Multichromatic Control of Gene Expression in *Escherichia coli*

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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 *Escherichia 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 pathway. 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 532-nm light less than 0.01 W/m². Expression of both sensors in a single cell allows two-color optical control of transcription both in 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 through different combinations of light wavelengths. This feature should aid precision single-cell and population-level studies in systems and synthetic biology.

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**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, light-dependent metabolic enzymes, signaling pathways, and protein-splicing enzymes; a light switchable protein dimerization system; and light-regulated neuronal ion channels and adrenergic receptors. These molecular genetic tools are unique in that they allow exquisite spatial and in some cases temporal control of cell states with minimal invasiveness.

Many biological and biotechnological applications require external control of cellular gene expression. To this point, all of the engineered light-regulated gene expression systems 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, 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. In another study, we fused the phytochrome Cph1 from *Synechocystis PCC6803* to the *Escherichia coli* histidine kinase EnvZ.

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to engineer a red light-regulated transcription system in *E. coli.* More recently, we used a phycobilisome/photodector interacting pair from *Arabidopsis* to rapidly engineer photoswitchable protein dimerization (seconds time scale) in mammalian cells. Other approaches have employed the blue light-responsive LOV (light, oxygen, or voltage) domain to control gene expression and signal transduction. In contrast to the photodetector-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.

Recently, a cyanobacterial two-component system has been shown to induce the expression of a phycobilisome-related gene in response to green light. The two-component system consists of 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. As in phytochromes, a bilin chromophore (in this case phycocyanobilin) binds to 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 535-nm (green) and 672-nm (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.

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 (pJT116; Supplementary Fig. S2) 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 (Supplementary Fig. S2). 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.

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 oxygen/reduction process. pJT118 and pPLPCB(S) were cotransformed into *E. coli* strain JT2, a derivative of the strain previously used for red light sensor experiments (RU1012) 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 medium for 10 cell divisions in the dark or under 0.080 W/m² 532-nm light as described previously. Miller assays were conducted to determine the abundance of β-galactosidase per cell under each condition. Dark-exposed bacteria produced 24.7 ± 1.3 Miller units (M.U.), while those exposed to green light produced 50.7 ± 3.1 M.U. (Fig. 2a, n = 4).

To determine whether the *E. coli* green light sensor functions as previously demonstrated *in vitro,* cells were then exposed to inactivating red light. Exposure to 0.080 W/m² 650-nm light resulted in a slight reduction in β-galactosidase levels as compared to dark-grown cells (Fig. 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 showed no response to green or red light (Fig. 2a). These gene expression data agree with *in vitro* assays indicating that in *E. coli,* the bilin-ligated (holo) form of CcaS is produced in the inactive green light-absorbing state (P₁) and is activated by green light and repressed by red light in a PCB-dependent manner.

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 Fig. 2a, expression of β-galactosidase was induced only in areas of green light (Fig. 2b). Because CcaS adopts the inactive ground state in the dark, the rates of phosphotransfer to CcaR and resulting transcription of lacZ were low in dark-exposed...
areas of the plate. However, in green light-exposed areas, CcaS kinase activity increased, increasing the abundance of β-galactosidase and the cleavage of its chromogenic black substrate in the medium (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 (Fig. 2b).

Construction of a red light-activated genetic circuit

Transcription from the output promoter of the previously constructed red light sensor (PompC) is inversely proportional to the intensity of red light. 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 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 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.

Characterization of spectral transfer functions

The transfer function describes the quantitative relationship between the input and output of a genetic circuit. 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 430 nm and 600 nm.

Fig. 1. Engineered two-color light induction system in E. coli. (a) Schematic representation of the system. The green sensor and chromophore biosynthetic pathways are as described in the main text. The red light-sensing protein Cph8 is expressed from the PompC promoter in the phosphorylated ground state. It is switched to the unphosphorylated state by 650-nm light and back to the phosphorylated state by 705-nm light. When phosphorylated, Cph8 passes a phosphoryl group to OmpR, which then binds to and activates transcription from PompC. 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 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.
For each wavelength in Fig. 2c, high levels (0.080 W/m²) of the respective wavelength were applied. In agreement with in vitro measurements of the absorbance of the CcaS holoprotein, the green light sensor shows transcriptional activation between 490 and 570 nm, with a maximum response near 535 nm. There is very little induction in 610 nm (orange) and the sensor is inactive in 650 nm (red) light. By contrast, the red sensor is strongly induced in the 610–650-nm range. As expected, the red sensor is inactive in the far-red region (730 nm). The red sensor also has a long tail into the blue regions of the spectrum, although the magnitude of the response decreases significantly below 610 nm.

Fig. 2 (legend on next page)
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 (Fig. 1 and Materials and Methods). The light intensity transfer functions of the three sensor combinations (green only, red only, and both) were then determined for 532-nm and 650-nm inputs. Cells expressing the green sensor show sharp activation between dark and 0.01 W/m² 532-nm light, above which point the response saturates (Fig. 3a). By contrast, bacteria expressing the red sensor show a small linear response to 532-nm light between dark and 0.080 W/m². Cells expressing both sensors have a transfer function very similar to that of cells expressing the green sensor alone, although the total Miller unit output is slightly lower (Fig. 3a). *E. coli* expressing only the red sensor are continuously induced by 650-nm light between dark and 0.01 W/m², after which point the response largely saturates (Fig. 3a). The shape of the red sensor transfer function to 650-nm light is similar to that of the green sensor to 532-nm light, and both sensors respond over similar light intensities. While cells expressing only the green sensor are slightly repressed by 650-nm light, the addition of the red sensor causes the cells to be induced by a transfer function similar to cells expressing the red sensor alone (Fig. 3a).

The transfer functions of the red and green sensors are nonadditive when combined in a single cell. For example, the decrease in Miller unit output of the green sensor in 650-nm 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 650-nm light (Fig. 3a). There are numerous direct or indirect interactions that could cause such nonadditivity. 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. Nonspecific effects such as competition for ribosomes or protein degradation machinery 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 phycobacterio- and constructing synthetic signaling pathways in bacteria in general.

The data in Fig. 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/m², just above the saturation point of the green sensor, so as not to trigger unwanted induction of the red light sensor (Fig. 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 by both colors of light (Fig. 3b).

The ribosome binding site upstream of lacZ in the red sensor was engineered to be weak (Supplementary Information), resulting in ~30-fold lower β-galactosidase output from the red sensor as compared to the green sensor (Figs. 2a and 3a). On plates, this causes green light-exposed areas to appear darker than red light-exposed areas (Fig. 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 nonadditivities that occur when the sensors are combined, the data in Fig. 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 coexpression 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. 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 the 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, 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\textsubscript{g} or P\textsubscript{r}). Picoseconds after absorption of the activating photon, isomerization of FCB drives a conformational rearrangement of the surrounding protein, which occurs on the order of milliseconds to seconds.\textsuperscript{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.\textsuperscript{25} When phosphorylated, the response regulator binds its cognate promoter and induces transcription. This occurs in minutes but can take on the order of 1 h to reach steady state.\textsuperscript{27} The light sensors should therefore allow reversible control of transcription on the minutes time scale. 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, although these systems do not have the benefit of reversibility.

Modern optical methods such as two-photon excitation or digital micromirror devices allow the projection of light patterns at subcellular resolution. If combined with such optics\textsuperscript{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 (Fig. 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 490 nm (Fig. 2c). Despite these overlaps, there are a variety of strategies for achieving independent control of the four sensor states. For example, intensities of 532-nm light less than ~0.01 W/m\textsuperscript{2} activate the green sensor while leaving the red sensor inactive (Fig. 3a). Far-red light (730 nm) can be applied concomitantly with 532 nm to inhibit the red sensor while activating the green sensor. To activate both sensors simultaneously, high levels (0.08 W/m\textsuperscript{2} or greater) of an intermediate wavelength such as 575 nm can be used (Fig. 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.\textsuperscript{28–30} 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.\textsuperscript{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 also be used to filter the responses of the existing light sensors. For example, a bistable genetic switch could be placed between\textsuperscript{27} the light sensors and the output genes. Bistable circuits cannot rest in intermediate output state but switch digitally from low to high output in response to continuous changes in input signal.\textsuperscript{32} Because the responses of the two light sensors decrease symmetrically with distance from the maximal inducing wavelength (Fig. 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 (Fig. 3a). Gradients of light can therefore be used to set different transcription levels across space in solid-phase experiments (Supplementary Fig. S1). 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.\textsuperscript{34} The ability to measure two-dimensional transfer functions in a single step could prove very useful in both systems\textsuperscript{34} and synthetic biology studies.\textsuperscript{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.\textsuperscript{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 ccsA-ccr cluster (chromosomal position 3399457–3405249) was amplified with the primers TACTA-GACTAGACTAGATGACGATCCATGAC-TAGACGATCGAGCTCCTAGCAGCGCAATGG
and TGTCAATGATCTGCAATTGACTGCTACTCT- 
TTGCCAC from purified genomic DNA and cloned into the 
pProTetE33 backbone (Clontech, Mountain View, CA) at 
the AatII and XbaI sites (underlined). The use of these sites 
removes the P1tet-O1 promoter, ribosome binding site, 6× 
His tag, and MCS while leaving the downstream transcription 
terminal. The endogenous Synechocystis promoters 
are therefore responsible for the expression of CcaS and 
CcrR in this plasmid. pJT116 was maintained with 34 μg/
ml chloramphenicol.

Construction of pJT118

The green light-inducible cpcG2 open reading frame in 
pJT116 was seamlessly replaced with lacZ using the 
MEGAWHOP protocol.54 lacZ was amplified from pEX- 
PlicZ (Invitrogen, Carlsbad CA) with the primers GATA- 
TACGTACGGGCCC and CCCCTTAGACCTAGGGCGTTCGGCTGCGG- 
TTGGCCTGGCAG and CACTTATAGATTTTGTCAGCCTTCAGCTTGGCTT- 
TACCCTTCTTTTGTCATGCCC (promoter and 
reading regions are underlined). This megaprimer was then used to extend
pJT116 in a MEGAWHOP to generate pJT106b. pJT106b was 
underlined). This megaprimer was then used to extend
pJT106b in a MEGAWHOP to generate pJT106b. pJT106b 
was maintained with 50 μg/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 
PLPCB39 carrying a spectinomycin resistance marker was 
constructed. To this end, the spectinomycin resistance 
cassette (including promoter, ribosome binding site, and 
specR gene) were amplified from plasmid pKD13 
using the primers AGAGCCTAGACATAGACATAGAATA- 
TAGTACGCGCCCAAGCAAGGAGCGAATCCGC and 
TATATTTTGACTTATACATCTATGGGGCC- 
TAGCTCTTTTGGCGAATACCTTGCC (primer binding 
sites are underlined) and cloned into pPLPCB using 
ApaI and SacI, which remove the kanamycin resistance 
cassette. pPLPCB(S) was maintained with 100 μg/ml 
spectinomycin.

Bacterial strains

Strain JT2 (RU1012 ΔompC-lacZ) was used for all experi-
ments. JT2 was constructed using the Datsenko–Wanner 
method40 to knock out the region of the RU1012 genome 
within which the lacZ gene was fused to the ompC open 
reading frame.55 The entire knocked out region contains, in 
order, the ompC promoter followed by the first 779 bp of the 
ompC gene, a translational fusion between the first 177 bp of the E. coli 
tryptophan synthase z subunit and lacZ, lacY, a truncated 
lacZ, and a second copy of the ompC promoter 
and the lacY gene, a translational fusion between the first 177 bp of the E. coli 
tryptophan synthase z subunit and lacZ, lacY, a truncated 
lacZ, and 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 marker. Although the embedded kanamycin 
resistance marker used to make this fusion was deleted in 
this step, the strand maintained resistance to kanamycin at 
50 μg/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 
50 μg/ml kanamycin for all experiments. The primers used 
to generate the knockout PCR fragment were

GAAAAATTTATGCTTTGAGTCTACTCCATCTGCAAAATATTTAAAAAGTGTGTTTTGCGTATGCCC (promoter and 
ph8 binding sequences are underlined). This PCR product was then used as a megaprimer in a 
MEGAWHOP reaction to clone the cassette downstream 
of the ccrR transcription terminator in pJT118. pJT118 was 
maintained with 34 μg/ml chloramphenicol.

Construction of pJT122

An expression cassette for the red light-responsive 
cph1/envZ chimera cph81 was added to the green light 
reporter plasmid pJT118 to generate plasmid pJT122. The 
P1tet-O1 promoter, ribosome binding site, and ph8 open 
reading frame were amplified using the primers 
GCCCTAGACCAGGGCGTTCCGCTCGGCCG- 
GAGCGGTATCA (underlined). pJT118 was seamlessly replaced with 
a 17-bp spacer, and the 
open reading frame was replaced with a consensus TTGACA site, 
and the start codon was replaced with a 6× His tag and MCS, 
leaving the downstream transcriptional terminator. The endogenous Synechocystis promoters 
are therefore responsible for the expression of CcaS and 
CcrR in this plasmid. pJT116 was maintained with 34 μg/
ml chloramphenicol.

Construction of pJT106b

pJT106b encodes a red light inter-WTer circuit driving a 
lacZ reporter gene. pJT106b is derived from pJT106, which 
carryes the PompC promoter Bba_R0082 driving the cl gene, 
the product of which represses the LuxR+3OC_hsl- 
activating, CI-repressed output promoter Bba_R0065.14 In 
pJT106b, R0065 is replaced by a LuxR+3OC_hsl-inde- 
pendent CI repressible promoter, Bba_J6467. To make 
J6467 luxR+3OC_hsl independent (and increase the 
overall transcription rate), the weak ~35 site of R0065 
(3TTACG) was replaced with a consensus TTTAGA site, 
the suboptimal 16-bp spacer between the ~35 and ~10 sites 
was replaced with a 17-bp spacer, and the ~16 nucleotide 
sequence was swapped from T to G. A megaprimer encoding these 
mutations was generated by amplifying the R0065 
region of pJT106b with the primers CGTACAGGTGGA- 
CACAAGAAAAATGCTTGTTATATC and CAT- 
TAAATGTGAGCAGTAACAAGCC (mutations are
in 1 mL of fresh LB (Lennox)+0.1 M Hepes (pH 6.6)+
appropriate antibodies, grown for 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 described before14 using the following band-
pass filters (Edmund Optics, Barrington NJ): 430 nm
NT43-160, 488 nm NT43-168, 532 nm NT43-174, 568 nm
NT43-179, 610 nm NT43-183, 650 nm NT43-189, and 730
nm NT43-195. Replicates were grown in parallel on a
darkened room with a Canon EOS Rebel SLR camera with
a macro lens and hood. Image levels, tone, contrast, 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 an EPP2000 UVN-SR
calibrated spectroradiometer (Stellarnet, Tampa, FL)
with a collection window ±30 nm from the reported
(peak) wavelength. The bandpass filters used in these
experiments have 10-nm transmission windows centered
on the peak emission wavelength.

**Plate assays**

Plate assays were conducted as described previously14
except that starter cultures were grown overnight in
unbuffered LB broth + appropriate antibiotics. The light
exposure step was carried out for 21 h, except in the case of
red sensor-only cells carrying the weak lacZ
binding site (plasmid pJT106b3), in which case, light
exposure was carried out for 48 h to allow the accumu-
lation of more black pigment.

The two-color mask used in Fig. 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 chilies were then
enhanced using Adobe Photoshop. Although the 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
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 spectro-
radiometer as described earlier.

**Imaging of 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.

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**Supplementary Data**

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jmb.2010.10.038.

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