Transgenic Expression of RecA of the Spirochetes *Borrelia burgdorferi* and *Borrelia hermsii* in *Escherichia coli* Revealed Differences in DNA Repair and Recombination Phenotypes

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*Borrelia burgdorferi*, a cause of Lyme borreliosis, and *Borrelia hermsii*, an agent of relapsing fever, are spirochetes, a group of bacteria that are phylogenetically distinct from other major bacterial groups, such as the firmicutes, actinomycetes, proteobacteria, and cyanobacteria (10). The spirochete clade itself is deep, and its genera are highly diverse (34). Besides parasitic genera like *Borrelia* and *Treponema*, the spirochete group includes a large number of free-living species, such as *Leptospira biflexa*. Members of the genus *Borrelia* are not free living but instead cycle between two sets of hosts: arthropod vectors and mammalian or avian reservoirs (3). Compared to what is known about other types of bacteria, such as *Escherichia coli* and *Bacillus subtilis*, little is known about the genetics and physiology of spirochetes.

The *B. burgdorferi* genome, which comprises the 1-Mb chromosome and several linear and circular plasmids, has been sequenced (6, 15). The limited biosynthetic capabilities demonstrated by the genome sequence are consistent with the obligately parasitic existence of *B. burgdorferi* in nature and the high nutritional requirements for cultivation in the laboratory (2). *B. hermsii* has a similarly limited genome size and restricted ecologic niche (3, 14). A cross-hybridization array study of the genomic DNA of *B. hermsii* indicated that this species has much the same genetic complement as *B. burgdorferi* (55).

The genus *Leptospira* includes both free-living and parasitic species, and in one study a parasitic species, like *Leptospira interrogans*, was more susceptible to DNA-damaging agents than a free-living species, like *L. biflexa* (42). As a constant resident of the interiors of either ticks or vertebrates, neither *B. burgdorferi* nor *B. hermsii* is likely to have much exposure to DNA-damaging UV radiation compared to the exposure of the facultatively free-living species *E. coli* and *L. biflexa*. *B. burgdorferi* thrives best under microaerophilic conditions (53), an environment in which oxidative damage to the DNA in the organism should be limited (33). As a consequence of this comparatively sheltered lifestyle, the DNA repair functions of the RecA protein may be less critical for the genus *Borrelia* than for bacteria that exist in the environment. On the other hand, another RecA function, homologous recombination, may be very important for the persistence of *B. burgdorferi* and *B. hermsii* in their mammalian hosts. Both species exhibit antigenic variation due to frequent gene conversion events involving the multiple alleles for surface lipoproteins (reviewed in reference 1). Similar antigenic variation through gene conversion in *Neisseria gonorrhoeae*, the cause of human gonorrhea, has been shown to be largely mediated by RecA (22).

The RecA protein of *B. burgdorferi* was identified previously and was shown to have many of the conserved motifs of other bacterial RecA proteins (11). To assess the role of RecA in the frequency of recombination at the VlsE locus in *B. burgdorferi* (54), we attempted to produce a knockout of the *recA* gene of this species by insertional inactivation (4). While experiments with positive controls demonstrated that transformation and insertional inactivation of another gene could be achieved in *B. burgdorferi*, no viable clones with insertions into the *recA* gene were obtained after multiple attempts (Putteet-Driver, unpublished data). This suggested to us that inactivation of *recA* of *B. burgdorferi* was either lethal or highly debilitating to the cell.
Although recA mutants with insertional inactivation have been obtained for a number of bacterial species, such as Brucella abortus (48), N. gonorrhoeae (21), and Xanthomonas oryzae (28), these organisms have usually been proteobacteria. In these cases both DNA damage repair and homologous recombination were usually impaired in the recA mutant cells (27). The much less common examples of organisms with RecA knockouts that either are apparently lethal or result in extremely slow growth have been members of other bacterial groups, such as the actinomycete Streptomyces lividans (29) or the spirochete L. biflexa (49).

Lacking a means of producing a recA mutant line of B. burgdorferi or B. hermsii, we instead characterized the function of spirochete RecA in E. coli. A previous study by Stamm et al. demonstrated that complementation of E. coli recA mutants with the recA gene of L. biflexa was feasible (43), even with the considerable genetic difference between a proteobacterium like E. coli and spirochetes (10). In the present study, recA mutant E. coli cells were transformed with plasmids expressing recombinant recA genes of the spirochetes B. burgdorferi, B. hermsii, and L. biflexa, and the abilities of the recombinant proteins to complement the recombination and repair functions of the host E. coli cells were assessed. While the three spirochete recA genes complemented defects in E. coli in a homologous recombination assay with nearly equal activities, B. burgdorferi RecA and B. hermsii RecA were less able to complement DNA repair defects in E. coli than either recombinant E. coli RecA or L. biflexa RecA was.

MATERIALS AND METHODS

Strains and growth conditions. B. burgdorferi strain B31 (= ATCC 35210), B. hermsii strain HS1 (36), Borrelia caridae (23), and Borrelia turicatae strain Oz1 (5) were grown in BSK II broth medium (2). For the genomic library of B. hermsii the spirochetes were first grown in mice, and then the population was expanded once by 1:100 dilution in brook medium as described previously (2). The spirochetes were harvested at centrifugation at 9,000 × g for 20 min at 23°C, and the cells were stored frozen in phosphate-buffered saline (pH 7.4) with 10% dimethyl sulfoxide at −80°C. The E. coli strains used in this investigation were RecA− strain JM105 with lacZ on F′ (50) and the following RecA− strains: JM109 with recA1 and lacZ on F′ (Stratagene, La Jolla, Calif.), Top10F′ with recA1 and lacZ on F′ (Invitrogen Life Technologies), and JC14604 (previously referred to as strain 286 tsx lacZ/ H9004 -2(Oc) 33 supE44 galK - (18)). The Star colonies were plated in a grid on plates, and the resultant colonies were transferred to Immobilon-Ny+ membranes (Millipore) as described previously (39). The membranes were prehybridized in 0.12 M Na2PO4, pH 7.2–0.25 M NaCl–7% sodium dodecyl sulfate in a shaking water bath at 60°C for 2 h. They were then probed with a PCR product of the B. burgdorferi recA gene, which spanned nucleotide positions 50 to 922 of the coding sequence (accession number AE001124) and was radiolabeled with a MegaPrime random prime labeling kit (Amersham Biosciences) and [α-32P]ATP in the same buffer at 60°C. The membranes were washed as described previously (37) and then exposed to X-ray film.

Colonial blot hybridization. Library clones were plated in a grid on plates, and the resultant colonies were transferred to Immobilon-Ny+ membranes (Millipore) as described previously (39). The membranes were prehybridized in 0.12 M Na2PO4, pH 7.2–0.25 M NaCl–7% sodium dodecyl sulfate in a shaking water bath at 60°C for 2 h. They were then probed with a PCR product of the B. burgdorferi recA gene, which spanned nucleotide positions 50 to 922 of the coding sequence (accession number AE001124) and was radiolabeled with a MegaPrime random prime labeling kit (Amersham Biosciences) and [α-32P]ATP in the same buffer at 60°C. The membranes were washed as described previously (37) and then exposed to X-ray film.

Northern blot analysis. Total RNA was extracted with the TRizol reagent (Invitrogen Life Technologies) from E. coli Top10F′ cells treated with isopropyl-β-D-thiogalactopyranoside (IPTG) as described above. One microgram of total RNA was separated on a 1% agarose denaturing gel, the bands were transferred to an Immobilon-Ny+ membrane (Millipore), and the blot was prehybridized in hybridization buffer as described previously (16). The probes for the blots were PCR products of positions 234 to 850 of B. burgdorferi recA (accession number AE001124), positions 227 to 840 of B. hermsii recA (AF395152), and the bla gene of the pBluescript II vector from position 2217 to position 2807. PCR products were radiolabeled by using a MegaPrime random prime labeling kit (Amersham Biosciences) and [α-32P]ATP. The membranes were hybridized in hybridization buffer as described previously (17). The blots were exposed to a phosphor screen (Molecular Dynamics) and scanned with a Personal FX phosphorimager (Bio-Rad Laboratories, Hercules, Calif.).

Cloning of recA fragments of other Borrelia species. The partial recA sequences of other Borrelia species were obtained by using primers whose sequences were conserved in the recA genes of B. burgdorferi and B. hermsii. The forward and reverse primers corresponded to positions 234 to 254 and 850 to 829, respectively, of the B. burgdorferi recA gene and were used under the conditions described above. The PCR products were ligated into a pCR 2.1 TA cloning vector (Invitrogen Life Technologies) and were transformed into competent E. coli Top10F′ cells.

DNA sequencing and analysis. Inserts of the plasmids were sequenced on both strands with a 377 automated sequencer (Applied Biosystems, Foster City, Calif.) by using M13 primers and custom primers based on the sequence. Expression of recA genes in E. coli. An Ndel restriction site was inserted into the pBluescript II KS plasmid (Stratagene, La Jolla, Calif.) by using the following primers: 5′ GTAACTATGTCATATGGTTCTCTGGTTG3′ and 5′ GCGCAA GCCGGCACATTAAACCCTACT3′. The recA coding regions of B. burgdorferi, B. hermsii, E. coli, and L. biflexa (accession number AF140431 [49]) were amplified from genomic DNA by using the forward and reverse primers with Ndel and BamH restriction sites (underlined), respectively, at their ends: for B. burgdorferi, 5′ GGAATTCCTATGTGCAAAGTGAAGGGAAAA GAA3′ and 5′ GGCGATATCCTAAATAGACATACAAACACACACAG3′; for B. hermsii, 5′ GGAATTCCTAATGTCATAAAGTTAAGGAAACCGAG3′ and 5′ GGCGATATCCTAAATAGACATACAAACACACACAG3′; for E. coli, 5′ GGCGATATCCTAAATAGACATACAAACACACACAG3′ and 5′ TTCCATATGTCATCGCAAAAAAACACACACAG3′; and for L. biflexa, 5′ GGAATTCCTAATGTCATAAAGTTAAGGAAACCGAG3′ and 5′ GGCGATATCCTAATGTCATAAAGTTAAGGAAACCGAG3′.
RESULTS

Cloning of recA genes of spirochetes in E. coli. In a preliminary study a recombinant plasmid containing the B. burgdorferi recA gene with its native promoter did not complement an E. coli recA mutant in the UV survival assay (data not shown).

Accordingly, we used an expression plasmid with the LacZ promoter for inducible expression of the recombinant recA genes in an E. coli Top10F cell strain. The different plasmids with recombinant RecA were constructed by using PCR-amplified coding regions from genomic DNA of B. burgdorferi, E. coli, and L. biflexa. NdeI sites were added before the start codon of each gene, and BamHI sites were added at the 3' ends of the PCR products 30 to 90 nucleotides after the stop codons. The products were cloned into the modified vector and then transformed into E. coli Top10F cells. The inserts were confirmed to be recA genes of the appropriate species by sequencing. The plasmids were designated pBb:recA, pEc:recA, and pLb:recA for the recA genes of B. burgdorferi, E. coli, and L. biflexa, respectively.

Cloning and sequencing of recA of B. hermsii and other relapsing fever species. The recA gene of B. hermsii had not been identified and cloned previously, and, accordingly, these steps were carried out in the present study. Genomic DNA of B. hermsii was digested with SpeI, and a Southern blot of the digested DNA that was probed with the PCR product of B. burgdorferi recA revealed a single hybridizing band at \( \sim 5 \) kb. Under the same hybridization conditions with the B. burgdorferi recA probe, we screened a threefold redundant genomic library of B. hermsii in the pUC18 vector. Southern blot analysis of three hybridizing transformants revealed that the clones contained overlapping fragments. Sequencing of these inserts and subsequent database searches identified the recA gene of B. hermsii within the inserts.

Figure 1 shows the recA gene and its flanking regions in B. hermsii. Upstream of recA on the same strand we identified a partial ORF corresponding to the 3' end of the transcription elongation factor gene greA. This partial sequence was 76% identical to the corresponding greA gene upstream of recA in B. burgdorferi. Downstream of the recA stop codon is an ORF that over its length is 55% identical to the partial sequence of BB0130, an ORF of B. burgdorferi, and 24% identical to TP0691, an orthologous ORF of Treponema pallidum. All three spirochete ORFs are similar to the ORFs encoding the CG1354 family of proteins with unknown functions in bacteria and archaea (http://www.ncbi.nlm.nih.gov/Structure/cdd/). In both genomes there were only a few nucleotides

FIG. 1. Physical map of B. burgdorferi and B. hermsii recA genes and flanking regions. The numbers in parentheses are the numbers of nucleotides between ORFs, the numbers below the map are the lengths (in kilobases), and the arrows indicate the direction of transcription. The solid lines represent the complete gene sequence, while the dashed lines represent partial sequences. The approximate extent of the B. burgdorferi recA probe used for library screening is shown. The partial sequence of the BB0130 ORF of B. hermsii is homologous to ORF BB0130 of B. burgdorferi.
between the coding sequences for the recA genes and the flanking ORFs.

As was done with the other recA genes, the recA coding region of B. hermsii was amplified by PCR, the product was cloned into the modified pBluescript vector to produce the pHbl:recA plasmid, and the sequence of the insert was verified.

On the basis of the conserved sequence in the recA genes of B. burgdorferi and B. hermsii, we designed degenerate primers to amplify the partial recA sequences of other relapsing fever and related species. With these primers we amplified ~0.6-kb fragments from B. coriaceae, B. miyamotoi, and B. turicatae. These fragments were then cloned into the pCR 2.1 TA cloning vector, and sequencing revealed a 572-bp insert for each of the three species. The fragments corresponded to amino acid residues 86 to 275 of B. hermsii RecA and residues 88 to 276 of B. burgdorferi RecA.

Comparison of deduced RecA proteins of Borrelia spp. The B. hermsii recA gene was 1,071 bp long, compared to the 1,095-bp recA gene of B. burgdorferi, and the nucleotide sequence was 78% identical to the B. burgdorferi recA sequence. The pairwise difference value for the nucleotide sequences of these two species of Borrelia is equal to the values obtained for different genera belonging to the gamma division of the class Proteobacteria. For example, the recA gene of E. coli K-12 (accession number NC_000913) is 79 and 77% identical to the orthologs in Yersinia pestis (NC_004835) and Shewanella oneidensis (NC_004347), respectively. The predicted 356-amino-acid protein was 78% identical to B. burgdorferi RecA, 54% identical to T. pallidum RecA, 51% identical to L. biflexa RecA, and 53% identical to E. coli RecA. Alignment of the partial RecA sequences of the five Borrelia spp. showed that the levels of pairwise amino acid identity between B. burgdorferi and the relapsing fever group of species were 89 to 91% and that the levels of pairwise identity between members of the relapsing fever group were 92 to 95%. The codon usages for the B. burgdorferi and B. hermsii recA genes were very similar, and like other Borrelia genes, these genes showed a bias toward A- and T-rich codons.

Figure 2 shows an alignment of conserved functional sites, as summarized by Lusetti and Cox (25), of the RecA sequences of E. coli (accession number NP_417179), L. biflexa, B. burgdorferi, B. hermsii, B. miyamotoi, and B. turicatae. The ATP-binding sites (residues 66 to 73 of E. coli RecA) of all the RecA proteins were identical, and the ATP hydrolysis sites (residues 141 to 150) were the same except for an 1142V substitution in B. miyamotoi and L. biflexa RecA and a V150T substitution in L. biflexa RecA. The sequences of the primary DNA binding sites (residues 157 to 164) were more divergent; compared to the E. coli and L. biflexa RecA proteins, there were three substitutions, M159I, Q163H, and I164M, in the B. hermsii recA gene was 1,071 bp long, compared to the E. coli recA gene was 1,095 bp long, compared to the B. burgdorferi recA gene. The secondary DNA binding sites (residues 195 to 209) of Borrelia spp. RecA proteins have polymorphisms at some positions, but these polymorphisms are conservative substitutions, such as an arginine at position 198 in B. burgdorferi in place of the lysine in other Borrelia spp. A less conservative substitution was the substitution of glutamine for lysine at position 152. This position is not in a defined functional site, but this junction between the ATP hydrolysis site and the primary DNA binding site is highly conserved among RecA proteins (19).

UV survival assays. The ability of the Borrelia spp. recA genes to complement a defective recombinational DNA repair pathway was investigated by using E. coli Top10F' cells containing plasmids pBluescript, pBh:recA, pBb:recA, pEc:recA, and pLb:recA. The cells were grown in the presence of IPTG before exposure to various doses of short-wavelength UV light after plating on solid agar without IPTG. For all the cells containing recombinant RecA, the survival of cells without IPTG induction was 5- to 10-fold less than the survival of cells treated with IPTG before UV exposure.

Figure 3 shows the mean levels of survival of the different cultures after different exposures. The survival of the recA mutant E. coli complemented by the L. biflexa recA gene was essentially the same as the survival of cells with E. coli recA provided on a plasmid. Although both B. burgdorferi recA and B. hermsii recA resulted in increased survival of the recA mutant host, the benefit was 102- to 103-fold less than the benefit provided by L. biflexa recA. The survival of E. coli cells with B. hermsii recA was 100-fold less than the survival of cells with B. burgdorferi recA and only 10- to 20-fold greater than the survival of the recA mutant host alone. The doses estimated to kill 99% of the cells were 17, 24, and 40 mL for E. coli harboring plasmids pBluescript, pBh:recA, and pBb:recA, respectively.

MMS and mitomycin C susceptibility assays. recA mutant cells are sensitive to the radiomimetic effects of MMS and mitomycin C (13). To determine if Borrelia spp. RecA proteins were capable of participation in the repair of DNA damage due to these chemicals, complementation studies with E. coli Top10F' cells were performed. IPTG-induced cells harboring one of the recA expression plasmids were grown in the presence of increasing doses of MMS or mitomycin C in the absence of IPTG. Figure 4 shows the survival and growth of colonies observed with different concentrations of MMS. In this experiment, the cells expressing L. biflexa RecA complemented the recA-deficient host as well as the E. coli recA-bearing plasmid. Even at the highest concentration (350 μg/
ml), the plating efficiency for pLb:recA-bearing cells was 90% of that for cells plated on agar without MMS. The population of cells with the B. hermsii recA gene was indistinguishable from the null plasmid control population in terms of susceptibility to MMS. The estimated concentrations of MMS necessary to reduce the numbers of colonies by 99% were 291, 286, and 350 μg/ml for pBluescript, pBh:recA, and pBb:recA, respectively.

With mitomycin C at concentrations less than 1.0 μg/ml there was no difference in survival and growth of the cells with the different plasmids. At a mitomycin C concentration of 1.0 μg/ml, the number of colonies of the recA mutant host strain with pBluescript was 0.05% of the number of colonies on plates without the agent, and the percentage of surviving colonies with the pLb:recA plasmid (0.31%) was more than 10-fold lower than the percentage of surviving colonies for the cells with E. coli recA (4.9%). The percentage of colonies with B. burgdorferi recA (0.29%) was approximately the same as the percentage of surviving colonies for the cells with pLb:recA, but no discernible increase in survival was provided by the B. hermsii recA plasmid.

To assess whether the observed differences between transgenic cells with B. burgdorferi recA and transgenic cells with B. hermsii recA in the DNA damage assays was attributable to pleiotropic effects of the plasmids, we measured the generation times of the different cell lines in the broth medium. The doubling times for cultures of Top10F’ cells harboring pBluescript, pBb:recA, pBh:recA, and pLb:recA were 66, 63, 66, 49, and 51 min, respectively. The difference between the doubling times for cells with B. burgdorferi recA and cells with B. hermsii recA was not statistically significant.

To determine if the lower level of complementation of the E. coli recA mutant by recombinant B. hermsii RecA was due to decreased expression of the gene in E. coli, a Northern blot analysis was performed with total RNA from E. coli Top10F’ cells containing plasmid pBluescript, pBl:recA, or pBluescript. The blots were probed with either a PCR product of the plasmid’s beta-lactamase gene, bla, or a 1:1 mixture of equivalently labeled PCR products of the recA genes of B. burgdorferi and B. hermsii. The results obtained with IPTG-induced cells are shown in Fig. 5. When we normalized for bla expression, there was not a detectable difference between transcription of the B. burgdorferi recA gene and transcription of the B. hermsii recA gene in E. coli. Similar results were obtained with uninduced E. coli cells (data not shown).

**red gam λ phage plaque production.** The results of the chemical susceptibility assays and the UV survival assay indicated that Borrelia RecA is capable of participating in the DNA repair pathway in E. coli but it participates at much lower levels than the RecA protein of L. biflexa. We next compared the activities of the different recombinant RecA proteins in recombination assays. We used red gam mutant λ phage, which in the absence of a suppressing recB or recC mutation lack the ability to form plaques on recA mutant E. coli strains (12). For the
mutant phage genomes were not produced from monomer increased 10-fold. This was an indication that multimers of the on plates of cells containing either pBb:recA, pBh:recA, or ing plasmid pLb:recA formed plaques. No plaques were seen estimated 65% of the phage added were capable of forming With IPTG-induced cells containing the pEc:recA plasmid, an experiment, red gam mutant phage were adsorbed to E. coli JM109 cells harboring the different recA plasmids, and the efficiency of plaque production was observed.

Table 1 shows the efficiency of plaque production in cells complemented with each of the recombinant recA plasmids. With IPTG-induced cells containing the pEc:recA plasmid, an estimated 65% of the phage added were capable of forming plaques, while 25% of the phage incubated with cells containing plasmid pLb:recA formed plaques. No plaques were seen on plates of cells containing either pBb:recA, pBh:recA, or pBlueScript, even when the number of phage adsorbed was increased 10-fold. This was an indication that multimers of the mutant phage genomes were not produced from monomer circles in the cells with Borrelia RecA.

**Homologous recombination assay.** The second recombination assay called for intramolecular homologous recombination between approximately 2.5-kb inverted repeats to produce a functional LacZ ORF in E. coli strain JC14604 (20). This strain contains a duplication of the lacZ region, and each copy possesses a different lacZ missense mutation. In the experiment the colonies growing on minimal agar plates with lactose as the carbon source were counted in triplicate after 7 days of incubation, and the frequency of recombinants was estimated by comparing the number of colonies on lactose plates with the number of colonies on glucose plates. Table 2 shows the results. Recombinants were undetectable on plates with lawns of the recA mutant cells with the vector plasmid alone. For cells bearing the pEc:recA plasmid the recombination frequency was approximately 25-fold higher than that for the cells bearing either pBb:recA or pBh:recA. In contrast to the DNA damage assays and plaque recombination assay, this recombination as say demonstrated that the complementation provided by Borrelia spp. RecA was nearly as great as that provided by L. biflexa RecA.

**Expression of recA in Borrelia species.** Expression of recA in B. burgdorferi and B. hermsii was examined next. The recA genes of B. hermsii and B. burgdorferi are preceded by σ70-type promoters. The putative −10 element was TATAAA in B. burgdorferi and B. hermsii. The sequences of the corresponding −35 elements were CCCCGA and TCCTGA in B. burgdorferi and B. hermsii, respectively. We first attempted to document the expression of recA in B. burgdorferi by Northern blot analysis using the flab gene as a positive control, but the level of transcription was below the level of detection of the assay (Putteet-Driver, unpublished data). We then analyzed the raw data from DNA array experiments to document recA expression in B. burgdorferi and B. hermsii and to assess whether the level of expression was affected by changes in environmental conditions (55). In these experiments a membrane-based DNA array of the ORFs of the genome of B. burgdorferi was probed with cDNA produced from RNA of B. burgdorferi growing at 23 or 35°C or RNA of B. hermsii growing in culture medium or the blood of infected mice. The raw data were adjusted for differences between ORFs in terms of the amount of hybridization by genomic DNA of either B. burgdorferi or B. hermsii and for differences in nucleotide identity.

Table 3 shows the results of this analysis. The ospC gene of B. burgdorferi was used a positive control for a temperature-regulated gene (40), and the expression of the flab gene of the two species was known not to vary at different temperatures or when in vitro cultivation conditions and in vivo cultivation conditions were compared (55). The recA genes of both B. burgdorferi and B. hermsii were expressed under all growth conditions examined but at a lower level than the flab genes of the species. Unlike the ospC gene, which showed eightfold-higher transcription at the higher temperature, the expression of recA in B. burgdorferi was not different at the two temperatures; these results confirm those of Ojaimi et al. (32). There was no detectable difference between the level of expression of recA in B. hermsii under in vitro conditions and the level of expression of recA in B. hermsii under in vivo conditions.

### Table 1. Efficiencies of plating of red gam λ bacteriophage on recA (JM105) and recA (JM109) E. coli cells containing expression plasmids bearing E. coli, L. biflexa, B. burgdorferi, and B. hermsii recA genes

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>recA gene</th>
<th>No. of PFU/plate</th>
<th>Mean no. of plaques (95% CI)</th>
<th>% Compared to JM105</th>
</tr>
</thead>
<tbody>
<tr>
<td>JM105/none</td>
<td>E. coli</td>
<td>500</td>
<td>514 (454–572)</td>
<td>100</td>
</tr>
<tr>
<td>JM109/pBluescript None</td>
<td>15,000</td>
<td>0</td>
<td>&lt;0.006</td>
<td></td>
</tr>
<tr>
<td>JM109/pEc:recA E. coli</td>
<td>1,500</td>
<td>979 (866–1092)</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>JM109/pLb:recA L. biflexa</td>
<td>1,500</td>
<td>374 (331–415)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>JM109/pB:recA B. burgdorferi</td>
<td>15,000</td>
<td>0</td>
<td>&lt;0.006</td>
<td></td>
</tr>
<tr>
<td>JM109/pBh:recA B. hermsii</td>
<td>15,000</td>
<td>0</td>
<td>&lt;0.006</td>
<td></td>
</tr>
</tbody>
</table>

* Estimated number of PFU per plate.

### Table 2. Frequencies of lactose-fermenting (Lac+) recombinants of E. coli strain JC14604 cells containing expression plasmids bearing E. coli, L. biflexa, B. burgdorferi, and B. hermsii recA genes

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>recA gene</th>
<th>Frequency of Lac+ colonies (10^-8) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBluescript None</td>
<td>E. coli</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>pEc:recA E. coli</td>
<td>48 (42–55)</td>
<td></td>
</tr>
<tr>
<td>pLb:recA L. biflexa</td>
<td>5.9 (5.6–6.2)</td>
<td></td>
</tr>
<tr>
<td>pB:recA B. burgdorferi</td>
<td>2.3 (2.1–2.5)</td>
<td></td>
</tr>
<tr>
<td>pBh:recA B. hermsii</td>
<td>2.6 (2.4–2.8)</td>
<td></td>
</tr>
</tbody>
</table>

* 95% CI, 95% confidence interval.

### Table 3. B. burgdorferi genome array analysis of recA expression in B. burgdorferi cells under two temperature conditions and in B. hermsii cells growing in culture medium or in the blood of infected mice

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gene</th>
<th>Growth conditions</th>
<th>Relative expression (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. burgdorferi flab</td>
<td>23°C</td>
<td>37 (29–45)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>flab</td>
<td>35°C</td>
<td>49 (31–67)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>recA</td>
<td>23°C</td>
<td>5.2 (2.3–8.0)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>recA</td>
<td>35°C</td>
<td>6.8 (6.1–7.4)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>ospC</td>
<td>23°C</td>
<td>6.5 (5.1–7.8)</td>
<td>&lt;10^-7</td>
<td></td>
</tr>
<tr>
<td>ospC</td>
<td>35°C</td>
<td>53 (40–58)</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

* 95% CI, 95% confidence interval.

Determined by two-tailed t test.
DISCUSSION

RecA was first identified in 1965 by Clark and Margulies through characterization of E. coli cells deficient in recombination (8). Soon after this, these and other investigators demonstrated that recA mutant cells were highly sensitive to UV irradiation, thus revealing that there is an association between the recombination and DNA repair processes (7). Subsequent studies over the last three decades, almost entirely with E. coli, have resulted in further characterization of RecA and its three major activities (reviewed in references 9 and 25). First, the RecA protein directly participates in recombination catalyzing DNA strand exchange reactions. Recombination occurs in conjunction with either of the two other recombination pathways: RecBCD, which mainly participates in repair of double strand breaks; and RecFOR, which mainly participates in repair of nicks or other damage present at stalled replication forks (25). Second, RecA promotes the autocatalytic cleavage of repressor proteins, such as LexA of the SOS response in E. coli and cI of bacteriophage λ. Third, RecA in conjunction with polymerase V allows mutagenic translesion synthesis of DNA after DNA damage.

With the exception of the genome of the endosymbiont Buchnera aphidicola (47), a recA ortholog has been identified in all bacterial genomes sequenced to date (http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=Genome), and the presence of a recA ortholog has been demonstrated in several other bacterial species belonging to diverse taxa. RecA function in spirochetes was first identified in the free-living spirochete L. biflexa by Stamm et al. through functional complementation of a recA mutant of E. coli (43). In that study, immunoprecipitation of cellular extracts of B. burgdorferi and T. pallidum with rabbit antiserum to E. coli RecA failed to detect RecA protein in these spirochetes. A recA gene of B. burgdorferi was later found by Dew-Jager et al., who showed that the deduced sequence of B. burgdorferi’s RecA protein had several substitutions, which, when individually present in E. coli RecA, altered or eliminated the function in that organism (11).

The sequence of the genome of B. burgdorferi confirmed the presence of a single ortholog of recA on the chromosome, as well as the presence of genes of the RecBCD pathway for double-strand break repair (15). These findings revealed the possible enzymatic basis for such recombination-mediated phenomena in B. burgdorferi as intramolecular gene conversions between multiple alleles of the vlsE sequences (54). On the other hand, the genome sequence did not reveal orthologs for the following genes: lexA, the gene for the SOS response repressor; umuC and umuD, the genes encoding the subunits of DNA polymerase V; and the recF, recO, or recR genes of the RecFOR pathway for gap repair (15). The DNA repair and recombination capabilities of B. hermsii are expected to be similar to those of B. burgdorferi since the genome sizes (14) and genetic complements of these organisms are similar (55). In the present study we found that the same genes that recA in B. burgdorferi also flank recA in B. hermsii.

To further define the functional capabilities of the RecA protein of Borrelia spp., we attempted to introduce gene disruptions within recA of B. burgdorferi, but we were unable to recover clones that contained the integrated plasmid (Putteet-Driver, unpublished data). This finding was the consequence of the lethality of such an event, as observed in Streptomyces lividans (51), or was obtained because the growth rate was greatly reduced, as occurred in the more robust organism L. biflexa (49). As an alternative approach, we characterized the DNA repair and recombination capabilities of recombinant RecA of B. burgdorferi and B. hermsii in recA mutant E. coli cells and compared these cells with cells expressing E. coli RecA or L. biflexa RecA from the same plasmid constructs under control of the Lac promoter. In addition, we identified, sequenced, and cloned into the same plasmid vector the recA gene of B. hermsii. The different plasmid constructs were compared with each other, as well as with recA mutant cells in which the E. coli RecA protein was expressed from the same plasmid vector. We demonstrated that recA was detectably transcribed in both B. burgdorferi and B. hermsii, but there was not a discernible difference in transcription at two different temperatures or between growth in broth medium and growth in mouse blood.

The recA genes of both B. hermsii and B. burgdorferi enhanced the ability of the host recA mutant E. coli cells to recover from UV irradiation and to carry out intramolecular recombination between inverted repeats to utilize the sole carbohydrate source in the medium. However, neither of these recA genes could match the transgenic recA of another spirochete, L. biflexa, or the recA gene of E. coli in either of these assays or in the promotion of plaque formation by red gam mutant λ bacteriophage. Overall, the RecA proteins of B. burgdorferi and B. hermsii appeared to be more competent to complement a recA mutant in an assay for intramolecular homologous recombination than in DNA repair assays.

In addition, we found significant differences between cells bearing B. hermsii recA and cells with B. burgdorferi recA in the DNA damage assays despite similar expression levels. While transgenic recA genes of both Borrelia spp. had equivalent activities in the assay of recombination between lacZ duplications, the transgenic cells with the B. hermsii recA gene exhibited only a 10-fold increase in survival after UV exposure compared to the survival of RecA- cells and no detectable increase in survival in the presence of the radiometric chemicals MMS and mitomycin C. The recA gene of B. burgdorferi was less effective than the recA gene of free-living L. biflexa in these assays but was still substantially more effective than the ortholog of B. hermsii.

Our findings for the expression of recombinant RecA of B. burgdorferi in E. coli were generally similar to those of Liveris et al. (24). In both studies Borrelia RecA proteins mediated homologous recombination more efficiently than they mediated recombinational DNA repair in E. coli. The P1 transduction assay of Liveris et al. demonstrated that B. burgdorferi RecA is able to promote intermolecular recombination in E. coli, while our studies of recombination between lacZ duplications demonstrated that intramolecular recombination is also promoted by Borrelia spp. RecA in E. coli. In both studies, the increase in survival compared with the survival of the vector control was 10^6- to 10^7-fold at the highest dose of UV irradiation. While we observed that recombinant B. burgdorferi RecA conferred a modest increase in mitomycin C resistance compared with that of the vector control, Liveris et al. observed complete or nearly complete complementation of a recA mutant E. coli strain in their mitomycin C assay. The
difference may be attributable to the continuing presence of mitomycin C during cultivation in our assay and the postexposure removal of mitomycin C by Liveris et al. before cultivation. Liveris et al. also observed that complementation with *B. burgdorferi* recA leads to induction of λ prophage and provided a measure of RecA’s coprotease activity; cleavage of the OQ represor protein permitted excision of the plasmid genome and subsequent entry into the lytic cycle.

The comparatively poorer complementation by *Borrelia* spp. recA than by *L. biflexa* recA in DNA damage repair assays could be attributable in part to lower expression of the borrelial RecA proteins in *E. coli*, but we do not think that this was a major determinant of the results. For one thing, all the recA genes were provided the same promoter at the same nucleotide distance from the start codon in the plasmid constructs, and we did not observe a difference in plasmid copy numbers between the cell lines (unpublished data). The *Borrelia* spp. recA genes have different codon usages than *E. coli* recA has, but even when the codon usages were the same, as they were for *B. hermsii* and *B. burgdorferi*, we observed a significant difference in activity between the recA genes of different species in some assays. Moreover, *L. biflexa* is as evolutionarily distant as either *Borrelia* species from *E. coli* (10, 34), yet its recA was demonstrably more effective in the *E. coli* background.

Finally, the recA genes of all three spirochete species functioned about the same in the homologous recombination assay. Thus, we concluded that *B. burgdorferi* RecA and especially *B. hermsii* RecA have relatively effective abilities to carry out homologous recombination but not DNA damage repair when they are expressed in a distantly related host species.

A discordance between homologous recombination and DNA repair was also observed with the recA gene of another obligate pathogen, *N. gonorrhoeae*, a member of the beta subdivision of the Proteobacteria (44). Stohl et al. reported that after UV irradiation *E. coli* cells expressing *N. gonorrhoeae* RecA had 10^3- to 10^4-fold lower levels of survival than cells expressing *E. coli* RecA had. In contrast, assays of homologous recombination with P1 transduction and Hfr conjugation showed that the activity of transgenic cells with *N. gonorrhoeae* recA was the same as that of cells with *E. coli* recA. Stohl et al. concluded that RecA of *N. gonorrhoeae* was able to interact with the RecBCD protein of *E. coli* as efficiently as *E. coli* RecA was. The findings of our lacZ duplication assay and the P1 transduction assay of Liveris et al. for homologous recombination are consistent with this interpretation (24).

However, the failure of the red gam mutant λ to produce plaques on cells expressing recombinant *Borrelia* spp. RecA and the results of the recombination assays with strain JC14604 suggest that the lack of plaque production by these phage is dependent upon more than the restoration of recombination and the interaction between RecA and RecBCD. Although the mechanism for the lack of plaque formation by red gam mutant λ in RecA-deficient *E. coli* remains to be established, one hypothesis is that recA-deficient cells cannot provide sufficient recombination for the production of multimers of the phage genome needed for packaging and successful multiplication of the phage (41). The finding that recBC mutations suppress recA mutations in assays of plaque formation by red gam mutant λ indicates that transduction and plaque assays assess different aspects of the interaction between RecA and RecBCD (52).

Stohl et al. attributed the poor complementation of the recA mutant in DNA damage assays to a failure of the RecA protein from *N. gonorrhoeae* to induce the SOS response as efficiently as *E. coli* RecA induces this response (44). *B. burgdorferi* does not appear to have an ortholog of the LexA repressor, and it is possible that *Borrelia* RecA has coprotease activity for Cl, as demonstrated by Liveris et al. (24), but does not have activity for LexA. An alternative or supplementary explanation is that there is alteration of loading of *Borrelia* or *Neisseria* RecA by RecFOR or another interaction between RecA and the RecFOR pathway in *E. coli*. *B. burgdorferi* does not have detectable orthologs of recF, recO, or recR (15). *N. gonorrhoeae* appears to have orthologs of recO and recR but not of recF (accession number NC_002946). It is possible that the RecA proteins of *Borrelia* and *Neisseria* cannot function in the key interaction between RecA and RecF in the SOS response in *E. coli* (35); consequently, these proteins would be expected to ineffectively complement a recA mutation in assays that mainly assess DNA damage repair.

We do not know why the RecA protein of *B. hermsii* is less efficacious than *B. burgdorferi* RecA in DNA damage repair in the *E. coli* genetic background. One possible explanation is the K152Q substitution found in the RecA protein of *B. hermsii* and other three relapsing fever species (Fig. 2). Among all other RecA sequences in the accessible databases, only the sequences of the RecA proteins of *T. pallidum* (accession number AAC65660) and *Chlorobium tepidum* (AAM73149) have a substitution at this position. This residue is located between the ATP hydrolysis site and a DNA binding site of *E. coli* RecA, and this region is involved in monomer-monomer interactions (25, 45, 46). Although an *E. coli* recA mutant with a K152Q substitution exhibited full activity in both DNA repair and coprotease assays (30), it is possible that this substitution has more adverse effects in conjunction with other substitutions in the protein.

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**REFERENCES**
