Small RNAs regulate gene expression of a mutualistic insect endosymbiont

Research interests

Determining the functional role of small RNAs in an obligate insect endosymbiont

*Buchnera aphidicola*

Abstract

Small RNAs (sRNAs) are recognized for their importance in the regulation of gene expression within all domains of life. Despite the prevalence these non-coding RNAs, the putative regulatory roles of many identified sRNAs remain to be determined. Bacterial genomes that are reduced in size from genetic drift have lost most regulatory elements. For example, *Buchnera aphidicola*, an unculturable obligate endosymbiont with a small genome that is harbored in the pea aphid (*Acyrthosiphon pisum*), has lost nearly all transcription factors. Nevertheless, a recent study from our lab discovered widespread expression of conserved sRNAs in four reduced *Buchnera* genomes that diverged an estimated 65 million years ago. It is unknown whether these sRNAs play a regulatory role in *Buchnera*, especially since *Buchnera* exhibits differential gene expression at the protein level. Some of the proteins that are differentially expressed are involved in the biosynthetic pathways of essential amino acids that are supplied to the aphid host. Further investigation on the functional roles of these conserved sRNAs will lead to novel insights on gene regulation in small genomes in addition to host-symbiont co-evolution. The main objectives of my research project were to 1) identify putative
regulatory sRNAs and their predicted target coding sequences (CDS) using multiple bioinformatic approaches, and to 2) determine the regulatory role of sRNAs in *Buchnera*. As *Buchnera* is unculturable, I helped develop a new dual plasmid vector system in *Escherichia coli* to co-express the target CDS and associated sRNA of unculturable symbionts. Green fluorescent protein (GFP) fused with the target CDS enables visualization and quantification of protein expression of the target CDS. I determined the function of antisense RNAs (asRNAs) for two genes *glyS* and *carB* in *Buchnera* with the dual plasmid vector system.

Original research project and questions

My research project is to functionally characterize sRNAs in the obligate insect symbiont *Buchnera aphidicola*, which supplies essential amino acids to its aphid host. *Buchnera* experienced massive loss of transcriptional regulators during the million-year co-evolution with its aphid host. The reduction in genome size compared to its free-living relative *E. coli* leads to a question about how a small genome, like that of organelles, regulates its gene expression. It was hypothesized that such regulation was unnecessary because of the homeostatic environment provided by the aphid host. However, previous work in my lab has found differential gene expression exists at the protein level but not the mRNA transcript level during two different life stages, embryo and bacteriocyte, in which the aphid host may have different nutrition needs (Hansen & Degnan 2014). The differential protein expression without corresponding mRNA transcript change suggests that post-transcriptional regulation may have occurred. In the same study, my lab identified many sRNAs that were conserved in four *Buchnera*
lineages that diverged about 65 million years ago. Small RNAs have been found to regulate gene expression in both bacteria and eukaryotes. Thus, we hypothesized that sRNAs in *Buchnera* regulate its gene expression.

**Accomplishments**

As an obligate insect symbiont, *Buchnera* is unculturable. Thus, I helped adapt a dual plasmid vector system developed by Urban & Vogel (2007) to co-express *Buchnera* sRNA and target CDS in *E. coli*. Green fluorescent protein (GFP) is fused with the target CDS to enable visualization and quantification of protein expression of the target CDS. By using this system, *Buchnera* sRNAs may bind to their predicted target CDS or transcripts and activate or repress (measured by the relative intensity of GFP fluorescence) the expression of their target proteins if they are regulatory at the post-transcriptional level.

**Final results**

Function of antisense RNAs (asRNA, a type of sRNA) of two genes, *glyS* and *carB* has been determined with the dual plasmid vector system I helped develop. Antisense RNA of *carB* has been found to upregulate expression of CarB protein, and the results have been included in a manuscript submitted for publication (Thairu et al 2017). Our work demonstrated that sRNAs regulate gene expression in an obligate insect symbiont with a highly reduced genome. I submitted my senior thesis and graduated with High Distinction in Integrative Biology.