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Illuminating the roles of the *Borrelia burgdorferi* adhesins

Jenifer Coburn¹, John Leong², and George Chaconas³

¹Division of Infectious Diseases, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI 53226 USA

²Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02114 USA

³Departments of Biochemistry & Molecular Biology and Microbiology, Immunology & Infectious Diseases, University of Calgary, Calgary, AB T2N 4N1, Canada

Abstract

The Lyme disease spirochetes, *Borrelia burgdorferi* (*sensu lato*), must cause persistent, disseminated infection to be maintained in the natural enzootic cycle. In human Lyme disease, spirochetes spread from the site of a tick bite to colonize multiple tissue sites, causing multisystem clinical manifestations. The Lyme spirochetes produce many adhesive surface proteins that collectively recognize diverse host substrates and cell types and are likely to promote dissemination and chronic infection in a variety of tissues. Recent application of state-of-the-art *in vivo* imaging technologies is illuminating mechanisms of interaction of *B. burgdorferi* with the host and the importance of multiple adhesins during mammalian infection.

Keywords

Lyme disease; *Borrelia burgdorferi*; adhesins; intravital imaging; dissemination; transmigration

Borrelia burgdorferi infection

Lyme disease is the most prevalent vector-borne infection in the Northern hemisphere, and leads to significant morbidity and health care costs in endemic regions due to the potential for long-term sequelae [1–4]. In North America, Lyme disease is caused by the spirochete *Borrelia burgdorferi*, while in Eurasia, two other species, *B. afzelii* and *B. garinii*, also cause Lyme disease. Additional *Borrelia* species are also agents of Lyme disease or similar clinical entities, although these cases are relatively rare. All of the Lyme borreliæ are zoonotic agents that are maintained in vector ticks and reservoir animals in nature. The ticks and reservoirs vary with geographic location, but for *B. burgdorferi*, the prototypic Lyme disease spirochete, *Ixodes scapularis* ticks and *Peromyscus leucopus* (white-footed mice) are the predominant and prototypical hosts in North America.

The Lyme disease *Borrelia* species must cause disseminated, persistent infection in immunocompetent hosts in order to propagate in its natural enzootic cycle, and this

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Corresponding author: Coburn, J. (jcoburn@mcw.edu).

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propensity for chronic systemic infection is reflected in human Lyme disease (Figure 1). After establishing infection in the skin at the site of inoculation, the spirochete is capable of spreading locally (giving rise to a characteristic skin rash) and systemically, resulting in a variety of both tissue-specific (e.g., arthralgia, carditis, or neuropathy) and constitutional signs and symptoms. The pathogen is able to establish stable infection in one or more of these tissues, resulting in chronic manifestations of Lyme disease, most notably arthritis and neuroborreliosis.

Although the *I. scapularis*–*P. leucopus* infectious cycle has been established in the laboratory, laboratory mice are generally used in place of the natural hosts (reviewed in [1]). For the purposes of laboratory investigation, *B. burgdorferi* can also be transmitted to both ticks and mice through injection, and in the case of ticks, by immersion [5]. *B. burgdorferi* infection of laboratory mice results in disseminated infection, and, depending on the mouse strain, development of several of the human Lyme disease manifestations, such as arthritis and carditis. This mouse strain-dependent variation has permitted application of murine genetic approaches to the investigation of disease pathogenesis [6, 7].

Reflecting its complex zoonotic life cycle, *B. burgdorferi* must negotiate a multiplicity of interactions within its two hosts, tick and mammal. These interactions, along with the notable motility of this spirochete, likely facilitate dissemination and survival in different tissue environments, with a further degree of flexibility imposed by the requirement for *B. burgdorferi* to utilize a diversity of vertebrate species as hosts. For example, adherence to host cell surface or extracellular matrix molecules can contribute to colonization of tissues; other binding activities might essentially ‘cloak’ the bacteria by shielding *B. burgdorferi* antigens with host-derived molecules, thereby protecting the bacteria from recognition by the host defenses [8]. Spirochetal attachment to host molecules may also trigger specific signaling pathways in the mammalian cell or alter host defense responses such as complement activation, and thus profoundly impact the nature of the host environment experienced by the bacterium [9–11].

As might be predicted by the above considerations (and similar to many other bacterial pathogens), *B. burgdorferi* apparently uses a large and diverse set of adhesins (see Glossary), several of which have been experimentally shown to contribute to the ability of the bacterium to cause infection and disease in mice (Table 1). Many *B. burgdorferi* adhesins have been identified over time, some only in the past few years. Most have been characterized only regarding the primary adhesion activities of recombinant versions *in vitro*. Therefore, critical gaps in our understanding remain, such as what is the repertoire of *bona fide* adhesins expressed by *B. burgdorferi*? And how do the diverse adhesins of *B. burgdorferi* each contribute to the essential abilities of the bacterium to disseminate from the tick bite and establish infection in multiple sites in the mammalian host? Complicating investigation of these questions is the likelihood that subsets of these multiple adhesins have overlapping activities and/or have been evolutionarily selected for contributing to the survival of the borreliae in diverse hosts (arthropod, mammalian, and avian, for example). Thus, addressing these questions requires the use of diverse approaches including *in vivo* imaging at the cellular and whole animal levels, and harnessing the limited power of *Borrelia* genetics.

Genetic manipulation of *B. burgdorferi* is possible (but challenging), and several selectable markers are available (reviewed in [12]), as well as one counter-selectable marker [13]. Nevertheless, major hurdles face investigators in the field, namely low transformation efficiency and a highly segmented genome [12, 14–17]. Loss of genome segments, commonly referred to as plasmids, during *in vitro* cultivation, plagues the research community. However, recent advances, namely a multiplex PCR protocol and a Luminex

bead-based system [18, 19], have facilitated assessing, at least at the population level, the retention of the parental clone's genome segments in mutant derivatives. All of these developments have only recently allowed those studying *B. burgdorferi* to develop tools such as transposon mutant libraries that researchers in other systems have been using for years to decades [20]. In addition, the use of strains that have been extensively passaged in the laboratory, and have already lost many or most of the plasmids and adhesin-encoding genes, has enabled the generation of gain-of-function derivatives that have shed considerable light on the roles of some of the adhesins in a less complex background [21].

Finally, a picture is worth a thousand words and recently developed imaging technologies such as intravital microscopy and whole animal bioluminescent imaging have provided considerable information regarding the roles of *B. burgdorferi* adhesins and dissemination in living animals and ticks. These studies have documented *B. burgdorferi* interactions *in vivo* with host animal structures and cell types, and over the time course of infection, revealing subtleties not previously discernable [22–29].

The history: identification of *B. burgdorferi* adhesins

A number of different approaches have been used to identify *B. burgdorferi* proteins that bind to mammalian substrates, including mining the genome for orthologs of adhesins in other bacteria, ligand blots, activity-based purification, and selection of clones with binding activity (e.g. [30–35]). Each approach has strengths and weaknesses, but a critical gap that persists in the field is that *in vitro* activity has not in all cases been proven relevant *in vivo* at this point in time. Given the difficulties of genetic manipulation of *B. burgdorferi*, the first genetic investigation of the significance of an adhesin-substrate interaction *in vivo* involved the use of decorin-deficient mice to assess the role of decorin binding by *B. burgdorferi* [32, 33, 36]. Fortunately, as the knowledge of the biology and genetics of *B. burgdorferi* has increased, the elegance of investigations into adhesin activity and relevance *in vivo* is also increasing. However, for some adhesins there is significant functional and in some cases genetic redundancy, so considerable future work is required to untangle their roles. Table 1 summarizes the *B. burgdorferi* adhesins identified to date and what is known of their activities *in vitro* and *in vivo*. For the purposes of this review, we will focus on innovative approaches used to understand the roles of a select set of adhesins that function in the mouse model of infection. These approaches should be useful for future evaluations of other adhesins in both ticks and vertebrate hosts, and are broadly applicable to other pathogen–host interactions as well.

Our fundamental hypothesis is that the numerous cell-binding pathways encoded by *B. burgdorferi* play important roles during the multiphasic and multisystemic infection process. As mentioned above, a complicating factor is that some of these binding activities may be partially overlapping, consistent with the observation that several mutants of *B. burgdorferi* that lack a single binding pathway have displayed no dramatic infection phenotype. Due to the multifactorial nature of *B. burgdorferi* cell attachment mechanisms, investigation of a particular adhesive pathway in isolation is unlikely to provide a critical assessment of this model. Thus, an integrative and systematic analysis of a large set of *B. burgdorferi* cell attachment pathways is required to organize the apparent chaos of seemingly disparate mechanisms into functional groups with overlapping activities.

Genetic approaches to identification and evaluation of *B. burgdorferi* adhesins

Two factors have rendered a genetic screen-based search for *B. burgdorferi* mutants defective in adhesin technically challenging or not feasible. First, *B. burgdorferi* encodes a

plethora of adhesins identified *in vitro*, some of which have overlapping or redundant functions. Examples include sets of adhesins that bind to fibronectin, glycosaminoglycans, or the complement regulator factor H. These host molecules, in turn, interact relatively promiscuously with other host and pathogen molecules. Second, genetic manipulations of *B. burgdorferi* remain labor-intensive and slow, particularly as investigators have become more rigorous as the field has evolved. The identification of high-passage *B. burgdorferi* strains that have lost many or most of the plasmids, many of which encode adhesins, enables a gain-of-function approach, which has proven critical in the identification of adhesin substrates and delineation of adhesin sequences required for function [21, 37, 38]. The strength of this approach is that it circumvents the confounding issue of functional redundancy of multiple *B. burgdorferi* adhesive pathways.

A critical prerequisite for the application of these genetic approaches is the identification of candidate adhesins. Lyme disease investigators have traditionally searched for adhesins by assessing the ability of spirochetes grown *in vitro* to bind cultured mammalian cell monolayers or extracellular matrix components (ECM). Biochemical approaches led to the identification of three well-characterized adhesins, the fibronectin-binding BBK32 and the decorin-binding proteins DbpA and DbpB [32, 35]. As genetic systems became available, these proteins were demonstrated to have roles *in vivo*. Mutants in *bbk32* show some attenuation in mice at low infectious doses [22, 39]. Mutants in the locus encoding DbpA and DbpB show more marked attenuation in mice, particularly at early stages of infection [22, 40, 41]. These results were confirmed in the recent screen of a signature-tagged transposon mutant library [20]. The gain-of-function approach was used to generate *B. burgdorferi* strains expressing DbpA, DbpB, and BBK32 as well as mutant versions or alleles originating from different *Borrelia* strains [24, 25, 37]. This experimental tactic demonstrated that both BBK32 and DbpA are bifunctional adhesins, each of them capable of binding to both glycosaminoglycans (GAGs) and to fibronectin (Fn) and decorin, respectively [21, 37, 38]. This work also demonstrated that the DbpA and DbpB proteins have distinct GAG-binding specificities.

Additional approaches in the hunt for *B. burgdorferi* adhesins have included searching the genome for orthologs of known adhesins of other bacteria, for motifs that are present in known adhesive proteins [9, 42], or testing *B. burgdorferi* proteins known to be exposed on the bacterial surface for the ability to bind to mammalian substrates [43]. For example, RevA and RevB, upon their identification as surface-exposed proteins, were shown to bind Fn [43]. However, the approach of testing surface proteins for adhesin activity has also identified a number of proteins that bind to notoriously sticky host proteins, e.g. plasminogen/plasmin, whose biological significance remains undetermined.

Another approach, phage display, which does not rely on homology to adhesin genes of other bacteria, or adhesin expression during *in vitro* culture, was used to identify additional adhesins of *B. burgdorferi*. This approach obviated concerns regarding homology assignments based on solely DNA sequence (as opposed to function), and concerns that *B. burgdorferi* gene expression *in vitro* is considerably different from that in the mouse or in the tick (reviewed in [44]). A *B. burgdorferi* phage display library was used to select for protein fragments encoded in the *B. burgdorferi* genome for adhesion to integrin $\alpha_5\beta_3$ and to the vasculature in living mice [30, 31, 45]. The integrin-binding adhesin P66 was selected using both approaches, and is essential for infection in mice, but not ticks [46]. OspC, which was previously demonstrated to be an important *B. burgdorferi* virulence factor [47], was identified as a candidate adhesin using this approach, the first hint of a possible biochemical activity for this protein. The *in vitro* binding of recombinant OspC to mammalian cells in culture confirmed the adhesion activity of the bacteriophage clones [30]. Thus, the different approaches used to identify *B. burgdorferi* adhesins have yielded a number of different

candidates, so it is particularly important to determine the importance of these proteins *in vivo*.

Imaging illuminates the roles of *B. burgdorferi* adhesins *in vivo*

Two methods of imaging have recently been applied to understanding the roles of *B. burgdorferi* during infection of live animals. The first method discussed here, bioluminescent imaging, allows the course of infection to be followed over time in a single animal, rather than euthanizing and harvesting tissues from multiple animals at the intervals of interest. *B. burgdorferi* strains expressing luciferase under the control of a constitutive promoter were recently used to provide new insights into the importance of the BBK32 and Dbp adhesins during infection of mice [22]. A *B. burgdorferi* *bbK32* knockout strain was mildly attenuated, giving rise to lower bacterial loads in different tissues at different times of infection. The effects were more apparent at a lower and likely more physiologically relevant dose. The *dbpBA* knockout was essentially avirulent in this bioluminescence study, whereas conventional analysis of a *dbpBA* mutant utilizing culture positivity or quantitative PCR as readouts revealed residual infectivity [40, 41, 48]. It is possible that the already significantly attenuated *dbpBA* knockout strain had a high fitness cost due to luciferase expression under the control of the constitutive promoter. Alternatively, differences in the mouse strains utilized for the two types of studies may explain differences in the tissue burden of bacteria, as Balb/C mice, which were used for the bioluminescent imaging analysis, are more resistant to colonization and pathology than C3H mice, which were used in the conventional infectivity studies [6, 49]. Nevertheless, this approach provided new insights into the roles of BBK32, DbpA, and DbpB in the kinetics of *B. burgdorferi* dissemination from the site of inoculation and the transient or stable nature of tissue colonization [22].

The second imaging technology, intravital microscopy, has allowed studies to move from assessing bacterial populations in a whole animal to assessing the interactions of single bacterial cells with host cells at high resolution. Whereas all previous cell attachment studies relied on endpoint analysis of cell binding *in vitro*, pioneering application of high-resolution intravital microscopy to the field of bacterial pathogenesis has opened a new approach to study a variety of pathogenic processes in their normal milieu [23–25]. Intravital microscopy allows for direct real-time visualization of a pathogen within a living animal. This approach permits, for the first time, insight into the relationship of a specific adhesin and specific events during colonization. Intravital microscopy has also been used to image *B. burgdorferi* motility in mouse skin [28] and *in situ* microscopy on isolated tick midguts and salivary glands has been used to study *B. burgdorferi* dissemination within the tick [26, 27]. Intravital imaging has also allowed for investigation of *B. burgdorferi* with cells of the host immune system [29].

In the case of vascular adhesion, the process being studied is subject to the tremendous shear forces of circulating blood and is akin to a spider trying to gain a foothold on the wall of a garden hose with the tap turned on full. Accurately reproducing such a scenario outside of the animal is difficult, if not impossible. The intravital approach allows for not only direct visualization, but quantitative analyses of recorded images of distinct modes of vascular interaction by the spirochete also provide mechanistic insights into pathogen–host interactions.

Vascular adhesion of *B. burgdorferi* in post-capillary venules has been studied using infectious green fluorescent spirochetes in the microvasculature of mouse flank skin and knee joint [23–25]. Thus far microscopic imaging has been limited to the initial interactions with the vasculature (Figure 2), but even this seemingly simple process turns out to be

incredibly complicated, and involves multiple steps (Figure 3). An insight that has already been gained from these studies is that penetration of *B. burgdorferi* across a vessel wall shares similarities with the migration of inflammatory cells across the same barrier [50]. Three types of spirochete interactions with the host vasculature were observed [23]. The shortest interactions (tethering) were defined as those interactions where the spirochete traversed 100 μm along the vessel in less than one second. Dragging interactions (1–20 seconds to traverse 100 μm) reflected bacterial creeping or crawling along the vessel wall, and stationary adhesions reflected spirochetes that were planted at a single site for 20 seconds or longer. In addition a small number of spirochetes were caught in the act of transmigrating across the vessel wall, i.e. extravasating. It is intuitively attractive to postulate, but not yet proven, that tethering, dragging, stationary adhesion and extravasation are temporally ordered steps during tissue colonization. Notably, tethering, dragging and stationary adhesions were observed in greatly reduced numbers in a non-infectious high passage *B. burgdorferi* strain, giving evidence for a biological role for these interactions.

Spirochetes displaying short-term interactions, i.e., tethering and dragging, were localized primarily (~90%) to cells, while stationary adhesions were found predominantly (70%) at cellular junctions, suggesting a fundamental difference in the mechanism of interaction [23]. The spirochetes observed transmigrating across the vessel wall were also confined to endothelial cell-cell junctions [23]. Furthermore, a mechanistic difference between tethering and dragging interactions was suggested by differential inhibition of dragging, but not tethering by GAGs, implicating these macromolecules as host ligands in vascular adhesion, with an important role at the dragging stage [25]. Host Fn was also found to contribute to *B. burgdorferi*–microvascular interactions; anti-Fn antibody inhibited tethering, dragging and stationary interactions. Moreover, the GAG-binding portion of Fn (the HepII peptide) but not the RGD-containing integrin-binding sequences were required for vascular interactions, indicating that Fn–GAG interactions, but not Fn–integrin interactions, are important in spirochetal–vascular adhesion.

Non-infectious high passage *B. burgdorferi* strains that do not interact efficiently with the vasculature provided a genetic spirochetal background for attempts to identify important adhesins by reconstituting vascular interactions through the construction of gain-of-function strains. Expression of the Fn- and GAG-binding protein BBK32 in the high passage strain completely restored tethering and dragging interactions to wild-type levels (quantified as described above) and re-established stationary adhesions (number seen per minute) to about 50% of the wild type frequency, identifying the first bacterial protein shown to mediate vascular interactions *in vivo* [25]. In contrast, a *B. burgdorferi* strain with a disrupted *bbk32* gene displayed only a small decrease in vascular adhesion, suggesting that BBK32 is not the only bacterial adhesin involved in promoting interactions between *B. burgdorferi* and the host vasculature, consistent with the observed redundancy of binding pathways encoded by this spirochete. However, the known Fn-binding adhesins RevA, RevB and the putative Fn-binding adhesin BB0347 did not confer significant increases in vascular adhesion in gain-of-function strains, suggesting that under shear flow, these proteins are not major players in vascular adhesion [24]. Therefore, while BBK32 appears to be the predominant Fn-binding vascular adhesin in *B. burgdorferi*, other adhesins that remain to be identified can contribute to vascular adhesion *in vivo* [24].

Recently, intravital microscopy coupled with genetic manipulation of the *bbk32* locus has been instrumental in defining the role of sequential BBK32 interactions with host Fn and GAGs in the vascular adhesion process [24]. Interactions of *B. burgdorferi* carrying mutated BBK32 variants specifically defective in either Fn or GAG binding were visualized (Figure 4). The results indicated that high affinity BBK32–Fn interactions modulate the short-lived tethering step of vascular adhesion and provide the molecular Velcro® to slow and stick

circulating spirochetes to vascular surfaces. This allows subsequent BBK32–GAG interactions of greater stability to be established, resulting in dragging interactions. Additional macromolecular interactions and mechanistic features required to establish stationary adhesions and to promote transmigration can now be investigated by systematic reconstruction of interactions that lead to the stable adherence required for tissue colonization by spirochetes in the bloodstream.

One caveat for the use of intravital microscopy to study vascular interactions is that the requirement for intravenous injection of large numbers of laboratory-grown *B. burgdorferi* does not strictly mirror the natural tick-transmitted process. Nonetheless, the results obtained seem to reflect biologically relevant events. For example, the attenuated colonization of BBK32 mutants observed after intradermal inoculation is reflected in the attenuated vascular interactions observed by intravital microscopy [22, 24, 25, 39]. Moreover, intravital imaging has revealed a wealth of mechanistic information on vascular interactions in a natural milieu that mimics the *bona fide* infectious process more accurately than *in vitro* studies, and this approach has greatly expanded our knowledge of essential steps in the colonization process.

Concluding remarks

A variety of biochemical and genetic approaches has identified a large collection of *B. burgdorferi* adhesins. Now a central challenge is to begin to functionally organize the universe of putative Lyme disease adhesins (Box 1). Functional definitions should extend beyond the simple characterization of substrate and cell attachment specificities to encompass detailed insight into the role of each adhesin in tissue colonization and survival within the mammalian host. A comprehensive approach to this ambitious problem will require the analyses of both gain-of-function and loss-of-function *B. burgdorferi* strains, and the combination of biochemical studies to define interactions and animal infection studies to test the significance of such interactions *in vivo*. The recent development of techniques to visualize spirochetal interactions with host tissue during infection of living animals has opened up new avenues to gain fundamental mechanistic insight into the spirochete–host interactions critical for disease. Ultimately, an integrated understanding that links the biochemical activities of each adhesin to the remarkable ability of *B. burgdorferi* to overcome host barriers and establish persistent, disseminated infection will reveal strategies to mitigate the human cost of Lyme disease.

Box 1

Outstanding Questions

- What are the best strategies to identify adhesins important in infection of animals and ticks as opposed to in the laboratory?
- How do adhesins, presumably acting in concert and/or in series, promote the complex steps required during spirochetal dissemination to multiple tissues?
- Do different adhesins contribute to colonization of different tissues?
- Are different adhesins important in different host species?
- Are dissemination mechanisms used by *B. burgdorferi* applicable to other pathogens?

Glossary

Adhesin	a bacterial protein that mediates attachment to a host molecule, whether that molecule is on the surface of the host cell, in the extracellular matrix, or is a soluble protein
Dragging interactions	<i>in vivo</i> interactions where <i>B. burgdorferi</i> traversed 100 µm along a blood vessel in 1–20 seconds
Extravasation	the process of exiting a blood vessel, e.g. bacteria or immune cells crossing the endothelial cell layer
Intravital microscopy	a high-resolution method to visualize biological processes in a living animal
Stationary adhesions	<i>in vivo</i> interactions where <i>B. burgdorferi</i> were adhered at a single site on a blood vessel for 20 seconds or longer
Tethering interactions	<i>in vivo</i> interactions where <i>B. burgdorferi</i> traversed 100 µm along a blood vessel in less than one second
Transmigration	the process of moving across a barrier, e.g. bacteria or immune cells crossing a layer of epithelial or endothelial cells
Zoonotic infection/zoonosis	an infection normally circulating among animals

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Highlights

- Innovative approaches contribute to identification and characterization of *Borrelia burgdorferi* adhesins.
- Novel techniques are required to define the importance of the large set of *B. burgdorferi* adhesins during infection.
- Intravital microscopy and bioluminescent imaging will contribute to assessment of the roles of *B. burgdorferi* adhesins during tissue colonization.

Figure 1.

The course of *Borrelia burgdorferi* infection and Lyme disease in humans. Certain strains of laboratory mice also develop arthritis and carditis.

Figure 2.

Intravital microscopy (IVM) of GFP-labeled *B. burgdorferi* in a post-capillary venule of mouse skin. Imaging was performed with a spinning disk laser confocal microscope. The blood vessel (red) was stained with PECAM-1 antibody conjugated to AlexaFluor555. The scale bar is 49 μm. This figure is reproduced from [25].

Figure 3.

Summary of the current state of knowledge regarding microvascular interaction and transmigration by *B. burgdorferi*. This figure is modified from [23] and reproduced with permission from [24].

Figure 4.

Two-step model for BBK32-mediated vascular adhesion. The left inset depicts the tethering step mediated by high affinity BBK32–Fn interactions through the Fn-binding domain. The right inset depicts dragging interactions mediated through BBK32–GAG binding sequences. This figure is reproduced with permission from [24].

Table 1

In vitro and *in vivo* activities of *B. burgdorferi* adhesins known or predicted to be exposed on the bacterial surface

Adhesin category and name	Genetic locus	<i>In vitro</i> adhesin activity	<i>In vivo</i> activity	Refs
Tick adhesins				
Osp (outer surface protein) A	<i>bbA15</i>	Binds to TROSPA (tick receptor for OspA)	Required for infection of ticks	[51–53]
OspC	<i>bbB19</i>	Binds to salivary protein (Salp) 15	Mutant disseminates in tick but does not survive in mammal	[26, 47, 54–56]
Mammalian complement regulator-binding adhesins				
CRASP (complement regulator-acquiring surface protein) CRASP-1 (CspA) CRASP-2 (CspZ) CRASP-3 (ErpP) CRASP-4 (ErpC) CRASP-5 (ErpA)	<i>bbA68, bbH06</i>	Bind to factor H and/or FHR-1 (factor H related) and/or FHL (factor H-like); CRASP-1 also binds some collagens, fibronectin, laminin, and plasminogen	Not determined	[57–60]
Erp (OspEF-related protein) A, C, P	<i>bbP38, bbI39, bbN38</i>	Bind to factor H and/or FHR-1 and/or FHL and plasminogen	ErpA mutants show slight attenuation in mice	[20, 57–59, 61]
Mammalian extracellular matrix-binding adhesins				
BBK32	<i>bbK32</i>	Binds fibronectin and glycosaminoglycans	Mutants show mild attenuation in mice, restores adhesive properties to high passage B31, distinct roles for vascular interactions for GAG and fibronectin-binding domains	[20, 22, 24, 25, 35, 38, 39, 42, 62–64]
Dbp (decorin-binding proteins) A and B	<i>bbA24, bbA25</i>	Bind decorin and dermatan sulfate glycosaminoglycans with somewhat different specificities	Mutants in operon show significant attenuation in mice but primarily early in infection	[20, 21, 40, 41, 48]
Bgp (<i>Borrelia</i> glycosaminoglycan-binding protein)	<i>bbO588</i>	Binds glycosaminoglycans and has nucleosidase activity	Attenuated in mice	[20, 65]
RevA	<i>bbM27, bbP27</i>	Binds fibronectin	Transposon mutant in <i>bbM27</i> is attenuated in mice but in <i>bbP27</i> is not, no role in vascular interactions in mice	[20, 24, 43]
RevB	<i>bbC10</i>	Binds fibronectin	No role in vascular interactions in mice, mild attenuation in mice	[20, 24, 43]
BB0347	<i>bbO347</i>	Not determined	No role in vascular interactions in mice	[24]
ErpX	<i>bbQ47</i>	Binds laminin	Not determined	[66]
Mammalian cell surface protein-binding adhesins				
P66	<i>bbO603</i>	Binds α_3 -chain integrins	Essential for infection of mice but not ticks, selected <i>in vivo</i> for adherence to vascular endothelium	[30, 31, 46]

Adhesin category and name	Genetic locus	<i>In vitro</i> adhesin activity	<i>In vivo</i> activity	Refs
BBB07	<i>bbB07</i>	Binds α_1 -chain integrins	Attenuated in mice	[9, 20]
Candidate adhesins				
OspC	<i>bbB19</i>	Binds mammalian cells	Required for initial infection of mice, and for colonization of certain tissues, selected <i>in vivo</i> for adherence to vascular endothelium	[20, 30, 47, 55, 56, 67]
VlsE	<i>bbF32</i>	Binds to mammalian cells	Required for later stages of infection in mammals; selected <i>in vivo</i> for adherence to vascular endothelium, transposon mutant attenuated in mice	[20, 30, 68–70]
Lmp1	<i>bb0210</i>	Not determined	Selected <i>in vivo</i> for adherence to vascular endothelium, required for persistent infection in mammals	[30, 71, 72]
Bmp family	<i>bb0382-0385</i>	Not determined	BmpD was selected <i>in vivo</i> for adherence to vascular endothelium, BmpC mutant attenuated in mice	[20, 30]
OspF family (OspF and G, ErpK and L)	<i>bbM38, bbO39, bbR42, bbS41</i>	ErpK binds mammalian cells	Selected <i>in vivo</i> for adherence to vascular endothelium, some transposon mutants attenuated in mice	[20, 30]