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Molecular characterization of the tick-*Borrelia* interface

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TABLE OF CONTENTS

1. ABSTRACT

Spirochetes in the genus *Borrelia* are responsible for tick-borne relapsing fever and Lyme disease. *Borrelia*-tick interactions are highly specific as each species of *Borrelia* is only transmitted by one or a few closely related species of ticks. *Borrelia* colonize the gut or salivary glands of ticks. Several *Borrelia* genes required for tick colonization or transmission have been identified. *Borrelia* genes required for transmission are induced by a pathway controlled by the alternate sigma factors RpoN (σ54) and RpoS (σS). A protein in the gut of *I. scapularis* ticks that functions as a receptor for *B. burgdorferi* has been identified. In addition, *Ixodes* tick saliva has proteins that alter host hemostasis and immunity, and some of these salivary proteins directly interact with *Borrelia* to facilitate transmission and host infection, whereas others appear to assist *Borrelia* indirectly by suppressing host defense mechanisms. The exciting discoveries on *Borrelia*-tick interactions are also being translated into novel preventive measures such as transmission blocking vaccines.

2. INTRODUCTION

Spirochetes are a diverse group of helical shaped bacteria that can be free-living, symbiotic or parasitic to many different host species. Spirochetes in the genus *Borrelia* have an especially complex life cycle as they are transmitted by arthropod vectors, primarily ticks, to different vertebrate hosts. The genus *Borrelia* can be further separated into genetically distinct groups related to the *Borrelia* that cause either Lyme disease or relapsing fever (Figure 1). Within each group some species of *Borrelia* cause disease in people and animals, whereas other species appear not to be pathogenic. *Borrelia*-tick interactions are highly specific as each species of spirochete is only transmitted by one or few closely related tick species. The whole genome sequence of several *Borrelia* species has been determined and this information has been used to profile the transcriptome and proteome of *Borrelia* at different stages of the life cycle. Moreover, new tools have been developed to genetically manipulate *Borrelia*, and it is now feasible to create and complement

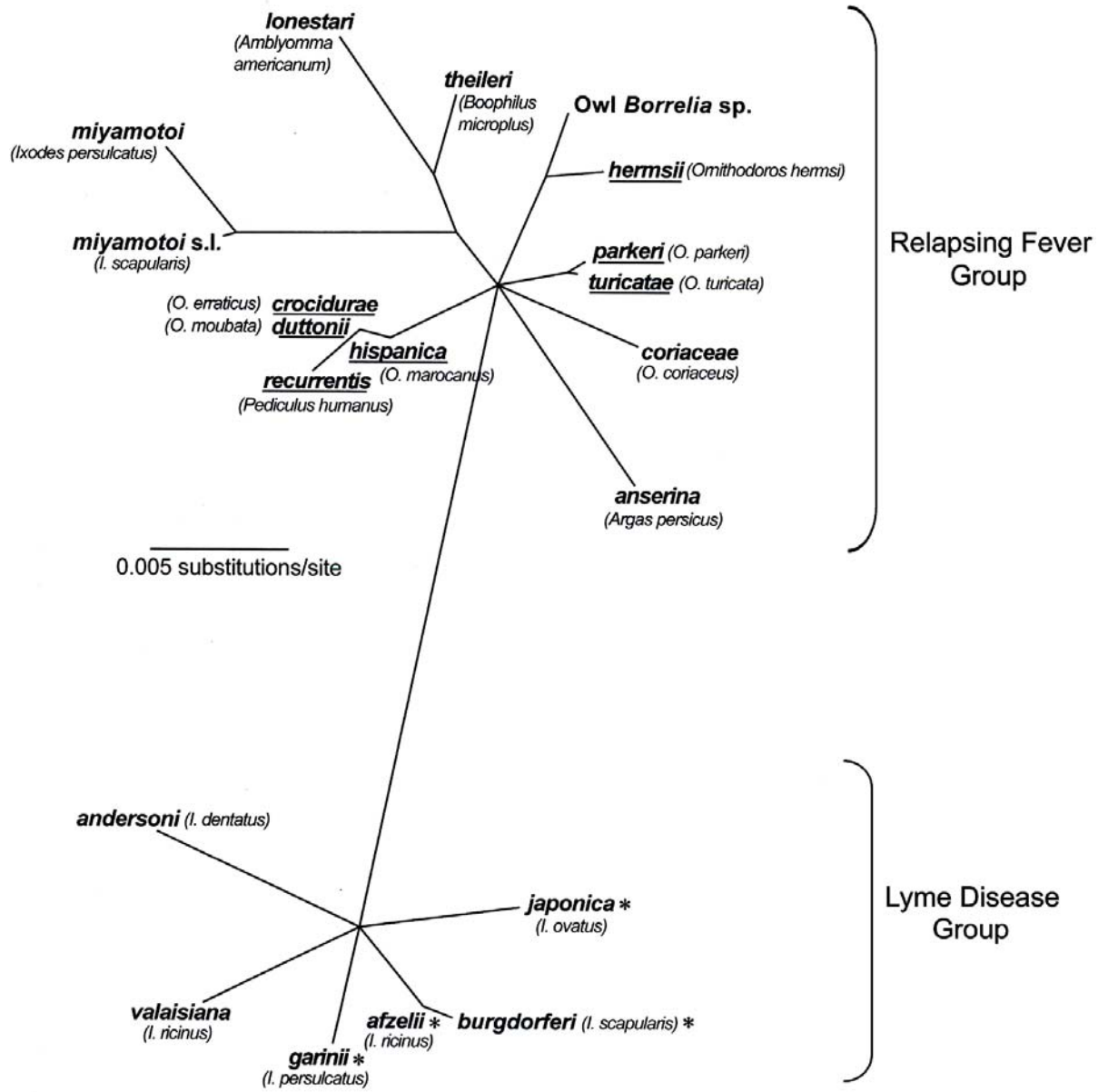

Figure 1. A phylogram of selected *Borrelia* species. The phylogram is labeled with the species of *Borrelia* and a known vector of each species. The *Borrelia* responsible for Lyme disease and relapsing fever form two distinct groups. Members of the relapsing fever group known to cause disease in people are underlined and members in the Lyme disease group known to cause disease in people are marked with an asterisk. The unrooted tree is based on 16S ribosomal RNA sequence and was originally published in Tick-borne diseases of humans. Editors: Goodman, Dennis and Sonenshine. Chapter 16, Relapsing Fever by Alan Barbour. ASM Press, Washington DC and reproduced here with permission from the publisher.

gene-specific mutants. As a result of these advances, we have gained novel insights into the biology of Borrelia-tick interactions, which are reviewed here. *(*Figure 1*: A phylogram of selected Borrelia species)*

3. THE LYME DISEASE *BORRELIA*

Lyme disease is an emerging infectious disease that has steadily increased in prevalence over the past 30 years (1). The disease is now common in parts of North America, Europe and Asia. Lyme disease is caused by at least 7 different species of closely related *Borrelia* that are transmitted by prostriate hard ticks (2). The three major species commonly associated with human infections are *B. burgdorferi sensu stricto*, distributed throughout the United States and Europe, and *B. afzelii* and *B. garinii*, which have overlapping ranges in Europe and parts of Asia (2).

3.1. Genome of *B. Burgdorferi*

The sequence of the *B. burgdorferi sensu stricto* strain B31M1 genome provided important clues about the physiology and complex life cycle of this spirochete (3).

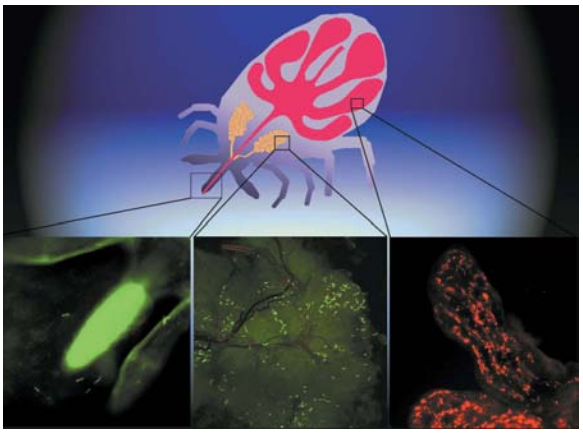

Figure 2. Distribution of *B. burgdorferi* within a feeding nymphal tick. Infected feeding ticks were dissected and the mouse skin attached to the hypostome (A), the tick's salivary glands (B) and the gut (C) were stained using fluorescently labeled antibodies against *B. burgdorferi*. In panel A the bright florescent structure is the hypostome, which the tick inserts into the hosts' skin during the blood meal. The hypostome is bright because it auto-fluoresces. When feeding ticks are removed from a mouse, often, a small piece of mouse skin remains attached to the hypostome. In panel A the fluorescein labeled spirochetes next to the hypostome are transmitted bacteria in the mouse's skin. Panel B is section of salivary gland that is heavily infected with *B. burgdorferi* and stained with a fluorescein conjugated antibody. Usually, spirochetes are found in the salivary glands after 48 to 60 hrs of tick feeding. Panel C represents a section of tick gut stained with a Texas Red conjugated antibody against *B. burgdorferi*. The tick gut remains infected with spirochetes before, during and after the blood meal. Figure was prepared by Jun Ohnishi, UNC-Chapel Hill.

The organism contains a segmented genome of approximately 1.5 megabases, consisting of a linear chromosome of 950 kb and 21 extra-chromosomal DNA elements (12 linear and 9 circular plasmids) ranging in size from 5 to 56 kb that add an additional 620 kb of DNA (3, 4). Interestingly, the organization and functional annotations of chromosomal and plasmid genes differ dramatically. The chromosome has fewer non-coding sequences and pseudogenes than the plasmids. The chromosomal DNA harbors a total of 853 genes that mainly code for house keeping proteins, such as those associated with cell cycle, transport, growth and metabolism. The plasmids encode 535 genes and 90% of these genes have no similarity to genes outside the *Borrelia* genus, suggesting that they perform specialized functions possibly related to the adaptation of the spirochete to different hosts. Some plasmids are unstable and readily lost during propagation of the bacteria *in vitro,* indicating they are required for specialized functions in the vector and/or host and not for growth in culture (5). It is difficult to genetically manipulate *Borrelia* because of the highly segmented, unstable genome and the presence of plasmid encoded restriction modification systems that degrade foreign DNA. Nevertheless, recent experimental efforts to introduce foreign DNA via allelic exchange or on shuttle vectors have been successful (5).

3.2. *B. burgdorferi* distribution within vector

Borrelia are maintained in nature through complex enzootic cycles that vary depending on the species and the geographical location (6, 7). In the Eastern United States, *Ixodes scapularis* is the main vector while *Peromyscus leucopus*, the white-footed mouse, is the primary reservoir host. Larval ticks acquire the pathogen while feeding on an infected mouse. The spirochetes are transstadially maintained in ticks through the molt to the nymphal and adult stages. The bacteria primarily colonize the luminal space of the gut, where they are found in association with the gut epithelium (8, 9). During a second blood meal, the spirochetes multiply in the gut, enter the hemocoel and disseminate to multiple organs including the salivary glands through which the bacteria enter the host (9-11) (Figure 2). The dissemination within the tick is transient and there is no evidence that bacteria can colonize any sites other than the lumen of the gut. Transmission is complex and requires approximately 48 to 60hrs following tick attachment to the host, during which period the spirochetes experience complex changes in gene expression. (Figure 2: Distribution of *B. burgdorferi* within a feeding nymphal tick.)

3.3.Regulation of *B. burgdorferi* gene expression at the vector-host interface

In contrast to many pathogenic bacteria, *B. burgdorferi* devotes large portions of its genome (more than 8% of coding genes, or approximately 150 genes) towards producing lipoproteins, many of which are exposed on the bacterial outer surface (3). Within the feeding tick, the spirochetes alter the expression of many genes in preparation for transmission and infection of a new host (12, 13). *B. burgdorferi* outer surface proteins (Osp) A and C have served as a paradigm for understanding the regulation of bacterial gene expression within feeding ticks. In ticks, *ospA* is predominantly expressed before the blood meal, whereas *ospC* is induced during the blood meal (14-17). The functions of these two proteins are consistent with their pattern of expression, where OspA is required for colonizing the vector and OspC is required for infecting the host (see below).

Physiochemical clues, such as temperature, pH and cell density act as signals for regulating the expression of *ospA* and *C* in culture and these signals are likely to play a role in the feeding tick as well (17-20). Proteomic and microarray studies with cultured spirochetes grown in "tick" (low temperature, high pH) or "host" (high temperature, low pH) like conditions have led to the identification of large subsets of *Borrelia* proteins and genes with "OspA-like" or "OspC-like" patterns of expression (19, 21-23). The bacterial signaling pathway regulating the expression of *ospC* and *ospC-like* genes has been characterized in some detail (24-27). The pathway is activated by a two component system consisting of a sensor with a histidine kinase domain (HK2) and a cytoplasmic response regulator protein (Rrp2) (28). Activated Rrp2 together with the alternative sigma factor, RpoN, induces

Table 1. Representative *B. burgdorferi* genes required at specific stages of the vector

Gene	Protein	Function of *Borrelia* protein in *Ixodes scapularis* ticks	References
BBA15	OspA	Attachment and colonization of the tick gut	(42, 44)
BBA16	OspB	Attachment and colonization of the tick gut	(45)
BBB19	OspC	Migration from gut to the salivary gland and transmission to host	(48, 49, 53, 79)
BBJ09	OspD	Persistence in tick between molts and blood meals	(80)
BBE16	BptA	Persistence in tick between molts and blood meals	(58)
BB0690	Dps	Persistence in tick between molts and blood meals	(59)
BB0365	La7	Survival in feeding and replete ticks	(Pal *et al in press*)
BB0450	RpoN	Regulates genes required for transmission from tick to host	(29)
BB0771	RpoS	Regulates genes required for transmission from tick to host	(25)

expression of the *rpoS* that encodes the second alternative sigma factor, RpoS (28), and possibly other *B. burgdorferi* genes (29). RpoS activates the transcription of *ospC* and *ospC*-like genes associated with tick transmission and host infection.

Less is known about the pathways and signals regulating *ospA* and *ospA*-like genes mainly expressed in the vector. Some studies indicate a simple reciprocal relationship where the signals and pathways that induce *ospC* within feeding ticks also decrease the production of *ospA* (16, 30). Other studies have found that even under ideal conditions for *ospC* expression, the bacterial population is heterogeneous with many spirochetes producing both proteins and others producing either one or neither of the two proteins, indicating a more complex regulation of *ospA* and *C* (31, 32). More recent studies have demonstrated that at the level of single bacterial cell, *ospA* and *ospC* are, indeed, reciprocally regulated by the RpoN/RpoS pathway (24, 25). In summary, the emerging view is that external signals and the RpoN/RpoS sigma factors play a key role in regulating gene expression at the vector/host interface, including the transcriptional activation of *ospC* and repression of *ospA*.

3.4. *B. burgdorferi* antigenic variation within ticks

Lyme disease spirochetes display high frequency antigenic variation that is driven by recombination at the *vls* locus on lp28-1. This locus has an expression site encoding a surface exposed lipoprotein, *vlsE*, and 15 silent cassettes located upstream of the expression site (33). Recombination between portions of the silent cassettes within a central variable domain of the expression site leads to the expression of novel *vlsE* alleles encoding antigenic variants of VlsE (34). Novel recombination events are detectable within days after infection of the mammalian host and likely help the spirochete evade host defense mechanisms (35, 36). When ticks feed on a mouse infected with *B. burgdorferi*, the ticks are colonized with a population of spirochetes that contain many different *vlsE* alleles (37). Studies have been conducted to determine if the *vlsE* locus recombines and generates novel antigenic variants within ticks as well. Investigators used artificial feeding methods to introduce clonal populations of spirochetes expressing a single allele of *vlsE* into ticks (37-39). When these infected ticks were fed on naïve mice, only the original, single *vlsE* allele was detected in the ticks, whereas multiple new *vlsE* alleles were detected in the mice within a few days of tick transmission. One explanation for these results is that the recombination frequency is similar in mice and ticks, but strong selective pressure in the host amplifies rare recombinants. Alternatively, it is possible that recombination at the *vlsE* locus is regulated and that the recombination rate is higher in mice compared to ticks.

3.5. *B. burgdorferi* genes of functional significance in the vector

The annotated genome of *B. burgdorferi* has approximately 1780 genes. Investigators have used gene expression patterns as a surrogate to understand the function of *Borrelia* genes. Specific genes that were selectively expressed in the vector or host were predicted to encode proteins of functional significance in the vector or host, respectively. Recently some of these predictions have been confirmed by creating *Borrelia* mutants and studying their phenotype in ticks or the host. Below, we summarize the studies that have led to the identification and genetic characterization of some B. burgdorferi genes required at different stages in the vector (Table 1: Representative *B. burgdorferi* genes required at specific stages of the vector)

3.5.1. *B. burgdorferi ospAB* operon

Two of the best characterized *B. burgdorferi* genes, *ospA* and *ospB,* constitute a single operon in the linear plasmid lp54, and are amongst the major outer membrane proteins produced by *B. burgdorferi* grown *in vitro.* These proteins show differential expression in the life cycle of spirochetes (13). *ospA* and *ospB* are expressed when spirochetes exit the infected mammalian host and enter feeding ticks (15, 40). Recombinant OspA and OspB specifically bind to the tick gut, and passive transfer of non-*Borrelia*cidal OspA or OspB antibodies in the murine host prevents tissue adherence of spirochetes in feeding ticks *in vivo (*41-43). This information provided the first clue that these proteins may possibly be involved in spirochete colonization of the tick gut. More convincing evidence was obtained when infection studies were performed with a mutant strain of *B. burgdorferi* containing an insertionally inactivated *ospAB* operon (44). The mutant without OspA and OspB remained fully infectious to mice but was unable to infect ticks. The *ospAB* mutant was able to migrate into the feeding ticks from an infected mouse, but the mutant failed to persist in the tick gut, indicating a critical role for the *ospAB* operon in the maintenance of *Borrelia* in ticks. An independent study using an OspB-deficient *B. burgdorferi* isolate indicated that like OspA, OspB is also actively involved in colonization and persistence of *B. burgdorferi* in the arthropod vector (45). Together, these experiments demonstrate the vital importance of OspA and OspB in mediating *B. burgdorferi* colonization of the tick gut. *B. burgdorferi* strains containing a copy of *ospA* fused to the constitutive *flaB* promoter have also been created and tested in mouse infection studies. Spirochetes constitutively producing

Table 2. *Ixodes* tick proteins likely to play a direct or indirect role in facilitating *B. burgdorferi* transmission

Protein	Activity	Borrelia Target	Effect on Borrelia	Ref
Anti-Hemostatic Salivary Proteins				
Salp14	Inhibits factor Xa preventing coagulation	---	Indirect	(81)
Ixolaris (member of Kunitz-domain containing protein family)	Inhibits the tissue factor pathway preventing coagulation	---	Indirect	(82, 83)
Penthalaris (member of Kunitz-domain containing protein family)	Inhibits the tissue factor pathway preventing coagulation	---	Indirect	(84)
Immunosuppressive Salivary Proteins				
Salp15	Inhibits IL-2 production and $CD4^+$ T-cell proliferation by binding the CD4 coreceptor	OspC	Directly facilitates transmission to the host	(49, 85, 86)
Isac and related family members	Inhibits the alternative complement pathway by dissociating the C3 convertase	---	Protects *Borrelia* from complement; Increases survival of *Borrelia* in ticks	(67, 68, 87, 88)
Sialostatin L and L2	Reduce T-cell proliferation by binding the serine protease, cathepsin L	---	Indirect	(89, 90)
Prostaglandin E_2	Inhibits IL-12 and TNF-alpha production from dendritic cells	---	Indirect	(91)
Iris	Inhibits lymphocyte proliferation and the production of IFN-gamma, IL-6, and TNF-alpha	---	Indirect	(92)
IL-2 binding protein	Inhibits T-cell proliferation by binding IL-2	---	Indirect	(93)
Non-salivary Proteins				
TROSPA	Expressed in tick gut; function unknown	OspA	Serves as receptor in tick gut for *B. burgdorferi* OspA	(65)

OspA failed to infect immuno-competent mice, indicating that *ospA* expression in the host is deleterious to the bacteria (46).

3.5.2. *B. burgdorferi ospC*

OspC is a prominent *Borrelia*l outer membrane protein that is swiftly upregulated by spirochetes during transmission from the feeding tick to the host (15, 17). Studies with *ospC* null mutants have unequivocally demonstrated that OspC is required for infecting mice (47, 48). These studies have also led to conflicting results about a role for the protein in the vector. One group created an *ospC* null mutant and observed a defect in tick salivary gland invasion as well as host infection (48). In a follow up study (49), the same group reported that OspC recruited a tick protein to the bacterial surface that facilitated salivary gland invasion and host infection (see below). Another group independently created *ospC* null mutants and used them in tick and mouse infection studies. They concluded that OspC was required for establishing an infection in a host but not for the migration or dissemination within the tick (47, 50-52). A recent study with *B. afzelii,* which is responsible for Lyme disease in Europe and Asia, supports the hypothesis that OspC is required for dissemination within the tick (53). The investigators complemented a natural *ospC* mutant and observed that the complemented strain invaded salivary glands more efficiently than the *ospC* mutant.

3.5.3. *B. burgdorferi* genes on lp25 required for tick infection

As mentioned previously, *B. burgdorferi* has at least 21 different linear and circular plasmids. As several plasmids are lost during *in vitro* culture, it is possible to assemble panels of isolates missing non-essential plasmids in order to determine if specific plasmids are required for infecting the vector and host. Linear plasmid 25 (lp25) has been found to be essential for both host and tick infection (54-57). *Bbe22* on lp25 encodes a nicotinamidase that is most likely required for the biosynthesis of NAD. *Bbe22* alone was sufficient to restore the ability of strains lacking lp25 to infect mice (54). However, complementation with *Bbe22* only partially restored tick infectivity, indicating that additional genes on lp25 were required for tick infection (56). Revel and colleagues recently used *in vitro* microarray expression data to predict genes on lp25 required in the vector (58). They focused on *bbe16*, as this gene appeared to be selectively expressed when *Borrelia* were grown *in vitro* under conditions mimicking the tick. By creating *bbe16* null mutant and complemented strains, they demonstrated BBE16 was required for persistent infection of ticks (58). BBE16 is most likely a surface exposed lipoprotein and the exact function of the protein remains to be elucidated. These studies indicate that both *bbe22* and *bbe16* on lp25 are required for tick infection.

3.5.4. Chromosomally encoded *Borrelia* genes required in the vector

As described earlier, the chromosomally encoded RpoN and RpoS sigma factors activate and repress many genes in feeding ticks, implying a role for the RpoN/RpoS pathway in *B. burgdorferi* tick transmission. Fisher *et al.* created a *rpoN* null mutant and demonstrated that mutant spirochetes successfully colonized tick guts but were unable to invade salivary glands and infect the vertebrate host, indicating that RpoN regulated genes were involved in transmission from the vector to the host (29).

Bb0690 is a chromosomally encoded gene that is selectively expressed to high levels in ticks and encodes a protein that bears strong similarity to a family of DNA-binding proteins induced during starvation (Dps proteins) of the bacteria (59). *B. burgdorferi* Dps expression is low throughout murine infection but increases during the tick inter-molt periods. A *B. burgdorferi* Dps null mutant was able to infect mice (59). When naïve ticks were allowed to engorge on these mice, the *dps* mutant was able to infect

the ticks but was unable to survive the extended period between tick molts. Dps protects bacterial DNA against starvation or oxidative stress-induced damage. Therefore, BB0690 may protect *B. burgdorferi* against DNA damage during the long inter-molt period between blood meals.

An infectious mutant of *B. burgdorferi* lacking another chromosomal gene, *bb0365*, has also recently been created (Pal *et al*, Manuscript *in press*). Spirochetes lacking BB0365, also known as p22 or lipoprotein LA7, were able to infect mice. When naïve ticks engorged on spirochete-infected mice, the *bb0365* mutant had a markedly decreased survival rate within ticks when compared to wild type *B. burgdorferi.* These studies suggest that BB0365 could be important for spirochete survival in feeding ticks. In summary, the ability to create specific mutants of *B. burgdorferi* has allowed researchers to identify *Borrelia* proteins that function at different stages in vector phase of the life cycle. This list, which includes both plasmid and chromosomally encoded proteins, is still relatively small and many more proteins required in the tick are likely to be identified in the near future.

3.6. Tick proteins that facilitate *Borrelia* transmission

Ixodes tick saliva has molecules with anti-hemostatic, anti-inflammatory, vasodilatory, and immunosuppressive activities, which assist the tick to feed on a host for prolonged periods without immune recognition and rejection (Table 2). Beyond serving the tick during feeding, salivary proteins can also directly contribute to the efficient transmission of *Borrelia* to a new host. *In vitro* studies have demonstrated that salivary gland extracts from *I. recinus* ticks reduce the phagocytosis and killing of *B. afzelii* by murine macrophages (60, 61). Other groups have also demonstrated that needle inoculation of *B. burgdorferi* into naïve mice in the presence of *I. recinus* or *I. scapularis* salivary gland extracts (SGE) allows more efficient survival and dissemination of the spirochetes as demonstrated by increases in the numbers of spirochetes present in various tissues when compared to mice needle inoculated with *B. burgdorferi* in the absence of SGE (62, 63). More recently, Lima and co-workers demonstrated that *B. burgdoferi* in tick salivary glands had an ID_{50} that was 10 times lower than *Borrelia* in the tick gut (64). Together these studies indicate that *I. scapularis* salivary components enhance the infectivity of *B. burgdorferi.*

(Table 2: Ixodes tick proteins likely to play a direct or indirect role in facilitating *B. burgdorferi* transmission)

3.6.1. Salp15- a tick protein that binds to OspC

Ramamoorthi and co-workers reported that *I. scapularis* salivary protein 15 (Salp15) bound to OspC and facilitated transmission of spirochetes from the tick to the host, suggesting that OspC may have a role in the vector as well as during early host infection (49) (Table 2). Through *in vitro* and *in vivo* binding assays, Salp15 was shown to directly bind to *B. burgdorferi* through OspC, and this binding protected *B. burgdorferi* from antibody mediated killing *in vitro*. When mice previously exposed to *B. burgdorferi* and displaying an antibody response against the spirochete were infected by needle inoculation with *B. burgdorferi* in the presence or absence of recombinant Salp15, the levels of spirochetes in the skin, joints, and bladder were significantly higher in mice that received Salp15. Additionally, when *salp15* was silenced in *I. scapularis* nymphs by RNA interference, the amounts of spirochetes transmitted to naïve mice were significantly reduced when compared to mock treated infected ticks (49). The results from this study indicate that Salp15 assists *B. burgdorferi* in transmission to and infection of the host during tick feeding.

The Salp15 binding to *Borrelia* might be responsible for a puzzling observation about OspC production by spirochetes moving from the tick to the host. We have reported that many *Borrelia* in the salivary glands and host skin are OspC negative by immuno- florescence microscopy, which argues against a role for this protein in transmission (32). Spirochetes in the salivary glands and host's skin may appear OspC negative because Salp15 masks OspC and prevents antibody from staining the bacteria.

3.6.2. The tick receptor for OspA (TROSPA)

Recent studies have identified a tick gut receptor that interacts with *B. burgdorferi* OspA (65). Pal *et al.* have established that *B. burgdorferi* OspA binds the tick receptor for OspA (TROSPA), which is expressed in the gut of *I. scapularis* ticks (65). *In vitro*, OspA specifically bound recombinant TROSPA, while TROSPA antisera partially blocked binding of OspA to tick gut extracts. Interestingly, *TROSPA* expression within the tick was upregulated during infection with *B. burgdorferi* and decreased during engorgement, correlating with *ospA* downregulation and transmission of the spirochetes to a new host. When uninfected ticks were allowed to feed on infected mice administered with TROSPA antisera, acquisition of *B. burgdorferi* by the ticks was significantly reduced when compared to infected mice treated with pre-immune sera. Similarly, RNA interference of *TROSPA* in nymphal ticks significantly diminished the ability of the ticks to acquire *B. burgdorferi* from infected mice. The results of Pal *et al.* demonstrate that *B. burgdorferi* colonizes the tick gut by adhering to TROSPA through OspA. This attachment allows the spirochete to persist within the tick until the next blood meal occurs and transmission begins. Although TROSPA has been shown to be actively engaged in supporting *B. burgdorferi* during its life cycle within the arthropod, the native, physiological function of TROSPA in ticks is currently unknown. *TROSPA* is not consistently expressed in all stages of *I. scapularis* ticks, and its expression is highly influenced by environmental signals, such as tick engorgement or *B. burgdorferi* infection, suggesting a developmental or transient rather than a housekeeping function. TROSPA bears weak amino acid sequence homology (31%) to anti-freeze glycoproteins (AFGP) in the protein database. AFGP have extensive sequence divergence across phyla and normally function by binding ice particles, thereby preventing ice crystal formation and freeze-induced tissue damage in plants, fish and arthropods. Studies addressing the role of TROSPA in tick physiology might contribute to a better understanding of *B. burgdorferi* pathogenesis in ticks.

3.6.3. The Isac family of proteins

Recently, researchers have identified a large family of *Ixodes scapularis* anti-complement (*Isac)* proteins that block the alternative complement pathway (66-68). At least one member of this family (Salp20) protects serum sensitive strains of *Borrelia* from lysis in *in vitro* assays, indicating this protein may protect *Borrelia* from host complement within the vector and during transmission (68). Furthermore, Soares *et al.* used RNA interference to silence *Isac* and related family members in ticks infected with *B. burgdorferi (*67). When control and *Isac* silenced nymphs were fed on mice, *Isac* treated ticks fed poorly and the *Borrelia* load within these ticks was lower than in the control ticks, indicating that *Isac* is needed for efficient tick feeding as well as for the growth of *Borrelia* within ticks. Taken together, these results demonstrate a possible role for the *Isac*-like family of proteins in *Borrelia* survival and transmission. As more work is done in this area, additional tick proteins required for *Borrelia* infection and transmission will be identified.

3.7. Tick immune system and *B. Burgdorferi*

Arthropods have innate immune systems that recognize and destroy invading pathogens. Surprisingly, little work has been done to determine how *Borrelia* evade tick immune responses. In one study, *B. burgdorferi* were injected into the hemocoel of *I. scapularis* and *Dermacentor variabilis*, which is not a competent tick vector of *Borrelia* (69). The spirochetes readily survived in the *Ixodes* hemolymph, whereas they were rapidly killed by hemocytes in the *D. variabilis* hemolymph. This study indicated that *Ixodes* ticks, unlike *Dermacentor* ticks, were immuno-tolerant towards *B. burgdorferi*.. Even though they appear immuno-tolerant and permissive to *B. burgdorferi* infection, *I. scapularis* and related ticks are not completely tolerant of *B. burgdorferi* as some tick genes are induced following infection. Ribeiro *et al.* recently identified several salivary genes that were up-regulated in infected *I. scapularis* nymphs when compared to uninfected nymphs (66), and these genes may encode proteins that limit *Borrelia* infection. Rudenko *et al.* have also demonstrated by subtractive hybridization that an *I. recinus* defensin-like gene is up-regulated in the gut after infection with *B. burgdorferi* (70, 71). Additionally, Rudenko *et al.* determined that putative genes potentially involved in an oxidative stress response are also up-regulated after infection. We currently do not understand the mechanism behind the relative immuno-tolerance of *I. scapularis* to *B. burgdorferi* and the role of tick immune responses in determining vector competence.

4. TICK-BORNE RELAPSING FEVER *BORRELIA*

Tick-borne relapsing fever (TBRF) is a rare but widespread disease present in the Americas, Europe, Africa and Asia. The *Borrelia* species responsible for relapsing fever are mainly transmitted by Argasid ticks, otherwise known as soft ticks. *B. recurrentis*, responsible for louse-borne relapsing fever, is transmitted by an insect vector. TBRF is characterized by episodes of fever separated by 1-2 week intervals of mild or no fever. Untreated disease can lead to serious neurological complications and a mortality frequency of 4 to 10%. Few studies have been done to determine the distribution of TBRF *Borrelia* within ticks. Burgdorfer compared *B. duttonii* distribution within the nymphal and adult *Ornithodorus* ticks and found that the spirochetes disseminated within both the nymphal and adult vectors within 24 hrs of infection and colonized multiple tissues including the salivary glands (72). Interestingly, nymphal ticks transmitted *B. duttonii* through the salivary glands whereas adult ticks appeard to transmit the organisms through coxial fluid. More recently, Schwan and Hinnebusch examined the distribution of *B. hermsii* in ticks and observed that in most cases, multiple organs including the salivary glands were colonized by the spirochetes (73). Thus, unlike the Lyme disease spirochetes, which mainly colonize the tick gut lumen, the relapsing fever spirochetes disseminate in the vector and colonize multiple tissues including the salivary glands.

4.1. Relapsing Fever *Borrelia* and tick interactions

In comparison to Lyme disease *Borrelia*, we know very little about the molecular interactions between relapsing fever *Borrelia* and their tick vectors. Relapsing fever spirochetes use multiple mechanisms to vary the dominant antigen expressed on the bacterial surface in mammals. Antigenic variation leads to cyclic spirochetemias in mammals that reach a density of 10^8 bacteria/mL of blood. Each new cycle of spirochetemia is characterized by the expression of a new antigenic variant on the surface. Schwan and colleagues compared the major surface proteins expressed by *B. hermsii* in mice and in ticks (73). They observed that the antigenic composition of the spirochetes within ticks was relatively stable, and most *B. hermsii* in the salivary glands expressed a single variable surface protein designated Vsp33. Moreover, the replacement of the variable surface antigens expressed in mice with the invariant Vsp33 appeared to be triggered by the low temperature encountered in the tick vector. Interestingly, *B. hermsii* Vsp33 is related to *B. burgdorferi* OspC at the amino acid level and antibodies against OspC cross react with Vsp33. Schwan has argued that Vsp33 and OspC have related functions in their respective *Borrelia* species and that the differences in the expression patterns of these proteins relate to the different feeding behaviors of the tick vectors (74). Lyme disease spirochetes infect hard ticks that feed continuously for several days, and there is ample time for the spirochetes to turn on the expression of *ospC,* which is required for transmission and host infection. In contrast, Argasid ticks only feed for a matter of minutes. Consequently, *B. hermsii* colonizing the salivary glands express *vsp33* and are poised for transmission during the short blood meal.

Projects are underway to sequence the genomes of relapsing fever spirochetes. DNA cross hybridization studies indicated that *B. hermsii* has at least 81% of the chromosomal and 43% of the plasmid encoded genes of *B. burgdorferi,* implying that these organisms have similar genomes despite the differences in the diseases they cause and the vectors they infect (75). The genetic tools developed for *B. burgdorferi* are also being used to study relapsing fever spirochetes. More comparative studies are needed to understand how Lyme disease and relapsing

fever spirochetes have adapted to enter, colonize and leave their respective vectors, which have markedly different life cycles and feeding patterns.

5. TICK-TRANSMISSION BLOCKING VACCINES

Studies on *Borrelia*-tick interactions are likely to contribute to the development of novel transmission blocking vaccines. Bacterial pathogens, such as *Borrelia*, that cause persistent infections in mammals have evolved sophisticated mechanisms of antigenic variation and differential gene expression to evade host immunity. As a result, antigens expressed in the host are, often, not good vaccine candidates. As the vector does not have an adaptive immune system, *Borrelia* antigens expressed in the vector are conserved and antigenic variation mechanisms appear to be inactive in the vector (39). In fact, the FDA-approved OspA Lyme disease vaccine induces antibodies in the host that enter the guts of infected feeding ticks and prevent the transmission of spirochetes from the vector to the host (14). Although an OspA-based vaccine is still in use to prevent *B. burgdorferi* infection in some animals, the human vaccine is no longer available. Therefore, further studies are needed to develop the next generation of vaccines for protecting people from *Borrelia* infections. As transmission blocking vaccines have the potential to block ticks from both acquiring and transmitting *Borrelia,* studies are also being conducted to determine if these vaccines can be used in reservoir hosts to reduce the proportion of infected ticks. In addition to bacterial antigens, tick proteins also have the potential to be developed into vaccines. Vaccines that target tick salivary proteins expressed early in the blood meal could inhibit tick feeding and block pathogen transmission. Alternatively, vaccines based on tick molecules that interact with *Borrelia* might be effective in blocking transmission without having any adverse effects on the vector.

6. SUMMARY, OUTSTANDING QUESTIONS AND FUTURE DIRECTIONS

Arthropod-borne diseases are fascinating biological systems where specific components from the vector, pathogen and host interact to enable pathogens to move between hosts. Since the discovery of the *Borrelia*l etiology of Lyme disease in 1982, much progress has been made in developing tools to study these spirochetes. These tools are now being applied to understand both *Borrelia*-host and *Borrelia*-vector interactions. As highlighted above, recent work has led to exciting discoveries of both *Borrelia* and tick molecules required at different stages in the vector. What is also clear is that we have only identified a very small subset of interactions and much remains to be discovered. We need to identify additional microbial as well as vector gene products required for the acquisition, maintenance and transmission of the microbe by the arthropod. How does *Borrelia* evade tick immunity or survive in the feeding gut when the bloodmeal is being digested by the vector? How does *Borrelia* persist in ticks through the intermolt periods dominated by temperature extremities and a scarcity of nutrients? Studies should also address details of how *B. burgdorferi* invades solid physical barriers, such as the chitinous gut peritrophic membrane, and then selectively migrates through the gut, hemocoel and salivary gland epithelia during its transmission to mammals. Comparative studies are needed with different species of *Borrelia* and ticks to identify common themes, as well as species-specific differences.

Given recent technological advances, the next few years are likely to be particularly productive with respect to defining the function of additional *Borrelia* genes in the vector and host. There are still formidable challenges to uncovering tick molecules regulating *Borrelia* infection. The long life span (approximately 2 years) and slow post-embryonic development of *Ixodes* ticks, coupled with limited genomic information severely blocks application of gene manipulation tools to tick research. Currently it is also not possible to analyze the entire tick transcriptome or proteome. In 2004, the *I. scapularis* Genome Project (IGP), a collaborative effort between the international community of tick researchers and two genome sequencing centers, was approved and is currently supported by the National Institutes of Health (76, 77). The goal of the IGP is to perform whole genome shotgun sequencing (WGS) to approximately six-fold coverage of the genome (76). Compared to sequenced fly genomes, *I. scapularis* has a larger genome (2.1×10^3 Mbp), which is uniquely organized into 27% highly repetitive, 39% moderately repetitive and 34% unique DNA (78). Currently, more than 18 million trace reads, representing approximately five-fold coverage of the genome, have been deposited at the National Center for Biotechnology Information (NCBI) trace archive (77). In addition, 20 *Ixodes* BAC clones, 370,000 BAC-end reads, and more than 80,000 ESTs have also been sequenced. The *Ixodes* mitochondrial genome has been assembled and annotated. By 2008, sequencing, assembly, and annotation of six-fold coverage of the *Ixodes* genome are expected. The tick genome will be best utilized if the community of tick researchers also develops tools for creating transgenic ticks.

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