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Alternate Sigma Factor RpoS Is Required for the In Vivo-Specific Repression of *Borrelia burgdorferi* Plasmid lp54-Borne *ospA* and *lp6.6* Genes

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While numerous positively regulated loci have been characterized during the enzootic cycle of *Borrelia burgdorferi*, very little is known about the mechanism(s) involved in the repression of borrelial loci either during tick feeding or within the mammalian host. Here, we report that the alternative sigma factor RpoS is required for the in vivo-specific repression of at least two RpoD-dependent *B. burgdorferi* loci, *ospA* and *lp6.6*. The downregulation of *ospA* and *lp6.6* appears to require either a repressor molecule whose expression is RpoS dependent or an accessory factor which enables RpoS to directly interact with the *ospA* and *lp6.6* promoter elements, thereby blocking transcription by RpoD. The central role for RpoS during the earliest stages of host adaptation suggests that tick feeding imparts signals to spirochetes that trigger the RpoS-dependent repression, as well as expression, of in vivo-specific virulence factors critical for the tick-to-mammalian host transition.

In order to be sustained within its enzootic life cycle, *Borrelia burgdorferi*, the causative agent of Lyme disease, must be able to adapt to the nutritional, physiological, and immunological stresses associated with growth within two strikingly different milieus, the *Ixodes* tick vector and the mammalian host. Presumably, *B. burgdorferi* accomplishes this by being able to sense signaling cues associated with tick feeding and growth within the host. The response to these signals, in turn, triggers the expression of factors that enable spirochetes to migrate from the tick midgut to the salivary glands and then, ultimately, to disseminate from the tick feeding site to host target tissues. Not unexpectedly, the entry of a bloodmeal into a *B. burgdorferi*-infected tick is accompanied by extensive changes in the borrelial transcriptome and proteome, a process referred to as mammalian host adaptation (1, 4, 43). While a number of studies have demonstrated that the expression of many borrelial loci can be modulated by the manipulation of in vitro growth conditions (1, 2, 5, 8, 10, 11, 17, 27, 32, 41, 48, 51, 58, 60, 63–65, 68), we and others have shown that as-yet-undefined mammalian host-specific cues are essential for triggering the genetic program(s) underlying host adaptation (1, 2, 8, 18, 27, 32, 46, 49, 51, 52, 63, 64, 66).

The majority of previously published reports have focused on increased borrelial gene expression both in vitro and in the context of the mammalian host. Recent microarray analyses, however, suggest that the ability of *B. burgdorferi* to repress the expression of select loci may be equally critical to host adaptation and disease pathogenesis (8, 21, 38, 39, 51, 61). The contributions of transcriptional induction and repression in *B. burgdorferi* are well exemplified by the reciprocal synthesis of *o*uter *s*urface *p*rotein A (OspA) and OspC. As first described by Schwan et al. (56) and subsequently confirmed by others (14, 20, 37, 40), the marked downregulation of OspA during tick feeding is accompanied by the increased expression of OspC. The OspA/OspC dichotomy has been recognized for a number of years, but its biological significance has only recently come to light. While there is still some debate regarding its role within the tick (21, 24, 45), OspC appears to be required for the establishment of infection (24), and consequently, the expression of this lipoprotein must be initiated prior to transmission (24, 40). OspA, on the other hand, appears to interact with a tick receptor, TROSPA, to anchor the spirochete within the midgut (42, 44, 69). The repression of OspA, therefore, is likely critical for the migration of spirochetes from the midgut. Studies examining the expression of these two lipoprotein genes have suggested two distinct mechanisms for their regulation. Expression of *ospC* is dependent on RpoS and is positively regulated by the Rrp2/RpoN/RpoS signaling cascade (30, 67). *ospA*, in contrast, is believed to be transcribed via the housekeeping factor RpoD (also referred to as σ^{70}) (3, 59) and is negatively regulated by an unknown mechanism in response to in vivo-specific signals.

In this report, we provide evidence demonstrating that the RpoS-dependent pathway is required for the repression of at least two lp54-borne loci, *ospA* and *lp6.6*. The downregulation of *ospA* and *Ip6.6* appears to require either a repressor molecule whose expression is RpoS dependent or an accessory factor which enables RpoS to directly interact with the *ospA* and *lp6.6* promoter elements, thereby blocking transcription by RpoD. The central role for RpoS during the earliest stages of host adaptation suggests that tick feeding imparts signals to spirochetes that trigger the RpoS-dependent repression, as well as expression, of in vivo-specific virulence factors critical for the tick-to-mammalian host transition.

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TABLE 1. Strains used in this study

Strain	Description	Reference(s)
CE162	*B. burgdorferi* wild-type virulent strain 297 clone	9, 16
AH200	*B. burgdorferi* strain 297 *rpoS* mutant clone; *rpoS*::*ermC* Ery[ra]	30
AH231	*B. burgdorferi* strain 297 *rpoN* mutant clone; *rpoN*::*ermC* Ery[r]	30
CE174	*B. burgdorferi* strain 297 *rpoS* mutant clone; *rpoS*::*ermC* Ery[r]	9, 16
CE467	CE174 complemented with pCE320-RpoS; Ery[r] Kan[rb]	9
CE998	CE174 *rpoS* chromosomal complement; Ery[r] Kan[r]	This work
CE56	CE162 containing pCE320/P_{flaB}-*gfp* reporter; Kan[r]	16
CE103	CE162 containing pCE320/P_{ospA}-*gfp* reporter; Kan[r]	This work
CE472	CE174 containing pCE320/P_{ospA}-*gfp* reporter; Ery[r] Kan[r]	This work

[a] Erythromycin resistance was selected for using 0.06 μg μl^{-1}.
[b] Kanamycin resistance was selected for using 400 μg μl^{-1}.

***B. burgdorferi rpoS* mutants fail to downregulate OspA and Lp6.6 in vivo.** *B. burgdorferi* cultivated within dialysis membrane chambers (DMCs) implanted into the peritoneal cavities of small mammals (i.e., rats and rabbits) (1, 57) undergoes many of the antigenic and physiological changes associated with mammalian host adaptation (1, 8, 9, 18, 23, 26, 46, 49, 51, 52). Most notably, DMC-cultivated spirochetes express little to no OspA in addition to expressing significantly higher levels of a number of borrelial lipoproteins than their in vitro-grown counterparts (1, 8, 9, 51, 57). During the course of our earlier studies examining gene expression in *B. burgdorferi rpoS* mutants cultivated within DMCs (9), we observed that the *rpoS* mutant AH200 (30) (Table 1) not only failed to express known RpoS-dependent lipoproteins but, quite surprisingly, also failed to downregulate OspA in vivo (Fig. 1A); this phenotype also applied to OspB, which was present at equally high levels in in vitro- and DMC-cultivated AH200 (Fig. 1A). A closer inspection of the polypeptide profiles of *B. burgdorferi* wild-type and mutant DMC-cultivated spirochetes by silver stain analysis revealed other differences, particularly within the lower-molecular-mass region (>20 kDa) (Fig. 1A), suggesting that the changes associated with the loss of RpoS during growth in vivo are not limited to OspA.

Previous studies by Hubner et al. (30) have demonstrated that the transcription of *rpoS* requires a second alternate sigma factor, RpoN, and as a consequence, *B. burgdorferi* isolates lacking RpoN exhibit lipoprotein expression phenotypes associated with the loss of *rpoS* (e.g., lack of OspC and DbpA) (21, 30). Fisher et al. (21), on the other hand, have proposed that RpoN can independently transcribe a number of *B. burgdorferi* loci. Thus, it was of interest to examine how the abrogation of RpoN affects the regulation of OspA. As shown in Fig. 1A, AH231 (Table 1), a *B. burgdorferi* strain 297 RpoN mutant, also failed to express OspC and downregulate OspA during growth within DMCs, indicating that the involvement of RpoN in this process is likely indirect and mediated through RpoS. Given that the *B. burgdorferi* genome contains only three annotated sigma factors (*rpoD*, *rpoN*, and *rpoS*) (22), the constitutive expression of OspA in both the *rpoN* and *rpoS* mutants also provides direct evidence that the transcription of this locus is entirely RpoD dependent.

To confirm that the failure to downregulate OspA during growth within DMCs was due to the loss of RpoS and not the result of a secondary mutation, we endeavored to restore *rpoS* by complementation. Because AH200 is refractory to transformation (9, 30), we were unable to introduce the previously

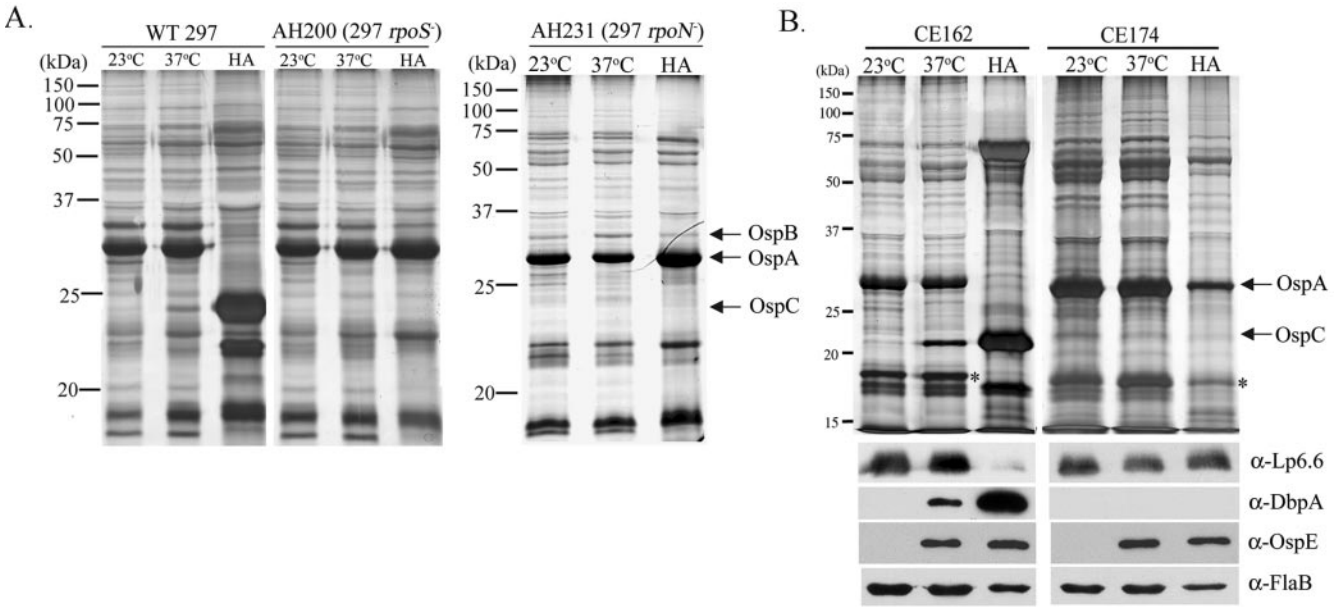

FIG. 1. *B. burgdorferi rpoS* and *rpoN* mutants fail to downregulate OspA in vivo. *B. burgdorferi* was cultivated in BSK-H medium at 23°C and following a temperature shift to 37°C and host adapted within DMCs (HA) as previously described (9). (A) Whole-cell lysates of the uncloned wild-type strain 297 (WT 297), *rpoS* mutant (AH200) (30), and *rpoN* mutant (AH231) (30) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then silver stained as previously described (9). (B) Whole-cell lysates (~10^7 per lane) for the wild-type clone CE162 (9, 16) and *rpoS* mutant clone CE174 (9, 16) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then either silver stained as previously described or immunoblotted using polyclonal sera directed against Lp6.6 (32), DbpA (25), OspE (1), or FlaB. Asterisks indicate the previously described truncated form of OspB (13). Molecular mass markers (kDa) are indicated.

TABLE 2. Primers used in this study

Primer	Sequence (5′–3′)	Use	Reference
FlaB-HIS 5′ BHI	5′-GGCGGATCCATGATTATCAATCATAATACATCAGC-3′	Histidine-tagged FlaB fusion for immunization	This work
FlaB-HIS 3′ RI	5′-GGCGAATTCTTATTATCTAAGCAATGACAAAACATATTG-3′	Histidine-tagged FlaB fusion for immunization	This work
ErmC1118R+BssHII	5′-GAGCGCGCAAACCGTGTGCTCTACGACCAAAAC-3′	*rpoS* chromosomal complement	This work
ErmC1F+BssHII	5′-GAGCGCGCCGATTCACAAAAAATAGGCACACG-3′	*rpoS* chromosomal complement	This work
T7+BclI	5′-GATGATCACATTATGCTGAGTGATATCCCG-3′	*rpoS* chromosomal complement	This work
T3+BclI	5′-GATGATCAAATTAACCCTCACTAAAGGG-3′	*rpoS* chromosomal complement	This work
PflgBF+KpnI	5′-GACTTGGTACCTAATACCCGAGCTTCAAGG-3′	*rpoS* chromosomal complement	This work
Kan3′R+KpnI	5′-GACTTGGTACCGGCGAATGAGCTAGCGCCGTC-3′	*rpoS* chromosomal complement	This work
RpoS-5′	5′-AAAACAAATCTTAAAAATAAAGAGGG-3′	*rpoS*::*ermC* cassette	9
RpoS-3′	5′-CTTGCAAATGCCTGAGTTATTGCAA-3′	*rpoS*::*ermC* cassette	9
P_{ospA}F+SphI	5′-GAGCATGCGCTTAATTAGAACCAAACTTAATT-3′	P_{ospA}-*gfp* reporter	This work
P_{ospA}R+SalI	5′-GAGTCGACAATATATTCTCCTTTTATATTAATATAAC-3′	P_{ospA}-*gfp* reporter	This work

described plasmid complement, *rpoS*/pCE320 (9), into this mutant. We, therefore, regenerated the *rpoS* mutation by electroporating CE162 (Table 1), a highly transformable, virulent *B. burgdorferi* 297 clone (9, 16), with a 3-kb amplicon fragment of AH200 genomic DNA containing the *rpoS*::*ermC* inactivation cassette as previously described (9, 16). The resulting erythromycin-resistant transformants were screened by PCR amplification for the presence of the *ermC* cassette within the endogenous copy of *rpoS* using primers rpoS-5′ and rpoS-3′ (Table 2) as previously described (9). Five *rpoS* mutants from two independent transformations, confirmed to contain the mutant *rpoS* allele using PCR, lacked expression of OspC and decorin binding protein A (DbpA) during temperature shift in vitro and within DMCs and also failed to downregulate OspA during cultivation within DMCs; representative results from the analysis of one transformant, CE174 (Table 1), are shown in Fig. 1B. Previous studies have demonstrated that a second borrelial lipoprotein, Lp6.6 (BBA62), exhibits a similar, if not identical, expression pattern to that of OspA, including downregulation within DMCs (1, 8, 32). To determine whether downregulation of Lp6.6 was similarly RpoS dependent, we immunoblotted lysates prepared from CE162 (wild type [wt]) and CE174 (*rpoS* mutant) cultivated in vitro and within DMCs using antiserum specific for Lp6.6 (32). Indeed, wild-type CE162 constitutively expressed Lp6.6 in vitro, while little to no Lp6.6 was detectable following DMC cultivation. As with OspA, the *rpoS* mutant CE174 continued to express Lp6.6 during DMC cultivation at levels similar to those observed in vitro (Fig. 1B). The upregulation of OspE in both the *rpoS* (Fig. 1B) and *rpoN* (data not shown) mutants following temperature shift confirmed that these isolates retained the ability to differentially express RpoD-dependent loci and, moreover, suggests that the defects in differential gene expression associated with these mutations are limited to the RpoN/RpoS pathway. These findings complement those reported earlier by Roberts et al. (52) for the expression of the Bdr-paralogous family in the same *rpoS* and *rpoN* mutants.

Complementation with *rpoS* restores the repression of OspA and Lp6.6 in vivo. Unlike AH200, CE174 was readily transformed with the complementing plasmid *rpoS*/pCE320 (9). The restoration of RpoS activity in the complemented transformant, CE467 (Table 1), was confirmed by its ability to express OspC and DbpA to wild-type levels following temperature shift in vitro (Fig. 2). This isolate also recently was shown to have restored infectivity for mice (9). Next, we cultivated CE162 (wt), CE174 (*rpoS* mutant), and CE467 (complemented *rpoS* mutant) within DMCs; the complementation of CE174 with *rpoS* resulted in the downregulation of both OspA and Lp6.6 to near wild-type levels (Fig. 2). Given that *rpoS* is not required for growth and survival within DMCs (9), one potential explanation for the residual expression of OspA and Lp6.6 in CE467 is the spontaneous loss of the complementing plasmid due to the lack of selection pressure in vivo. We, therefore, assayed DMC-cultivated CE467 spirochetes for the presence of *rpoS*/pCE320 by solid-phase plating in the presence and absence of antibiotic selection (53). In contrast to the high *rpoS*/pCE320 retention rates observed in vitro (≥90%) under antibiotic selection, the complementing plasmid was present in only 60% of CE467 spirochetes following DMC explantation and subsequent solid-phase plating in the absence and presence of kanamycin. To circumvent these stability issues, we initially attempted to take advantage of *B. burgdorferi*'s strict requirement for the plasmid lp25-encoded nicotinamidase (*pncA*) in vivo (49) by incorporating this locus into our pCE320 shuttle vector (15) and then transforming the resulting *rpoS*/pCE320+*pncA* plasmid into a variant of CE174 that had spontaneously lost the endogenous lp25 plasmid. While *B. burgdorferi* lacking lp25 is unable to survive in mice and within DMCs (18, 23, 49), we were surprised to find that (i) the addition of *pncA* conferred only a modest increase in the level of stability to the complementing plasmid in DMCs (data not shown) and that (ii) we were readily able to detect spirochetes lacking *pncA* following explantation (data not shown). Thus, while *pncA* is absolutely required to establish growth in vivo, once spirochetes make the physiological switch to growth within the mammalian host, the requirement for *pncA* appears to be markedly diminished or absent. It also is worth noting that Bockenstedt et al. (7) made a similar observation with antibiotic-treated mice.

As an alternative to plasmid complementation, we employed a more technically challenging chromosomal complementation strategy by inserting a wild-type copy of *rpoS*, including the upstream promoter region (using primers listed in Table 2), into the chromosomally located *ermC* gene in CE174. Briefly, the erythromycin resistance gene (*ermC*) from pGK12 (62) was PCR amplified using primers ErmC1118R+BssHII and ErmC1F+BssHII and cloned into BssHII-digested pBSII SK$^+$ (Stratagene,

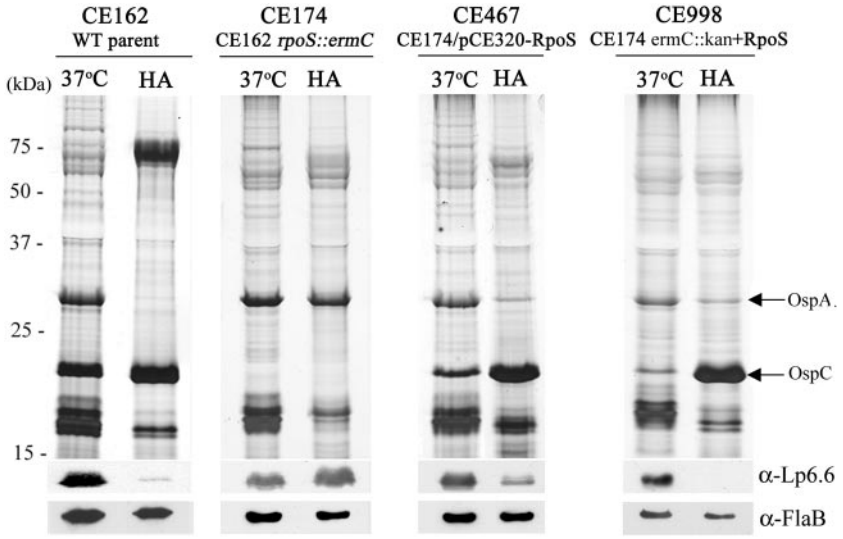

FIG. 2. Complementation with *B. burgdorferi* wild-type *rpoS* restores the downregulation of OspA and Lp6.6. Whole-cell lysates were prepared from the *B. burgdorferi* wild-type 297 strain CE162 (9, 16), the *rpoS* mutant CE174 (9, 16), and *rpoS* mutants complemented with wild-type *rpoS* on a circular plasmid (CE467) or on the chromosome (CE998) following a temperature shift in vitro (37°C) and within DMCs (HA). Lysate samples (~10^7 per lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then either silver stained or immunoblotted using polyclonal sera directed against Lp6.6 (32) or FlaB. Molecular mass markers (kDa) are indicated.

La Jolla, CA). The multiple cloning site was amplified from pBSII SK^+ using the commercially available T3 and T7 primers with BclI ends and introduced back into the BclI site of *ermC* (pCE665). The P_{flgB}::*kan* marker was amplified from pCE320 using the P*flgB*F+KpnI and *kan*3′R+KpnI primers and then cloned into the KpnI site in pCE665 to generate pCE671. Finally, the *rpoS* gene and 500-bp upstream region was PCR amplified using RpoS-5′ and RpoS-3′ (Table 2) and cloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA). The *rpoS*-complementing fragment was then cloned into pCE671 in the opposite orientation of the *kan* marker using BamHI and NotI to generate pCE697. Ten micrograms of pCE697 was introduced into CE174 by electrotransformation (53), and kanamycin-resistant transformants were selected as previously described (9, 16). One clone, CE998, was selected, and the integration of wild-type *rpoS* into chromosomal *ermC* was confirmed by PCR and sequencing (data not shown). As shown in Fig. 2, the expression of Lp6.6 was completely abrogated in DMC-cultivated CE998. Interestingly, however, the expression of OspA was significantly reduced, but as with the plasmid complement CE467, residual expression was detected. Taken together, the results from both complementation approaches clearly demonstrate that the failure to downregulate OspA and Lp6.6 during DMC cultivation is due to the loss of RpoS. However, subtle differences as to how this regulatory pathway influences the downregulation of individual genes may exist.

Repression of *ospA* by RpoS occurs at the level of transcription initiation and does not require localization of promoter sequences to a linear plasmid. Previous studies demonstrating that *ospA* is regulated at the transcriptional level have been dependent upon the detection or measurement of steady-state levels of message by Northern blotting (29, 34, 50), reverse transcription-PCR (28, 33, 40, 47, 50), or microarray analysis (8, 38, 39, 41, 51, 61). Thus, in order to determine whether the effect of RpoS on *ospA* downregulation is due to decreased transcription initiation or mRNA degradation, we decided to use a transcriptional reporter construct that would enable us to discriminate between these two possibilities. The selection of green fluorescent protein (GFP) as the reporter, in conjunction with flow cytometry, has the additional advantage of enabling one to assess gene expression by individual spirochetes within a population (6, 12, 15, 16, 54). For these studies, a 189-bp region of the upstream sequence containing both the *ospA* promoter and a poly(T) tract reported to be involved in the regulation of *ospA* transcription in vitro (59) was cloned upstream of a promoterless *gfp* contained on our cp32-based shuttle vector pCE320 (15). The resulting construct, P_{ospA}-*gfp* (Table 1), was then transformed into both *B. burgdorferi* wild-type and *rpoS* mutant isolates and assayed by flow cytometry following temperature shift and growth within DMCs, as previously described (16). These data are summarized in Table 3, while representative flow cytograms and micrographs are shown in Fig. 3. P_{ospA}-*gfp* expression in DMC-cultivated wild-type CE103 was significantly decreased compared to the levels observed at 37°C in vitro. To ensure that this low level of fluorescence is due to the downregulation of the *ospA* promoter

TABLE 3. P_{ospA}-*gfp* fluorescence levels from spirochetes grown at 37°C and within DMCs

Clone	Background	Promoter	37°C in vitro		DMC	
			MFI	% Fluor[a]	MFI	% Fluor
CE56	wt *rpoS*	*flaB*	239.6	79.9	395.9	82.1
CE103	wt *rpoS*	*ospA*	958.0	86.4	11.76	7.8[b]
CE472	*rpoS*::*ermC*	*ospA*	2,547.4	90.1	1,838.5	80.2

[a] % Fluor, percent fluorescence determined by flow cytometry, confirmed visually using fluorescence microscopy.

[b] At least 60% of the recovered spirochetes retained the shuttle vector as determined by solid-phase plating in the presence and absence of the selecting antibiotic.

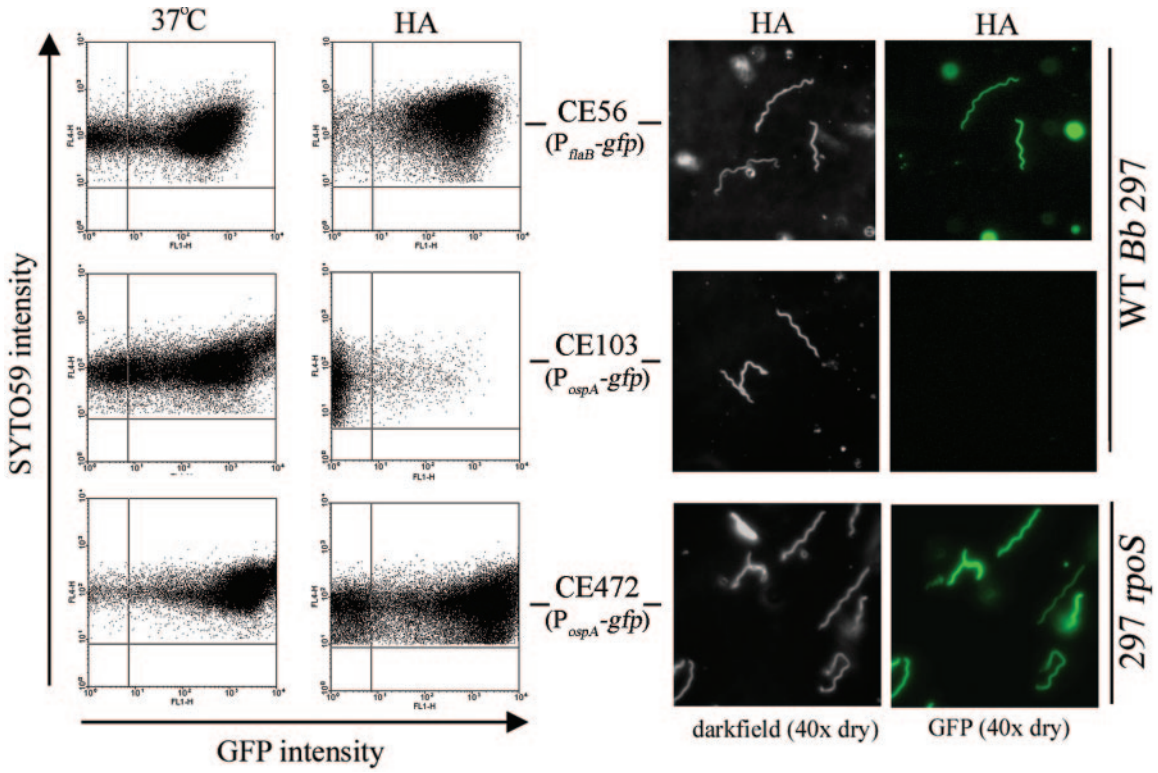

FIG. 3. RpoS-mediated repression of *ospA* occurs at the level of transcription initiation. Analysis of the *ospA* promoter (P_{ospA}) in wild-type and *rpoS* mutant *B. burgdorferi* cells using a GFP reporter system. (Left panels) Samples from wild-type strain 297 (CE103) and an *rpoS* mutant (CE472) transformed with the P_{ospA}-*gfp* reporter constructs cultivated in vitro following a temperature shift from 23°C to 37°C and within DMCs (HA) were analyzed by flow cytometry as described previously (12). Spirochetes were identified by forward- and side-scatter profiles and by staining with the nucleic acid stain SYTO59 (*y* axis; Molecular Probes). GFP intensity is measured on the *x* axis. Wild-type *B. burgdorferi* transformed with the constitutively expressed P_{flaB}-*gfp* (CE56) serves as a control. (Right panels) Dark-field and fluorescence microscopy images of spirochetes cultivated within DMCs.

and not spontaneous loss of the reporter shuttle vector in the absence of selective pressure, we assayed for reporter plasmid retention in CE103 following explantation as described above. More than 60% of DMC-cultivated CE103 spirochetes retained the P_{ospA}-*gfp* reporter as determined by solid-phase plating in the presence and absence of antibiotic and fluorescence microscopy; even after plasmid loss was taken into account, CE103 still expressed dramatically less GFP with respect to the percentage of fluorescent spirochetes and their average mean fluorescence intensity (MFI) than their in vitro-grown counterparts (Table 3). In contrast, the levels of GFP in the *rpoS* mutant CE472 remained high during DMC cultivation (Fig. 3 and Table 3). Thus, taken together, these data confirmed the following: (i) the P_{ospA}-*gfp* reporter faithfully reproduces the expression pattern of the native gene; (ii) the effect of RpoS on *ospA* downregulation in vivo occurs at the level of transcription initiation; (iii) the upstream sequences which mediate this effect remain operative when removed from neighboring plasmid lp54 sequences; and (iv) *ospA* transcription is entirely RpoD dependent. Regarding the last point, we observed that during in vitro growth, P_{ospA}-*gfp* expression in the *rpoS* mutant was consistently greater than that in the wild-type background (Table 3). In addition to further reinforcing the importance of RpoD for *ospA* transcription, these data raise the intriguing possibility that sigma factor competition for a limiting amount of RNA polymerase apoenzyme (19) could play a role in the regulation of *ospA* by providing a passive framework for modulating the expression of this locus.

Models for RpoS-dependent repression of *B. burgdorferi* loci in vivo. In this report, we demonstrate that RpoS is required for the repression, as well as the induction, of key borrelial virulence determinants during mammalian host adaptation. Two scenarios can be envisioned to explain this form of negative regulation (Fig. 4A). The first, and perhaps simplest, explanation is that RpoS controls the expression of a repressor molecule which in turn requires activation by some in vivo-signaling pathway in order to block transcription by RpoD. The existence of an *ospA* promoter repressor has been proposed in earlier studies (35, 36). Using a spontaneously occurring *B. burgdorferi* mutant that fails to express *ospA* in vitro, Margolis and Samuels (36) detected the binding of an unknown protein(s) to a region upstream of *ospA* in strain CA-11.2A that is almost identical to the sequence contained within our P_{ospA} reporter. In order for the same factor to be directly involved in the repression of both *ospA* and *lp6.6*, there must be common upstream sequences to which it can bind. It is worth noting, therefore, that sequences upstream of the *lp6.6* structural gene contain a poly(T) tract similar to that upstream of *ospA* (Fig. 4B). An alternative model, which cannot be eliminated using the data currently in hand, proposes that an in

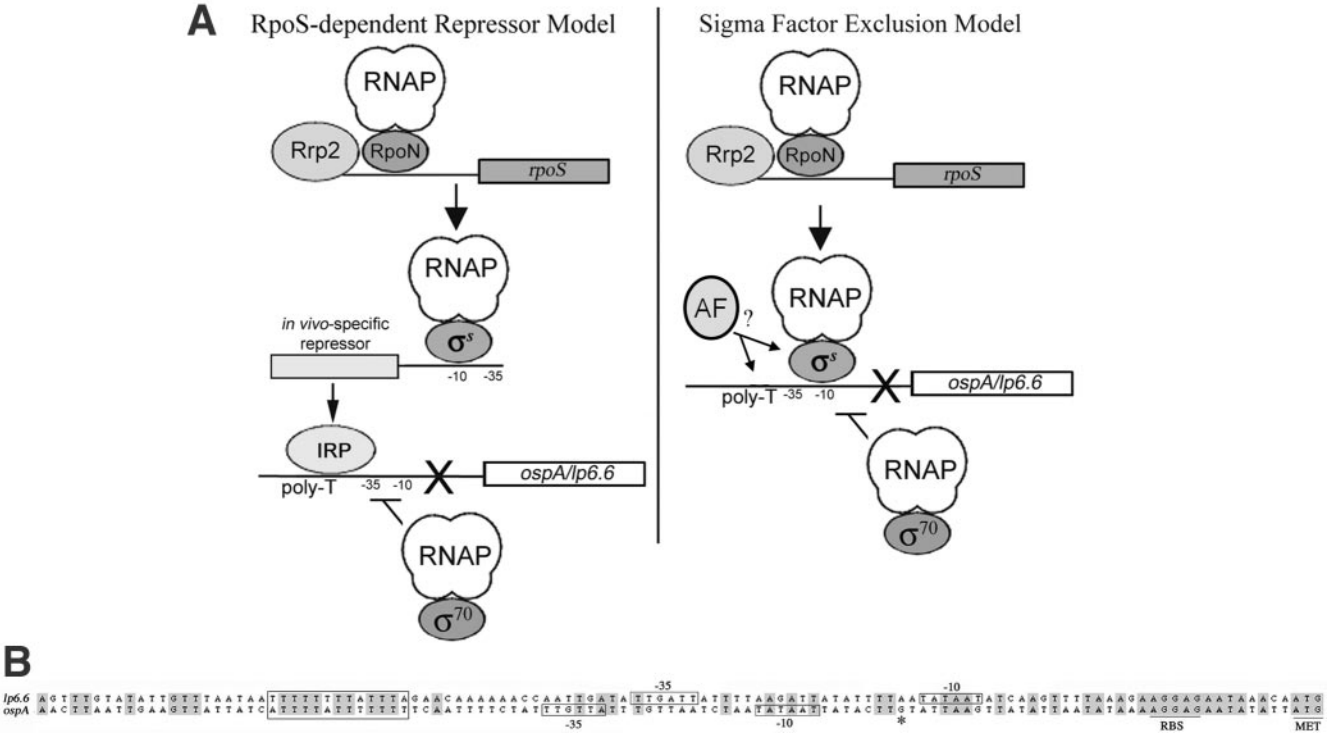

FIG. 4. (A) Proposed models for RpoS-dependent in vivo repression of *ospA* and *lp6.6*. (Left panel) RpoS controls the expression of a repressor protein which, upon activation by in vivo signals, binds to the *ospA* and *lp6.6* promoter regions, preventing subsequent transcription by the RpoD-containing RNAP holoenzyme. (Right panel) Modification by or association with an in vivo-specific accessory factor enables RpoS to bind to the *ospA* promoter and prevents the establishment of an open promoter complex, thereby blocking RpoD-dependent transcription initiation. Abbreviations: RNAP, RNA polymerase; Rrp2, response regulator 2; IRP, in vivo-specific repressor protein; AF, accessory factor; σ^s, RpoS; σ^{70}, RpoD. (B) Diagram of *ospA* and *lp6.6* upstream promoter regions including a poly(T) tract (boxed). The transcriptional start site for *ospA* (31) is designated by an asterisk. Putative −10/−35 regions, ribosome binding sites (RBS), and methionine start codons (MET) are indicated.

vivo-activated accessory factor either modifies or directly interacts with RpoS. This modification or interaction enables the alternate sigma factor to bind to the *ospA* and *lp6.6* promoters, thus blocking RpoD-dependent transcription, most likely by preventing the formation of an open complex at the promoter (Fig. 4A). For this regulatory mechanism to hold, a dramatic change in sigma factor recognition of the *ospA* promoter would have to occur. With either model, our finding that the presence of RpoS appears to diminish the RpoD-dependent expression of *ospA* is potentially significant because it suggests that *ospA* expression is subject to some constitutive level of basal inhibition and, as a consequence, is poised for repression. Support for this notion is provided by temporal studies demonstrating that the expression of *ospA* in *B. burgdorferi*-infected ticks is rapidly downregulated upon the acquisition of a bloodmeal (40, 55). Our prior studies have demonstrated that the expression of *rpoS* and RpoS-dependent genes occurs almost immediately after spirochetes enter exponential-phase growth following temperature shift (9); this rapid onset of RpoS-dependent gene expression presumably also occurs during the initial stages of spirochete expansion within *B. burgdorferi*-infected ticks. Thus, by integrating the alternate sigma factor RpoS into two distinct regulatory pathways involved in both the expression of OspC and the repression of OspA early during tick feeding, *B. burgdorferi* appears to have devised a strategy for ensuring the coordinated reciprocal regulation of these transmission-associated loci.

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All experimental procedures using animals were performed in accordance with protocols and guidelines established by the Institutional Animal Care and Use Committee.

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