Determination of lysine acetylation susceptibility of DksA in *Borrelia burgdorferi*

Armond J. Isaak[1], William K. Boyle[1], Hannah Sorensen[1], and Travis J. Bourret[1]

[1]Department of Medical Microbiology and Immunology, Creighton University, Omaha, NE, United States.

Travis J. Bourret
Creighton University
2500 California Plaza
Omaha, NE 68178
Phone: 402-280-3750
Email:
TravisBourret@creighton.edu

Abstract

For successful completion of its infectious cycle, the Lyme disease spirochete *Borrelia burgdorferi* must overcome a wide range of environmental stresses including shifts in temperature, pH, osmolarity, nutrient availability, as well as host-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS). Our long-term goal is to determine mechanisms underlying the ability of *B. burgdorferi* to sense and respond to the environmental stresses encountered during infection of mammals and *Ixodes spp.* Ticks. *B. burgdorferi* harbors limited repertoire of gene regulatory proteins and the molecular mechanisms underlying their ability to direct transcriptomic responses in response to changes in environment remain enigmatic. Recently, we described the global role for the DnaK suppressor protein (DksA) in the regulation of *B. burgdorferi* gene expression in response to nutrient limitation that is in accord with its canonical role in coordinating the stringent response of bacteria to conserve energy upon encountering environmental stresses. In a previously published study, a multitude of *B. burgdorferi* proteins, were identified as targets of lysine acetylation by endogenously produced acetyl-phosphate (Ac-PO_4) during *in vitro* growth. Lysine acetylation is a reversible post-translational modification (PTM) that contributes to the regulation of virulence gene expression in a variety of bacteria pathogens including *Salmonella enertica serovar Typhimurium. In silico* analysis of *B. burgdorferi* DksA suggests that five lysines (L118, L119, L121, L122, and L124) located in the C-terminus of DksA are likely targets for acetylation. The objective here is to determine the susceptibility of DksA to lysine acetylation and characterize the impact of this PTM on the gene regulatory activity of DksA required for *B. burgdorferi* to complete its infectious cycle in ticks and mice. Therefore, the hypothesis that DksA-dependent gene regulatory activity is modulated by lysine acetylation will be tested.

Introduction

Borrelia burgdorferi is the causative biological agent of Lyme disease, the most common vector-borne illness in North America and Europe. Currently, there is no Lyme disease vaccine on the market, and in effort to combat the disease, we look to *B. burgdorferi*. This agent experiences shifts in environmental stresses such pH, temperature, osmolarity, oxygen concentrations, nutrient availability as well as, reactive oxygen and nitrogen species during transmission (1). Our long-term goal is to determine mechanisms underlying the ability of *B. burgdorferi* to sense and respond to these environmental stresses during infection of its hosts, in particular *Ixodes spp*. ticks. One way *B. burgdorferi* responds to this shift in environment is by expressing genes controlled by the RNA polymerase (RNAP). Recently, the DnaK Suppressor Protein (DksA) was noted as a transcriptional regulator essential for energy conservation in response to the environmental stresses. In a previous study, various B. burgdorferi proteins were identified as targets for lysine acetylation, a reversible post-translational modification. The objective here is to determine if DksA is susceptible to lysine acetylation and characterize the impacts on its gene regulatory activity.

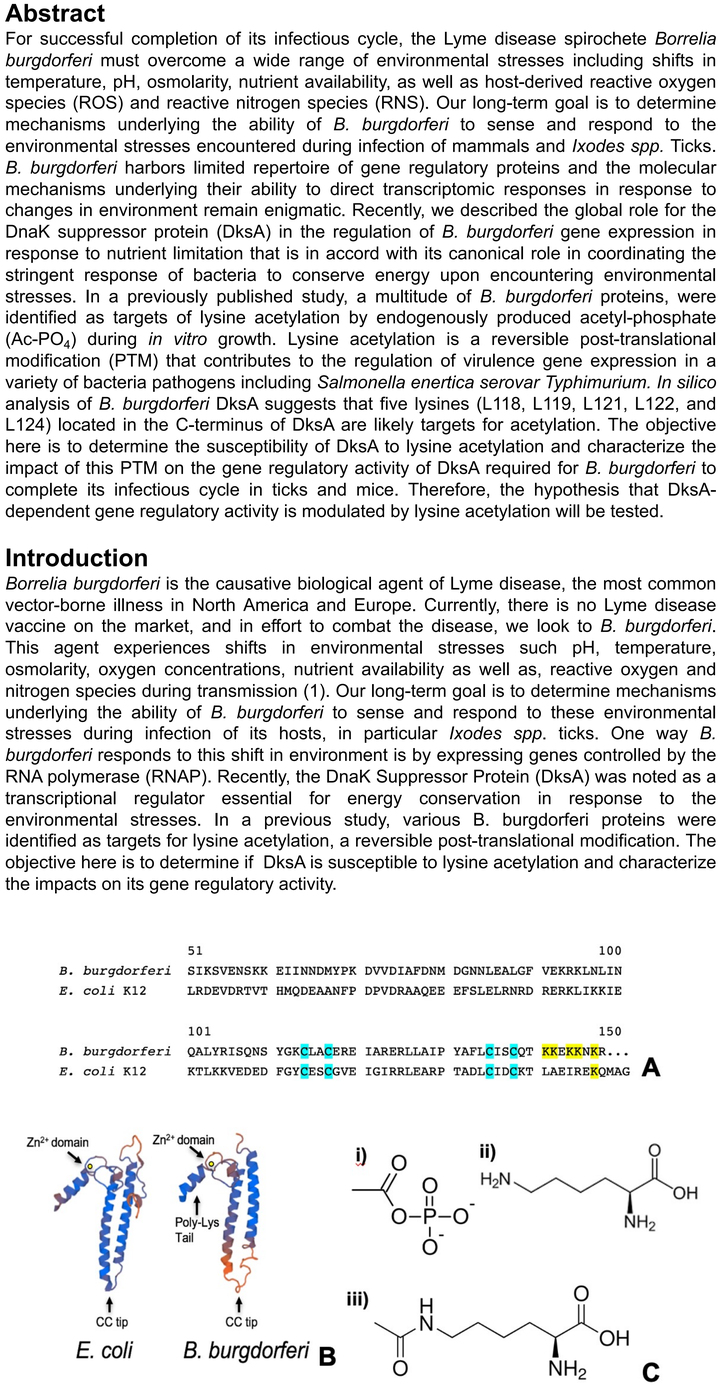

Figure 1. Protein structure of *B. burgdorferi* DksA. (A) Amino acid sequence alignment of *B. burgdorferi* and *E. coli* DksA proteins with conserved zinc finger encoding cysteines highlighted in blue and lysine residues highlighted in yellow. **(B)** SWISS-model of *E. coli* (left) and *B. burgdorferi* (right) DksA proteins illustrate predicted structural similarities based on a high-resolution crystal structure 1TJL. Color scale from blue (high) to orange (low) encodes Qmean score estimating model quality. Peptide N- and C-termini are indicated for each model. **(C)** The chemical structures of **i)** acetyl phosphate, **ii)** lysine, and **iii)** acetylated lysine.

Methods

Acetylation. Three different proteins of DksA, bovine serum albumin (BSA) and RNA polymerase were chosen to be acetylated. DksA and RNA polymerase were not diluted and remained at their given concentrations of 0.111mM and 0.001mM, respectively. BSA was diluted to 0.100mM. Acetyl-phosphate (AcP) was added in a dilution scheme of 100mM, 10mM, 1mM, 0.1mM and 0mM to each protein sample. Due to a low amount of RNA polymerase, there was not enough of the protein solution to prepare samples for 0.1mM and 0mM. For each sample 45 μL of the protein solution and 5 μL of the AcP concentrated solution was added and incubated at 37°C for three hours.

SDS-PAGE. 20 μL of each protein-AcP sample was mixed with 25 μL of 2X Laemmli Sample Buffer and 5 μL ethanol to prepare for SDS-PAGE. 10 μL of the resulting solution and the ladder (Spectra™ Multicolor Broad Range Protein Ladder) were each added to a lane. Two gels were used to accommodate the number of samples prepared. Gels were run for 20 minutes at 200V with 1X TGS running buffer in BIO-RAD set-up.

Immunoblotting. PVDF membranes were prepared by soaking in 200 proof methanol and 1X transfer buffer before adding to Trans-Blot system under each SDS-PAGE gel. Trans-Blot system was run in BIO-RAD Trans-Blot Turbo Transfer System for 30 min. The resulting membranes were then incubated with blocking solution of dehydrated milk solution for an hour and rinsed with 1X TBST buffer. Next, transferred to 1:10,000 anti-acetyllysine antibody solution (20mL 1X TBST buffer with 20 μL antibody) for six hours on rocker and later rinsed with 1X TBST buffer. Resulting image produced on BIO-RAD Gel Doc EZ Imager with ImageLab software.

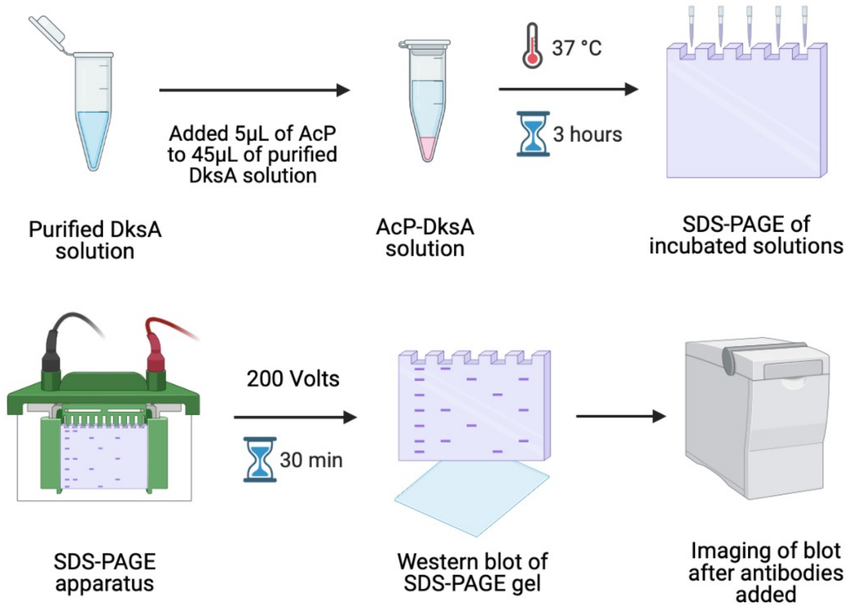

Figure 2. Overview of procedure. DksA was previously purified by Hannah Sorensen with 5 μL of each acetyl phosphate (AcP) solution added to a 45 μL aliquot of the protein solution to make dilution scheme. All dilution schemes for each protein were then incubated at 37°C for three hours before added to SDS-PAGE gels with 2X Laemmli Buffer-ethanol solution. The SDS-PAGE was run in apparatus for 30 minutes at 200 volts. SDS-PAGE gels were then added to Trans-Blot system with prepared membranes for 30 minutes. The membranes were then blocked with anti-acetyllysine 1:10,000 dilution solution for six hours and imaged using BIO-RAD Imaging software.

Acknowledgements

This work was made possible by support from the Creighton University Center for Undergraduate Research and Scholarship (CURAS) Faculty Research grant. Special consideration must also go to Dr. William Boyle for guiding me along this research project.

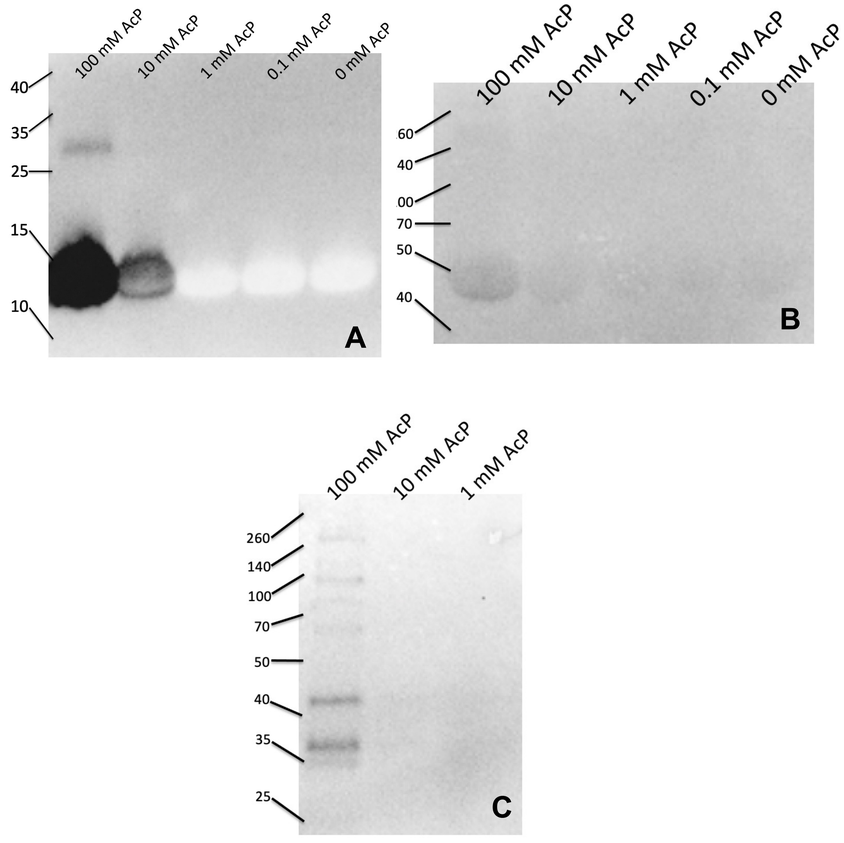

Figure 3. Western Blotting with anti-acetyllysine antibody. After SDS-PAGE was completed, proteins were transferred to PVDF membranes using the Trans-Turbo trans-blot system. The membranes were then blocked with TBST + 5% milk, and then incubated with 1:10,000 dilution of pan anti-acetyllysine antibody conjugated with HRP and images developed. 10 μL of Spectra™ Multicolor Broad Range Protein Ladder (260 kDa) was in the first lane for each gel. **(A)** Membrane of western blot of DksA protein under dilution scheme of acetyl-phosphate (AcP) with the fifth lane containing no AcP as a negative control. **(B)** Membrane of positive control bovine serum albumin (BSA) under dilution scheme of acetyl-phosphate. The negative control of 0mM AcP is also included in the fifth lane. **(C)** Membrane of western blot of *B. burgdorferi* RNA polymerase with same acetyl-phosphate dilution scheme as Figure 1A and B.

Summary

- **DksA is susceptible to lysine acetylation by acetyl-phosphate *in vitro,* likely at lysine residues in the C-terminal tail.**
- **For future work, determine whether acetylation of DksA occurs in B. burgdorferi during in vitro growth, and determine the impact of acetylation on B. burgdorferi gene regulatory function and virulence gene expression.**

References

1. **Bourret TJ, Boylan JA, Lawrence KA, Gherardini FC.** 2011. Nitrosative damage to free and zinc-bound cysteine thiols underlies nitric oxide toxicity in wild-type Borrelia burgdorferi. Mol Microbiol 81:259-273.
2. **Boyle WK, Groshong AM, Drecktrah D, Boylan JA, Gherardini FC, Blevins JS, Samuels DS, Bourret TJ.** 2019. DksA controls the response of the Lyme disease spirochete Borrelia burgdorferi to starvation. J Bacteriol 201:e00582-18.
3. **Bourret TJ, Boyle WK, Zalud AK, Valenzuela JG, Oliviera F, Lopez JE.** The relapsing fever spirochete Borrelia turicatae persists in the highly oxidative environment of its soft-bodied tick vector. Cell Microbiol. 2018:e12987. Epub 2018/11/30. doi: 10.1111/cmi. 12987. PubMed PMID: 30489694
4. **Bourret TJ, Lawrence KA, Shaw JA, Lin T, Norris SJ, Gheraidini FC.** The Nucleotide Excision Repair Pathway Protects Borrelia burgdorferi from Nitrosative Stress in Ixodes Scapularis Ticks. Front Microbiol. 2016;7:1397. doi: 10.3389/fmicb.2016.01397. PubMed PMID: 27656169; PMCID: 5013056.
5. **Weinert BT, Iesmantavicius V, Wagner SA, Scholz C, Gummesson B, Beli P, Nystrom T, Choudhary C.** Acetyl-phosphate is a critical determinate of lysine acetylation in E. coli. Mol Cell. 2013;51(2):265-72. Epub 2013/07/09. doi: 10.1016/j.molcel.2013.06.003. PubMed PMID: 9403685