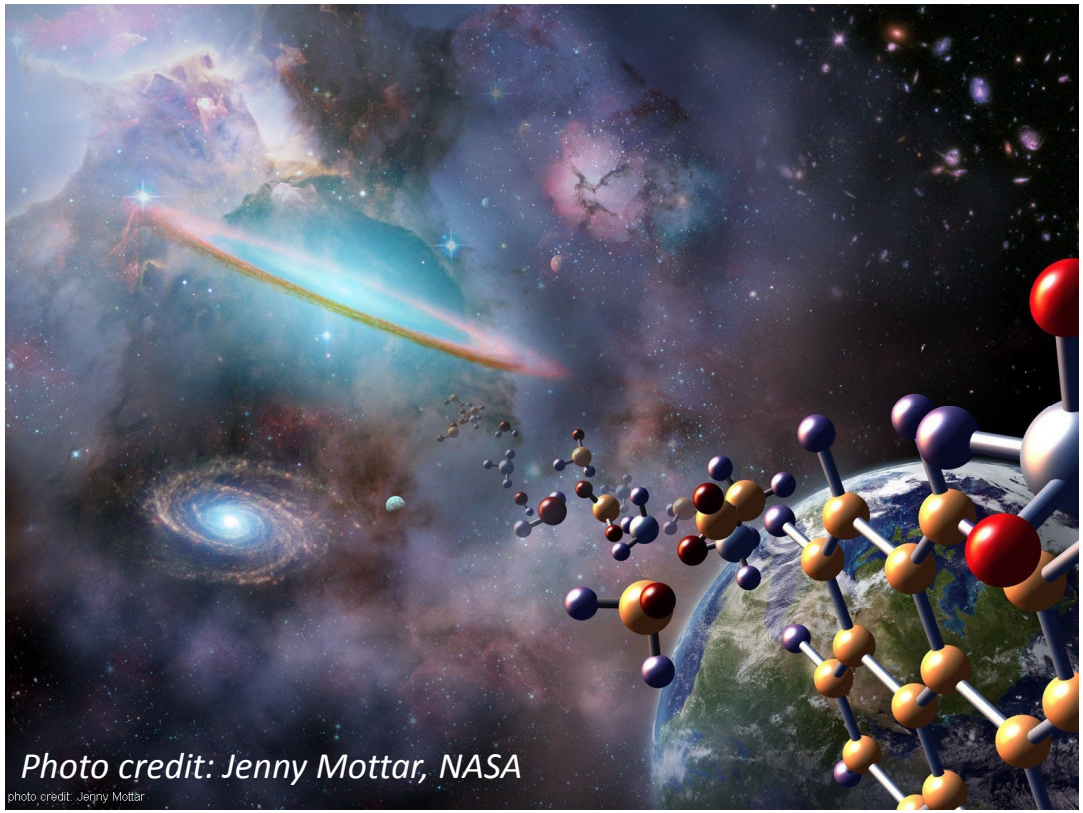
The molecular origins of life



L6 SoSe 2020

Zbigniew Pianowski

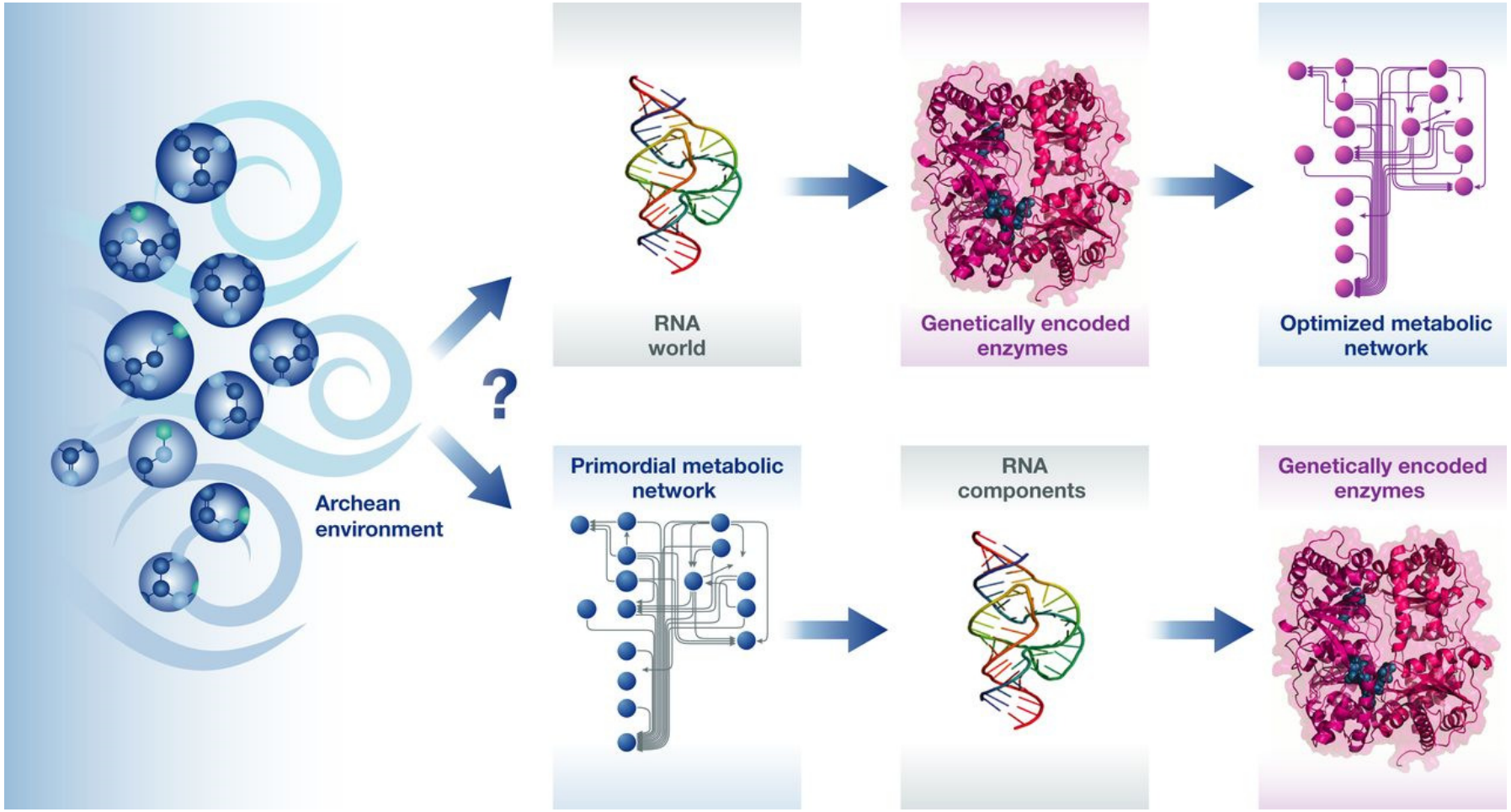
Origin of the Universe – stars, planets, elements

Origin of biorelevant monomers – primordial soup

Complex chemical processes on the way to living systems

Protocells and LUCA

Route to life by chemical networks



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

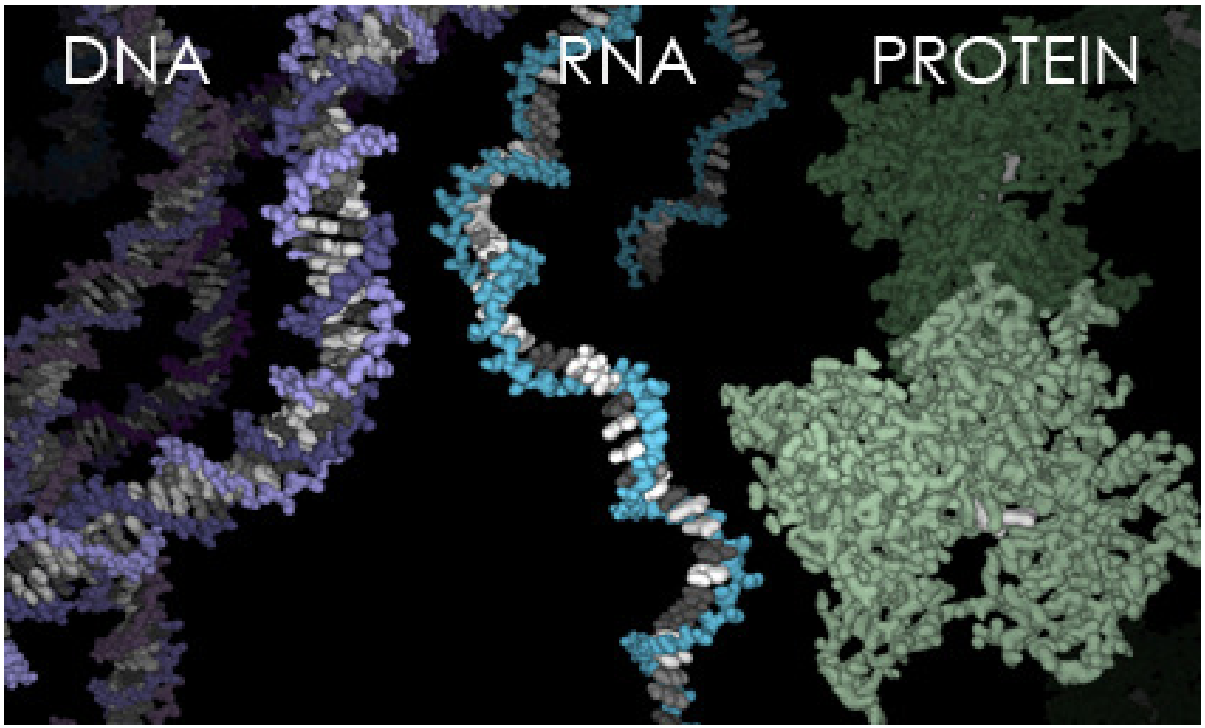
Metabolism-first vs. Genes-first

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.

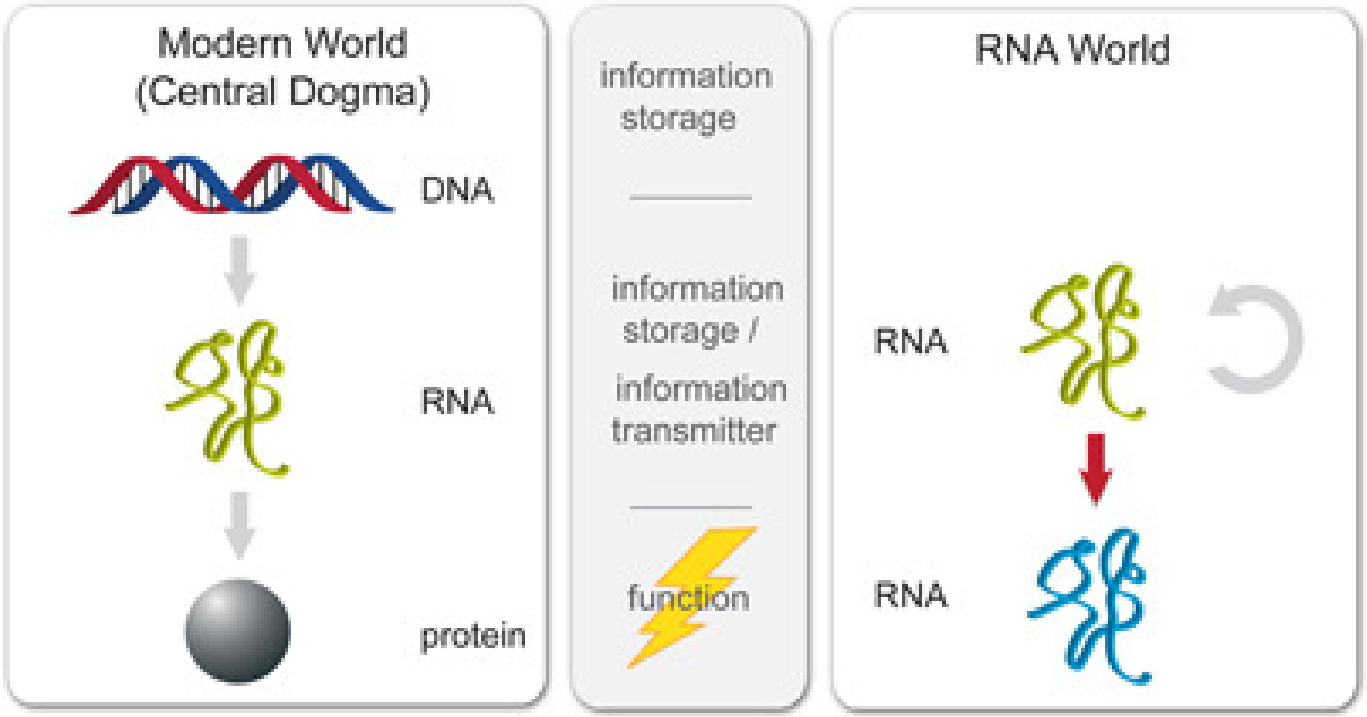
„Genes-first”



In modern cells, RNA (light blue, center) is made from a DNA template (purple, left) to create proteins (green, right).

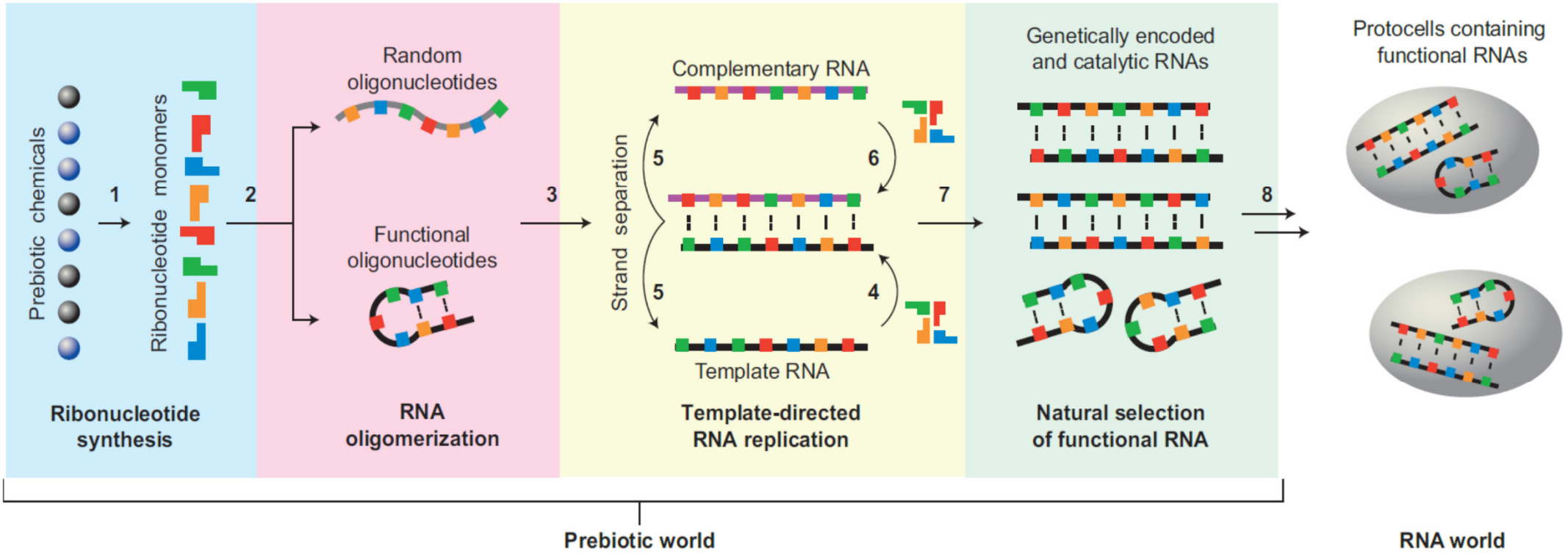
RNA folding is mediated by base-pairing interactions along different regions of a single-stranded RNA.

The RNA world

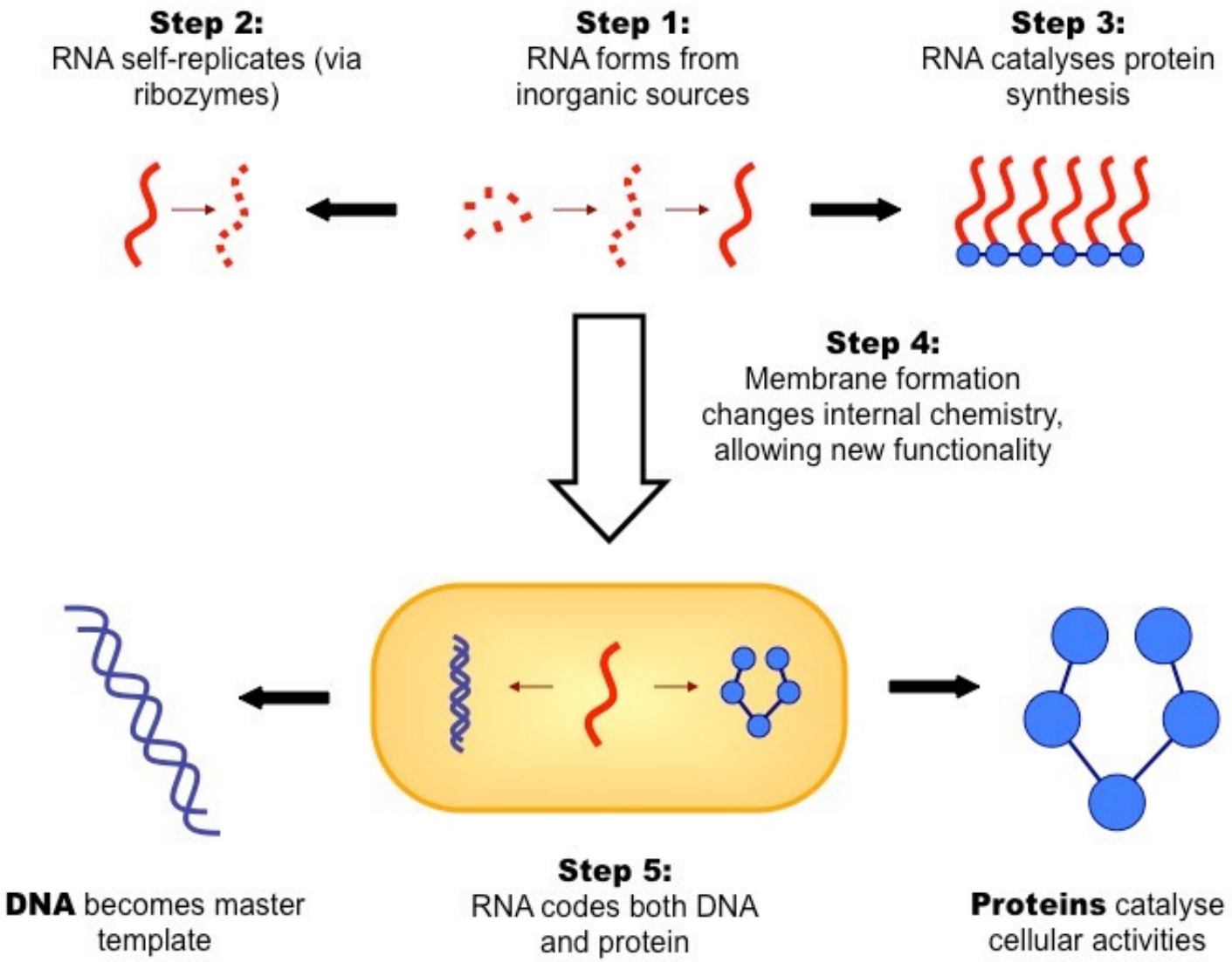


Conceptual idea that there was a period in the early history of life on Earth when RNA (or its structurally simplified analogue) carried out most of the information processing and metabolic transformations needed for biology to emerge from chemistry

The RNA world



The RNA world

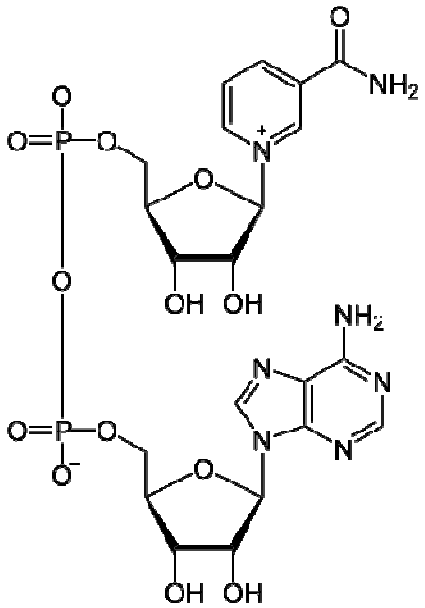


The RNA world

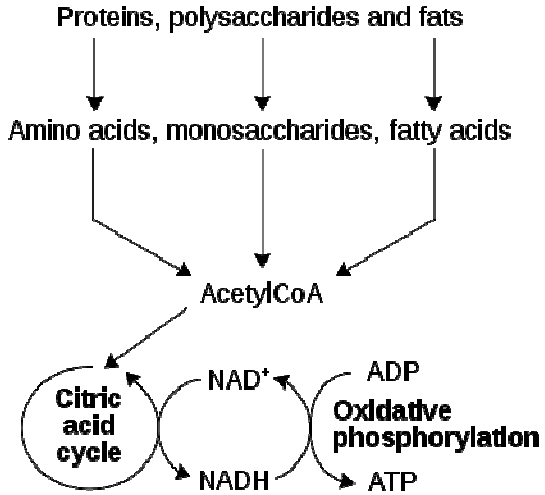
Crick, Orgel and Woese speculated in 1968 that, because RNA can form secondary structures, it has both a genotype and a phenotype and is a good candidate for the emergence of life

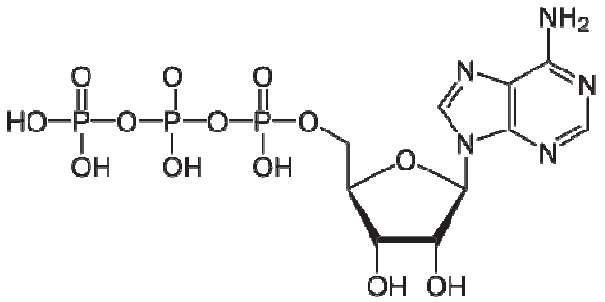
F. H. C. Crick *J. Mol. Biol.* **1968**, *38,* 367-379, L. E. Orgel *J. Mol. Biol.* **1968**, *38,* 381-393

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



Nicotinamide adenine dinucleotide (**NAD⁺**)



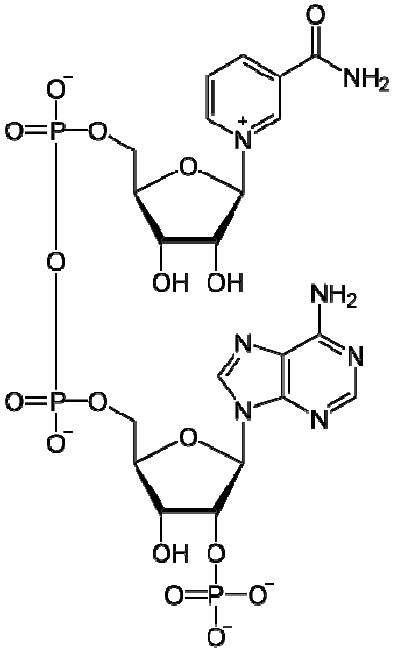


Adenosine triphosphate (**ATP**)

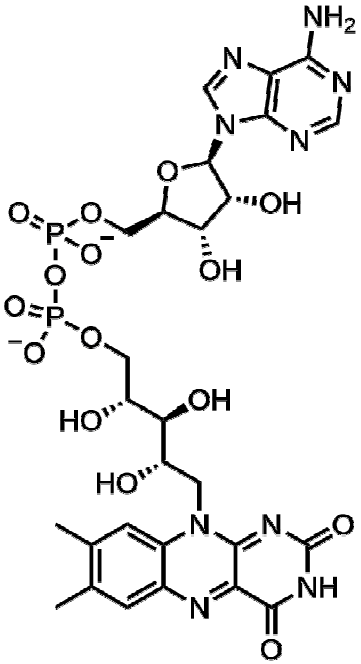
H. B. White III *J. Mol. Evol.* **1976**, *7,* 101-104

The RNA world

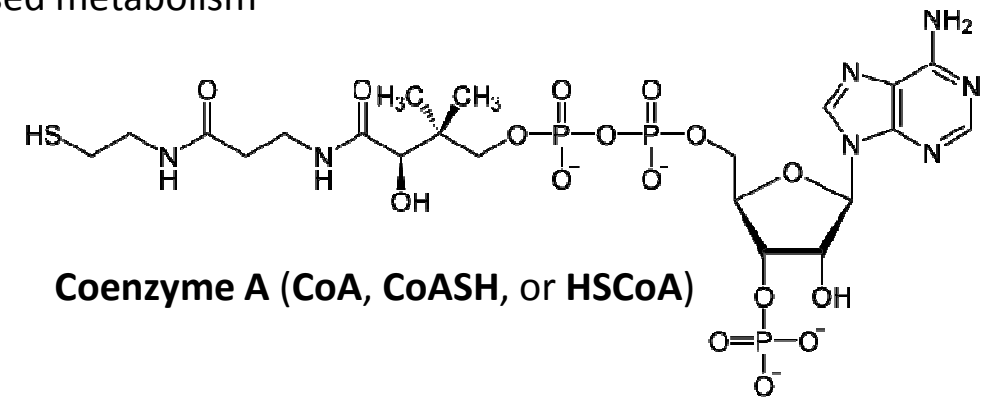
Ribonucleotide coenzymes now used by many proteins may be molecular „fossils” from the primoridal RNA-based metabolism



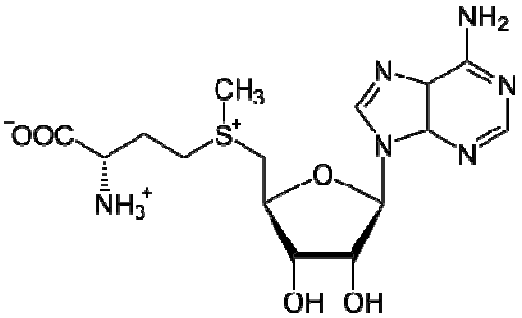
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)



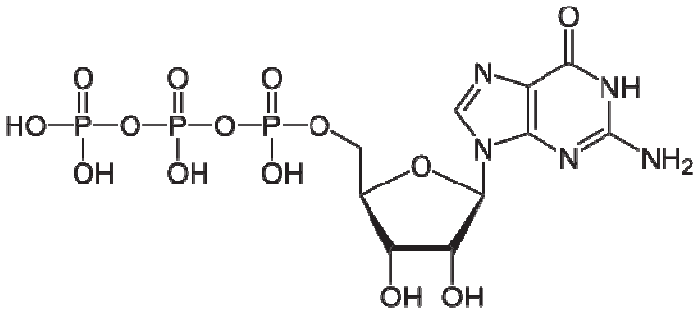
flavin adenine dinucleotide (FAD)



Coenzyme A (CoA, CoASH, or HSCoA)



***S*-Adenosyl methionine**

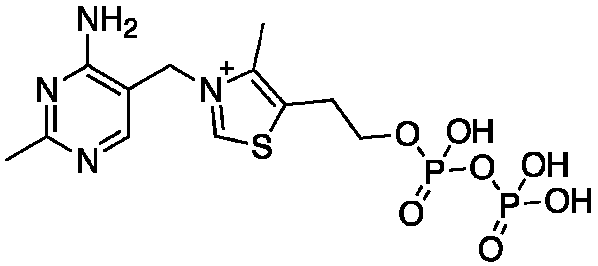


Guanosine-5'-triphosphate (GTP)

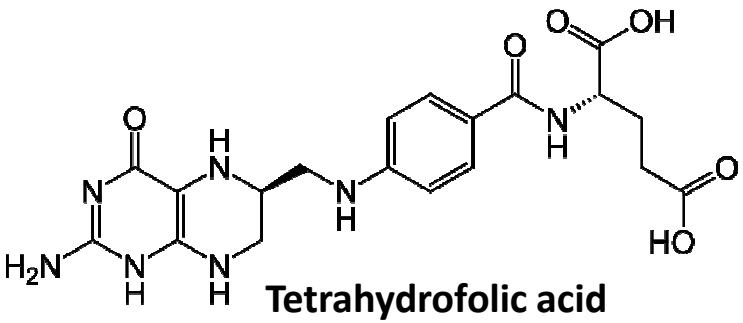
H. B. White III *J. Mol. Evol.* **1976**, *7*, 101-104

The RNA world

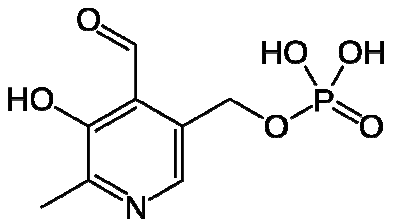
Other coenzymes contain cyclic nitrogen-containing bases that can also derive from nucleotides



Thiamine pyrophosphate (TPP or ThPP) – Vit. B_1



Tetrahydrofolic acid



Pyridoxal phosphate (PLP) – Vit. B_6

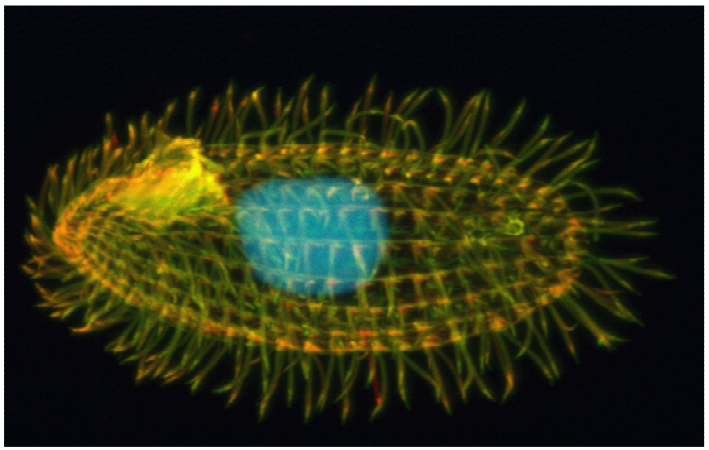
H. B. White III *J. Mol. Evol.* **1976**, *7*, 101-104

The RNA world

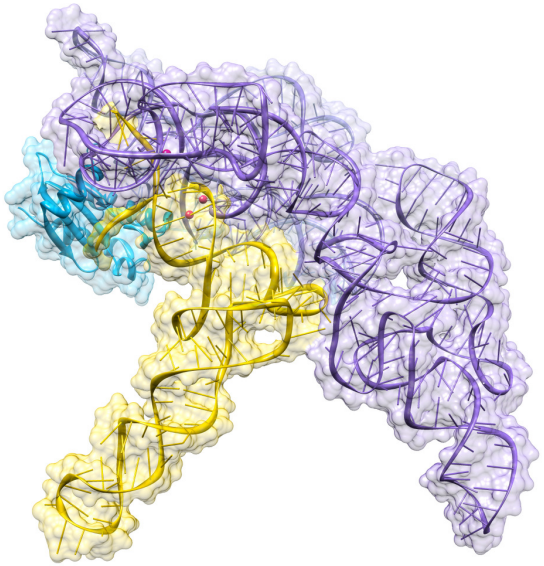
Ribozymes – Ribonucleic acid enzymes

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.



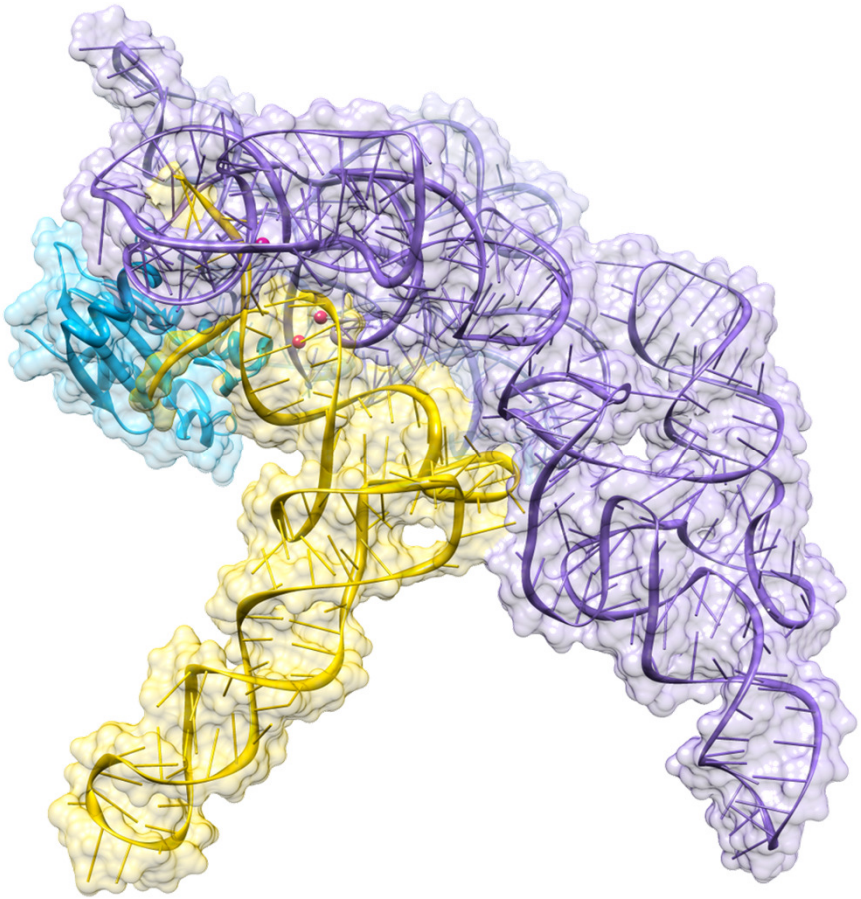
Tetrahymena thermophila



Bacterial RNAse P

The RNA world

Ribonuclease P

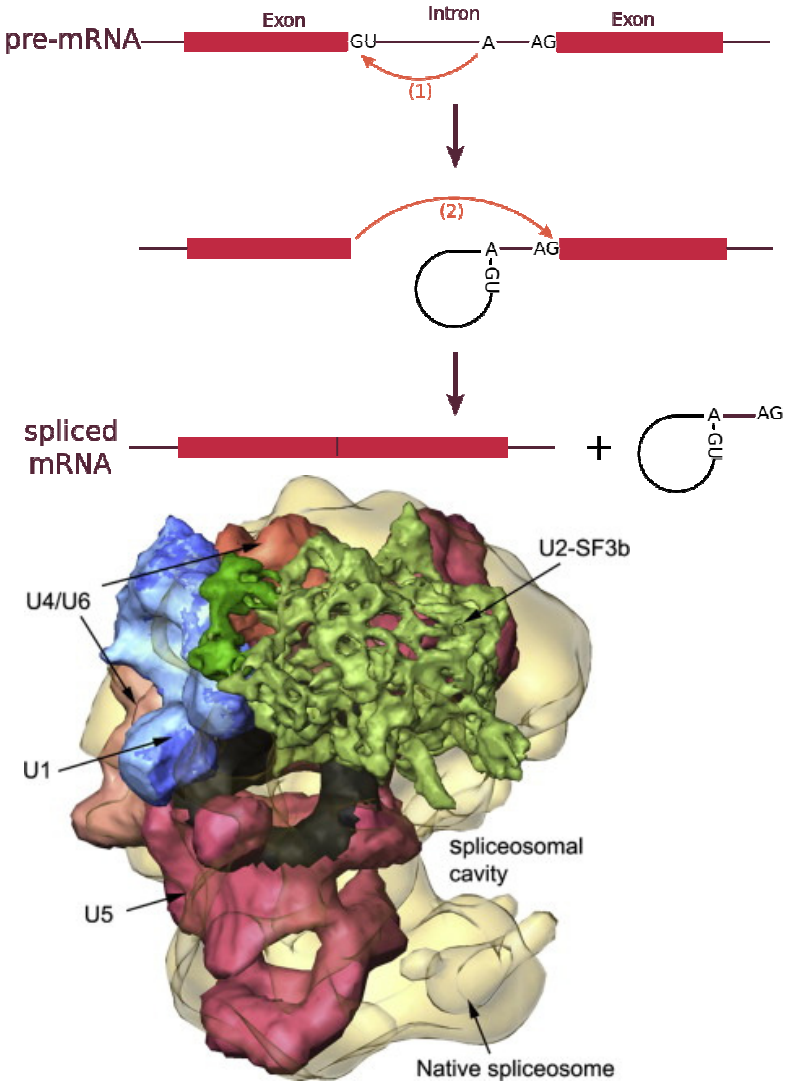


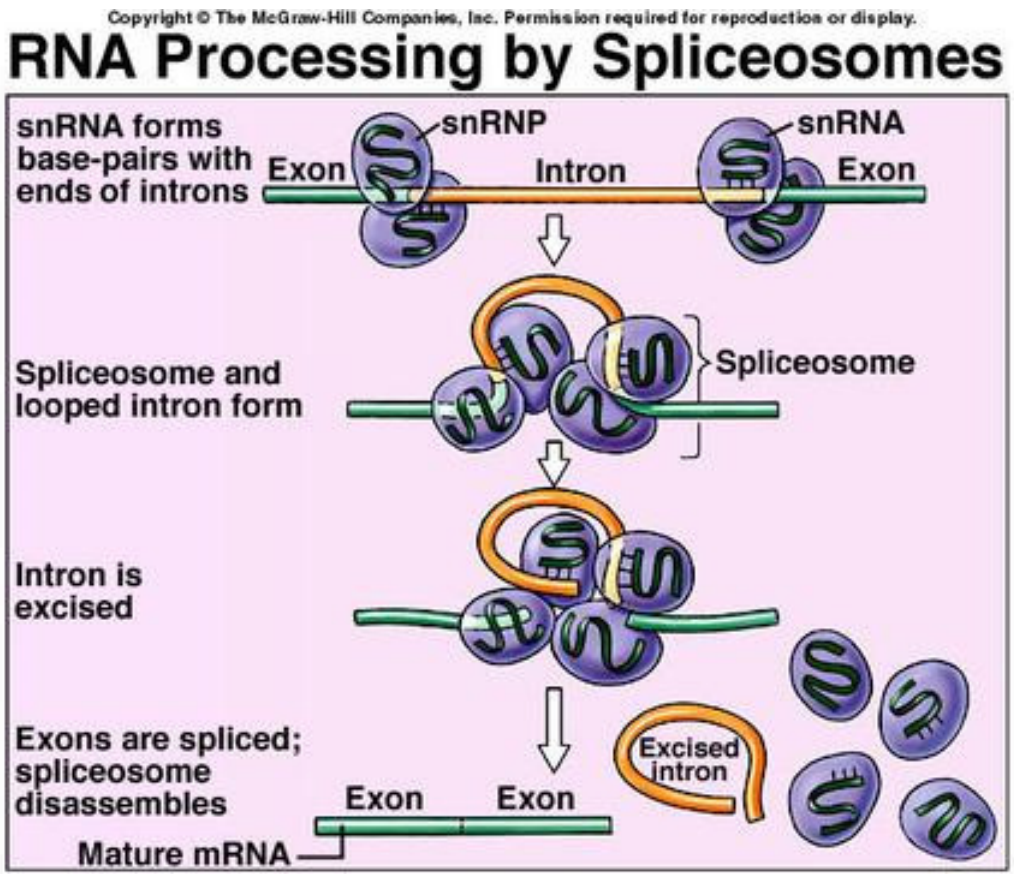
Ribonuclease P (RNase P) is a type of ribonuclease which cleaves RNA. RNase P is unique from other RNases in that it is a ribozyme – a ribonucleic acid that acts as a catalyst in the same way that a protein based enzyme would. Its function is to cleave off an extra, or precursor, sequence of RNA on tRNA molecules.

Bacterial RNase P has two components: an RNA chain, called M1 RNA, and a polypeptide chain, or protein, called C5 protein. *In vivo*, both components are necessary for the ribozyme to function properly, but *in vitro*, the M1 RNA can act alone as a catalyst. The primary role of the C5 protein is to enhance the substrate binding affinity and the catalytic rate of the M1 RNA enzyme probably by increasing the metal ion affinity in the active site.

Crystal structure of a bacterial ribonuclease P holoenzyme in complex with tRNA (yellow), showing metal ions involved in catalysis (pink)

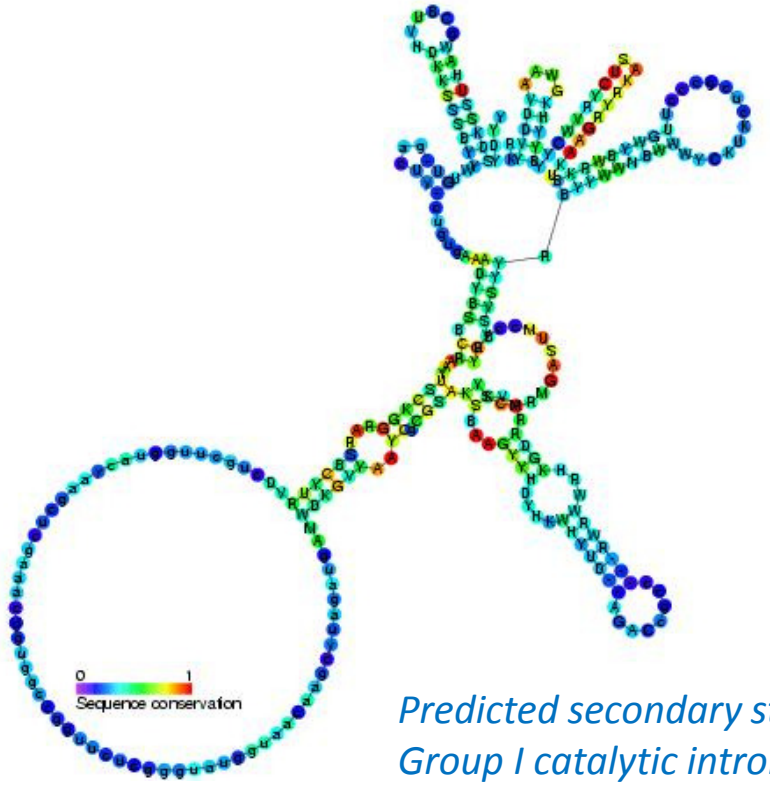
RNA splicing





Spliceosome – a complex of ribonucleoproteins

RNA splicing

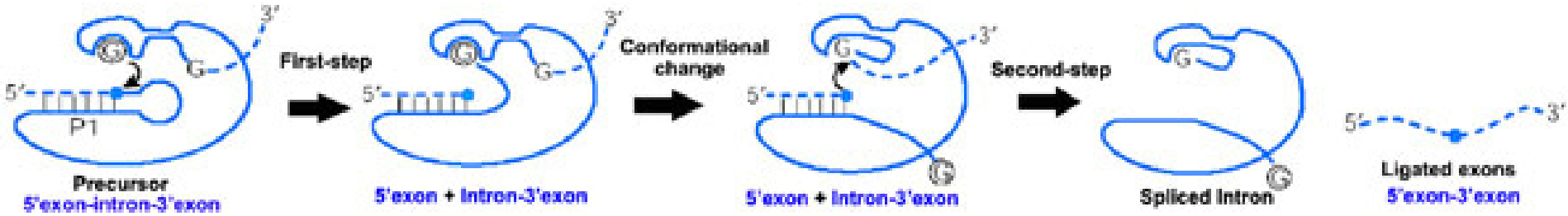


Predicted secondary structure and sequence conservation of Group I catalytic intron

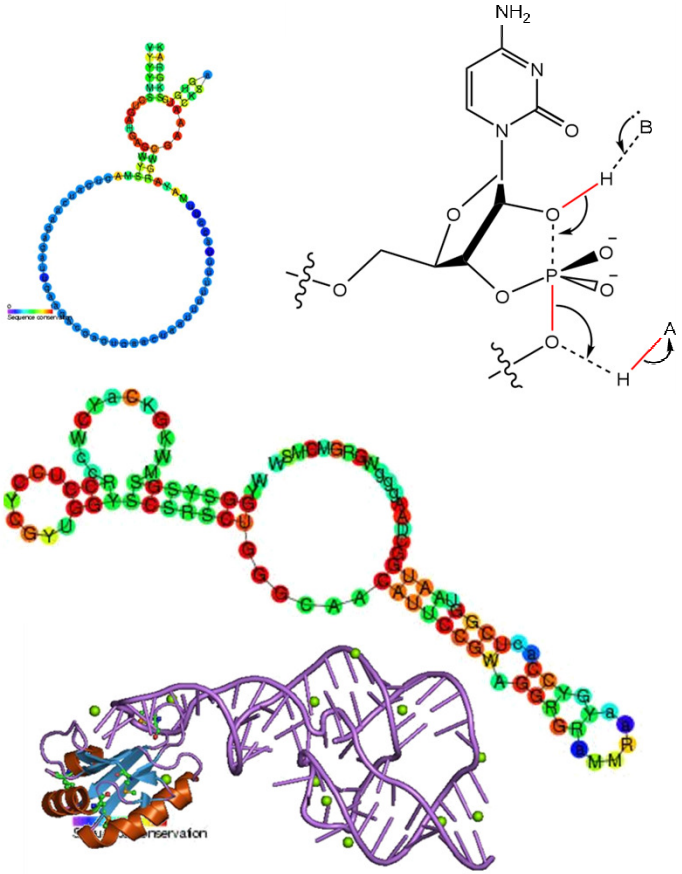
Self-splicing RNA introns

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



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)

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.

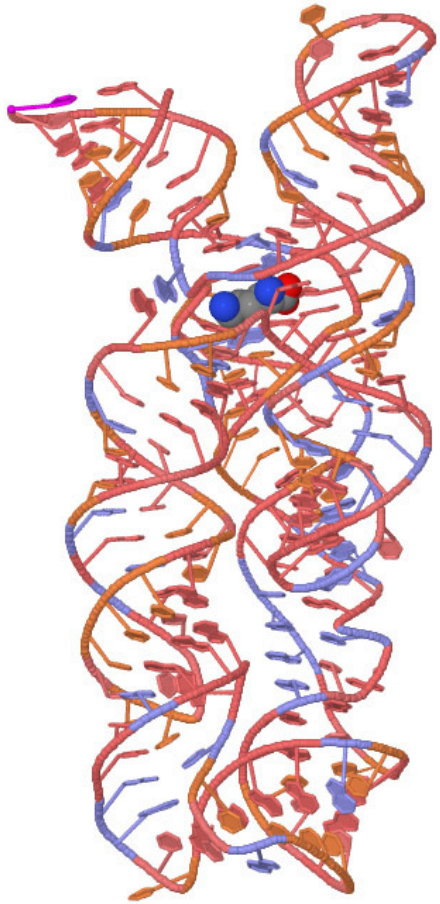
The ribozyme acts to process the RNA transcripts to unit lengths in a self-cleavage reaction. The ribozyme is found to be active in vivo in the absence of any protein factors and is the fastest known naturally occurring self-cleaving RNA.

Riboswitches

A riboswitch is a regulatory segment of a messenger RNA molecule that binds a small molecule, resulting in a change in production of the proteins encoded by the mRNA (bacteria, TPP riboswitch also in plants and funghi)

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

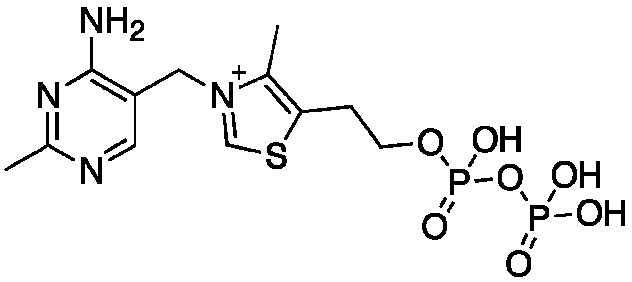
Before discovery of ***riboswitches*** only ***proteins*** were supposed to do so in the biological context.

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

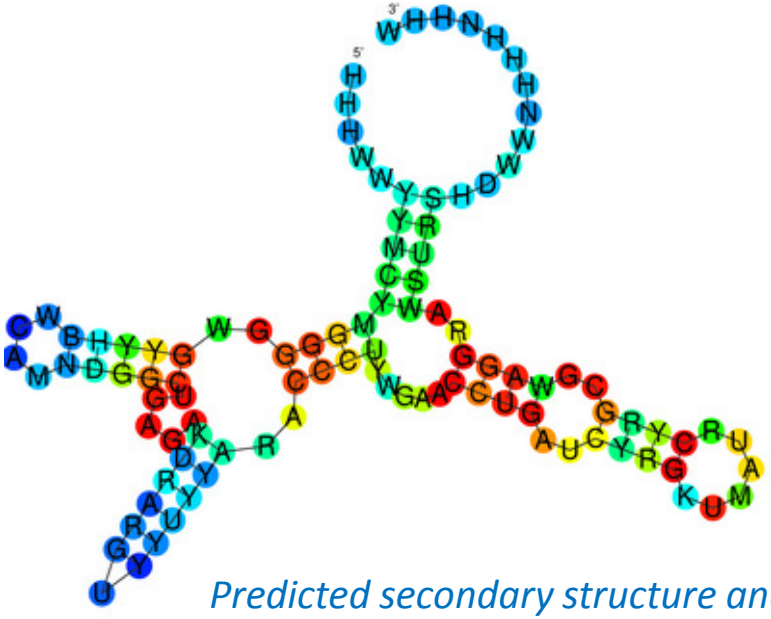
Riboswitches exist in all domains of life, and therefore are likely that they might represent ancient regulatory systems or fragments of ***RNA-world ribozymes*** whose binding domains remained conserved throughout the evolution

The TPP Riboswitch

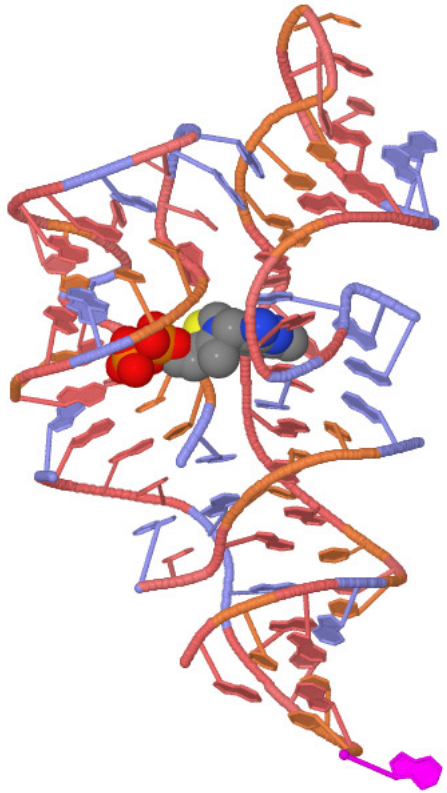
The **TPP riboswitch** (THI element and Thi-box riboswitch), is a highly conserved RNA secondary structure. It binds directly to thiamine pyrophosphate (TPP, a form of the vitamin B1, an essential coenzyme) to regulate gene expression through a variety of mechanisms in archaea, bacteria and eukaryotes.



Thiamine pyrophosphate TPP



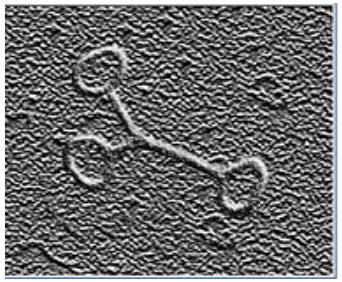
Predicted secondary structure and sequence conservation of TPP riboswitch



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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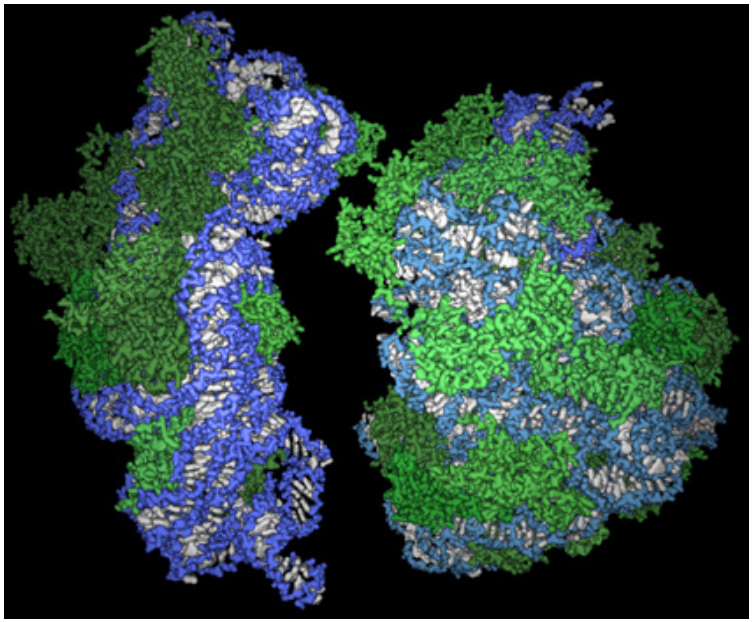
PSTVd-infected potatoes (right)

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

Ribosome – the ‚smoking gun'



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.

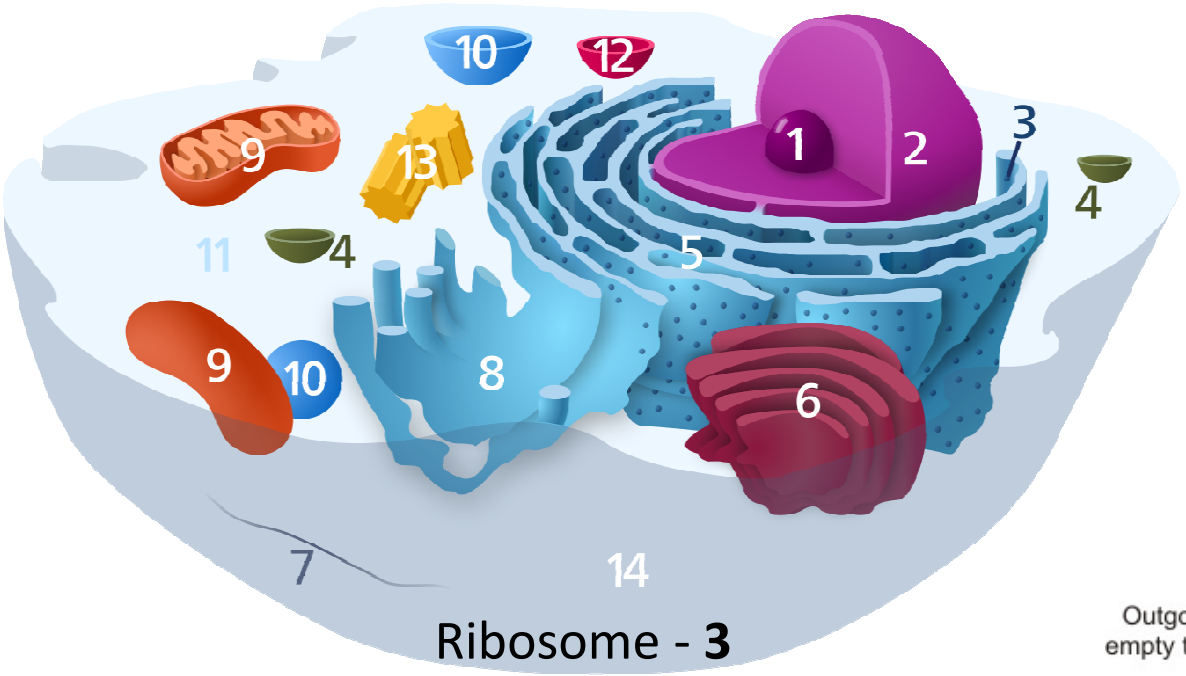
Ribosome is structurally highly conserved among all living species – most likely present in LUCA

Ribosomes:

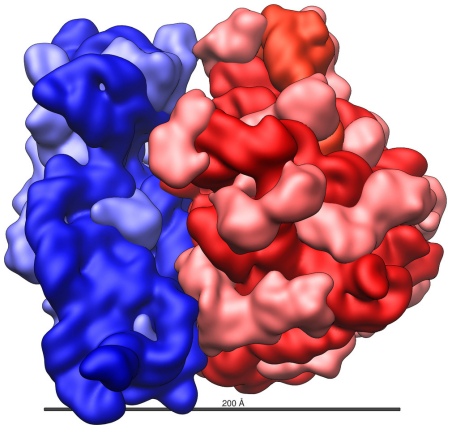
- the *small ribosomal subunit*, which reads the RNA
- the *large subunit*, which joins amino acids to form a polypeptide chain.

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

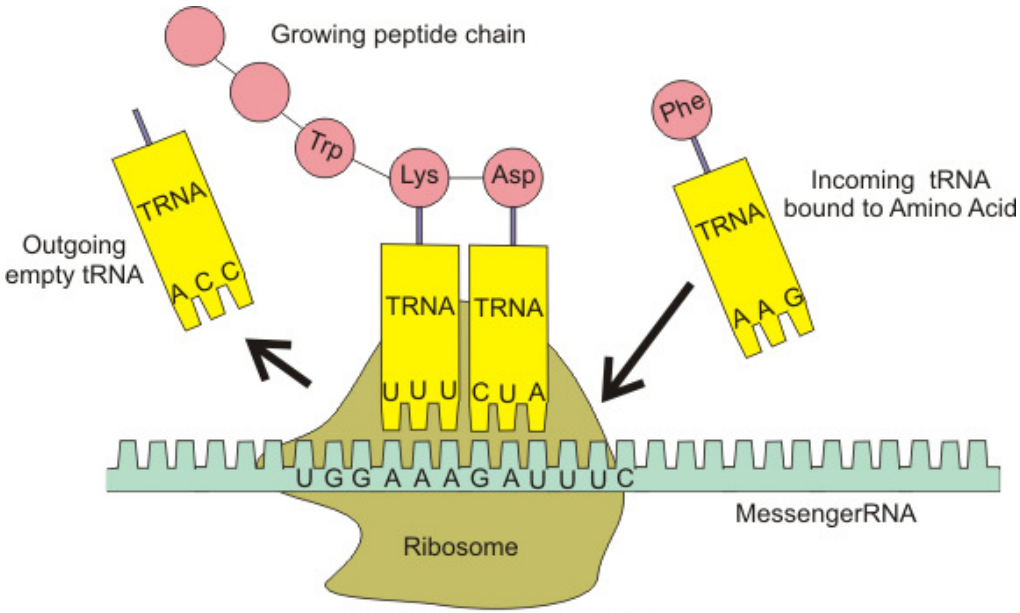
Ribosome – the ‚smoking gun'



Ribosome - **3**

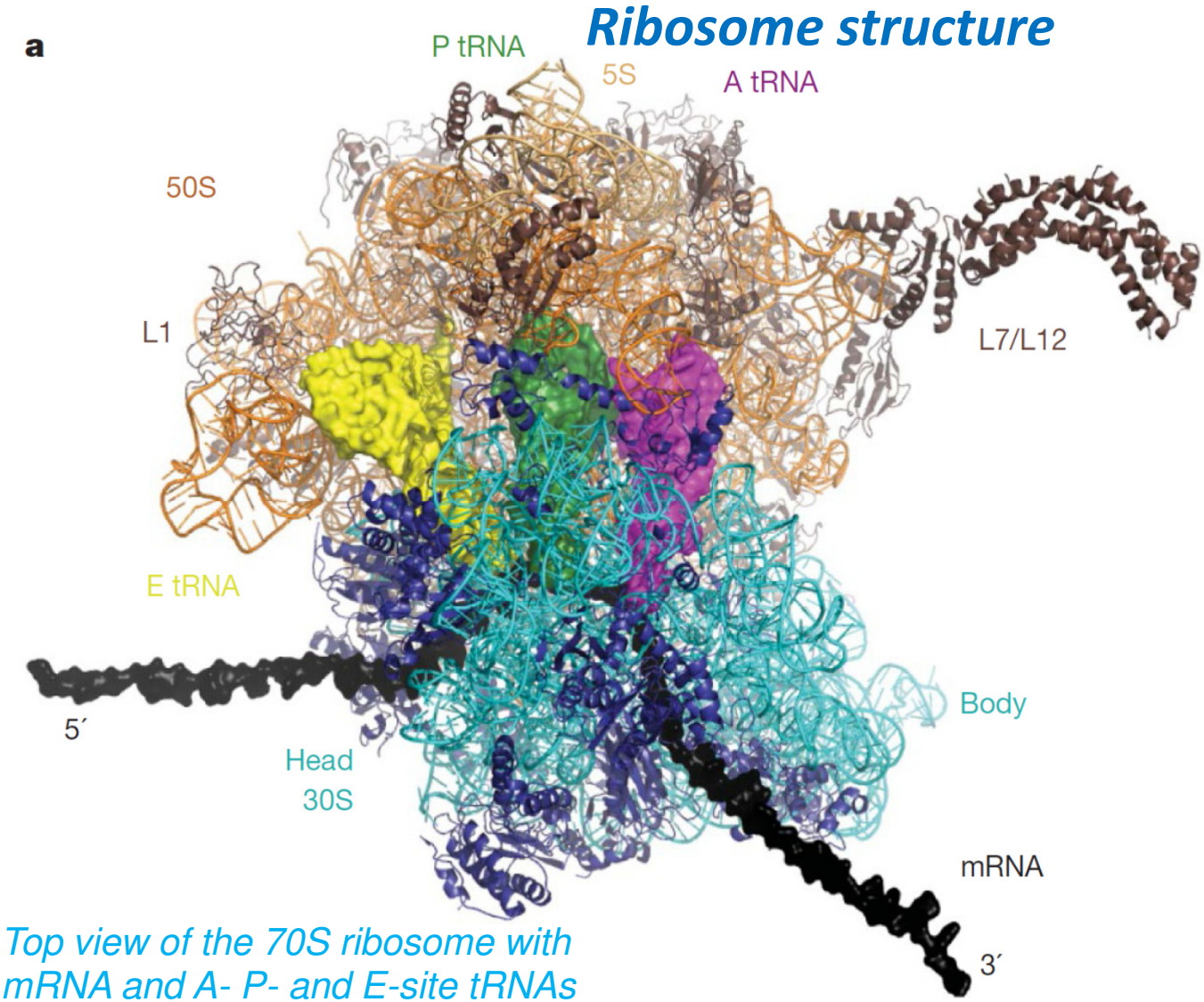


Large and small subunit

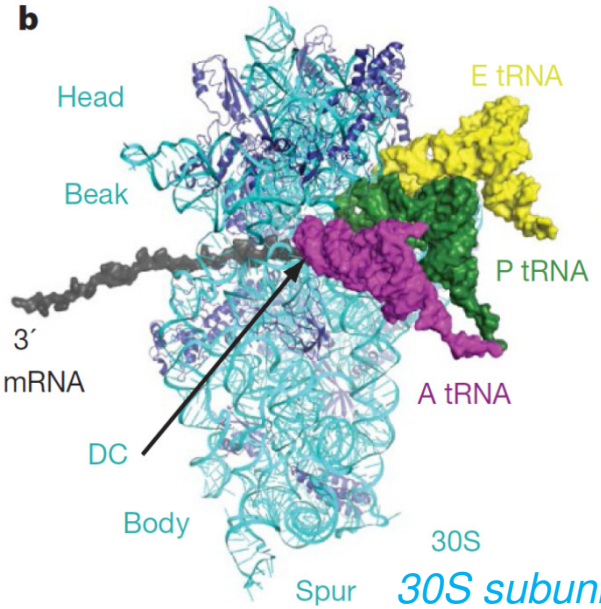


Peptide Synthesis

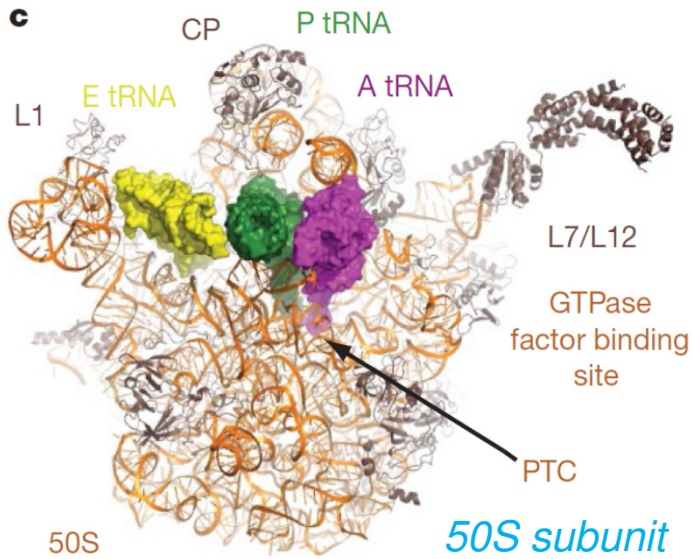
Ribosome structure



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



30S subunit

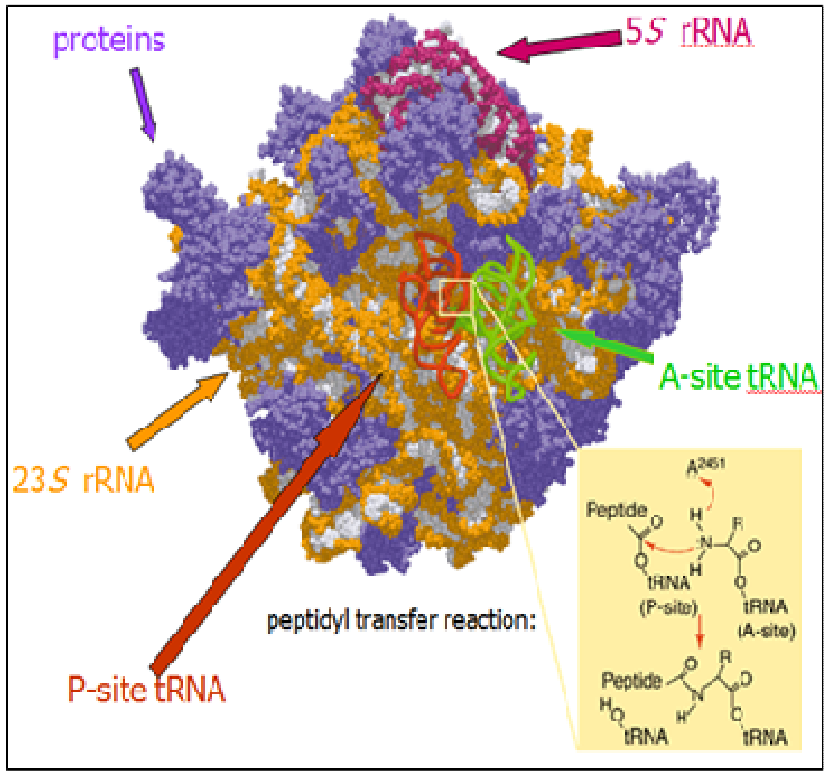


50S subunit

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!

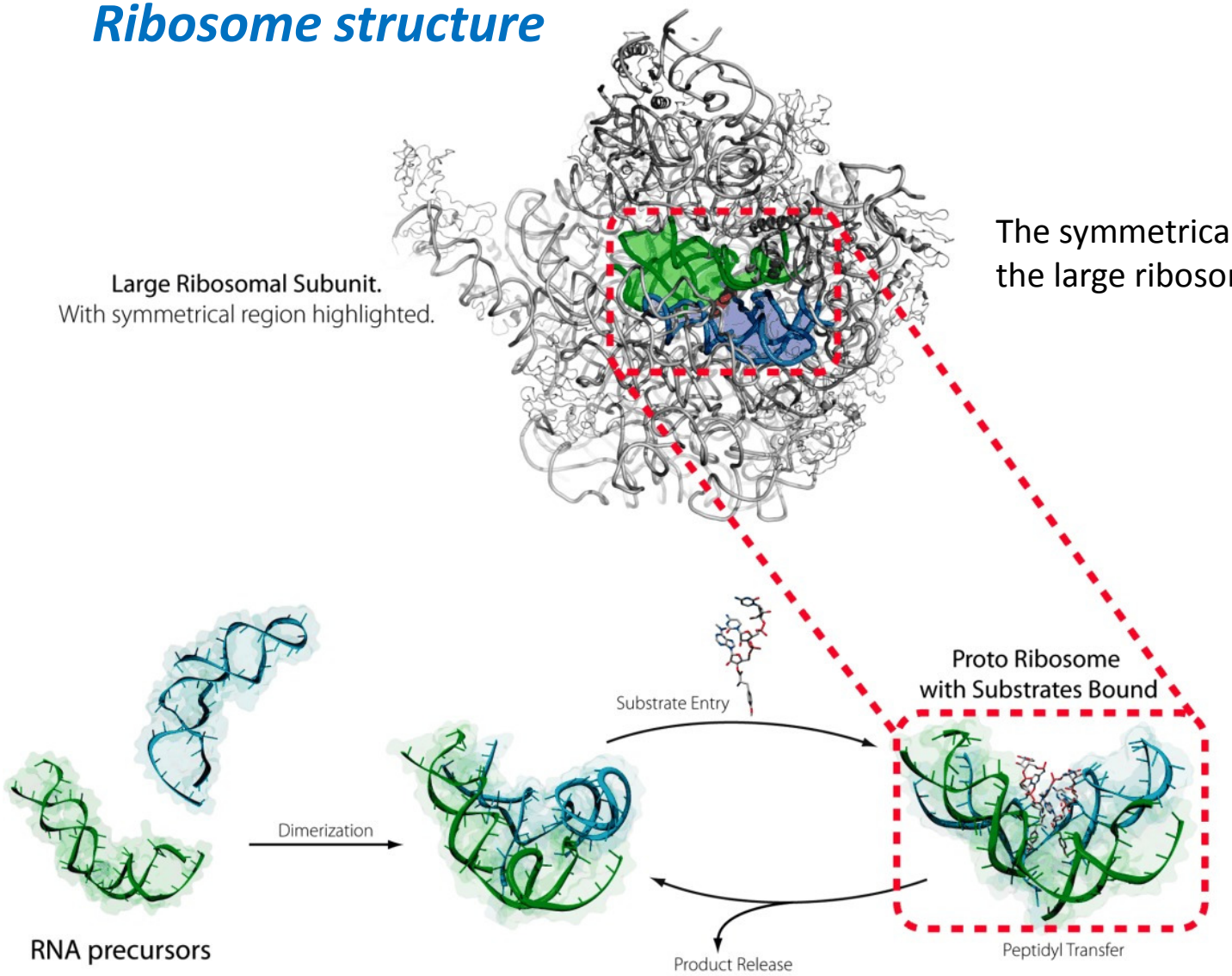
The ribosome may have first originated in an RNA world appearing as a self-replicating complex that only later evolved the ability to synthesize proteins when amino acids began to appear.

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

Ribosome structure



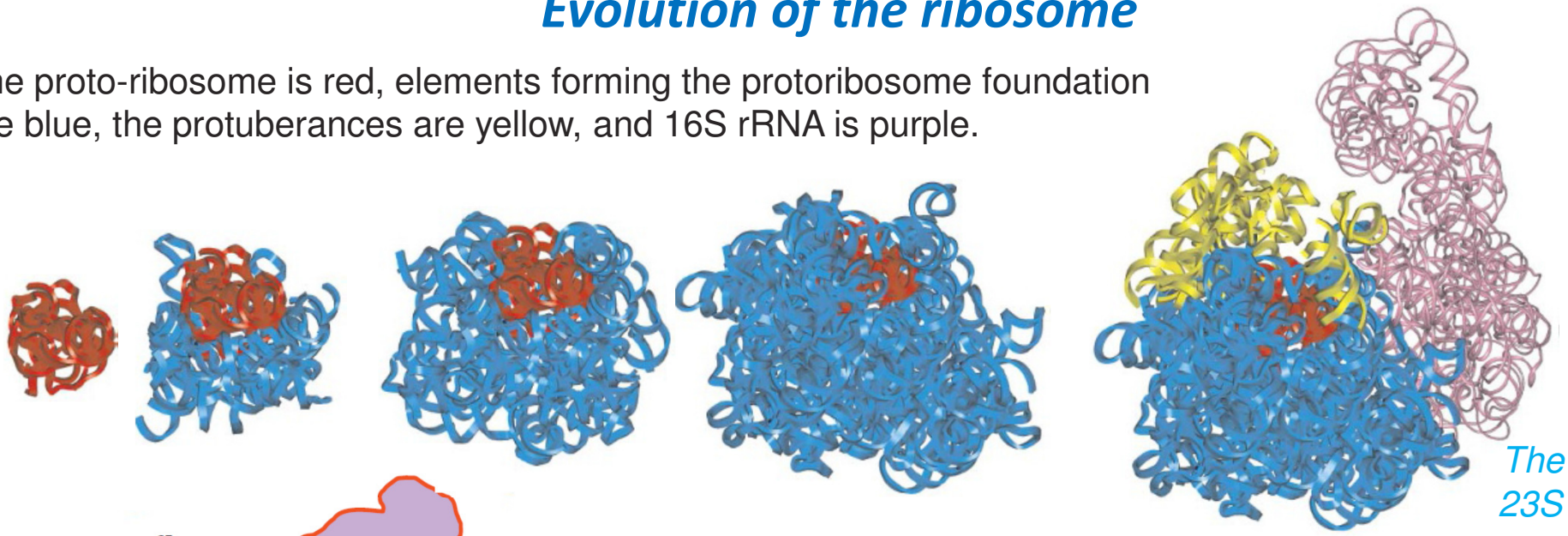
The symmetrical region within the large ribosomal subunit.

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

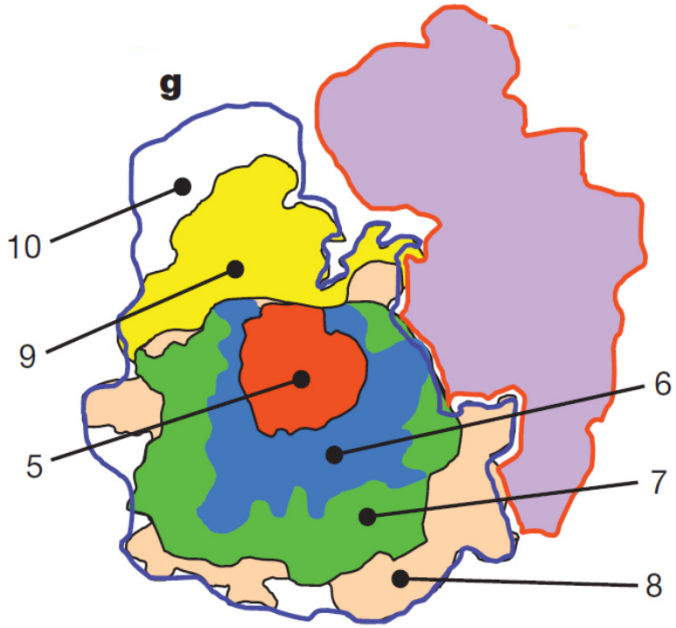
A. Yonath *et al. Israel Journal of Chemistry,* **2010***, 50,* 29-35

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.

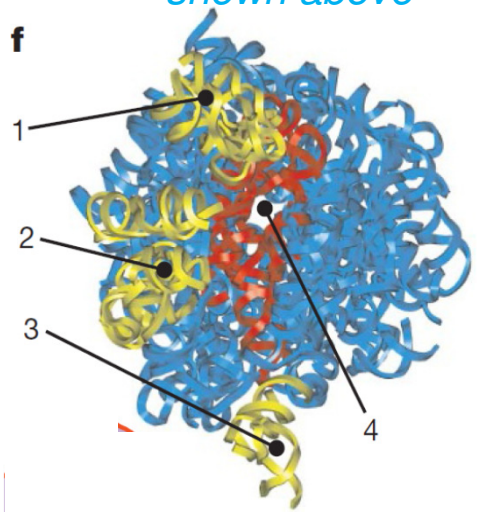


The top view of the 23S rRNA structure shown above



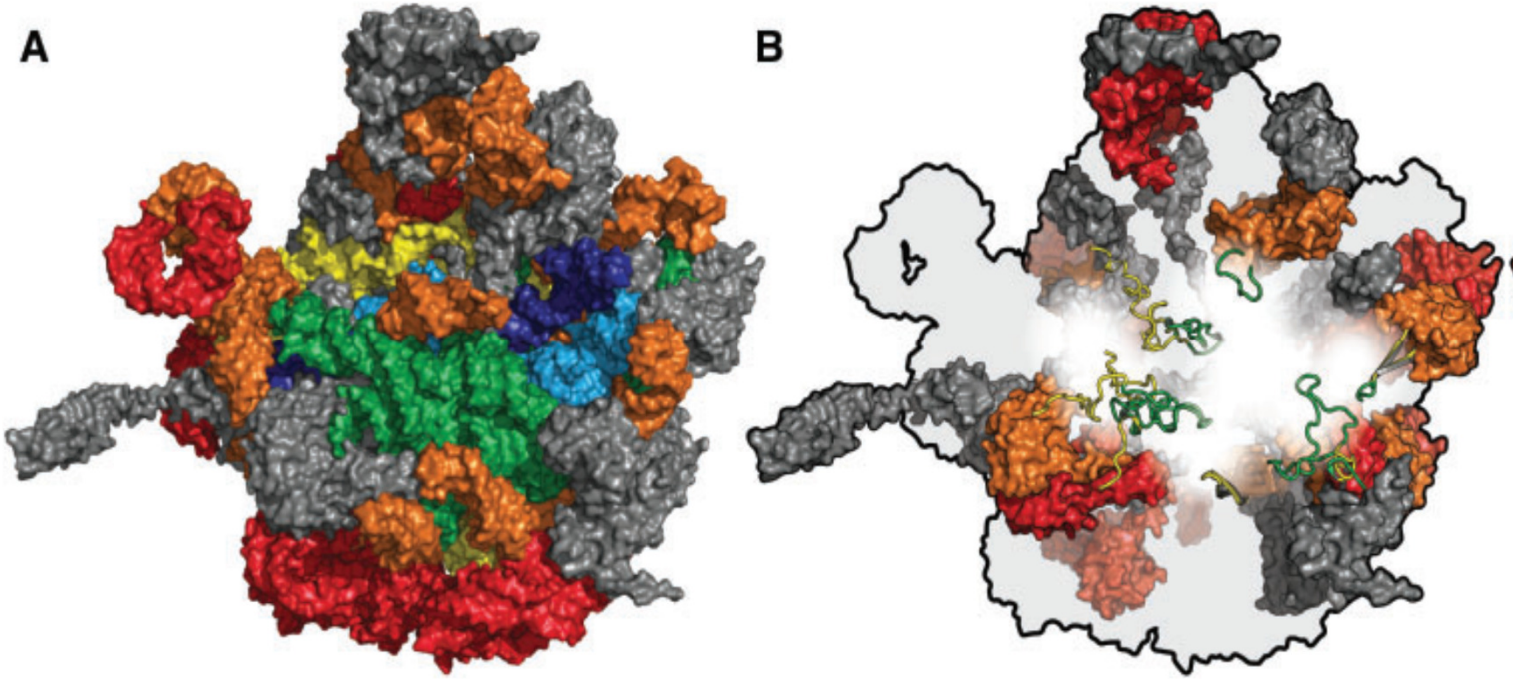
The positions of the parts of 23S rRNA shown above in the context of the whole ribosome. The structures of the 50S and 30S subunits are contoured by the blue and red line, respectively.

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

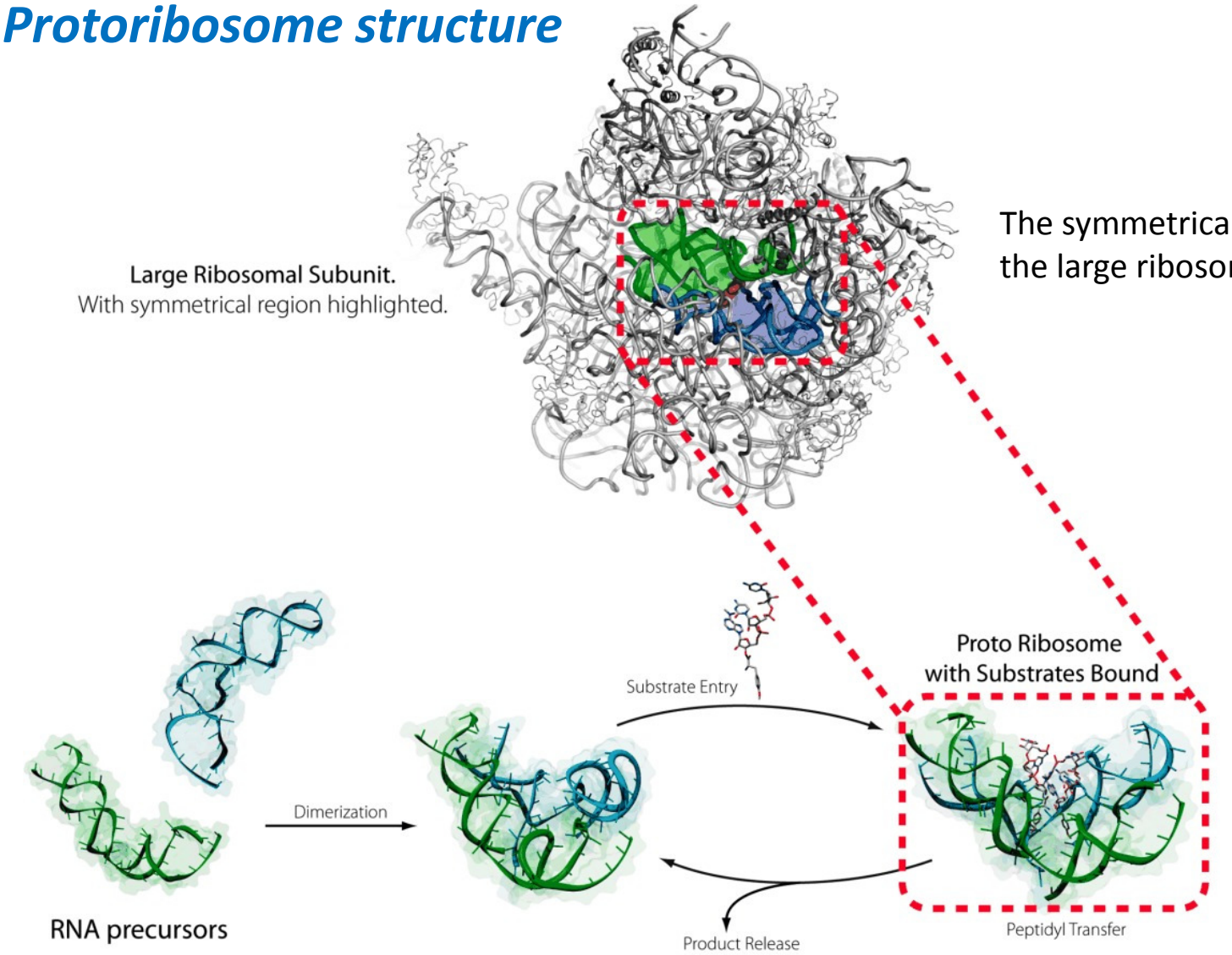


(A) The rRNA of the large subunit of the T. thermophilus ribosome colored by relative age. Phase 1, the most ancient phase, is dark blue. Phase 2 is light blue. Phase 3 is green. Phase 4 is yellow. Phase 5 is orange. Phase 6, the most recent prokaryotic phase, is red. rProteins are grey.

(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.

Protoribosome 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

The RNA world

RNA as catalyst

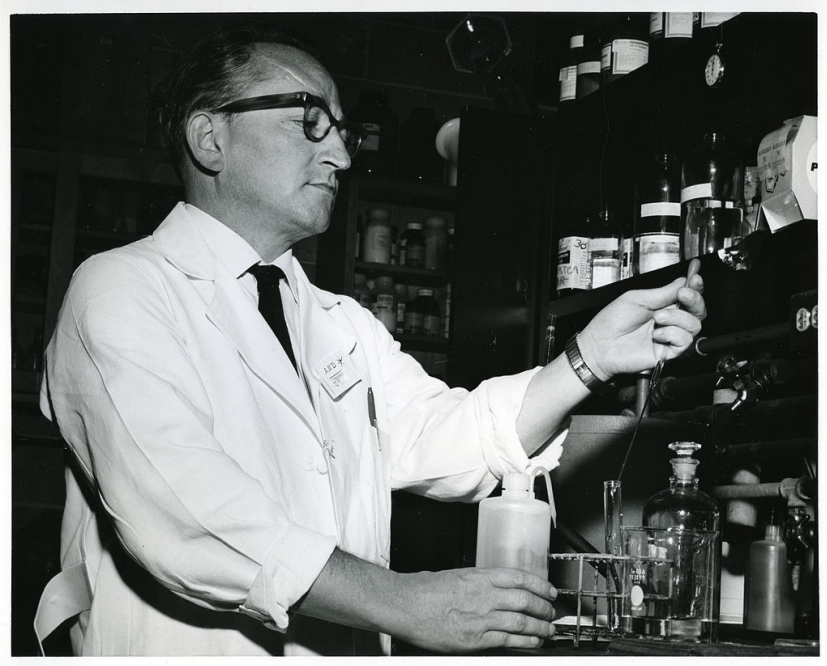
Currently known co-enzymes
Ribozymes
Ribosome

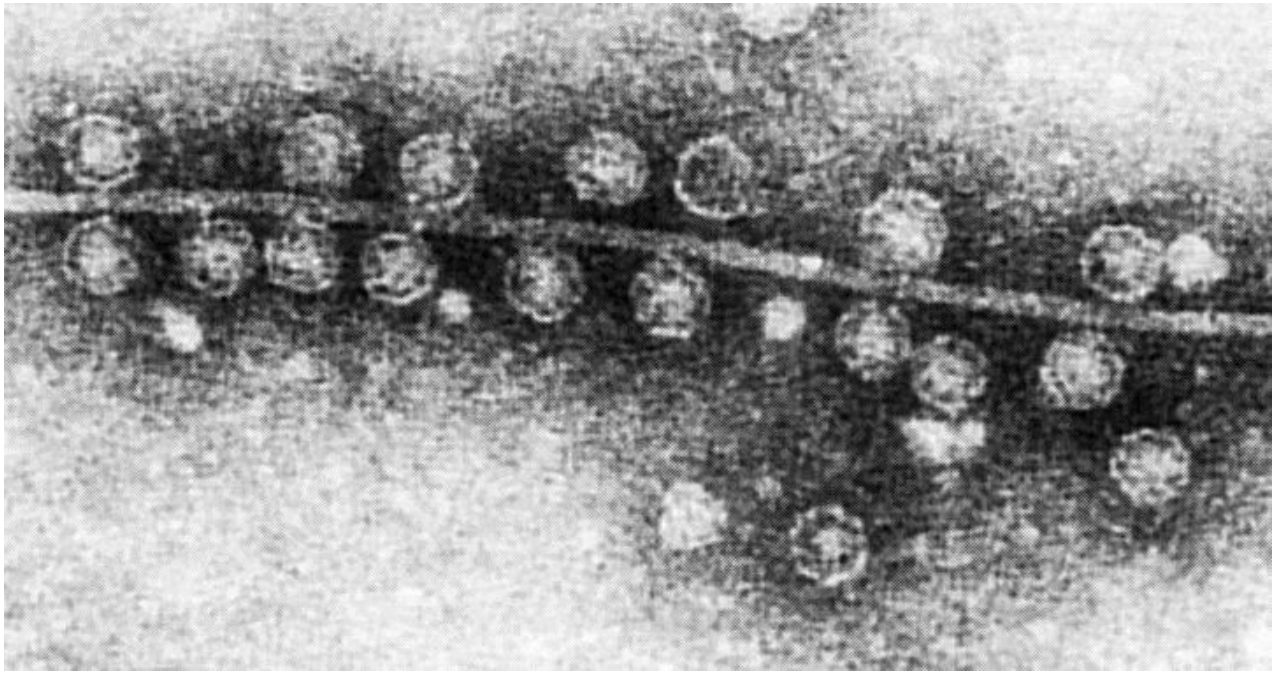
Can RNA evolve?

Can RNA replicate itself?

The RNA world

Can RNA evolve?



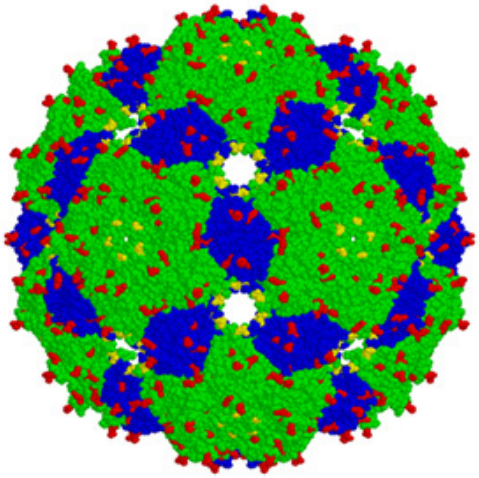


Spiegelman's monster

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!

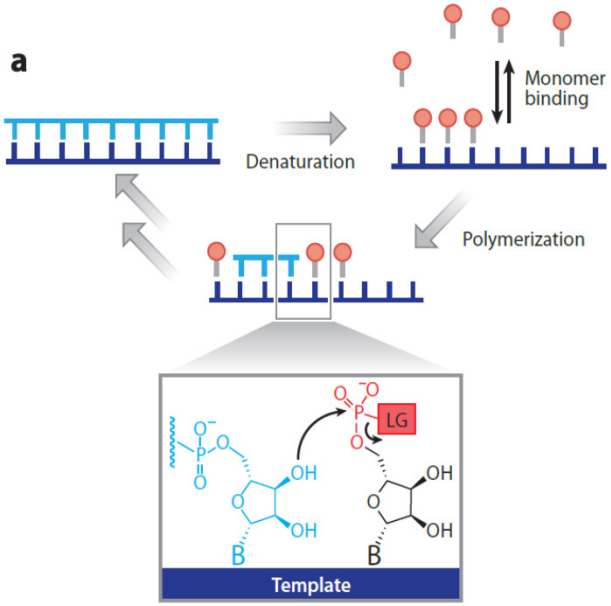
Sumper M., Luce R. *PNAS* **1975**, *72*, 162-166.

The RNA world

RNA self-replication

Nonenzymatic template-directed RNA polymerization

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

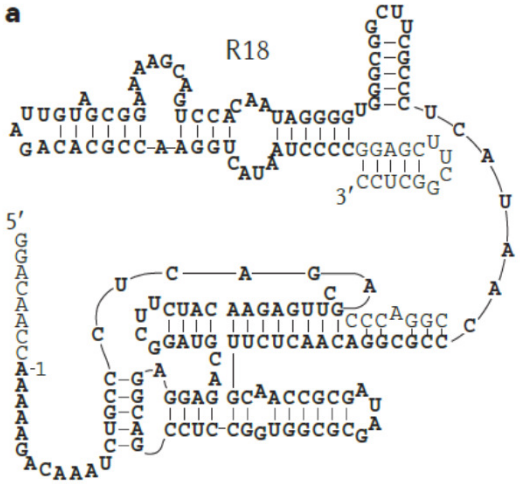


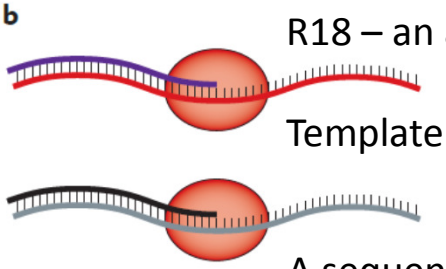
General RNA polymerase ribozyme (‚replicase')

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

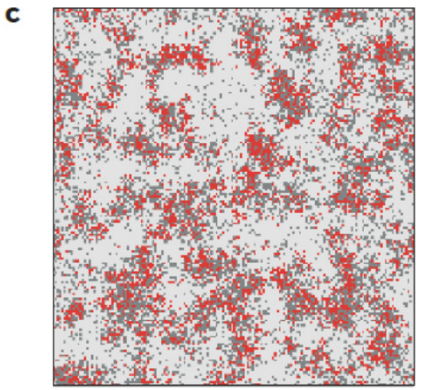


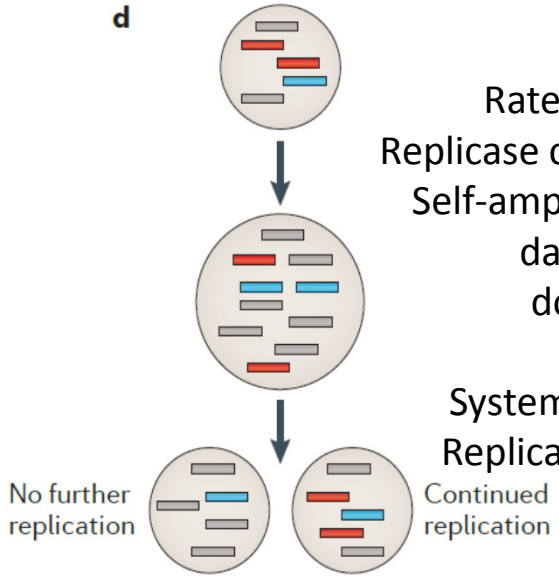


R18 – an artificial polymerase evolved from the class I ligase ribozyme.

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.

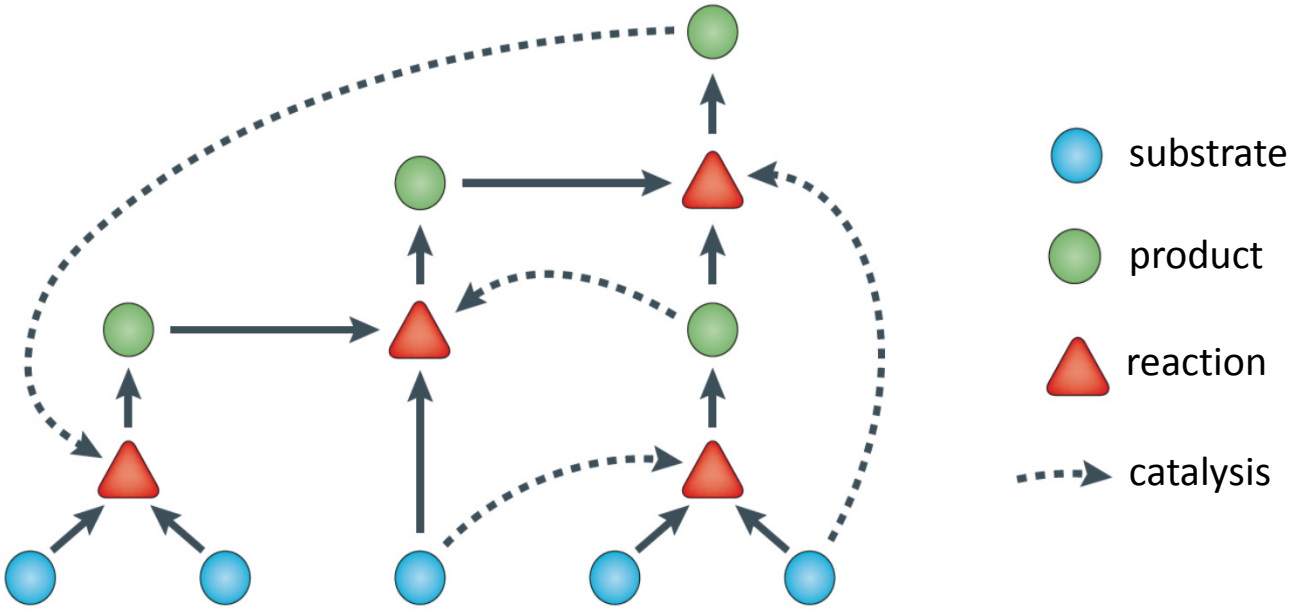
Attwater, J., Wochner, A. & Holliger, P. *Nature Chem.* **2013**, *5*, 1011–1018.

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?

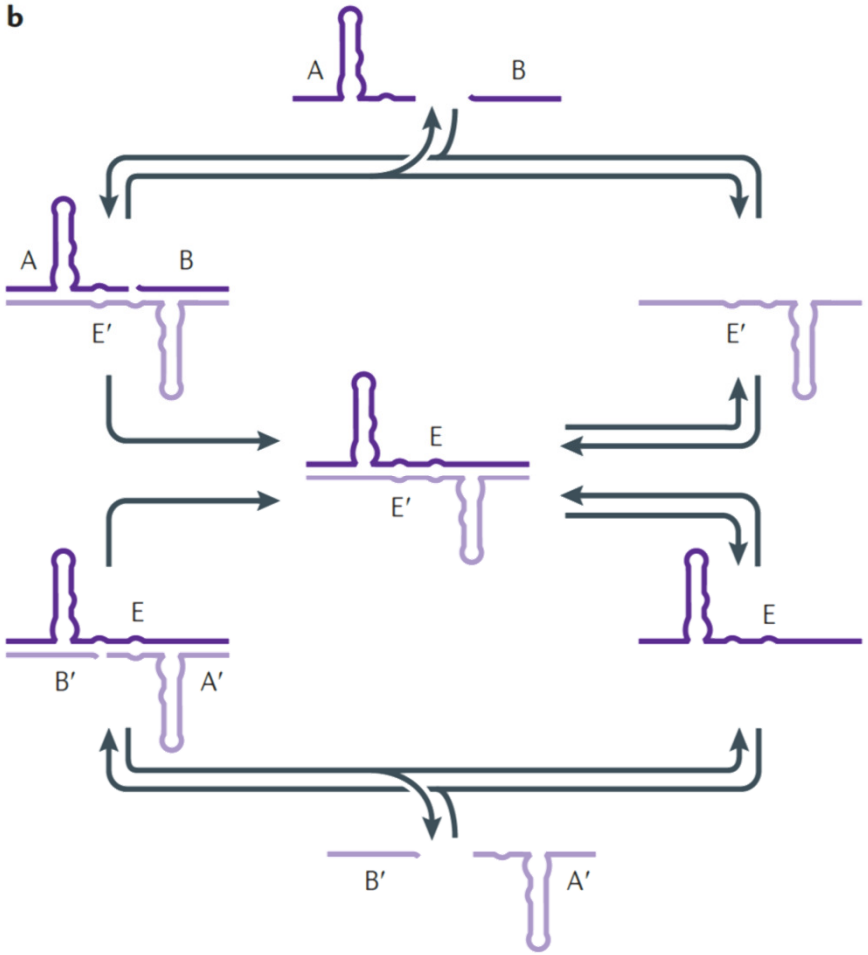
Possible solution – autocatalytic networks



No component can replicate without all the others

The RNA world

Mutually autocatalytic RNA networks

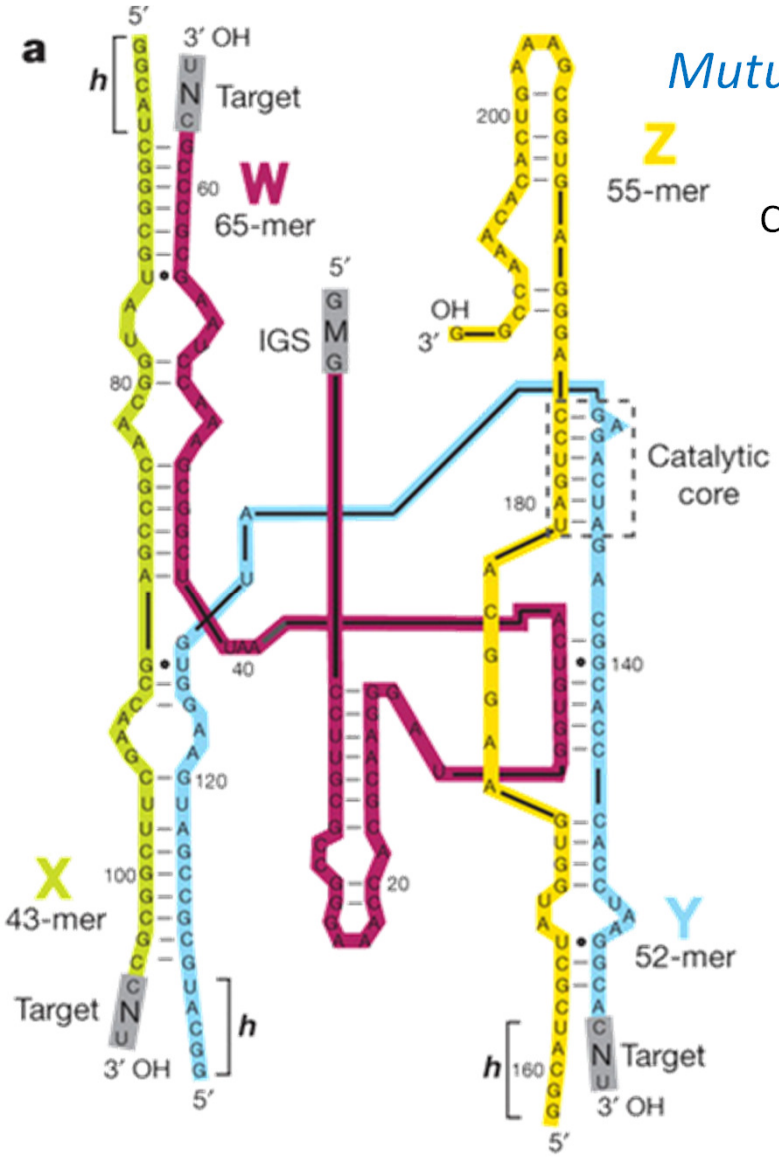


An autocatalytic set composed of two cross-catalytic ligases was demonstrated. RNA A and RNA B are ligated together by ribozyme E‘ to create ribozyme E, which can reciprocate and ligate RNA A‘ and RNA B’ to create ribozyme E‘.

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

The RNA world

Mutually autocatalytic RNA networks



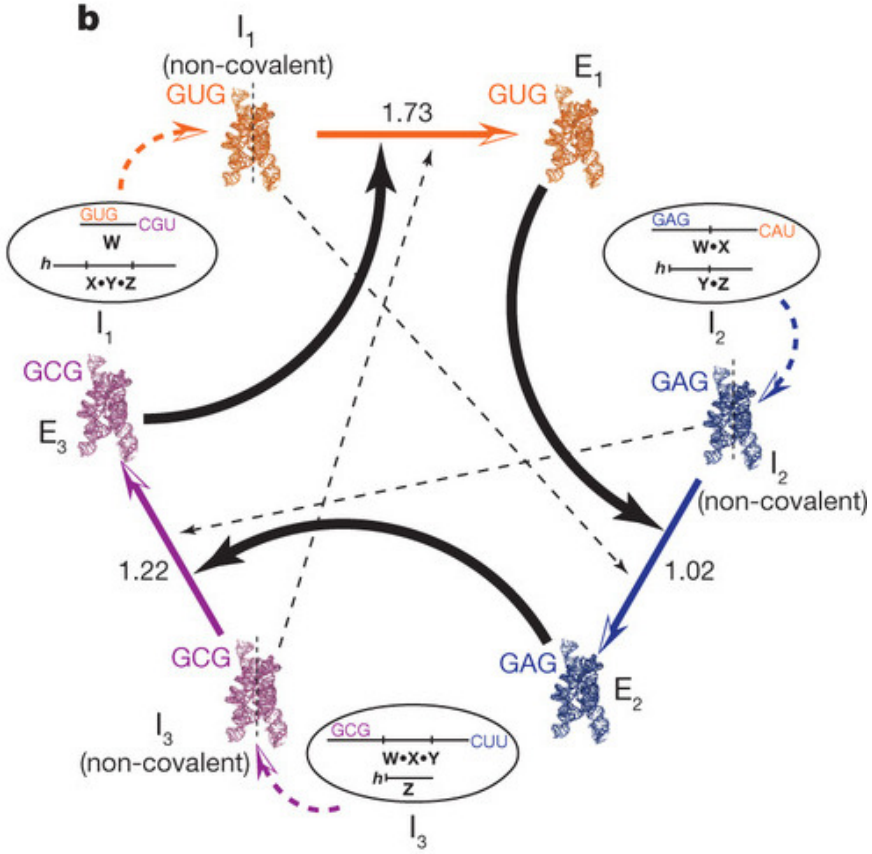
Cooperation between multiple strands that assemble to perform a single function.

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).

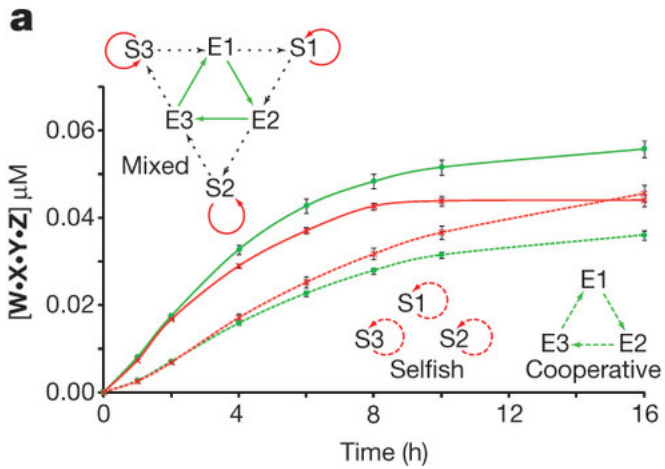
Vadia, N. *et al. Nature* **2012**, *491*, 72-77.

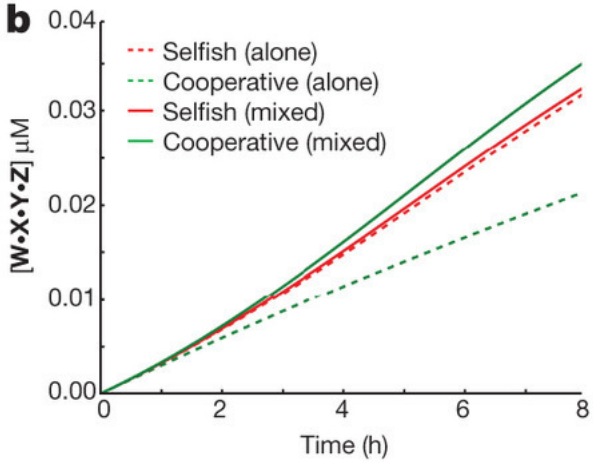
The RNA world

Mutually autocatalytic RNA networks



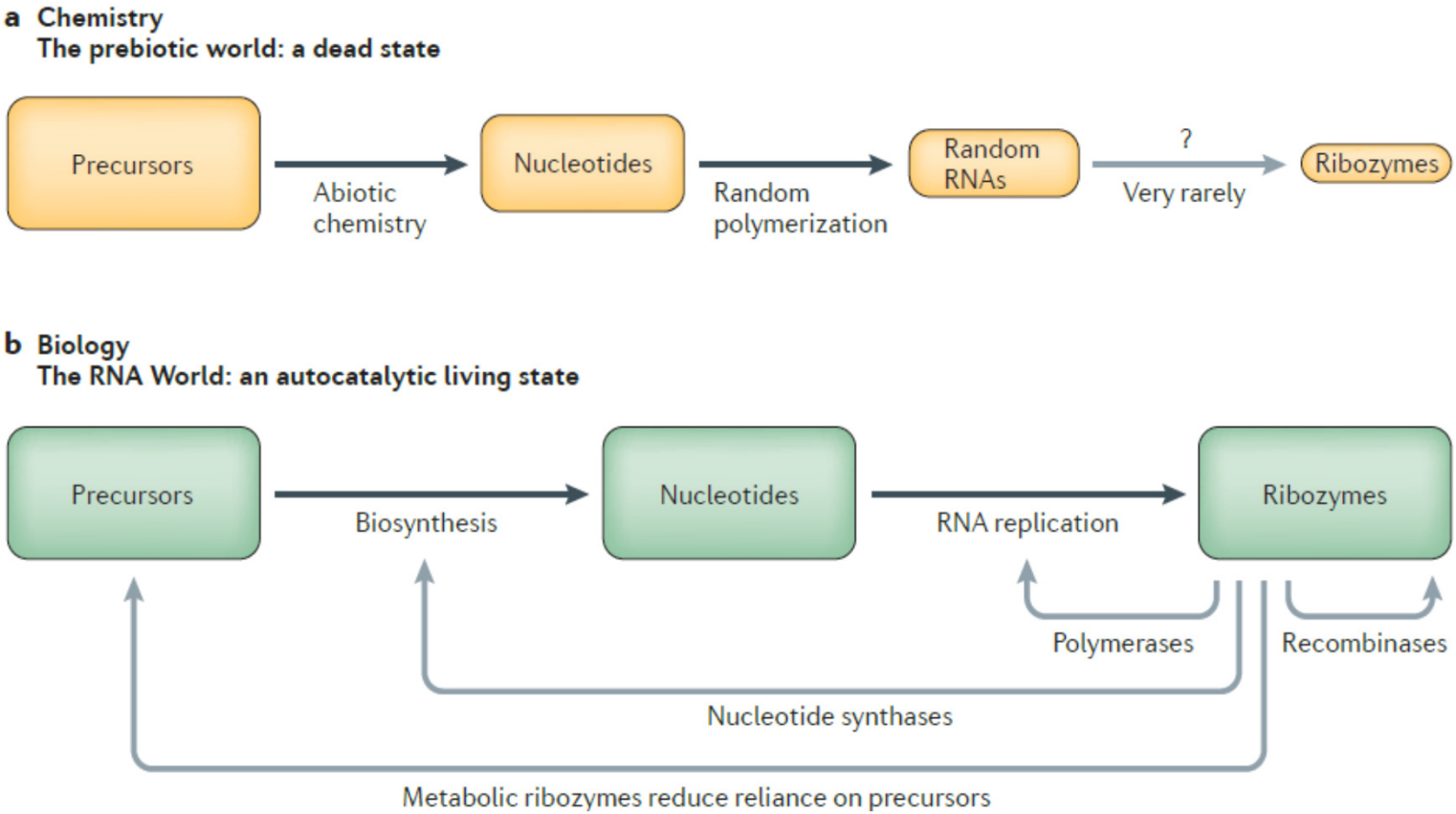
mixtures of RNA fragments that self-assemble into self-replicating ribozymes spontaneously form cooperative catalytic cycles and networks.





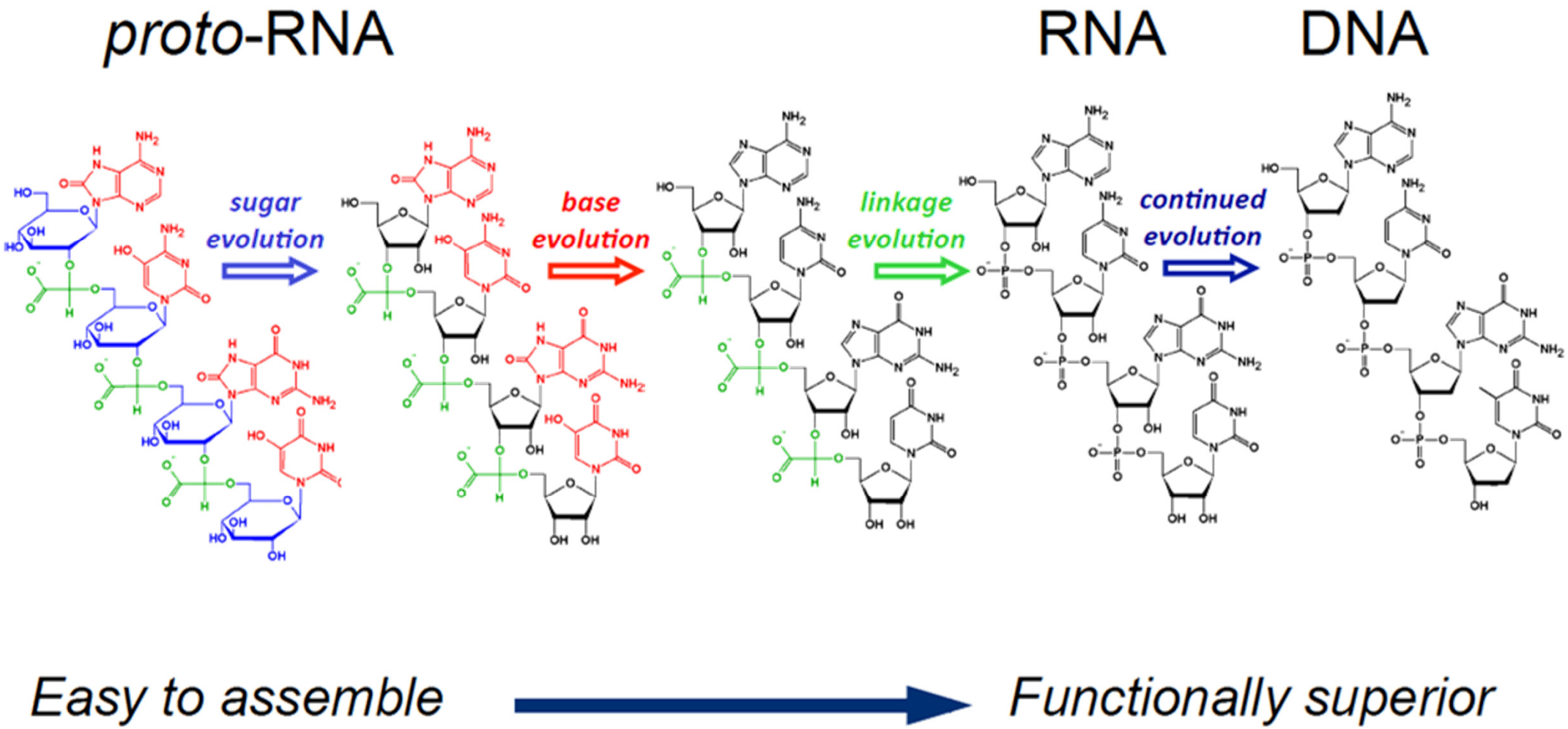
Vadia, N. *et al. Nature* **2012**, *491*, 72-77.

The RNA world



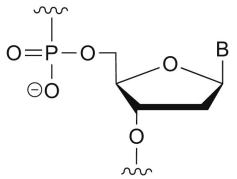
Transition from chemistry to biology involves autocatalytic feedbacks from ribozymes to all stages of the prebiotic chemistry

„RNA-second”

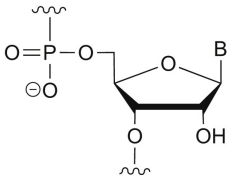


Proto-RNA evolution: According to the protoRNA theory, each of the components of RNA — sugar, base and phosphate backbone — may have originally taken different forms.

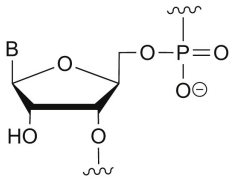
Artificial genetic polymers



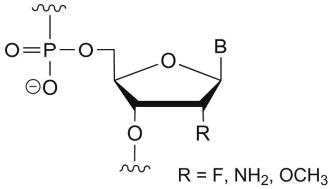
DNA



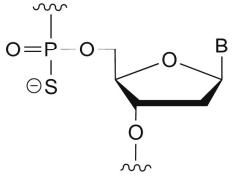
D-RNA
(natural)



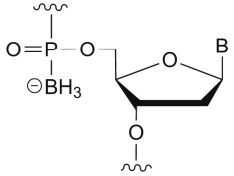
L-RNA
(unnatural)



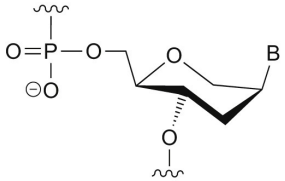
2'-modified RNA



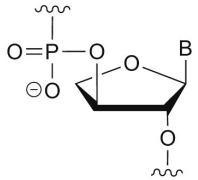
Phosphorothioate



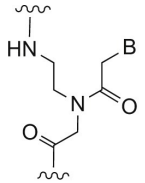
Boranophosphate



Hexitol Nucleic Acid
(HNA)

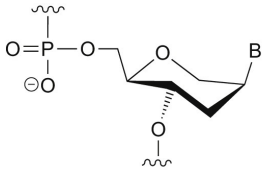


Threose Nucleic Acid
(TNA)

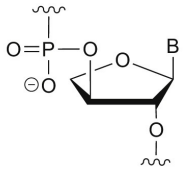


Peptide Nucleic Acid
(PNA)

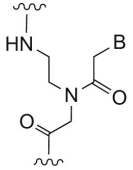
XNA – Xeno Nucleic Acids



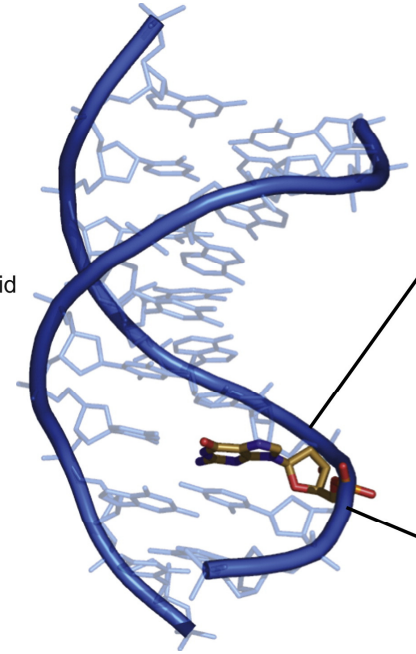
Hexitol Nucleic Acid
(HNA)

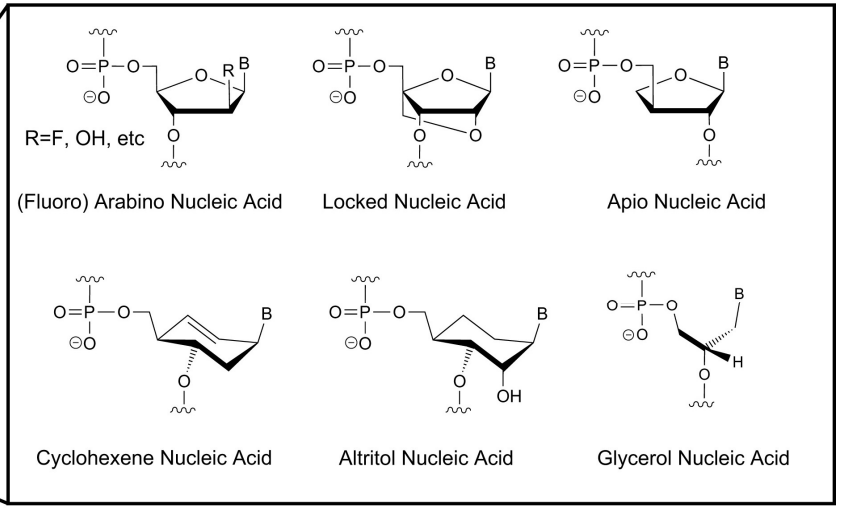


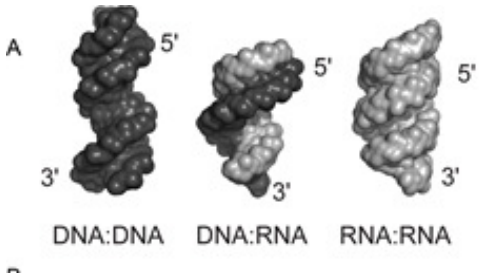
Threose Nucleic Acid
(TNA)

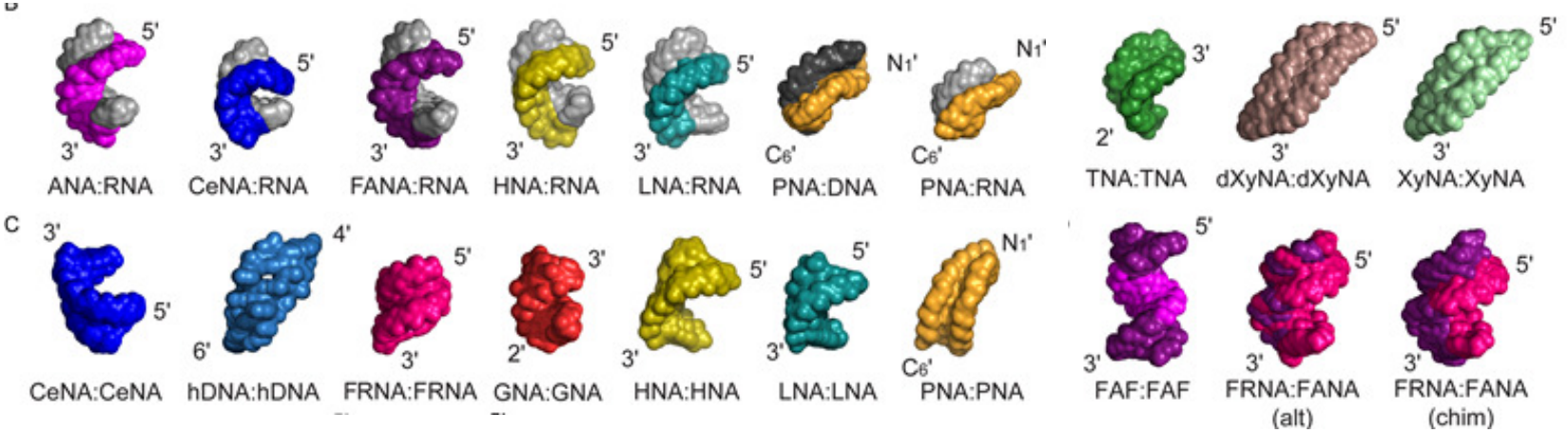


Peptide Nucleic Acid
(PNA)

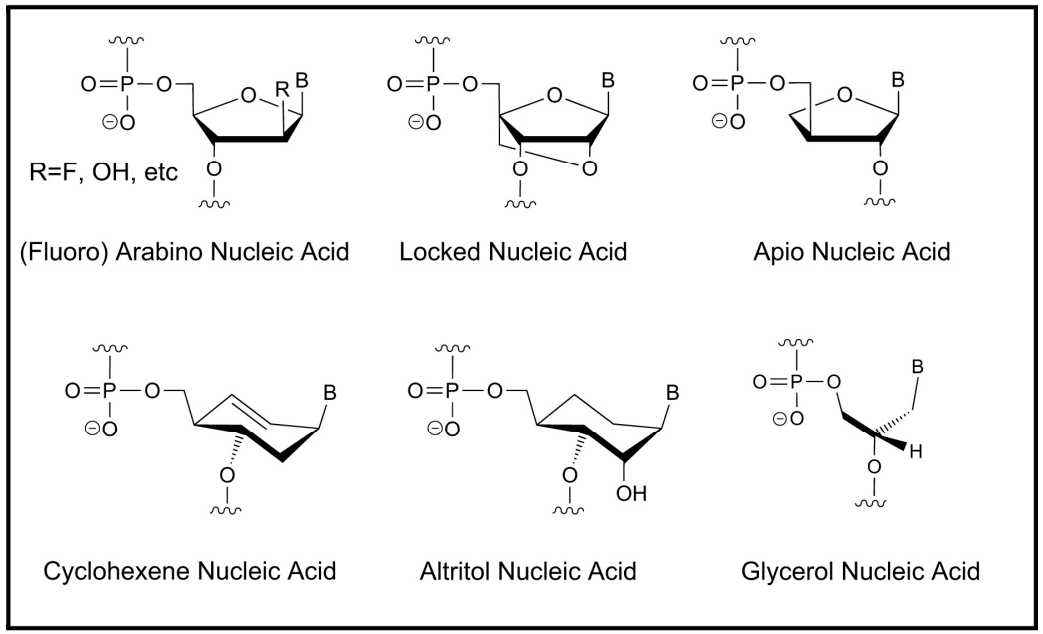


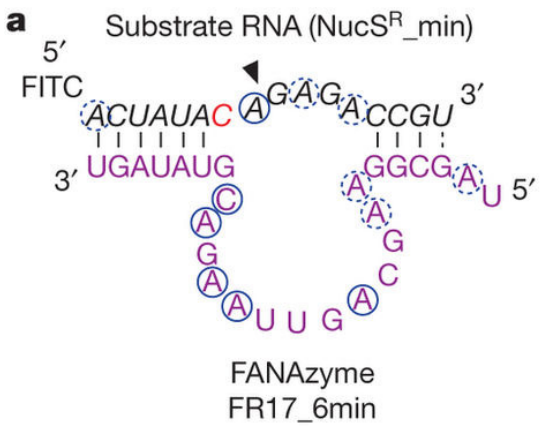


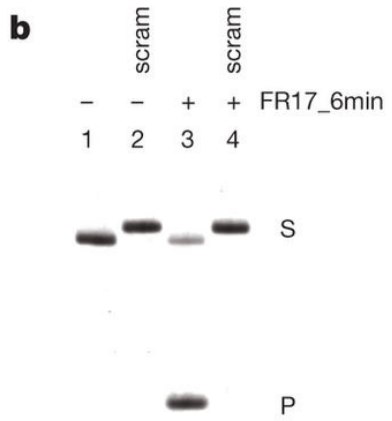


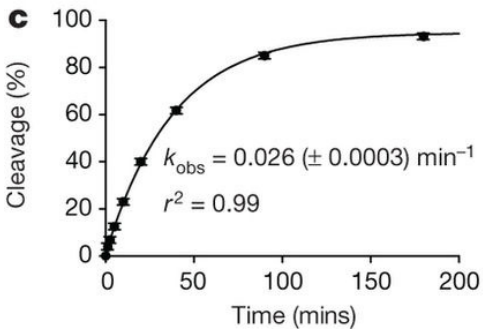


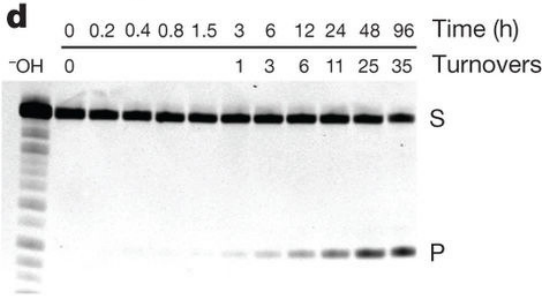
Chemical synthesis yields an active RNA endonuclease XNAzyme







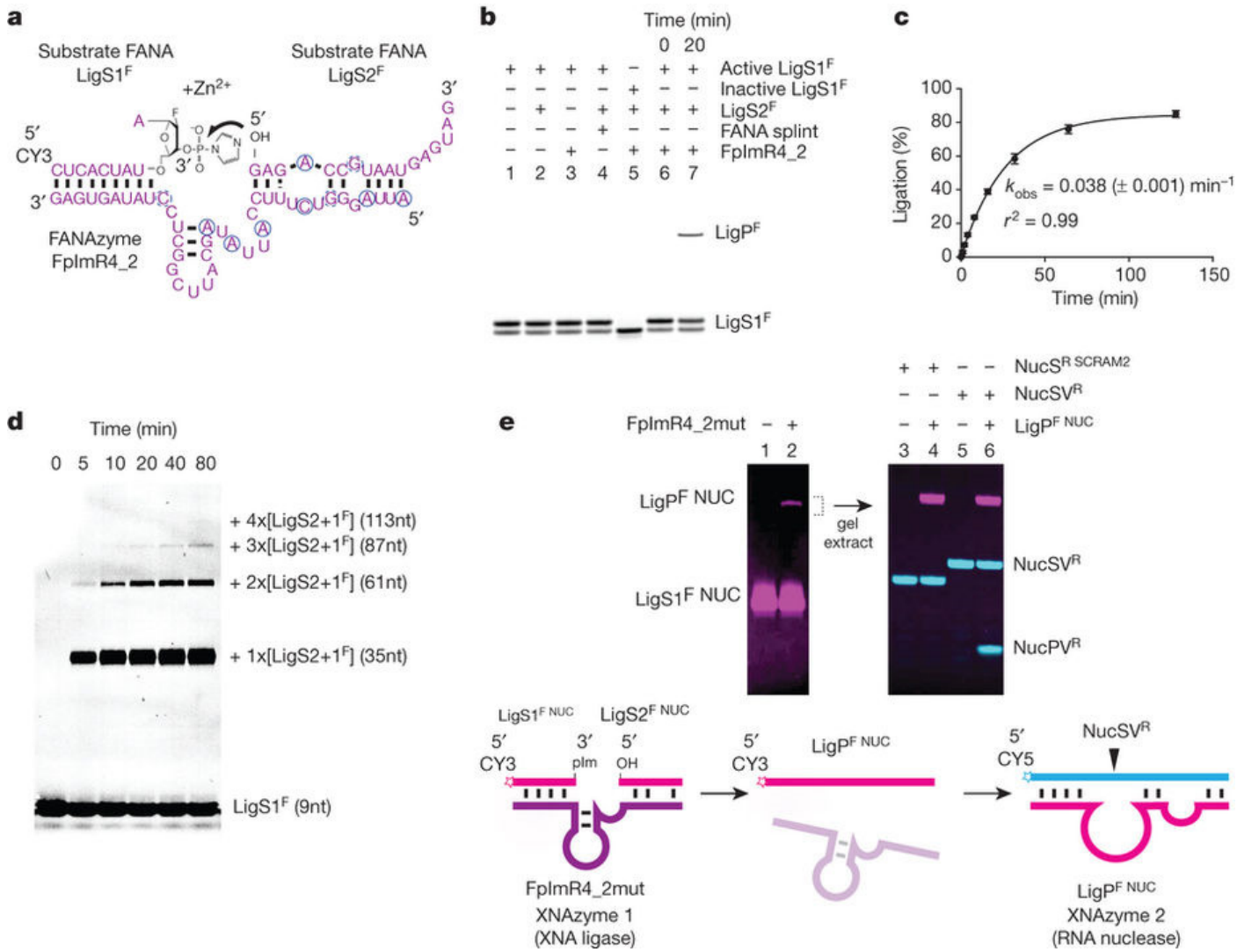




a, Secondary structure of truncated FANAzyme FR17_6 (FR17_6min, purple)
b, FR17_6min synthesized using FANA phosphoramidites cleaves cognate RNA substrate (NucSR_min; lanes 1 and 3), but not a scrambled RNA (NucSR SCRAM2; lanes 2 and 4), with...
c, essentially unchanged catalytic rate (k_{obs}) at 25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

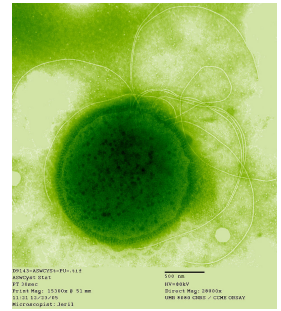
XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



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

Engineering XNA polymerases

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

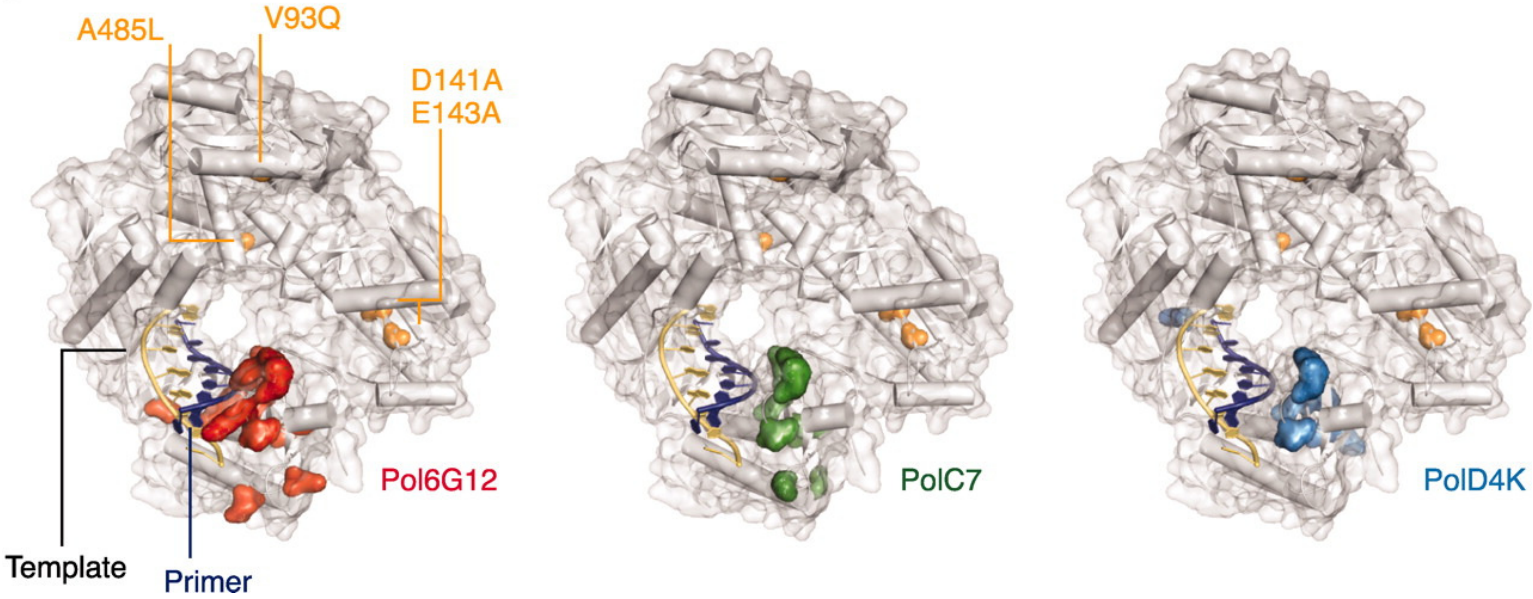


Thermococcus gorgonarius
(Angels Tapias)

A

	402	404	588	590	608	611	653	682	703	710	729	731
TgoT	YLD	..	FVT	..	LEIV	..	YEVPPEKLVIYEQITRDLKDYKATGPHVAV	..	VLKGSGRI	..	AEY	
Pol6G12	YLD	..	FAT	..	LKMV	..	YEVPPEQLVIYQPITKQLHDYRARGPHVSV	..	VPKGSGRI	..	AGY	
PolC7	YLD	..	FVT	..	LEIV	..	YQVPPQQLAIYQPITRALQDYKAKGPHVAV	..	VLKGSGKI	..	AEY	
PolD4K	YPD	..	FVT	..	LEIV	..	YEVPTQHLVIHKQITRALNDYKAIGPHVAV	..	VLKGSGRI	..	AEY	

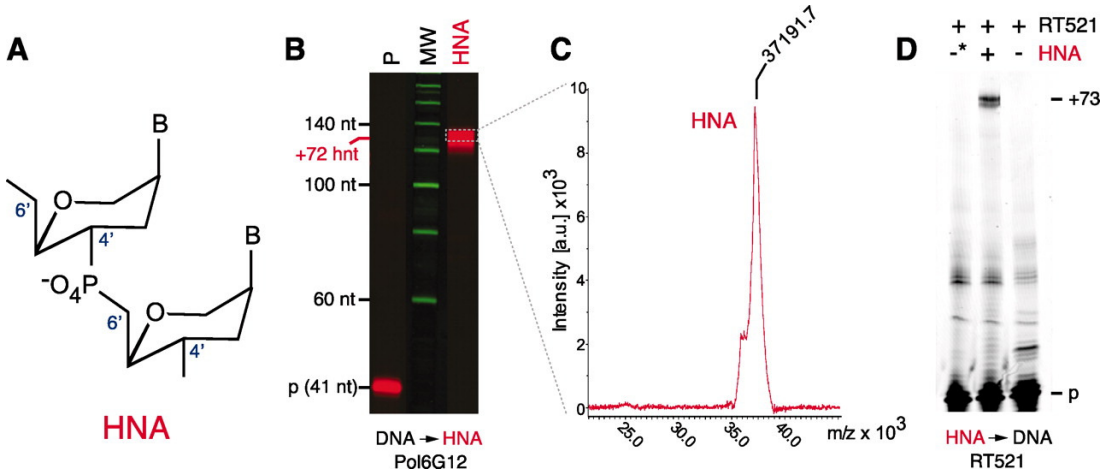
B



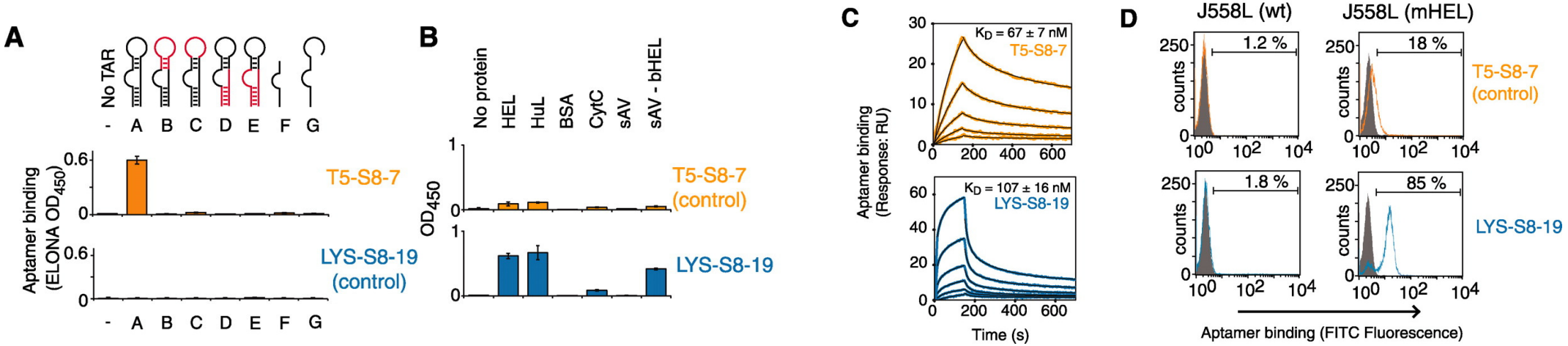
(A) Sequence alignments showing mutations from wtTgo in polymerases Pol6G12 (red), PolC7 (green), and PolD4K (blue).
(B) Mutations are mapped on the structure of Pfu (PDB: 4AIL).
Yellow - template; dark blue - primer; orange - mutations present in the parent polymerase TgoT

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

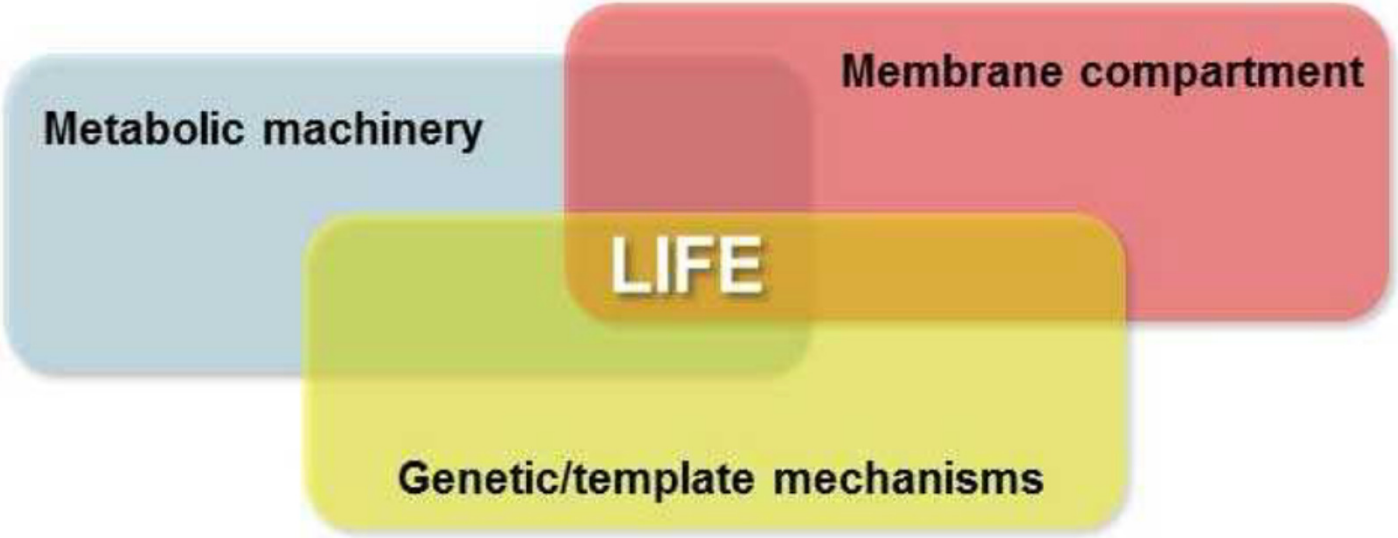
DNA-templated HNA synthesis and HNA-templated DNA synthesis



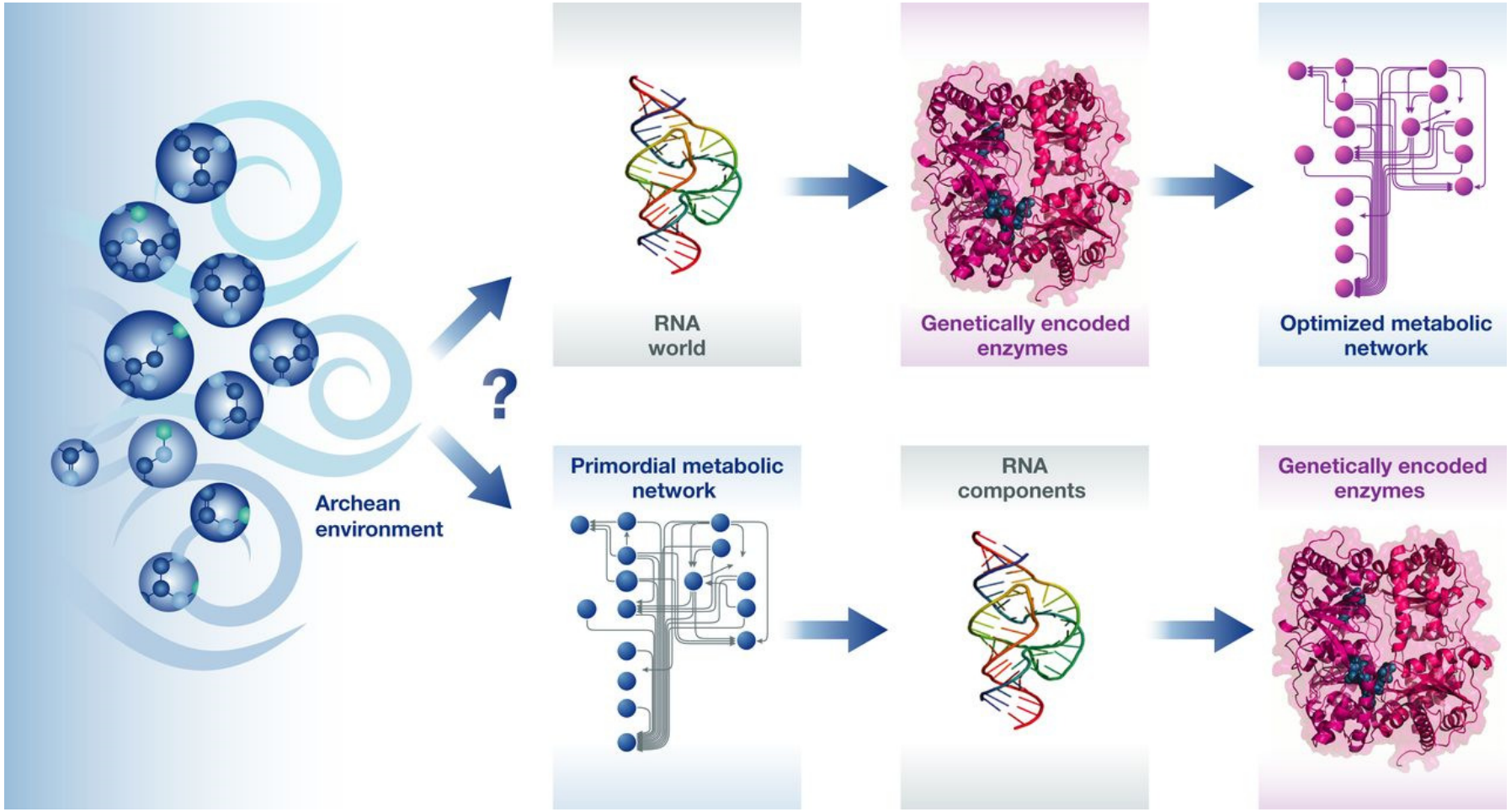
HNA aptamers



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


Membrane compartment
Metabolic machinery
LIFE
Genetic/template mechanisms

Route to life by chemical networks



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

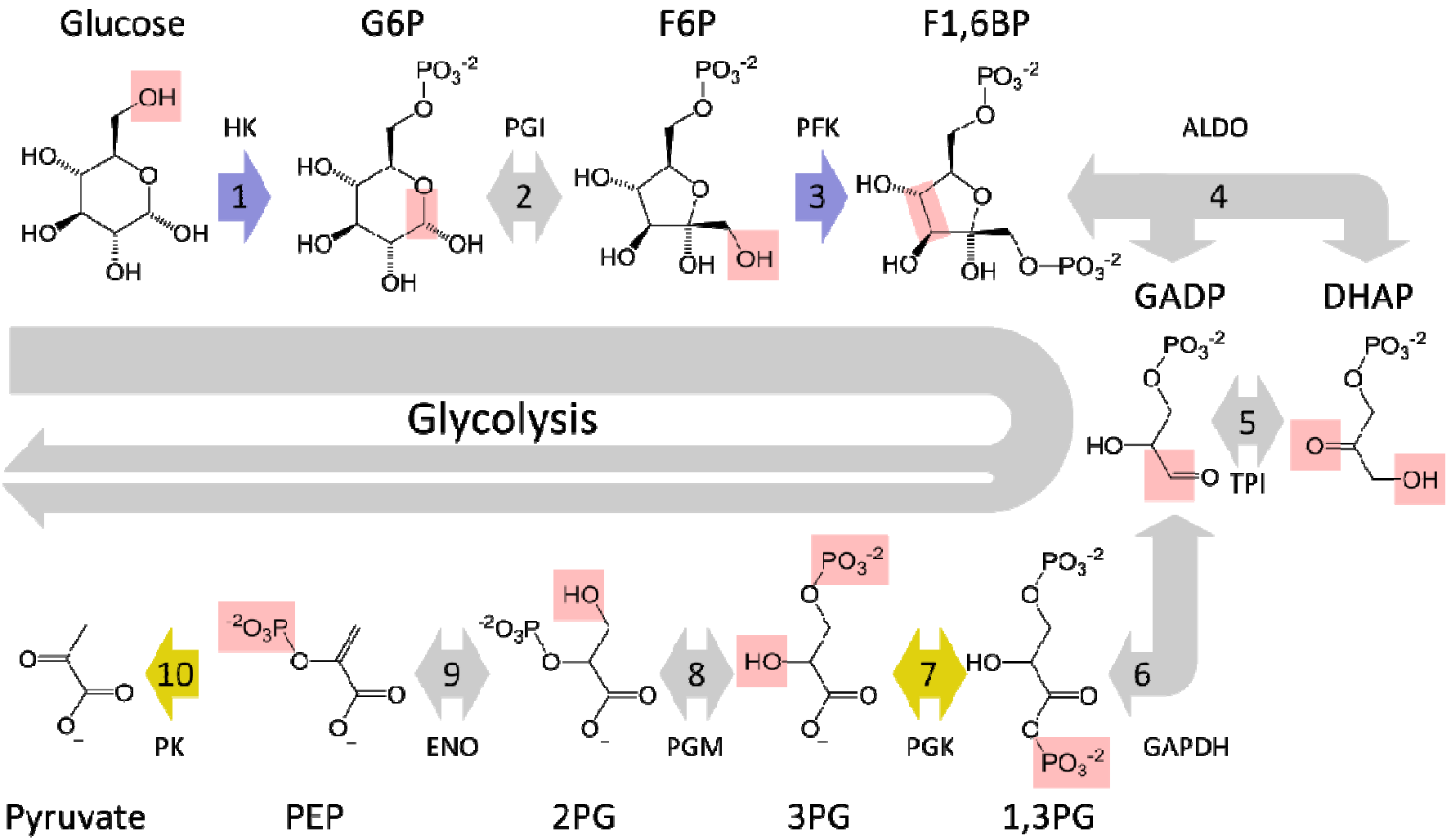
Metabolism-first vs. Genes-first

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.

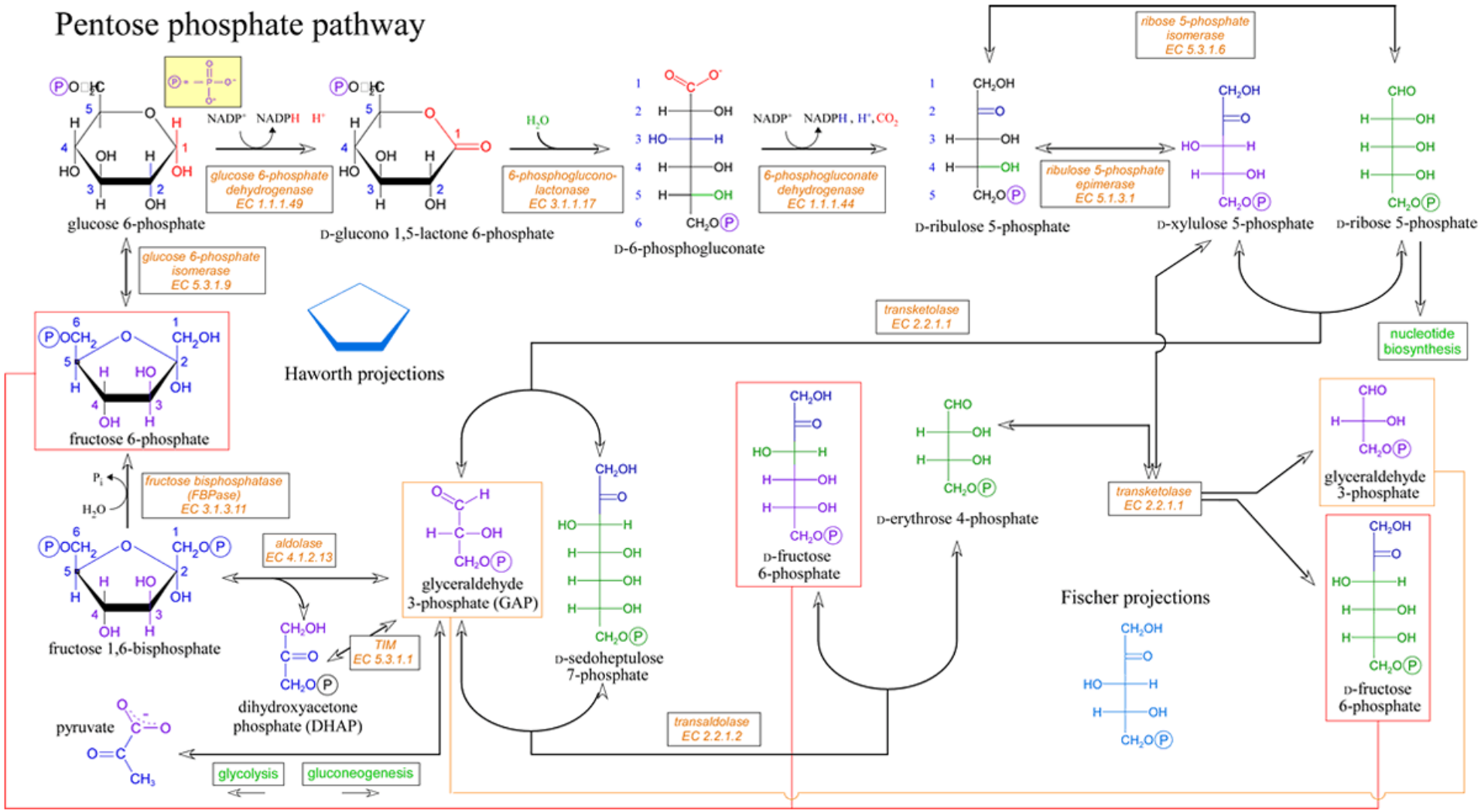
Metabolism-first: primitive metabolic cycles spontaneously assembled from simple prebiotic organic molecules or inorganic carbon sources as CO_2. And the cycles produced a set or more or less complex molecules needed for the replication process and construction of the genetic apparatus.

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.

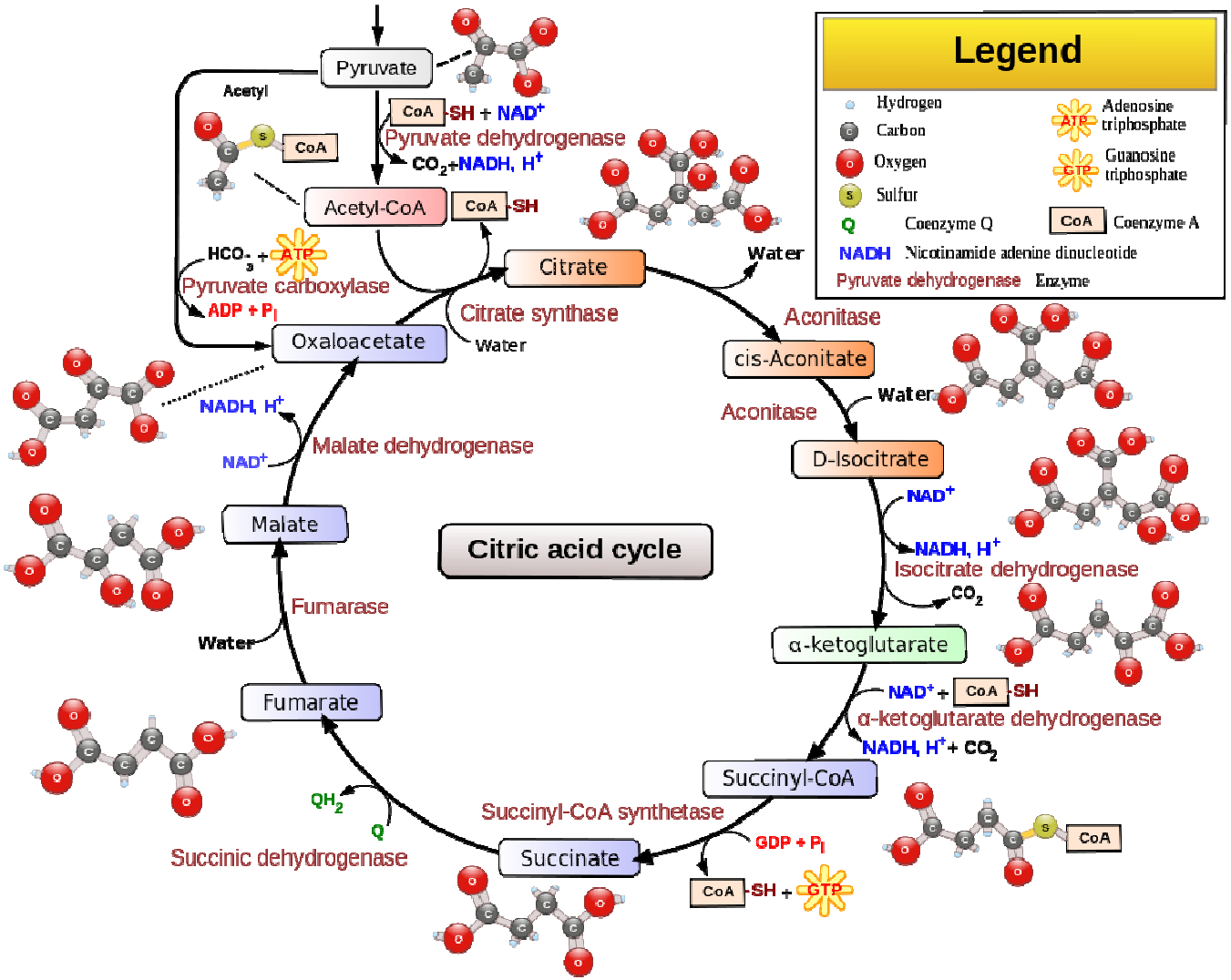
Glycolysis – energy from sugars



Pentose phosphate pathway



Citric acid cycle (Krebs cycle)



Metabolism-first - theories

Mathematical models:

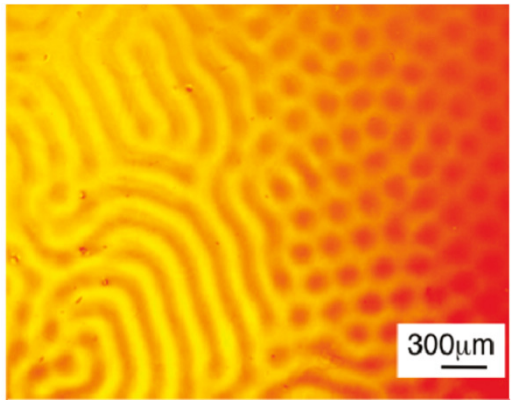
Dyson – modell based on catalytic oligomers (e.g. oligopeptides) and their monomers interacting inside isolated compartments (like protocells) permeable to monomers – solutions give two steady states („ordered/alive" + „disordered/dead") and the transition („creation"/"death") between them.
Per cell, the model requires 2000-20000 monomers of 9-11 kinds with the discrimination factor of the catalysis >60.
Problems: no experimental evidences, critical simplifications were later found out detrimental
Kaufmann – sufficient complexity leads to emergent properties of a system

Chemical models:

De Duve – proto-metabolism based on thioesters. *Problems: lack of experimental details*

Wächtershäuser – *the „Iron-Sulfur world"* – a system based on troilite/pyrite (FeS/FeS_2) system and H_2S, with CO/CO_2 as the carbon source; archaic metabolic cycles that involve thiol analogues of currently known metabolites „ignited" on the surface of pyrites starting with the reverse citric acid cycle in the absence of any enzyme or an organic catalyst. The theory expanded by Martin and Russell – metabolites confined inside compartments (not on the surface) which walls are made of pyrite, NiS and Co, Mn, W, Zn minerals, which expands the scope of possible catalysis. The „Fe-S" world would likely exist in proximity of hydrothermal vents – rich in minerals, volcanic gases and hot springs on the bottoms of oceans.

Self-organization of chemical networks



One of pre-conditions for life is to be far from thermodynamic equilibrium.

Life uses non-linear effects to amplify and stabilize minor environmental effects

Spatial and temporal synchronisation of reactive processes provides molecules with patterns of collective behavior. Under certain conditions far from thermodynamic equilibrium, heterogenous mixtures can trigger emergent properties at the collective level.

Oscilatory and autocatalytic processes are very common in biological systems. Examples include: metabolic cycles, immune response, or apoptosis.

Oscilatory reactions – importance for homeostasis. Provide positive and negative feedback loops to maintain the dynamic far-from-equilibrium state of the system.

Self-organization and self-assembly processes are under tight enzymatic control in all living organisms. However, oscilatory and autocatalytic behavior can appear sponateously in much simpler molecular systems.

Oscilatory reactions in biology

Endogenous processes - arise from feedbacks and internal loops between the different components of metabolic networks

ATP/ADP concentration in glycolytic cycle, circadian oscilations, metabolic rhytms, sleep-wake cycle

Exogenous processes – arise from external fluctuations in the environment

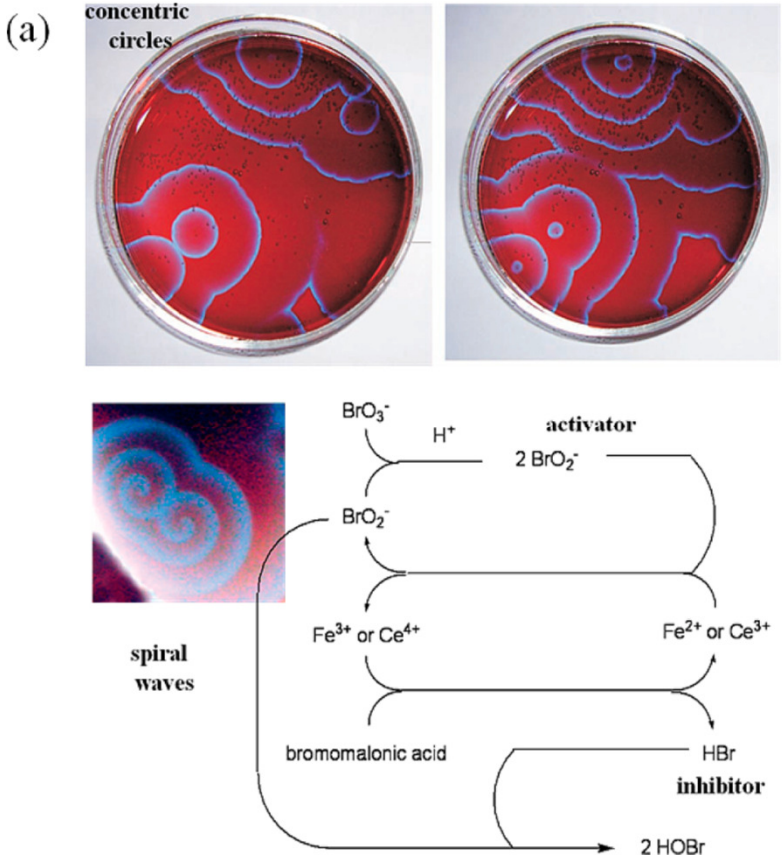
temperature, pH, humidity, illumination, UV irradiation, astronomic cycles

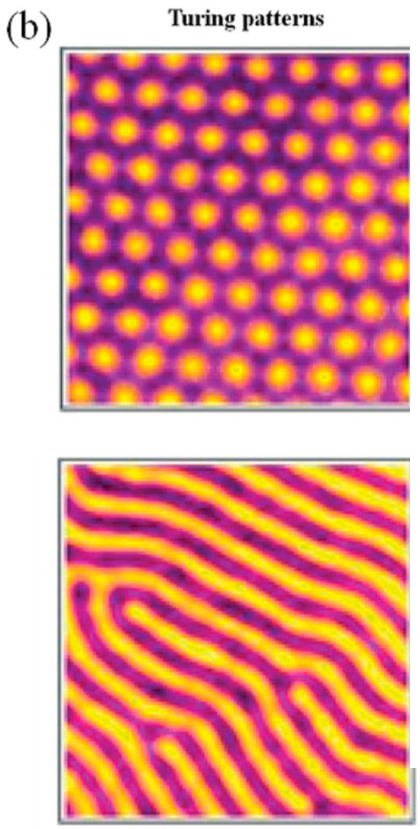
Chemical systems that mimic biological oscilations are studied as simple models

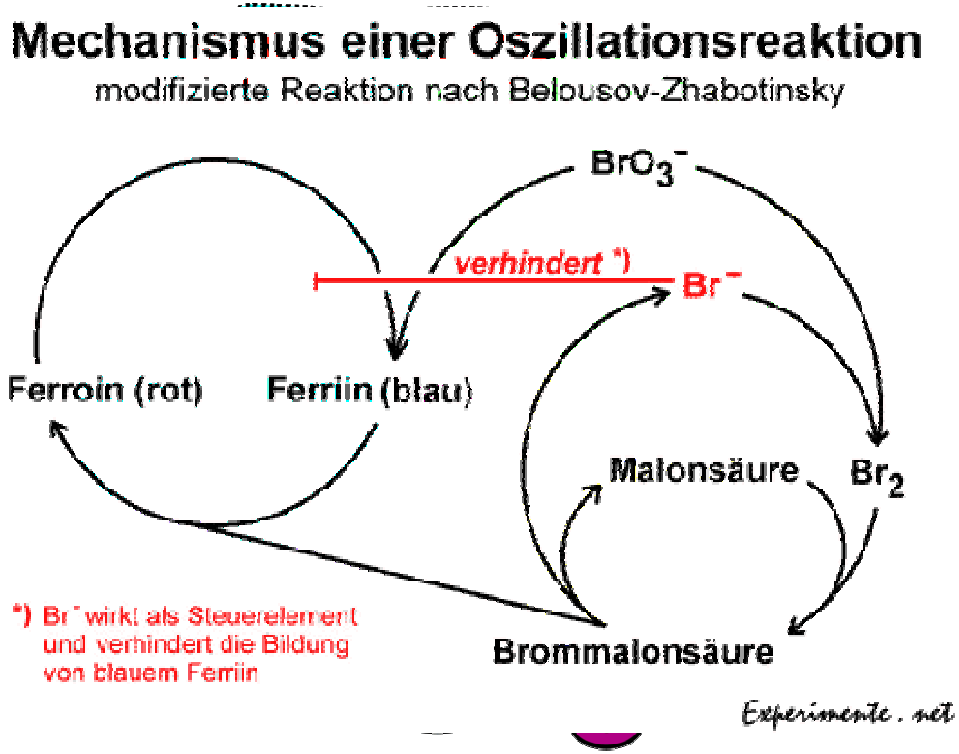
Belousov-Zhabotynski, CIMA reaction

Oscilatory reactions – activation and inhibition steps provide feedback loops to control the reaction speed. The most ancient protometabolic networks could have similar basic properties.

Belousov-Zhabotynski (BZ) reaction



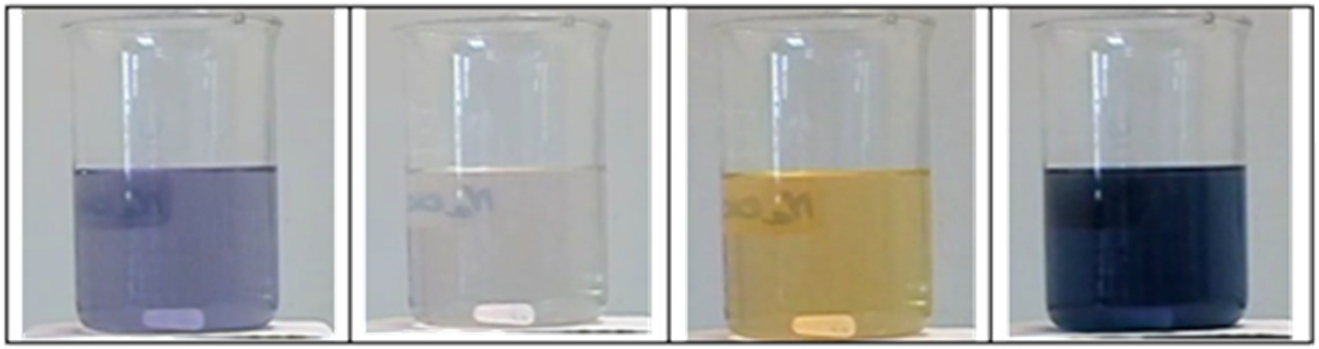




The reaction usually involves potassium bromate(VII) and malonic acid, optionally with cerium(IV) sulfate and citric acid. Ferroin is one of the common redox indicator

Briggs-Rauscher reaction

$$IO_3^- + 2\,H_2O_2 + CH_2(CO_2H)_2 + H^+ \rightarrow ICH(CO_2H)_2 + 2\,O_2 + 3\,H_2O$$

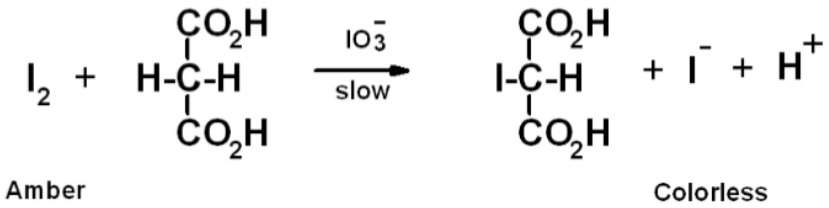


1. **The iodate ion is changed into iodine by hydrogen peroxide. The color changes to amber:**

$$2\,IO_3^- + 2\,H^+ + 5\,H_2O_2 \xrightarrow[\text{fast}]{Mn^+} I_2 + 5\,O_2 + 6\,H_2O$$

Colorless — Amber

2. **The free iodine reacts with malonic acid to produce iodide ions.**



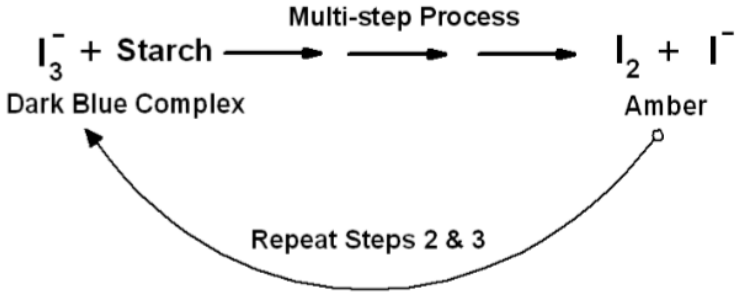
Amber — Colorless

3. **The free iodine combines with iodide very rapidly to form the negative ion I_3^-, which reacts with starch to form a dark blue complex:**

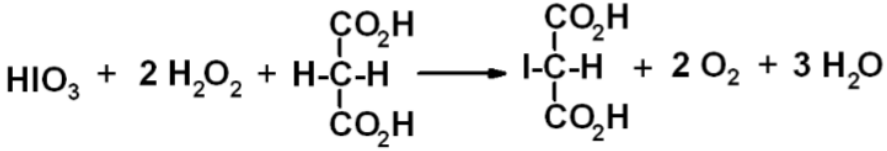
$$I_2 + I^- \xrightarrow{\text{fast}} I_3^- + \text{Starch} \xrightarrow{\text{fast}} \text{Dark Blue Complex}$$

Amber

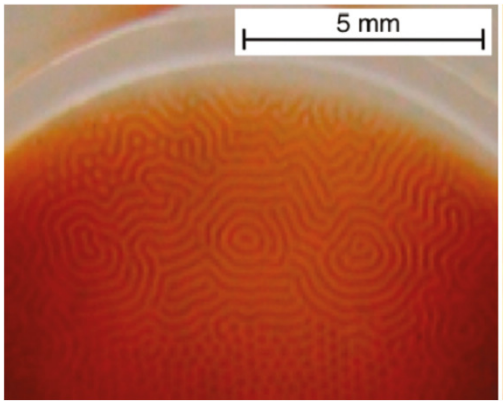
4. **After a period of time, the I_3^- ions are converted back into iodine and iodide ions, so the dark blue color disappears and the process repeats itself:**

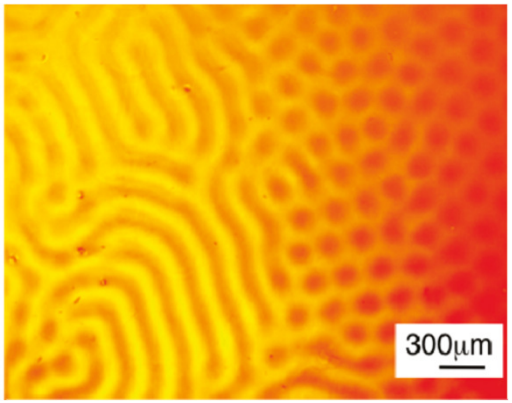


5. **Eventually the faster step 3 becomes dominant and the change of I_3^- back to iodine/iodide stops after about 15 cycles, so the solution remains dark blue. The overall chemical reaction is:**



Chlorite/iodide/malonic acid (CIMA) reaction





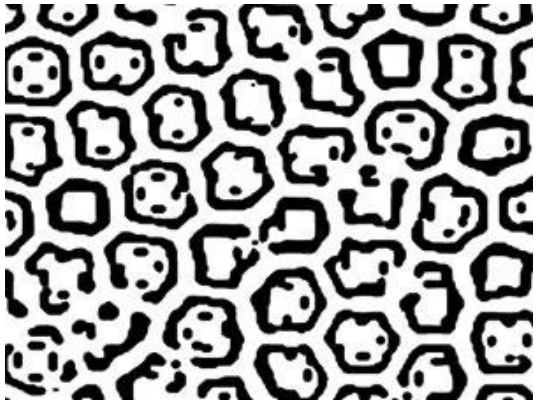
For the spontaneous generation of a Turing pattern, two intermediate species, an activator and an inhibitor, should be generated with the diffusion coefficient of the activator smaller than that of the inhibitor. The CIMA reaction that generates the activator, I^-, and inhibitor, ClO_2^-, was performed in an open gel reactor.

The mechanism of Turing pattern generation is also likely responsible for formation of stripes in certain mammals (e.g. zebra), or arrangement of leafs in plants

J. Phys. Chem. B 115(14):3959-63

Turing patterns also observed in metabolic reactions (glycolysis)

PLoS ONE 2007, 2(10):e1053



„Rosette" spots of a jaguar can be reproduced by two coupled activator/inhibitor processes

Autocatalytic processes

Inherent components of oscilatory reactions

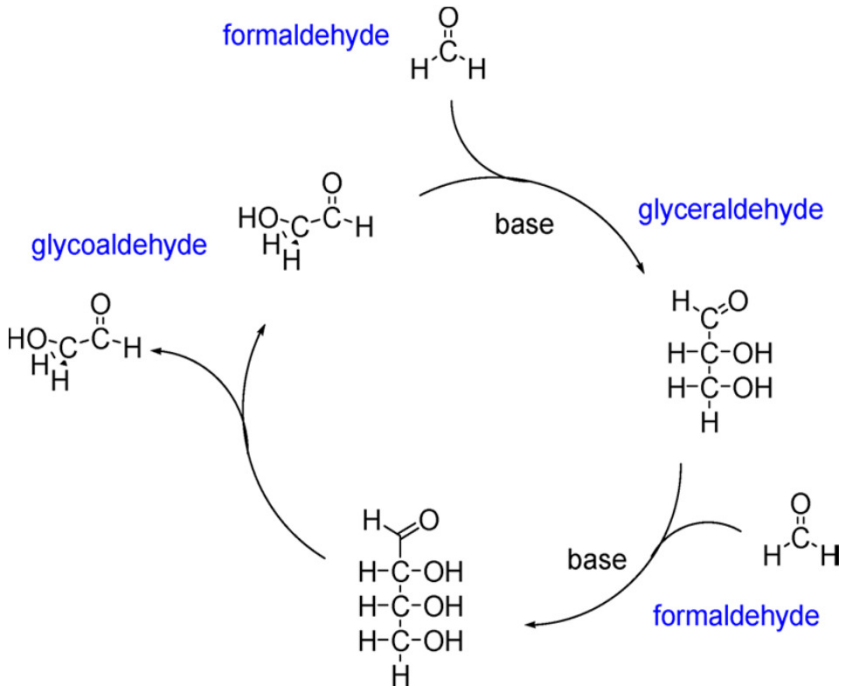
Explain the origin of homochirality

Fundamental concept for any system that grows and produces more copies of itself

Transition from chemical systems to biological ones inherently involves autocatalysis

Particularly interesting are links between chemistry and primitive metabolic pathways

Autocatalytic processes – formose reaction

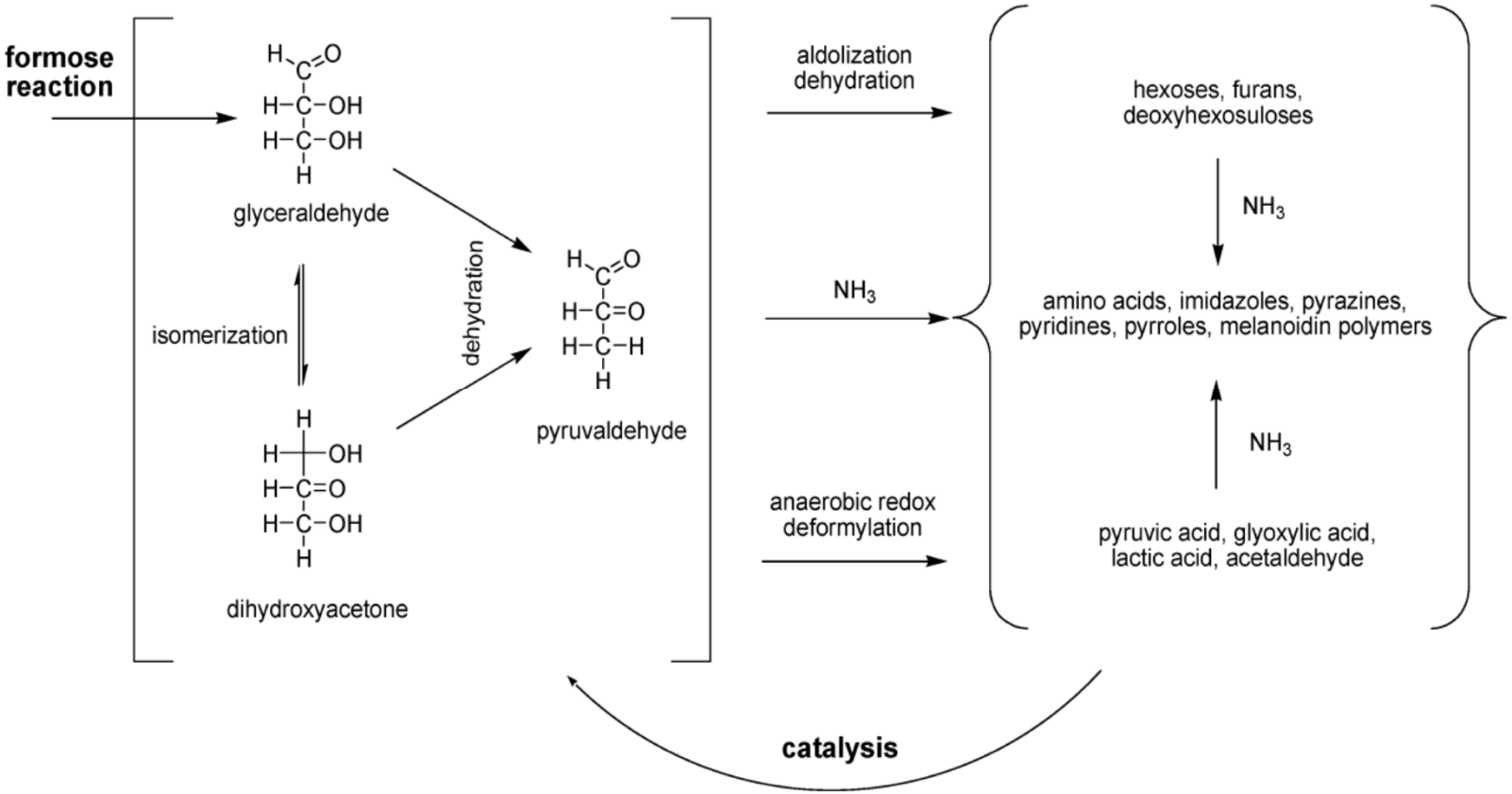


Formose reaction is one of the simplest autocatalytic cycles – two molecules of glycolaldehyde are produced from one.

Such unitary autocatalytic cycles would provide kinetic evolutionary advantage to evolving metabolic networks

More complex views on autocatalytic cycles

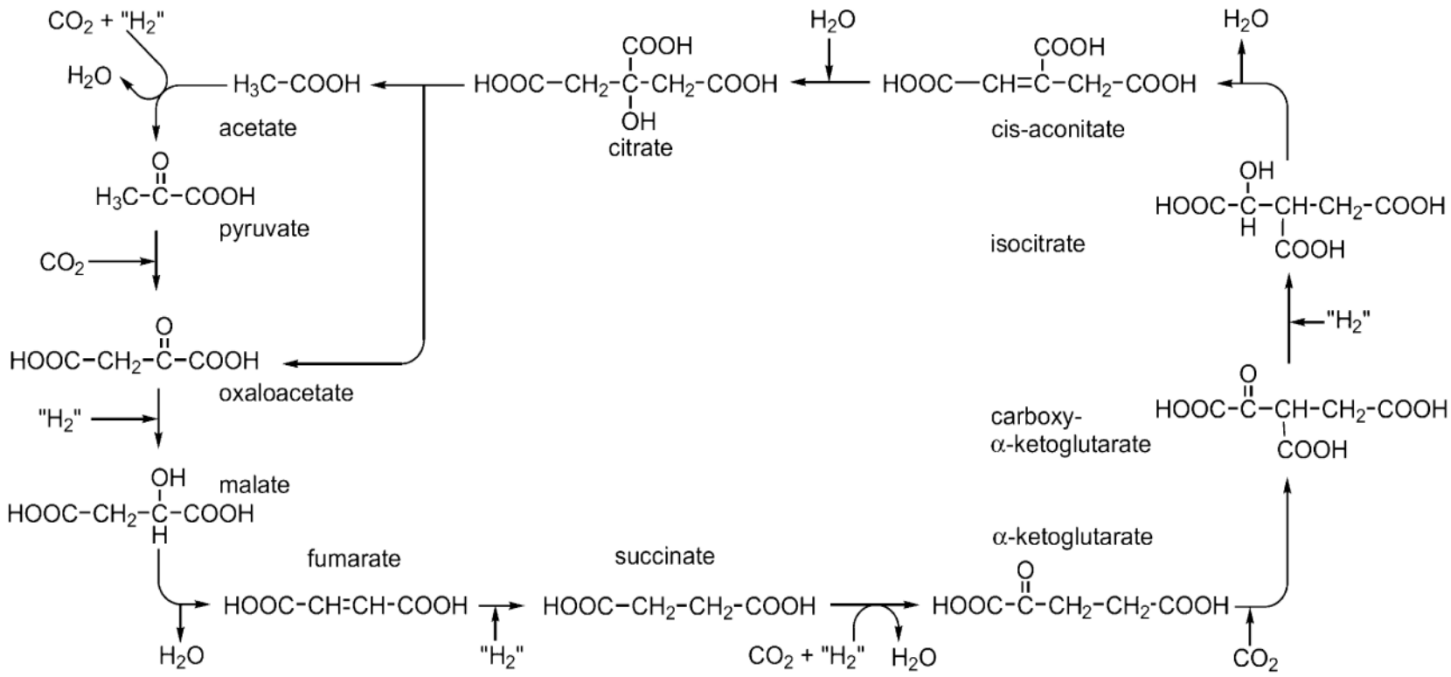
Coupling formose reaction with ammonia and thiols yields reactive α-hydroxy and α-aminothioesters, as well as numerous other aliphatic and aromatic compounds. Some of them enter another autocatalytic cycles.



This additionally suggests that glycolysis was the ancient metabolic pathway

Prebiotic variants of the reductive citric acid (Krebs/tricarboxylic acid) cycle

TCA/Krebs cycle is central for metabolism in aerobic forms of life.
The reverse citric acid cycle is used by some bacteria to produce complex carbon compounds from CO_2 and H_2O

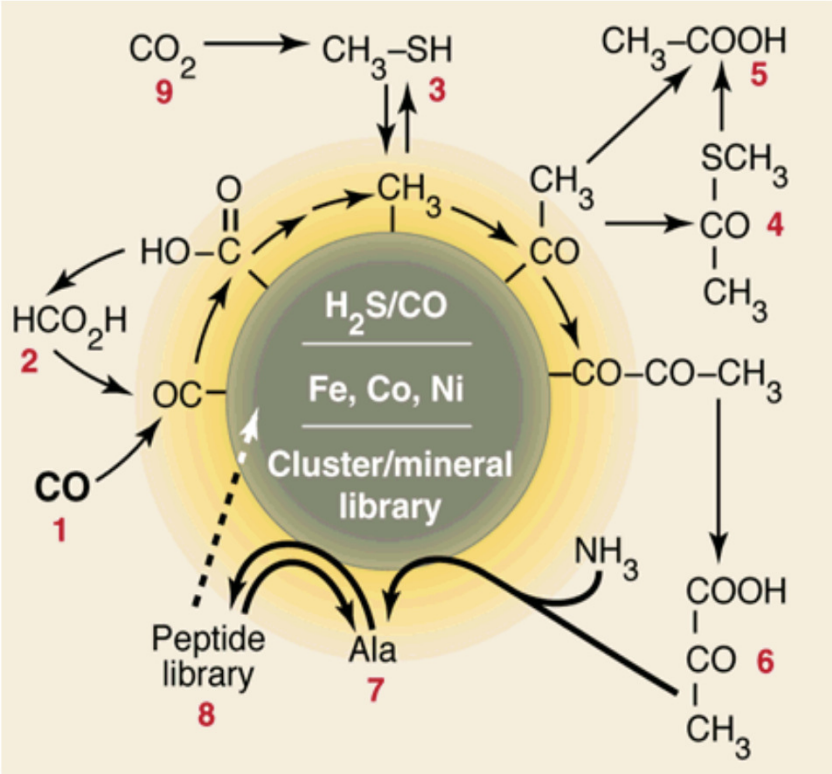


This catalytic cycle is claimed (Morowitz) to be able to run also in absence of enzymes (e.g. on mineral surfaces).
This could be the starting point for evolution of all other currently operating metabolic cycles.
However, no experimental demonstration of the full cycle under abiotic conditions delivered yet.

Problems: cross-reactivity, side reactions that drain active intermediates and energy until cycles stop.

Wächtershäuser' Iron-Sulfur World

The reverse citric acid cycle (Krebs' cycle)



$Ni(OH)_2 + HCN \rightarrow Ni(CN)_2$

$Ni(CN)_2 + CO \rightarrow \rightarrow$ glycolate/glycine
lactate/alanine
glycerate/serine
pyrruvic acid

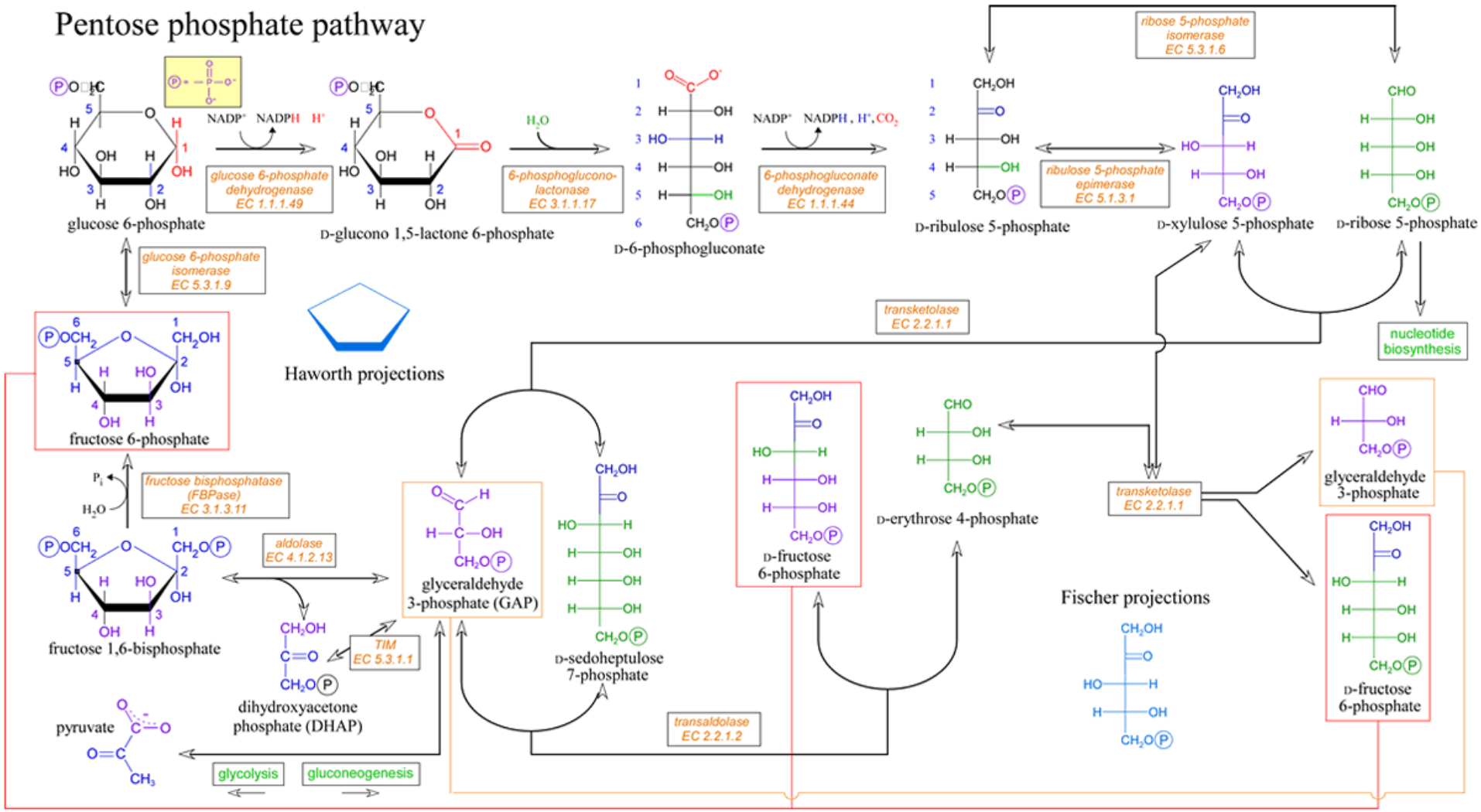
Ketoacids + NH_3 + $Fe(OH)_2$ + FeS (cat) + $H_2S \rightarrow$ aminoacids

Aminoacids + COS (or CO + H_2S) $\rightarrow$ oligopeptides

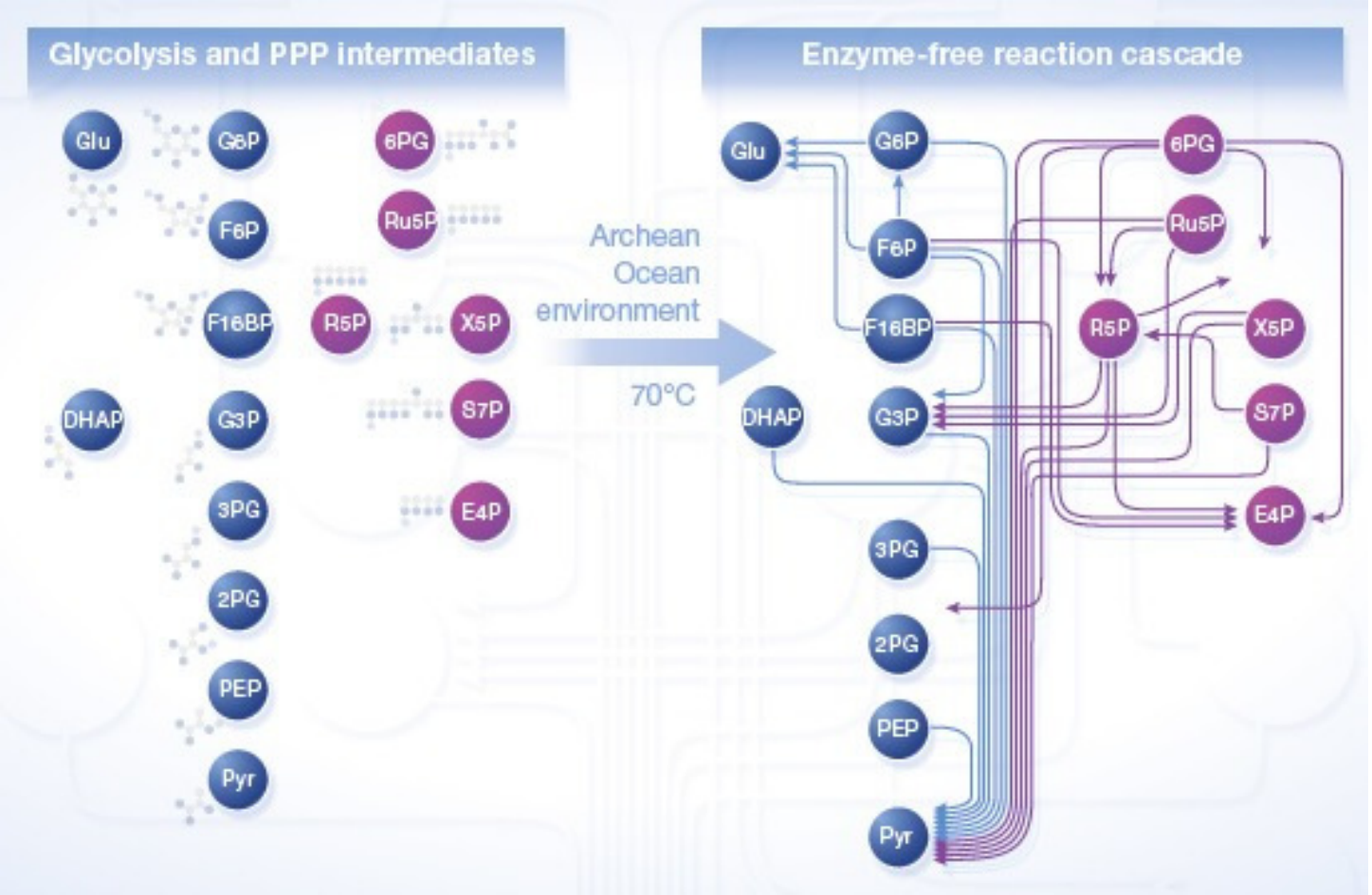
Currently, Krebs' cycle serves in organisms to degrade sugars into CO_2 and water and produce energy. In the „Iron-Sulfur World" the reverse Krebs' cycle would produce complex organic molecules out of CO_2 and energy from the hydrothermal vents

Metabolism may have started in our early oceans before the origin of life

Pentose phosphate pathway

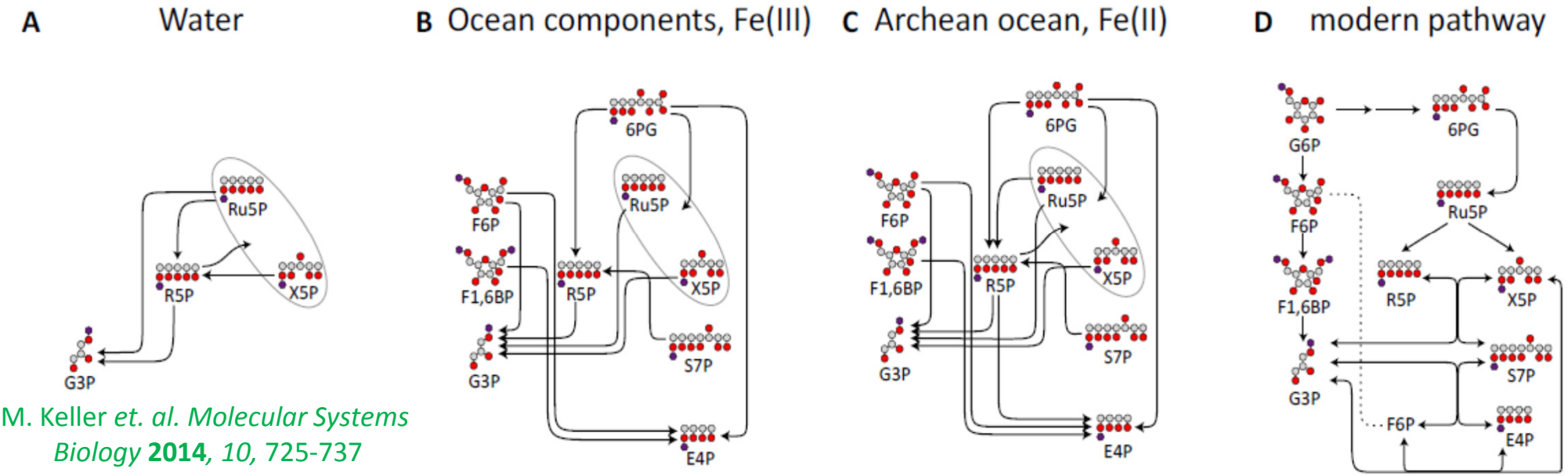


Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment



M. Keller *et. al. Molecular Systems Biology* **2014**, *10*, 725-737

Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment



M. Keller *et. al. Molecular Systems Biology* **2014**, *10*, 725-737

A Spontaneous reactivity of glycolytic and pentose phosphate pathway sugar phosphate intermediates as observed in water.

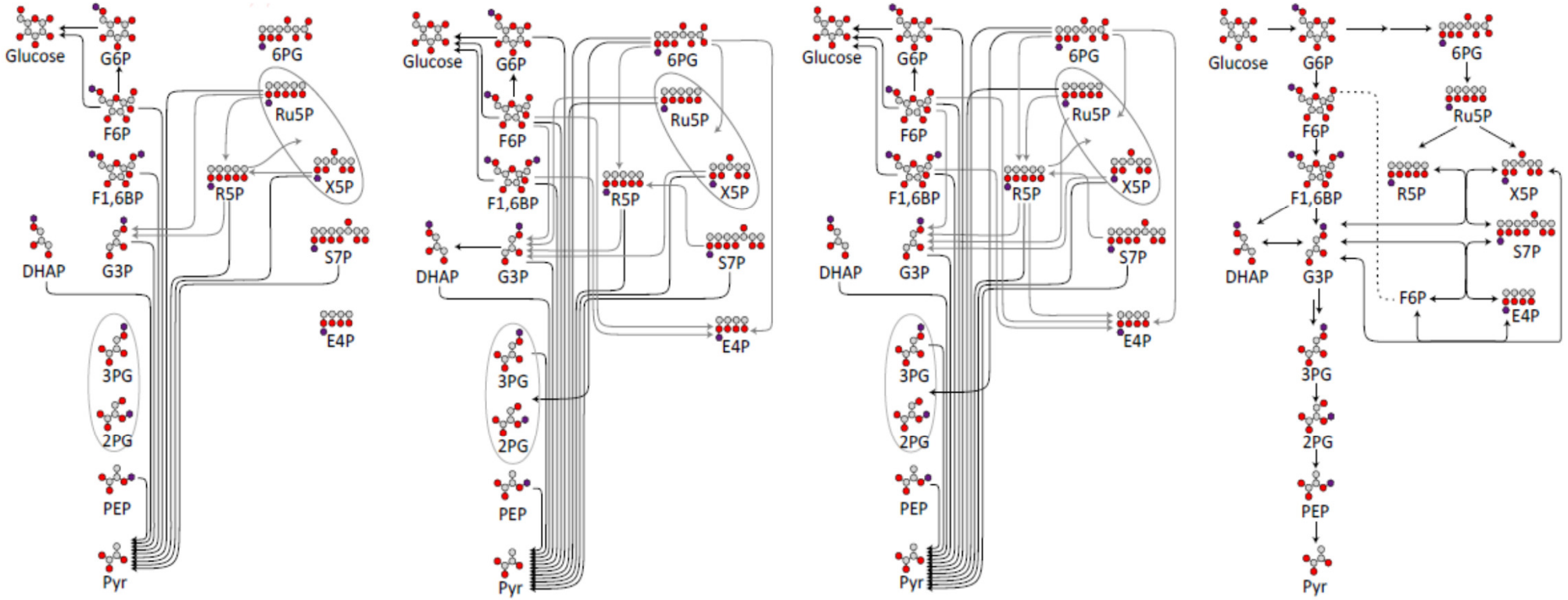
B The same reactions in solution with Fe^{III}, Co^{II}, Ni^{II}, Mo and phosphates simulating an Archean ocean.
In this milieu, 28 interconversion reactions among glycolytic and pentose phosphate pathway intermediates were observed.

C Iron maintained Fe(II) (as in reducing early oceans). *29 metabolite formation reactions were detected*. Differences to (B) concern additional interconversion of pentose phosphate metabolites, and fewer interconversions of 3-carbon metabolites.

D Network topology of modern glycolysis (canonical Embden-Meyerhof pathway) and the pentose phosphate pathway.

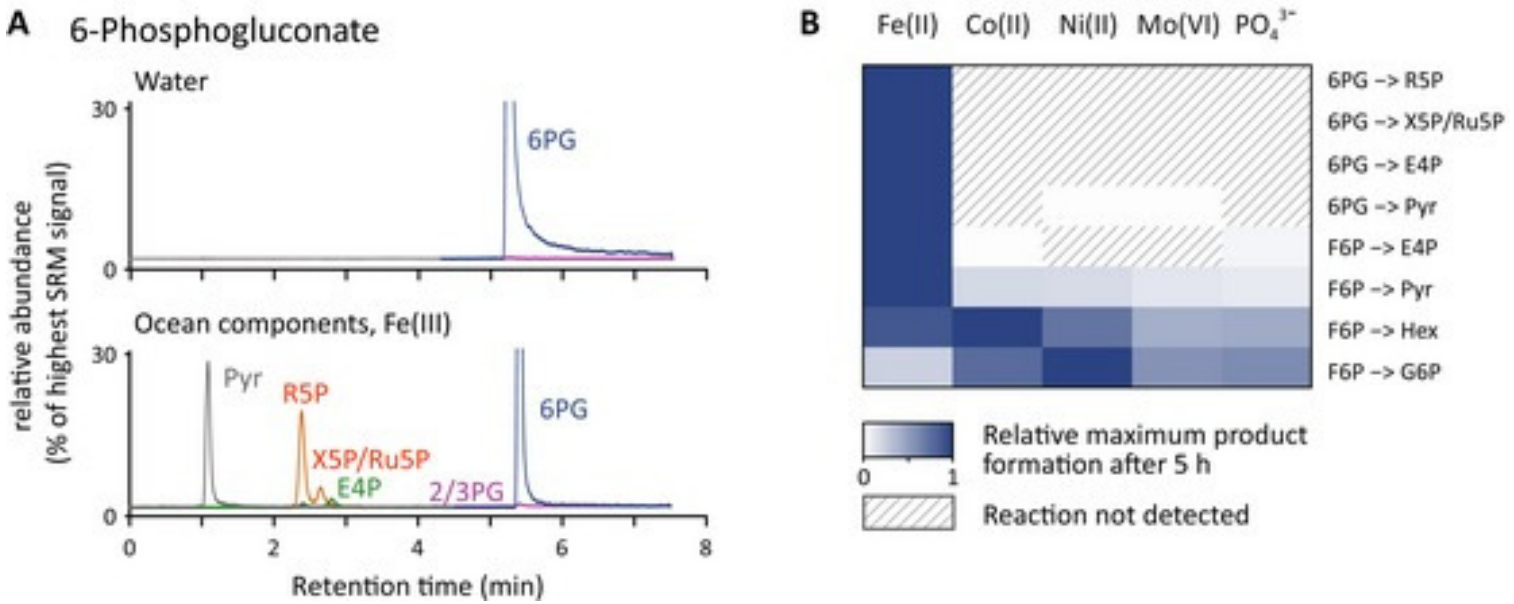
Pentose phosphate pathway: 6PG, 6-phosphogluconate; Ru5P, ribulose 5-phosphate; R5P, ribose 5-phosphate; X5P, xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; E4P, erythrose 4-phosphate.

Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment



Pentose phosphate pathway: 6PG, 6-phosphogluconate; Ru5P, ribulose 5-phosphate; R5P, ribose 5-phosphate; X5P, xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; E4P, erythrose 4-phosphate. **Glycolysis**: G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; F16BP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde 3-phosphate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate.

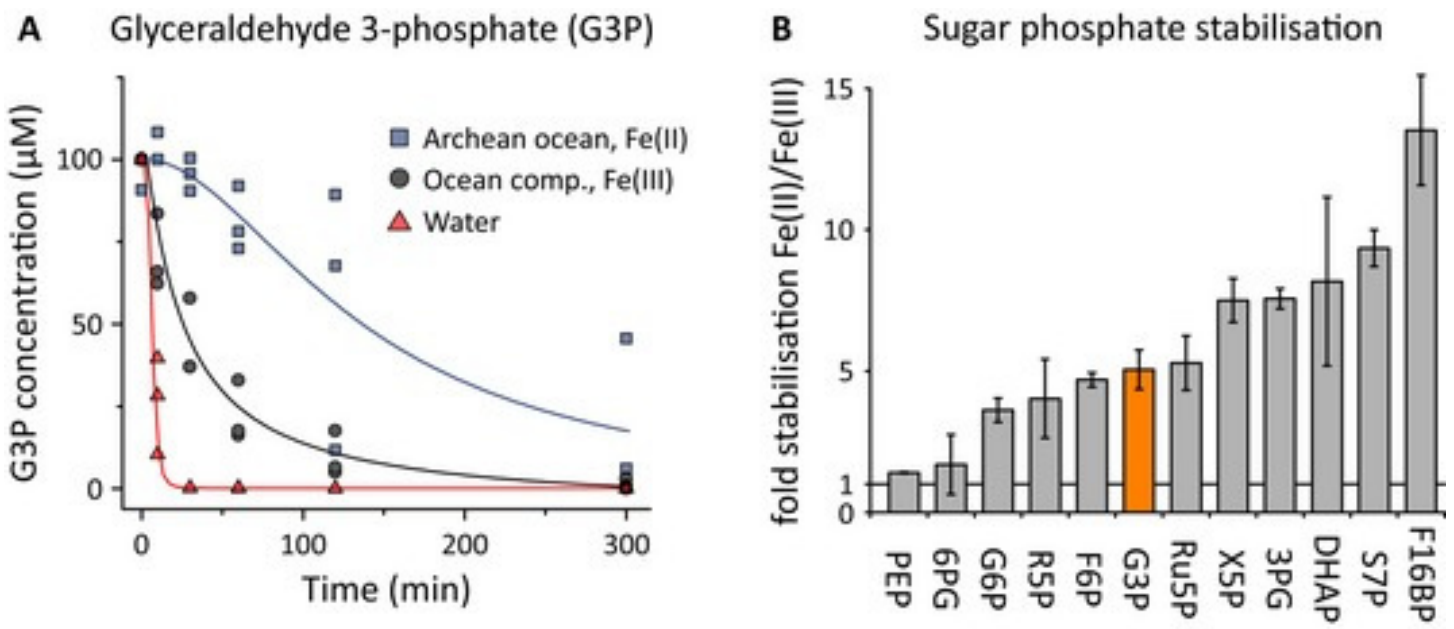
M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737



The Archean ocean ionic composition catalyses sugar phosphate interconversions. 6-phosphogluconate (6PG) was incubated at 70°C in water, or in the presence of Archean ocean plausible concentrations of Fe, Co, Ni, Mo and phosphate. The chromatograms illustrate an exemplary LC-SRM run targeting the glycolytic and pentose phosphate pathway intermediates recorded after 2 h. 6PG was stable in water (upper panel), but was interconverted into other pentose phosphate pathway intermediates and pyruvate as catalysed by the Archean ocean components (lower panel).

Iron is the predominant catalyst for pentose phosphate pathway interconversions. 6-phosphogluconate (6PG) and fructose 6-phosphate (F6P) were incubated at 70°C in the presence of the indicated Archean ocean constituents, and the formation of reaction products was monitored by LC-SRM over 2 h. Ferrous iron facilitated the interconversion of the metabolites into eight metabolic intermediates, whereas Co, Ni, Mo and phosphate together contributed to a subset of the reactions.

M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737



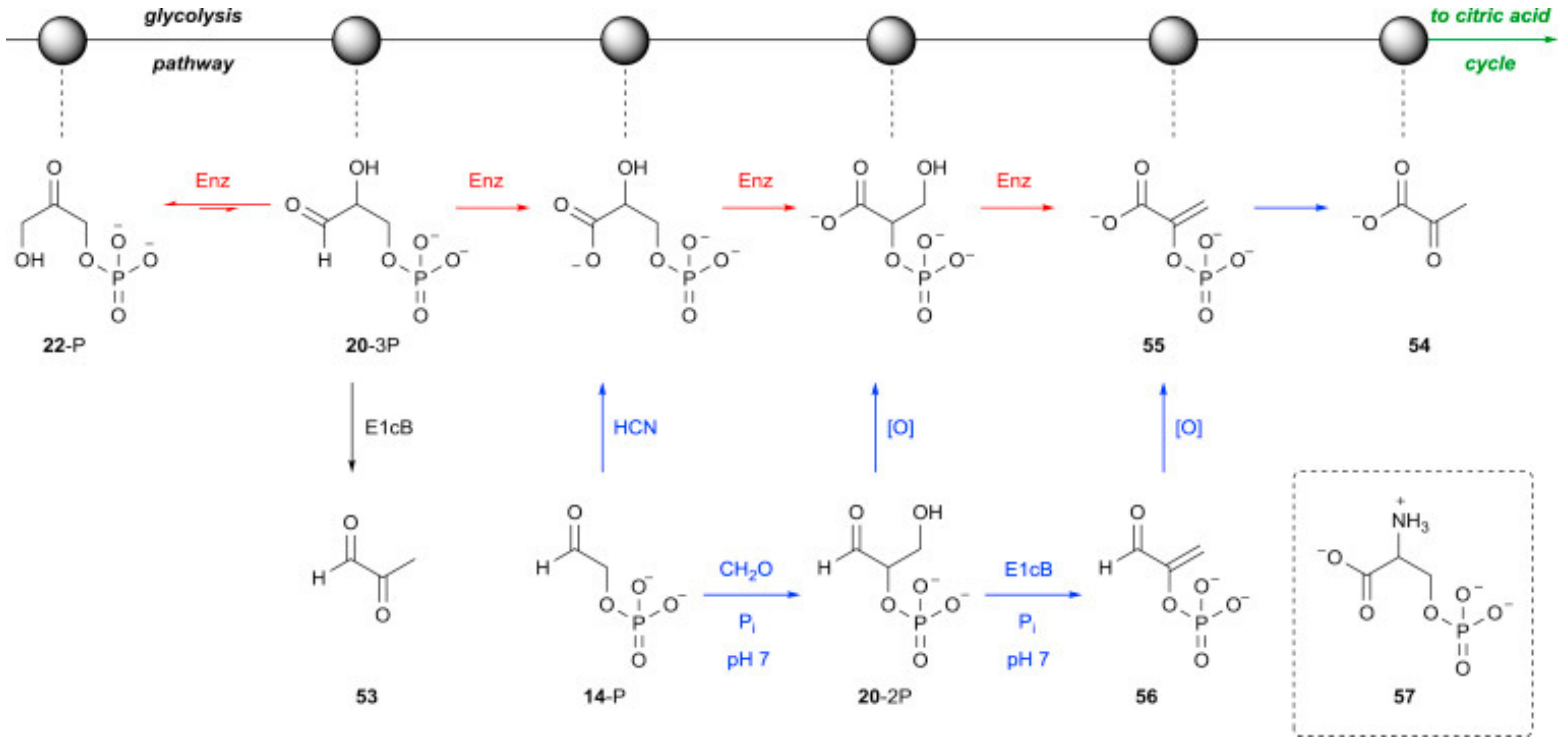
The stability of glyceraldehyde 3-phosphate (G3P) in Archean ocean simulations. G3P was diluted in water, or the Archean ocean mimetic containing Fe(III), Co, Ni, Mo and phosphate, or the analogous anoxic solution containing Fe(II). The solutions exposed to 70°C and monitored by LC-SRM for 5 h. G3P was degraded in water within minutes, was stabilized by the oxygenated, metal-rich ocean mimetic and remained detectable for more than 5 h in the ferrous iron-rich ocean simulation.

The ferrous iron-rich Archean ocean ionic composition favours stability of sugar phosphate intermediates. Glycolytic and pentose phosphate pathway intermediates were exposed to 70°C as in (A) and their concentration monitored over 5 h. Illustrated is the fold increase in stability in the Fe(II)-rich Archean ocean mimetic over the corresponding stability in the Fe(III)-rich isoionic solution. All sugar phosphate intermediates that constitute the PPP and glycolysis gained stability.

M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737

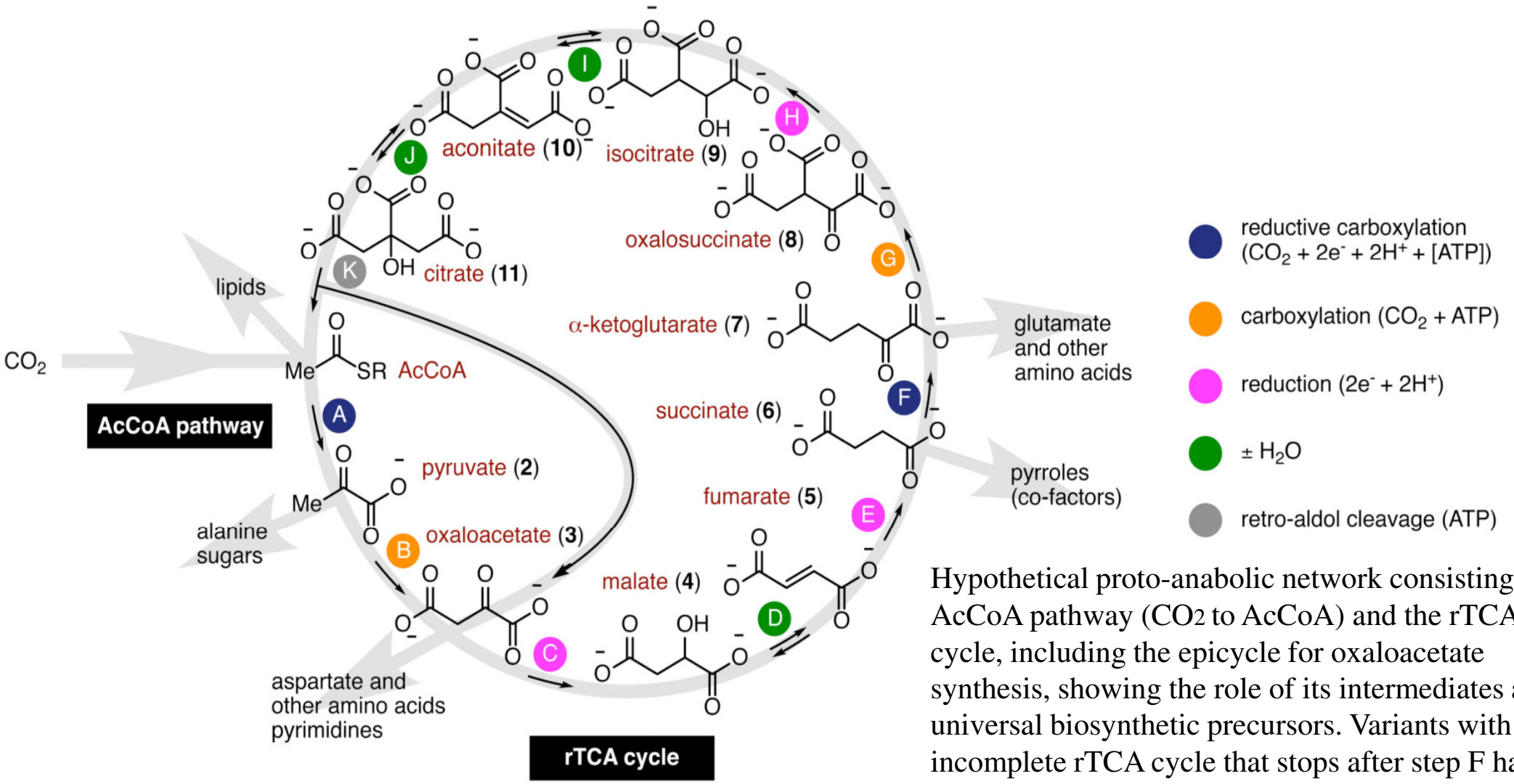
Prebiotic soup - summary

Prebiotic Reconstruction of the Triose Glycolysis Pathway by Selective α-Phosphorylation of the Simplest Sugars



S. Islam, M. W. Powner *Chem* **2017**, *2*, 470-501

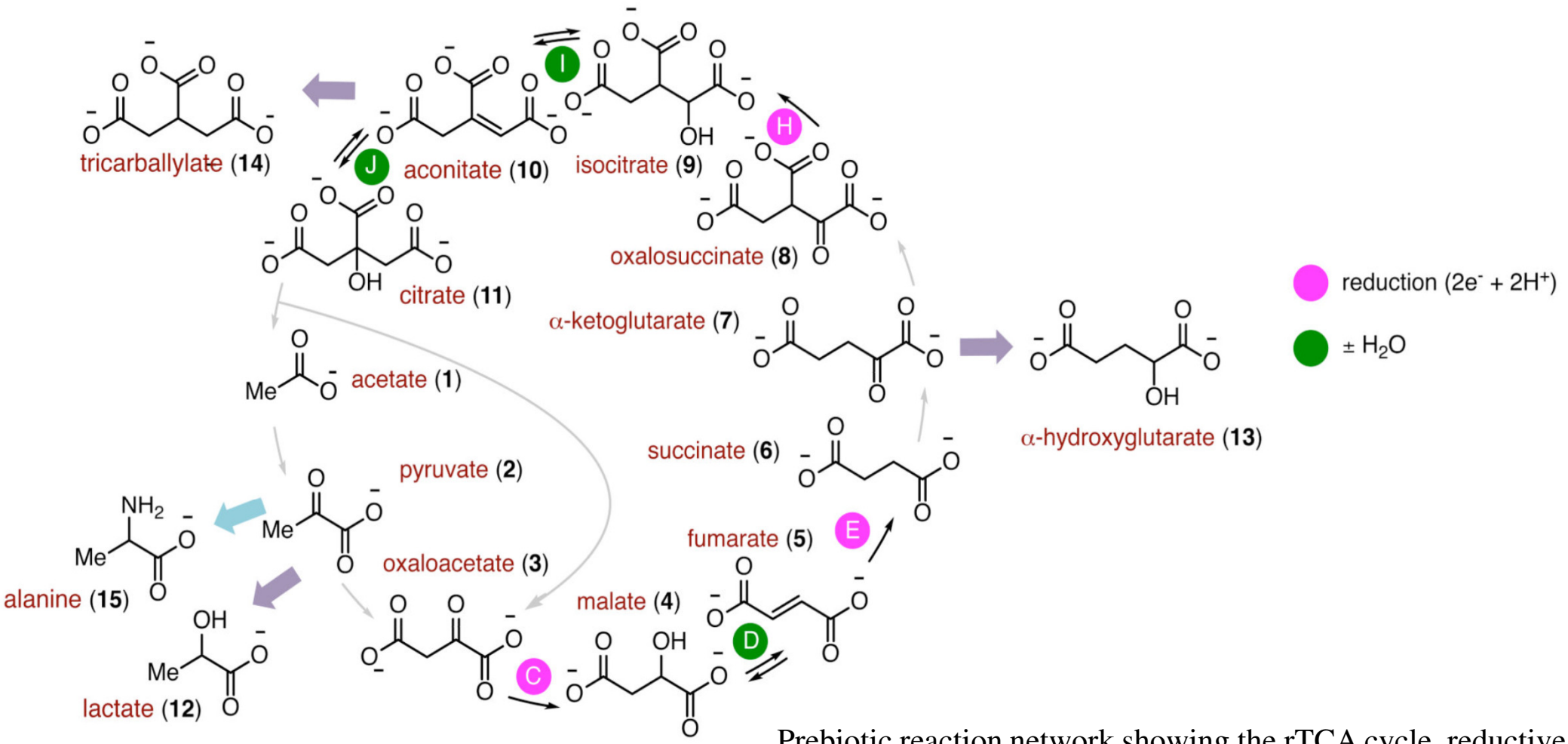
Metals promote sequences of the reverse Krebs cycle



Hypothetical proto-anabolic network consisting of the AcCoA pathway (CO_2 to AcCoA) and the rTCA cycle, including the epicycle for oxaloacetate synthesis, showing the role of its intermediates as universal biosynthetic precursors. Variants with an incomplete rTCA cycle that stops after step F have also been proposed

J. Moran *et al. Nat Ecol Evol.* **2017**, *1(11)*, 1716–1721

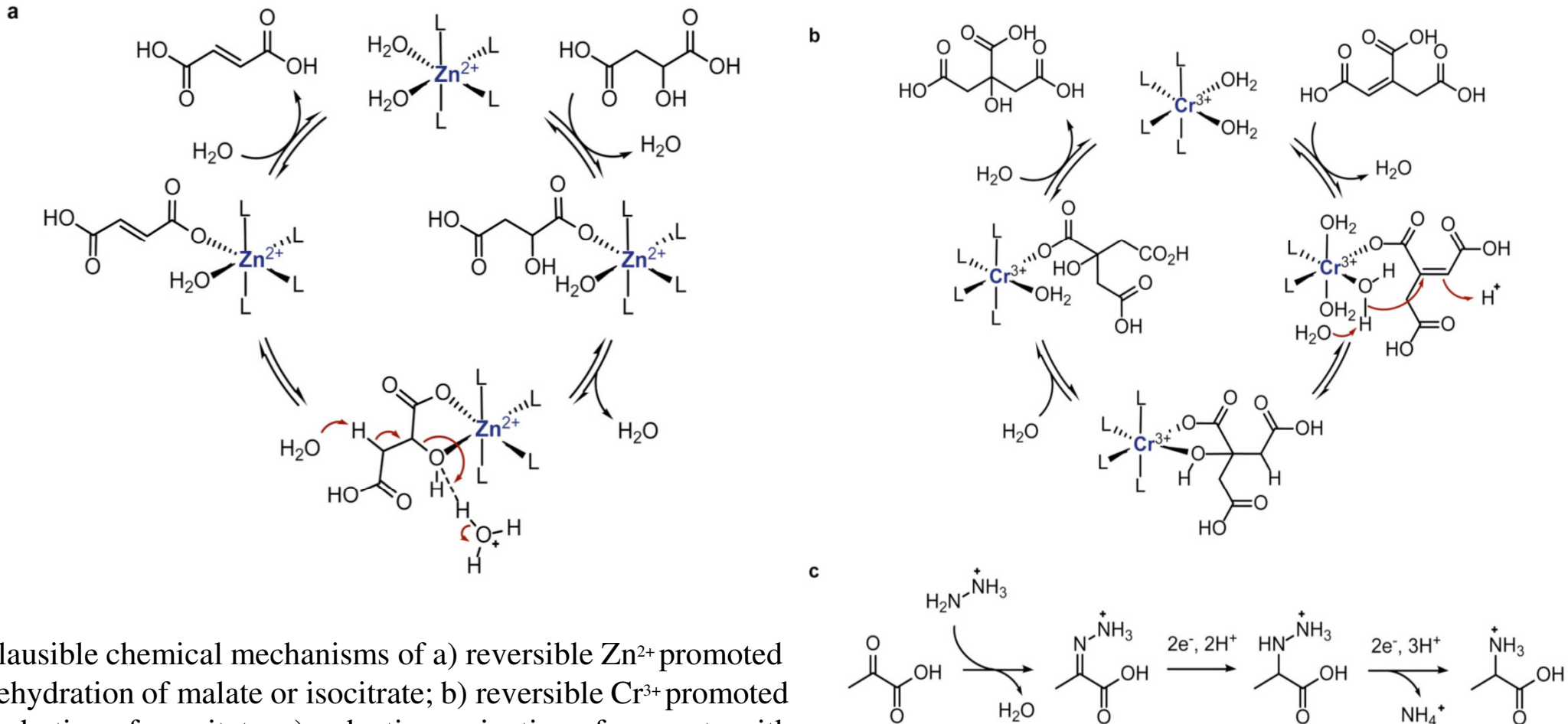
Metals promote sequences of the reverse Krebs cycle



Prebiotic reaction network showing the rTCA cycle, reductive amination (light blue arrow) and potential off-cycle reductions (mauve arrows).

J. Moran *et al. Nat Ecol Evol.* **2017**, *1(11)*, 1716–1721

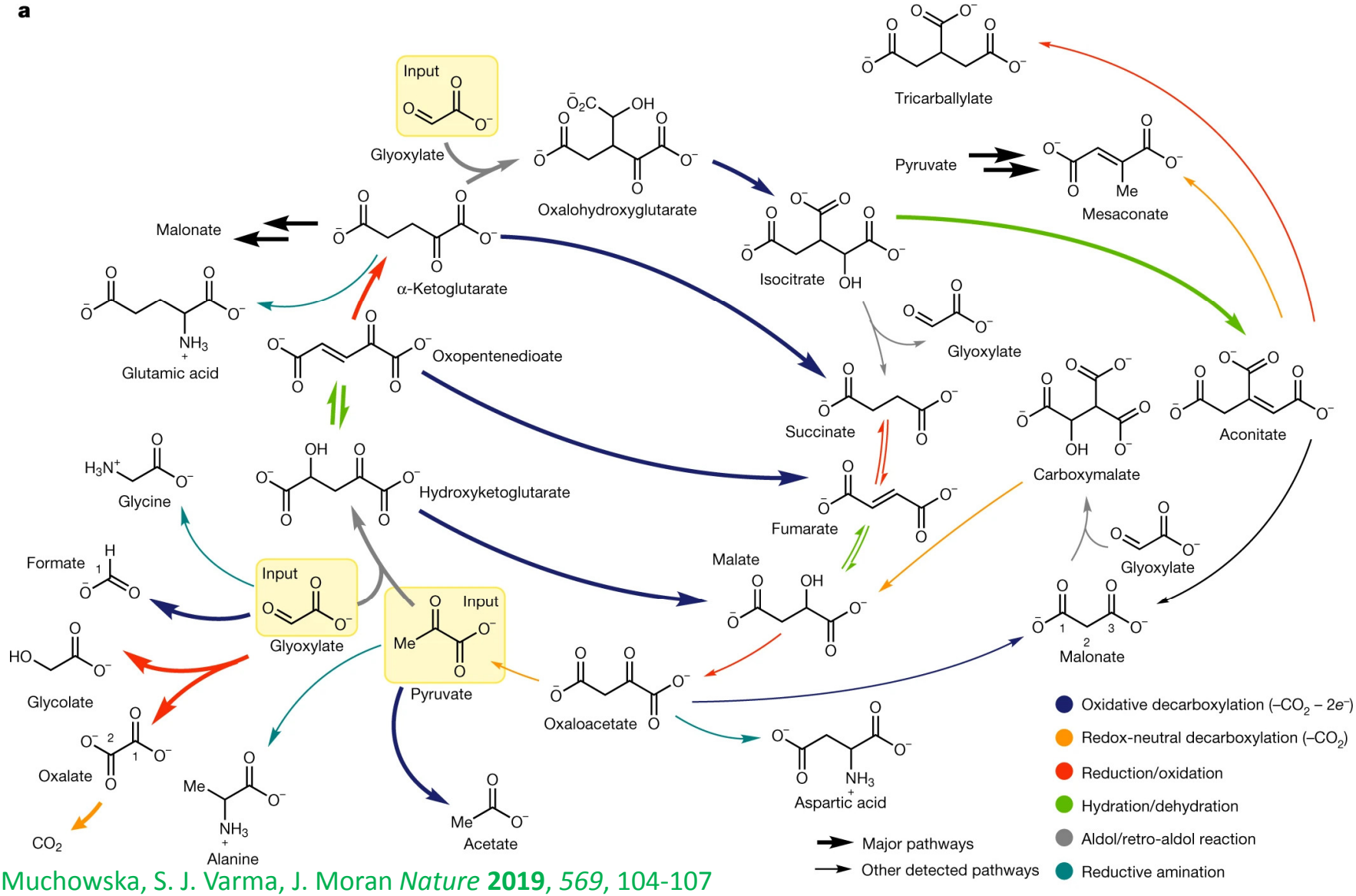
Metals promote sequences of the reverse Krebs cycle



Plausible chemical mechanisms of a) reversible Zn^{2+} promoted dehydration of malate or isocitrate; b) reversible Cr^{3+} promoted hydration of aconitate; c) reductive amination of pyruvate with hydrazine and subsequent reductive N-N bond cleavage to generate alanine. Metal complexes are depicted as mononuclear species for clarity. L = undefined ligand

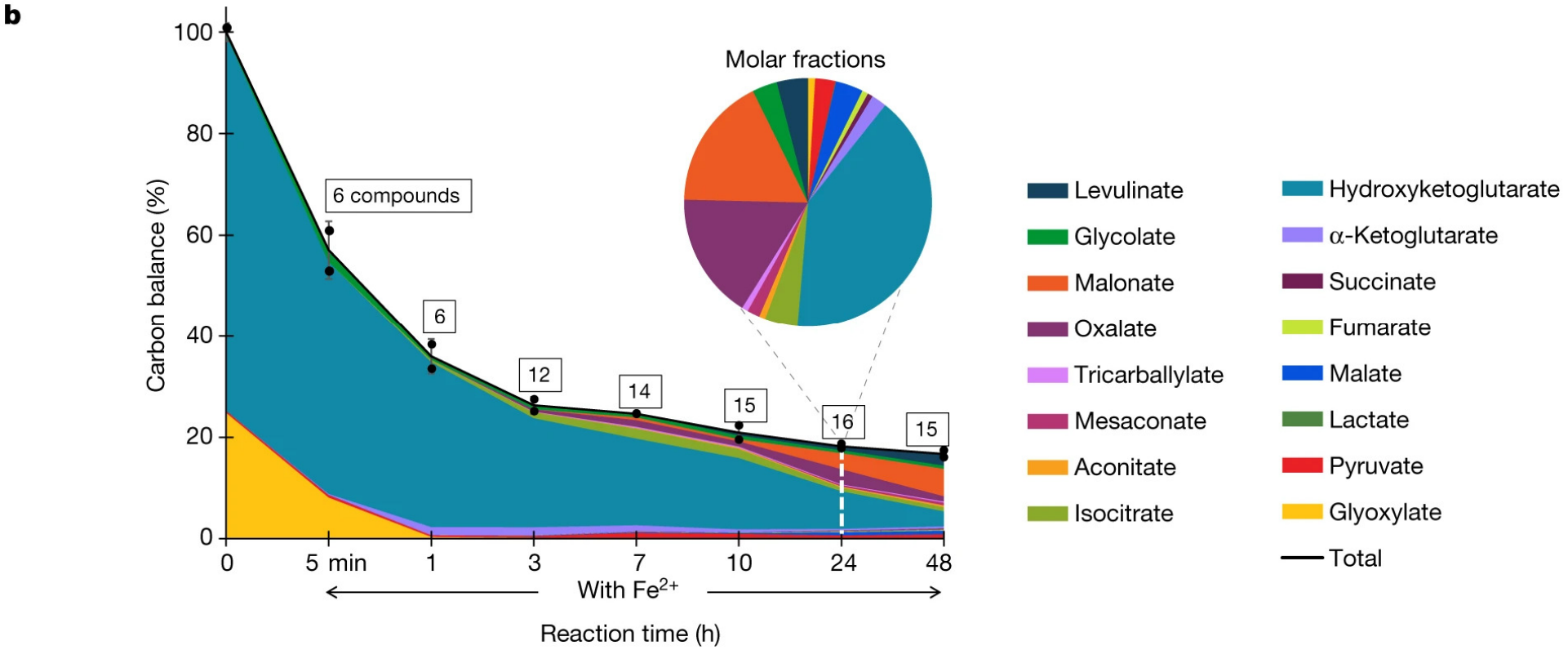
J. Moran *et al. Nat Ecol Evol.* **2017**, *1(11)*, 1716–1721

Synthesis and breakdown of universal metabolic precursors promoted by iron



K. B. Muchowska, S. J. Varma, J. Moran *Nature* **2019**, *569*, 104-107

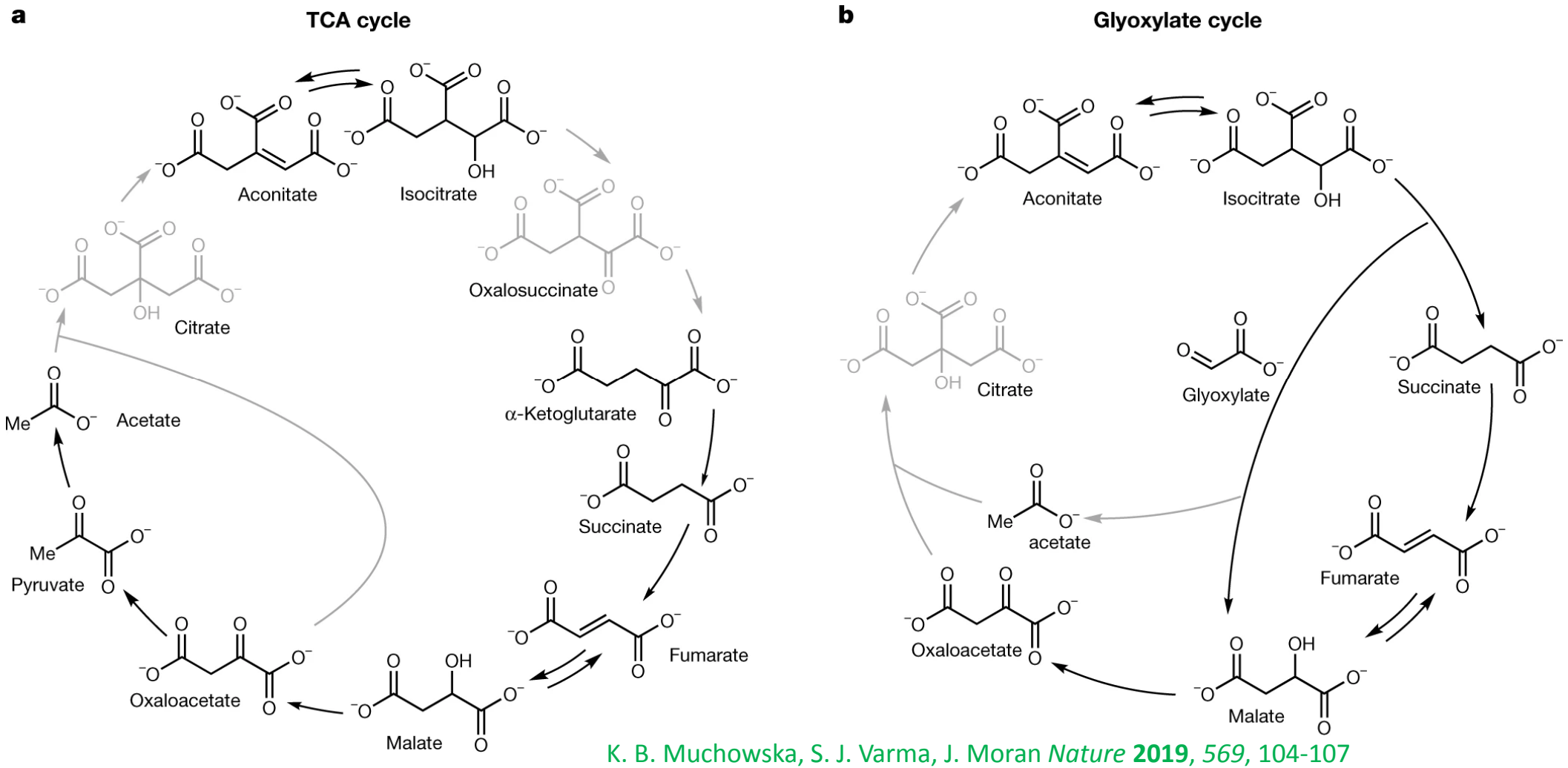
Synthesis and breakdown of universal metabolic precursors promoted by iron



K. B. Muchowska, S. J. Varma, J. Moran *Nature* **2019**, *569*, 104-107

Synthesis and breakdown of universal metabolic precursors promoted by iron

Comparison of the observed reaction network with the TCA and glyoxylate cycles. Intermediates and reactions found in both the biological cycle and the synthetic reaction network shown in black. Those found only in the biological cycle - in grey.



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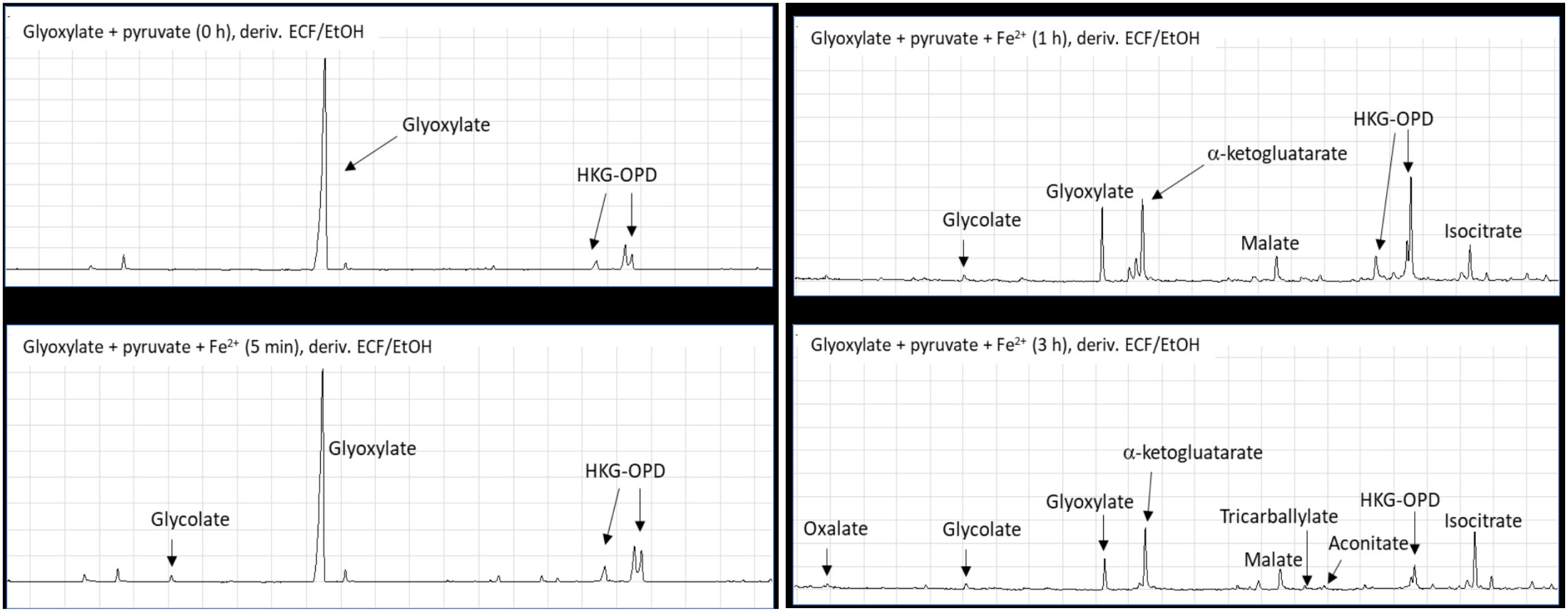

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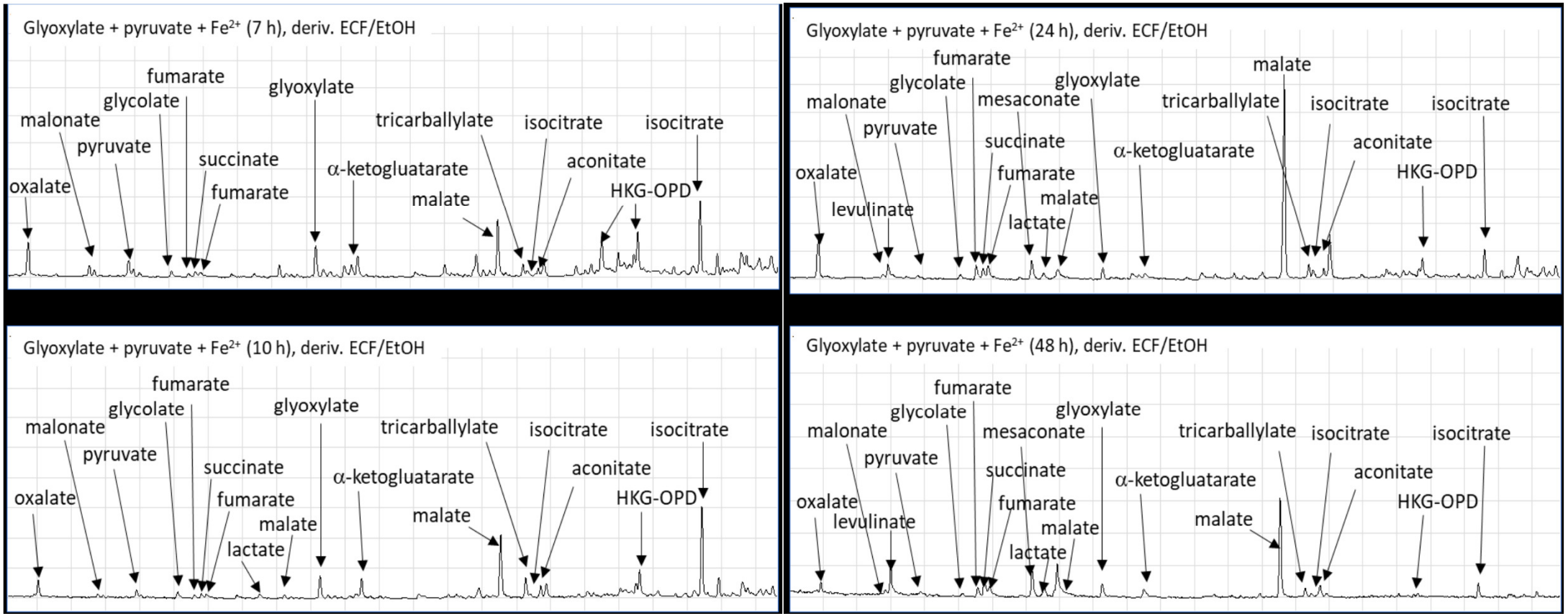

K. B. Muchowska, S. J. Varma, J. Moran *Nature* **2019**, *569*, 104-107

Metabolism-first - summary

Multiple components of contemporary metabolic cycles – reverse Krebs cycle and the pentose phosphate pathway can be successfully synthesized under prebiotically relevant conditions (iron ion catalysis, archaean ocean composition)

Unclear chemical nature of primordial metabolic cycles

Unclear how production of genetic molecules on the later stage provide evolutionary advantages

Evolution of such hypothetical networks requires multiple simultaneous mutations

In contrary, genetic polymers allow for additive accumulation of favorable mutations

A. Coggins, M. Powner *Nature Chem.* **2016**, *DOI: 10.1038/NCHEM.2624*