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In situ analysis of the higher-order genome structure in a single *Escherichia coli* cell

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1

Abstract

We developed a novel method for the in situ analysis of the higher-order structure of an individual genome from a single *Escherichia coli* cell using laser tweezers. Initially, condensed DNA was stably grasped by a laser without any chemical modification and without physical attachment to an artificial object such as micro plastic beads. Under optical transport, the trapped genome gradually unfolded in solution due to viscous friction. Interestingly, the nucleoid DNA from a log-phase cell is almost fully elongated, whereas in the stationary phase, unfolding of the nucleoid is characterized by step-wise elongation of 1.7-5.1 μm, corresponding to a size of 5-15 kbp, and a few tightly packed domains remain along the DNA chain. This suggests the coexistence of tightly packed and swollen domains in the genome in the stationary phase.

2

1. Introduction

Manipulation of the whole genome is a challenge in modern biotechnology and bioscience. Among the various methods used for manipulation, laser tweezers may be promising because of their ease of use and non-destructive application. Optical tweezers were first developed in the 1980s by Ashkin (Ashkin, 1997). In most previous studies on DNA manipulation by laser, optical tweezers have been used to trap beads that are tightly bound to DNA molecules, where the size of the beads is on the order of 1 μm (Bennink et al., 2001; Brower-Toland et al., 2002; Pope et al., 2002). Thus, the target of optical manipulation has been a plastic bead with a diameter of 1000 nm that is attached to DNA, where the double-strand structure has a diameter 2 nm. On the other hand, it is now clear that optical tweezers can stably trap and transport giant DNA (above the size of several tens kbp) under conditions where the DNA molecules are tightly compacted in the presence of condensing agents, such as, spermine or PEG (Katsura et al., 1998). With optical tweezers, we can selectively manipulate a desired DNA molecule on the glass plate of an optical microscope by using suitable staining with a fluorescent dye. It has also been suggested that optical tweezers may be suitable as a new methodology for gene delivery (Kubo et al., 2003). Since genome DNAs are generally very long, it would be useful to establish a method for the manipulation and observation of genome DNA larger than several hundreds of kilo base pairs, without fragmentation into short DNA chains.

There has been increasing interest in structure and function at the level of single macromolecules (Zlatanova and van Holde, 2006). Magnetic tweezers have been used to stretch

3

DNA (Leuba et al., 2003; Zlatanova and Leuba, 2002). By using DNA molecules that have been chemically attached to micro supermagnetic beads, stretching experiments have been performed on single DNA and single chromatin molecules (Cui and Bustamante, 2000; Murayama and Sano, 2001; Pope et al., 2004). The so-called comet assay has been frequently used to examine the genome structure (Nougayrède et al., 2006; Tice et al., 2000). The comet assay is a method for observing the breakage of DNA under agarose gel electrophoresis, and can be used to detect low levels of damage in genome DNA. To the best of our knowledge, no previous study has examined the stretching of genome DNA in different cell states without the use of micro beads.

In the present study, we analyzed the higher-order genome structure of samples obtained by the lysis of *E. coli*. To determine the detailed changes in the structure of the genome depending on the growth phase, we also performed the measurements with AFM.

2. Materials and Method

2. 1. Bacterial strains and growth conditions

A well-isolated colony of the *E. coli* strain K-12 on a LB agar plate was transferred to 2 ml of LB medium and cultured at 37 ^{0}C with rotation (30 rpm) to a certain cell density. The cell density was determined by the absorbance at a wavelength of 600nm.

2. 2. Cell lysis and sample preparation

For the lysis of *E. coli*, each strain was immersed in 2 ml of a buffer containing, 1 mM NaN$_3$ and 0.1 M NaCl for 5 min, and lysozyme was added to make the final concentration of 25 μg

4

/ ml.   After 2 min of incubation at 25 ^{0}C, a detergent solution containing Brij 58 (polyoxyethylene

hexadecylether) and sodium deoxycholate was added to give final concentrations, 0.25 mg / ml and

0.1 mg / ml, respectively. After 10 min, buffer was diluted 10-fold by pure water to minimize the

effect   of   detergent   on   the   higher-order   structure.   The   fluorescent   dye   4',

6-diamidino-2-phenylindole (DAPI) (to visualize DNA) and 2-mercaptoethanol (2-ME) (as an

antioxidant) were added to give final concentrations of 0.4 μM and 4 % (V / V), respectively. Nile

red was added to give a final concentration of 0.2 μM to visualize neutral lipids.

For the AFM analysis, cells were harvested from 100 μl of culture by centrifugation

(13000 xg, 1 min, at 4 ^{0}C) and washed once with 1 ml PBS (pH 7.2).   The cells were resuspended

in 250 μl of PBS and a 50-μl aliquot was placed on a round cover glass 15 mm in diameter. Extra

liquid was removed by blowing with a stream of nitrogen gas. Each strain was immersed in 2 ml of

a buffer containing 1 mM NaN$_3$ and 0.1 M NaCl for 5 min, and 25 μg / ml lysozyme was then

added. After 2 min of incubation at 25 ^{0}C, Brij 58 and sodium deoxycholate, were added to give

final concentrations of 0.25 mg / ml and 0.1 mg / ml, respectively.   After 10 min, the cover glass

was dried under a stream of nitrogen gas. The surface of the cover glass was gently washed with

distilled water and dried again for AFM analysis.

2. 3. Fluorescence microscopic observation and optical tweezers

The   sample   chamber   was   constructed   as   shown   in   Fig.   1   under   fluorescence

microscopy (OLYMPUS: IX-70). For optical trapping, a Nd: YAG laser (1064 nm) operating at 1W

was introduced into an oil-immersed objective lens (OLYMPUS, x100, numerical aperture = 1.30)

5

and focused on the stage of an inverted fluorescence microscope. An auto-stage (Sigma Koki) was used to control the movement of a trapped object on the horizontal plane (xy-plane) under the microscope. Fluorescence images were captured by an EB-CCD camera (Hamamatsu Photonics) and recorded on DV-video tapes. These experiments were conducted at $20\ ^0C$.

2. 4. AFM analysis

An atomic force microscope (Nanoscope IIIa, Digital Instrument) was used to image *E. coli* nucleoid structures in air at room temperature under a dynamic force mode with a 100 µm scanner. Probes made of a single silicon crystal with a cantilever length of 129 µm and a spring constant of 33–62 N/m (OMCL-AC160TS-W2, Olympus) were used for imaging. Data were collected in the height mode with a scanning rate of 0.5-1.0 Hz and a driving amplitude of 40–80 mV. Images were captured in a 512 x 512 pixels format and the captured images were flattened and plane-fitted before analysis. Image analysis was performed with the software that was provided with the imaging module (Digital Instrument, USA).

3. Results and Discussion

Figure 2 shows a typical fluorescence double-stained image of the single genome DNA in the *E. coli* cell lysate, where DNA is stained by DAPI and neutral lipids are stained by nile red. This image was captured after the DNA was moved ca. 1mm by laser tweezers, and arrows show the point trapped by the optical tweezers. In the DAPI image in Fig. 2, the major fluorescent object remains at the trapped position, and the connected smaller part trails behind. On the other

6

hand, in the image stained with nile red, the domain that corresponds to the major part in the DAPI image, can be seen, whereas no fluorescence due to nile red is seen for the small domain observed by DAPI. Neutral lipids effectively emit fluorescence when stained with nile red. A similar observation has been reported in the optical trapping of genome DNA from an Archaea (Oana et al., 2004). Thus, the major domain that is optically trapped is attributable to the consist of condensed DNA and neutral lipids.

Figure 3 shows the process of unfolding of single *E. coli* genomes during optical transport at a constant speed of 10 μm/s. Two different growth phases are shown: log phase on the left and stationary phase on the right. White triangles and arrows indicate the trapping point and direction of transport, respectively. In the log phase, the genome DNA smoothly elongated to over 100 μm, indicating that the packing is rather loose. In contrast, in the stationary phase, under viscous friction caused by optical transport, the genome showed only partial unfolding that likely reflected intrachain segregation.

The time-dependent changes in length in the log and the stationary phases (Fig. 4) illustrate a marked difference in the manner of physical unfolding. In the log phase, the genome is elongated at an almost constant speed. On the other hand, in the stationary phase, stepwise elongation of the genome was observed. These characteristic changes likely reflect a difference in the activity of the genes embedded in the genome.

In the log phase (Fig. 5, upper), DNA fibers were easily released out of the cell when the cell was lysed under physiological conditions. Close observation by AFM showed that fibers

7

were mostly composed of a bead structure, where a statistical analysis of the fiber width revealed

two populations at 40 nm and 80 nm (Kim et al., 2004). These fibers may be attributable to the thin

filaments and small fluorescent objects (Fig. 3 (D, E)). In the stationary phase (Fig. 5, lower), the

genome remained tightly packed even after lysis treatment. This tendency corresponds to the

results of the fluorescence observation.

In this study, we used a new experimental method with laser tweezers and found a

marked difference in the manner of genome packing in *E. coil* depending on the growth phase.

Although under fluorescence microscopy we can only observe DNA molecules with relatively low

resolution, we can obtain direct information on the structure in solution without any serious

physical treatments such as fragmentation by a restriction enzyme or modification with

micro-beads, etc., which may be useful for analyzing the state of giant genome DNA molecules as

they exist in cells. On the other hand, AFM revealed detailed information on DNA for a specimen

fixed on a solid substrate. Thus, the combination of these two methods should provide more

reliable information on what is actually occurring inside a living cell. Most importantly, we

showed that optical tweezers can be used in a non-destructive method for the remote handling of

single genomes. A preliminary report has shown the utility of laser manipulation of giant DNA

molecules from *Thermococcus kodakaraensis* (KOD1) (Oana et al., 2004), but that study did not

examine changes with the growth phase or cell stage. Another study has shown that global changes

of structure exists depending on growth phase and environmental stress in *E.coli* (Ohniwa et al.,

2006; Morikawa et al., 2006). Namely genome structure on the growth phase and environmental

8

stress may be related in gene expression.

This study found a large difference in the higher-order structure depending on the growth phase by the simple method of optical transport in buffer without any modification of the genome. This novel method may become a powerful tool for revealing the relationship between genome structure and gene activity.

9

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10

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11

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12

Figure Captions

Fig. 1. Schematic cross-sectional view of the experimental set-up. The single genome DNA in the

lysate of a *E. coli* is trapped and transported in buffer solution by optical tweezers.

Fig. 2. Fluorescence images of a double-stained cell in the stationary phase.

A: Image of a cell visualized by DAPI.

B: The same cell visualized by nile red.

C: The same cell before transport.

White triangles and arrows indicate the trapping point and direction of transport, respectively.

Fig. 3. Mechanical unfolding of a single *E. coli* genome DNA visualized by DAPI during transport

in buffer solution with optical tweezers. Pictures on the left are fluorescence microscopic images,

and those on the right are quasi-three-dimensional representations of the spatial distribution of

fluorescence intensity on the genome.

Fig. 4. Change in the long-axis length of genome during transport by optical tweezers for both the

log phase and stationary phase.

Fig. 5. AFM images of lysed *E. coli* in the log phase (upper) and stationary phase (lower). In the

log phase, the genome is mechanically unfolded into a thin filament, when the *E. coli* cells were

lysed. In stationary phase, most of the genome is tightly compacted. Scale bar: 500nm
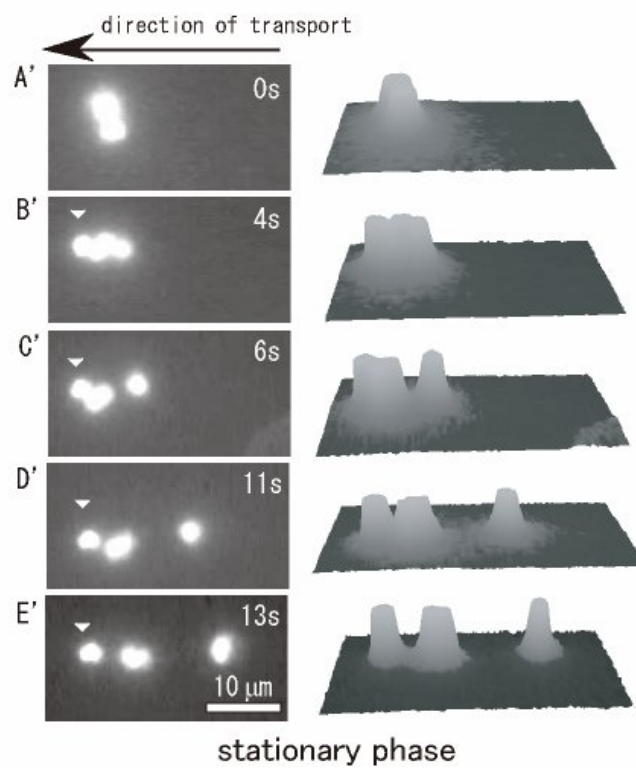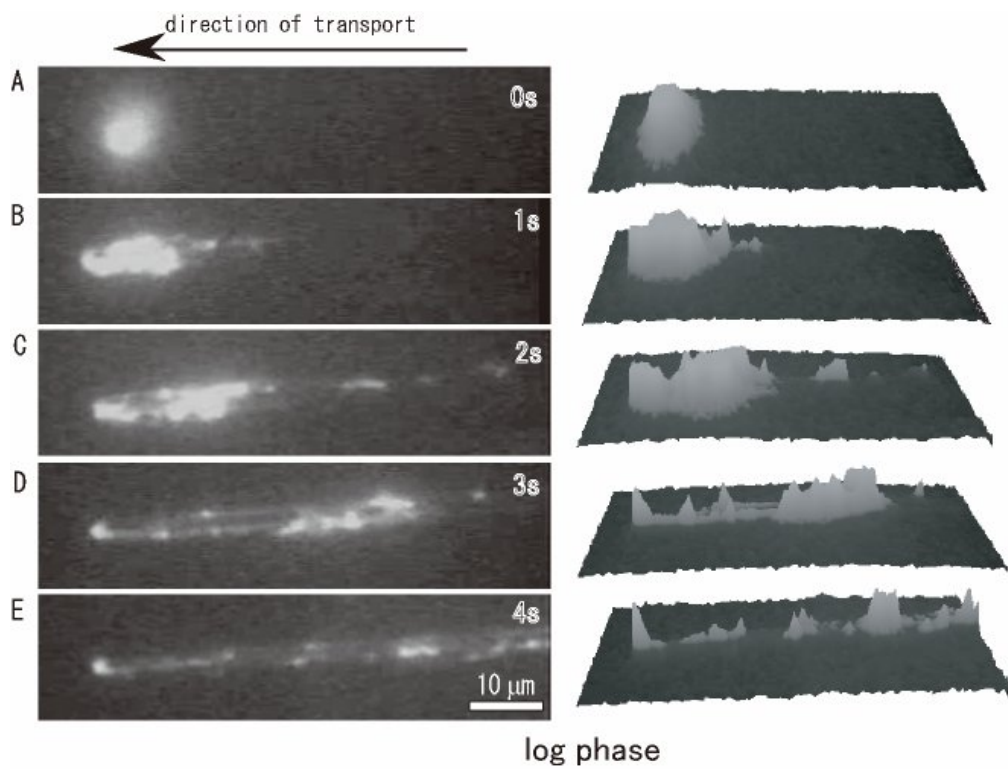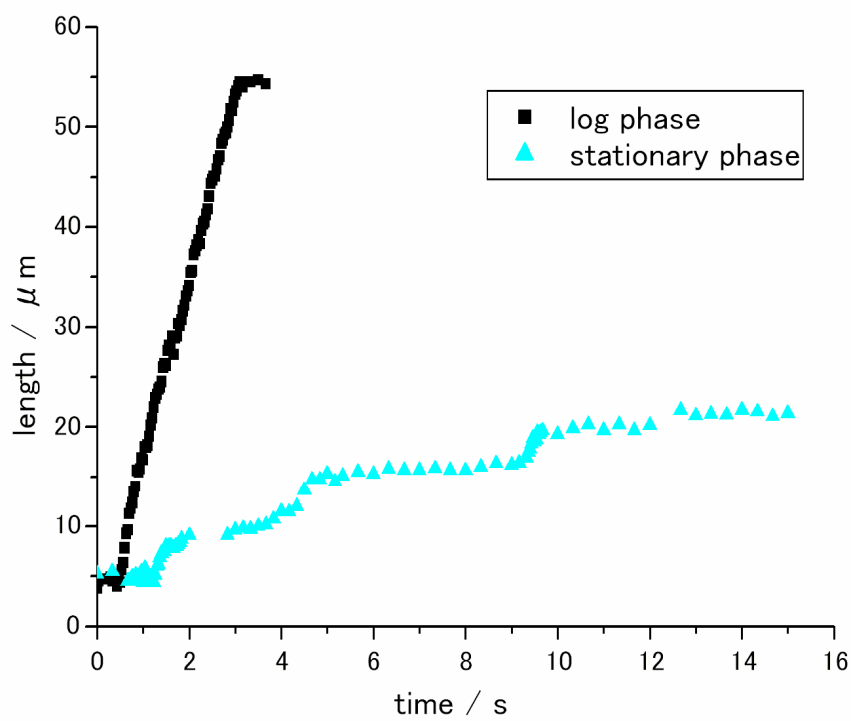
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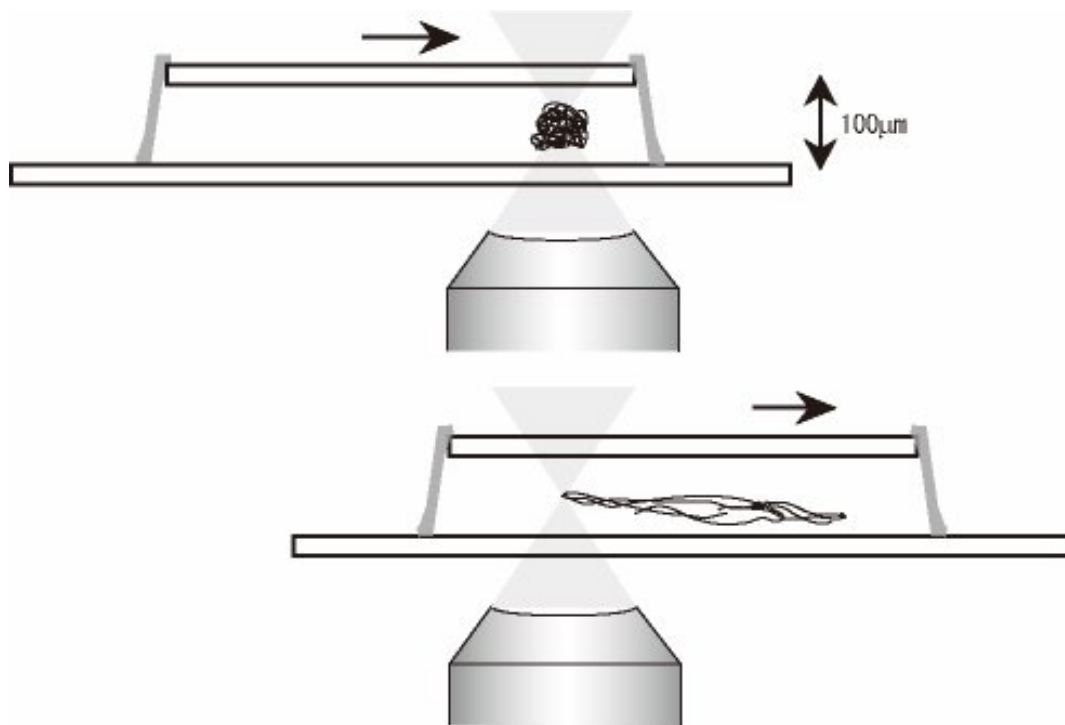
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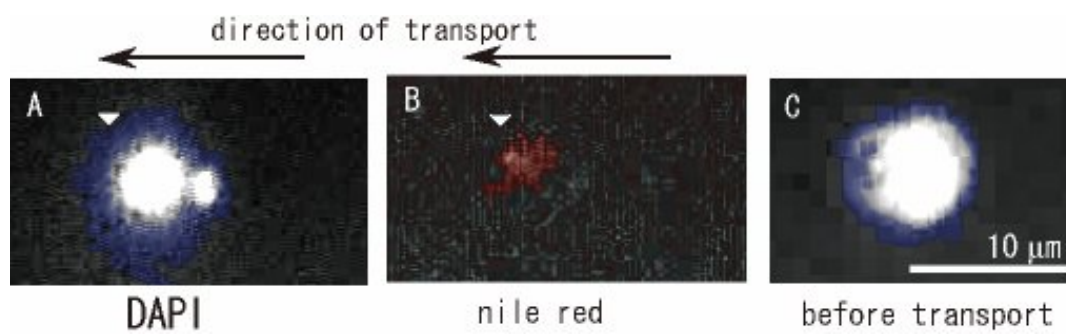
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