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Synechocystis was Engineered to have a Green-Light-Inducible Gene Expression Mechanism

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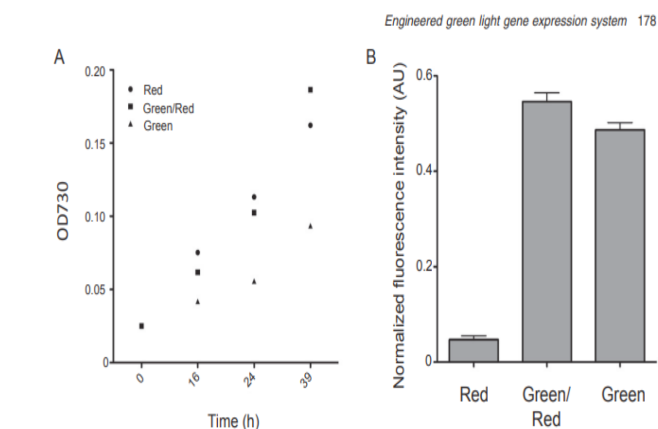
ABSTRACT

To develop a green light-regulated gene expression system unique to cyanobacteria, the CcaS histidine kinase, CcaR response regulator, and cpcG2 gene promoter of Synechocystis PCC6803 must be discovered (PcpcG2). Using a genetic controller to shine green light on CcaS and CcaR might activate the PcpcG2 gene. Researchers studied the capacity of the native PcpcG2 to react to green light using the reporter gene GFPuv. Because of this, it was evident that green light had a direct effect on both the amount of expression of PcpcG2 and the amount of expression of Ptrc, another cyanobacteria-specific constitutive promoter. The insertion of a ShineDalgarno-like motif into cpcG2's 5′ untranslated region increased CcaR expression. With this improved green-light detector in place, protein expression increased by a factor of 40 under green light irradiation. Bioprocesses that utilise novel cyanobacteria may benefit from the utilization of green-light gene expression systems.

INTRODUCTION

Using just sunlight, water, and a few inorganic components, cyanobacteria may directly convert carbon dioxide into the chemical needed to make biofuels and biomaterials. For biofuels and biomaterials production, cyanobacteria may be genetically altered with to achieve practically any desired trait. Researchers are using Escherichia coli and other species as building blocks to develop biosynthetic pathways Promoters may be incompatible with cyanobacteria, however this is unlikely given the differences in RNA polymerases between bacteria and plants (Huang and colleagues, 2010). They said that biotechnological methods for gene expression that do not rely on chemicals like IPTG and metal ions are becoming more prevalent .A novel expression technique is required for the cyanobacterial bioprocess. Different cyanobacteria light-sensing systems are used to prevent photodamage caused by strong or short-wavelength light, or to maintain a high level of photosynthetic efficiency Varying light-sensing systems may respond differently to different levels of illumination in order to regulate gene expression or enzyme activity for the production of second messengers or the phototaxis response (Narikawa, 2011; Song et al., 2011). Terauchi et al. (2004) reference two studies by Yeh et al (2004). The great majority of sensory systems are governed by a two-component paradigm.



At a wavelength of 730 nm, cells' fluorescence levels had stabilized after 39 hours of exposure to each kind of light. The intensity of fluorescence response regulator for histidine kinase after each culture was rinsed in phosphate buffered saline (Thermofisher Scientific, Waltham, MA). When light absorption stimulates the sensor protein's kinase activity, a signal is sent to the cognate response regulator.

Gene expression or flagella movement is triggered by a gene's promoter. CpcG2 gene expression in the marine bacterium Sync was shown to be controlled (Hirose et al., 2008). Green light induces phosphotransfer to CcaR and dephosphorylation of CcaR as a consequence of CcaSautophosphorylation, which is then reversed. Gene expression control was introduced

to cyanobacteria, which do not use photosynthesis as their primary energy source. Green light sensing system PCC6803 features CcaR and PcpcG2 response regulators, which were used to modify cyanobacterial PCC6803. By shining green light on CcaS and CcaR, you may activate the gene PcpcG2.

THOUGHTS ON HOW TO MAKE THINGS BETTER

The first table is called Table 1. (S1). Synechocystis sp. PCC6803 was transformed after preculturing the plasmid under red light. After the colonies had been incubated under red or green light for a period of time, the fluorescence intensity was evaluated. GFPuv (green fluorescent protein-derived fluorescence) fluoresced more brightly when exposed to red, green, or red-green light. In order to compare fluorescence intensities, we utilize the optical density (OD) of the culture at 730 nm (Fig. 1). In response to red light inhibition of CcaS, CcaS phosphatase has been demonstrated to dephosphorylate CcaR (Hirose et al., 2008). Even though red light is required for cell proliferation, green light may activate CcaS and lead to the transcription of PcpcG2. Yet, despite this, GFPuv shows a high degree of fluorescence.

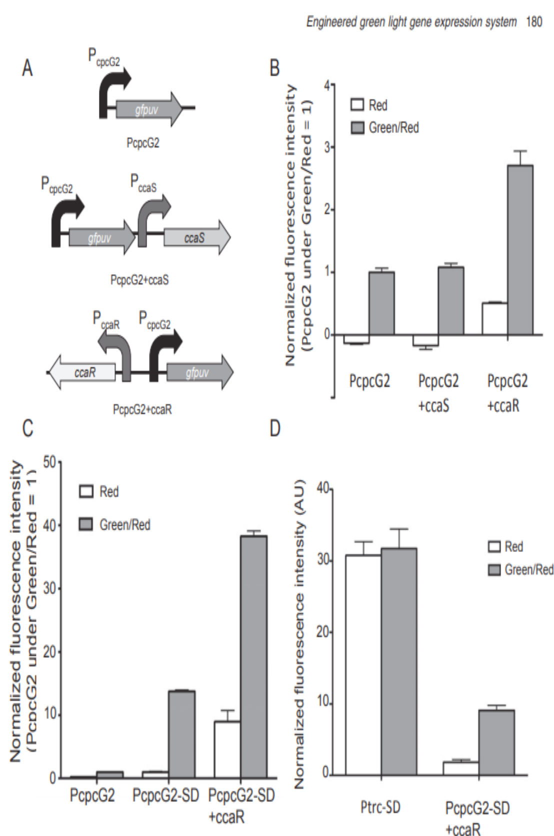
When the Ptc promoter was employed, gene expression was lower than when other promoters were used (Abe et al., 2013). It was originally tested to investigate whether CcaS gene dosage affected GFPuv expression in transfected cells. In Synechocystis sp., PCC6803, cells exposed to green and red light may not phosphorylate CcaS. Synechocystis sp. PCC6803 CcaS increased phosphorylation of CcaR may boost GFPuv expression. Synechocystis sp. PCC6803's native promoter was used to insert GFPuv and the ccaS gene onto plasmids and increase CcaS gene output and activity (Table S1). Endogenous and exogenous expression of CcaS had very equal GFPuv fluorescence intensity (Fig. 2B).

By increasing its phosphorylation of CcaR and decreasing its dephosphorylation of CcaR, CcaS responds to green light by increasing both. Because the kinase to dephosphorylase ratio did not alter, there was no change in CcaR phosphorylation. GFPuv expression was widely studied once the CcaR gene dosage was discovered. For this reason, it is possible that endogenous CcaR inhibits the full activation of PcpcG2 expressed in the wide-host-range pKT230 vector (Barth and Grinter, 1974). When the plasmid PcpcG2-GFPuv was co-transfected with ccaR from Synechocystis PCC6803, it was found that ccaR was more strongly produced under red light than green light (Fig. 2A).

Using green and red light, cells expressing GFPuv had three times the fluorescence intensity of those expressing CcaR. This is seen in Figure 2B. In the green-light gene expression system, CcaR's ability to induce gene expression was improved. The phosphorylation of CcaR by histidine kinases may increase fluorescence intensity when cells are exposed to red light rather than green light.

The presence of unphosphorylatedCcaR in cells exposed to red light may increase background expression. RBS, start codon RNA, antisense, and other trans elements regulate the production of protein. PcpcG2's promoter lacks SD sequences before the initiation codon. Most cyanobacterial genes have an SD-like motif, although only 26% of these genes are completely devoid of this pattern (Ma et al., 2002). The S1 ribosomal protein identifies AU-rich areas anterior to the initiation codon in a number of these genes' 5'UTR (Tzareva et al., 1994). Ribosomal protein S1 has numerous AU bases in its upstream sequence, and this may affect the expression of PcpcG2 in vivo. It was not expected that there would be a stable secondary structure in the area given the program's achievements, according to Mfold (Zuker, 2003). PcpcG2 had low amounts of GFPuv gene expression, thus an SD-like region was added to test whether it had any impact.

The 5′-UTRs of cyanobacterial genes were searched for SD-like sequences. C-phycocyanin and D1 encoded by psbA both have 5′-UTRs with SD-like parts. Synechocystis sp. 16S rRNA contains SD-like sequences. These two genes are found in other cyanobacteria, where they seem to be almost identical in structure and function. Synechococcus sp. PCC6803 has an SD-like gene sequence that seems to be important for the organism. PCC7002 Acquiring the SD-like sequence from the cpcB gene and inserting it into the PcpG2 promoter was done using Synechococcus PCC 7002 as a source (Table S1). In Escherichia coli prior to the SD sequence, AU-rich regions boosted expression (Komarova et al., 2002). Considering what we've learned, we believe that this functionality should be included.



Synechocystis PCC6803 was grown in the presence of red and green/red light in order to test a green-light detecting system.

Pcpg2 (GFPuv) and PcpcG2-GFPuv-PCcas (ccaS) as well as Pcpg2 (GFPuv) are shown schematically in this figure (ccaR).

As a result, PcpcG2+ccaS and ccaR were detected in Synechocystis PCC6803 using either red/green or green/red light. All other results are compared to PcpcG2 as the standard under a green light (set as 1).

S. PCC6803 compared to GFPuv+ccaR, GFPuv+ccaR-SD, and GFPuv+ccaR in terms of fluorescence intensity. In the presence of green light, other measures are normalized to PcpcG2 (set as 1).

When CPCB and PcpcG2-SD+ccaR replaced PtrC-SD in Synechocystis 6803, the relative normalized fluorescence intensity of 1.5 was achieved. the process through which genes are turned on and off to produce the color green.

The AU motif and the AU motif functioned together to increase protein translation. A pKT230-derived vector harboring PcpcG2-SD-GFPuv and ccaR under its native promoter was then produced." There is a table (S1) here. Speeding up transcription and translation might be achieved since CcaR has an SD-like region. Exogenous CcaR plasmid or SD-like sequences reduced GFPuv cells' fluorescence by 40 times compared to cells expressing GFP under red and green light (Fig. 2C). An SD-like sequence insertion (Fig. 2A) and the presence of exogenous CcaR (Fig. 2B) may have contributed to the almost threefold rise in CcaR (Fig. 2B) (Fig. 2C). The SD-like region of Ptrc reduced GFPuv expression three times more than the vector promoter (Fig. 2D). Because of its low ON/OFF ratio, PcpcG2-SD+ccaR for Synechocystis sp. PCC6803 provides an alternative to IPTG-induced inducible gene expression (Huang et al., 2010). A genetic instrument known as an induced promoter is the only technique to examine gene function and control of protein production. However, nothing is known about the inducible promoters of cyanobacteria. RNA polymerase structural modifications in Synechocystis PCC6803 render lactose promoter and derivatives ineffective (Huang et al., 2010). (Schneider and Hasekorn, 1988). In Synechocystis sp. PCC 6803, IPTG stimulation of the Ptrc promoter was greater than in other strains, according to Guerrero and colleagues (2012). When it comes to E. coli, IPTG is expensive to use, but other synthetic inducers are not.

To produce substantial amounts of biofuel or biomaterial, the nrsB nickel-inducible promoter in cyanobacteria is ineffective. Cyanobacteria must be grown in the dark before they can employ light-inducible promoters psbA1 and psbA2 to control protein production (Agrawal et al., 2001). Because of the high protein expression and high ON/OFF ratio, this study employed a green-light sensor device to control gene expression. Since photosynthesis does not need green light, we can grow cyanobacteria prior to gene activation with optimal growth. Because CcaS' phycocyanobilinchromophore is activated by red light and inhibited by green light, a minor but considerable increase in target gene expression was predicted (Hirose et al., 2008). Even in the presence of red light, we found that green light had a significant impact on the expression of the target gene. This green light sensor gadget may be useful in the production of biofuels and biomaterials.

In this study, researchers exploited an SD-like motif to enhance gene expression. Multiple RBSs of varied strengths may aid in optimizing enzyme expression levels in the biosynthetic process, hence decreasing undesired byproducts in the process. An SD-like sequence with variable intensity has been developed by Salis and his colleagues in 2009. Biofuel and biomaterials production requires the expression of a large number of genes. A single gene product can no longer be controlled by green light. There are transcriptional factors and gene products that govern cell viability. The amount of biofuel and biomaterial synthesis influenced by these parameters and gene products might vary greatly. All three genes in Synechocystis sp. PCC6803, ccaS and ccaR, make up the green-light detection mechanism in the organism. By exposing PcpcG2 to green and red light, we were able to increase the target gene's expression in this cell type. Cyanobacteria's gene expression may be altered by green light when red light is used to maintain appropriate growth conditions. Adding an SD-like region and increasing CcaR expression in the green-light sensor resulted in a 40-fold increase in the expression of the target gene. In a recombinant DNA study, the Ptrc promoter produced the same levels of expression as here. Innovative cyanobacterial bioprocesses may employ a green-light gene expression system to regulate gene expression.

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