The RNA World
New Discoveries Reflecting Ancient Biology

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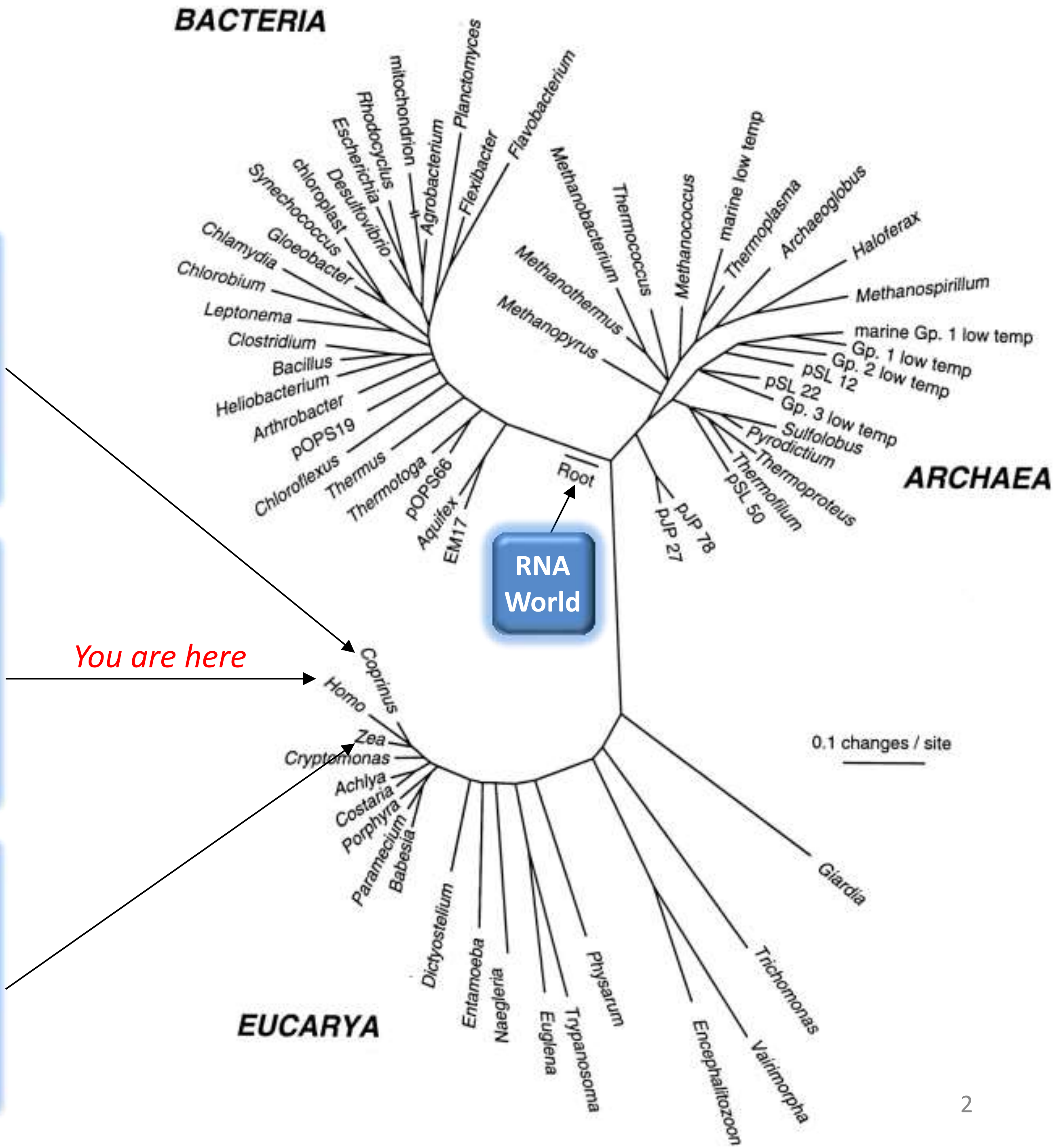
5 March 2014; CAPS

Tree of Life


You are here
BACTERIA
ARCHAEA
EUCARYA
RNA World
Root
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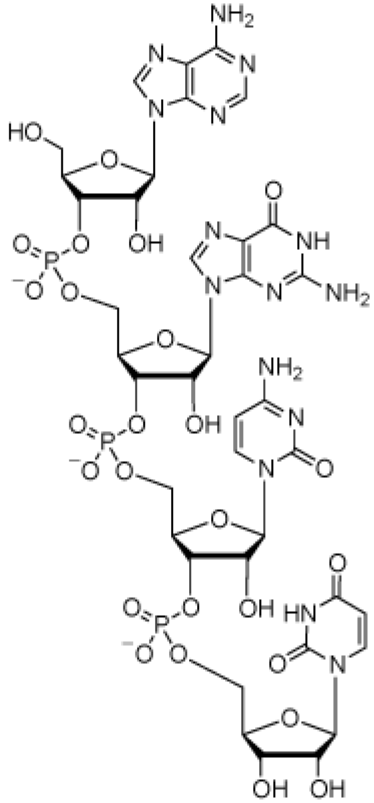
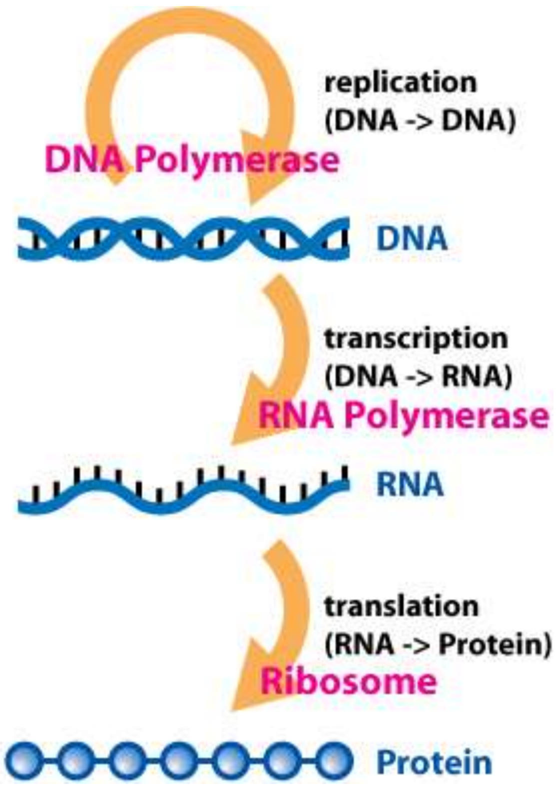
mitochondrion
Planctomyces
Flavobacterium
Rhodocyclus
Agrobacterium
Flexibacter
Escherichia
Desulfovibrio
chloroplast
Synechococcus
Gloeobacter
Chlamydia
Chlorobium
Leptonema
Clostridium
Bacillus
Heliobacterium
Arthrobacter
pOPS19
Chloroflexus
Thermus
Thermotoga
pOPS66
Aquifex
EM17
Methanobacterium
Thermococcus
Methanococcus
marine low temp
Thermoplasma
Archaeoglobus
Haloferax
Methanospirillum
Methanothermus
Methanopyrus
marine Gp. 1 low temp
Gp. 1 low temp
Gp. 2 low temp
pSL 12
pSL 22
Gp. 3 low temp
Sulfolobus
Pyrodictium
Thermoproteus
Thermofilum
pSL 50
pJP 78
pJP 27
Coprinus
Homo
Zea
Cryptomonas
Achlya
Costaria
Porphyra
Paramecium
Babesia
Dictyostelium
Entamoeba
Naegleria
Euglena
Trypanosoma
Physarum
Encephalitozoon
Vairimorpha
Trichomonas
Giardia

Why RNA?

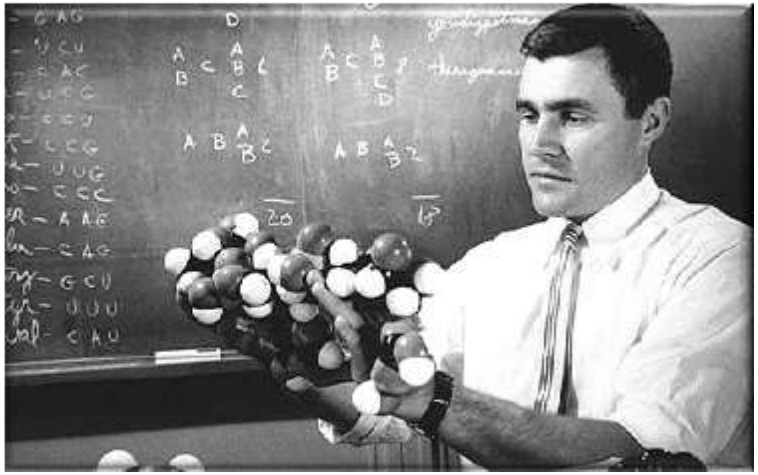
RNA



Biological Information Replication and Transfer

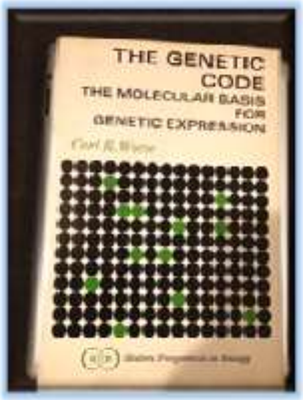


Origins of the RNA World Theory



Carl Woese

(1967) The Genetic Code, Harper and Row

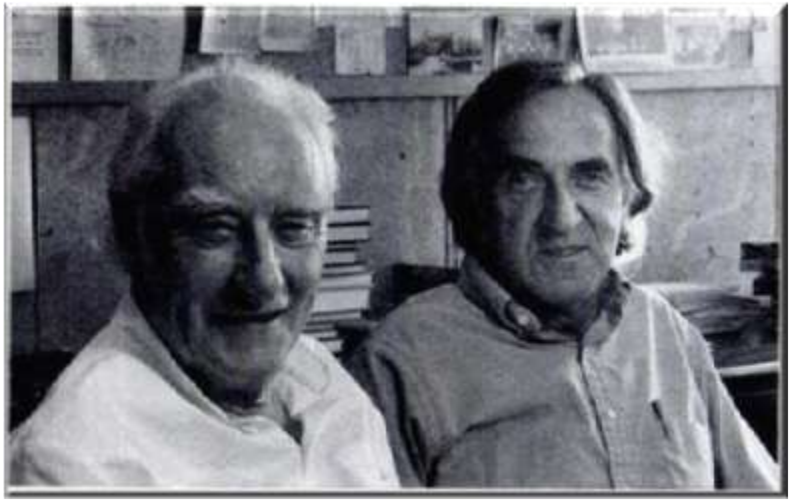


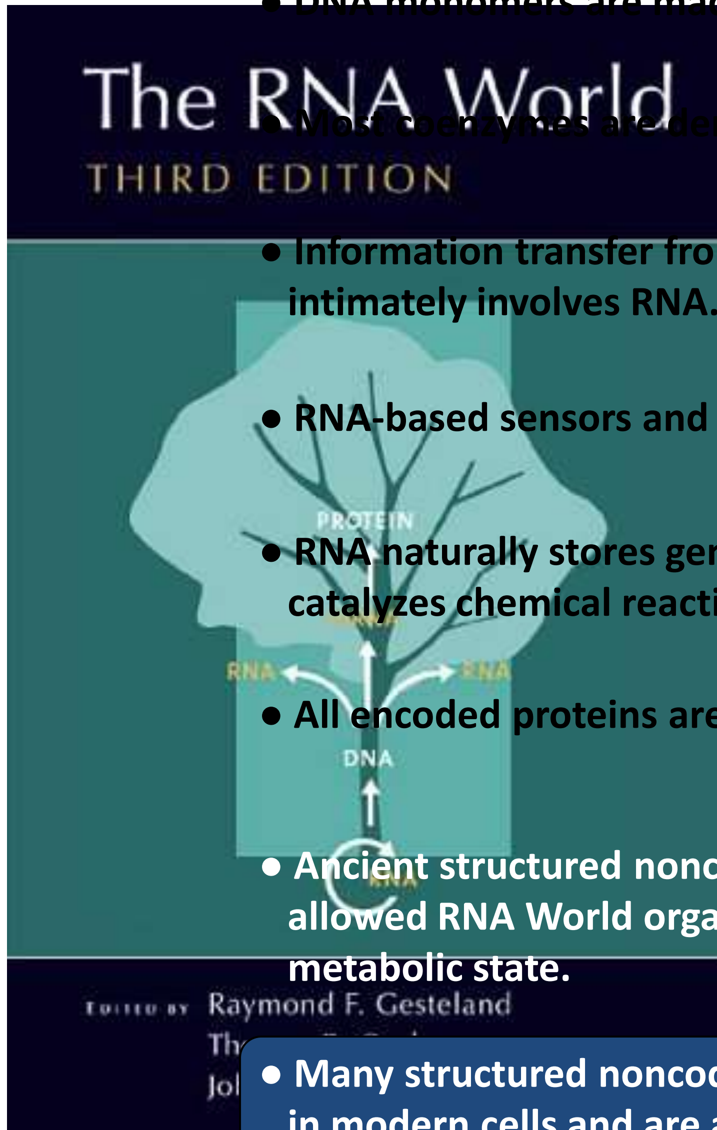
Leslie Orgel

(1968) Evolution of the Genetic Apparatus
J. Mol. Biol. 38:381

Francis Crick

(1968) The Origin of the Genetic Code
J. Mol. Biol. 38:367



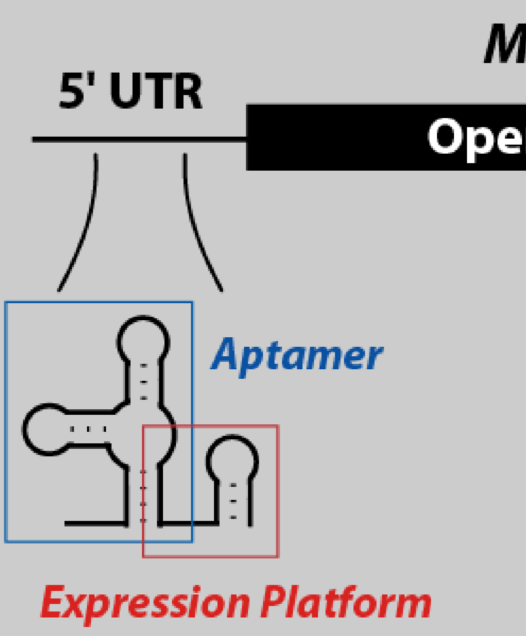


- DNA monomers are made from RNA monomers.
- Most coenzymes are derivatives of RNA.
- Information transfer from DNA to proteins intimately involves RNA.
- RNA-based sensors and switches exist (riboswitches).
- RNA naturally stores genetic information *and* catalyzes chemical reactions (ribozymes).
- All encoded proteins are made by ribozymes.
- Ancient structured noncoding RNAs could have allowed RNA World organisms to run a complex metabolic state.
- Many structured noncoding RNAs might exist in modern cells and are awaiting discovery.

Riboswitches

Natural RNA Elements that Bind Metabolites and Control Gene Expression

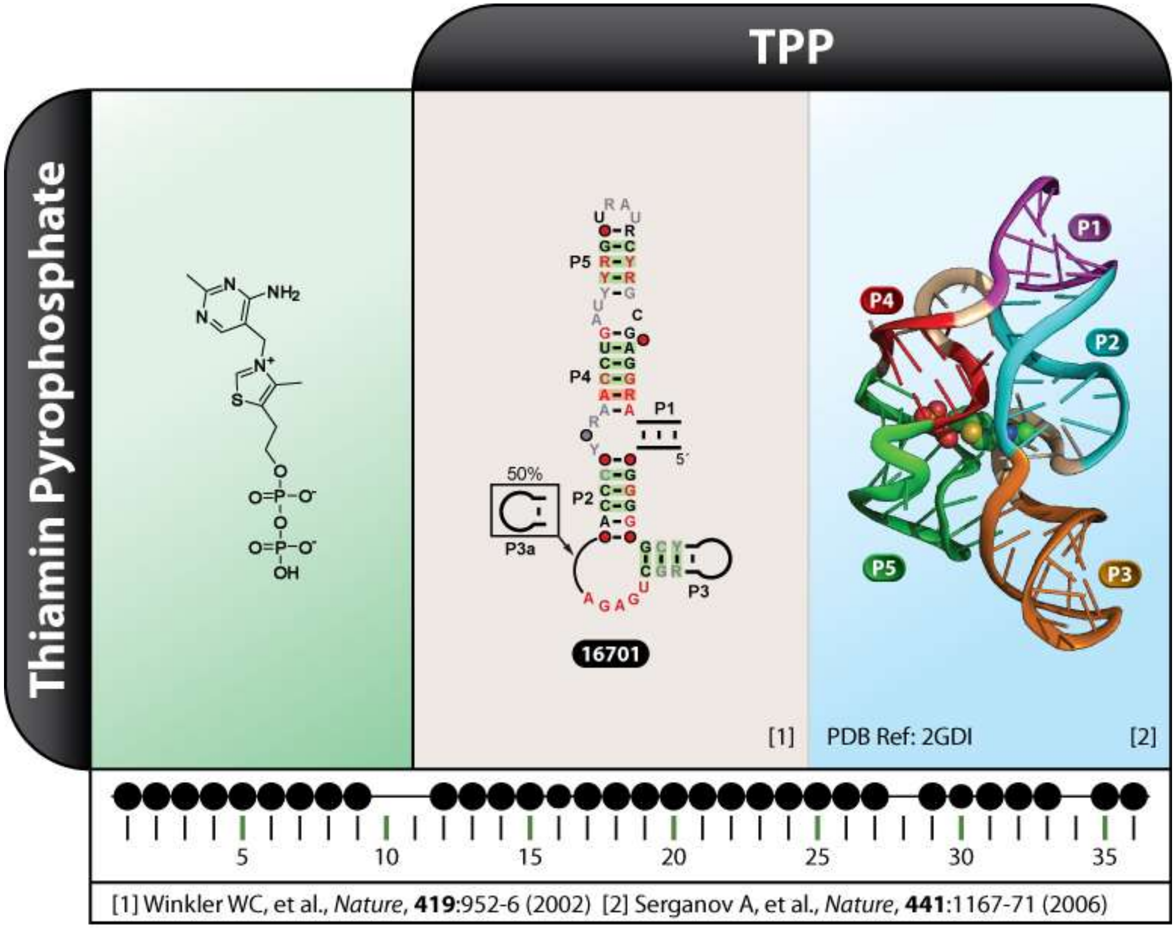
The Typical Architecture of Riboswitches in Bacteria



Aptamer selectively binds metabolites.

Expression Platform changes its folding pattern upon metabolite binding and controls gene expression.

TPP Riboswitches
Present in All Three Domains of Life



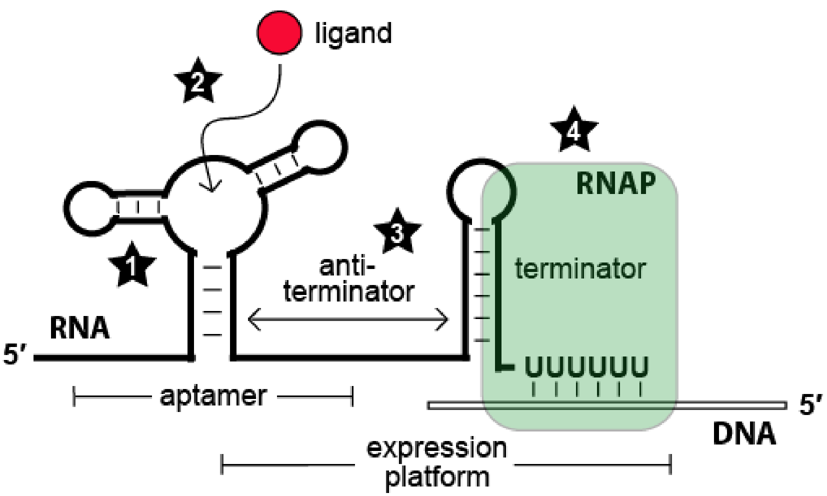
McCown, P.J., Breaker, R.R., unpublished

The Moving Parts of Riboswitches

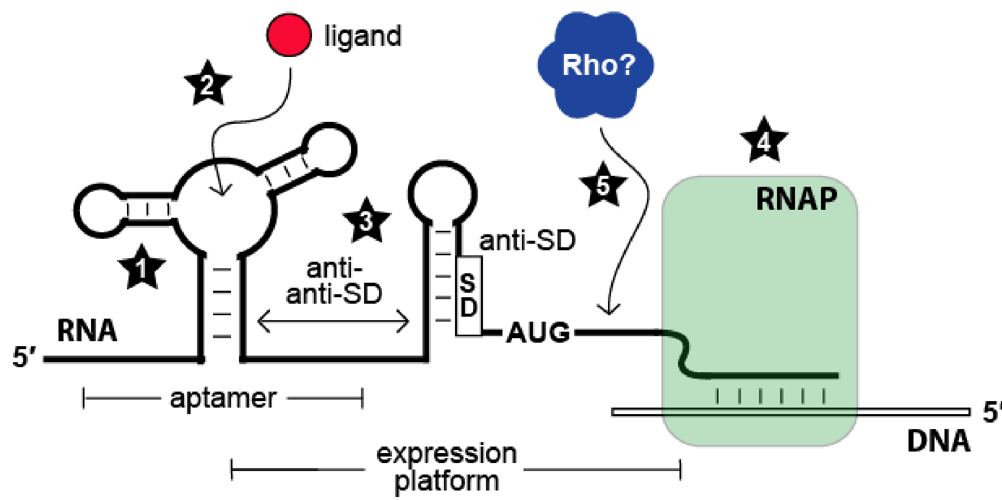
Aptamers and Expression Platforms

The two most common mechanisms of riboswitch gene control in eubacteria.

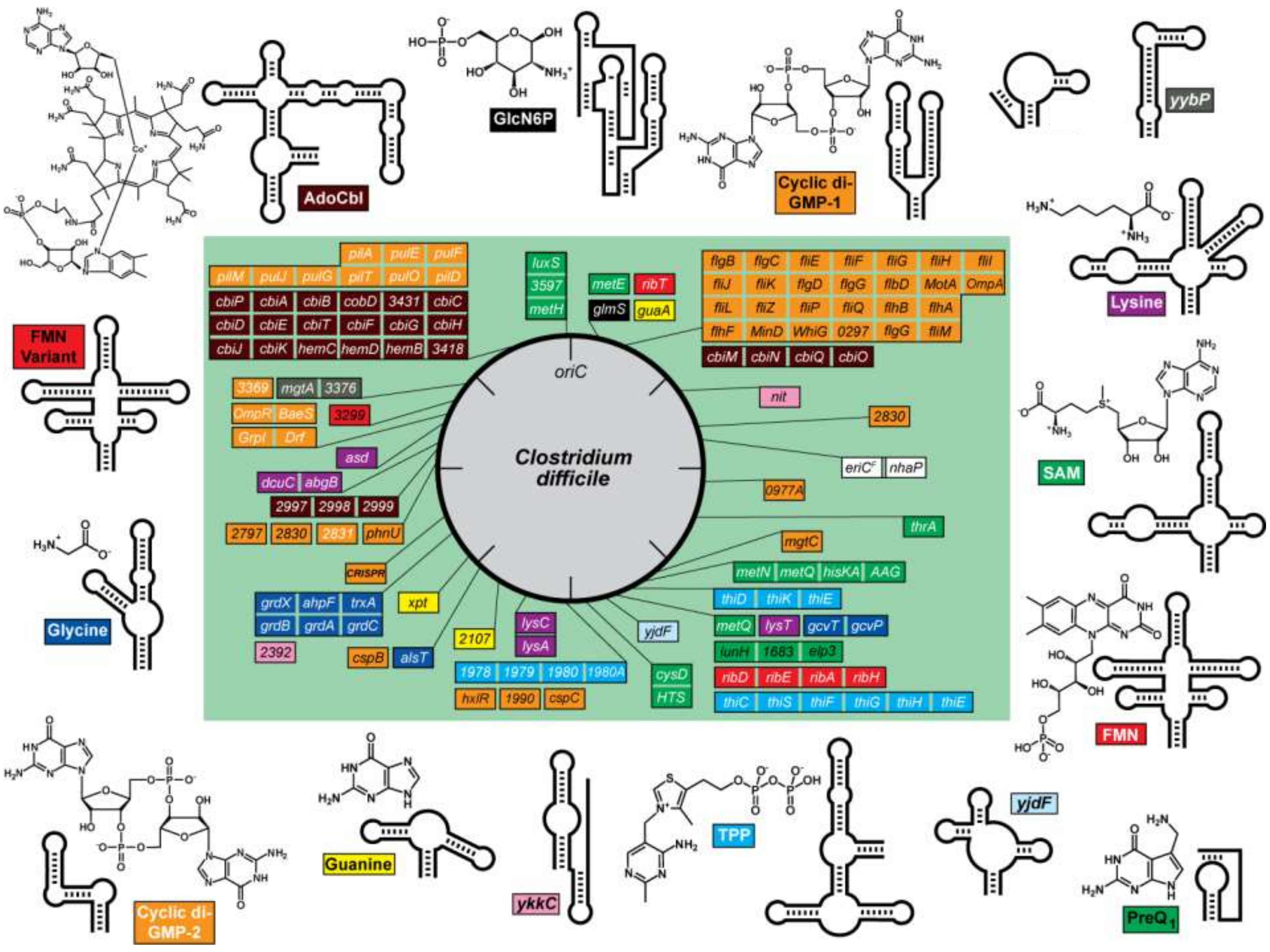
Transcription Control



Translation Control



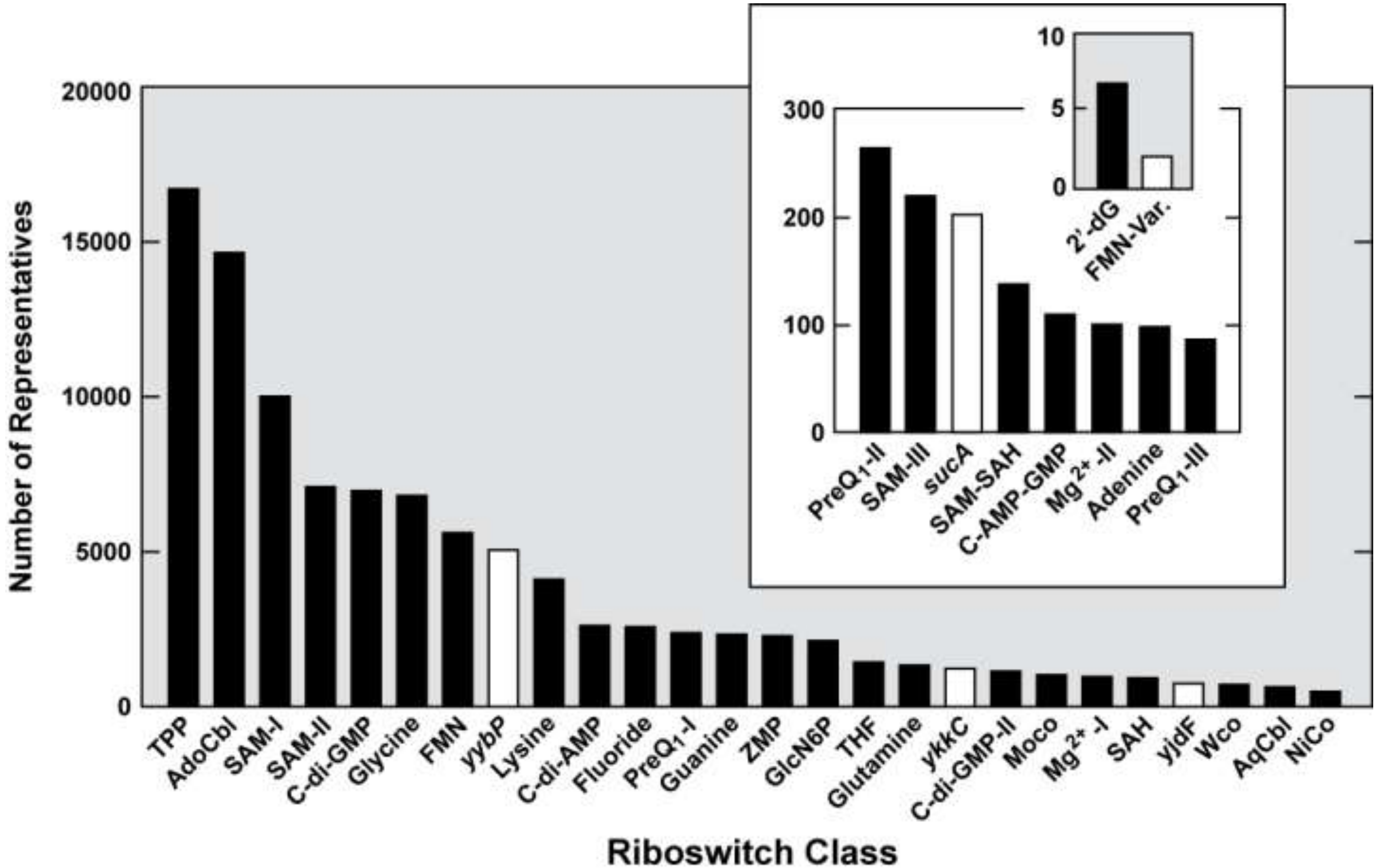
A stealth form of transcription control?



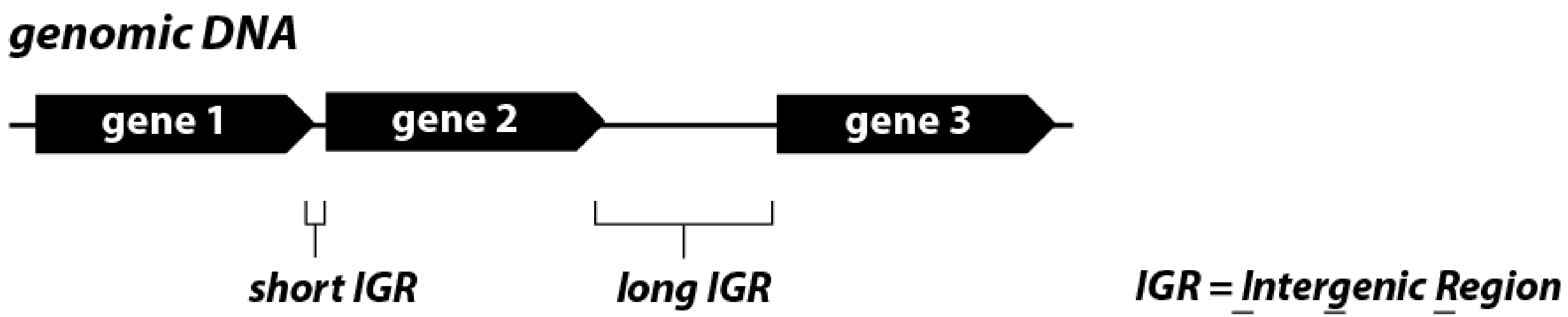
AdoCbl
GlcN6P
Cyclic di-GMP-1
yybP
Lysine
FMN Variant
SAM
Glycine
FMN
Cyclic di-GMP-2
Guanine
ykkC
TPP
yjdF
PreQ1
Clostridium difficile
oriC
pilA pulE pulF
pilM pulJ pulG pilT pulO pilD
cbiP cbiA cbiB cobD 3431 cbiC
cbiD cbiE cbiT cbiF cbiG cbiH
cbiJ cbiK hemC hemD hemB 3418
luxS
3597
metH
metE nrdT
glmS guaA
flgB flgC fliE fliF fliG fliH fliI
fliJ fliK flgD flgG flbD MotA OmpA
fliL fliZ fliP fliQ flhB flhA
flhF MinD WhiG 0297 flgG fliM
cbiM cbiN cbiQ cbiO
3369 mgtA 3376
OmpR BaeS 3299
GrpI Drf
asd
dcuC abgB
2997 2998 2999
2797 2830 2831 phnU
CRISPR
grdX ahpF trxA
grdB grdA grdC
xpt
2392
cspB alsT
2107
lysC
lysA
1978 1979 1980 1980A
hxlR 1990 cspC
cysD
HTS
yjdF
nit
2830
eriC^F nhaP
0977A
thrA
mgtC
metN metQ hisKA AAG
thiD thiK thiE
metQ lysT gcvT gcvP
lunH 1683 elp3
ribD ribE ribA ribH
thiC thiS thiF thiG thiH thiE

The Diversity and Distribution of Riboswitches

Data derived from the analysis of ~10,000 bacterial genomes



Noncoding RNA Discovery in Bacteria

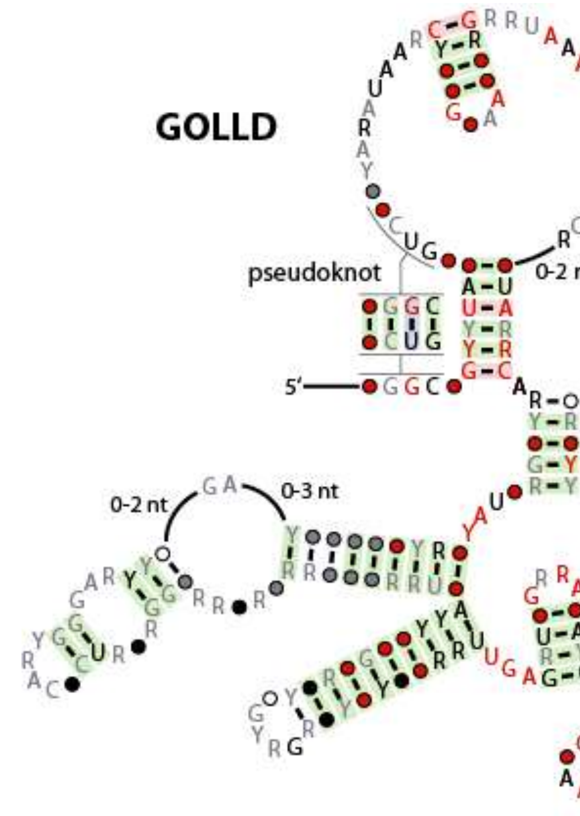


What we find…

- 100s of novel RNA structures are being discovered
- Approximately 80% appear to be protein binding sites or small noncoding RNAs
- Approximately 15% are predicted to be riboswitches
- Approximately 3% are small ribozymes or structured DNAs
- Approximately 2% are large structured RNAs of unknown function

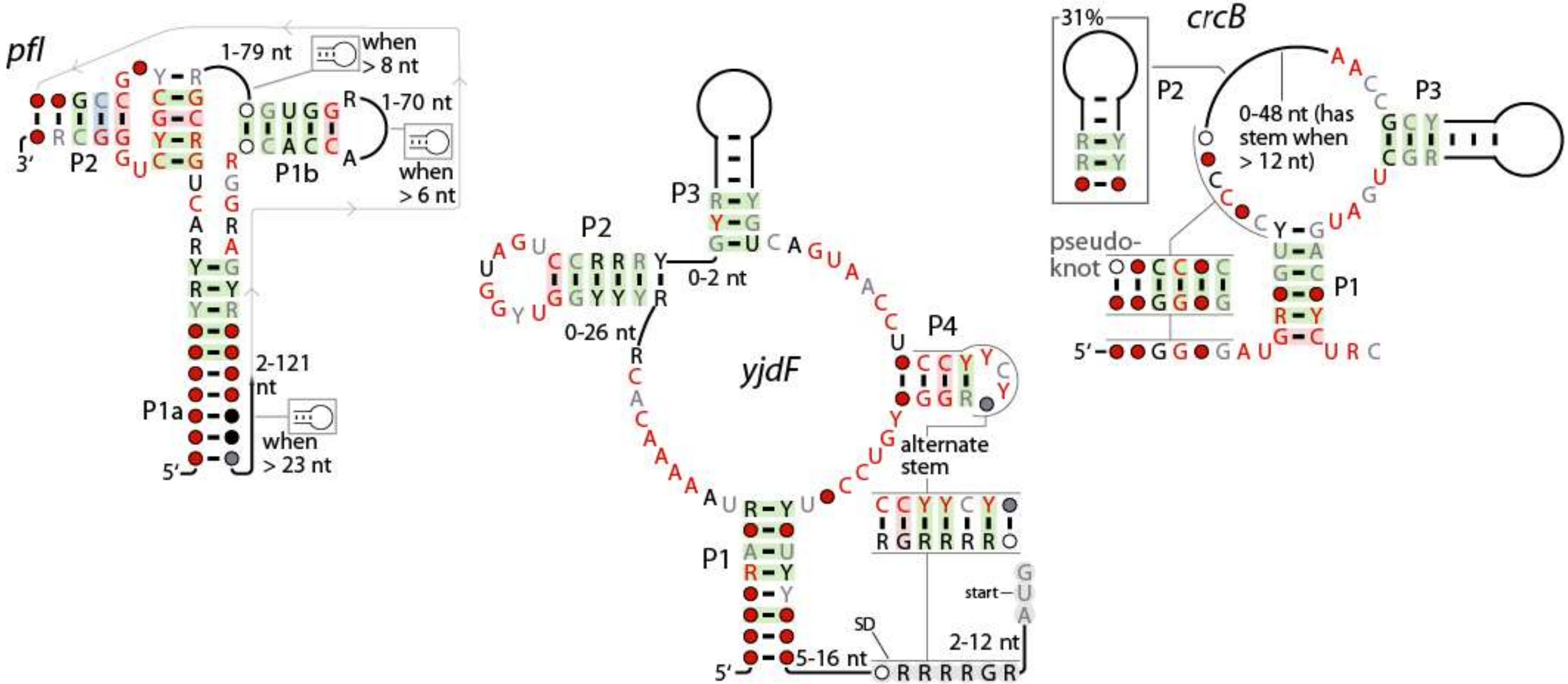
Predicting Functions for New RNAs

Large noncoding RNA?



New-found RNA Motifs

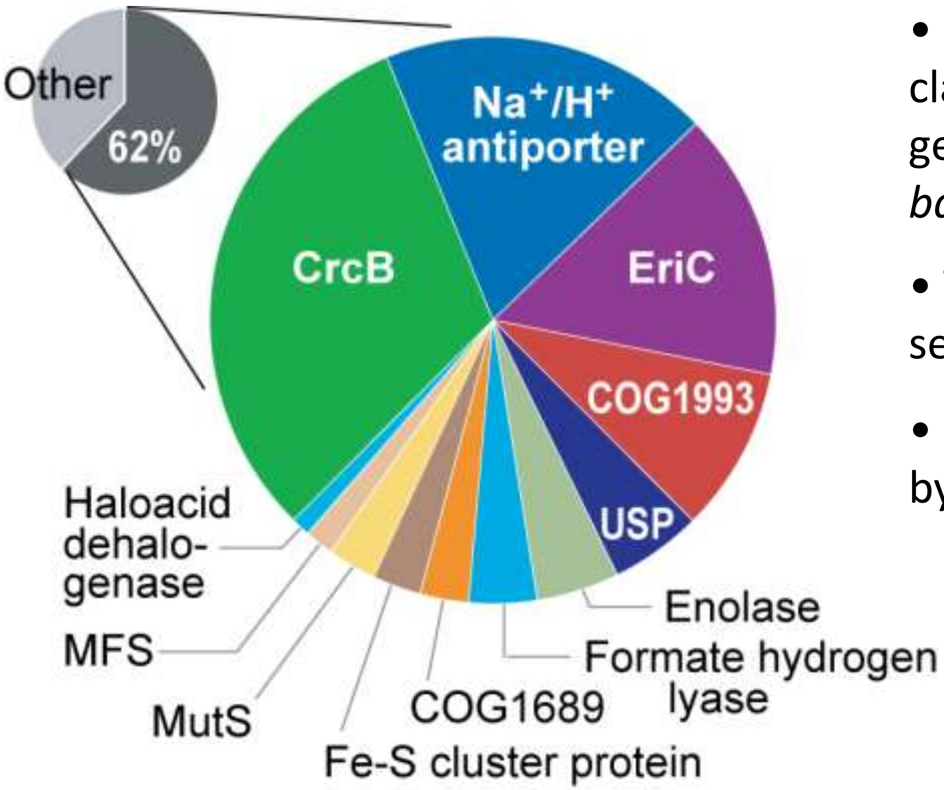
Some Recognizable, Others Mysterious



- Some RNA motifs have more complex structures that are associated with genes for specific biochemical pathways
- These likely function as metabolite- or ion-sensing riboswitches

Genes Associated with *crcB* Motif RNAs

Protein Functions Provide Clues to Riboswitch Ligand Identity



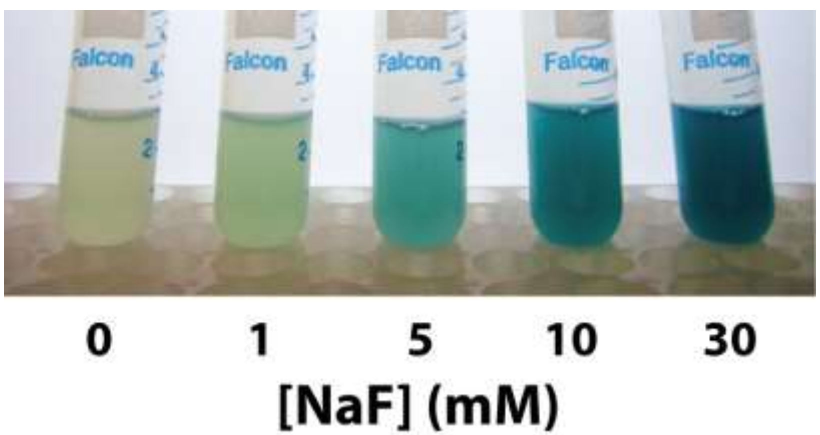
- Only two previously validated riboswitch classes are associated with a great diversity of gene types: *c-di-GMP-I, II riboswitches sense a bacterial second messenger*.
- Therefore, we chose to purchase and test several RNA dinucleotides (*e.g.* pApA).
- In-line probing gave a signal with pApA made by Oligos Etc.

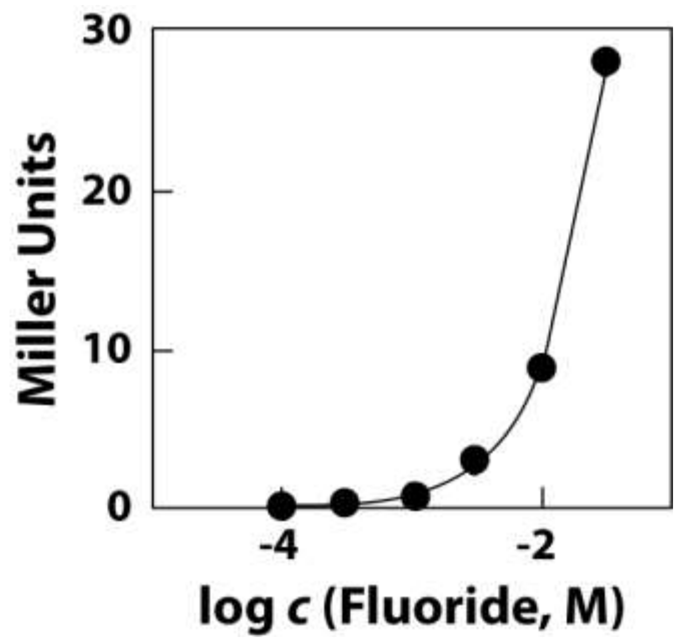
Baker, J.L. et al., 2012. Science 335:233-235

Regulation of Gene Expression by Fluoride

Riboswitch- Reporter Fusion Construct:

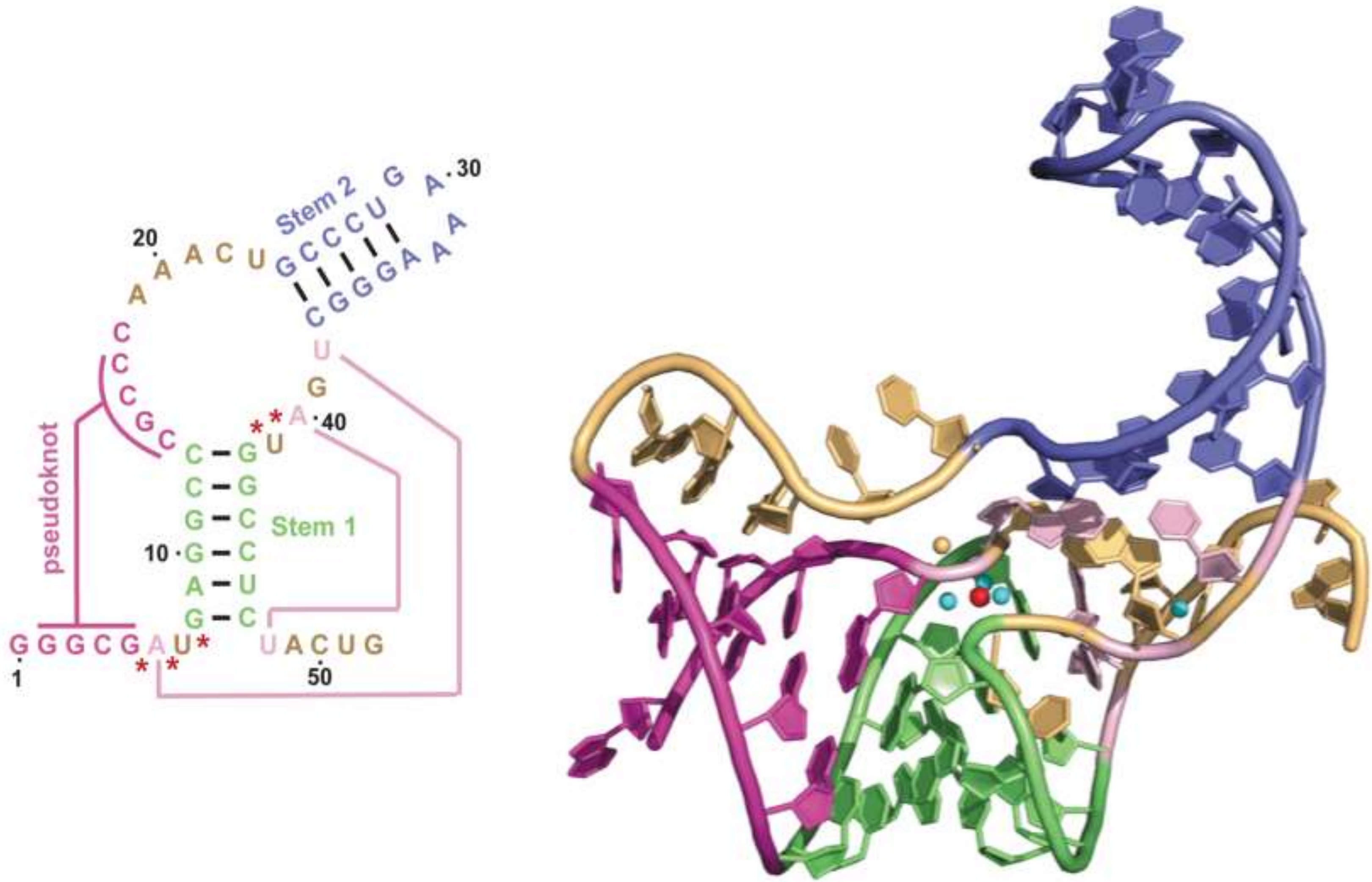
B. cereus riboswitch from the *crcB* gene fused *to E. coli lacZ* and expressed in *B. subtilis*…





The *crcB* Motif RNA from the *B. cereus crcB* Gene is a Fluoride Responsive Riboswitch.

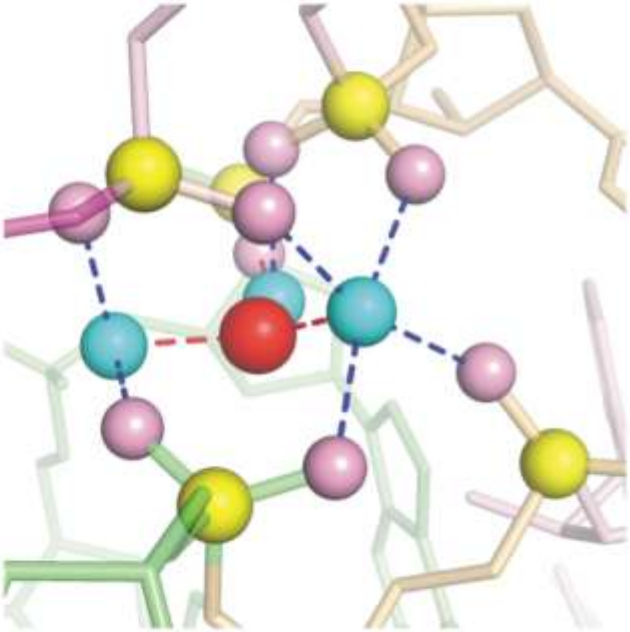
Fluoride Aptamer - The 3D Structure



Ren, A. et al., 2012. Nature 486:85-89.

The Fluoride Binding Pocket

The Riboswitch Pocket for Fluoride



$Mg_3(PO_4)_5F$

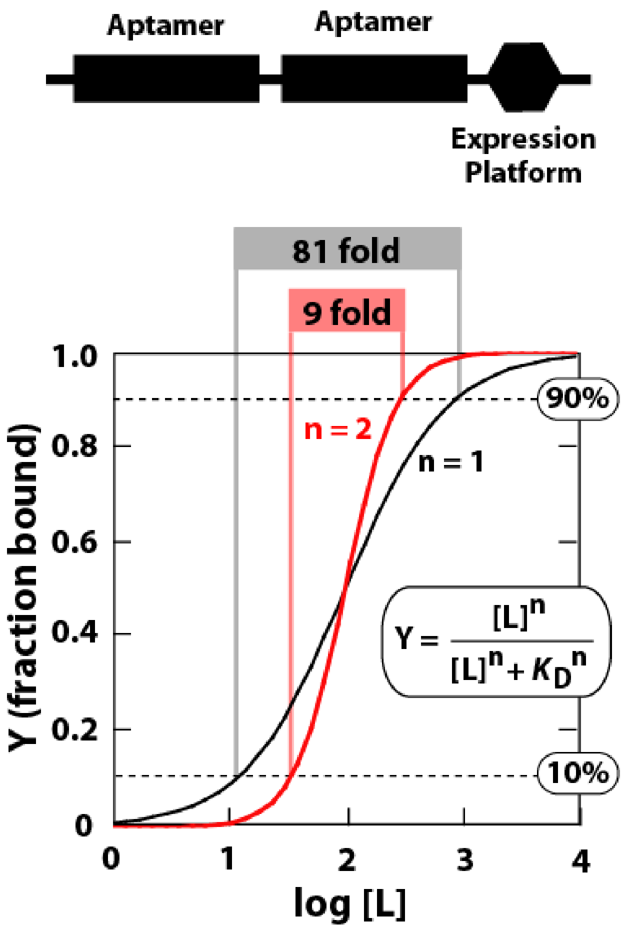
Fluorapatite

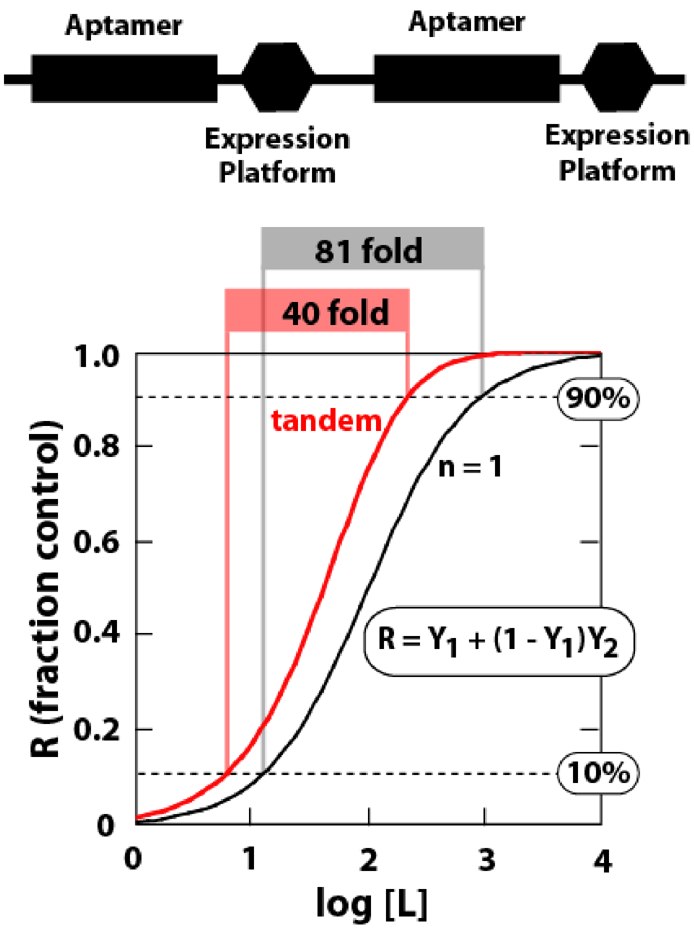
$Ca_5(PO_4)_3F$

RNA is forming a miniature gemstone

Simple Versus Complex Riboswitches

Two Architectures Yield Digital Gene Control

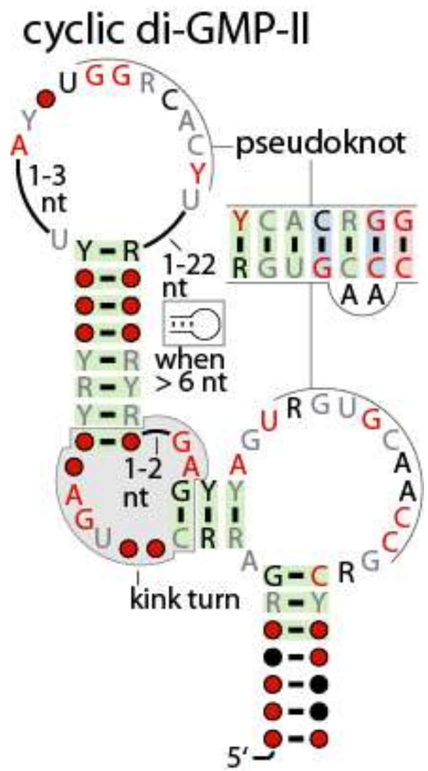




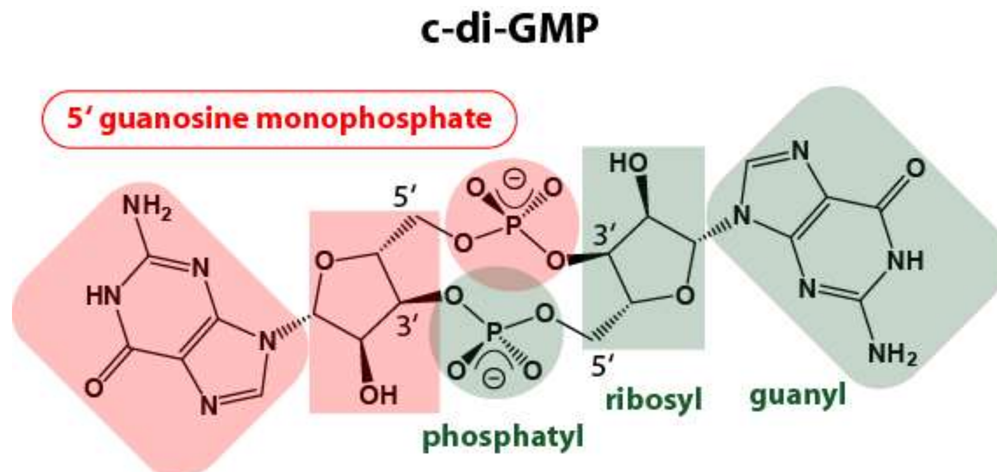
Welz, R., Breaker, R.R. 2007. RNA 13:573-582

A Riboswitch Class for c-di-GMP

Riboswitch Aptamer



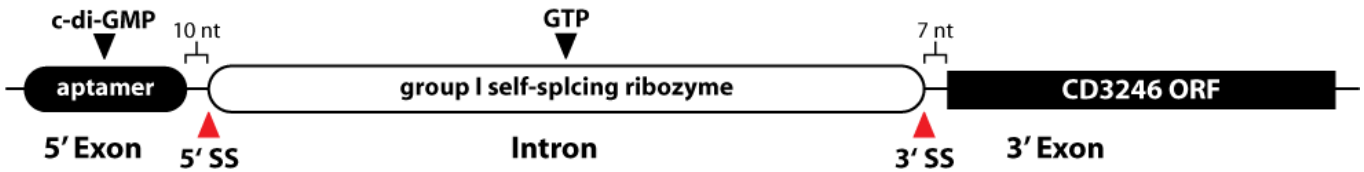
Riboswitch Ligand

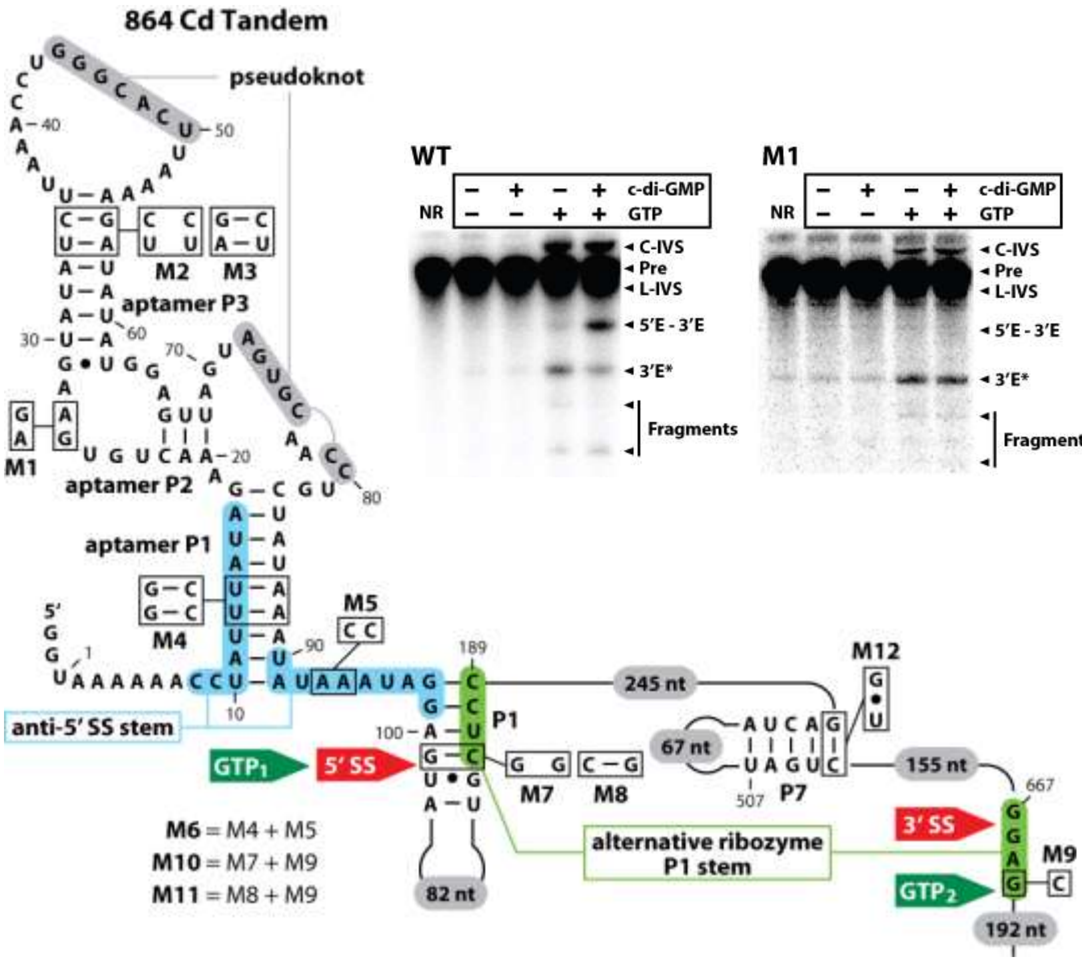


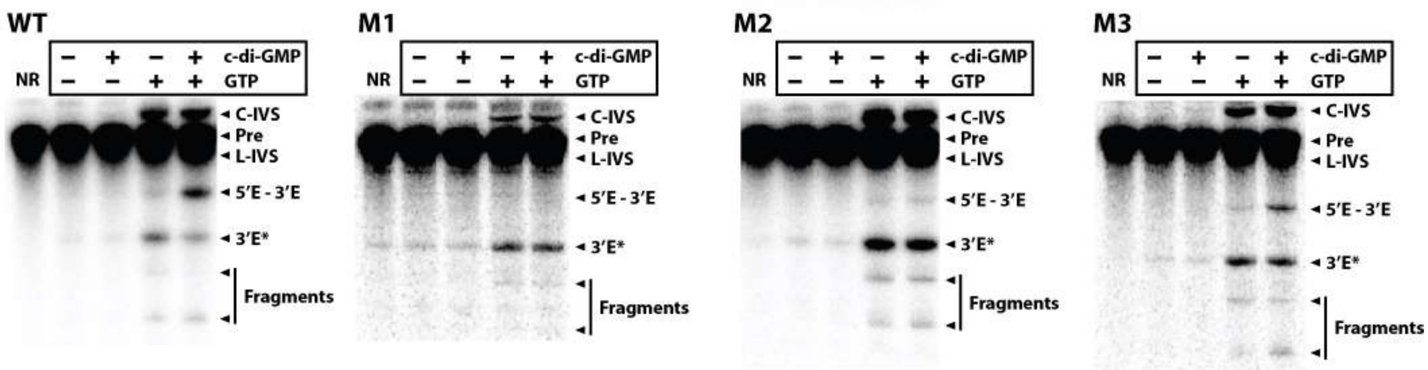
2 GTP ⟶ c-di-GMP

Lee, E.R., et al. 2010. Science 329:845-848

Modulation of Ribozyme Self-splicing by c-di-GMP



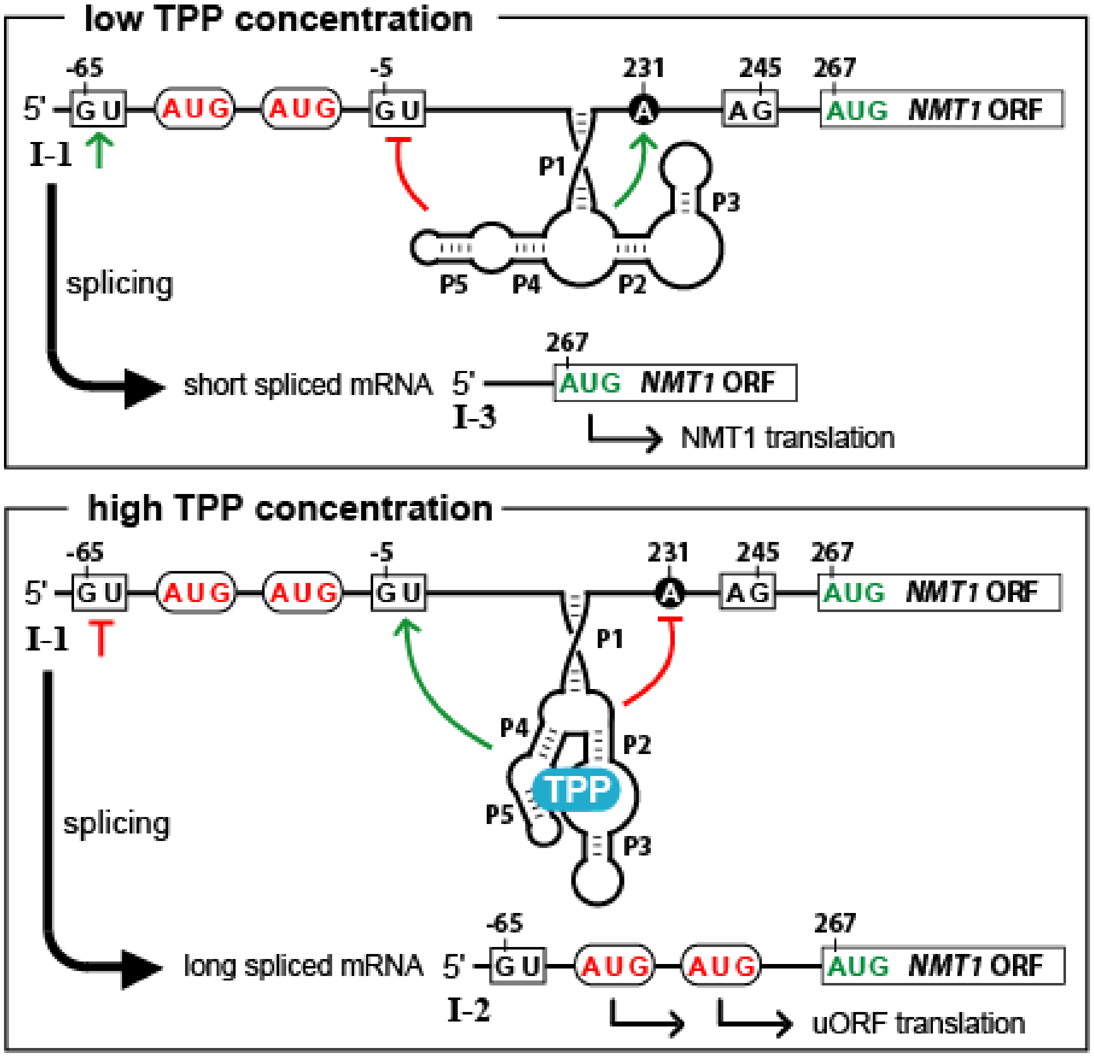


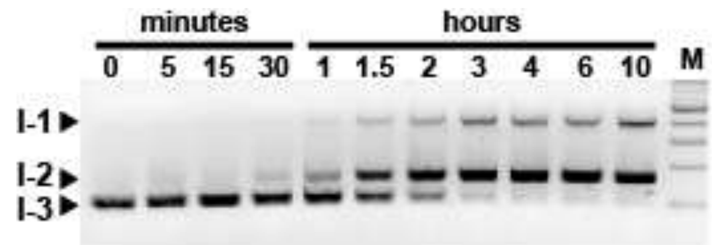


A two-input molecular logic gate?

2 GTP ⟶ c-di-GMP

TPP Riboswitch Mechanism for Modulating *NMT1* Alternative Splicing

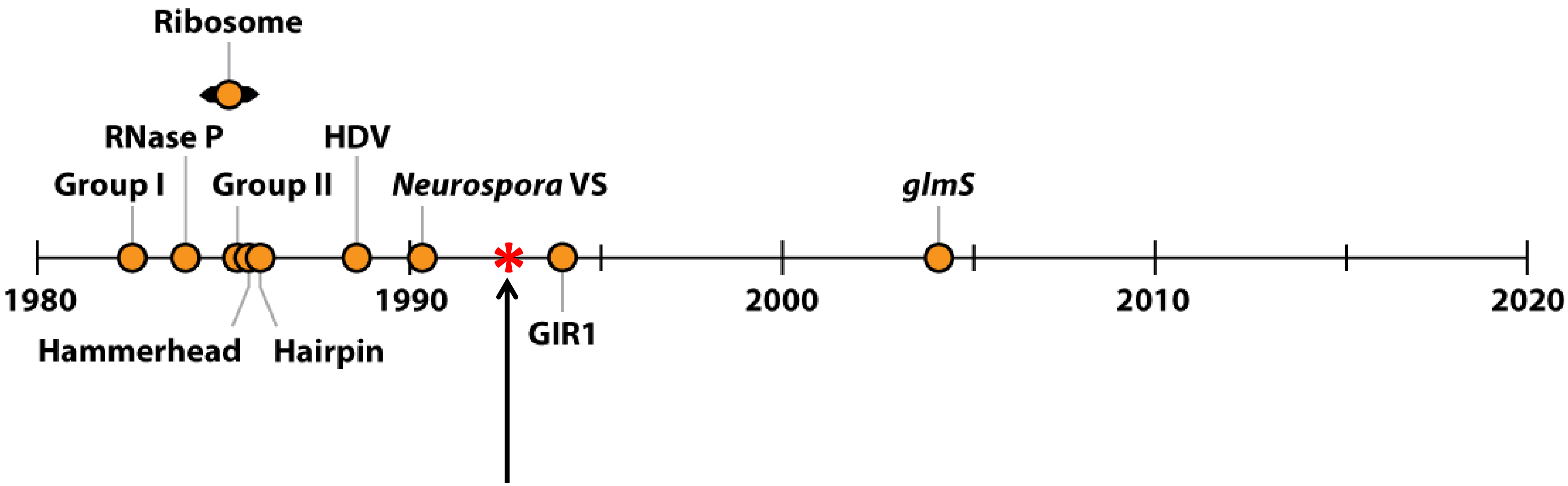




Thiamin addition results in reduced splicing activity and a redirection of splicing to yield an alternative mRNA configuration.

Cheah, M.T., Wachter, A., Sudarsan, N., Breaker, R.R. 2007. Nature 447:497-500

Ribozyme Classes: Timeline of Discoveries



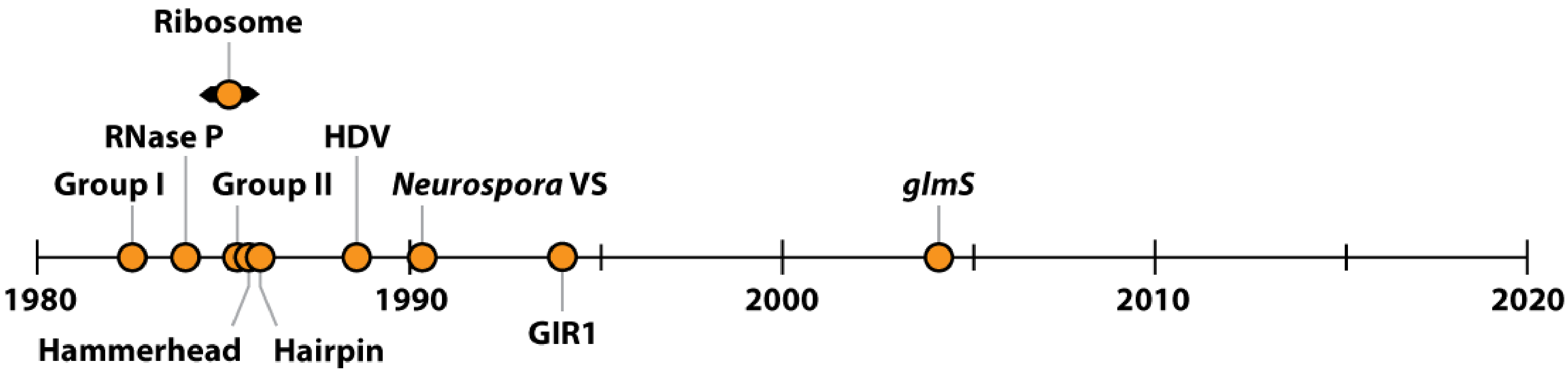
Leslie Orgel and Francis Crick

(1993) Anticipating and RNA World. Some Past Speculations on the Origin of Life: Where are They Today?

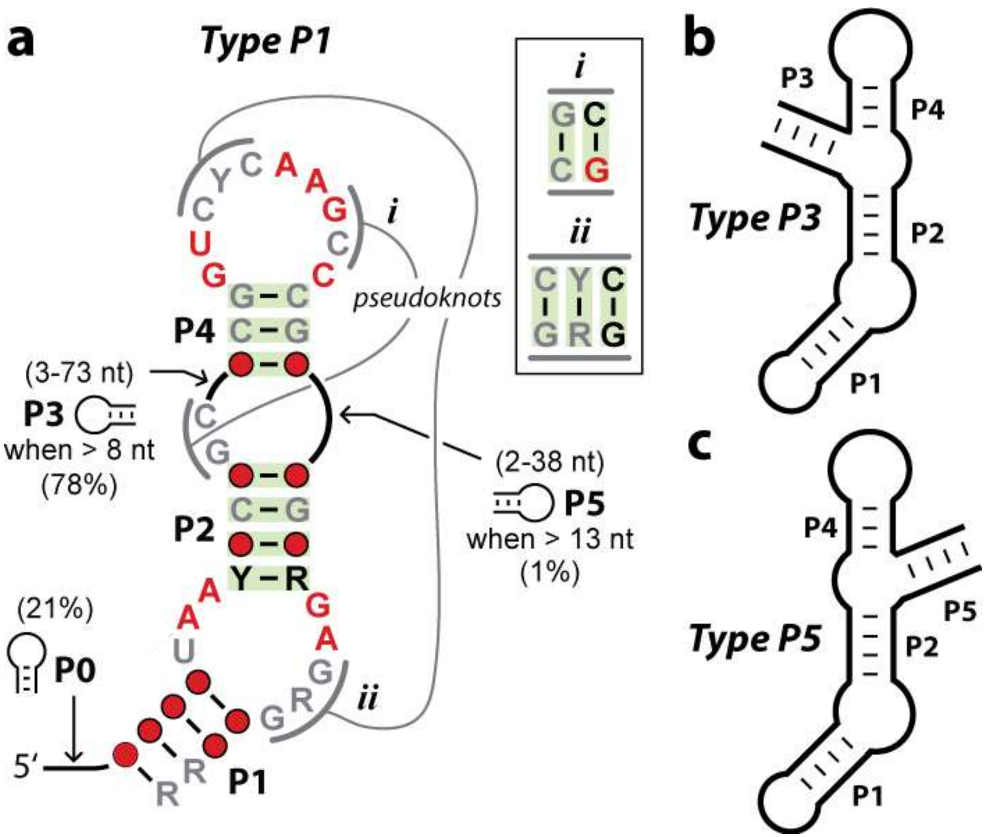
FASEB J. 7:238

"We took it for granted that RNA-based catalysis was necessarily less efficient than protein-based catalysis, and consequently that RNA catalysts had been superseded by protein enzymes in every case. The same assumption led us to underestimate the potential capacity of an RNA world."

Ribozyme Classes: Timeline of Discoveries



Twister: A Newfound ncRNA in Bacteria and Eukarya

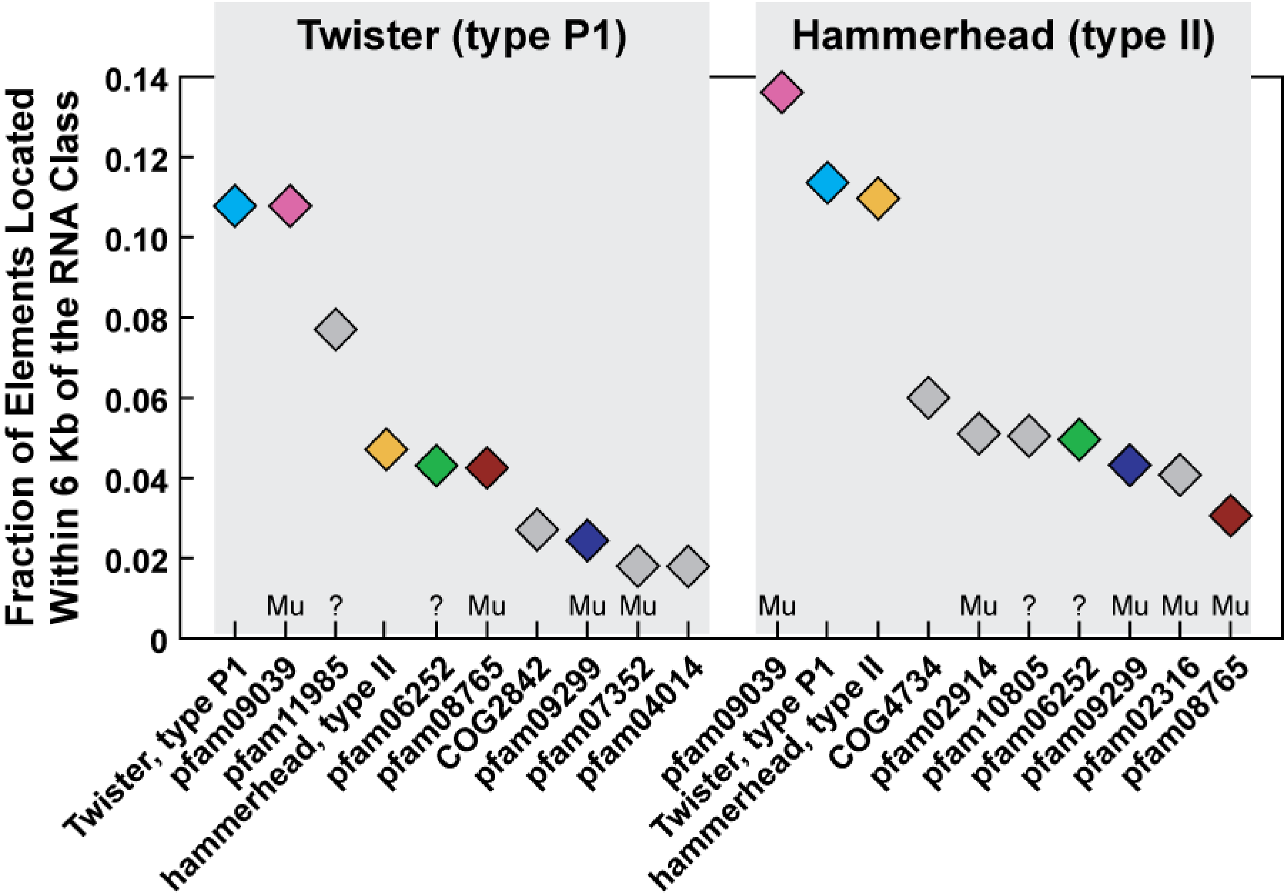


- The size and complexity of the RNA motif is similar to that of riboswitches
- Circularly-permuted riboswitches have never been found

- The secondary structure is identical to the Egyptian hieroglyph "twisted flax"

Roth, A. et al., 2013. Nat. Chem. Biol. 10:56-60.

Twister has a Familiar Genomic Distribution

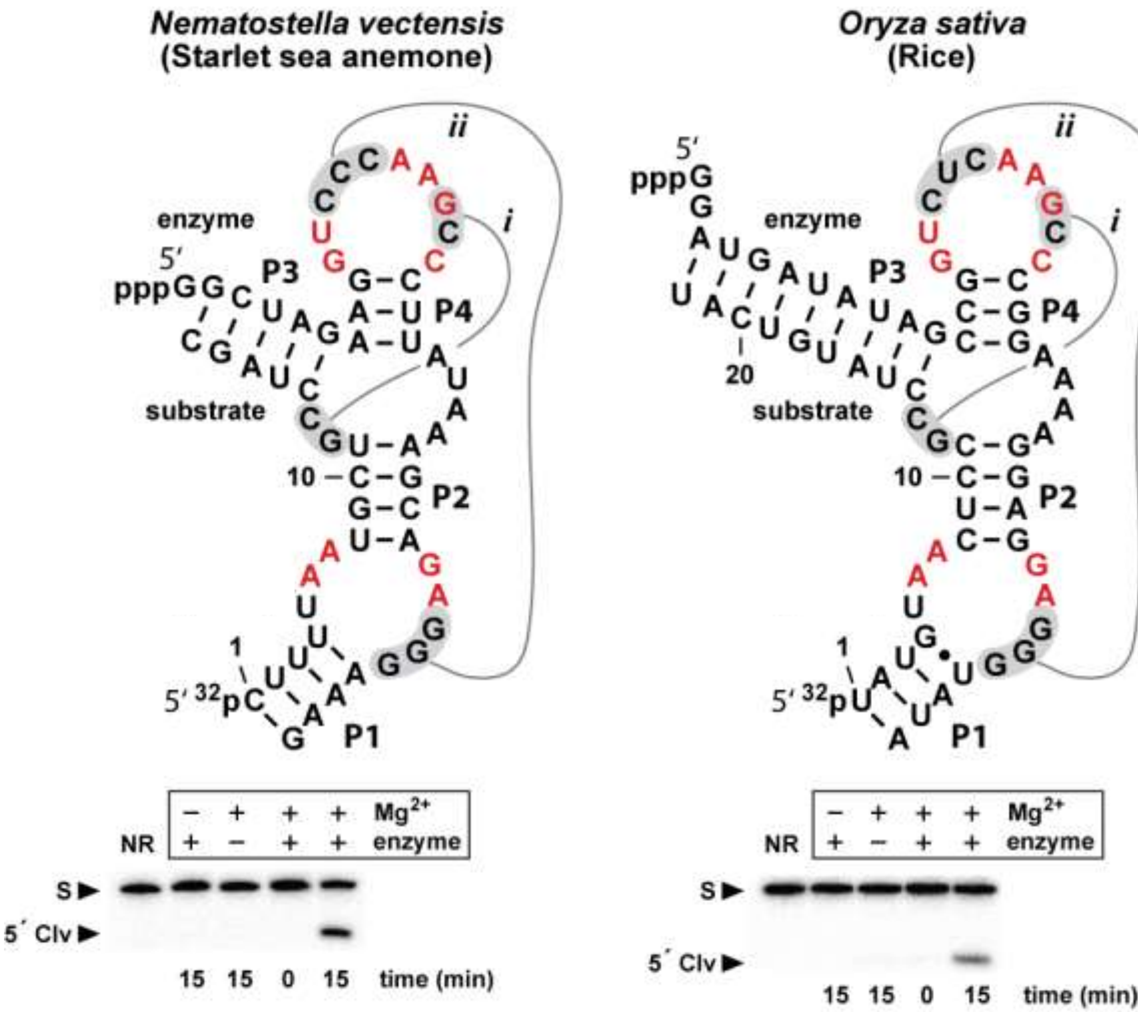


Mu = Mu phage gene
? = unknown function

- **Twister gene associations are very similar to that for hammerhead ribozymes**

Roth, A. et al., 2013. Nat. Chem. Biol. 10:56-60.

Bimolecular Twister RNAs Rapidly Cleave

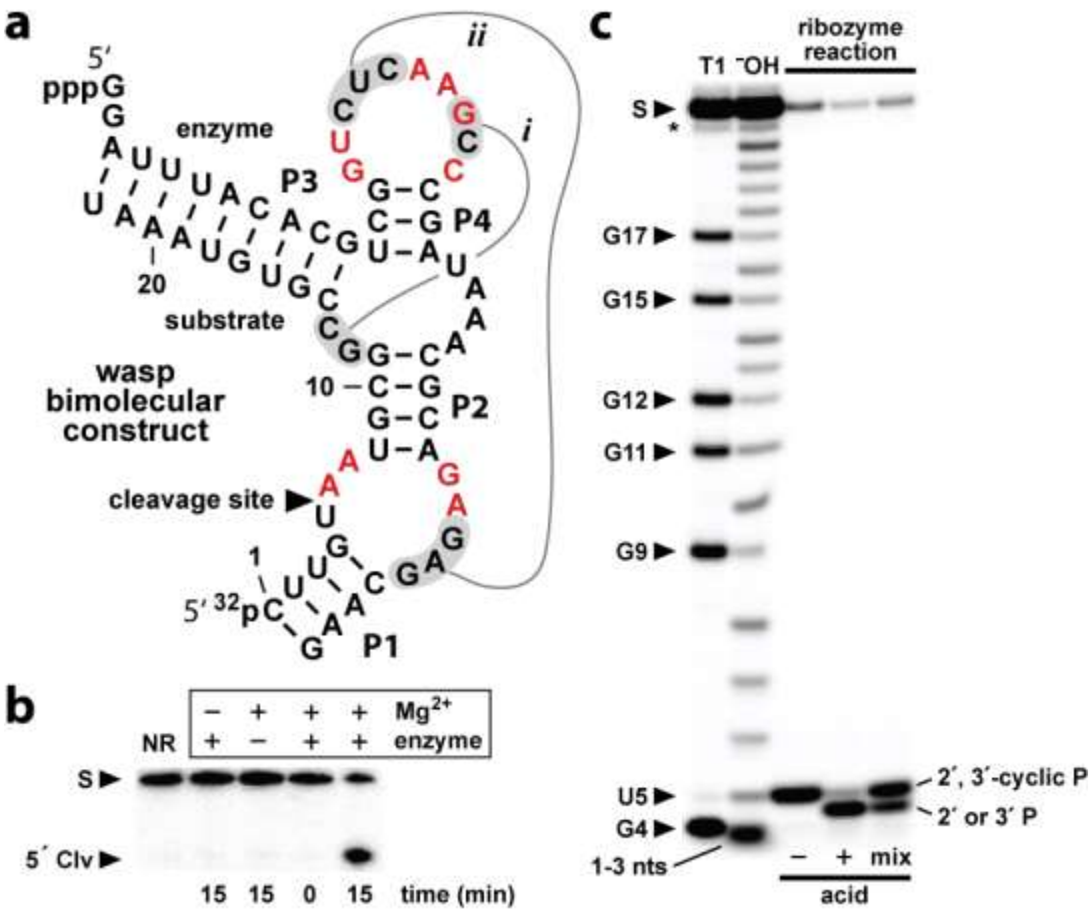


Nematostella vectensis

Oryza savita

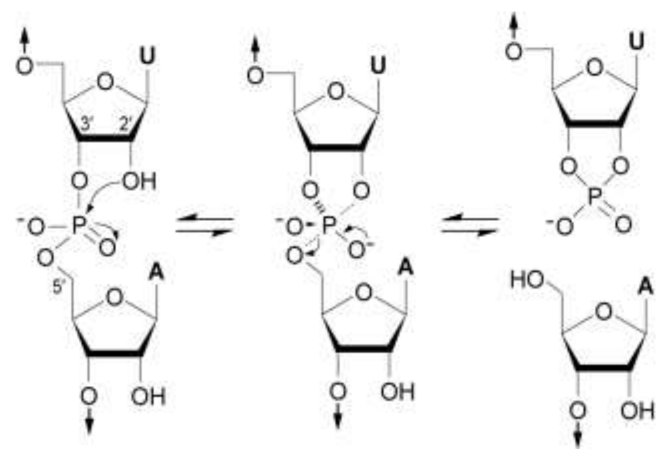
Roth, A. et al., 2013. Nat. Chem. Biol. 10:56-60.

Mapping the Ribozyme Cleavage Site



Nasonia vitripennis

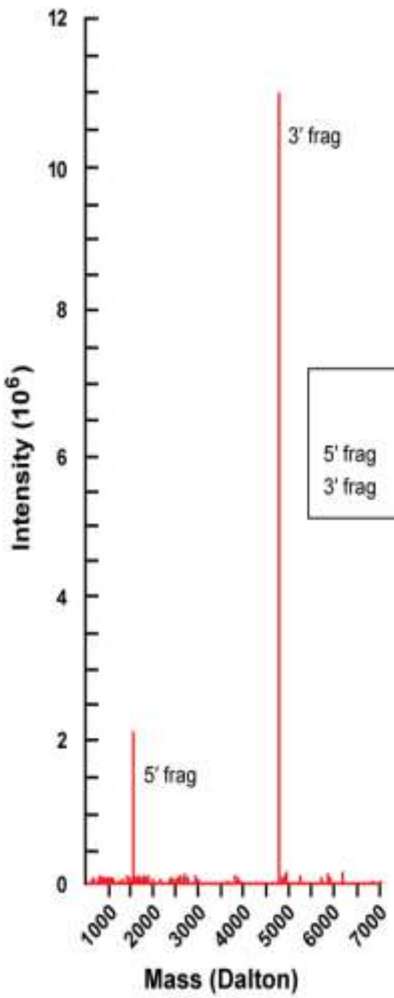
Ribozyme Mechanism



Roth, A. et al., 2013. Nat. Chem. Biol. 10:56-60.

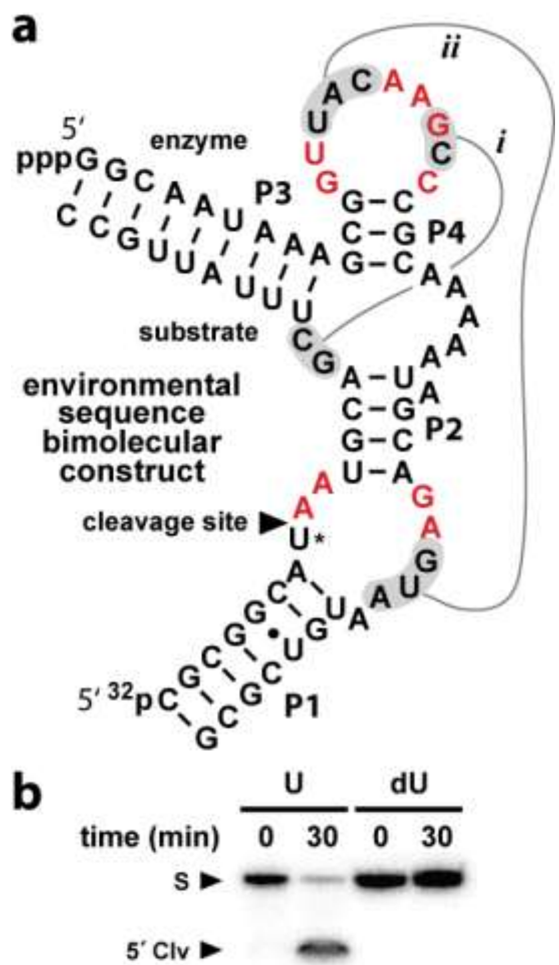
Mapping the Ribozyme Cleavage Site

Mass Spectrum Analysis

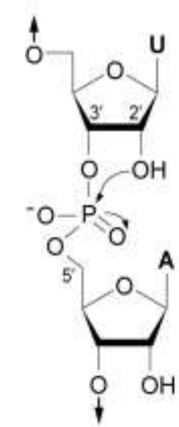


	Mass calcd. (Da)	Mass obsd. (Da)	Error (ppm) obsd. - calcd.
5′ frag	1528.1585	1528.157	-1.0
3′ frag	4770.7171	4770.717	-0.0

Nucleophile Deletion

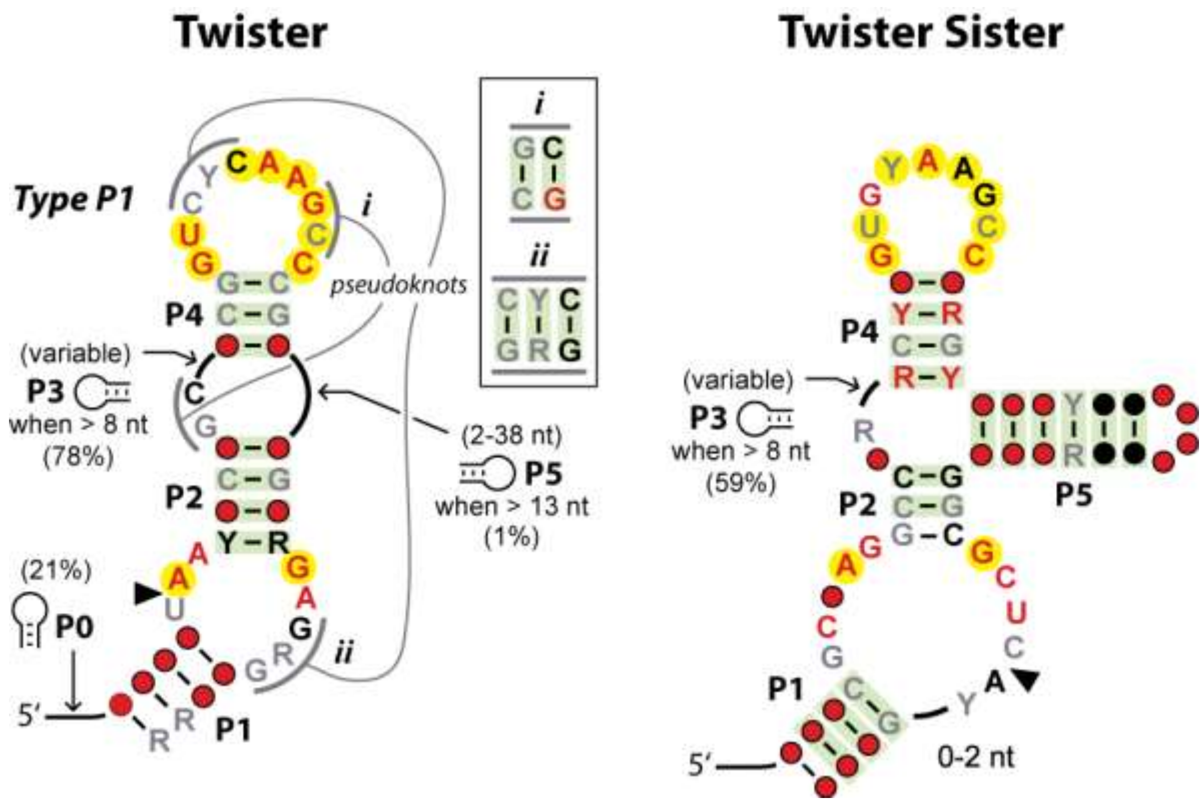


Environmental Sequence



Twister Sister

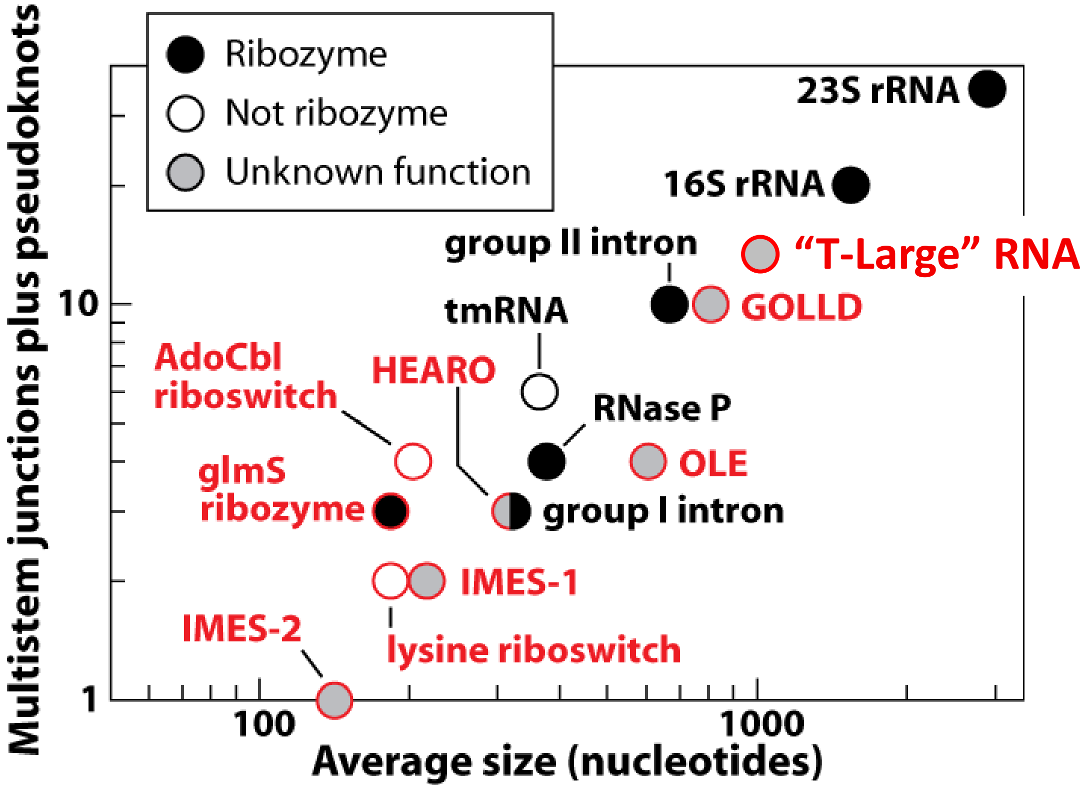
A Distinct Class of Natural Self-cleaving Ribozymes?



Ribozyme Search Strategy:

Look for new ribozymes where known ribozymes frequently reside

Large and Complex Bacterial Non-coding RNAs

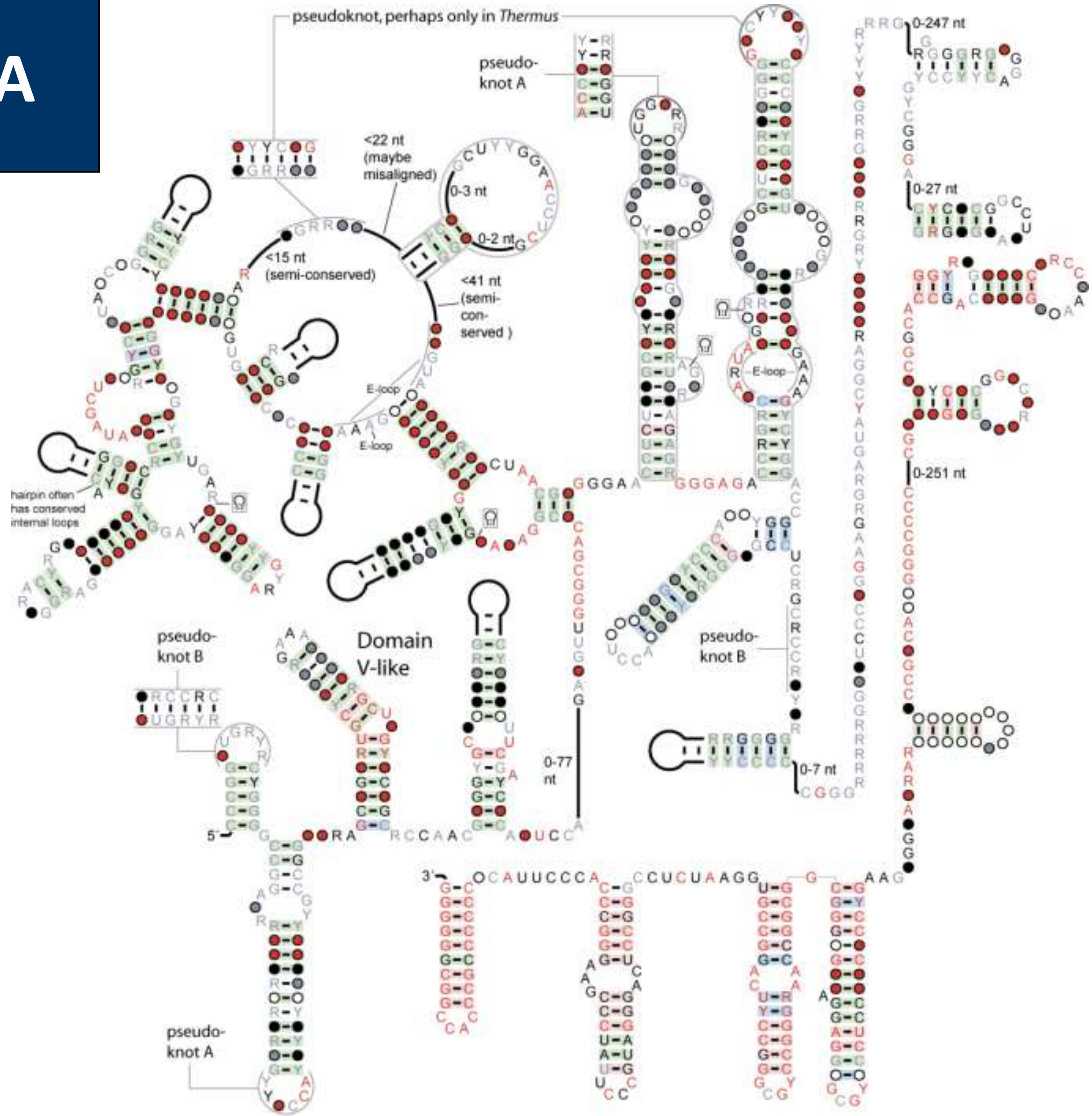


Red = Discovered within the last decade.

- Numerous large noncoding RNAs of unknown function remain to be discovered.
- Large structured noncoding RNAs are enriched for ribozymes.

Weinberg Z, Perreault J, Meyer MM, Breaker RR. 2009. Nature 462:656.

T-Large RNA

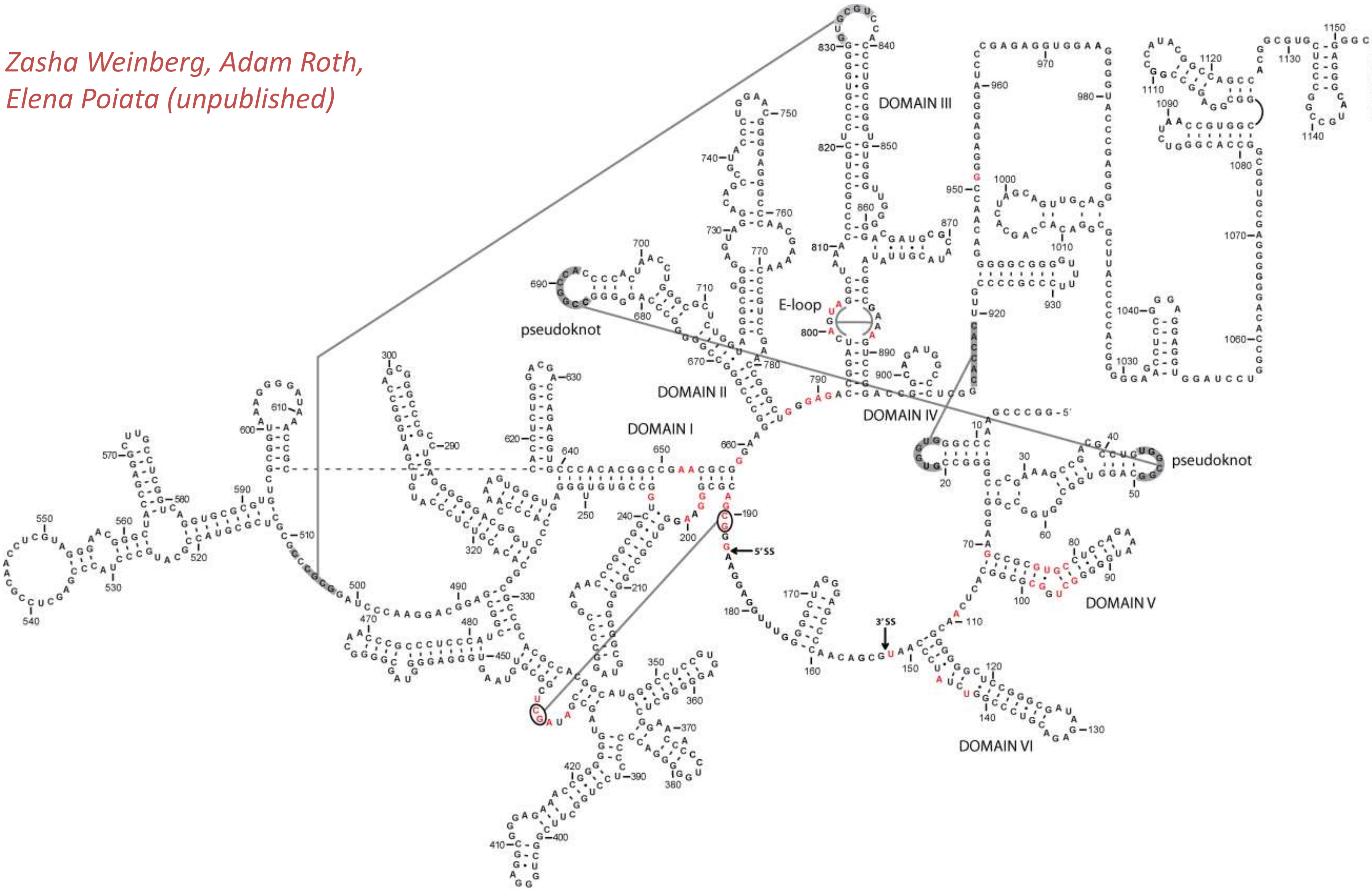


pseudoknot, perhaps only in Thermus
pseudo-knot A
<22 nt (maybe misaligned)
0-3 nt
0-2 nt
<15 nt (semi-conserved)
<41 nt (semi-con-served)
E-loop
hairpin often has conserved internal loops
Domain V-like
pseudo-knot B
0-77 nt
0-7 nt
0-247 nt
0-27 nt
0-251 nt
5'
3'

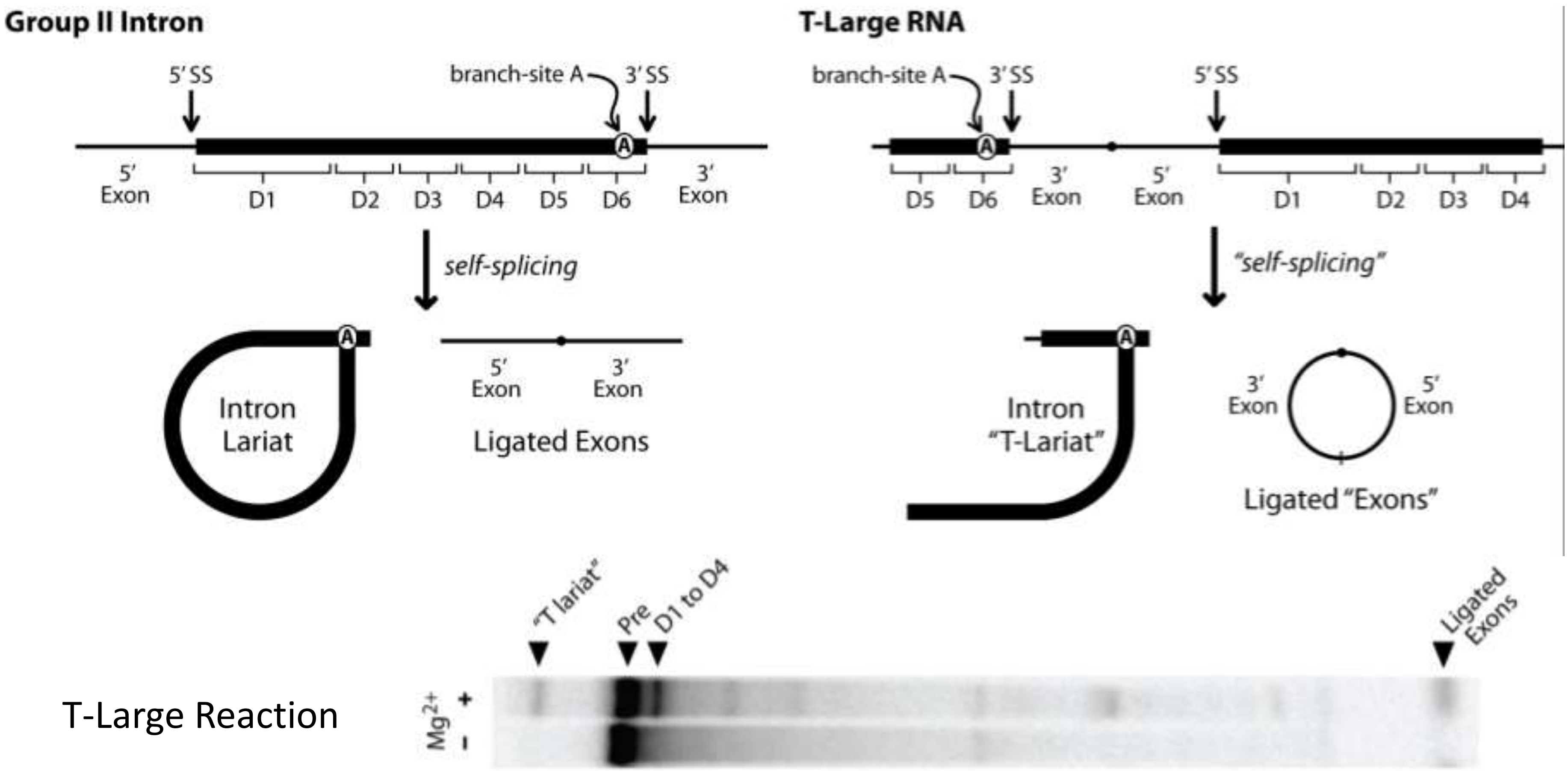
"T-Large" RNA

A Circularly Permuted Group IIA Ribozyme

Zasha Weinberg, Adam Roth, Elena Poiata (unpublished)

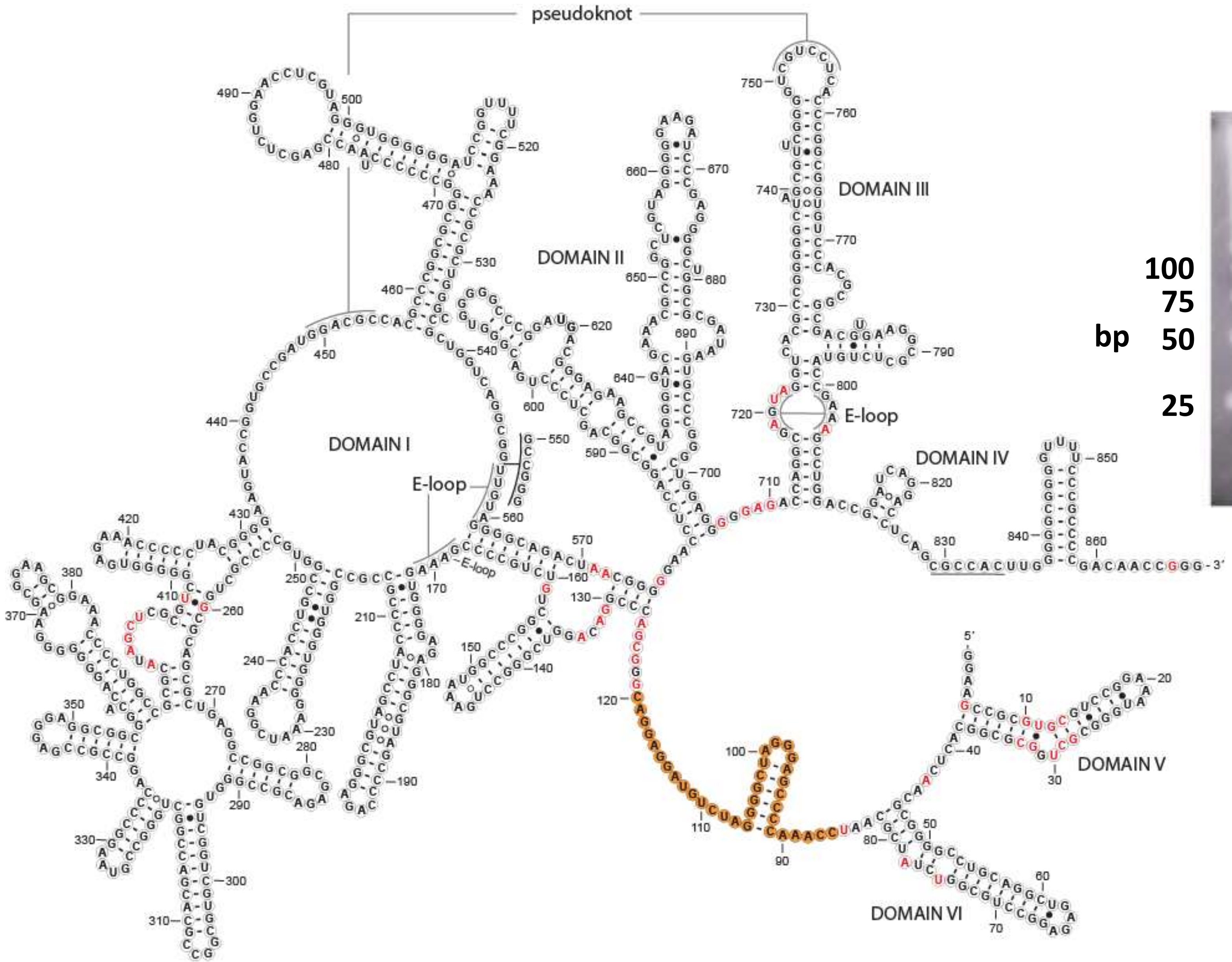


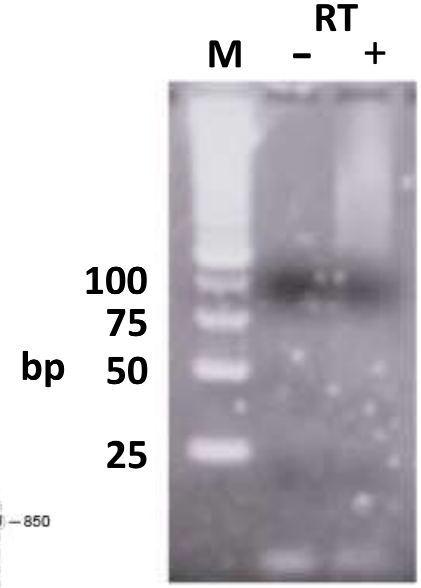
Normal Versus Permuted Group II Ribozymes
What are the Reaction Product Differences?



T-Large Reaction

RT-PCR and Sequencing Confirms a Circular Product

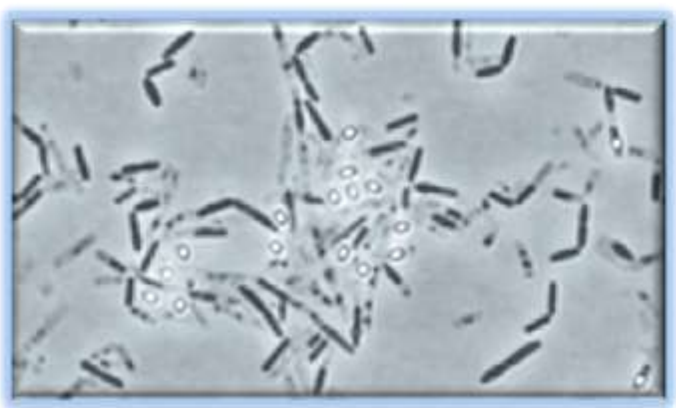




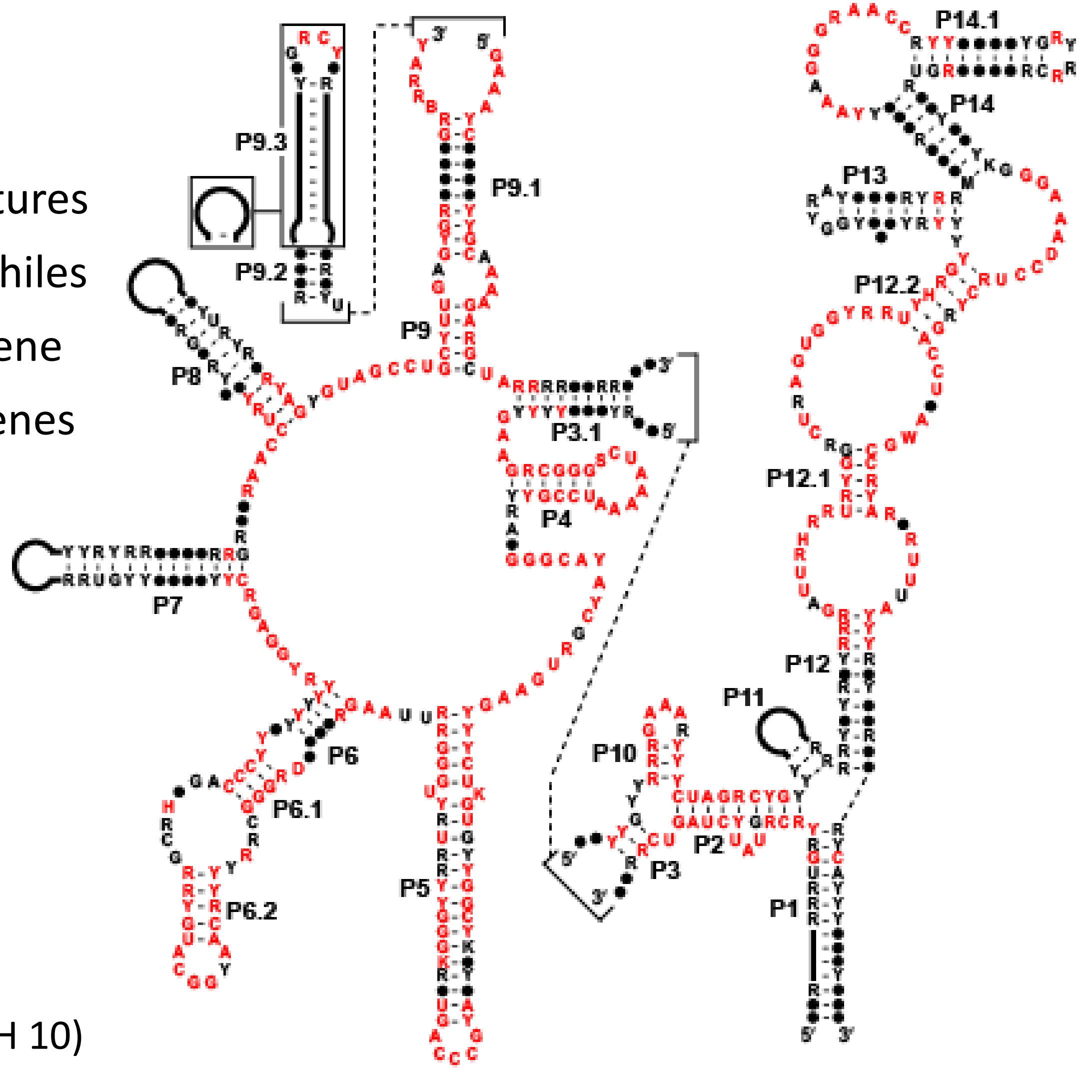
Ornate, Large, Extremophilic RNA

Strange Characteristics of OLE RNA

- Well conserved sequences and structures
- Found mostly in anaerobic extremophiles
- Syntenic with a membrane protein gene
- Usually by isoprenoid biosynthesis genes



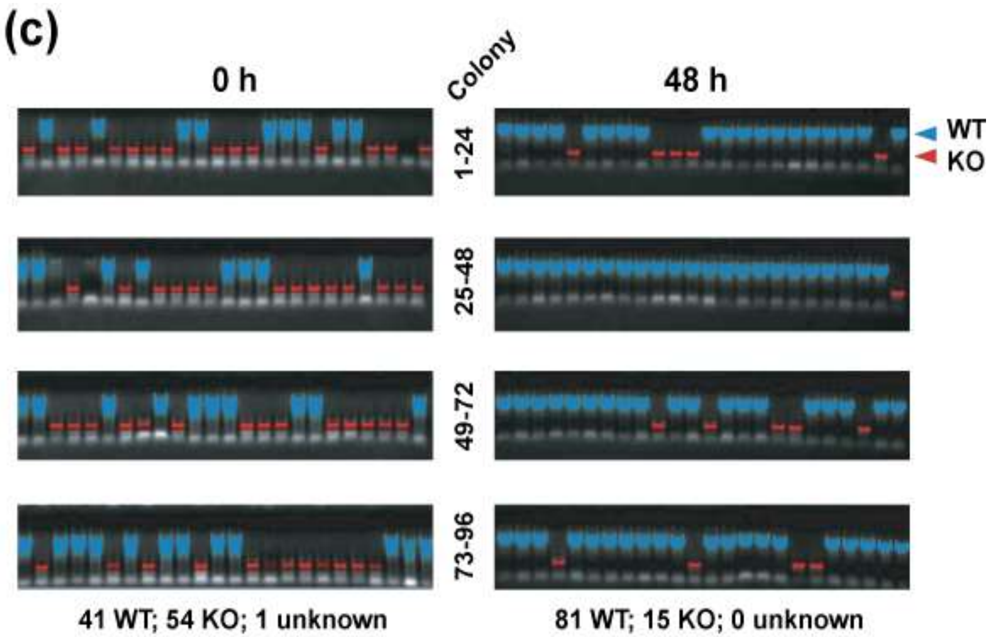
***Bacillus halodurans* C-125 Strain**
Gram-positive spore forming bacterium
Prefers growth under alkaline conditions (pH 10)



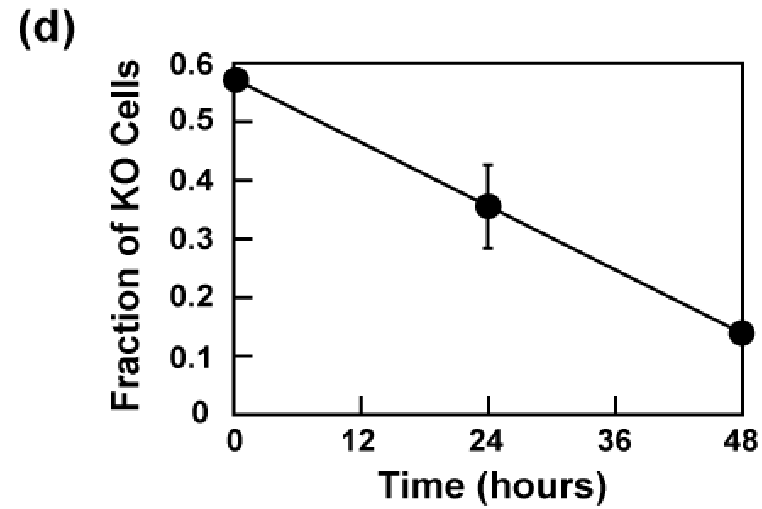
Puerta-Fernandez, E., et al. 2006. Proc. Natl. Acad. Sci. USA 103:19490-19495.

OLE RNA Knockout Cells are More Sensitive to Ethanol

Colony PCR



WT Cells Outcompete KO Cells

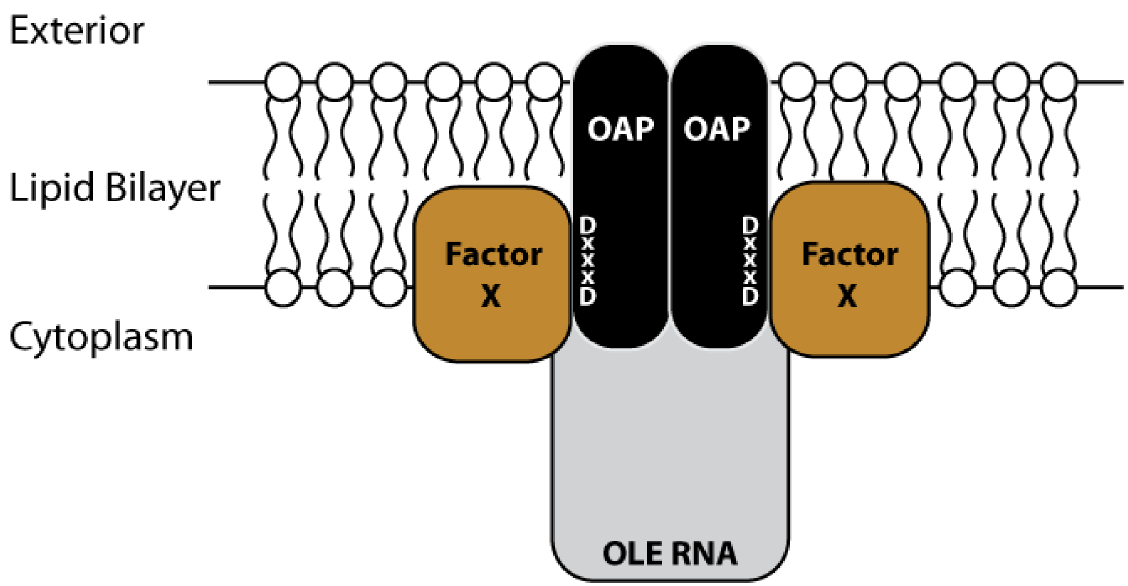


Growth in rich media plus 5% ethanol

OLE and OAP confer resistance to alcohol toxicity and heat stress. How?

Working Hypothesis for OLE-OAP Function

Architecture ?



Pathway ?

B confers some resistance to ethanol

D confers greater resistance to ethanol

OAP DxxxD mutant disrupts the ability of Factor X to make B

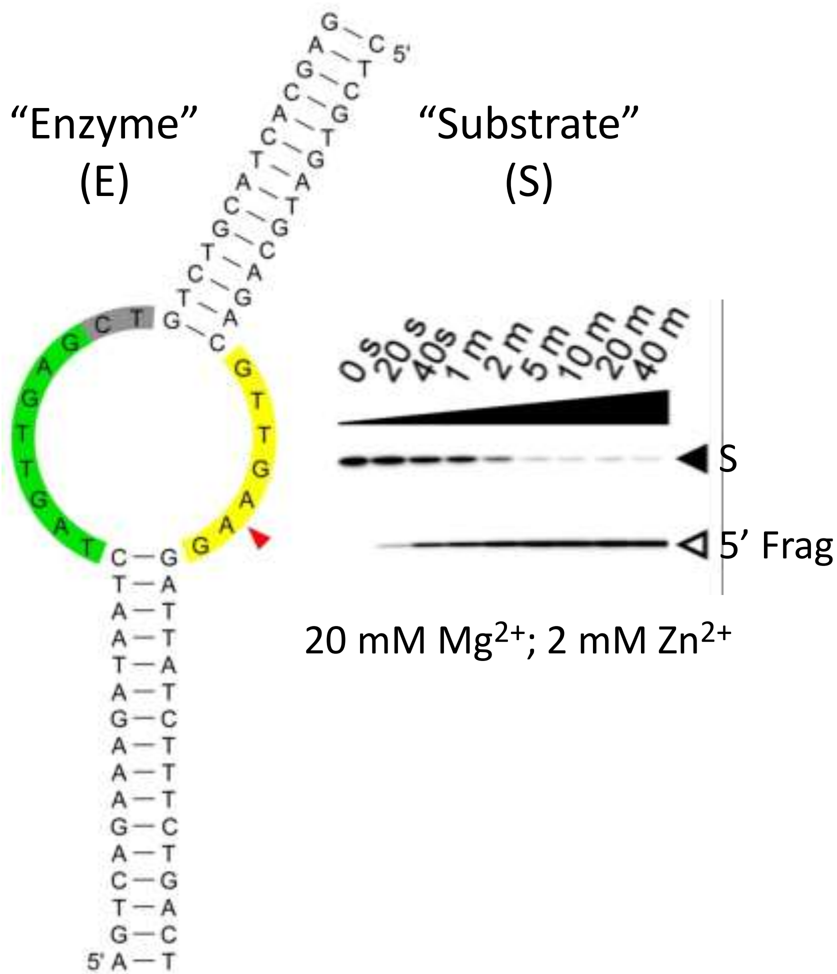
Cleaving DNA with DNA

High-speed Designer "DNase" Oligonucleotides

Small synthetic deoxyribozymes can be used to selectively cleave DNA target sequences

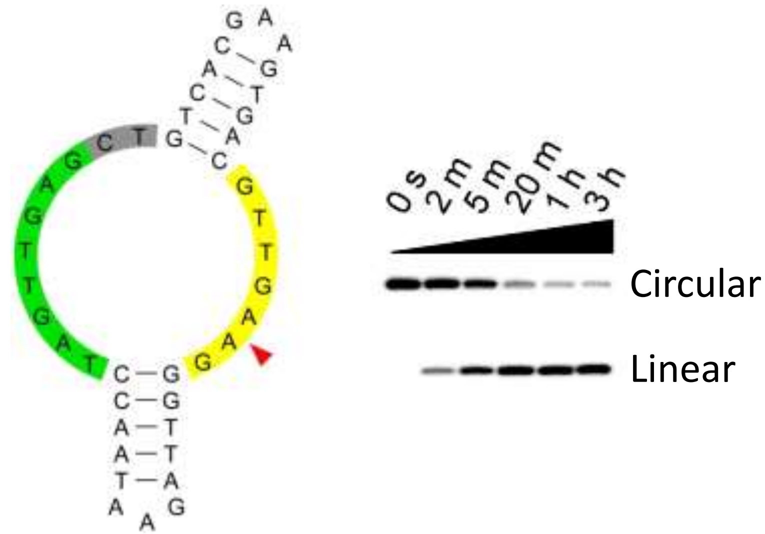
"Enzyme"
(E)

"Substrate"
(S)



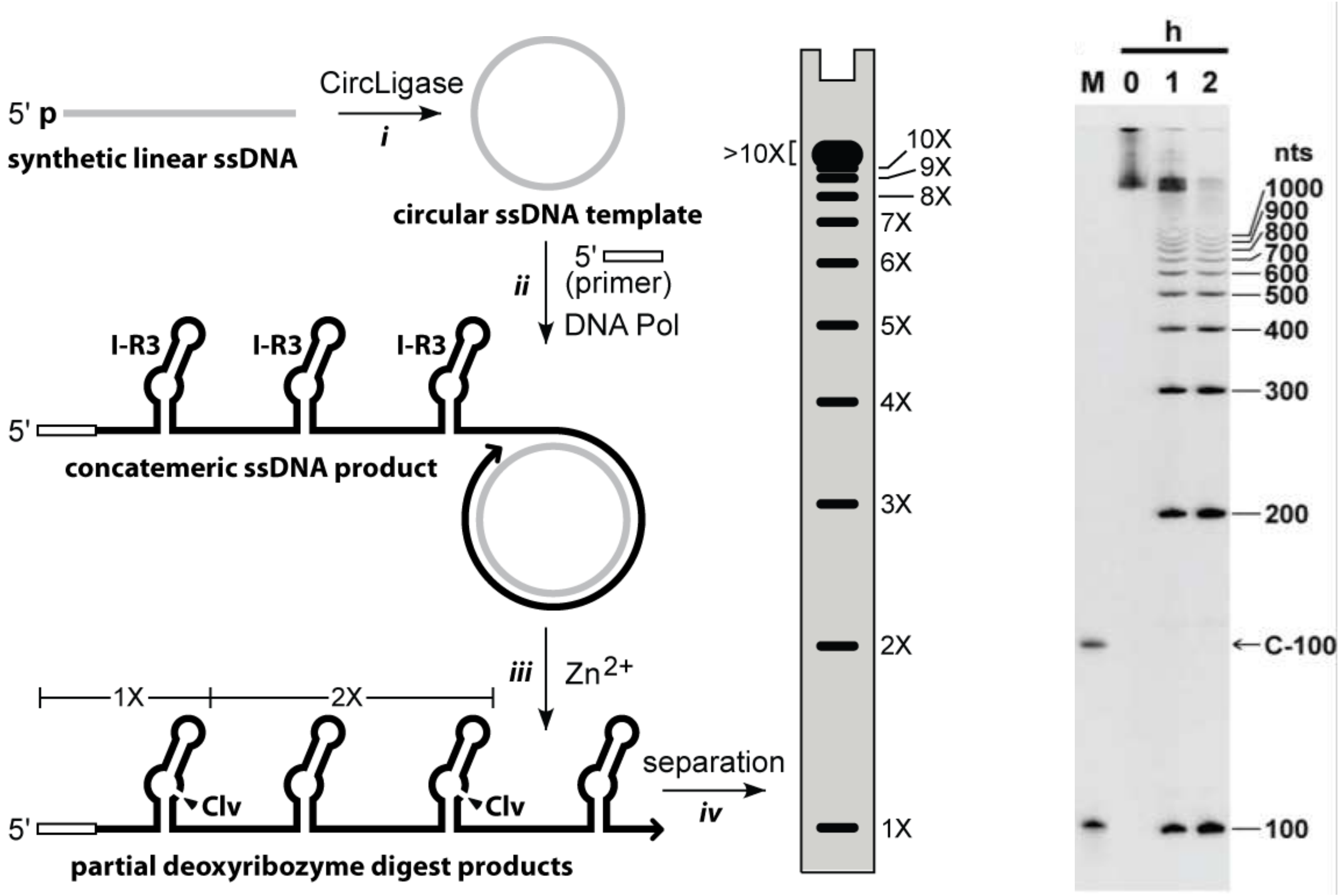
20 mM Mg^{2+}; 2 mM Zn^{2+}

43mer DNA Circle



Gu, H., et al., 2013. J. Am. Chem. Soc. 135:9121-9129.

Self-processing by a Simple ssDNA DNA “Genome”



Conclusions

- The chemical properties and the evolutionary history of RNA strongly support the view that an RNA World once existed.
- Biological components from the RNA World are not entirely extinct, and bioinformatics will continue to reveal ancient and new noncoding RNAs.
- Many new-found RNAs will be riboswitches, but more exceptional large and complex noncoding RNAs also will be found.
- Establishing functions of some RNAs will pose substantial challenges.
- Natural ribozymes and riboswitches can collaborate to create more sophisticated RNA devices, and some of these showcase the functions that may have been important in an RNA World.
- Establishing new RNA functions will reveal much more about RNA biochemistry and microbial life.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