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Light-Regulated mRNA Condensation by a Photosensitive Surfactant Works as a Series Photoswitch of Translation Activity in the Presence of Small RNAs

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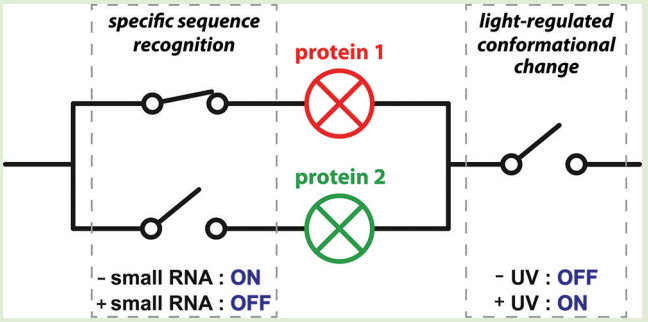
Supporting Information

ABSTRACT: AzoTAB, a photosensitive azobenzene cationic surfactant, which phototriggers translation activity through light-regulated condensation of mRNA, is added to a translation solution containing several mRNAs, which can be selectively silenced by specific small RNAs. We find that gene silencing by small RNAs remains functional regardless of AzoTAB concentration and UV illumination. In the absence of UV, the translation of all genes present in the medium is partially to fully inhibited depending on AzoTAB concentration. In contrast, the application of a short UV stimulus (365 nm for 1.5 min) results in the selective photoactivation of genes that are not silenced by small RNA. These results show that light-regulated condensation by AzoTAB works as a sequence-independent series photoswitch added to parallel sequence-specific regulation by small RNAs.



INTRODUCTION

In living cells, gene regulation results from several mechanisms, including the action of repressor and activator proteins, RNA interference system,[1] DNA methylation, and chromatin remodeling that modifies the accessibility of promoter DNA regions.[2] The rising progress in understanding natural gene control mechanisms and their interactions has been accompanied by the development of artificial approaches to control the gene expression activity, offering promising applications in nanotechnology[3,4] as well as in biological and medical research.[5] A first approach consists in the development of synthetic molecules able to influence gene expression such as DNA-binding proteins,[6,7] small noncoding RNAs,[1] and transcription factors.[8,9] A second approach focuses on the application of an external stimulus, such as temperature[10,11] and pH,[12] to control gene expression. Because of its facile implementation and high spatiotemporal resolution, light has attracted a particular attention and several approaches to control gene expression by light have been developed such as photo-uncaging of nucleic acids[13−15] or various regulating small molecules (e.g., small interfering RNA,[16] hormones,[17,18] antibiotics[19]), application of photosensitive nanorods,[20] and covalent grafting of photosensitive moieties to nucleic acids.[21−23] Most of these approaches are system-dependent and usually highly specific to a given transcription or translation machinery of interest. Recently, we have developed a method for photoregulation of gene expression at both transcriptional and translational levels with the advantage being nonspecific and directly applicable to various DNAs, RNAs, and polymerases.[24] This is based on the use of photosensitive azobenzene trimethylammonium bromide cationic surfactants, such as AzoTAB (Figure 1a)[25−31] and homologues.[27] When *trans*-AzoTAB is added to a reconstituted translation medium, mRNA condenses and translation is strongly hindered. After a short UV illumination (1−3 min at 365 nm), AzoTAB isomerizes to the *cis* configuration, which has a lower affinity for nucleic acids. As a result, mRNA unfolds and translation is partially to fully recovered depending on AzoTAB concentration.[24] Relying on light-induced condensation state of mRNA, this method has the advantage to be reversible and readily applicable to a large variety of gene sequences. However, using this method, all genes are activated (when mRNAs are

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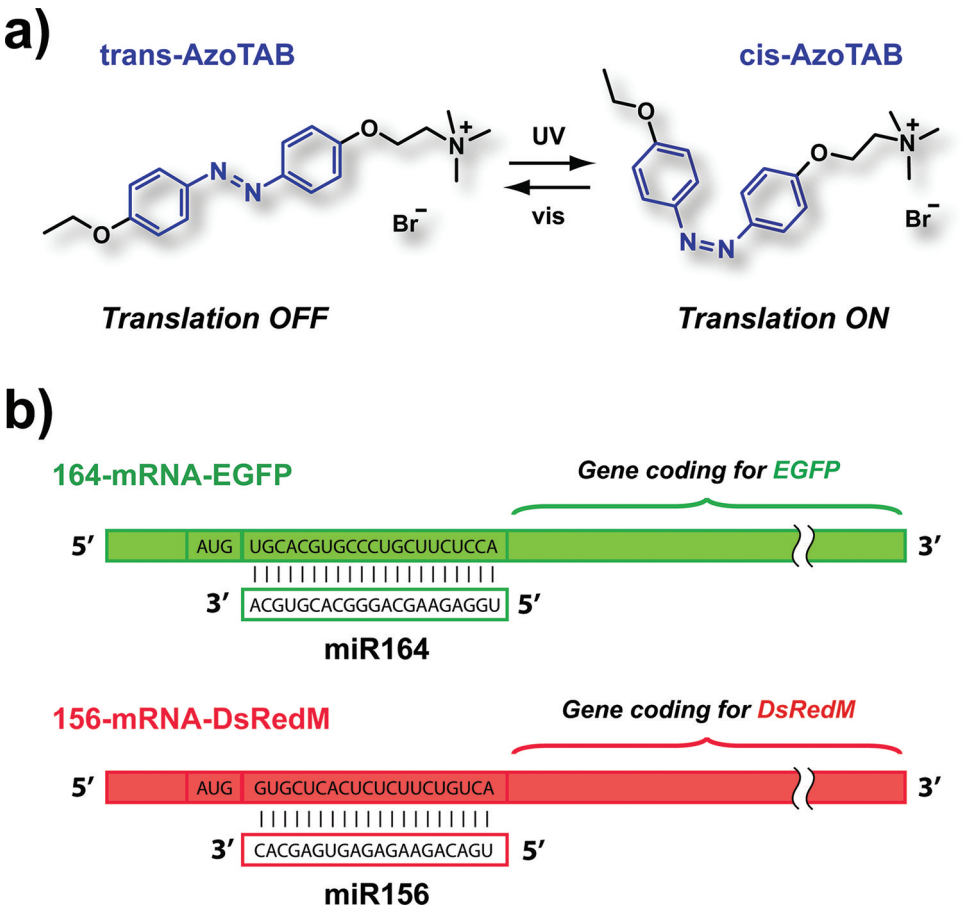

Figure 1. Experimental system. (a) Under visible light conditions (vis), *trans*-AzoTAB strongly binds to mRNA, mRNA condenses and translation is inhibited. After UV illumination (365 nm), AzoTAB undergoes a transition into *cis*-isomer, which is a weaker binder of mRNA, mRNA unfolds and translation is activated.[24] (b) We used two mRNAs, called 164-mRNA-EGFP and 156-mRNA-DsRedM, which code for EGFP and DsRedM proteins, respectively, and contain antisense sequences of two small RNAs (miR164 and miR156, respectively).

unfolded) or inhibited (when mRNAs are condensed) simultaneously so that selective photocontrol of a desired gene among others is impossible. Here we explore for the first time the concept of combining this sequence-independent method based on light-regulated condensation of mRNA with highly specific silencing strategy by small RNA. By using a simple *in vitro* translation model system composed of AzoTAB and two mRNAs that can be specifically silenced in the presence of small RNA(s), we study how these two orthogonal gene regulation strategies influence each other in terms of response to light stimulation and selectivity in the gene expression profile.

EXPERIMENTAL SECTION

Reagents and Solutions. Millipore water (18 Mohm·cm) was used for all experiments. AzoTAB synthesis was adapted from the method described by Hayashita et al.[27,32] The purity of the final product was checked by 250 MHz ^{1}H and ^{13}C NMR. A 20 mM AzoTAB stock solution in water was kept in the dark. The concentration of AzoTAB was calculated by weight. Its extinction coefficient obtained in water at 20 °C (25 558 ± 242 L mol^{-1} cm^{-1} at 357 nm) is in good agreement with that obtained by Le Ny and Lee (24 900 L mol^{-1} cm^{-1} at 357 nm).[25] Cell-free expression was performed using PURE System (PURESYSTEM classic II, BioComber Co., Ltd., Japan).[33,34] For calibrations, recombinant proteins EGFP and DsRedM were purchased from BioVision and Clontech, respectively. miR156 (20 bases: 5′-UGACAGAAGAGAGUGAG-CAC-3′, molecular weight 6516 Da) and miR164 (21 bases: 5′-UGGAGAAGCAGGGCACGUGCA-3′, molecular weight 6853 Da) small RNAs from *Arabidopsis thaliana* where purchased from Hokkaido System Science Co., Ltd., and additionally purified by polyacrylamide gel electrophoresis. Concentration of small RNA after purification was determined by absorption using NanoDrop1000 spectrophotometer.

Preparation of mRNAs. Two mRNAs were used for these experiments: one containing the miR164 antisense sequence inserted right after the AUG codon (...5′-AUG-UGCACGUGCCCUGCUU-CUCCA-3′...) in the open reading frame region and coding for enhanced green fluorescence protein (EGFP) referred to as 164-mRNA-EGFP, and the other one containing the miR156 antisense sequence (...5′-AUG-GUGCUCACUCUCUUCUGUCA-3′...) and coding for monomeric form of Discosoma sp. red fluorescent protein (DsRedM) and referred to as 156-mRNA-DsRedM.

DNA templates coding for 164-mRNA-EGFP and 156-mRNA-DsRedM were amplified by PCR from corresponding plasmid DNAs (Clontech) coding for EGFP and DsRedM proteins, respectively. Fragments of miR164 and miR156 antisense sequences were inserted to these DNAs in the forward primers during first PCR amplification (5′-AAGGAGATATACCAATG-TGCACGTGCCCTGCTTCTCCA-GTGAGCAAGGGCGAGGAG-3′ to insert miR164's antisense sequence (underlined) to EGFP-coding DNA and 5′-AAGGAGATA-TACCAATG-GTGCTCACTCTCTTCTGTCA-GGACAACACC-GAGGACGTCATC-3′ to insert miR156 antisense sequence fragment (underlined) to DsRedM-coding DNA. The reverse primers were 5′-TATTCATTACCCGGCGGCGGTCACGAA-3′ (EGFP rev.) and 5′-TATTCATTACTACTGGGAGCCGGAGTGG-3′ (DsRedM rev.), respectively. A 100 μL reaction solution was assembled in ice with the following final composition: 0.2 mM dNTPs, 4 ng of DNA

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template, 0.3 μM primers, 0.8 mM $MgSO_4$, and 1U DNA polymerase (KOD plus, TOYOBO) in KOD plus buffer. After a first step at 94 °C for 2 min, 20 cycles (94 °C for 15 s, 50 °C for 30 s, 68 °C for 60 s) were performed. Length and purity of amplified DNA were checked by 2% agarose gel electrophoresis. Finally, amplified DNA templates were purified using SeaPlaque GTG Agarose (FMC). Concentrations of amplified DNA was determined by absorption using a NanoDrop1000 spectrophotometer. After that, using this first PCR product, PCR amplification of both DNAs was performed again in the same conditions on the universal primer (5′-GAAATTAATACGACTCAC-TATAGGGAGACCACAACGG TTTCCCTCTAGAAA-TAATTTTGTTTAACTTTAAGAAGGAGATATACCA-3′) and the same reverse primers (EGFP rev. and DsRedM rev.). Then, separation and purification were performed in the same way as described above. Final DNA, which will be used for 164-mRNA-EGFP or 156-mRNA-DsRedM transcription, was obtained by the PCR amplification of the second PCR product using for each DNA T7-stem-loop uni. as a forward primer (5′-GAAATTAATACGACTCACTATAGGGAGAC-CACAACGGTTTCC-3′) and EGFP rev. or DsRedM rev. as reverse primers, respectively. The PCR reaction, separation, and purification were performed in the same way as described above, and the purification product was dissolved in water, followed by concentration measurement using a NanoDrop1000 spectrophotometer.

164-mRNA-EGFP and 156-mRNA-DsRedM (780 and 770 bases, respectively) were prepared from corresponding DNAs using a MEGAscript Kit (Ambion). A 20 μL reaction solution was assembled in ice (final composition: 7.5 mM NTPs, 1000 ng of DNA template, and 2 μL of enzyme mix in 10× reaction buffer) and incubated at 37 °C for 4 h. After transcription reaction, TURBO DNase (Ambion) was added to the solution to remove DNA. Thus, obtained mRNA was purified using RNeasy MinElute Cleanup Kit (QIAGEN) according to the manufacturer's instructions. Concentration of mRNA was determined by absorption using a NanoDrop1000 spectrophotometer.

UV Illumination. When UV irradiation was needed, the solution to be illuminated was placed for 1.5 min at 6 cm distance from an 8 W UVLMS-38 UV lamp (UVP, Upland, CA) working at 365 nm. According to previous measurements of transcription and translation activity,[24] UV illumination does not induce significant damage to nucleic acids and proteins under these conditions.

***In Vitro* Translation.** All experiments were performed under RNase-free conditions. Water, PURE System solution A (containing salts, tRNAs, and other agents), 164-mRNA-EGFP (40 nM), 156-mRNA-DsRedM (40 nM), one or both small RNA(s) (miR156, 800 nM; miR164, 800 nM) when needed, AzoTAB when needed, and PURE System solution B (containing enzymes and ribosomes) were assembled in this order to a final volume of 20 μL. As soon as the solution was assembled, the reaction mixture was divided into two 10 μL reactions. One solution was kept in the dark (−UV) while the other one was exposed to UV (+UV) for 1.5 min prior to incubation in the dark at 37 °C for 75 min for both solutions. The concentration of synthesized proteins was quantified from the 20-fold diluted reaction aliquots using a microplate reader (Infinite F200, Tecan), using excitation wavelength λ_{ex} = 485 nm and recording the fluorescence intensity at λ_{rec} = 535 nm for EGFP detection and λ_{ex} = 535 nm and λ_{rec} = 595 nm for DsRedM detection. Dilution and measurements were performed at room temperature. The background noise, which mainly corresponds to the fluorescence intensity of PURE System solution without mRNA nor AzoTAB, was subtracted to all measured values. EGFP and DsRedM translation activities where obtained by dividing the intensity of a given sample by that of the reference reaction ([AzoTAB] = 0 mM; −miR156; −miR164; −UV).

Calibrations of Fluorescence Intensity. *Effect of AzoTAB.* The determination of the influence of AzoTAB on the fluorescence of EGFP and DsRedM was performed under the same conditions as those during translation experiments. 10 μL of AzoTAB solution of increasing concentrations was distributed in the tubes, and half of the tubes was exposed to 365 nm UV light for 1.5 min. After 75 min of incubation of all tubes at 37 °C, the PURE System and the same amounts of either EGFP or DsRedM proteins was added in the tubes and the samples volume was adjusted to 200 μL followed by 15 min equilibration. The fluorescence intensity of proteins was measured using Infinite F200 (TECAN) microplate reader in the same conditions than after translation experiments.

Fluorescence in the Protein Mixtures. Water, PURE System, and various amounts of EGFP and DsRedM solutions were mixed to final volume 200 μL. The maximal concentrations of proteins corresponded approximately to the maximal fluorescence intensities of proteins detected after translation experiments. After 15 min of equilibration at room temperature, the fluorescence intensity of proteins was measured using Infinite F200 (TECAN) microplate reader in the same conditions than after translation experiments. No effect of one protein on the fluorescence intensity of another one was detected at the given concentrations.

RESULTS

Experimental System. We used AzoTAB, a photosensitive azobenzene cationing surfactant whose photodependent affinity for mRNA was exploited to regulate translation activity using light (Figure 1a). It was shown that, in the presence of one kind of mRNA, *trans*-AzoTAB induced mRNA condensation and inhibition of translation activity.[24] Upon a short UV illumination, AzoTAB isomerized to *cis*-configuration, resulting in a weaker binding to mRNA. As a result, mRNA unfolded and translation reaction was activated.[24] Here, we applied this method on a mixture of two mRNAs that can be specifically silenced by small RNAs (Figure 1b). Antisense sequences of miR164 and miR156 natural small RNAs were inserted into the open reading frames that code for enhanced green fluorescent protein (EGFP) and DsRed monomer protein (DsRedM), respectively (Figure 1b). We thus obtained two mRNAs: one coding for EGFP and containing miR164 antisense sequence right after AUG codon (164-mRNA-EGFP) and another one coding for DsRedM protein and containing miR156 antisense sequence (156-mRNA-DsRedM) (Figure 1b). The binding of the small RNAs to their complementary sequences in the open reading frame region inhibits the ribosome function, leading to the silencing of the protein synthesis. We have found that the presence of 10−20-fold molar excess of each small RNA totally and specifically inhibits the translation from the corresponding mRNA (data not shown). Translation reactions were carried out using PURE System, a cell-free *in vitro* translation system composed of purified components such as ribosomes, translation factors, aminoacyl-tRNA synthetases and other proteins, amino acids, and tRNAs.[33,34] A typical photocontrol experiment was conducted as follows. After assembling PURE System components, the two mRNAs (164-mRNA-EGFP and 156-mRNA-DsRedM), ±miR164, ±miR156, and AzoTAB, the reaction mixture was divided into two solutions: one exposed to UV irradiation at 365 nm for 1.5 min (+UV) while the other one was kept in the dark (−UV). After 75 min of incubation of both solutions in the dark at 37 °C, we measured the fluorescence intensity corresponding to EGFP (absorption/emission 485/535 nm), referred to as I_{EGFP}, and that corresponding to DsRedM (absorption/emission 535/595 nm), referred to as I_{DsRedM}.

To assess the relationship between fluorescence intensity and expression level for each protein, we first studied the effect of AzoTAB (*trans* or *cis*) on the fluorescence emission properties of EGFP and DsRedM proteins. We found that AzoTAB did not affect I_{EGFP} (Supporting Information Figure S1a) or I_{DsRedM} (Supporting Information Figure S1b) regardless of AzoTAB configuration (*trans* or *cis*) in the concentration range used in the photocontrol experiments ($0 \leq [AzoTAB] \leq 1.5$ mM).

Then, we studied the effect of the presence of one fluorescent protein on the fluorescence emission properties of the other protein. We found that, regardless of EGFP and DsRedM concentration, I_{EGFP} and I_{DsRedM} did not depend on the presence of the other protein (DsRedM and EGFP, respectively) (Supporting Information Figures S2a and S2c) and that I_{EGFP} (averaged for all DsRedM concentrations) and I_{DsRedM} (averaged for all EGFP concentrations) linearly increased as a function of [EGFP] and [DsRedM], respectively (Supporting Information Figures S2b and S2d). All these results show that, under our experimental conditions, there is no interference between EGFP and DsRedM and that each protein fluorescence emission intensity can be used as a quantitative measurement of the protein concentration, regardless of AzoTAB concentration for both *trans* and *cis* isomers.

Hence, translation activity (% translation) of a given protein, defined as the amount of protein produced in the sample solution divided by the amount produced in a reference solution ([AzoTAB] = 0 mM, −miR156, −miR164, −UV) was measured as a ratio of fluorescence intensities:

$$\%\ \text{translation} = (I_{\text{Sample}} - I_{\text{PURESystem}}) / (I_{\text{Reference}} - I_{\text{PURESystem}}) \times 100$$

where I_{Sample}, $I_{\text{PURESystem}}$, and $I_{\text{Reference}}$ are the intensities measured after 75 min of incubation at 37 °C for the sample solution, PURE System solution alone, and the reference solution, respectively. To quantify the efficiency of our photocontrol strategy, we measured the translation activity of EGFP and DsRedM as a function of AzoTAB concentration for different translation controlling factors: ±miR164, ±miR156, ±UV.

Photocontrol in the Absence of Small RNA. Figure 2 shows the translation activity of EGFP and DsRedM in the absence of small RNA. Without UV irradiation, increasing AzoTAB concentration strongly reduces the production of EGFP and DsRedM until complete inhibition, which is reached for [AzoTAB] = 1 and 1.5 mM for EGFP and DsRedM, respectively. In the presence of UV light for 1.5 min, the translation activity of both proteins also decreases but in a less abrupt manner, leading to a significant difference in translation activity between the UV-irradiated and nonirradiated samples.

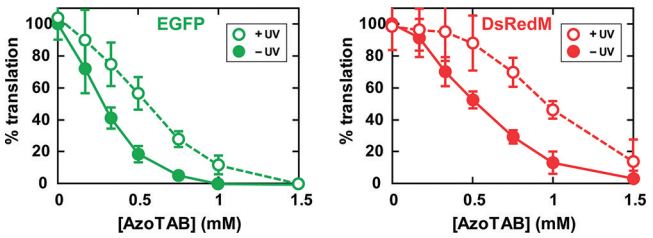

Figure 2. Translation activity (% translation) of EGFP (green, left panel) and DsRedM (red, right panel) as a function of AzoTAB concentration in the absence (−UV, filled circles) and in the presence (+UV, open circles) of illumination at 365 nm for 1.5 min. The experiments were performed in PURE System, with a mixture of 164-mRNA-EGFP (40 nM) and 156-mRNA-DsRedM (40 nM). Symbols are data points. Solid (−UV) and dashed (+UV) lines connecting symbols are guides for the eye. Symbols and error bars show mean values ± SD from triplicates. Error bars are not shown when they are smaller than the symbol size.

The recovery of translation activity upon UV illumination is particularly efficient for AzoTAB concentrations between 0.5 and 1 mM. For [AzoTAB] = 0.5 and 0.75 mM, UV exposure results in a 3.1-fold (from 18.5 ± 5.0 to 56.6 ± 10.1%) and 5.4-fold (from 5.1 ± 0.9 to 27.7 ± 4.9%) increase of EGFP production, respectively, and 1.6-fold (from 54.0 ± 5.2 to 87.9 ± 16.5%) and 2.2-fold (from 31.8 ± 4.0 to 70.2 ± 8.7%) increase of DsRedM production, respectively. For [AzoTAB] = 1 mM, EGFP is undetectable in the absence of UV but increased to 11.5 ± 6.0% after UV irradiation while DsRedM production is increased 2.9 times (from 16.3 ± 6.7 to 47.4 ± 5.3%). These results show that absolute levels of expression slightly differ between EGFP and DsRedM, but the global photocontrol pattern is very similar for both proteins and corresponds well to what was observed in the case of a solution containing only one type of mRNA.[24] For both systems (one or two mRNA(s)), translation efficiency for each protein strongly decreased upon addition of AzoTAB and is partially recovered by a short UV illumination. This shows that unspecific binding of AzoTAB to mRNA allows a photocontrol of translation activity that can be applied to different kinds of proteins, from a solution containing one or several mRNAs. Since AzoTAB interacts similarly with different mRNAs regardless of their sequence, this strategy alone cannot provide selectivity in the photoactivation of a gene of interest among others. Then, we have thus studied the situation in the presence of small RNAs that can specifically silence the translation of target gene(s).

Selective Photocontrol in the Presence of Small RNA. Figure 3 shows the translation activity of EGFP and DsRedM as a function of [AzoTAB], in the presence of miR156 or/and miR164. Figure 3a (left) shows that the presence of only miR156, which specifically binds to the mRNA coding for DsRedM, does not affect the AzoTAB-induced photocontrol of EGFP expression. At [AzoTAB] = 0.5, 0.75, and 1 mM, UV illumination results in an increase of EGFP production by a factor of 3.2 (from 16.4 ± 3.4 to 51.8 ± 5.9%), 6.7 (from 4.3 ± 0.1 to 28.7 ± 4.1%), and 68.5 (from 0.27 ± 1.26 to 18.5 ± 6.8%), respectively, which is very similar to the results in the absence of small RNA (Figure 2, left). In contrast, the expression of DsRedM (Figure 3a, right) is almost totally inhibited regardless of AzoTAB concentration and illumination conditions. These results show that the presence of AzoTAB does not affect the inhibition of DsRedM translation by miR156 binding. Analogous effect is observed in the presence of only miR164, which specifically binds to the mRNA coding for EGFP. Figure 3b (right) shows that the presence of miR164 induces a slight inhibition of DsRedM expression, but the photocontrol pattern is similar to that in the absence of small RNA (Figure 2, right). In this case, UV exposure results in a 1.8-fold (from 31.2 ± 8.2 to 56.1 ± 0.9%), 2.7-fold (from 19.5 ± 10.7 to 51.7 ± 8.5%), and 3.7-fold (from 8.7 ± 6.4 to 31.9 ± 10.4%) increase of DsRedM production for [AzoTAB] = 0.5, 0.75, and 1 mM, respectively. In contrast, the presence of miR164 totally inhibits EGFP production, regardless of AzoTAB concentration and illumination conditions (Figure 3b, left). Figure 3c shows the translation activity of EGFP and DsRedM in the presence of both small RNAs. In this case, expression of both proteins (EGFP and DsRedM) is almost fully inhibited, regardless of AzoTAB concentration and UV illumination conditions.

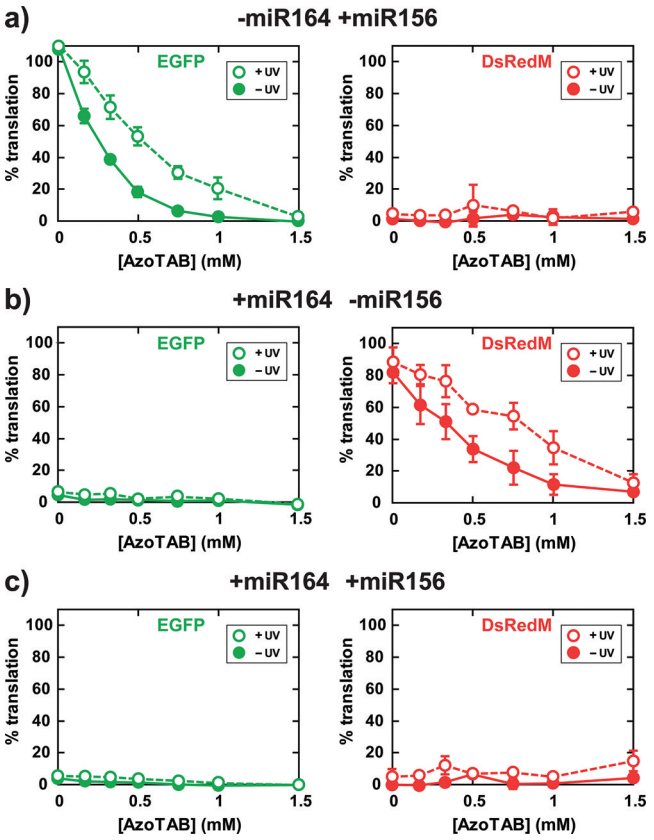

Figure 3. Translation activity (% translation) of EGFP (green, left panel) and DsRedM (red, right panel) as a function of AzoTAB concentration in the absence (−UV, filled circles) and in the presence (+UV, open circles) of illumination at 365 nm for 1.5 min, for different compositions in small RNA: (a) only miR156; (b) only miR164; (c) both small RNAs. The experiments were performed in PURE System, with a mixture of 164-mRNA-EGFP (40 nM) and 156-mRNA-DsRedM (40 nM). When added, the final concentration of each small RNA was 800 nM. Symbols are data points. Solid (−UV) and dashed (+UV) lines connecting symbols are guides for the eye. Symbols and error bars show mean values ± SD from triplicates. Error bars are not shown when they are smaller than the symbol size.

DISCUSSION

All these results confirm that small RNA binding to an antisense sequence present in coding mRNA induces silencing of protein translation with high gene selectivity when several mRNAs coding for different proteins are involved. Moreover, we found that, regardless of mRNA sequence: (i) AzoTAB-based photocontrol of translation from a given mRNA is fully operational in the absence of the small RNA specific to its antisense sequence; (ii) small RNA silencing is not affected by the presence of AzoTAB or application of UV illumination. This is thus the first demonstration that specific translation silencing by miRNA can be coupled with translation photocontrol using a photosensitive nucleic acid binder such as AzoTAB.

Figures 2 and 3, Supporting Information Figure S3, and Table 1 show that protein expression is partially to fully inhibited in the absence of UV illumination regardless of small RNA composition and that, depending on small RNA composition, UV exposure specifically leads to no protein expression, selective expression of one protein, or expression of both proteins. This system thus works as a superposition of two

Table 1. Qualitative Indication of Expression Levels of EGFP and DsRedM without (−UV) and with (+UV) UV Illuminations for 1.5 min for Different Compositions in Small RNAs[a]

		level of expression (−UV)		level of expression (+UV)	
miR156	miR164	EGFP	DsRedM	EGFP	DsRedM
+	+	low	low	low	low
+	−	low	low	high	low
−	+	low	low	low	high
−	−	low	low	high	high

[a]See Figures 2−3 for quantitative data and experimental conditions.

inhibitory systems connected through an "OR" logic gate or two activation systems connected through an "AND" logic gate. If at least one inhibitory component is present (−UV for all genes OR presence of a small RNA related to a target gene), expression of the corresponding gene(s) is inhibited. Similarly, expression of given gene(s) is activated only in the presence of UV AND absence of the corresponding small RNA(s). We suggest the following simple molecular picture as a possible mechanism (Figure 4a). Without small RNA, AzoTAB induces

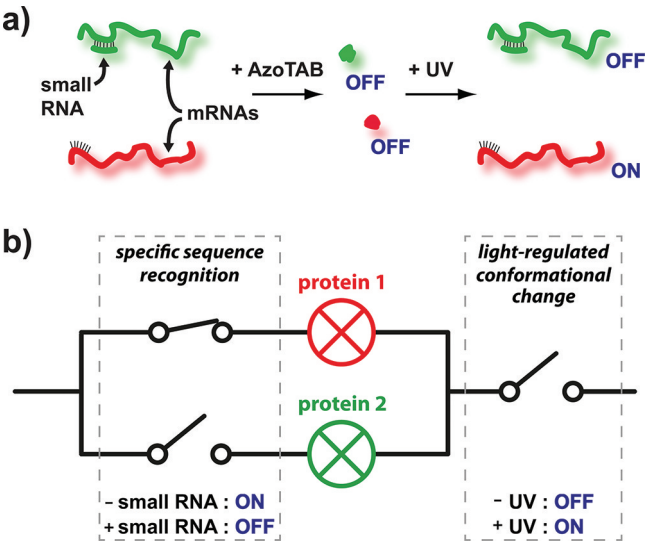

Figure 4. (a) Suggested molecular picture illustrating the mechanism of selective gene photoactivation. The presence of AzoTAB induces condensation and silencing of all mRNAs (OFF state). After UV illumination, all mRNAs unfold but small RNAs remind bound on their target sequence so that only genes that are not inhibited by small RNAs are selectively expressed (ON state). (b) Electrical analogy: small RNAs specifically silence target proteins in a sequence-dependent manner while light-induced RNA condensation works as a series photoswitch.

condensation of all mRNAs present in the medium leading to silencing of all genes. After UV illumination, all mRNAs unfold and all genes are expressed. This corresponds well to what has been observed when only one gene was present in the translation medium.[24] In the presence of small RNAs that specifically bind to their target sequences, sequence-specific gene silencing occurs regardless of mRNA condensation state, i.e., presence of AzoTAB or UV illumination. This is only possible because AzoTAB is a weak binder of mRNA and induces mRNA condensation through a cooperative process,

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which is typical of the interaction between cationic surfactants—including AzoTAB—and nucleic acids.[27,35,36] As a result, AzoTAB does not remove or replace any bound small RNA but induces global condensation of small RNA/mRNA complex. Therefore, in the case of a mixture of different mRNAs and AzoTAB, the presence of small RNAs can maintain silent target gene(s) regardless of mRNA condensation state while UV illumination and absence of specific small RNAs are necessary to both unfold mRNA and activate desired gene(s). Light-regulated condensation by AzoTAB thus works as a sequence-independent series photoswitch added to a parallel sequence-specific regulation by small RNAs (Figure 4b). Such a dual approach presents a practical interest for future bionanotechnological applications because it opens the possibility to place under photocontrol gene(s) of interest by adding appropriate small RNA(s) and AzoTAB without need of designing and synthesizing a particular photosensitive molecule specific to the target gene(s).

CONCLUSION

In summary, we have described a new method to selectively activate the translation of a gene of interest among others using light. Our method, which does not require any covalent grafting or chemical modification, consists in adding a photosensitive cationic surfactant, AzoTAB, to a translation solution containing one or several small RNAs, which can maintain silent some of the genes present in the medium. As a result, no gene was expressed in the absence of light and the application of a short UV stimulus resulted in the specific synthesis of one or several target proteins depending on the composition in small RNAs. We proposed a simple mechanism where light-regulated RNA condensation works as an additional series photoswitch of translation activity.

ASSOCIATED CONTENT

Supporting Information

Effect of AzoTAB (Figure S1) and protein (Figure S2) concentrations on EGFP and DsRedM fluorescence intensity; effect of microRNA composition on photocontrol of translation activity (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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