1-2012

Of Ticks, Mice and Men: Understanding the Dual-host Lifestyle of Lyme Disease Spirochaetes

Justin D. Radolf
University of Connecticut School of Medicine and Dentistry

Melissa J. Caimano
University of Connecticut School of Medicine and Dentistry

Published in final edited form as:
Nat Rev Microbiol. ; 10(2): 87–99. doi:10.1038/nrmicro2714.

Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes

Justin D. Radolf[1,2,*], **Melissa J. Caimano**[1], **Brian Stevenson**[3], and **Linden T. Hu**[4]

[1]Department of Medicine and Department of Pediatrics, University of Connecticut Health Center

[2]Department of Genetics and Developmental Biology and Department of Immunology, University of Connecticut Health Center, Farmington, Connecticut 06,030, USA

[3]Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky College of Medicine, Lexington, Kentucky 40536, USA

[4]Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center, Boston, Massachusetts 02067, USA

Abstract

In little more than 30 years, Lyme disease, which is caused by the spirochaete *Borrelia burgdorferi*, has risen from relative obscurity to become a global public health problem and a prototype of an emerging infection. During this period, there has been an extraordinary accumulation of knowledge on the phylogenetic diversity, molecular biology, genetics and host interactions of *B. burgdorferi*. In this Review, we integrate this large body of information into a cohesive picture of the molecular and cellular events that transpire as Lyme disease spirochaetes transit between their arthropod and vertebrate hosts during the enzootic cycle.

Since its original description, Lyme disease (Lyme borreliosis) has risen from relative obscurity to become a prototypical emerging infectious disease[1]. The path to notoriety began with a little noticed epidemic of oligoarthritis in the mid 1970s, mainly in children, in several rural communities clustered about the town of Lyme in southeastern Connecticut, USA[2]. Physicians misdiagnosed many of these children as having juvenile rheumatoid arthritis, leading two astute mothers to seek the assistance of investigators at nearby Yale University (New Haven, Connecticut)[1]. The observation that approximately one-quarter of patients developed an expanding, annular skin lesion before the onset of arthritis proved the key to solving the medical mystery. The pathognomonic 'bull's eye' rash, which is now called erythema migrans (FIG. 1), had been closely associated with the bite of the sheep tick (castor bean tick) *Ixodes ricinus* in Northern Europe and was widely believed to be caused by an unknown infectious agent, possibly a spirochaete[1]. These early field investigations prompted an intensive hunt that led to the isolation of the causative organism from a species of North American deer tick, *Ixodes scapularis*[3]. Shortly thereafter, a novel spirochaete was isolated from skin, blood and cerebrospinal fluid specimens obtained from patients with erythema migrans[4,5]. DNA–DNA hybridization studies revealed the isolate to be a new species within the genus *Borrelia*[6], and the species was subsequently named *Borrelia burgdorferi* after its co-discoverer, Willy Burgdorfer.

*To whom correspondence should be addressed: Justin D. Radolf, M.D., Department of Medicine, The University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3715, Phone: 860 679-8480; Fax: 860 679-1358, JRadolf@up.uchc.edu.

Lyme disease spirochaetes are widely distributed throughout the temperate zones of the Northern Hemisphere, and their range continues to expand as lands that were denuded for agriculture become reforested, creating new habitats for deer, ticks and infection-susceptible vertebrates[7]. Lyme disease accounts for >90% of all vector-borne disease in the United States, with nearly 30,000 confirmed cases reported in 2008 (Ref. 8). As many as 60,000 cases occur each year within the range of *I. ricinus*, the primary vector in Europe[9].

The expanding universe of *Borrelia burgdorferi*

Borrelia spp. fall into two major phyletic groups, one containing the causative agents of Lyme disease and the other containing the spirochaetes responsible for relapsing fever[7]. Phylogenetic analyses have led to the division of Lyme disease spirochaetes into numerous species, collectively referred to as *B. burgdorferi sensu lato* (*s.l.*). Among the more than 20 named and unnamed species in the *B. burgdorferi s.l.* complex, three genospecies predominate as human pathogens: *B. burgdorferi sensu stricto* (*s.s.*) in the United States and Western Europe, and *Borrelia garinii* and *Borrelia afzelii* in Eurasia[7]. Four other species, Borrelia valaisiana, Borrelia lusitaniae, Borrelia spielmanii and *Borrelia bissettii*, have also been isolated or detected by PCR in specimens from small numbers of patients[7]. Hereafter, *B. burgdorferi* is used to refer to *B. burgdorferi s.l.*

The enzootic cycle

The life cycle of Lyme disease spirochaetes is depicted in FIG. 1. *B. burgdorferi* is transmitted principally by four species of hard tick within the *I. ricinus* complex: *I. scapularis* and *Ixodes pacificus* (the western black-legged tick) in eastern and western North America, respectively, *I. ricinus* in Europe and *Ixodes persulcatus* (the tiaga tick) in Asia[7,10]. The tick larvae are uninfected when they hatch, as there is no transovarial transmission, and B. burgdorferi is acquired after feeding on an infected reservoir host. After moulting to the nymphal stage, the ticks transmit the pathogen to the animal that provides its next blood meal. Larvae of hard ticks other than *Ixodes* spp. inefficiently acquire Lyme disease spirochaetes from a blood meal and/or fail to maintain them through the trans-stadial moult[11]. The *Ixodes* spp. that transmit *B. burgdorferi* to humans tend to feed on diverse species of vertebrates, accounting for the geographical breadth of transmission cycles within the Northern Hemisphere. Their generalist feeding behaviour is also responsible for the incidental infection of humans[7,10]. Field studies in North America and Eurasia have identified a variety of small mammals and avian reservoirs in enzootic transmission cycles[7]. The white-footed mouse, *Peromyscus leucopus,* is considered to be the main reservoir in the northeastern United States, whereas rodents and migratory birds are the principal reservoirs in Europe for *B. afzelii* and *B. garinii*, respectively.

Outcomes of infection

Spirochaetes are deposited into the bite wound along with the tick saliva during tick feeding[12]. Infection rarely, if ever, occurs during the first 24 hours of nymphal feeding but becomes increasingly likely after the tick has been attached for 48 hours or longer[13]. The outcome of the inoculating event depends on the recipient. White-footed mice remain persistently infected without evidence of inflammation[14]. Although all laboratory strains of inbred mice are susceptible to infection, the development of organ system pathology (that is, arthritis and carditis) in immunocompetent mice is highly strain dependent[14,15] (BOX 1).

Box 1

Animal models of Lyme disease

One of the important early advances in the study of Lyme disease was the development of animal models of infection. As mice and other rodents are natural reservoirs for *Borrelia burgdorferi*, the causative agent, it is not surprising that wild *Peromyscus* spp. mice show no observable changes during infection with *B. burgdorferi*, as symptomatic disease could leave the infected animal with a survival disadvantage and/or limit the opportunities for transmission of spirochaetes to new cohorts of vectors[125]. However, when infected with *B. burgdorferi*, specific inbred strains of the laboratory mouse *Mus musculus* have been found to exhibit features similar to those of human Lyme disease[14,15]. For example, C3H and BALB/c mice develop both ankle joint arthritis and carditis on infection with *B. burgdorferi*, whereas C57BL/6 and DBA mice are more resistant to developing signs of infection and typically have only minimal inflammation in the heart and joints. The greater disease susceptibility of C3H mice does not seem to be due to greater control of the infection, as the spirochaete numbers are comparable to those in disease-resistant mouse strains. By contrast, in BALB/c mice, spirochaete numbers and signs of inflammation directly correlate with the inoculum size, suggesting that the mechanisms of arthritis and carditis might be different in this mouse strain. No strain of inbred mice develops erythema migrans, meningitis or encephalitis, and thus all are imperfect models for human Lyme disease. Also unknown is whether the genetic determinants controlling the development of arthritis and carditis in inbred mice are related to those responsible for the corresponding manifestations in humans.

Other animals have been used to model specific features of human Lyme disease. Dogs infected with *B. burgdorferi* can develop arthritis and facial nerve palsies[126]. Rhesus monkeys can also be infected with *B. burgdorferi* and are used as a model of neuroborreliosis because of their propensity for central nervous system infection, particularly when immunosuppressed with corticosteroids[14]. Rhesus monkeys also develop erythema migrans, mononeuritis multiplex and arthritis, making them the animal model that is most similar to humans for this disease[14]. However, for reasons of cost and ease of genetic manipulation, animal models other than mice have been studied sparingly.

The *B. burgdorferi* genome encodes no known toxins or the machinery that would be required to secrete them[16]; tissue damage, and hence disease, is believed to be mediated by the inflammatory response that is elicited in the mammalian host[15,17]. Although the natural history of Lyme disease in humans is variable and poorly defined[17], seroprevalence surveys in endemic areas have revealed that asymptomatic or subclinical infections occur frequently[18]. Erythema migrans, the most common clinical manifestation of borrelial infection, develops following an incubation period of 3–32 days[17]. Low-level spirochaetaemia probably occurs in the majority of untreated patients[19], occasionally affecting the peripheral or central nervous system, joints or heart[17]. In Europe, most dermatological manifestations are attributed to *B. afzelii*, and neuroborreliosis is caused mostly by *B. garinii*[20]. The relative infrequency of *B. burgdorferi s.s.* as a cause of disease in Europe is believed to explain why carditis and arthritis are less common sequelae of infection here than in North America[20]. Differences in infectivity have also been noted between isolates of *B. burgdorferi s.s.*[21,22].

The three decades since the discovery of *B. burgdorferi* have witnessed an impressive accumulation of knowledge on the molecular biology of this pathogen, its interactions with its arthropod vector, its virulence determinants, the immune responses it elicits during mammalian infection, and the mechanisms involved in immune evasion. In the remainder of

this Review, we integrate this knowledge into a cohesive picture of the molecular and cellular events that sustain the *B. burgdorferi* enzootic cycle.

Genomics and cellular architecture

Genomic complexity coupled with metabolic parsimony

Members of the genus *Borrelia* possess the most complex genomes of all known bacteria[16,23]. More than 20 distinct, naturally occurring genetic elements have been identified in the B31 type strain of *B. burgdorferi.* These include the ~1 Mb linear chromosome and an assortment of linear and circular plasmids totalling approximately 600 kb[16,23]. The chromosome carries the majority of housekeeping genes and is fairly constant in organization and content across the genus[23]. The plasmids, which encode most of the differentially expressed outer-surface lipoproteins, exhibit much greater variability in gene content, are not equally represented in all strains and are not all essential for maintenance of the enzootic cycle[23]. One of the most curious features of the borrelial genome is its degree of redundancy. This is reflected not only in the extraordinarily large number of paralogous gene families distributed mainly among its plasmid complement, but also in the cp32 plasmid family, members of which contain large stretches of essentially identical DNA interrupted by sequence-variable lipoprotein genes[23]. TABLE 1 summarizes the key genes that are currently believed to contribute to maintenance of the *B. burgdorferi* enzootic cycle.

The metabolic abilities of *B. burgdorferi* are extremely limited as a result of reductive evolution following the adoption of a lifestyle that involves parasitizing nutrients from its hosts[16,24]. The bacterium is an auxotroph for all amino acids, nucleotides and fatty acids. It also lacks genes encoding enzymes for the tricarboxylic acid cycle and oxidative phosphorylation, deriving energy instead from the fermentation of sugars to lactic acid via the Embden–Meyerhof pathway[24,25]. One of the metabolic oddities of *B. burgdorferi* is that it does not seem to require iron. Spirochaetes grown *in vitro* have no measurable iron content[26] and, with the possible exception of BB0690, a Dps-like bacterioferritin orthologue[27] (formerly annotated NapA), the genome does not encode orthologues for any known iron-requiring metalloproteins, such as cytochromes, catalase or superoxide dismutase[16,26].

Cell envelope

The structure, composition and physical properties of the cell envelope of *B. burgdorferi* diverge markedly from those of prototypical proteobacteria such as *Escherichia coli*[28] (FIGS 2,3). The outer membrane is a fluid and fragile bilayer that is devoid of lipopolysaccharide[16,29]. Its major lipid constituents are phosphatidylcholine, phosphatidylglycerol and the highly immunogenic but non-inflammatory glycolipids cholesteryl-6-*O*-acyl-β-D-galactopyranoside and cholesteryl-β-D-galactopyranoside[30], none of which is found in the typical proteobacterial outer membrane. The cholesterol glycolipids form raft-like microdomains that are required for the complement-independent bactericidal activity of antibodies directed against lipoproteins on the outer-surface of the spirochaete[29]. The borrelial outer membrane also contains a low density of proteins with membrane-spanning domains[28]. Some of these proteins possess channel-forming properties, although they lack sequence similarity to the better studied bacterial porins[28]. BesC, a TolC orthologue encoded by *bb0142*, forms part of an efflux system that is required for virulence and antimicrobial resistance[31].

From the standpoint of pathogenesis, the most notable difference between the outer membranes of *B. burgdorferi* and proteobacteria is the number and variety of lipoproteins that adorn the spirochaete surface[28] (FIGS 2,3). Characterization of differentially expressed outer-surface lipoproteins has been a major focus of research, including vaccine

development. How Lyme disease spirochaetes direct nascent lipoproteins to their surface is one of the mysteries of borrelial cell biology. *B. burgdorferi* contains a lipoprotein outer-membrane localization (LOL) system similar to that of proteobacteria for directing newly exported lipoproteins to the outer membrane, although the borrelial LOL system uses entirely different sorting rules to the *E. coli* prototype[28]. However, *B. burgdorferi* lacks any of the known pathways for translocating lipoproteins across the outer membrane to the outer surface[28]. A BamA (β-barrel assembly machinery A) orthologue[32] seems to be the central component of the *B. burgdorferi* outer-membrane-assembly machinery. The outer membranes of BamA-depleted spirochaetes are deficient in outer-surface lipoproteins as well as membrane-spanning β-barrels, suggesting that an as-yet-unidentified integral outer-membrane protein is involved in directing lipoproteins to the spirochaete surface.

Motility

B. burgdorferi moves by posteriorly propagating planar waves[33]. A defining morphological feature of *B. burgdorferi*, as with all spirochaetes, is that the organelles of motility — the flagella — are contained entirely within the periplasmic space[33] (FIG. 2d). The filaments are attached at each cell pole to linearly arranged flagellar motors (7–11 at each end), which are elaborate nanomachines that convert chemiosmotic potential into motive force[34]. Cryoelectron tomography has revealed that the flagellar filaments form flat ribbons that wind along the cell cylinder on top of the peptidoglycan layer and below the outer membrane[35] (FIG. 2d). Parallel and adjacent to the motors are arrays of methyl-accepting chemotaxis proteins[36] (FIG. 2b) that bind attractant and repellent molecules through their periplasmic domains, transmitting environmental signals to the motors by modulating the phosphorylation state of the chemotaxis (Che) two-component system, CheA–CheY[33].

Regulation of differential gene expression

Considering the extensive transcriptional changes that the spirochaete undergoes throughout the enzootic cycle, the apparatus for controlling gene expression seems surprisingly sparse. However, it is becoming increasingly apparent that layers of regulatory complexity do in fact exist[37,38]. Many genes that encode proteins required for transmission of spirochaetes between vector and vertebrate hosts are transcribed by an alternative RNA polymerase σ-factor, RpoS, which is transcribed by another alternative σ-factor, RpoN (also known as σ^{54} and NtrA)[37–41] (FIG. 4a). RpoN-dependent transcription of *rpoS* requires the formation of an open *rpoS* promoter complex, mediated by phosphorylated response regulatory protein 2 (Rrp2) and presumed metal-dependent DNA binding by a Fur–PerR orthologue, *Borrelia* oxidative stress regulator (BosR; also known as Fur), upstream of the *rpoS* promoter[37,38,42–47] (FIG. 4a). The phosphate group for Rrp2 phosphorylation seems to come from acetyl phosphate, the intermediate of the acetate kinase–phosphate acetyltransferase pathway that converts acetate to acetyl-CoA[48] (FIG. 4a). In addition, *rpoS* expression is post-transcriptionally regulated by the small RNA DsrA[49], the RNA chaperone Hfq[50] and the RNA-binding protein CsrA[51,52].

Three DNA-binding proteins (BpaB, EbfC and BpuR) are involved in regulation of the cp32-encoded *ospE*, *ospF*, and *elp* lipoprotein gene families (also collectively referred to as *erp*s)[38]. EbfC is also involved in regulating the expression of other borrelial genes, including those encoding components of the oligopeptide permease ABC transporter[53]. The DNA-binding protein Hbb has a role in regulating the expression of the gene encoding p66, a porin and adhesin[54]. As a final example, a two-component system composed of the histidine kinase Hk1 and the response regulator Rrp1 controls production of the second messenger molecule cyclic di-GMP (c-di-GMP) in response to stimuli received during both the larval and nymphal blood meals[55–57] (FIG. 4a). The fine-tuning of c-di-GMP levels is mediated by two phosphodiesterase (Pde) proteins, PdeA and PdeB[58,59] (FIG. 4a).

The *Borrelia*–tick interface

Larval acquisition

Lyme disease spirochaetes disseminate in their reservoir hosts and are then acquired by a naive larva taking a blood meal. Live imaging of disseminated borreliae in the dermis of mice revealed bacteria that appear to be searching randomly for the chemotactic stimuli that are provided, or elicited intracutaneously, by larval feeding[60]. Real-time visualization has also caught spirochaetes in the act of migrating directly towards the feeding site and rapidly entering the hypostome, whereas other, spirochaetes nearby seem oblivious to the same chemoattractants (L. Bockenstedt, personal communication). Larval acquisition of spirochaetes occurs rapidly. The bacteria can be detected in larval midguts by immunofluorescence within 24 hours of attachment and are plentiful by 48 hours, before significant amounts of blood have been imbibed[61].

During infection of the mammalian host, the borrelial Rrp2–RpoN–RpoS pathway is fully activated, promoting the expression of genes that are required within the mammalian host while repressing genes that are required within the tick[37,38,41] (FIG. 4b). By the time the tick larvae are fully engorged, this pathway is inactive, or nearly so, resulting in the upregulation of tick-phase genes, such as *ospA*, which are repressed by RpoS[38,62]. *B. burgdorferi* specifically binds the neuroendocrine stress hormones adrenaline and noradrenaline; it has been proposed that OspA expression is upregulated in response to the production of these hormones in the skin, which is in turn induced by the combined mechanical and pharmacological assault of larval feeding[63]. OspA-deficient spirochaetes cannot bind to tick receptor for OspA (TROSPA) on tick midgut epithelial cells and are eventually expelled from the larval digestive tract with the blood meal waste[64–66]. In addition, activation of the Hk1–Rrp1 pathway during larval feeding (FIG. 4b), with consequent production of c-di-GMP (FIG. 4a), is required for successful bacterial colonization of the larvae[55–57]. Hk1-deficient and Rrp1-deficient mutants are destroyed early during feeding[55,56], perhaps because they cannot remodel their surfaces to protect themselves against antimicrobial substances elaborated by the larval midgut.

The ingress of blood triggers a burst of spirochaete replication that continues for weeks through the moult[67]. Following tick drop-off, the spirochaete must turn to alternative carbon sources for glycolysis and phospholipid biosynthesis, as the glucose supply within the tick midgut lumen diminishes. The expression of genes within the *glp* operon, which encodes proteins involved in the uptake and utilization of glycerol, is upregulated during the tick phase of the spirochaete life cycle[56,68]. The induction of *glp* expression during larval acquisition of spirochaetes is driven by activation of the Hk1–Rrp1 pathway[56] and by the loss of RpoS-mediated gene repression[68] (FIG. 4b). I*xodes* spp. ticks surround the blood meal with an acellular matrix of glycoproteins and chitin, called the peritrophic membrane[67]. *N*-acetylglucosamine and chitobiose, secreted by epithelial cells for synthesis and remodelling of the peritrophic membrane, may be alternative carbon sources in addition to being essential for peptidoglycan biosynthesis[25,69].

The flat nymph

Spirochaetes within flat, or unfed, nymphs exist in a poorly understood metabolic state that enables them to endure prolonged periods of nutrient deprivation[67]. In this state, both the Rrp2–RpoN–RpoS and Hk1–Rrp1 pathways are inactive, as are mammalian-phase genes, while tick-phase genes are maximally expressed[37,38,67] (FIG. 4b). Despite appearances, spirochaetes residing within flat ticks are not dormant; the bacteria in dissected unfed midguts are motile, albeit sluggish[70]. Moreover, microarray analyses of spirochaetes cultivated *in vitro* under ‘unfed-tick’ conditions, as well as quantitative reverse transcriptase

PCR (qRT-PCR) of selected genes in borreliae residing in flat ticks, suggest that many borrelial genes are preferentially expressed in the anoxic environment of the unfed midgut[67,71–73]. A stringent response analogous to that observed in *E. coli* during amino acid starvation has been suggested to occur during this phase, as the levels of transcripts encoding a RelA–SpoT orthologue are higher in bacteria cultivated *in vitro* under conditions that mimic unfed ticks compared to those that mimic fed ticks and mammals[71]. Because the spirochaetes in unfed ticks are not replicating, they do not require *N*-acetylglucosamine. They do, however, require a carbon source to maintain subsistence levels of glycolysis. Glycerol, which is thought to be produced as a natural antifreeze by overwintering ticks[68], could permeate the outer membrane of the spirochaete and enter the bacterial cytoplasm via glycerol uptake facilitator GlpF, which is expressed by borreliae in unfed ticks[68]. In addition to OspA, the surface-exposed lipoprotein BptA[74] and BB690, a Dps-like bacterioferritin orthologue[27], are essential for prolonged residence in flat ticks.

The nymphal blood meal

The nymphal blood meal ends a long nutritional drought but also presents the bacterium with a new and complex set of challenges. To take advantage of the influx of nutrients, the spirochaete must sense the new environment and extensively revamp its substrate uptake mechanisms and intermediary metabolism from famine to feast mode[24,67]. Whereas a temperature shift during cultivation *in vitro* induces many of the antigenic changes associated with nymphal feeding in borreliae[37,38,41,67], temperature shifting of infected unfed ticks does not[75]. Metabolic changes in the spirochaete that are associated with nutrition from the blood meal, and possibly components of the blood meal itself, are required to fully activate the genetic programmes associated with transmission[48,73].

During the first 24 hours of nymphal feeding (the preparatory phase of the digestive process), little to no blood enters the midgut, and spirochaete numbers remain essentially unchanged from numbers in the unfed-tick state[70,76]. Activation of the Rrp2–RpoN–RpoS pathway at the outset of feeding (FIG. 4b) results in the transcription of genes that are required for mammalian infection and simultaneously begins the slow repression of tick-phase genes[37,38,62,67]. Nymphal feeding also induces the expression of genes which are regulated independently of RpoS and are dependent instead on σ^{70} (also known as RpoD). The products encoded by these genes include the outer-membrane-spanning protein p66, which acts as a porin and adhesin[77–79], and BbCRASPs (complement regulator-acquiring surface proteins), which are outer-surface lipoproteins that protect the bacterium against complement-mediated lysis by binding complement factor H and complement factor H-like protein 1 (Refs 80–83). As is the case during larval feeding, spirochaetes require the two-component system Hk1–Rrp1 to avoid being destroyed at the start of nymphal feeding[55,56] (FIG. 4b).

By 48 hours, bacterial replication and dissemination are well under way[67,70,76,84,85]. Although it has been suggested that spirochaetes traverse the tick midgut by downregulating OspA, detaching from epithelial cells, and then using their motility to migrate between cells and penetrate the basement membrane, two lines of evidence argue that this scenario is overly simplistic. First, OspA is highly expressed by spirochaetes distributed throughout the midgut, even at late time points during feeding[61,62,85]. Second, confocal microscopy has shown that the bacteria maintain a close association with the epithelial cells as they replicate, coalescing into networks of non-motile bacteria that literally surround the hypertrophying epithelial cells and progress towards the basement membrane[70,84]. Spirochaetes that reach the basolateral surface of the epithelium transition into motile organisms that cross the basement membrane and enter the haemocoel[70,86]. BBE31, a surface-exposed borrelial lipoprotein, may be required for spirochaetes to penetrate the midgut through its interaction with TRE31, a tick protein that is secreted by epithelial cells

during feeding[87]. Binding of host-derived molecules, such as plasminogen, to the bacterial surface probably facilitates penetration of the collagenous matrix of the basement membrane[88]. The salivary glands pose the final mechanical barrier to be overcome; only a handful of spirochaetes reach the interior of the gland, where they access the salivary stream[70,76,85].

Within the mammalian host

The bite site

The host factors in tick saliva that enhance the survival of *B. burgdorferi* and are inoculated into the cutaneous bite site (FIG. 5) include molecules that can impede various mammalian responses, including the generation of reactive oxygen species (ISL 1373), activation of complement (ISAC and SALP20), release of antimicrobial peptides (SALP15), chemotaxis of neutrophils (sialostatin L), and antibody-mediated killing, dendritic cell-mediated priming of T cells, and keratinocyte-mediated release of cytokines and antimicrobial peptides (SALP15)[67,89,90]. Expression of the bacterial protein OspC is crucial for the establishment of early infection[91–93], although there is controversy as to whether OspC promotes the penetration of salivary glands by spirochaetes disseminating within the tick or the survival of spirochaetes deposited at the bite site through binding of SALP15. As in the feeding nymph, borrelial proteins that bind to factor H- and factor H-like protein 1 protect the bacterium against complement-mediated killing during this early window of vulnerability[79–83].

Initial sensing of *B. burgdorferi* by the mammalian host probably occurs through pattern recognition receptors, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), on dendritic cells and sentinel macrophages within the dermis (FIG. 5). TLR2, which heterodimerizes with TLR1 to recognize triacylated lipoproteins, seems to be central to the induction of many inflammatory cytokines in response to *B. burgdorferi*. Engagement of TLR2–TLR1 heterodimers by borrelial lipoproteins results in activation of mitogen-activated protein kinase (MAPK) pathways, translocation of nuclear factor-κB (NF-κB) into the nucleus, and subsequent production and release of inflammatory mediators[15]. Other TLRs, such as TLR5, TLR7, TLR8 and TLR9, cooperate with TLR2–TLR1 to induce pro-inflammatory molecules, including type I interferons (IFNs)[94–97]. TLR-mediated release of chemokines and cytokines by resident phagocytes presumably attracts other inflammatory cells to the tick bite site. In mice, neutrophils are recruited early but disappear within 16 hours of inoculation and are followed by monocytes and macrophages, both of which are capable killers of *B. burgdorferi*[15]. However, *B. burgdorferi* is a highly motile organism and can move at speeds of up to 4 μm sec^{-1} in tissue[60], enabling it to evade capture by these comparatively sluggish professional phagocytes. Spirochaetes that are unable to evade phagocytes are ingested and rapidly degraded within phagosomal vacuoles[95]. The phagocytosis of borreliae and subsequent degradation within phagolysosomes further amplifies the release of inflammatory cytokines through activation of TLR signalling within phagolysosomes[95]. Internalization of spirochaetes in the absence of antibodies is mediated by at least two different integrins, αmβ2 integrin (also known as CR3 or CD18–CD11b) and α3β1 integrin[15,98]. Although the internalization of *B. burgdorferi* results in activation of the inflammasome, neither the course of disease nor the spirochaete burden is worsened appreciably in caspase 1-deficient mice[15]. In humans, recruitment of T cells heralds the development of the characteristic erythema migrans rash (FIG 1). Biopsies of erythema migrans lesions show infiltration by T cells ($CD8^+$ cells as well as $CD4^+$ cells), macrophages, plasmacytoid and monocytoid dendritic cells, and neutrophils in varying proportions, but no B cells[99,100]; a number of inflammatory cytokines and chemokines have also been identified in erythema migrans lesions[99,101].

Dissemination and immune evasion

After a delay of up to 2 days, *B. burgdorferi* begins to spread to distant tissues[102]. To disseminate, *B. burgdorferi* penetrates the matrix between cells and enters capillary beds. The spirochaete circumvents its inability to produce enzymes that are capable of digesting extracellular matrix components by appropriating host proteases such as plasminogen and its activator, urokinase[86,103]. *B. burgdorferi* also induces multiple host matrix metalloproteinases (MMPs), the major class of host proteases involved in the degradation of extracellular matrix components, from both phagocytic and non-phagocytic cells[15,79]. Entry into capillaries provides *B. burgdorferi* with access to the bloodstream. Egress from the circulation into tissues involves tethering and adhesion to vascular endothelium followed by extravasation[60]. *B. burgdorferi* expresses a variety of adhesins that could mediate attachment to host tissues through diverse receptors such as integrins (which can bind p66 and the outer-surface protein encoded by the locus BBB07)[77,104], proteoglycans (which can bind decorin-binding protein A (DbpA) and DbpB)[105], glycosaminoglycans[106], laminin (which can bind ErpX and BmpA)[107,108] and fibronectin (which can bind BBK32 and RevA)[79,109,110].

Carditis in mice and humans is predominantly caused by infiltration of monocytes or macrophages, with a minority of lymphocytes and neutrophils[15,17]. Arthritis in susceptible strains of mice, by comparison, shows a predominance of neutrophils with smaller numbers of macrophages and lymphocytes[15]. IFNγ appears to mediate carditis, but not arthritis, in susceptible mice[111,112], whereas type I IFNs may be particularly important for the development of arthritis[96]. *B. burgdorferi* is unique among pathogens in that its diacylglycerol glycolipid can directly activate invariant natural killer T cells[113]. These T cells might participate in clearing spirochaetaemia after being recruited by Kupffer cells that recognize *B. burgdorferi* in the liver[114]. In mice, invariant natural killer T cells might also be recruited to the heart during *B. burgdorferi* infection, where they could have a role in controlling infection and inflammation through augmenting phagocytosis[111].

Both the innate and adaptive immune systems are important for controlling infection and inflammation during the disseminated phase, as mice that are deficient in either system have greater bacterial burdens than wild-type mice[15]. T cells do not appear to be required for resolution of either arthritis or carditis in mice; in fact, the presence of T cells without B cells can worsen both[115]. $CD4^+$ T cells, however, can hasten resolution of carditis in the presence of B cells[115]. The development of the specific humoral response to *B. burgdorferi* is crucial for clearing the pathogen[15,116]. Early T cell-independent production of antibodies, predominantly immunoglobulin M, is crucial for the initial reduction of spirochaete burdens[117,118]. T cell-dependent production of immunoglobulin G by B cells is typically detectable by the second week of infection[15]. Antibody production does not seem to require TLR signalling, as both TLR2- and MYD88-knockout mice develop normal humoral responses to infection with *B. burgdorferi*[118–120]. Antibodies against many different borrelial proteins elicit strain-dependent reductions in pathogen burden or a reduction in carditis or arthritis[15]. In response, the bacterium is thought to downregulate lipoproteins, such as OspC, which are no longer required to establish or maintain infection[93,121]. *B. burgdorferi* also uses a system of antigenic variation to evade antibodies. VlsE is a 35 kDa lipoprotein that undergoes antigenic variation through the recombination of sequences from silent cassettes into the expressed *vlsE* locus[83,122,123]. The spirochaete may also exploit mechanisms used by the host to suppress inflammation as a means of limiting tissue damage, in effect making the mammalian host an unwitting accomplice to the spirochaete's strategy for persistence[124].

Concluding remarks and future directions

Two facets of the life cycle of *B. burgdorferi* — the bacterium's ability to adapt to markedly divergent host environments and its ability to evade the defences of its mammalian reservoir — account, to a large extent, for the extraordinary zoonotic success of this spirochaete and its continued expansion as a threat to public health. We are only just beginning to understand the mechanisms whereby this versatile bacterium coordinates changes in its transcriptome, cellular architecture and metabolism with the feeding behaviour and physiology of its arthropod vector. Marginally better understood are the stratagems that enable the spirochaete to navigate within the mammal, regularly gaining entry to niches that are impassable for other bacteria, when establishing a persistent infection. Despite its small genome, the Lyme disease spirochaete possesses deceptively complex machinery for tightly regulating gene and protein expression, with an unusual combination of components identified to date. The small number of orthologues for known transcription factors hints at the existence of novel post-transcriptional control pathways; it is noteworthy that the burgeoning interest in small RNAs among microbiologists has only recently made its influence felt in the field of borrelial research. Co-evolution of the spirochaete with its arthropod and mammalian hosts has enabled the bacterium to take advantage of host physiological processes with compensatory reductions in its own biosynthetic machinery. In so doing, the bacterium has developed ingenious and parsimonious strategies to obtain and utilize the nutrient sources available within the 'feast and famine' confines of its life cycle. Indeed, the nutrients themselves, especially carbon sources, and/or their metabolic by-products seem to provide regulatory as well as chemotactic signals that guide the spirochaete as it moves between hosts.

Beginning with the discovery that spirochaete lipoproteins are major inflammatory agonists, we have learned a great deal about the mechanisms by which the mammalian host senses the presence of live spirochaetes and mobilizes cellular and humoral defences to combat the intruder. By contrast, little is understood about the processes that occur late in infection and the mechanisms that enable the bacterium to persist in the face of the robust cellular and humoral immune responses that it elicits. In the past, the emphasis has been on immune evasion by the spirochaete, and this topic needs far more scrutiny. Nevertheless, attention also needs to be paid to the possibility that modulation of pathogen-sensing pathways and resultant responses by the host contribute to spirochaete persistence. We are just beginning to understand the polymorphisms in specific mouse genes that result in some inbred strains of mice being more prone to manifestations of infection than the natural *P. leucopus* host. Whether these genes are identical to those responsible for disease susceptibility and expression in humans is unknown. However, one can envision translational studies to answer the question of why some infected patients have only subclinical disease, whereas others develop overt manifestations. These and other advances will hopefully lead to a better understanding of the determinants of vector and host specificity for *B. burgdorferi*, and to the manipulation of these determinants to interrupt the cycle of transmission. Although eliminating Lyme disease spirochaetes from nature is unrealistic, diminishing their threat to humans would seem to be an achievable goal.

Acknowledgments

The authors gratefully acknowledge support from the US National Institutes of Health, National Institute for Allergy and Infectious Diseases (grants AI029735 and AI029735-20S1 to J.D.R. and M.J.C.; AI085248 to M.J.C.; AI044254 to B.S.; and AI071107, AI068799, AI082436 and AI080646 to L.T.H. The authors also thank S. Dunham-Ems, T. Petnicki-Ocwieja, E. Troy and C. Brissette for invaluable assistance with the figures and the table, many insightful comments and careful proofreading.

Glossary Terms

Oligoarthritis	Arthritis affecting more than one joint in an asymmetrical pattern
Pathognomonic	Characteristic for a particular disease
Spirochaete	A member of the ancient and deeply branching bacterial phylum Spirochaetes, which consists of bacteria that possess a helically coiled (spiral-shaped) or wave-like morphology and a distinctive mode of motility which enables them to penetrate viscous media and tissues
Genospecies	A term that is often used to describe different species of Lyme disease spirochaetes that tend to occur in particular geographic regions of the Northern Hemisphere
Transovarial	Passed from a female adult to the larva via the egg (as occurs with spriochaetes that cause relapsing fever but not those that cause Lyme disease)
Reservoir	In the context of Lyme disease, a vertebrate species that can be persistently and asymptomatically infected with spirochaetes and, therefore, can serve as a source of infection for naive feeding ticks, usually larvae
Trans-stadial	Transmitted to successive developmental stages of the tick
Enzootic	Existing in nature in animal reservoirs
Carditis	Inflammation of the heart; Lyme carditis is caused by spirochaete infection of the heart
Spirochaetaemia	Dissemination of spirochaetes through the bloodstream
Lipoprotein	A protein containing covalently bound fatty acids that, in bacteria, are typically at the amino terminus
Auxotroph	A bacterium that is unable to synthesize an essential nutrient
Dps	An oligomeric, ferritin-like protein that protects DNA against damage meditated by oxidative stress and starvation
TolC	A trimeric protein that forms outer-membrane channels and associates with inner-membrane pumps to export toxic molecules from the cell
Two-component system	A system for environmental sensing in bacteria, typically consisting of a sensor histidine kinase and a response regulator
Fur	(Ferric uptake regulation). A metal-dependent DNA-binding protein that binds Fe II and regulates iron transport and related metabolic processes
PerR	(Peroxide-sensitive repressor). A dimeric metalloprotein related to Fur. PerR orthologues contain two metal-binding sites per monomer: one site binds Zn II, and the other binds a regulatory metal, typically Fe II or Mn II
Hypostome	A barbed protuberance of the mouthparts that anchors the tick during the blood meal

Complement factor H and complement factor H-related protein 1	Serum proteins that prevent inadvertent activation of the alternative complement pathway. Binding of these proteins by CRASPs (complement regulator-acquiring surface proteins) on the surface of Lyme disease spirochaetes protects the bacteria against the lytic activity of complement generated via the alternative pathway
Basement membrane	A collagenous matrix that encloses the surface of the tick midgut facing the haemocoel
Haemocoel	The fluid-filled space that surrounds the tick midgut and contains the salivary glands
Plasminogen	The inactive form of a proteolytic enzyme (zymogen) that, on activation by urokinase, degrades many proteins in the blood, including fibrin
Pattern recognition receptors	Invariant (that is, germ-line encoded) components of the innate immune system that recognize exogenous molecules (typically of bacterial or viral origin)
Phagosomal	Within the intracellular membrane-bound compartment created when an exogenous particle is internalized by phagocytosis
Inflammasome	A macromolecular inflammatory signalling complex in macrophages; created when pattern recognition receptors within the macrophage cytosol of are activated by foreign substances or molecules (often of bacterial or viral origin)
Urokinase	A serine protease that activates plasmin, triggering a proteolytic cascade involved in thrombolysis or degradation of the extracellular matrix. Also called urokinase-type plasminogen activator
Matrix metalloproteinases	Zn-dependent endopeptidases that are capable of degrading extracellular matrix
Decorin	A proteoglycan component of connective tissue that binds to type I collagen fibrils in extracellular matrix
Invariant natural killer T cells	A heterogeneous group of CD1d-restricted T cells that recognize self and foreign lipids and glycolipids. These cells constitute approximately 0.2% of peripheral blood T cells

References

1. Benach, JL.; Garcia Monco, JC. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 7-26.
2. Steere AC, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. 1977; 20:7–17. This article describes the original epidemiological study of the outbreak of arthritis in and around Lyme, Connecticut. [PubMed: 836338]
3. Burgdorfer W, et al. Lyme disease—a tick-borne spirochetosis? Science. 1982; 216:1317–1319. This classic paper provides the first convincing evidence that Lyme disease is a tick-borne illness. [PubMed: 7043737]
4. Steere AC, et al. The spirochetal etiology of Lyme disease. N Engl J Med. 1983; 308:733–740. [PubMed: 6828118]

5. Benach JL, et al. Spirochetes isolated from the blood of two patients with Lyme disease. N Engl J Med. 1983; 308:740–742. This and reference 4 are the first two reports to describe the isolation of Lyme disease spirochaetes from patients. [PubMed: 6828119]
6. Hyde FW, Johnson RC. Genetic relationship of Lyme disease spirochetes to *Borrelia*, *Treponema*, and *Leptospira* spp. J Clin Microbiol. 1984; 20:151–154. [PubMed: 6490811]
7. Piesman, J.; Schwan, TG. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 251-278.
8. Hall-Baker PA, et al. Summary of notifiable diseases — United States, 2008. Morb Mortal Wkly Rep. 2010; 57:1–94.
9. O'Connell S, Granstrom M, Gray JS, Stanek G. Epidemiology of European Lyme borreliosis. Zentralbl Bakteriol. 1998; 287:229–240. [PubMed: 9563197]
10. Kurtenbach K, et al. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nature Rev Microbiol. 2006; 4:660–669. [PubMed: 16894341]
11. Mather TN, Mather ME. Intrinsic competence of three ixodid ticks (Acari) as vectors of the Lyme disease spirochete. J Med Entomol. 1990; 27:646–650. [PubMed: 2388239]
12. Ribeiro JM, Mather TN, Piesman J, Spielman A. Dissemination and salivary delivery of Lyme disease spirochetes in vector ticks (Acari: Ixodidae). J Med Entomol. 1987; 24:201–205. A definitive demonstration that spirochaetes are transmitted by the salivary route during tick feeding. [PubMed: 3585913]
13. Piesman J, Mather TN, Sinsky RJ, Spielman A. Duration of tick attachment and *Borrelia burgdorferi* transmission. J Clin Microbiol. 1987; 25:557–558. This classic study defines the ~48 hour window required for transmission of spirochaetes by feeding nymphs. [PubMed: 3571459]
14. Barthold, SW.; Cadavid, D.; Philipp, MT. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 359-411.
15. Weis, JJ.; Bockenstedt, LK. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 413-441.
16. Fraser CM, et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature. 1997; 390:580–586. A sequence analysis of the *B. burgdorferi* chromosome; one of the first genomic-sequence papers for this species. [PubMed: 9403685]
17. Radolf, JD.; Salazar, JC.; Dattwyler, RJ. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 487-533.
18. Fahrer H, et al. The prevalence and incidence of clinical and asymptomatic Lyme borreliosis in a population at risk. J Infect Dis. 1991; 163:305–310. [PubMed: 1988513]
19. Wormser GP, et al. Brief communication: hematogenous dissemination in early Lyme disease. Ann Intern Med. 2005; 142:751–755. [PubMed: 15867407]
20. Stanek G, Strle F. Lyme disease: European perspective. Infect Dis Clin North Am. 2008; 22:327–339. vii. [PubMed: 18452805]
21. Wormser GP, et al. *Borrelia burgdorferi* genotype predicts the capacity for hematogenous dissemination during early Lyme disease. J Infect Dis. 2008; 198:1358–1364. [PubMed: 18781866]
22. Earnhart CG, Buckles EL, Dumler JS, Marconi RT. Demonstration of OspC type diversity in invasive human Lyme disease isolates and identification of previously uncharacterized epitopes that define the specificity of the OspC murine antibody response. Infect Immun. 2005; 73:7869–7877. [PubMed: 16299277]
23. Casjens, SR.; Eggers, CH.; Schwartz, I. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 27-53.
24. Gheradini, F.; Boylan, J.; Lawrence, K.; Skare, J. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 103-138.
25. von Lackum K, Stevenson B. Carbohydrate utilization by the Lyme borreliosis spirochete, Borrelia burgdorferi. FEMS Microbiol Lett. 2005; 243:173–179. [PubMed: 15668016]

26. Posey JE, Gherardini FC. Lack of a role for iron in the Lyme disease pathogen. Science. 2000; 288:1651–1653. This article provides evidence for one of the most unusual physiologic properties of *B. burgdorferi*: its lack of a requirement for iron. [PubMed: 10834845]
27. Li X, et al. The Lyme disease agent *Borrelia burgdorferi* requires BB0690, a Dps homologue, to persist within ticks. Mol Microbiol. 2007; 63:694–710. [PubMed: 17181780]
28. Bergstrom, S.; Zuckert, WR. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 139-166.
29. LaRocca TJ, et al. Cholesterol lipids of *Borrelia burgdorferi* form lipid rafts and are required for the bactericidal activity of a complement-independent antibody. Cell Host Microbe. 2010; 8:331–342. A technically innovative study relating antibody killing of spirochaetes to the unique lipid composition and physical properties of the spirochaete outer membrane. [PubMed: 20951967]
30. Schroder NW, et al. Acylated cholesteryl galactoside as a novel immunogenic motif in *Borrelia burgdorferi* sensu stricto. J Biol Chem. 2003; 278:33645–33653. [PubMed: 12810705]
31. Bunikis I, et al. An RND-type efflux system in *Borrelia burgdorferi* is involved in virulence and resistance to antimicrobial compounds. PLoS Pathog. 2008; 4:e1000009. [PubMed: 18389081]
32. Lenhart TR, Akins DR. *Borrelia burgdorferi* locus BB0795 encodes a BamA orthologue required for growth and efficient localization of outer membrane proteins. Mol Microbiol. 2010; 75:692–709. [PubMed: 20025662]
33. Goldstein, SF., et al. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 167-187.
34. Liu J, et al. Intact flagellar motor of *Borrelia burgdorferi* revealed by cryo-electron tomography: evidence for stator ring curvature and rotor/C-ring assembly flexion. J Bacteriol. 2009; 191:5026–5036. [PubMed: 19429612]
35. Charon NW, et al. The flat-ribbon configuration of the periplasmic flagella of *Borrelia burgdorferi* and its relationship to motility and morphology. J Bacteriol. 2009; 191:600–607. [PubMed: 19011030]
36. Xu H, Raddi G, Liu J, Charon NW, Li C. Chemoreceptors and flagellar motors are subterminally located in close proximity at the two cell poles in spirochetes. J Bacteriol. 2011; 193:2652–2656. [PubMed: 21441520]
37. Skare, JT.; Carroll, JA.; Yang, XF.; Samuels, DS.; Akins, DR. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 67-101.
38. Samuels DS. Gene regulation in *Borrelia burgdorferi*. Annu Rev Microbiol. 2011; 65:479–499. [PubMed: 21801026]
39. Hubner, A., et al. Proc Natl Acad Sci USA. Vol. 98. 2001. Expression of *Borrelia burgdorferi* OspC and DbpA is controlled by a RpoN–RpoS regulatory pathway; p. 12724-12729.A ground-breaking demonstration of the unique control mechanism for rpoS transcription in *B. burgdorferi* and the role of this alternative σ-factor in controlling virulence gene expression
40. Fisher MA, et al. *Borrelia burgdorferi* σ54 is required for mammalian infection and vector transmission but not for tick colonization. Proc Natl Acad Sci USA. 2005; 102:5162–5167. [PubMed: 15743918]
41. Caimano MJ, et al. Analysis of the RpoS regulon in *Borrelia burgdorferi* in response to mammalian host signals provides insight into RpoS function during the enzootic cycle. Mol Microbiol. 2007; 65:1193–1217. [PubMed: 17645733]
42. Yang XF, Alani SM, Norgard MV. The response regulator Rrp2 is essential for the expression of major membrane lipoproteins in *Borrelia burgdorferi*. Proc Natl Acad Sci USA. 2003; 100:11001–11006. An elegant use of mutagenesis to demonstrate the essentiality of Rrp2 for the induction of RpoS-dependent gene regulation. [PubMed: 12949258]
43. Blevins JS, et al. Rrp2, a σ 4-dependent transcriptional activator of *Borrelia burgdorferi*, activates *rpoS* in an enhancer-independent manner. J Bacteriol. 2009; 191:2902–2905. [PubMed: 19201806]
44. Boardman BK, et al. Essential role of the response regulator Rrp2 in the infectious cycle of *Borrelia burgdorferi*. Infect Immun. 2008; 76:3844–3853. [PubMed: 18573895]
45. Burtnick MN, et al. Insights into the complex regulation of *rpoS* in *Borrelia burgdorferi*. Mol Microbiol. 2007; 65:277–293. [PubMed: 17590233]

46. Hyde JA, Shaw DK, Smith R III, Trzeciakowski JP, Skare JT. The BosR regulatory protein of *Borrelia burgdorferi* interfaces with the RpoS regulatory pathway and modulates both the oxidative stress response and pathogenic properties of the Lyme disease spirochete. Mol Microbiol. 2009; 74:1344–1355. One of the first investigations to demonstrate that the borrelial Fur–PerR orthologue, BosR, is required for expression of *rpoS*. [PubMed: 19906179]
47. Ouyang Z, Deka RK, Norgard MV. BosR (BB0647) controls the RpoN-RpoS regulatory pathway and virulence expression in *Borrelia burgdorferi* by a novel DNA-binding mechanism. PLoS Pathog. 2011; 7:e1001272. This impressive work defines the binding-site motif for BosR near the rpoS promoter. [PubMed: 21347346]
48. Xu H, et al. Role of acetyl-phosphate in activation of the Rrp2 RpoN RpoS pathway in *Borrelia burgdorferi*. PLoS Pathog. 2010; 6:e1001104. This study elegantly relates the concept of the acetate switch in *E. coli* to the activation of Rrp2 and differential gene expression in *B. burgdorferi*. [PubMed: 20862323]
49. Lybecker MC, Samuels DS. Temperature-induced regulation of RpoS by a small RNA in *Borrelia burgdorferi*. Mol Microbiol. 2007; 64:1075–1089. The first paper to present evidence for post-transcriptional regulation of *rpoS* in *B. burgdorferi*. [PubMed: 17501929]
50. Lybecker MC, Abel CA, Feig AL, Samuels DS. Identification and function of the RNA chaperone Hfq in the Lyme disease spirochete *Borrelia burgdorferi*. Mol Microbiol. 2010; 78:622–635. [PubMed: 20815822]
51. Karna SL, et al. CsrA modulates levels of lipoproteins and key regulators of gene expression critical for pathogenic mechanisms of *Borrelia burgdorferi*. Infect Immun. 2011; 79:732–744. [PubMed: 21078860]
52. Sze CW, Li C. Inactivation of *bb0184*, which encodes carbon storage regulator A, represses the infectivity of *Borrelia burgdorferi*. Infect Immun. 2011; 79:1270–1279. [PubMed: 21173314]
53. Medrano MS, et al. Regulators of expression of the oligopeptide permease A proteins of *Borrelia burgdorferi*. J Bacteriol. 2007; 189:2653–2659. [PubMed: 17237172]
54. Medrano MS, Policastro PF, Schwan TG, Coburn J. Interaction of *Borrelia burgdorferi* Hbb with the *p66* promoter. Nucleic Acids Res. 2010; 38:414–427. [PubMed: 19910373]
55. Caimano MJ, et al. The hybrid histidine kinase Hk1 is part of a two-component system that is essential for survival of *Borrelia burgdorferi* in feeding *Ixodes scapularis* ticks. Infect Immun. 2011; 79:3117–3130. The demonstration that the sensor kinase Hk1 is required for adaptation of the spirochaete to the tick host. [PubMed: 21606185]
56. He M, et al. Cyclic di-GMP is essential for the survival of *Borrelia burgdorferi* in ticks. PLOS Pathog. 2011; 7:e1002133. The discovery that the response regulator Rrp1 is required for adaptation of the spirochaete to the tick host. [PubMed: 21738477]
57. Kostick JL, et al. The diguanylate cyclase, Rrp1, regulates critical steps in the enzootic cycle of the Lyme disease spirochetes. Mol Microbiol. 2011; 81:219–231. The finding that Rrp1 is required for adaptation of the spirochaete to the tick host. [PubMed: 21542866]
58. Sultan SZ, et al. Analysis of the HD GYP domain cyclic di GMP phosphodiesterase reveals a role in motility and enzootic life cycle of *Borrelia burgdorferi*. Infect Immun. 2011; 79:3273–3283. [PubMed: 21670168]
59. Sultan SZ, Pitzer JE, Miller MR, Motaleb MA. Analysis of a *Borrelia burgdorferi* phosphodiesterase demonstrates a role for cyclic di guanosine monophosphate in motility and virulence. Mol Microbiol. 2010; 77:128–142. [PubMed: 20444101]
60. Moriarty TJ, et al. Real-time high resolution 3D imaging of the Lyme disease spirochete adhering to and escaping from the vasculature of a living host. PLoS Pathog. 2008; 4:e1000090. A landmark paper describing *in vivo* imaging of *B. burgdorferi* in mice, including remarkable images and movies of spirochaetes penetrating the vascular endothelium. [PubMed: 18566656]
61. Schwan TG, Piesman J. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. J Clin Microbiol. 2000; 38:382–388. [PubMed: 10618120]
62. Mulay VB, et al. *Borrelia burgdorferi bba74* is expressed exclusively during tick feeding and is regulated by both arthropod- and mammalian host-specific signals. J Bacteriol. 2009; 191:2783–2794. [PubMed: 19218390]

63. Scheckelhoff MR, Telford SR, Wesley M, Hu LT. *Borrelia burgdorferi* intercepts host hormonal signals to regulate expression of outer surface protein A. Proc Natl Acad Sci USA. 2007; 104:7247–7252. [PubMed: 17438273]
64. Pal U, et al. TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. Cell. 2004; 119:457–468. The identification of the receptor, expressed on tick midgut epithelial cells, that is critical for the tick phase of the spirochaete's enzootic life cycle. [PubMed: 15537536]
65. Yang XF, Pal U, Alani SM, Fikrig E, Norgard MV. Essential role for OspA/B in the life cycle of the Lyme disease spirochete. J Exp Med. 2004; 199:641–648. An elegant genetic demonstration that spirochaetes lacking OspA are virulent but unable to colonize larval midguts. [PubMed: 14981112]
66. Battisti JM, et al. Outer surface protein A protects Lyme disease spirochetes from acquired host immunity in the tick vector. Infect Immun. 2008; 76:5228–5237. [PubMed: 18779341]
67. Pal, U.; Fikrig, E. Borrelia: Molecular Biology, Host Interactions, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfold, UK: 2010. p. 279-298.
68. Pappas CJ, et al. *Borrelia burgdorferi* requires glycerol for maximum fitness during the tick phase of the enzootic cycle. PLoS Pathog. 2011; 7:e1002102. [PubMed: 21750672]
69. Tilly K, et al. Genetics and regulation of chitobiose utilization in *Borrelia burgdorferi*. J Bacteriol. 2001; 183:5544–5553. [PubMed: 11544216]
70. Dunham-Ems SM, et al. Live imaging reveals a biphasic mode of dissemination of *Borrelia burgdorferi* within ticks. J Clin Invest. 2009; 119:3652–3665. The first paper to use live imaging to track spirochaetes disseminating in feeding nymphs. [PubMed: 19920352]
71. Revel AT, Talaat AM, Norgard MV. DNA microarray analysis of differential gene expression in *Borrelia burgdorferi*, the Lyme disease spirochete. Proc Natl Acad Sci USA. 2002; 99:1562–1567. The first microarray analysis of the *B. burgdorferi* transcriptome, which cleverly uses *in vitro* conditions to emulate the tick and mammalian phases of the enzootic cycle. [PubMed: 11830671]
72. Ojaimi C, et al. Profiling of temperature-induced changes in *Borrelia burgdorferi* gene expression by using whole genome arrays. Infect Immun. 2003; 71:1689–1705. [PubMed: 12654782]
73. Tokarz R, Anderton JM, Katona LI, Benach JL. Combined effects of blood and temperature shift on *Borrelia burgdorferi* gene expression as determined by whole genome DNA array. Infect Immun. 2004; 72:5419–5432. [PubMed: 15322040]
74. Revel AT, et al. *bptA* (*bbe16*) is essential for the persistence of the Lyme disease spirochete, *Borrelia burgdorferi*, in its natural tick vector. Proc Natl Acad Sci USA. 2005; 102:6972–6977. [PubMed: 15860579]
75. Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. Proc Natl Acad Sci USA. 1995; 92:2909–2913. The seminal demonstration of the induction of OspC during the nymphal blood meal and the importance of temperature as a stimulus for differential gene expression. [PubMed: 7708747]
76. Piesman J, Schneider BS, Zeidner NS. Use of quantitative PCR to measure density of *Borrelia burgdorferi* in the midgut and salivary glands of feeding tick vectors. J Clin Microbiol. 2001; 39:4145–4148. [PubMed: 11682544]
77. Coburn J, Cugini C. Targeted mutation of the outer membrane protein P66 disrupts attachment of the Lyme disease agent, *Borrelia burgdorferi*, to integrin αVβ3. Proc Natl Acad Sci USA. 2003; 100:7301–7306. The first report to use targeted mutagenesis to show that a borrelial outer-membrane-spanning protein has a role in cytoadherence. [PubMed: 12748384]
78. Pinne M, et al. Elimination of channel-forming activity by insertional inactivation of the *p66* gene in *Borrelia burgdorferi*. FEMS Microbiol Lett. 2007; 266:241–249. [PubMed: 17233736]
79. Norris, SJ.; Coburn, J.; Leong, JM.; Hu, LT.; Hook, M. Borrelia: Molecular Biology, Host Interaction, and Pathogenesis. Samuels, DS.; Radolf, JD., editors. Caister Academic; Norfolk, UK: 2010. p. 299-331.
80. Hellwage J, et al. The complement regulator factor H binds to the surface protein OspE of *Borrelia burgdorferi*. J Biol Chem. 2001; 276:8427–8435. [PubMed: 11113124]
81. Kenedy MR, Akins DR. The OspE-related proteins inhibit complement deposition and enhance serum resistance of *Borrelia burgdorferi*, the Lyme disease spirochete. Infect Immun. 2011; 79:1451–1457. [PubMed: 21282413]

82. Stevenson B, El-Hage N, Hines MA, Miller JC, Babb K. Differential binding of host complement inhibitor factor H by *Borrelia burgdorferi* Erp surface proteins: a possible mechanism underlying the expansive host range of Lyme disease spirochetes. Infect Immun. 2002; 70:491–497. [PubMed: 11796574]
83. Kraiczy P, et al. Complement resistance of *Borrelia burgdorferi* correlates with the expression of BbCRASP 1, a novel linear plasmid-encoded surface protein that interacts with human factor H and FHL 1 and is unrelated to Erp proteins. J Biol Chem. 2004; 279:2421–2429. [PubMed: 14607842]
84. de Silva AM, Fikrig E. Growth and migration of *Borrelia burgdorferi* in *Ixodes* ticks during blood feeding. Am J Trop Med Hyg. 1995; 53:397–404. A detailed analysis of the route of spirochaete dissemination and the kinetics of spirochaete replication during the nymphal blood meal; this paper also provides additional evidence for salivary transmission of spirochaetes. [PubMed: 7485694]
85. Ohnishi J, Piesman J, de Silva AM. Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. Proc Natl Acad Sci USA. 2001; 98:670–675. A meticulous characterization of the expression profiles of OspA and OspC during tick dissemination. [PubMed: 11209063]
86. Coleman JL, et al. Plasminogen is required for efficient dissemination of *B. burgdorferi* in ticks and for enhancement of spirochetemia in mice. Cell. 1997; 89:1111–1119. A classic demonstration of how *B. burgdorferi* appropriates mammalian serum proteins to facilitate penetration of tick tissue barriers. [PubMed: 9215633]
87. Zhang L, et al. Molecular interactions that enable movement of the Lyme disease agent from the tick gut into the hemolymph. PLoS Pathog. 2011; 7:e1002079. [PubMed: 21695244]
88. Coleman JL, et al. *Borrelia burgdorferi* binds plasminogen, resulting in enhanced penetration of endothelial monolayers. Infect Immun. 1995; 63:2478–2484. [PubMed: 7790059]
89. Guo X, et al. Inhibition of neutrophil function by two tick salivary proteins. Infect Immun. 2009; 77:2320–2329. [PubMed: 19332533]
90. Kotsyfakis M, et al. Antiinflammatory and immunosuppressive activity of sialostatin L, a salivary cystatin from the tick *Ixodes scapularis*. J Biol Chem. 2006; 281:26298–26307. [PubMed: 16772304]
91. Pal U, et al. OspC facilitates *Borrelia burgdorferi* invasion of *Ixodes scapularis* salivary glands. J Clin Invest. 2004; 113:220–230. [PubMed: 14722614]
92. Grimm D, et al. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc Natl Acad Sci USA. 2004; 101:3142–3147. A definitive demonstration that OspC is a borrelial virulence determinant following infection of mammals via needle or tick. [PubMed: 14970347]
93. Tilly K, et al. *Borrelia burgdorferi* OspC protein required exclusively in a crucial early stage of mammalian infection. Infect Immun. 2006; 74:3554–3564. [PubMed: 16714588]
94. Petzke MM, Brooks A, Krupna MA, Mordue D, Schwartz I. Recognition of *Borrelia burgdorferi*, the Lyme disease spirochete, by TLR7 and TLR9 induces a type I IFN response by human immune cells. J Immunol. 2009; 183:5279–5292. The first report to show that borreliae activate host immune responses via endosomal TLRs. [PubMed: 19794067]
95. Cervantes JL, et al. Phagosomal signaling by *Borrelia burgdorferi* in human monocytes involves Toll-like receptor (TLR) 2 and TLR8 cooperativity and TLR8-mediated induction of IFN β. Proc Natl Acad Sci USA. 2011; 108:3683–3688. This paper confirms the degradation of *B. burgdorferi* within phagosomes as the principle mechanism for TLR signalling and demonstrates TLR2–TLR8 cooperativity. [PubMed: 21321205]
96. Miller JC, et al. A critical role for type I IFN in arthritis development following *Borrelia burgdorferi* infection of mice. J Immunol. 2008; 181:8492–8503. A landmark paper calling attention to the involvement of type I IFNs in Lyme disease. [PubMed: 19050267]
97. Shin OS, et al. Distinct roles for MyD88 and Toll-like receptors 2, 5, and 9 in phagocytosis of *Borrelia burgdorferi* and cytokine induction. Infect Immun. 2008; 76:2341–2351. [PubMed: 18378636]

98. Marre ML, Petnicki-Ocwieja T, DeFrancesco AS, Darcy CT, Hu LT. Human integrin α3β1 regulates TLR2 recognition of lipopeptides from endosomal compartments. PLoS ONE. 2010; 5:e12871. [PubMed: 20877569]
99. Salazar JC, et al. Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. J Immunol. 2003; 171:2660–2670. [PubMed: 12928420]
100. Duray PH. Histopathology of clinical phases of human Lyme disease. Rheum Dis Clin North Am. 1989; 15:691–710. [PubMed: 2685926]
101. Jones KL, et al. Higher mRNA levels of chemokines and cytokines associated with macrophage activation in erythema migrans skin lesions in patients from the United States than in patients from Austria with Lyme borreliosis. Clin Infect Dis. 2008; 46:85–92. [PubMed: 18171218]
102. Shih CM, Pollack RJ, Telford SR III, Spielman A. Delayed dissemination of Lyme disease spirochetes from the site of deposition in the skin of mice. J Infect Dis. 1992; 166:827–831. [PubMed: 1527418]
103. Brissette CA, et al. Borrelia burgdorferi infection-associated surface proteins ErpP, ErpA, and ErpC bind human plasminogen. Infect Immun. 2009; 77:300–306. [PubMed: 19001079]
104. Behera AK, et al. *Borrelia burgdorferi* BBB07 interaction with integrin α β1 stimulates production of pro-inflammatory mediators in primary human chondrocytes. Cell Microbiol. 2008; 10:320–331. [PubMed: 17822440]
105. Guo BP, Norris SJ, Rosenberg LC, Hook M. Adherence of *Borrelia burgdorferi* to the proteoglycan decorin. Infect Immun. 1995; 63:3467–3472. [PubMed: 7642279]
106. Parveen N, Caimano M, Radolf JD, Leong JM. Adaptation of the Lyme disease spirochaete to the mammalian host environment results in enhanced glycosaminoglycan and host cell binding. Mol Microbiol. 2003; 47:1433–1444. [PubMed: 12603746]
107. Brissette CA, Verma A, Bowman A, Cooley AE, Stevenson B. The *Borrelia burgdorferi* outer-surface protein ErpX binds mammalian laminin. Microbiology. 2009; 155:863–872. [PubMed: 19246757]
108. Verma A, Brissette CA, Bowman A, Stevenson B. *Borrelia burgdorferi* BmpA is a laminin-binding protein. Infect Immun. 2009; 77:4940–4946. [PubMed: 19703983]
109. Seshu J, et al. Inactivation of the fibronectin-binding adhesin gene *bbk32* significantly attenuates the infectivity potential of *Borrelia burgdorferi*. Mol Microbiol. 2006; 59:1591–1601. [PubMed: 16468997]
110. Brissette CA, Bykowski T, Cooley AE, Bowman A, Stevenson B. *Borrelia burgdorferi* RevA antigen binds host fibronectin. Infect Immun. 2009; 77:2802–2812. [PubMed: 19398540]
111. Olson CM Jr, et al. Local production of IFN γ by invariant NKT cells modulates acute Lyme carditis. J Immunol. 2009; 182:3728–3734. [PubMed: 19265151]
112. Brown CR, Blaho VA, Fritsche KL, Loiacono CM. Stat1 deficiency exacerbates carditis but not arthritis during experimental Lyme borreliosis. J Interferon Cytokine Res. 2006; 26:390–399. [PubMed: 16734559]
113. Kinjo Y, et al. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nature Immunol. 2006; 7:978–986. A remarkable biochemical and immunological study showing that borrelial glycolipids activate natural killer T cells. [PubMed: 16921381]
114. Lee WY, et al. An intravascular immune response to *Borrelia burgdorferi* involves Kupffer cells and iNKT cells. Nature Immunol. 2010; 11:295–302. [PubMed: 20228796]
115. McKisic MD, Redmond WL, Barthold SW. Cutting edge: T cell-mediated pathology in murine Lyme borreliosis. J Immunol. 2000; 164:6096–6099. [PubMed: 10843657]
116. Connolly SE, Benach JL. The versatile roles of antibodies in *Borrelia* infections. Nature Rev Microbiol. 2005; 3:411–420. [PubMed: 15864264]
117. Belperron AA, Dailey CM, Booth CJ, Bockenstedt LK. Marginal zone B cell depletion impairs murine host defense against *Borrelia burgdorferi* infection. Infect Immun. 2007; 75:3354–3360. [PubMed: 17470546]
118. Tunev SS, et al. Lymphoadenopathy during Lyme borreliosis is caused by spirochete migration-induced specific B cell activation. PLoS Pathog. 2011; 7:e1002066. [PubMed: 21637808]
119. Wooten RM, et al. Toll-like receptor 2 is required for innate, but not acquired, host defense to *Borrelia burgdorferi*. J Immunol. 2002; 168:348–355. [PubMed: 11751980]

120. Bolz DD, et al. MyD88 plays a unique role in host defense but not arthritis development in Lyme disease. J Immunol. 2004; 173:2003–2010. [PubMed: 15265935]

121. Liang FT, Jacobs MB, Bowers LC, Philipp MT. An immune evasion mechanism for spirochetal persistence in Lyme borreliosis. J Exp Med. 2002; 195:415–422. An elegant study demonstrating that OspC-specific antibodies select for spirochaetes that have downregulated expression of the lipoprotein during early mouse infection. [PubMed: 11854355]

122. Zhang JR, Hardham JM, Barbour AG, Norris SJ. Antigenic variation in Lyme disease *Borreliae* by promiscuous recombination of VMP-like sequence cassettes. Cell. 1997; 89:275–285. The first report to describe the *vls* system for antigenic variation by *B. burgdorferi*. [PubMed: 9108482]

123. Norris SJ. Antigenic variation with a twist–the *Borrelia* story. Mol Microbiol. 2006; 60:1319–1322. [PubMed: 16796669]

124. Petnicki-Ocwieja T, et al. NOD2 suppresses *Borrelia burgdorferi* mediated murine Lyme arthritis and carditis through the induction of tolerance. PLoS ONE. 2011; 6:e17414. [PubMed: 21387014]

125. Oliver JH Jr, et al. An enzootic transmission cycle of Lyme borreliosis spirochetes in the southeastern United States. Proc Natl Acad Sci USA. 2003; 100:11642–11645. [PubMed: 14500917]

126. Krupka I, Straubinger RK. Lyme borreliosis in dogs and cats: background, diagnosis, treatment and prevention of infections with *Borrelia burgdorferi* sensu stricto. Vet Clin North Am Small Anim Pract. 2010; 40:1103–1119. [PubMed: 20933139]

127. Radolf JD, Bourell KW, Akins DR, Brusca JS, Norgard MV. Analysis of *Borrelia burgdorferi* membrane architecture by freeze-fracture electron microscopy. J Bacteriol. 1994; 176:21–31. [PubMed: 8282698]

128. Rogers EA, et al. Rrp1, a cyclic di GMP-producing response regulator, is an important regulator of *Borrelia burgdorferi* core cellular functions. Mol Microbiol. 2009; 71:1551–1573. [PubMed: 19210621]

129. Pal U, et al. A differential role for BB0365 in the persistence of *Borrelia burgdorferi* in mice and ticks. J Infect Dis. 2008; 197:148–155. [PubMed: 18171298]

130. Pal U, et al. *Borrelia burgdorferi* basic membrane proteins A and B participate in the genesis of Lyme arthritis. J Exp Med. 2008; 205:133–141. [PubMed: 18166585]

131. Boylan JA, et al. *Borrelia burgdorferi bb0728* encodes a coenzyme A disulphide reductase whose function suggests a role in intracellular redox and the oxidative stress response. Mol Microbiol. 2006; 59:475–486. [PubMed: 16390443]

132. Eggers CH, et al. The coenzyme A disulfide reductase of *Borrelia burgdorferi* is important for rapid growth throughout the enzootic cycle and essential for infection of the mammalian host. Mol Microbiol. 2011; 82:679–697. [PubMed: 21923763]

133. Freedman JC, et al. Identification and molecular characterization of a cyclic di GMP effector protein, PlzA (BB0733): additional evidence for the existence of a functional cyclic di GMP regulatory network in the Lyme disease spirochete, *Borrelia burgdorferi*. FEMS Immunol Med Microbiol. 2010; 58:285–294. [PubMed: 20030712]

134. Pitzer JE, et al. Analysis of the *Borrelia burgdorferi* cyclic di GMP-binding protein PlzA reveals a role in motility and virulence. Infect Immun. 2011; 79:1815–1825. [PubMed: 21357718]

135. Salman-Dilgimen A, Hardy PO, Dresser AR, Chaconas G. HrpA, a DEAH-box RNA helicase, is involved in global gene regulation in the Lyme disease spirochete. PLoS ONE. 2011; 6:e22168. [PubMed: 21814569]

136. Chaconas G, Stewart PE, Tilly K, Bono JL, Rosa P. Telomere resolution in the Lyme disease spirochete. EMBO J. 2001; 20:3229–3237. An extraordinary demonstration that cp26-encoded ResT is required for telomere resolution during replication of the borrelial linear chromosome and plasmids. [PubMed: 11406599]

137. Jewett MW, et al. GuaA and GuaB are essential for *Borrelia burgdorferi* survival in the tick-mouse infection cycle. J Bacteriol. 2009; 191:6231–6241. [PubMed: 19666713]

138. Metts MS, McDowell JV, Theisen M, Hansen PR, Marconi RT. Analysis of the OspE determinants involved in binding of factor H and OspE-targeting antibodies elicited during

Borrelia burgdorferi infection in mice. Infect Immun. 2003; 71:3587–3596. [PubMed: 12761144]

139. Kraiczy P, et al. Immune evasion of *Borrelia burgdorferi*: mapping of a complement-inhibitor factor H binding site of BbCRASP 3, a novel member of the Erp protein family. Eur J Immunol. 2003; 33:697–707. [PubMed: 12616490]
140. Kawabata H, Norris SJ, Watanabe H. BBE02 disruption mutants of *Borrelia burgdorferi* B31 have a highly transformable, infectious phenotype. Infect Immun. 2004; 72:7147–7154. [PubMed: 15557639]
141. Purser JE, et al. A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of *Borrelia burgdorferi* in a mammalian host. Mol Microbiol. 2003; 48:753–764. [PubMed: 12694619]
142. Jewett MW, et al. The critical role of the linear plasmid lp36 in the infectious cycle of *Borrelia burgdorferi*. Mol Microbiol. 2007; 64:1358–1374. [PubMed: 17542926]
143. Fischer JR, LeBlanc KT, Leong JM. Fibronectin binding protein BBK32 of the Lyme disease spirochete promotes bacterial attachment to glycosaminoglycans. Infect Immun. 2006; 74:435–441. [PubMed: 16368999]
144. Blevins JS, Hagman KE, Norgard MV. Assessment of decorin-binding protein A to the infectivity of *Borrelia burgdorferi* in the murine models of needle and tick infection. BMC Microbiol. 2008; 8:82. [PubMed: 18507835]
145. Brown EL, et al. Resistance to Lyme disease in decorin-deficient mice. J Clin Invest. 2001; 107:845–852. [PubMed: 11285303]
146. Shi Y, Xu Q, McShan K, Liang FT. Both decorin-binding proteins A and B are critical for the overall virulence of *Borrelia burgdorferi*. Infect Immun. 2008; 76:1239–1246. [PubMed: 18195034]
147. Kumar M, Yang X, Coleman AS, Pal U. BBA52 facilitates *Borrelia burgdorferi* transmission from feeding ticks to murine hosts. J Infect Dis. 2010; 201:1084–1095. [PubMed: 20170377]
148. Promnares K, et al. *Borrelia burgdorferi* small lipoprotein Lp6.6 is a member of multiple protein complexes in the outer membrane and facilitates pathogen transmission from ticks to mice. Mol Microbiol. 2009; 74:112–125. [PubMed: 19703109]
149. Gilmore RD Jr, et al. The *bba64* gene of *Borrelia burgdorferi*, the Lyme disease agent, is critical for mammalian infection via tick bite transmission. Proc Natl Acad Sci USA. 2010; 107:7515–7520. [PubMed: 20368453]
150. Bykowski T, et al. Coordinated expression of *Borrelia burgdorferi* complement regulator-acquiring surface proteins during the Lyme disease spirochete's mammal-tick infection cycle. Infect Immun. 2007; 75:4227–4236. [PubMed: 17562769]
151. McDowell JV, et al. Evidence that the BBA68 protein (BbCRASP 1) of the Lyme disease spirochetes does not contribute to factor H mediated immune evasion in humans and other animals. Infect Immun. 2006; 74:3030–3034. [PubMed: 16622245]

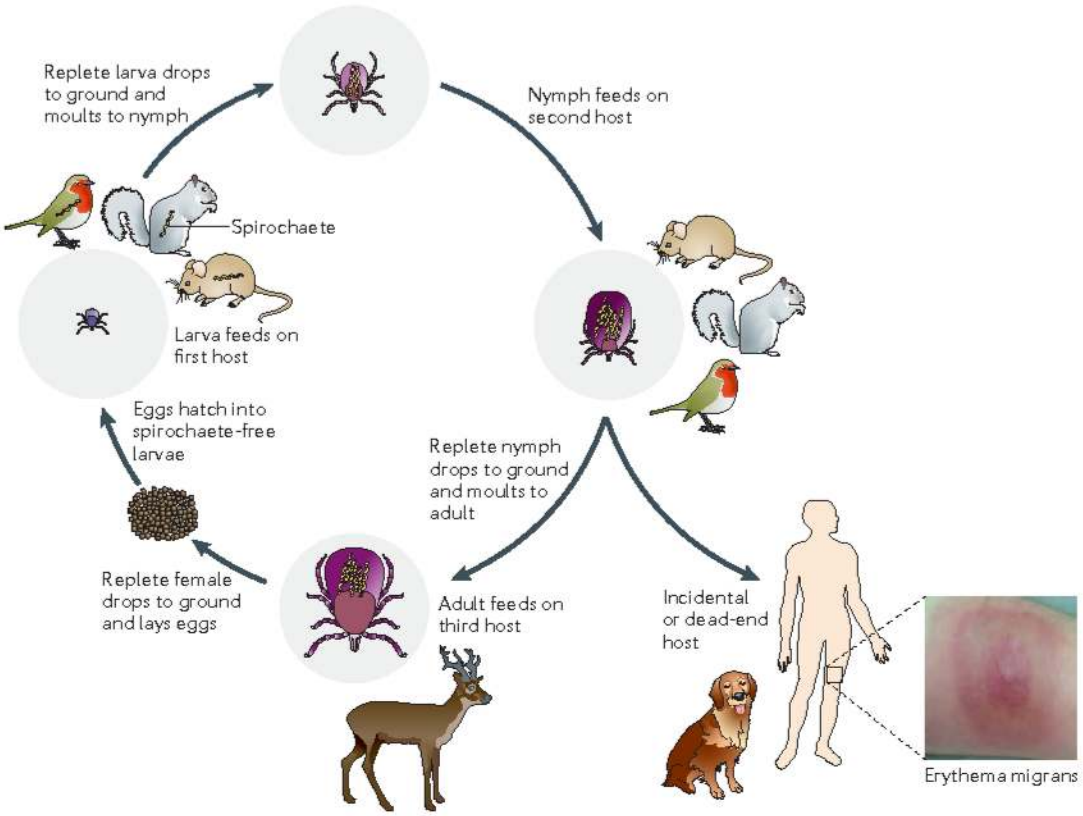

Figure 1.

The enzootic cycle of *Borrelia burgdorferi. Ixodes* spp. ticks undergo a three-stage life cycle — larva, nymph and adult — with one blood meal per stage. Although some *Borrelia* spp. that cause relapsing fever can be passed from adult to egg (transovarial transmission), this does not occur with *B. burgdorferi*, so each generation of tick must acquire a *B. burgdorferi* infection anew. Larval ticks feed on many different animals, including *Peromyscus* spp. mice, squirrels and birds. *B. burgdorferi* infection is acquired by feeding on an infected reservoir animal, and the bacterium is retained during the subsequent stages (that is, trans-stadially) after each blood meal and moult[7,10]. Nymphs feed on a similar range of hosts to larvae; transmission of spirochaetes to a competent reservoir host by a feeding nymph perpetuates the enzootic cycle for the next generation of larval ticks. Although small mammals are usually thought of as the primary reservoirs for Lyme disease spirochaetes, studies have called attention to the importance of migratory birds as disseminators of spirochaetes over large distances[7,10]. Adult ticks are not generally important for maintenance of *B. burgdorferi* in the wild, as they feed predominantly on larger animals such as deer, which are incompetent hosts for *B. burgdorferi*[7]. However, deer are important for maintenance of the tick population because adult ticks mate on them. Although all three stages of *Ixodes scapularis* can feed on humans, nymphs are responsible for the vast majority of spirochaete transmission to humans. It is unknown whether infected humans can transmit spirochaetes to feeding larvae, and humans are generally considered dead-end hosts and not part of the enzootic cycle. Dogs are probably incidental hosts and not part of the **enzootic cycle.**

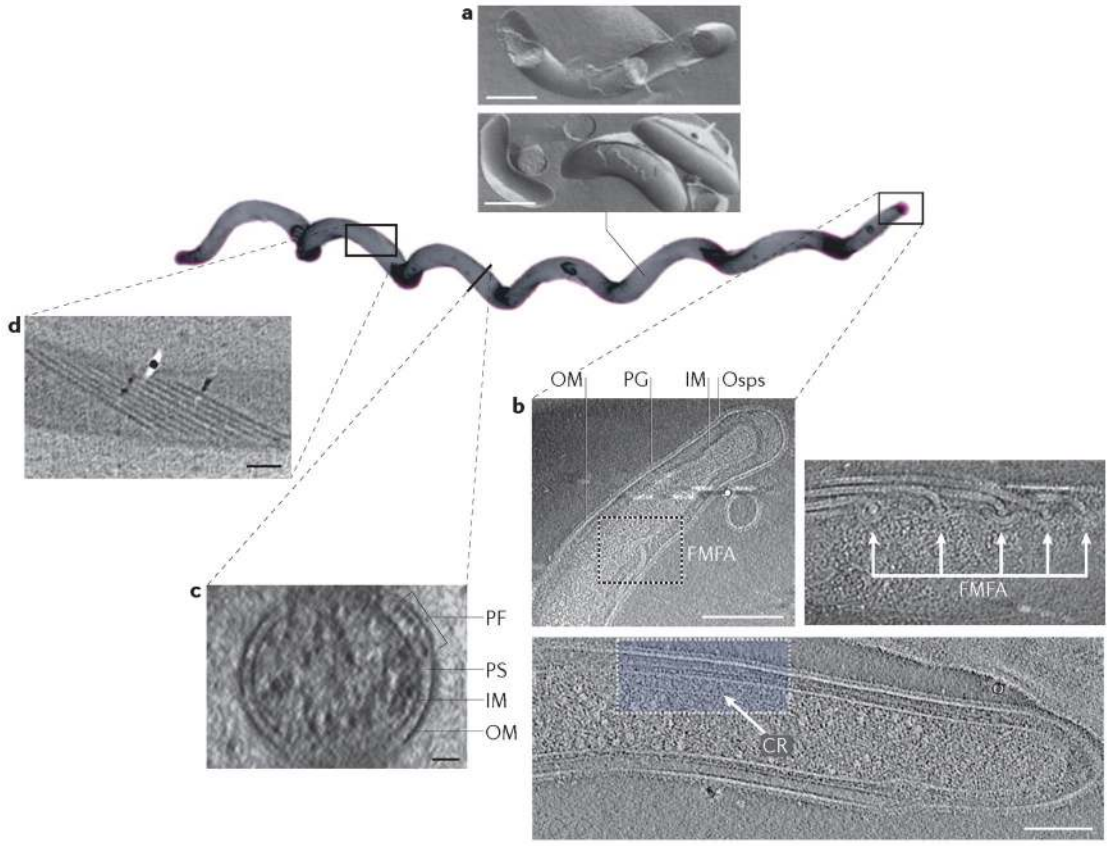

Figure 2. Cellular architecture of *Borrelia burgdorferi*

a | Freeze-fracture electron micrographs showing the convex and concave leaflets of the inner and outer membranes (IM and OM, respectively). Integral membrane proteins (particles) are considerably more abundant in the IM; the density of OM particles is much lower than that in a prototypical Gram-negative bacterium. Scale bars represent 500 nm. **b** | Cryoelectron tomograms of the ends of borrelial cells showing IM, OM, peptidoglycan (PG), flagellar motor and filament assemblies (FMFA), chemoreceptor arrays (CR) and an external layer comprising outer-surface lipoproteins (Osps). Upper scale bar represents 1 μm, lower scale bar represents 100 nm. **c** | Cryoelectron tomographic cross-section showing the IM, OM, periplasmic space (PS) and periplasmic flagella (PFs). Scale bar represents 50 nm. **d** | Longitudinal cryoelectron tomographic slice showing a ribbon of nine flagellar filaments wrapping around the IM in a right-handed helix. Scale bar represents 200 nm. Part **a** is reproduced, with permission, from REF. 127 c (1994) American Society for Microbiology (ASM). Part **b** is reproduced, with permission, from REF. 34 c (2009) ASM, and from REF. 36 c (2011) ASM. Parts **c**,**d** are reproduced, with permission, from REF. 35 c (2009) ASM.

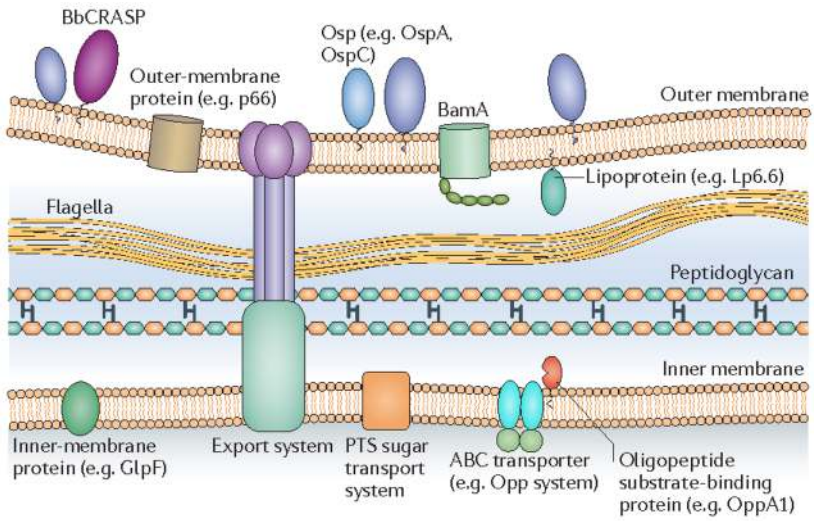

Figure 3. The borrelial cell envelope

This schematic of the borrelial cell envelope shows the outer membrane, flagellar filaments, peptidoglycan, and cytoplasmic inner membrane. The outer membrane contains outer-surface lipoproteins (Osps) in high density and β-barrel outer-membrane-spanning proteins such as BamA in low density. The inner membrane is rich in integral membrane proteins, many of which are transporters. BbCRASP, complement regulator-acquiring protein; OppA1, oligopeptide permease A1; PTS, phosphotransferase system.

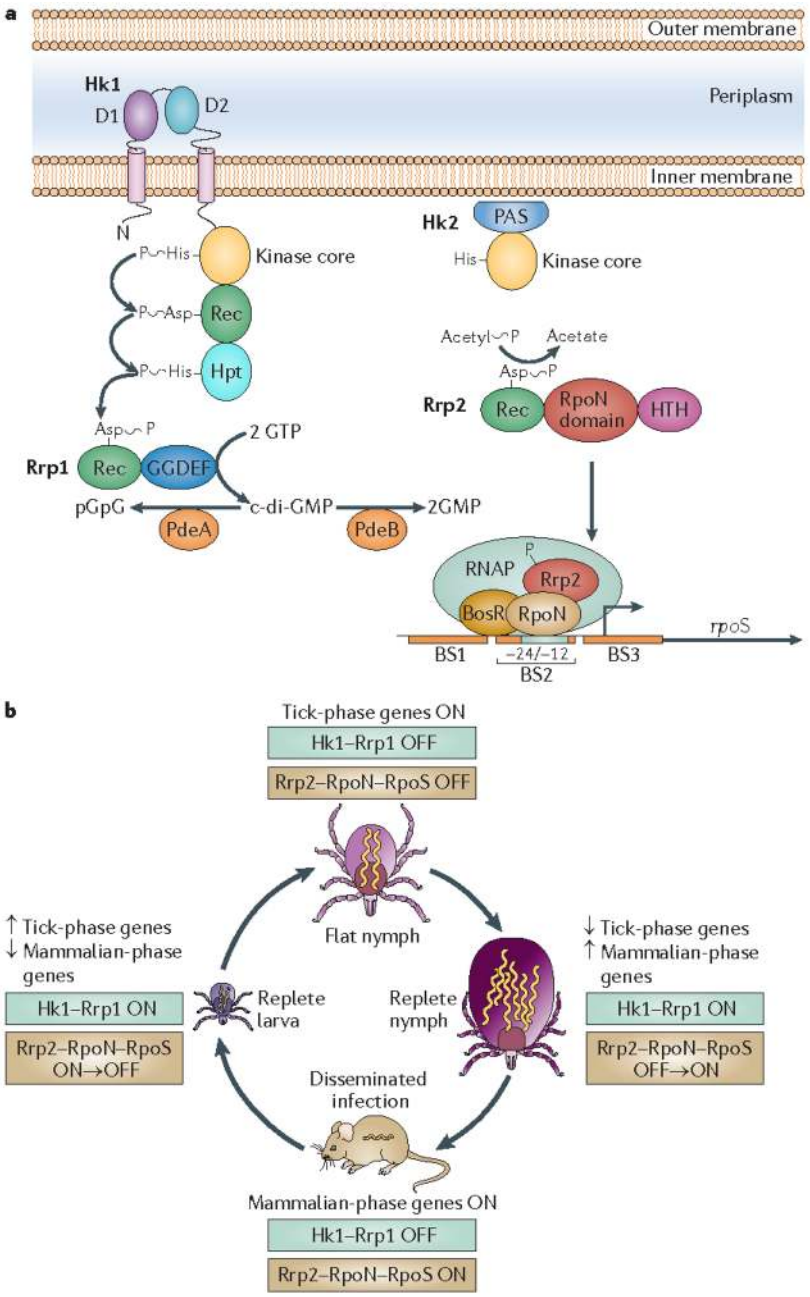

Figure 4. Regulation of gene expression in *Borrelia burgdorferi*

a | The histidine kinase 1 (Hk1)–response regulatory protein 1 (Rrp1) and alternative RNA polymerase σ-factor RpoS global regulatory systems. Binding of unidentified ligands to the periplasmic sensor domains (D1 and D2) of the hybrid histidine kinase Hk1 initiates a phosphorelay that activates the diguanylyl cyclase activity of Rrp1, resulting in the production of cyclic di-GMP (c-di-GMP)[55–57,128]. Phosphodiesterase A (PdeA) and PdeB degrade c-di-GMP to 5′-phosphoguanylyl-(3′– 5′)-guanosine (pGpG) and GMP, respectively[58,59]. Activation of Rrp2 *in vitro* and *in vivo* occurs via the high-energy phosphoryl donor acetyl-phosphate rather than by its presumptive cognate histidine kinase, Hk2 (REF. 48). The function of Hk2 is currently unknown. Phosphorylated Rrp2, *Borrelia* oxidative stress regulator (BosR) and RpoN initiate transcription of *rpoS*[37,38,42–47]. This is

depicted as a trimeric complex, but the precise interactions between these proteins have yet to be determined. Putative BosR-binding sites (BSs) containing the direct repeat sequence TAAATTAAAT are shown[47]; −24/−12 is the RpoN-binding site in the *rpoS* promoter[47]. RpoS in turn induces the expression of genes that are required during the mammalian-host phase of the spirochaete life cycle and represses the expression of tick-phase genes.**b |** Expression of the Hk1–Rrp1 and RpoS global regulatory systems during the *B. burgdorferi* life cycle[37,38,55–57,67,79]. In the flat nymph, both the Hk1–Rrp1 and the Rrp2–RpoN–RpoS systems are inactive and only tick-phase genes are expressed. The nymphal blood meal activates both the Hk1 Rrp1 and Rrp2–RpoN–RpoS pathways. Expression of mammalian-phase genes begins in concert with downregulation of tick-phase genes. Following inoculation into a mammalian host, the spirochaetes complete the process of adaptation; the Hk1–Rrp1 pathway is inactive, the Rrp2–RpoN–RpoS pathway is active, mammalian-phase genes are expressed and tick-phase genes are repressed. During larval acquisition of spirochaetes, Hk1–Rrp1 is activated, probably at the feeding site, whereas the Rrp2–RpoN–RpoS system is inactivated. Mammalian-phase genes are repressed, expression of tick-phase genes begins and ingested spirochaetes bind to the larval midgut epithelium via OspA and possibly other receptors[64–66]. GGDEF, a conserved motif present in diguanylyl cyclases[128]; Hpt, histidine-containing phosphotransfer domain[55]; HTH, helix–turn–helix doain; N, amino; PAS, putative sensor domain for Hk2; Rec, receiver domain.

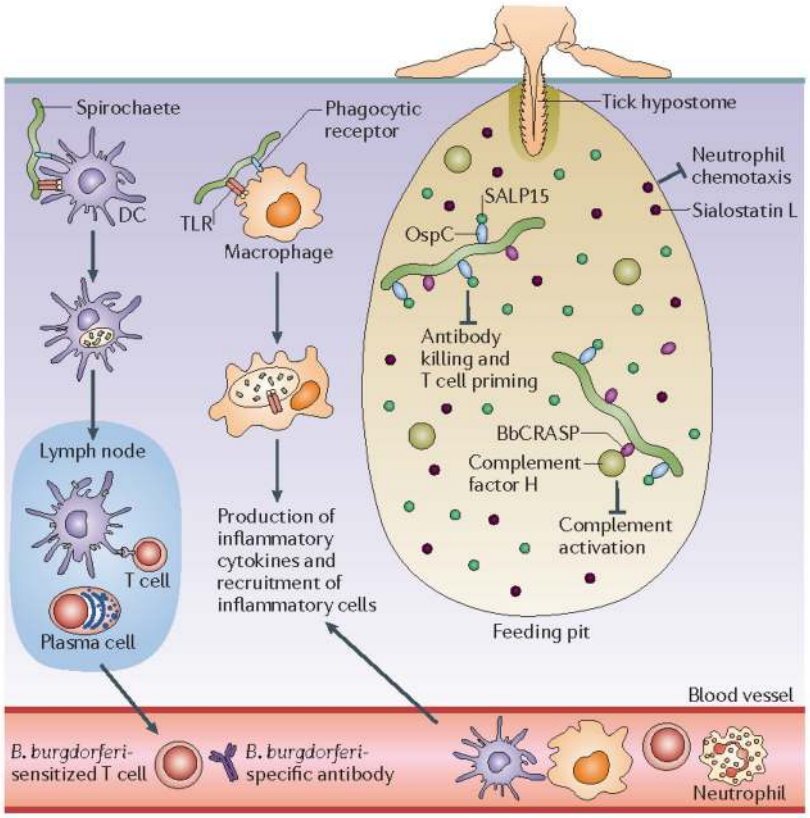

Figure 5. The tick–mammal interface

The tick creates a feeding pit with its mouthparts, using its hypostome (a barbed protuberance) as an anchor to the skin of its host. Initial salivary secretions form a cement cone around the hypostome that further anchors the tick during feeding. Subsequently, the tick produces copious amounts of saliva containing a plethora of bioactive agents that *Borrelia burgdorferi* exploits to help establish infection. The diagram shows just a few of these bioactive agents, including SALP15 (which binds to the spirochaetes and inhibits killing of the bacteria and T cell priming) and sialostatin L (which blocks neutrophil chemotaxis)[67,90]. A group of borrelial surface lipoproteins, collectively referred to as BbCRASPs (complement regulator-acquiring surface proteins), bind complement factor H, preventing activation of the alternative complement pathway [80–83]. *B. burgdorferi* cells are recognized by innate immune effector cells such as dendritic cells (DCs), neutrophils and macrophages, initially via surface-exposed pattern recognition receptors; activation of these cells increases following internalization and degradation of spirochetes within phagolysosomes. DCs that have taken up spirochetes migrate to the lymph nodes, where they present processed borrelial antigens to T cells and B cells. Sensitized T cells enter the circulation and are recruited to the site of infection. Plasma cells secrete specific antibodies that can kill *B. burgdorferi* via complement-dependent and –independent pathways. Production of pro-inflammatory cytokines by activated macrophages results in the recruitment of additional neutrophils, T cells, macrophages and DCs to the bite site, and eventually the development of erythema migrans[15]. OspC, outer surface lipoprotein C; TLR, Toll-like receptor. .

Table 1

Key genes thought to contribute to maintenance of the *Borrelia burgdorferi* enzootic cycle

Gene*	Function of encoded protein	Ref.
Chromosome		
glp operon (BB_0240–BB_0243)	Glycerol utilization within ticks	56,68
bb0365	Persistence in ticks	129
bmpA and *bmpB* (BB_0383 and BB_0382)	Laminin binding Establishment of joint infection	130
bb0690	Dps-like bacterioferritin orthologue Persistence in ticks	27
cdr (BB_0728)	Coenzyme A disulfide reductase Support of replication and protection against oxidative stress	131,132
hk1 and *rrp1* (BB_0420 and BB_0419)	Histidine kinase 1 and response regulatory protein 1 Two-component system required for survival within feeding ticks	55–57
plzA (BB_0733)	PilZ domain-containing cylic di-GMP effector protein Required for infection of ticks and mice	133,134
pdeA (BB_0363)	Phosphodiesterase A (EAL-type phosphodiesterase) Required to establish infection in mice	59
pdeB (BB_0374)	Phosphodiesterase B (HD-GYP-type phosphodiesterase) Required for survival in ticks and for tick-to-mammal transmission	58
p66 (BB_0603)	Integrin binding adhesin Porin	77,78
bosR (BB_0647)	*Borrelia* oxidative stress regulator Fur–PerR orthologue required for transcription of *rpoS*	46,47
csrA (BB_0184)	RNA-binding protein involved in post-transcriptional regulation of *rpoS*	51,52
rrp2 (BB_0763)	Response regulator protein 2 Required by RpoN for transcription of *rpoS*	42–45
rpoN (BB_0450)	Alternative σ-factor that transcribes *rpoS*	39,40
rpoS (BB_0771)	Alternative σ-factor that transcribes mammalian-phase genes and represses tick-phase genes	39–41
hrpA (BB_0827)	DEAH-box RNA helicase involved in global gene regulation	135
Plasmid cp26		
resT (BB_B03)	Telomere resolvase	136
ospC (BB_B19)	Dissemination within mice	91–93
guaA and *guaB* (BB_B18 and BB_B17)	GMP synthase and IMPdehydrogenase Involved in the purine salvage pathway	137
Plasmid cp32s		
ospE alleles such as *erpP* (BB_N38), *erpC* (GenBank acession AAC34910.1) and *erpA* (BB_L39 and BB_P38)	BbCRASP3, BbCRASP4 and BbCRASP5, respectively Complement inhibition Laminin binding Plasminogen binding	80–82, 103, 107,138, 139
Plasmid lp25		
bbe02	Restriction–modification system	140
bptA (BB_E16)	Persistence in ticks	74
pncA (BB_E22)	Pyrazinaminidase–nicotinamidase	141
Plasmid lp28-1		

Gene*	Function of encoded protein	Ref.
vls loci	Recombinatorial system for immune evasion	122,123
Plasmid lp36		
ade (BB_K17)	Adenine deaminase	142
bbk32	Fibronectin-binding protein	109,143
Plasmid lp54		
ospA and *ospB* (BB_A15 and BB_A16)	Colonization of tick midgut epithelium	64–66
dbpA and *dbpB* (BB_A24 and BB_A25)	Decorin-binding proteins	144–146
bbA52	Tick-to-mammal transmission	147
lp6.6 (BB_A62)	Lipoprotein 6.6 Persistence in ticks	148
bbA64	Antigen p35 Tick-to-mammal transmission	149
cpsA (BB_A68)	BbCRASP1 Outer-surface protein Complement inhibition	83,150, 151

IMP, inosine monophosphate.

*Locus identifiers are provided in brackets.