REGULATION OF OUTER SURFACE LIPOPROTEIN A IN THE LYME DISEASE SPIROCHETE *BORRELIA BURGDORFERI*

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ABSTRACT

Tara Lynn Oman

REGULATION OF OUTER SURFACE LIPOPROTEIN A IN THE LYME DISEASE SPIROCHETE BORRELIA BURGDORFERI

*Borrelia burgdorferi*, a bacterium which causes Lyme disease, is maintained in nature through a cycle involving two distinct hosts: a tick vector and a mammalian host. To adapt to these two diverse environments, *B. burgdorferi* undergoes dramatic alterations in its surface lipoprotein. Two essential lipoproteins, outer surface protein A (OspA) and outer surface protein C (OspC), are reciprocally regulated throughout the *B. burgdorferi* lifecycle. Very little is known about the regulation of OspA. These studies elucidate the regulatory mechanisms controlling the expression of OspA. Various truncations of the *ospA* promoter were created and then studied in our novel *in vitro* model of *ospA* repression or grown within the host-adapted model. A T-Rich region of the *ospA* promoter was determined to be a cis-element essential for both the full expression and full repression of *ospA*.

X. Frank Yang, Ph.D.- Chair
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<tr>
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<tr>
<td>AF</td>
<td>Accessory factor</td>
</tr>
<tr>
<td>Amp</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>BSK</td>
<td>Barbour-Stoenner-Kelly media</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>CAT</td>
<td>chloramphenicol acetyltransferase</td>
</tr>
<tr>
<td>CCLR</td>
<td>Cell culture lysis reagent</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control and Prevention</td>
</tr>
<tr>
<td>c-di-GMP</td>
<td>Bis-(3′-5′)-cyclic dimeric guanosine monophosphate</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>cp</td>
<td>circular plasmid</td>
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<tr>
<td>Ct</td>
<td>Cycle threshold</td>
</tr>
<tr>
<td>Dbp</td>
<td>Decorin-binding protein</td>
</tr>
<tr>
<td>DMC</td>
<td>Dialysis membrane chamber</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EBP</td>
<td>Enhancer binding protein</td>
</tr>
<tr>
<td>EPS</td>
<td>Electroporation solution</td>
</tr>
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<td>Kanamycin</td>
</tr>
<tr>
<td>Kb</td>
<td>Kilobases</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertani</td>
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<tr>
<td>Ip</td>
<td>linear plasmid</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>min</td>
<td>Minute</td>
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<tr>
<td>ml</td>
<td>Milliliter</td>
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<tr>
<td>ms</td>
<td>Millisecond</td>
</tr>
<tr>
<td>NtrC</td>
<td>Nitrogen regulatory protein C</td>
</tr>
<tr>
<td>Osp</td>
<td>Outer surface lipoprotein</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Quantitative reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>Rep</td>
<td>Repressor</td>
</tr>
<tr>
<td>Rept</td>
<td>Direct Repeat</td>
</tr>
<tr>
<td>RLU</td>
<td>Relative light unit</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RNAP</td>
<td>RNA polymerase</td>
</tr>
<tr>
<td>rps</td>
<td>Revolutions per second</td>
</tr>
<tr>
<td>Rrp1</td>
<td>Response regulator protein 1</td>
</tr>
<tr>
<td>Rrp2</td>
<td>Response regulator protein 2</td>
</tr>
<tr>
<td>SCID</td>
<td>Severe combined immunodeficient</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>Sec</td>
<td>Seconds</td>
</tr>
<tr>
<td>Strep</td>
<td>Streptomycin</td>
</tr>
<tr>
<td>TLR1</td>
<td>Toll-like Receptor 1</td>
</tr>
<tr>
<td>TLR2</td>
<td>Toll-like Receptor 2</td>
</tr>
<tr>
<td>TLR4</td>
<td>Toll-like Receptor 4</td>
</tr>
<tr>
<td>T-Rich</td>
<td>Thymine rich</td>
</tr>
<tr>
<td>VlsE</td>
<td>Vmp-like sequence E</td>
</tr>
<tr>
<td>Vmp</td>
<td>Variable major protein</td>
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<tr>
<td>X-gal</td>
<td>5-bromo-4-chloro-indolyl-β-D-galactopyranosid</td>
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CHAPTER ONE: INTRODUCTION

History of Lyme disease
Lyme disease is the most common arthropod-borne disease in the United States. In 1975, in Lyme, Connecticut, an unusual number of children presented symptoms of Juvenile Rheumatoid Arthritis (Steere et al., 1977). Although the definitive cause had not been confirmed, an infectious agent was suggested to be responsible for the arthritis displayed in the children. It was suspected that the unidentified infectious agent was being spread via an arthropod vector given that the patients lived near wooded areas and the highest frequency of outbreaks occurred in the summer (Steere et al., 1979; Steere et al., 1977). Around this same period, outbreaks of spotted fever spread by Rickettsia-infected ticks were occurring on Long Island, New York. In 1984, scientist Willy Burgdorfer was collecting tick samples in attempts to isolate a virulent strain of Rickettsia rickettsii. Incidentally, during this endeavor, Willy Burgdorfer discovered the spirochete Borrelia burgdorferi. It was suspected that this newly discovered bacterium was the causative agent of the arthritis cases in Lyme Connecticut (Burgdorfer, 2006; Burgdorfer et al., 1985). To confirm this notion, sera were taken from the patients in Lyme, Connecticut that were misdiagnosed with Juvenile Rheumatoid Arthritis and the presence of B. burgdorferi was confirmed.

Epidemiology of Lyme disease
B. burgdorferi is the primary causative agent of Lyme disease (Burgdorfer et al., 1985). Twelve of the thirty-seven known Borrelia species are capable of causing Lyme disease (Hengge et al., 2003). Lyme disease is an emerging infectious disease throughout Europe, Asia, South America, and Canada as well as the United States. According to the Center for Disease Control and Prevention (CDC), Lyme disease is the most common
vector-borne illness in the United States (2011). The number of reported Lyme disease cases has increased over the past 14 years. In 1996, there were 17,730 confirmed cases of Lyme disease while in 2010 there were 22,572 confirmed cases and 597 probable cases of Lyme disease in the United States as reported by the CDC (Figure 1).

Lyme disease is endemic in several areas of the United States, but is concentrated in the Northeastern and the upper Northeastern states (Figure 2). According to the CDC, 94% of the total Lyme disease cases were reported from just the following 12 states: Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Jersey, New Hampshire, New York, Pennsylvania, Virginia and Wisconsin (2010). Lyme disease is most prevalent in these states because they have a wooded, grassy environment which supports *B. burgdorferi*’s vector, the deer tick *Ixodes scapularis*, as well as the mammalian hosts required for the complete *B. burgdorferi* natural lifecycle.

**Natural Lifecycle of *B. burgdorferi***

In nature, *B. burgdorferi* is maintained via an enzootic cycle being transmitted between a tick vector, the *Ixodes* tick, and a mammalian host, usually small animals like the white-footed mouse, rabbit and some birds (Anderson, 1998). In the United States, *I. scapularis* is the main arthropod vector in the upper Midwestern and the Northeastern parts of the country while the Western blacklegged tick, *Ixodes pacificus*, is the main arthropod vector in the western part of the country. The tick’s lifespan is only two years. During their lifespan, the tick matures from an egg and then transforms into larval, nymph, and adult stages (Figure 3). The tick takes three blood meals throughout its entire life- once during each of its last three stages of development.
The adult female ticks lay eggs during the spring time, and then the eggs hatch during the summer into larvae. Upon hatching, the naïve larvae acquire *B. burgdorferi* when the tick larva takes a blood meal from a *Borrelia*-infected small mammal, usually a white-footed mouse. Because *B. burgdorferi* cannot be transmitted transovarially, infected ticks cannot pass the bacterium to its offspring and therefore the offspring must acquire the bacterium via the blood meal of an infected animal (Burgdorfer et al., 1985; Magnarelli et al., 1992; Piesman et al., 1986).

In the subsequent spring, the *Borrelia*-infected larva molts into a nymph. During the summer, the nymph is able to feed a second time. During this feeding, the nymph takes a blood meal from a small, uninfected mammal and will transmit the bacteria to the mammal thus completing the transmission cycle (Lane and Loye, 1991; Spielman, 1994; Steere et al., 2004). It is also during this nymphal stage that the infected tick is most likely to transmit *B. burgdorferi* to humans, which serve as accidental hosts (Lane and Loye, 1991). After molting into an adult, the tick feeds for the third and final time during the fall, usually on a large mammal such as a deer. Although deer are not competent reservoirs for spirochetes, larger animals are needed to feed a large number of adult ticks to support tick mating (Matuschka et al., 1993).
Figure 1. Reported Cases of Lyme Disease by Year in the United States, 1996-2010 (CDC Division of Vector-borne Infectious Diseases).

The number of confirmed cases and estimated probable cases of Lyme disease, as reported by the State Health Departments, shows an increase of incidences over time. There were 22,572 confirmed cases of Lyme disease in 2010, a 37% increase from 1996.
Figure 2. Reported Cases of Lyme Disease in the United States, 2009 (CDC Division of Vector-borne Infectious Diseases).

For each case of Lyme disease confirmed by the State Health Departments, one dot is placed randomly within the county of the patient's residence. Cases have been reported in nearly every state, but the county of residence is not necessarily the county in which the infection was acquired. The greatest number of cases was reported in the Northeastern and the upper Northeastern states.
Figure 3. The enzootic life cycle of *Ixodes scapularis*, the tick that spreads Lyme disease (CDC Division of Vector-borne Infectious Diseases).

The *Ixodes* tick has four stages of life: the egg, larva, nymph and adult. The egg is laid during the spring and hatches into larva during the summer. The larva takes a blood meal from a *B. burgdorferi*-infected small mammal or bird and then molts into a nymph during the subsequent spring. The infected nymph feeds another time during the spring or summer during which the tick transmits the bacteria to an uninfected mammalian host thus completing the *B. burgdorferi* transmission cycle. The nymph molts into an adult and the adult mates, takes a blood meal from a large mammal, and then lays eggs in the following spring to complete the lifecycle of the tick.
Clinical Manifestations of Lyme disease

The clinical manifestations of Lyme disease vary depending on its progression. Early localized infection (Stage One) is within days to weeks following initial infection with *B. burgdorferi*. These patients may present fever, headache, fatigue and Erythema Chronicum Migrans. Erythema Chronicum Migrans is the red macule at the site of the tick bite that expands to become an annular rash that has central clearing giving the appearance of a bulls-eye. Following the early localized infection is the early disseminated infection (Stage Two), which occurs weeks or months after initial inoculation. Symptoms in this stage include severe neurologic and cardiac complications such as meningitis, encephalitis, Bells’ palsy, and transient Atrio-Ventricular block. The last stage of the disease is late or persistent infection (Stage Three) which begins months to years after initial infection. Intermittent and migratory arthritis, as well as neurologic and musculoskeletal problems such as encephalitis and altered memory and speech are manifestations of persistent infection (Warinner, 2001).

*B. burgdorferi* Structure and Genome

*B. burgdorferi* belongs to a group of bacteria phylogenetically distinct from other main bacterial groups, the spirochetes (Woese et al., 1984). Characteristic of spirochetes *B. burgdorferi* is a long, thin helical shaped bacterium with endoflagella that give *B. burgdorferi* its characteristic helical shape (Figure 4). There are 7 to 11 flagella inserted near the poles of the spirochete that wind around the rod-shaped protoplastic cylinder, and overlap in the center, giving the spiral shape structure as well as motility to *B. burgdorferi* (Barbour and Hayes, 1986; Charon and Goldstein, 2002; Charon et al., 2009; Motaleb et al., 2000; Sartakova et al., 2001). *B. burgdorferi* lacks lipopolysaccharide and has an inner and outer membrane (Takayama et al., 1987). The outer membrane, or cytoplasmic cylinder, surrounds both the periplasmic flagella and the protoplastic
cylinder, which is composed of a peptidoglycan layer and an inner membrane enclosing the cytoplasmic contents (Barbour and Hayes, 1986; Johnson et al., 1984; Kudryashev et al., 2010; Rosa, 2005; Rosa, 1997).

The genetic composition of *B. burgdorferi* is complex and unusual, thus creating obstacles for genetic studies and genetic manipulation. The segmented genome is composed of a linear chromosome of approximately 910 kilobases (kb) along with eleven circular plasmids (cp) and twelve linear plasmids (lp) totaling approximately 610 kb (Casjens, 2000; Fraser et al., 1997). *B. burgdorferi* has the largest number of plasmids of any characterized genome. The plasmids are numbered according to their size in kb pairs. The sequenced *B. burgdorferi* strain B31 contains circular plasmids cp9, cp26, and nine homologous plasmids (cp32-1 to cp32-9); linear plasmids lp5, lp17, lp21, lp25, four homologous plasmids (lp28-1 to lp28-4), lp36, lp38, lp54, lp56; and the 910 kb linear chromosome (Fraser et al., 1997). The stability of these plasmids varies. Frequently, some plasmids are lost after only a few generations of *in vitro* growth while others are stable through continuous passage (Barbour, 1988; Byram et al., 2004; Grimm et al., 2003; Schwan et al., 1995; Xu et al., 1996).

Many of *B. burgdorferi*’s plasmids encode essential functions which are required for the spirochete to complete its natural infectious cycle (Labandeira-Rey et al., 2003; Labandeira-Rey and Skare, 2001; Lawrenz et al., 2002; Schwan et al., 1988; Xu et al., 1996). Two plasmids relatively unstable during *in vitro* culture growth, which are also essential for persistent infection within the mammalian host, are lp25 and lp28-1 (Labandeira-Rey et al., 2003; Purser and Norris, 2000). *B. burgdorferi* lacking lp25 cannot grow in wild-type mice, severe combined immunodeficient (SCID) mice, or within dialysis membrane chamber (DMC) implants suggesting a physiological defect. The lp25
plasmid contains the gene pncA, encoding for a nicotinamidase, which is likely to have a role for biosynthesis of NAD that is essential for \textit{in vivo} growth, but not required for \textit{in vitro} growth (Purser et al., 2003; Purser and Norris, 2000).

Similarly to lp25, the lp28-1 plasmid is also essential for the persistent infection in mice (Grimm et al., 2004a). In \textit{B. burgdorferi}, the lp28-1 plasmid contributes to the spirochete’s ability to persistently infect the host by encoding for VlsE (Vmp-like sequence E). Vmp stands for variable major protein and contributes to the antigenic variation of proteins in spirochetes (Barbour, 1993). VlsE is able to cause antigenic variation by undergoing an extensive genetic recombination mechanism at the \textit{vls}-locus (Coutte et al., 2009; Zhang and Norris, 1998).
Figure 4. The spirochete *B. burgdorferi* and schematic of its structure (Rosa et al., 2005).

(A) The scanning electron micrograph (left) showing the morphology of *B. burgdorferi* and the cross-sectional view of the transmission electron micrograph (right) showing the helical shape imparted by the periplasmic flagella. (B) Schematic of the spirochete illustrating the bundles of 7 to 11 flagella wound around the protoplasmic cylinder, overlapping in the middle, with the insertion points near the poles of the cell. The flagella are enclosed within the periplasm by the outer membrane. (C) The protoplasmic cylinder is enclosed by the cytoplasmic membrane and a peptidoglycan layer. The endoflagella are inserted into the cytoplasmic membrane and extend through the cell wall into the periplasm, the space between the cytoplasmic and outer membranes.
Lipoprotein Role in Virulence

*B. burgdorferi* is a Gram-negative-like bacterium that contains an inner and outer membrane, but unlike Gram-negative bacteria, *B. burgdorferi* lacks lipopolysaccharide (LPS) (Takayama et al., 1987). Rather than LPS, *Borrelia* contains an abundance of lipoproteins on its outer surface (Figure 5). LPS and lipoproteins are both major components of the outer membrane of bacteria. In fact, *B. burgdorferi* has an unusually large amount of lipoproteins; putative lipoproteins comprise as much as 14.5% of the genes encoded on *B. burgdorferi*’s plasmids, suggesting the lipoproteins play an important role in virulence (Casjens, 2000). Of all the open reading frames predicted in *B. burgdorferi*, lipoproteins account for 7.8% and this number is much higher than other bacterial genomes such as *Treponema pallidum*, 2.1%, or *Helicobacter pylori* containing 1.3% (Casjens, 2000; Fraser et al., 1997; Setubal et al., 2006).

Lipoproteins are membrane-anchored proteins found in *Borrelia* that are peripherally tethered to the lipid bilayer leaflets of the inner or outer membranes via the lipoprotein’s acyl group of the terminal cysteine. The lipoprotein begins as a prolipoprotein precursor within the cytoplasm. Next, the prolipoprotein is translocated through the inner membrane via a sec-dependent transport mechanism, and the ABC transporter-like complex, LolCDE, releases the outer membrane-targeted lipoproteins from the inner membrane (Yakushi et al., 2000). After release from the inner membrane, while in the periplasm, the lipids are modified to form mature lipoproteins containing a lipid-modified N-terminus. Lipoprotein maturation is suggested to occur in three steps: 1) diacylglyceroltransferase transfers a diacylglyceride to the sulfur of the cysteine side chain as specified by the signal sequence LXYC on the unmodified prolipoprotein; 2) signal peptidase II cleaves at the amino side of the cysteine residue to form a prolipoprotein; and 3) transacylase adds a third fatty acid to the new amino terminus via
an amide linkage to form the mature lipoprotein (Fraser et al., 1997; Hayashi and Wu, 1990; Juncker et al., 2003). The periplasmic chaperone, LolA, forms a complex with the lipoprotein and crosses through the periplasm (Yokota et al., 1999). Next, this complex interacts with LolB, a receptor on the outer membrane, mediating the anchor of lipoproteins to the inner leaflet of the outer membrane (Yokota et al., 1999). The lipoprotein is flipped across the outer membrane through an unidentified outer membrane module.

The most abundant lipoproteins are outer surface lipoproteins (Osp). Of these Osps, the most well-known of these outer surface proteins are OspA, OspB, OspC, OspD, OspE and OspF (Bergstrom et al., 1989; Burgdorfer et al., 1983; Howe et al., 1985; Lam et al., 1994; Wilske et al., 1993; Zumstein et al., 1992). Other lipoproteins include decorin-binding proteins (Dbp), *Borrelia* glycosaminoglycan-binding proteins (Bgp), and the VlsE. Interestingly Borrelial lipoproteins and Gram-negative bacteria’s LPS share similar roles: 1) they induce the host inflammatory response and are targets for bactericidal antibodies; 2) they contribute to the stability and structural integrity of the bacteria; and 3) they act as adhesions. Osps also have additional functions not shared by LPS: they are able use lipoproteins to 1) adapt to the various host environments; 2) evade phagocytosis by the host immune system; and 3) acquire nutrients (Liang et al., 2002).

Borrelial lipoproteins and Gram-negative bacteria’s LPS activate the mammalian host’s innate immune system. *Borrelia’s* activation of the host immune system is achieved via toll-like receptor II (TLR2) and toll-like receptor I (TLR1), whereas toll-like receptor IV (TLR4) recognizes the LPS of Gram-negative bacteria. TLR2 heterodimerizes with TLR1 to recognize triacyl-lipoproteins. The pattern recognition receptors of TLR2 recognize
Borrelial lipoproteins and activate the host’s inflammatory mediators to elicit the inflammatory response at the site of infection (Hirschfeld et al., 1999).

Also similarly to LPS, Osps play a role in the structural integrity of the bacteria. Throughout its lifecycle, *B. burgdorferi* must transition to and survive within many chemically different environments within its tick and the mammalian hosts. To respond to and adapt to these various environments, the bacteria undergo drastic adaptive changes through differential gene expression to alter its surface lipoprotein expression profile (Brooks et al., 2003; de Silva and Fikrig, 1997; Indest et al., 2000; Liang et al., 2002; Pal et al., 2004b; Seshu and Skare, 2000). The absence of Osps lead to a weak bacterial membrane, however addition of Osps is able to restore the structural integrity of the lipoprotein (Xu et al., 2008).

Another characteristic shared with LPS is the lipoprotein’s ability to function as an adhesion molecule used in bacterial transport and binding. Several lipoproteins have various adherence capabilities. Bgp is able to bind heparin sulphate and plays a role in mammalian infection (Parveen et al., 2003; Parveen and Leong, 2000). BBK32 is a lipoprotein that is able to bind fibronectin to promote *Borrelia* attachment to glycosaminoglycans and is important for dissemination (Probert and Johnson, 1998; Seshu et al., 2006). OspA is a lipoprotein that is able to attach to a protein within the tick gut, which is important for *Borrelia* colonization within the tick (Pal et al., 2000). Lipoproteins play a major role in *Borrelia* adhesion in a similar manner that LPS plays in adherence for Gram negative bacteria.
Both Gram-negative bacteria and *Borrelia* contain an inner membrane and outer membrane with a peptidoglycan layer located within the periplasmic space. Gram-negative bacteria contain LPS on the outer surface whereas *Borrelia* lacks LPS. Rather, *Borrelia* contains an abundance of lipoproteins on its outer surface.
Functions of the lipoproteins OspA and OspC

Two major Osps which are highly regulated are OspA and OspC; both are located on the outer membrane of *B. burgdorferi*. OspA and OspC are reciprocally regulated throughout the lifecycle of *B. burgdorferi*. *B. burgdorferi* residing within the midgut of unfed ticks have OspA present, while OspC is absent. However, upon a blood meal, the bacteria migrate from the tick midgut to the tick salivary glands to be transmitted to the mammalian host. During this transition, OspA is down-regulated and OspC is expressed (de Silva et al., 1996; Kobryn and Chaconas, 2001; Schwan and Piesman, 2000; Schwan et al., 1995). Some environmental and host signals affecting OspA and OspC expression include pH, cell density, temperature, and the presence of blood or other nutritional factors (Carroll et al., 2000; Carroll et al., 2003; Ramamoorthy and Scholl-Meeker, 2001; Revel et al., 2002; Schwan et al., 1995; Tokarz et al., 2004; Yang et al., 2004). For example, during *in vitro* growth cultivation at 23°C, OspC is not expressed, but a temperature shift to 37°C results in OspC being highly expressed. OspA, however, is unaffected by temperature: at both 23°C and 37°C, OspA is constitutively expressed which makes studying the repression of *ospA* difficult.

The presence of OspC is essential for 1) the migration of *B. burgdorferi* from the tick vector to the mammalian host; and 2) the infection of the mammalian host (Gilbert et al., 2007; Grimm et al., 2004b; Pal et al., 2004b; Tilly et al., 2006). Studies have shown that *ospC* mutant *B. burgdorferi* was not able to establish an infection in either wild-type mice or SCID mice. However, the *ospC* mutant was able to be transmitted to and establish infection within the tick. Therefore, OspC is not required to establish an infection within the tick, but OspC is essential for the invasion and infection of mammalian host (Yang et al., 2003a). To help facilitate the invasion of *Borrelia* into the mammalian host, OspC specifically binds Salp15. Salp15 is a tick salivary protein that blocks the mammalian
host’s CD4⁺ T-cell activation. This suppresses the host’s immune response at the site of the tick bite (Anguita et al., 2002; Ramamoorthi et al., 2005). In a study that compared \textit{B. burgdorferi} without Salp15 present in the environment to \textit{B. burgdorferi} with Salp15 present, it was found that \textit{B. burgdorferi} with Salp15 present have an increased bacterial burden and are better protected against antibody-mediated killing. This indicates that Salp15 assists \textit{B. burgdorferi} in evading the host immune system by blocking the host’s CD4⁺ T-cell activation thereby inhibiting the IgG antibody response (Anguita et al., 2002; Ramamoorthi et al., 2005).

OspC is required for the transmission and early infection of the mammalian host while OspA, on the other hand, is required for the colonization of \textit{B. burgdorferi} within the tick (Yang et al., 2004). OspA specifically binds the tick receptor TROSPA found within the midgut of the tick to facilitate attachment of the spirochete to the vector midgut (Pal et al., 2004a). The receptor in the tick gut serves as a ligand for tethering spirochetes via OspA binding (Pal et al., 2000; Pal et al., 2004a). While in the midgut of an unfed tick, spirochetes express high levels of OspA; together OspA and the flagellin proteins account for one-third of the total protein in \textit{B. burgdorferi} (Coleman and Benach, 1987).

When the infected tick feeds on a vertebrate host, the bacterium multiplies within the tick midgut and OspA is repressed while OspC is up-regulated (Schwan et al., 1995). Little or no OspA is present in the \textit{B. burgdorferi} transmitted to the mammalian host (Cassatt et al., 1998). The repression of \textit{ospA} is essential during mammalian infection, because even low levels of OspA can elicit a humoral response by the host to cause clearance of the bacterium or cause great immunological pressure on the pathogen (Strother et al., 2007; Xu et al., 2008). While OspA is not essential for early infection in mice, OspA is necessary for tick infection and maintenance in the midgut (Yang et al., 2004). The converse is true about OspC: OspC is essential for transmission to and early infection in
the vertebrate host, but is not necessary for the infection and maintenance within the tick midgut (Grimm et al., 2004b; Pal et al., 2004b).

**Reciprocal Production of OspA and OspC**

As mentioned previously, *B. burgdorferi* drastically alters its surface lipoproteins as a strategy to adapt to its two diverse host environments: the tick and the mammalian host (Anguita et al., 2002; Haake, 2000; Philipp, 1998; Schwan, 2003). In particular, during tick feeding, the two virulence factors OspA and OspC are reciprocally regulated and this coordinated regulation is believed to be important for the transmission of the spirochete between the tick and the mammalian host (Akins et al., 1998; de Silva et al., 1996; Montgomery et al., 1996; Stevenson et al., 1995). The reciprocal regulation has been shown in populations of cells as well as within individual cells using flow cytometry. Individual spirochetes coordinate the increase of OspC with the down-regulation of OspA (Srivastava and de Silva, 2008).

Most bacterial species have several sigma factors which are able to bind to an RNA polymerase core to form an RNA polymerase holoenzyme. The addition of a sigma factor to the RNA polymerase allows for promoter recognition specificity during the initiation of transcription. Most bacteria have a general housekeeping sigma factor (σ\(^{70}\)) which transcribes the majority of genes as well as several alternative sigma factors. The alternative sigma factors are able to distinguish different promoter sequences to direct the RNA polymerase to initiate transcription of select groups of genes in response to various environmental or developmental signals. *B. burgdorferi* contains only three sigma factors: RpoD (σ\(^{70}\)), and the alternative sigma factors RpoN (σ\(^{54}\)) and RpoS (σ\(^{5}\)) (Fraser et al., 1997).
The Response regulator protein II (Rrp2), in combination with RpoN and RpoS, forms the Rrp2-RpoN-RpoS regulatory network. During mammalian acquisition of *Borrelia*, Rrp2-RpoN-RpoS plays a central role in controlling the repression of OspA and the production of OspC as well as other differentially expressed genes in *B. burgdorferi* like decorin-binding protein A (DbpA), which facilitates *Borrelia* adherence to mammalian extracellular matrix, and the lipoprotein BBK32 that binds to mammalian fibronectin to facilitate spirochete acquisition (*Figure 6*) (Boardman et al., 2008; Caimano et al., 2007; Hubner et al., 2001; Yang et al., 2003a). The Rrp2-RpoN-RpoS pathway becomes activated through a cognate sensor histadine kinase Hk2 (gene BB0764) or acetyl phosphate (Xu et al., 2010) sensing environmental signals and activating Rrp2 (gene BBE0763) via phosphorylation. Signals such as low pH, elevated temperature and CO₂ concentration, high cell density and the presence of host signals are known to activate the pathway upon tick feeding (Akins et al., 1998; Burtnick et al., 2007; Caimano et al., 2007; Carroll et al., 1999; Indest et al., 1997; Stevenson et al., 1995; Yang et al., 2003b). Rrp2 is an NtrC-like bacterial two-component response regulator that functions as an enhancer binding protein (EBP) dependent on RpoN to activate transcription. Together, RpoN and the phosphorylated Rrp2 control the transcription of RpoS, a second alternative sigma factor. RpoS then up-regulates many genes, including *ospC*, and represses others, like *ospA*, via an unknown mechanism (Boardman et al., 2008; Caimano et al., 2007; Fisher et al., 2005; Ouyang et al., 2008; Yang et al., 2003b; Yang et al., 2005).

A study using *B. burgdorferi* grown within DMC’s implanted into rat peritoneal cavities (the “Host-adapted model”) demonstrated that wild-type bacteria had high OspC and low OspA levels, a profile expected for *B. burgdorferi* grown within a mammal (Caimano, 2005). However, *rpoS* mutants grown in the Host-adapted model were not able to
repress *ospA* nor express *ospC* implying that *ospA* repression and *ospC* activation are connected at the molecular level through an unknown mechanism involving RpoS.

There is a second two-component response regulator predicted to be encoded by the *B. burgdorferi* genome, Response regulator protein 1 (Rrp1, gene BB0419). While Hk2 and the Rrp2-Rpo-N-RpoS pathway promote spirochete transmission from the tick to the mammal and early infection of the mammal, the two-component system of histidine kinase Hk1 (gene BB0420) and response regulator Rrp1 promotes spirochete survival within the fed tick midgut (Caimano et al., 2011). It is predicted that Hk1 activates Rrp1 through phosphorylation. Rrp1, a guanylatecyclase, is responsible for the synthesis of the second messenger bis-(3'-5')-cycle dimericguanosine monophosphate (c-di-GMP) which affects the production and activity of Borrelial virulence factors such as those responsible for glycerol transport (He et al., 2011; Rogers et al., 2009; Ryjenkov et al., 2005). Because *ospA* becomes upregulated during transmission from the mammal to the tick, an Rrp1 activation condition, the Rrp1 pathway may contribute to the activation of *ospA*. 
Figure 6. The Rrp2-RpoN-RpoS regulatory network, $\sigma^{54}\sigma^{5}$ sigma factor cascade, controls the inverse production of OspA and OspC.

An unfed, infected tick takes a blood meal from a mammal and engorges with blood. In the fed, infected tick as well as the infected mammal, the Rrp2-RpoN-RpoS pathway is activated in *B. burgdorferi* upon response regulator Rrp2 phosphorylation by histadine kinase Hk2. Rrp2 in conjunction with RpoN activates transcription of RpoS. RpoS activates outer surface lipoprotein C (OspC) and OspC-related genes like decorin-binding protein A (DbpA) and the fibronectin-binding protein (BBK32). RpoS represses OspA via an unknown mechanism. The Rrp2-RpoN-RpoS pathway is not activated in the
unfed, infected tick. The mechanism of OspA expression is also not known (←, positive activation; ↑, negative activation).
**Genetic Regulation of ospA**

The signals and pathways by which ospA is expressed and the mechanism by which RpoS represses ospA have not been fully elucidated. OspA and OspB are encoded by a two-gene operon, the ospAB operon (genes BBA15 and BBA16), located on the lp54 plasmid (Figure 7) (Howe et al., 1986). The RpoD promoter drives the expression of the ospAB operon (Sohaskey et al., 1999). RpoD is a major sigma factor within *B. burgdorferi* and is constitutively expressed. While the expression of ospA has not been fully elucidated, it is known that the σ70-driven ospAB promoter contains three putative cis-elements: an inverted repeats (IR) region, a direct repeats (Rept) region, as well as a thymine rich (T-Rich) region (Sohaskey et al., 1999). Sohaskey et al. (1999) used the chloramphenicol acetyltransferase (CAT) reporter to show deletion of the T-Rich region of the ospAB promoter results in a decrease in reporter expression. Xu et al. (2010) demonstrated, by measuring ospA transcript of the ospA reporter within an ospAB mutant, that the Repeats and the T-Rich regions of the ospAB promoter are required for the full expression of ospA.

The alternative sigma factor RpoS, which is controlled through the Rrp2-RpoN-RpoS pathway, is required for ospA repression (Caimano et al., 2005; Yang et al., 2003a). It is postulated that RpoS represses ospA either indirectly or directly (Figure 8). A possible method by which RpoS could indirectly repress ospA is by controlling the expression of an unknown ospA repressor that binds to the ospAB promoter cis-element required for ospA repression. This would subsequently prevent the transcription of ospA by blocking access of the RpoD-containing holoenzyme to the ospAB promoter. Conversely, RpoS could bind directly to the ospAB promoter in the presence of an accessory factor that is specific to *in vivo* cultivation, the only condition in which ospA is repressed. RpoS could
then bind to the promoter blocking the RpoD-dependent transcription of ospA (Caimano et al., 2005).

In this study we seek to determine the cis-elements required for the repression and the activation of the ospAB promoter. A previous study by Sohaskey et al., used the transient CAT reporter system to evaluate which cis-elements were required for the activation of ospA. Their studies suggest that the T-Rich region is required for the full activation of ospA, however these studies were done using a transient, nonreplicating reporter. This reporter is transient because it does not replicate upon bacteria replication and is therefore an unstable reporter. Our goal is to develop a stable, replicating reporter system to more accurately determine which cis-elements play a role in the regulation of ospA. Another goal of these studies is to use our stable reporter system to identify the trans-factors required for the full activation and repression of ospA. And lastly, we want to develop an in vitro model to study ospA repression. Studying ospA repression has been proven tedious as the only way to acquire Borrelia with repressed ospA is to cultivate Borrelia in in vivo conditions. Cultivating Borrelia in vivo produces low yields of bacteria, so it is difficult to collect enough bacteria to use in experiments. The overall goal of our studies is to further elucidate the mechanisms by which ospA is regulated.
Figure 7. The ospAB operon the putative regulatory cis-elements in the upstream region of the ospA gene.

(A) Schematic of the ospAB operon located on the B. burgdorferi linear plasmid 54. (B) Inverted arrows (>>) and (<<) denote the inverted repeat element (IR); arrowheads (>>) denote the repeats element (Rept); asterisks (*) denote the T-Rich element (T-Rich). The -35 and -10 promoter elements, ribosomal-binding site (RBS), and the ATG start...
codon (*Met*) are underlined. Arrows (→) indicate the 3’ primer (P₃) and 5’ primers (P₅L; P₅IR; P₅IR,Rept; or P₅IR,Rept,T-Rich) used to create the four *ospAB promoter* constructs shown below. (C) Schematic representation of the *ospAB promoter* deletion constructs controlling luciferase or *ospA* expression. All four *ospAB promoter* constructs are denoted by the prefix P*ospAB* followed by the name of the corresponding deletion (indicated at the left) with the size of the resulting promoter (indicated at the right) in base pairs (bp). The full-length promoter (P*ospAB*-FL) contains all three putative cis-elements; P*ospAB*-ΔIR contains the Repeats and the T-Rich regions; P*ospAB*-ΔIR,Rept contains only the T-Rich element; P*ospAB*-ΔIR,Rept,T-Rich promoter does not contain any of the putative cis-elements; and the P*ospAB*-T-Rich-mut is the full-length *ospAB* promoter with a mutated T-Rich region.
Figure 8. Proposed models for the RpoS-dependent ospA repression.

(Left panel) RpoS controls the expression of an unknown ospA repressor protein. The unknown ospA repressor protein binds to the ospAB promoter at the T-Rich cis-element, which in turn prevents transcription of ospA by the RpoD-containing holoenzyme. (Right panel) Association with an in vivo-specific accessory factor allows RpoS to bind directly to the ospAB promoter thereby blocking RpoD-dependent transcription initiation. Abbreviations: RNA polymerase (RNAP), repressor (Rep), accessory factor (AF).
HYPOTHESIS

Based on the previous literature concerning ospA regulation, I propose the following:

First, I hypothesize that either the IR, Repeats, or T-Rich regions on the ospAB promoter are key components required for the full activation or repression of ospA. The rationale behind this hypothesis is based on previous literature which states these three regions are highly conserved suggesting that they are of importance (Sohaskey et al., 1999).

Second, ospA is being repressed either directly or indirectly by RpoS. Previous literature has shown that RpoS is required for ospA repression (Caimano et al., 2005). This suggests that either RpoS is able to directly bind to the ospAB promoter to prevent RpoD from initiating transcription or RpoS is able to control the expression of an unknown ospA repressor.

Third, I hypothesize that an in vitro model can be created to study ospA repression. ospA is constitutively expressed in vitro. In B. burgdorferi, the abrogation of ospAB results in the constitutive expression of RpoS (He et al., 2008). Since RpoS mediates the repression of ospA either directly or indirectly, an ospAB mutant would have RpoS constitutively expressed thereby creating ospA repression conditions. In order to measure ospA repression within the ospAB mutant, a luciferase reporter can be placed under the control of an ospAB promoter to measure the ospAB promoter activity and thus allow for a novel method to study ospA repression in vitro.
CHAPTER TWO: MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

The bacterial strains used in this study are described in Table 1. The TOP10 *E. coli* strain (Invitrogen, Carlsbad, CA) was used as the cloning host. *B. burgdorferi* clones 13A and BbAH130 as well as the *ospAB* mutant strain were described previously (He et al., 2008; Hubner et al., 2001; Xu et al., 2007; Yang et al., 2004). The B31 13A strain was isolated by serially diluting a wild-type *B. burgdorferi* strain B31 5A13 to identify a single clone which lost lp25 and lp56 but retained the remaining 19 plasmids of the *B. burgdorferi* genome. This clone, 13A, is more easily transformable because it lost the lp25 and lp56 plasmids which contain restriction enzymes that negatively affect transformation efficiency (Lawrenz et al., 2002). BbAH130 is an infectious strain derived from plating a low passage 297 strain on Barbour-Stoenner-Kelly (BSK) agar medium. *B. burgdorferi* 297 is an infectious strain which was isolated from the cerebrospinal fluid of a patient with Lyme disease (Yang et al., 2004). The *ospAB* mutant strain (Δ*ospAB*) was previously generated by electroporating the suicide vector, pXT-OspA-Strep, into the *B. burgdorferi* strain BbAH130. The suicide vector underwent homologous recombination with the wild-type *Borrelia* genome to insert the streptomycin-resistance gene (*aadA*) into the native *ospA* gene thus inactivating the *ospAB* operon.

*E. coli* cultures were grown with appropriate antibiotics at 37°C with aeration in Miller Difco Luria Bertani (LB) broth (Becton, Dickinson and Company, Sparks, MD) or grown on LB plates consisting of LB broth with 2% agar. Kanamycin concentrations for both LB broth and LB agar were 50 µg ml⁻¹ while ampicillin was 100 µg ml⁻¹. *Borrelia* were cultivated *in vitro* at 37°C with 5% CO₂ in BSK medium (Sigma, St Louis, MO) supplemented with 6% normal rabbit serum (Pel-Freez Biologicals, Rogers, AR).
Antibiotic concentrations used to select for mutants were 300 μg ml⁻¹ for kanamycin and 100 μg ml⁻¹ for streptomycin. Dark-field microscopy was used to enumerate spirochetes. Cultures were harvested at mid-logarithmic phase of growth (approximately 3×10⁷ spirochetes ml⁻¹), unless otherwise noted. For the norepinephrine studies, cultures were grown to mid-log in 10 ml BSK, split equally into two 5ml cultures and then centrifuged at 4000 x g for 20 minutes. The pellets were suspended into either BSK only or BSK with the addition of norepinephrine bitartrate (Sigma) to a final concentration of 10µM. After suspension, cultures were grown for 48 hours before harvesting.
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<tr>
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</tr>
<tr>
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<td>Source</td>
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</tr>
<tr>
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<tr>
<td>ΔospAB/pLuc-ΔIR,Rept,T-Rich</td>
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<td>This study</td>
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<td>ΔospAB/pLuc-T-Rich-mut</td>
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<td>ΔospAB with rrp2 point mutation</td>
<td>(He et al., 2008)</td>
</tr>
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<td>ΔospAB with inactivated rpoN</td>
<td>(He et al., 2008)</td>
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<td>ΔospAB with inactivated rpoS</td>
<td>(He et al., 2008)</td>
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**Generation of Native OspA Reporter Vectors**

The plasmid pOspAB is a shuttle vector containing origins of replication for both *E. coli* (ColE1) and *B. burgdorferi* (cp9) as previously described (He et al., 2008; Yang et al., 2004). pOspAB contains the native *ospA* gene as a reporter under the control of the full-length *ospAB* promoter containing all three of the putative cis-elements. The plasmid also has a kanamycin resistance marker. To construct shuttle vectors with various deletions of the *ospAB* promoter controlling the *ospAB* reporter, multiple polymerase chain reactions (PCR) were performed using the primer sets indicated in Table 2. pOspAB, containing the full-length *ospAB* promoter, served as the cloning template. Each PCR reaction used Taq DNA Polymerase (New England BioLabs) and the same 3’ primer, *ospAB*-Down-3’-SacI, but different 5’ primers. The 5’ primers were used in the reactions to create various length *ospAB* promoter fragments as noted in the ΔIR promoter removed the IR region from the full-length *ospAB* promoter; the ΔIR,Rept promoter removed the promoter segment encompassing the IR and the Repeat region of the *ospAB* promoter; and the ΔIR,Rept,T-Rich promoter removed the promoter segment encompassing each of the three putative cis-elements from the *ospAB* promoter.

During PCR amplification of the various promoters, the 3’ primer *ospAB*-Down-3’-SacI introduced a SacI restriction site to the 3’ end of the resulting fragments while each of the 5’ primers introduced an XbaI restriction site at the 5’ end (Table 2). The resulting PCR fragments (ΔIR, ΔIR,Rept, and ΔIR,Rept,T-Rich) were cloned into the pSC-A-amp/kan TA cloning vector (Stratagene, La Jolla, CA) to create the plasmids. Blue/white screening was used to select for transformants by spreading 40 µl of 40 µg ml⁻¹X-gal dissolved in dimethylformamide on top of the agar plates containing ampicillin. The three resulting plasmids and the parental plasmid pOspAB were digested with SacI and XbaI and then ligated together to create pOspAB-ΔIR, pOspAB-ΔIR,Rept, and pOspAB-Delta-IR.
IR, Rept, T-Rich (Table 1). These plasmids have various ospAB promoters site-directionally inserted upstream of the ospAB gene to control the transcription of ospAB. pOspAB has the full-length ospAB promoter containing all three of the putative cis-elements: the IR, Repeats and T-Rich regions. The pOspAB-ΔIR contains the Repeat and the T-Rich regions, but not the IR. The pOspAB-ΔIR,Rept contains the T-Rich region but lacks the IR and Repeats regions. pOspAB-ΔIR,Rept,T-Rich lacks all three of the putative cis-elements: IR, Repeats, and T-Rich.

Site-directed mutagenesis was performed by Genscript (Piscataway, NJ) to mutate the T-Rich region of the full-length ospAB promoter. The T-Rich region’s 10 bp sequence “TTATTTTTTT” of the plasmid pOspAB was mutated to the 10 bp sequence “CGCGGCGCGC” to create the plasmid pOspAB-T-Rich-mut. This plasmid contains ospAB expressed by the PospAB-T-Rich-mut promoter, which is the full-length ospAB promoter with a mutated T-Rich region.

To place the PospAB-T-Rich-mut promoter controlling luciferase expression, PCR amplification of the plasmid pOspAB-T-Rich-mut using the primers ospA-FL-BgIII and ospA-ATG-3-NdeI was performed (Table 2). The resulting promoter fragment and the pJD48 plasmid were digested with NdeI and BgIII and then ligated to create the plasmid pLuc-T-Rich-mut. This plasmid contains luciferase expressed by the full-length ospAB promoter with a mutated T-Rich region.
Table 2. Primers used in this study.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5' to 3')</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>ospAB- No-IR-XbaI</td>
<td>CATCTAGACATTAATCTAAGCTTAATTAGAAC</td>
<td>5' primer; PCR of the ospAB promoter for constructing pOspAB-ΔIR</td>
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<tr>
<td>ospAB- No-Repeat-XbaI</td>
<td>CATCTAGAACCAACTTAAATTGAAGTTATTAC</td>
<td>5' primer; PCR of the ospAB promoter for constructing pOspAB-ΔIR,Rept</td>
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<tr>
<td>ospAB- No-T-Rich-XbaI</td>
<td>CATCTAGAATTTTCTATTGTTATTTGTTAATC</td>
<td>5' primer; PCR of the ospAB promoter for constructing pOspAB-ΔIR,Rept,T-Rich</td>
</tr>
<tr>
<td>ospAB- Down-3'-SacI</td>
<td>CTGGAGGCTCCTAAGGACTTTTTTCCAGAAGTAA</td>
<td>3' primer; PCR of the ospAB promoter for constructing all of above listed plasmids.</td>
</tr>
<tr>
<td>ospA-FL-BglII</td>
<td>AGATCTAGACATTAACTTTTC</td>
<td>5' primer; PCR of the ospAB promoter for constructing pLuc-FL</td>
</tr>
<tr>
<td>ospA-No-IR-BglII</td>
<td>CTAGATCTCATTAATCTAAGCTTAATTAGAA</td>
<td>5' primer; PCR of the ospAB promoter for constructing pLuc-ΔIR</td>
</tr>
<tr>
<td>ospA-No-Repeat-BglII</td>
<td>CTAGATCTCCTAAACTTAAATGGAAGTTATTAT</td>
<td>5' primer; PCR of the ospAB promoter for constructing pLuc-ΔIR,Rept</td>
</tr>
<tr>
<td>ospA-No-T-Rich-BglII</td>
<td>CTAGATCTATTTTCTATTGTTATTTGTTAAT</td>
<td>5' primer; PCR of the ospAB promoter for constructing pLuc-ΔIR,Rept,T-Rich</td>
</tr>
<tr>
<td>ospA-ATG-3'-NdeI</td>
<td>CATATGATATTTCTCATTATATATATAACTT</td>
<td>3' primer; PCR of the ospAB promoter for constructing the four above listed plasmids.</td>
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<tr>
<td>FlaB-F</td>
<td>AGATCTTACCTGGATTTTACCCTAAGCCG</td>
<td>5' primer; PCR of the flaB promoter for constructing pLuc-PflaB</td>
</tr>
<tr>
<td>FlaB-R</td>
<td>CATATGATATTTCTCATTATATATAACTT</td>
<td>3' primer; PCR of the flaB promoter for constructing pLuc-PflaB</td>
</tr>
<tr>
<td>Q-HX-OspA-F</td>
<td>TAGCAGCCTTGGACGAGAAAACAG</td>
<td>5' primer; PCR of the ospA gene for qRT-PCR</td>
</tr>
<tr>
<td>Q-HX-OspA-R</td>
<td>TTATCAGAAGTTTCTTTTAACTCA</td>
<td>3' primer; PCR of the ospA gene for qRT-PCR</td>
</tr>
<tr>
<td>Q-HX-FlaB-F</td>
<td>ACCAGCATTCACTTTTACGGTCTCA</td>
<td>5' primer; PCR of the flaB gene for qRT-PCR</td>
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<tr>
<td>Q-HX-FlaB-R</td>
<td>CAGCAATAGGCTCATTCTTGGTTT</td>
<td>3' primer; PCR of the flaB gene for qRT-PCR</td>
</tr>
</tbody>
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*Restriction sites for purpose of cloning are highlighted in boldface letters
Generation of Luciferase Reporter Vectors

The previously described plasmid pJD48 was used to create the luciferase reporter vectors (Blevins et al., 2007). pJD48 is a shuttle vector containing a replication origin for B. burgdorferi (cp9) and for E. coli (ColE1). pJD48 contains an Ndel and BgIII cloning site upstream of the luciferase gene which allows for placement of a promoter to control the expression of the luciferase. In addition to luciferase, pJD48 also contains a kanamycin-resistance marker. To create luciferase reporter constructs with varying length ospAB promoters, serial truncations of the ospAB promoter were created via PCR using four various 5' primers and using the same 3' primer (PospA-ATG-3-Ndel). The 5' primer introduced a BgIII restriction site to the 5' PCR fragment end while the 3' primer introduced an Ndel restriction site to the 3' end of the PCR fragment; pOspA, containing the full-length wild-type ospAB promoter, served as the PCR template. Primer pairs are listed in Table 2. The resulting PCR fragments (FL; ΔIR; ΔIR,Repts; and ΔIR,Repts,T-Rich) were cloned into the pSC-A-amp/kan TA cloning vector. The resulting plasmids and pJD48 were digested with Ndel and BgIII and ligated together to create pLuc-FL, pLuc-ΔIR, pLuc-ΔIR,Repts, and pLuc-ΔIR,Repts,T-Rich (Table 1).

Genetic Manipulation of Borrelia burgdorferi

Borrelial electroporations were performed as previously described (Samuels, 1995; Yang et al., 2004). B. burgdorferi were recovered from -80°C frozen stock into 2mL of BSK medium. The culture was incubated at 34°C with 5% CO2 until the culture cell density was approximately 3×10^5 spirochetes ml^-1. The culture was then transferred to 50mL BSK medium with appropriate antibiotics and grown until the cell density reached more than 3×10^7 spirochetes ml^-1. For preparation of competent cells, the Borrelia culture was centrifuged at 4,000 rpm for 20 minutes at 4°C and the supernatant was decanted. The cells were washed twice with 30 ml cold saline (0.9% sodium chloride dissolved in
double-distilled water) and washed an additional three times with 30 ml cold Electroporation Solution (EPS) which consists of 9.3% sucrose and 15% glycerol dissolved in double-distilled water. After the final wash, the final pellet was gently suspended in 50 µl of EPS and transferred to a 1.5 ml eppendorf tube. About 5-20 µg of plasmid DNA was added to the resuspension and the mixture was transferred to a pre-chilled electroporation 0.2 cm cuvette (Bio-Rad) and allowed to chill on ice for at least 1 minute. To electroporate, the cuvette was placed in the gene pulser (Gene pulser, Bio-Rad) and a single exponential decay pulse of 2.5 kV, capacitance of 25 µF, and resistance of 200 Ω was allowed producing a time constant of 4 to 6 ms. Immediately after electroporation, 1 ml of BSK was added to the cuvette and then this was transferred to 35 ml BSK without antibiotics. The culture was incubated overnight at 34°C with 5% CO₂. Following overnight incubation, appropriate antibiotics were added and 220 µl of the 35 ml overnight culture was aliquoted into each well of a 96 well plate. The color of the medium in the plate wells were monitored; a change of color from red to yellow indicated that cells were growing, which was verified by microscopic examination of a sample of the cells from the well. If cell growth is confirmed, 10 µl of the positive culture was transferred to 2 ml or 15 ml BSK with appropriate antibiotics and then further analyzed and assayed.

Quantitative RT-PCR

Cultures were pelleted by centrifugation and the RNA extracted using the RNeasy mini kit (Qiagen, Valencia, CA) per the manufacturer’s instructions. Purified RNA was treated with DNase (Promega) and converted to cDNA using SuperScript III reverse transcriptase with random primers (Invitrogen, Carlsbad, CA) following the manufacturer’s instructions. Platinum SYBR green qPCR SuperMix-UDG (Invitrogen) along with primers specific for flaB and ospA were used to perform qPCR in triplicate on
the cDNA samples (Table 2). An absolute quantification method using qPCR was performed according to the manufacturer’s instructions (Stratagene) using the specific flaB primers and serial dilutions of the standard template, a cloning vector containing the flaB gene. Briefly, a standard curve was generated by plotting template quantity against the Ct values of the standards. The quantity of ospA transcript was determined by comparing the samples’ Ct values to the standard curve plot. The standards and samples were performed in triplicate using an ABI 7000 Sequence Detection System.

Luciferase assays

Luciferase assays were performed as previously described using the commercial luciferase assay system (Promega Corp., Madison, WI) (Blevins et al., 2007). After enumerating the bacteria via dark-field microscopy, bacterial cultures were centrifuged at 4,000 rpm for 20 minutes at 4°C. The supernatant was decanted and the pellet was washed twice with PBS, with centrifugation at 4,000 rpm for 20 min at 4°C after each wash. The final pellet was transferred to a 1.5 ml Eppendorf tube and was suspended in 100 µl of the cell culture lysis reagent (CCLR), which was supplied by the manufacturer. The debris were pelleted by centrifugation in a microcentrifuge at 15,000 rpm for 1 min. Ten microliters of lysate was aliquoted into luminometer tubes (Promega) and 50 µl of luciferase assay reagent was added immediately prior to beginning measurements. Luciferase activity was measured for 10 sec using a Centro LB 960 luminometer (Berthold Technologies, Oak Ridge, TN). The average background luminescence was subtracted from the readings and the measurement reported as relative light units (RLU).
Host-Adapted Spirochete Model

The insertion of DMC’s containing *B. burgdorferi* into the peritoneal cavities of rats was previously described (Akins et al., 1998). *Borrelia* were grown to mid-logarithmic and then diluted in BSK to $3 \times 10^3 Bb \text{ ml}^{-1}$. Five milliliters of this dilution was put into a 10 kDa Spectra/Por dialysis membrane (Spectrum Medical Industries Inc., Los Angeles, CA) and inserted into the peritoneal cavity of four- to six-week-old Sprague-Dawley rats (Harlan, Indianapolis, IN) using strict aseptic technique. Fourteen days after implantation, the DMCs were harvested and its contents removed by syringe aspiration. Spirochetes were enumerated via dark-field microscopy and then prepared for the luciferase assay as described.
CHAPTER THREE: RESULTS

Section I: The T-Rich region is required for the full activation of the ospAB promoter.

Deletion of T-Rich element in wild-type B. burgdorferi with luciferase reporter results in decreased luciferase expression

The ospAB promoter contains three putative cis-elements: the IR region, the repeats region, and the T-Rich region (Sohaskey et al., 1999). To determine which of these three putative cis-elements are required for the full activation of the ospAB promoter, the various length ospAB promoters PospAB-FL; PospAB-ΔIR; PospAB-ΔIR,Rept; and PospAB-ΔIR,Rept,T-Rich were created by PCR using pOspAB as a template. The four promoters are as follows: 1) the PospAB-FL promoter is the full-length ospAB promoter containing all three putative cis-elements; 2) the PospAB-ΔIR promoter lacks the IR region while retaining the Rept and T-Rich regions; 3) the PospAB-ΔIR,Rept promoter retains the T-Rich region but lacks the region encompassing the IR and the Rept elements; and 4) the PospAB-ΔIR,Rept,T-Rich promoter deletes the ospAB promoter region encompassing all three of the putative cis-elements. These promoters were site-directionally cloned upstream of the luciferase open reading frame in the pJD48 shuttle vector to control the expression of the luciferase reporter, and then the plasmids were electroporated into wild-type B. burgdorferi 13A to create the strains pLuc-FL; pLuc-ΔIR; pLuc-ΔIR, Rept; and pLuc-ΔIR,Rept,T-Rich, respectively.

These four strains were grown in vitro at 37°C, a condition in which ospA is constitutively expressed, and then harvested and prepared for the luciferase assay. The effect of the absence of the putative cis-elements on the ospAB promoter activity was determined
using the luciferase assay. The luciferase assay results indicate that there was no significant difference in luciferase activity between the *P*~*ospAB*-FL promoter and the *P*~*ospAB*-ΔIR or the *P*~*ospAB*-ΔIR,Rept promoters (Figure 9). However, there was a reduction in luciferase activity in the *P*~*ospAB*-ΔIR,Rept,T-Rich promoter, showing that deletion of the T-Rich element results in a decrease of *ospAB* promoter activation.
Figure 9. Influence of cis-elements on ospAB promoter activation.

The 13A strain electroporated with a shuttle vector containing the luciferase reporter under the control of either the constitutively expressed flaB promoter (PflaB), no promoter (none), the full-length ospAB promoter (PospAB-FL), the ospAB promoter lacking the IR region (PospAB-ΔIR), the ospAB promoter lacking the IR and Repeats regions (PospAB-ΔIR,Rept) and the ospAB promoter lacking all three putative cis-elements (PospAB-ΔIR,Rept,T-Rich). Strains were grown in BSK at 37°C and prepared for luciferase assay (*, p<0.05 using paired Student’s t-test).
Deletion of T-Rich element in the ospAB mutant B. burgdorferi with ospA reporter results in decreased ospA transcript.

Using various ospAB promoters controlling the luciferase reporter expression within the wild-type Borrelia 13A, it was determined that deletion of the T-Rich region results in decreased activation of the ospAB promoter. To confirm this finding, we used a similar approach to measure ospAB promoter activity, but employed a native ospAB reporter rather than using the luciferase reporter.

The various length ospAB promoters PospAB-FL; PospAB-ΔIR; PospAB-ΔIR,Rept; and PospAB-ΔIR,Rept,T-Rich were site-directionally cloned upstream of the ospAB open reading frame in the pOspAB shuttle vector to control the expression of the ospAB reporter. The plasmids were electroporated into ospAB mutant Borrelia to create the strains pOspAB-FL; pOspAB-ΔIR; pOspAB-ΔIR, Rept; and pOspAB-ΔIR,Rept,T-Rich, respectively. The resulting strains were grown in vitro at 37°C, a condition in which ospA is highly expressed. The cultures were harvested and then prepared for analysis by qRT-PCR.

The ospA transcript number was measured relative to the flagellin (flaB) transcript which is constitutively expressed. The results show that deletion of the IR or deletion of the IR and Rept regions together did not result in a significant decrease of ospA transcript compared to the full-length promoter (Figure 10). However, deletion of the IR, Rept and T-Rich regions combined showed a decrease in the ospA transcript level compared to the full-length promoter suggesting that the T-Rich region is required for the full activation of ospA. This result is in agreement with our previous finding using the luciferase reporter.
Figure 10. *ospA* transcript levels of the various *ospAB* promoters controlling expression of *ospAB* within the *ospAB* mutant *B. burgdorferi*.

The *B. burgdorferi* *ospAB* mutant with a shuttle vector containing the *ospAB* reporter under the control of either the full-length *ospAB* promoter (P*ospAB*-FL), the *ospAB* promoter lacking the IR region (P*ospAB*-ΔIR), the *ospAB* promoter lacking the IR and Repeats regions (P*ospAB*-ΔIR,Rept) and the *ospAB* promoter lacking all three putative *cis*-elements (P*ospAB*-ΔIR,Rept,T-Rich). Strains were grown in BSK at 37°C and prepared for qRT-PCR. Transcript relative to copies of flagellin, flaB (*, p<0.05 using paired Student’s t-test).
Mutation of the T-Rich element in wild-type *B. burgdorferi* with luciferase reporter results in decreased luciferase expression

Next, we wanted to further dissect the *ospAB* promoter. The promoters used to show that the T-Rich region is required for the full activation have multiple elements missing and not just the T-Rich region. To ensure that the effect we have seen is dependent solely on the T-Rich region and not a combination of the T-Rich element and the IR or Rept elements, we used site-directed mutagenesis to mutate only the T-Rich region.

Site-directed mutagenesis was used to replace the 10 bp sequence “TTATTTTTTT” of the T-Rich region with a different 10 bp sequence “CGCGGCGCG” to create the promoter *P*ospAB-T-Rich-mut. This promoter was site-directionally cloned upstream of the luciferase open reading frame in the pJD48 shuttle vector and then transformed into the 13A *B. burgdorferi* strain and grown *in vitro* at 37°C. The luciferase expression level of the *P*ospAB-T-Rich-mut promoter was compared to the *P*ospAB-FL. The *P*ospAB-T-Rich-mut promoter had significantly reduced levels of luciferase (*Figure 11*). The reduction in the *ospAB* promoter expression caused by the mutagenesis of the T-Rich region indicates that the T-Rich region alone is responsible for the full expression of *ospA*. Together, these studies show that the T-Rich region is required for the full activation of *ospA*. 
Figure 11. Influence of the T-Rich cis-element on ospAB promoter activation.

The 13A strain electroporated with a shuttle vector containing the luciferase reporter under the control of either the constitutively expressed flaB promoter (PflaB), the full-length ospAB promoter (PospAB-FL), and the PospAB-FL promoter with a mutated T-Rich region (PospAB-T-Rich-mut). Strains were grown in BSK at 37°C and prepared for luciferase assay (*, p<0.05 using paired Student’s t-test).
Section II: Establishment of an *in vitro* model of *ospA* repression

**Abrogation of *ospAB* results in constitutive activation of *ospC***

OspA and OspB are major surface lipoproteins in *B. burgdorferi*. During mammalian infection, OspC is produced while OspA is repressed. However, the inverse regulation is true during the infection of ticks: OspA is produced while OspC is not. A previous study by Yang et al. (2004) abrogated OspAB by disrupting the *ospAB* operon. Their studies demonstrated that the *ospAB* mutant was able to infect mice but was not able to colonize or replicate in ticks (Yang et al., 2004). In addition to this finding, we observed that the deletion of *ospAB* within *B. burgdorferi* results in the increased, constitutive expression of *ospC* (He et al., 2009).

To determine the influence of OspA and OspB on the expression of OspC, the protein profiles of the *ospAB* mutant and the wild-type parental strain BbAH130 were compared. The *ospAB* mutant and BbAH130 were grown *in vitro* at either 23°C, a condition in which OspC is not produced, or 37°C, a condition where OspC is produced. The cultures were harvested and the lysates were subjected to SDS-PAGE before the proteins were stained by Coomassie blue (*Figure 12*). As expected, at 23°C, the wild-type BbAH130 did not produce OspC; at low temperatures which mimic the tick environment, OspA is highly expressed whereas OspC is not. At both 23°C and 37°C, the *ospAB* mutant did not produce OspA, as expected, due to the disruption of the *ospAB* operon. However, the *ospAB* mutant exhibited OspC production at both 23°C and 37°C. OspC is normally expressed at 37°C, but not at 23°C. These results show that OspC is constitutively expressed in the *ospAB* mutant regardless of temperature.
In addition to determining the effect of \textit{ospAB} abrogation on OspC, the effect of \textit{ospAB} abrogation on other differentially expressed lipoproteins was also evaluated. Decorin-binding protein (DbpA), fibronectin-binding protein (BBK32), and multicopy lipoprotein-8 (Mlp8) are all lipoproteins which are known to be expressed at 37°C, but not at 23°C (He et al., 2007; Hubner et al., 2001; Yang et al., 2007). To determine if expression of these temperature-induced lipoproteins was affected by the abrogation of \textit{ospAB}, immunoblot assays were performed on the wild-type BbAH130 and \textit{ospAB} mutant whole cell lysates from cultures grown at 23°C. Antibodies specific to the differentially expressed lipoproteins DbpA, BBK32, Mlp-8 as well as OspC were used. FlaB, the constitutively expressed flagellin protein, served as the protein loading standard. The lipoproteins DbpA, BBK32 and Mlp-8 were not present or in low presence in the wild-type BbAH130, however, they were abundantly produced in the \textit{ospAB} mutant (Figure 13). These results demonstrate that in addition to \textit{ospC} being constitutively expressed in the \textit{ospAB} mutant grown at 23°C, \textit{dbpA}, \textit{bbk32} and \textit{mlp-8} are constitutively expressed or upregulated in the \textit{ospAB} mutant.
Figure 12. Abrogation of ospAB results in constitutive activation of ospC (He et al., 2009).

The wild-type clone BbAH130 (wt) and the isogenic ospAB mutant (ΔospAB) were grown in BSK medium at either 23°C (23) or 37°C (37). Cultures were harvested at mid-logarithmic phase, and cell lysates were subjected to SDS-PAGE before the protein was stained with Coomassie blue. The molecular mass markers are indicated at the left of the figure in kilodaltons (kDa). Protein bands corresponding to OspA and OspC are labeled on the right.
Figure 13. Abrogation of *ospAB* influences the production of lipoproteins (He et al., 2009).

The wild-type clone BbAH130 (wt) and the isogenic *ospAB* mutant (Δ*ospAB*) were grown in BSK medium at 23°C. Cultures were harvested at mid-logarithmic phase and whole cell lysates were probed with antibodies directed against the specific lipoprotein (indicated on left). FlaB is constitutively expressed and serves as the control for equal lysate amounts.
Mutation or inactivation of rpoS, rpoN or rrp2 abolishes constitutive expression of ospC in the ospAB mutant

In B. burgdorferi, the Rrp2-RpoN-RpoS pathway mediates the temperature-induced expression of ospC, dbpA, and bbk32 (Figure 6) (Eggers et al., 2004; Gilbert et al., 2007; He et al., 2007; Hubner et al., 2001; Yang et al., 2003a). Since abrogation of ospAB resulted in constitutive expression of ospC, dbpA and bbk32, and expression of these three lipoproteins is mediated through the Rrp2-RpoN-RpoS pathway, we hypothesized that the Rrp2-RpoN-RpoS regulatory pathway is constitutively activated in the ospAB mutant.

To determine if the Rrp2-RpoN-RpoS pathway is responsible for the constitutive expression of ospC within the ospAB mutant, mutations in the rrp2, rpoN, or rpoS genes were generated in the ospAB mutant. The ospAB mutant and the double mutant cultures were cultivated in BSK at 35°C, harvested at mid-log and the whole cell lysates subjected to SDS-PAGE before staining with Coomassie blue. The rrp2 point mutation, the inactivated rpoN and the inactivated rpoS in the ospAB mutant resulted in decreased constitutively expressed OspC production compared to the wild-type or the ospAB mutant (Figure 14). These results indicate that ospC is constitutively expressed due to the Rrp2-RpoN-RpoS pathway being constitutively expressed upon abrogation of ospAB.
Figure 14. Constitutive expression of OspC in the ospAB mutant is abolished upon mutation or inactivation of rpoS, rpoN or rrp2 (He et al., 2008).

The ospAB single mutant (ΔospAB), and the ospAB double mutants of rpoS (ΔrpoS), rpoN (ΔrpoN), and rrp2 (rrp2G239C) were cultured in BSK at 35°C, harvested at mid-log and the whole cell lysates subjected to SDS-PAGE before staining with Coomassie blue. The molecular mass markers are indicated at the left of the figure in kilodaltons (kDa).
Complementation of the ospAB mutant with a wild-type copy of ospAB or ospA alone restores repression of ospC at 23°C

The constitutive activation of the Rrp2-RpoN-RpoS pathway in the ospAB mutant could be due to spurious mutation locking the pathway in an active state rather than the constitutive expression being due to the abrogation of ospAB. To ensure that the constitutive activation of the Rrp2-RpoN-RpoS pathway was due to the loss of ospAB expression, the ospAB mutant was complemented with the shuttle vector pOspAB carrying a wild-type copy of native ospAB. The wild-type, ospAB mutant and the ospAB complement were cultivated in BSK at 23°C and whole-cell lysates were subjected to SDS-PAGE before staining with Coomassie blue. The ospAB mutant complemented with ospAB was not able to constitutively produce OspC at 23°C like the ospAB mutant (Figure 15). This suggests that the abrogation of ospAB is responsible for the constitutive activation of the Rrp2-RpoN-RpoS pathway.

In addition to complementing the ospAB mutant with native ospAB, the ospAB mutant was complemented with only ospA driven by the native ospAB promoter (pOspA) to determine if the absence of ospA was mainly responsible for the constitutive OspC production phenotype. The shuttle vector alone without ospAB or ospA, pBSV2, was used as a control to ensure the effects seen in pOspAB or pOspA were due to the shuttle vector, but rather to reintroducing ospAB and ospA. Complementation with ospA alone restored the temperature-dependent repression of ospC expression (Figure 15). Together, these data demonstrate that the abrogation of ospA, rather than spurious mutation, was responsible for the constitutive activation of the Rrp2-RpoN-RpoS regulatory pathway.
Figure 15. Complementing the *ospAB* mutant with *ospAB* or *ospA* alone restores *ospC* repression at 23°C (He et al., 2008).

The wild-type strain (wt), the *ospAB* mutant (∆*ospAB*), the *ospAB* mutant complemented with the shuttle vector alone (∆*ospAB*/pBSV2), and the *ospAB* mutant complemented with the shuttle vector carrying the native copy of *ospAB* (∆*ospAB*/pOspAB) or *ospA* (∆*ospAB*/pOspA) were cultivated at 23°C. Whole-cell lysates were subjected to SDS-PAGE before staining with Coomassie blue. The labeled bands on right correspond to OspA, OspB and OspC. The molecular mass markers are indicated at the left of the figure in kilodaltons (kDa).
Proposed model for the positive feedback circuit between the activation of the Rrp2-RpoN-RpoS regulatory pathway and the reduction of OspA

The constitutive production of OspC via the Rrp2-RpoN-RpoS regulatory pathway, which is constitutively activated upon abrogation of ospAB, suggests a positive feedback circuit (Figure 16). Feedback loops have been reported in other bacteria (Guespin-Michel and Kaufman, 2001), and such a feedback loop could be beneficial to B. burgdorferi’s transmission and mammalian infection. Given our results, we hypothesize that the Rrp2-RpoN-RpoS regulatory pathway is activated during tick feeding by an unknown signal. The activation of the Rrp2-RpoN-RpoS pathway results in the expression of an unknown transcriptional repressor regulated by RpoS, which is able to repress ospA expression. Upon repression of ospA, OspA production is decreased and there is a reduction in the surface-associated OspA. This reduction further activates the Rrp2-RpoN-RpoS pathway to achieve and maintain the maximal level of Rrp2-RpoN-RpoS activation to encourage the expression of OspC leading to B. burgdorferi’s migration from the tick midgut to establish infection in mammals.
Figure 16. Proposed model for the positive feedback circuit between the activation of Rrp2-RpoN-RpoS regulatory pathway and the reduction of OspA.

The activation of the Rrp2-RpoN-RpoS is hypothesized to be activated upon tick feeding by an unknown signal. The activation of this pathway results in expression of an unknown transcriptional repressor which is able to repress ospA. OspA production is decreased upon the repression of ospA causing a reduction in the surface-associated OspA. The reduction further activates the Rrp2-RpoN-RpoS pathway to achieve and maintain the maximal level of Rrp2-RpoN-RpoS activation, which in turn activates expression of ospC (←, positive activation; ↑, negative activation).
The proposal of an *in vitro* model of *ospA* repression

The expression of *ospC* is temperature-dependent during *in vitro* cultivation in BSK. Expression of OspA, however, is not temperature-dependent; OspA is abundantly expressed during *in vitro* cultivation at both 23°C and 37°C. Since OspA is not temperature-dependent like OspC, there has been a lack of an *in vitro* model for studying the regulation of *ospA*. Studying the downregulation of *ospA* can only be done *in vivo*, which is very difficult, therefore very little is known about the mechanism underlying the downregulation of *ospA*.

While the complete mechanism of *ospA* downregulation has not been fully elucidated, Caimano et al. discovered that the alternative sigma factor RpoS is required for the repression of *ospA* (2005). RpoS likely represses *ospA* by either 1) binding directly to the *ospAB* promoter; or 2) by controlling the expression of an unknown *ospA* repressor.

He and coworkers have shown that *rpoS* is constitutively activated in an *ospAB* mutant (He et al., 2008). Thus, the *ospAB* mutant can serve as a system to study the repression of the *ospAB* promoter *in vitro*. To develop this system, the pLuc-FL plasmid (a shuttle vector containing the luciferase reporter under the control of the full-length *ospAB* promoter) was electroporated into the *ospAB* mutant. In this construct, the *ospAB* mutant constitutively expresses *rpoS* and subsequently RpoS represses the full-length *ospAB* promoter (probably either directly or indirectly). The full-length *ospAB* promoter controls the expression of a luciferase reporter to measure the level of *ospA* repression by RpoS (Figure 17A).

The pLuc-FL plasmid was transformed into both the wild-type parental BbAH130 and the *ospAB* mutant. These strains were grown in BSK at 37°C and analyzed by the luciferase
assay. The *ospAB* mutant had reduced luciferase activity compared to the wild-type BbAH130 (Figure 17B). The reduced luciferase activity in the *ospAB* mutant indicates that RpoS was able to repress the *ospAB* promoter. This *ospAB* mutant containing the luciferase reporter under the control of the *ospAB* promoter can be used as a tool to study the repression of *ospA in vitro*. This is a novel *in vitro* model of *ospA* repression.
Figure 17. Establishment of an *in vitro* model of *ospA* repression.

(A) Schematic of proposed *in vitro* model of *ospA* repression. In *ospAB* mutant *B. burgdorferi* (Δ*ospAB*), RpoS expression is constitutive. RpoS is required for the repression of *ospA* via an unknown mechanism: either RpoS directly represses *ospA* or RpoS influences the expression of an unknown *ospA* repressor. The luciferase reporter expressed by the full-length *ospAB* promoter within the *ospAB* mutant can used to measure the level of *ospAB* promoter repression due to the overexpression of the *ospA* repressor. (B) BbAH130 (wt) and the *ospAB* mutant (Δ*ospAB*), both containing the shuttle vector with the full-length *ospAB* promoter controlling luciferase expression, were grown at 37°C. The constitutive flaB promoter and a promoterless promoter (none) controlling luciferase expression were used as controls.
Deletion of the T-Rich element results in increased luciferase expression using the *in vitro* model of *ospA* repression

We hypothesize that in *ospA* repression conditions, RpoS controls an unknown repressor (or repressors) which bind to the *ospAB* promoter at one or more of the three putative *cis*-elements (*Figure 16*). To determine which of the three putative *cis*-element(s) are required for the full repression of *ospA*, we employed the *in vitro* model of *ospA* repression where *rpoS* is constitutively expressed (*Figure 17*). Using this model, the *ospAB* mutant, which constitutively expresses the putative *ospA* repressor, was electroporated with a shuttle vector containing the luciferase reporter under the control of either 1) the full-length *ospAB* promoter, *PospAB-FL*; 2) the *PospAB-ΔIR* promoter which is lacking the IR element; 3) the *PospAB-ΔIR,Rept* promoter lacking the region encompassing the IR and Rept elements; or 4) the *PospAB-ΔIR,Rept,T-Rich* promoter which lacks all three putative *cis*-elements. These strains were grown at 37°C in BSK medium and then harvested and prepared for the luciferase assay. The *PospAB-ΔIR,Rept,T-Rich* strain had a significant increase in luciferase expression compared to the full-length, *PospAB-ΔIR*, and *PospAB-ΔIR,Rept* promoters (*Figure 18A*). This data suggests that the T-Rich region is required for full repression of *ospA* and that RpoS either directly binds to the T-Rich region or RpoS controls a repressor which binds to the T-Rich region.

To determine if the T-Rich region alone is responsible for the full repression of *ospA*, we used our full-length *ospAB* promoter with the mutated T-Rich region (*PospAB-T-Rich-mut*) controlling luciferase expression and placed the construct in the *ospAB* mutant *B. burgdorferi* and grown *in vitro* at 37°C. The luciferase expression levels of the *PospAB-T-Rich-mut* promoter was compared to the *PospAB-FL*. The *PospAB-T-Rich-mut* promoter had significantly increased levels of luciferase (*Figure 18B*). The reduced
*ospA* repression caused by the mutagenesis of the T-Rich region indicates that the T-Rich region alone is responsible for the full repression of *ospA*. 
Figure 18. Influence of cis-elements on luciferase repression using the in vitro model of ospA repression.

(A) Luciferase assay of the ospA mutant transformed with a shuttle vector containing the luciferase reporter under the control of either constitutively expressed flaB promoter (PflaB), promoterless (none), the full-length ospAB promoter (PospAB-FL), the ospAB promoter lacking the IR region (ΔIR), the ospAB promoter lacking the IR and Repeats regions (ΔIR,Rept) and the ospAB promoter lacking all three putative cis-elements (ΔIR,Rept,T-Rich). Strains were grown in BSK at 37°C and prepared for luciferase assay. (B) The ospAB mutant strain electroporated with a shuttle vector containing the
luciferase reporter under the control of either the constitutively expressed flaB promoter (PflaB), the full-length ospAB promoter (PospAB-FL), and the PospAB-FL promoter with a mutated T-Rich region (PospAB-T-Rich-mut). Strains were grown in BSK at 37°C and prepared for luciferase assay (*, p<0.05 using paired Student’s t-test).
**Addition of norepinephrine results in a decrease of repression using the *in vitro* model of *ospA* repression**

During mammalian infection, *ospA* is continually repressed. However, when an infected mammal is bitten by a naïve tick, *ospA* must be expressed in order for the *B. burgdorferi* to attach to colonize the tick midgut through OspA. The mechanism by which *ospA* is able to switch from repression to expression within the mammalian environment is not clear, although it is speculated that catecholamines from the host are able to signal the release of the repression of *ospA* (Scheckelhoff et al., 2007). Catecholamines, such as norepinephrine and epinephrine, are released by the mammalian host upon the presence of a stressor—such as a tick bite. To determine if the catecholamine norepinephrine is able to release *ospA* repression, *B. burgdorferi* were grown in the *in vitro* model of *ospA* repression in BSK containing either BSK only or BSK with the addition of norepinephrine. The full-length *ospAB* promoter (P*ospAB*-FL) and the *ospAB* promoter without any of the three putative *cis*-elements (P*ospAB*-ΔIR,Rept,T-Rich), both controlling the luciferase reporter, were cultivated.

The addition of norepinephrine to the *in vitro* model of *ospA* repression resulted in an increase of *ospA* expression, indicating release of *ospA* repression in both the *ospAB* promoter containing all three putative *ospA* cis-elements (P*ospAB*-FL) as well as the *ospAB* promoter lacking all three putative *ospA* cis-elements (P*ospAB*-ΔIR,Rept,T-Rich) (Figure 15). The release of repression in the promoter lacking all three putative *cis*-elements suggests that norepinephrine releases the repression of *ospA* through a mechanism independent of any of the three putative *cis*-elements.
Figure 15. Effect of norepinephrine on luciferase expression driven by various ospAB promoters. Luciferase assay of the ospAB mutant B. burgdorferi strain (ΔospAB) transformed with the various pLuc shuttle vectors containing either the constitutively activated promoter (PflaB), promoterless (None), full-length ospAB promoter (PospA-FL) or truncated ospAB promoter (PospA-ΔIR,Rept,T-Rich) were treated at 37°C with 10 µM norepinephrine (BSK+NE) or without norepinephrine (BSK Only) for 48 hours and harvested at late logarithmic phase (*, p < 0.05; **, p<.01 using ANOVA).
Section III. Host-adapted model for studying ospA repression

*B. burgdorferi* cultivated in dialysis membrane chambers exhibits reciprocal regulation: repression of OspA and expression of OspC

Studying the repression of *ospA* in the past has been difficult. During *in vitro* cultivation, *ospA* is constitutively expressed making the study of repression impossible; however, during *in vivo* conditions, *ospA* is constitutively repressed. The repression of *ospA* can be reproduced using the host-adapted spirochete model. The host-adapted spirochete model was developed by Akins et al. (1998) in order to obtain sufficient amounts of spirochetes from the vertebrate host environment for further assays and evaluations. In this method, *B. burgdorferi* is cultivated within DMC's of 10 kDa pore size to retain the bacteria within the chamber. The chamber is surgically implanted into the peritoneal cavity of a rat and incubated for two weeks before harvesting the DMC's to recover the *Borrelia*. *Borrelia* cultured in this manner display a similar antigenic composition to that of bacteria during mammalian infection. Specifically, *ospA* repression can be observed in *B. burgdorferi* cultured in this manner.

To establish the host-adapted model, the wild-type infectious strain BbAH130 used in these studies was cultivated within DMC's of Sprague-Dawley rats. The culture was incubated within the peritoneum for two weeks before harvest. OspA was expressed in the *in vitro* grown *B. burgdorferi*, as expected, because *ospA* is constitutively expressed *in vitro*. However, there was no OspA expressed in the host-adapted spirochete model culture (**Figure 19**). The repression of *ospA* within the host-adapted spirochete model shows that this model is an excellent tool that mimics the *in vivo* repression of *ospA*. 
Figure 19. Establishment of the host-adapted model.

BbAH130 (AH130) was either cultivated in BSK at 37°C (in vitro) or within DMC’s (10 kDa pore size) inside the peritoneal cavity of a Sprague-Dawley rat for two weeks. Whole cell lysates were subjected to SDS-PAGE before Coomassie blue staining. Protein bands corresponding to OspA and OspC are indicated on right. Protein size is indicated on left in kilodaltons (kDa).
Deletion of the T-Rich element results in increased luciferase expression using the host-adapted model

Using the *in vitro* model of *ospA* repression, it was determined that the T-Rich element was required for the full repression of *ospA*. To confirm the *in vitro* model of *ospA* repression, wild-type BbAH130 was electroporated with the shuttle vectors expressing the luciferase reporter under the control of either the full-length *ospAB* promoter or the *PospAB-ΔIR,Rept,T-Rich* promoters. These constructs were cultivated in the host-adapted model for two weeks and then harvested and prepared for luciferase assay. The *PospAB-ΔIR,Rept,T-Rich* had a higher amount of luciferase activity than the full-length *ospAB* promoter (*Figure 20*). This suggests that the *PospAB-ΔIR,Rept,T-Rich* promoter is lacking a cis-element essential for the repression of *ospA* within the host-adapted model. This result agrees with previous findings from the *in vitro* model of *ospA* repression.
Figure 20. Influence of host signals on luciferase expression of *B. burgdorferi* cultivated in the host-adapted model.

Luciferase assay of the wild-type *B. burgdorferi* strain BbAH130 transformed with shuttle vectors expressing luciferase under the control of either the full length *ospAB* promoter (FL) or the ΔIR,Rept,T-Rich *ospAB* promoter. The constitutively expressed *flaB* promoter (*PflaB*) was used as a control (*, p<0.05 using paired Student's t-test).
BB0219, a manganese transporter, is not essential for *B. burgdorferi* growth within the host-adapted model

*B. burgdorferi* is transmitted to mammals through the *Ixodes* tick vector. *Borrelia* must adapt to the unique environments of its two hosts to be able to establish infection. To establish infection, the bacterium needs to acquire essential nutrients and transition metals, like iron and magnesium, from its hosts. A metal transporter, BmtA (BB0219) has been recently identified as being important for transporting manganese and detoxifying reactive oxygen species (Ouyang et al., 2008).

A *bmtA* mutant was created by disrupting BB0219. The phenotype of the *bmtA* mutant displayed a decreased uptake in magnesium, an inability to establish an infection within mice and the need of *bmtA* for reactive oxygen species protection. We sought to determine if the *bmtA* mutant’s inability to establish an infection is due to a defect in the mutant’s growth. We cultivated the *bmtA* mutant within DMC’s implanted within the peritoneal cavity of a rat. Upon harvest, *B. burgdorferi* was enumerated and it was found that both the wild-type and the *bmtA* mutant had comparable growth within the DMC indicating that BmtA is not essential for growth within the host-adapted model (Table 3). Rather than BmtA affecting growth, BmtA must play a role in establishing infection in some other manner.
Table 3. Growth of wild-type, bmtA mutant and complement *Borrelia* within the DMC.

<table>
<thead>
<tr>
<th>Growth Condition</th>
<th>WT</th>
<th><em>bmtA</em> mutant</th>
<th>Complement</th>
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<tbody>
<tr>
<td><em>In vitro</em></td>
<td>++++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Mice</td>
<td>++++</td>
<td></td>
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<tr>
<td>DMC</td>
<td>++++</td>
<td>+++</td>
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Growth represented as “+”.
CHAPTER FOUR: DISCUSSION

The T-Rich Region is required for the full activation of the ospAB promoter

Previous studies on ospA regulation had used nonreplicating, transient reporters to measure the ospAB promoter efficacy. These studies failed to use reporters which are able to replicate in B. burgdorferi, due to their lack of a B. burgdorferi replication origin. Therefore these previous studies were not as accurate as would be if using a stable reporter construct. Unlike the previous studies, to ensure a more accurate measurement and evaluation of ospAB promoter activity, we 1) used shuttle vectors that could replicate within B. burgdorferi; and 2) used the luciferase reporter which is generally 100-fold more sensitive than the previously used CAT reporter.

In our studies, we the luciferase reporter which was codon-adapted to B. burgdorferi (Blevins et al., 2007). B. burgdorferi has a GC content of only 28.6%, so the luciferase gene was codon-adapted to ensure that the luciferase gene was accurately representing the transcriptional activity rather than having a codon bias (Fraser et al., 1997). To determine which of the three putative cis-elements was required for the full activation of ospA, the luciferase reporter was placed under control of the full-length ospAB promoter and its activity was compared to several truncated ospAB promoters which deleted various regions containing cis-elements. We had observed that the deletion of the ospAB promoter region spanning from the IR to the Repeat elements did not cause a significant decrease in ospAB promoter expression. However, when the ospAB promoter region spanning the IR, Rept and T-Rich regions was deleted, there was a significant decrease in ospAB promoter expression. This suggested that the T-Rich region was required for the full activation of ospA.
The same series of *ospAB* promoters used to control luciferase expression in a wild-type *Borrelia* strain were placed to control the native *ospA* reporter within an *ospAB* mutant strain to evaluate which putative cis-element was required for the full activation of *ospA*. The native *ospA* reporter demonstrated similar results to the luciferase reporter results: deletion of the IR and Rept region together did not cause a significant decrease in *ospA*, however, deletion of the IR, Rept, and T-Rich regions together resulted in a decrease in *ospA*. These *ospA* activation studies using a luciferase reporter and *ospA* reporter both suggested that the T-Rich region was required for the full activation of the *ospAB* promoter. However, we needed to ensure that the T-Rich region was solely responsible for the decrease in *ospAB* promoter activation, rather than the T-Rich region in conjunction with either the IR or Rept regions. To do this, site-directed mutagenesis was performed to mutate a 10 bp region of the T-Rich region of the full-length *ospAB* promoter to create P*ospAB*-T-Rich-mut. Like the other promoters tested, the P*ospAB*-T-Rich-mut promoter was placed controlling luciferase expression and evaluated using the luciferase assay. The P*ospAB*-T-Rich-mut promoter showed reduced luciferase expression compared to the full-length *ospAB* promoter indicating that the T-Rich region alone was responsible for the reduction in *ospAB* promoter activity observed in these studies. Our *ospA* expression findings were consistent with previous data in the literature demonstrating that the T-Rich region is required for the full activation of the *ospAB* promoter within a transient reporter system (Sohaskey et al., 1999). Our studies, which used stable reporters, showed that the T-Rich region is an important regulatory element required for the full activation of *ospA*.

**A Novel Approach to Studying *ospA* repression**

Studying *ospA* repression in *Borrelia* has proven to be a very difficult task as *ospA* is only expressed during *in vivo* cultivation and thus collecting enough bacterial sample
volume to do experiments has proven to be very laborious. Therefore, there is very little known about the repression of ospA other than that it is mediated by RpoS in an unknown manner (Caimano et al., 2005). To create an easier way to study ospA repression, we developed an in vitro model to study ospA repression. This was achieved by using the luciferase reporter under the control of the ospAB promoter within an ospAB mutant strain, which has RpoS constitutively expressed and thus mediating the repression of ospA. This advance in the field should make it easier for researchers to study ospA repression.

The T-Rich Region is required for the full repression of the ospAB promoter

Our in vitro model to study ospA repression was used to determine which cis-elements are required for the full repression of ospA. The luciferase reporter was placed under control of the full-length ospAB promoter and other truncated ospAB promoters so the cis-elements required for ospA repression could be determined. Deletion of the IR or IR Rept regions did not cause a significant change in ospAB promoter activity compared to the full-length ospAB promoter. However, deletion of the IR, Rept, and T-Rich regions resulted in an increase in promoter activity showing that the truncated promoter was not able to be fully repressed. This indicated that the T-Rich region was important for the full repression of ospA. To ensure that the decreased repression was due solely to the T-Rich region and not the T-Rich region in conjunction with the IR or Rept regions, we compared the PospAB-T-Rich-mut promoter, which has the full-length ospAB promoter with a mutated T-Rich region, to the full-length ospAB promoter. Our results had shown a decrease in repression which indicates that it is solely the T-Rich region responsible for the decrease in ospA repression. Our in vitro model of ospA repression demonstrated that the T-Rich region of the ospAB promoter was required for the full repression of the ospAB promoter.
To further study *ospA* repression, we explored the effects of norepinephrine on *ospA* repression. A previous study demonstrated that norepinephrine is able to de-repress *ospA* (Scheckelhoff et al., 2007). De-repressing *ospA* is required for the tick to acquire *Borrelia* from an infected mammal. In an infected mouse, *ospA* is repressed, however, upon tick feeding, *B. burgdorferi* must de-repress *ospA* in order to colonize the tick gut where OspA binds specifically to the tick’s TROSPA receptor. The previous study demonstrates that when a tick bites the mammal, the host releases norepinephrine and this contributes to de-repressing *ospA*. Given this, we wanted to determine if norepinephrine would affect *ospA* repression in our *in vitro* repression model.

Using our *in vitro* repression model, we cultured the *ospAB* mutant containing either the full-length *ospAB* promoter (P_{ospAB-FL}) or the *ospAB* promoter lacking the IR,Rept, and T-Rich region (P_{ospAB-ΔIR,Rept,T-Rich}) in BSK media only or BSK media containing norepinephrine. For both the P_{ospAB-FL} and the P_{ospAB-ΔIR,Rept,T-Rich} promoter, there was an increase in luciferase expression upon the addition of norepinephrine indicating that there was a de-repression of *ospA* for both promoters. From this study, we concluded that norepinephrine is able to de-repress *ospA*, which is agreement with the previous studies. Further advancing this, we were able to conclude that the de-repression of *ospA* is independent of the T-Rich region. There is likely another regulatory element between the T-Rich region and the *ospA* gene that plays a role in the de-repression of *ospA* via the addition of norepinephrine.

One last approach we used to study the repression of *ospA* was the host-adapted animal model in which *Borrelia* are cultivated within DMC’s implanted into the peritoneal cavity of a rat (Akins et al., 1998). We first established the host-adapted model within our lab. We observed that growing wild-type *Borrelia* within the DMC’s resulted in the
repression of ospA. Since ospA was able to be repressed, we were able to use the host-adapted model to confirm our ospA repression finding we observed in vitro. In the DMC’s, we grew the wild-type Borrelia strain with luciferase reporter expression under the control of either the full-length ospAB promoter or the PospAB-IR,Rept-T-Rich promoter. There was an increase in luciferase expression in the PospAB-IR,Rept-T-Rich promoter compared to the full-length ospAB promoter which mirrors our results from the in vitro studies that the T-Rich region is required for the full repression of ospA. One caveat of the host-adapted model is that, unlike in vitro cultivation, there is no antibiotic selection in order for the plasmids to be retained and replicating within Borrelia. However, it is probable that since both strains used the same parental vector and are identical except at the region of the ospAB promoter, that the plasmid would be lost at the same rate for both strains.

Previous studies in ospA repression had already shown that RpoS is required for the full repression of ospA. Our studies further dissected the mechanism of ospA repression by demonstrating that the T-Rich region of the ospAB promoter is an important cis-element required for the full repression of ospA. For ospA repression, Rrp2 becomes phosphorylated upon tick feeding. Then, Rrp2 in conjunction with RpoN activate RpoS. RpoS then represses ospA either by 1) associating with an in vivo-specific accessory factor that allows RpoS to directly bind to the T-Rich cis-element of the ospAB promoter; or 2) by controlling the expression of an unknown ospA repressor protein that binds to the T-Rich cis-element of the ospAB promoter to block RpoS-dependent transcription initiation. For ospA activation, on the other hand, RpoD is able to bind to the T-Rich region of the ospAB promoter to drive the transcription initiation of ospA.
CHAPTER FIVE: FUTURE DIRECTIONS

Potential role of BosR as a repressor of ospA

In *B. burgdorferi*, BosR (BB0647) has been found to encode a novel DNA-binding protein in the Fur/Per family of transcriptional regulators. BosR is required for the induction of RpoS (Hyde et al., 2010; Ouyang et al., 2009). RpoS represses ospA via an unidentified mechanism (Caimano et al., 2005). A recent study has discovered that BosR binds specifically to the rpoS gene at three distinct sites. By making specific mutations in the rpoS gene and measuring the binding capability of BosR, a novel direct repeat and inverted repeat sequence (TAAATTAAAT), which is critical for BosR binding, was identified (Ouyang et al., 2011). This direct repeat sequence which BosR specifically binds is similar to areas upstream of the T-Rich element found on the ospAB promoter. Since BosR regulates RpoS, and RpoS is required for the repression of ospA, it is plausible that BosR could play a key role in ospA repression through binding to a putative BosR binding site located on the ospAB promoter.

In an rpoS mutant, ospA is not able to be repressed (Caimano et al., 2005). Thus if BosR directly represses ospA, then inducing BosR in an rpoS mutant should restore ospA repression. These experiments can be performed in two different ospA repression conditions: 1) in the host-adapted model, or 2) in the ospAB mutant. For the host-adapted model study, either wild-type, rpoS mutant, or an rpoS mutant complemented with bosR can be electroporated with a shuttle vector containing the full-length ospAB promoter controlling luciferase expression. The wild-type *Borrelia* would display low luciferase expression because this condition supports the repression of ospA. The rpoS mutant would display high luciferase expression because previous studies have shown that ospA is not able to be repressed in rpoS mutants (Caimano et al., 2005). This is
likely due to the absence of a repressor controlled through RpoS. The rpoS mutant complemented with bosR would have low luciferase expression if BosR is able to specifically bind to the ospAB promoter to cause repression. If complementation with bosR results in high luciferase expression, this would indicate that BosR is not specifically binding to the ospAB promoter to cause repression and that repression of ospA is due to a trans-factor(s) controlled by RpoS.

A second method of determining if BosR is able to directly repress ospA would be by using the in vitro model of ospA repression. In this method, the same shuttle vector which contains luciferase under the control of the full length ospAB promoter would be electroporated into either 1) the BbAH130 wild-type strain; 2) the ospAB mutant strain; 3) the ospAB, rpoS double mutant; or 4) the ospAB, rpoS double mutant complemented with bosR. The strains would be cultivated at 37°C and analyzed by luciferase assay. The wild-type strain would expect to have high luciferase activity because ospA is highly expressed during in vitro cultivation. The ospAB mutant would be expected to have no luciferase activity because this is the in vitro model of ospA repression where the abrogation of ospAB causes ospA repression conditions. The ospAB, rpoS double mutant would show high luciferase activity because RpoS is required for the repression of ospA. Lastly, the ospAB mutant strain complemented with bosR would exhibit no luciferase activity if BosR is able to bind to the cis-elements of the ospAB promoter to cause repression. However, if BosR is not able to specifically bind to the ospAB promoter to repress its activity, then the strain would show high levels of ospA because ospA cannot be repressed in the absence of RpoS.
Identification of putative trans-factors via a knowledge-based approach

Previous studies have shown that *B. burgdorferi* *rpoS* mutants are not able to downregulate OspA even during *in vivo* cultivation when *ospA* is normally repressed. The precise mechanism by which RpoS controls *ospA* repression is not known. We hypothesized that an *ospA* repressor is regulated under the control of RpoS and that a knowledge-based approach can be used to help identify putative DNA-binding proteins controlled by RpoS.

Microarray data from Caimano et al. (2005) and Boardman et al (2008) identified genes being differentially regulated by RpoS within the host-adapted model. From this microarray data, a list was compiled which identified genes that appeared to be the most highly regulated by RpoS. From this list of RpoS-regulated genes, the genes with the highest transcript fold-change were next evaluated for their probability of binding to DNA. Their amino acid sequence obtained from Entrez Gene (NCBI) was entered on an online server (www.netasa.org/dbs-pred) to predict the probability of the protein binding to DNA. After determining the DNA-binding probability, we narrowed down the list to the top five gene candidates based on their: 1) fold change in the transcript levels between the RpoS mutant and the wild-type as determined from the microarray data; and 2) DNA-binding capability as predicted by the online server. The top five gene candidates are 1) BBL29; 2) BBD01; 3) BB0449; 4) BBJ01; and 5) BBJ02.

These putative DNA-binding proteins were cloned into a shuttle vector that placed their expression under the control of a constitutive promoter. Next, the shuttle vectors were electroporated into a wild-type *B. burgdorferi* strain. These strains were grown in *vitro* at 23°C, a condition in which *ospA* is expressed, to determine if the any of the five repressor candidates were able to repress *ospA*. If the gene is an *ospA* repressor, then
the overexpression of the gene in the WT strain would result in lower or no ospA. There was no difference in any of the five expressed genes indicating that genes did not have an effect on ospA repression and therefore are not ospA repressors.

In the future, other genes can be selected using our knowledge-based approach to determine if they are able to repress ospA. If the overexpressed gene does appear to repress ospA when overexpressed, an EMSA would need to be done to determine if the protein of interest is able to act as a trans-factor and bind to the ospAB promoter. Lastly to confirm that the gene is indeed an ospA repressor, the gene would need to be knocked out in B. burgdorferi and grown within a DMC. If ospA is not able to be repressed in this condition, then the gene would prove to be needed to repress ospA.

**Mass spectrometry identification of the putative trans-factors acquired using biotinylated ospAB promoter oligonucleotide**

The T-Rich region of the ospAB promoter has been identified as an important regulatory cis-element in both the expression and repression of ospA. The ospAB promoter can be used to isolate the trans-factor(s) binding to the ospAB promoter cis-elements by employing sequence-specific DNA affinity chromatography. In a method adapted from (Babb et al.), chemically synthesized biotin-labeled complementary oligodeoxynucleotides that encompassed the full-length ospAB promoter were purchased (2006). The full-length ospAB promoter was used because it contains the T-Rich region, which we found was crucial for the full activation of ospA, and thus the activator is likely binding to this region. Next, the biotin-conjugated oligodeoxynucleotides were affixed to streptavidin magnetic beads and protein extracts from either wild-type B. burgdorferi (for ospA expression conditions since wild-type has
high *ospA* expression) or *ospAB* mutant (for the *ospA* repression conditions since the
*ospAB* mutant has constitutive expression of an unknown *ospA* repressor) cultures were
added. The supernatant was removed and the beads coated with biotin-labeled *ospAB*
promoter were gently washed. Next, the proteins bound to the *cis*-element oligonucleotide
fragments were eluted from the magnetic beads and separated by SDS-
PAGE and visualized. Protein bands were extracted and analyzed by matrix-assisted
laser desorption ionization-time of flight (mass spectrometry) performed by the
Proteomics Core Facility at Indiana University School of Medicine (Indianapolis, IN). The
proteins were identified as shown in Table 4.

Of particular interest is gene BBJ02 (Table 4). This gene was identified in our
knowledge-based approach as being a putative *ospA* repressor due to its regulation by
RpoS and its DNA binding probability. BBJ02 was constitutively expressed in an
*ospA* activation condition to determine if it was able to act as an *ospA* repressor; it did not.

In the future, the isolation of the activator need to be redone. To improve these studies,
the bacterial lysis and washing steps need to be optimized to ensure less non-specific
binding. Also, the full-length *ospAB* promoter with the mutated T-Rich region (*P*ospAB-T-
Rich-mut) should be used as a control because it contains the same elements as the
full-length promoter except the T-Rich region. Since the activator is likely binding to the
T-Rich region, the *P*ospAB-T-Rich-mut should not have the activator present in the
proteins eluded from the promoter whereas the *P*ospAB-FL should have the activator
present.
Table 4. Proteins identified by mass spectroscopy to bind to the *ospAB* promoter.

The name of the protein or gene (protein/gene) on the left and its putative role in *B. burgdorferi* (comments). The number of peptides is noted (peptides) and its number of distinct sequence (# of distinct sequences).

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REFERENCES


CURRICULUM VITAE

Tara Lynn Oman

Education

Ph.D. in Microbiology & Immunology, Indiana University, Indianapolis, IN, 2012
Dissertation Title: “The Regulation of Outer Surface Protein A in the Lyme Disease Spirochete Borrelia burgdorferi”
Dissertation Advisor: Professor Xiaofeng Yang

B.S. in Biology, Indiana University, Bloomington, IN, 2006
Course highlights: Microbiology, Molecular Biology, Cellular Biology, Genetics, Statistical Techniques, Organic Chemistry I & II, Calculus I & II, Summer Flowering Plants, Spanish

Teaching and Mentoring Experience

Guest Lecturer, Indiana University School of Medicine, Indianapolis, IN
• Served as the mycology lecturer for the undergraduate level course “Microbiology for Nursing Majors” (J210)
• Created PowerPoint presentations, lectured, answered students questions and created questions for exams (3 semesters: Spring 2010 - Spring 2011; 150 students)

Graduate Teaching Assistant, Indiana University School of Medicine, Indianapolis, IN
• Taught laboratory sessions, improved experiments, created lecture notes, graded reports and created exam questions for the undergraduate level course “Microbiology for Nursing Majors” (Fall 2009; 32 students)

Undergraduate Tutor, Indiana University, Bloomington, IN, 2004 - 2006
• Tutored undergraduate and high school students in basic chemistry, human genetics, biology and finite math

Research Experience

Graduate Research Assistant, Indiana University, Indianapolis, IN, 2007 - 2012
• Investigated the regulation mechanism and function of the outer surface protein A (OspA) in the Lyme disease causative agent Borrelia burgdorferi
• Genetically manipulated Borrelia to create mutants to determine the cis-elements involved in ospA regulation
• Combined molecular and bioinformatic techniques to identify the trans-factor involved in ospA regulation
• Created the first in vitro model for studying ospA repression within Borrelia

Undergraduate Research Assistant, Indiana University, Bloomington, IN, 2005 - 2006
• Studied signal transduction of the Epidermal Growth Factor Receptor (EGFR) in Drosophila melanogaster and the effects of insulin production on cellular development
• Designed experiments, collected and analyzed data, genotyped drosophila, maintained insect stocks, prepared solutions and medias
Undergraduate Lab Technician, Indiana University, Bloomington, IN, 2004
• Studied solicitation pheromones in the burrower bug Sehirus cinctus
• Kept insect husbandry records, preparatory set-up for experiments, harvested clover seeds for insect feed

Awards and Honors

Fellowships
• Immunology and Infectious Disease Training Grant Fellowship, National Institutes of Health and Indiana University School of Medicine 2010 - 2012

Other distinctions
• IU School of Medicine’s Harold Raidt Graduate Student Teaching Award 2011
• Travel Award, 16th Annual Midwest Microbial Pathogenesis Meeting, Purdue University, IN 2010
• Indiana University College of Arts and Sciences Dean’s List 2003 - 2006
• Founders Day High Scholastic Achievement Honors 2003 - 2006
• National Society of Collegiate Scholars 2004 - 2005
• Phi Eta Sigma National Honor Society 2004
• Alpha Lambda Delta National Honor Society 2004

Professional Activities
• Graduate Student Representative, Indiana University School of Medicine Faculty Community Relations Committee, Indianapolis, IN 2007 - 2012
• Class representative for Organic Chemistry I & II, Indiana University Department of Chemistry, Bloomington, IN 2005-2006
• Class representative for Principles of Chemistry & Biochemistry, Indiana University Department of Chemistry, Bloomington, IN 2004 - 2005

Conferences and Presentations
• 17th Annual Midwest Microbial Pathogenesis Conference, St. Louis, MO (2010)
• Charon Symposium: Spirochetes, Microbial Motility and Chemotaxis, Morgantown, WV (2009)
• 16th Annual Midwest Microbial Pathogenesis Conference, West Lafayette, IN (2009)
• 14th Annual Midwest microbial Pathogenesis Meeting, Chicago, IL (2007)

Publications

Ming He, Tara Oman, Haijun Xu, Jon Blevins, Michael V. Norgard, and X. Frank Yang. “Abrogation of *ospAB* constitutively activates the Rrp2-RpoN-RpoS pathway (SigmaN-sigmaS cascade) in *Borrelia burgdorferi.*” *Molecular Microbiology,* 2008, 70(6):1453-64.