Spatiotemporal control of cell signalling using a light-switchable protein interaction

Anselm Levskaya1,2,3, Orion D. Weiner1,4, Wendell A. Lim1,5 & Christopher A. Voigt1,3

Genetically encodable optical reporters, such as green fluorescent protein, have revolutionized the observation and measurement of cellular states. However, the inverse challenge of using light to control precisely cellular behaviour has only recently begun to be addressed; semi-synthetic chromophore-tethered receptors1 and naturally occurring channel rhodopsins have been used to perturb directly neuronal networks2,3. The difficulty of engineering light-sensitive proteins remains a significant impediment to the optical control of most cell-biological processes. Here we demonstrate the use of a new genetically encoded light-control system based on an optimized, reversible protein–protein interaction from the phytochrome signalling network of Arabidopsis thaliana. Because protein–protein interactions are one of the most general currencies of cellular information, this system can, in principle, be generically used to control diverse functions. Here we show that this system can be used to translocate target proteins precisely and reversibly to the membrane with micrometre spatial resolution and at the second timescale. We show that light-gated translocation of the upstream activators of Rho-family GTPases, which control the actin cytoskeleton, can be used to precisely reshape and direct the cell morphology of mammalian cells. The light-gated protein–protein interaction that has been optimized here should be useful for the design of diverse light-programmable reagents, potentially enabling a new generation of perturbative, quantitative experiments in cell biology.

A quantitative understanding of living cells will require methods to perturb and control the activities of their constituent proteins at fine spatial and temporal resolutions. By measuring responses to precise perturbations, predictive models of cellular networks can be tested and iteratively improved4–6. A promising approach is to couple the activity of target proteins to light signals, either by incorporating photoactive allostERIC modulators semisynthetically4–6, or by exploiting naturally occurring light-sensitive domains7–9,10. A particularly useful light-sensitive interaction for creating a general genetically encoded light-control system for cell biology comes from the phytochrome signalling network of plants.

Phytochromes are photoreceptive signalling proteins responsible for mediating many light-sensitive processes in plants, including seed germination, seedling de-etiolation and shade avoidance11. They detect red and near-infrared light through the photoisomerization of a covalently bound tetrapyrrole chromophore such as phyocyanobilin (PCB)11. This photoisomerization event is coupled to an allosteric transition in the phytochrome between two conformational states called Pr (red-absorbing) and Pfr (far-red-absorbing) (Fig. 1a). In one well studied signalling pathway, upon stimulation with red (650 nm) light, the Arabidopsis thaliana phytochrome B (PhyB) protein binds directly to a downstream transcription factor, phytochrome interaction factor 3 (PIF3), translocates to the nucleus as a heterodimer and directly modulates the transcription of response genes. PIF3 binds only the red-light-exposed form of phytochrome, Pfr, and shows no measurable binding affinity for the dark- or infrared-exposed Pr state12. Thus, this interaction can be reversed by infrared

Figure 1 | The phytochrome–PIF interaction can be used to reversibly translocate proteins to the plasma membrane in a light-controlled fashion. a, apo-PhyB covalently binds to the chromophore phyocyanobilin (PCB) to form a light-sensitive holoprotein. PhyB undergoes conformational changes between the Pr and Pfr states catalysed by red and infrared light, reversibly associating with the PIF domain only in the Pfr state. b, This heterodimerization interaction can be used to translocate a YFP-tagged PIF domain to PhyB tagged by mCherry and localized to the plasma membrane by the C-terminal CAAX motif of Kras. c, Phytochrome and PIF domains functional in mammalian cells were tested for reversible light-dependent recruitment of YFP to the plasma membrane using confocal microscopy. Previously published PIF constructs either failed to show visible recruitment or showed irreversible recruitment. Only PhyB constructs harbouring tandem PAS repeats (unique to the plant phytochromes) showed detectable but reversible recruitment in vivo.

1The Cell Propulsion Lab, UCSF/UCB NIH Nanomedicine Development Center, 2Graduate Program in Biophysics, 3Department of Pharmaceutical Chemistry, 4Cardiovascular Research Institute, 5Howard Hughes Medical Institute and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94158-2517, USA.

©2009 Macmillan Publishers Limited. All rights reserved
light. This light-sensitive interaction has been mapped to the 650-residue amino-terminal photosensory core of PhyB and a conserved 100-residue N-terminal activated phytochrome binding (APB) domain of PIF3 (ref. 13).

In previous work, this light-sensitive interaction has been used in yeast to construct a photoreversible two-hybrid transcriptional activator to tune the expression level of a targeted reporter gene20, to target split intein domains to titrate the conditional protein splicing of a reporter gene24, and in vitro to target directly Cdc42 to its effector WASP to regulate actin nucleation25. Collectively this work suggests that the PhyB–PIF interaction can be functionally coupled to a wide variety of signalling processes through engineered fusion proteins.

So far, however, no reported system using the PhyB–PIF interaction has been demonstrated to enable fine spatiotemporal control of dimerization in vivo. Indeed, the relatively weak binding strength and slow reverse kinetics of the reported domains13,15 have prevented us from successfully applying these earlier interaction pairs for in vivo control of signalling. We have optimized the phytochrome interaction to enable its spatiotemporal control in experiments with live mammalian cells.

We first confirmed that PhyB could covalently bind externally supplied PCB chromophore in mammalian cells by using a PhyB mutant (Y276H) that fluoresces at far-red frequencies in the PCB-coupled state only16. NIH3T3 cells transfected with this construct show fluorescence after only 30 min of exposure to 5 μM PCB, confirming rapid autoligation at physiological conditions (Supplementary Fig. 1). Multiple potential phytochrome–PIF pairs were screened by a fluorescence translocation assay in NIH3T3 cells with confocal microscopy. We measured the red-light-induced translocation of yellow fluorescent protein (YFP) fused to PIF domains to co-expressed phytochrome domains fused through a flexible linker to mCherry and localized to the plasma membrane by a carboxy-terminal polybasic, prenylation sequence from Kras17 (Fig. 1b). Of all previously reported PIF domains13,15,16, only the N terminus of PIF6 is strong enough to cause significant translocation of YFP to the membrane (Fig. 1c). However, its interaction with the PhyB photosensory core (residues 1–650) is irreversible in infrared light. Assaying it against different variants of PhyB revealed that the tandem C-terminal PAS domains of plant phytochromes are necessary to confer rapid photoreversibility under infrared light, underlining the importance of a previously reported autoinhibitory interaction for phytochrome signalling25. We refer to the optimized, reversible PhyB–PIF6 interaction simply as the ‘Phy–PIF’ interaction.

Using this optimized Phy–PIF pair we observe rapid translocation to the plasma membrane under dilute red light (650 nm, 20 μmol m−2 s−1) and from the membrane under infrared light (>750 nm, 300 μmol m−2 s−1) (Fig. 2a and Supplementary Movies 1 and 2). Kinetic measurements of the Phy-induced cytoplasmic depletion of PIF–YFP under maximum illumination yield translocation time constants of 1.3 ± 0.1 s (s.d. n = 3) for membrane recruitment and 4 ± 1 s (s.d. n = 3) for membrane release (Fig. 2a and Supplementary Fig. 2), demonstrating second-timescale control. These rates are an order of magnitude faster than previous chemically induced translocation systems33 and are very near the physical limits for whole-cell diffusion (see calculation in Supplementary Information). The Phy–PIF translocation proved very robust—it could be cycled over a hundred times by alternating red and infrared illumination with no measurable decrease in recruitment ratios over time, despite many cycles of imaging at photon fluxes far higher than those phytochromes are exposed to in natural lighting conditions (Fig. 2b and Supplementary Movie 3).

The rapid forward and reverse kinetics of our Phy–PIF pair allow for fine spatial control of membrane recruitment by simultaneously exposing cells to patterned light at the two antagonizing wavelengths. In NIH3T3 cells co-expressing the above Phy–Kras(CAAX) and PIF–YFP recruitment pair, a nitrogen dye cell laser was used to deliver pulses of ‘activating’ red light (650 nm, 20 Hz) to a focused point on the sample plane, while the whole sample was bathed in continuous infrared light obtained by filtering the microscope bright-field source (>750 nm) (Fig. 3a). When the cell membrane is imaged by total internal reflectance (TIRF) microscopy we observe a sharp spot of membrane-localized YFP several micrometres in diameter around the irradiated point (Fig. 3c). The rapid ‘off’ kinetics of the Phy–PIF interaction traps the membrane-recruited YFP pool to this spot, as any YFP diffusing away is dissociated from the membrane by the surrounding infrared light. This spot of recruited YFP can be rapidly relocated across the cell by repositioning the point of incident light (Supplementary Movie 4).

We developed a second, fully automated method of controlling the distribution of both light frequencies on the cell membrane by using a digital micromirror array to project patterned light on to the sample plane of the microscope at micrometre resolutions23. By irradiating the sample with 650-nm and 750-nm light sources oriented to take advantage of both micromirror angle states, a complementary two-colour red/infrared pattern can be projected on to the sample plane, allowing one to ‘paint’ high-resolution inverse distributions of Phy and PIR phytochrome on to the membrane of the cell (Fig. 3b). We were able to project faithfully a simple pixel-based movie into the
systems21,23–25. For example, chemically induced membrane trans-
platform for small-molecule-induced chemical biology control
membrane recruitment systems have been successfully used as a
interactions that relocalize them to the membrane. Moreover, plasma
because many signalling proteins are, at least in part, activated by
changes, but with much higher spatial and temporal resolution.

We chose to focus on spatiotemporal control of the Rho-family
GTPases Rac1, Cdc42 and RhoA, given their central role in the
dynamic spatial regulation of the actin cytoskeleton at the polarized
edges of motile cells (Fig. 4a).

Gated-recruitment constructs were made from the isolated cata-
lytic modules (the DH–PH domain) of the RacGEF Tiam, the
Cdc42GEF intersectin and the RhoGEF Tim. The optimal construct
topologies for DH–PH activation were found by screening for Tiam-
DH-PH activity via the global morphological changes that occurred
in transfected, serum-depleted NIH3T3 cells when the entire field
was exposed to red light. Global recruitment of the optimal PIF–
Tiam–DH-PH chimaera caused a pronounced lamellipodial pheno-
type within 20 min in most (80%) co-transfected cells, compared
to PIF–YFP-only recruitment or control cells lacking the PCB chromo-
phore (Fig. 4b). This potent effect of recruiting the Tiam GEF activity to
the membrane is similar to that observed using chemical dimerizers21.
We further tested the generality of this construct topology by confirm-
ing that global RhoGEF recruitment induced cell body contraction in
fibroblasts (Supplementary Movie 9).

Given the strong global morphological effects of Tiam DH-PH
domain membrane translocation, we then tested the effects of spatially
localized light-activated translocation. Red laser stimulation was used
for localized recruitment of the Tiam DH-PH domain in serum-
depleted NIH3T3 cells (within a background of global repression by
infrared light), effecting within 5–10 min a localized lamellipodial

Figure 3 | Recruitment to the plasma membrane can be controlled spatially
by simultaneously irradiating cells with patterned red and infrared light.

3 μm and can be quickly moved by repositioning the laser. The final frame
shows that the YFP spot is not merely bleed-through of the excitatory laser
light, but genuine local fluorescent protein recruitment. d, TIRF movies of
structured membrane recruitment by programatically updating masks for
red and infrared light using a digital micromirror device as in b were
collected, revealing a faithful reproduction in the recruited YFP distribution
of a movie of the cellular automaton ‘game-of-life glider’ that was projected
(Supplementary Movie 5). e, Images show the raw traces of titrated input
650-nm light and recruited PIF–YFP. The plot (right panel) shows the
recruitment level as a function of 650-nm ratio for three typical experiments.
Inset shows the non-saturated regime. Scale bars, 20 μm.

membrane-recruited PIF–YFP distribution of a NIH3T3 cell. TIRF
imaging reveals fine features at five micrometres, demonstrating an
unprecedented degree of control over protein localization in living
cells (Fig. 3d and Supplementary Movie 5). Additionally, by dithering
the average amount of red light in the target mask through software,
we could smoothly titrate the fraction of active Phy and recruited
PIF–YFP, demonstrating effective ‘greyscale’ control of the chemical
potential (Fig. 3e and Supplementary Movie 6). Using this data,
we estimate the in vivo dissociation constant of the PhyB–PIF6 inter-
action to be approximately $K_d = 20–100 \text{ nM}$ (Supplementary Fig. 5).

We were motivated to engineer a membrane recruitment system
because many signalling proteins are, at least in part, activated by
interactions that relocalize them to the membrane. Moreover, plasma
membrane recruitment systems have been successfully used as a
platform for small-molecule-induced chemical biology control
systems21,23–25. For example, chemically induced membrane trans-
location of the Rho- and Ras-family small G proteins21,23 or the
guanine nucleotide exchange factors (GEFs) that activate them21
can generate global morphological changes. We reasoned that Phy–
PIF-induced translocation might generate similar morphological
changes, but with much higher spatial and temporal resolution.

We chose to focus on spatiotemporal control of the Rho-family
GTPases Rac1, Cdc42 and RhoA, given their central role in the
Rho-family G-protein signalling can be controlled by the light-activated translocation system. a, The catalytic DH-PH domains of RhoGEFs Tiam and intersectin (ITSN) activate their respective G proteins Rac1 and Cdc42, which in turn act through effector proteins to modify the actin cytoskeleton. b, Recruitable constructs with Tiam DH-PH domains were assayed for their ability to induce lamellipodia in NIH3T3 cells by exposing serum-depleted cells transfected with the indicated constructs to red (650 nm) light and counting the percentage of cells that produced lamellipodia within 20 min under live microscopy. Error bars indicate s.e.m. (n = 2, average 30 cells; P-value = 0.0004 for Tiam). c, Local induction and ‘bloom’ (Supplementary Movie 8). By slowly extending the point of activating light away from the cell, it is even possible to ‘draw out’ an extended process up to 30 μm from the main body of the cell that is stable after the light has been withdrawn. This indicates the future possibility of programatically specifying cell geometries and intercellular connections with light (Fig. 4c and Supplementary Movies 7 and 8).

We further verified the signalling activity of our PIF-DH-PH reagents by verifying that point induction causes local, transient increases of the active form of GTPase as measured by the membrane enrichment of biosensors—either mCherry-tagged GBD-binding domains from WASP (Fig. 4d and Supplementary Movie 11) or PAK (Supplementary Fig. 4)—by TIRF microscopy. Using these biosensors we see that GTPase activation occurs rapidly, within seconds, indicating that a subsequent signalling step is responsible for the typical delay of 5–10 min for lamellipodial and filopodial protrusions.

We have developed a genetically encoded, light-switchable Phy–PIF interaction module which, because it has a properly titrated tight but reversible interaction, has the potential to be applied to control any live cell process that is dependent on a recruitment event. Unlike classical uncaging techniques, photo reversibility allows our system to defeat diffusive spreading by using patterned light. Furthermore, the direct relationship between the recruited fluorescent fraction and signalling activity also enables measurable ‘dosage’ of signalling flux for quantitative perturbations. We show here that the system works robustly in mammalian cells with external PCB, extending previous demonstrations in yeast18 and its natural domain in plants, indicating that it is compatible with most eukaryotic cells. For genetically manipulable cells, it is, in principle, simple to include genes for enzymes that will generate PCB from haem or biliverdin.26

The high spatial and temporal resolution of light control allows this module to function as a novel analytical tool, in which highly complex spatial or temporal patterns can be used to drive a process. We have also demonstrated here how this module can be used as a high-resolution control module to sculpt cell shape in an unprecedented manner. Because of the generic nature of this interaction module, it is likely that it can be used to control an extremely broad range of cell biological processes without the need for laborious case-by-case protein engineering.

METHODS SUMMARY

Phycocyanobilin (PCB) purification. PCB was extracted by methanolysis at 70 °C from protein precipitates of Spirulina cell lysate (Seltzer Chemical) that were pre-washed to remove other tetrapyroles species. Free PCB was handled under a green safelight (λmax of 550 nm).

Light control experiments. NIH3T3 cells transiently transfected with the phytochrome and PIF constructs were pre-incubated in the dark with 5 μM PCB for 30 min and then washed before experiments. Non-coherent control-light frequencies were obtained by filtering white-light sources with 650-nm and 750-nm 20-nm band-pass filters (Edmund Optics) or a near-infrared RG9 glass filter (Newport). For morphology experiments, cells were serum-depleted (1% Bovine Calf Serum) for at least 12 h before imaging.

Received 8 July; accepted 24 August 2009.
Published online 13 September 2009.

1. Szobota, S. et al. Remote control of neuronal activity with a light-gated glutamate receptor. Neuron 54, 535–545 (2007).
2. Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G. & Deisseroth, K. Millisecond-timescale, genetically targeted optical control of neural activity. Nature Neurosci. 8, 1263–1268 (2005).

3. Han, X. & Boyden, E. S. Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. PLoS One 2, e299 (2007).

4. Mettetal, J. T., Muzzey, D., Gómez-Uribe, C. & van Oudenaarden, A. The frequency dependence of osmo-adaptation in Saccharomyces cerevisiae. Science 319, 482–484 (2008).

5. Bennett, M. R. et al. Metabolic gene regulation in a dynamically changing environment. Nature 454, 1119–1122 (2008).

6. Ghosh, M. et al. Cofilin promotes actin polymerization and defines the direction of cell motility. Science 304, 743–746 (2004).

7. Gorostiza, P. & Isacoff, E. Y. Optical switches for remote and noninvasive control of cell signaling. Science 322, 441–442 (2008).

8. Levskaya, A. et al. Synthetic biology: engineering Escherichia coli to see light. Nature 438, 438–442 (2005).

9. Lee, J. et al. Surface sites for engineering allosteric control in proteins. Science 322, 19–22 (2008).

10. Shimizu-Sato, S., Huq, E., Tepperman, J. M. & Quail, P. H. A light-switchable gene promoter system. Nature Biotechnol. 20, 1041–1044 (2002).

11. Quail, P. H. Phytochrome photosensory signalling networks. Nature Rev. Mol. Cell Biol. 3, 85–93 (2002).

12. Ni, M., Tepperman, J. M. & Quail, P. H. Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. Nature 400, 781–784 (1999).

13. Khanna, R. et al. A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix-loop-helix transcription factors. Plant Cell 16, 3033–3044 (2004).

14. Tyszkiewicz, A. B. & Muir, T. W. Activation of protein splicing with light in yeast. Nature Methods 5, 303–305 (2008).

15. Leung, D. W., Otomo, C., Chory, J. & Rosen, M. K. Genetically encoded photoswitching of actin assembly through the Cdc42-WASP-Arp2/3 complex pathway. Proc. Natl Acad. Sci. USA 105, 12797–12802 (2008).

16. Su, Y. & Lagarias, J. C. Light-independent phytochrome signaling mediated by dominant GAF domain tyrosine mutants of Arabidopsis phytochromes in transgenic plants. Plant Cell 19, 2124–2139 (2007).

17. Heo, W. D. et al. PI(3,4,5)P3 and PI(4,5)P2 lipids target proteins with polybasic clusters to the plasma membrane. Science 314, 1458–1461 (2006).

18. Al-Sady, B., Ni, W., Kircher, S., Schäfer, E. & Quail, P. H. Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. Mol. Cell 23, 439–446 (2006).

19. Genoud, T. et al. FHY1 mediates nuclear import of the light-activated phytochrome A photoreceptor. PLoS Genet. 4, e1000143 (2008).

20. Chen, M., Tao, Y., Lim, J., Shaw, A. & Chory, J. Regulation of phytochrome B nuclear localization through light-dependent unmasking of nuclear-localization signals. Curr. Biol. 15, 637–642 (2005).

21. Inoue, T., Heo, W. D., Grimley, J. S., Wandless, T. J. & Meyer, T. An inducible translocation strategy to rapidly activate and inhibit small GTPase signaling pathways. Nature Methods 2, 415–418 (2005).

22. Wang, S. et al. All optical interface for parallel, remote, and spatiotemporal control of neuronal activity. Nano Lett. 7, 3859–3863 (2007).

23. Castellano, F. et al. Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation. Curr. Biol. 9, 351–361 (1999).

24. Suh, B., Inoue, T., Meyer, T. & Hille, B. Rapid chemically induced changes of PtdIns(4,5)P2 gate KCNQ ion channels. Science 314, 1454–1457 (2006).

25. Inoue, T. & Meyer, T. Synthetic activation of endogenous PI3K and Rac identifies an AND-gate switch for cell polarization and migration. PLoS One 3, e3068 (2008).

26. Gambetta, G. A. & Lagarias, J. C. Genetic engineering of phytochrome biosynthesis in bacteria. Proc. Natl Acad. Sci. USA 98, 10566–10571 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank B. El-Sady, J. Tepperman, K. Thorn, G. Kapp and members of the Voigt, Weiner and Lim laboratories for assistance and discussion. We thank Molecular Devices and Photonics Instruments for the loan and customization of a Mosaic spatial light modulator. Data for this study were acquired at the Nikon Imaging Center at UCSF. This work was supported by a NSFGR fellowship (A.L.); NIH R01 GM084040 and Searles Scholar Award (O.D.W.); Packard Fellowship, the Howard Hughes Medical Institute, and NIH grants GM55040, GM62583 and EYO16546 (NIH Roadmap Nanomedicine Development Centers) (W.A.L.); Pew Fellowship, the Office of Naval Research, Packard Fellowship, NIH EYO16546, NIH A1067699, NSF BES-0547637, UC-Discovery and the SynBERC NSF ERC (C.A.V.).

Author Contributions Concept was conceived by A.L., W.A.L. and C.A.V.; all authors were involved in interpretation of results and preparation of the manuscript.

Author Information Plasmids will be available from Addgene (http://www.addgene.org). Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to W.A.L. (lim@cmp.ucsf.edu).