Published in final edited form as:

Chembiochem. 2015 January 19; 16(2): 254–261. doi:10.1002/cbic.201402576.

Light Control of Cellular Processes Using Photocaged Abscissic Acid

Catherine W. Wright^[a], Dr. Zhi-Fo Guo^[a], and Prof. Dr. Fu-Sen Liang^[a]

^[a] Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, NM 87131 (U.S.A.)

Abstract

Abscissic acid (ABA) was chemically modified with a photocaging group to promote photo-induced protein dimerization. This photo-controlled chemically induced dimerization (CID) method based on caged ABA enables dose-dependent light regulation of cellular processes, including transcription, protein translocation, signal transduction and cytoskeletal remodeling, without the need to perform extensive protein engineering. Caged-ABA can be easily modified to respond to different wavelengths of light. Consequently, this strategy should be applicable to the design of light-regulated protein dimerization systems and potentially be used orthogonally with other light-controlled CID systems.

Abstract

Keywords

Photocaging; Chemically induced dimerization; Abscissic acid; Signal transduction; Photochemistry

Introduction

Regulation of biological molecules and processes using light-controlled experimental platforms allows precise spatiotemporal interrogation of biological systems *in vivo*.^[1] Among these emerging technologies, light-inducible dimerization of photo-switchable protein pairs has great potential for achieving exquisite control of cellular processes.^[2] However, it is still difficult to induce dosage-dependent biological outcomes using photo-

F.S. Liang, fsliang@unm.edu.

Supporting information for this article is given via a link at the end of the document.

switchable proteins,^[2c] and repeated irradiations to switch proteins conformations can cause potential photo-damages to cellular structures.^[3] Furthermore, the number of available light-inducible interactive protein pairs is still limited and significant efforts would be required to engineer new ones with desired light response specificity. An alternative strategy of developing light controlled systems is using chemically induced dimerization (CID) methods in which protein-protein interactions are mediated by small molecule ligands.^[4] Several orthogonal CID systems have been described that enable simultaneous control of independent processes.^[5] However, only a few of these strategies are further developed to allow light-mediated controls, which can potentially provide precise spatial regulation of biological processes at cellular levels or in organisms.

Photocaging methods, which employ photo-removable groups to mask the activities of linked small molecules, have been used to give spatial control in regulating cellular processes.^[6] A key to the success of this strategy is the requirement of selectively installing a photo-labile group at a position on the small molecule that destroys its biological function. Light regulated CID methods developed thus far have employed caged-rapamycin systems to control protein synthesis and the activities of small GTPases and kinases.^[7] Compared to the approaches using rapamycin for photocaging, our method avoids the need for multistep synthesis to install caging groups at specific positions on rapamycin,^[7a] the extra effort to engineer the rapamycin-binding protein, or the introduction of additional extracellular proteins to achieve the caging effects.^[7b,c] Finally, to date no caged-CID systems other than the caged-rapamycin systems have been devised that provide the capability of orthogonal photo-regulation of two cellular processes.

We previously developed a new CID system comprised of the plant hormone, abscisic acid (ABA), and its associated binding proteins ABI and PYL.^[5g] The ABA-based dimerization system has many advantageous features, including rapid response kinetics, fast rate of reversibility, wide dose responsive range, no known toxicity issues and excellent bioavailability.^[5g] Moreover, we demonstrated that ABA and rapamycin systems are orthogonal and can be used simultaneously to control two independent cellular events.^[5g] In the study described below, we have developed a light-inducible ABA-based CID system using a photocaged ABA that enables dose-dependent photo-control of cellular processes (Figure 1).

Results and Discussion

Design of caged ABA

ABA binds with the PYL protein to form a complex that subsequently recognizes ABI. The results of crystallographic analysis show that ABA in the complex is enclosed within the PYL binding pocket.^[8] It is likely that structural modifications of ABA, especially those that involve incorporation of bulky groups, will disrupt its binding to PYL and, therefore, abolish its ability to promote subsequent PYL-ABI dimerization. Consequently, we believed that incorporation of a photo-removable group would result in an ABA derivative that would be incapable of binding to PYL. Furthermore, we speculated that the carboxylic acid moiety in ABA, which forms critical hydrogen bonds with PYL,^[8] would be an ideal site to attach chosen photo-removable groups.

Synthesis and photo-uncaging of ABA-DMNB

4,5-Dimethoxy-2-nitrobenzyl (DMNB) and other nitrobenzene groups have been used to cage small molecules because they can be removed by 365 nm light.^[9] In order to test our design outlined above, we synthesized the ABA derivative, ABA-DMNB, that contains a DMNB ester group (Figure 2A). Using HPLC analysis, ABA-DMNB was shown to be stable at 37 °C in biological buffer in the dark for 24 h (Figure S1). To examine the efficiency of the photo-uncaging process, solutions of ABA-DMNB (10 and 100 µM) were irradiated with 365 nm UV light emitted from a fluorescent microscope. HPLC analysis of the photolysate showed that the ABA-DMNB was cleaved to regenerate free ABA following irradiation for 120 sec (Figure 2B, S2). In addition to the expected ABA product, an additional product was generated at the same time in near equal amount during the photochemical process (Figure 2B). It has been reported that 2-*trans* isomer of ABA can be formed through photo-isomerization when exposed to the UV light.^[10] We purified the by-product by HPLC and confirmed by the mass spectrometry analysis that the by-product was indeed an ABA isomer, and the NMR data of this isomer supported that it is the 2-*trans* ABA isomer (see supporting information). Surprisingly, when uncaged ABA was subjected to the same photo-cleavage condition, only very little 2-*trans* isomer was produced, which suggested that the DMNB group may serve as a photosensitizing group to facilitate the isomerization of the ABA backbone under the applied irradiation condition (Figure 2B). The dimethoxynitrosobenzaldehyde released from the uncaging process of ABA-DMNB was not detected under the wavelength of 250 nm, which was used in the HPLC analysis. This is consistent with previous report that the nitrosobenzene derivatives have low absorptivity at 250 nm.^[11] The results of this effort show that photo-uncaging of ABA-DMNB occurs rapidly to produce ABA in biologically relevant concentrations.

Regulating gene expression by caged ABA and light

To confirm that the photo-regenerated ABA is biologically functional, we determined whether it can be employed to induce PYL-ABI dimerization in an ABA-inducible transcriptional activation system using an established HEK 293T inducible EGFP reporter cell line. The cell line was created with both the ABA-responsive split transcriptional activator DNA fragment (VP-PYL and GAL4DBD-ABI linked by IRES)^[5g] and an inducible EGFP DNA fragment (with 5xUAS and the IL2 minimal promoter) inserted into the genome (Figure 3A). EGFP expression is activated through the recruitment of VP16AD to the EGFP gene in the presence of ABA. 24 h after plating the cells, 10 µM of ABA, pre-irradiated ABA-DMNB products and non-irradiated ABA-DMNB were separately added to the cells and incubated for an additional 8 h. EGFP expression was then determined using a fluorescence microscopy. The results showed that both ABA and pre-photocleaved ABA-DMNB induced EGFP production (Figure 3B), which indicate that the uncaging of ABA-DMNB gives biologically functional ABA. In contrast, 10 µM of non-irradiated ABA-DMNB did not induce EGFP expression (Figure 3B). This finding demonstrates that ABA-DMNB would be stable in cells under the experimental condition and unable to induce PYL-ABI dimerization in the absence of light.

The cellular stability of ABA-DMNB was explored further using a more sensitive and quantitative luciferase assay system, which employs an inducible luciferase construct

(Figure 3A).^[5g] For this purpose, CHO cells were transfected with the ABA-inducible luciferase constructs for 24 h and then 10 μ M of ABA-DMNB was added and incubated in dark for 12 or 24 h. Cells were then harvested and analyzed by using the luciferase assay. The results showed that very little luciferase expression took place after a 12 h incubation period (Figure 3C), an observation that is consistent with the earlier finding that ABA-DMNB does not activate dimerization. Furthermore, the fact that only a minimal level of luciferase was induced following 24 h incubation demonstrates that ABA-DMNB is relatively stable under the cell culture condition despite possessing an ester that could potentially be hydrolyzed.

From the HPLC analysis of the ABA-DMNB photocleavage (Figure 2B), a near equal amount of 2-*trans* ABA isomer was formed during the uncaging process. The 2-*trans* isomer is reported to have a much lower biological activity.^[12] Based on the crystal structures of the ABA-PYL complex,^[8] it is expected that the *trans* isomer may not fit the PYL pocket as well as the *cis* isomer, which likely results in a lower dimerization efficiency and downstream activity. To confirm that the generated *trans* ABA isomer would not interfere with the dimerization induced by the regenerated *cis*-ABA, we tested the induced expression of luciferase as above by each or the mixture of ABA isomers. In this experiment, CHO cells were transfected with the ABA-inducible luciferase constructs for 24 h and then 10 μ M of the stock *cis*-ABA, photo-regenerated *cis*-ABA, photo-regenerated *trans* ABA isomer, or a mixture of regenerated *cis*- and *trans*-ABA (10 μ M each) was added and incubated for an additional 24 h. Cells were then harvested and subjected to the luciferase assay. As expected, the regenerated *cis*-ABA gave a similar activity as the stock ABA and the *trans* isomer has a much less activity (Figure S3). The mixture of the *cis* and *trans* isomers induced a comparable level of luciferase expression as the *cis*-ABA alone suggesting that the *trans* isomer does not attenuate the effects of the *cis*-ABA.

The ABA-inducible luciferase assay was also employed to determine if ABA-DMNB is effectively photo-cleaved in cell culture. Different quantities of ABA and ABA-DMNB (Figure 3D) were independently added to transfected CHO cells. The cells that received ABA-DMNB were either kept in the dark or irradiated using 365 nm UV light for 120 sec. After 12 h following compound addition and irradiation, cells were harvested and subjected to the luciferase assay. The results showed that cells that were treated with ABA or those with ABA-DMNB followed by irradiation induced luciferase expression (Figure 3D). Similarly, the HEK 293T inducible EGFP reporter cell line showed EGFP expression when cultures containing ABA-DMNB were irradiated (Figure S4). These results demonstrate that ABA-DMNB can be uncaged in cell culture to allow light-induced controls of transcriptional activation in live cells.

It is known that ABA gives dose-dependent induction of gene expression.^[5g] As expected, ABA generated by irradiation of ABA-DMNB also displays this property. Irradiation of transfected cells with the addition of increasing concentrations of ABA-DMNB lead to corresponding increases in the level of luciferase expression (Figure 3D). A concern of using UV-triggered reactions in cells is phototoxicity. However, based on cell morphology and the growth rate, no obvious cytotoxicity was observed under the irradiation conditions used to cleave ABA-DMNB. Finally, the results also demonstrated that the DMNB derived

photoproduct does not cause observable toxicity at the concentrations used in the experiments described above.

Photo Inducible Protein Translocation and Membrane Ruffling

The ability of using light to control protein translocation through ABA-DMNB uncaging was evaluated next. Plasmids expressing EGFP-tagged PYL and nuclear export sequence (NES) peptide-linked ABI (Figure 4A)^[5g] were transfected into CHO cells. The EGFP-PYL fusion protein was expected to have pan-cellular distribution. Its ABA-induced dimerization to the NES-ABI protein should trigger the localization of EGFP-PYL out of the nucleus. The transfected cells were then either treated or not treated with ABA, ABA-DMNB, or ABA-DMNB and irradiated for 60 sec. Fluorescence microscopy analysis was used to determine subcellular locations of EGFP fusion proteins. Cells treated with ABA or with ABA-DMNB followed by UV irradiation showed markedly decreased intensities of EGFP in the nuclei within 15 min (Figure 4B, 4C). On the other hand, cells that were either not treated with ABA-DMNB or with ABA-DMNB but not subjected to UV irradiation showed EGFP distribution throughout the whole cells. Furthermore, the observed nuclear export of EGFP fusion protein can be readily reversed by washing away the uncaged ABA using fresh media. These results show that protein translocation can be induced by light through photo-uncaged ABA and can be readily reversed.

To examine the reversibility of the photo-induced EGFP nuclear exportation process, cells that were treated with ABA or ABA-DMNB followed by UV irradiation were washed with fresh culture media not containing drugs and subjected to fluorescence analysis. The results showed that induced nuclear exportation of EGFP was reversed following three washes (within 30 min) (Figure 4B, 4C).

Next, we examined the induction of ruffle formation through the activation of the Rac1 signaling pathway in order to determine if the light-activated ABA system can be used to regulate a complex biological process. The GTP exchange factor, Tiam1, when presents at cell membranes, activates Rac1 to initiate a signaling pathway that leads to cytoskeletal remodeling and forms filopodia and lamellipodia.^[7b, 13] To activate Rac1 signaling and induce membrane ruffling, light and ABA-DMNB were employed to control membrane localization of Tiam1 (Figure 5A). A construct encoding a membrane localized ABI (myr-ABI)^[5g] and one expressing a constitutively active Tiam1^[7b] fused to EGFP and PYL were employed for this purpose (Figure 5A). Following the transfection of CHO cells with both plasmids for 24 h, 10 μ M of ABA-DMNB was added and the treated cell culture was irradiated with 365 nm light from a fluorescent microscope. Cells were then fixed on slides and analyzed under a fluorescence microscope. Cells were categorized as non-ruffled if they did not have observable cytoskeletal remodeling or as having ruffles if they displayed distinct lamellipodia and filopodia (Figure 5B). The cultures that received ABA or ABA-DMNB with irradiation showed a greater percentage of cells displaying ruffling compared to the cultures that were incubated with ABA-DMNB without irradiation or were given no drug (Figure 5C). Some background ruffling when overexpressing constitutively active Tiam1 was observed even when no drug was added, which is consistent with previous reports.^[14]

These observations demonstrate that the strategy of combining light and a caged ABA can be employed to control cytoskeletal remodelling through the initiation of Rac1 signalling.

Synthesis and analysis of ABA-DEACM for live cell studies

A confocal microscope is often used to achieve greater precision in light-induced processes and should be able to cleave caged ABA more precisely. However, a confocal microscope is commonly equipped with a 405 nm laser, which cannot efficiently uncage ABA-DMNB (Figure S5). To prepare another caged ABA that can be cleaved by 405 nm light, we conjugated ABA to the [7-(diethylamino)coumarin-4-yl]methyl (DEACM) group to give ABA-DEACM (Figure 6A).^[15] The design of ABA-DEACM demonstrates the flexibility of the small molecule caging system, in which different caging groups can be easily installed as needed by using simple chemical processes rather than lengthy protein engineering steps that are needed in other protein-based methods. Studies of photo-cleavage of ABA-DEACM using a 405 nm LED revealed that ABA-DEACM can be cleaved rapidly to give ABA within a few minutes (Figure 6B, S5). As observed in the case of ABA-DMNB uncaging, the isomerized ABA was also produced, which suggested that the addition of a caging group may in general sensitize and promoted the isomerization of ABA, although the mechanism is unclear. The absorptivity of the coumarin photo-cleavage product at the detection wavelength (250 nm) was measured and was shown to be low ($\epsilon=0.23$), and the coumarin by-product was therefore not seen in the HPLC analysis. Moreover, ABA-DEACM cannot be cleaved efficiently by 365 nm light (Figure S6), which is employed to activate the ABA-DMNB caging system. This observation suggests that DMNB and DEACM can potentially be utilized as orthogonal caging groups on orthogonal CID inducers to independently control two cellular events.

An evaluation of the stability of ABA-DEACM using HPLC and the luciferase assay showed that it is stable chemically and in cell culture (Figure S7, S8). Photo-uncaged ABA-DEACM also showed the ability to induce EGFP-PYL nuclear export (Figure S9) and Rac1 signaling activation/ruffle formation in cells (Figure S10). To test the uncaging process in live cell experiments, EGFP nuclear export experiments were carried out by transfecting CHO cells with EGFP-PYL and NES-ABI constructs. The cells were then either treated with 10 μ M ABA-DEACM and with or without 405 nm light irradiation under a confocal microscope, or with no drug but irradiated. The subcellular location of EGFP was followed for 20 min after irradiation. The nuclear export of EGFP fusion proteins was observed within a few minutes when treated with ABA-DEACM followed by irradiation, but not in the cases of no drug or no irradiation (Figure 6C, 6D). We also tested the use of ABA-DEACM in live cell imaging to induce cell morphology changes. We transfected CHO cells in a culture chamber with myr-ABI and EGFP-PYL-Tiam1 constructs. Cells that were irradiated in the absence of ABA-DEACM did not show any cytoskeletal remodeling (Figure 7A). On the other hand, cells that were incubated with ABA-DEACM and irradiated showed formation of filopodia and lamellipodia within 15 min (Figure 7A and 7B). These results demonstrate that the local uncaging of ABA-DEACM produced a sufficient level of ABA, which rapidly dimerized PYL- and ABI-fusion proteins before diffused away, to illicit desired biological responses.

Conclusions

In the investigation described above, we developed a new light-controlled CID system based on caged-ABAs that can be employed to regulate cellular processes, including transcription, protein translocation, signal transduction and cytoskeletal remodeling. We showed that dosage-dependent regulation can be achieved with this method. We anticipate that this new light-controlled ABA system can be used orthogonally with caged-rapamycin or other protein-based systems to independently regulate multiple cellular events using light of different wavelengths. As a result, the strategy should expand the repertoire of light-controlled methods that can be utilized to explore important biological systems.

Experimental Section

HPLC Analysis

Reversed-phase HPLC was performed on a Dionex Acclaim 120 (4.6 × 100mm) C18 column using an UltiMate 3000 pump system that included Variable Wavelength Detector 3100, Degasser 1210, and Autosampler SPS 3000. A mixture of water and acetonitrile containing 0.1% TFA was used as the eluent. Absorbance at 250 nm was used to monitor the elution of the molecules. The method used an increase in acetonitrile from 5% to 95% over 15 min to elute the molecules at a flow rate of 0.7 mL/min. The peaks for the molecules were integrated using Chromeleon software. The molar absorptivity of both free and caged ABA at 250 nm was measured, which was used to calculate the concentration of each species from the intensity of absorbance at 250 nm. The relative concentration of each compound was used to calculate the percent concentration of free ABA relative to the concentration of total ABA species (both caged and uncaged).

Cell Culture and Transfection

CHO cells and 293T EGFP reporter cells (provided by Dr. Gerald R. Crabtree) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) with 10% FBS (Atlanta Biologicals), 1x GlutaMAX (Gibco), and 1x penicillin/streptomycin (Pen/Strep; Gibco). Cells (15,000 to 50,000) were plated in wells of a 24- or 8-well plate for 24 h before transfection. DNA constructs (0.1 µg to 0.5 µg) were added to 50x (v/w) Opti-MEM (Gibco) and then 3x (v/w) PEI (Polysciences) was mixed with the DNA. The mixture was incubated for 20 min at the room temperature before adding it to cell cultures. The cells were grown for 1 d after transfection before experiments were performed.

DNA Plasmids Construction

The construction of the 5FL, 5IG, SV-VPiGA, NES-ABI, GFP-PYL, myr-ABI plasmids has been described previously.^[%g] The PYL-EGFP-Tiam1 construct was derived from pSV40-VP16-PYL-IRES-Gal4DBD-ABI by inserting codon optimized PYL fragment (PCR amplified by primers CCGACAGAAATTCGCCACCATGACCCAG-GACGAGTTT-ACCCAG and CCGACAGGCGCGCCGCTGCCGCCG-TTCATAGCCTCAGTAATGCT) using EcoRI and AscI sites, Tiam1-SG linker fragment (amplified by primers GCTATGAACGGCGCGCCA-AGTGCTGGTGGTAGTGCTGGT and CTAGAGTCGCGGCCGCTCAG-ATCTCAGTGTTC-AGTTTC) using AscI and NotI sites, and EGFP-SG-linker fragment

(amplified by primers CCGACAGGCGCGCCAG-GTGGATCTGGAGGTTTCAGGTGGATCTGGAGGTGTGAGCAAGGGCG AGGAGCTG and CCGACAGGCGCGCCCTTGTACAGCTCGTCCATGCC) using *AscI* site.

Photo-irradiation

Irradiation at 365 nm was performed using an Axio Observer (Zeiss) microscope with an HBO103 W/2 mercury arc lamp. Irradiation was performed using the DAPI filter set with peak excitation at 365 nm (power density 23 mW/cm²) and spectral width of 50 nm. No objective lens was used for whole-well irradiation, which created an area of illumination that nearly completely covers one well of a 24-well plate. Light was transmitted through the bottom of the well of polystyrene plate. Irradiation at 405 nm was performed using an Adjustable Focus Violet Purple Laser Pointer (LazerPoint SKU 0733579) with excitation wavelength of 405 nm and 1000 mW intensity positioned 8 cm above the bottom of either a 96- or 24-well plate and irradiated through the polystyrene lid. All samples for HPLC analysis were irradiated in DMSO to prevent evaporation of solvent and changes in sample concentration. Irradiation of cell cultures was in 24-well plates containing 500 μ L of culture media.

Luciferase Assay

Cells from 24-well plates were washed with PBS and lysed with 100 μ L of Reporter Lysis Buffer (Promega) by incubating and gently shaken at room temperature for 10 min after a freeze/thaw cycle. Cell lysates were centrifuged at 15,000 rpm in an Eppendorf Centrifuge 5424 and 25 μ L of lysate was used for luciferase assay. 100 μ L of luciferase assay reagent (5 mg luciferin (GoldBio) and 7 mg coenzyme A (Sigma) in 33 mL of Luciferase Assay Buffer [20 mM tricine, 1.07 mM (MgCO₃)₄Mg(OH)₂•5H₂O, 2.67 mM MgSO₄, 0.1 mM EDTA, 33.3 mM dithiothreitol, and 0.53 mM ATP in water) was added to lysates. Luciferase assay reagent was added through the auto-injector of GLOMAX-Multi Detection System (Promega), and the signal was detected by the instrument with a 1.5 s delay and 0.5 s integration time. All experiments were conducted in triplicate.

Slide Preparation

Cells were grown on glass coverslips in 24-well plates. The coverslips were washed with phosphate-buffered saline (PBS) and fixed with 300 μ L of 4% paraformaldehyde (PFA, prepared in PBS) at room temperature for 20 min. The cells were then washed twice with PBS and incubated with 1x DAPI in the dark at the room temperature for 5 min. After a final wash with PBS, the coverslips were mounted on a glass slide with Vectashield (VWR) mounting media and allowed to stand for 2 h in the dark before imaging.

Fluorescence Microscopy Imaging

Slides were imaged with Axio Observer (Zeiss) microscope or with Zeiss LSM 510 Meta confocal mounted on an AxioObserver inverted microscope using the 63x oil objective. Images were taken with DAPI and GFP channels.

Live Cell Confocal Microscopy Irradiation and Imaging

EGFP fluorescence of CHO cells was detected with a Zeiss LSM 510 Meta confocal mounted on an AxioObserver inverted microscope. The ABA-DEACM was uncaged using the 405 nm UV laser (25 mW) set to 25% power for around 3 sec. To image, fluorescence was excited with the 488 nm line of an argon laser (30 mW) with laser power attenuated to 50%. EGFP emission was collected with a FITC filter. Live cells were plated in 8-well coverslip-bottom culture chambers in 200 μ L media and maintained at 37 °C with an objective lens heater (Bioptics). Culture medium was exchanged to OptiMEM (Gibco) with caged ABA, or no drug prior to imaging. Images were acquired every 10 to 20 sec in different experiments with a 63x/ 1.2 NA water objective.

Statistical Analysis of Cell Population

Cells were categorized as displaying nuclear export of EGFP when the fluorescent intensity of the nucleus was less than 60% of the intensity of the cytoplasm. Cells were categorized as Ruffled when they displayed broad extensions identifiable as lamellipodia or filopodia from the GFP fluorescence from membrane localized EGFP-PYL-Tiam1. Cells were counted from three separate experiments with $N > 50$ for each experiment.

Image Analysis of Fluorescence Intensity in Nuclear Export Experiments

Images generated were analysed for fluorescent intensity using Slide Book v.6 software. Equal sized regions of interest were analysed from the cytoplasm and the nucleus to compare fluorescent intensity of EGFP in three cells for each condition from images taken every 20 sec for duration of 20 min.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Professor Gerald R. Crabtree for providing the EGFP reporter 293T cells, Professor Keith A. Lidke for the assistance and discussion of the uncaging experiments, the UNM Microscopy Facility for the assistance with the confocal and live cell imaging, and University of New Mexico for financial support.

References

- [1] a). Adams SR, Tsien RY. Annu. Rev. Physiol. 1993; 55:755–784. [PubMed: 8466191] b) Riggsbee CW, Deiters A. Trends Biotechnol. 2010; 28:468–475. [PubMed: 20667607] c) Lee H-M, Larson DR, Lawrence DS. ACS Chem. Biol. 2009; 4:409–427. [PubMed: 19298086] d) Mayer G, Heckel A. Angew. Chem. Int. Ed. 2006; 45:4900–4921. e) Deisseroth K. Nat. Methods. 2011; 8:26–29. [PubMed: 21191368]
- [2] a). Wu YI, Frey D, Lungu OI, Jaehrig A, Schlichting I, Kuhlman B, Hahn KM. Nature. 2009; 461:104–108. [PubMed: 19693014] b) Levskaya A, Weiner OD, Lim WA, Voigt CA. Nature. 2009; 461:997–1001. [PubMed: 19749742] c) Yazawa M, Sadaghiani AM, Hsueh B, Dolmetsch RE. Nature Biotech. 2009; 27:941–945. d) Konermann S, Brigham MD, Trevino A, Hsu PD, Heidenreich M, Cong L, Platt RJ, Scott DA, Church GM, Zhang F. Nature. 2013; 501:1–17.
- [3]. Pattison DI, Rahmanto AS, Davies MJ. Photochem. Photobiol. Sci. 2012; 11:38–53. [PubMed: 21858349]

- [4] a). Fegan A, White B, Carlson JCT, Wagner CR. *Chem. Rev.* 2010; 110:3315–3336. [PubMed: 20353181] b) DeRose R, Miyamoto T, Inoue T. *Pflugers Arch.* 2013; 465:409–417. [PubMed: 23299847] c) Gestwicki JE, Marinec PS. *Combi. Chem. High Throughput Screen.* 2007; 10:667–675.
- [5] a). Schreiber SL, Crabtree GR. *Immunol. Today.* 1992; 13:136–142. [PubMed: 1374612] b) Spencer DM, Wandless TJ, Schreiber SL, Crabtree GR. *Science.* 1993; 262:1019–1024. [PubMed: 7694365] c) Ho S, Biggar SR, Spencer DM, Schreiber SL, Crabtree GR. *Nature.* 1996; 382:822–826. [PubMed: 8752278] d) Belshaw PJ, Spencer DM, Crabtree GR, Schreiber SL. *Chem. Biol.* 1996; 3:731–738. [PubMed: 8939689] e) Bayle JH, Grimley JS, Stankunas K, Gestwicki JE, Wandless TJ, Crabtree GR. *Chem. Biol.* 2006; 13:99–107. [PubMed: 16426976] f) Zhao H-F, Boyd J, Jolicoeur N, Shen S-H. *Hum. Gene Ther.* 2003; 14:1619–1629. [PubMed: 14633404] g) Czlapinski JL, Schelle MW, Miller LW, Laughlin ST, Kohler JJ, Cornish VW, Bertozzi CR. *J. Am. Chem. Soc.* 2008; 130:13186–13187. [PubMed: 18788807] g) Liang F-S, Ho WQ, Crabtree GR. *Sci. Signal.* 2011; 4:rs2. [PubMed: 21406691] h) Miyamoto T, DeRose R, Suarez A, Ueno T, Chen M, Sun TP, Wolfgang MJ, Mukherjee C, Meyers DJ, Inoue T. *Nat. Chem. Biol.* 2012; 8:465–470. [PubMed: 22446836]
- [6] a). Ellis-Davies GCR. *Nat. Methods.* 2007; 4:619–628. [PubMed: 17664946] b) Kramer RH, Mourot A, Adesnik H. *Nat. Neurosci.* 2013; 16:816–823. [PubMed: 23799474]
- [7] a). Sadvoski O, Jaikaran ASI, Samanta S, Fabian MR, Dowling RJO, Sonenberg N, Woolley GA. *Bioorg. Med. Chem.* 2010; 18:7746–7752. [PubMed: 20427189] b) Umeda N, Ueno T, Pohlmeier C, Nagano T, Inoue T. *J. Am. Chem. Soc.* 2011; 133:12–14. [PubMed: 21142151] c) Karginov AV, Zou Y, Shirvanyants D, Kota P, Dokholyan NV, Young DD, Hahn KM, Deiters A. *J. Am. Chem. Soc.* 2011; 133:420–423. [PubMed: 21162531]
- [8] a). Miyazono K, Miyakawa T, Sawano Y, Kubota K, Kang HJ, Asano A, Miyauchi Y, Takahashi M, Zhi Y, Fujita Y, Yoshida T, Kodaira KS, Shinozaki KY, Tanokura M. *Nature.* 2009; 462:609–614. [PubMed: 19855379] b) Melcher K, Ng LM, Zhou XE, Soon FF, Xu Y, Suino-Powell KM, Park SY, Weiner JJ, Fujii H, Chinnusamy V, Kovach A, Li J, Wang Y, Li J, Peterson FC, Jensen DR, Yong EL, Volkman BF, Cutler SR, Zhu JK, Xu HE. *Nature.* 2009; 462:602–608. [PubMed: 19898420]
- [9] a). Callaway EM, Katz LC. *Proc. Natl. Acad. Sci. USA.* 1993; 90:7661–7665. [PubMed: 7689225] b) Bochet CG. *J. Chem. Soc. Perkin Trans. I.* 2002:125–152.
- [10] a). Cornforth JW, Mallaby R, Ryback G. *J. Chem. Soc. C.* 1968; 1968:1565–68. b) Lenton JR, Perry VM, Saunders PF. *Planta.* 1971; 96:271–80. [PubMed: 24493134] b) Mousseron-Canet M, Mani J. e. Daile JP, Olive JL. *Bull. Soc. Chim. Fr.* 1966; 12:3874–78. c) Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. *Annu. Rev. Plant Biol.* 2010; 61:651–679. [PubMed: 20192755]
- [11]. Tabei K, Nagakura S. *Bulletin of the Chem. Soc. of Japan.* 1964; 38:965–971.
- [12] a). Milborrow BV. *Planta.* 1966; 70:155–171. [PubMed: 24557943] b) Milborrow BV. *Ann. Rev. Plant Physiol.* 1974; 25:259–307.
- [13]. Michiels F, Habets GG, Stam JC, van der Kammen RA, Collard JG. *Nature.* 1995; 375:338–340. [PubMed: 7753201]
- [14]. Kunda P, Paglini G, Quiroga S, Kosik K, Cáceres A. *J. Neurosci.* 2001; 7:2361–2372. [PubMed: 11264310]
- [15]. Shembekar VR, Chen Y, Carpenter BK, Hess GP. *Biochemistry.* 2005; 44:7107–7114. [PubMed: 15882049]

Figure 1. Light activation of caged ABA can dimerize two proteins of interest (POIs) and induce downstream cellular processes. The induced proximity of any two POIs can lead to a wide variety of biological effects.

Figure 2.

Synthesis and photo-cleavage of Caged ABA. A) ABA was conjugated to the DMNB protecting group in a one-step synthesis. Irradiation with 365 nm light removes the DMNB group and results in the release of free ABA. B) HPLC analysis of the photo-cleavage reaction showed that irradiation of 100 μ M ABA-DMNB can regenerate free ABA within 2 min.

Figure 3.

Photo-triggered uncaging of ABA-DMNB can induce gene expression. A) Constructs and mechanism of ABA-dependent transcription initiation of a reporter gene. B) Induction of EGFP expression by 10 μ M ABA, ABA-DMNB or UV-irradiated ABA-DMNB in EGFP reporter 293T cells. Scale bar 100 μ m. C) Testing the stability of ABA-DMNB. CHO cells transfected with inducible luciferase expression constructs were incubated with ABA or ABA-DMNB for 12 and 24 h in the dark. D) Uncaging in cell culture and dosage-dependence of light-controlled luciferase expression. CHO cells transfected with inducible

luciferase constructs were treated with increasing dosages of ABA, or ABA-DMNB with or without UV irradiation. For C) and D), the relative luciferase expression fold changes were calculated based on transfected cells with no drug treatment. Error bars are SD (N=3).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4.

Photo-uncaging of ABA-DMNB can induce protein translocation. A) Constructs expressing EGFP-PYL and nuclear export sequence (NES)-tagged ABI. B) CHO cells were transfected with these constructs to test the light-induced EGFP translocation. Under conditions with no drug or with ABA-DMNB without irradiation, the EGFP-PYL was distributed throughout the cell. With the addition of ABA or the irradiation of added ABA-DMNB, the EGFP-PYL was exported out of the nucleus. After repeated washing with fresh media, the dimerization was reversed and EGFP-PYL diffused back into the nucleus. Scale bar 10 μ m. C) Quantitative analysis and statistics of EGFP-PYL translocation in EGFP expressing cells. Transfected and treated CHO cells were fixed and analyzed under a fluorescence microscope for nuclear export. Cells were categorized as showing nuclear export of EGFP when the intensity in the nucleus was less than 60% of that in the cytoplasm. Cells were counted from three separate experiments with $N > 50$ for each experiment.

Figure 5.

Caged ABA can be released to induce signal transduction to produce morphological changes. A) Constructs and mechanism of ABA-inducible membrane localization of Tiam1 and the initiation of Rac1 signalling. B) Examples of cell morphology classified as non-ruffled (top) or ruffled (bottom). Scale bar 10 μ m. C) Quantitative analysis and statistics of induced ruffling. Cells were counted and classified based upon the appearance of ruffled or non-ruffled morphology in EGFP expressing cells. Percentages of cells showing the ruffled morphology were calculated from three experiments with N > 50 for each experiment. Cultures that were given ABA or ABA-DMNB with irradiation showed a higher percentage of ruffled cell compared to cultures that were given ABA-DMNB without irradiation or no drug.

Figure 6.

ABA-DEACM for live cell experiments. A) Synthesis and photo-cleavage of ABA-DEACM by irradiation with 405 nm light. B) HPLC analysis of irradiated ABA-DEACM showed complete cleavage within 4 min. C) Quantitative analysis of EPFG-PYL nuclear export in live cell imaging under a confocal microscope. EGFP-PYL was monitored for 20 min and the ratio of the fluorescent intensity of the nucleus to the cytoplasm was calculated as a function of time. Grey bar indicates the period of irradiation with 405 nm laser equipped on the confocal microscope. D) Images of CHO cells incubated with ABA-DEACM before (0 sec) and after (620 sec) irradiation at 4 sec. Scale bar 10 μm . The data in C) and D) are representative data from three independent experiments.

Figure 7.

Photo-uncaged ABA-DEACM induced cytoskeletal remodeling. A) CHO cells were transfected with myr-ABI and EGFP-PYL-Tiam1 constructs and irradiated with 405 nm light with or without the presence of ABA-DEACM. No cytoskeletal remodeling was observed for cells that were irradiated without the presence of ABA-DEACM. On the contrary, the formation of filopodia and lamellopodia (arrows) was observed within 15 min of irradiation with 405 nm light in the presence of ABA-DEACM. Scale bar 10 μm. Images shown are representative results repeated in three wells for three independent experiments. B) The enlarged images of the outlined region in A) at different time points during the time course of imaging after uncaging. Obvious membrane ruffling can be observed starting at 5 min.