Overexpressing Two Helicobacter pylori Small RNAs from a Bacterial Pathogenicity-related Chromosomal Region to Investigate Their Regulation of Virulence Genes

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Helicobacter pylori: bacterial pathogen

Helicobacter pylori bacteria are human stomach pathogens infecting roughly 50% of humanity.[1]

- Resulting diseases include asymptomatic gastritis, stomach ulcers, gastric cancer, and MALT lymphoma[1,2]
- Of infected, 10-20% are symptomatic; 1-2% develop cancer[1]
- Regulates its genes to adapt to changing stomach environment (acidic, pH varies with food content, mechanical digestion, etc.)[3-5]

Helicobacter pylori virulence factor traits promote infection and cause disease symptoms.[1-5]

- The *cag* pathogenicity island (*cag*PAI): optional chromosomal region (DNA sequence) with pathogenicity-related genes
- The *cag*PAI encodes important virulence factors
- Strains *cag*PAI+ associated with severe disease and cancer[6,7]

How does *H. pylori* regulate its virulence genes, especially those located in the *cag*PAI?

- Few regulatory proteins compared to other bacteria[8]

All images created in BioRender.com

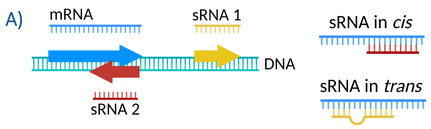
Small RNAs regulate gene expression

Small RNAs (sRNAs): short, non-protein-coding, regulatory RNA molecules.[9-11]

- 25–250 nucleotides long (average *H. pylori* gene: 945 nt)[12]
- Base-pair RNA molecules (or bind proteins)
- Target: antisense (in *cis*) or distant (in *trans*)
- Change conformation (shape) of target
- Increase/decrease/vary target gene expression

Helicobacter pylori possess >900 ncRNAs (sRNAs?); few are characterized.[9-11,13]

Do sRNAs from the *cag*PAI regulate *cag*PAI genes or virulence genes?



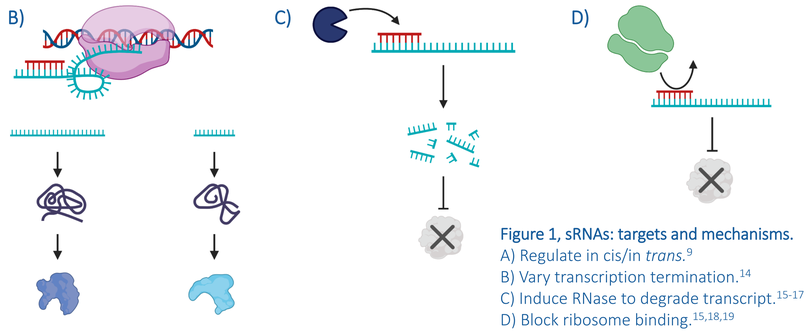


Figure 1, sRNAs: targets and mechanisms.
A) Regulate in cis/in *trans*.[9]
B) Vary transcription termination.[14]
C) Induce RNase to degrade transcript.[15-17]
D) Block ribosome binding.[15,18,19]

Investigating regulation by *H. pylori* sRNAs

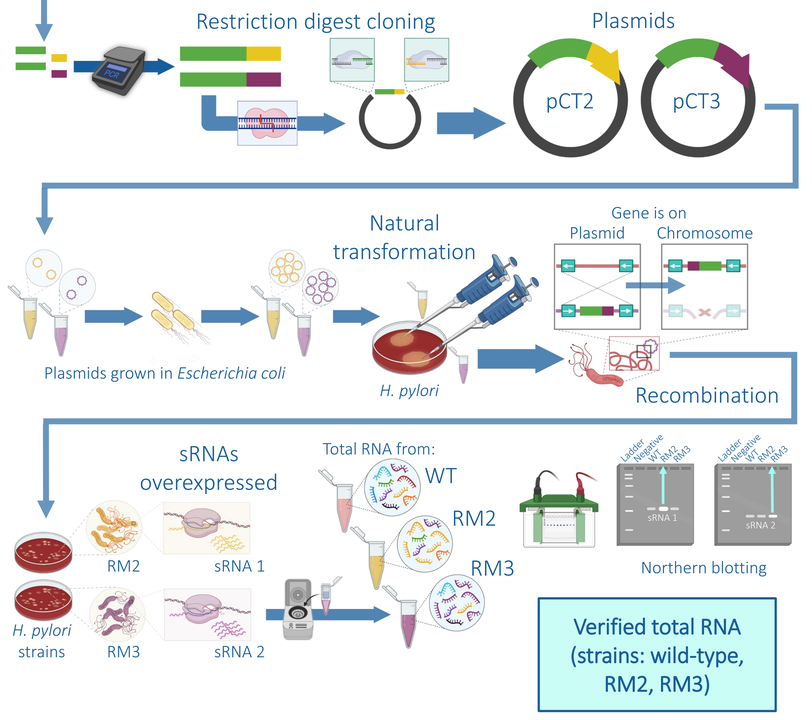
Objective: Identify and quantitate virulence gene RNA expression changes in *H. pylori* strains overexpressing (making excess) of two sRNAs from the *cag*PAI.

Hypothesis: In *H. pylori* overexpressing two sRNAs, we will observe significant changes of RNA expression (as transcript abundance) of ≥1 virulence and/or *cag*PAI genes.

Study overview: investigate virulence gene regulation by two *cag*PAI sRNAs

- Clone plasmids to carry sRNA genes
- Develop experimental *H. pylori* strains
- Grow *H. pylori*, isolate total RNA
- Identify differentially expressed virulence genes with RNA sequencing
- Measure/quantify expression changes

Cloning to overexpress small RNAs



Verified total RNA (strains: wild-type, RM2, RM3)

Figure 2, Methods. If RNA expression changes when *H. pylori* strains overexpress an sRNA versus in the unmodified wild-type strain, this indicates those gene(s) are regulated by that sRNA.

We used molecular and microbiology/culturing techniques to produce two experimental strains, each overexpressing a different sRNA.

Analyzing virulence gene regulation

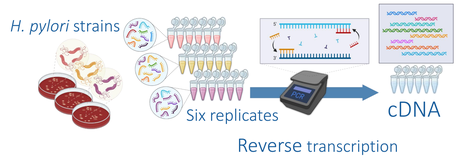
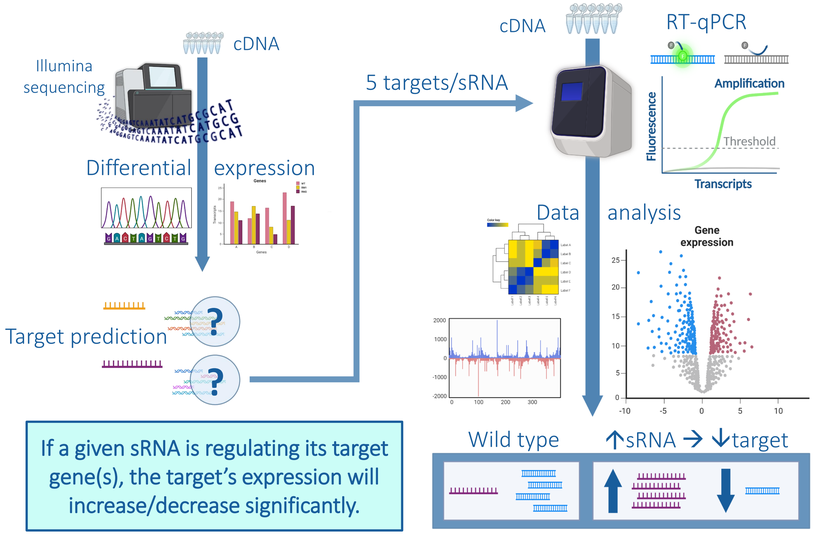


Figure 3, Analysis. RNA from strains (WT, RM2, RM3) will be analyzed with RNA-sequencing and reverse transcription quantitative polymerase chain reaction (RT-qPCR).



If a given sRNA is regulating its target gene(s), the target's expression will increase/decrease significantly.

Small RNAs in *H. pylori:* future directions

RNA-seq data/analysis

- Estimate all transcript abundances
- 18 samples (6 replicates per strain)
- Statistical analyses, plots for differential expression in experimental strains compared to WT

RT-qPCR data/analysis

- Quantify regulation directionality (up/down) and magnitude
- Five target genes/sRNA
- Normalized to rRNA and WT target
- Statistical analyses, plots

Conclusions

Identify virulence genes regulated by two *cag*PAI sRNAs, quantify such regulation

Future directions

- Investigate other sRNAs using protocols and materials developed here
- *In vitro* assays to determine phenotypic effects of sRNA overexpression
- Other targets of these sRNAs
- Molecular assays to determine mechanisms of regulation observed

Literature cited & acknowledgments

Brief citations

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Full citations

Acknowledgments

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