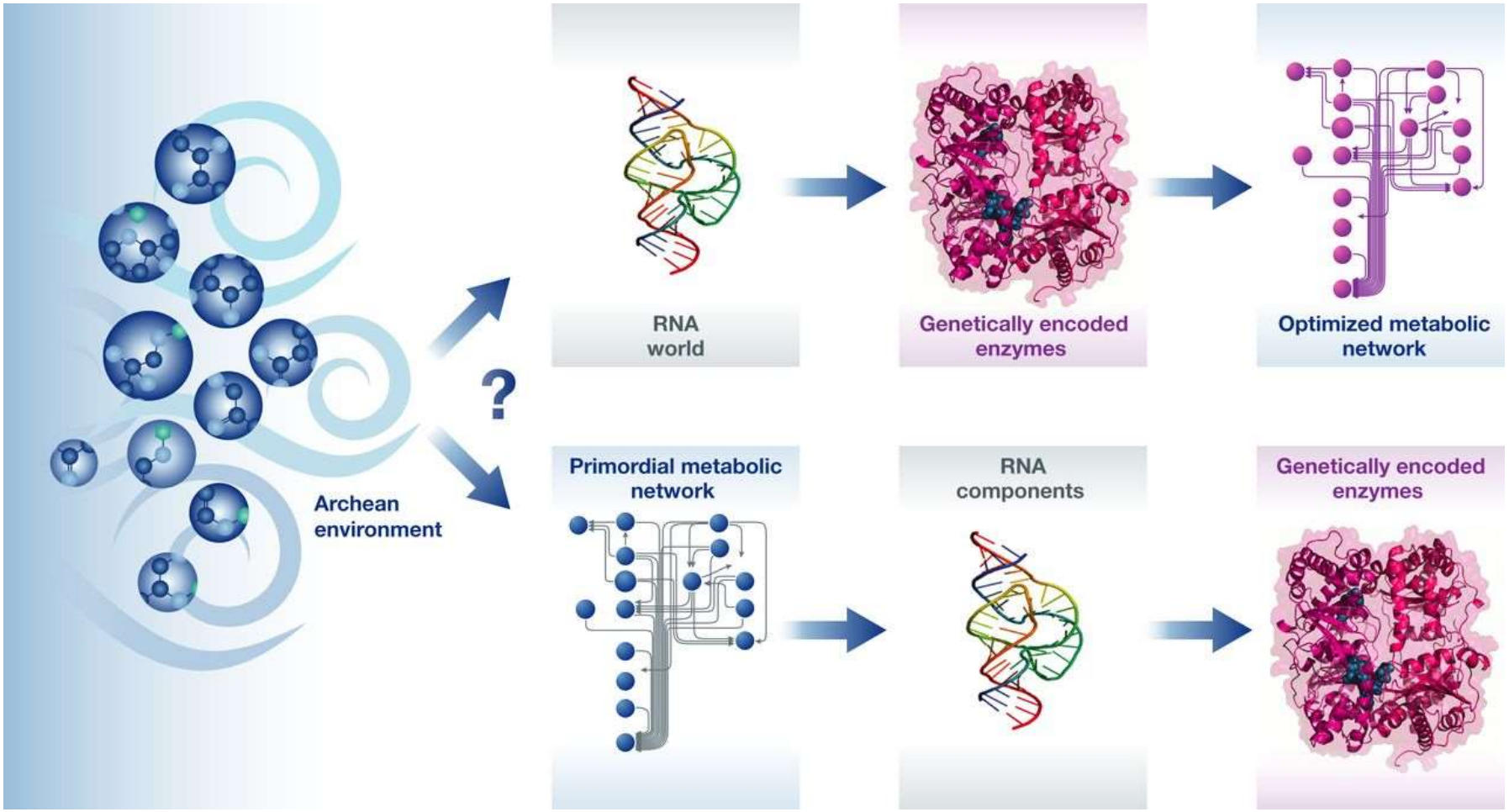
Route to life by chemical networks



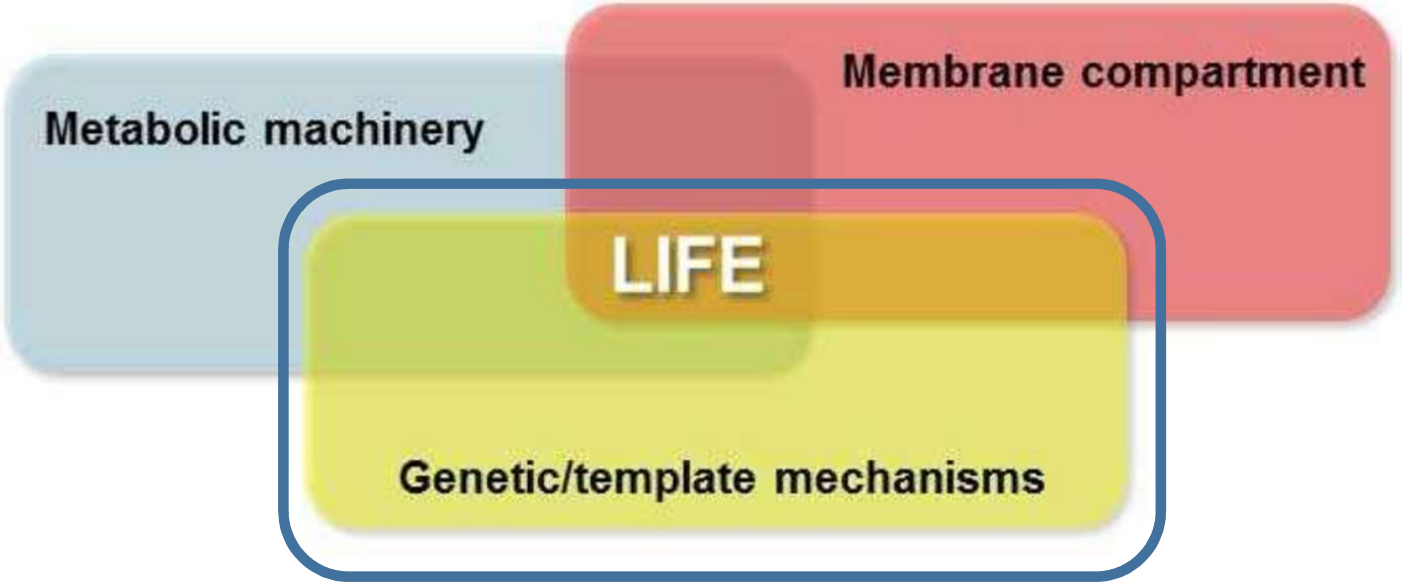
P. L. Luisi *Mol Syst Biol.* **2014,** *10*, 729

Metabolism-first vs. Genes-first

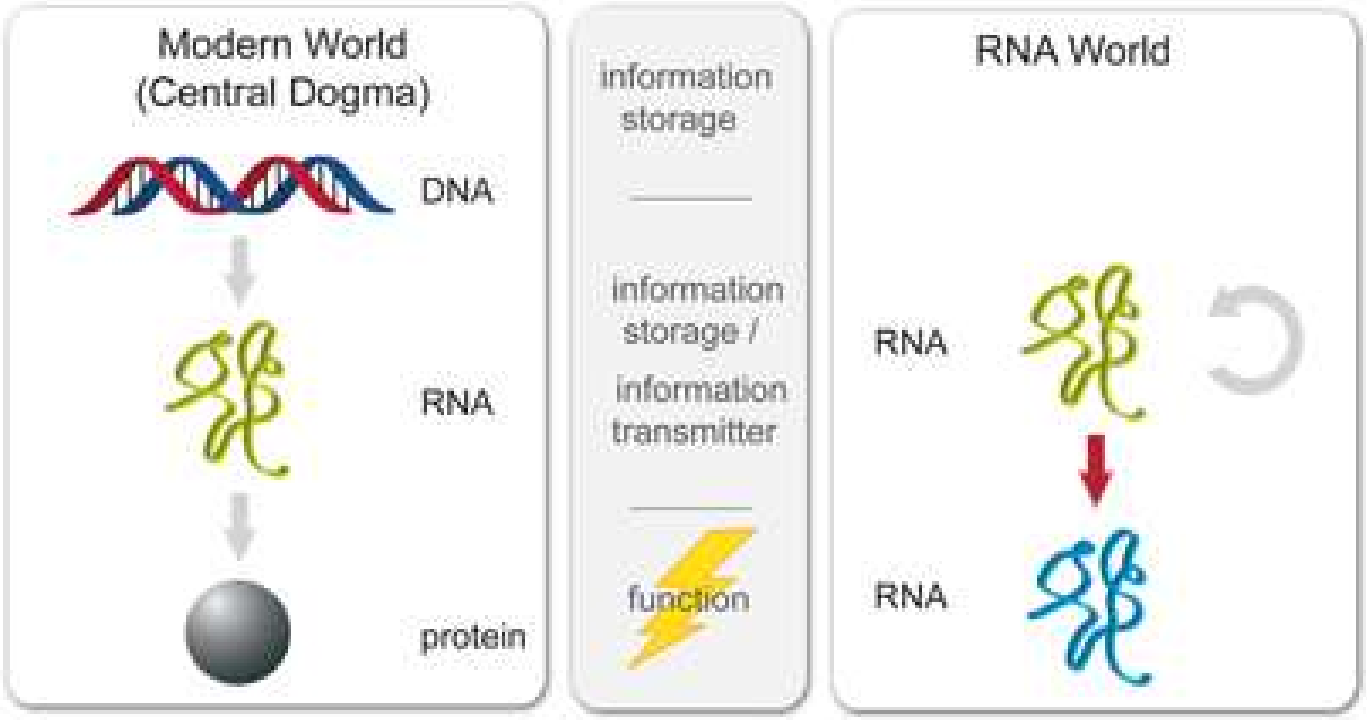
Genetics/replication-first: an information-carrying polymer capable of replication (RNA or something simpler) spontaneously arose from available prebiotic molecules available on early Earth. Metabolism incorporated later as a mean to receive energy from the surroundings in a controlled manner.

Metabolism-first: primitive metabolic cycles spontaneously assembled from simple prebiotic organic molecules or inorganic carbon sources as CO_2. And the cycles produced a set or more or less complex molecules needed for the replication process and construction of the genetic apparatus.

The supposed *proto-metabolism* would differ from the currently known one, because the chemical reactions were not catalysed by efficient enzymes, nor were aminoacid and peptide sequences determined by DNA.
The involved reactions were either spontaneous, or catalysed by inorganic catalysts or peptides.
Inorganic catalysts would be molecules, or ions, in solutions or on surfaces of solids such as clays or pyrites.
Peptides (or peptoids) formed either by random oligomerization or mutual catalysis.

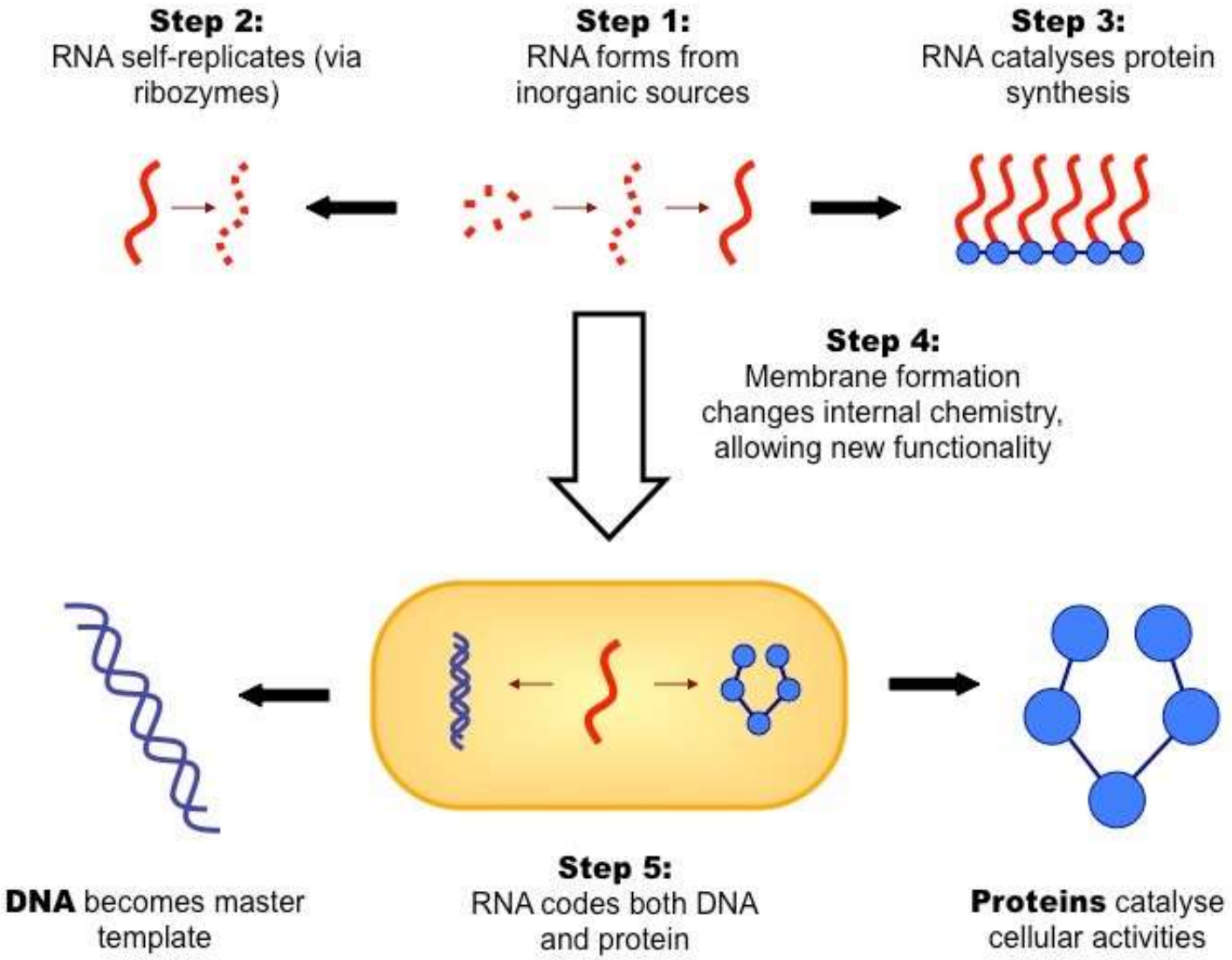

Membrane compartment
Metabolic machinery
LIFE
Genetic/template mechanisms

The RNA world



Conceptual idea that there was a period in the early history of life on Earth when RNA (or its structurally simplified analogue) carried out most of the information processing and metabolic transformations needed for biology to emerge from chemistry

The RNA world

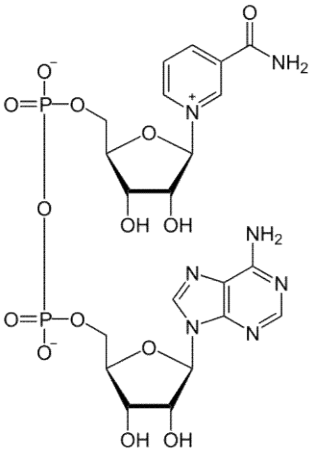


The RNA world

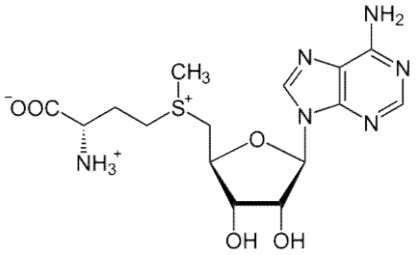
Crick, Orgel and Woese speculated in 1968 that, because RNA can form secondary structures, it has both a genotype and a phenotype and is a good candidate for the emergence of life

F. H. C. Crick *J. Mol. Biol.* **1968**, *38,* 367-379, L. E. Orgel *J. Mol. Biol.* **1968**, *38,* 381-393

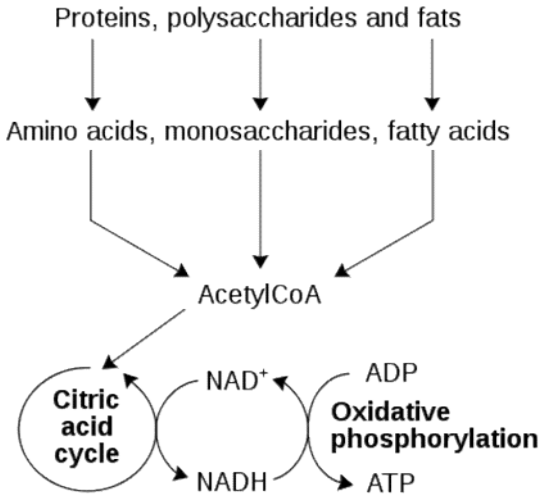
Ribonucleotide coenzymes currently used by many proteins may be molecular „fossils" from the primoridal RNA-based metabolism

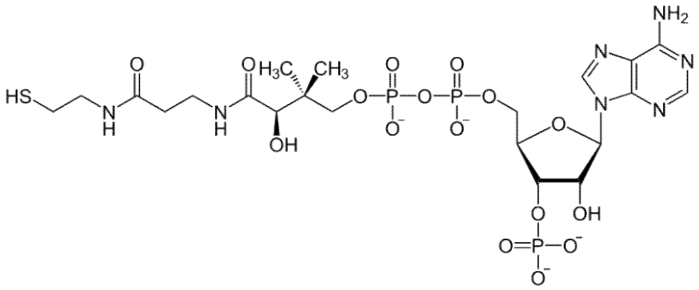


Nicotinamide adenine dinucleotide (NAD$^+$)

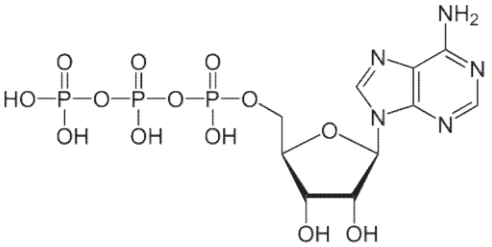


***S*-Adenosyl methionine**





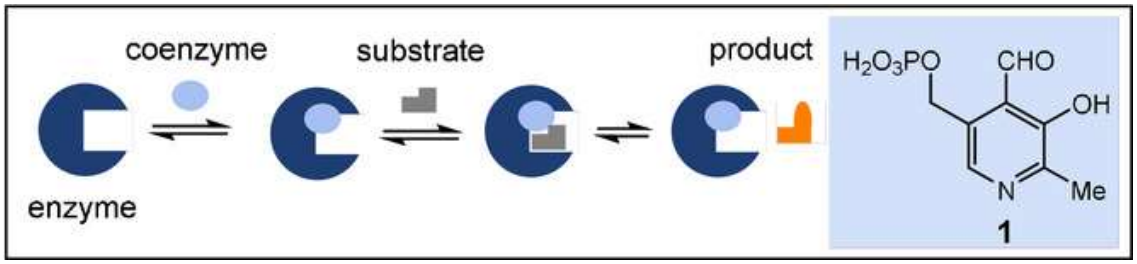
Coenzyme A (CoA, CoASH, or HSCoA)



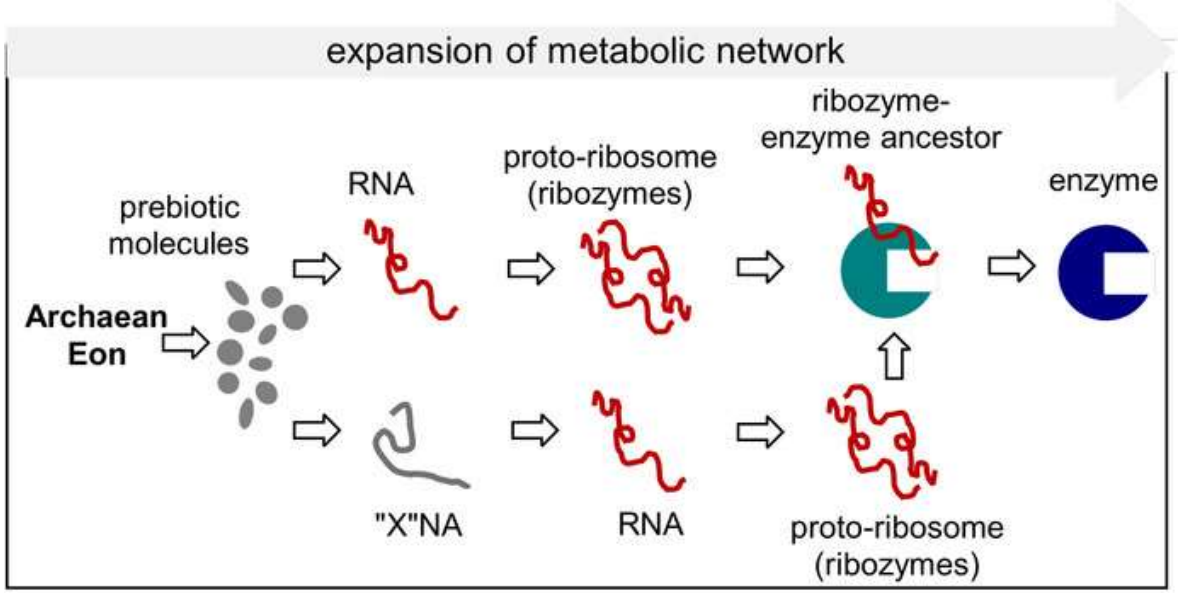
Adenosine triphosphate (ATP)

H. B. White III *J. Mol. Evol.* **1976**, *7,* 101-104

The RNA world



Ribonucleotide coenzymes currently enhance catalytic activity of proteins



Catalytic RNA chains that acquired aminoacids and oligoeptides to enhance their efficiency could be the sources of currently observed RN coenzymes

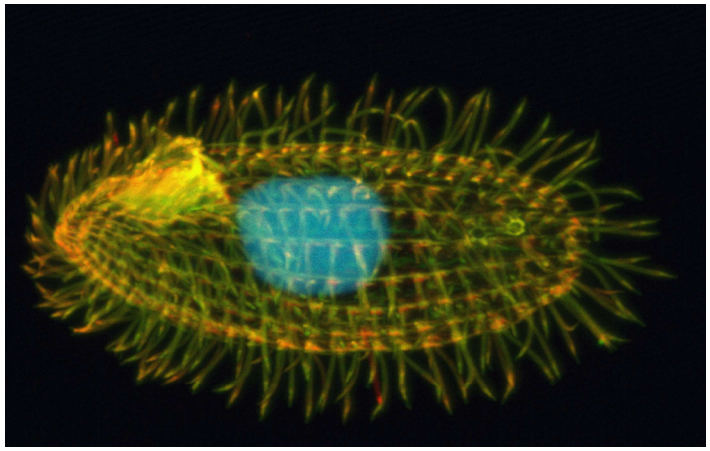
A. Kirschning *Angew. Chem. Intl. Ed.* **2021**, *60,* 6242-6269

The RNA world

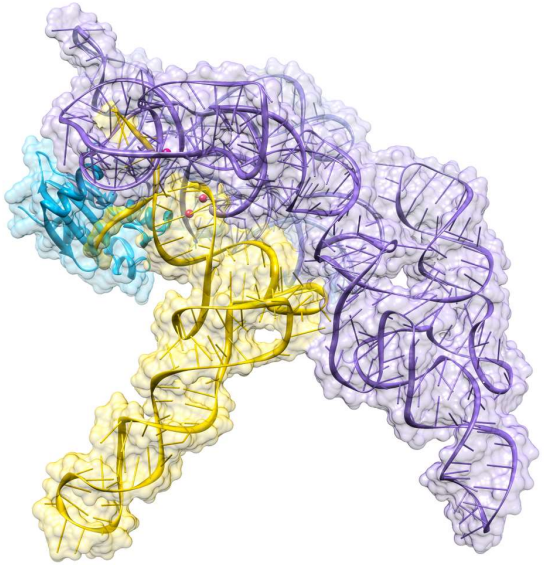
Ribozymes – Ribonucleic acid enzymes

1989 – Thomas Cech and Sidney Altman – Nobel Prize in chemistry for discovery of catalytic RNA

Thomas R. Cech was studying RNA splicing in the ciliated protozoan *Tetrahymena thermophila*
Sidney Altman and Norman Pace were studying the bacterial RNase P complex.

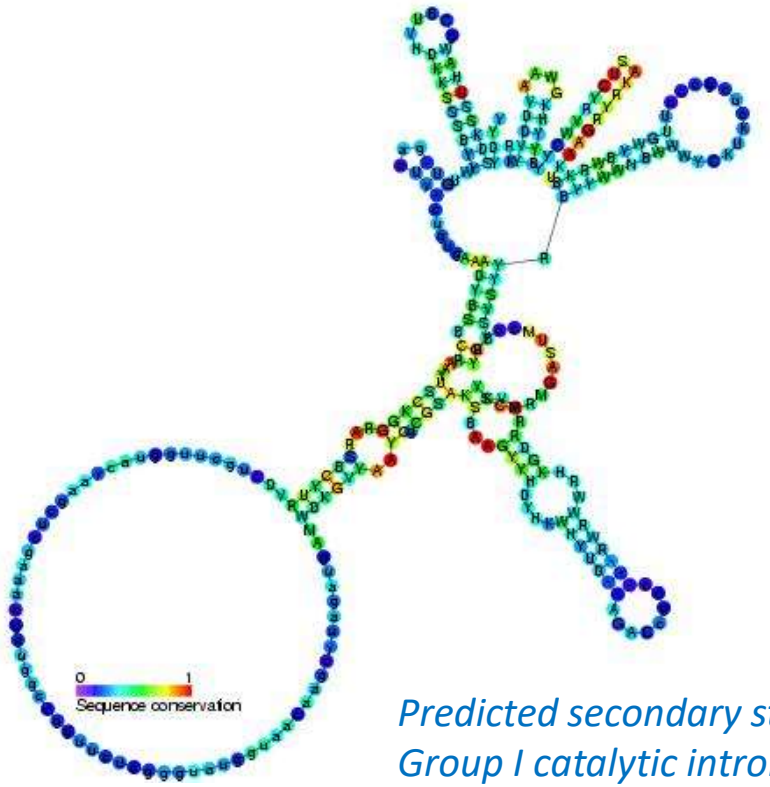


Tetrahymena thermophila



Bacterial RNAse P

RNA splicing

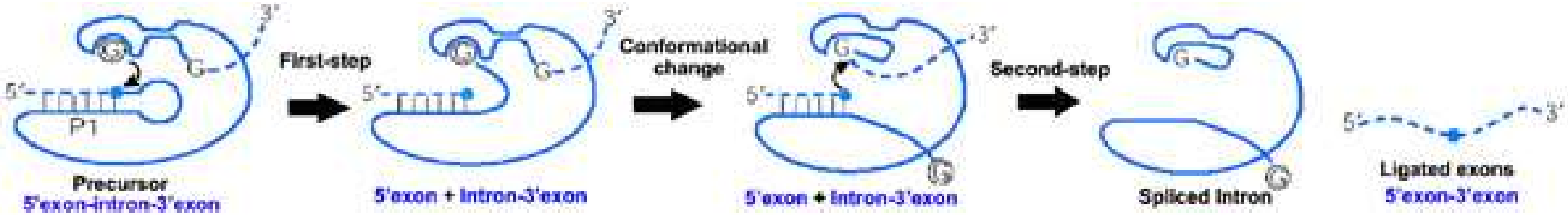


Predicted secondary structure and sequence conservation of Group I catalytic intron

Self-splicing RNA introns

RNA splicing in *Tetrahymena* was taking place also in absence of the spliceosome - the ‚negative control' obtained after protease digestion also spliced.

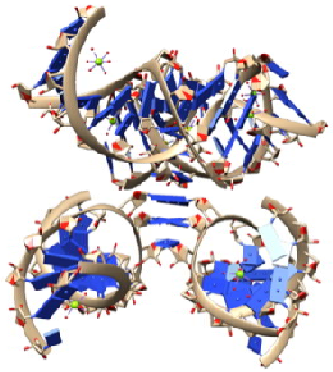
In contrary to the spliceosome, the ***catalytic*** motif ***does not*** contain protein part, ***only RNA***.
First known example of a ***ribozyme*** – ribonucleic acid-composed enzyme analogue.



Ribozymes and riboswitches

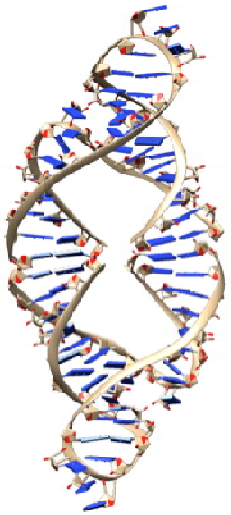
Ribozyme activities: cleavage/ligation of RNA and DNA, peptide bond formation, RNA processing reactions, including RNA splicing, viral replication, and transfer RNA biosynthesis.

Leadzyme

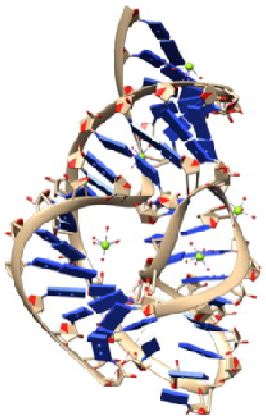


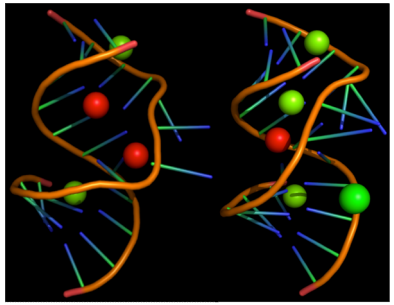
By Lucasharr - Own work, CC BY-SA 4.0,

Hammerhead ribozyme



Twister ribozyme





Leadzyme

Leadzyme catalyzes the cleavage of a specific phosphodiester bond in presence of lead (Pb^{2+})

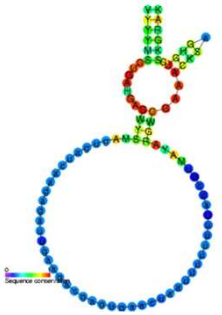
By Anarkalimahmood - Own work, CC BY-SA 3.0,

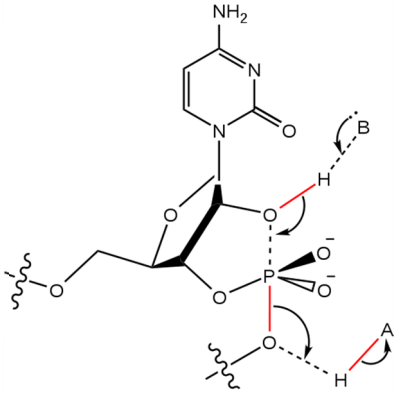
Twister ribozyme

The twister ribozyme is capable of self-cleavage. One of the fastest catalytic rates of naturally occurring ribozymes.

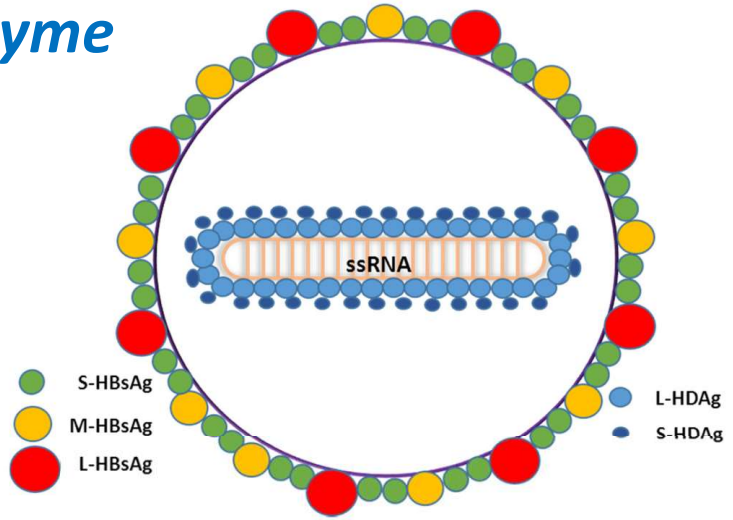
Hammerhead ribozyme

The hammerhead ribozyme is a RNA molecule motif that catalyzes reversible cleavage and joining reactions at a specific site within an RNA molecule (model system; targeted RNA cleavage experiments)





Hepatitis delta virus (HDV) ribozyme

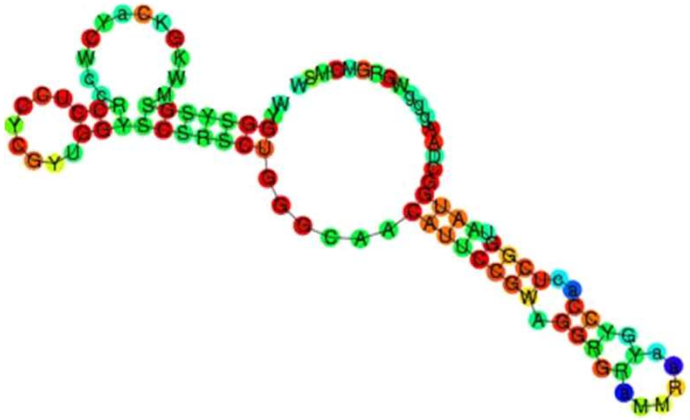


Hepatitis delta virus (HDV)

HDV is considered to be a **satellite** (a type of subviral agent) because it can propagate only in the presence of the hepatitis B virus (HBV). Transmission of HDV can occur either via simultaneous infection with HBV (coinfection) or superimposed on chronic hepatitis B or hepatitis B carrier state (superinfection).

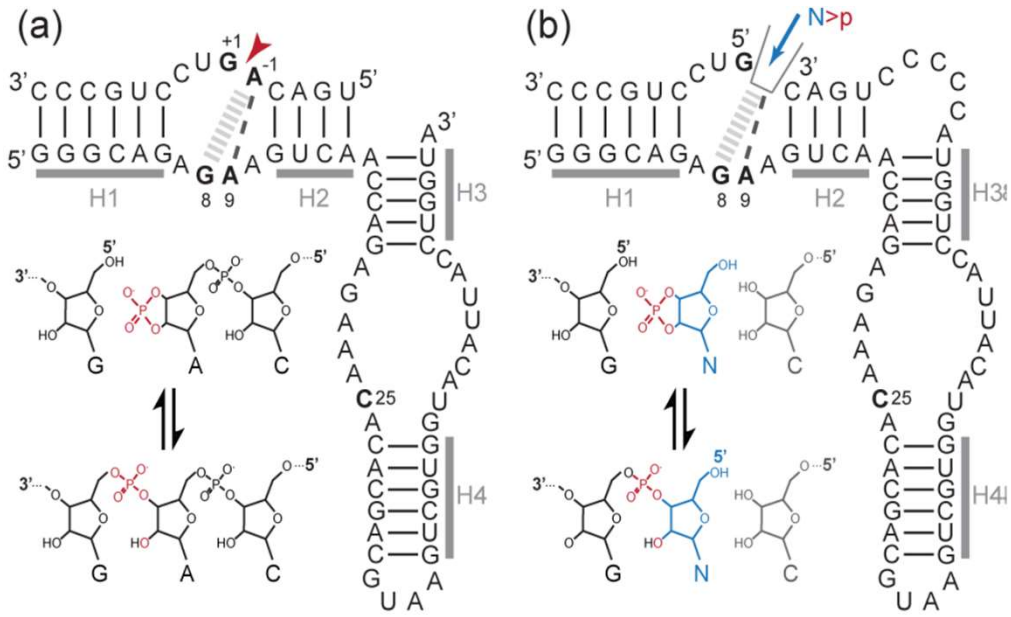
HDV ribozyme

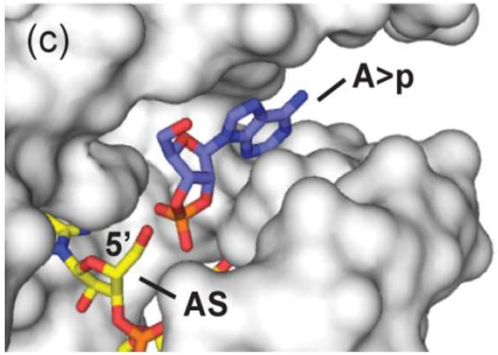
The hepatitis delta virus (HDV) ribozyme is a non-coding RNA found in the hepatitis delta virus that is necessary for viral replication and is thought to be the only catalytic RNA known to be required for viability of a human pathogen.

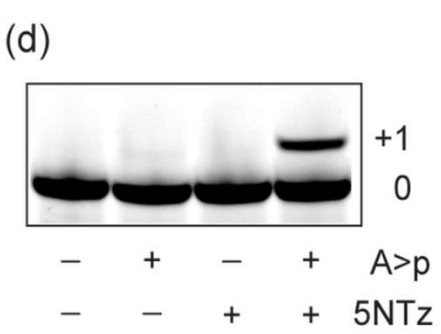


The ribozyme acts to process the RNA transcripts to unit lengths in a self-cleavage reaction. The ribozyme is found to be active in vivo in the absence of any protein factors and is the fastest known naturally occurring self-cleaving RNA.

Ribozyme-catalyzed primer extension







Design of a 5'-nucleotidyl transferase for N>p's.

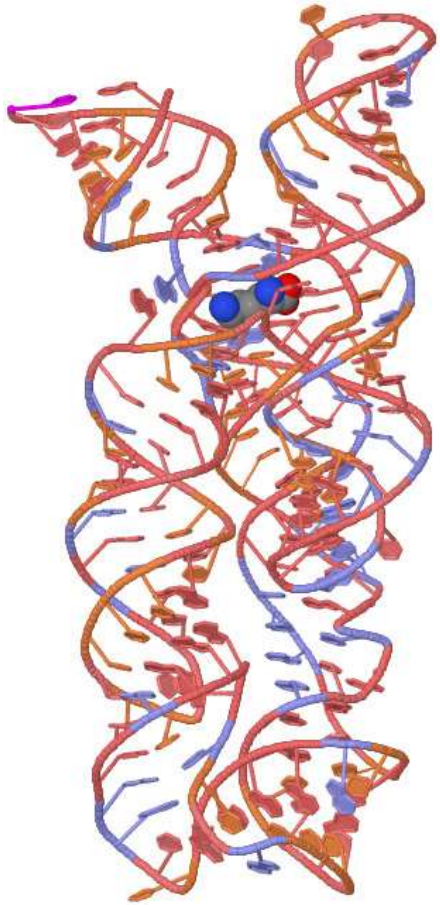
(a) Two-way junction HPz (small hairpin ribozyme), which catalyzes reversible RNA ligation using a 2′,3′-cyclic phosphate.
(b) Redesign of HPz into 5NTz (nucleotidyl transferase).
(c) Structural model of the substrate-binding pocket of 5NTz (based on PDB1M5V).
(d) 5NTz catalyzes 5′-adenylation in ice (2 mM A>p, 2 μM 5NTz, 1 μM 3′-FITC-labeled AS, 72 h in ice at −7 °C).

An engineered hairpin ribozyme catalyzes addition of all four N>p's (2′,3′-cyclic A-, G-, U-, and CMP) to the 5′-hydroxyl termini of RNA strands (eutectic ice phase formation at −7 °C). 5′ addition of 2′,3′-cyclic phosphate-activated β-nicotinamide adenine dinucleotide (NAD>p), as well as ACA>p RNA trinucleotide, and multiple additions of GUCCA>p RNA pentamers was also observed.

H Mutschler, P. Holliger *J. Am. Chem. Soc.* **2014,** *136,* 5193-5196

Riboswitches

2002 - (Breaker and Nudler) – discovery of a nucleic acid-based genetic regulatory element – ***riboswitch***.



The lysine riboswitch

Riboswitches - naturally occurring regulatory segments of mRNA that bind small molecules specifically. The binding results in a change in production of the proteins encoded by the mRNA

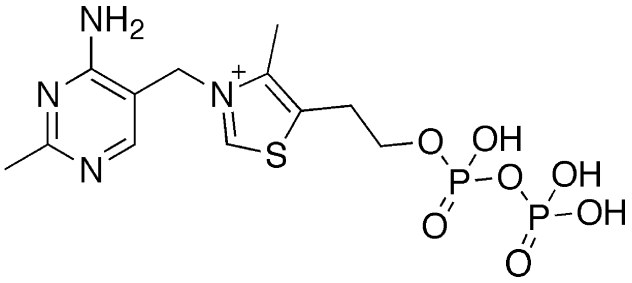
Before discovery of ***riboswitches*** only ***proteins*** were supposed to do so in the biological context.

Most known ***riboswitches*** occur in bacteria, but functional riboswitches of one type (the TPP riboswitch) have been discovered in archaea, plants and certain fungi.

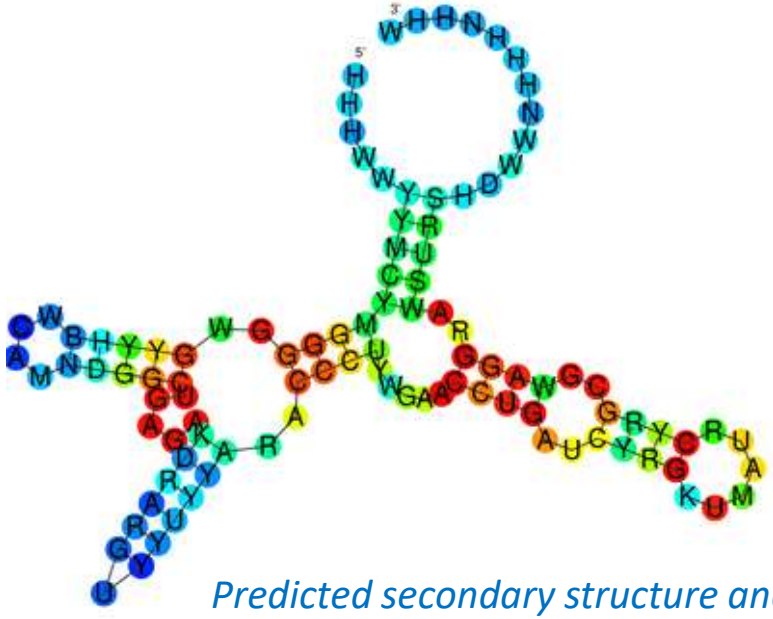
Riboswitches exist in all domains of life, and therefore are likely that they might represent ancient regulatory systems or fragments of ***RNA-world ribozymes*** whose binding domains remained conserved throughout the evolution

The TPP Riboswitch

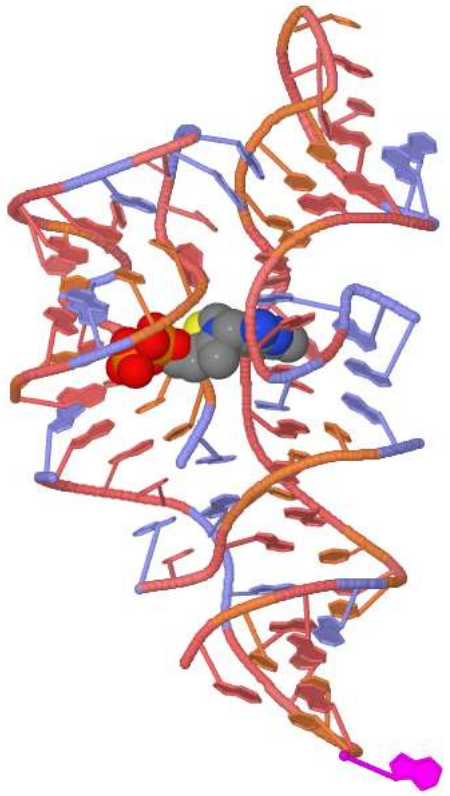
The **TPP riboswitch** (THI element and Thi-box riboswitch), is a highly conserved RNA secondary structure. It binds directly to thiamine pyrophosphate (TPP, a form of the vitamin B1, an essential coenzyme) to regulate gene expression through a variety of mechanisms in archaea, bacteria and eukaryotes.



Thiamine pyrophosphate TPP



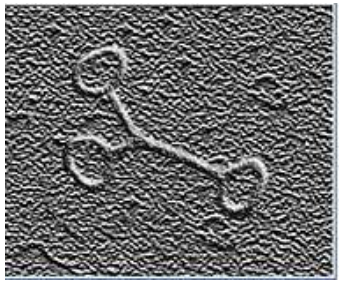
Predicted secondary structure and sequence conservation of TPP riboswitch

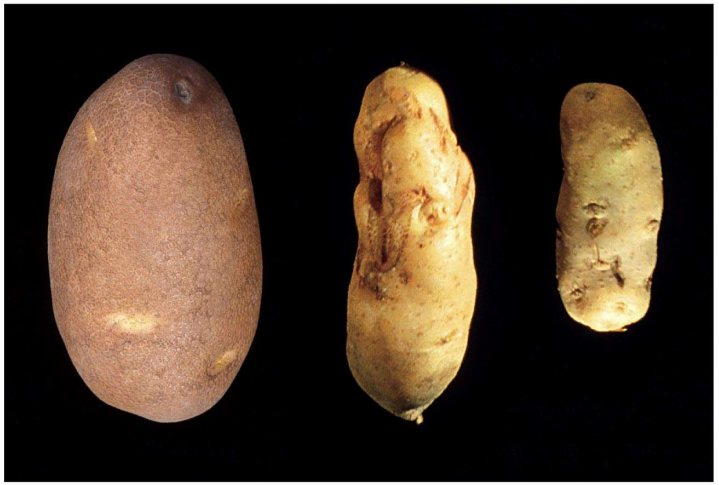


The 3D structure of TPP riboswitch (by Benjamin Schuster-Böckler)

Viroids

Viroids ("subviral pathogens„) are mostly plant pathogens, which consist of short stretches of highly complementary, circular, single-stranded, and non-coding RNA without a protein coat. Viroids are extremely small - 246 to 467 nucleobases (genomes of smallest viruses start from 2,000 nucleobases). Viroids are plausible "living relics" of the RNA world.





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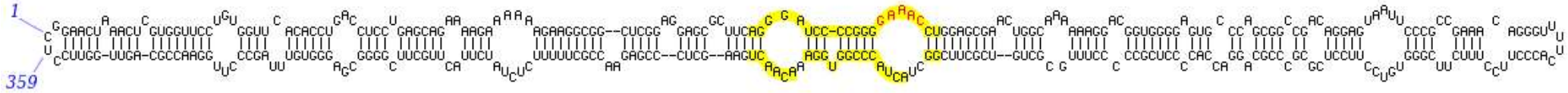
PSTVd-infected potatoes (right)

Viroid properties:

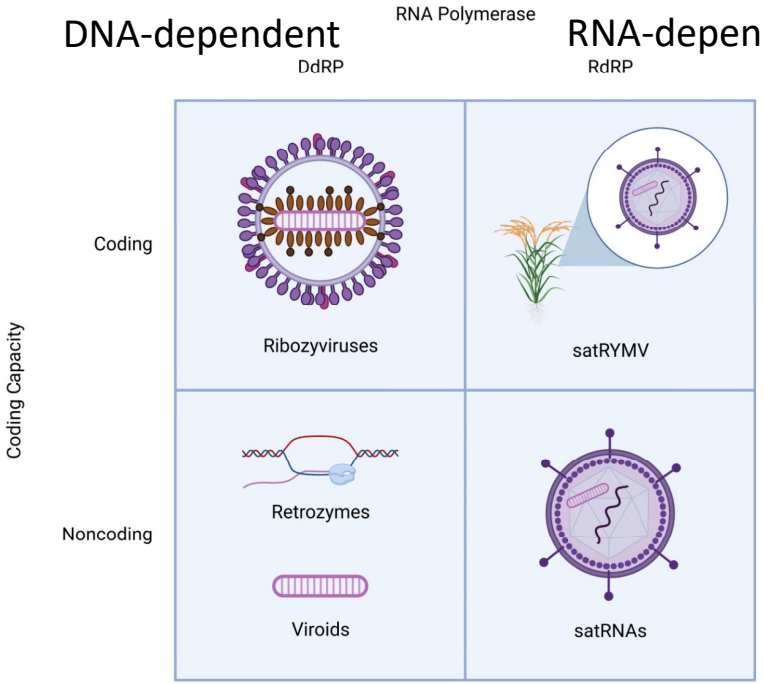
- small size (error-prone replication)
- high G-C content, (stability and replication fidelity)
- circular structure (complete replication without genomic tags)
- lack of protein-coding ability, consistent with a ribosome-free habitat; and replication mediated in some by ribozymes—the fingerprint of the RNA world.



Putative secondary structure of the PSTVd viroid

Viroids

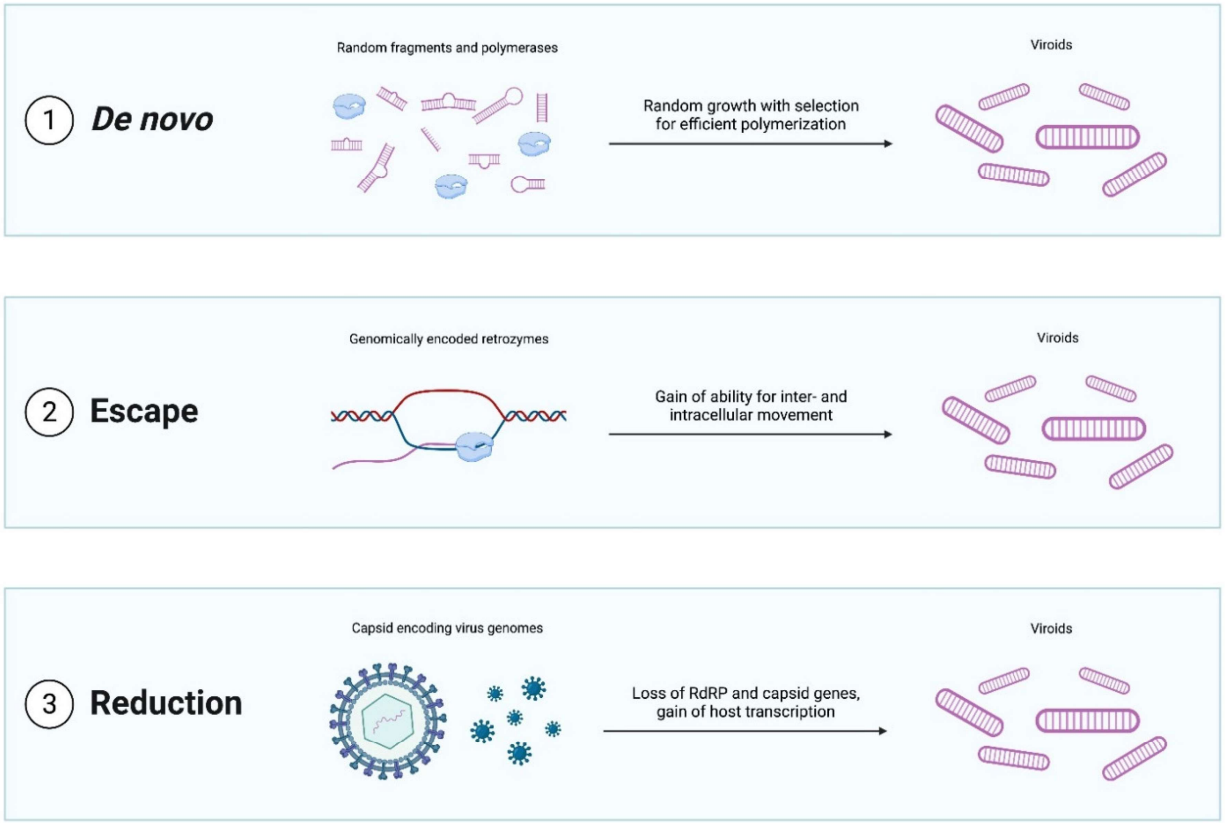
distinct classes of viroid-like RNAs



Viroid-like cccRNAs	Size	Host
Viroids	246–450 nt	Plants
Ribozyviruses	1547–1735 nt	Metazoans
Retrozymes	300–1116 nt	Eukaryotic genomes
satRNAs	220–457 nt	Plants

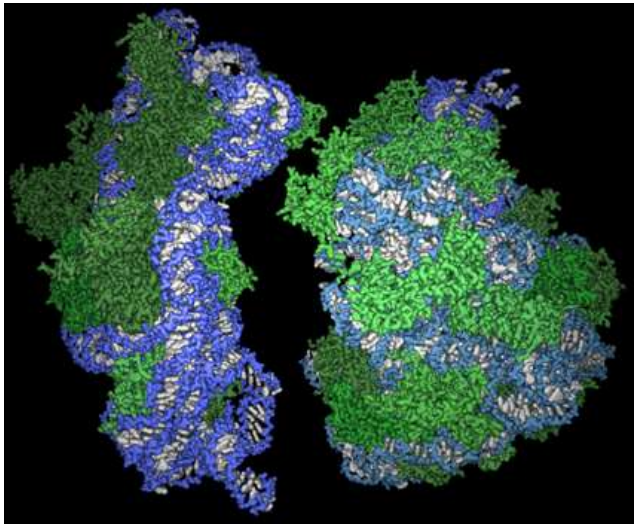
The satRNAs are encapsidated by the helper virus capsid proteins.

Evolutionary scenarios

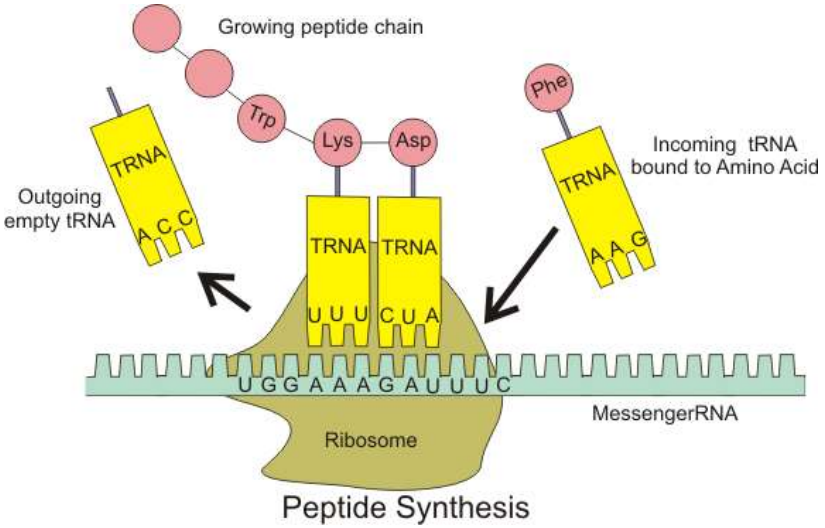


B.D. Lee, E. V. Koonin, *Life.* **2022**, *12,* 103

Ribosome structure and function



Ribosome: green - proteins, blue and white - RNA



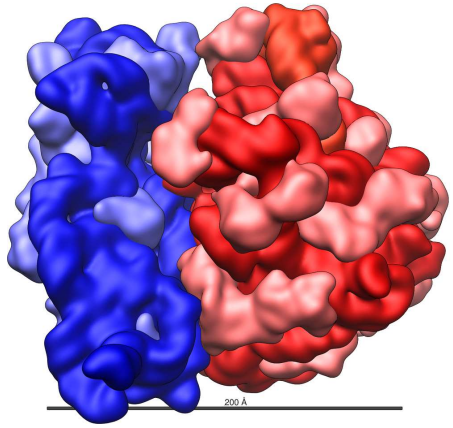
The ribosome is a molecular machine, found within all living cells, that serves as the site of biological protein synthesis (translation). Ribosomes link amino acids together in the order specified by messenger RNA (mRNA) molecules.

Ribosome is structurally highly conserved among all living species – most likely present in LUCA

Ribosomes:

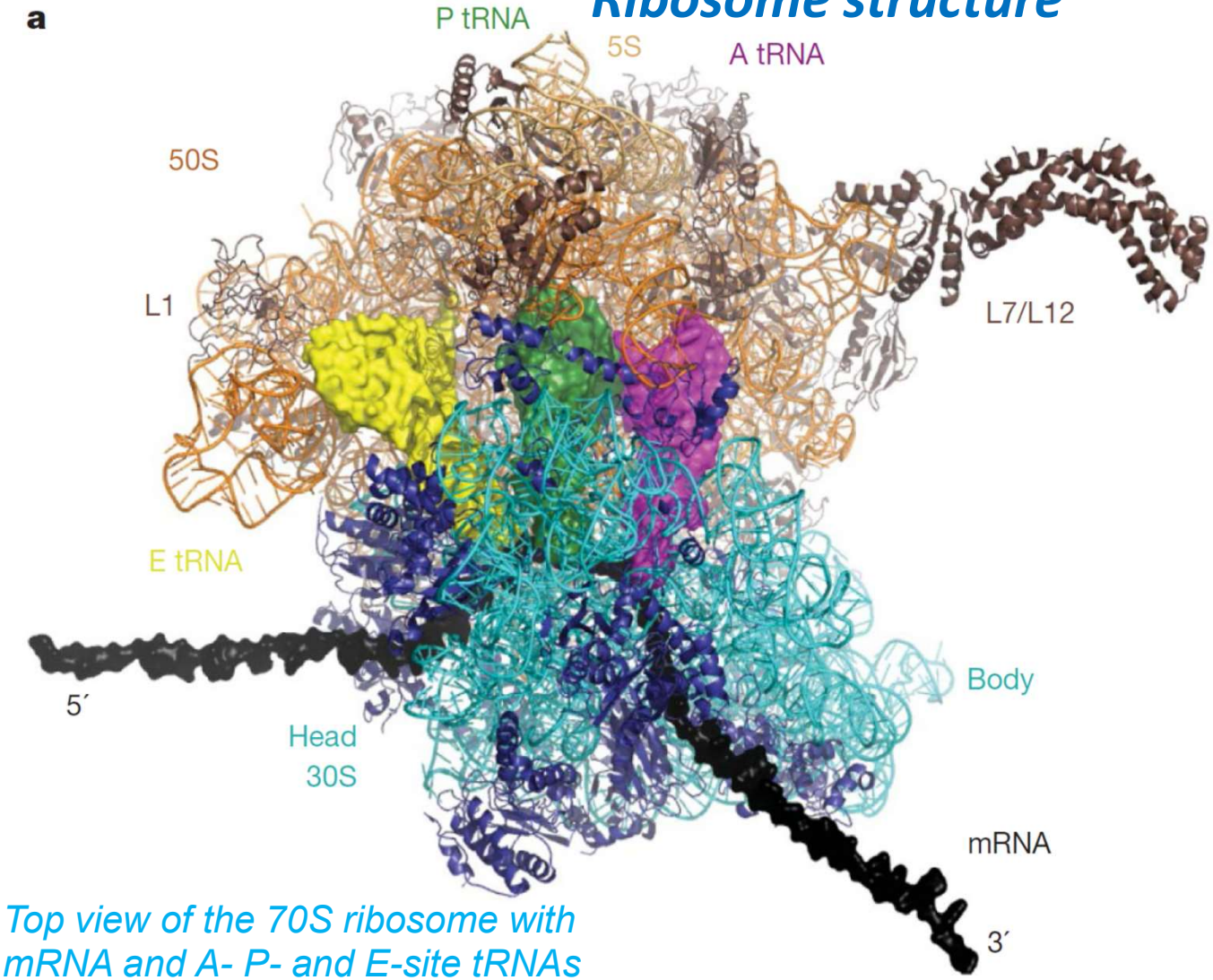
- the *small ribosomal subunit*, which reads the RNA
- the *large subunit*, which joins amino acids to form a polypeptide chain.

Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins.

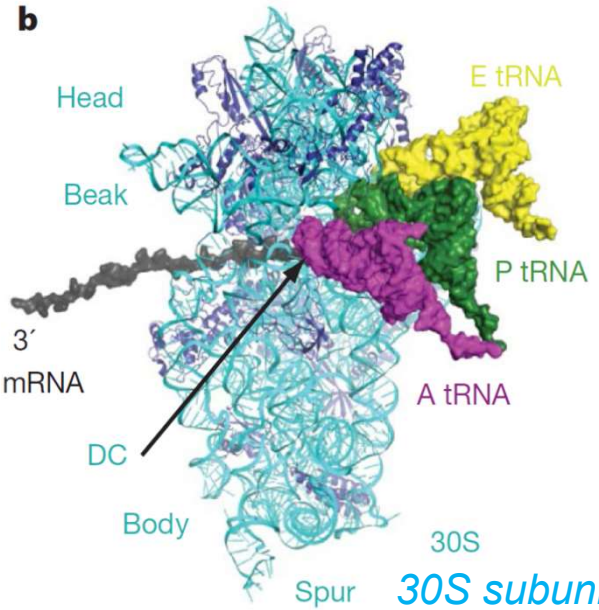


Large and small subunit

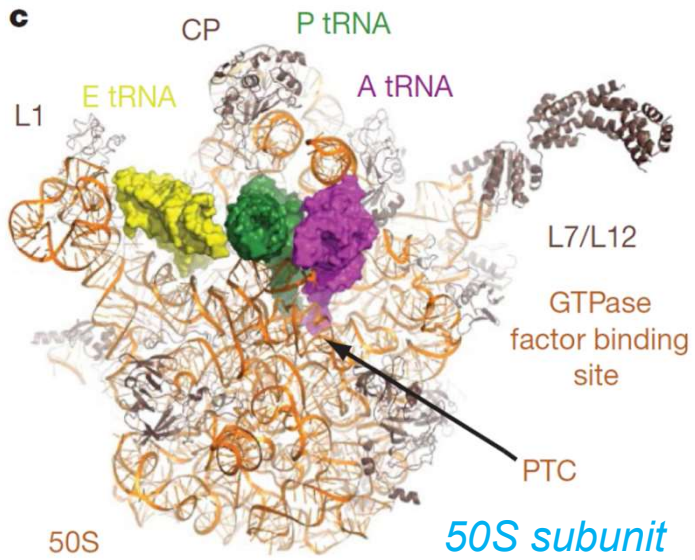
Ribosome structure



Top view of the 70S ribosome with mRNA and A- P- and E-site tRNAs



30S subunit

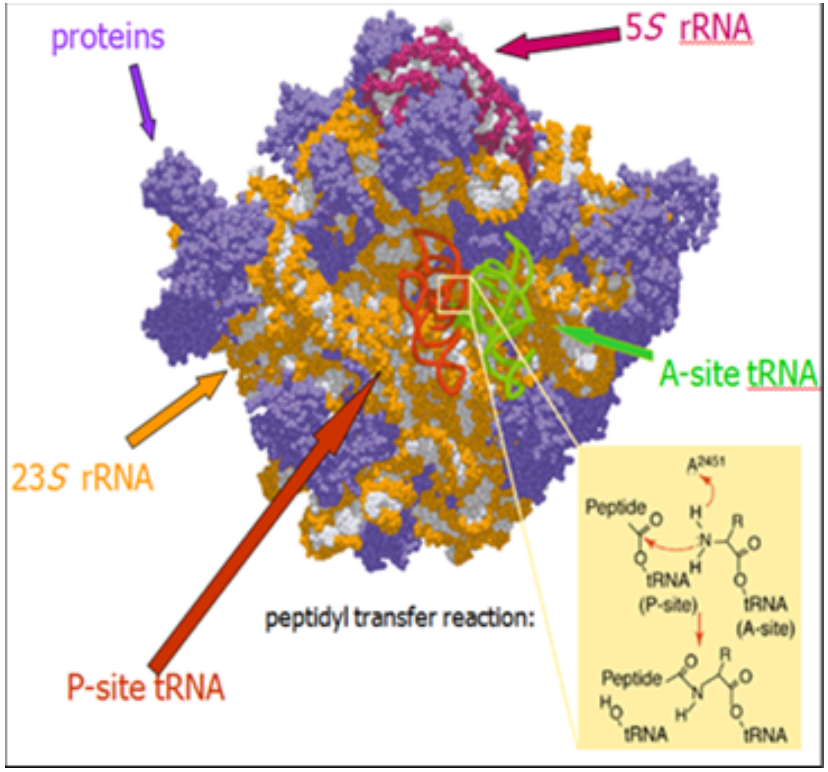


50S subunit

T. Martin Schmeing, V. Ramakrishnan *Nature.* **2009**, *461,* 1234-1242

Ribosome – the ‚smoking gun'

Ribosome is a ribozyme!



No protein is present within 18 Angstroms from the active site → proteins play a structural role, but DO NOT CATALYZE THE ACYL TRANSFER PROCESS

T. Cech *Science.* **2000**, *289,* 878-879

Ribosome – the ‚smoking gun'

Ribosome is a ribozyme!

The ribosome may have first originated in an RNA world appearing as a self-replicating complex that only later evolved the ability to synthesize proteins when amino acids began to appear.

Studies suggest that ancient ribosomes constructed solely of rRNA could have developed the ability to synthesize peptide bonds.

In addition, evidence strongly points to ancient ribosomes as self-replicating complexes, where the rRNA in the ribosomes had informational, structural, and catalytic purposes because it could have coded for tRNAs and proteins needed for ribosomal self-replication.

As amino acids gradually appeared in the RNA world under prebiotic conditions, their interactions with catalytic RNA would increase both the range and efficiency of function of catalytic RNA molecules. Thus, the driving force for the evolution of the ribosome from an ancient self-replicating machine into its current form as a translational machine may have been the selective pressure to incorporate proteins into the ribosome's self-replicating mechanisms, so as to increase its capacity for self-replication

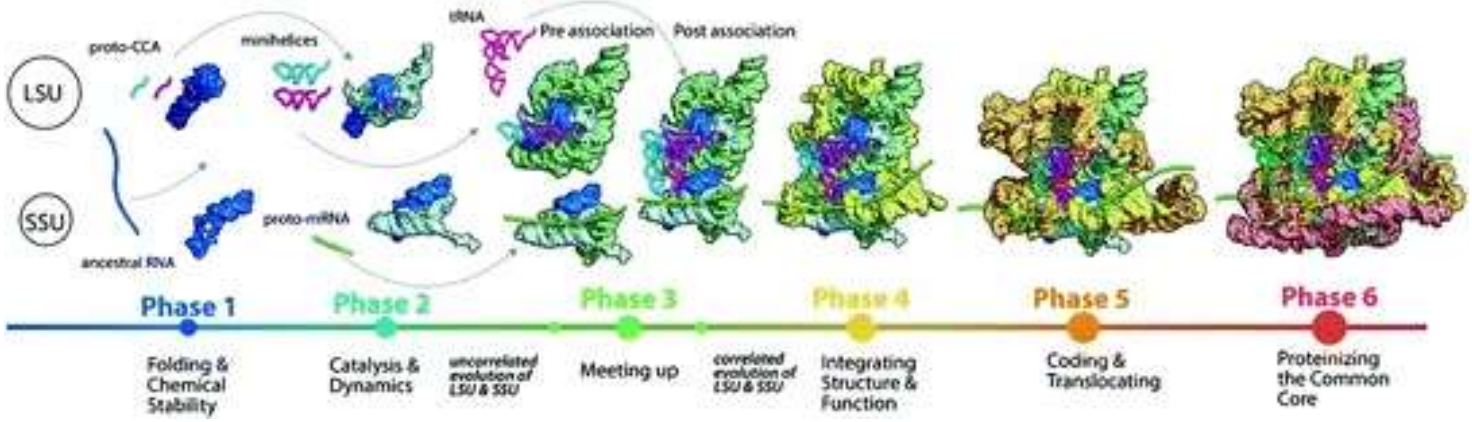
History of the ribosome and the origin of translation

The ribosome contains a record of its 4-billion-years-of-life history. Details of ribosomal RNA variation, observed by comparing three-dimensional structures of ribosomes across the tree of life, form the basis of our molecular-level model of the origins and evolution of the translational system.

The ribosome evolved by accretion, recursively adding expansion segments, iteratively growing, subsuming, and freezing the rRNA.

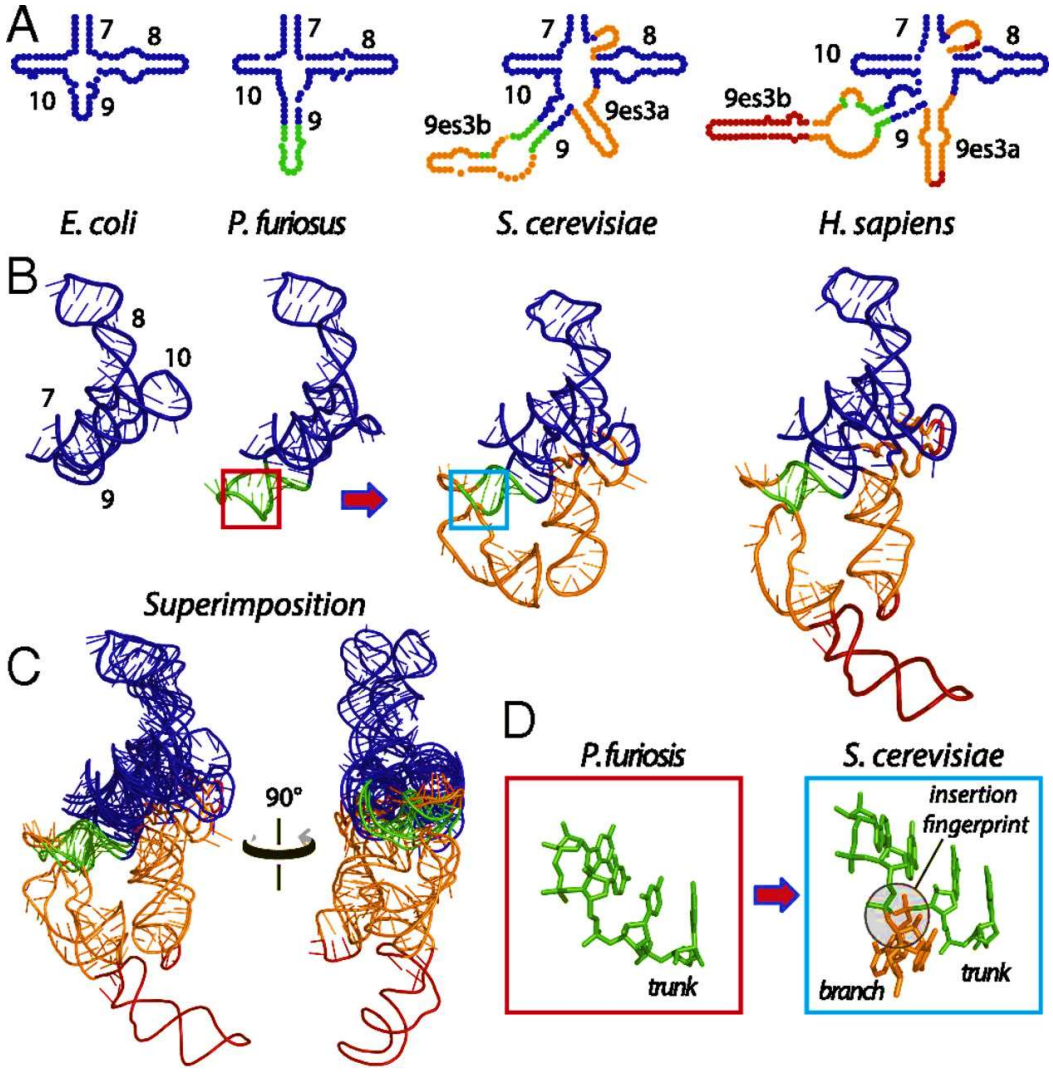
Functions of expansion segments in the ancestral ribosome areassigned by correspondence with their functions in the extant ribosome.

The model explains the evolution of the large ribosomal subunit (**LSU**), the small ribosomal subunit (**SSU**), **tRNA**, and **mRNA**.



Petrov AS, Gulen B, Norris A, Kovacs NA, Bernier CR, Lanier KA, Fox GE, Harvey SC, Wartell RM, Hud NV, Williams LD. Proc. Natl. Acad Sci USA. **2015**, *112*, 15396–15401

History of the ribosome and the origin of translation



Petrov AS, et al. Proc. Natl. Acad Sci USA. **2015**, *112*, 15396–15401

Accretion of SSU (small subunit) rRNA as illustrated by helices 7–10/es3 from species of increasing complexity.

A four-way junction at the surface of the common core, formed by helices 7–10, has expanded by accretion. Accretion adds to the previous rRNA core, leaving insertion fingerprints. (*A* and *B*)

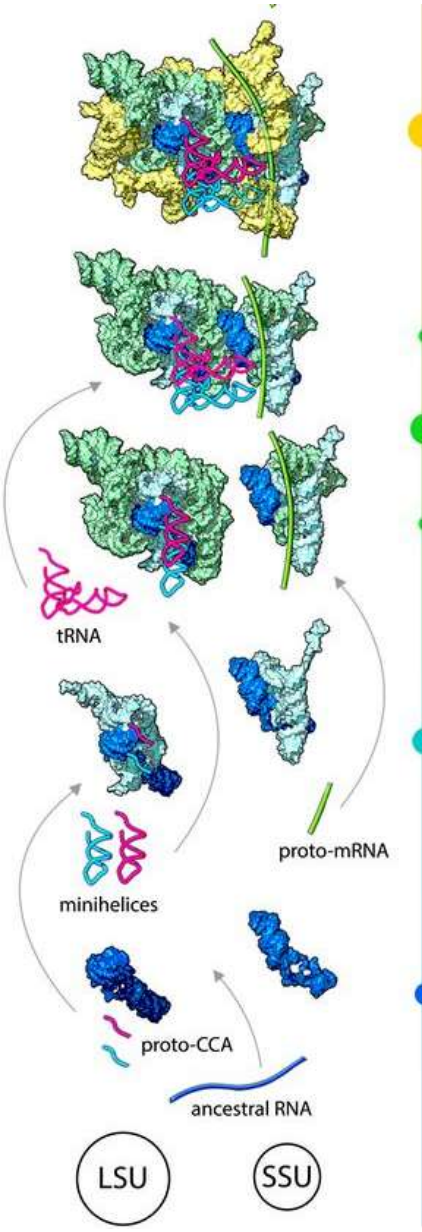
Secondary (*A*) and 3D (*B*) structures are preserved upon the addition of new rRNA.

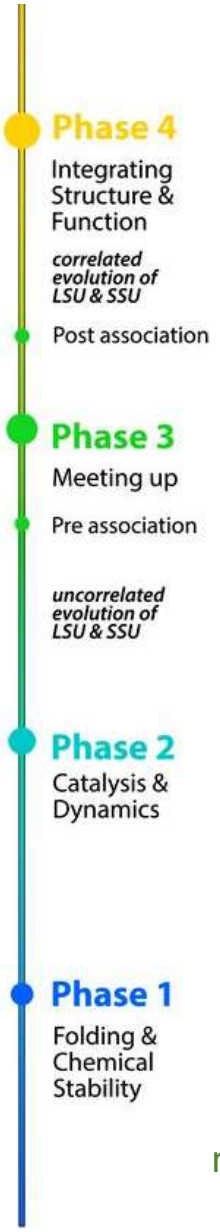
(*C*) Superimposition of the 3D structures highlights how new rRNA accretes with preservation of ancestral rRNA.

(*D*) A characteristic insertion fingerprint is shown in red and blue boxes.

The rRNA that approximates the common core is blue.
An expansion observed in archaea and eukaryotes is green.
An expansion that is observed only in eukaryotes is gold.
An additional expansion in mammals is red.

Phases of the accretion model of ribosomal evolution





Phase 1: ancestral RNAs form stem–loops and minihelices.

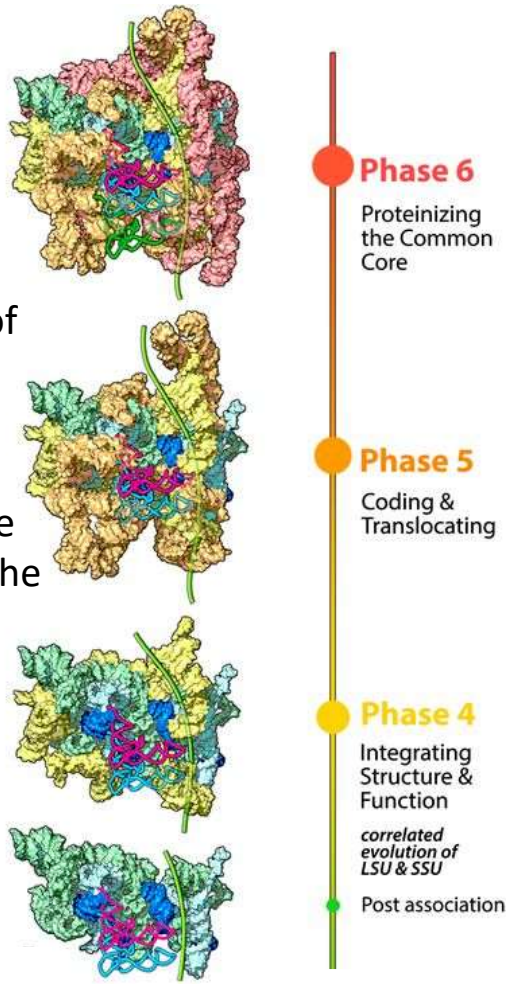
Phase 2: the LSU catalyzes the condensation of nonspecific oligomers. The SSU may have a single-stranded RNA-binding function.

Phase 3: the subunits associate, mediated by the expansion of tRNA from a minihelix to the modern L shape. LSU and SSU evolution is independent and uncorrelated during Phase 1–3.

Phase 4: evolution of the subunits is correlated. The ribosome is a noncoding diffusive ribozyme in which proto-mRNA and the SSU act as positioning cofactors.

Phase 5: the ribosome expands to an energy-driven, translocating, decoding machine.

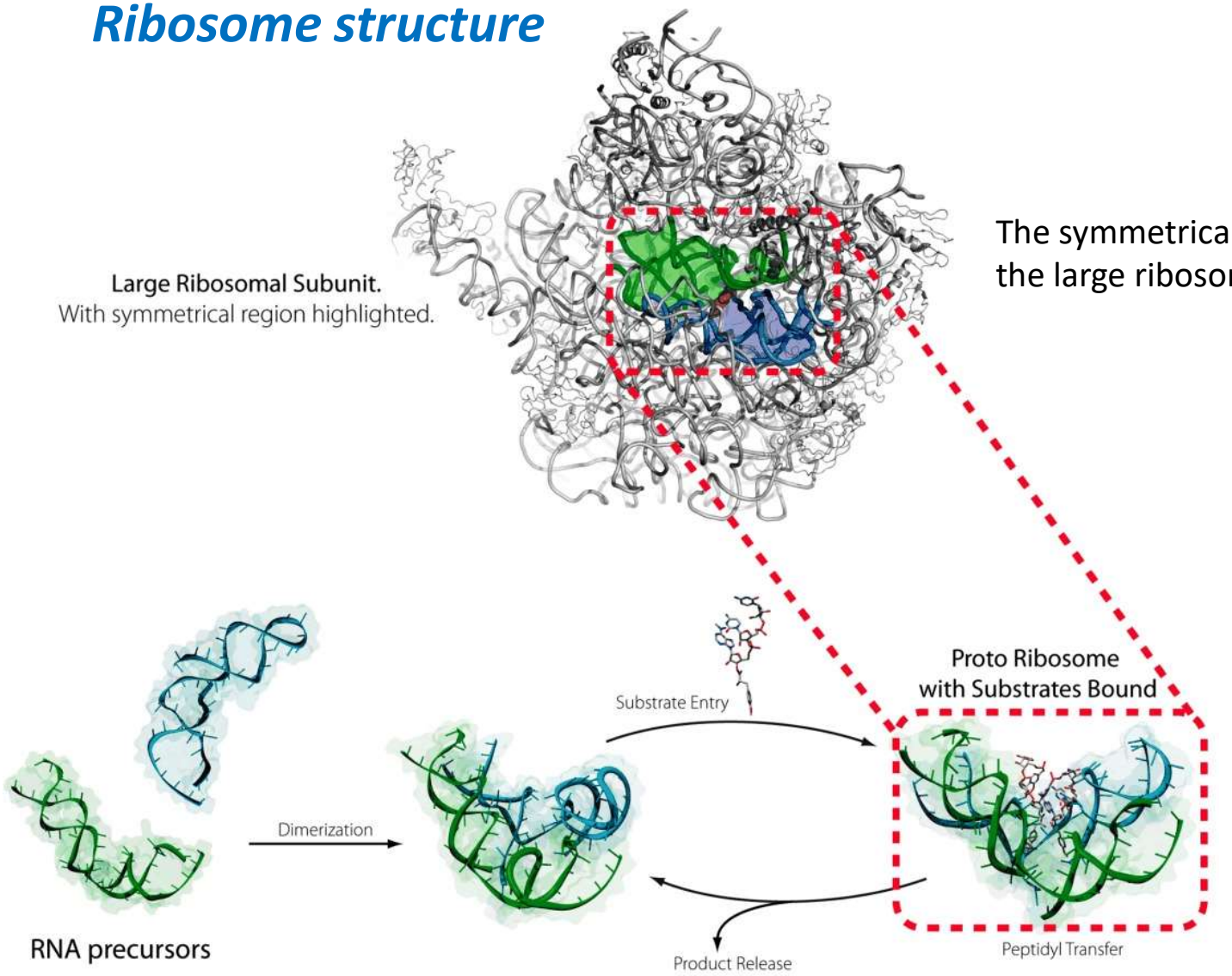
Phase 6 marks the completion of the common core with a proteinized surface (the proteins are omitted for clarity).



mRNA in light green. A-site tRNA - magenta, P-site tRNA -cyan, E-site tRNA - dark green.

Petrov AS, et al. Proc. Natl. Acad Sci USA. **2015**, *112*, 15396–15401

Ribosome structure



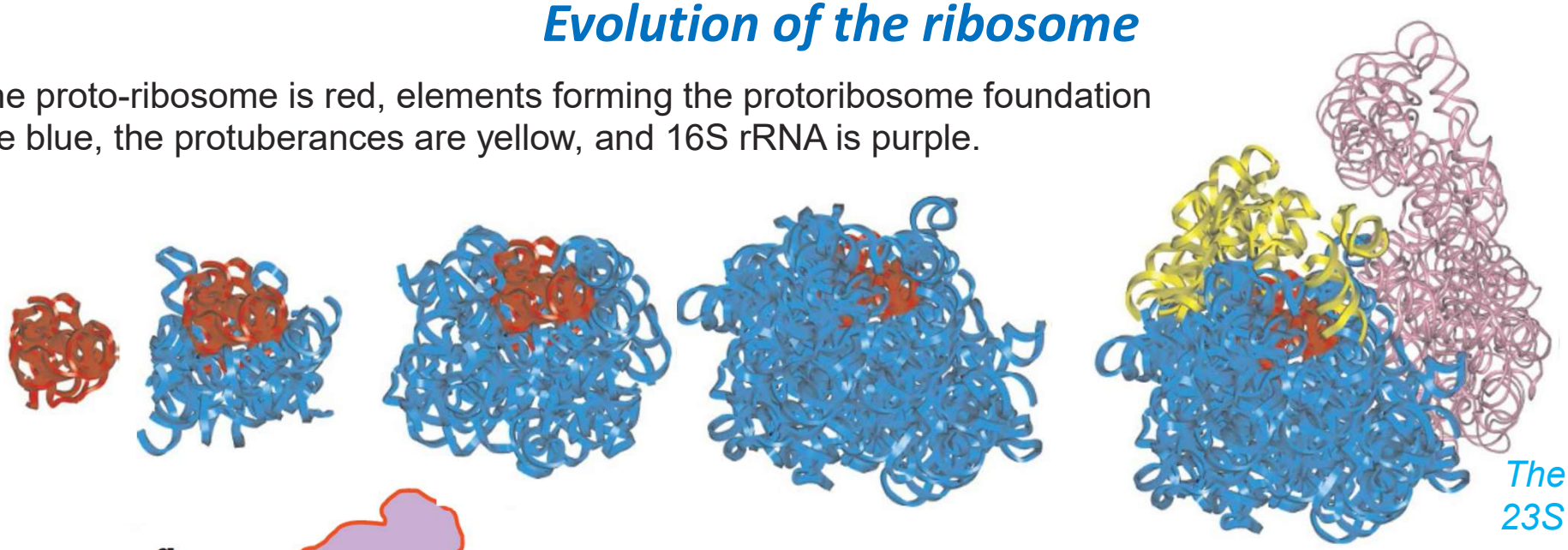
The symmetrical region within the large ribosomal subunit.

Schematic representation of "pocket-like" proto-ribosome formation from a RNA precursor, showing simple catalytic peptidyl transferase activity.

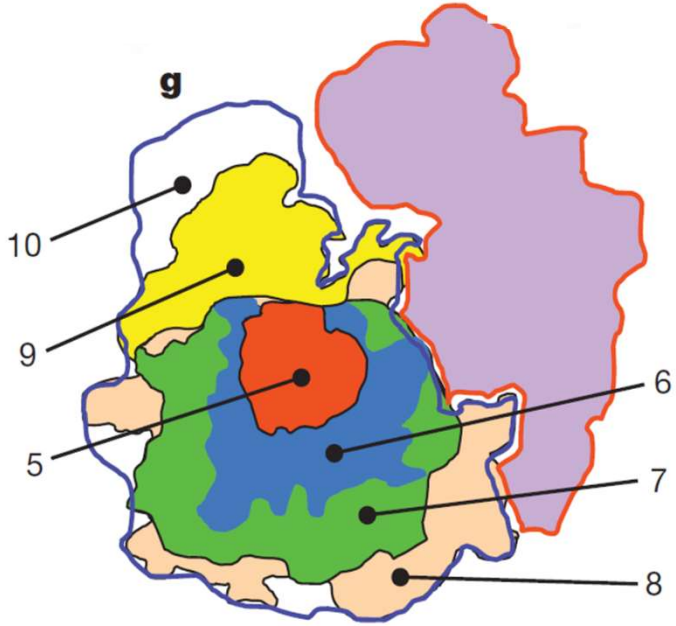
A. Yonath *et al. Israel Journal of Chemistry,* **2010***, 50,* 29-35

Evolution of the ribosome

The proto-ribosome is red, elements forming the protoribosome foundation are blue, the protuberances are yellow, and 16S rRNA is purple.

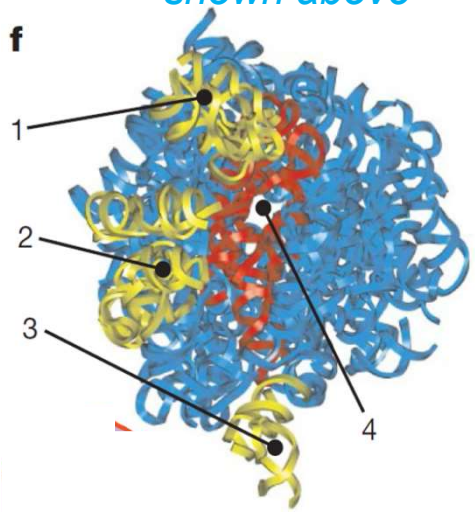


The top view of the 23S rRNA structure shown above



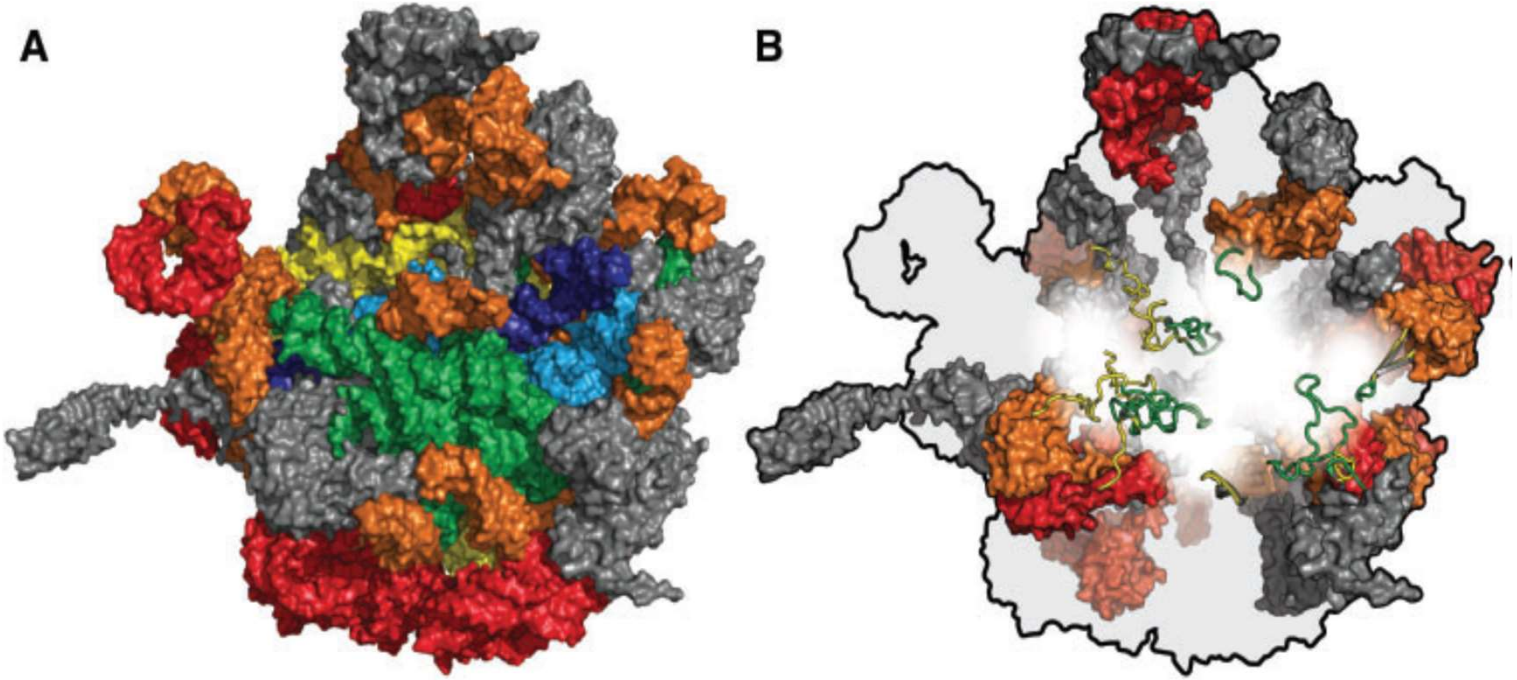
The positions of the parts of 23S rRNA shown above in the context of the whole ribosome. The structures of the 50S and 30S subunits are contoured by the blue and red line, respectively.

***1–3** are the L7/L12, central and L1 protuberances, respectively; **4** is the exit channel; **5–9** are the structures shown in a–e, respectively; **10** is the part of 50S subunit that does not include 23S rRNA. This part is formed by ribosomal proteins and 5S rRNA.*



K. Bokov, S. Steinberg Nature. **2009**, *457,* 977-980

Evolution of the ribosome



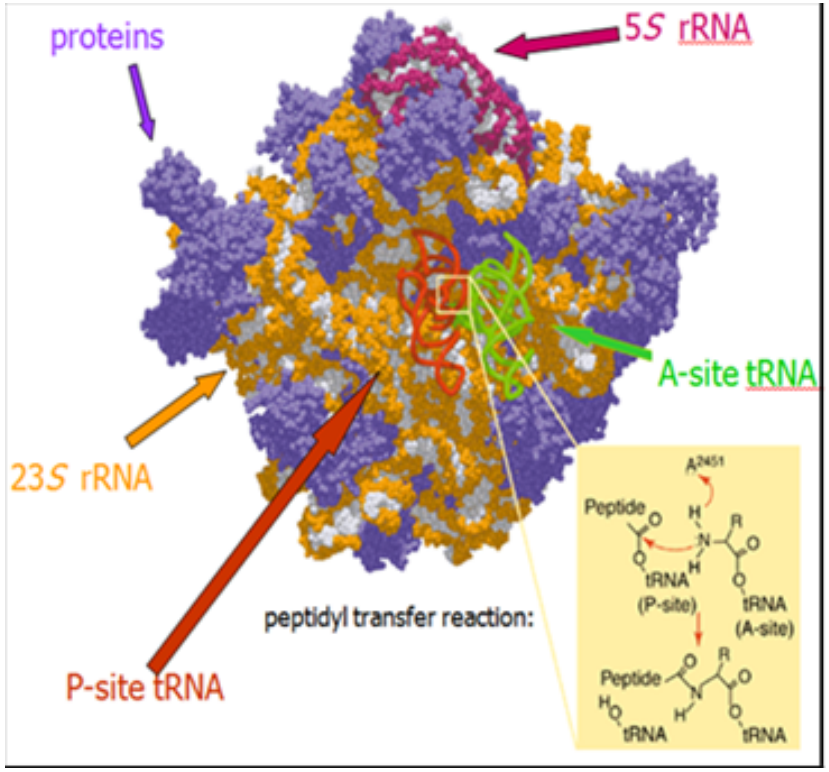
(A) The rRNA of the large subunit of the T. thermophilus ribosome colored by relative age. Phase 1, the most ancient phase, is dark blue. Phase 2 is light blue. Phase 3 is green. Phase 4 is yellow. Phase 5 is orange. Phase 6, the most recent prokaryotic phase, is red. rProteins are grey.

(B) The orientation is maintained but rRNA is colored in light grey, universal rProteins are colored by evolutionary phase, and bacterial rProteins are colored dark grey. Phases 3 (green) and 4 (yellow) are shown in cartoon representation. Phases 5 (orange) and 6 (red) are shown in surface representation. From PDB entry 1VY4

N. A. Kovacs *et al. Mol. Biol. Evol*. **2017** 34, 1252–1260.

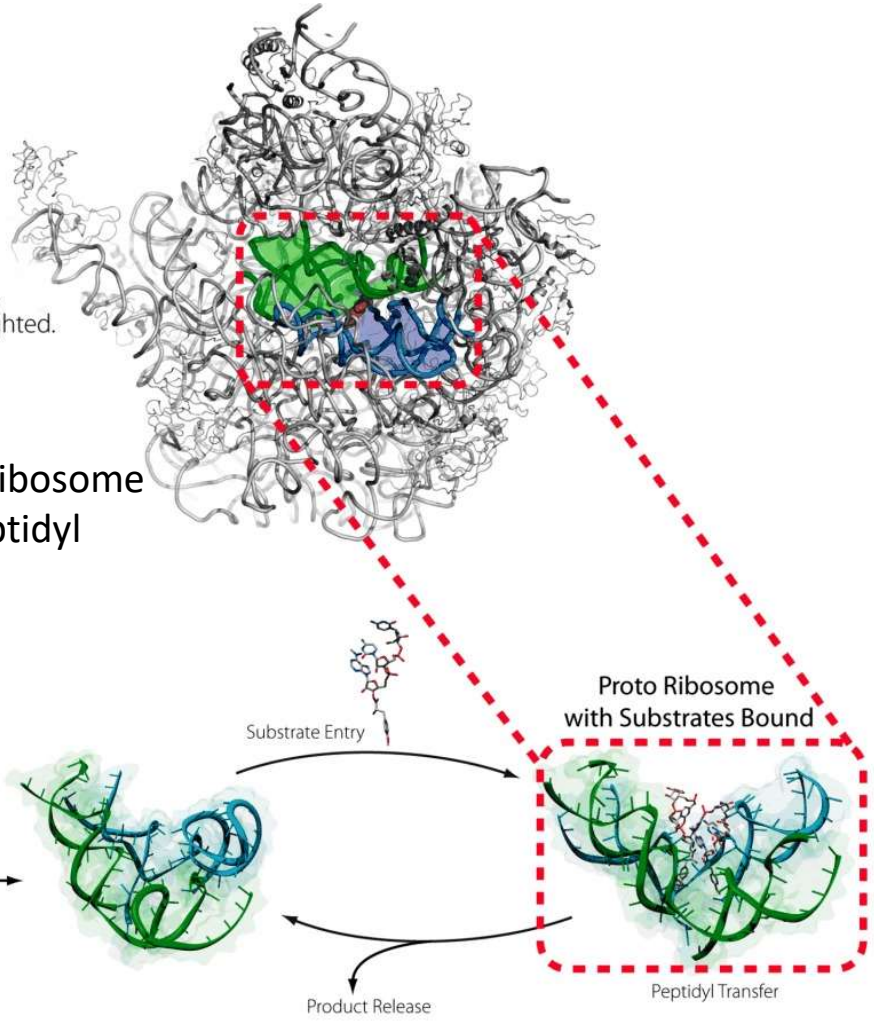
Ribosome – the ‚smoking gun'

Ribosome is a ribozyme!



No protein is present within 18 Angstroms from the active site → proteins play a structural role, but DO NOT CATALYZE THE ACYL TRANSFER PROCESS

T. Cech *Science.* **2000**, *289,* 878-879



"pocket-like" proto-ribosome
- simple catalytic peptidyl transferase activity.

A. Yonath *et al. Israel Journal of Chemistry,* **2010**, *50,* 29-35

The RNA world

RNA as catalyst

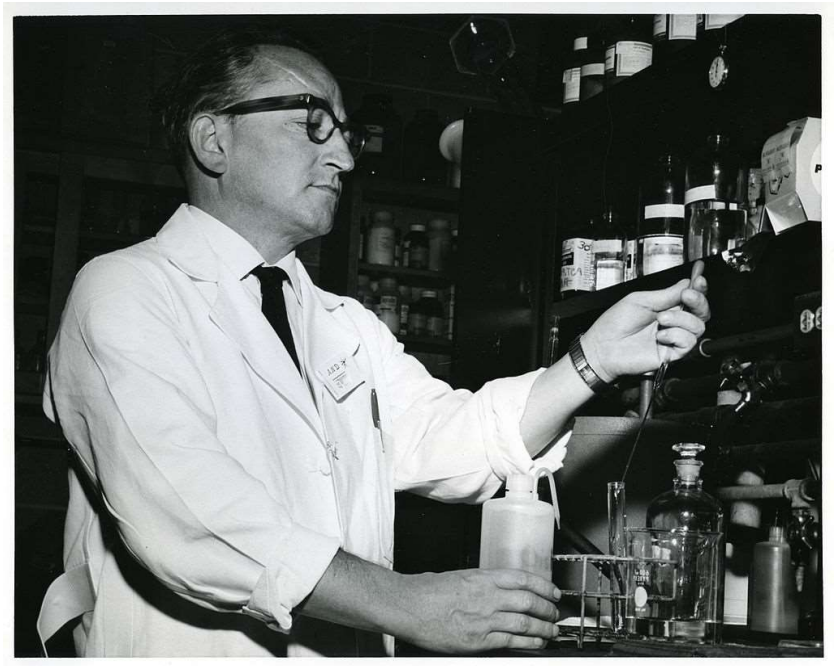
Currently known co-enzymes
Ribozymes
Ribosome

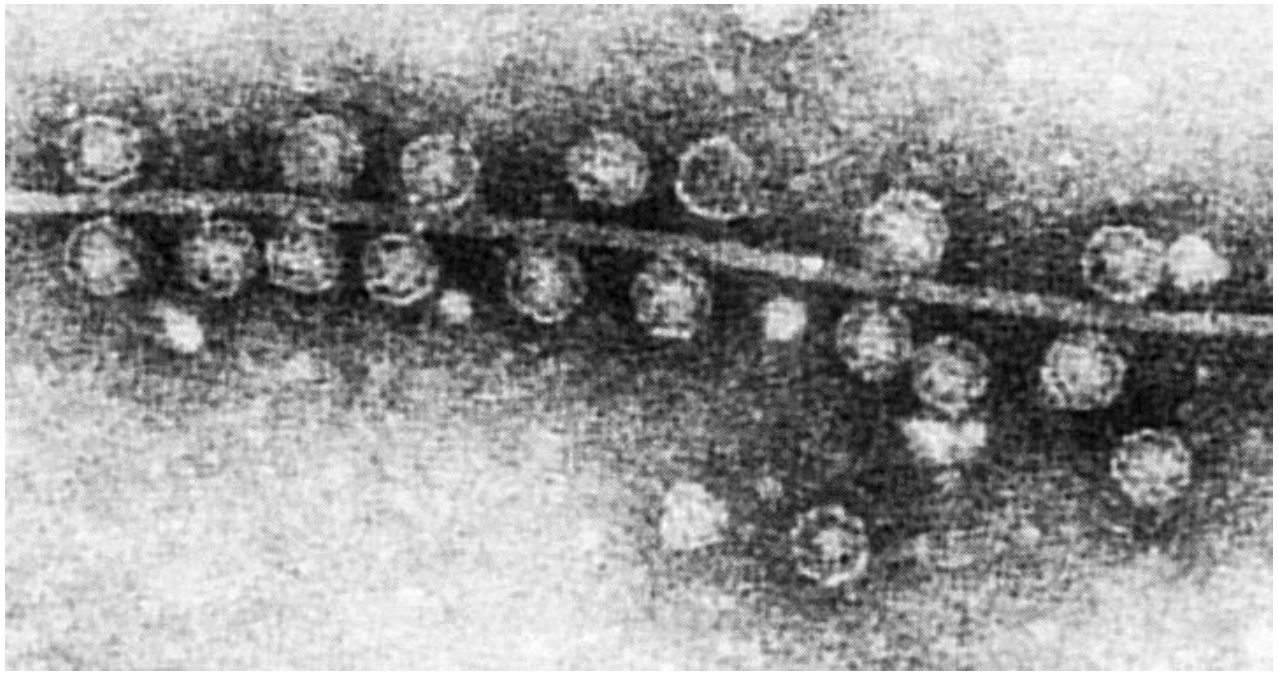
Can RNA evolve?

Can RNA replicate itself?

The RNA world

Can RNA evolve?



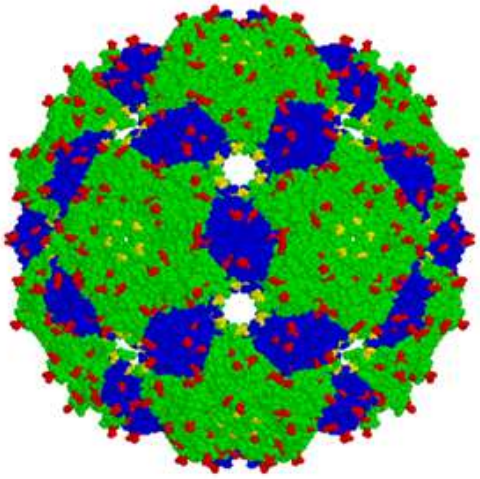


Spiegelman's monster

The RNA world

The bacteriophage Qβ – a virus containing RNA-dependent RNA polymerase (protein, enzymatic replicase)

Spiegelman's monster



Spiegelman mixed the Qβ RNA, the Qβ enzymatic replicase, mononucleotides and some salts (buffer). RNA replication begun.
An aliquot was transferred several times to a fresh solution without template.

Shorter RNA chains replicate faster. The selection in this system favors speed.
And no evolutionary pressure on pathogenicity was present anymore.
So the RNA became shorter and shorter due to random mutations during copying.

After 74 passages, the original 4500 nt RNA strand was reduced to 218 nt.
Such a short RNA chain replicated very quickly under these unnatural circumstances.
Of course, it lost all its genes and was unable to produce any useful proteins anymore.

First example of *in vitro* RNA evolution

Kacian D. L., Mills D. R., Kramer F. R., Spiegelman S. *PNAS* **1972**, *69*, 3038-3042.

Spiegelman's monster can be also formed by simple mixing of activated RNA monoers and the Qβ enzymatic replicase, in absence of any RNA template!

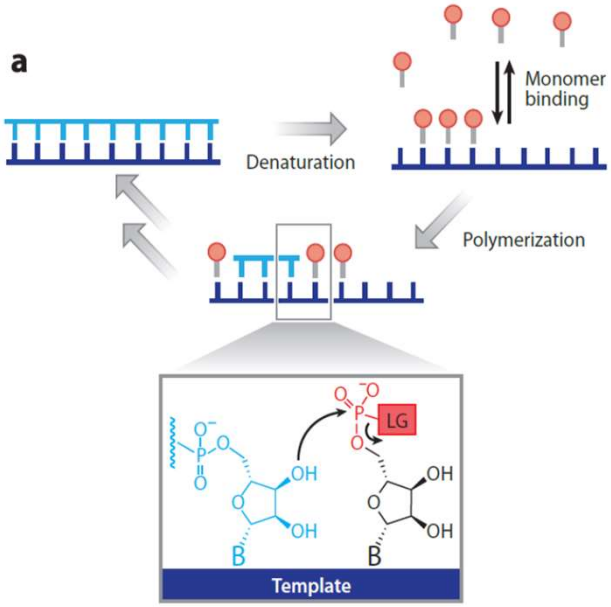
Sumper M., Luce R. *PNAS* **1975**, *72*, 162-166.

The RNA world

RNA self-replication

Nonenzymatic template-directed RNA polymerization

Maximally 30-50 nt extension, fidelity strongly sequence-dependent

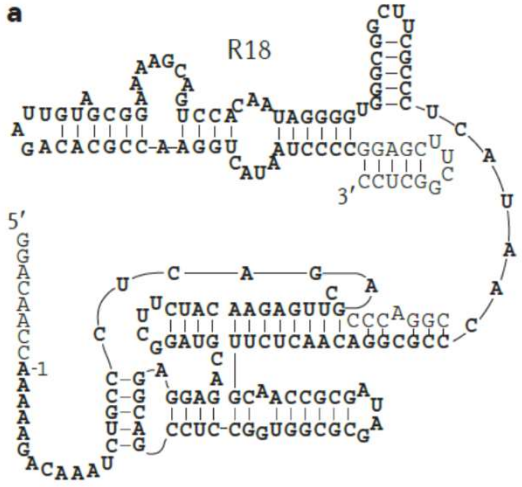


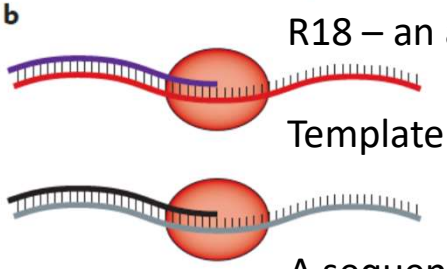
General RNA polymerase ribozyme (‚replicase')

Networks of RNA molecules that mutually catalyse their replication – autocatalytic replication of the whole network

The RNA world

RNA-dependent RNA polymerase ribozyme – Replicase - the ‚holy Grail' of the RNA world

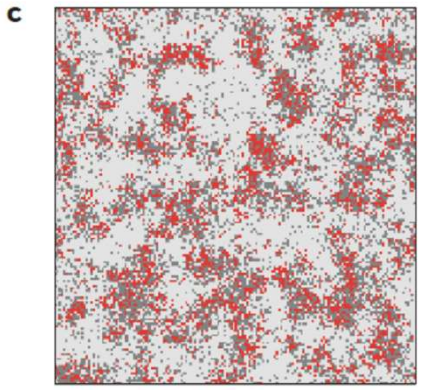


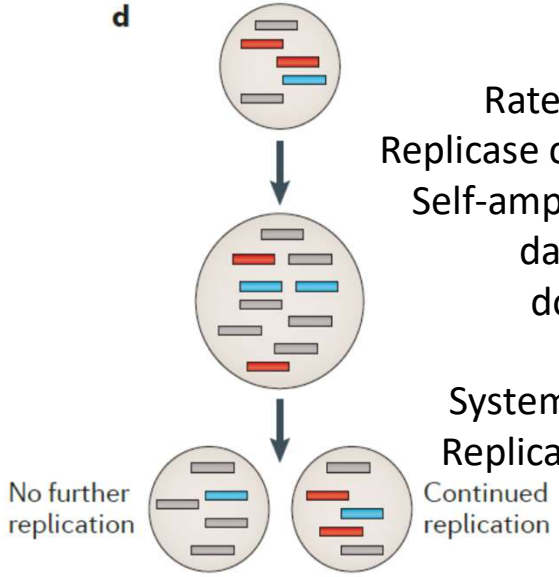


R18 – an artificial polymerase evolved from the class I ligase ribozyme.

Template: another copy of itself (red) or an unrelated sequence (grey).

A sequence of 206 nt was copied (fidelity 97.4%) at low temperatures by an engineered R18 mutant – first ribozyme capable to synthesize RNA oligomers longer than itself (though NO self-replication yet!)





Rate of replication not sensitive on the template's sequence.
Replicase could replicate other ribozymes (e.g. with metabolic functions).
Self-amplifying replicase needs a working complementary replicase – danger of paraistes (templates that copy themselves but do not contribute to the replication of the polymerase).

Systems of altruistic replicators are destroyed by parasites (grey). Replicators (red) can survive e.g. by diffusion on 2D surfaces (**c**) or selection inside compartments (**d**)

Johnston, W. K., Unrau, P. J., Lawrence, M. S., Glasner, M. E. & Bartel, D. P. *Science* **2001**, *292*, 1319–1325.

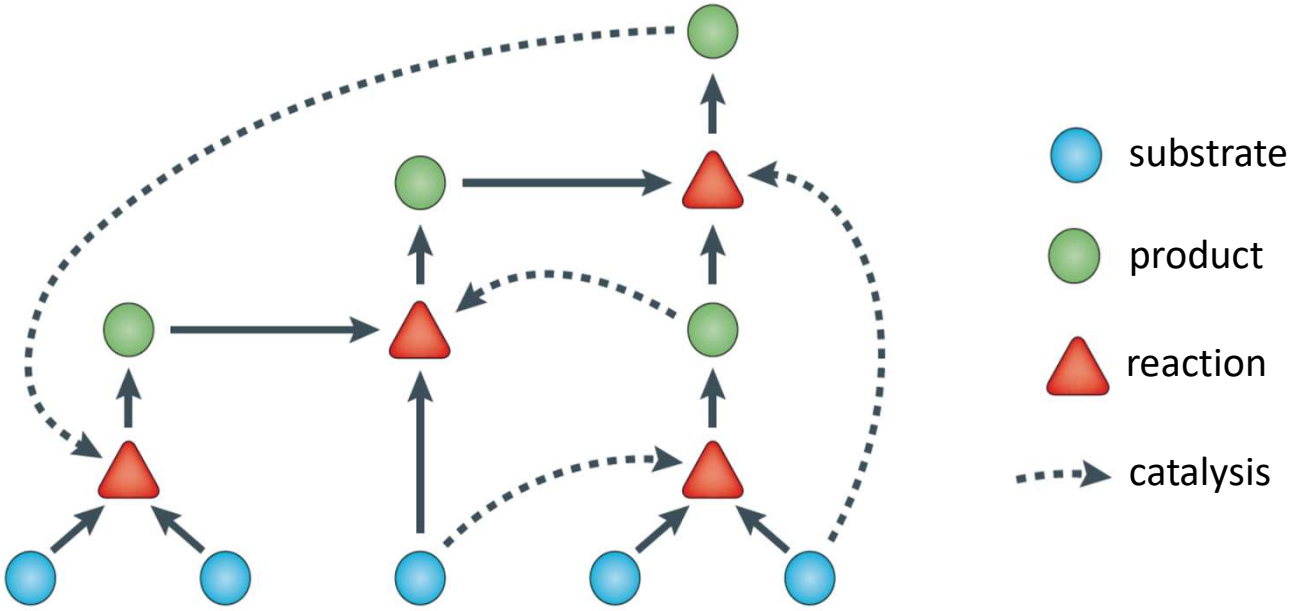
Attwater, J., Wochner, A. & Holliger, P. *Nature Chem.* **2013**, *5*, 1011–1018.

The RNA world

Replicase - problem

The replicase most likely needs to be long (> 200 nt) for the efficient replication –
How could such long fucntional RNA be spontaneously generated?

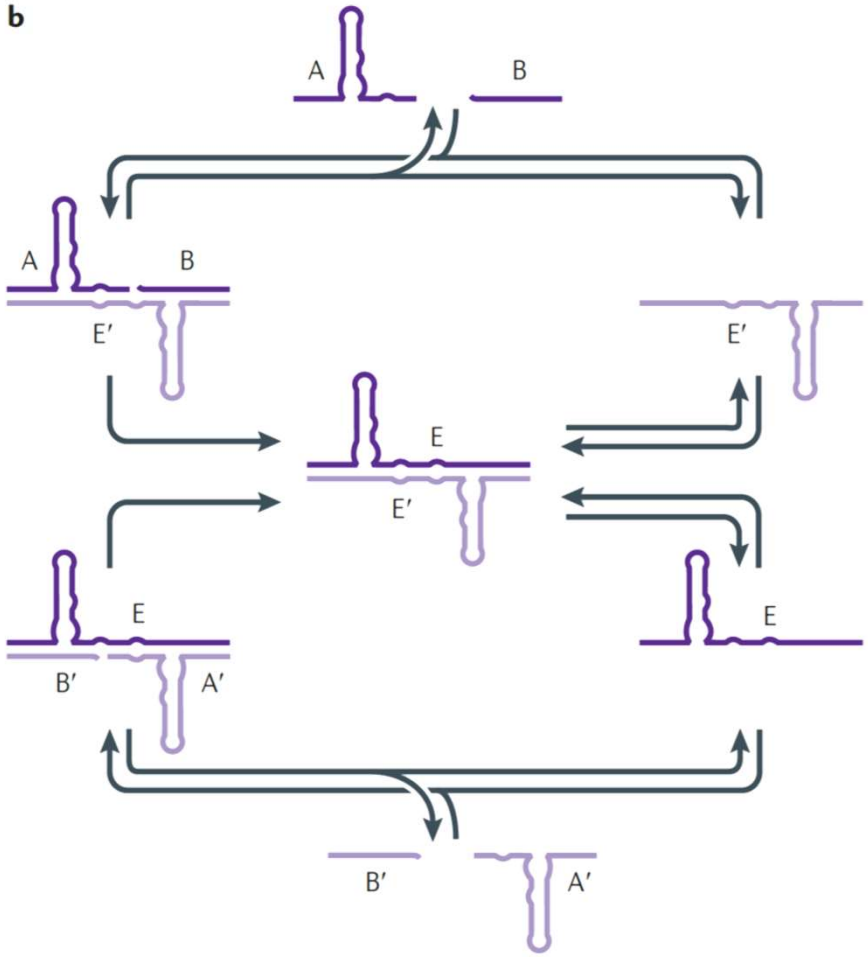
Possible solution – autocatalytic networks



No component can replicate without all the others

The RNA world

Mutually autocatalytic RNA networks

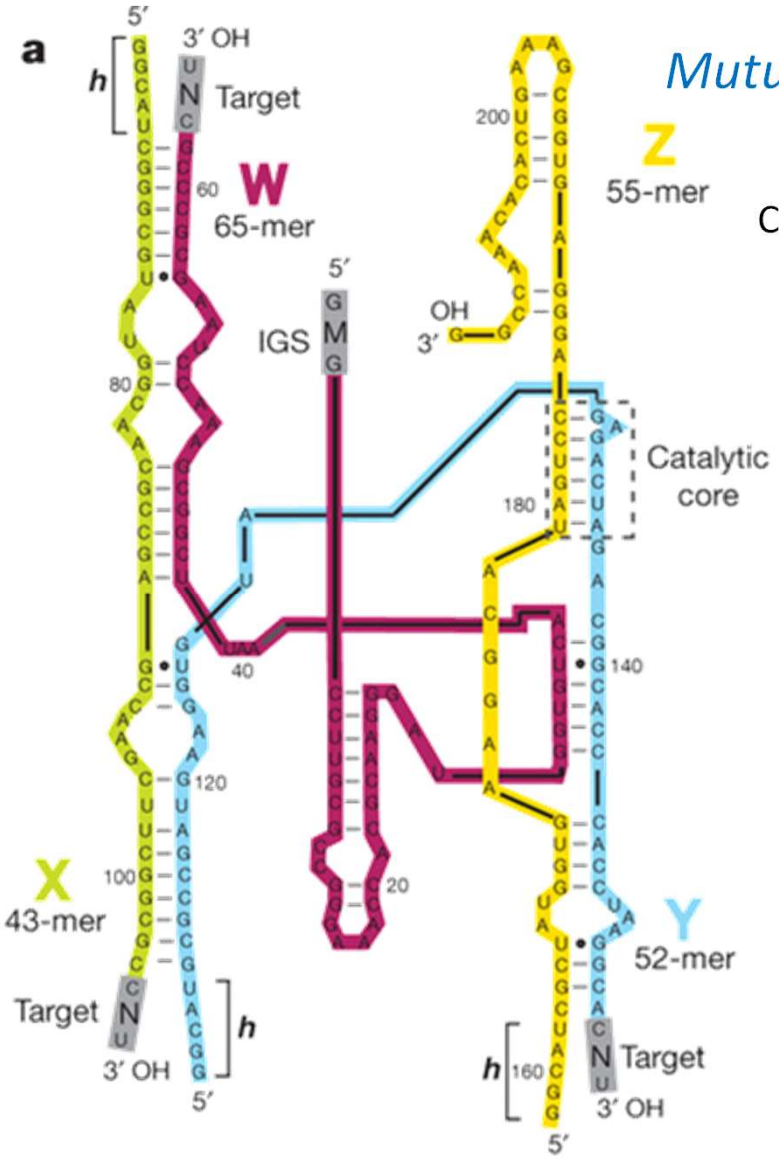


An autocatalytic set composed of two cross-catalytic ligases was demonstrated. RNA A and RNA B are ligated together by ribozyme E′ to create ribozyme E, which can reciprocate and ligate RNA A′ and RNA B′ to create ribozyme E′.

Lincoln, T. A. & Joyce, G. F. *Science* **2009**, *323*, 1229–1232.

The RNA world

Mutually autocatalytic RNA networks



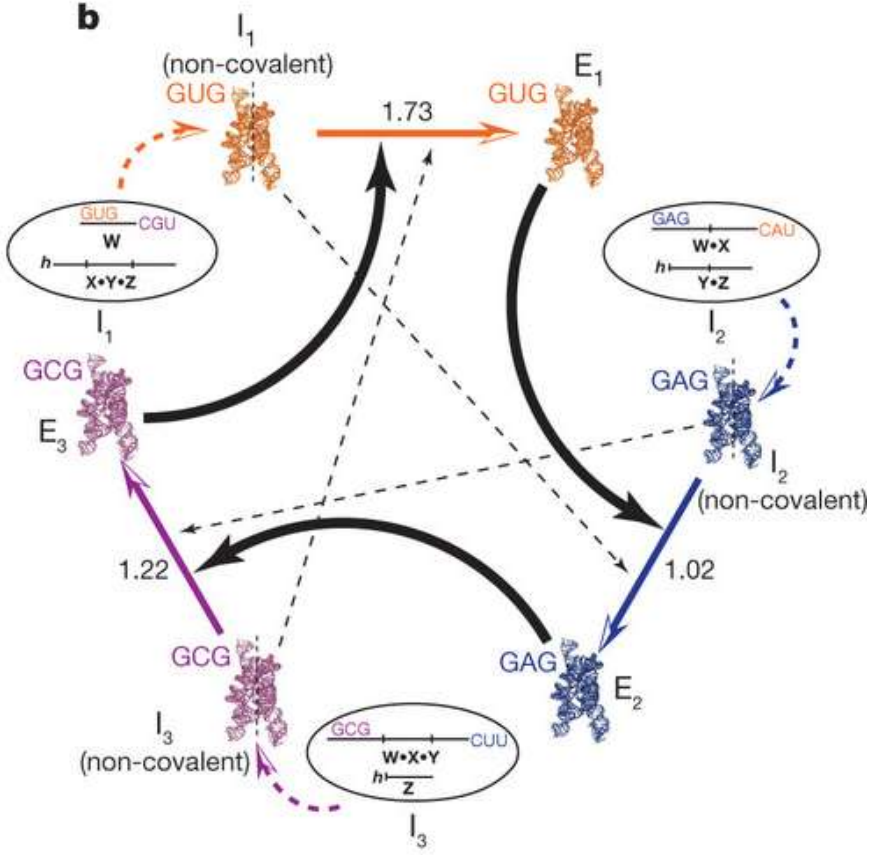
Cooperation between multiple strands that assemble to perform a single function.

Ribozymes, such as the *Azoarcus* recombinase, can be made from several short strands that assemble as a result of RNA secondary structure formation and information contained in internal guide sequences (IGSs) and complementary targets (grey).

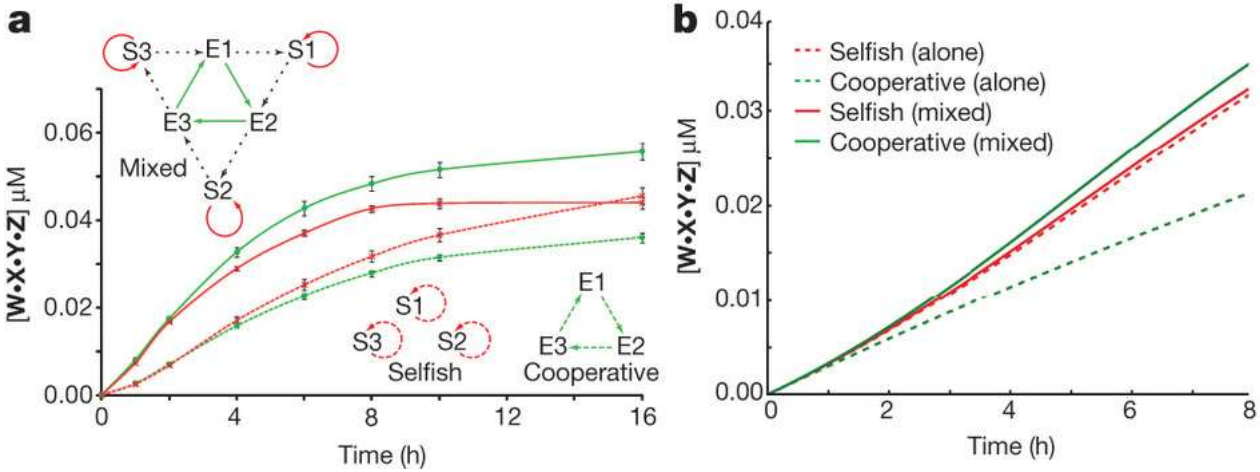
Vadia, N. *et al. Nature* **2012**, *491*, 72-77.

The RNA world

Mutually autocatalytic RNA networks



mixtures of RNA fragments that self-assemble into self-replicating ribozymes spontaneously form cooperative catalytic cycles and networks.



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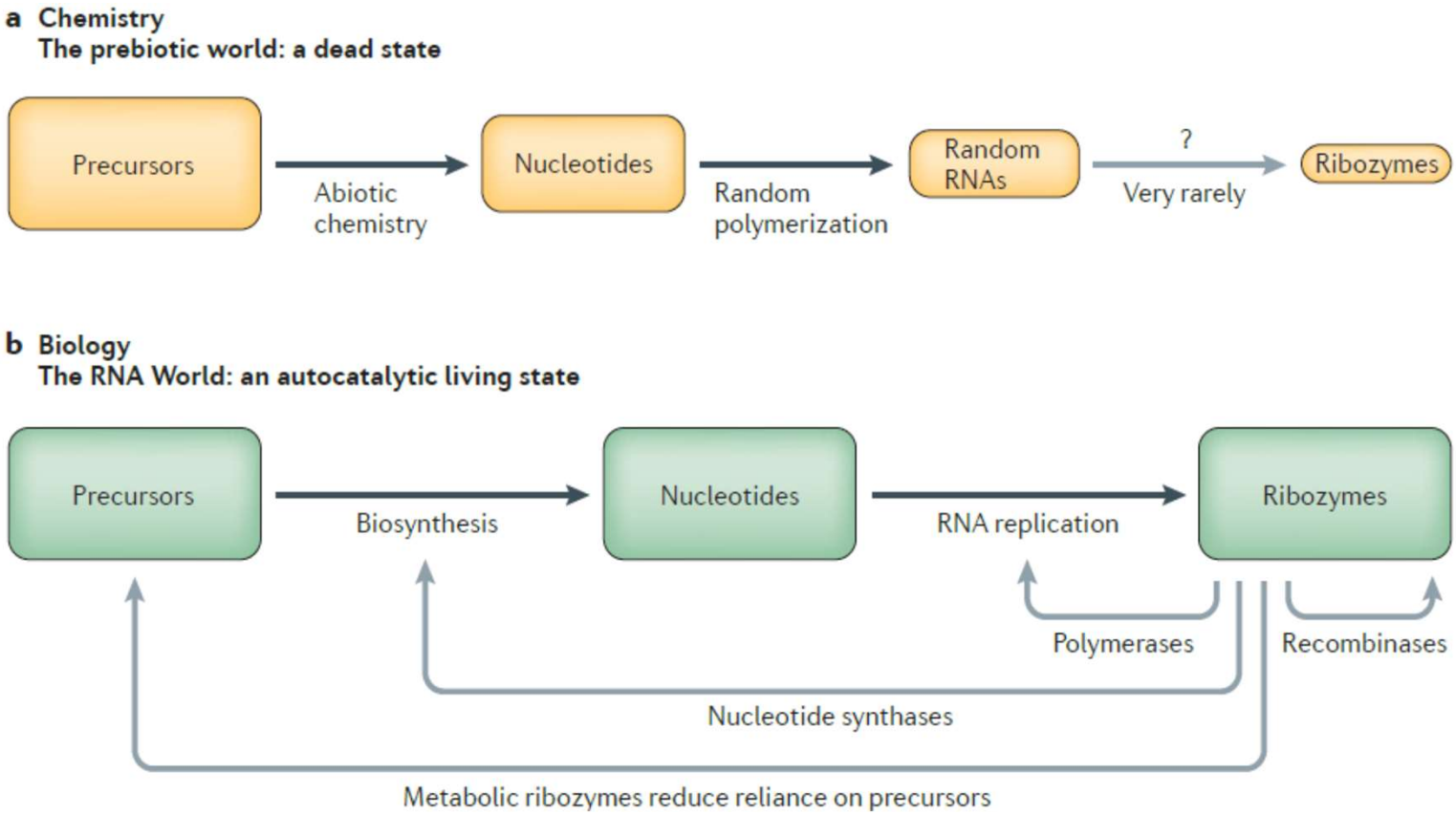
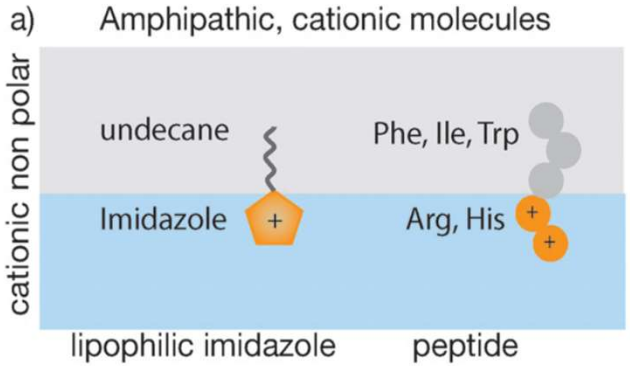
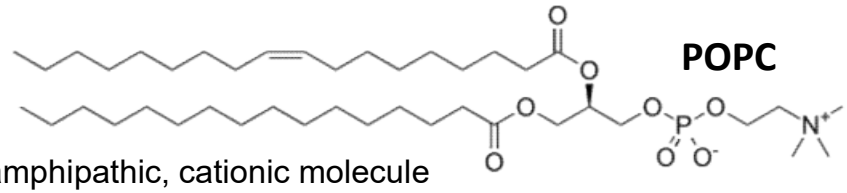
The RNA world



Transition from chemistry to biology involves autocatalytic feedbacks from ribozymes to all stages of the prebiotic chemistry

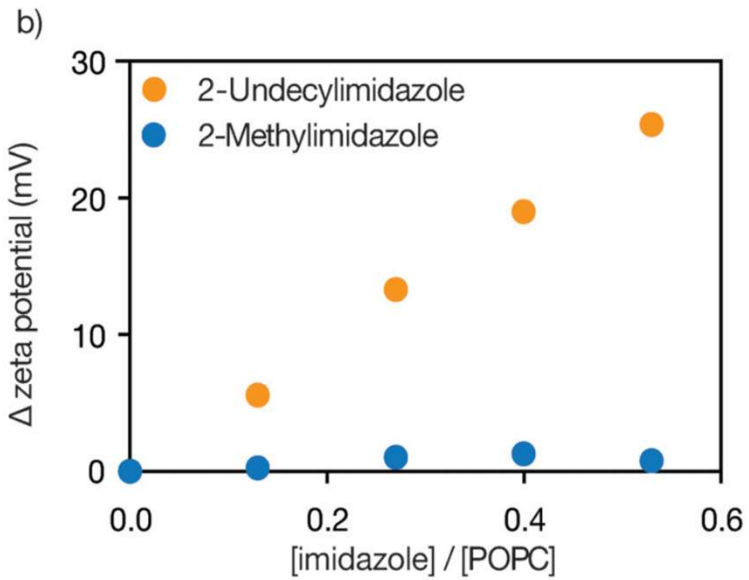
Noncovalent nucleotide association with membranes

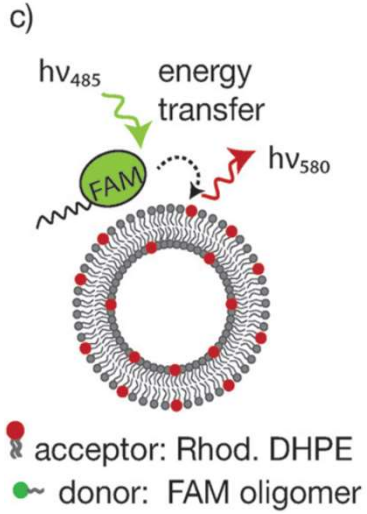


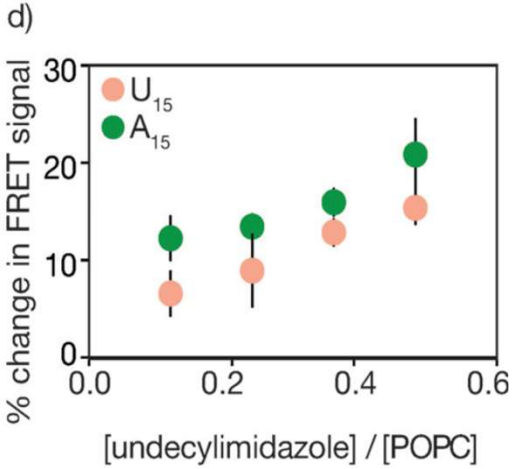


RNA localization with a model amphipathic, cationic molecule

a) Design of RNA-localizing molecules that include both nonpolar and cationic regions.
b) The change in zeta potential
c) Schematic of the FRET assay used to assess RNA localization to vesicle membranes
d) RNA (5'-FAM-U_{15} and 5'-FAM-A_{15}) shows increasing localization to POPC membranes that contain increased amounts of undecylimidazole.

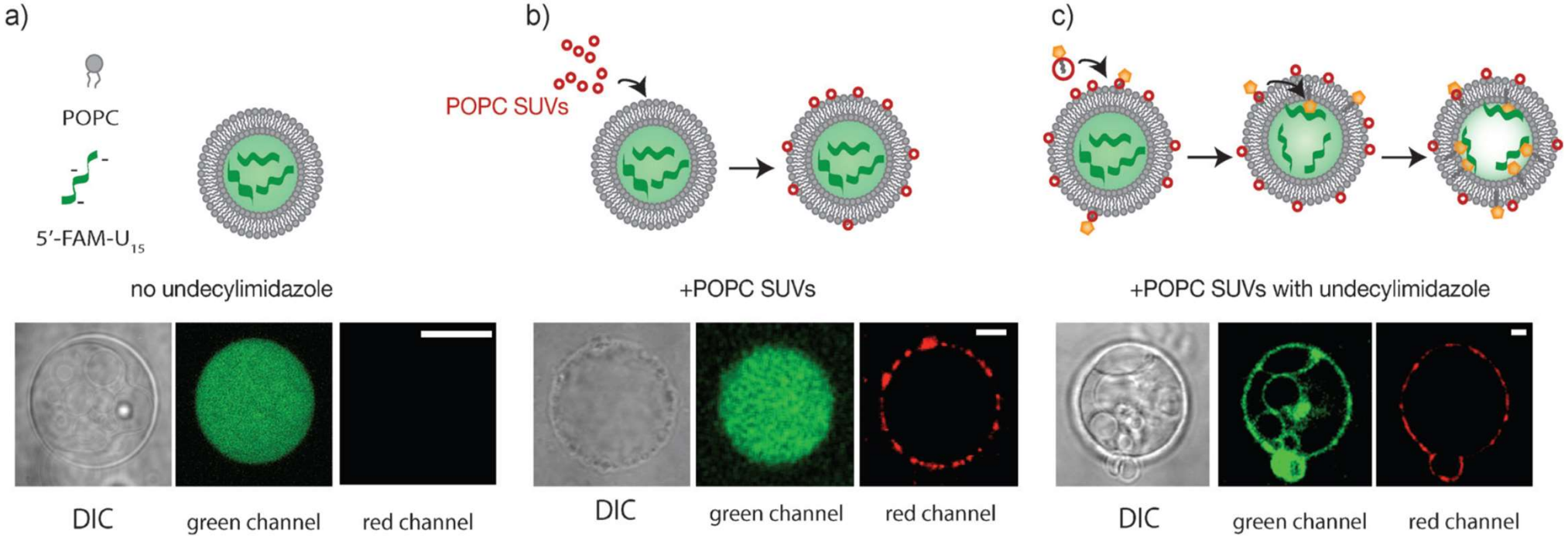






Neha P. Kamat, Sylvia Tobe, Ian T. Hill, and Jack W. Szostak *Angew. Chem. Int. Ed.* **2015**, *54*, 11735 –11739

Noncovalent nucleotide association with membranes



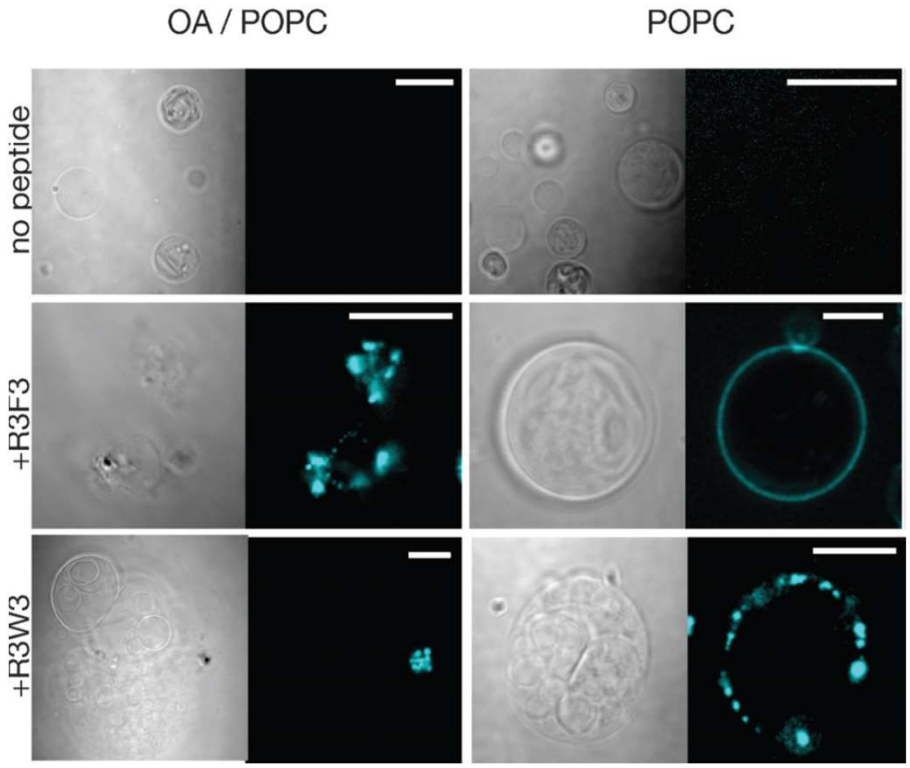
Microscopy of encapsulated RNA localization to POPC membranes with 2-undecylimidazole. Confocal images of 5'-FAM-U_{15} RNA (green) association with giant POPC vesicles membranes in the presence of 2-undecylimidazole. Differential interference contrast (DIC) microscopy images are shown for each vesicle.

a) RNA appears uniformly distributed in the interior of POPC GUVs.
b) The addition of SUVs containing a rhodamine-labeled lipid (red) leads to SUV aggregation and association with the giant vesicle membranes, but RNA (green) remains uniformly encapsulated in the vesicle interior.
c) The addition of SUVs containing a rhodamine-labeled lipid (red) and 40 mol% 2-undecylimidazole leads to SUV association with vesicle membranes and RNA (green) localizes to the vesicle surface. The scale bar is 20 mm.

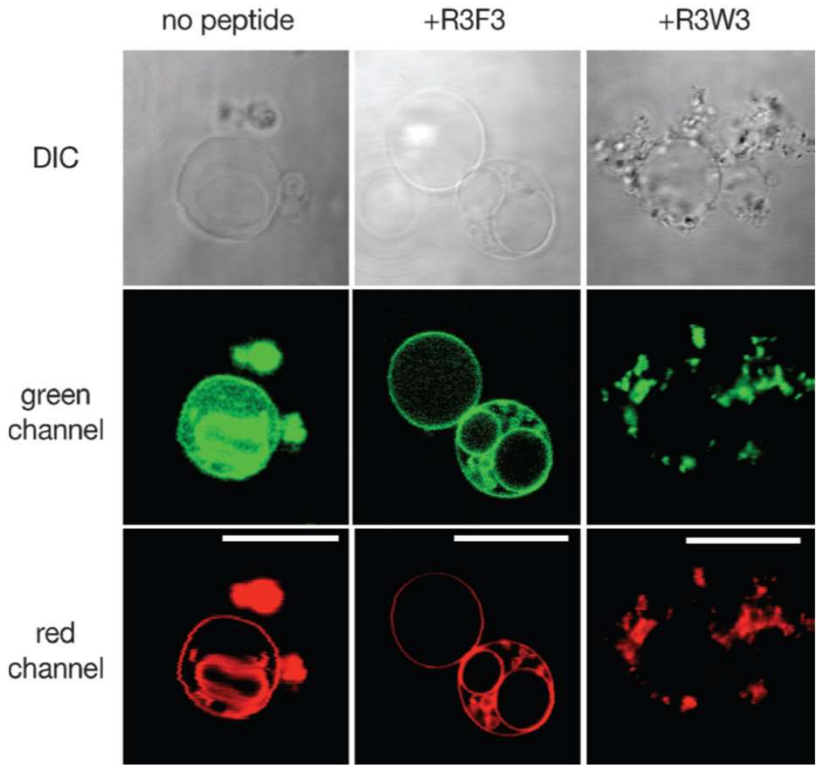
SUV – small unilamellar vescile ***GUV – giant unilamellar vescile (5-25 µm)***

Neha P. Kamat, Sylvia Tobe, Ian T. Hill, and Jack W. Szostak *Angew. Chem. Int. Ed.* **2015**, *54*, 11735 –11739

Noncovalent nucleotide association with membranes



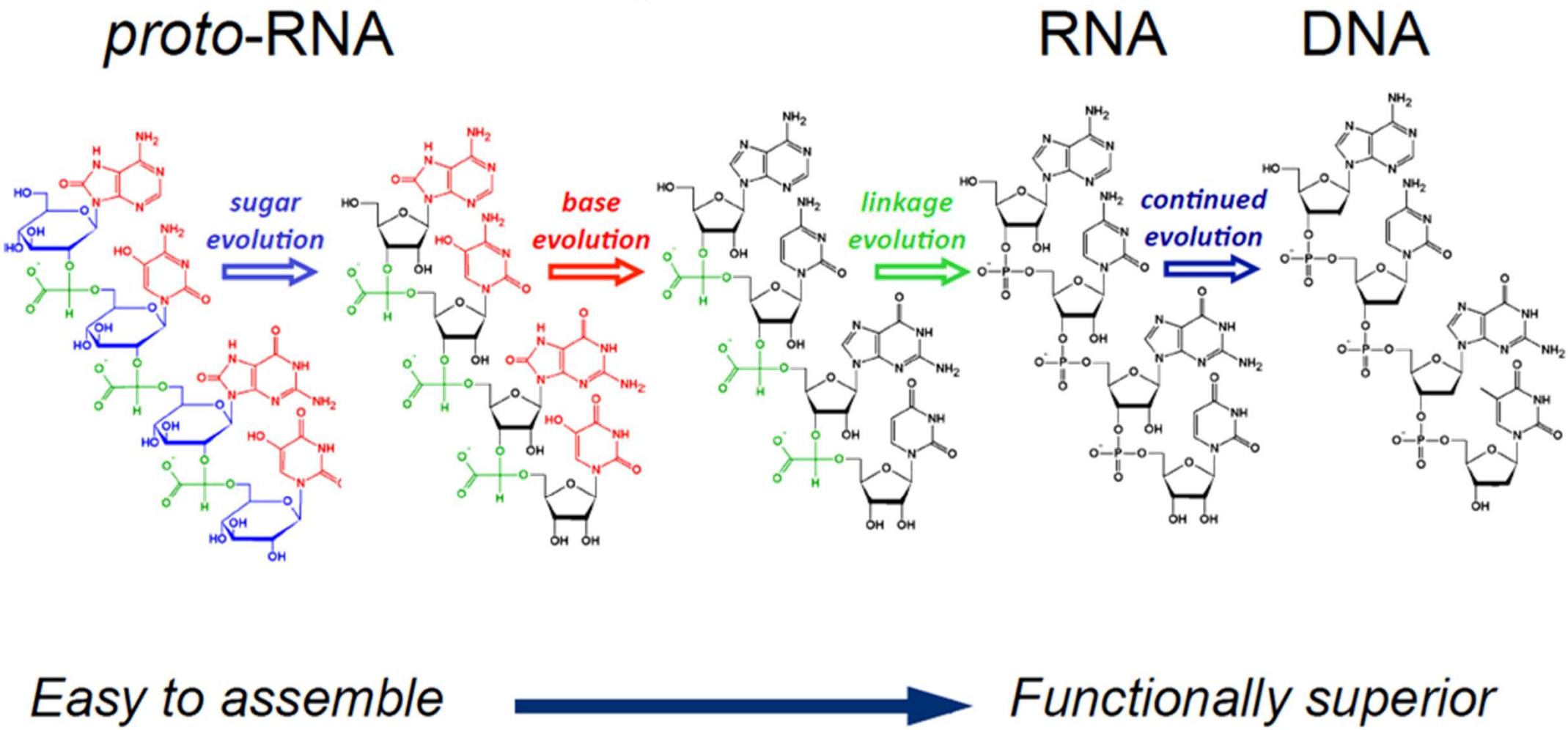
Microscopy of peptide-induced RNA–membrane association. Confocal images show RNA localization (5'-AlexaFluor647-labeled 15-mer, cyan) to the outside of oleic acid/POPC (90%/10%) and pure POPC membranes in the presence of R3F3 and R3W3 peptides. Control samples had no peptide added. For each image, the left panel shows the DIC image and the right panel shows AlexaFluor647 fluorescence. The scale bar is 20 mm.



Microscopy of encapsulated RNA localization to POPC membranes with peptides. Confocal images show that RNA (5'-FAMU$_{15}$, green) encapsulated in POPC vesicles (containing a rhodaminelabeled lipid, red) becomes localized to the membrane of certain vesicles after an overnight incubation with R3F3 and R3W3 peptides. The scale bar is 20 mm.

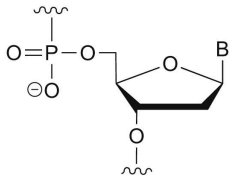
Neha P. Kamat, Sylvia Tobe, Ian T. Hill, and Jack W. Szostak *Angew. Chem. Int. Ed.* **2015**, *54*, 11735 –11739

„RNA-second”

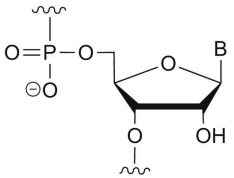


Proto-RNA evolution: According to the protoRNA theory, each of the components of RNA — sugar, base and phosphate backbone — may have originally taken different forms.

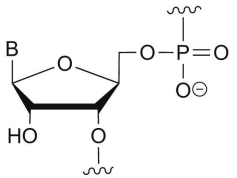
Artificial genetic polymers



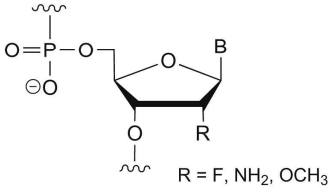
DNA



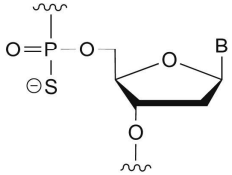
D-RNA
(natural)



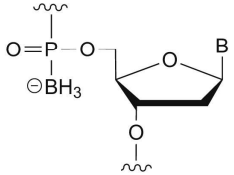
L-RNA
(unnatural)



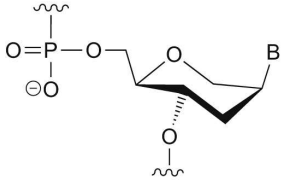
2'-modified RNA



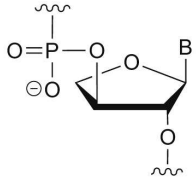
Phosphorothioate



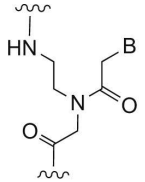
Boranophosphate



Hexitol Nucleic Acid
(HNA)

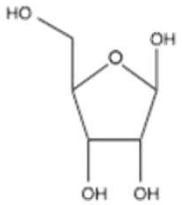


Threose Nucleic Acid
(TNA)

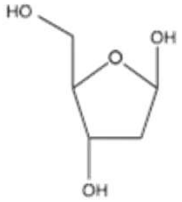


Peptide Nucleic Acid
(PNA)

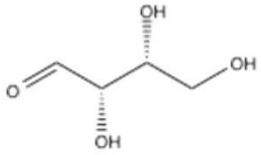
XNA – Xeno Nucleic Acids



Ribose



Deoxyribose



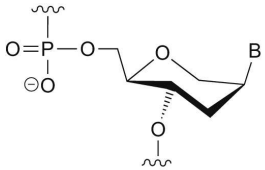
Threose

Cyclohexene

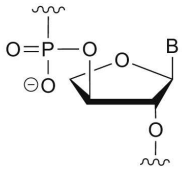
Glycol

Locked

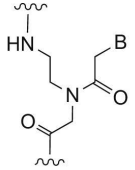
XNA – Xeno Nucleic Acids



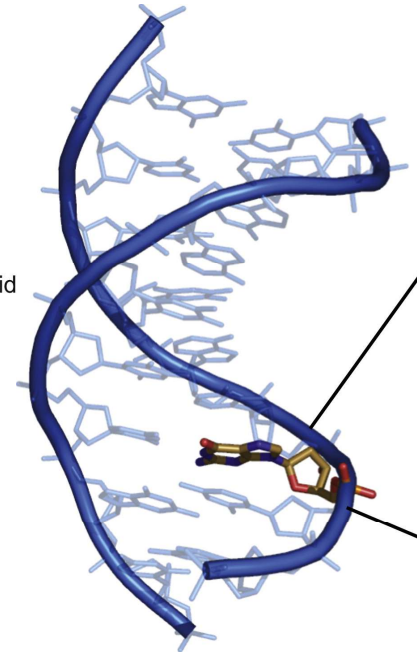
Hexitol Nucleic Acid
(HNA)

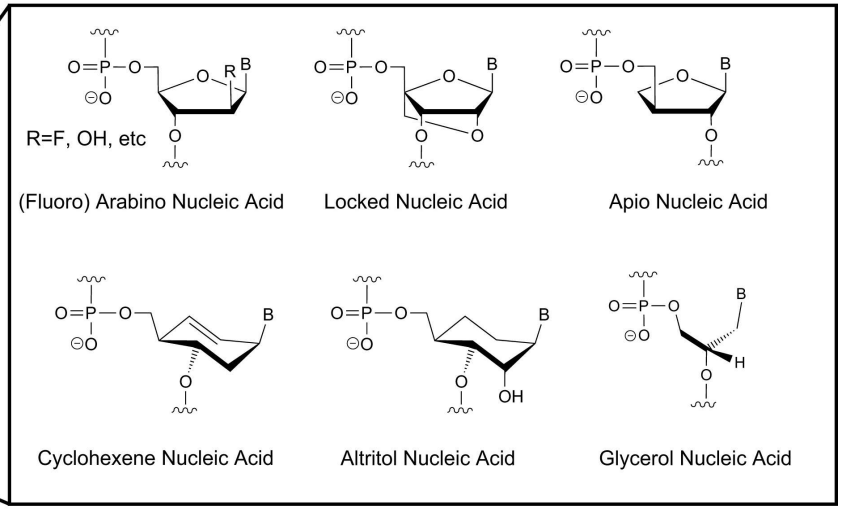


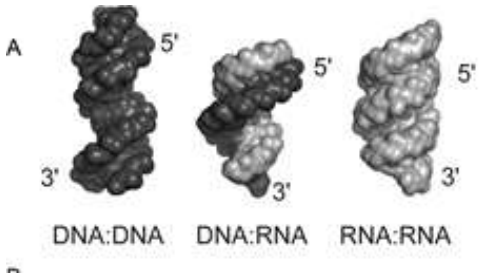
Threose Nucleic Acid
(TNA)

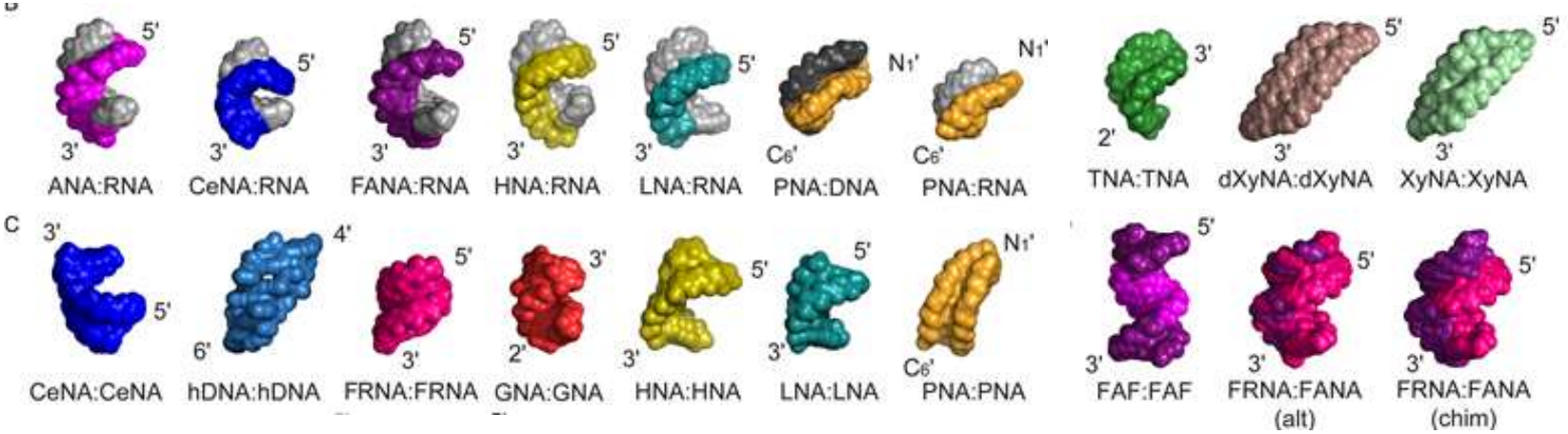


Peptide Nucleic Acid
(PNA)

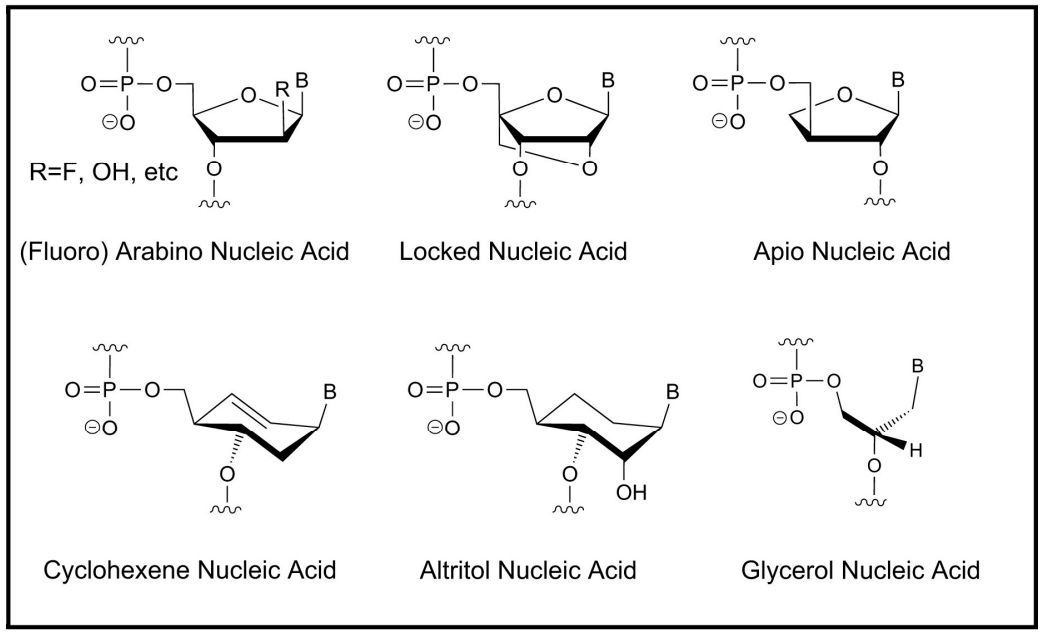


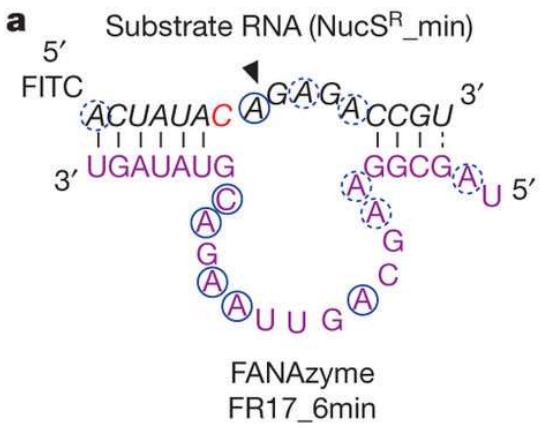


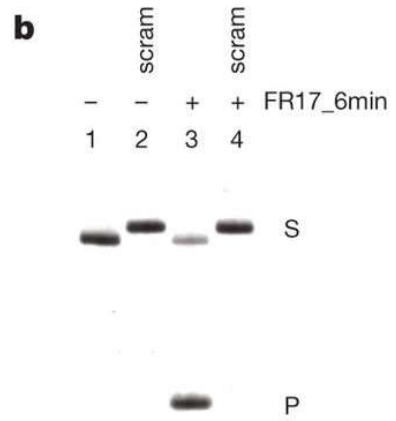


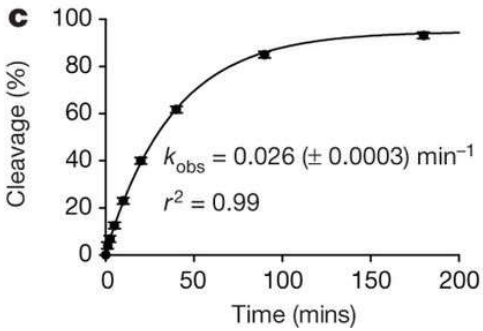


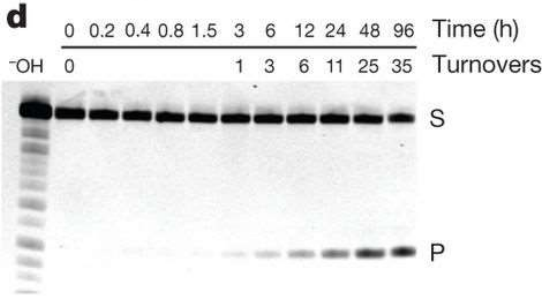
Chemical synthesis yields an active RNA endonuclease XNAzyme







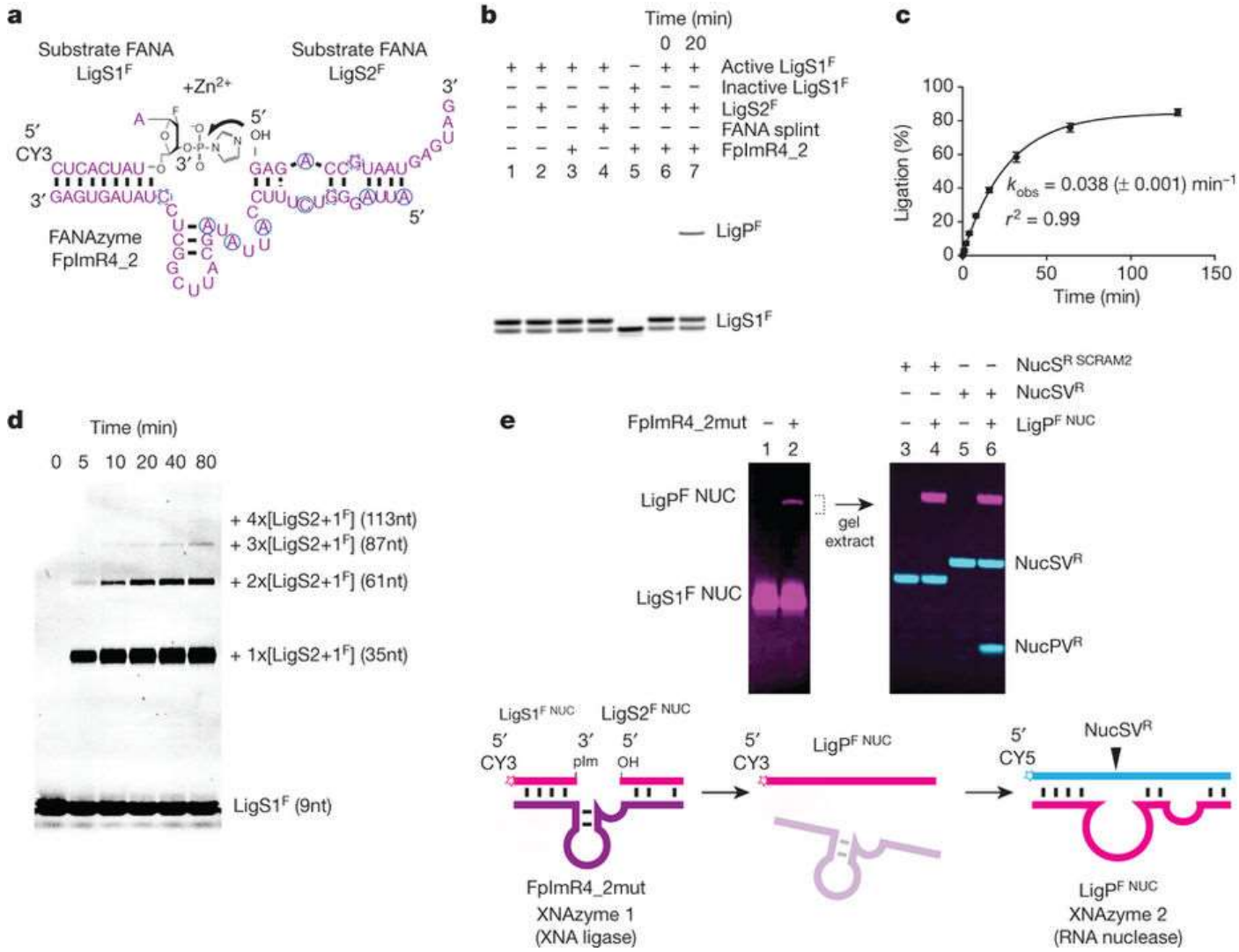




a, Secondary structure of truncated FANAzyme FR17_6 (FR17_6min, purple)
b, FR17_6min synthesized using FANA phosphoramidites cleaves cognate RNA substrate (NucSR_min; lanes 1 and 3), but not a scrambled RNA (NucSR SCRAM2; lanes 2 and 4), with...
c, essentially unchanged catalytic rate (k_{obs}) at 25 °C.
d, FR17_6min (10 nM) can perform multiple turnover cleavage of RNA NucSR_min (1 µM).

P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

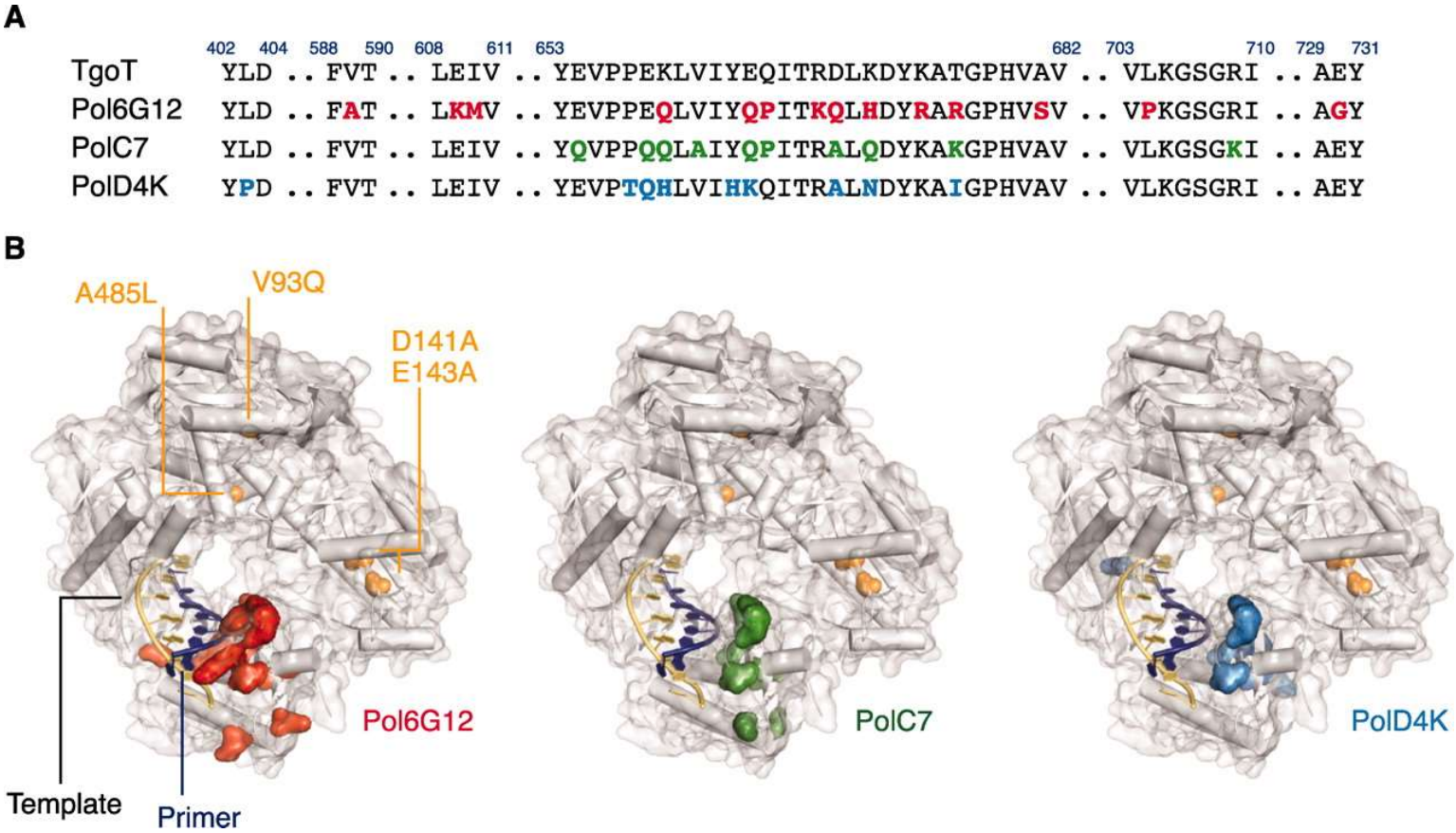
XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids

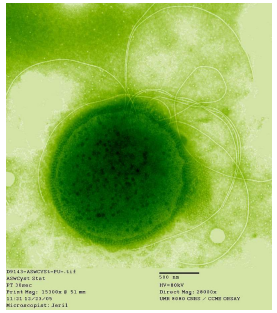


P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

Engineering XNA polymerases

TgoT, a variant of the replicative polymerase of *Thermococcus gorgonarius*





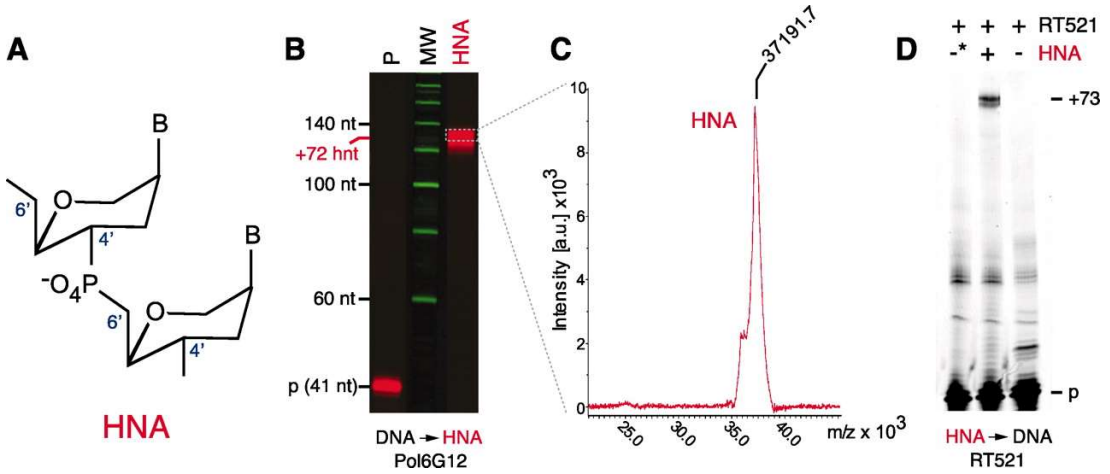
Thermococcus gorgonarius

(Angels Tapias)

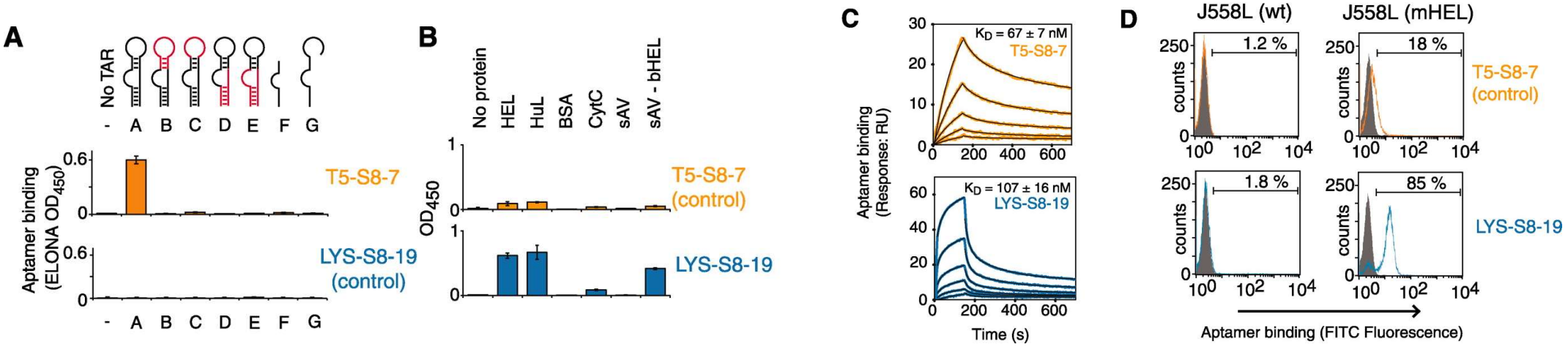
(A) Sequence alignments showing mutations from wtTgo in polymerases Pol6G12 (red), PolC7 (green), and PolD4K (blue).
(B) Mutations are mapped on the structure of Pfu (PDB: 4AIL).
Yellow - template; dark blue - primer; orange - mutations present in the parent polymerase TgoT

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336,* 341-344

DNA-templated HNA synthesis and HNA-templated DNA synthesis



HNA aptamers



P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336,* 341-344