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Transcribed Guanine-rich RNA Aggregates on Template DNA and Changes Its Conformation

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The direct visualization of transcribed RNA-dependent DNA structural change by atomic force microscopy revealed that guanine-rich RNA acts as a molecular switch for changing DNA conformation and the changed DNA structure is stably maintained, suggesting the possibility that DNA can memorize past transcription action in terms of shape.

DNA, encoding genetic information, is a flexible material that can change its conformation considerably. Although the conformational change of DNA is important in many biological reactions,[1] its precise mechanism has been insufficiently analyzed. Previously, we suggested that guanine-rich RNA (G-rich RNA) transcripts aggregate on template DNA, change the DNA conformation and accelerate several biological processes.[2,3] For example, immunoglobulin (Ig) switch-region RNA aggregates on template DNA after transcription, generates a large RNA and DNA complex (RNA/DNA complex), and relaxes the template DNA, which may be essential processes for the DNA recombination of Ig genes (class switch recombination) during B lymphocyte differentiation.[2] The precise structure of the RNA/DNA complex, however, has not been sufficiently clarified. Here, using two ribonucleases (RNase A and RNase H), we degraded aggregated RNA in a stepwise manner and examined the conformational change of template DNA by agarose gel electrophoresis and tapping-mode atomic force microscopy (AFM).

The plasmid DNA pGD231 contains the Ig switch-region DNA fragment. Using this plasmid, we generated G-rich RNA using T7 RNA polymerase in vitro (in vitro transcription) as reported previously.[2] The transcribed samples were initially treated with RNase A and then with RNase H. An aliquot of each sample was analyzed by agarose gel electrophoresis or AFM. Briefly, the samples for agarose gel analysis were run on a 1.5% gel in TAE buffer (40 mM Tris-Acetate, 1 mM EDTA) at 5 V cm^{-1}. The gel was stained with ethidium bromide and photographed under ultraviolet illumination (312 nm). For AFM analysis, the samples were purified using MicroSpin S-400HR columns (GE Healthcare). Then, the samples were diluted with 10 mM Tris-HCl (pH 7.9) and 1 mM NaCl to 1 ng μL^{-1} final concentration. After adding $MgCl_2$ (final concentration, 2.5 mM), 5 μL of each solution was deposited onto a freshly cleaved mica disk. After 5 min, the mica was blown dry with compressed air and rinsed twice with distilled water. AFM was carried out using an SPM-400 scanning probe microscope controlled by an SPI 3700 probe station (Seiko Instruments). A rectangular cantilever with a force constant of 35–40 N m^{-1} and a resonance frequency of 320–400 kHz (SI-DF40, Seiko Instruments) was used. All the images were obtained as height mode images in air at room temperature.

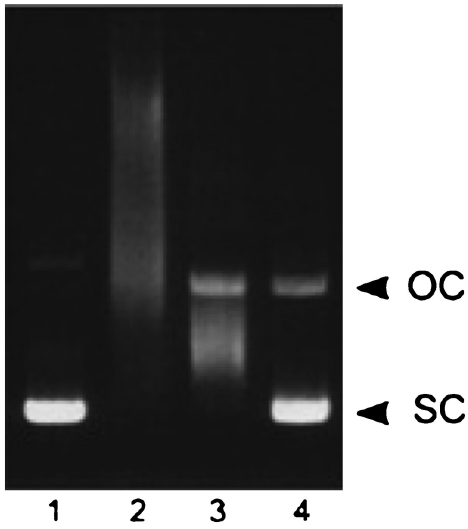

Figure 1. Agarose gel electrophoresis of reaction products. The plasmid samples, before (lane 1) and after in vitro transcription (lanes 2–4), were run on a 1.5% agarose gel. The samples were treated with RNase A alone (lane 3) or with both RNase A and RNase H (lane 4). OC, open circular form; SC, supercoiled form.

Figure 1 shows the results of the electrophoresis of the reaction products. Before in vitro transcription, the plasmid DNA migrated at the position of a supercoiled form (lane 1). After in vitro transcription, the DNA migrated more slowly and showed a smeared pattern (lane 2), suggesting RNA/DNA complex formation. After RNase A treatment, the plasmid DNA migrated at the positions of an open circular form and various relaxed forms, which appeared as a smear (lane 3). After adding RNase H, the plasmid DNA migrated mainly at the position of a supercoiled form (lane 4). Thus, the DNA conformation changes after the interaction with the transcribed RNA.

The results obtained by AFM were comparable to those obtained by electrophoresis, as shown in Figure 2. Before in vitro transcription or after the treatment with both RNase A and RNase H, plasmid DNA showed a supercoiled form (Figures 2A and 2D). After in vitro transcription, one large aggregate was deposited on each plasmid DNA, appearing like a pearl on a ring (Figure 2B, arrows), and the plasmid showed an open circular form as we reported previously.[2] The aggregate might be composed of transcribed RNA and a part of the template DNA, but its precise structure has been unknown. RNase A is an endonuclease that cleaves single-stranded RNA. After RNase A treatment, the aggregate became dissolved but the plasmid DNA still maintained a relaxed form and appeared like a ring without a pearl (Figure 2C). Interestingly, small DNA

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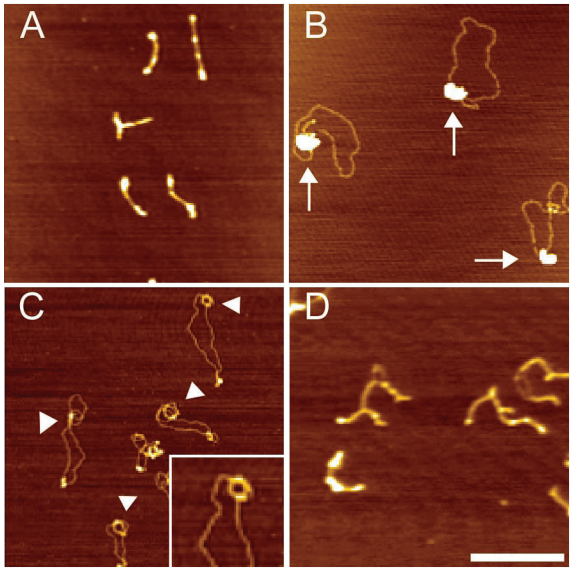

Figure 2. AFM analysis of reaction products. The plasmid samples, before (panel A) and after in vitro transcription (panels B–D), were visualized by AFM. The samples were treated with RNase A alone (panel C) or with both RNase A and RNase H (panel D). Arrows are large RNA aggregates (panel B). Inset, one enlarged image of the DNA loops suggested by arrow heads (panel C). Scale bar, 0.5 μm.

loop structures emerged after RNA digestion (Figure 2C, arrow heads and inset), suggesting that the dissolved aggregate was the transcribed RNA and the RNA aggregate was surrounded or wrapped by the DNA loops. RNase H is a ribonuclease that cleaves the RNA in an RNA/DNA duplex. After adding RNase H, the DNA loop disintegrated and the plasmid returned to a supercoiled form (Figure 2D). Thus, a part of the transcribed G-rich RNA hybridizes to the template DNA and generates an RNA/DNA duplex, which is essential for maintaining the DNA loop and relaxing the plasmid DNA. The residual single-stranded RNA that is cleavable with RNase A condenses in the center of the loop and generates large aggregates. Thus, AFM can be used to directly visualize the change in DNA conformation and the transcribed RNA can change the higher-order structure of template DNA.

Changes in higher-order chromatin structure are considered to be critical in dictating lineage-specific gene expression during development.[4] For example, cytokine gene and β-globin gene loci change their conformation in line with cell differentiation.[4,5] The precise mechanism of chromosomal conformation change, however, has not been fully elucidated. In this study, we showed that RNA is essential for determining DNA conformation. Namely, by direct visualization using AFM, we showed that the transcribed G-rich RNA hybridized with the template DNA, generated DNA loops, and markedly changed the plasmid DNA conformation. These are the direct lines of evidence that RNA is a molecular switch for changing the DNA conformation. Importantly, the newly discovered DNA loop structure may be essential for cell differentiation. The DNA loop formation (DNA looping) facilitates the relocation of two loci located far distance to close proximity and affects gene expression. Actually, in the class switch recombination of Ig genes, DNA looping is a prerequisite for the recombination and development of B lymphocytes.[6] It will be determined whether the RNA-dependent DNA looping is important for the chromosomal conformation change of other genomic loci.

The phenomenon of DNA looping suggests a new DNA function aside from maintaining genetic information: DNA, like a shape memory alloy, can memorize past transcriptional actions in the form of a loop. Once a DNA loop is generated, its shape may be maintained in the genome and contribute to lineage-specific gene expression. The physiological significance of this idea of "shape memory DNA" will also be examined in future experiments.

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