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Small molecules targeting RNA for novel antibiotic discovery

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Overview

- The need of discovering new drugs
- Bacterial non-coding RNA as the new drug target
- The potential druggability of bacterial riboswitch

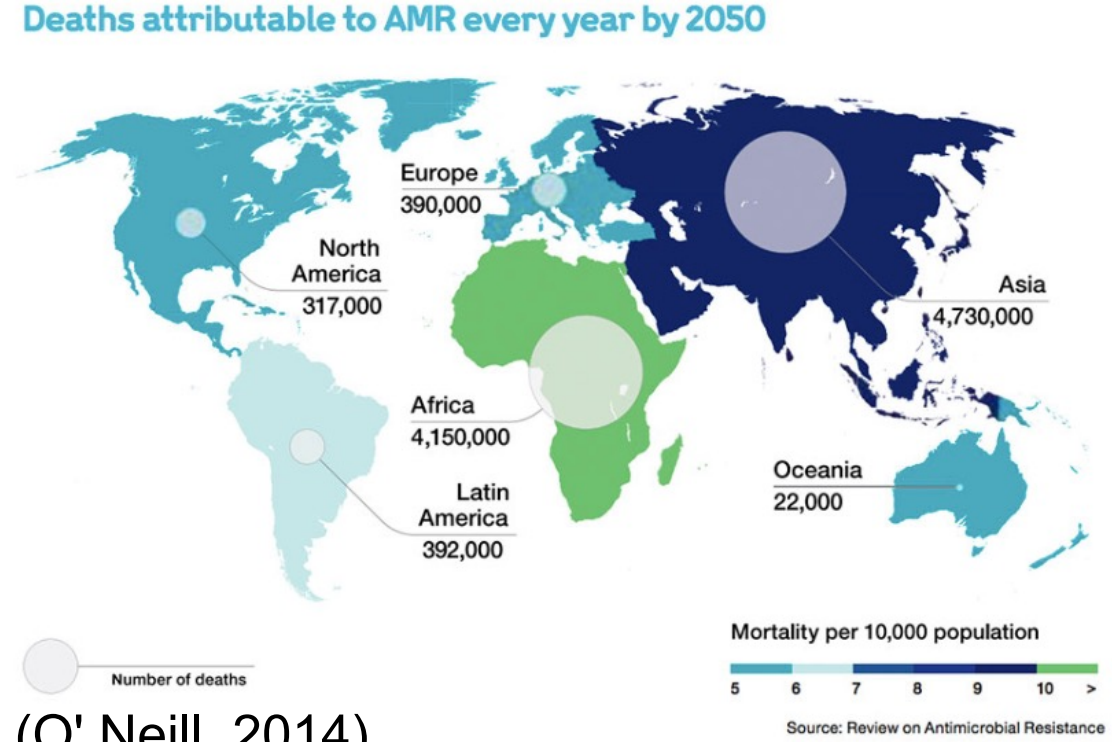
New drugs for global public health issues

New Disease e.g. COVID-19

Cumulative deaths: 6 602 552

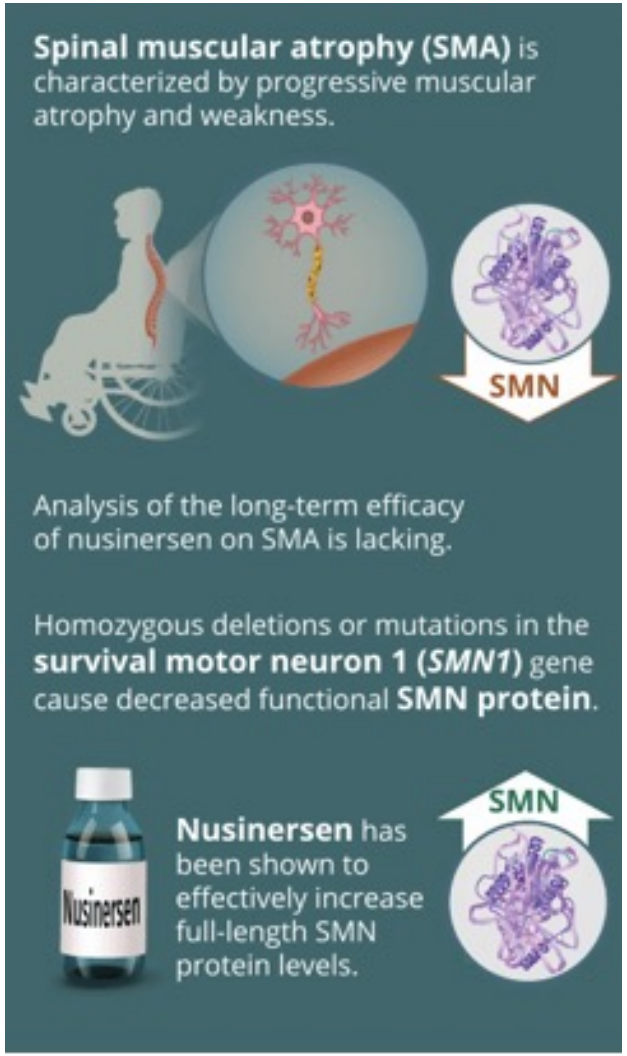
Data retrieved 23 Nov 2022
(WHO, 2020)

Antimicrobial resistance



(O' Neill, 2014)

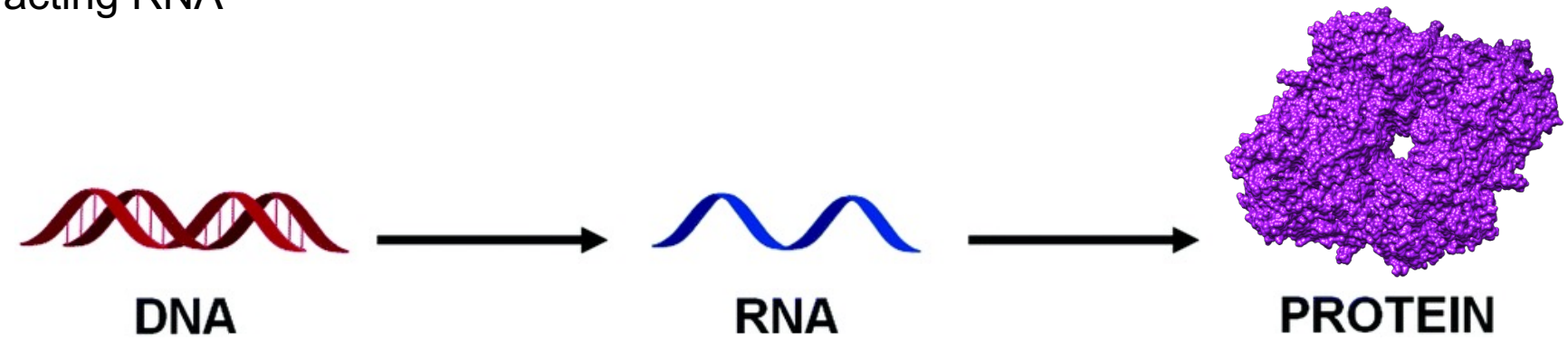
For currently untreatable disorder



(American Academy of Neurology, 2019)

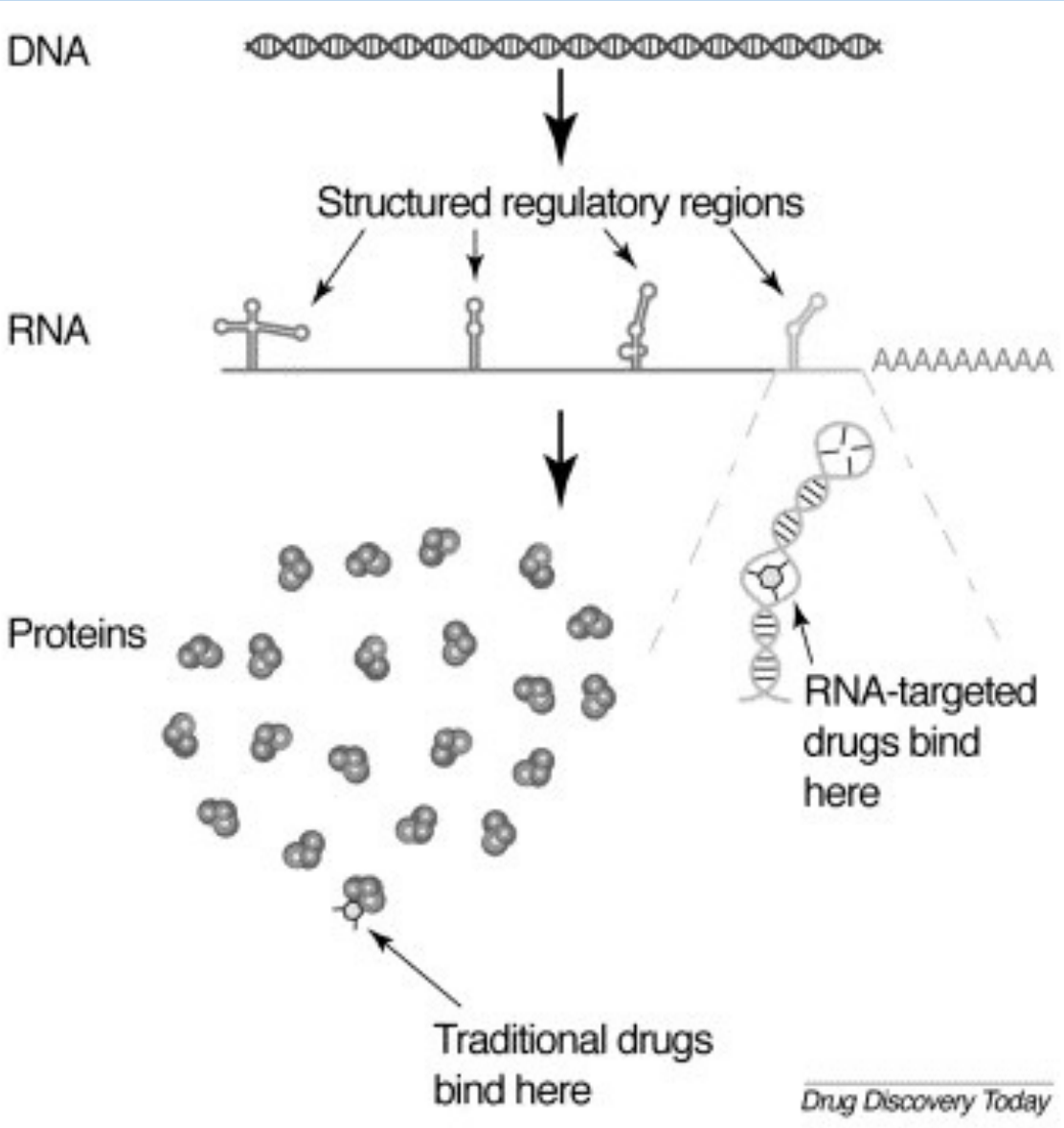
New potential drug targets from the discovery of non-coding RNA

lnc RNA	Long non-coding RNA
snoRNA	Small nucleolar RNA
miRNA	microRNA
snRNA	Small nuclear RNA
siRNA	Small interfering RNA
piRNA	Piwi-interacting RNA



Meyer, 2020

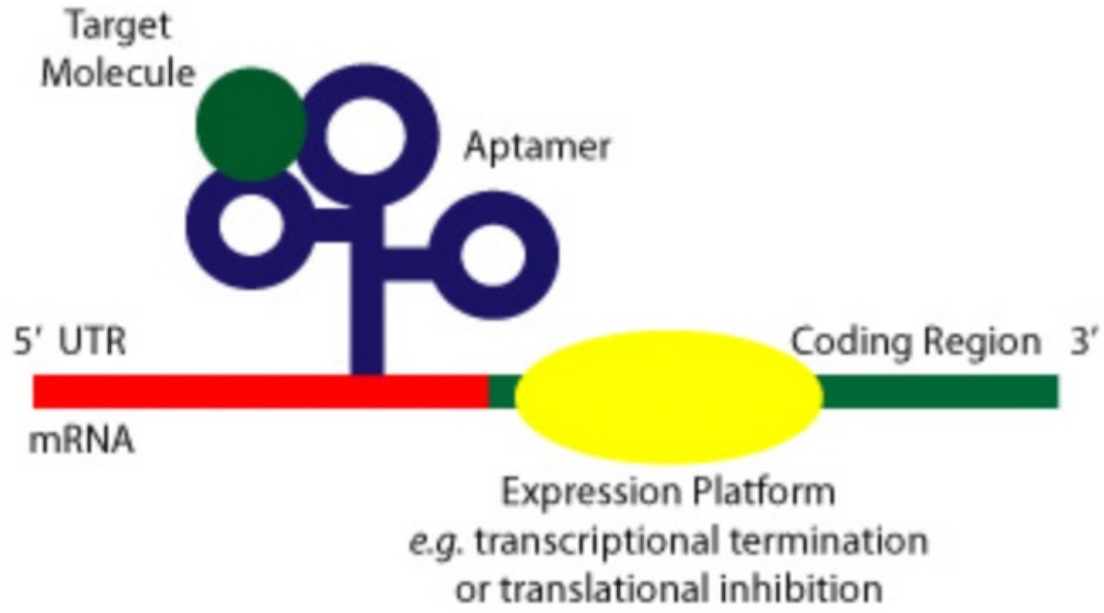
Bacterial non-coding RNA for drug targeting



Ecker and Griffey., 1999

Riboswitch

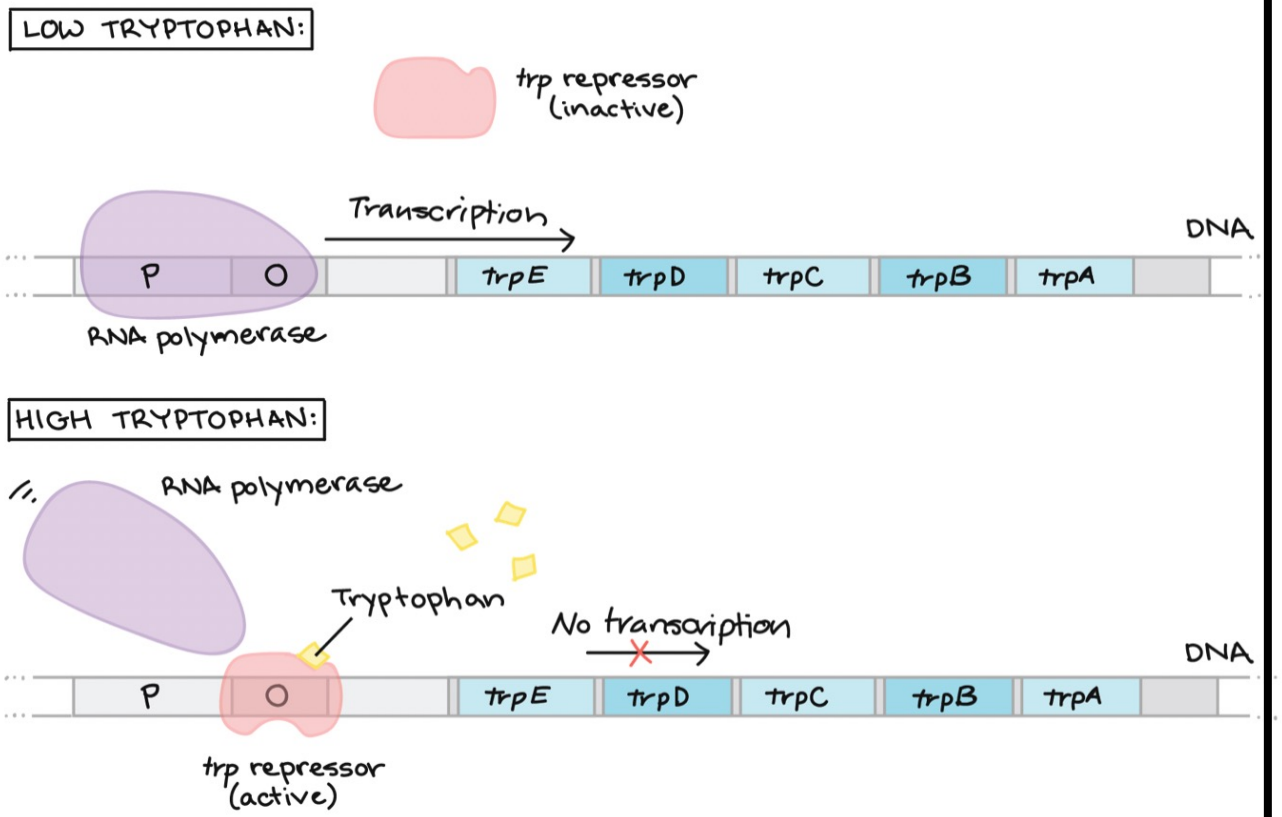
The element at 5’ untranslated region (UTR) of mRNA



Bacterial non-coding RNA regulating gene expression

Protein-mediated feedback control

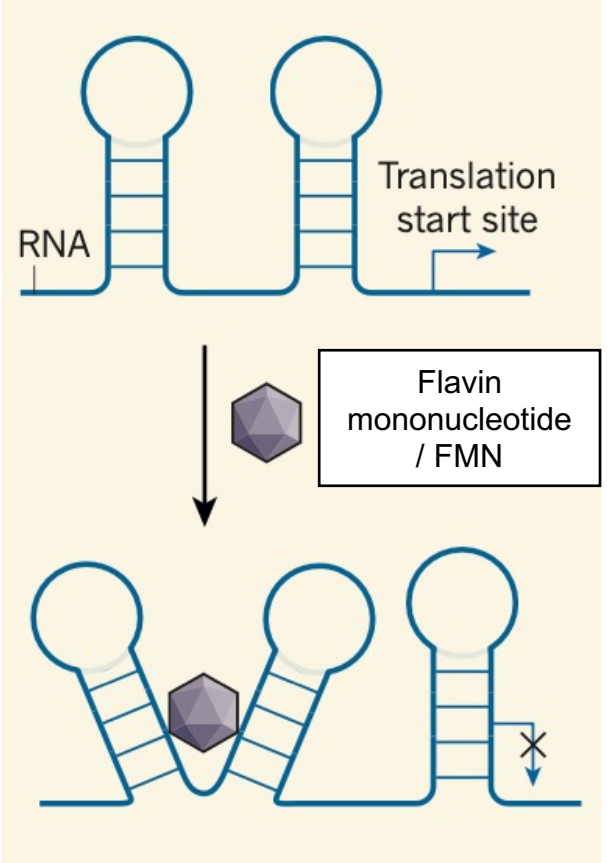
Example: Tryptophan (typ) biosynthesis



The excess tryptophan activates the transcription repressor (Babitzke and Gollnick., 2001).

RNA-mediated feedback control

Example: Riboflavin / vitamin B2 biosynthesis

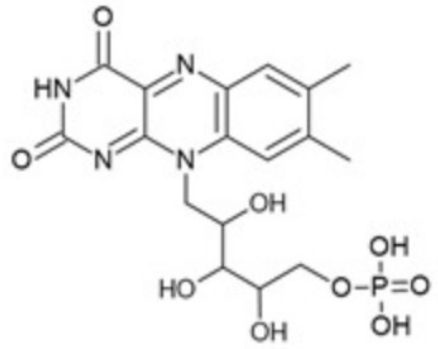
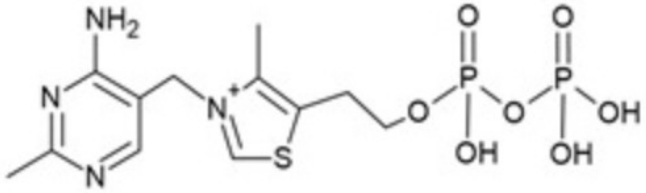


FMN binds to the RNA aptamer for transcription inhibition (Hermann., 2015).

Riboswitches discovered in human pathogens

1 to 4% of all bacterial genes are regulated by riboswitches.

More than 35 different classes of riboswitches for a diverse set of metabolites have been identified.

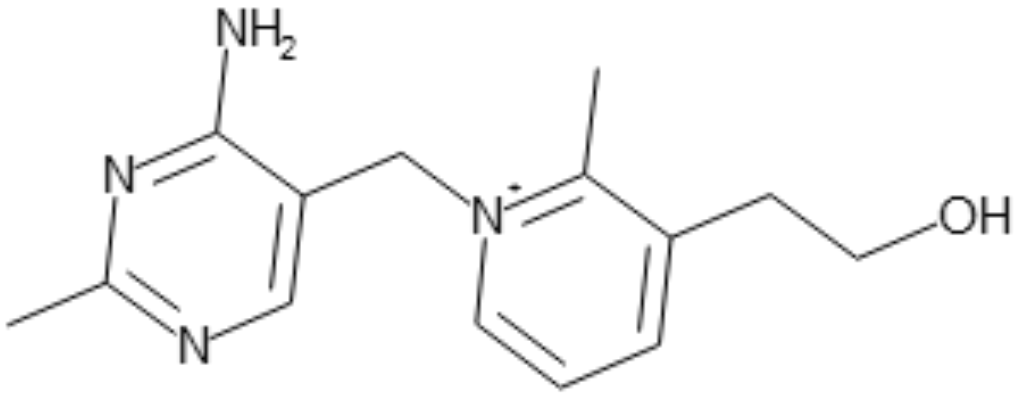
Riboswitch	Type	Cognate Ligand
FMN	Off	 flavin mononucleotide (FMN)
TPP	Off	 thiamine pyrophosphate (TPP)

FMN riboswitch identified in 41 pathogens
Acinetobacter baumannii, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*

TPP riboswitch identified in 48 pathogens: e.g.
A. baumannii, *P. aeruginosa*, *Mycobacterium tuberculosis*

Mironov and Gusarov, 2002 Winkler *et al*., 2002

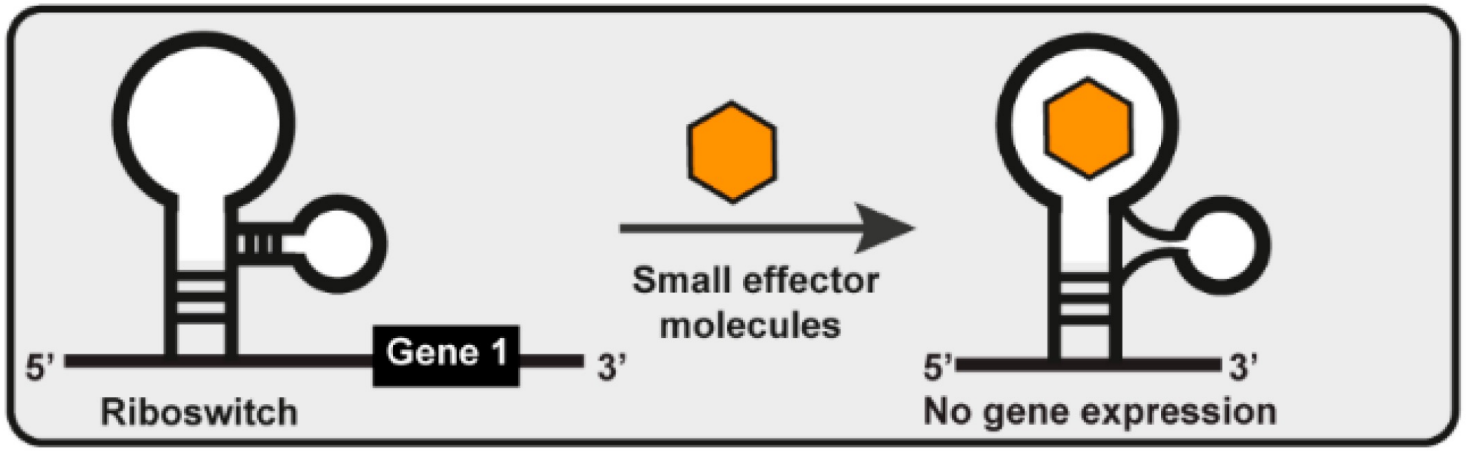
Disrupting bacterial riboswitch function for antibacterial activity



Pyrithiamine

Pyrithiamine is an antibiotic discovered in 1943 (Woodley and White., 1943).

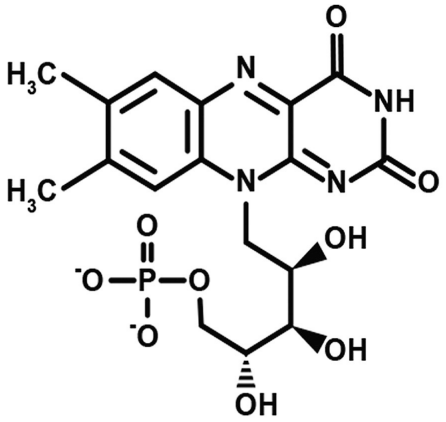
Recently proven to target bacterial TPP riboswitch (Sudarsan *et al*., 2005).



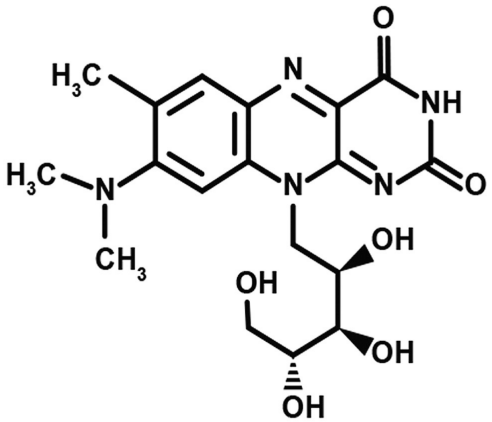
Proposed mode of action of inhibitors

Panchal and Brenk, 2021

Targeting FMN riboswitch by roseoflavin



FMN



Roseoflavin

Roseoflavin is a nature pigment produced in *Streptomyces davawensis*

Minimum inhibitory concentration (MIC)

MRSA RN4220: 0.06 µg/mL

MRSA COL: 0.06 µg/mL

Futher characterisation:

- Roseoflavin showed reduced bacterial burden in the murine infection model.
- Lethargy and riffled fur in mice were observed.

Lee *et al*., 2009

Wang *et al*., 2017

Concerns on the use of roseoflavin

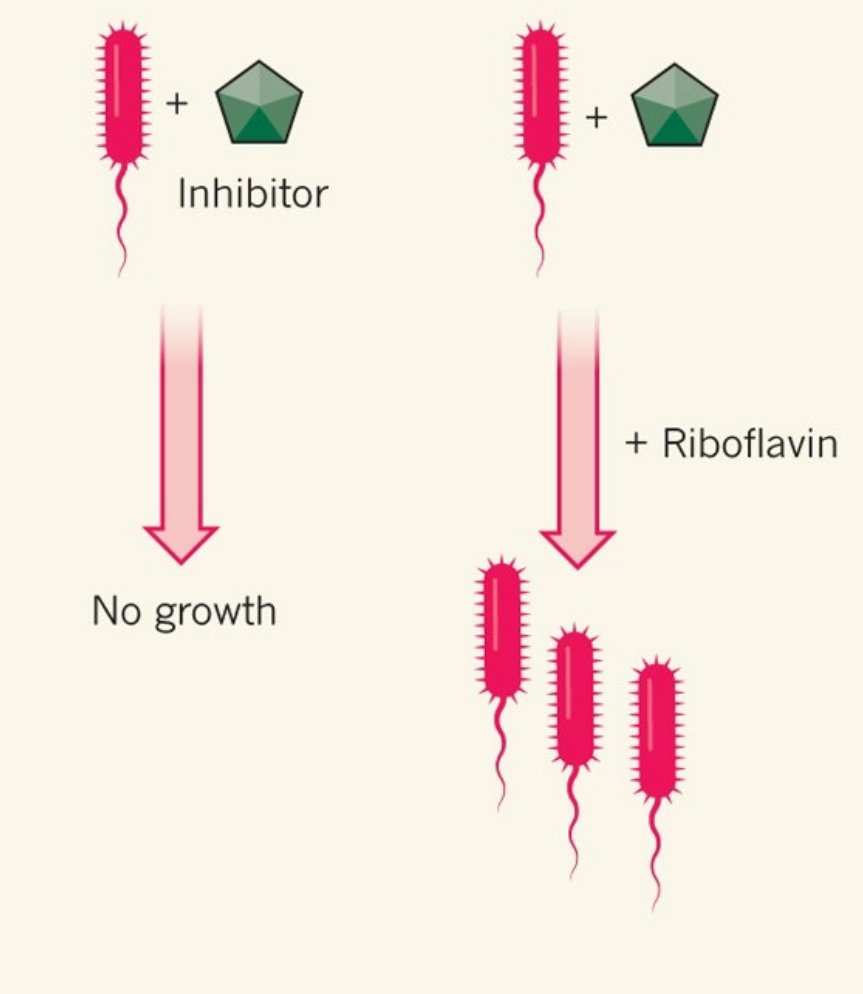
1. Off-target effects in the murine infection model.

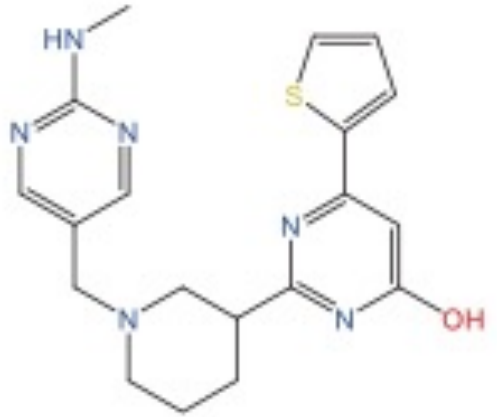
2. Structural and functional conservation between bacterial and human flavoenzymes.

Possible solution:
Search for a ligand that is not structurally similar to FMN.

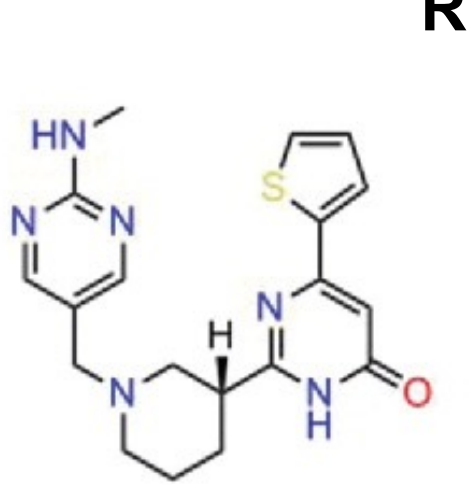
Howe *et al*., 2015

Inhibitor screening for different compounds

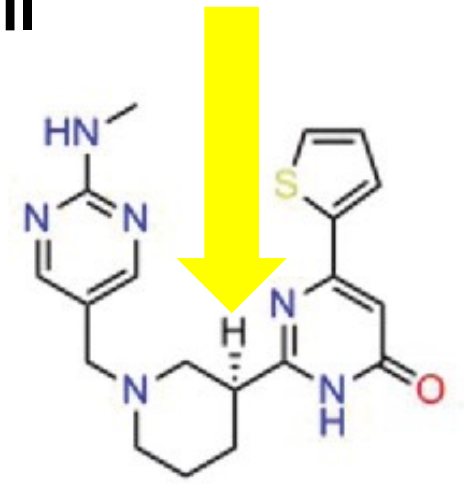




Ribocil



Ribocil-A

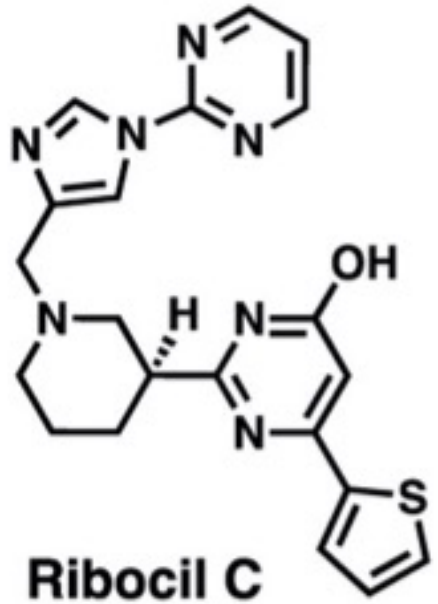


Ribocil-B

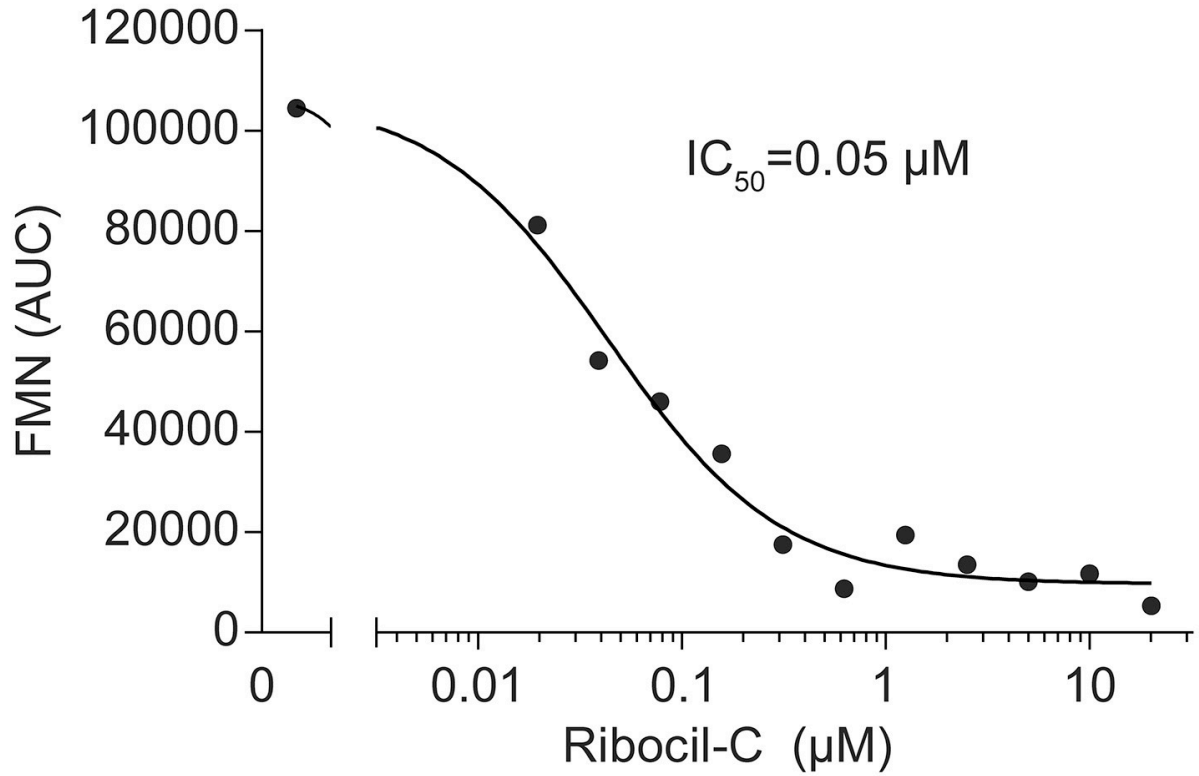
Screened by culturing *E. coli* and compounds with / without riboflavin supplementation.

Howe *et al*., 2015

Ribocil C showing antimicrobial effect on *E. coli*

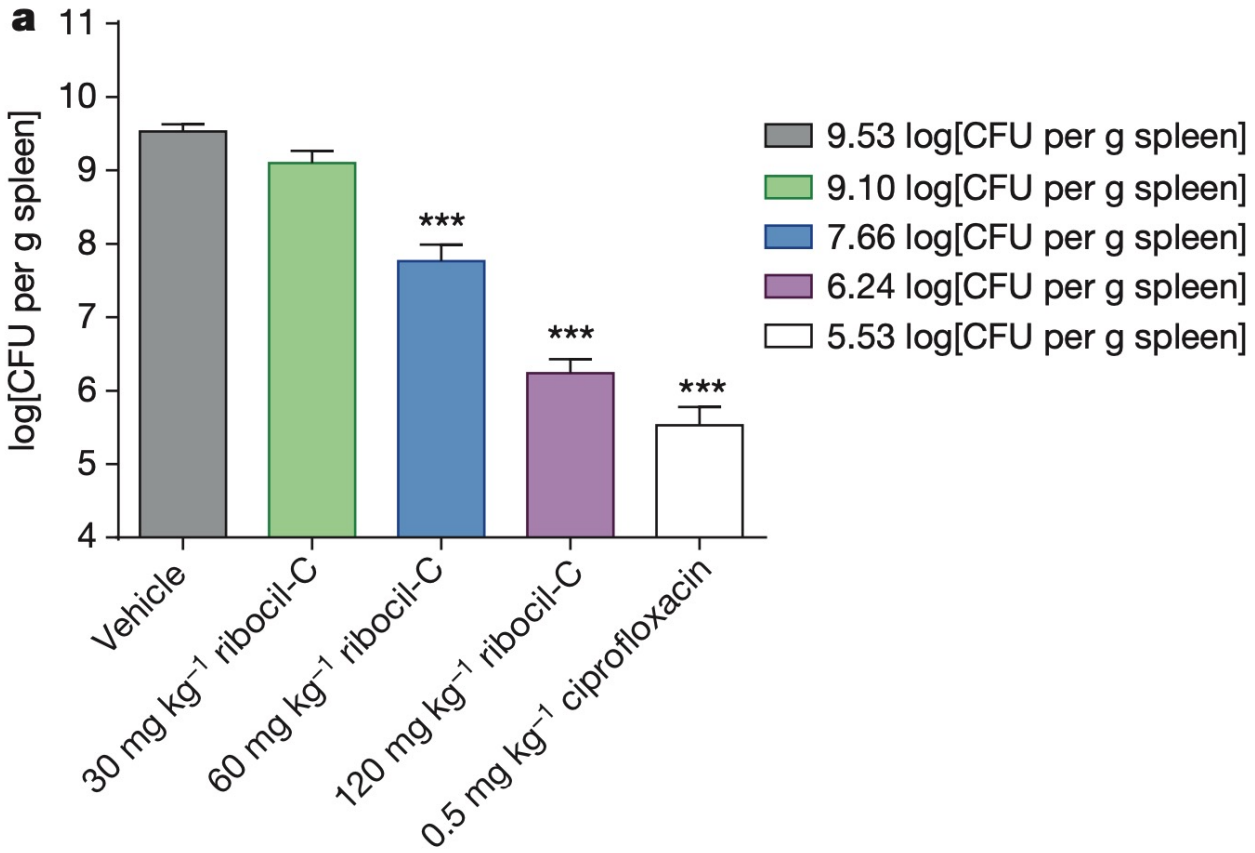


MIC against *E. coli.*
MB5746: 2 µg/mL
ΔtolC strain: 0.25 µg/mL



Howe *et al*., 2015

Ribocil C with antibacterial effect *in vivo*



Mice infected with *E. coli* MB5746

Infection: Intraperitoneal injection with *E. coli* MB5746.

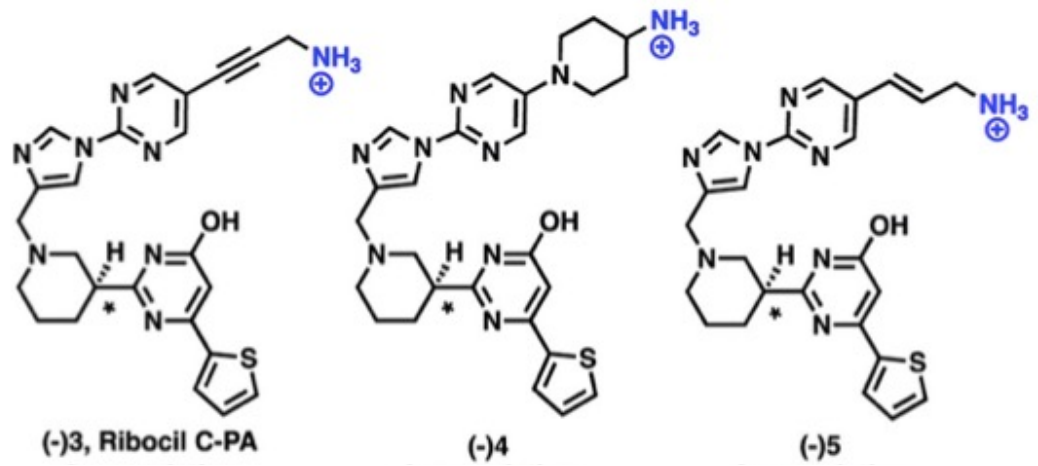
Treatment: Subcutaneous injection with ribocil-C or ciprofloxacin at 30 / 60 / 120 mg/kg.

Finding: Reduction in bacterial burden when ribocil C was administrated.

Limitation of ribocil C: No activity against wild type Gram-negative pathogens.

Howe *et al*., 2015

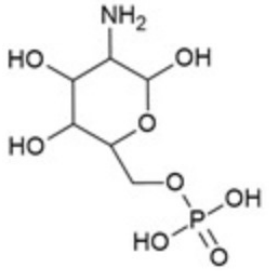
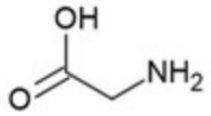
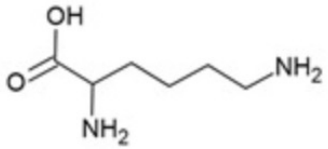
Ribocil C derivatives with improved antimicrobial activities against Gram-negative pathogens



	Compound MIC (μg/mL)	(-)3	(-)4	(-)5
MC *E. coli*	*E. coli* ΔrfaC JW3596	0.5	4	1
	E. coli ΔtolC JW5503	0.25	0.5	0.25
GN Pathogens	*E. coli* BW25113	4	8	8
	E. cloacae ATCC BAA-2341	4	8	4
	K. pneumoniae ATCC 27736	4	8	4
	A. baumannii ATCC 2093	64	>64	64
	P. aeruginosa PAO1	>64	>64	>64

Motika *et al.*, 2020

Other potentially druggable riboswitches

Riboswitch	Type	Cognate Ligand	
Fluoride	On	F^-	*A. baumannii, P. aeruginosa*, *E. faecium*
glmS	Off	 glucosamine-6-phosphate (GlcN6P)	*S. aureus*, *E. faecium*
Glycine	On	 glycine	*S. pneumoniae*, *Neisseria gonorrhoeae*, *S. aureus*, *A. baumannii, M. tuberculosis*
Lysine	Off	 lysine	*E. faecium*, *S. aureus*

Serganov and Nudler, 2014

Conclusion

- RNA can function more than an intermediate of DNA and protein.
- Bacterial non-coding RNA like riboswitch maybe the potential target for developing new antibiotics.
- Limitation: There are not enough knowledge about riboswitch.

Thanks for listening

Q&A

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