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.

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, a high affinity for cobalt and nickel metals.
- Direct purification of RNase Y from *B. burgdorferi*
  - avoid RNase contamination from heterologous species.

Outline of rny-10X-His genetic construct

![Image of genetic construct](image)

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

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 no-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.

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