

Functional Properties of *Borrelia burgdorferi* *recA*

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Functions of the *Borrelia burgdorferi* RecA protein were investigated in *Escherichia coli* *recA* null mutants. Complementation with *B. burgdorferi* *recA* increased survival of *E. coli* *recA* mutants by 3 orders of magnitude at a UV dose of 2,000 $\mu\text{J}/\text{cm}^2$. The viability at this UV dose was about 10% that provided by the homologous *recA* gene. Expression of *B. burgdorferi* *recA* resulted in survival of *E. coli* at levels of mitomycin C that were lethal to noncomplemented hosts. *B. burgdorferi* RecA was as effective as *E. coli* RecA in mediating homologous recombination in *E. coli*. Furthermore, *E. coli* λ phage lysogens complemented with *B. burgdorferi* *recA* produced phage even in the absence of UV irradiation. The level of phage induction was 55-fold higher than the level in cells complemented with the homologous *recA* gene, suggesting that *B. burgdorferi* RecA may possess an enhanced coprotease activity. This study indicates that *B. burgdorferi* RecA mediates the same functions in *E. coli* as the homologous *E. coli* protein mediates. However, the rapid loss of viability and the absence of induction in *recA* expression after UV irradiation in *B. burgdorferi* suggest that *recA* is not involved in the repair of UV-induced damage in *B. burgdorferi*. The primary role of RecA in *B. burgdorferi* is likely to be a role in some aspect of recombination.

The spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease, has a unique genomic organization consisting of a 910,725-bp linear chromosome and at least 21 linear and circular plasmids (6, 13). Less than one-half of the 1,689 annotated open reading frames have an identifiable database match (6, 13). In addition, very few genes have been characterized biochemically. Functional studies of *B. burgdorferi* genes in the postgenomic era have been hampered by this spirochete's slow growth in culture and by the limited availability of genetic tools. A potentially useful strategy for circumventing these difficulties is complementation of mutants in well-characterized bacteria, such as *Escherichia coli*. This strategy has been employed to elucidate the functions of a number of *B. burgdorferi* gene products (1, 5, 7, 8, 15, 20, 26, 32, 44, 49, 50).

Genetic recombination may play a role in *B. burgdorferi* persistence by generation of antigenic variation (2, 31, 37, 45, 47, 52, 56, 57). RecA is the key protein linking genetic recombination to DNA replication and repair in bacteria (23, 24). In *E. coli*, RecA promotes pairing and strand exchange between a single-stranded DNA molecule and a homologous double-stranded DNA molecule through a complex multistep pathway that requires a DNA-dependent ATPase function (29). In addition to this recombinase activity, an ATP-dependent coprotease activity has also been ascribed to RecA. In enterobacteria and *Bacillus* sp., the latter activity is part of a specialized and regulated form of DNA repair designated SOS repair (3). In *E. coli*, the SOS response involves the transcriptional repressor LexA, whose autocatalytic cleavage is enhanced by activated RecA following DNA damage (19). This results in the coordi-

nate expression of approximately 30 different genes, including *recA* and *lexA*.

The *B. burgdorferi* *recA* gene was originally described from isolate Sh-2-82 by Dew-Jager et al. (10). An orthologue of this gene from type strain B31MI has been annotated in the *B. burgdorferi* genome as BB0131 (13). The gene encodes a 365-amino-acid protein that exhibits 56% identity and 77% similarity with *E. coli* RecA. As a first step in elucidating the mechanism of recombination in *B. burgdorferi*, several activities of the *B. burgdorferi* RecA protein were studied in *E. coli* *recA* null mutants.

MATERIALS AND METHODS

Bacterial strains and plasmids. The following *E. coli* K-12 strains were employed in this study: C600 (*leuB6 thi-1 thr-1*), DH5 α (*recA1 gyrA thi-1 relA1*), HB101 (*recA13 leuB6 thi-1 proA*), and 25404 (wild type). A pUC18-based clone containing *B. burgdorferi* *recA* was obtained from the American Type Culture Collection (ATCC 631007). This plasmid contains a 1,410-bp DNA insert encoding the entire *B. burgdorferi* *recA* gene (1,095 bp), including 291 bp of 5' flanking sequences and 24 bp of 3' flanking sequences. This plasmid is designated pUC18-*recA*_{Bb}. An insertionally inactivated derivative of pUC18-*recA*_{Bb} was constructed by cleaving the plasmid at a unique EcoRV site in *recA* and inserting a 1,190-bp kanamycin cassette derived from transposon Tn1545. This antibiotic resistance cassette was PCR amplified from a kanamycin-resistant derivative of plasmid pGK12 (38) by using 5'GGGAATTCTGTTATGAGCCATATTTCAA CG3' and 5'GATTAGAAAACTCATCGAGC3' as the forward and reverse primers, respectively. A DNA fragment containing the *E. coli* *recA* gene along with 223 bp of 5' flanking sequence and 92 bp of 3' flanking sequence was generated by PCR by using wild-type *E. coli* C600 (*recA*⁺) DNA as the template and 5'GAGAAGCCTGTGCGCACCGTCTGG3' and 5'CTTGCTCACTGTG ATCGCGCACCG3' as the forward and reverse primers, respectively. This fragment was cloned into the unique SmaI site of pUC18 (54), and the resulting plasmid was designated pUC18-*recA*_{Ec}.

The gene orientation in the inserts relative to the pUC18 backbone and the sequences of the inserted fragments were determined by DNA sequencing (Davis Sequencing, LLC, Davis, Calif.). In all constructs, the *lacZ* promoter was located upstream of and in the same orientation as the inserted *recA* gene.

UV sensitivity measurements. *E. coli* DH5 α clones complemented with various plasmid constructs were tested for UV sensitivity by using the method of Miller (28). Cultures of these clones were grown overnight at 37°C in Luria-Bertani broth (LB) supplemented with 100 μg ampicillin per ml (LB-Amp). A 1:50

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dilution of each culture was regrown in LB-Amp to an A_{600} of 0.8. Samples (0.1 ml) of the *E. coli* cells were spread on LB-Amp agar plates at a 10^{-6} dilution and were subsequently irradiated with increasing doses of UV light (254 nm) with a Spectrolinker XL-1000 UV cross-linker (Spectronics, Westbury, N.Y.). The UV-treated bacteria were protected from room light, and colonies were counted after overnight incubation at 37°C. Identical plates which were not irradiated were used to measure the viable counts of the starting cultures.

Measurement of MMC sensitivity. Five-milliliter portions of exponentially growing *E. coli* cells were incubated for 12 h at 37°C in the presence of increasing amounts of mitomycin C (MMC) (Sigma) in LB-Amp. Prior to plating, the cultures were serially diluted in LB-Amp to obtain concentrations 10^4 , 10^5 and 10^6 cells/ml, and 0.1-ml portions of each dilution were plated on LB-Amp plates in duplicate and incubated overnight at 37°C. MMC sensitivity was measured by counting the surviving colonies. Untreated cells grown in an identical manner served as controls.

UV induction of lambda lysogens of *E. coli*. Wild-type bacteriophage lambda lysogens were produced by cross-streaking *E. coli* DH5 α preparations containing the various *recA* plasmid constructs with a high-titer phage lysate on LB agar (4). Colonies at the crossing points were purified and cross-streaked against both a wild-type phage lysate and a phage *cI* repressor-insensitive mutant phage (λ^{vir}) lysate. Colonies that were resistant to wild-type phage superinfection but sensitive to λ^{vir} lysis were employed in subsequent experiments.

Lysogenic *E. coli* strains were grown overnight at 37°C in LB-Amp and were serially diluted and plated on LB agar. The plates were exposed to increasing doses of UV light, and this was followed by overlaying with a liquid *E. coli* C600 culture. Phage plaques were counted after overnight incubation at 37°C. Untreated lysogens served as controls.

P1 transductions. Phage P1 transductions were performed by using the protocol of Miller (28). Both phage and *E. coli* 25404 were purchased from the American Type Culture Collection. Stocks of P1 were generated by plate lysis on LB agar. Approximately 10^6 phage were absorbed to the exponentially growing donor *E. coli* 25404 (Leu⁻) in LB supplemented with 5×10^{-3} M CaCl₂. The infected cells were plated on three LB agar plates in soft R-top agar. The lysates were scraped off the plates, pooled, and treated with chloroform. Cell debris was removed by centrifugation, and the lysate was stored in the presence of chloroform. The phage yield was measured by using liquid agar overlays and *E. coli* 25404 as the lawn. A lysate titer of 9.4×10^8 PFU/ml was obtained under these conditions.

Portions (0.1 ml) of overnight cultures of *E. coli* HB101 (Leu⁻) harboring the various pUC18-based plasmid constructs were transduced with 0.1 ml of lysate, and Leu⁺ colonies were selected on glucose minimal medium plates containing 100 μ g of ampicillin per ml. Lysate controls for bacterial contamination and host cell controls for Leu⁺ revertants were also included. Colonies which appeared after 48 h of incubation were serially patched three times on glucose minimal medium plates containing 100 μ g of ampicillin per ml. All experiments were performed in triplicate.

Determination of *B. burgdorferi* UV sensitivity. *B. burgdorferi* B31MI, an infectious isolate at passage 13, was grown in BSK-H medium at 33°C to a density of 1×10^8 organisms/ml. Cells were harvested by centrifugation and resuspended in 0.1 ml of phosphate-buffered saline (PBS) containing 1×10^7 cells. Spirochetes were rapidly transferred into and spread in culture dishes (32 by 10 mm) and then were exposed to UV irradiation at doses of 800 to 9,000 μ J/cm². After UV exposure the cells were kept in the dark. Then the cells were recovered by aspiration and serially diluted in BSK-H medium so that each plate contained approximately 100 organisms per 0.1 ml. Each 0.1-ml dilution was added to 2 ml of BSK-H medium containing 0.8% molten agarose and was overlaid onto plates (60 by 15 mm) containing solid BSK-H medium. The agar plates were incubated at 33°C for 9 days in a 5% CO₂ incubator, and colonies were counted. *B. burgdorferi* not exposed to UV irradiation served as a control.

Determination of *recA* expression by quantitative reverse transcription (RT)-PCR. A 40-ml culture of *B. burgdorferi* B31MI was grown to a density of 1×10^8 cells/ml in BSK-H medium at 33°C. Cells were harvested by centrifugation at 12,000 \times g for 10 min and were suspended in 1.2 ml of sterile PBS. Then 0.3-ml aliquots were placed into culture dishes (35 by 10 mm) and exposed to increasing doses of UV irradiation. Following irradiation, the cells were kept in the dark, 10 ml of BSK-H medium was added, and the cells were incubated at 33°C for 2 h.

Forty milliliters of LB was inoculated with an *E. coli* C600 overnight culture by using a 1:50 dilution, and the culture was grown at 37°C to an A_{600} of 1.0. Cells were harvested by centrifugation at 12,000 \times g for 10 min and were suspended in 4 ml of sterile PBS. One-milliliter aliquots were placed into culture dishes (100 by 15 mm) and exposed to increasing doses of UV. Following irradiation, the cells were protected from room light and following addition of 10 ml of LB were incubated at 37°C for 2 h.

B. burgdorferi or *E. coli* cells were recovered by centrifugation at 12,000 \times g for

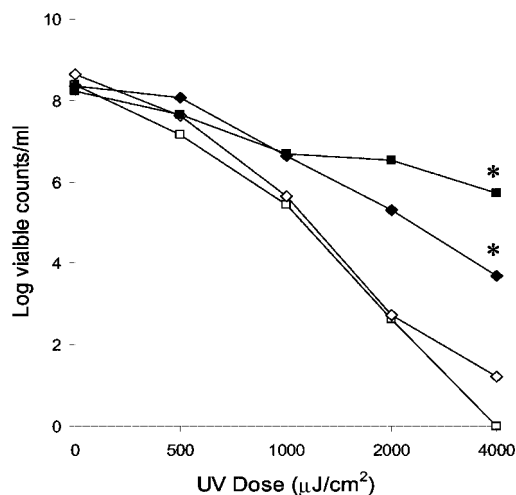


FIG. 1. Survival of DH5 α complemented with various pUC18-*recA* constructs after UV irradiation. ■, pUC18-*recA*_{Ec}; ◆, pUC18-*recA*_{Bb}; □, pUC18; ◇, pUC18-*recA*_{Bb}; Km^r. The enhanced survival of pUC18-*recA*_{Ec}- or pUC18-*recA*_{Bb}-complemented *E. coli* at a UV dose of 4,000 μ J/cm² relative to the survival of pUC18-complemented *E. coli* was statistically significant at a *P* value of 0.002 (indicated by asterisks).

10 min, and the pellets were washed twice with sterile PBS. RNA extraction was performed individually for each sample by using a PURESCRIPT kit (Gentra Systems, Minneapolis, Minn.) according to the manufacturer's protocol for gram-negative bacteria. Total RNA was treated with DNase (AMBION DNase kit), and RNA concentrations were determined spectrophotometrically at 260 nm. cDNA was generated by RT. Each reaction mixture contained first strand buffer, each deoxynucleoside triphosphate at a concentration of 0.4 mM, 10 U of RNase inhibitor, 500 ng of each random hexamer primer, 500 ng of total RNA, and 100 U of Superscript II. Following 1 h of incubation at 42°C, 5 ng of cDNA was retrieved, and RT-PCR was performed by using SYBR Green and a Lightcycler (Roche Diagnostics). The conditions used for RT-PCR amplification and the *recA*-specific primer sequences for *B. burgdorferi* have been described previously (27). The *E. coli recA* RT-PCR amplification conditions were essentially the same as those reported for *B. burgdorferi*, with the following exceptions. The PCR primer sequences employed for *E. coli recA* RT-PCR amplification were 5' ATGTGGAACCATCTCTACCGG 3' and 5' GTTGACTGCTTCAGGTTACC 3' for the forward and reverse primers, respectively, and an annealing temperature of 48°C was used. Each RT-PCR was performed in triplicate, and control mixtures containing no reverse transcriptase or no cDNA were included.

RESULTS

Complementation of *E. coli RecA* null mutants with *B. burgdorferi RecA*. The activity of the *B. burgdorferi RecA* protein was assessed by measuring survival of an *E. coli recA* null mutant complemented with plasmid-borne *B. burgdorferi recA* after UV irradiation. The data in Fig. 1 show that *B. burgdorferi recA* increased the survival of an *E. coli recA* mutant by 3 orders of magnitude at a UV dose of 2,000 μ J/cm². This complementation was about 10% that provided by the homologous *E. coli recA* gene expressed from the same vector construct at comparable UV doses. Complementation with a plasmid containing a variant of *B. burgdorferi recA* inactivated by insertion of a Km^r cassette did not result in increased survival. This clearly indicates that the spirochetal *recA* is functional in *E. coli*.

Complementation of MMC-treated *E. coli* by *B. burgdorferi recA*. UV irradiation results in mostly intrastrand thymine dimers in DNA, and repair of this damage reflects nucleotide excision (39). In order to assess the ability of *B. burgdorferi recA*

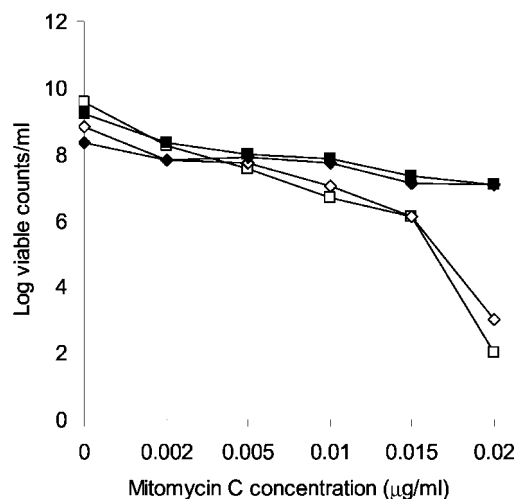


FIG. 2. Survival of DH5 α containing various pUC18-*recA* plasmids after 12 h of exposure to different doses of MMC. ■, pUC18-*recA*_{Ec}; ◆, pUC18-*recA*_{Bb}; □, pUC18; ◇, pUC18-*recA*_{Bb}::Km^r. Viable cells were counted by plating dilutions of MMC-treated and control cells on LB-Amp plates.

to promote repair of interstrand cross-links, similar survival studies were performed with cells exposed to increasing concentrations of MMC. Complementation of *E. coli* DH5 α with either wild-type *E. coli recA* or *B. burgdorferi recA* resulted in a 6-log increase in survival at the highest concentration of MMC (0.02 μ g/ml) (Fig. 2). In this case, the insertionally inactivated *B. burgdorferi recA* gene did not provide any protection. Together, the findings obtained with UV irradiation and MMC exposure demonstrated that *B. burgdorferi recA* can mediate repair of both intra- and interstrand DNA damage.

Phage induction by *B. burgdorferi* RecA. RecA activates the SOS response by cleavage of the LexA transcriptional repressor. Another substrate for the RecA coprotease activity is the λ phage cI repressor protein. In contrast to the rapid LexA cleavage by RecA, autocleavage of cI and other lambdoid phage repressors is slow and occurs at the high Mg²⁺ concentrations (>10 mM) often found in moribund cells (19, 36).

Phage λ cI repressor cleavage by *B. burgdorferi* RecA was measured by phage induction of *E. coli* lysogens. In this assay, the number of plaques obtained under each condition directly reflected the number of host cells that contained cI repressor

cleaved by RecA and that were subsequently converted into lytic centers, thereby producing detectable plaques. Table 1 shows the efficiencies of phage induction in *E. coli* DH5 α complemented with plasmids containing the various *recA* constructs. In uncomplemented *E. coli* DH5 α , no active phage production was observed since RecA is absolutely required for this process. Complementation with a plasmid containing either *E. coli recA* or *B. burgdorferi recA* resulted in production of active phage. Furthermore, exposure to increasing doses of UV irradiation resulted in significantly more plaques, as expected. Interestingly, even prior to UV irradiation, there was a 55-fold enhancement of λ phage induction in *B. burgdorferi recA*-complemented lysogens compared with that observed in cells complemented with the *E. coli recA* gene. This suggests that the *B. burgdorferi* RecA protein is able to induce the autocatalytic activity of the cI repressor even more efficiently than the homologous RecA is.

Recombinational activity of the *B. burgdorferi* RecA protein.

The ability of *B. burgdorferi* RecA to promote homologous recombination in *E. coli* was assayed by generalized P1 transduction for leucine prototrophs. In this case, HB101 was used as a surrogate host because it has a Leu⁻ phenotype. HB101 complemented with pUC18 did not yield any Leu⁺ transductants. In contrast, complementation with plasmids containing either wild-type *E. coli recA* or *B. burgdorferi recA* resulted in viable Leu⁺ recombinants (Fig. 3). In this case, *B. burgdorferi recA* disrupted with a kanamycin cassette could not provide the necessary RecA activity.

Effect of UV irradiation on *B. burgdorferi* viability. The studies described above demonstrated that the *B. burgdorferi* genome encodes a RecA protein that can participate in DNA repair and recombination. However, the *B. burgdorferi* genome apparently does not contain a LexA gene (13). Furthermore, the *B. burgdorferi* life cycle involves propagation in either a mammalian host or a tick vector and likely does not provide an opportunity for exposure to UV irradiation. In an initial effort to ascertain the possible role(s) of the RecA protein in *B. burgdorferi*, sensitivity to UV irradiation was measured.

A UV survival curve for *B. burgdorferi* B31MI is shown in Fig. 4. A UV dose of only 800 μ J/cm² resulted in a 76% loss of viability. At this UV dose, no reduction in viability was observed for wild-type *E. coli*; however, the response was similar to that observed with *E. coli recA* null mutants. Interestingly, a similar UV dose curve has been reported for *Leptospira biflexa*

TABLE 1. Production of λ infective centers in *E. coli* DH5 α , complemented with plasmids containing *recA*^a

Plasmid	Viable counts (cells/ml) ^b	PFU/ml with:		
		No UV irradiation	4,000 μ J of UV/cm ²	8,000 μ J of UV/cm ²
pUC18	$4.0 \times 10^8 \pm 1.4 \times 10^8$	0	0	0
pUC18- <i>recA</i> _{Bb}	$5.8 \times 10^8 \pm 3.0 \times 10^8$	$8.8 \times 10^7 \pm 4.5 \times 10^7$ ^c	$5.7 \times 10^8 \pm 1.3 \times 10^8$ ^d	$4.0 \times 10^8 \pm 1.3 \times 10^8$
pUC18- <i>recA</i> _{Bb} ::Km ^r	$4.6 \times 10^8 \pm 2.1 \times 10^8$	0	0	0
pUC18- <i>recA</i> _{Ec}	$8.3 \times 10^8 \pm 2.3 \times 10^8$	$1.4 \times 10^6 \pm 5.3 \times 10^5$ ^c	$6.9 \times 10^7 \pm 4.4 \times 10^7$ ^d	$1.8 \times 10^8 \pm 9.2 \times 10^7$
pUC18 (C600) ^e	$1.7 \times 10^9 \pm 2.2 \times 10^8$	$4.2 \times 10^7 \pm 1.8 \times 10^7$	$5.0 \times 10^8 \pm 4.0 \times 10^7$	$6.0 \times 10^8 \pm 1.3 \times 10^8$

^a The results are averages for three independent experiments.

^b Average \pm standard deviation.

^c With no irradiation the difference between the value for pUC18-*recA*_{Bb} and the value for pUC18-*recA*_{Ec} was statistically significant at a *P* value of 0.0005.

^d With 4,000 μ J/cm² the difference between the value for pUC18-*recA*_{Bb} and the value for pUC18-*recA*_{Ec} was statistically significant at a *P* value of 0.0108.

^e An *E. coli* C600 (RecA⁺) λ lysogen harboring vector pUC18 was employed to estimate the contribution of chromosomal *recA* to λ phage induction by UV irradiation.

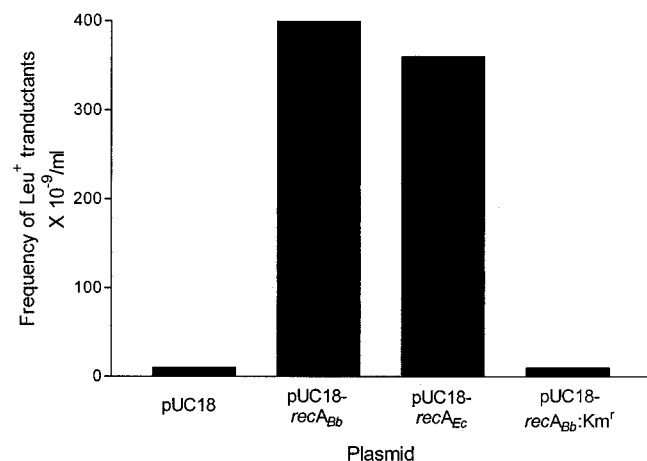


FIG. 3. Phage P1 transduction frequencies of HB101 complemented with various pUC18 *recA* constructs. The frequency was measured by determining the ratio of the number of Leu⁺ transductants to the total number of viable cells recovered at comparable multiplicities of infection.

(48). The rather steep slope of the survival curve indicates that the accumulated damage in *B. burgdorferi* DNA cannot be effectively repaired.

In *E. coli*, UV irradiation led to a dose-dependent increase in *recA* transcription (Fig. 5). Irradiation of *B. burgdorferi* with UV doses as high as 3,000 $\mu\text{J}/\text{cm}^2$ did not lead to any increase in *recA* expression. These UV exposure studies suggested that *recA* is not involved in the repair of UV-induced damage in *B. burgdorferi*.

DISCUSSION

Three different but interrelated functions of the *B. burgdorferi* RecA protein were measured in a complementation model system by using *E. coli recA* null mutants as surrogate hosts. The results of the present study suggest that *B. burgdorferi* RecA functions in the activation of both the *E. coli* SOS response and λ phage lysogen induction. *B. burgdorferi* RecA is also able to mediate homologous recombination in *E. coli*. Similar results for an *E. coli* complementation model have been reported by Putteet-Driver et al. (33). These findings are not surprising in view of the extensive studies demonstrating

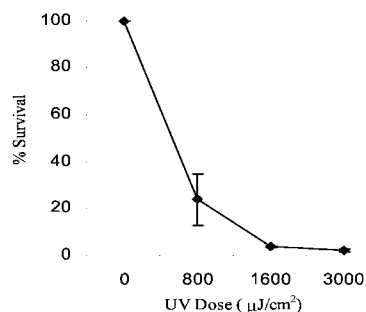


FIG. 4. Survival of *B. burgdorferi* B31MI after UV irradiation. The numbers of viable cells were determined by plating dilutions of UV-treated cells on BSK-H medium plates. The experiment was performed in triplicate.

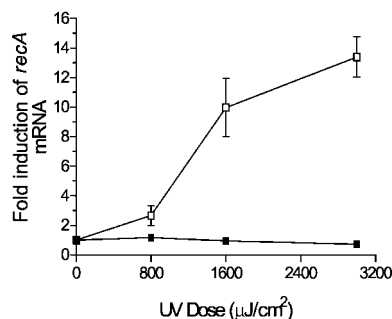


FIG. 5. Induction of *recA* mRNA after UV irradiation as determined by quantitative RT-PCR. \square , *E. coli* C600; \blacksquare , *B. burgdorferi* B31MI. The level of *recA* mRNA was measured by real-time RT-PCR as described in Materials and Methods. mRNA levels are expressed as the fold induction relative to the induction of unirradiated cells, which was defined as 1. The values are the averages obtained in three independent experiments.

complementation of *E. coli* with heterologous *recA* (21, 29, 43), which is likely the result of extensive conservation of this protein at the amino acid level. Alignment of the deduced amino acid sequences of the RecA proteins of *B. burgdorferi*, *Treponema pallidum*, and *L. biflexa* and *E. coli* RecA showed that *B. burgdorferi* RecA exhibits 56% amino acid identity and 77% similarity with *E. coli* RecA, 63% identity and 84% similarity with *T. pallidum* RecA, and 62% identity and 80% similarity with *L. biflexa* RecA.

The functional domains of the *E. coli* protein have been assigned by both structural and mutational studies (23). On the basis of sequence alignment and conservation, it is possible to deduce the identities of these functional domains in RecA proteins from other organisms. The nucleotide binding and hydrolysis regions defined by *E. coli* RecA residues G66 to T73 (A site), E96, D100, Y103, and I140 to D144 (B site) are invariant. The two domains designated loop 1 and loop 2 corresponding to residues E156 to D161 and Q194 to T210 in the *E. coli* are involved in DNA binding (23). Both loop sequences are completely conserved in *B. burgdorferi*, except for an E156D substitution. Individual RecA monomers interact with each other and single-stranded DNA, and filaments formed in this manner can participate in filament-filament interactions. These protein-protein interactions are required for all RecA functions (23). The residues that have been implicated in these interactions in *E. coli* RecA are highly conserved in the spirochetal proteins.

Although complementation with *B. burgdorferi* RecA conferred resistance to both UV irradiation and MMC exposure, the extent of complementation was different. Whereas complete repair of MMC-induced damage was observed in the complemented mutants, the level of survival after UV irradiation was only 10% of that observed in mutants complemented with the homologous gene. Essentially similar results were obtained by Putteet-Driver et al. (33), although only a moderate increase in survival following MMC treatment was observed. It is likely that the difference between the observed responses in the two studies was due to differences in the experimental design. UV exposure causes mostly intrastrand thymine dimers in DNA, the repair of which involves nucleotide excision. This pathway is induced by a rapid, transient,

RecA-mediated SOS response that removes the damage and quickly returns the cell to its normal preirradiation status (9). In contrast, growth in the presence of MMC induces inter-strand cross-links in the DNA, resulting in a more extended SOS response that may involve the differential expression of more than 1,000 genes (22). Thus, although SOS induction is the common outcome of both UV and MMC exposure in *E. coli*, the extent of the response is greater in MMC-treated cells. Therefore, it is possible that the rapid response after UV exposure cannot be fully provided by *B. burgdorferi* RecA in *E. coli*, whereas the longer-lived response to MMC can be fully complemented.

DNA damage-induced alterations in RecA structure promote an increased protein docking function which enhances the normally slow autocatalytic activity of LexA (25, 40). Rapid degradation of intracellular LexA by this coprotease activity of RecA in *E. coli* results in a 12-fold increase in *recA* expression and the coordinate induction of over 30 genes repressed by LexA (24). This is referred to as the SOS response (34). In *E. coli*, this response is induced by the persistence of single-stranded DNA (ssDNA), an indication of replisome inhibition at frequent DNA lesions (16, 24). This response appears to be controlled by competition for ssDNA between single-stranded binding protein (SSB) and RecA. Under normal in vivo conditions, SSB prevents RecA from polymerizing onto ssDNA; however, when replication forks are stalled due to DNA damage, SSB assists RecA binding to ssDNA (24). The functional form of RecA is a polymerized filament around an ssDNA molecule that presents a LexA docking site deep in its groove (19, 55). Binding of LexA to this site promotes rapid autocleavage, which results in coordinate activation of the genes in the SOS regulon.

The *B. burgdorferi* genome does not encode an orthologue of LexA (13), and a scan of the regions upstream of *recA* and other genes that comprise the SOS regulon in *E. coli* did not reveal any consensus LexA binding sites in *B. burgdorferi*. Despite this, the *B. burgdorferi* RecA protein was able to facilitate DNA repair in *E. coli*, indicating that it could interact with *E. coli* LexA. Furthermore, *B. burgdorferi* RecA was also competent for induction of λ phage, which requires RecA-mediated λ cI repressor autocleavage. Indeed, λ phage induction mediated by RecA was enhanced >50-fold compared with that observed in *E. coli* DH5 α complemented with the homologous RecA protein. This suggests that the *B. burgdorferi* protein harbors an enhanced coprotease activity. The presence of either of two point mutations, E39K and Q174K, in *E. coli* RecA results in increased coprotease activity (53). Interestingly, *B. burgdorferi* RecA contains both K39 and K174 in the wild-type sequence. These naturally occurring amino acid changes could explain the enhanced coprotease activity observed, as first suggested by Dew-Jager et al. (10).

The lack of a LexA protein has also been previously reported in *Bacillus fragilis* (17), *Thiobacillus ferrooxidans* (35), *Porphyromonas gingivalis* (12), and *Acinetobacter calcoaceticus* (18). In radiation-resistant *Deinococcus radiodurans* LexA is present, but cleavage is not enhanced by *recA* expression (30). The possible presence of an alternate protein(s) in *B. burgdorferi* that may provide LexA-like regulatory functions remains an open question.

The experiment whose results are shown in Fig. 4 demon-

strated that *B. burgdorferi* cannot effectively repair UV-induced damage. Furthermore, several findings suggest that the RecA function in *B. burgdorferi* is different from the role that this protein plays in *E. coli*. RecA-mediated DNA repair is exerted through its cleavage of LexA. As described above, no *lexA* orthologue has been identified in *B. burgdorferi*. Additionally, UV irradiation of *E. coli* results in increased expression of *recA*, but such induction does not occur in *B. burgdorferi* (Fig. 5). Thus, the role that RecA plays in normal *B. burgdorferi* biology remains to be clarified.

Little is known about the natural mechanism(s) of gene transfer, homologous recombination, and DNA repair in *B. burgdorferi*. Evidence suggests that *vls*, *erp*, and *ospC* loci undergo sequence variation that may require homologous recombination (45, 51, 57). Additionally, evolution of the *B. burgdorferi* genome by recombination between the linear chromosome and plasmids, as well as genetic exchange among various plasmids, has been suggested (6). Dykhuizen and Baranton estimated recombination frequencies for many *B. burgdorferi* isolates and concluded that this organism is mostly clonal and that there is limited genetic exchange between individual isolates (11). They hypothesized that *B. burgdorferi* contains a genetic transfer system that may permit the recombination of small (<1-kb) DNA fragments into its genome.

The results of the present study and those of Putteet-Driver et al. (33) demonstrate that *B. burgdorferi* RecA possesses both intermolecular and intramolecular recombination activities. It is, therefore, reasonable to suggest that this is the primary role of *recA* in *B. burgdorferi*. It is noteworthy that *B. burgdorferi* and *T. pallidum* both contain a reduced number of genes involved in recombination and DNA repair compared to *E. coli*. Interestingly, *B. burgdorferi* contains genes encoding only the RecBCD recombinational pathway, whereas only the RecF pathway genes are evident in *T. pallidum* (13, 14, 46). In *E. coli*, recombination is mediated primarily via proteins of the RecBCD pathway, and the function of the RecF pathway becomes evident only in *recBCD* mutants (23, 41, 42). The possible role that RecA may play in recombination in *B. burgdorferi* is currently under investigation.

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REFERENCES

1. Alekshun, M., M. Kashlev, and I. Schwartz. 1997. Molecular cloning and characterization of *Borrelia burgdorferi* *rpoB*. *Gene* 186:227-235.
2. Anguita, J., V. Thomas, S. Samanta, R. Persinski, C. Hernanz, S. W. Barthold, and E. Fikrig. 2001. *Borrelia burgdorferi*-induced inflammation facilitates spirochete adaptation and variable major protein-like sequence locus recombination. *J. Immunol.* 167:3383-3390.
3. Aravind, L., D. R. Walker, and E. V. Koonin. 1999. Conserved domains in DNA repair proteins and evolution of repair systems. *Nucleic Acids Res.* 27:1223-1242.
4. Arber, W. 1983. A beginner's guide to lambda biology, p. 381-395. In R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), *Lambda II*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. Boursaux-Eude, C., D. Margarita, J. Belfaiza, I. G. Old, and I. Saint Girons. 1998. Homologues of helicase genes *priA* and *ruvAB* of *Borrelia burgdorferi*, the Lyme borreliosis agent. *Res. Microbiol.* 149:235-245.
6. Casjens, S., N. Palmer, R. van Vugt, W. M. Huang, B. Stevenson, P. Rosa, R.

- Lathigra, G. Sutton, J. Peterson, R. J. Dodson, D. Haft, E. Hickey, M. Gwinn, O. White, and C. M. Fraser. 2000. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. *Mol. Microbiol.* **35**:490–516.
7. Cloud, J. L., R. T. Marconi, C. H. Eggers, C. F. Garon, K. Tilly, and D. S. Samuels. 1997. Cloning and expression of the *Borrelia burgdorferi lon* gene. *Gene* **194**:137–141.
8. Concepcion, M. B., and D. R. Nelson. 2003. Expression of *spoT* in *Borrelia burgdorferi* during serum starvation. *J. Bacteriol.* **185**:444–452.
9. Courcelle, J., and P. C. Hanawalt. 2001. Participation of recombination proteins in rescue of arrested replication forks in UV-irradiated *Escherichia coli* need not involve recombination. *Proc. Natl. Acad. Sci.* **98**:8196–8202.
10. Dew-Jager, K., W. Q. Yu, and W. M. Huang. 1995. The *recA* gene of *Borrelia burgdorferi*. *Gene* **167**:137–140.
11. Dykhuizen, D. E., and G. Baranton. 2001. The implications of a low rate of horizontal transfer in *Borrelia*. *Trends Microbiol.* **9**:344–350.
12. Fletcher, H. M., R. M. Morgan, and F. L. Macrina. 1997. Nucleotide sequence of the *Porphyromonas gingivalis* W83 *recA* homolog and construction of a *recA*-deficient mutant. *Infect. Immun.* **65**:4592–4597.
13. Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, and J. C. Venter. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* **390**:580–586.
14. Fraser, C. M., S. J. Norris, G. M. Weinstock, O. White, G. G. Sutton, R. Dodson, M. Gwinn, E. K. Hickey, R. Clayton, K. A. Ketchum, E. Sodergren, J. M. Hardham, M. P. McLeod, S. Salzberg, J. Peterson, H. Khalak, D. Richardson, J. K. Howell, M. Chidambaram, T. Utterback, L. McDonald, P. Artiach, C. Bowman, M. D. Cotton, and J. C. Venter. 1998. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* **281**:375–388.
15. Ge, Y., I. G. Old, I. Saint Girons, and N. W. Charon. 1997. Molecular characterization of a large *Borrelia burgdorferi* motility operon which is initiated by a consensus sigma70 promoter. *J. Bacteriol.* **179**:2289–2299.
16. Gigliani, F., C. Ciotta, M. F. Del Grosso, and P. A. Battaglia. 1993. pR plasmid replication provides evidence that single-stranded DNA induces the SOS system in vivo. *Mol. Gen. Genet.* **238**:333–338.
17. Goodman, H. J., J. R. Parker, J. A. Southern, and D. R. Woods. 1987. Cloning and expression in *Escherichia coli* of a *recA*-like gene from *Bacteroides fragilis*. *Gene* **58**:265–271.
18. Gregg-Jolly, L. A., and L. N. Ornston. 1994. Properties of *Acinetobacter calcoaceticus recA* and its contribution to intracellular gene conversion. *Mol. Microbiol.* **12**:985–992.
19. Horii, T., T. Ogawa, and H. Ogawa. 1981. Nucleotide sequence of the *lexA* gene of *E. coli*. *Cell* **23**:689–697.
20. Hubner, A., A. T. Revel, D. M. Nolen, K. E. Hagman, and M. V. Norgard. 2003. Expression of a *luxS* gene is not required for *Borrelia burgdorferi* infection of mice via needle inoculation. *Infect. Immun.* **71**:2892–2896.
21. Karlin, S., and L. Brocchieri. 1996. Evolutionary conservation of RecA genes in relation to protein structure and function. *J. Bacteriol.* **178**:1881–1894.
22. Khil, P. P., and R. D. Camerini-Otero. 2002. Over 1000 genes are involved in the DNA damage response of *Escherichia coli*. *Mol. Microbiol.* **44**:89–105.
23. Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer. 1994. Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* **58**:401–465.
24. Kuzminov, A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. *Microbiol. Mol. Biol. Rev.* **63**:751–813.
25. Lavery, P. E., and S. C. Kowalczykowski. 1992. A postsynaptic role for single-stranded DNA-binding protein in RecA protein-promoted DNA strand exchange. *J. Biol. Chem.* **267**:9315–9320.
26. Lin, B., S. A. Short, M. Eskildsen, M. S. Klempner, and L. T. Hu. 2001. Functional testing of putative oligopeptide permease (Opp) proteins of *Borrelia burgdorferi*: a complementation model in *opp⁻ Escherichia coli*. *Biochim. Biophys. Acta* **1499**:222–231.
27. Liveris, D., G. Wang, G. Girao, D. W. Byrne, J. Nowakowski, D. McKenna, R. Nadelman, G. P. Wormser, and I. Schwartz. 2002. Quantitative detection of *Borrelia burgdorferi* in 2-millimeter skin samples of erythema migrans lesions: correlation of results with clinical and laboratory findings. *J. Clin. Microbiol.* **40**:1249–1253.
28. Miller, J. H. 1992. A short course in bacterial genetics, p. 150–156 and 268–275. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. Miller, R. V., and T. A. Kojohn. 1990. General microbiology of *recA*: environmental and evolutionary significance. *Annu. Rev. Microbiol.* **44**:365–394.
30. Narumi, I., K. Satoh, M. Kikuchi, T. Funayama, T. Yanagisawa, Y. Kobayashi, H. Watanabe, and K. Yamamoto. 2001. The LexA protein from *Deinococcus radiodurans* is not involved in RecA induction following gamma irradiation. *J. Bacteriol.* **183**:6951–6956.
31. Ohnishi, J., J. Piesman, and A. M. de Silva. 2001. Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. *Proc. Natl. Acad. Sci.* **98**:670–675.
32. Purser, J. E., M. B. Lawrenz, M. J. Caimano, J. K. Howell, J. D. Radolf, and S. J. Norris. 2003. A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of *Borrelia burgdorferi* in a mammalian host. *Mol. Microbiol.* **48**:753–764.
33. Putteet-Driver, A. D., J. Zhong, and A. G. Barbour. 2004. Transgenic expression of RecA of the spirochetes *Borrelia burgdorferi* and *Borrelia hermsii* in *Escherichia coli* revealed differences in DNA repair and recombination phenotypes. *J. Bacteriol.* **186**:2266–2274.
34. Radman, M. 1975. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis, p. 355–367. In P. S. R. Hanawalt (ed.), *Molecular mechanisms for repair of DNA*. Plenum Press, New York, N.Y.
35. Ramesar, R. S., V. Abratt, D. R. Woods, and D. E. Rawlings. 1989. Nucleotide sequence and expression of a cloned *Thiobacillus ferrooxidans recA* gene in *Escherichia coli*. *Gene* **78**:1–8.
36. Roberts, J. W., C. W. Roberts, and N. L. Craig. 1978. *Escherichia coli recA* gene product inactivates phage lambda repressor. *Proc. Natl. Acad. Sci.* **75**:4714–4718.
37. Rosa, P. A., T. Schwan, and D. Hogan. 1992. Recombination between genes encoding major outer surface proteins A and B of *Borrelia burgdorferi*. *Mol. Microbiol.* **6**:3031–3040.
38. Sartakova, M., E. Dobrikova, and F. C. Cabello. 2000. Development of an extrachromosomal cloning vector system for use in *Borrelia burgdorferi*. *Proc. Natl. Acad. Sci.* **97**:4850–4855.
39. Sassanfar, M., and J. W. Roberts. 1990. Nature of the SOS-inducing signal in *Escherichia coli*. The involvement of DNA replication. *J. Mol. Biol.* **212**:79–96.
40. Siliaty, S. N., and J. W. Little. 1987. Lysine-156 and serine-119 are required for LexA repressor cleavage: a possible mechanism. *Proc. Natl. Acad. Sci.* **84**:3987–3991.
41. Smith, G. R. 1988. Homologous recombination in prokaryotes. *Microbiol. Rev.* **52**:1–28.
42. Smith, G. R. 1989. Homologous recombination in *E. coli*: multiple pathways for multiple reasons. *Cell* **58**:807–809.
43. Stamm, L. V., E. A. Parrish, and F. C. Gherardini. 1991. Cloning of the *recA* gene from a free-living leptospire and distribution of RecA-like protein among spirochetes. *Appl. Environ. Microbiol.* **57**:183–189.
44. Stevenson, B., and K. Babb. 2002. LuxS-mediated quorum sensing in *Borrelia burgdorferi*, the Lyme disease spirochete. *Infect. Immun.* **70**:4099–4105.
45. Stevenson, B., S. Casjens, and P. Rosa. 1998. Evidence of past recombination events among the genes encoding the Erp antigens of *Borrelia burgdorferi*. *Microbiology* **144**:1869–1879.
46. Subramanian, G., E. V. Koonin, and L. Aravind. 2000. Comparative genome analysis of the pathogenic spirochetes *Borrelia burgdorferi* and *Treponema pallidum*. *Infect. Immun.* **68**:1633–1648.
47. Sung, S. Y., J. V. McDowell, J. A. Carlyon, and R. T. Marconi. 2000. Mutation and recombination in the upstream homology box-flanked *ospE*-related genes of the Lyme disease spirochetes result in the development of new antigenic variants during infection. *Infect. Immun.* **68**:1319–1327.
48. Tchamadeu Kameni, A. P., E. Couture-Tosi, I. Saint-Girons, and M. Picardeau. 2002. Inactivation of the spirochete *recA* gene results in a mutant with low viability and irregular nucleoid morphology. *J. Bacteriol.* **184**:452–458.
49. Tilly, K., J. Fuhrman, J. Campbell, and D. S. Samuels. 1996. Isolation of *Borrelia burgdorferi* genes encoding homologues of DNA-binding protein HU and ribosomal protein S20. *Microbiology* **142**:2471–2479.
50. Tilly, K., R. Hauser, J. Campbell, and G. J. Osterheimer. 1993. Isolation of *dnaJ*, *dnaK*, and *grpE* homologues from *Borrelia burgdorferi* and complementation of *Escherichia coli* mutants. *Mol. Microbiol.* **7**:359–369.
51. Wang, G., A. P. van Dam, and J. Dankert. 1999. Evidence for frequent *ospC* gene transfer between *Borrelia valaisiana* sp. nov. and other Lyme disease spirochetes. *FEMS Microbiol. Lett.* **177**:289–296.
52. Wang, I. N., D. E. Dykhuizen, W. Qiu, J. J. Dunn, E. M. Bosler, and B. J. Luft. 1999. Genetic diversity of *ospC* in a local population of *Borrelia burgdorferi* sensu stricto. *Genetics* **151**:15–30.
53. Wang, W. B., E. S. Tessman, and I. Tessman. 1988. Activation of protease-constitutive RecA proteins of *Escherichia coli* by rRNA and tRNA. *J. Bacteriol.* **170**:4823–4827.
54. Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**:103–119.
55. Yu, X., and E. H. Egelman. 1993. The LexA repressor binds within the deep helical groove of the activated RecA filament. *J. Mol. Biol.* **231**:29–40.
56. Zhang, J. R., J. M. Hardham, A. G. Barbour, and S. J. Norris. 1997. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. *Cell* **89**:275–285.
57. Zhang, J. R., and S. J. Norris. 1998. Genetic variation of the *Borrelia burgdorferi* gene *visE* involves cassette-specific, segmental gene conversion. *Infect. Immun.* **66**:3698–3704.