

Transduction by ϕ BB-1, a Bacteriophage of *Borrelia burgdorferi*

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We previously described a bacteriophage of the Lyme disease agent *Borrelia burgdorferi* designated ϕ BB-1. This phage packages the host complement of the 32-kb circular plasmids (cp32s), a group of homologous molecules found throughout the genus *Borrelia*. To demonstrate the ability of ϕ BB-1 to package and transduce DNA, a kanamycin resistance cassette was inserted into a cloned fragment of phage DNA, and the resulting construct was transformed into *B. burgdorferi* CA-11.2A cells. The *kan* cassette recombined into a resident cp32 and was stably maintained. The cp32 containing the *kan* cassette was packaged by ϕ BB-1 released from this *B. burgdorferi* strain. ϕ BB-1 has been used to transduce this antibiotic resistance marker into naive CA-11.2A cells, as well as two other strains of *B. burgdorferi*. This is the first direct evidence of a mechanism for lateral gene transfer in *B. burgdorferi*.

Borrelia burgdorferi, the causative agent of Lyme disease (5, 32), has a complex genome that contains both linear and circular DNA (7, 13). A growing number of tools have been developed for studying the molecular biology of this organism, although elaborating a convenient manipulatable genetic system will require further effort. Despite indirect evidence for lateral gene transfer in these bacteria (19, 33, 38), natural mechanisms for the transfer of genetic material (including conjugative plasmids and transducing bacteriophages) remain elusive for *B. burgdorferi* and related spirochetes.

In a number of other bacteria, bacteriophages are well characterized, naturally occurring mechanisms for lateral gene transfer (22). Bacteriophages have been observed in association with many spirochetes (reviewed in references 10 and 11), including *Borrelia* spp. (3, 12, 14, 23), *Leptospira biflexa* (29), and *Brachyspira hyodysenteriae* (15, 27). VSH-1, a generalized transducing phage of *B. hyodysenteriae*, has been used to demonstrate the transfer of antibiotic resistance markers between two different strains of this spirochete (16). Recently, a prophage from *L. biflexa* was developed into a shuttle vector (28). To our knowledge, no transduction or any other naturally occurring mechanism for the introduction of exogenous DNA has been demonstrated in *B. burgdorferi* or related species.

We previously reported the preliminary characterization of a temperate bacteriophage (Fig. 1) (10–12), designated ϕ BB-1, which packages the 32-kb circular plasmids (cp32s) of the *B. burgdorferi* genome. The cp32s are a family of extrachromo-

somal elements, several of which can be maintained in a single cell (7, 8, 35). There are three regions of variability on these homologous plasmids: two that encode various lipoproteins, and one that may encompass a possible partitioning region (1, 6–8, 17, 24, 25, 33–36, 40, 42). Sequence analysis of these molecules suggests that they have evolutionarily undergone recombination, possibly from both endogenous and exogenous sources (6–8, 20, 33, 35, 38). Many features of the cp32 family are consistent with the hypothesis that these plasmids are temperate prophage genomes (7–11).

To elucidate a possible biological role for ϕ BB-1 as well as evaluate its use as a potential molecular tool, we have extended our characterization of this phage. Using a DNA fragment from a partial library of ϕ BB-1 DNA, we have constructed a recombinant cp32 (ϕ BB-1 prophage) carrying a kanamycin resistance cassette. Subsequently, ϕ BB-1 was used to transduce this recombinant cp32 to other strains of *B. burgdorferi*. The introduction of an antibiotic resistance marker into naive cells by ϕ BB-1 is the first direct demonstration of lateral gene transfer in *B. burgdorferi*.

MATERIALS AND METHODS

Bacterial strains and bacteriophage recovery. *B. burgdorferi* sensu stricto strains CA-11.2A (21) and high-passage B31-UM were part of our collection. High-passage *B. burgdorferi* sensu stricto strain 1A7, a clone of Sh2-82, was generously provided by Tom Schwan (Rocky Mountain Laboratories). Bacterial isolates were routinely cultivated in modified Barbour-Stoenner-Kelly (BSK-H) complete medium (Sigma, St. Louis, Mo.) at 34°C with a 5% CO₂ atmosphere. Culture densities were determined, and ϕ BB-1 particles were recovered from cultures induced with 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) as described previously (12). Phage particles were concentrated, stained, and visualized by transmission electron microscopy using a protocol described elsewhere (10).

Agarose gel electrophoresis. DNA for restriction digests was extracted as described previously (12) and digested with different restriction enzymes as instructed by the manufacturer (New England Biolabs, Beverly, Mass.). DNA samples were resolved in 0.8% agarose gels (SeaKem GTG; Bio*Whittaker Molecular Applications; Walkersville, Md.) in TBE (45 mM Tris-borate, 2 mM EDTA) at 80 V (4.3 V cm⁻¹). Field-inversion gel electrophoresis (FIGE) was performed at room temperature using a PPI-200 programmable power inverter (MJ Research, Waltham, Mass.) with optimal programs and times for separating the desired range of fragments determined using the GelTimes software supplied

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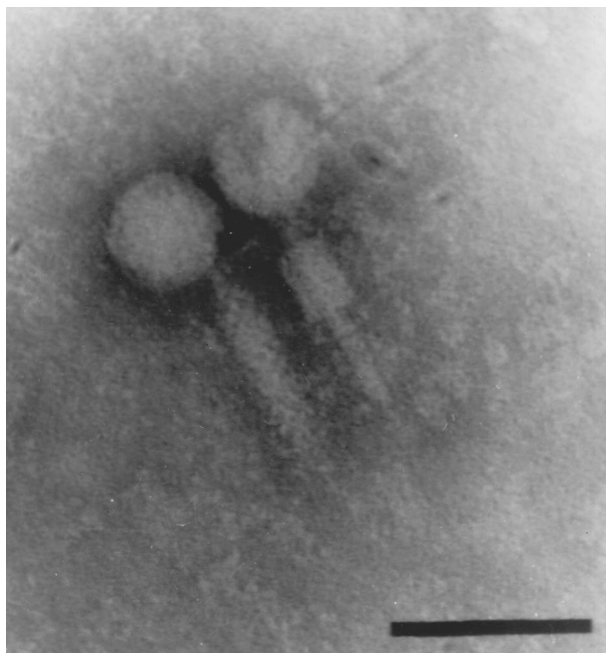


FIG. 1. *B. burgdorferi* phage particles. Samples were collected from polyethylene glycol-precipitated cell supernatants of an MNNG-treated culture of *B. burgdorferi* CA-11.2A and viewed by transmission electron microscopy. Although previously reported as having a simple noncontractile tail (12), subsequent modifications to the purification and preparation protocol (10, 11) reveal that all phage particles observed have intact contractile sheaths, seen here either extended (left) or contracted (right). Phosphotungstic acid stain. Bar, 45 nm.

by the manufacturer. The running buffer was supplemented with 100 mM glycine (as suggested by MJ Research), and the buffer was recirculated during electrophoresis. After electrophoresis, gels were stained with 0.5 μ g of ethidium bromide (EtBr) ml^{-1} for 0.5 to 1 h and destained in water for 1 to 2 h. The DNA was visualized on a UV transilluminator, and images were captured on a Gel Doc 1000 system (Bio-Rad, Hercules, Calif.). Two-dimensional gel electrophoresis and genomic DNA extraction were performed essentially as described previously (12, 31).

Southern hybridization. Agarose gels to be blotted were prepared and run as described above. After visualization, the gels were destained in water and then vacuum blotted to Immobilon-Ny⁺ (Millipore, Bedford, Mass.) as described elsewhere (18). Southern hybridization was performed as previously described (12). Approximate sizes of the major fragments were determined by comparison to markers of known sizes. Blots to be reprobated were stripped in increasingly stringent solutions as described elsewhere (2).

The conserved cp32-specific probes used for Southern hybridizations were probe 4 (8) and a probe that flanks an *NdeI* site (cp32SK12NdeI) in the paralogous family (PF) gene 50. Probes were generated from *B. burgdorferi* genomic DNA by PCR with *Taq* polymerase as described by the manufacturer (Sigma) using an annealing temperature of either 50°C (probe 4) or 44°C (probe cp32SK12NdeI). Additionally, the pOK12 plasmid was used as a probe for the *kan* cassette. All probes were labeled using the Prime-it II kit as instructed by the manufacturer (Stratagene, La Jolla, Calif.).

Variable region PCR. A ClustalW alignment (MacVector 6.5.1; Oxford Molecular, Madison, Wis.) of the available *B. burgdorferi* B31 cp32 sequences from GenBank (7) was performed to identify possible diagnostic variable regions. Oligonucleotides to highly conserved sequences flanking one variable region, designated VR1, were designed using MacVector (Table 1). The VR1s were amplified from either cellular cp32s or phage DNA using *Taq* polymerase and the cp32-VR1 primers with 25 cycles of 92°C for 1 min, 50°C for 1 min, and 72°C for 3 min. The PCR products were resolved on 0.8% agarose gels subjected to FIGE, as described above. The PPI-200 power inverter was programmed with a maximum resolution in the range of 2 to 6 kb. The sizes of the amplified VR1s were determined using Multi-Analyst Software 1.0 (Bio-Rad).

Construction of pCE210. *HindIII*-digested ϕ BB-1 DNA was ligated into the *HindIII* site of pBluescript II SK⁺ (Stratagene, La Jolla, Calif.), generating a

library that contained random phage DNA fragments of less than or equal to 4 kb. A plasmid with a 4-kb insert was selected and designated pCE100. The insert was found to contain one *NdeI* site approximately 1.1 kb from one end (Fig. 2A).

The 1.3-kb *flg* promoter-*kan* fusion conferring kanamycin resistance in *B. burgdorferi* was excised from pTAKanG (4) by *EcoRI* digestion. Both pCE100 linearized by digestion with *NdeI* and the purified *kan* cassette were treated with mung bean nuclease (New England Biolabs) to blunt the ends of the DNA as instructed by the manufacturer. The *kan* cassette was subsequently ligated into pCE100, resulting in pCE210 (Fig. 2A).

Transformation of *B. burgdorferi* CA-11.2A with the *kan* cassette. pCE210 was digested with *Bss*HII, and the insert containing the recombinant ϕ BB-1 DNA-*kan* cassette was gel purified with a Qiaex gel extraction kit (Qiagen, Valencia, Calif.). Purified insert DNA (10 μ l, \sim 2 μ g) was electroporated into competent *B. burgdorferi* CA-11.2A cells and plated in solid medium as described previously (30). Transformants were selected in solid BSK at 34°C in the presence of 500 μ g of kanamycin per ml. Colonies were screened by PCR using the cp32SK12NdeI primers (Table 1 and Fig. 2) as described above. PCR products were resolved on 1% agarose gels and stained with GelStar nucleic acid stain (Bio*Whittaker Molecular Applications).

Transduction assays. Phage particles were purified from the cell supernatants of the CA-11.2A/*kan*^R transformant (12) and treated with an additional 10% chloroform (CHCl_3) to eliminate possible cellular contamination, incubated at room temperature for 15 min, and then centrifuged at 14,000 \times g, and the aqueous phase was recovered. Sterile 1 M MgCl_2 was added to the sample to bring the final Mg^{2+} concentration to 16 mM, and 1 μ l of RQ1 DNase (Promega, Madison, Wis.) was added per 250 μ l of volume. After 0.5 h at 37°C, 100 μ l of the prepared phage sample (\sim 35 ng of phage DNA; the equivalent of \sim 10⁹ phage) was mixed with 10⁷ cells (100:1), and BSK-complete was added for a final volume of 1 ml.

After 16 h of incubation at 34°C, the phage-cell mixtures were plated, and potential transductants were selected on solid medium with 500 μ g of kanamycin per ml. Colonies were picked and screened by PCR with the cp32SK12NdeIF and Kan^R1207F primers (Table 1 and Fig. 2A) using the same parameters as for the cp32SK12NdeI primer pair. Positive clones were transferred into 10 ml of BSK-complete with 500 μ g of kanamycin per ml. The frequency of transduction was determined as the number of positive transductants divided by the total number of CFU.

When the putative transductants reached approximately 5×10^7 cells ml^{-1} , DNA was extracted from the culture and the clones were screened again by PCR for the *kan* cassette using the above primers. Additionally, using the Kan^R1207F primer and the cp32-VR1R primer, a 7.5-kb region of DNA was amplified (25 cycles of 92°C for 30 s, 50°C for 30 s, and 72°C for 6 min) with *Taq*Plus long (Stratagene) from each transductant.

Analysis of recombinants. Plasmid DNA was extracted from log-phase cultures of *B. burgdorferi* using Wizard Plus Midi preps (Promega). The concentration of DNA was determined by measuring the absorbance at 260 nm. A total of 500 ng of DNA was digested with either *EcoRV* or *XbaI*, and the DNA was resolved by FIGE. Following electrophoresis and staining, the gels were vacuum blotted, probed, stripped, and reprobated as described above.

RESULTS

Determining the minimum number of cp32s present in a population of cells or phage. Specific probes to individual *B. burgdorferi* B31 cp32s (8) did not hybridize to *B. burgdorferi* CA-11.2A cp32s (data not shown). To determine an approximate number of cp32s in both *B. burgdorferi* cells and ϕ BB-1 capsids, oligonucleotides that flank a diagnostic variable region (designated VR1) were designed (Table 1). VR1 encompasses

TABLE 1. Oligonucleotides generated for this study

Oligonucleotide	Sequence (5' to 3')	Application
cp32-VR1F	AAATAAAACTTAGGAGTTGGTTTTGAA	Diagnostic PCR
cp32-VR1R	TAACITTCCTAGCGTTAACTTCTGAT	Diagnostic PCR
cp32SK12NdeIF	ACTTTGTGTAGTGATTATTTGTTC	Screening; probe
cp32SK12NdeIR	GGGGAAAGAATTGTTGAAG	Screening; probe
Kan ^R 1207F	ATTACGCTGACTTGACGGG	<i>kan</i> cassette screening

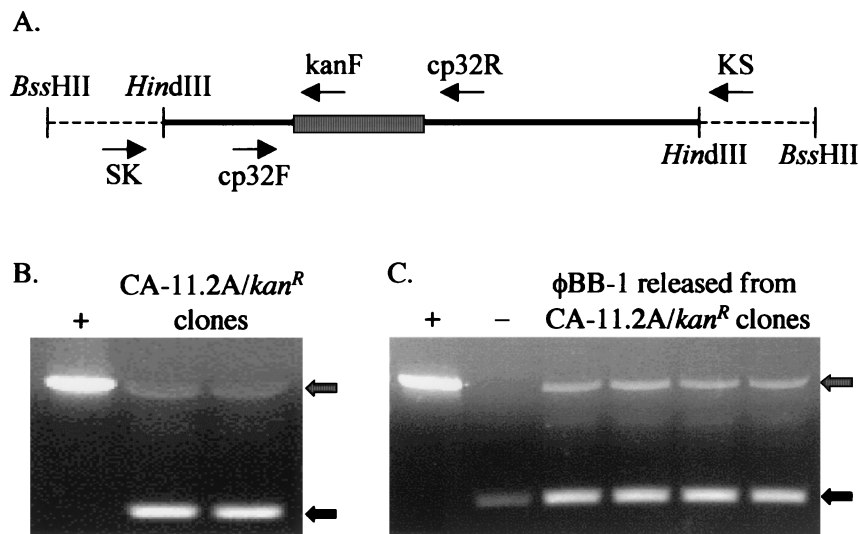


FIG. 2. Kanamycin-resistant transformants of *B. burgdorferi*. (A) pCE210 construct. A 4-kb fragment of phage DNA (solid line) was cloned into the *Hind*III site of pBluescript SK+ (dashed line). The *kan* cassette (hatched box) was cloned into a unique *Nde*I site on the phage fragment 1.1 kb from the SK primer location. The locations of the SK, KS, cp32SK12NdeIF (cp32F), cp32SK12NdeIR (cp32R), and Kan^R1207F (kanF) oligonucleotides are shown (not drawn to scale). Arrows indicate oligonucleotide site and orientation. (B) Transformed CA-11.2A colonies were screened by PCR using the cp32SK12NdeI primers. PCR products were resolved on a 1% agarose gel and stained with EtBr. Both a small product (no *kan* cassette; black arrow) and a larger product (integrated *kan* cassette; hatched arrow) were amplified from the transformants. (C) Products consistent with a population of cp32s both containing the *kan* cassette (hatched arrow) and lacking the *kan* cassette (black arrow) were also amplified from phage DNA collected from CA-11.2A/kan^R transformants. pCE210 DNA was amplified as a positive (+) control, and wild-type CA-11.2A phage DNA was used as a negative (-) control.

a portion of each cp32 where the *ospE/ospF/elp* genes (*erp* loci) are located (1, 6, 7, 20, 25, 35). The cp32-VR1R primer (27 nucleotides) is conserved on all the *B. burgdorferi* B31 cp32s and the cp32-like molecule integrated into lp56 (7). The cp32-VR1F primer (26 nucleotides) has one mismatch on cp32-9 and two mismatches on the cp32-like molecule integrated into lp56, but is conserved on all the other B31 cp32s. PCR amplification of VR1 with these oligonucleotides generates products of different sizes for several of the *B. burgdorferi* B31 cp32s whose sequences are available (Table 2).

Using the cp32-VR1 primers and FIGE for maximum resolution, we identified a minimum of four different cp32s and homologous molecules (i.e., lp56) present in *B. burgdorferi* CA-11.2A (Table 3; Fig. 3, lane 1) and also a minimum of four different cp32s (or homologous molecules) present in *B. burgdorferi* strain B31-UM (Table 3; Fig. 3, lane 3). The ϕ BB-1

phages released from CA-11.2A (Fig. 3, lane 2) and B31 cells (Fig. 3, lane 4) package cp32s that are represented by only three of the VR1s amplified from the respective hosts. The absence of the ~2.8-kb band (Table 3) from both ϕ BB-1 samples suggests that the phage does not package the cp32 that is integrated into lp56 (or any DNA that is packaged from the lp56 does not include the targeted region).

Inserting the kanamycin resistance cassette into a cp32. A construct containing a 1.3-kb *kan* gene integrated into a fragment of phage DNA (see Fig. 2A) was transformed into *B. burgdorferi* CA-11.2A cells. Colonies selected in 500 μ g of kanamycin per ml were screened by PCR using the cp32SK12NdeI primers (Table 1) that flank the site into which the *kan* cassette had been inserted (Fig. 2A and B). Partial sequence obtained from the fragment of phage DNA (data not

TABLE 2. Predicted fragment sizes^a of VR1 of the B31 cp32s

<i>B. burgdorferi</i> B31 plasmid	Amplicon size (bp)
cp32-1	3429 ←
cp32-3	2442
cp32-4	3377 ←
cp32-6	2507
cp32-7	3575
cp32-8	3429 ←
cp32-9	3367 ←
lp56	2796

^a As determined by MacVector software using GenBank accession numbers AE001575, AE001576, AE001577, AE001578, AE001579, AE001580, AE001581, and AE001584. Black arrows and hatched arrows indicate two pairs of VR1s that are indistinguishable by our methods.

TABLE 3. Amplification of the VR1s of CA-11.2A, B31, and ϕ BB-1^a

CA-11.2A		B31-UM		Predicted B31 plasmid
Total DNA	Phage DNA	Total DNA	Phage DNA	
		3,426	3,426	cp32-1 or 8
		3,343	3,343	cp32-9 or 4
3,047	3,047			
2,819		2,828		lp56
2,537	2,537			
		2,484 ^b	2,484	cp32-3 ^b
2,455	2,455			

^a Sizes determined by regression using Multi-Analyst software; predictions based on size estimates from ≥ 3 trials and applicable only to the B31 fragments. ^b Prediction confirmed by sequencing of VR1.

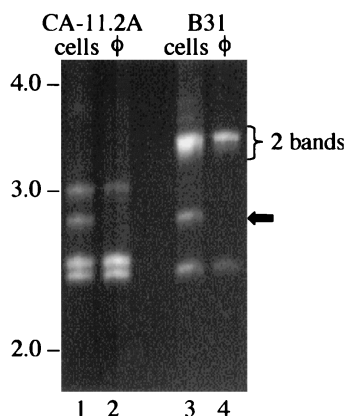


FIG. 3. Determining the minimum number of cp32 molecules in *B. burgdorferi* cells and ϕ BB-1 capsids by PCR amplification of VR1. Highly conserved primers that flank a variable region (VR1) of cp32 amplify four different size products from *B. burgdorferi* strains CA-11.2A and B31. The two VR1s from the cp32s of B31 at ~ 3.3 kb can be resolved with extended electrophoresis times (see Fig. 7). PCR of ϕ BB-1 released from both of these strains amplifies three of the VR1 products (lanes 2 and 4), but not the ~ 2.8 -kb amplicon (black arrow; see Table 2). Fragments were resolved on a 0.8% agarose gel by FIGE and stained with EtBr. Molecular sizes (in kilobase pairs) are indicated.

shown) indicates that the *kan* gene was inserted into the PF50 paralog of cp32. The cp32SK12NdeI primers amplify a product from both the recombinant cp32 (~ 1.4 kb) and the other homologous cp32 loci in the cells that do not contain the insert (~ 100 bp) (Fig. 2B). Both products are expected in a population of *B. burgdorferi* cells containing more than one cp32 if, as anticipated, the fragment bearing the *kan* cassette does not recombine with all of the cp32s. The ϕ BB-1 capsids collected from the supernatants of these CA-11.2A transformants include genomes containing the *kan* gene as well as uninserted parental phage genomes (Fig. 2C). These data are consistent with the results from the variable-region analysis, indicating that more than one cp32 is packaged in a population of ϕ BB-1 phage heads (Fig. 3, lanes 2 and 4).

The genomic location of the integrated *kan* cassette was determined by Southern hybridization (Fig. 4B and C) of total DNA resolved by two-dimensional electrophoresis (Fig. 4A). The blot of the gel was probed with cp32-specific probe 4 to localize the circular 32-kb molecules (Fig. 4B). The membrane was also probed with pOK12, the original source of the kanamycin resistance gene (4, 39) (Fig. 4C). pOK12 (the *kan* probe) hybridizes only to the CA-11.2A/*kan*^R transformant (Fig. 4C) and has the same hybridization pattern as the cp32-specific probe 4 (Fig. 4B), locating the integration site of the *kan* cassette to a cp32. Extracted phage DNA (Fig. 5A) was also analyzed. Probe 4 (Fig. 5B) hybridizes to phage DNA released from both the parental and the transformed CA-11.2A; however, the *kan* cassette probe hybridizes to only the ϕ BB-1 DNA packaged and released by CA-11.2A/*kan*^R (Fig. 5C).

Transduction. To evaluate the ability of ϕ BB-1 to transduce the *kan* gene into kanamycin-susceptible cells, bacteriophage from the CA-11.2A/*kan*^R transformant were incubated with CA-11.2A cells at an approximately 100:1 multiplicity of infection. After an overnight incubation, the cells were plated in 500

μ g of kanamycin per ml. We screened 10 of ~ 100 colonies by PCR. All the colonies screened contained the *kan* gene integrated into a cp32 (Fig. 6A and data not shown).

The frequency of transduction of ϕ BB-1 between CA-11.2A cells is $\sim 10^{-5}$ to 10^{-6} per cell. Alternatively, the frequency of transduction by ϕ BB-1 is $\sim 10^{-7}$ per phage particle (~ 100 transductants from $\sim 10^9$ phage particles). No colonies grew when either CA-11.2A cells without ϕ BB-1/*kan*^R or ϕ BB-1/*kan*^R phage without cells were plated in kanamycin. The addition of proteinase K to ϕ BB-1/*kan*^R prior to incubation with cells abrogated the transduction of the antibiotic resistance marker. Incubating washed, CHCl₃-killed CA-11.2A/*kan*^R cells

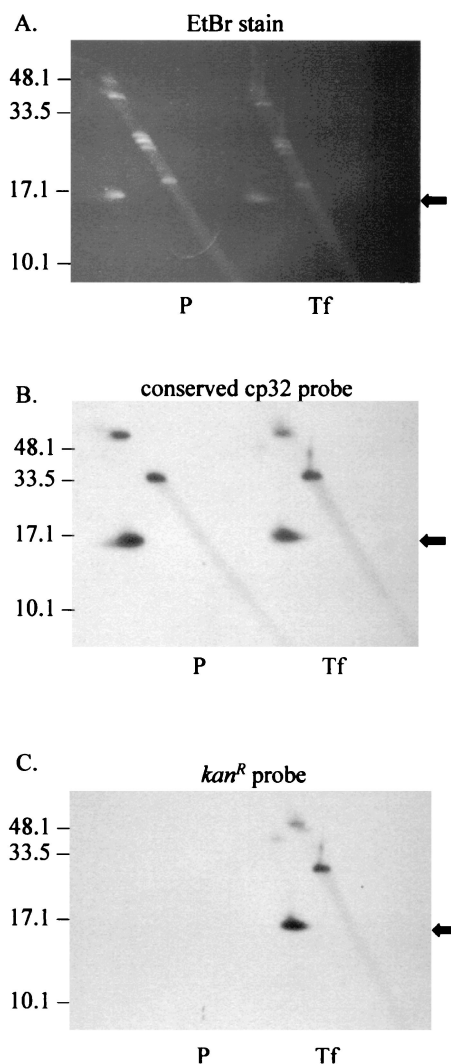


FIG. 4. Analysis of the genomic location of the kanamycin resistance cassette. Total DNA from both parental CA-11.2A (P) and a transformed clone of CA-11.2A/*kan*^R (Tf) was extracted and resolved by two-dimensional gel electrophoresis. The gel was stained with EtBr (A), blotted to nylon, and probed with the cp32-specific probe 4 (B) or pOK12, the source of the *kan* gene (C). The *kan* probe has the same hybridization pattern as the cp32-specific probe. The supercoiled form of cp32 is indicated by the black arrow, while the other hybridization sites likely represent the nicked (or lp56) and linearized forms of cp32 generated during DNA isolation. Molecular sizes (in kilobase pairs) are indicated.

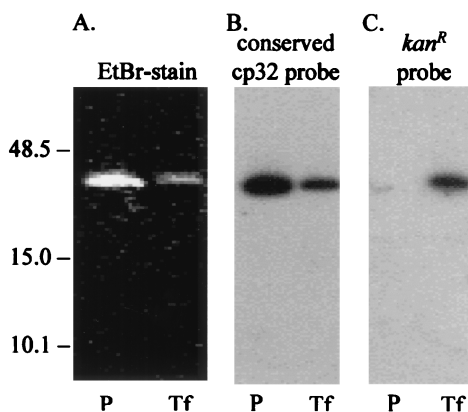


FIG. 5. Analysis of *kan* cassette packaging by ϕ BB-1. Phage particles were precipitated from both parental (P) and transformed (Tf) CA-11.2A cells. The DNA was extracted and resolved on a 0.5% agarose gel by conventional field electrophoresis. The gel was stained with EtBr (A), then blotted and probed with either probe 4 (cp32-specific; B) or pOK12 (*kan*; C). Molecular sizes (in kilobase pairs) are indicated.

with live CA-11.2A cells resulted in 0 to 3 colonies. However, no colonies grew when proteinase K was added to the dead transformant cells prior to mixing them with susceptible cells, implying that even the small amount of transfer between dead CA-11.2A/*kan*^R cells and live CA-11.2A cells requires protein. These data indicate that phage ϕ BB-1 mediated the lateral gene transfer.

ϕ BB-1/*kan*^R isolated from the CA-11.2A transductant (CA-11.2A/TR3) was incubated with several other strains of *B. burgdorferi*. The *kan* cassette was transduced by ϕ BB-1/*kan*^R (CA-11.2A) into *B. burgdorferi* strains B31 and 1A7 (a high-passage clone of *B. burgdorferi* Sh2-82) (Fig. 6A). No demonstrable DNA transfer occurred when dead CA-11.2A/*kan*^R cells were incubated with 1A7 and B31 cells. Long-range PCR using an internal *kan* primer (Kan^R1207F) and the cp32-VR1R primer, which lies outside the cloned fragment approximately 7.5 kb from the insertion site, was done to verify the integration of the *kan* cassette into the cp32 molecule (Fig. 6B). A partial sequence of the *ospC* gene from the transductant B31/TR1 confirmed that the strain (or at least the cp26) was derived from B31 (data not shown).

The frequency of transduction by ϕ BB-1/*kan*^R (CA-11.2A) into B31 is $\sim 10^{-6}$ to 10^{-7} and into 1A7 is $\sim 10^{-5}$ to 10^{-6} for individual clones of these strains. Neither the 1A7 transductant (1A7/TR5) nor the B31 transductant (B31/TR1) assumes the prolific CA-11.2A phage-producing phenotype, although both B31 and B31/TR1 spontaneously produce low levels of ϕ BB-1. ϕ BB-1 (B31) is also capable of packaging the *kan* cassette from B31/TR1 (data not shown). The *kan* marker can be efficiently transduced back into naive B31 using ϕ BB-1/*kan*^R (B31), but we have not yet been able to transduce the gene back into susceptible CA-11.2A cells using ϕ BB-1/*kan*^R (B31).

Variable-region analysis of the transduced cp32s. We have demonstrated a mechanism for lateral gene transfer in *B. burgdorferi* via transduction by ϕ BB-1. To analyze possible changes in the cp32 population of the transductants, the VR1s of the uncloned parent strains CA-11.2A, B31, and 1A7, as well as the transduced clones CA-11.2A/TR3, B31/TR1, and 1A7/TR5,

were amplified and resolved by FIGE (Fig. 7A). The region of the cp32 that we have designated VR1 is located ~ 5 kb from the site of the *kan* insertion (7).

The fragments generated by PCR from the VR1 regions of the CA-11.2A transductant (TR3; Fig. 7A, lane 2) are identical to those of parental CA-11.2A (Fig. 7A, lane 1). The B31 transductant (TR1; Fig. 7A, lane 4) has lost the smallest VR1 (2,484 bp; corresponding to cp32-3) of the parental B31 (Fig. 7A, lane 3) and has gained a VR1 that is the same size as the second smallest CA-11.2A VR1 (2,537 bp; Fig. 7A, black arrow). The B31 transductant has also lost the VR1 corresponding to lp56 (2,828 bp). In addition, 1A7 has a small VR1 (2,373 bp; Fig. 7A, lane 5) that is missing from 1A7/TR5 (Fig. 7A, lane 6), and the transductant has gained a VR1 that is also the same size as the second smallest CA-11.2A VR1 (2,537 bp; Fig. 7A, black arrow). This analysis suggests that the VR1 from the introduced cp32, which contains the 2,537-bp VR1 (data not shown), has replaced the smallest VR1 fragment of both B31/TR1 and 1A7/TR5.

Whether the loss of the VR1 from the transductants is due to displacement of a resident plasmid, recombination by the *kan* gene into an extant plasmid, or the loss of a plasmid during

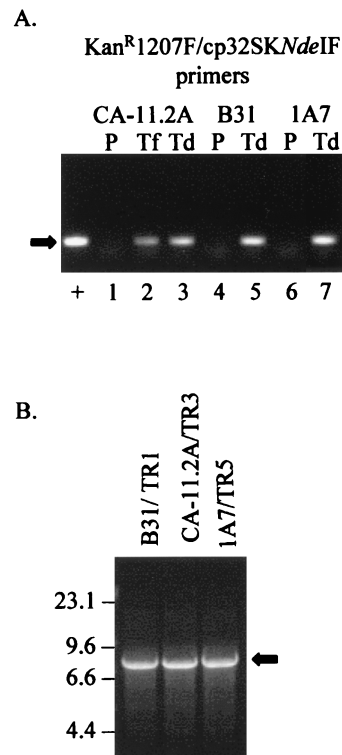


FIG. 6. PCR analysis of kanamycin-resistant transductants of *B. burgdorferi*. (A) Putative transductants from *B. burgdorferi* strains CA-11.2A, B31, and 1A7 were screened by PCR using the Kan^R1207F and cp32SK12NdeIF primers. pCE210 was amplified as a positive control (+), while the parental cells (P) of each strain served as negative controls (lanes 1, 4, and 6). Lane 2 is the CA-11.2A/*kan*^R transformant (Tf). PCR yields a ~ 125 -bp product from DNA containing the *kan* cassette (black arrow, lanes 2, 3, 5, and 7). The products were resolved on a 1% agarose gel and stained by EtBr. (B) The *kan* integration site in each transductant was verified by long-range PCR using the Kan^R1207F and cp32-VR1R primers.

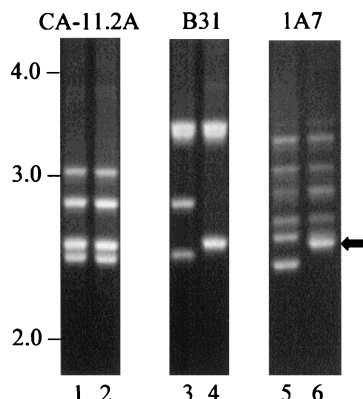


FIG. 7. Amplification of variable region VR1 from *B. burgdorferi* *kan* transductants. The cp32-VR1 primers were used to amplify the cp32 VR1s of parental CA-11.2A (lane 1), B31 (lane 3), and 1A7 (lane 5) as well as transductants CA-11.2A/TR3 (lane 2), B31/TR1 (lane 4), and 1A7/TR5 (lane 6). The 2,537-bp VR1 (black arrow) was found in all transductants. Amplification products were resolved on 0.8% agarose gels by FIGE and stained with EtBr. Molecular sizes are shown in kilobase pairs.

cloning and subsequent replacement with the ϕ BB-1/*kan*^R (CA-11.2A) prophage is not known. We have confirmed that the 2,537-bp VR1 is linked to the *kan* cassette on the transduced ϕ BB-1/*kan*^R (CA-11.2A) genome by amplifying the VR1 region from the *kan*^R/cp32-VR1R long-range PCR product (Fig. 6B and data not shown).

Restriction mapping of transductants. Plasmid DNA from CA-11.2A, CA-11.2A/*kan*^R (transformant), and CA-11.2A/TR3 (transductant) was extracted and digested with *Eco*RV to analyze changes in the restriction pattern of the cp32 molecules. The location of the *kan* cassette on cp32 was mapped using Southern hybridization (Fig. 8A).

There are four hybridization sites in the cellular cp32 population digested with *Eco*RV and probed with the cp32SK12NdeI probe (Fig. 8A, left panel). This includes a ~6.6-kb band (hatched arrow) in the parental CA-11.2A (Fig. 8A, lane 1). This *Eco*RV fragment is not present in either the CA-11.2A/*kan*^R transformant (Fig. 8A, lane 2) or the transductant, CA-11.2A/TR3 (Fig. 8A, lane 3). In the latter two strains, there is a ~8-kb fragment (Fig. 8A, black arrow) that is not found in the parental strain. The size difference is the same size as the *kan* cassette (~1.3 kb), suggesting that the *kan* cassette has been inserted into the 6.6-kb fragment. When the same blot is probed with the *kan* marker, the ~8-kb fragments in the CA-11.2A/*kan*^R transformant and in CA-11.2A/TR3 are the only hybridization sites (Fig. 8A, right panel, lanes 2 and 3, respectively). The ~6.6-kb fragment also corresponds to the major band in an *Eco*RV digest of ϕ BB-1 DNA probed with the cp32SK12NdeI probe (data not shown).

To compare the *kan* insertion site in the other transductants, plasmid DNA was extracted from parental CA-11.2A, B31, and 1A7 and the transductant CA-11.2A/TR3, B31/TR1, and 1A7/TR5 cells. The plasmids were digested with *Xba*I, and the DNA was resolved by FIGE. A blot of the gel was probed with both the cp32SK12NdeI PCR product and the *kan* cassette (Fig. 8B). When probed with the cp32SK12NdeI product (Fig. 8B, left panel), there is hybridization to a ~6.6-kb *Xba*I frag-

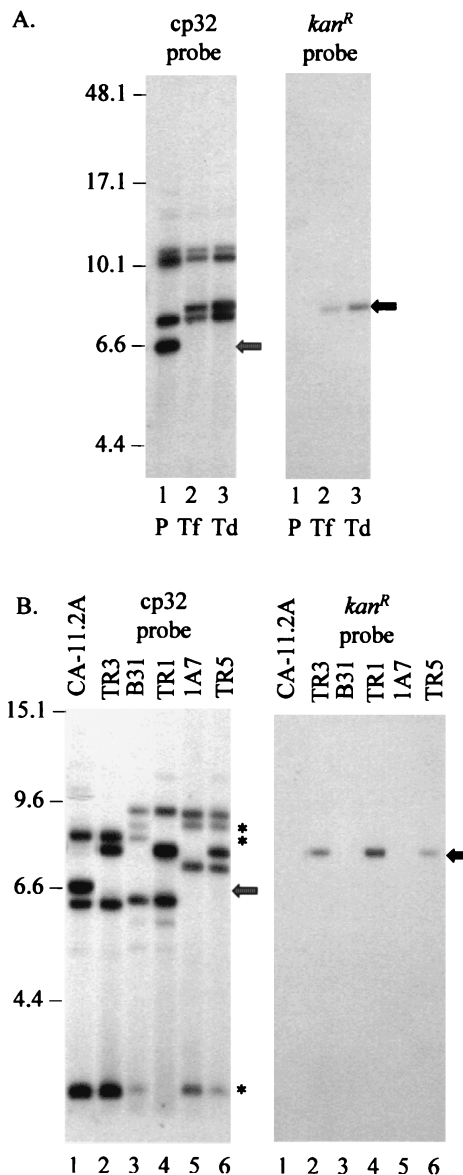


FIG. 8. Restriction mapping of the cp32s of *B. burgdorferi* *kan* transductants. (A) Comparison of the restriction maps of parental, transformant, and transductant CA-11.2A DNA. Plasmid DNA was extracted from CA-11.2A (lane 1; P), the CA-11.2A/*kan*^R transformant (lane 2; Tf), and CA-11.2A/TR3 (lane 3; Td), digested with *Eco*RV, and resolved by FIGE. A blot of the gel was probed either with the cp32SK12NdeI PCR product (left panel) or with *kan* (right panel). A unique ~6.6-kb fragment of CA-11.2A parental DNA is indicated by the hatched arrow. A ~8-kb band (black arrow) corresponding to the ~6.6-kb band plus the 1.3-kb *kan* insert is present in both the transformant and the transductant. (B) Comparison of the restriction maps of the cp32s of *B. burgdorferi* *kan* transductants. Plasmid DNA from CA-11.2A (lane 1), CA-11.2A/TR3 (lane 2), B31 (lane 3), B31/TR1 (lane 4), 1A7 (lane 5), and 1A7/TR5 (lane 6) was digested with *Xba*I and resolved by FIGE. A blot of the gel was probed with either the cp32SK12NdeI probe (left panel) or the *kan* probe (right panel). A ~8-kb band (black arrow) is found only in the transductants. The hatched arrow indicates the 6.6-kb fragment from the parental CA-11.2A strain (lacking the *kan* cassette). Three bands (*) appear in parental B31 but not B31/TR1. Molecular sizes are shown in kilobase pairs.

ment that is found exclusively in the parental CA-11.2A (Fig. 8B, lane 1; hatched arrow) and not in the parental B31 or 1A7. However, a \sim 8-kb fragment is present in all the transductant lanes (Fig. 8B, lanes 2, 4, and 6, black arrow). Hybridization with *kan* (Fig. 8B, right panel) verified that the \sim 8-kb fragment contained the *kan* cassette in all three transductants. This suggests that the *kan* cassette is on a cp32 in the transductants that is not a member of either of the parental B31 or 1A7 populations. The B31 parent has three bands that do not appear in B31/TR1 (Fig. 8B, left panel, lanes 3 and 4, asterisk), possibly due either to displacement of a cp32 or loss of plasmids (cp32 or lp56) during cloning. There are also three bands that are present in the B31 parent but absent from the transductant when plasmid DNA from these isolates is digested with *EcoRV* and probed with the cp32SK12NdeI probe (data not shown).

DISCUSSION

We recently identified ϕ BB-1, a temperate bacteriophage of *B. burgdorferi* (Fig. 1), whose prophage is in the cp32 family of circular plasmids (10–12). In this study we demonstrated that ϕ BB-1 can package all of the free cp32s within a population of cells (Fig. 3). To date, no known strain of *B. burgdorferi* carries fewer than three cp32s (7, 8, 26), and any or all of these cp32s may be prophages (associated with all of the necessary functions for lysogeny, progeny formation, and cellular lysis), or perhaps cryptic prophages, requiring other factors in *trans* for the successful propagation of ϕ BB-1. The effect that the protein products of the various cp32s (such as repressors) may have on each other in *trans* has not been studied.

Prior to this work, no mechanism for lateral gene transfer had been demonstrated in *Borrelia*. Previous evidence of recombination in at least one locus (*erp*, containing the *ospE*, *ospF*, and *elp* homologs) on the cp32s (33, 38) suggested that lateral gene transfer has occurred among these plasmids. Gene transfer could be between different cp32s within the same cell, by phage transduction of alternate cp32s within a population of cells, or by some other, as yet undefined, mechanism. In this study, the *kan* cassette was inserted into a fragment of phage DNA and electroporated into CA-11.2A cells, generating a recombinant cp32. To our knowledge, this is the first such integration into a member of the cp32 plasmid family.

Mapping and sequence data (not shown) indicate that the site of the *kan* cassette insertion is within the cp32–3 PF50 paralog (BBS34) of a CA-11.2A cp32, located near the variable region that includes the putative paralogous partitioning genes of the cp32s (7, 8, 36, 41). The cp32 containing the *kan* gene is stable over 20 passages (\sim 150 generations) in the absence of selection, suggesting that the disrupted open reading frame (ORF) (BBS34 homolog) either has no critical role in the molecular metabolism of this plasmid or has a function that can be supplied in *trans* by another cellular cp32. The PF50 paralog (BBC02) of the 9-kb circular plasmid (cp9), a derivative of cp32, is critical for the replication of a cp9-based vector (37), suggesting that the replication requirements for this smaller plasmid are different than those of the cp32s.

We have demonstrated transduction of the *kan* cassette by ϕ BB-1 into three different strains of *B. burgdorferi* (Fig. 6–8). This represents the first direct evidence of lateral gene transfer

in *B. burgdorferi*. The ability of ϕ BB-1 to transduce the antibiotic resistance marker into a strain is apparently unrelated to the ability of that strain to produce phage, since we have found that both low-passage B31 and high-passage 1A7 are transducible but not inducible (12; data not shown). Additionally, introducing the ϕ BB-1/*kan*^R (CA-11.2A) into these transducible strains does not confer the phage-producing phenotype of CA-11.2A on these strains.

The evidence suggests that the ϕ BB-1/*kan*^R genome is being introduced as a discrete plasmid into the cell during transduction. The host cell loses a VR1 marker concomitantly with gaining the ϕ BB-1 (CA-11.2A) VR1, suggesting the displacement of a host plasmid (Fig. 7). However, there are no obvious candidates discernible from the restriction maps (Fig. 8B). The PF50 paralog into which the *kan* cassette has been inserted is most similar to the PF50 paralog on cp32–3 of B31, the plasmid that is apparently absent in the transductant as determined by VR1 analysis. We hypothesize that the transduced cp32/*kan*^R from CA-11.2A phage displaced the resident cp32 homologs (carrying the smallest VR1s) in strains B31 and 1A7 (no cp32 data are currently available for the 1A7 parental strain Sh2–82). An alternate hypothesis is that prior loss of these cp32 homologs created populations of cells that were susceptible to transduction. B31 cp32–3 can be lost during cloning, and the loss of this plasmid has little or no effect on the infectivity of this isolate (26). Analysis of the plasmid profiles of several recent clones of CA-11.2A, B31, and 1A7 by PCR amplification of the VR1s suggests that the prior absence of the smallest VR1 is not required for transduction to occur, particularly in strain 1A7 (C. H. Eggers, B. J. Kimmel, and D. S. Samuels, unpublished data).

In this study, we have demonstrated a mechanism for lateral gene transfer that should be explored to establish its role within the infectious cycle or during the course of disease. Further analysis of ϕ BB-1, the first bacteriophage of *B. burgdorferi* described at a molecular level, could play a critical role in the investigation of plasmid genetics, the development of a genetic system, and the analysis of metabolic processes in these bacteria as a whole.

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