Received: 7 April 2018 | Revised: 11 June 2018 | Accepted: 14 June 2018

DOI: 10.1111/cmi.12885

SPECIAL ISSUE - REVIEW

WILEY

Borrelia burgdorferi protein interactions critical for microbial persistence in mammals

Quentin Bernard | Meghna Thakur | Alexis A. Smith | Chrysoula Kitsou | Xiuli Yang | Utpal Pal

Department of Veterinary Medicine, University of Maryland, College Park, Maryland

Correspondence
Utpal Pal, Department of Veterinary Medicine, University of Maryland, College Park, MD 20742.
Email: upal@umd.edu

Funding information
National Institute of Allergy and Infectious Diseases, Grant/Award Numbers: AI116620 and AI080615

Abstract

Borrelia burgdorferi is the causative agent of Lyme disease that persists in a complex enzootic life cycle, involving *Ixodes* ticks and vertebrate hosts. The microbe invades ticks and vertebrate hosts in spite of active immune surveillance and potent microbicidal responses, and establishes long-term infection utilising mechanisms that are yet to be unravelled. The pathogen can cause multi-system disorders when transmitted to susceptible mammalian hosts, including in humans. In the past decades, several studies identified a limited number of *B. burgdorferi* gene-products critical for pathogen persistence, transmission between the vectors and the host, and host–pathogen interactions. This review will focus on the interactions between *B. burgdorferi* proteins, as well as between microbial proteins and host components, protein and non-protein components, highlighting their roles in pathogen persistence in the mammalian host. A better understanding of the contributions of protein interactions in the microbial virulence and persistence of *B. burgdorferi* would support development of novel therapeutics against the infection.

KEYWORDS

Borrelia burgdorferi, host persistence, Lyme disease, microbial infectivity, protein interactions

1 | INTRODUCTION

Borrelia burgdorferi, the causative agent of Lyme disease, persists through a complex enzootic infection cycle between the tick *Ixodes* family, in particular *Ixodes scapularis*, and vertebrate animals (Radolf, Caimano, Stevenson, & Hu, 2012). Lyme disease is highly prevalent in the United States, Europe, and parts of Asia, although the rate of the disease is globally underestimated (Mead, 2015). Currently, a human vaccine against Lyme disease is unavailable; therefore the development of an effective prevention against the infection remains a warranted research goal. Once transmitted, the pathogen *B. burgdorferi* invades host tissues, whether in ticks or vertebrates, and it interacts with host molecules in order to establish infection (Radolf et al., 2012; Steere, Coburn, & Glickstein, 2004; Thakur et al., 2017). Because protein–protein interactions are highly specific and dynamic, events that can regulate critical cellular structure and/or physiological events (Blundell et al., 2000), such biomolecular interactions, may modulate *B. burgdorferi* infectivity. A variety of molecular tools are now available to study dynamic spirochete proteome (Norris, 2006; Yang et al., 2011) as well as protein–protein interactions in *B. burgdorferi* (Yang et al., 2018). These interactions are highly dynamic events that are likely to contribute unique surface structure of *B. burgdorferi*. Since protein–protein interactions may be particularly amenable as therapeutic targets, further studies are highly warranted to explore these novel areas of spirochete research. Herein, we broadly discuss protein–protein interactions within spirochete cells and ones involving vertebrate host ligands that impact some of the key physiological process in the microbe and in the host, such as localisation of outer membrane protein, cell division, chemotaxis, attachment to host cell or extracellular matrix molecules, and complement resistance. Due to space limitations, we are unable to discuss all relevant publications, rather we highlight a limited set of representative studies to exemplify how protein interactions support *B. burgdorferi* infectivity and persistence.

2 | PROTEIN INTERACTIONS INVOLVING SPIROCHETE PROTEINS

B. burgdorferi differentially produces specific proteins as the pathogen infects a host or cycles between arthropods and vertebrate hosts (de Silva & Fikrig, 1997). At the spirochete surface, an outer membrane (OM) has an atypical organisation amongst Gram-negative bacteria (Bergström & Zückert, 2010). Unlike Gram-negative bacteria, the spirochete OM lacks the classical lipopolysaccharides, instead it harbours numerous lipoproteins (Rosa, Tilly, & Stewart, 2005). These lipoproteins form distinct membrane protein complexes. Many of these protein complexes contain common or ubiquitous members, such as outer surface proteins like OspA, −B, −C, −D, and Lp6.6 (Yang et al., 2011). In contrast, other proteins, namely, P66 (Coburn, Leong, & Chaconas, 2013) and BB0405 (Kung et al., 2016), are constituents of specific functional complexes (Yang et al., 2011). Some of these functional complexes are involved in biomolecular interactions, including flagellar proteins that are likely crucial for the stability and the assembly of flagellar structure and motor, such as FlgE (Sal et al., 2008). Others, like the ones we highlight below, support microbial physiology and infectivity.

2.1 | BB0323 complex

The gene product BB0323 is part of an OM protein complex that is essential for cell fission (Stewart, Hoff, Fischer, Krum, & Rosa, 2004; Zhang, Yang, Kumar, & Pal, 2009). BB0323 is the substrate of at least two specific proteases in *B. burgdorferi*—a periplasmic serine protease called *B. burgdorferi* high temperature requirement protease A (HtrA) or BbHtrA (BB0104) and a C-terminal protease A, named as CtpA (Kariu, Yang, Marks, Zhang, & Pal, 2013; Ostberg et al., 2004; Ye et al., 2016). These proteases produce two discrete polypeptides with approximate MW around 27 and 12 kDa that encompass the N-terminal and C-terminal regions of BB0323, respectively. These polypeptides also form a homomeric complex (Kariu et al., 2013) while N-terminal polypeptide also interact with another spirochete protein, BB0238 (Groshong et al., 2014; Kariu et al., 2015; Thakur et al., 2017). The association of BB0323 and BB0238 proteins contributes to their mutual posttranslational stability (Kariu et al., 2015). More recently, it was shown that the interaction between BB0323 and a stretch of 11 amino acid residues of BB0238 may be essential in the successful establishment of spirochete infection and transmission to mammals (Thakur et al., 2017). The biologically relevant interaction between BB0323 and BB0238 supports the notion that spirochete protein–protein interactions are essential for vertebrate host infection, therefore constitute a viable target for future therapeutic interventions, for example, next-generation drug targets (Khan, Ahmad, Ahmad, Flynn, & Kumar, 2011; Lin et al., 2012) to thwart Lyme borreliosis.

2.2 | BB0795 complex

This heterooligomeric OM protein complex known as a β-barrel assembly machine, or BAM, has also been recently discovered in *B. burgdorferi* (Lenhart & Akins, 2009). Like other Gram-negative bacteria, a functional BAM complex is required for proper assembly of proteins into the spirochete OM. At least three proteins, BB0795, BB0324, and BB0028, were characterised as an integral structural and functional part of the complex (Lenhart, Kenedy, Yang, Pal, & Akins, 2012). A model for the spirochete BAM complex has been proposed, with BB0795, BB0324, and BB0028 being orthologs to BamA, BamB, and BamD, respectively, in Gram-negative bacteria (Kenedy, Lenhart, & Akins, 2012; Lenhart et al., 2012). The BAM complex plays an essential role in spirochete growth and infection, hence making its subunit interactions, such as BamA:BamB or BamA:BamD potential targets for novel inhibitors. More recently, Iqbal, Kenedy, Lybecker, and Akins (2016) have identified the inner membrane protein TamB, a constituent of the translocation and assembly module, as another subunit of the BAM complex (Iqbal et al., 2016). Thereby it indicates that the two complexes crosstalk and play an important role in OM biogenesis and supporting microbial infectivity.

3 | PROTEIN INTERACTIONS INVOLVING HOST LIGANDS

B. burgdorferi persists in an enzootic cycle where interactions of spirochete proteins with either host or vector proteins play a critical role in infectivity. We discussed interactions involving tick molecules in several of our past reviews (de Silva, Tyson, & Pal, 2009, Kung, Anguita, & Pal, 2013, Pal & Fikrig, 2003, Pal & Fikrig, 2010), so this section will essentially focus on interactions of spirochete proteins with mammalian ligands, as exemplified below:

3.1 | Extracellular matrix binding proteins

As soon as *B. burgdorferi* are deposited into the host dermis, spirochetes must move across the extracellular matrix (ECM) to reach blood vessels and disseminate to distant targeted organs, a process that is likely to rely on protein interactions. *B. burgdorferi* encodes a number of surface proteins that mediate spirochete dissemination and persistence through interactions with host ECM molecules (Coburn et al., 2013; Coburn, Fischer, & Leong, 2005). The most widely studied examples involve (a) interaction between spirochete decorin binding protein (DBP) A and B with host glycosaminoglycans containing ligands like decorin (Blevins, Hagman, & Norgard, 2008; Brown et al., 2001; Caimano, Eggers, Hazlett, & Radolf, 2004; Guo, Brown, Dorward, Rosenberg, & Hook, 1998; Hyde et al., 2011; Lin et al., 2014; Shi, Xu, McShan, & Liang, 2008; Weening et al., 2008), (b) binding of multiple *B. burgdorferi* proteins, such as BBK32, RevA/RevB, and BB0347, with host fibronectin (Brissette & Gaultney, 2014, Gaultney, Gonzalez, Floden, & Brissette, 2013, Hyde et al., 2011), (c) interaction of putative lipoproteins BBA33 and CRASP-1 with host collagens, especially with type-I/III and IV (Hallstrom et al., 2010; Zhi et al., 2015), (d) binding of ErpX and BmpA with host laminin (Brissette, Verma, Bowman, Cooley, & Stevenson, 2009; Verma, Brissette, Bowman, & Stevenson, 2009), (e) interaction of BbHtrA with several ECM molecules and most notably aggrecan, a major proteoglycan in joints (Russell & Johnson, 2013), and (f) binding of *B. burgdorferi* enolase, Erp proteins, OspA, OspC, and BBA70 with plasmin (ogen;

Floden, Watt, & Brissette, 2011; Fuchs, Wallich, Simon, & Kramer, 1994; Hallstrom et al., 2010; Koenigs et al., 2013; Lagal, Portnoi, Faure, Postic, & Baranton, 2006; Nogueira, Smith, Qin, & Pal, 2012; Onder et al., 2012; Toledo, Coleman, Kuhlow, Crowley, & Benach, 2011), a host protease able to degrade a variety of ECM components (Coleman, Roemer, & Benach, 1999) such as fibronectin, laminin, and vitronectin. Overall, a large number of spirochete surface proteins interact with a large array of ECM ligands to promote *B. burgdorferi* dissemination and colonisation while influencing host responses and ensuring microbial persistence in diverse mammalian tissues.

3.2 | Integrin binding proteins

B. burgdorferi can colonise multiple distant host tissues establishing long-term persistence in disseminated organs. Spirochete encodes specific proteins such as P66, BBB07, and BB0172 that can assist microbial colonisation via interaction with host ligands like specific integrin classes, a transmembrane receptor that facilitates adhesion to the ECM (Behera et al., 2008; Ristow et al., 2012; Ristow et al., 2015; Wood, Tamborero, Mingarro, & Esteve-Gassent, 2013). Amongst these interactions, for example, one between BBB07 and a host integrin α3β1 integrin leads to the modulation of proinflammatory mediators in chondrocytes (Behera et al., 2008). Another spirochete ligand BB0172 has also been shown to interact with the same α3β1 integrin in a metal ion-dependent manner (Wood et al., 2013). Therefore, interactions between a subset of integrin molecules and specific spirochete ligands may promote *B. burgdorferi* infection in multiple tissues, as well as triggering cellular proinflammatory responses in the host.

3.3 | Chemotaxis proteins

The transmission of spirochete requires its migration from the tick to the host dermis and then dissemination within the host from one organ to the other. Chemotaxis plays critical roles in supporting *B. burgdorferi* persistence in the enzootic infection cycle and this is partially mediated through chemotaxis (Che) proteins (Charon et al., 2012, Charon & Goldstein, 2002, Li, Motaleb, Sal, Goldstein, & Charon, 2000, Motaleb, Liu, & Wooten, 2015, Motaleb, Miller, Bakker, Li, & Charon, 2007, Sze, Zhang, Kariu, Pal, & Li, 2012). The chemotaxis signalling complex (Charon et al., 2012; Goldstein et al., 2010) is composed of three major components: methyl-accepting chemotaxis proteins (MCPs), the histidine kinase CheA, and the response regulator CheY. MCPs sense environmental and intracellular signals and control the activity of CheA after being coupled to it by CheW. Activated CheA then phosphorylates CheY, which interacts with the motor switch complex to regulate the flagella rotation. The *B. burgdorferi* chemotaxis pathway harbours multiple homologues of the coupling protein CheW ($CheW_1$/*bb0312*, $CheW_2$/*bb0565*, $CheW_3$/*bb0670*), histidine kinase CheA ($CheA_1$/*bb0567*, $CheA_2$/*bb0569*), and the response regulator CheY ($CheY_1$/*bb0551*, CheY2/*bb0570*, $CheY_3$/*bb0672*). The interaction of $CheW_1$ and $CheW_3$ with $CheA_2$, or $CheW_2$ with $CheA_1$, suggest the presence of two chemosensory pathways that could participate in different stages of the tick–mammalian cycle of the pathogen. Autophosphorylated histidine kinase CheA transfers the phosphate group to CheY, which in turn gets dephosphorylated by CheX/*bb0671* phosphatase (Djordjevic & Stock, 1998). The phosphatase activity of CheX is enhanced upon binding with CheD/*bb0606*. In addition to binding with CheX, CheD also interacts with two of the six methyl-accepting chemotaxis protein receptor (MCPs), indicating that CheD may also regulate the receptor activity (Moon, Hobbs, & Motaleb, 2016). In addition, CheY3 is shown to be phosphorylated by CheA2 and is a key regulator of spirochete movement (Motaleb et al., 2015; Novak et al., 2016).

3.4 | Complement binding proteins

The mammalian complement system represents an early and effective innate immune defence against invading pathogens like *B. burgdorferi*. But the spirochete is capable of counteracting the complement's response by producing several complement regulator-acquiring surface proteins (CRASPs) able to inhibit the complement system notably through the direct binding of the host complement regulatory/inhibitory molecules like factor H, the C7, or the C9 component (Caine & Coburn, 2016, de Taeye, Kreuk, van Dam, Hovius, & Schuijt, 2013, Kraiczy, 2016). Spirochete proteins capable of binding and thwarting the complement's action include CRASP1-5, CspA/Z, Erp Proteins, and OspE-related proteins. Besides, BBK32 has been shown to block the activation of the C1 complement complex (Garcia, Zhi, Wager, Hook, & Skare, 2016). Early in the process of dissemination and while interacting with the ECM, the interaction between spirochete protein BBA70 with plasminogen inhibits the host innate immune response through the degradation of complement proteins C3b and C5. OspC has also been shown to inhibit the classical and the lectin complement pathway by interacting with the C4b protein (Caine et al., 2017), The redundancy of these proteins allows *B. burgdorferi* to target and evade various steps of complement signal cascade, including its regulatory pathways ensuring microbial survival in the host (Koenigs et al., 2013).

3.5 | Interaction with other host proteins

B. burgdorferi encodes additional proteins that assist in microbial evasion of host immunity. For example, one of the *B. burgdorferi* surface proteins, annotated as Lmp1, is involved in various functions in spirochete infectivity, including host–pathogen interactions and evasion of host adaptive immunity by spirochetes (Antonara, Chafel, LaFrance, & Coburn, 2007; Yang et al., 2010; Yang et al., 2016; Yang, Coleman, Anguita, & Pal, 2009). More recently, the middle region of Lmp1 was shown to interact with host chondroitin-6-sulfate, which facilitates infection of mammalian hosts (Yang et al., 2016). Another prominent example is the spirochete surface protein, OspC, which binds a tick salivary gland protein, Salp15 (Anguita et al., 2002; Ramamoorthi et al., 2005); this complex then modulates the host immune response during the establishment of infection in mammals as the pathogen is transmitted from an infected tick. The OspC or Flagellin proteins are also known to interact with specific Toll like receptors in the host, specifically TLR2 or TLR5, respectively, again influencing the host immune response and modulating the expression of immune effectors, such as matrix metalloproteases (MMP) 9 (Gebbia, Coleman, & Benach, 2004).

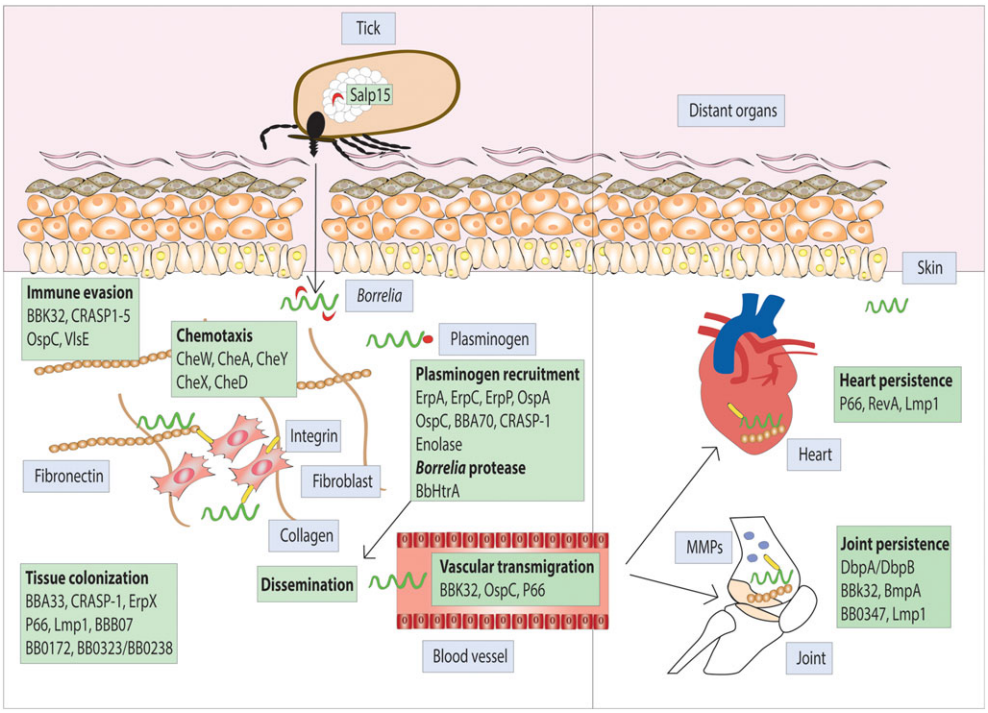

FIGURE 1 **Protein interaction involved in *Borrelia burgdorferi* infection of mammalian hosts transmitted via *Ixodes* ticks.** *B. burgdorferi* (Borrelia) is transmitted to the mammalian dermis at the tick-bite site. A spirochete outer surface protein (OspC), via interaction with a tick salivary gland protein (Salp15), carries the latter protein in the dermis where it assists the pathogen evasion of host immune insults. Protection from the vertebrate immune system, as well as dissemination through extracellular matrix or vasculature, is also mediated by interactions between additional sets of *B. burgdorferi* proteins, like BBK32, CRASP1–5, VlsE, Erp proteins, OspC, BBA70, and Enolase amongst others. The pathogen also uses its chemotaxis machinery system, including several Che proteins to disseminate into the host. The bacteria also use its own protease, BbHtrA, to degrade host extracellular matrix components and promote the infection. During and/or after dissemination to distant tissue locations, spirochetes interact with specific host molecules like matrix metalloproteases (MMP), collagen, or integrins and use specific adhesins like BBK32, DBPA/DBPB, P66, RevA, BBA33, BBB07, BB0172, Erp proteins, and Lmp1, amongst others to colonise the tissues and to facilitate its persistence in the infected organs

Once *B. burgdorferi* induces MMPs in host cells, these events might further assist the dissemination of the pathogen by remodelling the ECM (Behera et al., 2004; Behera, Hildebrand, Scagliotti, Steere, & Hu, 2005; Schramm et al., 2012). In addition, *B. burgdorferi* also encodes an elaborate antigenic variation system, via production of a surface protein VlsE, which interfaces with host adaptive immune response to ultimately help the spirochete to evade microbicidal responses (Zhang, Hardham, Barbour, & Norris, 1997); this interaction directly influences population dynamics and persistence of *B. burgdorferi* in the mammalian host and the tick (Brisson, Drecktrah, Eggers, & Samuels, 2012; Norris, 2014; Rogovskyy et al., 2015).

4 | CONCLUDING REMARKS

B. burgdorferi is a successful multi-host pathogen that developed elaborate defence strategies to combat host microbicidal responses so it can establish long-term infection in diverse array of tissues. As we demonstrated in this review, protein interactions play a critical role in microbial infectivity, pathogen entry, dissemination, and colonisation of various host or vector tissue milieus (Figure 1). Spirochetes demonstrate unique and highly specific interactions involving conserved spirochete proteins that are essential for infectivity in the vertebrate host. When interacting with host proteins, spirochetes use some redundancy, i.e. one involving the same host protein with multiple spirochete proteins or one between a specific spirochete protein and ubiquitous host ligands highlighting the importance of these biological events that ultimately facilitate microbial infectivity. Although these molecular interactions are likely to be complex, transient, and dynamic, they nevertheless represent novel molecular targets for therapeutics to control *B. burgdorferi* infection.

ACKNOWLEDGEMENTS

We are thankful to Gaelle Kolb for the critical reading of the manuscript. We also thank members of our laboratory for their assistance with the study. This work is supported by funding from the National Institute of Allergy and Infectious Diseases (Award Number AI080615 and AI116620 to U.P.). The authors declare that they have no competing financial interests.

ORCID

Utpal Pal http://orcid.org/0000-0002-7504-4628

REFERENCES

Anguita, J., Ramamoorthi, N., Das, S., Conze, D., Askenase, P., Rincón, M., … Fikrig, E. (2002). Salp15, an *Ixodes scapularis* saliva protein, inhibits CD4+ T cell activation. *Immunity*, *16*, 849–859.

Antonara, S., Chafel, R. M., LaFrance, M., & Coburn, J. (2007). *Borrelia burgdorferi* adhesins identified using *in vivo* phage display. *Molecular Microbiology*, *66*, 262–276.

Behera, A. K., Durand, E., Cugini, C., Antonara, S., Bourassa, L., Hildebrand, E., et al. (2008). *Borrelia burgdorferi* BBB07 interaction with integrin alpha3beta1 stimulates production of pro-inflammatory mediators in primary human chondrocytes. *Cellular Microbiology*, *10*, 320–331.

Behera, A. K., Hildebrand, E., Scagliotti, J., Steere, A. C., & Hu, L. T. (2005). Induction of host matrix metalloproteinases by *Borrelia burgdorferi* differs in human and murine lyme arthritis. *Infection and Immunity*, *73*, 126–134.

Behera, A. K., Thorpe, C. M., Kidder, J. M., Smith, W., Hildebrand, E., & Hu, L. T. (2004). *Borrelia burgdorferi*-induced expression of matrix metalloproteinases from human chondrocytes requires mitogen-activated protein kinase and Janus kinase/signal transducer and activator of transcription signaling pathways. *Infection and Immunity*, *72*, 2864–2871.

Bergström, S., & Zückert, W. R. (2010). Structure, function and biogenesis of the *Borrelia* cell envelope. In D. S. Samuels, & J. D. Radolf (Eds.), *Borrelia, molecular biology, host interaction and pathogenesis* (pp. 139–166). Norfolk, UK: Caister Academic Press.

Blevins, J. S., Hagman, K. E., & Norgard, M. V. (2008). Assessment of decorin-binding protein A to the infectivity of *Borrelia burgdorferi* in the murine models of needle and tick infection. *BMC Microbiology*, *8*, 82.

Blundell, T. L., Burke, D. F., Chirgadze, D., Dhanaraj, V., Hyvonen, M., Innis, C. A., et al. (2000). Protein-protein interactions in receptor activation and intracellular signalling. *Biological Chemistry*, *381*, 955–959.

Brissette, C. A., & Gaultney, R. A. (2014). That's my story, and I'm sticking to it—An update on *B. burgdorferi* adhesins. *Frontiers in Cellular and Infection Microbiology*, *4*, 41.

Brissette, C. A., Verma, A., Bowman, A., Cooley, A. E., & Stevenson, B. (2009). The *Borrelia burgdorferi* outer-surface protein ErpX binds mammalian laminin. *Microbiology*, *155*, 863–872.

Brisson, D., Drecktrah, D., Eggers, C. H., & Samuels, D. S. (2012). Genetics of *Borrelia burgdorferi*. *Annual Review of Genetics*, *46*, 515–536.

Brown, E. L., Wooten, R. M., Johnson, B. J., Iozzo, R. V., Smith, A., Dolan, M. C., et al. (2001). Resistance to Lyme disease in decorin-deficient mice. *The Journal of Clinical Investigation*, *107*, 845–852.

Caimano, M. J., Eggers, C. H., Hazlett, K. R., & Radolf, J. D. (2004). RpoS is not central to the general stress response in *Borrelia burgdorferi* but does control expression of one or more essential virulence determinants. *Infection and Immunity*, *72*, 6433–6445.

Caine, J. A., & Coburn, J. (2016). Multifunctional and redundant roles of *Borrelia burgdorferi* outer surface proteins in tissue adhesion, colonization, and complement evasion. *Frontiers in Immunology*, *7*, 442.

Caine, J. A., Lin, Y. P., Kessler, J. R., Sato, H., Leong, J. M., & Coburn, J. (2017). *Borrelia burgdorferi* outer surface protein C (OspC) binds complement component C4b and confers bloodstream survival. *Cellular Microbiology*, *19*, e12786.

Charon, N. W., Cockburn, A., Li, C., Liu, J., Miller, K. A., Miller, M. R., … Wolgemuth, C. W. (2012). The unique paradigm of spirochete motility and chemotaxis. *Annual Review of Microbiology*, *66*, 349–370.

Charon, N. W., & Goldstein, S. F. (2002). Genetics of motility and chemotaxis of a fascinating group of bacteria: The spirochetes. *Annual Review of Genetics*, *36*, 47–73.

Coburn, J., Fischer, J. R., & Leong, J. M. (2005). Solving a sticky problem: new genetic approaches to host cell adhesion by the Lyme disease spirochete. *Molecular Microbiology*, *57*, 1182–1195.

Coburn, J., Leong, J., & Chaconas, G. (2013). Illuminating the roles of the *Borrelia burgdorferi* adhesins. *Trends in Microbiology*, *21*, 372–379.

Coleman, J. L., Roemer, E. J., & Benach, J. L. (1999). Plasmin-coated *Borrelia burgdorferi* degrades soluble and insoluble components of the mammalian extracellular matrix. *Infection and Immunity*, *67*, 3929–3936.

de Silva, A. M., & Fikrig, E. (1997). Arthropod- and host-specific gene expression by *Borrelia burgdorferi*. *The Journal of Clinical Investigation*, *99*, 377–379.

de Silva, A. M., Tyson, K. R., & Pal, U. (2009). Molecular characterization of the tick-*Borrelia* interface. *Frontiers in Bioscience*, *14*, 3051–3063.

de Taeye, S. W., Kreuk, L., van Dam, A. P., Hovius, J. W., & Schuijt, T. J. (2013). Complement evasion by *Borrelia burgdorferi*: It takes three to tango. *Trends in Parasitology*, *29*, 119–128.

Djordjevic, S., & Stock, A. M. (1998). Structural analysis of bacterial chemotaxis proteins: Components of a dynamic signaling system. *Journal of Structural Biology*, *124*, 189–200.

Floden, A. M., Watt, J. A., & Brissette, C. A. (2011). *Borrelia burgdorferi* enolase is a surface-exposed plasminogen binding protein. *PLoS one*, *6*, e27502.

Fuchs, H., Wallich, R., Simon, M. M., & Kramer, M. D. (1994). The outer surface protein A of the spirochete *Borrelia burgdorferi* is a plasmin (ogen) receptor. *Proc. Natl. Acad. Sci. (U. S. A.)*, *91*, 12594–12598.

Garcia, B. L., Zhi, H., Wager, B., Hook, M., & Skare, J. T. (2016). *Borrelia burgdorferi* BBK32 inhibits the classical pathway by blocking activation of the C1 complement complex. *PLoS Pathogens*, *12*, e1005404.

Gaultney, R. A., Gonzalez, T., Floden, A. M., & Brissette, C. A. (2013). BB0347, from the lyme disease spirochete *Borrelia burgdorferi*, is surface exposed and interacts with the CS1 heparin-binding domain of human fibronectin. *PLoS one*, *8*, e75643.

Gebbia, J. A., Coleman, J. L., & Benach, J. L. (2004). Selective induction of matrix metalloproteinases by *Borrelia burgdorferi* via toll-like receptor 2 in monocytes. *The Journal of Infectious Diseases*, *189*, 113–119.

Goldstein, S. F., Li, C., Liu, J., Miller, M., Motaleb, M. A., Norris, S. J., et al. (2010). The chic motility and chemotaxis of *Borrelia burgdorferi*. In D. S. Samuels, & J. D. Radolf (Eds.), *Borrelia, molecular biology, host interaction and pathogenesis* (pp. 161–181). Norfolk, UK: Caister Academic Press.

Groshong, A. M., Fortune, D. E., Moore, B. P., Spencer, H. J., Skinner, R. A., Bellamy, W. T., & Blevins, J. S. (2014). BB0238, a presumed tetratricopeptide repeat-containing protein, is required during *Borrelia burgdorferi* mammalian infection. *Infection and Immunity*, *82*, 4292–4306.

Guo, B. P., Brown, E. L., Dorward, D. W., Rosenberg, L. C., & Hook, M. (1998). Decorin-binding adhesins from *Borrelia burgdorferi*. *Molecular Microbiology*, *30*, 711–723.

Hallstrom, T., Haupt, K., Kraiczy, P., Hortschansky, P., Wallich, R., Skerka, C., & Zipfel, P. F. (2010). Complement regulator-acquiring surface protein 1 of *Borrelia burgdorferi* binds to human bone morphogenic protein 2, several extracellular matrix proteins, and plasminogen. *The Journal of Infectious Diseases*, *202*, 490–498.

Hyde, J. A., Weening, E. H., Chang, M., Trzeciakowski, J. P., Hook, M., Cirillo, J. D., & Skare, J. T. (2011). Bioluminescent imaging of *Borrelia burgdorferi in vivo* demonstrates that the fibronectin-binding protein BBK32 is required for optimal infectivity. *Molecular Microbiology*, *82*, 99–113.

Iqbal, H., Kenedy, M. R., Lybecker, M., & Akins, D. R. (2016). The TamB ortholog of *Borrelia burgdorferi* interacts with the beta-barrel assembly machine (BAM) complex protein BamA. *Molecular Microbiology*, *102*, 757–774.

Kariu, T., Sharma, K., Singh, P., Smith, A. A., Backstedt, B., Buyuktanir, O., & Pal, U. (2015). BB0323 and novel virulence determinant BB0238: *Borrelia burgdorferi* proteins that interact with and stabilize each other and are critical for infectivity. *The Journal of Infectious Diseases*, *211*, 462–471.

Kariu, T., Yang, X., Marks, C. B., Zhang, X., & Pal, U. (2013). Proteolysis of BB0323 results in two polypeptides that impact physiologic and infectious phenotypes in *Borrelia burgdorferi*. *Molecular Microbiology*, *88*, 510–522.

Kenedy, M. R., Lenhart, T. R., & Akins, D. R. (2012). The role of *Borrelia burgdorferi* outer surface proteins. *FEMS Immunology and Medical Microbiology*, *66*, 1–19.

Khan, S. H., Ahmad, F., Ahmad, N., Flynn, D. C., & Kumar, R. (2011). Protein-protein interactions: Principles, techniques, and their potential role in new drug development. *Journal of Biomolecular Structure & Dynamics*, *28*, 929–938.

Koenigs, A., Hammerschmidt, C., Jutras, B. L., Pogoryelov, D., Barthel, D., Skerka, C., … Kraiczy, P. (2013). BBA70 of *Borrelia burgdorferi* is a novel plasminogen-binding protein. *The Journal of Biological Chemistry*, *288*, 25229–25243.

Kraiczy, P. (2016). Hide and seek: How Lyme disease spirochetes overcome complement attack. *Frontiers in Immunology*, *7*, 385.

Kung, F., Anguita, J., & Pal, U. (2013). *Borrelia burgdorferi* and tick proteins supporting pathogen persistence in the vector. *Future Microbiology*, *8*, 41–56.

Kung, F., Kaur, S., Smith, A. A., Yang, X., Wilder, C. N., Sharma, K., … Pal, U. (2016). A *Borrelia burgdorferi* surface-exposed transmembrane protein lacking detectable immune responses supports pathogen persistence and constitutes a vaccine target. *The Journal of Infectious Diseases*, *213*, 1786–1795.

Lagal, V., Portnoi, D., Faure, G., Postic, D., & Baranton, G. (2006). *Borrelia burgdorferi* sensu stricto invasiveness is correlated with OspC-plasminogen affinity. *Microbes and Infection*, *8*, 645–652.

Lenhart, T. R., & Akins, D. R. (2009). *Borrelia burgdorferi* locus BB0795 encodes a BamA orthologue required for growth and efficient localization of outer membrane proteins. *Molecular Microbiology*, *75*, 692–709.

Lenhart, T. R., Kenedy, M. R., Yang, X., Pal, U., & Akins, D. R. (2012). BB0324 and BB0028 are constituents of the *Borrelia burgdorferi* beta-barrel assembly machine (BAM) complex. *BMC Microbiology*, *12*, 60.

Li, C., Motaleb, A., Sal, M., Goldstein, S. F., & Charon, N. W. (2000). Spirochete periplasmic flagella and motility. *Journal of Molecular Microbiology and Biotechnology*, *2*, 345–354.

Lin, Y., Li, Y., Zhu, Y., Zhang, J., Li, Y., Liu, X., … Si, S. (2012). Identification of antituberculosis agents that target ribosomal protein interactions using a yeast two-hybrid system. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 17412–17417.

Lin, Y. P., Benoit, V., Yang, X., Martinez-Herranz, R., Pal, U., & Leong, J. M. (2014). Strain-specific variation of the decorin-binding adhesin DbpA influences the tissue tropism of the lyme disease spirochete. *PLoS Pathogens*, *10*, e1004238.

Mead, P. S. (2015). Epidemiology of Lyme disease. *Infectious Disease Clinics of North America*, *29*, 187–210.

Moon, K. H., Hobbs, G., & Motaleb, M. A. (2016). *Borrelia burgdorferi* CheD promotes various functions in chemotaxis and the pathogenic life cycle of the spirochete. *Infection and Immunity*, *84*, 1743–1752.

Motaleb, M. A., Liu, J., & Wooten, R. M. (2015). Spirochetal motility and chemotaxis in the natural enzootic cycle and development of Lyme disease. *Current Opinion in Microbiology*, *28*, 106–113.

Motaleb, M. A., Miller, M. R., Bakker, R. G., Li, C., & Charon, N. W. (2007). Isolation and characterization of chemotaxis mutants of the Lyme disease Spirochete *Borrelia burgdorferi* using allelic exchange mutagenesis, flow cytometry, and cell tracking. *Methods in Enzymology*, *422*, 421–437.

Nogueira, S. V., Smith, A. A., Qin, J. H., & Pal, U. (2012). A surface enolase participates in *Borrelia burgdorferi*-plasminogen interaction and contributes to pathogen survival within feeding ticks. *Infection and Immunity*, *80*, 82–90.

Norris, S. J. (2006). The dynamic proteome of Lyme disease *Borrelia*. *Genome Biology*, *7*, 209.

Norris, S. J. (2014). The vls antigenic variation systems of Lyme disease *Borrelia*: Eluding host immunity through both random, segmental gene conversion and framework heterogeneity. *Microbiol Spectr*, *2*.

Novak, E. A., Sekar, P., Xu, H., Moon, K. H., Manne, A., Wooten, R. M., & Motaleb, M. A. (2016). The *Borrelia burgdorferi* CheY3 response regulator is essential for chemotaxis and completion of its natural infection cycle. *Cellular Microbiology*, *18*, 1782–1799.

Onder, O., Humphrey, P. T., McOmber, B., Korobova, F., Francella, N., Greenbaum, D. C., & Brisson, D. (2012). OspC is potent plasminogen receptor on surface of *Borrelia burgdorferi*. *The Journal of Biological Chemistry*, *287*, 16860–16868.

Ostberg, Y., Carroll, J. A., Pinne, M., Krum, J. G., Rosa, P., & Bergstrom, S. (2004). Pleiotropic effects of inactivating a carboxyl-terminal protease, CtpA, in *Borrelia burgdorferi*. *Journal of Bacteriology*, *186*, 2074–2084.

Pal, U., & Fikrig, E. (2003). Adaptation of *Borrelia burgdorferi* in the vector and vertebrate host. *Microbes and Infection*, *5*, 659–666.

Pal, U., & Fikrig, E. (2010). Tick interactions. In D. S. Samuels, & J. D. Radolf (Eds.), *Borrelia, molecular biology, host interaction and pathogenesis* (pp. 279–298). Norfolk, UK: Caister Academic Press.

Radolf, J. D., Caimano, M. J., Stevenson, B., & Hu, L. T. (2012). Of ticks, mice and men: Understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nature Reviews. Microbiology*, *10*, 87–99.

Ramamoorthi, N., Narasimhan, S., Pal, U., Bao, F., Yang, X. F., Fish, D., … Fikrig, E. (2005). The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature*, *436*, 573–577.

Ristow, L. C., Bonde, M., Lin, Y. P., Sato, H., Curtis, M., Wesley, E., … Coburn, J. (2015). Integrin binding by *Borrelia burgdorferi* P66 facilitates dissemination but is not required for infectivity. *Cellular Microbiology*, *17*, 1021–1036.

Ristow, L. C., Miller, H. E., Padmore, L. J., Chettri, R., Salzman, N., Caimano, M. J., et al. (2012). The beta(3)-integrin ligand of *Borrelia burgdorferi* is critical for infection of mice but not ticks. *Mol Microbiol*.

Rogovskyy, A. S., Casselli, T., Tourand, Y., Jones, C. R., Owen, J. P., Mason, K. L., … Bankhead, T. (2015). Evaluation of the importance of VlsE antigenic variation for the enzootic cycle of *Borrelia burgdorferi*. *PLoS one*, *10*, e0124268.

Rosa, P. A., Tilly, K., & Stewart, P. E. (2005). The burgeoning molecular genetics of the Lyme disease spirochaete. *Nature Reviews. Microbiology*, *3*, 129–143.

Russell, T. M., & Johnson, B. J. (2013). Lyme disease spirochaetes possess an aggrecan-binding protease with aggrecanase activity. *Molecular Microbiology*, *90*, 228–240.

Sal, M. S., Li, C., Motalab, M. A., Shibata, S., Aizawa, S., & Charon, N. W. (2008). *Borrelia burgdorferi* uniquely regulates its motility genes and has an intricate flagellar hook-basal body structure. *Journal of Bacteriology*, *190*, 1912–1921.

Schramm, F., Kern, A., Barthel, C., Nadaud, S., Meyer, N., Jaulhac, B., & Boulanger, N. (2012). Microarray analyses of inflammation response of human dermal fibroblasts to different strains of *Borrelia burgdorferi* sensu stricto. *PLoS one*, *7*, e40046.

Shi, Y., Xu, Q., McShan, K., & Liang, F. T. (2008). Both decorin-binding proteins A and B are critical for the overall virulence of *Borrelia burgdorferi*. *Infection and Immunity*, *76*, 1239–1246.

Steere, A. C., Coburn, J., & Glickstein, L. (2004). The emergence of Lyme disease. *The Journal of Clinical Investigation*, *113*, 1093–1101.

Stewart, P. E., Hoff, J., Fischer, E., Krum, J. G., & Rosa, P. A. (2004). Genome-wide transposon mutagenesis of *Borrelia burgdorferi* for identification of phenotypic mutants. *Applied and Environmental Microbiology*, *70*, 5973–5979.

Sze, C. W., Zhang, K., Kariu, T., Pal, U., & Li, C. (2012). *Borrelia burgdorferi* needs chemotaxis to establish infection in mammals and to accomplish its enzootic cycle. *Infection and Immunity*, *80*, 2485–2492.

Thakur, M., Sharma, K., Chao, K., Smith, A. A., Herzberg, O., & Pal, U. (2017). A protein-protein interaction dictates borrelial infectivity. *Scientific Reports*, *7*, 2932.

Toledo, A., Coleman, J. L., Kuhlow, C. J., Crowley, J. T., & Benach, J. L. (2011). The enolase of *Borrelia burgdorferi* is a plasminogen receptor released in outer membrane vesicles. *Infection and Immunity*, *80*, 359–368.

Verma, A., Brissette, C. A., Bowman, A., & Stevenson, B. (2009). Borrelia burgdorferi BmpA is a laminin-binding protein. *Infection and Immunity*, *77*, 4940–4946.

Weening, E. H., Parveen, N., Trzeciakowski, J. P., Leong, J. M., Hook, M., & Skare, J. T. (2008). *Borrelia burgdorferi* lacking DbpBA exhibits an early survival defect during experimental infection. *Infection and Immunity*, *76*, 5694–5705.

Wood, E., Tamborero, S., Mingarro, I., & Esteve-Gassent, M. D. (2013). BB0172, a *Borrelia burgdorferi* outer membrane Protein that binds integrin alpha3beta1. *Journal of Bacteriology*, *195*, 3320–3330.

Yang, X., Coleman, A. S., Anguita, J., & Pal, U. (2009). A chromosomally encoded virulence factor protects the Lyme disease pathogen against host-adaptive immunity. *PLoS Pathogens*, *5*, e1000326.

Yang, X., Lenhart, T. R., Kariu, T., Anguita, J., Akins, D. R., & Pal, U. (2010). Characterization of unique regions of *Borrelia burgdorferi* surface-located membrane protein 1. *Infection and Immunity*, *78*, 4477–4487.

Yang, X., Lin, Y. P., Heselpoth, R. D., Buyuktanir, O., Qin, J., Kung, F., … Pal, U. (2016). Middle region of the *Borrelia burgdorferi* surface-located protein 1 (Lmp1) interacts with host chondroitin-6-sulfate and independently facilitates infection. *Cellular Microbiology*, *18*, 97–110.

Yang, X., Promnares, K., Qin, J., He, M., Shroder, D. Y., Kariu, T., … Pal, U. (2011). Characterization of multiprotein complexes of the *Borrelia burgdorferi* outer membrane vesicles. *Journal of Proteome Research*, *10*, 4556–4566.

Yang, X., Thakur, M., Koci, J., Smith, A. A., Singh, P., Zhuang, X., … Pal, U. (2018). Analysis of *Borrelia burgdorferi* proteome and protein-protein interactions. *Methods in Molecular Biology*, *1690*, 259–277.

Ye, M., Sharma, K., Thakur, M., Smith, A. A., Buyuktanir, O., Xiang, X., … Pal, U. (2016). HtrA, a temperature- and stationary phase-activated protease involved in maturation of a key microbial virulence determinant, facilitates *Borrelia burgdorferi* infection in mammalian hosts. *Infection and Immunity*, *84*, 2372–2381.

Zhang, J. R., Hardham, J. M., Barbour, A. G., & Norris, S. J. (1997). Antigenic variation in Lyme disease *Borreliae* by promiscuous recombination of Vmp-like sequence cassettes. *Cell*, *89*, 275–285.

Zhang, X., Yang, X., Kumar, M., & Pal, U. (2009). BB0323 function is essential for *Borrelia burgdorferi* virulence and persistence through tick-rodent transmission cycle. *The Journal of Infectious Diseases*, *200*, 1318–1330.

Zhi, H., Weening, E. H., Barbu, E. M., Hyde, J. A., Hook, M., & Skare, J. T. (2015). The BBA33 lipoprotein binds collagen and impacts *Borrelia burgdorferi* pathogenesis. *Molecular Microbiology*, *96*, 68–83.

How to cite this article: Bernard Q, Thakur M, Smith AA, Kitsou C, Yang X, Pal U. *Borrelia burgdorferi* protein interactions critical for microbial persistence in mammals. *Cellular Microbiology*. 2019;21:e12885. https://doi.org/10.1111/cmi.12885