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Plasmid Location of *Borrelia* Purine Biosynthesis Gene Homologs

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The Lyme disease spirochete *Borrelia burgdorferi* must survive in both its tick vector and its mammalian host to be maintained in nature. We have identified the *B. burgdorferi guaA* gene encoding GMP synthetase, an enzyme involved in de novo purine biosynthesis that is important for the survival of bacteria in mammalian blood. This gene encodes a functional product that will complement an *Escherichia coli* GMP synthetase mutant. The gene is located on a 26-kb circular plasmid, adjacent to and divergent from the gene encoding the outer surface protein C (OspC). The *guaB* gene homolog encoding IMP dehydrogenase, another enzyme in the purine biosynthetic pathway, is adjacent to *guaA*. In *Borrelia hermsii*, a tick-borne relapsing fever spirochete, the *guaA* and *guaB* genes are located on a linear plasmid. These are the first genes encoding proteins of known function to be mapped to a borrelial plasmid and the only example of genes encoding enzymes involved in the de novo purine biosynthesis pathway to be mapped to a plasmid in any organism. The unique plasmid location of these and perhaps other housekeeping genes may be a consequence of the segmented genomes in borreliae and reflect the need to adapt to both the arthropod and mammalian environments.

De novo purine biosynthesis is essential for infectivity, growth, and virulence of many bacteria in mammals (7, 11, 17, 20, 35). Purine biosynthesis is important for extracellular blood-borne pathogens to survive because purine levels in the blood are so low that purine salvage is usually inefficient. In fact, a purine auxotroph was used to identify *Salmonella typhimurium* genes in vivo that are necessary for virulence (21). In that system, the *purA* gene was used as a selectable marker for growth in mouse tissue.

De novo purine biosynthesis and interconversion of adenine and guanine compounds in the salvage pathway take place through the common precursor IMP. GMP is derived from IMP in two steps: oxidation of IMP to XMP is catalyzed by IMP dehydrogenase, encoded by the *guaB* gene, and subsequent amination of XMP to GMP is catalyzed by GMP synthetase, encoded by the *guaA* gene. The *Escherichia coli guaA* gene is in an operon with *guaB* (38), while the two genes are separate in *Bacillus subtilis* (23). All genes that encode enzymes involved in de novo purine biosynthesis reside on the chromosome in every organism studied so far (41). Furthermore, all steps in the biosynthetic pathway itself appear to be conserved in both prokaryotes and eukaryotes (28, 41).

We are interested in outer surface protein variation in *Borrelia burgdorferi* and have recently cloned the gene for the outer surface protein OspC from *B. burgdorferi* CA-11.2A (26). The *ospC* gene has been mapped to a 26-kb supercoiled circular plasmid in *B. burgdorferi* (24, 30). OspC synthesis in the CA-11.2A clone varies with in vitro passage and is inversely correlated with the synthesis of two other outer surface proteins, OspA and OspB (26). Analyses of the immune responses of Lyme disease patients to *B. burgdorferi* indicate that the most prominent early response is to OspC, whereas reactivities to OspA and OspB develop late in infection, if at all (8, 40). Vaccine studies in mice have demonstrated that immunization with OspA will elicit neutralizing antibodies that prevent infection by destroying spirochetes in the midgut of infected ticks, but that an anti-OspA immune response is ineffective against a previously established infection (10). These studies suggest that the expression of the *osp* genes may be modulated by the tick versus mammal environment. To begin a study of the regulation of *ospC*, we sequenced its 5′ flanking region, where we found the *B. burgdorferi* homologs of *guaA* and *guaB*, adjacent to and divergent from *ospC*.

MATERIALS AND METHODS

Strains. *B. burgdorferi* B31 (ATCC 35210), *B. burgdorferi* CA-11.2A (27), and *Borrelia hermsii* HS1 (ATCC 35209) were passaged in BSKII medium at 35°C as described previously (2). CA-11.2A is a clone derived from *Ixodes pacificus* tick isolate CA-11-90 from Sacramento County, California (33). Total genomic DNA was purified from *Borrelia* spp. as described previously (29).

Cloning and sequencing. Most of the *guaA* gene was sequenced on both DNA strands from the same recombinant plasmid that contained *ospC*, as previously described (26). Briefly, partial amino acid sequence of OspC tryptic peptides allowed the design of two degenerate oligonucleotides that were used in a PCR. The amplified fragment was used to identify and clone a segment of genomic DNA that contained the entire *ospC* gene. Sequencing the 3-kb *Pst*I insert containing *ospC* demonstrated that it also encoded the first 489 amino acids of GMP synthetase. By direct PCR cycle sequencing of CA-11.2A genomic DNA (GIBCO-BRL, Gaithersburg, Md.), we sequenced the 3′ end of *guaA* and the 5′ end of *guaB*, which were not included within the insert. The entire *guaB* gene was sequenced on both DNA strands from plasmid pDH68, described below. Sequence comparisons to protein sequence databases were performed by using the BLAST network service (National Center for Biotechnology Information, Bethesda, Md.) (1). The *guaA* and *guaB* sequences from *B. burgdorferi*, *Bacillus subtilis* (18, 23), and *E. coli* (37, 38) were aligned by using the MacVector 4.0 program (IBI, New Haven, Conn.).

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GuaA

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                 *         *         *         *         *         *         *         *
B. burg.  MM-N--AQAILVLDFGSQYSQLIARRIREIGVYTKVIPYYTPLKEIKNMNISGIILSGSPASVYSKEAPTLDMEIFNLKI 77
B. subt.  .t-klvnem.........n...t.....f...selh.htltae...k..pk......g.n...densfrc.ek..e.d. 79
E. coli   .te.ihkhr..i.......t..v...v..l...celwawdvteaq.rdf.p.......g.e.tteens.rapqyv.eagv 80
                 *         *         *         *         *         *         *         *
B. burg.  PVLGICYGMQIIVKLFGGLVSKDSKQEYGRSEIFLKDEKSL-------LFSELPNKFQII-MSHGDSIEKIPDNFK-QLA 148
B. subt.  .........lmthyl..k.eaatqr...kan.ri-egtpd-------..rd...e-.vvw.....lvvev.eg.tvda. 150
E. coli   ..f.v.....tmamql..h.easner.f.yaqvevvndsa.vrgieda.tadgkplldvw-.....kvta..sd.i-tv. 158
                 *         *         *         *         *         *         *         *
B. burg.  FTKNC-IASISNETQKIYGLQFHPEVTHSEFGDQILKNFVFKICQAQINWSLEGNLETIVKKIKLKVGSKKVILGLSGGT 227
B. subt.  tshh.pnsam.kgdk.wh.v......r...y.ndl......gv.esege..m.nfi.iemq..req..d.q.lc.....v 230
E. coli   s.es.pf.ima..ekrf..v........trq.mrm.er..rd...ceal.tpakiidda.ar.req..dd.........v 238
                 *         *         *         *         *         *         *         *
B. burg.  DSLVCALLIKKAINENLICVFVNTGLLRKNENKKIL-EL-KHQYDLNIKYIDASTKFLNRLKNISDPEEKRKIIGKEFVD 305
B. subt.  ..s.v.v..h...gdq.t.i..dh.....g.regvmktf-segfnm.vikv..kdr...k..gv....q......n..iy 309
E. coli   ..s.t.m.lhr..gk..t....dn....l..aeqv.-dmfgdhfg...vhvp.edr..sa.agen...a......rv..e 317
                 *         *         *         *         *         *         *         *
B. burg.  VFEKITLEDQNIEYLAQGTIYSDVIESKSKDSSSSK-IKSHHNVGGLPDKMSLKLLEPLNEFFKDEIIQIGINLGIKKES 384
B. subt.  ..ddeadklkg.d......l.t.i...-g-tataqt-...........ed.qfe.i....tl....vral.te...pd.i 386
E. coli   ..deea.kledvkw.......p.....aasatgkahv...........ke.kmg.v...k.l....vrk..le..lpydm 397
                 *         *         *         *         *         *         *         *
B. burg.  LYRHPFPGPGLAIRIIGEVTQEKINILQEADNILTEELFINDLYYQIRQAFVVLLPVKSVGVMGDQRTYEYTAVIRCVNT 464
B. subt.  vw.q.......g..vl....e..le.vr.s.a..r..ianhg.erd.w.y.t..pdir.......a...d..ies.rtsi 467
E. coli   ..........gv.vl...kk.ycdl.rr..a.fi...rka...dkvs...t.f...r.......g.k.dwvvsl.a.e. 477
                 *         *         *         *
B. burg.  QDFMTAEWTELPYSFLKKV-SSRIINEVRGINRVCYDISSKPPSTIEWE  512
B. subt.  dg-..sd.ari.wdv.e-.i.t..v...kh....v...t....a.....  513
E. coli   i.....h.ah...d..gr.-.n......n..s..v....g...a.....  525
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GuaB

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                 *         *         *         *         *         *         *         *
B. burg.                            MPNKITKEALTFDDVSLIPRKSSVLPSEVSLKTQLTKNISLNIPFLSSAMDTVTESQM 58
E. coli   mqsvtlcimprqyllttlveil.mlr.a.........l.v.ah.t...ntad.s.....t.r....m..a.......arl 80
B. subt.                          mwes.fs..g......l.v.a..e...hd.d.sve...tlk....vi.ag.......a. 59
                 *         *         *         *         *         *         *         *
B. burg.  AIAIAKEGGIGIIHKNMSIEAQ-------------------------------------------------------- 80
E. coli   ...l.q.....f........r.aeevrrvkkhesgvvtdpqtvlptttlrevkelterngfagypvvteenelvgiitgr 160
B. subt.  ...m.rq..l..........q.aeqvdkvkrsergvitnpffltpdhqvfdaehlmgkyrisgvpivnneedqklvgiit 139
                 *         *         *         *         *         *         *         *
B. burg.  --------------------RKEIEKVKTYKFQKTINTNGDTNEQKPEIFTAKQHLEKSDAYKNAEHKEDFPNACKDLNN 140
E. coli   dvrfvtdlnqpvsvymtp-kerlvtvregearevvlakmhekrve.alvvddef..igmitv.dfqka.ak......eqg 239
B. subt.  nrdlrfisdysmkisdvmtkeelvtasvgttldeaekilqkhkie.lplvddqnk.kgliti.di.kvie...ss..ihg 219
                 *         *         *         *         *         *         *         *
B. burg.  KLRVGAAVSIDIDTIERVEELVKAHVDILVIDSAHGHSTRIIELIKKIKTKYPNLDLIAGNIVTKEAALDLISVGADCLK 220
E. coli   r.......gagagne...da..a.g..v.l...s....egvlqr.retra...d.qi.g..va.aag.ra.aea.csav. 319
B. subt.  r.i.....gvtg..mt..kk..e.n..vi...t.....qgvlntvt..ret..e.ni....va.a..tra..ea...vv. 299
                 *         *         *         *         *         *         *         *
B. burg.  VGIGPGSICTTRIVAGVGVPQITAICDVYEACNNTNICIIADGGIRFSGDVVKAIAAGADSVMIGNLFAGTKESPSEEII 300
E. coli   .............t.........va.av..leg.g.pv...........ia.......sa..v.sml...e...g.iel 399
B. subt.  ............v............y.catearkhgkt.......k....it..l...gha..l.s.l...s...g.te. 379
                 *         *         *         *         *         *         *         *
B. burg.  YNGKKFKSYVGMGSISAMKRGSKSRYFQLENNEPKKLVPEGIEGMVPYSGKLKDILTQLKGGLMSGMGYLGAATISDLKI 380
E. coli   .q.rsy...r....lg..sk..sd....-sd.aad.........r.a.k.r..e.ih.qm...r.c..lt.cg..de.rt 478
B. subt.  .q.rr..v.r....va..ek...d....-.--.n..f.......rt..k.pveetvy..v...r.....c.skdlra.re 456
                 *         *         *         *         *
B. burg.  NSKFVKISHSSLKESHPHDVFSIT 404
E. coli   kae..r..gagiq...v...-t..kespnyrlgs 511
B. subt.  eaq.irmtgag.r.......-q..vhrnkalpglfgshqkktgfvydeccqsgffssd 513
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FIG. 1. Alignment of the deduced amino acid sequences of GMP synthetase and IMP dehydrogenase. Dots represent identical amino acids, dashes represent missing amino acids, and lowercase letters represent amino acid differences from the *B. burgdorferi* (*B. burg.*) sequence. Numbers to the right of each line represent the position of the last amino acid on that line. The *B. burgdorferi guaA* sequence continues in the same reading frame to another methionine 16 residues upstream of the indicated N-terminal methionine; however, this distal ATG codon is not close to a ribosome binding site and contains the apparent transcriptional start site of *guaA*. *B. subt.*, *Bacillus subtilis*.

Complementation. Plasmids pDH60 and pDH68 were derived from a library of *B. burgdorferi* B31 DNA partially digested with *Tsp*509I (New England Biolabs), cloned into vector λZAPII (Stratagene, La Jolla, Calif.), and screened with a *guaA* probe. The plasmids were obtained by in vivo excision as described by the manufacturer. PCR analysis and partial DNA sequencing showed that pDH60 contains an incomplete *guaA* gene in the same orientation as the vector-borne *lac* promoter, and pDH68 contains the entire *guaA* and *guaB* genes and upstream sequences extending to the start of the *ospC* gene in inverted orientation with respect to the *lac* promoter. Plasmids pDH60 and pDH68 were transformed into the *E. coli guaA*::Tn*10* strain ght1 (39). Transformants were selected on L ampicillin plates and individually tested for ability to grow on minimal plates without guanine (22).

Southern blot analysis. CA-11.2A DNA was cut with selected restriction endonucleases as instructed by the manufacturer (New England Biolabs, Beverly, Mass.), electrophoresed on a 0.8% agarose gel by field inversion gel electrophoresis for 16 h at 90 V on program 3 (PPI-200 Inverter; M. J. Research, Cambridge, Mass.), transferred bidirectionally to Biotrans filters (ICN Biochemicals, Cleveland, Ohio), and hybridized with a radiolabeled probe as previously described (29). Alternatively, uncut genomic DNA was electrophoresed on a 0.4% agarose gel at 50 V for 24 h and then transferred and probed as described above. The *guaA* probe was an 833-bp PCR product amplified from CA-11.2A DNA (primers, 5′-TAGC TAGTCCTGGGCCGGGA-3′ and 5′-ATCTCTTTTATTTTC AGAAC-3′), purified by filtration (Ultrafree-MC 30,000 NMWL filter unit; Millipore, Bedford, Mass.), and labeled with [α-^{32}P]dATP (New England Nuclear, Boston, Mass.), using a random primer kit (GIBCO-BRL). The *ospC* probe was a 476-bp PCR product (primers, 5′-ATCAAAACAATGC TTTAGGC-3′ and 5′-GCCAAGAAATCTTTCTTGAC-3′), purified and labeled as described above.

Primer extension analysis. Primer extension analysis was performed as described previously (12). Total RNA from *B. burgdorferi* CA-11.2A was isolated with a model 341 nucleic acid purification system (Applied Biosystems, Inc., Foster City, Calif.) as described previously (27); 30 μg of RNA was treated with 3 U of DNase I (GIBCO-BRL) for 30 min at 37°C and purified by phenol-chloroform extraction and ethanol precipitation before primer extension reactions were performed. For *ospC*, the primer 5′-TCCTGAATTATTACAAGATATAAA TA-3′, 43 bp downstream from the translational start site, was used. For *guaA*, the primer 5′-TCTCTAATTCTTCTTGCAA TTAGTTG-3′, 53 bp downstream from the translational start site, was used. Promoter analysis was performed with the MacTargesearch 2.0 program (13).

Nucleotide sequence accession numbers. The *guaA* and *guaB* sequences have been assigned GenBank accession numbers L25883 and U13372, respectively.

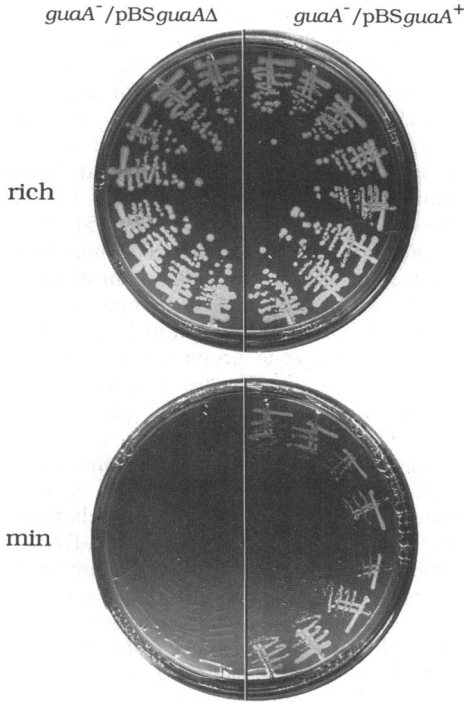

FIG. 2. Complementation of an *E. coli guaA* mutant with the *B. burgdorferi* gene. Eight transformants of the *guaA* mutant strain ght1 with pDH60 (left) or pDH68 (right) were streaked on L ampicillin (rich) or minimal without guanine (min) plates.

RESULTS AND DISCUSSION

We previously cloned and sequenced *ospC* from *B. burgdorferi* CA-11.2A, which exhibits variable expression of this gene (26). To better understand regulation of *ospC* transcription, we sequenced the 5′ flanking region of the *ospC* gene. Two hundred thirty-four base pairs upstream of the *ospC* initiation codon, we found an open reading frame that encoded a *B. burgdorferi* homolog of *guaA*, the gene encoding GMP synthetase. The deduced amino acid sequence of *B. burgdorferi guaA* has ≈50% amino acid identity with *guaA* from *Bacillus subtilis* (23) and *E. coli* (38) (Fig. 1). Conserved regions of identity were observed among these bacteria throughout the GMP synthetase sequences, including within the N-terminal sequence that is thought to be involved in the glutamine amidotransferase activity of the enzyme (38). A plasmid containing the *B. burgdorferi guaA* gene allowed an *E. coli* strain

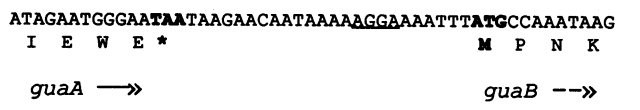

FIG. 3. Intergenic region between *guaA* and *guaB*. The sequence shown extends from the last four amino acids encoded by *guaA* (amino acid residues 509 to 512) through the first four amino acids encoded by *guaB*. Arrows adjacent to the gene designations indicate the direction of transcription. The translational stop codon for *guaA* (**TAA**) and the first codon of *guaB* (**ATG**) are indicated in boldface type, and a potential ribosome binding site for *guaB* is underlined.

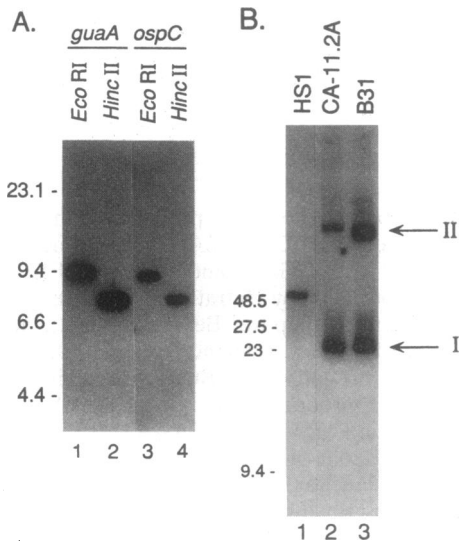

FIG. 4. Southern blot analysis of *Borrelia* DNA. (A) Digested *B. burgdorferi* DNA probed with *guaA* and *ospC* PCR products. CA-11.2A genomic DNA was cut with *Eco*RI (lanes 1 and 3) or *Hinc*II (lanes 2 and 4). Probes were as indicated above the lanes. DNA standards (in kilobases) are shown at the left. (B) *Borrelia* DNA probed with the *guaA* PCR product. An autoradiogram from a Southern blot containing genomic DNA from *B. hermsii* HS1 (lane 1), *B. burgdorferi* CA-11.2A (lane 2), and *B. burgdorferi* B31 (lane 3) is shown. Arrows point to different forms of the supercoiled plasmid DNA. DNA standards (in kilobases) are shown at the left.

with an insertion in its *guaA* gene to grow without exogenous guanine (Fig. 2); a partial *B. burgdorferi guaA* gene did not complement this *E. coli* mutant. These data demonstrate that the *B. burgdorferi* gene encodes an active product that confers the $GuaA^+$ phenotype in *E. coli*.

We found a *B. burgdorferi* homolog of *guaB*, the gene encoding IMP dehydrogenase, 24 nucleotides downstream from the *guaA* stop codon. The proximity of the two genes and the absence of promoter consensus sequences in the intergenic region suggest that the *B. burgdorferi guaA* and *guaB* genes form an operon (Fig. 3). If so, these two genes are in the opposite order from the operons in *E. coli* and *S. typhimurium*.

The deduced amino acid sequence of the first 80 residues of *B. burgdorferi* GuaB shares approximately 66% identity with homologous sequences in *E. coli* and *B. subtilis* GuaB proteins (Fig. 1) (18, 37). The carboxy-terminal two-thirds of the protein (residues 132 to 404) has 56% identity with *E. coli* and *Bacillus subtilis* sequences. This is approximately the same degree of similarity that *Bacillus subtilis* and *E. coli* sequences have with each other. However, a portion of *B. burgdorferi* GuaB encompassing residues 81 to 131 has no homology with, and is 58 residues shorter than, the corresponding region in GuaB of other organisms (Fig. 1). Within this more variable region there is only 37% identity between *E. coli* and *Bacillus subtilis* GuaB proteins. There is also heterogeneity at the amino and carboxy termini of the GuaB proteins from different organisms. The *B. burgdorferi guaB* gene was unable to complement an *E. coli guaB* mutant (data not shown); this does not prove, however, a lack of IMP dehydrogenase activity by the *guaB* gene product in *B. burgdorferi*. IMP dehydrogenases from other bacterial sources have been shown to vary widely with respect to allosteric properties, size, and subunit composition relative to the *E. coli* enzyme (37).

We performed Southern blot analysis to determine the number of copies of *guaA* in *B. burgdorferi*. When restriction digests of CA-11.2A genomic DNA were probed with *guaA* or *ospC* PCR fragments, single bands of identical size were recognized (Fig. 4A). This result indicates that the two probes recognized the same restriction fragment, as would be expected from the sequence data. Therefore, only one copy of *guaA* exists in the *B. burgdorferi* genome.

The *B. burgdorferi ospC* gene is located on a 26-kb circular plasmid (24, 30). To confirm that the *guaA* and *guaB* genes are located on this plasmid, undigested genomic DNA from *B. burgdorferi* CA-11.2A and B31 was probed with PCR products from each gene. *guaA*, *guaB*, and *ospC* mapped to the same 26-kb circular plasmid in these *B. burgdorferi* strains (Fig. 4B and data not shown). The results of these Southern blots and the genomic cloning indicate that *B. burgdorferi* contains one *guaA-guaB* locus located on the 26-kb circular plasmid adjacent to the gene encoding *ospC*. Additional evidence for this came from PCR amplification of total *B. burgdorferi* genomic DNA with degenerate primers from phylogenetically conserved regions of GuaA, in which only a single fragment, identical in size to the PCR product of the plasmid-encoded *guaA* gene, was amplified (data not shown). The *ospC* and *guaA* genes map to this plasmid in strains from each of the three recognized genospecies of Lyme disease spirochetes (data not shown).

Genomic DNA from *B. hermsii*, a causative agent of tick-borne relapsing fever, was probed with the *guaA* PCR product to ascertain the location of *guaA* in this species. The *guaA* homolog mapped to a >50-kb linear plasmid (Fig. 4B). *B. hermsii* contains several *ospC* homologs on linear plasmids, including the genes encoding the variable major proteins Vmp3 and Vmp24 (25, 26, 36). Rehybridizing the *guaA* blot with additional probes demonstrated that the >50-kb *B. hermsii* linear plasmid also contains *ospC* and *guaB* homologs (data not shown).

While *guaA* and *ospC* are adjacent on the *B. burgdorferi* 26-kb circular plasmid, we do not know if they are coordinately regulated. The intragenic region between *guaA* and *ospC* contains several potential promoters for *ospC* (based on primer extension analysis [24] and similarity to a consensus *E. coli* σ^{70} promoter sequence [26]), as well as two sets of overlapping inverted repeats, starting 63 and 78 nucleotides from the initiation codon of *ospC* (107 and 146 nucleotides from the presumed *guaA* start codon) (Fig. 5). Regulation of *guaA* expression in other bacterial species may be related to the potential stem-loop structures upstream of the *guaA* start codon (23, 38) and to intracellular guanine levels (41). The positions of the apparent transcriptional start sites of these genes indicate that the gene promoters are adjacent to and divergent from each other (Fig. 6), although analysis of the *ospC-guaA* intragenic region with the MacTargesearch 2.0 program did not reveal any consensus *E. coli* σ^{70} promoter sequences adjacent to the *guaA* transcriptional start site (Fig. 5). Transcriptional start sites in spirochetes growing in ticks and mammals may differ from those in spirochetes growing in culture. If these start sites are different in vivo, RNA secondary structures due to the overlapping inverted repeats may form and create targets for regulation of the two genes.

The location of the housekeeping genes *guaA* and *guaB* on a plasmid rather than the *B. burgdorferi* chromosome suggests unique circumstances in the acquisition or maintenance of these sequences. It is tempting to speculate that the spirochetes may have originally resided solely in the tick host, in which their populations were maintained by transstadial and transovarial transmission. Acquiring a plasmid containing the purine biosynthesis genes, and perhaps other linked genes,

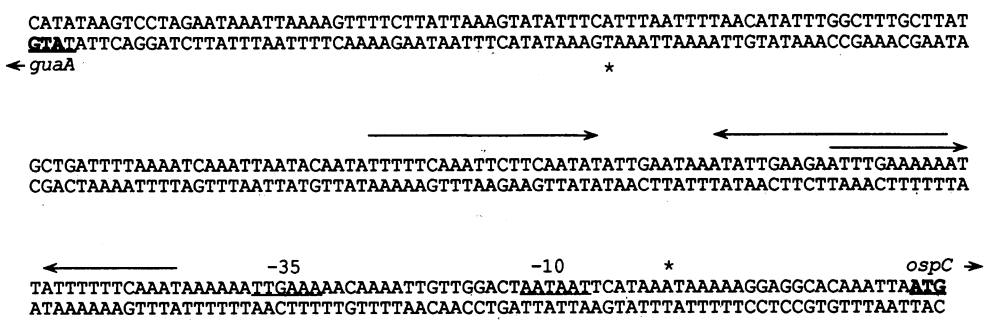

FIG. 5. Intragenic region between *ospC* and *guaA*. The sequence is from the first codon of *guaA* (GTA) to the first codon of *ospC* (ATG). Arrows displayed above the sequence represent inverted repeats. Asterisks are above and below transcriptional start sites of *ospC* and *guaA*, respectively. Predicted −35 and −10 promoter sites for *ospC* are shown.

could have allowed infection of a mammalian host. Concomitant or subsequent to this, *B. burgdorferi* would have lost the ability to be vertically transmitted within ticks. Natural horizontal transfer of the plasmid could convert avirulent, tick-confined species to virulence.

The location of the *guaA* and *guaB* genes on a plasmid allows an additional level of gene regulation that may be important for the complicated lifestyle of borreliae. In ticks, guanine levels can be very high; in fact, excreted guanine is an assembly pheromone that induces an immobilization response (34). Conversely, mammalian blood contains very low extracellular levels of purines and pyrimidines (6), requiring infectious bacteria to synthesize their own nucleotides (21). One explanation for the plasmid location of these genes in *Borrelia* species is that the spirochete may change the copy number of this plasmid when shuttling between ticks and mammals. While *B. burgdorferi* plasmid copy number in ticks and mammals has not been examined, *B. hermsii* linear plasmid copy number is lower in rich medium than in mice (19). Therefore, environmental guanine levels may be involved in the regulation of *guaA* and *guaB* expression in *Borrelia* species as they are in other bacteria (41).

The segmented nature and physical structures of the various DNA molecules that comprise the *Borrelia* genome are unusual among prokaryotes. As previously suggested, the distinction of chromosome and plasmid as defined in other bacteria may not be relevant to these spirochetal genetic elements (4, 5, 9, 14). Plasmids have been generally thought to be easily lost and gained, but the *gua* genes are likely to be important for growth in the guanine-poor environment of mammalian blood. In fact, the 26-kb circular plasmid contrasts with some other *B. burgdorferi* plasmids in that it has been retained in vitro, despite extensive passage in BSKII medium (3, 15, 30, 32). We suspect that the 26-kb circular plasmid may contain other essential genes in addition to the *gua* locus. Other species of bacteria have biosynthetic enzymes associated with smaller chromosomes or very large plasmids. Leptospires have a smaller 350-kb replicon in addition to a 4.6-Mb chromosome; although genes encoding many essential enzymes have been mapped to the chromosome, the smaller replicon contains the gene for at least one enzyme essential in amino acid and cell wall biosynthesis (42). *Rhizobium meliloti* contains two megaplasmids of approximately 1.5 Mb, each, as well as a 3.4-Mb chromosome. Symbiotic and catabolic genes are scattered among these three replicons (16). As in the case of *Borrelia* species, the designation of these genomic components as chromosome or plasmid is not straightforward.

Currently, a lack of genetic tools and growth in complex, undefined media make it impossible to test whether the *gua* genes, or any *B. burgdorferi* gene, are essential. However, the recent description of transformation of *B. burgdorferi* with an endogenous chromosomal marker (31) leaves promise that specific gene inactivation will be possible. The role of these and other genes in the infection and transmission cycle of *B. burgdorferi* between ticks and mammals can then be addressed.

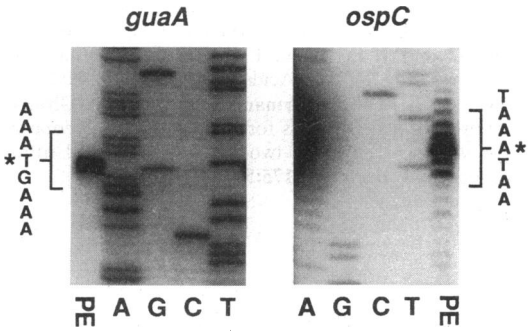

FIG. 6. Transcriptional start site mapping of *guaA* (left) and *ospC* (right). Sequencing reactions are shown (in lanes designated A, G, C, and T) next to the primer extension reactions (in lanes designated PE). The sequences adjacent to the primer extension lanes indicate the major transcriptional start sites (marked with an asterisk) and the surrounding sequence.

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