V 33 *Borrelia burgdorferi* cp32 prophages/plasmids, their *erp* loci, and mechanisms controlling *erp* transcriptional regulation during the Lyme spirochete's mammal-tick infection cycle

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All Lyme disease spirochetes naturally maintain 4 or more variants of a prophage which replicate episomally as circular plasmids known as cp32s. Each cp32 carries a mono- or bicistronic *erp* locus, encoding Erp outer-surface lipoproteins. *Borrelia burgdorferi* produce Erp proteins throughout mammalian infection, but repress their synthesis during tick colonization. Functions that have determined for Erp proteins include binding of host plasmin, laminin, complement factor H and factor H-related proteins, implicating roles in bacterial dissemination, colonization and evasion of host innate immune responses.

Transcriptional fusions with *gfp* were used to identify DNA elements necessary for regulation of *erp* transcription in *B. burgdorferi*. EMSA, DNA-affinity chromatography, and proteomics were used to identify borrelial cytoplasmic proteins that bind to *erp* regulatory DNA elements. Functions of those proteins were evaluated by in vitro transcription of *erp* promoters, and by over-expression of the proteins in *B. burgdorferi*.

A region of DNA immediately 5' of *erp* promoters, termed Operator 2, is required for regulation of *erp* transcription. Three proteins have been identified that bind *erp* Operator 2 DNA, which we named BpaB, EbfC, and BpuR. BpaB proteins are encoded on cp32 elements, and repress *erp* transcription. BpaB proteins also appear to play roles in plasmid replication/segregation. EbfC is encoded on the chromosome, and the *ebfC* gene is cotranscribed with *dnaX*, which encodes 2 subunits of DNA polymerase. EbfC competes with BpaB for binding to Operator 2, and functions as an *erp* antirepressor. Intriguingly, many other species of eubacteria encode orthologs of EbfC, and we demonstrated that the *E. coli* and *Haemophilus influenzae* homologs also bind DNA. BpuR appears to facilitate binding of EbfC to *erp* Operator 2 DNA and to inhibit binding of BpaB. BpuR contains PUR motifs, nucleic acid-binding motifs found in many other eubacterial, and eukaryotic proteins, and is the first bacterial PUR domain-containing protein to be demonstrated to bind DNA.

B. burgdorferi controls transcription of *erp* operons through binding of BpaB repressor, EbfC antirepressor, and BpuR modulator proteins. Homologs of EbfC and BpuR are encoded by a wide range of other bacterial species. Our studies are providing information about pathogenic and regulatory mechanisms of the Lyme disease spirochete as well as insights into the physiology of a diverse range of bacteria.