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Alternate Copies of D1 are Used by Cyanobacteria Under Different Environmental Conditions.

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Abstract: All cyanobacteria sequenced to date have multiple *psbA* genes, encoding the D1 protein. Some of these *psbA* genes have a series of mutations that would seem to render D1 incapable of binding the Mn_4CaO_5 metallocluster (Murray 2012). Nonetheless, these genes are expressed under specific environmental conditions, such as during N_2-fixation in unicellular diazotrophs of the genes *Cyanothece*. These genes emphasize the clever way that cyanobacteria have learned to deal with a constantly changing environment.

How smart are cyanobacteria? It's highly likely that they have learned many tricks during the more than 2.5 By that they have helped sculpt the biosphere, but can they decide when and which type of D1 they can add to Photosystem II (PSII)? Such is the hypothesis stated by James W. Murray in "Sequence variation at the oxygen-evolving centre of photosystem II: a new class of 'rogue' cyanobacterial D1 proteins" in *Photosynthesis Research* 110:177-184 (2012). (Murray 2012) provides a detailed *in silico* analysis of the amino acid changes in one class of *psbA* genes and highlights how these changes might affect the oxygen evolving complex (OEC). The D1 protein represents the main site for the OEC, which contains the Mn_4CaO_5 metallocluster and the Q_B site for quinone reduction. We would like to support the hypothesis, although not the terminology, and demonstrate that the alternate *psbA* genes are transcribed under specific conditions and represent a key strategy for many diazotrophic cyanobacteria.

As Murray indicates, the understanding that cyanobacteria possess and differentially express different *psbA* genes dates to the work of Golden and colleagues in *Synechococcus elongatus* sp. PCC 7942 (Golden, Brusslan et al. 1986; Schaefer and Golden 1989; Kulkarni and Golden 1994). They demonstrated that there were 2 forms of D1 and that form II was less susceptible to high light damage(Kulkarni and Golden 1994). We became involved with alternate D1 incorporation into PSII during our studies of low O_2 growth of several cyanobacteria (Summerfield, Toepel et al. 2008). We demonstrated that a cluster of genes that included *psbA1* was highly up regulated during growth of *Synechocystis* sp. PCC 6803 in low O_2, a surprising finding that was also identified in *Cyanothece* sp. ATCC 51142 and *Anabaena* sp. PCC 7120 (Summerfield, Toepel et al. 2008). We went on to show that the D1 protein is made and inserted into PSII, thus allowing a strain in which both *psbA2* and *psbA3* were deleted to grow

photoautotrophically. Thus, the alternative D1 (referred to as D1' (Salih and Jansson 1997) was inserted into the reaction center and was functional.

Simultaneously, we found that the unicellular diazotroph, *Cyanothece* sp. ATCC 51142, differentially expressed the *psbA* genes under nitrogen-fixing conditions, but only in the dark (Toepel, Welsh et al. 2008). In this case, *psbA4* was only transcribed to any extent during the early stages of the dark period, with the transcripts of *psbA4* going from less than 0.5% of the total *psbA* transcripts in the light to over 10% at D2. A semi-quantitative version of this data is shown in Figure 1. Although we do not have the tools in *Cyanothece* sp. ATCC 51142 to determine if the corresponding alternative D1 is inserted, the cell has a *psbA* gene (*psbA4* in Fig. 1) that has likely mutated to encode a protein that can replace D1 in PSII, but make it less likely that it can form a functional OEC and evolve O_2. We recently have obtained similar evidence for light-dark diazotrophic growth of *Cyanothece* sp. PCC 7822 in a microarray experiment and we have validated this finding as shown below (Fig. 1a). Therefore, both strains of *Cyanothece* we have studied are capable of expressing an alternate form of D1 during the dark, at the exact time that nitrogenase is being expressed, assembled and activated. This can be interpreted as an evolutionary response to the needs of diazotrophy vis-à-vis the integrity of PSII and the balance between the requirement to express the *nif* genes at dusk when light may still be activating photosynthesis.

Cyanothece Sp.PCC7822 and *Cyanothece* sp. strain ATCC 51142 were cultivated in nitrate depleted medium, at 30°C in 12-h light-12-h dark cycles, under 50 μmol photons m^{-2} s^{-1}, cells were harvest at D0, D4, L0 and L4, and also at D2 for *Cyanothece*

51142. RNA extraction was conducted with Tri-Reagent (Sigma-Aldrich) and then treated with DNase I (Invitrogen, Carlsbad, CA) before Reverse transcription (RT) was performed with Superscript II (Invitrogen, Carlsbad, CA) for semi-quantitative PCR. Each strain has several almost identical *psbA* genes that are transcribed at high levels throughout the day and common primers were constructed for these redundant genes.

For *Cyanothece* sp. PCC7822, the primers for each gene were:

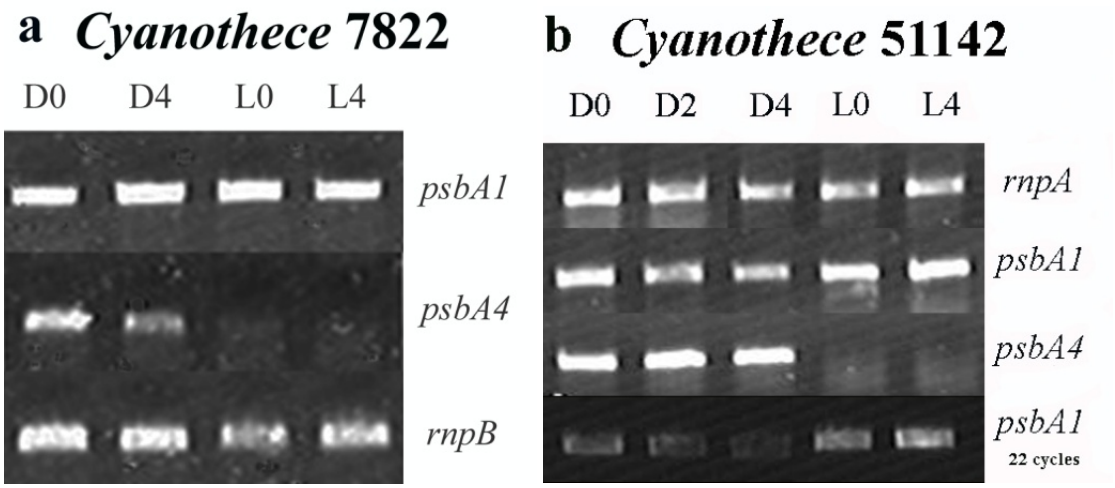
*psbA1*F CAGCAACGCGAAAGCGTTTCCGTA and *psbA1*R ACCGATAGCGTTGGAAGAAG
*psbA4*F GCGGAACGAGTTCCGCACCC and *psbA4*R TAACTCCCACCGCTAAGGTAGC
*rnpB*F CGTGAGGATAGTGCCACAGA and *rnpB*R AAACGGGACCGGTAAAAGAC

For *Cyanothece* sp. ATCC 51142, the primers for each gene were

*psbA1*F CTTAATCTACCCCATCGGAC and *psbA1*R AGGCCATGCACCTAAGAAGA
psbA4 F CTTCTCATCCCTAGCTTGGGC and *psbA4*R ACTGATCACTGTAGCCTTAC
*rnpA*F GGATTACCCAAACAACACCG and *rnpA*R CTTGACCACAATCACCACCT

PCR was carried out with 94°C for 1 min, cycles of 94°C for 30 s and 52°C for 30 s, and 72°C for 30 s to amplify regions of the genes.

Fig. 1. Semi-quantative PCR of (a) *Cyanothece* sp. PCC7822 at D0, D4, L0 and L4. PCR was performed for 30 cycles on *psbA*1 and *rnpB*, and 4 X template concentration was used for *psbA*4 (40 cycles), and (b) *Cyanothece* sp. PCC 51142 at D0, D2, D4, L0 and L4. PCR was performed for 25 cycles on *rnpA*, *psbA*1and *psbA*4, and 22 cycles for *psbA*1 for comparison.



It would seem that there are a series of different families of *psbA* genes and that the multiplicity of *psbA* genes in each cyanobacterial strain is not random, but based on environmental factors. Thus, one family is expressed under low O_2 conditions (see (Summerfield, Toepel et al. 2008) supplemental figure S3), another under high light and yet another under dark, diazotrophic conditions. Additional environmentally related parameters may still be found. Therefore, we consider the term rogue as semantically ambiguous and probably misleading: one definition for rogue is "to cheat" and that would be an appropriate designation for the dark-induced *psbA* genes and the corresponding D1 proteins. However, a more typical definition would be more negative—scoundrel or even becoming uncontrollable—and that would be a poor designation for these important genes. We concur in the importance of these genes, but believe that they deserve a more suitable nomenclature, one that is directly related to the environmental conditions. Thus, we propose that the genes be designated as follows: *psbA4*(dd) for dark, diazotrophic, and *psbA2*(LO_2) for induction under low O_2 conditions. Other environmental parameters may be identified in the future and similar designations may be appended to other *psbA* genes.

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