

Benchmarking of Optical Dimerizer Systems

Gopal P. Pathak,[†] Devin Strickland,^{‡,§} Justin D. Vrana,[†] and Chandra L. Tucker^{*,†}

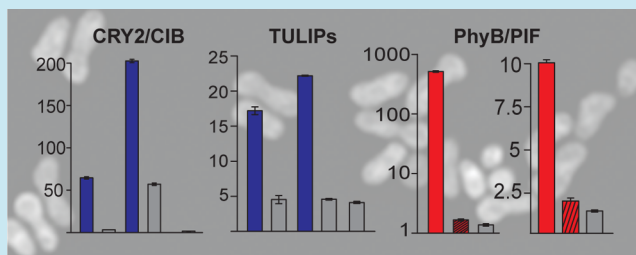
[†]Department of Pharmacology, University of Colorado School of Medicine, Aurora, Colorado 80045, United States

[‡]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637, United States

S Supporting Information

ABSTRACT: Optical dimerizers are a powerful new class of optogenetic tools that allow light-inducible control of protein–protein interactions. Such tools have been useful for regulating cellular pathways and processes with high spatiotemporal resolution in live cells, and a growing number of dimerizer systems are available. As these systems have been characterized by different groups using different methods, it has been difficult for users to compare their properties. Here, we set about to systematically benchmark the properties of four optical dimerizer systems, CRY2/CIB1, TULIPs, phyB/PIF3, and phyB/PIF6. Using a yeast transcriptional assay, we find significant differences in light sensitivity and fold-activation levels between the red light regulated systems but similar responses between the CRY2/CIB and TULIP systems. Further comparison of the ability of the CRY2/CIB1 and TULIP systems to regulate a yeast MAPK signaling pathway also showed similar responses, with slightly less background activity in the dark observed with CRY2/CIB. In the process of developing this work, we also generated an improved blue-light-regulated transcriptional system using CRY2/CIB in yeast. In addition, we demonstrate successful application of the CRY2/CIB dimerizers using a membrane-tethered CRY2, which may allow for better local control of protein interactions. Taken together, this work allows for a better understanding of the capacities of these different dimerization systems and demonstrates new uses of these dimerizers to control signaling and transcription in yeast.

KEYWORDS: optogenetics, light control, photoreceptor, phytochrome, cryptochrome, LOV domain, transcription



Optical dimerizers represent an emerging class of tools that allow control of cellular processes with light. These tools are similar in concept to well-established chemical dimerizer reagents, in that they provide a means to inducibly control protein–protein interactions, but use light rather than a small molecule for activation. As light can be delivered immediately with subcellular resolution, such tools allow exquisite spatial, temporal, and dose-dependent control of biological events. The basis of these tools is an interaction between two proteins or domains where one of the interacting partners is a photo-sensory protein or domain that interconverts between two states upon light excitation. The second protein or domain binds to the first in only one of the two states. If photostimulation is not maintained, all photosensory proteins naturally revert to their ground states over time (which varies from seconds to hours depending on the system), and thus, the binding interactions are naturally, and in some cases inducibly, reversible.

At present, several optical dimerization systems have been described based on different light-sensing domains of plants: phytochromes, cryptochromes, LOV domains, and UVR8.^{1–9} Two different systems based on *Arabidopsis* phytochrome B have been developed: phyB/PIF3¹ and phyB/PIF6.² In darkness or under far-red illumination (~730 nm), phyB exists in a Pr (red-light-absorbing) state that is unable to bind PIF family proteins, while absorption of red light shifts phytochrome into a Pfr far-red-light absorbing state that allows

binding. The phyB/PIF3 system has been used to control transcription^{1,10,11} and intein splicing¹² in yeast, but to date, it has not been successful for controlling interactions in mammalian cells, while the phyB/PIF6 dimerizers have been used to control protein localization and activity in mammalian cells and yeast.^{2,13–16} Naturally occurring blue-light-regulated dimerizers include those based on cryptochromes (*Arabidopsis* CRY2/CIB1)⁴ and LOV domains (*Arabidopsis* FKF/GIGANTEA).³ Dimerizers sensitive to UV light have also been developed, based on an *Arabidopsis* UVR8-UVR8 interaction⁶ or UVR8/COP1.^{7,8} While the above systems are based on natural plant protein interactions, engineered systems include one derived from the fluorescent protein Dronpa¹⁷ and several LOV-based systems, TULIPs, LOV-ipa, and LOV-SsrA.^{5,9}

Although different optical dimerizer systems can be used for similar purposes, they each have specific properties that may be advantageous for certain applications. Although some of the dimerization systems have been characterized in similar assays, there has been no systematic side-by-side comparison and it can be difficult for users to compare the different properties of each system. In this work, we set out to benchmark the properties of four different optical dimerizer systems, PhyB/PIF6, PhyB/

Special Issue: Synthetic Photobiology

Received: August 4, 2014

Published: October 28, 2014

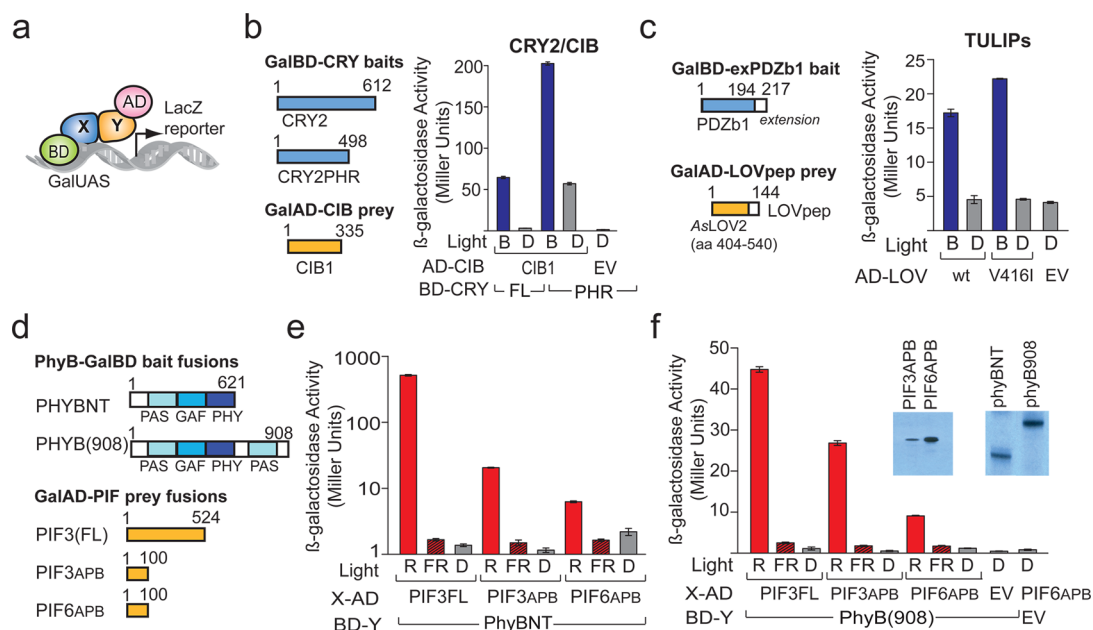


Figure 1. Comparison of optical dimerizer systems for control of DNA transcription. Yeast expressing Gal4BD-X and Gal4AD-Y fusions (where X and Y represent the light-interacting proteins) were tested by yeast two-hybrid with a β -galactosidase reporter (schematic shown in part a) after a 4 h incubation under different light conditions (D, dark; R, red light, 60 s pulse every 30 min, 2.8 mW/cm², 660 nm; FR, far-red light, 60 s pulse immediately after each red light exposure, 6.9 mW/cm², 730 nm; B, blue light, 700 ms pulse every 12 s for TULIPS and 1 s pulse every 3 min for CRY2/CIB, 5.8 mW/cm², 461 nm). EV indicates empty vector (Gal4BD or Gal4AD as indicated) control. Shown are constructs used and β -galactosidase reporter activity of yeast expressing (b) Gal4BD-CRY2 (FL or PHR) and GalAD-CIB1, (c) BD-ePDZb1 and AD-LOVpep (unmodified (“wt”) or V416I), (e) PhyBNT-GalBD and full length PIF3 (PIF3FL), the APB domain of PIF3 (aa 1–100, PIF3APB), or the APB domain of PIF6 (aa 1–100, PIF6APB). (f) PhyB(1-908)-GalBD (PhyB908) and PIF versions as in part e. Inset: Immunoblot analysis of GalAD and GalBD fusion proteins expressed in yeast. Schematics for constructs used in phytochrome experiments are shown in part d.

PIF3, CRY2/CIB, and TULIPS, side-by-side in the same assay, allowing quantitative evaluation of the light responses of each system. To compare the properties of the four systems, we used a yeast transcriptional assay. We further examined the blue light regulated systems, CRY2/CIB and TULIPS, for ability to regulate a yeast MAPK signaling pathway. Importantly, we demonstrate successful use of the CRY2-CIB technology for membrane recruitment using a membrane-bound CRY2 protein. This approach will allow more precise local control of intracellular processes, as compared with prior approaches in which CRY2 was cytosolic. Finally, we also describe an improved system for regulating transcription with blue light in yeast using CRY2/CIB dimerizers.

RESULTS AND DISCUSSION

Analysis of Optical Dimerizers Using a Yeast Transcriptional System. We used a split Gal4-based yeast two-hybrid assay to compare the ability of cryptochrome (CRY2/CIB),⁴ phytochrome (phyB/PIF3 and phyB/PIF6),^{1,2} and LOV domain (TULIPS)⁵ based dimerizer systems to regulate transcription with light (Figure 1). In this assay, the dimerizers bring together split halves of a transcription factor (separated into a binding domain, BD, and an activation domain, AD), leading to light-dependent induction of transcription of a β -galactosidase reporter (Figure 1a). As was previously reported,⁴ GalBD-CRY2 and GalAD-CIB1 yielded low levels of background in the dark but robust activation with light application, while a truncated CRY2PHR domain (residues 1–498 of CRY2) gave higher light-stimulated activity, but with significantly increased background in the dark (Figure 1b).

We examined the LOV-based TULIP system in the same assay, using identical vectors and light conditions. TULIPS consist of an *Avena sativa* LOV2- $\text{J}\alpha$ -helix domain with a peptide tag at the C-terminus of the $\text{J}\alpha$ -helix (LOVpep), which binds upon light excitation to an engineered erbin PDZ (ePDZ) domain.⁵ We tested LOVpep and two additional variants: a mutant (“3x-A”; T406A/T407A/I532A) that increases $\text{J}\alpha$ -helix docking to LOV2, and a V416I variant that maintains the lit state for an extended time ($t_{1/2}$ –370 s).⁵ Each of these was tested for interaction with a high affinity ePDZ domain, ePDZb1.⁵ We first generated GalBD-LOVpep and GalAD-ePDZb1 fusions, but yeast expressing GalAD-ePDZb1 showed severe toxicity phenotypes (very poor growth and deformed cells). During cloning, we identified a spontaneous frameshift mutant, exPDZb1, that extended the amino acid sequence beyond the ePDZb1 stop codon and suppressed the severe toxicity, but yeast expressing exPDZb1 in the GalAD vector still showed slow growth (Supporting Information Figure S1). Use of exPDZb1 in the lower-expressing GalBD vector (“GalBD-exPDZb1”) allowed normal yeast growth, and we used this configuration for all studies. While toxicity was not observed with the original TULIP system in either yeast or mammalian cells,⁵ the ePDZ constructs in that study also contained a C-terminal extension (containing a multiple cloning site linker), which extended the amino acid sequence beyond the PDZ domain.

Figure 1c shows interaction between wild-type or long-lived (V416I) LOVpep with exPDZb1. While we did not observe a substantial difference between LOVpep ($t_{1/2}$ ~ 30 s) or V416I LOVpep ($t_{1/2}$ ~ 370 s) when frequent pulses of light (every 12 s) were applied (Figure 1c), with less frequent light pulses we observed a reduction in light-dependent reporter activation

with wild-type compared with V416I (Supporting Information Figure S2a). The ("3xA") mutant showed poor light-dependent induction (Supporting Information Figure S2a) and was not further characterized. We observed background reporter activity in the dark that was also seen when GalBD-exPDZb1 was coexpressed with an empty vector control, and thus, it was not presumed to be due to dark state binding (Figure 1c). Indeed, when we switched the BD and AD fusion partners (generating GalBD-LOVpep and GalAD-exPDZb1) the reporter activation in the dark was eliminated (Supporting Information Figure S2b).

We next examined the interaction between *Arabidopsis* phyB and PIF3 or PIF6 (Figure 1d–f). Previously, a N-terminal fragment of phyB (phyBNT, residues 1–621) and PIF3 were used for light-inducible control of transcription in yeast,¹ while phyB908 (residues 1–908) and the APB domain of PIF6 (PIF6APB, residues 1–100) were used to regulate protein localization and function in mammalian cells.² Light sensitivity of phyB requires binding to a chromophore, phytochromobilin, or phycocyanobilin (PCB), that is not present in yeast or mammalian cells; thus, cells were supplemented with 10 μ M PCB. As previously demonstrated,^{1,11} we observed strong light-dependent induction of reporter activity using phyBNT-GalBD and full length PIF3 (GalAD-PIF3), with essentially no background activity in the dark (Figure 1e). Substitution of full length PIF3 with the APB domain (PIF3APB, residues 1–100), or substitution of phyBNT with a longer construct, phyB(908) (amino acids 1–908 of phyB) (Figure 1f) greatly reduced activity, suggesting these interactions have reduced affinity. The PhyB(1-908)/PIF6APB combination used to regulate activity and localization in mammalian cells² also showed reduced levels of activity compared with phyBNT/PIF3 (Figure 1f). With both forms of phyB, use of PIF3APB resulted in higher levels of reporter activity than PIF6APB despite lower expression, suggesting that substitution of PIF3APB for PIF6APB as a dimerizer module in mammalian cells could be preferable. In all cases, interaction was dependent on red light and reversed by far-red illumination.

Our preliminary studies suggested that dim broad-spectrum light did not excite CRY2 and TULIP sample preparations but could stimulate phyB. To quantitatively evaluate light sensitivity of each dimerizer system, we tested different room illumination conditions (Figure 2). In addition to testing dim ambient light

(equivalent to a dark closet with a door ajar, 590 μ W/cm²), we also tested exposure to bright room light (samples placed directly underneath a broad-spectrum fluorescent light source, 5120 μ W/cm²), to evaluate whether easily available fluorescent light sources could substitute for wavelength-specific LED lights that we typically use.¹¹ Both the CRY2 and LOV systems could be stimulated to the same degree by bright fluorescent room light as by wavelength-specific LEDs and were relatively insensitive to dim ambient light (Figure 2a). In contrast, room light did not activate phyB nearly as well as a red LED array, likely due to the presence of far-red light in the broad-spectrum fluorescent bulbs. PhyB was exquisitely sensitive to even dim light, which induced reporter activity to nearly the same amount as with bright room light (Figure 2a). To determine safe light conditions that would not activate phyB, we tested different levels of fluorescent or wavelength-specific LED light for stimulation of the PhyBNT/PIF3 interaction (Figure 2b). Even very dim room light that would not be sufficient for performing experimental manipulations (100 μ W/cm²) was sufficient to significantly activate phytochrome, while a dim blue LED (180 μ W/cm²) did not stimulate reporter induction.

We carried out a more detailed assessment of cross-reactivity of the different systems to different lighting conditions and also assessed crossreactivity with mismatched components (Supporting Information Figure S3). We tested CRY2 and TULIP (exPDZb1) baits for growth under red light and also for crossreactivity with PIF components. Each bait showed specific interaction under blue light with its counterpart bait, with no interaction under red light and no crossreactivity with other AD-fused preys. We did not test the phyB bait for stimulation by bright blue light, but note that any blue light stimulated phyB could be reverted by subsequent application of far-red light.

Generation of an Optimized Blue Light Regulated Transcription System. To develop a more robust blue light regulated transcriptional system comparable with the phyB/PIF3 system, we optimized the CRY2/CIB transcription system. We fused CRY2 to the LexA DNA binding domain (LexA-CRY2) and CIB1 to a stronger VP16 activation domain (VP16-CIB1) and examined activity of a LexA(8xop)-lacZ reporter, pSH18-34 (Figure 3). While background activity in the dark was minimal, reporter activity was stimulated \sim 100-fold with blue light application (Figure 3b). This construct shows the largest fold transcriptional activation in a blue-light stimulated system for yeast to date and should be useful for precise control of protein expression levels in any yeast strain and without the requirement for exogenous ligand.

Light-Inducible Activation of a MAP Kinase Pathway. The results from the transcriptional assays showed similar responses for TULIPs and CRY2/CIB dimerizer systems. We further examined these blue light regulated systems by comparing light induction in a completely different application, the yeast mating response MAP kinase pathway. The yeast MAP kinase pathway involved in mating response is a stereotypical G-protein coupled receptor signaling pathway that is initiated by ligand (α -factor) binding to a seven-transmembrane receptor coupled to a G-protein, Gpa1. Gpa1 activation results in recruitment of a scaffold protein, Ste5, to the plasma membrane, initiating a MAP kinase phosphorylation cascade leading to transcriptional changes and cell cycle arrest. Prior studies have demonstrated that simply recruiting Ste5 to the plasma membrane is sufficient to bypass the requirement

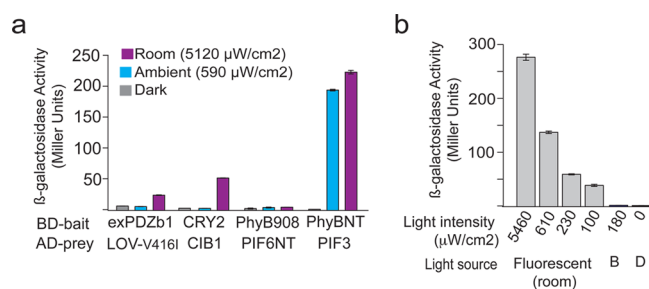


Figure 2. Light sensitivity of optical dimerizer systems. (a) Response of light-activated transcriptional systems to ambient or bright room light. Yeast expressing indicated GalBD (X-BD or BD-X) or GalAD (AD-Y) fusions were incubated under indicated room light for 4 h and assayed for β -galactosidase reporter activity. (b) Sensitivity of PhyB-PIF3 system to different room light conditions. Yeast expressing PhyBNT-GalBD and GalAD-PIF3 were incubated for 4 h in the dark ('D') or in the presence of fluorescent room light or blue light ('B', 461 nm) at indicated light intensities.

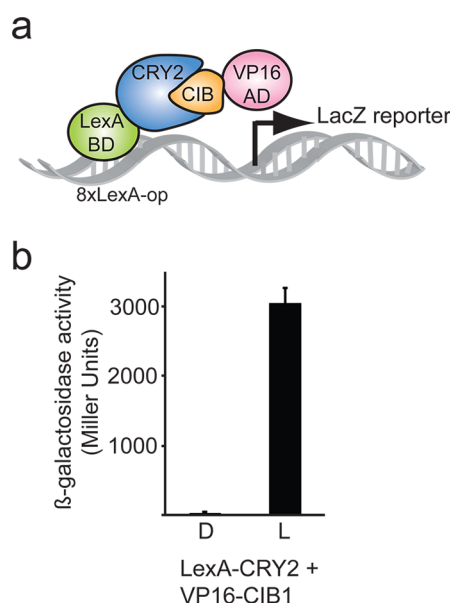


Figure 3. Optimized blue light transcriptional system. (a) Schematic showing indicated constructs used for the yeast transcriptional system. (b) β -galactosidase reporter activity of AH109 yeast expressing LexA-CRY2, VP16-CIB1, and a pSH18-34 reporter plasmid after 3 h incubation in blue light or dark.

for GPCR activation, resulting in MAP kinase pathway activation in the absence of α -factor stimulation.¹⁸

Previously, the TULIP dimerization system was used to induce translocation of the MAP kinase scaffold Ste5 to the plasma membrane, resulting in tight light control of the mating response in haploid yeast.⁵ In that work, the photoactivatable LOVpep was tethered to GFP, and targeted to the plasma membrane via fusion to the single-pass transmembrane protein Mid2, or to a version of Mid2 consisting of only the signal sequence and transmembrane helix, Mid2(SS/TM). Using a

similar approach, we fused CRY2-GFP or CRY2PHR-GFP to full length Mid2 or Mid2(SS/TM) (Figure 4a). We coexpressed the CRY2-Mid2 fusion constructs with CIB1-Ste5 or CIBN-Ste5 and assessed activation of the pathway using a P_{FUS1} -DsRed reporter. We found that CIBN-Ste5 was incapable of fully activating the pathway, as were CRY2 constructs tethered to the truncated Mid2 protein, which had been functional with the TULIP system (Supporting Information Figure S4). The highest lit-state activation and greatest fold activation were obtained using CRY2PHR fused to full-length Mid2 and CIB1-Ste5 (Supporting Information Figure S4 and Figure 4b,c). Surprisingly, use of CRY2PHR resulted in lower background (dark activity) than full length CRY2, a result that is in contrast with the transcriptional results in Figure 1b, in which use of CRY2PHR resulted in much higher background activity.

We directly compared the CRY2PHR/CIB1 combination with the corresponding TULIP constructs, as well as with α -factor mediated pathway activation (Figure 4b,c). Both CRY2/CIB and TULIPs showed higher dark-state pathway activation than control cells, although the dark-state activity was substantially lower for CRY2/CIB than for TULIPs. With both systems, the lit-state activation was essentially identical to α -factor-treated control cells. As seen in the transcriptional activation assays, CRY2/CIB and TULIPs have a comparable photoswitching dynamic range, although both systems require considerable context-dependent adjustment to avoid high dark-state background (always on) or low-lit state activation (always off).

Discussion. Here, we directly compared the properties of four optical dimerization systems, CRY2/CIB, TULIPs, phyB/PIF3, and phyB/PIF6. We find the phyB/PIF3 system shows significant advantages over the CRY2/CIB, TULIPs, and phyB/PIF6 systems for yeast transcriptional control, with higher fold levels of activation, extremely low background, and far-red reversible control. This system also shows exquisite light sensitivity, and care must be taken when working with these

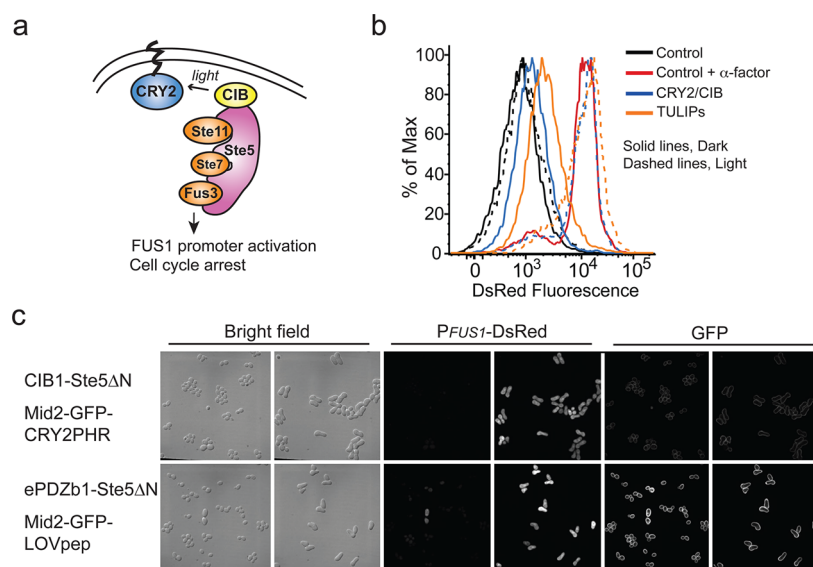


Figure 4. Comparison of CRY2/CIB and TULIP systems for activating a yeast map kinase pathway. (a) Schematic of dimerizer constructs. (b) Graph reporting DsRed fluorescence of cell populations expressing a P_{FUS1} -DsRed reporter gene and Mid2-GFP-CRY2PHR and CIB1-Ste5 Δ N(CRY2/CIB), Mid2-GFP-LOVpep and ePDZb1-Ste5 Δ N(TULIPs), control cells bearing only the reporter gene (control), or control cells stimulated with α -factor (control + α -factor). Samples containing dimerizers were either stimulated with light (solid lines) or left in the dark (dashed lines) for 5 h. (c) Images of yeast expressing CRY2/CIB or TULIP constructs as in part b.

constructs to avoid ambient light exposure. Surprisingly, we found the phyB/PIF6 system, which has worked well for control of protein localization in mammalian and yeast cells,^{2,14–16} to show much poorer ability to activate transcription in yeast compared with the robust phyB/PIF3 system. While the phyB/PIF3 system is superior for regulation of yeast transcription, a limitation is the requirement for exogenous chromophore, although notably a biosynthetic pathway for generating this chromophore has been reconstituted for mammalian cells, providing a major advance for users of phytochrome.¹⁹

The two blue light regulated systems, CRY2/CIB and TULIPs, showed similar levels of activity, light sensitivity, and fold activation in the yeast transcriptional assay. We further compared these two systems for ability to stimulate MAP kinase pathway activation through plasma membrane recruitment of the scaffold protein Ste5. Both systems activated the MAP kinase pathway to a similar extent with light treatment. In dark, activity in both cases was minimal, although we found the CRY2/CIB system showed slightly lower background than TULIPs. In deciding between the two systems, the CRY2/CIB system may be preferable if background activity is a concern. On the other hand, the TULIP fusion tags are much smaller than CRY2/CIB, and thus may be preferable for use with proteins that do not tolerate large fusions. A final caution with use of TULIPs, when working with these constructs for regulation of yeast transcription, we observed some constructs showing significant toxicity (growth defects and misshapen cells) that had not been observed in prior studies.⁵ Addition of C-terminal extensions to the sequences, as occurred with a spontaneous mutation, alleviated toxicity. The prior-described TULIP constructs⁵ also contained C-terminal extensions, which may explain why no toxicity was observed in these studies. Alternatively, differences in localization may be a factor: the proteins in this study were localized to the nucleus, compared to the cytosol in prior experiments.⁵

We used the CRY2/CIB dimerizers to develop a more robust light-activated transcriptional system for yeast. This system shows low background, high fold-activation with light, and can be utilized in any yeast strain to drive expression under LexA operator control. We expect this system may be orthogonal to the phyB/PIF3 system, allowing the possibility for multiplex control with red and blue light. In developing this work, we also demonstrate successful placement of a membrane-tethered CRY2 that retains ability to interact with a cytosolic CIB1. In the original report of light-inducible dimerization using CRY2/CIB, attempts were made to tether CRY2 to the plasma membrane via fusion to a prenylated EGFP, but CRY2 was unable to interact with CIB in this configuration.⁴ A tethered CRY2 that can be locally photoactivated will be useful for focal activation of signaling molecules at the plasma membrane or other subcellular sites.

Another notable outcome of this study is the observation that the behavior of these constructs in different applications is quite context-dependent. When we compared use of full-length CRY2 with truncated CRY2PHR for activation of the MAP kinase pathway, we found CRY2PHR to result in significantly reduced background activity. In contrast, we observed the opposite results with the transcriptional studies. Domain configuration, biological context, and construction details appear to have profound and unpredictable effects on photoswitching. Thus, users interested in conferring optical control to specific targets are advised to test not only different

optical dimerizer systems but also different configurations (i.e., N- vs C-terminal fusions), different truncations, and different linker lengths, as these may be important for optimal activity.

The past few years have led to a flurry of publications of new optical dimerizer systems, and additional protein modules and improved systems are steadily being added to the optical dimerizer toolset. We envision the assays described here may be useful for benchmarking these new systems as they become available, allowing users to gain a better understanding of their background, light sensitivity, fold-activation, and other quantitative parameters.

METHODS

Strains and Plasmids. Yeast strains used for two-hybrid assays were AH109 (*MAT α* , *trp1-901*, *leu2-3*, *112*, *ura3-52*, *his3-200*, *gal4 Δ* , *gal80 Δ* , *LYS2::GAL1_{UAS}-GAL1_{TATA}-HIS3*, *GAL2_{UAS}-GAL2_{TATA}-ADE2*, *URA3::MEL1_{UAS}-MEL1_{TATA}-lacZ*, *MEL1*), and Y187 (*MAT α* *ura3-52*, *his3-200*, *ade2-101*, *trp1-901*, *leu2-3*, *112*, *gal4 Δ* , *gal80 Δ* , *met-*, *URA3::GAL1_{UAS}-GAL1_{TATA}-lacZ*, *MEL1*) (Clontech). The yeast strain used for MAP kinase assays was S288c *MAT α* *PFUS1-DsRed-Max::TRP1*. For two-hybrid experiments, each photoreceptor domain was fused to a Gal4 binding domain (Gal4BD) and expressed in yeast strain AH109 (Clontech). phyBNT-GalBD (containing amino acids 1–621 of phyB fused at the N-terminus of the Gal4 DNA binding domain) in plasmid D153 and pGAD424-PIF3 were gifts from Peter Quail. To generate phyB908, D153-phyBNT was digested with Not I and phyBNT was removed and replaced by phyB(1-908) via homologous recombination, generating a fusion of phyB908 at the N-terminus of GalBD. LOVpep variants, ePDZb1, and CRY2 variants used in bait constructs were fused at the C-terminus of Gal4BD in plasmid pDBTrp²⁰ via homologous recombination. The exPDZb1 construct contained a “LGIRPPAAKLIPGEFLMIYDFYY” extension at the C-terminus of the erbin PDZb1, which came from a spontaneous mutation generated during homologous recombination in yeast (a frameshift insertion mutation occurring between the penultimate coding residue and the stop codon) that extended the coding sequence into the vector. The GalAD-PIF6APB prey was cloned by homologous recombination into pGADT7rec (Clontech) cut with *Sma*I, generating a construct containing the Gal4 activation domain, a GSSLGSGSGSGSSD linker (used in ref 2), followed by residues 1–100 of PIF6. We also generated and tested a GalAD-PIF6APB construct in pGAD424, where PIF6 was inserted in place of full length PIF3 at *Eco*RI and *Bam*HI sites. This construct showed similar results as the pGADT7rec PIF6 construct (data not shown). The GalAD-PIF3APB prey was cloned by homologous recombination in pGADT7rec, at the C-terminus of the Gal4AD. To generate GalAD-LOVpep and variants, the LOVpep insert was removed from pDBTrp-LOVpep at *Eco*RI/*Bam*HI sites and ligated into pGADT7rec. GalAD-ePDZb1 was cloned by ligation in pGADT7rec at *Nco*I and *Bam*HI sites. exPDZb1 was cut out of pDBTrp at *Eco*RI/*Bam*HI sites and cloned by homologous recombination into pGADT7rec digested with *Eco*RI and *Bam*HI. Generation of GalAD-CIB1 and pDBTrp-CRY2 constructs were previously described.⁴ pSH18-34 (Life Technologies), containing 8xLexA operator sites driving expression of *lacZ*, was used as a reporter for the experiment in Figure 3. For MAP kinase experiments, CIB-Ste5 Δ N and ePDZb1-Ste5 Δ N constructs under GAL1 promoter control were integrated at the URA3 locus in the S288c derived line.

The Mid2-GFP-CRY and Mid2-GFP-LOVpep constructs under GAL1 promoter control were integrated at the LEU2 locus.

β -Galactosidase Assays. AD and BD plasmids were transformed into Y187 and AH109 strains, respectively. Yeast were mated on YPD plates followed by selection of diploids on SD-Trp/-Leu plates. Yeast were grown overnight in SD-Trp/-Leu medium, followed by dilution to 0.2 OD₆₀₀ in SD-Trp/-Leu medium the next morning. Following an initial 3 h growth period in the dark, cultures were either kept in the dark or exposed to a red or blue LED light source for 4 h. For experiments with phytochrome, 10 μ M PCB was added during the initial dilution to 0.2, as this concentration was determined sufficient for maximal activation with a 4 h incubation time. After light treatment, cultures were harvested in log phase and lysed with Y-PER reagent (Thermo Scientific) and assayed for β -galactosidase activity using a standard protocol (Clontech Laboratories, protocol #PT3024-1) using ONPG (Sigma-Aldrich) as a substrate. Miller Units were calculated based on the following formula: $1000 \times OD_{420}/(t \times V \times OD_{600})$.²¹ Experiments were carried out at least three times with similar results to those shown.

Yeast Growth Assays. LOVpep and PDZb1 were coexpressed as GalDBD (bait) or GalAD (prey) fusion proteins in AH109 \times Y187 yeast. Cells were grown to log phase then diluted to 0.3 OD₆₀₀ and optical density was measured at indicated times during incubation at 30 °C.

Immunoblotting. Cells were lysed at 4 °C in 2 \times Laemmli sample buffer by glass bead lysis. Proteins were separated by electrophoresis on an SDS-PAGE gel and transferred to nitrocellulose membranes, followed by probing with Gal4BD (sc-577) or Gal4AD (sc-1663) antibodies (Santa Cruz Biotechnology).

MAP Kinase Experiments. S288c MATa PFUS1-DsRedMax::TRP1 yeast expressing Ste5 and Mid2 fusions were grown overnight in YPD at 30 °C with shaking, then aliquoted into microtiter plates. Dark plates were kept in the dark, while light exposed plates were placed under a benchtop fluorescent light (for flow cytometry experiments) or exposed to blue LED lights for 5 h with shaking. Cells were centrifuged and resuspended in 1 mL PBS and analyzed by flow cytometry, or mounted on coverslips and imaged using a confocal microscope.

■ ASSOCIATED CONTENT

● Supporting Information

Supplementary Figures S1–S4. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: chandra.tucker@ucdenver.edu.

Present Address

§Matrix Genetics, Seattle, WA 98102, United States

Author Contributions

D.S. and C.L.T. conceived and initiated this work. G.P. carried out transcriptional comparison studies. D.S. carried out MAP kinase benchmarking studies. G.P. and J.V. generated the improved blue light activated transcriptional system. G.P., D.S., and C.L.T. wrote and edited the paper.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Michael Glotzer, University of Chicago, for providing funds, equipment, and lab space for carrying out the MAP kinase studies, and for providing helpful comments on the manuscript. This work was funded by grants from the National Institutes of Health (GM100225 to C.L.T. and GM088668 to Michael Glotzer, University of Chicago) and by an American Cancer Society Postdoctoral Fellowship to D.S. (119248-PF-10-134-01-CCG).

■ REFERENCES

- (1) Shimizu-Sato, S., Huq, E., Tepperman, J. M., and Quail, P. H. (2002) A light-switchable gene promoter system. *Nat. Biotechnol.* 20, 1041–1044.
- (2) Levskaya, A., Weiner, O. D., Lim, W. A., and Voigt, C. A. (2009) Spatiotemporal control of cell signalling using a light-switchable protein interaction. *Nature* 461, 997–1001.
- (3) Yazawa, M., Sadaghiani, A. M., Hsueh, B., and Dolmetsch, R. E. (2009) Induction of protein–protein interactions in live cells using light. *Nat. Biotechnol.* 27, 941–945.
- (4) Kennedy, M. J., Hughes, R. M., Peteya, L. A., Schwartz, J. W., Ehlers, M. D., and Tucker, C. L. (2010) Rapid blue-light-mediated induction of protein interactions in living cells. *Nat. Methods* 7, 973–975.
- (5) Strickland, D., Lin, Y., Wagner, E., Hope, C. M., Zayner, J., Antoniou, C., Sosnick, T. R., Weiss, E. L., and Glotzer, M. (2012) TULIPs: Tunable, light-controlled interacting protein tags for cell biology. *Nat. Methods* 9, 379–384.
- (6) Chen, D., Gibson, E. S., and Kennedy, M. J. (2013) A light-triggered protein secretion system. *J. Cell Biol.* 201, 631–40.
- (7) Crefcoeur, R. P., Yin, R., Ulm, R., and Halazonetis, T. D. (2013) Ultraviolet-B-mediated induction of protein–protein interactions in mammalian cells. *Nat. Commun.* 4, 1779.
- (8) Müller, K., Engesser, R., Schulz, S., Steinberg, T., Tomakidi, P., Weber, C. C., Ulm, R., Timmer, J., Zurbriggen, M. D., and Weber, W. (2013) Multi-chromatic control of mammalian gene expression and signaling. *Nucleic Acids Res.* 41, e124.
- (9) Lungu, O. I., Hallett, R. A., Choi, E. J., Aiken, M. J., Hahn, K. M., and Kuhlman, B. (2012) Designing photoswitchable peptides using the AsLOV2 domain. *Chem. Biol.* 19, 507–517.
- (10) Pittman, A. M., Lage, M. D., Poltoratsky, V., Vrana, J. D., Paiardini, A., Roncador, A., Cellini, B., Hughes, R. M., and Tucker, C. L. (2012) Rapid profiling of disease alleles using a tunable reporter of protein misfolding. *Genetics* 192, 831–842.
- (11) Hughes, R. M., Bolger, S., Tapadia, H., and Tucker, C. L. (2012) Light-mediated control of DNA transcription in yeast. *Methods* 58, 385–391.
- (12) Tyszkiewicz, A. B., and Muir, T. W. (2008) Activation of protein splicing with light in yeast. *Nat. Methods* 5, 303–305.
- (13) Müller, K., Engesser, R., Metzger, S., Schulz, S., Kämpf, M. M., Busacker, M., Steinberg, T., Tomakidi, P., Ehrbar, M., Nagy, F., Timmer, J., Zurbriggen, M. D., and Weber, W. (2013) A red/far-red light-responsive bi-stable toggle switch to control gene expression in mammalian cells. *Nucleic Acids Res.* 41, e77.
- (14) Toettcher, J. E., Gong, D., Lim, W. A., and Weiner, O. D. (2011) Light-based feedback for controlling intracellular signaling dynamics. *Nat. Methods* 8, 837–839.
- (15) Yang, X., Jost, A. P.-T., Weiner, O. D., and Tang, C. (2013) A light-inducible organelle-targeting system for dynamically activating and inactivating signaling in budding yeast. *Mol. Biol. Cell* 24, 2419–30.
- (16) Toettcher, J. E., Weiner, O. D., and Lim, W. A. (2013) Using optogenetics to interrogate the dynamic control of signal transmission by the ras/erk module. *Cell* 155, 1422–34.
- (17) Zhou, X. X., Chung, H. K., Lam, A. J., and Lin, M. Z. (2012) Optical control of protein activity by fluorescent protein domains. *Science* 338, 810–4.

(18) Pryciak, P. M., and Huntress, F. A. (1998) Membrane recruitment of the kinase cascade scaffold protein Ste5 by the $G\beta\gamma$ complex underlies activation of the yeast pheromone response pathway. *Genes Dev.* 12, 2684–97.

(19) Müller, K., Engesser, R., Timmer, J., Nagy, F., Zurbriggen, M. D., and Weber, W. (2013) Synthesis of phycocyanobilin in mammalian cells. *Chem. Commun. (Camb)* 49, 8970–2.

(20) Tucker, C. L., Peteya, L. A., Pittman, A. M., and Zhong, J. (2009) A genetic test for yeast two-hybrid bait competency using RanBPM. *Genetics* 182, 1377–1379.

(21) Miller, J. H. (1972) *Experiments in Molecular Genetics*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.