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Who is the BosR around here anyway?

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Summary

Borrelia burgdorferi encodes a novel DNA-binding protein in the Fur/PerR family of transcriptional regulators termed BosR (BB0647). This issue of *Molecular Microbiology* contains two molecular genetic studies that help to clarify the function of BB0647 and resolve longstanding controversies. Loss of BB0647 appears to have a pronounced effect on borrelian gene expression and, in one study, caused significant *in vitro* growth defects. BB0647 also was found to be essential for infection of the mammalian host but not the tick vector. Both Ouyang *et al.* and Hyde *et al.* also demonstrate, quite unexpectedly, that BB0647 is required for induction of RpoS, an alternative sigma factor that controls a cadre of *B. burgdorferi* genes, most notably *ospC*, that enable the spirochetes to establish mammalian infection following tick inoculation. There are still many unanswered questions regarding the precise physiological role of BB0647, the most important of which relate to its Fur/PerR namesakes: to what extent does it regulate either the response to oxidative stress and/or transition metal uptake? The mechanism(s) whereby BB0647 interfaces with the Rrp2-RpoN-RpoS pathway also remains to be discerned. However, these two seminal papers establish BB0647 (aka BosR) as a central player in the molecular biology and physiology of *B. burgdorferi* as well as the pathogenesis of Lyme disease.

Borrelia burgdorferi, the causative agent of Lyme disease, lives in either an arthropod vector or a vertebrate host, usually a small mammal. The spirochete must adapt to the diverse conditions of these two environments; these responses are regulated by sensing environmental signals and altering gene expression programs accordingly during both transmission to the mammal and acquisition by the tick. The borreliologist's worldview of gene regulation during the enzootic cycle (Singh and Girschick, 2004; Fikrig and Narasimhan, 2006; Radolf and Caimano, 2008) evolved from the seminal observation that temperature influences the reciprocal production of two dominant outer surface lipoproteins, OspA and OspC (Schwan *et al.*, 1995). Hübner *et al.* (2001) provided the key mechanistic explanation by demonstrating that the alternative sigma factor RpoS is required for transcription of genes required for mammalian infection, in particular *ospC* (Grimm *et al.*, 2004; Pal *et al.*, 2004b). The conceptual converse, revealed by Caimano *et al.* (2005), is that RpoS is required to repress genes, such as *ospA*, that are essential for the tick phase of the spirochete's enzootic cycle (Pal *et al.*, 2004a; Yang *et al.*, 2004), but does so only in the presence of as yet undefined mammalian host-specific factor(s). Thus, RpoS has been proposed to be a molecular "gatekeeper" regulating genes required for the phases of the enzootic cycle (Caimano *et al.*, 2007).

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While we have learned a great deal in the intervening years about the intricate transcriptional and antigenic changes that Lyme disease spirochetes undergo as they cycle back and forth between their arthropod and mammalian hosts, we still know comparatively little about the molecular mechanisms that govern these changes. One of the puzzling features of the *B. burgdorferi* genome is that it encodes very few recognizable transcriptional regulators (Fraser *et al.*, 1997). Among these is BB0647, which originally was annotated as a homolog of Ferric uptake regulator (Fur), the well-characterized, iron-activated global regulator found in numerous Gram-negative species (Lee and Helmann, 2007; Carpenter *et al.*, 2009). Work from the Gherardini group showing that *B. burgdorferi* abstains from using iron (Posey and Gherardini, 2000) suggested that the designation Fur might be a misnomer. Boylan *et al.* (2003) subsequently reported that the BB0647 protein sequence more closely resembles that of PerR, a transcriptional repressor of the peroxide regulon involved in the oxidative stress response of *Bacillus* spp. (Mongkolsuk and Helmann, 2002; Lee and Helmann, 2007). They showed that BB0647 binds *in vitro* to the promoter region of the *dps* gene (*napA*; *bb0690*), which encodes a protein required during the tick phase of the enzootic cycle that is believed to protect borrelial DNA from oxidative damage at the time of feeding (Li *et al.*, 2007). DNA binding requires both Zn^{2+} as the only metal cofactor and the reduction of BB0647, although, somewhat inexplicably, oxidation also enhances binding. Their footprinting data indicated that BB0647 binds to a site that, for either a Fur or PerR ortholog, is relatively large (50 nucleotides) and centered unprecedentedly far upstream of the gene (162 nucleotides in the case of *dps*). They also used a surrogate *Escherichia coli* reporter system to show that BB0647 activated transcription of *dps*. Based on these findings, they renamed the protein “BosR” for *Borrelia* oxidative stress regulator, postulating that it functions as a transcriptional activator in the spirochete’s response to oxidative stress. Subsequent work supported this nomenclature by showing that BB0647 is a transcriptional activator of two other genes involved in oxidative defense, *sodA*, encoding superoxide dismutase (Seshu *et al.*, 2004), and *cdr*, encoding coenzyme A disulphide reductase (Boylan *et al.*, 2006). On the other hand, Katona *et al.* (2004) contended, based on electrophoretic mobility shift assays, that BB0647 binds to the consensus Fur box from *Escherichia coli* and the consensus Per box from *Bacillus subtilis*, in addition to the promoter regions of its own gene and *dps*; in their hands, binding did not require metals and was, in fact, inhibited by Zn^{2+} or oxidizing agents. They also identified, by computer analysis, 26 genes in *B. burgdorferi* that had putative Per boxes in their promoter regions. Because the protein bound to consensus Fur and Per boxes and oxidation inhibited its binding activity, they also proposed that BB0647 regulates oxidative stress response genes, but as a repressor rather than an activator, and they chose to retain the Fur annotation. The discrepancies in these two pioneering publications regarding the regulatory activity of BB0647 (BosR/Fur), its requirement for a metal cofactor, and the effect of oxidizing agents on its ability to bind DNA went unaddressed for a considerable period. The absence of iron in *B. burgdorferi* (Posey and Gherardini, 2000) means that BB0647 mechanistically is unlike Fur or PerR: Fur utilizes Fe^{2+} to bind DNA and PerR utilizes Fe^{2+} for metal-catalyzed oxidation, which is required for gene derepression (Lee and Helmann, 2007; Imlay, 2008; Giedroc, 2009). Another strategy for sensing peroxide, via thiol oxidation, is found in OxyR, a member of the LysR family and the functional equivalent of PerR in *E. coli* (Lee and Helmann, 2007; Imlay, 2008), although it lacks homology to BB0647. Clearly, a genetic approach was warranted to dissect the function of BB0647.

Despite monumental advances in our ability to genetically manipulate the spirochete in recent years (Rosa *et al.*, 2005; Samuels, 2006), *bb0647* defied exhaustive attempts of researchers to inactivate it. The first breakthrough came when Seshu *et al.* (2004) generated a null mutation in a high-passage non-infectious strain. A serious caveat to this study, however, is that *bb0647* from the “wild-type” parental strain had a point mutation that resulted in a conservative substitution of an arginine to a lysine (R39K); this single amino

acid change had a profound effect on the phenotype with respect to sensitivity to oxidative stress. Thus, the function of BB0647 *in vivo* remained elusive.

Two studies in this issue of *Molecular Microbiology* demonstrate that the genetic barrier for studying BB0647 has been successfully breached and that rapid progress can ensue towards understanding its function and mode of activity. The Norgard laboratory (Ouyang *et al.*, 2009b) and the Skare laboratory (Hyde *et al.*, 2009) have generated null mutations of *bb0647* in low-passage infectious backgrounds: experimental coups d'état. In addition, a recent report in another journal by one of these groups yielded similar results with an artificially inducible *bb0647* construct (Hyde *et al.*, 2010). The surprising and thought-provoking finding in both of these studies is that BB0647 influences the synthesis of RpoS and the expression of its regulon.

Ouyang *et al.* (2009b) demonstrated that *bb0647* is required for transmission and mammalian infection, but not tick infection, which makes sense as *rpoS* is not expressed in flat ticks (Caimano *et al.*, 2007). These authors also surveyed the genes regulated by BB0647 using a transcriptomic approach. Many of the BB0647-dependent genes were previously known to be RpoS-dependent; the most notable being *ospC*, the expression of which was decreased almost fortyfold in the mutant background. However, 50 BB0647-regulated genes were found to be RpoS-independent; these include some genes previously implicated in borrelial infectivity and eight of the 26 genes with putative Per boxes as identified by Katona *et al.* (2004). Loss of BB0647 had no effect on the levels of *dps* and *sodA* mRNA (i.e., less than twofold by qRT-PCR) despite the prior *in vitro* binding results (Boylan *et al.*, 2003; Katona *et al.*, 2004; Seshu *et al.*, 2004). Ouyang *et al.* (2009b) hypothesize that BB0647 influences RpoS levels either by directly binding to the *rpoS* promoter region or by stimulating phosphorylation of Rrp2, the response regulator that activates the RpoN-RpoS pathway (Yang *et al.*, 2003; Burtnick *et al.*, 2007; Boardman *et al.*, 2008; Ouyang *et al.*, 2008).

Hyde *et al.* (2009) also demonstrated a lack of RpoS induction as well as a loss of infectivity in mammals for the *bb0647* mutant. In addition, they showed that the *bb0647* mutant has a growth phenotype: the mutant reaches stationary phase at a lower cell density than wild type under microaerobic conditions (3.5 ppm O₂ and 1% CO₂) and has a longer lag phase under anaerobic conditions (<0.1 ppm O₂ and 5% CO₂). They also found that BB0647 activates the synthesis of Dps (NapA) and Cdr, but surprisingly not SodA, which protects against oxidative stress (Esteve-Gassent *et al.*, 2009). Enigmatically, in their study, BB0647 is synthesized at higher levels in the anaerobic compared to microaerobic cultures, a result that is hard to reconcile with their finding that spirochetes lacking BB0647 were approximately fourfold more susceptible to peroxide-mediated killing than wild type. They hypothesized that BB0647 senses the redox state, which they proposed varies between the tick and the mammal.

There are some noteworthy discrepancies between these two studies that need to be ironed out. However, directly comparing the gene expression results is complicated by the differences in growth conditions and expression assays. Ouyang *et al.* (2009b) grew their spirochetes at 37°C in 5% CO₂ whereas Hyde *et al.* (2009) grew theirs at 32°C in either 1% CO₂ (microaerobic) or 5% CO₂ with 3% H₂ (anaerobic). In particular, Ouyang *et al.* (2009b) induced RpoS with increased culture temperature (37°C), the classical *in vitro* cue (Schwan *et al.*, 1995), whereas Hyde *et al.* (2009) induced RpoS with CO₂ (Hyde *et al.*, 2007). Nevertheless, previous results from other laboratories using DNA-binding and heterologous reporter methods (Boylan *et al.*, 2003; Katona *et al.*, 2004) are supportive of Hyde *et al.* (2009) that BB0647 activates expression of *dps*. Perhaps the most striking disagreement between the two studies is that Hyde *et al.* (2009), but not Ouyang *et al.*

(2009b), note a growth defect of their respective *bb0647* mutant both microaerobically and anaerobically. The reason could be the slightly different strategies for mutagenesis (the selectable markers are in opposite orientations) or, more likely, the aforementioned different growth conditions. The important caveat here, and for all *in vitro* approaches, is that none of these are the actual conditions in which *B. burgdorferi* lives, which is a tick or a mammal. This is not to say that expression data obtained *in vitro* are without value, but there are well-documented instances in which the *in vitro* expression profiles of *B. burgdorferi* genes do not replicate those observed either in mammals or ticks (Cugini *et al.*, 2003; Mulay *et al.*, 2009).

So, the proverbial jury is still out on whether or not BB0647 controls an oxidative stress response regulon, including *dps*, and whether it plays any role in regulating transition metal homeostasis in *B. burgdorferi*. The latter issue is complicated by the unusual metal requirements of the spirochete along with an unfathomable repertoire of metal transporters that, with one notable exception (Ouyang *et al.*, 2009a), have mostly escaped detection by sequence gazing (Saier and Paulsen, 2000). BosR has become the accepted and widely used designation for BB0647, but how accurately this name depicts the function of this regulator in borrelian physiology is not yet clear from the currently available data. Structural analysis should help greatly in this regard. From the microarray data, BB0647 undeniably activates many genes and represses others (Hyde *et al.*, 2006; Ouyang *et al.*, 2009b). Curiously, Ouyang *et al.* (2009b) deployed neither a bioinformatics approach to analyze potential binding sites in the genome nor experimental assays of DNA-binding activity. The question therefore remains: how does BB0647 regulate these genes, specifically can BB0647 bind to operators of genes other than *dps*, *sodA*, *cdr*, and *oppA-IV* (Boylan *et al.*, 2003; Katona *et al.*, 2004; Seshu *et al.*, 2004; Boylan *et al.*, 2006; Medrano *et al.*, 2007)? Future work also undoubtedly will seek to decipher the mechanism by which BB0647 activates RpoS and the host milieu in which these two regulatory pathways intersect and overlap. RpoS regulation has already proven to be remarkably complex and versatile, entailing a second alternative sigma factor (Hubner *et al.*, 2001; Fisher *et al.*, 2005), an enhancer-binding protein/two-component signaling system (Yang *et al.*, 2003; Burtnick *et al.*, 2007; Boardman *et al.*, 2008; Ouyang *et al.*, 2008), and a small noncoding RNA (Lybecker and Samuels, 2007). Although Medrano *et al.* (2007) showed that *bb0647* was expressed at the highest level when *B. burgdorferi* had colonized the mammal, further research is required to determine the mechanics and kinetics of BB0647 activation, including the role of the metal cofactor *in vivo*. These two newsworthy manuscripts in this issue (Hyde *et al.*, 2009; Ouyang *et al.*, 2009b) have revealed a missing link in the regulatory circuitry that controls the transit of *B. burgdorferi* through its enzootic cycle and have thus laid the groundwork for defining the function of this intriguing transcription factor, hinting at a crucial connection between borrelian physiology and disease pathogenesis. They also underscore what is increasingly recognized in the Lyme disease field: namely that *B. burgdorferi* has developed, over evolutionary time, an impressive capacity to appropriate prototypical transcriptional regulators of model organisms and exploit them for its own paradigm-bending purposes in pursuit of its unique parasitic lifestyle.

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