS I complex. A basic patch formed by PsaD, PsaE and PsaA may be involved in the docking of the negatively charged ferredoxin via electrostatic interactions. This docking site was at first suggested based on the low resolution 6 Å structure of PS I [10]. Electron microscopy studies on cross-linked complexes between PS I and ferredoxin as well as between PS I and flavodoxin [11,12] and a large number of mutagenesis studies provide experimental evidence for this suggested docking site for ferredoxin (see [13] and references therein for a more comprehensive overview of mutant studies). It is remarkable that ferredoxin and flavodoxin bind to the same docking site, even though flavodoxin is much larger than ferredoxin and there is no sequence homology between the two proteins.

A crystal structure of the PS I–ferredoxin complex would be a final proof in the identification of the interaction sites. Recently, crystals from an active complex between PS I and ferredoxin were reported [14] (see Fig. 1D), but the quality of the co-crystals has to be improved before structural information will become available. Electron paramagnetic resonance (EPR) studies of the co-crystals confirmed formation of a photo-active complex between the PS I and the ferredoxin [14]. Interestingly, there is some evidence that the EPR features of the terminal FeS cluster, F_B, and its redox potential may be altered in the complex with ferredoxin, indicating the possibility for a conformational change upon ferredoxin binding.

It is surprising that the docking site for the electron acceptors is much more conserved during evolution than is the docking site for plastocyanin/cyt c_6. Ferredoxins isolated from plants and cyanobacteria show nearly the same second order rate constants for the electron transfer from cyanobacterial PS I [15].

3. The docking site for cyt c_6 and plastocyanin

Intensive structural and functional investigations by De la Rosa and coworkers have provided striking evidence that the docking site for the electron donors to PS I underwent rapid and large changes during evolution [6,16–21]. The authors hypothesized that cyt c_6 may be the older evolutionary electron donor, which was later replaced by the Cu-containing plastocyanin, due to iron limitations in the environment [6]. While green plants use plastocyanin as the only electron donor to the PS I, some cyanobacteria can use both plastocyanin and cyt c_6 as electron donors. Furthermore, the complex formation varies significantly among different organisms. In plants and green algae the PS I subunit, PsaF, contains an insertion of 25 amino acids in its luminal domain, which is responsible for formation of a tight complex between plastocyanin and PS I [22,23]. Most cyanobacteria do not form the tight complex; the interaction is purely diffusion-controlled. Despite the variability in the docking site of plastocyanin/cyt c_6 by PS I, the interaction site, where the electron transfer occurs, is strongly conserved among different organisms.

Plastocyanin and cyt c_6 bind to PS I at an indentation at the luminal side to re-reduce the primary electron donor $P700^+$. The major interaction site is formed by two helices, located at the luminal indentation close to $P700^+$. They provide a hydrophobic docking site for plastocyanin and cyt c_6. Fig. 2A shows a view of the potential docking site for plastocyanin and cyt c_6.

Both plastocyanin and cyt c_6 have hydrophobic faces that match the hydrophobic docking site of PS I [18]. Mutants that disturb this site are affected in binding and electron transfer [24]. A further increase in affinity can be provided by positively charged patches of both plastocyanin and cyt c_6 that may electrostatically drive their attractive movement toward PS I [24].

Two tryptophan residues partially exposed to the aqueous phase are further prominent features of the docking site (Fig. 2A,B). They may form an important part of the recognition site for the soluble electron donors and may even be involved in the electron transfer from plastocyanin/cyt c_6 to P700. Experimental evidence for this hypothesis was provided by recent mutagenesis studies on one of these tryptophans [25].

Whereas the structure of the purple bacterial reaction center with its electron donor, cyt *c*, was recently solved [26], no co-crystals have been reported for the complexes between PS I and cyt c_6 or plastocyanin. This is probably associated with the lack of tight complex formation in most cyanobacteria, with a few exceptions as, for example, in *Anabaena* PCC 7119 which shows a tight complex formation [27].

In this respect it is interesting that the chimeric mutant of PsaF in *Synechococcus elongatus*, containing the N-terminal domain of *Chlamydomonas* PS I, shows the same binding features for plastocyanin as PS I from green algae [28]. These results show that the N-terminus of PsaF is indeed responsible for the tight complex formation and therefore this mutant might be an interesting target for co-crystallization.

4. Interaction of PS I with its peripheral antenna systems

PS I is a complex protein–cofactor assembly, consisting of a fused reaction center and core antenna system. The core antenna consists of 90 chlorophylls and 22 carotenoids, which

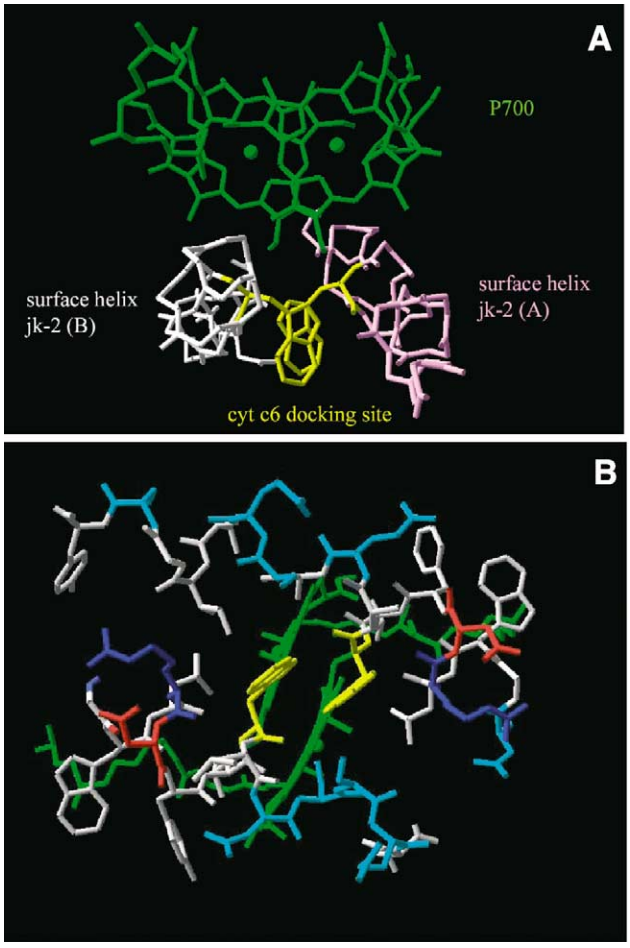

Fig. 2. the hydrophobic docking site for plastocyanin and cyt c_6. A: View parallel to the membrane plane. The two chlorophylls representing the primary donor P700 are depicted in green. The surface helices jk (2) of PsaA and PsaB are shown in light pink and white, respectively. The two tryptophan residues depicted in yellow may represent an important recognition site for the soluble electron transfer proteins. B: View from the luminal site onto the membrane plane. The same structure elements are shown as in A. Hydrophobic residues are depicted in gray, neutral residues are shown in light blue, positively charged residues are depicted in dark blue and negatively charged residues are shown in red. The two tryptophan residues are depicted in yellow.

are bound essentially to the same subunits, PsaA and PsaB, that coordinate most of the cofactors of the electron transfer chain (P700, A, A_0, A_1 and F_X). Surrounding the electron transfer chain, the core antenna of PS I has been conserved over millions of years of evolution and may therefore be quite similar in cyanobacteria and eukaryotes.

However, plant and cyanobacterial PS I differ in their quaternary structure, subunit composition and peripheral antenna systems. The major difference between the PS I from cyanobacteria and that from green algae (or higher plants) originates from the peripheral light-harvesting antennae. In cyanobacteria large membrane-extrinsic phycobilisomes function as peripheral antenna systems for both PS II and PS I [29]. Details of the interaction site between PS I and the phycobilisomes are so far unknown, although allophycocyanin is thought to link energetically the phycobilisomes and the photosystems.

However, there is some evidence from mutants lacking

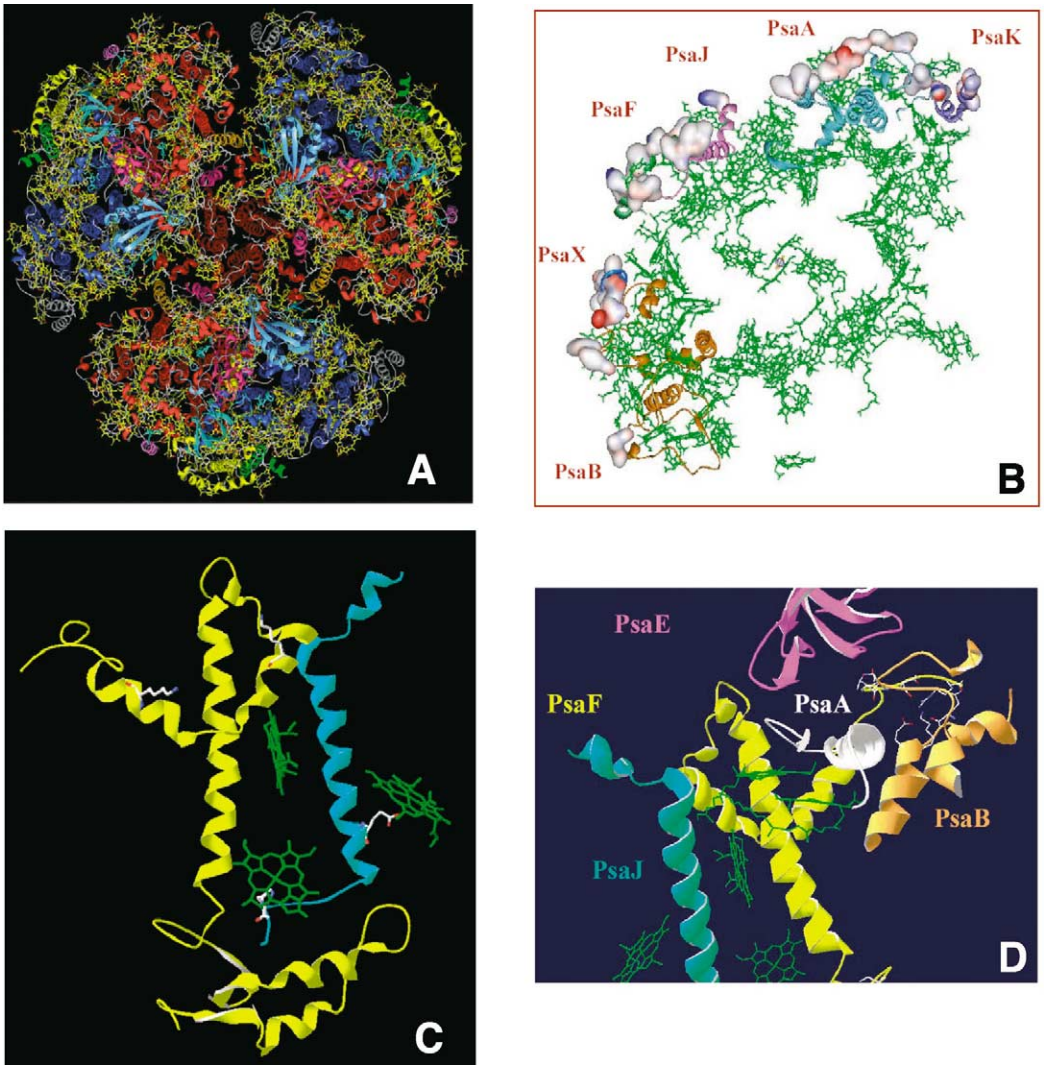

Fig. 3. Interaction sites of PS I with its peripheral antenna systems. A: Structure of the trimeric PS I from cyanobacteria at 2.5 Å resolution: view from the stromal side onto the membrane plane. The protein subunits are shown in a backbone representation (PsaA red, PsaB blue, PsaC purple, PsaD light blue, PsaE turquoise, PsaF yellow, PsaI orange, PsaJ green, PsaK gray, PsaL brown, PsaM dark red, PsaX violet). B: The chlorophylls of the core antenna system of PS I: the view is the same as for the upper-left monomer in Fig. 1A. The parts of the cyanobacterial proteins of PS I that are exposed to the membrane and may interact with membrane-intrinsic peripheral antenna complexes are highlighted. C: Structure of PsaF and PsaJ subunits of the cyanobacterial PS I. PsaJ binds three Chl *a* molecules coordinated by water/protein backbone (CL1301), Glu J28 (CL1303) and His J40 (CL1302). Positively charged surface-exposed Lys F108 and Lys F122 are potential docking sites for the peripheral antenna like the IsiA proteins in cyanobacteria or the LHCI complexes in plants. D: Interaction between PsaF and PsaA, PsaB, PsaE and PsaJ at the stromal side.

PsaF (ΔPsaF mutants) [28] that PsaF may be involved in the docking of the phycobilisomes. Under normal light intensities, where the phycobilisomes are attached mainly to PS II, these mutant cells grow like the wild type. Surprisingly, after being transferred to low light intensity the 'ΔPsaF cells' are unable to grow. Instead, they synthesize a large amount of allophycocyanin, which is supposed to link the phycobilisomes to the photosystems. After a period of 10 days the whole cells have a turquoise color which essentially resembles the spectrum of allophycocyanin, before the cells finally die (unpublished results of P. Jordan and P. Fromme). These results may be interpreted in terms of cells suffering from a lack of energy input from the phycobilisomes to PS I.

Under iron stress conditions, the phycobilisomes degrade and PS I acquires a ring of the IsiA proteins around the PS I trimer, forming a PS I–IsiA supercomplex with significantly increased light-harvesting capacity [7,8]. In these supercomplexes, tight structural coupling of the outer IsiA ring to the PS I trimer results in a strong energy coupling and faster transfer of excitation energy from the periphery of the complex to the PS I core [30,31]. The data pose the question of how the interaction of the PS I with this membrane-intrinsic peripheral antenna system is achieved. Fig. 3A illustrates the structure of the trimeric PS I, while Fig. 3B shows one monomeric unit in which the membrane-exposed regions of the PS I subunits are highlighted in gray with patches of negative (red) and positive (blue) charges. Inspection of the protein surface suggests that the following proteins may interact with the membrane-intrinsic antenna complexes: PsaA (amino acid regions comprised of A150–A180 and A236–A270), PsaB (region comprised of B226–B228 and B483–B492), PsaF (region comprised of F100–F130), PsaJ (region comprised of J2–J41),

PsaK (region comprised of K20–K34) and PsaX (region comprised of X7–X28). Again, PsaF together with PsaJ may form the major interaction site with the IsiA ring. The structure formed by these two proteins is shown in Fig. 3C.

PsaF has a very unusual folding. The N-terminus of PsaF is located in the lumen. The C-terminus of PsaF in the stromal region is involved in a strong interaction with regions of PsaJ, PsaA, PsaB and PsaE (see Fig. 3D). The PsaF subunit contains one transmembrane α-helix and two shorter α-helices that penetrate the membrane at an angle of ~30° in a V-shaped arrangement (see Figs. 3C and 3D). There are two lysine residues (Lys 108 and 122) in this region that protrude from the PS I complex into the membrane layer. They are conserved in nearly all cyanobacteria and may be responsible for strong interactions with the IsiA protein. It would be very interesting to see if this hypothesis can be proven by mutagenesis.

Neither IsiA nor phycobilisomes exist in green algae and higher plants. Instead, they contain the membrane-intrinsic Chl *a*/*b* binding antenna proteins, the light harvesting complexes I and II (LHCI and LHCII). The LHCI is the major peripheral antenna of PS I [9,32]. Although cyanobacterial PS I complexes assemble in thylakoids as a trimer, the eukaryotic PS I exists as a monomer associated with dimers of Lhca polypeptides [9,32]. So far an X-ray structure of any plant type PS I does not exist. Electron microscopy investigations identify 11–14 LHCI polypeptides attached to the monomeric eukaryotic PS I core [33,34]. The PsaF subunit was shown to play an important role in the interaction of the eukaryotic PS I with the LHCI complex [9]. The sequence comparison between eukaryotic and prokaryotic PsaF and PsaJ shows a high degree of sequence similarity for both proteins.

Of special importance is the conservation of one of the two lysine residues in nearly all plants and green algae in the region encoding the membrane-exposed V-shaped structure of PsaF. One might speculate that PsaF is the major recognition and interaction site for the external antenna complexes docking to both cyanobacterial and eukaryotic PS I.

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