Photochemical control of biological processes

Douglas D. Young and Alexander Deiters*

Received 9th November 2006, Accepted 7th December 2006
First published as an Advance Article on the web 20th December 2006
DOI: 10.1039/b616410m

Photochemical regulation of biological processes offers a high level of control to study intracellular mechanisms with unprecedented spatial and temporal resolution. This report summarizes the advances made in recent years, focusing predominantly on the *in vivo* regulation of gene function using irradiation with UV light. The majority of the described applications entail the utilization of photocaging groups installed either on a small molecule modulator of biomolecular function or directly on a biological macromolecule itself.

Introduction

In order to elucidate biological processes on a molecular level, precise external control over these processes is required.[1–4] Light represents an ideal external control element as it possesses several advantages over traditional modulators of gene function. Most importantly, light irradiation can be easily controlled in a spatial and a temporal fashion, conveying spatiotemporal control of biological activity to the system under study. Light irradiation is a non-invasive technique that results in minimal secondary perturbation of cellular processes, and the potential to regulate its amplitude enables the ability to tune the desired biological effect. Hence, the photochemical regulation of gene function is a rapidly advancing research field in the functional genomics area.

Several reviews regarding the photochemical activation of small molecules and biological macromolecules exist;[5–11] however, this *Emerging Area* report focuses predominantly on *in vivo* applications of light activatable molecules, with particular attention paid to the light-activation of gene function. It provides a non-comprehensive overview of recent developments in the application

North Carolina State University, Department of Chemistry, Campus Box 8204, Raleigh, NC 27695, USA. E-mail: alex_deiters@ncsu.edu; Fax: +1-919-515-5079; Tel: +1-919-513-2958

Douglas Young was born in Colorado, US, and in 2003 received BS degrees in Chemistry and Biology from the University of Puget Sound in Tacoma, Washington. He is currently a graduate student at North Carolina State University working under the supervision of Professor Alexander Deiters. He has received numerous awards and is currently a GAANN (Graduate Assistance in Areas of National Need) Biotechnology fellow. His current research interests include the application of solid-supported multicomponent reactions towards the preparation of libraries of biologically active compounds, and the development of photochemical regulation systems for the study of genes.

Douglas Young

Alexander Deiters

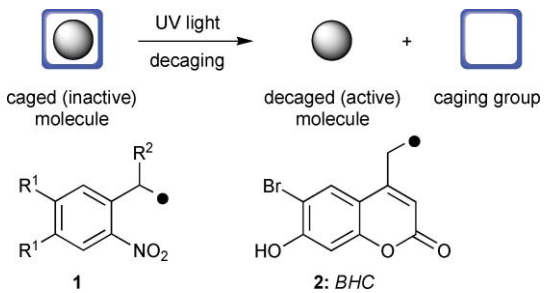
Alexander Deiters, born in Germany, studied Chemistry at the University of Münster, where he received his diploma degree in 1998 and his doctoral degree in 2000 for work in Professor Hoppe's group on novel cyclization reactions with enantioenriched allyllithium compounds. In 2001 he joined Professor Stephen Martin's lab at The University of Texas at Austin where he worked as a postdoctoral fellow on the total synthesis of indole alkaloids. In 2002 he began another postdoctorate in Professor Peter Schultz's lab at The Scripps Research Institute in La Jolla, where he was engaged in the in vivo *incorporation of unnatural amino acids into proteins. Since 2004 he has been an Assistant Professor of Chemistry at North Carolina State University and his current research interests include the development of novel chemical tools to answer biological questions with a particular emphasis on the photochemical switching of gene activity. He has received several awards in recognition of his research accomplishments, including fellowships from the German National Academic Foundation, the Fund of the Chemical Industry, the Alexander von Humboldt-Foundation, and the German Research Foundation. He was recently awarded a Basil O'Connor Starter Scholar Award from the March of Dimes Foundation.*

This journal is © The Royal Society of Chemistry 2007

of light towards controlling the activity of genes and proteins in a cellular environment.

Light activation of molecules: photocaging

Light-induced activation of biological processes is most commonly achieved through the initial deactivation of a particular molecule through installation of a photoprotecting group at a critical position. This renders the molecule inactive, in a practice known as "caging".[6] The photoprotecting group is removed upon irradiation with UV light, thus restoring the biological activity, in a practice known as "decaging" (Scheme 1). Several very effective caging groups are known, including derivatives of the 2-nitrobenzyl group **1** and the coumarin moiety **2**. Nitrobenzyl groups **1** are by far the most common caging groups due to their facile synthesis and easy installation on the molecule under study. They are typically decorated with electron donating groups (*e.g.* OCH_3) to shift the absorption maximum to a longer wavelength, allowing efficient decaging with non-photodamaging UV light of 365 nm (Scheme 1).

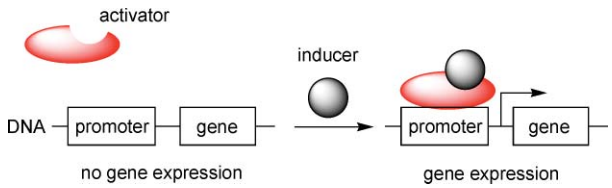


Scheme 1 General decaging reaction and caging groups mentioned in this report. Substituents on **1** include: $R^1 = H$, $R^2 = H$: *o*-nitrobenzyl (*ONB*); $R^1 = OCH_3$, $R^2 = H$: 4,5-dimethoxy-2-nitrobenzyl (*DMNB*); $R^1 = OCH_3$, $R^2 = CH_3$; (4,5-dimethoxy-2-nitrophenyl)ethyl (*DMNPE*). **2**: 6-bromo-7-hydroxycoumarin-4-ylmethyl (*BHC*).

Caged small-molecule modulators of gene function

Many mechanisms of gene regulation *in vivo* rely on small molecule inducers of gene expression. 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. Most commonly the small molecule binds to a protein, the activator, which subsequently binds to a promoter sequence on the DNA, thus turning on gene expression (Scheme 2). Examples of small molecule modulators of gene expression include doxycycline/tetracycline, lactose, β-estradiol, and ecdysone.[1–4] Several of these molecules have been caged to achieve photoregulation of expression systems.

The first example of employing a caged small molecule in an inducible gene control system was based on the estrogen receptor, and was reported by the Koh group.[12] The estrogen receptor (ER) is a nuclear hormone receptor that acts as a ligand-dependent transcriptional activator in eukaryotes. Upon binding of estradiol (**4**, Fig. 1), the ER undergoes a conformational change that leads to release from a binding protein. It then binds to a specific eukaryotic promoter, the estrogen response element, activating transcription (Scheme 2). Koh *et al.* synthesized the 3-OH photocaged estradiol



Scheme 2 General mechanism of many small molecule inducers of gene activation. Corresponding activator–promoter pairs discussed in this report are the estrogen receptor–estrogen response element and the reverse tetracycline-controlled transactivator–tetracycline responsive promoter element (Tet-ON system).

5 and exposed HEK293 cells harboring a luciferase reporter system under control of the ER to this compound. Cells irradiated with UV light showed 86% of maximum luciferase activity, in contrast to non-irradiated cells which only showed minimal background activity.

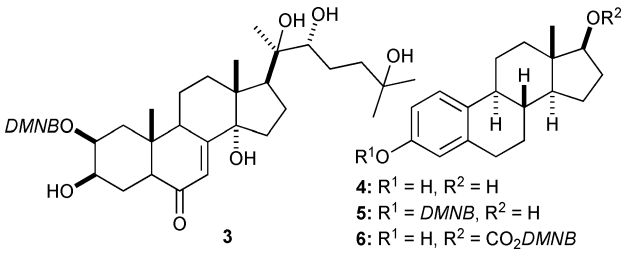


Fig. 1 Photocaged ecdysone **3** and estradiol **4–6**.

Subsequently, Lawrence *et al.* achieved photochemical control of an ecdysone-inducible gene expression system employing the same strategy.[13] Caging of ecdysone was accomplished on the most reactive 2-hydroxy position, leading to **3**. A luciferase reporter under control of the ecdysone inducible system was transfected into mammalian cells, and probed for luciferase expression with and without light irradiation. This was achieved in a spatial fashion *via* spot irradiation with a 100 W Hg lamp through a light microscope. Luciferase expression was detected at approximately 60% of the level induced by the natural molecule, and spatial control was achieved in tissue culture. Advantages of both systems include low basal expression and high inducibility upon light irradiation. Moreover, the ecdysteroids are exogenous to mammalian systems and appear to have no toxic effects.

A problem associated with both systems is the limited duration of transcriptional activation through diffusion of estradiol and ecdysone out of the cell. Recently, Koh *et al.* solved this problem by creating a permanent light-induced switching event through covalent bond formation between the activated ligand and the estrogen receptor (Fig. 2).[14] Here, they employed a tamoxifen selective mutant of the ligand binding domain of the ER fused to Cre recombinase. In combination with the photocaged tamoxifen aziridine **7**, which is decaged to **8** and then reacts with a cysteine residue in the binding pocket of the ER, recombination at a level of 26% of maximal activity (β-galactosidase reporter gene) was observed after 3 UV irradiations at 24 h intervals. Although the low level of gene activity, the necessity for multiple irradiations, and the potential diffusion of decaged **8** into neighboring cells prevents this from being a system with broad applicability, it represents a step further towards a general light-induced gene activation.

This journal is © The Royal Society of Chemistry 2007

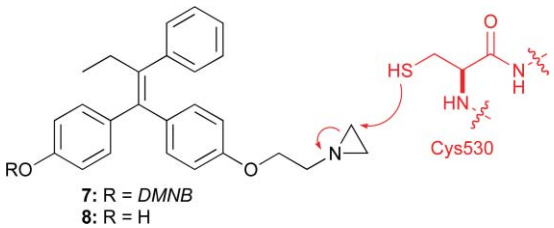


Fig. 2 Caged tamoxifen aziridine **7** for irreversible estrogen receptor activation. After decaging to **8**, a cysteine in the binding pocket of the receptor undergoes covalent bond formation.

As an application to showcase the utility of photocaged nuclear hormones in a model organism, Hayashi and co-workers recently synthesized the estradiol **6**, which is caged on the 17-OH group, and employed it towards gene regulation in transgenic *Arabidopsis* plants.[15] An estrogen receptor based transactivator system was employed to strongly express green fluorescent protein (GFP) in the presence of the small molecule activator. As a means of comparison the caged estradiol **5** was also employed, and in both cases GFP expression was regulated *via* irradiation with light; surprisingly, only compound **6** was capable of providing discrete spatial resolution (Fig. 3). The researchers employed a similar tactic to regulate a gene responsible for the development of the lateral root and root hairs. While limited by small molecule delivery and diffusion, the ability to use light to control developmental processes within complex organisms was demonstrated.

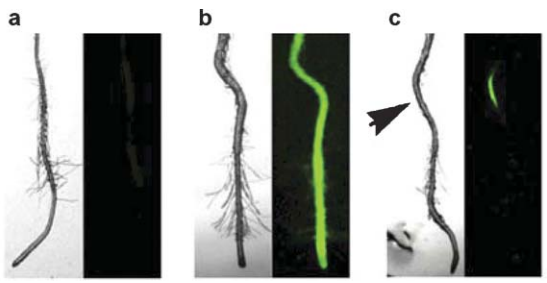


Fig. 3 Spatial control over GFP expression in *Arabidopsis* roots using **6**. (a) No light irradiation; (b) whole root irradiation; (c) spot irradiation. Reprinted from ref. 15, Copyright 2006, with permission from Elsevier.

The Tet system is a commonly employed conditional gene control system in eukaryotic cells and is functional in a wide range of model organisms.[3,4,16] Cambridge and co-workers recently developed a caged doxycycline molecule **9** (through the reaction of doxycycline with the commercially available diazo *DMNPE* **14** in 16% yield), which was used to regulate GFP expression under control of the Tet-ON system in cell culture (Fig. 4).[17] In the absence of light the doxycycline remained inactive, resulting in no GFP expression. However, after a brief irradiation, the caging group was removed, leading to the production of the transgenic GFP in cell culture. To demonstrate the ability to achieve spatial control in a higher organism, the system was employed in the regulation of β-glucuronidase (GUS) in transgenic tobacco leaves.[17] The GUS transgene was under the control of a Tet-ON regulated CMV (cytomegalovirus) promoter. After incubation of the plant tissue with the caged doxycycline, followed by partial irradiation, areas exposed to UV light expressed the GUS protein and thus displayed a blue pigmentation (Fig. 4).

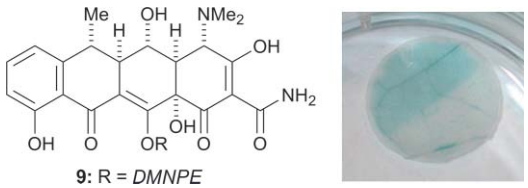
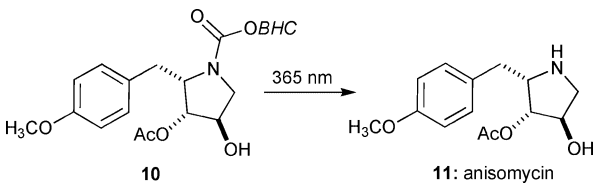


Fig. 4 Caged doxycycline **9** and its application in spatial control of gene expression in plant tissue using the Tet-ON system. Reprinted from ref. 17, Copyright 2006, with permission from Wiley-VCH.

While successful, the Tet-ON system does suffer intrinsically from a delayed induction, with maximal protein levels being detected after 12–14 h, and potential diffusion problems of decaged doxycycline. Nevertheless, this example displays photo-triggering of a significant gene expression system and demonstrates the feasibility of obtaining spatiotemporal control within cells and tissues.

Recently, Dore and co-workers employed light-activation of a small molecule towards the opposite aim: the inhibition of protein expression.[18] Although the caging of inhibitors of biological processes is not new,[9,19,20] inhibitory regulation in a spatial fashion was reported for the first time. The ribosome inhibitor anisomycin (**11**) was caged by installing a *BHC* caging group (Scheme 1) on the pyrrolidine ring *via* a carbamate linker (**10**). This caging group was superior to other, more commonly used caging groups (*DMNB* and *p*-nitrobenzyl), since it has a 10-fold greater quantum efficiency and a large absorption cross-section, making it amenable to two-photon decaging at 740 nm. Complete decaging of **10** was observed after a brief 2 s irradiation at 365 nm (Scheme 3). Its potential to control protein expression *in vivo* was successfully demonstrated in cell culture using GFP as a reporter gene. Irradiation of a subset of cells on a plate showcased the spatial control of the developed methodology; only irradiated cells exhibited decreased fluorescence, whereas surrounding non-irradiated cells showed constant or increased fluorescence. Future experiments will reveal if this approach is amenable to the study of biological processes within a multi-cellular model organism. It is surprising that no two-photon excitation[21] studies have been reported, since the *BHC* group is an excellent caging group for this purpose.



Scheme 3 Light-activation of protein synthesis inhibitor **11** through decaging of **10**.

In addition to the photochemical regulation of gene function, several reports describe the caging of low-molecular weight regulators of other biological processes, including Ca^{2+},[22] phosphatidic acid,[23] and nitric oxide.[24]

While most work towards photochemical gene regulation using caged small molecules involves their interaction with proteins, Deiters and Young recently achieved the photochemical regulation of ribozyme activity using caged theophylline as an exogenous allosteric regulator.[25] This light-activated hammerhead ribozyme

has the potential to be employed as a novel photochemical gene regulation tool.[26]

Caged biological macromolecules

A more direct means of obtaining control over biological systems is *via* the installation of caging groups directly on the active biomacromolecule.[6,7,9,27] Gene regulation can occur on the DNA, RNA and protein level, and all of these macromolecules have been investigated for photoregulation.

Proteins have traditionally been caged in a non-specific fashion (mostly on lysine residues) through isolation, installation of a reactive caging group *in vitro*, and subsequent purification. This approach affords little control over photochemical regulation as multiple caging groups are installed, and caging of essential residues is not guaranteed. Additionally, this is limited to accessible amino acid residues, and often to only those exposed at the surface of the protein. An alternative approach is the chemical synthesis of caged peptides and proteins and their subsequent introduction into the biological system.[28–30] The stepwise synthesis of proteins limits both their size and their quantity. These problems have been addressed through the native chemical ligation[31] allowing for the semi-synthesis of caged proteins.[32]

An alternative approach involves site-specific caging using unnatural amino acid mutagenesis with chemically synthesized misacylated tRNAs, as developed by Schultz and co-workers.[33] Although, this technology provides site-specific incorporation of caged amino acids into proteins and has been demonstrated to regulate the activity of ion channels in *Xenopus* oocytes by the groups of Dougherty and Lester,[34] a limiting factor is the laborious chemical synthesis of the misacylated tRNA. Recently, the Schultz group modified the translational machinery of yeast and bacterial cells by introducing a completely orthogonal tRNA–tRNA synthetase pair.[35] Two tRNA synthetases were engineered to only accept a caged tyrosine[36] **12** and a caged cysteine[37] **13** as the substrate (Fig. 5), and to charge the corresponding tRNA with these amino acids. This tRNA then allows for site-specific *in vivo* incorporation of the caged amino acid into proteins by the ribosome, in response to the amber stop codon, TAG. The *in vivo* incorporation of **12** was employed in the caging of a β-galactosidase reporter gene at the essential residue Tyr503. Cells incorporating the caged tyrosine displayed greatly reduced β-galactosidase activity, which was subsequently restored through UV irradiation at 365 nm (67% of wild-type activity, as demonstrated by a Miller assay). This technology removes limitations associated with the previous misacylation approach and enables the production of substantial quantities of site-specifically caged proteins *in vivo*. However, it is currently limited to tyrosine and cysteine amino acids and the employed *ONB* caging group is not optimal for irradiation with non-photodamaging UV light.

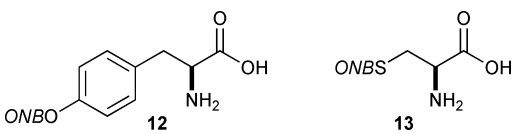
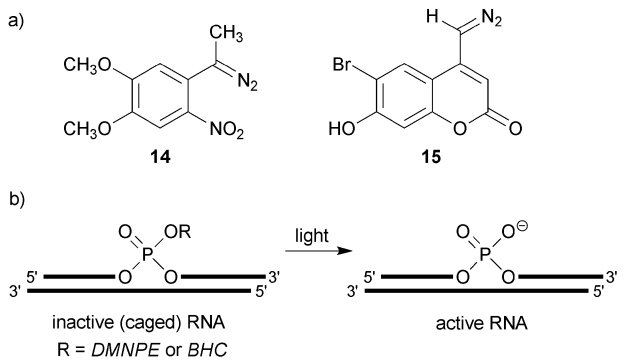


Fig. 5 Caged tyrosine **12** and caged cysteine **13**.

While proteins have been the traditional targets to achieve photoregulation of biological processes, more recently nucleic acid photocaging has been explored. Oligonucleotides have been found to be regulators of the expression of genetic information *via* a multitude of means, including siRNA, ribozymes, antisense, and aptamers.[38] The majority of nucleic acid photocontrol involves the statistical caging of the phosphorus backbone of both DNA and RNA.

Friedman and co-workers demonstrated the caging of a siRNA molecule targeting the GFP gene.[39] They conducted a non-specific caging of double stranded RNA using the diazo caging group **14** (Scheme 4). This reactive intermediate is readily generated from a commercially available kit (Invitrogen/Molecular Probes), and can be used to photoprotect a wide range of weak acids with a pK_a of 3–7, including carboxylic acids, phenols, and phosphates (Scheme 4).[11] They determined a caging efficiency of 3% with approximately 1.4 caging groups per RNA molecule. A significant difference in GFP expression was observed between the caged and irradiated siRNAs in mammalian cell culture; however, some GFP down-regulation was still observed with the caged siRNA. This was resolved by increasing the caging efficiency to 15%, but resulted in decreased photoinduced activation, potentially due to the lack of removal of all caging groups. Future work will reveal if this approach is amenable to the spatial control of RNA interference.



Scheme 4 (a) Diazo derivatives of caging groups **14** and **15**, and (b) their application in the non-specific caging of RNA.

The groups of Okamoto and Tsien recently reported light-activated gene expression in zebrafish embryos by employing chemically modified, caged mRNA.[40,41] Specifically, *in vitro* generated mRNA encoding GFP or β-galactosidase reporter genes was treated with diazo-modified hydroxycoumarin **15**, resulting in the formation of caged mRNA (Scheme 4). This RNA was injected into the cytoplasm of one-cell-stage zebrafish embryos which were subsequently incubated at 28 °C in the dark. After irradiation with UV light, two-thirds of the embryos showed expression of GFP, whereas non-irradiated zebrafish displayed little to no fluorescence (Fig. 6). Quantification of β-galactosidase activity indicated a 4.5-fold increased expression level after irradiation. Moreover, spatially restricted activation of caged mRNA was achieved by using a microscope equipped with a special illumination system. Although an interesting approach, the level of activation achieved is modest and the necessity to transcribe mRNA *in vitro*, followed by caging with varying efficiency, hampers a widespread application of this technology.

Light regulation can also be accomplished by specific installation of a caging group on a nucleotide base followed by

 This journal is © The Royal Society of Chemistry 2007

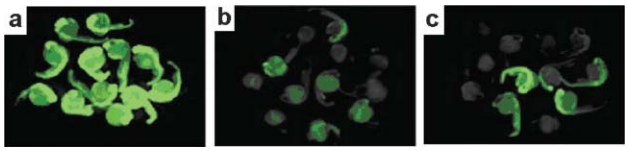
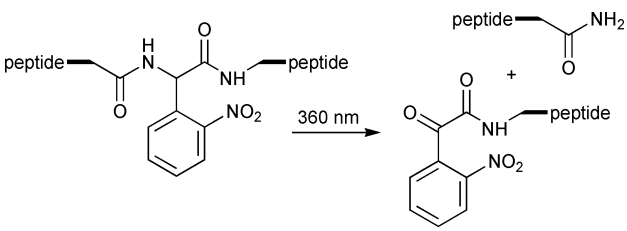


Fig. 6 Photochemical regulation of gene function in zebrafish through injection of caged mRNA. (a) wtGFP mRNA; (b) caged GFP mRNA; (c) caged GFP mRNA, followed by irradiation with UV light (365 nm, 10 s). Reprinted by permission from Macmillan Publishers Ltd from ref. 40, copyright 2001.

chemical synthesis of the nucleic acid oligomer. This principle was demonstrated in the caging of a DNAzyme,[33] and the caging of a DNA aptamer.[42,43] Although photoactivation assays were only conducted *in vitro*, both approaches have conceivable *in vivo* applications.[38,44]

Photocleavable biological macromolecules

Dougherty and co-workers demonstrated the photolytic cleavage of 2-nitrophenylglycine (Npg) incorporated into proteins *in vivo* (Scheme 5).[45] Specifically, this approach was employed to probe the structure–function relationships of ion channel domains. The Npg residue was site-specifically incorporated into intracellular, extracellular, and transmembrane positions of both a potassium ion channel and the nicotinic acetylcholine receptor by employing the tRNA misacylation approach. In all cases protein cleavage was observed after UV irradiation. While the cleavage efficiency was only approximately 50%, these studies were still effective in the assignment of vital structural motifs for ion channel function. This methodology provides an alternative means to photocaging when probing protein function with light; however, prolonged irradiation times of 4 h might not be compatible with most biological systems and prevent a high temporal resolution.

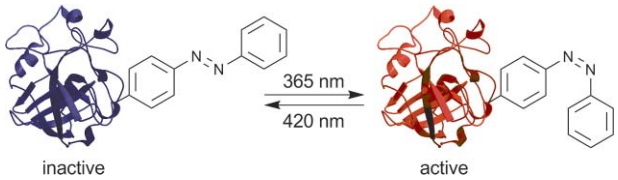


Scheme 5 Photochemical cleavage of a peptide/protein containing 2-nitrophenylglycine (Npg).

In vitro applications of the photochemical cleavage of a variety of biological macromolecules, including DNA,[46] proteins,[47] and peptides[48,49] have been reported as well.

Photoswitchable biological macromolecules

Although the vast majority of light-regulated biological processes involve the application of caging groups, the potential to photoregulate biological macromolecules through the incorporation of photoswitchable small organic molecules has also been realized.[50] Typically, this is achieved by incorporating a diazobenzene moiety, whose configuration can be reversibly switched from *trans* to *cis* through light irradiation (Scheme 6), into a polypeptide,[51] a protein,[52] double-stranded DNA,[53–55] or single-stranded RNA.[56]



Scheme 6 Configurational light-switching of a diazobenzene group attached to a biological macromolecule, thus converting the macromolecule from an inactive into an active form.

Recently, Loudwig and Bayley generated a protein pore which was modified with a single diazobenzene residue.[57] This was achieved by introducing a single cysteine residue into one subunit of the α-hemolysin heptamer, and subsequent bioconjugation of a thiol-reactive diazobenzene molecule. The pore was assembled within a lipid bilayer by combining this subunit with wild-type subunits. The photochemical switching event could be detected on the single molecule level by applying an electric potential and measuring the current across the bilayer. Isacoff, Trauner, and co-workers successfully applied the same approach *in vivo* using GluR6, a glutamate responsive ion channel.[58] Here, the diazobenzene was not only covalently linked to a cysteine residue which was engineered into the pore, but was also connected to a glutamate allosteric activator. Photochemical switching to the *cis* form allowed binding of the glutamate to the binding pocket of GluR6, thus opening the ion channel and allowing the flow of Na^+, K^+, and Ca^{2+}. This was conducted in transfected HEK293 cells and the cation flow into the cell was measured by Ca^{2+} imaging and whole-cell patch clamping. These experiments revealed that the engineered channel does not open completely upon irradiation with UV light of 380 nm, but it is fully closed when exposed to 500 nm light.

Engineering of cellular light receptors

A fundamentally different way to obtain regulation of gene expression on a transcriptional level has been achieved *via* the engineering of natural proteins which respond to light. This has been recently demonstrated by the groups of Quail, Ellington, and Voigt in the development of photoresponsive cells.[59,60]

Quail and co-workers reported a truly light-switchable cellular system which is based on the classical yeast two-hybrid technology, allowing the regulation of gene expression in eukaryotic cells.[60] Here, a phytochrome chromophore domain (Phy) is fused to the DNA binding domain of a GAL4 transcriptional activator. Through absorption of a red photon, the phytochrome is converted from its inactive form (Pr) into an active conformer (Pfr). This active conformer Phy(Pfr) then undergoes a specific interaction with a basic helix–loop–helix protein (PIF3), which itself is fused to the activating domain of the GAL4 transcriptional activator. Hence, irradiation with red light recruits the transcriptional machinery towards expression of a reporter gene (*lacZ* or *HIS3*) downstream of the GAL4 promoter sequence. Detectable increase of the *lacZ* reporter was present five minutes after a 60 s irradiation and reached a maximal 1000-fold increase after three hours. Furthermore, photochemical control of the *HIS3* reporter gene conveyed growth to a histidine auxotroph strain on histidine deficient media in the presence of light (Fig. 7). Upon irradiation

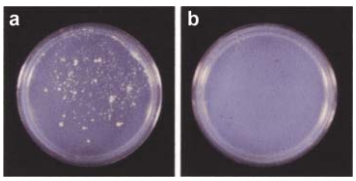
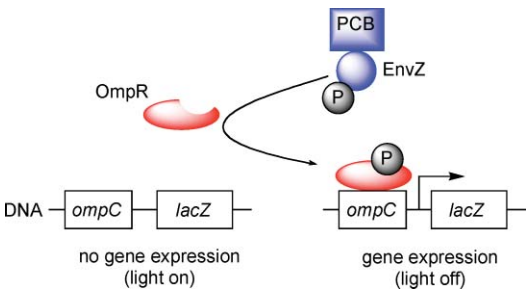


Fig. 7 Yeast two-hybrid assay employing a *HIS3* reporter gene on selective media. Cell growth is only observed in case of irradiation with red light for three days (a). No growth was observed in the dark (b). Reprinted by permission from Macmillan Publishers Ltd from ref. 60, copyright 2002.

with light of a different wavelength (far-red), it was possible to turn off gene expression instantly.

In a related approach, Ellington *et al.* created photoresponsive bacteria by fusing a membrane bound cyanobacterial photoreceptor (PCB) to a histidine kinase domain (EnvZ).[59] The kinase domain is responsible for phosphorylation of a transcriptional activator (OmpR), which then binds to a corresponding promoter region (*ompC* promoter), thus effectively turning on gene expression (Scheme 7). In the absence of light the kinase domain is active, promoting gene expression. However, upon light exposure a conformational change inhibiting kinase activity is induced, thus turning both phosphorylation of OmpR and gene expression off.



Scheme 7 Assembled multi-component system to achieve transduction of a light irradiation event to the deactivation of a reporter gene.

This method was employed in bacterial lithography using a β-galactosidase reporter system encoded by the *lacZ* gene, demonstrating the feasibility of obtaining excellent spatial control of gene expression using light (Fig. 8).

Fig. 8 Bacterial photographs using two different inverted image masks. Reprinted by permission from Macmillan Publishers Ltd from ref. 59, copyright 2005.

An intrinsic limitation of both systems is the need for the complete exclusion of ambient light, which interferes with the selective activation. Moreover, the phycocyanobilin cofactor is not naturally produced in yeast and bacterial cells, and needs to be added to the cell media. Ellington *et al*. elegantly solved this problem through the addition of the phycocyanobilin biosynthetic pathway to *Escherichia coli*.[59]

Conclusions

The photochemical regulation of biological processes can be achieved *via* multiple methodologies. These initial studies demonstrate the potential to successfully elucidate biological function through the spatiotemporal regulation of both genes and proteins with light. This photochemical regulation enables studies that are not possible with traditional methodologies, and paves the way for a bright future for this emerging field of research.

While *in vivo* applications of photoactivatable systems have been demonstrated by a variety of research groups, significant work must still be achieved to make photoregulation generally applicable, especially for use in multicellular organisms. Virtually all applications involve the regulation of simple reporter systems, and further studies must strive to probe endogenous gene function in a reliable fashion. Additionally, two-photon excitation has the potential to provide a higher level of spatial resolution with minimal photodamaging effects to the irradiated tissue. Finally, more general, and perhaps switchable, techniques for photochemical control of biological processes need to be developed.

Acknowledgements

We gratefully acknowledge financial support by the North Carolina State University Faculty Research and Development Fund, the American Chemical Society Petroleum Research Fund, the March of Dimes Birth Defects Foundation (grant 5-FY05-1215), and the Department of Education (GAANN Fellowship for DDY). We apologize to those researchers whose work could not be cited as a result of space limitations.

References

1 L. A. Banaszynski and T. J. Wandless, *Chem. Biol.*, 2006, **13**, 11–21.
2 A. R. Buskirk and D. R. Liu, *Chem. Biol.*, 2005, **12**, 151–161.
3 M. Lewandoski, *Nat. Rev. Genet.*, 2001, **2**, 743–755.
4 M. Padidam, *Curr. Opin. Plant Biol.*, 2003, **6**, 169–177.
5 M. Goeldner and R. Givens, *Dynamic studies in biology: phototriggers, photoswitches and caged biomolecules*, Wiley-VCH, Weinheim, 2005.
6 G. Mayer and A. Heckel, *Angew. Chem., Int. Ed.*, 2006, **45**, 4900–4921.
7 X. Tang and I. J. Dmochowski, *Mol. BioSyst.*, DOI: 10.1039/b614349k.
8 G. Dorman and G. D. Prestwich, *Trends Biotechnol.*, 2000, **18**, 64–77.
9 D. S. Lawrence, *Curr. Opin. Chem. Biol.*, 2005, **9**, 570–575; K. Curley and D. S. Lawrence, *Curr. Opin. Chem. Biol.*, 1999, **3**, 84–88.
10 S. R. Adams and R. Y. Tsien, *Annu. Rev. Physiol.*, 1993, **55**, 755–784.
11 J. A. McCray and D. R. Trentham, *Annu. Rev. Biophys. Biophys. Chem.*, 1989, **18**, 239–270.
12 F. G. Cruz, J. T. Koh and K. H. Link, *J. Am. Chem. Soc.*, 2000, **122**, 8777–8778.
13 W. Lin, C. Albanese, R. G. Pestell and D. S. Lawrence, *Chem. Biol.*, 2002, **9**, 1347–1353.
14 K. H. Link, Y. Shi and J. T. Koh, *J. Am. Chem. Soc.*, 2005, **127**, 13088–13089.
15 K. Hayashi, K. Hashimoto, N. Kusaka, A. Yamazoe, H. Fukaki, M. Tasaka and H. Nozaki, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2470–2474.
16 M. Mallo, *Front. Biosci.*, 2006, **11**, 313–327.
17 S. B. Cambridge, D. Geissler, S. Keller and B. Curten, *Angew. Chem., Int. Ed.*, 2006, **45**, 2229–2231.
18 M. Goard, G. Aakalu, O. D. Fedoryak, C. Quinonez, J. St Julien, S. J. Poteet, E. M. Schuman and T. M. Dore, *Chem. Biol.*, 2005, **12**, 685–693.
19 K. Curley and D. S. Lawrence, *Pharmacol. Ther.*, 1999, **82**, 347–354.
20 C. Jiang and E. M. Schuman, *Trends Biochem. Sci.*, 2002, **27**, 506–513.
21 E. Niggli and M. Egger, *Front. Biosci.*, 2004, **9**, 1598–1610.
22 W. Li, J. Llopis, M. Whitney, G. Zlokarnik and R. Y. Tsien, *Nature*, 1998, **392**, 936–941.

This journal is © The Royal Society of Chemistry 2007

23 J. Goedhart and T. W. Gadella, Jr, *Biochemistry*, 2004, **43**, 4263–4271.
24 C. M. Pavlos, H. Xu and J. P. Toscano, *Curr. Top. Med. Chem. (Hilversum, Neth.)*, 2005, **5**, 637–647.
25 D. D. Young and A. Deiters, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2658–2661.
26 A. U. Khan and S. K. Lal, *J. Biomed. Sci. (Basel)*, 2003, **10**, 457–467.
27 Y. Shigeri, Y. Tatsu and N. Yumoto, *Pharmacol. Ther.*, 2001, **91**, 85–92.
28 K. Zou, W. T. Miller, R. S. Givens and H. Bayley, *Angew.Chem., Int. Ed.*, 2001, **40**, 3049–3051.
29 W. F. Veldhuyzen, Q. Nguyen, G. McMaster and D. S. Lawrence, *J. Am. Chem. Soc.*, 2003, **125**, 13358–13359.
30 M. E. Vazquez, M. Nitz, J. Stehn, M. B. Yaffe and B. Imperiali, *J. Am. Chem. Soc.*, 2003, **125**, 10150–10151.
31 P. E. Dawson and S. B. Kent, *Annu. Rev. Biochem.*, 2000, **69**, 923–960.
32 M. E. Hahn and T. W. Muir, *Angew. Chem., Int. Ed.*, 2004, **43**, 5800–5803.
33 D. Mendel, J. A. Ellman and P. G. Schultz, *J. Am. Chem. Soc.*, 1991, **113**, 2758–2760.
34 K. D. Philipson, J. P. Gallivan, G. S. Brandt, D. A. Dougherty and H. A. Lester, *Am. J. Physiol.*, 2001, **281**, C195–206.
35 T. A. Cropp and P. G. Schultz, *Trends Genet.*, 2004, **20**, 625–630.
36 A. Deiters, D. Groff, Y. Ryu, J. Xie and P. G. Schultz, *Angew. Chem., Int. Ed.*, 2006, **45**, 2728–2731.
37 N. Wu, A. Deiters, T. A. Cropp, D. King and P. G. Schultz, *J. Am. Chem. Soc.*, 2004, **126**, 14306–14307.
38 R. R. Breaker, *Nature*, 2004, **432**, 838–845.
39 S. Shah, S. Rangarajan and S. H. Friedman, *Angew. Chem., Int. Ed.*, 2005, **44**, 1328–1332.
40 H. Ando, T. Furuta, R. Y. Tsien and H. Okamoto, *Nat. Genet.*, 2001, **28**, 317–325.
41 H. Ando, T. Furuta and H. Okamoto, *Methods Cell Biol.*, 2004, **77**, 159–171.
42 R. Ting, L. Lermer and D. M. Perrin, *J. Am. Chem. Soc.*, 2004, **126**, 12720–12721.
43 A. Heckel and G. Mayer, *J. Am. Chem. Soc.*, 2005, **127**, 822–823.
44 A. Heckel, M. C. Buff, M. S. Raddatz, J. Muller, B. Potzsch and G. Mayer, *Angew. Chem., Int. Ed.*, 2006, **45**, 6748–6750.
45 P. M. England, H. A. Lester, N. Davidson and D. A. Dougherty, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 11025–11030.
46 X. Tang and I. J. Dmochowski, *Angew. Chem., Int. Ed.*, 2006, **45**, 3523–3526; P. Ordoukhanian and J. S. Taylor, *Bioconjugate Chem.*, 2000, **11**, 94–103.
47 J. P. Pellois, M. E. Hahn and T. W. Muir, *J. Am. Chem. Soc.*, 2004, **126**, 7170–7171.
48 C. J. Bosques and B. Imperiali, *J. Am. Chem. Soc.*, 2003, **125**, 7530–7531.
49 J. P. Pellois and T. W. Muir, *Angew. Chem., Int. Ed.*, 2005, **44**, 5713–5717.
50 C. Dugave and L. Demange, *Chem. Rev.*, 2003, **103**, 2475–2532.
51 Z. H. Zhang, D. C. Burns, J. R. Kumita, O. S. Smart and G. A. Woolley, *Bioconjugate Chem.*, 2003, **14**, 824–829.
52 H. A. Lester, M. E. Krouse, M. M. Nass, N. H. Wassermann and B. F. Erlanger, *J. Gen. Physiol.*, 1980, **75**, 207–232.
53 Y. Liu and D. Sen, *J.Mol. Biol.*, 2004, **341**, 887–892.
54 S. Keiper and J. S. Vyle, *Angew. Chem., Int. Ed.*, 2006, **45**, 3306–3309.
55 M. Z. Liu, H. Asanuma and M. Komiyama, *J. Am. Chem. Soc.*, 2006, **128**, 1009–1015.
56 S. Roy, R. Nagarajan, P. C. Wu, K. Yang, F. F. Bruno, V. S. Parmar, S. K. Tripathy, J. Kumar and L. A. Samuelson, *J. Macromol. Sci., Pure Appl. Chem.*, 2001, **38**, 1383–1392.
57 S. Loudwig and H. Bayley, *J. Am. Chem. Soc.*, 2006, **128**, 12404–12405.
58 M. Volgraf, P. Gorostiza, R. Numano, R. H. Kramer, E. Y. Isacoff and D. Trauner, *Nat. Chem. Biol.*, 2006, **2**, 47–52.
59 A. Levskaya, A. A. Chevalier, J. J. Tabor, Z. B. Simpson, L. A. Lavery, M. Levy, E. A. Davidson, A. Scouras, A. D. Ellington, E. M. Marcotte and C. A. Voigt, *Nature*, 2005, **438**, 441–442.
60 S. Shimizu-Sato, E. Huq, J. M. Tepperman and P. H. Quail, *Nat. Biotechnol.*, 2002, **20**, 1041–1044.