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Changes in Bacterial Growth Rate Govern Expression of the *Borrelia burgdorferi* OspC and Erp Infection-Associated Surface Proteins

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The Lyme disease spirochete controls production of its OspC and Erp outer surface proteins, repressing protein synthesis during colonization of vector ticks but increasing expression when those ticks feed on vertebrate hosts. Early studies found that the synthesis of OspC and Erps can be stimulated in culture by shifting the temperature from 23°C to 34°C, leading to a hypothesis that *Borrelia burgdorferi* senses environmental temperature to determine its location in the tick-mammal infectious cycle. However, borreliae cultured at 34°C divide several times faster than do those cultured at 23°C. We developed methods that disassociate bacterial growth rate and temperature, allowing a separate evaluation of each factor's impacts on *B. burgdorferi* gene and protein expression. Altogether, the data support a new paradigm that *B. burgdorferi* actually responds to changes in its own replication rate, not temperature *per se*, as the impetus to increase the expression of the OspC and Erp infection-associated proteins.

Vector-borne pathogens, such as the Lyme disease spirochete, *Borrelia burgdorferi*, have overcome the challenges of persisting within two very different animal environments and possess mechanisms to efficiently transmit back and forth between vertebrate hosts and arthropod vectors. To facilitate this complex lifestyle, the bacterium produces specific proteins appropriate for each step in the infectious cycle. Considerable effort has been expended to identify the mechanisms by which *B. burgdorferi* senses its environment and accordingly regulates gene expression (1, 2). Such information both provides insight into pathogenic mechanisms and identifies new targets for preventative and curative therapies.

One of the first studies to delve into the mechanisms of *B. burgdorferi* gene regulation focused on OspC (outer surface protein C). The exact function of that protein remains to be elucidated, but it is necessary for the establishment of mammalian infection (3–10). In a landmark study in 1995, Schwan et al. demonstrated that synthesis of OspC is repressed in bacteria within unfed ticks, yet OspC production is induced as those ticks begin to feed (11). Furthermore, they showed that regulation of OspC synthesis can be recapitulated in culture: the protein is poorly expressed by bacteria cultured at 23°C but is abundantly expressed by bacteria that are first grown at 23°C, diluted into fresh medium, and then cultured at 34°C to 37°C (11).

Shortly thereafter, it was demonstrated that increasing the culture temperature from 23°C to 34°C enhances the production of several other antigenic proteins (12). Among these are a paralogous family of outer surface lipoproteins designated Erp (OspE/OspF-related proteins) (12–15). Erp proteins are expressed throughout vertebrate infection, adhere to various host factors, and appear to play roles in dissemination and colonization (16–25). As with *ospC*, *erp* transcription is repressed during tick colonization and induced during tick feeding and transmission (1, 2, 20, 26).

Since those initial reports, numerous studies have determined that a substantial number of *B. burgdorferi* genes can be regulated during cultivation by altering the incubation temperature (e.g., see references 2, 27, and 28). The premise behind examining culture temperature effects was that such changes were hypothesized to mimic conditions within a colonized tick: 23°C was thought to represent the ambient temperature of an unfed tick, and the change from 23°C to 34°C is comparable to that experienced by bacteria when a tick feeds on a warm-blooded animal (1, 11, 29, 30). As yet, there are no validated mechanisms by which *B. burgdorferi* can directly sense environmental temperature.

Increasing the culture temperature from 23°C to 34°C has profound effects on *B. burgdorferi* metabolism, a point that has largely been overlooked. Most notably, borreliae cultured at 34°C grow much faster than do those cultured at 23°C (12, 31). Several recent studies have linked *B. burgdorferi* metabolic activity with regulation of gene expression. As examples, acetate and the mevalonate pathway are involved in regulating the production of OspC and several other mammal-specific proteins (32, 33), while cellular concentrations of the *erp* antirepressor, EbfC, increase with the rate of bacterial replication (34, 35).

Prompted by those observations, we developed culture conditions that cause changes in bacterial growth rate independently of temperature. A new approach to studying the effects of a culture temperature shift was also employed. Results indicate that the rate of *B. burgdorferi* growth, rather than temperature, influences the expression of the OspC and Erp proteins.

MATERIALS AND METHODS

Bacteria and growth conditions. All studies utilized *B. burgdorferi* strain B31-MI-16, an infectious clone of the sequenced type strain B31 (20). Unless otherwise stated, *B. burgdorferi* bacteria were grown in complete Barbour-Stoenner-Kelly II (BSK-II) medium, which contains 6% (vol/vol) rabbit serum (36, 37). All media were preconditioned by incubation at 23°C or 34°C for 24 h prior to inoculation. In our experience, Lyme disease spirochetes grow and divide at optimal rates in artificial media when incubated near 34°C.

Densities of bacterial cultures were determined by microscopic enumeration using a Petroff-Hausser counting chamber (38). At each time

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point, cultures were counted in quadruplicate, and an average and standard deviation from this mean were calculated.

Temperature shift experiments from 23°C to 34°C were performed as described previously (11, 12). Briefly, *B. burgdorferi* was first cultured to late exponential phase (approximately 10^8 bacteria/ml) in BSK-II medium at either 23°C or 34°C. An aliquot of such a culture was diluted 1:100 into fresh BSK-II medium and then incubated at 23°C. Upon this culture attaining late exponential phase, an aliquot was diluted 1:100 into fresh medium and then incubated at 34°C. Late-exponential-phase cultures of the constant 23°C and the 23°C-to-34°C-shifted bacteria were harvested for analyses.

The effects of culture medium composition were assessed by using essentially the same technique as described above. Bacteria were grown to late exponential phase at 34°C in an incomplete medium. An aliquot of that culture was then diluted 1:100 into fresh, complete BSK-II medium and then incubated at 34°C. Both cultures were harvested at late exponential phase.

Two deficient media were used, each of which contains suboptimal concentrations of one or more essential metabolites. Complete BSK-II medium contains 6% rabbit serum, which provides the bacteria with lipids. Experiments were conducted to determine a concentration of rabbit serum that impaired borrelial growth yet permitted replication at 34°C at a rate comparable to that seen in complete medium at 23°C. As described here, *B. burgdorferi* bacteria were found to divide at a greatly reduced rate when cultured at 34°C in medium that contains only 1.2% (vol/vol) rabbit serum. BSK-II medium is a very complex, rich medium. Trials were undertaken with various concentrations of BSK-II medium diluted with phosphate-buffered saline (PBS) to determine a composition that facilitated slow borrelial growth. Severely reduced division rates were observed at 34°C in medium consisting of 25% (vol/vol) BSK-II medium diluted in PBS and containing 6% (vol/vol) rabbit serum.

To further test the hypothesis that an increased borrelial growth rate, rather than actual temperature, is responsible for the enhanced production of OspC and Erp proteins, *B. burgdorferi* bacteria were moved from −80°C to 23°C in complete BSK-II medium. Such temperature shifts were performed by diluting bacteria that had been frozen for >30 days at −80°C into fresh BSK-II medium and then culturing to late exponential phase at 23°C. To produce the frozen bacteria, glycerol was added to mid-exponential-phase cultures at a final concentration of 25%, and aliquots were then placed in a −80°C freezer.

Protein expression analyses. Cultures were harvested by centrifugation, washed at least twice with PBS, and then resuspended in PBS containing 1 mM phenylmethanesulfonyl fluoride (PMSF). Bacteria were lysed by immersion in boiling water for approximately 2 min. Total cellular proteins were separated by SDS-polyacrylamide gel electrophoresis. Specific proteins were detected by immunoblot analyses using previously described antibodies (34, 39–43). Murine monoclonal antibodies against *B. burgdorferi* RpoS were obtained from F. Yang (Indiana University, Indianapolis, IN) (43), and rat polyclonal antiserum against RpoS was obtained from M. Caimano and J. Radolf (University of Connecticut, Farmingdale, CT). The constitutively expressed FlaB protein served as a reference (12, 44). OspC, ErpA, ErpM, ErpY, BpaB, EbfC, OspA, and OspB immunoblot band intensities under each condition were quantified relative to the FlaB signal, using ImageJ (http://rsbweb.nih.gov/ij/).

Quantitative reverse-transcription PCR. Total RNA was extracted from cultured *B. burgdorferi* cells, and transcript levels were determined by quantitative reverse transcription-PCR (Q-RT-PCR), as described previously by Miller (45). Oligonucleotide primers are described in Table 1 (14, 46, 47). Each analysis was performed in triplicate, and means and standard deviations were determined. Levels of mRNA for *ospC*, *erpG*, and *rpoS* were standardized to levels of the constitutively expressed *flaB* message. A two-tailed Student *t* test was used to calculate statistically significant differences between culture conditions. GraphPad Prism v.5 (GraphPad, La Jolla, CA) was used to generate all graphs.

TABLE 1 Oligonucleotide primers used for Q-RT-PCR

Transcript	Primer	Sequence (5′–3′)	Reference
ospC	OSPCF-7	CAGGGAAAGATGCGAATACATCTGC	46
ospC	OSPCR-8	TAAGCTAAAGCTAACAATGATCC	46
erpG	E-33	TGCAAGATTGATGCG	14
erpG	E-34	ATTTTGAGGCTCTGC	14
rpoS	88-111	CTTGCAGGACAAATACAAAGAGGC	47
rpoS	245-220	GCAGCTCTTATTAATCCCAAGTTGCC	47
flaB	FLA3	GGGTCTCAAGCGTCTTGG	46
flaB	FLA4	GAACCGGTGCAGCCTGAG	46

RESULTS

Effects of culture medium composition on bacterial division rates. *B. burgdorferi* cells cultivated at 34°C in complete BSK-II medium with 6% rabbit serum divide approximately once every 12 h (Fig. 1). In contrast, bacteria grown in this medium at 23°C double approximately once every 32 h. We hypothesized that these differences in growth rate are responsible for the differences in *B. burgdorferi* expression patterns, independently of temperature. To test this prediction, culture media that lowered the rate of growth at 34°C were devised. The reduced genome of the obligately parasitic bacterium *B. burgdorferi* encodes very few anabolic enzymes, and the Lyme disease spirochete is an auxotroph for nearly all macromolecules (48). BSK-II medium is a very rich medium containing a mixture of defined and undefined ingredients (36, 37, 49). Complete BSK-II medium contains 6% rabbit serum, which provides sufficient fatty acids for optimal growth (36, 37, 49, 50). Reducing the amount of rabbit serum in the medium to 1.2% extended the doubling time to approximately 32 h (Fig. 1). Dilution of the components of BSK-II medium to one-quarter strength, with rabbit serum kept at 6%, impaired growth to approximately one doubling every 40 h (Fig. 1). These two media, designated "1.2% serum" and "25% BSK-II," respectively, were then used for studies on the effects of the *B. burgdorferi* growth rate on gene and protein expression. To do so, bacteria were first cultured at 34°C in either 25% BSK-II or 1.2% serum medium and then diluted 1:100 into complete BSK-II medium and cultured at 34°C. All bacteria passaged in complete medium grew with a doubling time of approximately 12 h (Fig. 1). These culture medium formulations then permitted the following studies on the effects of different growth rates at a constant temperature.

Effects of bacterial division rates on OspC and Erp protein and mRNA levels. The borrelial *ospC* and *erp* genes are carried on distinct genetic elements and are regulated through different mechanisms (31, 51). The *ospC* locus is carried on a small circular replicon, cp26, while *erp* operons are located on episomal prophages named cp32s (13, 52–55). Three different Erp proteins were assayed, which are encoded by separate operons, each on a different cp32 prophage: ErpA, encoded by two identical *erpAB* operons, one each on cp32-1 and cp32-8; ErpM, encoded by *erpLM* on cp32-7; and ErpY, encoded by *erpHY* on cp32-4 (54). Previous analyses demonstrated that Erp protein levels are regulated at the level of transcription and that cellular *erp* mRNA levels are directly proportional to Erp protein levels (14). Rather than be repetitive, transcript levels of a fourth *erp* locus, *erpG*, encoded on cp32-3, were examined by Q-RT-PCR. To enable comparisons of the current studies with previous reports,

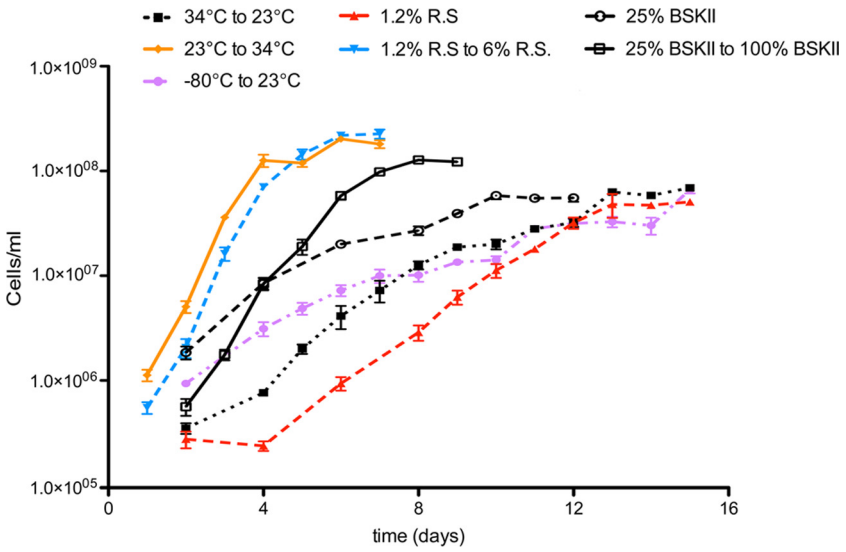

FIG 1 Effects of culture temperature and medium composition on *B. burgdorferi* growth. Culture densities were determined by enumerating cultured bacteria using a Petroff-Hausser counting chamber. Cultures were counted in quadruplicate at each time point. Culture conditions were as follows (unless otherwise noted, BSK-II medium contains 6% rabbit serum): 34°C to 23°C, grown to late exponential phase in BSK-II medium at 34°C, diluted into BSK-II medium, and then grown at 23°C; 23°C to 34°C, grown to late exponential phase in BSK-II medium at 23°C, diluted into BSK-II medium, and then grown at 34°C; −80°C to 23°C, stored at −80°C for >1 month, diluted into BSK-II medium, and then grown at 23°C; 1.2% R.S., grown to late exponential phase in BSK-II medium at 34°C, diluted into BSK-II medium plus 1.2% rabbit serum, and then grown at 34°C; 1.2% R.S. to 6% R.S., grown to late exponential phase in BSK-II medium plus 1.2% rabbit serum at 34°C, diluted into BSK-II medium, and then grown at 34°C; 25% BSK-II, grown to late exponential phase in BSK-II medium plus rabbit serum at 34°C, diluted into 25%-strength BSK-II medium, and then grown at 34°C; 25% BSK-II to 100% BSK-II, grown to late exponential phase in 25%-strength BSK-II medium at 34°C, diluted into full-strength BSK-II medium, and then grown at 34°C.

steady-state protein and mRNA levels were determined by immunoblotting and Q-RT-PCR.

Temperature shift experiments recapitulated previously reported results (11, 12, 14), with *B. burgdorferi* cultivated at 23°C producing less OspC and Erp proteins than did bacteria shifted from 23°C to 34°C (Fig. 2). In the analysis illustrated in Fig. 2, OspC protein levels were 1.5-fold higher in the bacteria shifted from 23°C to 34°C than in the bacteria maintained at 23°C. As previously observed, relative expression levels of different Erp proteins varied between alleles, apparently due to differences in promoter and operator strengths and the proportional utilization of the housekeeping RpoD and alternative RpoS sigma factors (14, 26, 56–60). For the experiment illustrated in Fig. 2, bacteria shifted in temperature produced 1.4-fold more ErpA, 3.4-fold more ErpM, and a slight, but statistically insignificant, increase in the ErpY level. Q-RT-PCR analyses mirrored protein results, with significantly higher levels of *ospC* and *erpG* mRNAs being produced by bacteria shifted from 23°C to 34°C than by those maintained at 23°C (Fig. 3).

Disassociation of bacterial growth rate and temperature was achieved by using the 1.2% serum and 25% BSK-II formulations of culture medium. *B. burgdorferi* cells were cultured at 34°C in either of the incomplete media and then diluted into complete medium and grown further at 34°C. Immunoblot analyses demonstrated differential protein expression patterns comparable to those observed with temperature shifts. In the experiment illustrated in Fig. 2, shifting bacteria from 1.2% serum to complete medium increased OspC levels 14-fold. *B. burgdorferi* bacteria cultured in 25% BSK-II medium did not produce detectable levels of OspC, whereas those shifted to complete BSK-II medium expressed high levels of that protein. The expression of Erp proteins was similarly induced by shifts from either 1.2% serum or 25% BSK-II medium to complete medium (Fig. 2). For the illustrated experiments, shifting *B. burgdorferi* from 1.2 to 6% serum increased ErpA levels 1.3-fold, ErpM 3.8-fold, and ErpY 1.3-fold. Changing from 25% to full-strength BSK-II medium increased ErpA levels 4.5-fold, ErpM 1.8-fold, and ErpY 1.9-fold. Q-RT-PCR of bacteria shifted from 1.2% to complete medium also showed that greatly enhanced *ospC* and *erpG* transcript levels accompanied an enhanced bacterial growth rate (Fig. 3). These differences in protein and mRNA levels are in line with analyses of temperature shift effects (see above) (11, 12, 14, 26).

Control studies indicated constitutive expression of the flagellin subunit FlaB under all culture conditions (Fig. 2). The OspA and OspB surface proteins were not affected to any significant extents by either changes in the culture temperature or BSK-II concentrations. However, a decrease in the OspA, but not the OspB, level was occasionally observed following an increase of the serum concentration from 1.2 to 6% (Fig. 2). Previous studies have shown mixed results for OspA regulation in culture, with some reports describing no effects on OspA following a temperature shift (11, 12) and others noting changes in culture (61, 62). The mechanisms controlling the *ospAB* operon are evidently complex and will require substantial further experimentation to unravel.

Effects of bacterial division rates on regulatory factors. OspC and Erp protein levels are regulated at the level of transcription (14, 63). Transcription of *erp* genes is controlled through two DNA-binding proteins and, to various extents, the RpoS sigma factor (34, 59, 60). BpaB represses *erp* transcription, while EbfC competes against BpaB for binding to the *erp* operator and functions as the antirepressor (34, 64). In contrast, transcription of

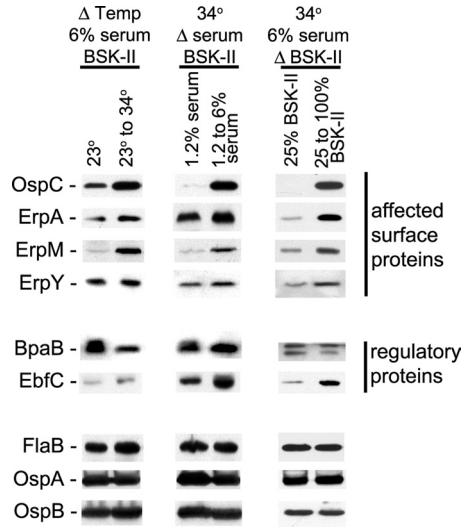

FIG 2 Increases in *B. burgdorferi* growth rate correlate with increased production of OspC and Erp proteins. Effects of changing culture conditions on *B. burgdorferi* protein expression profiles were examined by immunoblotting. For each column, two conditions were kept constant, while a third was varied. For all studies, bacteria were first cultured to late exponential phase under a condition that impaired growth (complete BSK-II medium at 23°C, BSK-II medium containing only 1.2% rabbit serum at 34°C, or 25%-strength BSK-II medium with 6% serum at 34°C) and then diluted 1:100 into fresh, complete BSK-II medium and cultured at 34°C. All cultures were harvested at late exponential phase. The constitutively expressed flagellar component FlaB served as a control, and fold changes of other proteins were calculated relative to the FlaB band intensities (44). Illustrated data for each condition are from analyses of the same paired bacterial lysates.

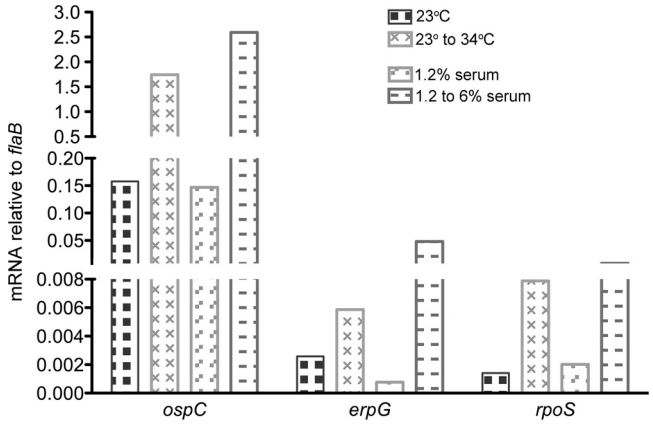

FIG 3 Further indications that changes in borrelial growth rates lead to alterations in gene expression. Shown are data from quantitative reverse transcription-PCR (Q-RT-PCR) analyses of bacterial *ospC*, *erpG*, and *rpoS* mRNAs. Results are presented relative to mRNA levels of the constitutively expressed *flaB* gene (45). All analyses were performed in triplicate. Error bars (±1 standard deviation) are below the resolution of the figure. Differences within paired conditions were all statistically significant ($P < 0.001$).

ospC is dependent primarily upon RpoS, with possible contributions from DNA supercoiling and an additional, as-yet-unidentified DNA-interacting factor(s) (62, 65–67).

Immunoblot analyses indicated that cellular levels of the *erp* repressor, BpaB, were decreased following changes from slow- to fast-growth conditions. In the studies illustrated in Fig. 2, BpaB levels decreased 2.2-fold, 1.1-fold, or 1.3-fold as a result of a shift to complete medium at 34°C from 23°C, 1.2% serum, or 25% BSK-II medium, respectively. These same changes in culture conditions also led to 1.3-fold, 1.7-fold, or 3.5-fold increases, respectively, in cellular levels of the *erp* antirepressor, EbfC (Fig. 2). These changes correspond with the known functions of BpaB and EbfC in regulating *erp* transcription (34).

RpoS is apparently expressed at relatively low levels under all examined conditions and could not be detected by immunoblotting using any of the anti-RpoS monoclonal or polyclonal antibodies. Although the cellular concentration of RpoS in actively growing *B. burgdorferi* bacteria is not known, *Escherichia coli* may contain between 0 and 100 molecules of each alternative sigma factor per cell (68). However, *rpoS* mRNA was detectable by Q-RT-PCR, which indicated that bacteria shifted from 1.2% to 6% serum increased *rpoS* mRNA levels by 4-fold (Fig. 3).

Actual culture temperature does not affect *B. burgdorferi* OspC or Erp protein levels. Previous 23°C-versus-34°C temperature shift studies used actively growing cultures to inoculate the 23°C culture (e.g., see references 11, 12, 14, and 69). Regardless of whether inoculating bacteria are first grown at 23°C or at 34°C, *B. burgdorferi* will produce low levels of OspC and Erp proteins when it is cultured at 23°C (11, 12, 14, 69). Considering the effects of the bacterial growth rate changes described above, and the observation that a temperature shift from 23°C to 34°C enhances the growth rate, we evaluated what the effect would be if 23°C cultures were started with bacteria that initially had even less metabolic activity. To do so, cultures that had been frozen at −80°C for over 1 month were used as inocula for cultures that were then incubated at 23°C. The hypothesis that environmental temperature directly influences OspC and Erp expression predicts that levels of those proteins will be the same in all 23°C cultures, independent of conditions previously experienced by the inoculum. Growth curve analyses of *B. burgdorferi* bacteria cultured at 23°C in complete medium indicated the same doubling times regardless of whether inocula were previously grown at 34°C or passaged directly from −80°C (Fig. 1).

Contrary to predictions of the temperature hypothesis, bacteria passaged from −80°C to 23°C produced appreciably larger amounts of OspC and Erp proteins than did bacteria passaged from 34°C to 23°C (Fig. 4). Comparing the cultures grown at −80°C to 23°C with those grown at 34°C to 23°C, the illustrated studies found 1.7-fold more OspC, 1.3-fold more ErpA, 4.6-fold more ErpM, and 1.8-fold more ErpY.

Cellular levels of BpaB, the *erp* repressor, were 1.6-fold higher in bacteria shifted from 34°C to 23°C than in bacteria shifted from −80°C to 23°C (Fig. 4). EbfC protein and *rpoS* mRNA were produced at low levels under both growth conditions (Fig. 3 and 4 and data not shown).

Control studies indicated that neither FlaB, OspA, nor OspB levels were affected by shifts from −80°C to 23°C or 34°C to 23°C (Fig. 4).

DISCUSSION

In order for the Lyme disease spirochete to infect a human or another vertebrate host, it is critical that bacteria within a tick detect when the arthropod is feeding on blood and then synthesize proteins appropriate for transmission and infection. In nature, *Ixodes* sp. ticks fast for several months between blood meals. Thus, *B. burgdorferi* bacteria within the midgut of an unfed tick experi-

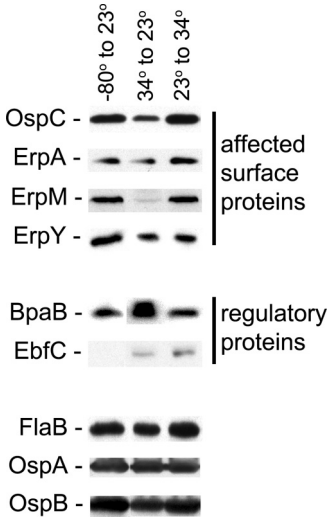

FIG 4 Absolute temperature does not control production of OspC or Erp proteins. *B. burgdorferi* bacteria were cultured in complete BSK-II medium under various temperature regimens, and protein contents were examined by immunoblotting. Conditions examined were cultures inoculated with bacteria that had been frozen for >1 month at −80°C and then cultured at 23°C (left lanes), cultures inoculated with bacteria that had been grown to late exponential phase at 34°C and then cultured at 23°C (center lanes), and cultures inoculated with bacteria that had been grown to late exponential phase at 23°C and then cultured at 34°C (right lanes). All cultures were harvested at late exponential phase. The constitutively expressed flagellar component FlaB served as a control, and fold changes of other proteins were calculated relative to the FlaB band intensities (44). Illustrated data for each condition are from analyses of the same paired bacterial lysates.

ence a nutrient-poor environment, and the bacteria probably do not grow or divide to any great extent (2). However, as the tick begins to feed on a host, nutrients in the ingested blood enable bacterial division at rates of approximately 1 to 2 h per cycle (1, 2, 70–74). This sudden change from low-level metabolism to rapid multiplication occurs only during transmission from tick to vertebrate host.

The initial hypothesis to explain the regulated expression of the *ospC* and *erp* operons proposed that bacteria sense environmental temperature, which changes from ambient to blood temperature during tick feeding (1, 11, 12). Cultivation at 23°C was hypothesized to represent the environment of an unfed tick, while a change in temperature from 23°C to 34°C represented the incoming warm blood meal. Results of the present studies contradict that notion and suggest that it be replaced with a new paradigm. The effects of changing culture conditions from −80°C to 23°C were similar to the effects of shifting temperatures from 23°C to 34°C, demonstrating that temperature *per se* cannot not be the primary cue controlling *ospC* and *erp* expression. However, both tested changes in culture temperatures increased the rate of bacterial growth and cell division. OspC and Erp production was also increased following shifts in culture medium composition that stimulated the growth rate, without any changes in temperature. Relating these observations to the natural infectious cycle, we hypothesize that *B. burgdorferi* uses changes in its own metabolic activity to determine when its vector tick is feeding on a vertebrate host. Since the change from slow to fast metabolism occurs only during the tick-to-vertebrate transmission stage, it would be an appropriate signal that *B. burgdorferi* must start producing factors involved in transmission through the tick and establishment of mammalian infection.

While the molecular mechanisms by which *B. burgdorferi* controls *ospC* and *erp* expression have not been completely elucidated, all current data support the hypothesized connection between levels of bacterial metabolism and gene expression. Transcription of *ospC* is dependent primarily upon the alternative sigma factor RpoS, which, in turn, is controlled by the Rrp2 response regulator, the Fur-like BosR DNA-binding protein, the RNA-binding protein CsrA, and an antisense RNA (47, 65, 75–84). Activation of Rrp2 is sensitive to levels of the metabolite acetate (32, 33). BosR responds to oxidizing conditions (79–81, 83, 85–87). The mechanisms controlling the antisense RNA or CsrA are not yet known, although in other bacterial species, CsrA is responsive to carbon source availability (82, 84). In addition, *rpoS* mutant *B. burgdorferi* bacteria are unable to transmit from feeding ticks to mammals, apparently due to defects in energy production (88). Borrelial *erp* transcription utilizes both RpoS and the housekeeping sigma factor RpoD, with some operons being dependent primarily upon RpoS and others not being detectably affected by this sigma factor (59, 60). *erp* transcription is also controlled by two DNA-binding proteins that compete for the *erp* operator: the repressor BpaB and the antirepressor EbfC (34, 58, 64, 89–91). *ebfC* is cotranscribed with *dnaX*, which encodes subunits of DNA polymerase, and both are positively regulated by the rate of bacterial multiplication (35). The present studies indicated that cytoplasmic concentrations of BpaB are inversely correlated with rates of bacterial replication (Fig. 2 and 4). Borrelial growth leads to production of 4,5-dihydroxy-2,3-pentanedione, also known as autoinducer-2, which positively affects production of Erp and other infection-associated *B. burgdorferi* proteins (42, 92–97). Taken together, these data support the hypothesis that the bacterial metabolic level is a key factor in the regulation of *B. burgdorferi ospC* and *erp* gene expression. Additional tests of this hypothesis are under way, including metabolic analyses of borreliae under various *in vitro* and *in vivo* conditions.

B. burgdorferi differentially expresses many proteins during its vertebrate-tick infectious cycle. We caution that bacterial metabolism levels may not be involved in the regulation of all borrelial proteins. Each gene and protein will need to be tested experimentally.

Evidence is accumulating that other pathogens control production of infection-associated factors in response to changes in their metabolism. As examples, *Salmonella enterica* senses its levels of ATP to control a virulence locus (98), while *Listeria monocytogenes* and *Legionella pneumophila* regulate virulence factor production in response to metabolites such as fatty acids and amino acids (99–101). The demonstrated effects of various metabolic activities in *B. burgdorferi* and other pathogens indicate that previously reported effects of temperature on other bacteria should be reexamined to determine the actual regulatory cue(s) (30).

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AUTHOR CORRECTION

Correction for Jutras et al., "Changes in Bacterial Growth Rate Govern Expression of the *Borrelia burgdorferi* OspC and Erp Infection-Associated Surface Proteins"

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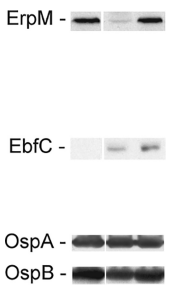
Volume 195, no. 4, p. 757–764, 2013, https://doi.org/10.1128/JB.01956-12. Page 761, Fig. 4: The published figure did not indicate splices introduced into the original image for several blots. In the corrected panels below, tooling has been added to indicate splicing.



Page 761, Fig. 4 legend: The following sentences should be added at the end: "For each protein immunoblot series, all of the illustrated lanes were taken from a single exposure of the same blot. Some intervening spaces were removed for clarity in the final figure, as indicated by white lines."

Correction of these errors does not change any of the results or conclusions of the article.

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