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REVIEW ARTICLE

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Comparative biochemistry of CO_2 fixation and the evolution of autotrophy

Summary Carbon dioxide fixation is a polyphyletic trait that has evolved in widely separated prokaryotic branches. The three principal CO_2-assimilation pathways are (i) the reductive pentose-phosphate cycle, i. e. the Calvin-Benson cycle; (ii) the reductive citric acid (or Arnon) cycle; and (iii) the net synthesis of acetyl-CoA from CO/CO_2, or Wood pathway. Sequence analysis and the comparative biochemistry of these routes suggest that all of them were shaped to a considerable extent by the evolutionary recruitment of enzymes. Molecular phylogenetic trees show that the Calvin-Benson cycle was a relatively late development in the (eu)bacterial branch, suggesting that some form(s) of carbon assimilation may have been operative before chlorophyll-based photosynthesis. On the other hand, the ample phylogenetic distribution of both the Arnon and the Wood pathways does not allow us to infer which one of them is older. However, different lines of evidence, including experimental reports on the NiS/FeS-mediated C–C bond formation from CO and CH_3SH are used here to argue that the first CO_2-fixation route may have been a semi-enzymatic Wood-like pathway.

Key words Calvin-Benson cycle · Arnon cycle · Wood pathway · Semi-enzymatic synthesis · Carbon dioxide assimilation

Introduction

Several different mechanisms for biological CO_2 fixation account for the diversity and evolutionary success of autotrophic life. According to the classical formulation of the heterotrophic theory of the origin of life [30], once the supply of abiotic organic compounds had become a limiting factor, primitive cells evolved other ways of obtaining carbon and energy. This led first to the development of photoautrophy, and afterwards to oxygen-releasing photosynthesis [30]. Several lines of evidence support the antiquity of the reductive pentose-phosphate pathway, or Calvin-Benson cycle. These include (i) the cyanobacteria-like microfossils in the 3.5 3 10^9 year old Australian Apex sediments, which suggest that the Calvin-Benson cycle appeared during early Archean times [35]; and (ii) the isotopic fractionation profiles of the early Archaean carbon cycle, which are consistent with the ribulose bisphosphate carboxylase/oxygenase (rubisco)-catalyzed carbon fixation process [15]. However, 16/18S rRNA-based universal phylogenies indicate that chlorophyll-based photosynthesis was a relatively late development in the (eu)bacterial branch [31, 45]. Thus, it is likely that the first autotrophs used chemical energy rather than light, and that the reductive pentose-phosphate pathway was preceded by alternative, older modes of CO_2-assimilation autotrophy.

Carbon dioxide assimilation is a widespread biological trait, but the biochemical dissimilarities between different pathways by which it takes place suggest that this ability evolved convergently in widely separated prokaryotes. In addition to the Calvin-Benson cycle, there are other CO_2-assimilation mechanisms, including (i) the reductive citric acid pathway, or Arnon cycle; (ii) the reductive acetyl-CoA cycle, i.e., the Wood pathway (sometimes also referred to as the Ljundahl-Wood pathway); and (iii) other less common mechanisms, such as the hydroxypropionate pathway first found in *Chloroflexus*, a green non-sulfur photosynthetic bacteria.

Twenty years ago it was argued that the ribulose-diphosphate cycle evolved via the ribulose monophosphate cycle in an ancestral heterotrophic population [32]. More recently, Wächtershäuser [41] has proposed a chemoautotrophic scheme of the origin of life in which pyrite formation is linked with early CO_2 fixation. In this paper we propose that none of these two alternatives is correct, and that the phylogenetic distributions of the Arnon and Wood pathways do not indicate by themselves which of these two cycles is the oldest. We also argue that energetic considerations and the experimental evidence on the NiS/FeS-mediated formation of C–C bonds from CO and CH_3SH [17] can be interpreted as supporting the hypothesis that a Wood-like semi-enzymatic pathway was the earliest biological carbon fixation route.

Biological carbon fixation can take place by different mechanisms

The reductive pentose-phosphate pathway, or Calvin-Benson cycle During the 1950s Calvin and his associates established, in a series of elegant experiments, the pathway by which CO_2 is assimilated by photoautotrophic eukaryotes [5]. The Calvin-Benson cycle, which originated in the cyanobacterial ancestors of chloroplasts, is the outcome of a process in which enzyme recruitment had a major role. As summarized in Table 1, in biochemical terms there are only two enzymes unique to the Calvin-Benson cycle, namely phosphoribulokinase, or ribulose 5-phosphate kinase (PRK), and ribulose bisphosphate (RuBP) carboxylase/oxygenase (rubisco). The other eleven chemical reactions are catalyzed by eight different enzymes (nine in plastids) that have additional roles in several heterotrophic pathways, such as glycolysis/gluconeogenesis and the pentose-phosphate oxidative route. Thus, a major portion of the Calvin cycle may be explained as the result of a patchwork assembly of a route [20] from pathways already extant in previously evolved heterotrophic anaerobes, such as the ability to synthesize pentoses from C_3- or C_6-compounds [29].

Analysis of the completely-sequenced genomes of *Methanococcus jannaschii* [4] and the closely related euryarchaeota *Archaeglobus fulgidus* [23] has led to the identification of ORFs which exhibit considerable levels of similarity with the rubisco large subunit (Table 1). Identification of rubisco-homologues in these euryarchaeotal genomes confirms previous reports of the presence of this enzyme in some non-retinal-containing species of halobacteria [1]. However, there is no report of Calvin-Benson cycle-based autotrophic growth in archaebacteria, and it has been suggested that the presence of rubisco-like sequences in archaeal genomes is due to horizontal transfer phenomena [22].

Primary structure comparisons do not indicate any obvious evolutionary relationships between rubisco and all the other

Table1 Enzymatic steps in the Calvin-Benson cycle

Enzyme	EC	Reaction	Other pathways	Distribution
phosphoribulokinase	2.7.1.19	ATP + D-ribulose 5-phosphate = ADP + D-ribulose 1,5 bisphosphate		B, E
ribulose bisphosphate carboxylase	4.1.1.39	D-ribulose 1,5-bisphosphate + CO_2 = 2,3-phospho-D-glycerate	glyoxylate & dicarboxylate metabolism	B, E (Small subunit) B, A, E (Large subunit)
phosphoglycerate kinase	2.7.2.3	ATP + 3-phospho-D-glycerate = ADP + 3-phospho-D-glyceroyl phosphate	glycolysis/gluconeogenesis	B, A, E
glyceraldehyde-3-phosphate dehydrogenase	1.2.1.13	D-glyceraldehyde 3-phosphate + phosphate + $NADP^+$ = 3- phospho-D-glyceroyl phosphate + NADPH		B, E
triosephosphate isomerase	5.3.1.1	D-glyceraldehyde 3-phosphate = glycerone phosphate	glycolysis/gluconeogenesis; fructose & mannose metabolism glycerolipid metabolism	B, A, E
fructose-bisphosphate aldolase	4.1.2.13	D-fructose 1,6-bisphosphate = glycerone phosphate + D-glyceraldehyde 3-phosphate	glycolysis/gluconeogenesis pentose phosphate cycle; fructose & mannose metabolism	B, E
fructose-bisphosphatase	3.1.3.11	D-fructose 1,6-bisphosphate + H_2O = D-fructose 6-phosphate + phosphate	glycolysis/gluconeogenesis pentose phosphate cycle; fructose & mannose metabolism	B, E
transketolase	2.2.1.1	Sedoheptulose 7-phosphate + D-glyceraldehyde 3-phosphate = D-ribose 5-phosphate + D-xylulose 5-phosphate	pentose phosphate cycle	B, A, E
ribulose-phosphate 3-epimerase	5.1.3.1	D-ribulose 5-phosphate = D-xylulose 5-phosphate	pentose phosphate cycle; pentose & glucuronate interconversions	B, A, E
ribose 5-phosphate epimerase	5.3.1.6	D-ribose 5-phosphate = D-ribulose 5-phosphate	pentose phosphate cycle	B, A, E

B= Bacteria; A= Archaea; E= Eukarya

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 1  prk  At   IVIG LAADSGCGK----ST FMRRLTSVFGGAAKP PKGGNPDSNTLISDT TTVICLDDYHSLD-- -RYGRKEQKVTALD- PRANDFDLMYEQVKA
 2  prk  Mc   IVIG LAADSGCGK----ST FMRRLTSVFGGAAEP PRGGNPDSNTLISDT TTVICLDDYHSLD-- -RTGRKEKGVTALD- PRANDFDLMYEQVKA
 3  prk  Ta   IVIG LAADSGCGK----ST FMRRLTSVFGGAAEP PKGGNPDSNTLISDT TTVICLDDYHSLD-- -RTGRKEKGVTALD- PKANDFDLMYEQVKA
 4  prk  So   IVIG LAADSGCGK----ST FMRRLTSVFGGAAEP PKGGNPDSNTLISDT TTVICLDDFHSLD-- -RNGRKVEKVTALD- PKANDFDLMYEQVKA
 5  prk  Cr   VVIG LAADSGCGK----ST FMRRMTSIFGGVPKP PAGGNPDSNTLISDM TTVICLDDYHCLD-- -RNGRKVKGVTALA- PEAQNFDLMYNQVKA
 6  prk  Ssp  VLIG VAADSGCGK----ST FLRRLTDLFG----- ------------EEF MTVICLDDYHSLD-- -RQGRKAAGVTALD- PRANNFDLMYEQIKT
 7  udk  Bs   VVIG IAGGSGSGK----ST VTRSIYEQFK----- ------------GHS ILMIQQDLYYKDQS- -HLPFEERLNTNYDH PLAFDNDYLIEHIQD
 8  udk  Ec   VIIG IAGASASGKSLIAST LYRELREQVG----- ------------DEH IGVIPEDCYYKDQS- -HLSMEERVKTNYDH PSAMDHSLLLEHLQA
 9  udk  Mg   ILVA ISGGSCSGK----TT VAEMIYQLLS----- -----------KKLK VAIICQDNYYKSYK- -NKPLLKRKTINFDH PDAFDWKLLRSHIED
 10 urk1 Sc   YIIG IGGASGSGK----TT VAAKIVSSIN----- ------------VPW TVLISLDNFYNPLGP EDRARAFKNEYDFDE PNAINLDLAYKCILN

 1  prk  At   LKNGIAVEKPIYNHV TGLLD--PPELIQPPKILVIEGLHPMFDER VRDLLDFSIYLDISN EVKFAWKIQRDMAER GHSLESIKASIE-ARKPDFDA
 2  prk  Mc   LKEGKAVEKPIYNHV TGLLD--APELIKPPKILVIEGLHPMFDSR VRDLLDFSIYLDISN EVKFAWKIQRDMAER GHSLESIKASIE-ARKPDFDA
 3  prk  Ta   IKEGKAIEKPIYNHV TGLLD--PAELIQPPKIFVIEGLHPMYDER VRELLDFSIYLDISN EVKFAWKIQRDMAER GHSLESIKASIE-ARKPDFDA
 4  prk  So   LKEGKAVDKPIYNHV SGLLD--PPELIQPPKILVIEGLHPMYDAR VRELLDFSIYLDISN EVKFAWKIQRDMKER GHSLESIKASIE-SRKPDFDA
 5  prk  Cr   LKEGKSVDKPIYNHV SGLID--APEKIESPPILVIEGLHPFYDKR VAELLDFKIYLDIST DIKFAWKIQRDMAER GHSLESIKSSIA-ARKPDFDA
 6  prk  Ssp  LKSGQSIMKPIYNHE TGLLD--PPEKVEPNKVVVIEGLHPLYDER VRELVDFGVYLDISE EVKINWKIQRDMAER GHTYEDILASIN-ARKPDFTA
 7  udk  Bs   LLNYRPIEKPIYDYK LHTRSE-ETVHVEPKDVIILEGILVLEDKR LRDLMDIKLYVDTDA DLRIIRRIMRDINER GRSIDSVIEQYVSVVRPMHNQ
 8  udk  Ec   LKRGSAIDLPVYSYV EHTRMK-ETVTVEPKKVIILEGILLLTDAR LRDELNFSIFVDTPL DICLMRRIKRDVNER GRSMDSVMAQYQKTVRPMFLQ
 9  udk  Mg   LLNGSIVNVPLYDYI NYTRAK-KTAKIGPIDVVILEGLMPWFDEK LSRLSKLKIFIETNG EERLIRRIERDWQ-R GRNIDSIIKQWREIVAPMYEI
 10 urk1 Sc   LKEGKRTNIPVYSFV HHNRVPDKNIVIYGASVVVIEGIYALYDRR LLDLMDLKIYVDADL DVCLARRLSRDIVSR GRDLDGCIQQWEKFVKPNAVK

 1  prk  At   FIDPQKQYA DAVIEVLPTTLIPDD NEGKVLRVRLIMKEG VKYFSPVYL------
 2  prk  Mc   YIDPQKQYA DAVIEVLPTQLIPGD NEGKVLRVRLIQKEG VQYFSPVYL------
 3  prk  Ta   FIDPQKQYA DAVIEVLPTQLIPDD NEGKVLRVKLIMKEG IKFFNPVYL------
 4  prk  So   YIDPQKQHA DVVIEVLPTELIPDD DEGKVLRVRMIQKEG VKFFNPVYL------
 5  prk  Cr   YIDPQKKDA DMIIQVLPTQLVPDD -KGQYLRVRLIMKEG SKMFDPVYL------
 6  prk  Ssp  YIEPQKQYA DVVIQVLPTRLIEDK -ESKLLRVRLVQKEG VKFFEPAYL------
 7  udk  Bs   FVEPTKRYA DIIIPEGGQNHVAID ---------LMVTKI QTILEQNAI------
 8  udk  Ec   FIEPSKQYA DIIVPRGGKNRIAID ---------ILKAKI SQFFE----------
 9  udk  Mg   FVEKMKRNA DLILPWSQRREVSTS ---------VLDVAI EHLFHKTVE------
 10 urk1 Sc   FVKPTMKNA DAIIPSMSDNATAVN LIINHIKSKLELKSN EHLRELIKLGSSPSQ
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Fig. 1 Multiple sequence alignment of a conserved phosphoribulokinase (ribulose 5-phosphate kinase) motif with its bacterial and eukaryotic uridine kinase/cytidine kinase homologues

Abbreviations: prk, phosphoribulokinase; udk, uridine kinase/cytidine kinase; At, *Arabidopsis thaliana*; Mc, *Mesembryanthemum crystallinum*; Ta, *Tritricum aestivum*; So, *Spinacia oleracea*; Cr, *Chlamydomonas reinhardtii*; Ssp, *Synechocystis* sp. PCC6803; Bs, *Bacillus subtilis*; Ec, *Escherichia coli*; Mg, *Mycoplasma genitalium*; Sc, *Saccharomyces cerevisiae*

sequences found in the available databases. On the other hand, phosphoribulosekinase (*prk*) is probably derived through gene duplication and divergence events from an ancestral, less specific kinase. This possibility is supported by a conserved 200-odd amino acid segment that phosphoribulokinase sequences share with uridine kinase/cytidine kinase (*udk*), a pyrimidine ribonucleotide biosynthetic enzyme that catalyzes the U(C)+ GTP = U(C)MP + GDP reaction (Fig. 1).

In every examined case, the carboxylating enzyme rubisco has been shown to perform the oxygenolytic cleavage of RuBP. This bifunctional character is the result of the competition between O_2 and CO_2 for the same catalytic site. From an autotrophic viewpoint, the inhibitory effect of oxygen on carbon assimilation (or photorespiration) appears to be a wasteful process. However, the ancestral carboxylase probably evolved in a CO_2-rich environment in which very little free oxygen was available. Throughout Earth's geological history this situation has been reverted, and rubisco appears as the outcome of an adaptative process that led to an increase in the ratio of substrate specificities CO_2/O_2 that compensated for the O_2 inhibitory effect. Analysis of the different strategies followed by the different photosynthetic groups that contain rubisco to reduce the impact of increasing oxygen pressures, such as the CO_2-concentrating mechanisms in cyanobacteria and algae, and the Hatch-Slack pathway in C4 plants, make this trend rather obvious [7].

Although it is possible that subterranean lithotrophs contribute significantly to biological carbon fixation [31], today the Calvin-Benson cycle appears to be responsible for the bulk of biological CO_2-fixation. It is present in all photosynthetic microorganisms, including cyanobacteria and purple bacteria, and also in some Gram-positive chemolithotrophs. As reviewed by Margulis [28], this appears to be the only autotrophic route acquired by eukaryotes through symbiotic events, involving either photoautotrophic or chemolithotrophic prokaryotes.

The Arnon cycle: the reductive citric acid pathway In 1966 Arnon and his coworkers proposed that carbon assimilation in the bacterium *Chlorobium limicola* (which photochemically disproportionates elementary sulphur to sulphide and sulphate), proceeds not by the standard Calvin-Benson cycle, but via a reductive citric acid cycle, or reverse Krebs cycle [3]. As summarized in Table 2, this pathway requires two additional enzymes from those involved in the cyclic oxidation of acetyl-CoA, namely ferredoxin-dependent 2-oxoglutarate, and ATP-citrate lyase*. Recent structural comparisons have shown that

* Our search for homologues of ATP -citrate lyase indicated a considerable level of sequence similarity with NCBI entry 482640 (pir A60956), which was originally deposited as a 300 amino acid fragment of the sea urchin embryonic ciliary dynein beta heavy chain [11]. However, detailed analyses of both dynein and ATP- citrate lyase sequences demonstrated that NCBI 482640 was misidentified, and that it is not a member of the dyneins but a ATP citrate lyase.

Table 2 Enzymatic steps in the reductive citric acid cycle (Arnon pathway)

Enzyme	EC	Catalyzed reaction	Other pathways	Distribution
2-oxoglutarate synthase	1.2.7.3	2-oxoglutarate + CoA + oxidized ferredoxin = succinyl CoA + CO_2 + reduced ferredoxin	citric acid cycle	B, A
isocitrate dehydrogenase ($NADP^+$)	1. 1.1.42	isocitrate + $NADP^+$= 2- oxoglutarate + CO_2 + NADPH	citric acid cycle; glutathione metabolism	B, A, E
aconitate hydratase	4. 2. 1. 3	citrate= cis-aconitate + H_2O	glyoxylate & dicarboxylate metabolism; citric acid cycle	B, E
ATP-citrate lyase	4. 1. 3. 6	citrate = acetate + oxacetate	citric acid cycle	B
malate dehydrogenase	1.1.1.37	(s)-malate + NAD^+ = oxaloacetate + NADH	citric acid cycle; piruvate, glyoxylate & dicarboxylate metabolism	B, A, E
fumarate hydratase	4.2.1.2	(s)-malate = fumarate + H_2O	citric acid cycle	B, A, E
succinate dehydrogenase	1.3.99.1	succinate + acceptor = fumarate + reduced acceptor	citric acid cycle; oxidative phosphorilation; butanoate metabolism	B, E
succinate-CoA ligase (ADP-forming)	6.2.1.5	ATP + succinate + CoA = ADP + succinyl CoA + phosphate	citric acid cycle; propanoate metabolism; C5-branched dibasic acid metabolism	B, A, E

B= Bacteria; A= Archaea; E= Eukarya

ATP citrate lyase belongs to an enzyme superfamily characterized by an unusual nucleotide-binding fold, i.e., the palmate- or ATP-grasp fold. This superfamily includes other ATP-dependent carboxylate-thiol ligases (succinate- and malate-CoA ligases), as well as enzymes endowed with carboxylate-amine ligase activity (glutathione synthetase, biotin carboxylase, and carbamoyl-phosphate synthetase) [13].

The reductive citric acid cycle is found in both bacterial and archaeal prokaryotes. It was first reported in the moderately thermophilic hydrogen-oxidizing *Hydrogenobacter thermophilus*, the aerobic *Aquifex pyrophilus*, and the sulphate reducer proteobacteria *Desulforobacter hydrogenophilus*, as well as in archeal species including members of the aerobically grown *Sulfolobus* genus, and *Thermoproteus neutrophilus* (when grown with H_2 and elemental suphur) [12, 34]. The wide distribution of this anabolic pathway and its modifications (such as the reductive acetyl-CoA or the reductive malonyl-CoA pathways) among anaerobic archaea and the most deeply rooted eubacteria strongly suggest that it evolved prior to the Calvin-Benson cycle [22]. This cycle is currently favored as the primordial metabolic pathway by the adherents of the pyrite-based chemoautotrophic theory of the origin of life [41].

The reductive acetyl CoA cycle (Wood pathway) Although A. F. Lebedeff had suggested in 1908 that direct assimilation of CO_2 is a widespread biological trait, it was not until sixty years later that Wood and his co-workers [26] demonstrated the fixation of atmospheric carbon dioxide into reduced organic compounds by the heterotrophic propionic acid bacteria [47, 48]. Further studies demonstrated that assimilation of CO_2 by the net synthesis of acetyl CoA could sustain autotrophic growth in *Clostridium thermoaceticum* [50]. Fixation by the autotrophic reductive acetate pathway is a simple process that involves the combination of two CO_2 (or CO) molecules to form a two-carbon compound, from which reduced organic components are formed by non-autotrophic or anaplerotic carboxylation processes and other typical heterotrophic reactions [48]. The first reaction in this pathway is the reduction of carbon dioxide to CH_3, which reacts as methyltetrahydrofolate with CoASH to form acetyl-CoA via a carboxyl donor such as CO or CO_2. Instead of the typical biotin carboxyl carrier protein found in *E. coli* and animals, the intermediates are carrier-bound organometallic complexes involving Ni, Fe, and S. In this cycle the breakage and formation of the thioester bond between CoASH and the C=O group of the acetyl moiety are both catalyzed by CO dehydrogenase/acetyl CoA synthase. This bifunctional enzyme is the central catalyst in this pathway, and mediates both the oxidation of CO to CO_2 and the synthesis of acetyl-CoA [33, 49].

The Wood pathway, which is the major CO_2 fixation mechanism under anaerobic conditions [49], also has an ample phylogenetic distribution, and is known to be used by acetogenic bacteria, methanogens, and sulphate-reducers for both anabolic and catabolic purposes [44]. The list includes *Acetobacterium woodii* and *Sporomusa* sp., as well as the sulphate reducers *Desulfobacterium autotrophicum* and *Desulfovibrio baarsii*. It is also widely distributed among methanogens, including *Methanobacterium thermoautotrophicum*, *Methanosarcina barkeri*, and in the early diverging hyperthermophilic genera *Methanopyrus*, *Methanococcus*, and *Methanothermus* [12, 34]. In spite of

the differences in formate utilization and the peculiar cofactors employed by methanogens, the first steps of acetyl-CoA synthesis are similar among these autotrophic archaea and the eubacteria [21, 43, 46], confirming the monophyletic origin of this pathway. Although no carbon monoxide dehydrogenase activity has been found in *Archaeoglobus profundis*, it has been reported in *A. lithotrophicus* [38]. This finding is consistent with the presence of the Wood pathway genes in the *A. fulgidus* genomes [23], where they are probably involved in the anaerobic oxidation of acetate to CO_2.

The hydroxypropionate pathway Several studies on the photoautotrophic growth of the thermophilic, green non-sulphur bacterium *Chloroflexus aurantiacus* indicated that, in some strains, CO_2 was assimilated into reduced organic compounds via a pathway different from those described above. Labelling experiments with cells grown in the presence of the aconitase-blocking fluroacetate, demonstrated that acetyl-CoA could be both an intermediate and a product of this CO_2-fixation pathway [16, 36]. This cyclic route involves the carboxylation of acetyl-CoA, which is then reductively converted to form 3-hydroxy-propionate [16]. This unusual intermediate is first reduced and carboxylated to form propionyl-CoA, which is converted through a second carboxylation into succinate and then to malyl-CoA [10, 36, 37]. It is possible that the starting point of succinate biosynthesis via this pathway, which is present also in the archaea *Thermoproteus neutrophilus* [36] and in a somewhat modified form in *Acidianus brierleyi* [19], involves glyoxylate derived from the reordination and escission of 2-methyl malonyl CoA through the glyoxylate shunt (Stanley L. Miller, personal communication).

Is autotrophic CO_2-fixation a primordial process?

Several autotrophic theories on the origin of life have been proposed which do not require preformed organic compounds of abiotic origin. Two of these theories tie the origin of CO_2-assimilation pathways to the appearance of life, i.e., they assume the first living systems were already endowed with the ability of fixing atmospheric carbon dioxide. One such theory involving non-enzymatic reactions was patterned after extant biochemical pathways of intermediate metabolism assumes that the citric acid cycle started with acetyl-CoA by two CO_2-fixations [14]. The development of such a system is envisoned to require UV light, clays, and transition state metals, all of which are likely components of the primitive environment. However, such cyclic pathways need to be very efficient, or they will stop working. One such example is precisely the Krebs cycle, which comes to a complete standstill unless the oxalacetate lost by non-enzymatic decarboxylation is replaced. In any case, this theory has not been examined in detail, and there is no experimental evidence supporting its basic assumptions [25].

Currently the most popular and elaborate autotrophic theory on the origin of life is that of Wächtershäuser [39–42]. According to this hypothesis, life began with the appearance of an autocatalytic two-dimensional chemolithotrophic metabolic system based on the conversion of iron sulphide into the stable crystalline mineral pyrite (FeS_2). Synthesis and polymerization of organic compounds are assumed to have taken place on the surface of FeS and FeS_2 in environments that resemble those of deep-sea hydrothermal vents. Replication followed the appearance of non-organismical iron sulfide two-dimensional life, in which chemoautotrophic carbon fixation is assumed to have taken place by a reductive tricarboxylic (TCA) or citric acid cycle of the type originally described for the photosynthetic green sulphur bacterium *Chlorobium limicola*. The ample phylogenetic distribution of this anabolic cycle and its modifications (such as the reductive acetyl-CoA and the reductive malonyl-CoA pathways) have been interpreted as evidence of its primordial character, which is assumed to have been primed by a carbon dioxide fixation process akin to the reductive acetyl-CoA pathway [22, 27, 41].

The reaction $FeS + H_2S = FeS_2 + H_2$ is highly exergonic (ΔG^0 = –9.23 kcal/mol, E^0 = –620 mV), and it has been demonstrated to take place under anaerobic conditions at neutral pH and 100°C [9]. The FeS/H_2S combination is a strong reducing agent, and it provides an efficient source of electrons for the reduction of organic compounds under mild conditions. The FeS/H_2S combination can produce molecular hydrogen, and reduce nitrate to ammonia, acetylene to ethylene, thioacetic acid to acetic acid, as well as more complex synthesis [27], including peptide-bond formation by activation with carbon monoxide on (Ni, Fe)S surfaces [18].

However, these experimental results are also compatible with a more general, modified model of the classical heterotrophic theory in which pyrite formation is recognized as an important source of electrons for the reduction of organic compounds [24]. It is possible, for instance, that under certain conditions atmospheric CO_2 would have been photoreduced by ferrous iron in solution, and pyrite formation on submerged rocks might have reduced nitrogen to ammonia [2] and organic compounds. The essential question in deciding between this chemoautotrophic theory and the heterotrophic hypothesis on the origin of life is clearly not pyrite-mediated organic synthesis, but whether direct CO_2 reduction and synthesis of organic compounds can take place by a hypothetical two-dimensional living system that lacks genetic information [24].

A semi-enzymatic model for the first CO_2-fixation pathway

Given the strong dissimilarities in carbon dioxide assimilation routes that have developed in widely separated autotrophs, it is important to understand which of them is oldest and

how it evolved from previous heterotrophic modes of nutrition. Answers to these questions should provide a coherent, non-teleological historical narrative consistent with (i) the phylogenetic distribution of autotrophic metabolisms; (ii) the geological conditions of the early Earth (such as its anoxic environment) and the availability of inorganic precursors and catalysts; (iii) recognition of the limited catalytic abilities of the first autotrophs, i.e., the earliest biological CO_2 assimilation pathways must have been relatively simple, and may have depended on spontaneous or semi-enzymatic carboxylation reactions leading to C–C bond formation.

Quayle and Ferenci [32] suggested a slow, stepwise development of the highly endergonic ribulose bisphosphate pathway in which the simpler ribulose-monophosphate cycle was assumed to be an intermediate stage in the evolution of rubisco-mediated CO_2 assimilation. However, this model is not consistent with the phylogenetic distribution of the ribulose biphosphate pathway, which appears to be a relatively late development in the (eu)bacterial branch. Thus, it is likely that an older form of chemoautotrophic carbon assimilation evolved before the appearance of chlorophyll-based photoautotrophy.

Adherents of the pyrite-based chemoautotrophic theory of the origin of life, on the other hand, have argued that the reductive TCA cycle, which has an autocatalytic nature and provides both archaeal and bacterial metabolisms with the starting material for practically all biosynthetic routes, was originally driven by pyrite formation and the very first anabolic pathway [22, 27, 41].

The ubiquity of Fe-S active centers in many ancient enzymes, including CO dehydrogenase, has been explained as the evolutionary outcome from this FeS/H_2S-mediated reduction of organic compounds [8, 41]. The archaeal acetyl-CoA synthase ($CH_3CO–SCoA$), like the *Clostridium thermoaceticum* enzyme [51], also includes Ni in its Fe-S reaction center [6, 8], and uses CO_2 or CO as precursors for acetyl-CoA [47, 48]. Based on these observations and on the key role of acetyl-CoA in manifold biosynthetic pathways (Eq. 1), Huber and Wächtershäuser [17] developed a non-enzymatic synthesis of $CH_3–CO–SCH_3$ from a mixture of CO and CH_3SH in a high-temperature reaction catalyzed by a mixture of co-precipitated NiS/FeS (Eq. 2):

$$CH_3\text{-corrin} + CO + HSCoA \xrightarrow{\text{acetyl-CoA synthase}} CH_3CO(SCoA) \quad (1)$$

HSCoA = coenzyme A

$$2CH_3SH + CO \xrightarrow{NiS/FeS} CH_3CO(SCH_3) + H_2S \quad (2)$$

Addition of selenium to the catalytic mixture NiS/FeS led to the synthesis of acetic acid and CH_3SH (Eq. 3),

$$CO + H_2S \xrightarrow{Se/NiS/Fes} CH_3COOH + CH_3SH \quad (3)$$

The abiotic C–C bond formation from CH_3SH and CO (Eq. 2), which is analogous to the metal-catalyzed industrial synthesis of CH_3COOH from CH_3OH and CO via the migration of a methyl group to a coordinated CO [8], demonstrates the feasibility of carbon monoxide fixation in a Wood-like reaction catalyzed by transition metal ions [17]. Although this reaction does not take place in a two-dimensional system as postulated by Wächtershäuser [39–42], the metal sulphide-catalyzed C–C bond forming process has been interpreted as evidence of a CO-assimilation process that would feed an archaic autocatalytic chemoautotrophic carbon-fixation cycle. As required by Wächsterhäuser's theory, such a cycle must have been a primitive variant of the reverse citric acid cycle, which once sparked by a C–C forming process akin to the Wood cycle, would become the starting point for all anabolic pathways. The appearance of the ancestral reductive TCA cycle would then be followed by the development of the reductive acetyl-CoA pathway [41].

However, there is an alternative intepretation to the results reported by Huber and Wächtershäuser [17], i.e., the reaction summarized in Eq. 2 suggests the possibility that the Wood pathway had preceeded the reductive citric acid cycle. It is possible, for instance, that a semi-enzymatic Wood-like cycle evolved in an ancestral heterotrophic population of limited catalytic abilities. According to this alternative interpretation, the utilization of metal sulphides as reducing agents also corresponds to an early step in biochemical evolution, i.e., acetyl-CoA synthase is the evolutionary outcome of a simple Ni-Fe-S catalyst with carbon monoxide dehydrogenase activity. The C_2-units generated by a reaction equivalent to that shown in Eq. 2 could be incorporated into cell material following a ferredoxin-dependent (or pyrite-dependent) reductive carboxylation. Corrin skeletons used first in the reduction of ribonucleotides in the RNA → DNA transition would be then selected as methyl-transfer molecules (CH_3-corrin), in a process originally mediated by broad-substrate primitive enzymes. This view is consistent with (i) the widespread distribution of the Wood pathway (Table 3); and (ii) the hypothesis that catalytic iron-sulphur clusters found in electron-transfer proteins have an ancient origin. However, it does not require a hot origin of life or an autotrophic emergence of living systems.

Conclusions

In this paper we have reviewed some of the biochemical characteristics of the basic CO_2-assimilation pathways. Sequence comparisons demonstrate that the patchwork assembly of catalysts has played a central role in the evolution of these different modes of carbon fixation. As underlined by Pace [31], phylogenetic distribution of the different types of energy metabolism and carbon dioxide fixation in universal molecular phylogenies does not follow a simple development

Table 3 Enzymatic steps in the reductive acetyl-CoA cycle (Wood pathway)

Enzyme	E C	Catalyzed reaction	Other pathways	Distribution
formate dehydrogenase	1.2.1.2	formate + NAD^+ = CO_2 + NADH	related to many other dehydrogenases	B, A, E
formyl tetrahydrofolate synthetase	6.3.4.3	ATP + formate + tetrahydrofolate = ADP + phosphate + 10- formyl tetrahydrofolate	glyoxylate & dicarboxylate metabolism	B, E
methenyl tetrahydrofolate	3.5.4.9	5,10-methylenetetrahydrofolate + H_2O = 10-formyl tetrahydrofolate	glyoxylate & dicarboxylate metabolism	B, E
methylene tetrahydrofolate dehydrogenase ($NADP^+$)	1.5.1.15	5,10-methylenetetrahydrofolate + $NADP^+$ = 5,10-methenyl tetrahydrofolate + NADPH	glyoxylate & dicarboxylate metabolism	B, E
5,10 methylene tetrahydrofolate reductase (FADH)	1.7.99.5 1.5.1.20	5-methyltetrahydrofolate + acceptor = 5,10-methylenetetrahydrofolate + reduced acceptor	dicarboxylate metabolism	B, A
carbon monoxide dehydrogenase	1.2.99.2	CO + H_2O + acceptor = CO_2 + reduced acceptor	methane metabolism	B, A, E

B= Bacteria; A= Archaea; E= Eukarya

and may indicate lateral gene transfer. However, it is obvious that some form of carbon assimilation, which is found even in strict heterotrophs, was operative before chlorophyll-based photosynthesis.

In contrast with the chemoautotrophic pyrite-based theory of the origin of life that assumes that the autotrophic reductive citric acid cycle was the first anabolic pathway [41, 42], we have proposed here that the experiments on metal sulphide-mediated C–C bond formation [17] can also be interpreted as an evidence that the Wood pathway is the most primitive carbon dioxide assimilation pathway. This alternative interpretation is consistent with (i) the ample phylogenetic distribution of the Wood cycle; (ii) the relative simplicity and energetically more favourable process of CO_2-assimilation mediated by the Wood pathway compared to other autotrophic routes [12]; and (iii) the possibility that the CO_2 assimilation was originally a semi-enzymatic pathway, in which the net synthesis of acetyl-CoA from CO/CO_2 was mediated by a simple NiS/FeS catalytic mixture ancestral to acetyl-CoA synthase [17].

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