Rrp2, a σ^{54}-Dependent Transcriptional Activator of *Borrelia burgdorferi*, Activates *rpoS* in an Enhancer-Independent Manner[∇][†]

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Rrp2 is the sole σ^{54}-dependent transcriptional activator present in the *Borrelia burgdorferi* genome. We showed that recombinant Rrp2 binds to DNA in a sequence-nonspecific manner. During infection, Rrp2 activates σ^{54}-dependent *rpoS* expression without an apparent upstream enhancer element commonly associated with other σ^{54}-dependent transcriptional activators.

Dramatic alteration of surface lipoprotein profiles is a key strategy which the Lyme disease pathogen *Borrelia burgdorferi* has evolved to persist within its natural enzootic cycle (19, 20). The Rrp2-RpoN-RpoS pathway (or the σ^{54}-σ^{S} cascade) plays a central role in modulating the differential gene expression involved in spirochete transmission from the arthropod vector (e.g., *Ixodes* species ticks) to a mammalian host (e.g., the white-footed mouse) (5, 6, 8, 10, 12, 25). In this pathway, the two-component response regulator Rrp2 acts in concert with the sigma factor RpoN (σ^{54} or σ^{N}) to directly control the production of another sigma factor, RpoS (σ^{S} or σ^{38}), which in turn governs expression of more than 10% of the genes annotated in *B. burgdorferi* (3, 6, 8, 14). This pathway appears to be activated in the spirochete at the onset of nymphal feeding and remains operative as the bacteria replicate in the mammal (6). Therefore, elucidating the mechanism by which the Rrp2-RpoN-RpoS pathway is activated is important to the understanding of host adaptation of *B. burgdorferi*.

σ^{54} is a unique alternative sigma factor which differs from the members of the σ^{70} family both in amino acid sequence and in transcription mechanism (13). σ^{54} recognizes a conserved $-24/-12$ promoter sequence, and the activation of the σ^{54}-polymerase holoenzyme requires specialized bacterial enhancer-binding proteins (EBP) (22). All members of the EBP family contain a highly conserved activation domain that interacts with σ^{54} for transcriptional activation. This domain also possesses ATPase activity that is essential for their activating function (23). In addition, a typical EBP contains a DNA-binding domain that binds to enhancer-like DNA elements and allows transcriptional activation far from the σ^{54} promoter (0.1 to 1.0 kb) via a DNA-looping mechanism (15, 17).

Whereas most bacterial genomes encode multiple EBP, the *B. burgdorferi* genome has only one predicted EBP, Rrp2 (9,

25). Rrp2 is an NtrC-like EBP which is comprised of an amino-terminal response regulator domain, a central σ^{54} activation domain, and a carboxy-terminal helix-turn-helix (HTH)-type DNA-binding domain (25). Like other EBP, Rrp2 was thought to activate *rpoS* transcription by binding to an enhancer site in the region of the *rpoS* gene (25). Surprisingly, a recent article by Burtnick et al. (5) reported that a *cat* transcriptional reporter containing only the minimal $-24/-12$ σ^{54}-type promoter of *rpoS* (with an additional 17 bp of upstream sequence) can be activated by endogenous Rrp2 in *B. burgdorferi*, suggesting that Rrp2 may be an unusual EBP which does not require an enhancer site for activating σ^{54}-dependent transcription. These observations raise several key questions. In particular, is the *rpoS* gene with the minimal promoter sufficient to fulfill the essential role of RpoS in mammalian infection by *B. burgdorferi*? If so, what is the function of Rrp2's putative DNA-binding domain?

***rpoS* expressed from its minimal promoter is sufficient for the production of RpoS, OspC, and DbpA.** To study the mechanism of Rrp2-dependent activation of *rpoS*, a previously described *rpoS* mutant of *B. burgdorferi* strain 297 (14) was complemented with the pJD44 shuttle vector (modified from pBSV2 [18]) carrying various *rpoS* regions (Fig. 1A): *rpoS* with more than 2 kb of flanking DNA (pJSB259), *rpoS* with the $-24/-12$ σ^{54}-type promoter but no flanking regions (pJSB296), or *rpoS* with a defective $-24/-12$ promoter (mutation of G-24T) (pJSB298) (18, 21). The primers used to amplify these regions are described in Table S1 of the supplemental material. The abilities of these *rpoS* constructs to restore the production of RpoS and the RpoS-dependent virulence factors OspC and DbpA in the *rpoS* mutant were then examined by performing immunoblot analyses on bacteria grown to postexponential phase in pH-adjusted medium (pH 6.8). The antibodies used in the immunoblot analyses have been previously described (2, 21).

As expected, complementation of the *rpoS* mutant with the construct carrying *rpoS* and 1,171 bp of contiguous upstream DNA (pJSB259), which contains the native $-24/-12$ region, restored production of RpoS, OspC, and DbpA (Fig. 1B). More importantly, the *rpoS* mutant complemented with *rpoS* expressed from only the minimal σ^{54}-dependent promoter
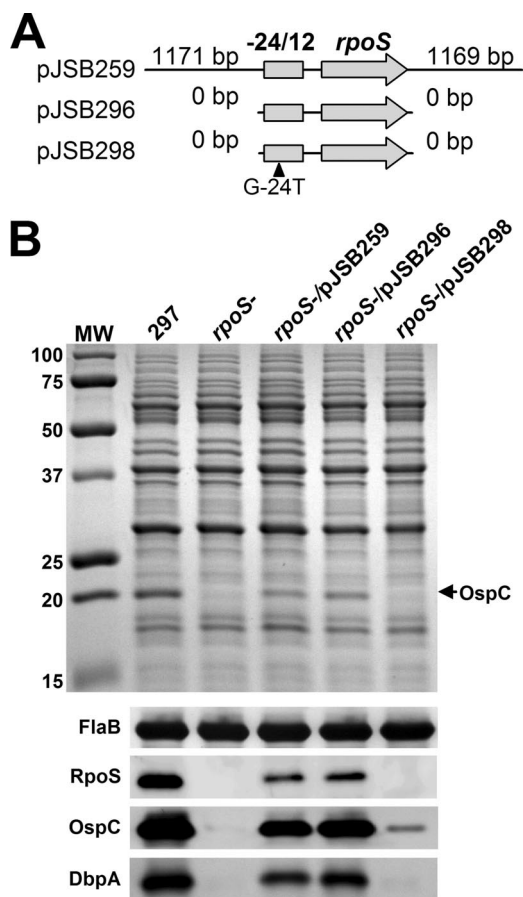
FIG. 1. Complementation of the *rpoS* mutant with a shuttle vector carrying various versions of the *rpoS* gene. (A) Diagram of the three shuttle vectors. Only the *rpoS* portion in each vector is shown. (B) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Coomassie blue stain) (top) and immunoblot assay (bottom) of whole-cell lysates of *B. burgdorferi* strains (labeled on top). Numbers at the left denote protein molecular mass markers (in kDa). The arrow at the right indicates the band corresponding to OspC. Antibodies used to detect the respective proteins are indicated to the left of the immunoblot results shown at the bottom of the panel.

(pJSB296) exhibited RpoS, OspC, and DbpA expression levels comparable to those observed in the pJSB259-complemented strain (Fig. 1B). In contrast, complementation with the construct carrying *rpoS* with the G-24T-mutated σ^{54}-type promoter (pJSB298) failed to restore expression of RpoS, OspC, and DbpA (Fig. 1B). Note that strains complemented with pJSB259 or pJSB296 exhibited levels of RpoS expression that were lower than those observed in the wild-type strain 297; this is likely due to the differences in the DNA topology of the plasmid-encoded and chromosomally encoded copies of *rpoS*. Alternatively, a possible difference in mRNA stability between the transcripts derived from the two forms of *rpoS* genes may contribute to the observed variation. It also is worth noting that OspC was detected in the *rpoS* mutant carrying pJSB298, albeit at a significantly reduced level. This presumably is due to the fact that the G-24T mutation in the promoter did not completely abolish *rpoS* expression, and therefore a very low level of RpoN-mediated *rpoS* transcription still occurred in this

strain (21). Nevertheless, these results support the conclusion, based on the data from the transcriptional reporter system employed by Burtnick et al. (5), that Rrp2 is capable of activating σ^{54}-dependent *rpoS* transcription in the absence of a specific upstream DNA *cis*-element under in vitro growth conditions.

***rpoS* with the σ^{54} minimal promoter is sufficient to restore infectivity of the *rpoS* mutant in mice.** To determine whether *rpoS* with only the σ^{54} minimal promoter is sufficient for mammalian infection, groups of five C3H/HeN mice were infected via intradermal needle inoculation with 10, 10^2, 10^3, 10^4, 10^5, or 10^6 spirochetes of wild-type strain 297, the *rpoS* mutant, or the *rpoS* mutant carrying pJSB296 (*rpoS* with the minimal promoter region). At 15 days postinoculation, mouse infection was assessed by culturing spirochetes from ear punch biopsy tissues. Whereas the *rpoS* mutant was completely avirulent in mice, the *rpoS* mutant complemented with pJSB296 had a 50% infectious dose (ID_{50}) value similar to the wild-type strain (Table 1). The *rpoS* mutant harboring pJSB296 also was able to cause chronic infection equivalent to the wild-type strain (examined at 28 weeks after infection) (data not shown). These results suggest that Rrp2, in conjunction with RpoN, can activate *rpoS* expression in the absence of an upstream enhancer under physiological conditions, and such activation is sufficient for spirochetes to establish and maintain infection in mice.

Properties of the recombinant Rrp2 proteins. To determine whether the predicted DNA-binding domain of Rrp2 is capable of binding DNA, we produced recombinant maltose-binding proteins (MBP) fused with either full-length Rrp2 (MBP-Rrp2) or amino acids 391 to 453 of the Rrp2 C-terminal domain (MBP-Rrp2Ct) using the pMAL system (New England Biolabs, Ipswich, MA). Recombinant proteins were expressed in *Escherichia coli* and sequentially purified from cell lysates using amylose-resin and heparin-Sepharose. Both MBP-Rrp2 and MBP-Rrp2Ct were capable of binding to heparin (data not shown), suggesting that the full-length and C-terminal recombinant Rrp2 protein might be able to bind DNA. Recombinant proteins were then further purified using a Mono Q HR5/5 ion exchange column (GE Healthcare, Chalfont St. Giles, United Kingdom). Purified MBP-Rrp2 and MBP-Rrp2Ct were soluble in solution, and chromatographic gel filtration analyses indicated that both recombinant proteins formed dimers in solution (data not shown). This latter observation suggests that one of the functions of the C-terminal domain (CTD) of Rrp2 likely is dimerization.

To further assess the physiological activities of the purified recombinant MBP-Rrp2 protein, we sought to determine

TABLE 1. The *rpoS* gene with a minimal $-24/-12$ σ^{54}-type promoter is sufficient to restore infectivity of the *rpoS* mutant in mice[a]

Strain or phenotype	No. infected/no. inoculated with indicated no. of bacteria						ID_{50}
	10^1	10^2	10^3	10^4	10^5	10^6	
Wild type	0/6	1/8	8/8	8/8	ND	ND	239
RpoS$^-$	ND	ND	ND	0/8	0/8	0/8	>10^6
RpoS$^-$/pJSB296	1/6	4/8	8/8	8/8	ND	ND	87

[a] The ID_{50} values were calculated using the method described by Reed and Muench (16). ND, not determined.

A Rrp2-Ct

0 170 500 1500 4600

B Rrp2-FL

0 6 19 56 170 500

C Rrp2-FL

0 6 19 56 170 500

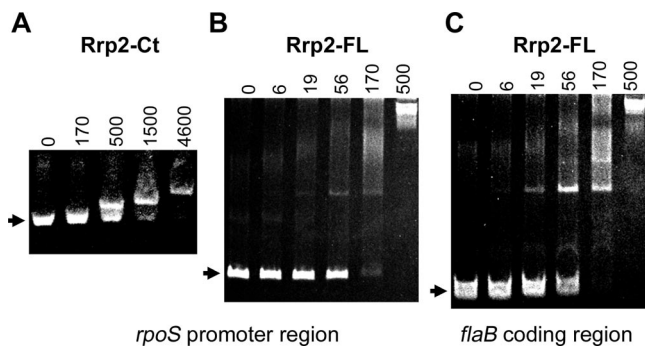*rpoS* promoter region   *flaB* coding region

FIG. 2. Electrophoretic mobility shift analyses of the DNA-binding activity of Rrp2. Various concentrations of recombinant C-terminal Rrp2 (MBP-Rrp2Ct) or full-length Rrp2 (MBP-Rrp2) (labeled on the top, in nM) were incubated with DNA fragments from either the *rpoS* promoter region (A and B) or the *flaB* coding region (labeled at the bottom) (C). Protein-DNA complexes were resolved from unbound DNA fragments on polyacrylamide gels and stained with ethidium bromide (0.5 μg/ml). The arrows indicate the positions of unbound DNA fragments.

whether purified recombinant MBP-Rrp2 protein had ATPase activity, as all σ^{54}-dependent regulators hydrolyze ATP when activated. Rrp2 is predicted to be activated by phosphorylation of its N-terminal receiver domain. It has been reported that BeF_3, but not $BeCl_2$ or NaF, mimics the Asp-phosphate of a receiver domain that activates NtrC and other response regulators (24). Therefore, MBP-Rrp2 (1.4 mM) was incubated in HEPES buffer (pH 7.3) with 0.8 mM ATP and 200 μM BeF_3, $BeCl_2$ or NaF at 37°C for 90 min. ATPase activities were measured by monitoring the release of P_i using the PiPer phosphate assay kit (Molecular Probes, Eugene, OR). We found that there was a fivefold increase in P_i release upon addition of BeF_3, but not $BeCl_2$ or NaF, to the reaction mixture (data not shown). This observation suggests that Rrp2 has phosphorylation-dependent ATPase activity.

Rrp2 binds DNA in a sequence-nonspecific fashion. The DNA-binding capacities of recombinant MBP-Rrp2 and MBP-Rrp2Ct were assessed using electrophoresis mobility shift assays. Reaction mixtures containing 10 nM of a 370-bp DNA fragment of the *rpoS* promoter region (200 bp upstream and 170 bp downstream of ATG), 50 mM HEPES (pH 7.5), 8 mM $MgCl_2$, 1 mM dithiothreitol, 10% glycerol, 2 μg/ml poly(dI-dC), and increasing amounts of protein were incubated at room temperature for 20 min. Protein-DNA complexes were resolved from unbound probe by electrophoresis on 5% poly-acrylamide–Tris-glycine gels. As shown in Fig. 2A and B, both the C-terminal domain and the full length of Rrp2 were capable of binding to the *rpoS* promoter region. The minimal concentration of full-length Rrp2 protein required to achieve maximum DNA binding in the presence of competitor poly(dI-dC) was 0.5 μM, which is comparable to that of recombinant *E. coli* NtrC binding to a single *glnAp2* site (0.25 μM) (7, 26). There appeared to be a difference in relative binding activities of MBP-Rrp2 and MBP-Rrp2Ct (approximately 10-fold), suggesting that other domains in Rrp2 may enhance the interaction of the C-terminal domain with DNA. Furthermore, phosphorylation of Rrp2 did not alter the DNA-binding pattern of Rrp2 (data not shown). Interestingly, the DNA-binding activity

of Rrp2 was not sequence specific, because Rrp2 also bound to a 227-bp DNA fragment of the *flaB* coding region (200 bp downstream of ATG) (Fig. 2C) or a 214-bp DNA fragment of the *kat* gene from *Neisseria gonorrhoeae* (data not shown).

Although the promiscuous DNA-binding activity of Rrp2 in vitro might be interpreted as a nonspecific interaction, several lines of evidence support the conclusion that Rrp2 possesses DNA-binding activity. First, Rrp2 has a predicted HTH DNA-binding domain (25). Second, recombinant Rrp2 binds heparin. Third, both recombinant full-length Rrp2 and the Rrp2 C-terminal domain bind to DNA in the presence of the non-specific competitor poly(dI-dC). Lastly, the relative DNA-binding affinity of full-length recombinant Rrp2 is comparable to that of recombinant *E. coli* NtrC binding to a single site (7, 26). Taken together, these results suggest that, although a specific enhancer-binding site is not required upstream of the $-24/-12$ region for Rrp2 to activate *rpoS* transcription, the predicted C-terminal domain of Rrp2 indeed possesses DNA-binding activity and that the interaction with DNA does not appear to be dependent upon a specific DNA sequence.

In an effort to determine the contribution of the DNA-binding activity of Rrp2 to transcriptional activation, we sought to take a genetic approach by constructing an *rrp2 B. burgdorferi* mutant strain that expressed either Rrp2 lacking the entire C-terminal domain of Rrp2 or Rrp2 with a truncated HTH DNA-binding motif. Following multiple rounds of transformation and recombination of the mutant construct into *B. burgdorferi*, no clones have been identified which carry a mutated *rrp2* allele that is missing either the entire CTD or the last 10 amino acids (corresponding to the last α-helix of the HTH motif). Because an analogous approach was used to generate our previously described Rrp2 G239C mutant of *B. burgdorferi* (3, 27), the inability to generate a strain carrying the CTD truncation or HTH mutation suggests that the CTD may provide a function that is essential for cell survival. This notion is consistent with the previous observation that deletion of *rrp2* appears to be lethal (5, 25).

In conclusion, the results from this study along with previous findings suggest that the *rpoS* gene, containing only the minimal σ^{54}-type promoter, is essential and sufficient to produce RpoS for mammalian infection of *B. burgdorferi*. This notion further supports the hypothesis that Rrp2 is an unusual σ^{54}-dependent activator that does not require an enhancer site for transcriptional activation. To this end, recent genomic sequence analyses have identified a group of σ^{54}-dependent activators that lack an apparent DNA-binding domain: FlgR in *Helicobacter pylori*, FlgR in *Campylobacter jejuni*, and CtcC in *Chlamydia trachomatis* (1, 11). Subsequent experimentation has confirmed that the FlgR proteins of *H. pylori* and *C. jejuni* are functional activators of σ^{54}-dependent genes (1, 4, 11). One common theme among FlgR, CtcC, and Rrp2 is that they are the sole σ^{54}-dependent activator present in each corresponding genome and, therefore, are responsible for activating all genes with a σ^{54}-type promoter in that genome. As a result, no enhancer site is required for these activators to discriminate σ^{54}-dependent genes for activation within the cell. The mechanism by which these activators that do not bind an enhancer site are recruited to σ^{54}-dependent promoter regions is not clear. One possibility is that they are capable of interacting with unbound σ^{54} prior to activation (e.g., in solution) and are

subsequently recruited to the promoter site by σ^{54}. However, Rrp2 has the predicted C-terminal DNA-binding domain that is not found in FlgR and CtcC. The sequence-nonspecific DNA-binding nature may be advantageous to Rrp2-dependent activation, because once recruited to the promoter by σ^{54}, Rrp2 would be capable of binding to any DNA sequence adjacent to the $-24/-12$ promoter site, which provides further stabilization to the Rrp2-σ^{54}-holoenzyme-DNA complex for transcriptional activation. One caveat of this study is that, despite intense efforts, the mutation of the C-terminal domain of Rrp2 to assess its role in the activation of *rpoS* transcription could not be achieved, potentially as a result of the essential nature of this domain to spirochetal survival. Further work, possibly through in vitro transcription analyses, is needed to study the role of the DNA-binding function of Rrp2 in transcriptional activation and the mechanism of recruitment of Rrp2 to the *rpoS* promoter region, which will provide insight into this novel form of σ^{54}-dependent transcriptional activation.

REFERENCES

1. Beck, L. L., T. G. Smith, and T. R. Hoover. 2007. Look, no hands! Unconventional transcriptional activators in bacteria. Trends Microbiol. **15:**530–537.
2. Blevins, J., K. E. Hagman, and M. V. Norgard. 2008. Assessment of decorin-binding protein A to the infectivity of *Borrelia burgdorferi* in the murine models of needle and tick infection. BMC Microbiol. **8:**82.
3. Boardman, B. K., M. He, Z. Ouyang, H. Xu, X. Pang, and X. F. Yang. 2008. Essential role of the response regulator Rrp2 in the infectious cycle of *Borrelia burgdorferi*. Infect. Immun. **76:**3844–3853.
4. Brahmachary, P., M. G. Dashti, J. W. Olson, and T. R. Hoover. 2004. *Helicobacter pylori* FlgR is an enhancer-independent activator of σ^{54}-RNA polymerase holoenzyme. J. Bacteriol. **186:**4535–4542.
5. Burtnick, M. N., J. S. Downey, P. J. Brett, J. A. Boylan, J. G. Frye, T. R. Hoover, and F. C. Gherardini. 2007. Insights into the complex regulation of *rpoS* in *Borrelia burgdorferi*. Mol. Microbiol. **65:**277–293.
6. Caimano, M. J., R. Iyer, C. H. Eggers, C. Gonzalez, E. A. Morton, M. A. Gilbert, I. Schwartz, and J. D. Radolf. 2007. Analysis of the RpoS regulon in *Borrelia burgdorferi* in response to mammalian host signals provides insight into RpoS function during the enzootic cycle. Mol. Microbiol. **65:**1193–1217.
7. Chen, P., and L. J. Reitzer. 1995. Active contribution of two domains to cooperative DNA binding of the enhancer-binding protein nitrogen regulator I (NtrC) of *Escherichia coli*: stimulation by phosphorylation and the binding of ATP. J. Bacteriol. **177:**2490–2496.
8. Fisher, M. A., D. Grimm, A. K. Henion, A. F. Elias, P. E. Stewart, P. A. Rosa, and F. C. Gherardini. 2005. *Borrelia burgdorferi* σ^{54} is required for mammalian infection and vector transmission but not for tick colonization. Proc. Natl. Acad. Sci. USA **102:**5162–5167.
9. Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J.-F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. Weidman, T. Utterback, L. Watthey, L. McDonald, P. Artiach, C. Bowman, S. Garland, C. Fujii, M. D. Cotton, K. Horst, K. Roberts, B. Hatch, H. O. Smith, and J. C. Venter. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature **390:**580–586.
10. Hubner, A., X. Yang, D. M. Nolen, T. G. Popova, F. C. Cabello, and M. V. Norgard. 2001. Expression of *Borrelia burgdorferi* OspC and DbpA is controlled by a RpoN-RpoS regulatory pathway. Proc. Natl. Acad. Sci. USA **98:**12724–12729.
11. Joslin, S. N., and D. R. Hendrixson. 2008. Analysis of the *Campylobacter jejuni* FlgR response regulator suggests integration of diverse mechanisms to activate an NtrC-Like protein. J. Bacteriol. **190:**2422–2433.
12. Lybecker, M. C., and D. S. Samuels. 2007. Temperature-induced regulation of RpoS by a small RNA in *Borrelia burgdorferi*. Mol. Microbiol. **64:**1075–1089.
13. Merrick, M. J. 1993. In a class of its own: the RNA polymerase sigma factor σ^N (σ^{54}). Mol. Microbiol. **10:**903–909.
14. Ouyang, Z., J. S. Blevins, and M. V. Norgard. 2008. Transcriptional interplay among the regulators Rrp2, RpoN, and RpoS in *Borrelia burgdorferi*. Microbiology **154:**2641–2658.
15. Popham, D. L., D. Szeto, J. Keener, and S. Kustu. 1989. Function of a bacterial activator protein that binds to transcriptional enhancers. Science **243:**629–635.
16. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27:**493–497.
17. Reitzer, L. J., and B. Magasanik. 1986. Transcription of *glnA* in *E. coli* is stimulated by activator bound to sites far from the promoter. Cell **45:**785–792.
18. Revel, A. T., J. S. Blevins, C. Almazan, L. Neil, K. M. Kocan, J. de la Fuente, K. E. Hagman, and M. V. Norgard. 2005. *bptA* (*bbe16*) is essential for the persistence of the Lyme disease spirochete, *Borrelia burgdorferi*, in its natural tick vector. Proc. Natl. Acad. Sci. USA **102:**6972–6977.
19. Rosa, P. A., K. Tilly, and P. E. Stewart. 2005. The burgeoning molecular genetics of the Lyme disease spirochaete. Nat. Rev. Microbiol. **3:**129–143.
20. Singh, S. K., and H. J. Girschick. 2004. Molecular survival strategies of the Lyme disease spirochete *Borrelia burgdorferi*. Lancet Infect. Dis. **4:**575–583.
21. Smith, A. H., J. S. Blevins, G. N. Bachlani, X. F. Yang, and M. V. Norgard. 2007. Evidence that RpoS (σ^S) in *Borrelia burgdorferi* is controlled directly by RpoN (σ^{54}/σ^N). J. Bacteriol. **189:**2139–2144.
22. Studholme, D. J., and R. Dixon. 2003. Domain architectures of σ^{54}-dependent transcriptional activators. J. Bacteriol. **185:**1757–1767.
23. Wedel, A., and S. Kustu. 1995. The bacterial enhancer-binding protein NtrC is a molecular machine: ATP hydrolysis is coupled to transcriptional activation. Genes Dev. **9:**2042–2052.
24. Yan, D., H. S. Cho, C. A. Hastings, M. M. Igo, S. Y. Lee, J. G. Pelton, V. Stewart, D. E. Wemmer, and S. Kustu. 1999. Beryllofluoride mimics phosphorylation of NtrC and other bacterial response regulators. Proc. Natl. Acad. Sci. USA **96:**14789–14794.
25. Yang, X. F., S. M. Alani, and M. V. Norgard. 2003. The response regulator Rrp2 is essential for the expression of major membrane lipoproteins in *Borrelia burgdorferi*. Proc. Natl. Acad. Sci. USA **100:**11001–11006.
26. Yang, X. F., Y. Ji, B. L. Schneider, and L. Reitzer. 2004. Phosphorylation-independent dimer-dimer interactions by the enhancer-binding activator NtrC of *Escherichia coli*: a third function for the C-terminal domain. J. Biol. Chem. **279:**36708–36714.
27. Yang, X. F., U. Pal, S. M. Alani, E. Fikrig, and M. V. Norgard. 2004. Essential role for OspA/B in the life cycle of the Lyme disease spirochete. J. Exp. Med. **199:**641–648.