Published in final edited form as:

Curr Opin Chem Biol. 2009 December ; 13(5-6): 678–686. doi:10.1016/j.cbpa.2009.09.026.

Light Activation as a Method of Regulating and Studying Gene Expression

Alexander Deiters

North Carolina State University, Department of Chemistry, Raleigh, NC 27695-8204, USA, (919) 513-2958 (phone), (919) 515-5079 (fax)

Alexander Deiters: alex_deiters@ncsu.edu

SUMMARY OF RECENT ADVANCES

Recently, several advances have been made in the activation and deactivation of gene expression using light. These developments are based on the application of small molecule inducers of gene expression, antisense- or RNA interference-mediated gene silencing, and the photochemical control of proteins regulating gene function. The majority of the examples employ a classical “caging technology”, through the chemical installation of a light-removable protecting group on the biological molecule (small molecule, oligonucleotide, or protein) of interest and rendering it inactive. UV light irradiation then removes the caging group and activates the molecule, enabling control over gene activity with high spatial and temporal resolution.

INTRODUCTION

The activity of genes is precisely orchestrated in a spatial and temporal fashion. Moreover, multiple genes can be wired to each other, thus creating highly complex genetic networks. In order to study the function of genes and gene networks, the genes need to be deactivated or activated with Nature's level of spatio-temporal resolution. Here, light irradiation represents a unique tool, since it can be easily and precisely controlled in timing, location, and amplitude.

This review focuses on recent developments (published mostly between January 2008 and May 2009) in the photochemical control of gene function in cell culture and multi-cellular organisms. Developments which did not involve the use of cellular systems are not discussed here, but several reviews complementing this one can be found in the literature [1–6].

Caging technology

A classical approach to render biologically active small molecules or biological macromolecules temporarily inactive is through chemical modification with a light-removable protecting group, so called “caging group” [6]. Irradiation, typically with non-photodamaging UV light of >360 nm, releases the biologically active molecule (from its cage) allowing it to perform its cellular function (Figure 1). This enables the switching of biological processes (on or off) using light. Besides precise spatial and temporal control over biological processes, caging technologies can also be used to produce rapid, repetitive release of effector molecules or finely graded changes in the magnitude of activation. Caged molecules are generated through

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the installation of a caging group, most often a 2-nitrobenzyl group, on an oxygen, sulfur, or nitrogen atom thereby inactivating the molecules biological function. Upon exposure of the caged compound to UV light, a photochemical reaction is initiated, restoring the biologically active molecule.

Light-activated small molecule inducers of gene expression

Several established control mechanisms of gene function rely on small molecule inducers of gene expression [7]. Due to their facile chemical manipulation, these organic ligands have become prime targets for the photochemical regulation of genes. In many of these cases, gene transcription is initiated or inhibited by a small molecule-protein interaction. Examples of caged small molecule inducers of gene expression include caged versions of estradiol, ecydson, tamoxifen, and isopropyl- β -D-thiogalactopyranoside [8–11].

The Tet-ON system is a commonly employed conditional gene control system in eukaryotic cells and is functional in a wide range of model organisms. Recently, a caged doxycycline molecule **1** was developed, which was used to regulate green fluorescent protein (GFP) expression under control of the Tet-ON system in cell culture using light irradiation [12]. This technology has now been extended to the photochemical control of GFP expression in brain slices from transgenic mice expressing GFP [13]. Moreover, surgically removed embryos from the same *CIG-tGFP* mouse line were incubated with **1** for 24 h in a mouse whole-embryo culture system. Irradiation (290–370 nm, 15 sec) was performed on either the whole embryo or localized to about three dorsal root ganglia. GFP fluorescence was imaged (Figure 3a), and only a low level of background fluorescence was detected in the non-irradiated embryo (Figure 3b), however, it should be noted that the embryo was exposed to a lower concentration of **1**. This low level of leakiness may be caused by **1** still binding to the Tet-repressor, since only a 58-fold reduced affinity of **1** compared to doxycycline was observed [13]. The irradiated embryo displayed GFP levels comparable to an embryo that was exposed to non-caged doxycycline (Figure 3a,c,d), especially when two subsequent irradiations were conducted. Spatial control of GFP activation was achieved as well (Figure 3e).

The Tet-ON/**1** system provides photochemical control of gene expression on the transcriptional level and thus needs several components (the TetR protein and a TetR DNA binding site) in addition to the small molecule to be functional. A simplified light-activated gene control system which only requires a short RNA sequence introduced into the 5' untranslated region (UTR) of the gene of interest, was recently reported [14]. This sequence encodes a full-length hammerhead ribozyme which has previously been shown to be active under physiological conditions [15], and leads to gene deactivation through self-cleavage and removal of the 5' cap from the mRNA, followed by inhibition of translation and mRNA degradation (Figure 4a). Toyocamycin, a natural antibiotic, efficiently inhibits ribozyme function *in vivo* at micromolar concentrations and thus affords gene expression. Importantly, the caged toyocamycin **2** is completely inactive and thus does not induce gene expression in mammalian cells until decaged through irradiation with UV light of 365 nm, as shown by a luciferase assay [14]. Moreover, it was possible to achieve spatially restricted activation of gene expression (GFP) in a monolayer of human embryonic kidney cells by localized irradiation using a fluorescence microscope (Figure 4). No diffusion and gene activation by the decaged toyocamycin in neighboring cells was observed.

Light-activated oligonucleotides for the regulation of gene expression

Most of the recently developed light-activation tools for the photochemical control of gene function are based on antisense and RNA interference approaches. Transient gene silencing represents one of the major approaches to study the function of particular genes, and antisense

agents provide powerful tools for the elucidation of gene activity during embryogenesis [16, 17]. Antisense agents can act via two mechanisms: 1) Degradation of target mRNA through enzymatic cleavage, where the enzymatic activity derives from recruited components, e.g. RNase H or the RNA-induced silencing complex (RISC), or is intrinsic to the antisense agent, e.g. in the case of a ribozyme or a deoxyribozyme. 2) Inhibition of translation by sterically blocking the mRNA from access by the ribosome without inducing cleavage. Antisense technologies have been employed in various cell cultures and every developmental model organism, targeting a wide range of genes.

Two different approaches to the photochemical regulation of antisense activity have been developed. The first approach involves the installation of caging groups directly on the gene silencing agent, e.g. phosphorothioate DNA (SDNA) [18], small interference RNA (siRNA) [19,20], or a fluoro-analog thereof (siFNA) [21]. The caging groups are either installed on the nucleotide bases or the phosphate backbone, in order to prevent hybridization of the oligonucleotide to mRNA and mRNA cleavage (Figure 5a). Installation on the phosphates has the advantage that the RNA can be purchased or transcribed and then chemically modified. The disadvantage is the resulting statistical caging which cannot be controlled in number or position of caging groups, leading to a pool of differently caged oligonucleotides. This can lead to partial gene silencing (leakiness) and incomplete oligonucleotide activation due to non-quantitative decaging. In contrast, caging of the nucleotide base enables precise control over the location and number of caging groups, as well as complete decaging, but requires the synthesis of the caged monomeric building blocks, followed by custom oligonucleotide synthesis.

The second approach involves the inhibition of the activity of the antisense agent with a hybridized complementary oligonucleotide which is connected through a photocleavable tether, thus forming a hairpin (Figure 5b). This approach has the advantage that both oligonucleotides can be purchased and that only one photolysis needs to occur for activation. Disadvantages are a less direct caging approach which requires careful design of the oligonucleotides [22], and the necessity to perform synthetic transformations on the two oligonucleotides.

Direct caging of a phosphorothioate antisense agent was recently accomplished [18], and it was demonstrated that hybridization of a 19-mer SDNA antisense agent targeting the *Renilla* luciferase gene to its mRNA complement could be completely suppressed through the incorporation of three caged thymidine nucleotides **3** into the oligomer. The complete inactivity of the caged SDNA was also verified by a luciferase reporter assay in mouse fibroblast cells (NIH 3T3). A brief irradiation with UV light (365 nm) fully restored antisense activity. Moreover, spatial control was achieved by conducting localized irradiations of a cellular monolayer (Figure 6).

Photochemical regulation of siRNA activity in mammalian cell culture was achieved by incorporating the caged thymidine **4** (Figure 7) at a crucial site in the central region of the siRNA duplex [19]. This completely abrogated gene silencing; however, a brief UV irradiation (366 nm, 40 min, 2.88 J/cm²) initiated RNA interference leading to the downregulation of GFP.

Photochemical regulation of RNAi was also demonstrated via the statistical caging of the phosphate backbone (and potentially of the nucleobases as well), as shown in **5** [23]. This work was recently improved upon by the specific caging of terminal phosphate groups of an siRNA duplex leading to up to a 6-fold decrease in gene expression in mammalian cells after UV irradiation [20]. However, the caged siRNA was not completely inactive, displaying some leakiness. The Monroe lab extended the phosphate backbone caging to siFNAs (2'-fluoro oligonucleotides) which induce RNAi while displaying a higher intracellular stability than

RNA duplexes. For the efficient light-regulation of RNAi, 8–9 caging groups (see **6**) were installed on a 21-mer siFNA [21]. Only 10% leakiness was detected before irradiation and a 6-fold restoration of RNAi after light exposure of transfected BHK-21 cells (365 nm, 40 J/cm²) was achieved. Importantly, this technology was also applied to the silencing of mosaic GFP expression in zebrafish embryos (Figure 8) [21].

A fundamentally different approach to the spatio-temporal control of RNAi involves the TAT-mediated endocytosis and endosomal trapping of fluorescently-labeled shRNA, followed by photochemical excitation of the fluorophore and activation of RNAi through release of the shRNA from the endosome [24]. However, compared to the well established caging approaches to the control of RNAi, the generality of this approach still needs to be documented.

Recently, the Chen and Dmochowski labs described the application of the photocleavable hairpin-inhibition approach shown in Figure 5b to the photochemical activation of morpholino (MO) and peptide nucleic acid (PNA) antisense agents in zebrafish embryos [25,26]. They targeted three different endogenous genes, *no tail (ntl)* (see Figure 9), *chordin (chrd)*, and *bozozok (boz)*, and demonstrated spatial and temporal control over gene silencing using UV irradiation of 365 nm. Subsequently, the same photocleavable hairpin-approach was applied to the control of SDNA antisense activity in mammalian cell culture. The *c-myc* gene was targeted and complete inhibition of SDNA antisense activity through hairpin formation was achieved. A brief UV irradiation (350 nm, 5 min) restored 50% of the antisense activity, compared to a non-caged control SDNA oligonucleotide [27]. These examples demonstrate the versatility of the photocleavable hairpin-inhibition approach to the regulation of antisense function; however, the development of general rules for the design and synthesis of the antisense-inhibitor oligomer and its light-cleavable tether seemed to be more challenging than anticipated [22].

Light-activated proteins for the regulation of gene expression

Proteins have traditionally been caged in a non-specific fashion (mostly on surface lysine residues) through isolation, installation of a reactive caging group, and subsequent purification [5]. This approach affords little control over photochemical regulation, as multiple caging groups are installed, and caging of essential residues is not guaranteed. More specific approaches include the semi-synthesis of caged proteins via native chemical ligation [28], and the application of chemically misacylated suppressor tRNAs delivering photocaged amino acids [29,30]. Recently, the protein biosynthetic machinery of bacterial, yeast, and mammalian cells has been engineered to incorporate caged analogs of tyrosine [31], cysteine [32], serine [33], and lysine [34].

This technology was applied to the activation and deactivation of genes in a spatio-temporal fashion through photochemical control of DNA recombination in human embryonic kidney cells (Figure 10) [35]. Importantly, the caged Cre recombinase enzyme was completely inactive before irradiation due to the caging of a tyrosine (Y324) hydroxyl group essential for catalysis. More than 70% of catalytic activity was restored through UV irradiation.

The Moffat lab reported the photochemical regulation of protein function without the need of installing caging groups. They took advantage of a naturally occurring light-receptor, the so-called Light, Oxygen, and Voltage (LOV) sensitive domain. LOV domains are found in phototropin proteins and utilize a non-covalently bound flavin-mononucleotide as a chromophore cofactor for the absorption of blue light. Light-absorption promotes the system into an excited triplet state and initiates covalent bond formation between the FMN and a cysteine residue of the LOV protein. The LOV domain then transduces a signal to the attached effector domain of the phototropin, e.g. a kinase or a transcription factor. The system thermally

reverts back to the initial non-signaling, non-adduct state. A fusion kinase YF1 was generated, by replacing the natural sulfate transport anti-sigma factor antagonist domain in YtvA with the histidine kinase domain of FixL, which exhibited a 1000-fold reduction in kinase activity when exposed to white light (Figure 11) [36]. Subsequently, a reporter construct expressing *lacZ* under control of the *B. japonicum* FixK₂ promoter to which phosphorylated FixJ binds was used to demonstrate the light-regulation of gene expression in *E. coli*. Upon introduction of plasmids expressing both YF1 and FixJ, β -galactosidase activity was suppressed 70-fold when the cells were exposed to white light.

An interesting application of the LOV domain towards the intracellular regulation of enzymatic activity of Rac1 was recently reported [37]. Rac1 is a member of the Rho family of small Ras-like GTPase, and is involved in the regulation of the actin cytoskeleton and cell adhesion. Fusing the LOV domain to Rac1 inhibits its activity in the dark due to steric blocking (Figure 12a). However, an irradiation with light of 458 nm induces a conformational change in the LOV domain that leads to unwinding of the adjacent α helix, thus unblocking Rac1 and restoring its activity. HeLa cells expressing the Rac1-LOV construct were quiescent until irradiated, which lead to the instantaneous formation of lamellipodial protrusions and membrane ruffles along the cell edges (Figure 12b). Moreover, the obtained reversible light-regulation of Rac1 activity could be used to generate directed cell movement with high spatial and temporal resolution.

Other recent applications of LOV domain fusions include the regulation of DNA binding by the Trp repressor [38], and dihydrofolate reductase activity [39]. A similar engineering of *E. coli* cells has previously been demonstrated to enable bacterial photography [40]. Due to the photochemical inactivation of protein function and thus gene expression, this approach is complementary to the light-activation of caged proteins.

Other approaches to the light-regulation of gene expression

Laser-mediated heating and gene expression in single cells of zebrafish embryos [41], and more recently *C. elegans* [42], has been reported. A localized increase in temperature, proportional to the power of the infrared laser was achieved (Figure 13a). By targeting single cells in transgenic *C. elegans* expressing GFP under control of a heat-shock promoter, it was found that irradiation with a laser power of 11 mW for 1 s was sufficient for gene induction in single seam cells [42]. Approximately 40% of targeted cells expressed GFP, with no expression visible in other cells (Figure 13b). At 13 mW, the frequency of GFP expression in targeted cells reached 60%, but expression was also observed in non-targeted cells.

Another fundamentally different approach to the light-activation of transgene expression in cell culture has been achieved through the light-triggered cellular delivery of transgenic DNA from diazobenzene containing vesicles [43]. If this approach proves to be generally applicable, it may be possible to photochemically release other cargo (e.g. siRNAs, antisense agents, proteins) as well.

CONCLUSIONS

Several advances towards the photochemical regulation of gene function with high spatial and temporal resolution have been recently made. These developments are driven by the need to precisely control biological processes inside live cells and organisms, in order to elucidate their detailed mechanisms. The discussed approaches use a wide range of classical gene regulation tools based on small molecule inducers, antisense agents, siRNAs, and proteins, and adapt them towards the photochemical regulation of gene function. Future developments will show if these approaches can be employed in hypothesis driven research projects. A minimal

background activity of the caged molecule and an excellent on/off ratio after light irradiation will be major factors in the applicability of these new technologies.

Acknowledgments

Financial support from the National Institutes of Health (R01GM079114) is acknowledged. AD is a Beckman Young Investigator and a Cottrell Scholar.

References

- Casey JP, Blidner RA, Monroe WT. Caged siRNAs for Spatiotemporal Control of Gene Silencing. *Mol Pharm* 2009;6:669–685. [PubMed: 19371085]
- Young DD, Deiters A. Photochemical control of biological processes. *Org Biomol Chem* 2007;5:999–1005. [PubMed: 17377650]
- Tang X, Dmochowski IJ. Regulating gene expression with light-activated oligonucleotides. *Mol Biosyst* 2007;3:100–110. [PubMed: 17245489]
- Mayer G, Heckel A. Biologically active molecules with a “light switch”. *Angew Chem Int Ed* 2006;45:4900–4921.
- Curley K, Lawrence DS. Light-activated proteins. *Curr Opin Chem Biol* 1999;3:84–88. [PubMed: 10021410]
- Adams SR, Tsien RY. Controlling cell chemistry with caged compounds. *Annu Rev Physiol* 1993;55:755–784. [PubMed: 8466191]
- Buskirk AR, Liu DR. Creating small-molecule-dependent switches to modulate biological functions. *Chem Biol* 2005;12:151–161. [PubMed: 15734643]
- Cruz FG, Koh JT, Link KH. Light-activated gene expression. *Journal of the American Chemical Society* 2000;122:8777–8778.
- Lin W, Albanese C, Pestell RG, Lawrence DS. Spatially discrete, light-driven protein expression. *Chem Biol* 2002;9:1347–1353. [PubMed: 12498888]
- Link KH, Shi Y, Koh JT. Light activated recombination. *J Am Chem Soc* 2005;127:13088–13089. [PubMed: 16173704]
- Young DD, Deiters A. Photochemical activation of protein expression in bacterial cells. *Angew Chem Int Ed* 2007;46:4290–4292.
- Cambridge SB, Geissler D, Keller S, Curten B. A caged doxycycline analogue for photoactivated gene expression. *Angew Chem Int Ed* 2006;45:2229–2231.
- Cambridge SB, Geissler D, Calejari F, Anastassiadis K, Hasan MT, Stewart AF, Huttner WB, Hagen V, Bonhoeffer T. Doxycycline-dependent photoactivated gene expression in eukaryotic systems. *Nat Methods* 2009;6:527–531. [PubMed: 19503080] A caged small molecule inducer of gene expression is used to control GFP expression in mouse and *Xenopus* embryos. Gene activation was achieved in a spatio-temporal fashion
- Young DD, Garner RA, Yoder JA, Deiters A. Light-activation of gene function in mammalian cells via ribozymes. *Chem Commun* 2009:568–570. The photochemical deactivation of a ribozyme located in the 5' untranslated region of luciferase and GFP enables the activation of these genes in mammalian cell culture. An advantage of this system is the small size of the RNA regulatory element
- Yen L, Svendsen J, Lee JS, Gray JT, Magnier M, Baba T, D'Amato RJ, Mulligan RC. Exogenous control of mammalian gene expression through modulation of RNA self-cleavage. *Nature* 2004;431:471–476. [PubMed: 15386015]
- Achenbach TV, Brunner B, Heermeier K. Oligonucleotide-based knockdown technologies: antisense versus RNA interference. *Chembiochem* 2003;4:928–935. [PubMed: 14523910]
- Scherer LJ, Rossi JJ. Approaches for the sequence-specific knockdown of mRNA. *Nat Biotechnol* 2003;21:1457–1465. [PubMed: 14647331]
- Young DD, Lusich H, Lively MO, Yoder JA, Deiters A. Gene silencing in mammalian cells with light-activated antisense agents. *Chembiochem* 2008;9:2937–2940. [PubMed: 19021142] Caging groups directly installed on a phosphorothioate antisense agent enable the photochemical regulation

of gene silencing in cell culture in a localized fashion. Excellent off/on ratios before/after light irradiation are obtained.

- 19•. Mikat V, Heckel A. Light-dependent RNA interference with nucleobase-caged siRNAs. *RNA* 2007;13:2341–2347. [PubMed: 17951332]A single caged nucleotide incorporated into an siRNA targeting GFP enabled efficient light-induced gene silencing in mammalian cell culture.
20. Shah S, Jain PK, Kala A, Karunakaran D, Friedman SH. Light-activated RNA interference using double-stranded siRNA precursors modified using a remarkable regiospecificity of diazo-based photolabile groups. *Nucleic Acids Res* 2009;37:4508–4517. [PubMed: 19477960]
- 21••. Blidner RA, Svoboda KR, Hammer RP, Monroe WT. Photoinduced RNA interference using DMNPE-caged 2'-deoxy-2'-fluoro substituted nucleic acids in vitro and in vivo. *Mol Biosyst* 2008;4:431–440. [PubMed: 18414741]Fluoro-modified siRNAs have been rendered light-activatable through the statistical installation of caging groups on the phosphate backbone. UV irradiation induced GFP silencing in cell culture and in a zebrafish embryo.
22. Richards JL, Tang X, Turetsky A, Dmochowski IJ. RNA bandages for photoregulating in vitro protein synthesis. *Bioorg Med Chem Lett* 2008;18:6255–6258. [PubMed: 18926697]
23. Shah S, Rangarajan S, Friedman SH. Light-activated RNA interference. *Angew Chem Int Ed* 2005;44:1328–1332.
24. Endoh T, Sisido M, Ohtsuki T. Spatial regulation of specific gene expression through photoactivation of RNAi. *J Control Release* 2009;137:241–245. [PubMed: 19376174]
- 25••. Shestopalov IA, Sinha S, Chen JK. Light-controlled gene silencing in zebrafish embryos. *Nat Chem Biol* 2007;3:650–651. [PubMed: 17717538]A light-activatable morpholino antisense agent was generated by inhibiting its function with a sense oligonucleotide, linked by a photocleavable tether. UV irradiation cleaved the tether, removed the inhibitor strand, and led to efficient downregulation of the *ntl* gene in zebrafish embryos.
- 26••. Tang X, Maegawa S, Weinberg ES, Dmochowski IJ. Regulating gene expression in zebrafish embryos using light-activated, negatively charged Peptide nucleic acids. *J Am Chem Soc* 2007;129:11000–11001. [PubMed: 17711280]A light-activatable PNA antisense agent was generated by inhibiting its function with a sense oligonucleotide, linked by a photocleavable tether. UV irradiation cleaved the tether, removed the inhibitor strand, and led to efficient downregulation of the *chrd* and *boz* gene in zebrafish embryos.
- 27•. Tang X, Swaminathan J, Gewirtz AM, Dmochowski IJ. Regulating gene expression in human leukemia cells using light-activated oligodeoxynucleotides. *Nucleic Acids Res* 2008;36:559–569. [PubMed: 18056083]A light-activatable phosphorothioate antisense agent was generated by inhibiting its function with a sense oligonucleotide, linked by a photocleavable tether. UV irradiation cleaved the tether, removed the inhibitor strand, and restored 50% of antisense activity.
28. Hahn ME, Muir TW. Photocontrol of Smad2, a multiphosphorylated cell-signaling protein, through caging of activating phosphoserines. *Angew Chem Int Ed* 2004;43:5800–5803.
29. Mendel D, Ellman JA, Schultz PG. Construction of a Light-Activated Protein by Unnatural Amino-Acid Mutagenesis. *J Am Chem Soc* 1991;113:2758–2760.
30. Philipson KD, Gallivan JP, Brandt GS, Dougherty DA, Lester HA. Incorporation of caged cysteine and caged tyrosine into a transmembrane segment of the nicotinic ACh receptor. *Am J Physiol Cell Physiol* 2001;281:C195–206. [PubMed: 11401842]
31. Deiters A, Groff D, Ryu Y, Xie J, Schultz PG. A genetically encoded photocaged tyrosine. *Angew Chem Int Ed* 2006;45:2728–2731.
32. Wu N, Deiters A, Cropp TA, King D, Schultz PG. A genetically encoded photocaged amino acid. *J Am Chem Soc* 2004;126:14306–14307. [PubMed: 15521721]
33. Lemke EA, Summerer D, Geierstanger BH, Brittain SM, Schultz PG. Control of protein phosphorylation with a genetically encoded photocaged amino acid. *Nat Chem Biol* 2007;3:769–772. [PubMed: 17965709]
34. Chen PR, Groff D, Guo J, Ou W, Cellitti S, Geierstanger BH, Schultz PG. A facile system for encoding unnatural amino acids in mammalian cells. *Angew Chem Int Ed* 2009;48:4052–4055.
- 35••. Edwards WF, Young DD, Deiters A. Light-Activated Cre Recombinase as a Tool for the Spatial and Temporal Control of Gene Function in Mammalian Cells. *ACS Chem Biol* 2009;4:441–445. [PubMed: 19413301]By employing unnatural amino acids mutagenesis, a tyrosine residue

- necessary for catalytic activity in Cre recombinase was replaced with a photocaged analog. Spatial control of DNA recombination using UV irradiation was demonstrated in mammalian cell culture.
- 36••. Moglich A, Ayers RA, Moffat K. Design and Signaling Mechanism of Light-Regulated Histidine Kinases. *J Mol Biol* 2008;385:1433–44. [PubMed: 19109976] A natural light-receptor was fused to a kinase engineered to achieve photochemical control over phosphorylation. This enabled the photochemical control of transcription in bacterial cells without the need for any caging chemistry.
- 37••. Wu YI, Frey D, Lungu OI, Jaehrig A, Schlichting I, Kuhlman B, Hahn KM. A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* 2009;461:104–108. [PubMed: 19693014] A fully genetically encoded fusion protein of a natural light-receptor and Rac1 enables spatial and temporal control of mammalian cell motility using light irradiation.
38. Strickland D, Moffat K, Sosnick TR. Light-activated DNA binding in a designed allosteric protein. *Proc Natl Acad Sci U S A* 2008;105:10709–10714. [PubMed: 18667691]
39. Lee J, Natarajan M, Nashine VC, Socolich M, Vo T, Russ WP, Benkovic SJ, Ranganathan R. Surface sites for engineering allosteric control in proteins. *Science* 2008;322:438–442. [PubMed: 18927392]
40. Levskaya A, Chevalier AA, Tabor JJ, Simpson ZB, Lavery LA, Levy M, Davidson EA, Scouras A, Ellington AD, Marcotte EM, et al. Synthetic biology: engineering *Escherichia coli* to see light. *Nature* 2005;438:441–442. [PubMed: 16306980]
41. Halloran MC, Sato-Maeda M, Warren JT, Su F, Lele Z, Krone PH, Kuwada JY, Shoji W. Laser-induced gene expression in specific cells of transgenic zebrafish. *Development* 2000;127:1953–1960. [PubMed: 10751183]
- 42••. Kamei Y, Suzuki M, Watanabe K, Fujimori K, Kawasaki T, Deguchi T, Yoneda Y, Todo T, Takagi S, Funatsu T, et al. Infrared laser-mediated gene induction in targeted single cells in vivo. *Nat Methods* 2009;6:79–81. [PubMed: 19079252] The application of an IR laser enabled the spatially restricted heating of single cells in the worm *C. elegans* and the activation of genes under control of a heat-shock promoter
43. Liu YC, Le Ny AL, Schmidt J, Talmon Y, Chmelka BF, Lee CT. Photo-assisted gene delivery using light-responsive catanionic vesicles. *Langmuir* 2009;25:5713–5724. [PubMed: 19435291]

Figure 1.

General decaging reaction, and a classical *ortho*-nitrobenzyl caging group. Chemical and photochemical properties of the caging group can be tuned with suitable substituents, including: $\text{R}^1 = \text{H}$, $\text{R}^2 = \text{H}$; $\text{R}^1 = \text{OCH}_3$, $\text{R}^2 = \text{H}$; $\text{R}^1 = \text{OCH}_3$, $\text{R}^2 = \text{CH}_3$. Adapted with permission the Royal Society of Chemistry from: *Org. Biomol. Chem.* 2007, **5**:999–1005.

Figure 2. Light-activated small molecule inducers of gene expression, caged doxycycline (1) and caged toyocamycin (2). The caging group (blue) is removed through UV irradiation.

Figure 3.

Photoactivated gene expression in a mouse embryo. (a) Fluorescence image of a control embryonic day 10.5 (E10.5) embryo (*CIG-tGFP*) incubated for 24 h with 20 μ M doxycycline, displaying widespread GFP fluorescence particularly in neurons of the spinal cord dorsal root ganglia, the developing heart and in trigeminal ganglia of the head. (b) Fluorescence image of an E10.5 embryo incubated with 2.6 μ M of **1** without irradiation. (c) E10.5 embryo after a single 15 sec pulse of UV light, and (d) after a second pulse of irradiation 3 h later. (e) Spatially restricted fluorescence in three dorsal root ganglia of an E10.5 embryo after localized irradiation at the tip of the tail with a spot size of about three ganglia (circle). Adapted by permission from Macmillan Publishers Ltd: *Nat. Methods* 2009, **6**:527–531, copyright 2009.

Figure 4.

A functional hammerhead ribozyme located in the 5' UTR of a gene leads to gene silencing through 5' cap removal. The irradiation of **2** leads to toyocamycin formation which inhibits ribozyme function and induces gene expression (e.g. luciferase or GFP). This enables spatial control of GFP activation in a monolayer of HEK293T cells. The area within the white circle was irradiated with UV light (360 nm, 30 sec), and the cells were imaged after 24 h to allow for GFP expression and maturation. Adapted with permission of the Royal Society of Chemistry from: *Chem. Commun.* 2009, 568–570.

Figure 5.
Two different approaches to the photochemical regulation of gene downregulation via antisense agents/RNA interference.

Figure 6. Caged thymidine nucleotide **3**. The caging group (blue) is removed through UV irradiation. a) Spatial regulation of *Renilla* luciferase expression using caged SDNA antisense agents. The cellular monolayer was only irradiated inside the white circle (365 nm, 5 min, 23 W), leading to localized silencing of luciferase in cells transfected with the caged SDNA. b) Cells which have been locally irradiated but not been transfected with the caged SDNA do not show luciferase silencing. Adapted with permission from: *ChemBioChem* 2008, **9**: 2937–2940. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

Figure 7. Base- and phosphate-caged nucleotides **4** and **5–6**, respectively. The caging group (blue) is removed through UV irradiation.

Figure 8.

Zebrafish embryos at 28 hours past fertilization (hpf); the box indicates the region in which image analysis was performed. Caged and control siFNA were co-injected with a GFP expression plasmid. Exposure to UV light (365 nm) was performed at 7 hpf, and photographs were taken at 28 hpf. Caged siFNA injections resemble control GFP mosaic expressions, but light exposure activated the siFNAs and resulted in the silencing of GFP expression. Adapted with permission of the Royal Society of Chemistry from: *Mol. Biosyst.* 2008, **4**:431–440.

Figure 9.

Zebrafish embryos at 24 hpf. a) *Ntl* null mutant displaying a characteristic phenotype with a greatly reduced tail and misshapen somites. b) An embryo injected with an anti-*ntl* MO shows the same phenotype, while c) an embryo injected with a caged anti-*ntl* MO shows a wild-type phenotype. d) Irradiation of embryos injected with the caged MO at the sphere stage results in a clean *ntl* mutant phenotype, thus validating light-activation of gene silencing. Adapted by permission from Macmillan Publishers Ltd: *Nat. Chem. Biol.* 2007, 3:650–651, copyright 2007.

Figure 10.

Cre recombinase was rendered inactive through site-specific incorporation of an *ortho*-nitrobenzyl protected tyrosine residue at position Y324. Irradiation with UV light (365 nm) restores activity and triggers DNA recombination. a) HEK293T cells uniformly expressing DsRed. b) Transfection of wild-type Cre recombinase leads to the excision of the DsRed gene and the activation of GFP expression. c) Caged Cre recombinase is completely inactive, until d) irradiated with UV light (365 nm, 5 min), thus catalyzing DNA recombination. Adapted with permission from: *ACS Chem. Biol.* 2009, **4**:441–445. Copyright 2009 American Chemical Society

Figure 11.

Kinase activity of fusion kinase YF1. a) The YF1 autophosphorylation and then transfer of the phosphoryl group to its substrate, the response regulator FixJ, is inhibited by light irradiation. b) This photochemical switching of activity is visualized on two gels which display a time course of FixJ phosphorylation with [γ - 32 P] ATP, catalyzed by YF1. Adapted from: *J. Mol. Biol.* 2008, **385**:1433–1444, with permission from Elsevier.

Figure 12.

a) Reversible light-regulation of the Rac1-LOV fusion protein. b) HeLa cells expressing Rac1-LOV showed lamellipodial protrusions and membrane ruffles within minutes after light irradiation. Adapted by permission from Macmillan Publishers Ltd: *Nature* 2009 **461**:104–108, copyright 2009

Figure 13.

a) Relationship between induced cellular temperature and power of the IR laser 5 s after the initiation of irradiation. b) Infrared-laser irradiation (11 mW, 1 s) induced cytoplasmic GFP expression, under control of a heat-shock promoter, in the targeted seam cell (arrow) of an L4 larva 3 h after irradiation. Adapted by permission from Macmillan Publishers Ltd: *Nat. Methods* 2009, **6**:79–81, copyright 2009.