Autotrophic carbon fixation in archaea

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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 CO_2 serves as sole carbon source (autotrophic = self-nourishing).

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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 temperatures[2]. Most cultivated autotrophic archaea are either anaerobes or can tolerate or use oxygen only at low concentrations. The inorganic substrates that these organisms can oxidize include H_2, H_2S, S, CO, NH_3, metal sulphides such as pyrite (FeS_2), and reduced metal ions. The electron acceptors that can be used include various oxidized inorganic compounds such as S, $S_2O_3^{2-}$, SO_4^{2-}, AsO_4^{3-}, NO_3^-, oxidized metal ions and even CO_2 (for anaerobic respiration). The use of O_2 (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 flow[3–5].

In general terms, the assimilation of CO_2 into cellular building blocks requires four reducing equivalents and an input of energy. A small organic molecule usually serves as a CO_2 acceptor, which is linked to CO_2 by a carboxylase; however, a large coenzyme or a prosthetic group on an enzyme can also function as a CO_2 acceptor, as is discussed below. All CO_2 acceptors must eventually be regenerated in a cycle in which CO_2 (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 CO_2 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 CO_2 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 CO_2. 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

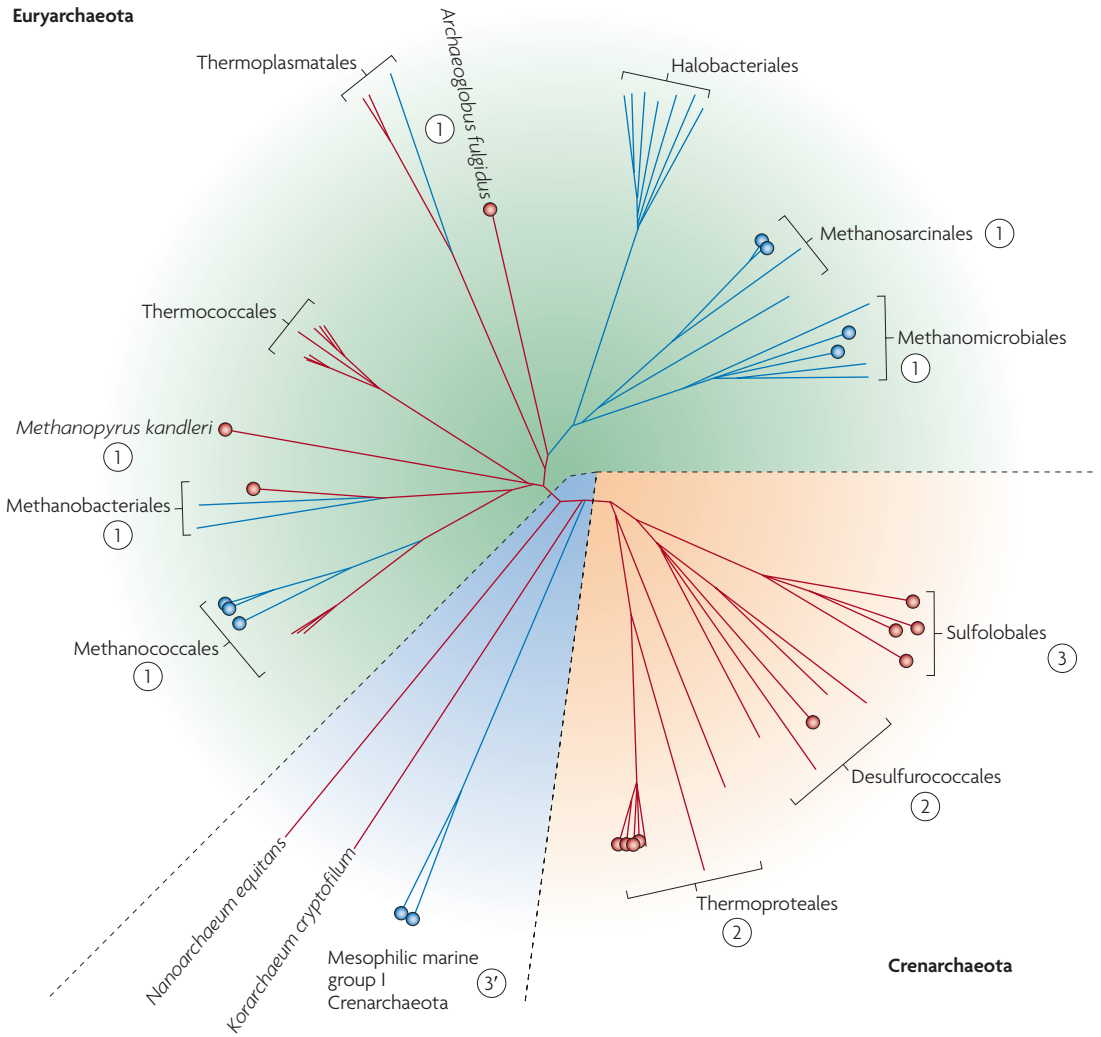

Figure 1 | **The phylogenetic (unrooted) tree of Archaea.** This phylogenetic tree is based on analyses of a concatamer of nine subunits of RNA polymerase, three transcription factors and 53 ribosomal proteins from all currently finished archaeal genomes (59 species). The numbers 1, 2, 3 and 3′ refer 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 CO_2 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 CO_2 using four molecules of H_2 to generate CH_4 or the disproportionation of acetate into CH_4 plus CO_2. The carbon assimilation pathway in these species — the reductive acetyl-CoA pathway — results in the fixation of two molecules of CO_2 to form acetyl-CoA, with a coenzyme and an enzyme metal centre as the CO_2 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 CO_2 to generate ATP. This pathway also operates in the sulphate-reducing euryarchaeal genera *Archaeoglobus*[11] and *Ferroglobus*[12].

Box 1 | **Autotrophic carbon fixation mechanisms**

Six mechanisms that assimilate CO_2 into cellular material have been identified (TABLE 1). Note that the different CO_2 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, CO_2 reacts with the five-carbon sugar ribulose 1,5-bisphosphate to yield two carboxylic acids, 3-phosphoglycerate, from which the sugar is regenerated[103]. 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 CO_2 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 proposal[14].

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 CO_2 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 CO_2 is reduced to the level of a methyl group, which is bound to a tetrahydropterin coenzyme. Another CO_2 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 CO_2, 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 CO_2 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 H_2 or CO restrict the reductive acetyl-CoA pathway to a limited set of anoxic niches.

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 CH_3COI to regenerate the metal catalyst. The CH_3COI reacts with water to generate acetic acid and regenerate HI.

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 H_2 to H_2S, but some grow under microaerobic or denitrifying conditions[16,17]. The dicarboxylate–hydroxybutyrate cycle can

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_3^-			
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; F_{420}, 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, ^{12}C is more weakly bonded and reacts more readily than ^{13}C because of its lighter mass. This means that organic matter tends to become enriched in ^{12}C (and depleted in ^{13}C; 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 $\delta^{13}C$ values as follows: $\delta 13C$ ‰ = [($^{13}C/^{12}C$sample - $^{13}C/^{12}C$standard) / ($^{13}C/^{12}C$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 CO_2 and one bicarbonate are transformed through C_4 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 CO_2 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 operate[20]. However,

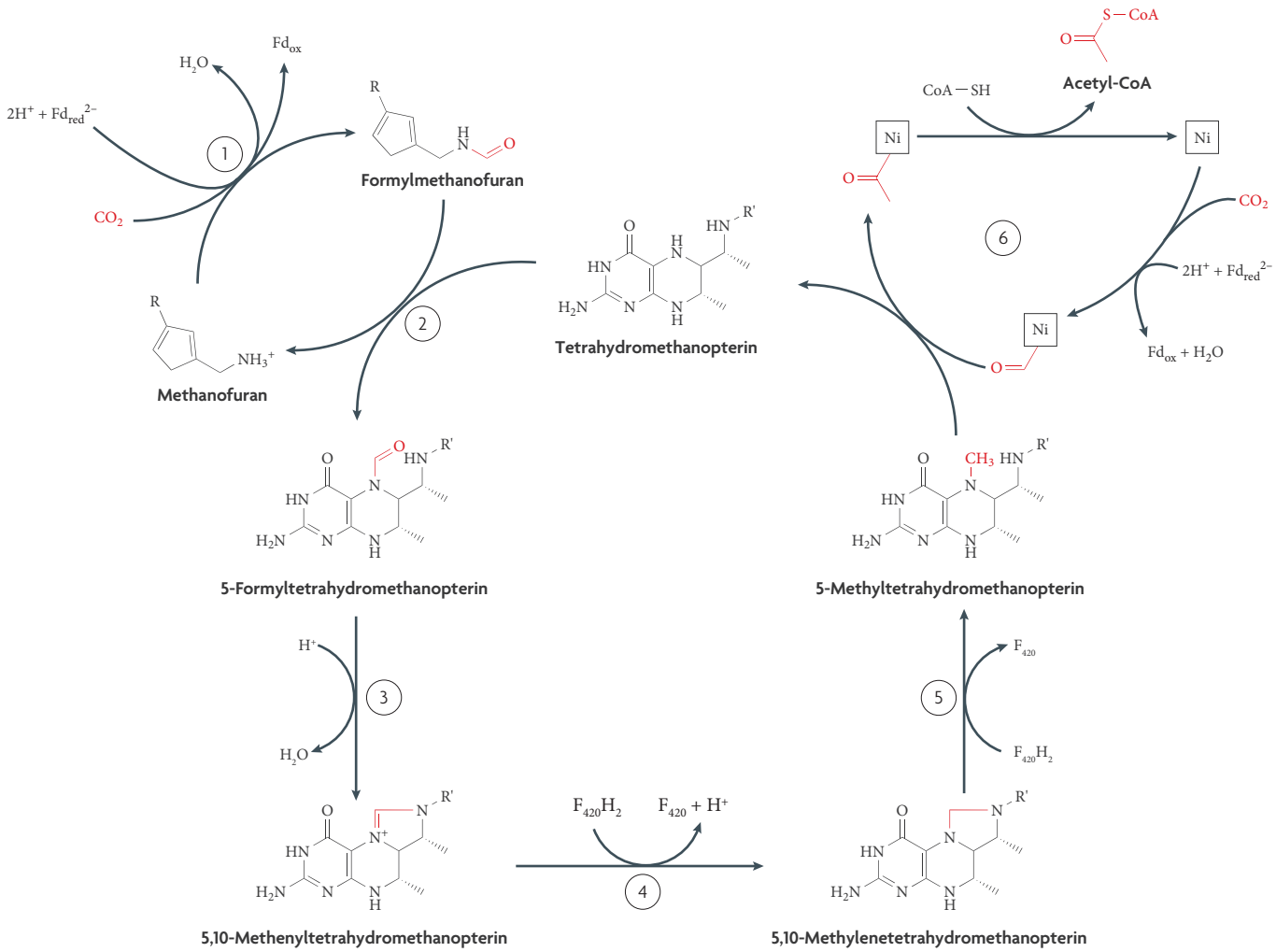

Figure 2 | **The reductive acetyl-coenzyme A pathway.** Two CO_2 molecules are reduced in total, one is reduced to CO bound to a nickel atom in the active centre of CO dehydrogenase and one to a methyl group bound to the carrier tetrahydropterin. Subsequently, a methyl-transferring corrinoid protein 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}); 1); formylmethanofuran: tetrahydromethanopterin formyltransferase (2); methenyl-tetrahydromethanopterin cyclohydrolase (3); methylene-tetrahydromethanopterin dehydrogenase (reduced deazaflavin factor 420 (F_{420}); 4); methylene- tetrahydromethanopterin reductase (reduced F_{420}; 5); and CO dehydrogenase–acetyl-CoA-synthase (probably Fd_{red}; 6). Note that in bacteria the pathway differs in that CO_2 is reduced to free formate, which becomes activated to N^{10}-formal-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 CO_2 species in the dicarboxylate–hydroxybutyrate cycle are CO_2 as the co-substrate for pyruvate synthase and bicarbonate (HCO_3^-) 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, Crenarchaeota[15].

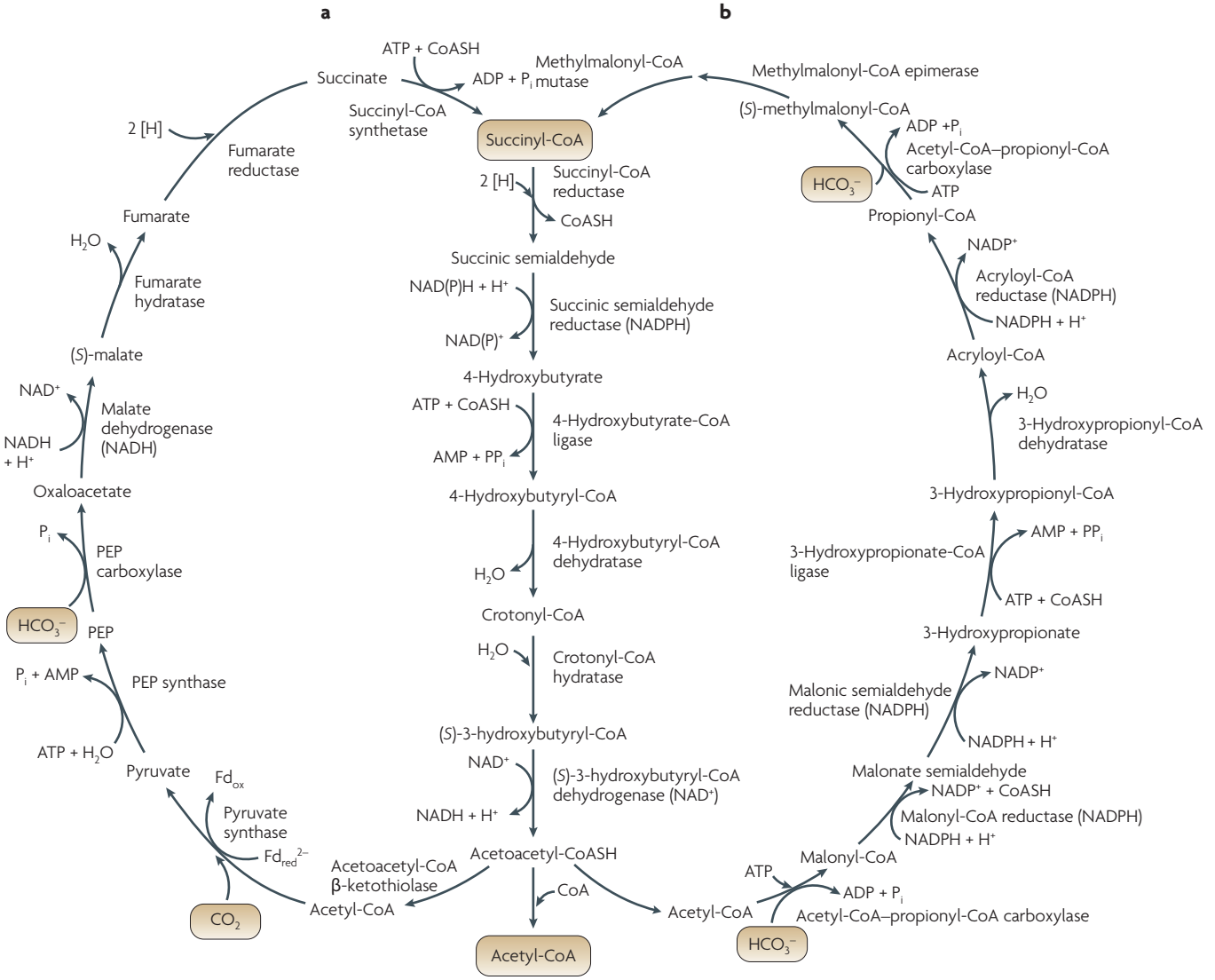

Figure 3 | **Pathways of autotrophic CO_2 fixation in Crenarchaeota.** The dicarboxylate–hydroxybutyrate cycle functions in Desulfurococcales and Thermoproteales (**a**) and the hydroxypropionate–hydroxybutyrate cycle functions in Sulfolobales (**b**). Note that succinyl-coenzyme A (succinyl-CoA) reductase 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 C_4 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 H_2 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 clostridia[21], it is sufficiently oxygen insensitive in Sulfolobales[15] 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 is also present in facultative anaerobic and even strictly anaerobic Sulfolobales species[15]. 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 archaea[29] 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 molecules[25]. 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 B_{12}-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 C_3 to C_6 compounds must therefore begin with acetyl-CoA. The first steps, the formation of pyruvate and PEP from acetyl-CoA and CO_2, 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 C_4 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 aldolase[49], and most lack a proven FBP aldolase-encoding gene. By contrast, the gene encoding an archaeal type V FBP phosphatase is present in many archaea[50]. It turned out that most archaea, except a few (late-evolved) mesophilic groups of the Euryarchaeaota (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 activity[51] (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

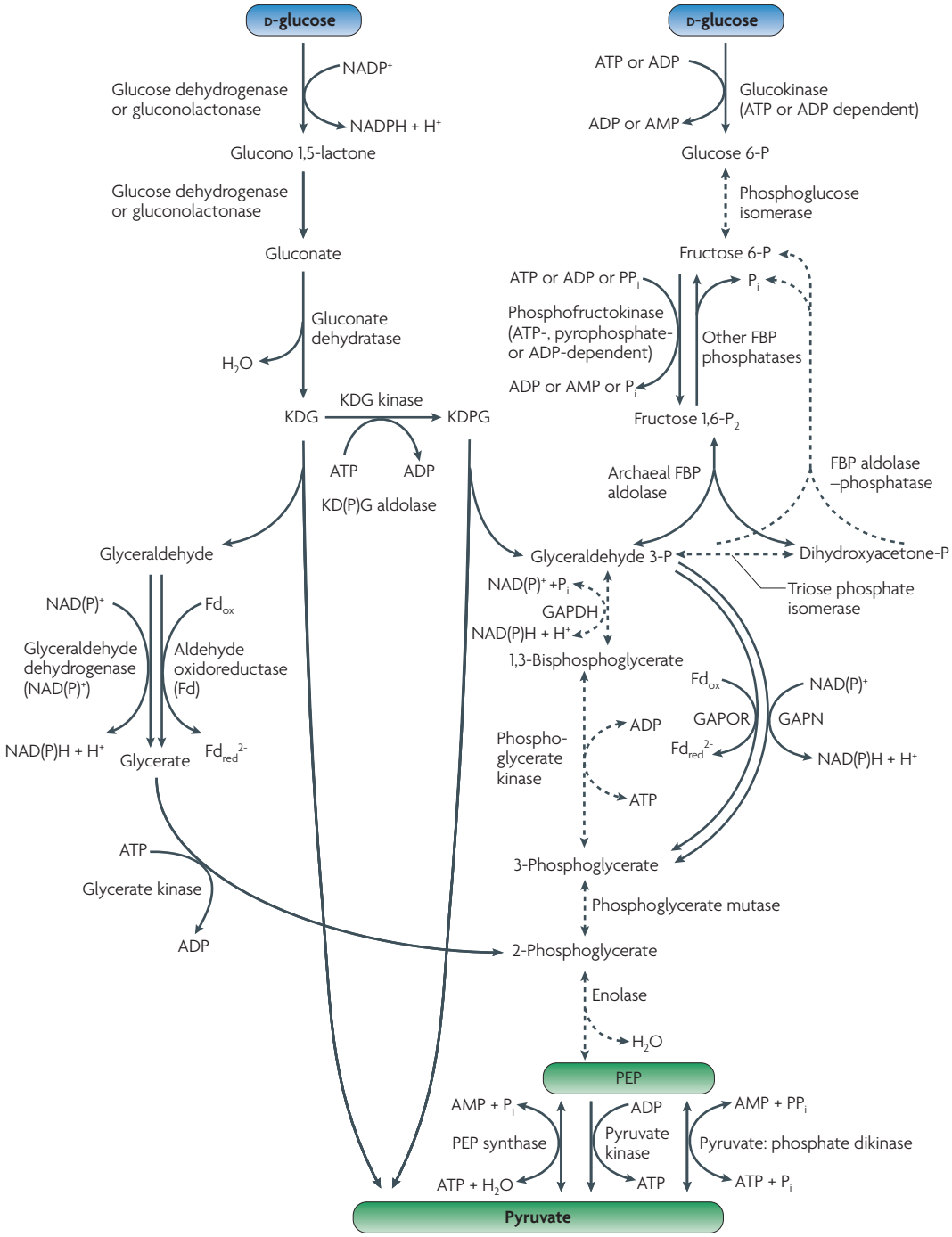

Figure 4 | **Central carbohydrate metabolism in archaea.** Pyruvate and phosphoenolpyruvate (PEP) formation differ in autotrophic organisms, but gluconeogenesis starting from PEP seems to be uniform (shown by the dashed arrows). In archaea and deep-branching bacterial lineages a bifunctional fructose 1,6-bisphosphate (FBP) aldolase–phosphatase displaces the reactions of the FBP aldolases and FBP phosphatase[51]. 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–phosphatase[50] (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 conditions[64] 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 metabolism[54], 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 tetrahydropterins[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 methylglyoxal[59].

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 CO_2. They often downregulate the enzymes that are specifically required for CO_2 fixation when organic substrates (such as acetate) are available. These regulatory effects can be dramatic[14], 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 Emden–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 growth[69], but the actual pathway of CO_2 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 pathway[71].

On the basis of genome analysis, areas for further research can be identified. If the genes encoding key enzymes of known autotrophic CO_2 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 CO_2 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 *Ferroglobus placidus* (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 metabolism[79]. A recent phylogenetic analysis suggested an archaeal origin of both RubisCO and RLP from the form III RubisCO in methanogenic Euryarchaeota[72] 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 genomes[86]. 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 C_1 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 C_1, and this choice is dictated by their energy metabolism. The reduction of C_1 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 C_1 unit-transforming machinery because they are specialized in the reduction of sulphur to hydrogen sulphide. Instead, they use a common anaerobic strategy to produce C_4 compounds from acetyl-CoA and two CO_2 and generate acetyl-CoA from the C_4-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 C_1 compounds

Autotrophic Euryarchaeota are strictly confined to anoxic conditions, generally specialized in metabolizing C_1 compounds and/or acetate, and their energy metabolism has low ATP yields. Therefore, they need much of the C_1-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 C_1 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, CO_2 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 archea, 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 CO_2. 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.

CO_2 species

As the bicarbonate (HCO_3^-) concentration in slightly alkaline water is much higher than the concentration of dissolved CO_2, autotrophs might profit from using bicarbonate instead of CO_2. 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 C_4 metabolism to increase the efficiency of photosynthesis[107]. 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 dimethylsulphoniopropionate[108]. It is possible that various widespread marine aerobic phototrophic bacteria have genes encoding a rudimentary 3-hydroxypropionate cycle for that purpose[43]. 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 compartments[91]. The common ancestor of life was probably a chemolithoautotrophic 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 pathway[85]. 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 CH_3SH using only Fe and Ni sulphides as catalysts[92].

Several aspects of the reductive acetyl-CoA pathway are unique, and this pathway might be close to the ancestral autotrophic carbon fixation pathway[83]. 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 CO_2 and H_2 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 C_1 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, H_2 and H_2S 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 matter[100]. 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 C_1 molecules that are partly reduced and more reactive than CO_2. 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 function[52]. 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.

1. Garrity, G. M. & Holt, J. G. *Bergey's Manual of Systematic Bacteriology, 2nd ed., vol. 1* (eds Boone, D. R., Castenholz, R. W. & Garrity, G. M.) 119–166 (Springer, New York, 2001).
2. Stetter, K. O. History of discovery of the first hyperthermophiles. *Extremophiles* **10**, 357–362 (2006).
3. Sapra, R., Bagramyan, K. & Adams, M. W. A simple energy-conserving system: proton reduction coupled to proton translocation. *Proc. Natl Acad. Sci. USA* **100**, 7545–7550 (2003).
4. Hedderich, R. & Forzi, L. Energy-converting [NiFe] hydrogenases: more than just H_2 activation. *J. Mol. Microbiol. Biotechnol.* **10**, 92–104 (2005).
5. Thauer, R. K., Kaster, A.-K., Seedorf, H., Buckel, W. & Hedderich, R. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Rev, Microbiol.* **6**, 579–591 (2008).
6. Taylor, G. T., Kelly, D. P. & Pirt, S. J. in *Microbial Production and Utilization of Gases* (eds Schlegel, H. G., Gottschalk, G. & Pfennig, N.) 173–180 (E. Goltze, K. G., Göttingen, 1976).
7. Ljungdahl, L. G. The autotrophic pathway of acetate synthesis in acetogenic bacteria. *Annu. Rev. Microbiol.* **40**, 415–450 (1986).
8. Wood, H. G. Life with CO or CO_2 and H_2 as a source of carbon and energy. *FASEB J.* **5**, 156–163 (1991).
9. Drake, H. L., Göβner, A. S. & Daniel, S. L. Old acetogens, new light. *Ann. N. Y. Acad. Sci.* **1125**, 100–128 (2008).
10. Ragsdale, S. W. Enzymology of the Wood-Ljungdahl pathway of acetogenesis. *Ann. N. Y. Acad. Sci.* **1125**, 129–136 (2008).
11. Vorholt, J. A., Kunow, J., Stetter, K. O. & Thauer, R. K. Enzymes and coenzymes of the carbon monoxide dehydrogenase pathway for autotrophic CO_2 fixation in *Archaeoglobus lithotrophicus* and the lack of carbon monoxide dehydrogenase in the heterotrophic *A. profundus*. *Arch. Microbiol.* **163**, 112–118 (1995).
12. Vorholt, J. A., Hafenbradl, D., Stetter, K. O. & Thauer, R. K. Pathways of autotrophic CO_2 fixation and of dissimilatory nitrate reduction to N_2O in *Ferroglobus placidus*. *Arch. Microbiol.* **167**, 19–23 (1997).
13. Huber, H. *et al.* A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic archaeum *Ignicoccus hospitalis*. *Proc. Natl Acad. Sci. USA* **105**, 7851–7856 (2008).
Reports the discovery of a dicarboxylate–hydroxybutyrate cycle in the archaeal order Desulfurococcales.
14. Ramos-Vera, W. H., Berg, I. A. & Fuchs, G. Autotrophic carbon dioxide assimilation in *Thermoproteales* revisited. *J. Bacteriol.* **191**, 4286–4297 (2009).
15. Berg, I. A., Ramos-Vera, W. H., Petri, A., Huber, H. & Fuchs, G. Study of the distribution of autotrophic CO_2 fixation cycles in Crenarchaeota. *Microbiology* **156**, 256–269 (2010).
16. Huber, H., Huber, R. & Stetter, K. O. in *The Prokaryotes, 3rd ed., vol. 3* (eds Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. & Stackebrandt, E.), 10–22 (Springer, New York, 2006).
17. Huber, H. & Stetter, K. O. in *The Prokaryotes, 3rd ed., vol. 3* (eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. & Stackebrandt, E.), 52–68 (Springer, New York, 2006).
18. Patel, H. M., Kraszewski, J. L. & Mukhopadhyay, B. The phosphoenolpyruvate carboxylase from *Methanothermobacter thermoautotrophicus* has a novel structure. *J. Bacteriol.* **186**, 5129–5137 (2004).
19. Ettema, T. J. G. *et al.* Identification and functional verification of archaeal-type phosphoenolpyruvate carboxylase, a missing link in archaeal central carbohydrate metabolism. *J. Bacteriol.* **186**, 7754–7762 (2004).
20. Strauss, G., Eisenreich, W., Bacher, A. & Fuchs, G. ^{13}C-NMR study of autotrophic CO_2 fixation pathways in the sulphur-reducing archaebacterium *Thermoproteus neutrophilus* and in the phototrophic eubacterium *Chloroflexus aurantiacus*. *Eur. J. Biochem.* **205**, 853–866 (1992).
21. Buckel, W. & Golding, G. T. Radical enzymes in anaerobes. *Annu. Rev. Microbiol.* **60**, 27–49 (2006).
22. Martins, B. M., Dobbek, H., Cinkaya, I., Buckel, W. & Messerschmidt, A. Crystal structure of 4-hydroxybutyryl-CoA dehydratase: radical catalysis involving a [4Fe-4S] cluster and flavin. *Proc. Natl Acad. Sci. USA* **101**, 15645–15649 (2004).
23. Ishii, M. *et al.* Autotrophic carbon dioxide fixation in *Acidianus brierleyi*. *Arch. Microbiol.* **166**, 368–371 (1997).
First report of the presence of a modified 3-hydroxypropionate cycle in Archaea.
24. Menendez, C. *et al.* Presence of acetyl coenzyme A (CoA) carboxylase and propionyl-CoA carboxylase in autotrophic *Crenarchaeota* and indication for operation of a 3-hydroxypropionate cycle in autotrophic carbon fixation. *J. Bacteriol.* **181**, 1088–1098 (1999).
25. Berg, I. A., Kockelkorn, D., Buckel, W. & Fuchs, G. A 3-hydroxypropionate/4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in Archaea. *Science* **318**, 1782–1786 (2007).
Reports the discovery of a hydroxypropionate–hydroxybutyrate cycle in Sulfolobales.
26. Huber, H. & Prangishvili, D. in *The Prokaryotes, 3rd ed., vol. 3* (eds Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. & Stackebrandt, E.), 23–51 (Springer, New York, 2006).
27. Auernik, K. S., Cooper, C. R. & Kelly, R. M. Life in hot acid: pathway analyses in extremely thermoacidophilic archaea. *Curr. Opin. Biotechnol.* **19**, 445–453 (2008).
28. Hallam, S. J. *et al.* Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. *PLoS Biol.* **4**, e95 (2006).
29. Karner, M. B., DeLong, E. F. & Karl, D. M. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409**, 507–510 (2001).
30. Norris, P., Nixon, A. & Hart, A. *Microbiology of Extreme Environments and its Potential for Biotechnology* (eds Da Costa, M. S., Duarte, J. C. & Williams, R. A. D.) 24–43 (Elsevier, London, 1989).
First report on the presence of an acetyl-CoA carboxylase in Archaea.
31. Burton, N. P., Williams, T. D. & Norris, P. R. Carboxylase genes in *Sulfolobus metallicus*. *Arch. Microbiol.* **172**, 349–353 (1999).
32. Hügler, M., Krieger, R. S., Jahn, M. & Fuchs, G. Characterization of acetyl-CoA/propionyl-CoA carboxylase in *Metallosphaera sedula*. Carboxylating enzyme in the 3-hydroxypropionate cycle for autotrophic carbon fixation. *Eur. J. Biochem.* **270**, 736–744 (2003).
33. Chuakrut, S., Arai, H., Ishii, M. & Igarashi, Y. Characterization of a bifunctional archaeal acyl coenzyme A carboxylase. *J. Bacteriol.* **185**, 938–947 (2003).
34. Alber, B. *et al.* Malonyl-coenzyme A reductase in the modified 3-hydroxypropionate cycle for autotrophic carbon fixation in archaeal *Metallosphaera* and *Sulfolobus* spp. *J. Bacteriol.* **188**, 8551–8559 (2006).
35. Kockelkorn, D. & Fuchs, G. Malonic semialdehyde reductase, succinic semialdehyde reductase, and succinyl-coenzyme A reductase from *Metallosphaera sedula*: enzymes of the autotrophic 3-hydroxypropionate/4-hydroxybutyrate cycle in *Sulfolobales*. *J. Bacteriol.* **191**, 6352–6362 (2009).
36. Alber, B. E., Kung, J. W. & Fuchs, G. 3-Hydroxypropionyl-coenzyme A synthetase from *Metallosphaera sedula*, an enzyme involved in the autotrophic CO_2 fixation. *J. Bacteriol.* **190**, 1383–1389 (2008).
37. Teufel, R., Kung, J. W., Kockelkorn, D., Alber, B. E. & Fuchs, G. 3-Hydroxypropionyl-coenzyme A dehydratase and acryloyl-coenzyme A reductase, enzymes of the autotrophic 3-hydroxypropionate/4-hydroxybutyrate cycle in *Sulfolobales*. *J. Bacteriol.* **191**, 4572–4581 (2009).
38. Holo, H. *Chloroflexus aurantiacus* secretes 3-hydroxypropionate, a possible intermediate in the assimilation of CO_2 and acetate. *Arch. Microbiol.* **151**, 252–256 (1989).
First hint of the role of 3-hydroxypropionate in autotrophic carbon fixation.
39. Strauss, G. & Fuchs, G. Enzymes of a novel autotrophic CO_2 fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. *Eur. J. Biochem.* **215**, 633–643 (1993).
40. Herter, S., Fuchs, G., Bacher, A. & Eisenreich, W. A bicyclic autotrophic CO_2 fixation pathway in *Chloroflexus aurantiacus*. *J. Biol. Chem.* **277**, 20277–20283 (2002).
41. Alber, B. E. & Fuchs, G. Propionyl-coenzyme A synthase from *Chloroflexus aurantiacus*, a key enzyme of the 3-hydroxypropionate cycle for autotrophic CO_2 fixation. *J. Biol. Chem.* **277**, 12137–12143 (2002).
42. Hügler, M., Menendez, C., Schägger, H. & Fuchs, G. Malonyl-coenzyme A reductase from *Chloroflexus aurantiacus*, a key enzyme of the 3-hydroxypropionate cycle for autotrophic CO_2 fixation. *J. Bacteriol.* **184**, 2404–2410 (2002).
43. Zarzycki, J., Brecht, V., Müller, M. & Fuchs, G. Identifying the missing steps of the autotrophic 3-hydroxypropionate CO_2 fixation cycle in *Chloroflexus aurantiacus*. *Proc. Natl Acad. Sci. USA* **106**, 21317–21322 (2009).
Shows the final steps of the 3-hydroxypropionate bicycle.
44. Eyzaguirre, J., Jansen, K. & Fuchs, G. Phosphoenolpyruvate synthetase in *Methanobacterium thermoautotrophicum*. *Arch. Microbiol.* **132**, 67–74 (1982).
45. Tjaden, B., Plagens, A., Dörr, C., Siebers, B. & Hensel, R. Phosphoenolpyruvate synthetase and pyruvate, phosphate dikinase of *Thermoproteus tenax*: key pieces in the puzzle of archaeal carbohydrate metabolism. *Mol. Microbiol.* **60**, 287–298 (2006).
46. Fuchs, G., Winter, H., Steiner, I. & Stupperich, E. Enzymes of gluconeogenesis in the autotroph *Methanobacterium thermoautotrophicum*. *Arch. Microbiol.* **136**, 160–162 (1983).
47. Jahn, U., Huber, H., Eisenreich, W., Hügler, M. & Fuchs, G. Insights into the autotrophic CO_2 fixation pathway of the archaeon *Ignicoccus hospitalis*: comprehensive analysis of the central carbon metabolism. *J. Bacteriol.* **189**, 4108–4119 (2007).
48. Schäfer, S., Barkowski, C. & Fuchs, G. Carbon assimilation by the autotrophic thermophilic archaebacterium *Thermoproteus neutrophilus*. *Arch. Microbiol.* **146**, 301–308 (1986).
49. Lorentzen, E., Siebers, B., Hensel, R. & Pohl, E. Mechanism of the Schiff base forming fructose-1, 6-bisphosphate aldolase: structural analysis of reaction intermediates. *Biochemistry* **44**, 4222–4229 (2005).
50. Rashid, N. *et al.* A novel candidate for the true fructose-1,6-bisphosphatase in archaea. *J. Biol. Chem.* **277**, 30649–30655 (2002).
51. Say, R. S. & Fuchs, G. Fructose 1,6-bisphosphate aldolase/phosphatase may be an ancestral gluconeogenic enzyme. *Nature* **464**, 1077–1081 (2010)
Discovery of the bifunctional FBP aldolase–phosphatase.
52. Van der Oost, J. & Siebers, B. in *Archaea: Evolution, Physiology and Molecular Biology* (eds Garrett, R. A. & Klenk, H.-P.) 247–259 (Blackwell, Malden, Massachusetts, 2007).
53. Siebers, B. & Schönheit, P. Unusual pathways and enzymes of central carbohydrate metabolism in Archaea. *Curr. Opin. Microbiol.* **8**, 695–705 (2005).
54. Ronimus, R. S. & Morgan, H. W. Distribution and phylogenies of enzymes of the Embden-Meyerhof-Parnas pathway from archaea and hyperthermophilic bacteria support a gluconeogenic origin of metabolism. *Archaea* **1**, 199–221 (2003).
55. Soderberg, T. Biosynthesis of ribose-5-phosphate and erythrose-4-phosphate in archaea: a phylogenetic analysis of archaeal genomes. *Archaea* **1**, 347–352 (2005).
56. Grochowski, L. L., Xu, H. & White, R. H. Ribose-5-phosphate biosynthesis in *Methanocaldococcus jannaschii* occurs in the absence of a pentose-phosphate pathway. *J. Bacteriol.* **187**, 7382–7389 (2005).
57. Orita, I. *et al.* The ribulose monophosphate pathway substitutes for the missing pentose phosphate pathway in the archaeon *Thermococcus kodakaraensis*. *J. Bacteriol.* **188**, 4698–4704 (2006).
58. Kato, N., Yurimoto, H. & Thauer, R. K. The physiological role of the ribulose monophosphate pathway in bacteria and archaea. *Biosci. Biotechnol. Biochem.* **70**, 10–21 (2006).
59. Grochowski, L. L. & White, R. H. Promiscuous anaerobes: new and unconvensional metabolism in methanogenic Archaea. *Ann. N. Y. Acad. Sci.* **1125**, 190–214 (2008).
60. Auernik, K. S., Maezato, Y., Blum, P. H. & Kelly, R. M. The genome sequence of the metal-mobilizing, extremely thermoacidophilic archaeon *Metallosphaera sedula* provides insights into bioleaching-associated metabolism. *Appl. Environ. Microbiol.* **74**, 682–692 (2008).
61. Podar, M. *et al.* A genomic analysis of the archaeal system *Ignicoccus hospitalis-Nanoarchaeum equitans*. *Genome Biol.* **9**, R158 (2008).
62. Auernik, K. S. & Kelly, R. M. Physiological versatility of the extremely thermoacidophilic archaeon *Metallosphaera sedula* supported by heterotrophy, autotrophy and mixotrophy transcriptomes. *Appl. Environ. Microbiol.* **76**, 2268–2672 (2010).
63. Zaparty, M. *et al.* DNA microarray analysis of central carbohydrate metabolism: glycolytic/gluconeogenic carbon switch in the hyperthermophilic crenarchaeum *Thermoproteus tenax*. *J. Bacteriol.* **190**, 2231–2238 (2008).

64. Chong, P. K., Burja, A. M., Radianingtyas, H., Fazeli, A. & Wright, P. C. Proteome and transcriptional analysis of ethanol-grown *Sulfolobus solfataricus* P2 reveals ADH2, a potential alcohol dehydrogenase. *J. Proteome Res.* **6**, 3985–3994 (2007).
65. DeLong, E. F. & Karl, D. M. Genomic perspectives in microbial oceanography. *Nature* **437**, 336–342 (2005).
66. Rusch, D. B. *et al.* The Sorcerer II Global Ocean Sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol.* **5**, e77 (2007).
67. Schleper, C., Jurgens, G. & Jonuscheit, M. Genomic studies of uncultivated archaea. *Nature Rev. Microbiol.* **3**, 479–488 (2005).
68. Allen, E. E. *et al.* Genome dynamics in a natural archaeal population. *Proc. Natl Acad. Sci. USA* **104**, 1883–1888 (2007).
69. Ferrer, M., Golyshina, O. V., Beloqui, A., Golyshin, P. N. & Timmis, K. N. The cellular machinery of *Ferroplasma acidiphilum* is iron-protein-dominated. *Nature* **445**, 91–94 (2007).
70. Hallam, S. J. *et al.* Genomic analysis of the uncultivated marine crenarchaeote *Cenarchaeum symbiosum*. *Proc. Natl Acad. Sci. USA* **103**, 18296–18301 (2006).
71. Hu, Y. & Holden, J. F. Citric acid cycle in the hyperthermophilic archaeon *Pyrobaculum islandicum* grown autotrophically, heterotrophically, and mixotrophically with acetate. *J. Bacteriol.* **188**, 4350–4355 (2006).
72. Tabita, F. R. *et al.* Function, structure, and evolution of the RubisCO-like proteins and their RubisCO homologs. *Microbiol. Mol. Biol. Rev.* **71**, 576–599 (2008).
Excellent review concerning the evolution of RubisCO and RLPs proposing their archaeal origins.
73. Ashida, H. *et al.* RuBisCO-like proteins as the enolase enzyme in the methionine salvage pathway: functional and evolutionary relationships between RuBisCO-like proteins and photosynthetic RuBisCO. *J. Exp. Bot.* **59**, 1543–1554 (2008).
74. Imker, H. J., Singh, J., Warlick, B. P., Tabita, F. R. & Gerlt, J. A. Mechanistic diversity in the RuBisCO superfamily: a novel isomerization reaction catalyzed by the RuBisCO-like protein from *Rhosdospirillum rubrum*. *Biochemistry* **47**, 11171–11173 (2008).
75. Maeda, N., Kanai, T., Atomi, H. & Imanaka, T. The unique pentagonal structure of an archaeal RuBisCO is essential for its high thermostability. *J. Biol. Chem.* **277**, 31656–31662 (2002).
76. Finn, M. W. & Tabita, F. R. Synthesis of catalytically active form III ribulose 1,5-bisphosphate carboxylase/oxygenase in archaea. *J. Bacteriol.* **185**, 3049–3059 (2003).
77. Finn, M. W. & Tabita, F. R. Modified pathway to synthesize ribulose 1,5-bisphosphate in methanogenic Archaea. *J. Bacteriol.* **186**, 6360–6366 (2004).
78. Kreel, N. E. & Tabita, F. R. Substitutions at methionine 295 of *Archaeoglobus fulgidus* ribulose-1,5-bisphosphate carboxylase/oxygenase affect oxygen binding and CO_2/O_2 specificity. *J. Biol. Chem.* **282**, 1341–1351 (2007).
79. Sato, T., Atomi, H. & Imanaka, T. Archaeal type III RuBisCOs function in a pathway for AMP metabolism. *Science* **315**, 1003–1006 (2007).
80. Mueller-Cajar, O. & Badger, M. R. New roads lead to RuBisCO in Archaebacteria. *BioEssays* **29**, 722–724 (2007).
81. Reysenbach, A, L. & Flores, G. E. Electron microscopy encounters with unusual thermophiles helps direct genomic analysis of *Aciduliprofundum boonei*. *Geobiology* **6**, 331–336 (2008).
82. Klenk, H.P. *et al.* The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature* **390**, 364–370 (1997).
83. Fuchs, G. in *Biology of Autotrophic Bacteria* (ed. Schlegel, H. G.) 365–382 (*Science Tech.*, Madison, Wisconsin, 1989).
84. Wächtershäuser, G. Evolution of the first metabolic cycles. *Proc. Natl Acad. Sci. USA* **87**, 200–204 (1990).
85. Russell, M. J. & Martin, W. The rocky roots of the acetyl-CoA pathway. *Trends Biochem. Sci.* **29**, 358–363 (2004).
Detailed discussion of the idea that acetyl-CoA pathway is an initial biochemical route.
86. Makarova, K. S., Sorokin, A. V., Novichkov, P. S., Wolf, Y. I. & Koonin, E. V. Clusters of orthologous genes for 41 archaeal genomes and implications for evolutionary genomics of archaea. *Biol. Direct* **2**, 33 (2007).
87. Fuchs, G., Stupperich, E. & Thauer, R. K. Acetate assimilation and the synthesis of alanine, aspartate and glutamate in *Methanobacterium thermoautotrophicum*. *Arch. Microbiol.* **117**, 61–66 (1978).
88. Ragsdale, S. W. Pyruvate ferredoxin oxidoreductase and its radical intermediate. *Chem. Rev.* **103**, 2333–2346 (2003).
89. Wächtershäuser, G. On the chemistry and evolution of the pioneer organism. *Chem. Biodivers.* **4**, 584–602 (2007).
The last update of Wächtershäuser's iron–sulphur world' theory of the chemolithoautotrophic origin of life.
90. Wächtershäuser, G. Before enzymes and templates: theory of surface metabolism. *Microbiol. Rev.* **52**, 452–484 (1988).
Discusses the basic concept of surface metabolism.
91. Martin, W., Baross, J., Kelley, D. & Russell, M. J. Hydrothermal vents and the origin of life. *Nature Rev. Microbiol.* **6**, 805–814 (2008).
92. Huber, C. & Wächtershäuser, G. Activated acetic acid by carbon fixation on (Fe, Ni)S under primordial conditions. *Science* **276**, 245–247 (1997).
93. Eschenmoser, A. Vitamin B_{12}: experiments concerning the origin of its molecular structure. *Angew. Chem. Int. Ed. Engl.* **27**, 5–39 (1988).
94. Schauder, R., Preuβ, A., Jetten, M. & Fuchs, G. Oxidative and reductive acetyl-CoA/carbon monoxide dehydrogenase pathway in *Desulfobacterium autotrophicum*. 2. Demonstration of the enzymes of the pathway and comparison of CO dehydrogenase. *Arch. Microbiol.* **151**, 84–89 (1989).
95. Thauer, R. K., Möller-Zinkhan, D. & Spormann, A. M. Biochemistry of acetate catabolism in anaerobic chemotrophic bacteria. *Annu. Rev. Microbiol.* **43**, 43–67 (1989).
96. Hattori, S., Galushko, A. S., Kamagata, Y. & Schink, B. Operation of the CO dehydrogenase/acetyl coenzyme A pathway in both acetate oxidation and acetate formation by the syntrophically acetate-oxidizing bacterium *Thermacetogenium phaeum*. *J. Bacteriol.* **187**, 3471–3476 (2005).
97. Cody, G. D. *et al.* Geochemical roots of autotrophic carbon fixation: hydrothermal experiments in the system citric acid, H_2O-(± FeS)-(± NiS). *Geochim. Cosmochim. Acta* **65**, 3557–3576 (2001).
98. Smith, E. & Morowitz, H. J. Universality in intermediary metabolism. *Proc. Natl Acad. Sci. USA* **101**, 13168–13173 (2004).
99. Eschenmoser, A. The search for the chemistry of life. *Tetrahedron* **63**, 12821–12844 (2007).
100. Kummer, C. *Der Glaube der Christen. Ein ökumenisches Handbuch* (eds Biser, E., Hahn, F. & Langer, M.) 25–44 (Pattloch Verlag, Munich, 1999).
101. Dagan, T., Artzy-Randrup, Y. & Martin, W. Modular networks and cumulative impact of lateral gene transfer in prokaryote genome evolution. *Proc. Natl Acad. Sci. USA* **105**, 10039–10044 (2008).
102. Zhaxybayeva, O. *et al.* On the chimeric nature, thermophilic origin, and phylogenetic placement of the Thermotogales. *Proc. Natl Acad. Sci. USA* **106**, 5865–5870 (2009).
103. Bassham, J. A. & Calvin, M. *The Path of Carbon in Photosynthesis* (Prentice Hall, Englewood Cliffs, 1957).
104. Evans, M. C. W., Buchanan, B. B. & Arnon, D. I. A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. *Proc. Natl Acad. Sci. USA* **55**, 928–934 (1966).
105. Tcherkez, G. G., Farquhar, G. D. & Andrews, T. J. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *Proc. Natl Acad. Sci. USA* **103**, 7246–7251 (2006).
106. Ellis, R. J. The most abundant protein on Earth. *Trends Biochem. Sci.* **4**, 241–244 (1979).
107. Keeley, J. E. & Rundel, P. W. Evolution of CAM and C_4 carbon-concentrationg mechanisms. *Int. J. Plant Sci.* **164**, S55–S77 (2003).
108. Todd, J. D. *et al.* Molecular dissection of bacterial acrylate catabolism - unexpected links with dimethylsulfoniopropionate catabolism and dimethyl sulfide production. *Environ. Microbiol.* **12**, 327–343 (2010)
109. Elkins, J. G. *et al.* A korarchaeal genome reveals insights into the evolution of the Archaea. *Proc. Natl Acad. Sci. USA* **105**, 8102–8107 (2008).
110. Brochier, C., Gribaldo, S., Zivanovic, Y., Confalonieri, F. & Forterre, P. Nanoarchaea: representatives of a novel archaeal phylum or a fast-evolving euryarchaeal lineage related to Thermococcales? *Genome Biol.* **6**, R42 (2005).
111. Brochier-Armanet, C., Boussau, B., Gribaldo, S. & Forterre, P. Mesophilic Crenarchaea: proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Rev. Microbiol.* **6**, 245–252 (2008).
112. Robertson, C. E., Harris, J. K., Spear, J. R. & Pace, N. R. Phylogenetic diversity and ecology of environmental Archaea. *Curr. Opin. Microbiol.* **8**, 638–642 (2005).
113. Quandt, L., Gottschalk, G., Ziegler, H. & Stichler, W. Isotope discrimination by photosynthetic bacteria. *FEMS Microbiol. Lett.* **1**, 125–128 (1977).
114. McNevin, D. B. *et al.* Differences in carbon isotope discrimination of three variants of D-ribulose-1,5-bisphosphate carboxylase/oxygenase reflect differences in their catalytic mechanisms. *J. Biol. Chem.* **282**, 36068–36076 (2007).
115. Sirevåg, R., Buchanan, B. B., Berry, J. A. & Troughton, J. H. Mechanisms of CO_2 fixation in bacterial photosynthesis studied by the carbon isotope fractionation technique. *Arch. Microbiol.* **112**, 35–38 (1977).
116. Preuβ, A., Schauder, R. & Fuchs, G. Carbon isotope fractionation by autotrophic bacteria with three different CO_2 fixation pathways. *Z. Naturforsch.* **44c**, 397–402 (1989).
117. House, C. H. *et al.* Carbon isotopic composition of individual Precambrian microfossils. *Geology* **28**, 707–710 (2000).
118. Holo, H. & Sirevåg, R. Autotrophic growth and CO_2 fixation in *Chloroflexus aurantiacus*. *Arch. Microbiol.* **145**, 173–180 (1986).
119. Ivanovsky, R. N. *et al.* Evidence for the presence of the reductive pentose phosphate cycle in a filamentous anoxygenic photosynthetic bacterium, *Oscillochloris trichoides* strain DG-6. *Microbiology* **145**, 1743–1748 (1999).
120. van der Meer, M. T., Schouten, S., de Leeuw, J. W. & Ward, D. M. Autotrophy of green non-sulphur bacteria in hot spring microbial mats: biological explanations for isotopically heavy organic carbon in the geological record. *Environ. Microbiol.* **2**, 428–435 (2000).
121. House, C. H., Schopf, J. W. & Stetter, K. O. Carbon isotopic fractionation by Archaeans and other thermophilic prokaryotes. *Org. Geochem.* **34**, 345–356 (2003).
122. Aoshima, M., Ishii, M. & Igarashi, Y. A novel biotin protein required for reductive carboxylation of 2-oxoglutarate by isocitrate dehydrogenase in *Hydrogenobacter thermophilus* TK-6. *Mol. Microbiol.* **51**, 791–798 (2004).
123. Miura, A., Kameya, M., Arai, H., Ishii, M. & Igarashi, Y. A soluble NADH-dependent fumarate reductase in the reductive citric acid cycle of *Hydrogenobacter thermophilus* TK-6. *J. Bacteriol.* **190**, 7170–7177 (2008).

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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=genomeprj
Aciduliprofundum boonei | *Ammonifex degensii* | *Archaeoglobus fulgidus* | *Chloroflexus aurantiacus* | *Ferroglobus 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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