acids in the distal part of CTD may constitute an added layer of gene regulation by mammalian RNA polymerases.

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A 3-Hydroxypropionate/4-Hydroxybutyrate Autotrophic Carbon Dioxide Assimilation Pathway in Archaea

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The assimilation of carbon dioxide (CO_2) into organic material is quantitatively the most important biosynthetic process. We discovered that an autotrophic member of the archaeal order Sulfolobales, *Metallosphaera sedula*, fixed CO_2 with acetyl–coenzyme A (acetyl-CoA)/propionyl-CoA carboxylase as the key carboxylating enzyme. In this system, one acetyl-CoA and two bicarbonate molecules were reductively converted via 3-hydroxypropionate to succinyl-CoA. This intermediate was reduced to 4-hydroxybutyrate and converted into two acetyl-CoA molecules via 4-hydroxybutyryl-CoA dehydratase. The key genes of this pathway were found not only in *Metallosphaera* but also in *Sulfolobus*, *Archaeoglobus*, and *Cenarchaeum* species. Moreover, the Global Ocean Sampling database contains half as many 4-hydroxybutyryl-CoA dehydratase sequences as compared with those found for another key photosynthetic CO_2-fixing enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase. This indicates the importance of this enzyme in global carbon cycling.

The dominant extant autotrophic pathway is the Calvin-Bassham-Benson cycle, which may have evolved late in evolution (*1*). Three additional autotrophic pathways are known: the reductive citric acid cycle (Arnon-Buchanan cycle), the reductive acetyl-CoA pathway (Wood-Ljungdahl pathway), and the 3-hydroxypropionate cycle. These pathways differ in several ways [e.g., with respect to energy demand, available reducing compounds, requirement for metals (Fe, Co, Ni, and Mo), usage of coenzymes, and oxygen sensitivity of enzymes]. These criteria determine the distribution of the pathways in autotrophic organisms in different habitats, in addition to their phylogeny.

In the thermoacidophilic Archaea *Sulfolobus*, *Acidianus*, and *Metallosphaera* spp., CO_2 fixation appears to proceed via a modified, but until now unresolved, variant of the 3-hydroxypropionate cycle (*2*–*6*) (Fig. 1). The 3-hydroxypropionate cycle was originally discovered in the phototrophic bacterium *Chloroflexus aurantiacus* (*7*, *8*); it involves the carboxylation of acetyl-CoA to malonyl-CoA by the biotin-dependent acetyl-CoA carboxylase. Because Archaea contain only trace amounts of fatty acids, if any, in their lipids, acetyl-CoA carboxylase cannot serve as the key enzyme of fatty acid synthesis. It seems that this enzyme functions in another metabolic pathway. Malonyl-CoA is reduced via malonate semialdehyde to 3-hydroxypropionate, which is further reductively converted to propionyl-CoA. Propionyl-CoA is carboxylated to (*S*)-methylmalonyl-CoA by a carboxylase, which is similar or identical to acetyl-CoA carboxylase. (*S*)-Methylmalonyl-CoA is isomerized to (*R*)-methylmalonyl-CoA, followed by carbon rearrangement to succinyl-CoA by coenzyme vitamin B_{12}–dependent methylmalonyl-CoA mutase. In *Chloroflexus*, succinyl-CoA is converted to (*S*)-malyl-CoA, which is cleaved by (*S*)-malyl-CoA lyase to acetyl-CoA (thus regenerating the CO_2-acceptor molecule) and glyoxylate (*8*). However, this succinyl-CoA conversion via (*S*)-malyl-CoA was not verified in Sulfolobales (*3*, *4*, *6*), leaving the problem of how acetyl-CoA is regenerated in this group of organisms.

A comparison of the genomes of autotrophic Archaea that have acetyl-CoA carboxylase genes revealed several groups, which differ with respect to the pattern of other characteristic genes and enzymes (table S1). One group comprising autotrophic members of the genera *Metallosphaera*, *Sulfolobus*, *Archaeoglobus*, and *Cenarchaeum* contains the genes for acetyl-CoA/propionyl-CoA carboxylase and methylmalonyl-CoA mutase. These organisms differ considerably in their energy metabolism and habitats. *Metallosphaera* and *Sulfolobus* spp. metabolize sulfur, pyrite, or hydrogen in volcanic areas (*9*); *Archaeoglobus* spp. are anaerobic sulfate reducers (*10*); and *Cenarchaeum* sp. is a symbiont of marine animals (*11*), which is closely related to *Nitrosopumilus* sp., a marine ammonia oxidizer (*12*). All these organisms contain the gene for 4-hydroxybutyryl-CoA dehydratase, a [4Fe–4S] cluster and flavin adenine dinucleotide–containing enzyme, which catalyzes the elimination of water from 4-hydroxybutyryl-CoA by a ketyl radical mechanism yielding crotonyl-CoA (*13*, *14*). This reaction is known to play a role in a few anaerobic bacteria such as *Clostridium aminobutyricum*, which ferment 4-aminobutyrate via 4-hydroxybutyryl-CoA (*15*).

We suspected that this group of Archaea is able to reduce succinyl-CoA to 4-hydroxybutyrate and convert it with the participation of 4-hydroxybutyryl-CoA dehydratase into two molecules of acetyl-CoA. Cell extracts of *M. sedula*, indeed, catalyzed a rapid (500 nmol min^{-1} mg^{-1} protein at 65°C) reduction of succinyl-CoA via succinate semialdehyde to 4-hydroxybutyrate with NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate ($NADP^+$) (*16*). Extracts also converted 4-hydroxy[1-^{14}C]butyrate to [^{14}C]acetyl-CoA at 50 nmol min^{-1} mg^{-1} protein at 65°C in the presence of Mg–adenosine triphosphate (MgATP), CoA, and nicotinamide adenine dinucleotide (NAD^+) (Fig. 2A). High-performance liquid chromatography (HPLC) analysis of the reaction course revealed labeled 4-hydroxybutyryl-CoA, crotonyl-CoA, and 3-hydroxybutyryl-CoA as intermediates (Fig. 2B). A plot of the relative amounts of radioactivity in the individual products versus time indicated that the order of intermediates was as expected and that acetyl-CoA was the end product (Fig. 2C). The organism grows at 75°C in a $H_2/O_2/CO_2$ mixture with a minimum generation time of 8 hours, which corresponds to a specific carbon fixation rate in vivo of 120 nmol min^{-1} mg^{-1} protein (*17*). Because two molecules of carbon are fixed in the proposed pathway, the minimal in

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vivo specific activity of enzymes in the cycle is 60 nmol min^{-1} mg^{-1} protein. All enzymes required for the postulated succinyl-CoA conversion to two acetyl-CoA were detected, and their specific activities met this expectation (see Table 1, which also summarizes the other enzyme activities; for cofactor specificity, see Fig. 1).

Some of the enzymes, for which no likely candidate gene could be identified in the genome, were purified, and the relevant genes were deduced from internal peptide sequences (Table 1). Succinyl-CoA reductase (NADPH) with a 39-kD subunit is identical to malonyl-CoA reductase, a paralog of aspartate semialdehyde dehydrogenase (*17*). Succinate semialdehyde reductase (NADPH) with a 40-kD subunit belongs to Zn-containing alcohol dehydrogenases. 4-Hydroxybutyryl-CoA synthetase [adenosine monophosphate (AMP)–forming] with a 50-kD subunit belongs to acyl-CoA synthetases. Similar genes were present in the sequenced Sulfolobales genomes (figs. S1 to S3).

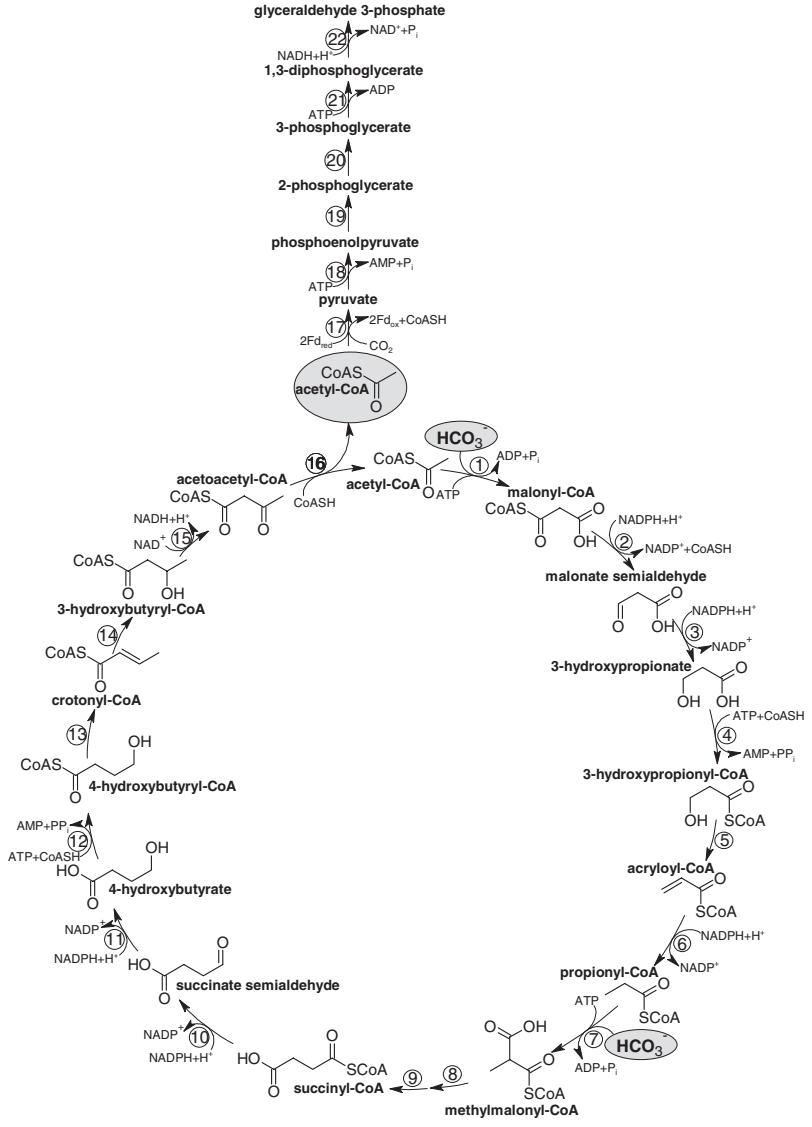

Fig. 1. Proposed autotrophic 3-hydroxypropionate/4-hydroxybutyrate cycle in *M. sedula*. Reactions of the cycle are shown. Enzymes: 1, acetyl-CoA carboxylase; 2, malonyl-CoA reductase (NADPH); 3, malonate semialdehyde reductase (NADPH); 4, 3-hydroxypropionyl-CoA synthetase (AMP-forming); 5, 3-hydroxypropionyl-CoA dehydratase; 6, acryloyl-CoA reductase (NADPH); 7, propionyl-CoA carboxylase; 8, methylmalonyl-CoA epimerase; 9, methylmalonyl-CoA mutase; 10, succinyl-CoA reductase (NADPH); 11, succinate semialdehyde reductase (NADPH); 12, 4-hydroxybutyryl-CoA synthetase (AMP-forming); 13, 4-hydroxybutyryl-CoA dehydratase; 14, crotonyl-CoA hydratase; 15, 3-hydroxybutyryl-CoA dehydrogenase (NAD^+); 16, acetoacetyl-CoA β-ketothiolase. The proposed pathway of glyceraldehyde 3-phosphate synthesis from acetyl-CoA and CO_2 is also shown. Enzymes: 17, pyruvate synthase; 18, pyruvate, water dikinase [phosphoenolpyruvate (PEP) synthase]; 19, enolase; 20, phosphoglycerate mutase; 21, 3-phosphoglycerate kinase; 22, glyceraldehyde 3-phosphate dehydrogenase. The activities of pyruvate synthase and PEP synthase at 75°C were 10 to 25 nmol min^{-1} mg^{-1} protein (*6*) and 25 nmol min^{-1} mg^{-1} protein, respectively.

Hence, the proposed 3-hydroxypropionate/4-hydroxybutyrate cycle, illustrated in Fig. 1, can be divided into two parts. The first transforms acetyl-CoA and two bicarbonate molecules to succinyl-CoA, and the second part converts succinyl-CoA to two acetyl-CoA molecules. In most Archaea, only one copy of genes for the acetyl-CoA/propionyl-CoA carboxylase subunits is present, which suggests that this is a promiscuous enzyme that acts on both acetyl-CoA and propionyl-CoA, as shown for *Acidianus brierleyi* (*18*) and *M. sedula* (*19*) and proposed for *Cenarchaeum symbiosum* (*20*).

A comparison of the proposed 3-hydroxypropionate/4-hydroxybutyrate cycle with the Calvin-Bassham-Benson cycle reveals important differences. The formation of one molecule of glyceraldehyde 3-phosphate from three molecules of CO_2 follows the equation: 3 CO_2 + 6 NAD(P)H + 9 ATP → 1 triosephosphate + 6 $NAD(P)^+$ + 9 adenosine diphosphate (ADP) + 8 inorganic phosphate (P_i). This does not account for the loss of energy and reducing power from the oxygenase activity of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO). Triosephosphate formation via the proposed cycle (Fig. 1) follows the equation: 2 bicarbonate + 1 CO_2 + 5 NAD(P)H + 2 reduced ferredoxin + 6 ATP → 1 triosephosphate + 5 $NAD(P)^+$ + 2 oxidized ferredoxin + 3 ADP + 4 P_i + 3 AMP + 2 inorganic pyrophosphate (PP_i). Hence, in energetic terms, the two processes are comparable [because ATP cleavage to AMP is equivalent to the loss of two energy-rich compounds, and reduced ferredoxin has a stronger reduction potential than NAD(P)H], but oxygenase activity of RuBisCO renders the Calvin-Bassham-Benson cycle energetically inferior.

The active "CO_2" species is CO_2 in the Calvin-Bassham-Benson cycle, whereas it is bicarbonate as cosubstrate for acetyl-CoA/propionyl-CoA carboxylase in the 3-hydroxypropionate/4-hydroxybutyrate cycle, with CO_2 as cosubstrate for pyruvate synthase (Fig. 1). The turnover number of RuBisCO (average: 5 s^{-1}) reflects a low catalytic efficiency that requires large amounts of enzyme (*21*, *22*). *M. sedula* acetyl-CoA/propionyl-CoA carboxylase catalyzing both carboxylation reactions has a turnover number at 65°C of 28 s^{-1} (*19*). The Michaelis constant (K_m) value of RuBisCO for dissolved CO_2 [the average value is 0.05 mM, but it may be as high as 0.34 mM in some marine cyanobacteria (*21*)] is lower than or even comparable to the apparent K_m value of archaeal acetyl-CoA/propionyl-CoA carboxylase for bicarbonate [0.3 mM (*19*)]. However, the CO_2 concentration in water equilibrated with air (1 atm pressure, 20°C, 370 parts per million CO_2) is 0.012 mM at pH 7.4 (which is the assumed intracellular pH), and the bicarbonate concentration under the same conditions is 0.26 mM. The concentration of bicarbonate will be even higher at pH 7.8 to 8.2 of seawater (apparent dissociation constant pK_{app} for CO_2/HCO_3^- is 6.3, 20°C). Thus, the acetyl-CoA carboxylase affinity for the

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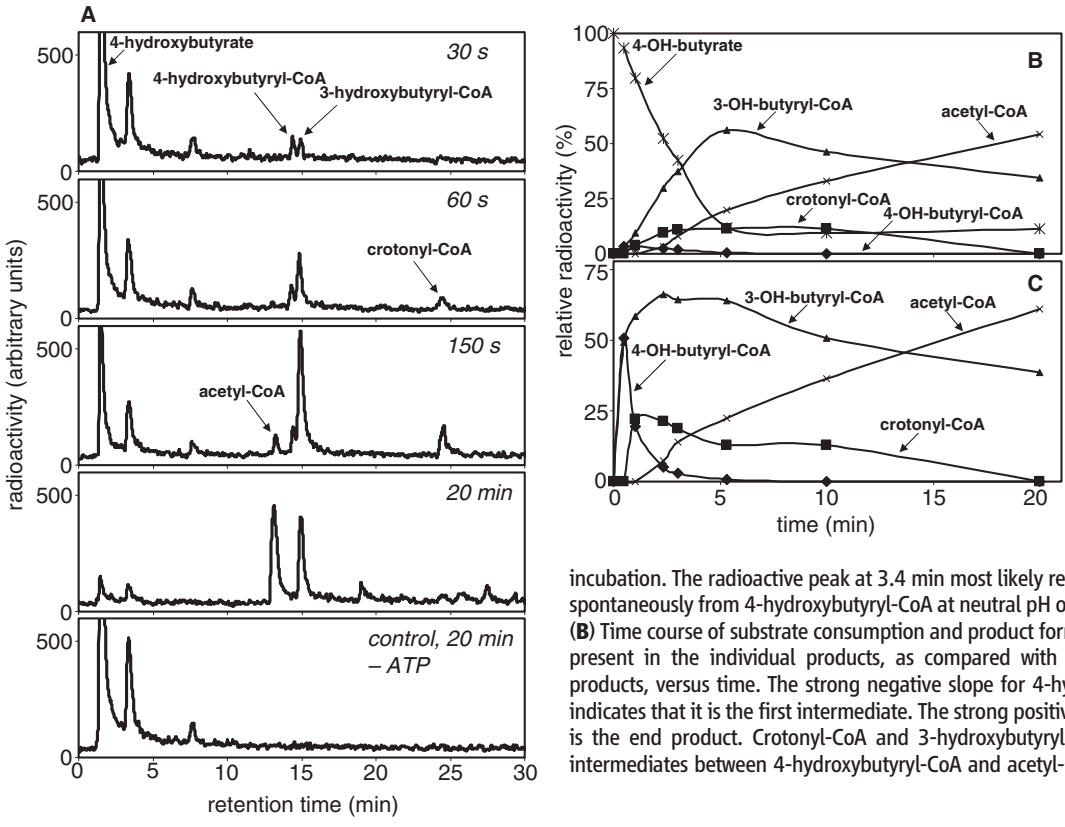

Fig. 2. Conversion of 4-hydroxy [^{14}C]butyrate to [^{14}C]acetyl-CoA at 65°C by cell extracts of *M. sedula*. The reaction mixture (0.5 ml) contained 100 mM Mops/KOH (pH 7.2), 3 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM 4-hydroxy[1-^{14}C]butyrate (160 kilobecquerels ml^{-1}), 3 mM ATP, 2 mM CoA, and 2 mM NAD^+. The reaction was started by the addition of 10 μl of cell extract (to the final concentration of 0.4 mg protein ml^{-1} in the assay). (**A**) HPLC separation of labeled substrate and products and ^{14}C detection by flow-through scintillation counting. The figure shows samples (50 μl) taken after 30-s, 60-s, 150-s, and 20-min incubation, as well as a control experiment lacking ATP after 20-min incubation. The radioactive peak at 3.4 min most likely represents γ-butyrolactone, which forms spontaneously from 4-hydroxybutyryl-CoA at neutral pH or from 4-hydroxybutyrate at acidic pH. (**B**) Time course of substrate consumption and product formation. (**C**) Percentage of radioactivity present in the individual products, as compared with the total radioactivity in all labeled products, versus time. The strong negative slope for 4-hydroxybutyryl-CoA (4-OH-butyryl-CoA) indicates that it is the first intermediate. The strong positive slope for acetyl-CoA indicates that it is the end product. Crotonyl-CoA and 3-hydroxybutyryl-CoA (3-OH-butyryl-CoA) behave like intermediates between 4-hydroxybutyryl-CoA and acetyl-CoA.

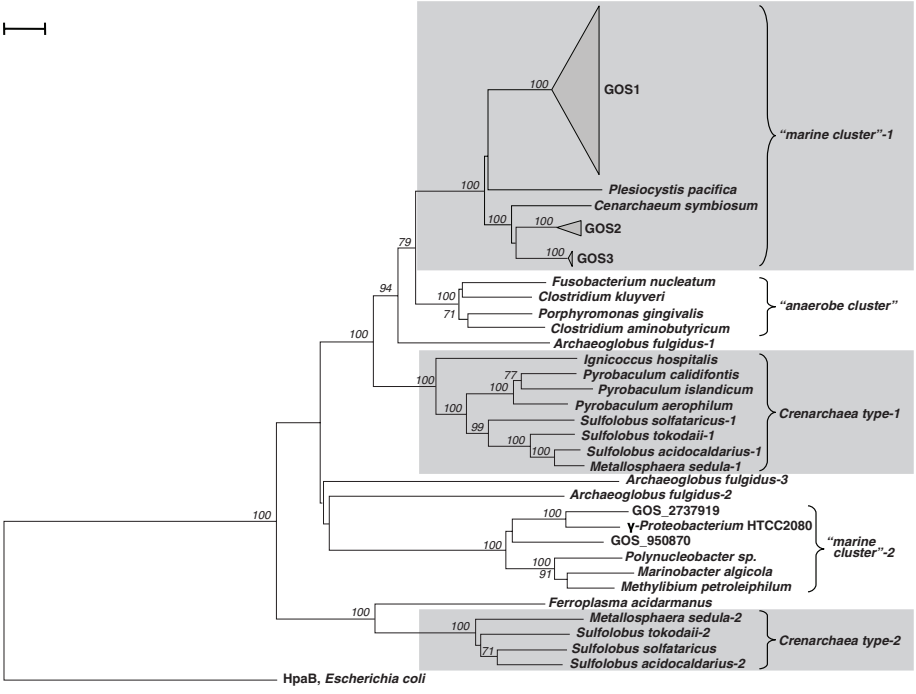

Fig. 3. Phylogenetic tree of representative 4-hydroxybutyryl-CoA dehydratase proteins. The tree is based on amino acid sequence analysis and rooted with 4-hydroxyphenylacetate 3-monooxygenase (HpaB) from *Escherichia coli*, which is a flavoprotein that resembles the flavin-binding domain of 4-hydroxybutyryl-CoA dehydratase. Tree topography and evolutionary distances are given by the neighbor-joining method with Poisson correction. The phylogenetic trees constructed by other algorithms (maximum parsimony, distance matrix, and maximum likelihood) resulted in identical topologies for principal clade grouping. The scale bar represents a difference of 0.1 substitutions per site. Numbers at nodes indicate the percentage bootstrap values for the clade of this group in 1000 replications. Only values above 70% were considered. The tree represents all full gene sequences from the GOS database and the sequences belonging to the major clusters on the tree. The accession numbers are listed in table S2, and the alignment is shown in fig. S4.

active "CO_2" species appears not to be inferior to that of RuBisCO. In addition, in volcanic gases and in animal hosts, the CO_2 concentration is much higher than in ambient air. Although 4-hydroxybutyryl-CoA dehydratase is slowly inactivated by oxygen, it may be sufficiently stable at low oxygen tensions to maintain activity, especially in the protected environment of the cell. There is no additional clue from the genome as to how these Crenarchaeota protect one of their key enzymes of CO_2 fixation from oxygen inactivation.

The 4-hydroxybutyryl-CoA dehydratase gene was not found in heterotrophic Crenarchaea, yet it is present in the genomes of autotrophic *Ignicoccus* and *Pyrobaculum* spp. (table S1), although these genera lack the genes required for part 1 of the proposed pathway. *I. hospitalis* forms succinyl-CoA from acetyl-CoA via pyruvate, phosphoenolpyruvate, and oxaloacetate (*23*) and may use the second half of the 3-hydroxypropionate/4-hydroxybutyrate cycle for the regeneration of the CO_2-acceptor acetyl-CoA from succinyl-CoA. *Pyrobaculum* spp. use a reductive citric acid cycle

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Table 1. Enzymes of the proposed 3-hydroxypropionate/4-hydroxybutyrate cycle in *M. sedula*. Because of the use of mesophilic coupling enzymes and the instability of some substrates, the indicated assay temperatures were used; the growth temperature was 75°C. The values are average values of several determinations, the mean deviations are about 10%, depending also on the batch of cells. The genes for 4-hydroxybutyryl-CoA synthetase, acryloyl-CoA reductase, succinate semialdehyde reductase, and one of the copies of 3-hydroxybutyryl-CoA dehydrogenase form a cluster in the *M. sedula* genome. ND, not determined.

Enzyme	Assay temperature (°C)	Specific activity (nmol min^{-1} mg^{-1} protein)		Genes in *M. sedula*
		Measured	Tentatively extrapolated to 75°C*	
Acetyl-CoA carboxylase	65	55–70 (*19*)	110–140	Msed_0147/Msed_0148/Msed_1375†
Malonyl-CoA reductase (NADPH)	65	≥210 (*17*)	≥420	Msed_0709
Malonate semialdehyde reductase (NADPH)	65	1,500	3,000	Unknown
3-Hydroxypropionyl-CoA synthetase (AMP-forming)	55	≥60 (*4*)	≥240	Msed_1456
3-Hydroxypropionyl-CoA dehydratase	55	≥60 (*4*)	≥240	Msed_2001
Acryloyl-CoA reductase (NADPH)	55	≥60 (*4*)	≥240	Msed_1426
Propionyl-CoA carboxylase	65	60–75 (*19*)	120–150	Msed_0147/Msed_0148/Msed_1375†
Methylmalonyl-CoA epimerase	55	Qualitatively shown by HPLC (*4*)	ND	Msed_0639
Methylmalonyl-CoA mutase	55	Qualitatively shown by HPLC (*4*)	ND	Msed_0638/Msed_2055†
Succinyl-CoA reductase (NADPH)	65	500	1,000	Msed_0709
Succinate semialdehyde reductase (NADPH)	65	2,500	5,000	Msed_1424
4-Hydroxybutyryl-CoA synthetase (AMP-forming)	65	150	300	Msed_1422
4-Hydroxybutyryl-CoA dehydratase	42	40	390	Msed_1220, Msed_1321
Crotonyl-CoA hydratase [(*S*)-3-hydroxybutyryl-CoA–forming]	42	1,500	15,000	Five possible candidates (Msed_0399, Msed_0384, Msed_0385, Msed_0336, Msed_0566)
(*S*)-3-Hydroxybutyryl-CoA dehydrogenase (NAD^+)	65	800	1,600	Four possible candidates (Msed_1423, Msed_0399, Msed_1993, Msed_0389)
Acetoacetyl-CoA β-ketothiolase	65	530	1,060	Seven possible candidates (Msed_0656, Msed_1647, Msed_1290, Msed_0396, Msed_0386, Msed_0271, Msed_0270)

*The specific activity at 75°C was calculated based on the assumption that a 10°C rise in temperature doubles the reaction rates. At high temperatures, enzymes may become inactivated in vitro, in contrast to the protected environment of the cell. †Subunits of multicomponent enzyme.

for CO_2 fixation, which results in acetyl-CoA formation from citrate (*6*); however, pyruvate synthase activity appears to be lacking in autotrophically grown cells (*24*). This enzyme is normally responsible for the reductive carboxylation of acetyl-CoA to pyruvate in autotrophic anaerobes. In the absence of pyruvate synthase, the second half of the proposed cycle may be used in the reverse direction to assimilate two molecules of acetyl-CoA into succinyl-CoA, to give a modified reductive citric acid cycle. Several heterotrophic Thermococcales and Halobacteriales harbor the characteristic genes for the first part of the cycle but lack those for the second part (table S1). Because their genomes appear to lack the genes for isocitrate lyase and malate synthase, which are key enzymes of the glyoxylate cycle, these organisms may thus use the first part of the cycle for the assimilation of acetyl-CoA, instead of the glyoxylate cycle, as well as for propionate assimilation.

4-Hydroxybutyryl-CoA dehydratase was originally described in a few strict anaerobes that use it in fermentation (*15*, *25*). These strictly anaerobic Bacteria form a separate "anaerobe cluster" in the 4-hydroxybutyryl-CoA dehydratase phylogenetic tree (Fig. 3). Two independent clusters are formed by hyperthermophilic Crenarchaea that are unable to ferment, with type 1 being more common than type 2. Another cluster is associated with mesophilic Crenarchaea such as *Cenarchaeum* sp., a member of the "marine group"–1 Crenarchaeota, as well as with many marine environmental metagenomes from the Global Ocean Sampling (GOS) database (*26*, *27*). The Basic Local Alignment Search Tool (BLAST) search identified 189 specific genes (18 complete genes and 171 fragments) of 4-hydroxybutyryl-CoA dehydratase in the GOS database, most of which (84%) group in the "marine cluster"–1 (Fig. 3). It is unlikely that the bearers of this gene conduct fermentation in the oxic seawater. The number of identified 4-hydroxybutyryl-CoA dehydratase gene sequences in the GOS database is comparable with that of the gene of RuBisCO large subunit (344 specific genes, including 26 complete genes and 318 fragments). This indicates that a group of abundant mesophilic autotrophic Crenarchaea in the sea (*28*–*30*) may use the proposed 3-hydroxypropionate/4-hydroxybutyrate pathway for CO_2 fixation; yet, such comparisons depend on the cloning efficiency of the required genes. Because these Crenarchaea probably use ammonia as an electron donor (*12*, *20*, *30*), their contribution to the carbon cycle should be much lower than that of phototrophs [~1% of annual marine production (*29*)].

It should be stressed that, in other Archaea, not all individual reactions of the proposed cycle may be catalyzed by the same kind of enzyme. For instance, various alcohol dehydrogenases, aldehyde dehydrogenases, acyl-CoA synthetases, or enoyl-CoA hydratases may fulfill the same

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function; yet, each superfamily of those enzymes comprises different enzyme families, from which genes may have been recruited. Also, the pathway of acetyl-CoA assimilation and phosphoenolpyruvate formation may differ. At last, one may be able to trace back the roots of the individual enzymes and come to tentative conclusions regarding the evolution of this cycle. Carboxyphosphate, which is used in the biotin-dependent carboxylase reactions, is an attractive model for carbon fixation during chemoevolution.

Several questions remain. What is the function of two different copies of 4-hydroxybutyryl-CoA dehydratase genes in autotrophic Sulfolobales? What are the functions of acetyl-CoA carboxylase and the enzymes of part 1 of the previously unrecognized pathway in various heterotrophic Archaea? Does the second part of the pathway, when operated in the reverse direction, serve as an acetyl-CoA assimilation route in some Archaea that lack the first part of the cycle? Which of the potential autotrophic pathways is operating in *Archaeoglobus*, which harbors the genes for the reductive acetyl-CoA pathway, for the proposed new pathway, and for RuBisCO (*31*, *32*)?

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Supporting Online Material

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Rev-erbα, a Heme Sensor That Coordinates Metabolic and Circadian Pathways

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The circadian clock temporally coordinates metabolic homeostasis in mammals. Central to this is heme, an iron-containing porphyrin that serves as prosthetic group for enzymes involved in oxidative metabolism as well as transcription factors that regulate circadian rhythmicity. The circadian factor that integrates this dual function of heme is not known. We show that heme binds reversibly to the orphan nuclear receptor Rev-erbα, a critical negative component of the circadian core clock, and regulates its interaction with a nuclear receptor corepressor complex. Furthermore, heme suppresses hepatic gluconeogenic gene expression and glucose output through Rev-erbα–mediated gene repression. Thus, Rev-erbα serves as a heme sensor that coordinates the cellular clock, glucose homeostasis, and energy metabolism.

Circadian rhythms are intrinsic time-keeping mechanisms conserved throughout the animal kingdom (*1*–*3*). Many aspects of mammalian behavior and physiology, including sleep-wake cycles, blood pressure, body temperature, and metabolic pathways are controlled by the circadian clock (*3*–*5*). At the molecular level, cellular rhythms are generated and maintained through interconnected transcriptional-translational feedback loops of clock genes, which are conserved in the central pacemaker and in peripheral tissues (*2*, *3*). The nuclear receptor Rev-erbα has been identified as a link between positive and negative loops of the circadian clock by controlling rhythmic expression of the *Bmal1* gene (*6*–*9*). This repression function is dependent on recruitment of the nuclear receptor corepressor–histone deacetylase 3 (NCoR-HDAC3) complex directly to the *Bmal1* gene.

Several genomewide expression studies have highlighted that genes involved in glucose metabolism, lipid metabolism, heme biosynthesis, and mitochondrial adenosine triphosphate (ATP) synthesis all exhibit a circadian pattern of expression (*1*, *10*–*12*). For example, gluconeogenic genes such as phosphoenol pyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) are typical clock-controlled metabolic-related genes (*12*). Furthermore, mice deficient in either *Bmal1* or *Clock* genes exhibit abnormal metabolic activities (*13*–*15*). Despite the evidence linking circadian clocks and metabolism, the mechanism by which the circadian clock is integrated into metabolic systems remains poorly understood.

To investigate the role of Rev-erbα in metabolic gene regulation, we first depleted Rev-erbα from HepG2 human hepatoma cells with small interfering RNA molecules (siRNAs). Expression of genes encoding gluconenogenic enzymes G6Pase and PEPCK was significantly increased when Rev-erbα amounts were reduced (Fig. 1A). By contrast, loss of Rev-erbα did not affect the expression of acetyl–coenzyme A (CoA) carboxylase or fatty acid synthase, which are involved fatty acid metabolism (fig. S1). Overexpression of full-length Rev-erbα in HepG2 cells decreased expression of G6Pase, PEPCK, and the known Rev-erbα target Bmal1 (Fig. 1B). Further, Rev-erbα repressed expression of a luciferase

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