Purification and staining of intact yeast DNA chromosomes and real-time observation of their migration during gel electrophoresis

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In the past few years, fluorescence microscopy has been used successfully to characterize the motion of intermediate-size DNA molecules (50–500 kbp) during steady- and pulsed-field gel electrophoresis. However, experimental difficulties had prevented the application of this technique to the direct observation of longer DNA chromosomes (1–2 Mbp). In the present study a particular procedure was followed for the purification and staining of chromosomal yeast DNA to protect it from shear forces. Also, a new highly fluorescent DNA-labelling dye, YOYO-1, was employed to improve brightness and contrast. Finally, the motion of such long DNA molecules (1–2 Mbp) was characterized under steady-field electrophoresis conditions. An accurate description of the molecular mechanisms of motion of such long molecules should provide the basis for a detailed analysis of the mechanisms responsible for DNA trapping.

INTRODUCTION

The design of improved methods of DNA size fractionation is one of the most challenging and important tasks in analytical biochemistry. In particular, efforts to obtain complete physical maps of human chromosomes require techniques capable of separating long DNA chromosomes whose dimensions differ in only a small percentage of their total length. Pulsed-field gel electrophoresis techniques are successfully applied to resolve intact chromosomal DNA molecules ranging in size between 50 and approx. 2000 kbp (0.05–2 Mbp) in size [1–5]. Usually, sharp bands are obtained and separation of such molecules is relatively fast, independently of the particular method used [6–12]. However, resolution of molecules larger than 2 Mbp is more difficult because these molecules are extremely sensitive to the intensity of the electric field [13–17]. Chromosomes of a few Mbp in size can fail to enter the gel, remaining trapped in the loading well when high-strength fields are employed, or can also become permanently immobilized (trapped) after travelling various distances into a gel, leading to band smearing [15–17]. Fo far, DNA trapping remains a practical barrier to high-resolution electrophoretic separation above a few Mbp.

In this context, the direct observation of the electrophoretic behaviour of very long DNA molecules and an accurate description of their molecular mechanisms of motion will provide the basis for a detailed analysis of the mechanisms responsible for DNA trapping. In previous work, fluorescence microscopy has been used successfully to investigate the dynamics of the motion of intermediate-size DNA molecules (50–500 kbp) during steady- and pulsed-field gel electrophoresis [18–23]. However, technical difficulties have limited the application of this technique to image longer DNA chromosomes (1–2 Mbp). First, long DNA molecules easily break into shorter fragments by mechanical shearing. The shear occurs during the preparation of the samples or the assembly of the microscope slide. Also, it has been very difficult to obtain high-quality images with standard fluorescent dyes like ethidium bromide or Acridine Orange [18–23]. In fact, the binding and spectroscopic characteristics of these dyes are such that relatively high DNA and dye concentrations must be employed. This leads to a high fluorescence background, owing to large amounts of free dye in the gel, and to confusion between different DNA molecules.

In the present study a particular procedure was followed for the preparation and staining of chromosomal yeast DNA, and special care was taken during the assembly of a microscope microchamber built specifically to run these experiments. This procedure allowed us to protect the DNA from shear forces and therefore to observe the dynamic behaviour of intact chromosomes. Also, to optimize the brightness and contrast of the fluorescence images, a new, high-affinity DNA-labelling fluorescent dye, 1,1-(4,4,7,7-tetramethyl-4,7-diazaundecamethylene)-bis{4-[3-methyl-2,3-dihydro(benzo-1,3-oxazole)-2-methylidene]}quinolinium tetraiodide (YOYO-1), was employed [24–28]. Finally, the motion of such long DNA molecules (1–2 Mbp) under steady-field electrophoresis conditions was characterized, and the effect on their motion of large voids present in the agarose gel, which become visible under the microscope when 'filled' with fluorescently labelled DNA, will be described.

MATERIALS AND METHODS

Yeast DNA staining with ethidium bromide

Preliminary microscopic electrophoresis experiments were performed with commercial *Saccharomyces cerevisiae* chromosomes (New England Biolabs) stained with ethidium bromide. The 16 yeast chromosomes (ranging in size between 200 kbp and approx. 2 Mbp, as indicated in Table 1) were separated by 120° pulsed-field gel electrophoresis. After separation, the different bands were cut with a razor blade and the gel slices, containing the different chromosomes, were stored in 0.5 × TBE buffer (45 mM Tris/45 mM borate/1 mM EDTA) and refrigerated at 5 °C.

Abbreviation used: YOYO-1, 1,1-(4,4,7,7-tetramethyl-4,7-diazaundecamethylene)-bis{4-[3-methyl-2,3-dihydro(benzo-1,3-oxazole)-2-methylidene]}-quinolinium tetraiodide.

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Table 1 Sizes of yeast DNA chromosomes employed for microscopic electrophoresis experiments

Chromosome	Size (kbp)
XII	1900
IV	1640
VII	1120
XV	1100
XVI	945
XIII	915
II	815
XIV	785
X	745
XI	680
V	610
VIII	555
IX	450
III	375
VI	295
I	225

Stock solutions of ethidium bromide (13 mM) were prepared by dissolving the solid dye in distilled water. The concentration was obtained by measuring the absorbance in water, taking ϵ_{481} as 5448 $M^{-1} \cdot cm^{-1}$. Staining of the yeast DNA samples was accomplished by incubating overnight a small part of the agarose gel slice containing the chromosome of interest with a large volume (typically 100 ml) of a solution of ethidium bromide (1.3 μM) in 0.5 × TBE. The DNA-containing gel was then melted at 60 °C in 1 ml of 1 % (w/v) ME SeaKem agarose gel containing 1.3 μM ethidium bromide and 2 % (v/v) 2-mercaptoethanol. Finally, 10 μl of this mixture was put on top of a microscope slide (where two parallel copper electrodes had previously been attached) pre-heated to 50 °C. A 22 mm × 22 mm coverslip was placed on top and sealed on two sides with fingernail polish. The gel was allowed to solidify for approx. 15 min at room temperature. The electrical connection was completed by dropping molten gel over the electrodes and 0.5 × TBE buffer was only occasionally added to keep it wet.

Purification of yeast DNA chromosomes and staining with YOYO-1

Yeast DNA chromosomes were first purified by using a protocol that was a modification of a procedure originally developed by Carle and Olson [29]. Yeast cells of *S. cerevisiae* (strain SF8381Dα, kindly donated by Professor Thomas Stevens, Institute of Molecular Biology, University of Oregon, Eugene, OR, U.S.A.) were grown overnight in 400 ml of YPD (1 % yeast extract, 2 % peptone, 2 % glucose) broth at 37 °C with continuous shaking. The resulting suspension was centrifuged, the supernatant discarded and the cells (approx. 3 ml) were washed with 10 ml of 0.05 M EDTA, pH 7.5, and centrifuged again. SCE buffer [1.0 M sorbitol/0.1 M sodium citrate/0.06 M EDTA (pH 7)] (1 ml) were mixed with 5 ml of molten 2 % (w/v) LMP agarose gel (FMC Bioproducts) and added to the tube containing the yeast cells kept at 50 °C. This suspension was loaded into a custom-made sprayer in which a stream of hot nitrogen was used to create very small (between 20 and 100 μm) beads of agarose containing several yeast cells each. The liquid agarose beads were allowed to solidify while still in the air and collected in 100 ml of SCE buffer. On centrifugation of this suspension approx. 2 ml of beads were collected and mixed with 3 ml of SCE buffer containing 20 mg of Zymolyase-20T (Seikagaku Corporation) and 2 ml of 0.5 M EDTA, pH 9.0. 2-Mercaptoethanol was added to a final concentration of 5 % (v/v). The tube was then incubated at 37 °C for 4 h. The supernatant was discarded and the agarose beads were resuspended in a solution prepared by mixing 4.5 ml of 0.5 M EDTA, pH 9.0, 0.5 ml of 0.1 M Tris buffer, pH 8.0, and 10 mg of Proteinase K (Gibco/BRL). Sodium *N*-laurylsarcosinate was added to a final concentration of 1 % (w/v). The tube was then incubated at 50 °C for 4 h. The solution was discarded and after the beads had been washed with 10 ml of 0.5 M EDTA, pH 9.0, they were stored in 2 ml of the same solution at 4 °C.

Approx. 50 μl of the suspension of these treated beads was then loaded into the side wells of a low-endosmosis agarose gel. A suspension of beads at 100-fold dilution was loaded into a central long well. The 16 yeast chromosomes were separated by 120 ° pulsed-field gel electrophoresis at 5 V/cm for 16 h, with pulse time increasing linearly between 80 and 180 s. The side lanes were then cut and stained in 0.5 × TBE containing 1.3 μM ethidium bromide. Individual bands in the wide central lane were then sliced and stored in 0.5 × TBE.

Staining of single DNA chromosomes was accomplished by incubating for about a week a small part of the agarose gel slice containing the chromosome of interest with a large volume (typically 100 ml) of a solution of YOYO-1 (0.1 μM) in 0.5 × TBE. Before use, a slice of this agarose containing the labelled DNA was destained for 1 h in 0.5 × TBE containing 4 % (v/v) 2-mercaptoethanol. The agarose slice was then placed into a custom-made electrophoresis microchamber, inside a glove box under a nitrogen atmosphere. Molten deoxygenated 1 % (w/v) FL agarose gel (FMC Bioproducts), also containing 4 % 2-mercaptoethanol, was poured around the slice. The gel was allowed to solidify for approx. 30 min and deoxygenated 0.5 × TBE buffer was added to the microchamber to complete the electrical connection. Finally the microchamber was sealed to minimize the diffusion of oxygen. A very low steady field of 1–2 V/cm was applied for approx. 2 h to pull the DNA out of the slice into the bulk gel.

The microscope–camera system used to observe the DNA molecules has been described elsewhere [27, 28]. The sample stained with ethidium bromide were irradiated with green light (510–560 nm) from a mercury or xenon lamp source; red wavelengths above 590 nm were selected for observation. When YOYO-1 was used to label the DNA, blue light (450–490 nm) was used for irradiation; green wavelengths above 520 nm were selected for observation.

RESULTS AND DISCUSSION

Preliminary observations of very long DNA molecules

As mentioned above, early microscope experiments with long DNA molecules were only partly successful because of several experimental limitations in the preparation and the staining of the DNA. First, it was very difficult to assemble micro-gels without breaking the DNA into shorter fragments by mechanical shearing. The shear occurs during the preparation of the samples or the assembly of the microscope slide. Also, standard fluorescent dyes such as ethidium bromide or Acridine Orange do not produce a good image contrast, especially when the DNA assumes very elongated conformations. It therefore becomes difficult to follow the whole contour of a very long molecule, expecially when part of it is out of the plane of focus. Moreover, owing to the relatively low binding affinity of these dyes for DNA, high DNA and dye concentrations have been employed, to favour the formation of the DNA–dye complex. This causes

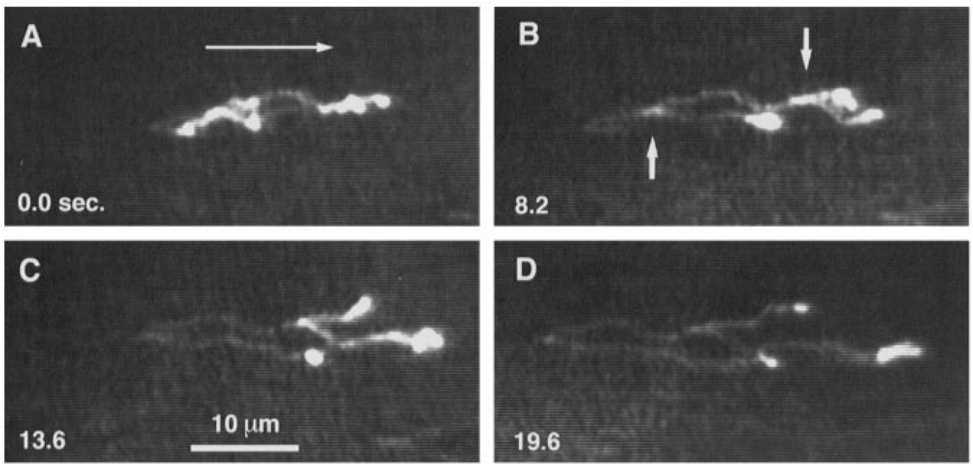

Figure 1 Ethidium bromide-labelled yeast DNA chromosome during steady-field electrophoresis

A time sequence of a yeast DNA chromosome (approx. 1 Mbp) undergoing steady-field electrophoresis is shown. The molecule is moving to the right as indicated by the arrow in (**A**). DNA was stained with ethidium bromide; the electric field was 8 V/cm.

great confusion between different molecules and also a high and heterogeneous fluorescence background.

Preliminary microscopic observations revealed that the motion of very long molecules (approx. 1 Mbp) during electrophoresis is qualitatively different from that of medium-sized molecules (50–500 kbp), as well as being more complex. Figure 1 shows a yeast chromosome (approx. 1 Mbp) stained with ethidium bromide and undergoing steady-field electrophoresis. This sequence illustrates features often found in these long molecules: (1) the leading end of the molecule branches out, forming several kinks that compete with one another to lead the chain (Figure 1B); (2) the molecule often remains entangled (trapped) around a gel fibre, forming a U-shape (Figure 1C); (3) one or both ends can divide into two or more domains, forming U-shapes within U-shapes (Figure 1D).

Imaging long DNA molecules (1–2 Mbp) by fluorescence microscopy

In search for a solution to the various experimental problems encountered during our preliminary experiments we considered recent developments in the DNA purification of yeast chromosomes for pulsed-field electrophoresis experiments [29] as well as searching for new high-affinity fluorescence dyes that would improve brightness and contrast in the imaging of single DNA molecules [24–28].

DNA breakage due to shear forces can be avoided by purifying the DNA from live yeast cells while protected inside spherical beads of regular agarose [29]. These beads are treated first with Zymolase, which creates fractures and holes in the cell walls, and then with a solution containing a detergent such as sodium *N*-laurylsarcosinate (to dissolve the plasma and nuclear membrane), Proteinase K to digest nuclear proteins and EDTA to inhibit any DNase activity. The naked DNA remains localized within the cavity previously occupied by the cell and is thus protected from shear. Then the beads are loaded in the side wells of an agarose gel while a more dilute suspension is loaded into a wide central well (Figure 2A) and the yeast chromosomes are separated by pulsed-field electrophoresis. After separation the side lanes are cut and stained with ethidium bromide (Figure 2A); the different bands in the central lane (containing the unlabelled DNA) are sliced and stored separately. A small part of the agarose gel slice containing the chromosome of interest is stained with YOYO-1 (Figure 2B) and then destained immediately before use (Figure 2C). The agarose slice is then placed into a custom-made electrophoresis microchamber (Figure 2D), built specifically to run these experiments, inside a glove box under a nitrogen atmosphere. A new agarose gel is then constituted around the slice and a low steady field is applied to pull the DNA into the bulk gel (Figure 2E). In this way individual yeast chromosomes of a few Mbp in size can be observed while they move through the gel, and their dynamic behaviour studied under the fluorescence microscope (Figure 2E) in conditions very similar to those used in macroscopic electrophoresis experiments.

During our investigation the contour length of several molecules was measured and found to be in good agreement with their theoretical size. Slight variation from the expected size can be attributed to partial intercalation of YOYO-1 between the DNA base pairs (tending to increase the DNA apparent length) and to the molecular intrinsic entropic elasticity (the molecules never totally stretch to their full contour length) and the zig-zag path that they are constrained to follow around the agarose fibres (tending to decrease the DNA apparent length). These effects usually balance one another and a size very close to the theoretical one is frequently measured. Only a small percentage (15–20%) of broken fragments was observed during our investigation.

In addition, we found during our experiments with gel electrophoresis that DNA molecules labelled with ethidium bromide or Acridine Orange appeared relatively bright under the microscope only if they were in a compact state. However, when the molecules assumed elongated conformations they appeared so dim that it was barely possible to follow their contour, expecially if they were partly out of the plane of focus. The quality of the images was also affected by the high and heterogeneous background due to the molecules out of the plane of focus and to the presence of an excess of free dye in solution. Therefore, while searching for new dyes that would optimize DNA brightness and image contrast, we decided to test a new series of highly fluorescent dyes recently designed and synthesized

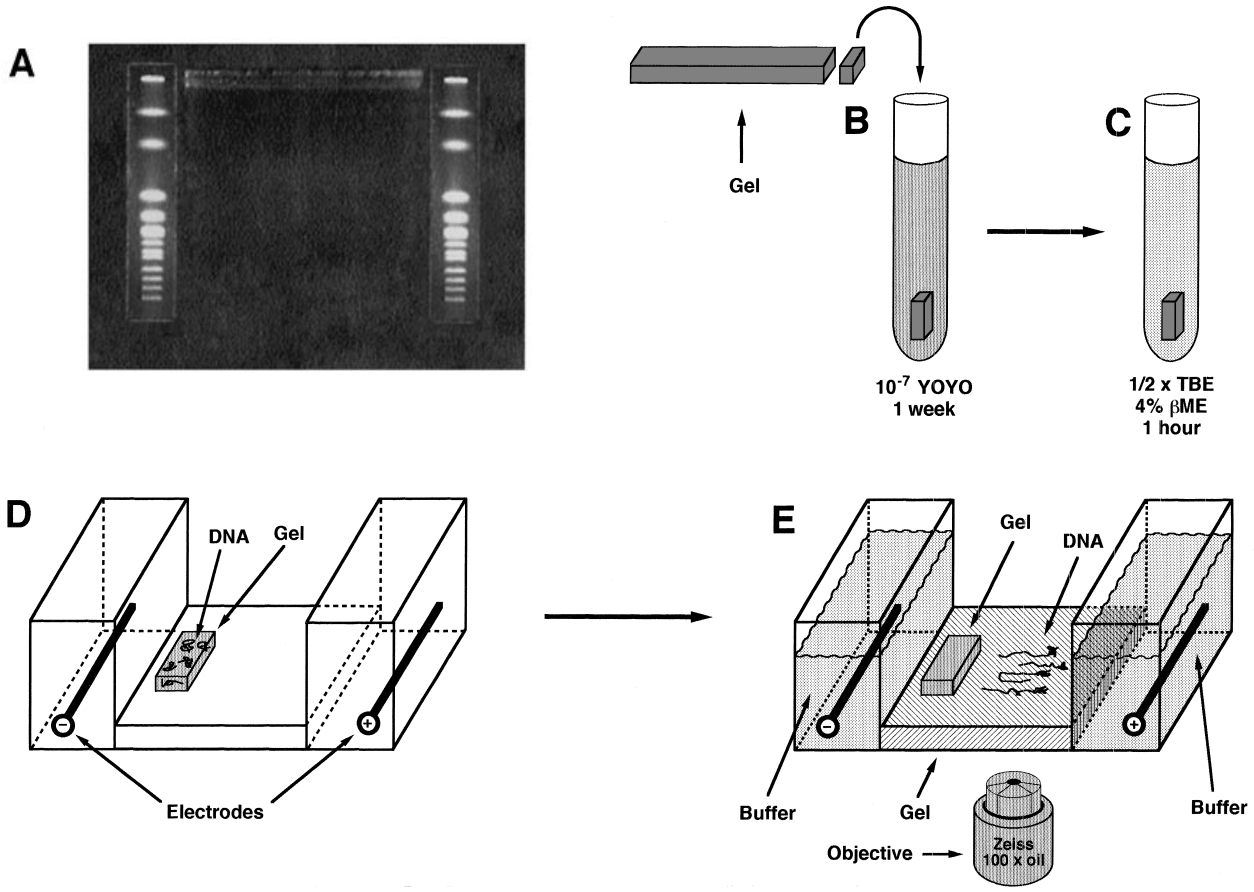

Figure 2 Separation and staining of yeast DNA chromosomes for microscopic electrophoresis experiments

In the procedure, the 16 yeast chromosomes are separated by 120° pulsed-field gel electrophoresis and the side lanes are cut and stained with ethidium bromide (**A**). Then a slice of gel containing the chromosome of interest in the central lane is cut and labelled with YOYO-1 (**B**). Immediately before the experiment, the gel slice is destained (**C**) and the electrophoresis microchamber is assembled (**D**). Finally, the DNA is subjected to electrophoresis into the bulk gel and its dynamic motion observed under the fluorescence microscope (**E**). Abbreviation: βME, 2-mercaptoethanol.

by Molecular Probes. In particular the use of a dimer of Oxazole Yellow, referred as YOYO-1, has markedly improved the quality of our DNA images [27, 28]. In fact, compared with ethidium bromide, YOYO-1 has a much higher binding affinity for DNA, a higher absorption coefficient, a higher fluorescence quantum yield and a greater increase in fluorescence on binding [27, 28]. T2 DNA molecules labelled with YOYO-1 appear very bright, also in conditions of maximum extension (see Figure 3E), against a very dark and homogeneous background.

However, to overcome the inevitable photosensitivity of the DNA–YOYO-1 complex under sustained conditions of high illumination [30], particular care was taken to deoxygenate all the solutions and to assemble the microscope slides under a nitrogen atmosphere in a custom-made glove box. A similar extensive strand breaking had previously been observed to occur with Acridine Orange [31]. DNA breaking becomes even more critical when imaging very long DNA molecules such as intact yeast chromosomes because the probability of a double-stranded break occurring anywhere within the molecule increases with the length of the chain. Therefore neutral-density filters were employed to reduce the excitation intensity and to increase as far as possible the durability of the experiments. This clearly leads to a decrease in the overall brightness of the molecules but allows optimal illumination conditions to be chosen depending on the particular experiment to be performed. With this procedure, in combination with the use of 2-mercaptoethanol, it was possible to minimize strand breaking and to extend the lifetime of the molecules to several minutes.

Also, by using long chromosomes of 1–2 Mbp in size (340–680 μm) it is not possible to image simultaneously the whole contour of a single molecule because it goes beyond the field of view of the microscope (approx. 50 μm) and because different parts of the chain are located at different depths in the gel. Therefore it was necessary to image separately different portions of the same chromosome and combine them together in a collage of 20–30 pictures. An example of the result of such time-consuming but effective elaboration is shown below in the time sequence of Figure 4, where the entire conformation of a 1.6 Mbp yeast chromosome is clearly visible.

Molecular mechanisms of electrophoretic migration of very long DNA molecules (1–2 Mbp)

Having solved the various technical problems mentioned above we have been able to make a further characterization of the dynamic behaviour of such large molecules during steady-field electrophoresis. As shown below, the general qualitative molecular motion of very long DNA molecules (of the order of few

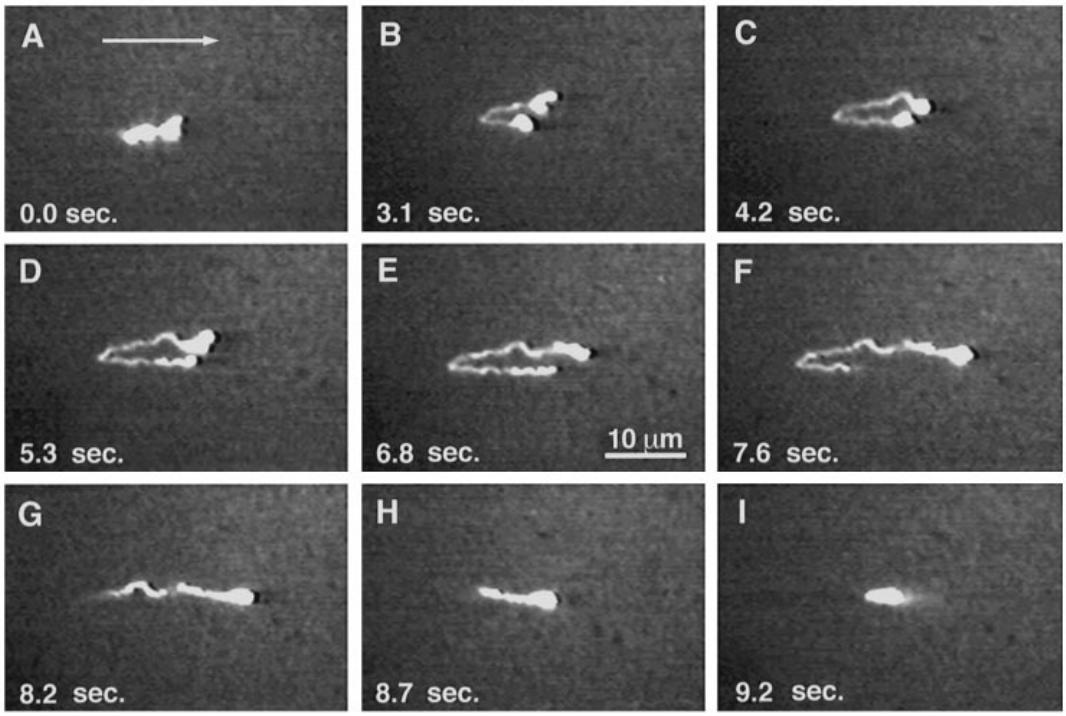

Figure 3 T2 DNA during steady-field electrophoresis

A time sequence of a T2 DNA molecule (164 kbp) labelled with YOYO-1 and undergoing steady-field electrophoresis is shown. The molecule is moving to the right as indicated by the arrow in (**A**). The electric field was 8 V/cm.

Mbp in size) is in several aspects similar to that of shorter ones (50–500 kbp).

As shown in Figure 3, medium-size molecules under the application of a steady electric field migrate through the gel by a cyclic motion of alternating elongated and compact conformations, resembling the motion of a caterpillar. Elongated states form because the molecules remain hooked around gel fibres (Figures 3A and 3B), extending both arms downfield, here named U-shapes (Figures 3C and 3D), and then slipping off, with the longer arm driving the chain (Figures 3E and 3F). The 'tail' then catches up with the 'head' (Figures 3G and 3H) and the molecule starts another 'caterpillar' cycle (Figure 3I). With increasing field strength the molecules become better aligned in the direction of the field and more elongated but never stretch totally to their theoretical contour length. This is due to the intrinsic entropic elasticity of the molecules and also to the zig-zag path through the agarose network. The leading end is usually bunched up, appearing brighter than other parts of the molecule, because it has to find its way through the gel pores. In contrast, the body of the DNA chain is usually stretched because the trailing end often remains wrapped around some gel fibres and, because it is already threaded through pores, just follows along the same path as the 'head'.

As shown in Figure 4, long DNA molecules also migrate through the gel displaying such elastic behaviour and remain hooked around agarose fibres, assuming characteristic U-shape conformations. However, there are some major differences between the molecular dynamics of very long and medium-size DNA molecules that should be highlighted. During each cycle long DNA molecules migrate through the gel for a greater distance, proportional to their length. As a result, it takes longer for molecules of this size to undergo a complete cycle of extension and contraction. Thus the cyclic caterpillar-like motion for such long molecules occurs with a frequency much lower than for shorter ones, depending strongly on the DNA size and electric field strength. At the beginning of a new cycle, the molecule appears in a very compact conformation and, because it is characterized by a high density of DNA, appears very bright under the microscope (Figure 4A). As the molecule moves through the gel, the leading end of the chain starts branching out in many loops or 'hernias' as it simultaneously threads several pores at once, sampling alternative paths through the gel (Figures 4B–4D). Such highly branched structures appear only in very large molecules because to a first approximation the number of branches should scale with the length of the molecules. Here, the main body becomes oriented along the field direction but a large number of doubled-up loops or hernias branch off in several points. For long chromosomes subjected to stronger electric fields, the body of the molecule, as well as the several branches, becomes better aligned in the direction of the field and more elongated (results not shown). As time progresses, the longer branches grow still longer, branching even further into a more ramified structure, at the expense of the shorter branches, which disappear (Figures 4E–4H). Eventually, even the few very long lateral branches disappear, pulled back by the ends of the molecule, to resolve into a fully elongated U-shape (Figure 4I). Because the total force acting on the molecules in a streched U-shape is proportional to its molecular size, very large molecules experience significantly higher tensions and appear more extended during each caterpillar cycle than shorter ones. The molecules seem to behave as though subdivided into several semi-autonomous regions, each advancing independently, until

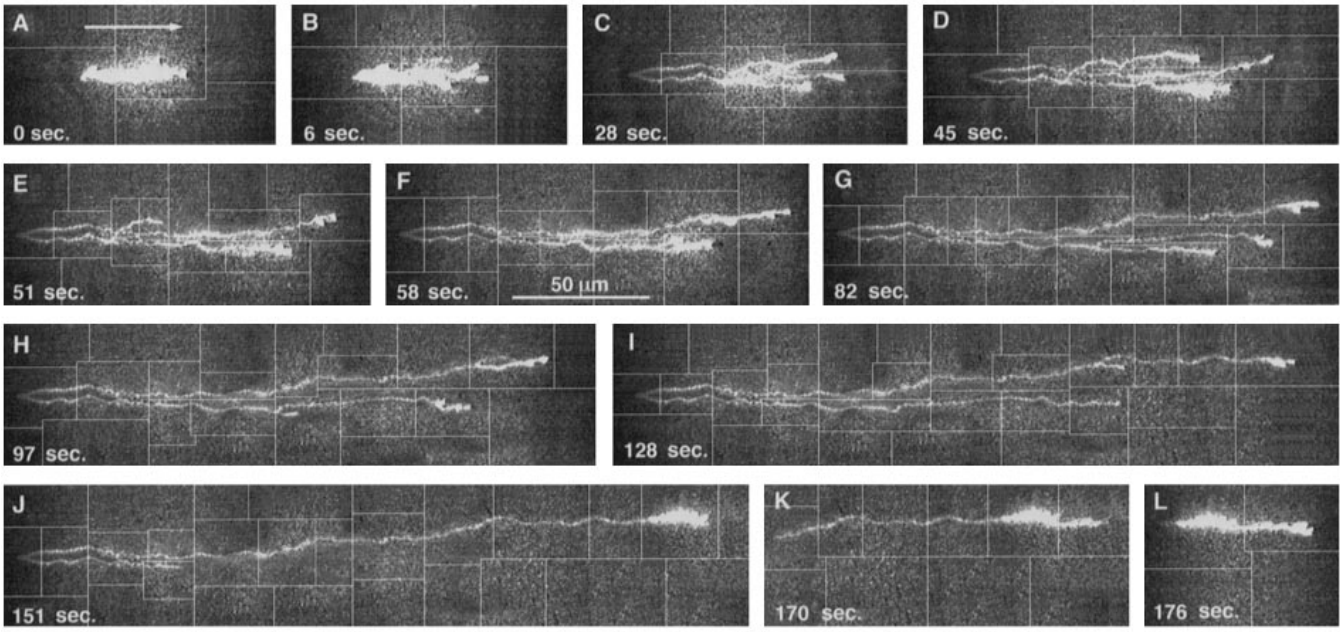

Figure 4 YOYO-labelled yeast DNA chromosome during steady-field electrophoresis

A time sequence of a yeast DNA chromosome (1.6 Mbp) undergoing steady-field electrophoresis is shown. The molecule is moving to the right as indicated by the arrow in (**A**). The DNA was stained with YOYO-1; the electric field was 5 V/cm.

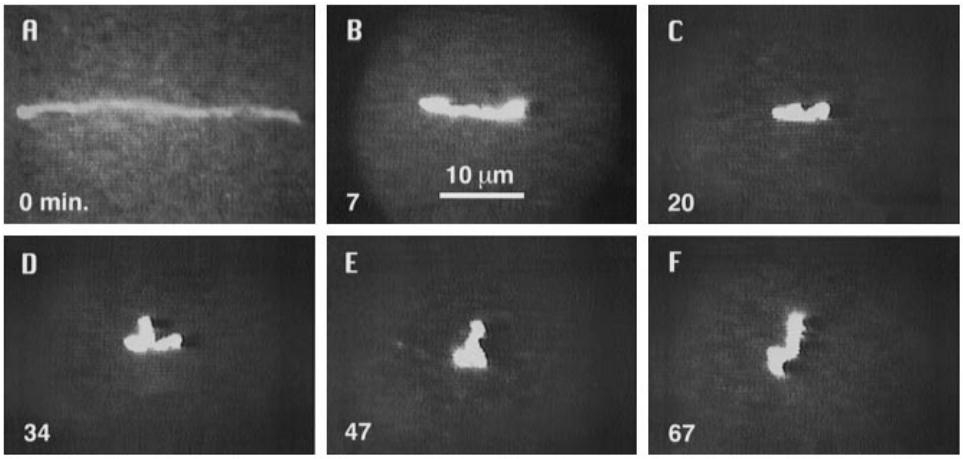

Figure 5 Field-off relaxation of a yeast DNA chromosome

A time sequence of a yeast DNA chromosome (approx. 1 Mbp) is shown for several minutes as it relaxes after the electric field has been turned off. Initially the molecule bunches up in large globs (**B**, **C**), then starts slowly moving in a vertical direction (**D**, **E**) and eventually loses its original alignment completely (**F**). The DNA was stained with ethidium bromide.

the constraint of contour length between these regions is reached. Also, during the undoing of a fully extended U-shape the tail end of the molecule slips off around a gel fibre very slowly (Figure 4J), before collapsing rapidily and trying to catch up with the head (Figures 4K–4L). Meanwhile, the leading end of the chain continues its motion through the gel and a new cycle begins. It soon becomes clear that end-effects play an ever decreasing role in larger DNA molecules, whereas kink formation and branching become dominant. In addition, the mechanism of motion described above (observed under the fluorescence microscope) seems to be very different from that predicted by certain computer simulation studies [32], where it is believed that these long molecules migrate through the gel in a ramified structure, the overall conformation is preserved during the motion and the formation of fully extended U-shapes is considered to be very unlikely.

Field-off relaxation of very long DNA molecules (0.5–2 Mbp)

Zimm [33,34] has suggested a model of the gel in which the matrix is made up of open spaces or 'lakes' connected by narrow 'straits'. By this model, the molecules will tend to fill these lakes

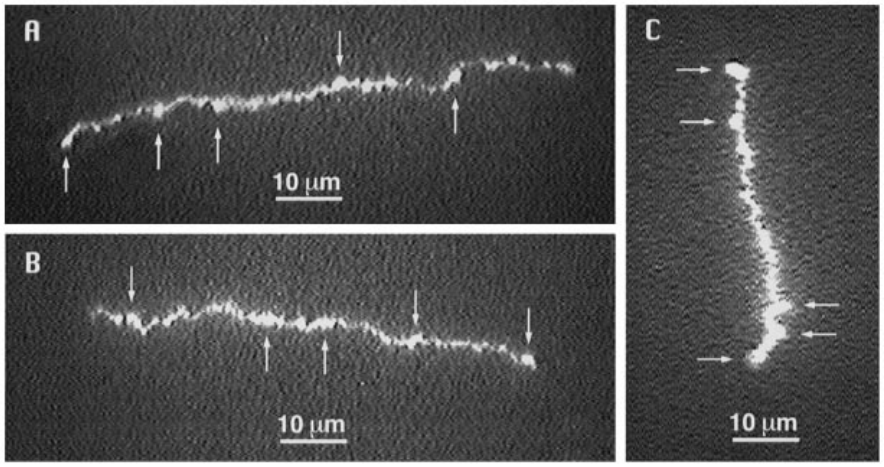

Figure 6 Images of partly relaxed yeast DNA chromosomes

The images were taken after the electric field had been turned off for several hours. The chromosomes (1.9 Mbp) bunch up in large beads (indicated by arrows), suggesting the presence of large cavities in the agarose structure. Before the field was turned off, the molecules in (**A**) and (**B**) had been moving horizontally, whereas the molecule shown in (**C**) had been moving in a vertical direction. The DNA was labelled with YOYO-1.

in a process that is entropically favourable. Indeed, fluorescence microscopy observations suggest that large voids in the agarose might have an important role in the dynamics of DNA molecules moving through agarose gels. As shown in Figure 5, when long DNA molecules (approx. 1 Mbp) are allowed to relax for prolonged periods in the absence of an electric field, they contract very rapidly as soon as the field is turned off (Figures 5A and 5B). Then the molecules keep contracting slowly while bunching up in widenings or 'globs' of few μm in size (Figures 5C and 5D). Eventually, depending on the relaxation time allowed, as well as the length of the molecule, very slowly the DNA can transfer between 'globs' and may even lose all alignment with the initial direction (Figures 5E and 5F). For longer DNA chromosomes (approx. 1.9 Mbp) the relaxation process becomes even slower: the molecules bunch up relatively quickly in wide 'globs' but maintain the direction of alignment even several hours after the electric field has been turned off (Figure 6). The DNA seems to be 'sucked' into these larger-than-average cavities in the gel structure [35]. The stained DNA acts then as a probe for large voids, filling them and thus making them visible. These cavities might correspond to the largest of the 'lakes' proposed by Zimm [33,34] and for this reason could be referred as 'super-lakes'. Such 'super-lakes' might also be major sources for kink or pseudopod formation. The molecules shown in Figure 6 may eventually lose 'memory' of their original alignment but on a much longer time scale.

Linear dichroism decay measurements on macroscopic gels [36] seem to support the results of our observations. There are at least three different relaxation time regimes: a fast one that appears related to the expansion–contraction mechanism of migration, a second regime that corresponds to the accumulation of chain segments in 'super-lakes' in the gel and a third, much slower, process corresponding to the global diffusion of the DNA molecule to a different region of the gel.

Conclusions

Clearly, improved molecular descriptions of the dynamics of very long DNA molecules will provide a way of understanding and eventually preventing molecular trapping; it would therefore be a major step towards the separation of larger molecules than is currently possible [37], possibly up to sizes of human chromosomal DNA (50–250 Mbp). A detailed analysis of the molecular mechanisms responsible for trapping long DNA molecules is currently under way in our laboratory. In addition, progress in understanding agarose properties [38,39] will eventually establish the ultimate limitations of electrophoresis in agarose gels.

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