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ON−OFF Switching of Transcriptional Activity of Large DNA through a Conformational Transition in Cooperation with Phospholipid Membrane

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Abstract: We report that structural transitions of DNA cause the ON−OFF switching of transcriptional activity in cooperation with phospholipid membrane in a reconstituted artificial cell. It has been shown that long DNA of more than 20−30 kilo base-pairs exhibits a discrete conformational transition between a coiled state and highly folded states in aqueous solution, depending on the presence of various condensing agents such as polyamine. Recently, we reported a conformational transition of long DNA through interplay with phospholipid membrane, from a folded state in aqueous phase to an extended coil state on a membrane surface, in a cell-sized water-in-oil microdroplet covered by phosphatidylethanolamine monolayer (Kato, A.; Shindo, E.; Sakaue, T.; Tsuji, A.; Yoshikawa, K. *Biophys. J.* **2009**, *97*, 1678−1686). In this study, to elucidate the effects of these conformational changes on the biologically important function of DNA, transcription, we investigated the transcriptional activity of DNA in a microdroplet. Transcriptional activity was evaluated at individual DNA molecule level by a method we developed, in which mRNA molecules are labeled with fluorescent oligonucleotide probes. Transcription proceeded on almost all of the DNA molecules with a coiled conformation in the aqueous phase. In the presence of a tetravalent amine, spermine, the DNA had a folded conformation, and transcription was completely inhibited. When the Mg^{2+} concentration was increased, DNA was adsorbed onto the inner surface of the membrane and exhibited an extended conformation. The transcription experiments showed that this conformational transition recovered transcriptional activity; transcription occurred on DNA molecules that were on the membrane.

Introduction

Long DNA molecules larger than 20−30 kilo base-pairs (kbp) have the characteristics of a semiflexible polymer and exhibit a large discrete conformational transition from a coiled state to a highly folded state in aqueous solution, in the presence of various condensing agents including cellular environmental factors such as polyamine.[1−6] This transition is discrete at an individual DNA molecule level and can be described in terms of a first-order phase transition under the criterion of Landau.[7,8] In the folded conformation, it has been reported that the biologically important function of DNA, transcription, is completely inhibited.[9,10]

The interaction of DNA with zwitterionic phospholipid such as phosphatidylethanolamine (PE) and phosphatidylcholine (PC) in the presence of divalent metal cations has been extensively investigated.[11−17] Recently, we found the conformational transition of DNA through interplay with phospholipid membrane, from a folded state in the aqueous phase to an extended coil state on the phospholipid membrane, in a cell-sized microdroplet (Figure 1A).[18] In a phospholipid-coated water-in-oil microdroplet[19−21] that encapsulated bacteriophage T4 DNA (166 kbp), the DNA was located in the aqueous phase with a folded conformation in the presence of a tetravalent amine, spermine (b). At a high Mg^{2+} concentration, DNA was adsorbed onto the membrane surface and exhibited an extended conformation when the membrane was composed of PE (c), but not when it was composed of PC. More interestingly, the adsorption of DNA

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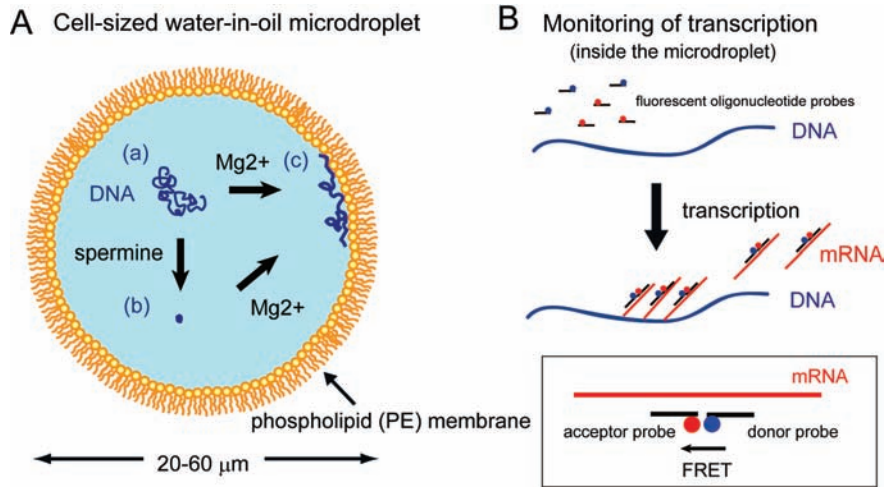

Figure 1. Experimental system. (A) Long DNA molecule has three states in a cell-sized water-in-oil microdroplet coated with phospholipid (PE) membrane: (a) coiled state in the aqueous phase, (b) folded state in the aqueous phase, and (c) extended state on the membrane surface. (B) mRNA synthesis from the DNA is monitored using two fluorescent oligonucleotide probes that hybridize to the mRNA. On the hybrid formed (inset), the distance between two fluorescence molecules is very close, resulting in FRET.

to the PE membrane surface accompanied by the conformational transition was observed only when DNA was encapsulated in a microdroplet of less than ~60 μm of diameter, but not in a microdroplet larger than ca. 100 μm, under a physiological ionic strength condition. The thermodynamic analysis showed that the translational entropy loss of a DNA molecule caused by adsorption, i.e., from being confined in a three-dimensional aqueous phase to being attached to a two-dimensional membrane surface, plays a key role in determining the localization and conformation of DNA under such cell-sized (micrometer space) confinement conditions.[18]

Over the past decade, the artificial cell studies, in which the cell-sized phospholipid vesicles encapsulating biochemical molecules are experimentally reconstituted and the molecular reactions and events inside the vesicle are investigated, have been progressing. In these studies, protein synthesis (transcription and translation), enzymatic reactions and other cellular molecular events are run under cellular environmental conditions (in a micrometer-scale aqueous space surrounded by a phospholipid membrane).[21−29] One of the purposes and goals of these studies is to examine and elucidate the characteristics and driving forces of the cellular molecular events in a micrometer-scale space, such as the reaction rate and the role of the phospholipid membrane as an interface, which are considered to be different from those on a macro-volume scale (test-tube scale). Our observation that the conformational transition of long DNA on the phospholipid membrane, which depends on the confinement volume, is considered to be a distinctive characteristic that appears to emerge under a cell-sized confinement condition.

The regulation of gene expression (transcription) is one of the most essential molecular events in living organisms. If we wish to elucidate the relation between these structural transitions of long DNA through interplay with phospholipid membrane and the transcriptional activity of DNA, the transcription reaction should be measured inside individual microdroplets. In a previous study, we developed a method to detect the transcriptional activity of a specific gene on DNA in a microdroplet (Figure 1B).[30] The *in vitro* transcription reaction in aqueous solution had been monitored using fluorescent oligonucleotide probes, such as the binary fluorescence resonance energy transfer (FRET) probes[31] or molecular beacon,[32] which hybridize to the specific mRNA synthesized. In the binary probe method, two fluorescent oligonucleotide probes, where one is labeled with the donor fluorescence molecule (donor probe) and the other with the acceptor fluorescence molecule (acceptor probe), hybridize to an adjacent location on the target mRNA. On the hybrid formed, the distance between two fluorescence molecules becomes very close, which results in FRET. These methods can also be used to visualize a specific mRNA in living cells under fluorescence microscopy.[33−42] We examined the binary FRET

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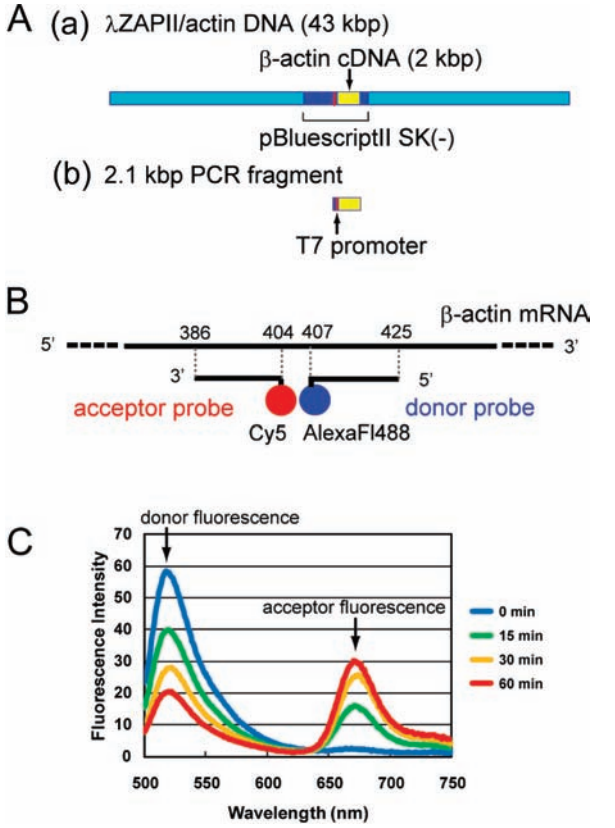

Figure 2. (A (a)) λZAPII DNA containing β-actin cDNA. β-actin cDNA with the terminator sequence of T7 RNA polymerase with the 3′-end was inserted into the multicloning site of λZAPII DNA.(A (b)) PCR fragment DNA from λZAPII/actin DNA. (B) Pair of fluorescent oligonucleotide probes that hybridize to a site of β-actin mRNA. (C) Fluorescence spectral changes with the progression of the transcription reaction from λZAPII/actin DNA (for details, see the Methods in the Supporting Information).

probe method to monitor a transcription reaction in a microdroplet. In the study, a plasmid DNA, fluorescent oligonucleotide probes for the mRNA, and other transcriptional reaction components were encapsulated in microdroplets, and the progress of the transcription reaction inside individual microdroplets could be monitored by measuring FRET fluorescence intensity under fluorescence microscopy.[30]

In this study, to elucidate the relation between the DNA conformational transition and its function in a cell-sized microdroplet coated with phospholipid membrane, we prepared a long DNA containing a gene transcribed under *in vitro* conditions (λZAPII DNA containing human β-actin cDNA, 43 kbp, Figure 2A). The DNA was encapsulated in microdroplets, in which the location and conformational state of DNA and transcriptional activity were investigated under fluorescence microscopy. The ON or OFF of transcriptional activity was evaluated at individual DNA molecule level, which showed that transcription is completely inhibited in the folded DNA molecule in the aqueous phase while it is reactivated when the DNA interacts with phospholipid membrane in the presence of higher concentrations of Mg^{2+}.

Results

Experimental System. We constructed the λZAPII vector DNA containing human β-actin cDNA (λZAPII/actin DNA, Figure 2A(a)). On the DNA, a single promoter sequence of T7 RNA polymerase is located upstream of the β-actin cDNA sequence. This DNA is ∼43 kbp in length and exhibits a conformational transition between a coiled state and a folded state in aqueous solution (described later). To examine the effects of DNA length in some experiments, we prepared a short (2.1 kbp) DNA fragment that contains the T7 promoter and β-actin cDNA sequence (Figure 2A(b)).

We also prepared a pair of fluorescent oligonucleotide probes for the 386-425 site of β-actin mRNA (antisense probe, Figure 2B). These are 19-mer 2′-*O*-methyl RNA, each of which is labeled with AlexaFl488 (donor) at the 3′-end or with Cy5 (acceptor) at the 5′-end.[30] These two probes hybridize to β-actin mRNA adjacent to each other, resulting in FRET.[30,33] We also prepared a pair of fluorescent oligonucleotide probes (that have the same sequence as the 386-425 site of β-actin mRNA), which do not hybridize to β-actin mRNA (sense probe).[30]

Figure 2C shows the fluorescence spectral changes of the reaction solution when transcription was carried out in the bulk aqueous solution with λZAPII/actin DNA and 300-fold amounts (molar ratio to DNA) of antisense fluorescent oligonucleotide probes. As in the case of a plasmid DNA containing β-actin cDNA,[30] donor fluorescence intensity (520 nm) decreased, and acceptor fluorescence intensity (670 nm) increased as incubation progressed, which indicates that the FRET efficiency increased with time. This result shows that β-actin mRNA synthesis occurred from λZAPII/actin DNA, which can be monitored by measuring FRET. When the transcription reaction was carried out with the 2.1 kbp DNA fragment, similar fluorescence spectral changes were observed (data not shown), which show that the 2.1 kbp DNA fragment also acts as a template for the transcription of β-actin mRNA.

DNA in Microdroplets. λZAPII/actin DNA molecules were labeled with fluorescent dye, YOYO-1, and those in the transcription buffer (6 mM Mg^{2+}) containing various concentrations of spermine were encapsulated in water-in-oil microdroplets composed of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). Figure 3 shows confocal microscope images of the microdroplets. At this Mg^{2+} concentration, DNAs were located in the aqueous phase. At a very low DNA concentration (0.25 ng/μL, which corresponds to less than 200 DNA molecules in a microdroplet with a diameter of 40 μm), each DNA molecule diffusing inside the droplet can be observed (Figure 3, c and f). In the absence of spermine (c), DNA had a coiled conformation, while in the presence of 0.6 mM spermine it had a highly compact (folded) conformation (f), as the case of T4 DNA we previously reported.[18,43] These results show that λZAPII/actin DNA exhibits a conformational transition between a coiled state and a highly folded state, depending on the concentration of spermine. Our previous studies show that the

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(43) The concentrations of Mg^{2+} and spermine at which λZAPII/actin DNA exhibits a conformational transition from a coiled state to a folded state are different from those for T4 DNA,[18] due to differences in DNA length (T4 DNA; 166 kbp, λZAPII/actin DNA; 43 kbp) and the ionic strength of the solution. The composition and concentrations of monovalent ions used in this study are those for the optimal activity of T7 RNA polymerase *in vitro*.

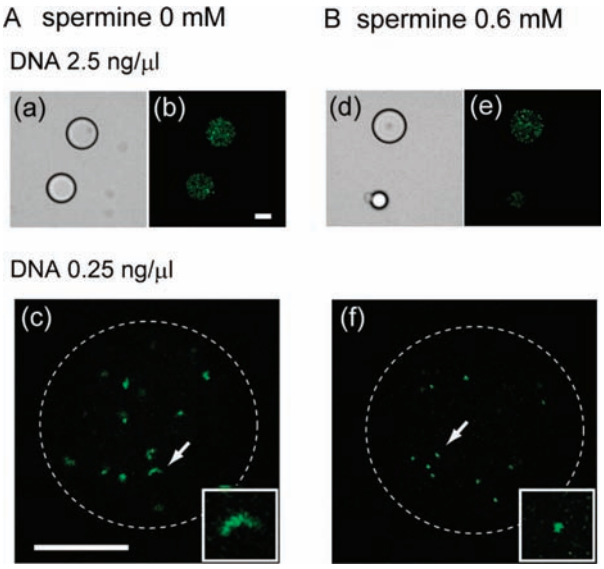

Figure 3. Confocal microscope images of DOPE microdroplets encapsulating YOYO-1-labeled λZAPII/actin DNA (6 mM Mg^{2+}). (a and d) Transmitted-light images (×20 objective). (b and e) Fluorescence images (×20 objective). (c and f) Fluorescence images (×100 objective). Each dotted circle in (c) and (f) indicates the periphery of a microdroplet. The arrow indicates a DNA molecule, the magnified image of which is shown in the inset. The image acquisition time was 33 ms. Scale bar: 20 μm.

conformational transition of long DNA (more than 20−30 kbp) induced by spermine is of the "all-or-none" (discontinuous) type at the individual DNA molecule level, and all of the DNA molecules have the folded state in the presence of sufficiently high concentrations of spermine.[18,44]

Transcription in Microdroplets. λZAPII/actin DNA (nonlabeled, 0.25 ng/μL) and fluorescent oligonucleotide probes (4×10^{-8} M, which corresponds to 4000-fold amount (molar ratio) of DNA) were encapsulated in DOPE microdroplets, and these were incubated on the microscope stage. To monitor the transcription inside the microdroplets, fluorescence images in three channels (FRET channel: acceptor fluorescence with donor excitation. Donor channel: donor fluorescence with donor excitation. Acceptor channel: acceptor fluorescence with acceptor excitation) were obtained sequentially at various incubation times.

In the absence of spermine, discrete FRET signals diffusing inside the microdroplet appeared in the FRET channel from 10−15 min of incubation (Figure 4a). The intensity of each FRET signal tended to become stronger as incubation progressed from 10 to 30 min, and did not change after 30−60 min. The number of discrete signals remained almost the same after 10−30 min. The similar discrete fluorescence signals appeared in the other two channels, the Donor channel and the Acceptor channel (Figure S2 in the Supporting Information), which indicates that multiple donor and acceptor probes were located in close proximity, giving a discrete fluorescence signal. When the sense probe was added to the reaction solution instead of the antisense probe, such discrete fluorescence signals did not appear in any of the three channels throughout the entire incubation period of more than 3 h (Figure 4b and Figure S2). Also, when incubation was run without RNA polymerase (with the antisense probe), these discrete signals were not observed (images not shown). These results show that the discrete signals were derived from β-actin mRNA synthesized inside the microdroplet.

In the last study, we performed experiments of transcription from a short plasmid DNA (pBluescript II SK(−) containing β-actin cDNA, 5 kbp) in the DOPE microdroplet.[30] When a high concentration of DNA was present (5 ng/μL, which corresponds to ∼30000 transcription sites in a microdroplet with a diameter of 40 μm), FRET fluorescence was diffusely observed throughout the microdroplet, and the progress of the transcription reaction was monitored by measuring the increase in FRET fluorescence intensity inside the microdroplet,[45] as in the case of bulk solution. At a very low DNA concentration, discrete FRET signals, which are similar to those in the present study, were observed inside the microdroplet, and FRET fluorescence in other parts of the microdroplet was within the background level.

In this method, each RNA molecule is labeled with a pair of fluorescent probes. Since a single fluorescence molecule diffusing in the aqueous solution is not detected as a discrete fluorescence signal under the imaging conditions, the discrete fluorescence signal observed should be considered not to correspond to a single RNA molecule diffusing in the aqueous phase but rather to "multiple RNA molecules". We conclude that each discrete fluorescence signal represents a "multiple RNAs on a single DNA", which means a DNA molecule that is being transcribed (more detailed discussion is given in the Footnote S1 in the Supporting Information).

Transcriptional Activity Is Inhibited by the Conformational Transition of DNA to a Folded State. Effects of Spermine. When the spermine concentration increased, the number of discrete fluorescence signals in a microdroplet decreased (Figure 4). The number of discrete FRET signals in the microdroplet images, each of which was obtained at a center confocal plane of the microdroplet, was counted at various spermine concentrations (Figure 5). The number of signals decreased to almost zero at more than 0.6 mM of spermine.

We also counted the number of YOYO-1-labeled DNA molecules in the microdroplet images at various spermine concentrations. These values were 14.0 ± 2.32, 12.8 ± 1.46, 13.1 ± 1.68, 12.4 ± 2.44, and 12.7 ± 1.67 at 0, 0.2, 0.4, 0.6, and 0.75 mM of spermine, respectively. These results show that the efficacy of encapsulation of λZAPII/actin DNA into DOPE microdroplets is independent of whether the DNA has a coiled state or a folded state at this very low DNA concentration, and there were almost the same numbers of DNA molecules in the microdroplet at spermine concentrations of 0−0.75 mM. According to our conclusion that each discrete fluorescence signal represents a DNA molecule that is being transcribed, almost all of the DNA molecules in the microdroplet were "transcriptional-active" in the absence of spermine, while at 0.4 mM of spermine, for example, only one-third of the DNA molecules

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(45) For λZAPII/actin DNA, ∼30000 transcription sites in a 40 μm microdroplet correspond to 43 ng/μL of DNA. It is actually difficult to encapsulate such high concentration of long λZAPII/actin DNA into microdroplets.

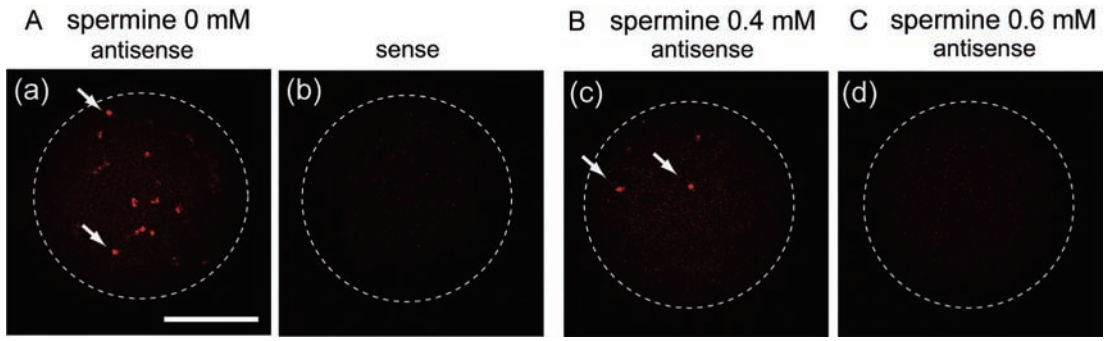

Figure 4. Confocal fluorescence microscope images (FRET channel) of DOPE microdroplet (6 mM Mg^{2+}). Representative images of 30-min incubation are shown. The dotted circle and arrows indicate the periphery of a microdroplet and discrete FRET signals inside the microdroplet, respectively. Scale bar: 20 μm.

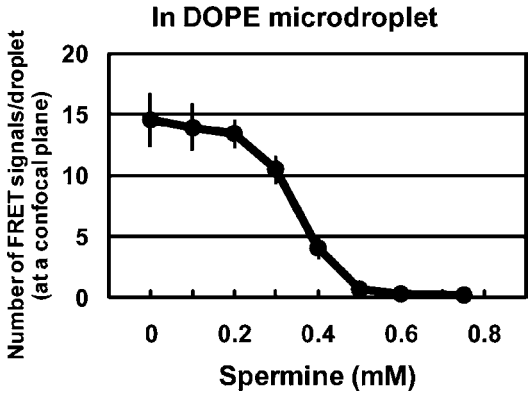

Figure 5. Number of discrete FRET signals formed by the transcription reaction in DOPE microdroplets with a diameter of 40−60 μm. For each microdroplet, image acquisition for 0.2 s was repeated three times at a center confocal plane of the microdroplet, and the number of discrete signals in the microdroplet was counted. More than 20 microdroplets were examined at each spermine concentration in two independent experiments.

were "transcriptional-active",[46] and none of the DNA was "active" at more than 0.6 mM of spermine.[47]

We also performed an experiment to examine the effects of spermine on the amount of RNA synthesis. Transcription was run for 30 min in the bulk solution, and synthesized RNA was purified (Figure 6A). Figure 6B also shows the results when short 2.1 kbp DNA, instead of λZAPII/actin, was used as a template for the transcription reaction. In λZAPII/actin DNA, as the spermine concentration increased, the amount of synthesized RNA decreased, and almost no RNA was detected at more than 0.6 mM of spermine. In contrast, with 2.1 kbp DNA, the presence of 0−0.3 mM of spermine did not affect RNA synthesis. When the spermine concentration increased to more than 0.3 mM, RNA synthesis decreased, but some RNA was detected even at 0.75 mM of spermine.

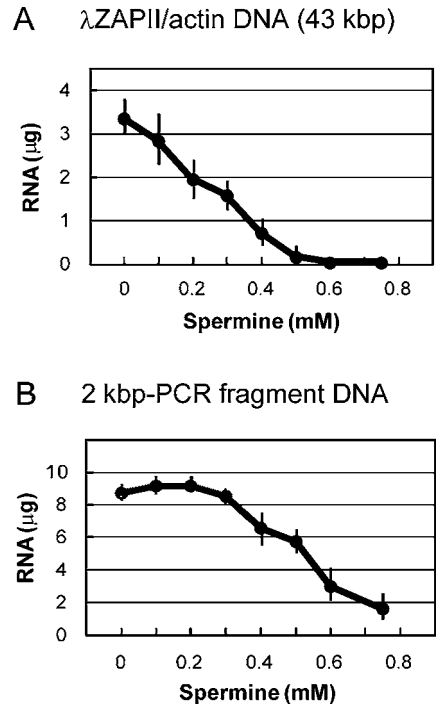

Figure 6. Dependence of RNA synthesis on the spermine concentration. The transcription reaction was performed with 1.25 ng/μL of λZAPII/actin DNA or 2.1 kbp DNA and various concentrations of spermine in 100 μL of bulk solution (6 mM Mg^{2+}) at 37 °C for 30 min. RNA was purified from the reaction solution, and the amounts were estimated.

In the transcription imaging of λZAPII/actin DNA in the microdroplet, almost all of the DNA molecules were "transcriptional-active" at a spermine concentration of 0−0.2 mM (Figure 5). On the other hand, the amount of RNA synthesized in the presence of 0.2 mM of spermine was less (~60%) than that in the absence of spermine (Figure 6A). These results suggest that fewer RNA molecules were synthesized from a DNA molecule during the incubation period. Since the amount of RNA synthesized from the 2.1 kbp DNA was almost the same under range of spermine concentrations of 0 to 0.2 mM (Figure 6B), the decrease in the amount of RNA synthesis on λZAPII/actin DNA is considered to be not a direct consequence of the effects of spermine on the transcription reaction but rather a consequence of the effects on the structure of long λZAPII/actin DNA. At 0.2 mM of spermine, λZAPII/actin DNA had a coiled conformation, not a folded conformation. Previously, we noted that the long-axis length of DNA molecules with a coiled conformation observed in the fluorescence images decreased

(46) As described above, the folding transition of long DNA is a first-order phase transition that is discrete at individual DNA molecule level.[7,8] Therefore, in the intermediate spermine concentration region, DNA molecules with a coiled conformation and those with a folded conformation coexist. The results and interpretation in Figure 5 are consistent with this fact.

(47) The average numbers of discrete FRET signals were not zero, but rather 0.27 and 0.20 at spermine concentrations of 0.6 and 0.75 mM, respectively. At these spermine concentrations, we observed several microdroplets that had only one signal. It is possible that the DNA was cut off to a shorter length during the experimental handling. If the cut-off DNA was too short to exhibit a conformational transition to a folded state, transcription might occur on this DNA (see later discussion on 2.1 kbp DNA). Therefore, we interpret our results to indicate that "none of the (long λZAPII/actin) DNA was "active" at more than 0.6 mM of spermine".

in aqueous solution when the concentration of polyamine or Mg^{2+} increased,[18,44] which is caused by the partial neutralization of the negative charges of the phosphate moieties of DNA by Mg^{2+} or spermine. In these "shrunken" coil structures, the rate of some steps in the transcription reaction, such as the binding of RNA polymerase to DNA, might become lower, which would result in a decrease in the reaction rate of RNA synthesis.

The most important result in Figure 6A is that only a very few RNA were detected at 0.5 mM of spermine, and none were detected at more than 0.6 mM of spermine. These results are consistent with those of transcription imaging at individual DNA molecule level, where no discrete FRET signal was observed at more than 0.6 mM of spermine (Figure 5). These results show that all of the DNA molecules with a folded conformation (Figure 3B) are "transcriptional-silent".

On a short 2.1 kbp DNA, RNA synthesis occurred in the presence of more than 0.6 mM of spermine (Figure 6B). When 2.1 kbp DNA was encapsulated in DOPE microdroplets and transcription was run, discrete FRET signals were observed even at 0.75 mM of spermine, although there appeared to be fewer signals than in the absence of spermine (images not shown).[48] These results support our conclusion that the complete inhibition of the transcription on long λZAPII/actin DNA in the presence of higher concentrations of spermine was due to the folding conformation of the DNA.

With 2.1 kbp DNA, the amount of synthesized RNA decreased when the spermine concentration became higher than 0.4 mM. When we observed fluorescent-labeled 2.1 kbp DNA in the microdroplets, the extensive aggregation and/or condensation of DNA molecules was not observed even at 0.75 mM of spermine. However, the small aggregates of several DNA molecules might be formed,[49] on which transcription would be inhibited or suppressed. Another possibility is that high concentrations of spermine directly affected the reaction rate in some steps of the transcription reaction.

Recovery of Transcriptional Activity on an Extended Coil Conformation on Phospholipid Membrane. The λZAPII/actin DNA in a DOPE microdroplet was adsorbed to the membrane inner surface at a high Mg^{2+} concentration (15 mM, Figure 7A(a−c)). On the membrane surface, the DNA molecule exhibits a quasi-two-dimensional coil state with intramolecular chain motion (movie S1 in the Supporting Information), as in the case of T4 DNA we reported.[18] The transcription reaction was run under this condition. From 10−15 min of incubation on the microscope stage, discrete fluorescence signals appeared on the membrane inner surface in all three channels. (An image in the FRET channel is shown in Figure 7B(a)). As discussed above, these signals are considered to be derived from multiple RNA molecules on a DNA molecule. These results show that RNA synthesis occurred on the DNA molecule which was located on the membrane surface and had an extended coil conformation.

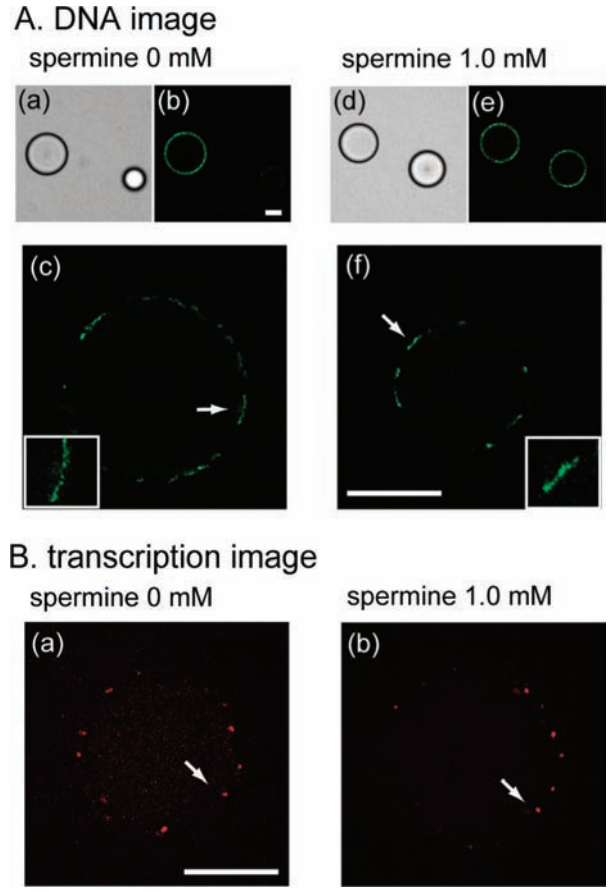

Figure 7. Confocal microscope images of DOPE microdroplets encapsulating λZAPII/actin DNA (15 mM Mg^{2+}). (A) DNAs labeled with YOYO-1 in a DOPE microdroplet. (a and d) Transmitted-light images (×20 objective). (b and e) Fluorescence images (×20 objective). (c and f) Fluorescence images (×100 objective). In (c) and (f), the arrow indicates a DNA molecule, the magnified image of which is shown in the inset. (B) λZAPII/actin DNA (nonlabeled) and fluorescent oligonucleotide probes were encapsulated in microdroplets, and the transcription reaction was run inside the microdroplets. Fluorescence images in the FRET channel at 30 min of incubation (×100 objective). The arrow indicates a discrete signal. Scale bar: 20 μm.

Next, we examined the transcriptional activity of DNA under the coexistence of high concentrations of Mg^{2+} and spermine. The transcription reaction was run with λZAPII/actin DNA and fluorescent oligonucleotide probes at various concentrations of Mg^{2+} and spermine in the bulk solution, and the reaction solution was then encapsulated in DOPE microdroplets. Discrete fluorescence signals similar to those observed when transcription was run inside microdroplets were observed. These discrete signals were not observed at high concentrations of spermine. The numbers of discrete FRET signals were counted (Figure 8). The concentration of spermine at which no discrete signal formed increased as the Mg^{2+} concentration increased. It has been reported that Mg^{2+} has a competitive effect on the folding transition of long DNA induced by spermine; DNA requires a higher spermine concentration for its conformational transition to the folded state in the presence of a higher concentration of

(48) We encapsulated the same concentration (0.25 ng/μL) of 2.1 kbp DNA in microroplets as was used for λZAPII/actin DNA; 0.25 ng/μL of 2.1 kbp DNA corresponds to more than 30000 molecules in a microdroplet with a diameter of 40 μm. Indeed, there were a large number of discrete FRET signals in the microdroplets. In addition, the diffusion of discrete signals in the aqueous phase was faster than in the case of λZAPII/actin DNA. Therefore, it was actually difficult to count the number of discrete FRET signals in the microdroplets.

(49) Under the present imaging conditions, it would be difficult to distinguish between a single 2.1 kbp DNA molecule and an aggregate of several DNA molecules, if it forms. This is due to the much lower signal-to-noise ratio of fluorescence images of a 2.1 kbp DNA diffusing in the aqueous phase. When compared to the long λZAPII/actin DNA, the short 2.1 kbp DNA shows a much faster translational diffusion because it does not exhibit an apparent coiled structural fluctuation. As a result, the residence time of the DNA on focus during an image acquisition (33 ms) becomes very short. Also, the number of fluorescent dyes (YOYO-1) included in a DNA molecule is smaller. These decrease the signal-to-noise ratio of fluorescence images of a 2.1 kbp DNA.

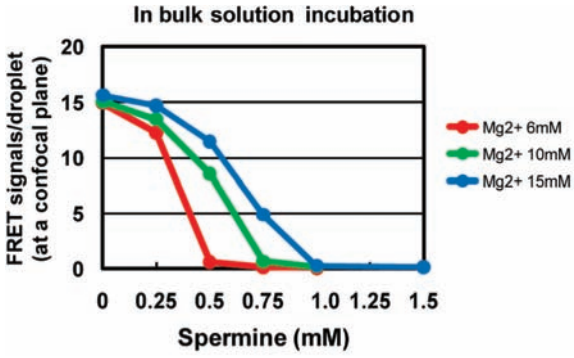

Figure 8. Effects of the Mg^{2+} and spermine concentrations on the number of discrete FRET signals formed by the transcription reaction in the bulk solution. The transcription reaction with λZAPII/actin DNA and fluorescent oligonucleotide probes was run in the bulk solution for 30 min, and the reaction solution was then encapsulated in DOPE microdroplets. The numbers of discrete FRET signals were counted.

Mg^{2+}, which is considered to be due to the competitive binding of Mg^{2+} and spermine to the phosphate moieties of DNA.[18] The results in Figure 8 are consistent with this fact when the results are interpreted to indicate that the conformational transition of λZAPII/actin DNA to a folded state occurred at a higher concentration of spermine in the presence of a higher concentration of Mg^{2+}. We also performed experiments to estimate the amounts of RNA synthesized under the conditions of Figure 8 (15 mM Mg^{2+}), which showed that RNA was not detected at more than 1.0 mM of spermine. These results show that all of the λZAPII/actin DNA molecules were "transcriptional-silent" (OFF) with 15 mM Mg^{2+} and 1.0 mM spermine in the bulk solution.

We observed λZAPII/actin DNA in DOPE microdroplets at 15 mM Mg^{2+} and 1.0 mM spermine (Figure 7A(d−f)). They were located on the membrane surface and exhibited an extended conformation as in the absence of spermine, which shows that the conformational transition of λZAPII/actin DNA from a folded state in the bulk solution to an extended coil state on the phospholipid membrane surface takes place. When the transcription reaction was run, the discrete fluorescence signals appeared on the membrane surface (Figure 7B(b)), which were very similar to those observed in the absence of spermine. These results show that transcription occurred under the condition in which it was completely inhibited in the bulk solution, when DNA was adsorbed to the PE membrane surface.

Discussion

In this study, the transcriptional activity of a long DNA in a cell-sized microdroplet coated with phospholipid membrane was investigated at individual DNA molecule level. The results are summarized in Figure 9. When the DNA was located in the aqueous phase with a coiled conformation, transcription was performed on the DNA (Figure 9a). In the presence of high concentrations of spermine, the DNA had a folded conformation in the aqueous phase. In this state, all of the DNA molecules were "transcriptional-silent" (Figure 9b). When the Mg^{2+} concentration increased, DNA was adsorbed onto the surface of the PE membrane and had an extended conformation. This conformational change of DNA through interplay with the phospholipid membrane was accompanied by the recovery of transcriptional activity (Figure 9c).

We previously reported that T4 DNA is not adsorbed to a membrane surface composed of PC at a Mg^{2+} concentration at

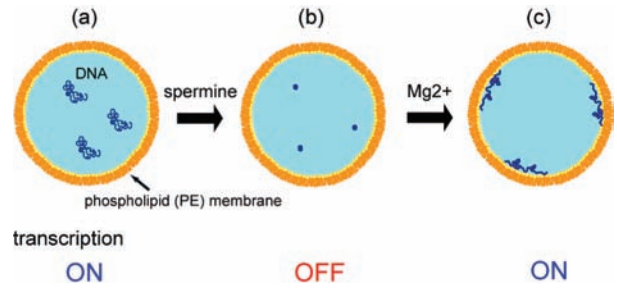

Figure 9. Summary of this study.

which it is adsorbed onto PE membrane.[18] We also observed that the adsorption of T4 DNA to the PE membrane surface does not occur when the microdroplet in which the DNA is encapsulated becomes large. This result is caused by the fact that the translational entropy loss of a DNA molecule upon adsorption increases as the microdroplet becomes larger, and that this entropy loss is not sufficiently compensated by the gain in free energy due to the DNA−phospholipid membrane interaction.[18] Our observation in this study that is the ON−OFF switching of transcriptional activity of DNA through interplay with phospholipid membrane should depend on the phospholipid composition of the membrane and the volume of the microdroplet in which DNA is confined.

In the experiments of gene expression, "transcriptional activity" has been usually evaluated in terms of amount of RNA synthesized from a large number of DNA molecules. However, there would be many cases that these estimates cannot provide the accurate information on the most essential matter regarding regulation of gene expression; whether the transcription of a specific gene on the DNA molecule is ON or OFF. Our method shown in this study, which directly evaluates whether or not the gene is transcribed at individual DNA molecule level, would provide very useful information for elucidating the characteristics of the regulation of gene expression.

In this study, we demonstrated the ON−OFF switching of function (transcriptional activity) through the structural transitions of DNA. In a cell, genome DNA forms a self-assembled complex with histone proteins (nucleosome), which further forms highly packed structures. The structural changes in these DNA−protein complexes (chromatin) are considered to be responsible for the regulation of gene expression for a number of genes that are located on a proximity region of DNA.[50,51] The regulation through structural changes/transitions is considered to act in an all-or-none manner for all of the genes located in the region, and its characteristics are much different than those of the regulation of individual genes by the binding and release of some regulatory proteins to a specific sequence on DNA, when it works in a cell. Since there is only a single gene and several to several hundred regulatory proteins for the regulation of individual genes in a cell, the binding and release between a regulatory site of the gene and its binding proteins are under thermodynamic fluctuation. In contrast, the structural changes are expected to provide the basis for more robust ON−OFF switching of transcription for a number of genes simultaneously.[52] The phenomena and experimental system shown in this study may be a good experimental model of the regulation

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of transcription through structural changes/transitions of DNA and DNA−protein complexes.

Our results are also very interesting from the perspective of native and artificial proto-cells. The present findings show that the ON−OFF switching of transcription is possible without regulatory DNA binding proteins and only requires a phospholipid membrane and some cellular environmental factors such as Mg^{2+} and polyamine. The conformational transition of DNA is induced by changes in the concentrations of Mg^{2+} and spermine under physiological ionic strength conditions, which accompanies the ON−OFF switching of transcriptional activity of DNA. Phase separation on phospholipid membrane to produce a PE domain may act as a trigger for the adsorption of DNA to the membrane and the recovery of transcriptional activity. In bacteria, changes in the lipid composition of the cell membrane in relation to the cell cycle have been reported.[53,54] Studies of the roles of phospholipid membrane in DNA function would give us novel and fruitful insights into regulation of DNA structure and function in living and artificial cells.

Supporting Information Available: Materials and Methods, additional footnote and figures, and a movie of λZAPII/actin DNA on the phospholipd membrane surface of the microdroplet. This material is available free of charge via the Internet at http://pubs.acs.org.

JA105154K

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