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Adhesion Mechanisms of *Borrelia burgdorferi*

Styliani Antonara, **Laura Ristow**, and **Jenifer Coburn**

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

Jenifer Coburn: jcoburn@mcw.edu

Abstract

The *Borrelia* are widely distributed agents of Lyme disease and Relapsing Fever. All are vector-borne zoonotic pathogens, have segmented genomes, and enigmatic mechanisms of pathogenesis. Adhesion to mammalian and tick substrates is one pathogenic mechanism that has been widely studied. At this point, the primary focus of research in this area has been on *Borrelia burgdorferi*, one agent of Lyme disease, but many of the adhesins of *B. burgdorferi* are conserved in other Lyme disease agents, and some are conserved in the Relapsing Fever *Borrelia*. *B. burgdorferi* adhesins that mediate attachment to cell-surface molecules may influence the host response to the bacteria, while adhesins that mediate attachment to soluble proteins or extracellular matrix components may cloak the bacterial surface from recognition by the host immune system as well as facilitate colonization of tissues. While targeted mutations in the genes encoding some adhesins have been shown to affect the infectivity and pathogenicity of *B. burgdorferi*, much work remains to be done to understand the roles of the adhesins in promoting the persistent infection required to maintain the bacteria in reservoir hosts.

3.1 Introduction

The spirochetes in the genus *Borrelia* cause relapsing fever and Lyme disease. This chapter focuses on the Lyme disease agents, and primarily on a single species, *Borrelia burgdorferi*, as this organism has been the primary object of study and the focus of relatively recent advances in approaches to understanding how these organisms cause infection and, in susceptible hosts, disease. *B. burgdorferi* is normally maintained in mammalian reservoir hosts and tick vectors, and the mechanisms by which *B. burgdorferi* causes infection remain poorly understood. In fact, how *B. burgdorferi* causes disease has been more thoroughly characterized by manipulation of the host rather than of the bacterium. Relatively recent advances in the genetic approaches that are possible in this organism have started to turn this tide, and have been applied to understanding the in vivo significance of the numerous adhesins that have been identified through in vitro studies.

There are a few oddities of *B. burgdorferi* that warrant introduction. First, the genome is relatively small, at approximately 1.5 Mbp, but is highly segmented, as approximately one third of the annotated genes are encoded on circular and linear plasmids (Fraser et al., 1997; Casjens et al., 2000). One of the “plasmids” is better thought of as a small chromosome

Correspondence to: Jenifer Coburn, jcoburn@mcw.edu.

(Byram et al., 2004). Second, *B. burgdorferi* encodes a large repertoire of lipoproteins, with approximately 7.8% of the genome encoding known or predicted lipoproteins (Setubal et al., 2006). Some of these lipoproteins have been identified as adhesins, but not all of the adhesins are lipoproteins. Finally, given the comparatively small genome size, a relatively large number of proteins that bind to mammalian or tick cells or extracellular matrix have been identified, and some of these have additional functions that may contribute to the life style of the bacterium.

Various laboratories have shown that *Borrelia burgdorferi* binds to an array of eukaryotic cells in vitro (Coburn et al., 1993; Comstock et al., 1993; Hechemy et al., 1989; Thomas and Comstock, 1989) and to components of the extracellular matrix (Guo et al., 1995; Leong et al., 1995, 1998a, b; Isaacs, 1994). Further studies have identified receptors on the surface of mammalian cells and particular molecules of the extracellular matrix to which the bacteria attach, and the *B. burgdorferi* proteins that serve as adhesins interacting with these molecules. Table 3.1 lists *Borrelia burgdorferi* adhesins, both known and candidate ones, as well as additional information on their respective host cell substrates and roles in *B. burgdorferi* infection. In this section, ECM-binding proteins will be reviewed; in later sections, those that bind to molecules specifically expressed on the mammalian cell surface, and those that bind to unknown substrates, will be described.

3.2 *Borrelia burgdorferi* Proteins That Promote Interaction with the Extracellular Matrix

3.2.1 Attachment to Fibronectin

Fibronectin (Fn) is present in both soluble and insoluble extracellular matrix forms, and is targeted by many bacterial adhesins due to its ubiquity, its multiple distinct functional binding domains, and its ability to interact with multiple substrates. These may also assist bacterial pathogens in establishment of infection. In normal physiology, Fn binds to several integrins and to other extracellular matrix components including collagen, fibrinogen and some proteoglycans. It plays a major role in cell adhesion, growth, migration and differentiation, and it is important for processes such as wound healing and embryonic development (reviewed in Kadler et al., 2008).

B. burgdorferi produces several Fn-binding adhesins (Table 3.1). Early work suggesting Fn binding activity by Szczepanski et al. (1990) and Grab et al. (1998) led to the identification of the best-characterized Fn-binding adhesin of *B. burgdorferi*, BBK32 (Probert and Johnson, 1998). The Fn-binding region of BBK32 was identified as a region of 32 amino acids that was common to all *Borrelia* strains tested (Probert et al., 2001). Elegant structure-function analyses revealed that BBK32 shares a mechanism of binding to Fn with Fn-binding adhesins of the Gram-positive pathogens *Staphylocccus aureus* and *Streptococcus pyogenes* (Probert et al., 2001; Raibaud et al., 2005). It also promotes the aggregation of plasma Fn to superFn (a higher order multimer of fibronectin) (Prabhakaran et al., 2009), supporting a role for this and other specific bacterial adhesins that goes beyond simple attachment. BBK32 is expressed by *B. burgdorferi* as the tick feeds and while the bacteria are in the mammalian host (Fikrig et al., 2000). Consistent with this pattern of expression,

deletion of *bbk32* increases the ID_{50} in mice (Seshu et al., 2006), but at high dose does not significantly attenuate virulence or tick colonization (Li et al., 2006).

Given that BBK32 deficient mutant strains still bind Fn, the identification of additional Fn-binding proteins came as no surprise. In fact, three additional Fn-binding proteins have been identified (Brissette et al., 2009a). The first of these proteins, RevA, was previously studied by other groups on the basis of differential gene expression in mammalian versus tick environments (Gilmore and Mbow, 1998; Carroll et al., 2001). In some *B. burgdorferi* strains, a second copy of *revA* is present on a different plasmid. A related gene, *revB*, has also been identified and, like recombinant RevA, recombinant RevB also binds Fn. RevA binds Fn with a slightly lower affinity than BBK32, but the affinity of RevB binding to Fn has yet to be determined (Brissette et al., 2009a). Experimental evidence for RevB binding indicates a more complex interaction with Fn in contrast to the dose-dependent binding of RevA to Fn (Brissette et al., 2009a). In addition, the function of a gene annotated in the genome sequence as encoding a putative Fn-binding protein, bb0347, has not been experimentally addressed. In the future, generation of *B. burgdorferi* strains in which the genes encoding Fn-binding proteins are inactivated could illuminate the roles of all of these proteins in the life cycle of *B. burgdorferi*.

3.2.2 Attachment to Decorin

Decorin is a proteoglycan that consists of a protein core composed of leucine rich repeats, and a glycosaminoglycan (GAG) chain consisting of either chondroitin sulphate or dermatan sulphate depending on the tissue in which it is expressed (Mcewan et al., 2006). Decorin is a component of connective tissue, binds to type I collagen and Fn and plays a role in matrix assembly. In addition to playing a role in the formation of the structural components of the extracellular matrix, the decorin core protein functions as a signaling mediator by interacting with the epidermal growth factor receptor (reviewed in Seidler and Dreier, 2008).

Due to the observations by several groups that *B. burgdorferi* is frequently seen in connective tissues in infected mammals and apparently in contact with collagen fibers, early investigations focused on direct adhesion to collagen. When no direct attachment to immobilized collagen was observed, attachment of the bacteria to the collagen-associated decorin was investigated. Two decorin-binding adhesins (DbpA and DbpB) were identified; the proteins are encoded on one of the linear plasmids in a bicistronic operon (Guo et al., 1998, 1995). A *dbpAB* mutant was deficient in colonization of the skin and other tissues, even in mice lacking adaptive immunity, suggesting that DbpA and DbpB proteins help the bacteria adhere to multiple tissues and are required for successful interactions with both innate and adaptive immune mechanisms (Weening et al., 2008). Both proteins were found to be important for the virulence of *Borrelia burgdorferi* in mice, yet contribute differently to colonisation and dissemination to various tissues (Shi et al., 2008a, b). Consistent with this, DbpA and DbpB also have distinct in vitro adhesion activities (Fischer et al., 2003). While the initial studies suggested that the Dbps require the intact proteoglycan for adhesion, subsequent studies indicated that these proteins also bind to glycosaminoglycans (GAGs) in the absence of the core protein (Fischer et al., 2003; Guo et al., 1995). Lysine residues (Lys-82, Lys-163, Lys-170) critical for decorin binding have been identified

(Brown et al., 1999; Pikas et al., 2003). The decorin-binding activity is uniquely tractable to investigation from the host perspective, as decorin-deficient mice were actually the first mutants used to investigate the role of any candidate virulence factors in *B. burgdorferi*. In decorin deficient mice, there were fewer bacteria present in hind tibiotarsal joints at low doses of the bacteria, and the arthritis was less severe (Brown et al., 2001).

Later work by a different group showed that *B. burgdorferi* does, in fact, bind to type 1 collagen lattices (Zambrano et al., 2004). This work highlights the importance of the purification methods in determining whether bacterial adhesion occurs to a particular substrate. Extracellular matrix proteins have limited solubility, and the plasma forms of some of these proteins, while soluble, may behave differently. At this point, the adhesin(s) responsible for collagen binding remain unknown.

3.2.3 Attachment to Glycosaminoglycans

While decorin is a proteoglycan, additional evidence for a more generalized proteoglycan (PG) binding activity of *B. burgdorferi* was also established. Binding to PGs accounts for some, but not all, of the *B. burgdorferi* cell attachment activity, depending on the cell line and bacterial strain examined. The PG-binding activity is largely determined by recognition of glycosaminoglycan (GAG) chains (Leong et al., 1995, 1998a, b). A *Borrelia* glycosaminoglycan binding protein (Bgp) was identified by Parveen and colleagues (Parveen and Leong, 2000) and shown to be surface-exposed in intact bacteria. The recombinant protein agglutinates erythrocytes, binds to the same glycosaminoglycans as whole bacteria, and competitively inhibits binding of *B. burgdorferi* to mammalian cells. Another previously identified *B. burgdorferi* adhesin, BBK32, which binds to Fn, was also found to bind to purified preparations of dermatan sulphate and heparin (Fischer et al., 2006). The fact that exogenous heparin had no effect on the binding affinity of the BBK32-expressing bacteria to Fn suggested that BBK32 can bind to multiple molecules independently, or that binding to soluble heparin is relatively weak. Different *B. burgdorferi* strains have different cell- and GAG-binding preferences, and binding to various cell types depends in part on the GAGs they express (Parveen and Leong, 2000). Host-adapted spirochetes show enhanced binding to GAGs (Parveen et al., 2003) suggesting their importance during in vivo infection. However, Bgp, BBK32, and DbpAB mutants of *B. burgdorferi* are all infectious in mice (Seshu et al., 2006; Shi et al., 2008a; Blevins et al., 2008; Weening et al., 2008; Parveen et al., 2006) although in some cases slightly attenuated, suggesting that these different proteins may be functionally redundant.

3.2.4 Attachment to Laminin

Laminin is a trimeric glycoprotein that is a component of extracellular matrices, playing an integral role in forming structural scaffolding in almost every tissue. Attachment to laminin has been demonstrated for many bacterial pathogens, including *B. burgdorferi*. The four paralogous Bmp proteins were found to bind laminin (Verma et al., 2009), as was one of the Erps (OpsE-related proteins, see below), ErpX (Brissette et al., 2009b). The four *bmp* genes are located on the chromosome and, although they are arranged in a cluster, they are differentially regulated (Bryksin et al., 2005; Dobrikova et al., 2001). Two have been shown to contribute to the development of arthritis in the mouse model of infection (Pal et al.,

2008). One of the Bmp proteins, BmpD, was selected for binding to vascular endothelium in mice by in vivo phage display (Antonara et al., 2007). Since laminin is a basement membrane protein, BmpD may also recognize additional mammalian substrates. The fragment of BmpD that was selected, however, contained sequences 3′ of the stop codon of the allele in the sequenced strain, B31. Thus it is possible that this fragment is available for binding mammalian substrates only in certain strains.

3.2.5 Erps and CRASPs: Binding to Complement-Regulatory Proteins Factor H and FHL

It has been shown that a large family of outer surface proteins, the Erps, is involved in the binding of host proteins. The Erps are encoded by a large multi-gene family, with different *erp* genes encoded on different plasmids, particularly the cp32 family of plasmids. In earlier literature in the field, these genes were known as *uhb* (upstream homology box) genes, due to the highly homologous 5′ non-coding regions (Marconi et al., 1996; Sung et al., 1998). Indeed, the Erp genes appear to be expressed in similar patterns, although there may be variations in timing and tissue-specific expression in some cases (Mcdowell et al., 2001; Stevenson, 2002). The Erp proteins can be divided into different functional categories: ErpX, which binds laminin, several that bind complement factor H and related complement regulators, and those for which adhesion activity has been demonstrated but the substrate(s) remain undefined. In general, Erp proteins are expressed during mammalian infection but are repressed during colonization of the tick (reviewed in Brissette et al., 2008).

The ErpA, ErpC and ErpP from various *Borrelia burgdorferi* strains (Alitalo et al., 2002; Hellwage et al., 2001; Metts et al., 2003) show significant affinity for factor H. Factor H is a complement regulatory protein that circulates in the human plasma and is bound to host cells, and protects host cells from attack by the complement system. Other bacteria have been shown to bind to factor H and in that way evade the complement activation which can lead to killing of the bacteria by opsonization (reviewed in Sjöberg et al., 2009). In addition, binding of factor H can help bacteria adhere to host cells since it binds to glycosaminoglycans that are present on the surface of cells (Brissette et al., 2008).

Complement regulator acquiring surface protein (CRASP)-1 and -2 function to evade the host immune system by binding components of the host immune system, factor H-like protein 1(FHL-1) and factor H (Alitalo et al., 2001; Hellwage et al., 2001; Kraiczy et al., 2001a, b; Mcdowell et al., 2001; Wallich et al., 2005). FHL-1 is encoded by the same gene as factor H, and has redundant function in controlling the alternative pathway of complement activation within host innate immunity, but has a unique 4 amino acid extension at the C-terminus (Kraiczy et al., 2001b). Both CRASP-1 and -2 bind to a region of factor H or FHL-1 that is buried in the unbound protein, but exposed when the C-terminal region is bound. Several Erps bind the C-terminal end of factor H, and so it is possible that CRASPs and Erps act in a complementary fashion (reviewed in Brissette et al., 2008). Strains completely deficient in Erps will need to be created in order to test the function of CRASPs alone, and vice versa. Recently, CRASP-1 has been shown to bind additional host components, including Fn, laminin, plasminogen and several types of collagen (Hallstrom et al., 2010). These additional functions expand the role of CRASP-1 beyond evasion of the immune system, and potentially into aiding colonization in the mammalian host.

In addition, a distinct subset of the Erps, those belonging to the OspF subfamily, were selected by in vivo phage display as candidate adhesins to the vasculature endothelium (Antonara et al., 2007). These are BBM38 (ErpK), BBO39 (ErpL), BBK2.10 and BBS41 (OspG), and none have been shown to bind factor H. Although the Erp proteins that were selected vary in sequence, they share significant common features. A recombinant protein expressing the selected portion of ErpK, which is also common to BBK2.10 and OspG, showed specific binding to the surface of particular cell lines, suggesting that the selected sequence may be responsible for binding of members of the OspF family to the surface of mammalian cells (Antonara et al., 2007).

3.2.6 Attachment to Mammalian Cell Surface Receptors

3.2.6.1 Attachment to Integrins—Although some of the substrates for *B. burgdorferi* attachment discussed above are also associated with the mammalian cell surface, this section will cover those substrates that are specific to the cell surface and not found in the extracellular matrix or body fluids. Integrins are obligate heterodimeric cation-dependent receptors, found on the surface of all mammalian cells except erythrocytes. They consist of two distinct chains: the alpha (α) and beta (β) subunits. The specificity of each integrin depends on the combination of the α and β subunits, but some bind multiple ligands. As is the case for several other pathogens, *B. burgdorferi* binds to integrins, including $\alpha_{IIb}\beta_3$ (Coburn et al., 1993), $\alpha_v\beta_3$, $\alpha_5\beta_1$ (Coburn et al., 1998), and $\alpha_3\beta_1$ (Behera et al., 2006). Different Lyme disease *Borrelia burgdorferi* strains bind preferentially to different integrins (Coburn et al., 1998). Binding of the bacteria to $\alpha_3\beta_1$ on chondrocytes activates inflammatory responses in a TLR-independent manner.

The *B. burgdorferi* ligand for the β_3 chain integrins is an outer surface integral membrane protein, P66 (Coburn et al., 1999; Coburn and Cugini, 2003). The principal ligand for integrin $\alpha_3\beta_1$ appears to be a putative outer surface protein, BBB07. Like intact *B. burgdorferi*, BBB07 stimulates human primary chondrocytes through integrin $\alpha_3\beta_1$ to secrete proinflammatory cytokines (Behera et al., 2008). While P66 also stimulates proinflammatory responses by chondrocytes, this does not require $\alpha_3\beta_1$.

3.2.7 Candidate Mammalian Substrate Adhesins of *B. burgdorferi*

Several additional candidate *B. burgdorferi* adhesins have been identified, but not yet validated as adhesins using additional approaches; for example, the putative mammalian receptors have not yet been identified. In each case, however, other work has demonstrated the proteins to be important in *B. burgdorferi* infection in mammals, specifically. All were enriched after in vivo selection for binding to vascular endothelium in living mice, but adhesion activity has not yet been demonstrated to be critical to their importance in the life of *B. burgdorferi*. One, OspC, was shown to be critical for *B. burgdorferi* to establish disseminated infection (Grimm et al., 2004a; Tilly et al., 2006). A second, VlsE, is better known for antigenic variation during infection (Zhang and Norris, 1998a, b; Lawrenz et al., 1999; Lin et al., 2009; Dresser et al., 2009). Finally, BB0210, annotated as Lmp1, was also highly selected, and is required for persistent infection in mice (Yang et al., 2009).

3.2.8 Interaction of *Borrelia burgdorferi* with the Arthropod Host

As mentioned above, *Borrelia burgdorferi* spends a large portion of its life cycle in the arthropod host. It has been shown that certain genes are expressed during the mammalian life cycle, and others are expressed when the bacteria are in the tick. Some of the genes expressed in the tick portion of the life cycle encode outer surface proteins, and the roles of the corresponding proteins in the tick have been investigated. One such protein, OspA, was shown to be an adhesin for tick tissues, and to bind to the "tick receptor for OspA" (TROSPA) (Pal et al., 2004). OspA can bind specifically to recombinant TROSPA in vitro, while in vivo studies showed that disruption of TROSPA by RNA interference diminished the ability of the ticks to acquire *Borrelia burgdorferi* from infected mice (Pal et al., 2004). A small number of *B. burgdorferi* were found to still bind with TROSPA activity abolished, leaving the door open to another adhesin-receptor interaction also mediating binding to the tick midgut.

Consistent with an important role for the life of *B. burgdorferi* in the tick, *ospA*, and the other gene in the bicistronic operon, *ospB*, are expressed when the bacteria leave the mammalian host and enter the feeding tick (Schwan and Piesman, 2000). The importance of these proteins for the maintenance of the bacteria in ticks was demonstrated when *ospAB* mutant bacteria that were fully infectious in mice were acquired by ticks, but unable to persist in the tick midgut (Yang et al., 2004). More recent work in the field has demonstrated that a primary role of OspA is protection of the bacteria already residing in the tick midgut from killing by host antibodies as the tick takes in a blood meal (Battisti et al., 2008), which would be critical for maintenance of the infection in the natural cycle between vertebrate animals and ticks.

A salivary protein of *Ixodes scapularis* ticks, Salp15, was shown to bind to a different *B. burgdorferi* protein, OspC (Ramamoorthi et al., 2005). Binding of the bacteria to Salp15, which inhibits T-cell activation, appeared to inhibit in vitro antibody mediated killing of the bacteria. Nonetheless, Salp15 does not seem to be required for *B. burgdorferi* to establish infection, since an infection can be established by needle inoculation of the bacteria into mice (Barthold et al., 1988). OspC however, is critical to the ability of *B. burgdorferi* to establish disseminated infection in mice (Grimm et al., 2004b; Tilly et al., 2006), and was selected as a candidate adhesin for the vascular endothelium in mice using in vivo phage display (Antonara et al., 2007).

3.3 Concluding Remarks

Borrelia burgdorferi expresses a number of adhesins, many with redundant functions. This redundancy suggests that these functions are critical to the ability of the bacterium to cause infection in mammals, but testing this hypothesis will require novel approaches, given the difficulties involved in the genetic manipulation of this organism. It will be especially interesting to further investigate *B. burgdorferi* interactions with the tick, which only become more complex as more is learned. It will also be interesting to see how *B. burgdorferi* adhesins interact with their host substrates at the molecular level, as many of the *B. burgdorferi* proteins have no homologs outside of the genus.With recent advances in

development of genetic tool sets, the in vivo roles of the *B. burgdorferi* adhesins should be discernable within the next few years.

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Table 3.1

Known and candidate *Borrelia burgdorferi* adhesins

Adhesin (genomic locus)	Comments, references
Adhesins that bind to mammalian extracellular matrix components	
Bgp (*bb0588*)	Binds to GAGs in vitro (Parveen and Leong, 2000), mutant is infectious in mice but shows some deficiency in tissue colonization (Parveen et al., 2006; Saidac et al., 2009), may also bind cell cell-surface proteoglycans
DbpA (*bbA24*)	Binds to decorin and GAGs in vitro (Fischer et al., 2003; Guo et al., 1998), adhesion specificity is different from that of DbpB, *dbpBA* mutants are attenuated in murine infection (Blevins et al., 2008; Shi et al., 2008a; Weening et al., 2008)
DbpB (*bbA25*)	Binds to decorin and GAGs in vitro (Fischer et al., 2003; Guo et al., 1998), binding specificity is different from that of DbpA, *dbpBA* mutants are attenuated in murine infection (Blevins et al., 2008; Shi et al., 2008a; Weening et al., 2008)
BBK32 (*bbK32*)	Binds to fibronectin and GAGs in vitro (Fischer et al., 2006; Probert and Johnson, 1998), these activities are important for *B. burgdorferi* interactions with the vasculature in vivo (Norman et al., 2008), *bbk32* mutants are attenuated in murine infection (Seshu et al., 2006)
RevA (*bbM27* & *bbP27*), **RevB** (*bbC10*)	Binds to fibronectin and laminin in vitro, some strains have two copies of the gene; there is a related gene (*revB*) that is present only in some *B. burgdorferi* strains (Brissette et al., 2009a)
Bmp family members A,B,C,D (*bb0382-0385*) **ErpX *(bbQ47)***	Bind to laminin in vitro (Verma et al., 2009); D was selected in vivo for adherence to vascular endothelium in living mice (Antonara et al., 2007); contributes to chronic joint infection (Pal et al., 2008) ErpX binds to laminin (Brissette et al., 2009b)
Erps: ErpA *(bbP38, bbI39)*, ErpC, ErpP *(bbN38)* **CRASPs: CRASP-1 (CspA, *bbA68)*, CRASP-2 (CspZ, *bbH06)*, CRASP-3=ErpP, CRASP-4=ErpC, CRASP-5=ErpA**	Bind to factor H and/or FHR-1 (factor H related) and/or FHL (factor H-like) (Alitalo et al., 2002; Hellwage et al., 2001; Metts et al., 2003)
Adhesins that bind to mammalian cell surface receptors	
P66 (*bb0603*)	Binds to integrins $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ (Coburn et al., 1999), knockout mutant is severely attenuated in mice but not in ticks (Coburn lab, unpublished data)
BBB07 (*bbB07*)	Binds to β1 integrins, stimulates proinflammatory signaling in chondrocytes (Behera et al., 2008)
Adhesins that bind to unidentified mammalian substrates (until further characterization is completed, these remain candidate adhesins)	
Lmp1 (*bb0210*)	Required for persistence and induction of disease manifestations in immunocompetent mice (Yang et al., 2009), selected in vivo for adherence to vascular endothelium in living mice (Antonara et al., 2007), host substrate(s) unknown
OspC (*bbB19*)	Essential for initiation of infection in mammals (Grimm et al., 2004b; Tilly et al., 2006, 2007) and for colonization of certain tissues (Xu et al., 2008), selected in vivo for adherence to vascular endothelium in living mice (Antonara et al., 2007), binds to cells in vitro, host substrate(s) unknown
VlsE *(bbF32)*	Required for persistent infection in mammals; (Dresser et al., 2009; Bankhead and Chaconas, 2007; Lin et al., 2009) selected in vivo for adherence to vascular endothelium in living mice (Antonara et al., 2007), binds to cells in vitro, host substrate(s) unknown
OspF family members: ErpK (*bbM38*), **ErpL** (*bbO39*), **OspG** (*bbS41*), **OspF** (*bbR42*)	OspF family members, all selected in vivo for adherence to vascular endothelium in living mice (Antonara et al., 2007), host substrate(s) unknown
Adhesins that bind to tick substrates	
OspA (*bbA15*)	Binds to TROSPA (tick receptor for OspA) (Pal et al., 2004)
OspC (*bbB19*)	Binds to Salp15 (Ramamoorthi et al., 2005)