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Iron deficiency induces a chlorophyll *d*-binding Pcb antenna system around Photosystem I in *Acaryochloris marina*

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Abstract

The prochlorophyte-like cyanobacterium *Acaryochloris marina* contains two *pcb* genes, *pcbA* and *pcbC*, which encode chlorophyll (Chl) *d*-binding antenna proteins PcbA and PcbC, respectively. Using real-time reverse transcriptase polymerase chain reaction (RT-PCR), it is shown that when *Acaryochloris* cells are grown in an iron-deficient medium, the transcription of the *pcbC* gene is up-regulated compared to that of *pcbA*. Biochemical and immunological analyses indicated that under the same iron-deficient conditions, the level of Photosystem I (PSI) decreased compared with that of Photosystem II (PSII). Electron microscopy revealed that concomitant with these changes was the formation of Pcb–PSI supercomplexes which, in their largest form, were composed of 18 Pcb subunits forming a ring around the trimeric PSI reaction centre core. Mass spectrometry indicated that the PcbC protein is the main constituent of this outer PSI antenna system. It is therefore concluded that in *Acaryochloris*, the PcbC protein forms an antenna for PSI when iron levels become limiting and in this way compensates for the drop in the level of PSI relative to PSII which occurs under these conditions.

Keywords: *Acaryochloris*; Iron stress; Photosynthesis; Pcb protein; Photosystem; Gene transcript level

1. Introduction

Iron is the most abundant transition metal in the crust of the earth and because of its key role in the redox reactions of electron transport, it is an absolute requirement for photosynthetic organisms. However, in the aquatic ecosystems, iron concentration can be sufficiently low to limit photosynthetic activity [1,2]. As a result, some cyanobacteria and other microorganisms have evolved a number of responses to cope with the frequently occurring condition of iron deficiency. One such response in cyanobacteria is to express two "iron stress-induced" genes, *isiA* and *isiB* [3,4]. The *isiB* gene encodes flavodoxin, which can functionally replace iron-containing ferredoxin as an electron acceptor for Photosystem I (PSI) [5]. The *isiA* gene encodes the IsiA protein, often called CP43′ because it has an amino acid sequence homologous to that of the inner antenna chlorophyll (Chl) *a* binding protein, CP43, of Photosystem II (PSII) [6]. The recent isolation of IsiA–PSI supercomplexes from cyanobacteria has revealed accessory light-harvesting functions for IsiA when cells are exposed to iron deficiency [7–9]. These supercomplexes are composed of a core of three PSI reaction centers (RCs) surrounded by a ring of 18 IsiA subunits, where the latter enhance the light-harvesting capacity of PSI by about 100% [10,11]. The formation of this supercomplex seems to compensate for the reduction in the number of PSI RCs compared with PSII RCs in response to a poor supply of iron.

IsiA is a member of a chlorophyll-binding protein family containing 6 membrane-spanning helices and includes not only CP43, but also CP47 of PSII and the products of the *pcb* genes (Pcb proteins) of prochlorophytes [6,12,13]. Prochlorophytes are cyanobacteria normally lacking phycobilisomes and possessing Chl *b* in addition to Chl *a*, of

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which there are three well known examples: *Prochloron didemni* [14], *Prochlorothrix hollandica* [15] and *Prochlorococcus marinus* [16]. In these organisms, the Pcb proteins bind both Chl *a* and Chl *b* and function as light harvesting antenna systems for PSI and PSII [8,17,18].

Acaryochloris marina is a novel prochlorophyte-like cyanobacterium that contains a high level of Chl *d*, rather than Chl *a* as in other cyanobacteria [19]. It is found on the undersurface of didemnid ascidians of tropical waters [20] and also cohabits with macrophytic red algae [21]. It is now clear that Chl *d* is the major pigment bound to the proteins of the PSI RC and PSII RC core complexes, where it not only serves as an inner-antenna light harvesting system [22], but is also involved in primary charge separation in the PSI RC [23] and possibly in PSII RC [24]. Like other cyanobacteria, *Acaryochloris* cells contain phycobiliproteins that act as a light harvesting antenna system for PSII [25,26]. However, this organism also has two *pcb* genes, *pcbA* and *pcbC* [27], and recently, a supercomplex of Pcb–PSII has been isolated, where the PcbA protein seems to associate preferentially with PSII [28]. Since some *pcb* genes have been found to be induced under low iron conditions in *P. marinus* MIT9313 [18], resulting in the formation of a 18-mer antenna ring around a trimeric PSI core similar to the IsiA–PSI supercomplex in cyanobacteria, it was of interest to investigate how *Acaryochloris* might respond to low iron conditions. To this end, we have investigated the transcript levels of its *pcb* genes and, with the aid of electron microscopy, the structure of antenna-reaction centre supercomplexes when *Acaryochloris* cells are grown under iron-deficient conditions.

2. Materials and methods

2.1. Culture and thylakoid membrane preparation

Cells of *A. marina* were cultured in 10 litres KES medium consisting of artificial seawater [29] supplemented with 8.0 mM Fe-EDTA at 28 °C under illumination at 30 E m^{-2} s^{-1} (+Fe cells). The iron-stress-induced *Acaryochloris* culture was grown under the same conditions as the normal nutrient culture, except that Mn-EDTA replaced Fe-EDTA. In order to establish the iron-stress conditions, *Acaryochloris* cells were sub-cultured at least twice into minus Fe (−Fe) medium before they were inoculated into the 10 litres iron-deficient medium. Both for +Fe and −Fe, the cultures was aerated continuously.

Cells were harvested by centrifugation at 7000×*g* and washed in buffer A: 50 mM MES pH 6.5; 20% (w/v) glycerol; 10 mM $CaCl_2$; 5 mM $MgCl_2$ with 1 mM each of benzamidine, amino-caproic acid, and phenylmethylsulfonyl fluoride. The cells were broken using a bead beater (BioSpec) with 0.1 mm glass beads, in buffer to which 2 mg/ml bovine serum albumin (BSA) was added, using 8 pulses of 30 s each, with 5 min cooling intervals in the dark in an ice bath. The beads were removed from the homogenate by centrifugation at 1000×*g* for 5 min, and then washed 3 times using buffer A. Unbroken cells were removed using centrifugation of 3000×*g* for 15 min. The thylakoid membranes were collected by centrifugation at 35,000×*g* for 30 min, then washed and resuspended in buffer B: 50 mM MES–NaOH pH 6.5; 20 mM $CaCl_2$; 2.5 mM $MgCl_2$; 500 mM betaine at a chlorophyll concentration of 1.0 mg/ml.

2.2. Determination of the transcript level of pcb genes using real-time RT PCR

The total RNA of *Acaryochloris* was isolated from cells grown under −Fe and +Fe conditions, respectively, using Tri-Reagent® (Astral Scientific Inc, Australia) according to the manufacturer's instructions. The quantity of RNA obtained was determined spectrophotometrically.

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed to analyze the transcript levels of the genes *pcbA*, *pcbC* and *psaB* (the latter encoding the PSI RC protein, PsaB) using a Light-Cycler Instrument (Mark II, Roche). The Kit-SYBR Green RT-PCR (QuantiTect #204243) was used according to the manufacturer's instructions. Primers used for amplification were as follows: forward 5′-GTGCTGGTGCTCTCTATG-3′ and reverse 5′-GCCAAATACCACC AGCGATA-3′ for *pcbA* to yield a 363-bp product by RT-PCR; forward 5′-AGCCGTC-CAAACTGATTTACC-3′ and reverse 5′-GAAAGTGTG-GAATAAACCG -3′ for *pcbC* to yield a 352-bp product by RT-PCR; forward 5′-GAGATCCTAGAAGCAC ACACCC-3′ and reverse 5′-CAAGAGCACGAGCAAGAACG -3′ for *psaB* to yield a 331-bp product by RT-PCR. All primers used were *Acaryochloris*-sequence specific. The primer specificity was monitored using melting curve data and was optimised for the reading temperature. The real-time RT-PCR was carried out in 10 μl using the following conditions: 1 cycle at 55 °C for 10 min (reverse transcription), 1 cycle at 95 °C for 60 s (denaturation), 35–40 cycles at 95 °C for 20 s, 52–56 °C for 20 s (annealing temperature changed via the primers), 72 °C for 20 s (amplification) and 74–80 °C for 15 s (measurement). The relative difference in transcript levels between two genes was calculated by normalization to one gene (gene1) crossing point (Cp) for relative transcript of the other (gene2) (i.e. $Cp_{gene1}-Cp_{gene2}$) so that gene 1 crossing point for the particular transcript equalled 0, the crossing point of gene 2, one cycle later, equalled −1, two cycles later equalled −2, etc. The relative transcript levels were calculated within each sample by assuming that a 1 cycle difference represented a 2-fold difference in message levels, which assumes 100% efficiency in amplification.

2.3. Electrophoresis and immunoblotting analyses

The polypeptide composition was analyzed by 10% polyacrylamide (Acrylamide:Bis acrylamide 32:1) slab gel

electrophoresis with 6 M urea and 5% stacking gels [30]. Gels were fixed for 30 min with 40% methanol and 7% acetic acid, stained with a solution containing 0.25% Coomassie brilliant blue R250 (Sigma), 45% methanol and 10% acetic acid.

Western blotting was conducted using a published procedure [31] with antibodies raised to the PSII reaction centre PsbA (D1) protein and to the extrinsic protein of PSI (PsaE) of *Synechocystis* 6803 (kindly provided by Dr. P. Nixon, Imperial College London and Professor J. Golbeck, Pennsylvania State University, USA, respectively).

2.4. Mass spectral analyses for peptide finger printing

The polypeptide bands of interest separated by electrophoresis were excised from the stained gel matrix and analyzed by mass spectral analysis [32] at the Department of Chemistry and Biochemistry, Arizona State University, USA. The known sequences of *pcb* genes (AY552463) [27] and *psaA/B* and *psbA-psbD* genes [33] of *Acaryochloris* were used to predict the finger-printing fragments digested by trypsin cleavage using the program "Peptide Cutter": (http://kr.expasy.org/tools.peptidecutter/), and then the fragments generated from experiments (mass spectral analysis) were compared to identify the excised polypeptides.

2.5. Isolation and image processing of supercomplexes

The iron-deficient and normal cultures of *Acaryochloris* were used to obtain the −Fe and +Fe supercomplexes. Isolated thylakoid membranes (−Fe/+Fe) were partially solubilized for 10 min in the dark at 4 °C using 1% β-dodecyl maltoside (DM). The dissolved complexes were separated on sucrose density gradients generated by the freeze–thawing technique [31] containing 50 mM MES pH 6.5; 500 mM betaine; 20 mM $CaCl_2$; 2.5 mM $MgCl_2$; and 0.03% DM and ultracentrifuged at 150,000×*g* for 18 h at 4 °C. The green fractions were carefully collected using a syringe.

2.6. Electron microscopy and image processing

Electron microscope (EM) analyses were carried out at room temperature by imaging samples stained with 1% uranyl acetate using a Philips CM100 electron microscope at a calibrated magnification of 51,500× as described previously [7]. Datasets for the lowest two bands (F4 and F5) on −Fe sucrose gradients (not shown) were built, consisting of 1836 and 4488 particles in total, where all possible single particles had been interactively selected from 5 and 10 electron micrographs, respectively, that displayed minimal astigmatism and drift as determined by analysis of their Fourier spectra. In a similar fashion, a data set for the +Fe F5 fraction was built and processed using 3355 particles [28]. All subsequent image processing was performed within the Imagic-5 software environment (Image Science Software GmbH, Berlin, Germany). After several rounds of iterative refinement involving multivariate statistical analysis and classification techniques, improved 2D class averages were obtained [34]. For modeling of X-ray data into projection maps, the 'O' software package [35] was used and the coordinates for PSII and PSI obtained for the structures designated 1S5L.pdb [36] and 1JB0 [37], respectively, under the RCSB Protein Data Bank code system (http://www.rcsb.org). Subunits attributed to Pcb protein were modeled using the coordinates assigned to the six transmembrane helices of CP43 in 1S5L.pdb given the high structural homology known to exist between these two protein families [6,12,13].

3. Results

3.1. Changes in pcbA and pcbC transcript levels monitored by real-time RT-PCR in +Fe and −Fe cells

As Fig. 1 shows, real-time RT-PCR revealed that the *pcbA* and *pcbC* transcripts of *Acaryochloris* change in response to whether iron is present or not in the culture medium. In contrast to +Fe cells, the *pcbC* gene is up-regulated by almost 10 times for −Fe cells compared with the *pcbA* gene. Relative to that of the PSI RC *psaB* gene, the transcript level of *pcbA* was down-regulated about 3 times, while the transcript level of the *pcbC* gene increased by nearly 15 times under iron-stress conditions. This therefore represents a very substantial up-regulation of the *pcbC* gene and down-regulation of the *pcbA* gene relative to the level of PSI under iron limiting conditions.

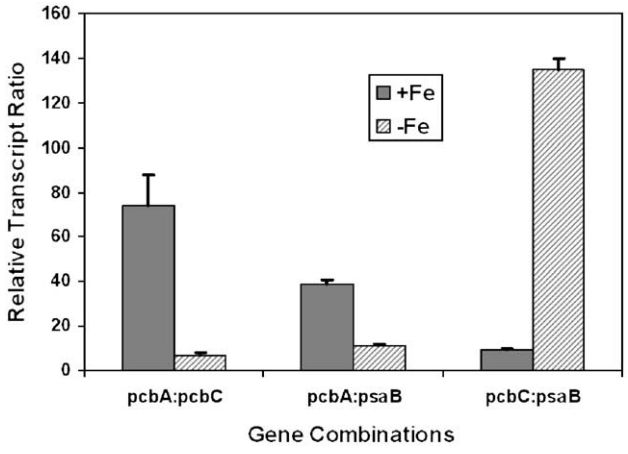

Fig. 1. Relative transcript levels of genes under +Fe and −Fe (i.e., normal culture conditions and iron-deficient culture conditions). Values are obtained as described in the Materials and Methods and represent the average of five individual RT-PCR experiments using isolated RNA from normal (+Fe) and iron-stressed (−Fe) cultured *Acaryochloris* cells, respectively. The iron stressed pcbA:psaB average amount calculated with three times individual RT-PCR results rather than 5 times.

3.2. Components of PSI and PSII of isolated thylakoid membranes from +Fe and −Fe cells

Thylakoid membranes were isolated from +Fe and −Fe *Acaryochloris* cell cultures to determine the levels of PSI to PSII protein subunits using gel electrophoresis (Fig. 2a) and Western-blotting with D1 and PsaE antibodies (Fig. 2b). Based on immunological analyses of thylakoid membranes, both PSI and PSII were decreased under −Fe conditions, as judged by loading equal Chl *d* concentrations onto the electrophoretic gels. In particular, the amount of PSI as judged by the levels of PsaE was significantly lower compared to that observed in +Fe thylakoids. The significant decrease in PSI in response to low iron can also be seen in the Coomassie brilliant blue-stained gel where the band at about 60 kDa, attributed to PsaA and PsaB proteins, is significantly reduced compared with that observed with +Fe cells. In contrast, the band corresponding to Pcb proteins at about 35 kDa increased in −Fe compared to +Fe thylakoids.

3.3. Isolation and structural characterization of antenna-reaction centre supercomplexes

To structurally localise the Pcb antenna proteins of *Acaryochloris*, isolated thylakoid membranes of +Fe and −Fe cells were mildly solubilized using β-dodecyl maltoside (DM) and subjected to sucrose density centrifugation. The resulting profile of the sucrose gradient fractions for the DM solubilized +Fe and −Fe thylakoid membranes consisted of five bands in both cases (F1 to F5; data not shown). All five bands were in similar relative positions except for the bottom fraction (F5), where the −Fe F5 band was higher in the gradient than the +Fe F5 band. Based on previous studies [7,8,17,18], it would be expected that the heaviest fraction (F5) contains supermolecular complexes comprising Pcb proteins and RC cores. EM analyses confirmed this (see Fig. 3a and b). These analyses revealed that the −Fe F5 fraction, generated from −Fe thylakoid membranes, contained large round-shaped particles about 280 to 320 Å in diameter (ringed in Fig. 2a). Single particle analysis performed on these particles suggested that they contain trimeric PSI RC in the centre surrounded by a density corresponding to Pcb proteins (Fig. 3b and c). Trimeric PSI RC complexes free of additional density were observed in both +Fe and −Fe F4 fractions of the sucrose gradients (see Fig. 3d) which corresponded in shape and size to the X-ray structure of the related PSI RC trimer of *Thermosynechococcus elongatus* [37]. The outlines of the PSI RC trimer and the monomer within it are shown in Fig. 3d and used to interpret the structures identified in the −Fe F5 fraction. In some cases, a complete ring of extra density was observed (Fig. 3c), and based on the X-ray structure of CP43 [36], we conclude that this ring consists of 18 Pcb subunits as is the case for similar Pcb–PSI supercomplexes isolated from *P. marinus* [7,18] and for the IsiA–PSI supercomplex of other cyanobacteria [6,8]. However, compared with previous studies [8,18], we found the 18-mer Pcb–PSI supercomplex, isolated from −Fe *Acaryochloris* cells, to be less stable giving rise to populations of PSI RC particles in −Fe F5, which did not have a complete set of Pcb subunits as typified in Fig. 3b. This partial loss of Pcb subunits occurs during DM solubilization of the isolated membranes as judged by the high level of free Pcb protein in F1 and trimeric PSI RC in the −Fe F4 fraction.

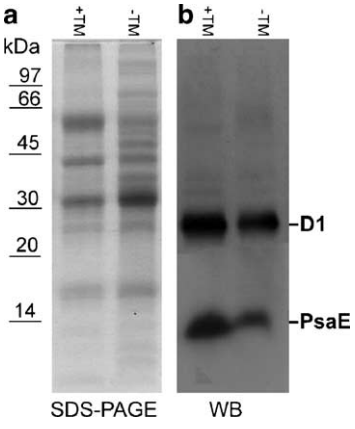

Fig. 2. Comparison of the polypeptide composition of thylakoid membranes isolated from *A. marina* and subsequent sucrose gradient separation. Analyses were made on two different preparations of thylakoid membranes isolated from +Fe/−Fe conditions (+TM/−TM respectively), loaded on a 1.2 μg Chl *d* per lane basis. (a) 10% SDS-PAGE. (b) Western blotting using PSII specific antibody (D1) and PSI specific antibody (PsaE) on these thylakoids.

In the case of +Fe F5 fractions, particles corresponding to the Pcb–PSI supercomplex were not observed. Instead, oblong shaped particles having a size of about 385 × 240 Å were seen (Fig. 3b). These particles have recently been shown to be Pcb–PSII supercomplexes containing two end-to-end PSII RC cores having 8 Pcb subunits on each side [28]. Therefore, it seems that in response to iron deficiency, *Acaryochloris* forms a Pcb–PSI supercomplex with a structural arrangement similar to that of other IsiA/Pcb–PSI supercomplexes reported previously [7–9,18]. Interestingly, we did not observe the large Pcb–PSII supercomplex isolated from +Fe cells in the −Fe F5 fraction or any other fraction, suggesting that this macromolecular PSII complex does not assemble in −Fe conditions or that it is more susceptible to disruption by DM when cells are iron-stressed.

Biochemical analysis by gel electrophoresis (Fig. 4) showed that the 35 kDa band attributed to Pcb proteins is clearly resolved in the −Fe F5 fraction (Fig. 4, lane −F5 marked “c”) and the mass spectroscopy protein fingerprinting revealed that this band corresponded to the product of the *pcbC* genes of *Acaryochloris* as well as identifying band “b” in −Fe F5 as the PsaA protein (see Table 1). As Table 1 shows, mass spectrometry also indicated that some PcbA protein was also present in −Fe thylakoids as judged

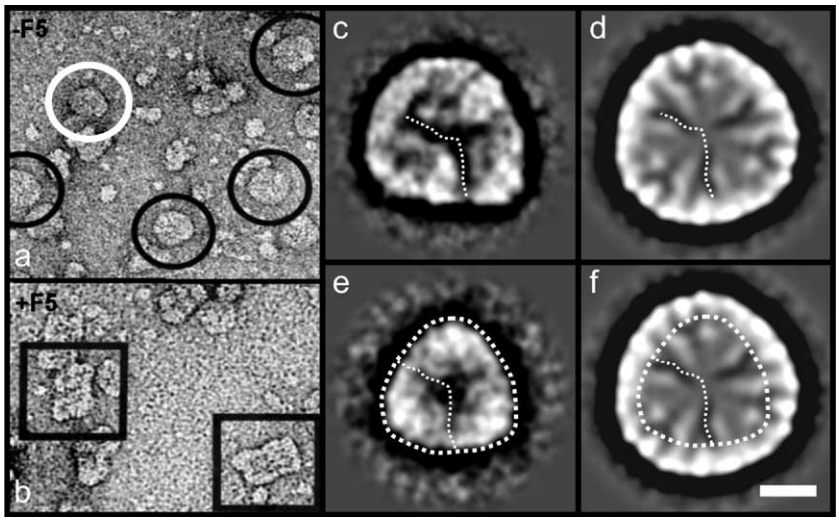

Fig. 3. (a,b) Electron micrographs (51,500×) of the antenna–reaction supercomplexes isolated in fractions +Fe F5 (+F5) or −Fe F5 (−F5), as labeled, obtained from sucrose density gradients (see Fig. 1c). There are obvious differences regarding the structures of the antenna–reaction centre supercomplexes present. (c) Post-image processing of the raw images leading to class averages obtained from subpopulations of particles attributed to broken Pcb–PSI supercomplexes (e.g. ringed in white in panel (a)); (d) the most intact Pcb–PSI supercomplexes observed (ringed in black in panel (a); (e) PSI trimers lacking Pcb protein components from +Fe F5. The likely three-fold symmetry axis has been marked on each class average, with (f) the outline of the *Acaryochloris* PSI trimer in panel (e) being overlaid onto the outline of the most intact supercomplex observed (d), indicating a typical 18 Pcb-subunit ring arrangement, surrounding a PSI trimer reaction centre core. Bar in panel (f) represents 10 nm, applicable to panels (c)–(f).

by its presence in the −Fe F1 fraction along with PcbC (Fig. 4, lane-F1 marked "a"). This analysis is consistent with real-time RT-PCR which also showed that two *pcb* genes are expressed under −Fe conditions although the *pcbC* gene is significantly up-regulated by iron deficiency with a concomitant down-regulation of *pcbA* (Fig. 1). As previously reported [28], mass spectrometry of the +Fe thylakoids also contained both *pcb* gene products but in this case, the PsbA level was higher than PsbC with the former being associated with PSII.

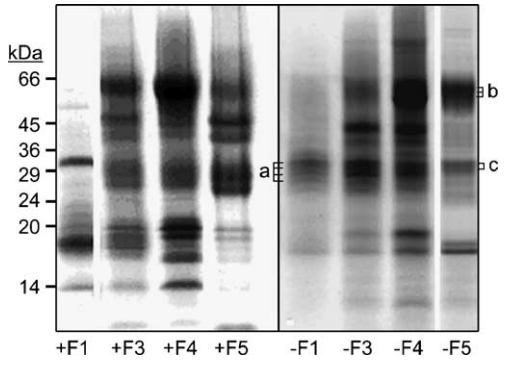

Fig. 4. SDS-PAGE analysis of the polypeptide composition for the five fractions isolated from both the +Fe (left panel) and −Fe (right panel) sucrose gradients. The labels a–c represent those bands analyzed by mass spectra finger printing. This analysis showed that band (a) containing PcbA and PcbC, encoded by the *pcbA* and *pcbC* genes. Band (b) was found to be composed of the PsaA and PsaB PSI RC core protein gene products. Band (c) was found to contain PcbC, encoded by the *pcbC* gene.

4. Discussion

There is considerable interest in *A. marina* because it uses Chl *d* in place of Chl *a* both in light harvesting [38], and in the RC of PSI [23], and possibly of PSII [24]. Here, we have identified the presence of a giant Pcb–PSI supercomplex in this novel organism when grown in media low in iron. This supercomplex was isolated by DM treatment of isolated thylakoid membranes and was present in the heaviest band (−Fe F5) of the sucrose density gradient. The most complete particle in the −Fe F5 fraction consisted of the PSI trimeric core surrounded by a ring of electron density interpreted to be 18 subunits of the Pcb protein. Therefore, *Acaryochloris* seems to be able to assemble a giant PSI supercomplex similar to that shown for cyanobacteria [7,9] and for prochlorophytes [8,18]. Real-time RT-PCR revealed that the expression of the *pcbC* gene is up-regulated under −Fe conditions and the resulting product, PcbC, is present in the same sucrose density fraction which contains the Pcb–PSI supercomplex. Thus, this gene product probably accounts for most of the extra PSI antenna of this supercomplex. Assuming that the PcbC protein of *Acaryochloris* binds 14 Chl molecules, as does CP43 [36], then the 18-mer Pcb ring increases the light harvesting ability of PSI by about 100%.

A ring of 18 IsiA antenna proteins around PSI was first observed in non-Pcb containing cyanobacteria [7,9]. The conditions for ring formation were induced under low iron conditions. As outlined in the Introduction, IsiA protein forms a family with Pcb antenna proteins. However in prochlorophytes (*P. didemni*, *P. marinus* and *P. hollandica*), the Pcb proteins bind Chl *a* and Chl *b* (and Mg-DVP in *P.*

Table 1
Protein mass finger-printing analysis

SDS-PAGE bands (Fig. 4)	Mass spectra reading fragments (Da)	Predicting mass of the genes (800–2800 Da)	Matching percent (%)	Identified proteins
Band a of lane-F1	891.4, 1069.3, 1279.6, 1713.7, 2265.9	890.9, 985.1, 1069.3, 1279.4, 1713.8, 2265.4, 2328.7	71.4 (5/7)	PcbA
	932.4, 1177.5, 1713.7, 1738.7, 1927.8, 2832.3	931.1, 1178.3, 1714.9, 1737.9, 1927.9, 2833.1 (6/6)	100 (6/6)	PcbC
Band b of lane-F5	848.3, 865.3, 872.3, 930.4, 1105.2, 1130.6, 1455.7, 1632.8, 2380.0, 2481.3	847.9, 865.0, 872.9, 929.0, 1105.2, 1130.3, 1454.7, 1632.7, 1946.2, 2100.3, 2380.7, 2480.7, 2858.4	76.9 (10/13)	PsaA
Band c of lane-F5	932.4, 1176.3, 1714.7, 1928.8, 2833.3	931.1, 1178.3, 1714.9, 1737.9, 1927.9, 2833.1	83.3 (5/6)	PcbC

Polypeptides generated from SDS-PAGE (Fig. 4) were digested by trypsin and the digested peptide fragments were analyzed by mass spectrometry (800–2000 Da) (mass finger printing). They were a good match (71–100%) with the predicted peptide masses from the relative genes (*psaA*: Ref. [31]; *pcbA/pcbC*: AY552463).

didemni and *P. marinus* [13]), while IsiA binds only Chl *a* unless the *cao* gene is artificially engineered into the cyanobacterial genome when Chl *b* can also be bound to IsiA [39]. It has been shown that there are a number of genes (1–8 copies) for Pcb proteins in various prochlorophytes [27,40,41] and at least some of these proteins are targeted to PSII to form a peripheral polymeric structure on PSII [17,18]. Furthermore, it has been shown that in *P. marinus* (strain SS120), the PcbG protein forms an 18-mer ring around its PSI trimer [8] while the same supercomplex is formed in *Prochlorococcus* (strain MIT9313) when the iron level is lowered in the culture medium [18], and in this case, the Pcb protein targeted to PSI is encoded by its *pcbB* gene. Interestingly, the SS120 strain up-regulates its *pcbC* gene in response to iron depletion, which suggests that PcbC may replace PcbG as an antenna protein in this low light strain of *Prochlorococcus* when the availability of iron is limited [18].

The demonstration of two different Pcb proteins being present in *Acaryochloris* [27] is therefore of considerable interest. The Pcb proteins of this organism bind mostly Chl *d*, together with a small amount of Chl *a* [42]. Previously, we have shown that the major, constitutive Pcb protein in *Acaryochloris* grown in +Fe conditions is encoded by its *pcbA* gene and forms a polymeric, peripheral structure with PSII (16 Pcb: 4 PSII RC) [28]. This supercomplex was observed in F5 fraction of the +Fe sucrose density gradient as shown in Fig. 3b, but it was not detected in the −Fe F5 fraction. Indeed, the *pcbA* gene was down-regulated in the absence of iron, although some PcbA protein could be detected in −Fe thylakoids. Therefore, *Acaryochloris* follows the same trend as previously described for *Prochlorococcus*, where, for example, in strain MIT9313, the product of its *pcbA* gene is targeted to PSII while its *pcbB* gene is expressed under −Fe conditions and its product targeted to PSI [18].

Acaryochloris seems to be quite widespread [20,21,43,44] and, along with *Prochloron*, inhabits didemnid ascidians that are found on tropical coral reefs and in tropical mangroves [45]. While *Prochloron* is an exosymbiont within the body of the ascidians, *Acaryochloris* may be, for the most part, an epibiont, on the underside of the ascidians, in a specialized environment about which little is known, except that it is greatly enriched in near infrared radiation [20]. Both organisms may suffer low iron conditions at times, since coral reefs are known to exist in marine waters that are very low in iron [46]. Since *Prochloron* is an exosymbiont, it is bathed in water that is drawn in by separate inhalant siphons to each individual of the colony, and therefore, its iron status is unlikely to be greatly modified by the ascidian colony. Thus, it may not be surprising that both *Prochloron* and *Acaryochloris* should retain the iron-sensitive *pcbC* gene. *P. hollandica* possesses three genes, *pcbA*, *B* and *C*, and the *pcbA* and *pcbC* genes are homologous with those of *Prochloron* and *Acaryochloris*. So it is possible that in *Prochlorothrix* also, the PcbC protein is induced under low iron conditions. Recently, an 18-mer Pcb–PSI supercomplex was observed in *P. hollandica*, although in this case, it is not yet clear which Pcb is associated with PSI [47].

The reason that iron deficiency induces the expression of light-harvesting antennae for PSI in classical cyanobacteria has been explained as a response to nitrogen limitation; iron acting as a proxy for nitrogen [48,49]. According to this reasoning, chlorophyll pigments are less expensive in terms of nitrogen than phycobiliproteins [13]. However, there is another explanation: Strzepek and Harrison [50] have observed that diatoms in the open ocean have a very different photosynthetic make-up to that of coastal and freshwater forms. Open ocean diatoms have reduced the number of PSI cores relative to PSII and have increased the antenna units to each core. This allows a greater photochemical turnover per PSI RC and therefore the same electron flux for fewer PSI RC complexes, while at the same time conserving the valuable iron located in the iron–sulfur centers and the cytochromes of redox active proteins of photosynthesis (including those of PSI) and respiration. The same argument can be applied to classical cyanobacteria, where iron starvation also leads to a drop in the PSI to PSII ratio with a concomitant increase in the antenna size of PSI. Here, we show that *Acaryochloris* follow the same pattern even though it might be expected that its major light-harvesting antenna should be associated with PSII under the low light conditions in which it lives [51]. However, *Prochlorococcus* MIT 9313, which live in an intermediate light climate, only produce an antenna ring around PSI under iron stress conditions [18] whereas the very

low light strain SS120 permanently has this additional PSI antenna system [7].

Acaryochloris appears to live under low light, enriched in near infrared [20] and this seems to be true for the other *Acaryochloris*-like organisms with Chl *d* [21]. It is therefore logical that the major Chl should be Chl *d* rather than Chl *a*, and that it is used by both PSI and PSII. However, there are significant quantities of phycobiliproteins present in *Acaryochloris* which act in a light-harvesting capacity [52,53]. The major pigment is phycocyanin [52], which harvests yellow-orange light. Its presence suggests that there is, at times, a yellow-orange light available to *Acaryochloris* in its natural habitat, which would also imply that there should be red light available too. If so, the small quantities of Chl *a* (up to 6%) would serve to harvest this light. The action spectra of oxygen production [53] suggest that this is true. Whether some of this Chl *a* is to be found attached to both PcbA and PcbC proteins is a matter for future investigation.

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