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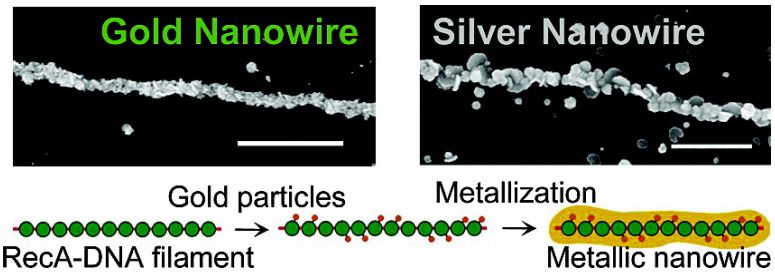

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Conductive Metal Nanowires Templated by the Nucleoprotein Filaments, Complex of DNA and RecA Protein

Taro Nishinaka,*,† Atsushi Takano,‡ Yuko Doi,† Makiko Hashimoto,† Akira Nakamura,† Yushu Matsushita,‡ Jiro Kumaki,† and Eiji Yashima*,†,§

Contribution from the Yashima Super-structured Helix Project, ERATO, Japan Science and Technology Agency, 101 Creation Core Nagoya, 2266-22 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-0003, Japan, Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan, and Institute for Advanced Research, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

Received January 25, 2005; E-mail: nishinak@yp-jst.jp; yashima@apchem.nagoya-u.ac.jp

Abstract: Development of preprogrammable conductive nanowires is a requisite for the future fabrication of nanoscale electronics based on molecular assembly. Here, we report the synthesis of conductive metal nanowires from nucleoprotein filaments, complexes of single- or double-stranded DNA and RecA protein. A genetically engineered RecA derivative possessing a reactive and surface accessible cysteine residue was reacted with functionalized gold particles, resulting in nucleoprotein filaments with gold particles attached. The template-based gold particles were enlarged by chemical deposition to form uniformly metallized nanowires. The programming information can be encoded in DNA sequences so that an intricate electrical circuit can be constructed through self-assembly of each component. As the RecA filament has higher degree of stiffness than double-stranded DNA, it provides a robust scaffold that allows us to fabricate more reliable and well-organized electrical circuitry at the nanoscale. Furthermore, the function of homologous pairing provides sequence-specific junction formation as well as sequence-specific patterning metallization.

Introduction

The next challenging goal targeted in the recent development of molecular-scale devices is integrating them into functional circuitry.[1,2] New approaches based on molecular recognition and self-assembly in biological systems have been explored as promising routes in fabricating nanometer-scale electrical devices.[3−9] The remarkable ability of DNA to self-assemble offers a creative approach in constructing nanoscale interconnections.[10−12] Metal nanowires consisting of silver,[3] gold,[5,13] platinum,[14] palladium,[15] or copper[16] have been formed by chemical modification and metal deposition onto DNA templates. Recently, Keren et al. realized a carbon nanotube field-effect transistor operating at room temperature by using DNA-based metal nanowires as interconnections and RecA protein as resists.[6] While DNA self-assembly provides a promising approach for constructing complex networks, molecular electronics requires robust wiring systems, and the physical and chemical instability and flexible properties of DNA molecules may hamper the formation of functional electronic circuitry. In the construction of molecular electronics using DNA, high temperatures, short wavelengths of light, and other harsh manufacturing conditions must be avoided to prevent decomposition, and interconnections should be much smaller than the persistence length of DNA[17−19] (50 nm, about 150 base pairs) to satisfy the linear connection requirements of each component. Fiber proteins such as polymerized amyloid[7] or F-actin[20] have greater stability and stiffness than DNA and have been used as

† ERATO, Japan Science and Technology Agency.
‡ Graduate School of Engineering, Nagoya University.
§ Institute for Advanced Research, Nagoya University.

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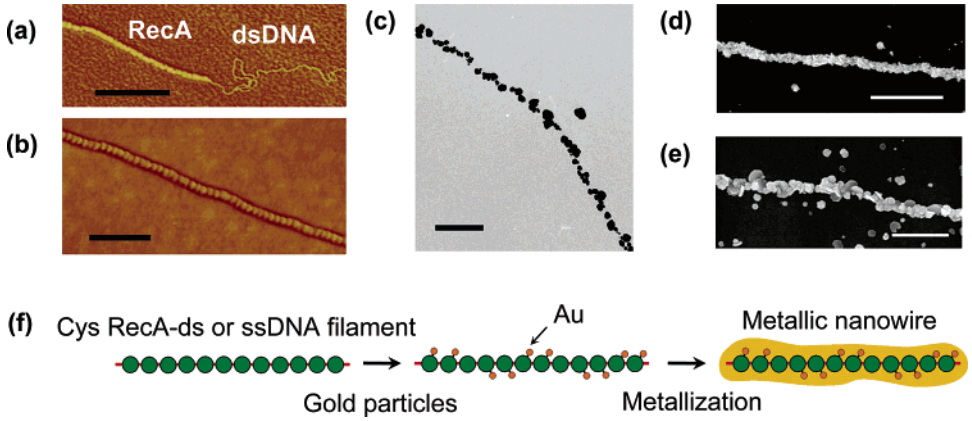

Figure 1. Metallic nanowire formation from nucleoprotein filaments. (a) Atomic force microscopy (AFM) image of the wild-type RecA protein polymerizing on λ dsDNA. Note the difference in flexibility between the RecA-DNA filament and a bare dsDNA. Scale bar: 250 nm. The filament formation reaction was interrupted after 2 min incubation at 37 °C, and then the AFM image was observed. (b) AFM image of cysteine derivative RecA protein (Cys RecA)-λ dsDNA filaments. Scale bar: 100 nm. Helical stripes of the Cys RecA-DNA filament (~10 nm) can be seen clearly. (c) TEM image of aligned gold particles after short-period gold enhancement. The Cys RecA-DNA filaments were gold-enhanced for 30 s on a carbon-coated electron microscopy grid before TEM measurements. Scale bar: 200 nm. (d) Scanning electron microscopy (SEM) image of a gold nanowire. The Cys RecA-DNA filaments were enhanced with GoldEnhance EM for 2 min on silicon. Scale bar: 1 μm. (e) SEM image of a silver nanowire. The Cys RecA-DNA filaments were silver-enhanced for 45 min on silicon. Scale bar: 600 nm. (f) Scheme for the synthesis of the metallic nanowires. The red line and filled circles (green) represent ssDNA (or dsDNA) and Cys RecA protein, respectively. The small protuberances on the second and third images are 1.4 nm-sized gold particles covalently linked to the sulfhydryl group of the Cys RecA. The shading on the final image indicates the continuous metal nanowire.

alternative template materials for the construction of metal nanowires. As compared to DNA, however, difficulties in programming wiring information and tailoring the nano-structured complex remain with fiber proteins.

A promising approach to circumvent these problems is the use of homologous recombination proteins, such as the RecA protein of *Escherichia coli*, for selective metal deposition and reinforcement of DNA molecules. In the presence of ATP or its poorly hydrolyzable analogue, adenosine 5′-*O*-(thiotriphosphate) (ATP-γ-S), RecA protein polymerizes around single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) to form a right-handed helical filamentous complex.[21−24] It contains about 19 bases or base pairs and about 6 RecA monomers per turn.[25,26] The ability of RecA protein to wrap around DNA and to self-assemble into a filamentous structure substitutes for recently developed DNA-templated metal nanowire technology. The RecA-DNA filaments have the advantage of greater stability and stiffness than that of DNA[18,19,25] (Figure 1a), and the protein provides an array of functionalities on the surface, which is available for the targets of chemical modification required for the metallization process. Furthermore, the RecA protein promotes homologous pairing and strand exchange reaction between ssDNA and dsDNA,[21−23,27−29] and the ability to pair two DNA molecules at their homologous sites raises the intriguing possibility of creating intricate nano-structured devices. In this study, we have successfully produced continuous metal nanowires with high conductivity from nucleoprotein filaments using a genetically engineered cysteine derivative RecA protein and DNA substrates. With the function of homologous pairing, a three-way junction and an insulating gap were also formed sequence-specifically on the target DNA substrates.

Results and Discussion

To produce metal nanowires, we first attached gold particles to the RecA filaments that had been genetically engineered to allow for chemical labeling, and then gold was deposited onto the gold particles. One additional cysteine residue was introduced to the C-terminus of a wild-type RecA protein (WT RecA) through site-directed mutagenesis to form cysteine derivative (353C, Cys RecA). Because the C-terminus is located far from the functional site of the RecA protein and is supposed to be accessible to exogenous reactant reagents, we expected the Cys RecA to have a high label efficiency and little effect on the specific RecA reaction. The purified Cys RecA protein expressed activity for the filament formation equivalent to that of the WT RecA (Figure 1b).[25,26] After the filament formation was completed, monomaleimido Nanogold (Nanoprobes, New York), a gold cluster complex with a core diameter of 1.4 nm, was added to the reaction mixture. Excess Nanogold was removed by gel filtration. Transmission electron microscopy (TEM) observation of the sample after a short-period gold deposition confirmed the attachment of the Nanogold particle to the Cys RecA filament (Figure 1c). Judging from the occurrence of gold-deposited particles in Figure 1c, we estimated that about 10% of Cys RecA monomers were labeled by Nanogold. Continuous gold or silver nanowires were obtained by treating the samples with gold or silver enhancer, by which metal ions are chemically deposited onto the gold particles, attached to the nucleoprotein filaments (Figure 1d and e; for a larger view, see Supporting Information Figure S1). The metallization took place uniformly over the entire nanowire, and the length corresponded well with the value expected from the DNA used. The diameter or height of the wires ranged from 80 to 500 nm, depending on the exposure time in the enhancement solution. The value can further be reduced by raising label efficiency or improving

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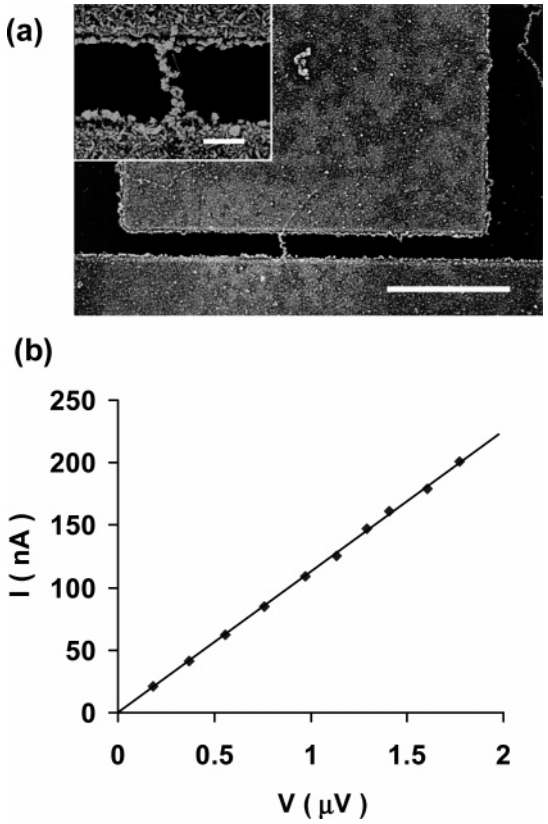

Figure 2. Electrical characteristic of a single gold nanowire. (a) SEM image of a nanowire bridging the 2 μm gap over a pair of electrodes. (b) $I-V$ curve corresponding to the Cys RecA-DNA-based gold nanowire shown in Figure 2a. Gold particle-attached RecA filaments were randomly placed over patterned electrodes and gold-enhanced for 25 min. The shape of the gold nanowire was somewhat distorted, probably because the growth of the gold nanowire was affected by a silicon nitride surface on the fabricated silicon chip. Scale bar: 10 and 1 μm (inset).

chemical deposition methods. No metal deposition was observed for the sample that had not been labeled by Nanogold, indicating that this metallization process was specific to the Nanogold labeling to the Cys RecA filaments.

The conductivity characteristics of the gold nanowires were determined by direct electrical measurements. We prepared silicon nitride-passivated silicon chips on which gold electrodes were lithographically patterned. Each electrode was spaced by a 2 μm gap. Nanogold attached Cys RecA filaments were deposited over silicon nitride membrane substrates with lithographically patterned electrodes, followed by gold enhancement to form metal nanowires. Conductivity was detected where single or multiple gold nanowires bridged over two electrodes, while no conductivity was detected where no gold nanowires bridged the gap. Figure 2a and b shows a gold nanowire bridging a 2 μm electrode gap and its $I-V$ curve measured by a four point probe method. The $I-V$ curve was linear with small deviations, indicating that the observed nanowire showed ohmic behavior ($R = 8.9\ \Omega$ for a single nanowire with a diameter of approximately 200 nm).

With the function of homologous pairing of RecA protein, a three-way junction in the metal nanowire was formed by the pairing of two DNA strands at a precise position (Figure 3). A 100-base probe having the DNA sequence of the center region (28813−28900) of λ DNA (48502 bp) was attached to one of the cohesive ends, enabling pair by the Cys RecA. The resulting nucleoprotein filament was metallized by gold deposition to produce a loop (19.7 kbp) nanowire conjugated with a three-way junction. Figure 3b and c shows an example of this type of the structure, and other examples are shown in the Supporting Information (Figure S2). Roughly 20% of molecules observed included rare examples of an open looped structure and, in most cases, examples of intertwined or knotted loop structures. Other molecules had truncated lengths of branched or linear-shaped structures. The loop length of the molecules observed was about 13 μm, which is in fairly agreement with the calculated length based on the base spacing of double-stranded DNA within the RecA filaments (0.51 nm, 1.5 times longer than that of B-form DNA).[24−26] This experiment demonstrates that an intricate electric wiring can be generated by preprogramming the design of circuitry within DNA sequences.

Because the Cys RecA protein can efficiently be labeled by Nanogold maleimide, while the labeling efficiency of the WT RecA is very low, sequence-specific patterning of the metal nanowire can be performed by successive filament formation with combination of the Cys and WT RecA proteins. Using λ DNA and its single-stranded homologous probe molecules, an approximately 1 μm gap was created in the middle of a 25 μm gold nanowire at a preprogrammed site, as shown in Figure 4. First, a 2027-base ssDNA probe, which has a homologous sequence to 23131−25157 region of 48502-bp λ DNA, was complexed with an equivalent amount of the WT RecA protein. The resulting WT RecA-DNA complex binds to the homologous site in the λ DNA substrate. An excess amount of the Cys RecA then was added to complete the nucleoprotein filament formation. Deposition of gold on the filament yielded gold nanowires, leaving a 2027-base section covered with the WT RecA, free from the metallization.

Recent studies by Keren et al. utilized RecA protein as a molecular "resist" in the DNA metallization process, which allowed them to produce sequence-specific patterning of DNA metal coating[5] and assemble molecular-scale devices on a DNA template to form an electric transistor.[6] In our study, cysteine derivative RecA protein was prepared and used as a scaffold for the metallization, and the wild-type RecA protein was used as a "resist" to prevent metallization of the protein. Our approach, which uses nucleoprotein as a template, can be incorporated easily with preceding studies of the DNA metallization,[3,5,6,13−16] yet includes several advantages over DNA-templated metal nanowires. First, the RecA filament is supposed to have a greater stability than DNA. In the RecA filament, the DNA molecule is wrapped around RecA proteins, and, therefore, it is protected from physical and chemical damages. Moreover, noncovalent binding of RecA-DNA complex can be covalently connected after cross-linking treatment, such as glutaraldehyde fixation. Manufacturing processes in silicon chip production demand the physical and chemical stability required for components to withstand harsh industrial conditions. Second, the RecA filament has a greater stiffness than DNA (Figure 1a). Rigid molecules can provide the robust scaffold of the type favored in manufacturing complex nanostructured devices. Interconnections connecting molecular devices should be much shorter than the persistence length of the molecules used so as to satisfy the linear connection requirement of each device. The persistence length of RecA filament is about

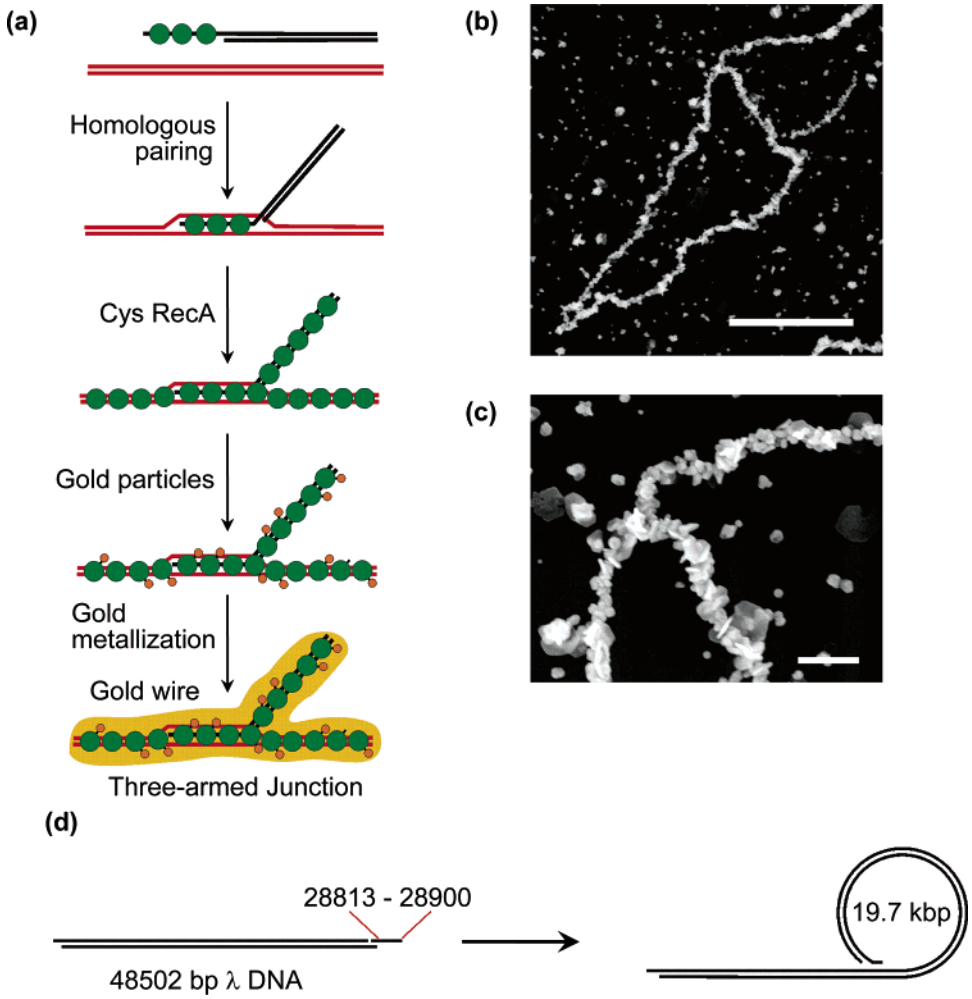

Figure 3. Sequence-specific three-way junction formation. (a) Scheme for the synthesis of the three-way junction. The red and black lines indicate DNA substrates. (b,c) SEM images of a three-way junction in different magnifications. Scale bar: 2 μm (b) and 300 nm (c). (d) Design of DNA substrates. The terminal of λ DNA was conjugated with the 28813−28900 region of λ DNA, resulting in 19.7 kbp loop formation.

0.6−1 μm, while that of double-stranded DNA is 50 nm.[17−19,25,30] Finally, our approach directly converts the product of homologous pairing to metal nanowires without requiring deproteinizing processes. This expands the practicability of fabricating further intricate nano-structured circuitry because, not only the self-annealing of complementary ssDNAs, but the unique RecA reaction pairing ssDNA and dsDNA at a precise homologous site can also be exploited for circuitry network formation. The homologous pairing can also be utilized for precise arrangement of molecular devices onto DNA strands to form functional electrical circuitry,[6] thus offering new possibilities for molecular lithography in the future.

Materials and Methods

Construction of Cysteine Mutants. For construction of *E. coli* RecA mutants, site-directed mutagenesis was performed using a commercially available kit, Mutan-Super Express Km (Takara). For a 353C mutant, a DNA fragment including *E. coli recA* gene was ligated into a vector, pKF19k-2, at a multi-cloning site, and this was used as a template. A DNA primer, 5′-ACTAACGAAGATTTTTGTTAATCGTCTTGTTG-3′ was synthesized (QIAGEN), and it was used as a mutation primer for the polymerase chain reaction (underline indicates the location of cysteine mutation). The PCR product was purified by QIAquick PCR purification kits (QIAGEN) and introduced into the *E. coli* MV1184 cells. The recA gene including the cysteine mutation was digested by *Cla*I and *Kpn*I endonucleases and ligated to pKK223-3-ECRecA, with the recA gene cloned into pKK223-3 (Amersham Biosciences) at the *Eco*RI and *Hind*III sites. The resulting plasmids for a 353C mutant were referred to as p353C. The entire mutant *rec*A gene was confirmed by DNA sequencing.

Purification of Cysteine Mutants. *E. coli* strain MV1184 was transformed by plasmid p353C. The cells were grown to $OD_{600} = \sim 0.7$ in 2 L of LB broth containing 50 μg/mL ampicillin, and then IPTG was added to a final concentration of 1 mM. After further incubation for 4 h, the cells were harvested by centrifugation and suspended in 50 mM Tris-Cl (pH 7.5) and 10% sucrose. A series of 1 s ultrasonic pulses was applied to the cell for 5 min, and then the cell lysate was centrifuged at 100 000*g* for 30 min at 4 °C. 10% polyethlenimine P-70 solution was added to the supernatant dropwise over a period of 15 min with gentle stirring until a final concentration of 0.5% was reached. After additional stirring for 30 min, the cell lysate was centrifuged at 15 000*g* for 10 min at 4 °C. The precipitate was suspended in 20 mL of R-buffer (20 mM Tris-Cl, pH 7.5, 1 mM DTT, 0.1 mM EDTA, and 10% glycerol), containing 150 mM ammonium sulfate, stirred with a glass rod for 30 min, and then centrifuged at 15 000*g* for 10 min at 4 °C. The precipitate was resuspended in the same buffer, stirred for 30 min, and centrifuged again under the same conditions. The precipitate

(30) Although a DNA molecule has a short persistence length in water solution, molecular combing approaches extend and fix DNA molecules in excess of their persistence length on a dry substrate. See: Bensimon, A.; Simon, A.; Chiffaudel, A.; Croquette, V.; Heslot, F.; Bensimon, D. *Science* **1994**, *265*, 2096−2098.

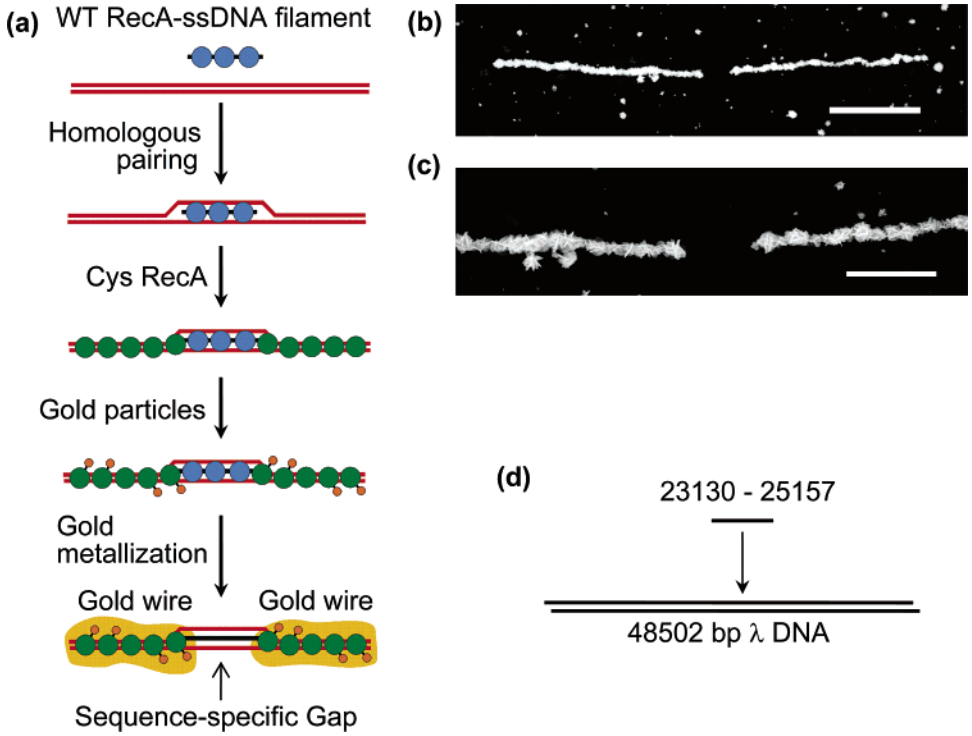

Figure 4. Sequence-specific gap formation. (a) Scheme for the synthesis of the sequence-specific gap. Blue and green filled circles indicate WT RecA and Cys RecA, respectively. (b,c) SEM images of a sequence-specific gap at different magnifications. Scale bar: 5 μm (b) and 2 μm (c). (d) Design of DNA substrates. A 2027-base ssDNA probe was localized on the 23130−25157 region of a 48502 bp λ DNA substrate, resulting in a ~1 μm gap in a ~25 μm gold nanowire.

was suspended in 20 mL of R-buffer containing 300 mM ammonium sulfate, stirred for 30 min, and centrifuged at 15 000*g* for 10 min at 4 °C. After re-extraction of the pellets with the same buffer, the supernatants were then combined. Finely grained ammonium sulfate was added to the solution at a final concentration of 0.28 g/mL over a period of 45 min. The precipitate was collected by centrifugation at 15 000*g* for 10 min at 4 °C and stored at −20 °C. The frozen precipitate was suspended in 5 mL of P-buffer (20 mM potassium phosphate, pH 6.8, 1 mM DTT, 0.1 mM EDTA, and 10% glycerol), containing 200 mM NaCl, and then dialyzed against the same buffer. The solution was applied to a 60-mL phosphor-cellulose column equilibrated with P-buffer containing 200 mM NaCl. The flow-through fraction was collected, and solid ammonium sulfate was added to a final concentration of 0.124 g/mL. Part of the fraction was loaded onto a 10-mL Butyl-Toyopearl column (Tosoh, Tokyo) equilibrated with P-buffer containing 0.124 g/mL ammonium sulfate. The 353C RecA was eluted with a stepwise gradient of ammonium sulfate (0.124−0 g/mL). Part of the fraction was loaded onto a 10-mL Hydroxyapatite column (BIO-RAD) equilibrated with P-buffer. The 353C RecA was eluted with a gradient of phosphate (0−0.5 M). The fraction containing 353C was collected and dialyzed against the buffer of 20 mM Tris-Cl, pH 7.5, 0.1 mM EDTA, and 50% glycerol. The purified protein was stored at −20 °C. The protein concentration was determined using the molar absorption coefficient, $\epsilon_{278} = 2.15 \times 10^4$ M^{-1} cm^{-1}.[31]

Attachment of Gold Particles to Nucleoprotein Filaments. Filament formation reactions were carried out at 37 °C for 20 min and were contained in 50 μL: 20 mM Tris-acetate (pH 7.5), 1 mM magnesium acetate, 2 mM ATP-γ-S, 10 μM λ DNA (Takara), and 3.3 μM 353C RecA. The concentration of DNA is expressed as moles of nucleotide residues. Monomaleimido Nanogold (Nanoprobes, New York) was added to a reaction mixture to a final concentration of 60 μM. After incubation for 5 min at room temperature, the reaction mixture was immediately loaded onto the gel filtration column (Sepharose 2B; Amersham Biosciences, Uppsala) equilibrated with 20 mM HEPES (pH 7.5), 1 mM magnesium acetate, and 1 mM ATP-γ-S. When fixation of the RecA filament was needed, the column fraction containing RecA filament was reacted with 0.5% glutaraldehyde for 8 min and the reaction was stopped by 0.1 M glycine, but this step can be omitted in many cases.

Metallization of Nucleoprotein Filaments. A droplet of sample solution containing the Nanogold-labeled 353C RecA filaments was placed onto a chip of silicon wafer for SEM or a copper grid coated with carbon membrane for TEM. The solution was incubated for 5−15 s and removed by filter paper. These samples were immediately gold enhanced by the method developed by Keren et al.[5] or by using GoldEnhance EM (Nanoprobes) solution. For silver enhancement, LI Silver solution (Nanoprobes) was used in accordance with manufacturer instructions. Exposure times varied from 30 s in the TEM experiments to 3−30 min in the SEM experiments. Usually, continuous nanowires of about 200 nm in diameter were obtained after 4 min of gold enhancement using the method of Keren et al.[5] To stop the reaction, the sample was dipped into 200 mL of water 1−3 times, in 50 mL of acetone (if necessary), and then dried at room temperature. SEM images were obtained using a Hitachi S-5000 scanning electron microscope with the accelerating voltage of 10 kV and emission current of 10 μA. TEM images were obtained with a Hitachi H-800 transmission electron microscope operated at an accelerating voltage of 100 kV. For AFM observation, a droplet of sample solution was placed onto (aminopropyl)triethoxysilane(AP)-mica, positively charged mica that had been functionalized with (aminopropyl)triethoxysilane. The solution was incubated for 5−15 s and removed by filter paper. AFM images were acquired with a NanoScope IV MultiMode system (Veeco Instruments Inc., Santa Barbara) in the tapping mode.

Conductivity Measurements. Resistance measurements of metal nanowires were carried out by a four-point probe method. Silicon chips fabricating 80 lithographically patterned electrodes with gold-stripes 30 μm wide by 400 μm long were prepared over a silicon nitride membrane (NTT Advance Technology, Ibaraki, Japan). Each electrode

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was separated by a 2 μm gap, although four of them were directly connected without any gap for use as reference. Nanogold-attached 353C RecA filaments were spread randomly over the patterned electrodes, and this was followed by gold enhancement to form metal nanowires. A Keithley 2182 nanovoltmeter and a Keithley 6485 picoammeter were connected to the electrodes through four probe needles, and the $I-V$ curve of the circuit was measured in the 0.2−2 μV range. To cancel the effect of thermoelectrical voltage, a pair of measurements with opposite polarity was set up, and the resulting values were averaged.

Junction Formation. The 100 mer DNA probe, GGGCGGCGACCTAATATCCATTGTTTCTTATATAAAGGTTAGGGGGTAAATCCCGGCGCTCATGACTTCGCCTTCTTCCCATTTCTGATCCTCTTCAAAA (purchased from QIAGEN), with a sequence complementary to a cohesive end and homologous to the 28813−28900 region of λ DNA, was mixed with λ DNA (48502 bp) under the solution condition of 20 mM Tris-acetate, pH 7.5, 2 mM magnesium acetate, and 50 mM NaCl. The final concentrations of the DNA probe and λ DNA were 1.25 μM and 8.3 nM, respectively. The DNA substrates were denatured at 65 °C for 5 min and cooled slowly to the room temperature. Excess DNA probes were removed by small-scale gel filtration (Chroma SPIN-1000, Clontech). 0.25 nM λ DNA with an annealed DNA probe was incubated with 0.44 μM 353C RecA in 25 mM Tris-acetate, pH 7.5, 5 mM phosphocreatine (SIGMA), and 20 U/mL creatine phosphokinase (SIGMA) at 37 °C for 10 min, and then 13 mM magnesium acetate and 1 mM dATP were added to the solution and the sample was further incubated for 60 min. Three volumes of solution mixture containing 25 mM Tris-acetate, pH 7.5, 3 mM ATP-γ-S, and 6.2 μM 353C RecA were added to the reacted solution, and the sample was incubated for 8 min at 37 °C. Metallization procedures were performed as described before.

Gap Formation. The gap formation was performed as per the method by Keren et al.[5] λ DNA was digested with *Hin*dIII, and the 2027 bp fragment (23130−25157 region of λ DNA) was separated on a 1% agarose gel, followed by purification with the QIAquick Gel Extraction Kit (QIAGEN). The DNA fragment was heated to 95 °C for 5 min and then put on ice for denaturation. Wild-type RecA was added to the DNA probe at a 1:3 molar ratio (1.46 μM RecA and 4.4 μM (bases) DNA probe) in 20 mM Tris-aceate, pH 7.5, and 1 mM magnesium acetate, and the sample was preincubated for 5 min at 37 °C. ATP-γ-S was then added to attain a final concentration of 1 mM, and the sample was further incubated for 15 min to ensure complete RecA polymerization on the DNA probe. To start homologous pairing, magnesium acetate concentration was raised to 4 mM, λ DNA was added to attain a final concentration of 0.12 nM, and the sample was incubated for 60 min at 37 °C. Finally, 5.1 μM 353C RecA was added to the sample, followed by 10 min incubation at 37 °C to complete RecA-dsDNA filament formation. Metallization procedures were performed as described before.

Supporting Information Available: Scanning electron microscopy image of gold nanowires and other examples of the three-way junction formation. This material is available free of charge via the Internet at http://pubs.acs.org.

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