Horizontally Acquired Genes for Purine Salvage in *Borrelia* spp.
Causing Relapsing Fever

Alan G. Barbour, * Adrienne D. Putteet-Driver, and Jonas Bunikis

Departments of Microbiology and Molecular Genetics and Medicine, University of California Irvine, Irvine, California 92697-4025

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Unlike *Borrelia burgdorferi*, the relapsing fever agent *Borrelia hermsii* and the related *Borrelia miyamotoi* had *purA* and *purB* genes of the purine salvage pathway. These were located among the rRNA genes. Phylogenetic analysis indicated that these genes had a different evolutionary history than those of orthologs in other spirochetes.

The spirochete genus *Borrelia* comprises several species of arthropod-transmitted, blood-borne pathogens that are divided into two groups on the basis of biological and genetic characteristics (2). One group includes the agents of human relapsing fever (RF), such as *B. hermsii*, as well as *B. anserina*, the cause of fowl spirochetosis, and *B. miyamotoi* sensu lato (1). The second group includes the agents of Lyme borreliosis (LB), such as *B. burgdorferi* and *B. garinii*. All *Borrelia* spp. studied to date either have been uncultivable or require a rich, complex medium for growth. The sequence of the genome of *B. burgdorferi* confirmed the limited biosynthetic capabilities for these organisms (5, 6).

As suggested by Schwan et al. (18), genomic differences between RF and LB species in their metabolic capabilities may account for some distinguishing features of the diseases caused by these groups of pathogens. For instance, RF organisms achieve very high densities in the blood, while LB species spirochetes are present in blood but undetectable by light microscopy (2, 17). Thus, our curiosity was piqued when we unexpectedly discovered by PCR assay ~3-kb insertions in the intergenic spacer (IGS) between the 16S and 23S rRNA genes during a study of IGS loci in RF species (4). The entire IGS of the “Connecticut” isolate of *B. miyamotoi* and *B. hermsii* HS1 was subsequently amplified using the following forward (F), reverse (R), forward-nested (Fn), and reverse-nested (Rn) primers: F, 5′-GCTACTCCTTTTCGCTCGCCAC (positions 5668 to 5690 of U03396); R, 5′-CTTCATGAAGTGGG AATCGCTAGT (2158 to 2181); Fn, 5′-TCCCCCTTCCGCTC GCCACTACT (5664 to 5685); and Rn, 5′-GAAGTGGGAA TCGCTAGTAACTC (2164 to 2185). The DNA was amplified using the Expand Long Template PCR System (Roche) with annealing at 60°C, as described previously (3). The PCR product from *B. miyamotoi* was cloned into the vector pCR2.1 TOPO (Invitrogen). The radiolabeled product from *B. hermsii* was used to probe a genomic library of *B. hermsii* in pUC18 as described previously (16), and hybridizing clones were isolated. Both sets of plasmid inserts were sequenced over both strands using custom primers on a Beckman CEQ 8000 sequencer. Homologous genes of other organisms were identified by blastx, blastn, and tblastx searches of GenBank databases (www.ncbi.nlm.nih.gov/BLAST).

What distinguished the two RF species from the two LB species was the presence in *B. hermsii* and *B. miyamotoi* of orthologs of the genes *hpt* (hypoxanthine-guanine phosphoribosyltransferase), *purA* (adenylosuccinate synthetase), and *purB* (adenylosuccinate lyase) (Fig. 1). There is a σ70-type promoter sequence 44 nucleotides (nt) upstream of the *hpt* start codon; the presumptive start of the *purB* gene overlaps the 3′ end of the *purA* gene by 15 nt. This putative hpt-*purA*-purB operon is on the strand opposite that carrying 16S RNA, alanine tRNA, isoleucine tRNA, a methylpurine-DNA glycosylase homolog (mag), a cofy hydrolase homolog, and 23S RNA. Partial sequencing of the IGS of the RF species *B. crocidurae* revealed the presence in that species of at least the *purB* gene (accession number AY884004).

The 16S-23S intergenic spacers of bacteria are notable for their varied lengths, intraspecies sequence polymorphisms, and the common occurrence of tRNA genes at the loci, but not typically for the presence of operational genes, such as *purA* and *purB* (10). The GC content of the hpt-*purA*-purB genes at 30.4% was similar to the overall GC content of the *B. burgdorferi* chromosome at 28.6% (6) and to the 29.6% GC content of 17,711 nucleotides of concatenated sequences of the following available sequences of *B. hermsii* chromosomal genes that have orthologs in other bacterial genera: *zwf*, *fruK*, *fruA2*, recC, recB, *reCD* (accession number AF169385), gpsA (AF06983), recG (AY146655), recA (AF395125), gyrB (AF098862), and flaB (M86838). On the other hand, the GC skew, i.e., (G – C)/(G + C), of 3,194 nucleotides of concatenated hpt, purA, and purB genes was 0.039, lower than the mean GC skew of 0.214 (95% confidence limits of 0.110 to 0.318) for the chromosomal genes listed above. The latter finding suggested the acquisition of an hpt-*purA*-purB gene cluster from another organism, but there was no evidence of a transposable element: the clusters in *B. hermsii* and *B. miyamotoi* were not flanked by inverted or direct repeats, and there was not within the insertion an open reading frame with discernible motifs of a transposase or DNA-binding protein.

The functional activity of the *purA* homolog of *B. hermsii* was
evaluated by complementation assay in a PurA- mutant of Escherichia coli. The purA gene was amplified using the forward primer 5'-GGATTCCATATGAAATTCTTACG CAGTTA-3', the reverse primer 5'-CGGATCCATTGCTTT ACTGGCATATCTTGA-3', and PCR conditions as described previously (16). After digestion with NdeI and BamHI, the products were ligated into a modified pBluescript II KS plasmid (16), and this construct was transformed into strain H1238 (purA54 thr flaA argF relA spoT argI) from the E. coli Genetic Stock Center, Yale University. The plasmid vector without an insert was transformed into the purA mutant as a negative control. Single colonies on Luria-Bertani medium plates were then cultivated in duplicate at 37°C in 6 ml of defined Dulbecco’s modified Eagle’s medium (Gibco) with adenine HCl (Sigma) at a final concentration of 0, 0.1, 1.0, or 10 μg/ml. The criterion for growth was absorbance of ≥0.2 optical density units by spectrophotometry at 595 nm and an indicator color change by 72 h; absence of growth was confirmed by phase microscopy. We found that the E. coli transformant with the B. henselii purA homolog grew in the absence of adenine supplementation, but the vector-only control detectably grew only in the presence of 1.0 or 10 μg/ml of adenine.

Adenylate synthetase and adenyllosuccinate lyase are purine salvage enzymes that catalyze steps in the formation of AMP from IMP (12). While both B. burgdorferi and B. henselii have orthologs for GMP synthetase (guaA) and IMP dehydrogenase (guaB) (14), which carry out analogous functions for the formation of GMP from IMP, B. burgdorferi had no discernable ortholog for purA and purB, or for purF, purC, and purG, which catalyze reactions for the formation of IMP from hypoxanthine (6). The facultatively pathogenic spirochete Leptospira interrogans has purA and purB orthologs. The oral spirochete Treponema denticola (AAS12016) has an ortholog of purB but not purA (19).

To further investigate the evolutionary origins of the purine salvage genes in RF Borrelia species, we carried out phylogenetic analysis of these sequences, as well as sequences from representative archaeabacteria, eukaryotes, and other phyla of eu-bacteria, for which whole-genome sequences were publicly available (www.ncbi.nlm.nih.gov/Genomes/index.html). Amino acid sequences were aligned using Clustal X version 1.83, and this alignment was the basis for a codon-based, gapped nucleotide alignment. To minimize effects of base composition bias across taxa, the third positions were excluded, and the evolutionary model of Galtier and Gouy was applied (7) using the PHYLO_WIN phylogenetic analysis program (8). Positions with gaps were ignored. Phylograms of purA and purB sequences with bootstrap values of ≥70% for nodes under distance (neighbor-joining), maximum likelihood, and maximum parsimony criteria are shown in Fig. 2.

The purA and purB genes of the spirochetes B. henselii, T. denticola, and L. interrogans cluster with high bootstrap support in separate monophyletic groups from each other. This is most apparent in the purB phylogram, B. henselii’s purB groups with orthologous sequences of actinobacteria, clostridial firmicutes, Fissobacterium, yeast, and animals, while the leptospire purB clusters with those of cyanobacteria, alphaproteobacteria, and Bacillus-Staphylococcus firmicutes, and the treponeme purB groups with those of gammaproteobacteria, plants, and two protists.

These findings indicate that purA and purB in RF Borrelia have a different evolutionary history than orthologous genes in leptospiral and treponemal spirochetes. The preceding phylogenetic analysis, the aforementioned differences between the hpt-purA-purB gene cluster and other chromosomal coding sequences in GC skew, and the unusual location of these operational genes among the rRNA genes lead us to conclude that the hpt-purA-purB locus was acquired by horizontal gene transfer (13). This could have happened before or in the last common ancestor for LB and RF Borrelia species, with subsequent selective loss of the locus from the LB species lineage, but a more parsimonious explanation is that it occurred in the RF lineage after the last common ancestor. Moreover, if there was a putative loss of the purA and purB functions in LB Borrelia spp., this would more likely, in our view, have been the consequence of in situ degradation of the genes, the manifestations of which would still be detectable as pseudogenes or sequence fragments (5), rather than a precise and complete deletion of the cluster.

Although the adaptive contributions of this acquisition for RF Borrelia in either vertebrate host or arthropod vector remain to be established, in the neuroinvasive K1 strain of E. coli...
the disruption of the purA gene was associated with decreased invasion of the blood-brain barrier (11), and PurA⁻ mutants of *Salmonella enterica* serovar Typhimurium had attenuated virulence (15).

**Nucleotide sequence accession numbers.** The 5,846-nt IGS of *B. hermsii* and the 5,814-nt IGS of *B. miyamotoi* were assigned GenBank accession numbers AY803734 and AY531879, respectively.

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**REFERENCES**


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