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The role of RNase Y in rpoS transcript processing in Borrelia burgdorferi

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The Role of RNase Y in *rpoS* Transcript Processing in *Borrelia burgdorferi*

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Abstract

Borrelia burgdorferi is the bacterial agent that causes Lyme disease. The pathogenic bacteria are transmitted to vertebrates through tick feeding and are maintained in nature in an enzootic cycle. The expression of outer surface protein OspC is essential for *B. burgdorferi* to move from the tick to a mammal. Alternative sigma factor RpoS is responsible for inducing gene expression for OspC production during the enzootic cycle. RpoS is encoded by two versions of RNA: a long one, which is hypothesized to be required for transmission from the tick, and a short one, which is thought to be involved in infection of the mammal. We seek to understand the mechanism of how the long *rpoS* RNA is generated so that RpoS protein can activate *ospC* gene expression during transmission.

Hypothesis

The long *rpoS* transcript is processed from an even longer *rpoS* transcript by a ribonuclease called RNase Y.

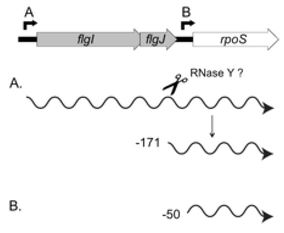

Figure 3. The long *rpoS* transcript (A) is hypothesized to function in transmission while the short *rpoS* transcript (B) is hypothesized for function during vertebrate infection.

Construction of a *B. burgdorferi* that produces an RNase Y-10X-His fusion

- Construct a recombinant *rny*, the gene encoding RNase Y, with a 10X-histidine tag
- Transform the recombinant *rny* into *B. burgdorferi*.

Purposes:

- Simplify RNase Y purification:
  - 10X-his tag, like a sticky handle, has a high affinity for cobalt and nickel metals.
- Direct purification of RNase Y from *B. burgdorferi*
  - avoid RNase contamination from heterologous species.

The enzootic cycle of *Borrelia burgdorferi*

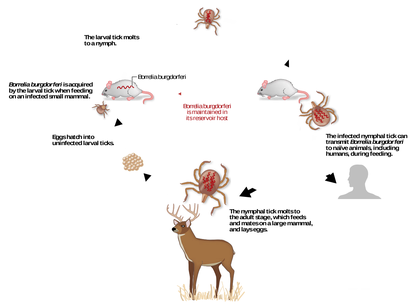

Figure 1. *B. burgdorferi* is transmitted from a tick vector to a vertebrate host. Figure from Brisson et al., 2012.

Purification of *B. burgdorferi* RNase Y from *Escherichia coli*

The expression of a recombinant RNase Y (*rnytrk* in pET28b+TEV) was optimized in *E. coli,* from which we purified RNase. Yield of the purified protein was assayed by Western blotting. RNase Y-*rpoS* cleavage assay was performed to observe RNase Y activity.

Outline of *rny*-10X-His genetic construct

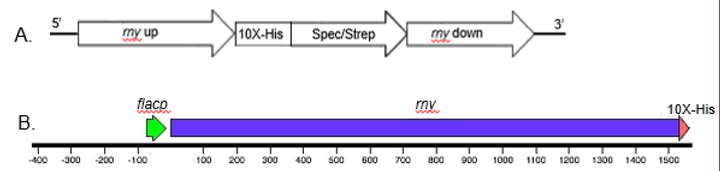

Figure 6. A. The *rny* construct with 10X-histidine tag was synthesized *in vitro* and transformed into *B. burgdorferi*. B. The artificial *flacp* promotor, like a switch, induces *rny* expression in the presence of isopropyl β-D-1-thiogalactopyraniside (IPTG).

Two *rpoS* transcripts

Sigma factors are a subunit of RNA polymerase, which transcribes DNA in the first step of gene expression. Sigma factors, such as RpoD, RpoS and RpoN, determine which groups of genes get expressed.

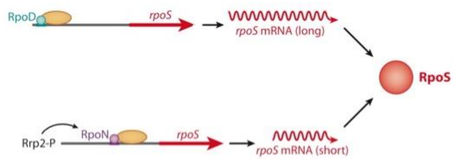

Figure 2. RpoS regulates expression of genes required for mammalian infection, like ospC. There are two versions of the rpoS transcript. The short version of the rpoS RNA requires RpoN for transcription. Transcription of the long version is RpoN-independent, but requires another sigma factor, RpoD. Figure from Samuels, 2011.

Purification of RNase Y

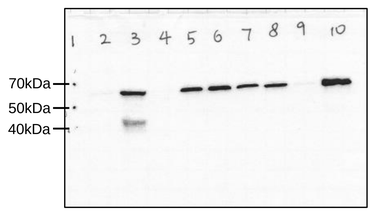

Figure 4. Western blot of RNase Y purification result. Lanes 5-8 show RNase Y in soluble fraction.

RNase Y activity assay

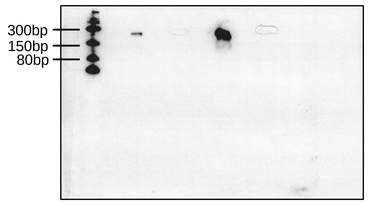

Figure 5. Cleavage assay result. The control experiments (1, 3) had no RNase Y added, to compare to the effect of RNase Y addition (3, 4).

Conclusions

- The cleavage assay did not show specific cleavage of the *rpoS* transcript by the recombinant RNase Y purified from *E. coli*. We hypothesize that there was a non-specific RNase contamination from *E. coli*.
- The recombinant *rny* with 10X-histidine tag was successfully constructed and transformed into *B.burgdorferi*.

Future directions

- Direct expression and purification of RNase Y from *B. burgdorferi* using the recombinant *rny* with 10X-His tag.
- Test the recombinant *rny* with 10X-His construct *in vivo*, using inducible gene system.

Acknowledgements
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