6

Adaptation to Glucosamine Starvation in *Borrelia burgdorferi* is Mediated by *recA*

Ryan G. Rhodes[1], Janet A. Atoyan[2] and David R. Nelson[2]
[1]*St. Bonaventure University, St. Bonaventure, NY*
[2]*University of Rhode Island, Kingston, RI*
USA

1. Introduction

Lyme borreliosis is a vector-borne disease confined primarily to the Northern hemisphere and caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato (s.l.) genospecies (Kurtenbach et al. 2006). The spirochetes are maintained exclusively within an enzootic cycle, alternating between a tick vector and vertebrate host. *B. burgdorferi* s.l. includes three pathogenic species and at least eight related species that are non-pathogenic or rarely cause infection in humans. The three pathogenic species include *B. burgdorferi* sensu stricto (s.s.), the only species found in North America, and *B. garinii* and *B. afzelii* the causative agents of borreliosis in Europe and Asia (Steere et al. 2004). These three species cause similar acute and chronic illness in humans, although regional differences do exist (Steere 2001).

B. burgdorferi is a limited genome organism and must scavenge most essential nutrients from its tick vector or vertebrate host (Das 2000; Saier MH 2000). The organism requires a complex medium for propagation in the laboratory, and specific addition of free N-acetyl-glucosamine (GlcNAc), a component of the bacterial cell wall, is necessary for cells to reach optimal cell densities in a single exponential phase (Barbour 1984; Tilly et al. 2001; Rhodes et al. 2009; Rhodes et al. 2010). In the absence of free GlcNAc *B. burgdorferi* can degrade chitin (a polymer of GlcNAc) to chitobiose and import this dimer through a dedicated chitobiose phosphotransferase system to satisfy its requirement for exogenous GlcNAc (Rhodes et al. 2010). However, in the absence of both free GlcNAc and chitin this spirochete exhibits a unique biphasic growth pattern in which the second exponential phase is dependent on the expression of the chitobiose transporter (Tilly et al. 2001; Rhodes et al. 2009; Rhodes et al. 2010). In the first exponential phase cells grow to approximately 2.0×10^6 cells ml^{-1} within three days by exhausting the small amount of free GlcNAc and/or GlcNAc oligomers from complex medium components such as rabbit serum and yeastolate. After utilizing these sources of GlcNAc cells exhibit a death phase in which cell density decreases below 1.0×10^5 cells ml^{-1} between days three and five. Finally, a second exponential phase is initiated between days five and seven in which cells grow to near optimal cell density (~5.0×10^7 cells ml^{-1}).

This peculiar growth pattern in the absence of free GlcNAc or chitin suggests there is a sequestered (or bound) source of GlcNAc within the medium that cells can access in the second exponential phase, but are unable to utilize in the first exponential phase. While this

source remains unknown, as well as the mechanism(s) used to obtain it, our data demonstrating the utilization of chitin by *B. burgdorferi* in the absence of free GlcNAc suggests that polymerized GlcNAc is not the source (Rhodes et al. 2010). In addition, it is unclear whether the adaptation to GlcNAc starvation (i.e. second exponential phase growth) is physiologic or genetic in nature. A previous report by Tilly et al. (Tilly et al. 2001) suggested that second exponential phase growth was a physiologic response, as cells from the second exponential phase exhibited biphasic growth after being transferred to fresh medium lacking GlcNAc. However, in that experiment starvation-adapted cells grew almost 10-fold higher in the first exponential phase as compared to cells that were not previously exposed to the starvation condition, leaving open the possibility that second exponential phase growth is due to the outgrowth of a mutant population.

Stress-induced mutagenesis occurring in bacteria under growth-limiting conditions is a phenomenon that has been intensively studied in recent years. Nearly two decades ago Cairns and Foster described adaptive reversion using an *Escherichia coli* Lac assay (Cairns & Foster 1991). In this system cells carrying a *lacI-lacZ* fusion gene with a +1 frameshift mutation in *lacI* were unable to utilize lactose, but when cultured with a non-lactose carbon source and then spread onto lactose plates, Lac+ revertants were obtained. Characterization of these stress-induced mutants revealed they were of two types: i) point mutants carrying a compensatory frameshift mutation in *lac* (Foster & Trimarchi 1994; Rosenberg et al. 1994) or ii) *lac*-amplified cells in which the leaky *lac* frameshift allele was amplified 20-50 times (Hastings et al. 2000; Powell & Wartell 2001; Kugelberg et al. 2006; Slack et al. 2006). Substantial work has been conducted to elucidate the underlying molecular mechanisms responsible for these two distinct types of stress-induced mutants. Formation of the Lac+ point mutants can occur via several stress-response and DNA repair and metabolism pathways, including i) homologous recombination via double-strand-break-repair (DSBR) proteins (Cairns & Foster 1991; Harris et al. 1994), ii) error-prone DNA replication (McKenzie et al. 2001), iii) the SOS response (McKenzie et al. 2000), iv) the RpoS starvation-stress response (Lombardo et al. 2004), and v) methyl-directed mismatch repair (MMR) (Harris et al. 1999). In contrast, *lac*-amplification does not require error-prone DNA replication or the SOS response (McKenzie et al. 2001), but does require the general stress-response regulator RpoS and homologous recombination via DSBR proteins such as RecA, RecBCD and RuvABC (Slack et al. 2006). While *B. burgdorferi* has a limited genome and lacks error-prone DNA polymerases and the SOS pathway, it is possible that stress-induced mutagenesis occurs in this organism and could be mediated by mismatch repair, RpoS and/or homologous recombination.

Several studies have been conducted in *B. burgdorferi* to evaluate the function of RecA both *in vitro* and *in vivo* (Liveris et al. 2004; Putteet-Driver et al. 2004; Liveris et al. 2008). Early attempts to generate a *recA* null mutant were unsuccessful; therefore transgenic expression of *B. burgdorferi* RecA in *E. coli* was used to determine its functional properties (Liveris et al. 2004; Putteet-Driver et al. 2004). In *E. coli* RecA has three major activities: i) it participates in homologous recombination by catalyzing DNA strand exchange, ii) it promotes the autocatalytic cleavage of LexA, a repressor of the SOS response, and iii) it directly facilitates bypass of DNA lesions by DNA polymerase V during the SOS response (Cox 2007). Complementation of an *E. coli recA* null mutant with *B. burgdorferi recA* restored the ability of the mutant to promote homologous recombination and activated the *E. coli* SOS pathway in response to DNA-damaging agents (Liveris et al. 2004; Putteet-Driver et al. 2004). In a

more recent study, Liveris *et al* (Liveris et al. 2008) successfully generated a *recA* mutant in a low passage infectious *B. burgdorferi* strain and showed that RecA mediates homologous recombination in this organism. However, this report demonstrated RecA is only minimally involved in DNA repair in response to DNA damaging agents, and does not mediate antigenic variation at the *vlsE* locus after mammalian infection.

As described above, a previous attempt to characterize *B. burgdorferi* second exponential phase growth in the absence of free GlcNAc has left unanswered questions as to whether this is a physiologic or genetic adaptation to the starvation condition. In this study we follow the growth of wild-type and *recA* mutant cells in the absence of free GlcNAc and suggest that growth in the second exponential phase is the result of a genetic adaptation that is, at least in part, mediated by RecA. Additionally, we characterize the *recA* transcriptional unit and provide evidence suggesting this gene is transcribed from two separate promoters.

2. Results

2.1 Adaptation to GlcNAc starvation

Wild-type cells cultured in the absence of free GlcNAc exhibit biphasic growth (Fig. 1A), with growth in the second exponential phase dependent on the expression of the chitobiose transporter *chbC* (Tilly et al. 2001; Rhodes et al. 2010). To determine if cells in the second exponential phase were adapted to grow in the absence of free GlcNAc we followed cell growth in BSK-II lacking GlcNAc. Cells were diluted to 1.0×10^5 cells ml^{-1} in the same medium at various time points along the biphasic growth curve, and growth of the new cultures was followed to determine if biphasic growth occurred (Fig. 1B and C). Diluting cells during the first exponential phase on day 2 (46 h) or at the peak of the first exponential phase on day 3 (72 h) into BSK-II lacking GlcNAc resulted in biphasic growth (Fig. 1B). In contrast, cells diluted from the second exponential phase on days 8-11 (191, 216, 237 and 263 h) into BSK-II lacking GlcNAc resulted in growth to high cell densities in a single exponential phase, with cell densities reaching $\geq 2.5 \times 10^7$ cells ml^{-1} (Fig. 1C). This cell density is equivalent to that reached by cells in the second exponential phase when cultured in BSK-II lacking free GlcNAc, though about one-tenth the cell densities reached by cells cultured in complete BSK-II. In some experiments cells diluted from the second exponential phase were followed for extended periods of time after reaching their peak cell density (~2.5×10^7 cells ml^{-1}) in a single exponential phase, and while there was a decline in cell density a second exponential phase was not observed as previously described (Tilly et al. 2001).

2.2 Adaptation to GlcNAc is the result of a mutational event

Two possible explanations for second exponential phase growth in a GlcNAc depleted medium are that: 1) second exponential phase growth occurs as a result of a physiological adaptation to GlcNAc starvation as cells induce genes to utilize sequestered GlcNAc; or that 2) second exponential phase growth occurs as the result of a mutational event that allows for the outgrowth of a mutant population able to obtain sequestered GlcNAc from a source they were previously unable to utilize. If second exponential phase growth was the result of a mutational event, it would be expected that cells removed from the second exponential phase would retain the ability to grow in a single exponential phase in BSK-II lacking

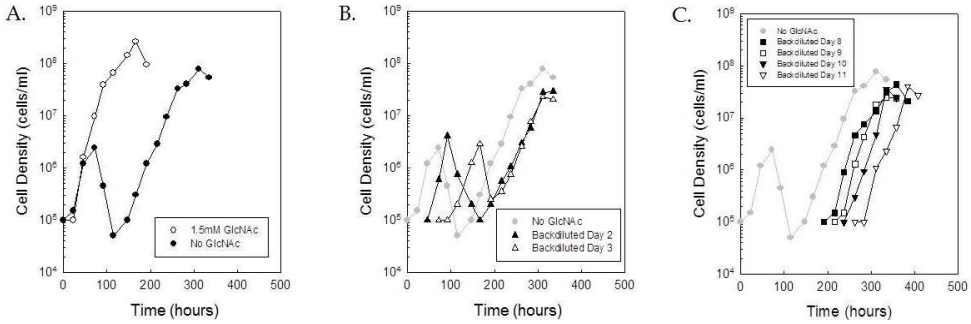


Fig. 1. Adaptation of B31-A to GlcNAc starvation. (A) Growth of B31-A in BSK-II with and without 1.5mM GlcNAc. Late-log phase cells were diluted to 1.0×10^5 cells ml^{-1} in complete BSK-II (open circle) or BSK-II lacking GlcNAc (closed circle), and cells were enumerated daily by darkfield microscopy. (B) Growth of B31-A in BSK-II without GlcNAc after dilution from the first exponential phase of a culture starved for GlcNAc. Cells starved for GlcNAc (closed gray circle) were diluted to 1.0×10^5 cells ml^{-1} at day 2 (closed triangle) and day 3 (open triangle) into BSK-II without GlcNAc, and growth was followed daily by darkfield microscopy. (C) Growth of B31-A in BSK-II without GlcNAc after dilution from the second exponential phase of a culture starved for GlcNAc. Cells starved for GlcNAc (closed gray circle) were diluted to 1.0×10^5 cells ml^{-1} at day 8 (closed square), day 9 (open square), day 10 (inverted closed triangle) and day 11 (inverted open triangle) into BSK-II without GlcNAc, and growth was followed daily by darkfield microscopy. These growth curves are representative of four independent growth experiments.

GlcNAc after serial passage in complete BSK-II. On the other hand, if a physiologic mechanism was responsible, then it would be expected that cells removed from the second exponential phase and subjected to serial passage in a complete medium would still exhibit biphasic growth when transferred back to a medium lacking GlcNAc. To test this, cells were diluted from the second exponential phase into complete BSK-II, serially passaged up to twelve times in this complete medium, and then re-evaluated for biphasic growth after being transferred back to BSK-II lacking GlcNAc (Fig. 2). Despite being cultured in the presence of free GlcNAc for up to twelve passages, cells exhibited only one exponential phase upon re-introduction to BSK-II lacking GlcNAc. Interestingly, cells diluted into BSK-II without GlcNAc after 7 and 12 serial passages in complete BSK-II grew to a higher cell density (~5-fold) than cells cultured in complete BSK-II for 1 or 2 passages.

To further demonstrate that adaptation to GlcNAc starvation is the result of a genetic event, cells were plated on BSK-II agar and the growth of wild-type or starvation-adapted clones was followed. Specifically, wild-type cells cultured in complete BSK-II and starvation-adapted cells serially passaged seven times in complete BSK-II (P. 7 No GlcNAc from Fig. 2; closed squares) were plated on BSK-II agar containing free GlcNAc to obtain isolated colonies. Ten clones from each were transferred to BSK-II broth lacking GlcNAc and growth was followed daily. All ten clones from wild-type cells not previously adapted to GlcNAc starvation exhibited biphasic growth when cultured in the absence of free GlcNAc (Fig. 3A). In contrast, eight of the ten clones obtained from cells previously adapted to GlcNAc starvation and serially passaged seven times in complete BSK-II grew to optimal cell densities in a single exponential phase when cultured in BSK-II lacking free GlcNAc (Fig. 3B).

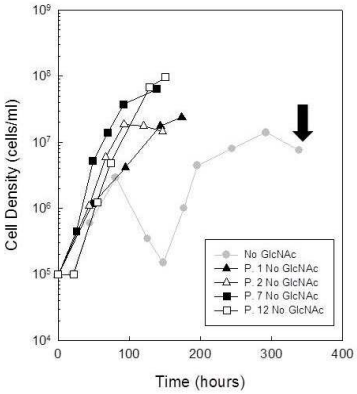


Fig. 2. Growth of starvation-adapted cells in BSK-II without GlcNAc after repeated subculture in complete BSK-II. B31-A cells grown in BSK-II without added GlcNAc (closed gray circle) were transferred (arrow indicates point at which adapted cells were sub-cultured) to complete BSK-II. After one passage in complete BSK-II cells were diluted to 1.0 × 10^5 cells ml^{-1} in BSK-II without GlcNAc (P. 1; closed triangle) and growth was followed daily by darkfield microscopy. Growth was also followed in BSK-II without GlcNAc after two (P. 2; open triangle), seven (P. 7; closed square) and twelve (P. 12; open square) serial passages in complete BSK-II. This is a representative experiment that was repeated twice.

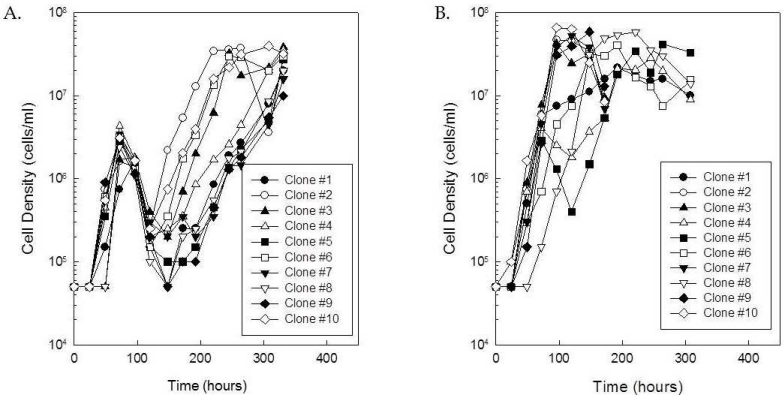


Fig. 3. Growth of GlcNAc starvation adapted and non-adapted clones in BSK-II without GlcNAc. (A) B31-A cells cultured in complete BSK-II (non-adapted) were plated on BSK-II agar (containing GlcNAc) and 10 clones were transferred to BSK-II broth without GlcNAc. Growth was followed daily by darkfield microscopy. (B) B31-A cells adapted to GlcNAc starvation and serially passaged in complete BSK-II were plated on solid medium and 10 clones were transferred to liquid BSK-II without GlcNAc. Growth was followed daily by darkfield microscopy.

It is possible that the two clones (clones #4 and #5 from Fig. 3B) from GlcNAc starvation-adapted cells exhibited biphasic growth as the result of a second-site mutation or it may be that a sub-population of non-adapted cells was able to persist in the absence of GlcNAc as

the result of the mutant population releasing sequestered GlcNAc from an as yet unknown medium component. In an attempt to clarify this point, cells from starvation-adapted clone #10 (Fig. 3B; designated RR45) were passaged twice in complete BSK-II, and then plated on BSK-II agar with GlcNAc. Ten clones were transferred to BSK-II broth without GlcNAc and growth was followed daily by darkfield microscopy. In addition, wild-type cells cultured in complete BSK-II were plated on BSK-II agar with GlcNAc, and the growth of ten clones was followed in liquid BSK-II without GlcNAc (Fig. 4A). As previously demonstrated, the ten wild-type clones exhibited biphasic growth (Fig. 4A). In contrast, all ten RR45 clones grew to optimal cell densities in a single exponential phase (Fig. 4B).

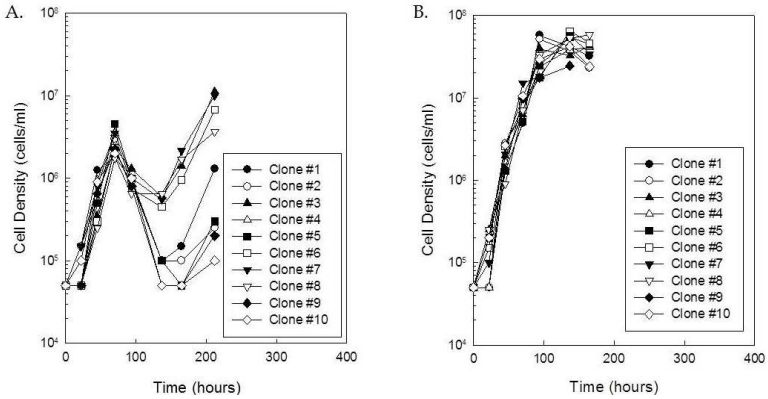


Fig. 4. Growth of GlcNAc starvation adapted RR45 and non-adapted wild-type clones in BSK-II without GlcNAc. (A) B31-A cells cultured in complete BSK-II (non-adapted) were plated on complete BSK-II agar and 10 clones were transferred to liquid BSK-II without GlcNAc. Growth was followed daily by darkfield microscopy. (B) RR45 cells (clone #10 from Fig. 3B) were plated on complete BSK-II agar and 10 clones were transferred to liquid BSK-II without GlcNAc. Growth was followed daily by darkfield microscopy.

2.3 Construction and characterization of a *B. burgdorferi recA* mutant and two complemented mutants

As double-strand-break-repair (DSBR) proteins, such as RecA, have been shown to be involved in stress-induced mutation in *E. coli* (He et al. 2006), we hypothesized that *recA* may play a role in the ability of *B. burgdorferi* to adapt to GlcNAc starvation. Therefore, we generated a *recA* deletion mutant in the B31-A background by allelic exchange, and designated this strain JA10 (see Methods). To confirm that RecA function was inactivated, we examined survival after exposure to DNA-damaging agents, as well as the ability to promote homologous recombination. Recently, Liveris et al. (Liveris et al. 2008) demonstrated that a low-passage infectious *B. burgdorferi recA* mutant was only moderately sensitive to DNA-damaging agents (UV irradiation and mitomycin C), but showed that RecA was essential for allelic exchange in this organism. We confirmed those results here using a high-passage non-infectious *recA* mutant (JA10) which showed no significant difference in sensitivity to DNA-damaging agents when compared to wild-type cells (data not shown), but was unable to promote homologous recombination (Table 1). Recombination frequency was determined by transforming each strain with pBB0450.1

	Transformation Frequency[a]		Recombination Frequency[b]	
Strain	*n*[c]	Average (±SD)	*n*[d]	Average (±SD)
B31-A	4	6.7×10^{-5} ($\pm 7.2 \times 10^{-5}$)	5	3.3×10^{-6} ($\pm 1.8 \times 10^{-6}$)
JA10	3	5.4×10^{-5} ($\pm 7.5 \times 10^{-5}$)	5	0 (± 0)
JA15	2	5.6×10^{-5} (ND[e])	4	0 (± 0)
JR1	7	3.4×10^{-7} ($\pm 2.6 \times 10^{-7}$)	7	6.5×10^{-6} ($\pm 1.2 \times 10^{-5}$)

[a] Transformation frequency is the number of transformants obtained after electroporation of cells with ErmC/pCE320 divided by the number of CFU obtained without antibiotic selection
[b] Recombination frequency is the number of recombinants obtained after electroporation of cells with pBB0450.1 (*rpoN*::*ermC*) divided by the number of CFU obtained without antibiotic selection
[c] Number of transformation experiments performed with ErmC/pCE320 to determine transformation frequency.
[d] Number of transformation experiments performed with pBB0450.1 to determine recombination frequency.
[e] ND, Not determined

Table 1. Transformation and recombination frequencies of the wild type (B31-A), *recA* mutant (JA10) and *recA* complemented mutants (JA15 and JR1).

(*ermC*::*rpoN*), a suicide vector carrying a mutant *rpoN* allele that confers erythromycin resistance. The wild-type strain exhibited a recombination frequency of 3.3×10^{-6} (Table 1), while no recombinants were observed for the *recA* mutant. It is possible, though unlikely, that the lack of recombination observed in the *recA* mutant was due to the inability of the plasmid to gain entry into the cell during electroporation. Therefore, we determined the transformation frequency of all strains by modifying the pCE320 *B. burgdorferi* shuttle vector to confer erythromycin resistance, and transformed cells with this new plasmid designated ErmC/pCE320. Transformation frequency was similar for both the wild-type and *recA* mutant strains (6.7×10^{-5} and 5.4×10^{-5}, respectively; Table 1), suggesting that the lack of recombinants observed after transformation with pBB0450.1 was due to the absence of a functional *recA* gene.

To provide further evidence that the *recA* gene in JA10 was inactivated, the mutant was complemented with plasmid pBB0131comp.2 which consists of the wild-type *recA* gene with 512 bp of upstream DNA, presumably encompassing its natural promoter, cloned into the *B. burgdorferi* shuttle vector pBSV2 (see Methods). This strain was designated JA15 and transformation and recombination frequencies were determined as described above. The JA15 complemented mutant exhibited a transformation frequency similar to that observed for the wild-type and *recA* mutant strains (5.6×10^{-5}; Table 1). However, this strain unexpectedly exhibited no homologous recombination, suggesting the 512 bp of DNA upstream of the *recA* translational start site may not be sufficient for expression of *recA* at wild-type levels. Therefore, we generated a second *recA* complemented mutant using plasmid pRecA.3 consisting of the wild-type *recA* gene fused to the constitutive *B. burgdorferi flaB* promoter and cloned into the shuttle vector pCE320. This strain was designated JR1 and transformation and recombination frequencies were determined. While

this strain exhibited a marked reduction in transformation frequency compared to the wild-type strain, RecA function was restored as JR1 demonstrated a 2-fold greater recombination frequency as compared to B31-A (Table 1). The reduction in transformation frequency observed in JR1 is likely due to the incompatibility of pRecA.3 and ErmC/pCE320 as they are both derivatives of the pCE320 shuttle vector.

2.4 Expression of *recA* determined by quantitative RT-PCR (qRT-PCR)

One possible explanation for the lack of recombination observed in the JA15 strain is that *recA* transcription was reduced, leading to decreased levels of functional RecA protein. To test this, we evaluated *recA* expression in B31-A, JA10, JA15 and JR1 using qRT-PCR (Table 2). RNA was extracted from late-log phase cells (~2.0×10^7 cells ml^{-1}) and the amount of *recA* transcript present in 10 ng of total RNA was determined. As expected, *recA* transcript levels in the JA15 complemented mutant were 61.5-fold lower than those measured in wild-type cells, possibly accounting for the lack of recombination observed in this strain. In contrast, *recA* transcript levels in the JR1 complemented mutant, in which *recA* expression was driven by a constitutive promoter, were 15.7-fold higher than the wild type. No *recA* transcription was detected in JA10 cells. These results may explain the 2-fold increase observed in recombination frequency in JR1 compared to the wild type. It is important to note that despite the differences in *recA* levels in these different strains, the growth rate between strains did not vary and the growth curves in complete BSK-II were indistinguishable (data not shown).

Strain	*recA* Copy Number Average[a] (±SD[b])	Fold Difference[c]
B31-A	8.49×10^5 ($\pm 1.57 \times 10^5$)	1
JA10	ND[d]	ND
JA15	1.38×10^4 ($\pm 2.03 \times 10^3$)	0.016
JR1	1.33×10^7 ($\pm 2.69 \times 10^6$)	15.7

[a] Average *recA* copy number in 10 ng of total RNA from three biological replicates
[b] SD, standard deviation
[c] Fold change in average copy number from wild-type levels
[d] ND, Not detected

Table 2. *recA* transcript levels.

2.5 Characterization of the *recA* transcriptional unit

Analysis of the *B. burgdorferi* genome reveals two genes (BB0132 and BB0133) immediately upstream of *recA* (BB0131) that are oriented in the same direction (Fig. 5A). The reduced expression of *recA* in the JA15 complemented mutant may be the result of regulatory elements encoded outside of the 512 bp of upstream DNA amplified to generate the complementation construct pBB0131comp.2. It is also possible, given the organization of the flanking genes, that *recA* is co-transcribed with one or both of the upstream genes as part of

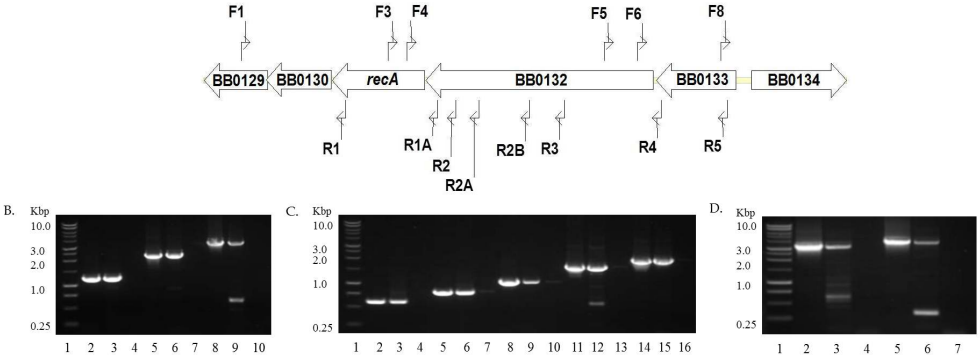


Fig. 5. Organization of *recA* and flanking genes. (A) Arrangement of the chromosomal region spanning genes BB0129 to BB0134 (7628 bp). Labeled arrows indicate the antisense and sense primers used for RT-PCR. (B) RT-PCR of B31-A RNA using a BB0129 antisense strand primer (F1) and sense strand primers R1 (lane 3), R1A (lanes 6) and R2B (lane 9). Positive control PCR reactions using B31-A genomic DNA as a template (lanes 2, 5 and 8) and no RT controls (lanes 4, 7 and 10) were also performed for each primer pair. (C) RT-PCR of B31-A RNA using a BB0131 (*recA*) antisense strand primer (F3) and sense strand primers R1A (lane 3), R2 (lane 6), R2A (lane 9), R2B (lane 12) and R3 (lane 15). Positive control PCR reactions using B31-A genomic DNA as a template (lanes 2, 5, 8, 11 and 14) and no RT controls (lanes 4, 7, 10, 13 and 16) were performed for each primer pair. (D) RT-PCR of B31-A RNA using a BB0131 (*recA*) antisense strand primer F3 with sense strand primers R4 (lane 3) and R5 (lane 6). Positive control PCR reactions using B31-A genomic DNA as a template (lanes 2 and 5) and no RT controls (lanes 4 and 7) were performed for each primer pair. A 1 kb ladder was used as a size standard (lane 1 of B, C and D).

an operon, and that the low level of expression observed in JA15 was due to a weak internal promoter. As previous studies have not evaluated the organization of the *recA* transcript in *B. burgdorferi*, we attempted to shed light on these questions by characterizing the *recA* transcriptional unit and identifying the *recA* promoter(s).

RT-PCR was used to determine if *recA* is co-transcribed with one or more of its neighboring genes (Fig. 5B-D). Antisense primers (F1 and F3; see Fig. 5) were used to generate cDNA from points originating in the downstream gene BB0129 and within the *recA* coding region. The cDNA was amplified in PCR reactions using the antisense primer and various sense primers to determine if mRNA transcripts spanned the junctions between adjacent genes. To determine if *recA* is co-transcribed with downstream genes, cDNA generated using primer F1 was PCR amplified using the same antisense primer and primers R1, R1A and R2B (Fig. 5B). All primer pairs produced products of the correct size when compared to positive controls, indicating the presence of an RNA transcript spanning genes BB0129-BB0132 and suggesting *recA* is co-transcribed with at least two downstream genes. In addition, the positive RT product obtained with primers F1 and R2B (lane 9; Fig. 5B) suggested that *recA* may be part of a larger operon encompassing one or both upstream genes, as the R2B primer was located 1.2 kb upstream of the *recA* translational start site. To confirm that *recA* is co-transcribed with BB0132, cDNA was generated using antisense primer F3 and PCR amplified using the same antisense primer and sense primers R1A, R2, R2A, R2B and R3

(Fig. 5C). An RT product of the correct size was obtained with all primer pairs when compared to the positive controls, confirming the results obtained with antisense primer F1 (Fig. 5B) and strongly suggesting that *recA* is co-transcribed with BB0132. To determine if BB0133 is also part of this transcriptional unit, cDNA generated with antisense primer F3 was PCR amplified using the same antisense primer and sense primers R4 and R5 (Fig. 5D). An RT product of the correct size was obtained with both primer pairs when compared to the positive controls, providing evidence that BB0133 is also part of this operon. Taken together, these RT results strongly suggest *recA* is co-transcribed with the upstream genes BB0132 and BB0133 and at least two downstream genes (BB0130 and BB0129).

2.6 5' RACE and promoter analysis

The RT results presented above strongly suggest that *recA* is co-transcribed with the upstream genes BB0132 and BB0133 (Fig. 5B-D); however, we did observe reduced expression of *recA* in our mutant complemented with the wild-type gene and 512 bp of upstream DNA (Table 2). Together, these results suggest two *recA* transcripts may exist, one beginning upstream of BB0133 and the other beginning within the 512 bp immediately upstream of the *recA* gene. Therefore, we used 5′ RACE to identify the two *recA* transcritptional start sites, and compared the promoter regions upstream of the start sites to previously identified RpoD, RpoS and RpoN-dependent promoter sequences in *B. burgdorferi* (Studholme & Buck 2000; Caimano et al. 2007; Rhodes et al. 2009).

To identify the transcriptional start site immediately upstream of the *recA* gene, total RNA was extracted from B31-A cells cultured to late log phase (~2.0×10^7 cells ml^{-1}) in complete BSK-II and cDNA was generated using antisense primer F3 (Fig. 5A) located within the *recA* coding region. The cDNA was column purified and a homopolymeric A-tail was added using terminal transferase. PCR amplification of the A-tailed cDNA with nested gene specific primer F4 and an oligo dT-anchor primer resulted in a product of approximately 250 bp (lane 2; Fig. 6A). The DNA band was extracted from the gel and sequenced with primer F4, and the transcriptional start site was determined to be 44 or 45 bp upstream of the *recA* translational start (Fig. 6C). We attempted to clarify the start site using G-tailed cDNA, but were unable to obtain reliable sequence data for this PCR product due to mispriming of the oligo dC-anchor primer to three guanosines (underlined in Fig. 6C) located 29-31 bp upstream of the *recA* translational start site (data not shown). Based on the sequence data obtained from the A-tailed reaction we identified the putative -10 and -35 promoter regions immediately upstream of the *recA* gene. Comparison of these putative promoter regions with previously described *B. burgdorferi* promoter consensus sequences revealed similarity to the RpoD-dependent consensus promoter, as homology was observed in five of the six bases within the -35 region and seven of the eleven bases within the extended -10 region (Fig. 7).

To determine the transcriptional start site upstream of BB0133, total RNA was extracted from B31-A cells cultured to late log phase (~2.0×10^7 cells ml^{-1}) in complete BSK-II, and cDNA was generated using antisense primer F5 (Fig. 5A) located within BB0132. A homopolymeric A-tail was added and PCR of the A-tailed cDNA with nested gene specific primer F6 and the oligo dT-anchor primer resulted in a 1.2 kb product (Fig. 6B). The DNA band was gel extracted and sequenced with nested primer F8, and the transcriptional start site was determined to be 45 or 46 bp upstream of the BB0133 translational start site (Fig. 6D). Both G-tailed and C-tailed cDNA were used to try and resolve this ambiguity, but PCR

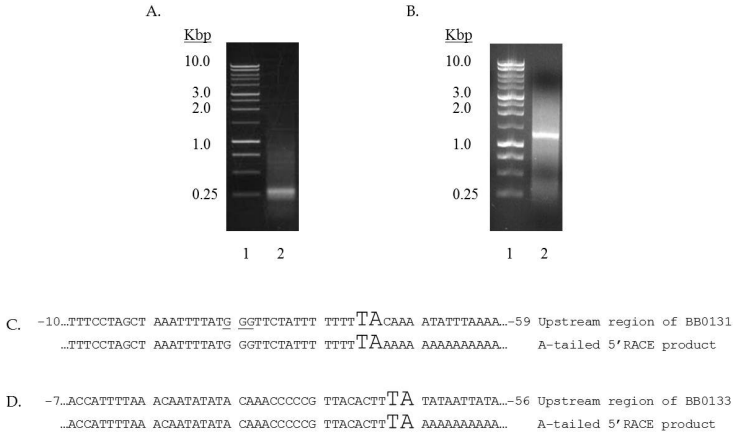


Fig. 6. Identification of the transcriptional start sites of the *recA* transcript. 5′ RACE was used to determine the approximate transcriptional start sites of the *recA* transcript. A-tailed PCR products obtained using primer F4 (A; lane 2) or primer F6 (B; lane 2) with the oligo dT-anchor primer were separated by gel electrophoresis on a 1% TAE agarose gel. A 1 kb ladder was used as a size standard (lane 1). (C) DNA sequence of the dA-tailed 5′ RACE product obtained with primer F4 showing the approximate transcriptional start site (enlarged font). Underlined guanosines indicate site of mispriming with the oligo dC-anchor primer and dG-tailed 5′ RACE product. (D) DNA sequence of the dA-tailed 5′ RACE product obtained with primer F6 showing the approximate transcriptional start site (enlarged font).

Promoter	-35 Region							Extended -10 Region											Reference
Predicted *recA* promoters																			
Upstream of *recA*	T	T	G	T	T	A	-10-	T	A	A	T	T	T	T	A	A	A	T	This Study
Upstream of BB0133	T	T	G	A	C	T	-11-	A	A	T	G	A	T	A	T	A	A	T	This Study
RpoD-dependent	T	T	g/a	a/t	a/c	A		T	A	T	G	a/t	T	A	T	A	a/c	T	[8]
RpoS-dependent	T	T	G	A	a/t	t/a		T	G	g/a	g/a	A	T	A	t/a	A	T	T	[26]
RpoS and RpoD -dependent	T	T	G	A	g/a	N		t/a	G	a/c	A	c/g	T	A	a/t	a/g	T	T	[8]
RpoN-dependent	T	G	G	C	A	C	N	N	N	N	N	T	T	G	C	W			[27]
		-24													-12				

Fig. 7. Identification of the putative *recA* promoters. Comparison of the putative *recA* promoters with the RpoD, RpoS and RpoN-dependent promoters in *B. burgdorferi*. Nucleotide positions in the predicted *recA* promoters that match the consensus RpoD-dependent promoter sequence are highlighted.

amplification with the nested primer F6 and the complementary-tailed anchor primers (C or G, respectively) did not produce a PCR product (data not shown). Therefore, we used the approximate transcriptional start site determined from the A-tailed cDNA to identify the putative -10 and -35 promoter regions. Comparison to *B. burgdorferi* consensus promoters suggests this operon is under the control of an RpoD-dependent promoter as homology was observed between five of the six bases within the -35 region and ten of eleven bases within the extended -10 region (Fig. 7).

2.7 Growth of the *recA* mutant in BSK-II without GlcNAc

Growth analysis of wild-type *B. burgdorferi* adapted to GlcNAc starvation suggests this organism undergoes a mutational event that allows growth to high cell densities in a single exponential phase in the absence of free GlcNAc (Figs. 2-4). Previous reports have demonstrated that DSBR proteins play a role in stress-induced mutations observed in other organisms (for reviews see (Galhardo et al. 2007; Hastings 2007)). Therefore, we evaluated the ability of a *recA* mutant in *B. burgdorferi* (JA10) to grow and adapt to GlcNAc starvation (Fig. 8). Late log phase *recA* mutant (JA10) cells cultured in complete BSK-II were diluted to 1.0×10^5 cells ml^{-1} in BSK-II lacking GlcNAc. Growth of the *recA* mutant prior to adaptation to GlcNAc starvation resulted in biphasic growth similar to wild-type cells that were not previously adapted to GlcNAc starvation (compare Fig. 8 open circles to Fig. 1A open circles). Similarly, when the *recA* mutant was diluted from the second exponential phase of a GlcNAc-starved culture (Fig. 8; 300 h) into the same medium biphasic growth was still observed (Fig. 8 open and closed triangles). This is in contrast to the single exponential phase typically observed in starvation-adapted wild-type cells that were cultured in the absence of GlcNAc (Fig. 1C).

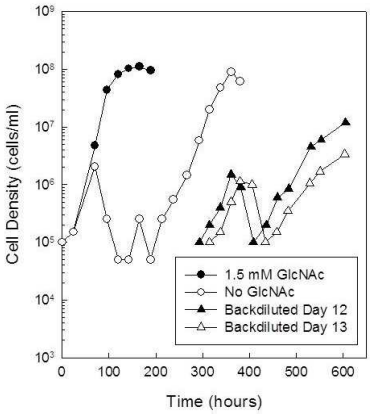


Fig. 8. Growth of JA10 (*recA* mutant) after adaptation to GlcNAc starvation. Growth of JA10 in BSK-II with and without 1.5 mM GlcNAc, and growth of JA10 cells after adaptation to GlcNAc starvation. Late log phase cells were diluted to 1.0×10^5 cells ml^{-1} in complete BSK-II (open circle) or BSK-II lacking GlcNAc (closed circle), and cells were enumerated daily by darkfield microscopy. Cells adapted to GlcNAc starvation were diluted (arrows) to 1.0×10^5 cells ml^{-1} at Day 12 (closed triangle) and Day 13 (open triangle) in BSK-II without GlcNAc, and growth was followed daily by darkfield microscopy.

Multiple growth experiments evaluating adaptation to GlcNAc starvation (i.e. dilution from the second exponential phase) were performed for each strain (Table 3). The growth experiment was repeated 15 times with *recA* mutant cells, and biphasic growth was observed each time cells were diluted from the second exponential phase of a culture starved for GlcNAc. This is in contrast to wild-type cells which exhibited biphasic growth only 3 times in 33 separate growth experiments, and reached high cell densities in a single exponential phase in 30 separate experiments.

Strain	Number of Growth Exp.[a]	Adapted[b]	Not Adapted[c]	Percent Adapted[d]
B31-A	33	30	3	91
JA10	15	0	15	0
JA15	17	5	12	29
JR1	13	3	10	23

[a] Number of independent growth experiments conducted in which cells were diluted from BSK-II lacking GlcNAc into the same medium
[b] Adapted – the number of times cells reached high cell density (1-2 × 10^7 cells ml^{-1}) in a single exponential phase
[c] Not Adapted – the number of times biphasic growth was observed
[d] Percent Adapted calculated as the number adapted (b) divided by the total number of experiments (a) multiplied by 100

Table 3. Adaptation to GlcNAc starvation.

To provide further evidence that RecA plays a role in the adaptation to GlcNAc starvation, we evaluated growth after GlcNAc starvation in the *recA* complemented mutants. Growth of the JA15 complemented mutant was followed in 17 separate experiments and single exponential phase growth to high cell densities in the absence of free GlcNAc was observed 5 times. Similarly, the JR1 complemented mutant was evaluated in 13 separate growth experiments and exhibited a single exponential phase in BSK-II lacking GlcNAc 3 times. Taken together, these results suggest RecA plays a role in adaptation to GlcNAc starvation and that the level of RecA expression may be important in the process.

3. Discussion

B. burgdorferi exhibits a unique biphasic growth pattern when cultured in the absence of free GlcNAc, with cells reaching optimal density in the second exponential phase. However, it had not been conclusively determined whether a physiologic or genetic adaptation mechanism is responsible for second exponential phase growth. This study was conducted to address this question as well as identify genes important to the process. We provide evidence that adaptation to GlcNAc starvation in *B. burgdorferi* is the result of a genetic event, and suggest a role for the homologous recombination protein RecA. In addition, we characterized the *recA* transcript and showed that transcription occurs from two different promoter sites.

In a previous report, Tilly et al (Tilly et al. 2001) suggested that the biphasic growth observed during *B. burgdorferi* cultivation in the absence of free GlcNAc was the result of a physiologic adaptation, as cells diluted from the second exponential phase into fresh medium lacking GlcNAc still exhibited biphasic growth. However, careful inspection of the growth curve from that study revealed that starvation-adapted cells grew nearly 10-fold higher in the first exponential phase after re-introduction into BSK-II lacking GlcNAc, than cells that were not previously exposed to the starvation condition (Tilly et al. 2001). To us, these results suggested that the adaptation that permitted second exponential phase growth was maintained by cells after passage in the same medium. We followed the growth of cells transferred from the second exponential phase into BSK-II lacking GlcNAc and found that

wild-type cells grew to a high cell density in a single exponential phase (Fig. 1C) in 30 out of 33 independent growth experiments (Table 3). This indicated that adaptation to the starvation condition was retained by the cells in nearly all of the experiments. However, it was not clear whether the adaptation was physiologic or genetic, as the starvation-stress condition was never removed (i.e. cells were only transferred from the second exponential phase directly into media lacking GlcNAc). Since other microorganisms exhibit stress-induced mutagenesis in response to nutrient deprivation and other stresses (for reviews see (Finkel 2006; Galhardo et al. 2007; Hastings 2007)), we hypothesized that *B. burgdorferi* may use similar mechanisms to adapt to GlcNAc starvation.

To determine if adaptation to GlcNAc starvation was physiologic or genetic, starvation-adapted cells were serially passaged in complete BSK-II to relieve the starvation-stress, then re-introduced to the starvation-stress condition (i.e. BSK-II lacking GlcNAc). If adaptation was physiologic then cells should exhibit biphasic growth; however, we found that after as many as twelve serial passages in a complete medium, cells still reached high cell density in a single exponential phase when re-introduced to a medium lacking free GlcNAc (Fig. 2). In addition, starvation-adapted cells were plated on BSK-II agar after seven serial passages in complete BSK-II and the growth of ten clones was followed in BSK-II broth lacking GlcNAc. Eight of the ten clones grew to optimal cell densities in a single exponential phase (Fig. 3B). It is possible that the two clones exhibiting biphasic growth were never adapted and only able to persist due to the release of GlcNAc from sequestered sources by the cells that had undergone adaptation. It is also possible that these two clones were originally adapted to GlcNAc starvation, but during serial passage in complete media became de-adapted, exhibiting the original wild-type phenotype (discussed below). In any case, re-plating of one of the adapted clones (RR45) resulted in all ten clones growing to a high cell density in a single exponential phase (Fig. 4B). These results strongly suggest that *B. burgdorferi* undergoes stress-induced mutagenesis that allows cells to adapt to GlcNAc starvation.

As discussed above, stress-induced adaptive mutagenesis in the *E. coli* Lac assay results in either point mutants or amplification of the "leaky" *lac* region that relieves the stress and allows cells to utilize lactose (Cairns & Foster 1991). This adaptive mutagenesis occurs by a number of different pathways (Cairns & Foster 1991; Harris et al. 1994; Harris et al. 1999; McKenzie et al. 2000; McKenzie et al. 2001; Lombardo et al. 2004), three of which are present in *B. burgdorferi*: i) MMR, ii) RpoS starvation-stress-response and iii) homologous recombination by DSBR proteins. The gene or genes directly involved in the utilization of sequestered GlcNAc in the second exponential phase remain unknown; therefore, it is difficult to determine if the genetic adaptation observed in *B. burgdorferi* is the result of a point mutation, amplification or both. However, data collected under GlcNAc starvation conditions relating to these three pathways in *B. burgdorferi* may shed some light on this question.

MMR is a highly conserved process that repairs mismatched bases that arise during replication, increasing the fidelity of DNA replication 100 to 1000-fold (reviewed in (Kunkel& Erie 2005)). DNA mismatches are recognized by the MutS and MutL proteins, and the mismatched base is removed from the unmethylated strand by the endonuclease activity of MutH. A reduction in MMR activity during stationary phase has been described and is one mechanism by which mutation frequency is increased, possibly resulting in advantageous point mutations (not amplifications) that allow for adaptation to certain

stresses (Feng et al. 1996; Harris et al. 1999). In addition, inactivation of MMR genes results in a mutator phenotype that has been observed in natural bacterial populations (LeClerc et al. 1996; Matic et al. 1997; Oliver et al. 2002). We evaluated our GlcNAc starvation-adapted clones for a mutation in *mutS* or *mutL* to determine if adaptation occurred due to inactivation of the MMR system. Genomic DNA was extracted from five starvation-adapted clones (Fig. 4, clones 1-5) and the *mutS* and *mutL* genes were sequenced along with 1 kb of flanking DNA on either side of the gene (data not shown). No mutations were observed in any of the clones, suggesting these clones have functional MMR systems and that adaptive mutation did not occur due to the absence of this important DNA repair pathway. It is unknown if MutS and MutL levels were reduced during GlcNAc starvation, but it may be of interest to determine these levels in future experiments.

The point mutants observed in the *E. coli* Lac assay require the presence of RpoS and the general stress response (Lombardo et al. 2004). It is unclear exactly how RpoS or components of the RpoS regulon regulate the formation of point mutations that confer the Lac+ phenotype, but it is known that RpoS promotes the use of the error-prone DNA polymerase DinB in DSBR (Ponder et al. 2005). As *B. burgdorferi* does not possess a DinB homologue or other error-prone DNA polymerases, it is unlikely this mechanism plays a role in adaptation of this spirochete to GlcNAc starvation. However, the RpoS regulon has also been shown to be necessary for adaptive amplification in the *E. coli* Lac assay (Lombardo et al. 2004), and previous studies conducted in our lab have demonstrated the importance of RpoS in GlcNAc starvation and biphasic growth (Rhodes et al. 2009). We reported a role for RpoS in the regulation of chitobiose utilization, and also demonstrated that an *rpoS* mutant was unable to initiate second exponential phase growth in the absence of GlcNAc until after 400 hours. In contrast, wild-type cells initiate a second exponential phase by 125 hours and reach optimal cell densities by 300 hours. It is possible the long delay observed in the *rpoS* mutant reflects the inability of these cells to amplify the gene(s) needed to obtain sequestered GlcNAc in the second exponential phase.

The DSBR protein RecA is also required for stress-induced point mutation and amplification in the *E. coli* Lac assay. With regard to point mutants, RecA most likely plays a role in increased mutagenesis through the up-regulation of the SOS response. As discussed above, *B. burgdorferi* does not possess the components for a functional SOS pathway, and so adaptive mutation in response to GlcNAc starvation by this mechanism is unlikely. However, homologous recombination and DSBR proteins such as RecA are also required for adaptation via amplification. Therefore, we evaluated the ability of a *recA* mutant to adapt to GlcNAc starvation by culturing cells in BSK-II without GlcNAc, and then diluting cells from the second exponential phase into the same medium. While the *recA* mutant exhibited biphasic growth similar to the wild type, these cells were unable to permanently adapt to GlcNAc starvation (Fig. 8) in any of the 15 independent experiments conducted (Table 3). These results suggest a role for RecA in the adaptation of wild-type cells to GlcNAc starvation, possibly through amplification of target gene(s) by homologous recombination.

Complementation of the *recA* mutant with two different complementation plasmids resulted in *recA* expression that was either 62.5-fold lower (JA15) or 15.7-fold higher (JR1) than wild-type expression (Table 2), and only partial restoration of the adaptive phenotype (Table 3). The partial restoration of the adaptive phenotype in the JA15 complemented mutant may be due to reduced levels of RecA protein resulting in less amplification of the target genes necessary for

obtaining sequestered GlcNAc. While only partial restoration of the adaptive phenotype in the JR1 complemented mutant may be due to a deamplification of the repeated target gene(s) because of abnormally high levels of RecA. It has been observed in the *E. coli* Lac assay that *lac*-amplified clones (Lac^+) plated on rich medium containing a color indicator for β-galactosidase give rise to both white (Lac-) and sectored colonies (blue colonies with white sectors) due to deamplification of the repeated *lac* region, and that deamplification is mediated by RecA (Tlsty et al. 1984). This deamplification mechanism could also account for the two clones that exhibited biphasic growth in BSK-II lacking GlcNAc after repeated passage in complete BSK-II (Fig. 3B), and the three GlcNAc starvation experiments in wild-type cells that did not result in the adaptive phenotype (Table 3).

As a result of the reduced *recA* expression in our complemented mutant with the wild-type *recA* gene and 512 bp of upstream DNA (Table 2; JA15) we characterized the *recA* transcriptional unit (Fig. 5) and identified two putative promoters for this gene (Fig. 6 and 7). RT-PCR analysis confirmed that *recA* was co-transcribed with both upstream genes (BB0132 encoding the transcription elongation factor GreA; and BB0133 encoding a protein with a tetratricopeptide repeat domain) and at least two of the downstream genes (BB0130 encoding a conserved hypothetical protein; and BB0129 encoding an RNA-uridine isomerase). The combination of our RT-PCR data with our expression data from JA15 suggested the possibility of two promoters, one just upstream of the *recA* coding region and the other upstream of BB0133. We confirmed this using 5′ RACE (Fig. 6), and identified two putative promoters based on comparison to previously identified *B. burgdorferi* RpoD, RpoS and RpoN-dependent promoters (Fig. 7) (Studholme & Buck 2000; Caimano et al. 2007; Rhodes et al. 2009). The promoter identified upstream of BB0133 is nearly identical to the RpoD consensus promoter indicating constitutive expression under normal conditions, while the putative promoter immediately upstream of the *recA* coding region shows the most, but only limited, homology to the RpoD consensus sequence. In light of these results and the role *recA* plays in adaptation to GlcNAc starvation, it is interesting to speculate that the promoter immediately upstream of *recA* may be involved in the expression of this gene under specific cellular conditions or stresses.

4. Methods

4.1 Bacterial strains and culture conditions

The bacterial strains and plasmids used in this study are described in Table 4. Wild-type and mutant *B. burgdorferi* strains were routinely cultured in modified BSK-II (Barbour 1984) medium supplemented with 7% rabbit serum (Invitrogen, Corp.; Carlsbad, CA) and any necessary antibiotics. BSK-II was modified from the original recipe by replacing 10x CMRL-1066 with 10x Media 199 (Invitrogen). In addition, free GlcNAc was left out of BSK-II for certain growth experiments. For selection and maintenance of antibiotic resistance in *B. burgdorferi*, streptomycin, kanamycin and erythromycin were used at concentrations of 100 $\mu g\ ml^{-1}$, 340 $\mu g\ ml^{-1}$ and 1 $\mu g\ ml^{-1}$ respectively.

E. coli DH5α was used to maintain plasmids during cloning procedures and was cultured in lysogeny broth (LB; 1% tryptone, 0.5% yeast extract, 1% NaCl) with the appropriate antibiotic(s). Antibiotics were used at the following concentrations for *E. coli* DH5α: streptomycin, 100 $\mu g\ ml^{-1}$; kanamycin, 50 $\mu g\ ml^{-1}$; ampicillin, 200 $\mu g\ ml^{-1}$.

4.2 Growth curve analysis

For analysis of growth in liquid cultures, late-log phase cells (5.0×10^7 cells ml^{-1} to 1.0×10^8 cells ml^{-1}) were diluted to 1.0×10^5 cells ml^{-1} in BSK-II with or without GlcNAc. In general, 6-12 µl of culture was used to inoculate 6 ml of fresh culture medium; therefore, negligible amounts of free GlcNAc were transferred with the inoculum when analyzing growth in medium lacking GlcNAc. Cells were enumerated daily by darkfield microscopy using a Petroff-Hauser (Hauser Scientific; Horsham, PA) counting chamber. Specifically, 2.5 µl of liquid culture was transferred to the counting chamber, and cells in all 25 squares were counted. Cells were diluted 1:10 in BSK-II prior to counting when the culture density was greater than 1.0×10^7 cells ml^{-1}. All growth experiments were carried out at 33°C in the presence of 3% CO_2. Unless otherwise stated, each growth curve presented in this study is representative of at least three independent trials, as growth data could not be pooled due to the length of experiments and the different times at which bacteria were enumerated.

4.3 Construction of a *recA* mutant in *B. burgdorferi*

The construct used to generate a *recA* (BB0131) mutation in the B31-A background was created as follows: i) A 2.1 kb fragment of the 3′ end of BB0131 and flanking DNA was PCR amplified with engineered restriction sites using primers (Table 5) 5′ BB0131 mutF1 (KpnI) and 5′ BB0131 mutR2 (XbaI); ii) the PCR fragment was TA cloned into pCR2.1 (Invitrogen) to generate plasmid pBB0131.1; iii) plasmids pBB0131.1 and pKFSS1 (Frank et al. 2003) (a *B. burgdorferi* shuttle vector conferring streptomycin resistance; Table 4) were digested with KpnI and XbaI and separated by gel electrophoresis; iv) the 2.1 kb band from pBB0131.1 was gel extracted and cloned into the gel extracted pKFSS1 fragment to generate pBB0131.2; v) the 2.1 kb fragment and flanking streptomycin resistance gene were PCR amplified from pBB0131.2 using primers 5′ BB0131 mutF1 (KpnI) and pKFSS1 R1; vi) the 3.6 kb amplicon was TA cloned into pGEM T-Easy (Promega, Corp.; Madison, WI) to generate pBB0131.3A or B (based on the orientation of the PCR product insertion); vii) plasmid pBB0131.3B was identified by restriction digest in which the 3′ end of the streptomycin resistance gene was adjacent to the XmaI site in the pGEM T-Easy vector; viii) the 5′ end of BB0131 and flanking DNA (1.8 kb) was PCR amplified with engineered restriction sites using primers 3′ BB0131 mutF1 (XmaI) and 3′ BB0131 mutR1 (SacII) and TA cloned into pCR2.1 to generate pBB0131.4; ix) plasmids pBB0131.3B and pBB0131.4 were digested with XmaI and SacII and separated by gel electrophoresis; x) the 1.8 kb fragment from pBB0131.4 was gel extracted and cloned into the gel extracted fragment from pBB0131.3B to generate the final construct pBB0131.5. All plasmids were confirmed by restriction digest and DNA sequencing. In summary, 877 of the 1098 bp constituting the *recA* coding region were deleted and replaced with the streptomycin resistance gene transcribed in the opposite orientation and under the control of the constitutive *B. burgdorferi flgB* promoter. Plasmid pBB0131.5 was confirmed by PCR using primers flanking the antibiotic insertion site (BB0131 mut confirm F1 and 5′ recA SalI) that resulted in a 1.7 kb fragment (Fig. 9, lane 3) compared to a 1.2 kb fragment generated with the wild-type genomic DNA as a template (Fig. 9, lane 2).

To generate the *recA* deletion mutant, competent B31-A cells were prepared and transformed with 15 µg of pBB0131.5 as previously described (Samuels et al. 1994). Briefly, transformed cells were resuspended in 10 ml of complete BSK-II and allowed to recover

overnight at 33°C. The transformation reaction was plated in solid BSK-II medium containing 100 µg ml^{-1} streptomycin to select for the allelic exchange event. Streptomycin resistant colonies were screened by PCR using primers that flanked the insertion site. One clone (JA10) was chosen for further experimentation and PCR of the genomic DNA from this strain with the primers BB0131 mut confirm F1 and 5′ recA SalI demonstrated the presence of the mutant *recA* allele (Fig. 9, lane 5). In addition, the insertion was confirmed by DNA sequencing of the PCR product.

Strain or Plasmid	Genotype and Description	Reference
Strains		
B . burgdorferi		
B31-A	High passage non-infectious wild-type	(Elias et al. 2000)
RR45	GlcNAc starvation adapted clone	This study
JA10	Str^R; B31-A *recA* mutant	This study
JA15	Str^R Kan^R; JA10 complemented with pBB0131comp.2	This study
JR1	Str^R Kan^R; JA10 complemented with precA.2	This study
E. coli		
DH5α	*supE*44 F^- Δ*lac*U169 (ϕ80*lac*Z ΔM15) *hsdR*17 *relA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1	(Hanahan 1983)
Plasmids		
pKFSS1	Str^R; *B. burgdorferi* shuttle vector, cp9 based	(Frank et al. 2003)
pBSV2	Kan^R; *B. burgdorferi* shuttle vector, cp9 based	(Stewart et al. 2001)
pCE320	Kan^R Zeo^R; *B. burgdorferi* shuttle vector, cp32 based	(Eggers et al. 2002)
ErmC/pCE320	Kan^R Zeo^R, Erm^R; *B. burgdorferi* shuttle vector, cp32 based	This study
pBB0450.1	Erm^R; *ermC*::BB0450	(Rhodes et al. 2009)
pBB0131.5	Str^R; *aadA*::BB0131 (*recA*)	This study
pBB0131comp.2	Kan^R; BB0131 complementation construct - *recA* with 512 bp of upstream DNA	This study
pRecA.3	Kan^R; BB0131 complementation construct –*recA* fused to constitutive *flaB* promoter	This study

Table 4. Strains and plasmids used in this study.

Primer Name	Sequence (5′→ 3′)
5′ BB0131 mutF1 (KpnI)	GCTAGGGTACCTGCTTATATCGCCTCAAAGCTCG
5′ BB0131 mutR2 (XbaI)	GCTAGTCTAGACTTAGCAAAGAAGTAGAACTTGC
pKFSS1 R1	TGATGAACAGGGTCACGTCGTC
3′ BB0131 mutF1 (XmaI)	GCTAGCCCGGGGCTTTTTCTATTTGAACTCTTGCAAGC
3′ BB0131 mutR1 (SacII)	GCTAGCCGCGGTATGGCCATGTCGGCTTTATCTCC
BB0131 mut confirm F1	CAAGGCTGATAGAGTAACTACCCA
BB0131 comp F1 (KpnI)	GCTAGGGTACCCAAGGCTGATAGAGTAACTACCCA
BB0131 comp R1 (XbaI)	GCTAGTCTAGATGCAGGCGAGAGAATGGAAATTGG
PflaB F1 (SalI)	GTCGACATATCATTCCTCCATGATAAAATTT
PflaB R1 (NotI)	GCGGCCGCTGTCTGTCGCCTCTTGTGG
5′ recA (SalI)	GTCGACATGTCAAAGTTAAAGGAAAAAAGAGAAAAAGC
BbrecA F1	CAAGGCTGATAGAGTAACTACCCA
ErmC F1	GGTTCCATAATATGTTCTCCCTTTCTCAG
ErmC R1	CCCAACGCTCGAATTTAAAGACCC
recA operon F1	CACGCTCTTTAAATAAGGGCTGAACC
recA operon F3	AATCTCAAGAGCTTGCTCTCCGGT
recA operon F4	CCAATGCCGAGAGCCTCATCTAAT
recA operon F5	TGGCCCATACTTCTCTGATTCCTG
recA operon F6	ACGTCCTTTAAATGCCTGCCAC
recA operon F8	CAAGGCTTTCAACTTGACCATTCCA
recA operon R1	CAGGCTCATGGTATTCATTGGGAG
recA operon R1A	TACTTGGGCCTTGGGAATCAAACC
recA operon R2	AAAGCTCGTGAACTTGGTGA
recA operon R2A	ATCGGTCTTAGAGGTCGTGAGAGA
recA operon R2B	AGGATTTGCATATTGAAGAAGAGATGC
recA operon R3	TATGGCCATGTCGGCTTTATCTCC
recA operon R4	GAAGATAGAGCACGTATTGATGAG
recA operon R5	CCGAAGAGGCTCATTCTCTTGA
oligo dT-anchor primer	GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTTTV
BB0131 F1	ACATCATCGCTTGAGCCCGATCTA
BB0131 R1	ACCACTACCGGTGGGAATGCTTTA

Table 5. Oligonucleotide primers used in this study

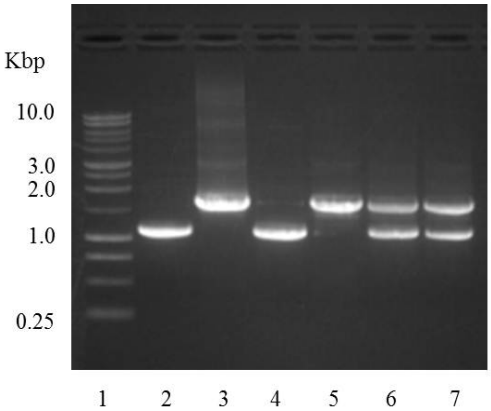


Fig. 9. PCR confirmation of the *recA* mutant and complemented mutant strains. PCR confirmation of JA10 (*recA* deletion/insertion mutant), JA15 (JA10 complemented with pBB0131comp.2) and JR1 (JA10 complemented with pRecA.3) using primers BB0131 mut confirm F1 and 5′ recA SalI which flank the insertion site. The larger PCR product is 1.7 kb (upper arrow) and contains the streptomycin resistance gene within *recA*. The smaller PCR product is 1.2 kb (lower arrow) and represents the wild-type *recA* gene. PCR reactions using the following templates were separated by electrophoresis: lane 1 – 1 kb ladder (Promega), lane 2 – B31-A genomic DNA, lane 3 – pBB0131.5 (*recA* mutation construct), lane 4 – pRecA.3 (complementation plasmid for JR1), lane 5 – JA10 genomic DNA (*recA* mutant), lane 6 – JA15 genomic DNA (JA10 complemented with pBB0131comp.2) and lane 7 – JR1 genomic DNA (JA10 complemented with pRecA.3).

4.4 Complementation of the *recA* mutant

Two approaches were used to complement the *recA* mutation in JA10. First, the *recA* gene and 512 bp of upstream DNA were PCR amplified using primers BB0131 comp F1 (KpnI) and BB0131 comp R1 (XbaI), and the 1.6 kb fragment was TA cloned into pCR2.1 to generate pBB0131comp.1. Plasmids pBB0131comp.1 and pBSV2 (Stewart et al. 2001) (*E. coli-B. burgdorferi* shuttle conferring kanamycin resistance), were digested with KpnI and XbaI and separated by gel electrophoresis. The 1.6 kb fragment from pBB0131comp.1 was gel extracted and ligated with the gel extracted fragment from pBSV2 to generate pBB0131comp.2. Fifteen micrograms of pBB0131comp.2 was transformed into competent JA10 cells and transformants were selected by plating on solid BSK-II medium containing 100 μg ml^{-1} streptomycin and 340 μg ml^{-1} kanamycin. Clones were screened by PCR using primers BB0131 mut confirm F1 and 5′ recA SalI. One clone, designated JA15, was selected for further experimentation and the presence of both the mutant and wild-type *recA* alleles was confirmed by PCR using 50 ng of genomic DNA as template (Fig. 9; lane 6).

We also generated a complementation construct in which we fused the wild-type *recA* gene to the constitutive *B. burgdorferi flaB* promoter. The *flaB* promoter was PCR amplified from B31-A genomic DNA using primers PflaB F1 (SalI) and PflaB R1 (NotI), and cloned into pCR2.1 to generate pPflaB. The *recA* coding region was PCR amplified from B31-A genomic DNA using primers 5′ recA (SalI) and BbrecA F1, and cloned into pCR2.1 to generate pRecA.1. Plasmids pPflaB and pRecA.1 were digested with SalI and NotI and separated by

gel electrophoresis. The 360 bp product from pPflaB was gel extracted and ligated into the gel extracted fragment of pRecA.1 to generate pRecA.2. The *flaB* promoter-*recA* fusion was removed from pRecA.2 by digestion with KpnI and XbaI (sites in pCR2.1 flanking the TA insertion site), separated by gel electrophoresis, gel extracted and cloned into like sites in pCE320 (Eggers et al. 2002) (a *B. burgdorferi* shuttle vector with a circular plasmid 32 (cp32) origin for replication that confers kanamycin resistance) to generate pRecA.3. The complementation construct pRecA.3 was concentrated and transformed into JA10 as described above. Transformants were screened by PCR using primers BB0131 mut confirm F1 and 5′ recA SalI, and one clone was designated JR1 and used for further experiments. PCR using 50 ng of JR1 genomic DNA resulted in amplification of both wild-type and mutant *recA* alleles (Fig. 9; lane 7).

4.5 Homologous recombination assay

A homologous recombination assay similar to that described previously by Liveris *et al* (Liveris et al. 2008) was used to determine transformation and recombination frequencies for the wild-type, *recA* mutant and *recA* complemented mutant strains. To determine transformation frequency we generated plasmid ErmC/pCE320 in which the pCE320 shuttle vector was modified to confer erythromycin resistance. Specifically, primers ErmC F1 and ErmC R1 were used to amplify the erythromycin resistance gene and its corresponding promoter from plasmid pBB0450.1, which was then TA cloned into pCR2.1 to generate pCR2.1/ErmC. Plasmid pCR2.1/ErmC was digested with BamHI and NotI, separated by gel electrophoresis, and the fragment containing *ermC* was gel extracted and cloned into like sites in pCE320 to generate ErmC/pCE320. Plasmid ErmC/pCE320 was concentrated to >1 μg μl^{-1} and cells were electroporated with 15 μg of plasmid as previously described (Samuels et al. 1994). After recovery overnight in BSK-II, transformants were selected on solid BSK-II containing 1 μg ml^{-1} erythromycin and transformation frequency was calculated by dividing the number of transformant colonies obtained by the total number of cells plated (CFU) as determined by dilution and plating without antibiotic selection. The presence of ErmC/pCE320 was confirmed in at least ten erythromycin resistant colonies from each experiment by colony PCR with primers specific for *ermC*.

The ability of *recA* expressing and non-expressing strains to mediate allelic exchange was determined by calculating the recombination frequency following electroporation with plasmid pBB0450.1 (*rpoN::ermC* with flanking DNA) (Rhodes et al. 2009). Transformation and plating with pBB0450.1 was carried out as described above for ErmC/pCE320 and recombinants were selected on solid BSK-II containing 1 μg ml^{-1} erythromycin. Recombination frequency was calculated by dividing the number of erythromycin resistant colonies by the total number of cells plated (CFU) as determined by dilution and plating without selection. Allelic exchange was confirmed in at least ten erythromycin resistant colonies from each experiment by colony PCR with primers flanking the insertion site in *rpoN*.

4.6 RNA extraction

RNA was extracted from wild-type cells (B31-A) for use in RT-PCR and qRT-PCR experiments. Briefly, cells were harvested during mid to late log phase (1.0 to 5.0 × 10^7 cells ml^{-1}) by centrifugation (10,000 × *g*, 12 min, 4°C). Pellets were resuspended in 500 μl of cold BSK-II and transferred to a 2 ml microcentrifuge tube. One milliliter of Bacteria RNAProtect

(Qiagen; Valencia, CA) was added, mixed by vortexing and incubated for 5 min at room temperature. Cells were collected by centrifugation at 5,000 × *g* for 10 min, the supernatant was decanted and pellets were stored at -80°C for up to 4 weeks prior to RNA extraction. RNA was extracted using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions and treated with RQ1 RNase-free DNase (Promega, Corp.) and RNasin (Promega, Corp.) to remove any contaminating genomic DNA. RNA was purified using the RNeasy Mini kit and following the RNA Clean-up protocol supplied by the manufacturer. RNA concentration (OD_{260}) and purity ($OD_{260/280}$) were determined by UV spectroscopy using the Nanodrop ND-1000 (Nanodrop products of Thermo Fisher Scientific; Wilmington, DE) and RNA integrity was verified by visualizing the intensity of the 16S and 23S rRNA bands on a 1% agarose gel. RNA was concentrated to greater that 1 μg μl^{-1} by isopropanol precipitation and stored at -80°C.

4.7 Quantitative RT-PCR (qRT-PCR) to determine *recA* expression

qRT-PCR was performed using the Mx3005P Multiplex Quantitative PCR System in conjunction with the Brilliant SYBR Green Single-Step qRT-PCR Master Mix kit (Stratagene; La Jolla, CA) according to the manufacturer's instructions. Briefly, a standard curve (10^1 to 10^7 copies per reaction) was prepared using a purified *recA* PCR product that was generated from B31-A genomic DNA using primers BB0131 F1 and BB0131 R1. Reactions (25 μl) containing 10 ng of total RNA extracted from mid-log phase cells (~1.0×10^7 cells ml^{-1}) were run under the following conditions: 1 cycle of 50˚C for 30 min and 95˚C for 15 min, followed by 40 cycles of 95˚C for 30 s and 58˚C for 30 s. Fluorescence was measured at the end of the 58˚C step every cycle. Triplicate samples for each experiment were averaged, and the average and standard deviations were calculated for three independent experiments. All qRT-PCR experiments included both no-reverse transcriptase (RT) and no-template controls. The *recA* copy number for each sample was calculated using the MxPro (Stratagene) data analysis software and the *recA* standard curve described above. Copy number in each sample was normalized based on the total RNA input (10 ng per reaction) and the fold difference in *recA* copy number was determined by comparing the expression in the *recA* mutant and complemented mutant strains to the expression in the wild-type strain.

4.8 RT-PCR to determine the *recA* transcriptional unit

RT-PCR was used to determine the organization of the *recA* transcriptional unit. Complementary DNA (cDNA) was generated from B31-A RNA using the SuperScript® First Strand Synthesis for RT-PCR kit (Invitrogen, Corp.) according to the manufacturer's instructions. Briefly, 0.5 μg of RNA was reverse transcribed with SuperScript II RT enzyme at 42°C for one hour using gene specific primers recA operon F1 or F3. The RT enzyme was heat inactivated and RNA was degraded from the RNA-cDNA hybrid using RNaseH. To confirm there was no genomic DNA contamination a separate no RT reaction was run in conjunction with each RT reaction. For PCR, antisense primers used to generate the cDNA were used in various combinations with the sense primers recA operon R1, R1A, R2, R2A, R2B, R3, R4 or R5. Three reactions were prepared for each primer set and the following templates were added: i) 50 ng of B31-A genomic DNA, ii) 2 μl of the appropriate cDNA reaction, and iii) 2 μl of the appropriate no RT control reaction. Templates were amplified using TaKaRa ExTaq (Fisher Scientific; Pittsburgh, PA) and the following thermal cycle: i) initial denaturation for 10

min at 95°C, ii) 35 cycles of 95°C for 30 sec, 58°C for 30 sec and 72°C for 1 to 4 min (depending on the size of the expected product), and iii) final extension at 72°C for 10 min. PCR products were separated on a 1% agarose gel and visualized using ethidium bromide.

4.9 5' Rapid Amplification of cDNA Ends (RACE) and promoter analysis

The approximate *recA* transcriptional start sites were determined using the 2nd Generation 5′/3′ RACE Kit (Roche Applied Science; Mannheim, Germany) according to the manufacturer's instructions. Briefly, two first-strand cDNA synthesis reactions were performed with 1 μg of total RNA and antisense primers recA operon F3 and recA operon F5 to determine the transcritptional start sites immediately upstream of BB0131 (*recA*) and BB0133. The two cDNA reactions were purified using the High Pure PCR Product Purification Kit (Roche Applied Science). The purified cDNAs were divided into two reaction tubes and a homopolymeric A or G-tail was added to the 3′ end using recombinant terminal deoxynucleotidyl transferase and dATP or dGTP. A PCR product was amplified from each tailed cDNA using the appropriate anchor primer (oligo dT-AP or oligo dC-AP) and a nested gene specific primer. Nested primer recA operon F4 was used to amplify tailed cDNA generated with recA operon F3, and nested primer recA operon F6 was used to amplify tailed cDNA generated with recA operon F5. The PCR products were subjected to electrophoresis and the bands were gel extracted using the QIAquick PCR Purification Kit (Qiagen). The sequence of the purified PCR products were determined using either recA operon F4 or recA operon F8 and aligned to the upstream sequence of *recA* or BB0133 to determine the transcriptional start sites. Promoter analysis was carried out by visual inspection and comparison of the region upstream of the approximate transcriptional start sites with previously described RpoD, RpoS and RpoN-dependent promoter sequences in *B. burgdorferi*.

4.10 Nucleotide sequencing and computer analysis

Nucleic acid sequencing was performed by the Rhode Island Genomics and Sequencing Center using a 3130xl Genetic Analyzer (Applied Biosystems; Forest City, CA). Sequencing reactions were prepared using the BigDye® Terminator v3.0 Cycle Sequencing Kit. Sequences were analyzed using DNASTAR Lasergene software (DNASTAR, Inc.; Madison, WI) and Vector NTI (Invitrogen, Corp.).

5. Conclusions

In this study we demonstrate that *B. burgdorferi* undergoes a genetic adaptation during GlcNAc starvation that allows cells to grow to a high cell density when re-introduced to the starvation condition. We also show that *recA* mutant cells do not respond to GlcNAc starvation the same as wild-type cells, suggesting a role for RecA in this process. Finally, we characterize the *recA* transcriptional unit and provide evidence that this gene is controlled transcriptionally from two separate promoters.

6. Acknowledgments

We thank P. Rosa and J. Radolf for providing strains and plasmids. This research is based in part upon work conducted using the Rhode Island Genomics and Sequencing Center, which

is supported in part by the National Science Foundation under EPSCoR Grant No. 0554548. This work was supported by NIH grant 5 R01AI03723010 awarded to DRN.

7. References

Barbour, A. G. (1984). "Isolation and cultivation of Lyme disease spirochetes." *The Yale Journal of Biology and Medicine* 57(4): 521-5.

Caimano, M. J., R. Iyer, C. H. Eggers, C. Gonzalez, E. A. Morton, M. A. Gilbert, I. Schwartz & 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." *Molecular Microbiology* 65(5): 1193-1217.

Cairns, J. & P. L. Foster (1991). "Adaptive Reversion of a Frameshift Mutation in *Escherichia coli*." *Genetics* 128(4): 695-701.

Cox, M. M. (2007). "Regulation of Bacterial RecA Protein Function." *Critical Reviews in Biochemistry and Molecular Biology* 42(1): 41-63.

Das, R., Hegyi H, Gerstein M (2000). "Genome analyses of spirochetes: a study of the protein structures, functions and metabolic pathways in *Treponema pallidum* and *Borrelia burgdorferi*." *Journal of Molecular Microbiology and Biotechnololgy* 2(4): 387-392.

Eggers, C. H., M. J. Caimano, M. L. Clawson, W. G. Miller, D. S. Samuels & J. D. Radolf (2002). "Identification of loci critical for replication and compatibility of a *Borrelia burgdorferi* cp32 plasmid and use of a cp32-based shuttle vector for the expression of fluorescent reporters in the Lyme disease spirochaete." *Molecular Microbiology* 43(2): 281-295.

Elias, A. F., J. L. Bono, J. A. Carroll, P. Stewart, K. Tilly & P. Rosa (2000). "Altered stationary-phase response in a *Borrelia burgdorferi rpoS* mutant." *Journal of Bacteriology* 182(10): 2909-2918.

Feng, G., H. Tsui & M. Winkler (1996). "Depletion of the cellular amounts of the MutS and MutH methyl-directed mismatch repair proteins in stationary-phase *Escherichia coli* K-12 cells." *Journal of Bacteriology* 178(8): 2388-2396.

Finkel, S. E. (2006). "Long-term survival during stationary phase: evolution and the GASP phenotype." *Nature Reviews Microbiology* 4(2): 113-120.

Foster, P. L. & J. M. Trimarchi (1994). "Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs." *Science* 265(5170): 407-409.

Frank, K. L., S. F. Bundle, M. E. Kresge, C. H. Eggers & D. S. Samuels (2003). "*aadA* confers streptomycin resistance in *Borrelia burgdorferi*." *Journal of Bacteriology* 185(22): 6723-6727.

Galhardo, R. S., P. J. Hastings & S. M. Rosenberg (2007). "Mutation as a stress response and the regulation of evolvability." *Critical Reviews in Biochemistry and Molecular Biology* 42(5): 399-435.

Hanahan, D. (1983). "Studies on transformation of *Escherichia coli* with plasmids." *Journal of Molecular Biology* 166: 557-580.

Harris, R. S., G. Feng, K. J. Ross, R. Sidhu, C. Thulin, S. Longerich, S. K. Szigety, P. J. Hastings, M. E. Winkler & S. M. Rosenberg (1999). "Mismatch repair is diminished during stationary-phase mutation." *Mutation Research* 437(1): 51-60.

Harris, R. S., S. Longerich & S. M. Rosenberg (1994). "Recombination in adaptive mutation." *Science* 264(5156): 258-260.

Hastings, P. J. (2007). "Adaptive amplification." *Critical Reviews in Biochemistry and Molecular Biology* 42(4): 271-283.

Hastings, P. J., H. J. Bull, J. R. Klump & S. M. Rosenberg (2000). "Adaptive amplification: an inducible chromosomal instability mechanism." *Cell* 103(5): 723-731.

He, A. S., P. R. Rohatgi, M. N. Hersh & S. M. Rosenberg (2006). "Roles of *E. coli* double-strand-break-repair proteins in stress-induced mutation." *DNA Repair* 5(2): 258-273.

Kugelberg, E., E. Kofoid, A. B. Reams, D. I. Andersson & J. R. Roth (2006). "Multiple pathways of selected gene amplification during adaptive mutation." *Proceedings of the National Academy of Sciences of the United States of America* 103(46): 17319-17324.

Kunkel, T. A. & D. A. Erie (2005). "DNA mismatch repair." *Annual Review of Biochemistry* 74(1): 681-710.

Kurtenbach, K., K. Hanincova, J. I. Tsao, G. Margos, D. Fish & N. H. Ogden (2006). "Fundamental processes in the evolutionary ecology of Lyme borreliosis." *Nature Reviews Microbiology* 4(9): 660-669.

LeClerc, J. E., B. Li, W. L. Payne & T. A. Cebula (1996). "High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens." *Science* 274(5290): 1208-1211.

Liveris, D., V. Mulay, S. Sandigursky & I. Schwartz (2008). "*Borrelia burgdorferi vlsE* antigenic variation is not mediated by RecA." *Infection and Immunity* 76(9): 4009-4018.

Liveris, D., V. Mulay & I. Schwartz (2004). "Functional properties of *Borrelia burgdorferi recA*." *Journal of Bacteriology* 186(8): 2275-2280.

Lombardo, M.-J., I. Aponyi & S. M. Rosenberg (2004). "General stress response regulator RpoS in adaptive mutation and amplification in *Escherichia coli*." *Genetics* 166(2): 669-680.

Matic, I., M. Radman, F. Taddei, B. Picard, C. Doit, E. Bingen, E. Denamur, J. Elion, J. E. LeClerc & T. A. Cebula (1997). "Highly variable mutation rates in commensal and pathogenic *Escherichia coli*." *Science* 277(5333): 1833-1834.

McKenzie, G. J., R. S. Harris, P. L. Lee & S. M. Rosenberg (2000). "The SOS response regulates adaptive mutation." *Proceedings of the National Academy of Sciences of the United States of America* 97(12): 6646-6651.

McKenzie, G. J., P. L. Lee, M.-J. Lombardo, P. J. Hastings & S. M. Rosenberg (2001). "SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification." *Molecular Cell* 7(3): 571-579.

Oliver, A., F. Baquero & J. Blázquez (2002). "The mismatch repair system *mutS*, *mutL* and *uvrD* genes in *Pseudomonas aeruginosa*: molecular characterization of naturally occurring mutants." *Molecular Microbiology* 43(6): 1641-1650.

Ponder, R. G., N. C. Fonville & S. M. Rosenberg (2005). "A switch from high-fidelity to error-prone DNA double-strand break repair underlies stress-induced mutation." *Molecular Cell* 19(6): 791-804.

Powell, S. C. & R. M. Wartell (2001). "Different characteristics distinguish early versus late arising adaptive mutations in *Escherichia coli* FC40." *Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis* 473(2): 219-228.

Putteet-Driver, A. D., J. Zhong & A. G. Barbour (2004). "Transgenic expression of RecA of the spirochetes *Borrelia burgdorferi* and *Borrelia hermsii* in *Escherichia coli* revealed differences in DNA repair and recombination phenotypes." *Journal of Bacteriology* 186(8): 2266-2274.

Rhodes, R., W. Coy & D. Nelson (2009). "Chitobiose utilization in *Borrelia burgdorferi* is dually regulated by RpoD and RpoS." *BMC Microbiology* 9(1): 108.

Rhodes, R. G., J. A. Atoyan & D. R. Nelson (2010). "The chitobiose transporter, *chbC*, is required for chitin utilization in *Borrelia burgdorferi*." *BMC Microbiology* 10: 21.

Rosenberg, S. M., S. Longerich, P. Gee & R. S. Harris (1994). "Adaptive mutation by deletions in small mononucleotide repeats." *Science* 265(5170): 405-407.

Saier MH, J., Paulsen IT (2000). "Whole genome analyses of transporters in spirochetes: *Borrelia burgdorferi* and *Treponema pallidum*." *Journal of Molecular Microbiology and Biotechnology* 2(4): 393-9.

Samuels, D. S., K. E. Mach & C. F. Garon (1994). "Genetic transformation of the Lyme disease agent *Borrelia burgdorferi* with coumarin-resistant *gyrB*." *Journal of Bacteriology* 176(19): 6045-9.

Slack, A., P. C. Thornton, D. B. Magner, S. M. Rosenberg & P. J. Hastings (2006). "On the mechanism of gene amplification induced under stress in *Escherichia coli*." *Public Library of Science: Genetics* 2(4): e48.

Steere, A. C. (2001). "Lyme Disease." *The New England Journal of Medicine* 345(2): 115-125.

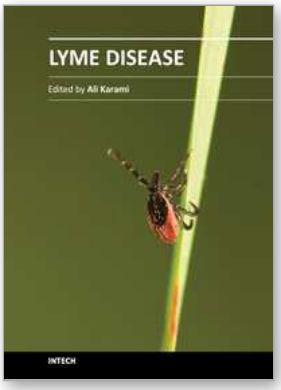
Steere, A. C., J. Coburn & L. Glickstein (2004). "The emergence of Lyme disease." *Journal of Clinical Investigation* 113(8): 1093-1101.

Stewart, P. E., R. Thalken, J. L. Bono & P. Rosa (2001). "Isolation of a circular plasmid region sufficient for autonomous replication and transformation of infectious *Borrelia burgdorferi*." *Molecular Microbiology* 39(3): 714-721.

Studholme, D. J. & M. Buck (2000). "The biology of enhancer-dependent transcriptional regulation in bacteria: insights from genome sequences." *Federation of European Microbiological Societies: Microbiology Letters* 186(1): 1-9.

Tilly, K., A. F. Elias, J. Errett, E. Fischer, R. Iyer, I. Schwartz, J. L. Bono & P. Rosa (2001). "Genetics and regulation of chitobiose utilization in *Borrelia burgdorferi*." *Journal of Bacteriology* 183(19): 5544-5553.

Tlsty, T. D., A. M. Albertini & J. H. Miller (1984). "Gene amplification in the *lac* region of *E. coli*." *Science* 37(1): 217-224.



Lyme Disease

Edited by Dr. Ali Karami

ISBN 978-953-51-0057-7
Hard cover, 160 pages
Publisher InTech
Published online 17, February, 2012
Published in print edition February, 2012

Lyme disease, or Lyme borreliosis, is an emerging infectious disease caused by bacteria belonging to the genus borrelia. Borrelia burgdorferi, in the strict sense. This book deals mostly with the molecular biology of the Lyme disease agent orrelia burgdorferi. It has been written by experts in the relevant field and is tailored to the need of researchers, advanced students of biology, molecular biology, molecular genetics of microorganism. It will also be of use to infectious disease experts and people in other disciplines needing to know more about Lyme borreliosis. The book contains chapters on the molecular biology of the Lyme disease agent, zoonotic peculiarities of Bb, advancement in Bb antibody testing, the serology diagnostic schemes in Bb, discovering Lyme disease in ticks and dogs, adaptation to glucosamine starvation in Bb, and porins in the genus borrelia.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Ryan G. Rhodes, Janet A. Atoyan and David R. Nelson (2012). Adaptation to Glucosamine Starvation in Borrelia burgdorferi is Mediated by recA, Lyme Disease, Dr. Ali Karami (Ed.), ISBN: 978-953-51-0057-7, InTech, Available from: http://www.intechopen.com/books/lyme-disease/adaptation-to-glucosamine-starvation-in-borrelia-burgdorferi-is-mediated-by-reca

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.