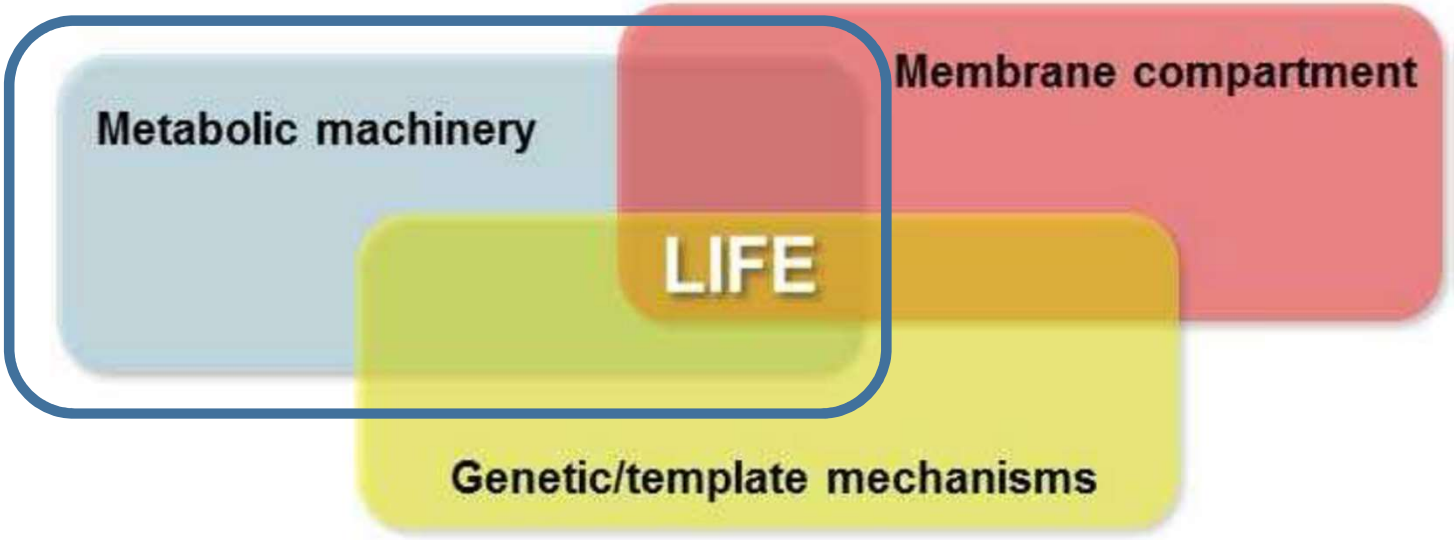
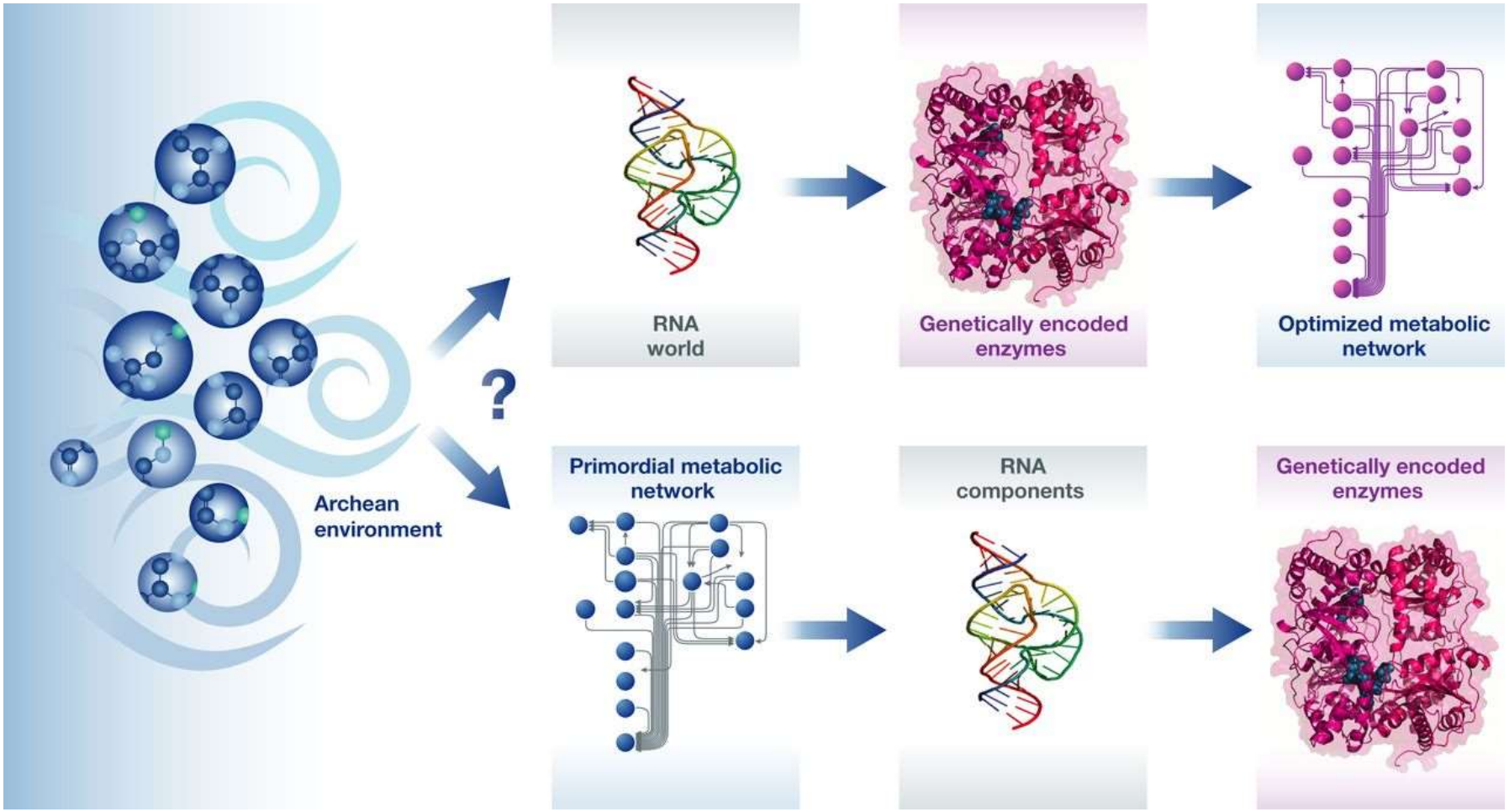

Metabolic machinery
Membrane compartment
LIFE
Genetic/template mechanisms

Route to life by chemical networks



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

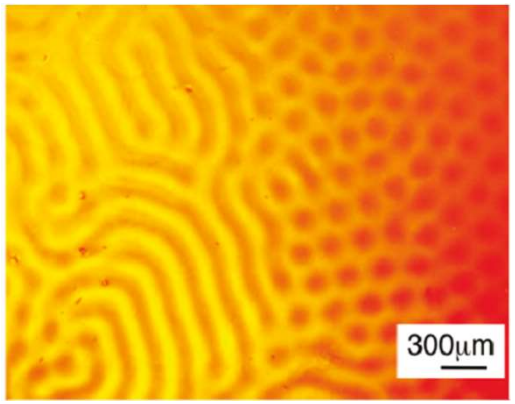
Metabolism-first vs. Genes-first

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 and 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

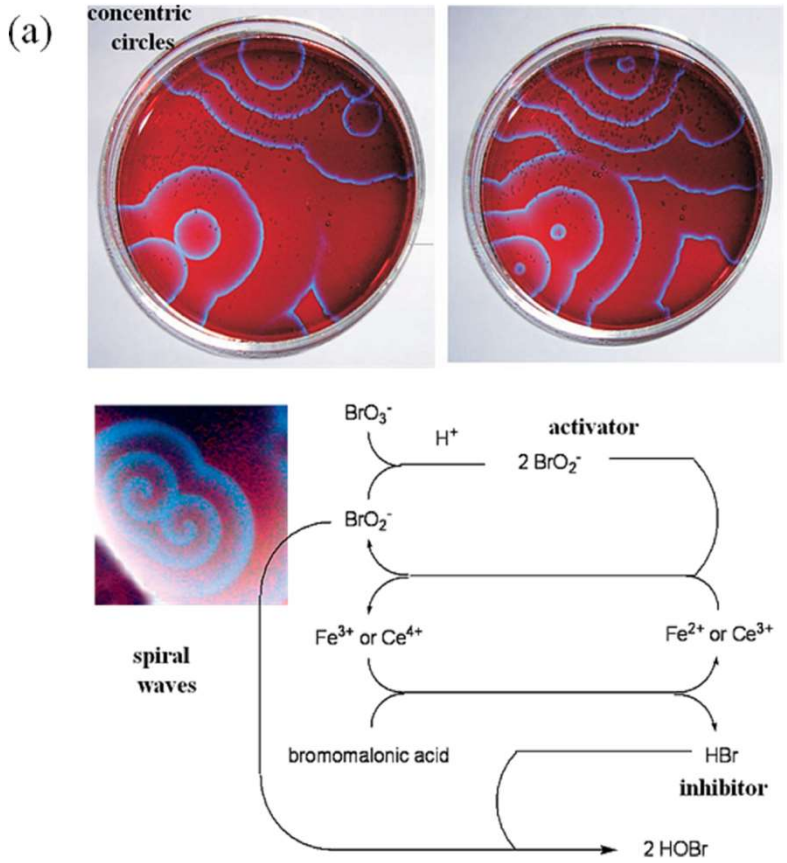
temperature, pH, humidity, illumination, UV irradiation, astronomic cycles

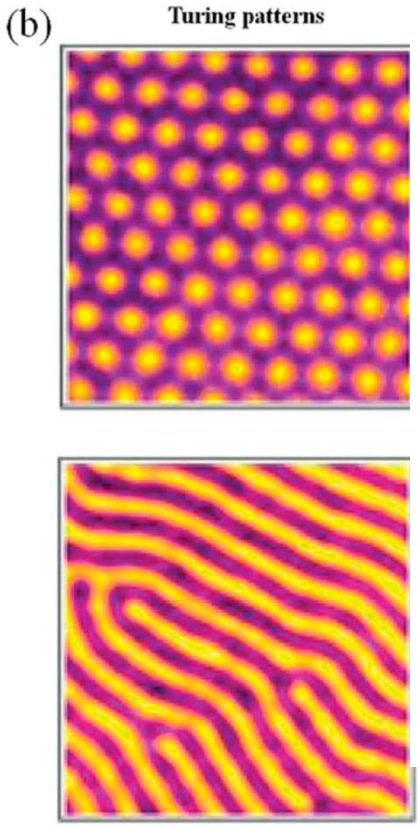
Chemical systems that mimic biological oscilations are studied as simple models

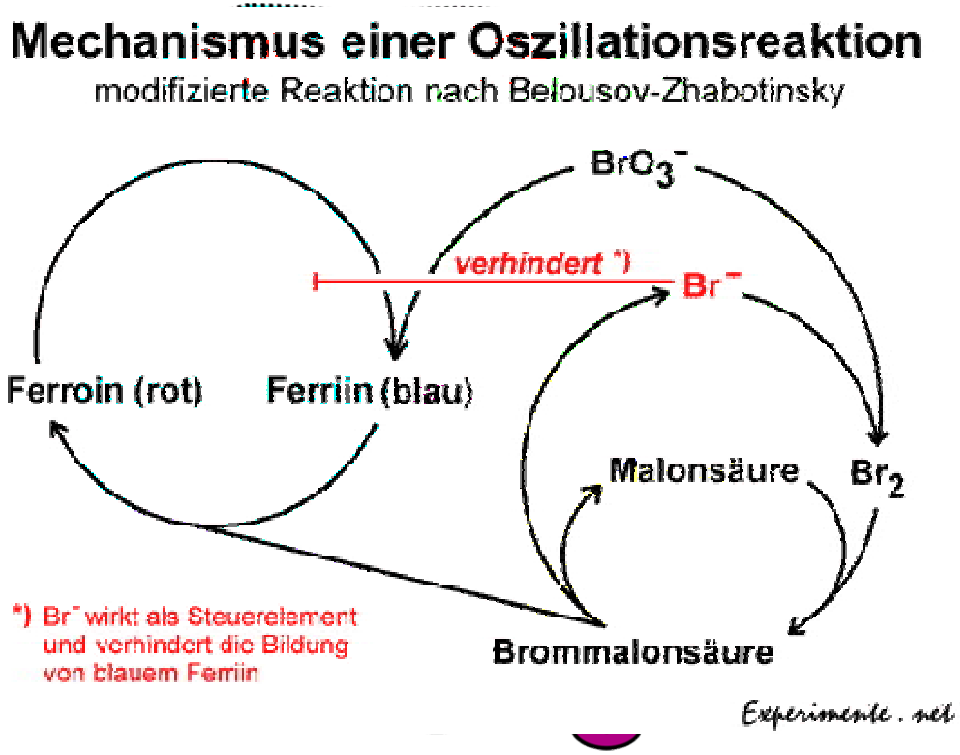
Belousov-Zhabotynski, CIMA reaction

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



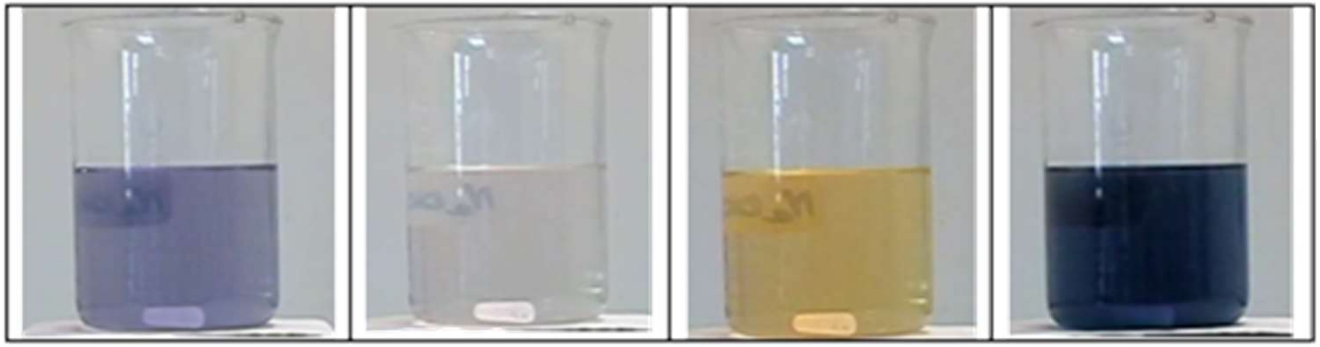




The reaction usually involves potassium bromate(VII) and malonic acid, optionally with cerium(IV) sulfate and citric acid. Ferroin is one of the common redox indicator

Briggs-Rauscher reaction

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

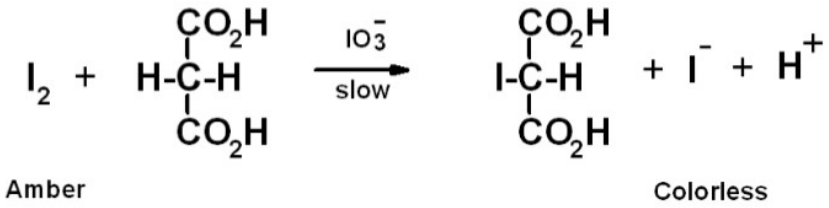


1. **The iodate ion is changed into iodine by hydrogen peroxide. The color changes to amber:**

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

Colorless — Amber

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



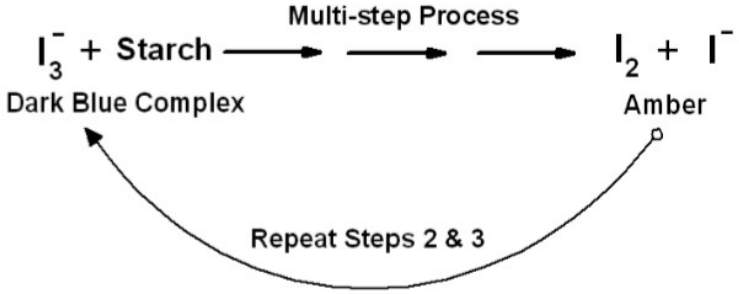
Amber — Colorless

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:**

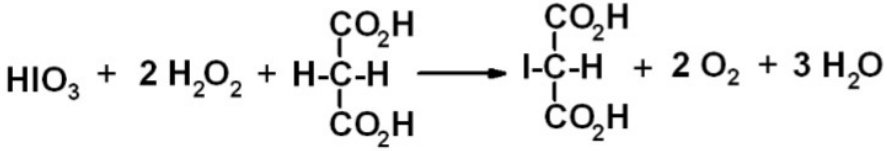
$$I_2 + I^- \xrightarrow[\text{fast}]{} I_3^- + \text{Starch} \xrightarrow[\text{fast}]{} \text{Dark Blue Complex}$$

Amber

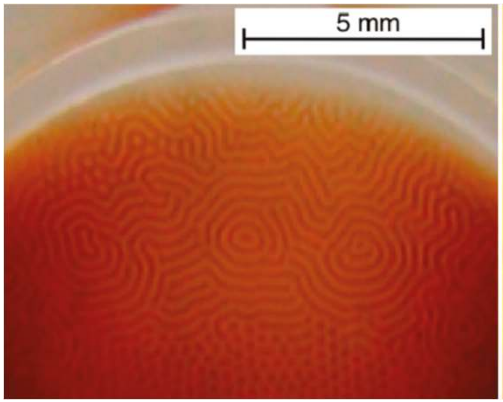
4. **After a period of time, the I_3^- ions are converted back into iodine and iodide ions, so the dark blue color disappears and the process repeats itself:**

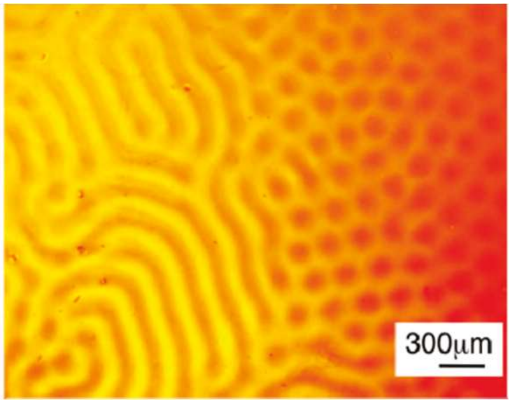


5. **Eventually the faster step 3 becomes dominant and the change of I_3^- back to iodine/iodide stops after about 15 cycles, so the solution remains dark blue. The overall chemical reaction is:**



Chlorite/iodide/malonic acid (CIMA) reaction





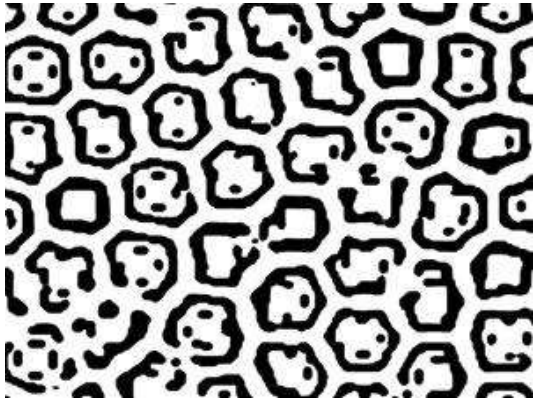
For the spontaneous generation of a Turing pattern, two intermediate species, an activator and an inhibitor, should be generated with the diffusion coefficient of the activator smaller than that of the inhibitor. The CIMA reaction that generates the activator, I^-, and inhibitor, ClO_2^-, was performed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

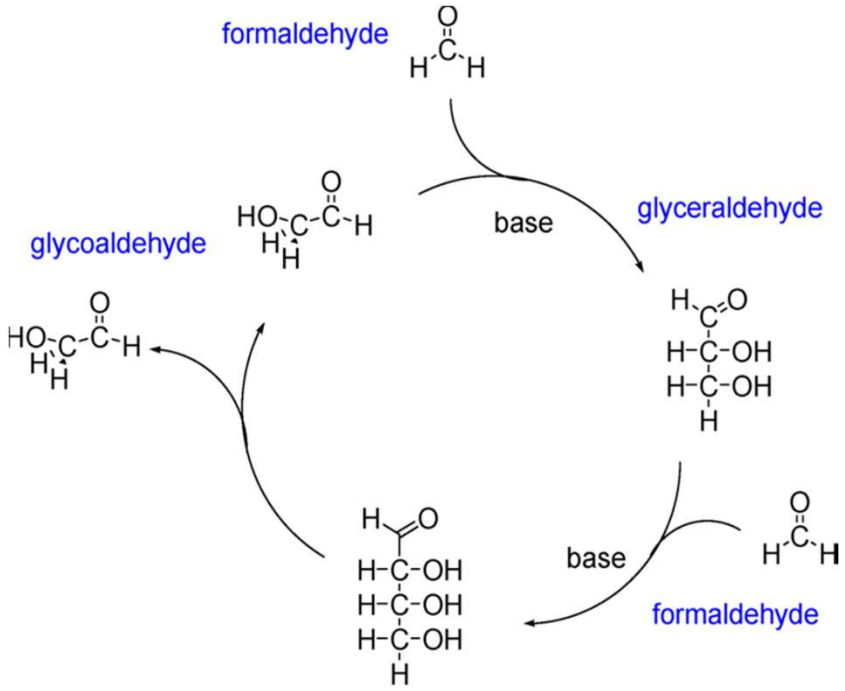
Explain the origin of homochirality

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

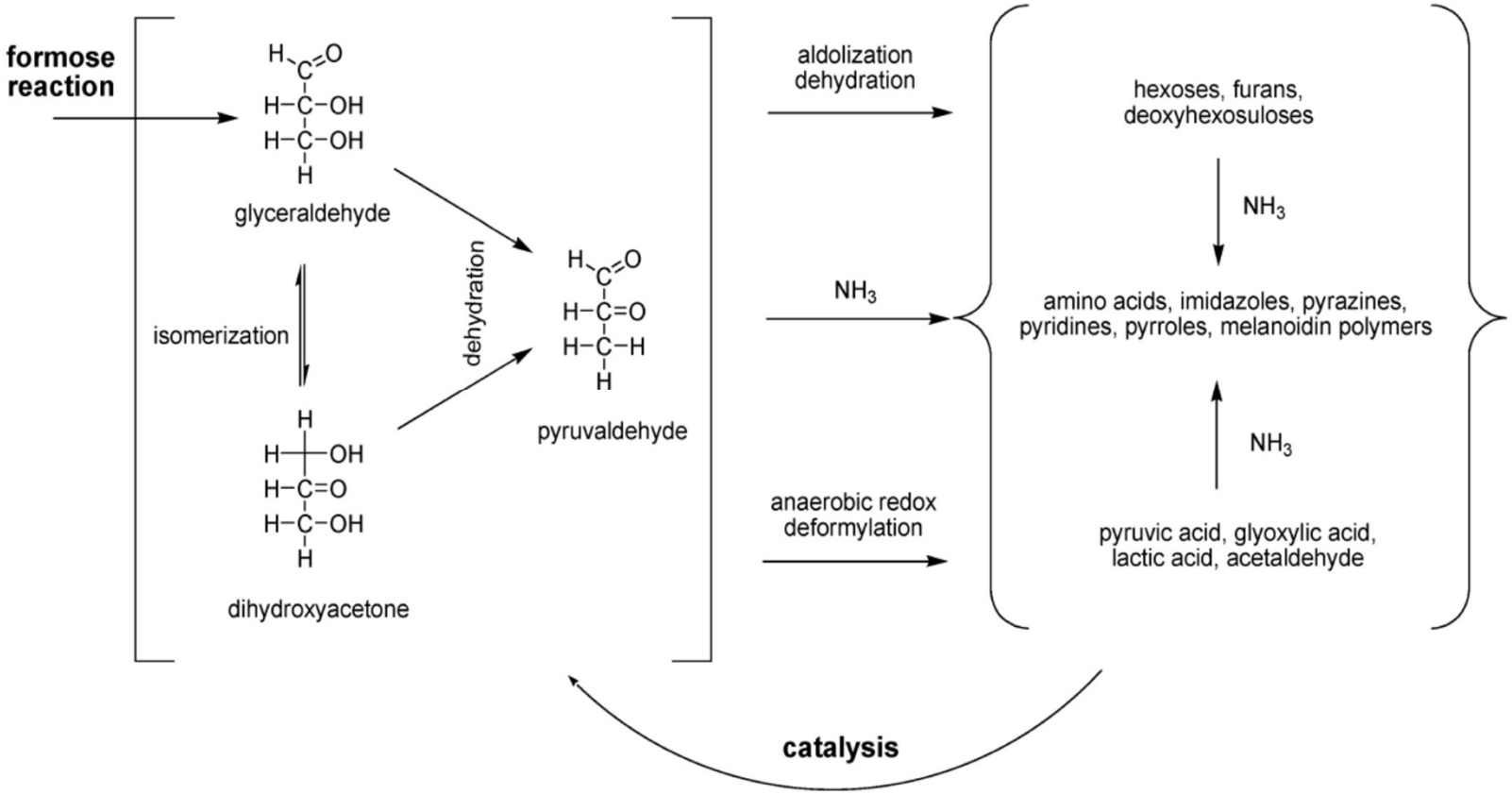


Formose reaction is one of the simplest autocatalytic cycles – two molecules of glycolaldehyde are produced from one.

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.

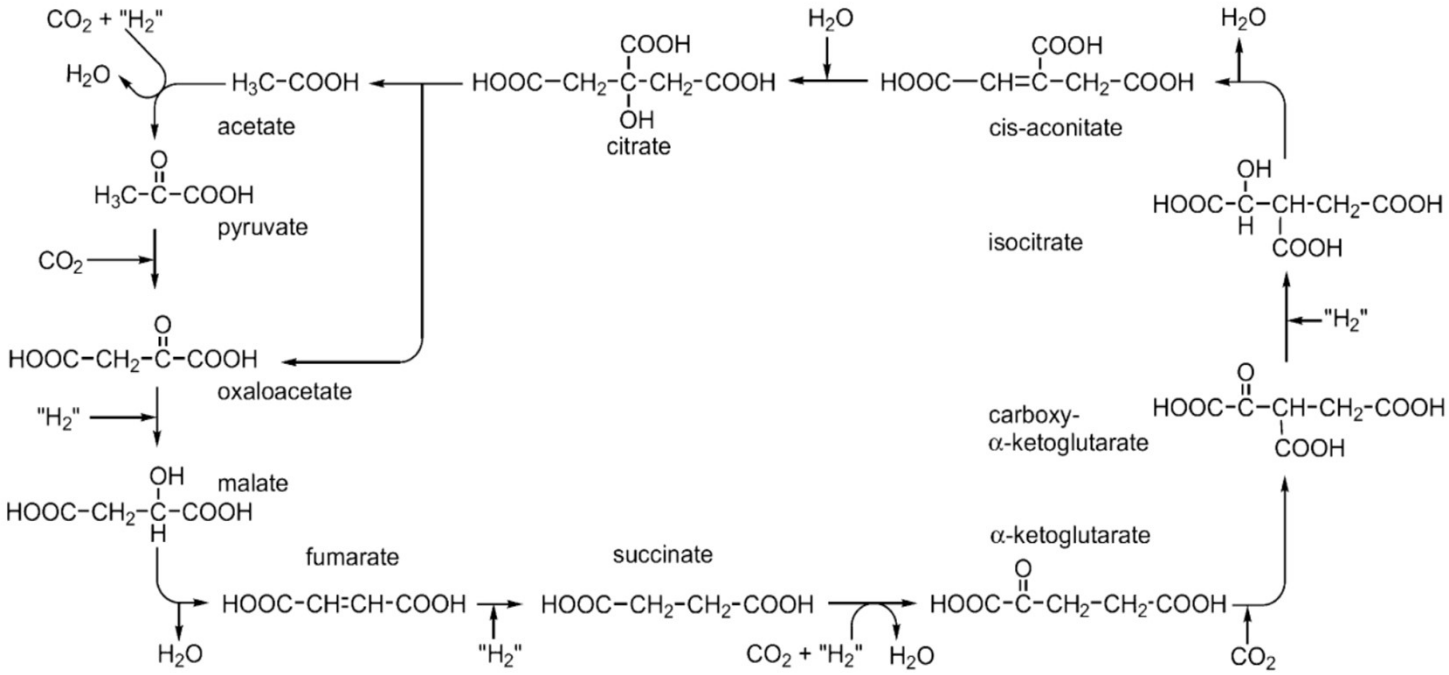


This additionally suggests that glycolysis was the ancient metabolic pathway

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



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 pathwaya 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

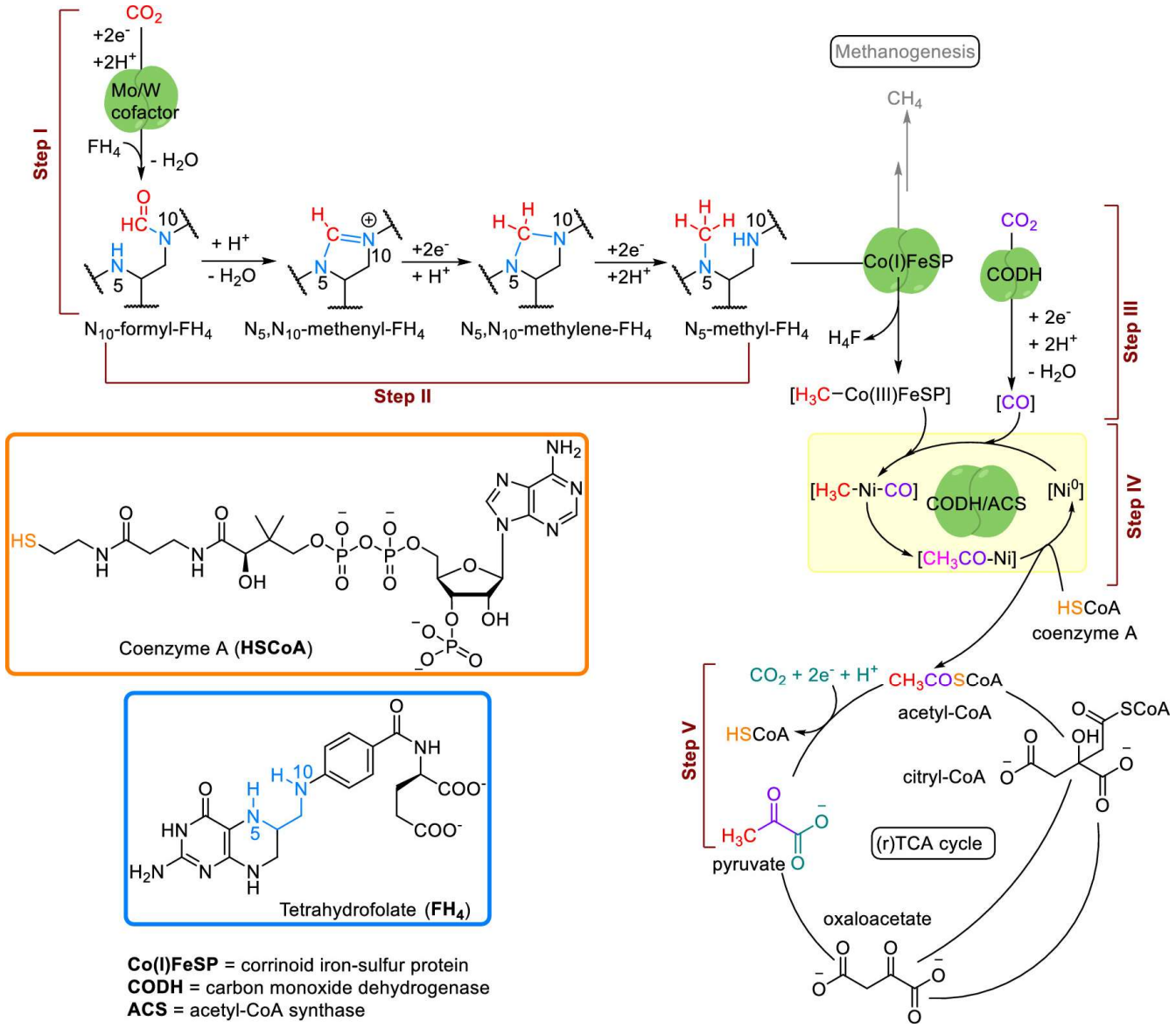
Protometabolic pathways

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



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**).

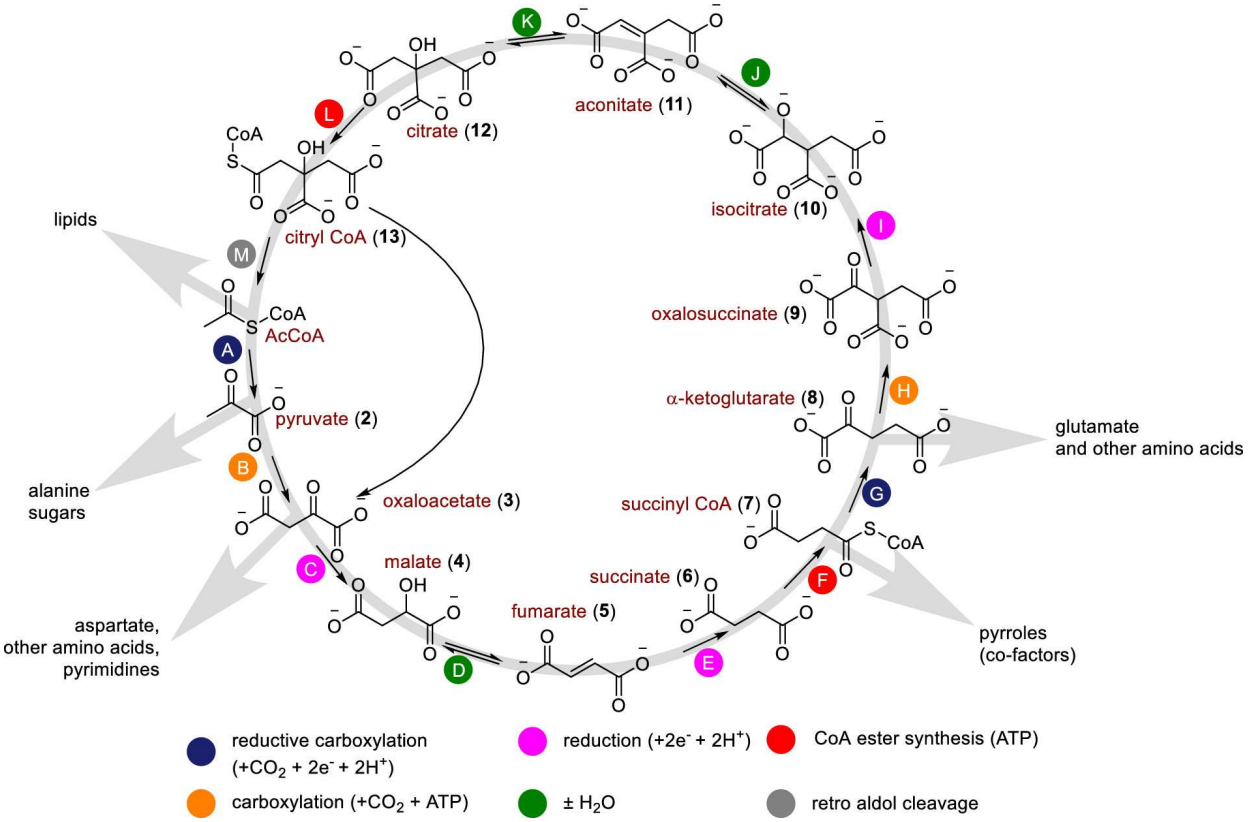
K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

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)



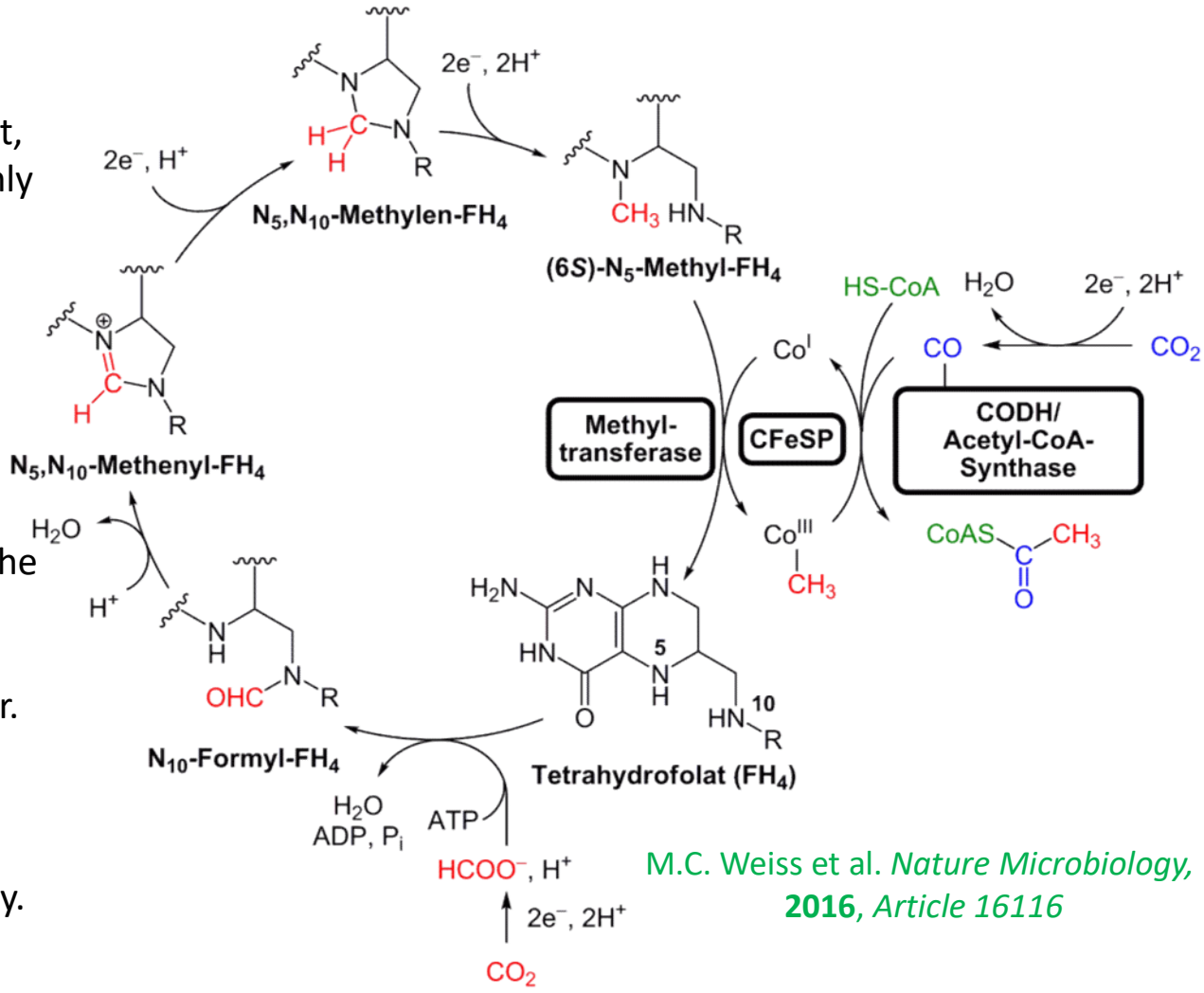
K. B. Muchowska, S. J. Varma, J. Moran *Chem. Rev.* **2020**, *120*, 7708−7744

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.

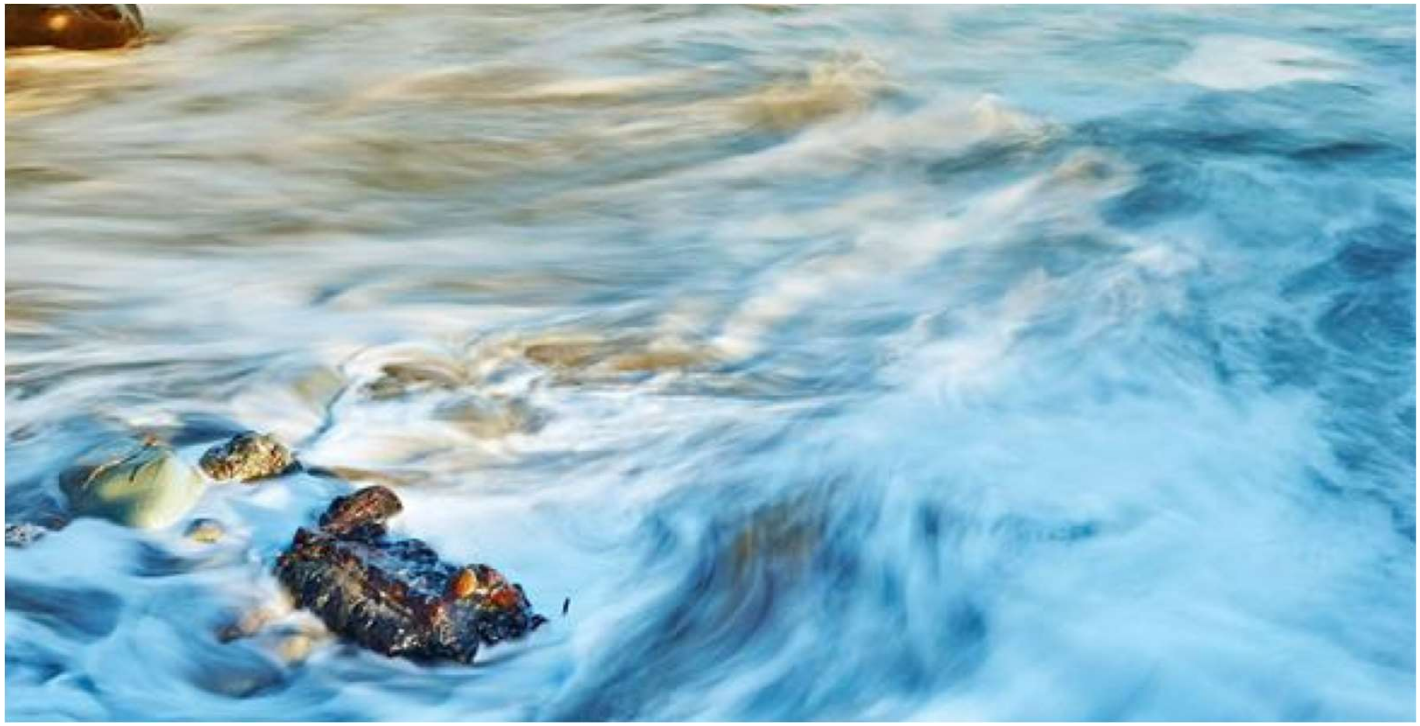
For all of these reasons, it is thought to be the most ancient CO_2 fixation pathway in life and is speculated to have its origins in prebiotic chemistry.



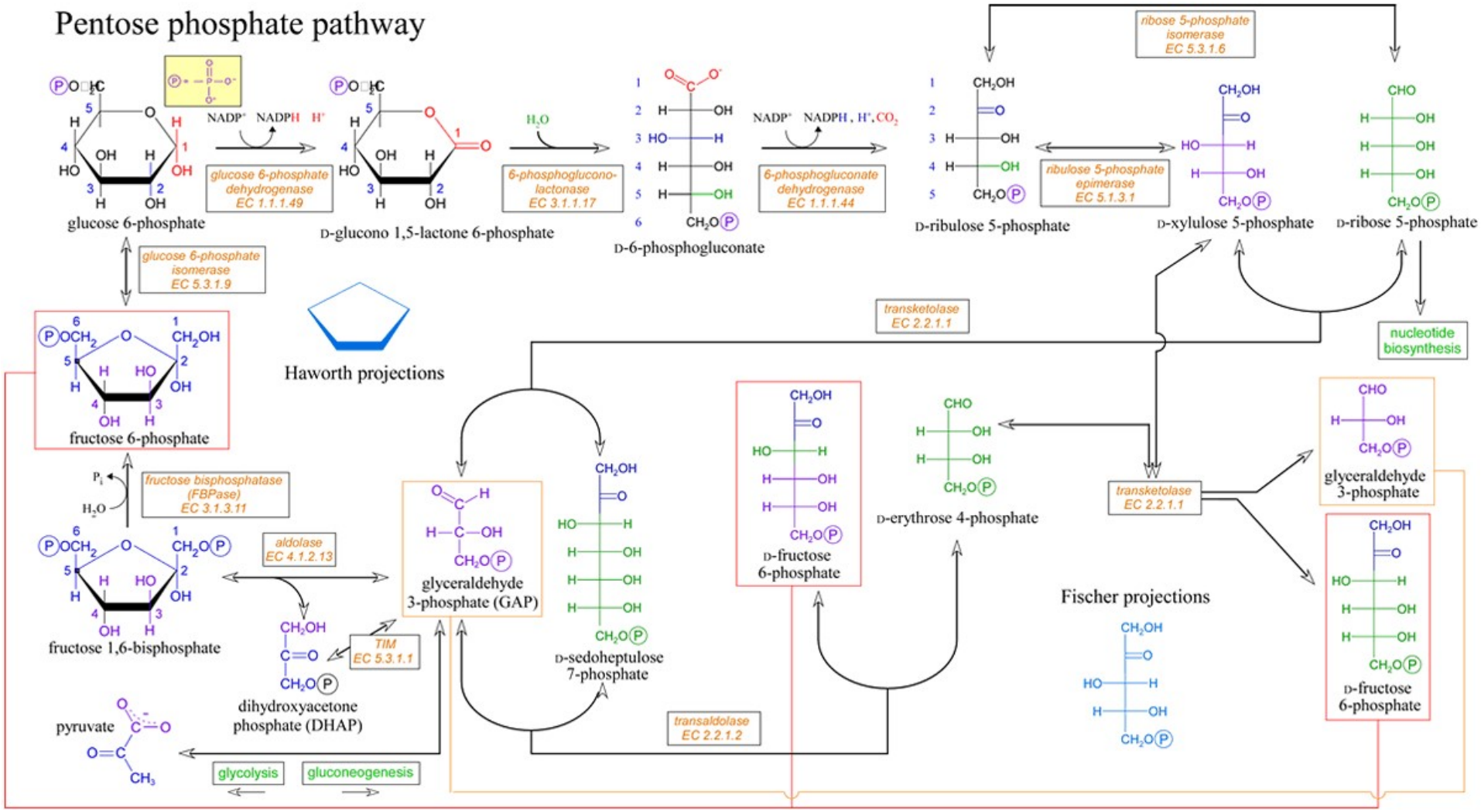
M.C. Weiss et al. *Nature Microbiology,* **2016**, *Article 16116*

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

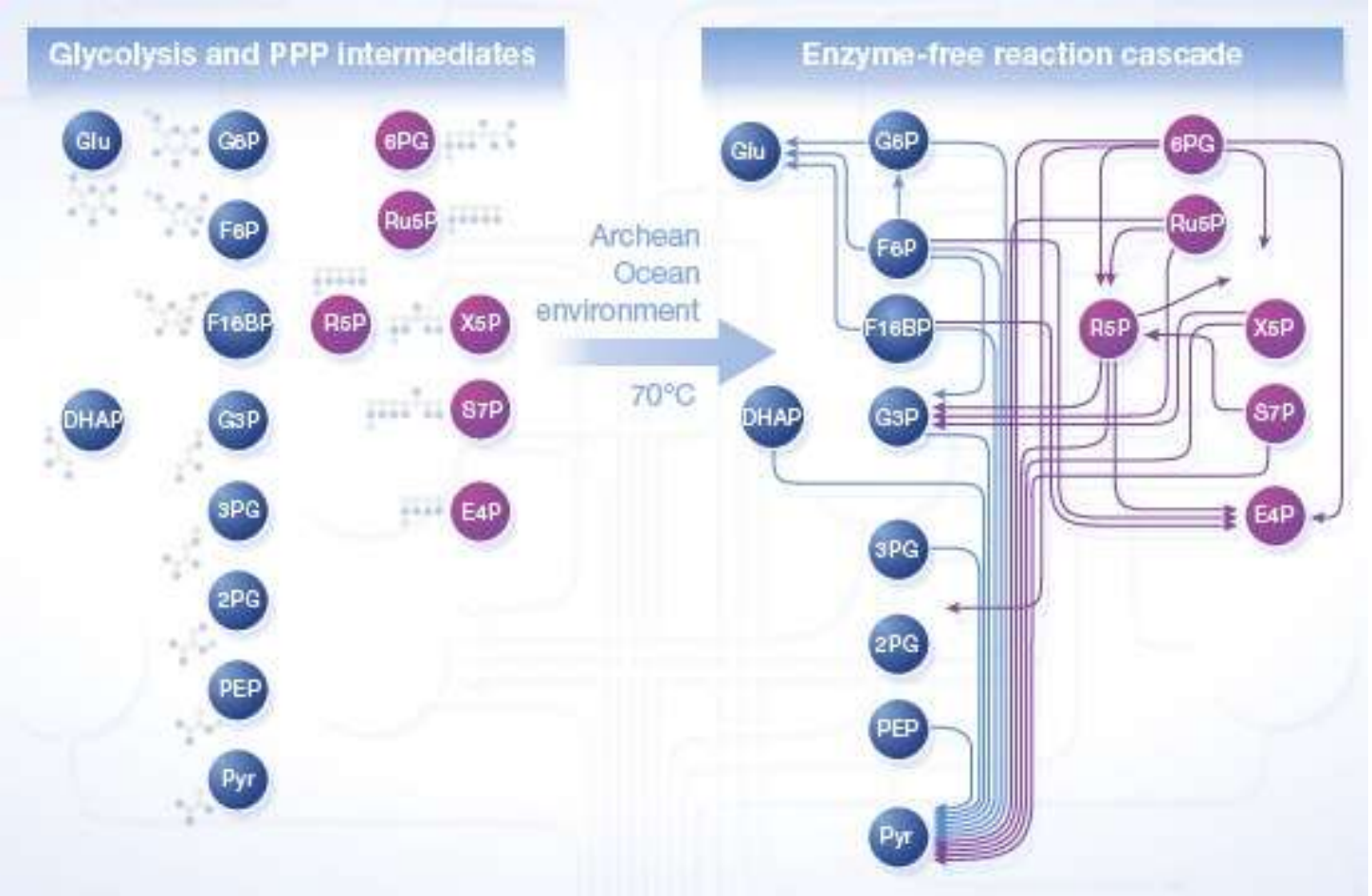
Metabolism may have started in our early oceans before the origin of life



Pentose phosphate pathway

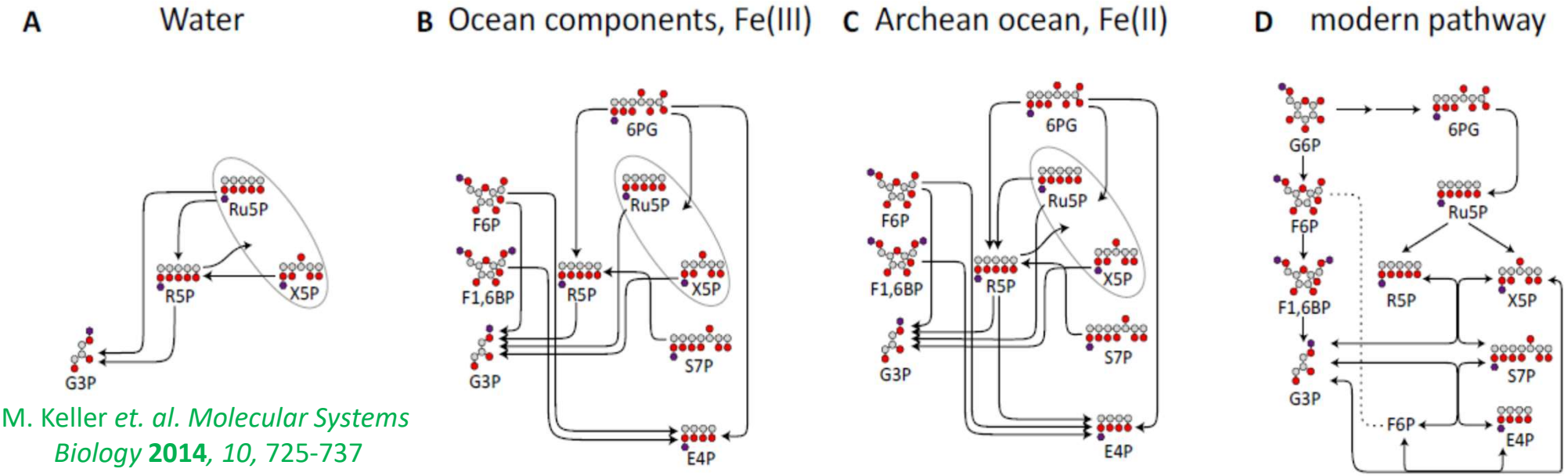


Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment



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.

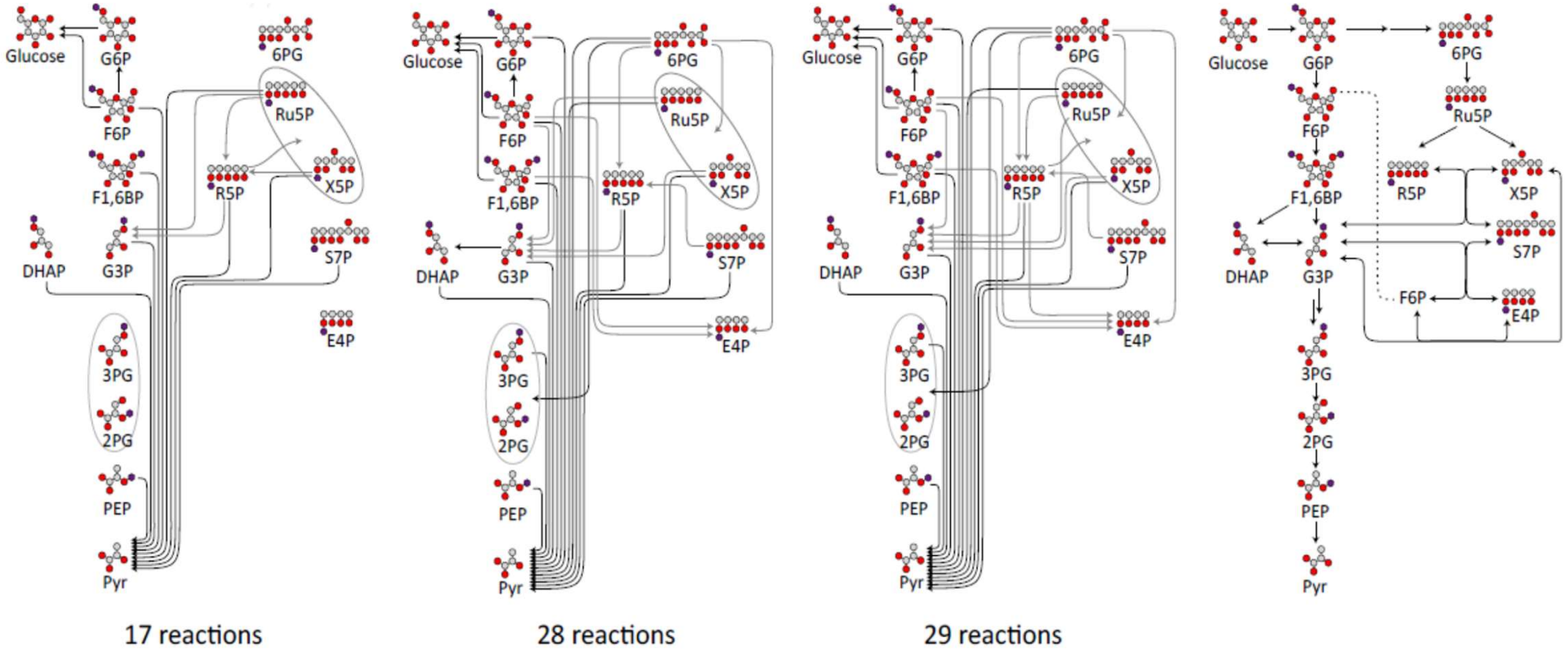
B The same reactions in solution with Fe^{III}, Co^{II}, Ni^{II}, Mo and phosphates simulating an Archean ocean.
In this milieu, 28 interconversion reactions among glycolytic and pentose phosphate pathway intermediates were observed.

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.

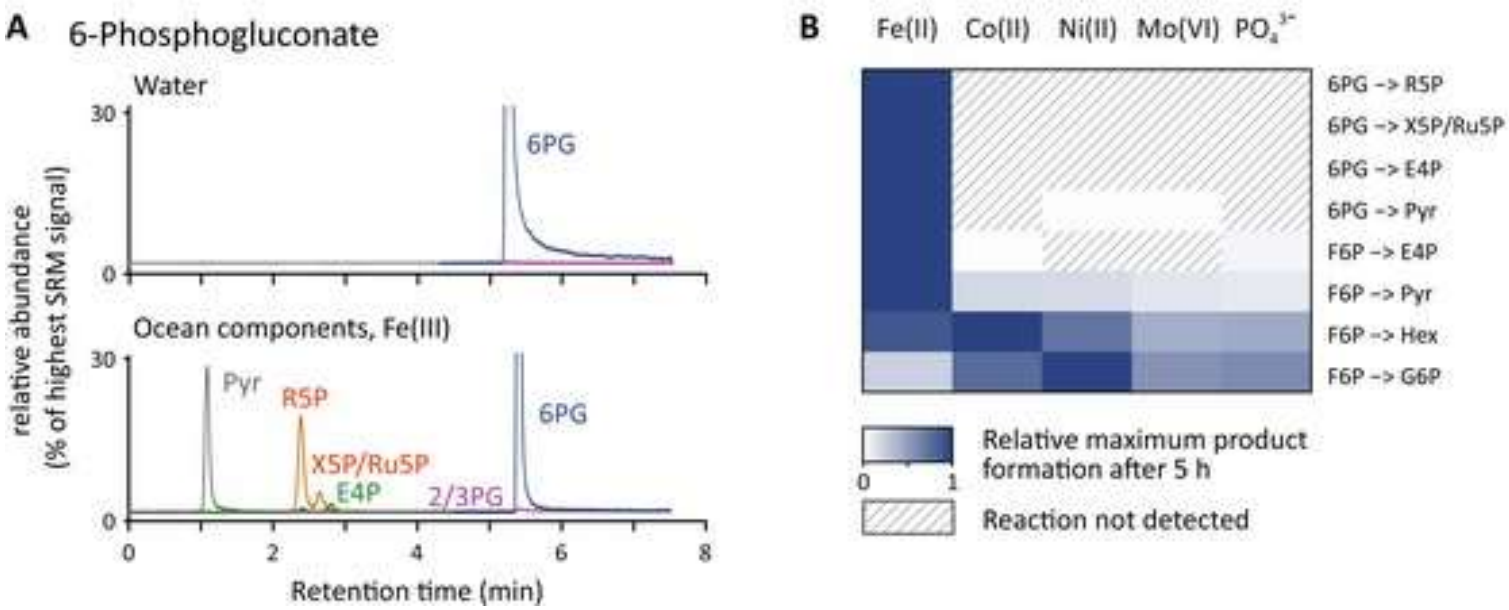
Pentose phosphate pathway: 6PG, 6-phosphogluconate; Ru5P, ribulose 5-phosphate; R5P, ribose 5-phosphate; X5P, xylulose 5-phosphate; S7P, sedoheptulose 7-phosphate; E4P, erythrose 4-phosphate.

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.

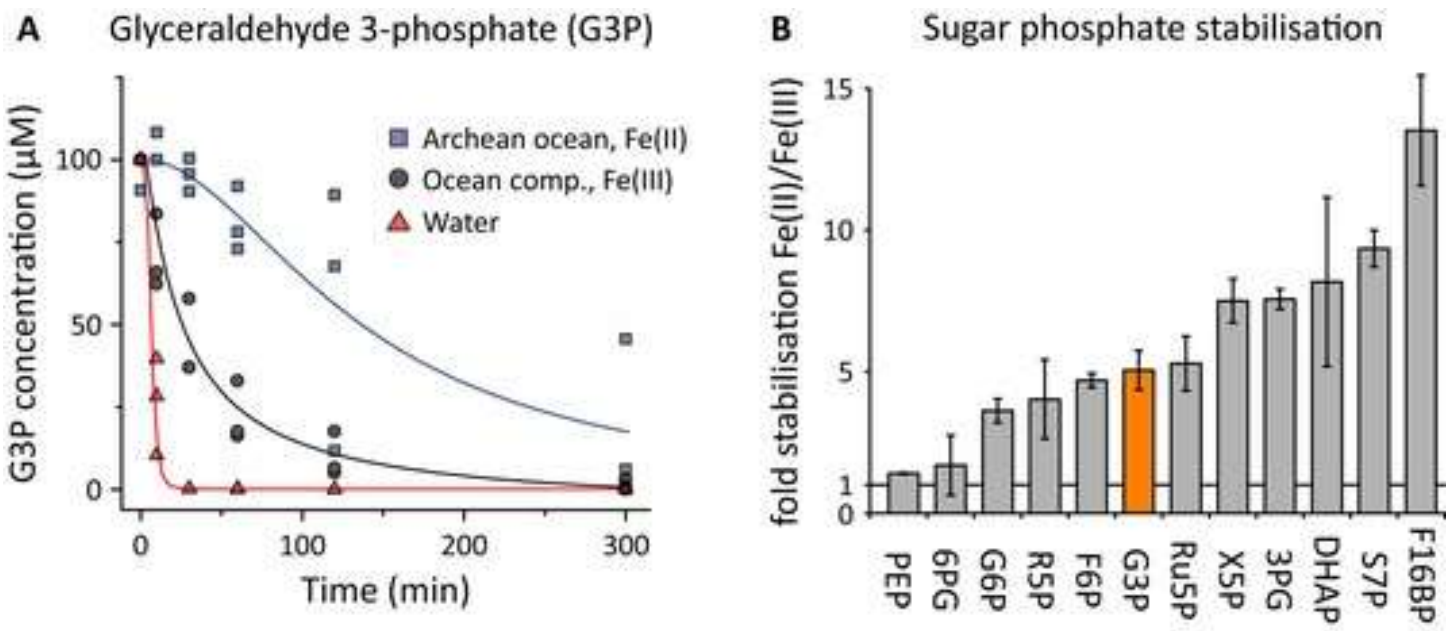
M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737



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.

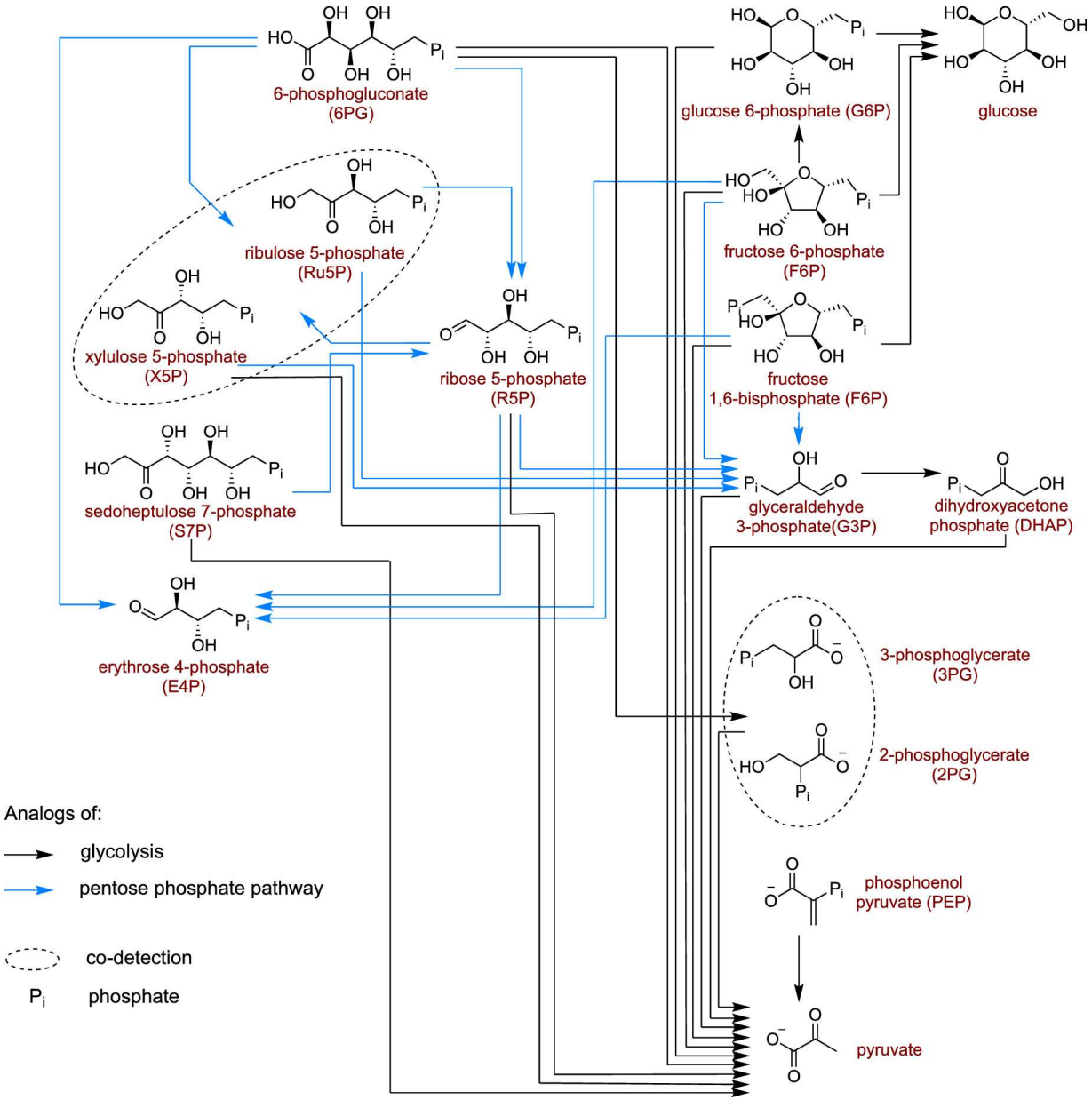
M. Keller *et. al. Molecular Systems Biology* **2014**, *10,* 725-737



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

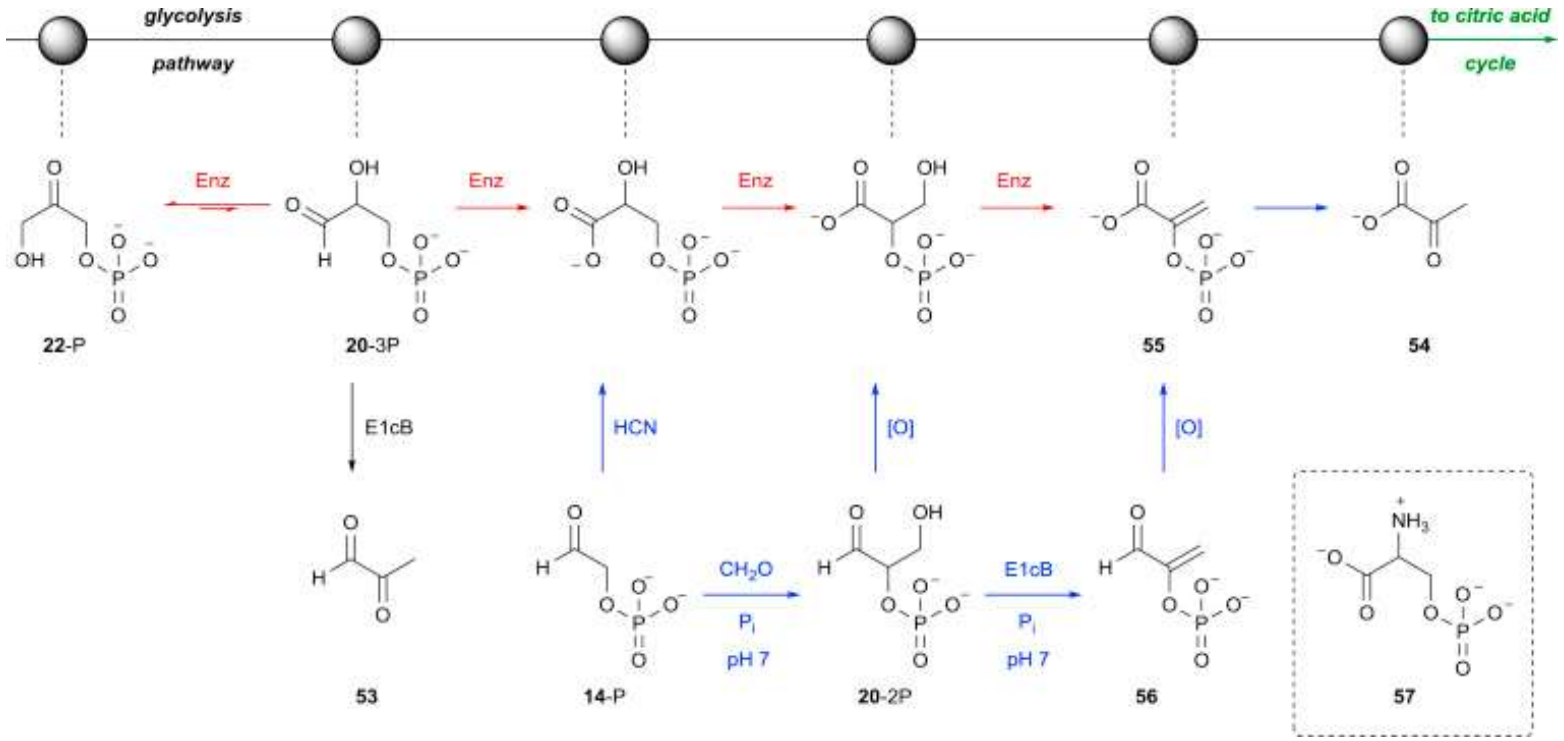


Nonenzymatic sugar phosphate interconversion in a plausible Archean ocean environment

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

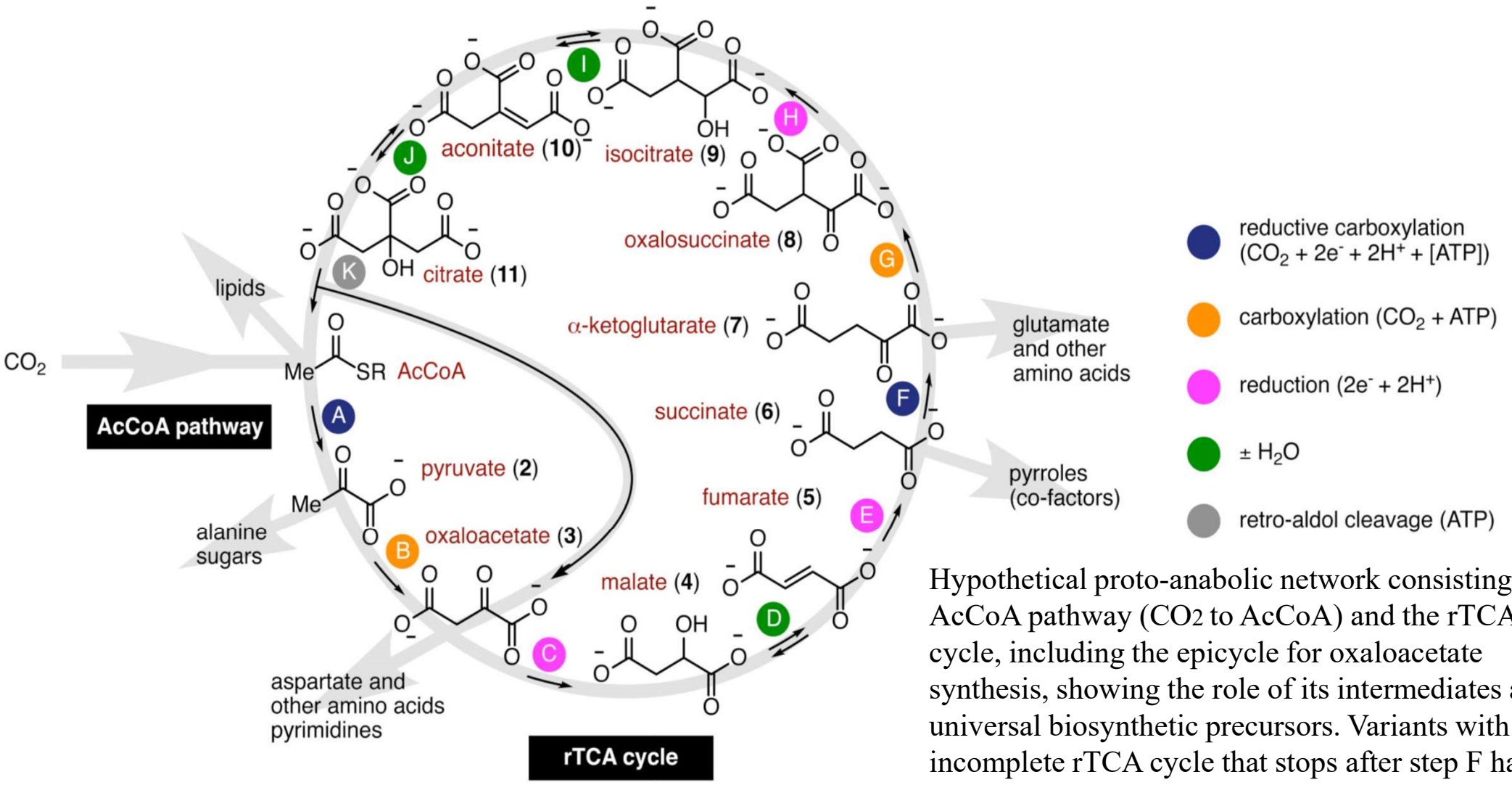
Prebiotic soup - summary

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



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

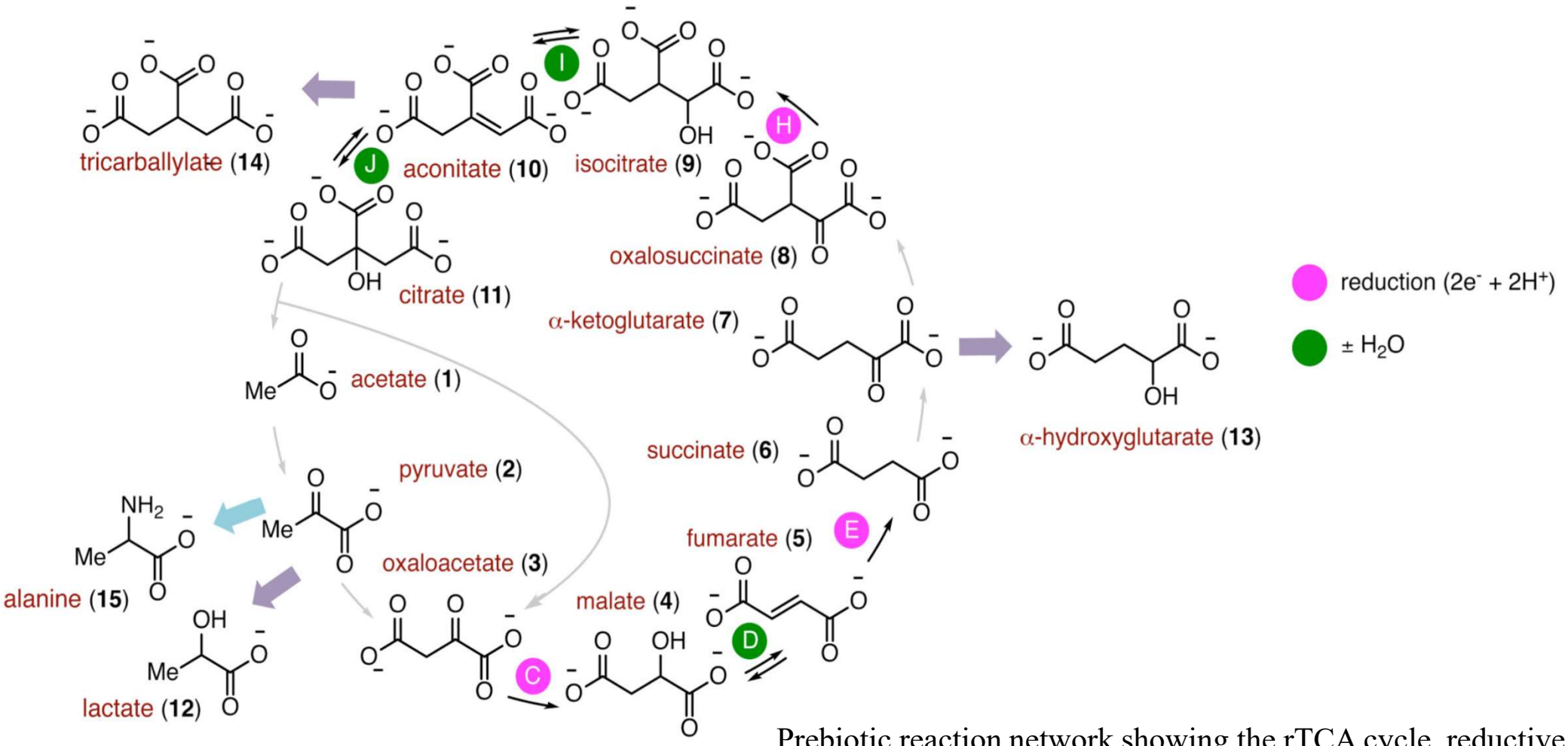
Metals promote sequences of the reverse Krebs cycle



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

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

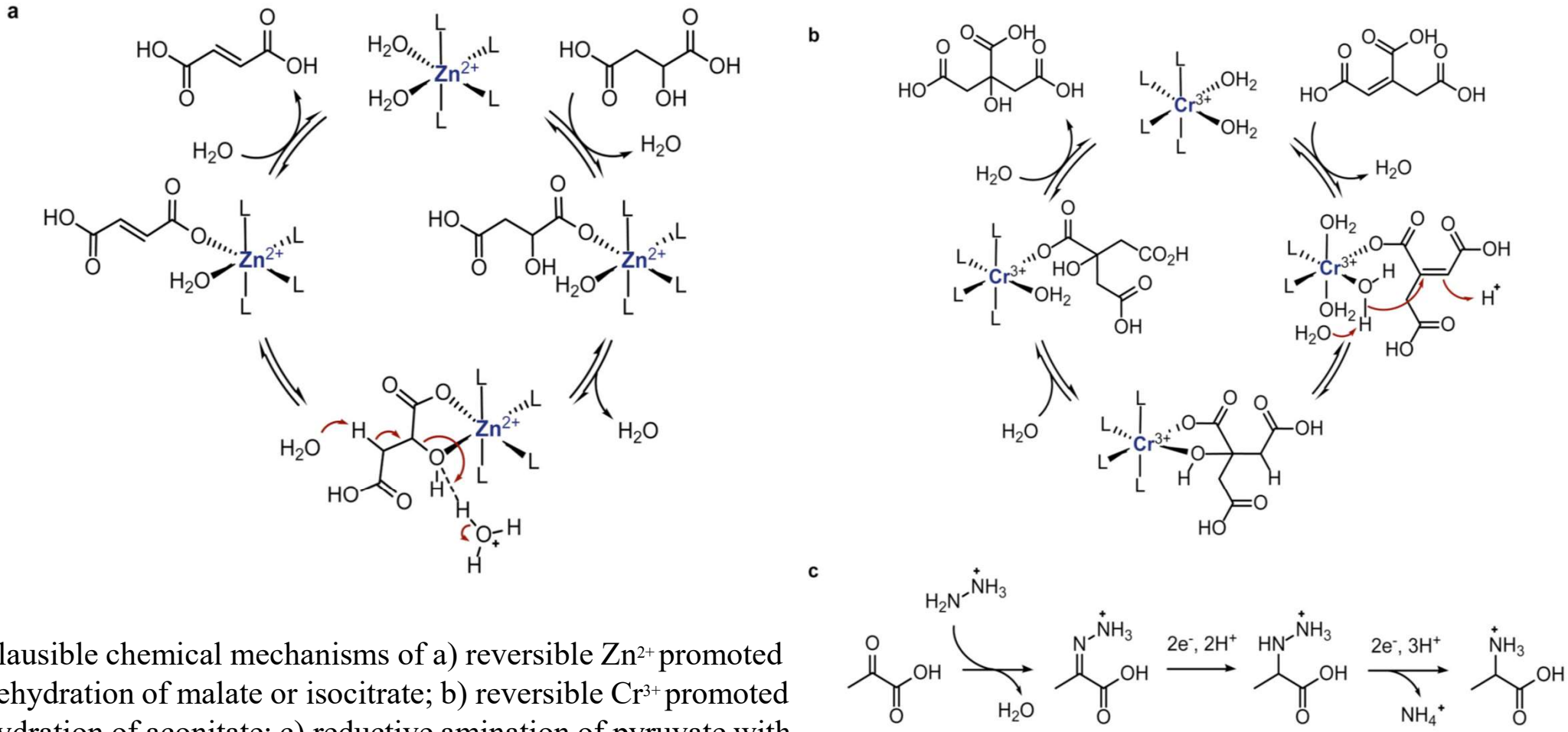
Metals promote sequences of the reverse Krebs cycle



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

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

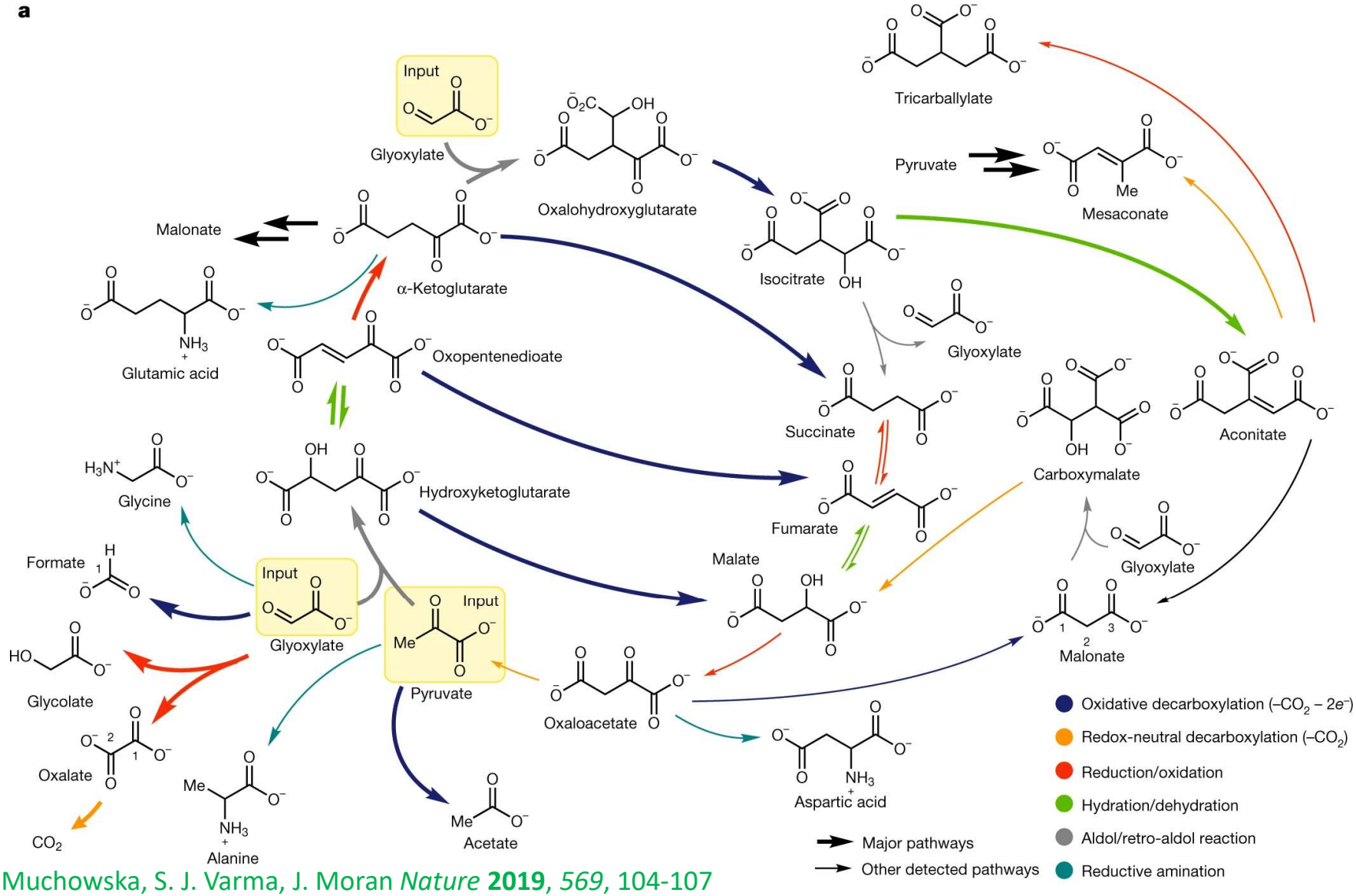
Metals promote sequences of the reverse Krebs cycle



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

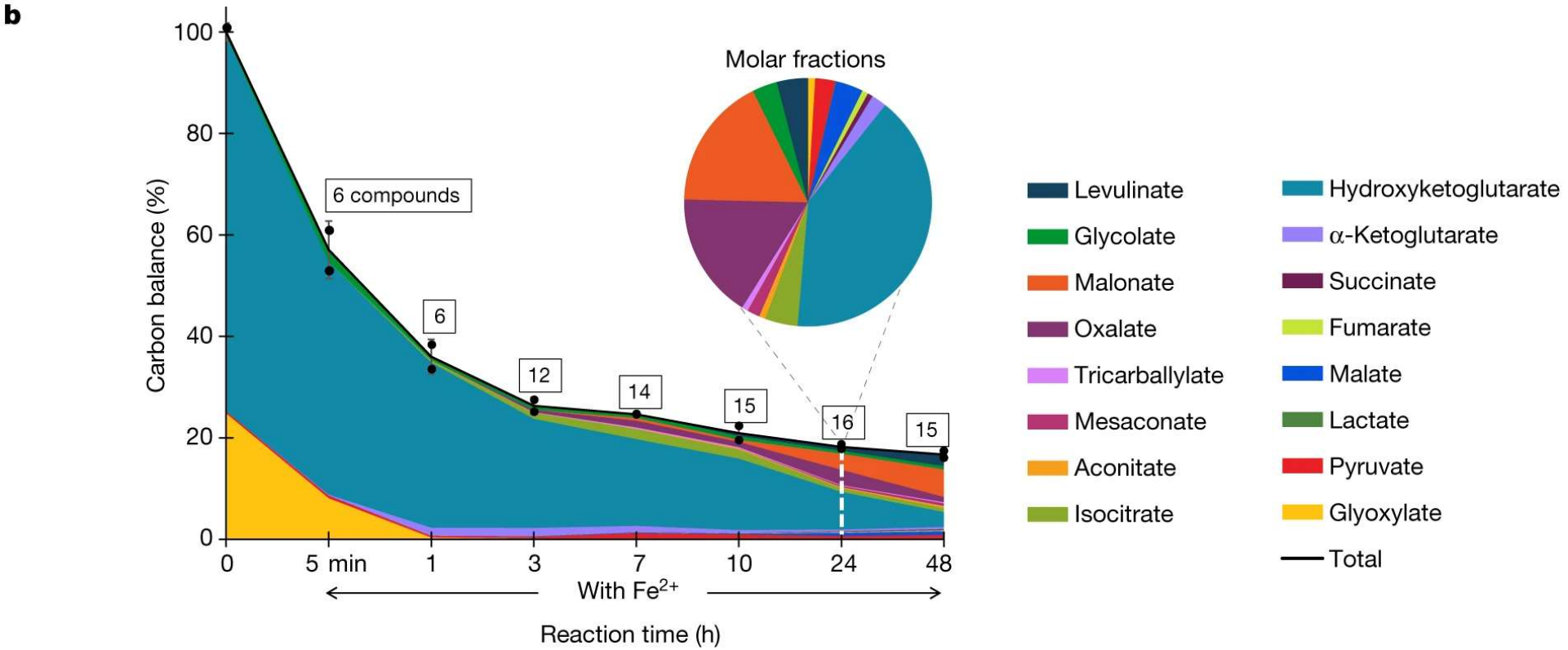
J. Moran *et al. Nat Ecol Evol.* **2017**, *1(11)*, 1716–1721

Synthesis and breakdown of universal metabolic precursors promoted by iron



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

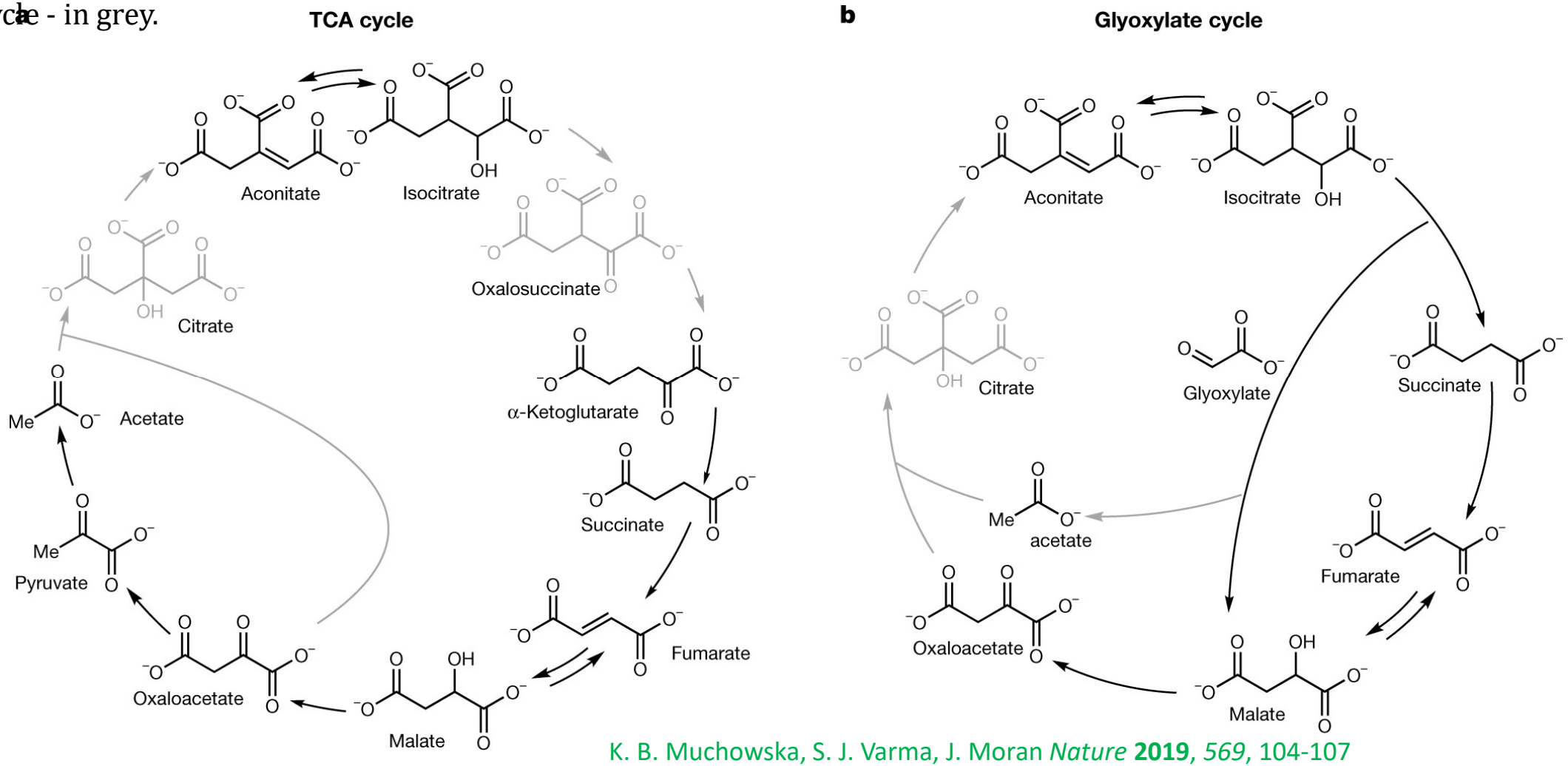
Synthesis and breakdown of universal metabolic precursors promoted by iron



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

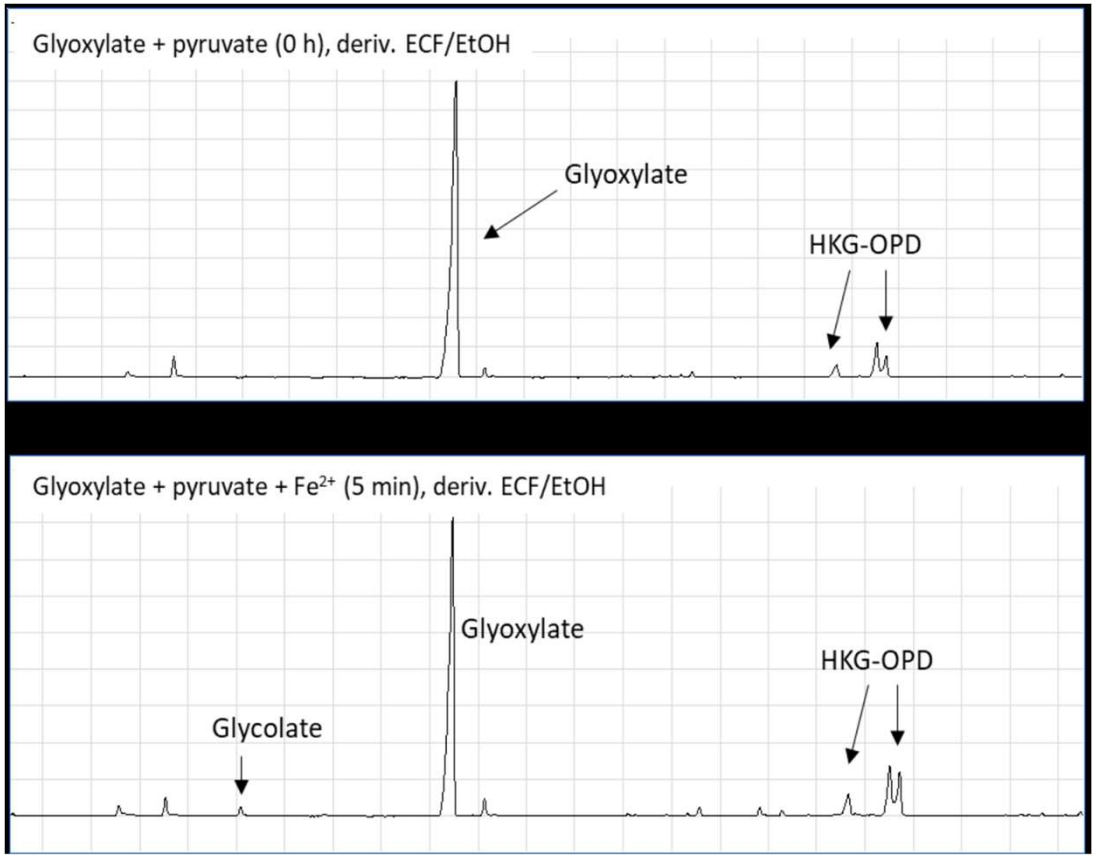
Synthesis and breakdown of universal metabolic precursors promoted by iron

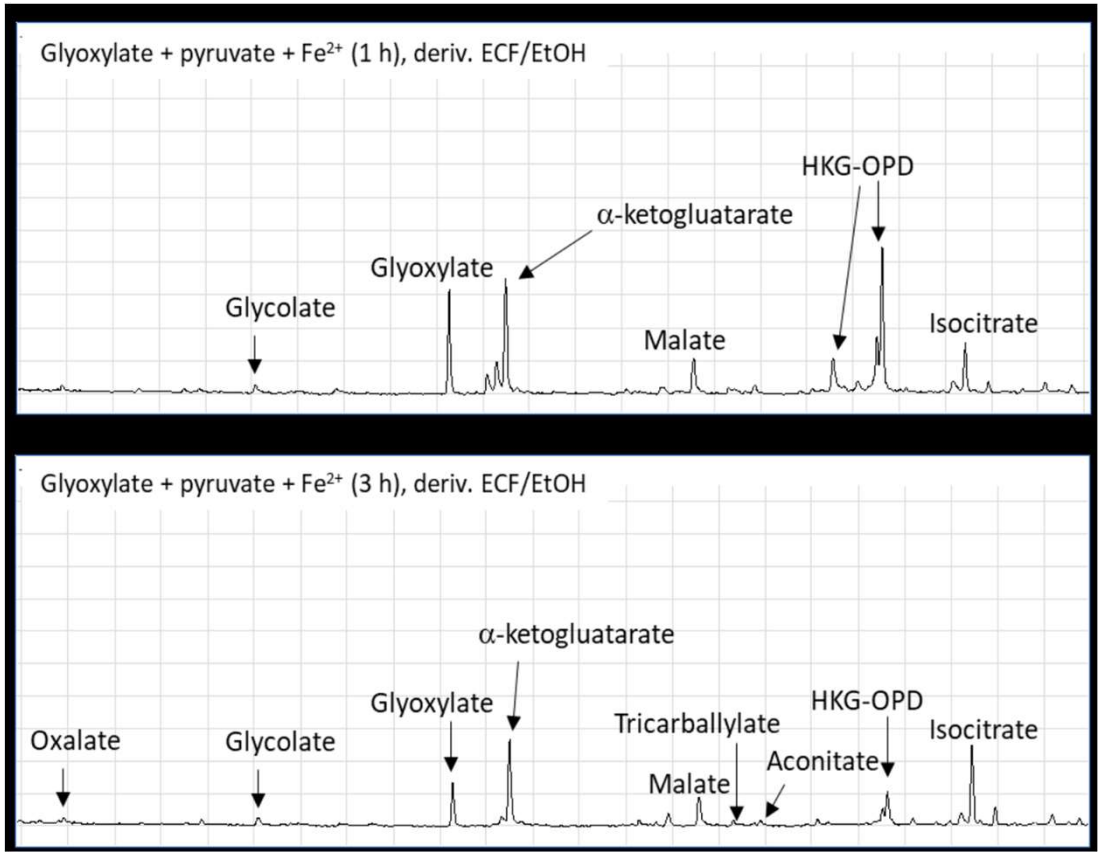
Comparison of the observed reaction network with the TCA and glyoxylate cycles. Intermediates and reactions found in both the biological cycle and the synthetic reaction network shown in black. Those found only in the biological cycle - in grey.



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

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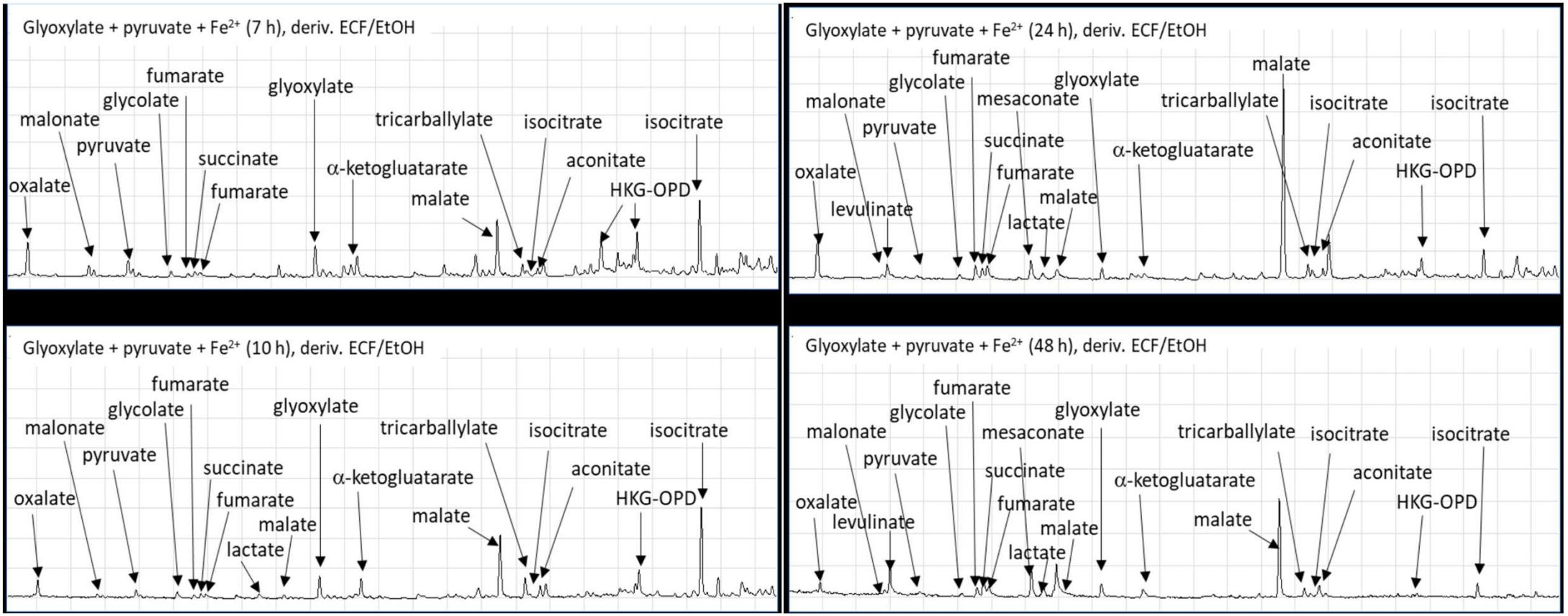

K. B. Muchowska, S. J. Varma, J. Moran *Nature* **2019**, *569*, 104-107

Metabolism-first - summary

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

Unclear chemical nature of primordial metabolic cycles

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

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