

# 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 <sup>[1]</sup>.

## Background/significance:

RuBisCO is encoded by the *rbc* operon in *Synechocystis* sp. PCC 6803 (Figure 1)<sup>[2]</sup>. 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).

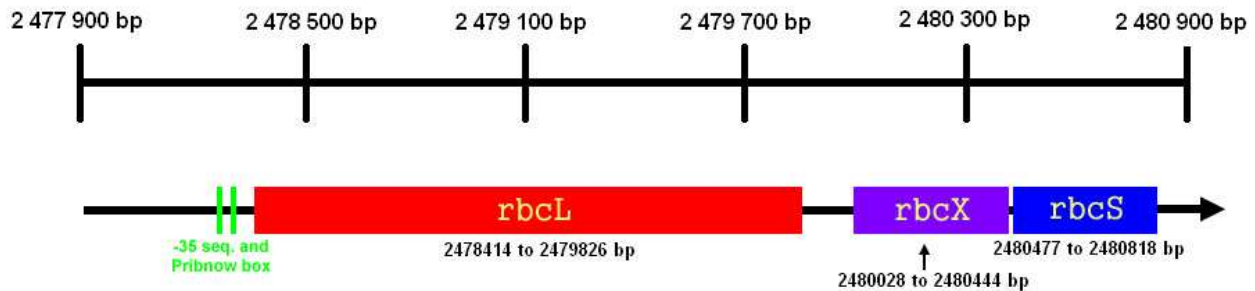


Figure 2: Organization of *rbcLXS* in *Synechocystis* sp. PCC 6803.

The *rbc* promoter is a 250 bp segment that regulates the transcription of all three genes in the operon <sup>[3]</sup>. 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 <sup>[3]</sup>. An A-T rich region, -250 to -215 upstream of the transcriptional start site, is a putative binding site of a cis-acting CO<sub>2</sub> response element that upregulates *rbc* expression at low CO<sub>2</sub> concentrations <sup>[4]</sup>. *rbcR*, the only *rbc* operon regulator identified in PCC 6803, is a LysR type transcription factor that activates *rbcLXS* expression <sup>[2,5]</sup>. LysR family transcriptional regulators recognize a T-N<sub>11</sub>-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 <sup>[6,7]</sup>. 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.

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1      TCTAATTAGA AAGTCCAAAA ATTGTAATTT AAAAAACAGT CAATGGAGAG CATTGCCATA
61     AGTAAAGGCA TCCCCTGCGT GATAAGATTA CCTTCAGAAA ACAGATAGTT GCTGGGTTAT
121    CGCAGATTTT TCTCGCAACC AAATAACTGT AAATAATAAC TGTCTCTGGG GCGACGGTAG
181    GCTTTATATT GCCAAATTTT GCCCGTGGGA GAAAGCTAGG CTATTCAATG TTTATGGAGG
241    ACTGACCTAG ATGGTACAAG CCAAAGCAGG GTTTAAGGCG GGCGTACAAG ATTATCGCCT
301    GACCTACTAT ACCCCCGACT ACACCCCCAA GGATACCGAC CTGCTCGCCT GCTTCCGTAT
361    GACCCCCCAA CCGGGTGTAC CTGCTGAAGA AGCCGCTGCT GCGGTGGCCG CTGAGTCTTC
421    CACCGGTACC TGGACCACCG TTTGGACTGA CAACCTAACT GACTTGGACC GCTACAAAGG
481    TCGTTGCTAT GACCTGGAAG CTGTTCCCAA CGAAGATAAC CAATATTTTG CTTTTATTGC
541    CTATCCTCTA GATTTATTTG AAGAAGGTTT CGTCACCAAC GTTTTAACCT CTTTGGTCGG
601    TAACGTATTT GGTTTTAAGG CTCTGCGGGC CCTCCGTTTA GAAGATATTC GTTTTCCCGT
661    TGCTTTAATT AAAACCTTCC AAGGCCCTCC CCACGGTATT ACCGTTGAGC GGGACAAATT
721    AAACAAATAC GGTTCGTCCTC TGCTTGGTTG TACCATCAAA CCCAAACTTG GTCTGTCCGC
781    CAAGAACTAC GGTTCGGGCTG TTTACGAATG TCTCCGGGGT GGTTTGGACT TCACCAAAGA
841    CGACGAAAAC ATCAACTCCC AGCCCTTCAT GCGTTGGCGC GATCGTTTCC TCTTCGTTCA
901    AGAGGCGATC GAAAAAGCCC AGGCTGAGAC CAACGAAATG AAAGGTCACT ACCTGAACGT
961    CACCGCTGGC ACCTGCGAAG AAATGATGAA ACGGGCCGAG TTTGCCAAGG AAATTGGCAC
1021   CCCCATCATC ATGCATGACT TCTTCACCGG CGGTTTCACT GCCAACACCA CCCTCGCTCG
1081   TTGGTGTCTG GACAACGGCA TTTTGCTCCA TATTCACCGG GCAATGCACG CCGTAGTTGA
1141   CCGTCAGAAA AACCACGGGA TCCACTTCCG GGTTTTGGCC AAGTGTCTGC GTCTGTCCGG
1201   CGGTGACCAC CTCCACTCCG GTACCGTGGT TGGTAAATTG GAAGGGGAAC GGGGTATCAC
1261   CATGGGCTTC GTTGACCTCA TGCGCGAAGA TTACGTTGAG GAAGATCGCT CCCGGGGTAT
1321   TTTCTTACC CAAGACTATG CCTCCATGCC TGGCACCATG CCCGTAGCTT CCGGTGGTAT
1381   CCACGTATGG CACATGCCCG CGTTGGTGGG AATCTTCGGT GATGATTCCT GCTTACAGTT
1441   TGGTGGTGGT ACTTTGGGTC ACCCCTGGGG TAATGCTCCC GGTGCAACCG CTAACCGTGT
1501   TGCTTTGGAA GCTTGTGTTT AAGCTCGGAA CGAAGGTCGT AACCTGGCTC GCGAAGGTAA
1561   TGACGTTATC CGGGAAGCCT GTCGTTGGTC CCCTGAGTTG GCCGCCGCCT GCGAACTCTG
1621   GAAAGAGATC AAGTTTGAGT TCGAGGCCAT GGATACCCTC TAAACCGGTG TTTGGATTGT
1681   CGGAGTTGTA CTCGTCCGTT AAGGATGAAC AGTTCCTTCGG GGTGAGTCTC GCTAATAAT
1741   TAGCCATTAA CAGCGGCTTA ACTAACAGTT AGTCATTGGC AATTGTCAAA AAATTGTTAA
1800   TCAGCCAAAA CCCACTGCTT ACTGATGTTT AACTTCGACA GCAATTTACC AATTACCGGG
1861   TAGAGTGTTT ATGCAAACCTA AGCACATAGC TCAGGCAACA GTGAAAGTAC TGCAAAGTTA
1921   CCTCACCTAC CAAGCCGTTT TCAGGATCCA GAGTGAATC GGGGAAACCA ACCCTCCCCA
1981   GGCCATTTGG TTAAACCAGT ATTTAGCCAG TCACAGTATT CAAAATGGAG AAACGTTTTT
2041   GACGGAATC CTGGATGAAA ATAAAGAAT GGTACTCAGG ATCCTGGCGG TAAGGGAAGA
2100   CATTGCCGAA TCAGTGTTAG ATTTTTTGCC CGGTATGACC CGGAATAGCT TAGCGGAATC
2161   TAACATCGCC CACCGCCGCC ATTTGCTTGA ACGTCTGACC CGTACCGTAG CCGAAGTCGA
2221   TAATTTCCCT TCGGAAACCT CCAACGGAGA ATCAAACAAC AACGATTCTC CCCCCTCTA
2281   ACGTAGTCAT CAGCAAGGAA AACTTTTTAAA TCGATGAAAA CTTTACCCAA AGAGCGCCGC
2341   TACGAAACCC TTTCTTACCT GCCCCCTTTA ACCGATCAAC AGATTGCTAA ACAGTTGAG
2400   TTTCTGTTAG ACCAGGGCTT TATTCCTGGC GTGGAATTTG AAGAAGACCC CCAACCCGAA
2461   ACCCACTTCT GGACCATGTG GAAACTGCC TTCTTTGGTG GTGCCACTGC CAACGAAGTT
2521   CTAGCCGAAG TACGGGAATG TCGTTCTGAG AATCCCAACT GCTACATTGC GGTGATTGGT
2581   TTCGACAATA TCAAACAGTG CCAGACTGTA AGCTTTATTG TCCACAAACC CAACCAAAC
2641   CAAGGCCGTT ACTAAGTTAC AGTTTTGGCA ATTACTAAAA AACTGACTTC AATTC

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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<sub>2</sub> 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<sub>2</sub> 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:**

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