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Homologues of helicase genes *priA* and *ruvAB* of *Borrelia burgdorferi*, the Lyme borreliosis agent

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SUMMARY

A DNA library of strain HB19 from *Borrelia burgdorferi* sensu stricto, an agent of Lyme borreliosis, was constructed in the cosmid pLA2917. Genes involved in initiation of DNA replication and resolution of recombination intermediates (Holliday junctions) were found on a 23-kbp region up to 0.7 kbp of the "left" extremity of the linear chromosome in representative species of *B. burgdorferi* sensu lato.

The potential *ruvB* gene, located at 22 kbp from the left telomere, was identified by the similarity of its deduced amino acid sequence to RuvB (helicases) of other bacteria. *B. burgdorferi ruvB* is part of an operon which comprises the homologues of *ruvA*, *queA* and *pfbB*. Expression of the *B. burgdorferi ruvB* and *ruvA* genes renders a wild-type *Escherichia coli* sensitive to UV light and mitomycin, indicative of negative complementation.

***priA*, which encodes the potential recognition factor for the primosome assembly site, was found at 15 kbp from the left telomere. RuvB and PriA sequences have motifs characteristic of helicases: a DExH box and an ATP binding site.**

Key-words: Borrelia burgdorferi, Helicase, Gene *priA*, Gene *ruvAB*; Telomere, Replication, Recombination, Primosome.

INTRODUCTION

Borrelia burgdorferi is the agent of Lyme borreliosis (Barbour and Hayes, 1986). The genome of this spirochaete is original in that it is essentially linear, comprising a small 1-Mbp linear chromosome with several linear and circular plasmids (Barbour and Garon, 1987; Baril *et al.*, 1989; Ferdows and Barbour, 1989). The structures of the *B. burgdorferi* chromosomal telomeres are not yet known; however, those of the linear plasmids consist of covalently closed ends (Hinnebusch and Barbour, 1991).

There are models for the replication of linear genomes such as animal viruses in which it is suggested that helicases may play a role (DeLange and McFadden, 1990). DNA helicases can be divided into two sets which play essential roles in DNA replication or recombination and repair (Kornberg and Baker, 1991). Genetic recombination depends upon the

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RecA protein to promote homologous recombination and recombinational repair *in vivo*. *In vitro*, the *Escherichia coli* RecA protein catalyses the formation of an intermediate called a Holliday junction, in which the two interacting duplexes are held together. The resolution of this intermediate into recombinant products requires the product of the *ruvA*, *ruvB* and *ruvC* genes (for a review see West, 1996). *E. coli ruv* mutants are sensitive to mitomycin C, UV light and various chemical mutagens (Otsuji *et al.*, 1974).

Another set of bacterial helicases such as PriA (formerly replication factor Y or protein n') and DnaB unwind DNA at the replication fork (Lebowitz and McMacken, 1986; Lee and Marians, 1987). PriA was first discovered as a protein essential for the conversion of single-stranded ϕX174 DNA to the duplex replicative form *in vitro*. PriA binds to a specific hairpin (primosome assembly site or PAS) on ϕX174 DNA coated with single-stranded DNA binding protein. PriA and primosome assembly play a role in *E. coli* DNA replication (for a review, see Zavitz and Marians, 1991) different from that of the "*oriC* type primosome".

Our ultimate goal was to identify the structures of the chromosomal telomeres from *B. burgdorferi*. As a first step towards this objective, we characterized the genes close to the "left" extremity. In this paper, we report the isolation and nucleotide sequence of 23 kbp which includes two helicase gene homologues, *priA* and *ruvB*, *ruvA* (specificity factor for RuvB), and also *queA* (S-adenosylmethionine tRNA ribosyltransferase isomerase), *truA* (pseudouridine synthase) and *pfbB* (pyrophosphate-dependent phosphofructokinase). The transcriptional organization of *ruvA* and *ruvB* was analysed and complementation of *E. coli ruv* mutants was explored.

MATERIALS AND METHODS

Bacterial strains

B. burgdorferi sensu stricto HB19 (Barbour *et al.*, 1983), of human origin and at passage 3, was used as source of DNA for the construction of the cosmid library. The ability of the clonal population of HB19 to infect CB17 SCID mice was confirmed by A. Barbour (personal communication). *E. coli* strains (table I) were grown in LB broth or on LB agar plates (Sambrook *et al.*, 1989) with ampicillin (100 μg/ml) or isopropylthio-β-D-galactoside (IPTG) at 0.5 mM or mitomycin (0.2/0.5 μg/ml) when needed.

Construction of a *B. burgdorferi* cosmid library. Nucleotide sequence of the 23-kbp left telomeric region

Total DNA from strain HB19 was prepared as described (Frey *et al.*, 1983) from 500 ml of culture at 10^8 bacteria/ml. DNA was partially digested with *Sau*3A and purified by sizing on a 10 to 40% sucrose density gradient. The 17-30-kbp long DNA fragments (1.4 μg) were ligated into the *Bam*HI-digested and alkaline-phosphatase-treated cosmid vector pLA2917 (1.6 μg) (Allen and Hanson, 1985) which had been purified on a "Wizard" DNA clean-up system (Promega, USA). The ligated mix was packaged into phage lambda particles as described by the supplier and used to infect *E. coli* HB101. The library was kept as individual clones in microtitre plates at −80°C.

Fragments from the chosen recombinant cosmids were cloned into a pGEM-7Zf(+) multicopy plasmid cloning vector (Promega, France) which has convenient sites for the construction of sets of nested deletions (Erase-a-base system, Promega, France). Nucleotide sequencing of the subsequent inserts was performed by dideoxynucleotide chain-termination reactions (Sanger *et al.*, 1977) on double-stranded templates with T7 DNA polymerase (Pharmacia, France), α(^{33}P) dATP and synthetic oligonucleotides. Nucleic acid and deduced amino acid sequences were analysed by "BLAST" and "FASTA" programs from the GCG (Genetics Computer Group) package (Devereux, 1991) and CLUSTAL software (Higgins and Sharp, 1988).

bp = base pairs.
IPTG = isopropyl β-D-thiogalactopyranoside.
kbp = kilobasepair.
kDa = kilodalton.
ORF = open reading frame.
RT-PCR = reverse transcription-polymerase chain reaction.
UV = ultraviolet.

Cloning of PCR fragments

A 2-kbp fragment containing both *ruvA* and *ruvB* was cloned into pCR-Script™ Amp SK(+) (Stratagene, USA), after PCR. The forward and reverse primers were A48 (5'-ATC CAT TAG GAA CTT TT-3', 251 bp upstream of the ATG from *ruvA*) and A43 (5'-CTA ACT TTT GAG TTA TT-3', downstream from the *ruvB* structural gene within *queA*) from recombinant plasmids carrying the D23 and I9 inserts, respectively (fig. 1). The recombinant plasmid with the *lac* promoter in the same orientation as the *ruvA* and *ruvB* genes was kept (insert R2). D13 was like D23, but the genes were under the control of the *lac* promoter of the pGEM-7Zf(+) vector.

RNA isolation and RT-PCR

Total cellular RNA was extracted from *B. burgdorferi* in exponential growth using "Tri Reagent" (Sigma, France). The RNA was incubated at 37°C for 1 h in the presence of RNase-free DNase (0.3 U/µg, Pharmacia, France) and further purified using the "RNeasy" kit from Qiagen (Germany).

To perform coupled reverse transcription and PCR amplification (RT-PCR), the "Access" RT-PCR system (Promega, France) was used following the instructions of the manufacturer. A sample of each reaction was analysed on a 2% agarose gel with TAE buffer (Tris-acetate 0.04 M, EDTA 0.001 M, pH8).

Complementation of *E. coli ruv* mutant

Growth was in LB broth or on LB agar plates (Sambrook *et al.*, 1989) with carbenicillin (100 µg/ml) and IPTG. *E. coli* AB1157 containing *B. burgdorferi ruvAB* or *ruvB* constructs (insert R2 and D13 respectively, fig. 1) or pCR-Script™ SK(+) or pGEM-7Zf(+) vectors as controls were exposed to a calibrated 254-nm UV source for 5 or 10 s. Appropriate dilutions of exposed and unexposed cells were plated on the same medium as above. The surviving fraction was counted after UV irradiation and overnight incubation in the dark at 37°C.

RESULTS AND DISCUSSION

Gene order within the left telomeric region of the linear chromosome of *B. burgdorferi*

B. burgdorferi sensu stricto strain HB19 was used as a source of DNA for the construction of a cosmid library. A 6.9-kbp BsF fragment (from strain 212) (Davidson *et al.*, 1992) bordered by a *Bss*HII site and the left telomere was used as a probe to screen the *B. burgdorferi* cosmid library by colony hybridization (Grunstein and Hogness, 1975). Four out of nine selected recombinant cosmids had inserts of approximately 20 kbp with several restriction fragments in common. Cosmid Db9 was kept for further study. After digestion of Db9 with *Eco*RI, the resulting six fragments (fig. 1) were cloned into pGEM-7Zf(+) (Promega, France).

Analysis of the nucleotide sequence of the inserts of the six recombinant plasmids derived from Db9 enabled the design of the potential genes present in the left telomeric region (fig. 1A). The fragment I12 located closest to the telomere (at 0.7 kbp) comprises several ORFs without any significant similarity with the content of the available data libraries. We had previously analysed insert D4, which comprises the *udk* gene at 15 kbp (Boursaux-Eude *et al.*, 1997) and in the following paragraphs, we analysed the inserts I2, D23 and I9, which are located at 13 and 15-23 kbp from the left telomere.

PriA and RuvB contain structural motifs characteristic of helicases

An open reading frame of 1,980 bp was identified as the potential coding region for PriA, the recognition factor for the primosome assembly site. This ORF begins at one extremity of fragment I2, continues into fragment D4 (fig. 1) and is oriented away from the left telomere and opposite *udk* (Boursaux-Eude *et al.*, 1997). The molecular mass of the *priA* gene product was calculated to be 77,316 Da. We found significant similarity between *B. burgdorferi* PriA and the published sequences of PriA from *E. coli* (28% identity), *Haemophilus influenzae* (28% identity) and PriA (URF2) from *Rhodospirillum rubrum* (26% identity) (Falk *et al.*, 1985; Fleischmann *et al.*, 1995; Lee *et al.*, 1990; Nurse *et al.*, 1990) (fig. 2). The *priA* gene had previously been mapped (Casjens *et al.*, 1995) at the left extremity of the chromosome in 20 representatives of *B. burgdorferi* sensu lato.

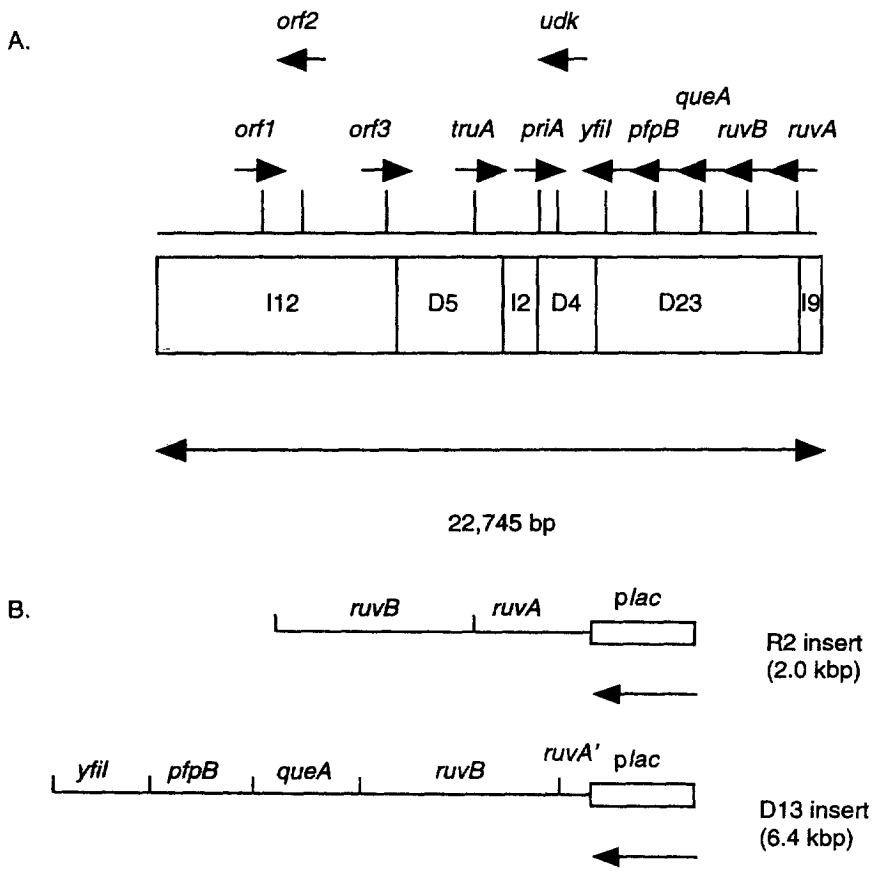

Fig. 1. A) Location of the potential genes originating from the 22,760-bp left telomeric region of the *B. burgdorferi* linear chromosome with the direction of transcription indicated by arrowheads.

The linear chromosome ends at approximately 0.7 kbp from the left of the figure. The vertical bars below the arrowheads are situated in the middle of the gene. The names of the 6 recombinant inserts carrying the genes or ORFs are boxed by *Eco*RI sites. Accession number AJ224476 has been attributed to the sequence of the 112 insert, while AJ224474 corresponds to the D5 insert with the *truA* gene. The sequence data of the potential genes *udk, priA* and *queA, ruvB, ruvA* have been assigned Genbank accession numbers X97449 and Y08885, respectively (inserts I2, D4, I9 and part of D23). AJ224475 corresponds to the rest of the D23 insert with the *pfpB* homologue.

B) Inserts R2 and D13, the construction of which is described in "Materials and methods", are indicated.

Direction of transcription is as above.

At one extremity of the 6.5-kbp *Eco*RI fragment (D23, see fig. 1), an ORF of 1,044 bp was identified as the potential coding region for RuvB, a helicase. A putative ribosome-binding site AGGA was located 7 bp upstream of the ATG start codon. The molecular mass of the *ruvB* gene product was calculated to be 38,981 Da. *B. burgdorferi* RuvB had a significant similarity of up to 50% when compared to nine eubacteria belonging to different phyla, namely Gram-negative, thermophiles, cyanobacteria, Gram-positive, *Mycoplasma* (all accession numbers are noted and six of the representative sequences are aligned in figure 3).

At the N-terminus of *B. burgdorferi* PriA and RuvB (figs. 2 and 3), there is a motif characteristic of an ATP binding site with the "P loop" consensus (GA)XXXXGK(ST) (Saraste *et al.*, 1990). RuvB from 6 bacterial species showed the motif GPPG(LV)GKT, while PriA from 4 bacterial spe-

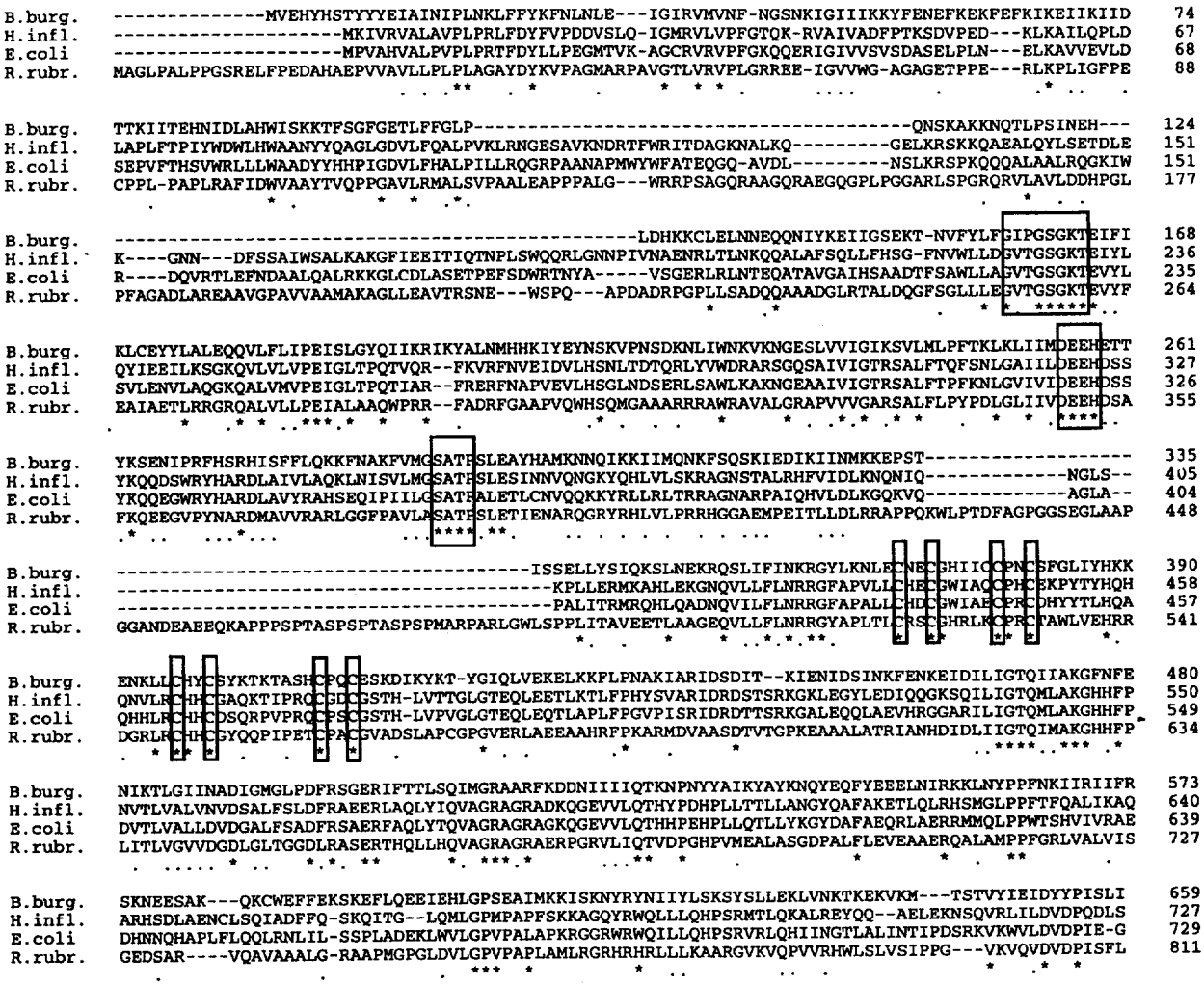

Fig. 2. Alignment of *B. burgdorferi* PriA with PriA from *H. influenzae* (P44647), *E. coli* (P17888) and *Rhodospirillum rubrum* (P05445).

The P loop and DExH motive are boxed at positions 157-164 and 255-258 from the *B. burgdorferi ruvB* sequence, respectively. The SATP motif is boxed at positions 291-294. The cysteine-rich zinc-binding motif $C(X)_2C(X)_5C(X)_2C(X)_{14}C(X)_2C(X)_9C(X)_2C$ is indicated by boxes around the cysteines from positions 369 to 412 of the *B. burgdorferi priA* sequence. The following is also valid for fig. 3. Identical residues are indicated by stars, similar residues by dots. A hyphen indicates a gap.

cies showed the motif G(IV)(PT)GSGKT. *B. burgdorferi* PriA is different at the second and third position of the latter motif (boxed in figs. 3 and 2, respectively). The second motif, shared by RuvB and PriA, is the DExH motif characteristic of helicases (Ouzounis and Blencowe, 1991) which is found in the first half of the molecule (second boxes in figs. 2 and 3). In addition, PriA possesses a SATP motif (positions 291-294, fig. 3) and in the second half of the molecule, 8 cysteines within 44 AA indicative of a cysteine-rich metal-binding motif (positions 369-412, fig. 3). The consensus $C(X)_2C(X)_5C(X)_2C(X)_{14}C(X)_2C(X)_9C(X)_2C$ is characteristic of zinc fingers found in proteins such as eukaryotic transcription factors and prokaryotic DNA binding proteins (Vallee *et al.*, 1991).

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B.burg.      MKDENSISFLSSNENYLYD-KSENELRPKV-FEDFKGQVNVKETLSIFIRASKERDEALDHVFLSGPPGLGKTTLAS   75
E.coli       MIEAD--RLISAGTTLPED-VADRAIRPKL-LEEYVGQPQVRSQMEIFIKAAKLRGDALDHLLIFGPPGLGKTTLAN   73
H.infl.      MIEAD--RIISGQAKVDED-VIDRAIRPKL-LADYVGQPQVREQMDIFIKAAKLRQDALDHLLIFGPPGLGKTTLAN   73
P.aeru.      MIEPD--RLISAVSGRERDEQLDRAIRPLK-LADYIGQPSVREQMELFIHAARGRQEALDHTLIFGPPGLGKTTLAN   74
M.pneu.      ----------------------MKLQIKPPNNFAEFVGKQEIINQIQLSIKASRINKAQLDHILLYGPPGVGKTTLAR   56
T.mari.      -----MSEFLTPERTVYDS--GVQFLRPKS-LDEFIGQENVKKKLSLALEAAKMRGEVLDHVLLAGPPGLGKTTLAH   69
                                    ..*     .. *. .  . . . *..     ***  .  ****.***  ***

B.burg.      IIAFEMNASIKITSAPAFDKPKDIIGILTGLDEKSVLFIDEIHRLRPIIEEMLCIAMEDYELDWVIGQGANARTVRM  152
E.coli.      IVANEMGVNLRTTSGPVLEKAGDLAAMLTNLEPHDVLFIDEIHRLSPVVEEVLYPAMEDYQLDIMIGEGPAARSIKI  140
H.infl.      IVANEMGVNIRTTSGPVLEKAGDLAAMLTNLEPHDVLFIDEIHRLSPAIEEVLYPAMEDYQLDIMIGEGPAARSIKL  150
P.aeru.      IIAQEMGVSIKSTSGPVLERPGDLAALLTNLEAGDVLFVDEIHRLSPIVEEVLYPAMEDFQLDIMIGEGPAGRSIKL  151
M.pneu.      LIASEMNTKLQIIQGGHLQRPSDFLNAVSLIKKGDVLFVDEIHAVAPSVMELMFPVMDDFRVQVLIGKDFNSKMVEM  133
T.mari.      IIASELQTNIHVTSGPVLVKQGDMAAILTSLERGDVLFIDEIHRLNKAVEELLYSAIEDFQIDIMIGKGPSAKSIRI  146
             ..* *.   ..        . *   .. .   ***.****  .  . *..  ..*. .  .**     . . .

B.burg.      PLPKFTLIGATTKPGKVTSPLYARFGITARFELYSEIELVEIIKRNSLILNIEIEEDAAFLLARSSRGTPRIANRLL  229
E.coli       DLPPFTLIGATTRAGSLTSPLRDRFGIVQRLEFYQVPDLQYIVSRSARFMGLEMSDDGALEVARRARGTPRIANRLL  227
H.infl.      DLPPFTLVGATTRAGSLTSPLRDRFGIVQRLEFYSVEDLTSIVARSAGCLNLELEQQAAFEVARRSRGTPRIANRLL  227
P.aeru.      DLPPFTLVGATTRAGMLTNPLRDRFGIVQRLEFYNVEDLATIVSRSAGILGLEIEPQGAAEIAKRARGTPRIANRLL  228
M.pneu.      KVNPFTWIGATTQFGKIINPLEDRFGMILNIDYYSNQEIERIVSIYGEQMELELKPEEITQITQHSKQTPRIAIRIV  210
T.mari.      DIQPFTLVGATTRSGLLSSPLRSRFGIILELDFYTVKELKEIIKRAASLMDVEIEDAAAEMIAKRSRGTPRIAIRLT  223
              .  ** .****. * .  **  ***.    . *   ..  *.      . .*.       ... .. ***** *.

B.burg.      RRIRDIAQVTGSLVITSDIVSIGLEMLRIDGEGLDEQDRNILRSLILKFNGGPVGVDTLAISVGETADSLEDFYEPY  306
E.coli       RRVRDFAEVKHDGTISADIAAQALDMLNVDAEGFDYMDRKLLLAVIDKFFGGPVGLDNLAAAIGEERETIEDVLEPY  304
H.infl.      RRVRDYADVRNGGIISINVAKQALSMLDVDDAGFDYLDRKLLSAVIERFDGGPVGLDNLAAAIGEERDTIEDVLEPY  304
P.aeru.      RRVRDFAEVRGQGDITRVIADKALNLLDVDERGFDHLDRRLLLTMIDKFDGGPVGIDNLAAALSEERHTIEDVLEPY  305
M.pneu.      KRLFEQKIVN--KKIDLAALFKSLMIYKN---GLQSIDVQYLKALNGQYE--PQGIKSICSMLGIDKSTVENKIEPF  280
T.mari.      KRVRDMLTVVKADRINTDIVLKTMEVLNIDDEGLDEFDRKILKTIIEIYRGGPVGLNALAASLGVEADTLSEVYEPY  300
             .*. .   *    *        . .      *   *   * .. .  * *. .. .     ..    **.

B.burg.      LIMKGFISRTHRGRKATEFAYLHLNLEMKEDGLNENQRVSF------                              347
E.coli       LIQQGFLQRTPRGRMATTRAWNHFGITPPEMP---------------                              336
H.infl.      LIQQGFLQRTPRGRIATSQTYRHFGLQKLSD----------------                              335
P.aeru.      LIQQGYIMRTPRGRVVTRHAYLHFGLNIPKRLGPGVTTDLFTSEDGN                              352
M.pneu.      LLRENMIQKTKKGRIITRTGRNYLTSC--------------------                              307
T.mari.      LLQAGFLARTPRGRIVTEKAYKHLKYEVPENRLF-------------                              334
             *.  . . .* .**  *
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Fig. 3. Alignment of *B. burgdorferi* RuvB with RuvB from *H. influenzae* (P44631), *E. coli* (AE000280), *Pseudomonas aeruginosa* (D83138), *Mycoplasma pneumoniae* (AE000028), *Mycobacterium leprae* (U00011) and *Thermus maritima* (U38840).

Alignments with the sequences of *Mycobacterium tuberculosis* (Z77724), *Mycoplasma genitalium* (U39720), *Synechocystis* PCC 6303 (U38892) and *Thermus aquaticus* (U22817) are not shown. The P loop and DExH motif are boxed at positions 64-71 and 115-118 from the *B. burgdorferi* RuvB sequence, respectively. See also legend, figure 2.

ruvB is the second gene of an operon comprising at least four genes: *ruvA, ruvB, queA* and *pfpB*

45 bp upstream from *ruvB*, we found the beginning of another ORF which continued into the *Eco*RI fragment I9 adjacent to D23 (fig. 1). This ORF was a *ruvA* homologue (encoding a specificity factor for RuvB) and consisted of 197 codons. The molecular mass of the *ruvA* gene product was calculated to be 22,702 Da. A significant identity was found between *B. burgdorferi* RuvA and RuvA from *Haemophilus influenzae* (44%), *E. coli* (42%), *Pseudomonas aeruginosa* (40%) and *Mycobacterium leprae* (37%) (Benson *et al.*, 1988; Fleischmann *et al.*,

1995; Hishida *et al.*, 1996; Philipp *et al.*, 1996; Shinagawa *et al.*, 1988) (data not shown).

The proximity of *ruvA* and *ruvB* suggested that these genes might be arranged in an operon. We tested the potential cotranscription of *ruvA* and *ruvB* using RT-PCR. A band of the expected size (690 bp) was found between *ruvA* and *ruvB* (fig. 4, lane 2). In addition, the potential *queA* gene (encoding S-adenosylmethionine tRNA ribosyltransferase isomerase) (Slany *et al.*, 1993) overlaps the end of *ruvB* by 35 bp. This gene is followed by a potential *pfpB* gene which could encode a pyrophosphate-dependent phosphofructokinase (Carlisle *et al.*, 1990). We checked whether the mRNA continued onwards from *ruvB*. Bands of the expected size were found between *ruvB* and *queA* (657 bp) and between *queA* and *pfpB* (612 bp) (fig. 4, lanes 4 and 6). These transcripts were not formed when reverse transcriptase was omitted from the reaction mix (fig. 4, lanes 3, 5 and 7), confirming that the RT-PCR products were derived from mRNA and not DNA.

The organization of *B. burgdorferi ruvA* and *ruvB* in an operon can be compared to the organization of *E. coli ruvA* and *ruvB* in a single LexA-regulated operon (Benson *et al.*, 1988; Shinagawa *et al.*, 1988). However, no SOS boxes could be identified in the upstream region of the *B. burgdorferi ruv* operon, and the latter is much larger, since it contains at least two additional genes. Large operons have been found in *B. burgdorferi* for genes involved in motility of the bacterium (Ge *et al.*, 1977) and this feature is perhaps related to its small genome size (Baril *et al.*, 1989; Ferdows and Barbour, 1989). In contrast to the situation found for *E. coli* and *B. burgdorferi*, the *ruvB* gene is a single transcription unit in thermophiles (Tong and Wetmur, 1996) and in the spirochaete *Treponema pallidum*, which is responsible for syphilis (S. Norris, personal communication). The *ruvA* and *ruvB* genes are adjacent in *H. influenzae* and *M. leprae*, but their organization as an operon has not been demonstrated experimentally (Fleischmann *et al.*, 1995; Philipp *et al.*, 1996).

The *ruvB* gene was also located in the left telomeric region of the chromosome (at 22 kbp from the extremity) for representatives of several *Borrelia* species, *B. burgdorferi sensu stricto* HB19, 212, B31, *B. garinii* 20047, *B. afzelii* VS461 and *B. japonica* HO14 (data not shown), as expected, since their genetic maps had been found to be very similar (Casjens *et al.*, 1995; Ojaimi *et al.*, 1994).

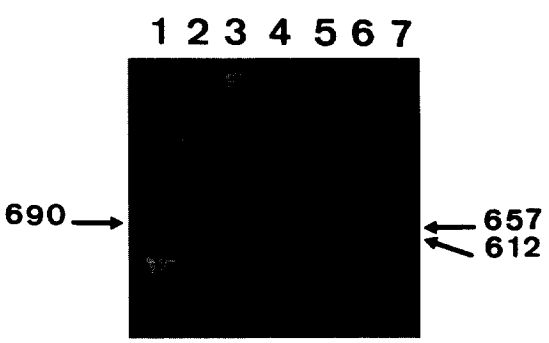

Fig. 4. RT-PCR products after agarose gel electrophoresis.

A sample of each reaction was analysed on a 2% agarose gel with TAE buffer (0.04 M Tris-acetate; 0.001 M EDTA pH8). The amplified products span the intergenic regions as follows: lane 2, *ruvA-ruvB*, lane 4, *ruvB-queA*, lane 6, *queA-pfpB*, lanes 3, 5 and 7, corresponding negative controls without reverse transcriptase. Lane 1: ΦX174 markers restricted by *Hae*III (bp).

Comparison of the left telomeric region of the chromosomes of B31 and HB19 strains

While this paper was in preparation, the preliminary sequence data of almost the whole linear chromosome of *B. burgdorferi* strain B31 (970,715 nucleotides) was made available by TIGR (ftp://ftp.tigr.org/pub/data/b_burgdorferi). The available sequence is not annotated; it is reported by TIGR to contain errors and is oriented with the right extremity corresponding to our left extremity. Comparison of the sequence closest to the telomere from strain HB19 (I12 fragment, fig. 1A) with the corresponding sequence from strain B31 revealed that there were 51 differences for 8,652 nucleotides.

The *priA* structural gene from strain HB19 is 99% identical to nucleotides 897970 to 895988 from strain B31 (http://www.ncbi.nlm.nih.

gov/cgi-bin/BLAST/nph-tigrbl) with four differences. The differences at nucleotides 372, 377, 453 and 1663 correspond to positions 897621, 897626, 897584 and 896274 and lead to one amino acid change from leucine to proline. This difference remains to be confirmed when the definitive B31 sequence from TIGR is deposited in the data banks.

The *ruvA* and *ruvB* structural genes from strain HB19 are 99% identical to nucleotides 887977 to 888516 and 888615 to 889658, respectively, from strain B31 (http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-tigrbl). The differences at positions 51, 156, 308 and 342 for *ruvA* correspond to nucleotides 888027, 888132, 888284 and 888318. The differences at positions 213, 657, 846 and 939 for *ruvB* correspond to nucleotides 888827, 889271, 889460 and 889553. These differences do not lead to any changes in the amino acid sequence.

The identity at 99% of the *ruvA*, *ruvB* and *priA* sequences for the two strains confirmed the sequence conservation within the same *B. burgdorferi* sensu stricto species.

Sensitivity of wild-type *E. coli* to mitomycin C and UV due to expression of *B. burgdorferi ruvA* and *ruvB* genes

To assess the functionality of the *B. burgdorferi* Ruv proteins we attempted to complement *E. coli ruv* mutants. Use was made of *ruvB* or *ruvA* or (*ruvA, ruvB*) *E. coli* mutants (table I) transformed by the construct bearing both *B. burgdorferi ruvB* and *ruvA* genes (insert R2; see fig. 1). No positive complementation (assayed by restoration of resistance to mitomycin C in the presence of IPTG) was detected. Although *B. burgdorferi* RuvB (and RuvA) are similar to other bacterial RuvB (and RuvA) regarding their amino acid sequence, the absence of complementation is not surprising, since spirochaetes, although members of the eubacteria (Woese, 1987), are phylogenetically distant from *Enterobacteriacae*. Only partial complementation was obtained in the case of RuvB from thermophilic eubacteria (Tong and Wetmur, 1996).

Surprisingly, expression of *B. burgdorferi ruvB* (and/or *ruvA*) in wild-type mitomycin-C- and UV-resistant *E. coli* renders the latter sensitive. When transformed by pCR-Script (R2) (fig. 1B), the corresponding wild-type strain AB1157 (table I) was more sensitive to mitomycin and UV than was the control AB1157 transformed with the plasmid vector pCR-Script alone (table II). Similar results were obtained when AB1157 was transformed with pGEM (D13) recombinant plasmid where the *ruvB* gene (followed by *queA, pfbB* and other genes; fig. 1B) is under the control of p*lac* (table II).

These results suggest that *B. burgdorferi ruvB* and *ruvA* gene products may interfere with the activity or formation of the complex multimeric structure of *E. coli* RuvAB (Parsons *et al.*, 1995). It has been shown that overproduction of *E. coli* RuvA has a profound negative effect in *E. coli*, amplified by the presence of an SOS-regulated

Table I. Characteristics of *E. coli* strains used in this study.

Strain	Genotype	Reference
AB1157	F- *thi-1 his-4 Δ(gpt proA)62 argE3 thr-1 leuB6 ara-14 kdgK51 rfbD1 lacY1 galK2 xyl-5 mtl-1 supE44 tsx-33 rpsL31*	(Bachmann, 1996)
HI24	Like AB1157, but *ruvB4*	(Otsuji *et al.*, 1974)
N2057	Like AB1157, but *ruvA60::Tn*10 with a polar effect on *ruvB*	(Sharples, 1990)
N3881	Like AB1157, but *ruvA200 eda*-51::*Tn*10	(Sargentini and Smith, 1989)

Table II. Effects of *B. burgdorferi ruvB* or *ruvA ruvB* expression on survival to UV light of *E. coli* AB1157 transformed by different constructs.

Plasmid vector	*B. burgdorferi* insert	Expression of: *ruvA*	*ruvB*	Relative survival 5"	10"
pCR-Script	O	–	–	0.2	0.03
pCR-Script	R2	+	+	0.02	0.006
pGEM-7f(+)	O	–	–	0.15	0.009
pGEM-7f(+)	D13	–	+	0.03	0.002

Experiments were reproducible and numbers correspond to the means of 2 to 3 experiments.

E. coli promoter (Sharples, 1990). Here, we demonstrate that overproduction of *B. burgdorferi* RuvB, or of both RuvA plus RuvB, has a modest negative effect. Similar results of negative complementation were obtained with *B. burgdorferi flgB*, which renders wild-type *E. coli* non-motile (Ge *et al.*, 1997).

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Homologues des gènes spécifiant des hélicases, *priA* et *ruvAB* de *Borrelia burgdorferi*, agent de la borréliose de Lyme

Une banque d'ADN de la souche HB19 de *Borrelia burgdorferi* sensu stricto, agent de la borréliose de Lyme, a été construite dans le cosmide pLA2917. Des gènes impliqués dans la mise en route de la réplication de l'ADN et la résolution des intermédiaires de recombinaison (jonctions Holliday) ont été trouvés dans une région de 23 kpb située à moins de 0,7 kpb de l'extrémité gauche du chromosome linéaire de diverses espèces de *B. burgdorferi* sensu lato.

Le gène *ruvB* potentiel, situé à 22 kbp du télomère gauche a été identifié grâce à la similitude de la séquence en acides aminés de son produit avec celle de RuvB (hélicase) d'autres bactéries. Le gène *ruvB* de *B. burgdorferi* fait partie d'un opéron comprenant les gènes homologues de *ruvA*, de *queA* et de *pfbB*. L'expression des gènes *ruvB* et *ruvA* de *B. burgdorferi* chez *Escherichia coli* de type sauvage rend la bactérie sensible à la lumière ultraviolette et à la mitomycine, ce qui indique une complémentation négative.

Le gène *priA*, qui spécifie le facteur de reconnaissance potentiel du site d'assemblage du primosome, est situé à 15 kpb du télomère gauche. Les séquences RuvB et PriA ont des motifs caractéristiques d'hélicases: une boîte DExH et un site de fixation de l'ATP.

Mots-clés: Borrelia burgdorferi, Hélicase, Gène *priA*, Gène *ruvAB*; Télomère, Réplication, Recombinaison, Primosome.

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