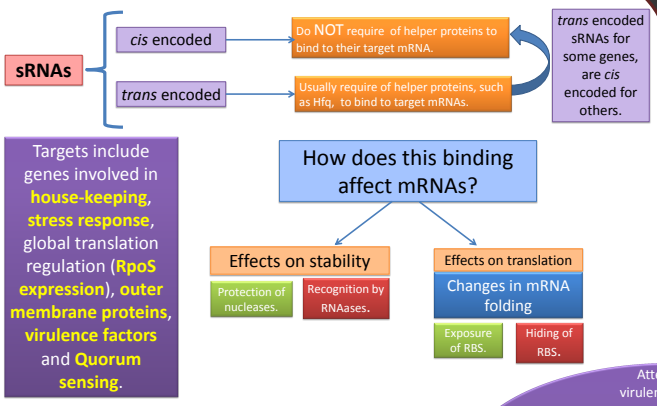
Role of sRNA regulation mediating Hfq in *Salmonella enterica* serovar Typhimurium pathogenicity pathways

Marc Emezue Lozano
Degree in **Genetics** - UAB
2013-14

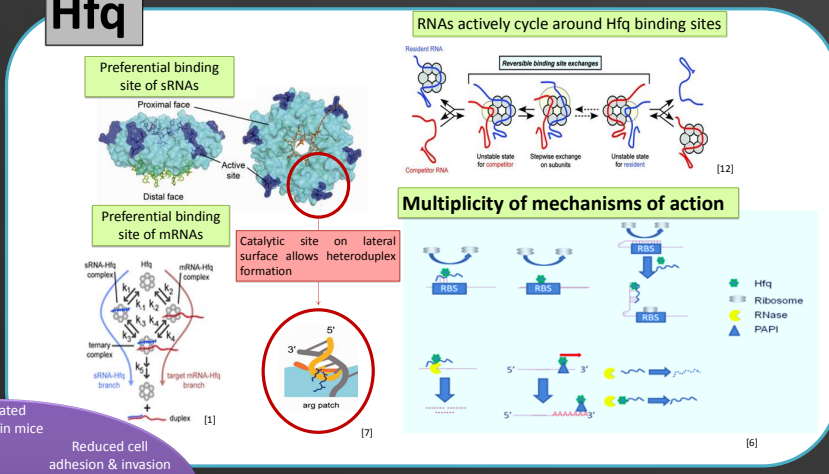
INTRODUCTION

Hfq is a Sm-like chaperone that acts as a global gene regulator in many bacteria. It can act both in a sRNA-dependent and independent manner in order to fulfill its purpose in regards to mRNA stability and subsequent translation. In this work, the role that Hfq plays in *Salmonella enterica* serovar Typhymurium's pathogenesis through a sRNA is reviewed. To accomplish this, it was needed to first acknowledge **the role that sRNAs play in regulatory pathways** as well as a thorough **understanding of Hfq**. Furthermore, insight into the **molecular mechanisms behind *S.* Typhimurium's pathogenesis** is required. Finally, the focus needed to be centered around **sRNAs**, so their **implication in the regulation** of the **T**ype **T**hree **S**ecretion **S**ystem encoded within ***S**almonella* **P**athogenicity **I**sland 1 (**T3SS-1** from now on), was covered.

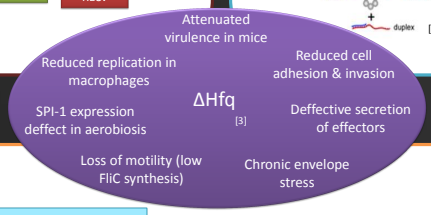
sRNAs are key elements in regulatory pathways



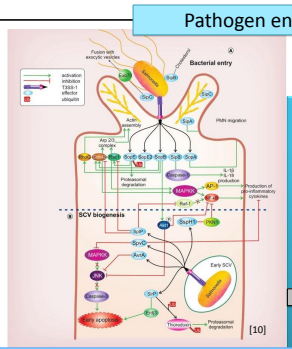
Hfq



Salmonella enterica serovar Typhimurium



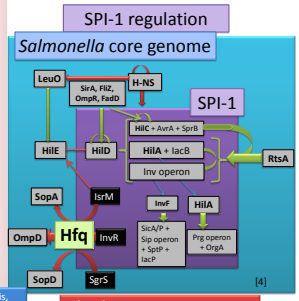
Pathogen entry into non-phagocytic cells



Entry into the cell: actin rearrangements to promote macropinocytosis, production of pro-inflamatory cytokines through the MAPK-AP-1/NF-κβ pathways and promotion of IL-1β & IL-18 synthesis, lead to bacterial internalization and leukocyte migration to the intestinal lumen.

Early SCV: cytoskeleton normalization, inhibition of the MAPK pathway through inhibition of Raf1, inhibition of genes under NF-κβ control, inhibition of early apoptosis and reduction of thioredoxin, promoting non-folded proteins accumulation in the ER which promote late apoptosis.

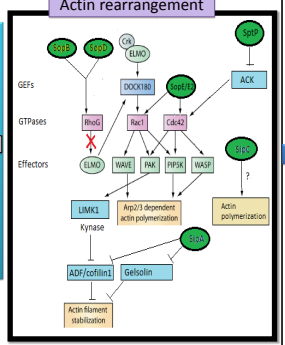
SPI-1 regulation



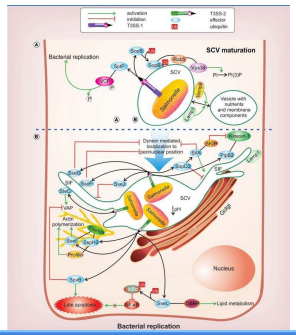
Induced:

- Stationary phase under anaerobic conditions.
- Late exponential phase under aerobic conditions.

Actin rearrangement



Intracellular survival and replication



SCV maturation: SopB allows for evasion of canonic endocytic pathway through recruitment of RabS and impeding other Rab GTP ases from joining the membrane. SptP increases bacterial replication. Activation of T3SS-2 thanks to environment acidification; this will allow *Salmonella* to keep its niche within the cell.

Late SCV: Migration to a perinuclear position thanks to SifA, PipB and SipC affecting vesicular traffic through interactions with dineine and kynesine Acquisition of nutrients is done thanks to endocytic vesicle intake mediating SIfs. 00

Apoptosis and dissemination

Hfq-sRNA interactions involved in *Salmonella* entry to the host cell

InvR and OmpD

InvR is a sRNA encoded within SPI-1 that is expressed in conditions akin to those in the gut, whereas OmpD is an outer membrane protein. When cells progress into stationary phase, its synthesis is inhibited. Its role in virulence is a matter of controversy, but some studies point towards a role in bacterial adherence to human cells.

SgrS and SopD

SgrS is a sRNA encoded within the genomic core that is up-regulated during the stress response towards G6P. SopD acts in concert with SopB to promote pro-inflamatory pathways and pathogen invasion. There are some studies that indicate that SopD plays a role in SNX-3 microtubule formation in a SopB-dependent manner; these in turn contribute to SCV formation. The biological reason underlying this mechanism is not clear yet, but it could be playing a role in the diminution of effector molecule levels.

IsrM and SopA

IsrM is a sRNA encoded within SPI-1 which is induced under moderately acidic pH and high osmolarity, conditions found within the stomach and, to a certain extent, the conditions found within the SCV. IsrM both inhibits SopA and HilE translation. Because of this, SopA is under negative direct control and positive indirect control of IsrM. High levels of SopA have been associated with early Salmonella exit to the cytosol, so its levels during infection must be under tight control. Furthermore, IsrM plays a role in replication inside macrophages, were the conditions are more acidic. Perhaps IsrM plays a role in controlling *Salmonella* exit when the cell dies by coordinating temporal expression of SPI-1 through inhibition of HilE. However, there is no proof that IsrM binding to HilE is Hfq dependent, so this falls outside the range of this study.

CONCLUSIONS

As we have seen, sRNAs are an integral part of Hfq's regulation. In the case of *S.* Typhimurium, a mutant defective on Hfq shows pleiotropic defects, hinting at its role as a global regulator. In the case of virulence, sRNAs play small yet important role. In regards to them, it is safe to say that they are important regulatory factors in some pathways and that they might be key in mediating host-pathogen interactions. Furthermore, due to the fact that only partial complementarity is needed for them to target a mRNA, they contribute to the regulation of horizontally acquired genes. Finally, it is important to note that they have been long overlooked in the studies performed in genetics. Thankfully, nowadays they are treated as important regulatory factors and this work tried to reflect such importance. However, more work on this issue needs to be addressed in the future.

References

1. Adamson DN, Lim HN, **Essential requirements for robust signaling in Hfq dependent small RNA networks**, *PLoS Computational Biology*, 2011 Aug;7(8):e1002138
2. Bakowski, M. A., theses, **Role of bacterial effectors SopD and SopB in pathogenicity of *Salmonella enterica* serovar Typhimurium**, 2009, University of Toronto
3. Chao Y., Vogel J., **The role of Hfq in bacterial pathogens**, *Current Opinion in Microbiology*, 2010 Feb;13(1):24-33
4. López-Garrido J., Casadesús J., **Regulation of *Salmonella enterica* Pathogenicity Island 1 by DNA Adenine Methylation**, *Genetics*, 2010; 184(3): 637–649
5. Meyer PN., Wilmes-Riesenberg MR., Stathopoulos C., Curtiss R 3rd, **Virulence of a *Salmonella* Typhimurium OmpD mutant**, *Infection and Immunity*, 1998 Jan;66(1):387-90
6. Munshaw, B., theses, **The Regulation of Prokaryotic Transposable Elements by the RNA Binding Protein Hfq**, 2012, University of Western Ontario
7. Panja S., Schu DJ., Woodson SA., **Conserved arginines on the rim of Hfq catalyze base pair formation and Exchange**, *Nucleic Acids Research*, 2013 Aug;41(15):7536-46
8. Papenfort K., Podkaminski D., Hinton JCD, Vogel J., **The ancestral SgrS RNA discriminates horizontally acquired *Salmonella* mRNAs through a single G-U wobble pair**, *PNAS*, 2012, E757-E764
9. Suárez M., Rüssmann H., **Molecular mechanisms of *Salmonella* invasion: the type III secretion system of the pathogenicity island 1**, *International Microbiology*, 1998 Sep;1(3):197-204
10. Van der Heijden, J., Finlay, B. B., **Type III Effector-Mediated Processes in *Salmonella* Infection**, *Future Microbiology*, 2012;7(6):685-703.
11. Vogel J., Luisi BF, **Hfq and its constellation of RNA**, *Nature reviews of Microbiology*, 2011 Aug 15;9(8):578-89
12. Wagner EG, **Cycling of RNAs on Hfq**, *RNA Biology*, 2013 Apr;10(4):619-26
13. Zhang, Y., Higashide, W. M., McCormick, B. A., Chen, J., Zhou, D., **The inflammation-associated *Salmonella* SopA is a HECT-like E3 ubiquitin ligase**, *Molecular Microbiology*, (2006) 62(3), 786–793