

RIBOZYMES STRUCTURE TYPES AND FUNCTIONS

RIBOZYMES

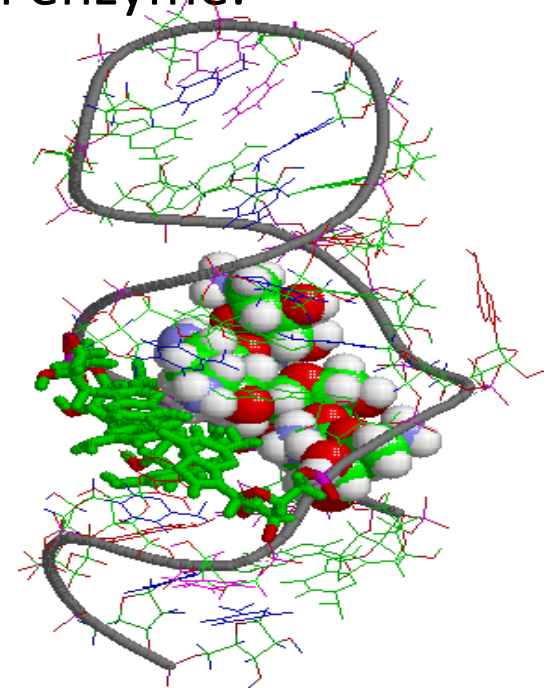
- Ribozymes are RNA molecules or catalytic enzymes that catalyze biochemical reactions.
- 1982: Ribozymes were first discovered by Thomas Cech and Sidney Altman.
- 1982: The term Ribozyme was introduced by Kelly Krugar et al.
- 1989: T. Cech and S. Altman shared the Nobel Prize in Chemistry for the discovery of RNA that could act as an enzyme.



S. Altman

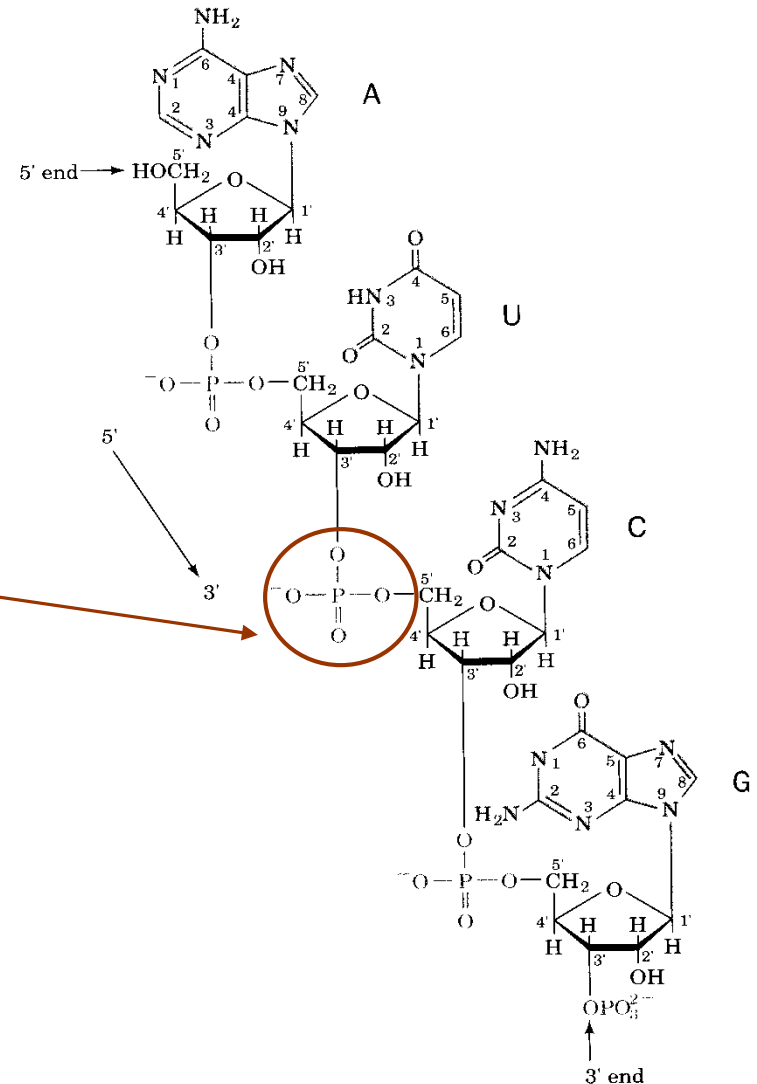


T. Cech



RIBOZYME:

- RNA possessing catalytic activity
- Increases the rate and specificity of:
 - phosphodiester bond cleavage
 - peptide bond synthesis
- Widespread occurrence in nature – from viruses to humans



CLASSES OF RIBOZYMES

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graph TD; A[CLASSES OF RIBOZYMES] --> B[LARGE RIBOZYMES]; A --> C[SMALL RIBOZYMES]; B --> D["➤ 100 – 3000 nucleotides  
➤ E.g self-splicing group I and group II introns and RNase P"]; C --> E["➤ 30 – 150 nucleotides  
➤ E.g hammerhead, hairpin, hepatitis delta ribozymes and varkud satellite RNA."];
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LARGE RIBOZYMES

- 100 – 3000 nucleotides
- E.g self-splicing group I and group II introns and RNase P

SMALL RIBOZYMES

- 30 – 150 nucleotides
- E.g hammerhead, hairpin, hepatitis delta ribozymes and varkud satellite RNA.

NATURALLY OCCURRING RIBOZYMES

- Self splicing introns: group I and group II
- Rnase P
- Hammerhead ribozyme
- rRNA peptidyl transferase
- Hairpin ribozyme
- Riboswitches
- Hepatitis delta virus
- Varkud satellite ribozyme
- Mammalian CPEB3 ribozyme

ARTIFICIAL RIBOZYMES

- Artificial ribozymes are synthesized in the laboratory based on the dual nature of RNAs as catalyst and an informational polymer.
- Synthesis of artificial ribozymes involves the mutation of natural ribozymes. The ribozymes are mutated by reverse transcribing them with reverse transcriptase into various cDNAs and amplified with mutagenic PCR.
- E.g. of artificial ribozymes include Leadzyme, ligase ribozyme and allosteric ribozyme.

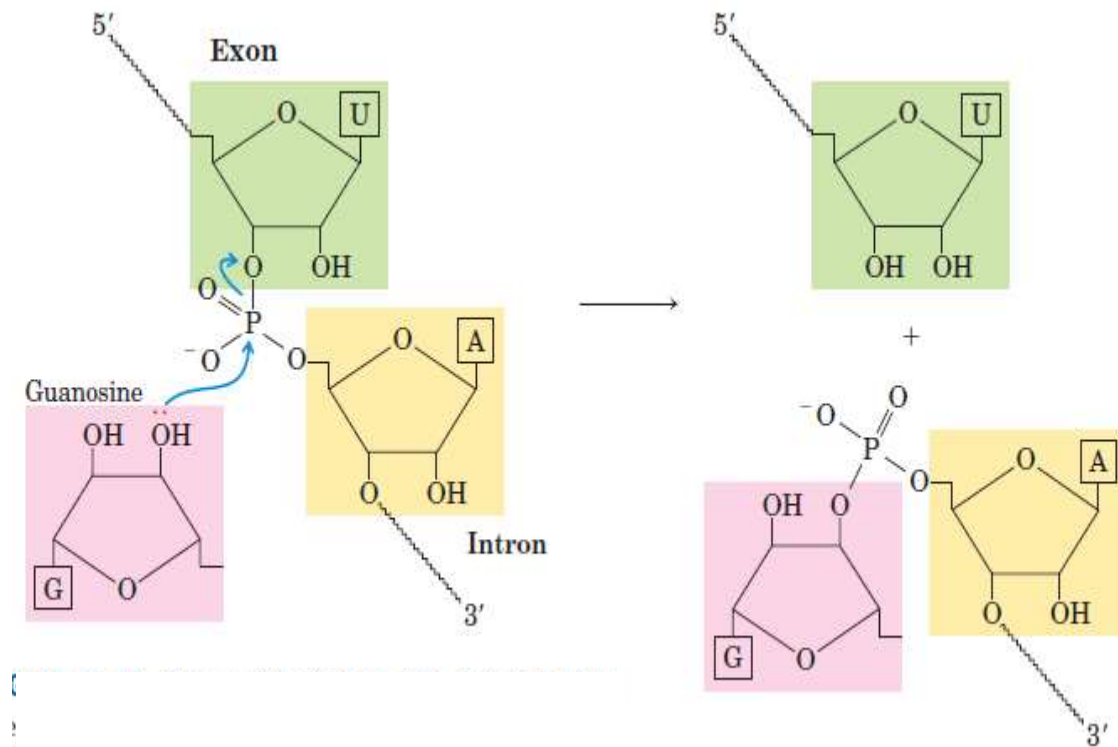
1. Self splicing introns: group I and group II

- **Group I introns** are abundant in fungal and plant mitochondria, but they are also found in nuclear rRNA genes, chloroplast DNA (ctDNA), bacteriophage, and in the tRNA of ctDNA and eubacteria. But, they are not found in higher eukaryotes such as in vertebrates.
- **Group II introns** found in bacteria and in organellar genes of eukaryotic cells mainly in mitochondrial and chloroplast RNAs of plants, fungi and yeast.

1. Self-splicing introns

This is a transesterification reaction in which the guanosine hydroxyl group attacks the phosphodiester bond between the 3' end of the first exon and the first nucleotide of the intron.

The guanosine remains attached to the 5' end of the intron. Then, the 3' end of the liberated exon attacks the extremity of base 413 from the intron to bring together the exon's two ends



GROUP I intron splicing

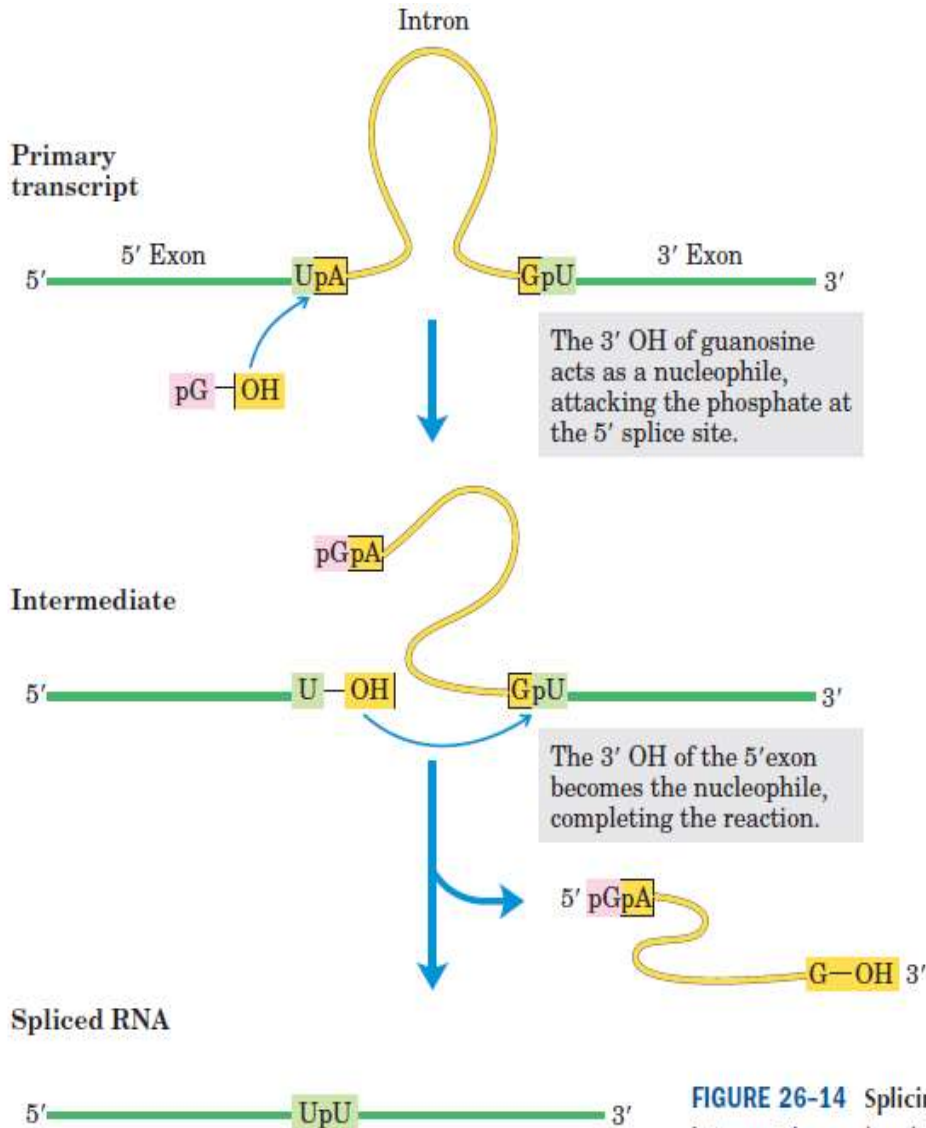


FIGURE 26-14 Splicing of introns. The nucleophile

GROUP II intron splicing

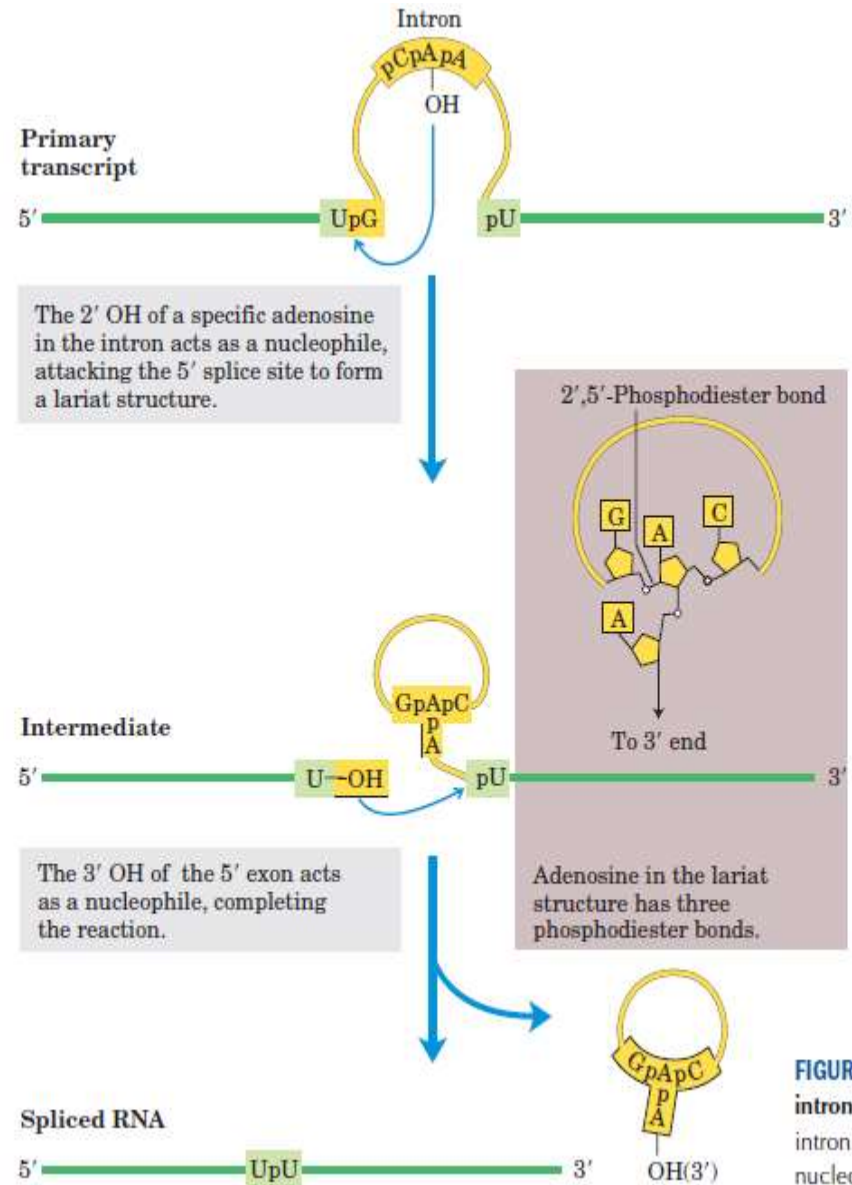


FIGURE
introns.
intron splicing
nucleop

2. RNase P

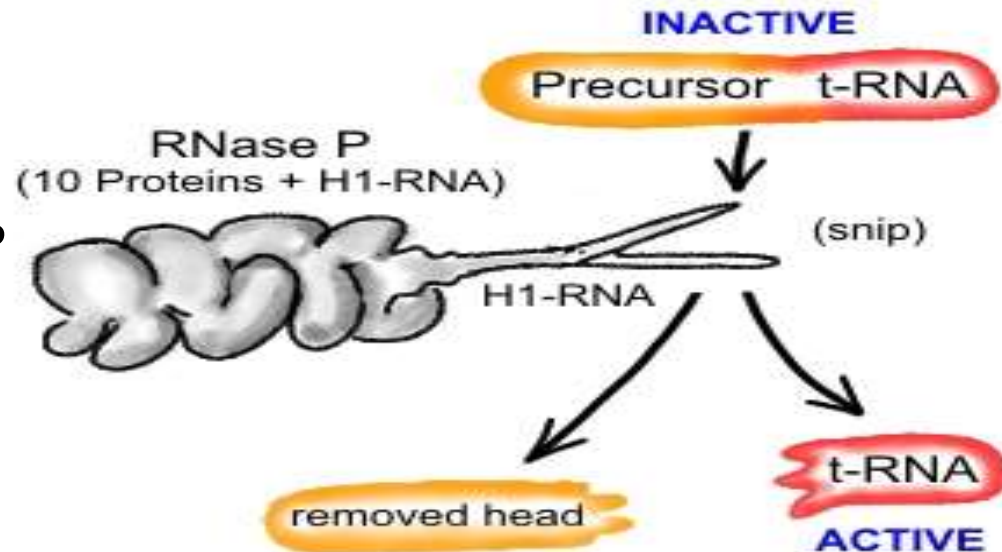
Ribonuclease P- cleaves a piece of RNA off a tRNA molecule

RNase P is able to selectively cut more than 60 tRNA precursors, which then become mature tRNA molecules capable of carrying amino acids during the translation of proteins.

RNase P catalyzes specific phosphodiester bond hydrolysis in pre-tRNAs to produce mature tRNA 5'-ends. Without RNase P this process would not be possible.

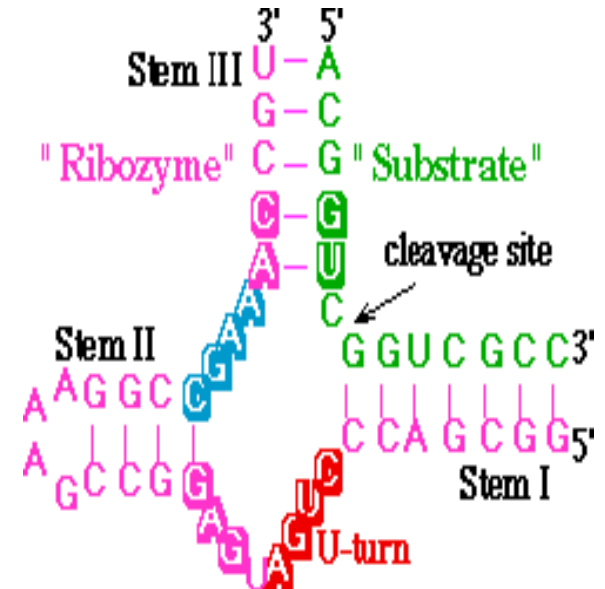
The enzyme is a ribonucleoprotein, although the RNA segment of the molecule has been shown to independently recognize and cleave the appropriate substrate both in vivo and in vitro.

The protein segment of the RNase P appears to allow the ribozymal segment to work at a faster hydrolytic rate and with less Mg^{2+} present.



3. Hammerhead ribozyme

- **Hammerhead RNAs** are RNAs that self-cleave via a small conserved secondary structural motif termed a hammerhead because of its shape.
- Most hammerhead RNAs are subsets of two classes of plant pathogenic RNAs: the satellite RNAs of RNA viruses and the viroids.
- Rolling circle replication initially produces a long strand of multiple copies of the virusoid RNA or plant RNA. Each copy contains a hammerhead motif that catalyzes strand breakage between itself and the next copy in transcript. Thus by virtue of HHRZ motifs, the long strand breaks itself into many individual molecules.
- Two properties of hammerhead ribozyme make them especially useful in gene therapy:
 - i. cleavage site specificity
 - ii. Catalytic activity

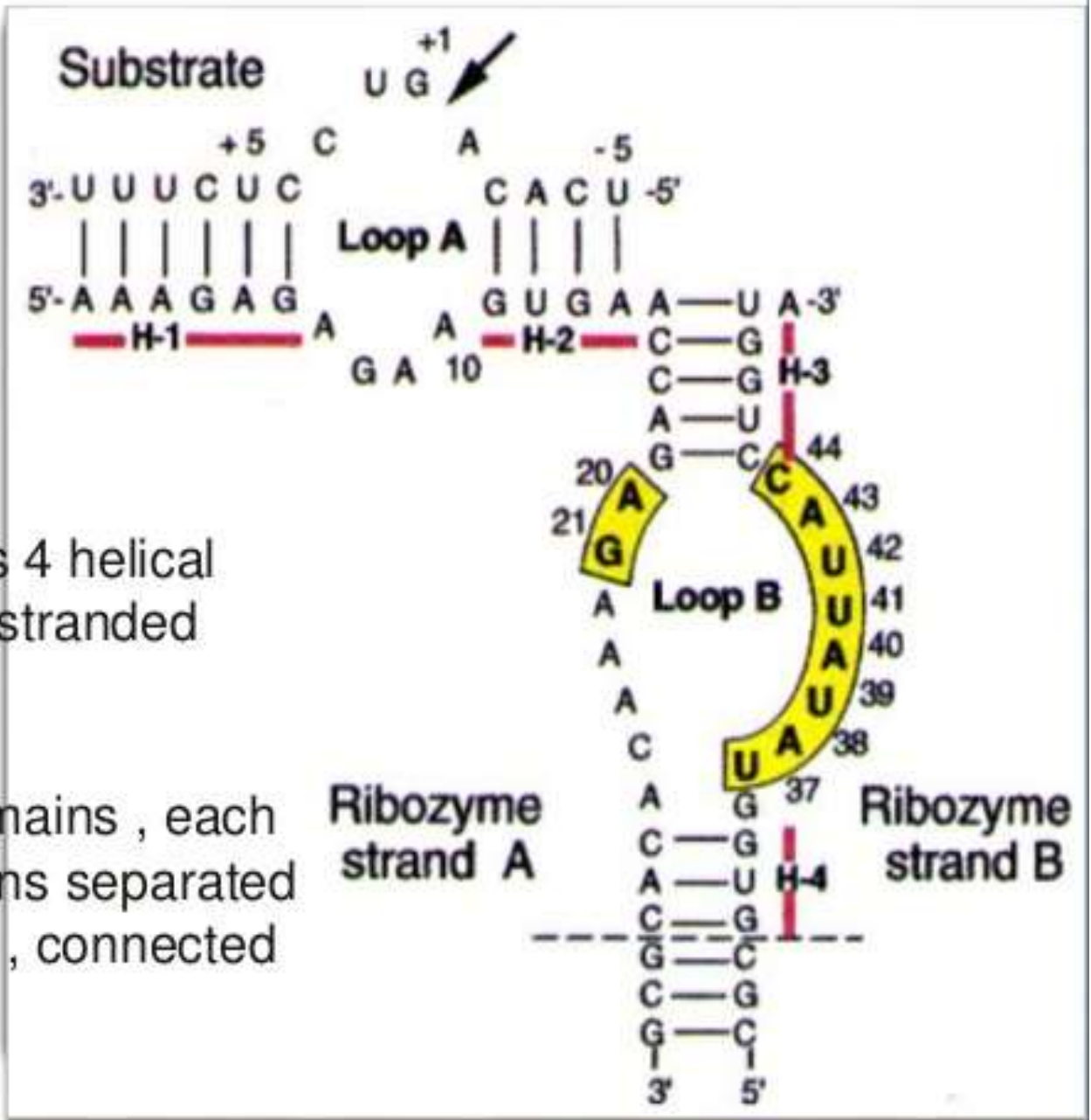


4. Hairpin ribozyme

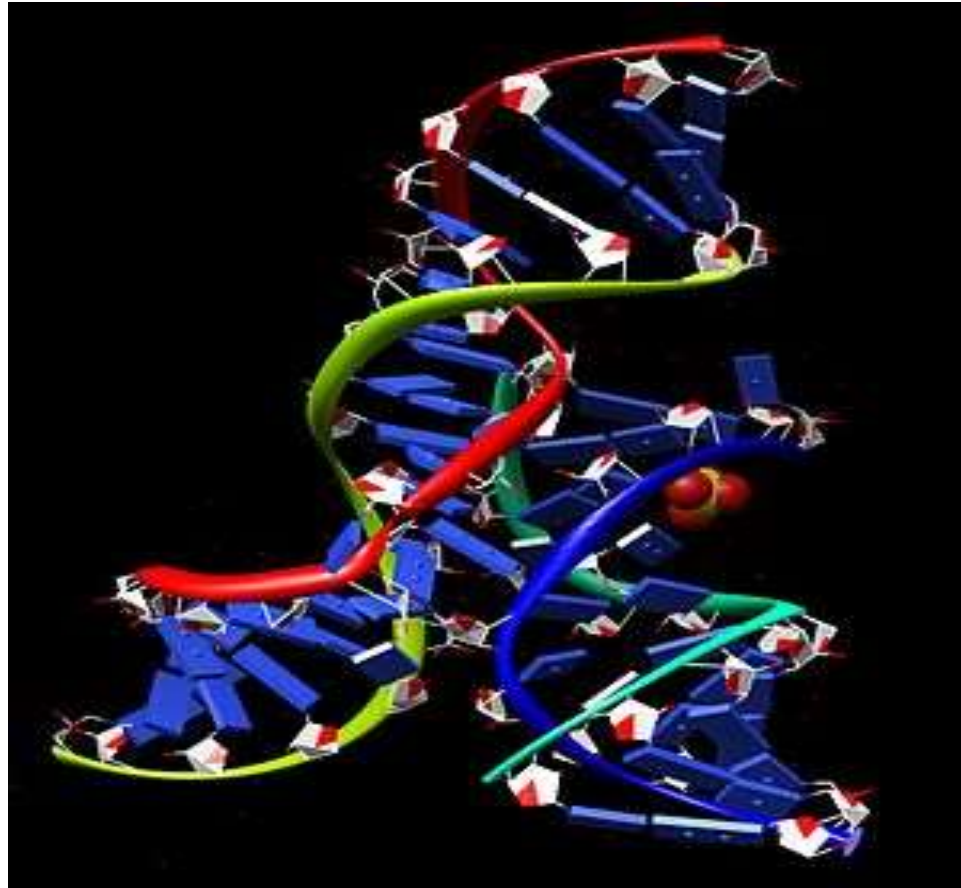
- Hairpin ribozyme is found in RNA satellites of plant viruses.
- It was first identified in the minus strand of the tobacco ring spot virus (TRSV) satellite RNA where it catalyzes self-cleavage and joining (ligation) reactions to process the products of rolling circle virus replication into linear concatameric and circular satellite RNA molecules, giving rise to a 2'-3'-cyclo phosphate and a free 5'-OH terminus.
- Catalytic activity of the hairpin ribozyme results from distortion and precise orientation of the substrate RNA and general acid-base catalysis by neighboring nucleotides without involvement of metal ions in catalysis.

STRUCTURE

- Hair pin contains 4 helical regions & 2 single stranded loops
- Consists of 2 domains, each with 2 helical regions separated by an internal loop, connected hinge region



- Hairpin ribozymes have been developed mainly for antiviral applications(Anti HIV Ribozyme)..
- When CD4+ lymphocytes from HIV-1 infected donors were transduced with an anti-HIV ribozyme vector and subsequently expanded, viral replication was delayed by 2-3weeks.



RIBOSWITCHES

- Riboswitches are cis-acting structural RNA molecules that directly control gene expression.
- Defined as mRNA elements that bind metabolites or ions as ligands and regulate mRNA expression by forming alternative structures in response to that ligand binding.
- Most occur in bacteria but functional riboswitches of one type (the TPP riboswitch) discovered in certain plants and fungi.
- The function of riboswitches is tied to the ability of RNA to form a diversity of structures.
- It is composed of two domains: the aptamer domain and the expression platform.
- The aptamer domain acts as a receptor that specially binds a ligand.
- A riboswitch can adopt different secondary structures to effect gene regulation depending on whether ligand is bound.

- Variety of ligands are sensed by riboswitches which includes magnesium ions, nucleic acid precursors, enzyme cofactors, and amino acid residues
- Riboswitches are most often located in the 5' untranslated region (5' UTR; a stretch of RNA that precedes the translation start site) of bacterial mRNA. where they regulate the occlusion of signals for transcription attenuation or translation initiation .
- However, not all riboswitches are at the 5' UTR; scientists have discovered that, in some eukaryotic mRNA, the thiamine pyrophosphate (TPP) riboswitch regulates splicing at the 3' end.

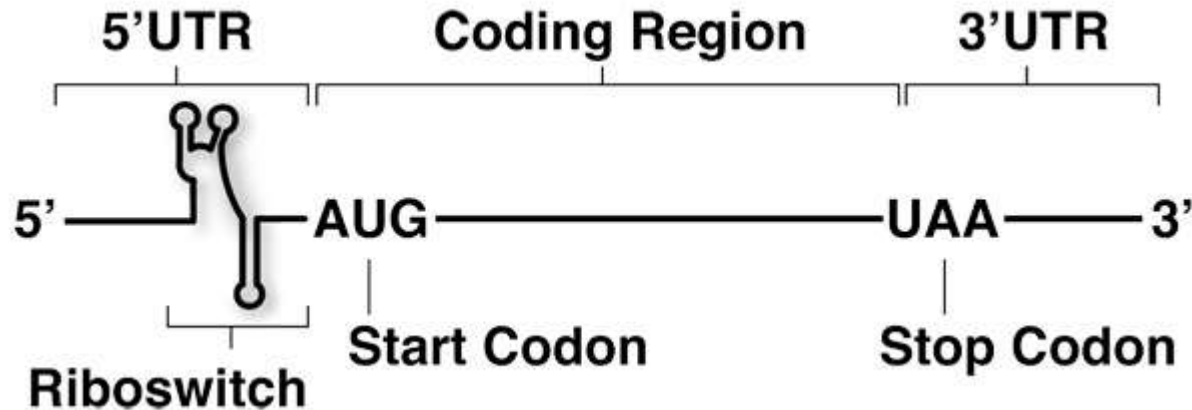


Fig.: mRNA anatomy: A typical bacterial mRNA transcript controlled by a riboregulatory element such as a riboswitch is composed of three sections: the 5' untranslated region (5' UTR), the protein-coding region beginning with the start codon (AUG) and ending with a stop codon (UAA), and the 3' untranslated region (3' UTR).

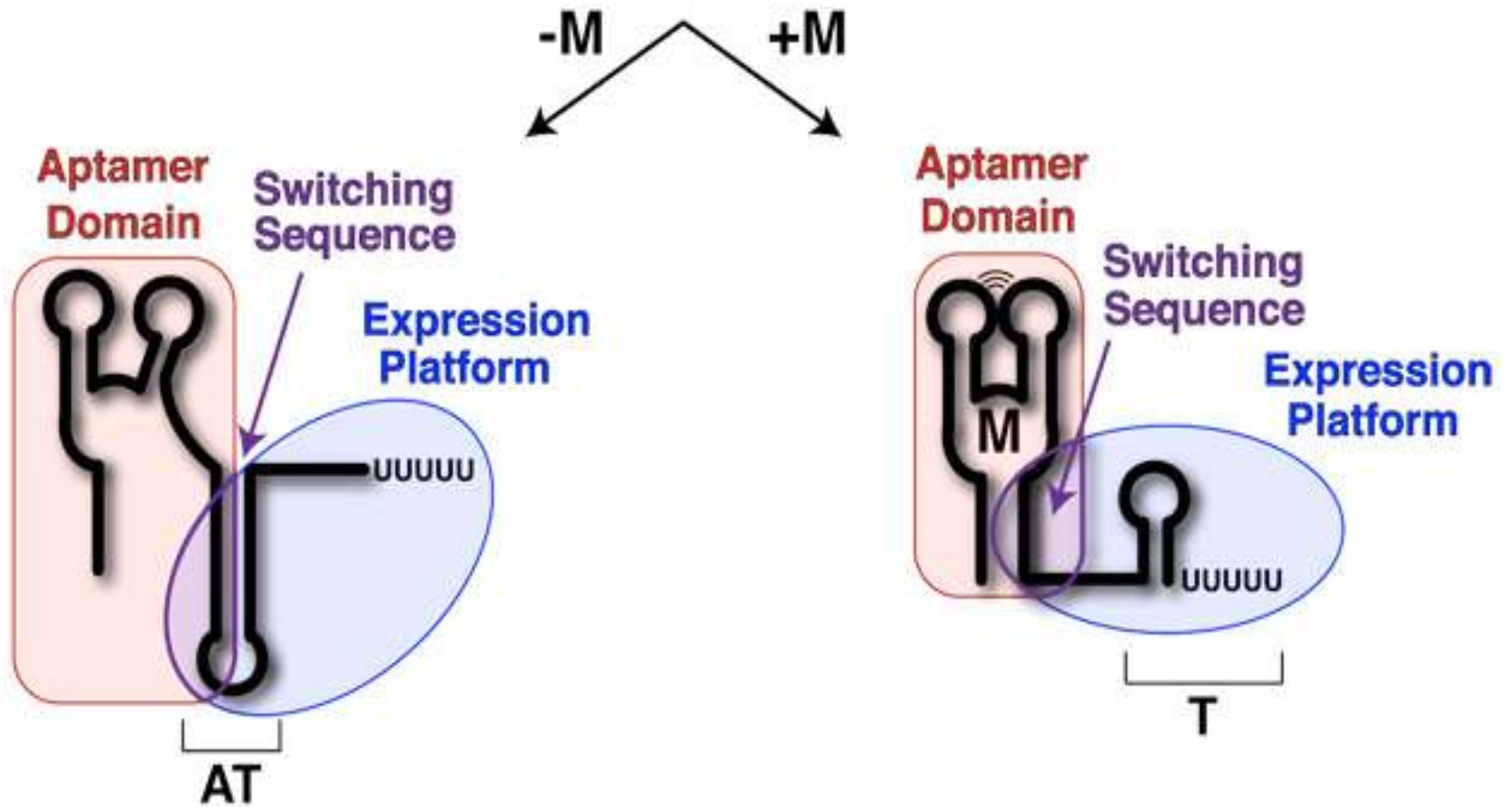


Fig. : Riboswitch domains

This schematic is an example of a riboswitch that controls transcription.

HEPATITIS DELTA VIRUS

- It is single-stranded circular RNA virus of approximately 1700 nucleotides.
- It is a [non-coding RNA](#) functions by [acid-base catalysis](#).
- It is an infectious agent that exists as a satellite RNA of the hepatitis B virus .
- Found in a satellite virus of hepatitis B virus, a major human pathogen.
- It is involved in a self cleavage process needed for processing the RNA transcripts and it is needed for viral replication.
- HDV ribozyme is the fastest known natural self cleaving RNA.
- The catalytic activity of HDV is more efficient in the presence of divalent cations.

- HDV ribozyme is composed of five helical segments connected by a double pseudoknot .
- Pseudoknot is a kind of tertiary interaction in RNA which can join distant parts of the same RNA strand.
- Mutagenesis and chemical studies shows that it consists of four helical regions, in many which the conserved nucleotides are located in helix 2 and its hairpin.
- Its structural motif neither resembles the hammered nor the hairpin ribozymes.
- The hepatitis delta ribozyme displays high resistance to denaturing agents like urea or formamide.
- Substrate recognition requires the formation of P1 stem.
- P1 ribozyme is a circular ribozyme and a hybrid of both genomic and anti-genomic HDV ribozymes.

- There is strong evidence that the catalytic mechanism of the HDV ribozyme involves the action of a cytosine base within the catalytic centre as a general acid-base catalyst.

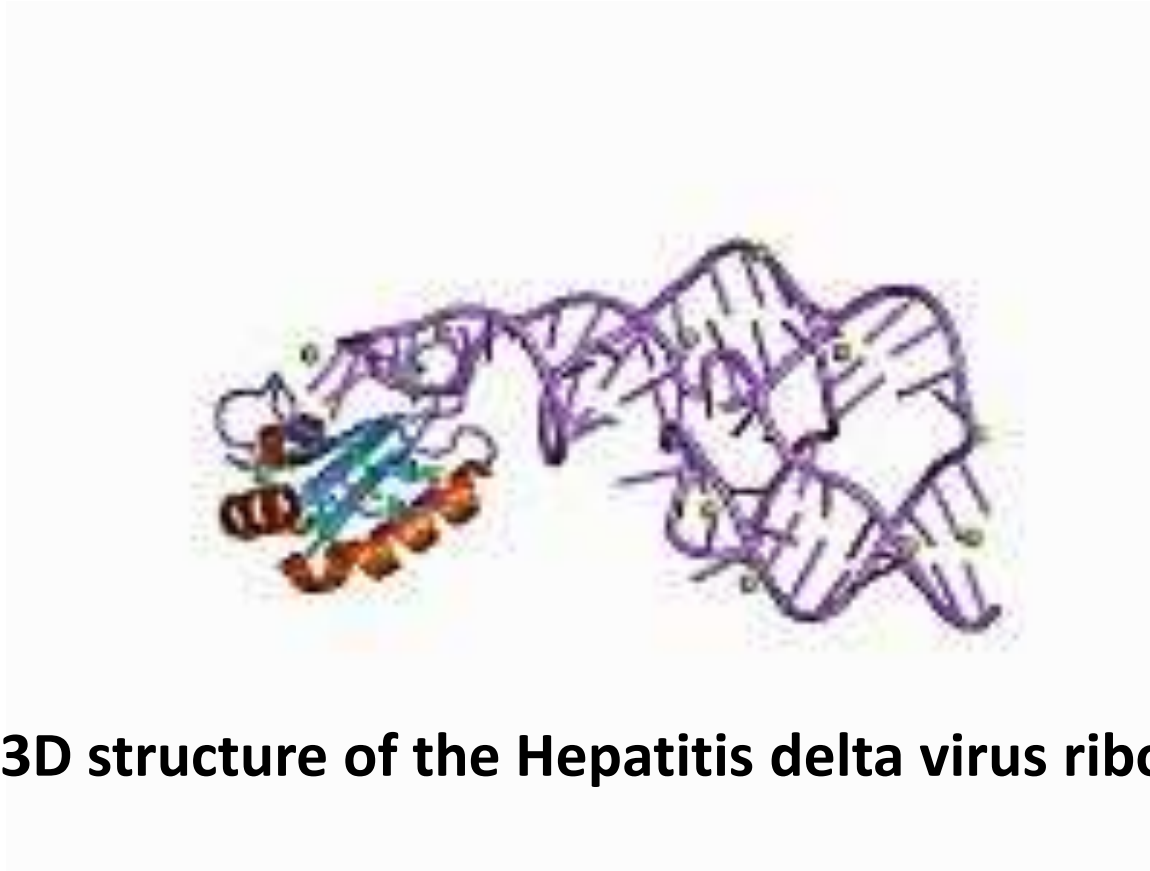


Fig.: 3D structure of the Hepatitis delta virus ribozyme.[\[1\]](#)

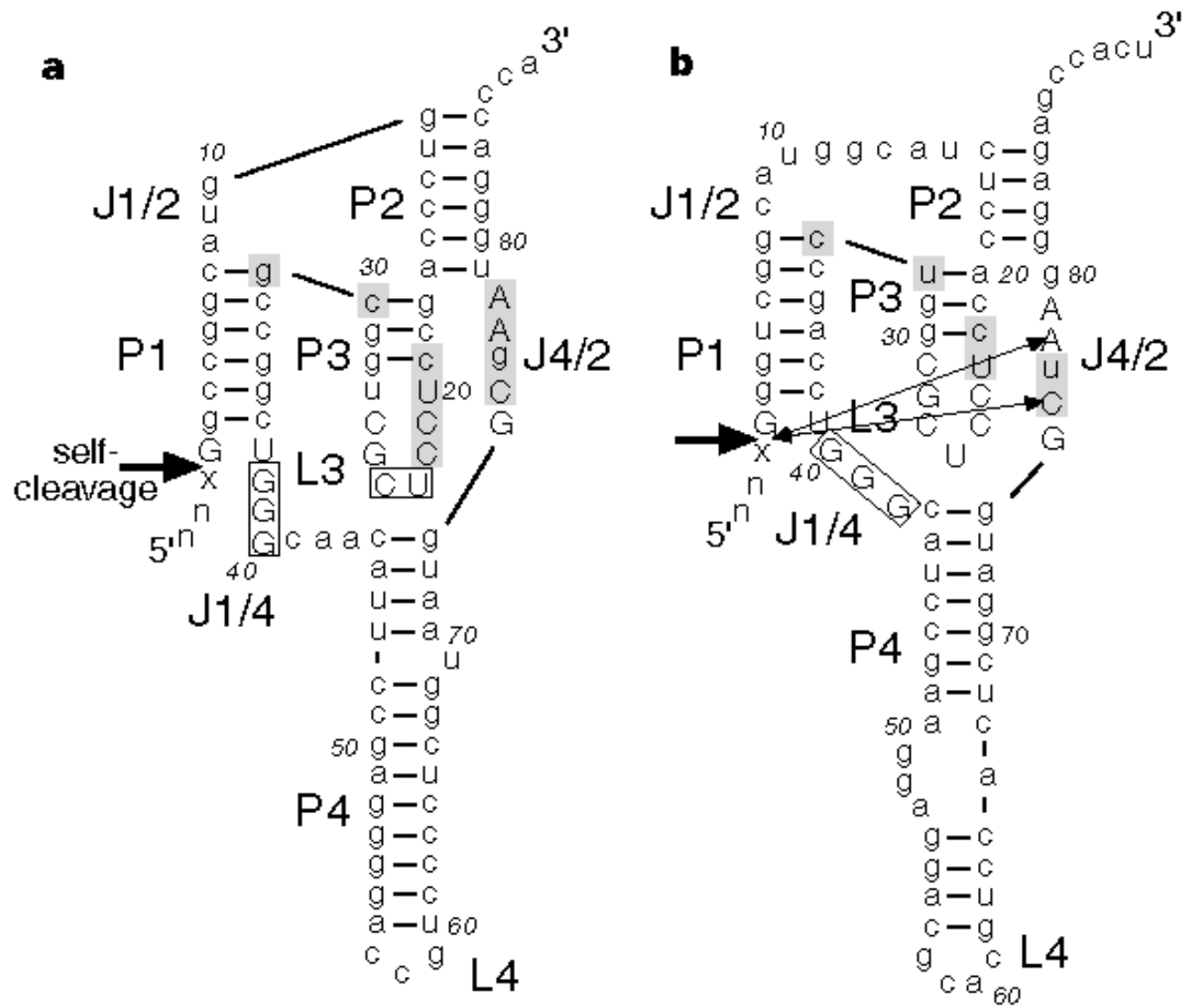


Fig.: Pseudoknotted secondary-structure models of **a**, genomic and **b**, antigenomic HDV ribozymes proposed by Perrotta and Been

VARKUD SATELLITE RIBOZYME

- VS ribozyme is an RNA enzyme that carries out the cleavage of a phosphodiester bond.
- It is important catalyst that is a 154 nucleotides long catalytic entity that is transcribed from a plasmid discovered in the mitochondria of certain strains of neurospora.
- VS RNA is a [long non-coding RNA](#) exists as a satellite RNA and is found in mitochondria of Varkud-1C and few other strains of [Neurospora](#).
- VS ribozyme contains features of both catalytic RNAs and group 1 [introns](#).
- VS ribosome has both cleavage and ligation activity and can perform both cleavage and ligation reactions efficiently in the absence of proteins.
- Binding of substrate is determined by tertiary interactions.

- VS RNA has a unique primary, secondary, and tertiary structure.
- The secondary structure of the VS ribozyme consists of six helical domains.
- Stem loop I forms the substrate domain while stem-loop II-VI forms the catalytic domain. When these 2 domains are synthesized in vitro separately, they can perform the self-cleavage reaction by [trans-acting](#) .

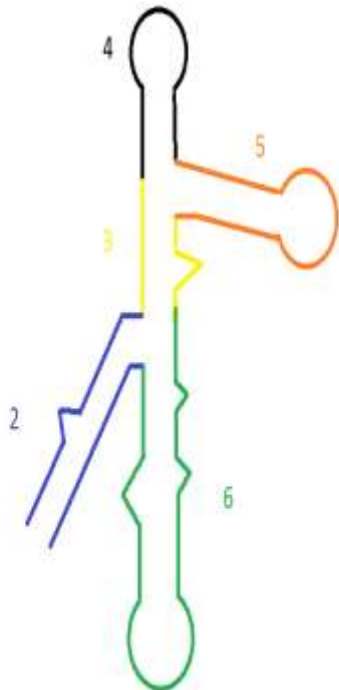


Fig.: Secondary Structure.

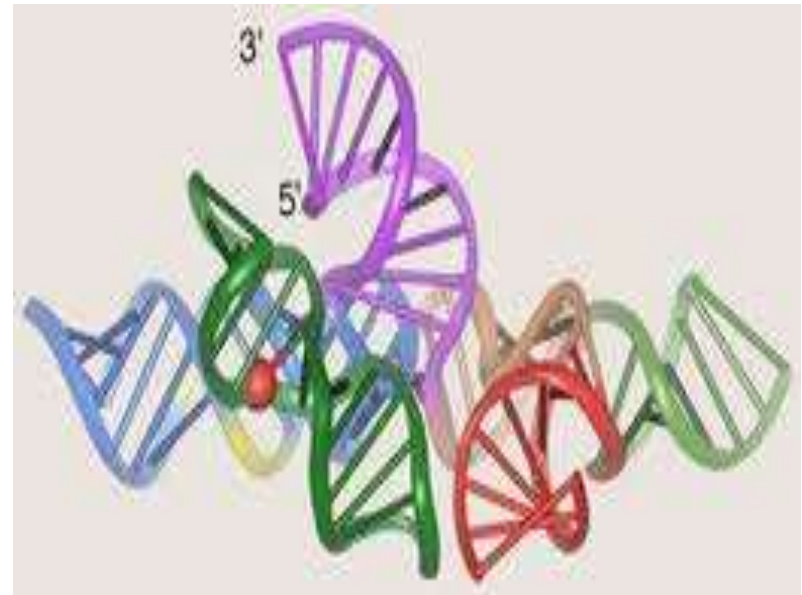


Fig.: 3D structure of VS ribozyme

- VS RNA is transcribed as a multimeric [transcript](#) from VS [DNA](#).
- VS DNA contains a region coding [reverse transcriptase](#) necessary for replication of the VS RNA.
- Once transcribed VS RNA undergoes a site specific cleavage.
- VS RNA self-cleaves at a specific phosphodiester bond to produce a monomeric and few mutimeric transcripts. These transcripts then undergo a self-ligation and form a circular VS RNA.
- The majority of VS RNA is made up of 881 nucleotides.

MAMMALIAN CPEB3 RIBOZYME

- It is a self-cleaving non-coding RNA.
- Located in the second intron of the cpeb3b gene which belongs to a family of genes regulating mRNA polyadenylation.
- It is highly conserved and found only in mammals.
- Exists as ribonucleoprotein complex.
- It is structurally and biochemically related to the human HDV ribozyme.

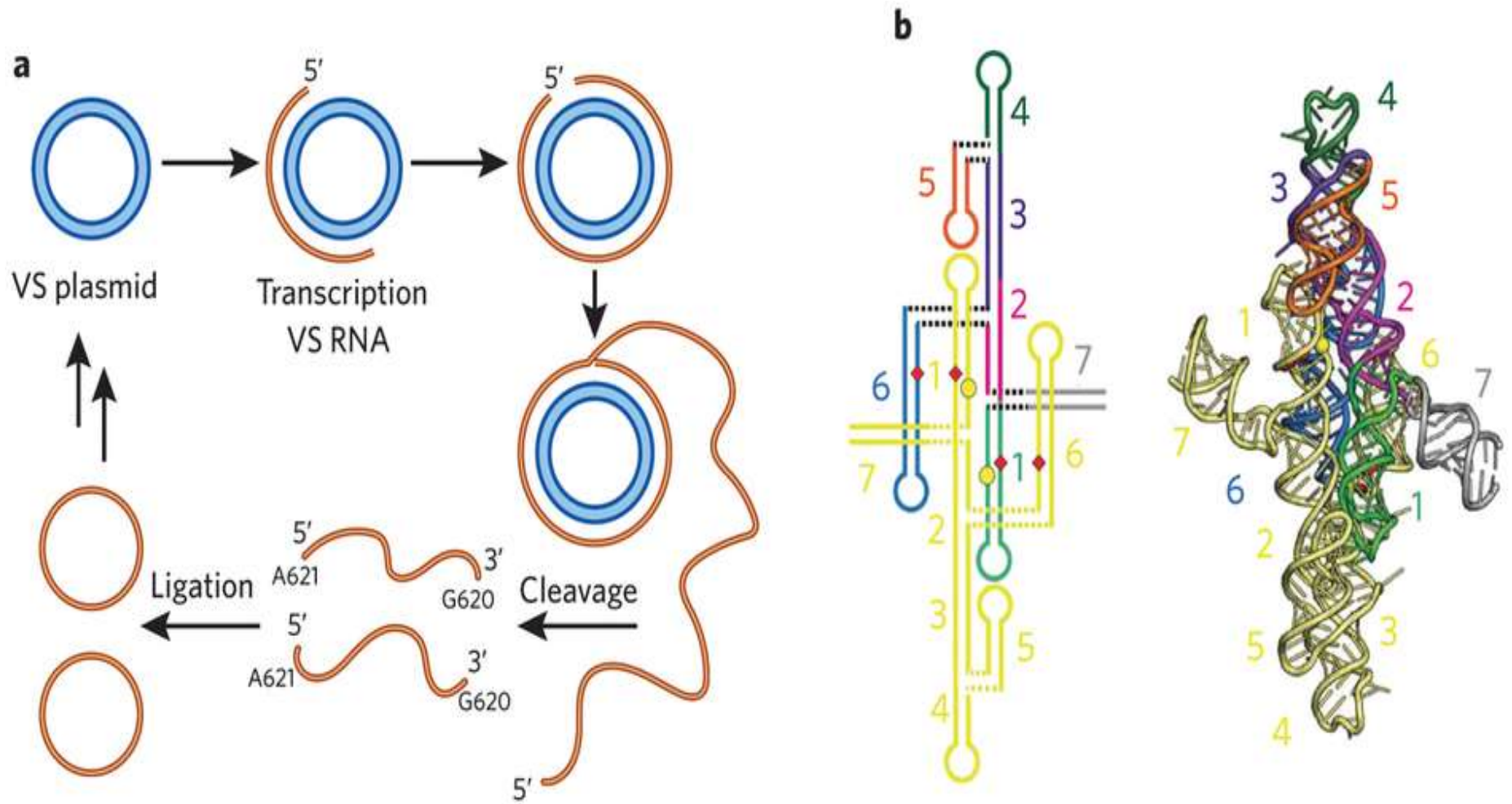


Fig.:(a) Diagram of the rolling circle replication of VS RNA.

(b) The secondary structure diagram and three-dimensional structure of the VS ribozyme.

GLMS RIBOZYME

- Self-cleaving
- Located in the 5' UTR of bacterial mRNAs that code for the production of glmS proteins which catalyzes the synthesis of glucosamine-6-phosphate.
- It is a recently discovered ribozyme that is unique in the world of naturally occurring ribozymes in two respects-
 - First, it is a ribozyme that is also a riboswitch.
 - Second, the regulatory effector of the ribozyme, glucosamine-6-phosphate, is actually a functional group that binds to the ribozyme active site and participates in the acid-base catalysis of RNA self-cleavage.

- The glmS ribozyme is derived from a self cleaving RNA sequence found in the 50 untranslated region of the glmS message; it cleaves itself, inactivating the message, when the cofactor glucosamine-6-phosphate binds.
- It responds to cell wall precursor GlcN6P and regulate the expression of GlcN6P synthetase gene(glmS).
- It controls mRNA stability in bacteria.
- Unlike every other riboswitch identified to date, the GlmS riboswitch does not appear to undergo any structural rearrangement upon effector binding, suggesting that the binding pocket is preformed in the absence of ligand.
- Identified within Actinobacteria, *Deinococcus thermus*, Tenericutes, Chloroflexi, *Thermobaculum ferenum* etc.
- Thus, glucosamine-6-phosphate production is regulated in many gram-positive bacteria via this ribozyme-mediated negative-feedback mechanism.
- The structure of the glmS ribozyme is thus, of particular interest both as a riboswitch and as an unusual catalytic RNA.
- It is also a potential antibiotic target as it is known to occur only in gram positive bacteria.

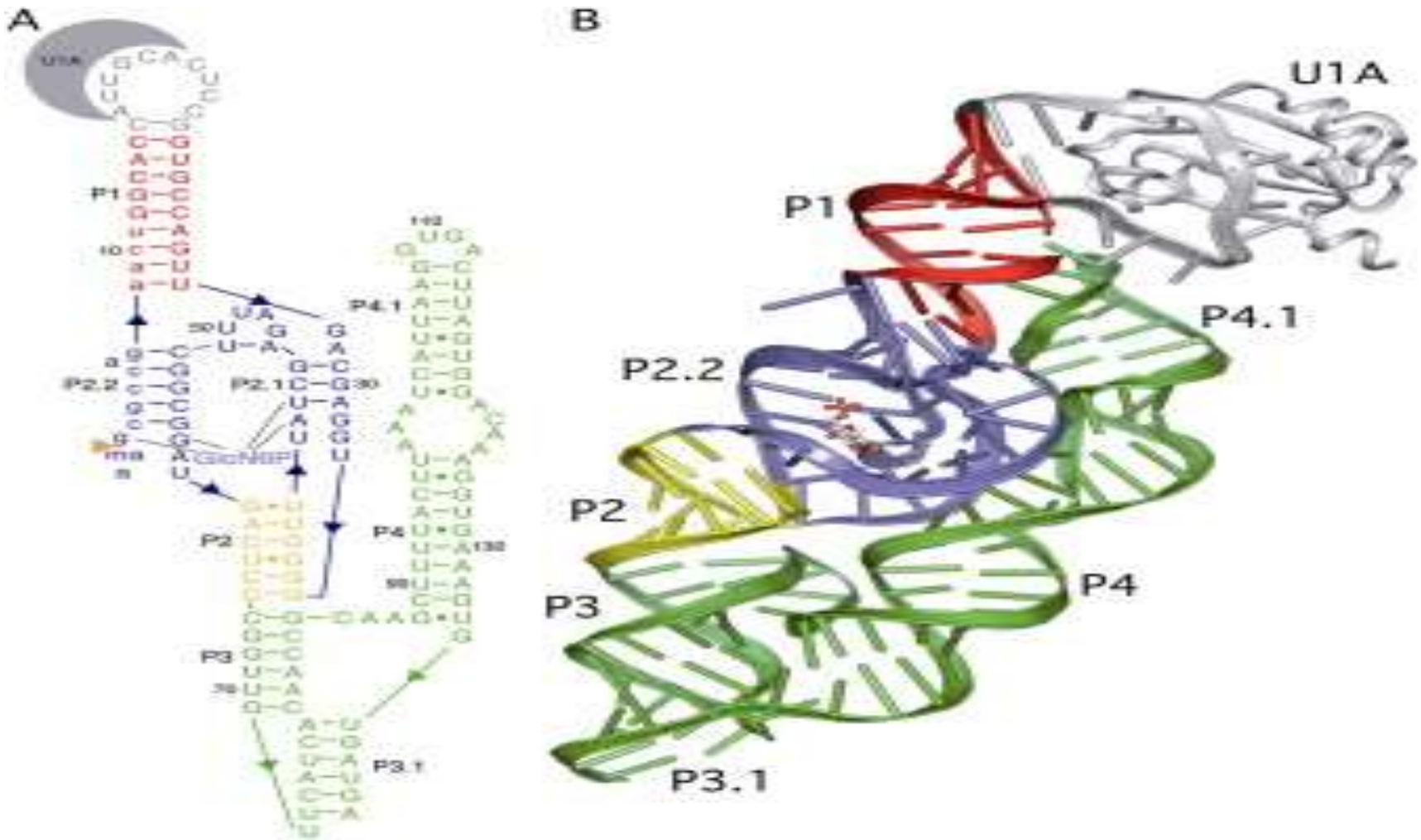


Fig.: Secondary and Tertiary Structures of the GlmS Ribozyme

(A) Secondary structure of the *B. anthracis* GlmS ribozyme.

(B) Tertiary structure depicted in ribbons format.

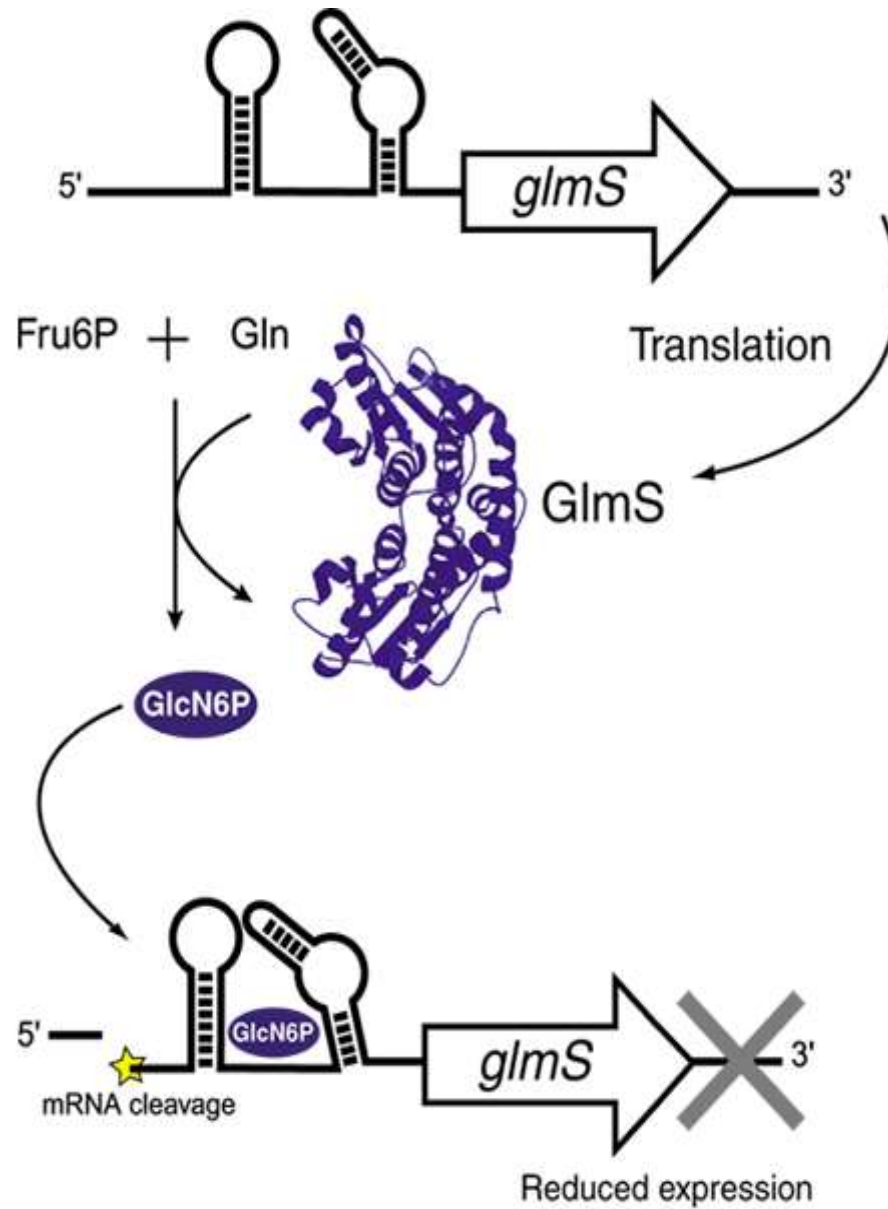


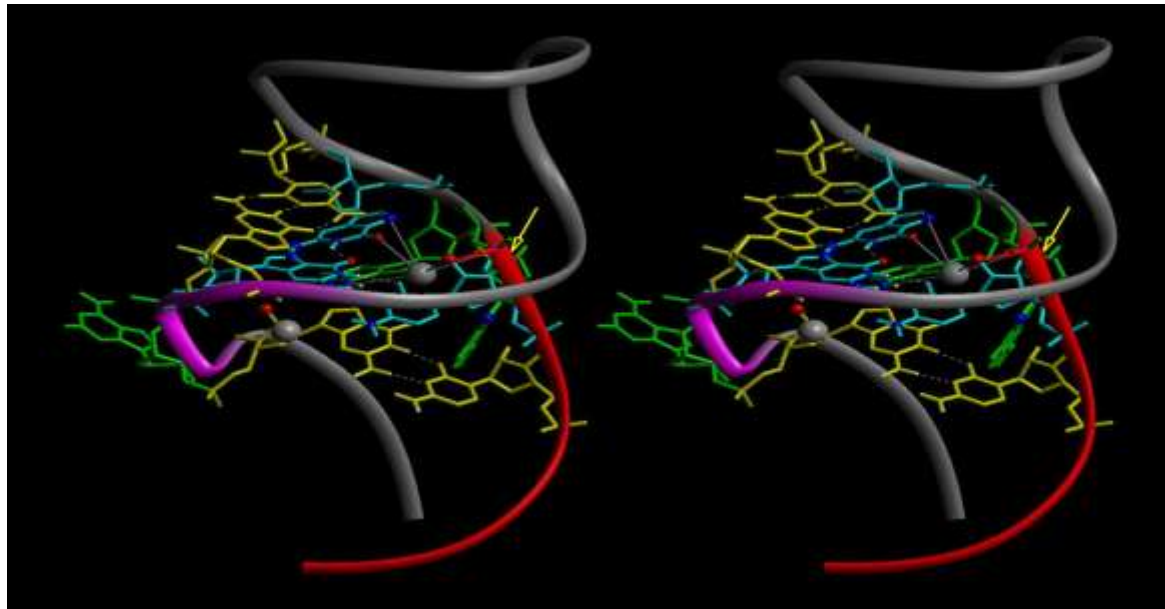
Fig: RNA-based feedback mechanism

ARTIFICIAL RIBOZYMES

- These ribozymes are synthesized in the laboratory based on the dual nature of RNAs as catalyst and an informational polymer.
- Synthesis of artificial ribozyme involves the mutation of natural ribozymes.
- It includes-
 - Leadzymes
 - Ligase ribozyme
 - Allosteric ribozyme

LEADZYMES

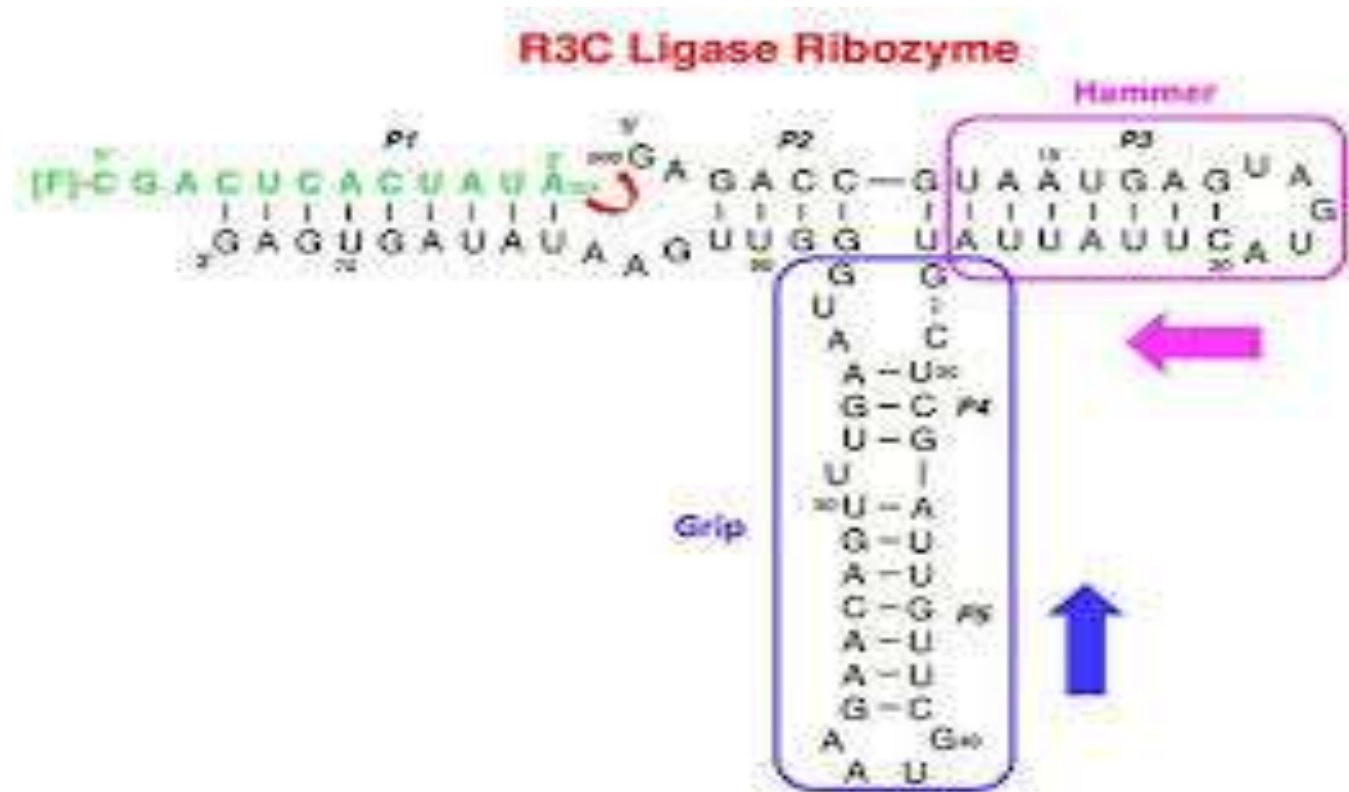
- Small ribozyme.
- Cleave RNA in presence of lead.
- For in vitro selection techniques.
- It has been proposed that leadzyme in 5S RNA may be important mechanism in lead toxicity.
- Structurally determined by x-ray crystallography.



LIGASE RIBOZYME

- Important class of ribozyme because they catalyse the assembly of RNA fragments into RNA polymers by forming phosphodiester bond, a reaction required of all extant [nucleic acid](#) polymerases and thought to be required for any self-replicating molecule.
- Ideas that the [origin of life](#) may have involved the first self-replicating molecules being ribozymes are called [RNA World hypotheses](#).
- Ligase ribozymes may have been part of such a pre-biotic RNA world.

- Michael Robertson and Andrew Ellington evolved a ligase ribozyme that performs the desired 5'-3' RNA assembly reaction, and called this the L1 ligase.



ALLOSTERIC RIBOZYME

- Allosteric ribozymes are a modified type of catalytic RNA whose activity can be regulated by external factors.
- Control of their cleavage activity is achieved by binding an effector molecule to an allosteric binding site .
- Acts as allosteric enzyme.
- Enhances catalytic rate of certain enzymes.

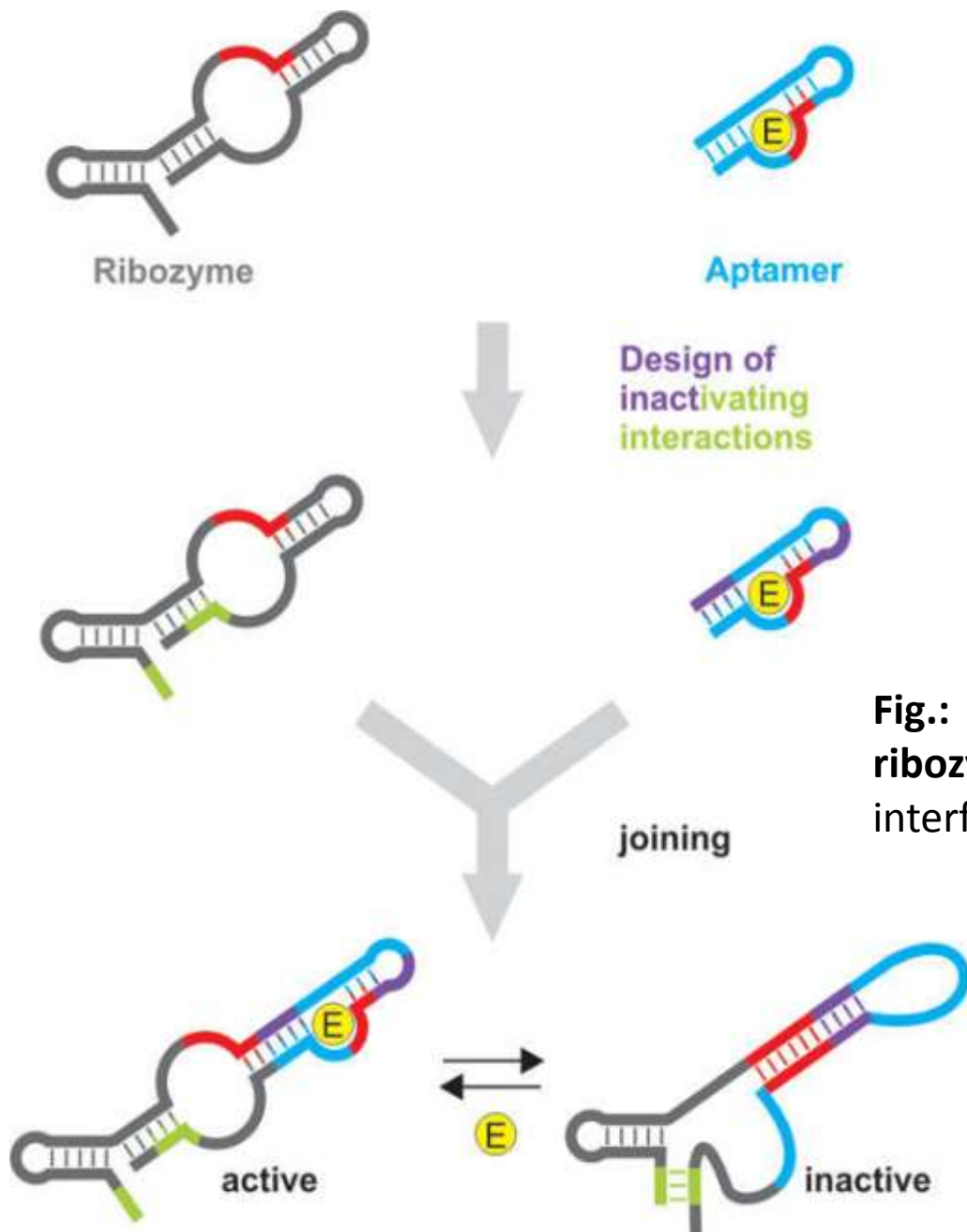


Fig.: Rational design of allosteric ribozymes operating by direct inactivating interference.

Table 1: The world of naturally occurring ribozymes

Catalytic RNA motifs	Catalytic reactions	Organisms
Group I introns	RNA splicing	Tetrahymena
Group II introns	RNA splicing	Plants, fungi, bacteria
Hammerhead	RNA cleavage	Plants pathogen
Hairpin	RNA cleavage	Plants pathogen
M1subunit of RNaseP	RNA cleavage	Eubacteria
Hepatitis delta virus	RNA cleavage	Human pathogen
Varkud satellite	RNA cleavage	Neurospora

NATURALLY OCCURRING RIBOZYMES

Ribozyme	Catalytic function	Biological function
rRNA, ?tRNA	Peptide bond formation	Protein synthesis
Ribonuclease P	Phosphodiester bond hydrolysis	Maturation of transfer RNA
Self-splicing group I and group II introns	Phosphodiester bond hydrolysis and ligation	Remove introns from precursor messenger RNA
Hairpin ribozyme Hammerhead HDV	Phosphodiester bond hydrolysis	RNA virus replication pathway

APPLICATIONS

➤ **Therapeutic applications**

- Treatment of cancer, infectious diseases and genetic disorders.

➤ **Ribozymes as a cofactors**

- RNA can act as a cofactor for amino acid residues.
- Amino acid complexes with RNA molecules during which the later functions as a cofactor, enhancing or diversifying the enzymatic capabilities of proteins.
- mRNAs have evolved from RNA molecules which catalyzes amino acid transfer to them

➤ **Chaperon like ribozymes**

- Like chaperon, RNA catalyzes protein folding. Such RNAs are called chaperon like ribozymes

➤ **Ribozymes as tools to study gene function and in target validation**

- Used to study the function, regulation and expression of genes

- Provide a unique tool for understanding gene function because they allow one to assess cellular responses to a rapid ablation of target gene expression.
- Inactivate specific gene expression, and thereby can be used to help identify the function of a protein or the role of a gene in a functional cascade..
- The use of ribozymes for target validation is critical for both basic biological research and drug discovery and development.
- Compared to other means of target validation such as use of transgenic animals, ribozymes offer specificity and ease of design and usage.

➤ **Riboswitches as antimicrobial agents**

RNA is a primary target of many antimicrobial compounds. They are targeted for novel antibiotics. For example, when the antibiotic pyrithiamine enters the cell, it is metabolized into pyrithiamine pyrophosphate. Pyrithiamine pyrophosphate has been shown to bind and activate the TPP riboswitch, causing the cell to cease the synthesis and import of TPP. Because pyrithiamine pyrophosphate does not substitute for TPP as a coenzyme, the cell dies.

THANKS