JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

Transcription Regulation System Mediated by Mechanical Operation of a DNA Nanostructure

Masayuki Endo,*[,†,§] Ryoji Miyazaki,[‡] Tomoko Emura,[‡] Kumi Hidaka,[‡] and Hiroshi Sugiyama*[,†,‡,§]

[†]Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Yoshida-ushinomiyacho, Sakyo-ku, Kyoto 606-8501, Japan.

[‡]Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan

[§]CREST, Japan Science and Technology Corporation (JST), Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan

Supporting Information

ABSTRACT: A transcription regulation system initiated by DNA nanostructure changes was designed and constructed. Using the toehold system, specific DNA strands induced the opening of the tubular structure. A transcription product from the purified tube-attached dsDNA template was observed by addition of DNA strands that were specific for opening the tubular structure.

Gene expression is controlled precisely by transcription factors that respond to ligands such as metabolically active molecules.[1] The dynamic reactions that occur during transcription are regulated by the interaction between RNA polymerase and DNA-binding proteins, which inhibits transcription; a simple example is their regulation by suppressors.[2] This means that the mechanical attachment and detachment of a suppressor to the regulation sites controls the on−off switching in the transcription system.

Mechanical control of DNA structural changes has been widely investigated in the field of DNA nanotechnology using DNA strand exchange, in which a target single-stranded DNA with an extra sequence attached to the terminal (called a toehold strand) is removed from the initial duplex by hybridization of a fully matched complementary strand.[3−5] The morphological change in the nanostructure can be controlled by strand exchange of toehold-containing DNA strands with specific fully matched complementary strands.[6] Therefore, this method can be applied to the regulation of transcription by controlling the 3D structural change that occurs around the promoter region.

In this study, we designed a tubular DNA nanostructure and introduced a double-stranded (ds) DNA template inside it, at the promoter region, to control RNA polymerase binding (Figure 1A). A tube-opening system using specific DNA strands was preinstalled in this tubular nanostructure. The six-helix bundled tubular structure was designed according to the rules described previously.[7,8] We used three scaffold strands and eight staple strands to form the tubular structure. In the geometry of the tube, the adjacent duplexes are connected at an angle of 120°, at which crossovers are introduced every 7 bp (Figure 1B).

The complex was formed by annealing the scaffold strands and staple strands in a buffer containing 20 mM Tris-HCl (pH 8.0) and 100 mM $MgCl_2$ from 85 to 15 °C at a rate of −1.0 °C/min. Formation of the complex was confirmed using gel electrophoresis (Figure 2A, lane 1). We observed a single band when all the strands were mixed in 1 equiv. AFM analysis revealed that this structure was a tubular nanostructure with a

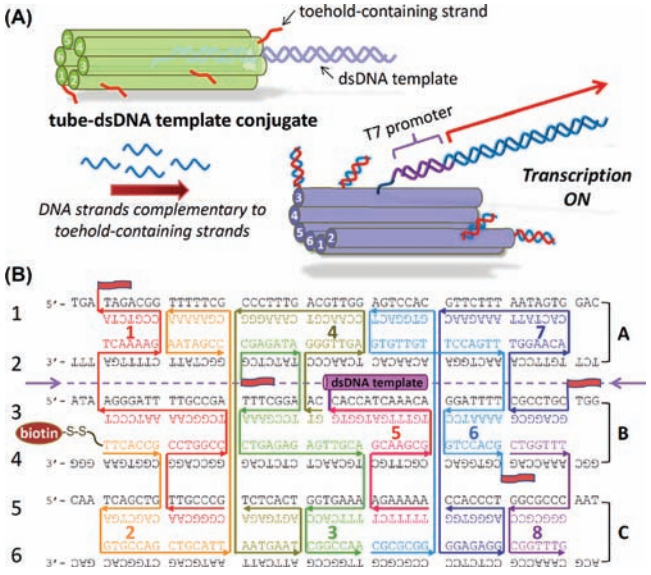

Figure 1. Transcription regulation system initiated by the nanostructure change of a DNA tube into an open form. (A) The T7 promoter region of the dsDNA template is wrapped by the tubular nanostructure. Operation of the tube opening is controlled by the toehold-containing strands (flags). (B) Design of the tubular structure and the sequences. Flags show the positions of the toehold strands. The numbers on the left side of the design represent the duplex number. The numbers of staple strands 1−8 are represented as colored letters. Addition of the four DNA stands complementary to the toehold-containing strands (staples 1, 3, 6, and 7) causes tearing of the tubular structure along the dotted line between the second and third duplexes. The dsDNA template is introduced onto the center of the third duplex (staple 5). For purification purposes, biotin with disulfide linkage is attached on the 5′-side of the staple in the fourth duplex (staple 2).

Received: August 9, 2011
Published: January 26, 2012

   dx.doi.org/10.1021/ja2074856 | J. Am. Chem.Soc. 2012, 134, 2852−2855

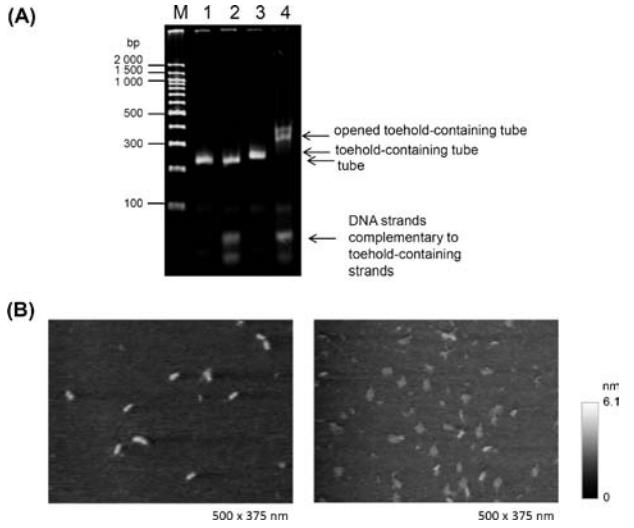

Figure 2. Complex formation and morphological change of the tubular DNA nanostructure to an open form. (A) Analysis using 4% native polyacrylamide gel electrophoresis (PAGE). Lane 1, assembly of a tubular structure; lane 2, addition of specific DNA strands to the tubular structure; lane 3, assembly of a tubular structure with toehold-containing strands; lane 4, addition of specific DNA strands to the toehold-containing tube. (B) AFM images of the closed form (left) and opened form (right) after addition of specific DNA strands that were complementary to the toehold-containing strands.

length of 19.1 ± 1.2 nm (expected length, 19 nm) and a diameter of ~5 nm (Figure S2C).

Next, we examined the opening of the tubular structure using the toehold system. The toehold-containing strands were introduced into the terminal of the four staple strands. After formation of the complex, four DNA strands complementary to the toehold-containing strands were added to open the tubular structure. Opening of the tube was confirmed using gel electrophoresis and AFM analysis. As shown in the gel image (Figure 2A), the smear band (lane 4) observed was retarded compared with the band corresponding to the closed tube (lane 3). The unmodified tube did not respond to the addition of the specific strands (lane 2). In solution, opened tubes should form flexible curved structures; thus, the opened tubes exhibited different sizes, resulting in smearing of the band in the gel. AFM also demonstrated that this structure was a tubular nanostructure that was opened to the single-layered structure by addition of the specific strands (Figures 2B and S2D). These results indicate that the formation and opening of the tube can be controlled using this toehold system.

Subsequently, we examined the effect of tube opening on the binding of RNA polymerase to the DNA tubular complex. A T7 promoter-containing dsDNA (40 bp) was attached to the DNA tube. After annealing, binding of T7 RNA polymerase to the closed and opened tubular structure was analyzed using native PAGE (Figure S3). The closed tubular complex was not affected by the addition of RNA polymerase. Conversely, the bands corresponding to the opened tubular complex shifted after addition of RNA polymerase at a concentration identical to that used for the closed structure. These results show that the closed tube prevents the binding of RNA polymerase to the promoter site and that opening of the tube induces RNA polymerase binding.

To construct a transcription-activation system, the PCR-amplified dsDNA template (~900 bp) was incorporated into the tubular structure. A mixture of the scaffold strands, staple strands, and dsDNA template with a staple strand sequence was annealed from 85 to 60 °C for 50 min, and then from 60 to 15 °C for 4.5 h, to allow complex formation. Migration of the band in the gel was slower than that of the dsDNA template (Figure

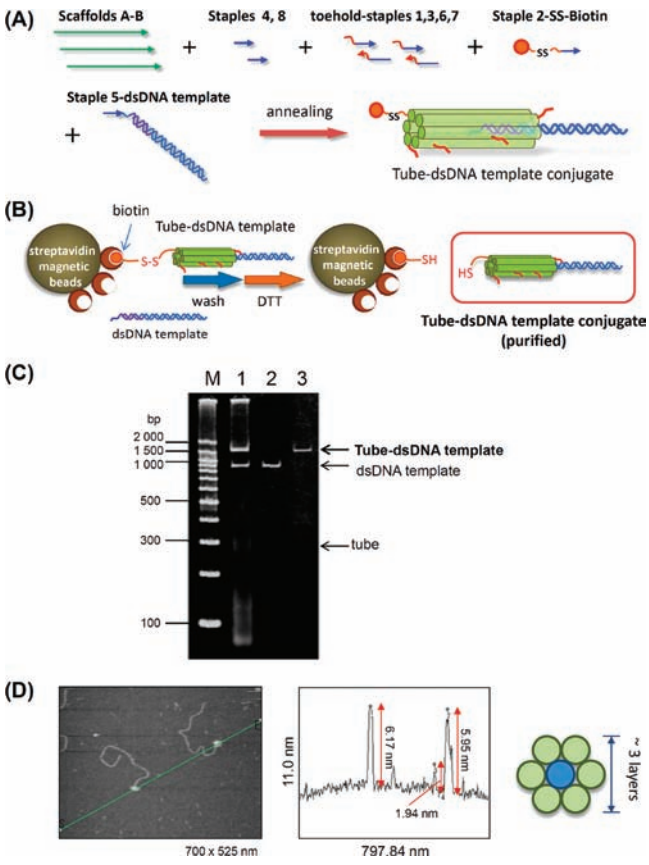

Figure 3. Preparation and purification of the tube–dsDNA template conjugate. (A) Scheme for assembling the DNA strands to prepare the tube–dsDNA template conjugate. (B) Scheme for purifying the tube–dsDNA template conjugate, which has a biotin and disulfide linker. (C) Native PAGE (4%) of the tube–dsDNA template conjugate. Lane 1, mixture; lane 2, supernatant after washing; lane 3, recovery after DTT reduction. (D) AFM image of the purified tube–dsDNA template conjugate and sectional analysis of the nanostructures along the green line in the AFM image. The illustration on the right is a sectional view of a nanostructure where a dsDNA template (blue) is included in the six-helix bundled tube (green).

3C, lane 1). Analysis of the mixture using AFM showed that the tubular structure appeared at the terminal of the dsDNA template (Figure S4E). Gel electrophoresis showed that the dsDNA template was incorporated into the tubular structure at a yield of 50–60%.

Construction of a gene expression system required the assembly and purification of the tube–dsDNA template conjugate. We introduced a biotin-labeled staple strand into the tubular structure (Figure 3A). After structure formation, the conjugate was incubated with streptavidin-conjugated magnetic beads, and the unattached dsDNA template was removed by washing (Figure 3B). The target structure was recovered by cleavage of the disulfide bond using DTT reduction. Gel electrophoresis showed that the target structure was purified and separated from the unincorporated dsDNA template (Figure 3C, lane 3). The recovered product was observed

dx.doi.org/10.1021/ja2074856 | J. Am. Chem.Soc. 2012, 134, 2852–2855

using AFM, and all dsDNA templates had the tubular structure at their terminal (Figure 3D). Sectional analysis showed that the amount of tubular structure was 3 times higher than that of the duplex structure, indicating that the six-helix-bundled tube wrapped the dsDNA template.

The tubular structure attached to the dsDNA template was opened using specific DNA strands that were complementary to the toehold-containing strands. The opened form was retarded in gel electrophoresis (Figure 4A). Sectional analysis

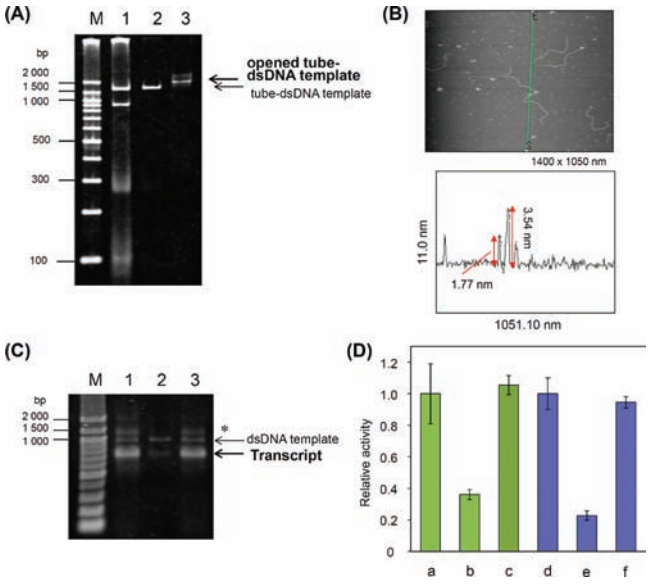

Figure 4. Transcription after opening of the tube–dsDNA template conjugate using DNA strands complementary to the toehold-containing strands. (A) Native PAGE (4%) of the opening of tube–dsDNA template conjugate by DNA strands complementary to the toehold-containing strands. Lane 1, mixture of tube–dsDNA template; lane 2, purified tube–dsDNA template conjugate; lane 3, after addition of complementary strands to tube–dsDNA template conjugate. (B) Sectional analysis of the tube–dsDNA template conjugate after addition of the complementary strands to the toehold-containing strands. (C) Agarose gel (1%) electrophoresis of the transcription mixture incubated at 34 °C. Lane 1, transcription from PCR-amplified dsDNA template; lane 2, transcription from the tube–dsDNA template conjugate without treatment with the complementary strands (closed); lane 3, transcription from the tube–dsDNA template conjugate after addition of the complementary strands (opened); M, DNA marker. Asterisk represents unidentified bands accompanying the transcription intensity. (D) Quantification of transcript at 37 (green bars) and 34 °C (blue bars): transcription using the dsDNA template (a,d) and transcription using the closed (b,e) and opened tubes (c,f). The values were normalized by the quantified band of transcript from the dsDNA template.

showed that the amount of conjugate in the tube region was twice that of the usual duplex, indicating that the tubular structure was changed to an open form (Figure 4B).

Finally, we examined *in vitro* transcription using T7 RNA polymerase. The dsDNA template comprised ~900 bp; thus, when transcription occurs, the switching can be monitored by the expression level of the RNA transcript. Before transcription, the tube–dsDNA template conjugate was incubated with DNA strands that were complementary to the toehold-containing strands at 30 °C for 15 min, followed by addition of T7 RNA polymerase. The reaction was performed at 34 and 37 °C for 5 min, after which the transcription level was analyzed using agarose gel electrophoresis. After the completion of the reaction at 34 °C, the transcription level of the sample to which the complementary strands were added was 5.5 times higher than that of the closed-tube structure (Figure 4C). The low-level transcription activity observed for the closed form may be attributed to imperfect prevention of RNA polymerase binding. The experiment of DNase cleavage of the tube conjugate revealed that the dsDNA template was slightly cleaved in the closed form (Figure S5). Therefore, the tube conjugate permitted partial access of RNA polymerase to the dsDNA template in the closed form, because the initial structure may contain a small portion in which the dsDNA template may be unwrapped by the tubular structure. The relative transcription levels of the closed and opened tube–dsDNA template conjugates were also dependent on temperature. Suppression of transcription by tube wrapping was more efficient at 34 °C than at 37 °C (Figure 4D). Although the suppression of transcription using the closed-tube structure was not still complete, transcription was controlled and recovered via the mechanical operation of the 3D structural change, by addition of specific DNA strands.

In conclusion, we have designed and prepared a tube-attached dsDNA and have controlled the mechanical opening of the tube using the toehold system. Transcription was controlled using this opening system. Basically, this system uses standard oligonucleotides and a PCR-amplified dsDNA template, thus differing from photocontrolled transcription, which uses photoresponsive synthetic oligonucleotides.[9,10] Specific DNA strands induced the 3D structural change and subsequent transcription. This method can be applied to mechanical switches for controlling the expression of various biological reactions.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, sequences of oligonucleotides, and additional AFM images. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

endo@kuchem.kyoto-u.ac.jp; hs@kuchem.kyoto-u.ac.jp

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Core Research for Evolutional Science and Technology (CREST) of JST and a Grant-in-Aid for Science research from the MEXT, Japan. Financial support from Iketani Science and Technology Foundation and Sumitomo Foundation to M.E. is also acknowledged.

■ REFERENCES

(1) (a) Patikoglou, G.; Burley, S. K. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 289. (b) Tan, S.; Richmond, T. J. *Curr. Opin. Struct. Biol.* **1998**, *8*, 41.

(2) McClure, W. R. *Annu. Rev. Biochem.* **1985**, *54*, 171.

(3) (a) Endo, M.; Sugiyama, H. *ChemBioChem* **2009**, *10*, 2420. (b) Rajendran, A.; Endo, M.; Sugiyama, H. *Angew. Chem., Int. Ed.* **2012**, *51*, 874.

(4) Bath, J.; Turberfield, A. J. *Nat. Nanotechnol.* **2007**, *2*, 275.

(5) (a) Yurke, B.; Turberfield, A. J.; Mills, A. P. Jr.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605. (b) Yan, H.; Zhang, X.; Shen, Z.; Seeman, N. C. *Nature* **2002**, *415*, 62.
(6) (a) Andersen, E. S.; Dong, M.; Nielsen, M. M.; Jahn, K.; Subramani, R.; Mamdouh, W.; Golas, M. M.; Sander, B.; Stark, H.; Oliveira, C. L.; Pedersen, J. S.; Birkedal, V.; Besenbacher, F.; Gothelf, K. V.; Kjems, J. *Nature* **2009**, *459*, 73. (b) Han, D.; Pal, S.; Liu, Y.; Yan, H. *Nat. Nanotechnol.* **2010**, *5*, 712.
(7) (a) Mathieu, F.; Liao, S.; Kopatsch, J.; Wang, T.; Mao, C.; Seeman, N. C. *Nano Lett.* **2005**, *5*, 661. (b) Kuzuya, A.; Wang, R.; Sha, R.; Seeman, N. C. *Nano Lett.* **2007**, *7*, 1757. (c) Wang, R.; Liu, W.; Seeman, N. C. *Chem. Biol.* **2009**, *16*, 862.
(8) (a) Douglas, S. M.; Chou, J. J.; Shih, W. M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 6644. (b) Douglas, S. M.; Dietz, H.; Liedl, T.; Högberg, B.; Graf, F.; Shih, W. M. *Nature* **2009**, *459*, 414.
(9) Kröck, L.; Heckel, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 471.
(10) Liu, M.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2006**, *128*, 1009.