BioGecko
ISSN: 2230-5807

Web of Science
Vol 09 Issue 03 2020

Synechocystis was engineered to have a green-light-inducible gene expression mechanism.

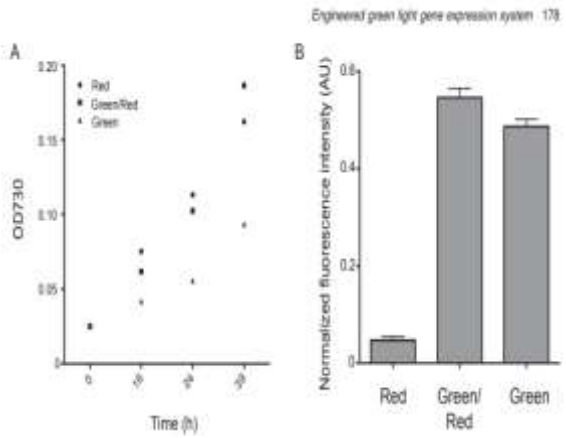
Zoe Wicomb,Etienne Leroux

Summary

Synechocystis sp. PCC6803's green-light detecting histidine kinase CcaS, the cognate response regulator CcaR, and the promoter of cpcG2 were described in order to build a green-light-regulated gene expression system for cyanobacteria (PcpcG2). Gene expression from PcpcG2 is activated by green-light illumination of CcaS and CcaR, which operate as a genetic controller. Native PcpcG2's ability to respond to green light was studied utilising GFPuv as a reporter gene placed into a broad-host-range vector. The expression of PcpcG2 was clearly induced by green-light illumination, however the amount of expression was much lower than that of Ptrc, which has previously been described to be a constitutive promoter in cyanobacteria. Since the 5′ untranslated region of the cpcG2 gene lacks the ShineDalgarno-like motif, an insertion into this area resulted in enhanced CcaR expression. As a consequence, under green-light illumination, the modified green-light detecting system produced 40-fold greater levels of protein expression than the wild-type promoter did. An designed green-light gene expression system might be used to regulate gene expression in new cyanobacterial bioprocesses.

Introduction

The capacity of cyanobacteria to directly transform carbon dioxide into the desired chemical, needing only sunshine, water, and a few inorganic substances, makes them a perfect host for the manufacture of biofuels or biomaterials. Because most cyanobacteria can be genetically modified, they can be used to produce biofuel and biomaterials more efficiently. There are a number of researchers who are trying to build biosynthetic pathways utilising components from Escherichia coli and other species (Atsumi et al., 2009; Liu and Curtiss, 2009; 2012; Oliver et al., 2013). Though it has been hypothesised that some of the promoters are incompatible with cyanobacteria (Huang et al., 2010), it is unlikely that this is the case due to variations in RNA polymerase between bacteria and plants (Schneider and Hasekorn, 1988). Traditional bacterial gene expression systems use chemicals like isopropyl -D-1-thiogalactopyranoside (IPTG) and metal ions, which are impractical for large-scale cyanobacterial cultivation, as well as the induction of specific genes (Briggs et al., 1990; Geerts et al., 1995; Lopez-Maury et al., 2002). The water recycling technique cannot use chemical inducers since they are difficult to remove from the growth media. The cyanobacterial bioprocess necessitates the development of a new gene expression system. In order to maintain an efficient photosynthesis or prevent photodamage from intense or short-wavelength light, cyanobacteria have a variety of light-sensing mechanisms. A variety of light-sensing systems regulate gene expression, enzymatic activity for the production of second messengers, or phototaxis response upon illumination with varying light, such as UV light; blue light (Yoshihara et al., 2004; Hirose and Yamamoto, 2008; 2010); green light (Terauchi et al., 2004; Hirose et al., 2008; 2010) and red light (Narikawa, 2011; Song et al., 2011). (Yeh et al., 1997; Terauchi et al., 2004). A two-component regulation model underpins the vast majority of sensing systems.



pKT230-PcpcG2-GFPuv-expressing Synechocystis sp. PCC6803 was able to control gene expression by exposing it to green light.

A . Growth curves in red, green, or both green and red light (green/red) are shown. The optical density at 730 nm was used to gauge growth.

B. After 39 h of incubation under each light irradiation, the fluorescence intensity of cells was normalised by their relative optical densities at 730 nm. A plate reader was used to assess the fluorescence intensity after each culture had been rinsed with phosphate buffered saline (Thermofisher Scientific, Waltham, MA) comprised of a sensor and response regulator for histidine kinase. As a result of the sensor protein's kinase activity being activated by light absorption, the signal is sent to the cognate response regulator.

Gene expression or flagella movement are activated when phosphorylated response regulator (PRR) attaches to a promoter's upstream region. This gene, CpcG2, has been shown to be chromatically controlled by the sensor Histidine Kinase CcaS and the cognate response regulator CcaR in Synechocystis PCC6803, a marine bacterium (Hirose et al., 2008). In the presence of green light, CcaS performs autophosphorylation, which is followed by phosphotransfer to CcaR and dephosphorylation of CcaR. A gene expression system was designed to control cyanobacteria's gene expression in response to green light, which is not a primary source of photosynthesis. Using the Synechocystis sp. PCC6803 green-light sensing system, we created a cyanobacteria-specific green-light-regulated gene expression system that included the green-light sensing histidine kinase CcaS, the cognate response regulator CcaR, and PcpcG2. Gene expression from PcpcG2 is activated by green-light illumination of CcaS and CcaR, which operate as a genetic controller.

Discussion and conclusions

GFPuv reporter protein gene was cloned from Synechocystis PCC6803 and placed upstream of the PcpcG2 promoter on the broad-host-range vector pKT230 as stated in Supplementary material (Bagdasarian et al., 1981) (Table S1). Under red-light illumination, plasmid was precultured and converted into Synechocystis sp. PCC6803. The fluorescence intensity of the cultures was then assessed after the cultures were incubated under continuous irradiation with green light, red light, or both. A rise in the fluorescence intensity of GFPuv-derived fluorescence was seen under green, red, and even red-green light illumination. When the culture's optical density (OD) at 730 nm was normalised, these fluorescence intensities were practically equal (Fig. 1). CcaS phosphatase activity has been shown to dephosphorylate CcaR as a consequence of red-light illumination repressing CcaS. (Hirose et al., 2008). We found that green light may activate CcaS, resulting in transcription via PcpcG2, even when red light is also being illuminated simultaneously, which is required for effective cell development. However, the GFPuv fluorescence intensity of expression.

In comparison to gene expression driven by the Ptrc promoter, the intensity was rather low (Abe et al., 2013). We initially focused on the gene dosage impact of CcaS on the GFPuv expression level in an effort to enhance gene expression. The phosphorylation of CcaR by CcaS may not occur in all Synechocystis sp., PCC6803 cells that are continually exposed with green and red light. Increasing CcaS in Synechocystis sp. PCC6803 may result in an increase in CcaR phosphorylation, which may lead to an increase in GFPuv expression in response to green-light illumination. Plasmids containing PcpcG2-GFPuv and the ccaS gene under the native promoter of Synechocystis sp. PCC6803 were produced to boost CcaS activity by raising its expression level (Fig. 2A) (Table S1). Expression of both endogenous and extracellular CcaS demonstrated approximately comparable GFPuv-derived fluorescence intensities (Fig. 2B).

The kinase activity of CcaS toward CcaR is increased by green light, but the dephosphorylation activity of CcaS toward CcaR is suppressed by red light. Because the ratio of kinase to dephosphorylation activity did not change as a

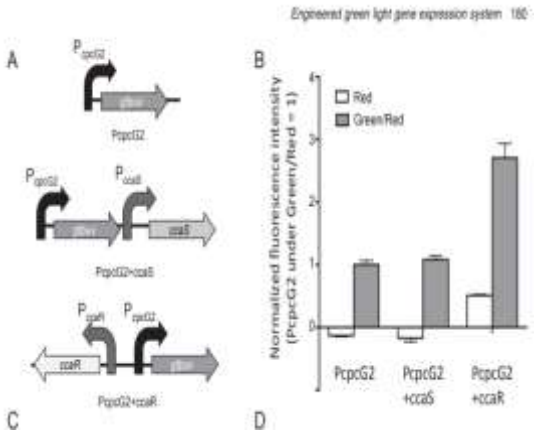
consequence of increased CcaS expression, the degree of CcaR phosphorylation remained steady. The impact of CcaR's gene dosage on GFPuv expression was then examined. Exogenously inserted PcpcG2, encoded in the broad-host-range vector pKT230, with a copy number more than 10, may not be completely activated by the green-light gene expression system since endogenous CcaR was used (Barth and Grinter, 1974). PcpcG2-GFPuv and the Synechocystis sp. PCC6803 ccaR gene were thus co-transfected into a plasmid (Table S1), and the GFPuv expression level was compared under green and red light illumination (Fig. 2A).

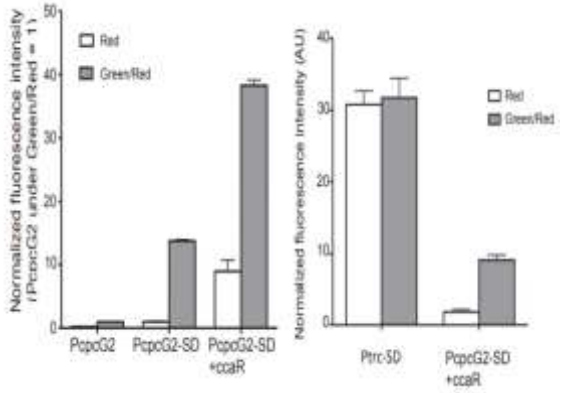
Because of this, GFPuv-derived fluorescence intensity was roughly three times greater under green and red light illumination compared to cells simply expressing CcaR. (Fig. 2B). In this study, it was shown that boosting the CcaR expression level increased the green-light gene expression system's ability to induce gene expression. Exogenously overexpressed CcaR is phosphorylated by certain endogenously present histidine kinases, which may be causing an increase in fluorescence intensity when the cells are illuminated with red light rather than green. The binding and activation of a similar promoter by several response regulators has also been described in unphosphorylated conditions (Schar et al., 2005; Kato et al., 2008).

Under red-light illumination, unphosphorylated CcaR may contribute to the background expression. A combination of promoter activity, ribosome binding site strength (RBS), mRNA structure around the RBS and start codon, and certain trans elements, such as antisense RNA, govern protein production levels primarily. Upstream of the start codon, the PcpcG2 promoter lacks a characteristic ShineDalgarno (SD) sequence. At reality, just 26% of cyanobacterial genes include an SD-like motif in the ideal place, making them devoid of SD-like sequences (Ma et al., 2002). S1 ribosomal protein detects AU-rich sequences in the 5′-untranslated region (UTR) upstream of the initiation codon in some of these genes that lack an SD-like sequence (Tzareva et al., 1994). PcpcG2 expression may possibly be controlled by the S1 ribosomal protein since the upstream region of PcpcG2 contains multiple consecutive AU bases (5′-UUAAG UUUAAUUACUAACUUUAUCU-3′). Mfold (Zuker, 2003) was unable to anticipate a stable secondary structure in the area, as indicated by the programme. It was decided to test the impact of inserting an SD-like region on GFPuv gene expression under PcpcG2 in light of the low levels of expression seen under PcpcG2.

The 5′-UTRs of cyanobacterial genes were screened for SD-like sequence possibilities. Cyanobacteria produce a number of photosynthesis-related proteins, including the psbA-encoded D1 protein and the cpcB-encoded c-phycocyanin, both of which include SD-like sequences in their 5′-UTRs. Synechocystis sp. PCC6803's 16S rRNA has an SD-like sequence that is entirely complementary to cpcB's SD-like sequence and is highly conserved throughout other cyanobacteria. That's why it seemed to us that the SD-like sequence of the CPC gene in Synechococcus sp. PCC6803 would have a major effect on the organism. The 5′-UAUAAGUAGGAGAUAAAAAC-3′ SD-like sequence obtained from the cpcB gene of Synechococcus sp. PCC7002 was inserted before the GFPuv start codon under the PcpcG2 promoter (Table S1). When GFPuv-derived fluorescence was lit with both red and green light, the SD-like sequence added to PcpcG2 increased its intensity by around 15 times (PcpcG2-SD in Fig. 2C).

Reverse transcription polymerase chain reaction study indicated that the GFPuv mRNA in PcpcG2-SD culture was twice as much as in PcpcG2 (data not shown), possibly due to improved binding of the ribosome to the mRNA. Because translation efficiency has increased, rather than transcription, GFPuv fluorescence has risen. The expression level in Escherichia coli was boosted by the AU-rich stretch upstream of the SD sequence (Komarova et al., 2002). As a result, we believe that the addition of





In Synechocystis PCC6803, a green-light detecting system was tested under red or green/red light, as shown in Fig. 2.

A . In this image, you can see a schematic depiction of each construct injected into pKT230, including Pcpg2 (GFPuv) and PcpcG2-GFPuv-PCcas (ccaS) and PcpcG2-GFPuv-PCcas (ccaR).

B. PcpcG2, PcpcG2+ccaS, or PcpcG2+ccaR in Synechocystis PCC6803 under green/red or red/green light, respectively. PcpcG2 normalised under green light is used as a reference point for all other values (set as 1).

C. PcpcG2, pKT230-PcpcG2-SD-GFPuv (PcpcG2-SD), and PcpcG2-SD-GFPuv+ccaR (PcpcG2-SD+ccaR) relative fluorescence intensity under red and green/red light for Synechocystis sp. PCC6803. PcpcG2 normalised under green light is used as a reference point for all other values (set as 1).

D . In Synechocystis sp. PCC6803 the SD-like sequence of cpcB and PcpcG2-SD+ccaR was substituted with the SD-like sequence of pKT230-Ptrc-SD-GFPuv (Ptrc-SD), resulting in a relative normalised fluorescence intensity of 1.5. Using the cell's optical density, each value is normalised. A gene expression method that produces green light

SD-like region had a synergistic impact on protein translation with the AU-rich motif. A pKT230-derived vector containing PcpcG2-SD-GFPuv and ccaR under the control of its native promoter was then created (Table S1). When we added an SD-like region to the CcaR gene, we hoped to see a synergistic impact of both accelerated transcription and enhanced translation. Cells producing GFPuv without the SD-like sequence or the exogenous CcaR plasmid had fluorescence roughly 40 times less than cells expressing GFPuv when illuminated in red and green (Fig. 2C). The nearly threefold rise from the exogenous CcaR (Fig. 2B) and the almost 15-fold increase from the insertion of the SD-like sequence (Fig. 2A) are virtually directly cumulative (Fig. 2C). The GFPuv expression level under the Ptrc promoter, which also contains an SD-like region, was three times lower than under the built vector (Fig. 2D). Due to a low ON/OFF ratio in Synechocystis sp. PCC6803 using IPTG as inducer (Huang et al., 2010), the PcpcG2-SD+ccaR system is an appealing option for an inducible gene expression system for Synechocystis sp. PC6803. The use of induced promoters, a kind of genetic tool, is critical in the study of gene function and the regulation of protein synthesis. However, nothing is known about cyanobacteria's inducible promoters. Traditional E. coli inducible promoters like lactose promoter or derivatives fail in Synechocystis sp. PCC6803 (Huang et al., 2010) due to structural changes in RNA polymerase (Schneider and Hasekorn, 1988). In Synechocystis sp. PCC 6803, Guerrero and colleagues (2012) found that IPTG induction of the PA1lacO1 promoter was superior to that of the Ptrc promoter. However, synthetic chemical inducers, such as IPTG, which is frequently employed in E. coli, are not cost-effective.

Because heavy metal ions are hazardous, nrsB, a nickel-inducible promoter in cyanobacteria that has a high ON/OFF ratio, cannot be used for large-scale production of biofuel or biomaterials. When using the light-inducible promoter psbA1 and psbA2 to regulate protein expression in cyanobacteria, dark culture is required before induction can occur (Agrawal et al., 2001). In order to regulate gene expression, we developed a green-light sensing system with a high protein expression level and a high ON/OFF ratio in this work. We can culture cyanobacteria with optimum development before gene activation since green light is not needed for photosynthesis. Because CcaS, which possesses a phycocyanobilin chromophore, is known to be activated by green light and inhibited by red light, we assumed that the simultaneous illumination with red and green light would cancel each other out, resulting in a very modest activation of target gene expression (Hirose et al., 2008). Even with red-light illumination, we discovered that green-light illumination had a substantial effect on the expression of target genes. Gene function analysis or biofuel or biomaterial manufacturing might both benefit from this green-light sensor device.

We used an SD-like motif to boost gene expression in this investigation. The use of several RBSs of varying

strengths in biofuel production applications might be beneficial in optimising the expression levels of the enzymes in the biosynthetic pathway to minimise unwanted byproducts. With the use of a programme created by Salis and colleagues (2009), a succession of SD-like sequences varying in strength may be easily generated. Gene expression for biofuel and/or biomaterials that need numerous genes to be synthesised will be the final aim. As of right now, we've been able to use green light to control the expression of only one gene product. Single gene expression, the transcription factor and/or gene product regulating cell survival, may alter biofuel and/or biomaterial production levels, even if just one gene expression is controlled. Finally, in Synechocystis sp. PCC6803, we discovered a green-light detecting system composed of the genes ccaS, ccaR, and the target promoter PcpcG2. By illuminating PcpcG2 with green light and red light, this method stimulated the target gene expression from PcpcG2. While maintaining suitable growth conditions with red light, green light may influence gene expression in cyanobacteria. Further engineering of the green-light sensor resulted in a 40-fold increase in target gene expression by boosting CcaR expression and introducing an SD like region. Ptrc, a powerful promoter employed in cyanobacteria recombinant DNA research, yielded levels of expression equivalent to those found here. An designed green-light gene expression system might be used to regulate gene expression in new cyanobacterial bioprocesses.

References

[1] Abe, K., Sakai, Y., Nakashima, S., Araki, M., Yoshida, W., Sode, K., and Ikebukuro, K. (2013) Design of riboregulators for control of cyanobacterial (Synechocystis) protein expression. Biotechnol Lett. doi: 10.1007/s10529- 013-1352-x.

[2] Agrawal, G.K., Kato, H., Asayama, M., and Shirai, M. (2001) An AU-box motif upstream of the SD sequence of lightdependent psbA transcripts confers mRNA instability in darkness in cyanobacteria. Nucleic Acids Res 29: 1835– 1843.

[3] Atsumi, S., Higashide, W., and Liao, J.C. (2009) Direct photosynthetic recycling of carbon dioxide to isobutyraldehyde. Nat Biotechnol 27: 1177–1180.

[4] Bagdasarian, M., Lurz, R., Ruckert, B., Franklin, F.C., Bagdasarian, M.M., Frey, J., and Timmis, K.N. (1981) Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in Pseudomonas. Gene 16: 237–247.

[5] Barth, P.T., and Grinter, N.J. (1974) Comparison of the deoxyribonucleic acid molecular weights and homologies of plasmids conferring linked resistance to streptomycin and sulfonamides. J Bacteriol 120: 618–630.

[6] Briggs, L.M., Pecoraro, V.L., and McIntosh, L. (1990) Copperinduced expression, cloning, and regulatory studies of the plastocyanin gene from the cyanobacterium Synechocystis sp. PCC 6803. Plant Mol Biol 15: 633–642.

[7] Geerts, D., Bovy, A., de Vrieze, G., Borrias, M., and Weisbeek, P. (1995) Inducible expression of heterologous genes targeted to a chromosomal platform in the cyanobacterium Synechococcus sp. PCC 7942. Microbiology 141 (Part 4): 831–841.

[8] Guerrero, F., Carbonell, V., Cossu, M., Correddu, D., and Jones, P.R. (2012) Ethylene synthesis and regulated expression of recombinant protein in Synechocystis sp. PCC 6803. PLoS ONE 7: e50470.

[9] Hirose, Y., Shimada, T., Narikawa, R., Katayama, M., and Ikeuchi, M. (2008) Cyanobacteriochrome CcaS is the green light receptor that induces the expression of phycobilisome linker protein. Proc Natl Acad Sci USA 105: 9528–9533.

[10] Hirose, Y., Narikawa, R., Katayama, M., and Ikeuchi, M. (2010) Cyanobacteriochrome CcaS regulates phycoerythrin accumulation in Nostoc punctiforme, a group II chromatic adapter. Proc Natl Acad Sci USA 107: 8854– 8859.

[11] Huang, H.H., Camsund, D., Lindblad, P., and Heidorn, T. (2010) Design and characterization of molecular tools for a synthetic biology approach towards developing cyanobacterial biotechnology. Nucleic Acids Res 38: 2577–2593.

[12] Kato, H., Chibazakura, T., and Yoshikawa, H. (2008) NblR is a novel one-component response regulator in the cyanobacterium Synechococcus elongatus PCC 7942. Biosci Biotechnol Biochem 72: 1072– 1079.

[13] Komarova, A.V., Tchufistova, L.S., Supina, E.V., and Boni, I.V. (2002) Protein S1 counteracts the inhibitory effect of the extended Shine-Dalgarno sequence on translation. RNA 8: 1137–1147.

[14] Liu, X., and Curtiss, R. (2009) Nickel-inducible lysis system in Synechocystis sp. PCC 6803. Proc Natl Acad Sci USA 106: 21550–21554.

[15] Liu, X., and Curtiss, R. (2012) Thermorecovery of cyanobacterial fatty acids at elevated temperatures. J Biotechnol 161: 445–449.

[16] Lopez-Maury, L., Garcia-Dominguez, M., Florencio, F.J., and Reyes, J.C. (2002) A two-component signal transduction

system Involved in nickel sensing in the cyanobacterium Synechocystis sp. PCC 6803. Mol Microbiol 43: 247–256.

[17] Ma, J., Campbell, A., and Karlin, S. (2002) Correlations between Shine-Dalgarno sequences and gene features such as predicted expression levels and operon structures. J Bacteriol 184: 5733–5745.

[18] Narikawa, R., Suzuki, F., Yoshihara, S., Higashi, S., Watanabe, M., and Ikeuchi, M. (2011) Novel photosensory two-component system (PixA-NixB-NixC) involved in the regulation of positive and negative phototaxis of cyanobacterium Synechocystis sp. PCC 6803. Plant Cell Physiol 52: 2214–2224. Oliver, J.W., Machado, I.M., Yoneda, H., and Atsumi, S. (2013)

[19] Cyanobacterial conversion of carbon dioxide to 2,3-butanediol. Proc Natl Acad Sci USA 110: 1249– 1254. Salis, H.M., Mirsky, E.A., and Voigt, C.A. (2009) Automated design of synthetic ribosome binding sites to control protein expression. Nat Biotechnol 27: 946–950.

[20] Schar, J., Sickmann, A., and Beier, D. (2005) Phosphorylation-independent activity of atypical response regulators of Helicobacter pylori. J Bacteriol 187: 3100– 3109. Schneider, G.J., and Hasekorn, R. (1988) RNA polymerase subunit homology among cyanobacteria, other eubacteria and archaebacteria. J Bacteriol 170: 4136– 4140.

[21] Song, J.Y., Cho, H.S., Cho, J.I., Jeon, J.S., Lagarias, J.C., and Park, Y.I. (2011) Near-UV cyanobacteriochrome signaling system elicits negative phototaxis in the cyanobacterium Synechocystis sp. PCC 6803. Proc Natl Acad Sci USA 108: 10780–10785.

[22] Terauchi, K., Montgomery, B.L., Grossman, A.R., Lagarias, J.C., and Kehoe, D.M. (2004) RcaE is a complementary chromatic adaptation photoreceptor required for green and red light responsiveness. Mol Microbiol 51: 567–577

[23] Tzareva, N.V., Makhno, V.I., and Boni, I.V. (1994) Ribosomemessenger recognition in the absence of the ShineDalgarno interactions. FEBS Lett 337: 189–194.

[24] Yeh, K.C., Wu, S.H., Murphy, J.T., and Lagarias, J.C. (1997) A cyanobacterial phytochrome two-component light sensory system. Science 277: 1505–1508.