Reconstruction of a chromatic response system in *Escherichia coli*

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Two-component signal transduction systems (TCS) are involved in widespread cellular responses to diverse signals from bacteria to plants. Cyanobacteria have evolved photoperception systems for efficient photosynthesis, and some histidine kinases are known to function as photosensors. In this study, we attempt to reconstruct the photoperception system in *Escherichia coli* to make an easily controllable ON/OFF switch for gene expressions. For this purpose, a CcaS-CcaR two-component system from *Nostoc punctiforme* was expressed with phycocyanobilin (PCB) producing enzymes in *E. coli* which carries a G-box-controlled reporter gene. We succeeded to endow *E. coli* with a gene activation switch that is regulated in a light-color dependent manner. The possibility of such a switch for the development of synthetic biology is pointed out.

**Key Words:** *Escherichia coli*; cyanobacteria; two-component system

**Introduction**

The two-component system (TCS) is a widespread signal transduction system from bacteria to plants. In *Escherichia coli*, 30 sets of TCS are functioning to respond to a variety of signals, independently or interactively (Oshima et al., 2002). By using TCS, *E. coli* can respond to several signals, such as those arising from metal ions, membrane stresses, metabolites, and chemicals. However, TCS-responding light signals have not been found in *E. coli*. On the other hand, cyanobacteria have evolved several types of photosensing TCS. For complementary chromatic acclimation, histidine kinases such as RcaE from *Fremyella diplosiphon*, and CcaS from *Synechocystis* sp. PCC 6803 and *Nostoc punctiforme*, are functioning as photoreceptors, called cyanobacteriochromes (Ikeuchi and Ishizuka, 2008).

In *Synechocystis* sp. PCC 6803, CcaS covalently binds phycocyanobilin at a conserved Cys residue of a GAF domain and undergoes reversible photoconversion between green- and red-absorbing forms with Z-E isomerization of the phycocyanobilin (Hirose et al., 2008). CcaS exhibits enhanced phosphorylation activity upon green light irradiation. CcaS directly transfers phosphate to its cognate response regulator CcaR which binds to the regulatory region of *cpeC-cpcG2-cpeR1* to activate them (Hirose et al., 2010). In filamentous cyanobacterium *Nostoc punctiforme*, CcaS is also bound by phycocyanobilin and regulated by a similar mechanism as *Synechocystis*. There is a conserved direct repeat of \([\text{CTTNTCNATTT}] \times 2\) (designated as the G-box) in the regulatory region of the *N. punctiforme* *cpeC* and *Synechocystis* *cpcG2* genes. The CcaR protein directly binds to the G-box and regulates their expressions (Hirose et al., 2010).

Because light is a tractable signal to control gene expressions, we have attempted to reconstruct the photosensing signal transduction and gene regulation system in *E. coli*. To this end, we employed CcaS-CcaR TCS from *N. punctiforme* to produce a genetic switch reactive to light wavelengths, we believe that the resultant *E. coli* equipped with such a switch should be useful for industrial and/or synthetic biological applications. The characterization and evaluation of the results obtained are addressed in this study.
Materials and Methods

Strains, media and growth conditions. The E. coli strain used was BW25113 (αGbox-lacZ). The strategy for the construction of a λ phage lysogen harboring a G-box-lacZ transcripational fusion gene was developed by Hirano et al. (1987). The DNA region containing a G-box followed by a cpeC promoter was amplified by PCR from a N. punctiforme chromosome using two primers: Nos. Gbox-U (ACATAAGCTTCAACTAAGAGAATTCGATAC) and Nos. Gbox-D (ATGTAAGCTTTGCCAAAGT-CATT). The amplified fragment was digested with HindIII and inserted into the HindIII site of pMS434HS carrying a promoter-less lacZ gene (Hirano et al., 1987). The resultant plasmid was transferred into H2017 (αF13) harboring a lysogenic page, αF13, and a λ phage lysate was prepared by UV irradiation. Strain BW25113 was lysogenized with the λ phage. Candidates for lysogens carrying the appropriate G-box-lacZ’ fusion gene were selected from blue plaques on agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). Purified lysogen was confirmed its phenotype and named BW25113 (αGbox-lacZ), and used in this study.

Strains were grown in Luria-Bertani (LB) medium without isopropyl β-D-1-thiogalactopyranoside (IPTG) at 37°C by reciprocal shaking under each light irradiation. Light was radiated at the following photon fluxes, using color fluorescent lamps from Panasonic Inc.: green light at 23 mol photons m–2 s–1 (lamp: FL20SL-B, peak at 430 nm); and white light at 24 mol photons m–2 s–1 (lamp: FL20SS-W/18).

Results and Discussion

Construction of E. coli strain producing CcaS, CcaR, and phycocyanobilin

To construct E. coli responsive to changes in light wavelengths, we designed the construction of a CcaS and CcaR two-component regulatory system from cyanobacterium, N. punctiforme in E. coli (Hirose et al., 2010). The outline of our design is schematically shown in Fig. 1. CcaS, a histidine kinase, is a green light receptor and chromatically regulates the expression of phycobilisome linker genes, cpeC and cpeG2, by the green light dependent phosphorylation of the response regulator CcaR. Phosphorylated CcaR binds to a cis-element, named the G-box, and activates its target genes. CcaS covalently binds the phycocyanobilin (PCB) at a conserved Cys residue in the GAF domain and undergoes reversible photoconversion between green- and red-absorbing forms with Z-E isomerization of the phycocyanobilin (Hirose et al., 2008). Phycocyanobilin (PCB)-dependent photoconversion causes the green light dependent activation of the G-box controlled genes in the cyanobacterium. To produce CcaS and CcaR proteins, both genes were cloned and controlled under lpp-lac promoters individually on the same plasmid vector pIN-III and the resultant plasmid was named pIN-III-ccaSR(lacI+) (Lunn et al., 1986). To synthesize PCB in E. coli, we used plasmid pKT271 that carries the PCB biosynthesis genes ho1 (encoding heme oxygenase) and pcyA (encoding phycocyanobilin: ferredoxin oxidoreductase) (Mukougawa et al., 2006). To monitor the photo-responsive output in E. coli, the DNA region upstream of the cpeC gene that contains the G-box was placed upstream of the lacZ gene and integrated at a λ att site on the E. coli chromosome.

The constructed E. coli, named BW25113 (αGbox-lacZ) was transformed with pKT271 and pIN-III-ccaSR(lacI+) and its β-galactosidase activity was monitored under various light conditions. As shown in Fig. 2, the cells cultured under green light expressed a high β-galactosidase activity whereas, under other light conditions such as white, red, blue, and dark, no-significant activity was detected (Fig. 2A). The slight activation found with both white and blue lights might be caused by a green light factor intrinsically included in both the white and blue fluorescent lamps used (data not shown).

Next, we confirmed the necessity of factors for the identified green light dependent activation of lacZ. As shown in Fig. 2B, the expression was detected only under both CcaS-CcaR, and pKT271 was co-transformed. No expression was detected when CcaS-CcaR or pKT271 was introduced with their cognate vector plasmids. Figure 2C shows that only CcaS, or only CcaR, is insufficient for full activation, suggesting both CcaS and CcaR are necessary for the activation. We confirmed that both ccaS+ and ccaR+ mRNAs were expressed from plasmid pIN-III-ccaSR(lacI+) in cells grown in LB medium without IPTG.
and the addition of IPTG did not affect the \(\beta\)-galactosidase activity under green light (data not shown). So we think a sufficient amount of CcaS and CcaR proteins, heme oxygenase and phycocyanobilin: ferredoxin oxidoreductase activity under green light (data not shown). So we think a sufficient amount of CcaS and CcaR proteins, heme oxygenase and phycocyanobilin: ferredoxin oxidoreductase activity under green light (data not shown). So we think a sufficient amount of CcaS and CcaR proteins, heme oxygenase and phycocyanobilin: ferredoxin oxidoreductase activity under green light (data not shown).

Based on these observations, we conclude that we have succeeded in reconstructing the green light dependent gene activation system in \textit{E. coli}.

Characterization of the nature of the photo-regulatory system

Light could be a non-erosive on-off signal for a transcriptional switch in \textit{E. coli}. To evaluate the switching nature of the reconstructed photo-sensory system, we analyzed the \(\beta\)-galactosidase activity after switching from green to red, and red to green, light. In Fig. 3A, cells were grown under red light, and, at time 0, the light was changed from red to green. The \(\beta\)-galactosidase activity increased after 1.5 h of switching in cells carrying pIN-III-ccaSR(lacI–), but not in cells carrying the vector plasmid, pIN-III-A1. On the other hand, cells grown under green light were changed to red light and the \(\beta\)-galactosidase activity was analyzed (Fig. 3B). The activity found under green light decreased after changing to red light. To evaluate the necessity of red light for shutoff of the promoter activity, we compared the response of the \(\beta\)-galactosidase activity between green to red, and green to dark, changes. As shown in Fig. 3C, the activity found under green light decreased in essentially the same way between changing to red light or to dark. This suggests that just turning off the green light is sufficient for a shutoff of the promoter activity.

In summary, we have succeeded in reconstructing the photo-responsive gene expression system in \textit{E. coli} by using cyanobacterial TCS. Because light is a non-erosive, and easy changeable signal, this system could be of use in gene regulation in various applications. Cyanobacteria have evolved a diverse set of photosensing proteins, cyanobacteriochrome, such as AnPixJ of \textit{Anabaena} sp.
PCC7120 (Fukushima et al., 2011), SyPixJ (Yoshihara et al., 2000), SyPixD (Okajima et al., 2005), TePixJ (Ishizuka et al., 2006), and CcaS proteins of Synechocystis sp. PCC 6803 and Nostoc punctiforme (Hirose et al., 2008, 2010). The control of gene expression in E. coli using the Cph1/EnvZ chimera sensor, Cph8 (Tabor et al., 2011), or CcaS from Synechocystis sp. PCC 6803, has been attempted and its utility has been shown (Levskaya et al., 2005; Schmidl et al., 2014; Tabor et al., 2011). Here, we also succeeded in reconstructing a photo-sensing system in E. coli by using CcaS-CcaR from N. punctiforme. The increase in the variety of photo-responsive gene expression systems in E. coli could extend the possibility of their use, not only in engineering applications, but also for the analysis of synthetic gene circuits in synthetic biological applications.

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