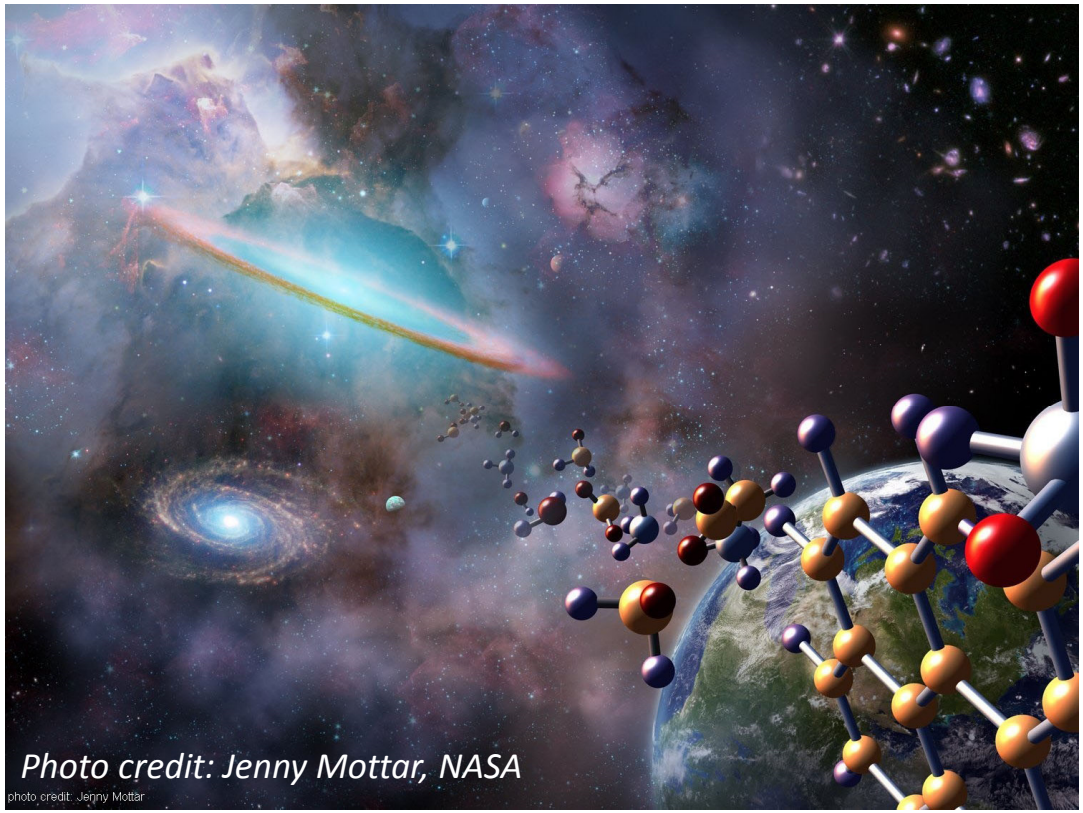
The molecular origins of life



Lecture 6, SoSe 2019 KIT

Zbigniew Pianowski

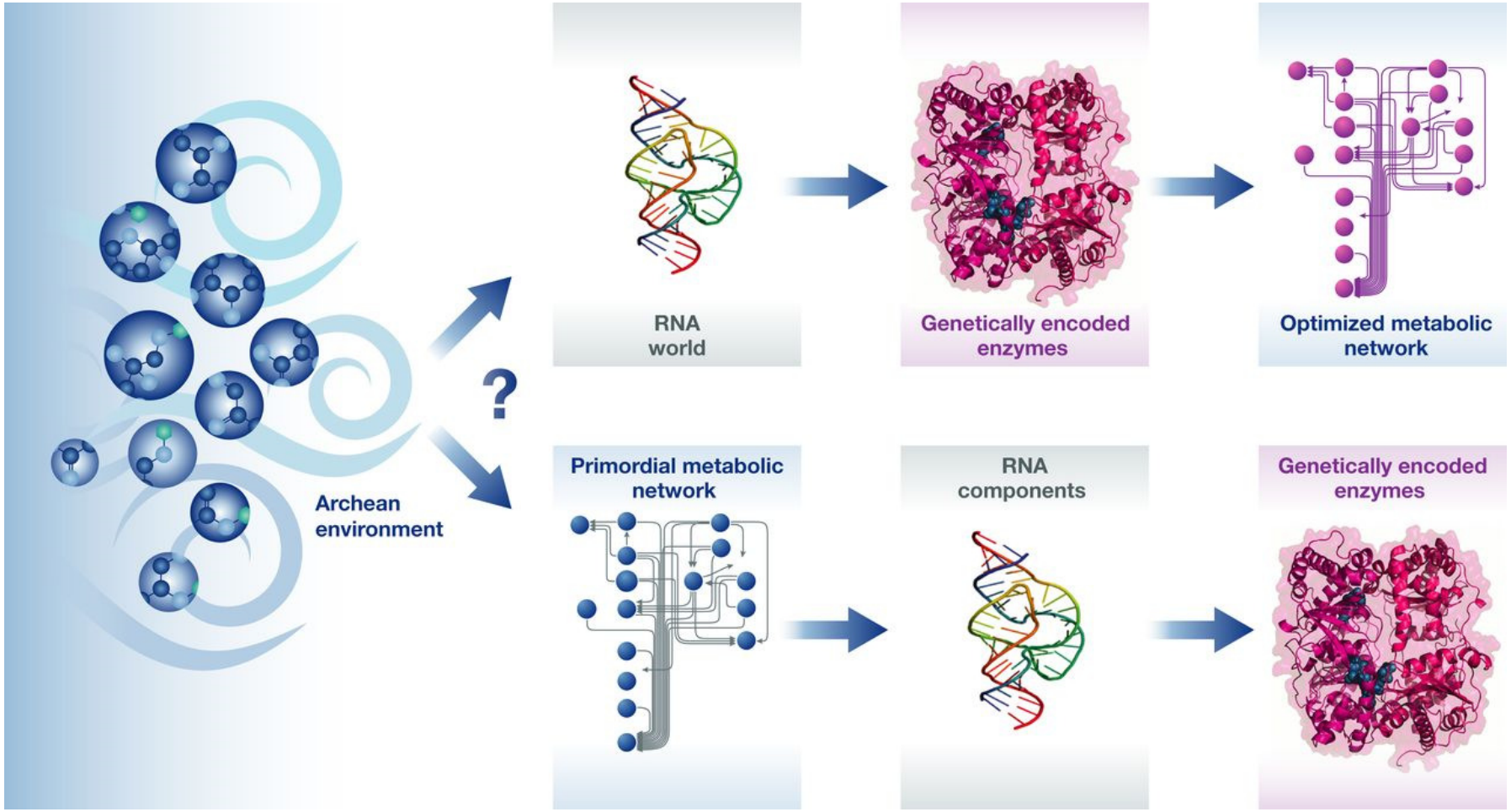
Origin of the Universe – stars, planets, elements

Origin of biorelevant monomers – primordial soup

Complex chemical processes on the way to living systems

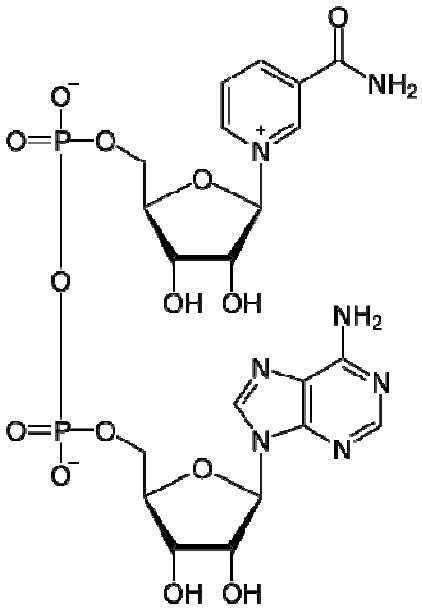
Protocells and LUCA

Route to life by chemical networks



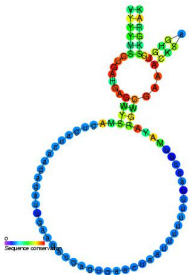
P. L. Luisi *Mol Syst Biol.* **2014,** *10,* 729

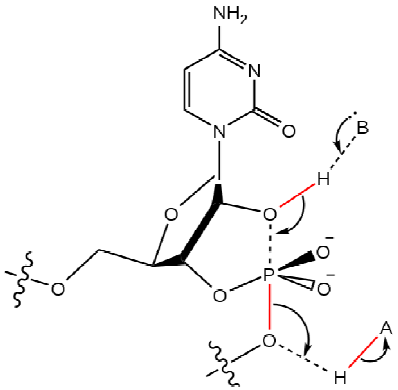
The RNA world



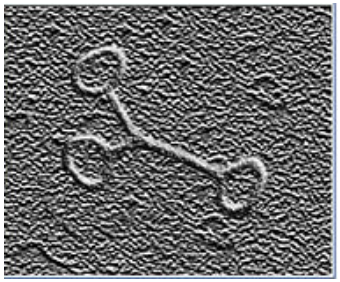
Nicotinamide adenine dinucleotide (NAD^+)

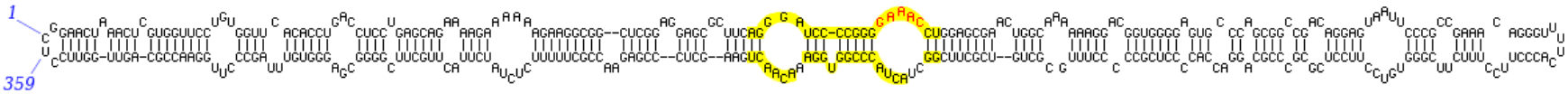
coenzymes



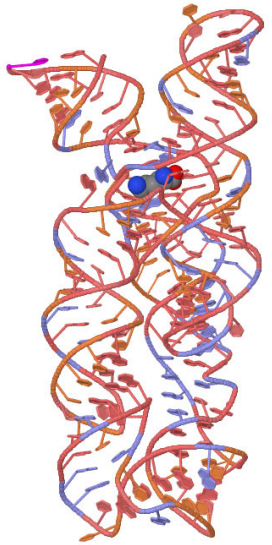


ribozymes



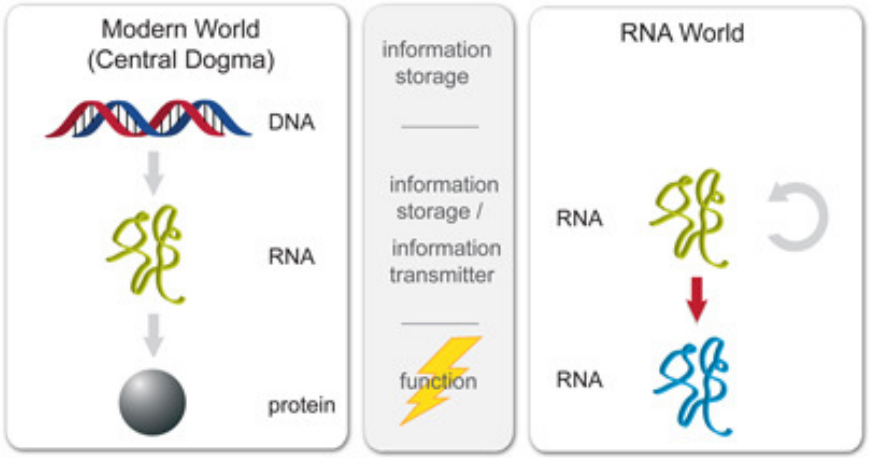


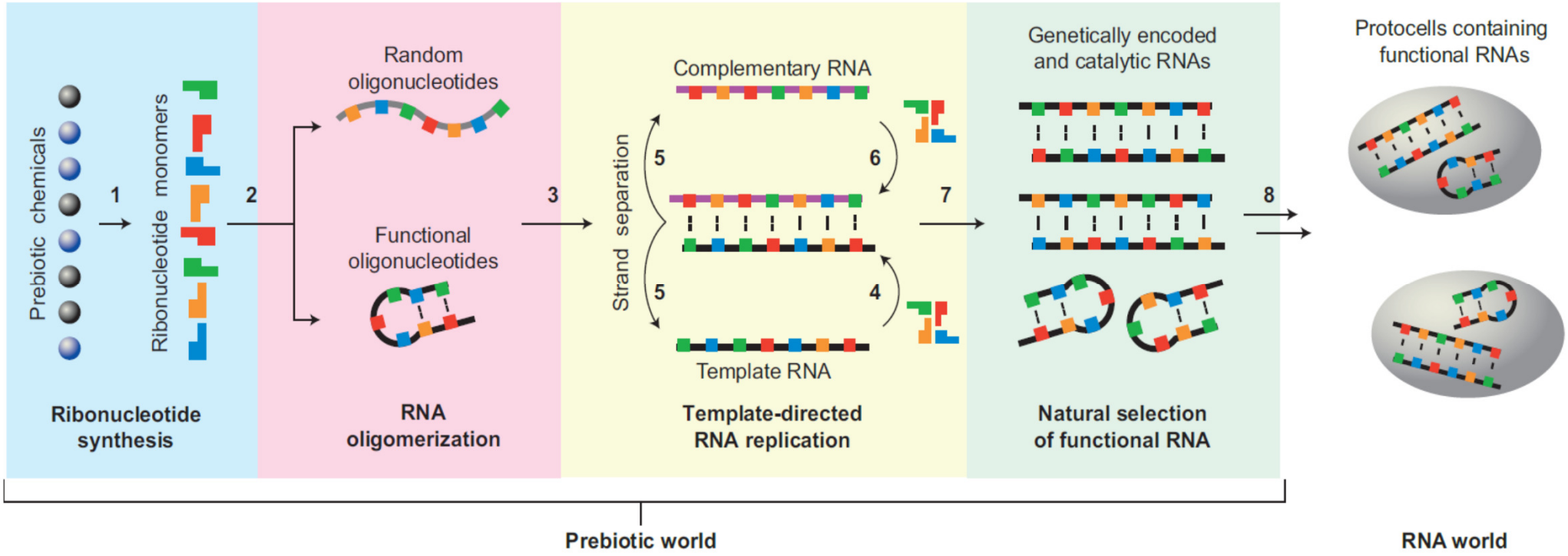
viroids



Riboswitches

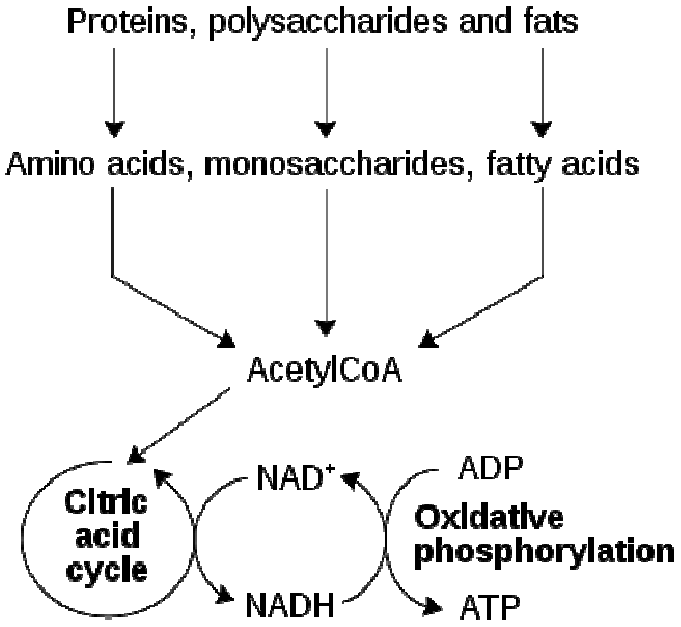
„Genes first" - the RNA world

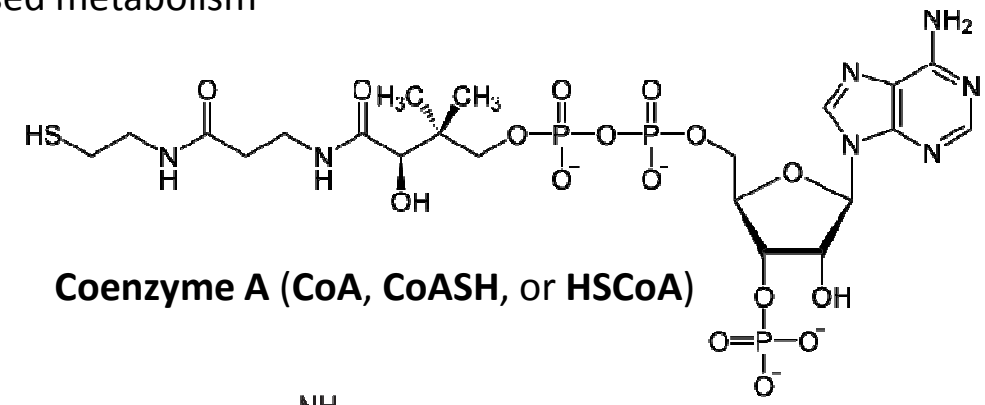




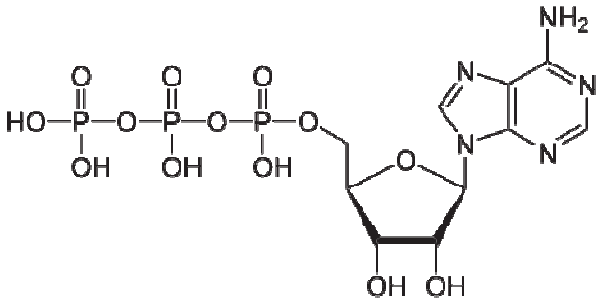
The RNA world

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

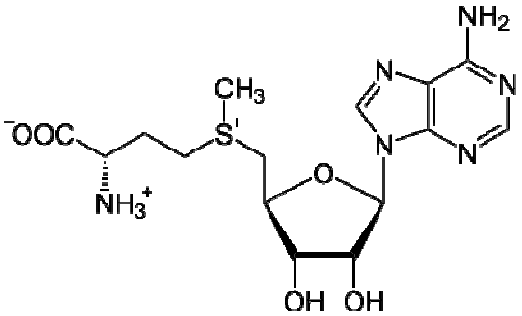




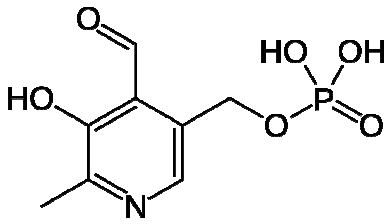
Coenzyme A (**CoA**, **CoASH**, or **HSCoA**)



Adenosine triphosphate (**ATP**)



***S*-Adenosyl methionine**



Pyridoxal phosphate (PLP) – Vit. B_6

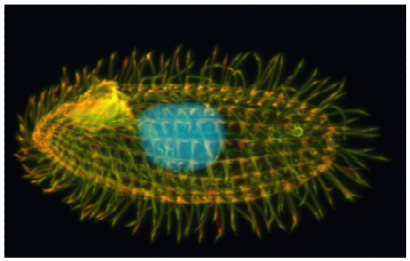
FAD, NADPH, GTP

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

The RNA world

Ribozymes – Ribonucleic acid enzymes

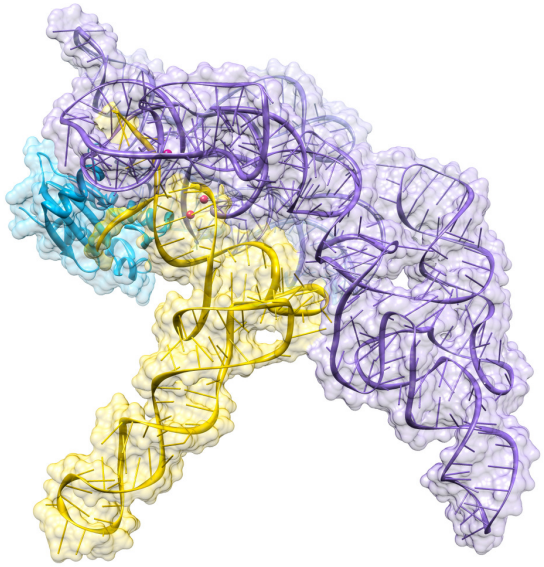
1989 – Thomas Cech and Sidney Altman – Nobel Prize in chemistry for discovery of catalytic RNA
Thomas R. Cech was studying RNA splicing in the ciliated protozoan *Tetrahymena thermophila*
Sidney Altman and Norman Pace were studying the bacterial RNase P complex.



Tetrahymena thermophila

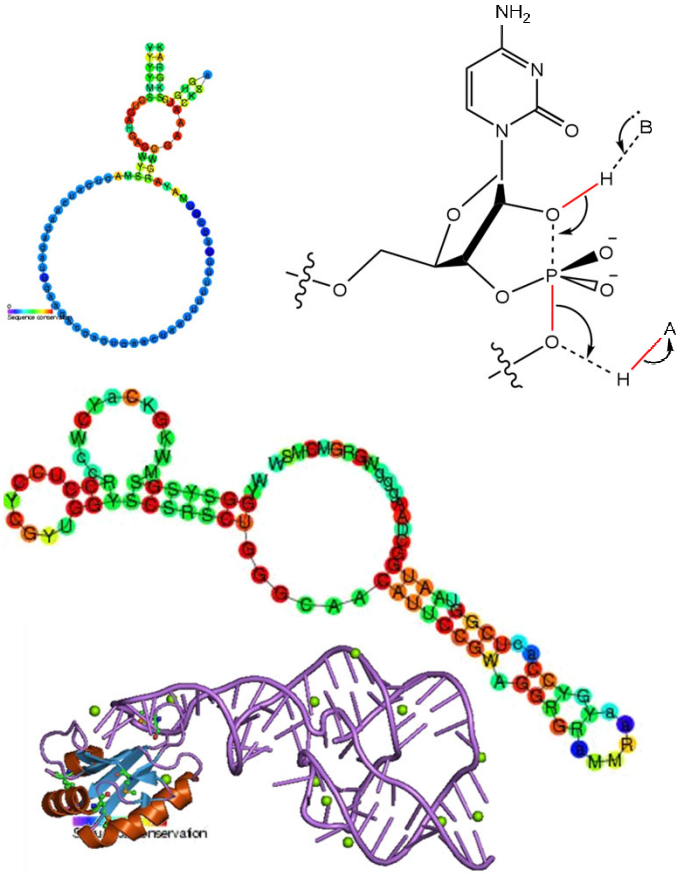
Self-splicing RNA introns

First known examples of a ***ribozyme*** – ribonucleic acid-composed enzyme analogue, whose the ***catalytic*** motif ***does not*** contain protein part, ***only RNA***.



Bacterial RNAse P

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

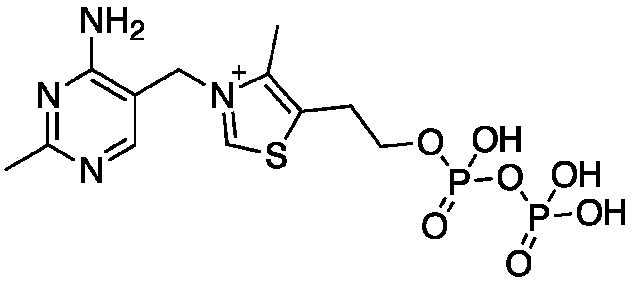
2002 - (Breaker and Nudler) – discovery of a nucleic acid-based genetic regulatory element – ***riboswitch***.

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

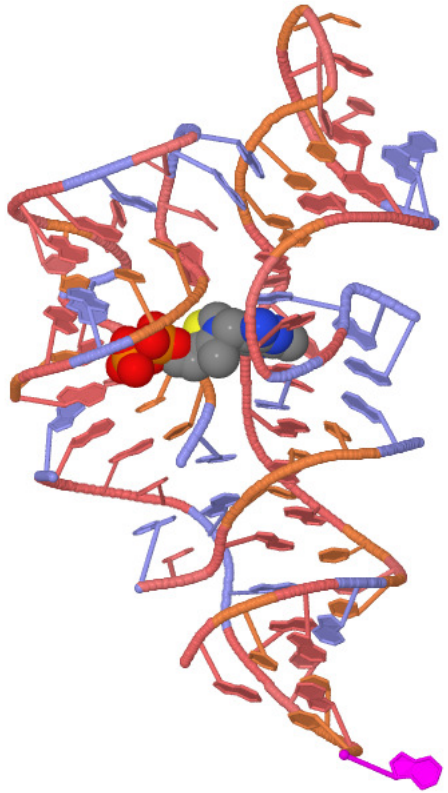
Before discovery of ***riboswitches*** only ***proteins*** were supposed to do so in the biological context.

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



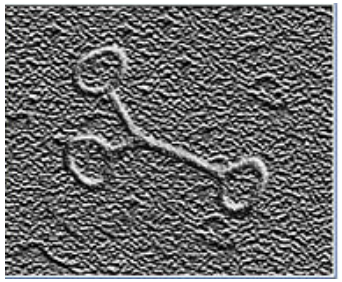
Thiamine pyrophosphate TPP



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)

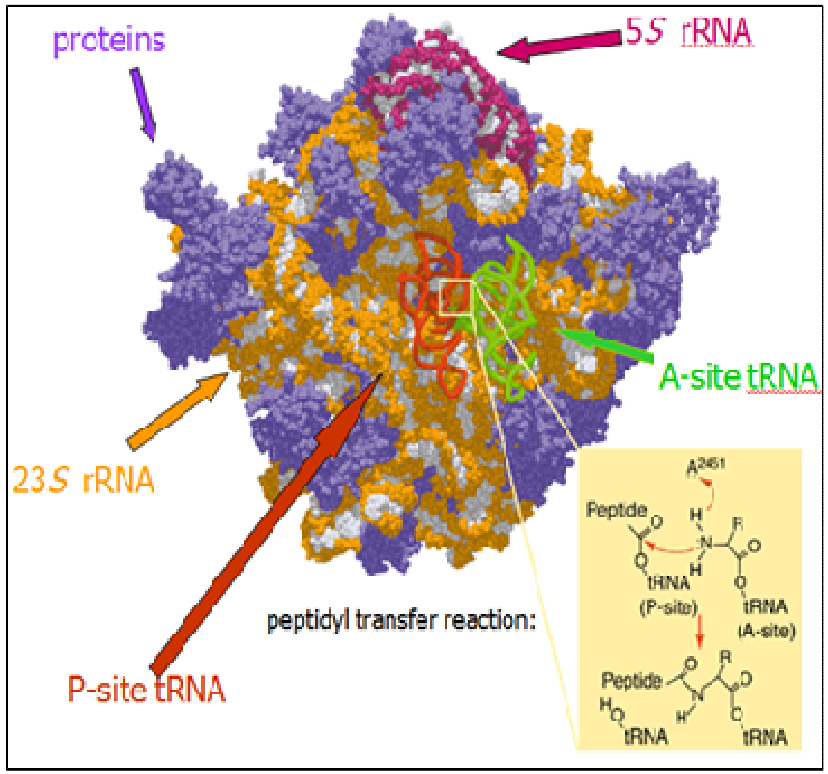
Viroid properties:

- 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 is a ribozyme!



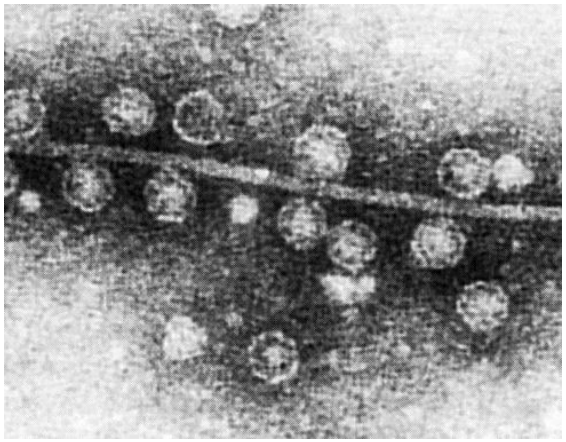
No protein is present within 18 Angstroms from the active site → proteins play a structural role, but DO NOT CATALYZE THE ACYL TRANSFER PROCESS

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

The RNA world

Can RNA evolve?

Spiegelman's monster



Spiegelman's Monster is the name given to an RNA chain of only 218 nucleotides that is able to be reproduced by the RNA replication enzyme RNA-dependent RNA polymerase, also called RNA replicase. It is named after its creator, Sol Spiegelman, of the University of Illinois at Urbana-Champaign who first described it in 1965.

Spiegelman introduced RNA from a simple bacteriophage Qβ into a solution which contained Qβ's RNA replicase, some free nucleotides, and some salts. In this environment, the RNA started to be replicated. After a while, Spiegelman took some RNA and moved it to another tube with fresh solution. This process was repeated.

Shorter RNA chains were able to be replicated faster, so the RNA became shorter and shorter as selection favored speed. After 74 generations, the original strand with 4,500 nucleotide bases ended up as a dwarf genome with only 218 bases. This short RNA sequence replicated very quickly in these unnatural circumstances.

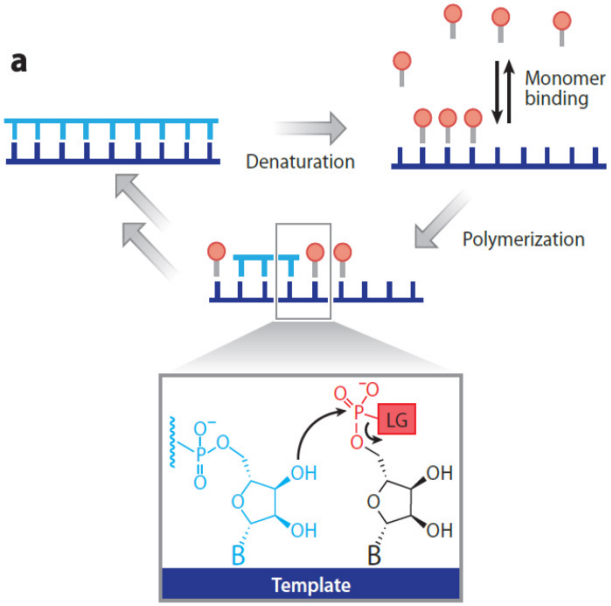
Kacian D. L., Mills D. R., Kramer F. R., Spiegelman S. *PNAS* **1972**, *69*, 3038-3042.

The RNA world

RNA self-replication

Nonenzymatic template-directed RNA polymerization

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

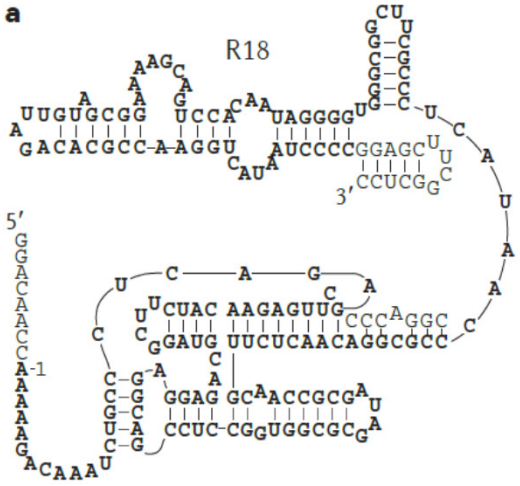


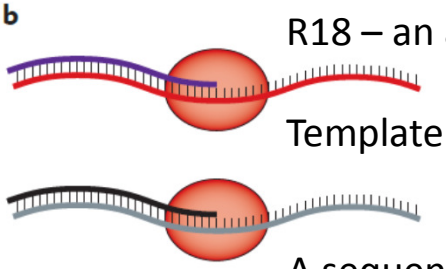
General RNA polymerase ribozyme (‚replicase')

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

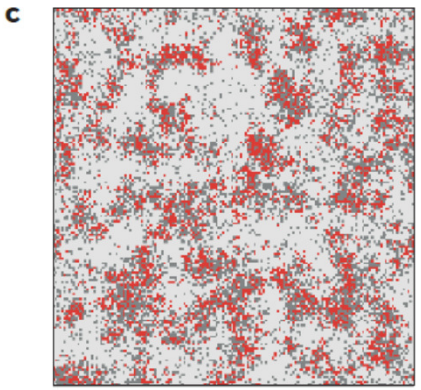


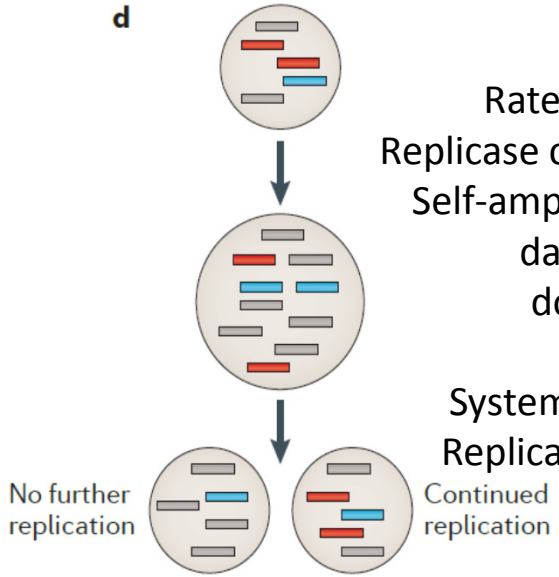


R18 – an artificial polymerase evolved from the class I ligase ribozyme.

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.

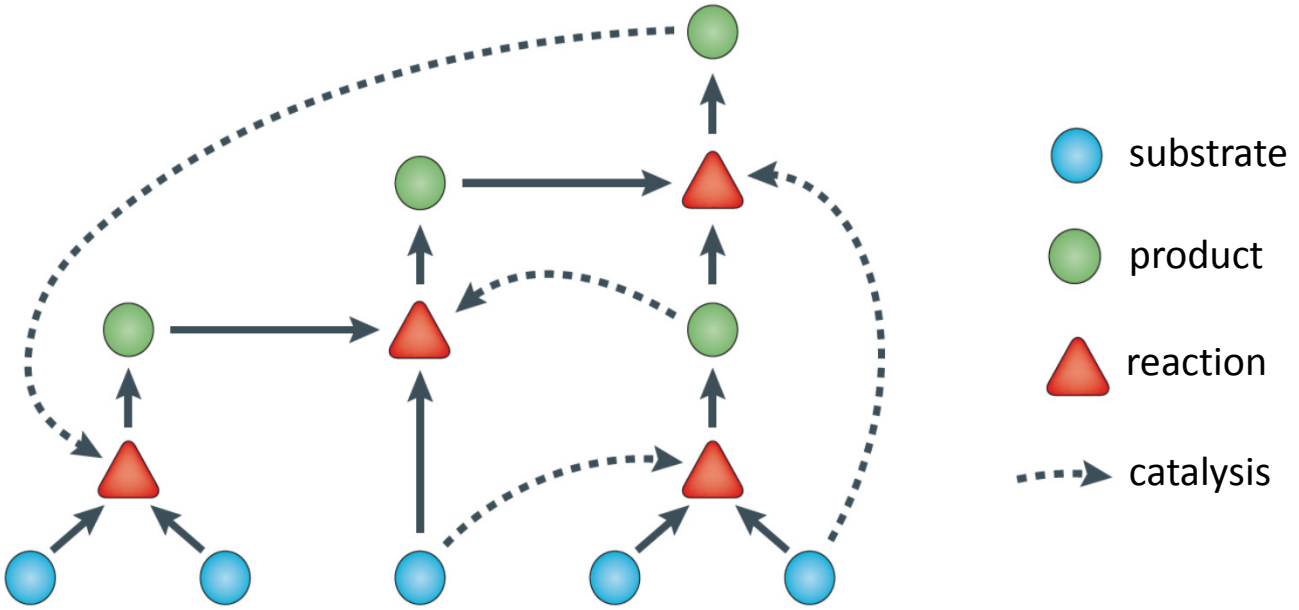
Attwater, J., Wochner, A. & Holliger, P. *Nature Chem.* **2013**, *5*, 1011–1018.

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?

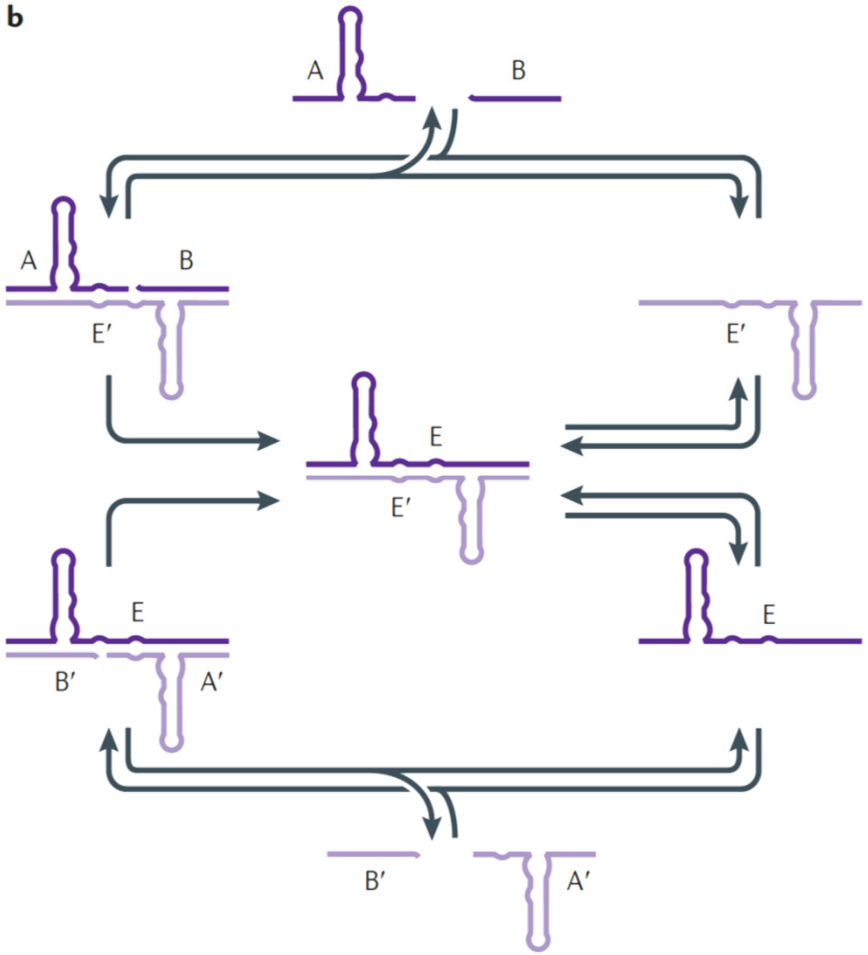
Possible solution – autocatalytic networks



No component can replicate without all the others

The RNA world

Mutually autocatalytic RNA networks

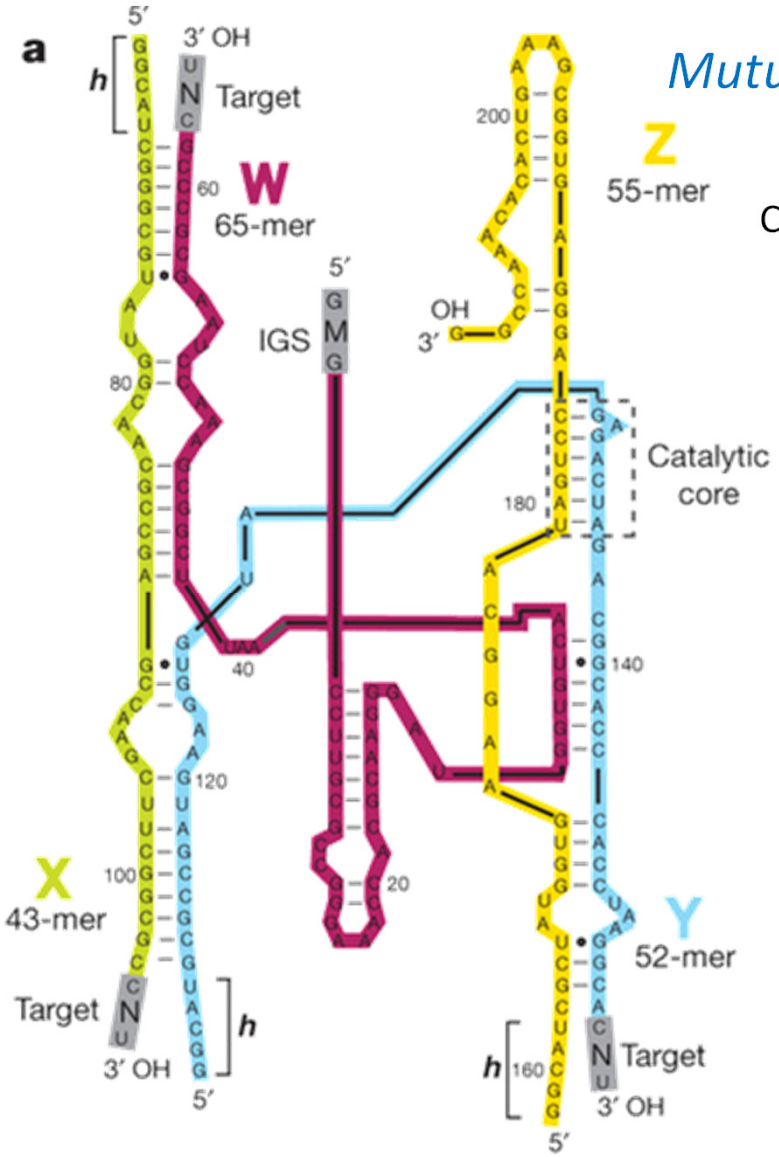


An autocatalytic set composed of two cross-catalytic ligases was demonstrated. RNA A and RNA B are ligated together by ribozyme E‘ to create ribozyme E, which can reciprocate and ligate RNA A‘ and RNA B’ to create ribozyme E‘.

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

The RNA world

Mutually autocatalytic RNA networks



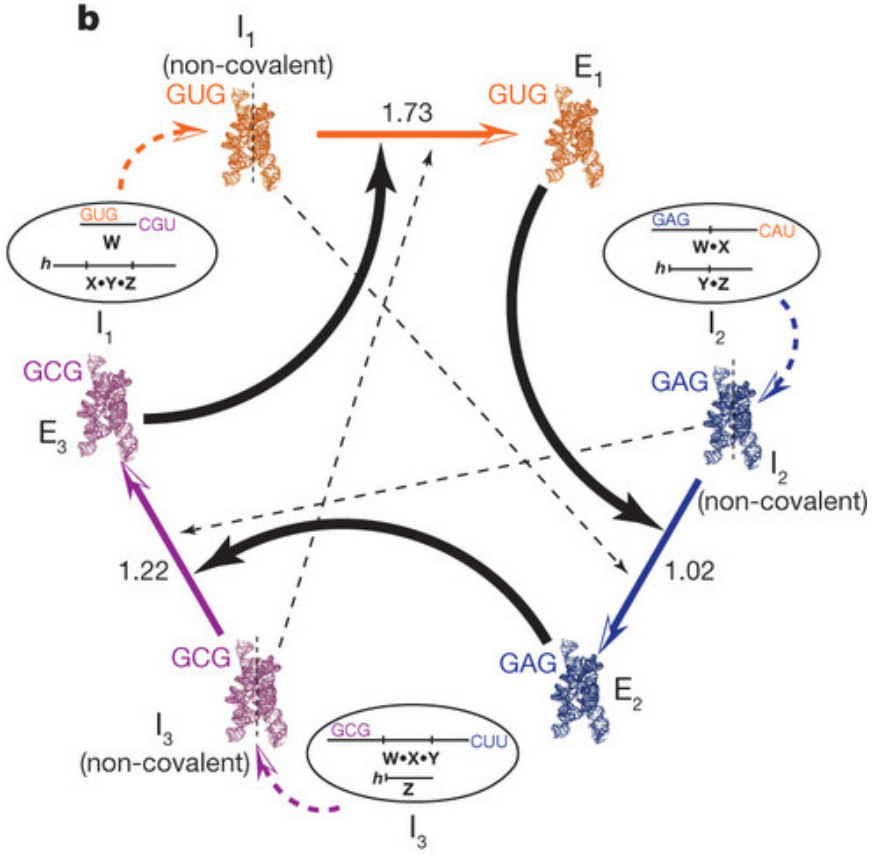
Cooperation between multiple strands that assemble to perform a single function.

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).

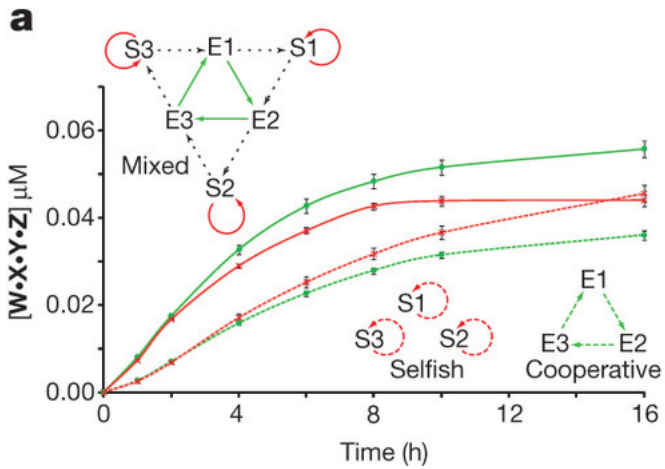
Vadia, N. *et al. Nature* **2012**, *491*, 72-77.

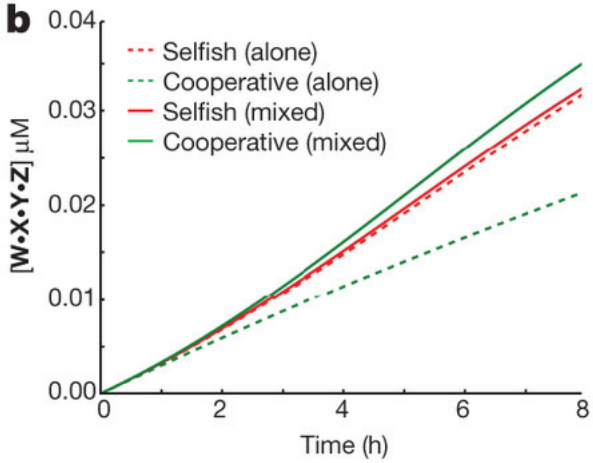
The RNA world

Mutually autocatalytic RNA networks



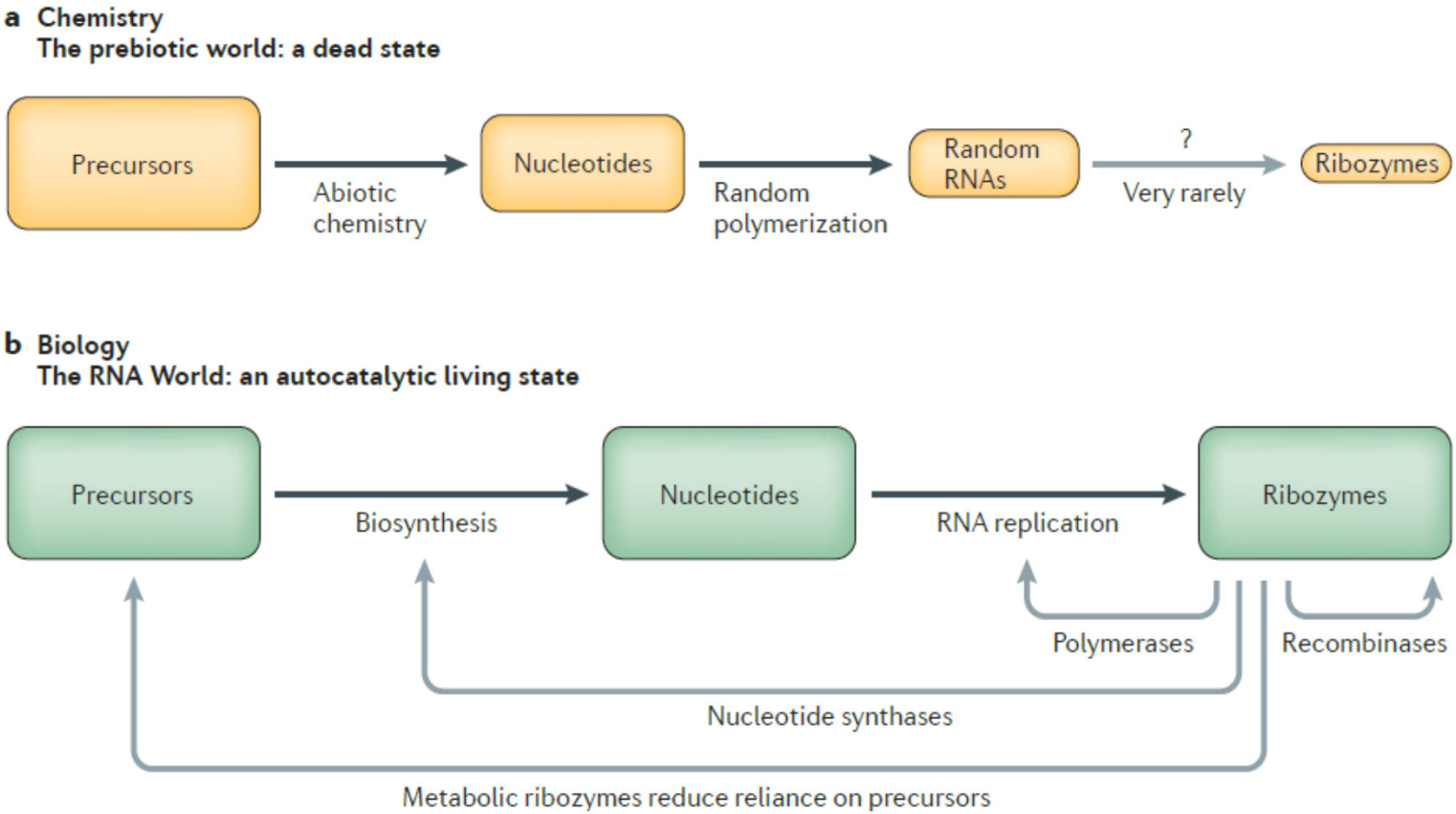
mixtures of RNA fragments that self-assemble into self-replicating ribozymes spontaneously form cooperative catalytic cycles and networks.





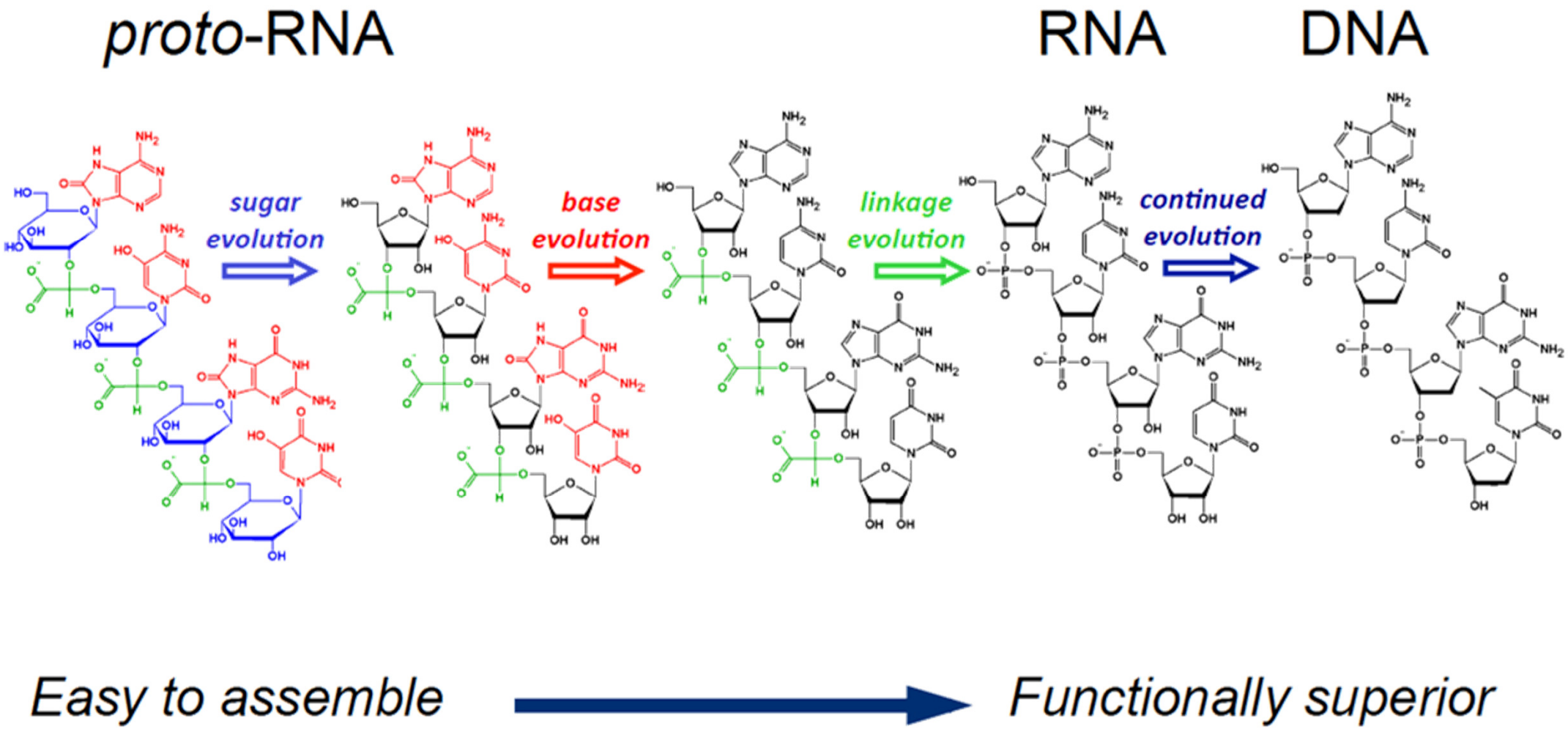
Vadia, N. *et al. Nature* **2012**, *491*, 72-77.

The RNA world



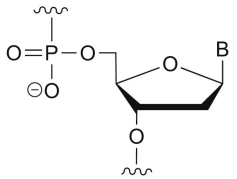
Transition from chemistry to biology involves autocatalytic feedbacks from ribozymes to all stages of the prebiotic chemistry

„RNA-second”

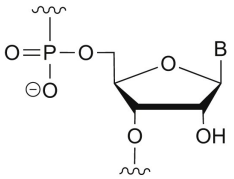


Proto-RNA evolution: According to the protoRNA theory, each of the components of RNA — sugar, base and phosphate backbone — may have originally taken different forms.

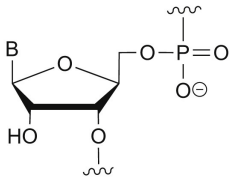
Artificial genetic polymers



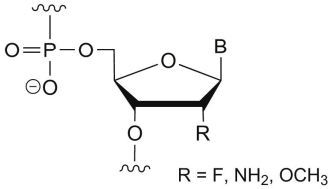
DNA



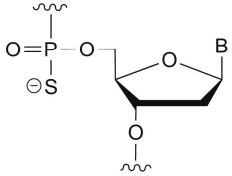
D-RNA
(natural)



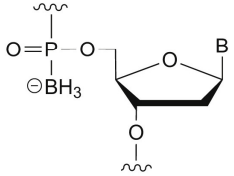
L-RNA
(unnatural)



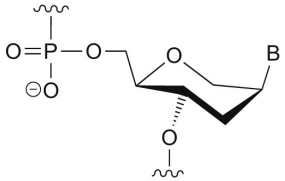
2'-modified RNA



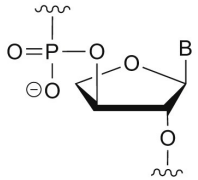
Phosphorothioate



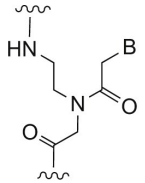
Boranophosphate



Hexitol Nucleic Acid
(HNA)

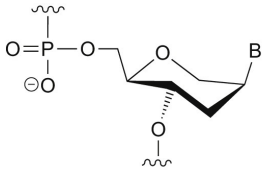


Threose Nucleic Acid
(TNA)

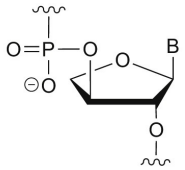


Peptide Nucleic Acid
(PNA)

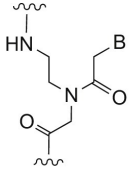
XNA – Xeno Nucleic Acids



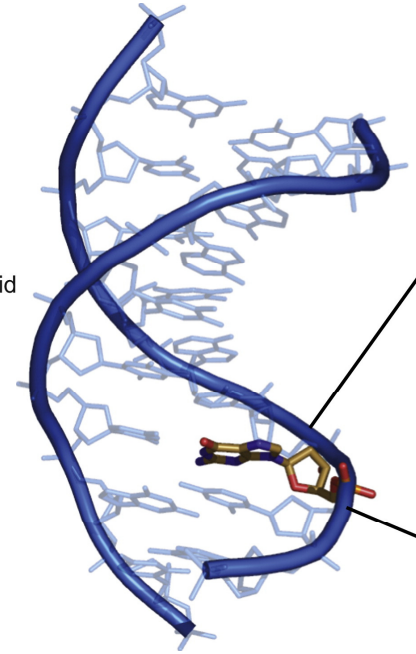
Hexitol Nucleic Acid
(HNA)

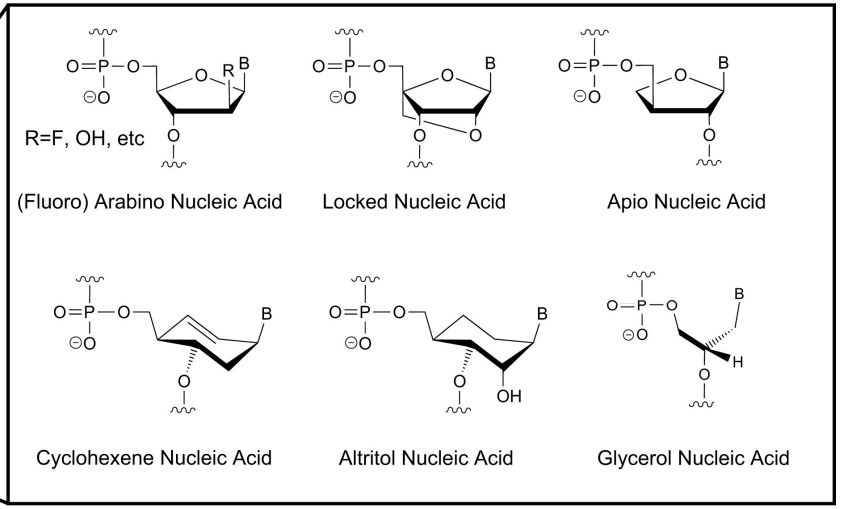


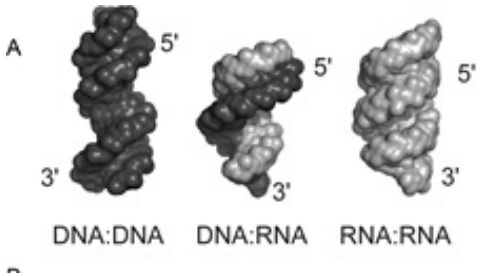
Threose Nucleic Acid
(TNA)

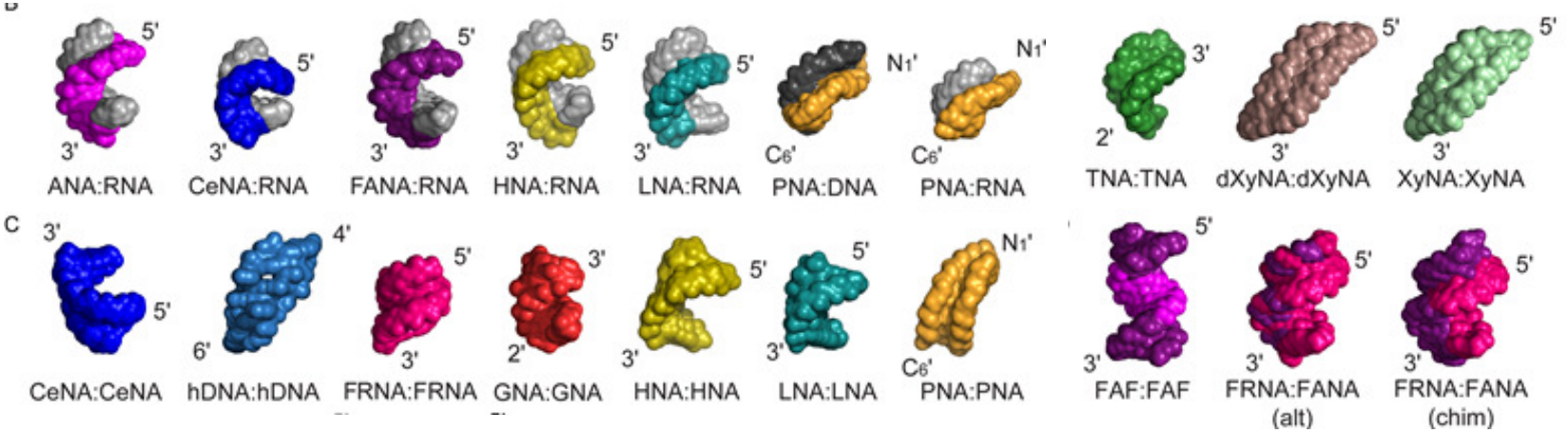


Peptide Nucleic Acid
(PNA)

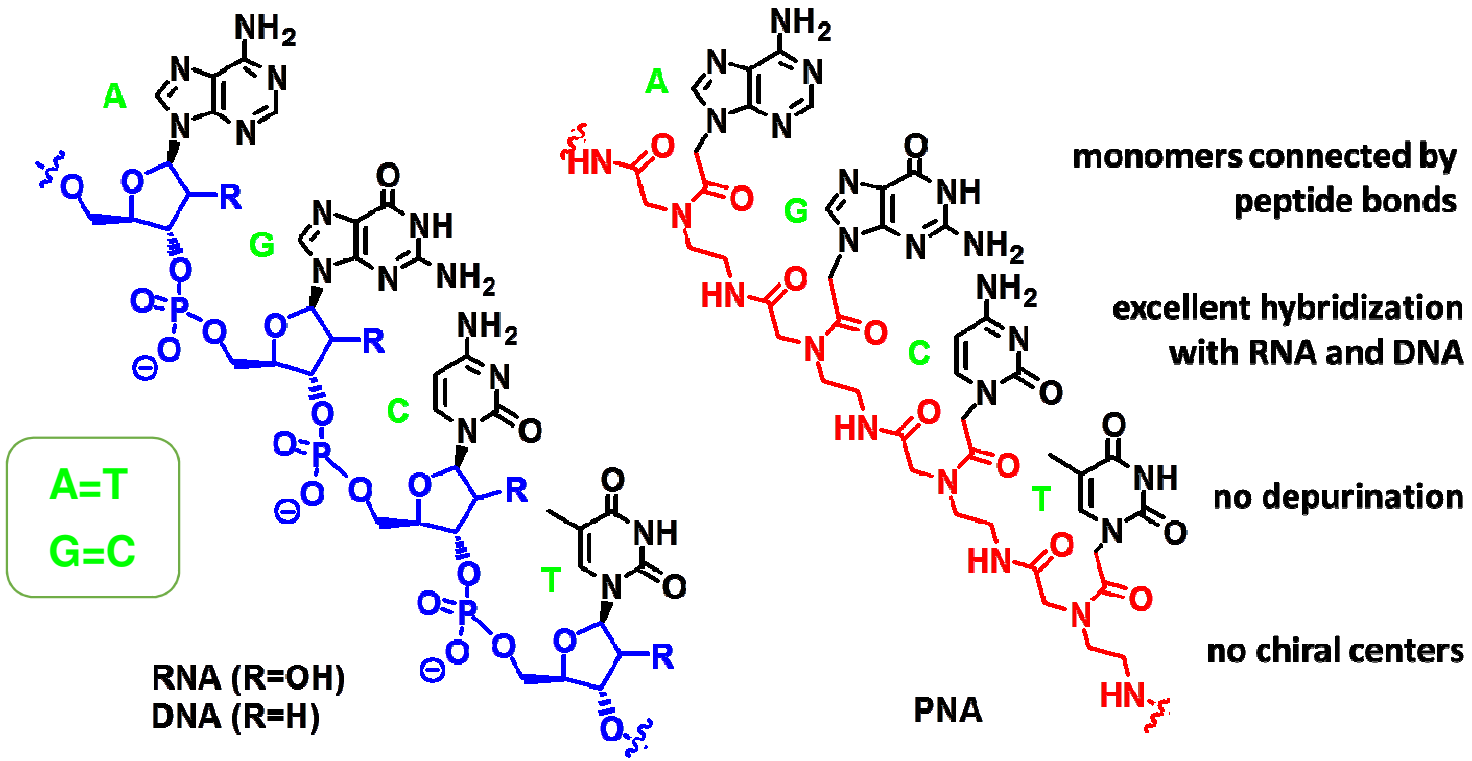


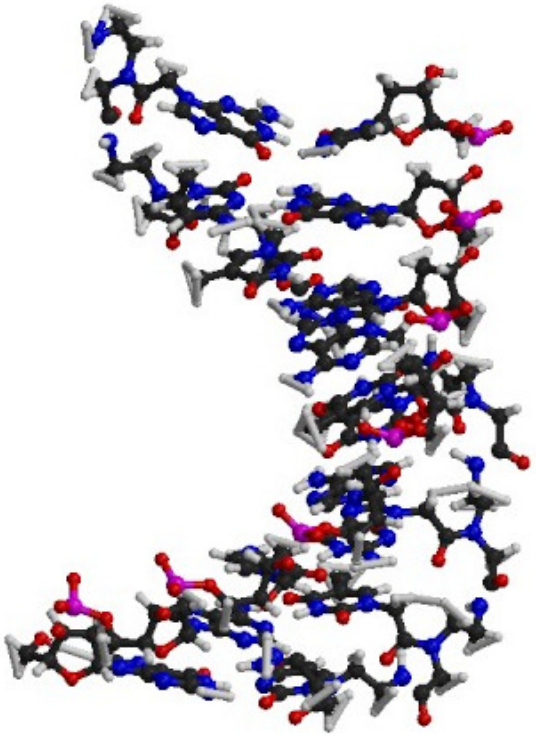






Peptidonucleic acids – functional DNA analogues



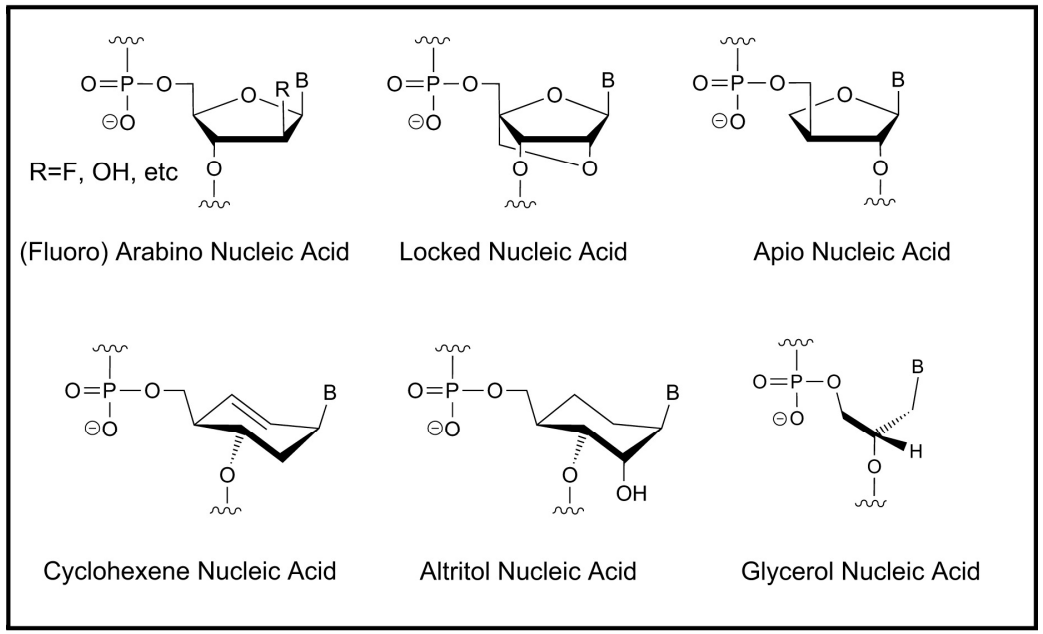


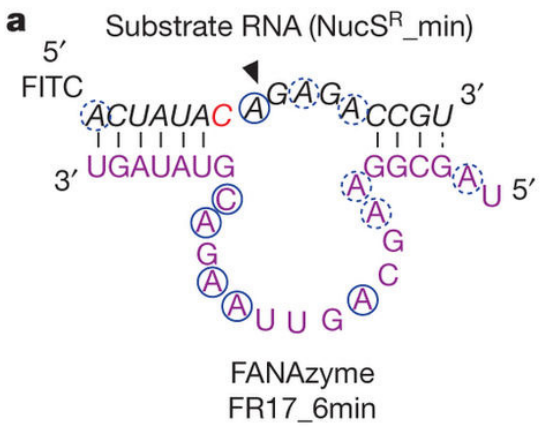
PNA-DNA duplex, NMR structure
PDB entry: 1PDT

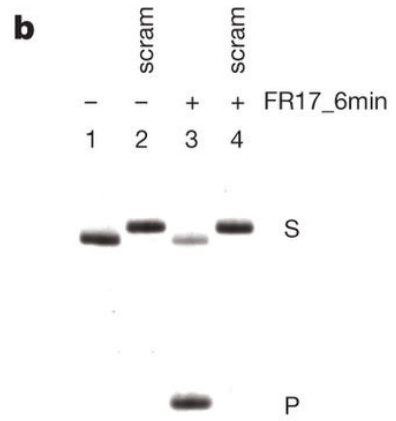
PNA – stable *ex vivo*, the backbone detected in cyanobacteria

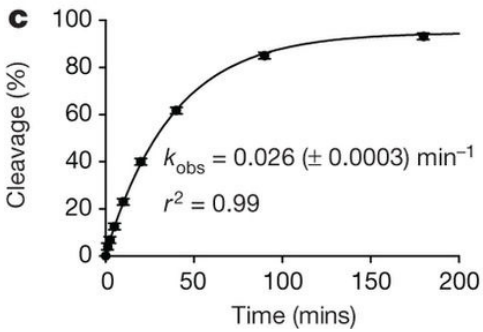
Applications: antigene, antisense agents; fluorescent DNA probes (FISH), anticancer, antiviral, antibacterial, antiparasitic agents; diagnostics, mol. biology

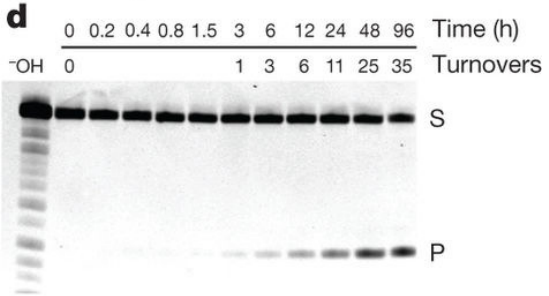
Chemical synthesis yields an active RNA endonuclease XNAzyme







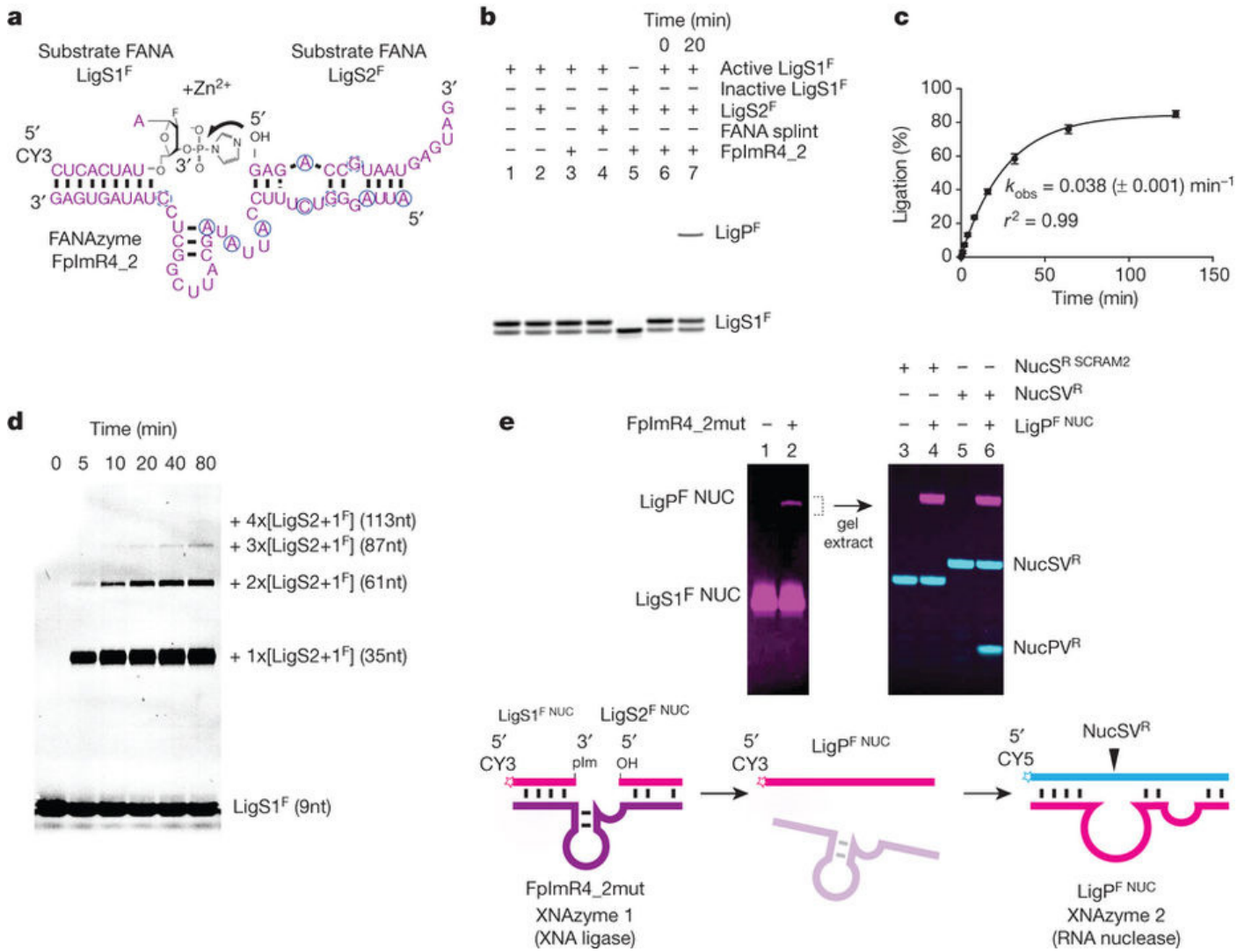




a, Secondary structure of truncated FANAzyme FR17_6 (FR17_6min, purple)
b, FR17_6min synthesized using FANA phosphoramidites cleaves cognate RNA substrate (NucSR_min; lanes 1 and 3), but not a scrambled RNA (NucSR SCRAM2; lanes 2 and 4), with...
c, essentially unchanged catalytic rate (k_{obs}) at 25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

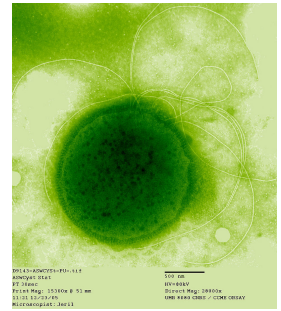
XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



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

Engineering XNA polymerases

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

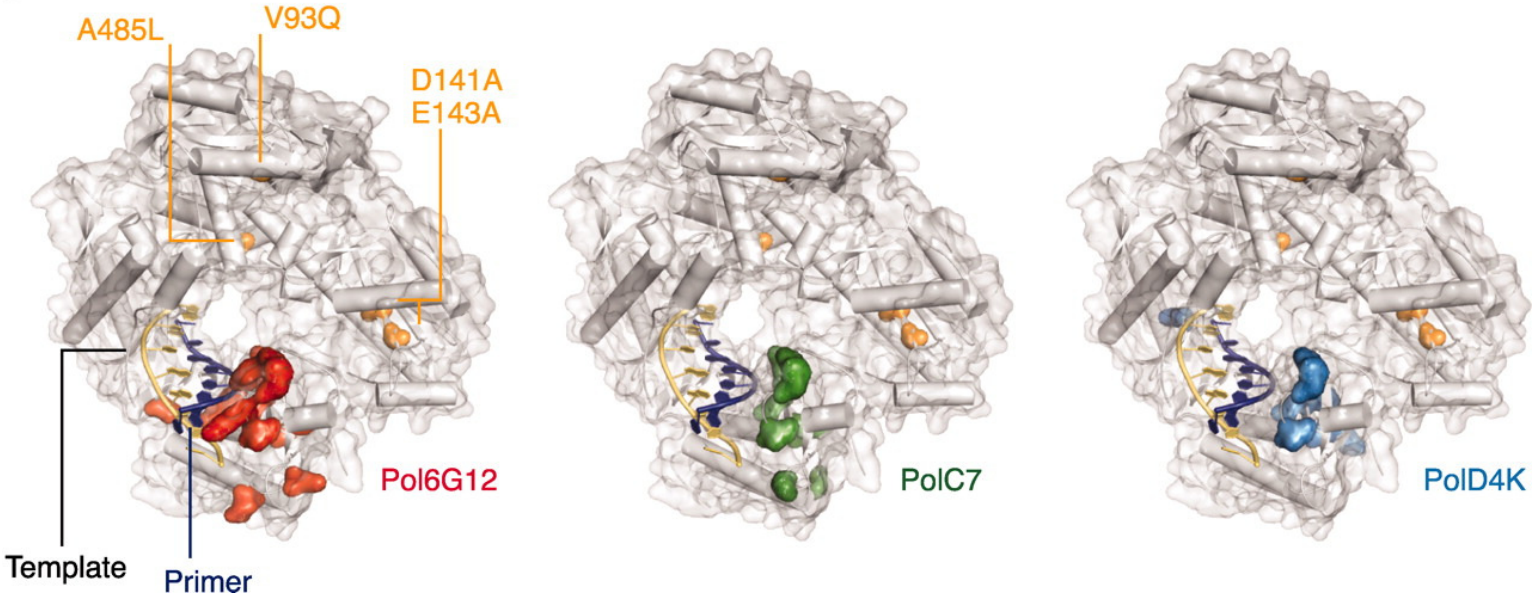


Thermococcus gorgonarius
(Angels Tapias)

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	

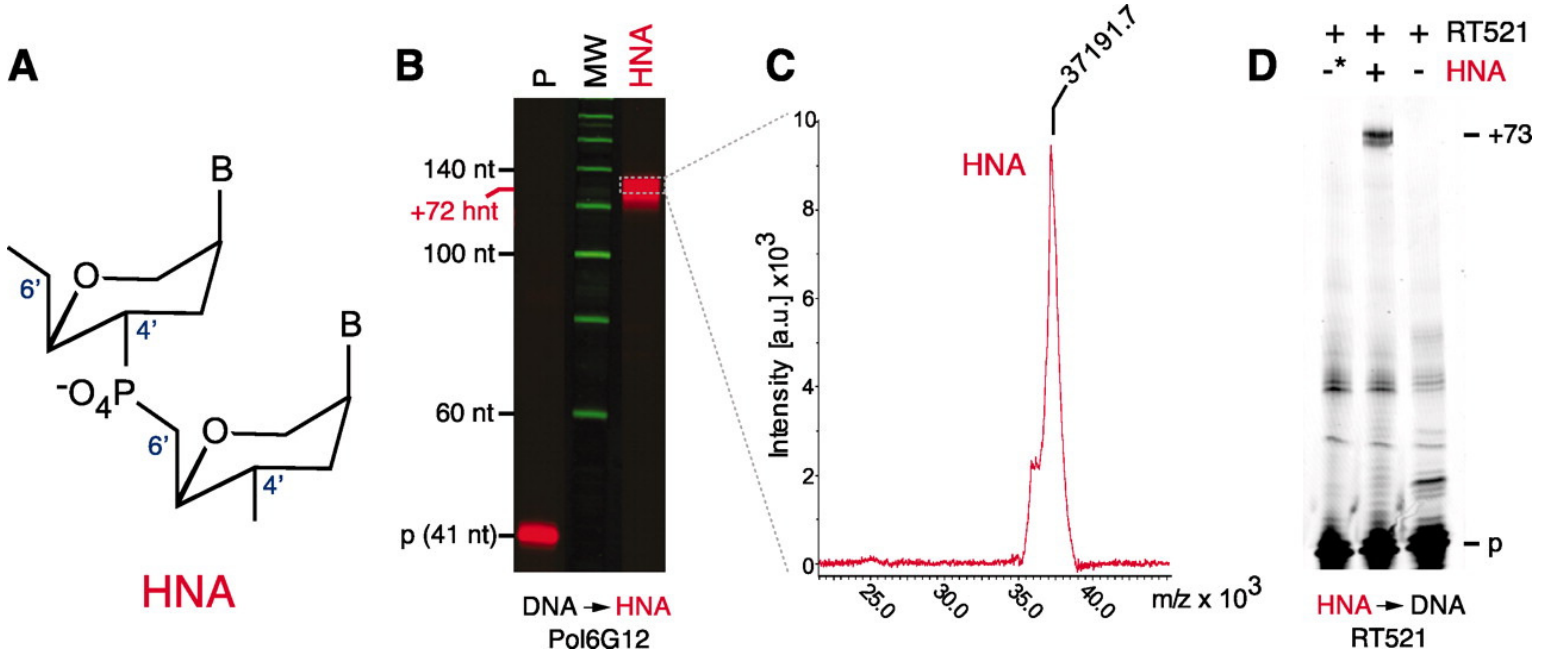
B



(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

HNA synthesis



(A) Structure of 1,5-anhydrohexitol (HNA) nucleic acids (B, nucleobase).
(B) Pol6G12 extends the primer (p) incorporating 72 hNTPs against template T1 to generate a full-length hybrid molecule with a 37,215-dalton expected molecular mass. (MW - ILS 600 molecular weight marker. P - primer-only reactions)
(C) MALDI-TOF spectrum of a full-length HNA molecule showing a measured HNAmass of 37,190 ± 15 daltons (n = 3 measurements). a.u., arbitrary units; m/z, mass-to-charge ratio.
(D) HNA reverse transcription (DNA synthesis from an HNA template). Polymerase-synthesized HNA (from template YtHNA4) is used as template by RT521 for HNA-RT (-* denotes a no HNA synthesis control to rule out template contamination).

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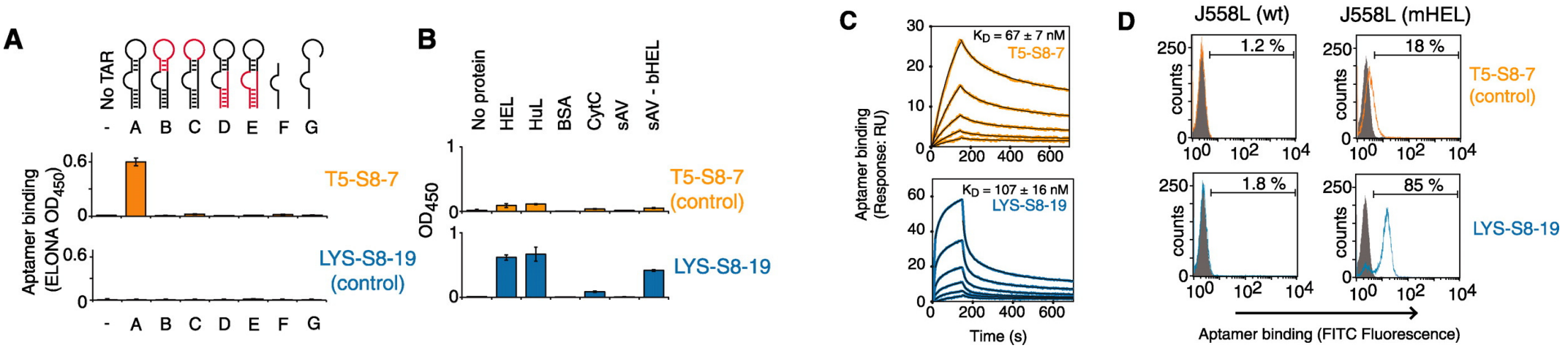
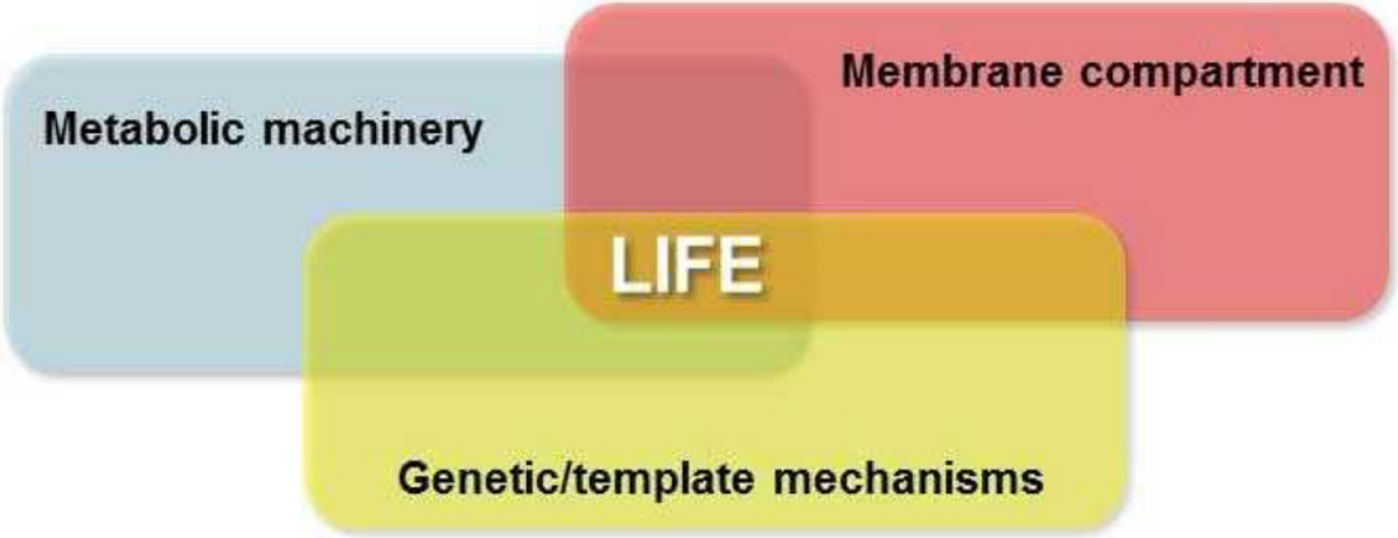
HNA aptamers



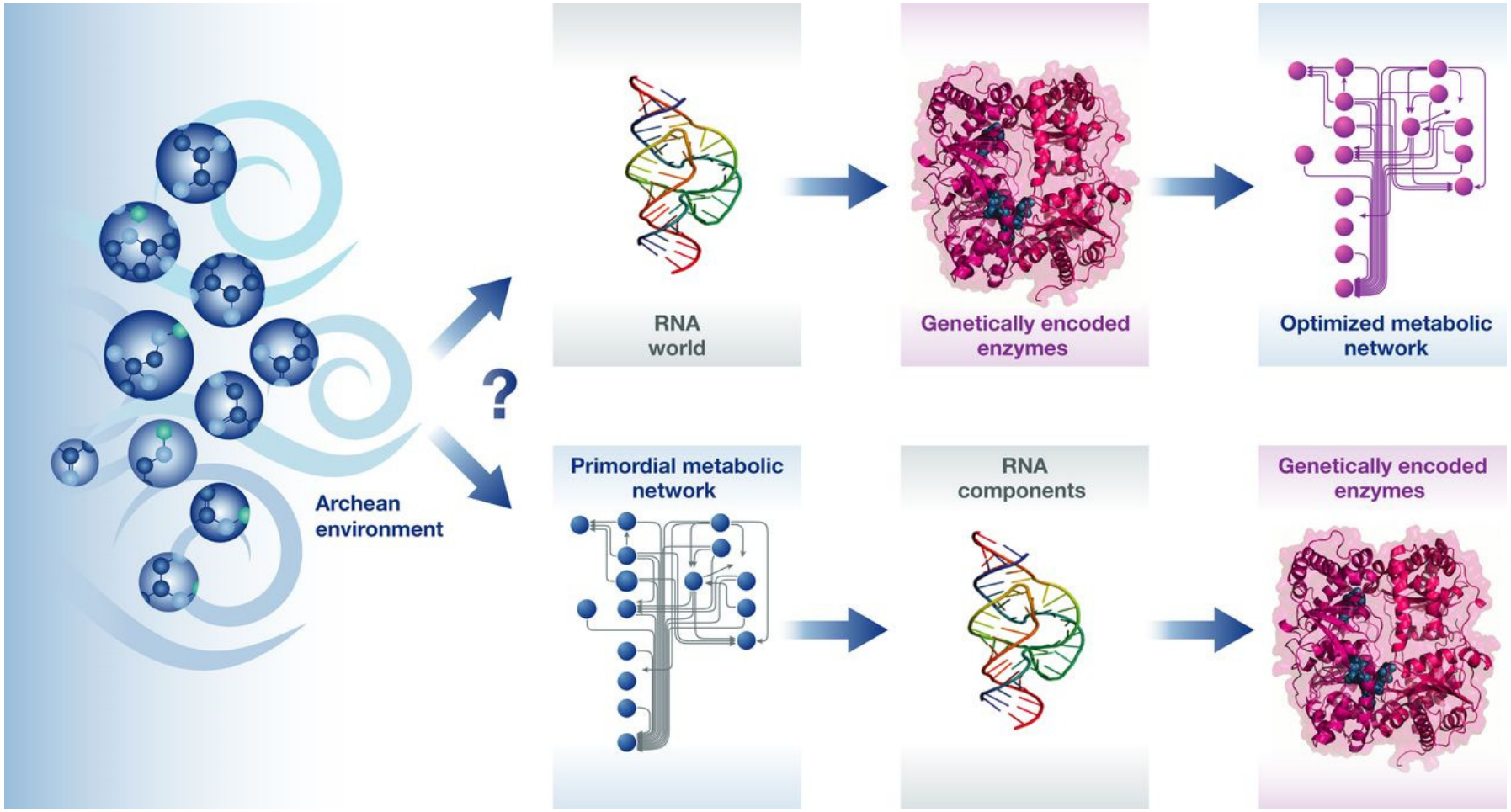
Characterization of HNA aptamers. Anti-TAR aptamer T5-S8-7 and anti-HEL aptamer LYS-S8-19.

(A and B) Aptamer binding specificity against TAR variants (red, sequence randomized but with base-pairing patterns maintained) and different protein antigens (human lysozyme, HuL; cytochrome C, CytC; streptavidin, sAV; biotinylated-HEL bound to streptavidin, sAV-bHEL). OD, optical density.
(C) Affinity measurements of aptamer binding by SPR. RU, response units.
(D) FACS analysis of fluorescein isothiocyanate (FITC)–labeled aptamers binding to plasmacytoma line J558L with and without expression of membrane-bound HEL (mHEL). wt, wild type.

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

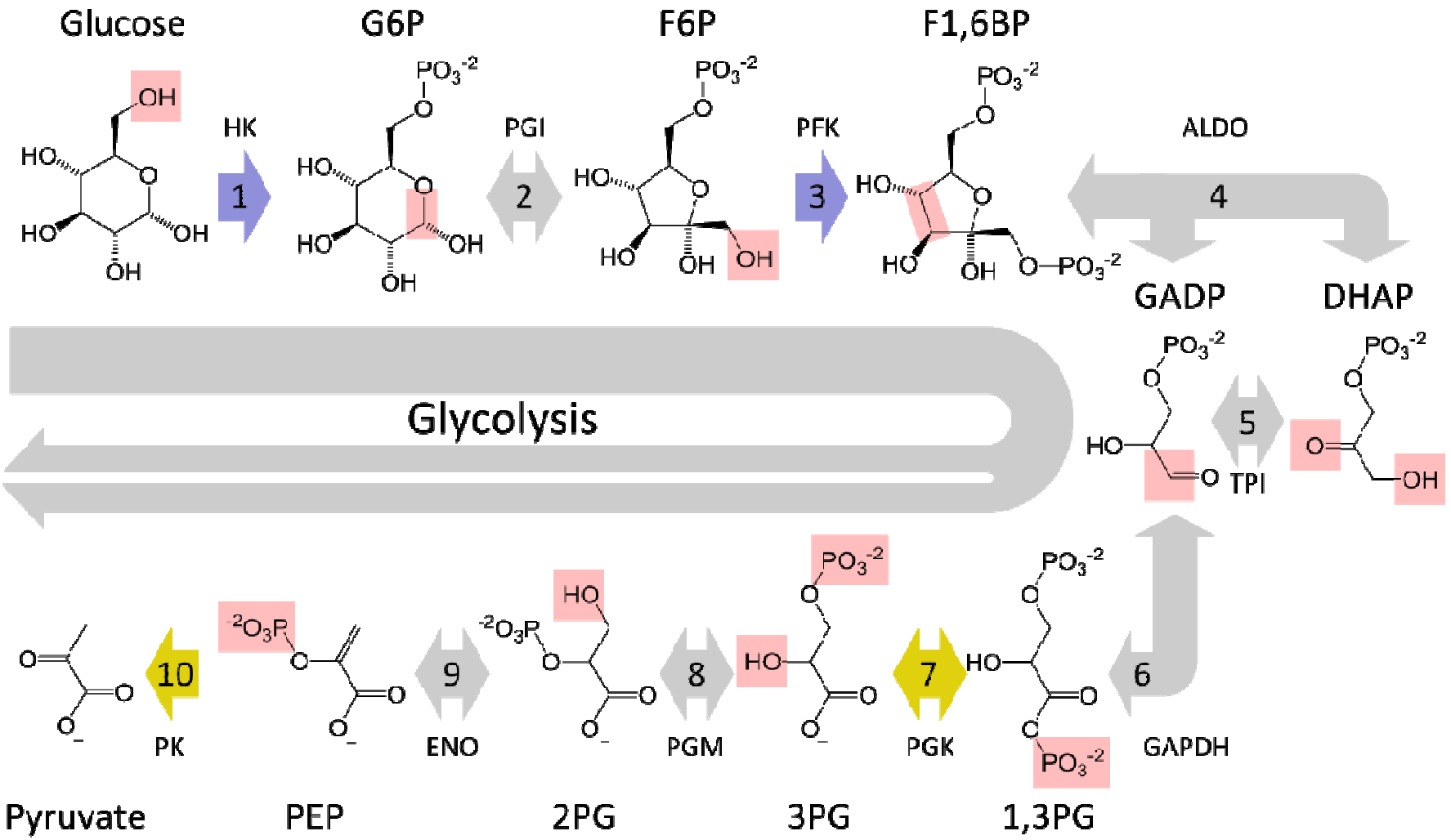
Metabolism-first vs. Genes-first

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.

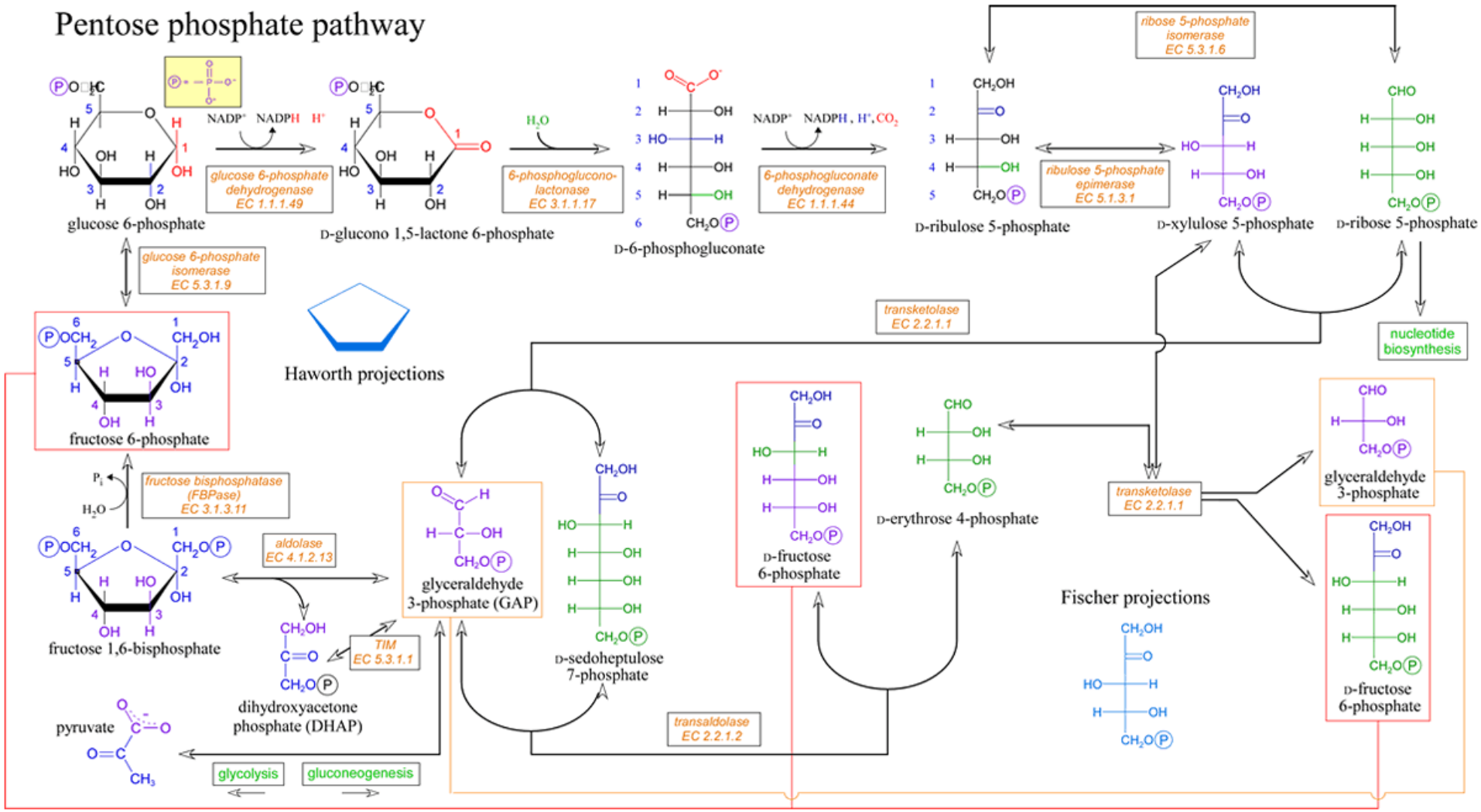
Metabolism-first: primitive metabolic cycles spontaneously assembled from simple prebiotic organic molecules or inorganic carbon sources as CO_2. And the cycles produced a set or more or less complex molecules needed for the replication process and construction of the genetic apparatus.

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.

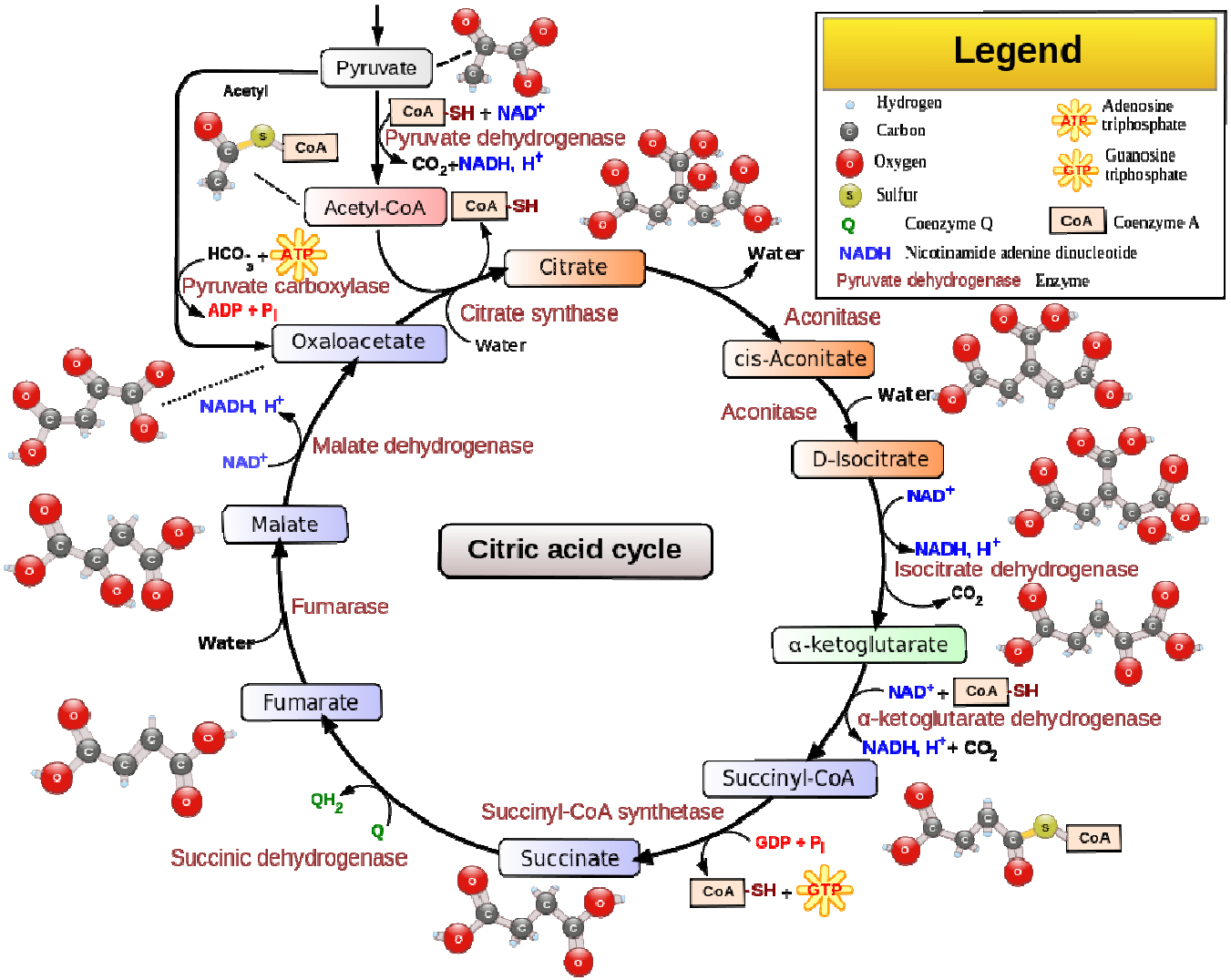
Glycolysis – energy from sugars



Pentose phosphate pathway



Citric acid cycle (Krebs cycle)



Metabolism-first - theories

Mathematical models:

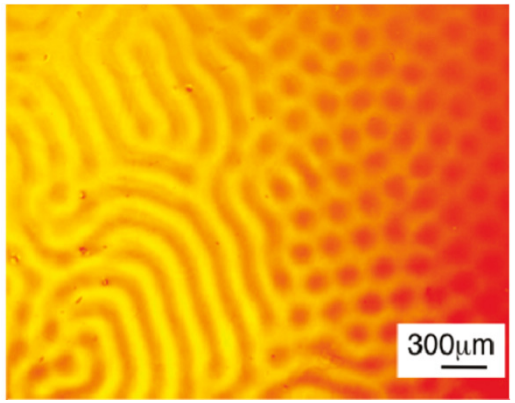
Dyson – modell based on catalytic oligomers (e.g. oligopeptides) and their monomers interacting inside isolated compartments (like protocells) permeable to monomers – solutions give two steady states („ordered/alive" + „disordered/dead") and the transition („creation"/"death") between them.
Per cell, 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

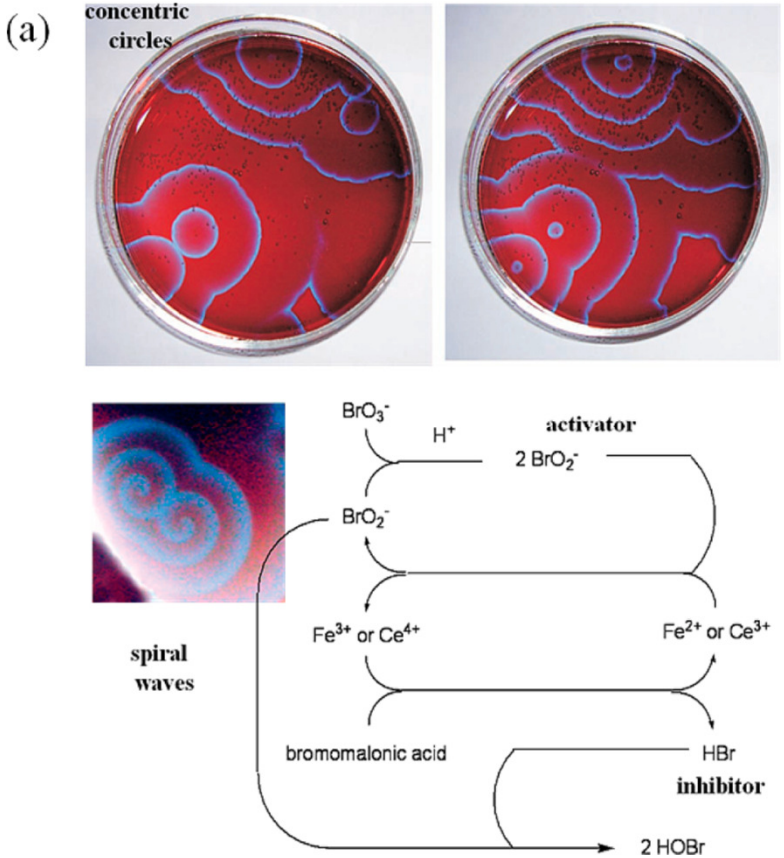
temperature, pH, humidity, illumination, UV irradiation, astronomic cycles

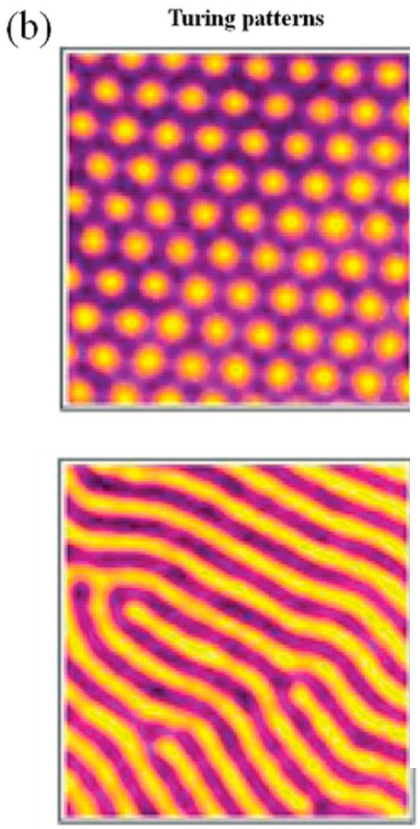
Chemical systems that mimic biological oscilations are studied as simple models

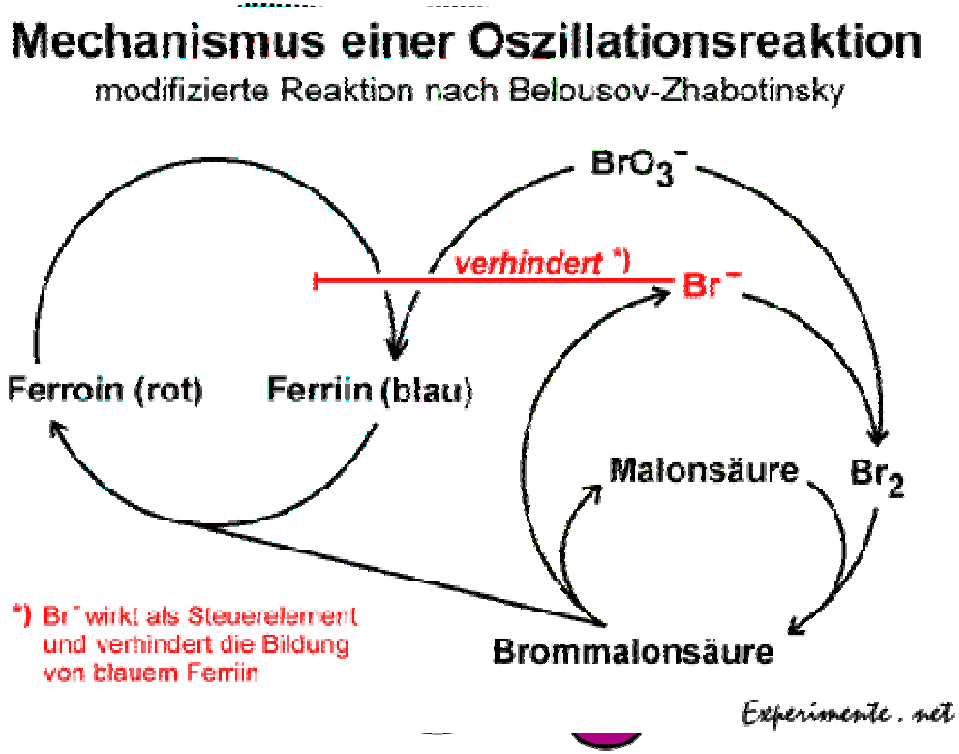
Belousov-Zhabotynski, CIMA reaction

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



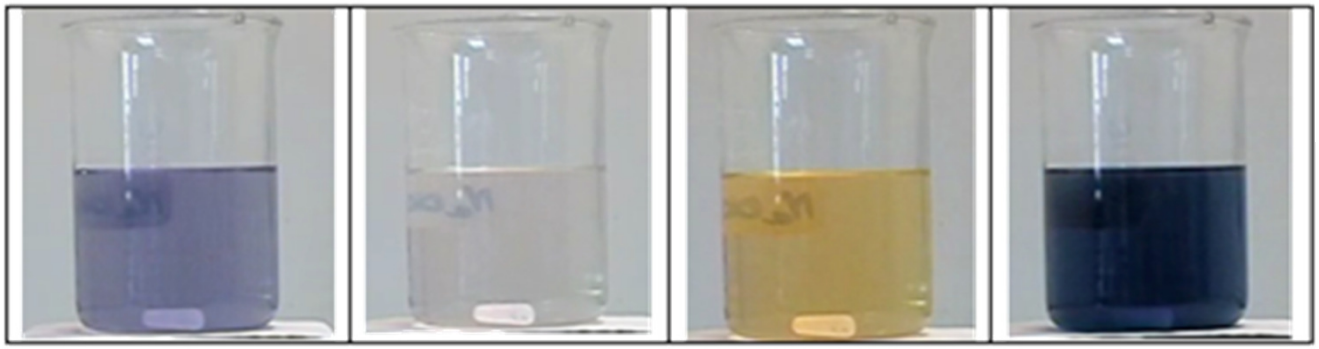




The reaction usually involves potassium bromate(VII) and malonic acid, optionally with cerium(IV) sulfate and citric acid. Ferroin is one of the common redox indicator

Briggs-Rauscher reaction

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

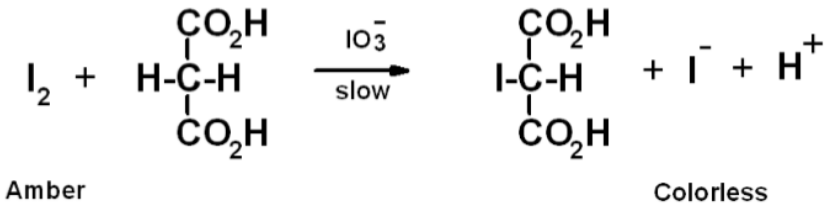


1. **The iodate ion is changed into iodine by hydrogen peroxide. The color changes to amber:**

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

Colorless — Amber

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



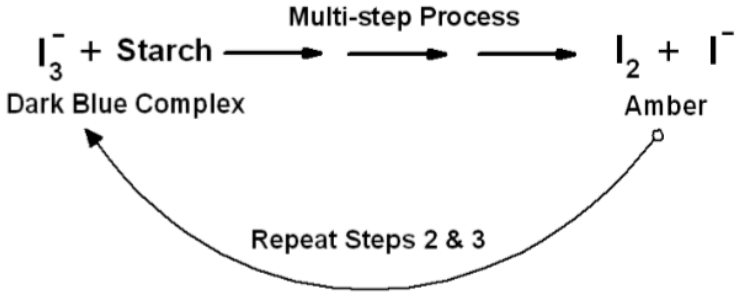
Amber — Colorless

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:**

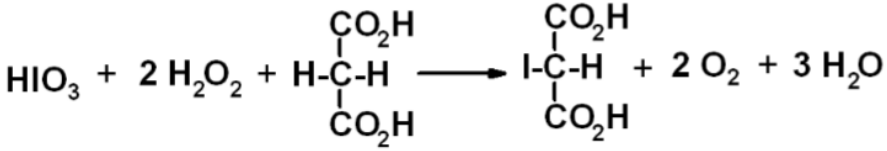
$$I_2 + I^- \xrightarrow{\text{fast}} I_3^- + \text{Starch} \xrightarrow{\text{fast}} \text{Dark Blue Complex}$$

Amber

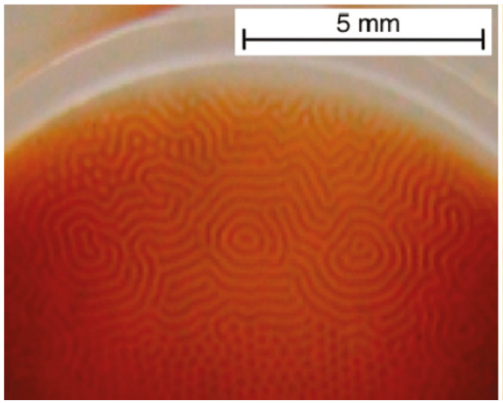
4. **After a period of time, the I_3^- ions are converted back into iodine and iodide ions, so the dark blue color disappears and the process repeats itself:**

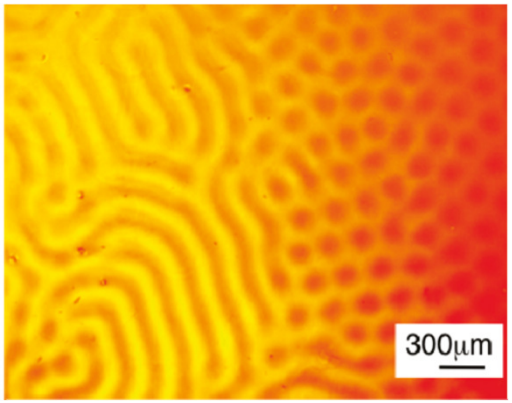


5. **Eventually the faster step 3 becomes dominant and the change of I_3^- back to iodine/iodide stops after about 15 cycles, so the solution remains dark blue. The overall chemical reaction is:**



Chlorite/iodide/malonic acid (CIMA) reaction





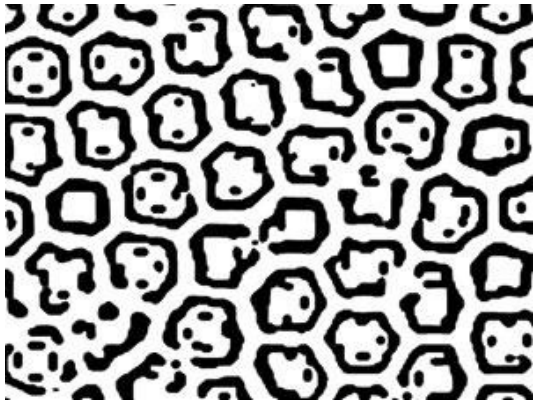
For the spontaneous generation of a Turing pattern, two intermediate species, an activator and an inhibitor, should be generated with the diffusion coefficient of the activator smaller than that of the inhibitor. The CIMA reaction that generates the activator, I^-, and inhibitor, ClO_2^-, was performed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

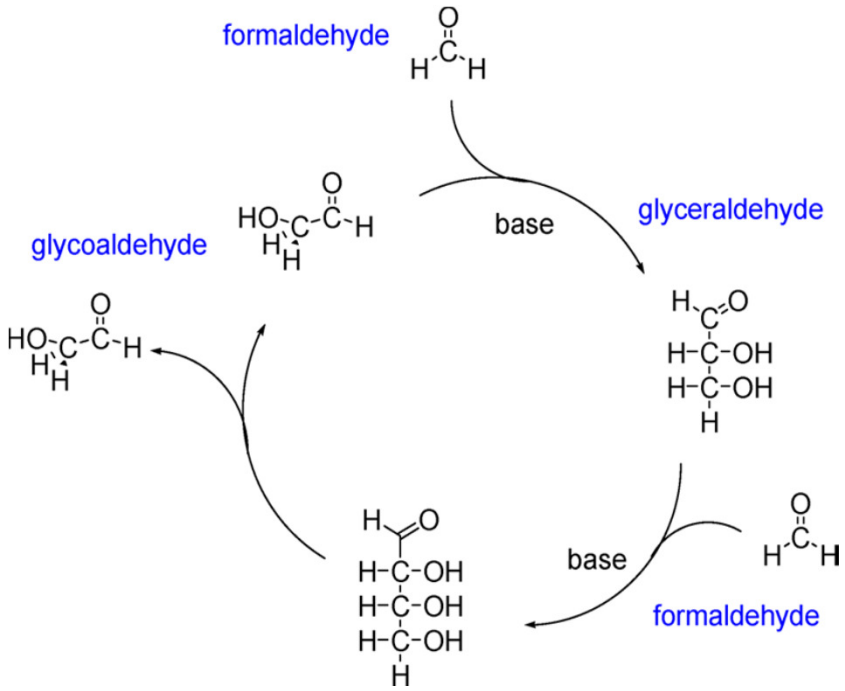
Explain the origin of homochirality

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

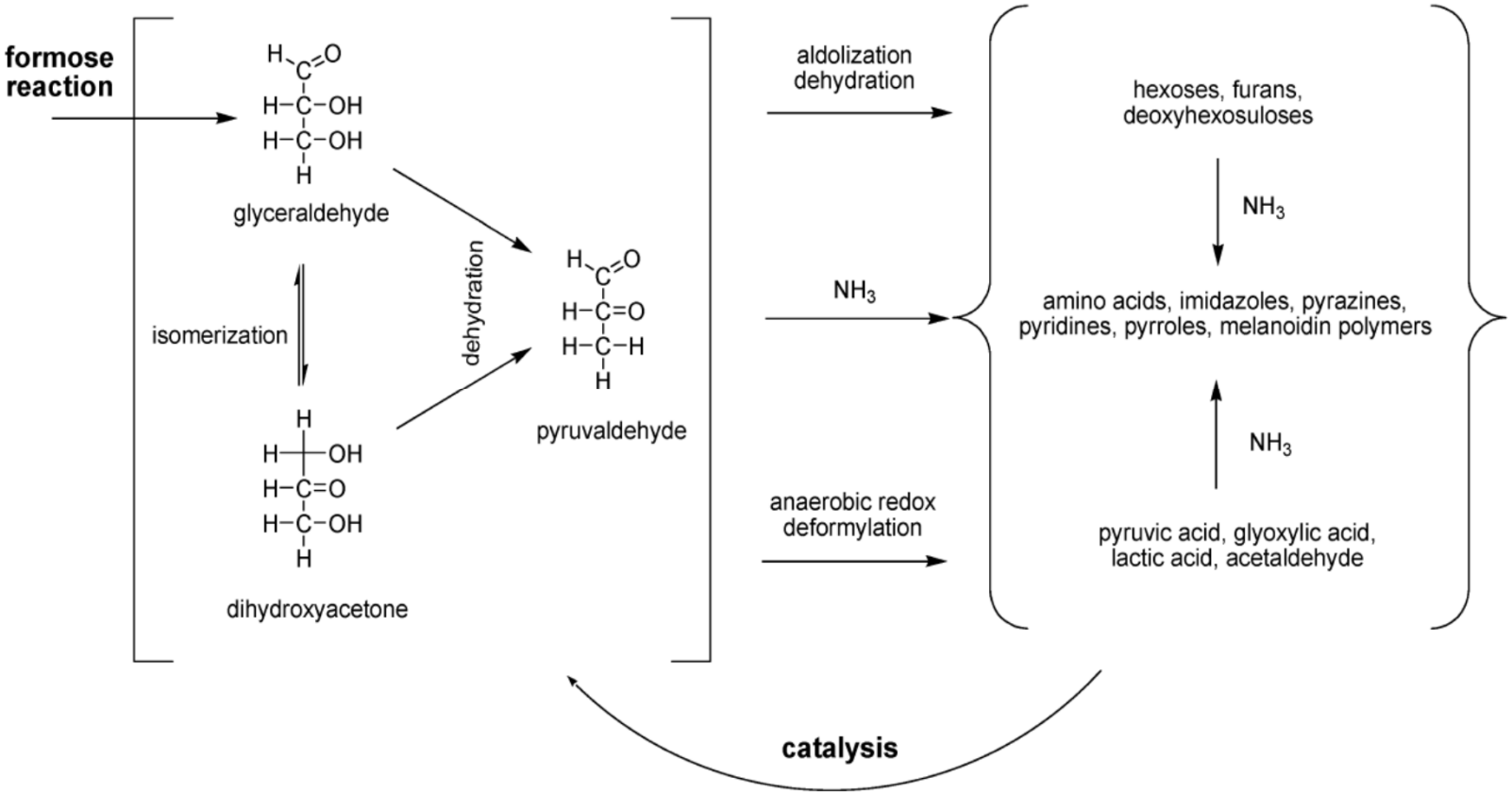


Formose reaction is one of the simplest autocatalytic cycles – two molecules of glycolaldehyde are produced from one.

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

More complex views on autocatalytic cycles

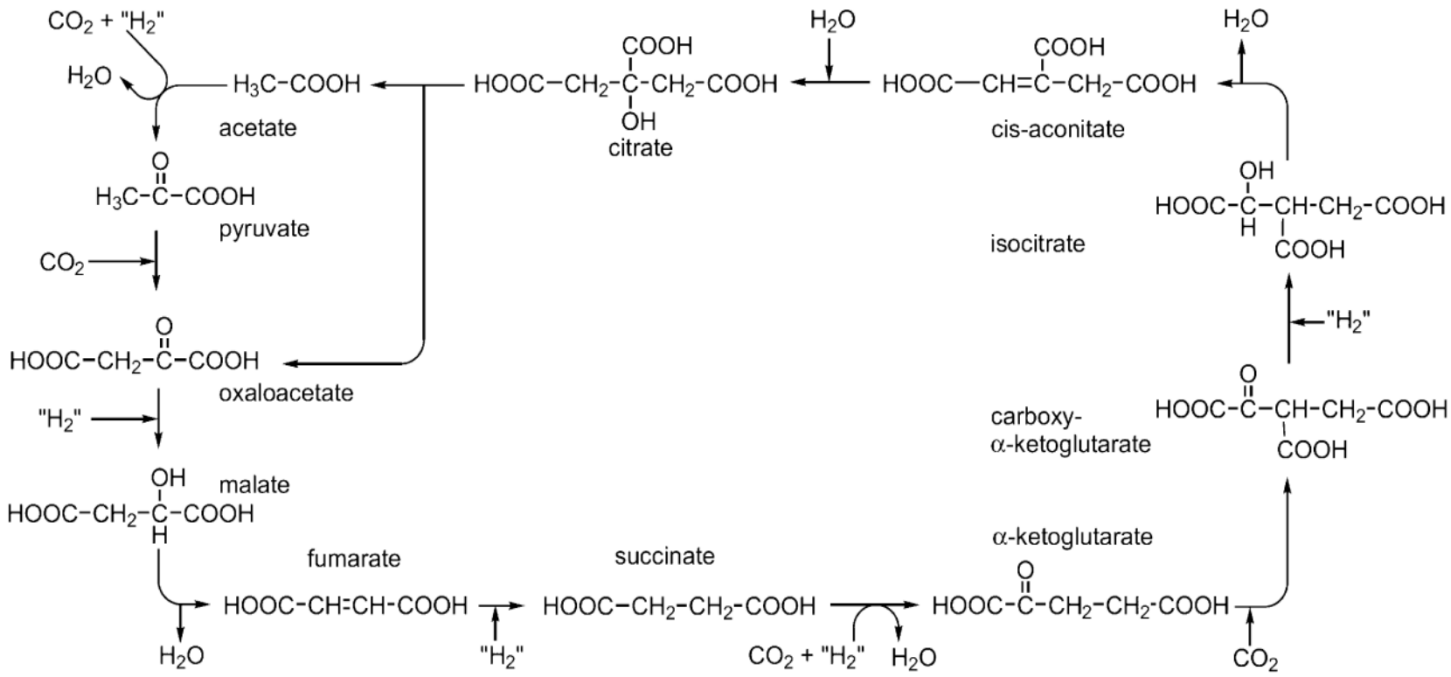
Coupling formose reaction with ammonia and thiols yields reactive α-hydroxy and α-aminothioesters, as well as numerous other aliphatic and aromatic compounds. Some of them enter another autocatalytic cycles.



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

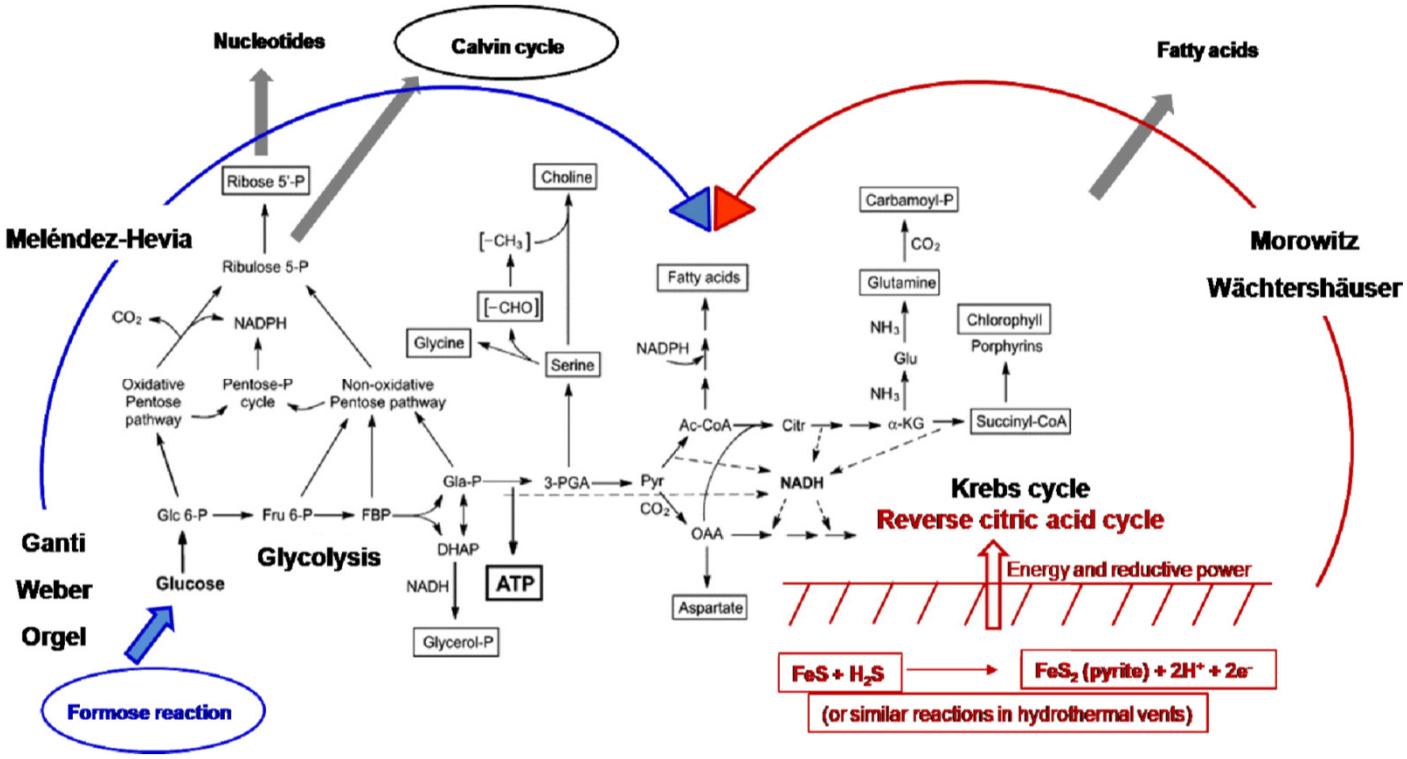


This catalytic cycle is claimed (Morowitz) to be able to run also in absence of enzymes (e.g. on mineral surfaces).
This could be the starting point for evolution of all other currently operating metabolic cycles.
However, no experimental demonstration of the full cycle under abiotic conditions delivered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

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

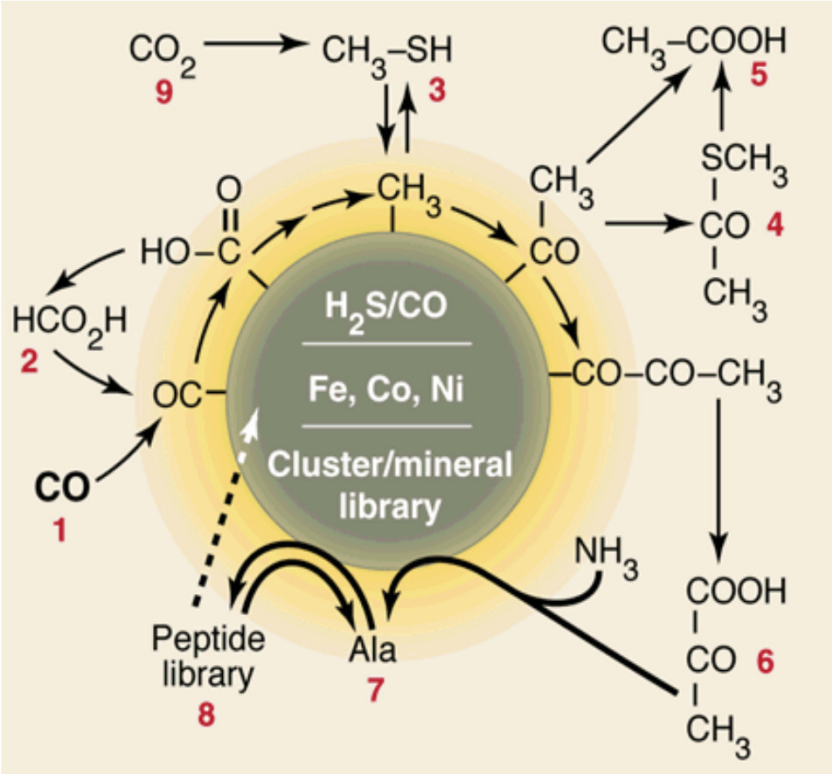
More complex views on autocatalytic cycles

(Black) Minimal metabolic map, constructed by simplifying present-day cellular metabolisms. (Blue) The clockwise sense of metabolic evolution in the scheme of Meléndez-Hevia et al.497 gives the formose reaction a prominent role as the first metabolic cycle, as Weber, Meléndez- Hevia, or Ganti proposed. (Red) The counterclockwise sense of metabolic evolution, according to the same scheme, would come from considering the reverse citric acid cycle as the first metabolic cycle, as Morowitz or Wächtershäuser have defended. In that case, energy and reductive power could be provided by redox reactions occurring on mineral surfaces (e.g., FeS, NiS) in hydrothermal vents, for instance.



Wächtershäuser' Iron-Sulfur World

The reverse citric acid cycle (Krebs' cycle)



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

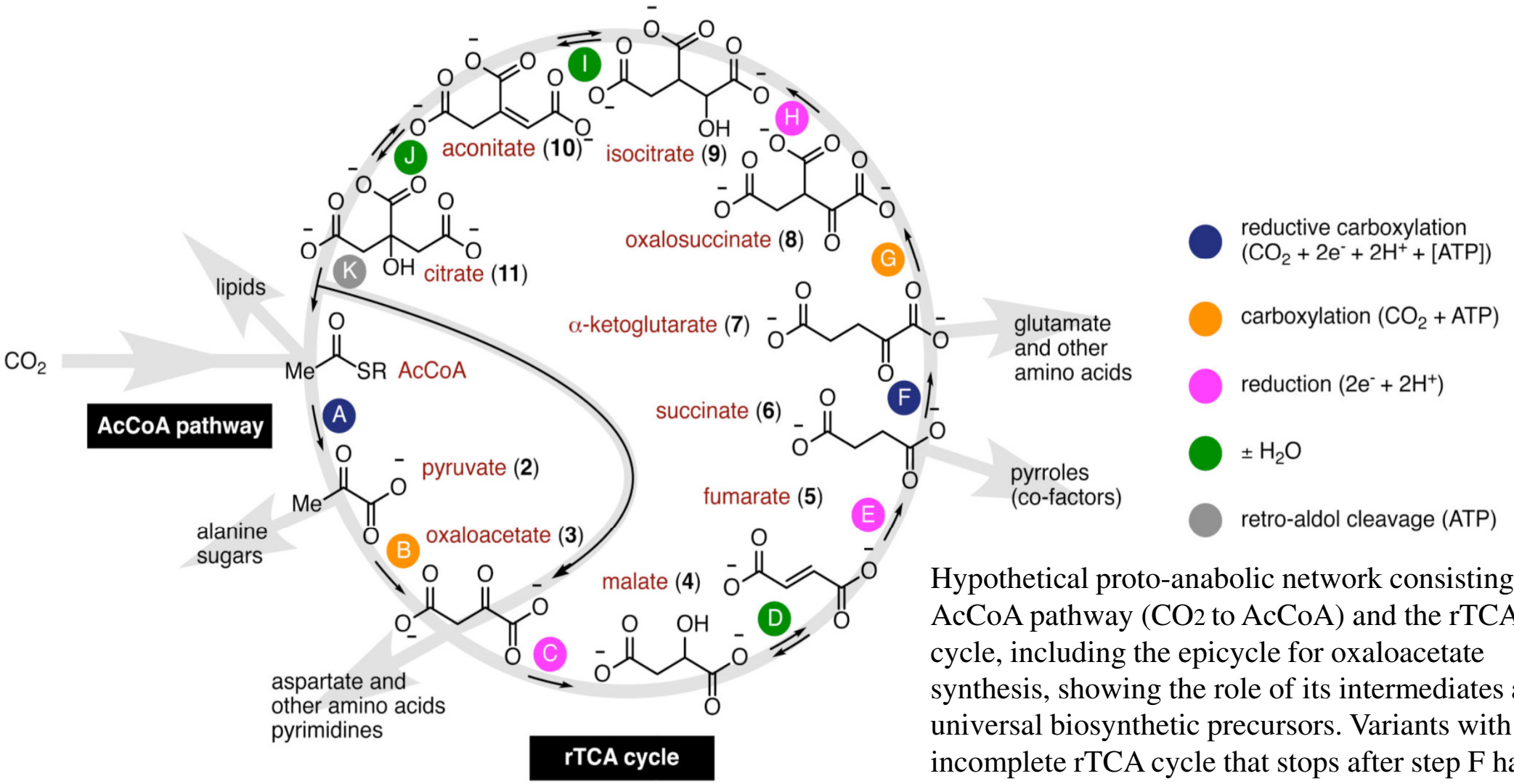
$Ni(CN)_2 + CO \rightarrow \rightarrow$ glycolate/glycine
lactate/alanine
glycerate/serine
pyrruvic acid

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

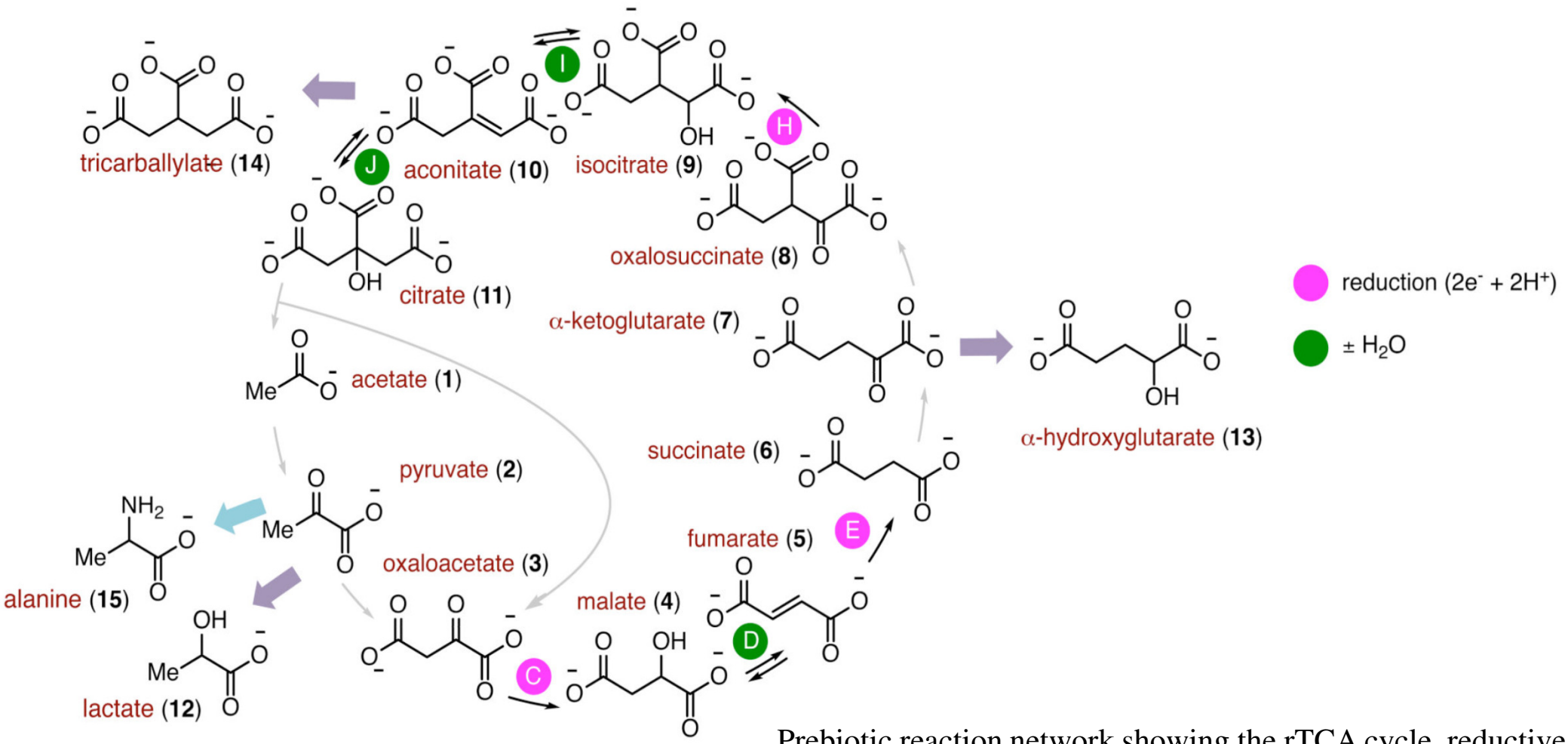
Metals promote sequences of the reverse Krebs cycle



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

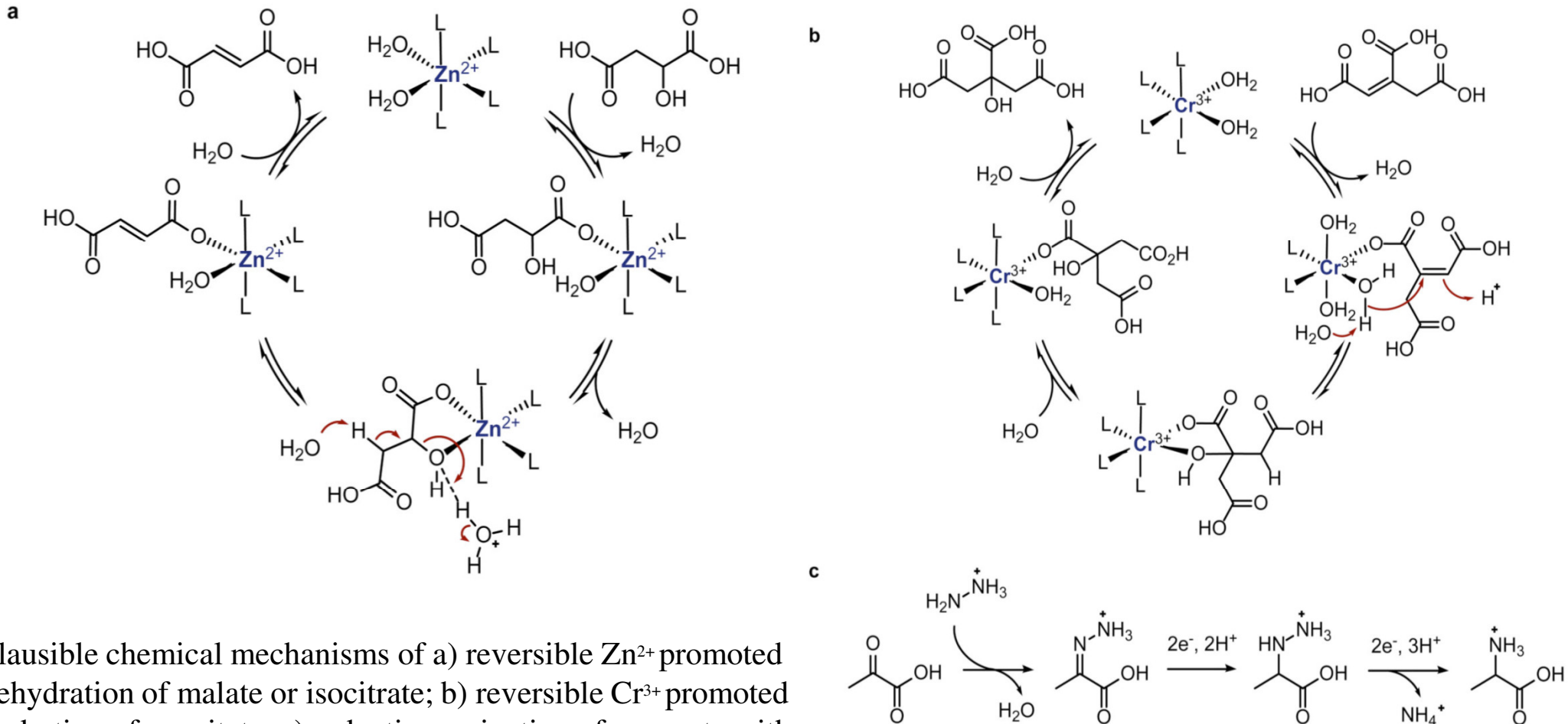
Metals promote sequences of the reverse Krebs cycle



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

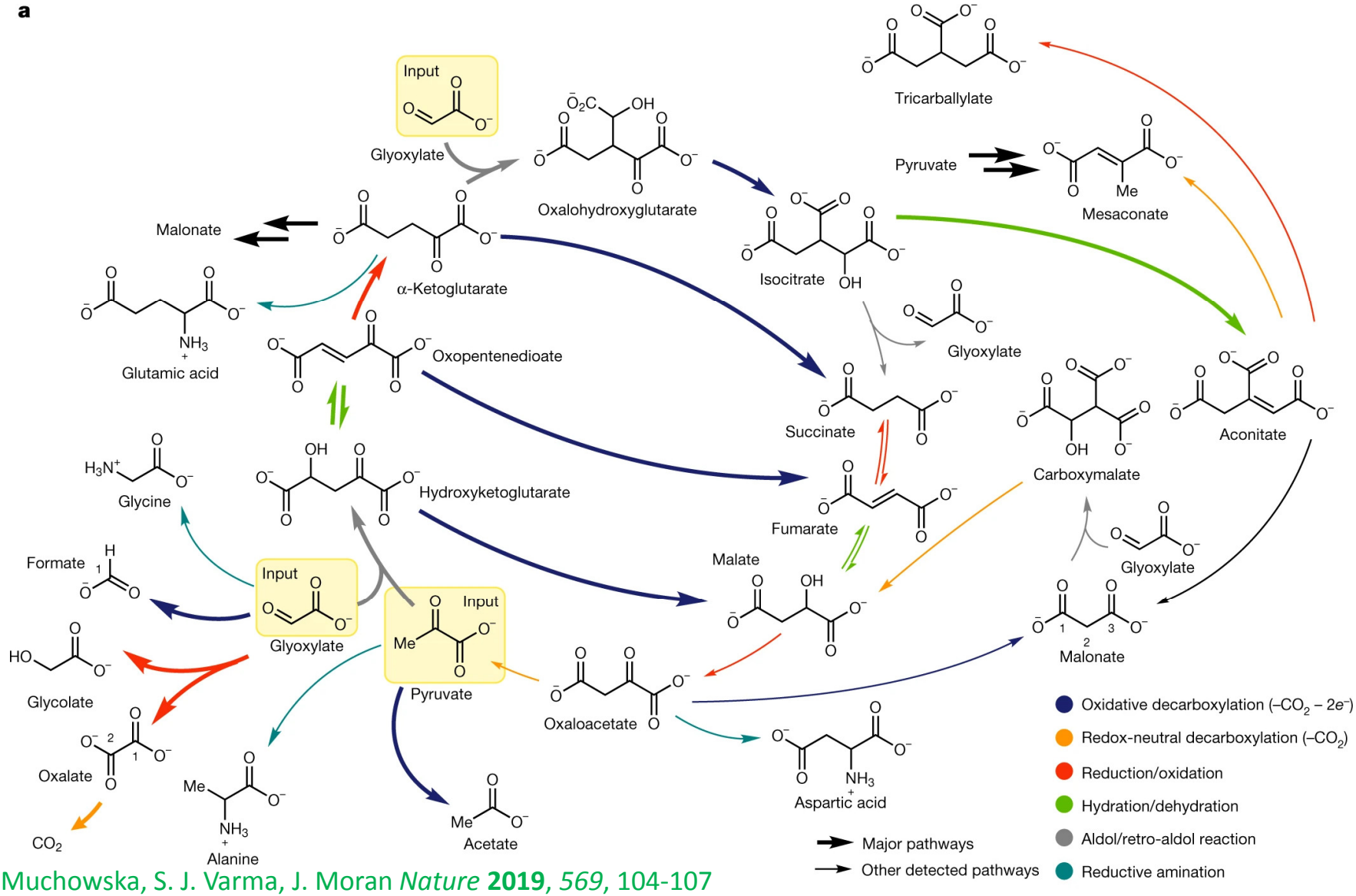
Metals promote sequences of the reverse Krebs cycle



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

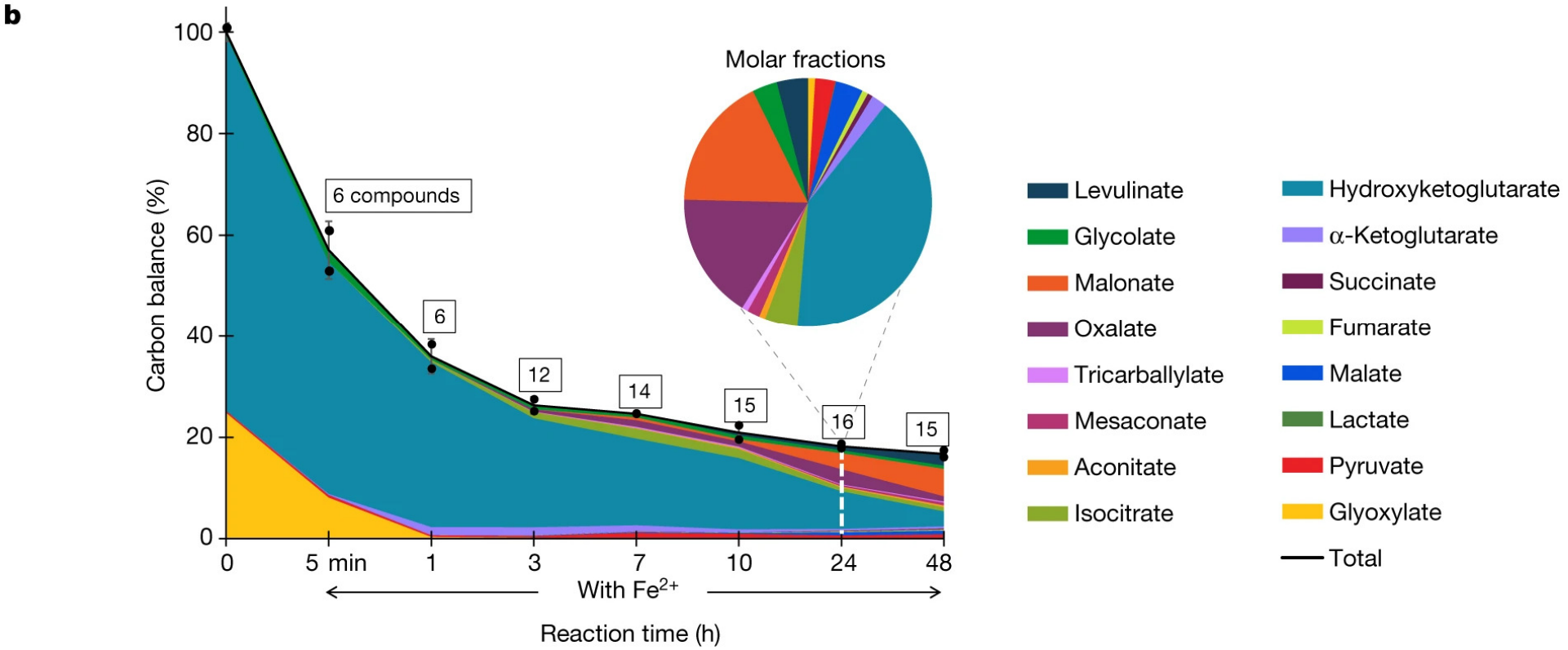
J. Moran *et al. Nat Ecol Evol.* **2017**, *1(11)*, 1716–1721

Synthesis and breakdown of universal metabolic precursors promoted by iron



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

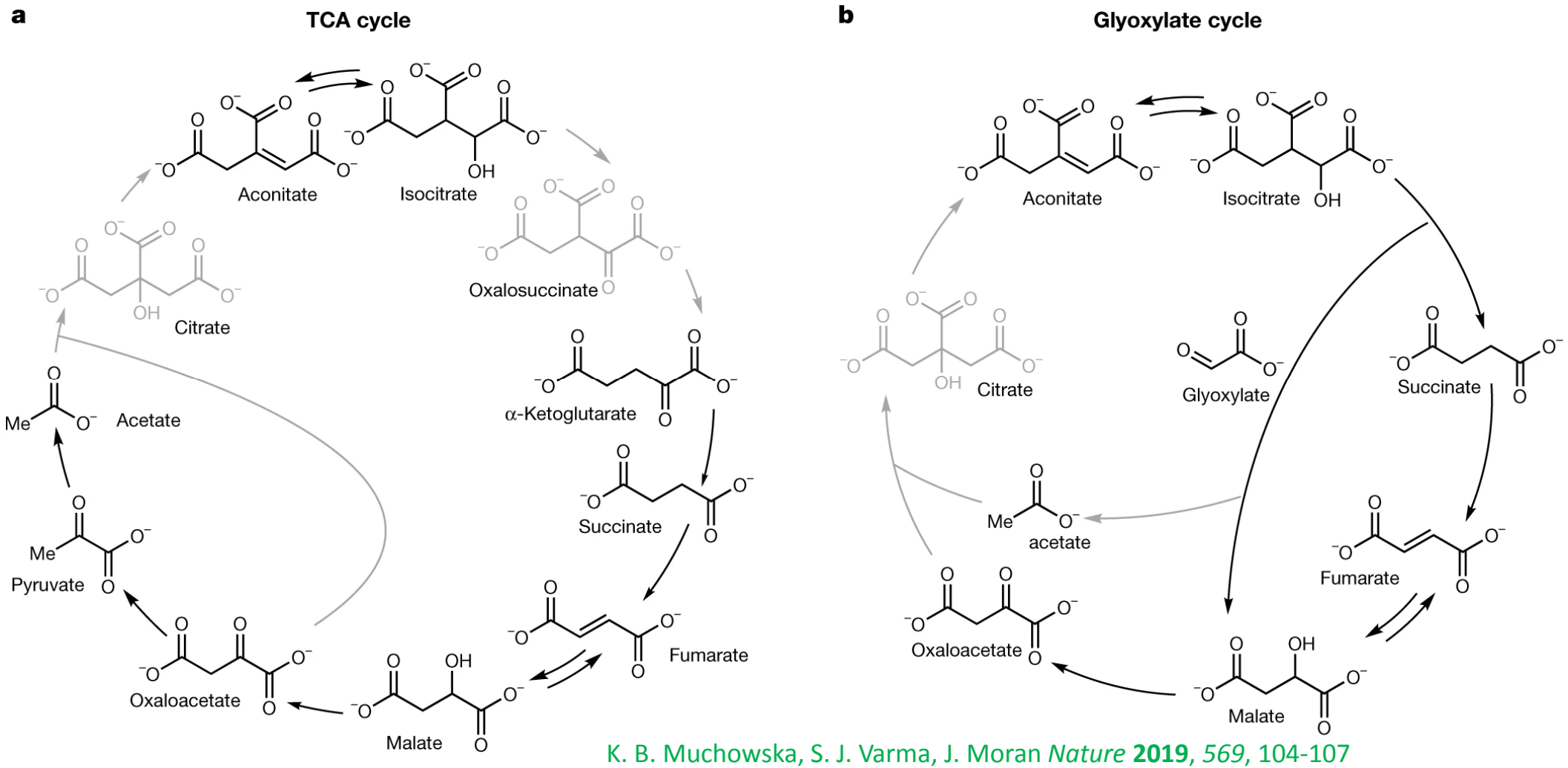
Synthesis and breakdown of universal metabolic precursors promoted by iron



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

Synthesis and breakdown of universal metabolic precursors promoted by iron

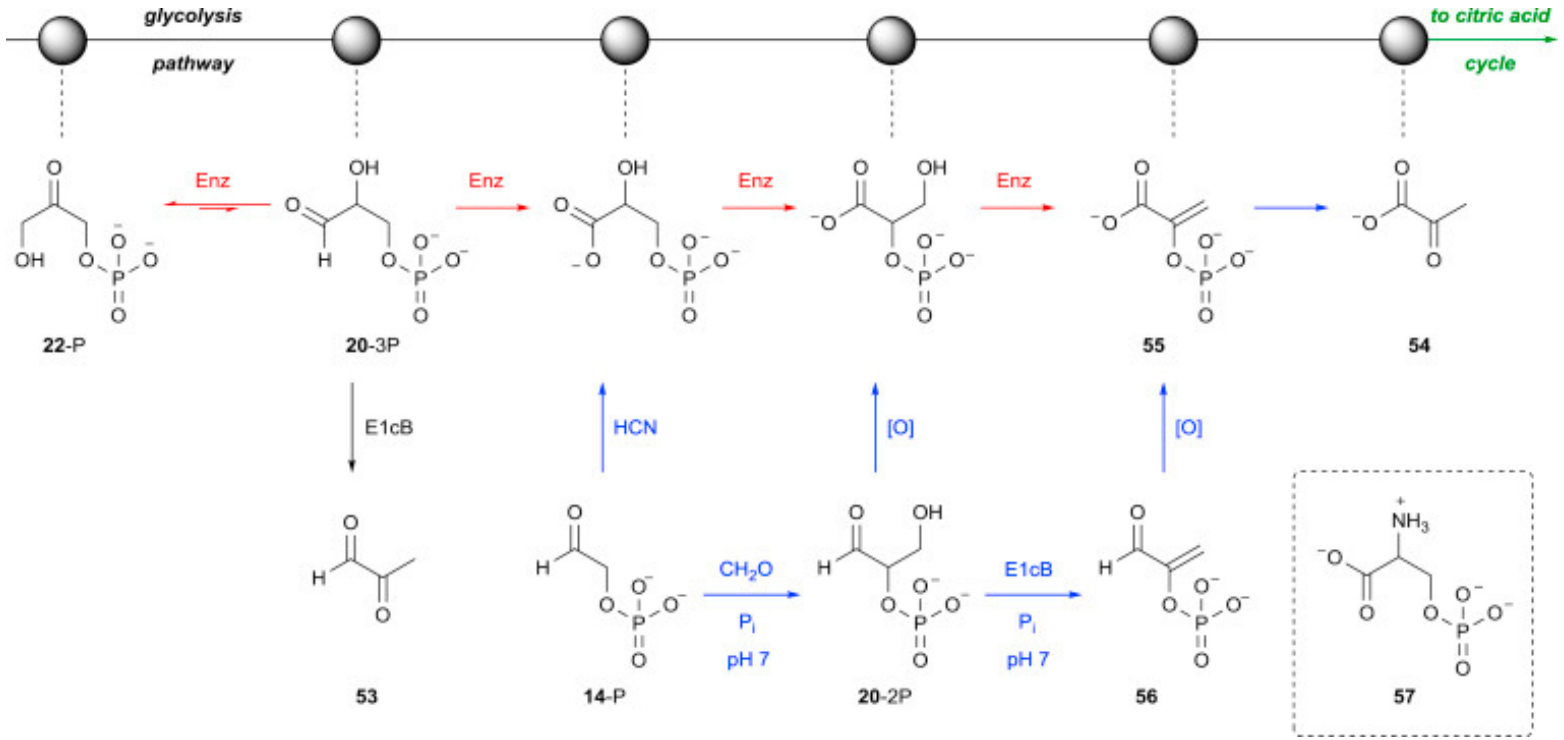
Comparison of the observed reaction network with the TCA and glyoxylate cycles. Intermediates and reactions found in both the biological cycle and the synthetic reaction network shown in black. Those found only in the biological cycle - in grey.



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

Prebiotic soup - summary

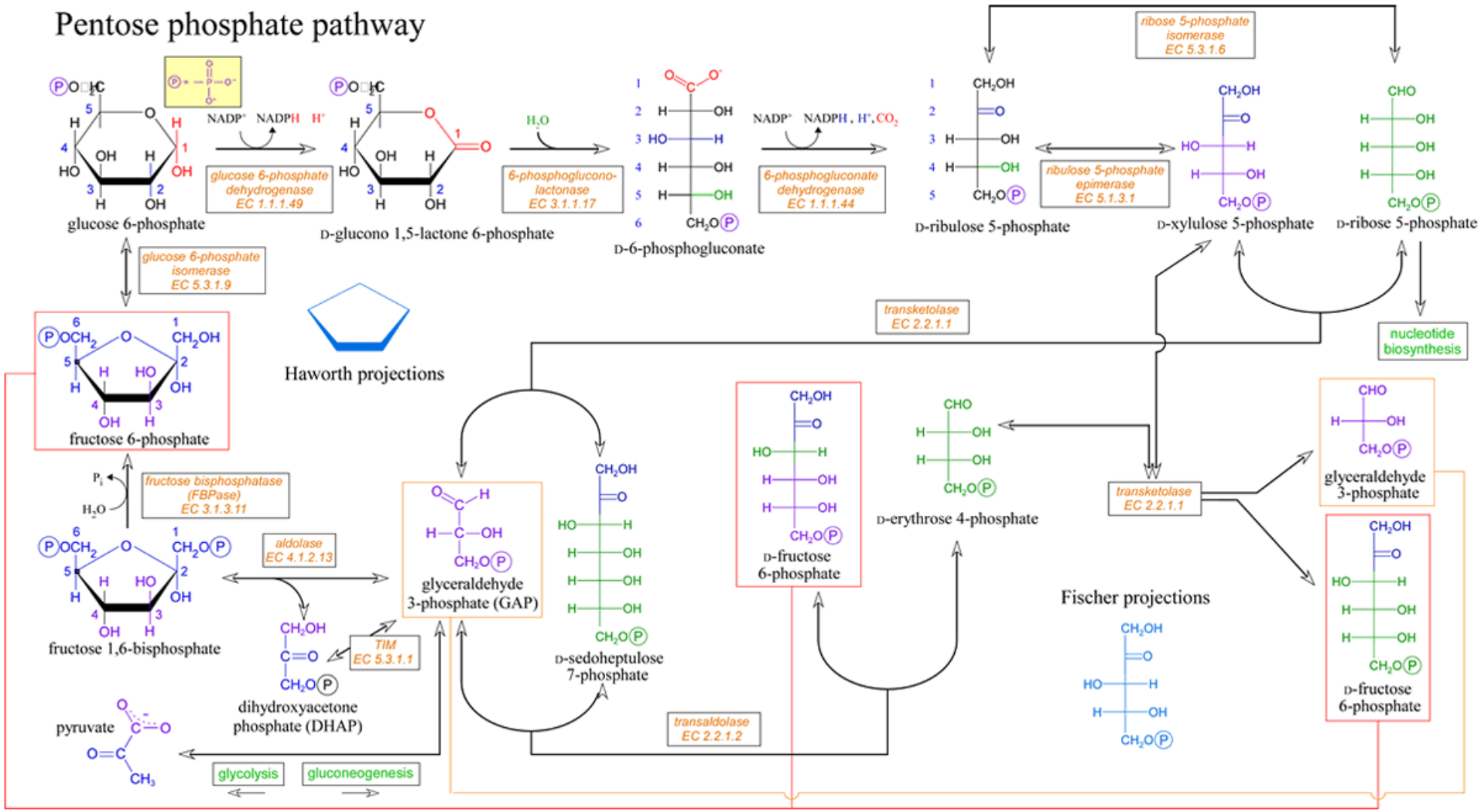
Prebiotic Reconstruction of the Triose Glycolysis Pathway by Selective α-Phosphorylation of the Simplest Sugars



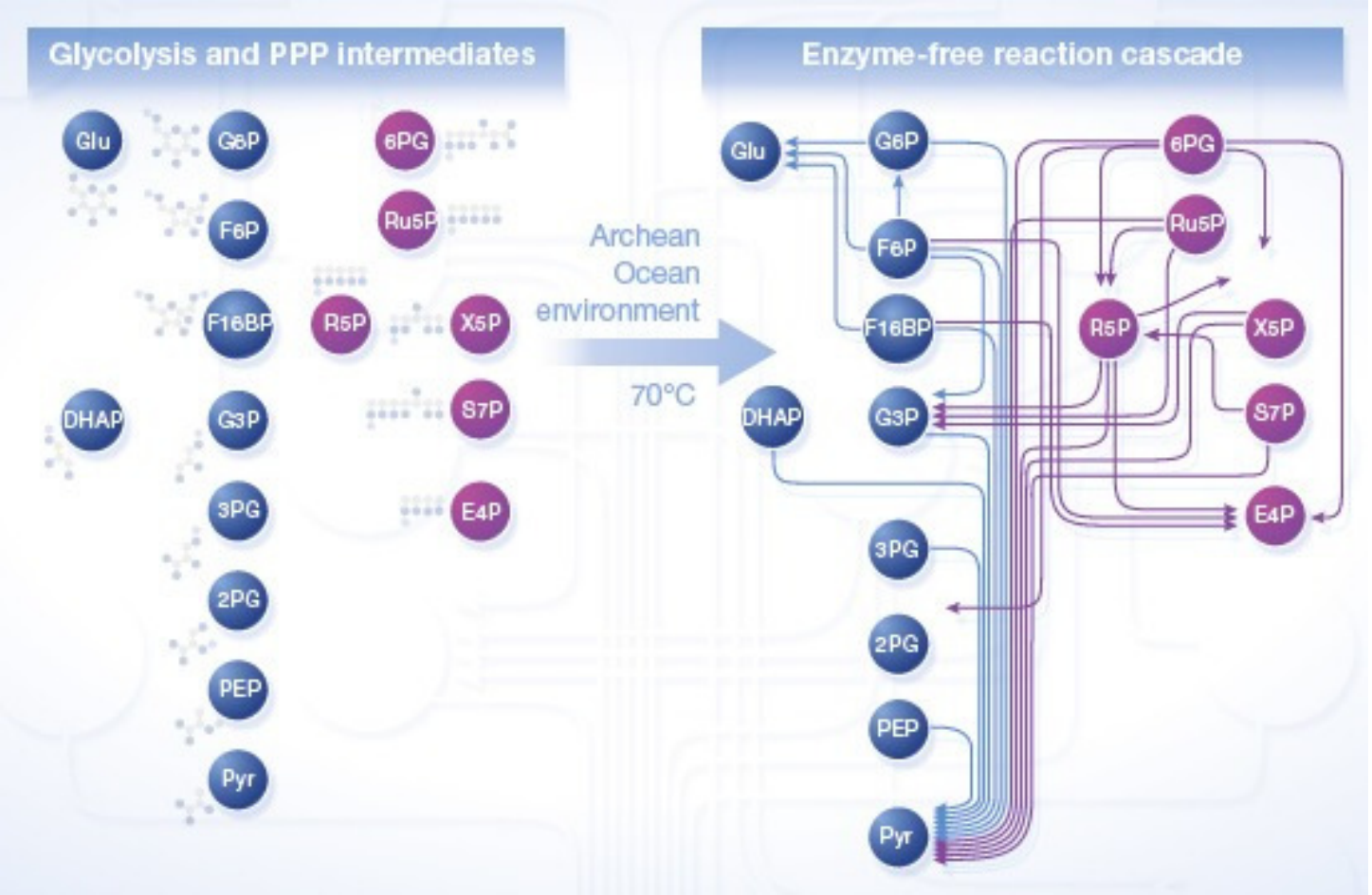
S. Islam, M. W. Powner *Chem* **2017**, *2*, 470-501

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

Pentose phosphate pathway

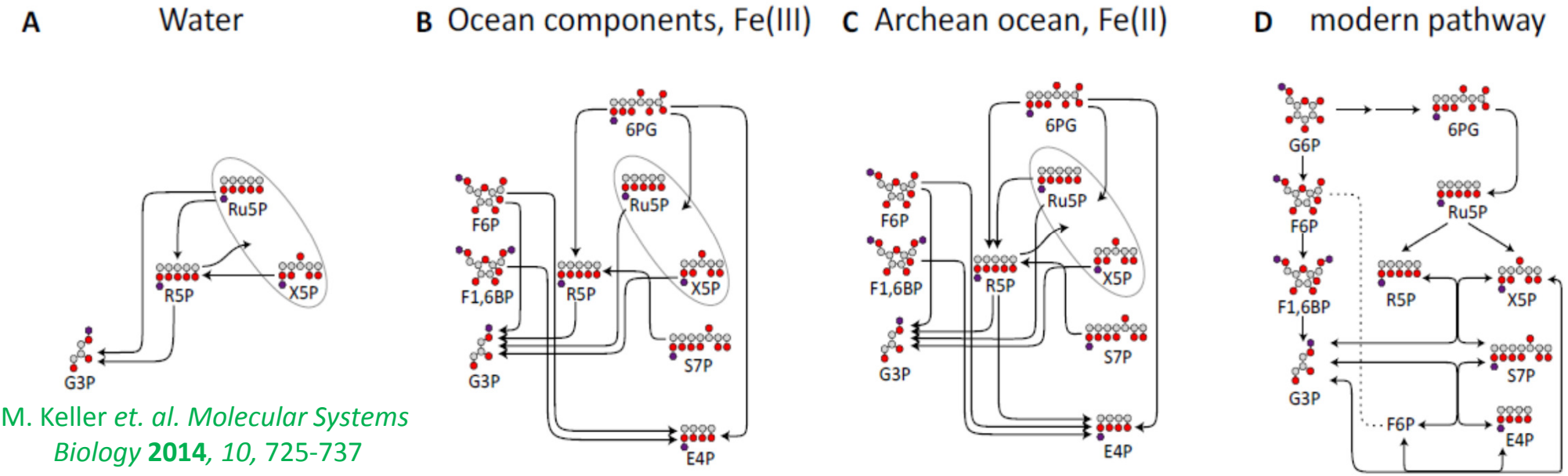


Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment



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.

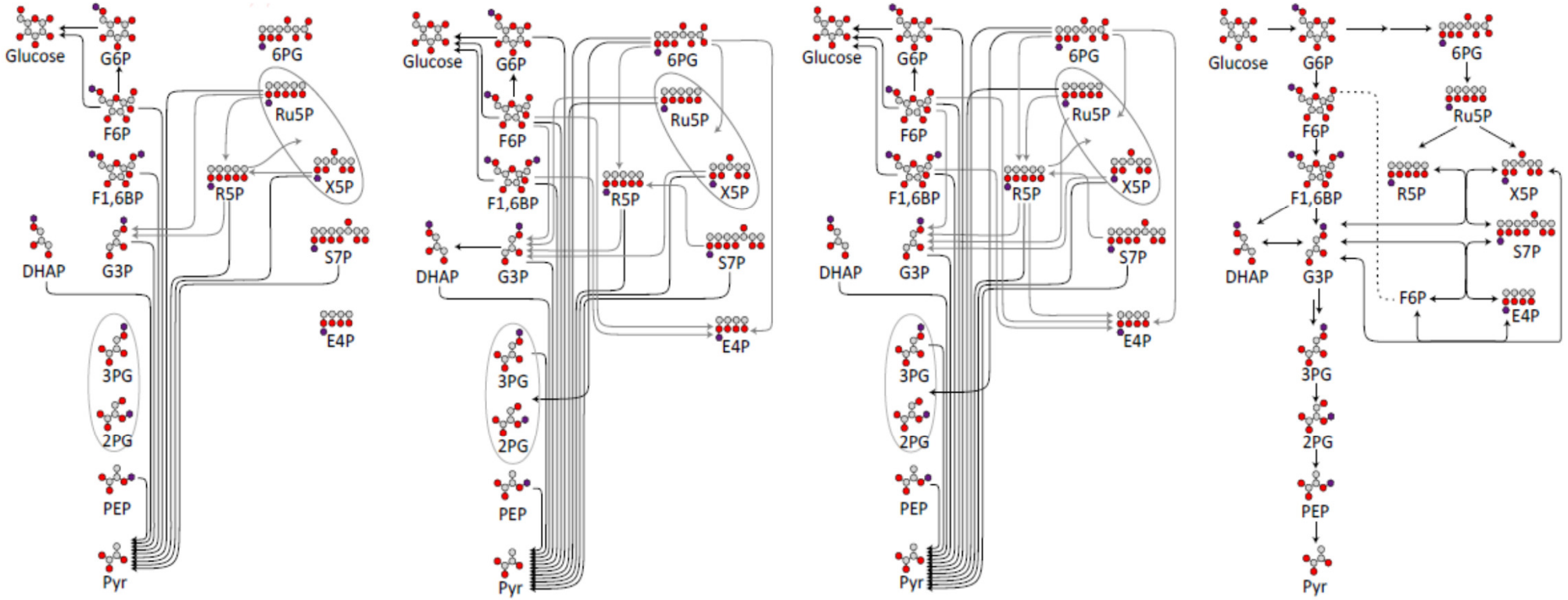
B The same reactions in solution with Fe^{III}, Co^{II}, Ni^{II}, Mo and phosphates simulating an Archean ocean.
In this milieu, 28 interconversion reactions among glycolytic and pentose phosphate pathway intermediates were observed.

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.

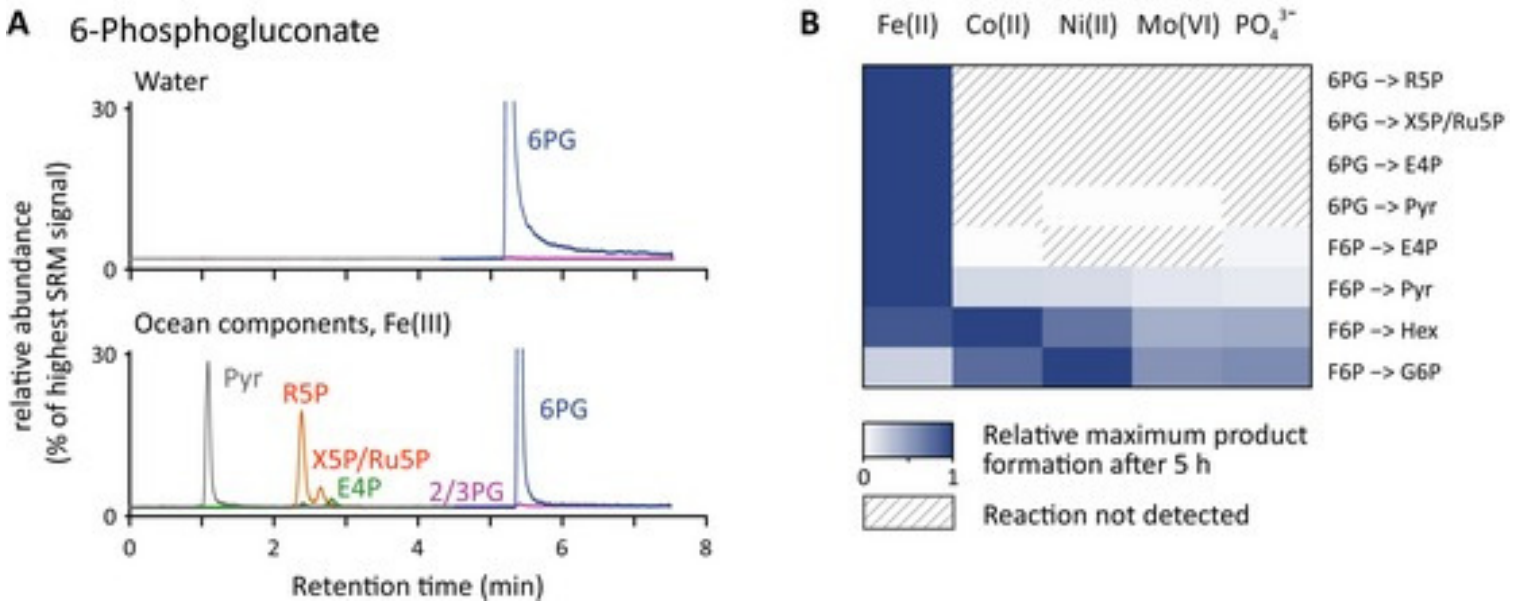
Pentose phosphate pathway: 6PG, 6-phosphogluconate; Ru5P, ribulose 5-phosphate; R5P, ribose 5-phosphate; X5P, xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; E4P, erythrose 4-phosphate.

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.

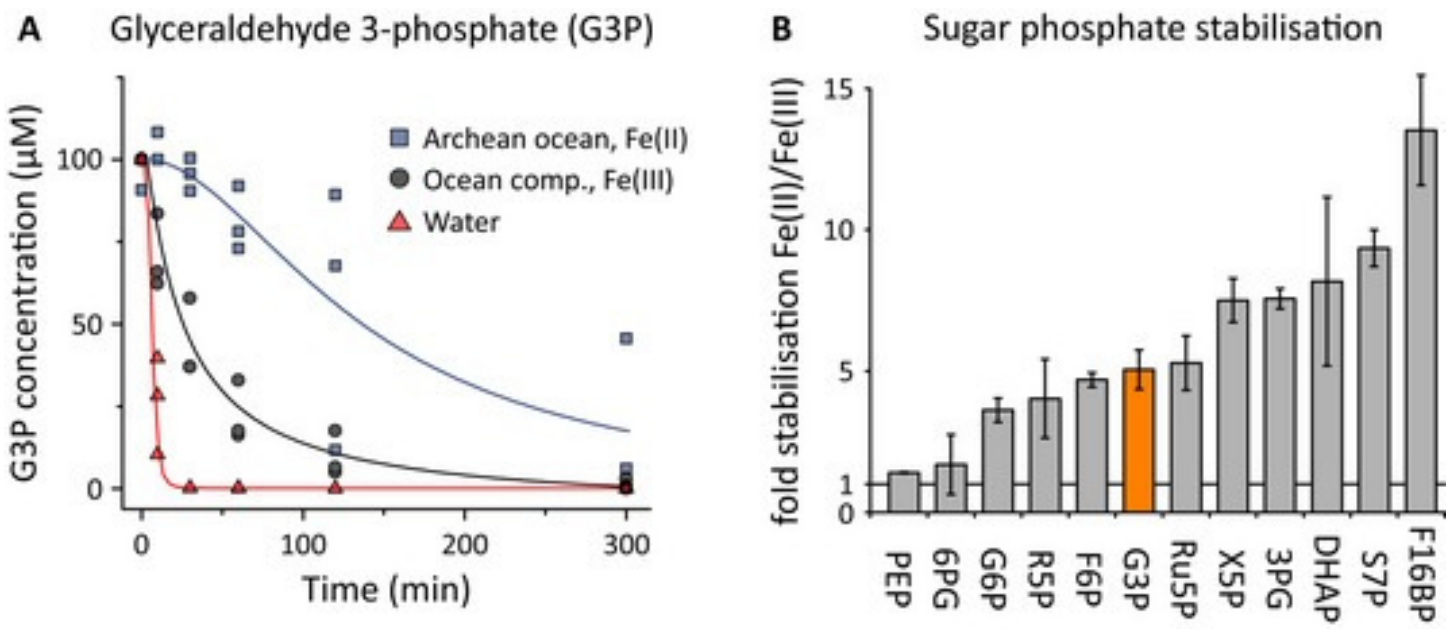
M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737



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

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)

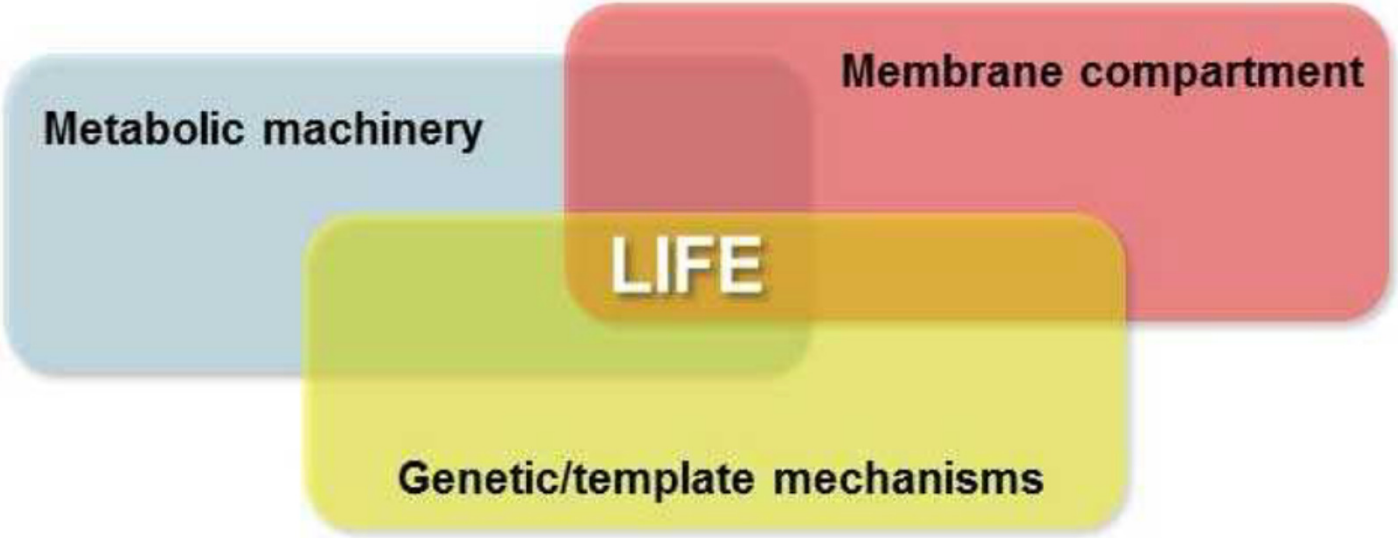
Unclear chemical nature of primordial metabolic cycles

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

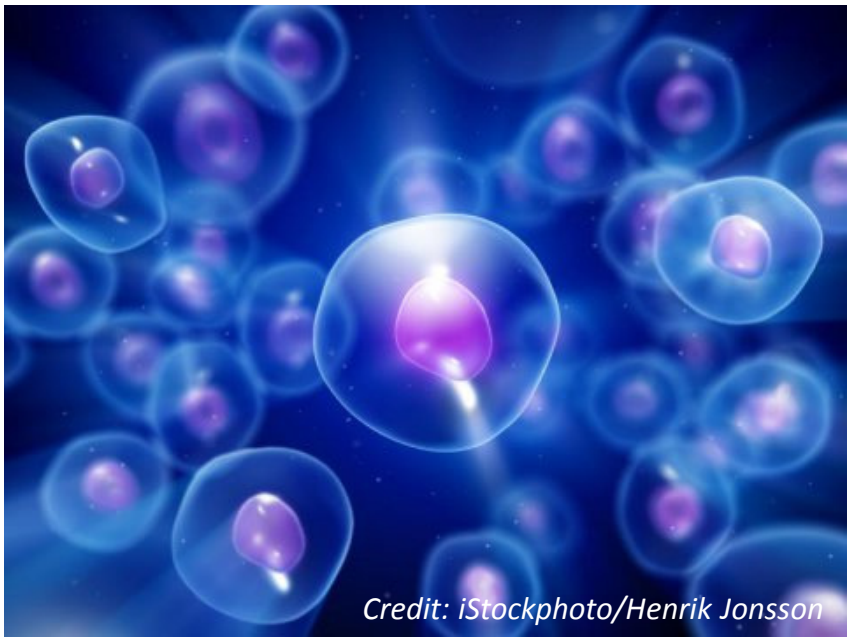
Evolution of such hypothetical networks requires multiple simultaneous mutations

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

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

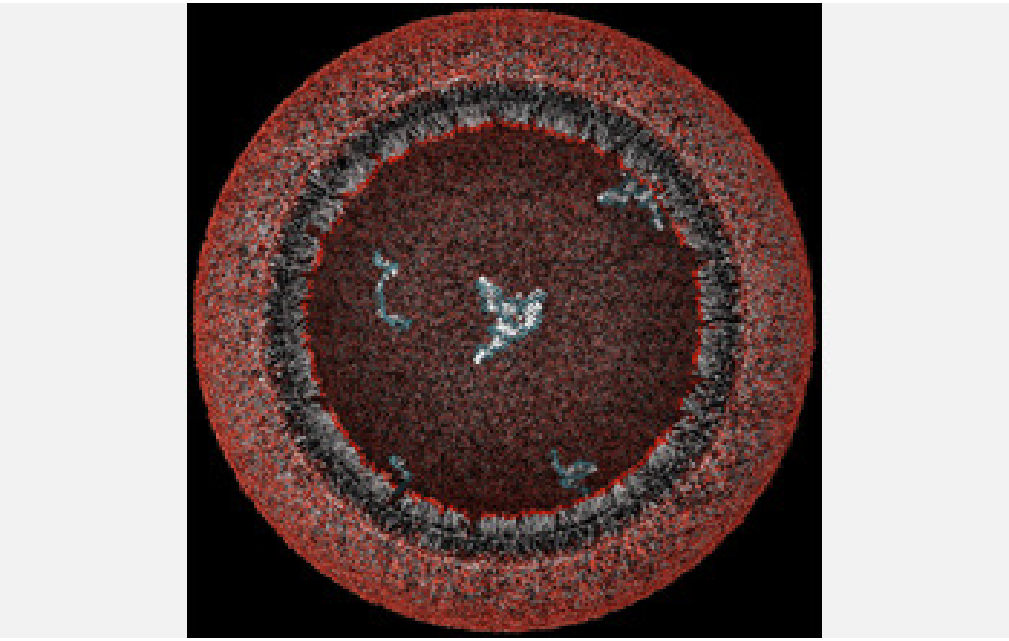

Membrane compartment
Metabolic machinery
LIFE
Genetic/template mechanisms

Encapsulation – essential for life



Membrane compartments

Assembly of amphiphilic monomers into protocellular compartments

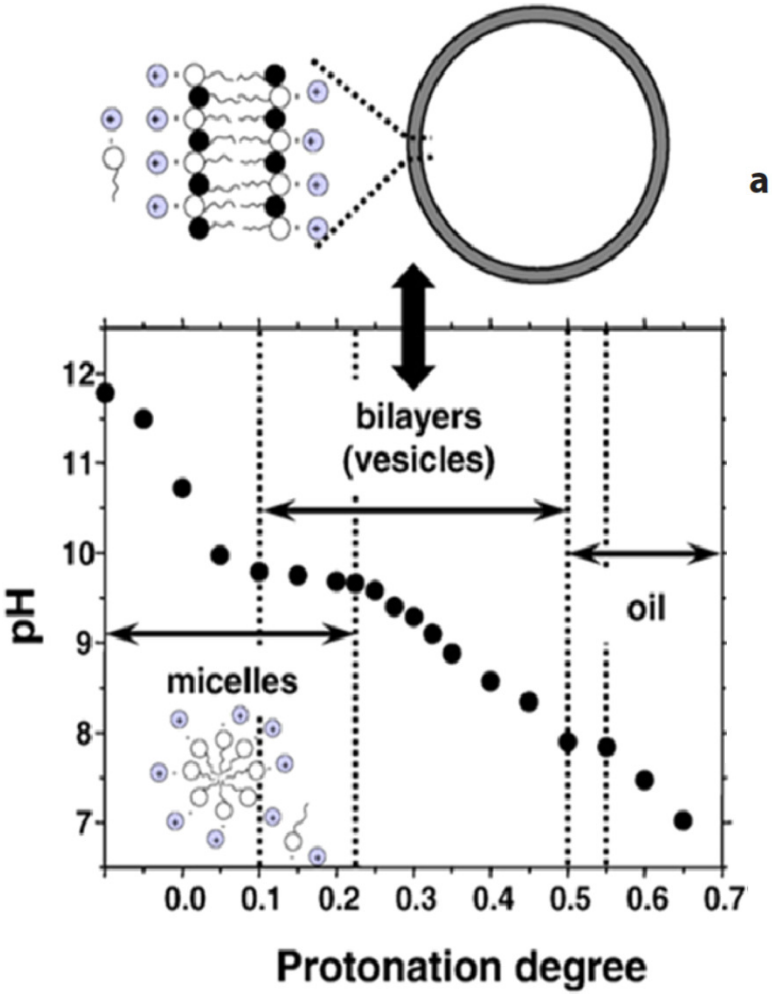


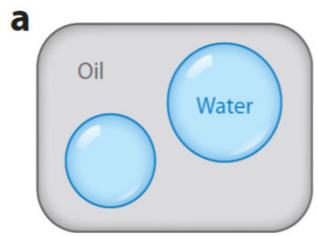
Credit: *Janet Iwasa*

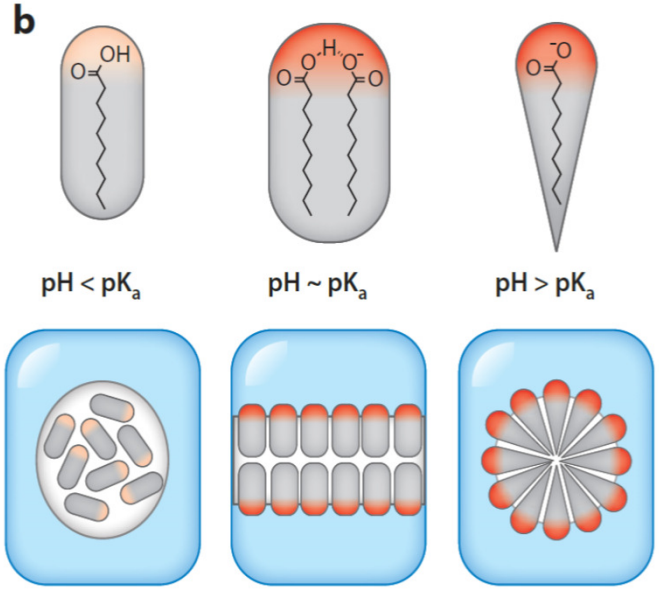
A three-dimensional view of a model protocell (a primitive cell) approximately 100 nanometers in diameter.

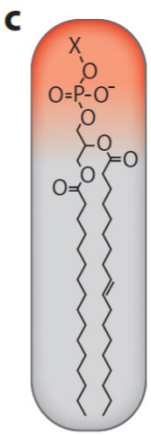
The protocell's fatty acid membrane allows nutrients and DNA building blocks to enter the cell and participate in non-enzymatic copying of the cell's DNA. The newly formed strands of DNA remain in the protocell

pH-dependent phase behavior of fatty acids in water



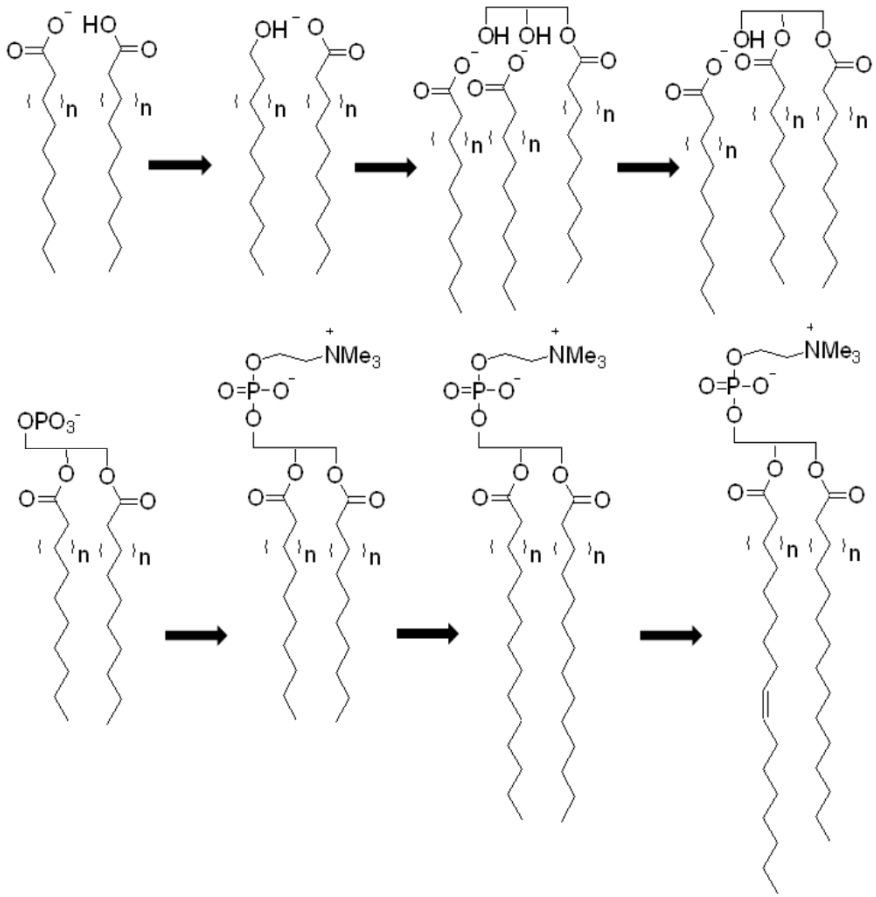




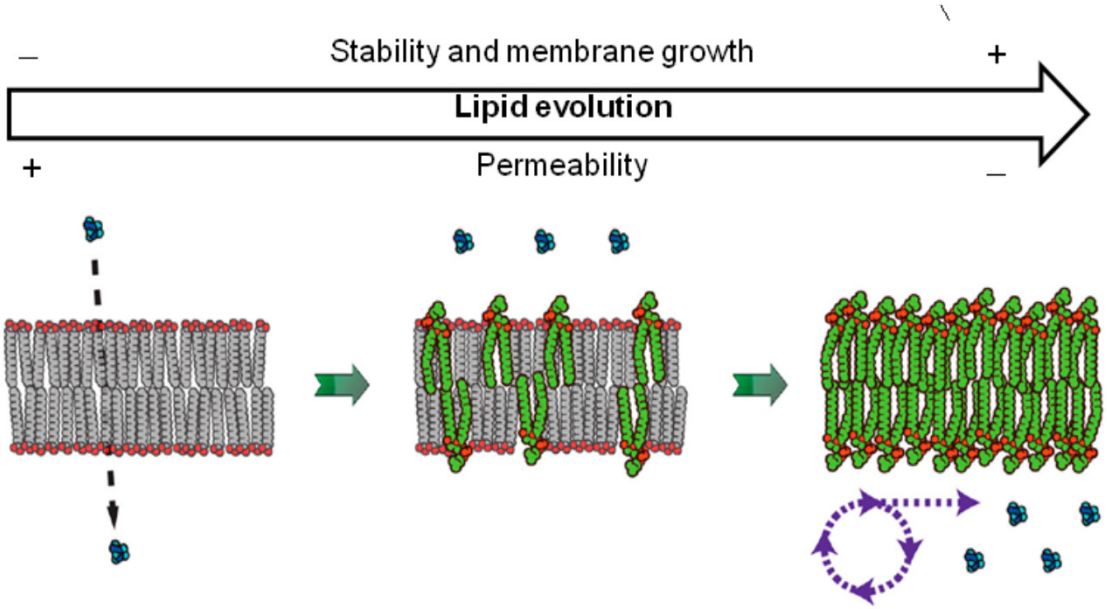


80 mM oleic acid/ sodium oleate in water

Scheme of the membrane evolution

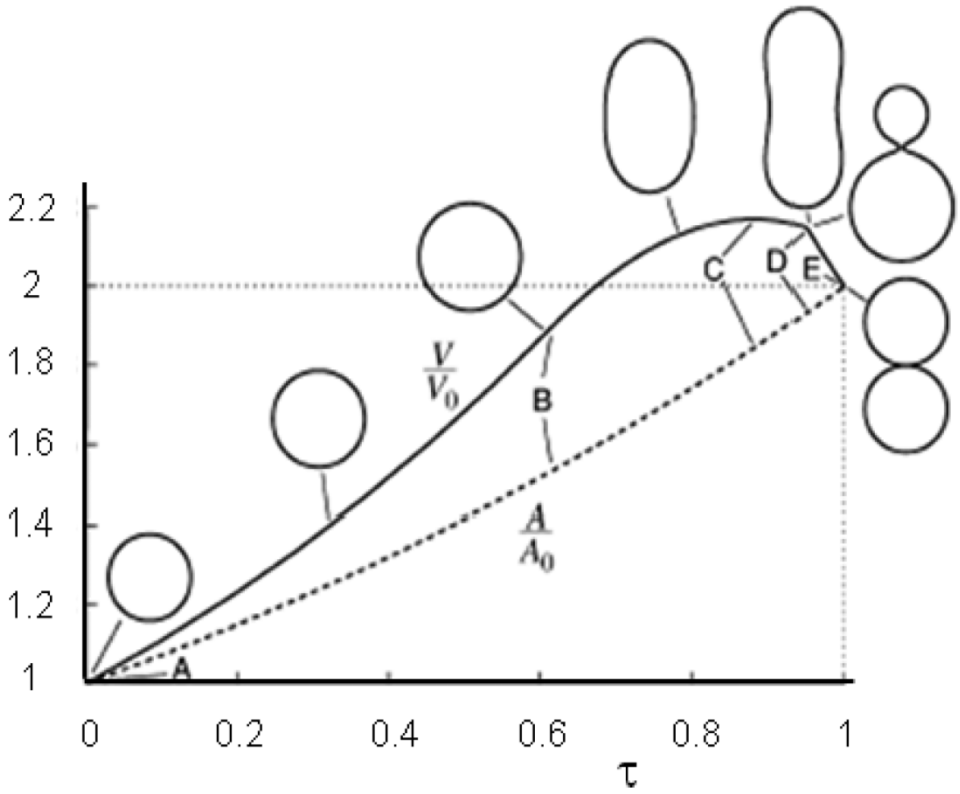


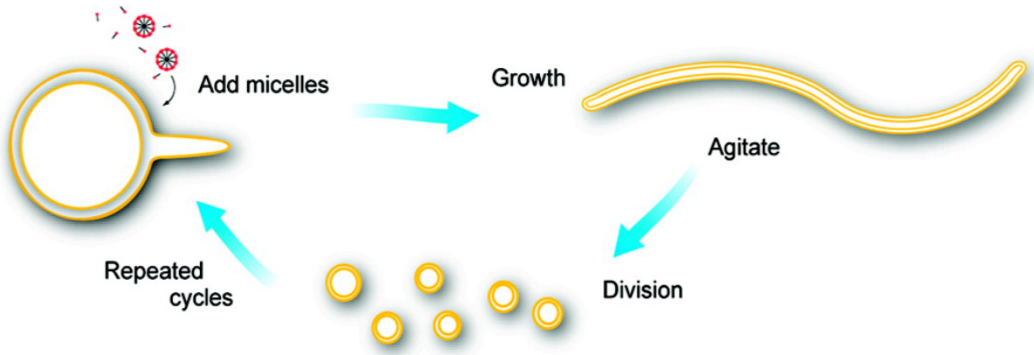
Chemical evolution of membrane components



More complex components lead to slower amphiphile desorption and thus faster growth of the protocell. Decreasing permeability is a selective pressure for the emergence of internalized metabolic and transport machinery in the system

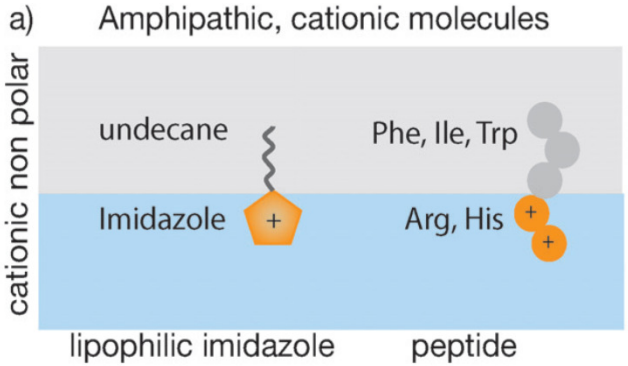
Growth and division of vesicles

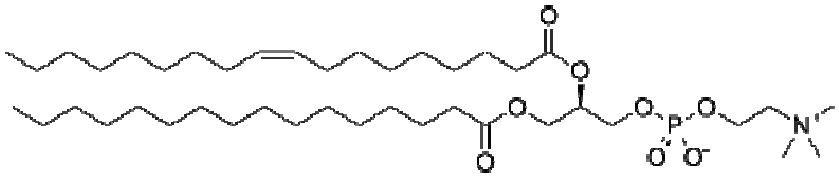


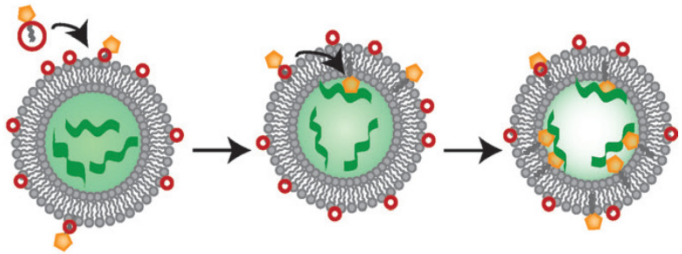


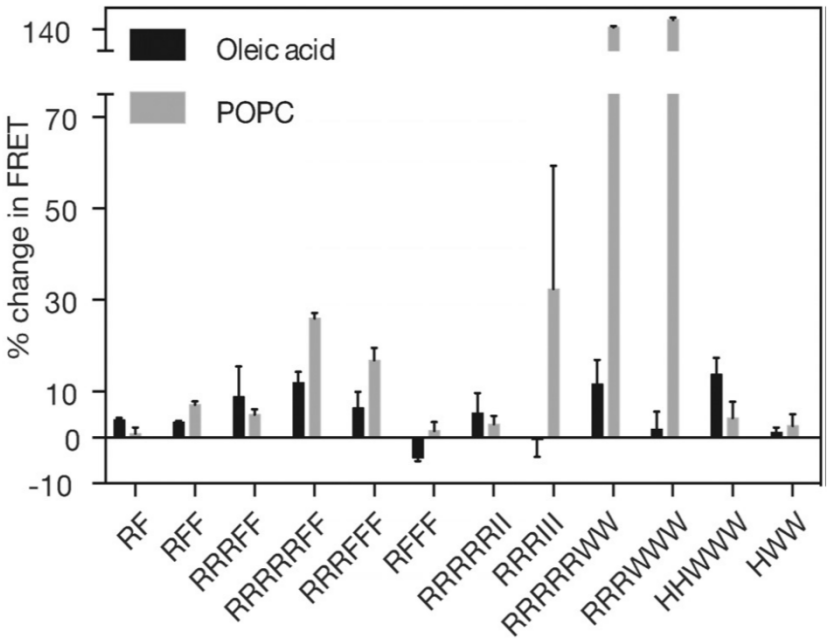
Ting F. Zhu, and Jack W. Szostak *J. Am. Chem. Soc.*, **2009**, 131 (15), 5705-5713

Noncovalent nucleotide association with membranes

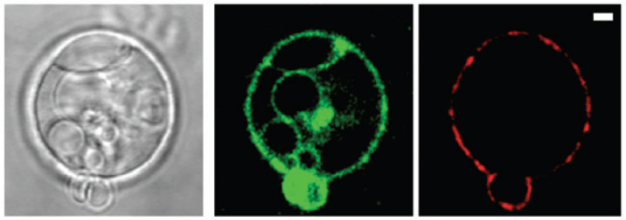








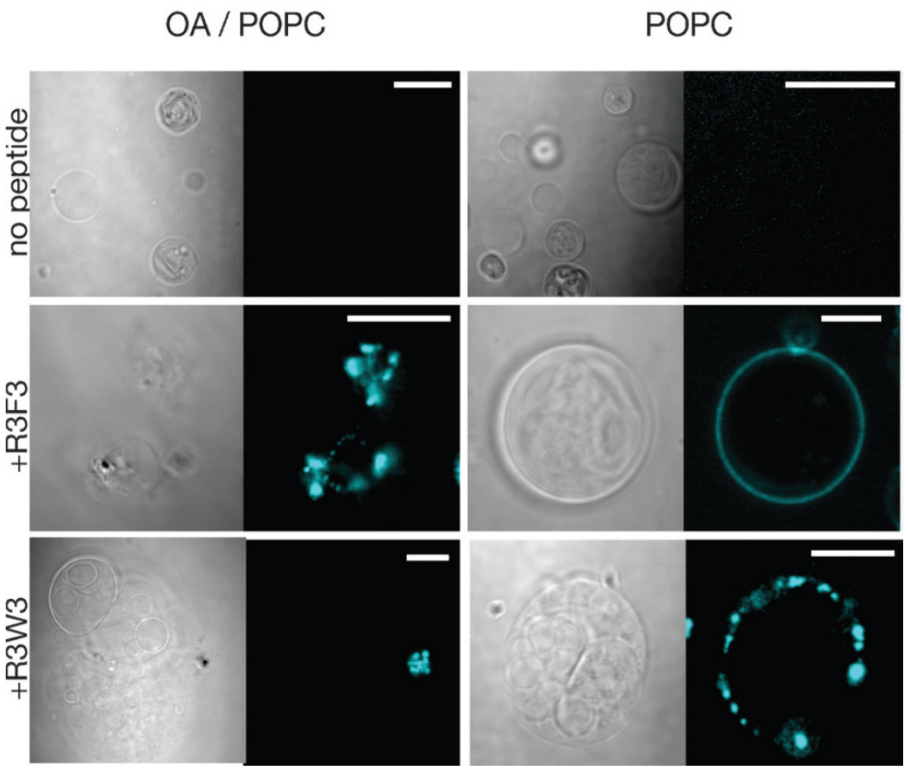
+POPC SUVs with undecylimidazole

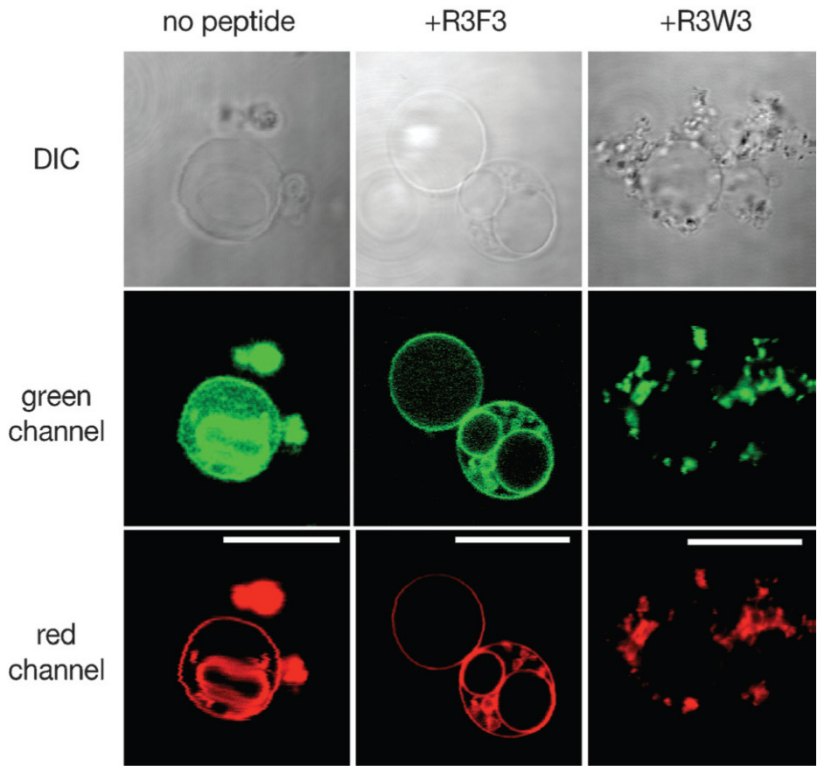


DIC green channel red channel

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

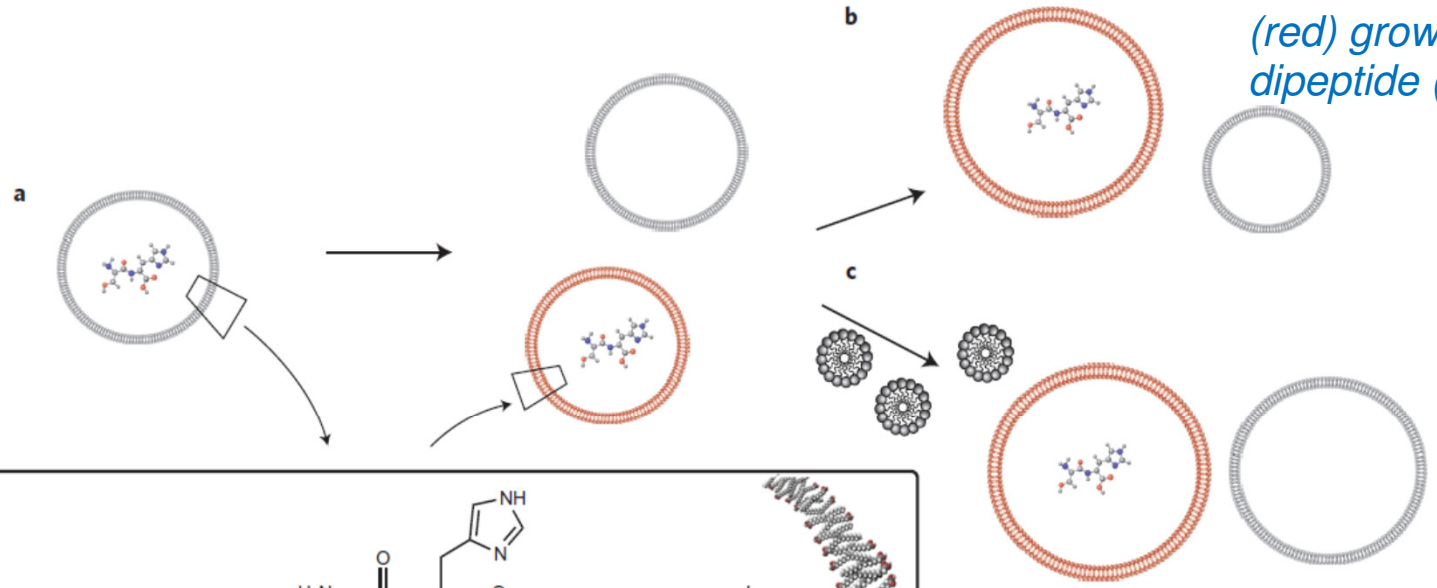




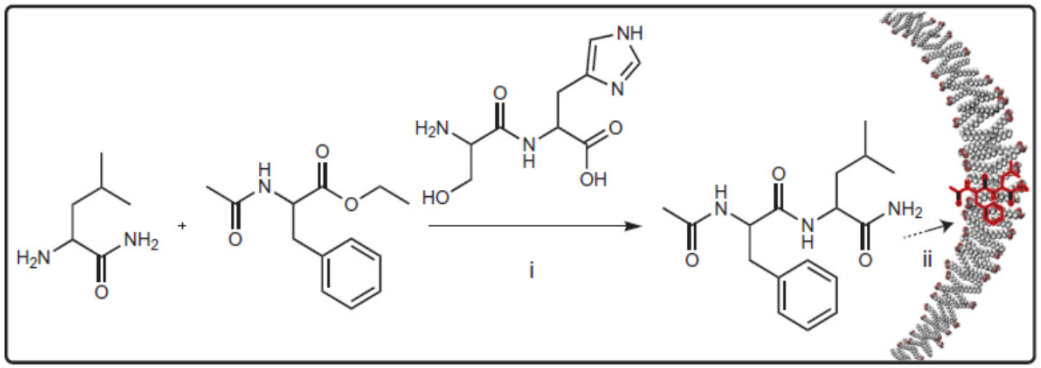
Neha P. Kamat, Sylvia Tobe, Ian T. Hill, and Jack W. Szostak *Angew. Chem. Int. Ed.* **2015**, *54*, 11735 –11739

Adaptive changes and competition between protocell vesicles

Vesicles with $AcPheLeuNH_2$ in the membrane (red) grow when mixed with vesicles without dipeptide (grey), which shrink

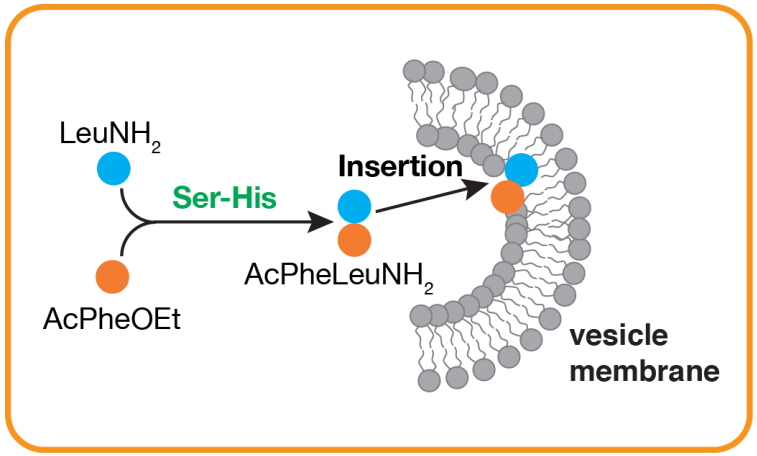


After micelle addition vesicles with $AcPheLeuNH_2$ in the membrane grow more than vesicles without the dipeptide.



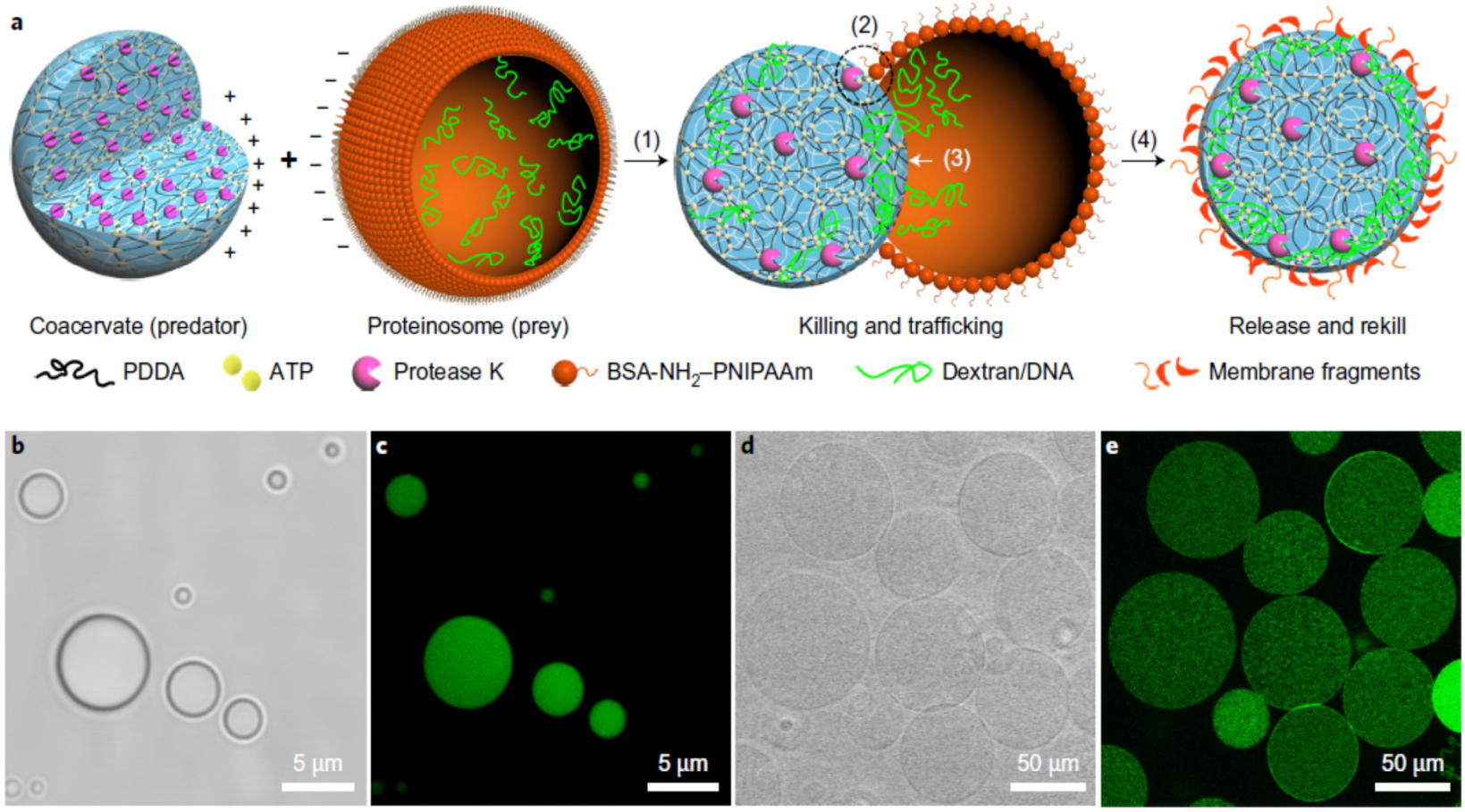
Synthesis of $AcPheLeuNH_2$ by catalyst encapsulated in fatty-acid vesicles. The dipeptide Ser-His catalyses the reaction between substrates $LeuNH_2$ and AcPheOEt (i), which generates the product of the reaction, $AcPheLeuNH_2$.

The product dipeptide $AcPheLeuNH_2$ localizes to the bilayer membrane



K. Adamala, J. W. Szostak *Nature Chem.* **2013**, *5,* 495-501

Predator/prey behavior



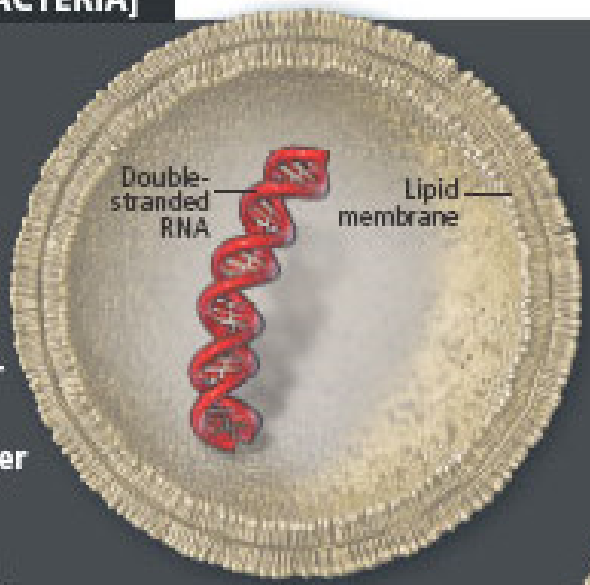
S. Mann *et al. Nature Chem.* **2016**, *DOI: 10.1038/NCHEM.2617*

From RNA world to bacteria

[FROM RNA WORLD TO BACTERIA]

Journey to the Modern Cell

After life got started, competition among life-forms fueled the drive toward ever more complex organisms. We may never know the exact details of early evolution, but here is a plausible sequence of some of the major events that led from the first protocell to DNA-based cells such as bacteria.

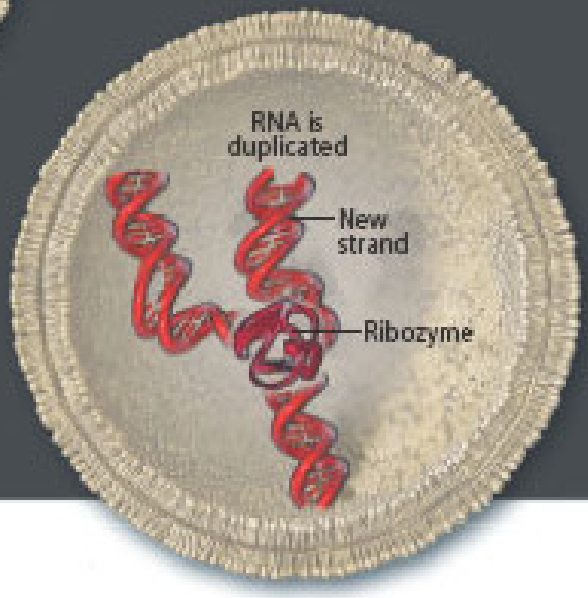


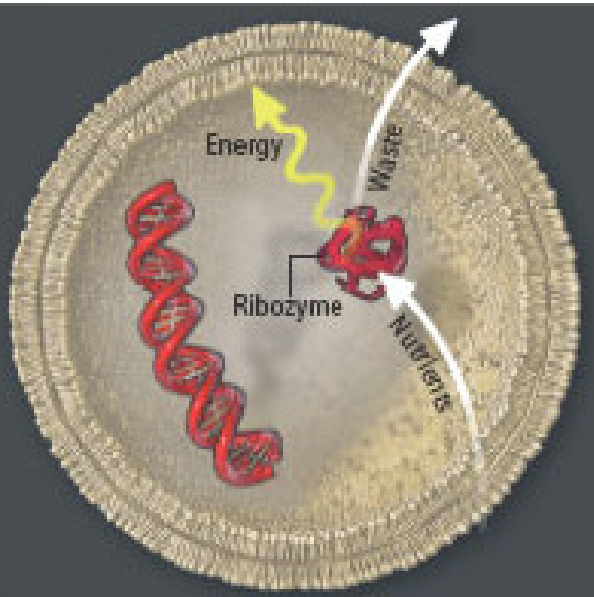
1 EVOLUTION STARTS ▲

The first protocell is just a sac of water and RNA and requires an external stimulus (such as cycles of heat and cold) to reproduce. But it will soon acquire new traits.

2 RNA CATALYSTS ▼

Ribozymes—folded RNA molecules analogous to protein-based enzymes—arise and take on such jobs as speeding up reproduction and strengthening the protocell's membrane. Consequently, protocells begin to reproduce on their own.





3 METABOLISM BEGINS ▲

Other ribozymes catalyze metabolism—chains of chemical reactions that enable protocells to tap into nutrients from the environment.

From RNA world to bacteria