Mutating the *Synechocystis* sp. PCC6803 RuBisCO promoter as a means of controlling levels of gene expression in cyanobacteria

Introduction:

Cyanobacteria are unique amongst bacteria in their ability to fix carbon in non-cyclic photosynthesis. RuBisCO, the enzyme responsible for carbon fixation in the Calvin-Benson cycle, is specific to the light metabolism, making the mechanism of its expression a prime target for light-dependent gene regulation [1].

Background/significance:

RuBisCO is encoded by the *rbc* operon in *Synechocystis* sp. PCC 6803 (Figure 1)[2]. The 2600 bp operon includes the genes for the large and small subunits of RuBisCO, *rbcL* and *rbcS*, as well as a chaperonin-like protein, *rbcX*, involved in the folding of rbcL (Figure 2).

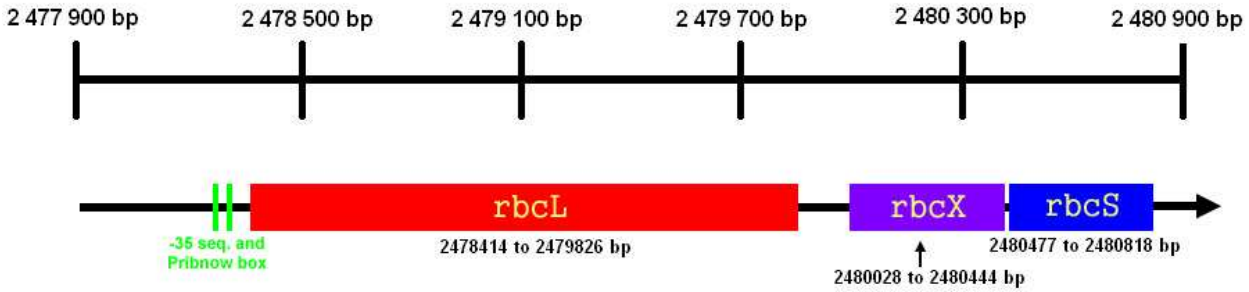

Figure 2: Organization of *rbcLXS* in *Synechocystis* sp. PCC 6803.

The *rbc* promoter is a 250 bp segment that regulates the transcription of all three genes in the operon [3]. Not much is known about the promoter, or its regulation. -35 and -10 consensus sequences, involved in the initiation of transcription, have been identified and are very similar to those other bacterial species [3]. An A-T rich region, -250 to -215 upstream of the transcriptional start site, is a putative binding site of a cis-acting CO_2 response element that upregulates *rbc* expression at low CO_2 concentrations [4]. rbcR, the only *rbc* operon regulator identified in PCC 6803, is a LysR type transcription factor that activates *rbcLXS* expression [2,5]. LysR family transcriptional regulators recognize a T-N_{11}-A DNA motif flanked by 3 bp inverted repeats upstream of the regulated gene.

The *rbc* promoter is a strong promoter previously targeted for its ability to effectively express foreign genes in cyanobacteria. It has been employed by *Escherichia coli*/cyanobacteria shuttle vectors such as pAQ-EX1 and pARUB19 [6,7]. Controlling the strength of the *rbc* promoter not only allows for the control of exogenous gene expression, but also creates a gradient promoter system with finer regulation than on/off promoters such as *lac* or *trp*. The gradient created by the various promoter strengths can also be used to measure the efficiency of other cyanobacterial promoters.

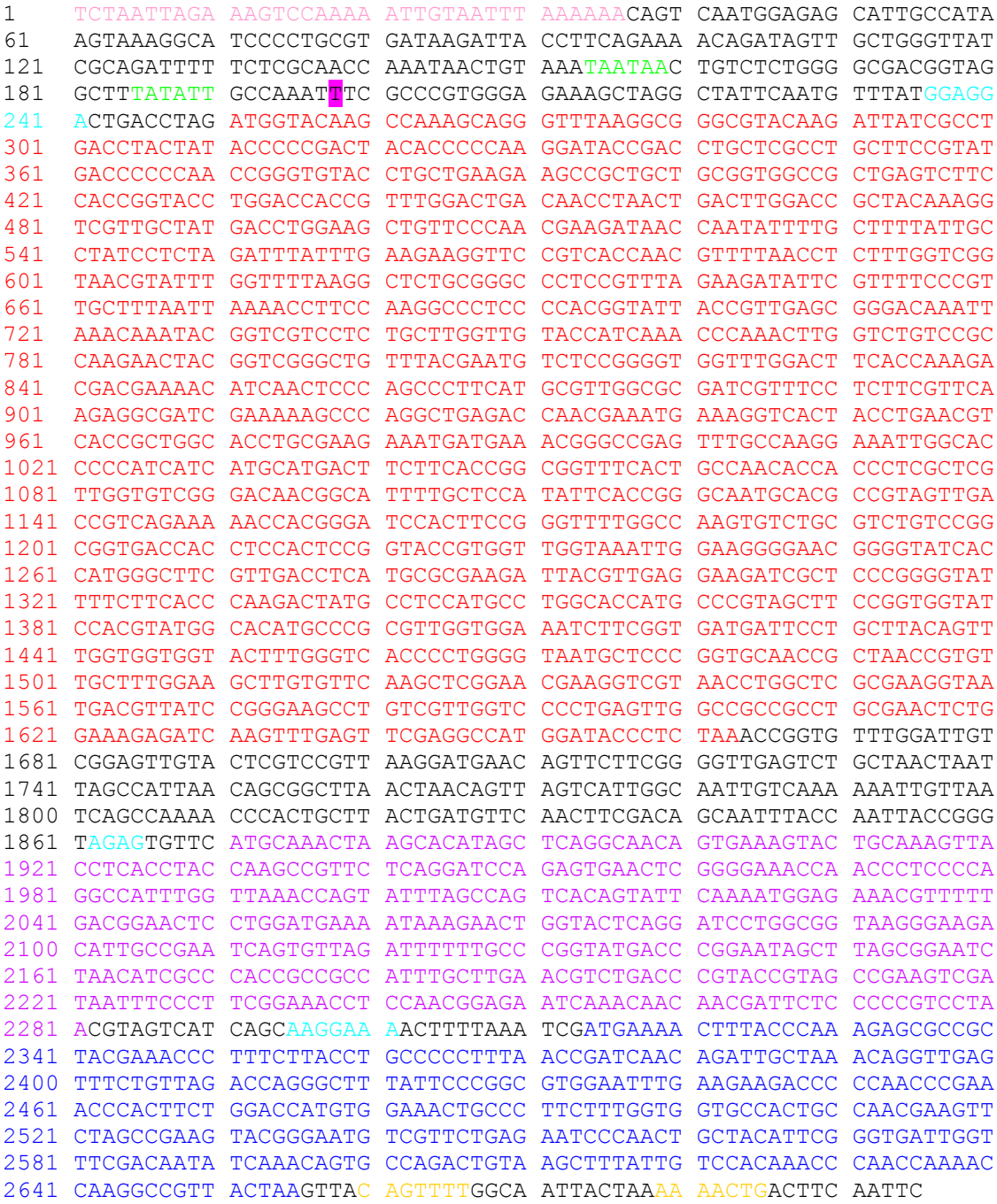

Figure 1: Nucleotide sequence of the *Synechocystis* sp. PCC 6803 *rbc* operon. The promoter sequence is located at 1 to 250. Elements of the *rbc* promoter include: CO_2 element (pink), -35 and -10 consensus sequences (green), transcriptional start site (highlighted in purple), and ribosomal binding site (light blue). Genes encoded by the *rbc* operon are *rbcL* (red), *rbcX* (purple), and *rbcS* (blue). 3' inverse repeats signal transcription termination (yellow).

Objective:

To create a gradient of down-regulated RuBisCO promoters in *Synechocystis* sp. PCC 6803 by mutating the -35 and/or -10 consensus sequences in the *rbc* promoter. Promoter strength will be measured two ways: directly, as PoPS (polymerase per second), and indirectly, by the intensity of fluorescence produced by the reporter gene, *luxCDABE*. The promoters (and the reporter gene, if not already available) will be created in BioBricks format.

Materials and Methods:

Step 1: Isolating and characterizing the *rbcLXS* promoter

The *rbc* promoter will be isolated from the 3.6Mb *Synechocystis* sp. PCC6803 genome using PCR and subcloned in *Escherichia coli*. To limit the number of factors affecting *rbc* promoter activity, a second promoter sequence will be isolated, without the 5' AT-rich region that allows for promoter regulation by CO_2 levels. Promoter strength will be assessed using a β-galactosidase assay. The truncated *rbc* promoter will be ligated with *lacZ* (BBa_I732017), inserted into pRL1383a, and conjugated into PCC6803. Efficiency of promoter repression by ___ in the absence of light will be tested by expressing this system in PCC6803 incubated in darkness. A Western blot will be performed to determine the absence/presence of *lacZ.* Promoter repression will be crucial if the promoter is to function effectively as an on/off switch for gene expression.

Step 2: Mutation of the -35 and -10 consensus sequences

The critical role played by the -35 and -10 consensus sequences make them prime targets for mutagenesis. Site-directed mutagenesis will be used to introduce single base pair substitutions in the -35 and/or -10 consensus sequences to alter promoter strength. Mutated promoters will be evaluated two ways:

1) By ligating the mutated promoters with a reporter gene and measuring the expression of that gene. *luxCDABE* will be used at this step, if available; if not, Cyan Fluorescent Protein, BBa_E0026, will be utilized. Alternatively, if a fluorometer is not yet available, levels protein expression will be measured using metal affinity chromatography (protein will be 6-His tagged).
2) By measuring PoPS.

Step 3: BioBricks

The two types of BioBricks created will be utilized to construct a light-controlled expression system in PCC6803 (Figure 3).

Figure 3: Proposed light-controlled expression system in *Synechocystis* sp. PCC6803.

In the proposed system, the *lux* operon (from *Photorhabdus luminescens*) will be inversely expressed compared to RuBisCO. In the presence of light, the *rbc* promoter will be activated in PCC6803, causing the expression of the lac repressor, *lacI*. LacI will repress the activity of the lac promoter, and in turn, the transcription of the *lux* operon. Although lacI will be tagged with a LVA degradation tag to prevent accumulation of the repressor, a lower strength *rbc* promoter is desired to limit system stress by unnecessary lacI production. In the absence of light, the *rbc* promoter will be turned "off," allowing for the expression of *luxCDABE*.

The indirect control of light production by the *rbc* promoter may not be as effective as proposed. The regulation by the *rbc* promoter is diluted through at least two different gene elements, and the success of the system is contingent upon both lacI and the *lac* promoter functioning exactly as intended.

Timeline:

References:

1. Stanier, R.Y. and Cohen-Bazire, G. "Prokaryotes: The Cyanobacteria." *Annual Review of Microbiology* 31: 225-274 (1977).

2. Synechocystis sp. PCC6803, complete genome. NCBI. 10 June 2008. <http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=NC_000911>.

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7. Takeshima, Y., Sugiura, M., and Hagiwara, H. "A Novel Expression Vector for the Cyanobacterium, *Synechococcus* PCC 6301." *DNA Research* 1: 181-189 (1994).