An Inverted Repeat in the *ospC* Operator Is Required for Induction in *Borrelia burgdorferi*

Dan Drecktrah1, Laura S. Hall1, Laura L. Hoon-Hanks1*, D. Scott Samuels1,2*

1 Division of Biological Sciences, The University of Montana, Missoula, Montana, United States of America, 2 Center for Biomolecular Structure and Dynamics, The University of Montana, Missoula, Montana, United States of America

**Abstract**

*Borrelia burgdorferi*, the spirochete that causes Lyme disease, differentially regulates synthesis of the outer membrane lipoprotein OspC to infect its host. OspC is required to establish infection but then repressed in the mammal to avoid clearance by the adaptive immune response. Inverted repeats (IR) upstream of the promoter have been implicated as an operator to regulate *ospC* expression. We molecularly dissected the distal inverted repeat (dIR) of the *ospC* operator by site-directed mutagenesis at its endogenous location on the circular plasmid cp26. We found that disrupting the dIR but maintaining the proximal IR prevented induction of *ospC* synthesis by DNA supercoiling, temperature, and pH. Moreover, the base-pairing potential of the two halves of the dIR was more important than the nucleotide sequence in controlling OspC levels. These results describe a cis-acting element essential for the expression of the virulence factor OspC.

**Citation:** Drecktrah D, Hall LS, Hoon-Hanks LL, Samuels DS (2013) An Inverted Repeat in the *ospC* Operator Is Required for Induction in *Borrelia burgdorferi*. PLoS ONE 8(7): e68799. doi:10.1371/journal.pone.0068799

**Editor:** Brian Stevenson, University of Kentucky College of Medicine, United States of America

**Received** November 19, 2012; **Accepted** June 3, 2013; **Published** July 3, 2013

**Copyright:** © 2013 Drecktrah et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was supported by Public Health Service grants AI051486 to D.S.S. and P20 GM103546 to the Center for Biomolecular Structure and Dynamics from the National Institutes of Health. L.L.H.-H. was supported by a Watkins Scholarship from The University of Montana, an Undergraduate Research Internship through the National Science Foundation EPSCOR program under Grant EPS-0701906, an Undergraduate Research Award from the Davidson Honors College, and an Honors Fellowship through the Montana Integrative Learning Experience for Students (MILES) program under Grant 52005905 from the Howard Hughes Medical Institute-Undergraduate Science Education Program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

* E-mail: scott.samuels@umontana.edu

* Current address: Colorado State University College of Veterinary Medicine & Biomedical Sciences, Fort Collins, Colorado, United States of America

**Introduction**

Lyme disease is caused by the spirochete *Borrelia burgdorferi*, which is transmitted via an *Ixodes* tick [1–3]. *B. burgdorferi* is maintained in an enzootic cycle between its tick vector and a vertebrate host reservoir [4–6]. Naïve larvae acquire *B. burgdorferi* when feeding on an infected animal and can then transmit the bacterium to uninfected hosts as nymphs during feeding, completing the cycle. *B. burgdorferi* encounters disparate, and hostile, environments as it transitions through the enzoonic cycle; the spirochete has evolved a repertoire of strategies, which involve the regulation of a variety of genes, to respond and adapt to these changes during its lifecycle [6–8].

The differential syntheses of outer surface lipoproteins (Osp), which are the interface between *B. burgdorferi* and its hosts, is paramount to the ability to infect, survive and replicate in both the tick and the vertebrate [6–10]. These regulated genes encode wide-ranging functions; for example, VhE mediates evasion from the vertebrate immune system [11–13], while OspA binds to a tick midgut protein and protects the spirochetes from the incoming blood meal [14–17]. One of the best-studied lipoproteins is OspC [18], which is required for transmission and the early stages of mammalian infection [19–22]. The *ospC* gene is carried on the conserved 26-kb circular plasmid cp26 [23,24]. The precise function of OspC remains elusive, but the outer membrane lipoprotein appears to have several activities [25], including providing initial protection from the innate immune system [26] and facilitating dissemination [27]. Additionally, OspC has a ligand-binding domain essential for its function [28] and binds the tick salivary protein Salp15 [29] as well as mammalian plasminogen [30,31], which may assist in transmission and dissemination, respectively. OspC is highly immunogenic, so its synthesis must be repressed for the spirochete to persist in the mammal [32–34]. *B. burgdorferi* that continue to produce OspC during infection of immunocompetent mice are cleared [34] and *ospC* expressed in trans from a shuttle vector is selected against during mammalian infection [35]. While *ospC* is present in all *B. burgdorferi* strains examined, the sequence is variable, with only strains carrying certain alleles capable of disseminating and establishing infection in humans [13,36–39].

OspC synthesis is induced in vitro in response to increased temperature, which presumably mimics a signal that occurs during tick feeding [40–42]. Subsequent studies showed that numerous factors such as pH [43,44], DNA supercoiling [42], oxygen [45], carbon dioxide [46], acetate [47], and transition metals [48] also control *ospC* expression. External signals are transmitted through a unique signaling pathway involving the sequential action of two alternative sigma factors, RpoN (σ54) and RpoS (σs) [49,50]. RpoN, in collaboration with the response regulator Rrp2 [51–54] and the transcription factor BosR [55–58], activates transcription of *rpoS*; RpoS, in turn, activates transcription of *ospC* [49] and other genes [54,59,60]. Another level of control of *ospC* expression is exerted during transition of *rpoS*. The small RNA DsrA Bb [61,62] and the RNA chaperone Hfq [63] control *rpoS* translation.
and subsequently ospC transcription. However, there is no evidence for post-transcriptional control of the ospC gene [42,44–49,52,61,64].

Previous studies identified an ospC operator consisting of two overlapping inverted repeats (IRs) 42 bp upstream of the major transcriptional start site [23,63–67]. The operator is highly conserved in B. burgdorferi sensu stricto [67], but there is some controversy about its role in ospC gene regulation. Eggers et al. [64] showed that the operator is required for full ospC expression in vitro, while Yang et al. [68] found the operator is dispensable for induction and repression. In addition, mutants lacking the operator were unable to persist in immunocompetent mice and were also cleared from SCID mice injected with transferred anti-OspC antibodies, suggesting that the operator is required to repress ospC expression during infection [69]. One caveat to these previous studies is that ospC was expressed in trans in an ospC null background strain. In the current study, we molecularly dissect the ospC operator in its native locus on cp26 and show that the distal IR is an important co-acting element controlling OspC expression.

Materials and Methods

Bacterial Strains and Culture Conditions

Low-passage B. burgdorferi strain 297 (BbAH130) [49] and all mutant strains were maintained in Barbour-Stoenner-Kelly II (BSK-II) liquid medium, pH 7.6, containing 6% rabbit serum [70]. In temperature shift experiments, cultures were passaged twice at 23°C, before inoculating cultures at 1 x 10^6 cells ml^-1 and growing at 23°C to late log phase (5 to 9 x 10^6 cells ml^-1) or inoculating cultures at 1 x 10^6 cells ml^-1 and growing at 34°C to late log phase. For temperature downshift experiments, cultures were grown to log phase at 34°C before inoculating cultures at 1 x 10^6 cells ml^-1 and growing at 23°C to late log phase. In experiments examining the effect of pH, cultures at 34°C in BSK II at pH 8.0 were passaged into BSK II at pH 7.0. To determine the effect of DNA supercoiling, cultures were grown to 23°C (inoculated at 1 x 10^6 cells ml^-1) in the presence of 10 ng ml^-1 coumermycin A1 (50 mg ml^-1 stock in DMSO) or DMSO (solvent control) until late log phase [42]. Cell density was determined using a Petroff-Hauser counting chamber [71].

Construction of ospC Promoter Mutants

Mutations in the ospC operator were generated by allelic exchange on cp26 [72]. Constructs containing the operator mutants were made by overlap extension PCR [73]. The 5’ portion of the upstream construct was amplified by PCR of genomic DNA using KOD polymerase (Novagen) with the primers ospC U866F and ospCp mutHup (297)R or ospCp mutHup (297)F (Table 1). The 3’ portion of the upstream construct, which also includes the ospC gene, was amplified by PCR using the primers ospC D673F and AatII + AgeI and ospCp mutHup (297)F (Table 1). PCR products were separated and purified in a 1% agarose gel. Paired 5’ and 3’ portions of the upstream construct were then combined and extended for six cycles in a thermal cycler. Next, the primers ospC U866F and ospC D673F + AatII + AgeI (Table 1) were added and the combined upstream construct was amplified by PCR. To generate the construct for the wild-type control strain with the antibiotic resistance cassette, PCR was done using primers ospC U866F and ospC D673F + AatII + AgeI with genomic DNA as a template. PCR products were separated in a 1% agarose gel, gel purified, polyadenylated, and cloned into pCR®2.1-TOPO. PCR of genomic DNA with the primers ospC D673F + AatII and ospC D1572R + AgeI (Table 1) was used to amplify the downstream construct, which was cloned into pCR®2.1-TOPO as described above. The accuracy of all DNA constructs was confirmed by sequencing. The downstream sequence was inserted into the upstream ospC operator mutation constructs using the synthetic AatII and AgeI restriction sites. Lastly, the kanamycin resistance cassette flgBp-aphI [74] was inserted downstream of the ospC gene into the engineered AatII site. The orientation of flgBp-aphI was determined by PCR using the primers kanR 488R and ospC D1572R + AgeI (primers a and b, respectively). The plasmid was linearized by digestion with AhdII and electroporated into competent B. burgdorferi [61,71,72]. Transformants were cloned in liquid BSK-II by diluting the electroporated cells to less than one cell per well of a 96-well plate in medium containing kanamycin (200 μg ml^-1) at 34°C and a 1.5% CO2 atmosphere [16]. Total genomic DNA was isolated from positive colonies and sequenced by the Murdock DNA Sequencing Facility at The University of Montana using the primer ospC U291F to confirm the site-directed ospC operator mutations.

RNA Isolation and qRT-PCR Analysis

RNA was isolated from 40-ml cultures of B. burgdorferi grown at 23°C containing 10 ng ml^-1 coumermycin A1 or DMSO as a solvent control using TRizol (Invitrogen) as previously described [61,75]. Samples were treated with Turbo DNase (Ambion) to remove contaminating DNA. Samples were screened by PCR to ensure that contaminating DNA had been removed using the primers flaB 423F and flaB 542R. cDNA was synthesized using 1 μg RNA with SuperScript® III for qRT-PCR (Invitrogen). flaB and ospC primers and probes were designed using Primer Express version 3.0 (Applied Biosystems). TaqMan absolute qRT-PCR was performed in 96-well plates using an Applied Biosystems 7300 Real-Time PCR System and standard curves for flaB and ospC were generated using a portion of the flaB ORF (nucleotides 278–551 of the ORF) cloned into pCR®2.1-TOPO and B. burgdorferi strain 297 (BbAH130) genomic DNA, respectively. Values represent the mean (±SEM) from three independent experiments.

SDS-PAGE and Immunoblotting

B. burgdorferi cultures were grown to late log phase at 23°C or 34°C and total cell lysates collected as previously described [61,72]. Equal amounts of protein were separated on pre-cast Novex 4–20% Tris-Glycine polyacrylamide gels (Invitrogen). Proteins were transferred by electrophoretic transfer to PVDF Immobilon™ membranes (Millipore) and membranes blocked in Blocking Buffer (138 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, 0.05% Tween 20, 4% dry milk, and 1% goat serum) overnight at 4°C. Membranes were incubated with rabbit anti-OspC antibodies (1:1000) [68,76] or anti-FlaB antibodies (1:1000) (a kind gift from Tim Schwan) in Blocking Buffer for 1 h at room temperature. Rabbit antibodies were detected by incubating membranes with goat anti-rabbit HRP-linked antibodies (Bio-Rad Laboratories) (1:20,000) in Blocking Buffer for 1 h at room temperature. HRP-linked antibodies were visualized by chemiluminescence using AmershamTM ECL Plus (GE Healthcare) and images were collected using an LAS-3000 Intelligent Dark Box (Fujiﬁlm Medical Systems USA). Images were processed using ImageJ [US National Institutes of Health and available at http://rsbweb.nih.gov/ij/] and Pixelmator (Pixelmator Team, Ltd).

Results

The ospC operator consists of a set of overlapping inverted repeats that are highly conserved in B. burgdorferi sensu stricto.
strains (Fig. 1) [65–67]. We hypothesized that the dIR has a specific role in responding to changes in DNA supercoiling, which is known to regulate ospC expression [42]. Increased negative DNA supercoiling can alter DNA structure, including extrusion of cruciforms from IRs, which can have a regulatory effect on transcription [77–79]. We generated site-directed mutations in the native ospC operator on cp26 that specifically disrupted the dIR (dIR–) and that changed the dIR sequence but retained complementarity (dIR+). Changes in the operator sequence of the mutant strains were confirmed by DNA sequencing; about one-third of the kanamycin-resistant clones contained the site-directed mutations.

The Role of the dIR in ospC Expression Induced by Relaxation of DNA Supercoiling

We have previously shown that a decrease in negative DNA supercoiling causes an increase in ospC transcription [42]. The antibiotic coumeryycin A1 relaxes DNA supercoiling by inhibiting DNA gyrase [80–83]. Wild-type and mutant strains were grown at 23°C in the presence of coumerycin A1 or DMSO (solvent only control) until late log phase to examine if the increase in ospC expression by the relaxation of supercoiling is mediated by the dIR of the operator. Transcript levels of ospC and flaB were measured by qRT-PCR. The fold increase in ospC transcript in the dIR+ strain grown in coumerycin A1, compared to the solvent control, was significantly greater than that seen in the dIR– strain (about thirteenfold compared to less than twofold) (Fig. 3). The change in flaB transcript levels was about twofold or less for both the dIR+ and dIR– strains (Fig. 3). These data suggest that the ability to form the dIR is an important regulator of coumerycin A1-mediated ospC expression.

To examine if the changes in ospC transcript levels in response to coumerycin A1 treatment were reflected in OspC protein levels, total cell lysates were analyzed by Western blot using polyclonal antibodies to OspC to determine the levels of OspC synthesis. OspC levels increased in the wild-type 297 strain in response to relaxation of supercoiling when grown at 23°C in the presence of coumerycin A1 compared to cultures grown in the DMSO control (Fig. 4). Very little OspC was produced when the dIR– strain was grown in coumerycin A1 compared with the wild-type strain and the dIR+ strain (Fig. 4). We have confirmed these results with a second independently constructed clone of each of the mutant strains (data not shown). Taken together, these results suggest that the ability to form the dIR is more important than the actual sequence, at least in regard to the nucleotides that we mutated. These data imply that the increase in OspC levels stimulated by relaxation of DNA supercoiling is mediated via the

### Table 1. Oligonucleotides used in this study.

| Name                  | Sequence (5’-3’)                                      |
|-----------------------|-------------------------------------------------------|
| ospC U866F            | AGCTTAATTTCCTACAAATTG                                  |
| ospC D697R+AatII+AgeI| ACCGTAATGACGGTCTAGCATTTATTGACCTTTTCTTTCCAG            |
| ospC D673F+AatII      | GAGTGGGAAAATAGAATTATAAATAGTCG                         |
| ospC D1572R+AgeI      | ACCGTTAATGAAAATTCTCAATATTG                            |
| ospC U291F            | ATTAGTGGCTATATGGG                                      |
| kanR 488R             | TCACCTGCAATACCAAACC                                   |
| ospCp mutHup(297)F    | TAAGACAAAAATAGAATTACAAATATTG                          |
| ospCp mutHup(297)R    | ATTGAAAGATTTTTACTAAATATTG                             |
| flaB 423F             | TTTCCTTTTTTTTTTTTTTTTTTTAAAATCGGGTTTAAATG            |
| flaB 542R             | TTGTTGTCAATAGAACGTC                                 |
| flaB probe            | 6-FAM-TACTTCTAGGCTCAAGGCTCCAGTGC-TAMRA               |
| ospC F                | CATGCGCAACTTTGGAATGA                                  |
| ospC R                | TTGCAGGTTCCTATCTGCTTTATAAT                           |
| ospC probe            | 6-FAM-TAAAGATAAGGGCGCTGACACCGAC-TAMRA                |

*Synthetic restriction sites are underlined.

doi:10.1371/journal.pone.0068799.t001

---

**Figure 1. The sequence of the *B. burgdorferi* strain 297 ospC operator.** Two overlapping inverted repeats (IR): proximal (pIR, dashed arrows) and distal (dIR, solid arrows) of the ospC operator. The promoter (−10 and −35) and ribosome-binding site (RBS) are in bold and the translational start site is in italics.

doi:10.1371/journal.pone.0068799.g001
The role of the dIR in temperature-regulated OspC synthesis

A temperature shift from 23°C to 34°C is commonly used in vitro to mimic an environmental signal during tick feeding that induces virulence gene expression [8,40]. To assess the role of the dIR in temperature-regulated OspC expression, cultures were grown at 23°C, shifted to 34°C and grown to late log phase. Total cell extracts were analyzed by Western blot. The wild-type 297 strain increased OspC synthesis upon a temperature shift (Fig. 5A). Similar to the coumermycin A1 treatment, OspC was not induced in response to temperature shift when the dIR was disrupted, while regenerating the complementarity of the dIR restored the ability to respond to increased temperature with increased OspC levels in the dIR⁺ strain (Fig. 5A). A second clone of each mutant, from an independent transformation, showed the same pattern of OspC levels during temperature shift (data not shown).

We next assayed if the dIR was also involved in reducing OspC levels at 23°C. Cultures were grown to late log phase at 34°C and then passaged and grown at 23°C to late log phase. Again, changing the dIR sequence but maintaining complementarity in the dIR⁺ strain allowed for the reduction in OspC levels similar to the wild-type strain shifted to 23°C (Fig. 5B).

The Role of the dIR in pH-regulated OspC Synthesis

OspC levels have also been shown to be regulated by pH [43,44], which is considered an environmental signal that changes during tick feeding: reducing the pH to 7.0 increases and raising the pH to 8.0 decreases OspC levels. To examine if pH-regulated OspC expression is mediated through the dIR, cultures were...
grow at 34°C in medium at pH 7.0 and then passaged into medium at pH 8.0. Total cell extracts were collected at late log phase and OspC levels were analyzed by Western blot. The dIR– strain did not show increased OspC levels at pH 7.0 and the dIR+ strain behaved like the wild-type strain in response to changing the pH (Fig. 6). Thus, all the signals examined, DNA supercoiling, temperature, and pH, control OspC levels through the dIR of the operator in vitro, suggesting a common mechanism, and the complementarity of the dIR may be more important than the specific sequence.

**Discussion**

Induction and repression of ospC transcription are crucial for *B. burgdorferi* to establish and maintain, respectively, an infection in mammals. A number of external factors, including temperature, pH, oxygen, carbon dioxide, acetate, and transition metals, regulate *ospC* expression [40–48,72,84]. Induction of *ospC* expression is generally accepted to be dependent on the RpoN-RpoS sigma factor cascade [49], which includes the regulatory proteins Rrp2 [51–54] and BosR [55–58]. Considerably less is known concerning the repression of *ospC* transcription, including the signals and accessory proteins involved. Here we show that the *cis*-acting dIR of the *ospC* operator functions to control expression and our data indicate that the base-pairing potential of the two halves of the inverted repeat, rather than the specific sequence, is essential for induction, thus providing a level of *ospC*-specific regulation downstream of RpoS.

The large intergenic region upstream of the *ospC* gene contains the operator and is highly conserved among *B. burgdorferi* sensu stricto strains, much more so than the *ospC* gene itself, likely indicating selective pressure on the regulation of *ospC* expression [65,67]. Certain features of the operator, including the proximal IR (pIR), are broadly conserved throughout *B. burgdorferi* sensu lato genospecies, but, inexplicably, the dIR does not overlap the pIR in *B. afzelii* strains and is not even obviously present in *B. garinii* strains [67], suggesting alternate modes of gene regulation. Although we and others previously had shown that deleting the operator has little effect on the regulation of *ospC* transcription [68,69], our current results more closely agree with Eggers et al. [64], who showed that the entire operator was required for full *ospC* expression in response to a temperature shift. These data suggest that the dIR plays a role in *ospC* regulation. This discrepancy may be explained by the differences in the experimental approaches between the studies. In the present work, we have utilized a more precise method to dissect the operator: site-directed mutations were generated in *cis* in the endogenous operator on cp26, while the other studies utilized truncated operator mutants in *trans* on a shuttle vector in an *ospC* null background. Thus, *ospC* expression in *trans* from a plasmid much smaller than cp26 (7 kb compared to 26 kb), albeit still circular, with a strong promoter fused to a selectable marker, may affect operator function, especially when DNA topology is likely involved [42,68,85]. In fact, OspC levels expressed in *trans* were elevated at

---

**Figure 4. The role of the dIR in OspC synthesis mediated by relaxation of DNA supercoiling.** Immunoblot analysis of whole-cell lysates from strains grown to late log phase at 23°C in 10 ng ml⁻¹ coumermycin A₁ (Cou) (+) or in DMSO as a solvent control (−). Membranes were probed with antibodies against OspC (upper panel) or FlaB (lower panel). doi:10.1371/journal.pone.0068799.g004

**Figure 5. The dIR is required for OspC synthesis regulated by temperature.** (A) Immunoblot analysis of whole-cell lysates from strains grown at 23°C and then temperature shifted to 34°C and grown to late log phase. The wild-type parental strain (297 WT) and the strain with a wild-type *ospC* operator linked to the antibiotic resistance cassette (WT) are controls. (B) Immunoblot analysis of whole-cell lysates from strains grown at 34°C and then temperature shifted to 23°C and grown to late log phase. Membranes were probed with antibodies against OspC (upper panels) or FlaB (lower panels). doi:10.1371/journal.pone.0068799.g005

**Figure 6. The role of the dIR in OspC synthesis mediated by pH increase.** Immunoblot analysis of whole-cell lysates from strains grown to late log phase at 34°C at pH 7.0 or shifted to pH 8.0. Membranes were analyzed by probing with antibodies against OspC (upper panel) or FlaB (lower panel). doi:10.1371/journal.pone.0068799.g006
23°C compared to wild type, even though the plasmid-borne ospC contained the entire operator region [60].

Mutations that disrupt the dIR, but maintain the pIR (dIR– strain) prevent an increase in OspC levels in response to temperature, pH or relaxation of supercoiling, suggesting that all these signals function through a similar mechanism. Thus, the dIR is required for an increase in the amount of OspC. The finding that the ospC induction by relaxation of supercoiling at 23°C with countermaya 3 (Fig. 3) depends on the dIR suggests that DNA topology has a regulatory role. These data imply that the regulatory element may be the DNA structure rather than the sequence, although we may not have mutated the nucleotides in the dIR strain that are important in regulation. We propose that the inverted repeats mediate the effect of DNA supercoiling, possibly by extruding a cruciform, or bind a trans-acting factor that recognizes an alternative DNA secondary structure. This provides a molecular mechanism for our previous observation that relaxation of supercoiling induces ospC expression [42].

Finally, we are aware of only a handful of studies in which site-directed mutations were introduced in cis into the genome of B. burgdorferi [51,73,86,87] and this work provides an important caveat for interpreting genetic experiments involving introduction of DNA in trans, albeit the methodology is considerably more convenient. Our results add another level of complexity to ospC regulation suggesting that the DNA structure of the operator serves to mediate the external signals affecting expression.

Acknowledgments

We thank Christian Eggers for thoughtful and critical reading of the manuscript; Meghan Lybecke, Steve Lodmell, Aaron Bestor, Kit Tilly, Paul Policastro, Melissa Hargreaves, Amanda Brinkworth, and Cassie Abel Simonich for helpful discussions; and Tom Schwan for anti-FlaB antibodies and Mike Minnick for assistance generating anti-OspC antibodies.

Author Contributions

Conceived and designed the experiments: DD DSS. Performed the experiments: DD LSH LLH-H. Analyzed the data: DD DSS. Contributed reagents/materials/analysis tools: DD LLH-H LSH. Wrote the paper: DD DSS.

References

1. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, et al. (1982) Lyme disease—a tick-borne spirochetal disease? Science 216: 1317–1319.
2. Benach JL, Bolser EM, Hanrahan JP, Coleman JL, Bast TF, et al. (1983) Spirochetes isolated from the blood of two patients with Lyme disease. N Engl J Med 308: 740–742.
3. Steepe AC, Grodecki RL, Kornbluth AN, Craft JE, Barbour AG, et al. (1983) The spirochetal etiology of Lyme disease. N Engl J Med 309: 733–740.
4. Lane RS, Pesman J, Burgdorfer W (1991) Lyme borreliae: relation of its causative agent to its vectors and hosts in North America and Europe. Annu Rev Environ 16: 587–609.
5. Pesman J, Schwanz TG (2010) Ecology of borreliae and their arthropod vectors. In: Samuels DS, editors. Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Norfolk, UK: Caister Academic Press. 251–278.
6. Piesman J, Schwan TG (2010) Ecology of borreliae and their arthropod vectors. In: Samuels DS, editors. Borrelia: Molecular Biology, Host Interaction and Pathogenesis. Norfolk, UK: Caister Academic Press. 251–278.
7. Singh SK, Girschick HJ (2004) Molecular survival strategies of the Lyme disease spirochaete Borrelia burgdorferi. Lancet Infect Dis 4: 575–583.
8. Samuels DS (2011) Gene regulation in Borrelia burgdorferi. Annu Rev Microbiol 65: 479–499.
9. Schwan TG (2003) Temporal regulation of outer surface proteins of the Lyme disease spirochaete Borrelia burgdorferi. Biochem Soc Trans 31: 108–112.
10. Kemeny MR, Lehman TR, Akira DR (2012) The role of Borrelia burgdorferi outer surface proteins. FEMS Immunol Med Microbiol 66: 1–9.
11. Zhang J-R, Hardham JM, Barbour AG, Norris SJ (1997) Antigenic variation in Lyme disease borreliae by promiscuous recombination of VLP-like sequence cassettes. Cell 90: 2711–2725.
12. McDowell JV, Sung S-Y, Hu LT, Marconi RT (2012) Time of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochetes. Nat Rev Microbiol 10: 87–99.
13. Singh SK, Girschick HJ (2004) Molecular survival strategies of the Lyme disease spirochaete Borrelia burgdorferi. Lancet Infect Dis 4: 575–583.
14. Samuels DS (2011) Gene regulation in Borrelia burgdorferi. Annu Rev Microbiol 65: 479–499.
15. Schwanz TG (2003) Temporal regulation of outer surface proteins of the Lyme disease spirochaete Borrelia burgdorferi. Biochem Soc Trans 31: 108–112.
16. Kemeny MR, Lehman TR, Akira DR (2012) The role of Borrelia burgdorferi outer surface proteins. FEMS Immunol Med Microbiol 66: 1–9.
17. Zhang J-R, Hardham JM, Barbour AG, Norris SJ (1997) Antigenic variation in Lyme disease borreliae by promiscuous recombination of VLP-like sequence cassettes. Cell 90: 2711–2725.
18. McDowell JV, Sung S-Y, Hu LT, Marconi RT (2012) Time of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochetes. Nat Rev Microbiol 10: 87–99.
19. Singh SK, Girschick HJ (2004) Molecular survival strategies of the Lyme disease spirochaete Borrelia burgdorferi. Lancet Infect Dis 4: 575–583.
20. Brison D, Breckhah D, Eggers CH, Samuels DS (2012) Genetics of Borrelia burgdorferi. Annu Rev Genet 46: 515–536.
21. Pal U, da Silva AM, Montgomery RR, Fish D, Anuita J, et al. (2000) Attachment of Borrelia burgdorferi within Ixodes scapularis meditated by outer surface protein A. J Clin Invest 106: 561–569.
22. Pal U, Li X, Wang T, Montgomery RR, Ramamoorthy N, et al. (2004) TROSPA, an Ixodes scapularis receptor for Borrelia burgdorferi. Cell 119: 457–468.
23. Yang XF, Pal U, Alani SM, Fikrig E, Nougard MV (2004) Essential role for OspA/B in the life cycle of the Lyme disease spirochaete. J Exp Med 199: 641–648.
24. Battisti JM, Bono JL, Rosa PA, Schrumpf ME, Schwan TG, et al. (2000) Outer surface protein A protects Lyme disease spirochetes from acquired host immunity in the tick vector. Infect Immun 72: 5234–5237.
25. Fuchs R, Jauris S, Lottspeich F, Preus-Marcus V, Wilde B, et al. (1992) Molecular analysis and expression of a Borrelia burgdorferi gene encoding a 22 kDa protein (pC) in Esh Hoch. Mol Microbiol 6: 503–509.
26. Grunow D, Tilly K, Eggers CH, Newn PE, Krumm JG, et al. (2004) Outer-surface protein C of the Lyme disease spirochaete; a protein induced in ticks for infection of mammals. Proc Natl Acad Sci USA 101: 3142–3147.
27. Pal U, Yang X, Chen M, Bockemhled LK, Anderson JF, et al. (2004) OspC facilitates Borrelia burgdorferi invasion of Ixodes scapularis salivary glands. J Clin Invest 113: 220–230.
28. Tilly K, Bestor A, Jewett MW, Rosa P (2007) Rapid clearance of Lyme disease spirochetes lacking OspC from skin. Infect Immun 75: 1517–1519.
Ouyang Z, Blevins JS, Norgard MV (2008) Transcriptional interplay among the regulators Rrp2, RpoN and RpoS in Borrelia burgdorferi. Mol Microbiol 48: 1665–1677.

Carroll JA, Garon CF, Schwam TG (1999) Effects of environmental pH on membrane proteins in Borrelia burgdorferi. Infect Immun 67: 3181–3187.

Yang X, Goldberg MS, Popova TG, Schoeler GB, Wikel SK, et al. (2000) Interdependence of environmental factors influencing reciprocal patterns of gene expression in virulent Borrelia burgdorferi. Mol Microbiol 37: 1470–1479.

Seshu J, Boylan JA, Gherardini FC, Skare JT (2004) Dissolved oxygen levels alter gene expression and antigen profiles in Borrelia burgdorferi. Infect Immun 72: 1580–1586.

Hyde JA, Trzcinskiowski JP, Skare JT (2007) Borrelia burgdorferi alters its gene expression and antigenic profile in response to CO2 levels. J Bacteriol 189: 437–445.

Xu H, Caimano MJ, Lin T, He M, Radolf JD, et al. (2010) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.

Troxell B, Ye M, Yang Y, Caimano MJ, Lin T, He M, Radolf JD, et al. (2010) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.

Boon J, Elas AF, Kopko III J, Stevenson B, Tilly K, et al. (2000) Efficient targetted mutagenesis in Borrelia burgdorferi. J Bacteriol 182: 2445–2452.

Barbour AG (1984) Isolation and cultivation of Lyme disease spirochetes. Yale J Biol Med 57: 521–525.

Fisher MA, Grimm D, Henion AK, Elias AF, Stewart PE, et al. (2005) Identification of an ospC operator critical for immune evasion of Borrelia burgdorferi. Mol Microbiol 64: 220–231.

Bly A, Yang X, Nolen DM, Popova TG, Radolf JD, et al. (2004) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.

Bly A, Yang X, Nolen DM, Popova TG, Radolf JD, et al. (2004) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.

Bly A, Yang X, Nolen DM, Popova TG, Radolf JD, et al. (2004) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.

Bly A, Yang X, Nolen DM, Popova TG, Radolf JD, et al. (2004) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.

Bly A, Yang X, Nolen DM, Popova TG, Radolf JD, et al. (2004) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.

Bly A, Yang X, Nolen DM, Popova TG, Radolf JD, et al. (2004) Role of acetyl-phosphate in activation of the Rrp2-RpoN-RpoS pathway in Borrelia burgdorferi. PLoS Pathog 6: e1000114.