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Review

On the origin of biochemistry at an alkaline hydrothermal vent

William Martin[1,*] **and Michael J. Russell**[2]

[1]*Institute of Botany, University of Düsseldorf, 40225 Düsseldorf, Germany*
[2]*Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA 91109, USA*

A model for the origin of biochemistry at an alkaline hydrothermal vent has been developed that focuses on the acetyl-CoA (Wood–Ljungdahl) pathway of CO_2 fixation and central intermediary metabolism leading to the synthesis of the constituents of purines and pyrimidines. The idea that acetogenesis and methanogenesis were the ancestral forms of energy metabolism among the first free-living eubacteria and archaebacteria, respectively, stands in the foreground. The synthesis of formyl pterins, which are essential intermediates of the Wood–Ljungdahl pathway and purine biosynthesis, is found to confront early metabolic systems with steep bioenergetic demands that would appear to link some, but not all, steps of CO_2 reduction to geochemical processes in or on the Earth's crust. Inorganically catalysed prebiotic analogues of the core biochemical reactions involved in pterin-dependent methyl synthesis of the modern acetyl-CoA pathway are considered. The following compounds appear as probable candidates for central involvement in prebiotic chemistry: metal sulphides, formate, carbon monoxide, methyl sulphide, acetate, formyl phosphate, carboxy phosphate, carbamate, carbamoyl phosphate, acetyl thioesters, acetyl phosphate, possibly carbonyl sulphide and eventually pterins. Carbon might have entered early metabolism via reactions hardly different from those in the modern Wood–Ljungdahl pathway, the pyruvate synthase reaction and the incomplete reverse citric acid cycle. The key energy-rich intermediates were perhaps acetyl thioesters, with acetyl phosphate possibly serving as the universal metabolic energy currency prior to the origin of genes. Nitrogen might have entered metabolism as geochemical NH_3 via two routes: the synthesis of carbamoyl phosphate and reductive transaminations of α-keto acids. Together with intermediates of methyl synthesis, these two routes of nitrogen assimilation would directly supply all intermediates of modern purine and pyrimidine biosynthesis. Thermodynamic considerations related to formyl pterin synthesis suggest that the ability to harness a naturally pre-existing proton gradient at the vent–ocean interface via an ATPase is older than the ability to generate a proton gradient with chemistry that is specified by genes.

Keywords: origin of life; thioesters; acetyl phosphate; formyl phosphate; carbamoyl phosphate; carboxyphosphate

1. INTRODUCTION

It was less than 30 years ago when submarine hydrothermal vents were discovered. Since their discovery, people have considered the idea, as one alternative to a prebiotic soup (Haldane 1929), that life might have originated at submarine hydrothermal vents (Corliss *et al.* 1981; Baross & Hoffman 1985). This idea is often dismissed by its critics on the grounds that submarine hydrothermal vents are simply too hot to have had anything to do with the origin of life. While it is true that life could not have arisen at approximately 400°C, it is not true that all hydrothermal vents are that hot. Some hydrothermal vents are much cooler than the famous originally discovered ones called black smokers. Such warm, alkaline vents, like Lost City near the Mid-Atlantic ridge, bear very H_2-rich water of about 40–90°C (Kelley *et al.* 2005; Proskurowski *et al.* 2006). Although such vents have existed for at least 30 000 years (Früh-Green *et al.* 2003), they have only been known for about 5 years, so biologists and geochemists have not had much time to think about their implications for the origin of life or to do experiments that simulate their conditions in the laboratory. One of us was thinking about the origin of life at warm, alkaline submarine vents before they were discovered. For example, Russell *et al.* (1994, p. 231) wrote:

> We propose that life emerged from growing aggregates of iron sulphide bubbles containing alkaline and highly reduced hydrothermal solution. These bubbles were inflated hydrostatically at sulphidic submarine hot springs sited some distance from oceanic spreading centers four billion years ago.

The Lost City vents (i) are located some distance from an oceanic spreading centre, (ii) bear alkaline, highly reduced water containing minor sulphide (Kelley *et al.* 2001), and (iii) form bubble-like microcompartments (Kelley *et al.* 2005). Thoughts

* Author for correspondence (w.martin@uni-duesseldorf.de).

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about the origin of life thus led to the inference of a particular kind of hydrothermal vent that was subsequently discovered; such a vent also could have existed 4 billion years (Gyr) ago as well.

The idea that the first kinds of organisms on Earth were autotrophs (satisfying their carbon needs from CO_2 alone) is slightly older than the idea of a prebiotic soup (Haldane 1929). The Russian biologist Mereschkowsky (1910, p. 360) while considering the nature of the first organisms inferred that they (i) were anaerobes, and (ii) had the

> 'ability to synthesize proteins and carbohydrates (the latter without the help of chlorophyll) from inorganic substances.' ['Fähigkeit, Eiweiße und Kohlenhydrate (letzteres ohne Vermittlung des Chlorophylls) aus unorganischen Stoffen zu bilden.']

Leduc (1911) and others (Hartman 1975; Fuchs & Stupperich 1985; Fuchs 1986; Morowitz *et al.* 2000) also suggested that the earliest forms of life were autotrophs. But, many scientists still prefer the idea of a prebiotic soup (Bada & Lazcano 2002) in which the first organisms would have survived from oxidation of the soup's reduced carbon compounds (de Duve 1991).

Regardless of how life originated and what the ancestral state of microbial metabolism is, the overall process of life's emergence must have been thermodynamically favourable, otherwise it would not have occurred. Hydrothermal vents are attractive in this respect, because hydrothermal H_2 interfaces with the CO_2 dissolved in the ocean from the early atmosphere (Osborn 1917; Goldschmidt 1952). Chemical equilibrium of the H_2/CO_2 system in hydrothermal conditions favours the synthesis of reduced carbon compounds, the essential constituents of life, regardless of the reaction path taken to get there (Shock 1990; Shock *et al.* 1998).

From the top-down (comparative biochemical) perspective, among those anaerobic core metabolic reactions of microbes that involve H_2 as an electron donor (Schönheit & Schäfer 1995; Amend & Shock 2001), methanogens and acetogens stand out, because they synthesize their adenosine triphosphate (ATP; table 1) by reducing CO_2. They do this with the help of the acetyl-coenzyme A (acetyl-CoA; Wood–Ljungdahl) pathway of CO_2 fixation (Fuchs 1986). The acetyl-CoA pathway has several biochemical features that point to its antiquity (Fuchs & Stupperich 1985), as listed by Fuchs (1989): (i) occurrence in anaerobes and extremophiles, (ii) occurrence in eubacteria and archaebacteria, (iii) minimal energy requirement, (iv) utilization of carbon monoxide (CO), formate, formaldehyde and methanol in addition to CO_2, (v) requirement for ubiquitous but varied coenzyme structures, and (vi) the importance of metals in the catalysis of CO_2 fixation and reduction.

Table 1. Abbreviations

Abbreviations	
4PH-L-Thr	4-(phosphohydroxy)-L-threonine
ACK	acetate kinase
ACS	acetyl-CoA synthase
AICAR	5-amino-4-imidazolecarboxamide ribonucleotide
AIR	5-aminoimidazole ribonucleotide
ATP	adenosine triphosphate
CO	carbon monoxide
CoA	coenzyme A
CODH	carbon monoxide dehydrogenase
CoFeSP	corrinoid iron–sulphur protein
COS	carbonyl sulphide
CPS	carbamoyl phosphate synthase
DX5P	1-deoxy-xylulose-5-phosphate
DXS	1-deoxy-xylulose-5-phosphate synthase
FAD	flavin adenine dinucleotide
FDH	formate dehydrogenase
GA3P	glyceraldehyde-3-phosphate
GAPOR	D-glyceraldehyde-3-phosphate oxidoreductase
GAR	glycinamide ribonucleotide
GS	glutamine synthase
GTP	guanosine triphosphate
H_4F	tetrahydrofolate
H_4MPT	tetrahydromethanopterin
MF	methanofuran
MoCo	molybdenum cofactor
MPT	methanopterin
NAD	nicotinamide adenine dinucleotide
PAT	phosphotransacetylase
PEP	phosphoenolpyruvate
PGA	phosphoglycerate
PLP	pyridoxal phosphate
PNP	pyridoxine 5′-phosphate
PPDK	pyruvate: pyrophosphate dikinase
RNA	ribonucleic acid
SRP	signal recognition particle
TCA cycle	tricarboxylic acid cycle
TPP	thiamine pyrophosphate

Figure 1. (*Opposite*.) Acetogenesis and methanogenesis. (*a*) Biochemical cartoon of acetogenesis (i) as redrawn from Müller (2003) and methanogenesis (ii) as redrawn from Schönheit & Schäfer (1995) including the structures of the salient pterin cofactors (iii, iv) and their relevant chemical intermediates (v) as redrawn from Maden (2002) with a brown dot to indicate the relevant moieties of the intermediates with respect to the complete structures. Ion pumping portions of the pathways are indicated schematically with green shading, whereby the coupling sites are known in some detail in methanogens (Schönheit & Schäfer 1995), but are not known with certainty in acetogens (Müller 2003), although it is certain that acetogens depend upon chemiosmosis for H_2/CO_2-dependent growth (Fuchs 1986; Müller 2003). The homologous CODH/ACS enzymes are indicated with purple shading, the ATPase with blue. The initial energy investment required in both pathways to generate a formyl pterin, but provided by different means, is indicated. The dependence upon the MoCo in steps leading to the formation of formyl pterin is indicated. The PAT and ACK steps of the methanogen pathway, recently uncovered by Rother & Metcalf (2004) under particular growth conditions, are bracketed and labelled with a question mark, because it is uncertain whether that growth mode is sustainable through substrate level phosphorylation alone (see text). (*b*) Some biologically relevant pterins (see text), including MoCo.

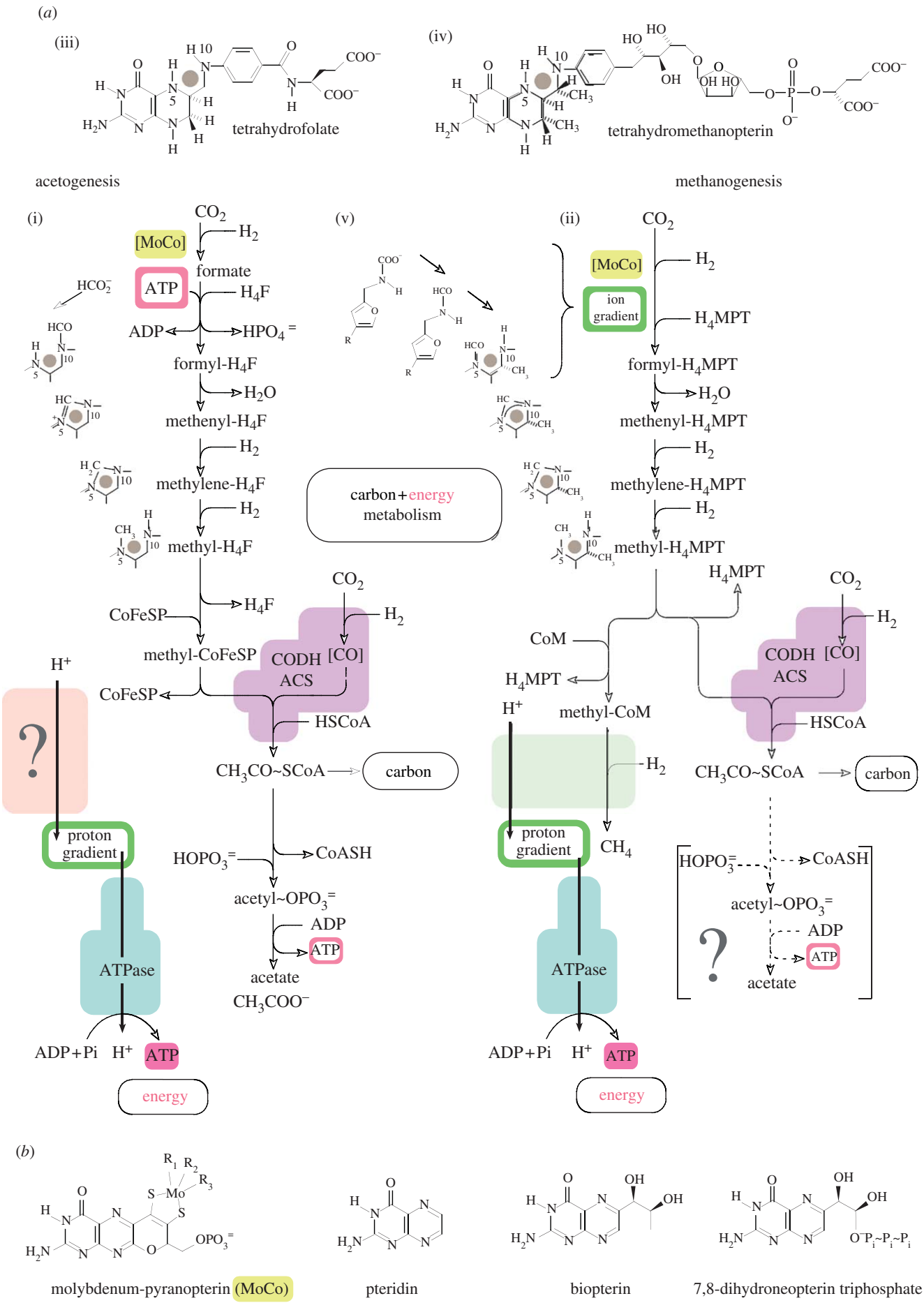


Figure 1. (*Caption opposite.*)

From the bottom-up (geochemical) perspective, hydrothermal vents provide a sustained source of chemical energy by virtue of the H_2/CO_2 chemical potential. Hydrothermal vent conditions would favour the synthesis of acetate in concentrations of approximately 5 mmol kg^{-1} at approximately 150°C (Shock 1992), also if hydrothermal fluid mixes with anoxic seawater at 40°C (Shock 1990; Shock *et al.* 1998). Furthermore, the microporous internal structure of hydrothermal vents provides a solution to the seemingly insurmountable problem of how it was possible to achieve sufficient concentrations of the organic building blocks of self-replicating systems so that anything like a self-replicating system could arise (Russell & Hall 1997; Martin & Russell 2003). This important issue of how life's chemical components could have achieved sufficient molarities to react is what de Duve (1991) has aptly termed the 'concentration problem'. Microporous internal structures at hydrothermal vents could, in principle, provide the concentrating mechanism needed at life's origin.

In previous papers, we have pursued the idea that life might have originated in structured iron monosulphide precipitates in an alkaline hydrothermal vent at a redox-, pH- and temperature gradient between sulphide-rich hydrothermal fluid and iron(II)-containing waters at the Hadean ocean floor (Russell *et al.* 1994; Russell & Hall 1997; Martin & Russell 2003). The naturally arising, three-dimensional compartmentation observed within fossilized seepage-site metal sulphide precipitates led to the idea that such inorganic compartments might have been the functional precursors of cell walls and membranes found in free-living prokaryotes (Russell & Hall 1997). The findings that FeS catalyses the synthesis of CH_3SH (Heinen & Lauwers 1996; Schulte & Rogers 2004) from CO_2 and H_2S, and that FeS and NiS together catalyse the synthesis of the thioester acetyl methyl sulphide from CO and methyl sulphide (Huber & Wächtershäuser 1997), furthermore suggested that analogous prebiotic syntheses could have occurred within such compartments at the vent. Compartmentation could have restrained the reaction products from diffusing into the ocean, providing sufficient concentrations of organic intermediates to allow something like a ribonucleic acid (RNA) world to arise. These naturally forming, catalytic-walled compartments could have housed the first self-replicating systems (Koonin & Martin 2005), with the precursors that support replication having been synthesized *in situ* geochemically and biogeochemically, and with FeS (and NiS) centres playing the decisive catalytic role. The universal ancestor of life so inferred would not have been a free-living cell, but confined instead to the naturally chemiosmotic, FeS compartments within which it arose (Martin & Russell 2003; Koonin & Martin 2005).

A hydrothermal origin (with concentration of the products from prebiotic syntheses catalysed by metal sulphides within naturally forming microcompartments at a warm, alkaline hydrothermal vent) is highly compatible with the view that the acetyl-CoA pathway of carbon fixation might be the most primitive biochemical pathway of CO_2 reduction (Fuchs & Stupperic 1985; Fuchs 1989). This is because, in addition to the features listed by Fuchs (1989) above, the acetyl-CoA pathway (i) is highly exergonic, (ii) involves no stereochemically defined intermediates, (iii) operates with very simple starting compounds (H_2, CO_2 and a thiol) that are present at hydrothermal vents (H_2 and thiols in the hydrothermal fluid, CO_2 in the ocean), (iv) involves many transition metal sulphide clusters as would also have been present at a hydrothermal vent, and (v) produces a highly reactive thioester (acetyl-CoA) as its initial product. This led to the notion that the hydrothermal mound in which life evolved might have produced acetate as a geochemical by-product before biochemistry got started (Russell & Martin 2004).

But the acetyl-CoA pathway needs a methyl group in order to operate, whose synthesis is (i) essential to produce the acetyl moiety and (ii) requires energy input in modern biochemistry (Thauer 1998; Maden 2000; Graham & White 2002; Müller 2003), which poses a bioenergetic problem in the context of autotrophic origins. Here, we consider the origin of the methyl group in modern manifestations of the acetyl-CoA pathway as it occurs in acetogens and methanogens and in terms of its prebiotic synthesis. We consider the entry of carbon, phosphate and nitrogen into metabolism and propose a simple core metabolic scheme that directly supplies the compounds from which the bases of RNA are synthesized in modern cells. We specify a curious bioenergetic problem related to the early synthesis of formyl pterins, to which the naturally chemiosmotic nature of alkaline vents could afford a possible solution.

2. ACETOGENESIS AND METHANOGENESIS: HOMOLOGOUS CHEMICAL CONVERSIONS

Acetogens and methanogens synthesize their ATP through the reduction of CO_2 with electrons that usually stem from H_2 and they employ the acetyl-CoA pathway. Since we will be exploring Fuchs's suggestion that the modern biochemistry of their CO_2 reduction might mirror the most ancient forms of carbon chemistry (Fuchs & Stupperich 1985; Fuchs 1989), it is necessary to briefly summarize acetogenic and methanogenic physiology.

Acetogens are organisms that reduce CO_2 to acetate via the Wood–Ljungdahl pathway (Ljungdahl 1994; Müller 2003), distinguishing them from organisms that produce acetate by other means. The overall design of the pathway is outlined in figure 1, which is modified from Müller (2003). It is a pathway of carbon and energy metabolism. One CO_2 is reduced to a cofactor-bound methyl group, formally via three hydride (H^-) transfers. This entails reduction of CO_2 to formate, an ATP-consuming step at the formyltetrahydrofolate (formyl-H_4F) synthetase reaction, two further reductions to methenyl-H_4F and methylene-H_4F, and methyl transfer to corrinoid iron–sulphur protein (CoFeSP) in the organisms termed Na^+ acetogens (Müller 2003) or, alternatively, a methyl transfer pathway involving cytochromes in the organisms termed H^+ acetogens (Müller 2003) that is not shown here.

The methyl group is donated to an enzyme that is crucial in the context of this paper, bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthase

(CODH/ACS), where it becomes bound to Ni at a Ni–Ni–[4Fe4S] cluster (the A cluster) of the enzyme (Doukov *et al.* 2002; Lindahl 2002; Darnault *et al.* 2003; Svetlitchnyi *et al.* 2004). At a different active site, a [Ni4Fe5S] cluster (the C cluster) of CODH/ACS reduces an additional CO_2 to CO by transfer of two electrons that typically stem from environmental H_2 via hydrogenases under autotrophic growth (CODH activity). CO from the C cluster traverses the enzyme and binds at the A cluster, presumably to the methyl-bearing Ni atom (Volbeda & Fontecilla-Camps 2006). The bound carbonyl is thought to be attacked by the methyl to yield a transition metal-bound acetyl moiety that is subsequently removed from the enzyme via thiolysis involving the free thiol (–SH) group of CoA (CoASH; Lindahl 2002; Svetlitchnyi *et al.* 2004; Volbeda & Fontecilla-Camps 2006). This releases acetyl-CoA.

Acetyl-CoA is a thioester. Thioesters are a very energy-rich class of compounds with high transfer potential to participate in other reactions (Lipmann 1941; de Duve 1991). Accordingly, the energy in the thioester bond can be used for many kinds of metabolic reactions. In a reaction catalysed by phosphotransacetylase (PAT), the acetyl moiety of acetyl-CoA is transferred to phosphate, producing another very energy-rich compound, the mixed anhydride acetyl phosphate. This in turn transfers the phosphoryl moiety to the β-phosphate of ADP, producing an energy-rich phosphoanhydride bond in ATP, a reaction catalysed by acetate kinase (ACK). Since one ATP is invested at the formyl-H_4F synthetase reaction and one ATP is gained at the ACK reaction, there is no net gain of ATP from this pathway yet.

The process of transferring electrons from H_2 to CO_2 to generate the methyl group involves one or more coupling sites that generate an ion gradient across the plasma membrane, although the precise coupling sites are not yet known (Müller 2003), as indicated with a question mark in figure 1. This chemiosmotic potential is harnessed by an ATPase (Na^+- or H^+ dependent, respectively) for net ATP synthesis. The overall acetogenic reaction is summarized as

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O, \quad (2.1)$$

with thermodynamic values of $\Delta G^{o}{}_{r} = -172.32$ and $-160.74\ \mathrm{kJ\ mol^{-1}}$ at 2 and 70°C, respectively (Amend & Shock 2001). At physiological conditions, the reaction is less exergonic

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 2H_2O, \quad (2.2)$$

with $\Delta G'_o = -104.6\ \mathrm{kJ\ mol^{-1}}$ (Thauer *et al.* 1977). The reaction to the level of the energy-rich thioester as written by Fuchs (1994)

$$2CO_2 + 8[H] + CoASH \rightarrow CH_3COSCoA + 3H_2O, \quad (2.3)$$

is exergonic with an estimated thermodynamic value of $\Delta G'_o = -59.2\ \mathrm{kJ\ mol^{-1}}$ if $2[H] = H_2$ and slightly endergonic with $\Delta G'_o = +13.2\ \mathrm{kJ\ mol^{-1}}$ if $2[H] = NADH$. The NADH reaction (slightly exergonic in the direction of CO_2) is not important here, but is one reason that the pathway is so versatile among microbes (Fuchs 1994; Zinder 1994).

Methanogens are organisms that produce methane from their core carbon and energy metabolism (Thauer 1998). The pathway of methanogenesis is summarized in figure 1, which is redrawn from Schönheit & Schäfer (1995). A cofactor-bound methyl group is synthesized from CO_2 via three reduction steps. The methyl-producing part of the pathway looks similar in overall design to acetogenesis, but—importantly—the enzymes of methyl synthesis in methanogens share no sequence similarity to those of acetogenesis that we could detect by database searching, and none has been reported in the literature. The pathway entails carbamate formation, carbon transfer and reduction to a formyl group via formylmethanofuran (formyl-MF) dehydrogenase, transfer of the formyl group to tetrahydromethanopterin (H_4MPT) via formyl-MF : H_4MPT formyl transferase, cyclization to form methenyl-H_4MPT and two further reductions to form methylene-H_4MPT and methyl-H_4MPT. This methyl group can go two ways: ATP synthesis for energy metabolism or acetyl-CoA synthesis for carbon metabolism. For ATP synthesis, the methyl group is transferred to coenzyme M and is ultimately reduced to yield CH_4, via reactions involving heterodisulphide reductase and methyl-CoM reductase (Ermler *et al.* 1997). Methane production itself is not important here, except that these terminal steps of methyl reduction to CH_4 involve ion pumping, generation of a proton gradient and harnessing of that chemiosmotic potential via an ATPase for net ATP gain (Thauer 1998) and furthermore involve cofactors (coenzymes B and M, see figure 2) that are always present in methanogens (Thauer 1998; Graham & White 2002) but not widely distributed among other prokaryotes (Chistoserdova *et al.* 2004). The overall methanogenic reaction for chemiosmotic ATP synthesis is summarized as

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O, \quad (2.4)$$

with $\Delta G'_o = -131\ \mathrm{kJ\ mol^{-1}}$ (Thauer 1998).

For carbon production in methanogenesis, the methyl group in methyl-H_4MPT is transferred to CODH/ACS as in the acetogens, and these enzymes (CODH/ACS) are related at the level of sequence similarity across acetogens and methanogens, although the family tree of CODH/ACS enzymes and their sequence families is complicated (Lindahl 2002). For the purposes of this paper, details of sequence similarity beyond its presence or absence are irrelevant. It is sufficient that their CODH/ACS enzymes share a common ancestor and to assume that the synthesis of CO as well as the final production of acetyl-CoA at CODH/ACS in methanogens probably follows similar mechanisms as in the acetogens. Here, more important than sequence similarity is the abundance of transition metal sulphide clusters in proteins associated with acetogenesis and methanogenesis. To illustrate this point for some of the methanogen proteins, formyl-MF dehydrogenase from *Methanosarcina* contains a subunit with eight [4Fe–4S] centres (Vorholt *et al.* 1996), the *eha* operon encoding energy converting FeNi hydrogenase (Ech) of *Methanobacterium thermoautotrophicum* encodes proteins with 6 [4Fe–4S] and 10 [4Fe–4S] centres, while a second operon for Ech (*ehb*) encodes a

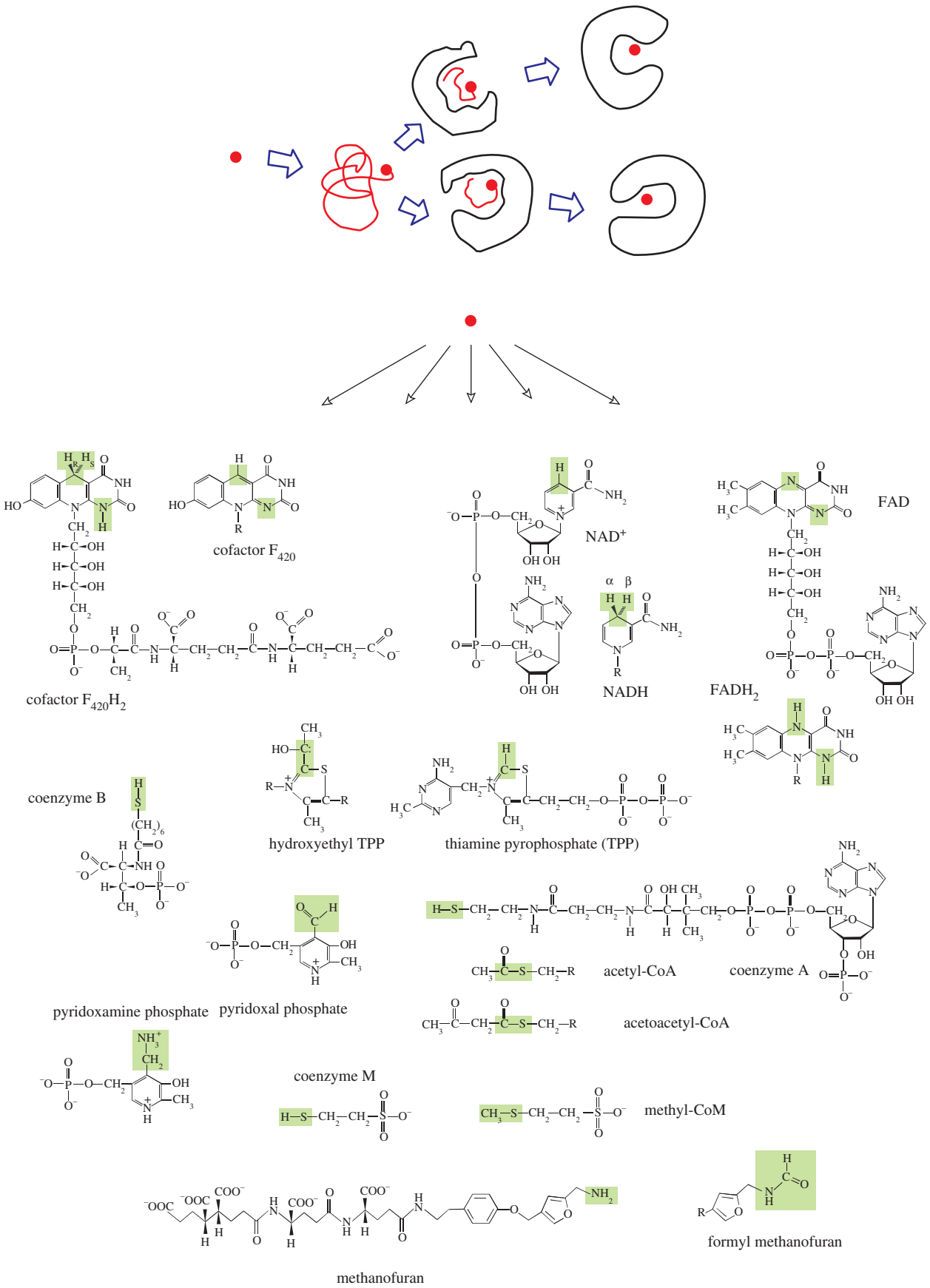


Figure 2. The RNA world in early formulations (White 1976) as discussed by Penny (2005). This envisages RNA-like cofactors performing essential catalyses early, with continued utility as part of larger RNA molecules, and with the original RNA scaffold of the ribozyme being replaced piece-by-piece by proteins over evolutionary time, sometimes more than once independently, with the cofactor still doing the catalytic job, but with better positioning of substrates and intermediates within a handed catalytic site of a protein. Some cofactors representing the red dot are redrawn from Graham & White (2002) and Stryer (1975). Note the conspicuous absence of chiral centres in the catalytically active moieties of the cofactors. NAD, nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide.

protein with 14 [4Fe–4S] centres (Tersteegen & Hedderich 1999); Ech is involved in the formyl-MF dehydrogenase reaction (Hedderich 2004). Some methanogen hydrogenases bear no FeS clusters, but use an $Fe(CO)_2$ centre instead (Lyon *et al.* 2004).

In summary, so far acetogenesis and methanogenesis are of similar design, share related enzymes at the thioester-producing step (CODH/ACS), but share nothing in common in the methyl pathway at the protein level (Fuchs 1994; Maden 2000). The cofactors involved (H_4F and H_4MPT) are very similar in structure (Thauer 1998; Maden 2000), as are the carbon intermediates on their way from the level of a formyl moiety to a methyl moiety as shown in figure 1*a*, as redrawn from Thauer (1998) and Maden (2000). Both H_4F and H_4MPT are pterins, but their functional moieties differ in important details. Thauer (1998, p. 2383) formulated it as follows (as corrected with reference to Thauer *et al.* 1996):

> The functionally most important difference between H_4MPT and H_4F is that H_4MPT has an electron-donating methylene group conjugated to N^{10} via the aromatic ring whereas H_4F has an electron-withdrawing carbonyl group in this position. As a consequence, the redox potential $E'_o = -390$ mV of the N^5,N^{10}-methenyl-H_4MPT/N^5,N^{10}-methylene-H_4MPT couple is by 90 mV more negative than the $E'_o = -300$ mV of the N^5,N^{10}-methenyl-H_4F/N^5,N^{10}-methylene-H_4F couple and the redox potential $E'_o = -320$ mV of the N^5,N^{10}-methylene-H_4MPT/N^5,N^{10}-methyl-H_4MPT couple is by 120 mV more negative than the $E'_o = -200$ mV of the N^5,N^{10}-methylene-H_4F/N^5,N^{10}-methyl-H_4F couple.

This means that methanogens can do a bit more additional biochemical work at the methenyl to methylene conversion step, by virtue of using MPT. Other examples of pterins are shown in figure 1*b*, including the molybdenum cofactor (MoCo), which is required in the methyl branch of both the pathways to get from CO_2 to the level of the formyl pterin: formate dehydrogenase (FDH) in the case of the acetogens and formyl-MF dehydrogenase in the case of the methanogens.

Methanogens usually do not produce acetate via PAT and ACK. An exception is *Methanosarcina acetivorans* C2A, which will grow and make substantial amounts of acetate when supplied with a diet of CO, rather than CO_2 and H_2 (Rother & Metcalf 2004). The physiology (termed carboxidotrophic acetogenesis) is not yet known in detail, but it does involve both PAT and ACK, because growth on CO with acetate production was blocked in genetically engineered PAT and ACK knockouts (Rother & Metcalf 2004). Until the report by Rother & Metcalf (but see also Bock & Schönheit 1995), acetogenesis had been a pathway unique to the eubacteria; its CO-dependent manifestation among methanogens has been suggested to indicate an ancient nature of acetogenesis (Ferry & House 2006).

3. CARBON DIOXIDE, THEN COFACTORS, THEN THE RNA WORLD

The RNA world (Gilbert 1986; Joyce 2002) is an essential element of any modern theory about early evolution, because it provides a conceptual framework for thinking about Darwinian selection among self-replicating molecules prior to the advent of fully fledged cells. Our proposal will also entail the existence of self-replicating and catalytic RNA molecules, just as proponents of the RNA world would see it (Penny 2005). However, the details of RNA self-replication are of no immediate importance here. Instead, we are concerned about the origin of the bases that make up the RNA world and, more specifically, about the origin of the reduced carbon and nitrogen species from which they were synthesized abiotically.

We embrace an important concept that existed even before the idea of an RNA world (Gilbert 1986; Joyce 2002) had been formulated, namely that the bases of nucleic acid enzymes themselves had catalytic ability and that they were by-and-by replaced by protein after the onset of translation (figure 2). As White (1976, p. 101) put it:

> A metabolic system composed of nucleic acid enzymes is proposed to have existed prior to the evolution of ribosomal protein synthesis. Vestiges of these nucleic acid enzymes persist in contemporary enzymes. This proposal rationalizes the fact that many coenzymes are nucleotides or heterocyclic bases which could be derived from nucleotides.

Under this view, ribozyme-catalysed reactions that required functional moieties lacking in the canonical bases G, A, C or U employed a different active base (a cofactor) for catalysis, and the utilization of such cofactors by some modern proteins can be viewed as a holdover from the RNA world (White 1976). Examples are the thiazole ring in thiamine pyrophosphate (TPP) for C2 transfers and a myriad of other reactions (Pohl *et al.* 2004), the hydride transfer potential of nicotinamide adenine dinucleotide (NAD^+), the one-electron-to-two electron transducing potential of flavin adenine dinucleotide (FAD), the transamination catalysis provided by pyridoxal phosphate (PLP) or other cofactors as outlined in figure 2. In modern biochemistry, many RNA molecules do indeed fold so as to bind various cofactors and other small molecules with exquisite specificity—riboswitches (Serganov *et al.* 2006; Montange & Batey 2006)—consistent with the idea of an RNA world.

In line with White's (1976) reasoning, a central aspect of our proposal will be that (i) methyl synthesis in methanogens and acetogens is chemically homologous (related by a common ancestral chemistry), even though the corresponding proteins in the modern pathways share no sequence similarity, (ii) the protein-aided pathway was preceded by a simpler pathway involving the pterin cofactors but without proteins, and (iii) it was, in turn, preceded by a pathway of methyl synthesis that operated spontaneously at a hydrothermal vent without pterins, using inorganic catalysts only. Hence, we view the pterins H_4F and H_4MPT (figure 1*a*) as RNA-related bases and as important intermediates in the evolutionary transition from inorganic-catalysed to protein-aided methyl synthesis.

4. AN INORGANIC START: HOW, AND WHERE, TO REDUCE CO_2?

In search of a prebiotic analogue of the methyl-generating branch of the acetyl-CoA pathway, operating with

inorganic chemistry only, and starting from CO_2, the experimental findings of Heinen & Lauwers (1996) are crucial. They were able to synthesize various small organic molecules from CO_2 and H_2S, including CH_3SH, using only FeS as the catalyst under rather mild laboratory conditions (50–100°C) in a few hours to a few days; an example of inorganically catalysed CO_2 reduction. Importantly, the chemical mechanism involved in the Heinen & Lauwers (1996) reaction is not understood. The direct transfer of electrons to CO_2 to produce formate—the first reduced carbon intermediate en route to methyl synthesis—is an extremely difficult chemical reaction that is not readily catalysed, even by FeS (Schoonen *et al.* 1999; p. 21 ff). This is because CO_2 is a very stable molecule that is unwilling to accept a hydride ion (H^-) to become formate ($HCOO^-$) if either H_2 or FeS minerals are the electron donors (Schoonen *et al.* 1999). The issue is whether the energies of the electrons in the lowest occupied molecular orbital in the electron donor (an FeS mineral in this case) and the highest unoccupied molecular orbitals of the electron acceptor (CO_2) overlap or not. Formate is the first critical intermediate towards the kinds of reduced carbon species as found in biochemistry. Formate (and the formyl group) is also the first critical intermediate in the methyl branch of the Wood–Ljungdahl pathway. Thus, we are obliged to suggest a plausible route for the production of formate that could operate under warm hydrothermal conditions.

In an alkaline hydrothermal system, there are two distinct times and places where the hardest chemical step—the initial reduction of CO_2—could, in principle, occur through (i) equilibrium processes when CO_2 reaches vent–ocean interface or (ii) serpentinization processes when the hydrothermal fluid is located deep in the crust. These different geochemical environments offer different kinds of conditions: the former is rich in transition metal sulphides, while the latter is not, but has the kinetic advantage of slightly higher temperature, and we need to consider both.

First, we consider the metal sulphide-rich vent–ocean interface. Schoonen *et al.* (1999) concluded that a possible explanation for the results reported by Heinen & Lauwers (1996) and for the results reported by Huber & Wächtershäuser (1997) could involve carbonyl sulphide (O=C=S, typically written as COS) as the critical intermediate in the carbon reduction process. COS is much more prone to accept electrons than CO_2 (Schoonen *et al.* 1999). Its synthesis can occur according to the reaction

$$CO_2 + H_2S \rightarrow COS + H_2O, \tag{4.1}$$

as suggested by Heinen & Lauwers (1996), who reported the occurrence of COS among their reaction products. The synthesis of COS could, in principle, occur under rather mild vent-like conditions involving metal sulphides where hydrogen sulphide interfaces with the CO_2-containing ocean. Schoonen *et al.* (1999) noted that some of the crucial thermodynamic values for COS and a related compound, carbon disulphide (CS_2) are lacking; hence, the thermodynamic values for reactions involving these compounds are not given. Reaction (4.1) does not involve redox chemistry, but when it comes to donating electrons to carbon, Schoonen *et al.* (1999, p. 28) surmise:

> A more facile electron transfer from pyrrhotite [note: an FeS mineral of the type Fe_7S_8] to either COS or CS_2 than to CO_2 means that the reaction pathway via C–S compounds is favoured over a pathway involving the reduction of CO_2 to HCOOH.

The reduction of COS would produce CO and sulphide. Schoonen *et al.* (1999) do not suggest a process by which this would occur. A possible solution to this problem is provided by an interesting side activity of CODH, an enzyme at the core of our considerations. Ensign (1995) reported that CODH from *Rhodospirillum rubrum*, which usually reduces CO_2 to CO under normal physiological conditions, very efficiently reduces COS to CO with electrons provided by dithionite, titanium(III) citrate or methyl viologen. The reaction characterized by Ensign (1995) is summarized as

$$COS + 2e^- + 2H^+ \rightarrow CO + H_2S. \tag{4.2}$$

The results of Ensign (1995) provide no cause to suspect that an active site other than the CO_2-reducing [$4FeNiS_5$] C-cluster of CODH is involved in this reaction. By analogy, we suggest that a very similar FeS-catalysed reaction might have occurred in the Heinen & Lauwers (1996) experiment (and hence could occur under hydrothermal vent conditions as well). In line with this suggestion, Seefeldt *et al.* (1995) reported that nitrogenase will also reduce COS to CO by reaction (4.2) and proposed a reaction mechanism. The active site of nitrogenase, which contains an [Fe_7MoS_9] cluster similar to a twin of the mineral greigite (Russell & Hall 2006), was implicated in the reaction mechanism (Seefeldt *et al.* 1995, 2004), suggesting that COS reduction to CO can be catalysed by transition metal sulphides. The hydration of CO produces formate (Schoonen *et al.* 1999):

$$CO + H_2O \rightarrow HCOOH. \tag{4.3}$$

The sum of reactions (4.1)–(4.3) is

$$CO_2 + 2e^- + 2H^+ \rightarrow HCOOH, \tag{4.4}$$

which constitutes a suggestion for the initial process of CO_2 reduction at the vent–ocean interface and in the reactions observed by Heinen & Lauwers (1996). The hydrogenase side activity of CODH (Menon & Ragsdale 2000) is also quite compatible with these considerations. We would assume the source of the electrons to be hydrothermal H_2. The thermodynamic value for the reaction written as

$$CO_2 + H_2 \rightarrow HCOOH, \tag{4.5}$$

has been calculated as slightly endergonic under certain conditions (Schoonen *et al.* 1999). However, the reaction involving bicarbonate and H_2 in the gaseous phase at pH 7 and 25°C is given as

$$HCO_3^- + H_2 \rightarrow HCOO^- + H_2O, \tag{4.6}$$

with $\Delta G_o' = -1.3$ kJ mol^{-1} (Thauer *et al.* 1977). Based on data from Amend & Shock (2001), Volbeda & Fontecilla-Camps (2006) have recently recalculated the

thermodynamic values for several reactions involving simple carbon compounds under slightly different conditions. Assuming the gases to be dissolved and at an activity of 1 mol kg^{-1} (requiring high pressure, which is present at a submarine hydrothermal vent), they estimate the reaction written as

$$CO_2 + H_2 \rightarrow HCOO^- + H^+, \tag{4.7}$$

to be considerably more exergonic, with $\Delta G_o' = -22.5$ kJ mol^{-1} (Volbeda & Fontecilla-Camps 2006). Yet, even assuming much lower reactant concentrations ($[CO_2] = 5.6$ mM, $[H_2] = 0.5$ mM and $[HCOO^-] = 1$ mM), at pH 7, 25°C and 1 bar, the thermodynamic value for equation (4.7) remains favourable with $\Delta G_o' = -7.9$ kJ mol^{-1} (Volbeda & Fontecilla-Camps 2006).

Hence, the reduction of CO_2 with H_2 to formate is not an insurmountable task in thermodynamic terms, but the route taken to get there is important, owing to the kinetic stability of CO_2. Luther (2004) has recently reported findings relevant to the issue, at least as far as the possible role of COS is concerned, by investigating in more detail the molecular orbital-dependent kinetics of COS reactivity, with regard to the experiment by Heinen & Lauwers (1996) and with regard to Schoonen *et al.*'s (1999) results. The results (Luther 2004) indicate that the lowest unoccupied molecular orbital for COS is a σ^* orbital, which, when filled, can readily break the C–S bond, releasing sulphide, and furthermore that the solid phase FeS should be able to activate or bond with the carbon atom in CO_2 so that the organic compounds could be produced under hydrothermal vent conditions. Luther (2004) writes the Lewis structure for COS as either O=C=S or O≡C–S. At the vent–ocean interface, H_2S would be interfacing with marine CO_2, so that the synthesis of COS at that site could be conceivable, although COS is extremely unstable under alkaline conditions (Rhodes *et al.* 2000).

Next, we consider serpentinization processes as the vent's deeper waters pass through the crust as a means of CO_2 reduction. Serpentinization is a geochemically very familiar process through which the magnesium–iron silicates that comprise the oceanic crust are hydrolysed and oxidized by ocean water in the downdraft of hydrothermal systems to produce magnetite (Fe_3O_4) and reduced alkaline hydrothermal fluid (Macleod *et al.* 1994; Palandri & Reed 2004; Schulte *et al.* 2006; Bach *et al.* 2006). The serpentinization reaction of olivine (magnesium–iron silicate) and water to serpentinite and iron-rich brucite, with further reaction of the latter with aqueous silica to serpentinite, magnetite and hydrogen, summarized by Bach *et al.* (2006) as

$$2Mg_{1.8}Fe_{0.2}SiO_4 + 3H_2O \rightarrow Mg_{2.85}Fe_{0.15}Si_2O_5(OH)_4 + Mg_{0.75}Fe_{0.25}(OH)_2, \tag{4.8}$$

$$57Mg_{0.75}Fe_{0.25}(OH)_2 + 30SiO_2(aq) \rightarrow 15Mg_{2.85}Fe_{0.15}Si_2O_5(OH)_4 + 23H_2O + 4Fe_3O_4 + 4H_2, \tag{4.9}$$

is an abundant source of geological reducing power. These reactions occur at depths of roughly 2–8 km under the ocean floor and at temperatures between approximately 80 and 200°C. Serpentinization is the source of H_2 in the fluid of submarine hydrothermal systems (Sleep *et al.* 2004). During the serpentinization reaction, Fe^{2+} reduces H_2O, yielding Fe^{3+} (in magnetite) and H_2. This process is quantitatively significant in geochemistry: 1 m^3 of olivine yields 500 mol of H_2 (Schulte *et al.* 2006) during serpentinization. Most of the oceanic crust consists of magnesium and iron silicates like olivine.

The water in hydrothermal fluid is not produced *de novo* in the crust; instead, it is drawn from the ocean deep into the crust in a convective current that resurfaces at hydrothermal vents (Shock 1992). Dinitrogen (N_2) dissolved in ocean water that is drawn into such a hydrothermal system is, like H_2O, also reduced and hence emerges at the vent to some extent as NH_3, for example, at calculated concentrations of up to approximately 40 mM in the kind of alkaline hydrothermal system (cf. path 8 in Shock 1992) at the seat of our considerations. Similarly, CO_2 that is dissolved in ocean water is also drawn into such a hydrothermal system and is, like H_2O and N_2, also reduced by this geochemical process.

On the global scale, Shock (1992) has estimated that early hydrothermal systems may have produced about 200 000 tonnes of reduced carbon species per year from CO_2. (Note that hydrothermal carbon flux reaches the ocean through specific points on the oceanic crust, vents, as opposed to raining down in a dispersed manner through the atmosphere.) The thermodynamic and experimental studies of McCollom & Seewald (2003) indicate that starting with 20 mM CO_2 in the presence of 40 mM H_2 at 350 bar (corresponding to about 3.5 km depth) and 175°C, the equilibrium concentration of formate in modern hydrothermal systems should be about 15 mM between pH 7 and 9. For the alkaline Lost City hydrothermal fluid (Kelley *et al.* 2001, 2005), formate was apparently not analysed, but because Lost City is hosted by serpentinizing rock, the prediction from thermodynamics (McCollom & Seewald 2003) is that formate should exist in the effluent. More recent studies of CO_2 reduction under hydrothermal conditions (Seewald *et al.* 2006), but in the absence of sulphur species, indicate that at 150°C and pH 6–10, the H_2-dependent reduction of CO_2 should proceed readily, yielding mainly formate and minor amounts of methanol.

However, when sulphur is included in thermodynamic simulations of H_2/CO_2 equilibria in hydrothermal systems, the results indicate that millimolar—or even greater—concentrations of methyl sulphide (CH_3SH, methanethiol) are expected in hydrothermal fluid (Schulte & Rogers 2004). Furthermore, the presence of CO (or its equilibrium product formate; M. Schulte 2006, personal communication) in such simulations leads to concentrations of methyl sulphide that are increased by orders of magnitude relative to CO_2-dependent concentrations (Schulte & Rogers 2004). Of course, the hydrothermal system that we assume would contain sulphide-bearing water (Russell & Hall 1997), because sulphide deposits are common in rocks of the

Early Archaean similar to those that would have hosted it (Lesher & Stone 1997).

With the present interest in alkaline hydrothermal vents as a possible starting point for the origin of biochemistry, Volbeda & Fontecilla-Camps (2006) have recently calculated the thermodynamic values for several relevant reactions involving carbon and sulphur species in the presence of H_2 (derived from serpentinization), including

$$CO_2 + H_2S + H_2 \rightarrow CH_3SH + 2H_2O, \quad (4.10)$$

with a notable $\Delta G'_o = -121.3\ \mathrm{kJ\ mol^{-1}}$.

Thus, given the reducing power of the hydrothermal system through serpentinization at depth and the thermodynamic studies of CO_2, H_2, formate and sulphur in hydrothermal systems discussed in this section, it appears that the hydrothermal fluid emerging at the vent–ocean interface should bear some formate and some methyl sulphide. This would not exclude the chemistry considered for the case that CO_2 reduction occurred primarily at the vent–ocean interface. The combination of transition metal sulphide-catalysed CO_2 reduction at the ocean–vent interface, for which the Heinen & Lauwers (1996) experiment serves as an experimental example, and reduced carbon species in the exhalate, derived during serpentinization (as with H_2 and NH_3), are not mutually exclusive.

Since CO is a crucial intermediate of the acetyl-CoA pathway, it assumes an important role in our present considerations, consistent with other present views on early bioenergetics (Ferry & House 2006). However, we would favour its delivery via hydrothermal formate, because formate, rather than CO, would appear to be the thermodynamically favoured species:

$$CO + H_2O \rightarrow HCOO^- + H^+, \quad (4.11)$$

with $\Delta G'_o = -33.6\ \mathrm{kJ\ mol^{-1}}$ for 1 M activity of the dissolved gas (Volbeda & Fontecilla-Camps 2006). Indeed, the source and amount of CO under early Earth conditions are presently debated. There have been recent suggestions that the early atmosphere may have contained substantial amounts of CO, and such atmospheric CO may have significantly contributed to organic synthesis (Miyakawa *et al.* 2002). There are microorganisms that can live anaerobically with CO as their sole source of carbon and energy (Ragsdale 2004), such as the Gram positive eubacterium *Carboxydothermus hydrogenoformans* (Svetlitchnyi *et al.* 2004). The physiology of *Carboxydothermus* is called hydrogenogenesis; the organism generates its energy from the reaction of CO with H_2O to produce CO_2 and H_2 with $\Delta G'_o = -20\ \mathrm{kJ\ mol^{-1}}$ (Soboh *et al.* 2002). Like acetogens and methanogens, it uses the Wood–Ljungdahl pathway (Wu *et al.* 2005). Organisms such as *C. hydrogenoformans* unite at least as many suspectedly primitive chemical traits as do acetogens and methanogens. But the hydrogenogenic reaction does not stand in the foreground here, because it generates CO_2 and H_2 as its end products, whereas at the origin of biochemistry, the synthesis of reduced carbon compounds is the issue.

Comets also can contain CO, in addition to other reduced carbon compounds (Povich *et al.* 2003). But on the very early Earth, there was no crust that was exposed to the atmosphere, but only ocean (Russell & Arndt 2005). Hence, whatever delivery of organic material to the early Earth that occurred by comets, interplanetary dust, meteorites or atmospheric chemistry would provide a very dilute solution of organics free to react in the oceans, and the concentration problem associated with getting those molecules to react would remain.

To summarize this section, two main paths stand in the foreground for initial CO_2 reduction in early alkaline hydrothermal systems: a COS-mediated process at the vent–ocean interface and the subsurface synthesis of formate and methyl sulphide that is delivered via hydrothermal fluid to the vent–ocean interface. Both processes would depend upon serpentinization as the source of reducing power. The paths are mutually non-exclusive. One further point concerning vents is important to keep in mind: even today, the entire volume of the global ocean is recycled through the sea floor via hydrothermal vents every approximately 100 000 years (Fisher 2005). On the early Earth, the widespread activity of hydrothermal systems would have surpassed the present rate. Hence, virtually all organic substances ever delivered to the ocean from space (Povich *et al.* 2003) or from syntheses in the atmosphere (Kasting & Brown 1998) will have been recycled through hydrothermal systems in some manner. The hydrothermal fluid at alkaline vents will have contained such compounds and/or their reaction products from higher temperature serpentinization processes, and such compounds could have been concentrated in microporous compartments at the vent. But concentration alone is not enough. Chemical reactions must take place, and the redox potential of such reduced carbon species with the H_2 couple could only be lower than that of marine CO_2. Hence, contributions of exogenous-reduced carbon to the origin of microbial physiology, as compared to *de novo* hydrothermal carbon reduction entailing the vast excess marine CO_2 supply, can probably be neglected. The serpentinization reaction represents an excellent source of sustained reducing power for early (bio)chemical reactions.

5. ACETYL PHOSPHATE, THE ORIGINAL PHOSPHORYL DONOR

From the foregoing section, it is reasonable to assume CH_3SH to be present at the vent, in addition to some CO from formate or COS. Following Huber & Wächtershäuser (1997), the transition metal sulphide-catalysed synthesis of acetyl thioesters should be possible too, since they were able to obtain substantial amounts of acetyl methyl sulphide (CH_3COSCH_3) from CH_3SH and CO in the presence of FeS, Fe/NiS or NiS (although a transition metal alone, $NiSO_4$, without sulphide, was an equally efficient catalyst). Assuming that the vent generates acetate as a stable waste product (Russell & Martin 2004), what happens with the energy difference en route from the acetyl thioester to free acetate? In a slightly different context, de Duve (1991) proposed that in addition to hydrolysis of the thioester bond, phosphorolysis was possible too, leading to the

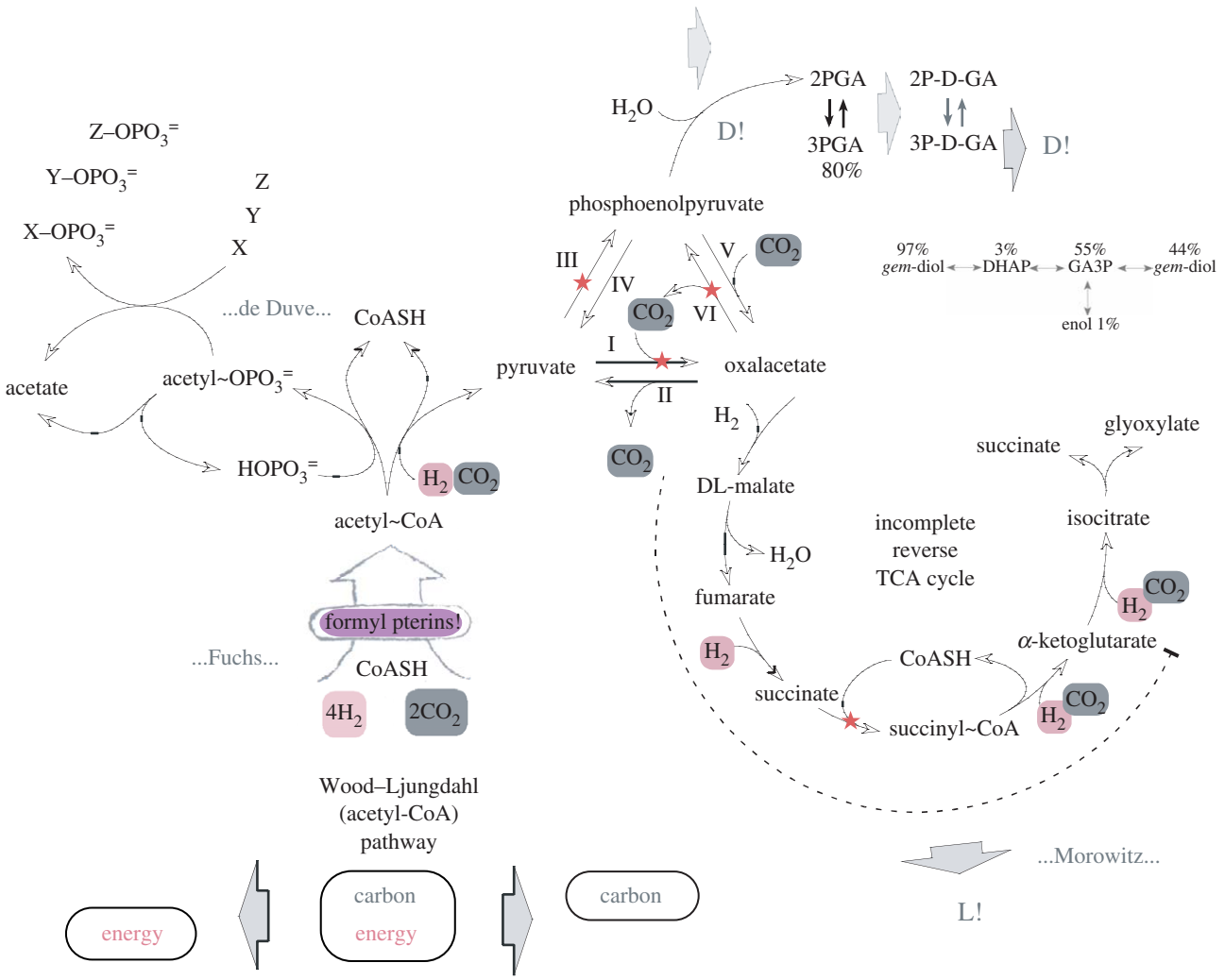


Figure 3. Schematic summary of some core reactions of carbon and energy metabolism relevant to modern biochemistry and possibly relevant to prebiotic chemistry. The names of the enzymes that catalyse the reactions labelled as I–VI interconnecting pyruvate, PEP and oxalacetate, as modified from O'Brien *et al.* (1977), are: I, pyruvate carboxylase; II, oxalacetate decarboxylase; III, PEP synthase (or alternatively pyruvate:orthophosphate dikinase); IV, pyruvate kinase (or alternatively pyruvate:orthophosphate dikinase); V, PEP carboxylase; VI, PEP carboxykinase. Reactions that are coupled to phosphoanhydride hydrolysis in GTP (or ATP) in the modern enzymatic reactions are labelled with a small red star. The exclamation point at formyl pterin involvement points to the bioenergetic problem of energy investment at the formyl pterin synthesis step of the Wood–Ljungdahl pathway as shown in figure 1 and as elaborated in §16. The 'D!' and 'L!' symbols indicate the points at which product chirality sets in among organisms that use the Wood–Ljungdahl pathway (see text). The references to Fuchs, de Duve and Morowitz in the figure underscore that we are merging mutually compatible aspects concerning early biochemistry that they have stressed previously into a common framework. The split from combined energy and carbon metabolism involving thioester formation from CO_2 and H_2 into committed energy and carbon metabolism for early biochemical evolution, both starting from the thioester acetyl-CoA (and simpler thiol analogues, such as acetyl methyl sulphide) is indicated (see text). PGA, phosphoglycerate; TCA cycle, tricarboxylic acid cycle. X, Y and Z indicate any substrate that can be phosphorylated by the acyl phosphate bond in acetyl phosphate. The isocitrate and glyoxylate steps in the modern incomplete reverse TCA cycle are apparently missing (see text). The box at right indicates aqueous equilibria in the triose phosphate system (Noltmann 1972).

(non-enzymatic, prebiotic) formation of acetyl phosphate and the free thiol. Specifically, he suggested (de Duve 1991, pp. 153–154):

> In substrate level phosphorylations, the thioesters synthesized oxidatively are attacked phosphorolytically by inorganic substrates, giving rise to the corresponding acyl phosphates: $R'SCOR + H_2PO_4^- \leftrightarrow R'SH + RCOPO_4H^-$ [...] the phosphorolysis of acetyl-coenzyme A to acetyl-phosphate represents this reaction. I submit that a reaction of this type may have signalled the primeval entry of inorganic phosphate into the fabric of life. Utilizing the energy stored in the thioester bond, it would have served to activate the phosphate ion into a highly reactive, energy rich, acyl-bound phosphoryl group. [...] It is conceivable that the same reaction occurred prebiotically with inorganic phosphate as the attacking agent and thereby gave rise to inorganic pyrophosphate, which, we have seen, very likely acted as the primitive carrier of high-energy phosphoryl groups before ATP became available.

The synthesis of acetyl phosphate, and its phosphorylation of various compounds X, Y and Z, releasing acetate, is shown in figure 3. With few exceptions (Ferry & House 2006), de Duve's (1991) suggestion for central role of acetyl phosphate at the very beginning of metabolism (whereby he assumed

Table 2. Approximate free energy of hydrolysis for the energy-rich bonds in some relevant compounds. (Values from Lehninger (1975)[a], Thauer *et al.* (1977)[b], Buckel & Eggerer (1965)[c] and Frey & Arabashi (1995)[d].)

PEP[a]	$\Delta G_o' = -62$ kJ mol^{-1}
1,3-bisphosphoglycerate[b]	$\Delta G_o' = -52$ kJ mol^{-1}
acetyl phosphate[a]	$\Delta G_o' = -43$ kJ mol^{-1}
carbamoyl phosphate[b]	$\Delta G_o' = -39$ kJ mol^{-1}
acetyl-CoA[c]	$\Delta G_o' = -32$ kJ mol^{-1}
ATP (to ADP)[a]	$\Delta G_o' = -31$ kJ mol^{-1}
glucose-1-phosphate[a]	$\Delta G_o' = -21$ kJ mol^{-1}
inorganic pyrophosphate[d]	$\Delta G_o' = -20$ kJ mol^{-1}
glucose-6-phosphate[a]	$\Delta G_o' = -14$ kJ mol^{-1}

Fe^{3+}-dependent glucose oxidations rather than CO_2 and H_2 as the thioester source) has received little attention in the literature. Acetyl phosphate is an excellent candidate source of primordial 'metabolic' energy: chemically much simpler than ATP and with higher phosphorylating potential (table 2).

6. CARBON METABOLISM, FIXING ONE MORE CO_2

The Wood–Ljungdahl pathway consists of two processes: (i) $H_2 + CO_2 \rightarrow$ methyl and (ii) methyl $+ CO_2 + H_2 +$ thiol $\rightarrow$ acetyl thioester. The assimilation of reduced carbon and harnessable chemical energy are united in the same compound, a thioester. At the next step in carbon assimilation in acetogens and methanogens, catalysed by pyruvate synthase, also called pyruvate:ferredoxin oxidoreductase (Fuchs 1989; Schönheit & Schäfer 1995; Furdui & Ragsdale 2000), a thioester's energy is spent, together with reducing potential from H_2, to incorporate one more CO_2, yielding pyruvate. At the acetyl phosphate step, the same thioester's energy is spent for the synthesis of energy currency. The split from the thioester towards acetyl phosphate and pyruvate in modern acetogen metabolism might correspond to an extremely ancient branch point in biochemistry, from coupled energy and carbon metabolism into committed energy metabolism and committed carbon metabolism, respectively, as sketched in figure 3.

The activation energy required to fix CO_2 at the pyruvate synthase step is supplied in part by the thioester hydrolysis and by the reducing power of H_2. The electrons for pyruvate synthase are supplied in the modern organisms via ferredoxin, although pyruvate synthase itself exhibits a hydrogenase side activity (Menon & Ragsdale 2000). The pyruvate synthase step is a paradigm for the biochemical utility of thioesters (de Duve 1988). The Wood–Ljungdahl pathway and the subsequent pyruvate synthase step constitute a unit, with pyruvate as a main intermediate product of carbon assimilation (Fuchs 1989; Furdui & Ragsdale 2000; Hügler *et al.* 2003). The reaction written as

$$5H_2 + 2H^+ + 3HCO_3^- \rightarrow \text{pyruvate}^- + 6H_2O, \qquad (6.1)$$

is exergonic with $\Delta G_o' = -57.3$ kJ mol^{-1} (Thauer *et al.* 1977). The reaction occurs in acetogens and methanogens and involves the participation of only two organic cofactors (a pterin and thiamine, allowing homology of CoA to a simple thiol) and many inorganic cofactors, such as Fe_2S_2, Fe_4S_4, Fe_4NiS_5, and other centres in the enzymes of the modern pathways (Baymann *et al.* 2003; Seefeldt *et al.* 2004; Volbeda & Fontecilla-Camps 2006). Notably, the methanogen version of the pathway from CO_2 to pyruvate does not involve a single phosphate-dependent reaction (but does involve chemiosmosis), while the acetogen version involves the hydrolysis of only one phosphoanhydride bond (which is generated by chemiosmosis) during methyl synthesis (figure 1). Pyruvate has been synthesized non-enzymatically under (somewhat too hot, in our view) hydrothermal vent conditions in the laboratory (Cody *et al.* 2000).

7. THE TRICARBOXYLIC ACID (KREBS) CYCLE: FORWARD, REVERSE AND INCOMPLETE REVERSE

The reverse (or reductive) tricarboxylic acid (TCA; table 1) cycle has long stood in the foreground of thinking about autotrophic origins (Hartman 1975), and it has favourable thermodynamics when H_2 is the source of reductant (Smith & Morowitz 2004). More heavily debated is the view that CO_2 fixation via the reductive TCA cycle was coupled to the synthesis of pyrite as a pulling reaction (Wächtershäuser 1990, 1998). The synthesis of pyrite

$$FeS + H_2S \rightarrow FeS_2 + H_2, \qquad (7.1)$$

is indeed exergonic with an estimated $\Delta G_o' = -38.4$ kJ mol^{-1} at pH 7 (Wächtershäuser 1992). But in our view, there are several problems with the idea of a pyrite-pulled reverse TCA cycle. Thermodynamics is not the main difficulty, for it is indeed favourable when linked to pyrite production

$$4HCO_3^- + 2H^+ + 7H_2S + 7FeS \rightarrow (CH_2\text{–}COO^-)_2 + 7FeS_2 + 8H_2O, \qquad (7.2)$$

with an estimated $\Delta G_o' = -429$ kJ mol^{-1} (Wächtershäuser 1990), although Schoonen *et al.* (1999) underscore various difficulties with the temperature dependence and kinetic properties of pyrite-dependent CO_2 reduction. One problem is that intermediates of the α-cycle initially proposed by Wächtershäuser (1990) contain too much sulphur in our view, as do compounds such as $CH_2{=}C(SH)COSH$, $HSOC\text{–}CHSHCH_2\text{–}COSH$, CH_3COSH and $(HS)_2CH\text{–}COSH$, that have been suggested as constituting the 'core of a primordial metabolism' (Huber & Wächtershäuser 1997). Another problem is pyrite itself, because the synthesis of pyrite is upheld as the main thermodynamic reaction at the seat of early biochemical reactions (Wächtershäuser 1998), even though some 'pyrite-pulled' reactions (Huber & Wächtershäuser 1997) occurred in the absence of both Fe^{2+} and sulphide (but $NiSO_4$ instead), and hence were altogether unable to produce pyrite. But from the standpoint of comparative biochemistry, the main problem that we see with the idea of a pyrite-pulled reverse TCA cycle as the primordial carbon-fixation pathway is that in modern metabolism the reverse (or reductive) TCA cycle is a specialized

and committed pathway of carbon fixation only, and it must be energetically supported by an independent energy metabolism (ATP synthesis). Phrased another way, no cells are known that satisfy their ATP needs via CO_2 reduction through the reductive TCA cycle alone. The Wood–Ljungdahl pathway to acetyl-CoA is (i) simpler and shorter than the reductive TCA cycle, (ii) linear, and (iii) carbon and energy metabolism in one; energetically, it supports itself.

Nonetheless, we would agree that the TCA cycle in either the forward or reverse direction (Hartman 1975; Smith & Morowitz 2004) is an ancient pathway, and we concur with Morowitz *et al.* (2000) that an ancient role of the TCA cycle in supplying precursors for (generally pyridoxal dependent) amino acid synthesis is reflected in modern amino acid biosynthetic routes. The biochemical relationship between the acetyl-CoA pathway and the *incomplete* reverse TCA cycle as it occurs in methanogens and acetogens (Fuchs 1989; Simpson & Whitman 1993; Furdui & Ragsdale 2000) is particularly consistent with this view. The incomplete reverse TCA cycle has the same chemical intermediates as the TCA cycle, except that it appears to lack citrate and isocitrate, such that it stops 'dead end' at α-ketoglutarate in the CO_2-fixing direction (Fuchs 1989; Simpson & Whitman 1993), thereby channelling pyruvate through oxalacetate into longer and more reduced carbon backbones for biosyntheses (Lengeler *et al.* 1999). Stated another way, during carbon fixation, the reverse TCA cycle produces acetyl-CoA, whereas the incomplete reverse TCA cycle converts acetyl-CoA (via pyruvate) into longer carbon backbones.

We have drawn a modified version of the incomplete reverse TCA cycle in figure 3 that goes two steps beyond α-ketoglutarate, including chemical conversions corresponding to those catalysed by isocitrate dehydrogenase (yielding isocitrate) and isocitrate lyase (yielding succinate and glyoxylate), even though the corresponding activities seem to be lacking in the modern manifestation of the pathway (Zeikus *et al.* 1977; Simpson & Whitman 1993). The reason for this is (i) that glyoxylate is a very important intermediate for various pathways of acetate assimilation in modern microbes, even though the underlying biochemistry seems to have many different facets in different modern organisms (Meister *et al.* 2005), and (ii) since our premises entail a geochemical setting involving acetate production, it seems reasonable to have glyoxylate on the map, even if it does not fit perfectly from the standpoint of comparative biochemistry. The importance of acetate itself in microbial metabolism has recently been reviewed (Wolfe 2005); transition metal sulphide-catalysed reactions in the context of early biochemistry have been recently reviewed by Cody (2004).

Thus, in modern acetogens and methanogens, the acetyl-CoA pathway plus pyruvate synthase funnels H_2 and CO_2 into pyruvate, which pushes on via one more (non-reductive but energy-consuming) CO_2 incorporation to oxalacetate, which feeds the incomplete reverse citric acid cycle resulting in the accumulation of malate, fumarate, succinate, succinyl-CoA and α-ketoglutarate for biosyntheses (Furdui & Ragsdale 2000). We suggest that the same chemical conversions, sketched in figure 3, were relevant at the origin of biochemistry. Today, these reactions are catalysed by enzymes. Like Smith & Morowitz (2004), we suggest that such reactions were possible without enzymes, but under hydrothermal conditions.

The reactions from acetyl-CoA to α-ketoglutarate shown in figure 3 require the hydrolysis of only two phosphoanhydride bonds in ATP or guanosine triphosphate (GTP) in modern biochemistry, at the steps generating oxalacetate and the thioester succinyl-CoA (Lengeler *et al.* 1999). Instead of ATP or GTP, acetyl phosphate would suffice in energetic terms because it has a higher free energy of hydrolysis than the anhydride bond in ATP that links the γ-phosphate to ADP (table 2). Furthermore, malate is the only intermediate with a chiral centre (one) and is widely found in biochemistry as both D and L forms (chiral centres in isocitrate are of no importance here). The general lack of chiral atoms in the conversions from acetyl-CoA to α-ketoglutarate in figure 3 would preclude the need to assume any form of stereoselective synthesis of starting compounds at the very earliest stages of biochemistry. Although the incomplete citric acid cycle provides important intermediates for amino acid (and nucleotide) biosynthesis today, it does not directly provide sugars, which is a more difficult topic.

8. DIFFICULT D-SUGAR PHOSPHATES

In contrast to the TCA cycle, the metabolic intermediates and chemical conversions in sugar phosphate metabolism are hardly conserved at all across all microbes (Verhees *et al.* 2003; Ahmed *et al.* 2005; Siebers & Schönheit 2005), leaving the early state of sugar phosphate conversions difficult to approach from the consideration of modern pathways. Given a hydrothermal vent setting, it is possible that ancestral gluconeogenic flux might have looked more like the pathway of *Pyrococcus furiosus* (Mukund & Adams 1995; Sapra *et al.* 2003) than like the Embden–Meyerhoff pathway as it occurs in yeast. For example, *P. furiosus* uses a single FeS-containing, ferredoxin- and pterin-dependent enzyme, D-glyceraldehyde-3-phosphate oxidoreductase (GAPOR), to interconvert D-glyceraldehyde-3-phosphate (D-GA3P) and 3-phospho-D-glycerate (Mukund & Adams 1995), consistent with an ancient role for FeS and pterins. The GAPOR reaction contrasts to the more widely familiar, two-step enzymatic conversion from 3-phospho-D-glycerate to 1,3-bisphospho-D-glycerate (an intermediate altogether lacking in the *P. furiosus* pathway) and onto D-GA3P as found in eukaryotes (Cerff 1982), reactions that are NAD- and ATP-dependent. In other words, the ATP-producing step of glycolysis in yeast, which requires the concerted action of D-GA3P dehydrogenase and 3-phospho-D-glycerate kinase, is catalysed by one FeS enzyme in *P. furiosus* that is pterin dependent, and does not consume ATP in the anabolic (sugar-synthesizing) direction (Mukund & Adams 1995; Sapra *et al.* 2003).

Traditionally, thoughts on the prebiotic synthesis of sugars centre around something similar to a formose reaction (Ricardo *et al.* 2004), whereby their phosphorylation is assumed to have occurred independently of their synthesis, perhaps with the help of inorganic

phosphate or nucleoside phosphates, i.e. the origin of sugars is traditionally viewed independently of the origin of sugar phosphates. De Duve's (1991) suggestion for the entry of phosphate into metabolism via acetyl phosphate would provide a suitable phosphoryl donor, but how to get from acetyl-CoA to sugars? In many organisms, this occurs via gluconeogenesis and involves the synthesis of phosphoenolpyruvate (PEP) in a manner that circumvents the reaction catalysed by pyruvate kinase, which is thermodynamically unfavourable in the direction of PEP

$$\text{pyruvate} + \text{ATP} \rightarrow \text{PEP} + \text{ADP}, \tag{8.1}$$

with $\Delta G'_o = +31.4$ kJ mol^{-1}. PEP assumes an important role in our considerations because a primitive proteinaceous precursor of the enzyme enolase consisting of handed peptides might have marked the origin of chirality in sugar phosphates (Martin & Russell 2003). Enolase catalyses the stereospecific addition of a water molecule to the double bond in PEP to produce 2-phospho-D-glycerate (2-PGA), and the stereochemistry at carbon atom 2 arising from that water addition is conserved in core metabolism, also in RNA. Sugar phosphate homochirality might have arisen this way (anabolically) in biochemical evolution. Many modern cells generate PEP from pyruvate via the TCA cycle intermediate oxalacetate, typically synthesized in a reaction catalysed by the enzyme pyruvate carboxylase

$$\text{pyruvate} + \text{CO}_2 + \text{ATP} \rightarrow \text{oxalacetate} + \text{ADP} + \text{P}_i, \tag{8.2}$$

with $\Delta G'_o = -2.1$ kJ mol^{-1}, which will proceed readily. Were this reaction coupled to acetyl phosphate hydrolysis ($\Delta G'_o = -43$ kJ mol^{-1}) rather than to ATP hydrolysis ($\Delta G'_o = -31$ kJ mol^{-1}), it would be more exergonic under standard conditions by about -12 kJ mol^{-1}. The conversion of oxalacetate to PEP is then typically catalysed by the enzyme PEP carboxykinase

$$\text{oxalacetate} + \text{GTP} \rightarrow \text{PEP} + \text{CO}_2 + \text{GDP}, \tag{8.3}$$

with $\Delta G'_o = +4.2$ kJ mol^{-1}, which is thermodynamically uphill, but the reaction proceeds readily in modern metabolism nonetheless, and would again be more favourable if coupled to acetyl phosphate rather than to GTP hydrolysis. The sum of reactions (8.2) and (8.3) is

$$\text{pyruvate} + \text{ATP} + \text{GTP} \rightarrow \text{PEP} + \text{ADP} + \text{GDP} + \text{P}_i, \tag{8.4}$$

with $\Delta G'_o = +2.1$ kJ mol^{-1}, and will tend to proceed if there is more pyruvate than PEP available (which is reasonable) and would furthermore be more favourable by about -24 kJ mol^{-1} if coupled to acetyl phosphate hydrolysis. The route via oxalacetate would get us to PEP in energetic terms. But in prokaryotes, the biochemical interconnections between pyruvate, PEP and oxalacetate are more direct (Sauer & Eikmanns 2005). In particular, the enzyme PEP synthase, which occurs in *Escherichia coli* (Cooper & Kornberg 1967) and *P. furiosus* (Hutchins *et al.* 2001), catalyses the reaction

$$\text{pyruvate} + \text{ATP} \rightarrow \text{PEP} + \text{AMP} + \text{P}_i, \tag{8.5}$$

with $\Delta G'_o = -12$ kJ mol^{-1} (Eyzaguirre *et al.* 1982), the favourable thermodynamic value being founded in the hydrolysis of two phosphoanhydride bonds during the reaction (Cook & Knowles 1985). Indeed, for each of the six possible direct interconversions of pyruvate, PEP and oxalacetate (figure 3), at least one enzyme is known that catalyses the reaction directly under physiological conditions (O'Brien *et al.* 1977; Sauer & Eikmanns 2005). Such tight interlocking of these three central intermediates in modern metabolism (Sauer & Eikmanns 2005), which connect autotrophic pyruvate production, the entry point of gluconeogenesis (PEP) and the entry point of the reverse TCA cycle (oxalacetate), would be compatible with the view that these are extremely ancient chemical reactions, regardless of the age of the enzymes by which they are catalysed.

In addition to the pyruvate synthase reaction, there is also a reaction catalysed by pyruvate:pyrophosphate dikinase (PPDK) that could, in principle, bridge the gap between pyruvate and PEP (Müller 1996)

$$\text{pyruvate} + \text{ATP} + \text{P}_i \rightarrow \text{PEP} + \text{AMP} + \text{PP}_i, \tag{8.6}$$

with $\Delta G'_o = +9.9$ kJ mol^{-1} (Eyzaguirre *et al.* 1982). The relevant chemistry of the reaction is the conversion of one phosphoanhydride bond into one enolphosphoester bond. Even though the reaction goes energetically uphill, it proceeds forward in modern metabolism to produce PEP; these unfavourable thermodynamics being 'pulled' by subsequent pyrophosphate hydrolysis. The acyl phosphate bond has a higher phosphorylating potential than the phosphoanhydride bond, so, in principle, the reaction of pyruvate and acetyl phosphate to yield PEP would be

$$\text{H}_3\text{CCOCOOH} + \text{CH}_3\text{COOPO}_3\text{H}_2 \rightarrow \text{CH}_2\text{C(COOH)OPO}_3\text{H}_2 + \text{CH}_3\text{COOH}, \tag{8.7}$$

with the relevant chemistry being the conversion of one acylphosphate bond into one enolphosphoester bond, energetically better than the PPDK reaction, which works (with the help of an enzyme).

Thus, the equilibrium thermodynamics under standard conditions of the chemical conversions linking pyruvate and oxalacetate to PEP is far from insurmountable. The presence of the simple phosphoryl donor (acetyl phosphate) plus a simple energy-releasing reaction (acetate production) could promote reactions to go forward. Acetyl phosphate is much more attractive than GTP or ATP as an early phosphoryl donor, because its hydrolysis product (acetate) is indeed a waste product that can be lost to the environment at no expense, whereas the loss of ADP would pose a serious problem of resource waste to a primordial chemical system. In this sense, phosphate appears in our considerations as a *cofactor*, a very simple one, whose recharging in terms of the mixed anhydride bond in acetyl phosphate would be favourable, given a sustained synthesis of acetyl thioesters.

Phosphoenolpyruvate is an important intermediate, because in organisms that use the Wood–Ljungdahl pathway, the enzyme enolase introduces the first chiral atom in sugar phosphate biochemistry by catalysing the stereospecific addition of a water molecule to the double bond in PEP:

$$\text{PEP} + \text{H}_2\text{O} \rightarrow \text{2-PGA}, \tag{8.8}$$

with $\Delta G_o' = +4.7$ kJ mol^{-1}. This uphill reaction works very well in modern metabolism, but it would be better if there were a 'pulling' reaction for 2-PGA product removal. The modern solution is the conversion of 2-PGA to 3-phospho-D-glycerate via the enzyme phosphoglyceromutase. This reaction is facile, as Wold (1971) writes:

> The phosphate ester of glycerate 2-phosphate is quite stable to hydrolysis in both acid and base, but at elevated temperatures an acid-catalysed migration takes place to give a mixture of the 2- and 3-phosphate ester of glycerate in a ratio of 4 : 1 in favour of the 3-phosphate.

This reaction would leave us one hydride transfer short of glyceraldehyde-3-phosphate (GA3P; the simplest sugar phosphate with a chiral centre) and sugar phosphate chemistry. If one were to get to sugar phosphates, their reactivity is such that various species would readily coexist in aqueous solution, as indicated in the small box at the right of figure 3 for the geminal diols of triosephosphate (Noltmann 1972), pulling products away from their source of synthesis, as in the case of 2-PGA and 3-PGA just mentioned above (Wold 1971). However, two issues bear heavily upon the foregoing considerations.

First, all of the thermodynamic values for the biochemical reactions mentioned in this section are for pH 7, 25°C and equilibrium, which do not exist at a hydrothermal vent on the ocean floor where our considerations are seated. There, variable temperatures and high pressures will prevail, and H_2 and CO_2 at hydrothermal vent conditions are far from equilibrium. The thermodynamics of the biochemical reactions considered here has not been calculated, to our knowledge, for simulated vent conditions. However, chemical equilibria for the CO_2, H_2, N_2 and H_2O systems have been calculated for hydrothermal vent conditions almost identical to those that we assume (Shock 1992; point 8 in path B, approximately 15 km off ridge and close to the ocean floor), where the predominant carbon product is acetate (approximately 5 mM), very much in line with our salient argument.

Second, modern biochemistry involves many uphill reactions, but it is the overall thermodynamics of a biological system (an open system) that allows some uphill reactions to proceed. The main reaction of acetogen metabolism

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 2H_2O, \qquad (8.9)$$

is highly exergonic with $\Delta G_o' = -104.6$ kJ mol^{-1} (Thauer *et al.* 1977), but the side reactions made possible by the main reaction are the ones that synthesize the fabric of life. Acetogens synthesize acetyl phosphate (and subsequently ATP) from thioester hydrolysis, even though some of the individual reactions are steeply uphill. This works because they grow when H_2 and CO_2, but little acetate, are present, whereby they 'take a cut' of both the energy and the carbon, as H_2 and CO_2 enter their cells and try to reach thermodynamic equilibrium with acetate (Schink 1997), their main waste product. Acetogen cell mass is a minor by-product of their main thermodynamically favourable reaction: acetate production. Organic synthesis at an alkaline hydrothermal vent can be viewed similarly.

9. A FOUNTAIN OF YOUTH?

The reader might ask whether we are really suggesting that this hypothetical hydrothermal vent is a fountain of chemical youth that spews up a constant supply of energy-rich thioesters from scratch and that the resulting reactants just fall into place according to the laws of thermodynamics, and that metabolism thus unfolds during that process. Yes, that is what we are suggesting, and it is not fundamentally different in basic content from what others have suggested previously (Morowitz *et al.* 2000), though differing in some details. Metabolic energy in the form of acetyl thioesters (acetyl-CoA) and acetyl phosphate is apparently free in this system. A hydrothermal mound has the same thing going for it that organisms using the acetyl-CoA pathway do; as Shock *et al.* (1998, p. 73) put it, in thermodynamic terms, organisms that use the Wood–Ljungdahl pathway

> are given a free lunch that they are paid to eat.

One could interject that a continuous supply of thioesters is interesting but of no use or relevance whatsoever because they would rapidly hydrolyse. We would counter that (i) the limiting principle is their synthesis, not their hydrolysis (or phosphorolysis), (ii) we have at hand a plausible source to replace the ones that are assumed to be lost, (iii) what could be better for early synthesis than highly reactive intermediates, and (iv) what reasonable suggestion in the literature for the origin of biochemistry does not involve water?

Traditional thinking on prebiotic syntheses usually avoids the use of any kind of modern metabolic energy, such as ATP. If acetyl phosphate is entertained as an intermediate with appreciable steady-state concentration, a larger spectrum of reactions could be entertained by virtue of its phosphorylating potential. Traditional thinking on prebiotic syntheses usually also avoids the introduction of genuinely biochemical starting compounds into experimental regimens. Approaches to purine synthesis have started with cyanide, for example, or other chemicals that have little to do with life as we know it. Maybe life's original chemical reactions would be easier to simulate without enzymes if one were to start with compounds similar to those that microbes use today. In addition, the classical biochemical pathways map that hangs on many laboratory walls reveals a particularly central position of acetyl-CoA, pyruvate, PEP and TCA cycle intermediates in biosyntheses. Like Morowitz *et al.* (2000), we suggest that this is because these are relics from the ancestral state of central metabolism, as very roughly sketched in figure 3.

10. PTERINS, GTP AND COFACTORS

Since they are essential to methyl synthesis in both the acetogen and the methanogen manifestations of the Wood–Ljungdahl pathway (figure 1), pterins play a particularly important role in this view of early biochemistry. Looking a bit closer into pterin

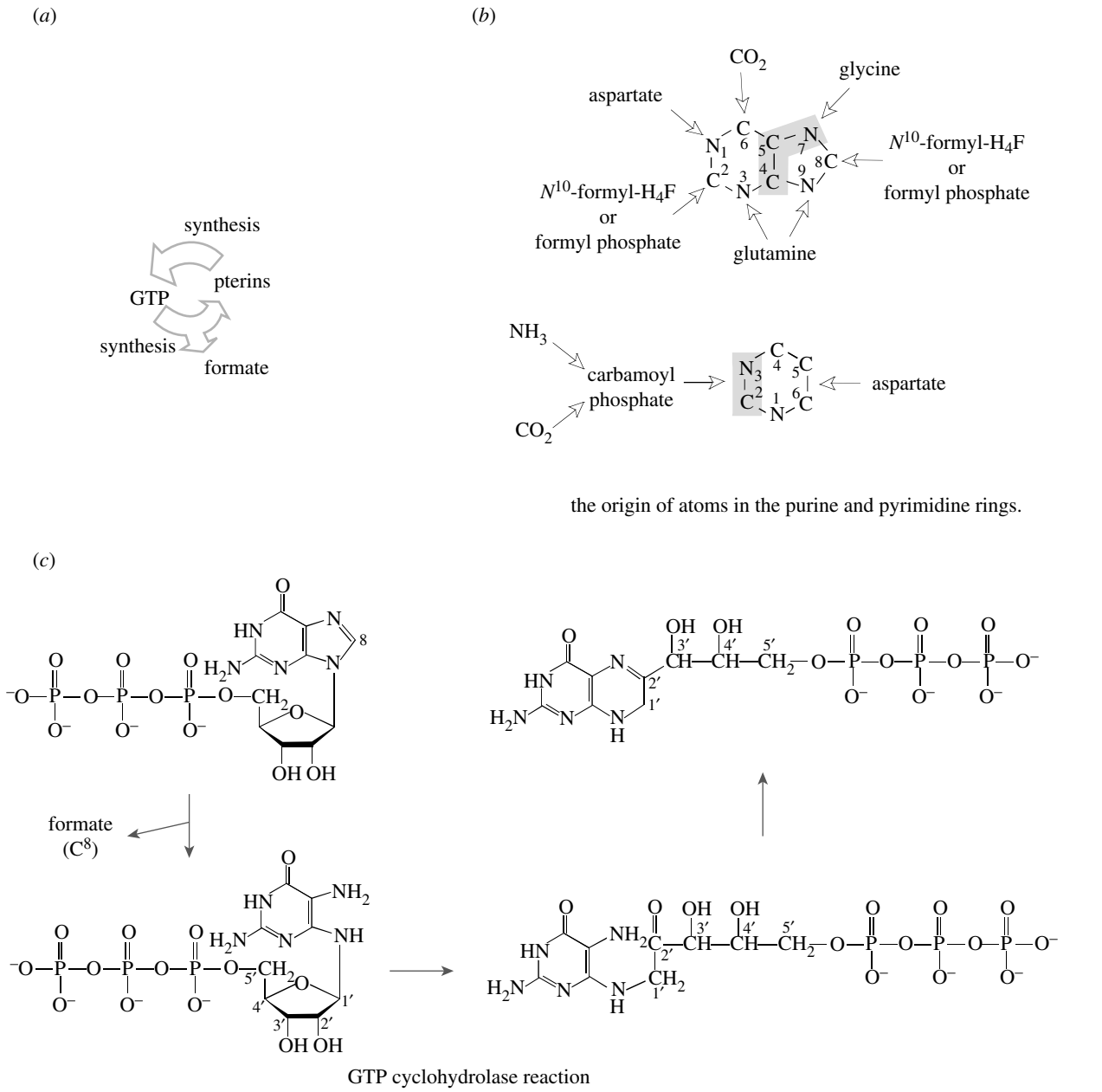


Figure 4. Pterins, purines and pyrimidines. (*a*) Schematic of the interdependence between GTP and pterin synthesis. (*b*) The origin of the atoms in the purine and pyrimidine rings, slightly modified from Stryer (1975) as it relates to microbes (Kappock *et al.* 2000), and including formyl phosphate-dependent reactions summarized by Ownby *et al.* (2005). H_4F, tetrahydrofolate. (*c*) The substrate, intermediates and product of the GTP cyclohydrolase reaction initiating pterin synthesis, redrawn from Wuebbens & Rajagopalan (1995).

biosynthesis reveals a curiosity, in that pterins are synthesized from GTP (figure 4*a*), but at the same time they are needed for purine synthesis (figure 4*b*). The first step of pterin biosynthesis is a complicated reaction between the guanine and the ribose moieties of GTP (Wuebbens & Rajagopalan 1995; Rebelo *et al.* 2003) catalysed by the enzyme GTP cyclohydrolase (figure 4*c*). It involves (i) addition of two water molecules, (ii) elimination of C^8 from the guanyl moiety as formate to produce a reaction intermediate that contains a fourfold substituted pyrimidine backbone, and (iii) condensation of the 2′ ribose carbon for ring closure, yielding the pterin 7,8-dihydroneopterin triphosphate, the structure of which is shown in figure 4*c*. But at the same time, the purine ring itself contains two carbons that are donated from a pterin, as shown in the inset of figure 4*b*, which is slightly modified from Stryer (1975, p. 514). It is possibly coincidence, but possibly not, that many enzymatic reactions involving pyruvate and CO_2 are GTP-dependent (sometimes ATP- or GTP-dependent), as is the synthesis of succinyl-CoA from succinate (figure 3), or protein synthesis itself. This tends to suggest to us an early role not only of pterins, but also of GTP in the biosynthetic pathways.

However, the product–synthesis relationship between pterins and GTP is not exceptional, as cofactors are often involved in their own synthesis. Examples are the biosynthetic pathways of thiamine pyrophosphate (TPP; Rodionov *et al.* 2002) and PLP (Drewke & Leistner 2001) (figure 5*a*). The biosynthesis of TPP starts from pyruvate and D-GA3P to yield

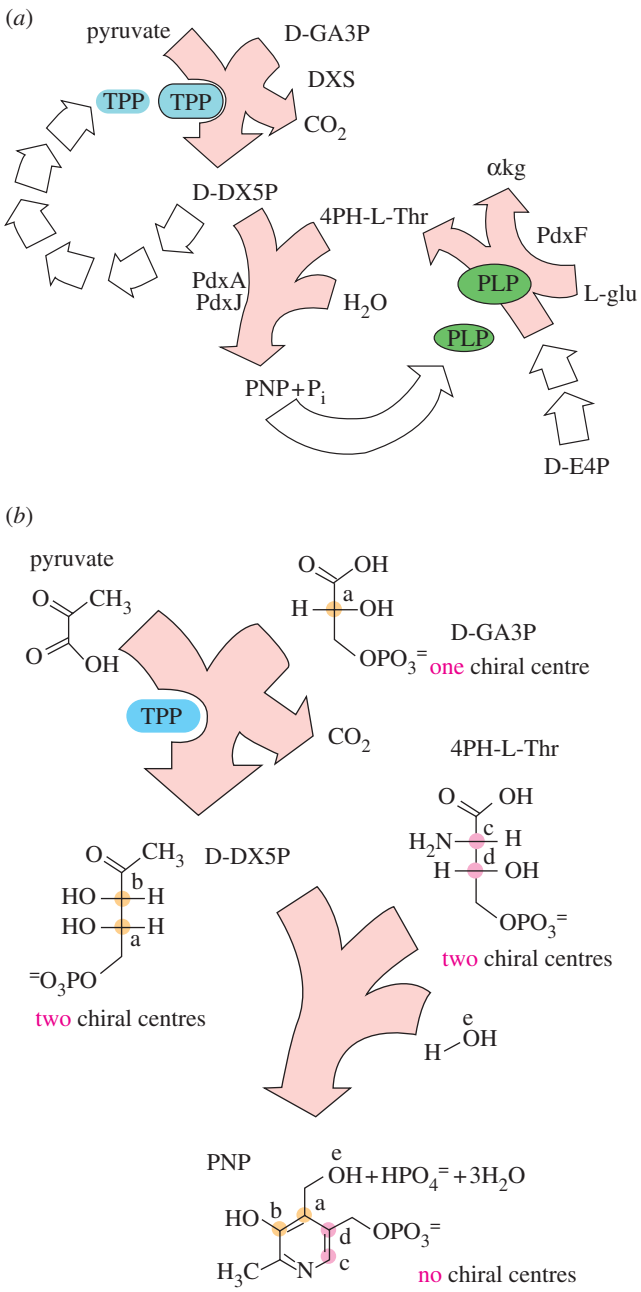


Figure 5. Chicken-and-egg synthesis and chirality loss. (*a*) Schematic of the circumstance that in some micro-organisms TPP (thiamine pyrophosphate) and PLP are required for their own synthesis (see text), but also positively feedback into their own synthesis in the sense of a chemical hypercycle (Hordijk & Steel 2004). (*b*) Disappearance of chiral centres through aromatization in PLP biosynthesis by PdxA and PdxJ according to the mechanism proposed by Laber *et al.* (1999), see also Eubanks & Poulter (2003). The chiral carbon atoms a–d in substrates and products are labelled. GA3P, glyceraldehyde-3-phosphate; DX5P, 1-deoxy-xylulose-5-phosphate; 4PH-Thr, 4-(phosphohydroxy)-threonine; glu, glutamate; αkg, α-ketoglutarate; E4P, erythrose-4-phosphate; PNP, pyridoxine phosphate.

1-deoxy-D-xylulose-5-phosphate (D-DX5P), in a TPP-dependent reaction. 1-Deoxy-xylulose-5-phosphate (DX5P) is condensed with 4-(phosphohydroxy)-L-threonine (4PH-L-Thr) to initiate PLP synthesis, but 4PH-L-Thr synthesis is PLP dependent (Drewke *et al.* 1996). Chicken-and-egg loops of this type are not an uncommon theme in the organization of critical biochemical pathways.

More important is what happens stereochemically during TPP or PLP synthesis in *E. coli*: four chiral centres (labelled a, b, d and e) are involved en route to the synthesis of PLP, which has none (figure 5*b*; redrawn from Laber *et al.* (1999) and Eubanks & Poulter (2003)). TPP also lacks chiral atoms (figure 2). The mechanisms involved in the 1-deoxy-xylulose-5-phosphate synthase (DXS) reaction (Eubanks & Poulter 2003) or pyridoxine 5′-phosphate (PNP) synthesis (Laber *et al.* 1999) indicate no fundamental need to start from chiral material for these syntheses. The newly discovered pathway of PLP biosynthesis in plants (Tambasco-Studart *et al.* 2005) is catalysed by an enzyme that condenses D-GA3P (one chiral atom) and D-ribulose-5-phosphate (two chiral atoms) together with ammonium donated from glutamine to pyridoxal 5′-phosphate (no chiral atoms). That the active moieties of cofactors (often aromatics) lack

chiral centres (figures 2 and 5), despite chirality of the adducts, suggests that prebiotic synthesis of aromatic cofactors could have occurred prior to the origin of homochirality. Proteins and RNA introduce chirality into modern biochemistry, and it is possible that homochirality arose that way.

Another curiosity is that TPP biosynthesis is intertwined with purine biosynthesis. TPP contains a pyrimidine moiety (figure 2) which, however, does not stem from pyrimidine biosynthesis. It stems instead from 5-aminoimidazole ribotide, an intermediate in *de novo* purine biosynthesis (Begley *et al.* 1999). Moreover, the pyrimidine moiety is generated via a complex rearrangement and insertion reaction, in which three carbon atoms of the pyrimidine ring of TPP are derived from the ribose carbons of 5-aminoimidazole ribotide (Begley *et al.* 1999), like pterin synthesis from GTP (Wuebbens & Rajagopalan 1995; figure 4*c*).

Coenzyme A is required for the reactions shown in figure 3, but not in a central manner. CoA is very complex (figure 2) and organic thiols would appear to suffice for early thioester formation. Noteworthy in CoA synthesis is the participation of a very simple and comparatively rare cofactor, the pyruvoyl group of pyruvoyl enzymes, at two decarboxylation steps that yields β-alanine from aspartate (Williamson & Brown 1979) and the active β-mercaptoethylamine moiety from cysteine (Yang & Abeles 1987). Modern CoA biosynthesis is not interlinked with that of GTP, PLP or TPP, although it does require methylene-H_4F (Genschel 2004). This latter requirement might suggest that pterins are older than CoA in biochemical evolution, similar to the conventional reasoning that RNA is older than DNA, because the synthesis of the latter requires the former. The circumstance that the pyruvoyl cofactor itself is synthesized from adjacent serine residues within polypeptide chains (Van Poelje & Snell 1988) would also speak for a later origin of CoA.

To summarize, pterins, TPP and GTP are the main organic cofactors required for the reactions shown in figure 3 to proceed. That their modern biosyntheses, and that of PLP, which will be important in a later section, are interlinked, tends to suggest their emergence from a set of coexisting chemical reactions, rather than from a stepwise series of synthetic inventions.

11. SOME IMPORTANT REACTIONS OF ACETYL PHOSPHATE OCCUR WITHOUT ENZYMES

Recently, de Duve (2003) asked: What could have catalysed the prebiotic synthesis of phosphoanhydride bonds from acetyl phosphate? New findings from de Zwart *et al.* (2004) show that Fe(II) ions very efficiently catalyse the synthesis of PP_i from acetyl phosphate and phosphate (25% yields at neutral pH and 12% in the presence of sulphide). Furthermore, FeS protected the PP_i so formed from hydrolysis of the anhydride bond (de Zwart *et al.* 2004). In the absence of enzymes, acetyl phosphate can also phosphorylate ADP to make ATP, which is another experiment that de Duve (2003) suggested. Kitani *et al.* (1991) reported the synthesis of ATP from ADP and acetyl phosphate at 25°C with 20% conversion and 100% selectivity on the ADP substrate using Fe(III) as a catalyst, and later reported that the Fe(III) ions were indeed catalytic (Kitani *et al.* 1995). Subsequent work using simulated hydrothermal vent conditions, but with trimetaphosphate ($P_3O_9^{3-}$) instead of acetyl phosphate as the phosphoryl donor, also produced good yields of ATP from AMP (Ozawa *et al.* 2004). But a plausible prebiotic source for $P_3O_9^{3-}$ (containing three phosphoanhydride bonds) is lacking, whereas a plausible prebiotic source for acetyl phosphate seems to be at hand. The conversion of acetyl thioesters to acetyl phosphate and subsequent phosphorylation reactions by the latter seem to work well in the absence of enzymes. The spontaneous non-enzymatic generation of an acyl phosphate bond from a thioester in the presence of phosphate was inferred to occur in aqueous solution (without enzymes) as an intermediate step en route to pyrophosphate production from thioesters (Weber 1981, 1982), although the acyl phosphate itself was not reported.

12. NITROGEN: CARBAMOYL PHOSPHATE AND A CRITICAL ACYL PHOSPHATE INTERMEDIATE

How did nitrogen enter metabolism? Thermodynamic simulations of chemical equilibria in early hydrothermal systems suggest that NH_3 would be the predominant form of soluble reduced nitrogen, and would have existed in hydrothermal fluid at concentrations approaching approximately 50 mM at the type of vent central to our considerations: peridotite and gabbro base, 15 km from the spreading zone (Shock 1992), similar to the setting at Lost City. Although nitrogen species at Lost City were not reported (Kelley *et al.* 2001), thermodynamics clearly suggests that NH_3 will be hydrothermally available.

Today, autotrophs incorporate nitrogen into biochemistry via NH_3. Nitrogenase is merely a source of NH_3 from N_2 and is a very complex protein that is of no crucial interest here, beyond its unusual Fe–Ni–Mo-sulphide centre (Seefeldt *et al.* 2004). From the standpoint of comparative biochemistry, glutamine and carbamoyl phosphate stand out as simple and highly conserved carriers of carbon–nitrogen bonds and are hence possible candidates for the entry of nitrogen into metabolism. Trotta *et al.* (1971) wrote:

> The findings suggest that glutamine-dependent carbam[o]yl phosphate synthetase (and perhaps other glutamine amidotransferases) arose in the course of evolution by a combination of a primitive ammonia-dependent synthetic enzyme and a glutaminase; this combination may have been associated with a change from ammonia to glutamine as the principal source of nitrogen.

Carbamoyl phosphate synthase (CPS) catalyses a most interesting set of conversions. In *E. coli*, four reactions are catalysed by CPS (Guy *et al.* 1997; with trivial names for the organic products given in brackets):

$$\text{glutamine} + H_2O \rightarrow \text{glutamate} + NH_3, \tag{12.1}$$

$$ATP + HCO_3^- \rightarrow ADP + HOCOOPO_3^{=} \quad \{\text{carboxyphosphate}\}, \tag{12.2}$$

$$HOCOOPO_3^{=} + NH_3 \rightarrow H_2PO_4^- + H_2NCOO^- \quad \{\text{carbamate}\}, \tag{12.3}$$

$$ATP + NH_2COO^- \rightarrow ADP + H_2NCOOPO_3^{=} \quad \{\text{carbamoyl phosphate}\}. \tag{12.4}$$

Glutamine serves merely as the source of NH_3 in the reaction, provided by a 40 kDa glutaminase subunit (Guy *et al.* 1997). The overall CPS reaction

$$2ATP + HCO_3^- + NH_3 \rightarrow 2ADP + H_2N(CO)OPO_3^{=} + H_2PO_4^-, \tag{12.5}$$

is exergonic with $\Delta G_o' = -23.2\ \text{kJ mol}^{-1}$ (Durbecq *et al.* 1997). CPS in *P. furiosus* is simpler as it requires no glutaminase and functions with NH_3 directly (Durbecq *et al.* 1997), entailing only reactions (12.2)–(12.4). *Pyrococcus furiosus* CPS is a 314 amino acid long protein with no obviously ancient traits, but is related to carbamate kinase, which Durbecq *et al.* (1997) suspect to be more ancient than CPS because it catalyses the reaction to carbamoyl phosphate by more direct means:

$$H_2NCOO^- + ATP \rightarrow ADP + H_2NCOOPO_3^{=}, \tag{12.6}$$

but in a steeply uphill direction with $\Delta G_o' = +8.9\ \text{kJ mol}^{-1}$ (Durbecq *et al.* 1997), which would, however, be thermodynamically more favourable by about $-12\ \text{kJ mol}^{-1}$ if coupled to acetyl phosphate hydrolysis (table 2). For comparison, the straight chemical equilibrium of bicarbonate and ammonia with carbamate (the substrate of the carbamate kinase reaction) is

$$NH_3 + HCO_3^- \rightarrow H_2NCOO^- + H_2O, \tag{12.7}$$

with $\Delta G_o' = -1.6\ \text{kJ mol}^{-1}$ (Durbecq *et al.* 1997). This reaction will also tend to go forwards, and hence is a suitable sort of reaction for early biochemistry.

The ATP-independent glutaminase reaction (12.1) of CPS is a paradigm for the central role of glutamine in modern nitrogen metabolism. NH_3 mainly enters modern metabolism as glutamine via glutamine synthase (GS), whose overall reaction

$$\text{glutamate} + NH_3 + ATP \rightarrow \text{glutamine} + H_2O + ADP + H_2PO_4^-, \tag{12.8}$$

involves two steps and γ-glutamyl phosphate (an acyl phosphate) as the intermediate (Wedler & Horn 1976):

$$\text{glutamate} + ATP \rightarrow \gamma\text{-glutamyl phosphate} + ADP, \tag{12.9}$$

$$\gamma\text{-glutamyl phosphate} + NH_3 \rightarrow \text{glutamine} + H_2O + H_2PO_4^-. \tag{12.10}$$

Interestingly, GS efficiently synthesizes glutamine from glutamate, carbamoyl phosphate and ADP (Tate *et al.* 1972), and also synthesizes ATP from acetyl phosphate and ADP (Tate *et al.* 1972). In metabolism, however, the amides alone are not enough, because amino acids usually stem from α-keto acids via reductive aminations and transaminations. Notably, both FeS and $Fe(OH)_2$ can catalyse reductive aminations in the presence of α-keto acids and ammonium to yield amino acids (Huber & Wächtershäuser 2003). Using these reactions germane to carbamoyl phosphate and glutamine synthesis, plus a steady supply of acetyl phosphate (instead of ATP, but with higher phosphorylating potential) and NH_3, we can construe a simple, but two-pronged, primordial nitrogen assimilation (figure 6*a*). One route would lead to carbamoyl phosphate via carbamate in analogy to the CPS or carbamate kinase reactions, but involving acetyl phosphate instead of ATP (figure 6*a*). The other, a GS-like reaction would require activated organic acids as analogues of γ-glutamyl phosphate, for which purpose acetyl phosphate in the simplest assumption could serve, yielding acetamide (or other amides from the corresponding organic acids) as a product, for reductive aminations and subsequent transaminations (figure 6*a*).

In figure 6*a*, these chemical conversions are suggested to have been possible, and to have occurred, without the help of genetically encoded proteinaceous catalysts. With a steady source of amides as organic amino donors, the synthesis of amino acids from the four α-keto acids in figure 3 could, in principle, be possible. Is such chemistry plausible in the absence of enzymes? Some examples from the literature indicate so. Hennet *et al.* (1992) and Marshall (1994) obtained simple amino acids from formate under (albeit somewhat unrealistic) vent conditions; Huber & Wächtershäuser (2003) obtained amino acids from α-keto acids under milder vent conditions, using Fe^{2+}. But a few examples also trace to older literature.

In the absence of enzymes, Nakada & Weinhouse (1953) reported the synthesis of glycine from glyoxylate (an α-keto acid) using aspartate, asparagine, glutamate or glutamine as the amino donor at room temperature, pH 7.4 and 10 mM reactant concentrations in phosphate buffer after 2 h. The conversion with glutamine yielded about 4% glycine after 2 h, but about 10% glycine (also with asparagine as the amino donor) after 24 h. Such reactions would correspond well to the kinds of conversions in figure 6*a*. However, pyruvate and α-ketoglutarate were not converted under those conditions (Nakada & Weinhouse 1953).

Morowitz *et al.* (2000) suggested that transaminations of α-keto acids involving PLP should be among the more ancient biochemical reactions, but will such reactions work in the absence of enzymes? Metzler & Snell (1952*a*) reported that most amino acids are efficiently converted by pyridoxal to the corresponding α-keto acids over a broad pH range at 100°C in the presence of Fe(II), Fe(III) or alum ($NH_4Al(SO_4)_2$) as catalysts, and that the reverse reaction works well too. Using 1 mM alum at pH 5, they showed greater than 40% conversion of pyridoxamine to pyridoxal with 10 mM α-ketoglutarate after 1 h at 100°C, and greater than 40% conversion of 10 mM pyridoxal to pyridoxamine with glutamate after 1 h at 100°C, without enzymes. The efficiencies remained similar at 2.5 mM concentrations of reactants. Similar efficiencies were reported for the pyridoxal/valine and pyridoxamine/α-ketoisocaproic acid after 1 h, pyridoxal/isoleucine

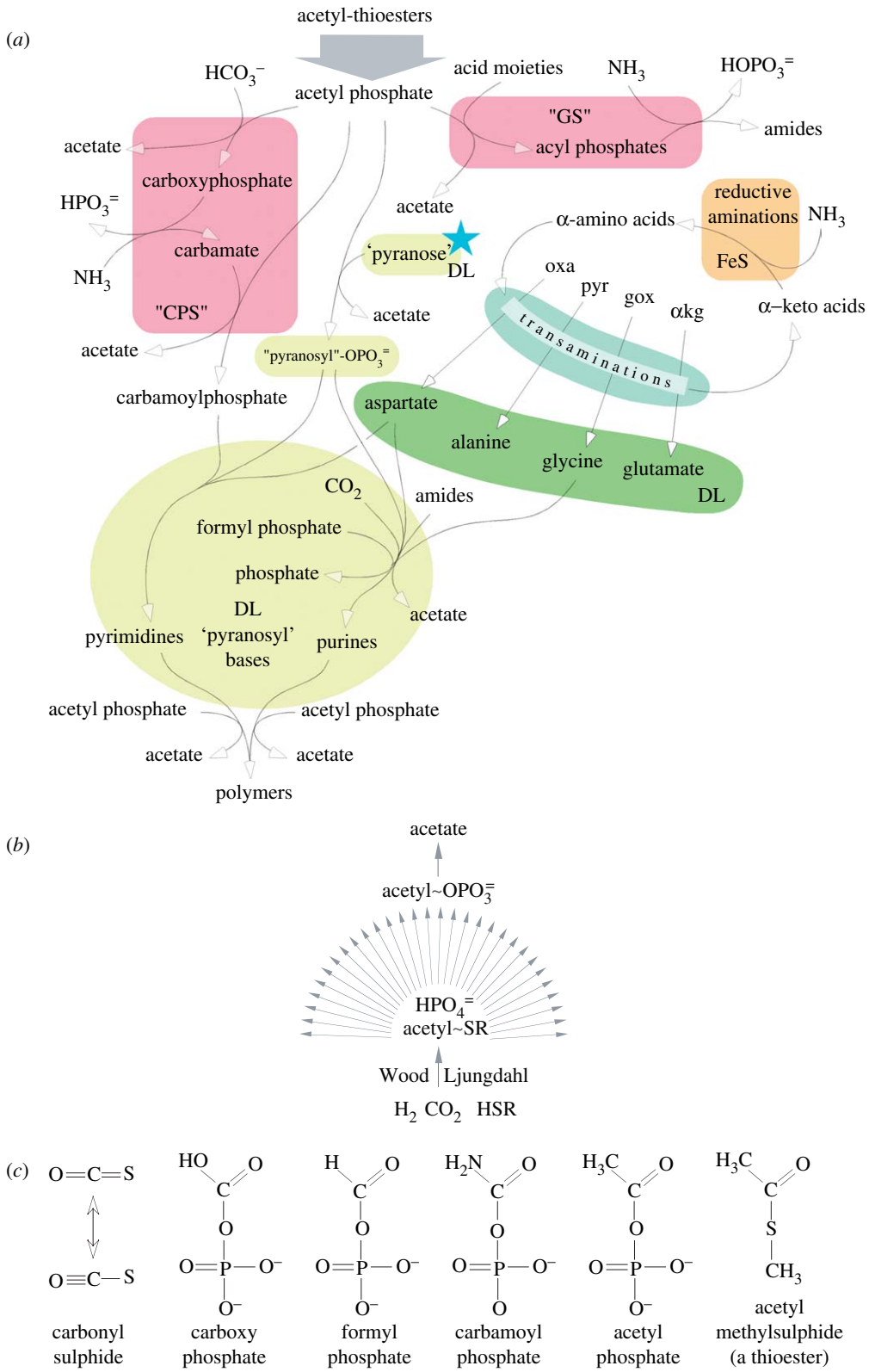


Figure 6. Speculations about early biochemistry. (*a*) Possible routes of nitrogen incorporation into metabolism, energetically feasible with acetyl phosphate owing to its higher energy of hydrolysis (table 2) than ATP, which is the modern phosphoryl donor in the enzymes today. Oxalacetate (oxa), pyruvate (pyr), glyoxylate (gox) and α-ketoglutarate (αkg) also occur on the left-hand side of figure 3. 'Pyranose' (Eschenmoser 2004) indicates that we do not specify the ancestral kind of sugar phosphate in the backbone of RNA-like polymers. The blue star indicates that we have not specified a mechanism of sugar phosphate formation here, although we suspect it to have proceeded via PEP and PGA, via the enolase reaction (figure 3). 'DL' indicates that pyranosyl (or other sugar) phosphate synthesis probably occurred without stereospecificity at first, and that a chemical hypercyclic feedback loop into a handed precursor of the enolase reaction as sketched in figure 7 tipped the homochirality scale for sugars. (*b*) A reminder that we are suggesting the synthesis of thioesters that are the 'food' for an autocatalytic chemical cycle (Hordijk & Steel 2004) to have been continuous and stable and to have proceeded initially with inorganic catalysts only. R indicates a simple aliphatic residue. (*c*) Structures of some of the simple reactive compounds that are central to the considerations in this paper. The Lewis structures for COS are from Luther (2004).

and pyridoxamine/α-keto-β-methylvaleric acid after 6 h, and so forth (Metzler & Snell 1952*a*). In a different report (Metzler & Snell 1952*b*), serine showed anomalous behaviour, being deaminated to pyruvate at greater than 50% efficiency within minutes in a reaction which required pyridoxal and metal salts as catalysts, but that did not convert pyridoxal to pyridoxamine. The efficiency of the Cu(II)-catalysed reaction increased dramatically above pH 8. They also showed approximately 50% conversion of cysteine to pyruvate, ammonia and H_2S with pyridoxal in the presence of alum (Metzler & Snell 1952*b*). This anomalous behaviour of serine (and cysteine) is exploited by serine dehydratase, which in most bacteria is not a PLP-dependent enzyme, but a [4Fe–4S] enzyme instead (Grabowski *et al.* 1993).

The point here is that the cofactor PLP alone will efficiently catalyse transamination (and other) reactions in the presence of inorganic catalysts, without enzymes (Metzler & Snell 1952*a*,*b*), and that some transamination reactions can proceed relatively efficiently (10% conversion) in the absence of the cofactor altogether. In this context, it is noteworthy that heating a dilute solution of NH_3 and glycoaldehyde gives a large family of pyridines substituted with the same functional groups as occuring in pyridoxin itself, aromatization stemming from water eliminations (Austin & Waddell 1999). The examples of PLP-promoted and facile α-keto acid/amino acid interconversions given here underscore the case regarding cofactors and simple chemistry, particularly in the context of the RNA world (figure 2), made by Penny (2005):

> In biochemistry the focus has been on macromolecules (especially proteins) catalysing reactions. However, weak catalysis is a property of many small molecules and metallic ions, albeit with much lower rates. For example, we think of the break down of hydrogen peroxide (H_2O_2) to water and oxygen being carried out by the protein enzyme catalase. However, catalase has a heme molecule as a coenzyme (cofactor) that is involved in this chemical reaction, and the heme molecule by itself (without the protein) is a weak catalyst of the reaction. Again, the metal at the active centre of heme, Fe^{2+}, is also a catalyst, even though even weaker than heme. Thus small molecules and ions are also catalysts. […] These principles are all important in the search for a good explanation for the origin of life, and equally perhaps for rejecting others that rely on untestable and unlikely events.

One could interject that whatever small amount of whatever product is formed under our assumed conditions will rapidly react or otherwise diffuse, but our proposal entails a continuous and uninterrupted geochemical supply of the basic starting compounds: acetyl thioesters and acetyl phosphate (figure 6*b*). The three-dimensional nature of the metal sulphide compartments at the vent provides a physical barrier to diffusion. Hence, whatever reactions occur, they can occur more or less continuously with the central and reactive intermediates being constantly replenished, and channelling into more complex organic molecules, but initially without stereospecificity.

13. THIS N INCORPORATION DELIVERS THE CONSTITUENTS OF PURINE AND PYRIMIDINE

Via the kinds of non-enzymatically catalysed transamination reactions described in the preceding section, glyoxalate, pyruvate, oxalacetate and α-ketoglutarate (the only four α-keto acids in figure 3) would yield glycine, alanine, aspartate and glutamate (figure 6*a*). The starting bouquet of four amino acids is the same as Copley *et al.* (2005) infer regarding the nature of the genetic code, but ours are yet racemic (glycine has no chiral centre anyway, and is among the carbon species expected in hydrothermal fluid (Shock 1992)).

Allowing the non-enzymatic GS-like reaction in figure 6*a* to be capable of yielding glutamine from glutamate (the synthesis of the γ-glutamylphosphate intermediate would be more favourable with acetyl phosphate than with ATP), these simple nitrogen incorporation routes yield all of the biochemical constituents of purine biosynthesis as it occurs in textbook biochemistry (Stryer 1975), as updated from newer studies on microbes (Kappock *et al.* 2000): glycine as the central backbone, CO_2, nitrogen from aspartate and glutamine, and two carbon atoms from a critical intermediate of our initial methyl synthesis branch in the Wood–Ljungdahl pathway—N^{10}-formyl-tetrahydrofolate (N^{10}-formyl-H_4F; figure 4*b*)—for which a much simpler formyl donor, formyl phosphate, can substitute in some modern organisms, as explained in the following passages.

The qualifier 'textbook' was added to the forgoing sentence because *Methanocaldococcus jannaschii*, a chemolithoautotrophic methanogen, was recently shown to use formate and ATP instead of the formyl group in an N^{10}-formylpterin for the origin of C^2 in the purine ring (Ownby *et al.* 2005). Notably, formyl phosphate (the chemically simplest acyl phosphate) could substitute efficiently for ATP and formate in that particular reaction of purine biosynthesis, catalysed by the folate-independent enzyme PurP (5-formaminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5′-monophosphate synthetase), indicating that formyl phosphate is formed during the reaction mechanism (Ownby *et al.* 2005).

Of particular interest, another reaction of purine biosynthesis involves the formation of formyl phosphate as a chemical intermediate in the modern enzymatic reaction (Ownby *et al.* 2005). This is glycinamide ribonucleotide (GAR) transformylase, the product of the *purT* gene, which uses ATP and formate to catalyse the synthesis of 5-phosphoribosyl-*N*-formylglycinamide in the third step of purine biosynthesis (Kappock *et al.* 2000), leading to the origin of C^8 in the purine ring (figure 4*b*). The formate- and ATP-dependent PurT reaction, which can proceed with the reaction intermediate formyl phosphate instead, and which is pterin independent, is an alternative in *E. coli* to the N^{10}-formyl-H_4F-dependent step catalysed by PurN (Marolewski *et al.* 1997). Similarly, the PurP reaction, together with PurO (a cyclase), is an alternative to the pterin-dependent PurH reaction.

Thus, the two pterin-dependent C1 transfers in purine biosynthesis can also occur in pterin-independent reactions that involve formyl phosphate (figure 4*b*).

In the search for a simple chemistry at the origin of bases, this might be significant. It prompted Ownby *et al.* (2005) to suggest that the formyl phosphate-dependent reactions in purine biosynthesis might represent a more primitive precursor chemistry that preceded the pterin-dependent pathway, a suggestion which we would emphatically second. The formate required for the PurP reaction is synthesized internally from CO_2 (Ownby *et al.* 2005).

It is also conceivable that formyl phosphate might represent a more primitive precursor of formyl pterins (formyl-H_4F and formyl-H_4MPT) in the methyl synthesis branch of the Wood–Ljungdahl pathway, as discussed in §16. The structures of some of the simple, but reactive, intermediates encountered in modern purine and pyrimidine biosynthesis, along with acetyl phosphate and a simple acetyl thioester are shown in figure 6*c*.

Another formyl phosphate-utilizing enzyme is formyl-H_4F synthetase, which is the product of the *purH* gene in *E. coli* that generates formyl-H_4F from formate and ATP, but which also catalyses the reaction corresponding to the ATP-consuming step of methyl synthesis in the Wood–Ljungdahl pathway of acetogens (figure 1; Smithers *et al.* 1987; Mejillano *et al.* 1989).

Some side reactions of these formyl phosphate-generating enzymes are of interest. Formyl-H_4F synthetase will accept carbamoyl phosphate as a substrate to generate ATP from ADP (Buttlaire *et al.* 1976; Smithers *et al.* 1987; Mejillano *et al.* 1989). GAR transformylase will also accept carbamoyl phosphate as a substrate to generate ATP from ADP, and catalyses the reverse reaction as well, in addition to catalysing the synthesis of acetyl phosphate and ADP from acetate and ATP in the forward and reverse reactions (Marolewski *et al.* 1997). We are not suggesting that the side reactions of these enzymes tend to reflect some special relationship between the proteins, but instead that they might implicate some relationship between the simple kinds of chemistry involved, entailing compounds such as formyl phosphate, carbamoyl phosphate and acetyl phosphate.

Furthermore, it is notable that when comparing the synthesis of 5-phosphoribosyl-*N*-formylglycinamide via the PurT reaction (formyl phosphate dependent) versus the reaction via PurN (formyl-H_4F dependent) (Marolewski *et al.* 1997), phosphate serves as a cofactor that is covalently bound to the formyl substrate, functionally replacing the H_4F moiety.

In addition to the starting compounds for purine synthesis, the starting compounds for pyrimidine synthesis also directly ensue from our consideration of simple reactions incorporating NH_3 into metabolism: carbamoyl phosphate and aspartate (figures 4*b* and 6*a*). Purines and pyrimidines are synthesized today from L amino acids, but the bases themselves lack chiral centres, and the only chiral atom that ends up in a base is the α-carbon of aspartate in pyrimidines; so, achiral amino acids would seem to suffice at the start. It is curious that modern *de novo* pyrimidine synthesis does not involve any reductive steps and only one oxidative step, at dihydroorotate dehydrogenase, where fumarate (an intermediate in figure 3) serves as the electron acceptor in anaerobes (Norager *et al.* 2003).

It is conceptually satisfying (but not necessarily significant) that a very simple route of CO_2 assimilation and the direct incorporation of NH_3 into the simplest α-keto acids, plus an additional simple route to carbamoyl phosphate, yields exactly the starting material that microbes use for the synthesis of purines and pyrimidines. The path from the Wood–Ljungdahl pathway via the incomplete reverse TCA cycle and simple NH_3 incorporation to modern nucleotide biosynthesis is surprisingly short in our view.

We admittedly neglect here the circumstance that, in modern biochemistry, the purines are synthesized *in toto*, the pyrimidines in part, on phosphoribosyl pyrophosphate (from which two carbons in pterins stem; figure 4c). However, the considerations in this section raise the possibility that the prebiotic synthesis of the bases of RNA did not entail anything vaguely similar to cyanide condensations, and that they furthermore did not occur independently of the primordial reduction of CO_2 and the primordial incorporation of NH_3 into metabolism, but rather as a by-product thereof. We stress that we have not suggested a primordial pathway for the synthesis of bases here, but we have suggested a primordial pathway for the synthesis of their constituents.

To conclude this section, we address the origin of C^6 in the purine ring from CO_2, which is of particular interest (figure 4*b*). It stems from the activity of 5′-phosphoribosyl-5-aminoimidazole carboxylase encoded in the *E. coli purEK* operon (Tiedeman *et al.* 1989), which catalyses the two-step reaction

$$\text{AIR} + CO_2 \rightarrow \text{carboxy-AIR}, \tag{13.1}$$

$$\text{carboxy-AIR} + \text{ATP} + \text{aspartate} \rightarrow \text{succino-AICAR} + \text{ADP} + P_i, \tag{13.2}$$

whereby AIR stands for 5-aminoimidazole ribonucleotide and AICAR stands for 5-amino-4-imidazolecarboxamide ribonucleotide (Lukens & Buchanan 1959). The carboxylating reaction (13.1) occurs via carboxyphosphate (Kappock *et al.* 2000), an intermediate in carbamoyl phosphate synthesis (12.2). The reaction with aspartate (13.2), which is also among our inferred N-incorporation products in figure 6*a*, adds N^1 to the purine ring (figure 4*b*) and the subsequent reaction

$$\text{succino-AICAR} \rightarrow \text{AICAR} + \text{fumarate}, \tag{13.3}$$

catalysed by adenylosuccinate lyase (Stone *et al.* 1993), the product of *E. coli purB* (Green *et al.* 1996), which also catalyses a very similar reaction in the last step of adenine biosynthesis (Stone *et al.* 1993), reduces the CO_2. The electrons that are donated to the growing purine ring, and that ultimately reduce the CO_2 at C^6, stem from aspartate. Thus, the C^6 carbon atom in purines stems from a CO_2-fixation reaction (figure 4*b*). In addition, the C^2 carbon of pyrimidines stems from carbamoyl phosphate (figure 4*b*), the carbon atom of which also stems in turn from CO_2 (figure 6*a*). Both these CO_2-incorporating reactions are highly conserved, even in humans (Stone *et al.* 1993; Summar *et al.* 2003). Hence, if one is so inclined, one could say

that the trace of an autotrophic origin is preserved in every purine and in every pyrimidine residue.

To summarize this section, the incomplete reductive TCA cycle and very simple nitrogen incorporations, involving carbamate and transaminations, supply the constituents of purine and pyrimidines in modern biochemistry. This might mirror prebiotic chemical reactions. Although the incorporation of carbon atoms at C^2 and C^8 in the purine ring usually requires formyl pterins, and hence would hardly seem primitive at first sight, the same incorporations can also occur without pterins *via* formyl phosphate in modern biochemistry. The formyl phosphate-dependent reactions might be relicts of a very early synthesis of purines, from which the pterins are derived. The origin of C^6 in purines from CO_2 and the orgin of C^2 in pyrimidines from CO_2 via carboxyphosphate might be the ancestral state of these reactions, and the biological constituents of bases today might be similar to, or the same as, the ones at the origin of biochemistry.

14. PUSHING OUT OF A RACEMATE AND INTO AN RNA WORLD

An RNA world requires a steady supply of bases. Our collection of chemicals (figures 3 and 6*a*), all derived from a steady supply of acetyl thioesters (figure 6*b*), could, in principle, provide the necessary starting material. But, two more things are needed for an RNA world: phosphorylating potential (e.g. in the form of acetyl phosphate) and homochiral sugar backbones. Deriving sugars for the RNA world is a problem (Ricardo *et al.* 2004; Springsteen & Joyce 2004). We have not suggested a simple chemical reaction that will reduce 3-phosphoglycerate (an acid phosphate) to GA3P (a sugar phosphate); alternative routes to sugar phosphates could also entail simpler phosphorylated compounds, such as glycoaldehyde phosphate (Pitsch *et al.* 1995). But because we assume that something like an RNA world existed, we also assume that the synthesis of sugars did occur and that it furthermore can (and will) occur under reducing hydrothermal conditions.

Pyranoses like the four carbon sugar threopyranose are attractive simpler solutions to ribose for the RNA world, but still work poorly as racemates (Eschenmoser 2004). Any of the various alternative routes to RNA synthesis, as outlined by Sutherland & Whitfield (1997), seem plausible too, whereby our system would have a replenishable and organic source of phosphorylating potential through acetyl phosphate for polymerization reactions. This is notable, because, as Schwartz (1998) has stressed, not only the source of bases and sugars is a problem for the RNA world, but also a reactive form of phosphate has been missing as well, with suggestions even ranging to phosphites—acetyl phosphate is a suitable reactive form.

The homochirality problem of sugars and amino acids is also challenging. Most solutions to the homochirality problem usually entail some kind of preformed achiral catalysts, for example, as sometimes found in meteorites (Pizzarello & Weber 2004), and generally start with sugars (for RNA), and more recently with amino acids. Tamura & Schimmel (2004) showed that a handed RNA molecule will undergo stereoselective aminoacylation, and thereby discriminate between D-Ala and L-Ala, and suggested that homochirality so initiated could be perpetrated, similar to our earlier suggestion (Martin & Russell 2003), which involved not a preformed but a formed handed catalyst instead (an initial peptidyl transferase). In a world of *de novo* synthesized organic material, some of it looking like RNA, some molecule evolved the ability to catalyse or promote a peptidyl transferase (ester hydrolysis, amide formation) reaction, possibly involving FeS-mediated (Keller *et al.* 1994) or COS-mediated (Huber & Wächtershäuser 1998; Leman *et al.* 2004) peptide bond synthesis. That molecule (the initial peptidyl transferase) need not have been the ribosome or even self-replicating, although it would seem very likely that the ribosome was a triplet RNA replicator (a 'triplicase') before it started catalysing the synthesis of peptide bonds (Penny 2005). The initial peptidyl transferase of the ribosome would accept either D- or L-amino acids, not both (Martin & Russell 2003), as illustrated with the example of the modern ribosome (figure 7). Let us assume that it accepted L, which would channel handed amino acids into handed peptides.

Here, one might interject that the mirror image of the handed catalyst would have done the same for the D form. But with an organic mixture of sufficient complexity, an exact mirror image would be exceedingly unlikely to arise. For example, a racemate of a modern peptide, 26 amino acids long, consists of 2^{25} (3.3×10^7) molecules, if they all have exactly the same sequence, and if only stereochemistry at the α-carbon is considered, and if all peptide bonds involve the proper amino and carboxyl moieties. If we, however, relax those assumptions a bit and assume a random sequence for 20 amino acids, a racemate consisting of $3.3\times10^7\times6.7\times10^{33}=2.2\times10^{41}$ peptide molecules ensues, corresponding to about 40 000 000 megatonnes of peptide. This racemate will never arise. Reducing the number of amino acids in our 26-mer to four instead of 20, still requires about 25 g of peptide for a racemate of identical sequences. If other kinds of bonds other than α-carbon peptide bonds are allowed, an occasional organic impurity other than an amino acid, shorter and longer molecules, different sequences, etc., it quickly becomes clear that a racemate of anything moderately complex cannot readily be synthesized under abiotic conditions if there are polymerization reactions going on, for example, peptide bond synthesis between amino acids (Huber & Wächtershäuser 1998; Leman *et al.* 2004). With sugars, the racemate situation is much worse, because sugars have more chiral carbons that are able to form acetal or phosphoester bonds.

In other words, starting with a mixture of D and L amino acids, and given a mechanism like COS-mediated *N*-carboxyanhydride intermediates (Leman *et al.* 2004) to generate peptide bonds between them, handed products would simply be unavoidable. A simple-handed peptide is the kind of handed catalyst that we suggested as the precursor of the modern enzyme enolase (Martin & Russell 2003), which adds a water molecule to PEP in a stereospecific manner to produce 3-phospho-D-

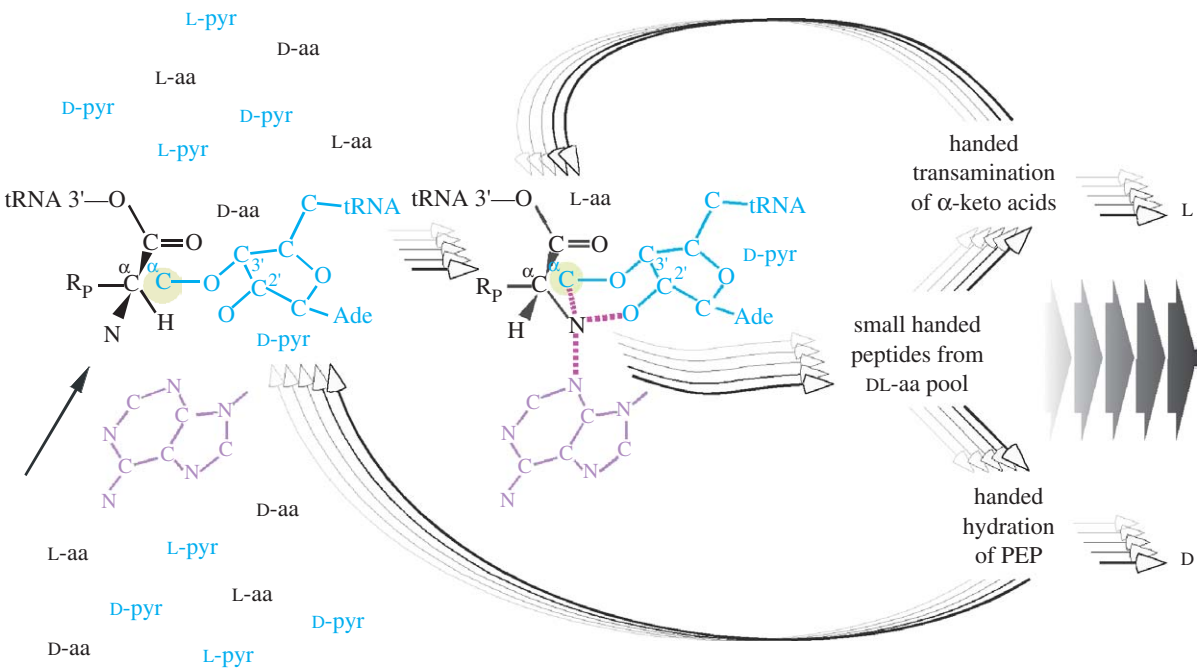


Figure 7. Suggestion for the origin of homochirality (see text) via an autocatalytic cycle in the sense of Hordijk & Steel (2004). From a mixture of L and D amino acids, only those of one α-carbon configuration are incorporated into protein by virtue of the chance stereochemistry of the initial peptidyl transferase reaction catalysed by a protoribosome. Handed peptides (also possible to synthesize initially by chance, owing to the complexity of racemates with many chiral centres, see text) with some enolase activity produce more D sugars, possibly as pyranose (pyr), leading to more stereoselective peptide synthesis at the peptidyl transferase site of the ribosome as redrawn from Steitz (2005), because only activated amino acids of one configuration will polymerize. This is schematically indicated by the fit of an activated L-amino acid into the modern peptidyl transferase site (centre), where the α-amino group of the next amino acid to be polymerized is coordinated by the active site so as to attack the C-terminal carbonyl carbon of the tRNA-bound growing peptide chain, whereas the D-configuration (left) leaves the amino group in the wrong spot (arrow) for peptidyl transfer. While the translation process can filter one configuration into peptides, it cannot synthesize the L-configuration. But handed peptides with PLP-dependent transaminase activities can (arrows leading to L), feeding back into the autocatalytic cycle by promoting more of both the stereochemically homogeneous enolase and the transaminase activity. Note that the autocatalytic cycle requires a sustained source of new precursors ('food') in order to operate (Hordijk & Steel 2004).

glycerate, instead of the L form (figure 3). This would, in our view, be enough to tip the homochirality scale (figure 7) via propagation through sugar stereochemistry, promoting synthesis of a particular stereochemical configuration in RNA sugars—whether three, four or five carbons long (Eschenmoser 2004)—and thereby promoting the polymerization of preferentially L or D amino acids via handedness of the peptidyl transferase active site (figure 7). Earlier feedback loops with different stereochemistry are, of course, imaginable, but a simple two-channelled process of this type, with stereoselective synthesis at the enolase reaction and stereoselective filtering at the peptidyl transferase reaction, would seem sufficient to make the transition from a mix of DL sugars and amino acids into stereoselective syntheses without the need for pre-existing achiral catalysts.

This would get one, in principle, to good substrates for an RNA world—not necessarily via the shortest route, but via a thermodynamically favourable one, fuelled by thioesters and acetyl phosphate (figure 6). The most valuable next stereochemical step would seem to be a handed peptide that would promote stereospecific transamination of α-keto acids (figure 5*a*,*c*), ideally involving the achiral cofactor PLP (Smith & Morowitz 2004). An RNA world seems essential to early evolution (figure 2), but it would probably also contain peptides synthesized from amino acids non-stereoselectively at first. At the level of base-containing RNA-like polymers that can act as template for their own replication, provided that a steady supply of precursors is maintained (figure 6*a*), a dramatic transition in the nature of the chemistry at the vent would take place, because natural selection sets in, with the non-identical self-replicating contents of different compartments evolving independently (Koonin & Martin 2005) within the mound. We admittedly avoid the origin of the genetic code altogether here, but stress the suggestion of Copley *et al.* (2005) that the genetic code might have arisen via the synthesis of amino acids from α-keto acids that were covalently bonded to RNA, and that the syntheses involved the catalytic properties of two bases in the RNA, giving rise to a two-letter code that initially specified the same amino acids as in figure 6 (glycine, alanine and aspartate/glutamate) plus valine. The series of arrows in figure 5c are drawn to look like a chemical hypercycle (Eigen 1992), and recent theoretical work indicates that autocatalytic networks may be much simpler to evolve than one might have thought (Hordijk & Steel 2004; Mossel & Steel 2005), provided that there is a sustained source of carbon and energy.

15. CARBAMATE, MOLYBDENUM COFACTOR AND CARBONYL SULPHIDE: BIOCHEMICAL RELICTS?

In methanogens, the initial step of CO_2 reduction, catalysed by formyl-MF dehydrogenase, starts with the spontaneous (non-enzymatic) synthesis of a carbamate via the condensation of CO_2 and the primary amine of methanofuran (MF; Vorholt & Thauer 1997; Bartoschek *et al.* 2000). The reaction proceeds readily at pH 8, because carbamate synthesis requires the non-protonated form of the amine (Bartoschek *et al.* 2000), which is favoured under alkaline conditions, as we assume for the vent, where bicarbonate would, however, predominate over CO_2, the substrate for carbamate formation (Bartoschek *et al.* 2000). The circumstance that the very first chemical reaction of CO_2 *en route* to modern methane synthesis, carbamate formation, occurs without an enzyme (Bartoschek *et al.* 2000) and furthermore that no enzymatic activity could be found to speed the reaction rate of carbamate formation (Bartoschek *et al.* 2000) might represent a relic of a primordial biochemistry.

Methyl synthesis in acetogens and methanogens (figure 1) is MoCo dependent, because FDH in acetogens (Yamamoto *et al.* 1983) and formyl-MF dehydrogenase in methanogens both require either Mo or W in MoCo (Karrasch *et al.* 1990). FDH requires either Mo or W in the MoCo in various organisms (Hille 2002). It can be Mo dependent (Boyington *et al.* 1997), W dependent (de Bok *et al.* 2003), or accept either Mo or W (Brondino *et al.* 2004). In methanogens, formyl-MF dehydrogenase, like FDH, requires Mo or W (Schmitz *et al.* 1992; Hochheimer *et al.* 1998), probably as pterin complexes (Hochheimer *et al.* 1995), further implicating the possible involvement of these metals in early formyl-synthesizing chemistry. The presence of Mo and/or W in these formyl-generating steps is possibly significant from the geochemical perspective because these metal ions are soluble in alkaline hydrothermal solution as $Mo^{IV}S_4^{2-}$ or $W^{IV}S_4^{2-}$, but not at neutral pH (Nekrasov & Konyushok 1982; Russell *et al.* 1994). Although coordinated by a pterin in modern biochemistry, the modern dependence of formyl pterin synthesis upon these transition metals hints that Mo- or W-containing inorganic catalysts may be more efficient with regard to CO_2 reduction to formate than FeS, as used by Heinen & Lauwers (1996). Sarkar & Das (1992) have synthesized a functional model of the W-dependent FDH in the laboratory using simple organic ligands; the reduced catalyst, $[W^{IV}O(S_2C_2(CN)_2)_2]^{2-}$, was reported to fix CO_2. Alkaline conditions would accommodate the presence of these crucial heavy metal sulphide species at the vent in soluble form, which might also represent biochemical relics from earliest times.

The alkaline vent that we assume focussed not only the interface of H_2 with CO_2, but also the interface of HS^- with CO_2 on the early Earth, suggesting that it would be a probable site for COS synthesis at that time. COS might have been a crucial intermediate in the transition from inorganic to organic chemistry (formate synthesis), as outlined in §4. Some methanogens (and acetogens) might even be able to undergo sustained growth on COS, but as with methanogen growth on CO, the question remains whether such growth is possible via substrate level phosphorylation alone (Rother & Metcalf 2004; Ferry & House 2006).

COS is a significant trace gas in the atmosphere today, with estimated 2.3 million tonnes of COS produced and consumed each year by modern ecosystems, much of the production occurring in soil (Conrad 1996), whereby barley and chickpea roots convert COS to CO and H_2S (Ren 1999), not to CO_2 and H_2S according to the carbonic anhydrase reaction suspected to support microbial growth on COS (Conrad 1996). Furthermore, some prokaryotes have been found that can grow on COS (or CS_2) as the sole energy (but not energy and carbon) source (Jordan *et al.* 1997; Kelley 1999). These examples suggest to us that COS may have a more prominent role in modern biochemistry than is presently suspected. Like Conrad (1996), we presume that the source of modern environmental COS is biological; if generated anaerobically, the underlying biochemistry might be of interest in the context of early biochemistry.

16. THE EARLY FORMYL PTERIN PROBLEM

Going back to the methyl synthesis branch of the acetyl-CoA pathway in figure 1, we return to the highly endergonic steps that: generate the pterin-bound formyl group; differ in acetogens and methanogens; and require energy input. These steps bear heavily upon the transition from inorganic methyl synthesis to pterin-dependent methyl synthesis in our proposal.

For acetogens, Maden (2000) has estimated that the synthesis of N^{10}-formyl-H_4F from formate and H_4F is endergonic with $\Delta G_o' = +22\ \mathrm{kJ\ mol^{-1}}$. This is an extremely steep bioenergetic barrier. The reaction as catalysed by N^{10}-formyl-H_4F synthetase goes forward only because it is coupled to ATP hydrolysis. Specifically, the ATP hydrolysis—by analogy to *E. coli* N^{10}-formyl-H_4F synthetase (Mejillano *et al.* 1989; Smithers *et al.* 1987)—at the N^{10}-formyl-H_4F synthetase step would appear to involve the synthesis of formyl phosphate as the active intermediate. In the absence of enzymes, formyl phosphate will spontaneously formylate H_4F specifically at the N^5-position (Jahansouz *et al.* 1990), but the ATP required for formyl phosphate synthesis to generate formyl-H_4F comes from chemiosmotic coupling (Müller 2003).

In methanogens, the synthesis of formyl-MF from the carbamate, catalysed by formyl-MF dehydrogenase, is also highly endergonic ($\Delta G_o' = +16\ \mathrm{kJ\ mol^{-1}}$; de Poorter *et al.* 2003). The subsequent transfer of the formyl group to H_4MPT by formyl-MF : H_4MPT formyltransferase is slightly exergonic, with $\Delta G_o' = -3.5\ \mathrm{kJ\ mol^{-1}}$. Even though free formate does not occur in the methanogen pathway, Maden (2000) has estimated that the synthesis of N^5-formyl-H_4MPT from formate is endergonic with $\Delta G_o' = +9\ \mathrm{kJ\ mol^{-1}}$. The reaction mechanism of formyl-MF dehydrogenase is dependent upon about 2–4 sodium ions traversing the membrane from the outside of the cell to the inside (Kaesler & Schönheit 1989; Schönheit & Schäfer 1995; de Poorter *et al.* 2003). This ion gradient is generated by the later steps of methane synthesis, which involve chemiosmotic coupling.

Thus, in both the acetogen and the methanogen pathway, the synthesis of the N^{10}- or N^5-pterin-bound formyl groups requires energy input that, today, ultimately depends upon a proton-pumping mechanism and harnessing of that chemiosmotic energy (figure 1).

What is the problem? The problem is that if CO_2 reduction with H_2 to acetate is thermodynamically favourable (which it is) and if acetyl phosphate were produced via substrate level phosphorylation as the initial energy currency (as we are suggesting), then why do acetogens and methanogens require chemiosmosis in order to grow from the reduction of CO_2 with H_2? The activation energy of CO_2 can be overcome at CODH/ACS to make CO; that is not the problem. Cracking H_2 into electrons and protons with metal sulphides is also not the problem: the active site of Fe-only hydrogenase has recently been synthesized in the laboratory as an active and soluble iron sulphide (Tard *et al.* 2005).

The activation energy and the initial uphill reaction to get from CO_2 to the level of the formyl pterin are apparently the crux. The overall reaction to acetate will pull the formyl-generating step, and both acetogens and methanogens use chemiosmosis (and the overall downhill reaction to acetate or methane) to overcome the first hurdle; the former indirectly (ATP expense) and the latter directly (ion influx).

The problem is this: before early metabolic systems could harness chemiosmosis, how were they actually synthesizing formyl pterins, and worse, *why did microbes not take that chemiosmotic-independent mechanism of formyl pterin synthesis along when they left the vent*? We call it the early formyl pterin problem.

Why is this so important? It has to do with thermodynamics. First, it is clear from the acetate-producing reaction that

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 2H_2O, \quad (16.1)$$

with $\Delta G_o' = -104.6$ kJ mol^{-1} (Thauer *et al.* 1977) and an energy conservation efficiency of approximately 50% in anaerobes (Thauer *et al.* 1977), that only one ATP can be made from the reaction directly via substrate level phosphorylation, the one that is invested by acetogens to produce formyl-H_4F. For methanogens, the thermodynamic considerations are similar. The reaction

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O, \quad (16.2)$$

with $\Delta G_o' = -131$ kJ mol^{-1} (Thauer 1998) could yield two phosphoanhydride bonds with an energy conservation efficiency of 50%, but not at 25% (Thauer *et al.* 1977). The observation is that methanogens growing from reaction (16.2) do not employ substrate level phosphorylation; instead they gain all of their energy from chemiosmosis (Thauer 1998), indicating that energy conservation efficiency in methanogens is below 50%, consistent with the view that $\Delta G_o'$ of about -70 kJ mol^{-1} is required for ATP synthesis (Thauer *et al.* 1977; Schink 1990). If the energy conservation efficiency of methanogens would permit the synthesis of two phosphoanhydride bonds, microbial ingenuity would surely have found a way to substitute an FDH-related (and phosphoanhydride dependent) analogue of the formyl-H_4F reaction for the reaction catalysed by MF dehydrogenase. The observation is that microbes apparently did not find such an alternative; hence, it is probably not thermodynamically feasible.

Modern cells have no formyl pterin problem, because they have chemiosmosis. But prior to the origin of chemiosmotic coupling as a means to synthesize high-energy bonds, how was it possible to synthesize formyl pterins from CO_2 while satisfying all metabolic energy needs from acetyl phosphate (or any other energy currency), if the synthesis of the methyl group in acetyl phosphate (or acetyl thioesters) consumes the one phosphoanhydride bond that it has to offer? Put another way, prior to the advent of genes, translation, proteins and chemiosmotic ATP synthesis (which allows biochemistry to store energy in increments that require much less than -70 kJ mol^{-1} per proton pumped), formyl pterin synthesis from CO_2 presents a thermodynamic hurdle that appears insurmountable without some help from geochemistry. There appear to be three possible solutions to this problem.

The first entails geochemical methyl synthesis deep in the vent, for example, via serpentinization. If methyl groups are delivered to the vent–ocean interface, for example, in the form of methyl sulphide, as discussed in §4, then for every methyl group delivered, one acetyl phosphate can be synthesized which is free to do chemical work other than synthesis of another methyl group for the next molecule of acetyl phosphate. In other words, geochemically delivered methyl groups permit net organic synthesis from CO_2 via acetyl phosphate (or acetyl thioesters). However, in this case, the biochemical system proposed here would remain strictly dependent upon geochemically provided methyl groups up until the advent of (protein dependent) chemiosmotic harnessing. This delegates the thermodynamic hard work involved in the synthesis of the formyl pterin to serpentinization, but ties the origin of biochemistry to the serpentinization process via a kind of umbilical cord consisting of chemically accessible methyl groups (for example, methyl sulphide) dissolved in the hydrothermal fluid.

The second possible solution entails the synthesis of pyrophosphate at the vent in a (protein independent) manner that inorganically harnesses the natural pH and redox gradient at the vent–ocean interface as explicated previously (Russell & Hall 1997, 2006). This would constitute a protein- and acetyl phosphate-independent source of chemical energy, although the free energy of hydrolysis of pyrophosphate (-20 kJ mol^{-1}; Frey & Arabshahi 1995) is very close to the amount required at the step catalysed by N^{10}-formyl-H_4F synthetase ($\Delta G_o' = +22$ kJ mol^{-1}).

A third possible solution would be a direct, sustained, geochemical and acetyl phosphate-independent synthesis of formyl phosphate, or other compound thermodynamically capable of directly N-formylating a pterin (Jahansouz *et al.* 1990), at the vent–ocean interface. But we can presently offer no plausible suggestion for such a chemistry.

In all the three cases, the premise that the Wood–Ljungdahl pathway is the primordial pathway

of carbon incorporation leads to the inference that the origin of biochemistry was inextricably tied to a geochemical process in or on the Earth's crust, for bioenergetic reasons related to the steeply uphill synthesis of a formyl pterin starting from CO_2.

The advent of chemiosmotically coupled high-energy bond synthesis would solve the early formyl pterin problem, as outlined in §17. But the observation that methanogens and acetogens invented two fundamentally different, but chemiosmosis dependent, solutions to the problem of formyl pterin synthesis (figure 1) suggests that these solutions originated (i) independently and (ii) in an environment that offered naturally pre-existing and harnessable chemiosmotic potential. This would be consistent with the view that the self-replicating but non-free-living ancestors of the archaebacterial and eubacterial lineages became genetically distinct after the advent of protein synthesis on ribosomes but prior to the origin of protein-based chemiosmotic coupling mechanisms (Martin & Russell 2003; Koonin & Martin 2005). The considerations in this section furthermore suggest that the ability to self-replicate with CO_2 as the sole carbon source (autotrophy), while still likely a physiological attribute of the first free-living cells, was probably not an attribute of the universal ancestor—an evolving and translating, but physically confined and geochemically dependent, chemical system—from which the first genuinely autotrophic free-living cells arose. Owing to the early formyl pterin problem, our proposal is slightly but significantly different from the family of models called autotrophic origins (starting biochemistry straight from CO_2 alone), but it might belong to the family of hydrothermal origins, and would conform to Fuchs's (1989) suggestion that the use of C1 compounds more reduced than CO_2 might reflect an ancient attribute of the acetyl-CoA pathway.

17. BIOENERGETIC STEPS FROM ROCKS TO MICROBIAL COMMUNITIES

To summarize in terms of a sequence of bioenergetic events, we propose that life evolved at a pH-, temperature-, and redox gradient at an alkaline hydrothermal vent (Russell *et al.* 1994; Russell & Hall 1997; Martin & Russell 2003), and that the source of energy originally stems from the thermodynamic disequilibrium of hydrothermal H_2 originating from serpentinization in the Earth's crust with marine CO_2 originating from volcanoes (figure 8*a*). Precipitated metal sulphides at the vent, comparable to those found at Tynagh, Ireland (Russell & Hall 1997, 2006; figure 8*b*) with internal microcompartments similar to those of the Irish ore deposits (figure 8*c*–*e*), or similar to those with low metal sulphide content found at Lost City (Kelley *et al.* 2001; figure 8*f*) could provide a concentrating mechanism for organic compounds formed.

Within those microcompartments, acetyl thioester and acetyl phosphate formation from CO_2 reduction in the presence of thiols could be the first organic source of high-energy transfer potential (figure 9*a*). The initial synthesis of acetyl thioesters and acetyl phosphate would have been purely inorganic, involving transition metal sulphides and, probably, a methyl group of geological origin, for example, methyl sulphide (Heinen & Lauwers 1996). Sustained synthesis of acetyl thioesters (Huber & Wächtershäuser 1997) could generate acetyl phosphate via phosphorolysis (de Duve 1991). With methyl groups and NH_3 supplied by serpentinization (figure 9*b*), acetyl phosphate would be a source of both carbon for synthesis and phosphorylating potential to promote chemical reactions that otherwise would be slow to occur. Acetyl phosphate would provide a replenishable source for phosphoanhydride bonds, and could serve as the universal energy currency for the origin of bases that comprise the RNA world, in principle, even up to the advent of genes and proteins.

We assume that biochemical evolution eventually reached the level of self-replicating RNA and genetically encoded proteins, where biological invention and Darwinian mechanisms set in, with proteins (and their cofactors) playing the central role. The crucial step from the standpoint of bioenergetic novelties would involve a protein that could harness the natural, pre-existing chemiosmotic potential at the vent–ocean interface in the form of chemical energy (figure 9*c*). This could have been a simple protein like the single-subunit H^+-pyrophosphatase (Baltscheffsky *et al.* 1999) to start. Once invented, a coupling pyrophosphatase could immediately harness the naturally pre-existing proton gradient at the hydrothermal vent, because the vent we assume was alkaline (pH approximately 9–10) inside, like modern alkaline vents (Kelley *et al.* 2001, 2005), and acidic (pH approximately 5–6) outside owing to CO_2 and Lewis acids in the oceans (Russell & Hall 1997), and convert it into biochemically utilizable phosphoanhydride bonds. This would have been followed eventually by the appearance of rotor–stator type ATPase, which, with a few rare exceptions in some fermenters and parasites, is just as universal among prokaryotes as the genetic code (Shirakihara *et al.* 1997; Lolkema *et al.* 2003).

With a mechanism to harness the naturally pre-existing pH gradient at the vent–ocean interface in the form of high-energy chemical bonds, acetyl thioesters would no longer be needed as a source of phosphoanhydride bonds, and formyl pterin synthesis would no longer pose a bioenergetic problem because it could become dependent upon chemiosmotic coupling (figure 9*d*). One might complain that proton influx as shown in figure 9*c* would rapidly lead to the loss of chemiosmotic potential, but we recall that a constant physical flux of alkaline water towards the ocean is present at the vent. One might also complain about the appearance of hydrophobic compounds in figure 9*c*, that we suggest stems from simple thioester condensations, as in isoprene, fatty acid and polyketide synthesis (de Duve 1991). One might furthermore complain that the presence of a membrane protein requires a mechanism to insert proteins into hydrophobic layers, which today requires the signal recognition particle (SRP) that threads nascent polypeptide chains into a hydrophobic layer as they emerge from ribosomes. The SRP, with its notable RNA component, is interpretable as a relict of the RNA world

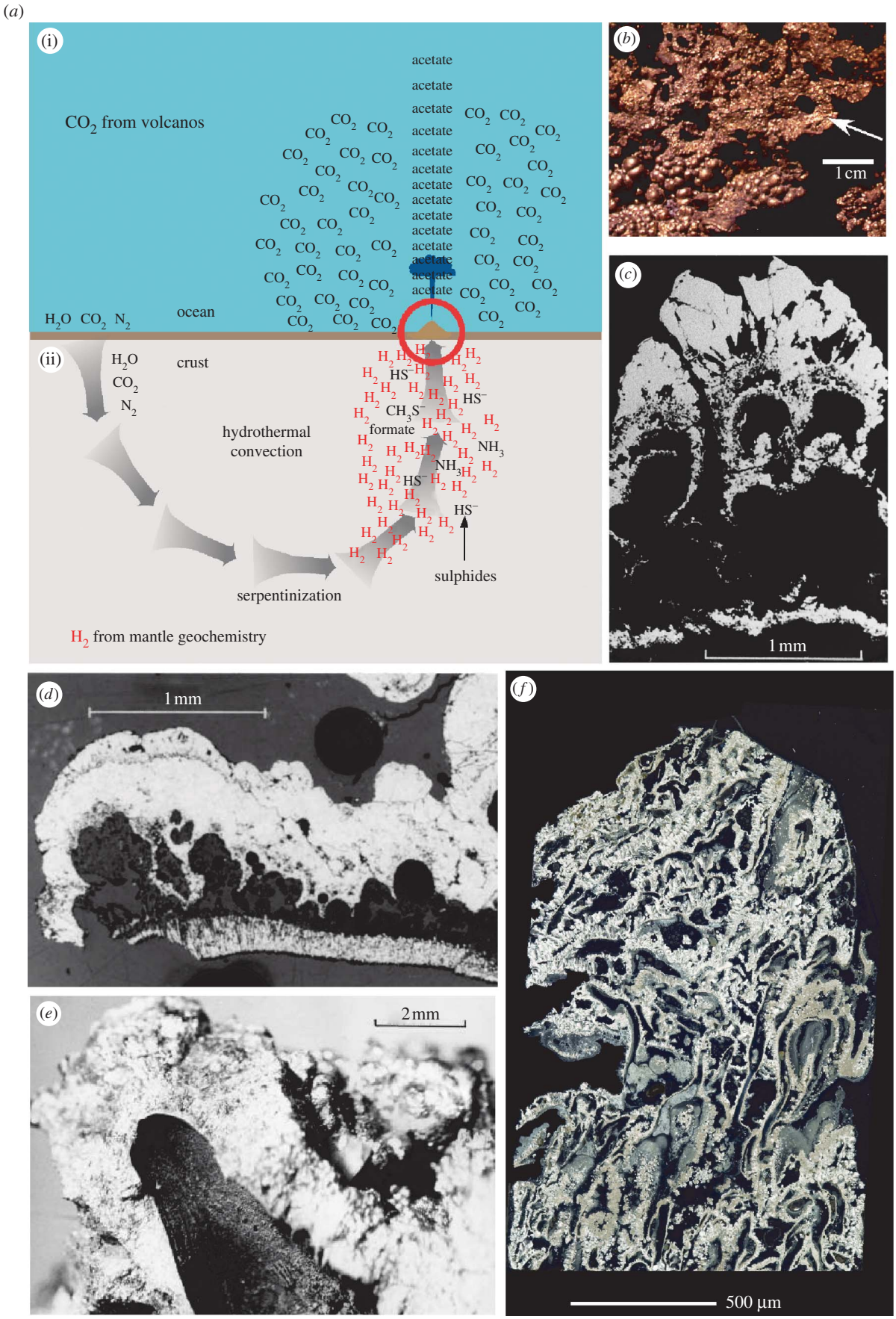


Figure 8. Hydrothermal starting conditions for biochemical origins. (*a*) Redox gradient in the early ocean, with (i) CO_2 from volcanoes meeting hydrothermal sulphide and (ii) H_2 at an alkaline vent with acetate production (Russell & Martin 2004) The red circle indicates a hydrothermal mound. (*b*) A metal sulphide orebody with botryoids (bubbles) of FeS owing to inflation of freshly precipitated FeS by vent water, the arrow indicating a small chimney in cross-section (Russell & Hall 1997). (*c*)–(*e*) Electron micrographs through botryoids (see Russell & Hall 1997). (*f*) Electron micrograph of a section from the Lost City alkaline vent (Kelley *et al.* 2001), kindly provided by Deborah Kelley.

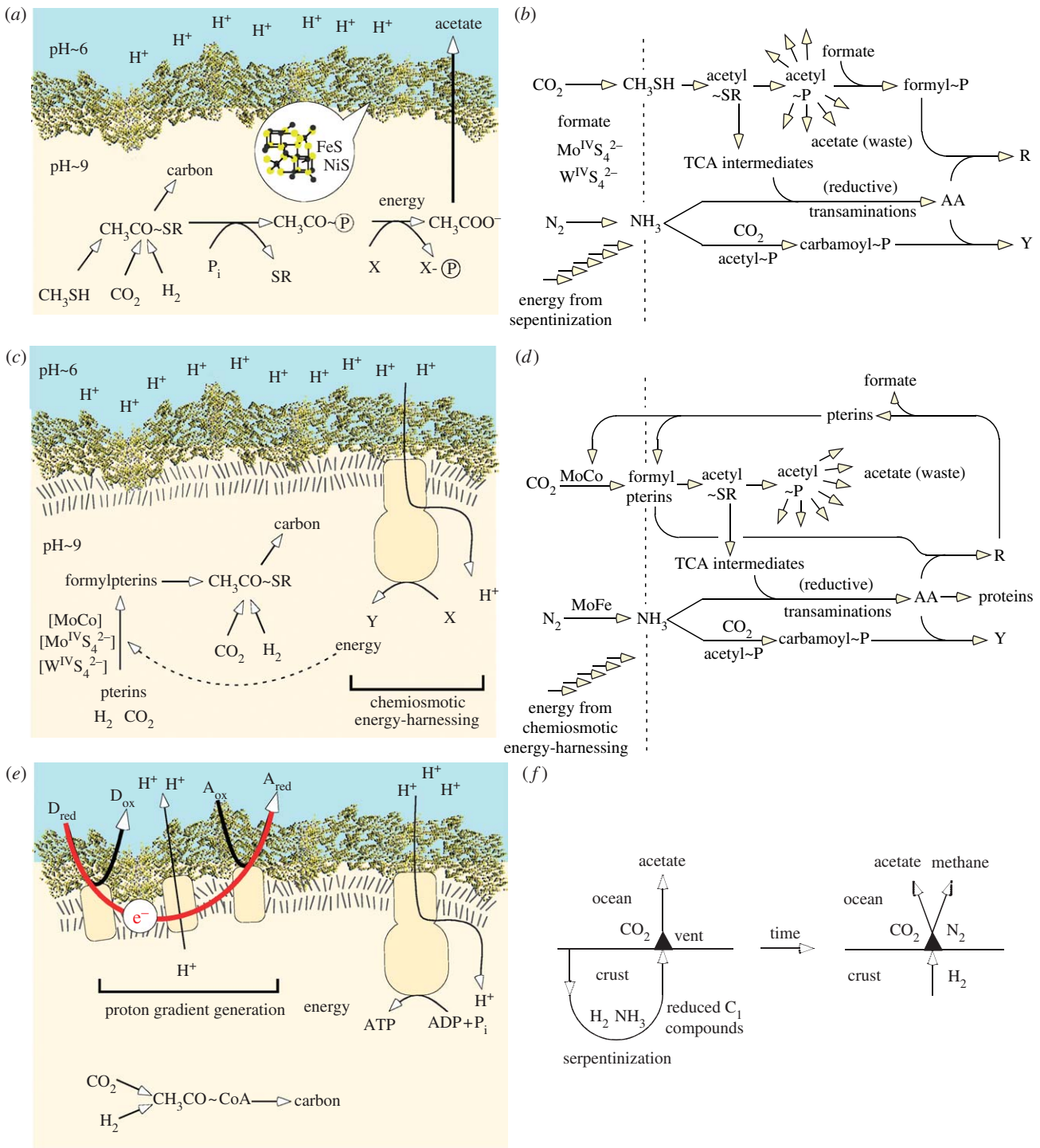


Figure 9. Possible stages in the early evolution of carbon and energy metabolism. (*a*) Carbon assimilation from methyl sulphide and CO_2 to acetyl thioesters and energy metabolism through acetyl phosphate via substrate level phosphorylation. This could, in principle, fuel the evolution of self-replicating systems into the world of genes and proteins. (*b*) Hypothetical biochemical map of early biochemistry at a stage that is still dependent on geochemically reduced C1 compounds. AA, amino acids; R, purines; Y, pyrimidines; P_i, inorganic phosphate; ~P, organic phosphate. Reducing power from H_2 is required at almost all steps (not shown). (*c*) With the advent of protein synthesis, harnessing the pre-existing proton gradient at the vent via simple proteins that conserve energy permits the use of pterin-dependent CO_2 fixation as in the modern Wood–Ljungdahl pathway (see text). The energy conserving reaction X→Y is kept general, because it can indicate pyrophosphate synthesis, ATP synthesis or Ech-type energy conservation (Hedderich 2004) as in formyl-MF dehydrogenase (see text). (*d*) Hypothetical biochemical map of early biochemistry at a stage that is independent of geochemically reduced C1 compounds by virtue of chemiosmotic energy harnessing. MoFe indicates the Mo-Fe-S centre of nitrogenase. Abbreviations as in (*b*). (*e*) With the advent of proteins that catalyse membrane-associated electron transport coupled to proton pumping, chemiosmotic potential can be generated autogenously, a prerequisite for the free-living lifestyle among autotrophs. Proton-pumping systems like Ech, that operate as a single, membrane-associated complex, without the help of quinones or analogues, such as methanophenazine, would possibly precede cytochrome-type (Schütz *et al.* 2000) proton-pumping systems. (*f*) Autotrophy equates to achieving independence from reduced carbon and nitrogen species supplied geochemically by serpentinization, and requires mechanisms for both autogenous proton pumping and chemiosmotic energy harnessing.

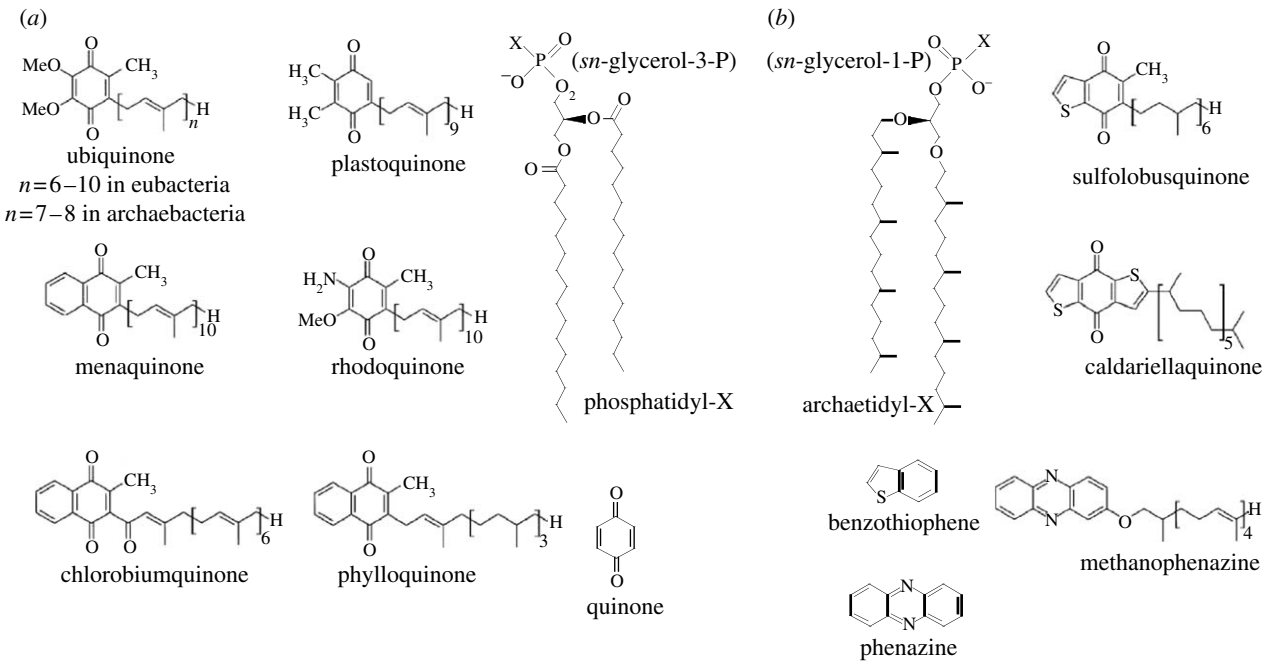
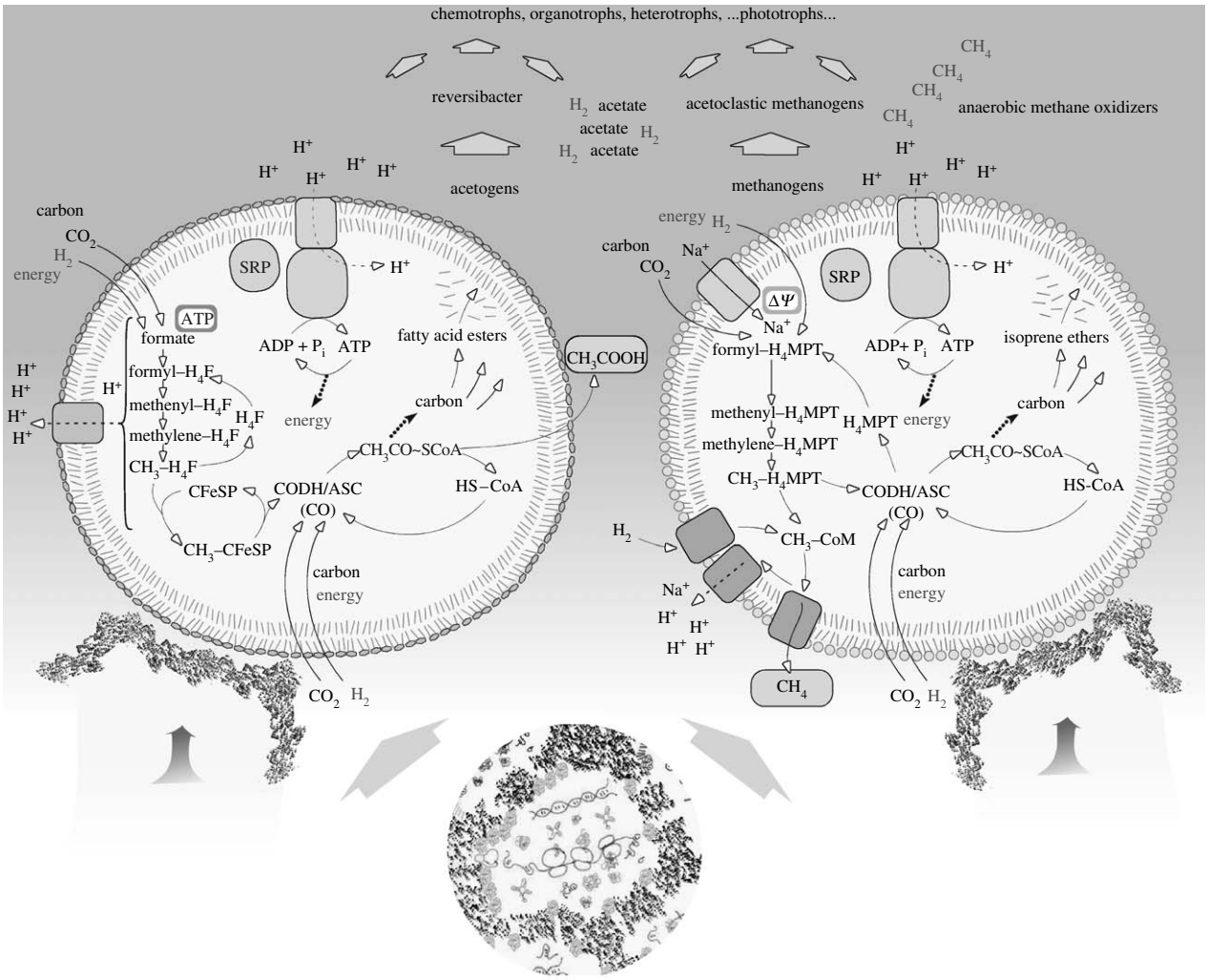


Figure 10. Quinones, quinone analogues and lipids. Structures from Berry (2002) and Lengeler *et al.* (1999). (*a*) Compounds common among or specific to eubacteria (with the exception of ubiquinone). (*b*) Compounds specific to archaebacteria. Note the difference in glycerol configuration (Koga *et al.* 1998). There has been a claim that archaebacteria synthesize eubacterial-type phospholipids (Pereto *et al.* 2004), but reading of the original literature behind that claim (Gattinger *et al.* 2002) reveals that the archaebacteria in question were grown on yeast extract and/or with fatty acid supplements; the methanogens contained the lipids (Gattinger *et al.* 2002), the inference that they synthesized them (Pereto *et al.* 2004) is tenuous.

(Cavalier-Smith 2002), and could have been present at this stage. Cytochrome *bc*-type (and Rieske-type) proteins are almost as universally distributed among cells as the ATPase is (Schütz *et al.* 2000), and such proteins are also possible candidate early SRP substrates. The early formyl pterin problem suggests that chemiosmosis-harnessing ATPases are probably older than formyl pterin synthesis from CO_2 alone, and hence predate autotrophy in the strict sense.

The next important step, in bioenergetic terms, would be the origin of simple kinds of proteinaceous proton-pumping redox chemistry (figure 9*e*): biologically portable means to generate a harnessable ion gradient via chemistry that is specifiable by genes. Perhaps, the simplest—and possibly the oldest—modern proton-pumping system is the energy converting hydrogenase Ech, which (i) catalyses the reversible cleavage of H_2 into protons and electrons, (ii) can generate transmembrane ion gradients without the participation of either quinones or their methanophenazine analogues among methanogens, (iii) can harness transmembrane ion gradients to promote thermodynamically difficult redox reactions, and (iv) is also essential for the formyl-MF dehydrogenase reaction in methanogens (reviewed by Hedderich 2004). Most other proton-pumping systems involve quinones or their analogues (Berry 2002) and entail several complexes for the transfer of electrons from an available reduced donor (designated in figure 9*e* as D_{red}) such as H_2 to an available oxidized acceptor (designated in figure 9*e* as A_{ox}) such as CO_2, to yield a reduced end product, such as acetate or methane. Such complexes would require the (genetically encoded) synthesis of specific membrane-soluble two electron carriers, like quinones (in acetogens) or methanophenazine (in methanogens), and eventually the lipids themselves—isoprenoid ethers versus fatty acid esters of different glycerol stereochemistry (Koga *et al.* 1998)—as outlined in figure 10. The circumstances that the membrane-soluble electron carriers, the lipids, the reduced end products of proton-pumping redox chemistry, and the chemiosmosis-dependent mechanisms of formyl pterin synthesis differ in acetogens and methanogens, respectively, suggest to us that these genetically encoded attributes arose in independent genetic lineages that were spatio-temporally differentiated within the confines of the vent and that were not yet free-living prokaryotes (Martin & Russell 2003). Even the chemical nature of cell walls that make prokaryotic plasma membranes turgor resistant (Kandler & König 1998) and the mechanisms of DNA maintenance differ among eubacteria and archaebacteria (Leipe *et al.* 1999; Poole & Logan 2005). Such fundamental differences can be attributed to independent origins of the underlying genes via the same spatiotemporal differentiation process (Koonin & Martin 2005). Differentiated H_4F and H_4MPT biosynthetic pathways eventually became established in their modern forms (Graham & White 2002), as did pathways for other cofactors that tend to distinguish the two main groups of prokaryotes, for example, MF (figure 2), coenzyme B, coenzyme M, F_{420} (figure 2) and cobamids (Graham & White 2002), or even for the biosynthesis of some cofactors that are universal but are synthesized by unrelated proteins in archaebacteria and eubacteria, such as acetyl-CoA (Genschel 2004). The genetic invention of Mo-containing nitrogenases to synthesize NH_4^+ from N_2, instead of utilizing geochemically supplied NH_3, would probably also be a prerequisite



a universal ancestor that was physically confined within three-dimensional transition metal sulphide compartments at vent

Figure 11. Cutting loose. Escape from the vent was only possible when genetically encoded lipid synthesis and cell wall synthesis had been achieved, and when autogenous formyl pterin synthesis as well as *ab initio* ion-pumping mechanisms had been developed for bioenergetic reasons relating to energy conservation efficiency (see text), but in independent lineages of energetically sustainable and genetically replicating ensembles within the network of FeS compartments (see also Russell & Hall 1997; Martin & Russell 2003; Koonin & Martin 2005). The ancestral state of eubacterial physiology would be acetogenesis and that of archaebacterial physiology would be methanogenesis, followed by anaerobic microbial communities (Schink 1997) utilizing H_2, acetate, methane and similar small organic compounds.

for departure from the vent. In a sense, the origin of autotrophy could be seen as a process of making the hard chemistry of CO_2 and N_2 reduction, which was initially provided by serpentinization, locally specifiable by discrete collections of genes and hence portable (figure 9*f*).

The next step would be the emergence of free-living chemoautotrophs (figure 11). We suggest that the first free-living eubacteria survived from acetogenesis, that the first free-living archaebacteria survived from methanogenesis (figure 11), and that these stem lineages emerged independently from the inorganic confines of the vent at which they arose. Recent evidence for biological methane production at 3.45 Gyr ago (Ueno *et al.* 2006) would be compatible with the view presented here. Those chemolithoautotrophic lifestyles would be self-sustaining starting points for further biochemical evolution, through both descent with modification and lateral gene transfer (Doolittle 1999) in the two prokaryotic domains. New and specialized inventions in the seemingly boundless diversity of biochemical machineries that generate chemiosmotic potential (Schäfer *et al.* 1999; Baymann *et al.* 2003) would follow. The antiquity that we attribute to acetogenesis and methanogenesis stems from the specific circumstance that these organisms synthesize their ATP at the expense of reducing CO_2.

Utilizing the waste products produced by other microbes (figure 11), the first microbial communities would surely have been anaerobic and might have entailed acetoclastic methanogenesis (Daniels 1993), *Reversibacter*-like metabolism in which acetogenesis runs in the reverse direction (Zinder 1994), and anaerobic methane oxidation (Krüger *et al.* 2003; Shima & Thauer 2005). In terms of their metabolic starting and end products, ancestral microbial communities might have looked very similar to modern anaerobic syntrophic

communities (Fenchel & Finlay 1995; Schink 1997), except that there were no eukaryotes, all of which are heterotrophs (Martin & Müller 1998) and hence arose much later in evolution (Rivera & Lake 2004), after the origin of α-proteobacteria, because all known eukaryotes are ancestrally mitochondrion-bearing (Embley & Martin 2006).

18. CONCLUSION

The overall chemical design of methanogenesis and acetogenesis suggests that simple organic reactions catalysed by transition metal sulphides, as in CODH/ACS and pyruvate synthase, are the starting point of biochemistry, that its prime product was an acetyl thioester, and that the pterin-dependent methyl synthesis branch came to supplant pre-existing inorganic methyl synthesis. The main early flux of carbon into metabolism appears to have occurred via the Wood–Ljungdahl pathway, entailing a mechanism of formyl pterin generation that was dependent upon geochemical processes linked in some manner to the Earth's crust. Early carbon metabolism might have taken root at acetyl-CoA, proceeding via pyruvate and oxalacetate into the incomplete reductive TCA cycle, giving rise to central, but mostly achiral, carbon backbones for further essential biosyntheses, just as it occurs in acetogens and methanogens today. The synthesis of acetyl phosphate via phosphorolysis of acetyl thioesters might be the ancestral state of phosphoryl transfer potential and phosphoanhydride bonds in biochemistry, as de Duve (1991) inferred previously, but assuming very different starting conditions. The chemiosmosis-dependent nature of CO_2 fixation in methanogens and acetogens indicates that the ability to harness proton gradients in these pathways must be older than the ability to generate them with a chemistry that is specified by genes.

Phosphorylation via acetyl phosphate, derived from acetyl thioesters, seems to be sufficient to support the sustained evolution of a very complicated and advanced self-replicating system, one that would have had a modern genetic code, ribosomes and proteins, but that would not have been free living, rather confined to its inorganic housing instead.

One might interject that in early times, there could have existed forms of life that differed in fundamental biochemical architecture from the forms of life that exist today, and that they became extinct without having left a trace. While this cannot be excluded, it would not address the origin of the life forms that are observed today; hence it appears to be irrelevant to the problem. By assuming that early biochemistry was fundamentally different from today's, one can obtain more freedom to think about the problem, but at the steep price of losing all logical constraints on the topic that would link it stringently to the explanandum (modern biochemistry). The premise that there is a trace of evolutionary history to be deciphered in modern biochemistry is what de Duve has called 'congruence' and Morowitz called 'historical continuity'.

A geological setting other than an alkaline hydrothermal vent for the origin of biochemistry might lead to a better model that more thoroughly accounts for available observations. Alternatives must, however, also be thermodynamically favourable. There are many aspects of our considerations that are not substantiated by evidence at all; for example, the exact source of RNA-like bases. But at the same time, we are suggesting that it may not be possible to efficiently synthesize bases or essential cofactors in the laboratory without compounds that belong to the realm of biochemistry; hence, that approach might have not yet received sufficient experimental attention. The chemical environment that we suggest for the synthesis of the RNA world contains sulphide and active methyl, acetyl, formyl and carbamoyl moieties, and it is possible that the chemical nature of some base modifications in tRNA and rRNA, such as 2-thiocytidine, 2-thiouridine, 5-methyl-2-thiouridine, 5-carbamoylmethyluridine, N^2, N^2-dimethylguanosine, N^4-acetylcytidin, 5-formylcytidine and the like (Limbach *et al.* 1994; Sprinzl *et al.* 1998), might represent biochemical relicts from a time when base synthesis was not as precise as it is today.

The synthesis of universal cofactors in early biochemistry (such as pterins, thiamine, pyridoxal and the like) might be more critical in getting an ancient but very complex biochemistry going than has traditionally been presumed. Most present approaches to prebiotic biochemistry strive to obtain end products of biochemical pathways, perhaps with the notion that if enough of them are synthesized, they will eventually react in a retrograde manner so as to generate a core metabolism (Horowitz 1945). Perhaps, the synthesis of cofactors (or their analogues) is more important than the main products, because if the cofactors can perform catalysis without enzymes for a few important reactions, as in the old transamination experiments with PLP (Metzler & Snell 1952*a*,*b*) or glutamine alone (Nakada & Weinhouse 1953), then carbon flow would be improved.

Cofactors often contain the essential moieties of catalysis; they channel brunt carbon flow to the building blocks of life while allowing the overall redox reaction that makes life possible to occur more rapidly. The catalytic role of base-like cofactors other than G, A, C and U has always been a conceptually satisfying element of the RNA world (White 1976). But the catalytic roles of bases and base analogues that are not part of information storage, retrieval and expression (cofactors) would seem to assume the most crucial position in early biochemistry, because at some point in time, the four main bases have to be synthesized rather specifically and in large amounts, a process that would clearly be aided by cofactors. It seems to us that the four main bases are not the starting point of biochemical evolution, but instead the most utilitous substances that biochemistry ever invented.

Carbon and energy metabolism need not have evolved in the manner that we have suggested here. But had they done so, we would be able to recognize that imprint in the biochemistry of modern microbes, and biochemical maps would harbour clues as to how it occurred.

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NOTICE OF CORRECTION

The citation for and text of the quotation in the left column of page 7 are now correct, and the details of the citation (Thauer *et al.* 1996) have been added to the reference list on page 38.

5 June 2007