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RNA Used to Control a DNA Rotary Nanomachine

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Abstract

The PX-JX₂ device is a robust sequence-dependent DNA nanomachine. It is controlled by the addition of DNA strands to the solution. The ability to control such devices with RNA will enable the machine to respond to signals generated by transcriptional logic circuits *in vitro*, or possibly *in vivo*. The PX-JX₂ device does not respond properly to RNA strands, so we have adapted the signaling method by using a cover-strand strategy. This approach entails using DNA control strands that contain sequence regions to set the device to either state. An RNA strand is used to mask one of these two regions, enabling the machine to assume the state corresponding to the unmasked region.

Keywords

DNA Nanomechanical Devices; RNA; Cover Strands; Unusual DNA Motifs

A large number of DNA-based nanomechanical devices have been described, controlled by a variety of methods: These include pH changes and the addition of other molecular components, such as small molecule effectors, proteins and DNA strands.¹ The most versatile of these devices are those that are controlled by DNA strands: This versatility results because they can be addressed specifically by strands with particular sequences; these strands can be added to the solution directly, or perhaps they can result from another process ongoing within the local environment. The PX-JX₂ device is a robust sequence-dependent DNA-based rotary nanomachine.² We have reported the use of a pair of these devices in a larger construct that converts DNA sequences to polymer assembly instructions.³ Recently, we have developed a cassette that incorporates this device into 2D DNA arrays, thereby enabling the use of a variety of devices as individual robotic arms, potentially capable of nano-patterning and of performing nano-manufacturing operations.⁴

A series of operations by this device can be effected by the addition of strands to the solution, as performed with the polymer assembly system. However, a more general type of control would include the ability of the device to respond to signals generated within the solution. For example, these signals could report some condition within the solution, such as the RNA output of *in vivo*⁵ or *in vitro*⁶ transcriptional logic circuits. The presence of the signals would then enable the system to respond to this condition, either by active nanomechanical motion, or perhaps, as in the polymer assembly system, by recording the existence of the condition as an historical output record.

To behave in this fashion, it is necessary for the PX-JX₂ system to respond properly to RNA. The machine cycle of the PX-JX₂ device² is shown in Figure 1a. The control strands, shown in green and pink, set the structural state of the device; they contain a toehold⁷ that enables their removal from the complex to leave a naked frame; addition of the new control strands to

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the naked frame sets the state of the next cycle. We have not been successful in our attempts to substitute RNA directly for the DNA control strands. This is not unexpected, because the helical structures of RNA and DNA differ substantially (e.g., ref. ⁸), even though the nominal components of the PX state and the RNA double helix both contain 11 nucleotides per helical repeat unit. The data demonstrating this problem are illustrated in Figure 2a. The single bands in lanes 1 and 2 illustrate the results of successful formation of the two device states using the control strands in an all-DNA system. By contrast, the smear in lane 3 demonstrates that the PX state is not formed well at all when an RNA strand replaces the DNA strand. The JX₂ state is formed more successfully when RNA is used; this is not too surprising, since achieving this state entails only the formation of linear duplex structures in the control region, rather than the unusual PX motif. Consequently, we ascribe the failure to the incompatibility of RNA and DNA structures in the context of the PX motif.

Figures 1b and 1c illustrate our solution to this problem. It is well-known that RNA and DNA hybridize successfully to each other in a linear context. Thus, we have chosen to use the robust parts of the two systems, the successful response of the PX-JX₂ device to conventional DNA strands and the ability of RNA to pair successfully with single-stranded DNA to yield an RNA-DNA hybrid duplex molecule. The approach adopted here is to use RNA as a 'cover strand' that masks particular oligonucleotides within a longer DNA single strand. The strategy entails using a DNA strand that contains control regions for both states. However, in the presence of the controlling RNA strands, only one of the control regions is exposed. The individual states are illustrated in Figure 1b. The control strands are the same, but their coverage by different RNA strands (drawn squiggly) results in different functions for them. Thus, when the green portions of the strands are exposed, they bind to the device setting it to the PX state; likewise when the pink portions of the strands are exposed, they bind to the device setting it to the JX₂ state. Figure 1c illustrates the whole machine cycle. The PX starting state is at the left; the green portion of the control strand pair is exposed and sets the state by binding to the frame. Proceeding clockwise, PX cover strands are added, thereby covering the ability of the control strands to set the PX state, leaving a naked device frame, and two completely hybridized RNA-DNA duplex molecules. The next step in the cycle is the removal of the JX₂ cover strands with biotinylated anti-cover strands, so that the control strand can now pair with the frame to produce the JX₂ state, seen at the right. Analogous to the original PX-JX₂ device, the cover-anti-cover duplex is removed by magnetic streptavidin beads. The process cycles through analogous steps seen at the bottom of the diagram, to regenerate the PX state. The sequences used in the experiments described below are shown in the supplementary information.

Gel electrophoretic data that support the operation of the device are shown in Figures 2b and 2c. Panel b shows both the formation of the correct states of the device, and their successful transitions to the opposite states. Lane 1 contains a molecule annealed to form the JX₂ state, and lane 2 contains the results of transforming it to the PX state by means of the cover strand strategy. Lane 3 contains a molecule annealed to form the PX state, and lane 4 contains the results of transforming it to the JX₂ state in the same way. Panel c shows that the system can be cycled successfully. Lane 1 contains a molecule annealed to produce the PX state in the device. Lanes 2, 3 and 4, respectively, illustrate the successful transitions of the same device to the JX₂ state, then the PX state, and then the JX₂ state again. Both panels show single bands of the expected mobilities, indicating that the device has formed and functioned as designed. Although the data shown are from gels where formation of the system was done slowly, operation of the device can be done much more quickly. We can perform each of the four steps of the transformation successfully in about 30 minutes, with 10 minutes for streptavidin magnetic bead waste removal, for a 2.3 hr isothermal cycle. Trying to accelerate the system more than that (by a factor of three, to emulate the all-DNA device), leads to non-robust transformations (data not shown).

In addition to gel electrophoretic data, we also present atomic force microscopy (AFM) data to demonstrate the operation of the device using RNA cover strands. This is similar to the system used previously.² The basis of the system is shown schematically in Figure 3. The device connects a series of DNA trapezoids made of three edge-sharing⁹ DNA triangles that correspond to a half-hexagon. When the devices are in the PX state, the trapezoids point in the same direction. By contrast, when the devices are in the JX₂ state, alternating trapezoids point in opposite directions. These two different arrangements are readily visible in the AFM, as seen in Figure 4a, which shows both of them in control experiments. Figure 4b illustrates a series of connected devices starting in the PX state, converting to the JX₂ state and then converting again to the PX state. The reverse experiment is shown in Figure 4c, which contains a three- state series beginning with the JX₂ state, switching to the PX state, and then switching back to the JX₂ state. In both situations, the conversions are clear, indicating that the method we introduce here works effectively.

We have shown that the state of the robust DNA-based sequence-dependent PX-JX₂ nanomechanical device can be controlled by RNA strands, using a cover-strand strategy. The data to support this capability include both gel mobility data and direct visualization of the results of state change by atomic force microscopy. As noted above, we have used multiple PX-JX₂ devices in a rotationally-based translation machine.³ The data here suggest that the cover-strand approach could be used to produce translation products that reported the state of the environment of the translation machine, reflecting the results of environment-sensitive transcription. In a similar vein, we have described a cassette that incorporates the PX-JX₂ device into an array, and we have shown that it is functional in that context.⁴ The presence of a variety of PX-JX₂ device cassettes incorporated into an array could report on a large number of different environmental factors, if those factors led to differential transcriptional products. Thus, we have produced a method of coupling a robust sequence-dependent DNA nanomechanical device to a transcriptional signal. Transcriptional control of the Yurke *et al.* tweezers⁷ has been reported by Dittmer and Simmel.¹⁰ The tweezers is a less robust device than the PX-JX₂ device, but its structure entails only linear RNA-DNA hybrids and thus does not require the cover-strand strategy necessitated by the unusual PX motif.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1. The PX-JX₂ Device Directed by DNA and by RNA. (a) The Machine Cycle of the Device Directed by DNA. The Device is shown in the PX state at the left. The green set strands are removed by unset strands in step I. They bind to the toeholds on the set strands (drawn horizontal), and the duplex complexes are removed by magnetic streptavidin beads that bind to 5' biotin groups (black dots). Removal of the green PX-state set strands leaves a naked frame, capable of binding the pink JX₂-state set strands (step II) to put the device in the JX₂ state. (b) The Cover Strand Strategy for RNA. RNA molecules are drawn with squiggly lines. A single set strand is capable of setting either state, depending on which half of it is exposed. When the pink halves of the set strands are covered by RNA cover strands and the green halves are exposed, the device will be set to the PX state (left); when the green halves are covered RNA cover strands and the pink halves are exposed, the device will be set to the JX₂ state (right). (c) The Machine Cycle of the Device Directed by RNA. Starting from the left, in the PX state,

RNA PX cover strands (brown) cover the green portion of the set strands, leaving a naked frame and two RNA-DNA hybrid molecules (upper left). At the next step, biotinylated JX₂ anti-cover strands (the biotin is indicated by a black dot on their 5' ends) remove the blue RNA cover strands, exposing the pink portions of the set strands (upper right). These then combine with the frame to set the device to the JX₂ state. Analogous processes along the bottom half of the diagram return the system to the PX state at left.

Figure 2.

Gels Showing the Behavior of the System Controlled by RNA. (a) LM contains a 10 nucleotide pair linear marker. Lane 1 contains annealed PX molecules, with DNA set strands. Lane 2 contains annealed JX₂ molecules with DNA set strands. Lane 3 contains annealed PX molecules with RNA set strands. Lane 4 contains annealed JX₂ with RNA set strands. This is an 8% non-denaturing polyacrylamide gel, run at 20 °C and stained with stains-all dye (Sigma E-7762); all nucleic acid species are present in concentrations of 1 μM. Device strand sequences have been designed using the program SEQUIN.¹¹ All the DNA strands are synthesized by routine phosphoramidite techniques¹² and gel purified in RNase-free conditions. All the RNA strands are ordered from IDT (Coralville, IA). Set strands and the framework of the device are hybridized from 90 °C to 20 °C for 48 hrs in a solution containing 40mM Tris base, 20mM acetic acid, 2mM EDTA and 12.5mM magnesium acetate, pH 8.1. Note the smear band in lane 3 indicating the device assembled with RNA PX set strands is not well formed. (b) The components of the device in operation. This is a 6% non-denaturing polyacrylamide gel, run at 20 °C and stained with stains-all dye (Sigma E-7762). The lane LM contains a 10 nucleotide pair linear length marker ladder. Device strand sequences have been designed using the program SEQUIN.¹¹ All RNA strands obtained and prepared as described in (a). Lane 1 contains the device [1 μM] assembled with JX₂ set strands and lane 3 contains the device [1 μM] assembled with PX set strands. Lane 2 contains the products transformed from the material in lane 1 by the set strands via adding JX₂ cover strands and removing the PX cover strands by adding PX anti-cover strands (see Figure 1c). Likewise, lane 4 contains the products transformed from the material in lane 3 by removing the set strands via adding PX cover strands and removing the JX₂ cover strands by adding JX₂ anti-cover strands (Figure 1c). Note the absence of extraneous products in lanes 2 and 4; this finding indicates that these transformations are robust, i.e., neither breakdown products nor multimers result. (c) Cycling the device. The gel shows the cycling of the device through 4 steps. Lane 1 contains material in the initial PX conformation, and lanes 2 to 4, respectively, show alternating transformations to the other state. Cover strands were added to the preformed molecules in the PX or JX₂ states at 20 °C and kept at 20 °C for 120 min. At that time, JX₂ or PX anti-cover strands were added at 20 °C for 6 hrs; at the same time, the uncovered JX₂ or PX set strands form JX₂ or PX molecules by combining with the DNA frame molecules. After the transition of device conformation is

finished, the mixture was treated with streptavidin beads at 20 °C for 30 min to remove the cover-strand/anti-cover-strand duplexes. The addition of cover strands, followed by anti-cover strands, was then repeated twice.

Figure 3.

A System to Visualize Changes of State by Atomic Force Microscopy. The top part of the diagram shows a series of half-hexagon DNA trapezoids connected by a series of PX-JX₂ devices controlled by the RNA cover strand technique; the devices are in the PX state, so they are all parallel. The bottom shows that when the system is converted to the JX₂ state the trapezoids form a zigzag arrangement. The system is large enough to be visualized in by the AFM.

Figure 4.

Atomic Force Microscopy Observation of the PX-JX₂ Device Cycling when Controlled by the RNA Cover-Strand Method. White scale bars are all 40 nm in length. The DNA frame molecules of the initial species are produced by annealing their constituent single strands from 90 °C to 20 °C in a Thermos over a period of 5 days. Meanwhile, the set strands are hybridized from 90 °C to 20 °C for 2 days. After annealing, the set strands and the DNA frame are mixed, heated to 37 °C and cooled to 20 °C for 12 hrs. The one-dimensional arrays of these half-hexagon-plus-device units cohere by way of 11-nucleotide sticky ends. RNA cover strands were added to the preformed PX or JX₂ at 20 °C and kept at 20 °C for 120 min. Then JX₂ or PX anti-cover strands were added at 20 °C for 6 hrs; at the same time, the uncovered JX₂ or PX set strands could form JX₂ or PX molecules with the DNA frame molecules. After the transition of the device conformation is complete, the mixture is treated with streptavidin beads at 20 °C for 30 min to remove the cover-strand/anti-cover-strand duplexes. (a) These images contain control molecules, not devices, that are constrained to be in the PX or JX₂ motifs. AFM samples are prepared by placing 5 μ l of a solution containing 0.5 μ M DNA on a piece of freshly cleaved mica (Ted Pella, Inc.), blowing it dry, and washing several times with nuclease-free distilled water. Images are obtained in isopropanol by scanning with a Nanoscope VI in contact

mode. The upper panels show PX linear arrays in a parallel arrangement, and the lower panels show JX₂ linear arrays in a zigzag arrangement. (b) and (c) Cycling the Device. These panels show a full cycle of the operation of the device, sampling aliquots from each cycle. In (b), the system originates in the PX state, and is then converted (left to right) to the JX₂ state, and back to PX. In (c), the system originates in the JX₂ state, and is then converted (left to right) to the PX state, and back to JX₂. The PX linear arrays are clearly in the parallel arrangement, and the JX₂ linear arrays are clearly in the zigzag arrangement.