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Review

Adaptation of *Borrelia burgdorferi* in the vector and vertebrate host

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Abstract

Borrelia burgdorferi sensu lato is the causative agent of Lyme disease, which afflicts both humans and some domestic animals. *B. burgdorferi*, a highly evolved extracellular pathogen, uses several strategies to survive in a complex enzootic cycle involving a diverse range of hosts. This review focuses on the unique adaptive features of *B. burgdorferi*, which are central to establishing a successful spirochetal infection within arthropod and vertebrate hosts. We also discuss the regulatory mechanisms linked with the development of molecular adaptation of spirochetes within different host environments.

Keywords: Borrelia burgdorferi; Ticks; Adaptation

1. Introduction

Borrelia burgdorferi sensu lato is the group of spirochetes causing Lyme disease and includes at least 10 genospecies [1]. The three genospecies commonly associated with human infections include *B. burgdorferi* sensu stricto, which is widespread in both the USA and Europe, and *B. afzelii* and *B. garinii*, which are primarily distributed in Europe [2]. The clinical manifestations of Lyme borreliosis differ in North America and Eurasia, possibly due to the genetic diversity among different *B. burgdorferi* genospecies. *B. garinii* is associated with neurologic diseases, while *B. burgdorferi* sensu stricto and *B. afzelii* are more likely to cause arthritis and cutaneous symptoms, respectively [3–5]. Different genospecies seem to vary in their ability to survive in a given host: for example, *B. burgdorferi* sensu stricto and *B. garinii* are reported to persist in birds, whereas *B. afzelii* fails to survive in avian hosts [6].

B. burgdorferi sensu lato is transmitted by *Ixodes* ticks [7–9]. The nature of the enzootic cycle of *B. burgdorferi* in specific geographic areas influences the incidence of human infections. In the northeastern and north central United States, *B. burgdorferi* sensu stricto is principally maintained in a cycle involving larval and nymphal *Ixodes scapularis* ticks and white-footed mice [2]. Occasionally, *I. scapularis* nymphs or adults feed and transmit infection to a wide range of vertebrates, including humans. *Ixodes* species in other parts of the United States also harbor Lyme spirochetes, but few ticks are infected in comparison to areas with a high incidence of human Lyme disease [8,10]. Differences in tick-host preferences may possibly explain the low prevalence of such infections. For example, *I. pacificus* in the west and *I. scapularis* in the southeast feed on reptiles, which are not susceptible to spirochete infection. *I. neotomae*, another potential vector, feeds on rodents but rarely bites humans [8,10]. In Europe and Asia, the Lyme disease pathogen is generally maintained between small rodents and *I. ricinus* or *I. persulcatus* [11].

2. Structure, genome, gene expression and regulation

The structure of *B. burgdorferi* sensu lato is typical of a spirochete: a spiral or coil-shaped cell that is generally 20–30 μm in length and 0.2–0.5 μm in width. Individual spirochetes, however, can vary in length, diameter, tightness and regularity of the coils. The protoplasmic cylinder containing the cytoplasm with its organelles and flagellar apparatus is covered by a periplasm and a lipoprotein-based outer surface membrane [12]. Complete genome sequencing of *B. burgdorferi* sensu stricto (strain B31M1) disclosed several unique adaptive features of the spirochete [13]. The genome size is relatively small, approximately 1.5 megabases, consisting of a linear chromosome of 950 kilobases and at least 21 extra-

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Table 1
Examples of differentially expressed *B. burgdorferi* lipoprotein gene products with suggested functions

Gene product	Expression	Receptor	Function	Reference
DbpA and DbpB	Mammal	Decorin	Colonization	[40]
Bgp	Mammal	GAGs	Colonization	[102]
P66	Mammal	Integrins	Colonization	[72]
Erp (OspE/F)	Mammal	Factor H	Host defense	[62]
ErpT (Arp)	Mammal	?	Colonization?	[103]
P47 (Bbk32)	Mammal	Fibronectin	Colonization	[68]
OspC	Mammal/tick	?	Transmission	[51]
OspA	Tick	A gut protein	Colonization	[91]

chromosomal DNA elements or plasmids (620 kilobases within nine linear and 12 circular plasmids) [16]. *B. burgdorferi* has the largest numbers of plasmids known for any bacterium to date. Plasmid genes are of special interest, as they contain many of the genes associated with spirochete pathogenicity. The chromosome contains 853 genes coding for a basic set of known eubacterial proteins that drive cell cycle, growth and metabolism, with an unusual absence of genes responsible for cellular biosynthetic reactions [13]. Plasmids encode another 535 genes, and 90% of the genes have no convincing similarity to genes outside *Borrelia* genus, suggesting that they perform specialized functions possibly related to spirochete adaptation. Further experiments demonstrated that some of the plasmids can be lost during propagation of the bacteria in vitro, and loss of infectivity in mice often parallels the loss of specific plasmids [14–18]. Most of the spirochete genome consist of linear DNA with covalently closed hairpin ends or telomeres. *B. burgdorferi* has evolved highly efficient enzymes to replicate such telomeric DNA structures. Recently ResT, a new class of telomere resolvase, has been identified to be encoded by *B. burgdorferi* locus *bbb03*, which performs a highly efficient but complex two-step DNA transesterification during replication to generate covalently closed hairpin telomeres [19].

B. burgdorferi has evolved remarkable abilities to survive in a wide range of organisms such as arthropods, and vertebrates like birds and mammals. In contrast to other pathogenic bacteria, *B. burgdorferi* has devoted a large portion of its genome (more than 8% of coding genes or 150 genes) towards producing lipoproteins [13,16]. Studies have demonstrated the ability of the bacterium to alter its surface structure by differential lipoprotein gene expression at various stages of its life cycle in mammals and ticks, which is likely to aid in host adaptation and immune evasion [20–27]. A few host molecules were also identified that are involved in interactions with spirochete ligands and thus aid in bacterial survivability in diverse host environments (Table 1). In addition to the transcriptional activation of selective genes, events such as variable recombination also contribute to the alterations of spirochete structure. As an example, the *B. burgdirferi vlsE* locus consists of an active telomeric expression site flanked by a number of upstream silent *vls* cassettes [28]. Recombination events between the *vlsE* gene and *vls* cassettes occur in mammals [29] and most likely in feeding ticks [30], producing a genetically diverse population of spirochetes. Apart from controlling transcription or recombination events, spirochetes were also reported to generate antigenetically diverse populations by modulating intracellular (surface vs. periplasmic) translocation of lipoproteins [31].

A series of in vitro studies have identified a number of environmental signals that are thought to regulate gene expression in spirochetes and includes temperature [27,32], pH [33], cell density [34,35] and host factors [36,37]. Interestingly, many of these regulated genes are differentially expressed in vivo and include *hsp*, *mlp*, *dbpA*, *bbk 32*, *erp* (*ospE/F* -related), *ospC* and *ospA* [21,22,36,38–44]. Induction of lipoproteins belonging to large gene families such as Mlp [45], Erp [44] as well as OspC [27,43] at higher temperature may mimic temperature-induced changes in gene expression that occur during tick feeding, when the spirochetes are exposed to warm host blood. Likewise, lowered pH changes the expression of a few spirochete genes in vitro [33,46], and these genes may represent regulated genes during the tick blood meal, because the pH of the tick gut drops during the feeding process. However, the regulation seems complex and is likely to be multifactorial. For example, temperature alone does not appear to be a sufficient signal for *ospC* induction, because unfed ticks exposed to higher temperatures do not induce *ospC* expression [27]. A recent study suggested that the same environmental stimuli seem to coregulate spirochete proteins like OspA and OspC in vitro, suggesting that regulatory pathways of differentially expressed genes are interlinked [46]. However, little is known about the mechanism or key regulatory networks that govern spirochete gene regulation in vivo. The *B. burgdorferi* genome surprisingly contained relatively few homologs of known eubacterial regulatory proteins [13]. For example, although *B. burgdorferi* displays a classical heat-shock response, the genome does not contain a known heat-shock sigma factor [13]. A recent microarray analysis also failed to identify significant evidence of adaptive changes in clusters of spirochete regulatory genes under different host or growth conditions, whereas the same conditions caused significant changes in lipoprotein gene expression [37]. It is proposed that *B. burgdorferi* adapts to exploit relatively minor changes in the expression of regulatory genes in order to affect downstream target gene expression, so that relatively small alteration in regulatory gene expression can control production of large numbers of target lipoproteins [37]. *B. burgdorferi*

constitutively expresses RpoN, a sigma subunit, which is regulated via a post-transcriptional mechanism [47]. RpoN controls the expression of RpoS, an alternative sigma factor, which in turn, is thought to regulate the transcription of several lipoproteins like OspC, OspF, Mlp-8 and DbpA [46,48]. An external or environmental signaling event is proposed to induce an RpoN activator protein which is likely to bind to an enhancer region upstream of where RpoN complexed with the RNA polymerase holoenzyme (–24/–12 region), leading to the synthesis of *rpoS* mRNA. RpoS then mediates the synthesis of target lipoproteins of *B. burgdorferi* [48].

3. Adaptation in vertebrates

B. burgdorferi is transmitted to vertebrates, mostly to mammals, during tick feeding. An *Ixodes* tick takes approximately 3–4 d to complete the engorgement process, during which a pronounced multiplication of spirochetes takes place in the gut of the tick [49]. The spirochete numbers are reported to increase several hundredfold [50], and the differential gene expression as well as the variable recombination may contribute to the production of new molecules on the spirochete surface [30]. The newly synthesized proteins are believed to aid in the transmission from tick gut via salivary gland to the dermis of the host [51]. Although spirochetes evolve their own mechanism to fight host immune defense, the transmission via tick saliva provides them with certain adaptive advances. A feeding tick secretes molecules that influence the host immune system. For example, tick saliva inactivates the host complement system [52] and inhibits phagocyte function [53], which in turn, could help the adaptation of the spirochete to its new environment. Recently, an *I. scapularis* salivary protein, Salp15, has been shown to modulate CD4+ T cells [54]. After transmission, *B. burgdorferi* remains localized into the host skin for several days. The spirochetes are reported to invade distant skin sites or a number of organs and are found in high concentrations in the spleen, urinary bladder, joints and heart [8]. Spirochetes can cross the blood-brain barrier in several experimental models, most notably primates, and colonize the nervous system [55–57]. It is interesting to note that the bacteria are able to establish a chronic infection even in the face of the sophisticated immune system that exists in mammals. How does the pathogen adapt itself to this challenging environment?

Like many other invasive pathogens, *B. burgdorferi* uses a variety of mechanisms for protection against components of the host innate immune system. The alternate pathway of the complement system is a major primary host defense [58]. The pathway activates with an initial deposition of the C3 protein to the pathogen surface, followed by several amplification loops, ultimately resulting in the formation of membrane attack complex, which kills the pathogen. Some of the Erp (OspE/F-related) proteins have been reported to be synthesized by *B. burgdorferi* during early mammalian infection [59–61]. Studies have revealed that Erps contribute to the ability of *B. burgdorferi* to infect mammals by blocking host complement-mediated killing [62,63]. The spirochetes are capable of producing different Erps on the surface, and each Erp is reported to exhibit different relative affinities for the complement inhibitors of various potential vertebrate hosts [64]. Therefore, the presence of multiple Erps on the surface can allow for a single *B. burgdorferi* to resist complement-mediated killing in a wide range of hosts. All known *B. burgdorferi* strains can bind C3, although the deposition of the downstream components of complement system like C5b to C9 varies in different strains, resulting in a vast number of strains being resistant to complement attack [65]. The mechanism of resistance to complement is mediated by the binding of two host-derived complement control proteins: factor H and factor H-like protein-1 / reconectin [62,66]. *B. burgdorferi* surface protein OspE has been identified to bind the complement regulatory factor H [62]. OspE interacts with carboxy-terminal of factor H; therefore, the amino-terminal domain of the complement inhibitor remains free to exert its regulatory activities. The spirochete has also evolved other interesting survival strategies against innate immune attack. *B. burgdorferi* was shown to bypass the need for physiological iron for its growth and survival [67]; many of the iron-containing enzymes are well-known targets for oxidative host defenses against pathogens.

A large number of genes have been identified in *B. burgdorferi* which are selectively expressed, when spirochetes are inside a mammalian host milieu. The differentially expressed genes are likely to contribute to spirochete dissemination and colonization of target tissues. For example, *B. burgdorferi* synthesizes DbpA, DbpB and BBK 32 early in mammalian infection, which bind to host extracellular matrix proteins like decorin or fibronectin [40,68,69]. Spirochete adhesins such as DbpA or BBK 32 are thought to play critical roles in the early stage of Lyme disease by mediating the tissue adherence of *B. burgdorferi*. Local adherence ability in the skin extracellular matrix proteins could facilitate the survival of extremely small numbers of spirochetes (estimated to be between 1 and 10 [70]), which first enter the host at the site of tick feeding. *B. burgdorferi* is thought to replicate in the skin before endovascular dissemination towards distant organs. Theoretically, binding of soluble extracellular matrix proteins by spirochetes may also provide this organism with a mechanism for establishing persistent infection in wide host tissues. As shown with *B. crocidurae* [71], the ability of *B. burgdorferi* to bind bulky, host-derived proteins, such as fibronectin, may also mask recognition of the spirochete by the host immune system. *B. burgdorferi* surface molecule P66 has been shown to bind β_3-chain integrins [72], which are expressed in a variety of host locations. Thus, multiple adhesin mechanisms through such widespread host receptors as decorin, fibronectin or integrins contribute to the virulence of the organism and aid in the ability of the pathogen to establish chronic infection in multiple tissues. However, little is known about specific host receptors expressed in a local-

ized or tissue-specific manner and that bind to *B. burgdorferi*. The spirochetes are reported to use host-derived plasmin to facilitate invasion, as demonstrated in cell cultures and in mice genetically deficient in plasminogen [73,74]. Identification of the *guaA* gene encoding GMP synthetase in *B. burgdorferi*, an enzyme responsible for de novo purine biosynthesis, has also been implicated in the survival of bacteria in mammalian blood [75].

A number of recent studies involving microarray analysis of spirochete gene expression within chamber-implants [76] or in hosts at initial or chronic phases of murine infection address the question of how *B. burgdorferi* evades adaptive immunity in mammals [36,37]. Studies indicated that *B. burgdorferi* is able to generate multiple phenotypes during the transmission and earlier phases of murine infection [30,77]. Transcription and recombination events have been shown to occur in the feeding tick gut to generate a diverse population of spirochetes with antigenic and genetic variability [30]. Antibodies generated in the infected host selectively eliminate the targeted immunodominant phenotypes of spirochetes, while the adapted spirochetes continue to survive by downregulation of a selected set of proteins targeted by host antibody. A recent study demonstrated that *B. burgdorferi* expresses a set of approximately 116 lipoprotein genes during early mammalian infection and, as a part of adaptive immune response, will downregulate more than 80 of these genes [36]. This adaptation is proposed to be fundamental for *B. burgdorferi* to survive in mammals and establish persistent infection. In addition, *B. burgdorferi* has been proposed to develop alternate strategies to remain hidden from the immune system, such as masking immunodominant surface antigens [78] or persisting in close association with cells at immune privilege sites [79]. *B. burgdorferi* has been speculated to generate specific mechanisms to inhibit phagocytosis or ingestion by host cells [80]. For long-term persistence in host tissues, *B. burgdorferi* has also been proposed to modulate a wide range of host cytokines such as TNF-alpha [81], IFN-gamma [82,83], IL-6 [84,85], and IL-12 [86]. IFN-gamma-mediated events have actually been shown to promote *vls* locus recombination to generate a diverse population of spirochetes [87], which could potentially help to evade host immune response.

4. Adaptation in ticks

B. burgdorferi faces an entirely different environment when transmitted from mammals to *Ixodes* ticks. Studies have shown that although ticks usually feed for 96 h, both larval and nymphal ticks rapidly acquire spirochetes during the first 24 h of attachment [43], even before ticks are engorged with significant amounts of blood. The number of organisms within fed ticks continues to increase during and after the blood meal, possibly by continual entry of spirochetes during the blood meal and by replication [43,88]. The bacterium must avoid being digested with the tick blood meal and has to survive in significant temperature extremities of the poikilothermic organism, and also periods of limited nutritional resources and metabolic activity. During a subsequent blood meal, spirochetes must also have to sense the appropriate stimuli, and cross the gut barrier to travel to the salivary gland at the right time for transmission to a new vertebrate host. This crucial event requires coordination between *B. burgdorferi* development and *Ixodes* host activities that are likely to be mediated by the expression of both pathogen and vector gene products.

Several proteins that *B. burgdorferi* synthesizes in ticks have been identified. These include OspA, OspB, OspC, ErpT, MlpA, Rev, Erp homologs and Lp 6.6 [20,39,89,90]. Although little is known about the functional role of genes expressed during acquisition into or transmission from the tick, a few studies have focused on the function of OspA in ticks. OspA first appears on the surface of *B. burgdorferi* during larval acquisition and subsequent colonization of the tick gut [43]. *B. burgdorferi* continues to produce abundant OspA during its persistence within the gut of the tick until the next blood meal and subsequent transmission back to a new host. Recent studies demonstrated that OspA is a ligand that allows for the spirochetes to bind and colonize the tick gut [91,92]. Blood digestion in ticks occurs intracellularly, as hemolyzed blood is taken inside a gut cell by receptor-mediated endocytosis [93]. OspA-mediated adherence of spirochetes to tick gut cells could have implications to prevent internalization of spirochetes during the initial phases of blood meal digestion. OspA also binds itself [91], which could further promote OspA-based adherence in ticks and might explain the reported aggregation of *B. burgdorferi* in vitro, and in the gut of *Ixodes* ticks [94]. *B. burgdorferi* remains tightly attached to the gut epithelium soon after its appearance in the gut and appears to remain attached during the intermolt period, which can last for several weeks to months.

B. burgdorferi has evolved mechanisms to sense the environmental stimuli when a tick starts to feed. The spirochete migrates from the tick gut to the salivary glands before its transmission to a new host location (Fig. 1). In nymphs, dispersal to the salivary gland occurs over a period of several days of feeding, with the maximum number of spirochetes found in the saliva at 72 h after the commencement of tick feeding [49,50]. When a tick starts feeding, many spirochetes downregulate *ospA* and upregulate *ospC* [43,89,95]. This inverse relationship between OspA and OspC leads to the hypothesis that while downregulation of *ospA* favors *B. burgdorferi* exit from the gut, *ospC* is needed for their efficient migration from the gut via the salivary gland and transmission to the new host [20,43,51]. Recent antibody blocking experiments using either OspA [92] or OspC [51] support this hypothesis. During feeding, the tick can produce soluble factors, which in turn can aid spirochetes during the migration from the gut to the salivary gland via the hemocoelic cavity. In fact, a recent report showed that tick salivary gland

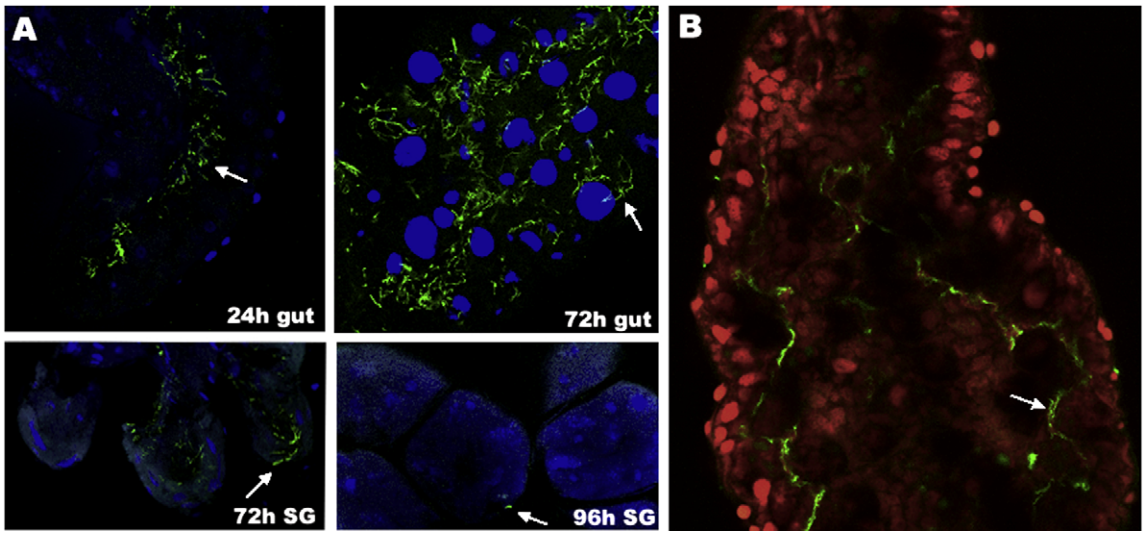

Fig. 1. Transmission and colonization of *B. burgdorferi* within *I. scapularis*. Images from multiple fluorescent chambers were recorded at a magnification of 400× in a confocal laser scanning microscope and are presented as a single image for clarity. (A) Transmission of *B. burgdorferi* from *I. scapularis* during feeding on mice. Ticks generally feed for 96 h, while spirochetes at the tick gut multiply extensively and migrate to the vertebrate host via the salivary gland. At 24 h of feeding, most of the spirochetes are confined within the lumen of the tick gut (upper right panel), while at 72 h they are dispersed through the gut, possibly for efficient migration (upper left panel). Salivary gland invasion peaks at 48–72 h of feeding (lower left panel), and at 96 h, fewer numbers of spirochetes are visible at the salivary gland (lower right panel). The spirochetes (arrows) are stained with an FITC-labeled goat anti- *B. burgdorferi* antibody (green), and the nuclei of gut or salivary gland epithelial cells are stained with TO-PRO-3 iodide (blue). Note the hypertrophy of tick nuclei during late stages of feeding. (B) Colonization of *B. burgdorferi* in resting *I. scapularis*. The spirochetes (arrow) remain closely associated with gut epithelial cells (stained red with propidium iodide).

extracts enhanced chemotactic migration of spirochetes [96]. At present there is no information about the regulation of genes that facilitate spirochete survival or transmission in ticks. Within a feeding gut, signals that induce the switch for the expression of genes necessary for transmission to new host, as for example, from to *ospA* to *ospC* [27] in *B. burgdorferi*, are likely to be external, which might then trigger factors inside the bacterium to regulate target protein expression. When ticks feed, apart from direct contact with the components of the ingested blood, spirochetes encounter a major alteration in the physical environment in the gut. For example, the pH of the midgut decreases from 7.4 to 6.8 [46], temperature increases from ambient (23 °C) to 37 °C, and spirochete cell density increases by replication [50]. All these physical parameters are in fact shown to act interdependently to promote reciprocal expression of *ospA* and *ospC* [46].

Future investigations will examine how *I. scapularis* ticks favor colonization and transmission of *B. burgdorferi* in contrast to other phylogenitically related ticks. *I. scapularis* could produce ligands that participate in *B. burgdorferi* colonization and survival within ticks. A number of studies also addressed whether variations in the tick innate immune responses play a role in supporting the *B. burgdorferi* life cycle in various tick species [97–99]. Antimicrobial peptides have been detected in the *Dermacentor variabilis* hemolymph [99], a species that does not support the spirochete life cycle. It has also been shown that experimentally inoculated spirochetes can survive within *I. scapularis* but are readily killed in the case of *D. variabilis* [98].

5. Concluding remarks

B. burgdorferi is exquisitely adapted to persist in a diverse range of hosts. Extensive literature has focused on identifying a wide array of genes that are expressed at particular host sites and regulated on a tight spatial and temporal basis. Much work is needed to understand the pathogenic roles of *B. burgdorferi* proteins with no assigned functions, which may prove to play important roles in spirochete adaptation in mammals or in *Ixodes* ticks. It is also necessary to understand the regulatory signaling mechanisms, both external and internal, that ultimately govern expression of essential gene products that aid in bacterial adaptation to diverse environments. Spirochete numbers increase immediately before and after transmission to new host locations. Many bacteria use quorum-sensing mechanisms to regulate gene expression at the population level and synchronize production of specific proteins that are needed for the infection process. The *B. burgdorferi* genome encodes a variety of enzymes involved in quorum-sensing, like LuxS, MetK and Pfs [13]. It has been shown that cultured spirochetes responded to the addition of autoinducer-2 by altering production of a large number of proteins, indicating that an LuxS-mediated quorum might operate in *B. burgdorferi* [100]. *B. burgdorferi oppA* gene encodes a novel surface exposed oligopeptide permease, which is proposed to utilize peptide pheromones for adaptation in the changing environments of ticks and mammals [101]. Spirochetes invade and colonize a variety of tissues within a given host or vector, which could involve

distinct sets of host receptors at different tissues. Identification of such receptors at the *B. burgdorferi* -host interface will establish the paradigms for exploring both how these interactions support long-term persistence of spirochetes, as well as new strategies to interrupt spirochete transmission.

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