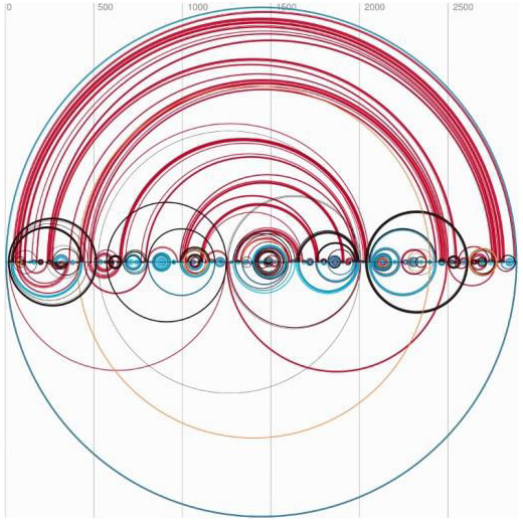
Graduate Student Seminar

Revisiting the Central Dogma

The role of **Small RNA** in **Bacteria**



The Chinese University of Hong Kong
Faculty of Medicine
Department of Microbiology

Supervisor : Prof. Margaret Ip
Student : Helen Ma (PhD student)
Date : 2014-12-15

Content

- (I) Introduciton
  - Revisiting the Central Dogma
  - Types of RNA / Small RNAs in Bacteria
- (II) Functions and Mechanisms of Small RNA
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  - (B) Riboswitches
  - (C) Intergenic RNAs
- (III) Small RNA Prediction Softwares and Databases
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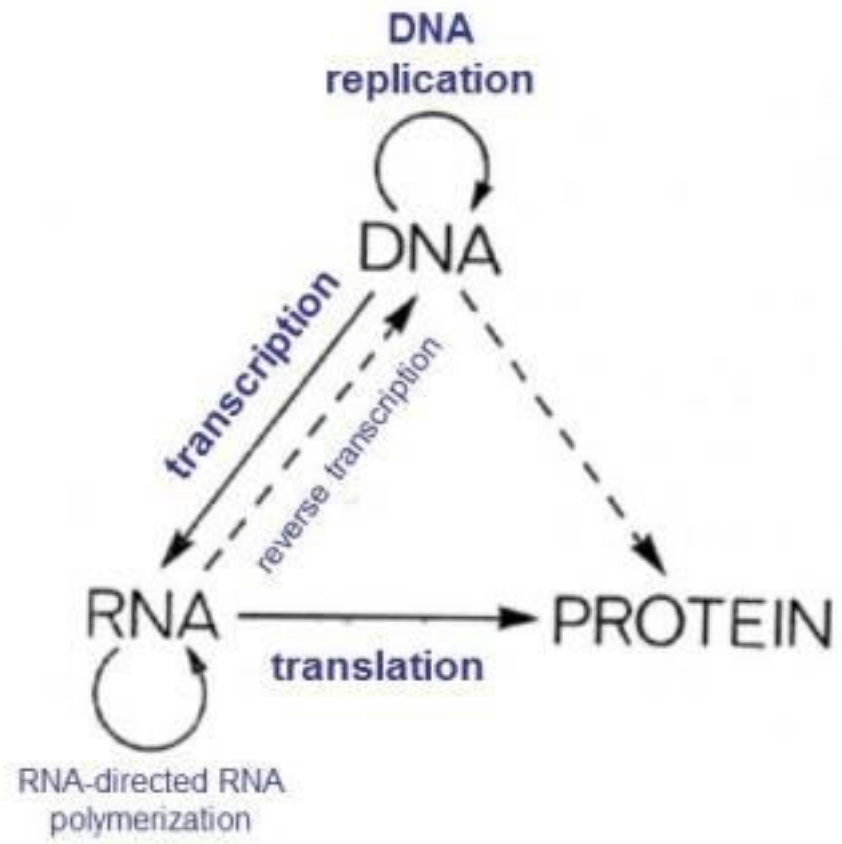
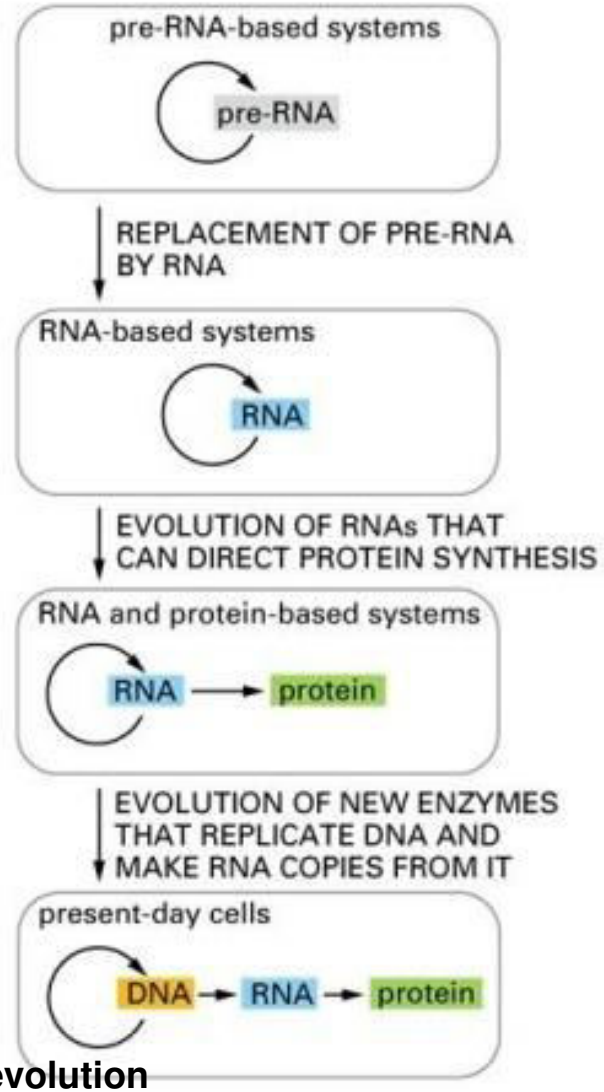
  - BSDR - Bacterial Small Regulatory RNA Database
- (IV) Future Perspectives
  - Hijacking nature's design - Artificial small RNAs

Revisiting the Central Dogma

Flow of information

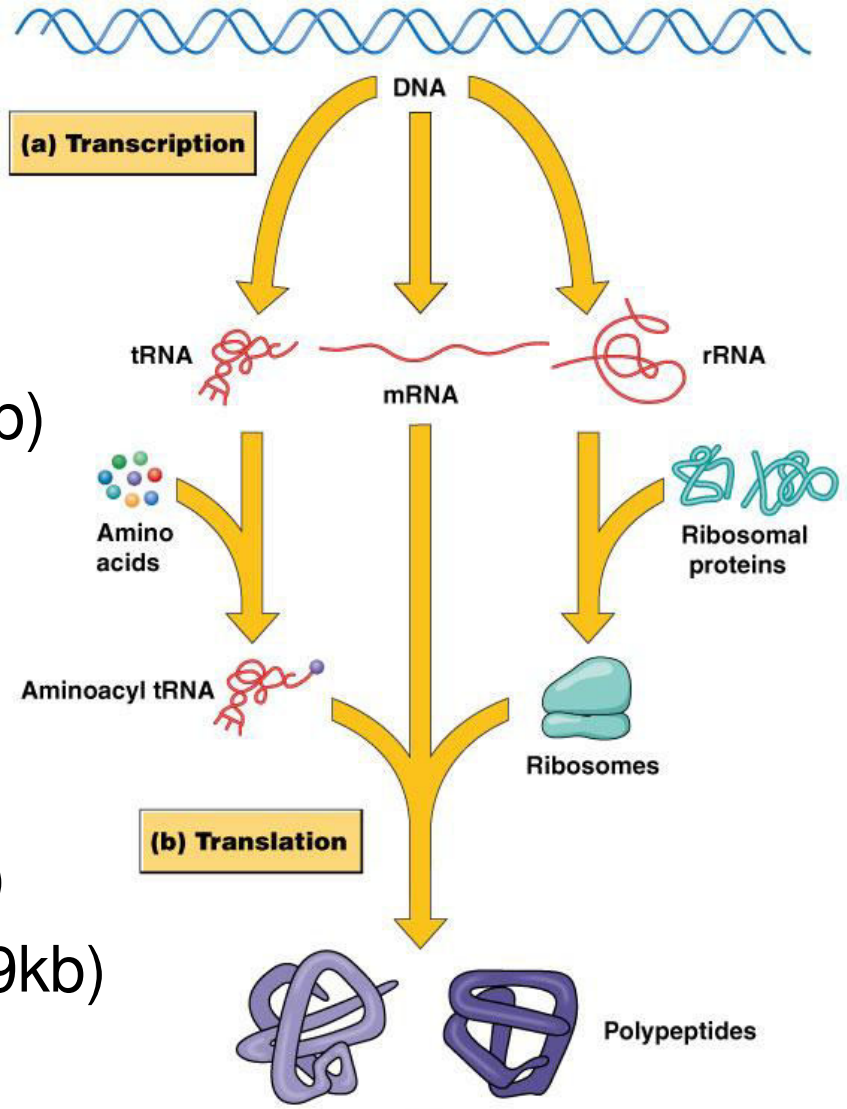


RNA World Hypothesis



Types of RNA in Bacteria

- **Coding RNA**
  - code for / translated to a protein
- Messenger RNA (mRNA, ~1-2kb)
- **Non-coding RNA (ncRNA)**
  - does not code for / translated to a protein
- Transfer RNA (tRNA, ~60-95bp)
- Ribosomal RNA (rRNA, ~1.5-2.9kb)
- **Small RNAs (~30-500nts)**

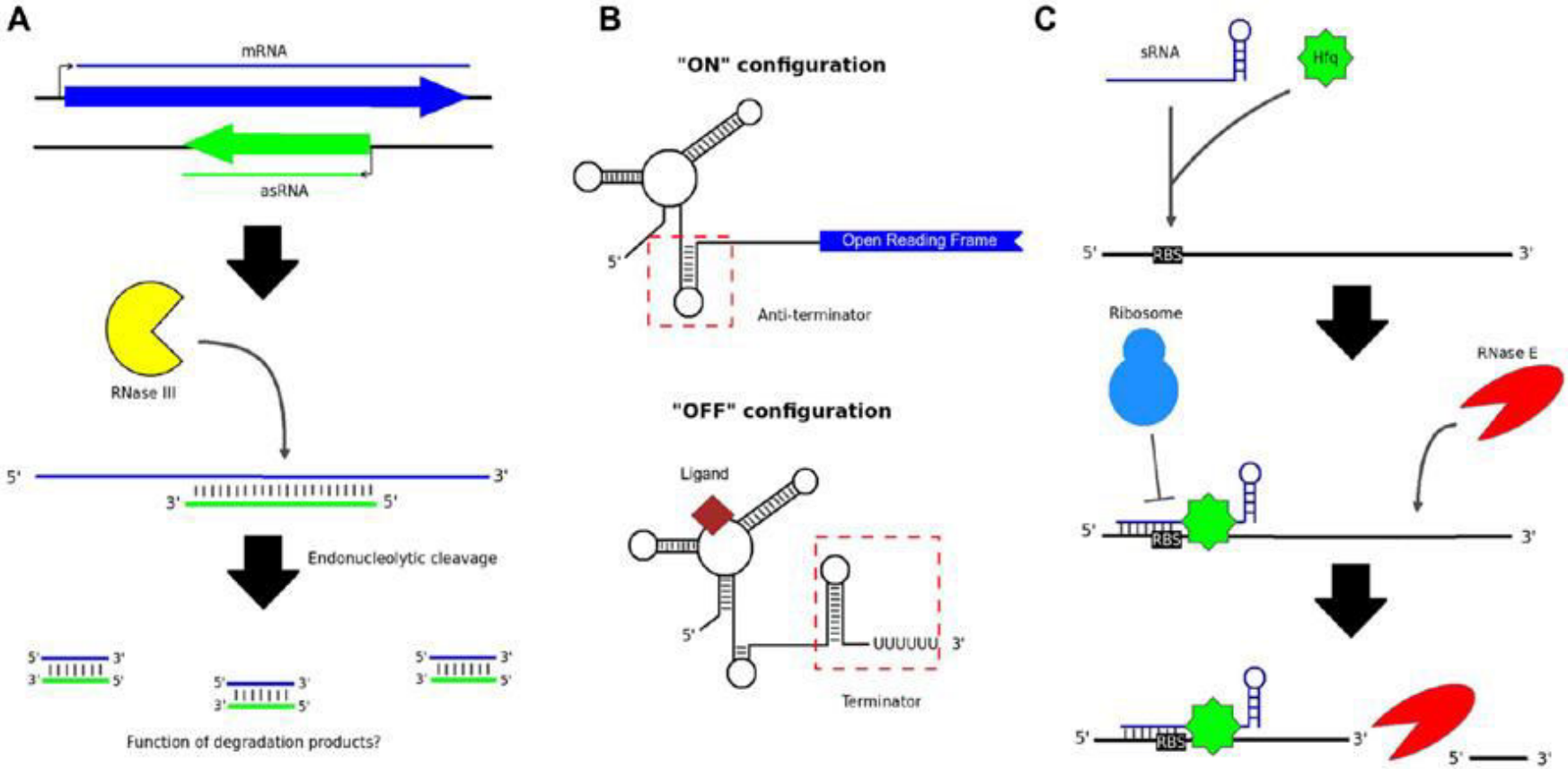


Principles of Cell Biology
Becker's World of the Cell, 8th edition Hardin, Bertoni & Kleinsmith, 2012
http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-21/CB21.html

(I) Introduction

Types of Small RNA in Bacteria

(A) Antisense RNAs (asRNA) (B) Riboswitches (C) Intergenic Small RNAs



Dinan A.M. Loftus B.J. (Non-)translational medicine: targeting bacterial RNA
Front. Genet., 08 November 2013 | doi: 10.3389/fgene.2013.00230
http://journal.frontiersin.org/Journal/10.3389/fgene.2013.00230/full

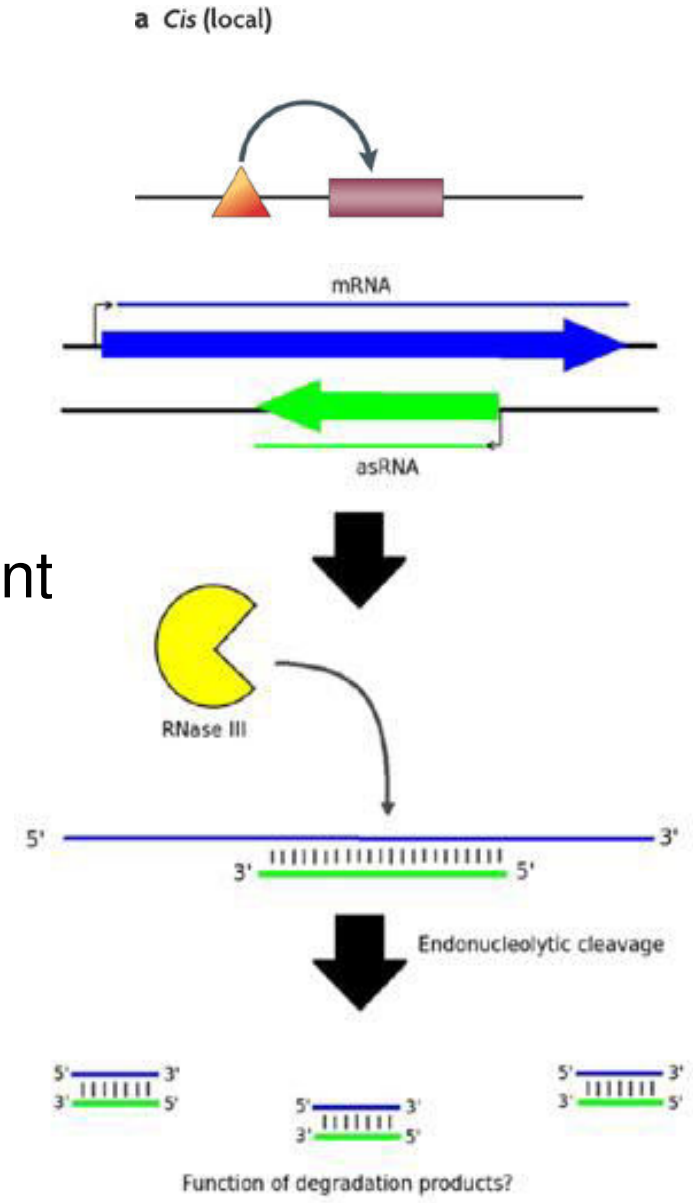
(II) Functions and Mechanisms

(A) Antisense RNAs

- Study in *E. coli* since 1960s
- Transcribed from opposite strand
- Maintain plasmid number
- Against bacteriophage development
- Blocks ribosome for protein expression
- Down-regulate gene expression

  e.g. outer membrane proteins OmpF(efflux pump),

  DicF (cell division), etc.

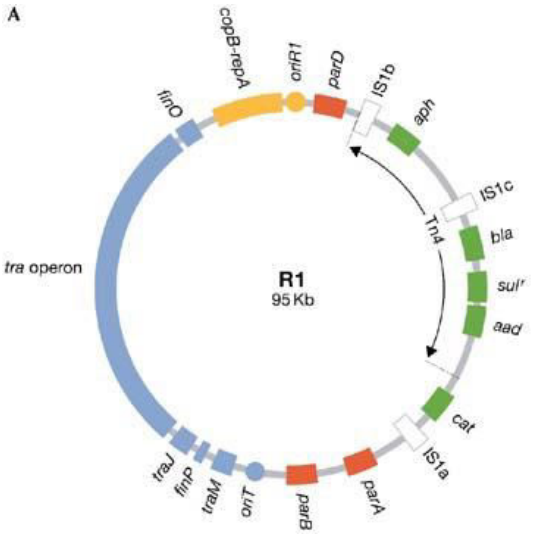


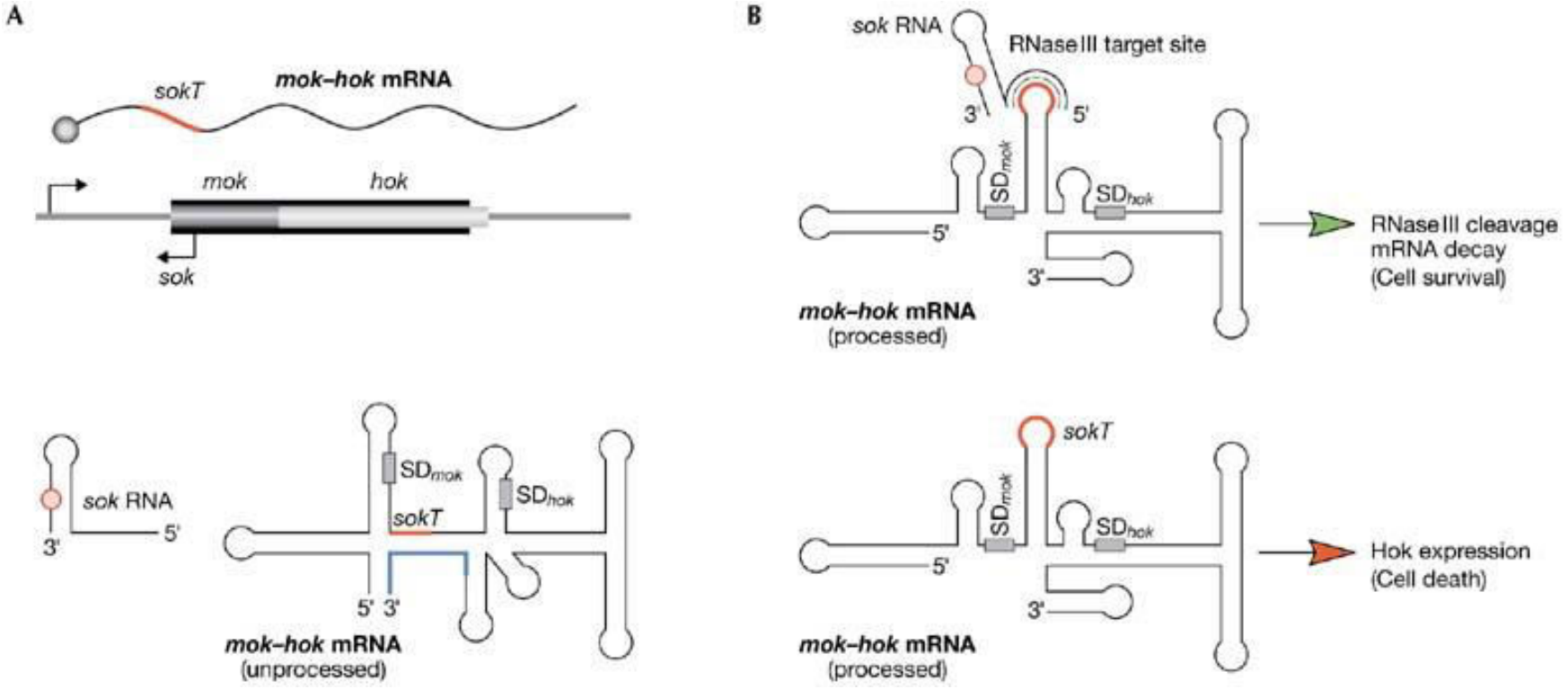
Wager E.G.H. and Simons R.W. ANTISENSE RNA CONTROL INBACTERIA, PHAGES, ANDPLASMIDS. Annu. Rev. Microbiol. 1994. 48.'713-4

(II) Functions and Mechanisms

(A) Antisense RNAs

- Post-segregation killing system in plasmid R1
- **Stem-loop structures** of translated **parB** gene
- **hok** : **ho**st **k**illing
- **mok** : **m**ediator **o**f **k**illing
- **sok** / **sokT** : **s**upression **o**f **k**illing

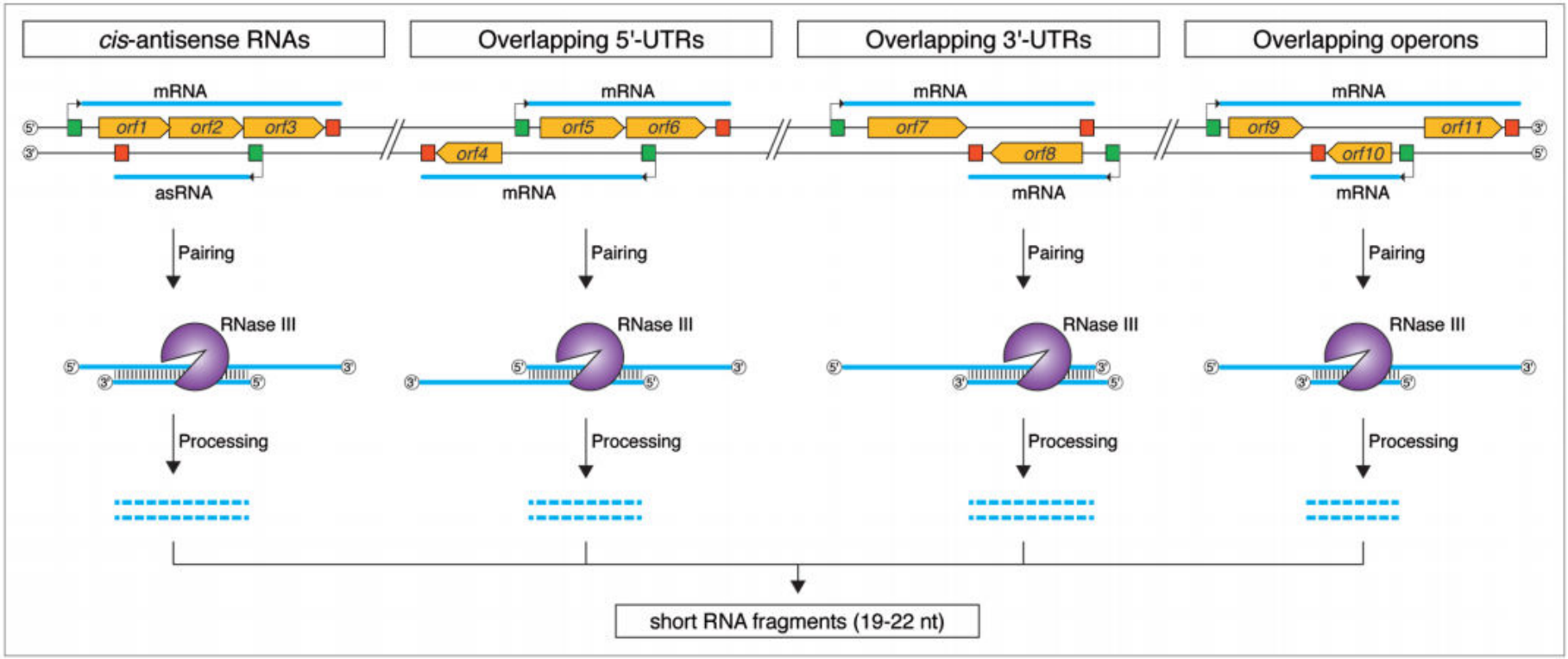




Guillermo de la Cueva-Méndez, Belén Pimentel Gene and cell survival: lessons from prokaryotic plasmid R1EMBO reports: 8 (5)01 May 2007 | pp 421 - 515

(II) Functions and Mechanisms

(A) Antisense RNAs



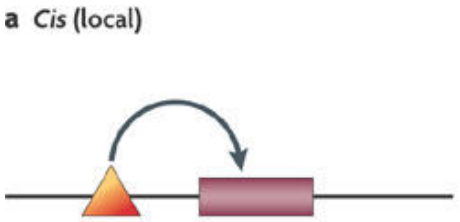
Hypothesis :

- (1) Antisense RNA acts as a threshold level to control level of sense RNA, to prevent over reaction to enviromental stimuli
- (2) Control over genes in the same operon by one antisense RNA molecule
- (3) Potential role of short RNA fragments on target gene expression

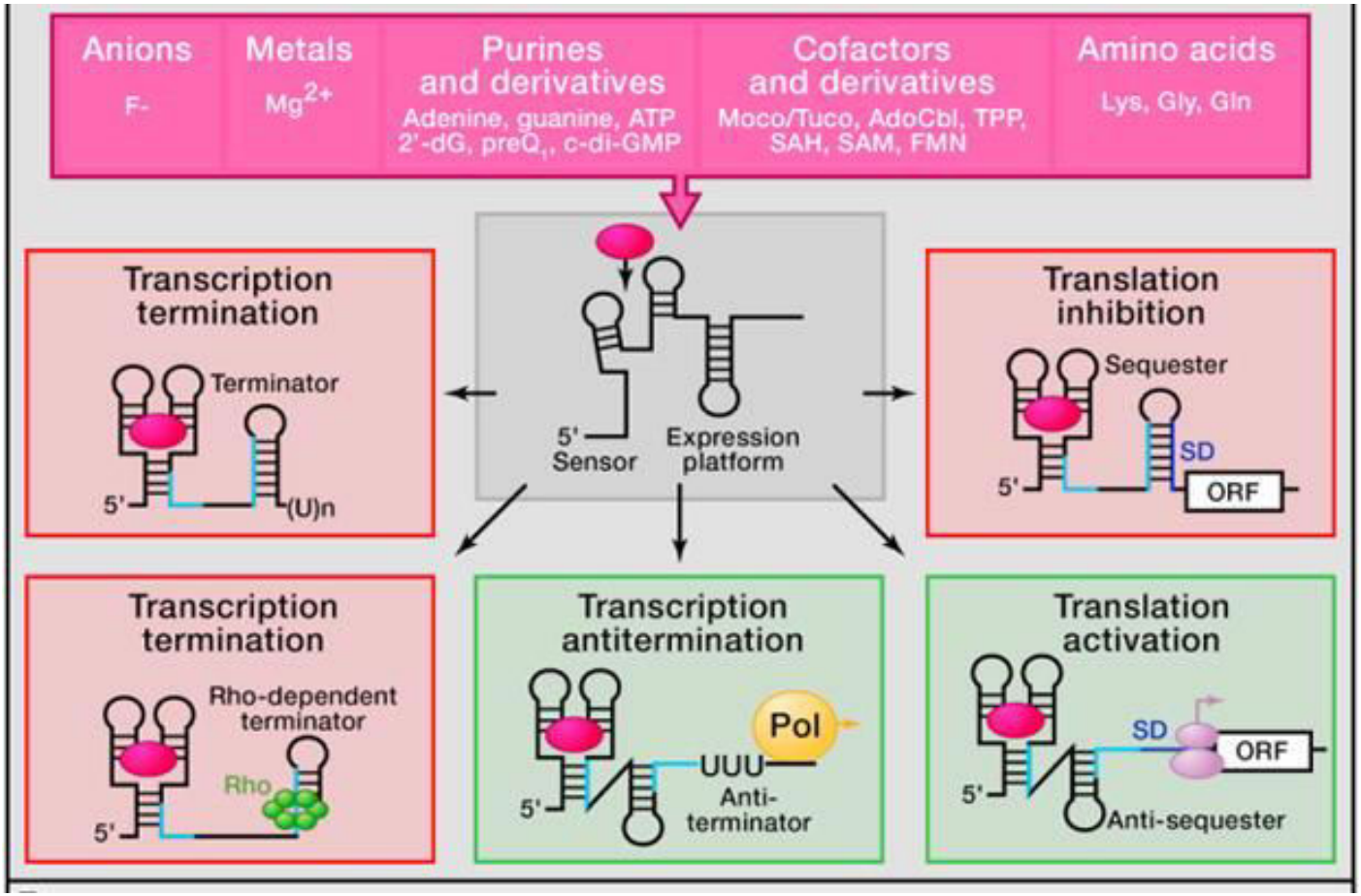
Lasa S. et. al., An effort to make sense of antisense transcription in bacteria. RNA Biology 9:8, 1039-1044; August 2012; © 2012 Landes Bioscience

(II) Functions and Mechanisms

(B) Riboswitches



- Discovered in 2000s
- RNA-based intracellular sensors
- Stem-loop structures

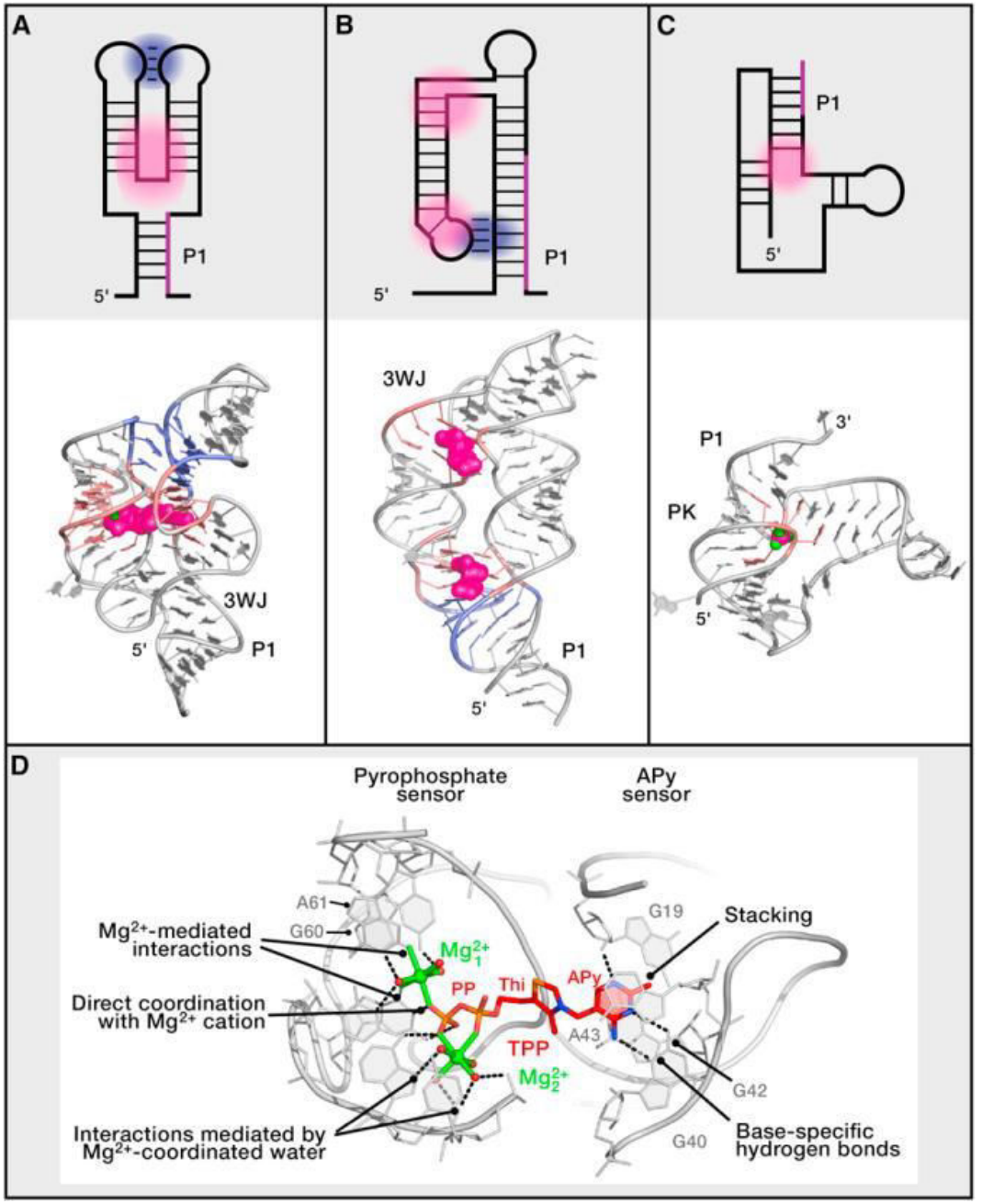


Serganov A. and Nudler V. A Decade of Riboswitches. Cell. Jan 17, 2013; 152(0): 17–24.

(II) Functions and Mechanisms

(B) Riboswitches

- "Junctional" riboswitches
- Position of metabolic sensors
- Stability of **stem-loop structures**
- Class Ia (A) e.g. purines
- Class Ib (B) e.g. vitamine derivatives
- Class II (C) e.g. Mg2+

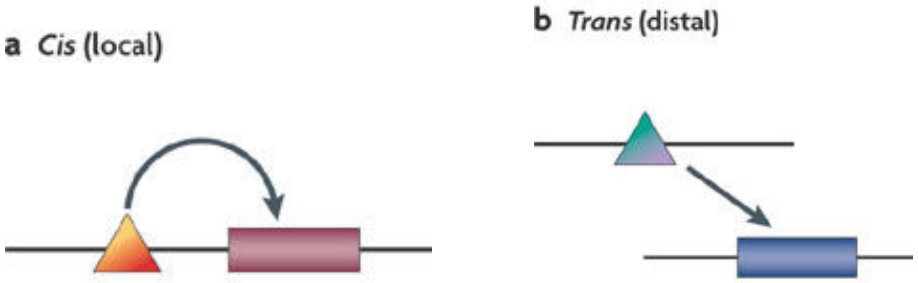


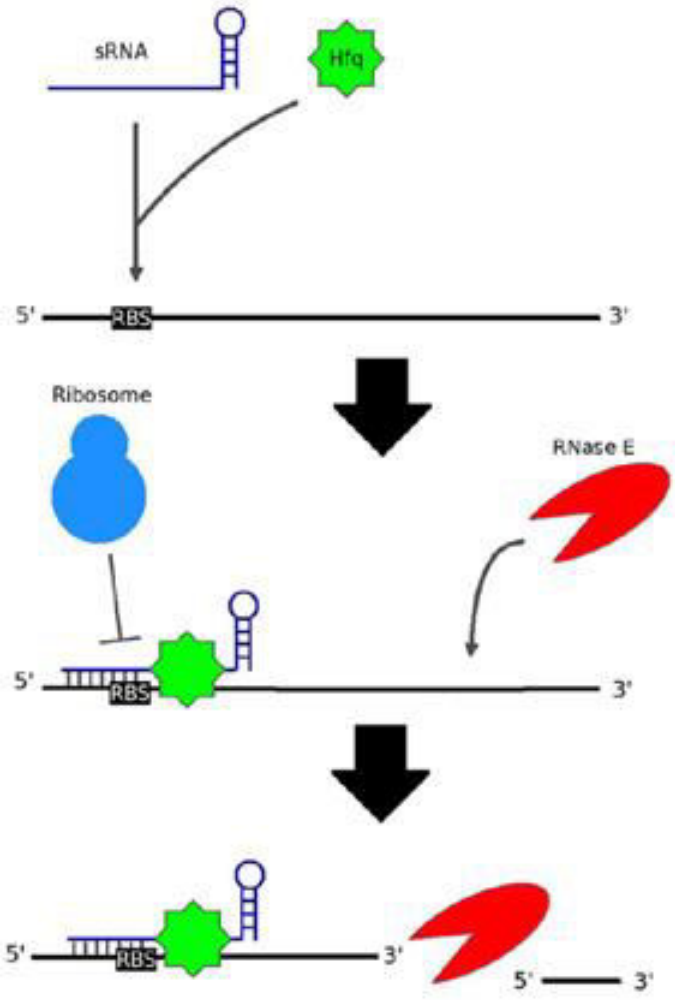
Serganov A. and Nudler V. A Decade of Riboswitches. Cell. Jan 17, 2013; 152(0): 17–24.

(II) Functions and Mechanisms

(C) Intergenic small RNAs

- Binds to target mRNA in short stretches (7-12nt)
- generally overlapping with / close to ribosomal binding site (RBS)
- **Stem-loop structures** block 70S ribosome formation and translation initiation





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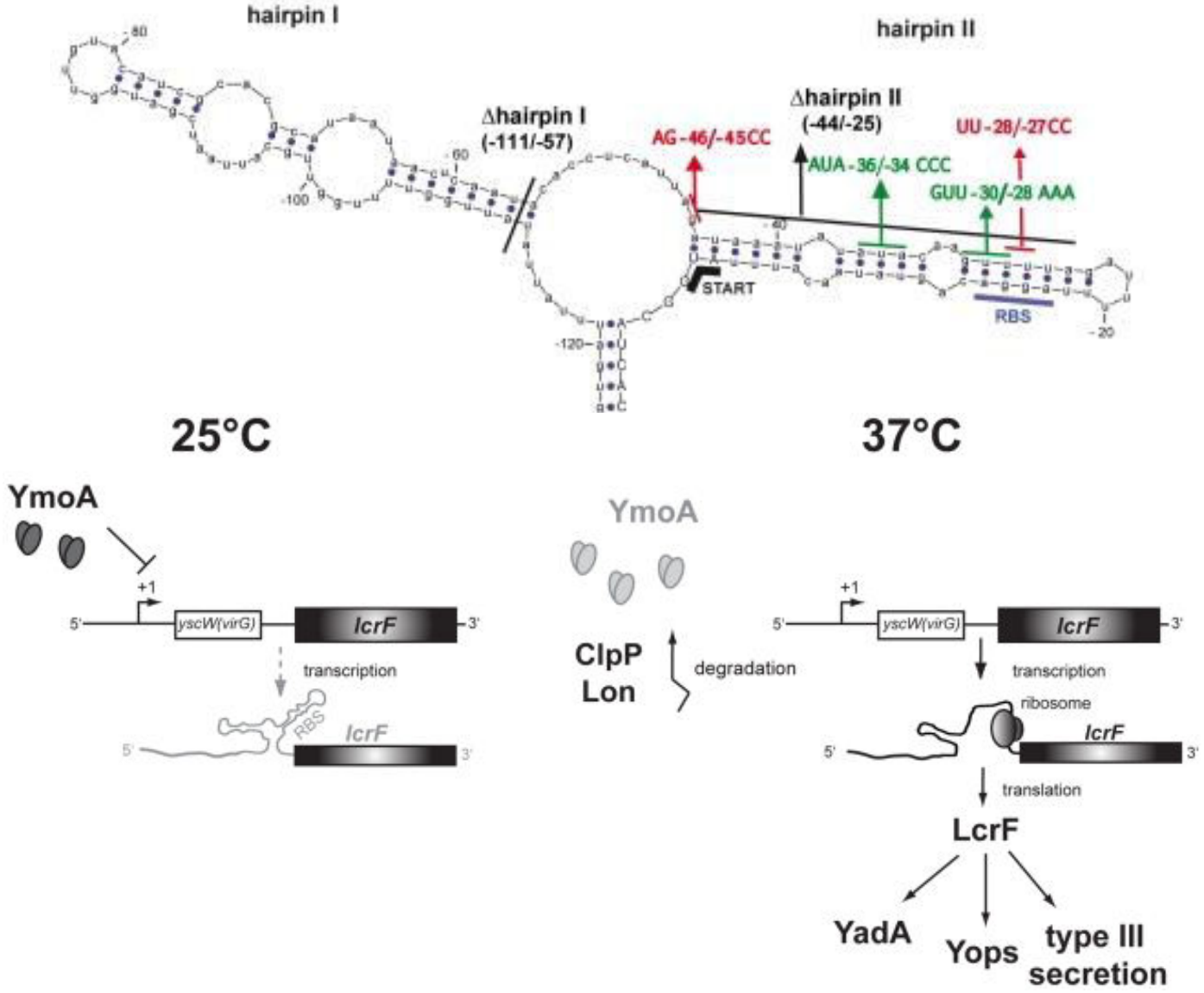
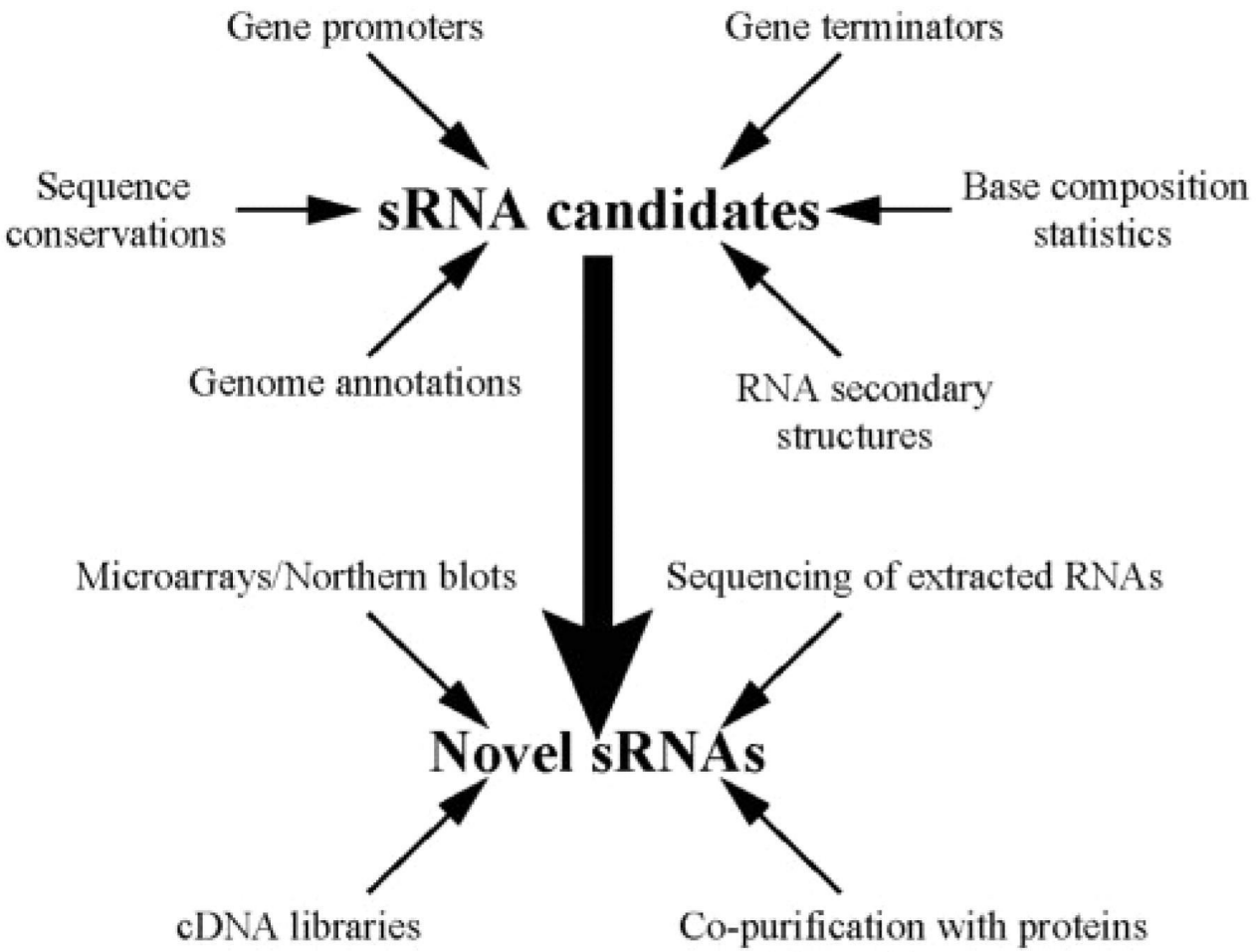
(II) Functions and Mechanisms

- Virulence regulation by thermosensor in *Yersinia*



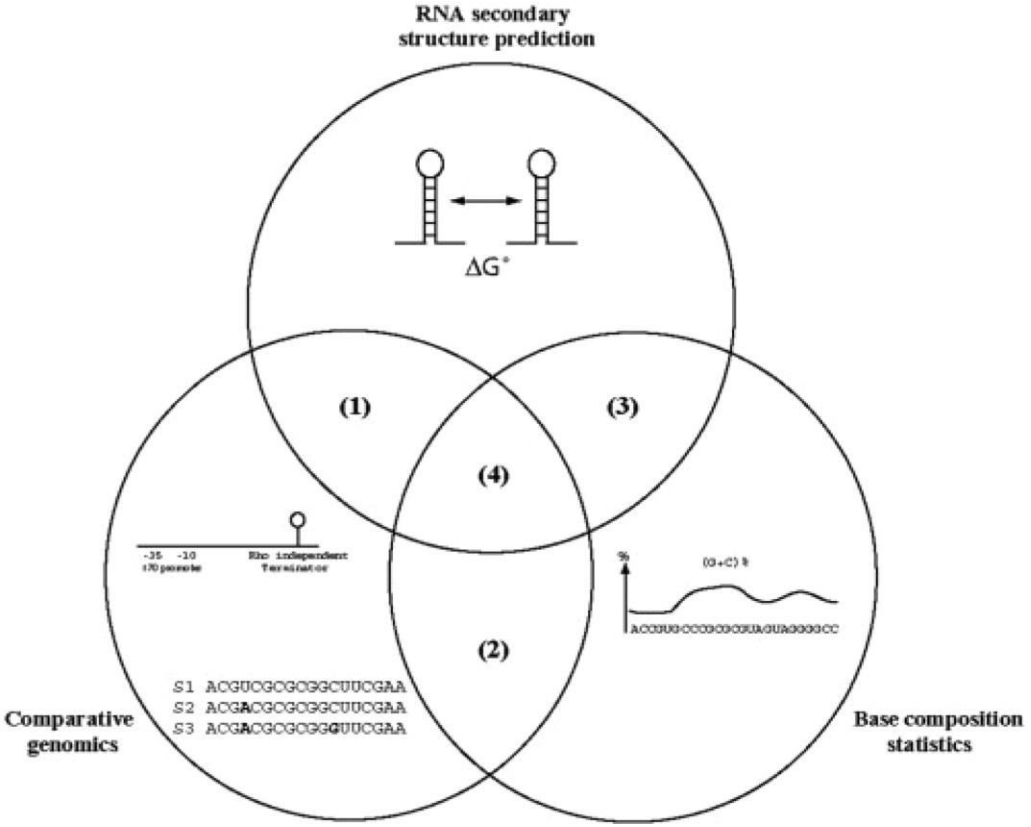
Boheme K. et. al. Concerted Actions of a Thermo-labile Regulator and a Unique Intergenic RNA Thermosensor Control Yersinia Virulence. PLoS Pathog. Feb 2012; 8(2): e1002518.

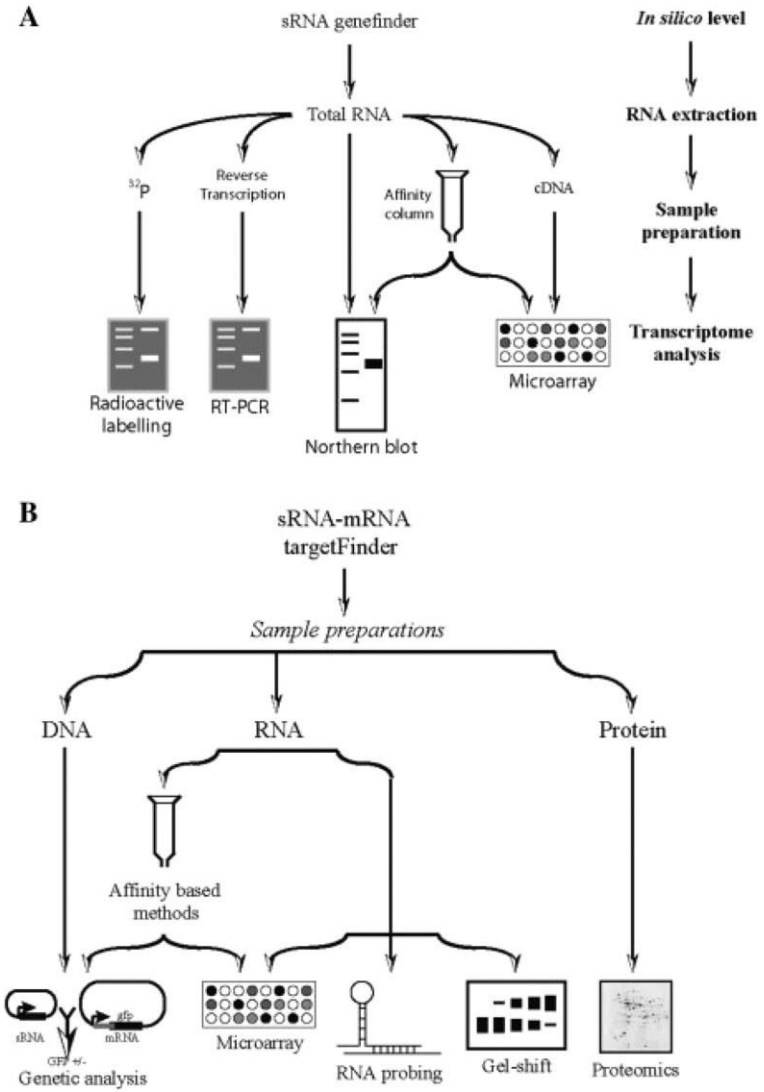
(III) Small RNA Prediction Softwares and Databases



Pichon C. and Felden B. Small RNA gene identification and mRNA target predictions in bacteria.Bioinformatics. 2008 Dec 15;24(24):2807-13.

(III) Small RNA Prediction Softwares and Databases

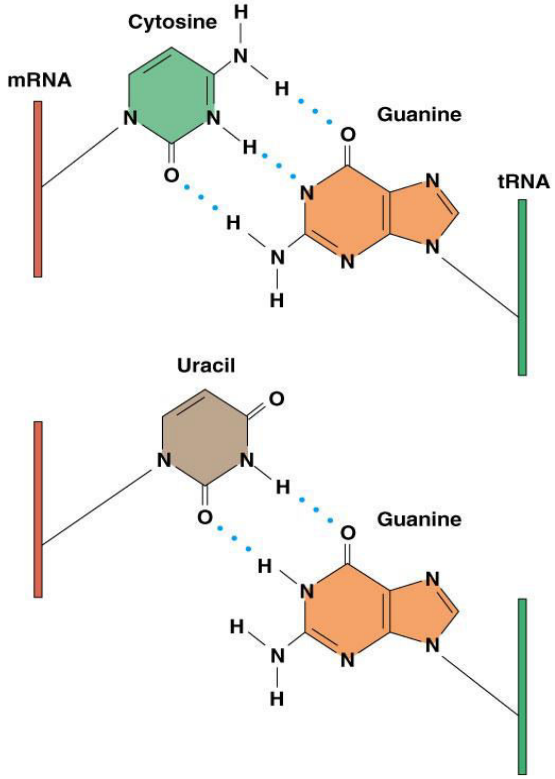


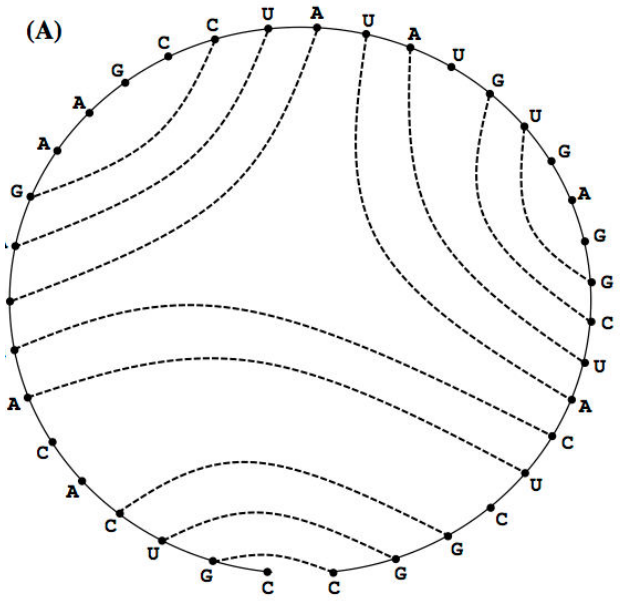


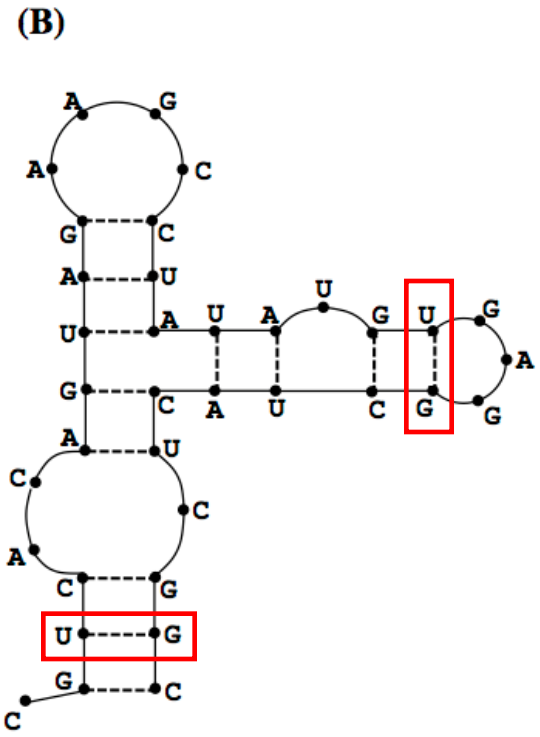
Pichon C. and Felden B. Small RNA gene identification and mRNA target predictions in bacteria.Bioinformatics. 2008 Dec 15;24(24):2807-13.

(III) Softwares and Databases

- DNA base pairing : A-T , G-C
- RNA base pairing : A-U , G-C, G-U...
- **Sample RNA string:**
  CGUCACAGUAGAAGCCUAUAUGUGAGGCUACUCGGC (36nt)







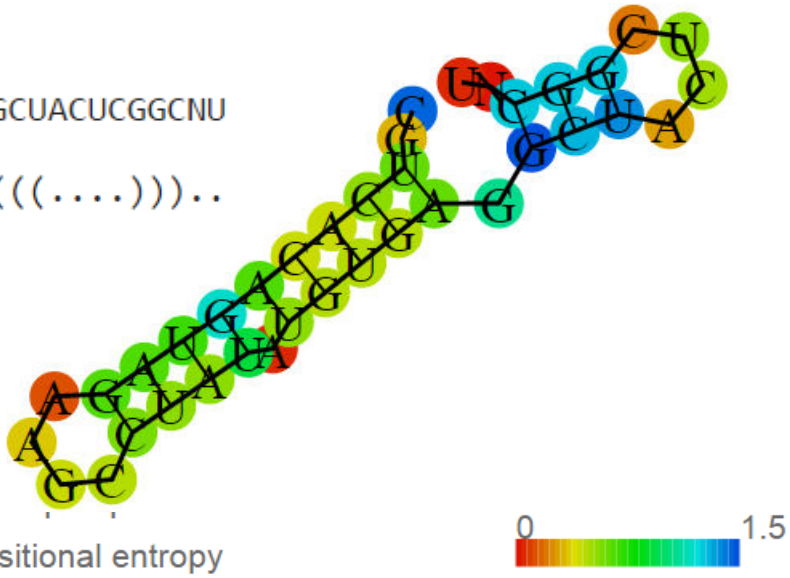
Wobble Bonding and RNA Secondary Structures http://rosalind.info/problems/rnas/

(III) Softwares and Databases

RNAfold

CGUCACAGUAGAAGCCUAUAUGUGAGGCUACUCGGCNU

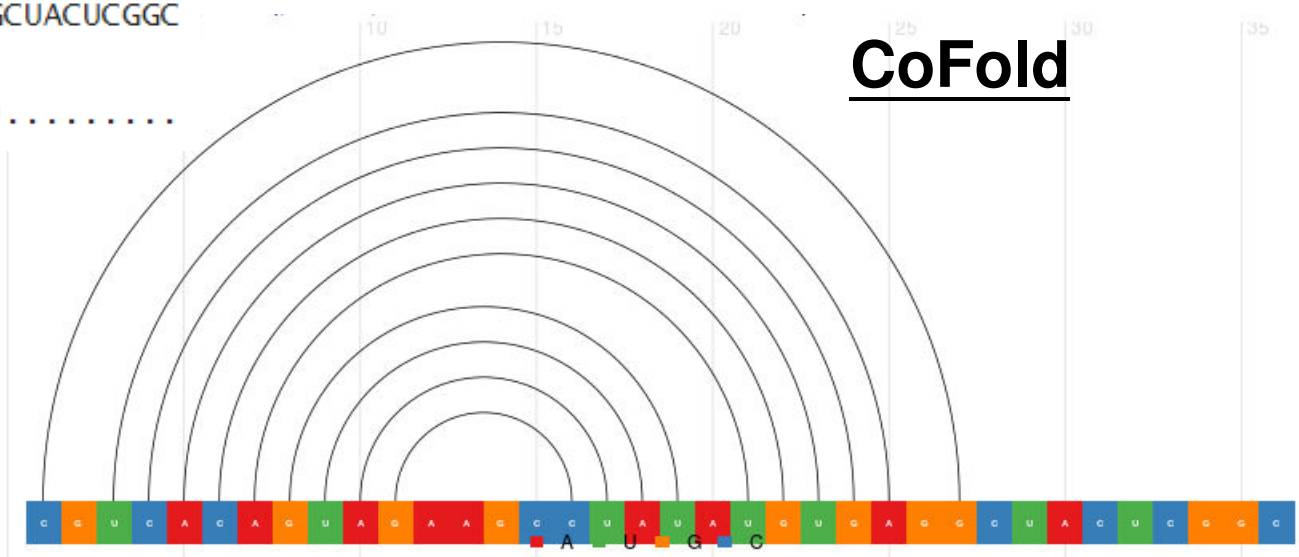
..((((((((((....))))).))))).(((....)))..



CGUCACAGUAGAAGCCUAUAUGUGAGGCUACUCGGC

(.((((((((((....))))).))))).).........

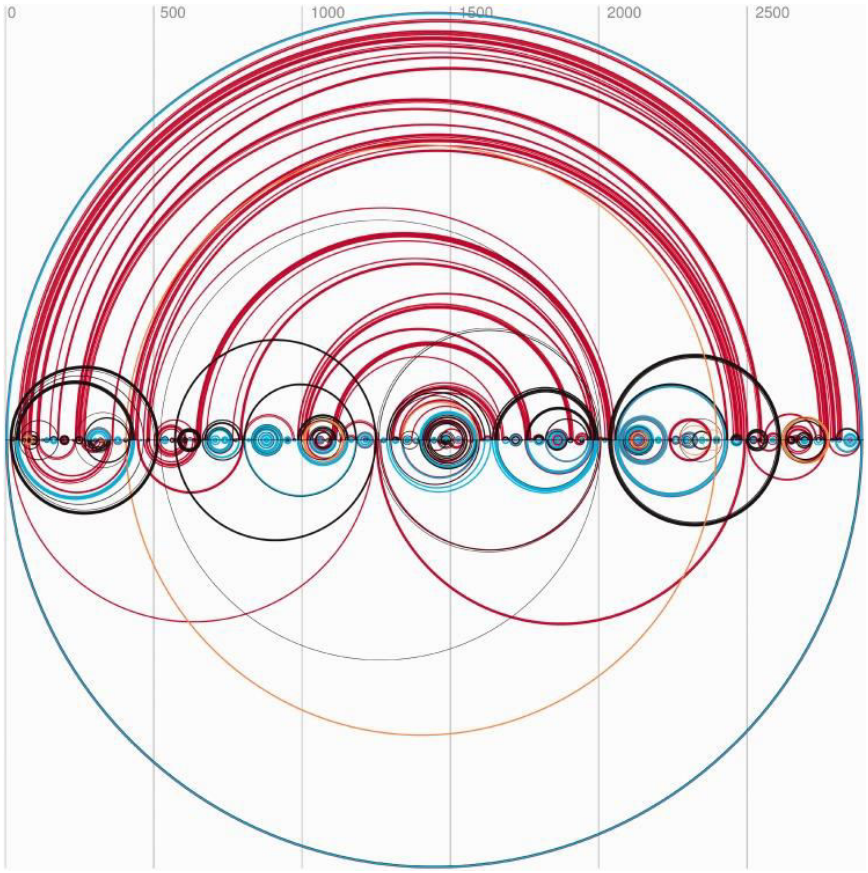
CoFold



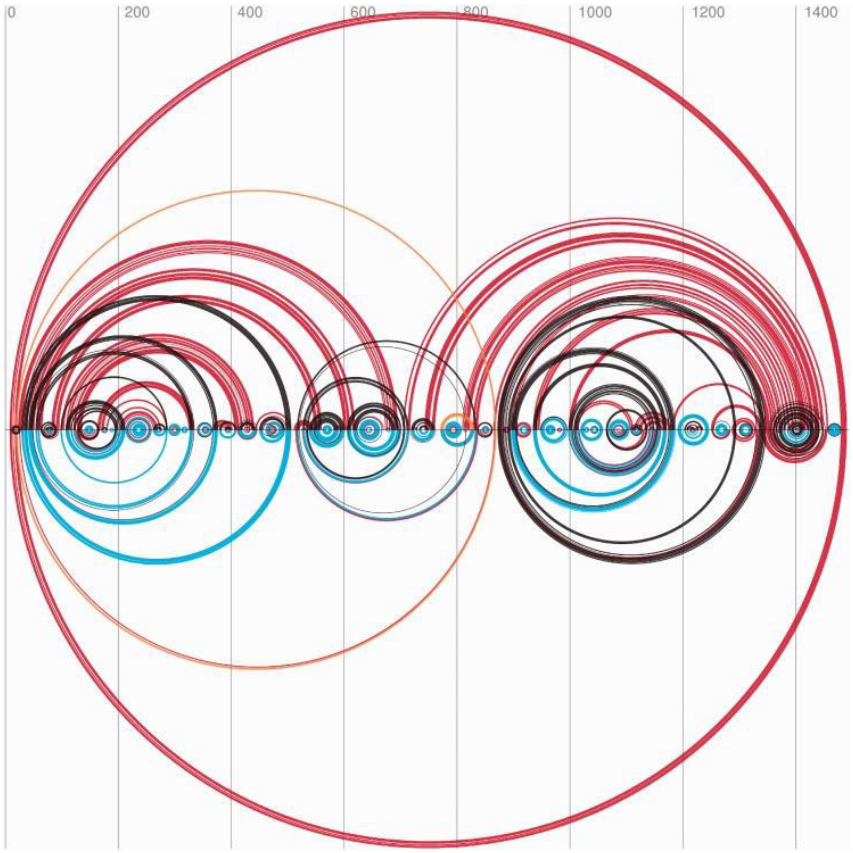
RNAfold http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
CoFold http://www.e-rna.org/cofold/

(III) Softwares and Databases

- RNAfold vs. CoFold



23S rRNA of *P. aeruginosa* (2893nt)

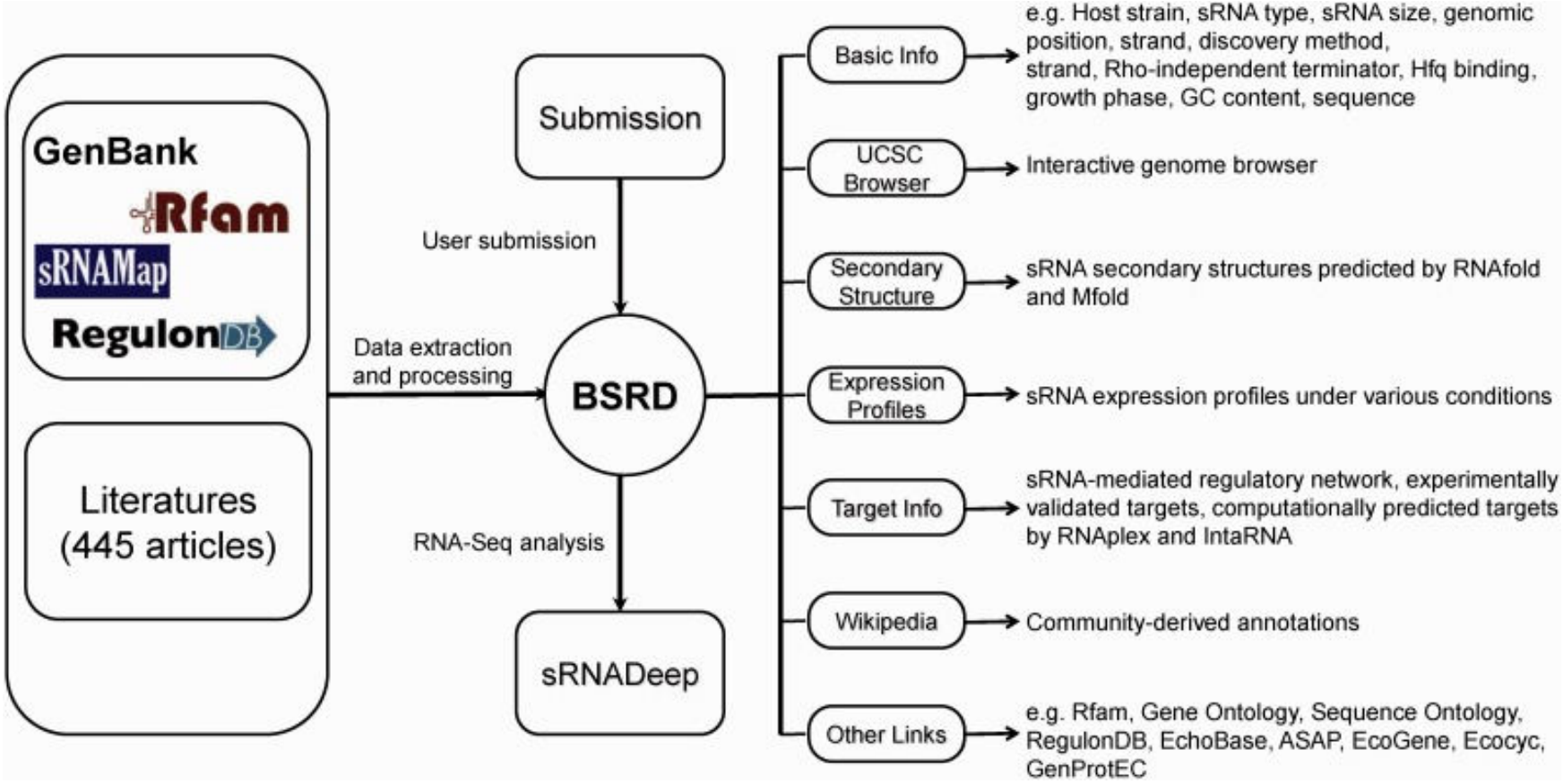


16S rRNA fresh water algae *Cryptomonas sp.* (1493bp)

Proctor J. R. and Meyer I. M. CoFold: an RNA secondary structure prediction method that takes co-transcriptional folding into account. Nucleic Acids Res. May 2013; 41(9): e102.

(III) Small RNA Prediction Softwares and Databases

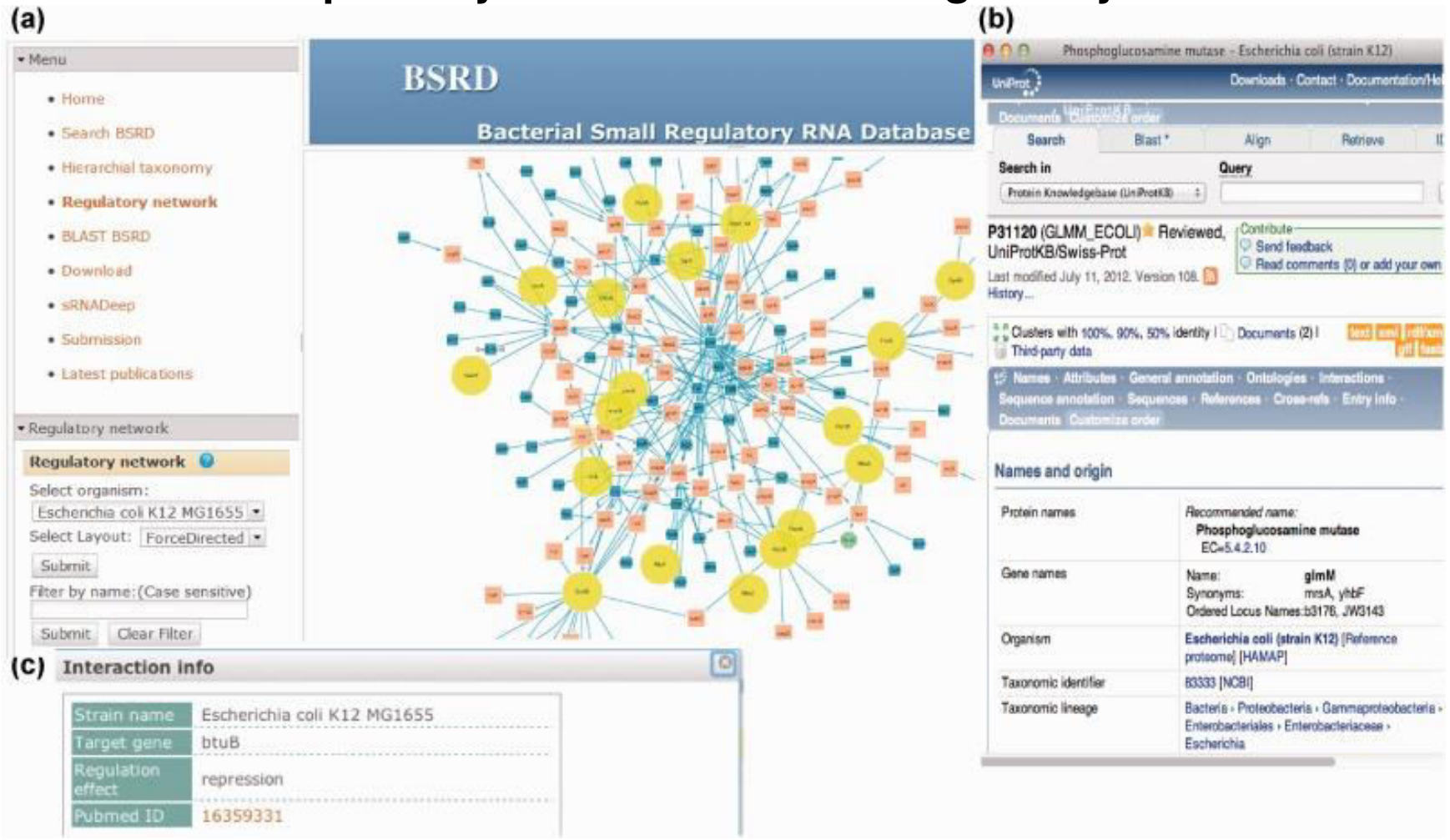
- **BSRD: a repository for bacterial small regulatory RNA**



Li L. et. al. BSRD: a repository for bacterial small regulatory RNA. Nucleic Acids Res. Jan 2013; 41(Database issue): D233–D238.

(III) Small RNA Prediction Softwares and Databases

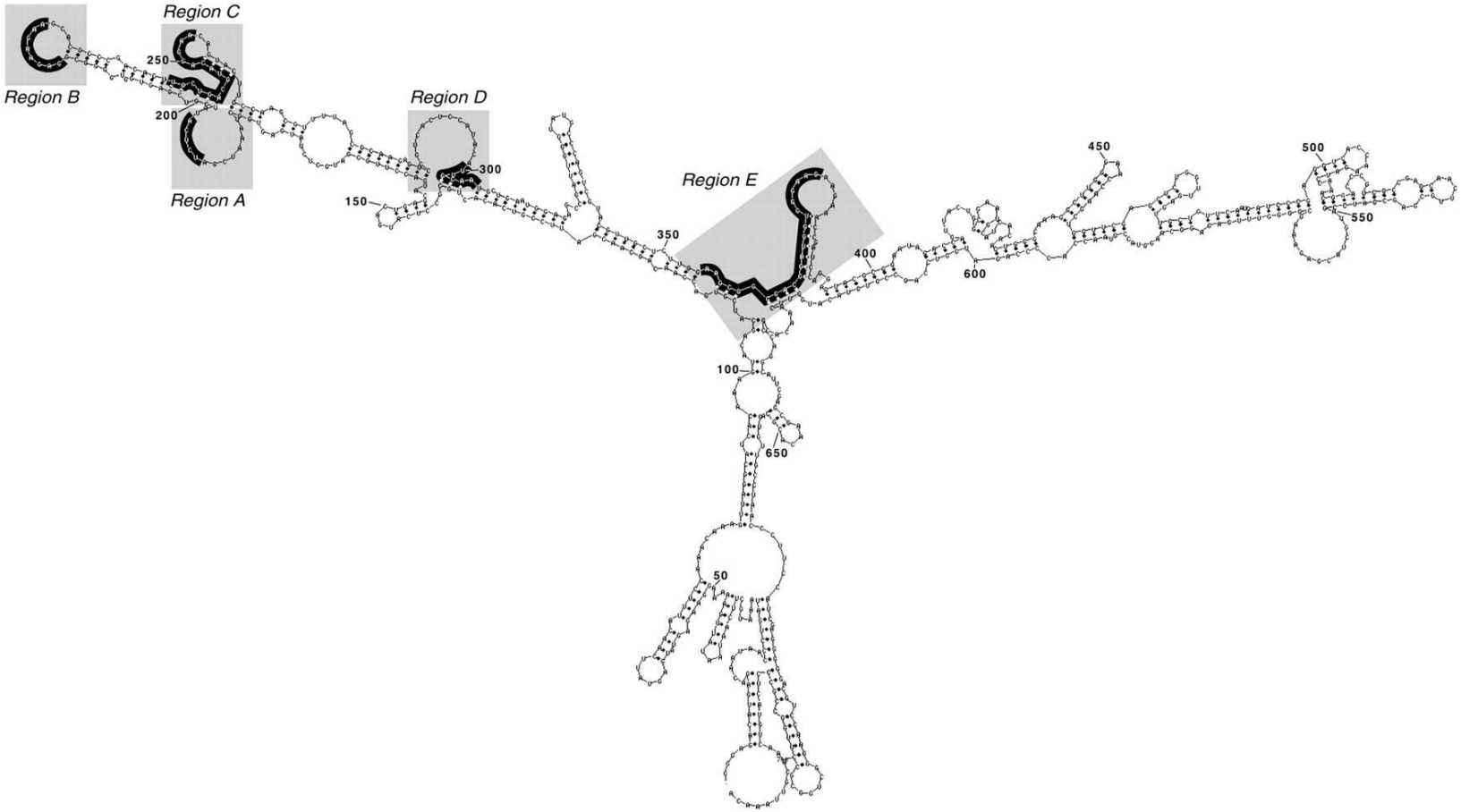
- **BSRD: a repository for bacterial small regulatory RNA**



Li L. et. al. BSRD: a repository for bacterial small regulatory RNA. Nucleic Acids Res. Jan 2013; 41(Database issue): D233–D238.

(IV) Future Perspectives

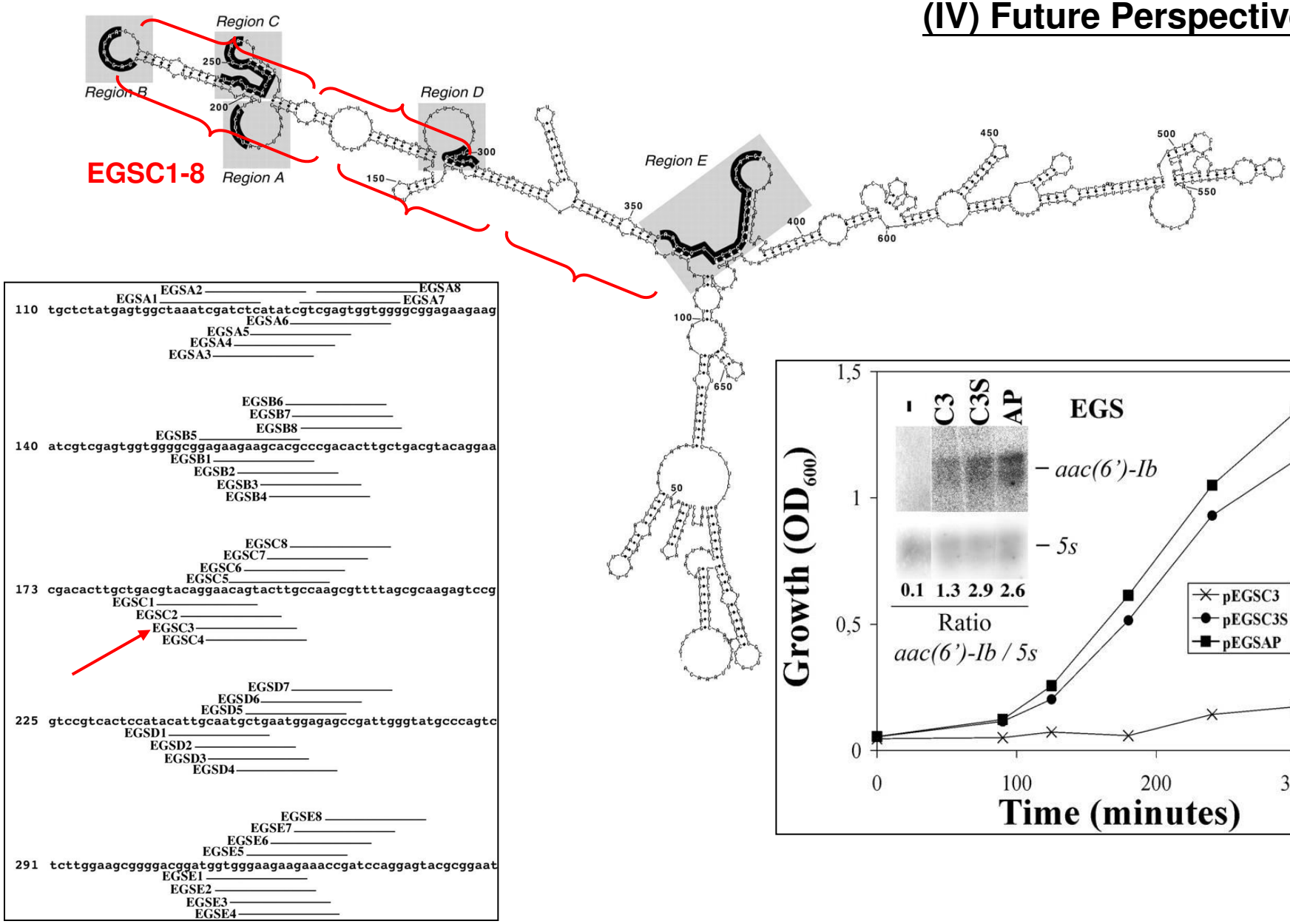
- **Hijacking nature's design - Artificial small RNAs**
- Switching off the aminoglycoside resistance gene, aac(6′)-Ib



Sarno R. et. al. Inhibition of Aminoglycoside 6′-N-Acetyltransferase Type Ib-Mediated Amikacin Resistance by Antisense OligodeoxynucleotidesAntimicrob. Agents Chemother. October 2003 vol. 47 no. 10 3296-3304

Ramirez M. S. et. al. Rise and dissemination of aminoglycoside resistance: the aac(6′)-Ib paradigm. Front. Microbiol., 17 May 2013 | doi: 10.3389/fmicb.2013.00121

(IV) Future Perspectives



Solar Bistue A. J. C. et. al. External Guide Sequences Targeting the aac(6')-Ib mRNA Induce Inhibition of Amikacin Resistance. Antimicrob. Agents Chemother. June 2007 vol. 51 no. 6 1918-1925

Ramirez M. S. et. al. Rise and dissemination of aminoglycoside resistance: the aac(6')-Ib paradigm. Front. Microbiol., 17 May 2013 | doi: 10.3389/fmicb.2013.00121