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Characterization of a *Helicobacter pylori* Small RNA by RT-PCR

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Abstract

- *Helicobacter pylori* is a human gastric bacterial pathogen that uses small RNAs (sRNAs) for posttranscriptional regulation of gene expression (e.g., motility, adhesion, urease activity).
- RT-PCR was used to characterize sRNA Hpnc2665.
- RT-PCR results for Hpnc2665 are unclear, representing longer-than-expected transcripts of variable lengths.
- sRNA Hpnc2665 occurs downstream of another predicted sRNA. The results may represent two overlapping (possibly relatively large) transcripts.
- Northern blotting should resolve unclear RT-PCR results.

Introduction

- *Helicobacter pylori* is a human gastric bacterial pathogen producing gastritis, ulcers, and gastric cancer [5].
- Over 200 *H. pylori* small RNAs (sRNAs) are identified [3], with some characterized in mechanism and RNA transcript target (e.g., genes affecting motility, adhesion, urease activity, etc.) [5,6,7].
- An sRNA molecule is a small noncoding RNA (generally <300 nucleotides) operating in posttranscriptional regulation by base-pairing RNAs, affecting downstream gene expression [1,2,5].
- Using a promoter trap system, promoters were previously identified for 3 sRNAs antisense to the *cag* pathogenicity island (a set of virulence genes that encode a Type-IV secretion system) including sRNA Hpnc2665 [4].
- Previous work in this lab (interrupted by the pandemic) was begun to characterize this sRNA with RT-PCR (reverse transcription polymerase chain reaction) and *in silico* prediction of an intrinsic terminator [1,2].
- RT-PCR uses total RNA extracted from *H. pylori* and custom oligonucleotide DNA primers to explore the length (and thus sequence location) of an sRNA based on complementarity to the primers.

Literature

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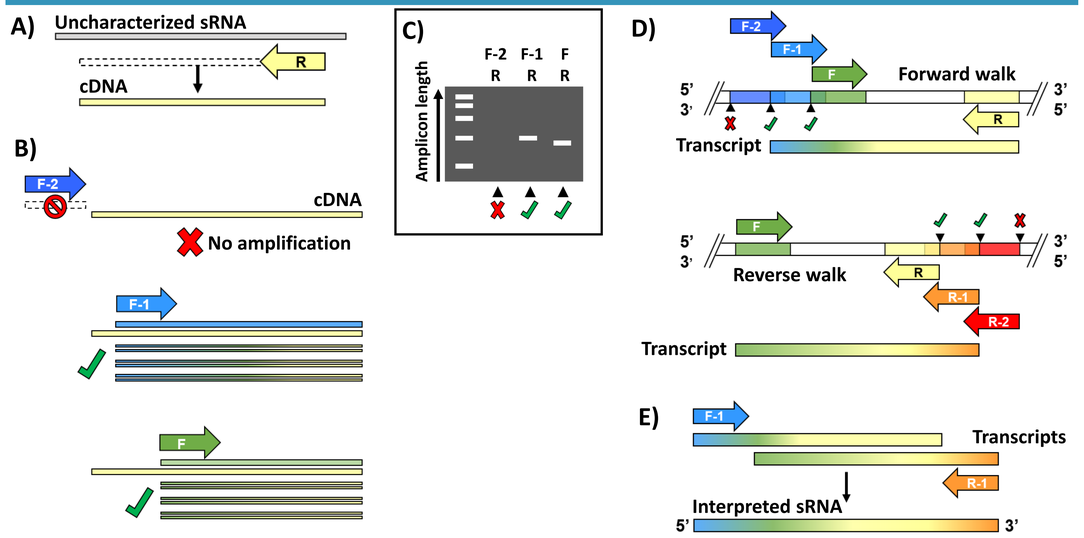
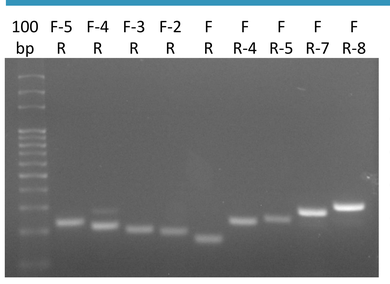
3. Sharma, C.M. *et al.* 2010. *The primary transcriptome of the major human pathogen* Helicobacter pylori. Nature, 464:250-255.
4. Ta, L.H. *et al.* 2012. *Conserved transcriptional unit organization of the cag pathogenicity island among* Helicobacter pylori. Front. Cell. Inf. Microbio., 2:46.
5. Tejada-Arranz, A., De Reuse, H. 2021. *Riboregulation in the major gastric pathogen* Helicobacter pylori. Front. Microbiol., 12:712804.
6. Vannini, A., Roncarati, D., Danielli, A. 2016. *The cag-pathogenicity island encoded CncR1 sRNA oppositely modulates* Helicobacter pylori *motility and adhesion to host cells.* Cell. Mol. Life Sci., 73:3151-3168.
7. Wen. Y., Feng, J., Sachs, G. 2013. Helicobacter pylori *5' ureB-sRNA, a cis-encoded antisense small RNA, negatively regulates ureAB expression by transcription termination.* J. Bacteriol., 195:3.

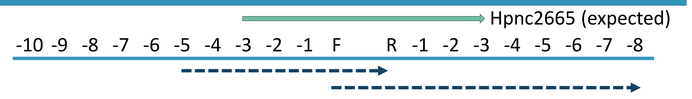
Methods



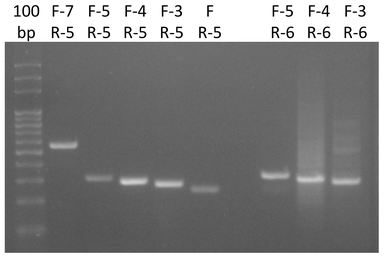
RT-PCR: A) Reverse transcription from *H. pylori* total RNA with reverse primer complementary to sRNA Hpnc2665 produces complementary DNA (cDNA). **B)** "Primer walking" PCR with forward primers (e.g., F-2, F-1, F) complementary to cDNA only amplifies DNA if forward primer is on the cDNA produced from the sRNA. **C)** Agarose gel electrophoresis displays a band if amplification occurred, indicating that the primer pairs were on the sRNA. **D)** Forward and reverse walks are performed with different forward and reverse primer pairs. **E)** Interpretation of example shows length of sRNA according to which primer pairs had a band on the gel.

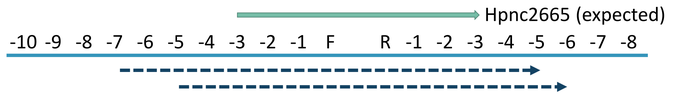
Results



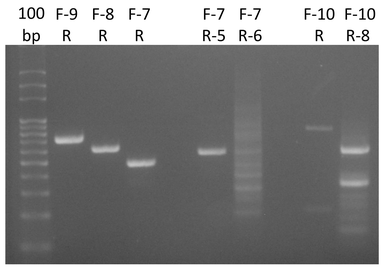


- F-5 to R is on a transcript (expected sizes).
- F to R-8 is on a transcript (expected sizes).
- F-5 is in the expected promoter region.
- (Some intermediate results not shown; negative controls were always run on original gels).





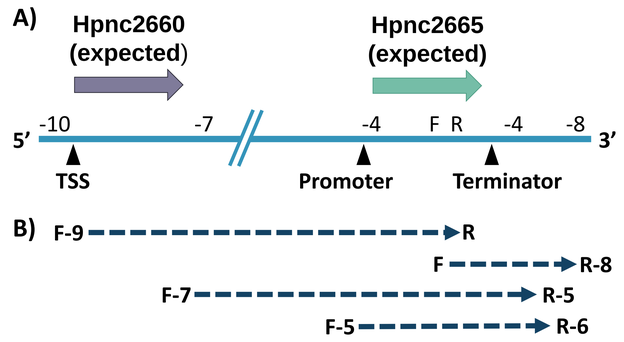
- F-7 to R-5 is on a transcript (expected sizes).
- F-5 to R-6 is on a transcript (expected sizes).
- F primers beyond F-5 did not form product with R-6 (not shown).



- F primers up to F-9 are on a transcript with R (expected sizes).
- F-7 produces a transcript with R-5 but not R-6.
- F-10 and R have one correct size band and one incorrect band.
- F-10 and R-8 (entire region) do not produce a single transcript.

Discussion

- Results are unclear. Transcripts are too long for sRNAs and extend far past expected promoter and terminator regions.
- However, DNA contamination was ruled out and negative controls were clear.
- Does the Hpnc2665 region contain more than one transcript, one or both of which are larger than expected for sRNAs?
- sRNA Hpnc2665 occurs downstream of Hpnc2660, which is expected to occur between F-10 and F-7 (approximately 261 base pairs) [3,4].
- The span of Hpnc2665 was predicted to lie between F-4 and R-4 (approximately 237 base pairs) [1].
- This may indicate two or more transcripts, one stretching from F-10 to at least R and one from at least F to R-8.
- R-6 may contain a terminator structure based on the variability of primer pairs with it.
- Further RT-PCR on sRNA Hpnc2665 is unlikely to be helpful considering these confounding factors.



A) Schematic of sRNAs Hpnc2660 and Hpnc2665, showing the transcription start site for Hpnc2660 [3] and predicted promoter and terminator regions for Hpnc2665 [1]. **B)** Transcripts suggested by results, possibly representing two or more total transcripts of unusual length for sRNAs.

Future directions

- RT-PCR on the other two antisense sRNAs found in Ta *et al.*'s 2012 promoter trap study [4].
- Northern blotting with a fluorescent probe that binds in the overlap region of Hpnc2660 and Hpnc2665.
- If the Northern blotting shows two bands of sizes that correlate with RT-PCR results for Hpnc2660 and Hpnc2665, this would suggest these are two very long transcripts.

Acknowledgments

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