Targeting Expression with Light Using Caged DNA*  

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In this report, we describe the inactivation and site-specific light induction of plasmid expression using a photosensitive caging compound. Plasmids coding for luciferase were caged with 1-(4,5-dimethoxy-2-nitrophenyl)diazoethane (DMNPE) and transfected into ~1-cm diameter sites of the skin of rats with particle bombardment. Skin sites transfected with caged plasmids did not express luciferase. However, subsequent exposure of transfected skin sites to 355-nm laser light induced luciferase expression in proportion to the amount of light. Liposome transfection of HeLa cells with DMNPE-caged green fluorescent protein (GFP) plasmids showed similar results. Caging DNA with DMNPE blocks expression at the level of transcription, since in vitro production of mRNA from linearized GFP plasmid was also blocked by caging and subsequently restored by exposure to light. Under the reaction conditions of these experiments, our absorbance data indicate that each DMNPE-caged GFP plasmid contains ~270 caging groups. In addition to inhibition and subsequent restoration of plasmid bioactivity, the presence and photocleavage of this relatively small number of cage groups also alters electrophoretic mobility of plasmids and optical absorption characteristics. This light-induced expression strategy provides a new means to target the expression of genetic material with spatial and temporal specificity.

The unrealized goal of in vivo gene therapy is the controlled expression of exogenous genes exclusively within a target cell population. Successful in vivo gene therapy must overcome two significant challenges: 1) delivery of transgenes to the specific target cell population and 2) subsequent expression only within these cells. Viral and nonviral technologies for delivery of transgenes to specific target cells are currently under development, but none of these techniques is suitable in its current form for targeted delivery of transgenes in vivo.

Nonspecific delivery of “silent” genes followed by site-specific induction is one potential targeting strategy. Several postdelivery expression strategies have been described (1). These may be broadly classified into induction by tissue-specific promoters and induction by changes in the cellular environment. Tissue-specific promoters (2, 3) remain a promising technique but must be developed for each target cell population. Most current induction strategies add factors to the cellular milieu to control expression. These factors include metal ion concentration (4, 5), tetracycline (6, 7), hormones (8–10), and recently RNA-binding aptamers (11). However, targeted expression with environmental induction agents is difficult, since it requires limiting the in vivo distribution of the induction agents solely to the target cell population. To achieve site-specific expression, a strategy permitting spatially targeted induction is needed.

In this report, we describe a targeting strategy based on “cage” chemistry. Caged compounds have a covalently attached group that is rapidly cleaved upon exposure to near-UV light. Attachment of the caging compound renders the bioactive molecule inert, until photolysis releases it in its bioactive form (12, 13). Caged compounds have been used in a number of temporal biological studies to examine cell motility, the chemistry of muscle contractility, active transport proteins, biological membranes, and other intracellular responses (14, 15). Cage compounds have also been used in the caging of nucleotide analogs (16), in the synthesis of biochip oligonucleotides (17), and most recently to temporally control ribozyme reactions by including caged adenosine within synthesized RNA oligonucleotides (18). Application of caging chemistry to plasmid DNA offers the possibility of light-activated expression after delivery to cells, and furthermore, since light exposure can be spatially controlled, this photoactivation approach has the potential to produce targeted expression.

EXPERIMENTAL PROCEDURES

Plasmid Caging with DMNPE—5 mg of 4,5-dimethoxy-2-nitroacetophenone hydrazone and 50 mg of manganese(IV) oxide were gently agitated in 1 ml of Me6SO at 25 °C for 20 min (Molecular Probes, Inc., Eugene, OR). Manganese oxide was removed from the activated caging compound by filtering the solution through 100 mg of Celite™ (Blak-Ray, San Gabriel, CA; model B 100 AP). Spectrographic characterization of this lamp confirmed that the emission spectrum is 365 ± 8 nm (Triax-180 spectrograph; Instruments S.A., Edison, NJ). Approximately 5 min after the light exposure ended, the cuvette contents were rescanned, and the postlight absorbances were compared with the prelight spectra.

The abbreviations used are: DMNPE, 1-(4,5-dimethoxy-2-nitrophenyl)diazoethane; MOPS, 4-morpholinepropanesulfonic acid; GFP, green fluorescent protein.
Calculation of Caging Efficiency from Absorbance Measurements—

The extinction coefficient ($\epsilon_{355 \text{ nm}}$) of the bound caging DMNPE group was calculated from the local absorbance peak at 355 nm before exposure to 365-nm light of commercially available DMNPE-caged ATP (Molecular Probes). The extinction coefficient for DMNPE-ATP ($\epsilon_{355 \text{ nm}}$) was obtained from the expression,

$$\epsilon_{355 \text{ nm}} = \frac{A_{355 \text{ nm}}}{c b} \left(\frac{0.89(\mu CVm)}{1 \text{ cm}}\right) = 3540 \text{ m}^{-1} \text{ cm}^{-1} \quad (\text{Eq. 1})$$

where $A_{355 \text{ nm}}$ represents absorbance at 355 nm, $c$ represents molar concentration of solution, and $b$ represents path length of the cuvette (1 cm). The concentration value (75 mM) in this calculation was adjusted to account for the 89% of ATP that was reported to be caged (19). The extinction coefficient was then used to determine the molar concentration of DMNPE in the caged plasmid samples by measuring the absorbance at 355 nm. From these data, an average number of DMNPE caging groups per plasmid was then calculated.

DNA Gel Electrophoresis—150 ng of plGreenLantern-1 plasmid per well was run in 1% agarose in Tris acetate buffer (4 mM Tris acetate, 0.1 mM EDTA, pH 8.5) at 5 V/cm for 1 h 45 min. Gels were stained after electrophoresis with 1× SYBR-Gold nucleic acid gel stain (Molecular Probes) in 1× Tris acetate buffer for 15 min. The line profile analysis tool in Image Pro Plus Software (Media Cybernetics, Silver Spring, MD) was used to measure band intensities.

In Vitro Transcription—BamHI-linearized plGreenLantern-1 plasmid was used as the template for the MEGAscriptTm In Vitro Transcription Kit (Ambion Inc., Austin, TX, catalog no. 1330). 1 μg of DMNPE-caged and control plasmid templates were incubated for 4 h at 37 °C in the enzyme mixture supplied with the kit. Immediately prior to incubation, one caged sample was exposed to 365-nm light as described above. Gels were stained after electrophoresis with 1× SYBR-Gold nucleic acid gel stain (Molecular Probes) in 1× MOPS buffer at 4 V/cm for 25 min. 5 μl of RNA product from the kit was run in 1.5% agarose-formaldehyde in 1× MOPS buffer at 4 V/cm for 1 h. For caged samples, 10 μl of kit product was loaded to improve visualization of the caged versus caged light-exposed bands. Gel band intensities were quantified using the summation statistics tool in Image Pro Plus Software (Media Cybernetics, Silver Spring, MD). Intensity values for each pixel were summed for each lane of the gel. Background values were measured over three separate empty lanes of the gel (not shown), averaged, and subtracted from all other measured values.

Caged Green Fluorescent Protein (GFP) Expression in HeLa Cells—

HeLa cells were liposome- transfected with plasmids coding for GFP. 1 μg of plGreenLantern-1 plasmid was added to the caged group containing 100 μl of Opti-MEM medium (Life Technologies) and used for transfection. Cationic liposomes (Dioleoyl-3-Trimethylammonium-Propane Dioleoyl Phosphatidylethanolamine, 1:1) were prepared by vacuum evaporation followed by extrusion to yield unilamellar liposomes of 0.1 μm as described previously (20). HeLa cells were seeded onto 35-mm Petri dishes (Fisher) at 20,000 cells/cm² 18–18 h before transfection. 1 ml of the DNA-liposome complex in Opti-MEM was added to the cell culture dish for 3.5 h, after which the solution was replaced with 1 ml of Opti-MEM.

Both pre- and post-transfection effects of light were investigated. To study light-induced plasmid damage, matched cultures were transfected with plasmids that had been exposed to 5 J/cm² of light with the lamp described in the spectral scanning protocol, or no light before transfection. In a second group of cultures, after liposomal transfection with unexposed plasmids, culture dishes were individually exposed to 0.5, 2.6, or 5.6 J/cm² of light from the same light source. A third group of samples was transfected with unexposed DMNPE-caged pGFP and exposed to light in the same manner as the second group.

Following light exposure, Opti-MEM medium in all culture dishes was replaced with 2 ml of Dulbecco's modified Eagle's medium plus 10% calf serum. 48 h after transfection, cells were washed with cold calcium- and magnesium-free phosphate-buffered saline twice, trypsinized (0.15%) and paraformaldehyde-fixed (1%) for 5 min at room temperature. The fixed cells were then washed with calcium- and magnesium-free phosphate-buffered saline containing 1% formaldehyde three times, resuspended in calcium- and magnesium-free phosphate-buffered saline containing 1% formaldehyde, and stored at 4 °C for flow cytometry analysis. Transfection samples were analyzed by a FACSCalibur (Becton-Dickinson) flow cytometer equipped with an argon laser exciting at a wavelength of 488 nm. For each sample, 20,000 gated events were collected by list-mode data consisting of side scatter, forward scatter, and fluorescence emission centered at 530 nm (FL1), 580 nm (FL2), and 610 nm (FL3). Determination of positive events for GFP expression was made using a standard gating technique (20). Cytometric results from a nontransfected control sample were displayed on a dot plot of FL3 versus FL1 fluorescence intensity. A gate was drawn along a line of maximum detected FL1 intensity for the control events. This gate was kept constant through analysis of all subsequent measurements. The percentage of each transfection group attached to ATP and pGFP was determined. The number of events within this gate divided by the total number of events collected.

Particle-mediated Gene Delivery—The plasmid used for in vivo rat studies was the luciferase expression plasmid pCEP4 coding for luciferase and containing the SV40 PolyA signal (Promega, Madison, WI). The luciferase expression plasmid vector was obtained from Ref. 21. Briefly, plasmid DNA was precipitated on gold particles (Agracetus, Inc., Elmhurst, IL). After the particles adhered to the tubing, it was cut into ½-inch segments such that 0.5 μg of DNA-coated gold particles would be delivered with each dose. Prepared tubing segments were stored at –20 °C in a sealed, desiccated container. At the time of the experiment, the DNA-gold-coated tubes were brought to room temperature, loaded into the cylinder of the delivery device, and accelerated into the tissue by a rapid release of a pulse of helium with an electrically controlled valve at 400 p.s.i. Particles were deposited in a circular pattern with a diameter of ~0.8 cm. Male Harlan Sprague Dawley rats (250–300 g; Harlan Sprague Dawley, Indianapolis, IN) were housed in the Vanderbilt Veterans Affairs animal care facility maintained according to the American Association for Accreditation for Laboratory Animal Care Standards. The animals were allowed food and water ad libitum. All transfection and surgical procedures are carried out under general anesthesia with ketamine and xylazine. All animal procedures were approved under the guidelines of the local animal care and use committees. Cutaneous transfections were carried out with the animal under general anesthesia. Two rows of three sites per row were transcribed across the dorsal surface of each rat with a single dose of the expression construct or caged DNA. The site was prepared by clipping the pelts and by treatment with the depilatory agent, Neet (Reckitt and Colman Inc., Wayne, NJ) according to the manufacturer's recommendations. The plasmid-gold complexes were then transfected into the skin at the selected pressure by placing the muzzle of the gene gun in contact with the site. Within 1 h of particle-transfection, site groups of 6 rats were exposed to targeted pulses of light with a Nd:YAG laser tuned to 355 nm (Vanderbilt University Free Electron Laser Facility). The total dosage for each site was either 1 or 10.8 J/cm². Light was delivered in 10-mJ pulses at 10 Hz. The light pattern was circular and matched the dimensions of the transfection region. 24 h after transfection, animals were killed by carbon dioxide poisoning, pelts were removed, and tissue from transfection sites was isolated and halved. One half was sectioned, imaged, and analyzed in Image Pro Plus to determine average bead depth. The other half of each transfection site was homogenized in a lysis buffer containing 100 mM potassium phosphate, pH 7.8, and 1.0 mM dithiothreitol, 0.1% Triton X-100, and 10 mg/ml phenylmethylsulfonyl fluoride at 4 °C. Luciferase activity was measured in 20–μl aliquots of tissue lysate, with a 100-μl luciferase assay buffer consisting of 5 mM ATP, 15 mM MgCl₂, and 1 mM β-luciferin as provided by Promega using a liquid scintillation counter (Packard Instrument Co.) set for single photon counting. The luciferase activity was expressed as counts/min.

RESULTS

Spectral Scans—Fig. 1 shows the similarity of the absorbance spectra of DMNPE-caged pGFP plasmid prepared for these studies (top) and DMNPE-caged ATP obtained from Molecular Probes (bottom). The upper solid curves are absorbance spectra before caged materials were exposed to light, and the lower dotted curves are spectra obtained 5 min after exposure to 20 min of 365-nm light. Light produced small shifts in absorbance at 355 nm for both DMNPE-caged plGFP and commercially available DMNPE-caged ATP. After exposure to 365-nm light, increases in absorbance at 390 nm are observed for both compounds. This is presumably produced by photolysis of the caging group attached to ATP and pGFP. Native DNA plasmids
and ATP do not absorb in the region from 300 to 450 nm (lower solid curves).

**DNA Gel Electrophoresis**—Electrophoresis of caged plasmids shows characteristic changes in mobility corresponding to the addition and removal of the caging groups (Fig. 2). Typical plasmid conformation bands are observed; the bands with greatest mobility correspond to multiple conformations observed with supercoiled bands (S), the least mobile bands correspond to the nicked conformations (N), and the bands that appear between these two groups are presumed to be relaxed (R) (22). The markers in lane A are from an EcoRI digest of λ DNA. Lane B is caged plasmid. Lane C contains caged light-exposed plasmid exposed to 20 min of 365-nm light. Lane D contains plasmid that was exposed to the caging reaction conditions but without the caging compound. Typical plasmid conformation bands are observed; the bands with greatest mobility correspond to multiple conformations observed with supercoiled bands (S), the least mobile bands correspond to the nicked conformations (N), and the bands that appear between these two groups are presumed to be relaxed (R) (22).

**In Vitro Transcription Results**—Fig. 3 shows a denaturing agarose-formaldehyde gel (1.5%) of mRNA from *in vitro* transcription reaction of caged and caged light-exposed plasmids. BamHI-linearized Green Lantern plasmid was used as the template for the MEGAscript In Vitro Transcription Kit (Ambion Inc., Austin, TX; catalog no. 1330). B, mRNA product for native pGFP plasmids; C, caging reaction controls; D, caged pGFP; E, caged light-exposed pGFP. Lanes D and E were loaded with twice as much of the transcription kit product to improve visualization of the caged versus caged light-exposed bands.

**GFP Expression in HeLa Cells**—The expression level of native pGFP was 43 ± 3.9% (n = 11, mean ± S.E., gray bars, Fig. 4). Within each experiment, the percentages of GFP-positive cells were normalized with this positive control group. Without
exposure to light (i.e., with 0 J/cm² of 365-nm light) the fraction of HeLa cells that express caged pGFP (solid bar) is about one-fourth of the level of expression of native material (25.8%, n = 7). After exposure to 0.25 or 0.5 J/cm² of light, expression of the caged material increases to 50% of control. The asterisks indicate significant difference from expression of caged plasmids that received no light exposure (p < 0.05, Bonferroni’s t test). Cultures transfected with caged pGFP and treated with 2.8 and 5.6 J/cm² of light showed decreasing expression levels of 20 and 10%, respectively. Native GFP expression levels also decreased with increasing post-transfection light exposure, from a normalized 100% with no light exposure to 81, 24, and 10% with a light exposure of 0.5, 2.6, or 5.6 J/cm², respectively. Cultures exposed to 2.8 or 5.6 J/cm² of light after transfection showed significantly lower levels of expression than those that received no light (denoted by crosses, p < 0.05, Bonferroni’s t test). Plasmids exposed to the highest dose of light (5.6 J/cm²) before transfection (bar labeled Pre-Flash) express at levels equal to control plasmids that received no light.

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demonstrate the feasibility of light-induced expression and its potential for targeting expression. Plasmids coding for luciferase were caged with 1-(4,5-dimethoxy-2-nitrophenyl)diazoethane and transfected into −1-cm diameter sites of rat skin with gold particle bombardment (21). The presence of the DMNPE cage groups on plasmids blocks expression, sites transfected with caged plasmids have expression levels equal to nontransfected skin sites (Fig. 5). However, luciferase expression is induced at sites transfected with caged plasmid and subsequently exposed to 355-nm laser light. A low dose of 355-nm light (1 J/cm²) increases luciferase expression to 6 ± 3% of positive control. Increasing the light dosage by an additional factor of 10 and 20 produces additional increases in luciferase expression to 12 ± 4 and 17 ± 6% of positive control. Caged plasmids subjected to 1.6 J/cm² of light before particle-mediated delivery result in skin sites with the highest expression and are 40 ± 12% of positive control.

Results from cytometric analysis of HeLa cells liposome-transfected with DMNPE-caged GFP plasmid also show that caging can reversibly block expression. Cells transfected with caged pGFP do not express GFP at the levels of cultures transfected with native pGFP. However, with exposure to 0.5 J/cm² light, GFP expression is induced and increases from 25 to 50% of positive controls (Fig. 4). When doses of light greater than 0.5
J/cm² were applied to the transfected cultures, expression levels decreased. This decrease was further characterized in tests of 365-nm light exposure on native pGFP expression. As shown by the gray bars in Fig. 4, increasing the light dose decreases the percentage of GFP-expressing cells transfected with native pGFP. Light levels above a threshold level 0.5 J/cm² produced significantly lower levels of expression of native pGFP plasmid. In cultures transfected with native pGFP that had been given the highest exposure of light prior to transfection, expression levels were identical to nonexposed controls. This latter observation suggests that the decrease in expression is not due to plasmid damage caused by light exposure.

Subsequent in vitro investigation suggests that the mechanism of expression blockade occurs at the transcriptional level. In an in vitro transcription assay, caging DNA blocks mRNA production, but after light-induced photo-activation, mRNA transcription is restored. The pattern of light-induced in vitro transcription (Fig. 3) is very similar to the pattern of light-induced expression observed in the expression study in rat skin. As seen in the rat skin study, caged DNA has near background levels of transcription; mRNA produced by the transcription of a caged pGFP linear template is only 3.7% of positive control. Further parallel ing the in vivo expression results, the exposure of caged templates to 365-nm light increases the mRNA production 5-fold to 19% of control. As seen in vivo, complete restoration of bioactivity was not achieved despite matching the concentration of controls and caged products by absorbance at 260 nm. However, matching plasmid concentration using optical absorbance does not take into account the conformational state of the plasmids, which may be important for expression (23).

Indeed, a DNA gel comparison with native plasmids or control plasmids (Fig. 2) shows that a substantial proportion of the caged plasmid is nicked, which is not seen in native plasmid. Furthermore, electrophoretic comparison of caged plasmids and caged plasmids exposed to light also indicates that there is a reversible alteration in caged plasmid structure produced by exposure to light. The DMNPE-caged plasmids exhibit lower mobility, resulting in characteristic band shifts seen in agarose gel electrophoresis (Fig. 2). The greatest light-induced change is seen in the pair of relaxed conformation bands with apparent linear molecular size of 10 kilobases. In the caged state, the distribution of plasmid between the two relaxed bands is approximately equal. After exposure to light, 70% of the relaxed form appears in the more mobile band. These shifts are consistent with the addition of the nonpolar DMNPE groups that retard plasmid mobility by neutralizing negative charges on the phosphate linkages of the DNA backbone.

The characteristic absorbance of the DMNPE cage groups provides a convenient means to compare caged plasmids with other caged moieties. For example, light-induced changes in the absorbance of DMNPE-caged plasmid are similar to those observed with commercially available caged ATP (Fig. 1). The absorbance spectrums of DMNPE-caged ATP (Molecular Probes) and DMNPE-caged pGFP plasmid show similar peaks in absorbance at 355 nm (lower curves). Absorption at this wavelength is consistent with the presence of DMNPE caging groups, since native ATP and DNA do not absorb in this region. Approximately 5 min after a 20-min exposure to 365-nm light, similar increases in absorbance at 390 nm are observed for both compounds as a result of photolysis of the caging group (upper curves). These characterize the end result photoproducts and do not compare measurement of rapid photolysis events, such as those previously reported for caged ATP (12, 16).

Although no structural studies have been completed, a probable location of the reactive site on DNA is at the negatively charged phosphate backbone, with the most likely configuration illustrated in Fig. 6. The choice of this site is consistent with the attachment of this caging group to other moieties, which occurs at weak oxy acids, such as carboxylic acids and phosphates (16).

Based on the extinction coefficient of DMNPE-ATP, the number of caging groups per plasmid was determined from increases in 355-nm absorption after caging (Fig. 1). Although the pGreenLantern plasmid has ~10,000 phosphate sites available for caging, these calculations suggest that only ~270 cage groups are present. These caging reaction conditions apparently produce a low rate of reaction, since this is less than 3% of available phosphate sites.

Expression levels of the flashed caged plasmids in vivo (Fig. 4) and in vitro mRNA transcription levels (Fig. 3) both indicate that the plasmids remain viable after the addition and removal of the caging group. Furthermore, this does not appear to be a plasmid-specific phenomenon, since the caging technique is effective with several reporters (pGreenLantern, Fig. 2; pCEP4 Luciferase, Fig. 4; β-galactosidase, data not shown (24, 25)). However, under these experimental conditions, expression of these caged plasmids exposed to light never achieved that same level as native plasmid expression.

In the in vivo experiment, one possible explanation for incomplete restoration is that the optical properties of the skin attenuate 355-nm light, and increased light energy at the skin surface is needed to completely restore activity. Reduced light delivery to the gold particles due to light absorption by the skin prevents full re-expression, since this does not explain the similar expression percentage observed in the in vitro transcription assay or the HeLa cell expression studies. In this regard, it is important to note that no selection method was employed to separate caged intact plasmids from noncaged or damaged plasmids. After the caging reaction, plasmid structures probably range from plasmids with no bound cage groups to plasmids with a large number of cage groups as well as plasmids damaged during the cage reaction. This may offer an additional explanation of only partial restoration by light exposure. Further optimization by selecting only intact plasmid with favorable expression conformations should improve the
efficiency of light-induced gene expression from caged plasmids.

Previous reports suggest that the bioactivity of the released nitrosoketone from the photolyzed caged compound inside the target cell may be harmful (12, 16, 27). Most biological preparations that use caged compounds employ relatively high (millimolar) concentrations of the caged compounds to elicit the desired light-induced effect. At these concentrations, it has been reported that the reactive nitrobenzyl groups can form covalent adducts with reactive sulfhydryls, such as cysteine residues on proteins (12, 16, 27). However, an important difference between most caging applications and this one is that the concentration of released photoproduct from caged plasmid is much lower than the $10^{-3}$ M used in most other caging applications. In the in vitro transcription assay, the concentration of released photoproduct is calculated to be $\sim 1 \times 10^{-7}$ M. Based on our previous HeLa transfection studies (28), the total copies of pGFP are less than $10^5$/cell, which would produce less than $3 \times 10^{-17}$ mol of cage after photolysis, yielding an upper limit estimate of photoproduct concentration of $10^{-6}$ M. In the rat skin studies, this is more difficult to determine, but it is expected to be much lower than $10^{-3}$ M. While we cannot completely rule out the possibility of released photoproduct inhibiting re-expression, these inhibitory effects would be expected to be significantly lower than those seen in other biological studies using caged compounds.

Light at 355 nm falls into the UVA classification and is thought to be far less damaging to cells than UVB light (28, 29). The maximum light dosage used to uncage plasmids in these experiments is less than half of the UVA exposure that might be received in a single visit to a tanning parlor (28). However, little is known about the inhibitory effects of 355-nm light on plasmid expression. At this wavelength, light effects would not be expected, since increased expression is produced by increased light exposure in the in vivo studies. However, our GFP cytometry data from cultured HeLa cells after exposure to 365-nm light (Fig. 4) appear to contradict the in vivo findings and indicate some inhibitory effects on expression. Further investigation is necessary to determine how plasmid expression in cultured cells is affected by 365-nm light.

Interestingly, biochemical inactivation of DNA has a biological precedent. It perhaps has closest functional homology with DNA methylation, which occurs under cellular control to regulate transcription (30, 31). Although the mechanism of regulated expression by methylation is not well understood, DNA methylation and gene expression have a strong inverse correlation. The addition of a methyl group is apparently sufficient to block transcription. Similarly, our data suggest that the attachment of the somewhat larger DMNPE caging compound also inactivates DNA by blocking transcription.

In summary, this report is the first description of the application of photosensitive caging compounds and light to target the spatial and temporal expression of genetic materials. If this strategy proves useful, additional applications based on this caging technology could be developed to possibly prolong plasmid expression by protecting plasmids from methylation and degradation after delivery to cells or perhaps to protect antisense oligonucleotides from enzymatic degradation.

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REFERENCES


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