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The Divergence and Natural Selection of Autocatalytic Primordial Metabolic Systems

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Abstract The diversity of the central metabolism of modern organisms is caused by the existence of a few metabolic modules, combination of which produces multiple metabolic pathways. This paper analyzes biomimetically reconstructed coupled autocatalytic cycles as the basis of ancestral metabolic systems. The mechanism for natural selection and evolution in autocatalytic chemical systems may be affected by natural homeostatic parameters such as ambient chemical potentials, temperature, and pressure. Competition between separate parts of an autocatalytic network with positive-plus-negative feedback resulted in the formation of primordial autotrophic, mixotrophic, and heterotrophic metabolic systems. This work examined the last common ancestor of a set of coupled metabolic cycles in a population of protocells. Physical-chemical properties of these cycles determined the main principles of natural selection for the ancestral Bacteria and Archaea taxa.

Keywords Autocatalytic network · Feedback · Chemical potentials · Phase diagrams · Natural selection · Ancestral metabolism · Last common bacterial (archaeal) ancestor

Introduction

The metabolism of living systems parallels the biochemical homeostatic networks organized by negative-plus-positive feedback. In these networks, the primary metabolism corresponds with a minimal feedback system (Tsokolov 2010). The organization of biological feedback loops has been previously described (Eigen and Schuster 1979; Kauffman 1993), and they are believed to be the common denominator for all subsequent forms of life following their appearance (Lahav et al. 2001). Theoretical models of ancestral biochemical autocatalytic systems have been developed for more than 30 years (e.g. Eigen 1971; King 1978; Dyson

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1982; Gánti 1984; Kauffman 1986, 1993; de Duve 1987; Segré et al. 2000; Shenhav et al. 2007). However, very few positive-plus-negative feedback loops have actually been constructed for early life forms in primordial chemical systems.

Autocatalytic processes are key functions in the metabolic systems of extant organisms. Autotrophic metabolism is considered primordial and is found in prokaryotes which are the closest to the root of the phylogenetic tree of life. Modern models of the origin of autotrophic metabolism biochemistry uses CO_2 chemoautotrophic fixation as the basis of emergence and evolution for primordial intermediary metabolism. The citrate cycle (the tricarboxylic acid cycle, the citric acid cycle, the Krebs cycle) is able to function in both oxidative and reductive pathways and plays a key role in intermediary metabolism (Hartman 1975). The reductive citrate (RC) cycle of CO_2 fixation (Arnon-Buchanan cycle) has become the basis for the theory of primordial metabolism (Wächtershäuser 1988, 1990). In addition, it has set the groundwork for the conception of intermediary metabolism as a universal autocatalytic chemical network (Morowitz 1999; Morowitz et al. 2000; Trefil et al. 2009). The RC cycle exhibits specific features that favor chemical self-organization: (1) it is an autocatalytic chain comprised of a single short loop; (2) its synthesis steps are redundant; and (3) all its reactions are first order in cycle intermediates (Smith and Morowitz 2004).

We have also suggested that the 3-hydroxypropionate (3-HP) cycle, which occurs in thermophilic prokaryotes, is another intermediary core metabolism (Marakushev 2008). According to this proposal, the general system of archaic CO_2 fixation developed as a coupled 3-HP and RC cycle that contained a succinate ↔ fumarate redox pair which was able to switch electron flow between the forward or reverse direction depending on the geochemical environment redox potential (Marakushev 2008; Marakushev and Belonogova 2009). The geochemical composition of the hydrothermal fluid, mineral environment, pressure, and temperature determined the stability of the components involved in the autocatalytic bi-cycle of CO_2 archaic fixation. This also raised the possibility of self-organized cycles. According to King (1977), a combination of protometabolic cycles can be described by the term symbiosis which originates from Ancient Greek (συμβίωσις [sumbíōsis]: "living together"). Thus, the coupled 3-HP and RC cycles can be considered a symbiosis of cycles in which the 3-HP cycle functions at low chemical hydrogen potentials (μ_{H2}) and the RC cycle operates at high μ_{H2} (Marakushev and Belonogova 2011).

Numerous papers deal with the problem of natural selection and evolution of primordial pre-biochemical systems (e.g., Fernando and Rowe 2007 and Fry 2011). The present paper suggests the hypothesis that life emerged through the evolution of autocatalytic metabolic cycles comprised of small carboxylic acid molecules. We also propose that the autocatalytic feedback effect may exhibit variations that result in self-regulation and variable reproduction and which constitute the most general basis for competition, natural selection, and evolution. The suggested proto-metabolic systems of positive-plus-negative feedback could help elaborate on the main principles of divergence, natural selection, and evolution of the first ancestral taxa of prokaryotes.

Autocatalytic Cycles with Positive Feedback

Autocatalytic cycles with positive feedback consist of a set of metabolites that are reproduced each turn of the cycle according to the following equation:

$$S + A_i \rightarrow 2A_i \quad (1)$$

Substrate S converts to metabolite A_i, which is also an autocatalyst due to its presence on both sides of Eq. (1). In other words, metabolite A_i becomes the catalyst for a new cycle and serves as the catalyst for its own production (King 1978; Blackmond 2009).

Figure 1 shows the proposed scheme of the archaic metabolic (AM) network as a tri-cycle of carboxylic acids. The biomimetically reconstructed archaic oxidative citrate (AOC), reductive citrate (ARC), and 3-hydroxypropionate (A3-HP) cycles are combined by their common sequence of components: malate ↔ fumarate ↔ succinate. These reactions proceed in the forward direction for the A3-HP and AOC cycles and in the reverse direction for the ARC cycle. The succinate–fumarate equilibrium ($(CH)_2(COOH)_2+H_2=(CH_2)_2(COOH)_2$) is a redox switcher, changing the flow of electrons in the direction of different cycles (Marakushev and Belonogova 2009, 2011). Thus, the redundant AM network as a whole provides substantially higher stability against changes in environmental redox conditions. Evidence of this modularized network can be found in relict structural mosaics of these three metabolic pathways in some prokaryotic taxa located near the root of the phylogenetic tree of life.

Typically, the modularity of complex biochemical networks contributes to the robustness, flexibility, and evolvability of all organisms (Spirin et al. 2006; Samal et al. 2006). The idea that the ARC cycle should be a member of a network of concatenated homologous cycles

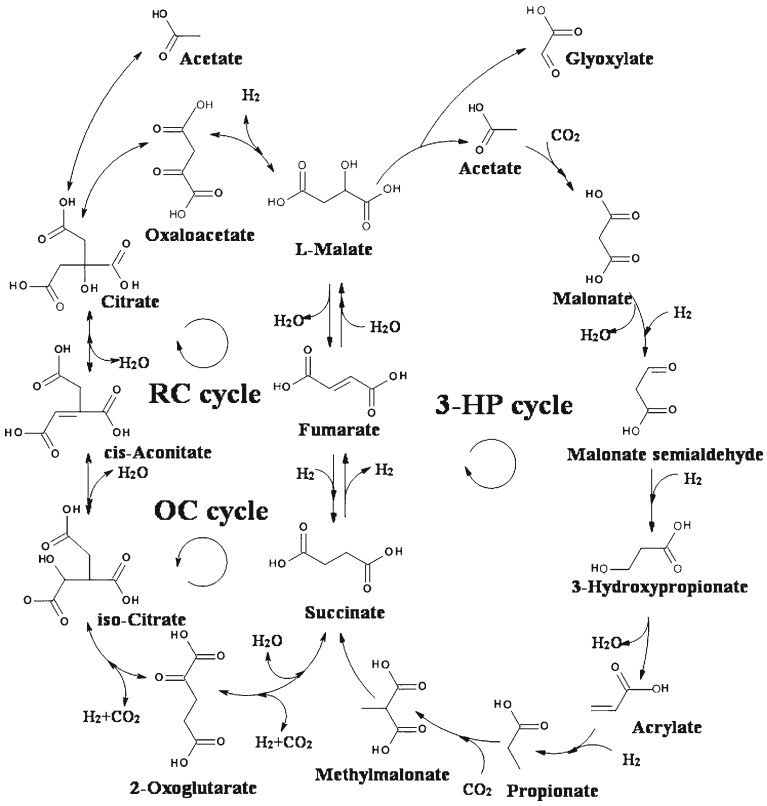

Fig. 1 The biomimetically reconstructed archaic metabolic (AM) network (AM tri-cycle) of the last bacterial common ancestor (LBCA). The AM tri-cycle is presented in the form of coupled autocatalytic loops of archaic reductive citrate (ARC), oxidative citrate (AOC), and 3-hydroxypropionate (A3-HP) cycles. Acetate, glyoxylate, and CO_2 are the waste products formed when autocatalysts are irreversibly removed from the cycle

was suggested for the first time by Günter Wächtershäuser (Wächtershäuser 1988, 1990). Subsequently proposed a mechanism of evolution called "grafting" where previously independent autocatalytic systems symbiotically interact in a manner that increases the growth rate of both systems, thereby leading to their mutual interdependence (Lindahl 2004).

In the ARC cycle, two CO_2 molecules are consumed to produce acetate by disproportioning citrate into acetate and oxaloacetate. In the A3-HP cycle, two CO_2 molecules are fixed to form glyoxylate by disproportionating malate into glyoxylate and acetate (Fig. 1). These two loops are self-reproduced autocatalytic systems. In addition, molecular hydrogen is the source for both energy and reduction equivalents (electrons) for both these cycles. In contrast to the autotrophic ARC and A3-HP cycles, the autocatalytic AOC cycle is heterotrophic. In this cycle, the insertion of one acetate and two water molecules results in the formation of two CO_2 and four H_2 molecules.

The autocatalytic nature of the ARC CO_2 fixation cycle has been previously demonstrated (Morowitz 1999; Smith and Morowitz 2004). The ARC cycle has been proposed to be statistically favored among competing redox relaxation pathways under early-earth conditions, and this feature may have driven its emergence while also accounting for its evolutionary robustness and universality. Its cycle intermediates are a source of sugars, lipids, purines, pyrimidines, coenzymes, and amino acids (Smith and Morowitz 2004; Eschenmoser 2007; Trefil et al. 2009). The autocatalytic nature of the 3-HP cycle as well as the RC cycle has also been described (Marakushev and Belonogova 2011). Without the regeneration of glyoxylate→3-hydroxipropionate, the 3-HP cycle would be a synthetic pathway for $2CO_2+2H_2+3$-hydroxipropionate→glyoxylate$+2H_2O+3$-hydroxipropionate. This would only require first-order reactions of environmental hydrothermal H_2 and CO_2 via usage of 3-hydroxipropionate as a network catalyst. Glyoxylate→3-hydroxipropionate synthesis provides the possibility of positive feedback in the form of $3CO_2+6H_2+3$-hydroxipropionate→$3H_2O+2$ 3-hydroxipropionate (one 3-hydroxipropionate→two 3-hydroxipropionate) or, equivalently, $4CO_2+7H_2+$methylmalonate→$4H_2O+2$ methylmalonate. This would, consequently, result in network autocatalysis and prevent the intermediates from participating in parasitic side reactions. The autocatalytic components of the autotrophic ARC and A3-HP proliferating chemical systems are then reproduced exponentially and attain macroscopic quantity.

The stability of positive feedback autocatalytic cycles has been observed in different metabolic network topologies (Reznik and Segre 2010). Superpositions of cycles in principle provide a branched network of catalytic pathways, and competition between the pathways generates additional distributed robustness in the system as a whole (Goldstein 2006). Moreover, a superposition of cycles that results in the appearance of negative feedback would provide the system with a new quality. In particular, it would provide the ability to adapt to the environment as the result of natural selection and survivability.

Competition and Natural Selection of Negative Feedback Autocatalytic Cycles

The coupling of autocatalytic cycles, accompanied by chemical oscillations due to a negative feedback (Gánti 1984, 2003), was experimentally studied in detail for the Belousov - Zhabotinskii reaction (Zhabotinskii 1964). This reaction was theoretically analyzed as a possible model for similar reactions under prebiotic Earth conditions (Tsokolov 2010). Theoretical calculations of switching between oscillations and homeostasis demonstrated that networks with negative and positive feedback motifs can undergo bifurcation to a state where a stable fixed point and stable limit cycle coexist (Li et al. 2012).

The proposed biomimetic combinatorial AM tri-cycle (Fig. 1) includes the general sequence of the intermediate reactions malate ↔ fumarate ↔ succinate of the AOC, ARC and A3-HP cycles. These three intermediates of the tri-cycle are the branching points in cycle direction for the reactions. Succinate is one of the nodal intermediates of metabolic systems in modern prokaryotes. In addition, the metabolic systems of most anabolic ancestors are also derived from succinate and its derivatives (Estelmann et al. 2011).

The following reactions have succinate as a bifurcation point:

(a). Succinate→fumarate+H_2 ($f(\sigma_1)$ – AOC, A3-HP cycles) or
(b). Succinate+CO_2+H_2→2-oxoglutarate+H_2O ($f(\sigma_1, \sigma_2)$ – ARC cycle),

where σ_i are the homeostatic parameters of those transformations that may serve as a general characteristic for feedback systems, and $f(\sigma_i)$ is a homeostatic function that defines the reaction route.

Figure 2 shows these reactions in the AM tri-cycle in the context of CO_2 and H_2 chemical potentials. This diagram was plotted according to Marakushev and Belonogova (2009) by using the aqueous constants determined under standard conditions (see Table in Supplementary material). The fully ionized forms of carboxylic acids (the autocatalysts of the cycles) are presented therein. The lines in the diagram show the two-phase equilibria of the autocatalysts and separate the fields of thermodynamic stability (facies) for succinate, fumarate, and 2-oxogutarate. Here, the H_2 and CO_2 chemical potentials are homeostatic parameters σ_1 and σ_2, respectively. The decrease in H_2 chemical potential (reaction *a*) results in the formation of fumarate, which occurs independently of the CO_2 chemical potential, and the initiation of the AOC and A3-HP cycles ($f(\sigma_1)$). The increase in both H_2 and CO_2 chemical potentials (reaction *b*) forms 2-oxogutarate ($f(\sigma_1, \sigma_2)$) and initiates the ARC cycle (see Fig. 1). This well-pronounced negative feedback of the autocatalytic AM tri-cycle

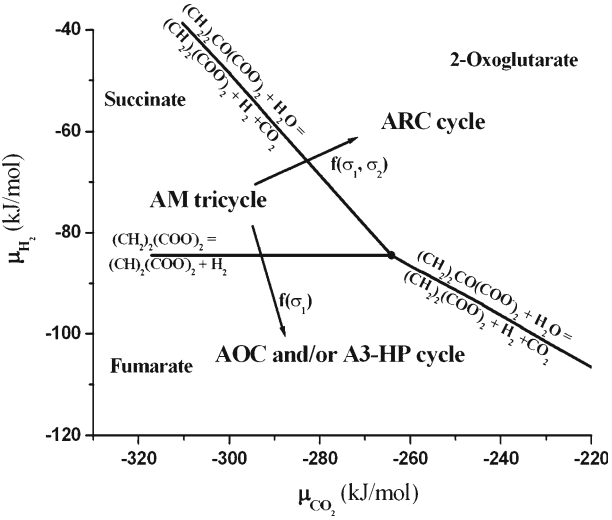

Fig. 2 The bifurcation point of succinate (2-oxoglutarate←succinate→fumarate) into the ARC (2-oxoglutarate facies), AOC, and A3-HP cycles (fumarate facies) on a diagram of H_2 and CO_2 chemical potentials (μ_{H2}=RTlnα_{H2} and μ_{CO2}=RTlnα_{CO2}) under standard aqueous conditions. α—Activities of aqueous H_2 and CO_2. Lines—monovariant equilibria, point—nonvariant equilibrium. $f(\sigma_i)$ is the homeostatic function that determines the direction of the AM tri-cycle reactions; σ_1 is the chemical potential of H_2; and σ_2 is the chemical potentials of CO_2. Constants can be found in the Supplementary Material

causes its divergence into these two directions depending on the chemical potentials of the hydrothermal environment. These potentials are the driving forces for natural selection in autocatalytic proto-metabolic systems.

Abiogenic chemical systems have been used to study the appearance of properties akin to natural selection in biological systems via physical-chemical kinetic analysis (Parmon 2001, 2002). The kinetic irreversibility conditions (i.e., a large deviation from thermodynamic equilibrium; Eq. 1) used in self-reproduced chemical systems (Glansdorff and Prigogine 1971) cannot provide natural selection among autocatalysts (Parmon 2001, 2002). Natural selection only occurs if reaction reversibility and the autocatalyst A_i termination reaction are considered:

$$S + A_i \rightleftarrows 2A_i(k_i, k_i) \tag{2}$$

$$A_i \rightarrow W(k_w) \tag{3}$$

where W is a waste product formed when autocatalyst A_i is irreversibly withdrawn from the autocatalytic cycle, and k_i, k_{-i} and k_w are the forward, reverse, and waste production rate constants, respectively, at the stages of autocatalyst amplification and autocatalyst termination. A stationary state with positive A_i valuses is only possible if the reverse reaction (k_{-i}) is involved (Laurenzi 2000; Parmon 2002; Arslan and Laurenzi 2008). At the same time, kinetic irreversibility is necessary for autocatalyst termination (the formation of waste products). Several populations of autocatalysts can coexist at high stationary rate of substrate inflow, but a single population exists at a low rate and does not disappear at any positive substrate flow (Parmon 2001).

In the AM tri-cycle, fumarate autocatalyst is formed only at decreasing H_2 chemical potentials (Fig. 2) when the AOC and A3-HP cycles are initiated and the ARC cycles disappear. In this case, the autocatalytic system approaches the vicinity of partial chemical equilibrium between substrate S (H_2) and autocatalysts A_i (succinate, fumarate). Thus, natural selection among autocatalyst populations of the AM network is, apparently, determined by the conditions at which chemical processes proceed near the thermodynamic equilibrium of autocatalysts. Figure 2 shows the transition (reactions *a* and *b*) of the stationary system from thermodynamic equilibria of the substrates (H_2 and CO_2) and autocatalysts (succinate, fumarate, 2-oxoglutarate) to non-equilibrium areas (facies) of autocatalysts stability. Ordering in chemical systems of microscopically conjugated reactions also occurs near chemical equilibrium (Galimov 2004).

The final products (W) in the autocatalytic AM tri-cycle can be CO_2, acetate, or glyoxylate (Fig. 1). CO_2 can revert to the AM system through the autotrophic ARC and A3-HP cycles. Acetate reverts to the ARC cycle through carboxylation: first to pyruvate and then to oxaloacetate. In modern metabolic systems, glyoxylate also reverts to the 3-HP cycle (Friedmann et al. 2006; Zarzycki et al. 2009), and a biomimetic model of this process can be presented as follows: glyoxylate+propionate→(β-methylmalate→D-citromalate)→pyruvate+acetate. This type of autocatalytic systems evolution can be described as waste-conversion (Lindahl 2004). This term implies that there are no waste products since the "waste" reverts to the metabolic cycles and stimulates cycle expansion. The term 'No Molecule Left Behind' has been proposed for this process by Srinivasan and Morowitz (2009b), and biochemical systems that exhibit such effectiveness are competitively advantageous.

In additional to chemical potentials, the most important factors defining the reaction route in negative feedback autocatalytic systems are homeostatic parameters such

as temperature and pressure. The malate bifurcation point (Fig. 1) illustrates the effect of these factors:

(c) malate→glyoxylate+acetate ($f(\sigma_3, \sigma_4)$ – A3-HP cycle) or
(d) malate→fumarate+H_2O ($f(\sigma_5)$ – ARC cycle) or
(e) malate→oxaloacetate+H_2 ($f(\sigma_1)$ – AOC cycle)

These reactions branch towards all three cycles. Reaction (*c*) is independent of chemical potentials but is governed by ambient temperature and pressure (σ_3 and σ_4) while Reaction (*d*) and (*e*) depend on the chemical potential of water (σ_5) and H_2 (σ_1), respectively. The chemical potential of the environment's hydrogen ions (pH) must also be taken into account. For example, the free energy of Reaction (*c*) (malate→glyoxylate+acetate) reverses the sign from positive to negative at 544 K and 405 K for non-ionized and fully ionized acids, respectively. The latter only exist under hydrothermal alkaline environmental conditions.

Figure 3 shows bifurcation reactions (*c*) and (*e*) for fully ionized autocatalysts in a phase diagram of temperature (T, K) and molecular hydrogen chemical potential (μ_{H2}). In this case, hydrogen turns into an intensive parameter from extensive one, and the three-component C–H–O system becomes two components. According to Gibbs' phase rule, the association of four phases (acetate, glyoxylate, malate, and oxaloacetate) in this system is nonvariant (Gibbs 1878; Marakushev and Belonogova 2009). This corresponds to a defined temperature and chemical potential (denoted by 405 K). The temperature increase is accompanied by the formation of facies by acetate-glyoxylate paragenesis (A3-HP cycle). Decreasing the H_2 chemical potential shifts the system towards facies of oxaloacetate (AOC cycle). Pressure (σ_3) is also a factor in this natural selection since the malate ⇄ glyoxylate + acetate equilibrium shifts to higher temperatures as pressure increases. Thus, the negative feedback in this system determines its divergence in the two directions ($f(\sigma_3, \sigma_4)$ and $f(\sigma_1)$). This is controlled by temperature, pressure, and H_2 chemical potential and affects the natural selection for the AOC and A3-HP cycles.

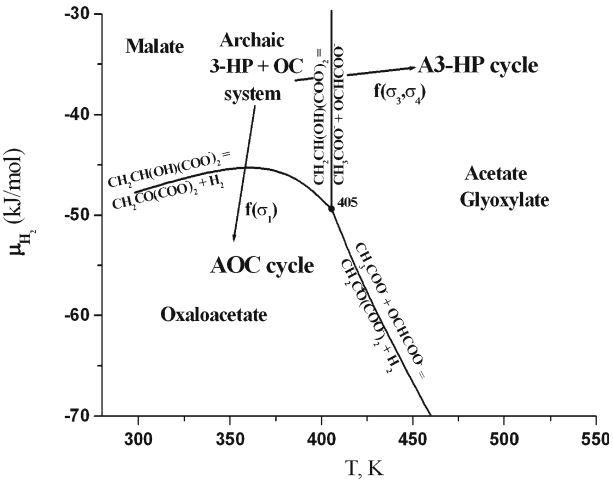

Fig. 3 The bifurcation point of malate (oxaloacetate←malate→acetate+glyoxylate) into the AOC (oxaloacetate facies) and A3-HP (acetate+glyoxylate facies) cycles on a diagram of the chemical potential of H_2 (μ_{H2}=RTlnα_{H2}) and temperature (K) at the saturated vapor pressures. σ_1, σ_3, and σ_4 are the chemical potentials of H_2, temperature, and pressure, respectively. For other abbreviations, see Fig. 2

Autocatalytic reversibility (Eq. 2) and waste-conversion are needed to maintain the stationary state of chemical autocatalytic systems while negative feedback, influenced by environmental homeostatic parameters (σ_i) (Figs. 2 and 3), is required for further system evolution. 'Primitive metabolism' denotes a set of similar and complex reactions that are mutually regulated and coordinated for survival and replication (Lifson 1997). Thus, the AM tri-cycle is a primordial metabolic C−H−O system that contains an autocatalytic negative feedback network of its chemical reactions. This tri-cycle provides a basis for nascent Darwinian natural selection via hydrothermal environment (i.e., the chemical potentials of H_2, pH, CO_2, and H_2O, temperature, and pressure). As early as 1977, evolution has been suggested as arising from successive symbioses resulting in change from simple, spontaneously arising, autocatalytic particles to complex prokaryotic cells (King 1977).

Divergence of Ancestral Bacterial Metabolic Systems

Most researchers assume that the last bacterial common ancestor (LBCA) should be considered a pool of genes that are shared among a great number of primordial organisms. However, this work reports on a more primitive version of the LBCA: a set of coupled metabolic cycles that exist among the protocell population of the incipient Bacteria domain. Because autocatalysis is an exceedingly conservative process, extant cells repeat the history of their successive symbioses in its internal cycles (King 1977).

The number of organic compounds necessary for the emergence of an organized metabolic network core is surprisingly low. According to Srinivasan and Morowitz (2009b), a minimal autotrophic metabolome core involves about 65 carboxylic acids. Figure 1 shows a version where the core intermediary metabolism involves only 18 carboxylic acids comprised of 13 C−H−O compositions. We suppose that the above combinatory system of three archaic metabolic cycles (the AM tri-cycle) formed the basis for primordial intermediary metabolism at the level of LBCA. This paved the way for the evolution of the first autonomic protocells on the ancient Earth.

As an integral metabolic system, the AM tri-cycle exists in a certain phase space of chemical potentials (e.g., Fig. 2). Ionized carboxylic acids of this metabolic network exist under highly alkaline hydrothermal environmental conditions that, possibly, dominated the early Archaean ocean (Shibuya et al. 2010). Stationary systems (namely the ARC, AOC, and A3-HP cycles) compete with each other and cannot coexist in changing hydrothermal environments. The suitability of the cycles to the changing conditions was a criterion for their selection, and this resulted in a distribution of primary metabolic systems among groups of emerging protocells. Natural selection then resulted in the divergence of the LBCA and the formation of cells with various metabolic systems (Fig. 4). This phenotypic diversification was governed mainly by these selection factors; temperature, pressure, and H_2, pH, CO_2, and H_2O chemical potentials.

One population of protocells that utilized the AM tri-cycle occurs in geochemical environments that correspond to the phase space of 2-oxoglutarate facies (Fig. 2). These protocells retained only the ARC cycle of the AM tri-cycle and evolved into species of the nascent bacterial phylum: ancestral Aquificae (Fig. 4 - I).

An analysis of small subunit (16S) rRNA gene sequences shows that Aquificae represent the first line of divergence in the Bacteria domain (Burggraf et al. 1992; Pitulle et al. 1994; Barion et al. 2007). *Aquifex aeolicus*, the modern representative of this line, is an obligate hyperthermophilic chemolithoautotroph, and has one of the smallest genomes among free-living autotrophic bacteria (Deckert et al. 1998). The RC cycle is a conservative intermediary of

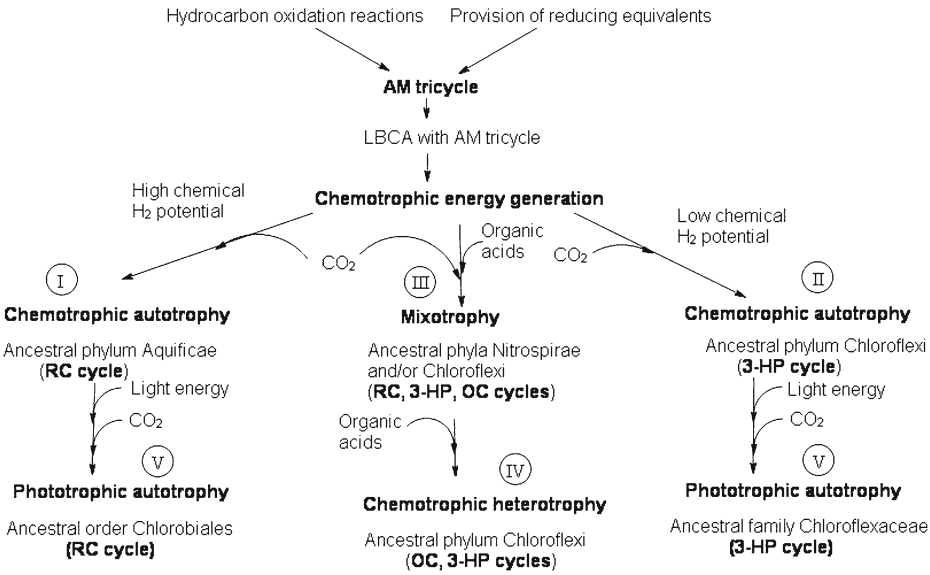

Fig. 4 Divergence scheme of negative feedback on the AM tri-cycle that forms the ancestral systems of chemoautotrophic, photoautotrophic, mixotrophic, and heterotrophic metabolism. AM – archaic metabolic; RC, 3-HP and OC – reductive citrate, 3- hydroxypropionate, and oxidative citrate, respectively. Mixotrophic organisms use several metabolic strategies simultaneously or switch between different strategies (Hügler and Sievert 2011)

Aquifex aeolicus core metabolism and is used to model the archaic anabolic intermediary metabolism of the LBCA (Srinivasan and Morowitz 2009a, b). This model possesses a minimal metabolome that contains all the properties of reductive chemoautotrophic bacterium metabolism. The hydrogen and sulfide energetics of the ARC cycle (Wächtershäuser 1990; Kalapos 2007; Marakushev and Belonogova 2009) correspond with the energy metabolism of extant bacteria existing in hyperthermophilic hydrothermal conditions (Nakagawa and Takai 2008). We suppose that the reductive carboxylation of succinate and the formation of 2-oxoglutarate in the first stages of the ARC cycle (Figs. 1 and 2) were catalyzed by ferredoxin-like Fe−S mineral clusters. The clusters then further evolved into contemporary carboxylases such as 2-oxoglutarate: ferredoxin oxidoreductase, which is a key enzyme of the RC cycle (Yamamoto et al. 2010).

Another population of protocells that utilized the AM tri-cycle occurs in geochemical environments that correspond to the phase space of fumarate facies (Fig. 2). These protocells retained the A3-HP cycle of the AM tri-cycle and evolved into the ancestral Chloroflexi clade (Fig. 4 - II). The 3-HP cycle (with the sequence of succinate→fumarate→malate reactions) is the only pathway for CO_2 fixation in extant thermophilic phototrophic anoxygenic non-sulphur green bacteria of the Chloroflexaceae family (Chloroflexi phylum). These bacteria are capable of the autotrophic growth (Holo and Sirevåg 1986; Strauss and Fuchs 1993; Ugol'kova and Ivanovsky 2000; Klatt et al. 2007; Zarzycki et al. 2008, 2009) and use molecular hydrogen as electron donors.

Chloroflexus belongs to the group of phototrophic bacteria on the earliest branch of the microorganism evolution (Woese 1987). Typical siliceous hot spring microbial mats are formed by either filamentous *Chloroflexus* alone or by *Chloroflexus* and cyanobacteria. The analysis of the fractionation of stable carbon isotopes demonstrated that these mats are

modern analogs of ancient formations of Precambrian stromatolites (van der Meer et al. 2000). These stromatolites were formed by either filamentous *Chloroflexus* or their simpler extinct ancestral bacterial branch that may have existed ca. 3–3.5 Gyr ago (Schopf 2006). Filamentous microfossils from Precambrian (3.235 Gyr) hydrothermal sulfide deposits (Rasmussen 2000) are also similar to extant 'filamentous anoxic phototrophs' that belong to the *Chloroflexaceae* family. Transition analysis and membrane chemistry and topology show that the root of the bacterial tree of life is behind or within ancestral Chloroflexi (Cavalier-Smith 2006, 2010). Structural analysis of polarizing indels (inserts and deletions) also argues that the root of the tree of life is near Chloroflexi (Valas and Bourne 2009), and according to phylogenetic analyses Chloroflexi should be located close to Aquificae (Boussau et al. 2008).

Extant prokaryotes can be divided into various physiological groups such as chemo-autotrophs, photoautotrophs, mixotrophs, and heterotrophs. At low H_2 chemical potential (Fig. 2), the ARC cycles are dying and there is the possibility of functioning in the AOC cycles (see Fig. 1). In this scenario, mixotrophic (Fig. 4 - III) or heterotrophic (Fig. 4 - IV) rather than autotrophic metabolism occurred. This situation arises when acetate, succinate, and other organic acids (intermediates and substrates of the OC cycle) are formed from endogenous hydrocarbons in hydrothermal systems (Marakushev 2008; Marakushev and Belonogova 2009). Abyssal emission of hydrocarbons with simultaneous development of alkaline magmatism exists (Kropotkin 1985; Gold 1992; Marakushev and Marakushev 2008, 2010) and is well pronounced in hydrothermal systems of the mid-ocean ridges (Konn et al. 2009; Lang et al. 2010), cold seeps, and mud volcanoes (Jørgensen and Boetius 2007; Marakushev and Marakushev 2008).

Almost all strains of *Chloroflexus* can grow heterotrophically (mixotrophically) using various organic substances (e.g., organic acids) and under either aerobic or anaerobic conditions (Hanada and Pierson 2006). In extant *Chloroflexus aurantiacus*, all enzymatic activities of a complete OC cycle have been shown to occur (Krasil'nikova et al. 1986).

In mixotrophic metabolism, organic components assimilate in additional to CO_2 fixation (Fig. 4-III). However, the RC and 3-HP cycles are also able to use organic acids (acetate, succinate, propionate) as a carbon source since they are anaplerotic (compensatory) intermediates of these metabolic pathways (Hügler and Sievert 2011). Remarkable examples of the mixotrophic lifestyle are chemoautotrophic Fe(II)–oxidizing Leptospirillum (Levicán et al. 2008; Goltsman et al. 2009) and nitrite–oxidizing Nitrospira (Lücker et al. 2010), a deeply branching lineage in the bacterial phylum Nitrospirae. Biochemical molecular mechanisms found in Nitrospirae are able to switch electron flow towards the RC and OC cycles. Therefore, Nitrospirae are able to assimilate either carbon dioxide or organic acids (e.g., acetate, pyruvate, and formate; Lücker et al. 2010). The RC cycle is thought to be an ancestor of the OC cycle (Aoshima 2007). However, the data above suggest that both metabolic systems could occur at the level of the LBCA. The biochemistry of Chloroflexaceae and Nitrispirae indicate the strong possibility of a conserved ancestral AM network of mixotrophic metabolism in extant bacteria (Fig. 4-III).

Thus, physical and chemical conditions of the hydrothermal environment have provided ecological niches that favor the development of ancient Chloroflexi, Aquificae, and Nitrospirae taxa due to their metabolic systems. For instance, ancestral *Aquifex* settled under strongly reductive conditions of deep-sea sulfide hydrotherms while ancestral *Chloroflexus* and *Nitrospira* spread in more oxidative non-sulphur high-temperature areas.

Extant *Chloroflexus* genetically differ from other photosynthetic organisms, and its ancestor likely acquired metabolic and anoxygenic photosynthesis genes as a result of the geodynamic shift of deep hydrotherms into a photic zone (Fig. 4 – V, right). This occurred

via horizontal gene transfer from green sulfur bacteria (bacteriochlorophyll) and purple proteobacteria (photosynthetic reaction centers) to ancestral forms of Chloroflexus, followed by the formation of extant green non-sulfur anoxygenic phototrophic bacteria (Blankenship 1992; Xiong et al. 2000). It is possible that extinct ancestral Aquificae taxa similarly evolved to extant phototropic sulfur bacteria which assimilate CO_2 in the RC cycle (Fig. 4 – V, left).

The distribution of the above metabolic systems corresponds with the phylogenetic tree of CO_2 fixation pathways (Hügler and Sievert 2011) and is presented in Fig. 5. Different bacterial communities are distributed by different geochemical type along modern hydrothermal systems of mid-ocean ridges. Hydrothermal chimneys are globally dispersed seafloor habitats associated with mid-ocean ridge spreading centers. The extant Aquificeae taxa are widespread in East Pacfic Rise (21°N; Reysenbach and Shock 2002) and Broken Spur (Voordeckers et al. 2008) acidic sulfide hydrothermal black smoker chimneys. In contrast, Chloroflexi and Nitrospirae are typical representatives of the communities inhabiting alkaline carbonate chimney walls in the Lost City hydrothermal field (Brazelton et al. 2010).

Above describes the obvious correlations between physical-chemical properties of metabolic negative feedback loops and the observed divergence of metabolism in ancestral bacterial taxa. These correlations cannot be accidental. Next, a feedback mechanism is proposed for the branching of metabolic systems that occurred in ancestral metabolism in the nascent archaeal taxa.

Divergence of Ancestral Autotrophic Archaeal Metabolic Systems

Recently, two new pathways of autotrophic carbon dioxide assimilation have been discovered in the Archaea domain (Crenarchaeota subdomain): reductive dicarboxylate /4-hydroxybutyrate (RD/4-HB) and 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4-HB) cycles (Berg et al. 2007; Huber et al. 2008; Teufel et al. 2009; Berg et al. 2010a). The cycles appear to be autocatalytic reaction pathways. The combination of metabolic modules generates certain autotrophic systems for CO_2 (HCO_3^-) fixation in extremophilic Archaea (Huber et al. 2008; Berg et al. 2010b). We have proposed that the CO_2 fixation in ancestral Archaea is based on the

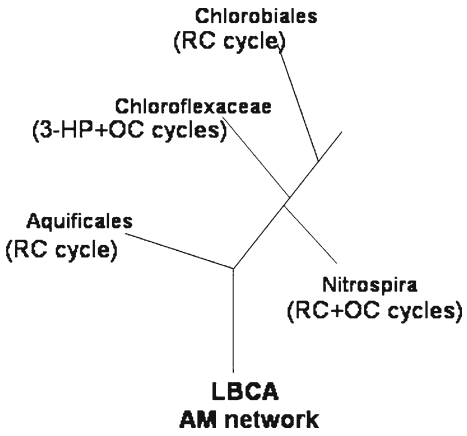

Fig. 5 Schematic phylogenetic tree depicting the distribution of ancestral core metabolic pathways among first phylogenetic bacterial lineages. A modified version of phylogenetic tree is presented according to (Hügler and Sievert 2011). LBCA is the last bacterial common ancestor

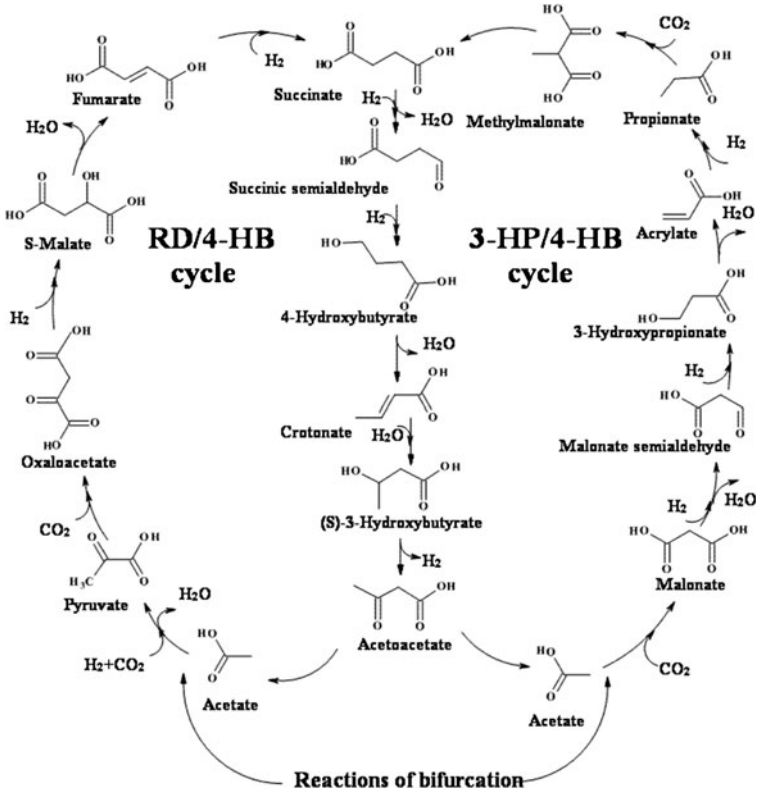

Fig. 6 The biomimetic scheme of archaeal chemoautotrophic CO_2 fixation pathways as coupled autocatalytic loops of archaic reductive dicarboxylate/4-hydroxybutyrate (ARD/4-HB) and 3-hydroxypropionate/4-hydroxybutyrate (A3-HP/4-HB) cycles. Bifurcation points are shown

RC, 3-HB, 3-HP/4-HB, and RD/4-HB cycles (CO_2 archaic fixation tetracycle; Marakushev and Belonogova 2010). This combination was stipulated by data on the existence of the RC cycle in the Thermoproteales and Desulfurococcales orders (Hu and Holden 2006) and of the 3-HP cycle in the Sulfolobales order (Alber et al. 2006). However, the latest biochemical and phylogenetic studies refute the existence of the RC and 3-HP cycles in the Archaea domain (Ramos-Vera et al. 2009; Berg et al. 2010a; Sato and Atomi 2011).

Most of the Thermoproteales and Desulfurococcales orders are obligate anaerobes. The RD/4-HB cycle reduces elementary sulfur via molecular hydrogen to hydrogen sulfide. The 3-HP/4-HB cycle operates in the autotrophic Sulfolobales order (extreme thermoacidophils). Most Sulfolobales can grow chemoautotrophically on sulfur, metal sulfides, or molecular hydrogen in microaerobic conditions.

The RD/4-HB and 3-HP/4-HB cycles have the same reaction sequence: succinyl-CoA→semialdehyde succinate→4-hydroxybutyrate→4-hydroxybutyryl-CoA→crotonyl-CoA→3- hydroxybutyryl-CoA→acetoacetyl-CoA→acetyl-CoA (Berg et al. 2010b). Like the combination of the RC and 3-HP cycles, the combination of these cycles transforms positive to positive-plus-negative feedback in this coupled autocatalytic system. This combination can be presented as a biomimetic thermodynamic C−H−O system with a general sequence of

reactions: succinate→semialdehyde succinate→(4-hydroxybutyrate→crotonate→3-hydroxybutyrate)→acetoacetate→acetate (Fig. 6). Two different carboxylation reactions occur at the bifurcation point (acetate) of the bi-cycle: i) $CH_3COOH+H_2+CO_2=CH_3COCOOH+H_2O$ (ARD/4-HB cycle) $f(\sigma_1, \sigma_2)$ and ii) $CH_3COOH+CO_2=CH_2(COOH)_2$ (A3-HP/4-HB) $f(\sigma_2)$.

The bifurcation point of the archaic archaeal bi-cycle is shown on the diagram of H_2 and CO_2 chemical potentials (Fig. 7) that was plotted using aqueous constants under standard conditions. The lines in the diagram are two-phase equilibria of the autocatalysts that separate the facies of acetate, pyruvate, and malonate. The increase in H_2 and CO_2 chemical potentials results in pyruvate formation ($f(\sigma_1, \sigma_2)$)and initiation of the ARD/4-HB cycle reactions. An increase in CO_2 chemical potential alone results in malonate formation ($f(\sigma_2)$) and initiation of the A3-HP/4-HB cycle reactions. This negative feedback determines the divergence of the archaic bi-cycle in the two directions as determined by the chemical potentials of the hydrothermal environment. Consequently, this results in natural selection for autocatalytic archaeal proto-metabolic systems of CO_2 fixation. Thus, like the archaic bacterial network, the direction of electron flow in the archaeal archaic bi-cycle is governed by H_2 and CO_2 chemical potentials.

This bi-cycle of archaic chemoautotrophic CO_2 fixation is proposed as a basis for the metabolism of the last archaeal common ancestor (LACA) of the Crenarchaeota subdomain. Crenarchaeota are near the root of the archaeal phylogenetic branch of the tree of life (Brochier-Armanet et al. 2011). Natural selection by the hydrothermal environment resulted in the divergence of the LACA cells into the ancestral Thermoproteales and Desulfurococcales orders and the Sulfolobales order which use the RD/4-HB and 3-HP/4-HB cycles, respectively (Fig. 8). The function, community structure, and productivity of extant chemolithotrophic Archaea are primarily controlled by variations in the geochemical composition of hydrothermal fluids (Takai and Nakamura 2011). Undoubtedly, these same factors participate in the divergence of the LACA into ancestral archaeal taxa.

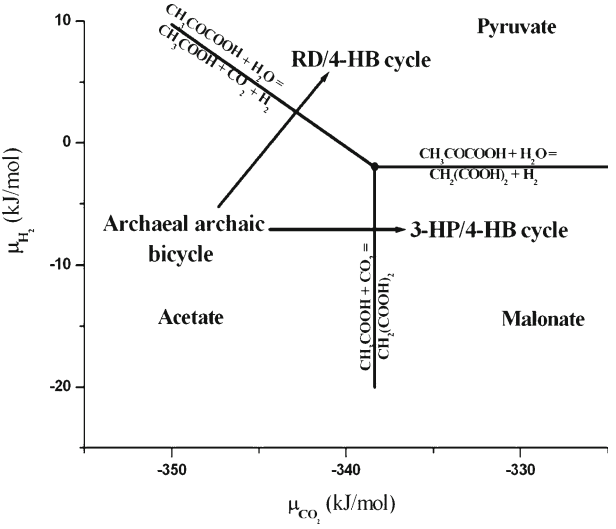

Fig. 7 The bifurcation point of acetate (pyruvate←acetate→malonate) into the ARD/4-HB (pyruvate facies) and A3-HP/4-HB (malonate facies) cycles on a diagram of H_2 and CO_2 chemical potentials under standard aqueous conditions. The carboxylic acids are presented in the nonionized form. For other abbreviations, see Fig. 2

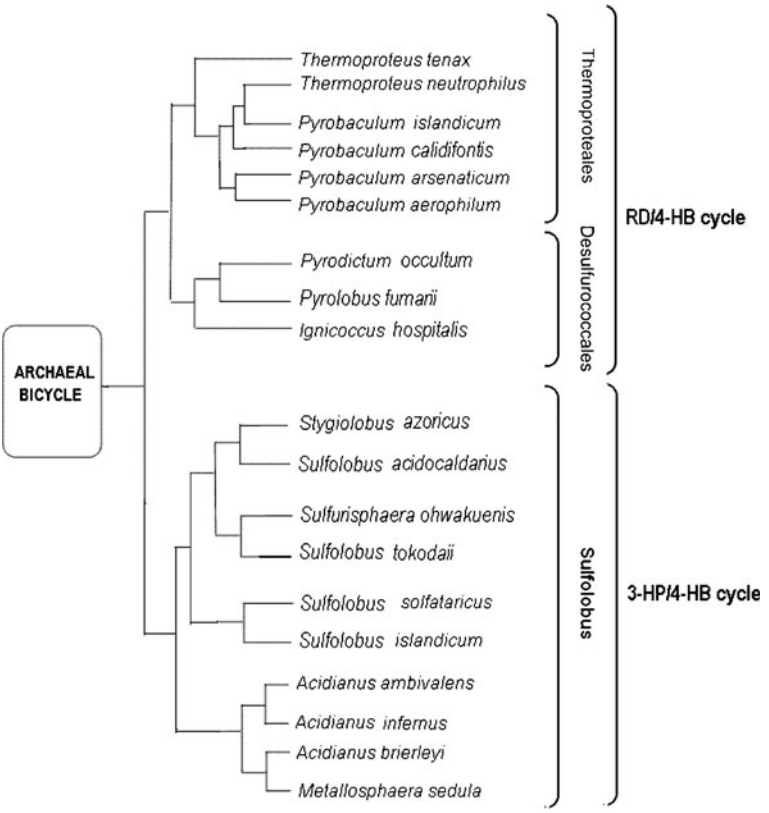

Fig. 8 Evolutionary divergence of the ancestral archaeal bi-cycle into two autotrophic CO_2 fixation cycles in archaeal subdomain Crenarchaeota. The phylogenetic tree of autotrophic Crenarchaeota species is based on ribosomal 16S RNA gene sequences (according to Berg et al. 2010a). The chemoautotrophic Thermoproteales and Desulfurococcales orders use the reductive dicarboxylate/4-hydroxybutyrate (RD/4-HB) cycle. The chemoautotrophic Sulfolobales order uses the 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4-HB) cycle

Conclusion

Among current theories of "metabolism first", archaic CO_2 fixation is typically based on the acetyl-CoA pathway (Wood-Ljungdahl path) which functions as the primary core of intermediary metabolism (Martin and Russell 2003, 2007; Russell and Martin 2004). In this case, acetate is the product of an archaic autotrophic acetyl-CoA pathway of CO_2 fixation in low-temperature hydrothermal vents whereas methane is formed in high-temperature environments. This results in the divergence from the last common ancestor into acetogens and methanogens which then formed the domains Bacteria and Archaea, respectively (Martin and Russell 2007; Nitschke and Russell 2009). However, the acetyl-CoA path is unlikely to be the primary metabolic system due to its rather complex mechanism of CO_2 fixation (high-carbon isotope fractionation, (House et al. 2003)) and due to the need for complex cofactors for methanogenesis and acetogenesis catalysis. Additionally, the recent phylogenetic indel data (Lake et al. 2009) excluded methanogenesis as the first primitive metabolism. Fundamental studies of membrane chemistry and topology (Cavalier-Smith 2006; Cavalier-Smith 2010) have also proven that thermophilic taxa of the domain Bacteria are closest to the root of the phylogenetic

tree of life while the domain Archaea occurs much later on this tree. Nevertheless, two bifurcation points ("electron bifurcation") of the acetyl-CoA path have been considered (Martin 2012). Analysis of these branching reactions created a separate problem, however, as it was a much more complex system comprised of five components (C-H-O-N-S) rather than three (C-H-O).

Currently, experimental data on CO_2 fixation in autocatalytic chemical systems does not exist. Huber et al. (2012) experimentally demonstrated the possibility of various autocatalytic metabolic systems formation using C1-nutrients (CO, COS, HCN, CH_3SH) and CO and H_2 reductants. These gave rise to a self-expanding set of richly functionalized and evermore-complex organic compounds in conditions similar to volcanic hydrothermal settings (Huber et al. 2012). They showed that positive feedback ("feedforward effect") and metabolic evolution emerged through the rate-promotion of one synthetic reaction by the products of another. At the same time, exactly CO_2 fixation takes place in extant chemoautotrophic organisms in both extreme and in standard conditions. Therefore, a physical-chemical analysis of the CO_2 fixation autocatalytic biomimetic models (including those based on negative feedback that leads to the natural selection of specific metabolic systems) is absolutely essential. In general, intermediates of cycles can be formed from various simple substances (e.g. from endogenous hydrocarbons (Marakushev and Belonogova 2009)). However, subsequently the intermediates form autocatalytic networks of CO_2 fixation with arising positive-plus-negative feedback.

Life is a product of chemistry that obeys deterministic laws and natural selection (de Duve 2007). This applies to the origin of life as well and has been emphasized by Lifson (1997, p. 1): "theories of self-organization without natural selection are refuted".

The problem of the origin and evolution of metabolic pathways is usually solved by either developing or already developed genetic machinery (see, for example, Fani and Fondi 2009). Therefore, the solution to the origin and development of primordial biochemistry at the first stages of life is appreciably complicated. The present work was performed in the framework of "metabolism first" theories. We made an attempt to show that physical-chemical properties of simple positive-plus-negative feedback autocatalytic chemical systems, which function as primitive metabolic systems, could be the main factors driving natural selection and evolution of ancestral forms of life on the ancient Earth.

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