Molecular Stretching of Long DNA in Agarose Gel Using Alternating Current Electric Fields

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ABSTRACT We demonstrate a novel method for stretching a long DNA molecule in agarose gel with alternating current (AC) electric fields. The molecular motion of a long DNA (T4 DNA; 165.6 kb) in agarose gel was studied using fluorescence microscopy. The effects of a wide range of field frequencies, field strengths, and gel concentrations were investigated. Stretching was only observed in the AC field when a frequency of ~10 Hz was used. The maximal length of the stretched DNA had the longest value when a field strength of 200 to 400 V/cm was used. Stretching was not sensitive to a range of agarose gel concentrations from 0.5 to 3%. Together, these experiments indicate that the optimal conditions for stretching long DNA in an AC electric field are a frequency of 10 Hz with a field strength of 200 V/cm and a gel concentration of 1% agarose. Using these conditions, we were able to successfully stretch *Saccharomyces cerevisiae* chromosomal DNA molecules (225–2,200 kb). These results may aid in the development of a novel method to stretch much longer DNA, such as human chromosomal DNA, and may contribute to the analysis of a single chromosomal DNA from a single cell.

INTRODUCTION

Recent developments in whole-cell analysis have made it possible to detect a single-molecule probe in a single living cell (Byassee et al., 2000). Based on optical properties of materials, a combination of fluorescence-image microscopy and capillary electrophoresis can be used to perform cell separation and metabolic cytometry at the single cell level (Krylov et al., 1999; Krylov and Dovichi, 2000). New techniques, such as optical mapping (Schwartz et al., 1993) or fiber-fluorescence in situ hybridization approaches (Wiegant et al. 1992; Fidlerova et al., 1994), have attempted to speed up physical mapping steps. Using the dynamic molecular combing method of total genomic DNA, Michalet et al. (1997) performed direct measurement of deletions involving the TSC2 gene region. Using recently developed microfabrication technology for bioanalytical systems, Hirano et al. (1999) have succeeded in recovering a specific DNA fragment cut from a single DNA molecule by a pulsed N_2 laser. Using a combination of these new techniques, our goal is to establish a method for analyzing specific DNA fragments on specific chromosomal DNA from specific target cells. A critical component of this method is the ability to stretch long DNA in solutions. It is impossible, however, to stretch long DNA in solutions by the dynamic molecular combing method. Therefore, in our previous work (Ueda et al., 1997, 1999), we developed another method to stretch long DNA in polymer solutions using alternating current (AC) electric fields. Based on this stretching method, Oana et al. (1999) demonstrated the visualization of restriction mapping in solution at the level of a single λ DNA molecule. To use the mapping technique for longer chromosomal DNA, we have developed a DNA stretching method using agarose gel instead of polymer solutions. In this paper, we investigate the experimental conditions of stretching long DNA in agarose gel under AC electric fields and discuss the mechanism of stretching long DNA in polymer solutions and in agarose gel, based on a simple tube model (Doi and Edwards, 1986). Stretching long DNA in agarose gel can potentially provide a powerful tool for applications like fiber-fluorescence in situ hybridization of post-pulsed field gel electrophoresis.

MATERIALS AND METHODS

Experimental apparatus

An electrophoretic miniature cell on a microscope (Fig. 1) was used for direct observation of DNA motions. Two platinum (Pt)-electrodes were placed with the distance of 1 or 0.5 cm from each other in the miniature cell. Sinusoidal electric fields generated by a multifunction synthesizer (model number 1942, NF Corporation, Yokohama, Japan) were applied between the electrodes through a high-speed power amplifier bipolar power supply (model number 4020, NF Corporation). An inverted fluorescence microscope (Axiovert 135TV, Carl Zeiss, Tokyo, Japan) equipped with a 100× oil-immersion lens (Plan-Neofluar, numerical aperture = 1.3) and a high pressure Mercury lamp were used to visualize the cell. The fluorescence images were observed with a SIT (silicon-intensified target) camera (model number C2400-08, Hamamatsu Photonics, Hamamatsu, Japan) and an EB (electron bombardment)-CCD camera (model number C7190-21, Hamamatsu Photonics, Hamamatsu, Japan). The DNA images and the voltages of the electric field were recorded directly on the computer by an image-capture board (Himawari and Wave-in 32, Library, Inc., Tokyo, Japan). The maximal length and the center of mass of DNA molecules were calculated by image processing software (Cosmos32, Library, Inc.).

Materials

T4 DNA (165.6 kb, Nippon Gene, Tokyo, Japan), stained with a fluorescent dye, YOYO-1 (Molecular Probes, Inc., Eugene, OR), was injected into

Received for publication 7 May 2001 and in final form 20 September 2001.

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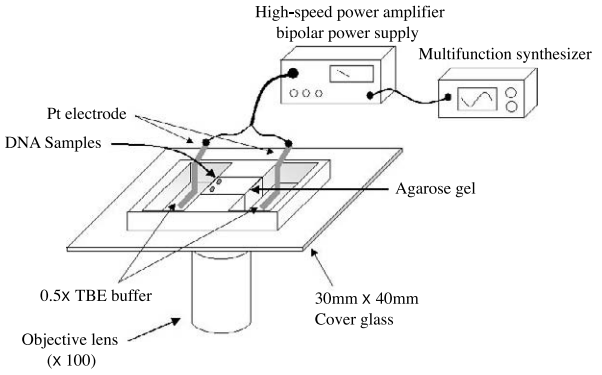

FIGURE 1 Schematic diagram of experimental setup. Two Pt-electrodes were placed with the distance of 1 or 0.5 cm in the miniature cell. Sinusoidal electric fields generated by a multifunction synthesizer were applied between the electrodes through a high-speed power amplifier bipolar power supply.

agarose gel (Agarose NA, Amersham Pharmacia Biotech, Buckinghamshire, UK) in 0.5 × TBE buffer (45 mM Tris-borate, 1.25 mM EDTA, pH 8.0) under steady electric fields (5 V/cm) for a few minutes. To remove oxygen before DNA observations, the agarose gel was exposed to a solution containing 4% (v/v) 2-mercaptoethanol, 2.3 mg/ml glucose, 0.1 mg/ml glucose oxidase, and 0.018 mg/ml catalase. Otherwise, samples were prepared as described in previous work (Ueda et al., 1997, 1999).

Saccharomyces cerevisiae chromosomal DNA (225–2,200 kb, Bio-Rad Laboratories, Hercules, CA) was purchased as a gel plug. The gel was cut into small pieces (~3 × 2 × 1 mm) with a razor blade and stored at 4°C for 1 week in 0.5 × TBE buffer containing 0.1 μM YOYO-1 and 4% (v/v) 2-mercaptoethanol (Gurrieri and Bustamante, 1997). The gel pieces were then incubated in a melted agarose solution on a mini-electrophoretic cell. After the agarose solidified, the gel was exposed to a solution containing 4% (v/v) 2-mercaptoethanol, 2.3 mg/ml glucose, 0.1 mg/ml glucose oxidase, and 0.018 mg/ml catalase identical to that used for T4 DNA. The chromosomal DNA was injected into the working agarose gel from the gel plug under steady electric fields (1–2 V/cm) for 1 h. The buffer solutions in the reservoirs were then replaced with fresh buffer solutions, and DNA stretching experiments were started.

RESULTS

Stretching process

Fig. 2 (*A* and *B*) show fluorescence images of T4 DNA (165.6 kb) in 1% agarose gel without an electric field and in an AC electric field (sinusoidal AC electric field was applied in the horizontal direction), respectively. This figure shows that long DNA can be stretched with AC electric fields in a gel system as well as in polymer solutions. Fig. 3 *A* shows the fluorescence images of stretching T4 DNA, where an AC field (10 Hz, 200 V/cm) is applied at time 0 s. Fig. 3 *B* shows the change in the maximal length R_l of the DNA. As shown in Fig. 3 *B*, R_l increases gradually and reaches an asymptotic limit after ~20 s. In this case, the value of R_l fluctuates ~40 μm, whereas the native contour length of T4 DNA is ~55 μm. Note that due to the heterogeneity of the pore size in agarose gel (Zimm, 1991), DNA is occasionally trapped in an intermediate state at ~10 s (Fig. 3 *B*). During the stretching process in agarose gel, DNA can be repeatedly trapped in an intermediate state. After several trials, the DNA could usually find an effective path in the agarose gel and reach the asymptotic limit of the maximal length. A few molecules, however, were never stretched in gel systems under AC fields. After switching AC fields to steady fields, these molecules still did not move and were irreversibly trapped in gel fibers (Viovy et al., 1992). This irreversible trapping is never observed in polymer solutions.

External frequency dependence

Fig. 4 shows the average maximal length $\langle R_l \rangle$ as a function of the frequency of the electric field. In Fig. 4, closed circles indicate the maximal length of $\langle R_l \rangle$ time averaged for 10 s with one to five different molecules in each frequency.

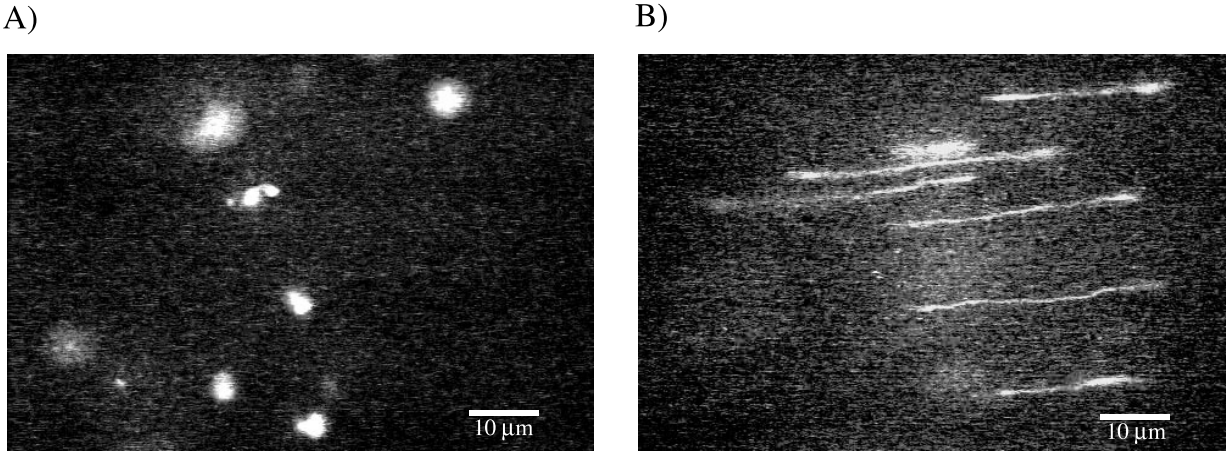

FIGURE 2 Fluorescence images of T4 DNA in 1% agarose gel. (*A*) In the absence of an external electric field. (*B*) Under a sinusoidal AC electric field (200 V/cm, 9 Hz). The electric field was applied in the horizontal direction.

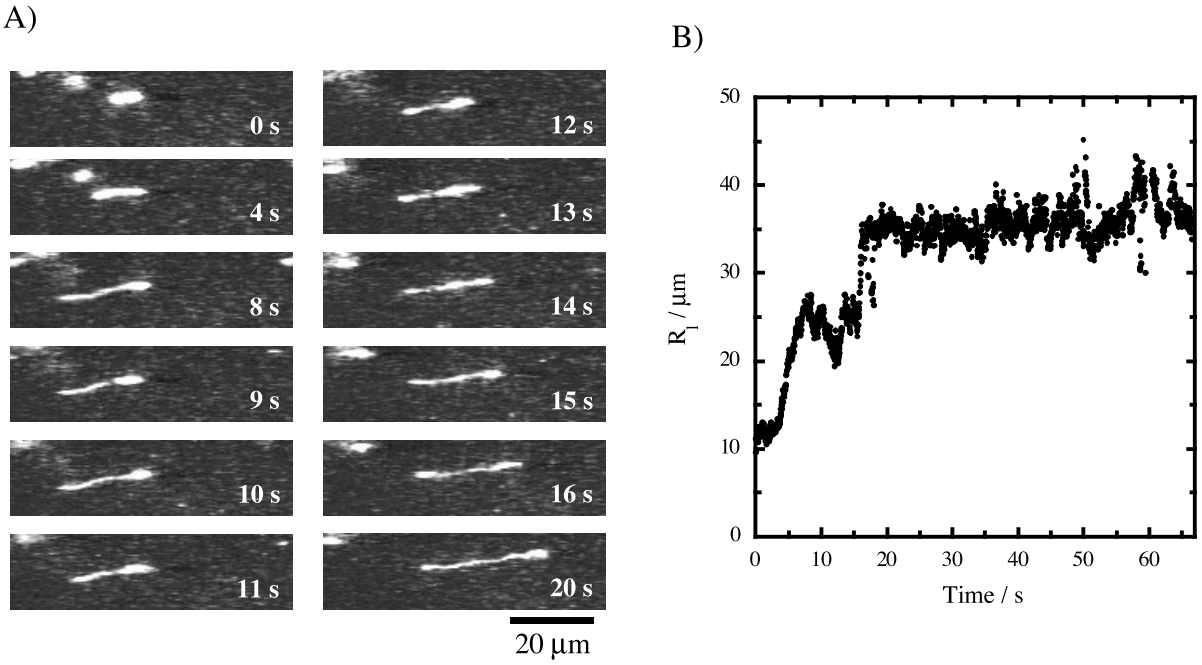

FIGURE 3 Stretching process of T4 DNA in 1% agarose gel under an AC electric field. (*A*) Sequential fluorescence images of T4 DNA stained with YOYO1. At 0 s, the electric field (200 V/cm, 10 Hz) was applied to a random coiled DNA in the horizontal direction. (*B*) Plots of the maximal length R_l of the T4 DNA (illustrated in *A*) after application of the AC electric field.

Open circles indicate the maximal length of $\langle R_l \rangle$ time averaged for 2 s (0.1 Hz) and 7 s (0.5 Hz). Time averages for 2 and 7 s were used because the migration distances of the DNA in these conditions are larger than the observed view area. DNA molecules that were irreversibly trapped in gel fibers were not counted. The horizontal, dashed line indicates the equilibrium value of T4 DNA in a random coil state without an external field.

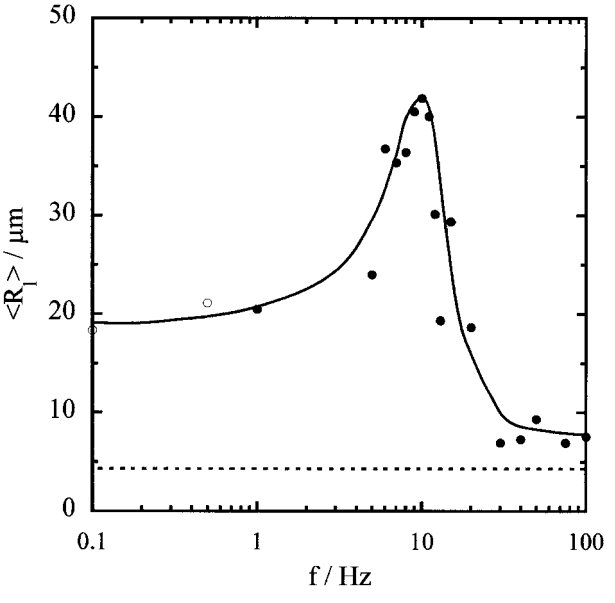

FIGURE 4 Average maximal length $\langle R_l \rangle$ of T4 DNA versus the frequency f of the electric field. Maximal length R_l of each molecule was time averaged for 10 s (●) and for ~7 s (○) with one to five different molecules in each frequency. The horizontal, dashed line indicates the equilibrium value of T4 DNA in a random coil state without an external electric field.

Although we cannot classify the frequency into distinct regions (e.g., linear motion, antiresonance, stretching, orienting) as is possible in polymer solutions (Ueda et al., 1999), it is possible to characterize the frequency regions in Fig. 4 by using time courses of the maximal length R_l and the x component of center of mass G_x during DNA motion. When the fluctuation level of G_x is less than the time-averaged R_l, DNA is expected to undergo tube-like motion in agarose gel. On the other hand, when the fluctuation level of G_x is greater than R_l, DNA performs more complex motions (except for the linear motion of DNA in concentrated polymer solutions (Ueda et al., 1999)).

Fig. 5 shows the time courses of R_l and G_x for the frequencies of 1, 10, and 100 Hz (200 V/cm), respectively. In these figures, G_x is rescaled so that the average value corresponds to zero. At 1 Hz, both R_l and G_x oscillate around the average values. G_x simply correlates with the external field, whereas R_l fluctuates in a more complex manner as shown in Fig. 5 *A*. At 10 Hz, both R_l and G_x are constant, as shown in Fig. 5 *B*. DNA is stretched and does not change position. At 100 Hz, both R_l and G_x are constant as shown in Fig. 5 *C*. In this condition, DNA is oriented but stretched. Although at low frequencies (~0.1 Hz) linear motion of DNA does not occur in agarose gel (Ueda et al., 1999), the response of DNA to other external frequencies is

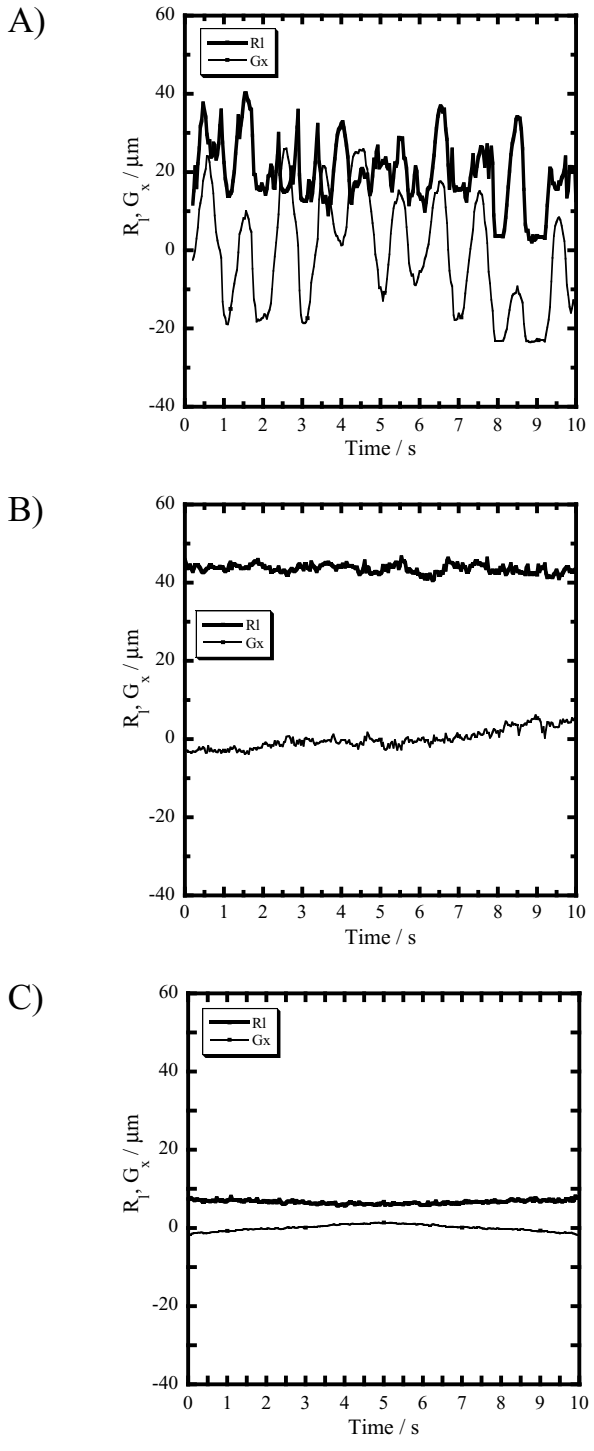

FIGURE 5 Time evolution of maximal length R_l and x component of center of mass G_x of T4 DNA in 200 V/cm under different external frequencies. The thick lines represent R_l and the thin lines represent G_x. (*A*) 1 Hz; (*B*) 10 Hz; (*C*) 100 Hz.

similar to that in polymer solutions. It should be noted that the average maximal length $\langle R_l \rangle$ has an apparent maximum ~10 Hz both in agarose gel and in polymer solutions (Ueda et al., 1999). However, the one-half value width of the peak shape in agarose gel is ~10 Hz, smaller than that in polymer solutions.

External field strength dependence

Fig. 6 *A* shows the maximal length R_l and the horizontal component G_x of the center of mass of T4 DNA in different electric field strengths (150, 50, and 250 V/cm). Fig. 6 *B* shows the fluorescence images corresponding to Fig. 6 *A*. The bright spot at the left end of the DNA in Fig. 6 *B* indicates a coiled or bent structure. The coiled structure at the terminal was not appreciably stretched in any field strength.

As shown in Fig. 7, the average maximal length of DNA is nearly proportional to the strength of the external electric field between 50 and 300 V/cm. Between 400 and 600 V/cm, it gradually decreases. The vertical dashed line in Fig. 7 indicates the boundary between stretching and non-stretching regions. We distinguish the high electric field region from the stretching region for the following reason. Fig. 8 shows the time evolution of the maximal length R_l and the horizontal component G_x of the center of mass of T4 DNA in 10 Hz. In the stretching region, both the R_l and the G_x fluctuate only slightly and with no relation to the strength of the external field (Fig. 8, *A* and *B*). The averaged values for 10 s are $R_l = 36.4 \pm 0.9$ (μm) and $G_x = 0.0 \pm 0.7$ (μm) in 50 V/cm, $R_l = 43.6 \pm 1.1$ (μm) and $G_x = 0.0 \pm 2.4$ (μm) in 200 V/cm, respectively. Essentially, both R_l and G_x are constant in the stretching region. The fluctuation (0.7 and 2.4 μm) in G_x is 20 times smaller than the average maximal length (36.4 and 43.6 μm) and does not disturb the stretched conformation of DNA. On the other hand, in the case of 600 V/cm, both R_l and G_x show large fluctuations (Fig. 8 *C*). The average values for 10 s are $R_l = 21.0 \pm 4.4$ (μm) and $G_x = 0.0 \pm 9.9$ (μm), respectively. The standard deviation of G_x (9.9 μm) is large enough to disturb the DNA conformation compared with the average maximal length (21.0 μm). Therefore, there is a qualitative difference between DNA motion in the region under 450 V/cm and in the region over 450 V/cm. In high electric fields (500 and 600 V/cm), DNA molecules move a great distance compared with the maximal length of the DNA. As a result, the conformation of the DNA is always disturbed with cross-points of agarose gel. Thus, it does not move in a tube-like motion and cannot be stretched.

Agarose concentration dependence

Fig. 9 shows the relationship between agarose concentration and the average maximal length of DNA. The average maximal length of DNA is essentially constant within a wide range of agarose concentrations from 0.5 to 3%. The pore size of the gel at these concentrations is 800 to 260 nm (Maaloum et al., 1998). At lower concentrations (<0.5%),

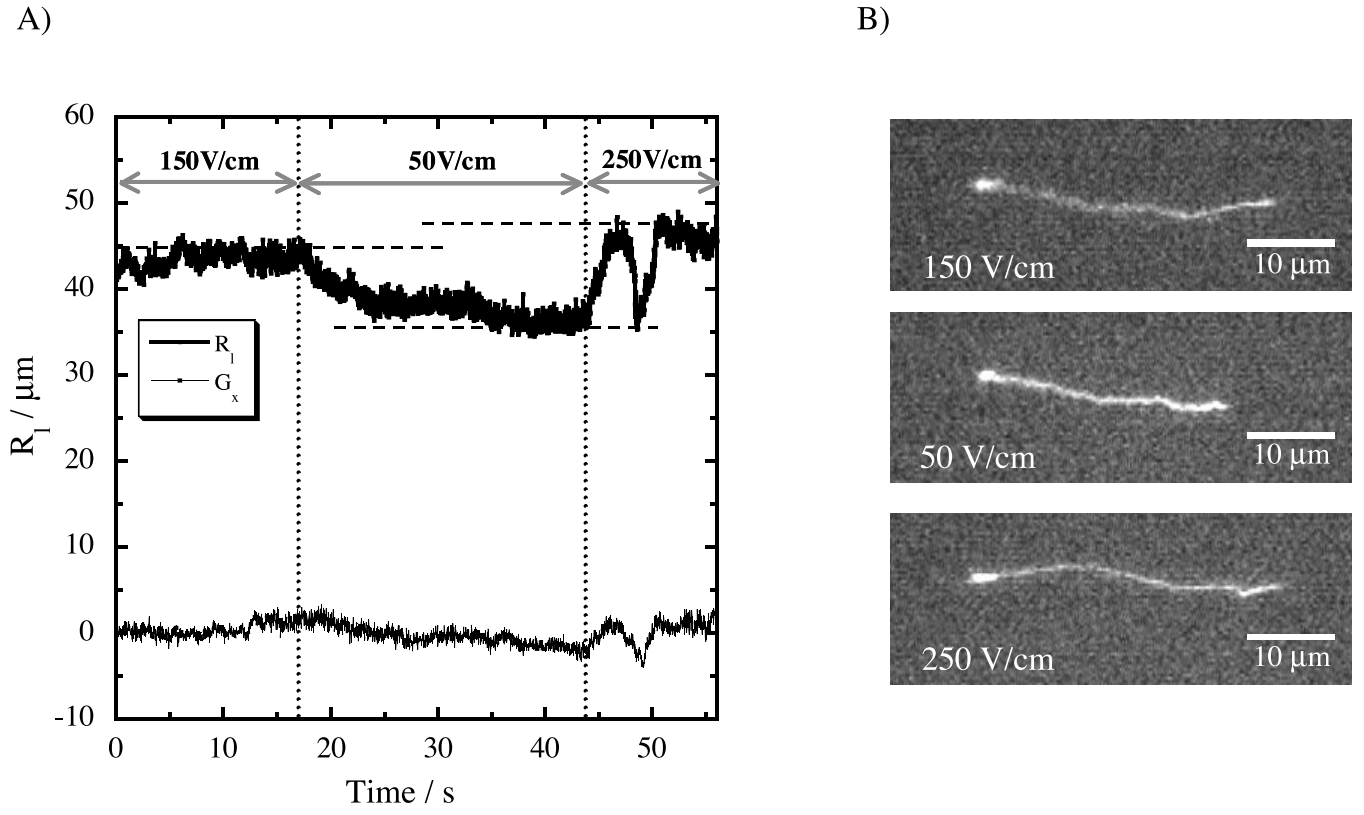

FIGURE 6 Maximal length R_l and x component of center of mass G_x of stretched T4 DNA in different electric field strengths. (*A*) Plots of R_l and G_x under electric fields of 150, 50, and 250 V/cm. (*B*) Fluorescence images of T4 DNA under electric fields of 150, 50, and 250 V/cm.

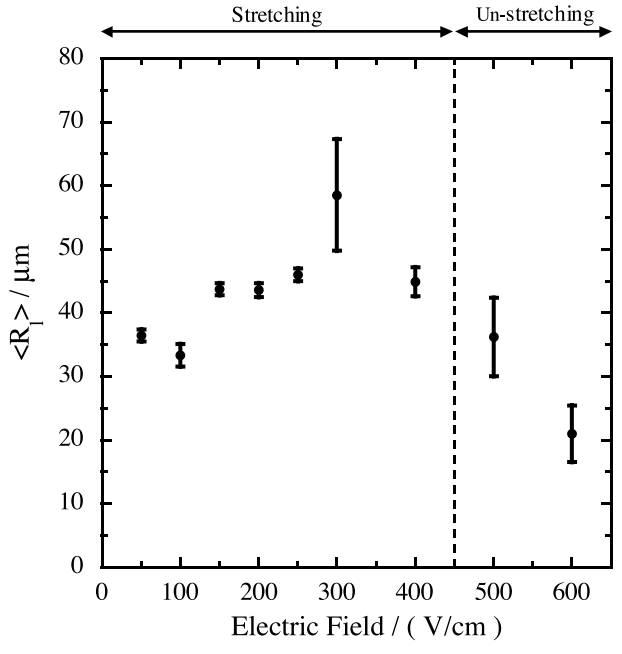

FIGURE 7 Average maximal length $\langle R_l \rangle$ of T4 DNA versus electric field strength. The dashed line indicates the boundary between stretching and nonstretching regions.

the gel is too soft to make up a working gel system for DNA stretching. The dashed line in Fig. 9 at ~3.5 μm shows the average maximal length of T4 DNA obtained in low molecular weight solutions such as 58% saccharose under the same electric field conditions (Ueda et al., 1997). It should be noted that DNA is never stretched in low molecular weight solutions. Therefore, confinement of DNA motion by entanglement with gel fibers is needed for DNA stretching. It means that stretching occurs as a result of tube-like motion. Fig. 9 shows that even a pore size of 800 nm (0.5% agarose gel) is small enough to confine the molecular motion of T4 DNA in a tube-like motion.

Molecular weight of DNA dependence

As discussed above, the best condition for T4 DNA stretching is a 1% agarose gel with a 10-Hz and 200-V/cm electric field. In our previous work, we have shown that the conditions for DNA stretching in polymer solutions are essentially the same for various sizes of DNA (48.5 kb–1 Mb) (Ueda et al., 1999). Similarly, in a gel system, we can stretch various sizes of DNA in the same conditions (1% agarose gel, 10 Hz and 200 V/cm). Fig. 10 *A* shows stretching of *S. cerevisiae* chromosomal DNA. The fluorescence image is composed of three images. The maximal length is

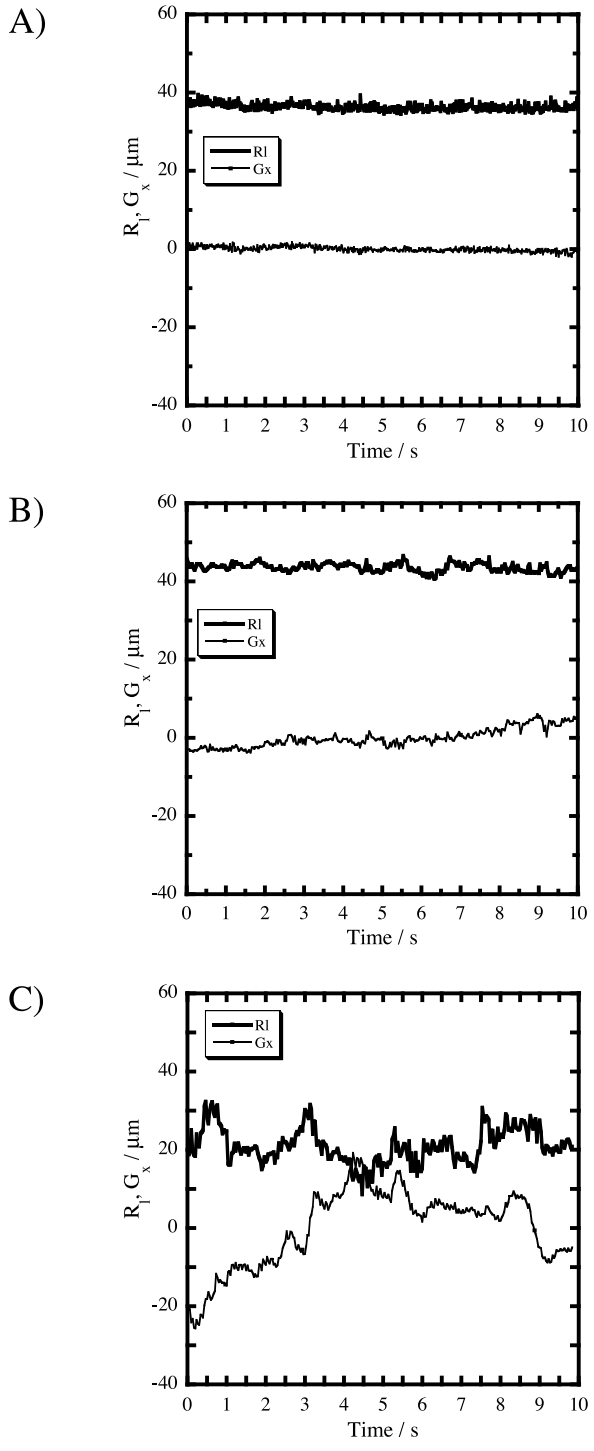

FIGURE 8 Time evolution of maximal length R_l and x component of center of mass G_x of T4 DNA in 1% agarose gel. The thick lines represent R_l, and the thin lines represent G_x. (*A*) 50 V/cm, 10 Hz. (*B*) 200 V/cm, 10 Hz. (*C*) 600 V/cm, 10 Hz.

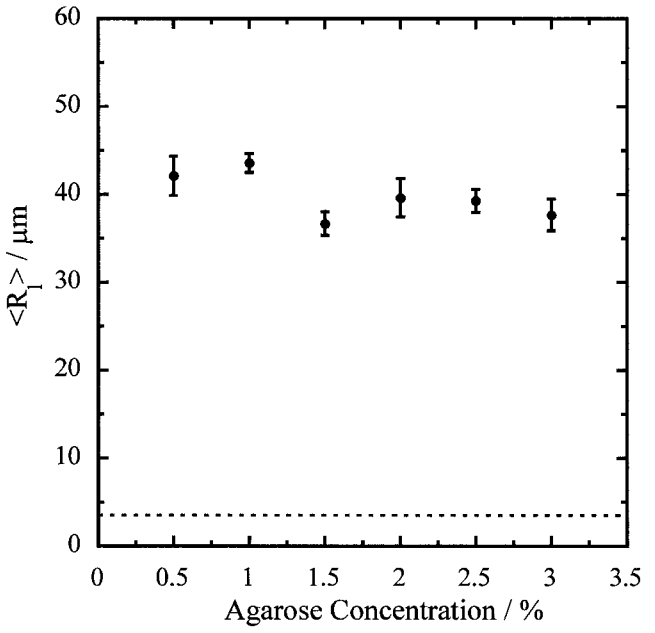

FIGURE 9 Average maximal length $\langle R_l \rangle$ of T4 DNA versus agarose gel concentration. The dashed line shows the average maximal length of T4 DNA in a low molecular weight solution (Ueda et al., 1997).

~100 μm. Fig. 10 *B* shows a trace of the fluorescence image. The arrows labeled "coil" in Fig. 10 *B* indicate coiled parts of DNA, which are not stretched completely in this state. The arrow labeled "bent" in Fig. 10 *B* shows bent structure, which appears occasionally near the end of DNA. The apex of the bent structure did not move during stretching in agarose gel. When DNA is stretched in polymer solutions, asymmetrical motion of the apex characterizes the stretching process (Ueda et al., 1999).

DISCUSSION

Irreversible trapping

Occasionally, a few molecules of DNA were never stretched in gel systems under AC fields. To investigate whether they were irreversibly trapped in gel fibers, we applied steady electric fields (1–100 V/cm) to them. Under these electric fields the DNA molecules did not move, indicating that they were irreversibly trapped in gel fibers. As in the case of crossed-field gel electrophoresis (Viovy et al., 1992), irreversible trapping implies the formation of "tight knots" of DNA around gel fibers. The authors suggested that the trapping threshold N_c scales as $N_c \sim E^{-2}$, in which E is electric strength and N_c is trapping threshold that can be defined arbitrarily. For example, we can choose as N_c the DNA size corresponding to 50% trapping for a few minutes. Therefore, lower electric fields are preferable to prevent irreversible trapping for longer DNA. On the other hand, from the point of view of the trapping mechanism, two dangling ends of DNA are required to orient in the same direction from the hanging point at the gel fiber. However, after stretching out once, DNA adopts a linear conformation in which both ends remain separated. Therefore, trapping

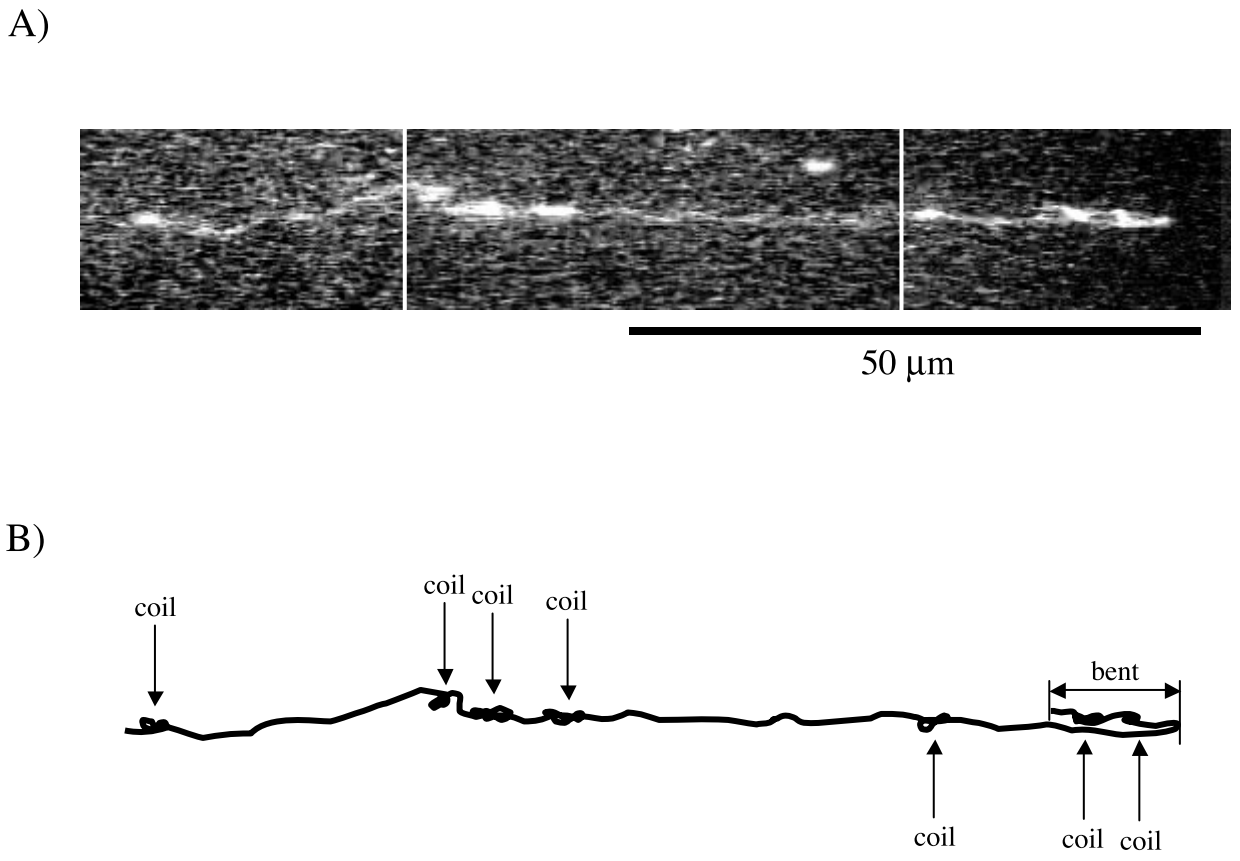

FIGURE 10 Stretching of *S. cerevisiae* chromosomal DNA, consisting of 225 to 2,200 kb fragments under an AC field (200 V/cm, 10 Hz) in 1% agarose gel. (*A*) Fluorescence image of *S. cerevisiae* chromosomal DNA. (*B*) Schematic drawing corresponding to the fluorescence image.

would be expected to occur during the early stage of DNA stretching. To avoid irreversible trapping in stretching DNA, we should apply electric fields to DNA with low field strength (10 V/cm) at the start and gradually increase the field strength up to 200 V/cm.

Mechanism of stretching in polymer solutions and in gels

Optimized electric conditions for stretching DNA in polymer solutions and in gels are essentially the same (200 V/cm, 10 Hz). This fact implies that the mechanisms of DNA stretching are similar in polymer solutions and in gels. In our previous work (Ueda et al., 1999), we proposed an asymmetric kink model to explain DNA stretching in polymer solutions. The model was based on the observation that kinking parts of DNA moved only toward the apex of the kink in polymer solutions. Fig. 11 shows this kind of asymmetric kink motion in a polymer solution. However, in the gel system, we have never observed asymmetric motion of the kink (or bent part). As shown in Fig. 12, in the gel system, a dangling end of DNA progresses toward the direction to which the end points. Assuming that long DNA undergoes tube-like motion in the stretching condition in both polymer solutions and in gels, we explain the difference between DNA motion in these two media as follows.

Fig. 11 shows the asymmetric kink motion in a 7% linear polyacrylamide (PA) solution. Shear flow was applied in the direction of the white arrow shown in the first panel. After DNA was initially stretched in the direction of the arrow, an electric field with frequency of 10 Hz was applied in the horizontal direction. Two major kinks moved in opposite directions, but the apex of the kink could move only in the direction that the apex pointed to. We explain this as follows. Shown in Fig. 13 is the simplest case in which DNA has only one kink. The thick lines represent DNA within a thin tube. When an electric force is applied in the same direction as the apex points (Fig. 13 *A*), both the short and long arms move in the direction of the electric force. Then, the apex moves out of the tube with a distance of R_{out1}. On the other hand, when an electric force is applied in the opposite direction to which the apex points (Fig. 13 *B*), the long arm pulls the shorter arm through. Then, the long arm escapes from the tube with the distance R_{out2}, and the apex is held in the same position. Therefore, the effect of a symmetric external field on the kink of DNA should be asymmetric.

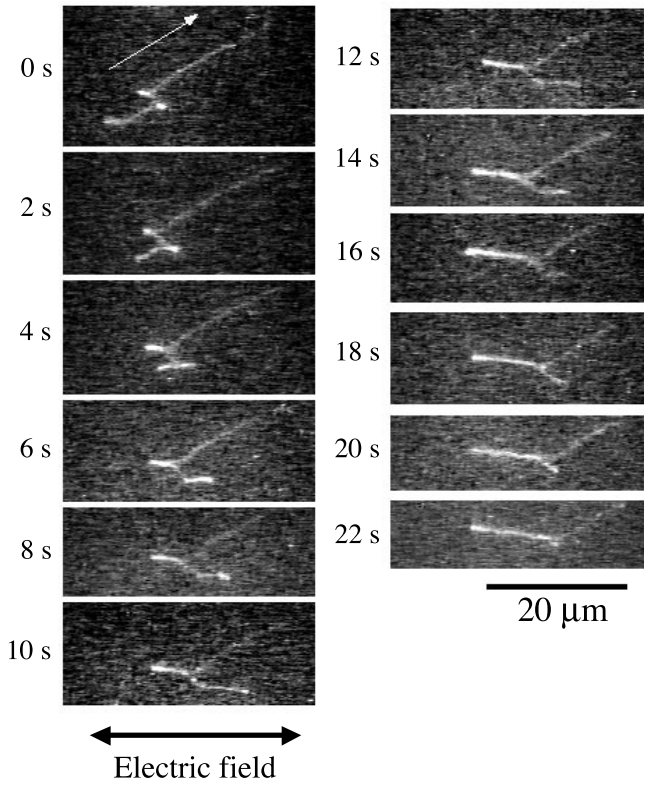

FIGURE 11 Stretching process of T4 DNA in 7% linear PA. To observe the initial stretching process, DNA was first elongated by shear flow in 7% PA. Shear flow was applied in the direction of the white arrow shown in the first picture. An electric field (10 Hz) was applied in the horizontal direction. Each image was taken at 2-s intervals. Two major kinks are shown traveling in opposite directions. This figure is reprinted from our previous paper (Ueda et al., 1999) with permission.

We estimated that 0.1 to 0.6 μm is the minimal distance between kinks that undergo asymmetric motion in 7% PA (Ueda, 1999a). The mesh size of 7% PA is ~2 nm, and the diameter of the tube wrapping DNA in 7% PA is similar (Doi and Edwards, 1986; Wu et al., 1996). Because the diameter of the tube is much less than the minimal distance between the kinks, the kinks perform distinct asymmetric motion in polymer solutions. On the other hand, the pore size in 1% agarose gel is ~500 nm (Maaloum et al., 1998), and the diameter of the tube wrapping DNA is similar. Because the tube is wide, compared with the DNA diameter, the segments of DNA are not constrained by the tube completely and move in a complex manner. As a result, one would expect the asymmetric kink motion to disappear in agarose gel. Fig. 14 shows schematic diagrams representing this type of situation. In the wide tube, DNA adopts a rather coiled conformation, and the DNA segments can move more freely than would be possible in a thin tube. We explain DNA motion in a wide tube with one kink in the following four steps: 1) segments of long and short arms move in the tube toward the electric force; 2) a segment pool is generated at the apex of the tube

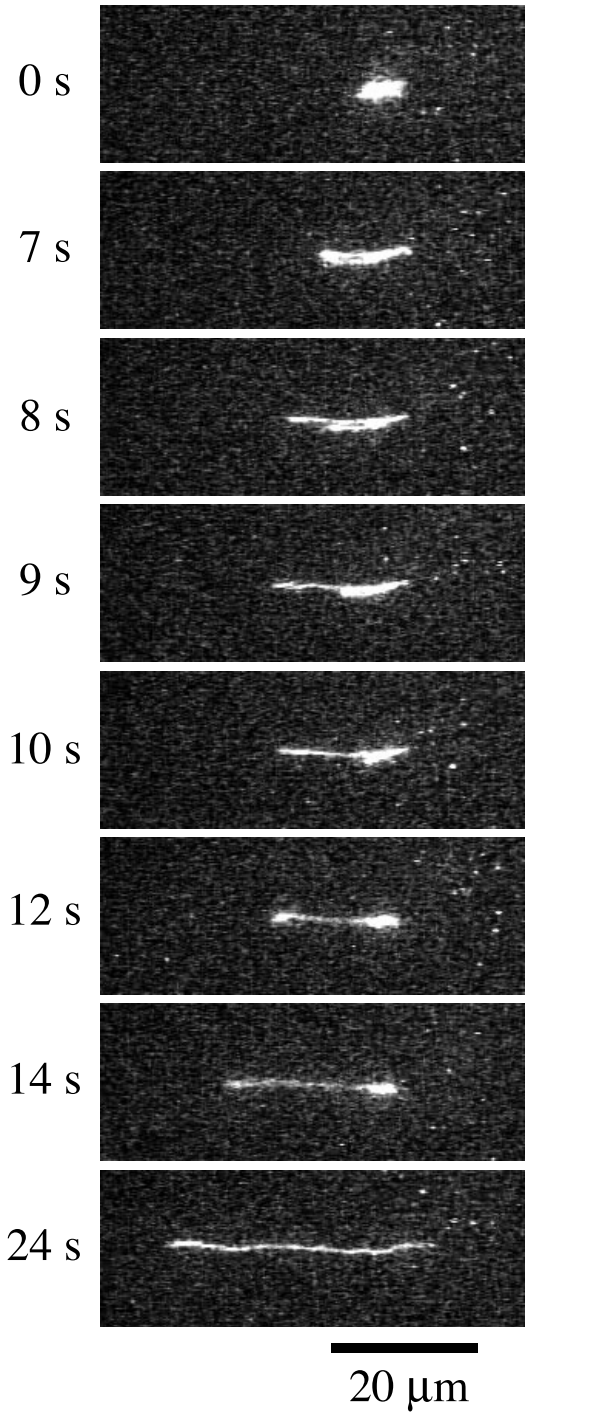

FIGURE 12 Stretching process of T4 DNA in 1% agarose gel. An electric field (10 Hz) was applied in the horizontal direction. The kinked part forms a brighter spot suggesting a segment pool (see Fig. 14). When the DNA is stretched in the gel, dangling ends of DNA progress to elongated, whole DNA chains.

because DNA segments prefer to form coiled conformations in a wide tube rather than overcoming the potential wall of the wide tube; 3) when the electric force is inverted, all of the DNA segments change their direction

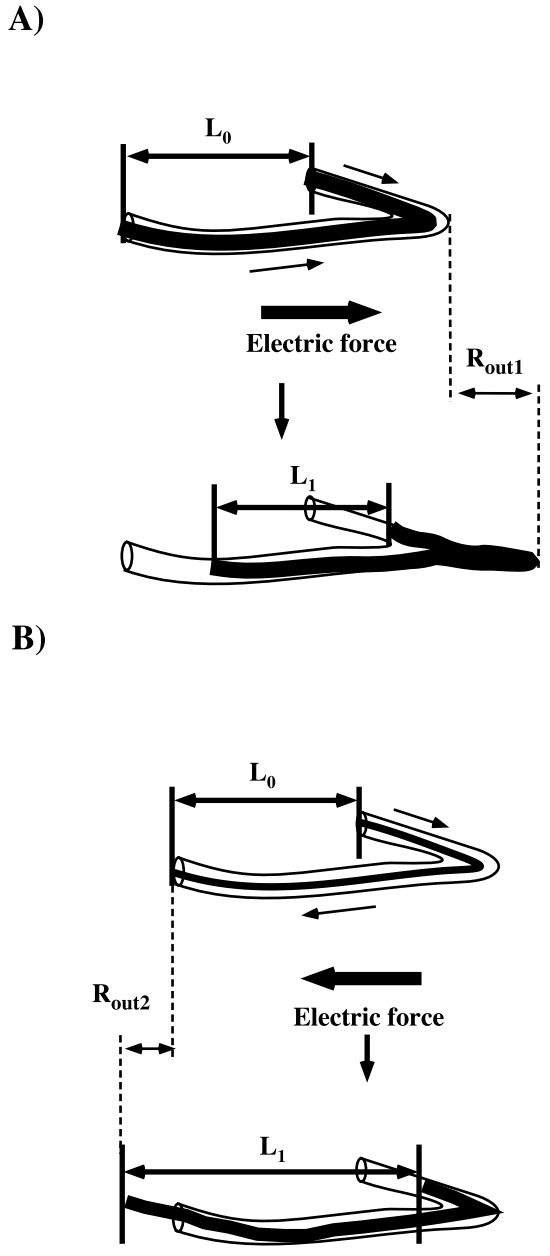

FIGURE 13 Schematic diagram of a kink of DNA in a thin tube. These figures are reprinted from our previous papers (Ueda et al., 1997, 1999; Ueda, 1999b) with permission. (*A*) The apex of the kink points in the direction of the electric force. Both the shorter and longer arms move in the tube in the direction of the electric force. Then, the apex breaks the tube and extends out to a distance defined as R_{out1}. (*B*) The apex of the DNA kink points in the opposite direction of the electric field. The longer arm pulls the shorter arm and escapes from the old tube to a distance defined as R_{out2}. The apex is held in the same position.

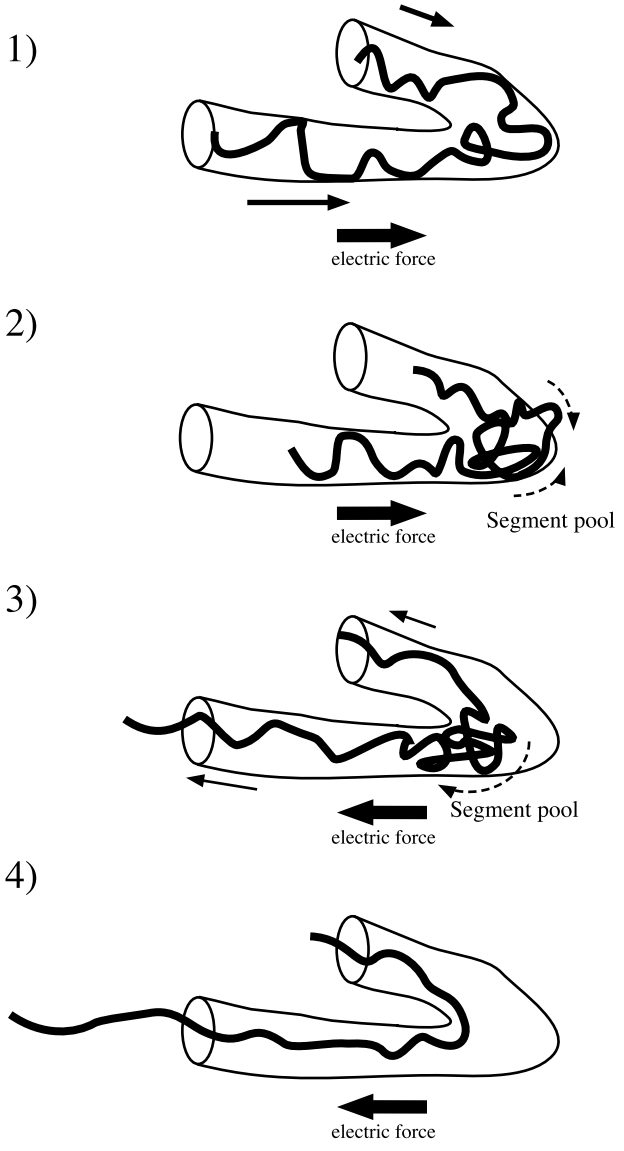

FIGURE 14 Schematic diagram of sequential steps in the DNA stretching process in a wide tube. In a wide tube, DNA has a rather coiled conformation allowing the segments of DNA to move more freely than in a thin tube. DNA motion in a wide tube with one kink can be divided into the following four steps. (*1*) Segments of longer and shorter arms move in the tube toward the electric force. (*2*) Because DNA segments tend to form coiled conformations instead of extending through the wall of the wide tube, a segment pool is generated at the apex of the tube. (*3*) When the electric force is inverted, all of the DNA segments change direction and move toward the electric force. Note that the segment pool can move as a group in a wide tube. In Fig. 14, the segment pool travels in the tube of the longer arm. (*4*) As the segment pool is stretched, a V shape is formed. Then the longer arm is extended even longer.

of motion toward the electric force. Note that the segment pool can move as a group in a wide tube. Fig. 14 shows the segment pool traveling through the long arm of the tube. 4) Finally, as the segment pool is stretched, a V shape is formed. Then the long arm extends outward and becomes much longer.

This explanation is based on our observations of the DNA stretching process (Fig. 12). However, we cannot explain why the segment pool prefers to move toward the long arm at step 3. Although we do not yet understand the complete mechanism, it should be noted that symmetry breaking also occurs at the kinked part in a wide tube. The tube diameter

determines whether the asymmetric kink motion occurs. Therefore, tube-like motion is essential for DNA stretching. We propose that the motion of DNA segments confined in the tube induces the stretching of complete DNA. Furthermore, to explain the optimized electric field condition (10 Hz, 200 V/cm), we should qualitatively analyze the tube-like motion with the mobility of a smaller segment of DNA (for example, persistence length of ~50 nm).

For stretching longer DNA

We pointed out that the optimal stretching conditions (200 V/cm, 10 Hz, 1% agarose in 0.5 × TBE buffer) are effective for longer DNA like *S. cerevisiae* chromosomal DNA (225–2,200 kb). However, it should be noted that in the case of *S. cerevisiae* chromosomal DNA, the leading time for the almost stretched state is longer than that for T4 DNA because longer DNA has a greater probability of being trapped in intermediate states. In fact, Fig. 10 *A* shows such an intermediate state revealing coiled and bent parts along the DNA chain (indicated by arrows in Fig. 10 *B*). To shorten the leading time, we should "anneal" the intermediate states using external perturbations like low frequency electric fields (Ueda, 1999b). For example, the frequency and the strength of the electric field should be adjusted gradually according to the initial and intermediate conformation of DNA. Therefore, we should construct a system in which we can adjust the "annealing" parameters for the conformation of an individual DNA. Using such a system, it will be possible to further investigate the stretching process of long DNA.

In summary, we have proposed a novel method for stretching a DNA molecule in gel with AC electric fields. Using fluorescence microscopy, the effects of a wide range of field frequencies, field strengths, and gel concentrations were investigated. From these experiments we have determined the optimal conditions (200 V/cm, 10 Hz, 1% agarose in 0.5 × TBE buffer) for practical use of the stretching method. Using the conditions optimized for T4 DNA, we succeeded in stretching *S. cerevisiae* chromosomal DNA (225–2,200 kb). The results reported here may aid in the development of a novel method to stretch much longer DNA, such as human chromosomal DNA and may contribute to the analysis of a single chromosomal DNA from a single cell.

The present work was partially supported by Yamada Science Foundation, a grant of Core Research for Evolutional Science and Technology (CREST) from Japan Science and Technology Corporation (JST), a grant from New Energy and Industrial Technology Development Organization (NEDO) of the Ministry of Economy, Trade and Industry, Japan, a grant-in-aid for Scientific Research from the Ministry of Health, Labor and Welfare, Japan, and a grant-in-aid for Scientific Research from the Ministry of Education, Science and Technology, Japan.

Figs. 11 and 13 are reprinted from our previous papers (Ueda et al., 1997, 1999; Ueda, 1999b). Our special thanks are due to The Society of Polymer Science (Japan) for permission to use the photographs and the figures.

One (M.U.) of the authors started this research based on private communications with Ms. Yumi Ito (Hokkaido University). I wish to thank her for the thoughtful and helpful discussions.

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