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# Azobenzene-Tethered T7 Promoter for Efficient Photoregulation of Transcription

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***Abstract:*** Azobenzene was additionally introduced into side chain of T7 promoter for the photocontrol of transcription reaction by T7 RNA polymerase (T7 RNAP). When a single azobenzene molecule was introduced into the T7 promoter either at the loop-binding region of the RNAP (−7 to −11 position) or at the unwinding region (−1 to −4 position), transcription was suppressed in the trans-form but proceeded faster in the cis-form. The amount of transcripts after UV irradiation with respect to that in the dark was 1.5−2.0-fold. Kinetic analysis of the transcription reaction revealed that the photoregulatory mechanism was different in these positions. The photoisomerization of an azobenzene at the loop-binding region primarily affected $K_m$. On the other hand, the isomerization of an azobenzene at the unwinding region mainly affected $k_{cat}$. Still more clear-cut photoregulation was achieved when two azobenzenes were introduced into both loop-binding and unwinding regions, respectively: transcription proceeded 7.6-fold faster after UV irradiation than that in the dark. This synergistic effect was observed only when two azobenzenes were introduced into these two different regions, respectively, and introduction of them into the same loop-binding region drastically lowered the transcription activity. The cooperation of two azobenzenes at loop-binding and unwinding regions would contribute to the clear-cut photoregulation of transcription.

## Introduction

Artificial control of biofunction is one of the currently important and attractive themes for post-genome era.[1] Recently, much attention has been focused on the artificial control of gene expression because of their potential applications to nanotechnology as well as cell biology and pharmacology.[2] Various external stimuli, such as light, heat, electric field, and pH, were applied to control gene expression.[3] Among these stimuli, photocontrol of gene expression has been investigated due to its advantages on controlling the gene expression spatially and temporally; photocaged nucleic acids and other ligands,[4] or a plant-phytochrome-based light-switchable gene promoter system, were applied to the photoregulation.[5] Previously, we have synthesized azobenzene-tethered DNA, and primer elongation by DNA polymerase or RNA digestion by RNase H has been successfully photoswitched.[6] These are based on the photoregulation of the formation and dissociation of DNA duplex. As evidenced by NMR, *trans*-azobenzene intercalates between the base-pairs and thus stabilizes the duplex by stacking interaction.[7] On the contrary, nonplanar *cis*-azobenzene significantly destabilizes the duplex, which is dissociated to the two single strands. This dynamic change of hybridization is suitable for the photoregulation of gene expression based on the antisense strategy.

In the present paper, we propose another strategy for the photoregulation of gene expression with the azobenzene-tethered DNA. Azobenzene is introduced into the promoter region of RNA polymerase (RNAP), and the transcription reaction by RNAP is photoregulated by trans−cis isomerization of the

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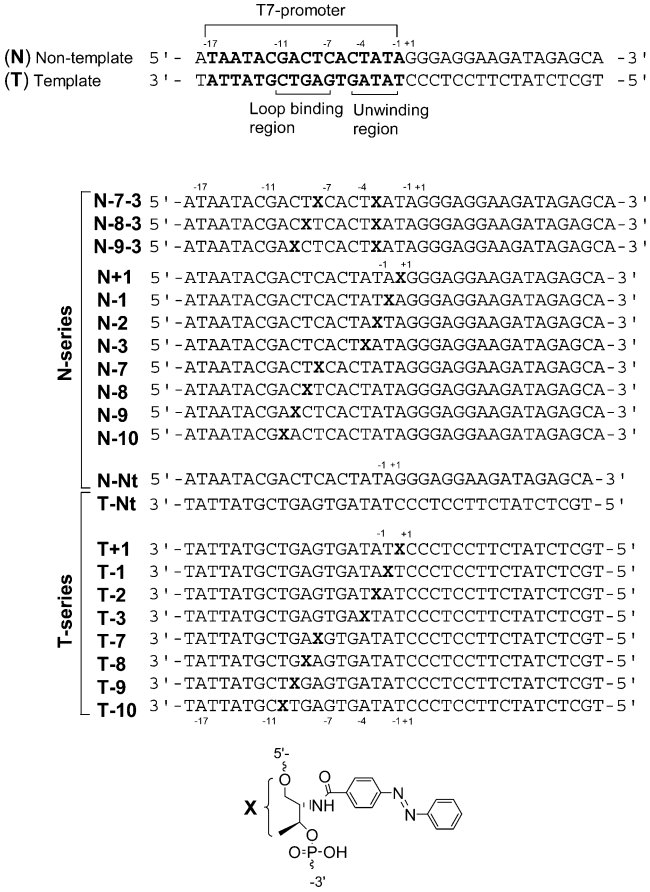
incorporated azobenzenes. This strategy is based on the change of local structure of DNA duplex around azobenzenes by trans−cis isomerization, not on the basis of the photoregulation of hybridization. Far below the $T_m$, dissociation of the duplex does not occur even in the cis-form, but the local structure of the duplex around azobenzene changes by the trans−cis isomerization, which would affect the interaction between DNA and the enzyme. In a preliminary communication,[8] we have demonstrated that this strategy worked well for the photoregulation of transcription: transcription is suppressed in the trans-form and is accelerated in the cis-form with a simple half duplex as a model system. Here, we use full duplex for the transcription of T7 RNAP, and photoregulatory efficiency was investigated in detail by changing the position of an azobenzene in the promoter. Kinetic analysis revealed that photoregulatory mechanism depended on its position. On the basis of this analysis, a new modified promoter involving two azobenzenes was designed for the efficient photoregulation. It was found that clear-cut photoregulation was achieved with two azobenzenes introduced into both loop-binding and unwinding regions, respectively.

## Results

**Introduction of Azobenzene into T7 Promoter for Photoregulation of Transcription.** Azobenzene was additionally introduced into either the nontemplate strand (N-series) or the template strand (T-series) on D-threoninol as shown in Scheme 1.[8] In this study, various azobenzene-tethered T7 promoters were used by combination of the N-series and T-series strands (Scheme 1). The azobenzene moiety in the T7 promoter duplex reversibly isomerized either by UV or by visible light irradiation under the reaction conditions (see Figure S1 in the Supporting Information). For example, almost all of the incorporated azobenzene moiety of **N-9/T-Nt** took the trans-form in the dark or after visible-light irradiation, whereas 32% of azobenzene was isomerized to the cis-form by UV irradiation.[9] Because the $T_m$ values of these promoter duplexes were around 56 °C, trans→cis isomerization did not induce dissociation of the duplex at 37 °C. Most of the photoregulatory transcription experiments were carried out under these conditions.

T7 promoter consists of two functional regions, RNAP recognition region (from the −11 to −4 site) and unwinding region (from the −4 to −1), as shown in Scheme 1. Especially, the bases from −11 to −7 are known to be bound by loop in T7 RNAP (loop-binding region in Scheme 1). The duplex in the unwinding region will be melted by T7 RNAP. Our strategy is to introduce azobenzene into these two functional domains for effective photoregulation of transcription. A typical time course of the transcription by T7 RNAP either in the dark (UV −) or after UV irradiation (UV +) is shown in Figure 1 with **N-9/T-Nt** tethering an azobenzene between the positions −9 and −10 on the nontemplate strand. Although transcript increased with time in both UV + or UV −, its rate was different: transcription proceeded 1.5-fold faster after UV irradiation than in the dark.[10] Thus, **N-9/T-Nt** tethering *cis*-azobenzene has higher activity than the trans-form. It should be noted that UV irradiation did not at all affect the transcription reaction with natural T7 promoter that has no azobenzene (see natural **N-Nt/T-Nt** promoter in Figure 2).

***Scheme 1.*** Sequences of T7 Promoters and Modified DNAs Synthesized in This Study




**Photoregulation of Transcription with Modified Promoter Tethering Single Azobenzene to the Loop-Binding Region.** Similar acceleration of transcription by trans→cis isomerization was also observed when an azobenzene was introduced from −10 to −7, where the specificity loop of RNAP was reported to be bound strongly (see the top of Scheme 1).[11] Irrespective of the position of azobenzene, the transcription process was accelerated by UV irradiation as shown in Figure 2. However, photoregulatory efficiency of transcription α, defined as the ratio of the amount of transcript after UV irradiation with respect to that in the dark after 2 h incubation, depended on the position. Highest α was observed for **N-10/T-Nt**, although transcription activity itself was about one-fifth of natural promoter (**N-Nt/T-Nt**). With **N-7**, **N-8**, and **N-9**, relatively high transcription activity was maintained, and their α was around 1.5−2.0. Photoregulated transcription was also observed when an azoben-

(8) Asanuma, H.; Tamaru, D.; Yamazawa, A.; Liu, M. Z.; Komiyama, M. *ChemBioChem* **2002**, *8*, 786−789.
(9) Although photoisomerization showed sequence dependency, about 20−40% of the azobenzene was isomerized to the cis-form by UV irradiation under the conditions employed for all of the azobenzene-tethered T7 promoters used in this study.
(10) As depicted in Figure 1a, T7 RNAP produced two main transcripts. The lower band corresponded to the normal 17-mer transcript, and the upper band corresponded to the 18-mer, which involved an uncoded extra base at the terminal. In the present study, the amount of transcript was calculated as the sum of both bands, because this extra base will not affect the translation step. This one-base extension is always observed with T7 RNAP: (a) Milligan, J. F.; Groebe, D. R.; Witherell, G. W.; Uhlenbeck, O. C. *Nucleic Acids Res.* **1987**, *15*, 8783−8798. (b) Milligan, J. F.; Uhlenbeck, O. C. *Methods Enzymol.* **1989**, *180*, 51−62. (c) Pleiss, J. A.; Derrick, M. L.; Uhlenbeck, O. C. *RNA* **1998**, *4*, 1313−1317.
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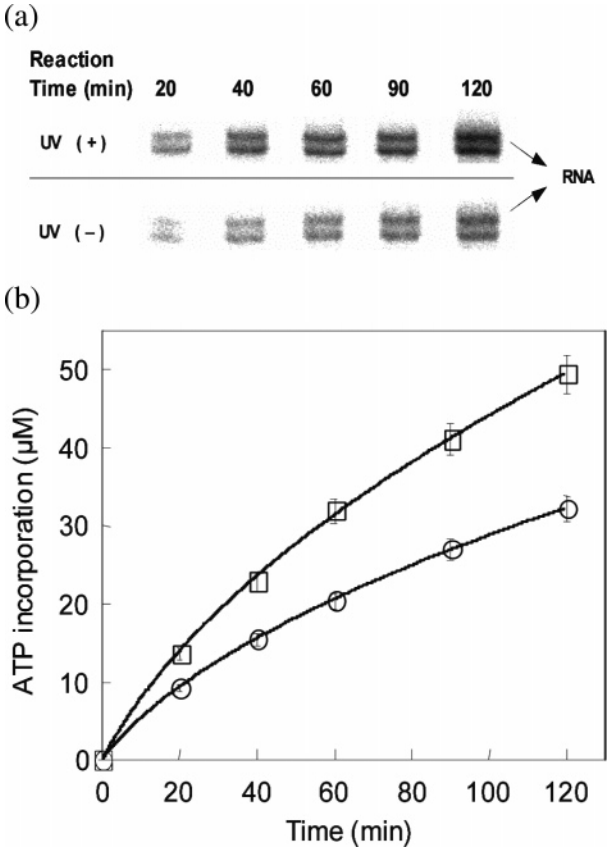


***Figure 1.*** Time course of the transcription at 37 °C with a combination of **N-9/T-Nt** either in the dark or after UV irradiation. (a) PAGE patterns of main products of RNA in the dark (UV −) or after UV irradiation (UV +). (b) Quantitative plots of the amount of incorporated $^{32}$P-labeled ATP in the dark (○) or after UV irradiation (□).

zene was incorporated into the template strand of the promoter (Figure 2b, T-series). In contrast with **N-7** and **N-8**, transcription activity was lowered when an azobenzene was introduced into the opposite strand of the same position (compare **N-7/T-Nt**, **N-8/T-Nt** with **N-Nt/T-7**, **N-Nt/T-8** in Figure 2).

**Incorporation of Azobenzene in the Unwinding Region.** Figure 3 shows the transcription by RNAP with a promoter tethering an azobenzene downstream from −4 to +1 (an azobenzene between the positions −1 and +1) where the duplex is reported to be melted by RNAP.[11] Interestingly, when an azobenzene was introduced into the nontemplate strand of this region, transcription was hardly suppressed in the cis-form as compared to the natural **N-Nt/T-Nt** promoter (see gray bars in Figure 3a). Rather, the activity of **N-3/T-Nt** was a little bit higher than natural promoter without an azobenzene. On the contrary, transcription was interfered in the trans-form, especially with the **N-3** tethering an azobenzene between −3 and −4. As a result, the largest photoregulatory efficiency ($\alpha = 1.5$) was observed with **N-3/T-Nt**. Unlike the promoter tethering an azobenzene to the nontemplate strand (N-series), the transcription activity rather decreased in case that an azobenzene was introduced into the template strand (see Figure 3b). Furthermore, photoregulation was not observed for **T-2** and **T-3** at all.

**Kinetic Analysis of the Photoregulation of Transcription.** As described in the previous sections, transcription activity was higher in *cis*-azobenzene than in the trans-form. Here, kinetic assays were performed with **N-9/T-Nt** and **N-3/T-Nt** as well as natural **N-Nt/T-Nt** by Michaelis−Menten plots of a transcription rate as a function of the concentration of the promoter.[12] Interestingly, the curves of these two promoters were entirely different (compare Figure 4a with b), indicating that an azobenzene in **N-9** and **N-3** played a different role of photoregulation (see Figure S2 for the plots of **N-Nt/T-Nt**). From these plots, $K_m$ and $k_{cat}$ in both the trans- and the cis-forms could be calculated as in Table 1. Comparison of **N-9** with **N-Nt** revealed that $K_m$ of **N-9** was 1 order larger than that of **N-Nt**, whereas $K_m$ of **N-3** was almost the same with **N-Nt**, demonstrating that tethering an azobenzene at a specific loop-binding region strongly interfered the binding of RNAP. Furthermore, photoisomerization changed different kinetic parameters in each promoter: for the **N-9/T-Nt**, trans→cis isomerization did not change $k_{cat}$, but changed $K_m$. In contrast, $k_{cat}$ doubled by trans→cis isomerization with a smaller change

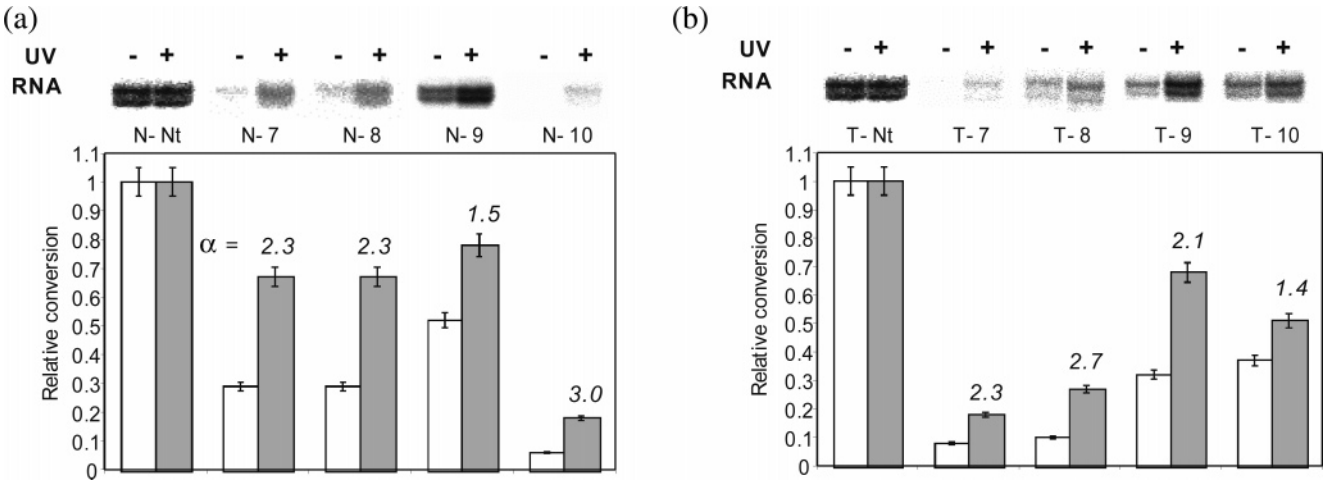


***Figure 2.*** Photocontrol of transcription reaction with a promoter tethering single azobenzene to the (a) nontemplate (**T-Nt** as a template strand) and (b) template strand (**N-Nt** as a nontemplate strand) in the T7 RNAP loop-binding region in the dark (UV −, white bars) and after UV irradiation (UV +, gray bars) at 37 °C after 2 h incubation. Photoregulatory efficiency α, defined as the ratio of the amount of transcript after UV irradiation with respect to that in the dark after 2 h incubation, was also listed in the figure. Upper parts show the PAGE pattern of the main products, and lower parts show their quantitative analyses. Relative conversion is defined as the ratio of the amount of RNA produced with respect to that of the natural promoter (**N-Nt/T-Nt**) in the dark at 37 °C after 2 h incubation.

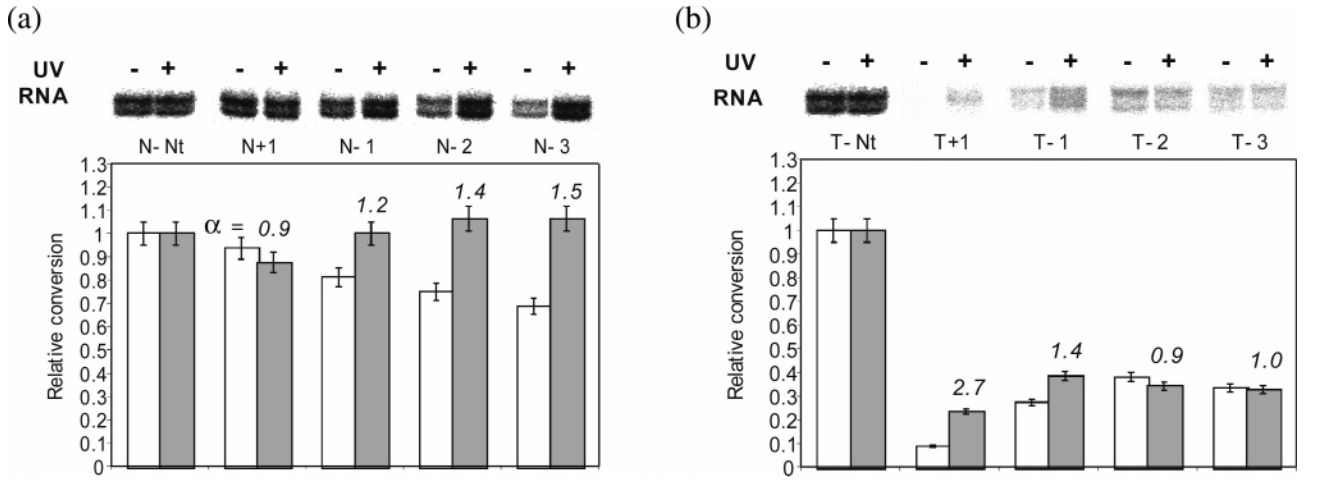


***Figure 3.*** Photocontrol of transcription reaction with a promoter tethering single azobenzene to the (a) nontemplate (**T-Nt** as a template strand) and (b) template strand (**N-Nt** as a nontemplate strand) in the T7 RNAP unwinding region in the dark (UV −, white bars) and after UV irradiation (UV +, gray bars) at 37 °C after 2 h incubation. Photoregulatory efficiency α, defined as the ratio of the amount of transcript after UV irradiation with respect to that under dark after 2 h incubation, was also listed in the figure. Upper parts show the PAGE pattern of the main products, and lower parts show their quantitative analyses. Relative conversion is defined as the ratio of the amount of RNA produced with respect to that of the natural promoter (**N-Nt/T-Nt**) in the dark at 37 °C after 2 h incubation.

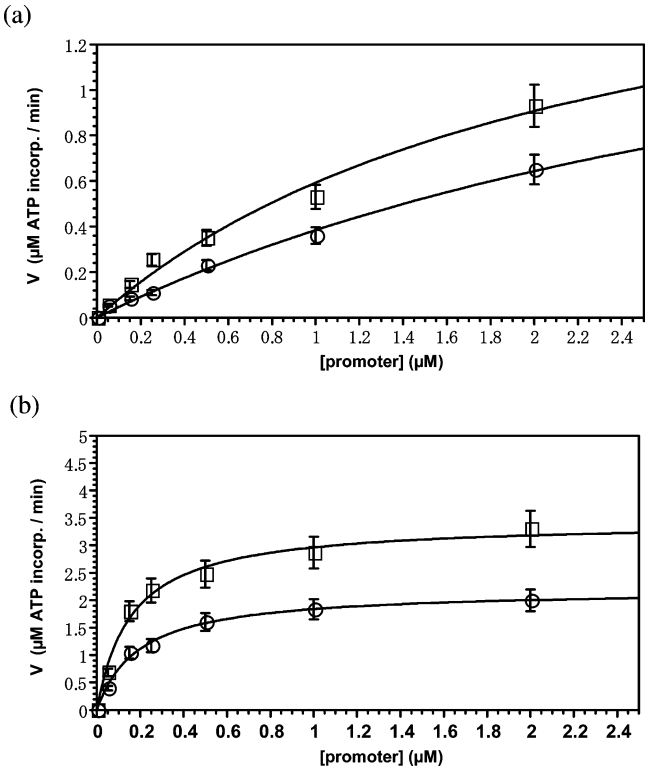


***Figure 4.*** Michaelis−Menten plots of the transcription reaction (ATP incorporation rate) by T7 RNAP as a function of the concentration of promoter with (a) **N-9/T-Nt** and (b) **N-3/T-Nt** in the dark (○) and after UV irradiation (□) at 37 °C. The concentration of T7 RNAP was kept 0.15 $\mu$M. From a set of the data, a single set of the best-fit values for $K_m$ and $k_{cat}$ was obtained as listed in Table 1. In each figure, Michaelis−Menten curves, predicted from these sets of $K_m$ and $k_{cat}$, are also superimposed.

of $K_m$ for **N-3/T-Nt**. These results demonstrate that an azobenzene at the loop-binding region of the promoter controlled the binding affinity and an azobenzene at the unwinding region changed the transcription rate constant (NTP incorporation rate).

**Incorporation of Two Azobenzenes into T7 Promoter for Still More Effective Photoregulation.** Although photoregulation of transcription was possible, photoregulatory efficiency α was at most 2.0 with **N-7/T-Nt** or **N-8/T-Nt**. For the practical applications, still more clear-cut photoregulation of transcription should be required. The above kinetic analysis revealed that an azobenzene in a different region of the promoter played a different role in the photoregulation, which prompted us to introduce two azobenzenes into loop-binding and unwinding regions, respectively. This combination significantly raised the α with reasonable transcription activity as shown in Figure 5a. For example, **N-9-3/T-Nt** involving two azobenzenes (see Scheme 1 for the sequence) in the nontemplate strand interfered transcription strongly under dark (UV −): the amount of transcript was below one-tenth with respect to that of natural **N-Nt/T-Nt**. However, transcription fairly accelerated after UV irradiation, resulting in high photoregulatory efficiency (α = 7.6). It should be noted that the cis-form kept a reasonably high transcription activity (see gray bar of **N-9-3/T-Nt** in Figure 5a). Similarly, other combinations (**N-7-3/T-Nt**, **N-8-3/T-Nt**, and **N-3/T-9** involving two azobenzenes in both nontemplate and template strands, respectively) also attained high photoregulatory efficiency.

Photoregulatory efficiency was also high even when two azobenzenes were introduced into the loop-binding region only (see Figure 5b). However, transcription activity after UV irradiation was so small that it was about one-tenth of the natural promoter. These results indicate that high photoregulatory efficiency was compatible with reasonable activity only when two azobenzenes were introduced into both specific recognition

***Table 1.*** Kinetic Parameters for **N-9/T-Nt** and **N-3/T-Nt** Promoters Determined from the Michaelis−Menten Plots of Figure 4

| promoter | azobenzene | $K_m/10^{-6}$ M | $k_{cat}/s^{-1}$ | $k_{cat}/K_m/10^6$ M$^{-1}$ s$^{-1}$ |
|---|---|---|---|---|
| **N-Nt/T-Nt**[a] | | 0.30 | 0.34 | 1.1 |
| **N-9/T-Nt** | trans-form | 4.13 | 0.22 | 0.053 |
| | cis-form | 2.26 | 0.21 | 0.093 |
| **N-3/T-Nt** | trans-form | 0.19 | 0.24 | 1.3 |
| | cis-form | 0.14 | 0.37 | 2.6 |

[a] See Figure S2 for the actual Michaelis−Menten plots.

(12) Here, simple steady-state kinetic formalism depicted in Scheme S1 was assumed according to the literature. (a) McClure, W. R. *Annu. Rev. Biochem.* **1985**, *54*, 171−204. (b) Martin, C. T.; Coleman, J. E. *Biochemistry* **1987**, *26*, 2690−2696.

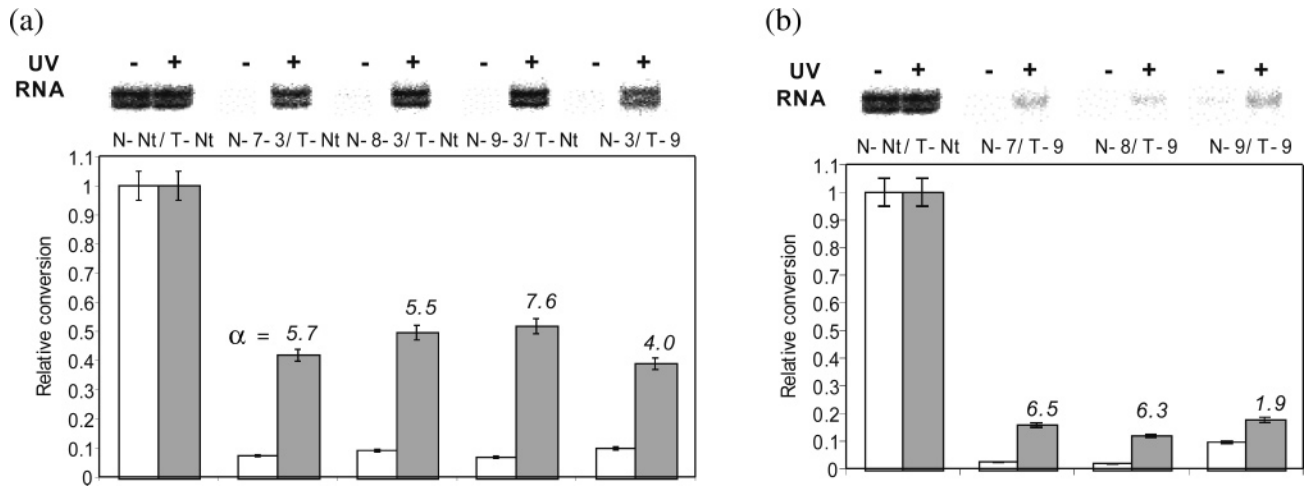


***Figure 5.*** Photocontrol of transcription reaction with a promoter tethering two azobenzenes (a) to both the loop-binding and the unwinding region, respectively, or (b) to only the loop-binding region either in the dark (UV −, white bars) or after UV irradiation (UV +, gray bars) at 37 °C after 2 h incubation. Photoregulatory efficiency α, defined as the ratio of the amount of transcript after UV irradiation with respect to that in the dark after 2 h incubation, was also listed in the figure. Relative conversion is defined as the ratio of the amount of RNA produced with respect to that of the natural promoter (**N-Nt/T-Nt**) in the dark at 37 °C after 2 h incubation.

and unwinding regions, respectively, each of which plays a different role in the transcription.

## Discussion

**Photoisomerization of Azobenzene in the T7 Promoter.** Photoregulatory transcription was achieved by introducing azobenzene(s) into the T7 promoter, and its efficiency was quantitatively analyzed. In each experiment, almost all of the azobenzene in the promoter took the trans-form in the dark or after visible light irradiation, whereas about 30% was isomerized to the cis-form under the present photoirradiation conditions.[9] Therefore, all of the quantitative data for "*cis*-azobenzene" are apparent values because they include 70% of "*trans*-azobenzene". Far below the $T_m$, trans→cis isomerization was rather suppressed due to the stacking interaction. To raise the cis-isomerization efficiency, UV irradiation at higher temperature (especially higher than $T_m$) or much stronger UV irradiation (such as a Xenon lamp as a light source) is effective.

**Effect of the Position of Azobenzene in the T7 Promoter on the Transcription. (i) Loop-Binding Region.** Most of the photoresponsive promoter tethering an azobenzene showed trans-off and cis-on switching irrespective of the position in the promoter. However, photoregulatory efficiency and transcription activity fairly depended on the position. Furthermore, kinetic analysis revealed that the photoregulatory mechanism was different in the position of an azobenzene as summarized in Table 1. In the case of **N-9/T-Nt**, tethering an azobenzene at the loop-binding region, trans−cis isomerization did not change $k_{cat}$ but $K_m$, indicating that affinity of RNAP to the promoter was photoregulated. Steitz et al. reported the high-resolution crystal structure of T7 RNAP promoter complex,[11] demonstrating that three amino acids in the specificity loop strongly interact with G-11 (nontemplate), G-9 (template), and A-8 (template) from major groove through hydrogen bonds (see Figure S3a). An azobenzene in **N-9**, located between G−C at −9 and A−T at −10 positions, interrupted the binding of T7 RNAP due to the steric hindrance either in the trans- or in the cis-form, and thus $K_m$ increased as compared to **N-Nt/T-Nt**.

According to the previous NMR analysis of azobenzene-tethered DNA duplex,[7] planar *trans*-azobenzene intercalated between the adjacent base-pairs and fairly stabilized the duplex. Further NMR analysis revealed that nonplanar *cis*-azobenzene also intercalated between the base-pairs[13] and interfered with the adjacent base-pairing (see Figure S4). These analyses suggest that *cis*-azobenzene induces much larger disordering of the duplex than does the trans-form. Yet $K_m$ of *cis*-**N-9** was smaller than that of the trans-form; *cis*-**N-9** bound to the RNAP stronger than did the trans-form. In the trans-form, an azobenzene was strongly intercalated between the adjacent base-pairs, and thus binding of the specificity loop to this region, such as recognition of G-9 at template strand by R756 in the loop, was considerably inhibited as schematically illustrated in the left of Figure 6. In case of the cis-form, presumably loosely intercalated *cis*-azobenzene would be forced to flip out by RNAP, or forced to change its position in a manner that RNAP could fit (see right of Figure 6). As a result, trans→cis isomerization decreased $K_m$ and thus accelerated the transcription.[14]

**(ii) Unwinding Region.** When an azobenzene was incorporated into the template strand (**T+1** to **T-3** in Figure 3b), both the trans- and the cis-forms significantly deactivated the transcription. According to Steitz et al,[11] base-pairs from −4 to −1 (unwinding region) are melted by T7 RNAP and the template strand is directed into the catalytic site of transcription (see Figure S3b). Because the template strand at the unwinding region was strongly bound by the RNAP, azobenzene in this strand would interfere the binding either in the trans- or in the cis-form.

In contrast, introduction of azobenzene into the nontemplate strand (**N+1** to **N-3** in Figure 3a) did not much suppress the transcription activity in the *cis*-form as compared to natural promoter, whereas the trans-form suppressed transcription. A kinetic study on **N-3/T-Nt** revealed that this photoregulatory effect was attributed to $k_{cat}$, not $K_m$. Our result is similar with the previous kinetic study carried out by Martin et al., reporting that deletion of the nontemplate as far upstream as position −1

(13) Distinct NOE between *cis*-azobenzene and the imino proton of the adjacent base-pair was observed. Furthermore, these imino proton signals were broadened due to the steric hindrance of intercalated *cis*-azobenzene. See Figure S4.

(14) A similar photoregulatory mechanism will work for a series of **N-*n*** ($7 \leq n \leq 10$) promoters tethering an azobenzene at this region.

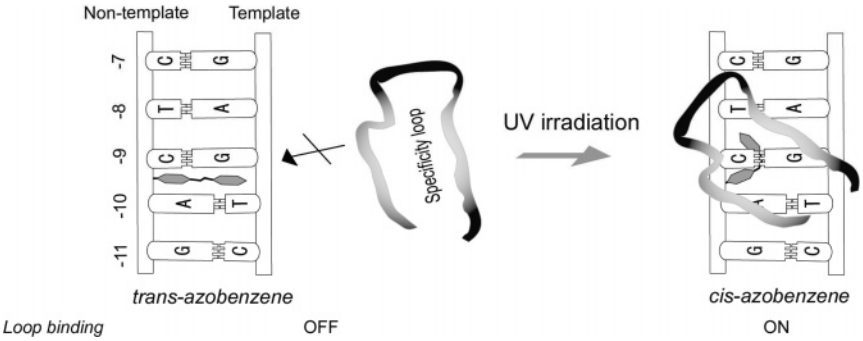


***Figure 6.*** Proposed mechanism for the change of $K_m$ by trans→cis isomerization of azobenzene at the specificity loop-binding region (**N-9/T-Nt**) of the promoter. In the trans-form (left), strongly intercalated azobenzene interfered the binding of RNAP, whereas *cis*-azobenzene (right) would be forced to flip out and facilitated the binding of RNAP.

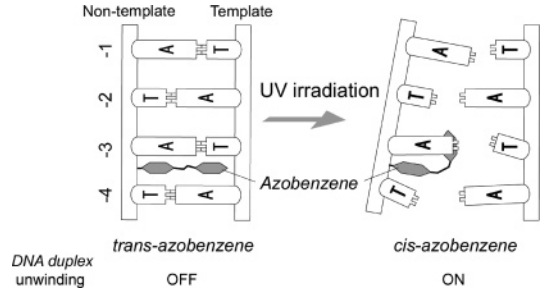


***Figure 7.*** Proposed mechanism for the acceleration of transcription by trans→cis isomerization of azobenzene, which is tethered at the unwinding region of the promoter. In the trans-form (left), duplex is firmly formed and unwinding by RNAP is interrupted. On trans→cis isomerization (right), melting of this region is facilitated due to the steric hindrance of nonplanar *cis*-azobenzene.

does not much affect the binding ($K_m$), but leads to a 2-fold increase in $k_{cat}$.[15] In other words, dissociation of the nontemplate strand at around the unwinding region facilitates NTP incorporation without changing the affinity of RNAP to the promoter. According to our previous study on the azobenzene-tethered DNA, *trans*-azobenzene stabilizes the duplex while *cis*-azobenzene destabilizes it.[7] Trans→cis isomerization facilitates the dissociation of the duplex. Assumedly, the duplex would be firmly formed in the trans-form that prevented the melting, while melting of unwinding region was facilitated in the cis-form to accelerate the transcription (NTP incorporation), as illustrated in Figure 7.

**Synergistic Effect of the Two Azobenzenes in T7 Promoter on the Photoregulation.** Clear-cut photoregulation was achieved when two azobenzenes were introduced into both the loop-binding and the unwinding regions, respectively. Photoregulatory efficiency α of **N-9-3/T-Nt** expected from the product of those of **N-9/T-Nt** and **N-3/T-Nt** (α = 1.5 for both promoters) was about 2. Yet the experiment result (α = 7.6) far exceeded this estimation. Thus, the synergistic effect of two azobenzenes for the efficient photoregulation is conclusive. Because two azobenzenes were introduced into the different regions, they did not conflict with each other but cooperated the photoregulation. In this case, reasonably high transcription activity was maintained in the cis-form. This cooperated photoregulation was mainly attributed to the strong suppression of transcription in the trans-form. Assumedly, strongly intercalated two *trans*-azobenzenes disordered the promoter duplex, and thus binding of RNAP would be strongly interfered. Yet trans→cis isomerization facilitated the binding of RNAP (loop-binding region) and the melting of the unwinding region, which switched the transcription on.[16] When two azobenzenes were introduced at the loop-binding region such as **N-7/T-9** combination, they significantly disordered the original promoter duplex at the loop-binding region where RNAP strictly recognizes. Therefore, binding of RNAP to the promoter would be strongly interfered either in the trans- or the cis-form, and thus transcription was suppressed.

In conclusion, we have devised novel photoresponsive artificial promoters. By using the azobenzene-tethered promoter, transcription can be efficiently and reversibly photoregulated. The cis−trans isomerization of azobenzene by UV or visible light provides two different states of the promoter, which allows on−off switching of RNA production. These photoresponsive promoters would be powerful tools for application in genetic therapy and photoswitching nanodevices, as well as mechanistic analysis of protein−DNA interaction.

## Experimental Section

**Materials.** All of the conventional phosphoramidite monomers, CPG columns, the reagents for DNA synthesis, and Poly-Pak cartridges were purchased from GLEN RESEARCH Co. T7 RNAP was purchased from Takara Bio Inc. Enzyme concentration was determined by optical density at 280 nm with an extinction coefficient of $1.4 \times 10^5$ $M^{-1}$ $cm^{-1}$.[17]

**Synthesis and Purification of Promoter DNAs.** All promoter DNAs used in this study were synthesized using an automated DNA synthesizer (Applied Biosystems model 394) by typical phosphoramidite chemistry. The synthesized oligonucleotides were purified by Poly-Pak cartridges followed by gel electrophoresis, and then desalted by reversed-phase HPLC (Merck LiChrospher 100 RP-18(e) column). Modified DNAs tethering the azobenzene moiety were synthesized from the corresponding phosphoramidite monomers.[6b] The concentration of oligonucleotides was determined by measuring the absorbance at 260 nm and using the integrated extinction coefficient of bases at 260 nm: dA = 15 200, dT = 8400, dG = 12 010, dC = 7050 and azobenzene residue = 4100 $M^{-1}$ $cm^{-1}$. All of the modified oligonucleotides used were characterized by MALDI-TOFMS (Bruker AutoFLEX mass spectrometer in positive ion mode).

**N-Nt.** Obsd: 10 866.6 (Calcd: 10 867.4). **T-Nt.** Obsd: 10 624.0 (Calcd: 10 626.0). **N+1.** Obsd: 11 239.1 (Calcd: 11 242.5). **N-1.**

(15) (a) Maslak, M.; Martin, C. T. *Biochemistry* **1993**, *32*, 4281−4285. (b) Ujvári, A.; Martin, C. T. *Biochemistry* **1996**, *35*, 14574−14582.

(16) In the double-incorporated azobenzene case, such as **N-9-3/T-Nt**, we think that the most active species for the transcription after UV irradiation is the doubled cis isomer (cis−cis isomer) whose population is about 10% of the total promoter. However, the possibility that only one cis isomer (cis−trans or trans−cis), which exists in about 40% of the population, also contributes to the effective transcription cannot be ruled out.

(17) King, G. C.; Martin, C. T.; Pham, T. T.; Coleman, J. E. *Biochemistry* **1986**, *25*, 36−40.

Obsd: 11 248.2 (Calcd: 11 242.5). **N-2.** Obsd: 11 238.8 (Calcd: 11 242.5). **N-3.** Obsd: 11 241.9 (Calcd: 11 242.5). **N-7.** Obsd: 11 238.6 (Calcd: 11 242.5). **N-8.** Obsd: 11 243.1 (Calcd: 11 242.5). **N-9.** Obsd: 11 241.5 (Calcd: 11 242.5). **N-10.** Obsd: 11 242.7 (Calcd: 11 242.5). **N-7-3.** Obsd: 11 618.3 (Calcd: 11 617.6). **N-8-3.** Obsd: 11 618.1 (Calcd: 11 617.6). **N-9-3.** Obsd: 11 618.1 (Calcd: 11 617.6). **T+1.** Obsd: 11 001.0 (Calcd: 11 001.1). **T-1.** Obsd: 11 000.9 (Calcd: 11 001.1). **T-2.** Obsd: 11 000.7 (Calcd: 11 001.1). **T-3.** Obsd: 11 001.5 (Calcd: 11 001.1). **T-7.** Obsd: 11 000.7 (Calcd: 11 001.1). **T-8.** Obsd: 11 000.9 (Calcd: 11 001.1). **T-9.** Obsd: 10 999.5 (Calcd: 11 001.1). **T-10.** Obsd: 10 998.3 (Calcd: 11 001.1).

**Transcription Reaction by T7 RNAP.** Conditions for the T7 RNAP reaction were as follows: [T7 RNAP (from TaKaRa)] = 50 unit in 20 $\mu$L (corresponding to 0.15 $\mu$M), [[$\alpha$-$^{32}$P]ATP] = 2 $\mu$Ci in 20 $\mu$L, [each NTP] = 0.5 mM, [each strand of the promoter] = 2.0 $\mu$M, and [spermidine] = 2 mM. Tris-HCl buffer (40 mM, pH 8.0) containing dithiothreitol (5 mM), $MgCl_2$ (24 mM), and NaCl (2 mM) was used. First, a mixture of template and nontemplate strands was annealed in 10 mM Tris-HCl buffer (pH 8.0) with 10 mM NaCl by heating at 95 °C for 3 min and cooling to 37 °C for 30 min. The mixture was then further cooled on ice, and stock solution involving NTPs and [$\alpha$-$^{32}$P] ATP was added. After the addition of T7 RNAP, the reaction mixture was incubated at 37 °C for 2 h to achieve transcription. During the reaction, a small amount of this mixture was sampled at desired intervals, and transcription was stopped by adding dye solution containing 80% formamide, 50 mM EDTA, and 0.025% bromophenol blue (in the same volume as the sampled reaction mixture). This mixture was then subjected to electrophoresis on 20% polyacrylamide 7 M urea gel. The full-length RNA separated on the gel was analyzed with a FLA-3000 bio-imaging analyzer (Fuji Photo-Film).

For the Michaelis−Menten analysis, transcription was carried out by changing the concentration of promoters. Data analysis and presentations were carried out with Kaleidagraph (Synergy Software) or DeltaGragh (SPSS Inc.). In this experiment, the concentration of T7 RNAP was kept constant at 0.15 $\mu$M. Other conditions were the same as described above.

**Photoisomerization of the Azobenzene Tethered to T7 Promoters.** The trans−cis photoisomerization was achieved by using the UV light from a 6W UV-A fluorescent lamp (FL6BL-A, Toshiba) to irradiate the sample through a UV-D36C filter (from Asahi Technoglass) for 1 min. The intensity of the UV light was below 100 $\mu$J $s^{-1}$ $cm^{-2}$. The cis→trans isomerization was achieved by irradiation with visible light from a Xenon lamp (UV Spot Light Source: HAMAMATSU PHOTONICS) for 1 min through a L-42 filter (from Asahi Technoglass). For the photoregulatory experiments, UV light was irradiated before the incubation was started at 37 °C.

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**Supporting Information Available:** Supplemental Figure S1 (UV−Vis spectra of the azobenzene-tethered T7 promoter duplex), Figure S2 (Michaelis−Menten plots of the transcription reaction with **N-Nt/T-Nt**), Figure S3 (interaction between T7 promoter and T7 RNAP), Figure S4 (local structure of DNA duplex around azobenzene), and Scheme S1 (kinetic model for runoff in vitro transcription by T7 RNAP). This material is available free of charge via the Internet at http://pubs.acs.org.

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