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A Tightly Regulated Molecular Motor Based upon T7 RNA Polymerase

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ABSTRACT

Controlled movement of materials or molecules within the nanometer range is essential in many applications of nanotechnology. Here we report the capture, movement, and release of cargo molecules along DNA by a modified form of T7 RNA polymerase (RNAP) in a manner that is controlled by the sequence of the DNA. Using single-molecule methods, we visualize the assembly and manipulation of nanodevices and the ability to harness rotary and linear forces of the RNAP motor.

Many applications in nanotechnology require the precise movement and positioning of components within the nanometer scale range. Biological systems also require such capacities, and biomolecular motors such as F_1-ATPase and kinesin have been adapted to power the rotary and linear movement of nanodevices, respectively.[1−6] Although these motors may be switched on and off in response to changes in substrate or reaction conditions, they cannot be controlled in a precise, incremental fashion. In contrast, the movement and positioning of RNA polymerase (RNAP) and other nucleotide polymerases may be controlled in an information-dependent manner, depending upon the sequence of the template. Molecular motors based upon the latter enzymes may therefore provide important advantages for applications in nanosystems requiring controlled movement.

The mechanism of action of RNAP during transcription suggests that it might be utilized as a molecular stepper motor. As the polymerase advances, it unwinds the DNA at the leading edge of the transcription complex and rewinds the DNA at the trailing edge, resulting in both linear and rotary movement of the complex with respect to the helical axis of the DNA. Each step of nucleotide (nt) incorporation corresponds to 0.34 nm of linear translocation and 36° of rotation, and it has been shown that the progress of the RNAP may be controlled in a stepwise manner by withholding or adding appropriate substrates, as directed by the sequence of the template.[7,8] The movement of the RNAP may therefore be regulated according to the design of the template. Importantly, single-molecule studies indicate that the multisubunit *E. coli* RNAP can exert linear forces up to 30 pN and rotary forces of 5 pN·nm,[9−11] which are in the range

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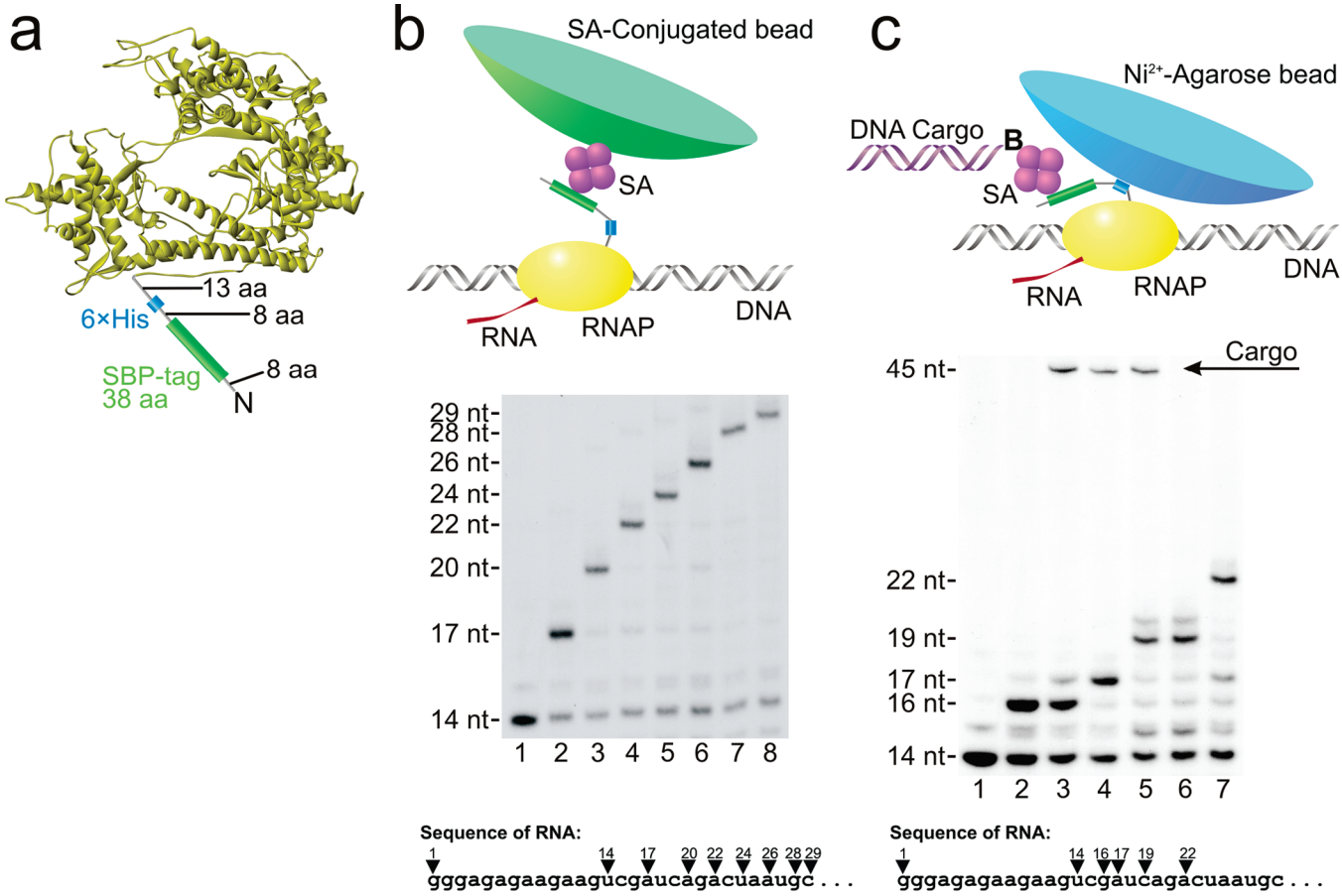

Figure 1. Movement of the RNAP motor and bound cargo. (a) Structural representation of SBP−His-RNAP. (b) SBP−His-RNAP ECs halted at position +14 were formed by incubating SBP−His-RNAP with DNA (template 1, see Supporting Information), GTP, α-(^{32}P)-ATP, and UTP, and immobilized on SA-beads (upper panel and lane 1). The ECs were advanced downstream by the addition of limited mixtures of substrate and washing, as indicated below, to positions +17 (CTP, GTP, ATP; lane 2), +20 (UTP, CTP, ATP; lane 3), +22 (GTP, ATP; lane 4), +24 (CTP, UTP; lane 5), +26 (ATP, lane 6), +28 (UTP, GTP; lane 7), and +29 (CTP, lane 8). Samples were withdrawn after each step and analyzed by gel electrophoresis. (c) SBP−His-RNAP ECs were formed as in part b and immobilized on Ni^{2+} beads (upper panel and lane 1). The ECs were advanced to position +16 by addition of CTP and GTP (lane 2). A 45 bp SA-biotin-conjugated ^{32}P-DNA cargo molecule (cargo 1, see Supporting Information) was added (lane 3, upper band) and transported downstream to positions +17 (ATP, lane 4) and +19 (CTP and UTP, lane 5). The cargo was eluted by the addition of biotin (lane 6), and the EC was advanced to position +22 (ATP and GTP, lane 7).

exerted by other biomolecular motors (e.g., F_1-ATPase, kinesin, myosin).

In this work, we have utilized the single subunit RNAP encoded by bacteriophage T7 to explore the potential of this class of enzymes as molecular motors. Although force measurements have not been conducted for this enzyme, they are likely to be similar to those of the multisubunit RNAPs due to similarities in the transcription mechanism. T7 RNAP (and the RNAPs of related phages) offer particular advantages for such studies due to their ease of genetic manipulation, high specificity, and processivity.

For many applications involving controlled movement by an RNAP motor, it would be desirable to immobilize the enzyme or the template along which it moves to a solid surface. Crystal structures of T7 RNAP indicate that the N-terminus of the enzyme is solvent exposed and far from the polymerization domain,[12−15] and a variety of modifications to this region of the RNAP have been made without affecting catalytic activity.[16−18] Consistent with these observations, it has been shown that the fusion of a histidine$_6$ tag to the N-terminus (His-RNAP) allows transcription elongation complexes (EC) to be attached to a solid surface (Ni^{2+}-agarose beads) and "walked" along a DNA template during repeated cycles of nucleotide addition and removal.[8]

To confer reversible cargo-binding capabilities to T7 RNAP, we modified His-RNAP to include a streptavidin-binding protein (SBP) domain[19] (SBP−His-RNAP; Figure 1a). As shown in Figure 1b, SBP−His-RNAP transcription complexes may be immobilized on streptavidin (SA) conjugated beads and advanced along the template in increments from 1 to 3 nt during repeated cycles of polymerization, as had previously been demonstrated for His-RNAP.[8] In subsequent experiments we demonstrated controlled movement over much greater distances (see, for example, Figure 2).

The ability of the SBP−His-RNAP to capture, transport, and release a cargo molecule was examined as shown in Figure 1c. Transcription complexes halted 14 nts downstream from the promoter were immobilized on Ni^{2+}-agarose beads and washed to remove unincorporated substrates (lane

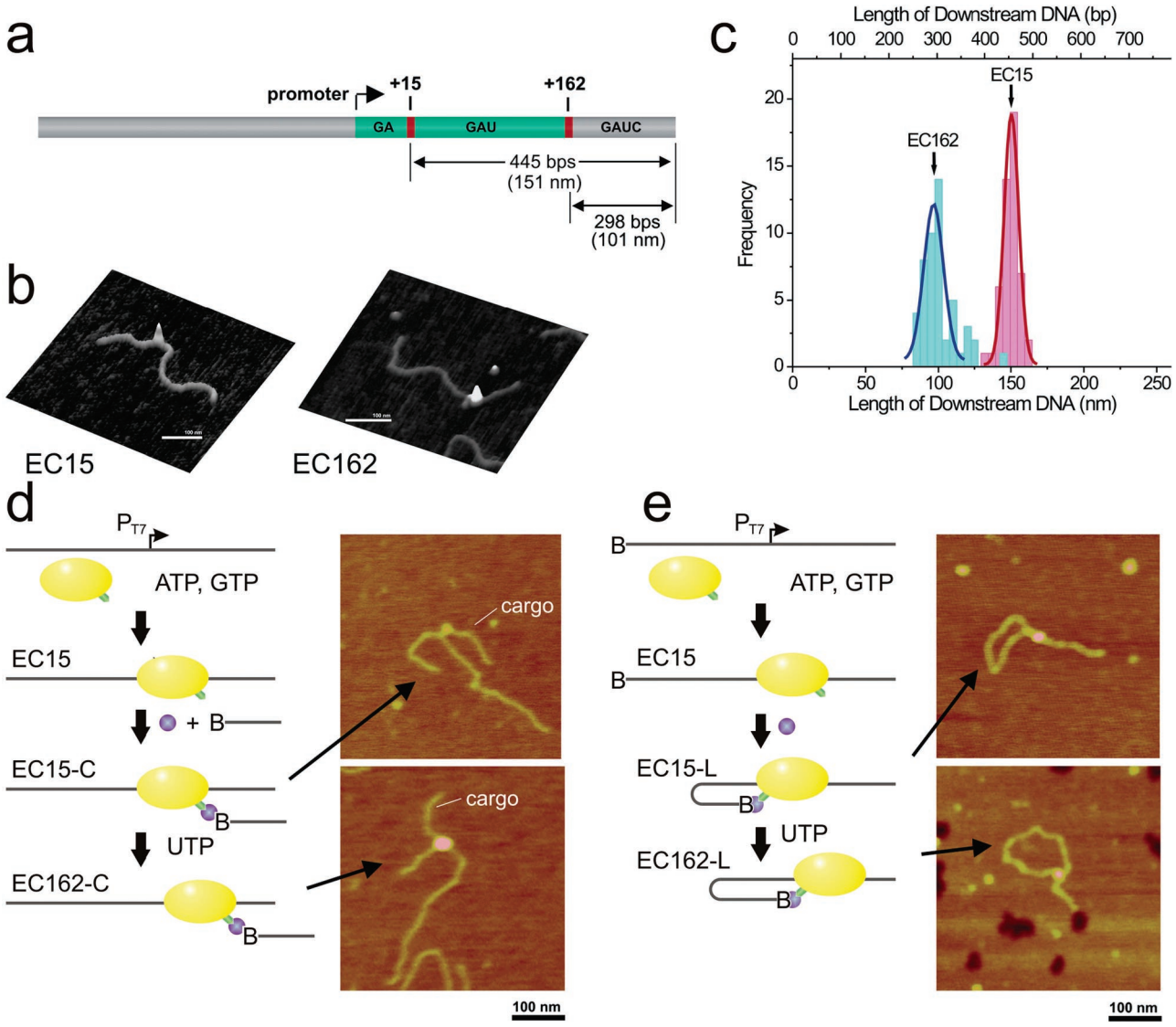

Figure 2. Assembly and manipulation of DNA nanodevices. (a) A DNA template (template 2) was constructed that allows the formation of an EC15 in the presence of GTP and ATP and advance of the EC to +162 upon addition of UTP. (b) AFM images of ECs halted at positions +15 (left) and +162 (right) (bar = 100 nm). (c) Downstream DNA contour length distributions of complexes (n = 50) halted at positions +15 (red) and +162 (blue). (d) and (e) SBP-His-RNAP ECs halted at +15 (EC15) were coupled to a biotinylated DNA molecule through an SA bridge (blue sphere) using either a separate cargo molecule (cargo 2, see Supporting Information) to create an intermolecular device (EC15-C; panel d) or using a template (template 2B) that was biotinylated at the upstream end, thereby forming an internal loop in the template (intramolecular device; EC15-L, panel e). The devices were visualized by AFM when the ECs were halted at position +15 (upper panels) and after advancement to +162 (lower panels).

1). The complexes were advanced 2 base pairs downstream by the addition of CTP and GTP (lane 2), and an SA-conjugated ^{32}P-DNA cargo molecule 45 base pairs in length was then added. The cargo was retained by the complex after washing, as indicated by the upper band in lane 3. The cargo was advanced downstream in two sequential steps: first by 1 base pair (lane 4) and then by 2 base pairs (lane 5). Finally, the cargo was eluted by the addition of biotin (lane 6), and the polymerase was advanced another 3 base pairs downstream (lane 7).

The experiment above suggests that RNAP might be utilized to assemble and manipulate novel DNA nanodevices. The construction of simple inter- and intramolecular devices and their responses to the addition of substrate were visualized by atomic force microscopy (AFM) as shown in Figure 2. To construct an intermolecular device, we incubated an SBP−RNAP EC with SA and a separate biotinylated DNA cargo molecule (similar to the approach shown in Figure 1c) (Figure 2d). To assemble an intramolecular device, the template DNA was biotinylated at its upstream end; attachment of this end to the halted SBP−RNAP via an SA tetramer bridge resulted in the formation of an internal DNA loop that extends from the end of the template to the polymerase (Figure 2e). In both cases, the design of the template was such that addition of UTP results in advancement of the halted EC by 147 base pairs (or 50 nm; i.e., from position +15 to +162). Analyses of 50 elongation complexes by AFM (Figure 2c) indicates that nearly all complexes were advanced along the DNA in the expected manner (consistent with results obtained with immobilized

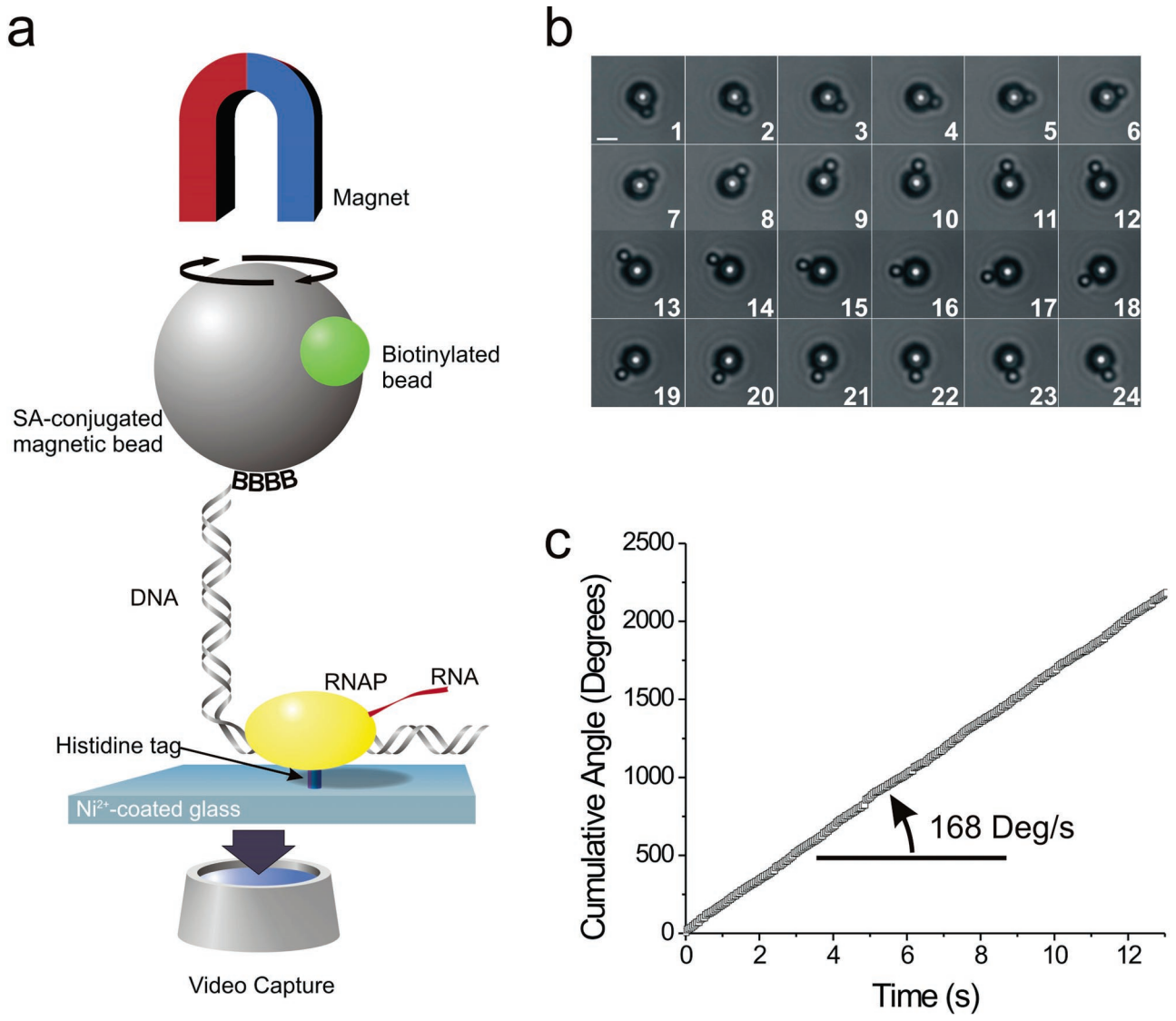

Figure 3. Visualization of reporter bead rotation by the RNAP motor. (a) His-RNAP ECs were assembled on a 4 kb biotinylated DNA template (template 3) that was tethered to a SA-conjugated magnetic doublet reporter bead. The complexes were immobilized on a Ni^{2+}-conjugated surface, and a magnetic force of 0.1 pN was applied to suspend the reporter beads above the surface. (b) Transcript elongation was resumed by the addition of NTPs, and rotation of the beads was visualized by video microscopy and recorded at 20 frames·s^{-1}; the frames shown here represent 0.1 s intervals. The bar in panel 1 represents 2 μm. (A movie is available in Supporting Information). (c) A plot representing the cumulative angle (degrees) of the rotating reporter doublet bead over time (s).

complexes, Figure 1). In the case of the intermolecular device, the bound cargo was observed to advance 50 nm along the contour length of the DNA (Figure 2d); in the case of the intramolecular device, movement of the polymerase along the template resulted in an enlargement of the loop by a corresponding interval (Figure 2e).

To demonstrate the ability of the RNAP to generate rotary forces, we fixed the RNAP to a solid surface and visualized the rotation of a magnetic bead tethered to the downstream DNA using magnetic tweezers (Figure 3).[11] The angular velocity of the bead was mostly uniform, corresponding to a transcription rate of 5 nt·s^{-1}. Within the limits of resolution of the experiment, there was no evidence for halting or nonspecific pausing, as has been reported for *E. coli* RNAP;[11] however, the length of the DNA template linking the EC to the bead in this experiment was 4 kilobases, and this may result in significant damping of irregular movement. A similar observation concerning the uniform rate of movement of single molecules of T7 RNAP was recently reported by Thomen et al.[27]

Controlling the movement and position of components within nanosystems remains a major challenge. For many applications, it would be useful to position RNAP motors with bound cargos on a grid of DNA templates and to move the cargos in a controlled manner. Other investigators have demonstrated that DNA bridges may be assembled between two locations on a surface,[20,21] and it has been shown that T7 RNAP may form active transcription complexes on DNA molecules stretched at near contour length by molecular "combing".[22] The experiment shown in Figure 4 demonstrates linear movement of a single RNAP along an immobilized DNA molecule. The enzyme was covalently bound to a fluorescent nanodot, and transcription by individual RNAPs on combed DNA was visualized by fluorescence

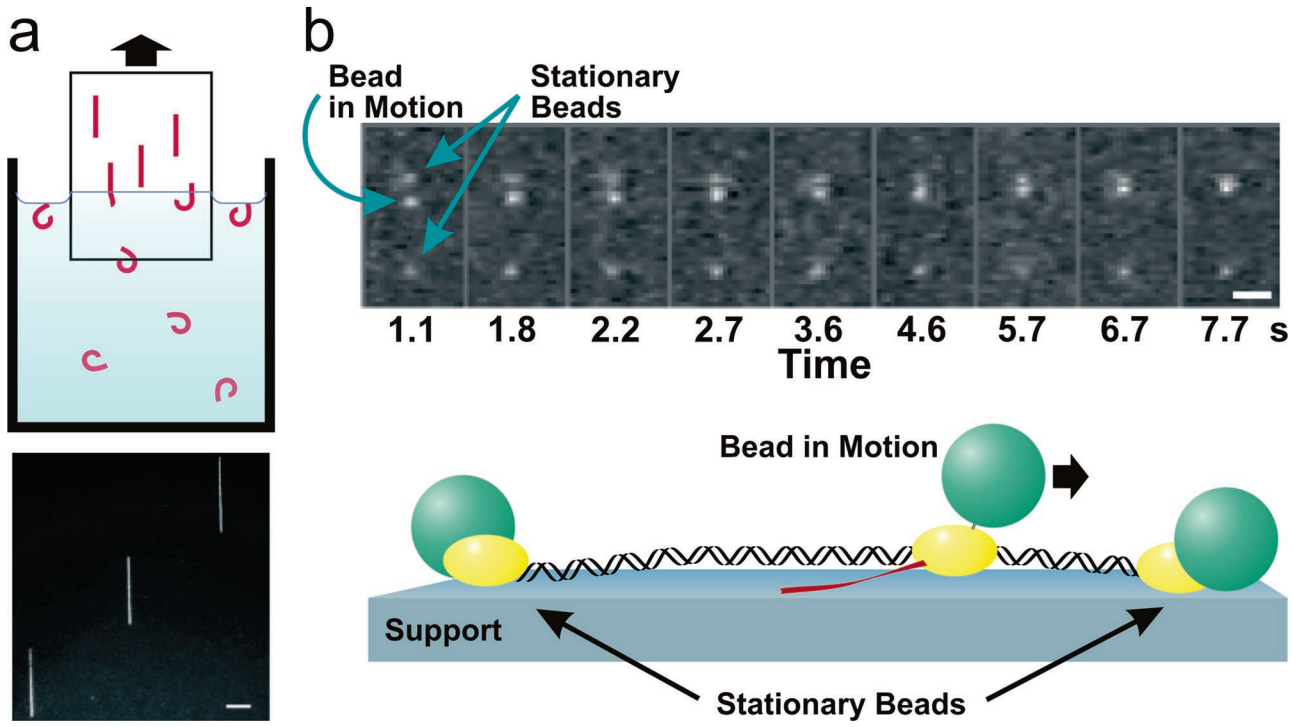

Figure 4. Visualization of RNAP movement on immobilized DNA. (a) T7 DNA (38 kb) was deposited at near contour length on a PMMA-coated glass surface by controlled withdrawal of a cover slip through an air−solvent meniscus, as previously described[22] (upper panel) and visualized by staining with YOYO-1 (lower panel; bar = 5 μm). (b) Transcription by T7 RNAP conjugated to fluorescent nanodots was visualized by video fluorescence microscopy; the images shown were captured at the indicated time intervals (bar = 3 μm). Nonspecific DNA surface interactions lead to the immobilization of some RNAP molecules (or fluorescent beads) while allowing movement of others (cartoon).

microscopy. While many complexes were observed to move in a regular fashion in response to the addition of substrate, some beads remained stationary. The interaction of combed DNA with the surface is not uniform along its length,[22] and the latter observation may be due to surface interactions that lead to transcription arrest or to nonspecific adsorption of the nanodots. This will require further investigation.

In this report, we demonstrate that bacteriophage T7 RNAP may be harnessed to move and position target molecules along DNA in a manner that depends on the sequence of the template. We believe that this technology may have potential applications in nanorobotics, molecular manufacturing, and nanoelectromechanical systems. Several related single-subunit phage RNAPs (e.g., T3, SP6, K11) with distinct promoter specificities are available, permitting the construction of multiple RNAP motors that may each be directed to a specific position on the DNA template and moved independently from one another. Coupling of the RNAP to evolved ligand-specific peptides or to RNA and DNA aptamers would allow the capture and manipulation of a wide variety of organic and inorganic molecules or structures. Last, we anticipate that assembly of DNA grids on solid surfaces would allow the construction of novel and adjustable molecular platforms to support movement of the RNAP motors. While other nucleotide polymerases could, in principle, be utilized as molecular stepper motors, the phage RNAPs have particular advantages due to their ease of genetic manipulation, high processivity, and specificity.

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Supporting Information Available: Experimental procedures, DNA sequences, and video files. This material is available free of charge via the Internet at http://pubs.acs.org.

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