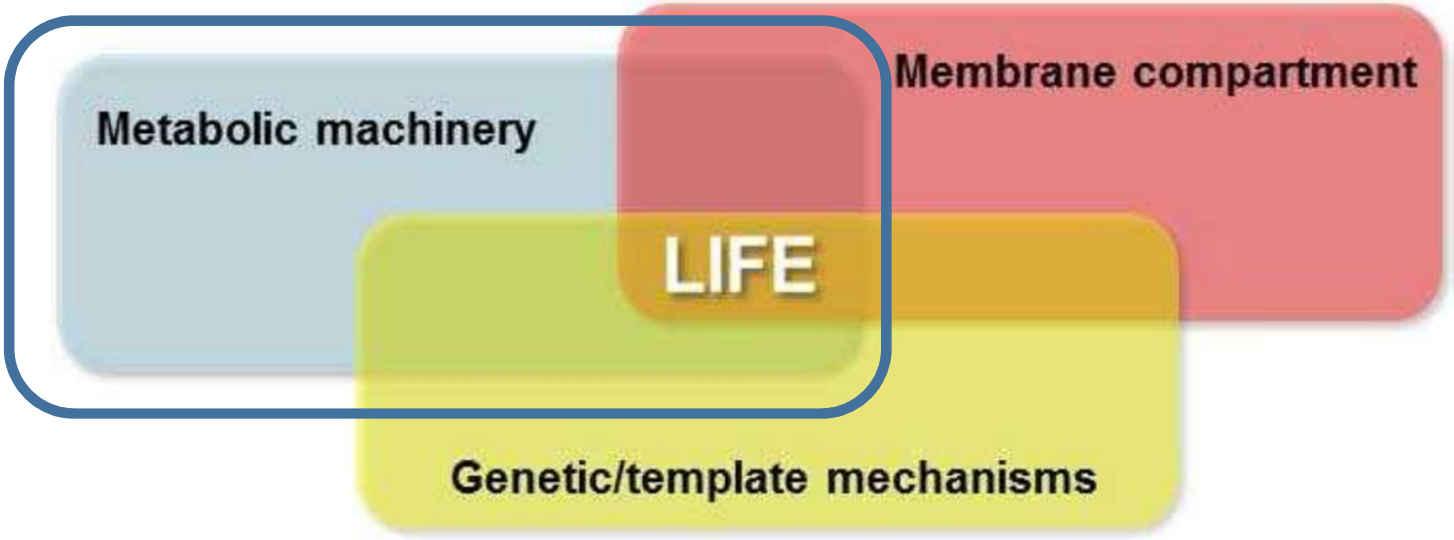
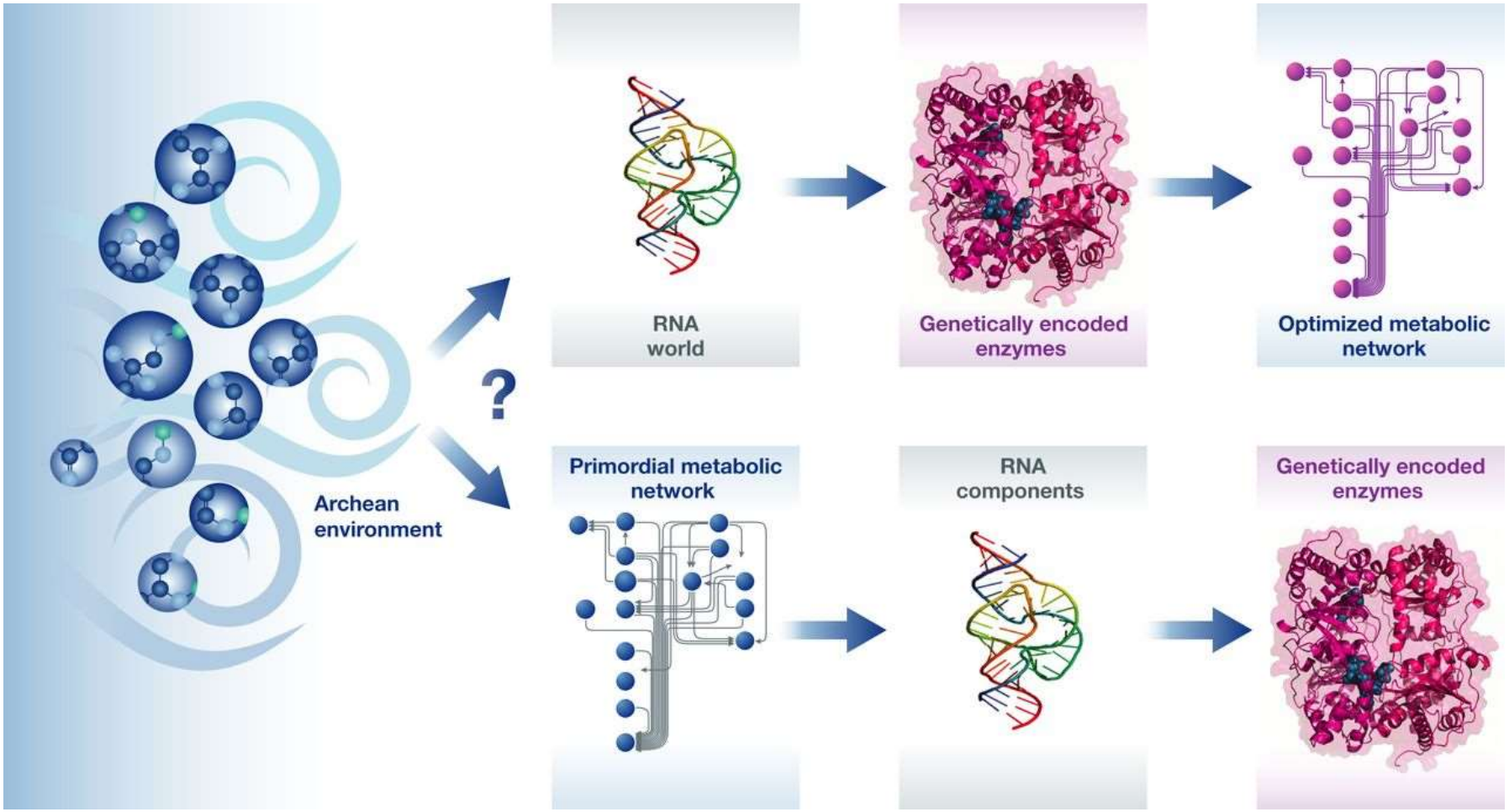

Metabolic machinery
Membrane compartment
LIFE
Genetic/template mechanisms

Route to life by chemical networks



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

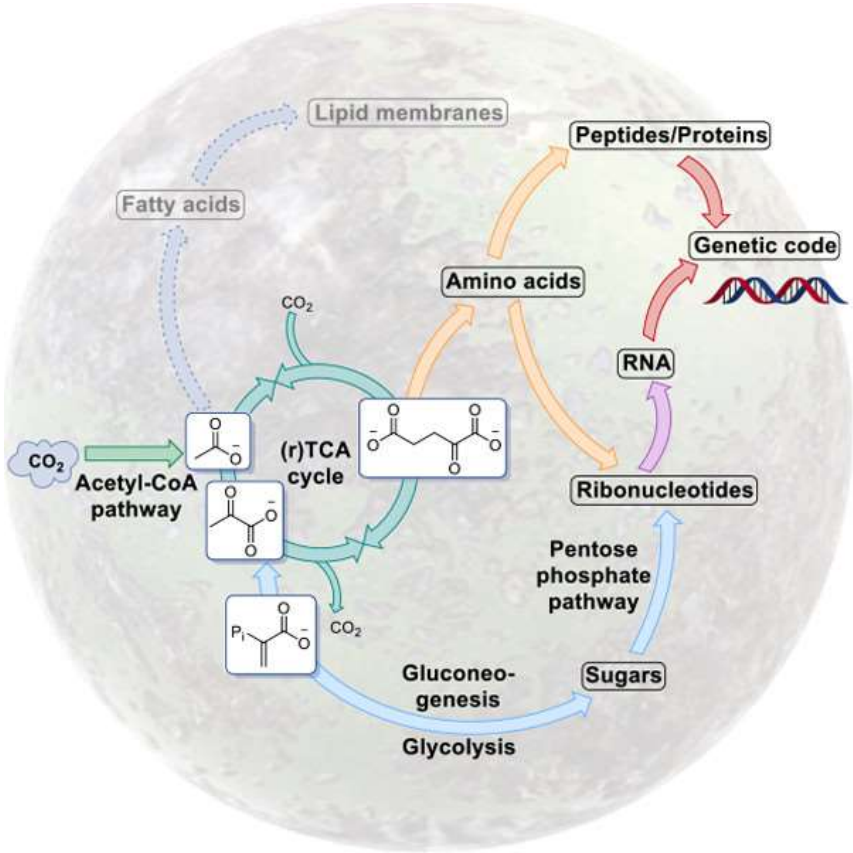
Metabolism-first vs. Genes-first

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.

Metabolism-first



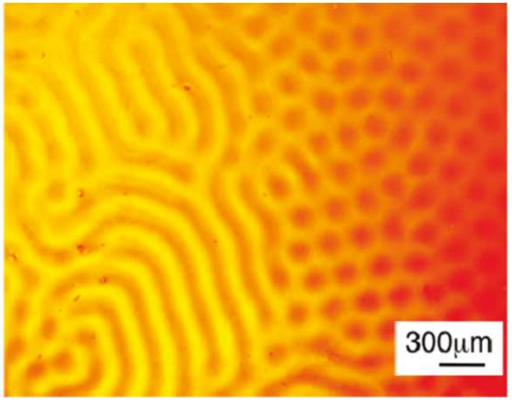
Protometabolism provides building blocks for construction of genetic systems

K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708–7744

Metabolism and self-organization of chemical networks

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

Life uses non-linearity (autocatalysis, oscillatory systems) 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.

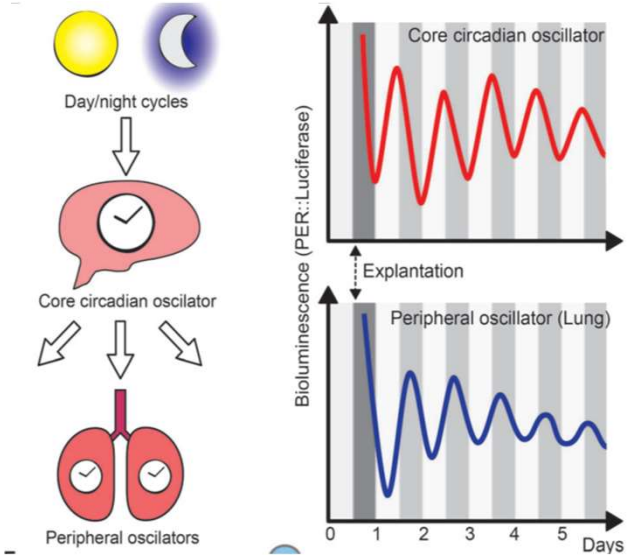
Metabolism and self-organization of chemical networks

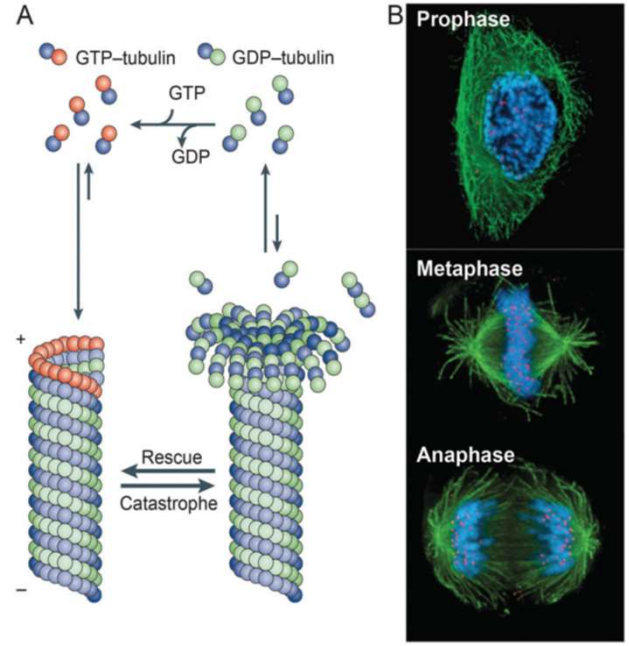
Oscilatory and autocatalytic processes are very common in biological systems. Examples include: metabolic cycles, day/night cycles, immune response, microtubule dynamics, or apoptosis.

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.

Simple oscillatory networks can also be designed.





R. Merindol, A. Walther *Chem. Soc. Rev.*, **2017**, *46*, 5588--5619

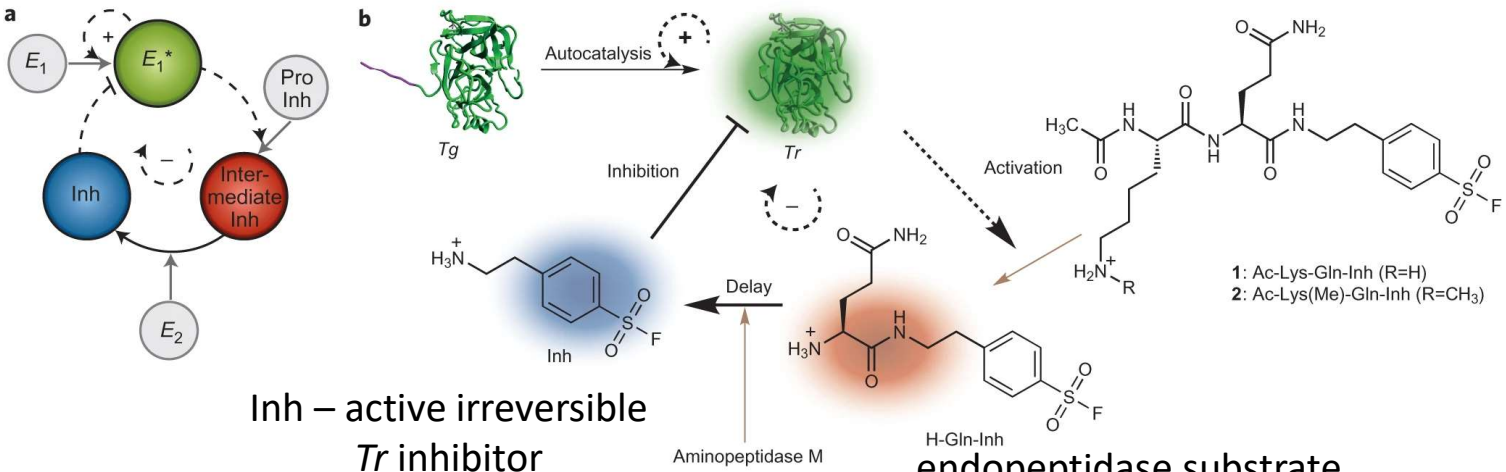
Enzymatic oscillator

a Enzymatic oscillator based on autocatalytic production and delayed inhibition of an enzyme. The combination of positive and negative feedbacks results in an oscillating system

trypsinogen (*Tg*)
enzymatically inactive

Trypsin (*Tr*)

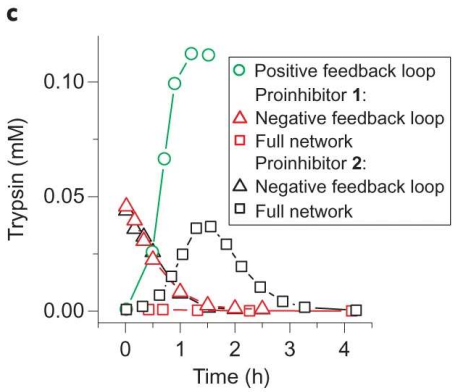
Tr cleaves the Lys residue of the proinhibitor

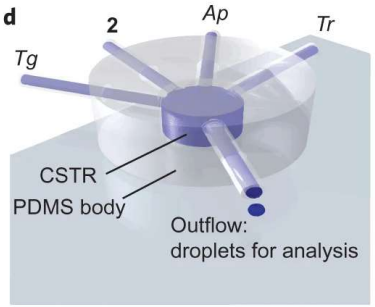


Inh – active irreversible *Tr* inhibitor

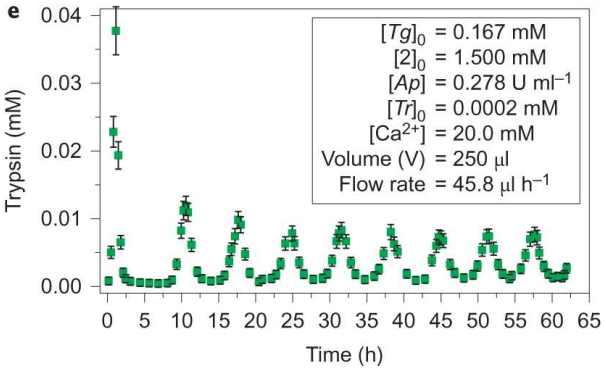
endopeptidase substrate

c, *Tr* from *Tg* (green), inhibition of *Tr* by Inh (red/black triangles) the complete network (red/black squares).





d, the flow reactor



e, [*Tr*] vs. time under flow conditions at 23 °C.

S. N. Semenov et al. *Nature Chem.* **2015**, *7*, 160–165

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

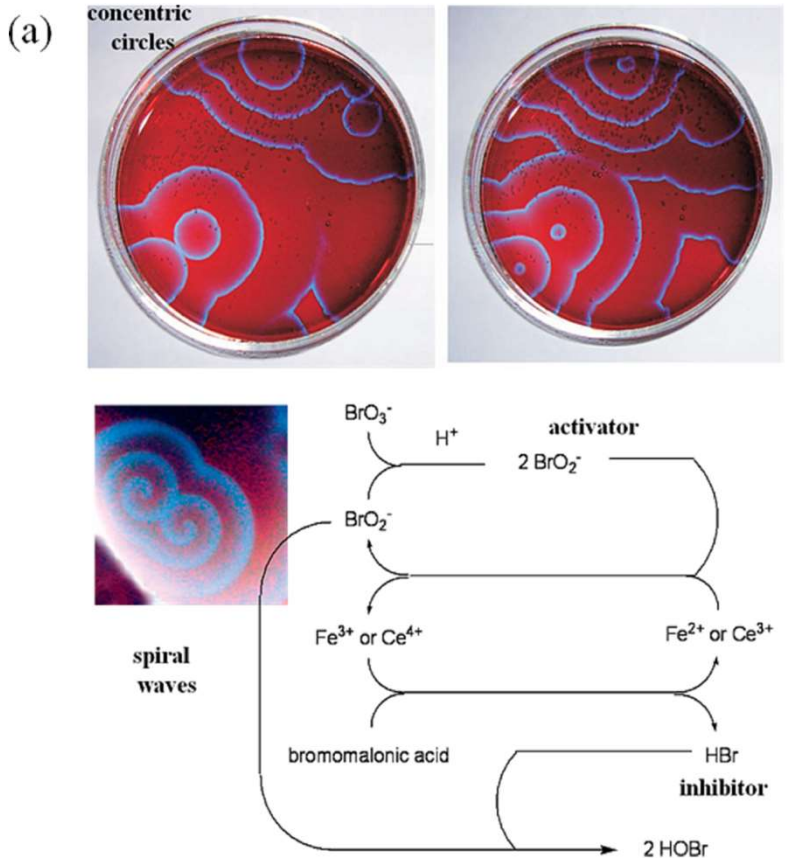
temperature, pH, humidity, illumination, UV irradiation, astronomic cycles

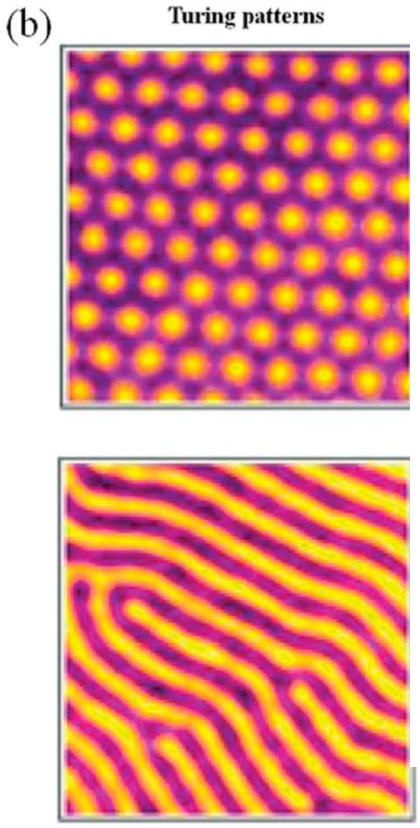
Chemical systems that mimic biological oscilations are studied as simple models

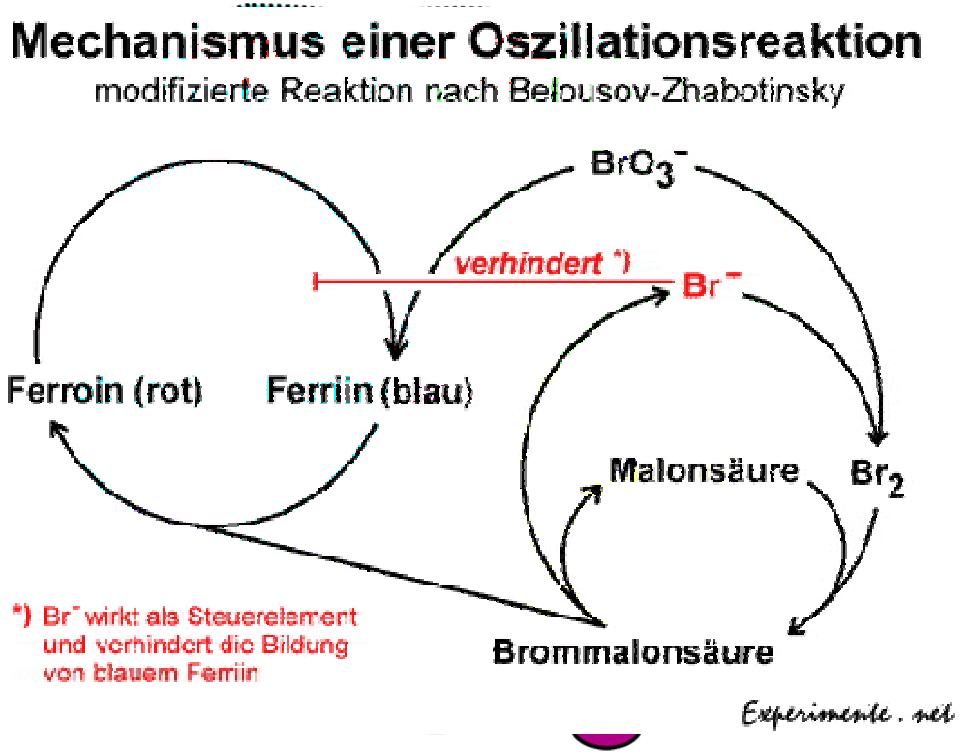
Belousov-Zhabotynski, CIMA reaction

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



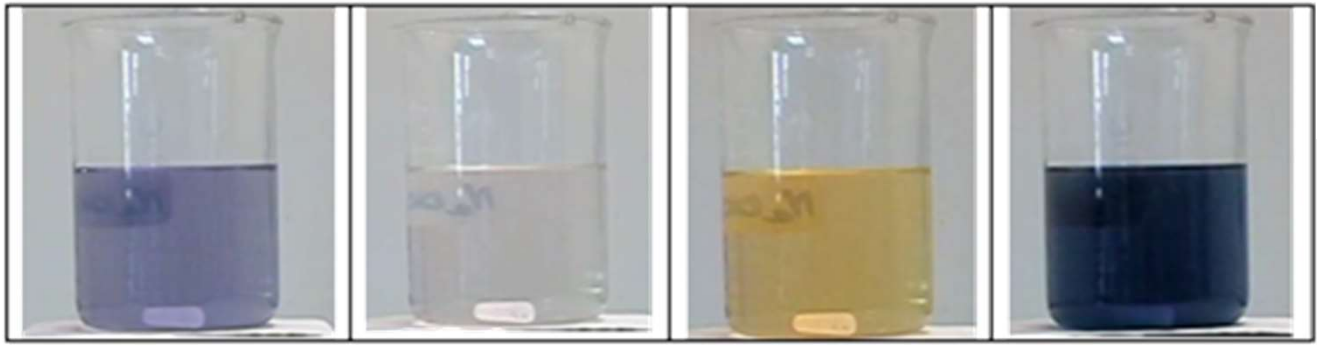




The reaction usually involves potassium bromate(VII) and malonic acid, optionally with cerium(IV) sulfate and citric acid. Ferroin is one of the common redox indicator

Briggs-Rauscher reaction

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

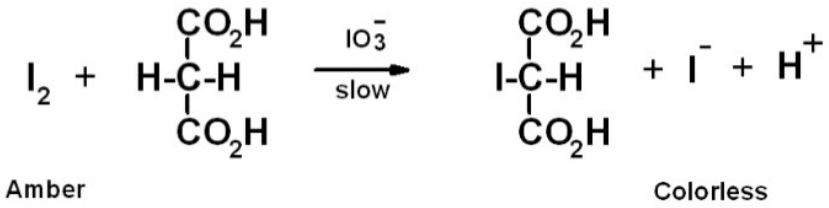


1. **The iodate ion is changed into iodine by hydrogen peroxide. The color changes to amber:**

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

Colorless — Amber

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



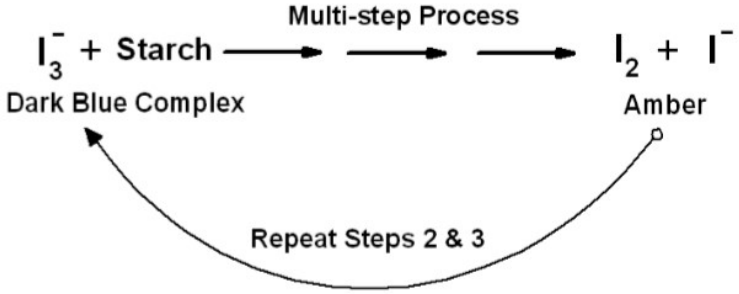
Amber — Colorless

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:**

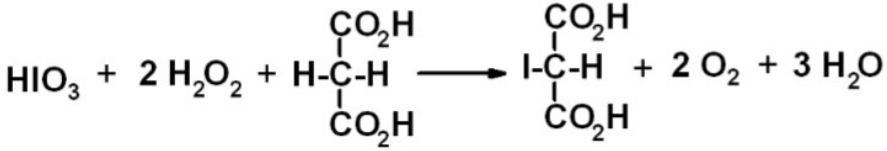
$$I_2 + I^- \xrightarrow[\text{fast}]{} I_3^- + \text{Starch} \xrightarrow[\text{fast}]{} \text{Dark Blue Complex}$$

Amber

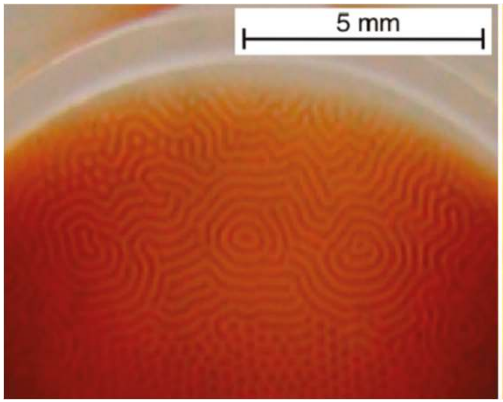
4. **After a period of time, the I_3^- ions are converted back into iodine and iodide ions, so the dark blue color disappears and the process repeats itself:**

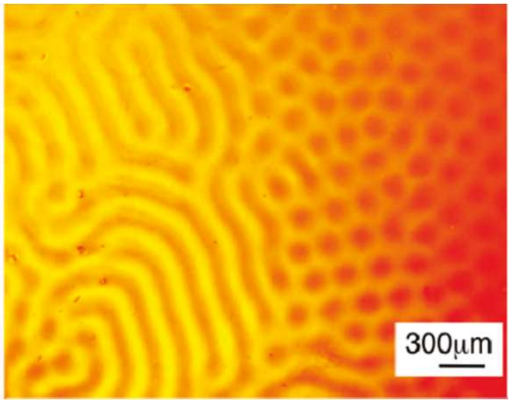


5. **Eventually the faster step 3 becomes dominant and the change of I_3^- back to iodine/iodide stops after about 15 cycles, so the solution remains dark blue. The overall chemical reaction is:**



Chlorite/iodide/malonic acid (CIMA) reaction





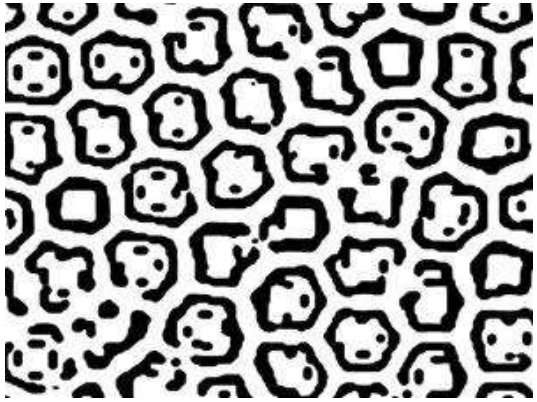
For the spontaneous generation of a Turing pattern, two intermediate species, an activator and an inhibitor, should be generated with the diffusion coefficient of the activator smaller than that of the inhibitor. The CIMA reaction that generates the activator, I^-, and inhibitor, ClO_2^-, was performed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

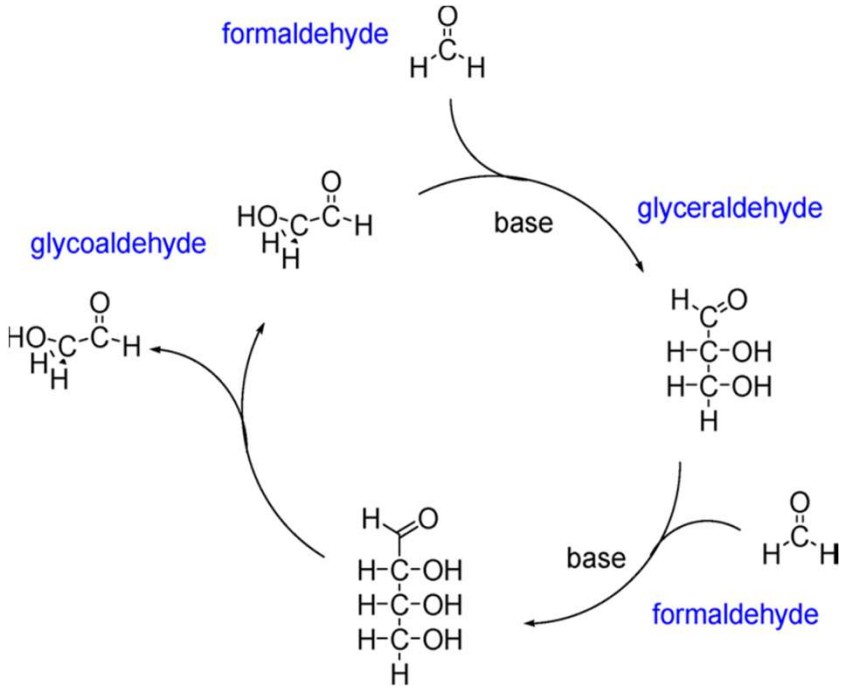
Explain the origin of homochirality

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

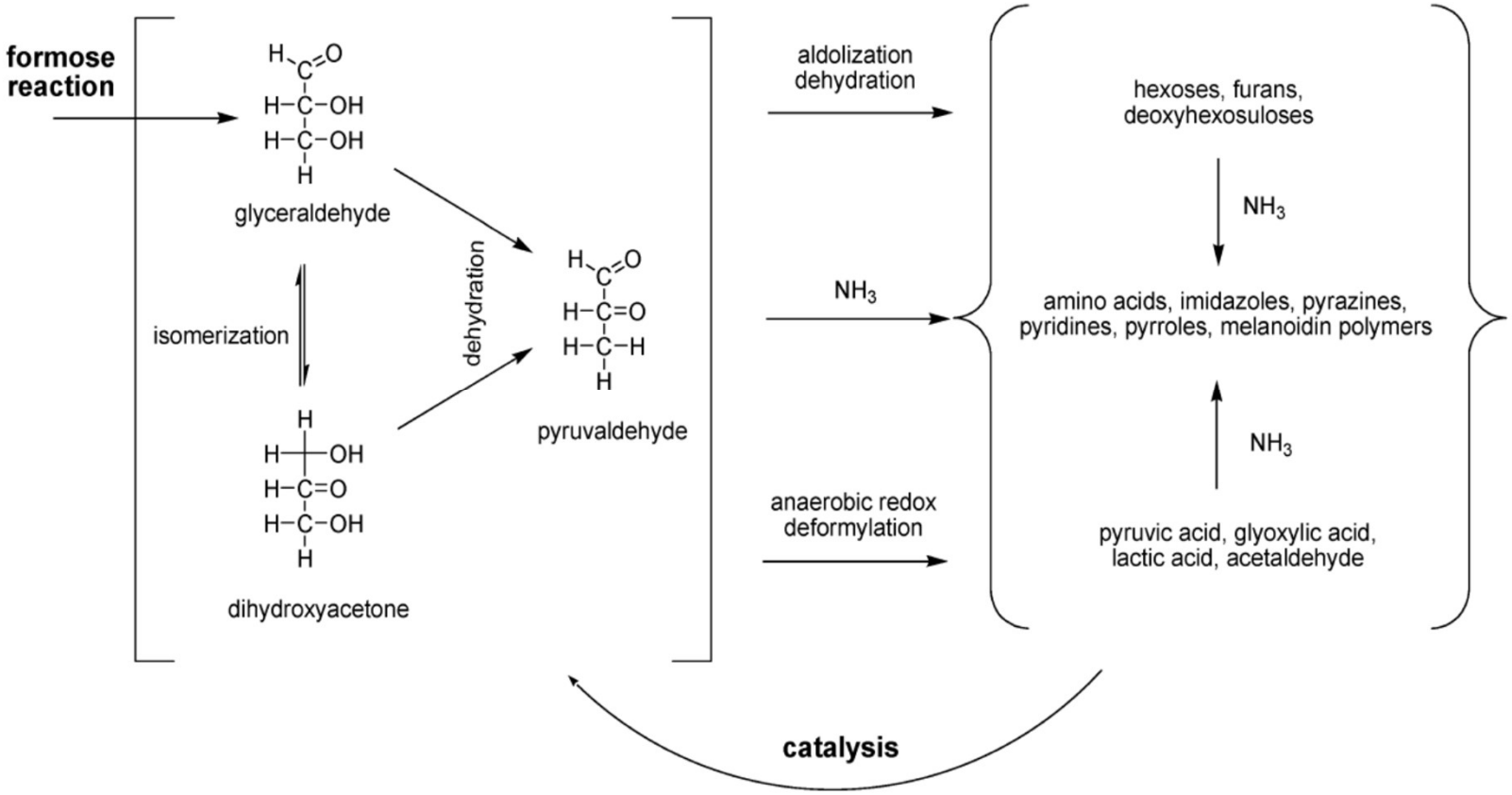


Formose reaction is one of the simplest autocatalytic cycles – two molecules of glycolaldehyde are produced from one.

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.



Origin of metabolism - hypotheses

Horowitz (1945) – retrograde evolution - an organism capable of Darwinian evolution already existed before the emergence of the metabolic pathway in question. The positive evolutionary selection pressure for this metabolite leads to the emergence of an enzyme catalyzing its synthesis from abiotially available substrates.

Granick – stepwise evolution - biochemical pathways became extended one step at a time; every intermediate was once a functional end-product; each innovation within a metabolic pathway produces useful metabolites.

Yčas and Jensen - biochemical pathways might have evolved from common ancestors that utilized promiscuous enzymes leading to different end-products.

Lazcano and Miller - patchwork assembly - Promiscuous enzymes catalyze multiple reactions in multiple pathways, meaning that if a pool of such enzymes was available, certain enzymes might be recruited from existing pathways to help evolve a new one

Tawfik – integrated metabolite–enzyme co-evolution model - side products originating from either the activity of promiscuous enzymes or from nonenzymatic reactions ("underground metabolism") provide evolutionary stepping stones for the emergence of specialized enzymes that make these products. Nonenzymatic reactions are likely to have helped new enzymatic pathways emerge both at the origin of life and at later evolutionary stages. New pathways emerge from pre-existing enzyme-free transformations → simultaneous invention of multiple new enzymes is no longer required. **"one enzyme at a time**" - the enzyme that catalyzes the rate-determining step emerges first, thus providing the biggest advantage.

K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

Protometabolic pathways

At the earliest steps in the origin of life - **proto-metabolic pathways** that predated enzymatic biochemistry, and which would, by definition, have been **entirely nonenzymatic.**

The basic chemistry of biochemical pathways is older than the enzymes that catalyze it.

Enzymes began to emerge from the energy dissipating protometabolism to accelerate or increase specificity for reactions that benefit the network's persistence by channeling them away from nonproductive thermodynamic dead-ends

The metabolisms of all the organisms within an ecosystem: life reductively builds up its molecules from CO_2 (**anabolism**) and oxidatively breaks them back down to CO_2 again (**catabolism**), giving rise to the global biological carbon cycle.

The metabolisms of autotrophic organisms are models for early biochemistry because of their relative simplicity.
Autotrophs can use either chemical energy (chemoautotrophs) or light (photoautotrophs) as energy source.
Most biological and geological evidence support a later emergence of photosynthesis
→ **early life was very likely CO_2-fixing and chemotrophic**

K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

Protometabolic pathways

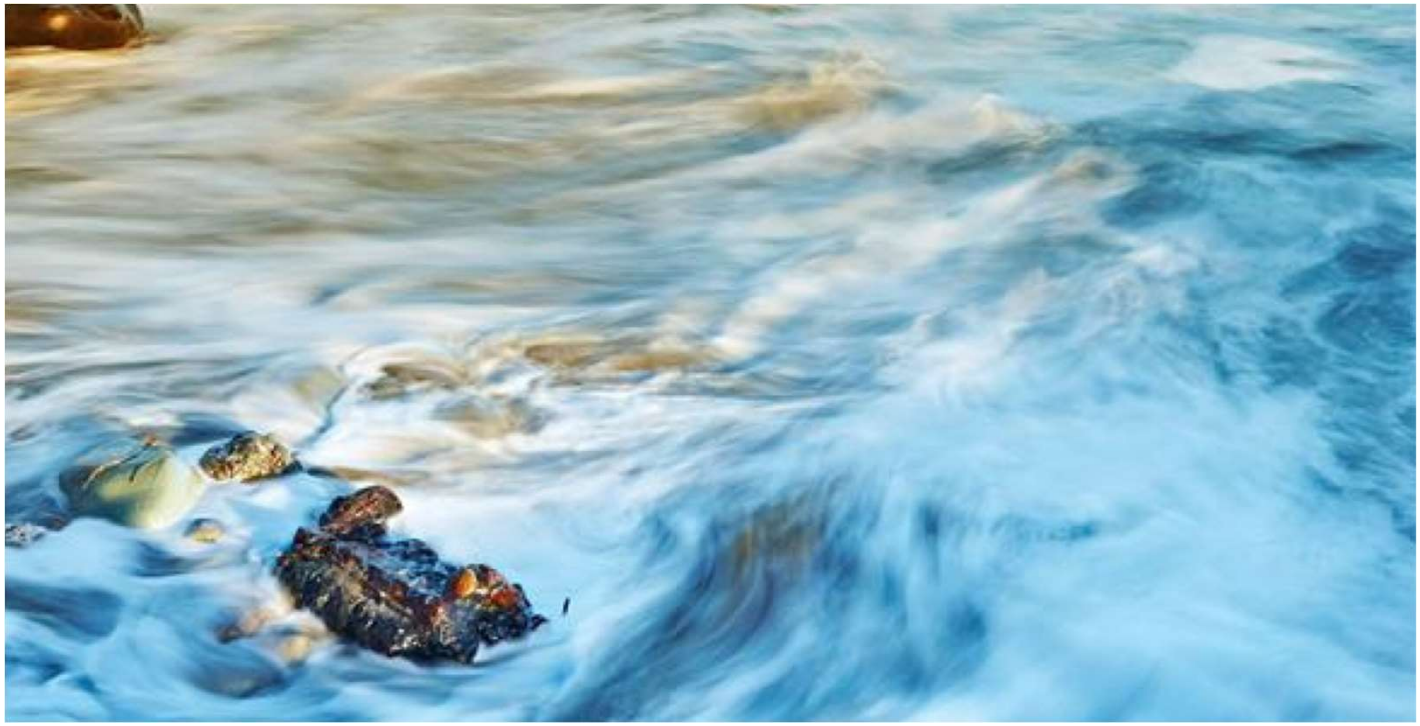
Living organisms always build their biochemistry from a small collection of carboxylic acids that can be interconverted to generate the five precursors to all other metabolic pathways:

(1) **acetate**, or acetyl when it is bound to a cofactor, is the biosynthetic precursor to lipids and terpenoids,
(2) **pyruvate** is the precursor to sugars and various amino acids,
(3) **oxaloacetate** is the precursor to various amino acids and pyrimidines,
(4) **succinate** is the precursor to various cofactors, and
(5) **α-ketoglutarate** is the precursor to various amino acids.

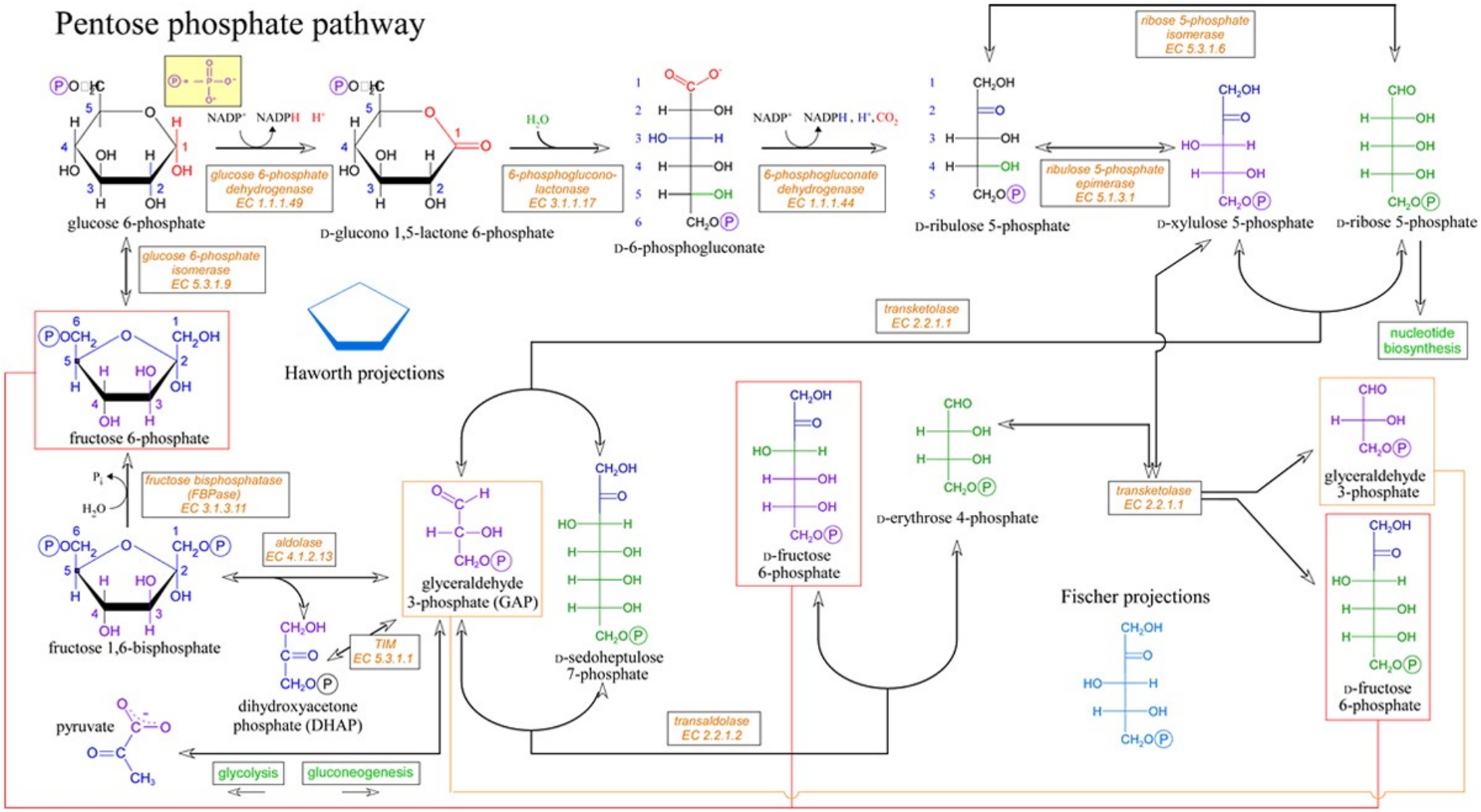
The central role of these compounds in building all life's chemistry suggests they were likely involved in prebiotic chemistry

K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

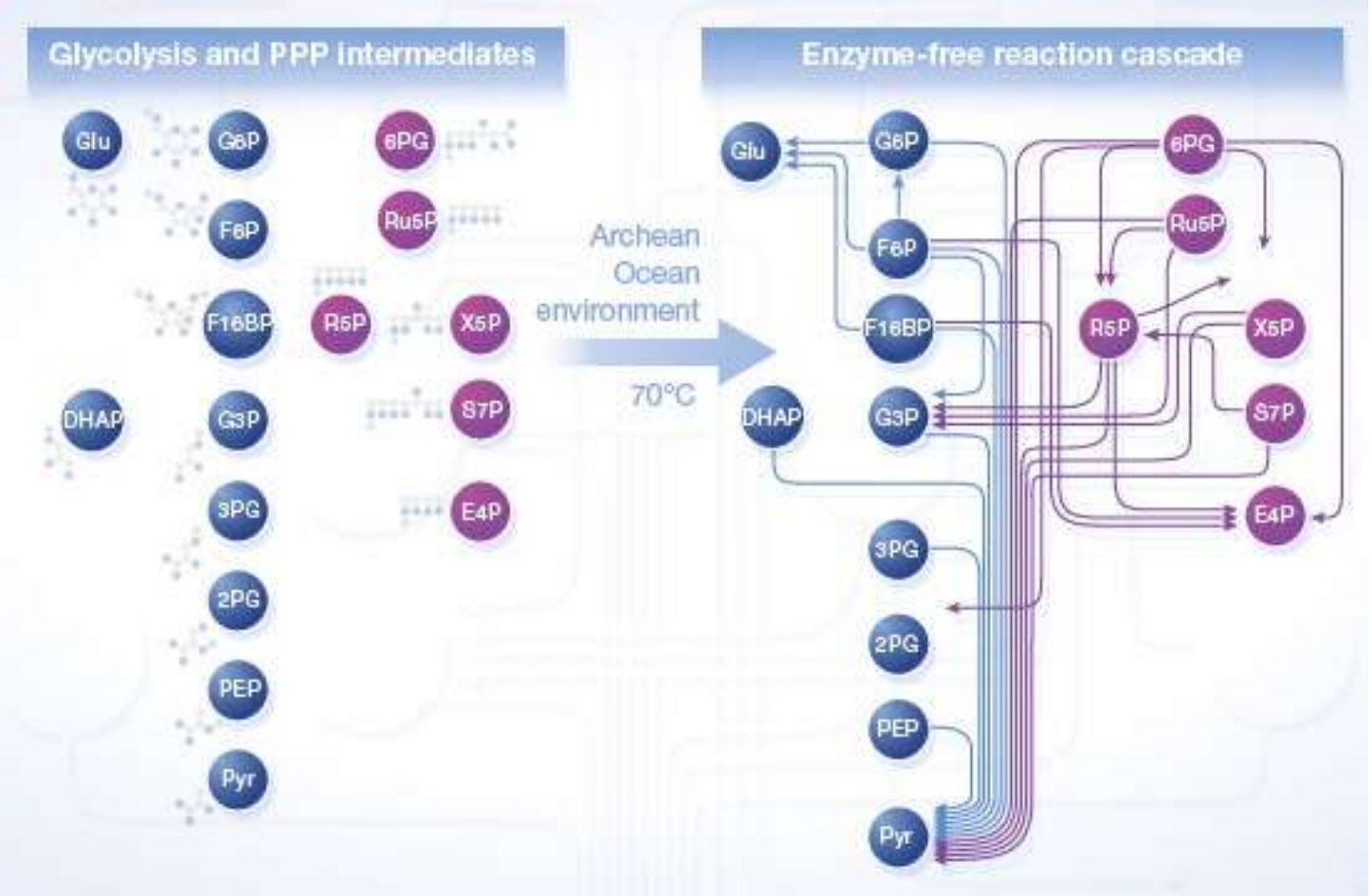
Metabolism may have started in our early oceans before the origin of life



Pentose phosphate pathway

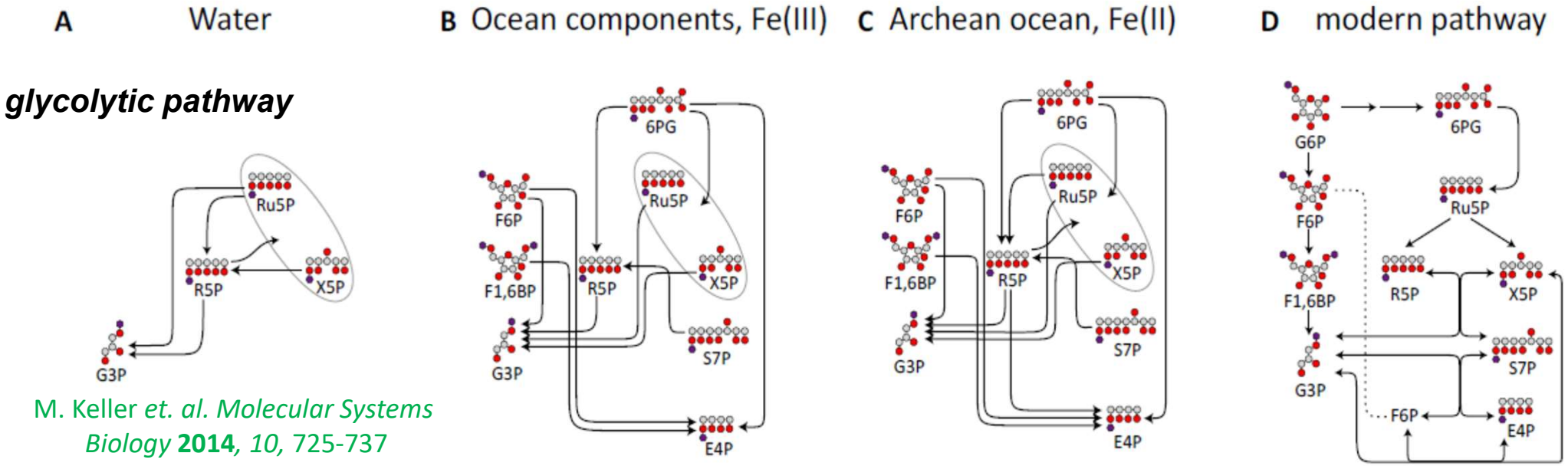


Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment



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.

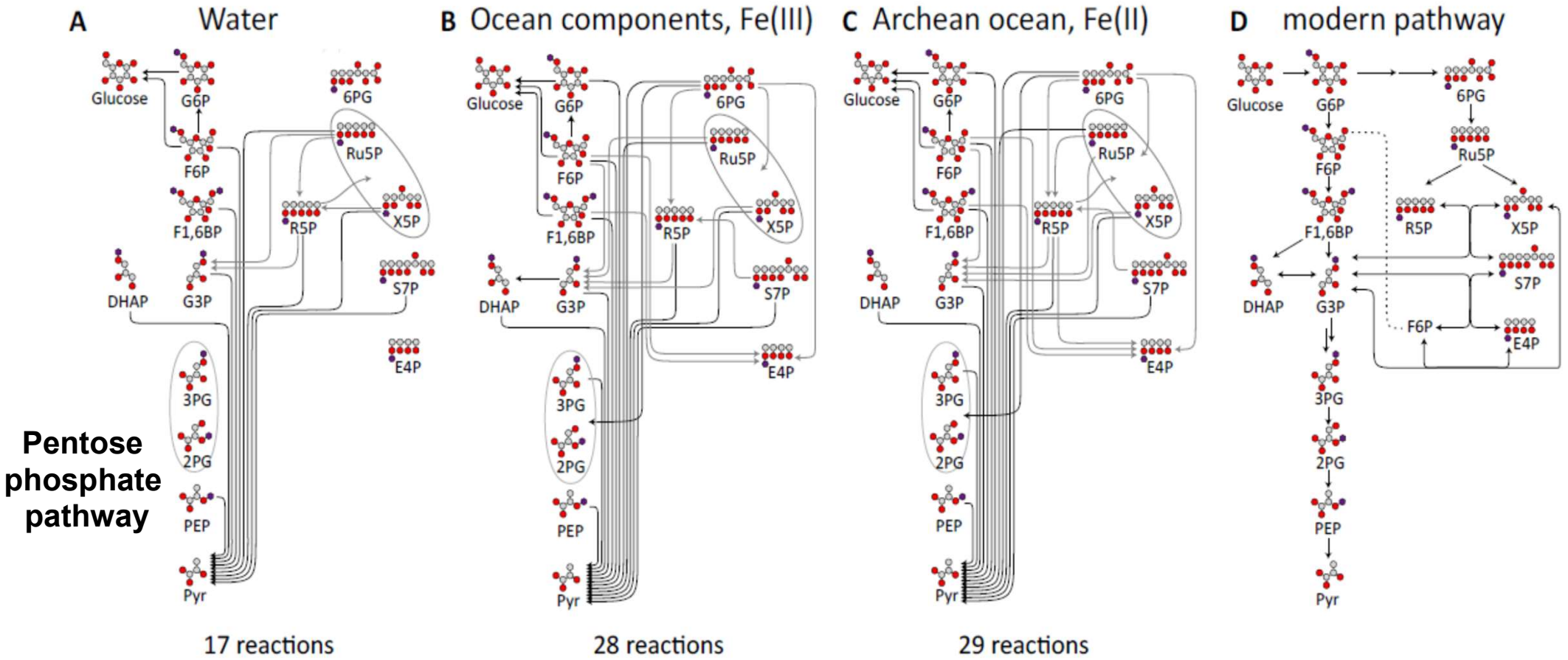
B The same reactions in solution with Fe^{III}, Co^{II}, Ni^{II}, Mo and phosphates simulating an Archean ocean.
In this milieu, 28 interconversion reactions among glycolytic and pentose phosphate pathway intermediates were observed.

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.

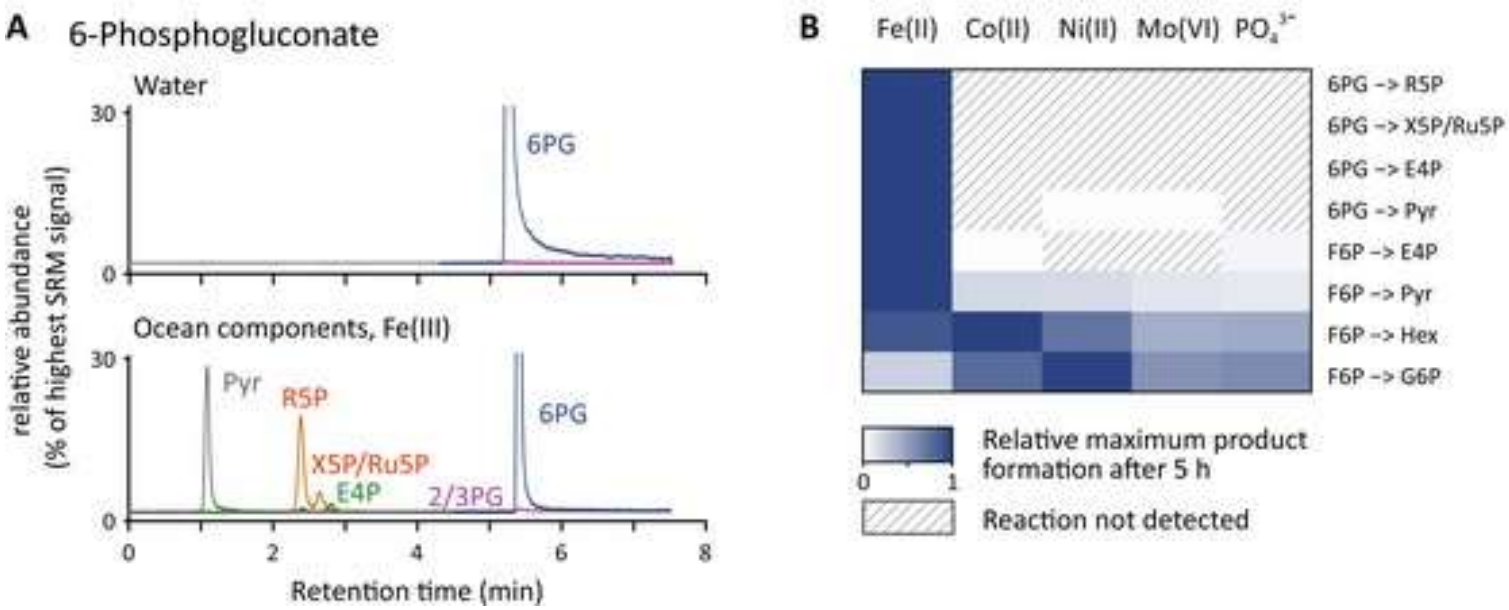
Pentose phosphate pathway: 6PG, 6-phosphogluconate; Ru5P, ribulose 5-phosphate; R5P, ribose 5-phosphate; X5P, xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; E4P, erythrose 4-phosphate.

Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment



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.

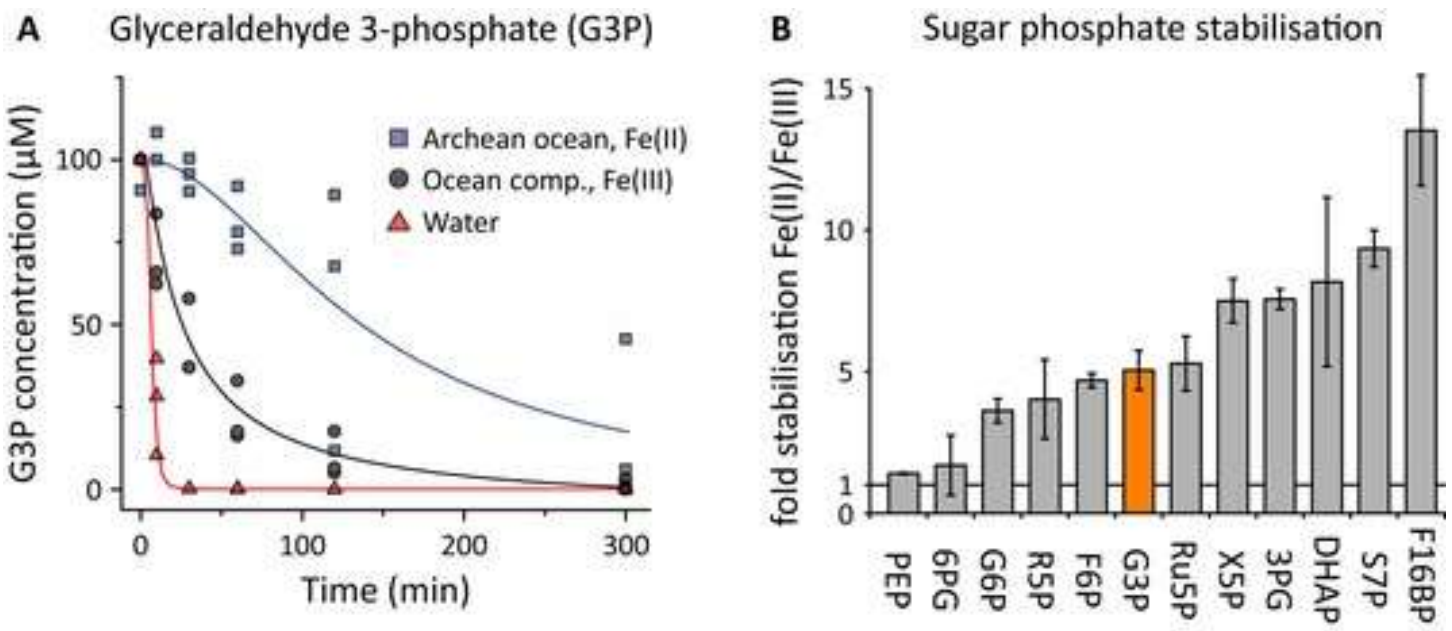
M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737



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.

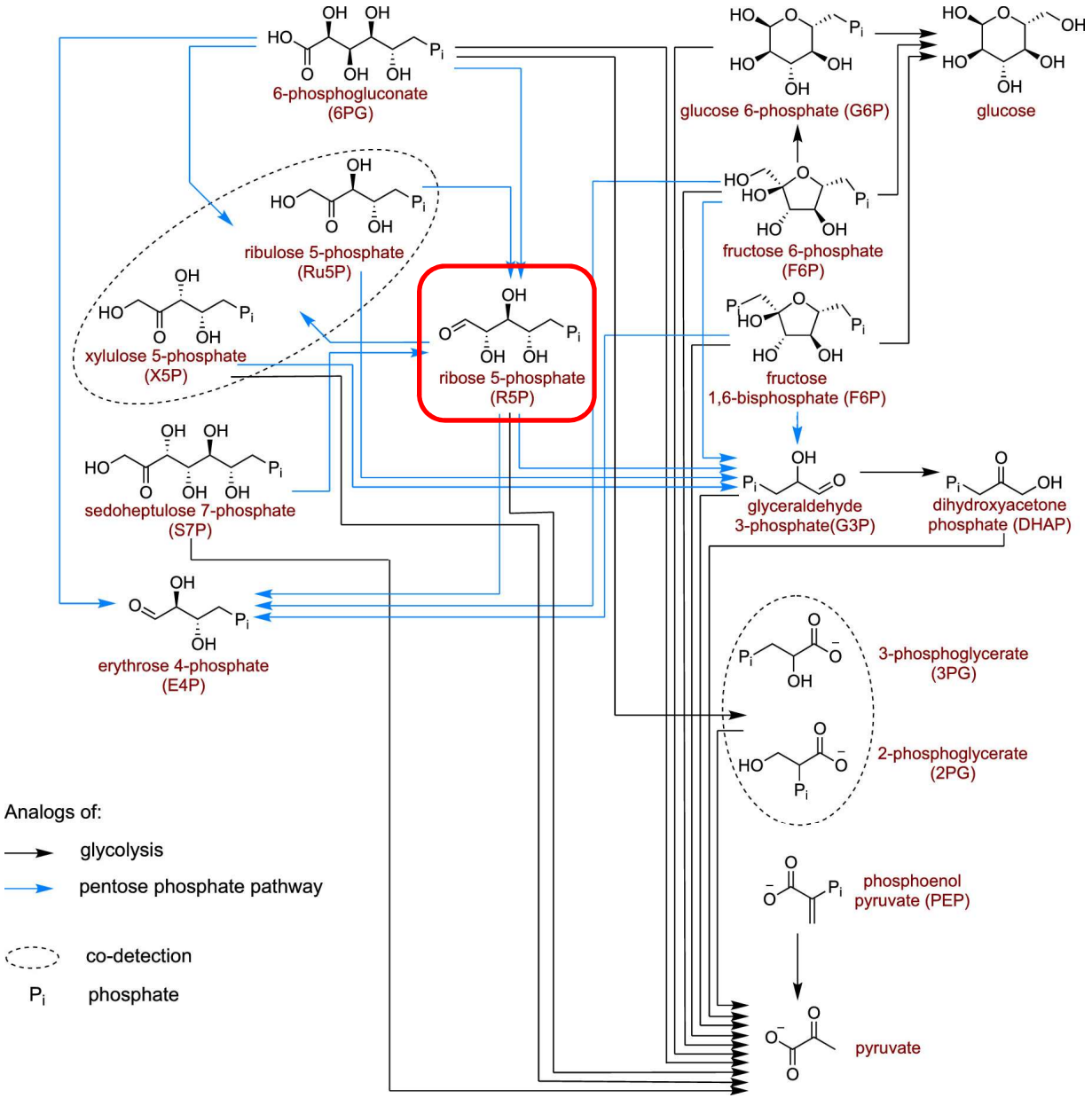
M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737



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

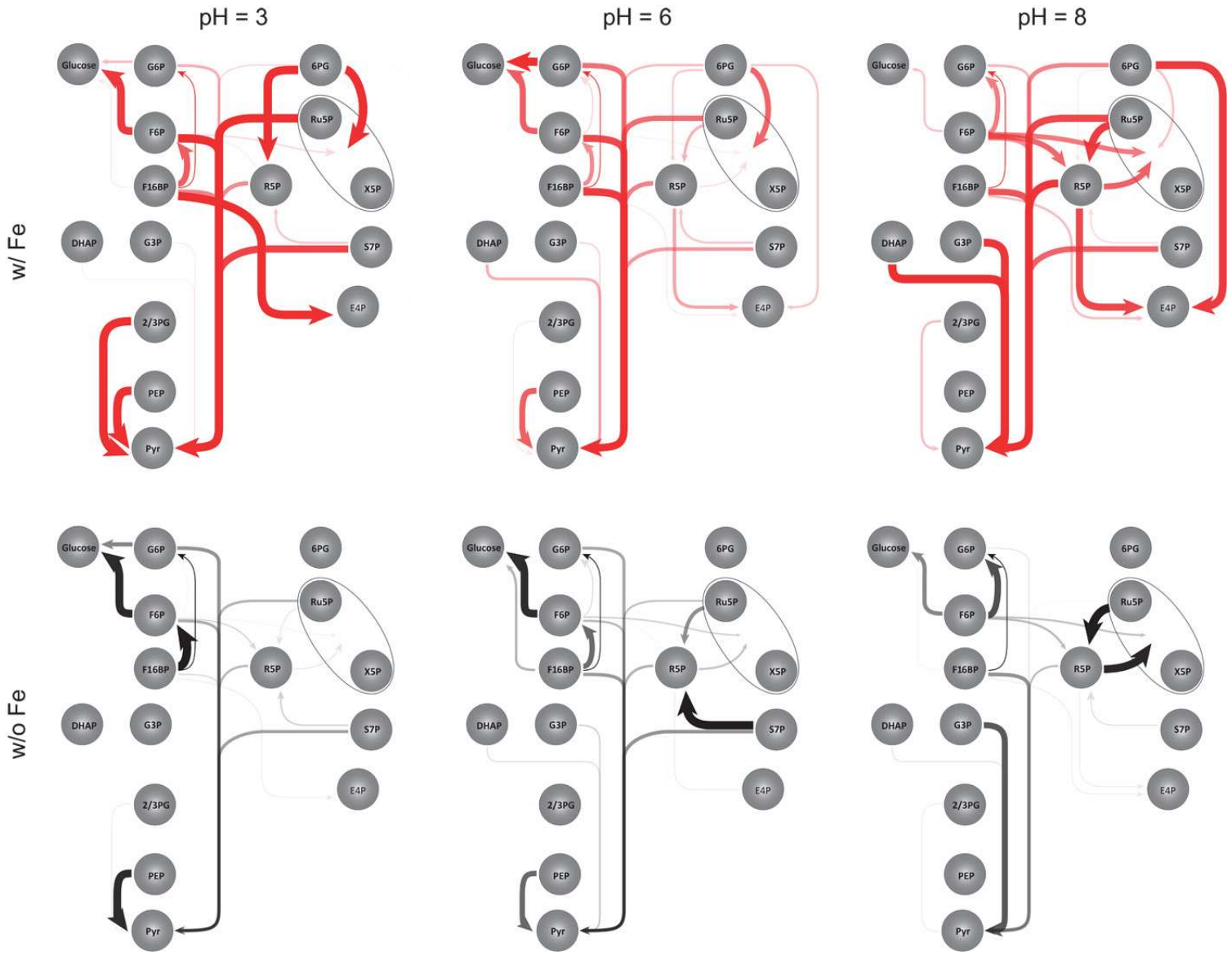


Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment

Ribose-5-phosphate is one of the products (81% selectivity of observed products at steady state at 20 ºC)

Fe^{2+}, 70 ºC, 5 h

M. Keller *et. al. Sci. Adv.* **2016**, *2*, e15012



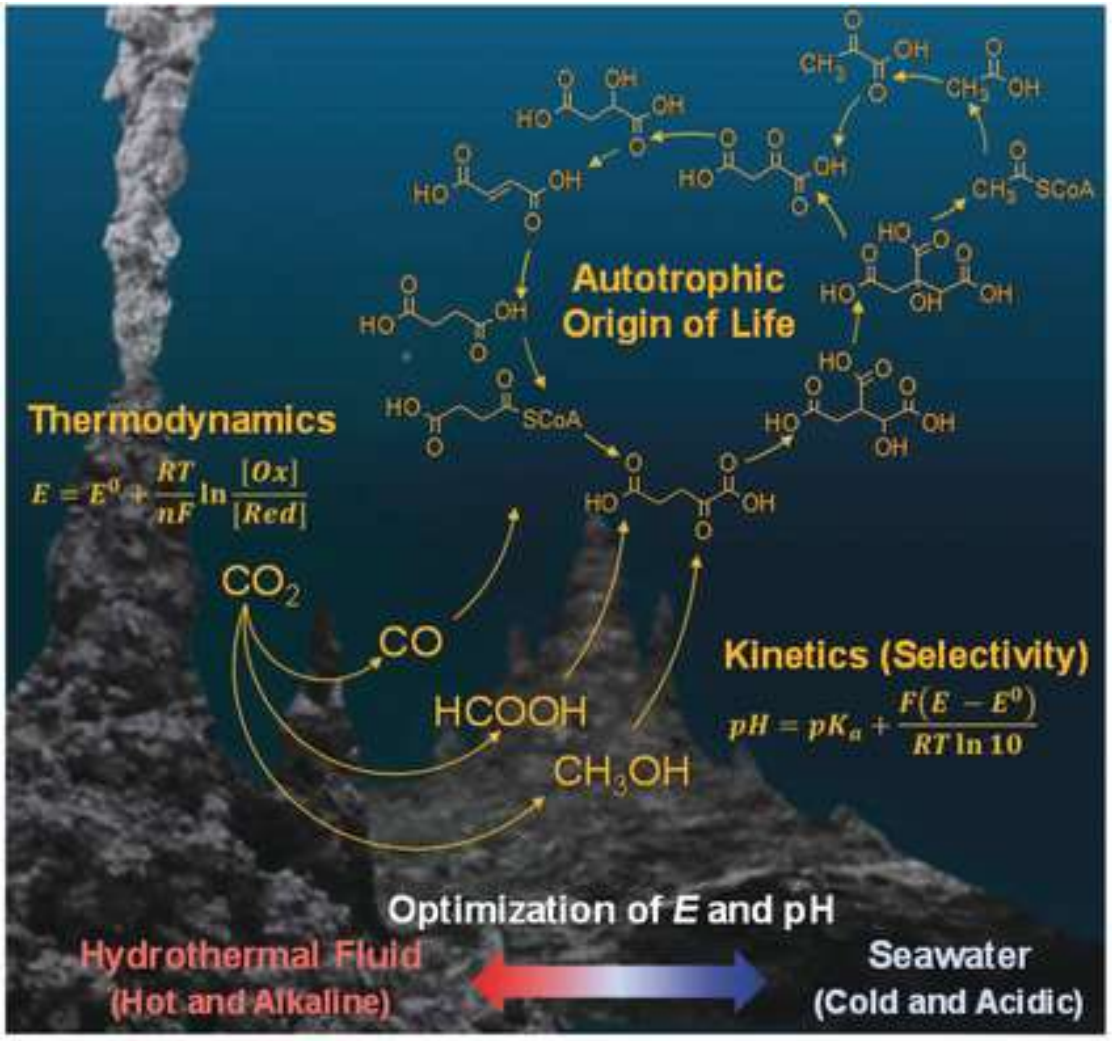
Iron and pH-dependent activity of a non-enzymatic glycolysis and PPP

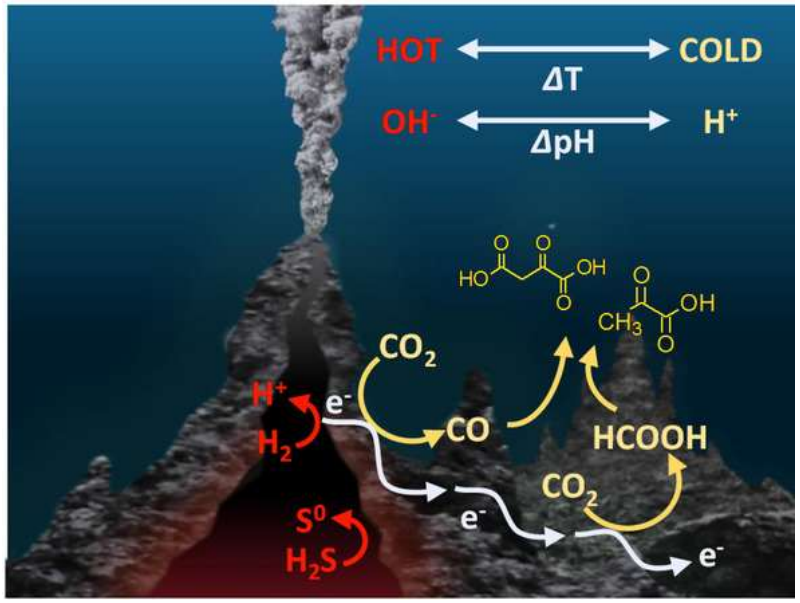
Reaction at pH 3, 6, and 8 and in the presence (top, red) and absence (bottom, black) of Fe(II).

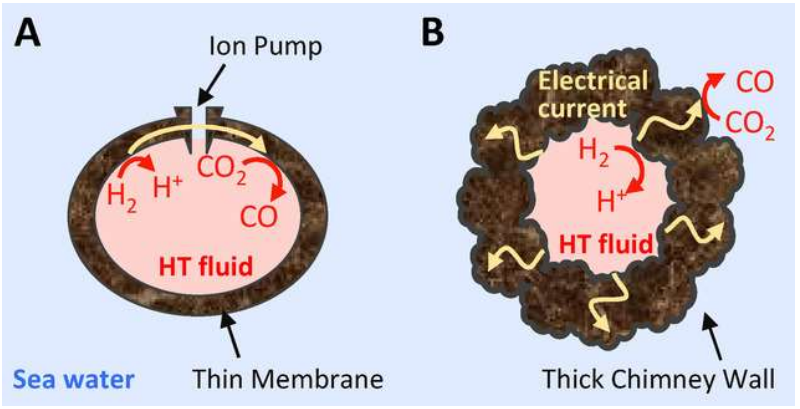
At **neutral** or slightly acidic pH, multistep reactions representing **glycolysis** are mostly active, whereas little reactivity in the PPP is observed. At **alkaline pH, the PPP** and lower glycolysis gain activity. **Strongly acidic** pH instead favors **pentose isomerization** and the formation of nonphosphorylated Glu and Pyr.

M. Keller *et. al. Sci. Adv.* **2016**, *2,* e15012

Non-enzymatic reactions corresponding to the core metabolism







H. Ooka, S. E. McGlynn, R. Nakamura, *ChemElectroChem* **2019**, *6*, 1316.

Carbon-metal bonds: rare and primordial in metabolism

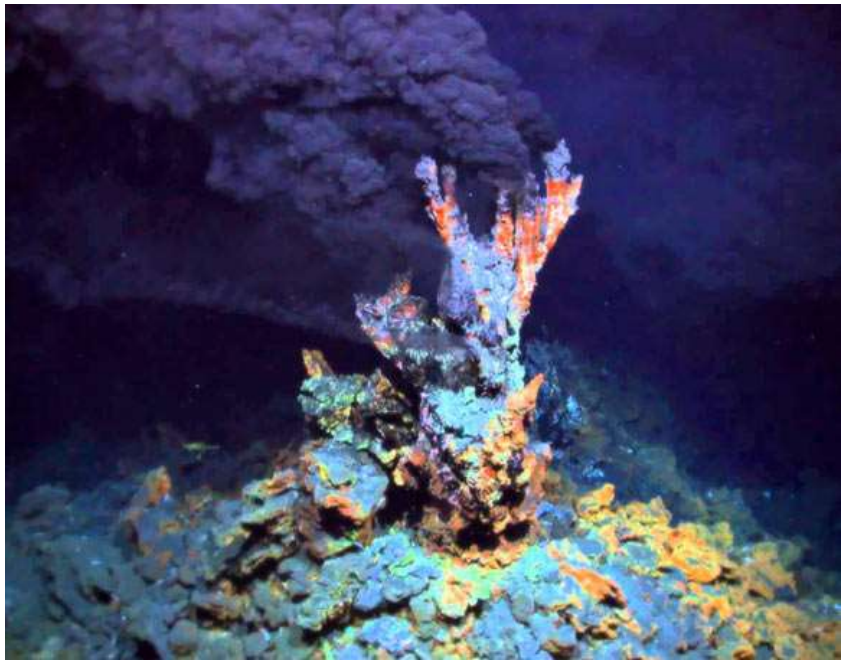
There is a clear record of geochemical origins preserved in metabolism

Submarine **hydrothermal vents** are rich in hydrogen (**H_2**), an ancient **source of electrons and chemical energy** for life (Serpentinization: $Fe + H_2O \rightarrow Fe_xO_y + H_2$).

Reactions involving **H_2 and CO_2** in hydrothermal systems **generate abiotic methane and formate**; these reactions resemble the core energy metabolism of methanogens and acetogens.
These organisms are strict anaerobic autotrophs that harness energy via H_2-dependent CO_2 reduction.

Native metals can also reduce CO_2 to formate and acetate in the laboratory.

The enzymes that channel H_2, CO_2, and N_2 into methanogen and acetogen metabolism are the backbone of the most ancient metabolic pathways. Their **active sites share carbon–metal** bonds which, although rare in biology, are conserved relics of primordial biochemistry present at the origin of life.



A deep-sea hydrothermal vent.

W. F. Martin *Trends Biochem. Sci.* **2019**, *44*, 807-818

Carbon-metal bonds: rare and primordial in metabolism

Catalysis mediated by organic cofactors, thioesters, and iron sulfide (FeS) clusters presumably has preceded enzymes in evolution.

Cofactors – often present in the catalytic core of enzymes, and perform the catalysis, while enzymes provide pre-organization, hydrophobic pocket and substrate recognition; **RNA-originating cofactors** had protein enzymes „grown" around them (RNA World).

Thioesters are central to metabolism, highly reactive and energetic (free energy of hydrolysis: thioester bond –43 kJ/mol, ATP –31 kJ/mol, ATP often generated from thioesters). Thus, thioesters might have preceded phosphates as energy currencies in evolution. Thioesters can be synthesized in the laboratory from CO and methyl sulfide in the presence of FeS.

FeS clusters are traditionally viewed as primitive catalysts in metabolism because they are completely inorganic and because metal sulfides would have been common on the early Earth.
Reduced FeS clusters are also a currency of chemical energy, similar to thioesters and ATP.

Enzymes with which autotrophic anaerobes access CO_2, N_2, and H_2 at the interface between the environment and biology can provide insights into the nature of primordial metabolism.

W. F. Martin *Trends Biochem. Sci.* **2019**, *44*, 807-818

Carbon-metal bonds: rare and primordial in metabolism

CO_2 was the starting point for biological carbon

Electrons to reduce CO_2:
Acetogens and methanogens – from inorganic sources: H_2, (H_2S, FeS,...)
Rest of autotrophes – from chlorophyll-based photosynthesis

Among modern microbes, there are six known pathways of biological CO_2 fixation, Among those six, the **acetyl-CoA pathway** (the Wood-Ljungdahl pathway) is the only one that occurs in both archaea and bacteria, thus probably the most ancient one. Yet, it seems to have evolved in two different ways: different C1 carriers and enzymes of the methyl synthesis branch (between bacteria and archea).
This pathway also generates ion gradients used by ATPases to produce ATP.

Currently, acetogenes and methanogenes use this pathway mainly for energy generation (only c.a. 5% of the fixed carbon stays in the cell mass, the rest is excreted as acetate or methane)

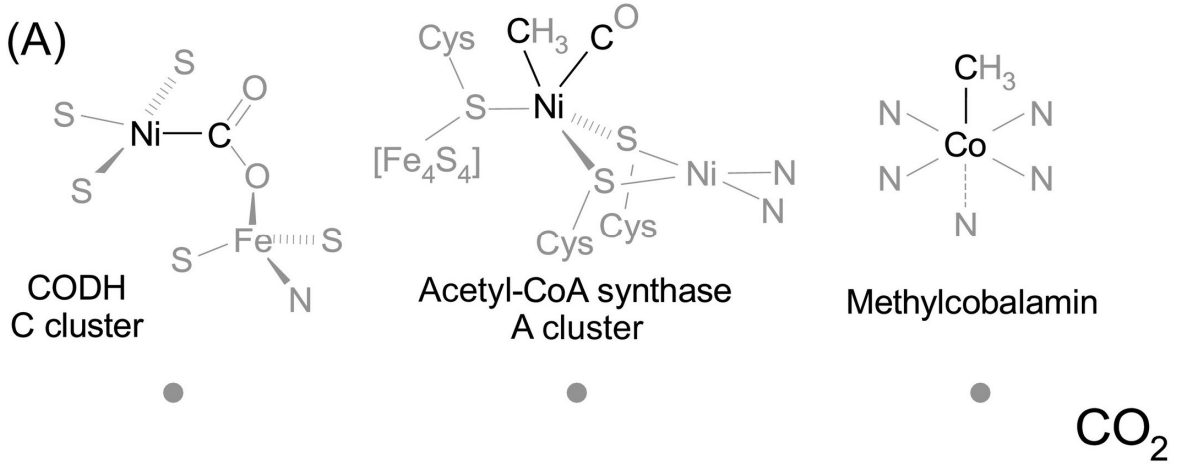
W. F. Martin *Trends Biochem. Sci.* **2019**, *44*, 807-818

Carbon-metal bonds: rare and primordial in metabolism

Three key enzymes of the W-L pathway have organometallic compounds as their active sites

CO dehydrogenase (CODH) synthesizes CO from CO_2
Acetyl-CoA synthase (ACS) condenses a methyl group and CO to an acetyl group that is covalently bound to the enzyme and removed by the thiol of coenzyme A → the energy-rich thioester acetyl-CoA
The methyl group is donated to ACS via methylcobalamin in the **corrinoid FeS protein, CoFeS**, which performs an unusual metal-to-metal methyl transfer reaction.

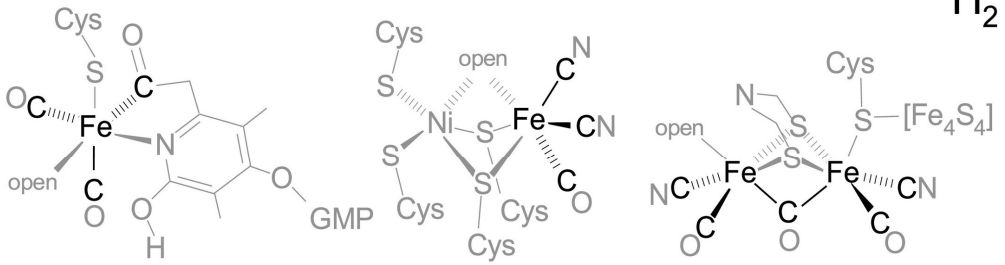
The acetyl-CoA pathway can even be emulated by reactions between native metals and CO_2 in laboratory experiments.



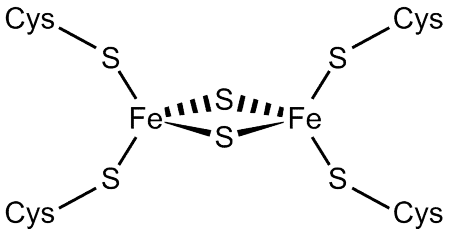
W. F. Martin *Trends Biochem. Sci.* **2019**, *44*, 807-818

Carbon-metal bonds: rare and primordial in metabolism

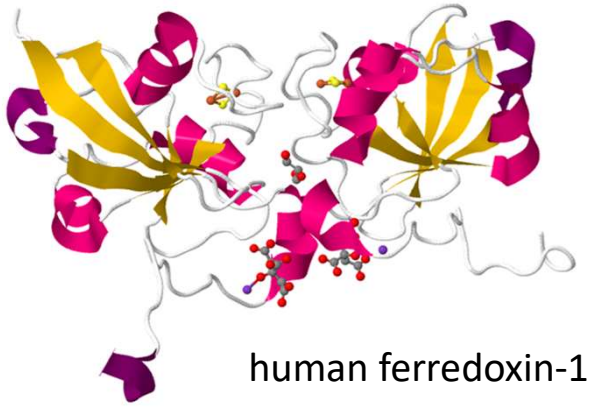
In cells that harness H_2 as a source of electrons for carbon and energy metabolism, electrons enter metabolism via **hydrogenases** and a soluble FeS protein called **ferredoxin**. The generation of reduced **ferredoxin** from H_2 is an energetic challenge in its own right and involves the process of flavin-based electron bifurcation, which is the most recently recognized mechanism of biological energy conservation.



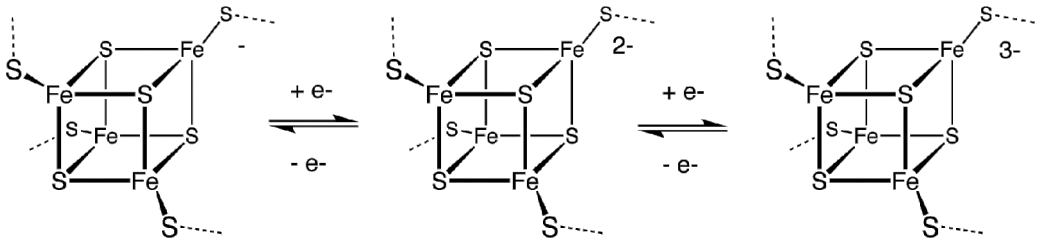
[Fe] Hydrogenase [Fe–Ni] Hydrogenase [Fe–Fe] Hydrogenase H cluster



Fe_2S_2 cluster



human ferredoxin-1



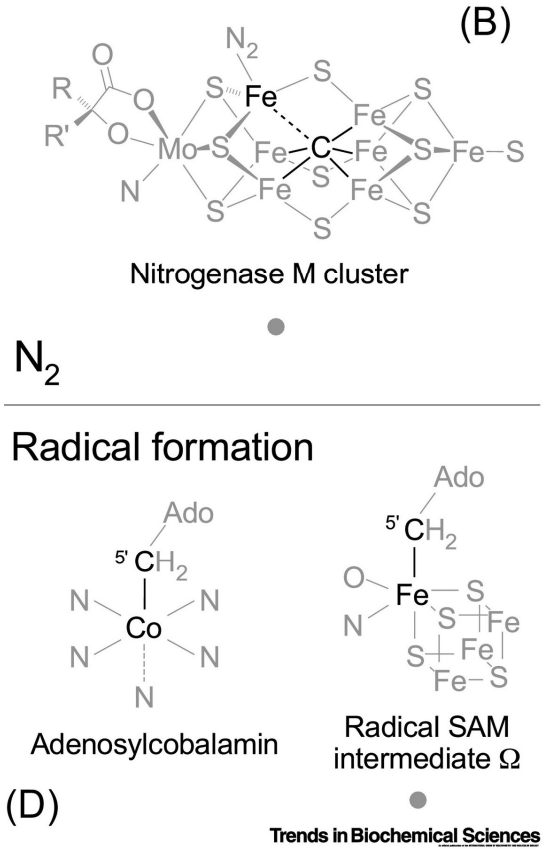
W. F. Martin *Trends Biochem. Sci.* **2019**, *44*, 807-818

redox reactions for Fe_4S_4 clusters

Carbon-metal bonds: rare and primordial in metabolism

Nitrogen fixation: **nitrogenase**

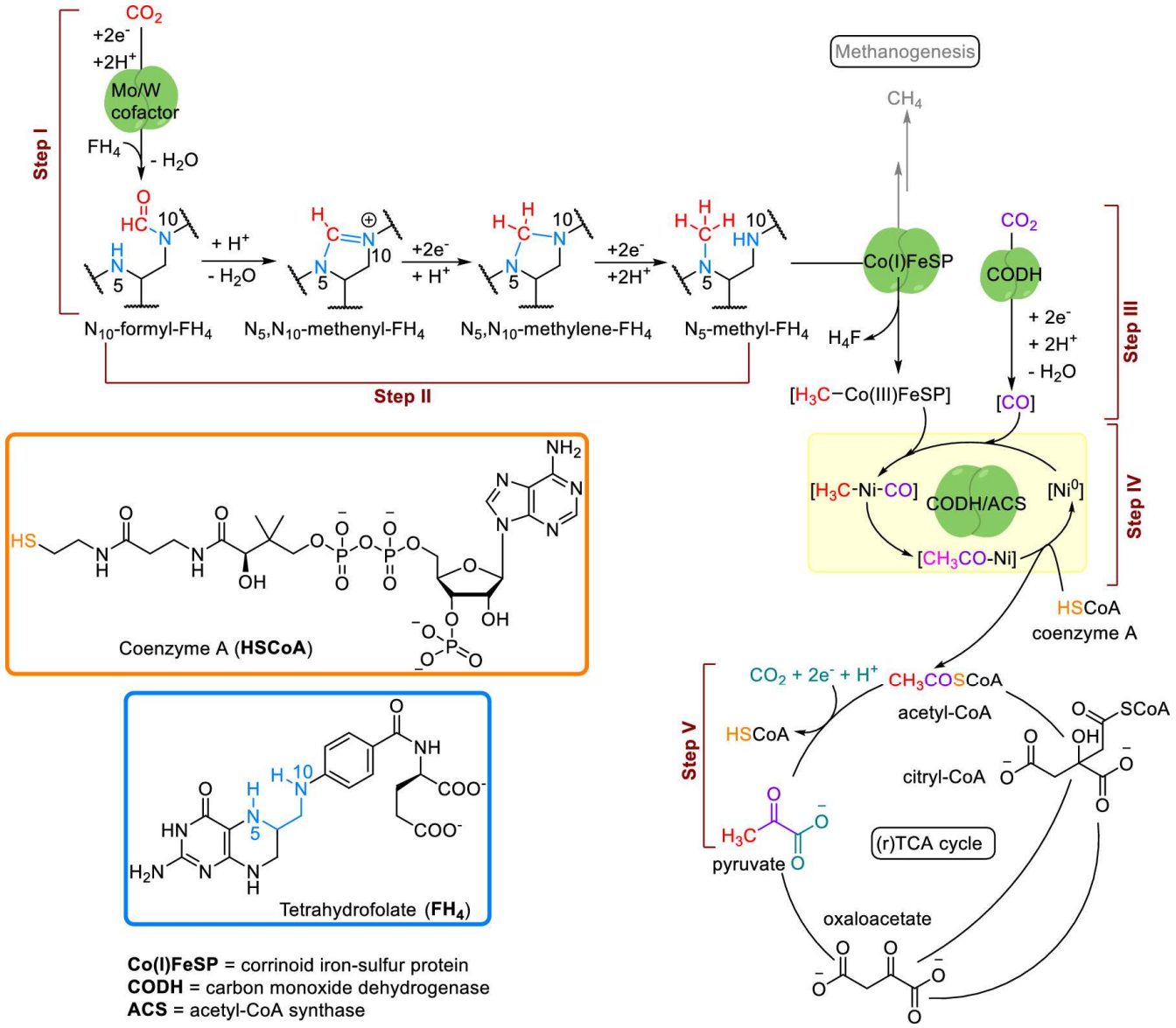
The center of the **nitrogenase** active site harbors a carbide carbon atom that is complexed by iron - the only biological carbide described to date. Its insertion starts from the methyl group of SAM. It is the biological model for the Haber–Bosch process, it also catalyzes industrially relevant reactions involving carbon, e.g. Fischer–Tropsch reactions, as well as reactions of various organic and inorganic nitrogen compounds. Nitrogenase accepts electrons from low potential ferredoxin,



Radical formation: **adenosylcobalamin** and **SAM-dependent enzymes**

Adenosylcobalamin and iron–carbon bond in the **radical SAM** reaction intermediate Ω are **cofactors** involved in **generating radicals** for enzymes that catalyze reactions with a radical-dependent mechanism, including reactions that are particularly common in cofactor biosynthesis.

W. F. Martin *Trends Biochem. Sci.* **2019**, *44*, 807-818



Protometabolic pathways- acetyl-CoA pathway

Autotrophic organisms build themselves from CO_2.

There are only six known CO_2 fixation pathways used by autotrophs. One of these, the Calvin cycle, is related to photosynthesis, which is thought to be a later development.

Chemoautotrophs use at least one of the other five pathways. Of these five, the simplest and most ancient is the **acetyl-CoA pathway**, which is short and linear and produces two of the universal precursors (**acetate** and **pyruvate**).

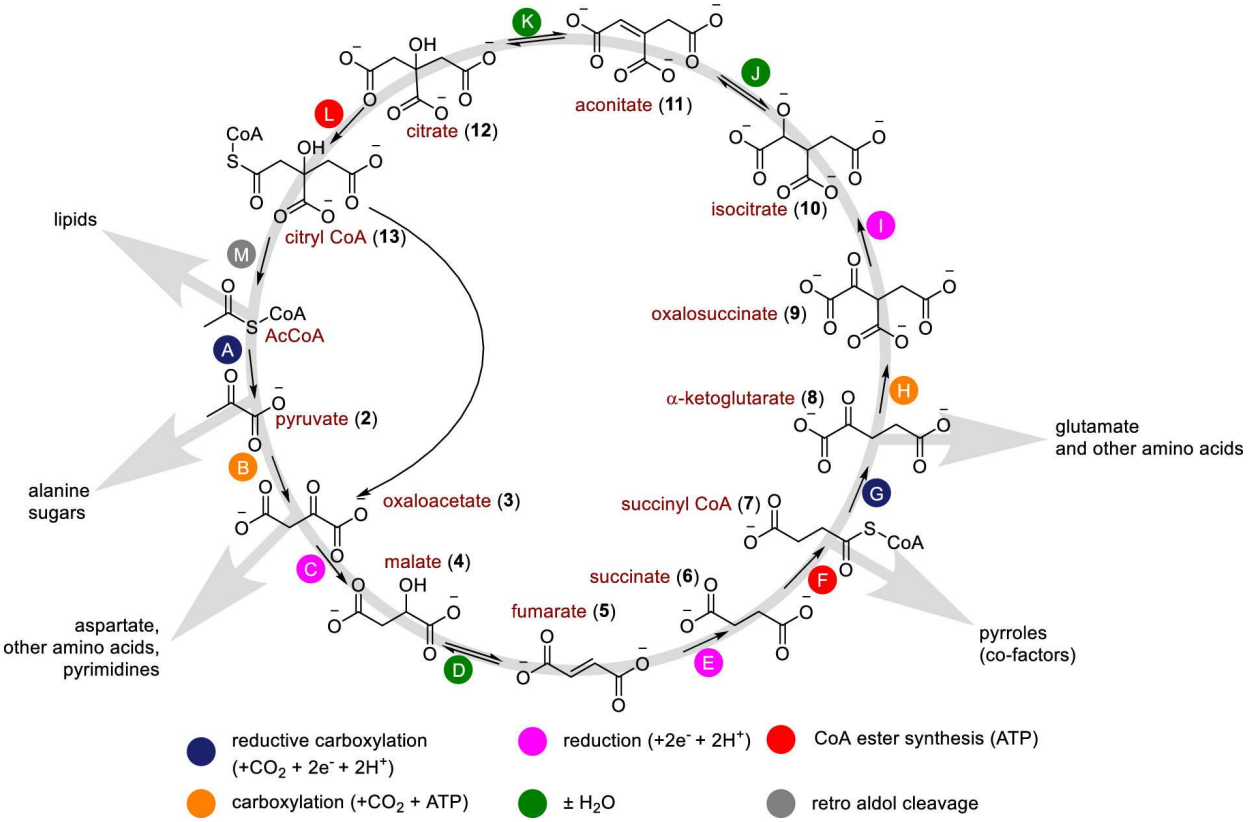
K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

Protometabolic pathways

The remaining four anabolic pathways:
the rTCA cycle,
the 3-hydroxypropionate bicycle,
the dicarboxylate-hydroxybutyrate cycle, and
the 3-hydroxypropionate-4-hydroxybutyrate cycle
share many similarities. All four pathways are autocatalytic. They also all either contain the five universal metabolic precursors or make them from intermediates of the cycle by no more than two steps.
Thus, **the essential function of these four pathways is to generate the five universal precursors to metabolism**.

In contrast, carbon catabolism, with CO_2 as end-product, mostly converges to the oxidative TCA cycle or its parts, also providing the same universal metabolic precursors.

reverse tricarboxylic acid cycle (rTCA)



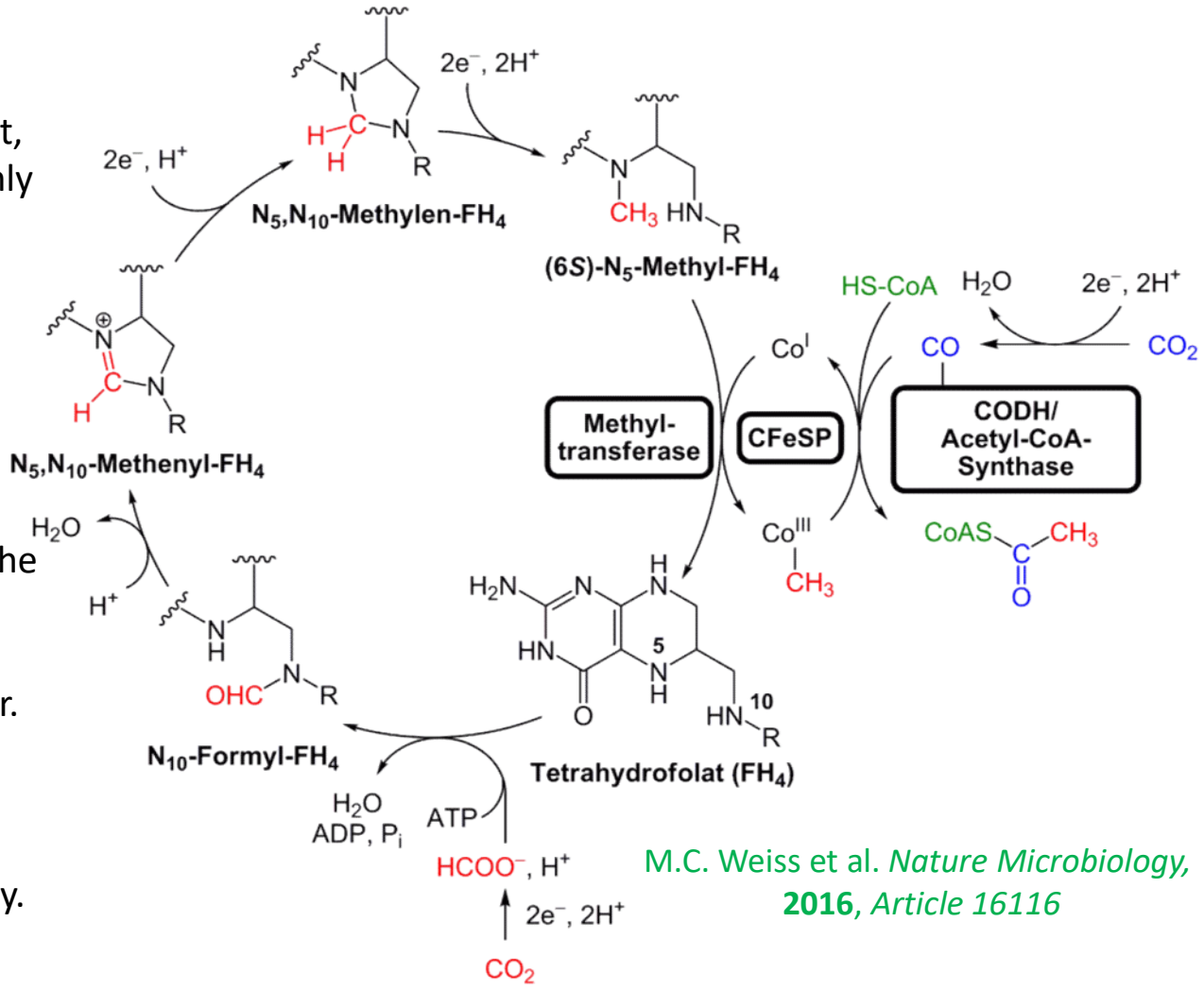
K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

Protometabolic pathways - Wood-Ljungdahl (WL) (acetyl-CoA) anaerobic carbon fixation

Of the six autotrophic CO2 fixation pathways, the acetyl-CoA pathway (also known as the Wood−Ljungdahl pathway) is the simplest, shortest, most dependent on transition metals and is the only pathway whose potential to generate ATP is equivalent to the amount of ATP it consumes.
It is the starting point for carbon and energy metabolism in ancient anaerobic organisms - the **acetogens** and the **methanogens.**

The overall function of the pathway is to produce acetyl CoA, the precursor to lipids, and pyruvate, the precursor to sugars and some amino acids.
This pathway is also unique among the six carbon fixation pathways because it is not cyclic, but linear.

For all of these reasons, it is thought to be the most ancient CO_2 fixation pathway in life and is speculated to have its origins in prebiotic chemistry.



M.C. Weiss et al. *Nature Microbiology,* **2016**, *Article 16116*

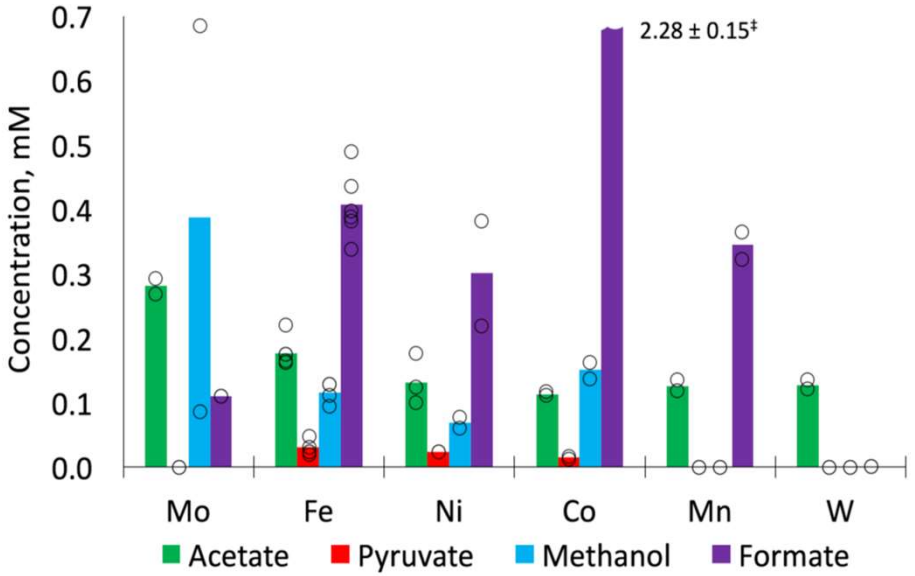
K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

Prebiotic WL analogue –native metals

how could primitive metabolic systems have fixed CO_2 before the origin of proteins?

Native transition metals (Fe^0, Ni^0 and Co^0) selectively reduce CO_2 to acetate and pyruvate—the intermediates and end-products of the AcCoA pathway—in near millimolar concentrations in water over hours to days using 1–40 bar CO_2 and at temperatures from 30 to 100 °C.

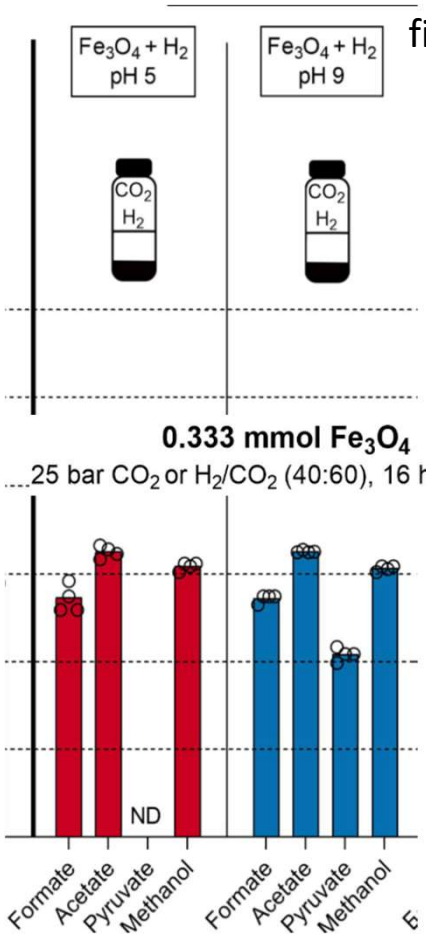
100 ºC, 35 bar, 16 h
1 mmol/mL 1 M KCl in H_2O



Geochemical CO_2 fixation from native metals could have supplied critical C_2 and C_3 metabolites before the emergence of enzymes.

S.J.Varma, K.B. Muchowska, P. Chatelain, J. Moran, *Nat Ecol Evol* **2018**, *2,* 1019–1024.

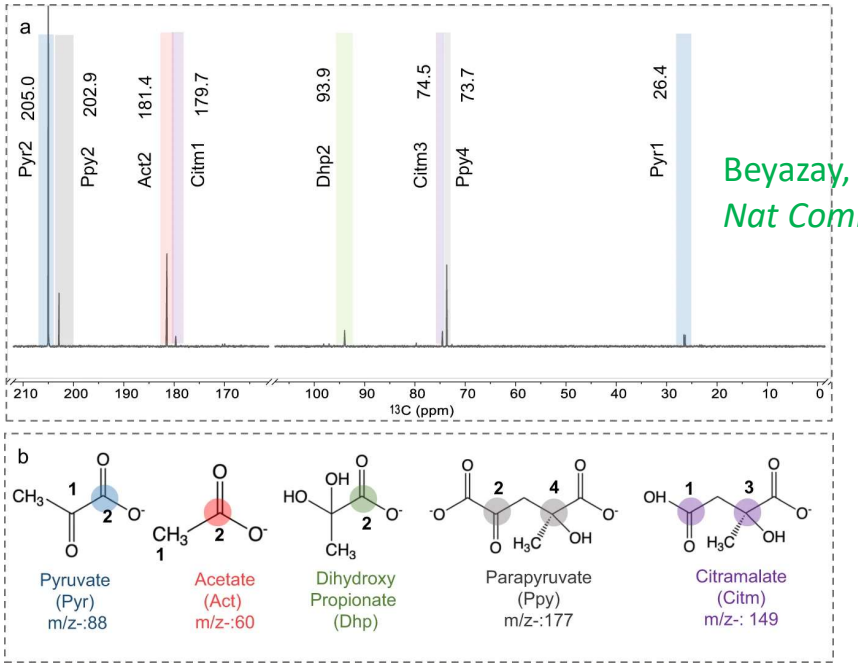
Prebiotic WL analogue – nanoparticles and minerals



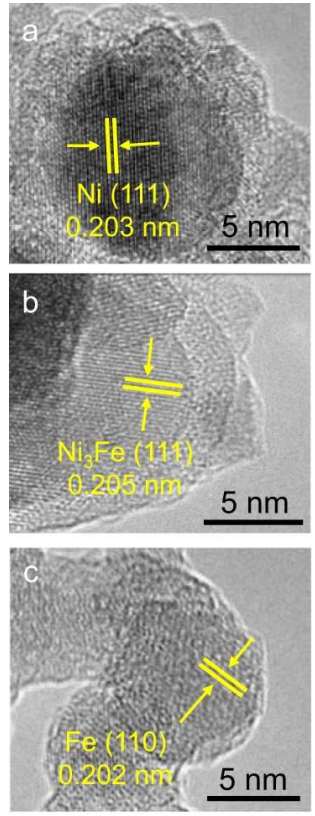
Three hydrothermal minerals—greigite (Fe_3S_4), magnetite (Fe_3O_4) and awaruite (Ni_3Fe)—catalyse the fixation of CO_2 with H_2 at 100 °C under alkaline aqueous conditions. The product spectrum includes formate (up to 200 mM), acetate (up to 100 µM), pyruvate (up to 10 µM), methanol (up to 100 µM) and methane

Preiner, M., Igarashi, K., Muchowska, K.B. *et al. Nat Ecol Evol* **2020**, *4,* 534-542.

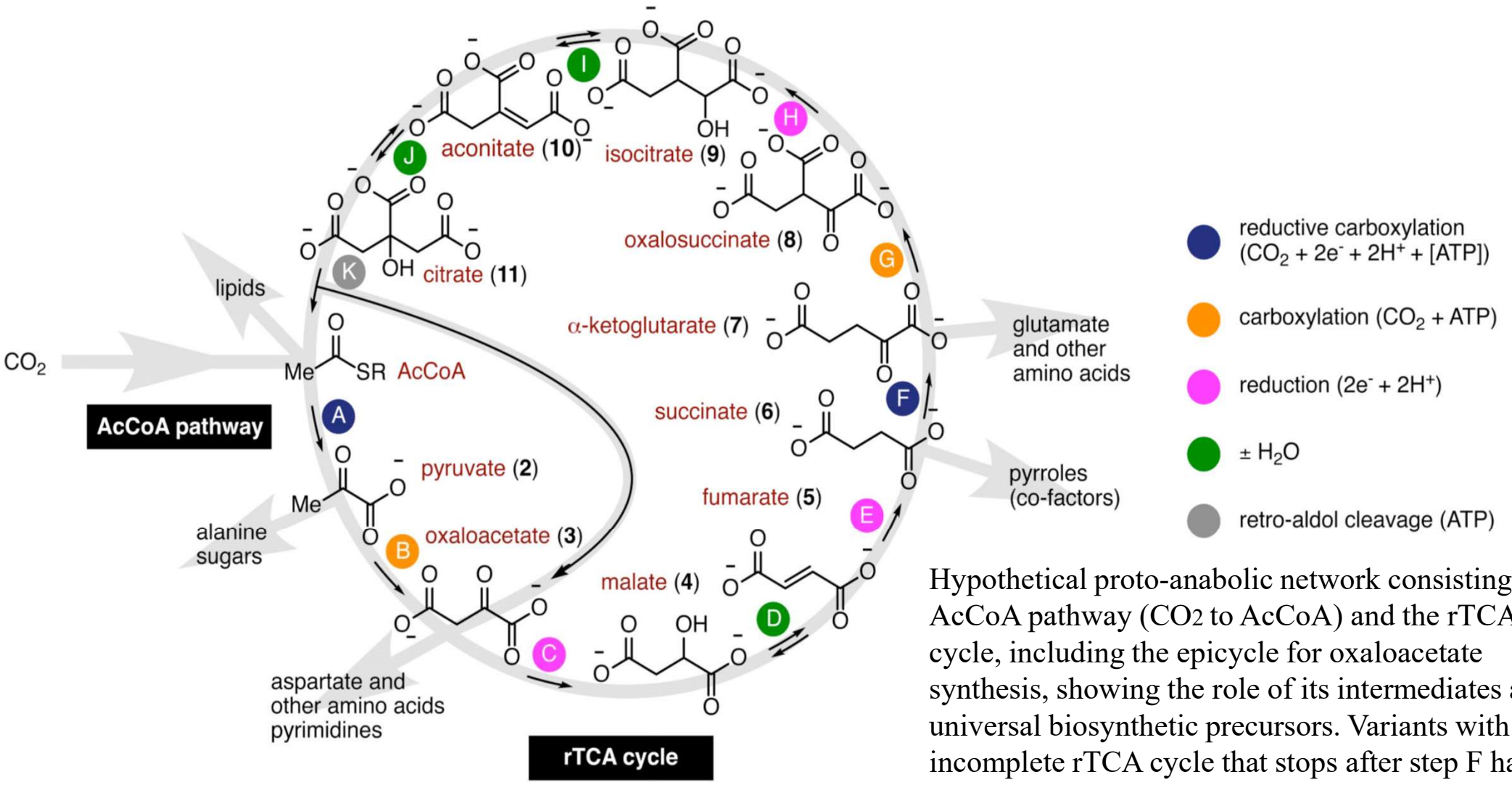
Ni_3Fe nanoparticles prepared via the hard-templating method catalyze the conversion of H_2 and CO_2 to formate, acetate and pyruvate at 25 °C under 25 bar. (+ parapyruvate, and citramalate) - all at very moderate reaction conditions without organic catalysts.



Beyazay, T., Belthle, K.S., Farès, C. et al. *Nat Commun* **2023**, *14,* 570



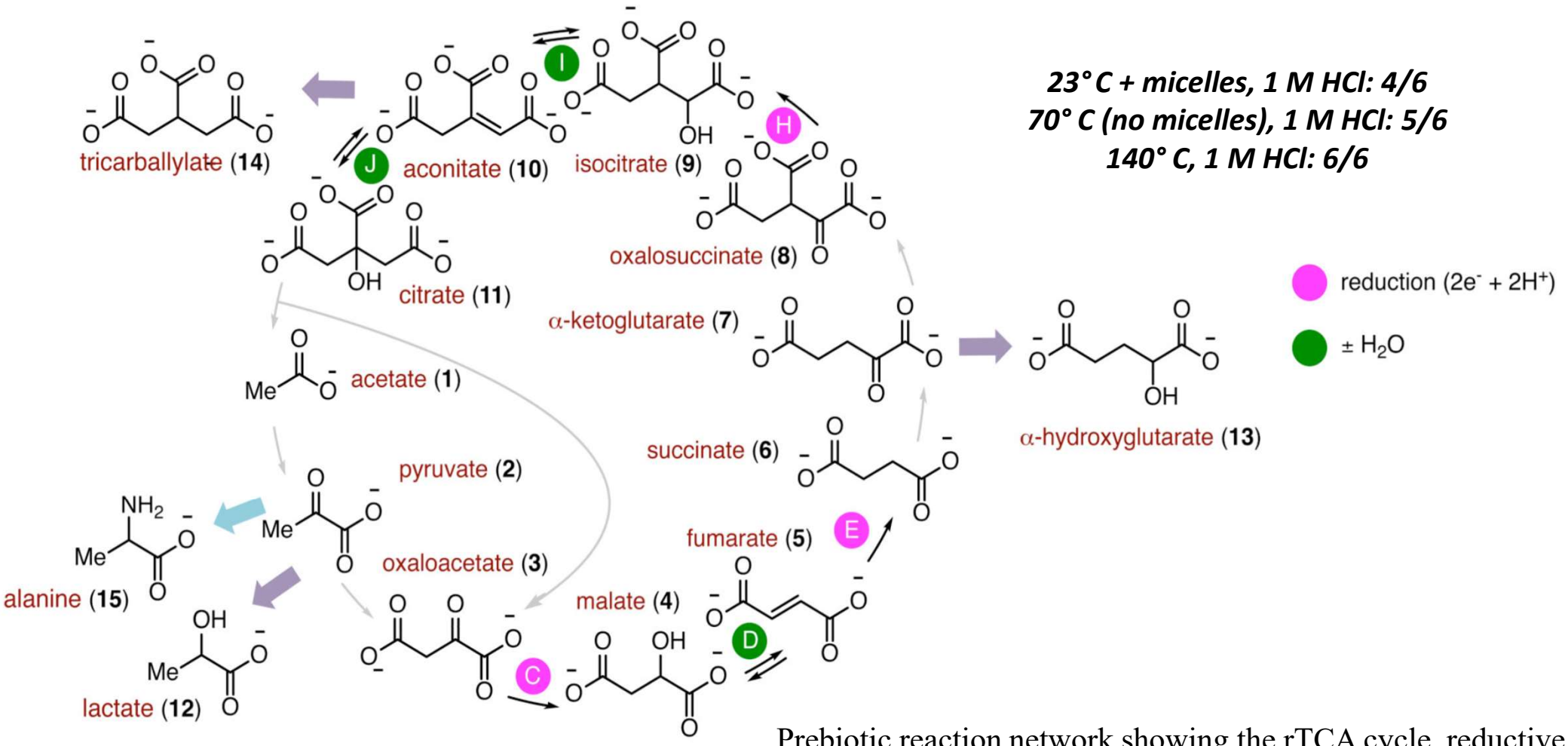
Metals promote sequences of the reverse Krebs cycle



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

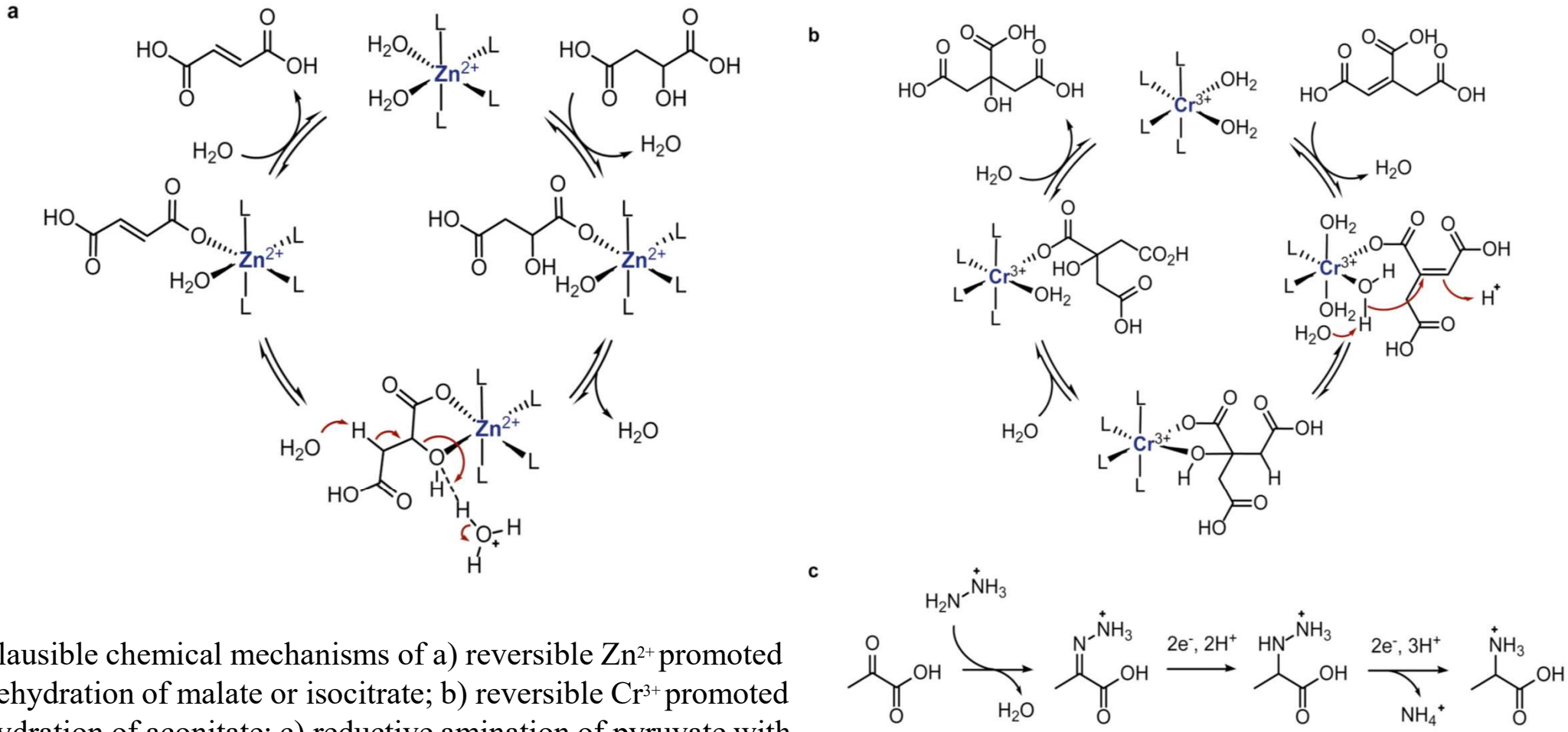


23° C + micelles, 1 M HCl: 4/6
70° C (no micelles), 1 M HCl: 5/6
140° C, 1 M HCl: 6/6

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

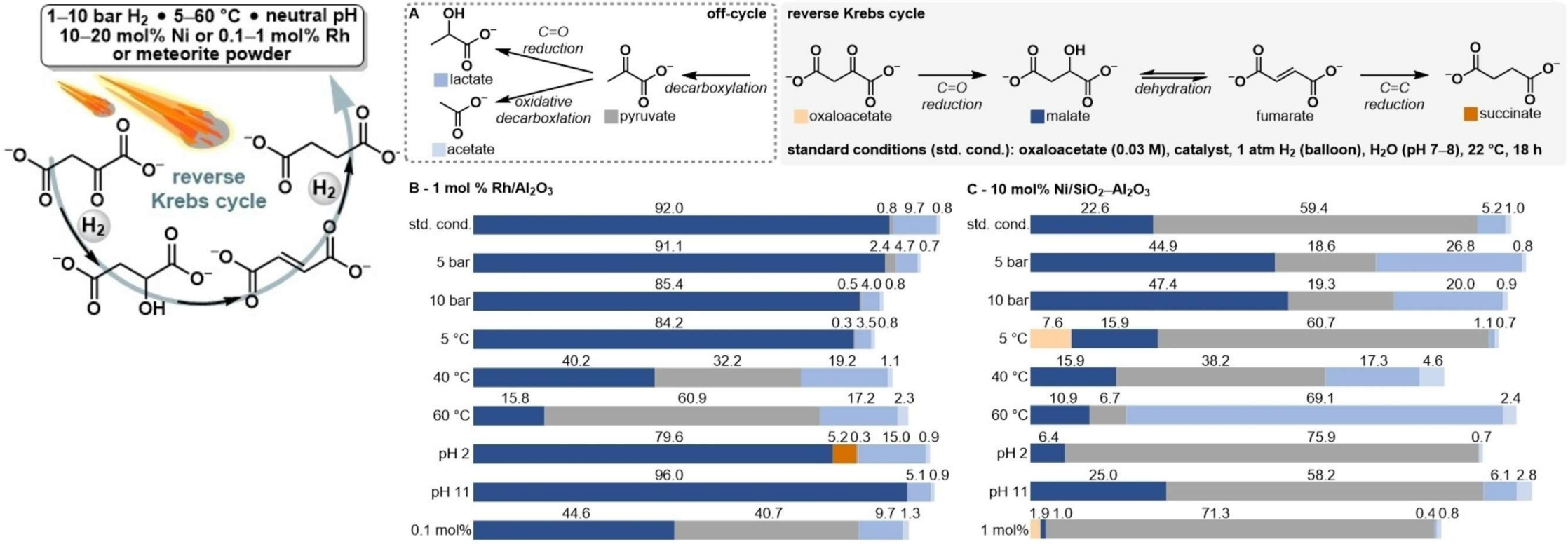
Metals promote sequences of the reverse Krebs cycle



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

Meteorite catalysis in the non-enzymatic reverse Krebs cycle analogue



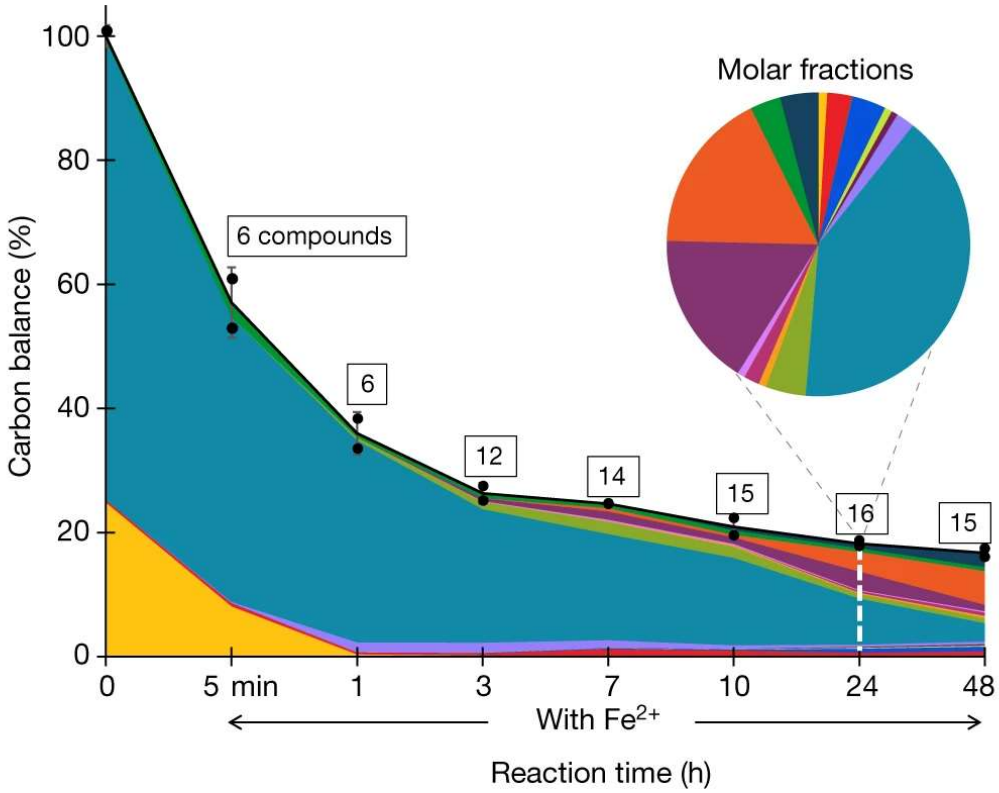
Meteorites also work!

(Campo del Cielo, Gibeon, Sikhote Alin)

S. A. Rauscher, J. Moran, *Angew. Chem. Int. Ed.* **2022**, *61*, e202212932

Synthesis and breakdown of universal metabolic precursors promoted by iron

b



Initial conditions: pyruvate, glyoxylate, Fe^{2+}, 70 ºC

All 5 universal precursors, 9/11 TCA intermediates
Similar results with Mn^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+};
HCl or KCl aren't catalysts

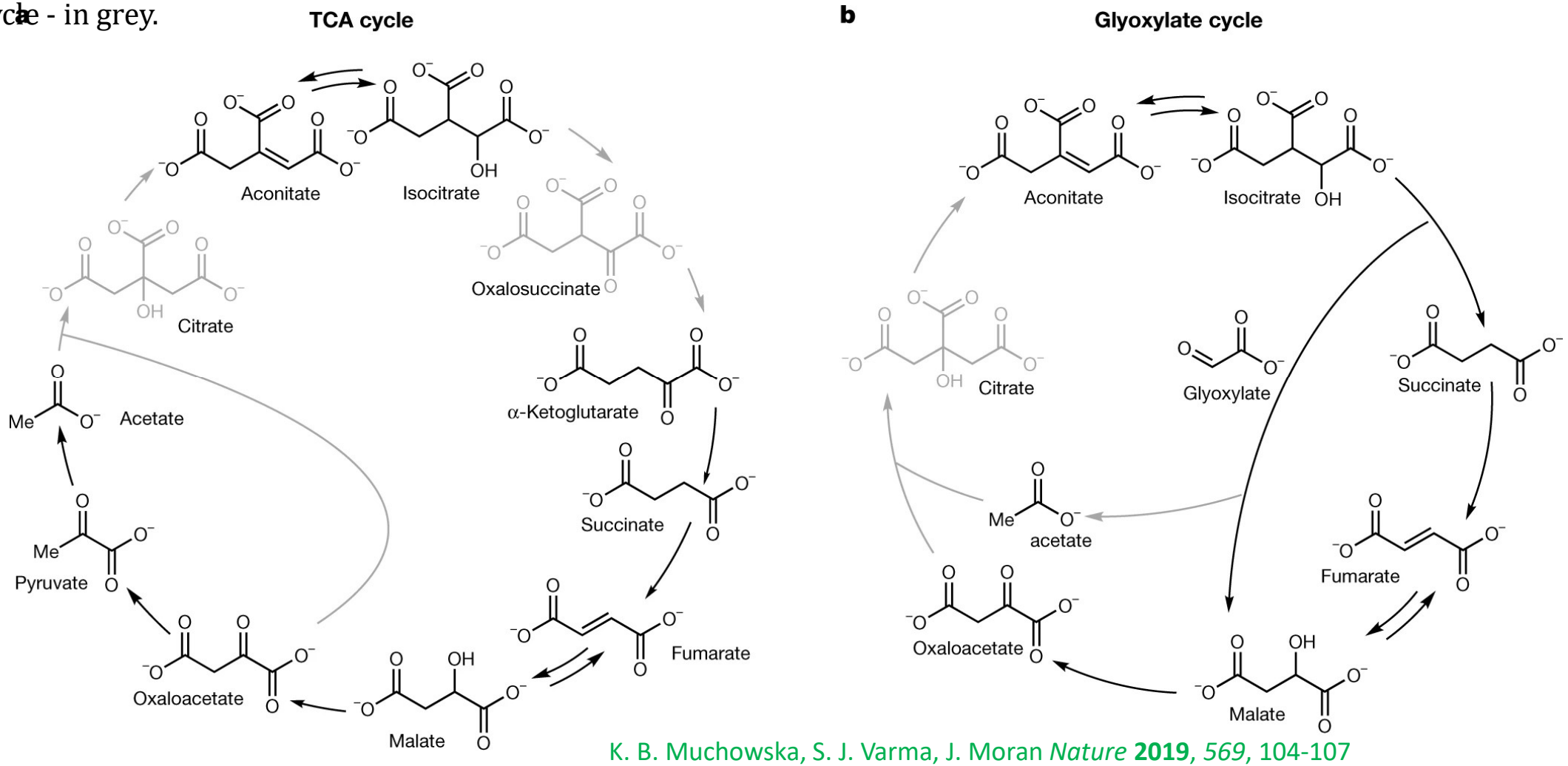
- Levulinate
- Glycolate
- Malonate
- Oxalate
- Tricarballylate
- Mesaconate
- Aconitate
- Isocitrate
- Hydroxyketoglutarate
- α-Ketoglutarate
- Succinate
- Fumarate
- Malate
- Lactate
- Pyruvate
- Glyoxylate
- Total

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

See also (0.5 M phosphate, 50ºC):
Stubbs, R.T., Yadav, M., Krishnamurthy, R., Springsteen, G. *Nat. Chem.* **2020**, ***12***, 1016–1022.

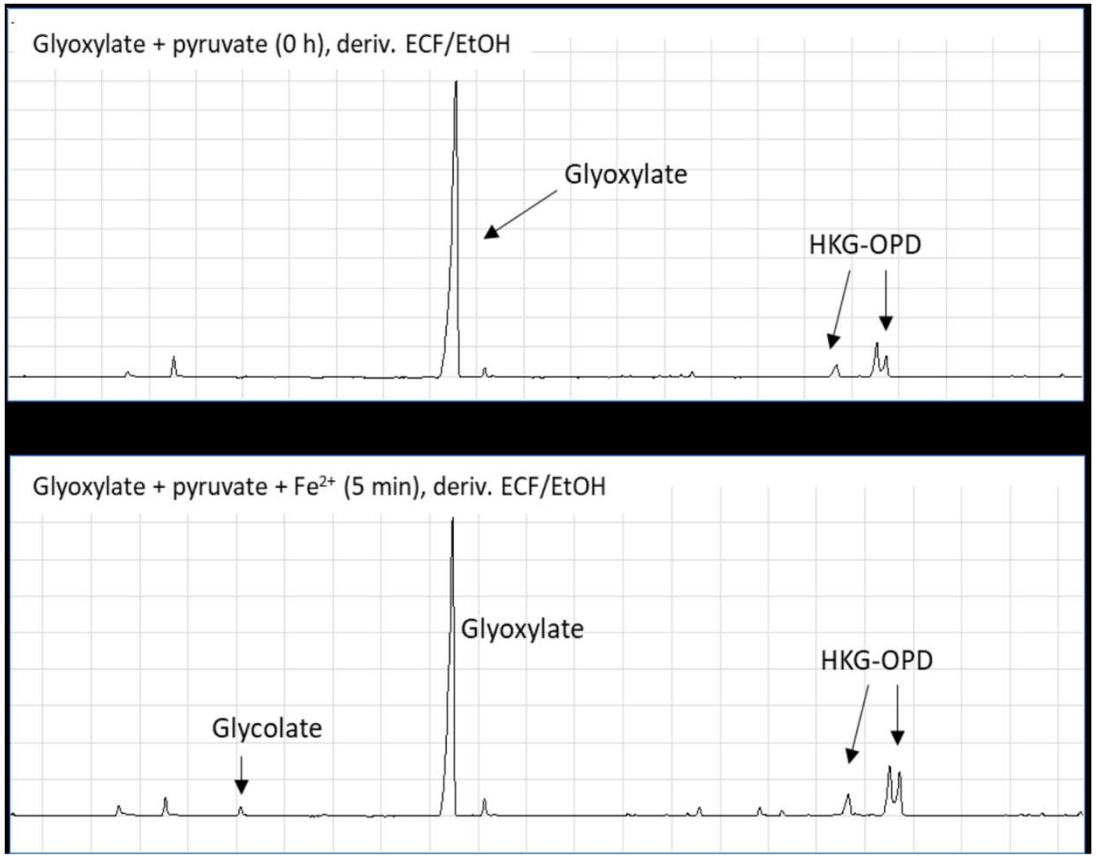
Synthesis and breakdown of universal metabolic precursors promoted by iron

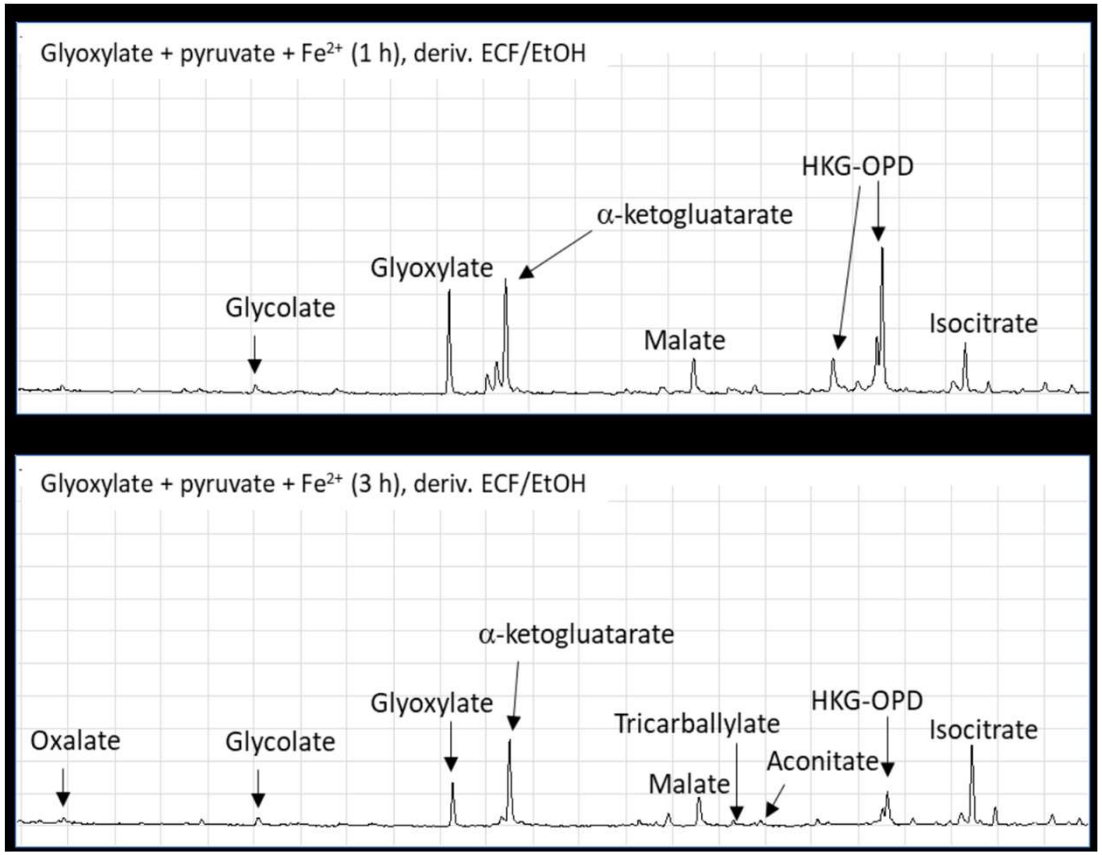
Comparison of the observed reaction network with the TCA and glyoxylate cycles. Intermediates and reactions found in both the biological cycle and the synthetic reaction network shown in black. Those found only in the biological cycle - in grey.



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

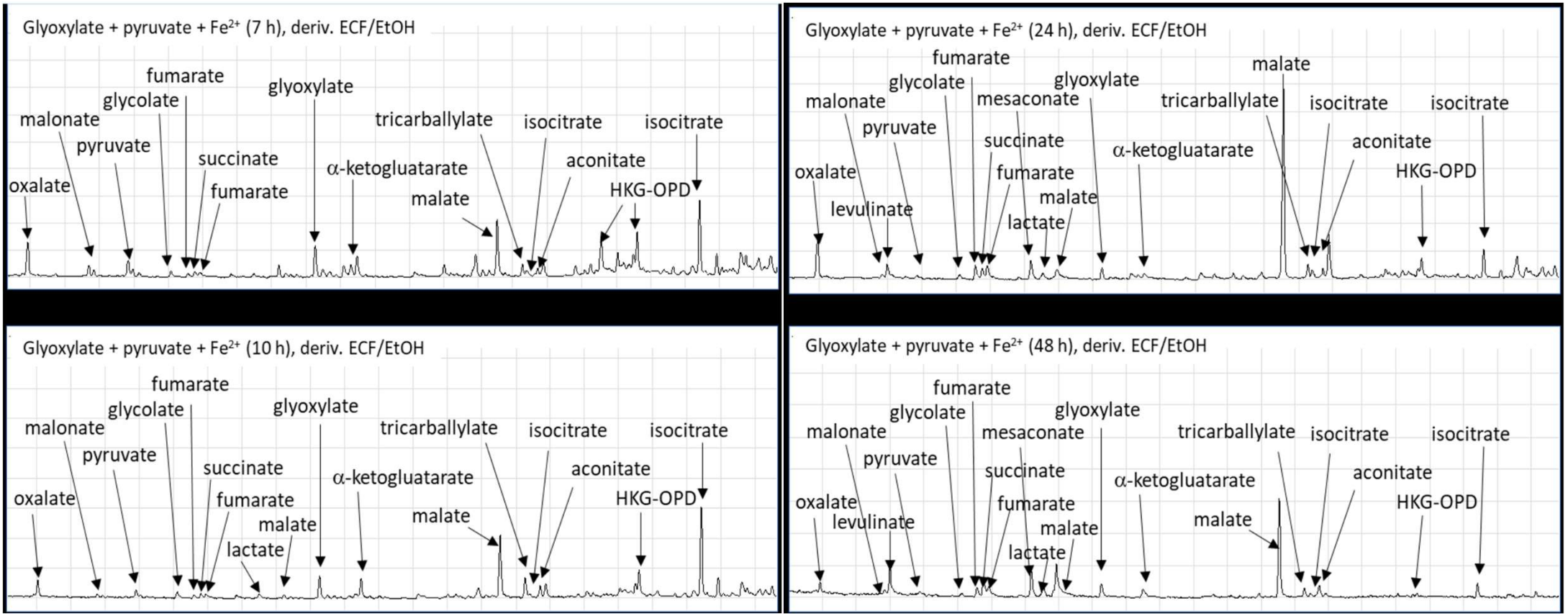
Synthesis and breakdown of universal metabolic precursors promoted by iron





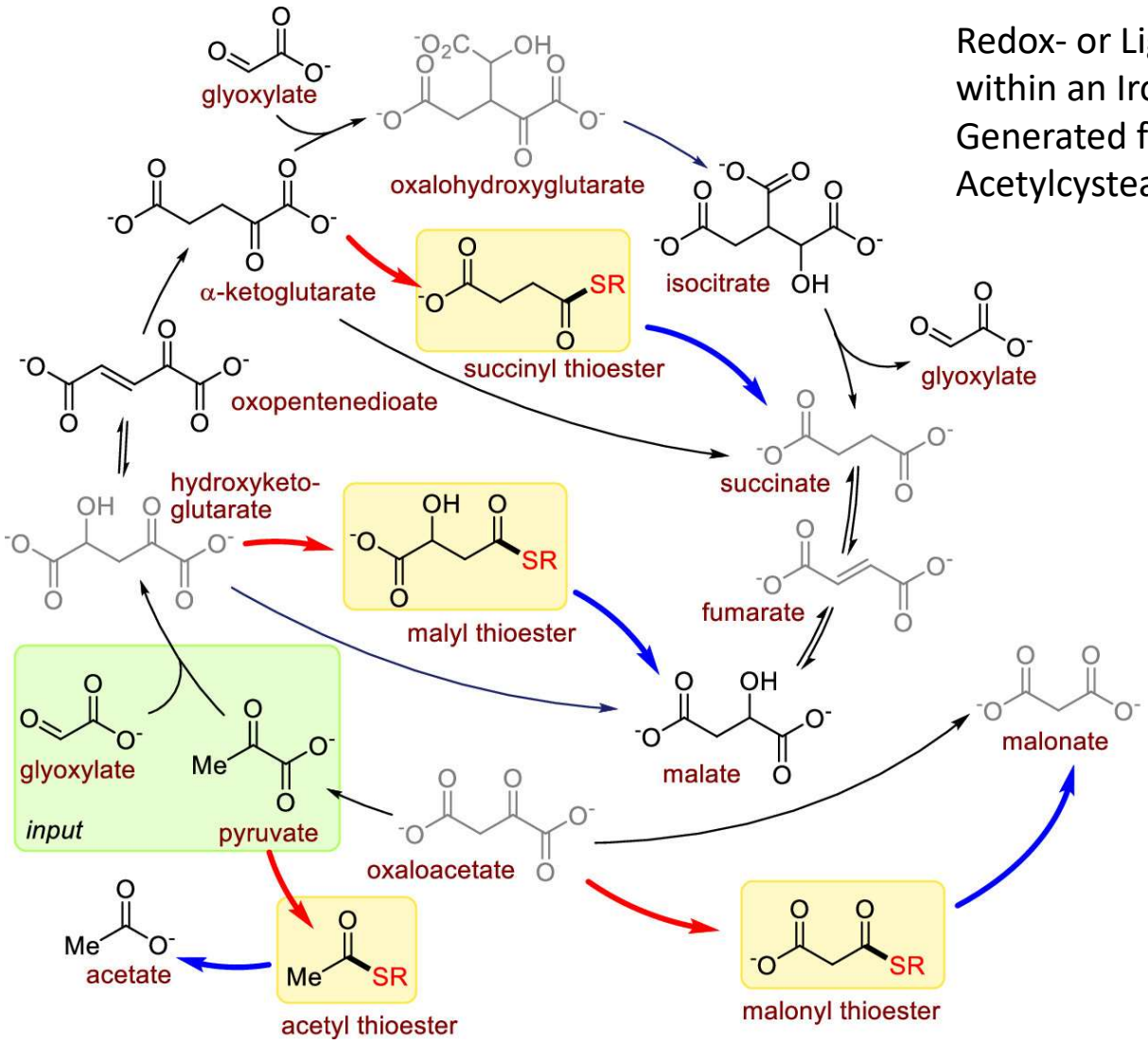
K. B. Muchowska, S. J. Varma, J. Moran *Nature* **2019**, *569*, 104-107

Synthesis and breakdown of universal metabolic precursors promoted by iron

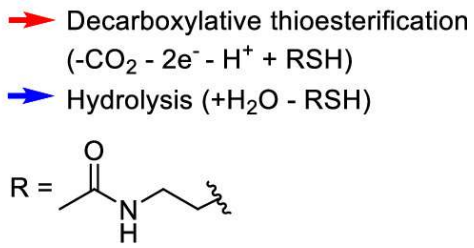


K. B. Muchowska, S. J. Varma, J. Moran *Nature* **2019**, *569*, 104-107

Universal metabolic precursors → thioesters

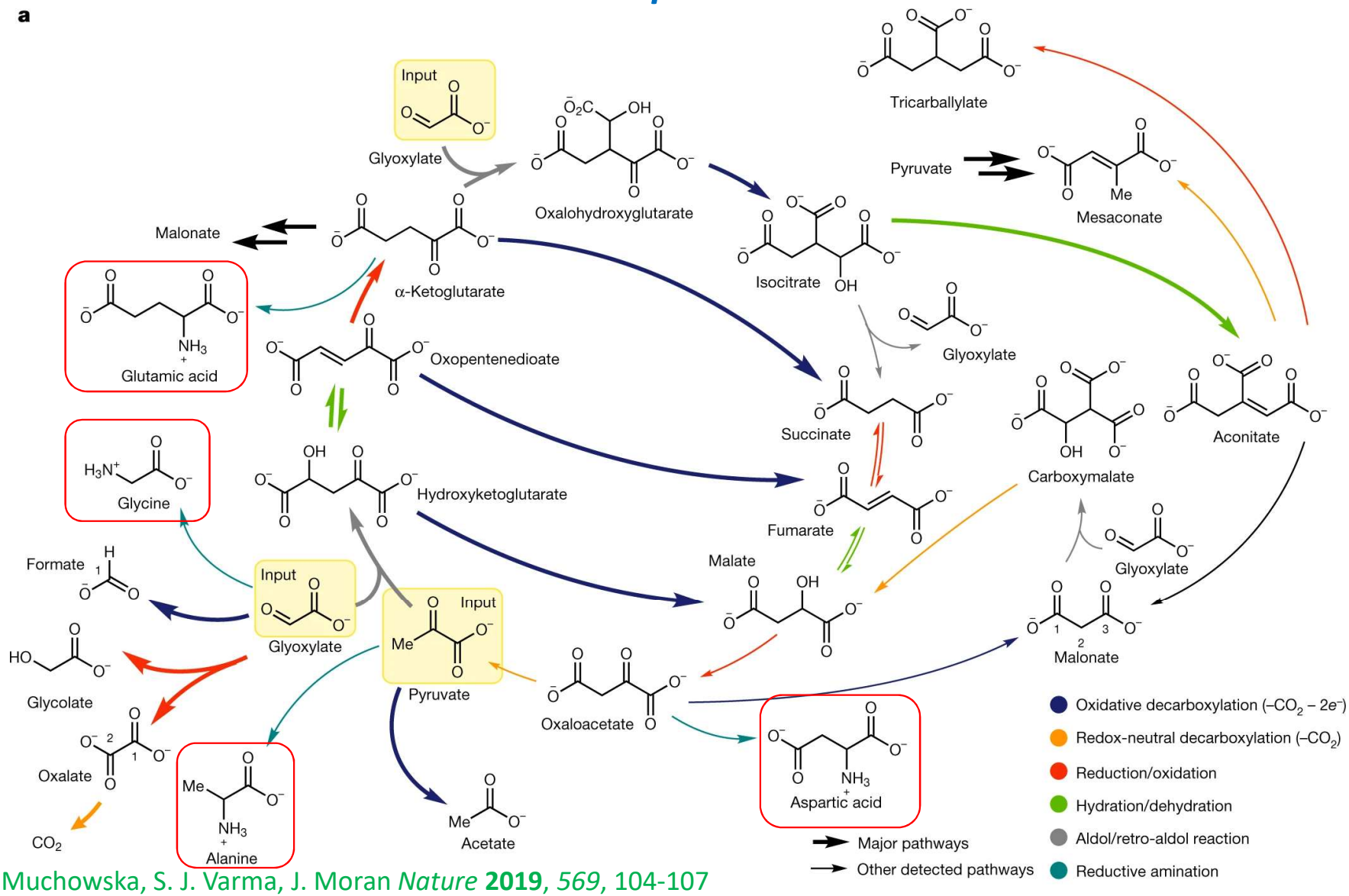


Redox- or Light-Promoted Thioester Formation within an Iron-Catalyzed Reaction Network, Generated from Pyruvate, Glyoxylate, and *N*-Acetylcysteamine



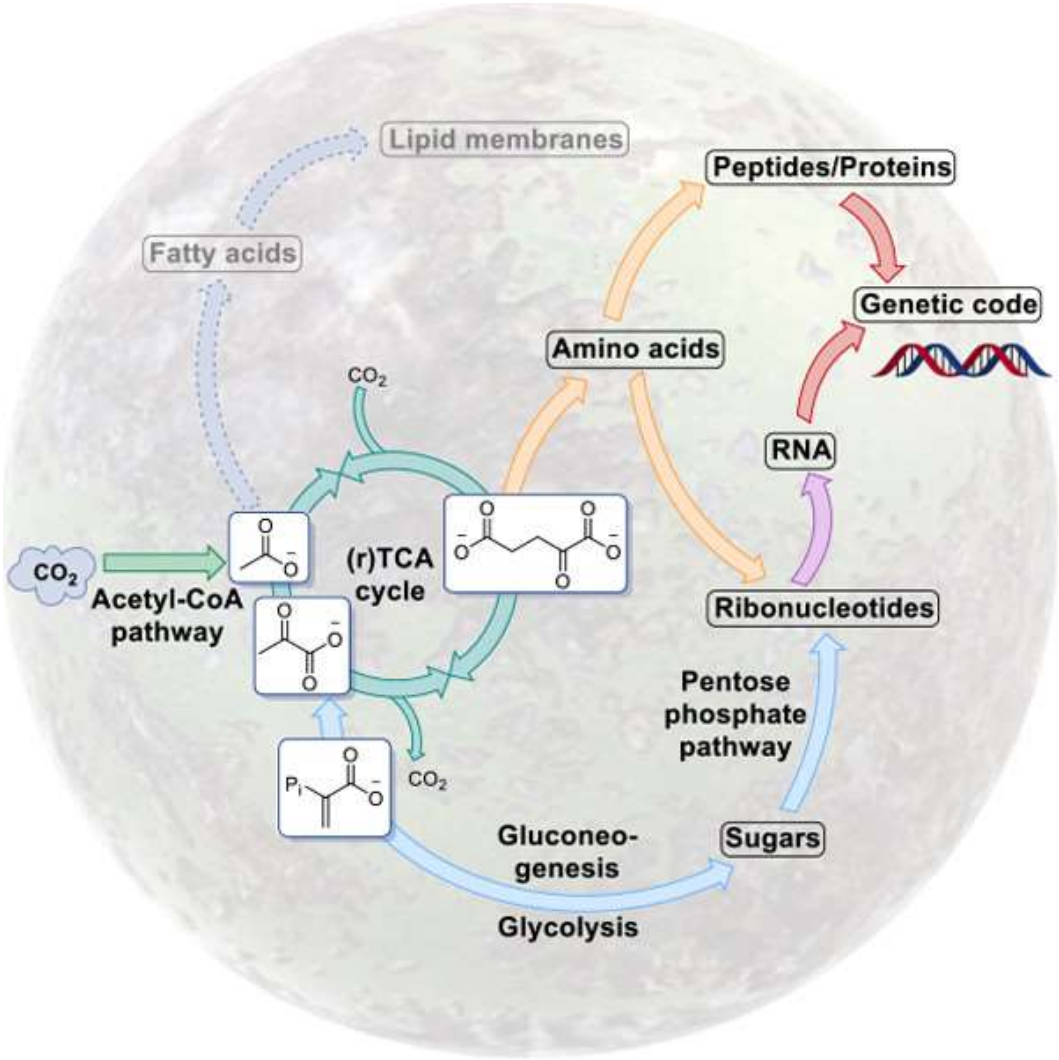
Chevallot-Beroux, E.; Gorges, J.; Moran, J.
ChemRxiv **2019**, 8832425
10.26434/chemrxiv.8832425.v1

Universal metabolic precursors → aminoacids



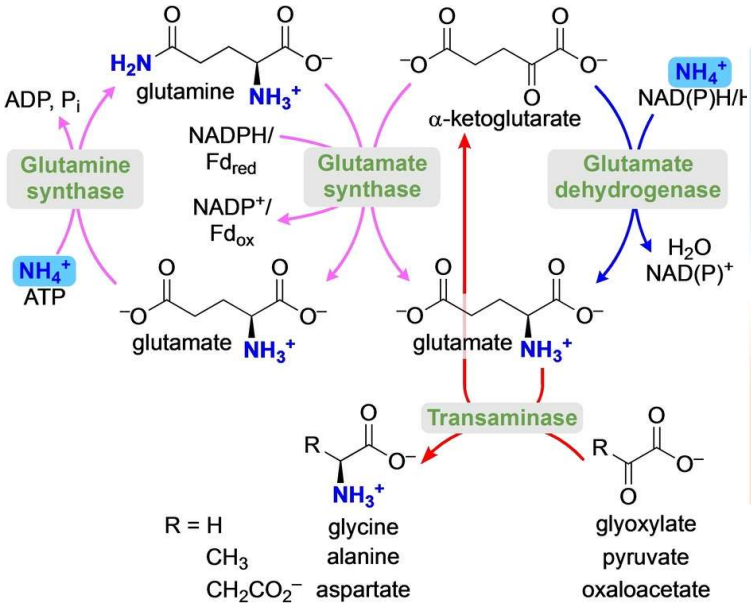
K. B. Muchowska, S. J. Varma, J. Moran *Nature* **2019**, *569*, 104-107

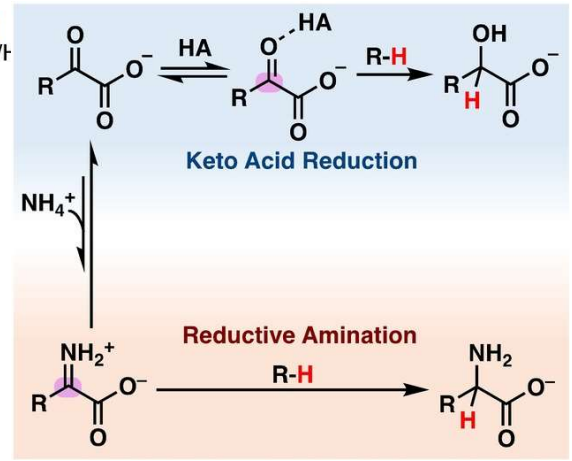
Core protometabolism map

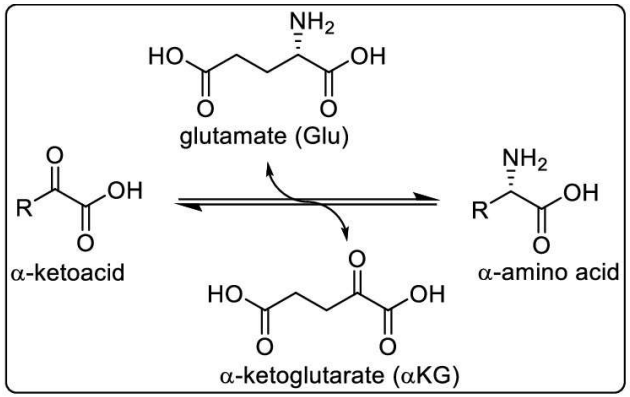


K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708–7744

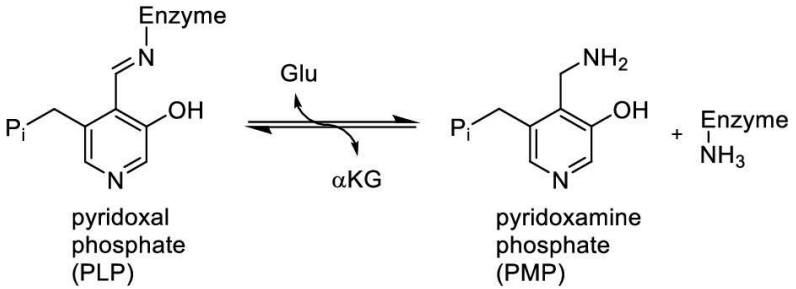
Universal metabolic precursors – nonenzymatic aminoacid synthesis

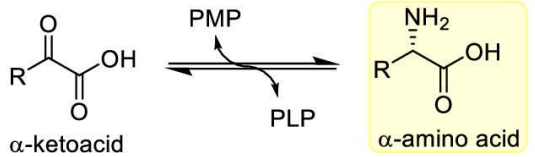






Mechanism:



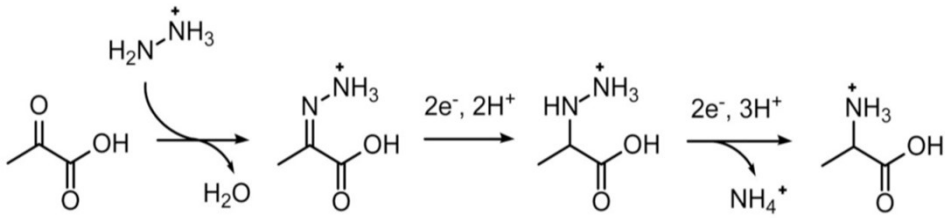


The flow of ammonia in amino acid biosynthesis proceeds through reductive amination for α-ketoglutarate but through transamination for pyruvate and oxaloacetate .

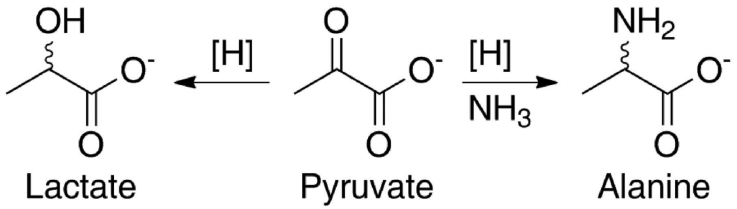
R. J. Mayer, J. Moran, *Angew. Chem. Int. Ed.* **2022**, *61*, e202212237

K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708–7744

Universal metabolic precursors – nonenzymatic aminoacid synthesis

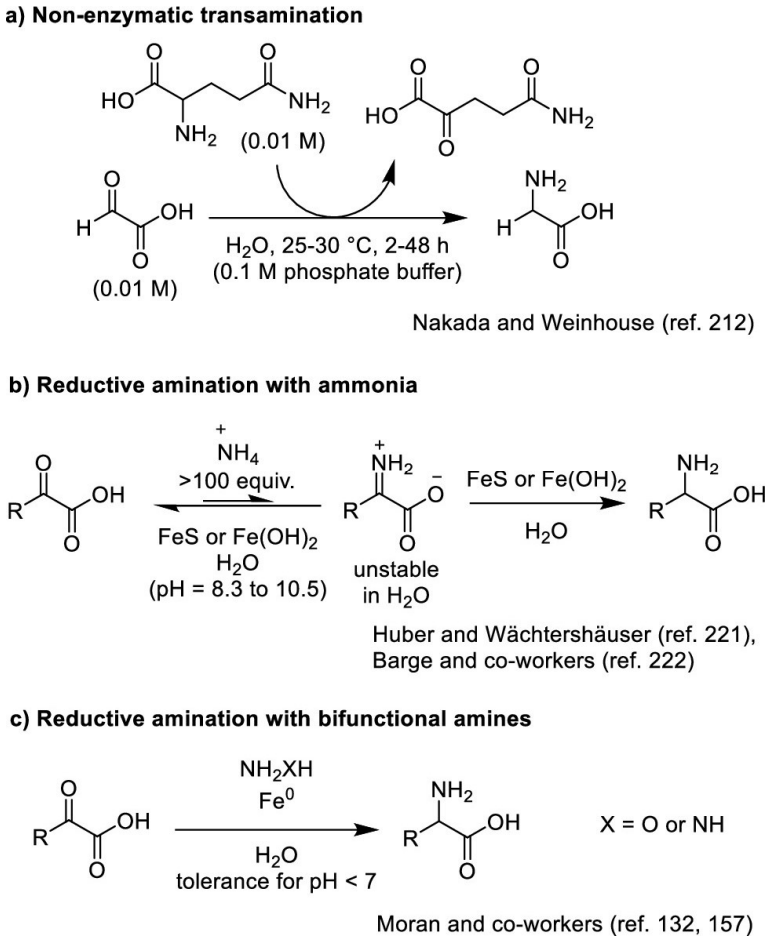


J. Moran *et al. Nat Ecol Evol.* **2017**, *1(11)*, 1716–1721



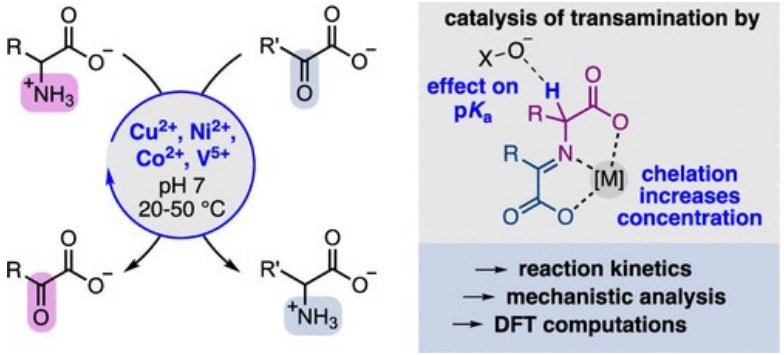
Pyruvate can undergo reductive amination in the presence of mixed-valence iron oxyhydroxides to form alanine,as well as the reduced product lactate

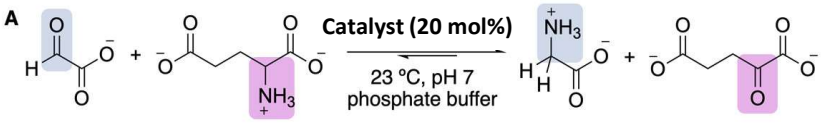
Barge, L. M.; Flores, E.; Baum, M. M.; van der Velde, D. G.; Russell, M. J. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 4828– 4833,



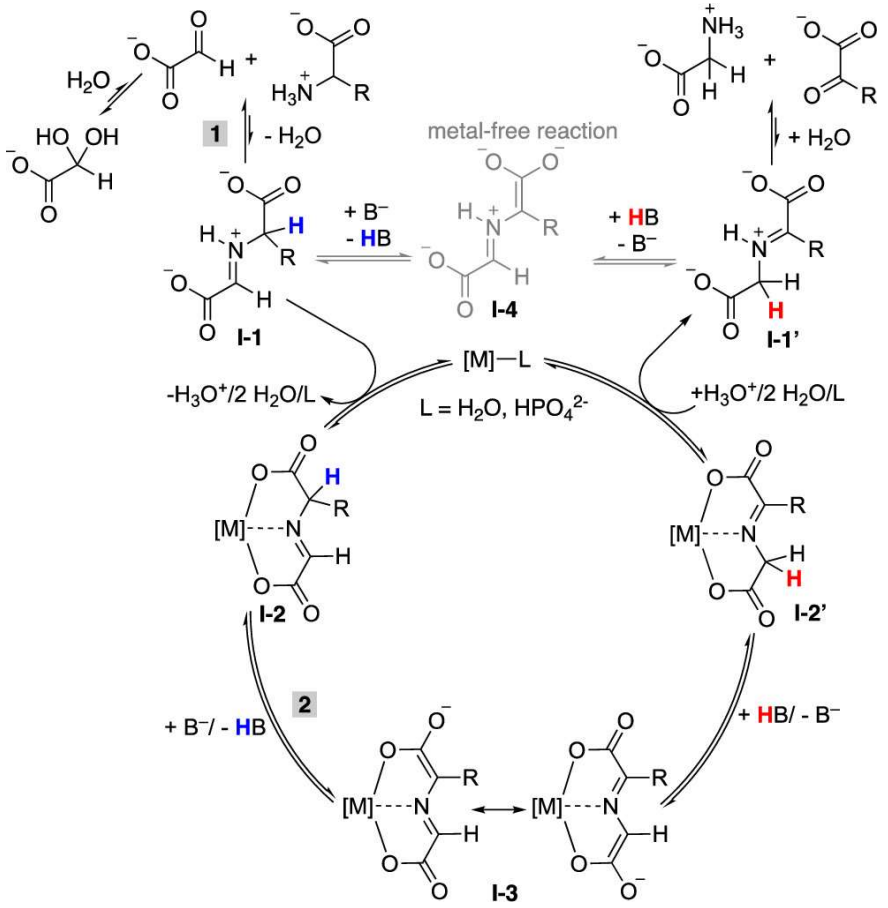
R. J. Mayer, J. Moran, *Angew. Chem. Int. Ed.* **2022**, *61*, e202212237

Universal metabolic precursors – nonenzymatic aminoacid synthesis



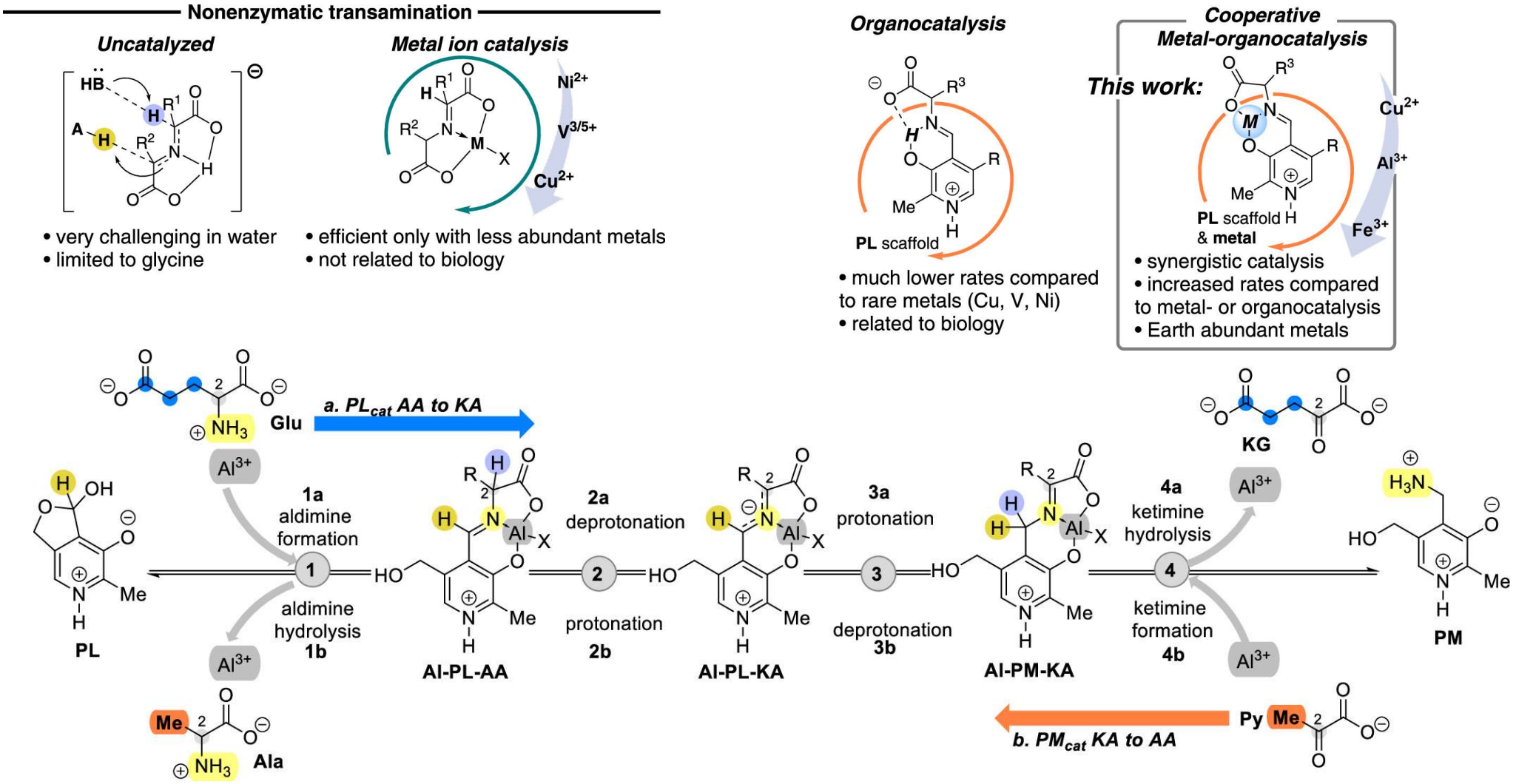


entry	catalyst	yield (%)	entry	catalyst	yield (%)
1	NiCl2	90.5 ± 1.2	12	VOSO4	1.2
2	CuCl2	86.6 ± 1.3	13	CrCl2	0.7
3	CoCl2	60.9 ± 6.9	14	MgCl2	0.5
4	NaVO3	49.0 ± 4.0	15	WCl6	0.5
5	FeCl3	25.5 ± 2.9	16	MoCl3	0.4
6	FeCl2	20.2	17	MnCl2	0.3
7	AlCl3	8.3	18	CrCl3	0.3
8	ZnCl2	7.2	19	CeCl3	0.3
9	KAl(SO4)2	6.7	20	CaCl2	0.3
10	PdCl2	5.8	21	none	0.4 ± 0.1
11	HgCl2	5.6	22	enzyme	99.3b



R. J. Mayer, H. Kaur, S.A. Rauscher, J. Moran, *J. Am. Chem. Soc.* **2021**, *143,* 19099-19111.

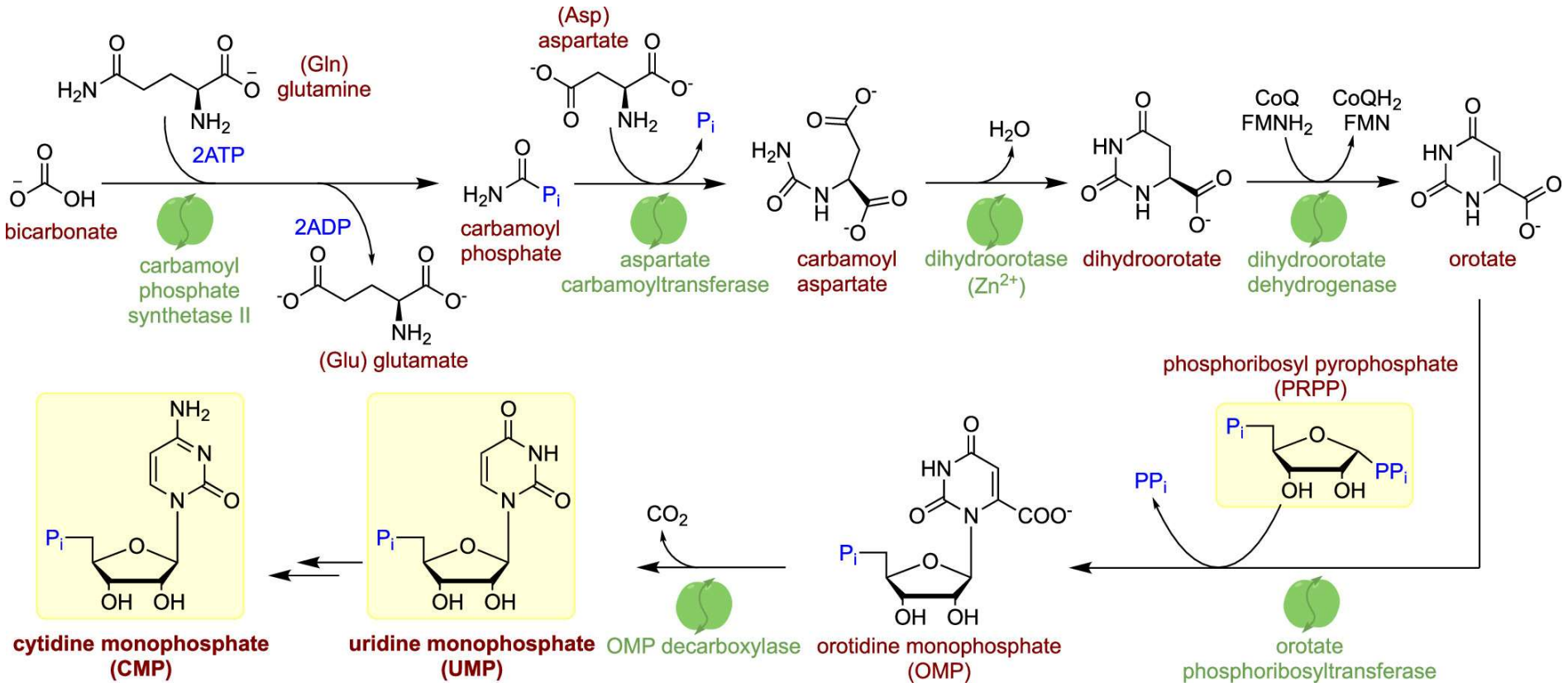
Universal metabolic precursors – nonenzymatic aminoacid synthesis



Q. Dherbassy. R. J. Mayer, K. B. Muchowska, J. Moran, *J. Am. Chem. Soc.* **2023**, *145,* 13357-13370.

Universal metabolic precursors – nonenzymatic pyrimidine nucleobase synthesis

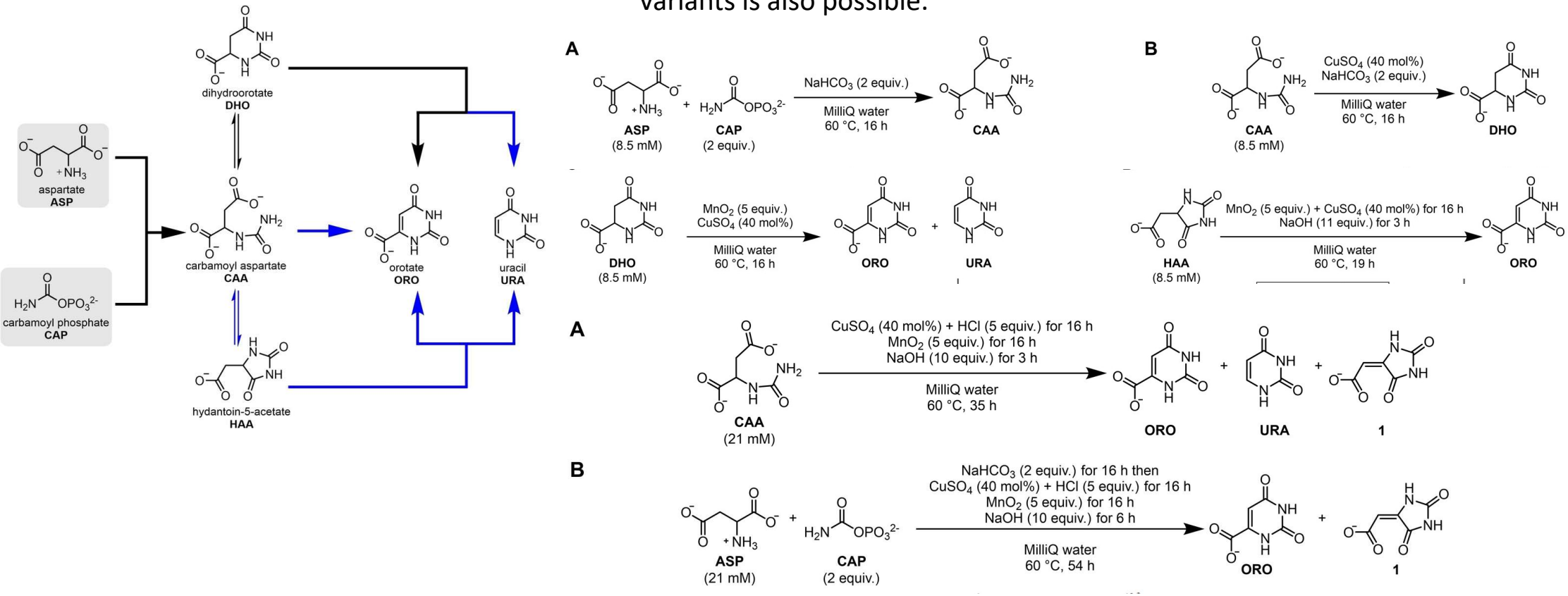
Biosynthesis of Pyrimidine Ribonucleotides



K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708–7744

Universal metabolic precursors – nonenzymatic pyrimidine nucleobase synthesis

All three reactions of pyrimidine nucleobase biosynthesis that convert aspartate to orotate proceed at 60 °C without photochemistry under aqueous conditions in the presence of metals such as Cu^{2+} and Mn^{4+}.Combining reactions into one-pot variants is also possible.



Life may not have invented pyrimidine nucleobase biosynthesis from scratch, but simply refined existing nonenzymatic reaction channels

J. Yi *et al. Angew. Chem. Int. Ed.* **2022**, *61,* e202117211

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)

Phylogenetic analysis of primitive organisms can be helpful in deciphering the origin of metabolism

Metal ions in aqueous conditions can emulate Ac-CoA (WL) pathway of CO_2 fixation, as well as provide numerous central metabolic intermediates that can further assemble into rTCA cycle, as well as become substrates for abiotic synthesis of aminoacids and pyrimidine nucleobases.

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