

IOWA STATE UNIVERSITY

ISU McNair Program

Non-coding RNAs in *Agrobacterium tumefaciens*

Presenter: Chris Hernandez

Mentor: Dr. Kan Wang

Acting Mentor: Dr. Keunsub Lee

Outline

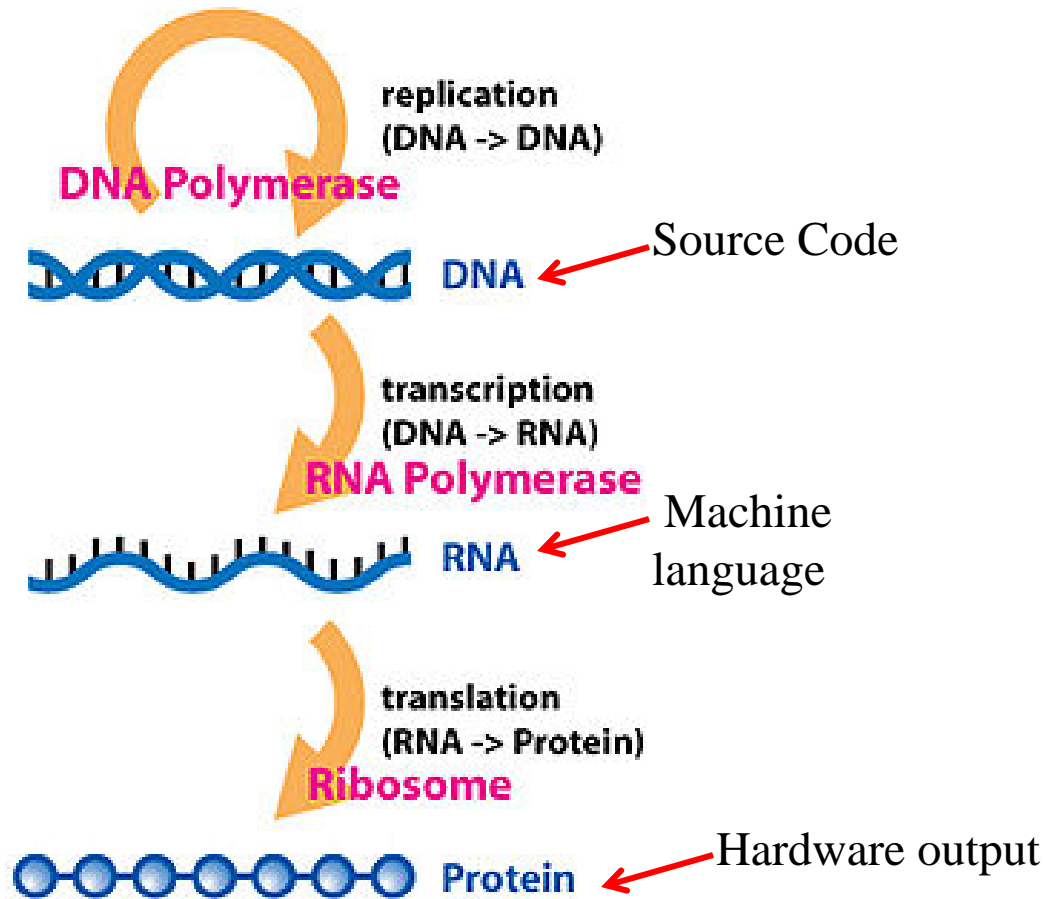
1. *Agrobacterium*
2. Central dogma review
3. The world of ncRNAs
4. The role of ncRNAs In *Agrobacterium*?
5. Experimental design
6. Results
7. Future directions
8. Acknowledgements

Agrobacterium: plant terrorist or biologist's tool?

- Microbe responsible for crown gall tumors
- Hijacks plant machinery to make food by inserting new code (DNA) into existing plant program
- Can be used by biologists for genetic engineering



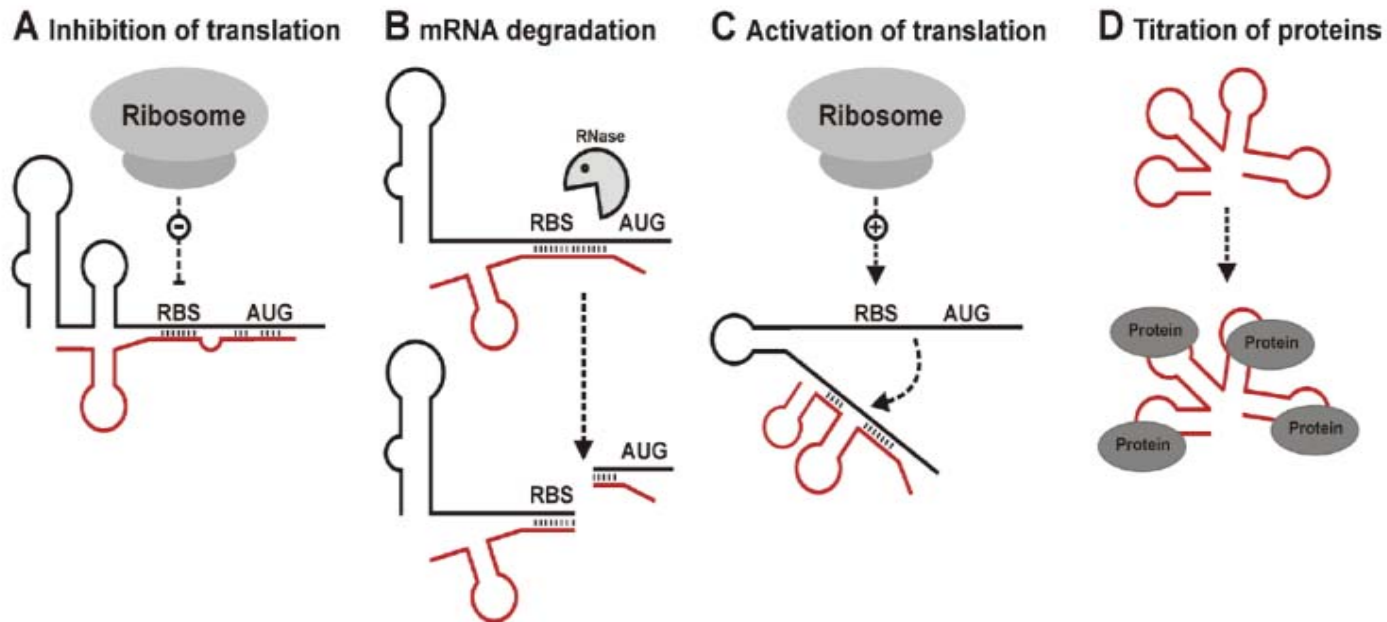
The Central Dogma



- RNA traditionally thought of as only an intermediate

ncRNAs:

- RNA is more than just an intermediate
- Many non-coding RNAs (ncRNAs) have been identified
- ncRNAs are involved in regulatory roles



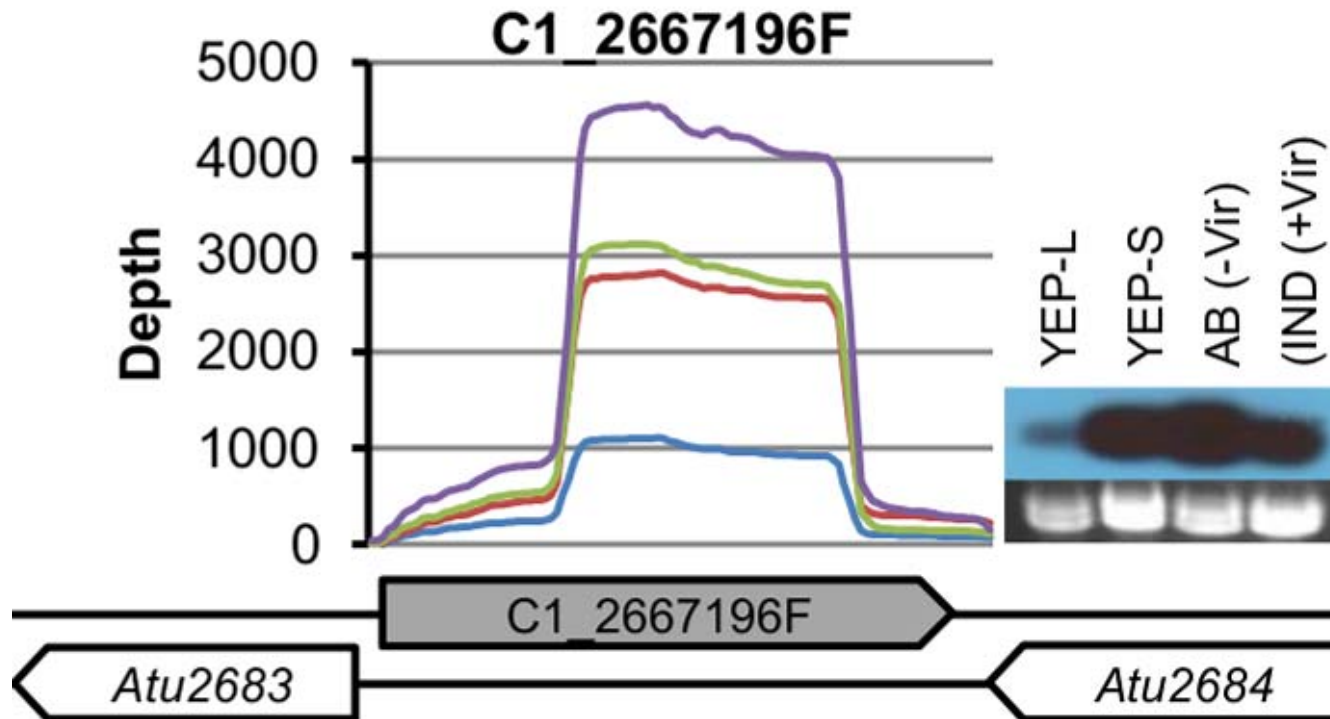
ncRNAs in *Agrobacterium tumefaciens*

- Initial work has identified regulatory RNAs in *Agrobacterium* that are differentially expressed (Lee *et al.* 2013)
- Question: What are the roles of these ncRNAs in *Agrobacterium* ?

Lee et al. A Genome-wide Survey of Highly Expressed Non-coding RNAs and Biological Validation of Selected Candidates in *Agrobacterium tumefaciens*. PLOS One 8: e70720. doi:10.1371/journal.pone.0070720 (2013)

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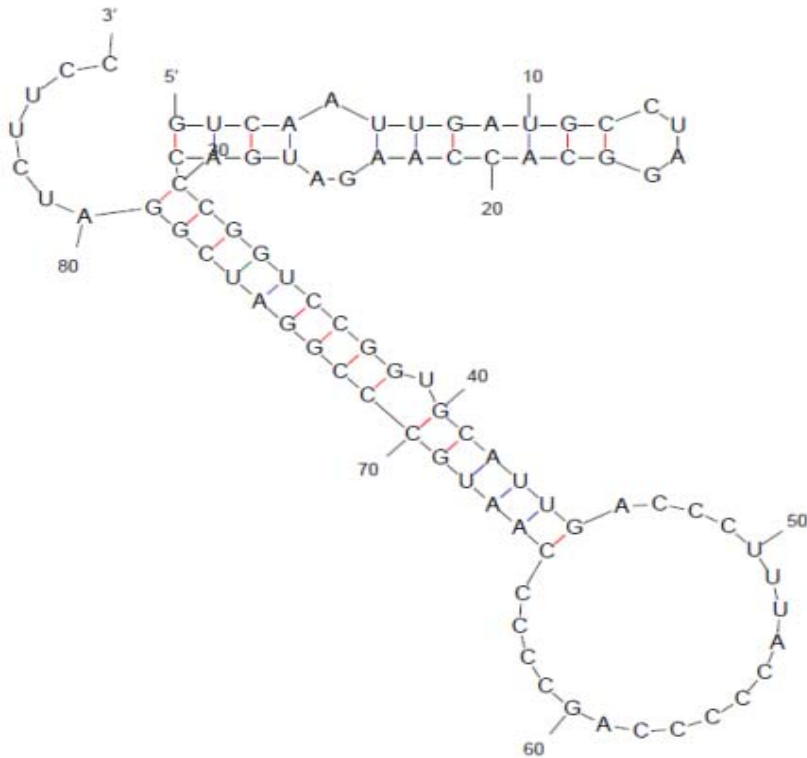
7-1: Putative ncRNA regulator



Lee et al. A Genome-wide Survey of Highly Expressed Non-coding RNAs and Biological Validation of Selected Candidates in *Agrobacterium tumefaciens*. PLOS One 8: e70720. doi:10.1371/journal.pone.0070720 (2013)

7-1 Con't

Predicted Secondary Structure*



dG = -35.60 [Initially -33.40] 12Aug08-14-44-04

Id - mRNA	mRNA description
<i>phbC</i>	poly-beta-hydroxybutyrate synthase
<i>Atu1751</i>	hypothetical protein
<i>pfs</i>	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
<i>xynA</i>	endo-1,4-beta-xylanase
<i>mgsA</i>	methylglyoxal synthase
<i>Atu2228</i>	diguanylate cyclase

*predicted by: <http://rna.informatik.uni-freiburg.de/IntaRNA/Input.jsp>

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Experimental Design

+AS

(induced)

C58
 $\Delta 7-1$

C58
 $\Delta 7-1$

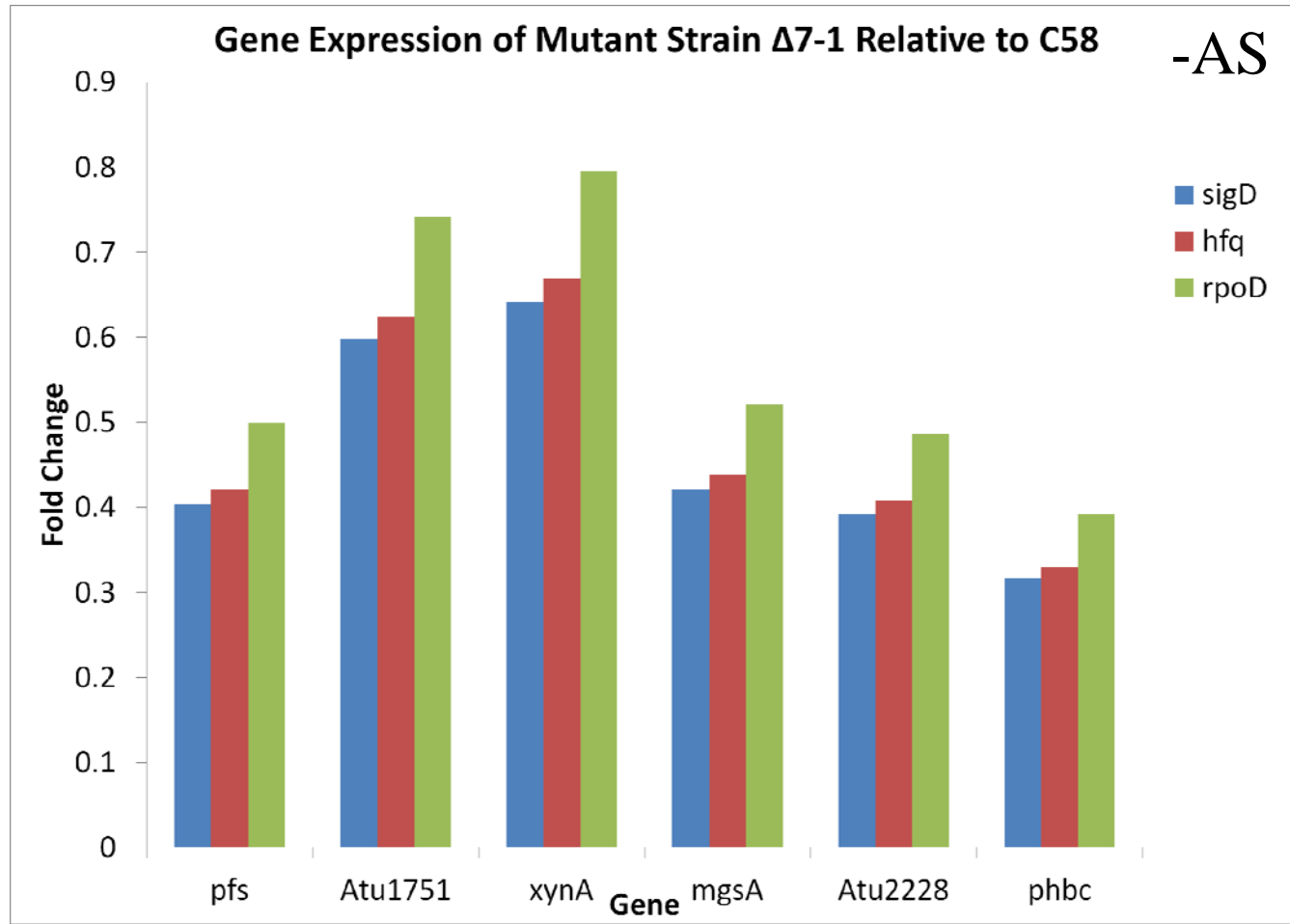
Extract
RNA

qPCR

Analyze
results

-AS

Results



Summary and Future Directions

- Gene expression of six potential ncRNA target genes were measured in both wild type strain (C58) and ncRNA mutant strain ($\Delta 7-1$) by quantitative PCR. No marked differences were observed
- Future work would include replications, different growth conditions, and will assay more genes.
- cDNA libraries could be constructed for the mutant strains and RNA-seq will be used to identify differentially expressed genes

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