



The Consistent Tick-Vertebrate Infectious Cycle of the Lyme Disease Spirochete Enables *Borrelia burgdorferi* To Control Protein Expression by Monitoring Its Physiological Status

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ABSTRACT The Lyme disease spirochete, *Borrelia burgdorferi*, persists in nature by alternately cycling between ticks and vertebrates. During each stage of the infectious cycle, *B. burgdorferi* produces surface proteins that are necessary for interactions with the tick or vertebrate tissues it encounters while also repressing the synthesis of unnecessary proteins. Among these are the Erp surface proteins, which are produced during vertebrate infection for interactions with host plasmin, laminin, glycosaminoglycans, and components of the complement system. Erp proteins are not expressed during tick colonization but are induced when the tick begins to ingest blood from a vertebrate host, a time when the bacteria undergo rapid growth and division. Using the *erp* genes as a model of borrelial gene regulation, our research group has identified three novel DNA-binding proteins that interact with DNA to control *erp* transcription. At least two of those regulators are, in turn, affected by DnaA, the master regulator of chromosome replication. Our data indicate that *B. burgdorferi* has evolved to detect the change from slow to rapid replication during tick feeding as a signal to begin expression of Erp and other vertebrate-specific proteins. The majority of other known regulatory factors of *B. burgdorferi* also respond to metabolic cues. These observations lead to a model in which the Lyme spirochete recognizes unique environmental conditions encountered during the infectious cycle to “know” where they are and adapt accordingly.

KEYWORDS *Borrelia*, spirochetes

All organisms require mechanisms to detect their environment, in order to produce only those proteins that are useful in specific conditions. This is particularly important for vector-borne pathogens, which must survive in two distinct host types while also facilitating transmission back and forth between those hosts. An understanding of a pathogen’s sensory and signal transduction mechanisms may reveal critical components that can be targeted for development of novel therapies.

Lyme disease (Lyme borreliosis) is a potentially debilitating disease of humans and domestic animals. The incidence of this disease has been steadily increasing, in part due to expanding ranges of vector ticks (1). Lyme disease can manifest in many tissues and organs, with symptoms that include arthritis, meningitis, facial nerve paralysis, and atrioventricular nodal block. Failure to treat this infection promptly and adequately can result in persistent debilitating effects or, sometimes, death (2–4).

The infectious agent of Lyme disease, *Borrelia burgdorferi sensu lato* (hereafter called *B. burgdorferi*, for simplicity), does not produce classical toxins or other recognizable virulence factors that directly cause host damage. Instead, disease symptoms are due

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to overly robust immune system reactions to bacterial components (5–10). Thus, to cause clinical disease, *B. burgdorferi* needs only to colonize and survive in the patient's body.

B. burgdorferi is transmitted to humans and other vertebrates through the bites of infected *Ixodes* species ticks. Transmission is a complex, multistep process (5, 11, 12). First, bacteria within the midgut of a tick detect when the tick vector is imbibing a blood meal, change from a relatively inert metabolism to one of rapid replication and movement, and then alter the composition of their outer membrane proteins from tick-specific adhesins to proteins that are optimal for vertebrate infection. Next, bacteria penetrate the tick midgut wall, invade the salivary glands and ducts, and pass with saliva into the bite wound. Following inoculation into the vertebrate host, *B. burgdorferi* migrates through solid host tissues and in the bloodstream and then establishes long-term colonization of a variety of organs and tissues. These complexities require that *B. burgdorferi* efficiently determines its location in the tick-vertebrate infectious cycle and produces only those proteins and other factors that are essential for that time and place.

The Lyme disease spirochete encodes only two regulatory two-component systems (13, 14). Only one of those, Hk1-Rrp1, includes a signal receptor/histidine kinase that passes through the inner membrane and can, therefore, detect an external signal(s) (15–19). The other two-component system, Hk2-Rrp2, has a cytoplasmic receptor/histidine kinase, implying response to an internal signal(s) (19–25). However, investigations have revealed that *B. burgdorferi* employs a complex signaling network to control production of proteins (26–28). With the exception of Hk1-Rrp1, all known regulatory factors are controlled by rates of bacterial replication and/or levels of cytoplasmic factors (27–29). That realization begs the question of why *B. burgdorferi* can coordinate differential expression of numerous proteins during its complex infectious cycle by sensing internal cues.

Herein, we describe the elucidation of signaling pathways that are used by *B. burgdorferi* to control production of a family of infection-associated proteins, along with a hypothesis of how that network meshes with the Lyme spirochete's natural tick-vertebrate infectious cycle.

B. BURGENDORFERI AS A MODEL VECTOR-BORNE PATHOGEN

In addition to being the causative agent of a significant human disease, *B. burgdorferi* has emerged as a valuable model organism for studies of vector-borne bacteria and of the *Spirochaetota* phylum in general. The following features of *B. burgdorferi* contributed to completion of the studies described in the remainder of this review and which continue to advance our understanding of this infectious bacterium and the pathogenesis of Lyme disease.

B. burgdorferi is readily cultivated in the laboratory (30, 31). Although currently used culture media are complex and contain undefined ingredients, analyses of bacterial physiological responses can be readily observed in response to variations in concentrations of medium ingredients, temperature, pH, osmotic strength, or other factors (19, 29, 32–34). For instance, replacement of glucose with other compounds enabled characterization of carbohydrate utilization by *B. burgdorferi* (35).

Additionally, the entire infectious cycle of the Lyme disease bacterium can be replicated in the laboratory, using ticks and model vertebrate hosts such as mice, rats, rabbits, and macaques (36–39). Thus, functions for proteins and other bacterial factors can be precisely assessed during infection processes.

B. burgdorferi is a member of *Spirochaetota*, a phylum of bacteria that diverged many millennia ago from the ancestors of the more commonly studied *Proteobacteria* and *Firmicutes* (40, 41). Initial attempts to genetically manipulate *B. burgdorferi* were unsuccessful, due to a combination of commonly used selectable markers not being transcribed by the spirochete and the inability of commonly used plasmids to replicate (42, 43). However, a stepwise approach, from recombination of an antibiotic resistance-encoding mutation into the chromosome, followed by use of that locus to integrate circular DNA via single crossover, led to the development of numerous selectable

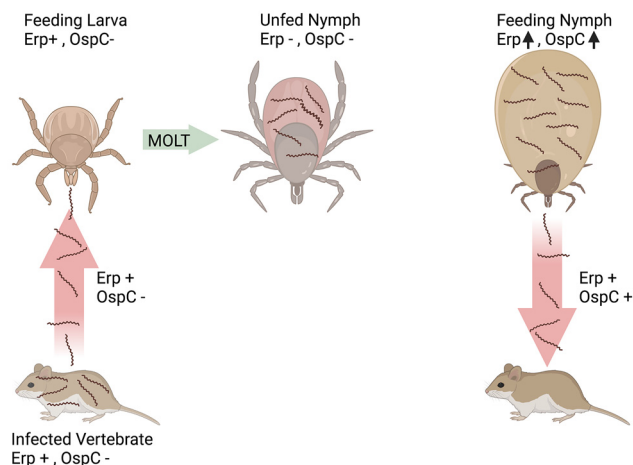


FIG 1 Natural infectious cycle of the Lyme disease spirochete. There are three postembryonic life stages of *Ixodes* species ticks: larva, nymph, and adult (not shown). Each stage feeds once and then molts to the next stage. Adult females feed once, lay eggs, and then die. In areas where Lyme disease is endemic, larvae and nymphs feed on the same vertebrate species, while adults feed on different hosts and therefore do not play major roles in the maintenance of *B. burgdorferi*. There is essentially no transmission of *B. burgdorferi* through tick eggs, so larvae are uninfected upon hatching. Larvae acquire *B. burgdorferi* if they feed on an infected reservoir vertebrate. Bacteria colonize the tick's midgut and persist through the molt into the nymph stage. When a nymphal tick begins to feed, bacteria in the midgut undergo significant metabolic changes, penetrate the midgut wall, enter the salivary glands, and are injected into the vertebrate host with the tick's saliva. *B. burgdorferi* in persistently infected vertebrates produces Erp surface proteins but not OspC. The spirochetes cease production of Erps after acquisition by feeding larvae, such that unfed nymphal ticks are colonized by bacteria that lack both Erps and OspC. When the tick begins to feed, OspC and Erps are induced, and transmitted bacteria produce both classes of surface proteins. *B. burgdorferi* spirochetes cease production of OspC within a few days of vertebrate infection but continue to express Erps on their outer surfaces.

markers, cloning vectors, fluorescent tags, and other genetic tools (42–60). CRISPR-interference (CRISPRi) methods were recently developed and have been exploited to knock down expression levels of *B. burgdorferi* proteins (61).

B. burgdorferi naturally contains multiple, distinct replicons, some of which encode essential infection-associated genes, and extended cultivation may result in spontaneous loss of one or more of those replicons (13, 14, 62–64). However, there are simple methods to screen transformants for the presence of all replicons, adding a step to the generation of mutants in infectious bacteria but a step that is usually not insurmountable (65). Studies of cultured *B. burgdorferi* can be simplified by using clonal strains with minimal, yet highly stable, genomes (66, 67). To date, all known regulatory factors of Lyme disease *Borrelia* spp. are encoded on replicons that are never, or rarely, lost during cultivation (27, 28).

INITIAL OBSERVATIONS OF GENE/PROTEIN REGULATION AND SUBSEQUENT MODELS

Despite the infectious agent of Lyme disease having been identified in 1982 (68, 69), the first investigations of gene and protein regulation by *B. burgdorferi* were not published until 1995. *B. burgdorferi* requires a specific outer surface protein, OspC, for the initial stages of vertebrate infection (70–81). In their seminal studies, Schwan and colleagues observed that *B. burgdorferi* within the midguts of unfed ticks did not produce OspC, while the onset of tick feeding induced the bacteria to produce substantial quantities of OspC (32) (Fig. 1).

Schwan et al. further observed that *B. burgdorferi* cultured at 23°C produced significantly less OspC protein than did bacteria that had been transferred from 23 to 37°C (32). This change is not a heat shock response but requires several generations of bacterial replication at the warmer temperature (33). Such temperature shift studies are generally accomplished in the laboratory by diluting a mid-exponential phase 23°C culture 1:100 into fresh medium, incubating the new aliquot at 35 to 37°C to mid-exponential

phase, and then harvesting both cultures at the same time (32, 33). Simultaneous harvesting is possible because *B. burgdorferi* replicates several times faster when cultured at 35 to 37°C than it does at 23°C (33).

The choices of 23°C and 35 to 37°C were made because they mimic temperatures experienced by *B. burgdorferi* in unfed and feeding ticks, respectively (32). Some other vector-borne bacteria are known to modulate their proteomic profiles in response to temperature (82, 83). Those results led to hypotheses that *B. burgdorferi* possesses mechanisms to sense temperature, which then conveys a signal(s) to affect transcription of target genes. To date, a thermosensitive regulator has not been experimentally demonstrated to exist in *B. burgdorferi*.

Subsequent studies revealed that it is actually the change in bacterial replication rate that results in changed levels of OspC and several other surface proteins (29). Moreover, differences in replication rates also affected levels of the regulatory proteins known to control *ospC* and other loci. This was demonstrated through the use of two incomplete formulations of culture medium that support *B. burgdorferi* replication at 35°C at rates that are similar to growth in complete medium at 23°C (29). *B. burgdorferi* was then cultured to mid-exponential phase in either of the incomplete media at 35°C and then diluted into complete medium and cultured to mid-exponential phase at 35°C. All of the slow-replication conditions (either of the incomplete media at 35°C or complete medium at 23°C) resulted in substantially lower levels of OspC protein than after passage to the fast-replication condition (complete medium at 35°C) (29). Further indication that temperature, *per se*, was not the signal for OspC repression/induction was observed when *B. burgdorferi* that had been stored at –80°C was inoculated into complete medium and grown at 23°C. Bacteria passaged from –80 to 23°C produced OspC at levels comparable to those normally observed after passage from 23°C to 35 to 37°C (29). Thus, the condition before passage to 23°C impacted the expression of OspC. Note that a mechanism that strictly detects temperature would not be affected by the preceding conditions. The significance of *B. burgdorferi*'s ability to detect changes in replication rate and respond by altering its proteome are discussed further below.

Since the initial observations of Schwan et al. in 1995 (32), numerous studies have been undertaken to elucidate mechanisms by which the Lyme disease spirochete senses its environment and controls expression of genes and proteins. While genetic and biochemical analyses have identified several DNA-binding proteins and other factors that have impacts on gene expression (26–28), few published studies have examined their mechanisms of action at a molecular level. To date, the most completely defined mechanism is that which controls production of *B. burgdorferi* Erp proteins. Analyses of that gene and protein family have provided important insights on borrelial sensory and regulatory mechanisms, as follows.

B. BURGDORFERI *erp* GENES AND Erp PROTEINS

Individual Lyme disease spirochete cells naturally maintain numerous *erp* operons, each of which is carried on an episomal cp32 prophage (14, 64, 84–92). The original isolate of the *B. burgdorferi* type strain, B31, contained at least 11 cp32-derived episomes, 10 of which carry mono- or bicistronic *erp* operons, and encoded 13 distinct Erp outer surface lipoproteins (13, 14, 85, 86). Some borrelial *erp* genes have been given various other names, including *ospE*, *ospF*, *elp*, *bbk2.10*, *bbk2.11*, and *p21*, but we consider it easier to view them as a single family of genes, since all possess highly conserved features such as (i) genomic location, (ii) promoter and operator DNA sequences, and (iii) regulatory mechanisms and (iv) encode surface-localized lipoproteins with conserved leader and N-terminal amino acid sequences (91, 93).

All Erp proteins are surface-exposed lipoproteins (94, 95). Dual-labeling studies found that all of the Erp proteins of *B. burgdorferi* type strain B31 are simultaneously coexpressed (96). Immunofluorescence microscopy and quantitative reverse transcription (RT)-PCR determined that *B. burgdorferi* produces little to no detectable amounts of any Erp protein during colonization of unfed tick midguts, but the genes and

proteins are induced upon tick feeding (97, 98). The *erp* genes and Erp proteins are expressed during transmission from tick to vertebrates, throughout vertebrate infection, and continue to be expressed during transmission from infected vertebrates to naive ticks (Fig. 1) (98, 99). Consistent with that expression profile, all identified functions of Erp proteins involve interactions between *B. burgdorferi* and its vertebrate hosts, including binding to complement factors H, C1r, and C1s, plasmin, laminin, and glycosaminoglycans (100–104).

As noted above, almost all Lyme disease borreliae naturally contain numerous *erp* operons, each located on a separate cp32 prophage. Why do individual bacteria contain multiple, distinct *erp* genes? One early hypothesis suggested that *erp* loci might recombine during vertebrate infection as a mechanism to generate antigenic diversity. However, analyses of clonal strains before, during, and after passage through mice revealed absolute conservation of *erp* sequences (105, 106). A report suggesting genetic variation was later found to have undoubtedly detected PCR artifacts (106, 107). At present, the most reasonable explanation for the simultaneous coexpression of numerous Erp proteins with distinct sequences is due to the broad host range of vector ticks. For example, *Ixodes scapularis* in the northeastern United States might feed on rodents, birds, or other vertebrates (108). Only the nymph and adult stages of *Ixodes* species ticks can transmit Lyme disease spirochetes, and each stage feeds only once (Fig. 1). For *B. burgdorferi* to successfully infect a vertebrate, the bacteria must be able to interact with the new host's tissues while also fending off immune system responses. Those features often differ considerably between the various potential hosts of *B. burgdorferi*. Continuing our example, *B. burgdorferi* in an *I. scapularis* tick in the northeastern United States cannot "predict" the type of host that the tick is feeding on, and so Lyme spirochetes express a wide variety of host-interactive surface proteins during transmission to increase the probability that their surface proteomes are adequate to infect the vertebrate into which they are deposited. Supporting this hypothesis, different Erp proteins exhibit different affinities for the complement factor H proteins of different potential vertebrate hosts (109, 110). This hypothesis can also explain why *B. burgdorferi* simultaneously produces numerous other polymorphic protein families, such as the CspA/pFam54, Rev, and Mlp outer surface proteins (111–118).

MECHANISMS CONTROLLING TRANSCRIPTION OF *erp* OPERONS

DNA sequences 5' of all *erp* operons are highly conserved, and a single transcriptional start site was mapped to 14 bp 5' of the initial *erp* gene's start codon (66, 119). To identify *cis* sequences required for regulation of *erp* transcription, *erp* 5' DNA sequences were fused to *gfp*, and then independently replicating plasmids with each construct were introduced into *B. burgdorferi* (66). As noted above, *erp* operons are transcribed at greater levels when *B. burgdorferi* are cultured at 35 to 37°C than at 23°C. Thus, transformants were cultured at both temperatures, and bacterial green fluorescent protein (GFP) levels were determined by flow cytometry (66). Successive deletions of 5' DNA revealed that an approximately 145-bp region 5' of the transcriptional start site is necessary for regulation of transcription. Deletion of that region resulted in constitutive, high-level transcription (66). This regulatory region was designated the *erp* operator.

Electrophoretic mobility shift assays (EMSAs) with *B. burgdorferi* cytoplasmic extracts detected that protein(s) bound specifically to *erp* operator DNA (66). DNA affinity chromatography/pulldown was then employed to purify proteins that bound to the *erp* operator (120, 121). Three proteins were detected, which were subsequently identified by mass spectrometry. All three are novel nucleic acid-binding proteins: BpaB (borrelial ParB analog) (122), BpuR (borrelial PUR domain) (123), and EbfC (erp-binding factor, chromosomal) (120).

All small borrelial replicons, including the cp32s, carry a *bpaB* gene. In most replicons, *bpaB* is located 3' of a putative *parA*. Biochemical studies indicate that BpaB is analogous to the ParB proteins of other bacterial replicons. Each *B. burgdorferi* replicon contains a unique *parA-bpaB* locus, which is presumed to enable the compatibility of multiple replicons within individual cells (13, 14, 64, 86, 124). However, the various *parA-bpaB* pairs are maintained across different isolates. For example, 12 distinct *parA-bpaB* pairs have been identified in the cp32 prophages of Lyme disease spirochete

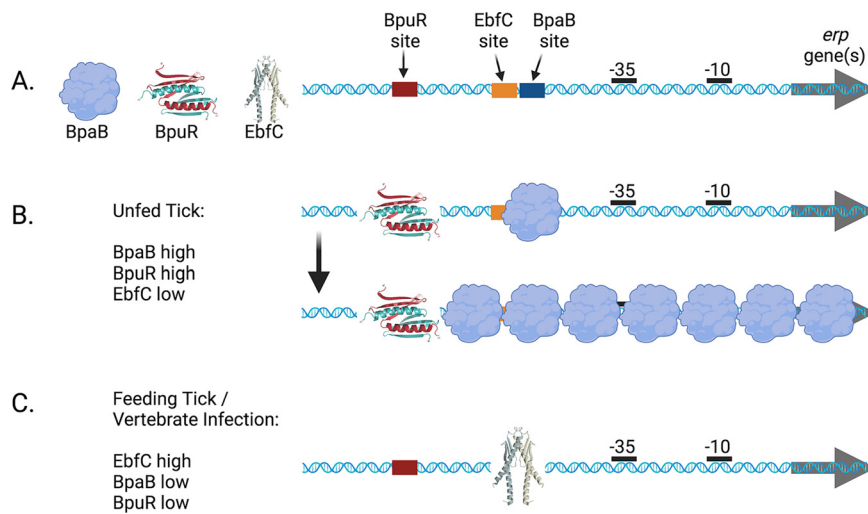


FIG 2 Mechanism by which *B. burgdorferi* controls transcription of *erp* operons. (A) All *erp* operons include a 5' operator that consists of a BpaB-binding site, two to three Ebfc-binding sites, and a BpuR-binding site. The BpaB- and Ebfc-binding sites are adjacent, and the two proteins compete for binding to the DNA. (B) In the midgut of an unfed tick, or under slow-replication culture conditions, BpaB and BpuR levels are elevated, while Ebfc levels are low. BpaB binds to its high-affinity binding site and then spreads along the DNA, stabilized by protein-protein interactions. The presence of BpuR enhances transcription repression by BpaB, possibly by influencing the direction of BpaB multimerization. (C) In a feeding tick, during vertebrate infection, or during culture under rapid replication conditions, Ebfc levels are high, while BpaB and BpuR levels are low. Binding of Ebfc to *erp* operator DNA blocks binding by BpaB, freeing the promoter for recognition by RNA polymerase. Promoter -35 and -10 elements are indicated by solid black bars 5' of the open reading frames (ORFs). The illustrated structures of BpuR and Ebfc are adapted from the solved and modeled proteins (123, 128, 131).

isolates collected throughout the world (64, 91, 124, 125). All cp32-encoded BpaB proteins contain a conserved amino acid sequence that is not found in any other type of BpaB, and that domain is required for binding to *erp* operator DNA (122). As a consequence, BpaB proteins from any cp32 will bind to the *erp* operator elements of all of the *erp* loci in that bacterial cell (122). This cross talk would facilitate the observed simultaneous coexpression of all *erp* operons in a bacterium (96).

BpaB serves as the repressor of *erp* transcription. A high-affinity BpaB-binding site is located 5' of all *erp* promoter elements (Fig. 2). BpaB bound to this site facilitates binding of additional BpaB proteins to adjacent DNA. The lower affinity for those DNA sequences is apparently offset by protein-protein interactions between the BpaB molecules. BpaB spreads along DNA from the initial nucleation site and evidently prevents RNA polymerase from interacting with *erp* promoter sequences (122, 126). Consistent with BpaB being the *erp* repressor, cp32 *bpaB* genes are transcribed at higher levels during tick colonization than they are during vertebrate infection (126).

BpuR is encoded on the *B. burgdorferi* main chromosome and so is considered to be a host protein that is coopted by cp32 prophages for regulation of their own genes (120, 123). The *erp* operator elements contain a single BpuR-binding site, 5' of the BpaB-binding sequence (Fig. 2). BpuR binding to that site enhances the repressive activity of BpaB, and it thus serves as a corepressor (123). Correlating with that activity, *bpuR* is expressed at greater levels in unfed ticks than it is during vertebrate infection (127).

Homologs of BpuR are encoded by some other species of bacteria and, intriguingly, by all multicellular eukaryotes (123, 128, 129). The hallmark of these proteins is a structurally conserved "PUR" domain. All assessed PUR proteins bind to double-stranded DNA, single-stranded DNA, and RNA. Identified RNA targets of BpuR include its own mRNA, creating a feedback loop in which BpuR represses its own translation (Fig. 3) (127, 130). Additional binding sites have been detected throughout the borrelial transcriptome, and numerous physiological effects are apparent when BpuR is dysregulated (127).

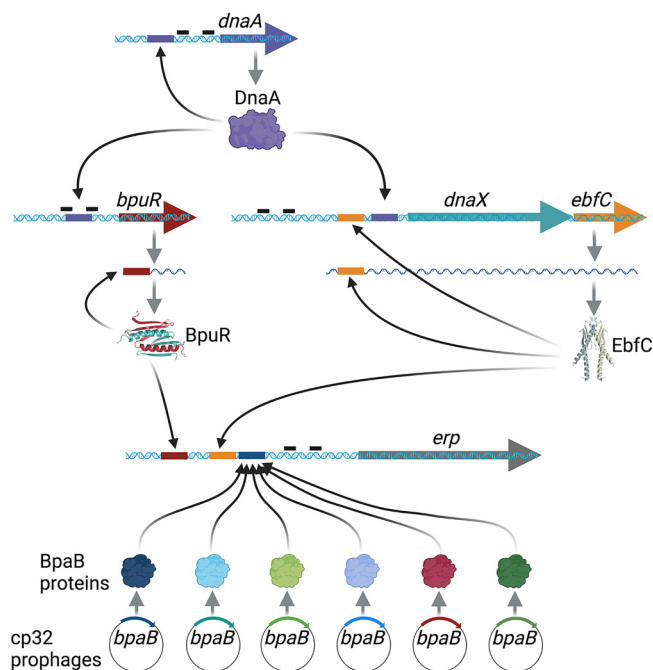


FIG 3 Current understanding of *erp* regulatory circuits. Each cp32 produces a distinct BpaB. However, all cp32-encoded BpaB proteins contain a conserved amino acid motif that binds to *erp* operator DNA. That feature permits cross talk and coordinated repression/derepression of all *erp* operons in a bacterium. BpuR can bind its own mRNA and inhibit translation. DnaA binds to *bpuR* promoter DNA between the -10 and -35 sequences. Ebfc is cotranscribed with *dnaX*, which encodes the τ subunit of DNA polymerase III. Both DnaA and Ebfc bind to sequences between the *dnaX-ebfC* promoter and the initiation codon of *dnaX*. Ebfc also binds to the 5' untranslated region of the *dnaX-ebfC* mRNA. DnaA binds to its own 5' DNA. Promoter -35 and -10 elements are indicated by solid black bars 5' of the ORFs. The illustrated structures of BpuR and Ebfc are adapted from the solved and modeled proteins (123, 128, 131).

Ebfc is also encoded on the main chromosome (120). All *erp* operons contain 2 to 3 Ebfc binding sites adjacent to their BpaB-binding site (Fig. 2). Ebfc and BpaB compete for binding to the *erp* operator (131). Ebfc can inhibit the transcriptional repression of BpaB and thus serves as the antirepressor (126). The *ebfC* gene is more highly transcribed during vertebrate infection than during tick colonization, paralleling the expression pattern of the *erp* genes (126).

Almost all known species of eubacteria encode a well-conserved homolog of Ebfc, which has also been referred to as "ORF-12" or "YbaB" (132–137). We and others subsequently examined several Ebfc homologs and determined that they, too, bind DNA (134, 135). Through studies that included visualization of GFP-tagged Ebfc in *B. burgdorferi*, we determined that Ebfc colocalizes with borrelial chromatin and fits the criteria of being a bacterial nucleoid-associated protein ("histone-like" protein) (138).

The mechanisms by which BpaB, BpuR, and Ebfc control transcription of *erp* operons is diagrammed in Fig. 2. During colonization of the tick midgut, BpaB and BpuR are relatively highly expressed, while Ebfc is repressed. As a result, BpaB binds to the high-affinity site in the *erp* operator and spreads along the DNA, enhanced by BpuR. When the tick begins to feed on a vertebrate host, BpaB and BpuR levels decrease, while Ebfc is induced, resulting in Ebfc outcompeting BpaB for the *erp* operator and facilitating promoter recognition by RNA polymerase.

DnaA BINDS TO THE PROMOTER REGIONS OF THE *bpuR* AND *ebfC* OPERONS

Subsequent studies of BpaB, BpuR, and Ebfc found that each regulates transcription and/or translation of numerous other genes and proteins (127, 138, 139). The majority of investigations have focused on the chromosomally encoded BpuR and Ebfc

proteins, since they control bacterial genes in addition to being coopted by the cp32 prophages for *erp* regulation.

The *bpuR* gene is located in a monocistronic operon (Fig. 3) (13, 123). The master regulator of chromosomal replication, DnaA, binds over the *bpuR* promoter (Fig. 3) (127). The apparent involvement of DnaA in the expression of *bpuR* is hypothesized to explain how BpuR concentration is coordinated with the rate of bacterial replication.

In *B. burgdorferi*, *ebfC* is the second gene in a bicistronic operon, following *dnaX*. The *dnaX* gene encodes the tau (τ) subunit of the DNA polymerase III holoenzyme. Both DnaA and EbfC proteins bind specifically to sites between the promoter and the *dnaX* start codon (A. C. Krusenstjerna, T.C. Saylor and B. Stevenson, unpublished data). As with the *bpuR* operon, binding of DnaA to the *dnaX-ebfC* operon may connect EbfC levels with the rate of bacterial replication. Involvement of DnaA in the expression of tau would also coordinate synthesis of the DNA polymerase III holoenzyme with DNA replication. Nearly all eubacteria encode a homolog of EbfC, and the majority also cotranscribe *ebfC* with *dnaX* (138). Little is known about how those other species regulate transcription of their *dnaX-ebfC* operons, other than there being evidence of a protein-binding site 5' of *dnaX* in *Escherichia coli* and *Caulobacter crescentus* (140–143). Thus, detailed studies of the *B. burgdorferi dnaX-ebfC* operon may provide insight into a wide range of bacteria.

OTHER REGULATED GENES AND PROTEINS

Other than the *erp* operons, the *B. burgdorferi ospC* and *rpoS* operons are the best studied, but our understanding of their regulatory mechanisms is far from complete. Both *rpoS* and *ospC* are repressed during colonization of unfed ticks and are greatly enhanced upon tick feeding (32, 144). Both genes are also influenced by the bacterial division rate in culture, their levels increasing upon shifts to conditions of rapid division (29). The effects of replication rate in culture parallel the effects on *erp* operons, as was described above.

Regulation of the *ospC* gene requires the RpoS alternative sigma factor (145, 146). Yet, other factors are undoubtedly at play with regulation of *ospC*, since that gene is repressed early during vertebrate infection while RpoS continues to be produced (147). A DNA sequence(s) 5' of the *ospC* promoter is necessary for transcriptional regulation (148–150). The role(s) of that upstream sequence has yet to be fully examined.

RpoS plays critical roles in adapting *B. burgdorferi* for transmission from tick to vertebrate and for vertebrate infection (151). The *rpoS* gene is induced during tick feeding, but the molecular mechanisms underlying control of RpoS synthesis have yet to be defined. Another alternative sigma, RpoN, is necessary for *rpoS* induction, as is the activated form of the Rrp2 unit of the Hk2-Rrp2 two-component regulatory system (21–23, 145, 152–154). But at least one additional promoter is involved with transcription of *rpoS*, which is dependent upon the housekeeping sigma RpoD, and that transcript is influenced by the small RNA DsrA and the chaperone Hfq (155, 156). Two proteins, BosR and BadR, bind DNA sequences 5' of *rpoS*, but their modes of action have not been fully elucidated (157–164). Another protein, BBD18, appears to affect RpoS through a posttranscriptional mechanism (165–168).

Despite uncertainties about the mechanisms controlling *OspC* and RpoS, it is clear that both are upregulated during tick feeding and in culture under conditions of rapid bacterial replication (29, 32, 147, 151, 169).

METABOLIC CUES DIRECT *B. BURGDORFERI* PROTEIN PRODUCTION

It is important to recognize that even though the tick-vertebrate infectious cycle involves a large number of distinct interactions between *B. burgdorferi* and its hosts, the cycle never varies. The bacteria move from a feeding tick into a vertebrate, and then from that vertebrate into another tick, in a back-and-forth dance that has persisted for millennia. The absence of variation means that *B. burgdorferi* does not require the contingency systems that are found in free-living bacteria. Moreover, its

predictable routine has evidently permitted *B. burgdorferi* to refine its regulatory system to detect conditions that occur only at specific times.

After *B. burgdorferi* is acquired by a feeding tick larva, and the tick completes digestion of that blood meal, the spirochetes enter an extended period of limited nutritional resources. Quantification of *B. burgdorferi* in tick midguts shows essentially no bacterial replication after the blood meal has been digested (12, 170). This period of low metabolic activity may last for many months. The tick's next blood meal brings a rapid and dramatic change to the Lyme spirochete's metabolism, with a doubling time of approximately 2 h (12, 170–173). The only time during *B. burgdorferi*'s infectious cycle where bacterial metabolism quickly shifts from slow to rapid is when the tick feeds on a vertebrate.

In the laboratory, shifting *B. burgdorferi* from a condition of slow replication to a condition of rapid replication mimics conditions in a tick as it initiates feeding and results in significant increases of Erp, EbfC, RpoS, and OspC proteins (29). Under those same conditions, levels of BpaB and BpuR decrease significantly (29). The evident roles of DnaA in controlling EbfC and BpuR, and ultimately Erp, link chromosomal replication and bacterial division to production of surface proteins that are involved with vertebrate infection. Continued bacterial replication during vertebrate infection would maintain the signals to produce Erp proteins.

We further hypothesize that other signals that affect borrelial gene regulation are also tied to the bacterium's invariant life cycle (29, 147). Among known environmental signals are pH, osmolarity, carbon sources, redox potential, metal ions, and carbon dioxide (34, 157, 158, 164, 174–186). Many of those conditions change during tick feeding or differ between tick and vertebrate tissues. Further studies are needed to determine how each of those environmental conditions correlates with the *B. burgdorferi* infectious cycle. Similarly, it is likely that the consistent infectious cycles of other vector-borne pathogens may also be regulated by detection of physiological changes that occur only at a specific step in the cycle.

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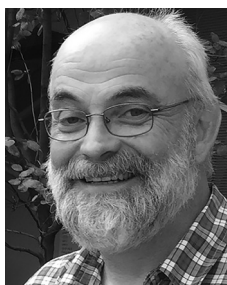
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