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***Borrelia burgdorferi* and tick proteins supporting pathogen persistence in the vector**

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Abstract

Borrelia burgdorferi, a pathogen transmitted by *Ixodes* ticks, is responsible for a prevalent illness known as Lyme disease, and a vaccine for human use is unavailable. Recently, genome sequences of several *B. burgdorferi* strains and *Ixodes scapularis* ticks have been determined. In addition, remarkable progress has been made in developing molecular genetic tools to study the pathogen and vector, including their intricate relationship. These developments are helping unravel the mechanisms by which Lyme disease pathogens survive in a complex enzootic infection cycle. Notable discoveries have already contributed to understanding the spirochete gene regulation accounting for the temporal and spatial expression of *B. burgdorferi* genes during distinct phases of the lifecycle. A number of pathogen and vector gene products have also been identified that contribute to microbial virulence and/or persistence. These research directions will enrich our knowledge of vector-borne infections and contribute towards the development of preventative strategies against Lyme disease.

Keywords

Borrelia burgdorferi; *Ixodes*; ticks; Lyme disease; vector–pathogen interaction

Lyme disease, caused by the bacterial pathogen *Borrelia burgdorferi*, is currently regarded as the most prevalent vector-borne infection in the USA and parts of Europe and Asia [1–4]. The microbe survives in a complex enzootic cycle involving tick vectors belonging to the *Ixodes ricinus* species complex and a variety of mammalian hosts, usually small rodents [5–8]. In North America the primary tick vector is *Ixodes scapularis*. During the blood meal engorgement, *B. burgdorferi* migrate from the vertebrate host dermis into the tick gut, where they continue to persist through the intermolt period. As infected ticks feed on a subsequent host, spirochetes exit the gut, invade the salivary glands and, along with tick saliva, transmit to the skin. From the dermis, *B. burgdorferi* disseminate to several internal organs, including the joints, heart and nervous system [9–12]. According to the US CDC, arthritis is a frequent

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clinical condition of Lyme disease that is reported in at least 30% of untreated and confirmed human cases. However, borreliac infection is also responsible for other complications, such as a pathognomonic skin rash, carditis and various neurological symptoms [13–18]. *Borrelia* species commonly associated with Lyme disease are very diverse – both genetically and antigenically – and include a number of genospecies, with the most well known being *B. burgdorferi sensu stricto* (prevalent throughout the USA and Europe), and *Borrelia afzelii* and *Borrelia garinii* (primarily distributed in Europe and parts of Asia) [2]. Despite significant strain diversity, Lyme disease pathogens are only transmitted by a single group of ticks – the *Ixodes ricinus* complex – thereby suggesting the evolution of a highly specific relationship between the vector and the pathogen [19,20]. Due to their unique evolutionary divergence from related arthropod and bacterial species, respectively [21], *B. burgdorferi* and *I. scapularis* cannot be understood by applying many traditional concepts of microbiology or host–pathogen interaction and, therefore, warrant in-depth experimental studies. Availability of targeted gene inactivation and gene silencing tools for the pathogen [22] and vector [20,23], as well as the completion of the *I. scapularis* genome sequencing project [24–26], have provided researchers with increasing opportunities to study *Borrelia*–tick interactions in the vector and at the interface with the mammalian host.

Revealing novel features of the vector–pathogen interaction will have a direct impact on translational studies for the effective management of *B. burgdorferi* infection. Despite the fact that the incidence of Lyme disease has been increasing [4] and that inherent difficulties exist in achieving timely diagnosis and administering appropriate treatment, a vaccine to prevent the infection in humans is still unavailable [27]. Therefore, some of the current efforts are directed towards a better understanding of the distinct phases of the borreliac lifecycle, especially within the vector, as well as the identification of pathogen or vector proteins as potential vaccine candidates [20]. The subject of *B. burgdorferi* interactions with its host or vector has been discussed in past publications [6,8,9,19,20,28–34], therefore, the purpose of the current review is to summarize the most recent developments in the field, including studies that are pertinent to highlighting the basic concepts of vector–pathogen interaction and emphasizing the identification of borreliac and tick proteins that are important for pathogen survival in the vector.

***B. burgdorferi*: a tick-borne pathogen**

B. burgdorferi is unique among bacteria even compared with related pathogenic spirochetes *Leptospira* and *Treponema* [35–37]. Although described as Gram-negative because of its double membrane, *B. burgdorferi* has different cellular organization and membrane composition from other diderms [21]. In particular, its outer membrane contains a low density of membrane-spanning proteins and lacks classical lipopolysaccharides, which help stabilize and protect bacterial membranes. Rather, the *B. burgdorferi* cell membrane displays a chemically distinct form of peptidoglycan [38], low molecular weight glycolipids and numerous triacylated lipoproteins, some of which interact with host components [39]. Another unique feature of *B. burgdorferi* is its highly segmented and unstable genome that is difficult to genetically manipulate [40,41]. In addition to a linear chromosome that is fairly conserved and carries the majority of the housekeeping genes [41], up to 21 linear and circular plasmids [40,41] encode numerous proteins without homology to known proteins, which potentially perform specific functions and respond to different environments during the spirochete's infectious cycle [19]. Remarkably, despite its relatively small size (1.5 mb), the genome exhibits notable structural and functional redundancy, reflected by the presence of a significant number of paralogous gene clusters [40,41], as well as experimental evidence that many borreliac genes are nonessential for infectivity [22]. Nearly 5% of the chromosomal and 15% of the plasmid DNA produces a diverse set of 130 potential

membrane lipoproteins [40,41], many of which are surface exposed and potentially involved in host–pathogen interactions. Transcriptional or post-transcriptional regulation and recombination at specific loci further affect the differential expression of many proteins, including outer membrane antigens, that contribute to the successful persistence of *B. burgdorferi* in a complex enzootic cycle [20,42–45].

The tick–mammal infection cycle of *B. burgdorferi*

B. burgdorferi thrives in nature in an intricate tick–mammal infection cycle. Given the lack of transovarial transmission, the pathogen must be acquired at one of the vector life stages when ticks engorge on infected mammals, primarily wild rodents [8,46]. Spirochetes disseminate along with the blood meal from the infected host to the tick, and colonize the gut. They remain in the gut until the next blood meal, when a fraction of the spirochetes exit the gut, invade the salivary glands and transmit to a new host [47]. Therefore, maintenance of *B. burgdorferi* in the enzootic cycle requires successful persistence in multiple developmental stages of the arthropod, as well as coordinated dissemination through tick tissues to the new host. Major phases of the *B. burgdorferi* lifecycle in the vector are briefly highlighted in the following paragraphs.

Acquisition by feeding ticks

Although a detailed timeline of the molecular events remains unknown, larval or nymphal ticks acquire *B. burgdorferi* through blood during the first 36 h of attachment and feeding on infected hosts [20], such as *Peromyscus leucopus* (the white-footed mouse), a common but not the primary reservoir host in the USA [48–53]. Spirochetes migrate from the murine dermis, enter the tick and can be detected by quantitative reverse-transcription PCR within 1 day of tick attachment to the mouse [54]. *Ixodes* ticks form a transient peritrophic membrane surrounding the blood meal [20]; however, how this acellular barrier influences spirochete acquisition and colonization of tick gut epithelium remains enigmatic. It is also currently unknown how a certain fraction of *B. burgdorferi* avoids being digested with the blood meal in the gut and successfully bypasses potential tick innate immune defense mechanisms. In other vectors, such as mosquitoes, major losses in *Plasmodium* numbers occur during their invasion of the *Anopheles* gut, sometimes resulting in complete elimination of ingested parasites, most probably due to robust innate immune responses mounted by the vector [55,56].

Persistence in the unfed vector

B. burgdorferi continues to persist in the gut lumen through the next developmental phase of the tick [57]. This is one of the most remarkable adaptive features of Lyme disease spirochetes, as survival within unfed or intermolt ticks probably poses significant challenges owing to several factors, including temperature extremes caused by daily or seasonal changes, as well as nutritional stress. The latter results from the fact that the lumen of an unfed gut is poor in nutrients, primarily because gut epithelia store macromolecules within cellular endosomes, that would be difficult for extracellular pathogen to access [58]. Notably, mass spectrometry-based proteomics of ticks have demonstrated the persistence of certain mammalian blood components within the tick gut [59].

Transmission from ticks to hosts

For transmission to mammals, spirochetes must first sense events linked to tick–host association and blood meal engorgement. *B. burgdorferi* rapidly multiplies in the lumen of the gut while the tick is taking a blood meal, generating a phenotypically diverse population of spirochetes [60], a fraction of which are known to cross the gut epithelial barrier and disseminate to the hemocoel for transmission to the host via the salivary glands [61,62].

Although the precise mechanism by which *B. burgdorferi* crosses the tissue barrier is unknown and could involve active migration through gut epithelial tissue, the movement of nonmotile spirochetes from apical to basal epithelium via adherence to gut epithelial cells has been proposed [63]. The identities of tick and borrelial proteins that are likely to assist in dissemination of spirochetes from the gut to the salivary glands and eventually to the host have also been suggested [64–68]. Once the *B. burgdorferi* migrate from the gut to the hemocoelic space, they then rapidly traverse the hemolymph [63], invade the salivary glands and eventually transfer to the host dermis. More recent studies suggested that RpoS-dependent expression of selected genes plays an important role in the transmission of spirochetes by feeding nymphs [69].

***B. burgdorferi* proteins supporting pathogen persistence**

In the following sections is a summary of studies that have examined the roles of specific *B. burgdorferi* plasmids or individual proteins in spirochete infection of the tick vector. All plasmid names are given in reference to B31. Other strains carry varied genomes of differing plasmid contents.

Plasmids—Studies involving *B. burgdorferi* isolates missing select plasmids reinforce the role of plasmid-encoded gene products in the spirochete lifecycle. For example, the linear plasmid lp25 was shown to be essential for both mouse and tick infection [70–75]. In addition, *B. burgdorferi* missing lp28-4 displays a reduced ability to persist in ticks and transmit to mice [74]. Other plasmids, such as lp36, have been identified as playing nonessential roles in spirochete survival in ticks, but being important for mammalian infectivity [76].

Gene products integral to regulatory pathways—The details of borrelial gene regulation have recently been discussed [9,43]. Chromosomally encoded alternate σ factors, RpoN and RpoS, along with a borrelial two-component system, Hk2–Rrp2, globally activate and repress many genes required for different phases of the spirochete infection cycle. For example, genes within the RpoN/RpoS regulon have been shown to promote tick transmission and early mammalian infection, with the pathway being active only in feeding nymphs [77–82]. RpoN (σ 54)-dependent transcription of RpoS requires the formation of an open promoter complex mediated by an activator of the RpoN/RpoS cascade [77,81,83], the response regulator Rrp2 that is phosphorylated by a metabolic intermediate, acetyl phosphate [84]. Attempts to disrupt Rrp2 have been unsuccessful [83], most probably because Rrp2 is essential for cell growth [85]. The *Borrelia* oxidative stress regulator, previously known as Fur, also binds upstream of the RpoS promoter and plays an important role in infectivity [86–90]. In addition, RpoS expression is thought to be post-transcriptionally regulated by the RNA-binding protein CsrA, which plays a critical role in infectivity [91,92]. Overexpression of CsrA resulted in a significant reduction in FlaB levels [93]. As FlaB expression is essential for infection [94], regulation by CsrA could be an important factor contributing to *B. burgdorferi* infectivity in mice [91,93] and possibly ticks. Notably, RpoS can also be transcribed from an unknown σ 70-dependent promoter, and this form of the transcript is subject to small RNA DsrA-dependent post-transcriptional regulation [95]. A more recent study shows that RpoS-driven gene expression is especially important for borrelial transmission, as RpoS-deficient *B. burgdorferi* were confined to the tick gut lumen, where they were transformed into an unusual morphology during later stages of the blood meal [69].

Although no report, to date, demonstrates that Rrp1 is the cognate response regulator for histidine kinase, Hk1, the location of Hk1 and Rrp1 on the borrelial chromosome and the phenotype of their mutants suggest the Hk1–Rrp1 pathway [96–99] constitutes a potential

two-component system essential for *B. burgdorferi* survival during larval and nymphal blood meals. Spirochetes deficient in either protein were virulent in mice and able to migrate out of the bite site during feeding, but were killed within the gut following acquisition by ticks [96,98,100]. While a Rrp1 mutant remained infectious in mammals but could not survive in ticks [98,100], constitutive expression of the glycerol metabolic (*glp*) operon fully rescued the defect, allowing spirochete survival in BSK-glycerol, but only partially restoring persistence in ticks [98]. Therefore, additional factors for complete restoration of *B. burgdorferi* survival in ticks remain to be identified. These studies suggest that the *glp* operon is a target of a second messenger, cyclic dimeric GMP (c-di-GMP) [98,101]. On the other hand, spirochetes overexpressing Rrp1 displayed normal motility patterns and chemotactic responses, but were noninfectious in mice [100]; however, the bacteria could persist in ticks and survive a natural blood meal. This response regulator synthesizes c-di-GMP that regulates tick-specific borreliar infection by modulating the expression and/or activity of gene products required for survival within feeding ticks [96,98–100]. BB0374, also known as PdeB, specifically hydrolyzes c-di-GMP [102]. PdeB mutants exhibited significantly increased flexing motion and reduced ability to survive in ticks, and were subsequently unable to transmit to the host [102]. Altogether, these results indicate that Rrp1 and c-di-GMP are not required for murine infection, but are important for *B. burgdorferi* colonization of ticks.

Additional genes integral for borreliar motility & chemotaxis contribute to the invasiveness & infectivity of the pathogen—

BB0733, also known as PilZ, is the only PilZ domain-containing protein in *B. burgdorferi* that binds c-di-GMP with high specificity [97,103] and supports borreliar persistence in fed ticks [103]. The phosphodiesterase BB0363, which hydrolyzes c-di-GMP [104], also plays a critical role in spirochete motility and infection [104]. Finally, a recent study suggested that cheA2, a gene encoding a histidine kinase essential for chemotaxis of *B. burgdorferi*, is required for tick-transmitted infection of murine hosts [105].

Proteins

Outer surface proteins: A number of *B. burgdorferi* outer surface proteins (Osps) are known to be important for spirochete persistence in and transmission through ticks [20]. OspA and OspB, highly abundant proteins expressed by cultured borreliar cells, primarily display vector-specific expression *in vivo* and play critical roles in borreliar persistence in the tick gut [57,106–108]. OspA is shown to interact with a tick protein in the gut that is required for spirochete colonization of the gut epithelium [109]. This highly studied borreliar protein is also reported to bind plasminogen [110] and, in addition, protect spirochetes in the feeding tick gut from host-derived bactericidal antibodies [111]. OspC, another well-studied antigen, is induced in feeding ticks during borreliar transmission to the host [112]. While there are conflicting reports regarding the role of OspC in spirochete dissemination through tick tissues [65,67,69,113], the gene product is required by spirochetes to establish early murine infection. OspC is also known to bind plasminogen [114,115], which could assist spirochete migration through ticks. Another Osp, annotated OspD, is shown to be upregulated in a narrow time frame corresponding to tick feeding, and plays either a secondary or nonessential role in *B. burgdorferi* persistence in ticks [116,117].

Dps: A *B. burgdorferi* protein similar to the DNA-binding protein from starved cells (Dps) family is shown to protect the spirochete against DNA damage. Dps expression is low-throughout murine infection, but increases during tick intermolt periods [118]. The protein is likely to be important for protection of the spirochete against starvation or oxidative stress-induced damage, conditions that are pronounced during the long intermolt period between

blood meals [118]. The crystal structure of Dps has recently been determined [119], which could be helpful in understanding the exact function of the protein.

VlsE: *B. burgdorferi* encodes a major surface lipoprotein that undergoes antigenic variation in mammalian hosts via recombination of sequences from silent cassettes into the expressed vlsE locus [44,120–123]. These intragenic and promiscuous recombination events are also reported, but minimal, in feeding ticks [60,124].

Lipoproteins: Several putative lipoproteins have been implicated in supporting *B. burgdorferi* persistence in ticks or their transmission through feeding ticks; however, in most cases, their function in spirochete biology is unknown. *Lp6.6* is not expressed during mammalian infection, but is selectively induced as pathogens enter ticks and persist in the vector; the protein is shown to play a role in transmission from ticks to mammals, as deletion of *lp6.6* impairs the ability of spirochetes to migrate from feeding ticks to naive hosts [54]. This outer membrane antigen lacks surface exposure [125], yet is highly abundant and exists in multiple protein complexes [54,126]. Another subsurface membrane protein, LA7 (also annotated p22 or BB0365), may be important for spirochete survival in feeding ticks [127]. The lipoprotein, BptA, encoded by the locus *bbe16*, is required for persistent infection of ticks by spirochetes [128]. More recent studies have suggested that BBA07 is potentially expressed on the spirochete surface and regulated by the Rrp2–RpoN–RpoS pathway, supporting *B. burgdorferi* transmission from infected ticks to murine hosts [129]. Finally, *bbe31* that encodes an outer surface lipoprotein shown to interact with a tick protein and facilitate spirochete migration through the hemolymph during feeding [68].

Complement regulator-acquiring surface proteins: At least three classes of genes encode several BbCRASPs, that are differentially expressed during the mammal–tick infection cycle [130,131]. BbCRASP-2 function is shown to be nonessential for spirochete infectivity [132]. Similarly, BbCRASP-1 may not play a vital role in mammalian infection [133], but could be important for spirochete survival in feeding ticks.

BB0323: This protein plays critical roles in the organization of the spirochete outer membrane, cell fission and infectivity [134,135]. As BB0323 is one of the few outer membrane proteins that is consistently required by *B. burgdorferi* throughout the tick–rodent infection cycle, further understanding its function in spirochete biology may identify possible therapeutic targets to prevent Lyme disease.

BBA52 & BBA64: BBA52 is an outer membrane protein that is exposed on the microbial surface and may exist as a homo-oligomer [136]. The gene product is only expressed in the vector and facilitates *B. burgdorferi* dissemination from feeding ticks to mice [66]. BBA52-specific antibodies block pathogen transmission from infected ticks to naive hosts [136], making this antigen a potential component of Lyme disease vaccines. Similarly, BBA64 has also been shown to play an important role in the persistence of spirochetes and their transmission from ticks to mammalian hosts [137].

BmtA: BmtA (BB0219) is probably a metal transporter, as mutants are sensitive to ethylenediaminetetraacetic acid chelation, and has been shown to be essential to the *B. burgdorferi* infection cycle [138].

Enzymes: The locus *bbe22* on lp25 encodes a nicotinamidase, which is most likely required for NAD⁺ biosynthesis and plays a critical role for spirochete infectivity of mice and ticks [72,75]. A gene located on the plasmid lp36 annotated *bbk17* (*adeC*) encodes an adenine deaminase that is also shown to contribute to spirochete infectivity in rodents, however,

AdeC function may not be important for borrelial persistence in ticks [76]. Finally, *bb0728* is dually transcribed by $\sigma 70$ and RpoS to produce a coenzyme A disulfide reductase shown to play important roles during spirochete multiplication in the enzootic cycle [139].

Two key enzymes integral for purine salvage pathways were also studied: IMP dehydrogenase and GMP synthase, both of which are encoded by genes in the *guaAB* operon [140]. The enzymatic activities of the proteins are thought to be essential for *B. burgdorferi* mouse infectivity and to provide a survival advantage to spirochetes in the tick [140].

More recent studies examined *B. burgdorferi* gene products involved in the glycolytic pathway found to have roles in supporting persistence of the spirochete in ticks. As mentioned earlier, the *B. burgdorferi* gene *glpD* probably encodes a glycerol-3-phosphate dehydrogenase that contributes to the vector-specific phase of the spirochete lifecycle [101]. A number of studies have demonstrated the occurrence of certain glycolytic enzymes, such as enolase, on the pathogen's surface or in the outer membrane [141–143]. *B. burgdorferi* enolase in its recombinant form binds plasminogen while retaining its enzymatic activities and is shown to facilitate pathogen survival in feeding ticks [143].

A recent study suggested that BB0646, which demonstrated strong lipase and hemolytic activity integral for spirochete infectivity [144], is also likely to be important for spirochete transmission from the vector to mammalian hosts.

Although these studies prove that a specific set of genes, located either on plasmids or the chromosome, are of functional significance, several other *B. burgdorferi* gene products are shown to play redundant functions in infection. Several investigations involved mutants lacking certain gene products nonessential for spirochete persistence in the tick–rodent infection cycle: LuxS was implicated in the quorum sensing pathway [145], ChbC involved in the transport of chitobiose [146], MalQ an amylomaltase potentially involved in sugar utilization [147] or BB0844 [148] and BBA05 [149], two highly regulated proteins with an unknown function. In addition, targeted deletion of the *bba01* to *bba07* locus of plasmid lp54 did not significantly affect the ability of *B. burgdorferi* to persist in the tick–rodent infection cycle [150].

***I. scapularis* proteins supporting the spirochete infection cycle**—Identification of *I. scapularis* proteins that influence *B. burgdorferi* persistence in and transmission through the vector will probably generate novel information that may provide insights into the molecular mechanisms that support pathogen survival within the tick [20]. These studies may also contribute to the development of effective preventative strategies to combat *B. burgdorferi* infection and may serve as a paradigm that could help in the understanding of other tick-borne diseases [46,151–153]. As the tick gut and salivary glands are the major organs associated with borrelial persistence and/or transmission, a number of studies have focused on these two tissues and identified several important proteins, which are highlighted below. While some of these proteins display expression, others are expressed organ-specific in multiple tissues.

Tick saliva or salivary gland proteins—Several studies, including those taking global approaches [154–156], cataloged putative proteins in tick saliva or the salivary glands and assessed their intrinsic properties [157–159]. Some of the salivary components possess characteristics that allow the tick to feed on a host without immune recognition and/or rejection, an ability that directly or indirectly facilitates the transmission of pathogens such as *Borrelia*, as well as fostering their ability to establish infection soon after reaching the host [19,160]. Immunization of hosts with *I. scapularis* salivary gland proteins expressed

during early feeding has been shown to impair *B. burgdorferi* transmission from ticks to hosts [154]. The potent immunomodulatory properties of tick saliva have been evidenced by the downregulation of chemokines and antimicrobial peptides along with other experimental demonstrations [159,161,162]. Apart from saliva, a number of specific salivary gland proteins were identified to play a role in *B. burgdorferi* infection, as discussed in the following sections.

Salp15: A feeding- and *B. burgdorferi*-induced salivary gland protein, termed Salp15, was shown to have remarkable host immunosuppressive properties, inhibiting CD4⁺ T-cell activation [163]. Salp15 binds to *B. burgdorferi* through OspC, facilitating the survival of spirochetes within the infected host [164–166]. This tick protein is an immunoprotective antigen, as Salp15 antiserum can significantly protect mice against *B. burgdorferi* challenge [167]. The family of proteins is reported in many tick species and has been suggested to be under positive selection [168].

Salp25D: An abundant salivary protein, Salp25D, is likely to play an important role during tick acquisition of *B. burgdorferi*. The protein is immunodominant and functions as an antioxidant that detoxifies reactive oxygen species to allow the spirochete to survive at the tick–host interface [169,170].

Salp20: This salivary gland protein has been shown to protect *B. burgdorferi* from *in vitro* lysis and may protect pathogens from components of the complement pathway during transmission [171]. Salp20 is related to proteins in the *I. scapularis* anticomplement family that are responsible for blocking the host alternative complement pathway [155].

Tick histamine release factor: This constituent of tick saliva is upregulated during rapid feeding phases of ticks, as well as during *B. burgdorferi* infection [172]. Silencing the gene by RNAi, actively immunizing mice with recombinant protein or passively transferring antiserum significantly interfered with tick feeding and decreased the *B. burgdorferi* burden in mice [172].

Tick salivary lectin pathway inhibitor: More recently, a tick salivary protein, termed P8, was identified [173]. Silencing p8 or immunization with the protein impaired *B. burgdorferi* transmission from ticks to mammals, acquisition by ticks and persistence in the tick gut.

Tick receptor for OspA: A receptor for *B. burgdorferi* OspA, termed tick receptor for OspA (TROSPA) has been identified in the tick gut [109]. TROSPA expression is upregulated during *B. burgdorferi* infection and downregulated during tick engorgement. The receptor has been identified in other *Ixodes* or *Rhipicephalus* species [174,175], and a gene encoding TROSPA was also overexpressed in *Babesia*-infected ticks [175]. Binding of OspA to TROSPA allows *B. burgdorferi* to colonize the gut, but the receptor's native physiological function is unknown [109]. TROSPA has weak amino acid sequence homology to antifreeze glycoproteins.

TRE31: This is a tick gut protein identified as interacting with *B. burgdorferi* BBE31 [68]. TRE31 is likely to be important for borrelial dissemination through ticks, as silencing the gene decreased *B. burgdorferi* burden in the tick hemolymph [68].

The studies discussed in paragraphs above – investigating a number of *B. burgdorferi* and *I. scapularis* gene products that support pathogen persistence in and transmission through the vector – are summarized in Table 1.

Tick immune system influencing pathogen persistence

Our understanding of arthropod innate immune responses, primarily involving the fruit fly and mosquito, has advanced over the past decades. However, the subject of tick immune responses, especially how *I. scapularis* responds to invading pathogens, remains underexplored. The environment of the feeding tick gut [58,176], which pathogens such as *B. burgdorferi* encounter upon their arrival from an infected host, is likely to be hostile. As spirochetes adapt to the luminal space of the gut and colonize the epithelial tissues, the bacteria must avoid tick innate immune defense mechanisms. Although gastric digestion in ticks is primarily intracellular [58,176], degradation of blood components such as hemoglobin could create peptides with antimicrobial activities [177]. In addition, a number of studies in model arthropods have illustrated the existence of pathogen recognition molecules that induce Toll (against bacteria, viruses or fungi) and/or immune deficiency (targeting Gram-negative bacteria) pathways [178–185]. However, how these networks operate in *I. scapularis*, as well as their influence on spirochete persistence, remains unclear. Nevertheless, ticks probably produce classical antimicrobial peptides [186] in the gut, as tick genomes encode several putative antimicrobial peptides. In *Ixodes* ticks, the gene encoding a defensin-like peptide [187] is upregulated in the gut after infection with *B. burgdorferi* [188]. Several studies have reported the development of annotated lists of organ-specific transcripts [155,189], including immune-related genes, which will impact our understanding of tick biology and vector-pathogen interaction. While *I. scapularis* appears to be immunotolerant of *B. burgdorferi*, a set of tick genes are induced after spirochete infection, including those potentially involved in oxidative stress response [188]. Interestingly, spirochetes survived after injection into *Ixodes* hemolymph but not that of *Dermacentor variabilis*, which is not a vector of *B. burgdorferi* [190,191]. However, despite these studies, the precise mechanism by which *B. burgdorferi* persists in the gut or evades tick innate immune responses, such as the potential generation of reactive oxygen species, antimicrobial peptides or phagocytosis, requires further investigation.

Vaccination strategies interfering with *B. burgdorferi* lifecycle

The incidence of Lyme disease continues to increase, and its endemic areas are growing. Owing to clinical manifestations shared with other diseases and individual patient variations in immune response, proper diagnosis of Lyme disease remains challenging. Antibiotic treatment is available but not always successful, therefore, development of a vaccine is a major focus of Lyme disease research. Although the search for effective prevention has been underway for several decades, there is currently no Lyme disease vaccine available for humans [27]. Earlier studies led to the development of a US FDA-approved vaccine based on the recombinant form of OspA. However, it was withdrawn from the market owing to poor sales and other patient-related complications. Not only is early diagnosis of Lyme disease especially difficult [192,193], but also treatment may incur high costs [194] and sometimes fails to clear the infection [11,18]. Other preventative strategies, such as tick control via pesticides or avoidance of tick bite(s) using protective clothing, have proven ineffective [27]. As proteins on a cell surface are instrumental to host–pathogen interactions and may serve as immunogens that confer protection as targets for neutralizing antibodies, novel vaccination strategies being proposed are based on multiple surface-exposed *B. burgdorferi* tick antigens or a combination of both. Two major vaccination strategies that target the pathogens, either in the reservoir hosts or feeding vector, are discussed in the following paragraphs.

Reservoir-targeted vaccines—Considering that one of the major epidemiological factors influencing the incidence of zoonoses, such as Lyme disease, is the availability of infected reservoir hosts, the development of wildlife-targeted vaccines is a potentially safe and effective strategy to prevent the infection. In fact, reservoir-targeted vaccine approaches

have been successfully used in the past to eradicate rabies from wildlife [195,196]. Since the discovery of the protective efficacy of OspA in mice and humans [197–199] and initial studies showing that immunization of wild mice with OspA reduces *B. burgdorferi* prevalence in a confined geographic area [200], a number of new studies have explored the use of the antigen for reservoir-targeted vaccines with several bacterial and viral delivery platforms [201–204]. For example, a vaccinia virus-based oral vaccine expressing OspA reportedly protects laboratory mice against infection by feeding ticks and clears ticks of infection [201]. White-footed mice developed a year-long systemic OspA-specific response that protected against tick challenge and cleared *B. burgdorferi* from significantly decrease the transmission the tick [205]. Vaccination with the oral bait delivery system protected 89% of mice and reduced pathogen load in ticks eightfold. Given that an effective deployment of oral vaccines specifically targeted to wildlife reservoirs is possible, such an immunization program is likely to disrupt the transmission cycle of Lyme disease pathogens.

Immunization of hosts with *I. scapularis* subolesin has been shown to confer protection against tick bite and decrease acquisition of multiple pathogens [206–209]. A recent study reported the utility of the protein as an oral reservoir-targeted vaccinia virus-based vaccine for tick engorgement and *B. burgdorferi* transmission from ticks to murine hosts [210]. A single dose of the vaccine induced a robust antibody response against subolesin, which dramatically reduced subsequent tick infestation, thereby exerting significant influence on either tick acquisition of spirochetes from infected mice or spirochete transmission from infected ticks to naive hosts [210].

Transmission-blocking vaccines—A number of reasons justify the fact that targeting the pathogen in the vector by blocking transmission could be an innovative and effective method to combat vector-borne infections such as Lyme disease [19]. First, it is conceivable that *B. burgdorferi* antigens expressed in the vector face less immune selection pressure than those expressed in the host. In addition, many borrelial gene products that are expressed in the vector are encoded by stable or core sections of the borrelial genome [66]. Finally, antigenic variation mechanisms appear to be minimally active in the vector [211]. Therefore, identification of additional surface-exposed *B. burgdorferi* proteins that are expressed in the tick as immunogenic candidates in the feeding gut is the first step toward the development of potential vaccines [212]. Host antibodies to these proteins in the feeding gut may block or of spirochetes from ticks to mammals [136,212]. As pathogen neutralization occurs within the feeding vector, the development of a successful transmission-blocking vaccine requires that the antigen induce high and long-lasting circulating antibody titers in immunized hosts. Recently, our laboratory identified BBA52 as a potential candidate for a component of transmission-blocking Lyme disease vaccines [136]. Mice vaccinated with BBA52 were significantly less susceptible to subsequent tick-borne infection, and passive transfer of BBA52 antibodies to ticks completely blocked transmission of *B. burgdorferi* to mammalian hosts.

In addition to borrelial antigens, surface-exposed tick gut proteins may also form the basis of Lyme disease vaccines. Immunization with tick proteins required for pathogen survival has the potential to block the acquisition and transmission of *B. burgdorferi*. If vaccination interferes with specific arthropod survival or development, the strategy can potentially prevent multiple infections that are often cotransmitted by a single tick species. For example, immunization of hosts using the *I. scapularis* protein subolesin significantly interfered with the ability of ticks to transmit multiple pathogens, including *Anaplasma* and *Babesia* [207]. In addition to its effect on tick oviposition, subolesin immunization also reduces tick weight and survival [206,213]. Vaccination with a gut antigen of *Rhipicephalus microplus*, Bm86, interfered with tick feeding and, therefore, prevented effective pathogen

transmission [214]. The antigen has been developed into successful bovine vaccines, commercialized, and is currently marketed as Gavac and TickGARD [215–217].

Future perspective

B. burgdorferi persists in a complex enzootic infection cycle involving *I. scapularis* ticks, and much progress has been made in the field since the spirochete was discovered to be the cause of Lyme disease. Our understanding of the biology of host–pathogen interaction, primarily involving model insects, such as the fruit fly or mosquito, has advanced over the past decades. However, our knowledge of tick biology, especially the molecular interaction of *I. scapularis* with the pathogen it maintains and transmits, and the mechanism(s) by which the *Ixodes* immune response influences invading pathogens, remains insufficient. These areas are understudied but important and warrant future investigation. A limited number of spirochete and tick proteins have been shown to be required at different stages of infection, including for tick-to-rodent transmission, and identification of additional candidates or further exploration of how identified proteins facilitate pathogen persistence and/or transmission can contribute to the development of novel vaccines to prevent Lyme disease. As *Ixodes* ticks transmit a wide array of serious human and animal pathogens, the information gathered from studying *Ixodes–Borrelia* interactions can be extrapolated to other tick-borne infections.

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Website

301. J Craig VenterTM Institute. www.jcvi.org

Executive summary

Borrelia burgdorferi

- Although described as Gram-negative, *Borrelia burgdorferi* possesses unique cellular organization and membrane composition.
- The genome of *B. burgdorferi* is highly segmented and unstable.
- Many genes are differentially expressed and a substantial portion encode for membrane proteins potentially involved in host–pathogen interactions.

The tick–mammal infection cycle

- *B. burgdorferi* survives in a complex vector–host infection cycle.
- During acquisition by the vector, spirochetes migrate from an infected host to ticks and colonize the gut.
- The pathogen continues to persist in the gut lumen through the intermolt stages.
- During transmission, a fraction of the spirochetes move to the salivary glands and migrate to a new host.

***B. burgdorferi* & *Ixodes scapularis* proteins**

- A select set of *B. burgdorferi* plasmids and proteins and *Ixodes scapularis* gene products have been found to play role(s) in spirochete persistence in and/or transmission through the tick.

Tick immune system

- Tick innate immune defense mechanisms exist, but how these pathways operate to control invading pathogens remain largely unknown.
- Immunotolerance of *B. burgdorferi* by *Ixodes* ticks.
- Differential expression of tick genes in response to infection.

Vaccination strategies

- A Lyme disease vaccine for humans is highly warranted but currently absent.
- Potential use of reservoir-targeted vaccines for the control of Lyme disease.
- Use of transmission-blocking vaccines to prevent Lyme disease.

Table 1

Borrelia burgdorferi and *Ixodes scapularis* proteins important for spirochete survival in ticks and/or transmission from the vector to the hosts.

ORF/plasmid/gene name	Major attribute(s)	Ref.
<i>Borrelia burgdorferi</i>		
<i>bb0240-bb0243 (glp)</i>	Glycerol utilization	[98,101]
<i>bb0323</i>	Organization of outer membrane and cell fission	[134,135]
<i>bb0337 (eno)</i>	Enolase; glycolysis and binds plasminogen	[141–143]
<i>bb0363 (pdeA)</i>	Phosphodiesterase; hydrolyzes c-di-GMP	[104]
<i>bb0365 (LA7/p22)</i>	Unknown	[127]
<i>bb0374 (pdeB)</i>	Phosphodiesterase; hydrolyzes c-di-GMP	[102]
<i>bb0419 (rrpI)</i>	Response regulatory protein, diguanylate cyclase	[96–101]
<i>bb0420 (hklI)</i>	Histidine kinase	[96,98,99]
<i>bb0450 (rpoN)</i>	Alternate σ factor of RpoS	[77–85]
<i>bb0647 (bosR/fur)</i>	Oxidative stress regulator; binds RpoS promoter	[86–90]
<i>bb0690 (dps)</i>	Binds DNA	[118,119]
<i>bb0728 (cdt)</i>	Coenzyme A disulfide reductase	[139]
<i>bb0733 (plzA)</i>	Binds c-di-GMP	[97,103]
<i>bb0763 (rrp2)</i>	Response regulatory protein, activator of RpoS	[77,81,83–85]
<i>bb0771 (rpoS)</i>	Alternate σ factor	[69,77,78,80–84,86,88,89,95]
<i>bba07</i>	Unknown	[129,150]
<i>bba15 (ospA)</i>	Interacts with tick gut receptor (TROSPA), binds plasminogen and protects against host antibodies in the gut	[107–111]
<i>bba16 (ospB)</i>	Unknown	[106,108]
<i>bba52</i>	Unknown	[66,136]
<i>bba62 (lp6.6)</i>	Unknown	[54,125]
<i>bba64</i>	Antigen p35	[137]
<i>bbb17 (guaB)</i>	IMP dehydrogenase	[140]
<i>bbb18 (guaA)</i>	GMP synthase	[140]
<i>bbb19 (ospC)[†]</i>	Interacts with Salp15 and binds plasminogen	[65,67,69,70,82,113–115]
<i>bbe16 (bptA)</i>	Unknown	[128]
<i>bbe22 (pncA)</i>	Nicotinamidase	[73,75]
<i>bbe31</i>	Interacts with tick protein TRE31	[68]
<i>lp28-1 (vlse)</i>	Undergoes genetic recombination	[44,45,60,70,71,124,211]
<i>lp28-4</i>	Unknown	[74]
<i>bbj09 (ospD)</i>	Unknown	[116,117]
<i>bb0646[‡]</i>	Lipase and hemolytic activities	[144]
<i>Ixodes scapularis</i>		
<i>salp15</i>	Interacts with OspC	[163–168]
<i>salp20</i>	Potential inhibitor of host complement pathway	[171]

ORF/plasmid/gene name	Major attribute(s)	Ref.
<i>salp25D</i>	Antioxidant; detoxifies ROS	[169,170]
<i>tHRF</i>	Tick histamine release factor	[172]
<i>TROSPA</i>	Tick receptor for OspA	[109,174]
<i>TRE31</i>	Interacts with spirochete protein BBE31	[68]
<i>TSLPI</i>	Tick salivary lectin pathway inhibitor	[173]

ORF and attributes are based on annotations in the database [301] and publications indicated by references.

[†]Conflicting role(s) of gene products in ticks.

[‡]Speculative role(s) of gene products in ticks.

c-di-GMP: Cyclic dimeric GMP; ORF: Open reading frame; ROS: Reactive oxygen species.