Non-enzymatic metabolic reactions and life's origins

Kamila B. Muchowska,[1*] Sreejith J. Varma,[1] and Joseph Moran[1*]

[1]University of Strasbourg, CNRS, ISIS UMR 7006, 67000 Strasbourg, France

*Correspondence: muchowska@unistra.fr, moran@unistra.fr

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ABSTRACT: Prebiotic chemistry aims to explain how the biochemistry of life as we know it came to be. Most efforts in this area have focused on provisioning compounds of importance to life by multi-step synthetic routes that do not resemble biochemistry. However, gaining insight into why core metabolism uses the molecules, reactions, pathways, and overall organization that it does requires us to consider molecules not only as synthetic end goals. Equally important are the dynamic processes that build them up and break them down. This perspective has led many researchers to the hypothesis that the first stage of the origin of life began with the onset of a primitive non-enzymatic version of metabolism, initially catalyzed by naturally occurring minerals and metal ions. This view of life's origins has come to be known as "metabolism first". Continuity with modern metabolism would require a primitive version of metabolism to build and break down ketoacids, sugars, amino acids, and ribonucleotides in much the same way as the pathways that do it today. This review discusses metabolic pathways of relevance to the origin of life in a manner accessible to chemists, and summarizes experiments suggesting several pathways might have their roots in prebiotic chemistry. Finally, key remaining milestones for the protometabolic hypothesis are highlighted.

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BIOGRAPHY

Kamila B. Muchowska

Kamila B. Muchowska is a CNRS research scientist at the Institut de Science et d'Ingénierie Supramoléculaires (University of Strasbourg & CNRS), associated with the Laboratory of Chemical Catalysis. She obtained her MSc Eng degree in chemical technology from the Gdansk University of Technology (Poland) in 2011. In 2015, she was awarded her PhD in physical organic chemistry (Prof. Scott Cockroft, University of Edinburgh, UK). After 3.5 years as a postdoctoral researcher with Prof. Joseph Moran, where she studied metabolism-like reactions potentially related to the origin of life on Earth, she joined the CNRS (French National Center for Scientific Research) in 2019 in the molecular biology/biochemistry section. Her research interests include prebiotic chemistry, evolutionary (bio)chemistry and complex systems.

Sreejith J. Varma

Sreejith J. Varma was born in Kochi, India. He completed his integrated BS-MS dual degree from IISER Pune, India. Shortly afterwards, he joined the group of Prof. Joseph Moran at the Institut de Science et d'Ingénierie Supramoléculaires (University of Strasbourg & CNRS) to pursue his doctoral studies. In 2019, he obtained an FRC Thesis Prize for his PhD thesis on the chemical origins of metabolic pathways. Currently, he is a postdoctoral researcher with Prof. Markus Ralser at the Charité Universitätsmedizin Berlin.

Joseph Moran

Joseph Moran is a Professor and director of the Laboratory of Chemical Catalysis at the Institut de Science et d'Ingénierie Supramoléculaires (University of Strasbourg & CNRS). Originally from Montréal, Canada, he completed his undergraduate studies and Ph.D. in chemistry at the University of Ottawa under Prof. André Beauchemin. After six months as a visiting researcher with Prof. John Pezacki at NRC Canada and two years as an NSERC postdoctoral fellow with Prof. Michael Krische at the University of Texas, he joined the Institut de Science et d'Ingénierie Supramoléculaires as an assistant professor in 2012 and was promoted to full professor in 2018. His research interests include systems chemistry that mimics metabolism, supramolecular catalysis, and the use of vibrational strong coupling in organic chemistry.

1. INTRODUCTION

Understanding how and why geochemistry transitioned into biochemistry at the origins of life is one of the grand challenges for the chemical sciences in the 21st century.[1] Over the past 65 years, "top-down" analysis of biological metabolism and genetics, as well as "bottom-up" experimental approaches involving simple chemistry have both tried to address this question. The former has advanced appealing theories and the latter has uncovered intriguing chemical hints. However, a lack of overlap between the two approaches has left those researching the chemical origins of life struggling to agree on a path forward. Each scientist working on the origins problem enters the area with their own viewpoints biased by their prior training, the present authors included. These diverging viewpoints have polarized researchers in prebiotic chemistry around three central fault-lines: 1) Whether genetics or metabolism began first and gave rise to the other; 2) The need for continuity between prebiotic chemistry and biochemistry; 3) Whether prebiotic chemistry began as a reaction network in which reactions were kinetically coupled or whether prebiotic synthesis scenarios involving sequential chance events should also be considered. Although these three points are, in principle, distinct, opinions on the first point tend to dictate those on others.

Those who follow a "genetics-first" approach to the origin of life assume that Darwinian selection is the most ancient principle of life. In this view, prebiotic chemistry should start with the synthesis of a genetic molecule, which then begins to self-replicate with change within a compartment. RNA is usually the genetic molecule of interest in this regard.[2–6] In addition to transmitting genetic information, it is thought that some variants of the early genetic molecule will begin to catalyze other chemical reactions, allowing some sequences to gain a selective advantage. Working together, a collection of genetic molecules eventually give rise to a metabolism resembling the one we know today. Proteins eventually replace RNA as the catalysts of choice and life continues to evolve from there. In this purest form of the "RNA World" view of the origin of life, all of life's biochemistry and metabolism that we know today, except for the structure of RNA, is invented relatively late as a by-product of RNA self-replication. If these big assumptions are correct, there is little need for invoking continuity between prebiotic chemistry and biochemistry before RNA-replication is up and running. Prebiotic chemistry might be very different from biochemistry as we know it. Indeed, it has been argued that since "biology almost always relies on chemistry that does not proceed efficiently in the absence of catalysis" and since "prebiotic chemistry must proceed of its own accord", that prebiotic chemistry "must generally be different from the underlying chemistry used in biology."[7] This analysis logically implies that naturally abundant catalysts wouldn't have been available for prebiotic chemistry. In this working hypothesis, a system needs to be continuously provided with the building blocks of RNA and of compartments until it evolves the ability to build its own components. In principle, the prebiotic synthesis of these building blocks could be delivered by discontinuous processes that are contingent upon chance events, but they would need to occur in a regular, sustained manner over long periods of time. Aware of these limitations, prebiotic chemists working in an RNA world framework generally accept scenarios in which different streams of reactants perform different chemistries, each flowing together at just the right time and rate to enable a sequence of mutually incompatible chemical reactions. Ultimately, this chain of improbable events gives rise to a synthetic sequence producing the building blocks of RNA or compartments.[8,9] A large fraction of prebiotic chemistry research published over the past 65 years, even if not explicitly performed in the guise of an RNA world scenario, fits into this broad conception and has been reviewed many times from different angles.[10–13]

There has been no shortage of criticism for the approach to the origin of life described above, and by proxy, much experimental research on prebiotic chemistry.[14-16] Some is related to the fact that, by prioritizing one single characteristic of life above all others, namely the ability to self-replicate and undergo Darwinian selection, "genetics-first" theories provide very little explanatory power about why other aspects of life work the way they do. Below, we briefly explore these considerations.

First, focusing exclusively on the synthesis of the building blocks of RNA and cellular compartments fails to capture several of life's other equally essential characteristics. Life is not just a collection of compounds, but also a collection of processes, which are collectively described as metabolism.[17] These include synthesis (anabolism), breakdown (catabolism), and energy capture and transmission (energy conservation), among others. Life seems to manifest itself in almost endless varieties, but just as remarkable is the extent to which certain aspects of biochemistry show regularities or have eluded innovation despite roughly four billion years of evolution. Why does life ultimately build itself and break itself down through only five metabolic intermediates all made of C, H, and O? Why are there only six pathways of autotrophic CO_2 fixation, with four of them being very similar? Why are the enzymes in some of these pathways almost totally reliant on transition metals for catalysis? Why does nitrogen enter metabolism through glutamic acid and glutamine and not the other 18 proteinogenic amino acids? Why are amino acids built through transamination of ketoacids and not through the Strecker reaction? Why are sugars built through gluconeogenesis and not the formose reaction, which is in many ways much simpler? Why are nucleobases built from amino acids and not by oligomerization of HCN? It is not yet clear whether the answers to these questions lie in chance "frozen accidents" of prebiotic chemistry that were too difficult to displace in a complex system[18,19] or whether they became ubiquitous due to convergence towards an optimal chemical solution.[20] It could well be that the processes by which life makes and breaks down its transient metabolic intermediates are just as fundamental to its nature and origin as RNA or cellular compartments. Viewed from this perspective, molecules like ribonucleotides may not be an end-goal of metabolism so much as a happy byproduct that turned out to be functional. If this is the case, then some biochemical pathways should be more ancient and more likely to have emerged from prebiotic chemistry. Synthetic chemists wishing to tackle the origins problem should therefore be aware of biosynthetic pathways and the latest thinking with regards to their evolution. An understanding of biochemistry and its context within biological evolution is critical to understanding the nature of the problem to be solved.

Second, a focus on stepwise synthesis fails to capture life's dynamic kinetic nature. Life's chemical processes form complex networks in which its subsystems are kinetically coupled to each other, even if the different chemistries happen in different physical locations within an organism. For this reason, many researchers are looking for prebiotic chemistry with subsystems that can network and interact in a similar way, which requires its components to remain kinetically coupled. To be kept in a non-equilibrium state, the environment would need to

be subject to, for example, gradients of redox potential, pH, temperature, or pressure. It is important to distinguish such a non-equilibrium environment from scenarios in which stochastic events or step-changes in conditions are required, such that each chemical sub-system reaches equilibrium before proceeding to the next stochastic event or before interacting with another sub-system. These "sequential chance event" scenarios cannot achieve kinetic coupling between sub-systems, and thus lack one of life's critical features.[14] They are also unlikely to occur without human intervention.[21] Some have argued that highly unlikely sequences of events should not be ruled out because life itself might be a rare occurrence,[22] but ignoring constraints on probabilities makes it impossible to rationally discriminate between competing hypotheses.

A predominant alternative view of life's origins is known as the "metabolism-first" hypothesis.[23–31] In this view, a self-organized complex reaction network, likely aided by naturally occurring metal or mineral catalysts, emerged as a means to dissipate free energy in its environment.[32] This chemical network would have provided a foundation from which polymer-based self-replicators, catalysts and organic compartments later emerged. This network should have some character that allows for its self-amplification.[33,34] This review is written from the specific perspective that the reaction network that gave rise to biological metabolism would have needed to be similar in terms of its "substrates, reaction pathways, catalysts or energy coupling".[35] Such a protometabolism would provide an up-front explanation for why life's biochemistry is the way it is. It would also make the transition from a protometabolism to metabolism quite straightforward.[36] It is, by definition, chemistry that is compatible with metabolism and already provides the starting materials and reactions on which catalysts, whether RNA- or protein-based, could eventually act and be selected (more on this in Section 2.1).[37] This is not only theory. There is historical evidence that it is hard to transition to a fundamentally different reaction network – life hasn't ever done it as far back as we have evidence. Evolutionary biologists might describe this phenomenon as a "frozen metabolic accident",[19,38] a variation of the "frozen accident" term historically coined by Crick to describe the universality of the genetic code.[39] In short, once a (bio)chemical system begins to harbor multiple connected subsystems, it becomes essentially unalterable, because a significant change would reverberate through all interconnected subsystems. This would be true for purely chemical complex reaction networks as for biological ones. Estimates for the appearance of the Last Universal Common Ancestor to all life (LUCA) date to around at least 3.5 billion years ago,[40] if not earlier.[41] Attempts to extract LUCA's genes using "big data" approaches, although still debated,[42] suggest that its core biochemistry does not seem unfamiliar, in the sense that LUCA's metabolism involved a hydrogen-dependent CO_2-fixation pathway and used cofactors and reactions widely present in biology today.[43] If the chemistry underlying metabolism is so easy to fundamentally change on short timescales, such that it is unrecognizable from modern biochemistry, it is puzzling why it would have changed so little in the subsequent 3.5 billion years. The alternative is that it has not drastically changed.

From an experimental standpoint, the "metabolism-first" approach to prebiotic chemistry involves the search for naturally occurring catalysts and conditions that allow chemistry resembling core metabolic pathways to occur without enzymes. Ideally, these should occur under mutually compatible conditions, that might plausibly exist along a non-equilibrium environment. The end-goal of this approach would be to reconstruct a complete non-enzymatic metabolism that produces the main sub-systems of biochemistry, including genetic molecules such as RNA. Compared to the "genetics-first" hypothesis, much less experimental work has been done along the lines of the "metabolism-first" view of the origin of life. Neither approach has yet to demonstrate how it leads to the properties of life considered to be the most fundamental by the other viewpoint: experiments done in a "metabolism-first" context have not demonstrated the emergence of genetic molecules, nor have experiments done in a "genetics-first" context demonstrated the emergence of metabolism. For a chemist entering the origin of life field, it can be difficult to distinguish between competing hypotheses when none yet offer a complete account or have yet to be fully developed experimentally. However, it is good to keep in mind that the goal of prebiotic chemistry is to explain the origins of biochemistry as we know it, rather than a hypothetical alternative. In other words, when chemistry that is very different from the biochemistry we know today is put forward as the chemistry that led to life, the burden of proof lies with the claimant to demonstrate experimentally how the transition to the biochemistry we know today occurred. Without demonstrating such a transition and the "missing links", experiments do not carry any explanatory power with regards to the origin of life and remain exclusively proofs of principle in the chemical domain. Similar scrutiny applies to those who argue that prebiotic chemistry mirrored modern biochemistry. Experiments showing that such chemistry is possible without enzymes must be demonstrated.[44] However, once such experiments exist, the explanatory power with regards to the origin of life is significantly easier to support.

The goal of this review is to aid newcomers to the "metabolism-first approach" by describing the biological pathways that are most likely to have their roots in prebiotic chemistry, summarizing the experimental work that has been done to assess those ideas, and outlining the key places where future work could bring increased insight. We have tried our best to do this in a way accessible to chemists by keeping biochemical jargon and acronyms to a minimum and by focusing on the reactivity patterns and metabolic intermediates that life uses to build up its biochemistry. We discuss the minimal subset of metabolic subsystems that, in our view, must have existed to give rise to genetics and later Darwinian selection, including central carbon metabolism and the biosynthesis of amino acids, sugars and ribonucleotides. We then summarize the chemical experiments that probe the prebiotic plausibility of these pathways and that bring insight into their origins. Finally, we try to chart a path forward for how chemists can advance the field in a biologically meaningful way.

Biochemistry is vast, and we must naturally focus on some of its aspects and omit others. Notably, we omit discussions of the biosynthesis of fatty acids. Despite several works describing the potentially prebiotic Lipid World,[45–50] predominantly relying on Fischer-Tropsch-like geochemical processes furnishing long-chain amphiphiles and hydrocarbons,[51] experimental work that recapitulates non-enzymatic fatty acid biosynthesis has been scarce. Another reason for the omission of fatty acids is that their potential contribution to the compartmentalization observed in cells, if it was required at the origin of life, might have been achieved by other means, including inorganic compartments in rocks or minerals or by other types of organics present in a protometabolism.[52–57] Certainly, this should not discourage the readers from undertaking experimental efforts into

biochemistry-inspired abiotic fatty acid synthesis! Additionally, we do not discuss the biosynthesis of cofactors, whether organic or inorganic,[58,59] as this has been covered in a prebiotic context in a recent review.[60] Finally, we omit the geological context of "metabolism-first" theories of the origin of life, as this has been reviewed and speculated upon many times.[61–63]

2. TERMINOLOGY AND MODERN THINKING ON THE EVOLUTION AND ORGANIZATION OF METABOLISM

2.1. Mechanisms for the evolution of biochemical pathways

How biochemical pathways emerged and evolved has been widely discussed and several hypotheses proposed. This has recently been reviewed in detail by Tawfik and co-workers,[64] and will be briefly summarized here.

The earliest model—called retrograde evolution—was put forward by Horowitz in 1945.[36] The Horowitz hypothesis assumes that an organism capable of Darwinian evolution already existed before the emergence of the metabolic pathway in question. In such a case, a metabolite that provides a useful function to the organism will be depleted by this function. The resulting positive evolutionary selection pressure for this metabolite leads to the emergence of an enzyme catalyzing its synthesis from a compound already existing in the surrounding environment, which Horowitz assumed would be produced by some abiotic means.[36] The repetition of this process eventually leads to the emergence of a new metabolic pathway in the reverse (retrograde) direction to its synthesis (Figure 1a). However, it remains unclear how and why an abiotic process would have produced all of the starting materials, intermediates and end-products for the pathway in the first place. Overall, it seems that retrograde evolution would be most likely to occur at a stage of biological evolution after the origin of life.

In a later forward pathway evolution model, Granick proposed the stepwise recruitment of subsequent steps of a pathway.[65] In this paradigm, biochemical pathways became extended one step at a time, from smaller biosynthetic chains, and that every intermediate was once a functional end-product (Figure 1b). To create evolutionary selection pressure, the Granick hypothesis requires that each innovation within a metabolic pathway produces useful metabolites. Subsequently, Yčas[66] and Jensen[67] noticed that biochemical pathways might have evolved from common ancestors that utilized promiscuous enzymes leading to different end-products ("generalists-to-specialists", Figure 1c). In this scenario, chemically identical transformations do not necessarily imply a shared evolutionary precursor, as transformations might have evolved independently due to being chemically essential. For this reason, phylogenetic analyses are indispensable to decipher the evolutionary history of a pathway.[64] Finally, the phenomenon of promiscuous enzymes was employed in the "patchwork assembly" model by Lazcano and Miller.[68] 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 (Figure 1d). The current view leans towards the patchwork hypothesis being the most prevalent in extant biochemistry, since most metabolic pathways employ enzymes of different evolutionary origins. However, this does not always imply that the patchwork hypothesis was applicable to the pathways' prebiotic beginning.[64]

To settle the apparent discrepancies between the above hypotheses, Tawfik and co-workers proposed a new, integrated metabolite-enzyme co-evolution model. According to their hypothesis, side products originating from either the activity of promiscuous enzymes or from non-enzymatic reactions (the so-called "underground metabolism"[69]) provide evolutionary stepping stones for the emergence of specialized enzymes that make these products (Figure 1e). Non-enzymatic reactions are particularly important here, as they are likely to have helped new enzymatic pathways emerge both at the origin of life and at later evolutionary stages. The assumption that new pathways emerge from pre-existing enzyme-free transformations means that the simultaneous invention of multiple new enzymes is no longer required. On the contrary, Tawfik and co-workers bring up the stepwise, gradual optimization ("one enzyme at a time"), where the enzyme that catalyzes the rate determining step emerges first, thus providing the biggest advantage. Consequently, in the modern view on biochemical pathway evolution, the four classical hypotheses (Horowitz, Granick, Yčas and Jensen, and Lazcano and Miller, as highlighted above) do not have to be seen as mutually exclusive but can be considered as complementary.[64]

The five models for the evolution of metabolic pathways described above all involve situations where Darwinian selection mechanisms are in place, which would already imply the existence of a highly complex biochemistry. However, in the context of the earliest steps in the origin of life, we need to consider proto-metabolic pathways that predated enzymatic biochemistry, and which would, by definition, have been entirely non-enzymatic (Figure 1f). Non-enzymatic reactions are more common in biology than is typically appreciated by chemists.[70] The impressive rate accelerations caused by enzymes[71] are usually benchmarked against uncatalyzed reactions, but this fails to capture the substantial rate accelerations that can be observed simply from small molecule catalysts such as metal ions, minerals or organocatalysts. Along these lines, it has been argued that "enzymes do not perform feats of magic; they just accelerate and add specificity to reactions that tend to occur anyway. That is, it suggests that the basic underlying chemistry of the [biochemical] pathway is older than the enzymes that catalyze it."[72] Consequently, as enzymes began to emerge from the energy dissipating protometabolism, the strongest evolutionary selection pressures would likely direct them to accelerate or increase specificity for reactions that benefit the network's persistence[1,73] by channeling them away from non-productive thermodynamic dead-ends.[35,72,74,75]

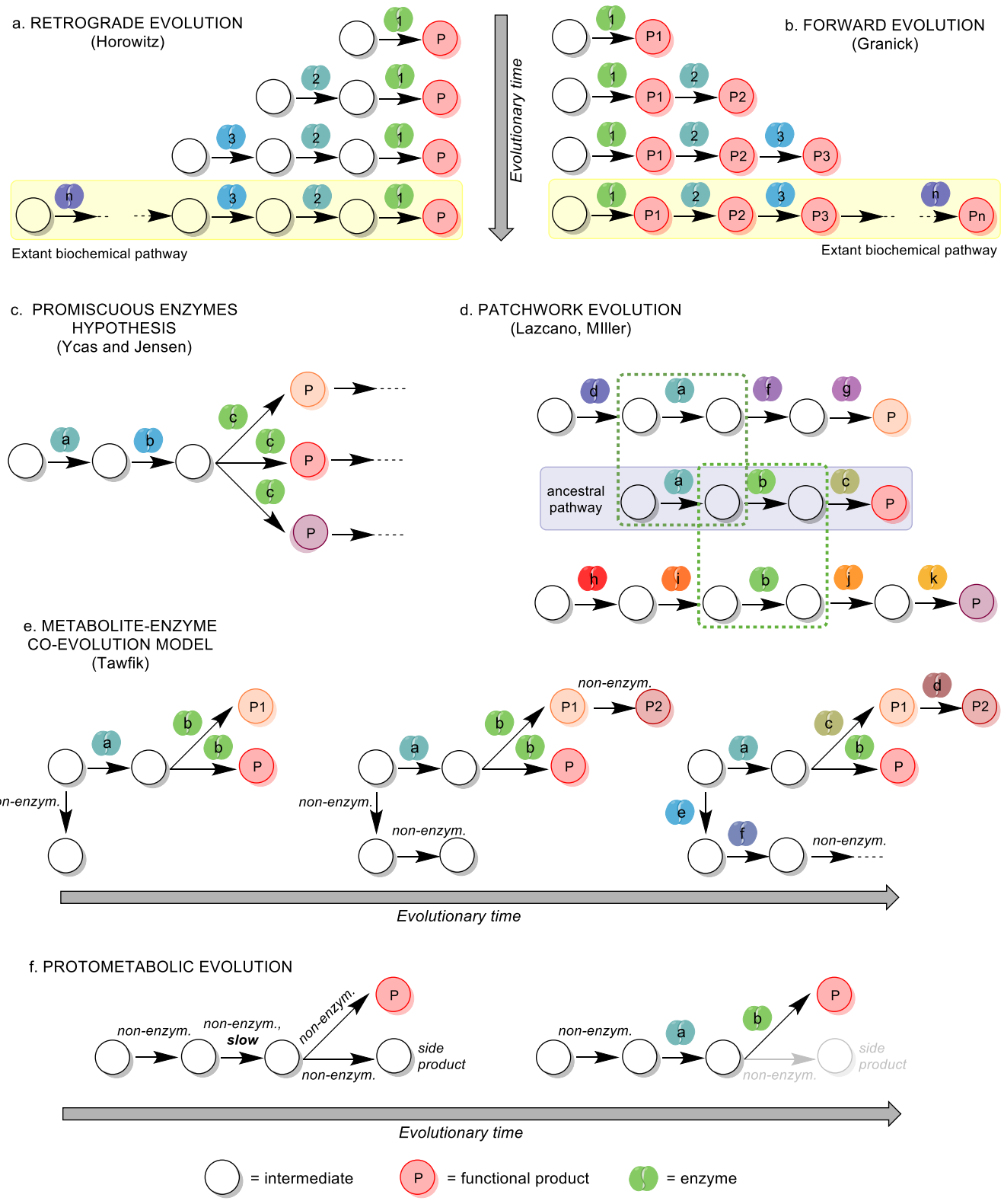


Figure 1. A simplified overview of models for biochemical pathway evolution. Identical enzymes are denoted by the same colors.

2.2. Ecology and energy sources for metabolism

Individual organisms either make all of their own metabolites from inorganic sources (autotrophic organisms) or need to take metabolites from other organisms in order to survive (heterotrophic organisms). When the metabolisms of all the organisms within an ecosystem are taken together and the metabolic reactions summed up, the net result is that 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.[76] Other essential atoms for life, such as N, S and P are also subject to such redox cycles.[77–81] The metabolisms of autotrophic organisms are often used as models for early biochemistry because of their relative simplicity. Within autotrophs, another distinction can be made based on the source of energy for their metabolism. This can be either chemical energy (chemoautotrophs) or light (photoautotrophs). Most biological and geological evidence support a later emergence of photosynthesis,[82] and thus suggest a picture of early life that is CO_2-fixing and chemotrophic (chemoautotrophic),[83] meaning it harnessed inorganic electron donors to drive chemosynthesis, though dissenting views also exist.[84,85]

2.3. Five universal metabolic precursors

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; 5) α-ketoglutarate is the precursor to various amino acids. The central role of these "metabolic pillars" in building all of life's chemistry suggests they were likely to be involved in prebiotic chemistry.[76] Following this concept popularized by Morowitz,[76,86] we will refer to these five compounds as "universal metabolic precursors" throughout this review.

2.4. A primer on core carbon metabolism

Like ecosystems as a whole, autotrophic organisms build themselves from CO_2. Perhaps surprisingly, given nearly four billion years of evolution, there are only six known CO_2 fixation pathways used by autotrophs.[87] One of these, the Calvin cycle, is related to photosynthesis, which is thought to be a later development.[88] 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). The remaining four 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, meaning they have a self-amplifying network structure (in short: a cyclic sequence of reactions which allows the cycle to double the number of molecules within itself every time it turns over). 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[89]—also providing the same universal metabolic precursors. We will discuss several of these pathways in sections 3 and 4.

3. THE ACETYL-CoA PATHWAY

3.1. Overview

Of the six autotrophic CO_2 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.[90,91] A recent phylogenetic study has concluded it operated in the last universal common ancestor (LUCA) to all life.[43] It is the starting point for carbon and energy metabolism in anaerobic organisms that branch deeply within the tree of life, the acetogens and the methanogens.[72] In acetogenic microorganisms, the pathway is important for biosynthesis and energy metabolism, while in methanogens it is only used for the latter.[92] From a biosynthetic standpoint, 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. The acetyl-CoA pathway is also unique among the six carbon fixation pathways since 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.[72,93,94]

3.2. Enzymes and mechanism

The mechanisms and enzymology of the pathway have been well-studied (Figure 2).[95] The first step comprises a reversible two-electron reduction of carbon dioxide to a formyl group (step I) and is carried out by an enzyme called *formate dehydrogenase*. In anaerobic organisms, this enzyme may consist of oxygen-sensitive metal centers containing W, Mo, or Fe. With the aid of tetrahydrofolate or methanopterin cofactors, the formyl moiety undergoes a dehydration and further reductions to become a methyl group (step II).[96] Meanwhile, a Ni-containing metalloenzyme called *carbon monoxide dehydrogenase* (CODH) catalyzes the reversible reduction of a second molecule of CO_2 to CO (step III).[97] The two processes join up when a Co-based enzyme known as *corrinoid iron sulfur protein* (CoFeSP) transfers the methyl group from its organic cofactor to the reduced active site of a Ni-based enzyme called *acetyl-CoA synthase* (ACS) (step IV). Here, CO and methyl group combine to form an acetyl-Ni species, which is then trapped by coenzyme A, a thiol, to produce acetyl CoA, a thioester.[95] Much of the acetyl CoA produced undergoes a further enzyme-catalyzed reductive carboxylation to furnish pyruvate (step V). Some biochemists consider this last step to be part of the pathway, while others do not.[90] Organisms using this pathway get the required electrons from reducing molecules found in their environment, usually dihydrogen. Interestingly, the electrons from H_2, when split equally, are not sufficiently reducing to enable some of the key steps in the pathway. A complex mechanism known as electron bifurcation is required to generate such electrons, which are temporarily stored in the form of highly reduced Fe-based cofactors.[98]

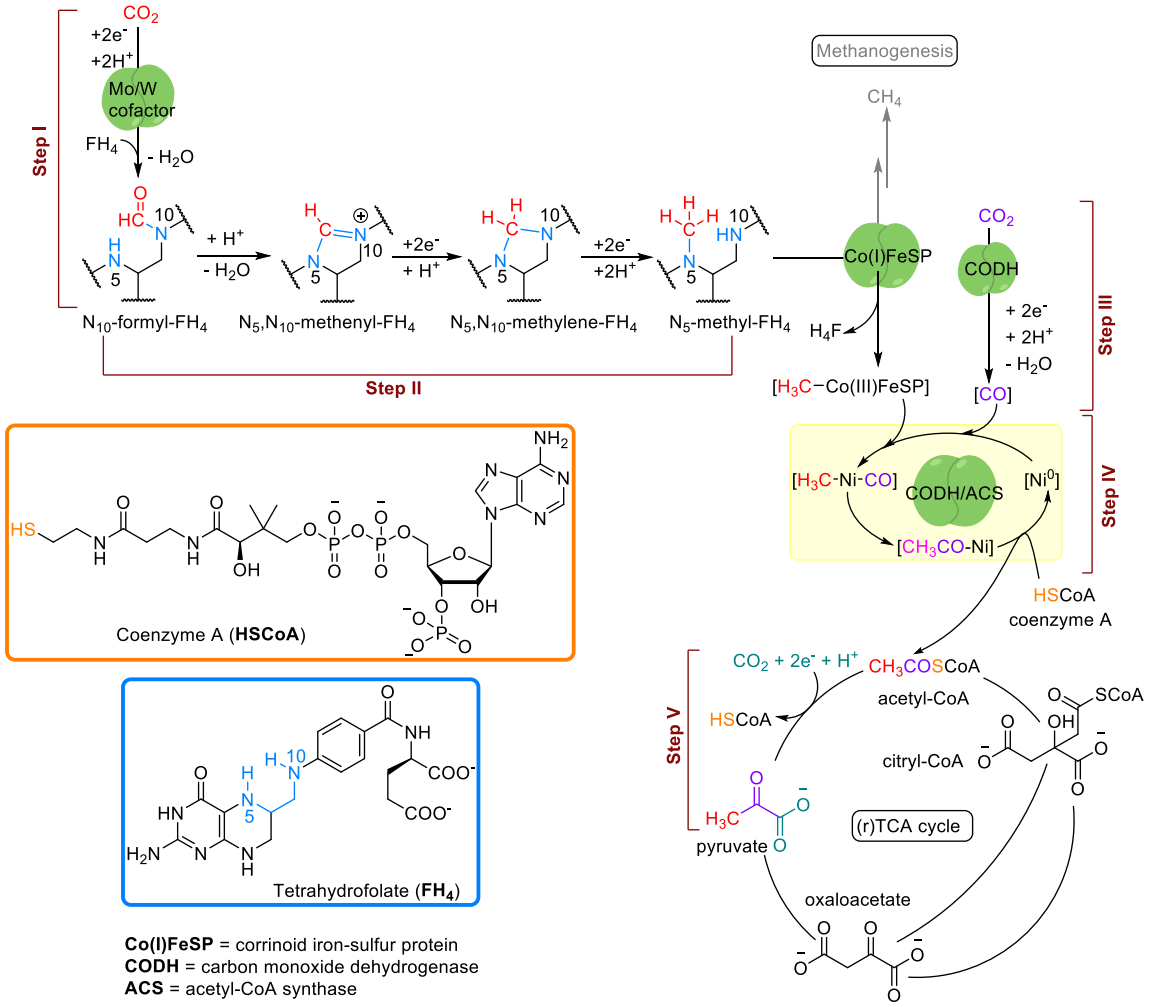


Figure 2. The metalloenzymes and cofactors of the acetyl-CoA pathway.

3.4. The search for a prebiotic analog of the acetyl-CoA pathway

Given the ancient nature of the acetyl-CoA pathway and its reliance on transition metals, several researchers in the origins of life field have tried to discover non-enzymatic variants starting from CO_2 or HCO_3^-, as well as analogs based on more reduced C_1 sources such as CO and HCOOH. Researchers specializing in CO_2 fixation, while not focused on origins research, have also reported relevant chemistry.[99] Generally speaking, reports using CO_2 or HCO_3^- as C_1 source require an external reducing agent, such as a reduced metal, a reducing electrode, or hydrogen gas, while those employing reduced C_1 sources can themselves act as the reducing agent. In 1997, Huber and Wachtershäuser showed that acetate could be produced from the reaction of methyl thiol and CO at 100 °C. Some mechanistic experiments suggested that an activated acetyl species, probably a thioacid or thioester, was a likely intermediate (Table 1, entry 1)[100] A few years later, Cody and co-workers detected acetate and pyruvate in micromolar concentrations after heating a gold tube containing neat formic acid, nonyl thiol and FeS at 250 °C under very high pressure (entry 2).[101] While those specific reaction products parallel those seen in the Acetyl-CoA pathway, the use of neat formic acid as solvent, which decomposes to a mixture of H_2O, H_2, CO_2 and CO under the extreme reaction conditions, is geologically unrealistic. In 2006, Yamasaki and co-workers described the reduction of CO_2 to formate using an alloy of Fe and Ni at 300 °C, but acetate and pyruvate were not detected (entry 3).[102]

In 2010, Feng and co-workers described the production of both formate and acetate from CO_2 using freshly prepared iron nanoparticles at 200 °C under moderate pressures, indicating that abiotic CO_2 fixation to acetate is not only a property of metal sulfur compounds, but one that can also be accomplished by zero-valent metals (entry 4).[103] Herschy and co-workers obtained micromolar quantities of formate and nanomolar quanitites of formaldehyde from H_2 and CO_2 in a mackinawite (Ni-doped FeS) reactor simulating an alkaline hydrothermal vent.[104] In 2015, chemists working outside a prebiotic context showed that *N*-doped nanodiamond electrodes operating at highly reducing potentials fix CO_2 to acetate in water (entry 5).[105] Another electrochemical report, this time carried out in a prebiotic context by de Leeuw and co-workers, used greigite (Fe_2S_4) electrodes under bubbling CO_2 to furnish formate, acetate and pyruvate at ambient temperature (entry 6).[106] Although the materials for this experiment are geochemically plausible, the required potential of −1.1 V is more reducing than any known natural environment. In 2018, our team reported that simply placing metallic Fe powder in water under CO_2 between 30-100 °C resulted in the formation of formate, acetate and pyruvate in the near millimolar range, with the latter two being the major products at the lower end of that temperature range.

Table 1. Non-enzymatic acetyl-CoA pathway analogs. Percentages in brackets correspond to yields calculated with respect to the carbon donor as the limiting reagent. [a] Thiol as the limiting reagent. [b] Metal as the limiting reagent. [c] Faradaic efficiency.

Entry	Carbon source	Reducing agent /catalyst	Reaction conditions	Product Yields				Ref.
				Formate	Methanol	Acetate	Pyruvate	
1	CO (1 bar), CH_3SH (8 mM)	FeS-NiS (1:1),	pH 8, 100 °C, 7 d	-	-	3.28 mM (41%)[a]	-	100
2	HCOOH (110 μmol), thiol	FeS	500-2000 bar, 250 °C, 6 h	-	-	5.5 x 10^{-5} mmol (0.05%)	7.7 x 10^{-5} mmol (0.07%)	101
3	CO_2 (22.5 mmol)	FeNi (98.75:1.25),	300 °C, 6 h	1.4 mM (2.3%)	-	-	-	102
4	CO_2 (14 bar), H_2O	Fe^0 (5 mmol)	200 °C, 72 h	8.5 mM (0.0085%)[b]	-	3.5 mM (0.0035%)[b]	-	103
5	$NaHCO_3$ (0.5 M aq)	N-doped nanodiamond (3.68 %) electrode, −0.55 - −1.30 V	rt, 1 h	1.2 mM (0.24% / 0.01%[c])	-	16.1 mM (3.2% / 0.07%[c])	-	105
6	CO_2	Greigite electrode (−1.1 V)	pH 6.5, 120 h, rt	1.3 μmol (1.5%)[c]	0.35 μmol (1.2%)[c]	0.57 μmol (2.6%)[c]	0.48 μmol (2.8%)[c]	106
7	CO_2 (1-35 bar)	Fe^0	H_2O, 30-100 °C, 16 h	0.41 mM (0.014%)	0.12 mM (0.026%)	0.18 mM (0.054%)	0.03 mM (0.012%)	107
8	CO_2 (15-25 bar)	Ni_3Fe (also Fe_3O_4, or Fe_3S_4), H_2	H_2O, pH 4-10, 100 °C, 16 h	332 mM	0.12 mM	0.56 mM	10 μM	108

Ni and Co were also found to produce acetate and pyruvate at 100 °C, while Mn, Mo and W gave only acetate (entry 7).[107] It is notable that the three metals that furnish both acetate and pyruvate are also the same three metals that are essential to the functioning of the Acetyl-CoA pathway. A follow-up study showed that similar results can be obtained using hydrogen gas as the reducing agent, in close analogy with the biological pathway, with hydrothermal minerals (awaruite—Ni_3Fe, magnetite—Fe_3O_4, and greigite—Fe_3S_4) as catalysts under neutral to alkaline conditions.[108] The yield of the CO_2 reduction products was increased by two to three orders of magnitude in the presence of H_2, indicating the role of hydrogen in the reaction mechanism.

3.5. Summary and future directions

A wide range of reducing conditions and catalysts are capable of reducing CO_2 to acetate and pyruvate in a manner closely emulating the acetyl-CoA pathway. It is therefore highly plausible that the acetyl-CoA pathway emerged from geochemistry on the early Earth, and that over time a suite of enzymes evolved to make the reactions more efficient. Elucidating the mechanisms of the CO_2 fixation reactions that mimic the acetyl-CoA pathway will be important to understand how the system may have evolved from one mediated by a simple mineral to one mediated by metalloenzymes. It should be noted that the systems described so far only capture the biosynthetic aspect of the acetyl-CoA pathway. The bioenergetic aspect of the pathway, for example the trapping of acyl intermediates as energy-rich thioesters or acyl phosphates starting from CO_2, has yet to be demonstrated in a non-enzymatic manner.

4. THE TRICARBOXYLIC ACID CYCLES

The rTCA cycle (reductive tricarboxylic acid cycle, reductive Krebs cycle, Arnon cycle) and the TCA cycle (tricarboxylic acid cycle, Krebs cycle) have been proposed to be an outgrowth of prebiotic chemistry, or at least a very early development in life, due to their central places in biochemistry. They comprise 13 intermediates, ten of which are simple carboxylic acids and three of which also contain a thioester. The two cycles are roughly the reverse of each other, with the TCA cycle having a net catabolic function and being oxidative, and the rTCA cycle having a net anabolic function and being reductive. The catalytic machineries that mediate their reactions share many similarities. Regardless of the redox direction in which they run, both cycles supply the five universal precursors to biological metabolism. They are connected by six different reaction types: 1) reductive carboxylation and oxidative carboxylation, 2) carboxylation and decarboxylation, 3) reduction and oxidation, 4) hydration and dehydration, 5) thioesterification and thioester hydrolysis, and 6) retro-aldol and aldol reactions. Some biochemists consider the thioesterification step to be part of the preceding or subsequent step, in which case the cycles are referred to as having only eleven steps and five distinct reaction types. The set of three reactions that interconvert oxaloacetate and citryl-CoA in the TCA cycle, referred to as an epicycle, is also often considered separately.

4.1. Biochemistry of the reductive tricarboxylic acid (rTCA) cycle

In 1966, Arnon and co-workers discovered that some organisms living in reducing environments run a version of the well-known TCA cycle in the reductive direction, to the astonishment of many in the biochemistry community (Figure 3).[109] Organisms running the rTCA cycle use a source of reducing electrons in their environment such as H_2, H_2S or Fe^{2+} to drive the cycle forward. It consists of four C-C bond-forming reactions. Two of the C-C bond forming reactions are reductive carboxylations of CoA thioesters (acetyl CoA, step A; succinyl CoA, step G). The other two C-C bond forming reactions are ATP-dependent α-carboxylations of the ketoacids pyruvate (step B) and ketoglutarate (step H), which are thought to proceed through the intermediacy of carboxyphosphate.[110] The cycle also includes one C-C bond-breaking reaction involving a thioester, the retro-aldol reaction of citryl CoA (step M). ATP is required to convert succinate to succinyl CoA (step F) and citrate to citryl CoA (step L). The remaining reactions are reductions (steps C, E and I) and reversible hydrations/dehydrations (steps D, J and K). In total, four of the 13 reactions of the rTCA cycle consume ATP.

The specific mechanisms and enzymes involved in the rTCA cycle have recently been reviewed in the prebiotic chemistry context and will not be discussed in detail here,[111] but it is notable that metalloenzymes play an important role. In particular, Fe-based enzymes or cofactors mediate the reductive carboxylations and the reversible hydrations/dehydrations (five reactions).

4.2. Could the rTCA cycle have originated in prebiotic chemistry?

Of the four autocatalytic CO_2 fixation pathways used by chemoautotrophs, only the rTCA cycle contains all five universal precursors directly on the cycle.[112] A parsimony-maximizing analysis of the network structure of the four pathways concluded that the rTCA cycle is likely the evolutionary ancestor to the other three,[87] although it is not certain whether the rTCA cycle was the earliest pathway to produce the same intermediates. Today, the biological rTCA cycle is regulated by no fewer than ten distinct enzymes, but evidence exists that steps with similar mechanisms were once catalyzed by a smaller, more promiscuous set of enzymes.[113]

Subsequently, it has been proposed that the rTCA cycle could represent not just ancient biochemistry, but prebiotic chemistry enabled by a small number of naturally occurring catalysts.[25,86,114] Many of the enzymes of the rTCA cycle rely on metal-based cofactors in their active sites, which may provide a clue as to how a prebiotic precursor to the cycle could have originated. In fact, hydration and dehydration reactions of closely related substrates to those of the rTCA cycle had already been demonstrated under acid, base or metal ion catalysis, leading to specific mechanistic hypotheses for some enzymatic steps even before the enzymology of the cycle was elucidated.[115–117] In light of the widespread occurrence of FeS clusters in several rTCA enzymes and cofactors, Wächtershäuser proposed that a prebiotic sulfur-based analogue of the rTCA cycle once existed, catalyzed by FeS minerals.[25,118] Smith and Morowitz later proposed that a more biology-like rTCA cycle may have operated before enzymes, possibly catalyzed by metal ions and driven by inorganic pyrophosphate.[119]

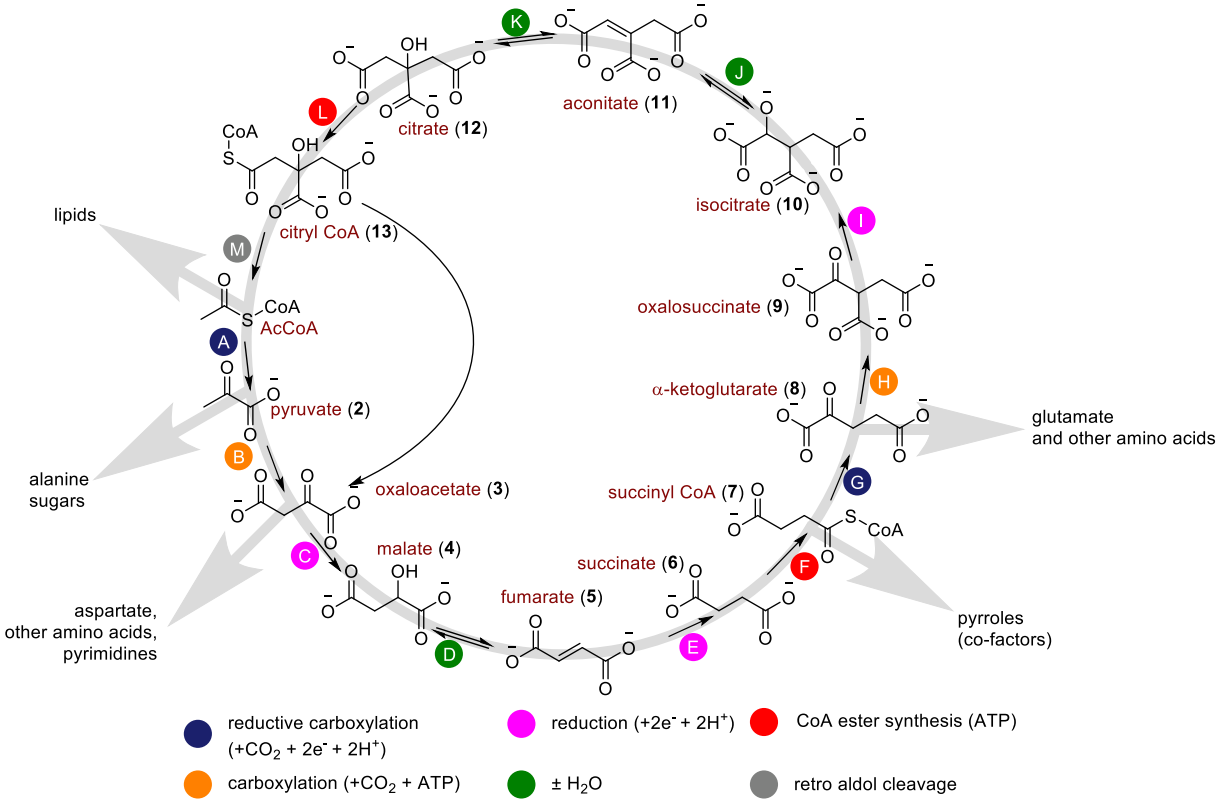


Figure 3. The rTCA cycle and its central place in biosynthesis.

Which form of the rTCA cycle might represent a good model for prebiotic chemistry—if any at all[120]—has been debated. The full rTCA cycle is only found in one of the two most ancient branches of the tree of life (bacteria) and not the other (archaea). Thus, it has been argued that it is unlikely to have been operating in its full form in early life or in prebiotic chemistry.[30] Rather, a short linear sequence of reactions from acetyl-CoA to α-ketoglutarate, known as the horseshoe rTCA (Figure 3, steps A-F), has been suggested to be ancestral and possibly prebiotic (see also section 4.5). Furthermore, Orgel pointed out that a full autocatalytic prebiotic version of the rTCA cycle would be implausible due to parasitic reactions, such as unwanted reduction reactions leading off-cycle.[121] This would cause the cycle to die out faster than it could self-amplify. Consequently, a short, linear pathway would be more plausible. In this vein, a hybrid pathway consisting of the acetyl-CoA pathway (CO_2 to pyruvate)[122] and the horseshoe rTCA cycle (pyruvate to ketoglutarate) has also been proposed to have been prebiotic[30] and potentially promoted by Fe(Ni)S minerals.[123] Another analysis of the rTCA cycle was based on a set of hypothetical reactions that could be derived from a small selection of plausible mechanisms.[124] It concluded that the rTCA cycle is likely just one of several possible optimal ways of producing the five universal intermediates, and that other prebiotic chemistries should not be ruled out. A computational analysis of the structure of (r)TCA intermediates suggested that they are not "especially unusual in the context of chemical space",[125] and that higher order factors than simply a chemical optimization might be at play concerning the topology of the modern (r)TCA cycle. Only experiments can settle these conflicting ideas.

4.3. Experimental search for a prebiotic analog of the rTCA cycle

The first systematic experimental studies regarding a search for a non-enzymatic analog of the rTCA cycle were reported by Cody and co-workers in 2001, who looked at the non-enzymatic decomposition of citrate under FeS and NiS catalysis at 200 °C.[126] Although an ATP-independent version of the desired retro-aldol fragmentation indeed appeared to happen under these extreme conditions, it was unsurprisingly accompanied by a number of rapid and competing decomposition reactions that do not occur in biochemistry. In 2006, Zhang and Martin showed that two of the three reduction reactions of the rTCA cycle (oxaloacetate to malate and fumarate to succinate) could be carried out in high yield under ZnS UV photocatalysis using sulfide as the source of electrons.[127] This was followed by more detailed studies of efficiency and kinetics conducted by Guzman and Martin.[128,129] Wang and co-workers reported that FeS in the presence H_2S could reduce oxaloacetate to malate in yields up to 6% at 100 °C and pH 7-10.[130] Kitadai and co-workers showed that the same reduction reactions of the rTCA cycle (oxaloacetate to malate and fumarate to succinate) may be effected under alkaline hydrothermal vent conditions with FeS that has been partially electrochemically reduced at −0.7 V.[131] Our laboratory demonstrated that by combining metallic iron (as reducing agent), Zn^{2+} and Cr^{3+} in acidic water, oxaloacetate could be converted to succinate, through the intermediacy of malate and fumarate, in a spontaneous sequence of reduction, dehydration and reduction reactions. The reaction sequence required temperatures between 80-140 °C, however it could be carried out at ambient temperature in the presence of tocopherol-based nano-sized micelles, which—although not prebiotic—highlighted the potentially important role of compartmentalisation. The same combination of metals, this time only at 140 °C, enabled the conversion of oxalosuccinate to citrate, through the intermediacy of isocitrate and aconitate, in a spontaneous sequence of reduction, dehydration and hydration reactions (Figure 4). Thus, a single combination of metals may drive six rTCA cycle reactions under the same conditions.[132]

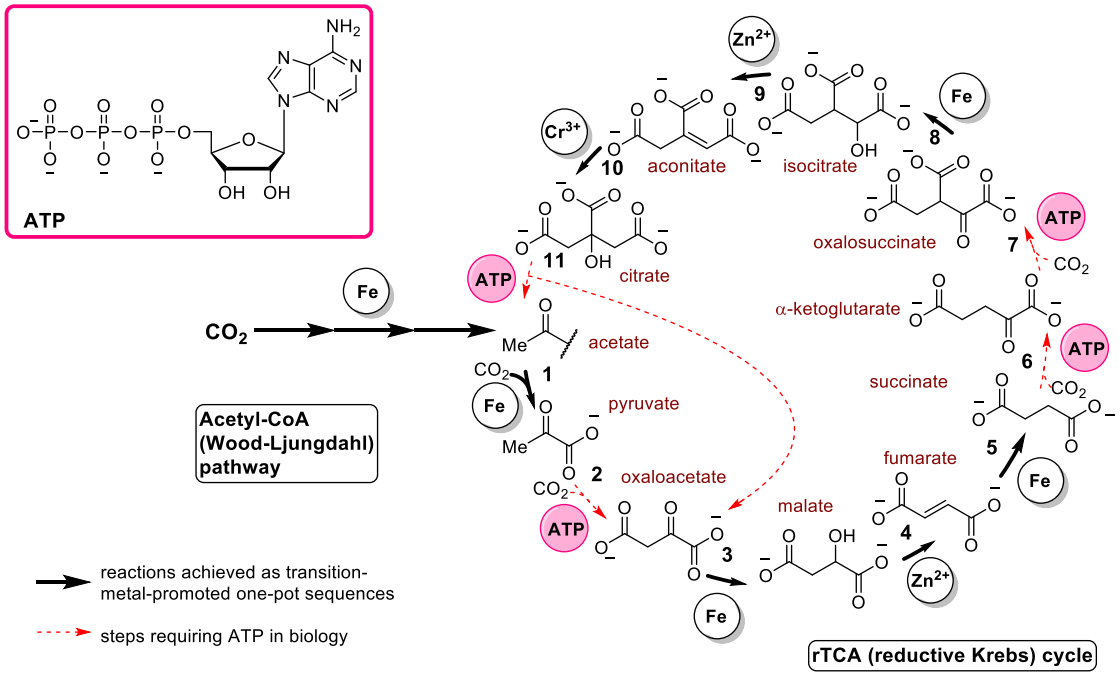


Figure 4. Non-enzymatic reactions of the acetyl-CoA pathway and the rTCA cycle reported to date (bold arrows). Reactions 3, 5 and 7 were achieved photochemically (ref. 127), while individual reactions 3 and 5 (ref. 131), as well as one-pot sequences 3-4-5 and 8-9-10 (ref. 132) were found to be promoted by transition metals. Reactions requiring ATP in biochemistry are highlighted

In the same study, a quantitative evaluation of the potential parasitic reduction reactions in the presence of metallic Fe^0 revealed that about twice as much material prefers to move forward on-cycle than off-cycle. This means that while Orgel's efficiency requirement[121] for sustaining an autocatalytic rTCA cycle remains relevant, the problem may not be so severe that it could not be overcome by a hybrid network in which the acetyl-CoA pathway feeds into and stabilizes the rTCA cycle.[87] This would be consistent with Braakman and Smith's theoretical analysis that concluded that the reductive acetyl-CoA pathway and the rTCA cycle once functioned together in a primordial form of carbon fixation.[87] However, these suggestions are difficult to confirm because the evolutionary history of the rTCA cycle and acetyl-CoA pathway is extremely hard to deconvolute in primitive microorganisms.[133] Of greater relevance to the potential prebiotic nature of the rTCA cycle would be the experimental demonstration of efficient non-enzymatic versions of its C-C bond forming reactions, which were not accomplished in the above studies.

4.4. Biochemistry of the oxidative tricarboxylic acid (TCA) cycle

Due to its importance in human metabolism, the most well-known central catabolic pathway is the TCA cycle (also known as the Krebs cycle or the citric acid cycle), discovered in 1937.[134] Like the rTCA cycle, it generates the five universal precursors to metabolism, but through one C-C bond-making steps, (an aldol reaction) and four C-C bond-breaking steps. The latter includes two oxidative decarboxylations that generate energy-rich thioesters, one of which is coupled to ATP production (Figure 5).

The first step of the TCA cycle is a thermodynamically favored aldol reaction (-37.6 kJ mol^{-1})[135] between acetyl CoA and oxaloacetate to give citrate and free CoA (via a citryl-CoA intermediate), catalyzed by *citrate synthase*. The subsequent steps are essentially the reverse of those in the rTCA cycle (see section 4.1) and involve several FeS or Fe^{2+}-dependent enzymes.[136] Overall, each turnover of the TCA cycle yields two CoA thioester molecules and one ATP (or GTP) molecule as a net outcome. Additionally, the biochemical reducing agents NADH and $FADH_2$ obtained in the TCA cycle are also fed into oxidative phosphorylation pathways (electron transport chain). Detailed mechanisms of all these transformations have been described elsewhere and will not be repeated here.[136]

4.5. Could the TCA cycle have originated in prebiotic chemistry?

Due to its early discovery and central role in metabolism, the TCA cycle has been suggested to have very ancient roots. Hartman proposed that the TCA cycle, operating together with pyruvate carboxylation and the acetyl-CoA pathway, may have started as prebiotic chemistry.[137] Nowadays, a standalone TCA cycle is generally thought to be a later development in the origin of life compared to parts of the rTCA cycle,[138,139] often due to indications from the geological record that little molecular oxygen was available for oxidative reactions on the early Earth.[82] On the other hand, oxidations may well have been achieved through UV light,[140] inorganic oxidants,[141] or by strongly oxidizing hydroxyl radicals from deep-Earth water radiolysis.[142] Recent discoveries of deep-branching bacteria running a bidirectional rTCA cycle raise the possibility that a precursor to the TCA and rTCA cycles may have also been operating this way, using oxidative and reductive processes coupled together (we will discuss this in section 4.7).

4.6. Experimental search for a prebiotic analog of the oxidative TCA cycle

In early work by Waddell and co-workers, attempts were made to see whether sunlight could promote reactions of the TCA cycle without enzymes.[143–145] Though these photolysis experiments indeed produced intermediates of the TCA cycle, mechanistic parallels with the biological TCA cycle remain to be established. In closer mechanistic analogy to the reactions of the TCA cycle, Wang and co-workers reported the oxidation of malate to oxaloacetate using an $FeS/S^0/H_2S$ system in less than 2% yield.[130] Indeed, intermediates of the TCA cycle undergo non-enzymatic oxidation and oxidative cleavage reactions,[146] usually in the presence of strong oxidants.[147,148] In 2017, Ralser and co-workers demonstrated that a combination of FeS and $S_2O_8^{2-}$ generates the products of the TCA cycle starting from almost any TCA cycle intermediate.[141] The sulfate radical mediated process was reported to proceed in up to 90% carbon efficiency. Some aspects of this finding have been criticized, followed by a response from the authors.[149–151] The resulting debate highlights the need for communication between disciplines and the need for carefully chosen and validated analytical techniques. Nonetheless, the collection of results presented in this section indicate that oxidative biochemistry also follows a chemical path of least resistance that enzymes could later have accelerated and made more selective.

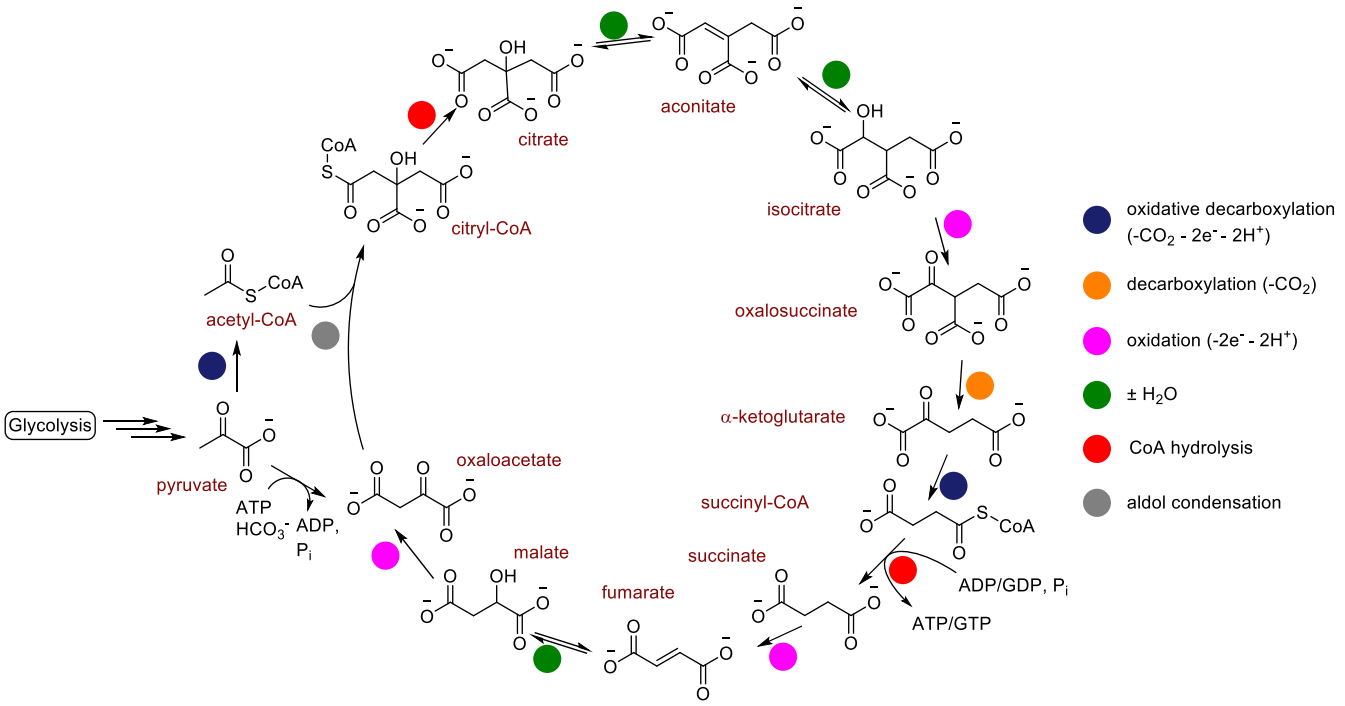


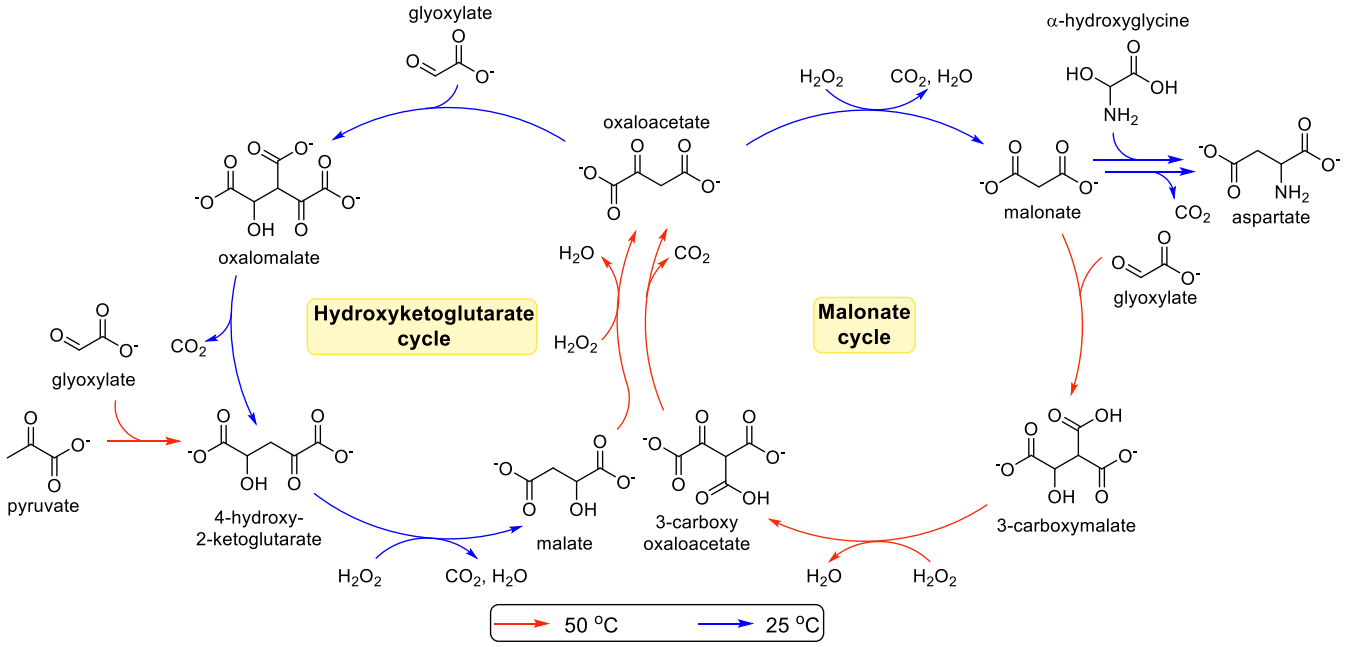
Figure 5. Reactions of the enzymatic oxidative TCA cycle.

4.7. Prebiotic carbon metabolism without ATP

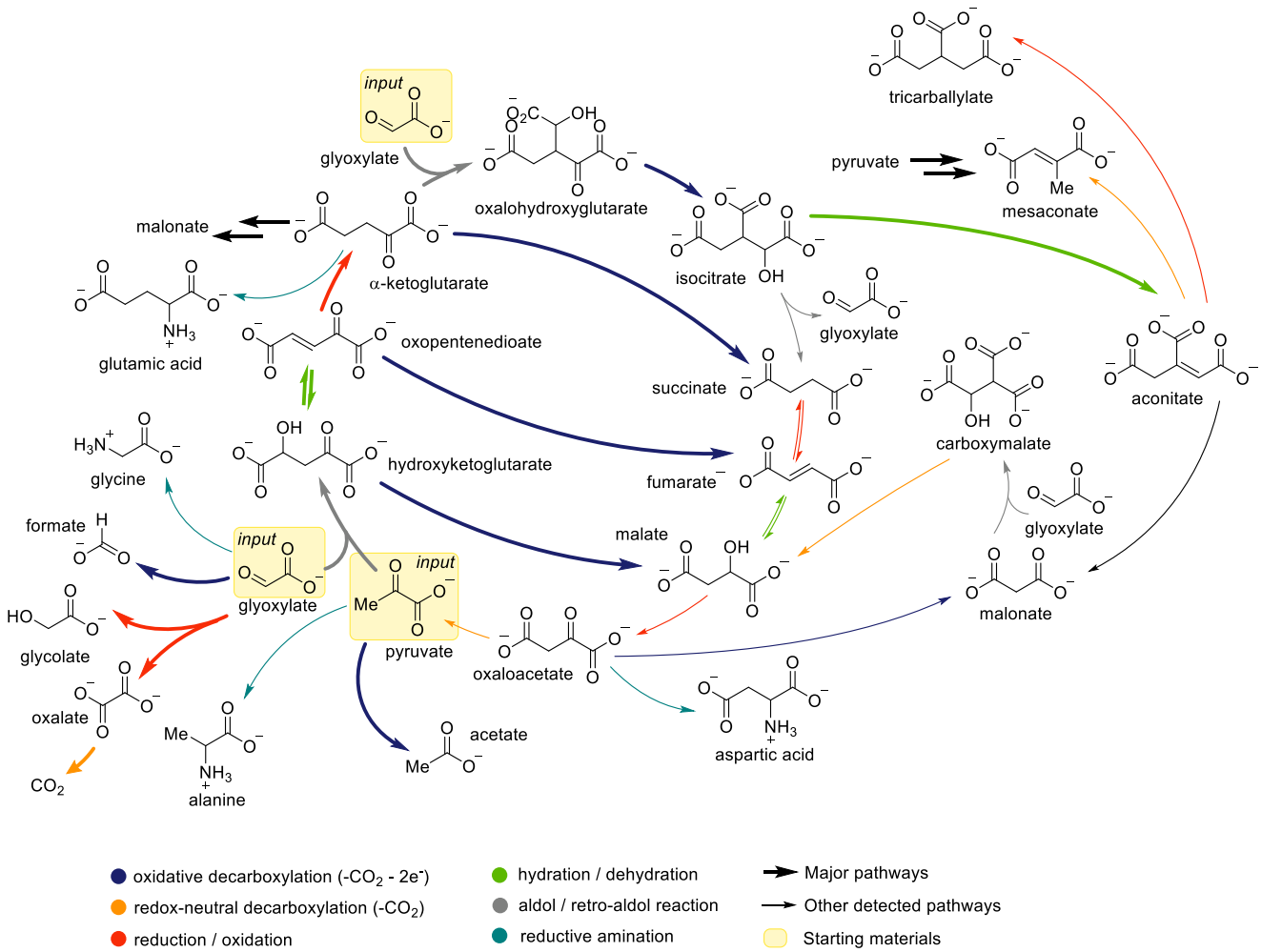
If protometabolism was continuous with metabolism, it becomes hard to imagine how prebiotic anabolic processes, other than the AcCoA pathway, could have operated without ATP or some simpler inorganic analog (for example a polyphosphate[152]). However, no ATP-dependent C-C bond forming reactions have thus far been shown to have prebiotic analogs. It remains unclear whether this is because it is extremely difficult without enzymes from a kinetic perspective, or simply because such an experimental approach to prebiotic chemistry is fairly new. Recently, Segrè and co-workers performed a computational analysis suggesting that once phosphate-dependent reactions are abstracted from the entirety of known biochemical transformations, what remains forms a strongly interconnected network that relies on thioester chemistry and FeS-containing enzymes (see section 5 for a discussion of the role of thioester in non-enzymatic energy conservation processes).[20,153] This network is also highly reliant on pyruvate and glyoxylate. Indeed, the importance of glyoxylate as a building block in prebiotic chemistry was described before,[154] such as in Eschenmoser's "glyoxylate world" hypothesis.[155] Reinforcing the role of these two simple biomolecules in potentially prebiotic, metabolism-mimicking cycles was the 2018 study by Springsteen, Krishnamurthy and co-workers. The authors reported an oxidative decarboxylation-driven bicyclic reaction network, seeded by the aldol reactions of glyoxylate with pyruvate, oxaloacetate, or malonate, in the presence of H_2O_2.[156] At 25 °C, oxaloacetate and glyoxylate react to ultimately produce malate, another intermediate of the TCA cycle. Oxaloacetate can also be oxidized to malonate, which can react with α-hydroxyglycine, an adduct formed from ammonia and glyoxylate, to produce the amino acid aspartate. At 50 °C, malonate itself can react with glyoxylate, leading to another intermediate of the TCA cycle, oxaloacetate. Oxaloacetate can also be obtained from the oxidation of malate. The net result at this temperature is the formation of a reaction network with bicyclic structure in which oxaloacetate is the pivotal link.

Linking the information obtained from the top-down[20] and bottom-up[156] approaches, we recently reported a non-enzymatic reaction network that arises from glyoxylate and pyruvate in Fe^{2+}-rich water at 70 °C.[157] The network does not require strong oxidants to drive it forward. Instead, the C-C bonds are formed by aldol reactions between reactive keto intermediates and broken down by decarboxylation and retro-aldol reactions. Oxidations and reductions are likely mediated by the Fe^{2+}/Fe^{3+} redox shuttle or by Oppenauer oxidation/Meerwein-Ponndorf-Verley reduction. The types of reactions and the intermediates found to be operating in the reaction network recapitulate most of the biological TCA cycle (9/11 intermediates, 7/11 reactions) and glyoxylate cycle (8/9 intermediates, 5/8 reactions), including all of the universal precursors to metabolism (Scheme 2). It is notable that the Fe^{2+}-promoted network is not unidirectional in terms of its underlying redox reactions. Furthermore, the intermediates of the reaction network can be pushed towards other classes of biomolecules. The addition of metallic iron and hydroxylamine to the reaction mixture converts glyoxylate, pyruvate, oxaloacetate, and α-ketoglutarate to four biological amino acids, glycine, alanine, aspartate, and glutamate, respectively (this will be described in more detail in section 6). The same ketoacids can be converted to thioesters, as will be discussed in section 5. Based on this study and the one from Springsteen and Krishnamurthy,[156] it seems plausible that both the (r)TCA and glyoxylate cycles may have had a common precursor that originated as prebiotic chemistry. The experiment also suggests that a non-enzymatic protometabolism could have continuously built up and broken down biomolecules, as life does today at the ecosystem level. It appears a matter of time for reaction networks similar to the ones above to be integrated in a continuous manner with other metabolic sub-systems.

Scheme 1. Two linked non-enzymatic cycles arising from glyoxylate and pyruvate, oxaloacetate or malonate at ≤ 50 °C in the presence of H_2O_2.[156]



Scheme 2. Reaction network arising from pyruvate and glyoxylate at 70 °C in water in the presence of Fe^{2+}. Reductive amination of α-ketoacids to the corresponding amino acids occurs upon the addition of hydroxylamine and Fe^0 to the reaction mixture.[157]



4.8. Summary and future directions

Much of the rTCA and TCA cycles can be enabled without enzymes. In the case of the rTCA cycle, notably lacking are the ATP-dependent steps, including all of the C-C bond forming and breaking reactions. The only exception is perhaps the conversion of acetyl to pyruvate, which was proposed to be an intermediate step in acetyl-CoA pathway-like CO_2 fixation chemistry.[90,107,108] A serious setback for the prebiotic rTCA cycle hypothesis, whether in its full or horseshoe form, is the absence of experimental evidence for its non-enzymatic C-C bond-forming reactions. A major unanswered question is therefore whether inorganic polyphosphates[152] might have predated the function of ATP in a protometabolic rTCA cycle. Still, even if only ATP-independent reactions of the rTCA cycle or TCA cycle were operational as prebiotic chemistry, their non-enzymatic versions likely paved the way for the subsequent evolution of functional enzymatic pathways (see Figure 1f).[74]

It is completely possible that anabolic (typically reductive) and catabolic (typically oxidative) processes needed to be linked from the very beginnings of prebiotic chemistry.[158] Such chemical space would resemble the "bowtie" architecture known in biochemistry, where anabolism and catabolism are mutually dependent and relayed via the intermediate of the (r)TCA cycle.[159] What this means for prebiotic chemistry is that non-enzymatic protometabolism would have necessarily emerged in a formation-destruction regime.[160] A requirement for readily available electron donors and acceptors means the system would have needed to be part of, for instance, a geochemical redox cycle. It would also have had to be replenished by a constant supply of small building blocks, to remain in a disequilibrated state. More specifically, a reductive anabolic network would build up larger carbon compounds from small molecules such as CO_2, and an oxidative catabolic pathway would remove these products by breaking them down. While these pathways might superficially look to be the reverse of each other, they must be driven thermodynamically by different processes and proceed through different transition states.[160] Autocatalysis of the anabolic pathways could offer control over an evolutionary "combinatorial explosion" while maintaining a source of complexity even before the onset of modern-type genetics,[161] whereas the oxidative pathways would recycle reduced material, allowing the system to escape equilibrium.[162] Experimental systems embodying these properties should be highly sought-after in the future.

5. PREBIOTIC THIOESTER CHEMISTRY AND THE EMERGENCE OF BIOENERGETICS

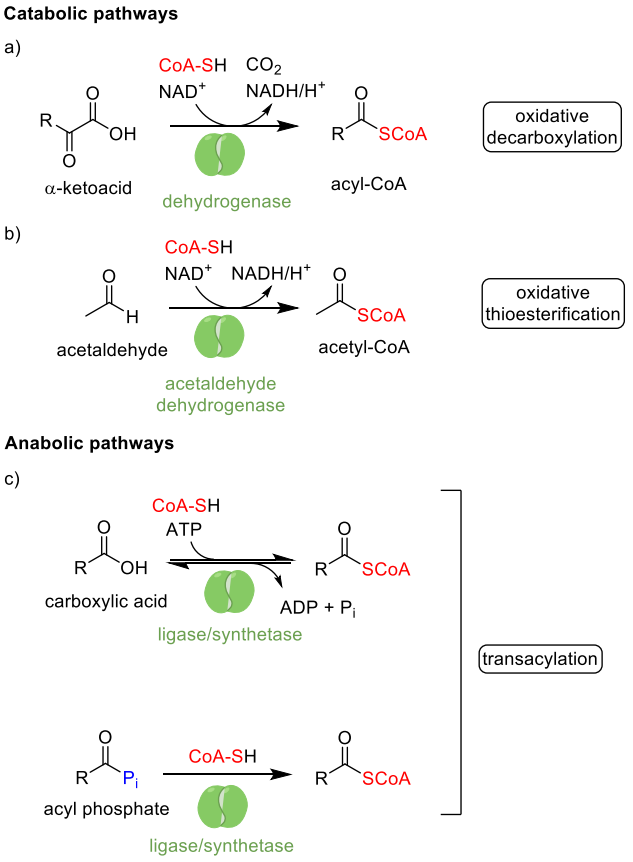
In biology, phosphate chemistry and thioester chemistry are closely intertwined. It is not clear whether and how these processes became coupled in prebiotic chemistry, whether it was before or after the emergence of enzymes, and whether they arose independently or at once. This knowledge is, however, essential for a full understanding of the bioenergetic aspects of extant life. Evolutionary aspects of bioenergetics form a very broad topic and have been discussed in depth, from various perspectives[163–166] and will not be included here. Instead, we will give a brief summary of thioester-forming processes relevant to bioenergetics and highlight experimental attempts to recreate it without enzymes, linking them to existing hypotheses on thioester chemistry in early metabolism.

5.1. Thioesters in biological energy conservation

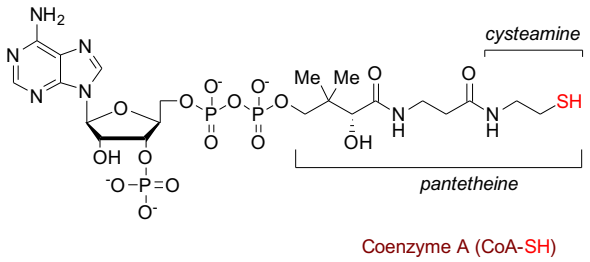
The most prominent catabolic processes that produce thioesters as intermediates involve oxidative decarboxylation of α-ketoacids (Scheme 3a) or oxidation of aldehydes (Scheme 3b) sourced from central carbon metabolism. The biological thiol that is most frequently employed is cofactor A (CoA, Scheme 4), which generates important biological thioesters such as acetyl-CoA or succinyl-CoA. However, in some cases a thiol moiety of a cysteine residue produces transient enzyme-bound thioesters in the course of catalysis. These types of mechanisms are involved in pathways such as the TCA cycle and glycolysis and the products play pivotal roles in fatty acid, carbohydrate, and protein metabolism. Despite the apparent simplicity of the net chemical transformations, enzymatic thioesterifications are often surprisingly mechanistically complex. For instance, the production of acetyl-CoA from pyruvate requires a multi-step reaction catalyzed by a three-enzyme couple, pyruvate dehydrogenase complex, with a thiamine diphosphate cofactor (TPP).[136]

In most anabolic pathways, the reactions that generate thioesters tend to do so from carboxylic acids via transacylation reactions that depend on ATP (Scheme 3c). Acyl phosphates can also undergo transacylation with thiols to give thioesters. These types of reactions occur in pathways such as the rTCA cycle and gluconeogenesis. Here we do not consider cases where existing thioesters undergo carbon chain elongation, such as in fatty acid synthesis. A notable exception in which thioesters are formed without recourse to ATP is the key step of the acetyl CoA pathway described in section 3.

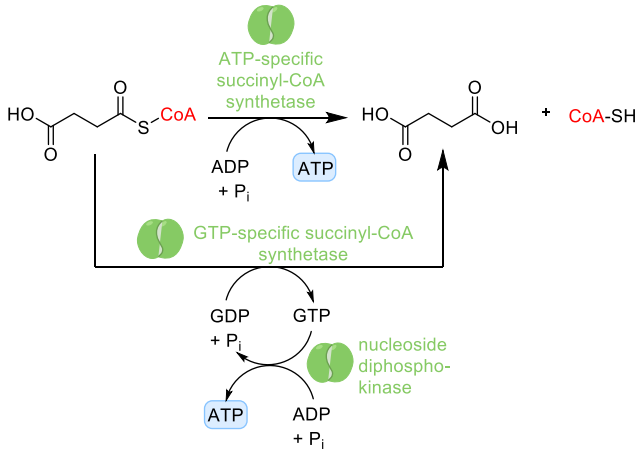
Scheme 3. Typical thioester-generating processes in metabolism: a, from α-ketoacids; b, from aldehydes; c, via acyl transfer.



Scheme 4. Structure of coenzyme A (CoA).



Scheme 5. Coupling of succinyl-CoA hydrolysis to the production of ATP (TCA cycle).



The energy-rich thioester bond (-31.5 kJ mol^{-1} in acetyl-CoA) provides an efficient energy source for the phosphorylation of ADP to ATP (or GDP to GTP, in the case of succinyl-CoA), making the sulfur-based energy relay indispensable for biological energy conservation (Scheme 5). Similar chemistry is performed by several other important enzymes, for instance acetyl-CoA synthetase or long-chain-fatty-acid—CoA synthetase. Complete biochemical mechanisms of these transformations have been described in detail elsewhere,[17,136] and will not be repeated here.

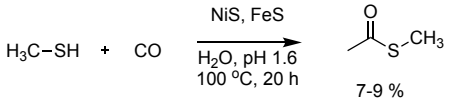
5.2. Prebiotic thioester chemistry in the context of bioenergetics

As we have seen, thioesters such as CoA are deeply engrained in biosynthesis and bioenergetics in all organisms. For this reason, it has been hypothesized that their incorporation into the chemistry of life occurred very early on life's evolutionary timeline, likely before enzyme-mediated biochemistry emerged. It has also been proposed that CoA itself is likely to have had ancient roots.[91,167] Thus, de Duve proposed a "Thioester World" period during the transition from prebiotic chemistry to biochemistry, which was later explored by others.[114,168–172] The plausibility of an ancient, thioester-dependent metabolism was recently reinforced by a computational analysis of thermodynamic properties and topologies of all known metabolic transformations. Using network expansion algorithms, Segrè and co-workers obtained a hypothetical metabolic network resembling a primitive (r)TCA cycle, that serves as a hub for the synthesis of acetyl, malonyl, malyl and succinyl thioesters.[153] Structurally simpler CoA subunits such as pantetheine (Scheme 4) were suggested as primitive CoA predecessors.[153,167] Nonetheless, experimental reports on prebiotically feasible thioester syntheses have been scarce. A notable example includes the low-yielding synthesis of acetyl thioester from CO and MeSH, which can be viewed as an analog of the Acetyl CoA pathway (Scheme 6a).[100] Other examples produce thioesters from aldehydes (analogous to Scheme 3b), such as the reaction of glyceraldehyde and *N*-acetylcysteine,[173] or the photochemical oxidation of aldehydes with disulfides under solvent-free conditions.[174] (Scheme 6b)

Recently, our group reported the oxidative formation of thioesters from α-ketoacids using the thiol *N*-acetylcysteamine, a truncated version of CoA. Acetyl, malonyl, malyl and succinyl thioesters were produced from pyruvate, oxaloacetate, 4-hydroxy-α-ketoglutarate and α-ketoglutarate, respectively, in yields up to 31%, with remaining material being mostly unreacted ketoacid or carboxylic acids resulting from thioester hydrolysis (Scheme 6c). The oxidative process could be enabled under aqueous conditions by the presence of sulfate radicals (generated thermally at 70 ºC or photochemically at ambient temperature) or directly by UV light.[140] One of the unique aspects of this decarboxylative ketoacid-to-thioester reaction is its ability to be integrated with the ketoacid-forming reaction network shown in Scheme 2.[157]

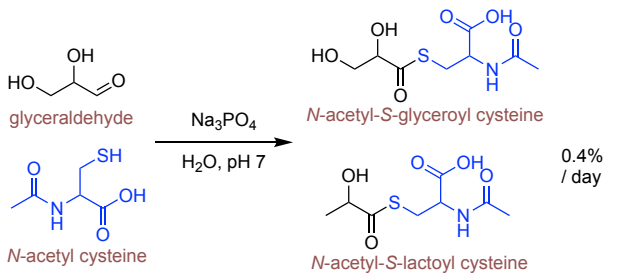
Scheme 6. Examples of prebiotically-feasible thioester synthesis.

a) synthesis of thioesters from thiols

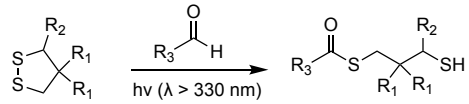


Huber and Wächtershäuser (ref. 100)

b) synthesis of thioesters from aldehydes

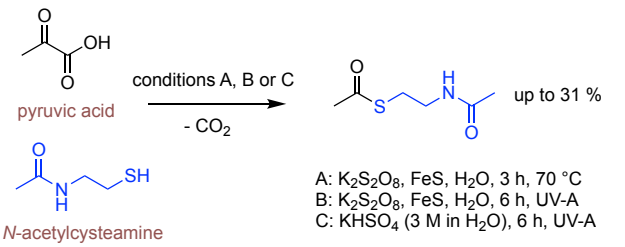


Weber (ref. 173)



Takagi and co-workers (ref. 174)

c) synthesis of thioesters from α-ketoacids



Moran and co-workers (ref. 140)

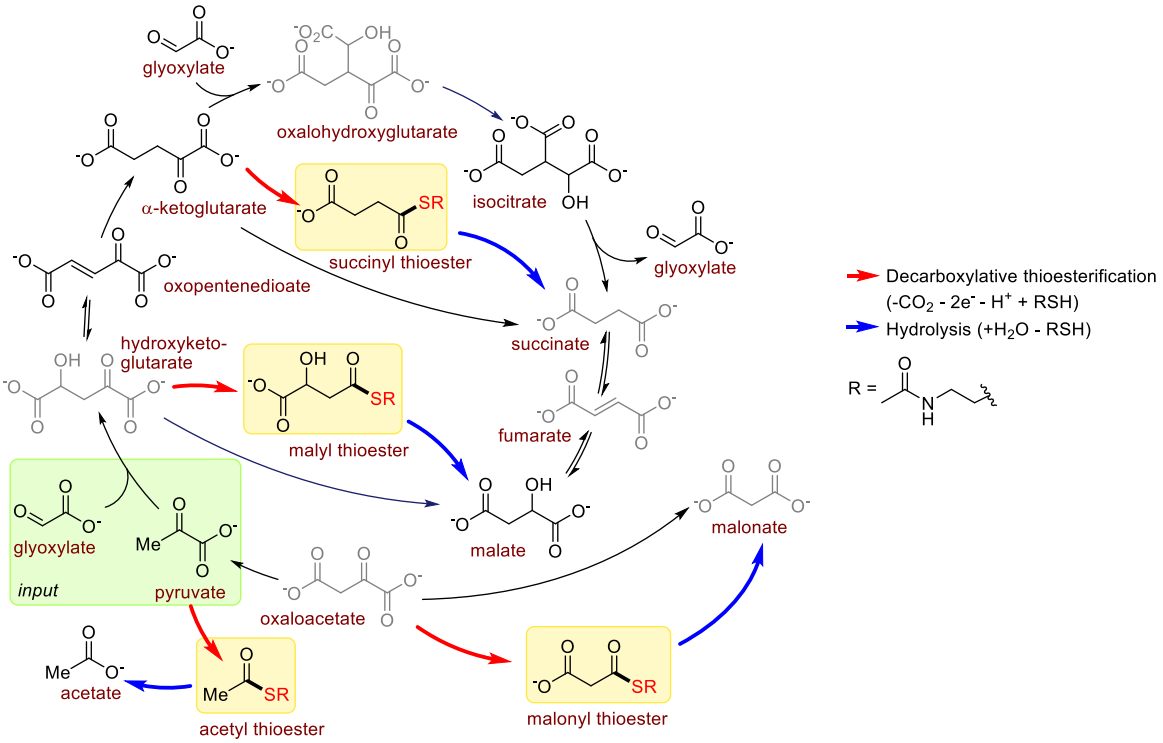
Thus, mixing glyoxylate and pyruvate in the presence of Fe^{2+} and *N*-acetylcysteamine at 70 ºC produces a variety of ketoacids, which upon exposure to the sulfate radical precursor $K_2S_2O_8$, produce acetyl, malyl and succinyl thioesters in a single pot experiment (Scheme 7).[140] This non-enzymatic reaction network embodies several of the key features of the biological TCA cycle, including the ability to form C-C bonds via aldol reactions and to couple the oxidative decarboxylation of ketoacids to the formation of high energy thioesters.

5.3. Summary and future directions

Experimental evidence now supports conjecture that thioester synthesis and life's catabolic processes may have been linked at a stage as early as prebiotic chemistry. The convergence between computational predictions of a primitive thioester-based metabolism[153] and the experimental observation of thioesters generated within a complex reaction network support the idea that top-down and bottom-up approaches to the origin of metabolism can, and should, eventually meet.[175]

What is currently missing is the link between thioester chemistry and phosphate chemistry in the context of energy conservation. The efficient and reversible interconversion of thioesters and acyl phosphates or other activated acyl species is a key aspect of bioenergetics that has yet to be demonstrated experimentally under prebiotic conditions. This difficulty may be because simple thioesters tend to hydrolyze faster than they undergo useful intermolecular reactions.[176] Indeed, Lane and co-workers demonstrated that only thioacids and not simple thioesters could produce acyl phosphates in the presence of inorganic phosphate,[177] in line with Liu and Orgel's earlier demonstration that thioacetic acid can react with phenylphosphate under oxidative conditions to generate acetyl phenylphosphate at room temperature.[178] A key question in this regard is the structural impact of the thiol moiety on thioester reactivity. CoA, the biological thiol cofactor, is not a simple thiol, but also contains three phosphate groups (Scheme 4). In the absence of structure-activity relationship studies,[179] it remains unclear why life ended up using such a complex cofactor if a simpler one could have offered similar function. In fact, this same question could be asked of many of life's cofactors. It has been suggested that certain structural elements of cofactors, such as phosphate or ribonucleotide moieties, may have served as molecular recognition sites that pre-existing enzymes could bind, thus enabling the evolution of co-factors and enzymes via chemistry-driven divergence.[180] In the case of CoA, the cofactor may have needed its complex structure in order to execute its core functions, namely the ability to interconnect thioester and acyl phosphate chemistry. It is possible that thioester and polyphosphate-driven chemistries were both required from the outset in order to circumvent thermodynamic bottlenecks.[181] Prebiotic sources of phosphate and prebiotic means of phosphorylation have been the subject of investigation for a long time and have recently been reviewed.[182-184] However, studies on non-enzymatic versions of the phosphorylation reactions found in core metabolic pathways remains rare, with a notable exception being the phosphorylation of adenosine, AMP or ADP to ATP.[177,185–188] Further experimental investigations will be required to understand the potential functional roles of thioesters in a prebiotic context and their relationship with phosphate.

Scheme 7. Redox- or light-promoted thioester formation within an iron-catalyzed reaction network, generated from pyruvate, glyoxylate and *N*-acetylcysteamine.[140] Molecules observed in the presence of Fe^{2+}, thiol and oxidant are shown in black, while those observed in the presence of Fe^{2+} only are shown in grey.



Another important question is the link between thioester chemistry and the biochemistry of nitrogen. Thioesters of amino acids are known to easily polymerise.[189–192] Thus, it has been suggested that non-ribosomal peptide synthesis, a non-coded thioester-dependent form of peptide synthesis that is still important to life today, may have given rise to coded peptide synthesis via the aminoacyl tRNA.[171] This idea should provide an interesting approach towards bio-inspired peptide synthesis.

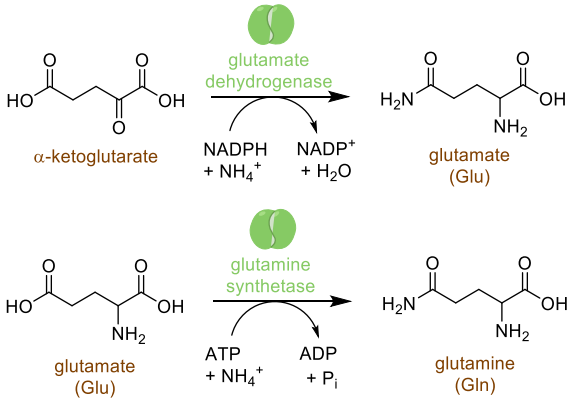
6. AMINO ACIDS

Amino acids in the form of peptides constitute a significant share of cellular mass (45% w/w of dry mass in *Saccharomyces cerevisiae*).[193] Amino acids are the building blocks that make up enzymes, which in turn catalyze amino acid formation. This chicken-and-egg relationship presents a causality problem for prebiotic chemistry. Owing to the central role of amino acids in life, their prebiotic synthesis is one of the most thoroughly explored areas of origin of life chemistry. Such was the appeal of amino acids as a biosignature that their detection, for example in the famous Miller-Urey spark discharge experiment,[194] used to be equivocated with the undeniable relevance of an experiment to abiogenesis. Amino acids were also detected in meteorites,[195,196] in tails of comets,[197] and potentially in interstellar space,[198] which has since stirred up debate on the prebiotic relevance of extraterrestrial delivery of these building blocks to Earth.[199] Popular approaches to prebiotic amino acid synthesis employ such chemistries as the Strecker reaction (with α-aminonitrile intermediates)[200] or the Bücherer–Bergs synthesis (with hydantoin intermediates),[201] and have recently been reviewed elsewhere.[11] However, the above strategies differ strongly from how biology makes amino acids—which is from α-ketoacids, sometimes over several steps. Neither the spark discharge experiments, the meteoritic delivery or the prebiotic syntheses mentioned above explain why and how life arrived at making amino acids the way it does. However, amino acids can also be built by prebiotic chemistry in the same way that life does it now, from α-ketoacids, before there were enzymes. Which prebiotic means of making amino acids are more relevant to the origins of life? Which better accounts for the amino acid inventory used by life today? To help frame these questions, we will present a brief overview of biological amino acid synthesis, touch on how these pathways relate to the genetic code, and review potentially prebiotic amino acid syntheses inspired by biology. These points suggest that prebiotic amino acid synthesis likely occurred from α-ketoacids, as it does in biology, within a protometabolism that furnished them.

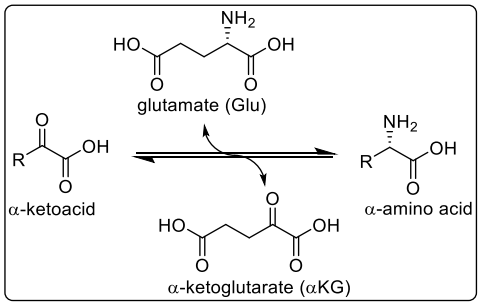
6.1. Amino acid biosynthesis and its relationship to the genetic code

How nitrogen enters metabolism shows, perhaps surprisingly, very little variation throughout life. Ammonia from N_2 or nitrate reduction becomes incorporated into two amino acids: glutamate and glutamine. These amino acids are the major hubs for the biosynthetic entry of nitrogen into metabolism.[202] Glutamine is biosynthesized when glutamate reacts with ammonia and ATP (Scheme 8) and serves mainly as a nitrogen donor in the biosynthesis of pyrimidine nucleobases (this will be described in section 8). The main role of glutamate is to transfer nitrogen to α-ketoacids in transamination reactions. This process is mediated by a class of enzymes called *aminotransferases* or *transaminases* that require a cofactor, pyridoxal phosphate. The mechanism proceeds in two steps. First, an amine group is transferred from glutamate to pyridoxal phosphate. The second step involves the transfer of the amino group from pyridoxal phosphate to the target α-ketoacid to generate the amino acid (Scheme 9).[136] Glycine, alanine, valine, and aspartate are synthesized this way directly from their corresponding α-ketoacids (Figure 6). Glutamate can also be synthesized from α-ketoglutarate, by transamination from glutamine.

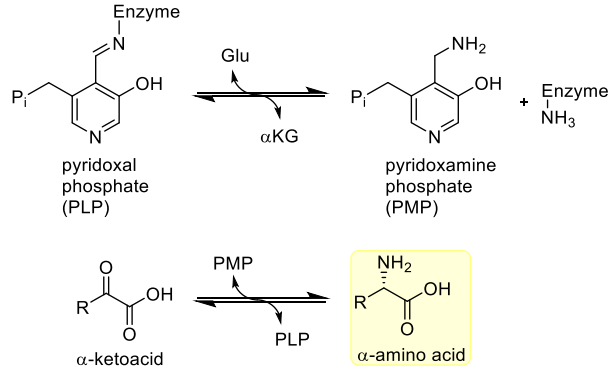
Scheme 8. Entry of nitrogen into metabolism: biosynthesis of glutamate and glutamine



Scheme 9. Biochemical mechanism of amino acid synthesis via transamination



Mechanism:



Other amino acids require a more complex biosynthesis, but their nitrogen is usually relayed by glutamate at some point in their biosynthetic pathway. Closer examination of the 20 proteinogenic amino acids reveals that 16 are biosynthesized in 1-12 steps from three TCA cycle intermediates: pyruvate, oxaloacetate and α-ketoglutarate. The four others are also ultimately derived from the TCA cycle but are much further away (over 20 steps) (Figure 6). Within the working hypothesis that a non-enzymatic (r)TCA cycle, or something that produced the same key ketoacids, was the core of prebiotic chemistry, we might consider the 16 amino acids synthesized in 1-12 steps to be the most ancient, whereas the four synthesized in >20 steps would be later developments.

The biosynthesis of amino acids bears strong evolutionary ties to the biosynthesis and evolution of genetics that might date back to prebiotic chemistry.[203–208] The code contains many unexplained patterns, most notably a strong correlation between the first two bases in a codon and the biosynthetic pathway used to make the coded amino acid.[209] The third base of most codons is highly redundant and therefore contains less information than the first two bases. By omitting the third base, a simplified doublet code was proposed which can be viewed as a set of biosynthetic instructions for the coded amino acid.[210,211] Of the 16 "ancient" amino acids, those whose codon starts with cytidine (C) are derived from ketoglutarate, while for adenine (A) it is oxaloacetate, and for uracil (U) it is pyruvate (Figure 6). The second base of the codon is also predictive of the later transformations in amino acid biosynthesis.[211]

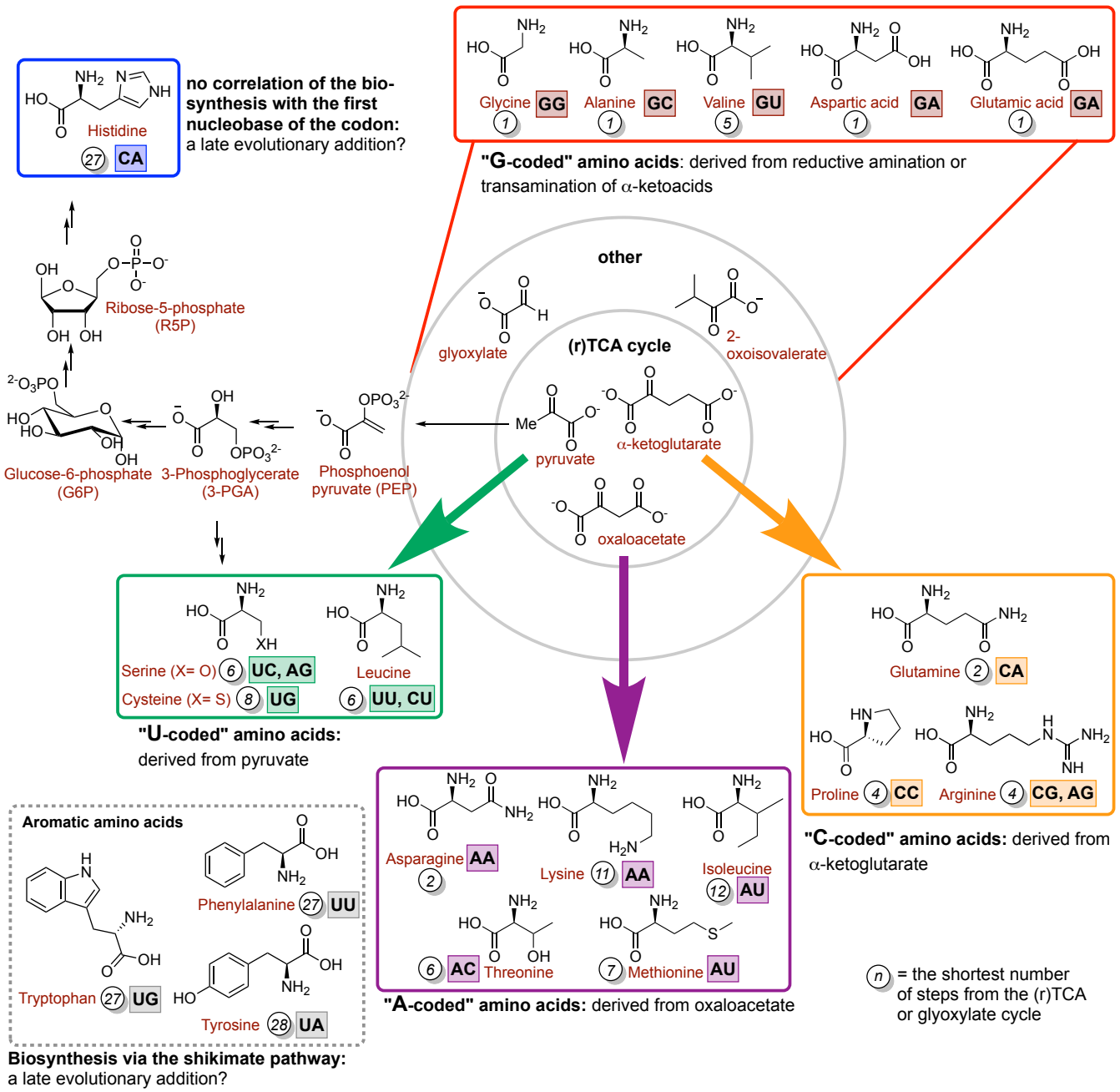


Figure 6. Biosynthesis of amino acids, highlighting correlations between the first two bases in RNA codons and their biosynthetic origins.

Why should the code be more strongly correlated with the amino acid's biosynthetic history than it is with its chemical properties? Copley and co-workers suggests that, within a protometabolism similar to the one described in the previous section, diribonucleotides would become covalently bound to amino acid precursors and induce intramolecular catalysis of specific, sequential steps of amino acid biosynthesis.[210] In other words, amino acids would be made right on the diribonucleotide, producing the associations now found in the genetic code that correlates with the amino acid's synthetic history. The authors proposed a set of organocatalytic mechanisms for the alleged intramolecular catalysis, which has yet to be tested experimentally. Although the specific mechanisms proposed seem unlikely in our view, ideas along these lines hold great promise as starting points to understand the emergence of the genetic code from a protometabolism.

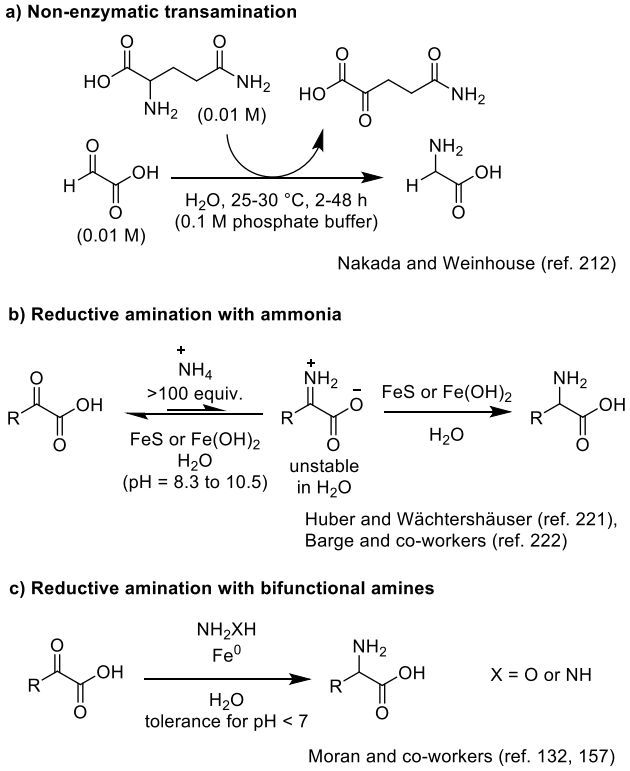
6.2. Prebiotic synthesis of amino acids from α-ketoacids

Plausibly prebiotic examples of amino acid synthesis that occur in ways reminiscent of biosynthesis have been known for a long time, though they are relatively few. Non-enzymatic transamination of ketoacids was reported as early as 1953, when Nakada and Weinhouse obtained glycine from glyoxylic acid using a glutamine/glutamate couple at ambient temperature in aqueous phosphate buffer[212] (Scheme 10a). Similar transamination reactions of other ketoacids were later shown to be promoted by Fe^{2+} and other metal ions.[213,214]

In 1975, Nakajima and co-workers synthesized an α-ketoacid, pyruvate, using the FeS-containing Schrauzer's complex, and subsequently converted it to alanine by transamination with pyridoxamine in 0.3% yield.[215] This method was later improved by the same authors to utilize ammonia as nitrogen donor, giving yields up to 73% for phenylalanine obtained from phenylpyruvate.[216] Following up on earlier similar reports,[217–220] this approach was later adopted by Huber and Wächtershäuser, who used freshly prepared FeS and $Fe(OH)_2$ with NH_3 as nitrogen donor to obtain alanine, glutamate, phenylalanine and tyrosine in yields up to 30% (Scheme 10b).[221] Recently, two research teams reported improved conditions for this type of reactivity. Barge and co-workers proposed a mixed-valence iron oxyhydroxide mineral system, where alanine could be obtained from pyruvate in 70% yield at 70 °C and pH 10. However, this reaction environment was incompatible with other α-ketoacids such as oxaloacetate.[222] Subsequently, Kitadai and co-workers reported reductive amination of several α-ketoacids (glyoxylic, pyruvic, oxaloacetic and α-ketoglutaric) to amino acids (glycine, alanine, aspartic acid and glutamic acid) promoted by partially electroreduced FeS (FeS-Fe^0) under alkaline conditions.[131] Building on the work of Zhang and Martin using ZnS photocatalysis to enable reactions of the rTCA cycle,[127] Su and co-workers showed that the same system could enable reductive amination.[223] All of the above reports describe experiments conducted under basic conditions (to ensure the nucleophilicity of the nitrogen atom) and require a large excess of ammonia (typically ≥ 100 equivalents of ammonia[131,221,222]) due to the unfavorable equilibrium for the formation of an NH-imine in water (Scheme 10b). Not only does this raise doubts about the geochemical plausibility of an environment that is highly concentrated in ammonia, but also presents a potential problem for amino acid synthesis in those metabolic origins scenarios that require neutral or acidic aqueous environments. Under such conditions, ammonia would occur in a mostly protonated form and therefore would not be nucleophilic.

Recently, we reported the synthesis of alanine from pyruvate using a stoichiometric quantity of hydrazine as a nitrogen source. This was inspired by the biological dinitrogen reduction pathway, where hydrazine is one of the intermediates. In an acidic environment, hydrazine is protonated, like ammonia, but protonation of the second nitrogen atom is avoided due to electrostatic repulsion. This leaves a nucleophilic nitrogen available for reductive amination, even under strongly acidic conditions (Scheme 10c). Metallic iron was used as reducing agent for C-N double bond and N-N single bond cleavage. For pyruvate as starting material, a binary mixture of alanine (97%) and lactate (3%) was observed.[132] In a follow up study performed under much milder acidic conditions (pH 4.5-5.7), hydroxylamine was used as nitrogen donor for reductive amination of α-ketoacids. Hydroxylamine is an intermediate in natural nitrogen cycles,[77] and a potentially prebiotic feedstock.[224,225] Consequently, four biological amino acids (glycine, alanine, aspartic acid and glutamic acid) were obtained from their respective α-ketoacid precursors (glyoxylic acid, pyruvic acid, oxaloacetic acid and α-ketoglutaric acid), directly from a carboxylic acid complex network (Scheme 10c, see also Scheme 2).[157] However, the relevance of bifunctional amines such as hydroxylamine and hydrazine to a broader protometabolism is also questionable, for example, due to their likely incompatibility with electrophilic functional groups such as thioesters and acyl phosphates. They also do not give insight into why life uses ammonia, rather than bifunctional amines, in its biochemistry.

Scheme 10. An overview of prebiotically-relevant examples of amino acid synthesis that mimic biological mechanisms.



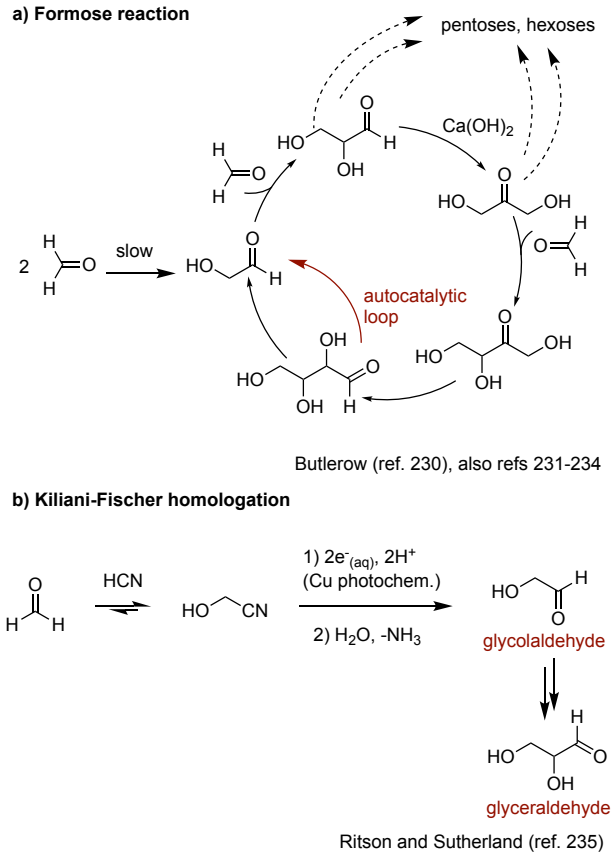
6.4. Summary and future directions

Organic chemistry offers countless ways to make amino acids under plausibly prebiotic conditions. As shown in this section, some of these, particularly the non-enzymatic transamination reactions, closely parallel amino acid biosynthesis. Placing prebiotic synthesis of amino acids in the context of a protometabolism similar to core carbon metabolism gives a concise explanation for why nature selected the 20-or-so proteinogenic α-amino acids that it did: they are the ones that could be easily made from the available ketoacids. This contrasts with other attempts to explain this selection, especially in light of the fact that many potentially prebiotic syntheses also furnish β and γ-amino acids, or α-amino acids bearing alternative complex side chains.[226–228] The same question pertains to the composition of primordial polypeptides. Were they based on the same set of amino acids as modern proteins or on a smaller subset? Recent studies indicate that proteins composed solely of the "early" amino acids would indeed have still been functional.[229] Is life's way of transporting ammonia solely a result of physical considerations in contemporary biology (ie. its rapid diffusion through membranes), or does it also trace back to prebiotic chemistry? What is lacking is an experimental demonstration from prebiotic chemistry that explains why biochemistry incorporates ammonia through the intermediacy of just two amino acids, and then builds the other amino acids in the ways that it does. Furthermore, experimental evidence is necessary to test ideas that the close relationship between amino acid biosynthesis and the first one or two letters of the genetic code is a result of their common evolutionary history.

7. CARBOHYDRATE METABOLISM

Classically, prebiotic chemists have focused on sugar syntheses involving the formose reaction (the polymerization of formaldehyde),[230–234] (Scheme 11a), or the Kiliani–Fischer-type homologation of hydrogen cyanide with cyanohydrin intermediates (Scheme 11b).[235] However, nature uses no such chemistry and leaves no trace that it ever did. Consequently, these chemistries give little insight into how and why biological carbohydrate synthesis came to operate in the way that it does. Sugar biosynthesis starts from pyruvate and follows a pathway known as gluconeogenesis. Sugars are broken down by biological pathways known as glycolysis and the pentose phosphate pathway. Most intermediates in these pathways are phosphorylated and gluconeogenesis appears to be conserved across all life. Why does life do things this way, when alternative chemistry to make sugars would be much simpler? Phosphate binding is the driving force for the evolution of nearly all the most ancient enzyme families,[236] and this key role likely predated enzymes altogether. Like the other metabolic pathways described in this review, sugar metabolism likely started as a subset of a larger, non-enzymatic reaction network. To frame the discussion, below we present the pathways of sugar anabolism and catabolism used by nature, summarize current opinions on the evolutionary origins of these pathways, and review the prebiotic chemistry attempts to recreate them, highlighting those that are the most similar to biosynthesis.

Scheme 11. An overview of non-biological approaches to sugar synthesis.



7.1. Biological sugar metabolism

Gluconeogenesis is the anabolic pathway that builds sugar phosphates from pyruvate (Figure 7). It is present in all archaea and displays a high degree of uniformity.[237] The "central trunk" of gluconeogenesis, the fragment between phosphoenol pyruvate and glyceraldehyde-3-phosphate (Figure 7, red arrows), contains very similar enzymes across the clades of primitive organisms. This means that these enzymes are universal for life and likely share their evolutionary history.[238] In contrast, glycolysis is much more varied across different species of archaea, where it can be carried out by a variety of pathways, including the Embden-Meyerhof-Parnas (EMP) pathway, the Entner-Doudoroff (ED) pathway, fragments of the pentose phosphate pathway, or their combinations. These various pathways employ different enzymes and different intermediates (Figure 7).[90,239] Consequently, gluconeogenesis is typically considered more ancient than glycolysis.[90,239,240] However, the types of chemical mechanisms involved in all of the abovementioned pathways, whether anabolic or catabolic, are quite similar. They include phosphorylations and dephosphorylations, isomerizations, hydrations and dehydrations, and aldol and retro-aldol reactions (Figure 7).[136]

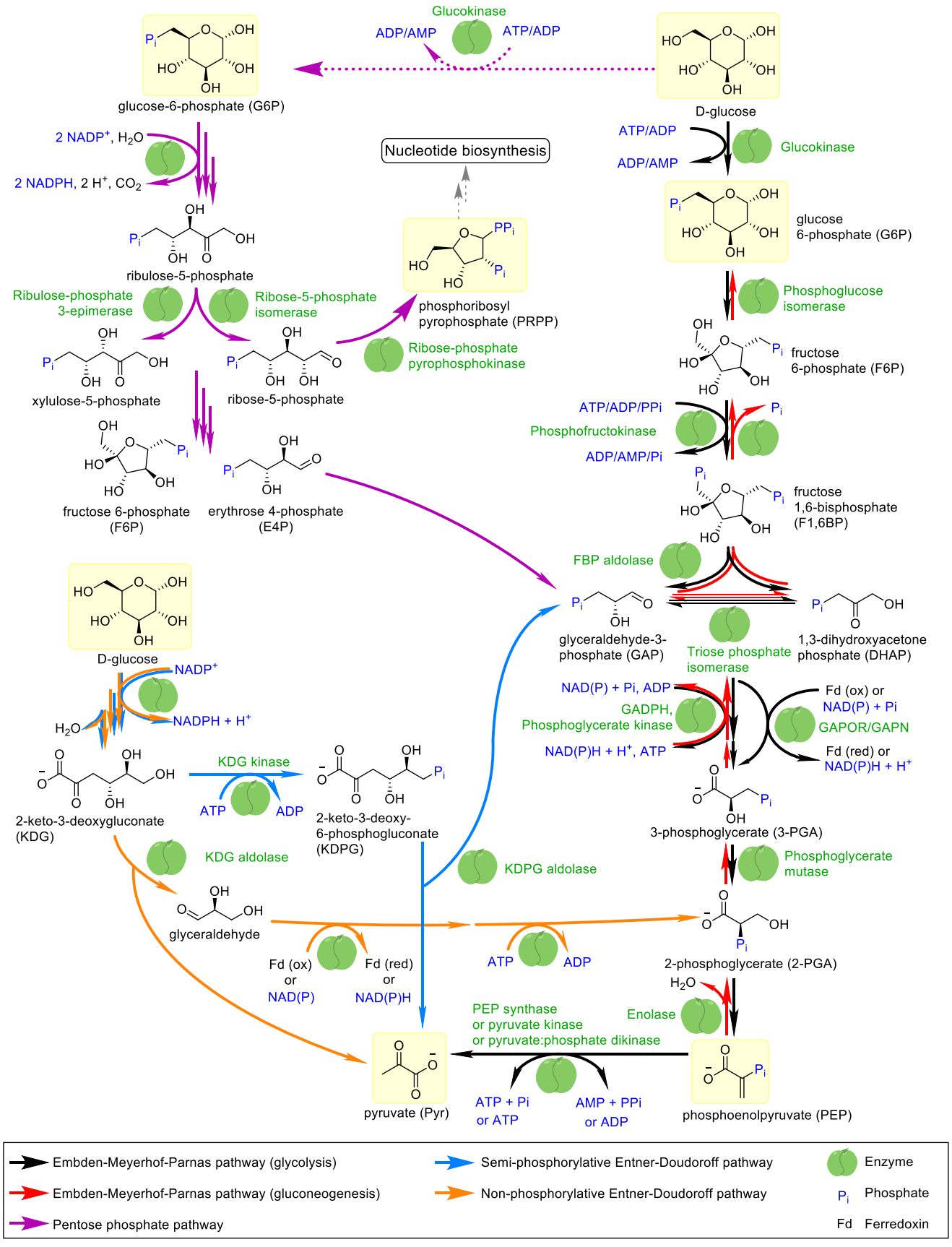
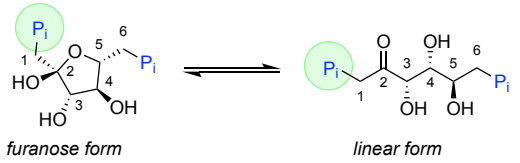


Figure 7. An overview of biological carbohydrate synthesis and breakdown.[90,139] Only key intermediates and enzymes are shown. Major biochemical hubs are highlighted by yellow boxes.

Gluconeogenesis involves the phosphorylation of pyruvate to phosphoenolpyruvate (PEP), followed by hydration of the resulting alkene to give 2-phosphoglycerate (2-PGA). Intramolecular phosphate transfer to the more stable terminal primary alcohol then occurs to give 3-phosphoglycerate (3-PGA). Phosphorylation of the carboxylate moiety of 3-PGA results in an acyl phosphate intermediate (1,3-bisphosphoglycerate, not shown) that is reduced to an aldehyde to give glyceraldehyde-3-phosphate (GAP). Some of the GAP isomerizes to the ketone form, 1,3-dihydroxyacetone phosphate (DHAP), which then undergoes a crossed aldol reaction with remaining GAP to give fructose-1,6-bisphosphate (F1,6BP). The phosphate at the 1-position is then selectively hydrolyzed to fructose-6-phosphate (F6P). It is important to note that the two phosphates in F1,6BP are not chemically equivalent. In the open form of F1,6BP, the phosphate to be hydrolyzed at the 1-position is adjacent to a reactive ketone group, which is additionally activated by two adjacent electronegative oxygen groups that render it significantly more electrophilic through inductive effects. F6P then isomerizes from the furanose to its pyranose form, glucose-6-phosphate (G6P) (Scheme 12). Hydrolysis of the remaining phosphate to give glucose, completes the pathway.

Scheme 12. Cyclic and linear form of F1,6BP.



fructose 1,6-bisphosphate (F1,6BP)

Glycolysis is much more varied across species than gluconeogenesis. Although it proceeds through the same intermediates as gluconeogenesis, but in the reverse direction, it should not be mistaken for being the microscopic reverse of the latter, since it has a different thermodynamic driving force. Depending on the organism, some variations of glycolysis involve phosphate to different extents (Figure 7, black vs blue vs orange arrows).

Finally, the pentose phosphate pathway occurs in parallel to glycolysis—it does not replace it. The major purpose of this pathway, apart from the generation of NADPH, is converting glucose into a variety of phosphorylated pentose sugars. Notably, ribose-5-phosphate becomes further phosphorylated to furnish a furanose, phosphoribosyl pyrophosphate (PRPP), which is then funneled towards ribonucleotide biosynthesis (the role of PRPP as a ribonucleotide building block will be described in section 8).

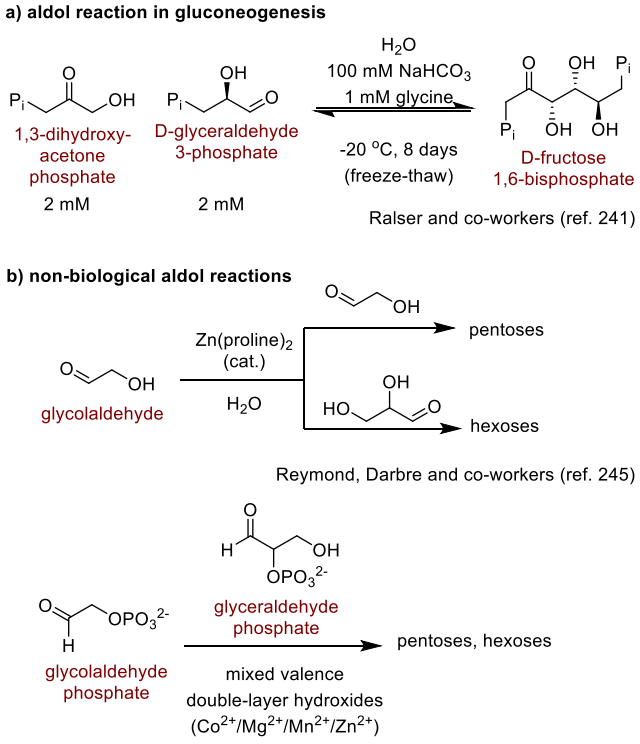
7.2. Prebiotic synthesis and breakdown of carbohydrates mirroring metabolism

Sugar biosynthesis via gluconeogenesis is driven thermodynamically by the consumption of ATP. Non-enzymatic conditions that enable the key phosphorylation reaction of pyruvate to phosphoenolpyruvate have yet to be described. However, the key C-C bond forming aldol reaction of gluconeogenesis has recently been explored. Ralser and co-workers found that the aldol reaction between two unstable intermediates, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, can be promoted simply by freezing them to give a stable hexose, fructose 1,6-bisphosphate (Scheme 13a, see also gluconeogenesis in Figure 7, red arrows). Although the level of diastereoselectivity of the aldol reaction was not established, the crossed aldol reaction between GAP and DHAP was found to be faster than the self-condensation reaction. The reaction rate is increased by concentrating the solution in interstitial micro-channels in ice. The reaction was also found to be accelerated by the amino acids glycine and lysine, which may hint at the early mechanisms of enzyme evolution.[241] This conceptually resembles previous work in which amino acids and dipeptides were found to bias the stereoselectivity of aldol reactions forming trioses, tetroses and pentoses from glycolaldehyde and glyceraldehyde in water.[242–244]

Other ways to accelerate aldol reactions in the context of prebiotic chemistry have been studied, though they do not directly mimic reactions of gluconeogenesis. Complexes of metal ions and amino acids have been shown to accelerate aldol reactions in water.[245] Mineral surfaces have also been used to preorganize substrates of aldol reactions in water, producing tetrose, pentose and hexose phosphates from formaldehyde, glycolaldehyde, and glyceraldehyde (Scheme 13b).[246,247]

Apart from the templating effect,[248] mineral surfaces may have also played a role in phosphorylation of gluconeogenesis intermediates.[249] For example, the phosphorylation of ribose to phosphoribosyl pyrophosphate (PRPP) appears straightforward under dry-down conditions on silica,[250] (the relevance of this reaction to non-enzymatic ribonucleotide synthesis will be discussed in section 8, see also Scheme 13). However, in order to make phosphoenol pyruvate (PEP), a very strongly activated phosphate donor would be required.

Scheme 13. Aldol reactions in prebiotic sugar synthesis



The search for simple compounds that can accomplish the same activating and phosphorylating role as ATP is a major open question. Simple thioacids, acyl phosphates, or inorganic polyphosphates or cyclic phosphates[251] could possibly play this role, although some of these molecules may be too prone to hydrolysis to be chemically useful.[177] An extensive summary of the potentially prebiotic phosphorylation hypotheses developed to date can be found in recent reviews.[182–184]

The hydrolysis of phosphate esters occurs in both gluconeogenesis and glycolysis. The specific phosphate ester hydrolysis reaction found in gluconeogenesis, the hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate, has no known non-enzymatic equivalent. However, other closely related non-enzymatic reactions have been described. Huang and Zhang reported that iron oxide nanoparticles in water as well as aged solutions of iron salts promote the hydrolysis of glucose-6-phosphate (G6P), 2-phosphoglycerate (2-PGA), ribose-5-phosphate (R5P) and fructose-1-phosphate (F1P) leading to the idea of "inorganic phosphatases".[252,253]

Unlike the reactions of gluconeogenesis, glycolysis does not need to be driven by an activated phosphate donor, and therefore the search for a non-enzymatic variant appears to be more straightforward. Ralser and co-workers reported a complex system of reactions resembling glycolysis and the pentose phosphate pathway, occurring in aqueous solution of Fe^{2+} salts (Figure 8, Scheme 14).[254,255] The specific conditions (soluble Fe^{2+}, 70 ºC) were chosen in order to simulate oceanic environments thought to be representative of the Archaean geologic eon. The iron salts were found to increase the stability of labile intermediates and accelerated catabolic reactions compared to pure water. Up to 29 of the interconversions present in glycolysis and the pentose phosphate pathway were observed to occur under the standard conditions, with pyruvate being the ultimate end-product. However, not all of the reactions map directly onto the biological pathway and some reactions could not be definitively characterized due to difficulties in chromatographic separation of certain intermediates. Some of the reactions were found to be Fe-dependent, whereas others were not. The extent of pH dependence and the optimal pH was found to be different for each of the individual reactions in the network. Those that were Fe-dependent showed different extents of Fe-concentration dependence. The observation that each reaction responds differently to pH and Fe is significant in that it explains why tailored enzymatic catalysts would be beneficial to the optimization of the overall pathway once Darwinian selection mechanisms were in place. The conditions identified in this study are notably very similar to those optimal for other non-enzymatic variants of carbon metabolism discovered later (see sections 4 and 5).

7.3. Summary and future directions

Much of glycolysis and the pentose phosphate pathway can be enabled without enzymes, promoted by Fe^{2+}. These discoveries give deep insight into how the reactions of these pathways might have originated and why they evolved. Much less work has been done on non-enzymatic analogs of gluconeogenesis—only a single aldol reaction has thus far been demonstrated. A graphical summary of known non-enzymatic reactions that recapitulate biological gluconeogenesis and the pentose phosphate pathway, leading from pyruvate to PRPP, is shown in Figure 8. Although many are lacking, there is sufficient precedent for mineral or metal catalysis of related reactions to warrant optimism that all of the pathways could have worked together before enzymes. As has previously been pointed out by Wächtershäuser, it is possible that the anionic phosphate or carboxylate groups present in all of the intermediates in gluconeogenesis are a remnant of the mechanistic constraints of its prebiotic origins, serving as essential molecular recognition elements to concentrate, pre-organize and activate metabolites on cationic minerals,[24] and later as anchors binding the substrates to the earliest enzymes.[236] Critical to the development of a non-enzymatic gluconeogenesis will therefore be the discovery of activated phosphate donors, likely polyphosphates, and the identification of suitable mineral catalysts. A mechanism for how such polyphosphates might be regenerated in a continuous manner would also be necessary. Though challenging, these are worthy goals because they would yield fundamental insight into why sugar metabolism works the way it does.

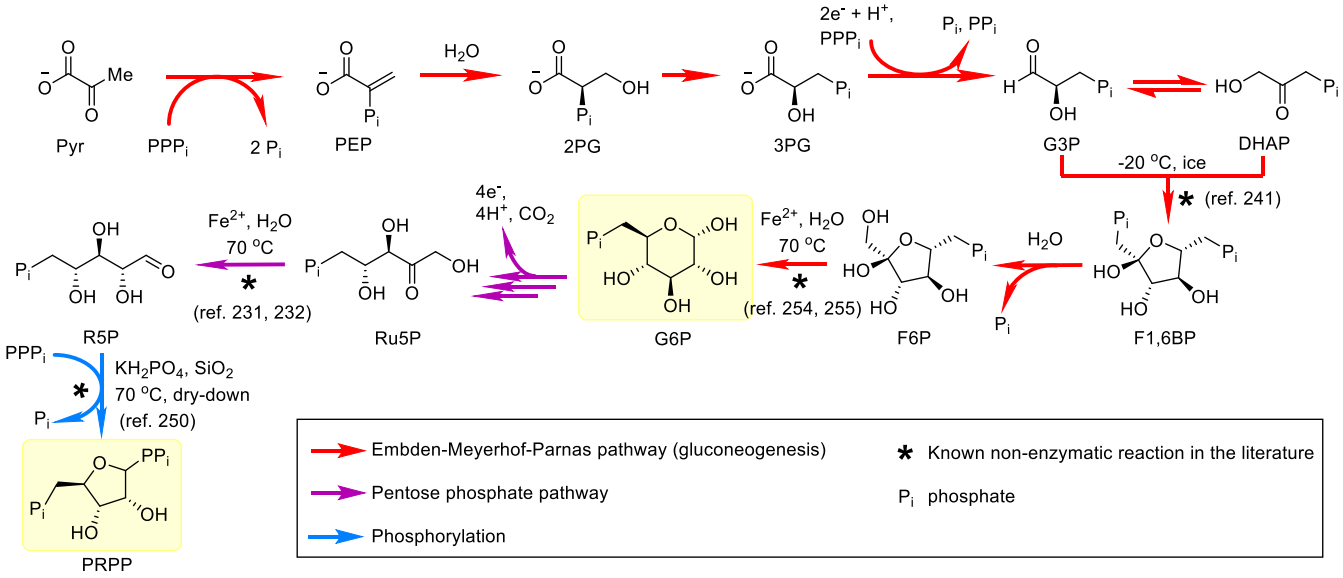
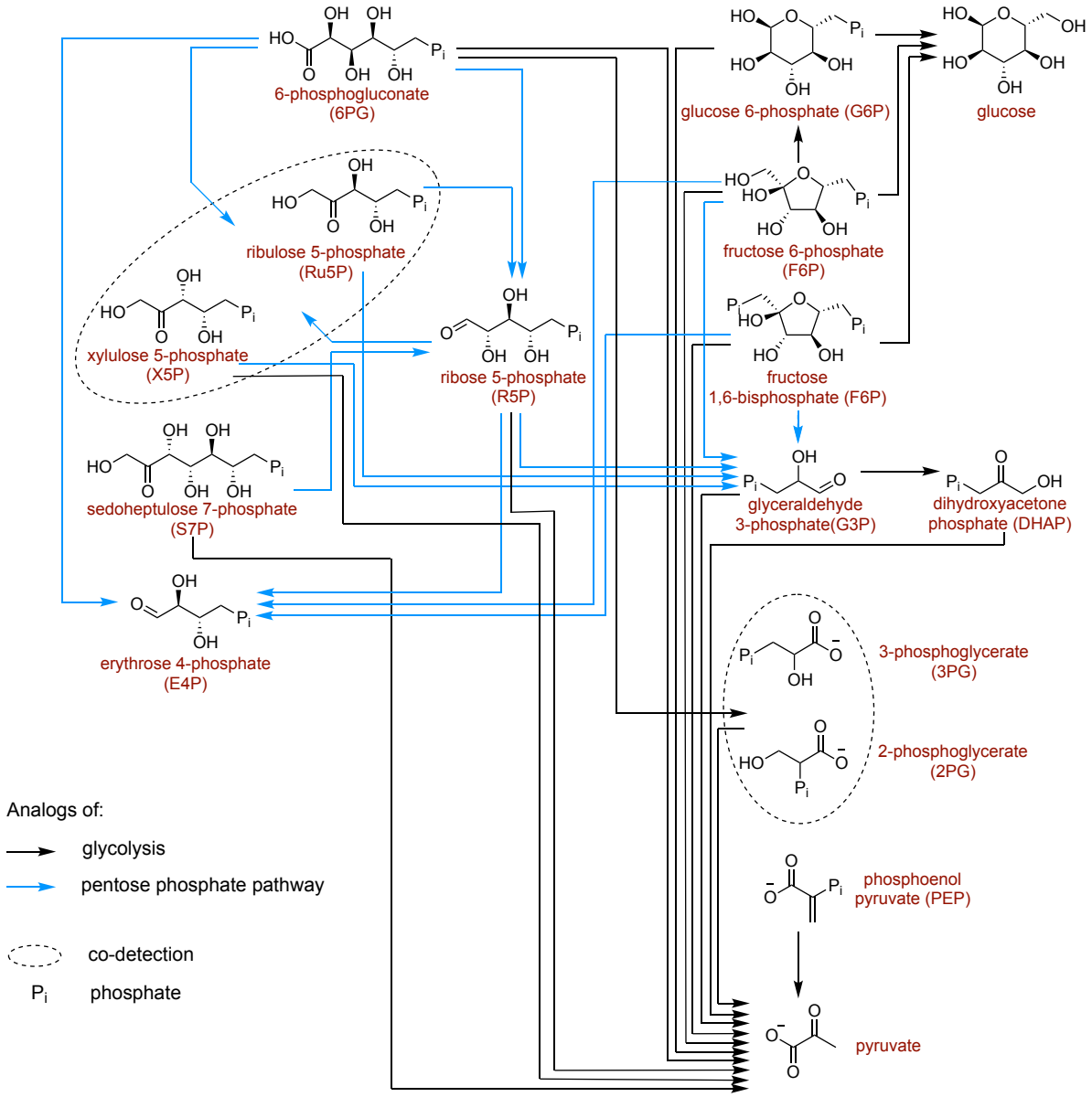


Figure 8 .Overview of reactions from pyruvate to phosphoribosyl pyrophosphate (PRPP). Established non-enzymatic reactions are indicated by an asterisk.

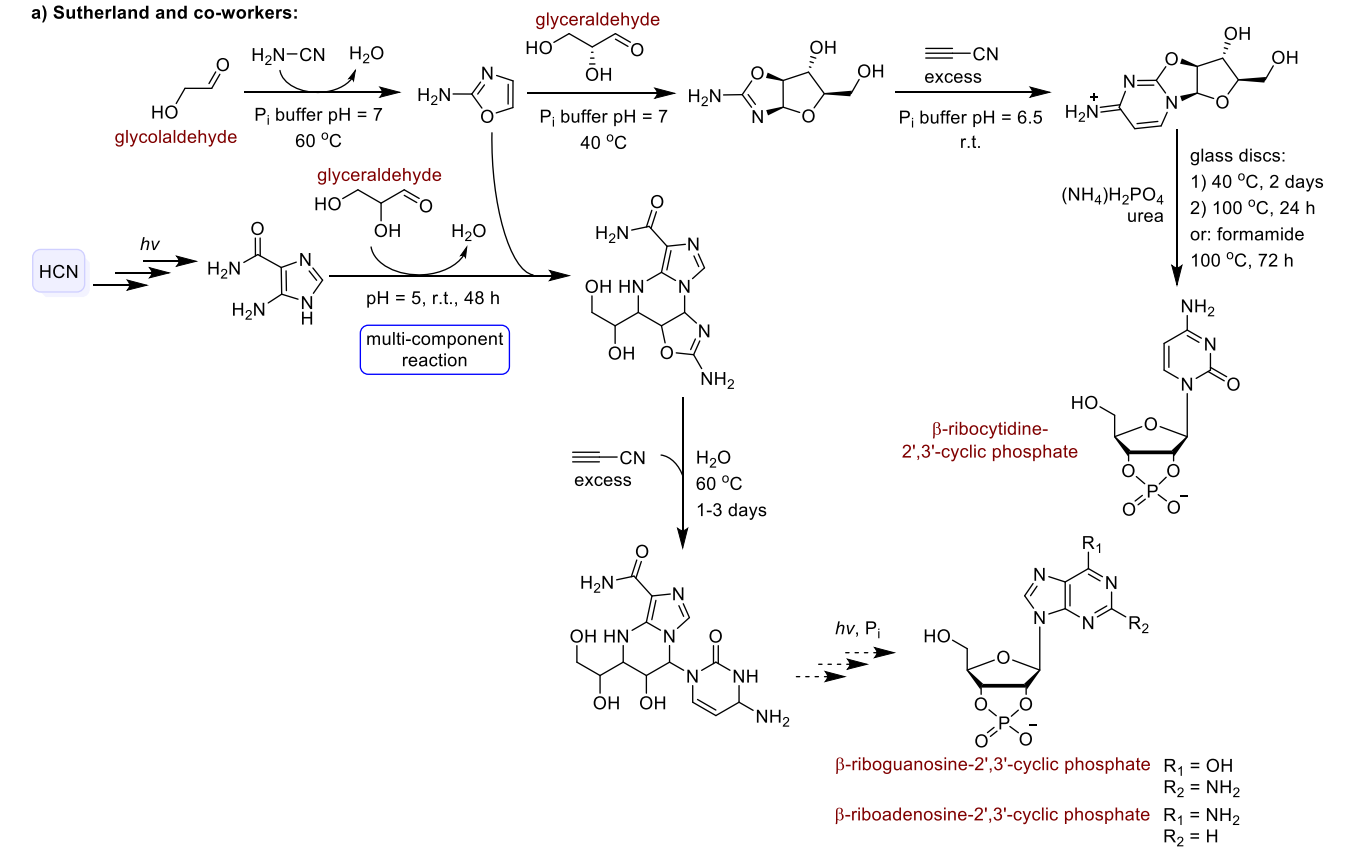
Scheme 14. Network of non-enzymatic analog of glycolysis (black arrows) and pentose phosphate pathway (red arrows) reported by Ralser and co-workers in Fe^{2+}-rich warm water.[254,255]

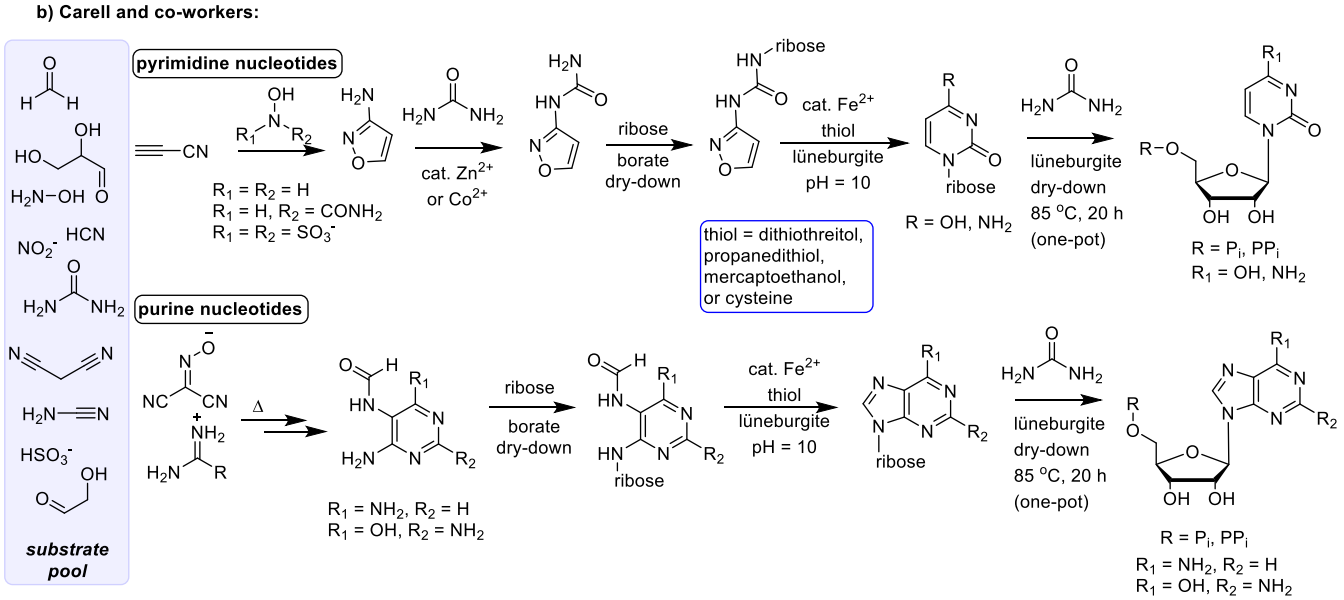


8. GENETICS AS AN OUTGROWTH OF PROTOMETABOLISM

Nucleic acids contain the universal informational code of all life. According to some theories, the first step of abiogenesis involved the prebiotic formation of RNA building blocks and the development of their oligomerization/polymerization mechanisms.[256] These popular theories have come to be known as "genetics-first" theories for the origin of life, the most prominent among them being the "RNA world" scenario.[4–6] Consequently, the empirical pursuit of prebiotic ribonucleotide synthesis has become a major occupation of chemists interested in the origin of life. Over the past decades, most approaches to the prebiotic syntheses of ribonucleotides were based on reactions of hydrogen cyanide and its derivatives. These advances have been reviewed in detail elsewhere and will not be elaborated on in the present review.[11,12,257,258] To highlight what are, in our view, two critical limitations to this approach, we will discuss two representative case studies (Scheme 15).[8,259–263] Although the syntheses are very different, they both start from compounds that are distinct from those found in metabolism today and proceed through synthetic routes that are conceptually different from the way that life assembles ribonucleotides. To varying extents, they both require sequential changes to the reaction conditions (reagents, solvent, pH, temperature, catalysts) that must be carefully temporally controlled in order to ensure the reaction proceeds down the desired path. The Sutherland synthesis of pyrimidine cyclic phosphates[260] requires four sequential well-timed changes to the conditions, in addition to the syntheses of glycolaldehyde and 2-aminooxazole. However, purine cyclic phosphates are made in a completely different way using different conditions.

Scheme 15. a) Highlights of the HCN-based syntheses of ribonucleotides reported by Sutherland and co-workers.[259,260] b) Unified purine and pyrimidine ribonucleotide synthesis promoted by dry-down cycles over minerals reported by Carell and co-workers).[263]





Without even taking into account the syntheses of the building blocks (glyceraldehyde, 2-aminooxazole and 5-aminoimidazole-4 carboxamide), at least three sets of conditions are required (Scheme 15a).[259] In contrast, the Carell synthesis requires four to five changes to reaction conditions, two of which can be carried out in a single pot. In this case, the sequential transformations are similar for both purine and pyrimidine ribonucleotides (Scheme 15b).[263] These approaches, however elegant, bear more similarity to a stepwise total synthesis of a natural product than to biosynthesis, where compounds are formed and react in a continuous manner. There is no doubt that these reports are beautiful feats of synthetic prowess. However, interpreted in light of the goal of prebiotic chemistry, which is to explain how life's biochemistry came to be the way that it is, the explanatory power of these works responds to these criteria to different extents. Sutherland's pyrimidine synthesis is completely different from biosynthesis. Carell's synthesis of pyrimidine ribonucleotides loosely resembles their biosynthesis, in the sense that ribose is coupled to a pre-existing heterocycle. However, in Carell's purine biosynthesis, ribose is coupled to a pre-formed aminopyrimidine, rather than building the heterocycle directly on the sugar scaffold.

Although it has been argued that the prebiotic chemistry that gave rise to ribonucleotides must have been distinct from biosynthesis,[7] both approaches leave to the imagination how a proto-biological system with dramatically different chemistry would have transformed itself into the biochemistry we know today. The typical response to these concerns is that evolution over long time periods would have slowly enabled such a transition.[264] Still, evolution is not a synonym for magic. For specific prebiotic chemistry to have any explanatory power, its transition into biochemistry must be demonstrated experimentally. In addition to these criteria, if multiple prebiotic chemistries appear plausible, Occam's razor should be applied to distinguish between the competing possibilities.

What is the alternative? Following the logic of continuity with biochemistry presented throughout this review, Harrison and Lane argued that prebiotic ribonucleotide synthesis might also imitate its biosynthesis. "To understand the origin of life, we would be foolish to ignore life as a guide".[35] To frame the discussion, we therefore present the biosynthetic pathways towards ribonucleotide synthesis and highlight non-enzymatic examples that parallel it.

8.1. Biosynthesis of ribonucleotides

Ribonucleotides are the monomeric building blocks of RNA. They are comprised of a pentose sugar, a nitrogen-containing nucleobase (purine or pyrimidine), and at least one phosphate unit. Non-phosphorylated analogs of nucleotides are known as nucleosides, which are intermediates of nucleic acid catabolism. In ribonucleotide biosynthesis, the pentose sugar, ribose, is always supplied as phosphoribosyl pyrophosphate (PRPP, sourced from the pentose phosphate pathway—see section 7), however the biosynthesis of purines and pyrimidines differs substantially. The biochemical origin of purine and pyrimidine carbon and nitrogen atoms is shown in Figure 9.[30]

The biosynthesis of pyrimidine ribonucleotides is described in Scheme 16. They are constructed from the coupling of PRPP and orotate, the latter of which is built from aspartate and carbamoyl phosphate, to give orotidine monophosphate (OMP), followed by decarboxylation to give uridine monophosphate (UMP). Further phosphorylation, capture with ammonia (from glutamine) and partial dephosphorylation gives cytidine monophosphate (CMP).

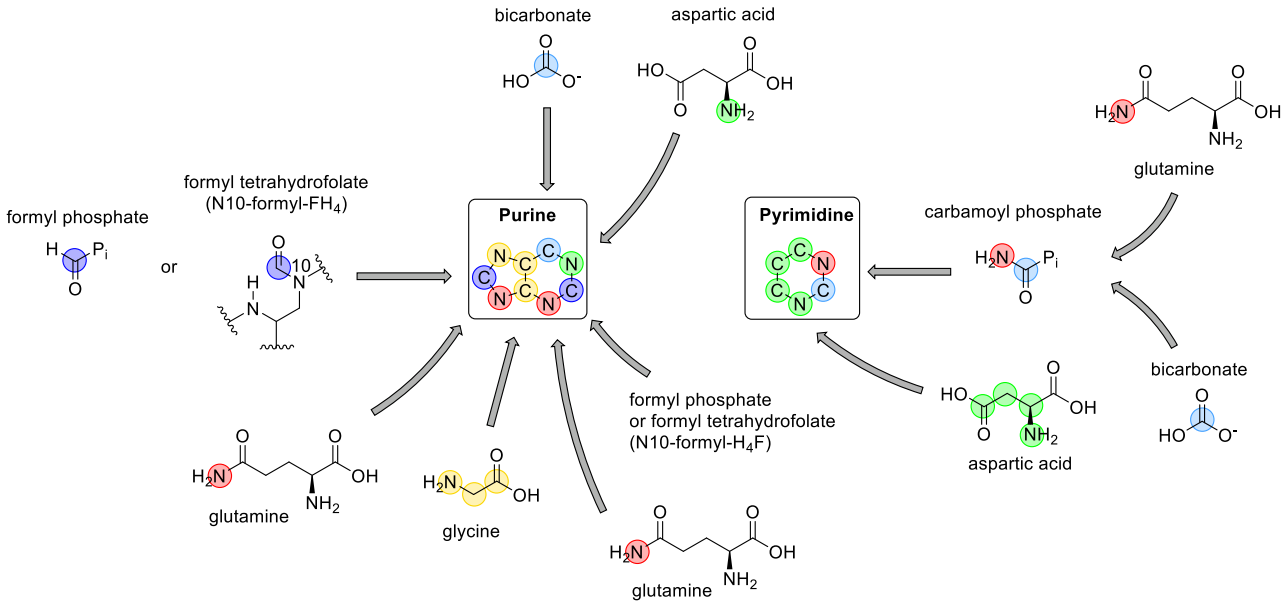
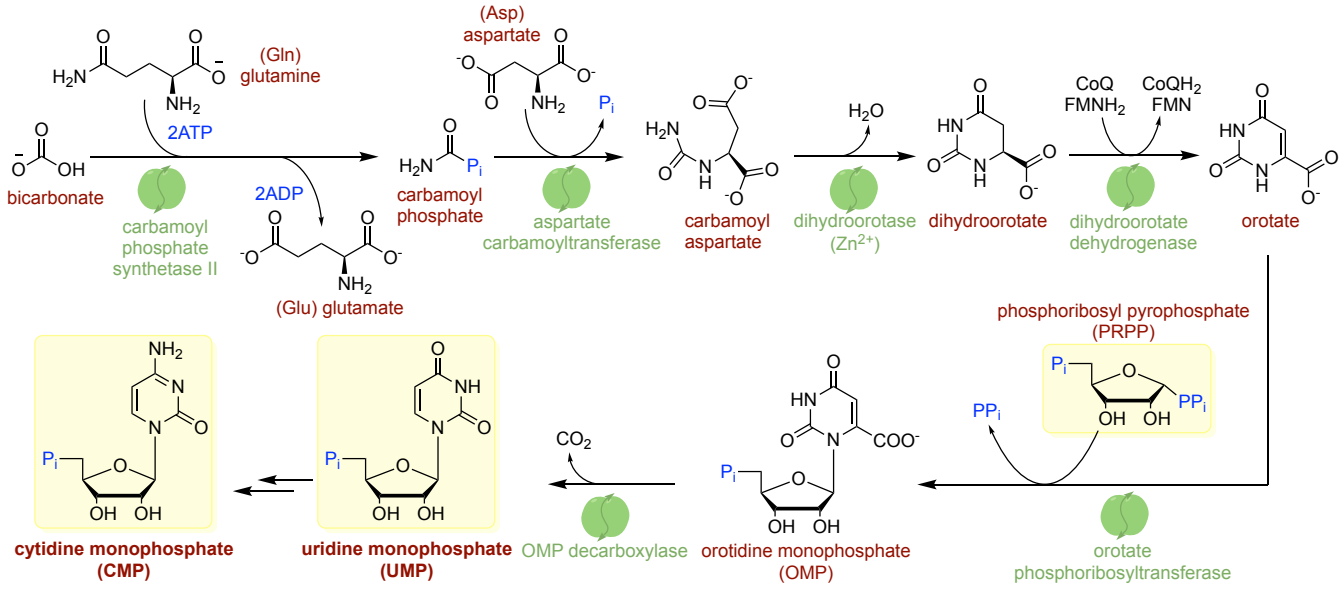


Figure 9. Biosynthetic origin of purine and pyrimidine nucleobase scaffolds.[30]

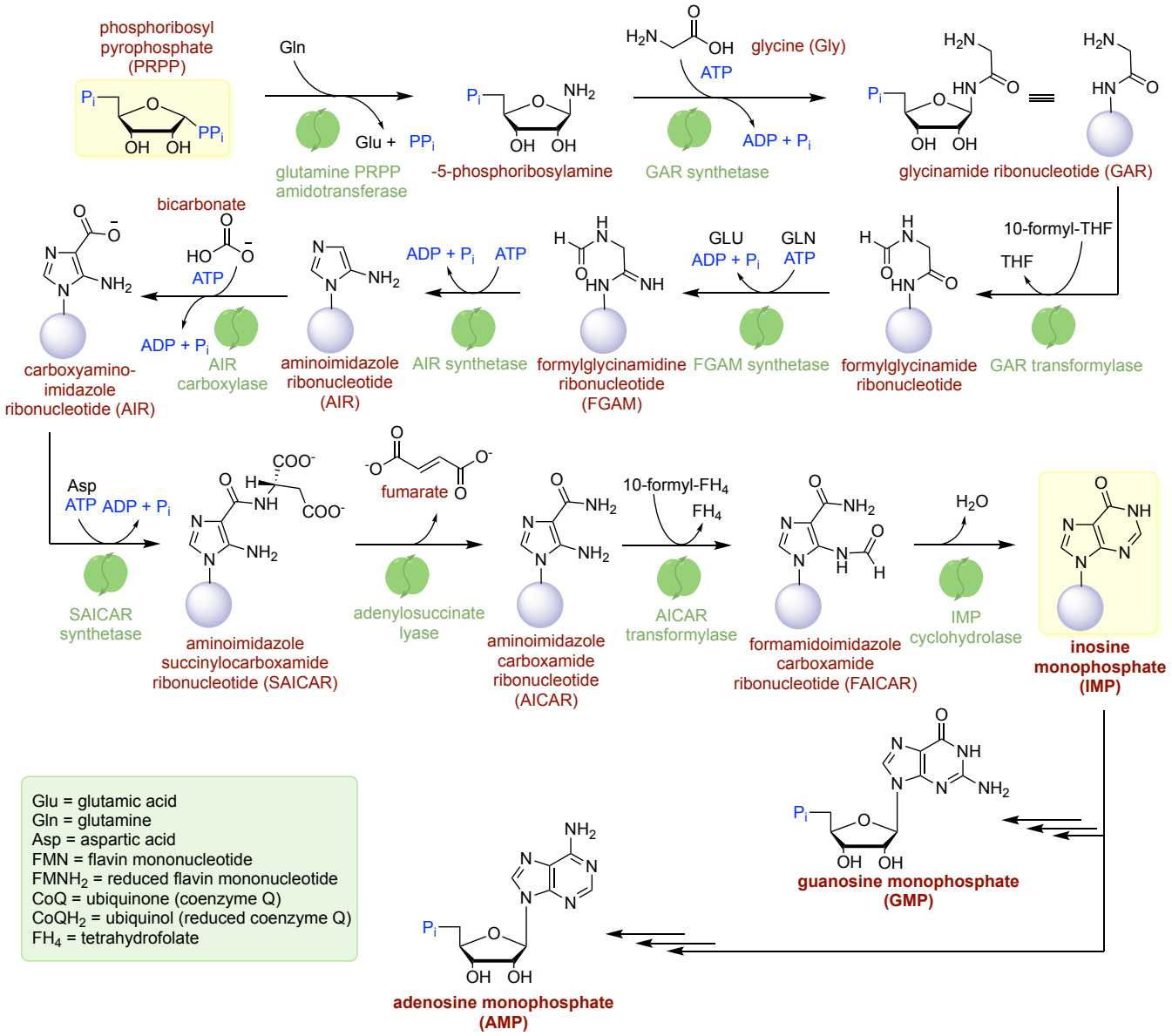
The biosynthesis of purine ribonucleotides is described in Scheme 17. They are constructed from the coupling of PRPP and glycine, followed by a series of formylation, amination, cyclization, and carboxylation reactions to give inosine monophosphate (IMP). GTP-driven amination of IMP leads to adenosine monophosphate (AMP). Alternatively, if IMP is oxidized prior to polyphosphate-driven amination, guanosine monophosphate (GMP) is obtained. Herein lies yet another of life's intriguing "chicken and egg" problems. ATP is needed to synthesize GMP from IMP, while GTP is consumed in the synthesis of AMP from IMP.[136] This poses some interesting questions about what could have been the primordial source of polyphosphates preceding ATP and GTP biosynthesis.

Several general points can be made regarding ribonucleotide biosynthesis. First, all three amino acid building blocks of nucleobases can be obtained from TCA/rTCA cycle intermediates (aspartate from oxaloacetate, and glutamine from α-ketoglutarate via glutamate), or from closely related bypasses (glycine from, for example, glyoxylate). This would suggest that ribonucleotide biosynthesis emerged alongside a metabolism that could supply all of these building blocks.[265] Second, PRPP becomes incorporated into the nucleotide structure at different points in the biosynthesis of purine ribonucleotides compared to pyrimidine ribonucleotides. Purine nucleobases are gradually built on the sugar scaffold, while pyrimidine nucleobases are pre-fabricated as orotidine, and then appended onto PRPP prior to decarboxylation. Third, in addition to the *de novo* syntheses of ribonucleotides, a variety of salvage pathways exist to recycle free nucleobases. Purine ribonucleotides are regenerated by coupling adenine or guanine to PRPP. Pyrimidine ribonucleotides are regenerated by coupling uracil to ribose-1-phosphate, which is then phosphorylated at the 5-position and then undergoes further transformations identical to those of *de novo* biosynthesis.[17]

Scheme 16. Biosynthesis of pyrimidine ribonucleotides.



Scheme 17 Biosynthesis of purine ribonucleotides



8.2. Prebiotic synthesis of nucleobases and nucleotides that parallel biosynthesis

Surprisingly, a recent 40-page review on prebiotic nucleotide synthesis by Krishnamurthy and co-workers reveals that barely any experimental work has been done on approaches that directly parallel *de novo* ribonucleotide biosynthesis.[12] A 2012 study by Springsteen and co-workers shows a biosynthesis-inspired route to purines starting from glycine, however, this synthesis—unlike biology—relies on nitrile chemistry in its subsequent steps.[266] Slightly more work has been done on non-enzymatic analogs of ribonucleotide salvage pathways.[267] Why hasn't more effort been expended in this direction? We suspect it is not because it is chemically impossible, but because very few researchers have attempted it. This is not for lack of ideas. Wächtershäuser has long proposed that mineral catalysis assisted in the prebiotic synthesis of nucleotides along biosynthetic lines.[118] Building on this, Fontecilla-Camps proposed that the roles played by organic cofactors in modern nucleotide biosynthesis were once fulfilled by mineral catalysts that were later replaced (Scheme 18).[268] The pathways suggested by Fontecilla-Camps use similar building blocks to the extant biochemical syntheses of purines and pyrimidines: glycine, aspartic acid, bicarbonate or formate, mostly supplied via the universal metabolic precursors. Lewis-acidic mineral surfaces are proposed to play a role analogous to phosphorylation by ATP in biochemistry—adsorption and chelation of small-molecule substrates and intermediates by the mineral should activate (increase the electrophilicity) of their carbonyl moieties, facilitating the nucleophilic attack of nitrogen-containing groups. Binding to the mineral surface may be compared, in this case, to substrate-protein binding in extant enzymatic reactions. Certainly, these ideas need to be put to the test.

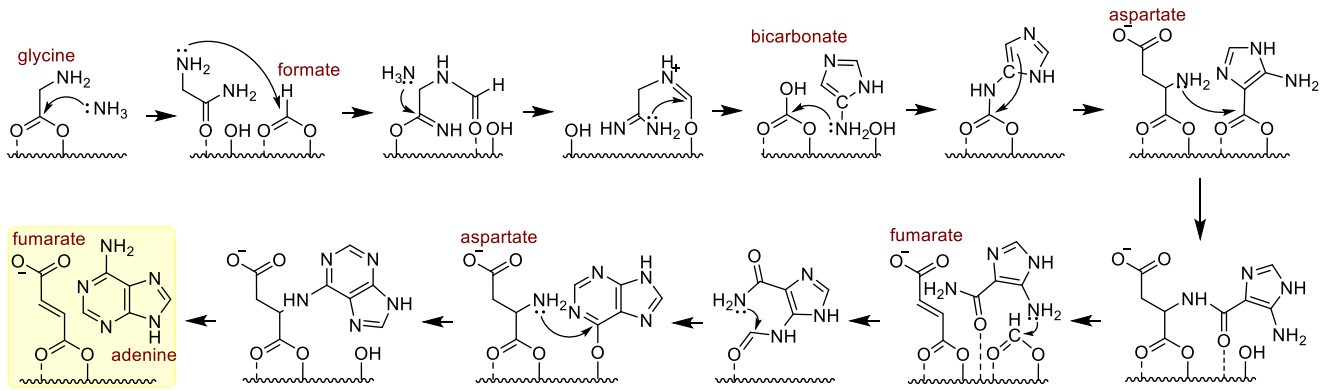
Isolated earlier experimental reports have appeared along these lines. In 1961, Fox and Harada disclosed that heating malic acid and urea in polyphosphoric acid at 130 °C produced uracil in one pot in 14% yield (Scheme 19a).[269] Nearly 30 years later, Yamagata and co-workers showed orotate could be synthesized from aspartate and urea under UV light irradiation, albeit in low yields (0.44%).[270] Although these are not exactly the

reactions of *de novo* pyrimidine biosynthesis, the uracil presumably forms through the same *N*-carbamoylaspartate intermediate used in the biosynthesis of orotate, illustrating that nucleobases can be generated abiotically from simple metabolites. Analogously, Schwartz and Chittenden showed that β-alanine and urea solutions evaporated and heated in the presence of different clays gave dihydroxyuracil, which could then be photodehydrogenated to uracil.[271,272] A more complex example comes from Dose and co-workers, who showed that ribonucleotide-like structures were generated simply by dry-heating mixtures of three amino acids at 180 °C (scheme 19b).[273] However, a wider variety of analytical techniques may be required to corroborate the proposed structures.

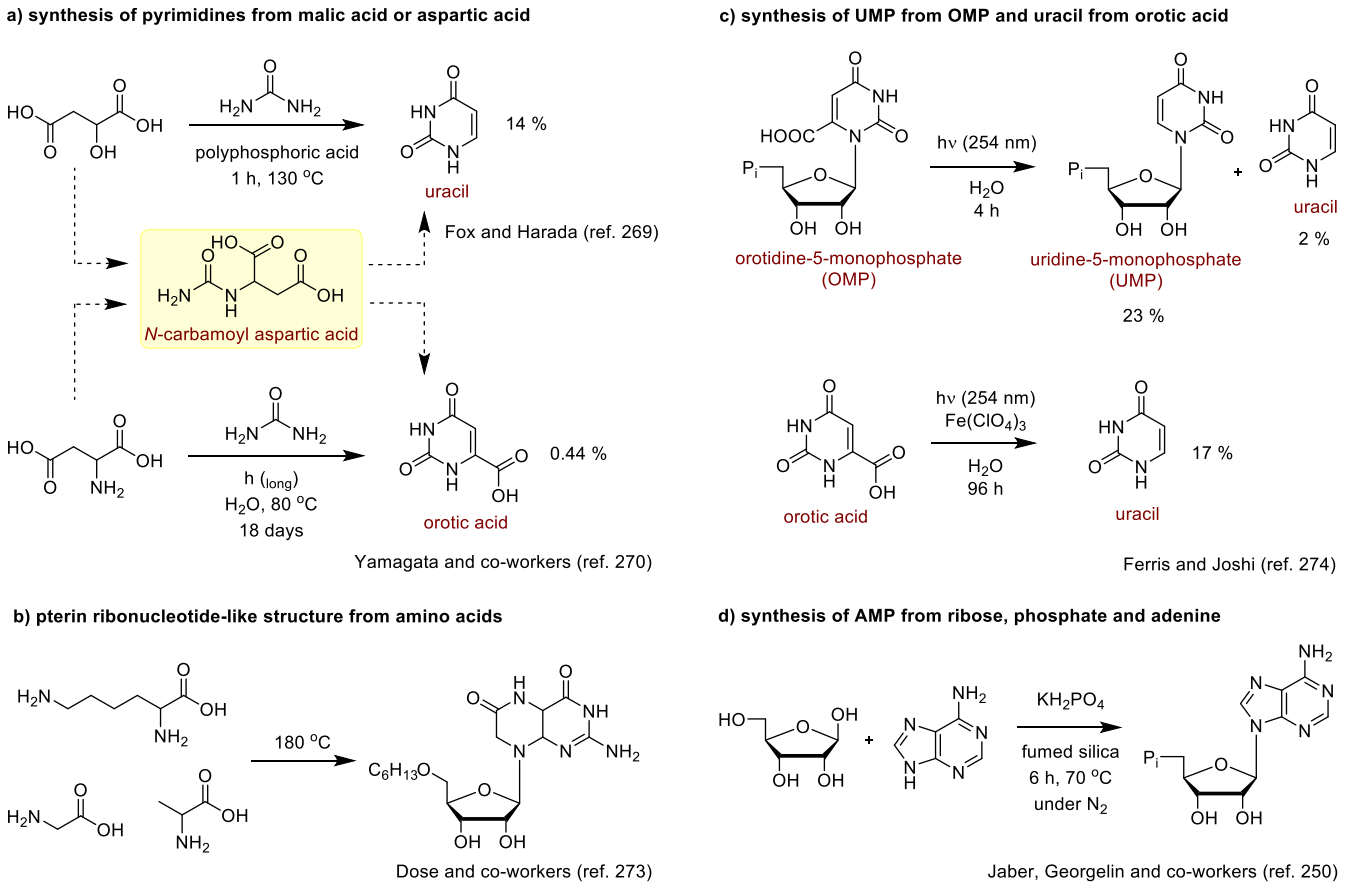
One of the steps in pyridimidine ribonucleotide biosynthesis where enzymes provide the biggest rate acceleration compared to the uncatalyzed reaction (10^{17}-fold) is during the decarboxylation of orotidine monophosphate (OMP) to uridine monophosphate (UMP).[274] With such a large rate acceleration, it might be tempting to assume that this reaction would be impossible to achieve without enzymes. Indeed, the low uncatalyzed rate of this reaction has recently been used as an argument against the feasibility of a prebiotic ribonucleotide synthesis that parallels biosynthesis.[275] However, Ferris and Joshi had already shown in 1979 that the conversion of OMP to UMP occurs at room temperature in water under UV irradiation (Scheme 19c).[276] Similarly, orotate undergoes decarboxylation to uracil when irradiated in the presence of Fe^{3+} salts, which appear to play the role of a photoredox catalyst. Thus, other strong oxidants should equally be able to promote this transformation, with or without light.

In contrast to *de novo* ribonucleotide biosynthesis, much more work has recently been done towards mimicking their synthesis by salvage pathways. Jaber, Georgelin and co-workers recently reported a mineral-mediated assembly of AMP from ribose, phosphate and adenine by drying these compounds at 70 °C on a fumed silica surface (Scheme 19d).[250] On the basis of ^{31}P NMR, the authors suspect that both ribose-5-phosphate (R5P) and PRPP are formed as intermediates in the reaction. Similarly, Cronin and co-workers reported the dehydrative condensation of R5P with adenine, guanine and cytidine to give a variety of derivatives of adenosine, guanosine and cytosine when heating in water at 90 °C under acidic conditions (pH = 2.5).[277] The distribution of nucleotide products was found to be changed by the presence of glycine in the reaction mixture. Similar transformations were found to be induced by aqueous microdroplets, as reported by Zare and co-workers.[278,279] Other reports involving the condensation of sugars and nucleobases, but that less closely mimic the salvage pathways of ribonucleotide biosynthesis, have been recently reviewed.[12]

Scheme 18. Hypothetical mineral-surface-mediated synthesis of adenine proposed by Fontecilla-Camps.[268]



Scheme 19. Biological ribonucleotide synthesis under prebiotic conditions.

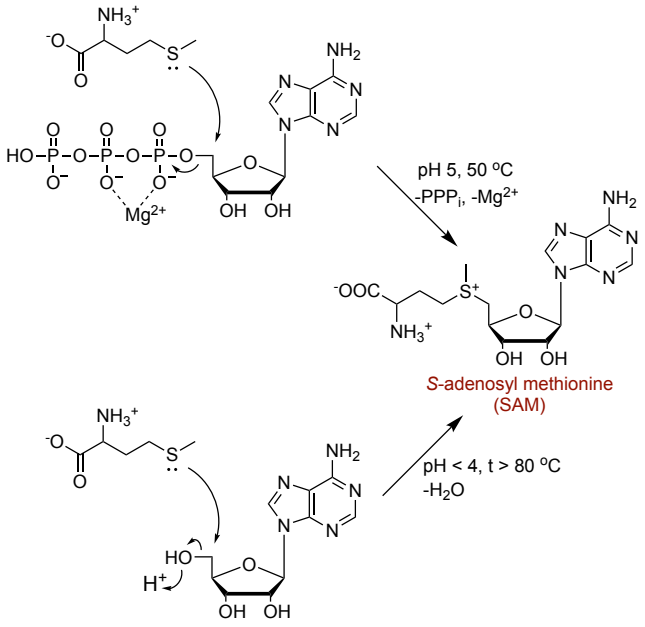


Many important biological cofactors are derived from ribonucleotides, and their non-enzymatic synthesis following biosynthetic pathways is also of great interest. Laurino and Tafwik recently demonstrated that the synthesis of *S*-adenosylmethionine (SAM), the cofactor responsible for biological methylation, occurs spontaneously in the absence of enzymes starting from ATP and methionine upon heating to 50 °C in pH 5 Mg^{2+}-rich water, or from adenosine and methionine upon heating to ≥ 80 °C in pH ≤ 4 (Scheme 20).[280] Cofactors produced by non-enzymatic analogs of biosynthetic pathways could have played the role of small molecule organocatalysts, before there were complex enzymes, and aided the oligomerization of ribonucleotides, a concept that has recently begun to be explored by Richert and co-workers.[281,282]

8.3. Summary and future directions

Especially when compared to the large body of work on the prebiotic synthesis of ribonucleotides through alternative chemistries, relatively little work has been done towards mimicking their *de novo* biosynthesis. Though several papers that mimic ribonucleotide salvage pathways have appeared, there are still none that mimic the entirety of *de novo* ribonucleotide biosynthesis starting from PRPP or R5P. This would be particularly compelling if it could be carried out in a continuous manner that proceeds without human intervention to guide the reaction outcome. In light of the reactions summarized in Scheme 19, criticisms that some of the reactions of the biological pathways are impossible without enzymes appear unwarranted. A non-enzymatic version of pyrimidine ribonucleotide biosynthesis seems like a tangible goal, although it admittedly appears more difficult for the purines.

Scheme 20. Synthesis of *S*-adenosyl methionine under prebiotic conditions (Tawfik and co-workers).[280]



Integrating undirected abiotic ribonucleotide synthesis with non-enzymatic pathways that generate its building blocks would be a landmark achievement in the field, as the "metabolism first" and "RNA world" scenarios would cease to be mutually exclusive.[283] A non-enzymatic reaction network, similar to biological metabolism, that spontaneously produces ribonucleotides would have much more explanatory value than current discontinuous approaches.

Finally, it may be worthwhile to consider the biosynthesis of other nucleic acids as being potentially prebiotic. A recent one-pot report from Trapp and co-workers furnished deoxyribonucleosides following the aldol reaction between acetaldehyde and glyceraldehyde.[284] This nearly recapitulates one of the minor biosynthetic pathways for 2-deoxyribose-5-phosphate, via the aldol reaction of acetaldehyde and glyceraldehyde-3-phosphate, suggesting that the point of entry of DNA into biochemical space could have dated back to prebiotic chemistry,[285,286] in line with the ideas of some biologists.[287]

9. CONCLUSIONS AND OUTLOOK

Regardless of how it emerged, biology is constrained by the laws of chemistry and physics and didn't become the way it is solely by accident, but by a combination of necessity and chance events controlled by physicochemical constraints.[288] Researchers in prebiotic chemistry are starting to try to understand and mimic life's chemical processes rather than just making its molecules. Consequently, more experimental reports are appearing that deal not only with individual syntheses, or individual classes of biomolecules, but with entire complex reaction networks, some of which bear real similarities to life's metabolic pathways. Reaction networks, in turn, must be considered as a set of transformations whose behavior is necessarily kinetically coupled, both at the microscopic and macroscopic scales, creating a dynamic complex system, not simply an interconnected synthetic scheme when drawn on paper.[21,162] From here, a link can be made to the global characteristics of life, which itself is an out-of-equilibrium, dissipative system,[289] and whose emergence depended on its disequilibrated environment.[14] For this reason, good theoretical models are essential to help understand the kind of complex systems that would have led to the emergence of life.[290] Another question for origins of life research which remains unaddressed is why life has settled for μM-mM concentrations of its metabolites, while these concentrations could have, in principle, been significantly higher or lower. Organisms have grown in size and scale, but this has not affected metabolite concentrations. Could scaling principles from chemical process engineering give insight?

The study of non-enzymatic versions of biological reactions was sparse until recently likely because fundamental aspects of metabolism were themselves only recently uncovered. It has been further inhibited by the new analytical challenges faced when exploring true networks of reactions. The number of analytes that require characterization and quantification increases dramatically with the complexity of the system.[291] Moreover, intermediates that are consumed faster than they are produced become very difficult to detect because of their very low steady-state concentrations. The requirements for limits of detection also become more exigent.[292] With these tools now relatively widespread, we expect further major advances in the coming years. Beyond the demonstration of non-enzymatic versions of more metabolic pathways, subsequent advances will come from exploring how biocatalysts emerged from and influenced these networks. We hope this review outlined those that are the most pressing to explore.

However, sometimes the most difficult thing to change is how we think. Here we restate three aspects touching on the origin of life that warrant reconsideration. First, we may need to reconsider old conceptual divisions within prebiotic chemistry. The need for metabolic pathways to work together as a whole means that constraints on the non-enzymatic chemistry of one reaction or pathway become relevant to the others and, ideally, all must be considered simultaneously (Figure 10).

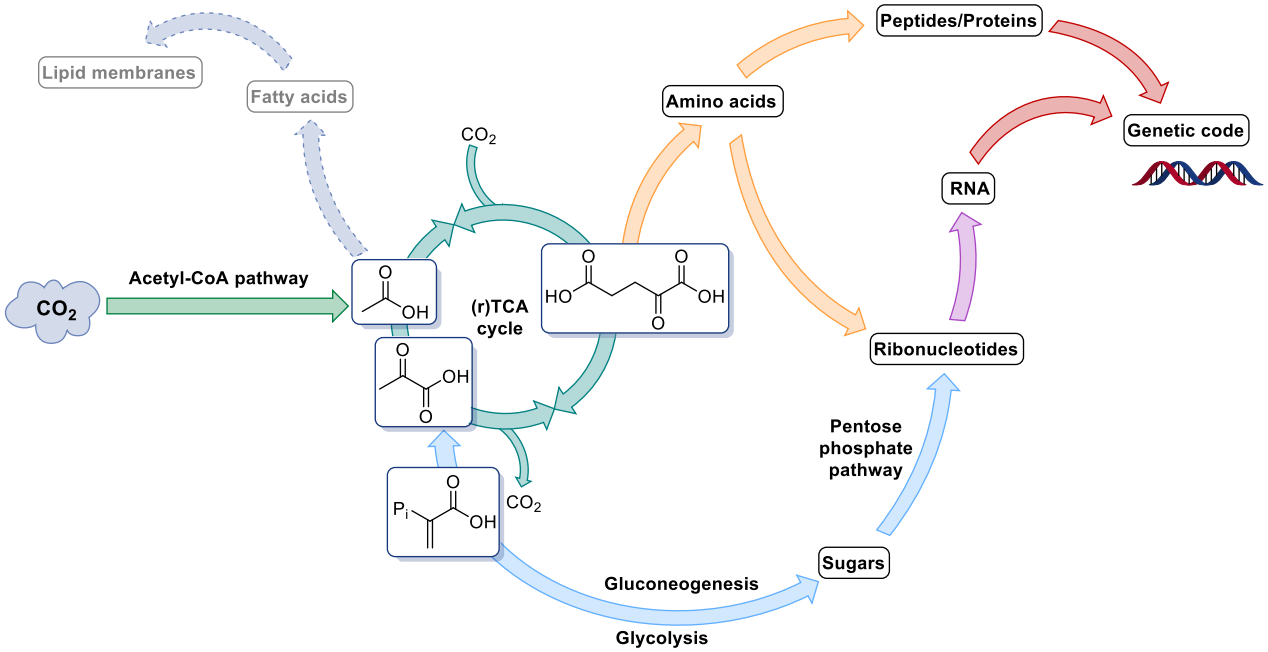


Figure 10. Relationships between different biochemical subsystems.

This inevitably forces us to adopt systems approaches to prebiotic chemistry.[8,13] The above also means the once-prominent divide between the major trends in the field is gradually closing, and the classical "genetics first" and "metabolism first" theories should cease to be seen exclusively in terms of an "either/or" relationship.[293,294] This is important also in the context of the onset of Darwinian evolution within a primitive metabolic system. Once genetics was in place, biochemical pathways could have evolved and expanded rapidly.[64] The existence of a prebiotic non-enzymatic pathway, which already provides intermediates and end-products, is an excellent starting point for enzyme evolution.[74]

Second, we may need to reconsider perceived gaps between prebiotic chemistry and biochemistry. In 2018, Krishnamurthy warned against taking cues from biological pathways and translating them in a prebiotic context,[295] using Orgel's 2004 conclusion[296] that "the direct generation of nucleosides from a fully formed nucleobase (e.g. adenine and uracil) and ribose – has been inefficacious" and pointing out that this problem has been circumvented by alternative chemistries such as those described in section 8. Questions about whether these problems have actually been circumvented aside, he argued that this hints "at the possibility that extant biomolecules might have been created through prebiotic pathways that are very different from what is observed in extant biochemistry". We agree with the spirit of this statement – it is indeed possible that some aspects of biochemistry do not perfectly mirror those in prebiotic chemistry. However, the points raised in the current review suggest a number of caveats. Abandoning links between prebiotic chemistry and biochemistry can easily lead to a total lack of constraint on origins of life research—or at least to an abundance of studies that will always remain in the proof-of-principle domain. One has to be careful not to neglect the possibility that a biology-inspired solution appears ineffective simply because appropriate conditions for the missing chemical link have not yet been identified. Indeed, between 2017 and 2018, three different groups reported the direct coupling of canonical nucleotides and ribose,[250,277-279] showing that a biology-like approach to this problem was possible after all. Perceived gaps between prebiotic chemistry and biochemistry may not be gaps at all, but simply a result of the difficulty of finding the right solution within the vastness of chemical space. That said, despite our emphasis on metabolism in this review, we are not advocating that all relevant prebiotic chemistry, without exception, must have mimicked today's biochemical pathways. Other chemistries may also have made important contributions.

Finally, we may have to question our own conception of life. In this review, we have dealt with prebiotic chemistry relevant to the AcCoA pathway of CO_2 fixation, the rTCA and TCA cycles, thioester bioenergetics, amino acid metabolism, sugar metabolism and ribonucleotide biosynthesis (Figure 10). We have neglected to consider fatty acids mostly because, to the best of our knowledge, there are not yet reports of non-enzymatic versions of the way biochemistry constructs fatty acids. We do not know yet if this is simply because they have not been investigated, because the right conditions have not yet been identified, or because it might be a case where prebiotic chemistry does not resemble metabolism. It remains completely possible that fatty acid biosynthesis also operated non-enzymatically, and it is well known that fatty acids can lead to the formation of vesicles.[297] On the other hand, the metabolic perspective may cause us to question the preconception that cells are the fundamental unit of life. As individuals ourselves, we naturally perceive individuality, embodied by cellularity, as a fundamental element that would have been necessary from the earliest stages of prebiotic chemistry. We characterize the search for the origin of life as being eponymous with the search for the first cell. This might be anthropomorphism. Looking beyond the individual, an ecosystem- or biosphere-centric view of life should be considered. From this perspective, cellularity is only a means of partitioning an ecosystem- or planetary-scale metabolism to make optimal use of local environments, which might vary greatly across space.[76] Could the chemistry of the ecosystem be more essential than the cellularization that partitions it? Navigating these fundamental questions about the nature of life will undoubtedly make for exciting years of work ahead.

AUTHOR INFORMATION

Corresponding Author

* muchowska@unistra.fr, moran@unistra.fr

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

2-PGA, 2-phosphoglycerate; A, adenine; AcCoA, acetyl-coenzyme A; ACS, acetyl-CoA synthase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; ASP, aspartate/aspartic acid; C, cytidine; CMP, cytosine monophosphate; CODH, carbon monoxide dehydrogenase; CoFeSP, corrinoid iron-sulfur protein; CoQ, coenzyme Q (ubiquinone); $CoQH_2$, reduced coenzyme Q (ubiquinol); DHAP, 1,3-dihydroxyacetone phosphate; DNA, deoxyribonucleic acid; F1,6BP, fructose 1,6-bisphosphate; F1P, fructose 1-phosphate; FAD, flavin adenine dinucleotide; FADH2, flavin adenine dinucleotide (reduced hydroquinone form); FH_4, tetrahydrofolate; FMN, flavin mononucleotide; $FMNH_2$, flavin mononucleotide (reduced form); G, guanine; G6P, glucose 6-phosphate; GAP, glyceraldehyde-3-phosphate, GDP, guanosine diphosphate; GLN, glutamine; GLU, glutamate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; IMP, inosine monophosphate; LUCA, last universal common ancestor; NAD^+, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); OMP, orotidine monophosphate; P_i, inorganic phosphate; PEP, phosphoenol pyruvate; PLP, pyridoxal phosphate; PRPP, phosphoribosyl pyrophosphate; R5P, ribose 5-phosphate; RNA, ribonucleic acid; rTCA, reductive tricarboxylic acid cycle; SAM, *S*-adenosylmethionine; TCA cycle, tricarboxylic acid cycle; THF, tetrahydrofurane; TPP, thiamine diphosphate cofactor; U, uracil; UMP, uridine monophosphate.

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