

Autotrophic carbon fixation in archaea

Ivan A. Berg*, Daniel Kockelkorn*, W. Hugo Ramos-Vera*, Rafael F. Say*, Jan Zarzycki*, Michael Hügler**, Birgit E. Alber*§ and Georg Fuchs*

Abstract | The acquisition of cellular carbon from inorganic carbon is a prerequisite for life and marked the transition from the inorganic to the organic world. Recent theories of the origins of life assume that chemoevolution took place in a hot volcanic flow setting through a transition metal-catalysed, autocatalytic carbon fixation cycle. Many archaea live in volcanic habitats under such constraints, in high temperatures with only inorganic substances and often under anoxic conditions. In this Review, we describe the diverse carbon fixation mechanisms that are found in archaea. These reactions differ fundamentally from those of the well-known Calvin cycle, and their distribution mirrors the phylogenetic positions of the archaeal lineages and the needs of the ecological niches that they occupy.

Thermophilic

An organism that grows best at temperatures exceeding the ambient temperature. Extreme thermophiles (hyperthermophiles) have optimal growth temperatures above 80 °C.

Chemolithoautotroph

An organism that derives energy from a chemical reaction (chemotrophic) based on inorganic substrates as electron donors (lithotrophic), and CO₂ serves as sole carbon source (autotrophic = self-nourishing).

*Mikrobiologie, Fakultät Biologie, Universität Freiburg, Schänzlestrasse 1, D-79104 Freiburg, Germany.

†Present address: Water Technology Center (TZW), Karlsruher Strasse 84, D-76139 Karlsruhe, Germany.

§Present address: The Ohio State University, Department of Microbiology, 484 West 12th Avenue, 417A Biological Science Building, Columbus, Ohio 43210-1292, USA. Correspondence to G.F. e-mail: georg.fuchs@biologie.uni-freiburg.de

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Archaea form the third domain of life, alongside the other two domains, the Bacteria and Eukarya. The distinct archaeal lineages that have been identified include the Crenarchaeota (from the Greek 'crenos' for spring or origin) and the Euryarchaeota (from the Greek 'euryos' for diversity)¹ (FIG. 1). Archaea resemble Bacteria in their structural organization and metabolism, whereas their genetic information system (the process of transcription) shares many traits with Eukarya.

The early branching lineages of the archaea and bacteria harbour many thermophilic chemolithoautotrophs, a fact that was taken as an indication for a chemolithotrophic origin of life at high temperatures². Most cultivated autotrophic archaea are either anaerobes or can tolerate and use oxygen only at low concentrations. The inorganic substrates that these organisms can oxidize include H₂, H₂S, S, CO, NH₃, metal sulphides such as pyrite (FeS₂), and reduced metal ions. The electron acceptors that can be used include various oxidized inorganic compounds such as S, S₂O₃²⁻, SO₄²⁻, AsO₄³⁻, NO₃⁻, oxidized metal ions and even CO₂ (for anaerobic respiration). The use of O₂ (for aerobic respiration) is rare and is considered a late adaptation that reflects the increasing oxygen content of the atmosphere after the emergence of oxygenic photosynthesis. The generation of ATP follows a chemiosmotic mechanism: electron flow from the reduced inorganic substrate to the oxidized inorganic electron acceptor is coupled to the transduction of H⁺ or Na⁺ across the cytoplasmic membrane, and the archaeal H⁺ ATP synthase uses the resultant proton-motive force for ATP synthesis. Reducing power for biosynthesis is also provided by the oxidation of reduced inorganic substrates, although

the reduction of NAD or NADP and ferredoxin might require an energy-driven reverse electron flow³⁻⁵.

In general terms, the assimilation of CO₂ into cellular building blocks requires four reducing equivalents and an input of energy. A small organic molecule usually serves as a CO₂ acceptor, which is linked to CO₂ by a carboxylase; however, a large coenzyme or a prosthetic group on an enzyme can also function as a CO₂ acceptor, as is discussed below. All CO₂ acceptors must eventually be regenerated in a cycle in which CO₂ (oxidation state + 4) is reduced to cellular carbon (average oxidation state 0). The energetically unfavourable steps of this pathway can be driven by ATP hydrolysis, and the reduction steps are driven by low-potential reduced coenzymes, usually NADPH; occasionally, however, reduced ferredoxin or the reduced deazaflavin factor 420 is used. The product of such a metabolic cycle is a central cellular metabolite, from which polymer building blocks can be derived. None of the chemolithoautotrophic archaea seems to use the Calvin cycle for CO₂ fixation (BOX 1), even though in some species one of the key enzymes, ribulose1,5-bisphosphate carboxylase-oxygenase (RubisCO), is present. Instead, these organisms use diverse CO₂ fixation mechanisms to generate acetyl-coenzyme A (acetyl-CoA), from which the biosynthesis of building blocks can start.

This Review discusses the autotrophic carbon fixation pathways in archaea, two of which were discovered only recently. Archaeal carbon fixation strategies have in common the synthesis of acetyl-CoA from CO₂. As many archaea lack a functional fructose 1,6-bisphosphate (FBP) aldolase, which catalyses the last, controlled step in gluconeogenesis, this raises the question of how

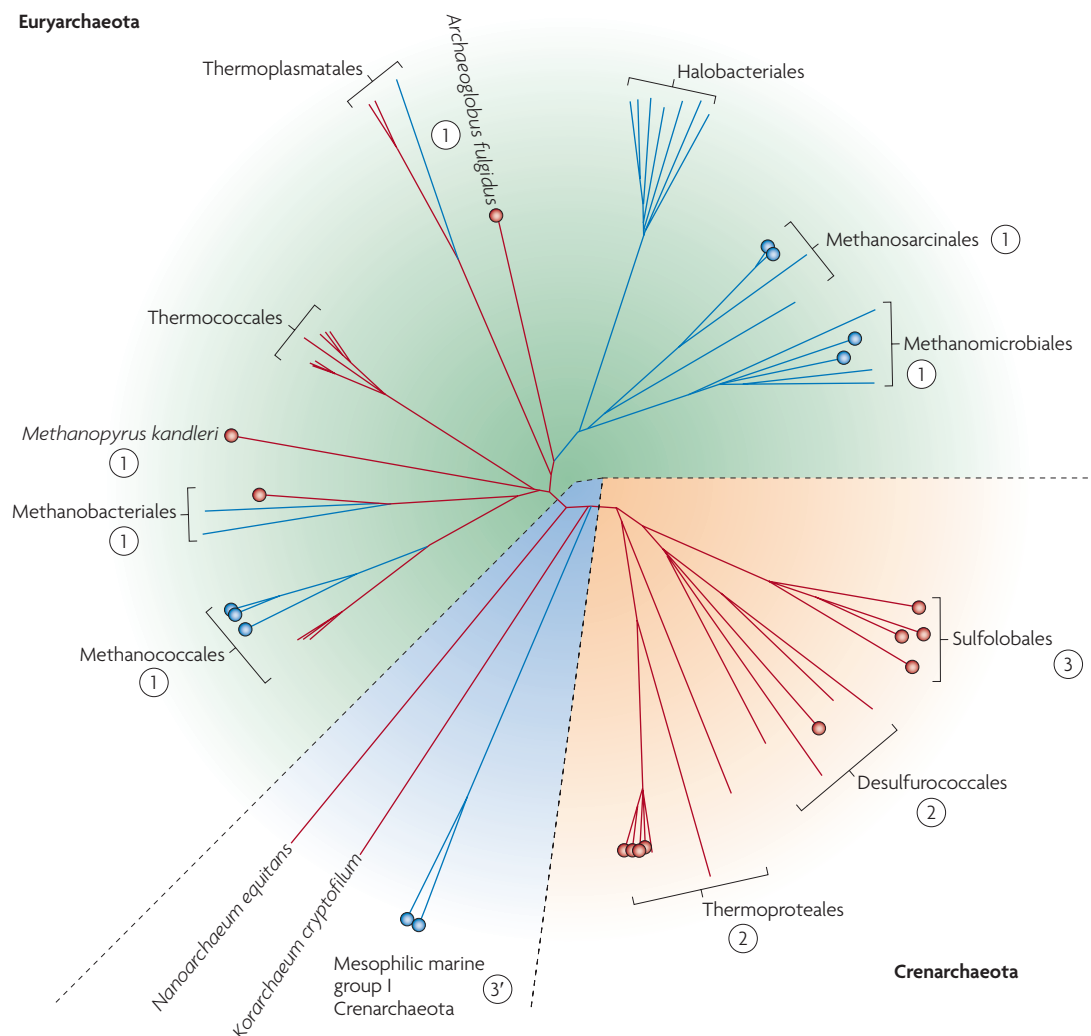


Figure 1 | The phylogenetic (unrooted) tree of Archaea. This phylogenetic tree is based on analyses of a concatamer of nine subunits of RNA polymerase, three transcription factors and 53 ribosomal proteins from all currently finished archaeal genomes (59 species). The numbers 1, 2, 3 and 3' refer to the presence of the reductive acetyl-coenzyme A (acetyl-CoA) pathway (1), the dicarboxylate–hydroxybutyrate cycle (2), the hydroxypropionate–hydroxybutyrate cycle (3) and possibly a modified hydroxypropionate–hydroxybutyrate cycle (3'). The lineages in red represent hyperthermophilic or thermophilic archaea with an optimal growth temperature that is equal to or higher than 65°C, and the lineages in blue represent archaea with an optimal growth temperature that is lower than 65°C. Autotrophic members are marked by a circle at the end of the lineage. Note that the phylogenetic position of the Nanoarchaeota, Korarchaeota and 'marine group I' Crenarchaeota is currently being debated^{109–112}.

gluconeogenesis functions in these organisms. Recently, a new type of bifunctional FBP aldolase–phosphatase was discovered that might be the ancestral gluconeogenic enzyme. We then discuss some of the reasons for the occurrence of the observed metabolic diversity in archaea and the rationale behind the distribution of the existing mechanisms. Finally, we raise some open questions that must be addressed in future studies and touch on the question of whether the extant autotrophic pathways can serve as models for an ancestral metabolism.

The reductive acetyl-CoA pathway

The reductive acetyl-CoA pathway is an interesting pathway in the Euryarchaeota that may indeed be a model for primordial CO₂ fixation. Methanogenic archaea

probably constitute a monophyletic but diverse group within the Euryarchaeota (FIG. 1). They are strict anaerobes that derive energy mainly from two processes: the reduction of CO₂ using four molecules of H₂ to generate CH₄ or the disproportionation of acetate into CH₄ plus CO₂. The carbon assimilation pathway in these species — the reductive acetyl-CoA pathway — results in the fixation of two molecules of CO₂ to form acetyl-CoA, with a coenzyme and an enzyme metal centre as the CO₂ acceptors. It was elucidated by the laboratories of Wood, Ljungdahl, Thauer and others^{6–10} as a pathway that is used by acetogenic bacteria to synthesize acetate from CO₂ to generate ATP. This pathway also operates in the sulphate-reducing euryarchaeal genera *Archaeoglobus*¹¹ and *Ferroplasma*¹².

Box 1 | Autotrophic carbon fixation mechanisms

Six mechanisms that assimilate CO₂ into cellular material have been identified (TABLE 1). Note that the different CO₂ fixation mechanisms lead to different carbon isotope fractionation values in biomass (TABLE 1).

Calvin cycle

In the Calvin–Benson–Bassham cycle, which was discovered about 50 years ago, CO₂ reacts with the five-carbon sugar ribulose 1,5-bisphosphate to yield two carboxylic acids, 3-phosphoglycerate, from which the sugar is regenerated¹⁰³. This cycle operates in plants, algae, cyanobacteria, some aerobic or facultative anaerobic Proteobacteria, CO-oxidizing mycobacteria and representatives of the genera *Sulfobacillus* (iron- and sulphur-oxidizing Firmicutes) and *Oscillochloris* (green sulphur bacteria). An autotrophic symbiotic cyanobacterium conferred the CO₂ fixation machinery on a eukaryotic cell giving rise to the chloroplasts of plant cells. The presence of the key enzyme, ribulose 1,5-bisphosphate carboxylase–oxygenase (RubisCO), is often considered to be synonymous with autotrophy. Phylogenetic analysis and general considerations denote the Calvin cycle as a late innovation^{72,83,84}.

Reductive citric acid cycle

In 1966, Arnon, Buchanan and co-workers proposed another autotrophic cycle for the green sulphur bacterium *Chlorobium limicola*, the reductive citric acid cycle (also known as the Arnon–Buchanan cycle)¹⁰⁴. This cycle is less energy-consuming than the Calvin cycle, involves enzymes that are sensitive to oxygen and is therefore found only in anaerobes or in aerobes growing at low oxygen tensions. These include some Proteobacteria, green sulphur bacteria and microaerophilic bacteria of the early bacterial phylum Aquificae. Initially, the reductive citric acid cycle was also proposed to operate in certain archaea (notably *Thermoproteus neutrophilus*)²⁰, but recent findings refute this proposal¹⁴.

Reductive acetyl-coenzyme A pathway

At the start of the 1980s, a third autotrophic pathway was found in certain Gram-positive bacteria and methane-forming archaea, the reductive acetyl-coenzyme A (acetyl-CoA) or Wood–Ljungdahl pathway^{6–10}. In these strict anaerobic organisms that now also include some Proteobacteria, Planctomycetes, spirochaetes and Euryarchaeota, one CO₂ molecule is reduced to CO and one to a methyl group (bound to a carrier); subsequently, acetyl-CoA is synthesized from CO and the methyl group (FIG. 2). Although this pathway is the most energetically favourable autotrophic carbon fixation pathway (TABLE 1), it is restricted to strictly anaerobic organisms.

3-Hydroxypropionate bicycle

The 3-hydroxypropionate bicycle occurs in some green non-sulphur bacteria of the family Chloroflexaceae^{38–40,43}. This seems to be a singular invention, and the pathway has not been found elsewhere. The conversion of acetyl-CoA plus two bicarbonates to succinyl-CoA uses the same intermediates as in the hydroxypropionate–hydroxybutyrate cycle, but most of the enzymes are completely different. Furthermore, the regeneration of acetyl-CoA proceeds by the cleavage of malyl-CoA, yielding acetyl-CoA and glyoxylate. The assimilation of glyoxylate requires a second cycle (hence the name bicycle).

Hydroxypropionate–hydroxybutyrate cycle

The hydroxypropionate–hydroxybutyrate cycle occurs in aerobic Crenarchaeota (Sulfolobales and possibly marine Crenarchaeota group I)²⁵ (FIG. 3b). Although some of the intermediates and the carboxylation reactions are the same as in the 3-hydroxypropionate bicycle in Chloroflexaceae, the archaeal cycle probably has evolved independently.

Dicarboxylate–hydroxybutyrate cycle

The dicarboxylate–hydroxybutyrate cycle occurs in the anaerobic crenarchaeal orders Thermoproteales and Desulfurococcales^{13–15}. The hydroxypropionate–hydroxybutyrate and dicarboxylate–hydroxybutyrate cycles are described in the main text (FIG. 3).

One molecule of CO₂ is reduced to the level of a methyl group, which is bound to a tetrahydropterin coenzyme. Another CO₂ molecule is reduced to CO bound to nickel in the reaction centre of CO dehydrogenase (FIG. 2). CO dehydrogenase also acts as an acetyl-CoA synthase. It accepts the methyl group from the methylated tetrahydropterin through a methylated corrinoid protein, combines it with CO to form an enzyme-bound Ni-acetyl group, and releases this group with CoA to form acetyl-CoA. This key enzyme is therefore referred to as a CO dehydrogenase–acetyl-CoA synthase and probably has common roots in Bacteria and Archaea. This is in contrast to the enzymes involved in the formation of methyltetrahydropterin from CO₂, which differ considerably in Bacteria and Archaea.

The reductive acetyl-CoA pathway can be considered a biological equivalent of the industrial Monsanto process, in which acetate is produced from CO and methanol through metal catalysis. There are many

variants of the reductive acetyl-CoA pathway, which differ in the use of coenzymes or electron carriers. Among the autotrophic CO₂ fixation pathways, the reductive acetyl-CoA pathway has the lowest energetic costs, requiring probably less than one ATP to make pyruvate (TABLE 1). However, the demanding requirements for metals, cofactors, anaerobiosis and substrates with low reducing potential such as H₂ or CO restrict the reductive acetyl-CoA pathway to a limited set of anoxic niches.

The dicarboxylate–hydroxybutyrate cycle

The dicarboxylate–4-hydroxybutyrate cycle (shortened to the dicarboxylate–hydroxybutyrate cycle) functions in the anaerobic or microaerobic autotrophic members of the crenarchaeal orders Thermoproteales and Desulfurococcales^{13–15} (FIG. 1). Many grow as strict anaerobes by reducing elemental sulphur with H₂ to H₂S, but some grow under microaerobic or denitrifying conditions^{16,17}. The dicarboxylate–hydroxybutyrate cycle can

Monsanto process

An important method for the manufacture of acetic acid. The feedstock methanol is combined catalytically with CO to give acetic acid. The reaction is catalysed by a metal (rhodium) catalyst. Methanol reacts with catalytic amounts of HI to give methyl iodide. The reaction cycle is completed by the loss of CH₃COI to regenerate the metal catalyst. The CH₃COI reacts with water to generate acetic acid and regenerate HI.

Table 1 | Pathways for autotrophic carbon fixation

Pathway*	ATP equivalents for synthesis of one pyruvate	Reductants for synthesis of one pyruvate (10 [H])	CO ₂ -fixing enzymes	Active CO ₂ species	Intermediates that can be used for biosynthesis	Carbon isotope fractionation [‡]	Key enzymes
Reductive pentose phosphate cycle (Calvin–Benson–Bassham cycle)	Seven	Five NADH or NADPH	RubisCO	CO ₂	3-Phosphoglycerate, triose phosphates and sugar phosphates	–20 to –30 ‰ ^{113,114}	RubisCO and phosphoribulokinase
Reductive citric acid cycle (Arnon–Buchanan cycle)	Two [§]	Two NADH or NADPH, one unknown donor and two ferredoxin	2-Oxoglutarate synthase	CO ₂	Acetyl-CoA, pyruvate, PEP, oxaloacetate, succinyl-CoA and 2-oxoglutarate	–2 to –12 ‰ ^{115,116}	2-Oxoglutarate synthase and ATP-citrate lyase
			Isocitrate dehydrogenase	CO ₂ [§]			
			Pyruvate synthase	CO ₂			
			PEP carboxylase	HCO ₃ [–]			
Reductive acetyl-CoA pathway (Wood–Ljungdahl pathway)	Approx. one	Three ferredoxin and two F ₄₂₀ H ₂ (in methanogens)	Acetyl-CoA synthase–CO dehydrogenase	CO ₂	Acetyl-CoA and pyruvate	< –30 ‰ ^{83,115,117}	Acetyl-CoA synthase–CO dehydrogenase and enzymes reducing CO ₂ to methyltetrahydropterin
			Formylmethanofuran dehydrogenase (in methanogens)	CO ₂			
			Pyruvate synthase	HCO ₂			
3-Hydroxypropionate bicycle	Seven	Six NADH or NADPH, but one FAD is reduced	Acetyl-CoA and propionyl-CoA carboxylase	HCO ₃ [–]	Acetyl-CoA, pyruvate and succinyl-CoA	–12.5 to –13.7 ‰ ^{118–120}	Malonyl-CoA reductase, propionyl-CoA synthase and malyl-CoA lyase
3-Hydroxypropionate–4-hydroxybutyrate cycle	Nine	Six NADH or NADPH, but one FAD is reduced	Acetyl-CoA and propionyl-CoA carboxylase	HCO ₃ [–]	Acetyl-CoA and succinyl-CoA	–0.2 to –3.8 ¹²¹	Acetyl-CoA–propionyl-CoA carboxylase, enzymes reducing malonyl-CoA to propionyl-CoA, methylmalonyl-CoA mutase and 4-hydroxybutyryl-CoA dehydratase
Dicarboxylate–4-hydroxybutyrate cycle	Five	Two or three ferredoxin , one or two NADH or NADPH, and one unknown donor	Pyruvate synthase	CO ₂	Acetyl-CoA, pyruvate, PEP, oxaloacetate and succinyl-CoA	–0.2 to –3.8 ¹²¹	4-Hydroxybutyryl-CoA dehydratase
			PEP carboxylase	HCO ₃ [–]			

CoA, co-enzyme A; F₄₂₀, deazaflavin factor 420; FAD, flavin adenine dinucleotide; PEP, phosphoenolpyruvate; RubisCO, ribulose 1,5-bisphosphate carboxylase–oxygenase. *Alternative name of pathway is provided in brackets. †In biological processes, when inorganic carbon is used to make organic compounds, ¹²C is more weakly bonded and reacts more readily than ¹³C because of its lighter mass. This means that organic matter tends to become enriched in ¹²C (and depleted in ¹³C; therefore negative sign) relative to the reservoir of inorganic carbon from which it has been drawn. Carbon stable isotopic fractionations are measured relative to a fossil belemnite standard (the PDB standard). Isotopic fractionations are normally small and so values are measured in parts per thousand (‰) and expressed as δ¹³C ‰ = [(¹³C/¹²C)_{sample} – (¹³C/¹²C)_{standard}] / (¹³C/¹²C)_{standard} × 1000. ‡The presence of biotin-dependent 2-oxoglutarate carboxylase in, for example, *Hydrogenobacter thermophilus*¹²², can increase the energy requirements of the cycle. †NADH in *Hydrogenobacter thermophilus*¹²³. †Note that reduction of ferredoxin may be energy driven^{3–5}, which would increase the energy demands of the ferredoxin-dependent pathways.

be divided into two parts: in the first part, acetyl-CoA, one CO₂ and one bicarbonate are transformed through C₄ dicarboxylic acids to succinyl-CoA, and in the second part, succinyl-CoA is converted through 4-hydroxybutyrate into two molecules of acetyl-CoA (FIG. 3a). One acetyl-CoA can be used for biosynthesis and the second serves as a CO₂ acceptor for the next round of the cycle.

The dicarboxylate–hydroxybutyrate cycle starts with the reductive carboxylation of acetyl-CoA to

pyruvate, a reaction that is catalysed by pyruvate synthase (also known as pyruvate:ferredoxin oxidoreductase). This oxygen-sensitive enzyme is common in strict anaerobes, bacteria and archaea. Pyruvate is converted to phosphoenolpyruvate (PEP), followed by carboxylation of PEP to oxaloacetate, which is catalysed by an archaeal PEP carboxylase^{18,19}. The subsequent reduction to succinyl-CoA involves an incomplete reductive citric acid cycle. Originally, a complete reductive citric acid cycle was thought to operate²⁰. However,

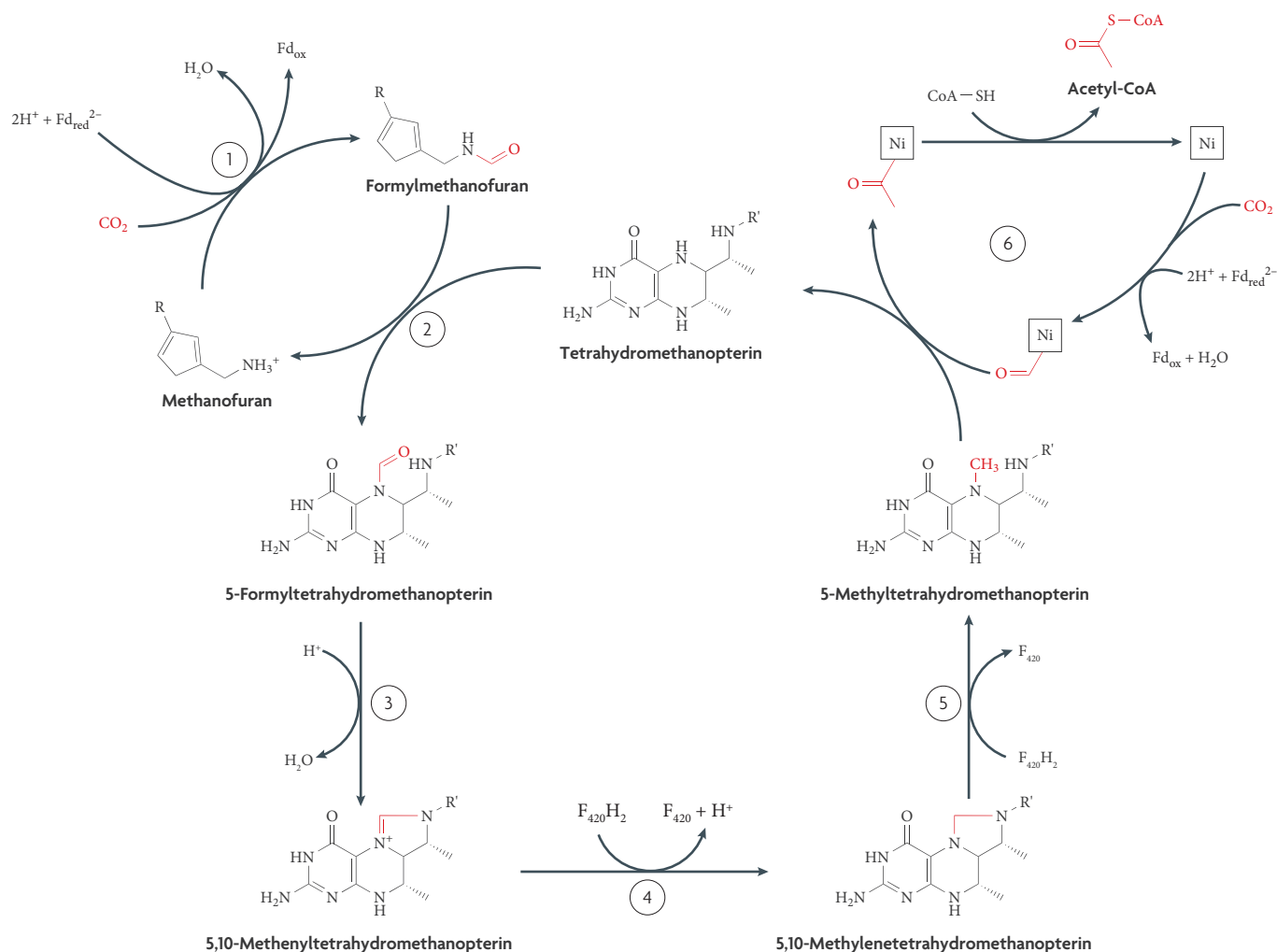


Figure 2 | The reductive acetyl-coenzyme A pathway. Two CO₂ molecules are reduced in total, one is reduced to CO bound to a nickel atom in the active centre of CO dehydrogenase and one to a methyl group bound to the carrier tetrahydropterin. Subsequently, a methyl-transferring corrinoid protein functions in methyl transfer, and acetyl-coenzyme A (acetyl-CoA) is synthesized from CO and the methyl group. The enzymes involved in each reaction are: formylmethanofuran dehydrogenase (reduced ferredoxin (Fd_{red}²⁻); 1); formylmethanofuran: tetrahydromethanopterin formyltransferase (2); methenyl-tetrahydromethanopterin cyclohydrolase (3); methylene-tetrahydromethanopterin dehydrogenase (reduced deazaflavin factor 420 (F₄₂₀H₂); 4); methylene-tetrahydromethanopterin reductase (reduced F₄₂₀H₂; 5); and CO dehydrogenase–acetyl-CoA-synthase (probably Fd_{red}²⁻; 6). Note that in bacteria the pathway differs in that CO₂ is reduced to free formate, which becomes activated to N¹⁰-formyl-tetrahydropterin in an ATP-dependent reaction. The tetrahydropterin also differs. Fd_{ox}, oxidized Fd.

succinyl-CoA is not converted to 2-oxoglutarate but is further reduced to succinic semialdehyde and then to 4-hydroxybutyrate. 4-Hydroxybutyrate is then converted into two acetyl-CoA molecules, a process that requires 4-hydroxybutyryl-CoA dehydratase, a key enzyme in the dicarboxylate–hydroxybutyrate cycle. 4-Hydroxybutyryl-CoA dehydratase contains a 4Fe–4S centre and flavin adenine dinucleotide and catalyses the elimination of water from 4-hydroxybutyryl-CoA by a ketyl radical mechanism^{21,22}. Its product, crotonyl-CoA, is converted into two molecules of acetyl-CoA through a normal β -oxidation reaction.

The active CO₂ species in the dicarboxylate–hydroxybutyrate cycle are CO₂ as the co-substrate for pyruvate synthase and bicarbonate (HCO₃⁻) as the

co-substrate for PEP carboxylase. Pyruvate formation in this cycle requires five ATP equivalents, and one energy-rich pyrophosphate is formed (the fate of which is unknown); this is compared with the seven ATP equivalents per pyruvate in the Calvin cycle (TABLE 1). A comparison with the 3-hydroxypropionate–4-hydroxybutyrate cycle (shortened to the hydroxypropionate–hydroxybutyrate cycle; discussed below) (FIG. 3b) reveals that the dicarboxylate–hydroxybutyrate cycle preferentially uses reduced ferredoxin instead of NADH or NADPH as the reductant. The oxygen sensitivity of some of its enzymes (for example, pyruvate synthase) and electron carriers (for example, ferredoxin) restricts this cycle to anaerobic, or at best microaerobic, Crenarchaeota¹⁵.

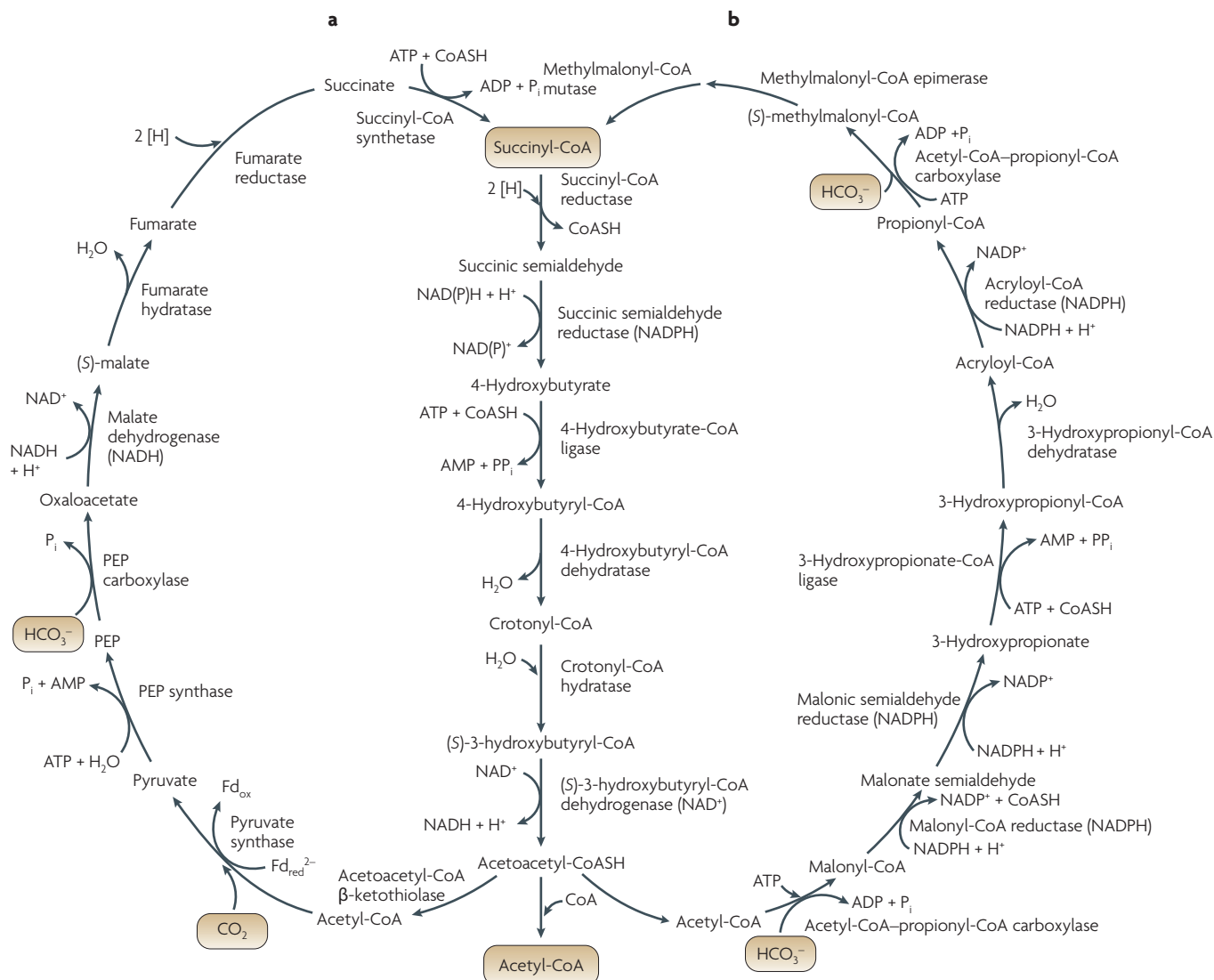


Figure 3 | Pathways of autotrophic CO₂ fixation in Crenarchaeota. The dicarboxylate–hydroxybutyrate cycle functions in Desulfurococcales and Thermoproteales (**a**) and the hydroxypropionate–hydroxybutyrate cycle functions in Sulfolobales (**b**). Note that succinyl-coenzyme A (succinyl-CoA) reductase in Thermoproteales and Sulfolobales uses NADPH^{14,35} and reduced methyl viologen (possibly as a substitute for reduced ferredoxin) in Desulfurococcales^{13,15}. In Sulfolobales, pyruvate might be derived from succinyl-CoA by C₄ decarboxylation. CoASH, coenzyme A; Fd_{red}²⁻, reduced ferredoxin; Fd_{ox}, oxidized ferredoxin; PEP, phosphoenolpyruvate.

The hydroxypropionate–hydroxybutyrate cycle

The hydroxypropionate–hydroxybutyrate cycle functions in the autotrophic crenarchaeal order Sulfolobales^{15,23–25} (FIG. 1). This group comprises extreme thermoacidophiles from volcanic areas that grow best at a pH of around 2 and a temperature of 60–90 °C. Most Sulfolobales can grow chemoautotrophically on sulphur, pyrite or H₂ under microaerobic conditions^{26,27}. The enzymes of the hydroxypropionate–hydroxybutyrate cycle are oxygen tolerant. One of the key enzymes, 4-hydroxybutyryl-CoA dehydratase, is also found in fermenting clostridia, in which it plays a part in γ -aminobutyrate fermentation. Although it is inactivated by oxygen in clostridia²¹, it is sufficiently oxygen insensitive in Sulfolobales¹⁵ to operate under microoxic or even oxic conditions. Therefore, the hydroxypropionate–hydroxybutyrate cycle fits well

with the lifestyle of aerobic Crenarchaeota, although it should be noted that it is also present in facultative anaerobic and even strictly anaerobic Sulfolobales species¹⁵. These species might have returned to an anaerobic lifestyle while retaining enzymes that are associated with an aerobic environment. The presence of genes encoding key enzymes of the hydroxypropionate–hydroxybutyrate cycle in the mesophilic marine group I Crenarchaeota^{25,28} (FIG. 1) suggests that these abundant marine archaea²⁹ also use this cycle.

In the hydroxypropionate–hydroxybutyrate cycle, one molecule of acetyl-CoA is formed from two molecules of bicarbonate. The key carboxylating enzyme is the bifunctional biotin-dependent acetyl-CoA–propionyl-CoA carboxylase^{30–33}. In Bacteria and Eukarya, acetyl-CoA carboxylase catalyses the first step in fatty acid

biosynthesis. However, Archaea do not contain fatty acids, so this enzyme obviously has a different metabolic role in these organisms.

The hydroxypropionate–hydroxybutyrate cycle can be divided into two parts (FIG. 3b). The first transforms acetyl-CoA and two bicarbonate molecules through 3-hydroxypropionate to succinyl-CoA^{23–25}, and the second converts succinyl-CoA through 4-hydroxybutyrate to two acetyl-CoA molecules²⁵. The product of the acetyl-CoA carboxylase reaction, malonyl-CoA, is reduced to malonic semialdehyde and then to 3-hydroxypropionate^{34,35}, which is further reductively converted to propionyl-CoA^{36,37}. Propionyl-CoA is carboxylated to (S)-methylmalonyl-CoA by the same carboxylase^{32,33}. (S)-methylmalonyl-CoA is isomerized to (R)-methylmalonyl-CoA, followed by carbon rearrangement to succinyl-CoA by coenzyme B₁₂-dependent methylmalonyl-CoA mutase. Succinyl-CoA is then converted to 4-hydroxybutyrate and then to two acetyl-CoA molecules^{25,35}; this second reaction sequence involving 4-hydroxybutyrate is apparently common to the autotrophic Crenarchaeota.

Acetyl-CoA–propionyl-CoA carboxylase uses bicarbonate as a co-substrate. Pyruvate is probably formed from succinyl-CoA through decarboxylation of malate or oxaloacetate, which requires one and a half turns of the cycle to build succinyl-CoA from four molecules of bicarbonate. The hydroxypropionate–hydroxybutyrate cycle requires nine ATP equivalents to make pyruvate (generating three molecules of pyrophosphate). Pyrophosphate might serve as energy source or might be hydrolysed by pyrophosphatase. Although the 3-hydroxypropionate part of this cycle resembles the first part of the 3-hydroxypropionate bicycle that functions in *Chloroflexus aurantiacus* (a phototrophic green non-sulphur bacterium)^{38–40}, the enzymes used to synthesize propionyl-CoA from malonyl-CoA are not homologous, although the intermediates are the same^{25,41,42}. Furthermore, in *C. aurantiacus* acetyl-CoA is regenerated by malyl-CoA cleavage, requiring an additional cycle to assimilate glyoxylate, the second product of this cleavage reaction^{40,43}. Therefore, these pathways that superficially seem to be similar might have evolved independently in Sulfolobales and Chloroflexi.

Gluconeogenesis from acetyl-CoA

All autotrophic pathways in archaea lead to the production of acetyl-CoA. The biosynthesis of C₃ to C₆ compounds must therefore begin with acetyl-CoA. The first steps, the formation of pyruvate and PEP from acetyl-CoA and CO₂, differ between archaea. In strict anaerobes, the ferredoxin-dependent pyruvate synthase catalyses the reductive carboxylation of acetyl-CoA, and pyruvate conversion to PEP uses PEP synthase (also known as pyruvate:water dikinase) or pyruvate:phosphate dikinase^{44,45} (FIG. 4). PEP carboxylase generates C₄ compounds. In the aerobic Sulfolobales, most of the intermediate succinyl-CoA is withdrawn from the carbon fixation cycle to serve as a precursor for biosynthesis, and pyruvate and PEP formation probably proceeds through oxidation of succinate to malate

or oxaloacetate. By contrast, gluconeogenesis starting from PEP seems to be uniform in different archaea.

Initially, all the enzyme activities and genes of the Embden–Meyerhof–Parnas gluconeogenic pathway, which is necessary to form FBP from PEP, were thought to be present in archaea (including glycerate 3-phosphate kinase, glyceraldehyde 3-phosphate dehydrogenase and FBP aldolase). This is in contrast to the great diversity that is seen in the archaeal glycolytic pathways, which mostly use different enzymes and intermediates (FIG. 4). However, experimentally, it proved difficult or impossible to detect FBP aldolase activity in archaea. In many cases this enzyme activity could only be measured in the direction of FBP formation, whereas the reverse reaction, FBP cleavage, failed^{46,47}. This discrepancy was mysterious as the reaction that is catalysed by FBP aldolase is freely reversible. However, tracer studies in several autotrophic archaea revealed a hexose labelling pattern that was consistent with the classical gluconeogenic route involving FBP aldolase^{47,48}.

Only a small group of archaea contain a proven archaeal FBP aldolase⁴⁹, and most lack a proven FBP aldolase-encoding gene. By contrast, the gene encoding an archaeal type V FBP phosphatase is present in many archaea⁵⁰. It turned out that most archaea, except a few (late-evolved) mesophilic groups of the Euryarchaeota (most Methanosarcinales and Methanomicrobiales as well as the (heterotrophic) Halobacteriales (FIG. 1)), contain a bifunctional FBP aldolase–phosphatase, which showed similarly high FBP aldolase and FBP phosphatase activity⁵¹ (FIG. 4). This pace-making enzyme catalyses the conversion of two triose phosphate molecules directly to fructose 6-phosphate and inorganic phosphate. Interestingly, this enzyme is also present in the deep-branching, mostly thermophilic bacterial lineages (Aquificae, Thermotogae, Chloroflexi, Deinococcus–Thermus and Clostridia–Firmicutes), whereas it is missing in most other bacteria and in Eukarya. This highly conserved, heat-stable, bifunctional FBP aldolase–phosphatase might represent the ancestral gluconeogenic enzyme. Its distribution pattern and unidirectional catalytic activity suggest that the Embden–Meyerhof–Parnas pathway evolved first in the direction of gluconeogenesis.

Furthermore, FBP aldolase–phosphatase guarantees a unidirectional gluconeogenic pathway under conditions in which the carbon flux does not need to be switched to sugar degradation. Its combination with the modified Entner–Doudoroff pathway (notably the non-phosphorylated variant) might even allow simultaneous and instantaneous use of growth substrates that require either glycolysis or gluconeogenesis without the burden of transcriptional regulation. Its bifunctionality and high substrate affinity ensure that heat-labile triose phosphates are quickly removed and trapped in stable fructose 6-phosphate. Early life forms probably contained little carbohydrate (compared with the cellulose-containing plants, the most important primary producers of carbohydrates) making sugars rare organic growth substrates. The great diversity of glycolytic pathways in heterotrophic archaea^{52,53} might be the result of

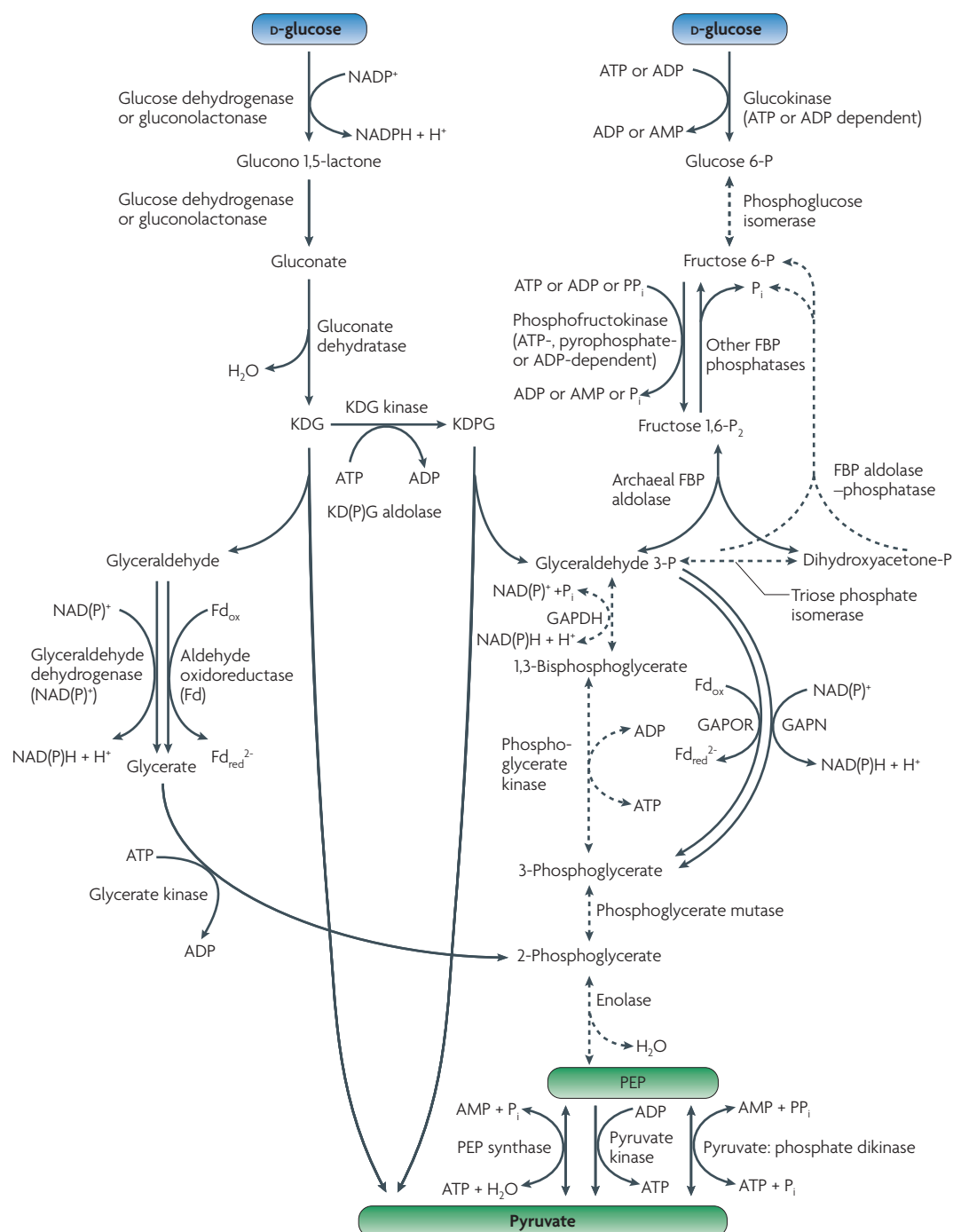


Figure 4 | **Central carbohydrate metabolism in archaea.** Pyruvate and phosphoenolpyruvate (PEP) formation differ in autotrophic organisms, but gluconeogenesis starting from PEP seems to be uniform (shown by the dashed arrows). In archaea and deep-branching bacterial lineages a bifunctional fructose 1,6-bisphosphate (FBP) aldolase-phosphatase displaces the reactions of the FBP aldolases and FBP phosphatase⁵¹. Archaea that can degrade sugars use modifications of the Embden-Meyerhof-Parnas (for example, *Thermococcus kodakarensis*; right) or Entner-Doudoroff (for example, *Sulfolobus solfataricus*; left) pathways^{52,53}. KDG (2-keto-3-deoxy-D-gluconate) and KDPG (2-keto-3-deoxy-6-phosphogluconate) are intermediates of the non-phosphorylative or semi-phosphorylative Entner-Doudoroff pathway, respectively. As FBP is only an intermediate of the Embden-Meyerhof-Parnas pathway, the archaea that use this pathway for sugar degradation must strictly regulate the expression of FBP aldolase-phosphatase⁵⁰ (or might contain a different FBP aldolase). By contrast, the archaea that use the Entner-Doudoroff pathway can express this enzyme under both glycolytic and gluconeogenic conditions⁵⁴ without the risk of a futile FBP-fructose 6-phosphate cycle. Figure is modified, with permission, from REF. 52 © Blackwell Publishing (2007) and REF. 53. © Elsevier (2005). Fd_{ox}, oxidized ferredoxin; Fd_{red}²⁻, reduced Fd; GAPDH, normal NAD(P)-dependent glyceraldehyde 3-phosphate dehydrogenase; GAPN, non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase; GAPOR, non-phosphorylating glyceraldehyde 3-phosphate oxidoreductase; P_i, inorganic phosphate.

convergent evolution and even late adaptation to sugar metabolism⁵⁴, when large quantities of cyanobacterial and finally plant cell walls could serve as the main growth substrates. This change in global sugar supply might have caused the loss of the ancestral unidirectional enzyme in heterotrophic bacteria.

Archaea generate pentose phosphates by many different routes. A modified oxidative pentose phosphate pathway probably functions in *Halobacterium* spp.⁵⁵. However, the most common route is the reversal of a pathway of formaldehyde assimilation that is found in some methylotrophic bacteria. In this reverse reaction sequence, fructose 6-phosphate is converted to pentose phosphate and formaldehyde. Formaldehyde can be scavenged by oxidation or by addition to tetrahydropyrimidines^{55–58}. Another option (identified in *Thermoplasma* spp.) is the use of transaldolase and transketolase, which catalyse the reversible interconversion of triose phosphates with fructose 6-phosphate to various sugar phosphates, thus providing pentose phosphates (as well as tetrose phosphates for aromatic amino acids)⁵⁵. Note that a different strategy for aromatic biosynthesis exists in archaea that does not start from erythrose 4-phosphate and PEP, but from hexoses, aspartate semialdehyde and methylglyoxal⁵⁹.

Regulation of autotrophic carbon metabolism

Many autotrophic archaea are facultative autotrophs or they can co-assimilate organic substrates into cellular building blocks even though they do not use organic substrates as an energy source by oxidizing them to CO₂. They often downregulate the enzymes that are specifically required for CO₂ fixation when organic substrates (such as acetate) are available. These regulatory effects can be dramatic¹⁴, and little is known about the transcriptional regulation of those genes. Another example for the need of regulation is the threatening futile cycling of glycolysis and gluconeogenesis, which occurs even in heterotrophic archaea. Specifically, some heterotrophic archaea form FBP aldolase–phosphatase constitutively, which would be deleterious during growth on sugars if a normal Embden–Meyerhof–Parnas pathway were used because the enzyme would reconvert triose phosphates into fructose 6-phosphate and inorganic phosphate. However, if glycolysis proceeds through variants of the Entner–Doudoroff pathway (especially the non-phosphorylated version, in which triose phosphates, fructose 6-phosphate or FBP are not involved), the antagonistic carbon fluxes do not share common metabolites. Under such conditions, the anabolic and catabolic pathways might coexist without mutual disturbance and be formed constitutively. In other cases they need to be strictly regulated, and only a few studies have dealt with this aspect (FIG. 4).

Genomic analysis of carbon fixation

Although only a limited number of species have been studied biochemically, approximately 90 archaeal genomes are now available in the NCBI database, considerably extending our ideas about the distribution of metabolic pathways in archaea. For example, although

all enzyme activities of the hydroxypropionate–hydroxybutyrate cycle have been shown only in *Metallosphaera sedula* and *Stygiolobus azoricus*^{15,25}, all sequenced autotrophic Sulfolobales have the corresponding genes. Moreover, genome sequence data inspired the recent elucidation of the new carbon fixation cycles in *M. sedula*^{25,60} and *Ignicoccus hospitalis*^{13,61}. Genome sequence data will be highly valuable for identifying new pathways and for the analysis of metabolic regulation of existing pathways^{62–64}. Such data might also tell us whether the distributions of the pathways outlined in this Review can be generalized, and findings from metagenomic approaches should allow to deduce the distribution of a particular pathway in nature^{65–67}.

The identification of a gene encoding putative 4-hydroxybutyryl-CoA dehydratase in autotrophic members of the genus *Ferroplasma*⁶⁸ is of particular interest. It seems to be expressed during autotrophic growth⁶⁹, but the actual pathway of CO₂ assimilation has yet to be confirmed. Genome analysis provides an opportunity to study uncultivated or slow-growing species. Autotrophic members of the mesophilic marine group I Crenarchaeota, which include the ammonia-oxidizing sponge symbiont *Cenarchaeum* spp. and free-living *Nitrosopumilus* spp., are thought to use the hydroxypropionate–hydroxybutyrate cycle. This conclusion is based solely on the presence of the genes encoding all key enzymes of this cycle and the coincident absence of genes encoding key enzymes of other autotrophic pathways^{25,28,70}; further experimental evidence for its operation is necessary.

However, genomic data should be interpreted with caution. For example, the substrate specificity of ordinary enzymes belonging to large families cannot simply be predicted from sequence comparison. One telling example is succinyl-CoA reductase, which is different in Sulfolobales, Thermoproteales and Desulfurococcales^{13–15,25,35}. The presence of conserved enzymes of a pathway, which all catalyse mechanistically difficult reactions, should be used as an indicator for the presence of a particular metabolic pathway, but not as proof of its existence. Moreover, genome analysis can be inconsistent with biochemical data, as is the case for *Pyrobaculum islandicum*, a close relative of *Thermoproteus neutrophilus*; genomic data (available from the DOE Joint Genome Institute website) suggest the presence of the dicarboxylate–hydroxybutyrate cycle, whereas enzymatic studies suggest the presence of a different pathway⁷¹.

On the basis of genome analysis, areas for further research can be identified. If the genes encoding key enzymes of known autotrophic CO₂ fixation pathways are lacking, it is possible that another autotrophic pathway exists. For example, *Pyrobaculum arsenaticum* lacks the gene encoding 4-hydroxybutyryl-CoA dehydratase, but genes coding for components of an alternative pathway have not been identified.

The role of RubisCO. Genome sequence data have revealed some insights into the potential function of RubisCO. Four forms of RubisCO have been identified,

with forms I–III being true carboxylating RubisCO enzymes. Form IV is referred to as RubisCO-like protein (RLP) and is found in many bacteria and archaea. Although structurally related to the true RubisCOs, RLPs do not function as RubisCO enzymes, but instead catalyse different reactions in sulphur metabolism^{72–74}. Form III RubisCO is only found in archaea, and its metabolic role is unclear^{72,75–80}. RubisCO has been found in many archaea that were not reported to be able to grow autotrophically and also in some Euryarchaeota that grow autotrophically but use the reductive acetyl-CoA pathway for CO₂ fixation. Phosphoribulokinase, the second key enzyme of the Calvin cycle, is also absent in archaea. The exceptions are a few methanogens (for example, *Methanosaeta thermophila*), *Aciduliprofundum boonei* and *Ferroplasma acidiphilum* (genomes available from the DOE Joint Genome Institute website), which contain genes encoding phosphoribulokinase and form III RubisCO^{80,81}. Likewise, the genome of *M. thermophila* seems to contain genes encoding both subunits of an ATP citrate lyase. It might well be that under some conditions these archaea run a functional Calvin cycle. The genome of *Archaeoglobus fulgidus* (a member of the Euryarchaeota) contains genes encoding the key enzymes not only of the reductive acetyl-CoA pathway, but also of the hydroxypropionate–hydroxybutyrate cycle, the dicarboxylate–hydroxybutyrate cycle and RubisCO^{11,76,82}, raising the question of whether only one or multiple pathways are functioning in this species, depending on the growth conditions.

Although some of the archaea that contain RubisCO might have other options to form ribulose 1,5-bisphosphate^{72,77,79}, the function of RubisCO remains elusive; however, it has been proposed to have a role in AMP metabolism⁷⁹. A recent phylogenetic analysis suggested an archaeal origin of both RubisCO and RLP from the form III RubisCO in methanogenic Euryarchaeota⁷² and a late appearance of the Calvin cycle in evolution^{83–85}. Interestingly the genome of the autotrophic Gram-positive *Ammonifex degensii* (available from the DOE Joint Genome Institute website) contains genes encoding the archaeal phosphoribulokinase and form III RubisCO, suggesting lateral gene transfer from the Archaea to Firmicutes.

Which autotrophic pathway is used?

What are the rules that govern the distribution of autotrophic pathways? Obviously, which metabolic pathway is used depends on both the genetic predisposition (the phylogeny) of an organism and the constraints of the occupied niche (the ecology). The discrete allocation of the archaeal autotrophic pathways to distinct groups mirrors these restraints. For a discussion of some of the ecological determinants of autotrophic pathways in general, see BOX 2. With regard to phylogeny, the phylogenetic tree seems to suggest that the common ancestor of all archaea was an anaerobic thermophilic chemolithoautotroph (FIG. 1). The same conclusion has been made from the analysis of archaeal genomes⁸⁶. Consequently, anaerobic

thermophilic chemolithoautotrophic archaea should share some inherited metabolic traits. By contrast, aerobic, non-thermophilic or heterotrophic extant archaeal species should represent a derived evolutionary stage, be it aerobic sulphur oxidizers, heterotrophs or halophiles using light as additional energy source.

In fact, all autotrophic pathways that are known, except the Calvin cycle, start biosynthesis from acetyl-CoA, which requires the generation (or regeneration) of acetyl-CoA. Anaerobic autotrophic Euryarchaeota and Crenarchaeota have a common heritage of carboxylating enzymes that are essential for acetyl-CoA assimilation: ferredoxin-dependent oxygen-sensitive pyruvate synthase^{87,88} and PEP carboxylase, respectively^{18,19}. Incidentally, these two carboxylases are also essential for the reductive citric acid cycle. It would seem that the primordial energy metabolism of archaea was based on either anaerobic C₁ or sulphur chemistry causing methanogens on one side and Thermoproteales and Desulfurococcales on the other to use different methods of acetyl-CoA formation.

Methanogens form acetyl-CoA *de novo* from C₁, and this choice is dictated by their energy metabolism. The reduction of C₁ to methane allows the formation of acetyl-CoA by the addition of only two more enzymes: a methyl transferase with a methyl-accepting corrinoid protein and a CO dehydrogenase–acetyl-CoA synthase. The anaerobic autotrophic Crenarchaeota, however, do not have this C₁ unit-transforming machinery because they are specialized in the reduction of sulphur to hydrogen sulphide. Instead, they use a common anaerobic strategy to produce C₄ compounds from acetyl-CoA and two CO₂ and generate acetyl-CoA from the C₄-compound succinyl-CoA. The derived aerobic autotrophic Crenarchaeota (specifically, Sulfolobales) still use this acetyl-CoA-regenerating machinery. However, because of the oxygen sensitivity of ferredoxin and pyruvate synthase, they developed another option to transform acetyl-CoA to succinyl-CoA that uses acetyl-CoA–propionyl-CoA carboxylase. The use of carboxyphosphate as an intermediate in this reaction is also an attractive model for carbon fixation during chemoevolution.

The autotrophic marine Crenarchaeota (which are adapted to aerobic life, mesophilic conditions and aerobic ammonia oxidation) seem to use the same mechanism of carbon fixation as the Sulfolobales. However, they might have arrived independently at the same result. Almost all enzymes that are involved in the hydroxypropionate–hydroxybutyrate cycle seem to have been recruited from different gene pools and to belong to large enzyme families that include carboxylic acid-CoA ligases, enoyl-CoA hydratases, as well as alcohol, aldehyde, acyl-CoA and 3-hydroxyacyl-CoA dehydrogenases. This is not surprising considering the ease with which the substrate specificity of these enzyme families can be changed by mutations. One example is malonyl-CoA reductase: in the Sulfolobales this enzyme originated by duplication of the gene encoding aspartate semialdehyde dehydrogenase, an enzyme that is required for threonine and methionine

Box 2 | **Benefits of the different autotrophic pathways under different conditions**

The pros and cons of the different pathways should be considered. Given that organisms that use the Calvin cycle have come to dominate most aerobic ecosystems, they presumably have some advantages. Still, five other options exist that obviously can pre-empt such advantages. Balancing the different requirements of the autotrophic pathways might be what determines whether a non-Calvin-type autotrophic organism can successfully compete.

Oxygen, metals and supply of C₁ compounds

Autotrophic Euryarchaeota are strictly confined to anoxic conditions, generally specialized in metabolizing C₁ compounds and/or acetate, and their energy metabolism has low ATP yields. Therefore, they need much of the C₁-transforming machinery for their energy metabolism. The reductive acetyl-coenzyme A (acetyl-CoA) pathway ideally copes with such constraints. Also, essential metals are more available under anoxic conditions owing to the higher solubility of the reduced forms of most metals. In Crenarchaeota, the oxygen-sensitive dicarboxylate–hydroxybutyrate cycle is restricted to the anaerobic Thermoproteales and Desulfurococcales, whereas the oxygen-insensitive hydroxypropionate–hydroxybutyrate cycle is restricted to the mostly aerobic Sulfolobales and possibly marine Crenarchaeota. The two lifestyles presuppose different electron donors with different redox potentials and different oxygen sensitivity of cofactors and enzymes. In a nutshell, energy cost-effective but oxygen-sensitive mechanisms cannot exist in aerobes because the enzymes would be inactivated by oxygen; and not all anaerobes have C₁ substrates at their disposal.

Energy demands

The different pathways require different amounts of ATP to make the cellular precursor metabolites. The costs for synthesizing all auxiliary, CO₂ fixation-related enzymes also differ, which might determine the energy costs involved. The synthesis of the catalysts itself can require a huge amount of energy as well as nitrogen and sulphur sources, especially if the pathways involve many auxiliary enzymes. Carboxylases with low catalytic efficiency must be synthesized in large amounts, as is the case for ribulose 1,5-bisphosphate carboxylase–oxygenase^{105,106}. So, energy limitation exerts a strong selective pressure in favour of energy-saving mechanisms, and the energy costs are largely spent for the synthesis of autotrophy-related enzymes.

Metabolic fluxes

In bacteria and archaea, the need for sugar phosphates in the biosynthesis of cell walls is lower than in plants, which also synthesize huge amounts of cellulose and lignin that is derived from erythrose 4-phosphate and phosphoenolpyruvate (PEP). The main metabolic fluxes are diverted from acetyl-CoA, pyruvate, oxaloacetate and 2-oxoglutarate, and their synthesis from 3-phosphoglycerate is partly connected with a loss of CO₂. Therefore, in bacteria autotrophic pathways directly yielding acetyl-CoA are more economical. Still, most facultative aerobic bacteria use the Calvin cycle, the regulation of which is almost detached from the central carbon metabolism and therefore may be particularly robust.

CO₂ species

As the bicarbonate (HCO₃[−]) concentration in slightly alkaline water is much higher than the concentration of dissolved CO₂, autotrophs might profit from using bicarbonate instead of CO₂. The usage of bicarbonate is a special feature of PEP carboxylase and biotin-dependent carboxylases (that is, acetyl-CoA–propionyl-CoA carboxylase). This property of PEP carboxylase is used in plants in crassulacean acid and C₄ metabolism to increase the efficiency of photosynthesis¹⁰⁷. The same might be true for acetyl-CoA–propionyl-CoA carboxylase, and the higher bicarbonate concentration could potentially make up for a lower bicarbonate affinity.

Co-assimilation of organic compounds

Many autotrophic bacteria and archaea living in aquatic habitats probably encounter carbon oligotrophic conditions and grow as mixotrophs. Co-assimilation of traces of organic compounds might pay off. A complete or even a rudimentary hydroxypropionate–hydroxybutyrate cycle, for instance, allows the co-assimilation of numerous compounds. These include fermentation products and 3-hydroxypropionate, an intermediate in the metabolism of the ubiquitous osmoprotectant dimethylsulphoniopropionate¹⁰⁸. It is possible that various widespread marine aerobic phototrophic bacteria have genes encoding a rudimentary 3-hydroxypropionate cycle for that purpose⁴³. Similarly, the dicarboxylate–hydroxybutyrate cycle allows the co-assimilation of dicarboxylic acids and substrates that are metabolized through acetyl-CoA.

biosynthesis, whereas the corresponding enzyme in *Chloroflexus* spp. is derived from completely different sources. The gene encoding the postulated marine crenarchaeal malonyl-CoA reductase has not been identified yet by BLAST search and therefore seems to differ from the known genes.

A model for primordial metabolism?

The topic of autotrophic carbon fixation invites the consideration of some evolutionary scenarios. The ‘metabolism first’ theory assumes that life started in a hydrothermal-vent setting in the Hadean ocean with catalytic metal sulphide surfaces^{89,90} or compartments⁹¹. The common ancestor of life was probably a chemolitho-autotrophic thermophilic anaerobe^{86,89–91}. According to

this theory, inorganic carbon fixation proceeded on minerals and was based on catalysis by transition metal sulphides. Given the structural and catalytic similarity between the minerals themselves and the catalytic metal or Fe–S-containing centres of the enzymes or cofactors in the acetyl-CoA pathway, one attractive idea is that minerals catalysed a primitive acetyl-CoA pathway⁸⁵. There is experimental support for this idea; for example, both the thioester acetyl methylsulphide and its hydrolysed product, acetate, can be produced from CO and CH₃SH using only Fe and Ni sulphides as catalysts⁹².

Several aspects of the reductive acetyl-CoA pathway are unique, and this pathway might be close to the ancestral autotrophic carbon fixation pathway⁸³. First, it uses CO, a common volcanic gas with strong

reduction potential, as an intermediate. Second, it has minimal energy requirements and intimately links anabolism and catabolism, as the product of the pathway, acetyl-CoA, can be converted to acetate — acetate formation from CO₂ and H₂ is the energy-yielding process in acetogenic bacteria — with the formation of ATP. Third, the pathway makes extensive use of coenzymes (tetrahydropterin, cobalamin and others, depending on the systematic position of the organism), metals (Fe, Co, Ni, Mo or W) and Fe-S centres. Coenzymes probably preceded the more complex proteins as catalysts^{90,93}. Fourth, it enables the assimilation of volatile CO, formaldehyde, methanol, methylamine or methylmercaptane. Such C₁ units occur (or might have occurred) in volcanic exhalations and they react spontaneously with cofactors of the reductive acetyl-CoA pathway. Fifth, this pathway depends on strict anoxic conditions as it uses low-potential electron donors, and some of its enzymes, notably CO dehydrogenase–acetyl-CoA synthase, are inherently highly oxygen sensitive. The required metals are preferentially water soluble in the reduced oxidation state, which also requires anoxic conditions. Sixth, the process can be simulated in the laboratory to make not only acetylthioesters, but also derived products by simply incubating CO, H₂ and H₂S or methylmercaptane with Ni and Fe salts; these inorganic metals form mixed Ni–Fe sulphides that act as catalysts^{89,92}. Finally, the pathway is not restricted to methanogenic archaea, but occurs in several strictly anoxic groups of bacteria. It can even be reversed and used for the oxidation of acetyl-CoA instead of the citric acid cycle^{94–96}.

One can advance similar but less coherent arguments in favour of the other pathways that are used by anaerobes: the reductive citric acid cycle (BOX 1) and the dicarboxylate–hydroxybutyrate cycle^{13,84,97,98}. Both require Fe–S-containing proteins, such as ferredoxin, and thioesters to facilitate chemical reactions. Such features fit well into a simple primordial carbon fixation scheme that is postulated by the ‘iron-sulphur’ theory^{89,90}. However, these cycles are restricted to either bacteria or archaea and might be specific (although ancient) innovations in these groups. In addition, both groups use enzymes that are also required for the assimilation of acetyl-CoA regardless of how it is made.

If we extrapolate the basic features of contemporary anaerobic autotrophic metabolism down to the level of its primitive non-enzymatic beginning, can we identify the prevalent prebiotic chemistry? Obviously not, as there is no infallible single criterion for a primitive type of metabolism, even though the biochemical unity that underlies the living world makes sense only if most of the central metabolic intermediates and pathways already existed in the common ancestor. The origins of life cannot be discovered, they must be reinvented^{89,99}. In other words, the phylogenetic reconstruction of ancestral metabolism requires the separation of life processes into parts that can be explained abiotically, followed by their reconstitution.

Progress in this endeavour requires contributions from different disciplines. Biologists must follow Ariadne’s thread of life from the most complex forms back to the least complex and finally to the point at which life emerged for the first time from inorganic matter¹⁰⁰. Microbiologists can contribute the extant metabolic repertoire of bacteria and archaea, such as autotrophic pathways leading to the set of central metabolites and building blocks, from which the least complex living beings are made; not to mention the universal coenzymes or cofactors, which might have preceded the more complex replicable polymer catalysts (RNA and protein) for assisting autocatalytic metabolic cycles. Prebiotic inorganic carbon can also mean CO, COS, HCN, HCHO and other C₁ molecules that are partly reduced and more reactive than CO₂. A prerequisite for a functioning metabolic cycle is its linkage to any kind of energy-providing process and to the generation of a primitive information-processing system. Only such a self-reproducing entity can enter biological evolution.

Bioinformaticians can shed light on early evolution by analysing the phylogenetic origins of genes. The genomes of archaea are full of examples in which gene duplication has resulted in two paralogues, one of which maintains its original function and another that is relieved from selection pressure and can rapidly evolve and eventually reach another function⁵². Alternative mechanisms of diversification based on lateral gene transfer may be even more important^{101,102}. Determining the phylogenetic roots of key enzymes of autotrophic pathways, including RubisCO, RLPs, ATP citrate lyase, CO dehydrogenase–acetyl-CoA synthase, acetyl-CoA–propionyl-CoA carboxylase and 4-hydroxybutyryl-CoA dehydratase, will be especially challenging. Which of these genes belonged to the basic equipment of Archaea and Bacteria, or Archaea only, and which genes in Archaea were derived from Bacteria, and vice versa? Answering these questions will eventually help to understand the early evolution of life and lead to a hypothetical chronology of events that led from chemoevolution to cellular carbon metabolism.

Earth scientists can contribute information on the most likely physicochemical scenario for the prebiotic geochemical environment, detailing boundary conditions such as gas composition, atmospheric pressure and temperature. On the basis of the map of the known metabolic landscape and the presumed physicochemical boundary conditions, chemists can then test all chemically conceivable molecular systems deemed to have a potential for self-assembly and self-replication.

The biological options are now open for dispute and — even more importantly — for experimental demonstration of their potential as candidate primordial reactions. Obviously, with six extant autotrophic carbon fixation cycles, the map of the metabolic landscape is more diverse than previously thought. Perhaps we do not yet have a complete picture, but we can begin to connect the dots.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomemap>
Acidilobus profundus boonei | *Ammonifex degensii* | *Archaeoglobus fulgidus* | *Chloroflexus aurantiacus* | *Ferroplasma placidus* | *Metallosphaera sedula* | *Methanosaeta thermophila* | *Pyrobaculum arsenaticum* | *Pyrobaculum islandicum* | *Thermoproteus neutrophilus*

FURTHER INFORMATION

DOE Joint Genome Institute website: <http://img.jgi.doe.gov>
 Georg Fuchs' homepage: <http://portal.uni-freiburg.de/ag-fuchs>

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