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Genetics

Pulsed-field electrophoresis: Application of a computer model to the separation of large DNA molecules

(biased reptation theory/DNA chain stretching/yeast chromosomes)

MARC LALANDE[†‡], JAAN NOOLANDI[§], CHANTAL TURMEL[†§], JEAN ROUSSEAU[§¶], AND GARY W. SLATER[§]

[†]National Research Council Canada, Biotechnology Research Institute, 6100 Royalmount Avenue, Montréal, Québec H4P 2R2; [‡]Department of Pathology and Centre for Human Genetics, McGill University, Montréal, Québec; [§]Xerox Research Centre of Canada, 2660 Speakman Drive, Mississauga, Ontario, L5K 2L1; and [¶]Guelph–Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry, University of Waterloo, Waterloo, ON N2L 3G1, Canada

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ABSTRACT **The biased reptation theory has been applied to the pulsed-field electrophoresis of DNA in agarose gels. A computer simulation of the theoretical model that calculates the mobility of large DNA molecules as a function of agarose pore size, DNA chain properties, and electric field conditions has been used to generate mobility curves for DNA molecules in the size range of the larger yeast chromosomes. Pulsed-field electrophoresis experiments resulting in the establishment of an electrophoretic karyotype for yeast, where the mobility of the DNA fragments is a monotonic function of molecular size for the entire size range that is resolved (200–2200 kilobase pairs), has been compared to the theoretical mobility curves generated by the computer model. The various physical mechanisms and experimental conditions responsible for band inversion and improved electrophoretic separation are identified and discussed in the framework of the model.**

The ability to resolve large [>100 kilobase pairs (kbp)] fragments of DNA using pulsed-field electrophoresis represents a major advance in molecular genetics. Schwartz and Cantor (1) demonstrated that yeast chromosomes could be separated in agarose gels by the application of alternately pulsed perpendicularly oriented electric fields. This apparatus as well as modifications of orthogonal field alternating gel electrophoresis systems (2–4) have been used to obtain electrophoretic karyotypes of several microorganisms (5–10) and to perform long-range restriction mapping of DNA fragments (11–16). Other electrophoretic configurations that have been developed to resolve large DNA molecules include the periodic inversion of a single electric field (17), the use of transverse fields in a vertical electrophoresis apparatus (18), the application of contour-clamped homogeneous electric fields (19), and the use of a rotating gel system (20). In all the pulsed-field electrophoretic systems used thus far, the pulse times have been determined empirically. In the present report, preliminary data are presented describing the separation of large DNA fragments based on a quantitative model of pulsed-field electrophoresis.

Slater and Noolandi (21, 22) derived a theoretical model describing the dynamics of DNA molecules electrophoresed in agarose gels. Briefly, the theory considers that the DNA molecule undergoes one-dimensional worm-like motion, termed reptation (23), in a "tube" formed by the consecutive pores housing the molecule in the three-dimensional mesh of the gel. Under the influence of the electric field and the thermal agitation (Brownian motion), the DNA chain drifts through the tube and new tube sections are continuously formed by the end segment leaving the original tube. Lateral movements of DNA in the gel matrix are not considered, as they cannot lead to net center-of-mass displacement. The equations describing the dynamics of the chain in its tube have been given in previous articles (21, 22, 24, 25). The motion of the chain is further reduced to discrete jumps of length a along the tube axis, where a is the average pore size of the agarose gel. The time duration of a jump is a function of the instantaneous distance in the field direction, h_x, between the ends of the molecule. The direction taken by the jump in the tube (forward or backward) is also a function of h_x and includes an element of randomness due to thermal agitation. A computer simulation has been performed with the rules derived from the equations of the biased reptation model (24, 25). At each step, the end-to-end distance h_x is calculated, and the choice of a forward or backward jump and of the direction of the leading end segment is carried out according to biased probability distributions and two random numbers. The time duration of the jump is computed and added to the total time. The mobility of the DNA molecule is then calculated from the displacement of the center-of-mass and the total time elapsed (after typically 10^5–10^7 jumps of length a).

Initially, we attempted to separate large DNA molecules by applying an intermittent electric field in which the DNA was subjected to successive rounds of stretching and relaxation during the on and off electric field cycles, respectively. In practice, the DNA chain relaxation times for very large (>300 kbp) DNA molecules are much too long to be used experimentally. The simple electric-field geometry used to establish the field inversion gel electrophoresis (17) for separating large DNA molecules prompted us to attempt to apply the theoretical model to experimental data obtained from a similar electrophoretic system. The results of the application of the computer simulation to the field inversion electrophoresis of yeast chromosomal DNA are presented below.

MATERIALS AND METHODS

Sample Preparation. DNA samples were prepared in plugs of 0.5% agarose by diluting the sample in the appropriate buffer (see below) and adding an equal volume of 1.0% (wt/vol) low melting point agarose (Bethesda Research Laboratories) maintained either at 42°C or at 45°C in the case of yeast cells. The mixture was then immediately aspirated into previously autoclaved silicone tubing (inner diameter, 3/32 inch; 1 inch = 2.54 cm) (6411-63; Cole Parmer, Chicago, IL) and placed at 4°C as described (18). The solidified agarose was cut into 0.5-cm lengths (plugs). The λ phage Charon 21A of 41.7-kbp monomer size (26) was amplified in LE392 bacteria in liquid culture by standard techniques (26). Multimers of phage DNA were prepared from the DNA extracted from the amplified phage (11). Plugs were prepared from DNA resuspended at 10 mg/ml in TE buffer (10 mM Tris·HCl, pH 7.5/100 mM EDTA) for 24 hr at room temperature and stored as described above. The yeast strains A364a, DCO4α (both provided by D. Y. Thomas, Biotechnology

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Research Institute, Montreal), and YP80 (provided by P. Hieter, Johns Hopkins University, Baltimore) were cultured by standard techniques and were harvested in stationary growth phase. Yeast cells were entrapped in agarose using modifications of published procedures (1). Briefly, 1 ml of 1% low melting point agarose containing 60 μg of Zymolase per ml was added to 1 ml of cells resuspended in 0.5 M EDTA for preparation of the plugs, which were then incubated in 0.5 M EDTA/0.01 M Tris·HCl, pH 7.5/7.5% 2-mercaptoethanol, at 37°C overnight. After rinsing twice in 0.5 M EDTA/0.01 M Tris·HCl, pH 7.5, the plugs were incubated in 0.01 M Tris·HCl, pH 7.5/0.5 M EDTA/proteinase K (2 mg/ml)/1% Sarcosyl, for 48 hr at 50°C. After rinsing three times, the plugs were stored at 4°C in 0.5 M EDTA.

Preparation of Agarose Gels. Agarose (type NA; Pharmacia, Uppsala, Sweden) was dissolved in electrophoresis buffer (89 mM Tris base, pH 8.0/89 mM boric acid/2 mM EDTA) by boiling. After cooling to ≈60°C, 300 ml of 0.8% (wt/vol) agarose solution was poured into a gel bed (20 × 25 cm) equipped with a 20-well comb (Bethesda Research Laboratories). The plugs (0.5 cm long) containing the DNA samples were inserted into the gel slots using a spatula. The gel was then submerged in 2.5 liters of electrophoresis buffer in a horizontal gel electrophoresis apparatus (model H1, Bethesda Research Laboratories).

Pulsed-Field Electrophoresis. Electrophoresis was performed by applying a periodic sequence of field pulses having the following pattern: a "forward" (direction of migration) polarity electric field, E_{+2}, of +2 V/cm was first applied for a fixed period of time T_{+2}, followed by a second field of "inverse" polarity, E_{-1}, of −1 V/cm for a fixed period of time T_{-1}. The ratio $T_{+2}/T_{-1} = 2.5$ was kept constant for all experiments. Our rationale for selecting these experimental conditions is based on an analytical study of the biased reptation model, which indicates that the mobility of a DNA fragment of size K (in kbp) should increase by a factor of $(R_T R_E - R_E^2)/(R_T R_E - 1)$, when the pulse duration T_{+2} is increased from zero to infinity. Here $R_T = T_{+2}/T_{-1}$ and $R_E = |E_{+2}/E_{-1}|$ are the pulse time and field ratios, respectively. Moreover, this increase is predicted, using the parameters defined in the legend of Fig. 3, to begin at a critical pulse duration $T^*_{-1} \approx 0.041\ K(R_T R_E - 1)/E^2_{-1}(R_E - 1)$ sec. Choosing voltages of $E_{+2} = +2$ V/cm and $E_{-1} = -1$ V/cm ($R_E = 2$) and a time ratio $R_T = 2.5$ results in a predicted increase in mobility of ≈20% and a critical pulse duration of 0.164 K·sec (e.g., 16 sec for 100 kbp or 164 sec for 1 Mbp). The times T^*_{-1} are size dependent and separation is expected for molecules in the range of size K if $T_{-1} \approx T^*_{-1}(K)$. The ratios $R_E = 2$ and $R_T = 2.5$ were chosen to have reasonable fields and time durations and good electrophoretic separation for yeast chromosomes.

In a typical experiment, a pulse of +2 V/cm was applied for $T_{+2} = 150$ sec followed by a pulse of −1 V/cm lasting $T_{-1} = 60$ sec; this sequence of total time duration $T_{+2} + T_{-1} = 210$ sec was then repeated for 65 hr. In another experiment, three different pulse sequences were applied for equal durations during the electrophoresis. The temperature of the buffer during the electrophoresis was found to increase by only 2°C–3°C and, therefore, buffer cooling and recirculation were not required. The power supply was built and designed at the Xerox Research Centre of Canada. It is a self-contained direct current power supply with two built-in timers to control the intervals T_{-1} and T_{+2} for each cycle. A switch is provided to select the polarity of the output during the second part of the cycle. The electrophoresed DNA was stained in the gel with ethidium bromide (1 μg/ml) and was visualized using an ultraviolet transilluminator equipped with a standard gel photography apparatus.

Southern Blot Hybridizations. Gels containing electrophoresed DNA were twice treated with 0.25 M HCl for 10 min to depurinate the DNA. Following a 45-min denaturation treatment in 0.5 M NaOH and 0.5 M NaCl, the gels were allowed to renature for 2 hr in 1 M Tris (pH 7.6) and 0.6 M NaCl. The DNA was transferred to nitrocellulose filter paper by the method of Southern (27). The filters were then baked for 2 hr at 80°C. Prehybridization and hybridization of the filters were performed as described elsewhere (28). The yeast-specific DNA probes consisted of YRp7, which is specific for the *TRP1* gene (29) located on chromosome IV (30); Mp8HIS3, which is specific for the *HIS3* gene (M. Whiteway, personal communication) located on chromosome XV (30); pADI7/*Bgl*II$_2$, which is specific for a gene encoding acyl coenzyme A oxidase adjacent to the *KEX1* gene (D. Y. Thomas, personal communication), which is located on chromosome VII (30); and the ribosomal DNA probe, pSZI, which is specific for chromosome XII (J. Szostak, personal communication). DNA probes were ^{32}P-labeled by nick-translation (31). After hybridization, the filters were washed as described (28), air-dried, and exposed to Kodak XAR-2 film at −70°C.

RESULTS

Effects of Pulse Time on Electrophoresis of Yeast Chromosomes. Using the preliminary estimates of experimental parameters derived from the biased reptation model (see *Materials and Methods*), agarose plugs containing DNA from the yeast strains A364a, DCO4α (Fig. 1 *A*, *B*, and *C*) and YP80 (Fig. 1*C*) were subjected to electrophoresis for different pulse times T_{+2} as shown. As the time duration T_{+2} is gradually increased from 50 to 150 sec, the number of electrophoretic bands that can be resolved for strains A364a and DCO4α increases from 3 at the shorter pulse duration (Fig. 1*A*) to 13 at the longer pulse duration (Fig. 1*C*). The electrophoretic banding pattern of the different yeast strains for $T_{+2} = 150$ sec (Fig. 1*C*) is similar to those observed using other pulsed-field electrophoresis systems (3, 4, 10, 17), although Southern blotting (Fig. 1*D*) indicates that the mobility of chromosome IV [≈1600 kbp (30)] as detected using probe YRp7 is greater than that of the smaller sized chromosome VII [≈1100 kbp (30)], which can be identified using probe pADI7/*Bgl* II$_2$. The yeast strain YP80 has a deletion of the chromosome IV-specific *TRP1* gene for which YRp7 is a probe and, hence, the chromosome IV electrophoretic band of YP80 is not detected using YRp7 (Fig. 1*D*).

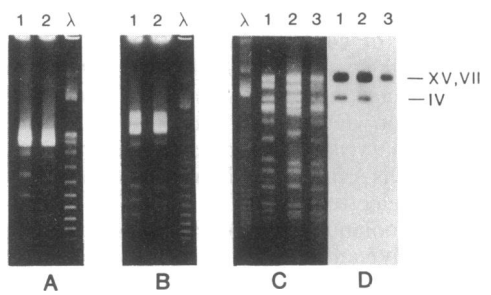

FIG. 1. Effect of lengthening pulse durations on the electrophoretic separation of yeast chromosomes. Multimers of the λ phage Charon 21A (λ lane) of monomer size 41.7 kbp and chromosomal DNA from strains A364a, DCO4α, and YP80 were prepared as described. The time durations T_{+2} and T_{-1} of the +2 V/cm and −1 V/cm pulses were $T_{+2} = 50$ sec and $T_{-1} = 20$ sec (*A*), $T_{+2} = 75$ sec and $T_{-1} = 30$ sec (*B*), and $T_{+2} = 150$ sec and $T_{-1} = 60$ sec (*C*). Electrophoresis was carried for a total of 65 hr (*A* and *B*) and 68 hr (*C*). The electrophoresed DNA for the $T_{+2} = 150$ sec and $T_{-1} = 60$ sec pulse time (*C*) was transferred to nitrocellulose filter paper and hybridized using the ^{32}P-labeled DNA probe YRp7, which is specific for chromosome IV (*D*). The same filter was then hybridized with DNA probe pADI7/*Bgl* II$_2$, which is specific for chromosome VII (*D*). In both cases, exposure times for the autodiograms were 24 hr.

The nature of the apparently anomalous mobility of the chromosome IV band relative to that of the band containing chromosomes VII and XV (which comigrate in the yeast strains analyzed here; see Fig. 1 *C* and *D*) was further investigated by increasing the pulse times (Fig. 2). In Fig. 2*A*, chromosome IV (which hybridizes to YRp7) demonstrates a lower electrophoretic mobility (Fig. 2*B*) than that of chromosome XV (which is detected using Mp8HIS3), indicating that the expected band order for chromosomes IV and XV has been obtained by increasing the pulse time durations to T_{+2} = 225 sec. In Fig. 2*C*, T_{+2} has been increased to 300 sec and the electrophoretic mobility of the chromosome XV fragment is again observed to be greater than that of the chromosome IV fragment (Fig. 2*D*). Also, the physical separation between the two electrophoretic bands (Fig. 2*D*) is increased relative to the shorter T_{+2} = 225 sec pulse (Fig. 2*B*), indicating that a better resolution of the two bands can be achieved by lengthening the pulse duration.

Biased Reptation Model of Pulsed-Field Electrophoresis. A computer simulation of the biased reptation model was performed to test the suitability of the reptation theory in describing the mobility of large DNA fragments in pulsed-field conditions. Ten parameters enter the simulation: the two fields (E_{-1} and E_{+2}) and their time duration (T_{-1} and T_{+2}) in the pulse sequence, the size of the DNA molecule, the total (size-dependent) friction coefficient (ξ) of the molecule in the tube, the average pore size (a) of the gel, the persistence length (p) of DNA, the screening factor (σ) related to the strength of the buffer, and the temperature.

Fig. 3 presents the mobility curves obtained from the computer simulation of the biased reptation theory as the pulse duration T_{+2} is increased from 20 to 1000 sec (T_{+2}/T_{-1} = 2.5) for three DNA fragment sizes; 1100, 1600, and 2200 kbp, which correspond, respectively, to the sizes of chromosome XV and VII, chromosome IV, and chromosome XII. The curves show three general features predicted by the biased reptation model: the mobility of large fragments is a slowly increasing function of molecular size for small values of T_{+2}; the mobility increases monotonically with the pulse duration (T_{+2}), and separation as well as the correct band order can be obtained by a suitable choice of T_{+2}.

For smaller DNA molecules (<300 kbp), there is excellent agreement between the measurements of the mobilities of the smallest two chromosomes (200–300 kbp) in Fig. 1*A* for all values of T_{+2} and the theoretical curves, which are not shown here. In particular, the mobility is observed to increase monotonically with T_{+2} and separation is observed for $T_{-1} \approx$

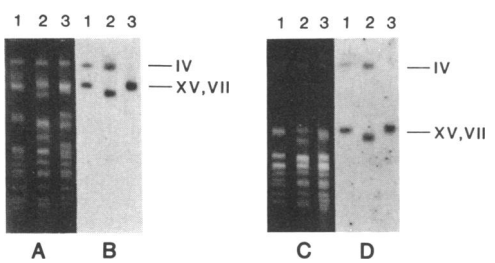

FIG. 2. Effect of lengthening pulse durations on the electrophoretic band order of yeast chromosomes IV and XV. Chromosomal DNA from yeast strains A364a (lane 1), DCO4α (lane 2), and YP80 (lane 3) was electrophoresed under conditions similar to those of Fig. 1 except that the time durations of the forward (+2 V/cm) and inverse (−1 V/cm) pulses were increased to T_{+2} = 225 sec and T_{-1} = 90 sec (*A*) and T_{+2} = 300 sec and T_{-1} = 120 sec (*C*). The electrophoresis was carried out for a total of 65 hr in both cases. The electrophoresed DNA (*A* and *C*) was transferred to nitrocellulose filter paper and hybridized sequentially with probes YRp7 and Mp8HIS3 (*B* and *D*), which detect the electrophoretic bands corresponding to chromosomes IV and XV, respectively. The autoradiograms were exposed for 24 hr.

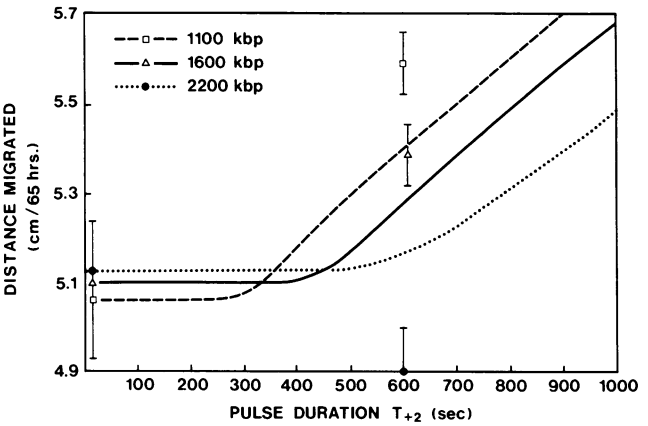

FIG. 3. Theoretical mobility curves predicted by the computer simulation of the biased reptation model. The y axis gives the total distance migrated by each molecule after 65 hr of electrophoresis, and the x axis gives the time duration T_{+2} of the +2 V/cm electric field pulse. The simulation was performed with fields of +2 V/cm and −1 V/cm, a ratio of time intervals T_{+2}/T_{-1} = 2.5, and a temperature of 20°C, which are the values used in the electrophoresis experiments. We have used p = 50 nm and a = 110 nm, as suggested by our previous study of reptation with small DNA molecules (37). A screening factor of σ = ½ corresponds to typical values found in the literature (32). The number of pores housing a molecule—i.e., the number of segments corresponding to a given fragment size (in kbp)—was evaluated using the procedure suggested in ref. 33 and the parameters given above. Finally, the value of $2\sigma eM/3\xi = 12.5 \times 10^{-4}$ cm^2/V·sec (where M is the size of the molecule in base pairs and $e = 1.6 \times 10^{-19}$ C is the charge of one electron; $2\sigma eM$ is the total charge of the molecule, and ξ is its total friction coefficient) was chosen for the 2200-kbp DNA molecule (about the size of chromosome XII) to have the experimentally observed velocity with T_{+2} = 20 sec and T_{-1} = 8 sec pulses—i.e., ≈5.1 cm/65 hr.

$T^*_{-1}(K)$ for K = 200–300 kbp. The biased reptation theory, therefore, offers a satisfactory description of the physics that govern the mobility DNA fragments less than ≈300 kbp.

For larger (>300 kbp) yeast chromosomes, theory and experiment differ qualitatively with respect to the monotonic increase predicted by the biased reptation model. Examination of the effect of pulse duration T_{+2} on the mobility of the larger yeast chromosomes reveals that physical mechanisms other than reptation may also affect the migration of the larger DNA molecules. For short pulse time durations (T_{+2} = 2.5 sec and T_{+2} = 10 sec; not shown here), all chromosomes comigrate at 51 ± 2 mm after 65 hr of electrophoresis. The two smallest chromosomes (≈200 and ≈225 kbp), as well as the λ phage multimers (multiples of 41.7 kbp) are observed to migrate at increasing distances (>51 mm) for T_{+2} > 50 sec (see Fig. 1). The largest chromosomes (>300 kbp) initially display a decreased mobility as T_{+2} increases (see Fig. 1*C*). For much longer pulses (T_{+2} = 3000 sec), it was observed (data not shown) that the chromosomes comigrate at 65 ± 3 mm after 65 hr of electrophoresis, in which case the electrophoresis can be considered as made of a series of continuous field experiments. The larger yeast chromosomes (>300 kbp), therefore, show a sudden decrease in mobility in a distinct molecular size-dependent range of pulse durations but otherwise follow the general increase in mobility shown by smaller (<300 kbp) DNA fragments when T_{+2} increases, as predicted by the reptation model.

Applications to Genetic Mapping in Yeast. To maintain a monotonic relationship between electrophoretic mobility and DNA fragment size, while preserving the separation of all the bands in the yeast electrophoretic karyotype, the pulse times (T_{+2}/T_{-1} = 2.5) were varied over a 72-hr electrophoresis time. A T_{+2} = 75 sec pulse was applied over the first 24 hr of electrophoresis to resolve the smaller yeast chromosomes.

During the second 24 hr of electrophoresis, a T_{+2} = 150 sec pulse was used to resolve the yeast chromosomes of intermediate size. A T_{+2} = 300 sec pulse was applied during the final 24 hr of electrophoresis to separate the larger chromosomes. The results of this experiment are summarized in Fig. 4*A*. Southern blotting (Fig. 4 *B* and *C*) using the yeast chromosome-specific DNA probes allows the assignment of electrophoretic bands to, in order of increasing mobility, chromosomes XII (≈2200 kbp) using pSZ1, IV (≈1600 kbp) using YRp7, and XV (≈1100 kbp) using Mp8HIS3, in three strains A364a, DCO4α, and YP80. The expected DNA size versus band mobility relationship is therefore observed in Fig. 4, and minor differences in band length are due to karyotypic differences between the three yeast strains that can be resolved using the pulsed-field electrophoresis technique. This sequence of pulses applied during the electrophoresis results in a high-resolution electrophoretic karyotype for yeast, where the mobility is a monotonic function of DNA size for all fragments resolved in Fig. 4 (data not shown).

DISCUSSION

Several elegant experimental systems have been developed (1, 3, 17–20) with the purpose of separating large (>100 kbp) molecules of DNA. There has been no previous attempt, however, to quantitate the mobilities of the DNA fragments separated in these apparatuses, nor has there been any formal discussion of the physics that underlie the mechanisms by which separation was achieved. The results in Fig. 3 represent an attempt to apply a theoretical model to pulsed-field electrophoresis. The theoretical curves (Fig. 3) agree with the experimental data with respect to two critical observations in pulsed-field electrophoresis: firstly, the model predicts that large fragments do not necessarily migrate in order of molecular size (Fig. 1*C*); secondly, the mobility of all fragments increase by a common factor when pulse duration times are increased.

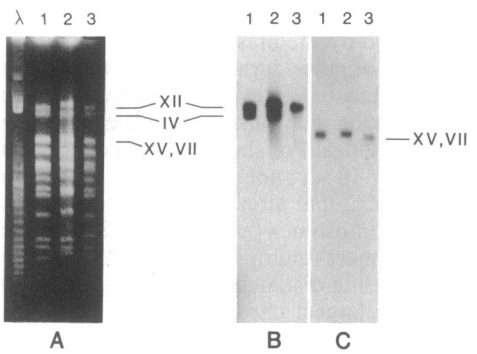

FIG. 4. Electrophoretic karyotype for yeast. Multimers of the λ phage Charon 21A and chromosomal DNA from yeast strains A364a (lane 1), DCO4α (lane 2), and YP80 (lane 3) were electrophoresed by applying a sequence of pulse times during a 72-hr electrophoresis experiment (*A*). The time durations of the forward (+2 V/cm) and inverse (−1 V/cm) pulses were T_{+2} = 75 sec and T_{-1} = 30 sec during the first 24 hr of electrophoresis. The pulse times were increased to T_{+2} = 150 sec and T_{-1} = 60 sec during the second 24 hr, and to T_{+2} = 300 sec and T_{-1} = 120 sec during the final 24-hr period. The electrophoresed DNA was transferred to nitrocellulose filter paper and hybridized sequentially with probes YRp7 and pSZI (*B*), which detect the electrophoretic bands corresponding to chromosomes IV and XII, respectively. Autoradiographic exposure times (*B*) were 24 hr. The samples run in parallel in the same electrophoresis apparatus yielded an identical pattern of band mobilities (data not shown) and were similarly transferred and hybridized using the chromosome XV-specific DNA probe Mp8HIS3 (*C*), and the autoradiogram was exposed for 6 days.

That a DNA molecule of larger size can display a mobility greater than that of a smaller-sized molecule was explained, in the case of continuous field electrophoresis, by a physical phenomenon that we have termed DNA "self-trapping" and for which we have developed a theoretical argument and presented experimental evidence in a separate report (25). Briefly, DNA molecules in a certain size range that are constrained in a gel can be trapped for extended periods of time in conformations of near-zero mobility, which minimize the distance h_x between the ends of the DNA molecule. Since the mobility of a DNA molecule is determined by h_x, fragments that display a circular loop conformation, where h_x is small, will not move until a change in conformation occurs as a result of thermal fluctuations. In fact, strong band inversion is observed for fragments <100 kbp in pulsed-field conditions, as predicted by the biased reptation model. This effect can be greatly reduced by a good choice of pulse conditions (unpublished data).

A nonreptation mechanism also adds a resonance-like decrease in mobility in a size-dependent range of pulse durations for large (>300 kbp) fragments (Figs. 1 and 2); moreover, this decrease in mobility may also be accompanied by a nonmonotonic mobility–size relationship (Fig. 1). The wide electrophoretic separation of yeast chromosomes (Figs. 1 and 2) is, therefore, related both to the increase in mobility predicted by the biased reptation theory with the use of unequal fields ($R_E \neq 1$) and to the decrease due to this nonreptation mechanism. It should be noted that no reptation-driven separation is predicted by the biased reptation model if the two fields are of equal intensity ($R_E = 1$) such that $(R_T R_E - R_E^{-2})/(R_T R_E^{-1}) = 1$ (see *Materials and Methods*). The separation of DNA molecules achieved in the FIGE system (17) where equal fields are used ($R_E = 1$) will be due only to the nonreptation mechanism, defined as resonance (17), and will not be further enhanced by reptation, as is the case here, where $R_E \neq 1$.

The reason that there is not perfect agreement between our experiments (Figs. 1 and 2) and biased reptation theory (Fig. 3) may be related to an effect where pulsed fields enhance DNA self-trapping by coupling to the intramolecular modes of relaxation and result in anomalously small mobilities (Fig. 1). Although this cannot formally be referred to as a "resonance" effect since the polymer chain behaves as an overdamped oscillator, intramolecular modes can be important on the scale of molecular sizes and pulse conditions used here. In the original version of the biased reptation model (22), the chain is assumed to be in equilibrium in its "tube"; the model chain is therefore considered to be rigid on the time scale of field-driven chain motion. The relaxation time for the intramolecular modes is given by the Rouse time (34) T_R, while the field-driven disengagement time of the chain from its tube is given by $T_E = L/v_\ell$, where L is the tube length and v_ℓ is the field-driven longitudinal chain velocity (22). The biased reptation model is therefore valid if $T_R << T_E$, which in the experimental conditions here (see legend to Fig. 3) reduces to $K > 500$ kbp for the DNA fragment size limit above which the biased reptation model does not completely describe the mobility. This is in reasonable agreement with the value of ≈300 kbp observed to be the minimum molecular size leading to a "resonance-like" decrease in mobility (Fig. 1).

In the case where the time scale of the tube disengagement is less than that of intramolecular modes of relaxation ($T_E < T_R$), the DNA chain is no longer in equilibrium in its tube. Accordingly, if the pulse duration T_{+2} is long enough for the chain to completely disengage from its tube at least once during a cycle ($T_E < T_{+2} < T_R$), large effects of tube fluctuations on chain mobility and DNA self-trapping are expected. In our experimental conditions, such effects are predicted, therefore, for values of T_{+2} ranging from 90 to 400

sec for $K = 2200$ kbp. These time scales for T_{+2} compare well to those values observed in Figs. 1 and 2.

A better agreement between experimental data (Figs. 1 and 2) and the theoretical curves (Fig. 3) may also necessitate better estimates of certain physical parameters involved in the electrophoresis of DNA in agarose gels. For example, physical measurements of the agarose gel pore size distribution will allow improvements in the results of the computer simulation in which a single average pore size (Fig. 3) is presently assumed. Optical measurements (35, 36) have recently confirmed the alignment and stretching of DNA in the field direction that is predicted by reptation theory. These experimental techniques (35, 36) may also allow more direct measurements of parameters, such as the number of segments corresponding to a given fragment size, which are required for the computer simulation (Fig. 3).

A quantitative model of DNA gel electrophoresis such as the one that we are developing will offer distinct advantages related to the construction of long-range restriction maps of genetic loci, such as the major histocompatibility complex (13) and the Duschenne muscular dystrophy gene (14–16), which span large regions of the human genome. Firstly, given the difficulty in accurately sizing DNA molecules by comparing their mobilities to those of size standards, it would be very useful to determine the size of the DNA molecule directly from the measured mobility making use of universal theoretical curves such as those presented in Fig. 3. Secondly, the optimal pulse conditions for resolving DNA molecules in a given size range can be predicted using the quantitative model. This should permit the construction of restriction maps with the highest possible degree of resolution for DNA fragment lengths from 1 kbp to >2000 kbp using simple experimental conditions such as those used to obtain the electrophoretic yeast karyotype shown in Fig. 4. By using scaling laws derived from the biased reptation model, it will be possible to further improve the experimental system by reducing the total electrophoresis time, which is 72 hr in Fig. 4. Finally, a fundamental understanding of the electrophoresis of large DNA fragments permits the quantitative description of DNA self-trapping and band-inversion and allows these effects, which may very seriously complicate long range restriction mapping of genomic DNA, to be identified and eliminated by using appropriate experimental parameters, which can be derived from the theoretical model.

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1. Schwartz, D. C. & Cantor, C. R. (1984) *Cell* **37**, 67–75.
2. Carle, G. F. & Olson, M. V. (1984) *Nucleic Acids Res.* **12**, 5647–5664.
3. Carle, G. F. & Olson, M. V. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3756–3760.
4. McPeek, F. D., Coyle-Morris, J. F. & Gemmill, R. F. (1986) *Anal. Biochem.* **156**, 274–285.
5. Van der Ploeg, L. H. T., Schwartz, D. C., Cantor, C. R. & Borst, P. (1984) *Cell* **37**, 77–84.
6. Van der Ploeg, L. H. T., Smits, M., Ponnudurai, T., Vermeulen, A., Meuwissen, J. H. E. & Langsley, G. (1985) *Science* **229**, 658–661.
7. Holmes Giannini, S., Schittini, M., Keithly, J. S., Warburton, P. W., Cantor, C. R. & Van der Ploeg, L. H. T. (1986) *Science* **232**, 762–765.
8. Pologe, L. G. & Ravetch, J. V. (1986) *Nature (London)* **322**, 474–477.
9. Bernards, A., Kooter, J. M., Michels, P. A. M., Moberts, R. M. P. & Borst, P. (1986) *Gene* **42**, 313–322.
10. DeJonge, P., De Jongh, F. C. M., Meijers, R., Steensma, H. Y. & Scheffers, W. A. (1986) *Yeast* **2**, 193–204.
11. Van der Bliek, A. M., Van der Velde-Koerts, T., Ling, V. & Borst, P. (1986) *Mol. Cell. Biol.* **6**, 1671–1678.
12. Brown, W. R. A. & Bird, A. P. (1986) *Nature (London)* **322**, 477–481.
13. Hardy, D. A., Bell, J. I., Long, E. O., Lindsten, T. & McDevitt, H. O. (1986) *Nature (London)* **323**, 453–455.
14. van Ommen, G. J. B., Verkerk, J. M. H., Hofker, M. H., Monaco, A. P., Kunkel, L. M., Ray, P., Worton, R., Wieringa, B., Bakker, E. & Pearson, P. L. (1986) *Cell* **47**, 499–504.
15. Burmeister, M. & Lehrach, H. (1986) *Nature (London)* **324**, 582–585.
16. Kenwrick, S., Patterson, M., Speer, A., Fischbeck, K. & Davies, K. (1987) *Cell* **48**, 351–357.
17. Carle, G. F., Frank, M. & Olson, M. V. (1986) *Science* **232**, 65–68.
18. Gardiner, K., Laas, W. & Patterson, D. (1986) *Somatic Cell Mol. Genet.* **12**, 185–195.
19. Chu, G., Vollrath, D. & Davis, R. W. (1986) *Science* **234**, 1582–1585.
20. Anand, R. (1986) *Trends Genet.* **2**, 278–283.
21. Slater, G. W. & Noolandi, J. (1985) *Phys. Rev. Lett.* **55**, 1579–1582.
22. Slater, G. W. & Noolandi, J. (1986) *Biopolymers* **25**, 431–454.
23. Lerman, L. S. & Frisch, H. L. (1982) *Biopolymers* **21**, 995–997.
24. Slater, G. W., Rousseau, J. & Noolandi, J. (1987) *Biopolymers* **26**, 863–872.
25. Noolandi, J., Rousseau, J., Slater, G. W., Turmel, C. & Lalande, M. (1987) *Phys. Rev. Lett.* **58**, 2428–2431.
26. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
27. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
28. Lalande, M., Kunkel, L. M., Flint, A. & Latt, S. A. (1984) *Cytometry* **4**, 101–107.
29. Struhl, K., Stinchcomb, D. T., Scherer, S. & Davis, R. W. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1035–1039.
30. Mortimer, R. K. & Schild, D. (1985) *Microbiol. Rev.* **49**, 181–212.
31. Rigby, P. W., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
32. Ross, P. D. & Scraggs, L. (1964) *Biopolymers* **2**, 231–236.
33. Lumpkin, O. J., Déjardin, P. & Zimm, B. H. (1985) *Biopolymers* **24**, 1573–1593.
34. Doi, M. & Edwards, S. F. (1986) *The Theory of Polymer Dynamics* (Oxford Univ. Press, Oxford).
35. Äkerman, B., Jonsson, M. & Nordén, B. (1985) *J. Chem. Soc. Chem. Commun.*, 423–424.
36. Hurley, I. (1986) *Biopolymers* **25**, 539–554.
37. Slater, G. W., Rousseau, J., Noolandi, J., Turmel, C. & Lalande, M. (1987) *Biopolymers*, in press.