Closing the gaps on the viral photosystem-I psaDCAB gene organization

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Summary

Marine photosynthesis is largely driven by cyanobacteria, namely Synechococcus and Prochlorococcus. Genes encoding for photosystem (PS) I and II reaction centre proteins are found in cyanophages and are believed to increase their fitness. Two viral PSI gene arrangements are known, psaJ→C→A→B→K→E→D and psaD→C→A→B. The shared genes between these gene cassettes and their encoded proteins are distinguished by %G+C and protein sequence respectively. The data on the psaD→C→A→B gene organization were reported from only two partial gene cassettes coming from Global Ocean Sampling stations in the Pacific and Indian oceans. Now we have extended our search to 370 marine stations from six metagenomic projects. Genes corresponding to both PSI gene arrangements were detected in the Pacific, Indian and Atlantic oceans, confined to a strip along the equator (30°N and 30°S). In addition, we found that the predicted structure of the viral PsaA protein from the psaD→C→A→B organization contains a lumenal loop conserved in PsaA proteins from Synechococcus, but is completely absent in viral PsaA proteins from the psaJ→C→A→B→K→E→D gene organization and most Prochlorococcus strains. This may indicate a co-evolutionary scenario where cyanophages containing either of these gene organizations infect cyanobacterial ecotypes biogeographically restricted to the 30°N and 30°S equatorial strip.

Introduction

Cyanobacteria play a key role in oceanic photosynthesis and contribute to the global carbon cycle and oxygen supply (Li et al., 1993; Liu et al., 1997; Partensky et al., 1999). Genes encoding for photosystem-II (PSII) reaction centres (the D1 and D2 proteins encoded by the psbA and psbD genes, respectively) are found in cultured and uncultured phages that infect marine cyanobacteria (Mann et al., 2003; Lindell et al., 2004; 2005; Millard et al., 2004; Sullivan et al., 2005; 2006; Zeidner et al., 2005; Sharon et al., 2007), are expressed upon infection (Lindell et al., 2005; 2007; Clokie et al., 2006), and it was suggested that this increases phage fitness (Bragg and Chisholm, 2008; Hellweg, 2009). See Puxty and colleagues (2014) for a recent review on viral ‘photosynthesis’.

Using environmental metagenomics, uncultured cyanophages were recently found to contain gene cassettes coding for photosystem-I (PSI) reaction centres (Sharon et al., 2009). Two viral PSI gene organizations are currently known (Sharon et al., 2009; Béjà et al., 2012), psaJ→C→A→B→K→E→D and psaD→C→A→B. The psaJ→C→A→B→K→E→D cassette contains a gene fusion between the psaJ and psaF and is characterized by a low %G+C content of around 40%, while the four gene cassette, psaD→C→A→B, tends to have a higher %G+C content, ranging from 42% to over 50%. The fused PsaJF protein from the low %G+C cassette was hypothesized to be able to accept electrons not only from PSII (via plastocyanin or cytochrome c6) but to also work with other electron donors like soluble cytochrome c that usually function as electron donors to cytochrome oxidase (Sharon et al., 2009). This was recently shown using a heterologous Synechocystis

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system mimicking the cyanophage system (Mazor et al., 2014).

Our knowledge on the psaD→C→A→B gene cassette is based solely on two metagenomic scaffolds originating from the Global Ocean Sampling (GOS) expedition (Sharon et al., 2009; Béjà et al., 2012) (upper Fig. 1). These scaffolds contain a partial psaD gene, the highly conserved psaC gene, the beginning of psaA, and one clone also contains the far end of psaB gene. In addition, we have recently shown, using polymerase chain reaction (PCR) with primers amplifying the unique viral gene organization psaC→psaA (found in both gene cassettes), that psaA genes coming from the viral psaD→C→A→B cassette are diverse (Hevroni et al., 2015).

The goal of this study was to expand our knowledge regarding PSI genes carrying phages, with a special emphasis on phages carrying the psaD→C→A→B gene organization. We wanted to better understand why such two different viral PSI gene organizations exist, whether they are capable of different functions and who are the potential cyanobacterial hosts. For this 370 marine stations from six metagenomic projects were analysed for the presence of viral PSI genes. Numerous viral scaffolds were found from both PSI gene organizations, and the analysis of the scaffolds matching the psaD→C→A→B gene organization enabled us to close the gaps in missing parts of the gene sequences. In addition we were able to model the viral PsaA protein encoded by the psaD→C→A→B gene organization and to find substantial structural differences to its PsaA counterpart from the viral psaJF→C→A→B→K→E→D organization.

Results and discussion

To date, numerous low %G+C viral PSI cassette sequences have been identified (Sharon et al., 2009; Alperovitch-Lavy et al., 2011). In contrast, only two high %G+C viral PSI scaffolds are currently known (upper Fig. 1). To increase our understanding of the high %G+C viral PSI gene arrangement and fill up the sequence gaps in the psaD→C→A→B cassettes, we have examined the GOS (Rusch et al., 2007; Yooseph et al., 2007), Pacific Ocean Virome (POV) (Hurwitz and Sullivan, 2013), Tara Oceans (Karsenti et al., 2011; Brum et al., 2015; Sunagawa et al., 2015), C-MORE:BULA (Hewson et al., 2009), Moore Virome Project (The Gordon and Betty Moore Foundation Marine Microbial Initiative genomes), and Hawaii and Line Islands metagenomic datasets using the viral protein sequences of PsaD, PsaA and PsaB as queries.

Fig. 1. Schematic gene organization of GOS and Tara Oceans scaffolds containing viral PSI genes from the psaD→C→A→B cassette. PSI genes are coloured according to their %G+C content; the calculation was performed on each gene separately. Grey boxes represent viral ORFs. Two GOS clones previously reported are boxed at the top. DNA sequences can be found in Appendix S1. gp23 – major capsid protein; gp17 – terminase large subunit; DNApol – DNA polymerase; regA – translation regulator. For clarity, not all detected scaffolds are shown.
etc., our results show that viral PSI gene cassettes are widespread. The presence of scaffolds matching the \( \text{psaD} \rightarrow \text{C} \rightarrow \text{A} \rightarrow \text{B} \) cassette was observed in the Pacific and Indian oceans, and for the first time also detected in the Atlantic Ocean. To the same extent, it is worth noting the existence of different picocyanobacterial clades or ecotypes that occupy distinct environmental conditions (see Scanlan et al., 2009, for a review). For instance, within the \( \text{Synechococcus} \) subcluster 5.1 (Dufresne et al., 2008; Scanlan et al., 2009), to which most marine \( \text{Synechococcus} \) belong, just a few clades such as clade II and III (Scanlan et al., 2009) and clades CRD1 and CRD2 (Sohm et al., 2015) have been reported between 30°N and 30°S. On the contrary, clades I or IV are found in coastal and/or temperate mesotrophic open ocean waters largely above 30°N and below 30°S. The same geographical restrictions are valid for \( \text{Prochlorococcus} \) clades such as the HLII clade occupying strongly stratified surface waters between 30°N and 30°S, or the contrary case clade HLI living in more weakly stratified surface waters, particularly between 35° and 48°N and 35° and 40°S, just to mention a few examples (Scanlan et al., 2009). Therefore, it seems quite logical to assume that the distribution of cyanophages containing PSI-viral genes would fit with the distribution of their cyanobacterial hosts, and in this particular case we suggest that cyanophages containing PSI-viral genes are restricted to infect picocyanobacterial ecotypes living in the belt defined between 30°N and 30°S.

The newly discovered scaffolds from the high \%G + C gene organization allowed us, for the first time, to construct PsA, PsD and PsB phylogenetic trees containing more than one viral high \%G + C entity. As previously observed (Béjà et al., 2012), partial sequences of viral proteins from the high \%G + C gene organization cluster together, within the marine \( \text{Synechococcus} \) clade, while partial proteins from the low \%G + C gene organization group cluster separately forming their own clade. Having longer sequences originated from environmental scaffolds made it possible to construct full-length PsB and PsD phylogenetic trees (Fig. 3), based on all 756 and 193 amino acids positions of PsB and PsD respectively. These trees show a different topology to the previously reported, with both low and high \%G + C gene organizations forming monophyletic clades outside of the \( \text{Prochlorococcus} \) and \( \text{Synechococcus} \) clades.

Some of the new high \%G + C scaffolds from GOS and Tara Oceans contained several viral genes other than PSI genes. These genes (e.g. DNA polymerase or \( \text{regA} \) genes) resemble genes from cyanomyophages (T4-like phages; see Fig. S1), suggesting a possible \( \text{Myoviridae} \) origin for these scaffolds. In all viral scaffolds where sequences reaching beyond the photosynthetic gene arrangement borders were available, there were always viral genes constraining the arrangement (Fig. 1). This supports the notion that there are no other neighbouring photosynthetic genes accompanying the \( \text{psaD} \rightarrow \text{C} \rightarrow \text{A} \rightarrow \text{B} \) gene arrangement. It is important to remark that the
retrieved viral psaD sequences were always flanked by a non-photosynthetic viral open reading frame (ORF), either upstream or downstream in the high %G + C and low %G + C gene organizations respectively. The same occurs downstream to the high %G + C psaB and upstream the psaJF (which can be found only in low %G + C sequences). Furthermore, the sequences retrieved consistently match the psaJF→C→A→B→K→E→D or psaD→C→A→B gene organizations, accordingly to their %G + C content, which might indicate that viral PSI genes are found solely in the two previously described cassettes. However, as metagenomic data are fragmented by nature, we cannot rule out the presence of standalone PSI photosynthetic genes (e.g. the psaJ gene;...

Fig. 3. Maximum likelihood phylogenetic trees of (A) PsaD – based on 193 amino acids positions, and (B) PsaB – based on 756 amino acids positions. Circles represent bootstrap values higher than 90%. Phage name colours represent %G + C classification according to the colour index in Fig. 1, purple stands for low %G + C sequences, green and blue for high %G + C. The scale bar indicates the average number of amino acid substitutions per site.
Based on data from the environmental scaffolds, we were able to assemble a full-length viral PsaA protein sequence from the high %G+C family by overlapping partial sequences. Structure prediction of the assembled viral PsaA was then compared with that of PsaA proteins from *Synechococcus*, *Prochlorococcus* and with the viral low %G+C version. As shown in Fig. 4A, the overall structure of the four proteins is conserved except for a small loop (boxed in Fig. 4A) facing outside the membrane. This loop is in close proximity to the hydrophobic binding site of plastocyanin/cytochrome c₅ (Sommer et al., 2004; Mazor et al., 2012), therefore potentially influencing the electron transfer between the electron donor and P700 in PSI. To further confirm the existence of this loop, we designed a set of degenerate primers based on a PsaA protein’s sequence alignment and used them to amplify the gene from viral concentrates collected from the Line Islands. Positive overlapping *psaA* PCR products were successfully retrieved (GenBank #s KP411049-KP411210), and their %G+C content was similar to viral low and high %G+C groups. This loop is found in the viral high %G+C PsaA, in *Synechococcus*, and also in low light adapted (LL) *Prochlorococcus* MIT9313 and MIT9303, and is missing in PsaA proteins of other LL *Prochlorococcus*, high light adapted (HL) *Prochlorococcus*, and in the viral low %G+C version. Interestingly, the high %G+C viral version of the loop is different from the marine cyanobacterial loop, containing a conserved arginine residue (Fig. S2); the viral loop is therefore positively charged compared with the cyanobacterial versions.

Fig. 4. Structure modelling of PsaA proteins from cyanobacteria and cyanophages. (A) PsaA from *Synechococcus* (in cyan), HL *Prochlorococcus* (green), low %G+C viral (purple) and from the reconstructed high %G+C viral (red). The loop missing in PsaA from *Prochlorococcus* (except in LL *Prochlorococcus* MIT9313 and MIT9303) and the low %G+C viral, but present in *Synechococcus*, LL *Prochlorococcus* MIT9313 and MIT9303, and in the high %G+C viral PsaA is boxed. (B) Electrostatic potential of the luminal side of PsaA proteins boxed in panel A. Red and blue indicate negative and positive potentials respectively. The loop sequences alignment can be found in Fig. S2.
cassette. This could indicate that cyanophages containing potentially be cyanobacteria which have the lumenal loop in PsaA and psaD leading to the assumption that phages carrying the originating from the viral psaJF protein. The fused PsaJF protein found in the viral psaJF→C→A→B→K→E→D cassette, namely being promiscuous for its electron donors and being able to accept electrons from electron donors other than plastocyanin or cytochrome c₆ (Mazor et al., 2014).

We suspect that cyanophages carrying high %G+C PSI genes infect hosts with similar protein structures, leading to the assumption that phages carrying the psaD→C→A→B gene organization might infect cyanobacteria which have the luminal loop in Psaa and have a similar geographical distribution. The hosts could potentially be Prochlorococcus MIT9313 and MIT9303 [belonging to an LL Prochlorococcus clade (clade LLIV)], which is considered to be closely related to Synechococcus and is a clade widely distributed within the 40°N to 35°S latitude range, largely restricted to the deep euphotic zone (Scanlan et al., 2009; Biller et al., 2015), or Synechococcus from clades CRD1 and CRD2 which are present in similar latitudes, 40°N to 30°S (Sohm et al., 2015).

The luminal loop is missing in Psaa protein versions originating from the viral psaJF→C→A→B→K→E→D cassette. This could indicate that cyanophages containing the psaJF→C→A→B→K→E→D gene organization might infect different cyanobacterial hosts as compared with phages containing the psaD→C→A→B gene organization. Likewise, these cyanophages would only infect cyanobacterial species or ecotypes latitudinally restricted to the strip defined between 30°N and 30°S. However, we cannot rule out the possibility that these two kinds of phages infect the same hosts but perform differently to achieve a similar outcome, namely PsaaJF and the luminal loop in Psaa could perform similar functions regarding the docking of electron donors to PSI.

Our Psaa modelling suggests that the Psaa protein from the psaD→C→A→B gene organization might function differently from Psaa versions of the potential hosts and the other viral gene organization, presenting a new kind of PSI complex. Evolutionary studies regarding PSII proposed that a minimal complex composed by the Psaa, Psab, Psac and Psad proteins could theoretically form a functional reaction centre (Nelson, 2011). Therefore, characterizing phages with the psaD→C→A→B gene set might shed light on PSI evolution and lead to a better understanding of PSI light reactions, as this might be the only extant case of a minimal, functional PSI that comprised only four subunits.

**Experimental procedures**

**Metagenomic data analysis**

Microbial and viral metagenomic datasets were downloaded from CAMERA (Seshadri et al., 2007), IMicrobe database (http://imicrobe.us) or MG-RAST (Meyer et al., 2008).

Microbial metagenomes from the GOS expedition project (Venter et al., 2004; Rusch et al., 2007), Hawaii and Line Islands, Biogeochemistry of the Upper Ocean: Litudinal Assessment (C-MORE:BULA) project (Hewson et al., 2009), Tara Oceans expedition (Sunagawa et al., 2015), and viral metagenomes from the POV project (Hurvitz and Sullivan, 2013), Moore Virome project, and Tara Oceans virome (Brun et al., 2015) were analysed using BLAST v2.2.28 tools (see Table S1 for the metagenomic datasets).

First a collection of amino acid sequences from low and high %G+C viral PSI genes psaa and psab (Table S2) was used as query for a tblastn search (e-value 0.1) against the metagenomes. Contigs and reads matching PSI proteins and their paired-end mates were further screened using BLASTX (e-value 10e-10) against the NCBI non-redundant (nr) protein database to identify those that were likely to have a viral origin according to the top score hit of taxonomy assignment and presence of viral genes on the contig or read mate.

**psaa amplification and cloning**

Degenerate primers were designed against a Psaa protein multiple sequence alignment of a wide variety of organisms, including eukaryotes, prokaryotes and viral Psaa proteins obtained from GenBank (Primers TTTTV[IV][V]_fw, ACNASCNCTTGGRTNTGGAA; HHIHAF_rev, RAANGC RTGDATRTGRTG; MPPY[PA]Y_fwd, ATGCNCCNTA; TTTV[AS]FF_rev, RAARANBWCCANGTNGT; with 512, 192, 256 and 1536 degeneracy respectively). Two PCR reactions (Reaction B: TTTTV[IV][V]_fw – HHIHAF_rev; Reaction L: MPPY[PA]Y_fwd – TTTV[AS]FF_rev) were performed directly on viral concentrates from the Pacific Southern Line Islands [collected in April 2009 and in November 2013 from Caroline island (Millennium Island) and in October 2013 from Vostok Island]. Viral concentrates were prepared according to Haas and colleagues (2014). The PCR reactions B and L were performed using BIO-X-ACT™ Short mix (Bioline, London, UK), in a total volume of 30 µl containing 1 µl of phage concentrate as template, OptiBuffer (1×), 2 µM primers (each), 0.8 mM dNTPs, 2 mM MgCl₂ and 2.4 U BIO-X-ACT™ Short DNA polymerase. The PCR conditions were the following: Reaction B – 95°C, 5 min; 40 cycles of 95°C, 30 s; 53°C, 30 s, 72°C, 100 s; and Reaction L – 95°C, 5 min; 40 cycles of 95°C, 30 s; 50°C, 30 s, 72°C, 70 s. Reaction L PCR was also performed using the Tiangen 2× Taq PCR MasterMix (Tiangen Biotech, Beijing), in a total volume of 25 µl containing 1 µl phage concentrate as template, 1.2 µM primers (each) and Master Mix (1×). The PCR amplification conditions were as previously described. The PCR products

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(Reaction B – 1500 bp approximately; Reaction L – 1100 bp approximately) were cloned using the PCRII-TOPO vector (Invitrogen, San Diego, CA) according to the manufacturer’s specifications and sequenced using Sanger sequencing (Macrogen Europe, Amsterdam, NL). Sequences retrieved were checked against published psaC→A viral sequences using k-mers analysis in the overlapping region between the amplicons (Hevroni et al., 2015).

Phylogenetic tree construction and analysis

PsAB and PsAD sequences from the Tara Oceans and GOS projects were obtained by translating the scaffold DNA sequence according to the correct open reading frame and aligned along with sequences from Prochlorococcus and Synechococcus (retrieved from GenBank). Multiple sequence alignments were constructed using CLUSTALX v2.1 (Larkin et al., 2007). Maximum likelihood phylogenetic trees were constructed using the phylology.fr pipeline (Dereeper et al., 2008), which included PhyML v3.0 (Guindon et al., 2010) and the WAG substitution model for amino acids (Whelan and Goldman, 2001). One hundred bootstrap replicates were conducted for each analysis. See Appendices S2–S7 for the alignments used to construct the trees.

PsAA protein structure models

Structural models for the viral, Prochlorococcus and Synechococcus PsA proteins were predicted and folded according to the Protein Data Bank 1JB0 record (Jordan et al., 2001) using the HHpred software v2.0.16 (Soding et al., 2005) and MODELLER v9.11 (Sali et al., 1995). Protein models were visualized and the electrostatic potential calculated using PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.). For each protein model, we performed several protein predictions and selected one representative sequence (in bold) for Fig. 4 (Synechococcus WH7803, WH8102, WH7805, WH8109, CC9902, RS9917, RS9916, CC9311, RCC307; HL-Prochlorococcus CCMP1986, MIT9515, MIT9301; LL-Prochlorococcus NATL2A, MIT9313, MIT9211; low %GC viral GO268816; high %GC viral psaA sequences from Fig. 1 and five different sequences of PCR reactions B and L, among them KP411157.1 and KP411207.1).

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Fig. S1.** Maximum likelihood phylogenetic trees of (A) DNApol, (B) gp17, (C) gp23 and (D) RegA. Circles represent bootstrap values higher than 90%. Phage sequences retrieved in this study are coloured in red. The scale bar indicates the average number of amino acid substitutions per site.

**Fig. S2.** Multiple sequence alignment of the loop area in partial PsaA proteins. The arginine conserved in high %G + C viral sequences is marked in blue. Conserved negative amino acids are coloured in red. Names of the viral sequences represent reads/scaffolds or PCR products retrieved in this study (except for GQ268816).

**Table S1.** Metagenomic datasets analysed.
**Table S2.** Sequences used as query for the TBLASTN analysis.
**Appendix S1.** DNA sequences from Fig. 1.
**Appendix S2.** Protein alignment used to construct the PsaD phylogenetic tree (Fig. 3A).
**Appendix S3.** Protein alignment used to construct the PsaB phylogenetic tree (Fig. 3B).
**Appendix S4.** Protein alignment used to construct the DNApol phylogenetic tree (Fig. S1A).
**Appendix S5.** Protein alignment used to construct the gp17 phylogenetic tree (Fig. S1B).
**Appendix S6.** Protein alignment used to construct the gp23 phylogenetic tree (Fig. S1C).
**Appendix S7.** Protein alignment used to construct the RegA phylogenetic tree (Fig. S1D).