

Evolution of the metabolic pathways in the Archaean

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Abbreviations

TCA	TriCarboxylic Acid
RC	Respiratory Chain
PPP	Pentose Phosphate pathway
CoA	Co-enzyme A
EMP	Embden-Meyerhof-Parnas
ED	Entner-Doudoroff

Abstract

Research for evolution has many angles. Gene analysis, amino acid comparison are some examples. In this paper, I will look at it from the metabolic point of view. In this paper I investigated the relationship between the evolution of the metabolic pathways and the circumstances and substrates available on Earth. Furthermore, a literature search has been done to see if photosynthesis could be originated from Archaean nitrogen reduction. And finally, the metabolism of contemporary organisms given as examples of the early pathways is examined.

1. Introduction

Is the metabolic evolution, as described by Kooijman e.a. ^(21,22) corresponding with the conditions on Earth and the substrates available? Can photosynthesis be originated in nitrogen reduction? Are the proposed intermediates as mentioned in the metabolic evolution correct? In this paper, I will try to give an answer to these questions. This paper starts with a description of the four metabolic pathways and an introduction about metabolic evolution. Then the conditions of Earth during the Archaean and the energy substrates available for organisms are reviewed and the origin and consequences of photosynthesis is shortly discussed. Organisms mentioned as examples for intermediates in the metabolic evolution are analysed.

1.1 Metabolic pathways

To understand the quantitative aspects of metabolism, the interactions between larger biochemical modules must be understood ⁽²¹⁾. Larger biochemical modules are organized in metabolic pathways. There are four important metabolic pathways.

The Pentose Phosphate pathway (PPP) is a series of extra-mitochondrial transformations by which glucose-6-phosphate is oxidized with the formation of carbon dioxide. It reduces NADP and ribulose 5-phosphate. Some of this latter compound is subsequently transformed to sugar phosphates with 3 to 7 or 8 carbon atoms, whereby glucose 6-phosphate is regenerated. Some ribulose 5-phosphate is also used in the synthesis of nucleotides and amino acids. The PPP is primarily used to interconvert sugars as a source of precursor metabolites and to produce reductive power.

The Glycolytic pathway converts glucose 6-phosphate to pyruvate or (anaerobically) lactate, ethanol or glycerol, with the formation of 2 ATP. The transformation occurs extra-mitochondrial in the cytoplasm. In some organisms, they are localized in an organelle, the glycosome. Most pyruvate is converted to acetyl and bound to coenzyme A (CoA).

The TriCarboxylic Acid (TCA) Cycle is also known as the citric acid or the Krebs cycle. It oxidizes (without the use of dioxygen) the acetyl group of Acetyl-CoA to two carbon dioxide molecules, under the reduction of 4 molecules NAD(P) to NAD(P)H. In eukaryotes that contain them, these transformations occur within their mitochondria. Some organisms have a variant of the TCA cycle, the glyoxylate cycle, which converts pyruvate to glyoxylate and malate with another pyruvate. Since pyruvate can also be obtained from fatty acids, this route is used for converting fatty acids originating from lipids into carbohydrates. Some plants possess the enzymes of the glyoxylate cycle in specialized organelles, the glyoxysomes.

The Respiratory Chain (RC) oxidizes the reduced coenzyme NAD(P)H, and succinate with dioxygen, which leads to ATP formation through oxidative phosphorylation. Similarly to the TCA cycle, it occurs inside mitochondria. Amitochondriate eukaryotes process pyruvate through pyruvate-ferrodoxin oxidoreductase rather than through the pyruvate dehydrogenase complex. If the species can live anaerobically, the respiratory chain can use fumarate, nitrate or nitrite as electron acceptors in the absence of dioxygen.

1.2 Metabolic evolution

To overcome the consequences of changing living conditions, individuals adapted their metabolism by changing the metabolic system through mutation, organization, selection, gene exchange and internalization of other organisms ⁽²¹⁾. Archaea internalized Eubacteria, which turned into mitochondria and most of the Eubacterial genes were transferred to the host ⁽³²⁾. Prokaryotes exchange metabolic properties with each other by lateral gene transfers and Eukaryotes specialized in symbiotic relationships and internalization of other organisms to acquire new metabolic properties ⁽²²⁾. Metabolic organization is acquisition and loss of new metabolic pathways and recombination of existing pathways. In symbiosis, the boundaries of individuals are crossed and individuals are often hard to recognize. Because Eukaryotes evolved from Prokaryotic gatherings, the metabolism of Eukaryotes developed from interacting Prokaryotic modules ⁽²¹⁾.

The evolution of metabolism followed two paths, an Archaeal and a Eubacterial. The Archaeal path starts with an organism with inverse acetyl-CoA metabolism and it acquires first an inverse TCA cycle and later also an inverse Glycolysis (Gluconeogenesis). The Eubacterial path starts with an organism with an inverse PPP and acquires inverse RC. Then the two paths merge into an organism with inverse RC, inverse PPP, Glycolysis, TCA and RC, which evolves in an organism with PPP, Glycolysis, TCA and RC ⁽²²⁾. Besides the mentioned differences, the Archaea and the Eubacteria have also different membrane metabolism. Archaea possess an isoprenoid-ether metabolism and Eubacteria a fatty acid metabolism.

The first three pathways (PPP, Glycolysis, TCA), in combination with their substrates provide almost all the building blocks an organism needs. These pathways are universal but also superficial because the enzymes can differ essentially.

The RC is a later advanced feature because the dioxygen it created was rare, or absent when life evolved on earth. The central role of glucose during the early evolution of life is questionable, because its degradation is coupled with dioxygen and as a permanent source of substrate, glucose as a monomer gives osmotic problems. The central role of glucose and carbohydrates likely evolved after oxygenic phototrophy evolved. It is likely that early life forms were anaerobic chemolithoautotrophic. These organisms fix inorganic carbon, CO₂ through the TCA cycle. Nowadays, this cycle is linked to the respiratory chain (RC), which is not found in any Archaea. Glycolysis is also not found in eukaryotic Eubacteria. Archaea possess the Embden-Meyerhof pathway.

So, the inverse TCA cycle is placed at the origin of metabolism. It provides building blocks, including pyruvate. But the TCA cycle is complicated and likely had a precursor that was less complex, such as a linear Acetyl-CoA pathway. For instance, homoacetogens have such a pathway and use organic and C1 compounds as an electron donor.

2. The early Earth and available energy substrates.

2.1 Early Earth

Organisms always want to use as less energy as possible. Therefore, they strive after the most energetic favourable situation and they adapted their metabolic pathways on the environment they are living in. In this part, the circumstances on Earth during the Archaea are reconstructed using the literature available.

The formation of Earth happened about 4.6⁽²⁸⁾ - 4.5 Ga⁽¹⁷⁾ ago. The period from the formation of Earth until 3.8 Ga ago is called the Hadean, the Archaean is from 3.8-2.5 Ga ago. During the Hadean and the early Archaean, the atmosphere contains CH₄, H₂, N₂, H₂O and CO₂ gasses and is depleted of dioxygen⁽³³⁾. The sun is as young as the Earth and approximately 10% to 30%^(17, 28) weaker as it is today. If the modern Earth were airless, its surface temperature would be -17 °C⁽²⁴⁾. Because the greenhouse effect, the actual surface temperature is 33 °C warmer and thus sustains liquid water⁽²⁸⁾. Thus, during the Archaea, the weaker sun could not have given the warmth Earth needs to keep the water unfrozen.

Earth was suffering frequently from heavy meteorite impacts that caused the temperature to rise and there was a lot of volcano activity^(17, 18, 28). Volcano's releases H₂ and CO₂. According to Kasting⁽¹⁸⁾, with no dioxygen to form water, hydrogen could accumulate in the atmosphere and oceans in concentrations high enough for methanogens to use⁽¹⁸⁾. In absence of essential nutrients, such as phosphorus and nitrogen, it was thermodynamically possible for methanogens to use most of the available H₂ to make methane⁽¹⁸⁾. The methanogens would have made enough methane to keep the planet warm⁽¹⁸⁾. As the temperature rises, more fast-growing, heat-loving specialists would survive⁽¹⁸⁾. When atmospheric methane becomes more abundant than CO₂, the reaction of methane towards sunlight will change. Instead of oxidizing to CO or CO₂, it polymerizes to form complex hydrocarbons, which condense into particles, forming an organic haze⁽¹⁸⁾. Organic haze absorbs incoming sunlight and reradiates it into the space, thereby reducing the total amount of radiation that reaches the surface, cooling down the surface of Earth⁽¹⁸⁾. The methane levels in the atmosphere created a greenhouse effect what could have been exactly what the planet needed to prevent the water from freezing⁽¹⁸⁾. This theory is unlikely because the methane needed to cause this greenhouse effect is supposed to be produced by methanogens, and thus is biogenic. In his article⁽¹⁸⁾, he refers to a moon of titan, which also has high methane levels and an organic haze. Since there is no live on Titan, as far as we know, the methane in its atmosphere is not biogenic. That leaves the question, why the methane in Earth's atmosphere is supposed to come from organisms and that from Titan not?

Furthermore, for a methane smog to screen the planet, the CH₄:CO₂ ratio of the air must have been high⁽²⁹⁾.

The heavy meteorite bombardments the Earth was suffering, is, according to Nisbet and Sleep⁽²⁸⁾, an event that could heat the oceans sometimes to more than 110 °C or even the ~350 °C needed to convert the whole ocean to steam (Nisbet&Sleep). The high temperature needed to convert the whole ocean to steam, water boils at 100 °C, is

probably necessary because the pressure in the atmosphere builds up when more water is turned into steam.

The meteorite bombardment⁽²⁸⁾ was also capable initially of ejecting matter into orbit from any of the inner planets. Thus if life were present on any inner planet with liquid water, it could have spread quickly across that planet, such that cells were present everywhere where conditions are suitable. Thereafter, each significant impact event producing huge numbers of ejecta would have been likely to have thrown a few rocks into space that carried undamaged cells. Some of these ejecta would have crossed to other inner planets⁽²⁸⁾. Thus, according to Nisbet and Sleep⁽²⁸⁾, life could have originated from other planets than Earth, and travelled to Earth on a meteorite.

This could suggest that, if life came from an other planet, it started on Earth during the meteorite bombardments. Since these bombardments were at its heaviest at 3.8 Ga, life possibly arrived at that time. There are several studies and theories that predict life has begun earlier than 3.8 Ga, perhaps even 4.2 Ga ago⁽³³⁾. Because it is plausible that life also could have been originated on Earth, and all organisms have the same basic building blocks, life as we know it should have started from one common ancestor. So, life coming from a meteorite is not a logical explanation. Furthermore, if life has started on an other planet in one of the other inner planets of our solar system, why is there no evidence for it on the planets? And from all the planets, the habitat of Earth is the friendliest for life.

It is possible that some UV radiation from the relatively young sun would have not only destroyed organic molecules and ammonia, but also generated very low concentrations of dioxygen and ozone from water and carbondioxide in the atmosphere^(3, 6).

If there was a high partial pressure of CO₂, the Hadean Ocean was acidic and such CO₂ pressure would also induce a strong greenhouse effect and high ocean temperatures, between 85 and 110 °C⁽¹⁵⁾. Unfortunately, the pressure of CO₂ in the Archaean atmosphere can not be proved until a rock is found with Archaean air locked in itself. Perhaps abiotic Earth was indeed glaciated, with occasional meteorite impact melting events, and pools of water around volcanoes. The ocean may have been covered with sea ice, with some open leads and thin ice were the ice melted away⁽²⁸⁾ and because life has an aqueous nature, it follows that life itself must have begun in water, presumably in the Hadean ocean⁽³³⁾.

2.2 Energy substrates

In the early Archaean, the atmosphere was almost depleted of dioxygen, and in anoxic conditions, the thermodynamically stable molecules are H₂O, CO, CH₄ and CO₂⁽³³⁾.

Ammonia would have come from the mantle via degassing during volcano outburst and/or partial dissolution of ammonium-bearing minerals such as feldspars⁽³³⁾. Some of this could have entered early cells by the passive diffusion of NH₄⁺ across the membrane⁽³³⁾. Because of its nature, NH₄⁺ is the most likely nitrogen source for the early organisms, but UV radiation breaks it down⁽²⁸⁾. But with a thin ozone layer, possibly not all the ammonia has been broken down. Smog in the atmosphere that absorbed UV radiation could also allow ammonia to exist below^(34, 42). It is also possible that some UV radiation from the relatively young sun would have not only destroyed organic molecules and ammonia, but also generated very low concentrations of dioxygen and ozone from

water and carbodioxide in the atmosphere ^(3, 6). The amount of NH_4^+ a very thin ozone layer could protect is unknown.

The lightning in the atmosphere, cosmic radiation and meteorite trails contributed in the formation of nitrate and sulphate. In high-energy fixation, nitrogen and sulphur are combined with oxygen form nitrates and sulphates, which are carried to the earth's surfaces in the rainfall as acids. Because sulphur is a weaker electron acceptor than nitrogen, it is more likely that more sulphur was oxidized than nitrogen, and thus sulphate was more abundant as nitrate. Lightning discharge accounts for 10^{12} - 10^{13} gram fixed nitrogen per year. The amount of biologically fixed nitrogen produced today is in excess of 2×10^{13} g/year ⁽³¹⁾. Note that lightning discharges account for minimum 5%, but maximum 50% of all fixed nitrogen. The fixed nitrogen by lightning discharge can be a huge source of energy substrates for (early) organisms.

Early Archaean life would have had access to redox contrast between a more oxidized atmosphere-ocean system, open at the top to space, and the more reduced fluids in contact with mantle-derived magmas. An early atmosphere rich in sulphur gases, with CO_2 partial pressure exceeding 2 bars, could have provided some warmth and protection against UV radiation ⁽¹⁶⁾. But such a CO_2 pressure is never proved.

As a carbon source, CO_2 was used by the early organisms ^(30, 14).

The anaerobic waste products pyruvate, lactate, acetate, ethanol, propanol of organisms still contain a lot of energy, but this energy was not available for the organisms. With the rise of oxygen levels, organisms with aerobic assimilation were able to extract the energy of these anaerobic waste products ⁽⁷⁾. In the Precambrian, oxygenic bacteria caused the rise in dioxygen levels, producing the modern atmosphere, allowing modern respiration ⁽⁸⁾, and forming the ozone layer, that functions as UV protection ⁽²⁷⁾.

3. Photosynthesis and the rise of oxygen.

The current idea is that photosynthesis has its origin among Eubacteria, because no Archaeobacteria are completely photosynthetic. However, the extreme halophiles (e.g. *Halobacterium salinarium*) have acquired the capacity to use light to run various molecular pumps ⁽⁴⁰⁾.

At a Reactiono centre, photons excite electrons, which are then passed down a redox chain to a final electron acceptor. The reduced compounds produced in this process store chemical energy for later use. There are two types of phototrophic mechanisms in the Bacteria: reaction centre type 1 (RC1) has core and core antenna domains that are parts of a single polypeptide; whereas reactions centre type 2 (RC2) is composed of short core proteins without antenna domains ⁽²⁷⁾. RC1 uses Fe-S as an electron acceptor and RC2 uses quinone as an acceptor ⁽⁹⁾. Only organisms that contain RC1 and RC2 are able to strip H₂O into O₂, H⁺ and electrons (27). The function and structure of the core proteins of RC1 and RC2 argues for homology, but the evolutionary relationship is still improvable ^(26, 36).

Bacteriorhodopsins, first observed in the Archaea, act as light-activated proton pumps but do not produce reduced compounds that store chemical energy ⁽²⁷⁾. In cyanobacteria, RC2 is associated with separate core antenna proteins that are homologous to the core antenna domains of RC1 ⁽²⁷⁾. The RC2s in cyanobacteria co-operate with antenna proteins that perform a similar function of that of the antenna domain of RC1 core proteins ⁽³⁶⁾. Plants chloroplasts and cyanobacteria use both RC1 and RC2 to oxidize water, producing O₂ as waste. It is possible that phototrophic eukaryotes have acquired their RCs by the endosymbiotic incorporation of a cyanobacterium within a eukaryotic cell ⁽¹⁰⁾.

The energy of light, in the form of photons, is the energy that drives a RC. This energy is absorbed by chlorophylls and bacteriochlorophylls ⁽¹⁾. Recently, a link has been discovered between nitrogenase proteins and bacteriochlorophyll b ⁽⁴¹⁾. Nitrogenase is an ATP-hydrolyzing, redoxactive complex of two proteins, dinitrogenase $\alpha_2\beta_2$ heterotetramer (where α =NifD and β =NifK proteins) and dinitrogenase reductase γ_2 homodimer (NifH protein). The α subunit contains the active site for dinitrogen reduction, which is a MoFe₇S₉ metal cluster ⁽³¹⁾. The fixation of dinitrogen takes place in aerobic and anaerobic environments ⁽³¹⁾. Nitrogenases contain iron-sulphur clusters that are essential for catalytic activity. Exposition to dioxygen irreversibly inhibit the protein ⁽¹¹⁾. It is one of the most metabolically expensive enzyme and it hydrolyzes 16 ATPs per N₂ fixed ⁽³¹⁾. There are strong structural and functional similarities between *bchB/chlB* and the *nifD/nifK* gene demonstrated ⁽⁴¹⁾. A phylogenetic tree made of the gene coding for a subunit of bacteriochlorophyll b/chlorophyll b, *bchB/chlB* and for subunits of the nitrogenase protein, *nifK, nifD, nifE* and *nifN* showed that the *nif* genes can be used as outgroup to root the photosynthetic *bchB/chlB* subtree ⁽⁴¹⁾.

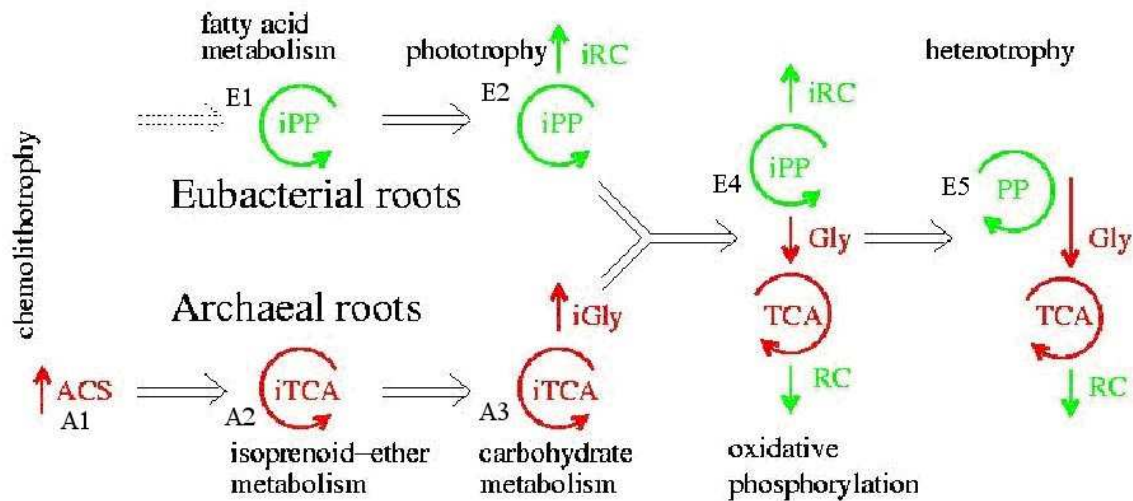
An other link between the nitrogen metabolism and photosystem has been discovered. In nitrite oxidation, the nitric oxide reductase (Nor) reduces NO to N₂O. NO is the product of nitrite oxidation and immediately after it is formed, it is reduced to N₂O, because NO is very toxic. In most denitrifying bacteria, this enzyme is a membrane complex of cytochrome b (*norB*) and cytochrome c (*norC*). This enzyme receives electrons from cytochrome c ⁽²⁾. Cytochromes transfer electrons in the RC.

A homolog of cytochrome b, named qNor because it has a quinol-binding site, is present in the Archaea *Pyrobacterium aerophilum*. There are also NO reductases found in the Archaea's *Sulfolobus solfataicus* and in *Methanosarcina mazei*. The NO reductase in *Sulfolobus solfataicus* has a heme binding site ⁽²⁾.

The qNor enzyme can be the ancestor of cNor, because it is likely that NO reduction has originated early in evolution because of its toxicity ⁽²⁾.

4. Intermediate steps in the evolution of metabolism.

The evolution of metabolism is summarized in the figure below. The metabolic evolution followed two paths, an Archaeal and an Eubacterial path. The Archaeal path starts with an organism with inverse acetyl-CoA metabolism and it acquires first an inverse TCA cycle and later also an inverse Glycolysis (Gluconeogenesis). The Eubacterial path starts with an organism with an inverse PPP and acquires inverse RC. Then the two paths merge into an organism with inverse RC, inverse PPP, Glycolysis, TCA and RC, which evolves in an organism with PPP, Glycolysis, TCA and RC ⁽²²⁾. For every intermediate step, organisms are suggested in ⁽²²⁾ as representatives of these steps. For these representatives, a literature research was done to check their metabolic properties and if they are a good match.



The example given for A1 is the *Methanococcus* genus, as a representative of organisms with only inverse acetyl-CoA metabolism and no Glycolysis, Gluconeogenesis or enzymes of the (reductive) TCA cycle.

Methanococcus maripaludis is a member of this genus and is a strictly anaerobe, methane-producing archaeon. It utilizes CO₂ as the sole carbon source during autotrophic ^(30, 14) with a maximum growth temperature of 88 °C ⁽²⁵⁾. Carbon monoxide dehydrogenase/acetyl-coenzyme A synthase fixes CO₂ and forms acetyl-CoA. Acetyl-CoA is then synthesized by Acetyl-CoA synthetase ⁽¹⁴⁾. Once acetyl-CoA is produced, it is converted by the incorporation of another CO₂ into pyruvate by a multisubunit pyruvate:ferredoxin oxidoreductase and then to oxaloacetate by pyruvate carboxylase. Oxaloacetate enters the TCA cycle, which proceeds in the reductive direction ⁽¹⁴⁾. In *M. maripaludis*, all of the enzymes for the reductive arm of the TCA cycle are present, leading from oxaloacetate to 2-oxoglutarate. The oxidative branch of the TCA cycle is absent ⁽¹⁴⁾.

Additionally, in at least 2 of the members of the *Methanococcus* genus, *M. jannaschii* and *M. maripaludis*, most of the genes for Glycolysis and Gluconeogenesis are present^(14, 39). These genes include those for two unusual phosphoglycerate mutases⁽¹²⁾, an ADP-dependent enzyme with both glucokinase and phosphofructokinase activities⁽³⁵⁾, and an Archaeal-type fructose bisphosphate aldolase⁽³⁷⁾. Genes for glycogen synthesis and degradation are also present, including glycogen synthase⁽¹⁴⁾.

M. maripaludis can meet its nitrogen needs from several sources, including ammonia assimilation, the fixing of diatomic nitrogen, and the assimilation of alanine^(37, 14).

M. maripaludis has a nitrogenase operon that contains the nitrogenase complex and proteins that participate in the synthesis of the nitrogenase cofactor, as well as proteins that regulate nitrogenase activity^(14,19,20).

The presence of the enzymes for the reductive TCA cycle, Glycolysis and Gluconeogenesis places the genus *Methanococcus* somewhere near place A2/A3 in the figure for the evolution of metabolism.

The *Thermoproteus* genus is mentioned as a representative of organisms on place A2, that have an inverse TCA cycle, but no Glycolysis or Gluconeogenesis. *T. tenax* possesses a variant of the reversible Embden-Meyerhof-Parnas (EMP) pathway and a variant of the Entner-Doudoroff (ED) pathway for carbohydrate metabolism⁽³⁸⁾. *T. tenax* can grow on glucose, starch, glycerate, glycerol, ethanol and malate⁽³⁸⁾. *T. tenax* has no evidence of a PPP pathway, which places this genus closer to place A3 in the figure than on A2, its original place.

The *Sulfolobus* genus is given as an example for the intermediate organisms, placed on A3. These organisms should have an inverse TCA cycle and Gluconeogenesis.

Sulfolobus solfataricus can metabolise glucose and galactose by a non-phosphorylative variant of the Entner-Doudoroff pathway. It can produce acetyl-CoA⁽²³⁾ and its citric acid cycle is coupled to oxidative phosphorylation⁽⁵⁾. These properties place this member of the *Sulfolobus* genus between A3 and E4 in the figure for metabolic evolution.

The *Nitrosomonas* genus is placed at place E2 in the figure for metabolic evolution.

Nitrosomonas europaea is an ammonium-oxidizing organism that oxidizes ammonium with oxygen to nitrite⁽⁴⁾. Genes for the enzymes common to Gluconeogenesis and Glycolysis and the TCA cycle are present, suggesting that complete oxidation of simple sugars and organic acids should be possible⁽⁴⁾.

The hydrolysis of fructose 1,6-bis-phosphate may be carried out by a pyrophosphate-dependent 6-phosphofructokinase. *N. europaea* does not synthesize glycogen or α -hydroxybutyrate as storage products but does accumulate polyphosphate when growth is limited by low values of pH⁽⁴⁾.

The activity of the TCA cycle enzyme α -ketoglutarate dehydrogenase was not detected or was very low, in contrast to other activities of the TCA cycle. However, genes for subunits E1, E2, and E3 of the enzyme α -ketoglutarate dehydrogenase (which are shared by pyruvate dehydrogenase) are present. So, it is not certain if genes for an α -ketoglutarate dehydrogenase are expressed⁽⁴⁾.

The presence of the enzymes of the TCA cycle and the genes for enzymes of the Glycolysis and Gluconeogenesis suggested that this member of the genus *Nitrosomonas* should be more close to place E4 on the figure for metabolic evolution.

The genus *Chloroflexus* is mentioned as an example for the intermediate organisms on place E3 in the metabolic evolution.

Chloroflexus aurantiacus fixates inorganic carbon via the 3-hydroxypropionate pathway. This pathway is a cyclic inorganic carbon fixation mechanism in which acetyl-CoA is carboxylated and reductively converted via 3-hydroxypropionate to propionyl-CoA. The first enzymes of this pathway are the same enzymes as the reductive TCA cycle has.

Propionyl-CoA is carboxylated and converted to malyl-CoA, which is cleaved yielding the first inorganic carbon acceptor molecule acetyl-CoA and glyoxylate ⁽²⁶⁾.

Sugars in *C. aurantiacus* are probably synthesized via the reversed Embden-Meyerhof pathway, which forms glucose from 2-phosphoenolpyruvate (PEP) molecules. Labelling studies have indicated that the C-1, C-2, C-5, and C-6 from glucose are most likely derived from acetyl-CoA, whereas the C-3 and C-4 of glucose are mainly derived from newly fixed inorganic carbon. Labelling studies with ¹³C-labeled substrates other than acetate suggest that glucose may be formed from malate via oxaloacetate and pyruvate ⁽²⁶⁾. Although this member of the *Chloroflexus* genus has no TCA cycle, its 3-hydroxypropionate pathway contains several enzymes that resemble those of the TCA cycle. The existence of the TCA cycle and the ability of this organism to create glucose place him at closer to E4 than to E3 in the figure for metabolic evolution.

Because *Chloroflexus aurantiacus* has TCA genes, I searched for other organisms that could be placed at E3. I found *Chlorobium tepidum*, but soon it was clear that this organism also possessed TCA proteins ⁽⁹⁾. This organism can grow only phototrophic on acetate and pyruvate but is unable to grow of glucose, which indicates that it does not have a full Glycolysis. It uses NH₄ and N₂ as nitrogen source and both sulphide and thiosulfate can be used as source of sulphur and as electron donors for photosynthesis ⁽⁹⁾.

Planctomycetes

The *Planctomycetes* are a different kind of organisms that are not mentioned in the figure. A literature research for these organisms was done, to see if there were any clues of their origin. One of its members, the *pirelulla* sp. strain 1, possesses the genes for the enzymes of the Glycolysis, TCA cycle, oxidative phosphorylation and pentose-phosphate cycle. The glyoxylate bypass and the Entner-Doudoroff pathway are not found ⁽¹³⁾. The *pirelulla* sp. strain 1 has mechanisms to reduce the damaging effects of UV radiation. Its genome harbours 110 genes encoding proteins with significant similarity to prokaryotic (75%) and eukaryotic (25%) sulphates ⁽¹³⁾. It also seems to be capable of synthesizing all amino acids ⁽¹³⁾. Its genome harbours all genes required for biosynthesis of lipid A, the major consistent of the lipopolysaccharide (LPS) layer in Gram-negative bacteria ⁽¹³⁾.

5. Conclusion and Discussion

5.1 Conclusion

During the Archaean, the surface of the Earth was glaciated, but around volcanoes and on places where heavy meteorites had smacked into the surface, liquid water was present. A thin ozone layer may have existed. As nitrogen source is NH_4^+ the most logical, but it is quickly broken down by UV radiation. An ozone layer would protect some NH_4^+ , but not certain is how much it could protect. Lightning discharges provide another source of nitrogen, they fix about 10^{12} - 10^{13} gram nitrogen per year, probably in the form of nitrate. The metabolic evolution as mentioned by Kooijman⁽²¹⁾ is in agreement with the circumstances and substrates available on Earth.

With the rise of oxygen, more substrates became available for the organisms. Although Archaea do not possess a full photosynthetic system, it looks like some parts of it have its origin in Archaean nitrogen metabolism, namely the cytochromes in nitric oxide reductases and (bacterio) chlorophyll in nitrogenases.

The intermediates in the metabolic evolution tree have been analysed. In at least one member of every genus mentioned as the A1, A2, A3, E1 and E2 examples of intermediates in the evolution of metabolism, genes are found of the Glycolysis, Gluconeogenesis and (inverse) TCA cycle. Because the Archaean (A1, A2, A3) are suggested to have evolved separately from Eubacteria (E1, E2), I suggest that their common ancestor possibly already had genes for TCA, Glycolysis and Gluconeogenesis before it split into a group of Archaea and Eubacteria. This makes it easier to place the planctomycetes in the evolutionary tree, since they also have genes for TCA cycle, Glycolysis and Gluconeogenesis⁽¹³⁾. It is possible that the common ancestor of Archaea and Eubacteria did not split in 2, but in 3 branches, the Archaea, Eubacteria and the Planctomycetes.

5.2 Discussion

How the circumstances were on Earth during the Archaean or other era is almost impossible to know, especially the atmosphere. Unless a bubble of air is found inside a rock with a nice label on it, you never know for sure how it was. And even if you know it for a particular time, that is also a fraction of the time.

The intermediate examples of the metabolic evolution path possess more metabolic paths than was thought they have. Although it is possible that they indeed had these properties when they originated as the particular organism they are, it is also possible that they acquired these metabolic paths later and that their ancestor has extinct. This is not rare, a lot of intermediate organisms have led go.

Some assumptions are based on phylogenetic trees. Those trees are often built for one, or for a group of enzymes. It is assumed that those enzymes are evolutionary stable, and therefore can give a realistic image of evolution. But it is not for sure that enzymes are evolutionary stable and it is also possible that organisms acquired enzymes via other

routes, by gene exchange or phagocytoses e.g. These trees say something about the evolution of the particular enzyme, of group of enzymes and thus not always something about the evolutionary path of the organism it is made from.

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