Identification of an Oxygenic Reaction Center $psbADC$ Operon in the Cyanobacterium $Gloeobacter violaceus$ PCC 7421

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Abstract

$Gloeobacter violaceus$, the earliest diverging oxyphotobacterium (cyanobacterium) on the 16S ribosomal RNA tree, has five copies of the photosystem II $psbA$ gene encoding the D1 reaction center protein subunit. These copies are widely distributed throughout the 4.6 Mbp genome with only one copy colocalizing with other PSII subunits, in marked contrast to all other $psbA$ genes in all publicly available sequenced genomes. A clustering of two other $psb$ genes around $psbA3$ (glr2322) is unique to $Gloeobacter$. We provide experimental proof for the transcription of a $psbA3DC$ operon, encoding three of the five reaction center core subunits (D1, D2, and CP43). This is the first example of a transcribed gene cluster containing the D1/D2 or D1/D2/CP43 subunits of PSII in an oxygenic phototroph (prokaryotic or eukaryotic). Implications for the evolution of oxygenic photosynthesis are discussed.

Key words: $Gloeobacter violaceus$, photosystem II, type 2 reaction center.
transcript pool in control, UVB, and high light conditions. This work has shown that the psbA gene in question here is transcribed and is also the most transcribed psbA gene under control and stress conditions.

Reverse transcriptase–polymerase chain reaction (RT-PCR) was employed to experimentally confirm whether the psbA3DC genomic region forms an operon as predicted via sequence analysis. Gloeobacter-culturing conditions and RT-PCR methods are detailed in Supplementary Material online. The RT-PCR results in figure 2B confirm the existence of a polycistronic mRNA transcript covering psbA3, psbD, and psbC. The long RT-PCR products (fig. 2, lane 7) confirm that the polycistronic mRNA spans all three psbA3, psbD, and psbC genes. These results validate the statistical prediction of a unique psbA3DC operon in Gloeobacter. To our knowledge, this is the only example of the cotranscription of psbA and psbD, encoding D1 and D2 reaction center core subunits.

We note that psbA and psbD are frequently found in bacteriophages that infect marine cyanobacteria. In these phage genomes, psbA and psbD genes are in close proximity and in the same direction due to the small phage genome size (Mann et al. 2003; Millard et al. 2004). However, the strong homology between psbA copies in Gloeobacter (>83%) and the lack of a second psbDC operon decreases the likelihood that the psbADC operon is an artifact of a phage infection.

The origin and evolution of the oxygenic type II reaction centers are still areas of scientific debate. Theories have been proposed based on a variety of criteria from the chemistry of the water oxidation reaction (Blankenship and Hartman 1998; Dismukes et al. 2001) to the sequence and structure of the PSII subunits (Mulkidjanian and Junge 1997; Schubert et al. 1998) and other more general criteria such as 16S rRNA (Olsen et al. 1994). Based on sequence and cofactor similarity, it has been generally accepted that the four core chlorophyll-binding subunits of PSII (D1, D2, CP43, and CP47) are the result of acquisition and evolution of two RC1 antenna domains (N-terminal domain of psaA) and the L (pufL) and M (pufM) RC2 subunits (Schubert et al. 1998; Baymann et al. 2001; Raymond and Blankenship 2004). D1 is functionally equivalent to L (binding to the second quinone acceptor Qb), and D2 is functionally equivalent to M (binding to the primary quinone acceptor Qa) (Lockhart et al. 1996), whereas CP43 and CP47 are functionally equivalent to the N-terminal domain of homodimeric RC1 psaA (antenna-binding proteins with identical α-helix number and arrangement) (Schubert et al. 1998; Baymann et al. 2001).

We argue that if D1 and D2 were evolved from L and M, they should also retain a genomic arrangement similar to that of the L and M subunits in which the corresponding genes (pufL and pufM) are organized in an operon (fig. 3). This pufLM operon is a conserved feature of the purple and green nonsulfur bacteria (Blankenship and Hartman 1998; Sauer and Yachandra 2002). In other words, psbA and psbD may also have been arranged in an operon in the ancestral
precursor. Furthermore, if CP43 and CP47 are both evolved from the N-terminal domain of the homodimeric RC1 psaA, a common ancestor existed that we refer to herein as "CP precursor."

These arguments are the basis for an evolutionary hypothesis presented in figure 3 in which we propose that the PSII precursor was a four-subunit complex (D1, D2, and two identical "CP precursor" proteins). These four subunits were encoded by a psbAD operon and "psbC" (encoding "CP precursor") whose reaction center stoichiometry was twice that of D1 and D2. This PSII precursor may have been capable of photochemical oxidation of ferrous or manganous minerals (e.g., Fe²⁺ / Fe⁴⁺ (EpH 7.5 / 0.2 V) or Mn²⁺(HCO₃⁻)/Mn³⁺(CO₃²⁻) (EpH 7 / 0.55 V)). In Step 2, psbD and psbC became joined in an operon—an arrangement maintained in modern oxygenic phototrophs. Also in this step, the formation of a catalytic site on D1 but not D2 led to the asymmetrical development of the two CP proteins resulting in CP43 and CP47. This protein environment enabled the formation of the Mn₄CaO₅ cluster and water oxidation. In Step 3, the need for faster turnover of D1 protein due to water oxidation damage resulted in the dissociation of the psbADC operon into psbA and psbDC.

Water oxidation chemistry causes unavoidable production of radicals that damage the surrounding D1 protein causing it to be removed and replaced much faster than all other subunits of PSII (Mulo et al. 2009). Consequently, this damage may have led to the need for faster gene turnover, which provides a selection pressure for the breakup of the psbADC operon. Contemporary Gloeobacter maintains the psbADC operon structure but has four other copies of psbA in the genome to allow independent expression of psbA genes relative to other PSII-related genes (Sicora et al. 2008). All other cyanobacterial genomes sequenced to date have separated this operon (psbA + psbDC) to more rapidly or efficiently repair the damaged psbA gene product.

In conclusion, the identification of a psbADC operon in Gloeobacter, not observed before in other oxygenic phototrophs, may have important implications in the evolution of oxygenic photosynthesis. Although the hypothesis presented here is supported by the present data, further studies are needed to confirm this model. Nevertheless, the presence of a psbADC operon in Gloeobacter is a unique characteristic among cyanobacteria.

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