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Photoresponsive 5′-cap for the reversible photoregulation of gene expression

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ABSTRACT

Photoresponsive 5′-caps that can be reversibly *cis–trans* isomerized by light irradiation were developed for the reversible photoregulation of gene expression. The 8-naphthylvinyl cap (8NV-cap) in the *trans* form completely inhibited translation of mRNA, whereas the *cis* form yielded protein with the same efficiency as mRNA capped with the normal-cap, a 26-fold higher efficiency than that of the *trans* form. The 8NV-capped mRNA could be switched between a translating state (ON state) and a non-translating state (OFF state) in a reversible fashion by alternately irradiating with monochromatic 410 nm or 310 nm light.

Living processes are managed through the precise control of 'when, where, and how long (or how frequently)' genes are expressed. For example, expression of the *bicoid* protein over a period of several hours at the anterior tip of an oocyte syncytium organizes the anterior development of drosophila embryos.[1] The cyclic expression of the *Hes7* protein in the presomitic mesoderm (PSM) occurs every 2 h during somitogenesis and accompanies formation of the periodic structure of the somite.[2] Oscillatory expression of the *Hes1* protein contributes to diverse differentiation responses in embryonic stem (ES) cells.[3] To control such important biological events using external stimuli is a long-cherished dream for biologist. The most promising external trigger is photoirradiation because it allows accurate and easy control of the location and time at which an event occurs. One common approach to the photoregulation of gene expression involves installation of a photoprotecting group that can be removed by photoirradiation.[4] Okamoto et al. described a mRNA caging system that used a caging compound 6-bromo-4-diazomethyl-7-hydroxycoumarine (Bhc-diazo).[5] They introduced the Bhc group into mRNA at a rate of 30 phosphate sites per 1 kb mRNA by mixing in DMSO. Bhc-caged mRNA displayed severely reduced translational activity, whereas illumination of Bhc-caged mRNA with ultraviolet light enabled recovery of the translational activity. However, the recovery rate of gene expression in the in vitro experiment was low, 23% compared with intact mRNA, due to RNA cleavage by Bhc-mediated caging. Furthermore, this approach required cumbersome processes after in vitro transcription, such as introduction of Bhc group and purification. Moreover, this strategy allowed for only a single off-to-on regulation event because the uncaged mRNA produced upon photoirradiation could not be re-caged; once the Bhc group was removed by photoirradiation, the uncaged mRNA continued to be translated until it was degraded by nucleases. These problems prohibited control over the magnitude and period of gene expression. Additional efforts are, therefore, needed to develop reversible photoregulation methods that allow precise control over 'when, where, and how long (or how frequently)' gene expression occurs. Here, we report a reversible method for photoregulating translation using the *cis–trans* photoisomerization of a photoresponsive 5′-cap. Recently, we developed several photochromic nucleosides (PCNs) that could reversibly change their photochemical and physical properties upon *cis–trans* photoisomerization under external photostimulus.[6] In our strategy, we focused attention on the translation initiation mechanism. Translation begins with binding between 7-methylguanosine (5′-cap) at the 5′-end of mRNA and the eukaryotic initiation factor 4E (eIF4E).[7] Several eukaryotic initiation factors then associate continuously and cooperatively. Finally, the ribosome assembles onto the mRNA complex and translation begins. Translation efficiency depends on the affinity of interaction between the 5′-cap and eIF4E.[8] Therefore, if we substitute the photoresponsive 5′-cap (7-methylated PCN) for the native 5′-cap, translation can be reversibly controlled by photoregulation of the interaction between the mRNA and eIF4E via *cis–trans* photoisomerization of the photoresponsive 5′-cap (Fig. 1).

We designed three photoresponsive 5′-caps, 8-styryl cap (8ST-cap), 8-naphthylvinyl cap (8NV-cap), and 8-fluorenylvinyl cap (8FV-cap) such that the affinity for eIF4E could be modulated by steric hindrance. The photoresponsive 5′-caps were synthesized

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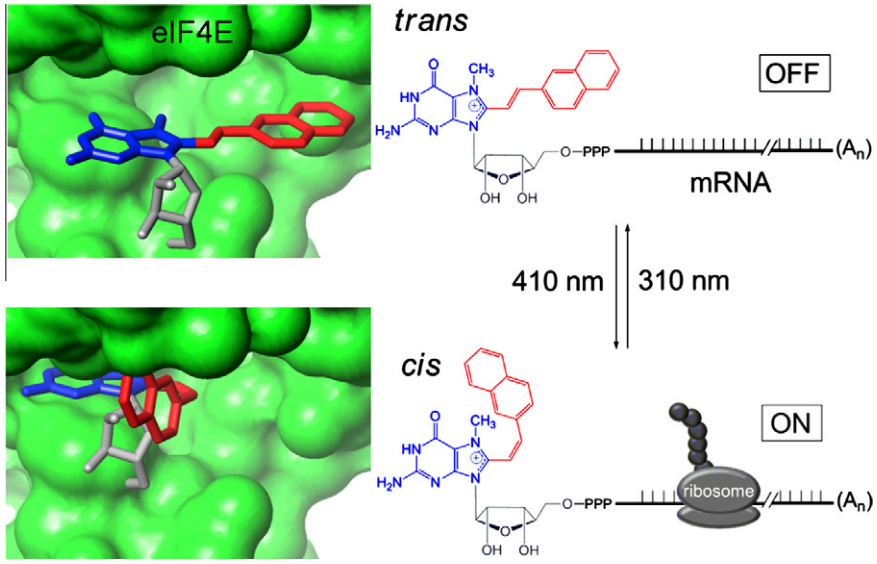
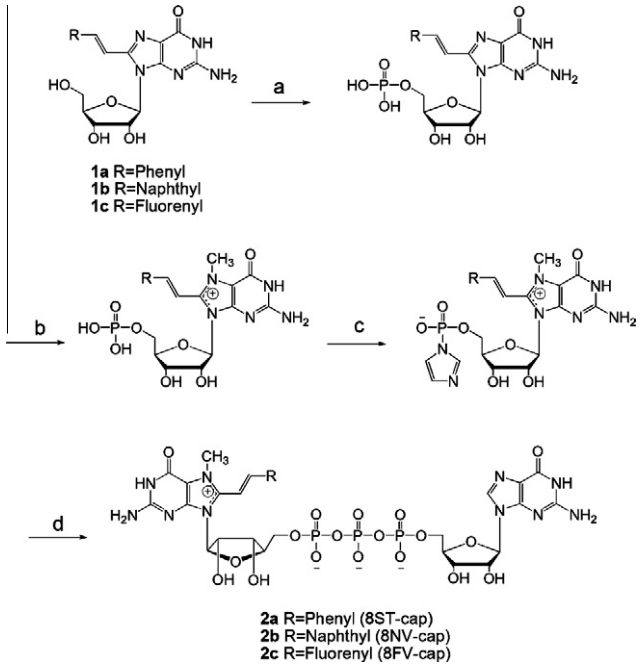


Figure 1. Reversible photoregulation of the translation by *cis–trans* photoisomerization of photoresponsive 5′-cap.

according to Scheme 1. The starting compounds, PCNs (**1a**–**c**), were prepared as described previously.[6a] PCN was converted to the corresponding 5′-monophosphate by stirring PCN with phosphorus oxychloride and trimethyl phosphate at 2 °C. After quenching with 1 M TEAB, the precipitate was filtered. Treatment of the residue with methyl iodide in DMSO afforded the corresponding N7-methylated 5′-monophosphate. The methylated 5′-monophosphate was converted to the corresponding imidazolide salt in the presence of imidazole, triphenylphosphine, triethylamine, and aldrithiol. Finally, the coupling reaction between the resulting imidazolide salt and GDP in the presence of the zinc chloride catalyst gave the corresponding photoresponsive 5′-cap (**2a**–**c**). Pure 8ST-cap, 8NV-cap and 8FV-cap were obtained as a mixture of isomers (cis:-trans = 1:99, 8:92 and 2:8, respectively, as determined by HPLC peak area). The structures of the photoresponsive 5′-caps were characterized by ^{1}H and ^{31}P NMR and mass spectroscopy.



Scheme 1. Reagents and conditions: (a) $POCl_3$, $(OMe)_3P$, 2 °C, 16 h; (b) MeI, DMSO, 20 h; (c) imidazole, aldrithiol, PPh_3, Et_3N, DMF, 15 h; (d) GDP (TEA-salt), $ZnCl_2$, DMF, 70 h.

As reported previously,[6a] PCNs show rapid and highly efficient reversible *cis–trans* photoisomerization upon alternate irradiation with monochromatic light. In these PCNs, both *cis* and *trans* isomers were thermally stable. They showed no thermal isomerization even at 80 °C for 20 h. Methylation at the N7 position of PCN produced only small changes in the photoisomerization properties. Photoisomerization of the photoresponsive 5′-caps was performed at 25 °C by using a 300 W Xenon lamp (MAX-302, Asahi spectra Co., Ltd). The reaction mixture was held from the light source to 2 cm and irradiated with 410 nm (103 mW/cm^2) for 2 min for *cis* to *trans* photoisomerization and 310 nm (81 mW/cm^2) for 2 min for *trans* to *cis* photoisomerization. The *trans* forms of 8ST-cap, 8NV-cap, and 8FV-cap were photoisomerized to the *cis* forms by irradiation at 410 nm, with 89%, 88%, and 95% conversion, respectively, as determined by HPLC peak area. Subsequent irradiation at 310 nm yielded the *trans* forms with 64%, 81%, and 92% conversion, respectively. To estimate the capping efficiency, short capped RNA (5′-cap-GGGAGA-3′) was synthesized using the MAXIscriptTM kit (Ambion, Inc.). 8ST-cap produced optimum efficiency as compared to the normal-cap. However, the efficiency decreased as the size of R-moiety become large. Introduction of the photoresponsive 5′-cap to the 5′-end of the green fluorescence protein (GFP) mRNA was accomplished by in vitro transcription using the MEGAscript kit (Ambion, Inc.). The reaction solution, containing four ribonucleotide triphosphates, the photoresponsive 5′-cap, and T7 RNA polymerase, was incubated at 37 °C for 4 h in the presence of the DNA template prepared by PCR amplification of the plasmid DNA, which contained a 30-base poly(A) tail and a T7 promoter site. After DNase digestion of the DNA template, the transcribed products with 5′-caps were purified using the MEGAclearTM kit (Ambion, Inc.).

The difference in mRNA translational efficiency for mRNA containing either the *trans* or *cis* forms of the 8ST-cap, 8NV-cap, or 8FV-cap, was investigated by in vitro translation of the capped GFP-mRNA using Transdirect insect cells (Shimadzu, Co.). Translation of mRNA was more efficient for photoresponsive 5′-caps in the *cis* form than in the *trans* form, as shown in Figure 2. From the molecular modeling, this effect was most likely due to different degrees of steric hindrance between the R-moiety and the active site

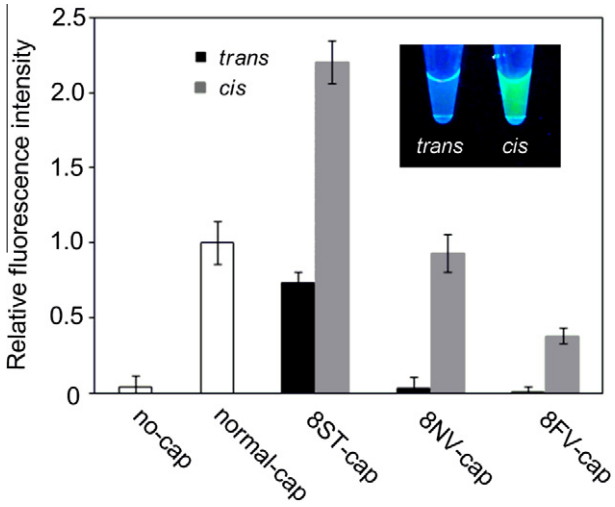


Figure 2. Relative fluorescence intensity of translational GFP after 10 h from photoirradiation (excitation: 480 nm, emission: 509 nm). The reaction mixture was irradiated with 410 nm for 2 min for *cis* to *trans* photoisomerization and 310 nm for 2 min for *trans* to *cis* photoisomerization at room temperature. Inset: fluorescence image of GFP translated from 8NV-capped mRNA.

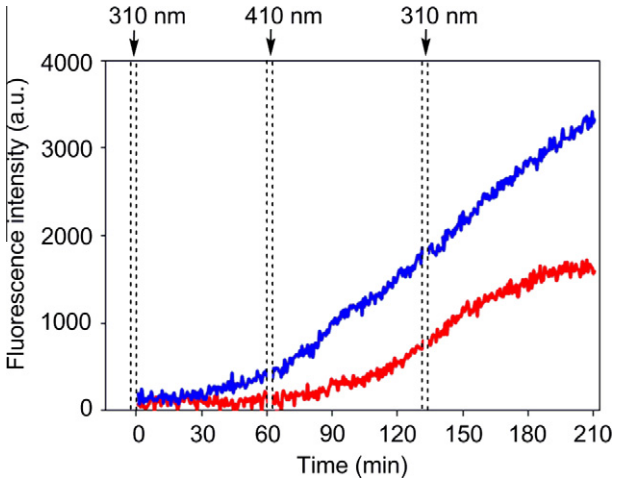


Figure 3. The time course of fluorescence intensity of GFP (excitation: 480 nm, emission: 509 nm). Blue line: normal-capped mRNA, red line: 8NV-capped mRNA. The reaction mixtures were irradiated with 310 nm for 2 min at 0 min and 130 min time points, and with 410 nm for 2 min at 60 min time point.

of eIF4E (see Supplementary data). Although the *cis* forms of the photoresponsive 5′-caps could interact with eIF4E, the bulky substituents, such as fluorene in the 8FV-cap, introduced steric hindrance in the cavity of eIF4E, even in the *cis* form. Thus, translation was suppressed regardless of the isomer adopted. Translation efficiency of 8ST-capped mRNA in *cis* form was 2.2 times higher than the normal-capped mRNA. This is probably because photoresponsive 5′-caps cannot be incorporated in the reverse orientation as anti-reverse cap analogs (ARCAs).[9] The 8NV-cap exhibited the highest photomodulation efficiency. The 8NV-cap in the *trans* form completely inhibited translation of mRNA, whereas the *cis* form yielded GFP translation with the same efficiency as mRNA capped with the normal-cap, a 26-fold higher efficiency than that of the *trans* form.

Next, we reversibly photoregulated translation using the 8NV-capped mRNA. Figure 3 shows the time course of fluorescence intensity during in vitro translation. The reaction solution containing mRNA capped with the normal cap or the 8NV-cap was irradiated with 310 nm light for 2 min, at the 0 min and 130 min time points, and with 410 nm for 2 min at the 60 min time point. The fluorescence intensity of the sample containing normal-capped mRNA increased at a constant rate irrespective of photoirradiation. In contrast, translation of 8NV-capped mRNA was not observed between 0 min and 60 min, during which time the 8NV-cap was in the *trans* form due to illumination with 310 nm at 0 min (OFF state). After photoisomerization to the *cis* form by 410 nm illumination, the fluorescence intensity gradually increased, indicating that the mRNA was translated (ON state). Subsequent illumination with 310 nm light displayed a low increase rate of fluorescence and no increase finally, suggesting that translation was again inhibited (OFF state). The increase in fluorescence observed in the second OFF state derived from GFP synthesized before 310 nm illumination at 130 min time point because maturation of GFP need tens of minutes.[10]

In summary, we synthesized three photoresponsive 5′-caps and successfully developed a method for the reversible photoregulation of translation using the *cis–trans* photoisomerization of the photoresponsive 5′-cap. The photoresponsive 5′-caps showed rapid and highly efficient reversible *cis–trans* photoisomerization upon illumination at specific wavelengths. The mRNA containing the 8NV-cap at the 5′-end could be switched between a translating state (ON state) and a non-translating state (OFF state) in a reversible fashion by alternately irradiating with monochromatic 410 nm or 310 nm light. Therefore, our method enable us to control 'when, where, and how long (or how frequently)' gene expression occurs. For the application to in vivo photoregulation of important biological events, introduction of additional techniques such as multiphoton exitation and photochromic nucleobases photoisomerized by longer wavelength will be needed and is now in progress.[6]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.119.

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