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Multichromatic control of gene expression in *Escherichia coli*

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Abstract

Light is a powerful tool for manipulating living cells because it can be applied with high resolution across space and over time. We previously constructed a red-light sensitive *E. coli* transcription system based on a chimera between the red/far red switchable cyanobacterial phytochrome Cph1 and the *E. coli* EnvZ/OmpR two-component signaling pathways. Here we report the development of a green light inducible transcription system in *E. coli* based on a recently discovered green/red photoswitchable two-component system from cyanobacteria. We demonstrate that transcriptional output is proportional to the intensity of green light applied and that the green sensor is orthogonal to the red sensor at intensities of 532nm light less than 0.01W/m^2. Expression of both sensors in a single cell allows two-color optical control of transcription in both batch culture and in patterns across a lawn of engineered cells. Because each sensor functions as a photoreversible switch, this system should allow the spatial and temporal control of the expression of multiple genes though different combinations of light wavelengths. This feature should aid precision single cell and population-level studies in systems and synthetic biology.

Keywords

Light-regulated promoter; synthetic biology; two-component system; phytochrome; cyanobacteriochrome

Introduction

Several genetically encoded tools have been developed for the optical regulation of molecular interactions inside of living cells. These include light regulated transcriptional regulatory systems in bacteria [1; 2] and yeast [3], light-dependent metabolic [4], signaling [5] and protein-splicing [6] enzymes, a light switchable protein dimerization system [7], and light regulated neuronal ion channels [8] and adrenergic receptors [9]. These molecular genetic tools are unique in that they allow exquisite spatial and in some cases temporal control of cell states with minimal invasiveness.

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Many biological and biotechnological applications require external control of cellular gene expression. To this point all of the engineered light-regulated gene expression systems [1; 2; 3] have been monochromatic: transcription from a given promoter is regulated (reversibly or irreversibly) by one set of light wavelengths. The development of multichromatic gene regulatory systems, where different light wavelengths regulate the expression of different genes, will allow more advanced control of synthetic and natural gene regulatory networks.

Phytochromes, a ubiquitous family of proteins that switch between active and inactive signaling states in response to red and far red light [10], have previously been used for synthetic control of living cells. In the first reported example, a phytochrome/phytochrome binding protein pair was adapted to a classical two-hybrid system to construct a light regulated promoter in yeast [3]. In another study, we fused the phytochrome Cph1 from *Synechocystis* PCC6803 to the *E. coli* histidine kinase EnvZ to engineer a red light regulated transcription system in *E. coli* [1]. More recently we used a phytochrome/phytochrome interacting pair from *Arabidopsis* to engineer rapidly photoswitchable protein dimerization (seconds timescale) in mammalian cells [7]. Other approaches have employed the blue-light responsive LOV (light oxygen voltage) domain to control gene expression and signal transduction [2; 5]. In contrast to the phytochrome-based tools, however, LOV-based systems respond unidirectionally to light exposure with dark-dependent relaxation of signaling occurring on the order of minutes to hours [11].

Recently, a cyanobacterial two-component system has been shown to induce the expression of a phycobilisome-related gene in response to green light [12]. The two-component system comprises the membrane-associated histidine kinase CcaS and its response regulator CcaR. CcaS is a member of the cyanobacteriochrome family of proteins, a cyanobacteria-specific relative of the phytochromes with blue-shifted absorption spectra [13]. As in phytochromes, a bilin chromophore (in this case phycocyanobilin), binds at a conserved cysteine within an N-terminal GAF (cyclic GMP phosphodiesterase, Adenylyl cyclase, FhlA) domain and imparts reversible photoactivation of signaling activity with maximal responses to 535nm (green) and 672nm (red) light. Absorption of green light increases the rate of CcaS autophosphorylation, phosphotransfer to CcaR, and transcription from the promoter of the phycobilisome linker protein *cpcG2*, while absorption of red light reverses this process [12].

Because they share a common chromophore and light absorption mechanism but have different chromatic specificities and transcriptional outputs, we hypothesized that CcaS/R may be able to function alongside our previously constructed red sensor (Cph8) for multichromatic control of gene expression in *E. coli*. Because CcaS is inactivated in the red band to which the Cph1/EnvZ chimera Cph8 responds, green and red light could be differentially applied to specifically induce transcription from each system. Moreover, because both sensors are photoreversible, such a system would allow multiplexed spatiotemporal control of gene expression.

Results

Cloning of the *ccaS/ccaR* cluster

To investigate whether the green light-inducible two-component system could function in *E. coli*, a plasmid expressing CcaS and CcaR and carrying a *lacZ* reporter fused to the P_{cpcG2} promoter (pJT118, Supplemental Figure 2) was constructed. To this end the *ccaS/ccaR/cpcG2* cassette was amplified from the genome of *Synechocystis* PCC6803 and cloned into a multicopy vector, generating plasmid pJT116 (Supplemental Figure 2). The open reading frame of the output gene *cpcG2* was then seamlessly replaced with that of *lacZ* (Materials and Methods). The product of *lacZ*, β-galactosidase, was chosen as a reporter because it has

previously proven tractable in both batch culture and plate-based light regulation experiments [1; 14].

The plasmid pPLPCB(S) (Materials and Methods) was used to produce the chromophore PCB for green light sensor experiments. pPLPCB(S) carries the *Synechocystis* PCC6803 genes *ho1* and *pcyA*, which convert heme into PCB via a two-step oxidation/reduction process. pJT118 and pPLPCB(S) were co-transformed into *E. coli* strain JT2, a derivative of the strain previously used for red light sensor experiments (RU1012) [15] from which a genomic fusion between the *ompC* promoter and *lacZ* was deleted (Materials and Methods).

Green light induced gene expression in *E. coli*

Green light induced transcription from P_{cpcG2} was assayed by growing *E. coli* expressing CcaS/R in liquid media for 10 cell divisions in the dark or under 0.080 W/m^2 532nm light as described previously [14]. Miller assays were conducted to determine the abundance of β-galactosidase per cell under each condition. Dark exposed bacteria produce 24.7 ± 1.3 Miller units (M.U.) while those exposed to green light produce 50.7 ± 3.1 M.U (Figure 2a, n = 4).

To determine whether the *E. coli* green light sensor functions as previously demonstrated *in vitro* [12], cells were then exposed to inactivating red light. Exposure to 0.080 W/m^2 650nm light results in a slight reduction in β-galactosidase levels as compared to dark grown cells (Figure 2a). To determine whether the green light-dependent increase in gene expression is a specific effect of light absorption by the CcaS chromophore, the experiments were repeated in a strain lacking PCB. This strain shows no response to green or red light (Figure 2a). These gene expression data agree with *in vitro* assays [12] indicating that in *E. coli*, the bilin-ligated (holo) form of CcaS is produced in the inactive green light absorbing state (P_g) and it is activated by green light and repressed by red light in a manner dependent upon PCB.

Solid-phase light exposure experiments were then conducted to determine if the green light response could be visualized as patterns of gene expression across a lawn of cells. In agreement with the data in Figure 2a, expression of β-galactosidase is induced only in areas of green light (Figure 2b). Because CcaS adopts the inactive ground state in the dark, the rate of phosphotransfer to CcaR and resulting transcription of *lacZ* are low in dark exposed areas of the plate. However, in green light exposed areas, CcaS kinase activity increases, increasing the abundance of β-galactosidase and the cleavage of its chromogenic black substrate in the media (Materials and Methods). Because the output signal is black, this results in a negative print of the projected image on the bacterial plate. Plate-based bacterial films expressing the green sensor do not respond to red images and are dependent on PCB (Figure 2b).

Construction of a red light-activated genetic circuit

Transcription from the output promoter of the previously constructed red light sensor (P_{ompC}) is inversely proportional to the intensity of red light [1]. For many applications, including an initial demonstration of two-color optical gene regulation, a sensor that is activated by red light (analogous to the green sensor) is desirable. For this purpose, a genetic inverter [16] was placed between the red light sensor and *lacZ*. Similar to our previously reported inverted red sensor [14], the CI repressor from phage λ is expressed as the output of P_{ompC}, and *lacZ* is expressed under the control of a CI repressible promoter (Figure 1). Dark exposure therefore results in high-level production of CI repressor and repression of *lacZ* transcription while exposure to red light relieves this repression.

The performance of the red sensor was examined using Miller Assays. Cells were grown for 10 generations in the dark or under 0.080 W/m^2 650nm light (Materials and Methods). Dark exposed cells produce 0.58 +/− 0.01 M.U. while red light exposed cells generate 1.41 +/−

0.03 M.U (Figure 2a). This 2.4-fold induction is similar to green light response and is dependent on PCB (Figure 2a). Unlike the green sensor, which remains inactivated in red light, the red sensor shows a minor response to high levels (0.080 W/m^2) of green light (532nm, Figure 2a). Lawns of bacteria expressing the red sensor print images of red light as negatives but do not respond significantly to images of green light (Figure 2b).

Characterization of spectral transfer functions

The transfer function describes the quantitative relationship between the input and output of a genetic circuit [17; 18; 19; 20]. In the case of the light sensors, the input can be light wavelength or light intensity. The spectral transfer functions of the green and red sensors were determined by measuring transcriptional output relative to dark exposed cells at different wavelengths of light between 430nm and 730nm (Figure 2c). For each wavelength in Figure 2c, high levels (0.080 W/m^2) of the respective wavelength were applied. In agreement with *in vitro* measurements of the absorbance of the CcaS holoprotein [12], the green light sensor shows transcriptional activation between 490 and 570nm with a maximum response near 535nm. There is very little induction in 610nm (orange) and the sensor is inactive in 650nm (red) light. By contrast, the red sensor is strongly induced in the 610-650nm range. As expected the red sensor is inactive in the far red region (730nm). The red sensor also has a long tail into the blue regions of the spectrum, though the magnitude of the response decreases significantly below 610nm (Figure 2c). Despite the slight overlap, we determined that the separation in the action spectra of the two sensors could allow them to be combined for multi-wavelength optical control of gene expression in a single cell.

Two-color optical control of gene expression

To investigate whether the two light sensors can function simultaneously in a single cell, a plasmid expressing both the green and red sensors (pJT122) was constructed (Figure 1 and Methods). The light intensity transfer functions of each of the three sensor combinations (green only, red only and both) were then determined for 532nm and 650nm inputs. Cells expressing the green sensor show sharp activation between dark and 0.01W/m^2 532nm light, above which point the response saturates (Figure 3a). By contrast, bacteria expressing the red sensor show a small linear response to 532nm light between dark and 0.080 W/m^2. Cells expressing both sensors have a transfer function very similar to cells expressing the green sensor alone, though the total Miller Unit output is slightly lower (Figure 3a).

E. coli expressing only the red sensor are continuously induced by 650nm light between dark and 0.01 W/m^2, after which point the response largely saturates (Figure 3a). The shape of the red sensor transfer function to 650nm light is similar to that of the green sensor to 532nm light and both sensors respond over similar light intensities. While cells expressing only the green sensor are slightly repressed by 650nm light, the addition of the red sensor causes the cells to be induced by with a transfer function similar to cells expressing the red sensor alone (Figure 3a).

The transfer functions of the red and green sensors are non-additive when combined in a single cell. For example, the decrease in Miller Unit output of the green sensor in 650nm light would be expected to offset the increase in Miller output from the red sensor, but this is not observed in the data. In fact, the presence of the green sensor leads to a greater increase in Miller output by the red sensor in response to 650nm light (Figure 3a). There are numerous direct or indirect interactions that could cause such non-additivity. For example, the red sensor could more effectively compete for chromophore, diminishing the response of the green sensor when both are present in a single cell. Alternatively, the kinase domain of the red sensor could dephosphorylate CcaR, the response regulator of the green sensor pathway, reducing signaling through the green pathway. Non-specific effects such as

competition for ribosomes [21] or protein degradation machinery [22] could also affect the expression level of a given sensor when the other is overexpressed. Follow-up investigations of these effects could inform future efforts in engineering phytochromes and constructing synthetic signaling pathways in bacteria in general.

The data in Figure 3a demonstrate that appropriate dosing of light wavelengths and intensities allows independent control of the sensors in a single cell. This was then demonstrated by projecting a composite green-red image onto agarose embedded films of engineered bacteria. The intensity of projected green light was set at 0.02 W/m^2, just above the saturation point of the green sensor, so as not to trigger unwanted induction of the red light sensor (Figure 3b). When a strain expressing only the green sensor is exposed to this two-color image, β-galactosidase abundance increases sharply in the green areas and within regions of white light but not in the red areas. Conversely, a strain expressing the red sensor is induced for β-galactosidase in red areas but only very slightly in green regions. Finally, in a strain expressing both sensors, β-galactosidase expression is induced both colors of light (Figure 3b).

The ribosome binding site upstream of *lacZ* in the red sensor was engineered to be weak (Supplemental Information), resulting in ~30-fold lower β-galactosidase output from the red sensor as compared to the green sensor (Figure 2a, 3a). On plates, this causes green light exposed areas to appear darker than red light exposed areas (Figure 3b). The translation of color information to differences in monochrome intensity results in grayscale effects which compensate for the lack of visually distinct (color) outputs. Despite the slight non-additivities that occur when the sensors are combined, the data in Figure 3 demonstrate that the expression of a second sensor does not significantly change the response of a given sensor to its cognate light wavelength, and that co-expression of the two sensors allows two-color optical control of gene expression in a single cell.

Discussion

Several light regulated transcriptional regulatory systems have previously been constructed [1; 2; 3]. By combining an *E. coli* red light sensor with a recently discovered green light sensor from *Synechocystis*, we have engineered a multichromatic gene regulatory system where different promoters are controlled by different wavelengths in a single cell. This system has several unique properties. Because most gene regulatory systems rely on the addition of chemicals to the growth medium, modulation of gene expression is often unidirectional with reversal depending on decay or dilution of the effector compound. By contrast, both sensors reported here function as switches that can be toggled between states by different light wavelengths [12; 23; 24], a feature that allows more precise temporal control of gene expression.

The reversible behavior of the green and red light sensors begins at the phycocyanobilin (PCB) chromophore. After ligation to PCB, the holoprotein adopts a stable green or red absorbing ground state (P_g or P_r). Picoseconds after absorption of the activating photon, isomerization of PCB drives a conformational rearrangement of the surrounding protein which occurs on the order of milliseconds to seconds [25]. Structural changes in the light-sensing domains are then transmitted to the kinase domains, activating phosphosignaling. Phosphotransfer from the histidine kinase to its response regulator then occurs in milliseconds [26]. When phosphorylated, the response regulator binds its cognate promoter and induces transcription. This occurs in minutes but can take on the order of one hour to reach steady state [27]. The light sensors should therefore allow reversible control of transcription on the minutes timescale. Because of the relatively slow nature of gene expression and protein decay, however, time periods on the order of hours will be required

to switch between ON and OFF steady states. This would also be the case for chemically regulated transcription systems, though these systems do not have the benefit of reversibility.

Modern optical methods such as two-photon excitation or digital micromirror devices (DMDs) allow the projection of light patterns at subcellular resolution. If combined with such optics [7] and fluorescent or luminescent reporter genes, the two-color system described here should allow real-time control and observation of the expression of multiple genes in individual cells within a larger population. This would enable facile external patterning of genotypes and studies of time-dependent multicellular phenomena such as biofilm formation.

The action spectra for the two light sensors in this study partially overlap (Figure 2c). The maximum inactivating wavelength of the green sensor is effectively the same as the maximum activating wavelength of the red sensor. The result is that there are only three 'cognate' control wavelengths for four possible states. The red sensor also has a long blue tail, showing induction in response to wavelengths as low as 490nm (Figure 2c). Despite these overlaps, there are a variety of strategies for achieving independent control of the four sensor states. For example, intensities of 532nm light less than ~0.01W/m^2 activate the green sensor while leaving the red sensor inactive (Figure 3a). Far red light (730nm) can be applied concomitantly with 532nm to inhibit the red sensor while activating the green sensor. To activate both sensors simultaneously, high levels (0.08 W/m^2 or greater) of an intermediate wavelength such as 575nm can be used (Figure 2c).

To improve performance, the action spectra of the light sensors themselves could also be engineered. A number of mutations in the light sensing domains of phytochrome-related proteins have been generated and shown to alter the absorbance spectra [28; 29; 30]. As expected, mutations in the chromophore binding pocket affect absorption, but other more dramatic mutations in domain architecture likely play a role as well. Indeed, the green sensing cyanobacteriochrome protein used here has several binding pocket mutations in conserved residues as well as domain organization differences as compared to the red sensor [12]. Because we have linked the sensors to gene expression outputs in E. coli, standard laboratory evolution methods targeting critical amino acids in the chromophore binding domain, or even the domain shuffling methods altering the overall architecture of the phytochrome could potentially be used to rapidly generate new light sensors or to narrow the spectral sensitivities of existing sensors.

Synthetic gene circuits could be also used to filter the responses of the existing light sensors. For example, a bistable genetic switch could be placed between [32] the light sensors and the output genes. Bistable circuits cannot rest in intermediate output states, but switch digitally from low to high output in response to continuous changes in input signal [33]. Because the responses of the two light sensors decrease symmetrically with distance from the maximal inducing wavelength (Figure 2c), a bistable switch could cut off responses below a certain threshold, effectively narrowing the action spectra of the sensors.

The output of the two light sensors reported here changes continuously with input (Figure 3a). Gradients of light can therefore be used to set different transcription levels across space in solid-phase experiments (Supplemental Figure 1). The light sensors could be connected to genetic circuits and one or two-dimensional light gradients could be applied to determine circuit transfer functions over a continuous range of inputs in a single experiment. The ability to measure two-dimensional transfer functions in a single step could prove very useful in both systems [34] and synthetic biology studies [35; 36].

The system reported here represents the first engineered multichromatic gene regulatory system, whereby the expression of different genes can be controlled by different wavelengths of light. Multi-channel optical regulation of neuronal membrane potentials stands to revolutionize neurobiology by allowing unprecedented temporal control of neuronal activity *in vivo* [37]. The multiplexed optical control of gene expression should find broad utility in scientific, engineering and industrial applications.

Materials and Methods

Plasmid construction

Construction of pJT116. The fragment of the *Synechocystis* PCC6803 genome bearing the *ccaS-ccaR* cluster (chromosomal position 3399457-3405249) was amplified with the primers TACTAGACTAGACTAGATCAGAGTACGATCAGTCATGACTAGACGATCGGACGTCCTAAGCTCGAGGCAAATGG and TGTCATGTATCGTCAATGGTACTGACTCTACTCAATACGTTCTAGATCTTCTAGACTAGTTTTTCCCTTGGCAC from purified genomic DNA and cloned into the pProTet.E333 backbone (Clontech, Mountain View, CA) at the AatII and XbaI sites (underlined in the primers). The use of these sites removes the $P_{LTetO-1}$ promoter, ribosome binding site, 6x HIS tag and MCS while leaving the downstream transcription terminator. The endogenous Synechocystis promoters are therefore responsible for the expression of CcaS and CcaR in this plasmid. pJT116 was maintained with 34μg/mL chloramphenicol.

Construction of pJT118. The green light inducible *cpcG2* open reading frame [12] in pJT116 was seamlessly replaced with *lacZ* using the MEGAWHOP protocol [38]. *lacZ* was amplified from pEXP*lacZ* (Invitrogen, Carlsbad CA) with primers GATATAACAGTATAGATTTTGTCAGCCTTCAGCTTGGCTTTACCGTCAAAAAAATTAGACTCGAGCGGCCGC and CACATACCAGTTATTGGCTGGACATTAAACAACTTTTAAGTTTAATTACTAACTTTATCTATGATAGATCCCGTCGTTTTACAACG to generate the green light responsive reporter plasmid pJT118 (lacZ binding regions underlined). pJT118 was maintained with 34μg/mL chloramphenicol.

Construction of pJT122. An expression cassette for the red-light responsive *cph1*/*envZ* chimera *cph8* [1] was added to the green light reporter plasmid pJT118 to generate plasmid pJT122. The $P_{LTetO-1}$ promoter, ribosome binding site and *cph8* open reading frame [1] were amplified using primers GCCCTAGACCTAGGGCGTTCGGCTGCGGCGAGCGGTATCACCTTTCGTCTTCACCTCGAG and GTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTTACCCTTCTTTTGTCATGCCC (promoter and *cph8* binding sequences underlined respectively). This PCR product was then used as a megaprimer in a MEGAWHOP reaction to clone the cassette downstream of the *ccaR* transcription terminator in pJT118. pJT122 was maintained with 34μg/mL chloramphenicol.

Construction of pJT106b. pJT106b encodes a red light inverter circuit driving a *lacZ* reporter gene. pJT106b is derived from pJT106, which carries the P_{ompC} promoter BBa_R0082 driving the *cI* gene, the product of which represses the LuxR + $3OC_6HSL$-activated, CI-repressed output promoter BBa_R0065 [14]. In pJT106b, R0065 is replaced by a LuxR + $3OC_6HSL$-independent CI repressible promoter BBa_J64067. To make J64067 LuxR + $3OC_6HSL$-independent (and increase the overall transcription rate), the weak −35 site of R0065 (TTTACG) was replaced with a consensus TTGACA site, the suboptimal 16bp spacer between the −35 and −10 sites was replaced with a 17bp spacer and the −16

nucleotide was swapped from T to G. A megaprimer encoding these 4 mutations was generated by amplifying the R0065 region of pJT106b with the primers CGTACAGGTTGACAACAAGAAAATGGTGTGTTATAGTCG and CATTAAATGTGAGCGAGTAACAACCCG (mutations underlined). This megaprimer was then used to extend pJT106 in a MEGAWHOP to generate pJT106b. pJT106b was maintained with 50μg/mL ampicillin.

Construction of pPLPCB(S). Because strain JT2 bears native kanamycin resistance, pJT118 and pJT122 bear chloramphenicol resistance and pJT106b bears ampicillin resistance, a variant of plasmid pPLPCB [39] carrying a spectinomycin resistance marker was constructed. To this end, the spectinomycin resistance cassette (including promoter, ribosome binding site and $spec^R$ gene) were amplified from plasmid pKD13 [40] using primers AGAGCCTAGACCATAGACATAGAATATACGTACGGGCCCAGCAAGCGAACCGGAATTGCC and TATATTGACTCTAGCTCTAACTCTATGGGCTCTAGAGCTCTTATTTGCCGACTACCTTGG (primer binding sites underlined) and cloned into pPLPCB using ApaI and SacI which remove the kanamycin resistance cassette. pPLPCB(S) was maintained with 100μg/mL spectinomycin.

Bacterial strains

Strain JT2 (RU1012 ΔP_{ompC}-*lacZ*) was used for all experiments. JT2 was constructed using the Datsenko-Wanner method [40] to knock out the region of the RU1012 genome within which the *lacZ* gene was fused to the *ompC* open reading frame [15]. The entire knocked out region contains in order: the *ompC* promoter followed by the first 789bp of the *ompC* gene, a translational fusion between the first 177bp of the *E. coli* tryptophan synthase alpha subunit and *lacZ, lacY*, a truncated *lacA*, a second copy of the *ompC* promoter driving a second copy of the *ompC* gene which is internally disrupted by a Tn5 transposon carrying a kanamycin resistance maker. Though the embedded kanamycin resistance marker used to make this fusion was deleted in this step, the strain maintained resistance to kanamycin at 50μg/mL. This suggests that at least one additional, unannotated kanamycin resistance marker is present in the genome of RU1012. Strain JT2 was grown in the presence of 50μg/mL kanamycin for all experiments. The primers used to generate the knockout PCR fragment were: GAATTATTATTGCTTGATGTTAGGTGCTTATTTCGCCATTCCGCAATAATCTTAAAAAGTGTGT AGGCTGGAGCTGCTTC and TTGTACGCTGAAAACAATGAAAAAAGGGCCCGCAGGCCCTTTGTTCGATATCAATCGAGAATTCC GGGGATCCGTCGACC which bear homology to the region immediately upstream of the *ompC* promoter and immediately downstream of the end of the *ompC* ORF.

Miller Assays

Overnight cultures were grown in 3mL unbuffered LB broth (Lennox formulation) + appropriate antibiotics to OD_{600} ~3-4. These cultures were diluted to OD_{600} = 0.001 in 1mL fresh LB (Lennox) + 0.1M HEPES pH = 6.6 + appropriate antibiotics, grown 10 cell divisions (to OD_{600} = 1.0) and subjected to Miller Assays as described previously 14. Light was projected onto the growing cultures as before [14] using the following bandpass filters (Edmund Optics, Barrington NJ): 430nm NT43-160, 488nm NT43-168, 532nm NT43-174, 568nm NT43-179, 610nm NT43-183, 650nm NT43-189, 730nm NT43-195 Replicates were grown in parallel on a single day while data for different light intensities and wavelengths were collected on different days.

Determination of light intensity

The intensity of light was measured in power units of Watts per square meter using a EPP2000 UVN-SR calibrated spectroradiometer (Stellarnet, Tampa, FL) with a collection window ±30nm from the reported (peak) wavelength. The bandpass filters used in these experiments have 10nm transmission windows centered around the peak emission wavelength.

Plate assays

Plate assays were conducted as described previously [14] except that starter cultures were grown overnight in unbuffered LB broth + appropriate antibiotics. The light exposure step was carried out for 21 hours except in the case of red sensor only cells carrying the weak *lacZ* ribosome binding site (plasmid pJT106b3) in which case light exposure was carried out for 48 hours to allow the accumulation of more black pigment.

The 2-color mask used in Figure 3b was generated by taking a photograph of chili peppers (Whole Foods, San Francisco, CA) with a Canon EOS Rebel SLR camera with a macro lens and hood. The background was made black and the RGB characteristics of the chilis were then enhanced using Adobe Photoshop. Though color composition of the chili regions of the image was greater than 90% red, the stems contained significant red, green and blue components. To remove the red and blue components, the stems were isolated using the magic wand function and their color balance was minimized away from red and blue (toward cyan and yellow), and toward green (away from magenta). A color enhanced tiff file was then used as the template to fabricate a 35mm slide (Oscar's Photo Lab, San Francisco, CA), which was used to mask a white light projector as reported previously [14]. Light transmission through the stem and chili regions of the mask was verified to be almost exclusively green and red respectively using a spectroradiometer as above.

Imaging the agarose plates

Agarose plates were placed face up on a white fluorescent light box, and photographs were taken in a darkened room with a Canon EOS Rebel SLR camera with a macro lens and hood. Image levels, tone, contrast and shadowing were adjusted using Adobe Photoshop (Adobe Systems Inc., San Francisco, CA) to more accurately represent the appearance of the agarose plates to the naked eye.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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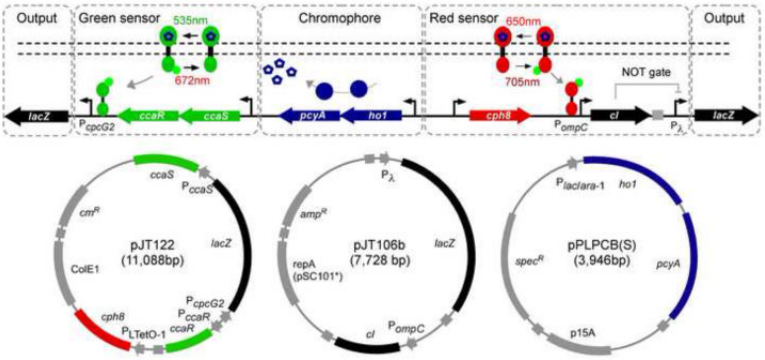

Figure 1.

Engineered two-color light induction system in *E. coli*. (A) Schematic representation of the system. The green sensor and chromophore biosynthetic pathway are as described in the main text. The red light sensing protein Cph8 is expressed from the $P_{LTetO-1}$ promoter in the phosphorylated ground state. It is switched to the unphosphorylated state by 650nm, and back to the phosphorylated state by 705nm light [39]. When phosphorylated, Cph8 passes a phosphoryl group to OmpR which then binds to and activates transcription from P_{ompC}. Because it is inactivated by red light, Cph8 can be considered a logical (NOT red) sensor. A genetic inverter, or logical NOT gate is used to invert the response of the (NOT red) sensor to that of a red light sensor. (B) Plasmid maps of the green + red sensor plasmid pJT122, the red light inverter plasmid pJT106b and pPLPCB(S), a variant of pPLPCB [39] in which the kanamycin resistance cassette has been replaced by a spectinomycin resistance cassette (Materials and Methods). Note that the true configuration of the DNA encoding this system is represented by the plasmid maps while the version shown atop this Figure is slightly simplified for clarity.

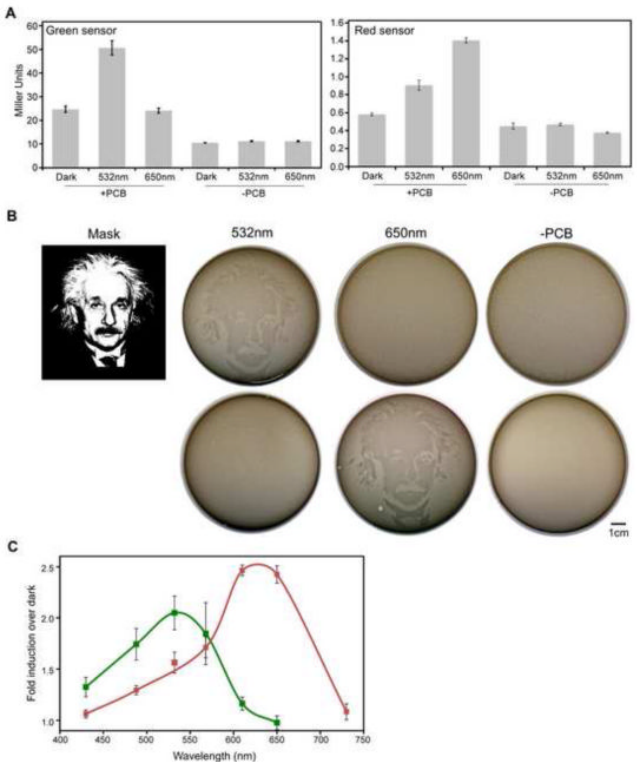

Figure 2.

Transcriptional response of green and red sensors to different light conditions. (A) *E. coli* cultures were grown in the dark, under 0.080W/m^2 532nm light or 0.080W/m^2 650nm light. (+PCB) strain JT2 carrying the green (pJT118) or red sensor (pCph8 + pJT106b3) plasmids and pPLPCB(S). (−PCB) JT2 carrying only the green or red sensor plasmids. Each data point represents the average of four separate cultures grown and measured in parallel on a single day. Data taken under different light conditions were collected on different days. Miller Assays are conducted as reported previously [14]. Error bars represent ± one standard deviation. (B) Plate-based assays of green and red sensors. The mask shown was used to project an image of 532nm or 650nm filtered light onto an agarose embedded film of bacteria expressing the green (top) or red (bottom) sensors. The chromogenic substrate S-gal (Sigma) and ferric ammonium citrate are added to the agarose media such that the product of *lacZ*, ß-galactosidase, produces a visible black pigment when expressed. 0.030 W/m^2 532nm and 0.080W/m^2 650nm red light were projected through the mask for all trials. A slightly lower 532nm intensity was used because the red sensor shows a minor response to 0.080W/m2 532nm light (Figure 2a, 3a). The green sensor strain is the same as Figure 2a. The red sensor strain is JT2 carrying pCph8, pPLPCB(S) and pJT106b (a variant of pJT106b3 with a stronger ribosome binding site upstream of lacZ) for higher pigment production on plates. The −PCB condition indicates a given strain lacking pPLPCB(S) exposed to its cognate light wavelength. After 21 hours, the bacterial plates produce images that can easily be seen by eye with no further image enhancement. (C) Spectral transfer functions. *E. coli* carrying the green or red sensor (strains as in Figure 2a) were exposed to saturating levels of a given light wavelength and Miller Assays were conducted as in Materials and Methods. Data are reported as fold induction over dark exposed cells. This is calculated by dividing the Miller Unit (M.U.) value of the light exposed cells by the M.U. value of the same strain grown in the dark. Each data point represents the average of four separate cultures grown and measured in parallel on a single day. Data at different light wavelengths (or dark) were

collected on different days. Error bars represent ± one standard deviation. Miller Assays are conducted as reported previously [14].

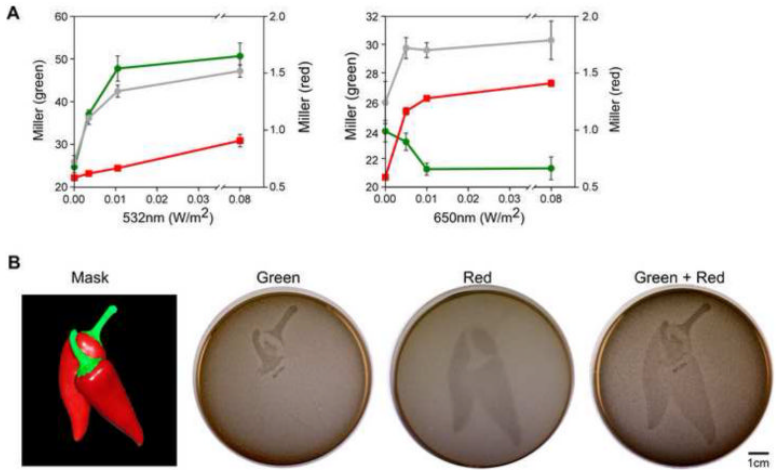

Figure 3.

Two-color optical control of gene expression in *E. coli*. (A) Light intensity transfer functions of strains carrying each sensor alone or both sensors. Strains expressing the green sensor only (green circles), red sensor only (red squares) or both (grey circles) were exposed to varying intensities of 532nm or 650nm light and Miller Assays were conducted as in Materials and Methods. The green and green + red data (circles) obey the left axis while the red sensor data (squares) obey the right axis. Two axes were used because the absolute Miller Unit output of the RBS weakened red sensor is low compared to the green sensor. Error bars represent ± one standard deviation. (B) Two color bacterial photography. A two-color mask was generated from a color-enhanced photograph of chili peppers. Green light passing through the stem regions of the image was set at 0.02 W/m^2, slightly above the saturation point of the green sensor. At these illumination levels the mask transmits 0.02-0.025 W/m^2 650nm light, above the saturation point of the red sensor. The same light intensities were used for all three plates. Green and red sensor only strains are as in Figure 2a. Green + red strain is JT2 carrying plasmids pJT122, pJT106b3 and pPLPCB(S).