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Megabase-sized linear DNA in the bacterium *Borrelia burgdorferi*, the Lyme disease agent

(bacterial chromosome/electrophoresis/spirochete/plasmid)

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ABSTRACT **Using pulsed-field gel electrophoresis we examined the genome of *Borrelia burgdorferi*, a eubacterium of the spirochete phylum and the agent of Lyme disease. A population of this species' cells was lysed *in situ* in agarose blocks. An abundant DNA form that behaved as a linear duplex molecule under different electrophoretic conditions was found. The estimated size of the molecule was 950 kilobases. DNA from two other genera of spirochetes did not enter the gel under these conditions. These studies indicate that *Borrelia* spirochetes, perhaps uniquely among prokaryotic organisms, have linear chromosomes.**

Spirochetes have been assigned their own phylum among eubacteria on the basis of their unique architecture and ribosomal RNA sequences (1, 2). Members of the spirochete genus *Borrelia* are transmitted by arthropods from one vertebrate host to another. Included in this genus are species that cause relapsing fever and *Borrelia burgdorferi*, the cause of Lyme disease (reviewed in ref. 3). In *Borrelia hermsii*, a relapsing fever species, the genes for major outer membrane proteins are arrayed on linear plasmids (4). Investigations of similar plasmids of *B. burgdorferi* revealed that the linear molecules had covalently closed ends (5). Although replicons with this structure are unusual in prokaryotic organisms (5, 6) and despite evidence to date that bacteria have circular chromosomes (7–9), we wondered whether the chromosome of a borrelia might be linear. This possibility was studied with pulsed-field gel electrophoresis. The experiments described herein are first steps in the structural characterization of a borrelia's genome. The results indicate that *B. burgdorferi* contains novel forms of linear duplex DNA of a size consistent with a bacterial chromosome.

METHODS

Strains and Culture Conditions. *B. burgdorferi* strain B31 (ATCC 35210) was cultivated in BSK II medium under conditions previously described (10). *Spirochaeta aurantia* was provided by P. Greenberg (University of Iowa) and cultivated in YTG medium (11); *Treponema denticola* was provided by S. Holt (University of Texas Health Science Center, San Antonio) and grown in GM-1 medium (12).

Preparation of DNA. Agarose blocks of DNA were prepared by a modification of the method of Smith *et al.* (13). Spirochetes were grown to a cell density of 10^8 cells per ml. Cells were harvested by centrifugation at 8000 × *g* for 15 min at 20°C, washed with 50 mM Tris/150 mM NaCl, pH 8.0 (TN buffer) at 20°C, and then resuspended in TN buffer at 37°C. An equal volume of molten 1% low-melting-point agarose (SeaKem Incert; FMC) in TN buffer was added to the cell suspension, and the mixture was poured into acrylic casting wells. After the blocks solidified, they were first immersed in the lysis solution, which in its complete form was 50 mM Tris/50 mM EDTA/1% $NaDodSO_4$, pH 8.0, with 1 mg of proteinase K (Boehringer Mannheim) per ml, and then incubated at 50°C for 16–24 hr. The DNA concentration in melted blocks was determined by microfluorimetry (Hoefer); calf thymus DNA in agarose was the standard. Agarose blocks were stored in 10 mM Tris/1 mM EDTA, pH 8.0 (TE buffer) at 4°C until use. Bacteriophage λ DNA (BRL) was formed into open circles by incubating 1.5 μg of linear DNA in 1.0 ml of 10 mM sodium phosphate/100 mM NaCl/1 mM EDTA, pH 7.4, first at 75°C for 15 min and then at 45°C for 8 hr (14).

Transverse Alternating-Field Electrophoresis (TAFE). DNA blocks were washed twice with TE buffer and then loaded into wells of 1% agarose gels (Seakem GTG; FMC) containing TAFE running buffer. Concentrated (20×) running buffer was composed of 24.2 g of Tris base (Research Organics), 2.9 g of free acid EDTA, 5 ml of glacial acetic acid, and sufficient deionized water to yield 1 liter. The TAFE procedure was a modification of the method of Gardiner *et al.* (15) and was carried out in a GeneLine system (Beckman) at 14°C with buffer recirculation. Unless otherwise stated, the electrophoresis parameters were a pulse time of 60 sec, a constant current of 150 mA, buffer volume of 3.5 liters, and a duration of 18 hr. Start and final voltages were 250 V and 220 V, respectively. Size estimates in kilobases (kb) for *Saccharomyces cerevisiae* strain 334 chromosomes were those provided by the supplier (Beckman). Ladders of phage λ multimers were obtained from FMC; molecular weight standards for linear duplex DNA ranging in size between 9 and 48 kb were from BRL. Gels were stained in ethidium bromide (200 ng/ml).

Nuclease Treatments. Blocks were washed twice at 20°C with TE buffer containing 1 mM phenylmethylsulfonyl fluoride, 3 times with TE buffer alone, and then equilibrated in the appropriate nuclease buffer specified by the supplier for 1 hr. Restriction endonuclease digestion was performed for 14 hr with 0.2–0.5 unit of enzyme per microliter. DNase I (20 ng/ml) digestion of the blocks was at 37°C for 1 hr; RNase A (40 μg/ml of TE buffer) treatment was at 37°C for 2 hr.

Intrinsic Radiolabeling of DNA. BSK II medium (12 ml) containing [^{3}H]thymidine (0.5 mCi; 1 Ci = 37 GBq) was inoculated with 5×10^7 *B. burgdorferi* cells. After 3 days of incubation, the 10^9 cells composing the culture were harvested, washed with TN buffer, and resuspended in 0.3 ml of TN buffer. To this suspension was added 0.3 ml of 1% molten low-melting-point agarose. Agarose blocks were incubated in lysis solution, washed with TE buffer, and subjected to TAFE. The gel was stained with ethidium bromide, and excised gel slices were melted in water at 95°C; scintillation fluid at 65°C was added to the melted gel slices before liquid scintillation counting.

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Abbreviation: TAFE, transverse alternating-field electrophoresis.

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Direct Gel Hybridization. Agarose gels were immersed in 0.2 M HCl for 10 min, rinsed with water, immersed in 0.5 M NaOH/1.5 M NaCl for 1 hr, rinsed again with water, placed in 1 M Tris/0.5 M NaCl, pH 7.5, for 1 hr, and then dried onto a nylon membrane (5). *B. burgdorferi* DNA in blocks was labeled *in situ* with ^{32}P by nick translation using a commercial kit (BRL). Reagents were added to molten (37°C) agarose blocks that contained 2 μg of DNA. The mixture was incubated at 15°C for 2 hr during which it solidified. The block was melted again at 68°C and applied to a Sephadex G-50 column in TE buffer to recover the probe. The conditions for hybridization and washing were those previously described (5).

Density Gradient Centrifugation. Total DNA of *B. burgdorferi*, which had been extracted as described (16), was subjected to ethidium bromide/CsCl density gradient centrifugation (154,000 × *g* for 6 hr) (5). The fractions were examined by TAFE and by constant-field agarose electrophoresis, in which the running buffer was 90 mM Tris/90 mM borate/2 mM EDTA, pH 8.0.

UV Light and Ethidium Bromide Treatment of DNA. After *B. burgdorferi* DNA blocks had equilibrated in 10 mM Tris/50 mM EDTA/0.3 M NaCl, pH 8.0, they were transferred to this solution supplemented with 0.5 μg of ethidium bromide per ml and placed in the dark for 1 hr. Blocks were then exposed for different periods of time to 254-nm UV light (Ultraviolet Products, San Gabriel, CA) at an intensity of 330 $\mu W/cm^2$ of block surface. After UV exposure, the blocks were heated to either 95°C or 60°C for 10 min and then 60°C for 10 min. The suspension was transferred to a 0°C bath to solidify the agarose, and TAFE was performed.

Two-Dimensional Agarose Gel Electrophoresis. A modification of the method of Serwer and Hayes was used (17). A mixture of circular and linear phage λ DNA was loaded into a horizontal 1% agarose gel and subjected to constant-field electrophoresis at 1 V/cm for 4 hr in TAFE buffer. The gel was removed, and other wells were filled with agarose blocks of *B. burgdorferi* or *S. cerevisiae*. The first dimension was TAFE as described above. For the second dimension, the gel was removed from the vertical apparatus and placed back in a horizontal electrophoresis with the anode and cathode perpendicular to the first-dimension electrodes. Constant-field electrophoresis (6 V/cm) was then carried out for 45 min in TAFE buffer. The gel was stained with ethidium bromide after the last electrophoresis.

RESULTS

TAFE Analysis of Borrelia DNA. Previous studies of *B. hermsii* and *B. burgdorferi* revealed linear duplex DNA plasmids of 16–53 kilobases (kb) (5, 18). To provide further resolution of larger linear plasmids and to study chromosomes of borreliae, we examined total DNA of *B. burgdorferi* using the pulsed-field gel electrophoresis system of TAFE. To prevent shearing of large forms of DNA, the cells were lysed *in situ* in agarose blocks. Initial experiments established conditions for *in situ* lysis of spirochetes in the blocks. Fig. 1 *Left* shows the migration patterns of total DNA of *B. burgdorferi* prepared with different lysis solutions and then subjected to TAFE; *S. cerevisiae* chromosomes served as size markers. Buffered $NaDodSO_4$ alone or in combination with proteinase K was sufficient to lyse the cells in the blocks; the expected linear plasmids with sizes of 49, 29, and 16 kb (5) were seen in the gel. In the absence of $NaDodSO_4$ the DNA remained at the origin.

In those lanes demonstrating cell lysis, a thick band that had an apparent size as duplex linear DNA of ≈1000 kb (or one megabase) was observed (Fig. 1 *Left*). There was also a more diffuse band that migrated in the TAFE gel as if it had a linear duplex length of 800 kb. These ethidium bromide-stained bands were insusceptible to RNase A but digestible

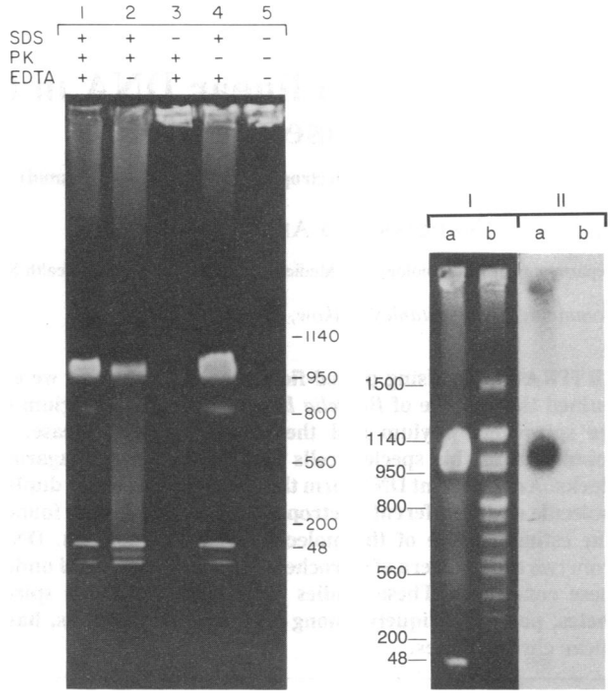

FIG. 1. TAFE and direct gel hybridization of *B. burgdorferi* strain B31. (*Left*) Lysis of cells under different conditions. Agarose blocks were incubated in complete lysis solution (lane 1) or in lysis solution lacking EDTA (lane 2), $NaDodSO_4$ (SDS; lane 3), proteinase K (PK, lane 4) or all three components (lane 5). Numbers on the right refer to sizes in kb of selected *S. cerevisiae* chromosomes (200–1140 kb) and a linear molecular weight standard (48 kb) used in the gel. (*Right*) Direct gel hybridization. Agarose blocks of *B. burgdorferi* (lanes a) or *S. cerevisiae* (lanes b) were subjected to TAFE. Lanes: I, ethidium bromide-stained TAFE gel; II, autoradiograph of the gel hybridized with radiolabeled total DNA of *B. burgdorferi*. Numbers on the left refer to sizes in kb of selected *S. cerevisiae* chromosomes (200–1500 kb) and a linear molecular weight standard (48 kb) used in the gel.

with DNase I (data not shown). When EDTA was deleted from the lysis solution, this DNA degraded. Although absence of proteinase K from the mixture did not appear to affect either the electrophoretic migration or stability of the bands, we subsequently used the complete lysis solution to minimize the effect of residual cellular proteins on experimental outcomes. (Hereafter the term "megabase-sized" refers to material in TAFE gels that had an apparent size as a linear duplex DNA molecule of about 1000 kb.)

The contribution of the megabase-sized DNA to total DNA in the blocks was estimated by intrinsic radiolabeling with thymidine and by DNA hybridization. The megabase-sized material was the greatest peak of radioactivity that migrated into the gel. Bands of megabase-sized DNA, DNA migrating with an apparent size of 800 kb, and 49-kb linear plasmid were excised from each of four individual lanes of the gel and assayed for radioactivity; the mean cpm (± SEM) were 37,659 ± 1797, 4993 ± 524, and 2215 ± 74, respectively. Mean radioactivity in the remainder of each of the four lanes of the gel was 22,368 ± 525 cpm. The megabase-sized DNA accounted for 57% of the labeled thymidine that entered the gel. When direct gel hybridization was carried out with total *B. burgdorferi* DNA as a probe, the megabase-sized band contained the majority of hybridizable material present in the gel; little hybridizable DNA remained at the gel origin (Fig. 1 *Right*).

Fig. 2 shows DNA fragments that were obtained when the agarose blocks of *B. burgdorferi* DNA were incubated with restriction enzymes *Mlu* I and *Sma* I. The megabase-sized DNA was cleaved into *Mlu* I fragments of 440, 170, 160, 55,

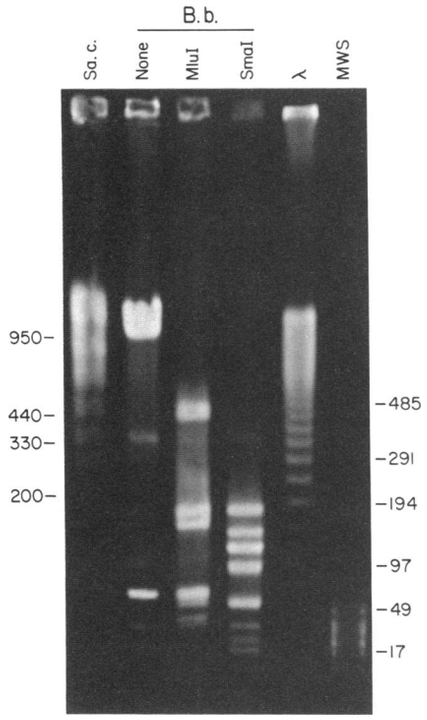

FIG. 2. TAFE of restriction enzyme fragments of *B. burgdorferi* total DNA. *B. burgdorferi* (lanes B.b.) DNA in agarose blocks was digested with *Mlu* I or *Sma* I. Linear duplex DNA size standards were *S. cerevisiae* chromosomes (lane Sa.c.), a phage λ multimer ladder (lane λ), and high molecular weight standards (lane MWS). The numbers to the left and right indicate selected size standards in kb. Serially increasing pulse times of 1, 2, 5, 10, 15, 20, 30, 40, 45, 50, and 60 sec for 2 hr each over a run of 22 hr were used.

50, and 40 kb and *Sma* I fragments of 170, 140, 125, 120, 110, 105, 55, 50, and 10 kb. There were no *Mlu* I sites in the linear or circular plasmids; *Sma* I cut only the 49-kb linear plasmid to a large fragment of 25 kb and smaller fragments of <10 kb. Total sizes of *Mlu* I and *Sma* I fragments of genomic DNA, exclusive of linear and circular plasmids, were estimated to be 915 and 885 kb, respectively. The results of digests with these infrequently cutting enzymes indicated that the chromosome size of *B. burgdorferi* was ≈900 kb.

With fewer *B. burgdorferi* cells in the blocks, the size of the large DNA was more accurately estimated. This DNA of *B. burgdorferi* had an estimated linear equivalent length of 950 kilobase pairs when its TAFE migration was compared with that of the *S. cerevisiae* chromosomes (Fig. 3). This figure was within 7% of the genome size estimated with restriction enzymes. The figure also shows that the DNA of the spirochetes *S. aurantia* and *T. denticola* did not enter the gel under the same conditions. Effective release of *S. aurantia* and *T. denticola* DNA from cells was demonstrated by the susceptibility of their DNAs to DNase and restriction enzymes after, but not before, treatment of the blocks with complete lysis solution (data not shown).

Structure of Megabase-Sized DNA. Of interest was the structure of the megabase-sized DNA of *B. burgdorferi* seen in TAFE gels. Using electrophoresis under different conditions, we examined these DNA molecules to determine if they were supercoiled circular, open circular, or linear.

Strain B31 has stably maintained, supercoiled circular plasmids with average circumferences of ≈28 kb (5). These supercoiled plasmids were separated from the 16- to 49-kb range linear plasmids of this strain by a plasmid enrichment procedure followed by ethidium bromide/CsCl density gradient centrifugation (5). The supercoiled and linear plasmid fractions so obtained were then compared with total DNA of

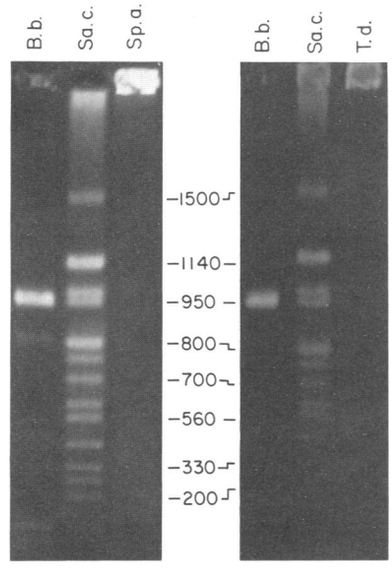

FIG. 3. TAFE of DNA of *in situ* lysed *B. burgdorferi* (lanes B.b.), *S. cerevisiae* (lanes Sa.c.), *S. aurantia* (lane Sp.a.), and *T. denticola* (lane T.d.) cells. TAFE conditions are described in the text. Numbers in the middle refer to sizes in kb of selected *S. cerevisiae* chromosomes (200–1500 kb).

B. burgdorferi and *S. cerevisiae* in TAFE gels run in the absence of ethidium bromide (Fig. 4 *Left*, "Pre-EB"). The 28-kb supercoiled plasmid fraction (lane a) migrated in the TAFE gel at the same speed as the band with an apparent

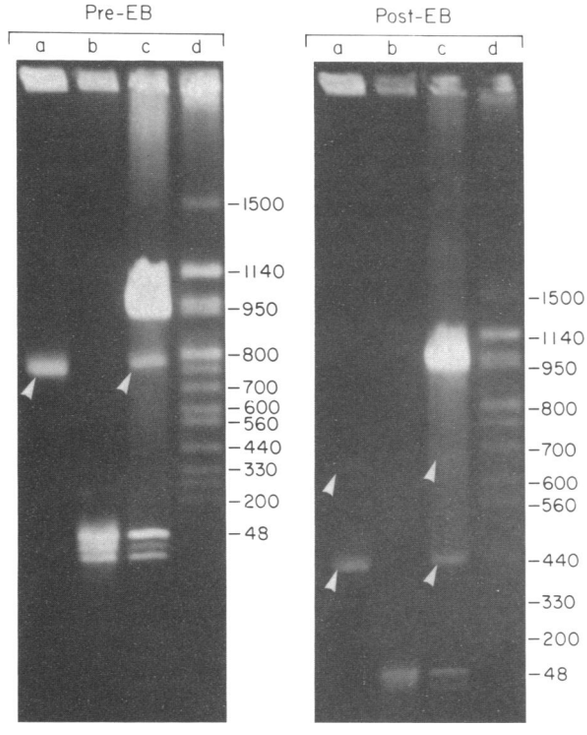

FIG. 4. TAFE migration of *B. burgdorferi* DNA in absence (*Left* "Pre-EB") and presence (*Right* "Post-EB") of ethidium bromide. Lanes: a, purified supercoiled plasmid; b, linear plasmids; c, total *B. burgdorferi* DNA; d, *S. cerevisiae* chromosomes. (*Left*) TAFE was carried out for 18 hr. Arrowheads indicate the location of supercoiled plasmids. (*Right*) The gel was stained with ethidium bromide and subjected to TAFE for an additional 11 hr. Arrowheads indicate two forms of more rapidly migrating DNA in lanes a and c. Numbers on the right refer to sizes in kb of selected *S. cerevisiae* chromosomes (200–1500 kb) and a linear molecular weight standard (48 kb) used in the gel.

linear size of 800 kb in the *in situ* lysis block (lane c). The migration of bands in the linear plasmid fraction (lane b) identified the location of the linear plasmids in the *in situ* lysis preparations (lane c).

After this TAFE gel was stained with ethidium bromide, it was subjected to electrophoresis under the same conditions. The migration of the supercoiled plasmid changed when compared with the other DNA bands in the gel (Fig. 4 *Right*, "Post-EB"). Whereas the supercoiled plasmid initially had an apparent size of 800 kb, the apparent size of this plasmid after ethidium bromide treatment was 440 kb (lower arrowheads in Fig. 4 *Right*). The stained gel also showed fainter bands in lanes b and c (upper arrowheads in Fig. 4 *Right*) with an apparent size of 650–680 kb; these minor bands also appear to derive from the supercoiled plasmids and may be nicked forms of the plasmids. In contrast to the supercoiled plasmid fraction, the abundant DNA form and the linear plasmids of *B. burgdorferi* did not change in migration with respect to the linear yeast chromosomes.

This experiment demonstrated that the "800-kb" band released from the *B. burgdorferi* blocks in TAFE gels was the supercoiled plasmid of *B. burgdorferi*. The lack of effect of ethidium bromide on the relative migration of the megabase-sized DNA suggested that it, like the yeast chromosomes, was linear and not supercoiled circular DNA. Additional evidence for the latter conclusion was provided by experiments in which the pulse time for the TAFE was changed from 60 to 30 sec. Under these conditions the supercoiled plasmid had an apparent size of 510 kb instead of 800 kb; the large form of *B. burgdorferi* DNA had the same apparent size (i.e., 950 kb) with respect to the yeast chromosomes (data not shown). Inasmuch as alterations of pulse times in pulsed-field gels affect the mobilities of linear DNA molecules to a much greater extent than supercoiled circles (19), it was more likely that the yeast chromosomes and the large *Borrelia* DNA rather than the supercoiled plasmids changed in migration rate.

In another study of the structure of the megabase-sized DNA we used ethidium bromide and UV irradiation to produce single-strand breaks in the DNA (20) and examined the effect of subsequent heat denaturation (Fig. 5). We supposed that if the megabase-sized DNA were actually an abundant supercoiled molecule, introduction of nicks to form open circles would change the migration of the DNA (20). Agarose blocks containing total DNA of *B. burgdorferi* were first treated with ethidium bromide and then exposed to short-wave UV light for 2 min (lanes a and b), 5 min (lanes c and d), or 10 min (lanes e and f). Other blocks (lanes g and h) were not subjected to this UV exposure. The heat denaturation step provided confirmation that single-strand breaks had been introduced. With longer periods of exposure of the ethidium bromide-treated DNA to UV light, greater breakage was evident upon heat denaturation (lanes d and f). However, introduction of several single-strand breaks did not alter the migration of undenatured large DNA (lanes c and e). Of additional note was the apparent ability of infrequently nicked large DNA to rapidly reanneal after heat denaturation (lanes b and g).

These experiments indicated that megabase-sized *B. burgdorferi* DNA was not a supercoiled circle. Although evidence to date suggests that open circular DNA migrates through a pulsed-field gel system, such as TAFE, very slowly if at all (19, 21), we addressed the possibility that megabase-sized DNA of *B. burgdorferi* was an open circle by using two-dimensional agarose gel electrophoresis. Open circular phage λ DNA served as a control; it along with linear phage λ DNA were driven a short distance into the agarose gel with a constant electrical field of low voltage. Agarose blocks of *B. burgdorferi* and *S. cerevisiae* were then loaded, and TAFE was performed in the first dimension. Constant-field electrophoresis was conducted in the second, perpendicular direction by using a high-voltage gradient (Fig. 6). The open circles of phage λ DNA, indicated by "a" in Fig. 6, entered the agarose gel during low-voltage constant-field electrophoresis but failed to migrate any further during TAFE or high-voltage electrophoresis. In contrast, the linear form of phage λ DNA ("b") migrated in both dimensions. The putative chromosome ("c"), supercoiled circular plasmids ("d"), and linear plasmids ("e") of *B. burgdorferi* also migrated in both dimensions as did most of the chromosomes of *S. cerevisiae*. Of note was the slower migration in the second dimension of the supercoiled plasmid of *B. burgdorferi* compared to the megabase-sized DNA of this species. This phenomenon presumably was a function of agarose concentration and field strength (20, 22). The chromosomes of *S. cerevisiae* of 950 kb and above slowed or arrested in migration in the second direction.

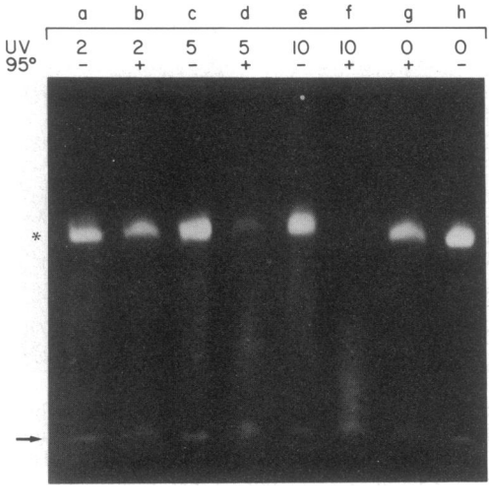

FIG. 5. Effect of single-strand nicks and heat denaturation on the TAFE migration of *B. burgdorferi* DNA. Ethidium bromide-treated blocks (lanes a–h) were exposed to UV light for various lengths of time (min). DNA in some blocks was then denatured at 95°C (+). The asterisk indicates DNA with an apparent size of 950 kb. The arrow indicates the 49-kb linear plasmid of *B. burgdorferi*.

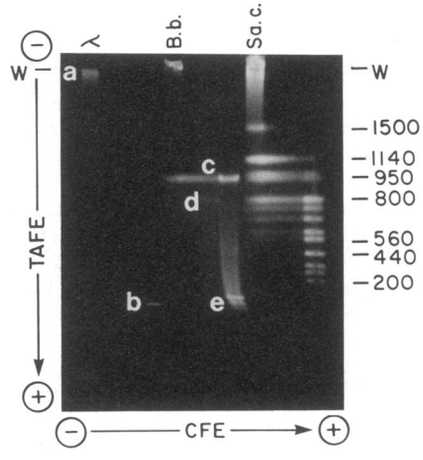

FIG. 6. Two-dimensional gel electrophoresis of open circular and linear forms of bacteriophage λ DNA (λ), total *B. burgdorferi* DNA (B.b.), and *S. cerevisiae* chromosomes (Sa.c.). W indicates the line of wells in the agarose gel. First dimension: TAFE. Second dimension: constant-field electrophoresis (CFE). The lower-case letters indicate the following: a, phage λ open circular DNA; b, phage λ linear DNA; c, the large DNA of B.b; d, supercoiled plasmids of B.b.; and e, linear plasmids of B.b. The relative migrations in the first dimension of selected Sa.c. chromosomes are shown on the right.

DISCUSSION

When the cells of *B. burgdorferi* were lysed in agarose and the preparation was subjected to pulsed-field electrophoresis, the bulk of DNA entered the gel and migrated with an apparent size of 900–1000 kb. This phenomenon has not, as far as we know, been noted when the genomes of other eubacteria have been examined by pulsed-field electrophoresis. Studies of *Escherichia coli* (13), *Hemophilus influenzae* (23), and mycoplasmas (24) showed that their chromosomes migrated in pulsed-field gels only if the DNA had been first fragmented with restriction enzymes or DNase. The estimated sizes of the chromosomes of these bacteria ranged from 900 kb for the mycoplasma *Ureaplasma urealyticum* (24) to 4700 kb for *E. coli* (13). The failure of intact bacterial chromosomes to enter the gel was attributed to the circularity of eubacterial chromosomes (13). Movement in the pulsed-field gel was thought to be impeded because open loops of the chromosome are impaled on spurs of agarose (17). The comparative ease with which the large DNA of *B. burgdorferi* traveled the gel suggested that it was either a supercoiled closed circle or a linear molecule. Subsequent experiments were designed to determine if the large form of DNA was linear or circular.

A means to precisely define the chromosome of a borrelia is lacking at present; a borrelia's genome size had not been estimated by other means. There are no borrelia genes that are known to be chromosomal and have been cloned. Even a primitive map of a borrelia's genome has not been done; mutants have yet to be isolated. Our conclusion that the large form of DNA was probably the borrelia's chromosome was based on these findings: (*i*) the majority of thymidine-labeled or hybridizable DNA in the lysed cells was accounted for by the large DNA band in the TAFE gels, and (*ii*) digests with infrequently cutting restriction enzymes indicated that *B. burgdorferi* has a chromosome size of ≈900 kb.

The migratory behavior of the DNA under different electrophoretic conditions and after various treatments indicated that the megabase-sized band was linear duplex DNA. In all cases the presumed chromosome of *B. burgdorferi* behaved like the linear chromosomes of *S. cerevisiae* (25) and not like supercoiled circular molecules (19). We confirmed that open circular DNA even as small as 47 kb in circumference would not migrate in the TAFE gels or under high-voltage gradient conditions (17, 19, 21).

It is possible that the megabase-sized DNA represented a circular chromosome that had been sheared during the lysis treatment or electrophoresis. However, we think this was unlikely for the following reasons: (*i*) The DNA bands observed were discrete; there were not preceding or trailing smears of smaller or larger fragments. (*ii*) The band was the same size when different preparations were examined and when other *in situ* lysis procedures, including the method used to lyse *E. coli* cells in agarose blocks (13), were used. The large *Borrelia* DNA also had the same migration relative to yeast chromosomes when examined by field-inversion gel electrophoresis and clamped homogeneous electric field electrophoresis (unpublished observations). Nevertheless, we cannot rule out the possibility that the *Borrelia* chromosome is truly circular in the cell and that during lysis either a protein binding the two ends together is released or an infrequently cutting nuclease severs a circular chromosome once. Further study of the termini of these large linear molecules should help to determine whether they are circularly permuted and whether they have covalently closed ends like *Borrelia* linear plasmids (5).

The finding in a *Borrelia* sp. of linear DNA molecules ranging in size from 16 to ≈1000 kb leads to consideration of whether it would be more appropriate to use the term "minichromosome" rather than "plasmid" when referring to the smaller linear replicons. We earlier suggested that the linear plasmids were proviruses or the remnants of *Borrelia* viruses (5, 26). This may still be the case, but an argument that the shorter linear molecules derived from the chromosome itself can now be made.

A broad survey of different types of bacteria to see if their chromosomes migrate in TAFE or other forms of pulsed-field electrophoresis systems has not been done. It is possible that more bacterial species have linear, chromosome-sized DNA molecules. These structures of *B. burgdorferi*—and perhaps other bacteria—provide both opportunities and challenges to those studying the origins and evolution of prokaryotic and eukaryotic organisms.

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