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Transcriptional Control of DNA-Based Nanomachines

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ABSTRACT

The construction of autonomous DNA-based nanomachines is an important challenge. To this end we have combined DNA tweezers with the transcription machinery of prokaryotic organisms and created a gene that codes for the production of an mRNA strand to bring about a conformational change in the tweezers. Gel electrophoresis and FRET experiments demonstrate that the transcription process successfully reads out the gene and automatically brings the machine to the desired state.

Precise nanoscale movements can be achieved reversibly and reproducibly with molecular machines based on DNA. A variety of motion, including stretching [1−4] and twisting,[5,6] can be performed by these devices in well-defined steps. Recently, a DNA machine that binds, carries, and releases a single protein molecule upon instruction has been developed.[7] Generally, these machines operate through the manual addition of a "fuel" strand, consisting of single stranded DNA (ssDNA) that affects a conformational change in the device.[3] The machine is returned to its original configuration by the introduction of a "removal" strand fully complementary to the fuel strand. To a large extent, DNA in these studies is regarded as a structural material with controllable self-assembly properties rather than as a biological molecule. The sequence and highly specific base pairing rules in DNA are used for designing the type of motion the nanomachine performs rather than as a genetic code. The fact that DNA is part of a highly evolved system for protein synthesis in organisms has been largely irrelevant. In this letter we take advantage of the properties of nucleic acids as a genetic material, which can be synthesized and controlled by the gene expression machinery, to genetically program the automatic "closing" of DNA-based tweezers.

Gene expression consists of two processes: transcription and translation. The product of transcription is a messenger RNA (mRNA) molecule, which then can serve as a template for protein synthesis during translation. It is during transcription that gene regulation occurs through a complex series of switches based on responses to stimuli or the cell's environment. So far, nanomachines have been controlled with DNA rather than RNA, although this nucleic acid has similar self-assembly capabilities and base pairing rules (except instead of adenine−thymine, there is adenine−uracil) to DNA. This is mainly due to the ready availability of DNA and the greater effort required to protect RNA from RNAse digestion, which is ubiquitous. We have engineered a gene to encode for the production of a fuel strand in the form of mRNA (Figure 1a). This mRNA fuel strand, when it encounters a DNA tweezers, is able to hybridize with the DNA and bring about closing of the tweezers (Figure 1b).

The DNA tweezers in its "open" state is constructed from three strands of ssDNA, which hybridize to produce two stiff arms of double-stranded DNA hinged by a short segment of ssDNA forming a V configuration with two segments of ssDNA at each end of the V (Figure 1). The sequence and structure of this tweezers and its accessory strands are similar to those published in ref 3. The fuel gene consists of a promoter and start sequence for the T7 RNA polymerase,[8] the fuel sequence, and a non-looping transcription termination sequence.[9] In vitro transcription was initiated by combining the gene with a transcription solution consisting of T7 RNA polymerase, ribonucleotide triphosphates (rNTPs), RNAse inhibitor, and a transcription buffer with the proteins, salts, and pH necessary for efficient transcription, and incubated at 37 °C for 3 hours.[10]

To determine whether the mRNA transcripts produced by T7 RNA polymerase are full length and behave similarly to normal ssDNA fuel, non-denaturing gel electrophoresis under RNAse-free conditions was conducted. In Figure 2a the polyacrylamide gel electrophoresis (PAGE) of the operating cycle of mRNA fuel is compared with that of ssDNA fuel. To the tweezers in its relaxed open state (lane a) was added ssDNA fuel, resulting in the disappearance of the band corresponding to open tweezers and the emergence of a

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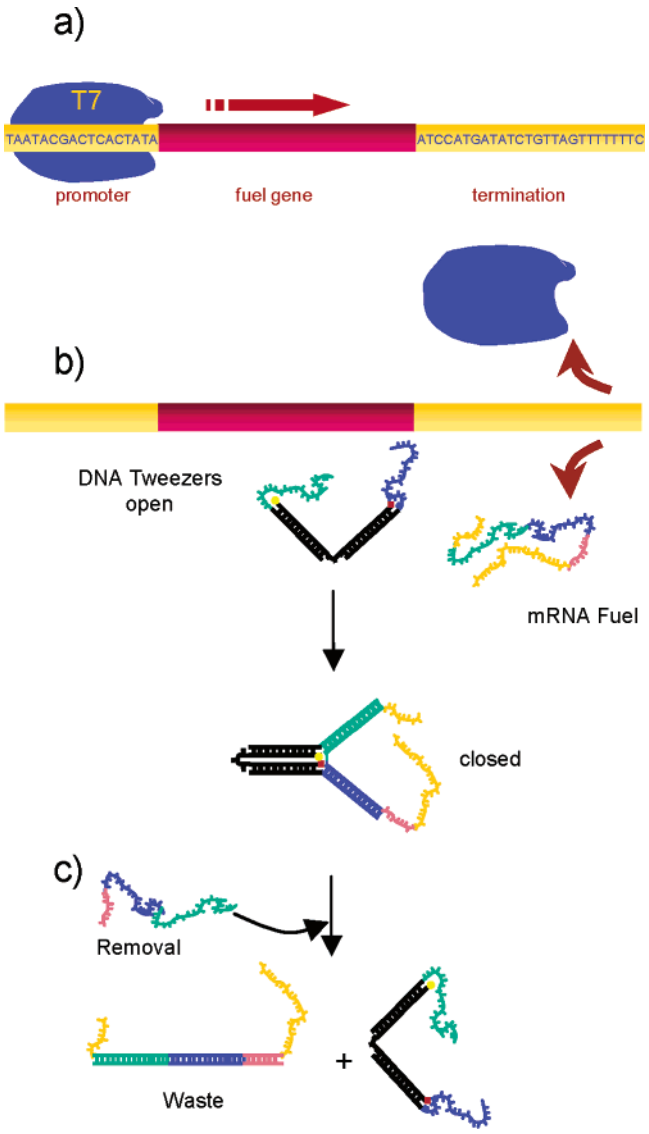

Figure 1. (a) Schematic of the double-stranded DNA template used to transcribe the fuel mRNA. The promoter and termination sequence employed are shown. (b) Transcription produces a mRNA strand that causes the tweezers, labeled with two dyes, to change configuration. (c) The tweezers can be returned to its original state by the addition of a removal strand.

slower band for closed tweezers (lane b). When ssDNA removal is added, the tweezers returns to its open state and an additional band is apparent for the fuel-removal duplex waste product (lane c). Two bands are visible in the transcription reaction after 3 h, one corresponding to the DNA gene and the other to the transcribed mRNA, which is slower than its template because of the tendency of RNA to form secondary structures through non-Watson−Crick base pairing (lane e). When open tweezers were added to this completed transcription reaction (Figure 1b), the mRNA band vanishes and a band corresponding to the closed tweezers develops (lane f). This tweezers-mRNA hybrid is slower than its DNA-only counterpart because the transcription start and termination, in addition to the fuel sequences, are inserted into the mRNA by the T7 RNA polymerase. The ssDNA removal strand can also hybridize with the mRNA to restore the tweezers to its open state and produces mRNA-removal waste (lane g). The small residual of closed tweezers is a consequence of shorter transcripts being written that can close the tweezers but do not contain the toehold sequence (Figure 1c, pink segment) to allow the removal strand to grip on to the fuel segment and initiate branch migration. Otherwise, the mRNA encoded by the fuel gene is observed to perform the same function as ssDNA fuel.

To bring about automatic switching of the tweezers, the nanomachine must be present during all stages of gene expression. In this way the tweezers is automatically instructed by information programmed in the fuel gene to open rather than by external coercion. A transcription reaction was completed with the open tweezers present in solution from the start. The corresponding PAGE experiment exhibits a band for the closed tweezers and the mRNA band is absent, indicating successful closure of the tweezers through gene expression (Figure 2b, lane i). The tweezers can be opened once again with the manual addition of ssDNA removal strand, and mRNA-DNA waste is produced (lane j). Thus, the presence of the DNA machine during transcription does not cause premature termination by binding of the tweezers to the growing mRNA strand or disturb the process in any other way.

The motion of the tweezers during transcription can be monitored in real time using fluorescence resonance energy transfer (FRET) experiments. Similar to ref 3, the tweezers were labeled with Oregon Green 488 and Blackhole Quencher 1 (Figure 1b). In the open state of the tweezers, energy transfer is inefficient due to the large separation between dye and quencher. When the tweezers are closed, the dye and quencher are brought closer to a distance smaller than the Förster radius and quenching is efficient. A measurement of the fluorescence of a transcription reaction consisting of labeled tweezers at 33 °C exhibits a decrease in fluorescence with time, consistent with the closing of tweezers as the fuel gene is transcribed into mRNA (Figure 3). The reaction is completed after approximately 2.5 h, which is significantly longer than the few minutes required for the tweezers to close when DNA fuel is added. To eliminate the possibility that the quenching results from binding of the RNA polymerase, transcription proteins, or fuel gene to the tweezers, a transcription reaction with tweezers was conducted without rNTPs. The corresponding FRET experiment displays little change in fluorescence over 2 h. Moreover, the manual addition of removal ssDNA to the functional transcription reaction after 2.5 h results in almost a complete recovery of the fluorescence, signifying the expression of full-length, operational mRNA transcripts.

We have shown that a program for the operation of DNA-based nanomachines can be coded genetically. Using an artificially created gene and the transcription machinery of prokaryotic cells, automatic closing of DNA tweezers was brought about by a messenger RNA fuel strand. Because mRNA and transcription conditions are compatible with DNA nanomachine function, it will also be possible to get the nanodevice to perform a complex series of tasks, such as alternatively opening and closing a fixed number of times, by encoding in a gene the instructions and taking advantage

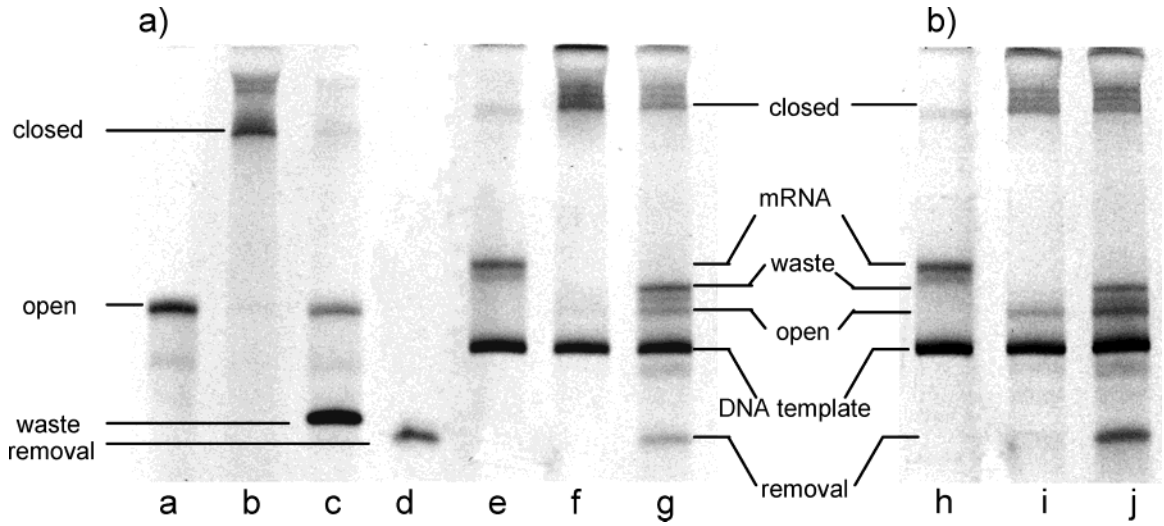

Figure 2. Analysis of tweezers operation by non-denaturing PAGE. The lanes are as follows: (a) a, tweezers; b, tweezers + fuel; c, (tweezers + fuel) + removal; d, removal; e, transcription reaction; f, transcription reaction + tweezers; g, (transcription reaction + tweezers) + removal (b) h, transcription reaction; i, transcription reaction with tweezers present from start; j, to the previous reaction was added manually removal strand.

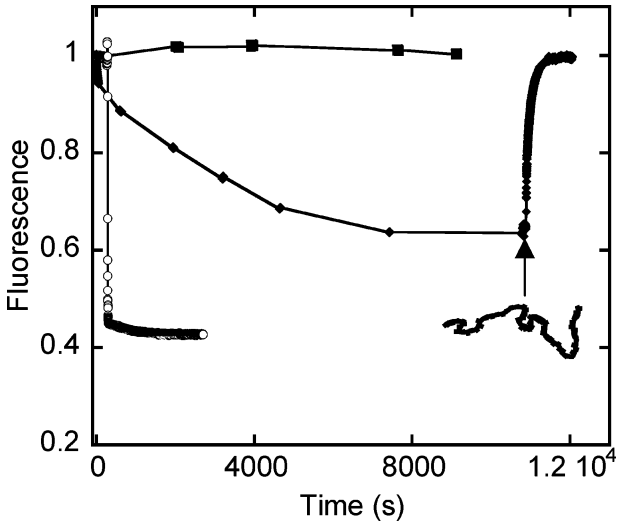

Figure 3. FRET experiments of the closing of tweezers with time as mRNA is transcribed (solid diamonds). At the completion of transcription, removal strand was manually added to the former reaction to return tweezers to their open state. The same reaction in the absence of rNTPs (solid squares) and the closing of tweezers by the addition of ssDNA fuel (open circles) are shown.

of the wide range of regulation motifs that exist for turning on and off genes in series and in parallel.[11]

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References

(1) Li, J. W. J.; Tan, W. H. *Nano Lett.* **2002**, *2*, 315−318.
(2) Feng, L. P.; Park, S. H.; Reif, J. H.; Yan, H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4342−4346.
(3) Yurke, B.; Turberfield, A. J.; Mills, A. P.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605−608.
(4) Simmel, F. C.; Yurke, B. *Appl. Phys. Lett.* **2002**, *80*, 883−885.
(5) Yan, H.; Zhang, X. P.; Shen, Z. Y.; Seeman, N. C. *Nature* **2002**, *415*, 62−65.
(6) Mao, C. D.; Sun, W. Q.; Shen, Z. Y.; Seeman, N. C. *Nature* **1999**, *397*, 144−146.
(7) Dittmer, W. U.; Reuter, A.; Simmel, F. C. *Angew. Chem., Int. Ed.* **2004**, in press.
(8) Imburgio, D.; Rong, M. Q.; Ma, K. Y.; McAllister, W. T. *Biochemistry* **2000**, *39*, 10419−10430.
(9) Lyakhov, D. L.; He, B.; Zhang, X.; Studier, F. W.; Dunn, J. J.; McAllister, W. T. *J. Mol. Biol.* **1998**, *280*, 201−213.
(10) Sambrook, J.; Russell, D. W. *Molecular Cloning − A Laboratory Manual*, 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 2001.
(11) Lee, T. I.; Rinaldi, N. J.; Robert, F.; Odom, D. T.; Bar-Joseph, Z.; Gerber, G. K.; Hannett, N. M.; Harbison, C. T.; Thompson, C. M.; Simon, I.; Zeitlinger, J.; Jennings, E. G.; Murray, H. L.; Gordon, D. B.; Ren, B.; Wyrick, J. J.; Tagne, J. B.; Volkert, T. L.; Fraenkel, E.; Gifford, D. K.; Young, R. A. *Science* **2002**, *298*, 799−804.

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