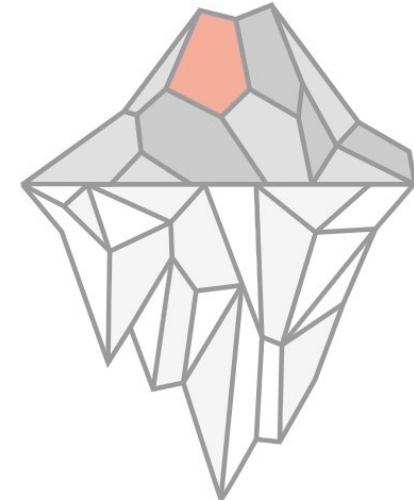


MICROBIOLOGY OF EXTREME ENVIRONMENTS

CHEMOLITHOAUTOTROPHY 1: CARBON FIXATION PATHWAYS



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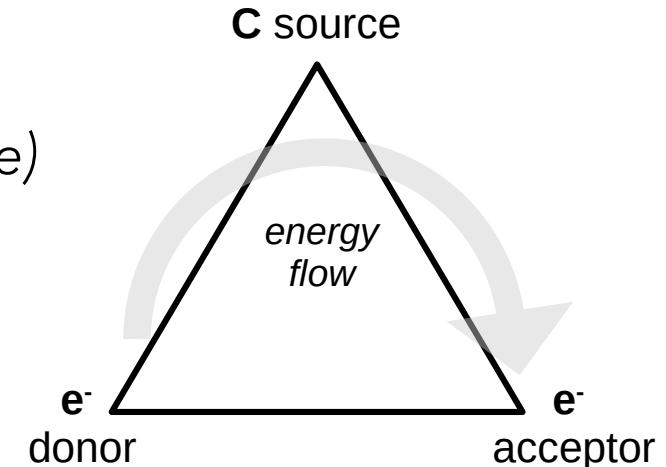


Metabolism 101

An **electron donor** (also known as energy source)

A **carbon source** (for biosynthesis)

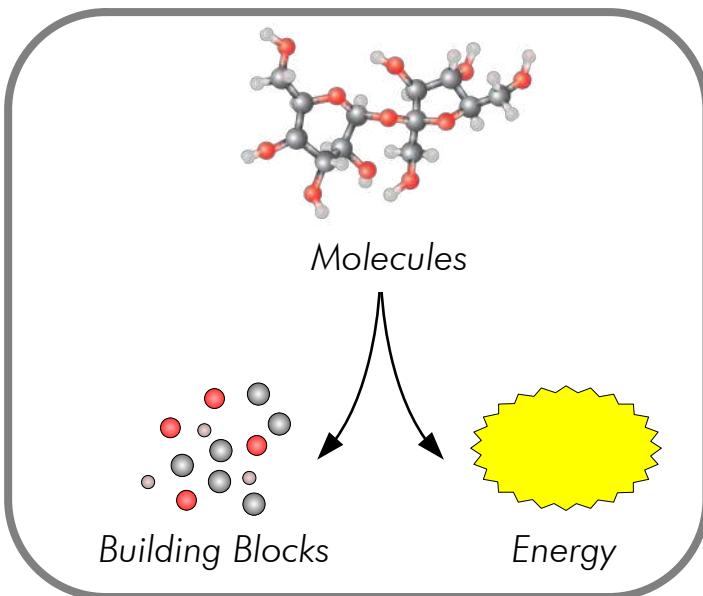
An **electron acceptor**



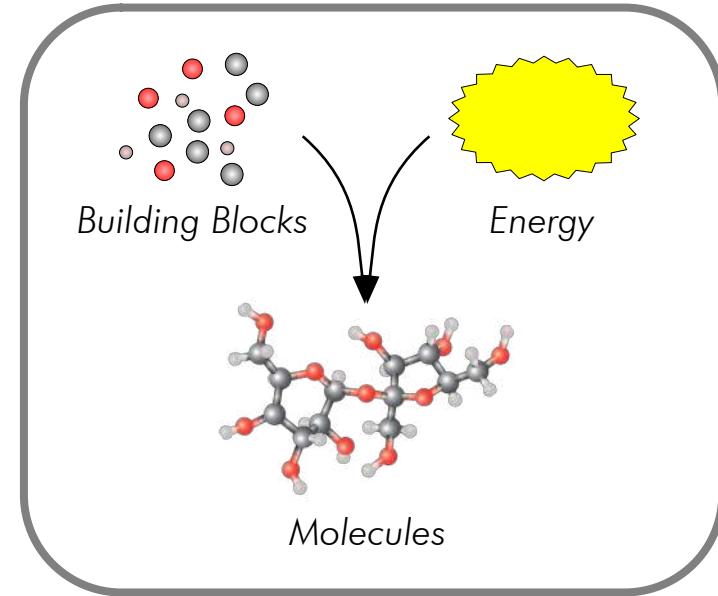
All type of metabolism, requires these three basic elements.

An electron donor (the source of reducing power used to carry out redox reactions), a carbon source used as a donor of carbon for biosynthetic purposes, and an electron acceptor, used to dispose of excess reducing equivalents.

Catabolism vs Anabolism



Catabolism is the set of metabolic pathways that breaks down molecules to smaller subunits, releasing energy to be used in anabolic reactions



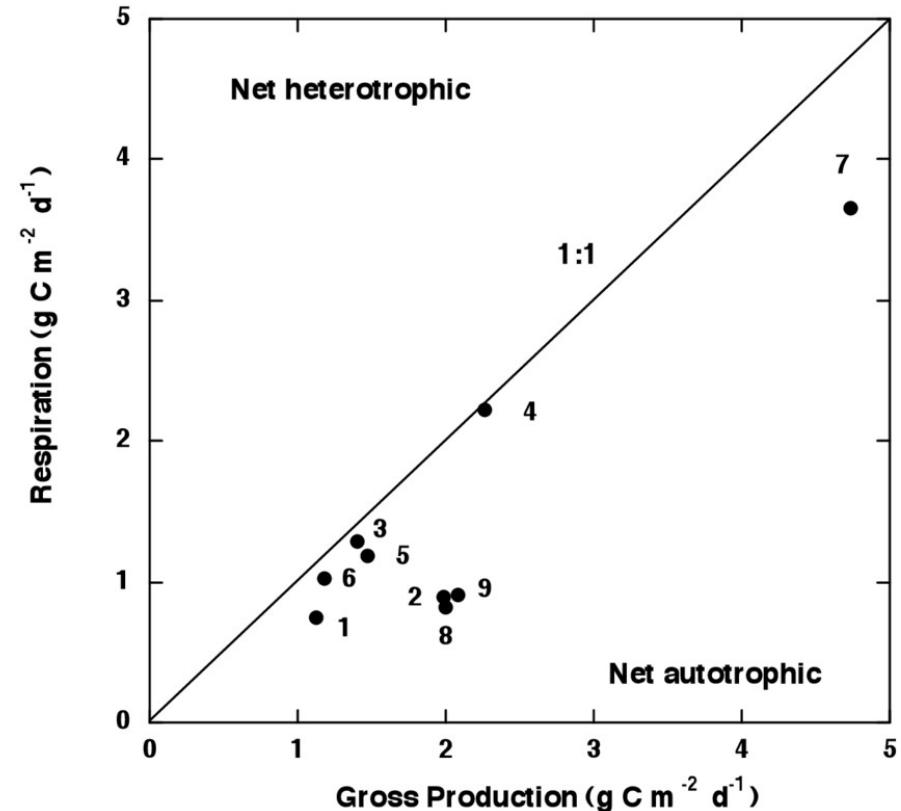
Anabolism is the set of metabolic pathways that build molecules from smaller subunits, requiring energy to form new molecular bonds

Net productivity

Considering the primary carbon source, every organism can be (mainly) classified in heterotroph or autotroph. Similarly can be done for ecosystems.

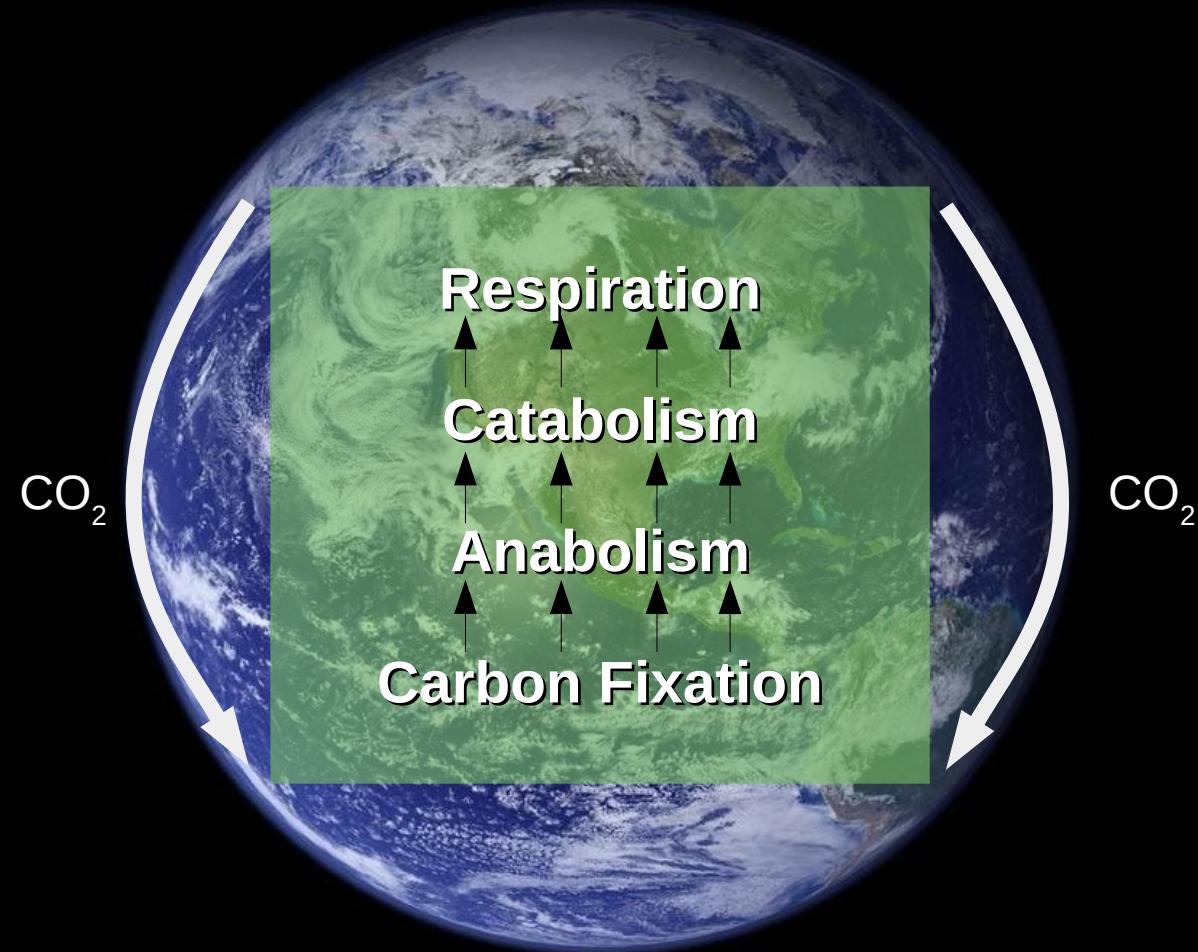
Classically, the rate of respiration vs the rate of gross photosynthetic productivity have been used to classify ecosystems based on their net productivity.

This is used to identify ecosystems as Net Heterotrophic or Net Autotrophic





*Our planet is globally **Autotrophic***



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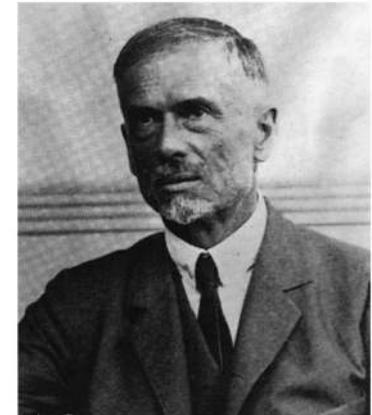
The history of Chemolithotrophy

In 1885 **Winogradsky** entered the laboratory of Anton deBary at the University of Strassburg. Winogradsky was assigned to confirm deBary's position that bacteria manifested fixed, stable, and definable properties and could thus indeed be subjected to a botanical style system of taxonomy.

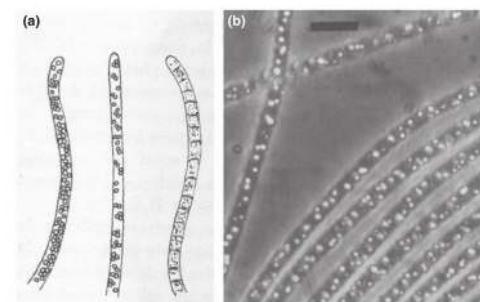
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In his memoirs, in a more informal fashion Winogradsky described the moment that he arrived at this hypothesis. 'Then one day as I was following the Île canal on my way home after a tiring day of chemical work which also involved hydrogen sulfide and sulfur, it suddenly occurred to me that sulfur might be oxidized by *Beggiatoa* to sulfuric acid. I could at once appreciate all the significance and implications of my conjecture, having no doubts that it offered the solution to my problem... The work was humdrum, it dragged on and on sluggishly, and all of a sudden it developed into an interesting result and was finished. All the beating around suddenly made sense, and I matured in my own eyes. Even so, I could not see that my discovery would become an epoch-making discovery, would determine the course of all my future work, and that it would open a new chapter in microbiology and physiology.' (Zavarzin, 1989). What a wonderful description of one of those 'aha!' moments that make scientific research such a unique thrill.

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S.N. Winogradsky
1856-1953



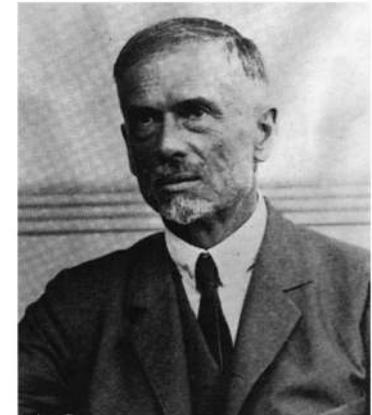
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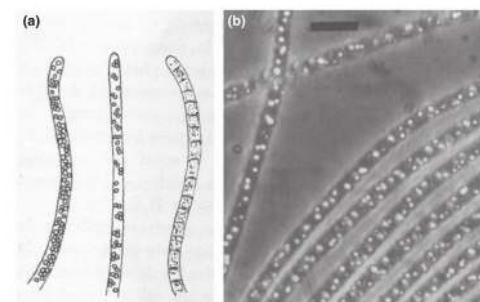
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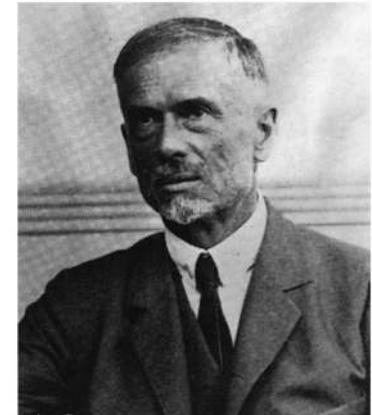
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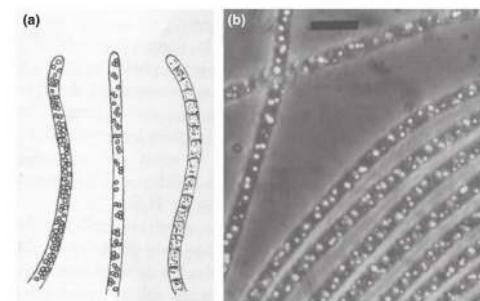
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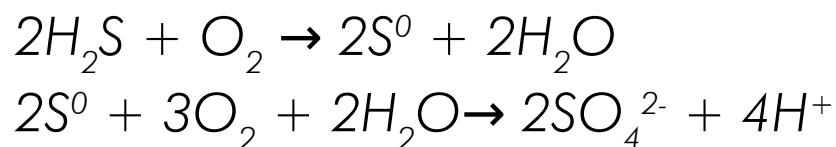


Beggiatoa is a genus of bacteria in the class Gammaproteobacteria, in the Proteobacteria phylum.

They are named after the Italian medic and botanist F. S. Beggiato.

Beggiatoa oxidized hydrogen sulfide (H_2S) as an energy source, forming intracellular sulfur droplets. Oxygen is the terminal electron acceptor and CO_2 is used as carbon source.

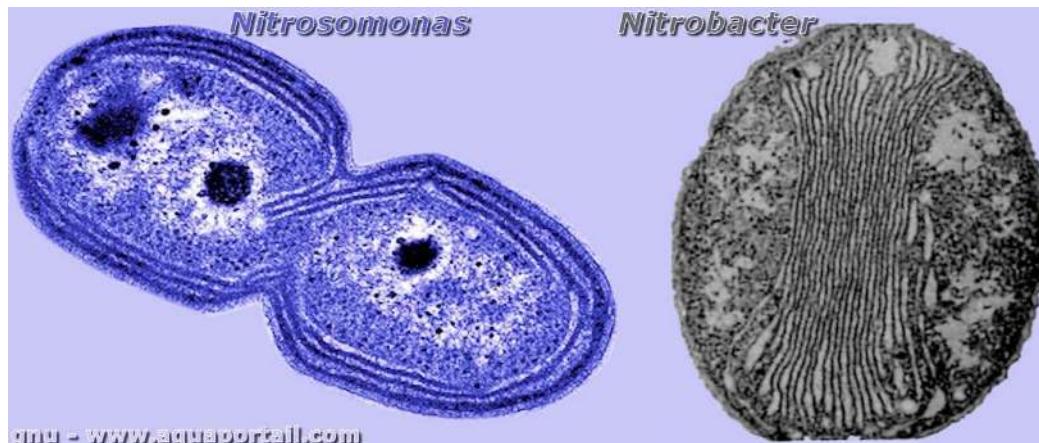
Winogradsky referred to this form of metabolism as inorgoxidation (oxidation of inorganic compounds).



The history of Chemolithotrophy

After discovering chemolithotrophy, Winogradsky moved to Zurich to study the process of Nitrification, i.e. the conversion of ammonia to nitrate

The process, carried out in two step by two distinct bacteria, required a series of problem to be solved, the last of which was the carbon source of the organism. At the time the only known autotroph were pigment containing photosynthetic organisms



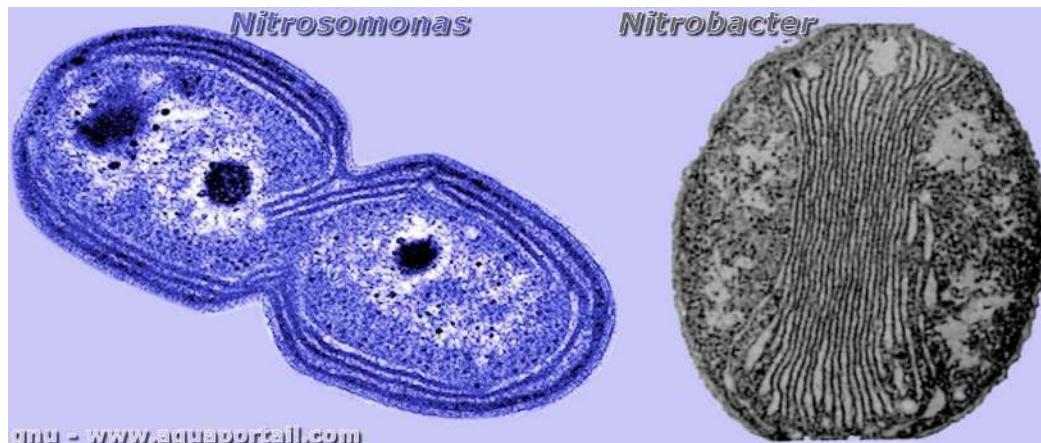
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To determine if indeed the organisms were growing and synthesizing their cell material in the absence of added organic material, he proceeded to rigorously exclude any organic material from his media. Accordingly he then used double distilled water, calcined salts and acid-washed glassware. Having done so, he then grew the nitrifying cultures, combusted the total organic material in the culture, collected and measured the CO₂ thus generated, subtracted the amount of CO₂ generated from the uninoculated culture material, and concluded that the data conclusively demonstrated that substantial net organic material had been synthesized. He concluded that a new truth of general physiological importance has been generated, namely '...a complete synthesis of organic material by the action of living organisms has been accomplished on our planet independent of solar energy.' (Winogradsky, 1890b).

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Carbon Fixation Pathways

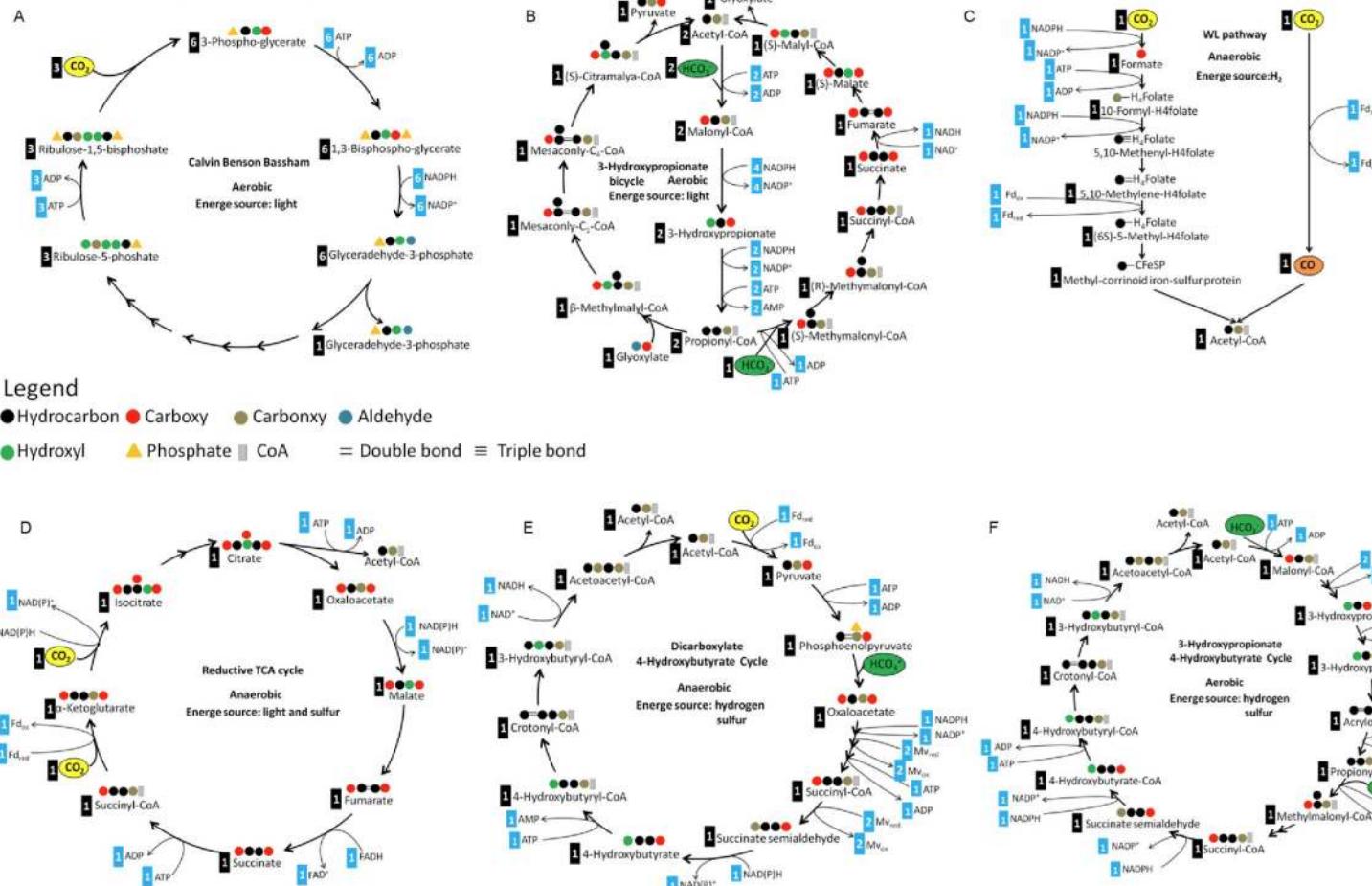


Figure 1 Six natural CO_2 -fixation pathways. A, Calvin cycle; B, 3-hydroxypropionate cycle; C, Wood-Ljungdahl pathway; D, reductive TCA cycle; E, dicarboxylate/4-hydroxybutyrate cycle; F, 3-hydroxypropionate/4-hydroxybutyrate cycle.

Gong et al. 2016 Sci Chi Life Sci

Carbon Fixation Pathways

As of today we know 7 distinct carbon fixation pathways, plus anaplerotic reactions and a reversible version of the oxidative tri-carboxylic acid cycle

Of the known six pathways, 5 are cycles and 1 is a linear pathway where intermediates are not recycled within the pathway but are produced elsewhere in the central metabolism

The energetic and carbon molecules requirements for each pathway vary greatly, as well as their efficiency, greatly influencing their distribution in the environments and within the Tree of Life

Namely the six pathways are:

- **Calvin Benson Bassham Cycle**, also known as the Calvin Cycle
- **Arnon Buchanan Cycle**, also known as the reductive TCA Cycle
- **Wood-Ljungdahl pathway**, also known as the reductive acetyl-CoA pathway
- **3-Hydroxypropionate cycle**
- **Dicarboxylate/4-Hydroxybutyrate cycle**
- **3-Hydroxypropionate/4-Hydroxybutyrate bicycle**
- **Wolf Cycle**

Carbon Fixation Pathways: their discovery

Calvin Benson Bassham Cycle was discovered in 1950 by Melvin Calvin, James Bassham, and Andrew Benson at the University of California, Berkeley by using the radioactive isotope carbon-14 and a culture of a Green Algae (Chlorophyceae)

Arnon Buchanan Cycle was discovered in 1966 in photosynthetic green sulfur bacteria (Chlorobi), and it was the first of the new carbon fixation pathways to be identified

Wood-Ljungdahl pathway was discovered in 1986 in acetogenic bacteria, and was later reported in methanogenic archaea and in sulfate-reducing bacteria

3-Hydroxypropionate bicycle was first described in *Chloroflexus antarticus* in 2002 by Herter and colleagues. It was the first of a series of new carbon fixation pathways identified in Archaea

Carbon Fixation Pathways: their discovery

Dicarboxylate/4-Hydroxybutyrate cycle was discovered by Huber and colleagues in the archaeum *Ignicoccus hospitalis* a in 2008

3-Hydroxypropionate/4-Hydroxybutyrate cycle was discovered in 2007 by Berg and colleagues in the Sulfolobales *Metallosphaera sedula*

Wolfe Cycle was originally proposed in 1988, when Rouvière and Wolfe suggested that production of methane by hydrogenotrophic Archaea was carried out in a cycle. The nature of the cycle was confirmed only in 2012 by Lie and colleagues

Forming Carbon-Carbon bonds

Carbon–carbon bond formation is the key reaction for organic synthesis to construct the carbon framework of organic molecules

The assimilation of CO₂ (oxidation state of +4) into cellular carbon (average oxidation state of 0, as in carbohydrates) requires four reducing equivalents (electrons)

CO₂ is organicated via one of two mechanisms: carboxylation, in which CO₂ is attached to an existing metabolite, or reduction, in which CO₂ is converted to formate or carbon monoxide before further assimilation

Enzymes responsible for CO₂ incorporation are redox enzymes (oxidoreductases) that use cofactors to lower the energy of activation. These cofactors are often metals

Forming Carbon-Carbon bonds

An input of energy is also required for the reductive conversion of CO₂ to cell carbon and is provided by ATP hydrolysis

Anaerobes often use low-potential electron donors like reduced ferredoxin for CO₂ fixation, whereas aerobes usually rely on NAD(P)H as a reductant

The CO₂ taken up by the organism generates relatively reductive pyruvate or acetyl-CoA, both of which are important intermediate metabolites for cell growth.

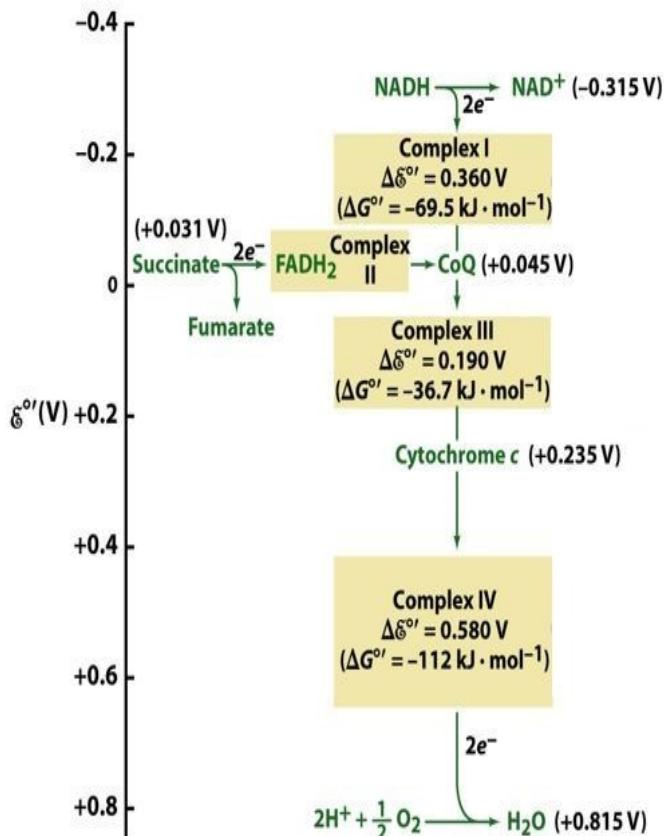
Since the carbon atom in a CO₂ molecule is in the highest positive valence state. Consequently, the changes of the Gibbs free energy (ΔG) of these CO₂ reductive reactions are usually positive, thus thermodynamically unfavorable and challenging.

Energy and reduction potential

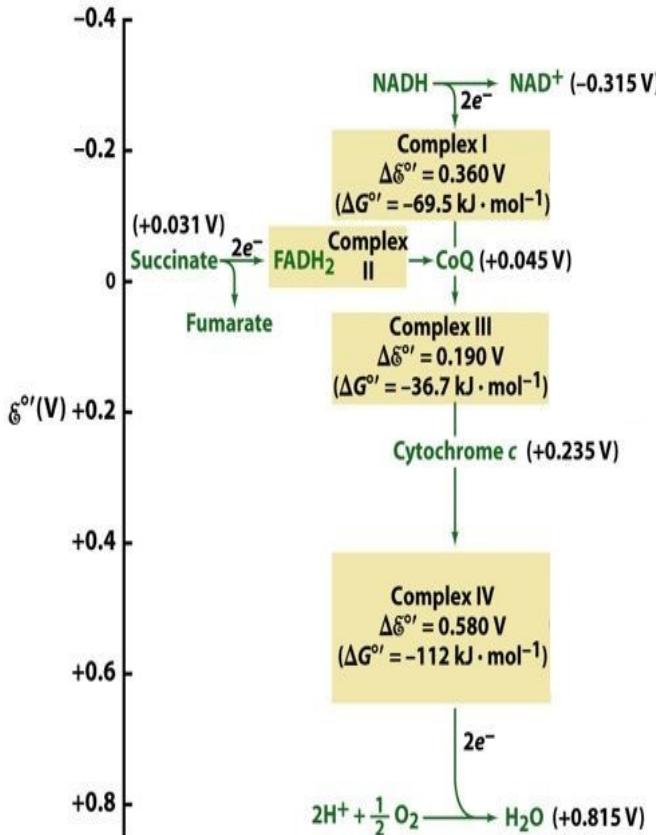
Table 1 Reaction energy diagram and glossary

Reaction energy diagram	Glossary
<p>The diagram illustrates a reaction coordinate plot where Energy is on the vertical axis and The process of reaction is on the horizontal axis. A solid black line represents the reaction with an enzyme catalyst, while a dotted red line represents the reaction without a catalyst. The initial state is labeled 'substrate' and the final state is 'product'. Two activation states are indicated by dashed horizontal lines. The activation energy without catalyst ($\Delta E'$) is the height from the substrate level to the peak of the dotted curve. The activation energy with enzyme (ΔE_2) is the height from the substrate level to the peak of the solid curve. The reaction activation energy (ΔE) is the difference between $\Delta E'$ and ΔE_2. The Gibbs free energy change (ΔG) is the vertical distance from the substrate level to the product level.</p>	<p>ΔE Reaction activation energy, minimum energy required to start a chemical reaction, related to the substrates and catalyst.</p> <p>ΔE_1 Reaction activation energy without catalyst</p> <p>ΔE_2 Activation energy with enzyme</p> <p>$\Delta E'$ Reduced activation energy by catalyst</p> <p>ΔG The change of Gibbs free energy, a thermodynamic function to judge the direction of a process in chemical thermodynamics, determined by the free energy of substrates and products.</p> <p>$\Delta_r G$ The change of Gibbs free energy of a specific reaction.</p> <p>$\Delta_r G'$ The change of Gibbs free energy of a specific reaction under some condition</p> <p>$\Delta_r G'^m$ The change of Gibbs free energy of a specific reaction under physiological condition</p> <p>$\Delta_r G'_c$ The change of Gibbs free energy of per mol carbon under certain conditions</p> <p>$\Delta_r G'^m_c$ The change of Gibbs free energy of per mol carbon for a specific reaction under physiological conditions</p> <p>$\Delta_t G'^m_c$ The total change of Gibbs free energy of per mol carbon for a CFP</p>

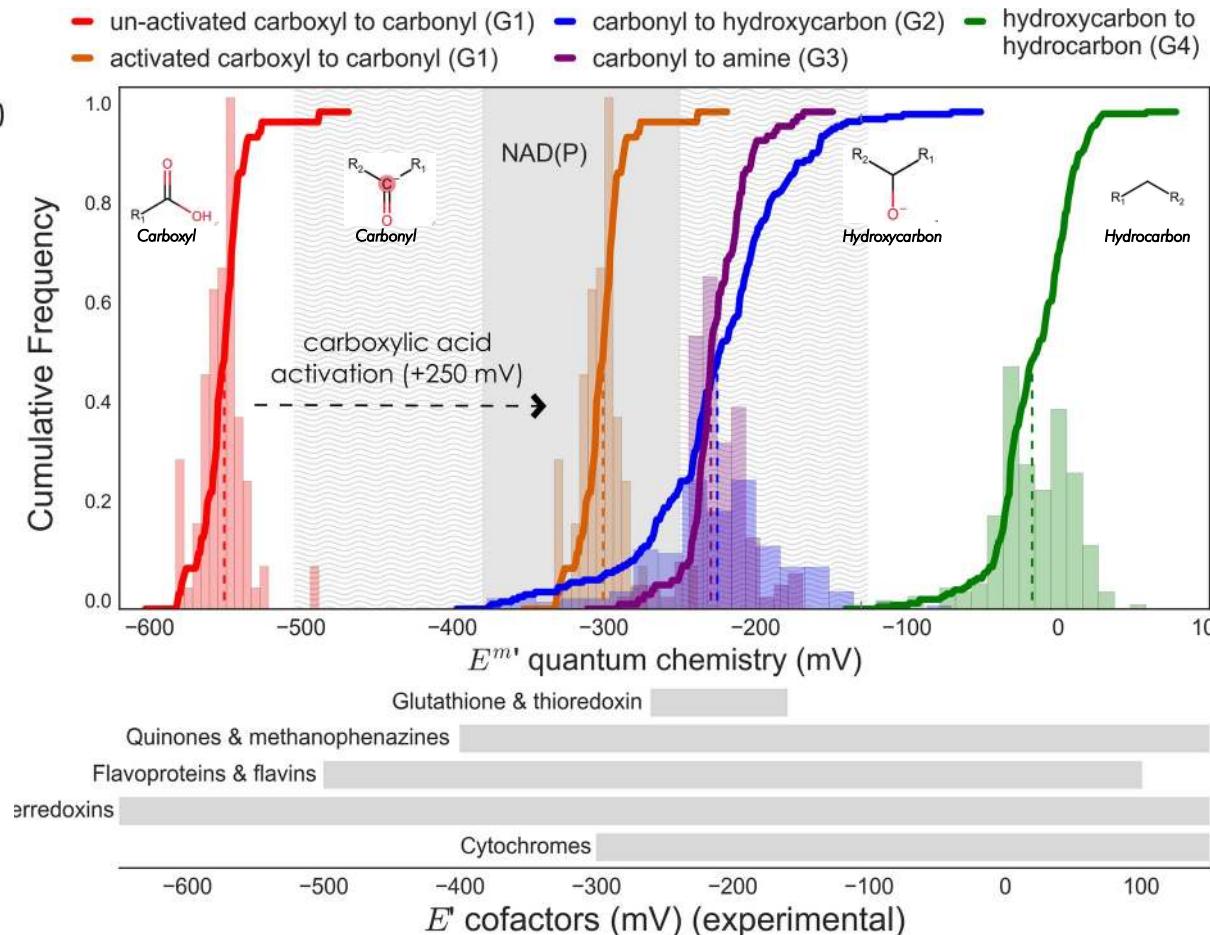
The need for reduction potential



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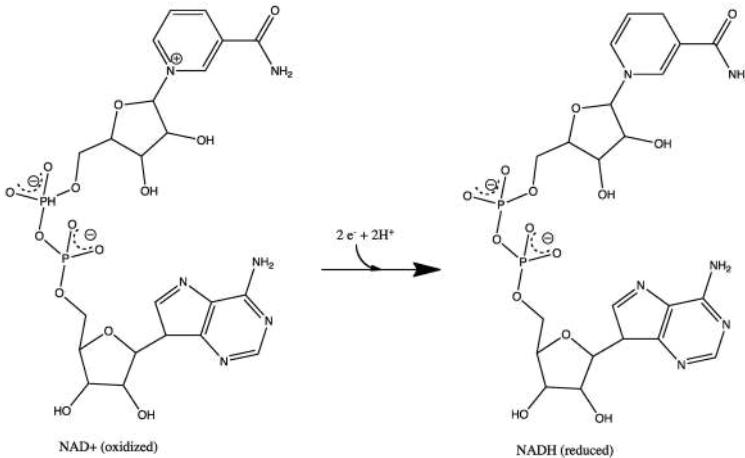


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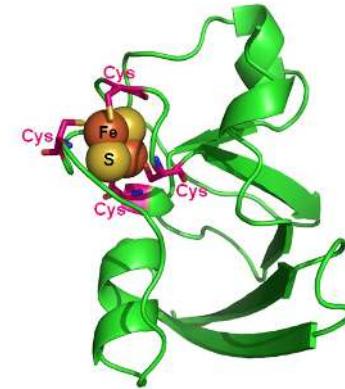
XXXXX

The need for reduction potential



NADH
-320 mV

NADH is a coenzyme consisting of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine nucleobase and the other nicotinamide



Ferredoxin
from -320 to -600 mV

Ferredoxins are iron–sulfur proteins that mediate electron transfer in a range of metabolic reactions

Low potential electrons and electron bifurcation

The autotrophic pathways operating under anaerobic (or microaerobic) conditions involve highly oxygen-sensitive reduction steps that are driven by reduced ferredoxin. These steps include reactions that range from -420 mV to -520 mV

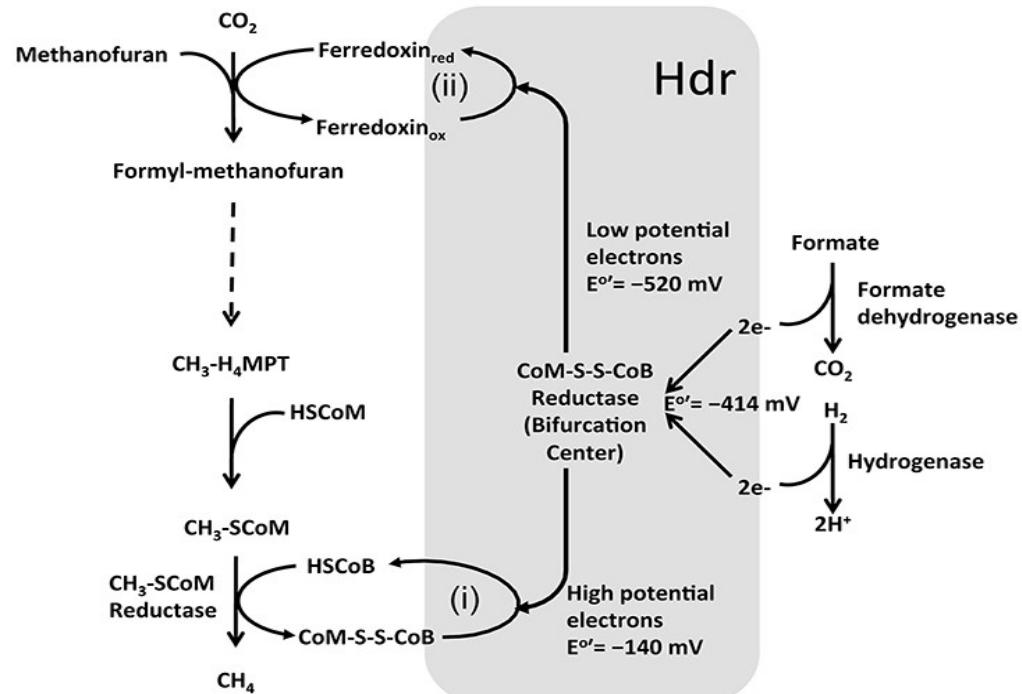
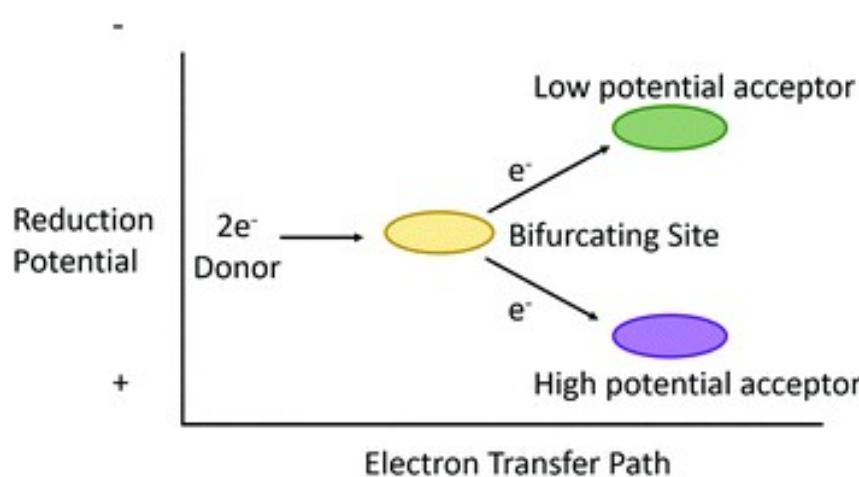
Molecular hydrogen (H_2) is often used by anaerobes as preferred electron donor, given its low potential (-414 mV). Despite this, low potential needs to be achieved through a complex mechanism know as **electron bifurcation**

A variety of different mechanism exist under this name:

Ferredoxin Reduction by NADH in mbrane-Bound Rnf Complex and Soluble Electron-Bifurcating Enzymes, Electron Bifurcation by Soluble [NiFe]-Hydrogenase and Heterodisulfide Reductase, Membrane-Bound Energy-Converting [NiFe]-Hydrogenases and Soluble Bifurcating [FeFe]-Hydrogenases

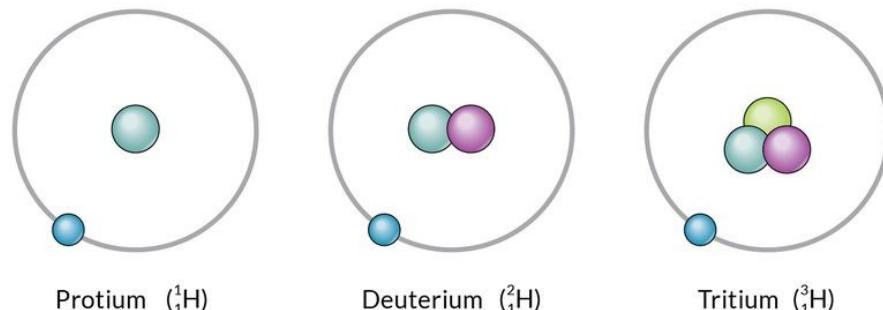
Low potential electrons and electron bifurcation

In its essence, electron bifurcation oxidizes a two-electron donor, using the electrons to reduce cofactors on two separate electron-transfer redox chains. The coupling of these redox reactions allows one of the electrons to move thermodynamically uphill, leveraging the downhill flow of the other electron.



Mass Dependent Isotopic Fractionation

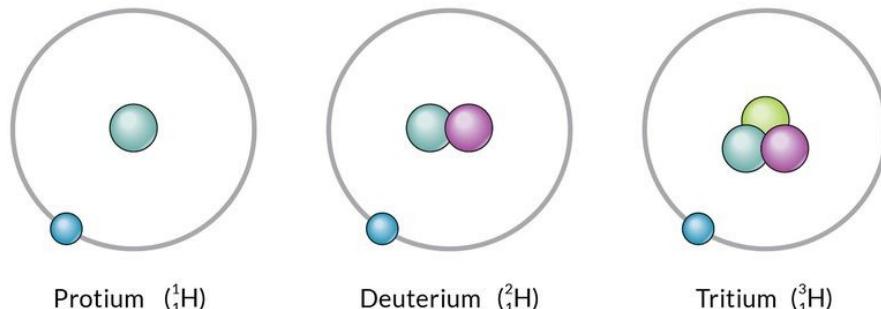
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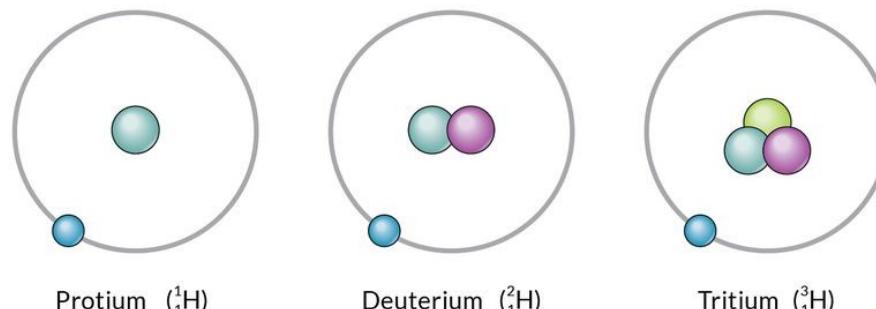
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$$\delta^A X = 1000 \times \frac{^A R_{\text{sample}} - ^A R_{\text{std}}}{^A R_{\text{std}}}$$

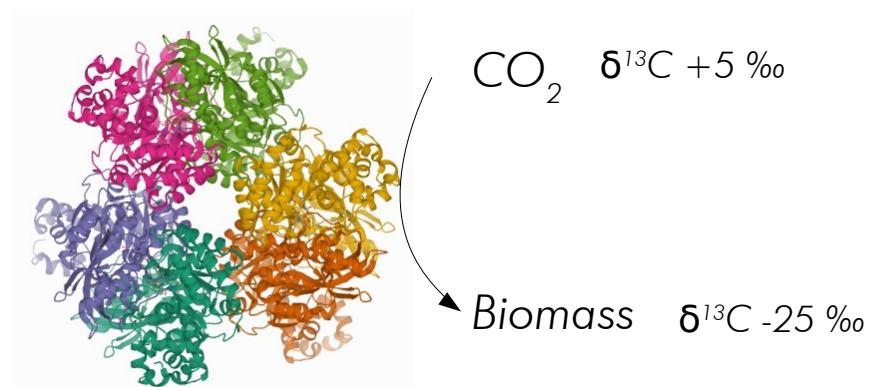


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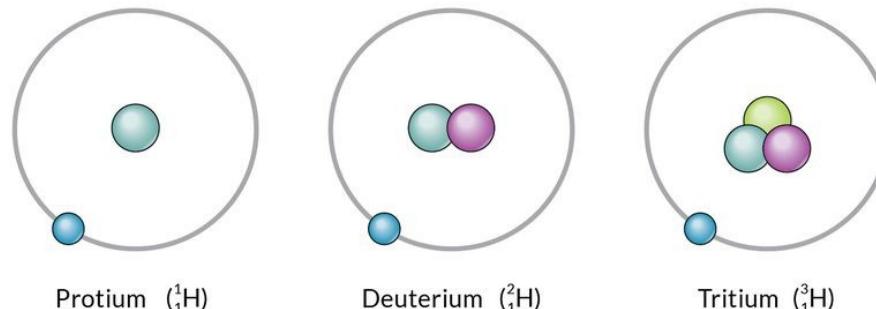


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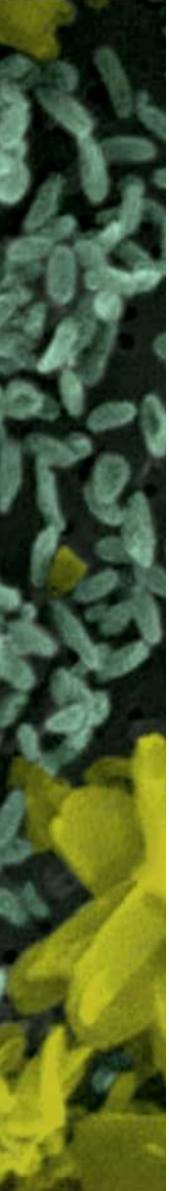
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$\text{CO}_2 \ \delta^{13}\text{C} +5 \text{ ‰}$

$\Delta^{13}\text{C} -30 \text{ ‰}$

Biomass $\delta^{13}\text{C} -25 \text{ ‰}$



PATHWAYS OF CARBON FIXATION

Calvin-Benson-Bassham Cycle

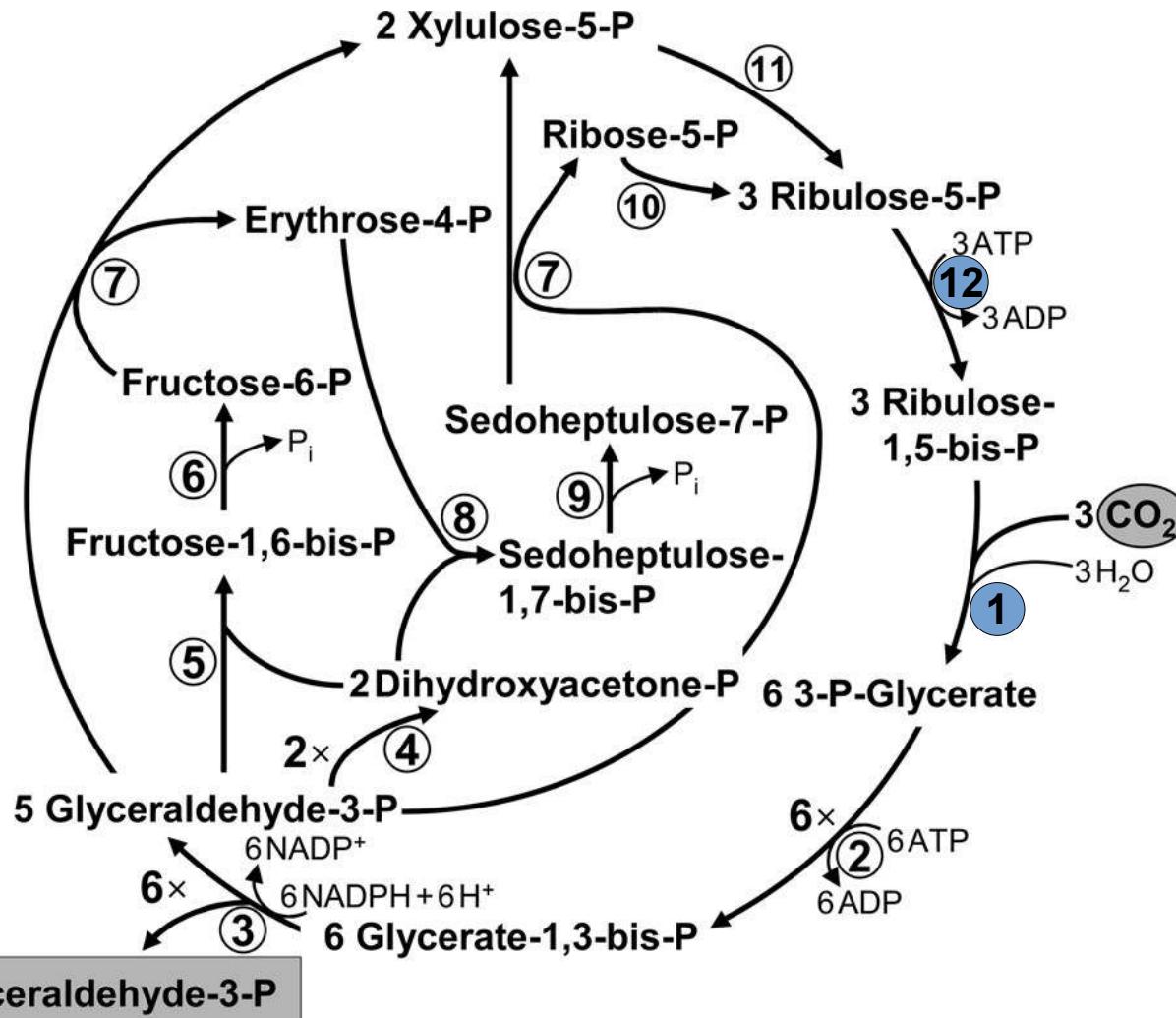
Discovered in 1950 in Green Algae, the Calvin Cycle is the dominant cycle in the extant biosphere, and dominated autotrophic processes in the surface of our planet

The key enzymes are the Ribulose-1,5 Bifosphate Carboxylase/Oxygenase (RuBisCo) and phosphoribulokinase

The cycle requires 9 ATP equivalents and 6 NADPHs for the synthesis of 1 glyceraldehyde-3-phosphate molecule, and is one of the most energetically demanding carbon fixation pathways

The Calvin Cycle operates in plants, algae, Cyanobacteria, and many aerobic or facultative aerobic proteobacteria belonging to the Alpha, Beta, and Gamma subgroups, and found in iron and sulfur-oxidizing members of the Firmicutes, some Mycobacteria and Chloroflexi

Calvin-Benson-Bassham Cycle



1. ribulose-1,5-bisphosphate carboxylase/oxygenase
2. 3-phosphoglycerate kinase
3. glyceraldehyde-3-phosphate dehydrogenase
4. triose-phosphate isomerase
5. fructose-bisphosphate aldolase
6. fructose-bisphosphate phosphatase
7. transketolase
8. sedoheptulose-bisphosphate aldolase
9. sedoheptulose-bisphosphate phosphatase
10. ribose-phosphate isomerase
11. ribulose-phosphate epimerase
12. phosphoribulokinase

RuBisCo

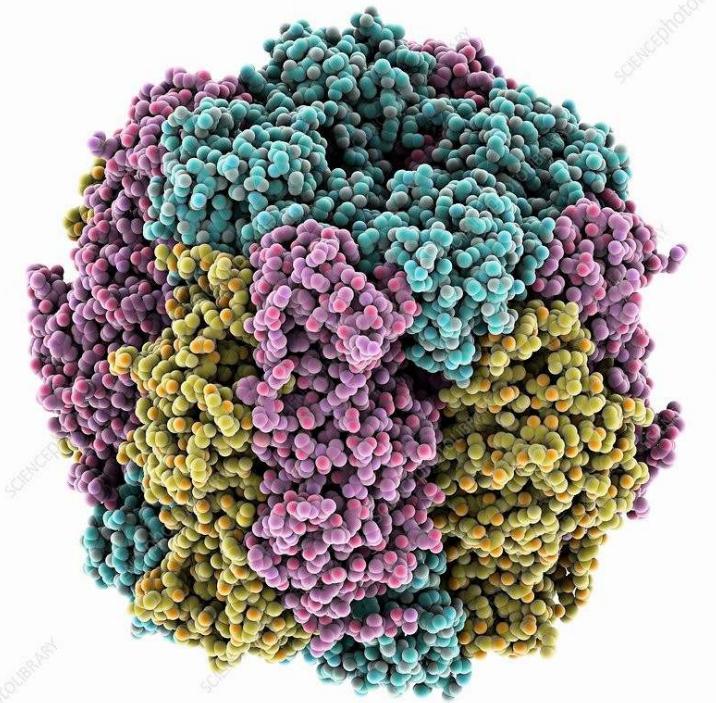
RuBisCo is a large (500 kDa) heterodimeric enzymes formed by a large and a small subunit

Different forms of RuBisCo enzymes are known and widely distributed on the Tree of Life

It is inhibited by oxygen, and it is a relatively slow and inefficient enzyme

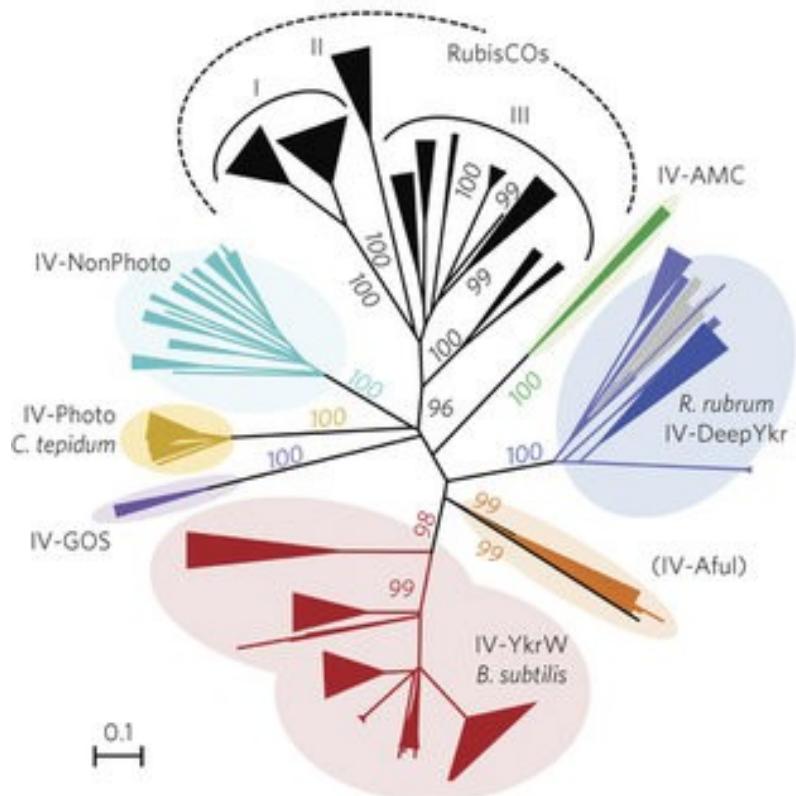
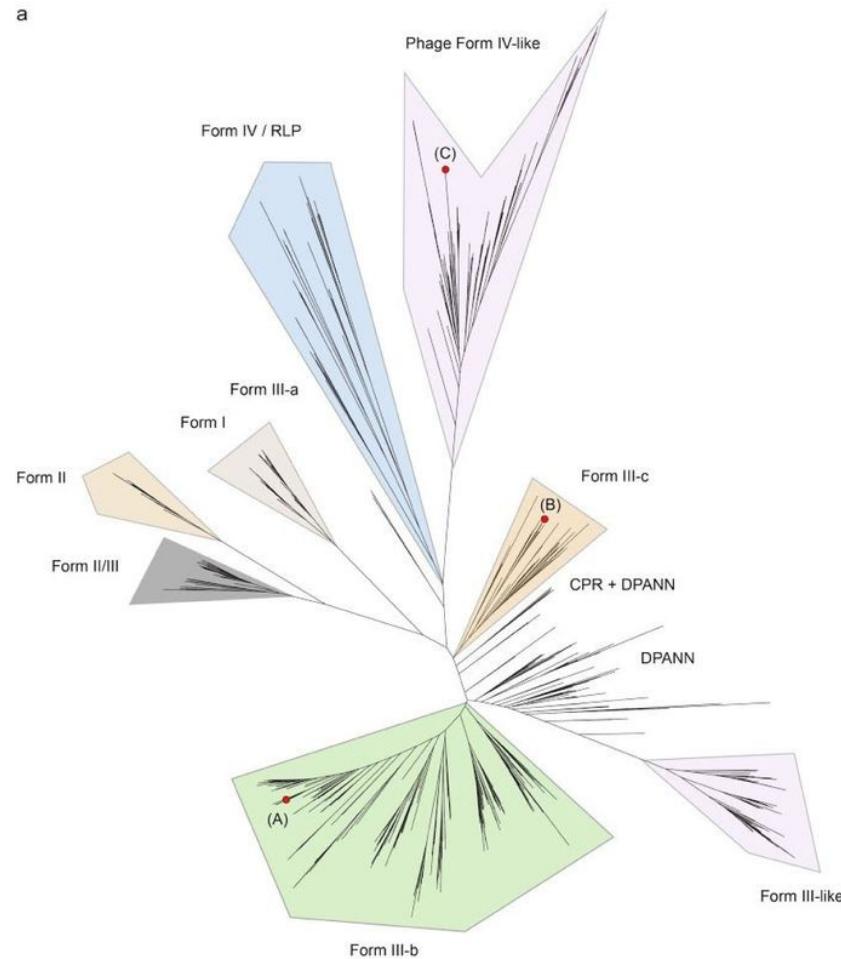
Can make up to 50% of chloroplast protein, and is probably the most abundant protein on Earth

Fractionates significantly Carbon, with a $\Delta^{13}\text{C}$ between -10 and -37‰ , and vary significantly between Bacteria, C3, C4 and CAM plants

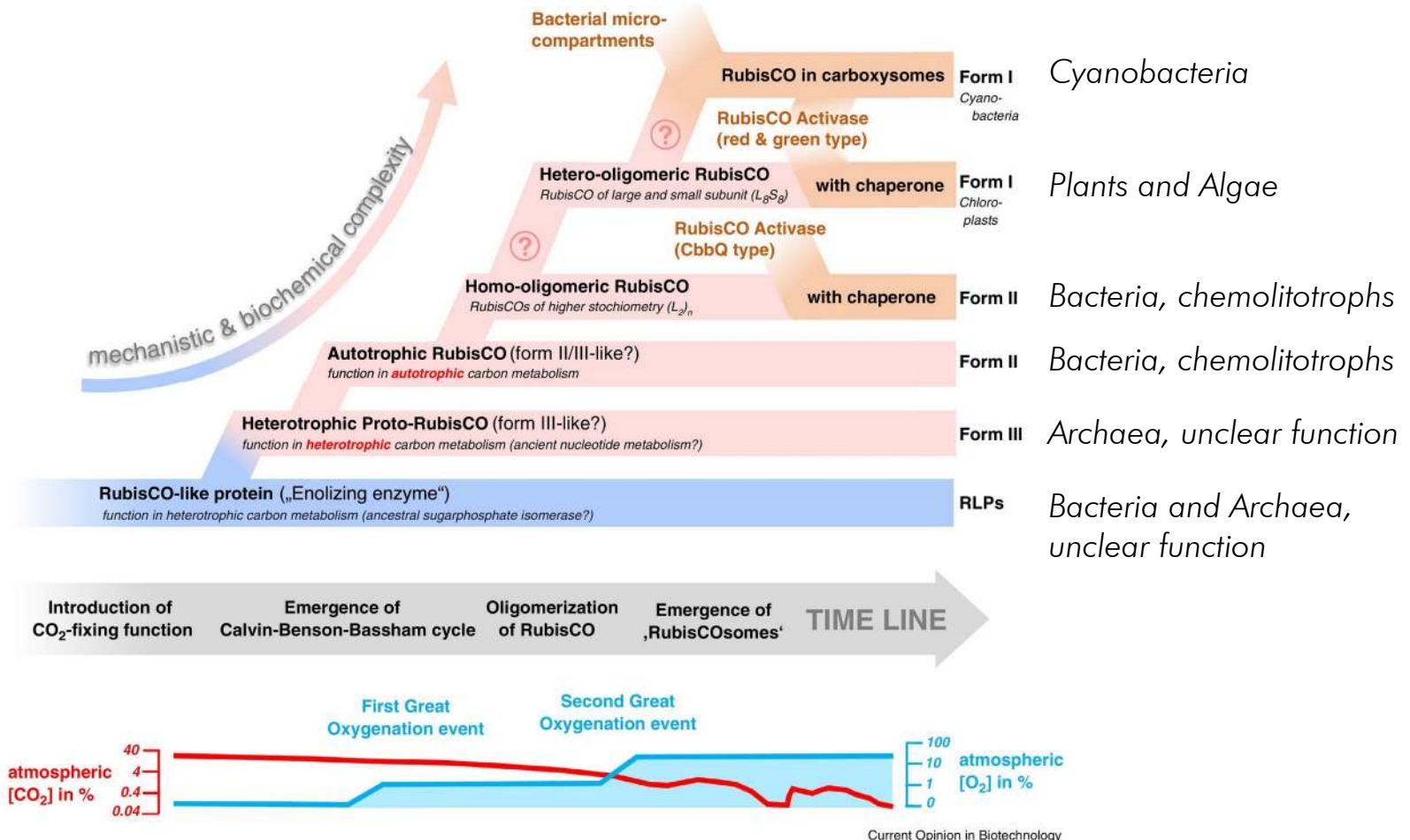


RuBisCo and RLP phylogeny

a



Evolution of RuBisCo



Arnon-Buchanan Cycle

Also known as the reductive TCA cycle, was discovered in 1966 in photosynthetic green sulfur bacteria (Chlorobi), it was the first of the new carbon fixation pathways to be identified

It is abundant in numerous anaerobic chemolithoautotrophic bacteria, some anaerobic chemolithoautotrophic archaea and some anoxygenic photoautotrophs

Two major variants exist, evolutionary linked

ATP-citrate lyase, Succinyl-CoA carboxylase and 2-Oxoglutarate synthase are the key enzyme for its functioning in reverse and are extremely oxygen sensitive

3 CO₂ and 2-3 ATP are used to generate 1 molecule of Pyruvate, making the rTCA cycle a very energy efficient carbon fixation pathway

$\Delta^{13}\text{C}$ isotopic fractionation is between –8 and –20 ‰

Arnon-Buchanan Cycle: acceptance of new discoveries is sometime very slow...

When first reported, the rTCA cycle was met with wide skepticism by the research community when first reported. At the time the Calvin Cycle was considered the only autotrophic pathway

"Looking back, I believe the widely held view that autotrophy was synonymous with Rubisco was the major barrier to acceptance of the cycle by the scientific community. Once confirmed, the Chlorobium work was the death knell for this long-standing dogma. Thus, some 25 years after our publication in the Proceedings of the National Academy of Sciences (Evans et al. 1966) the Arnon–Buchanan cycle began to appear in textbooks of microbiology and related fields (for example, see Madigan et al. 1996)." Buchanan



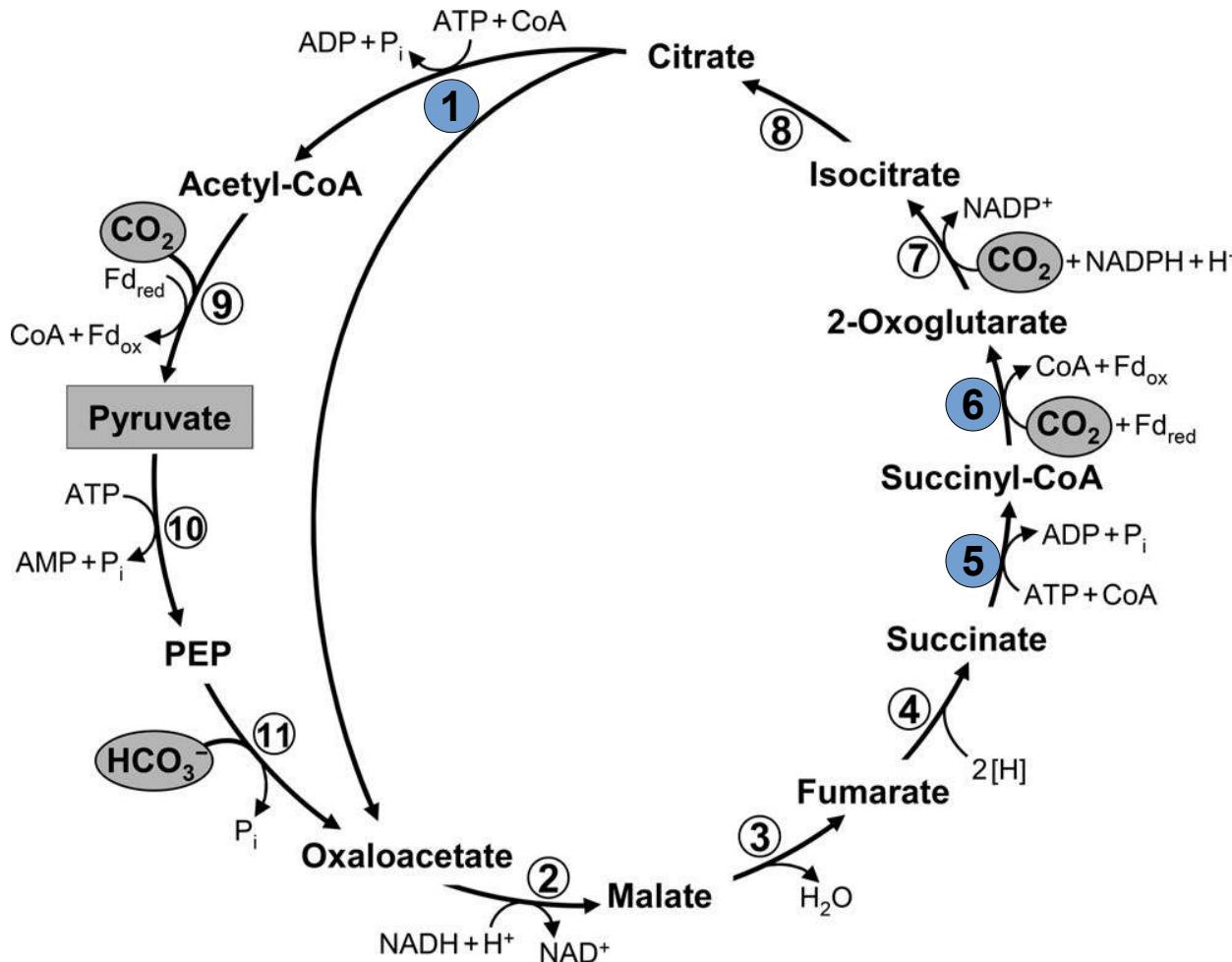
Arnon-Buchanan Cycle: acceptance of new discoveries is sometime very slow...

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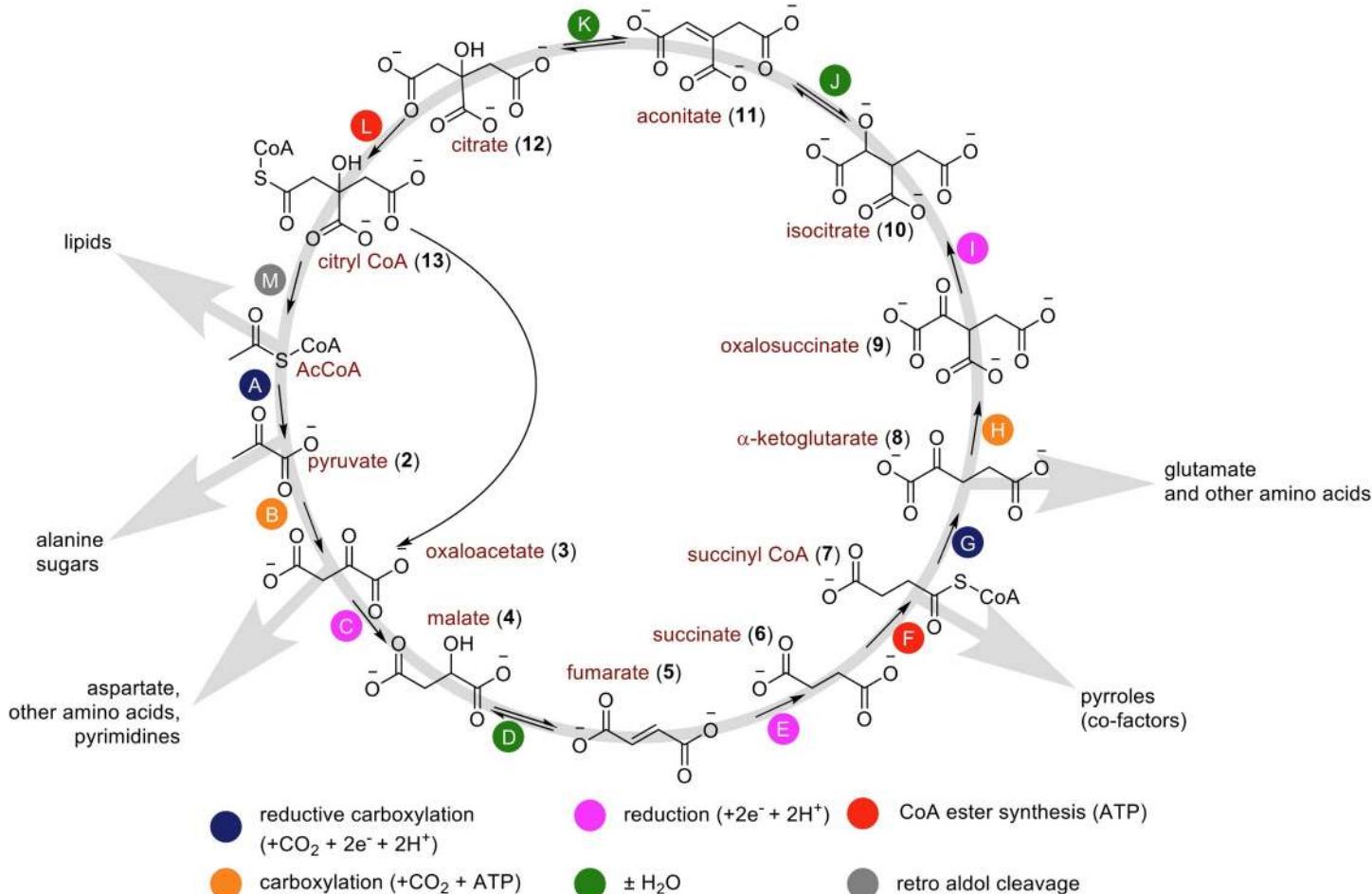
Buchanan

Arnon-Buchanan Cycle



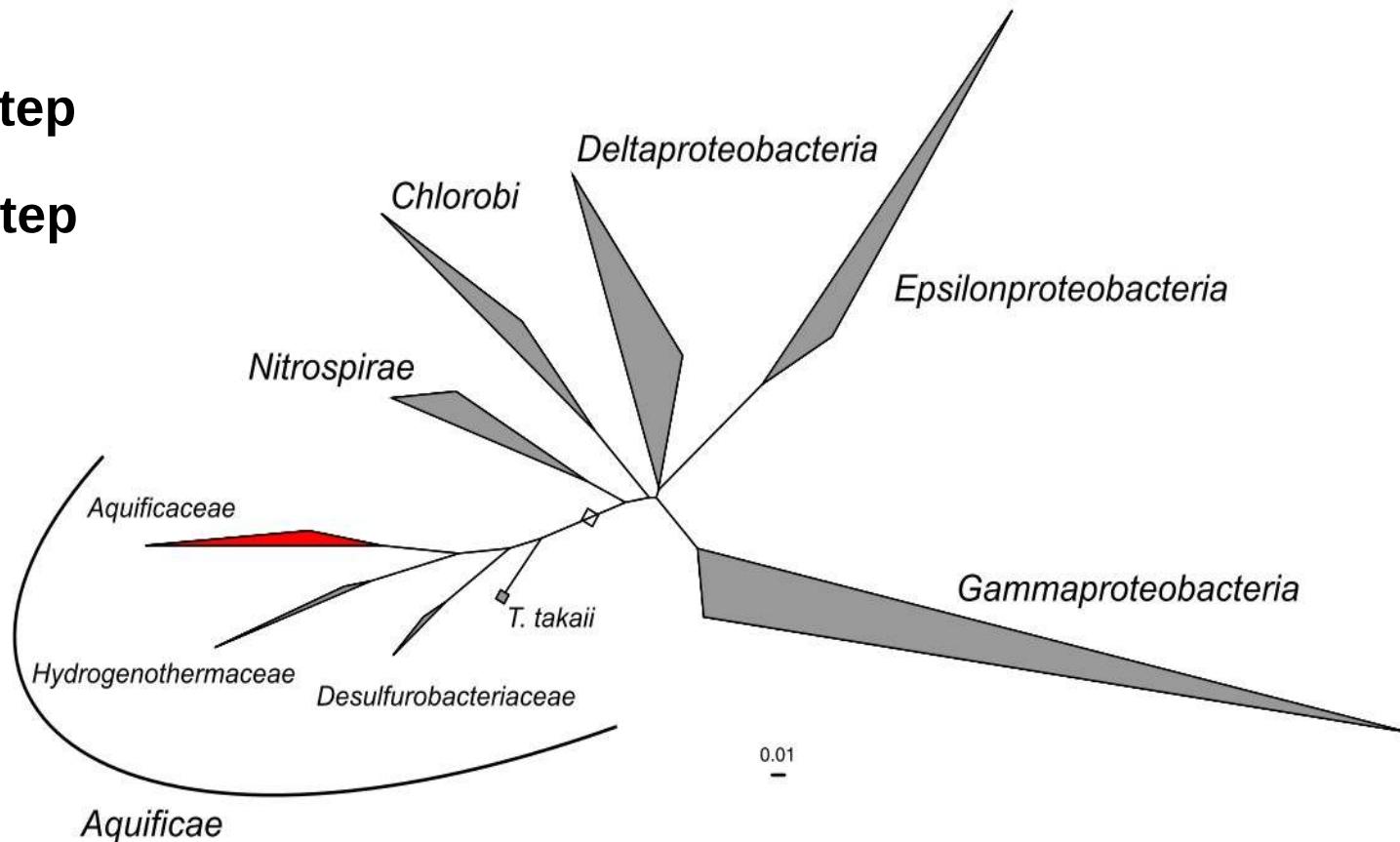
1. ATP-citrate lyase
2. malate dehydrogenase
3. fumarate hydratase
4. fumarate reductase
5. succinyl-CoA synthetase
6. ferredoxin (Fd)-dependent 2-oxoglutarate synthase
7. isocitrate dehydrogenase
8. aconitate hydratase
9. Fd-dependent pyruvate synthase
10. PEP synthase
12. PEP carboxylase

Arnon-Buchanan Cycle: centrality of intermediates



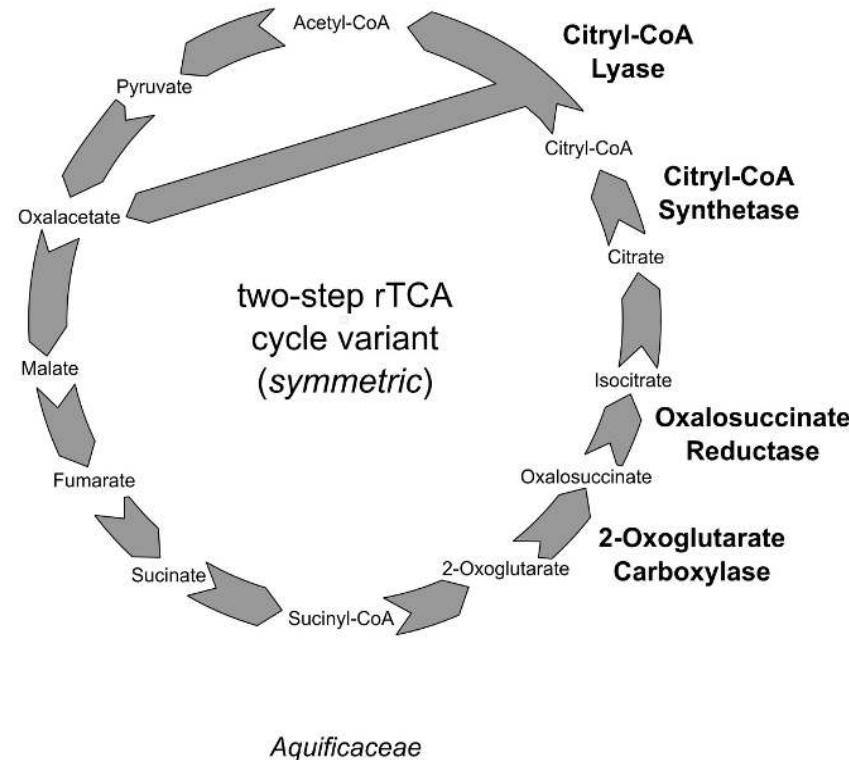
Arnon-Buchanan Cycle

- █ two-step
- one-step

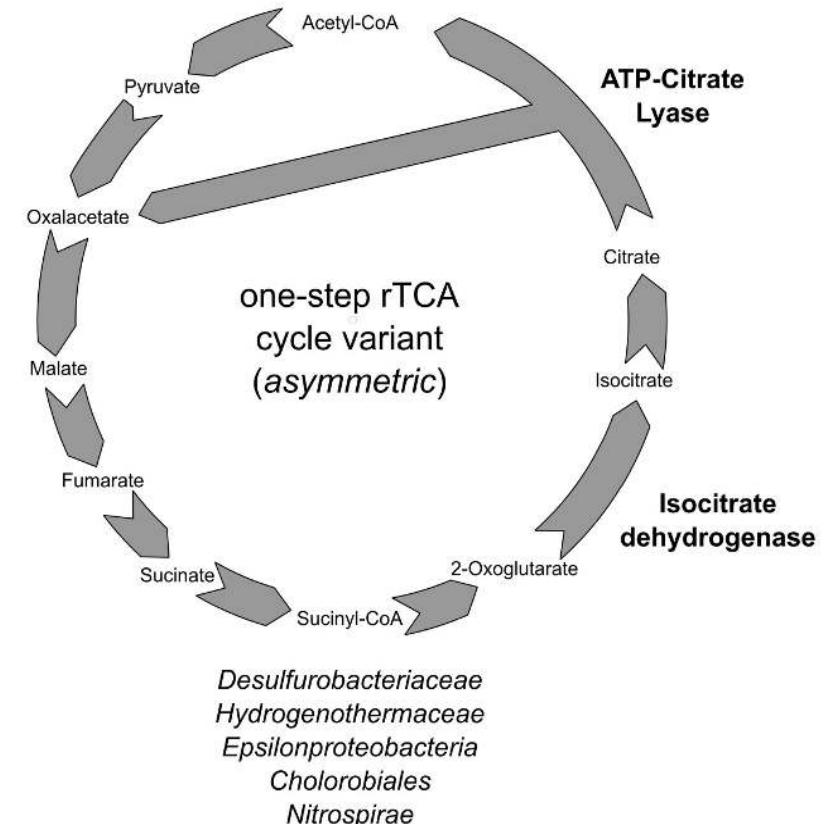


Arnon-Buchanan Cycle: two different variant

A



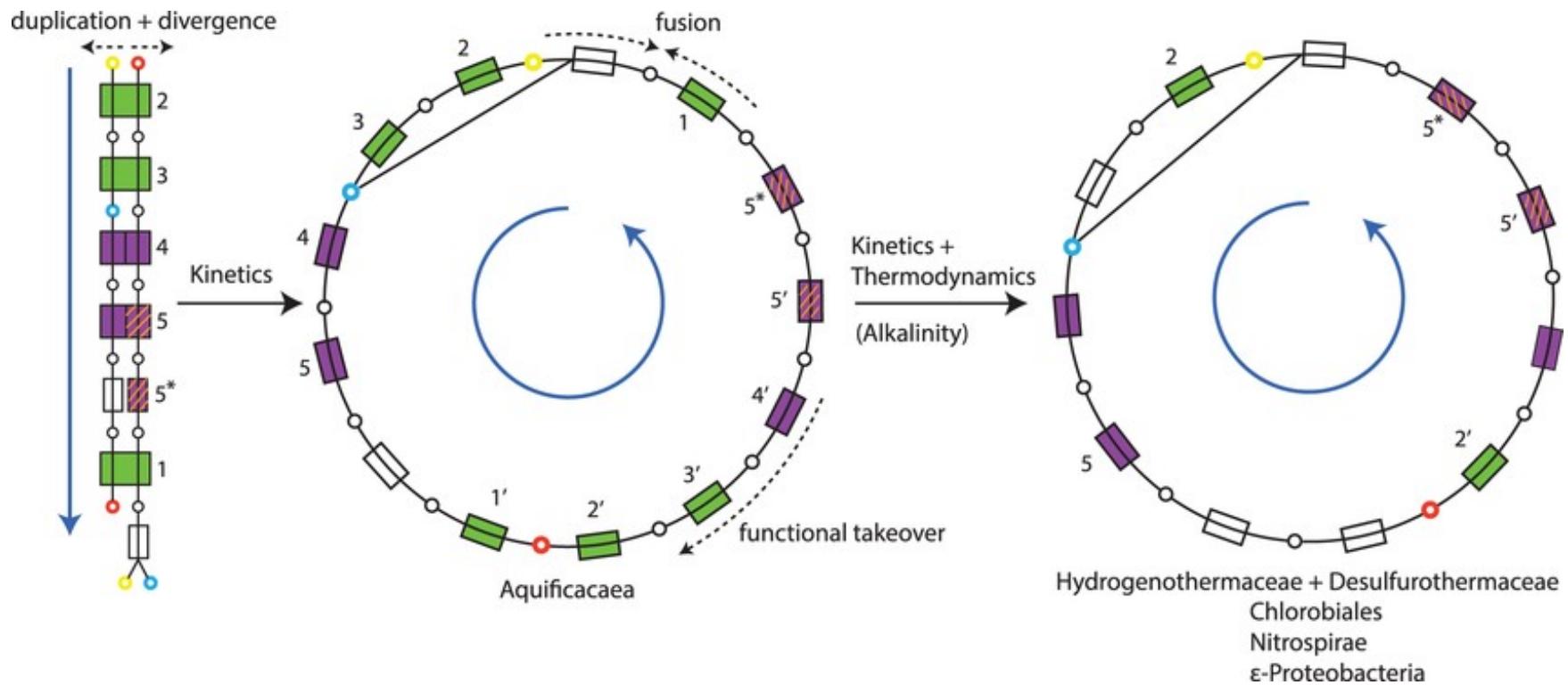
B



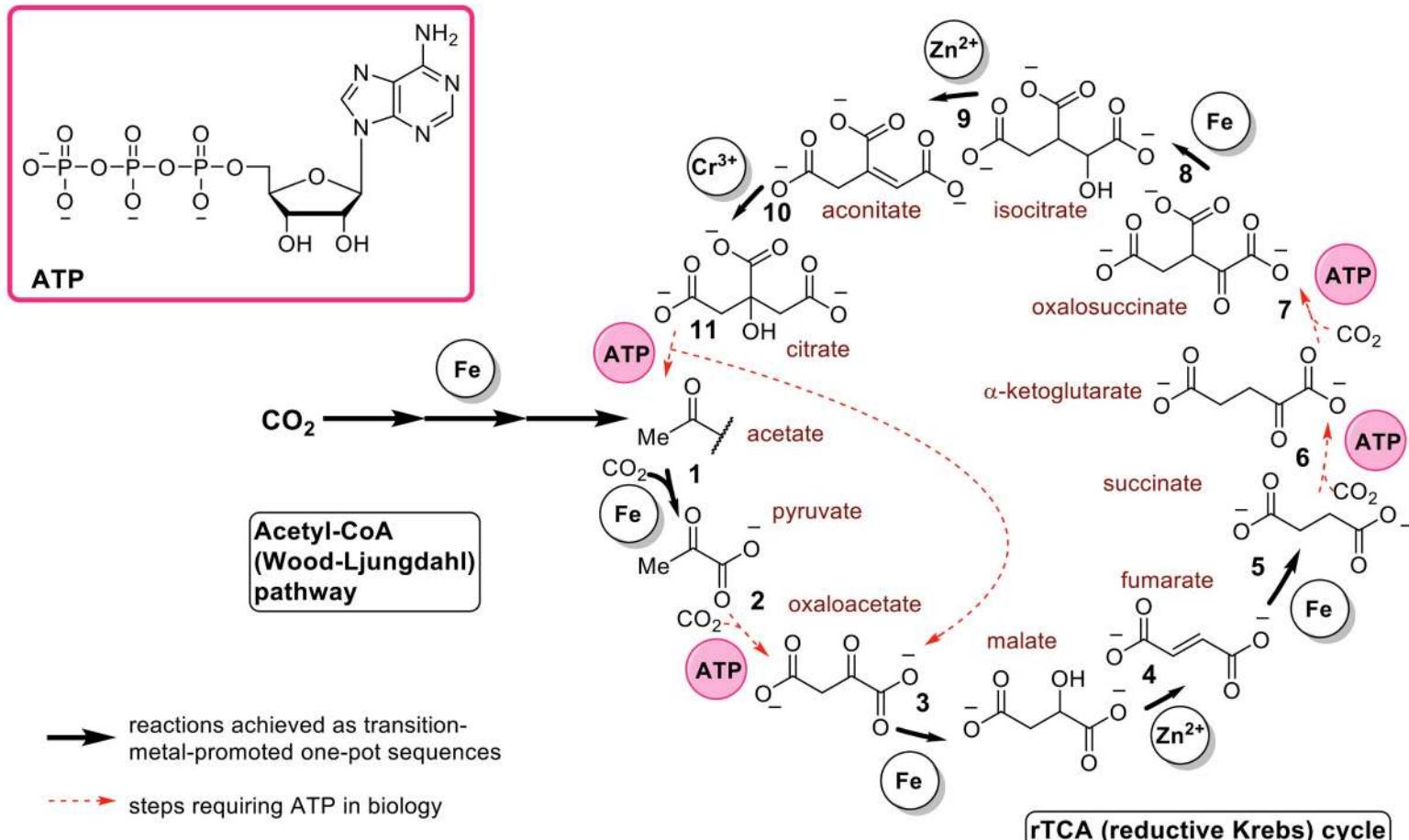
Aquificaceae

Desulfurobacteriaceae
Hydrogenothermaceae
Epsilonproteobacteria
Chlorobiales
Nitrospirae

Arnon-Buchanan Cycle: Evolution



Arnon-Buchanan Cycle: at the Origin of Life



Wood-Ljungdahl pathway

The reductive Acetyl-CoA pathway, discovered in 1986 in acetogenic bacteria, and later reported in methanogenic archaea and in sulfate-reducing bacteria is the only linear pathway among the known carbon fixation pathways

The pathway is divided in two branches, producing the methyl group and the carbonyl group of the Acetyl-CoA molecule, respectively

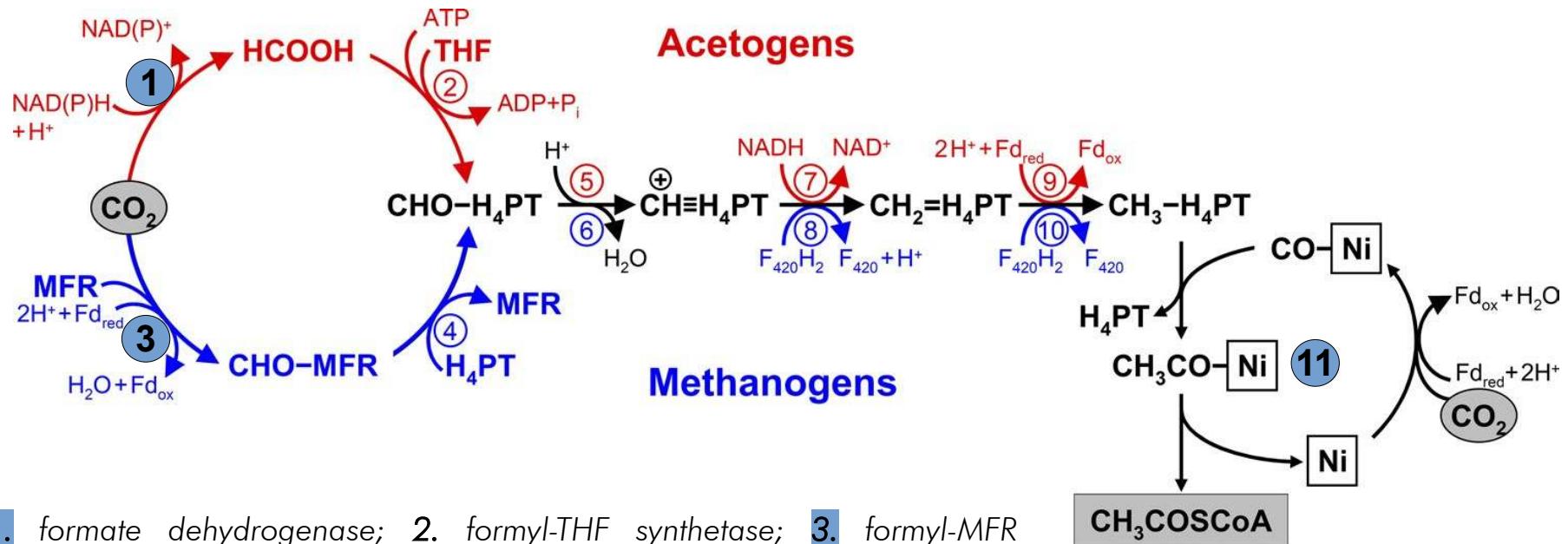
The methyl branch is functionally similar but not homologous between Bacteria and Archaea, while the carbonyl branch is homologous, this complicates all evolutionary work

The key enzymes is a bifunctional heterotetramer formed by the carbon monoxide dehydrogenase and the acetyl-CoA synthase

It has been proposed as the possible ancestral c-fixation pathway

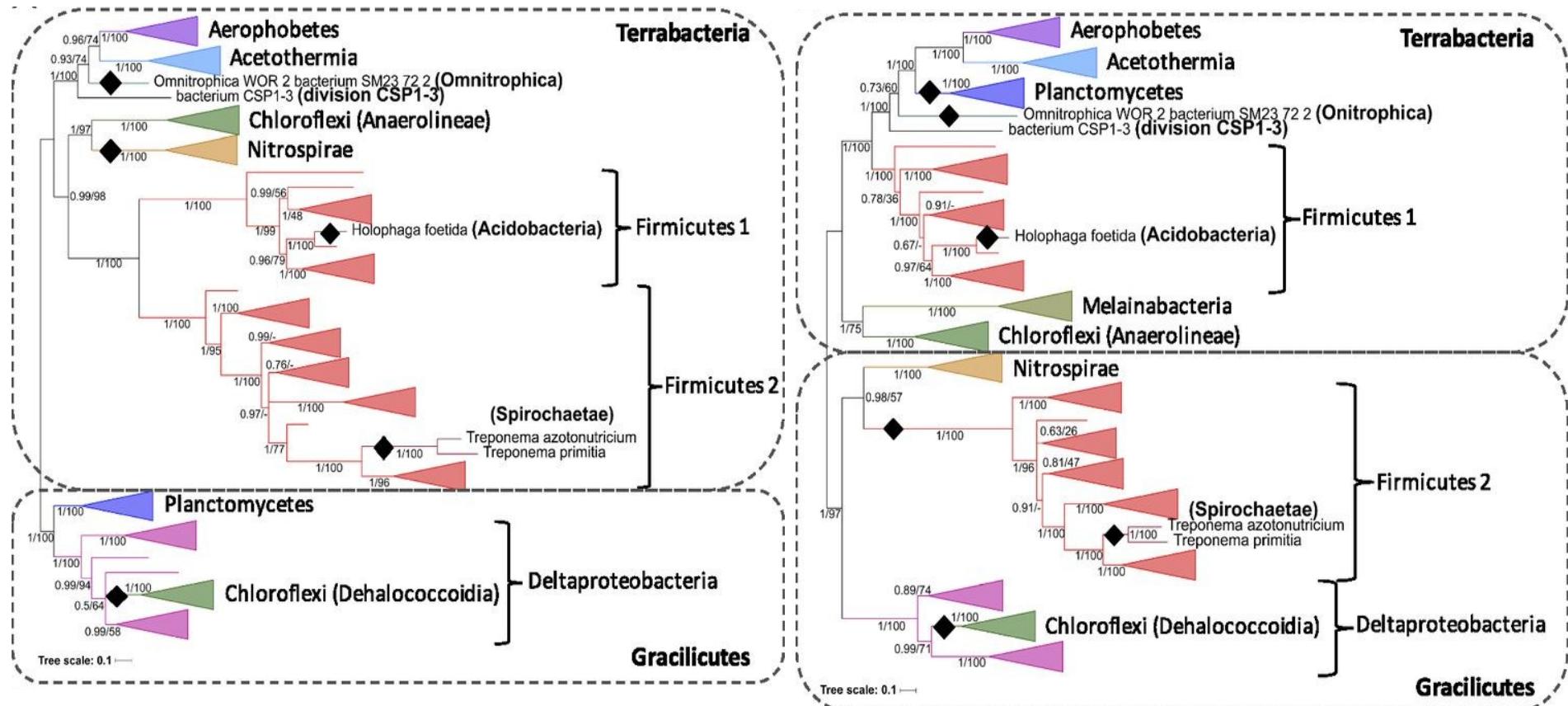
$\Delta^{13}\text{C}$ isotopic fractionation is variable and between -15 and -80 ‰

Wood-Ljungdahl pathway

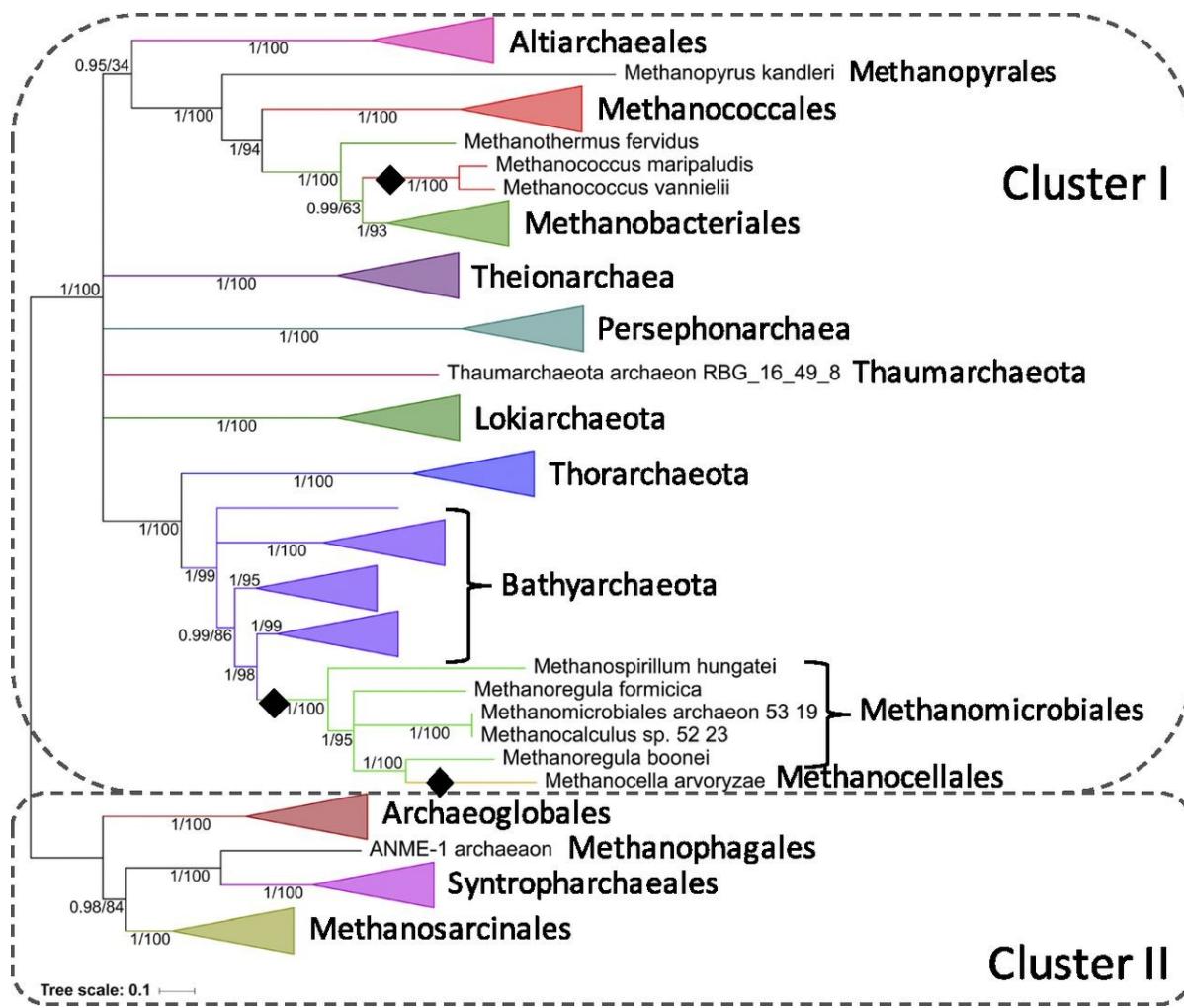


1. formate dehydrogenase; 2. formyl-THF synthetase; 3. formyl-MFR dehydrogenase; 4. formyl-MFR:tetrahydromethanopterin formyltransferase; 5. methenyl-THF cyclohydrolase; 6. methenyl-tetrahydromethanopterin cyclohydrolase; 7. methylene-THF dehydrogenase; 8. methylene-tetrahydromethanopterin dehydrogenase; 9. methylene-THF reductase; 10. methylene-tetrahydromethanopterin reductase; 11. CO dehydrogenase/acetyl-CoA synthase

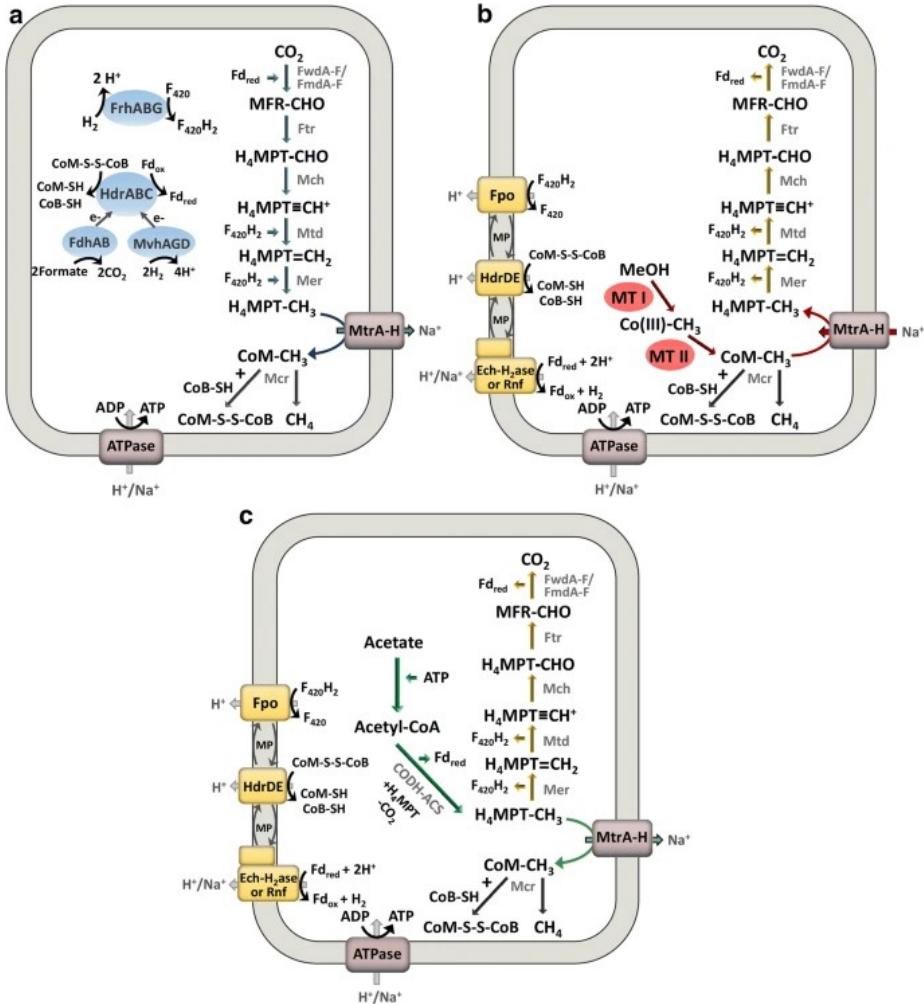
Wood-Ljungdahl pathway: distribution



Wood-Ljungdahl pathway: distribution



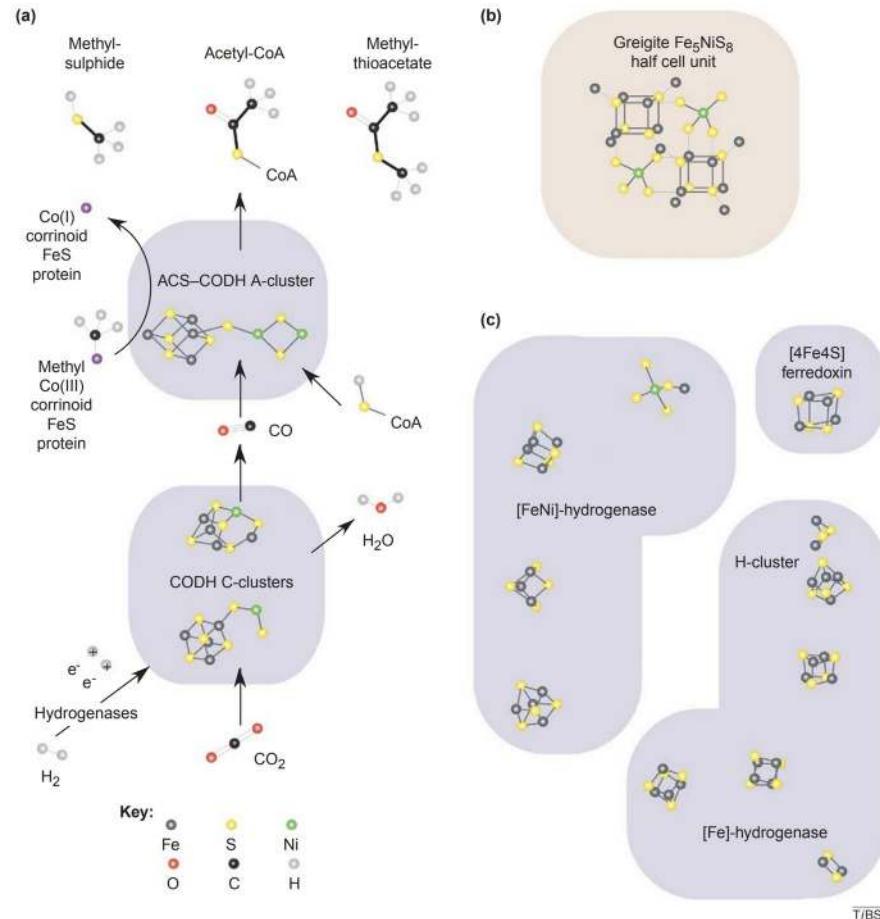
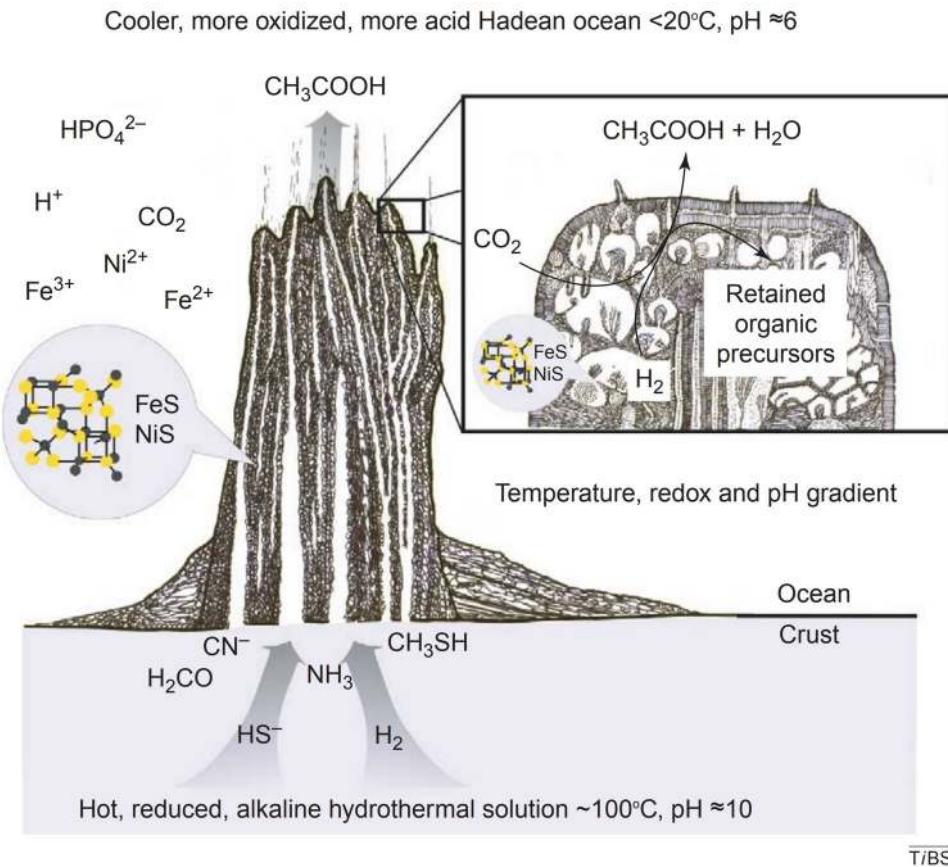
Wood-Ljungdahl pathway: Methanogens



Methanogens use the reductive Acetyl-CoA pathway and couple it to the Methyl coenzyme M reductase (*mcrA*) to produce methane (CH_4)

Methanogens can be either Hydrogenotrophic (a), Methylotrophic (b) and Aceticlastic (c) methanogenesis pathways

Wood-Ljungdahl pathway: at the Origin of Life



Wolf Cycle

Wolf cycle was originally proposed in 1988, when Rouvière and Wolfe suggested that production of methane by hydrogenotrophic Archaea was carried out in a cycle rather than a pathway

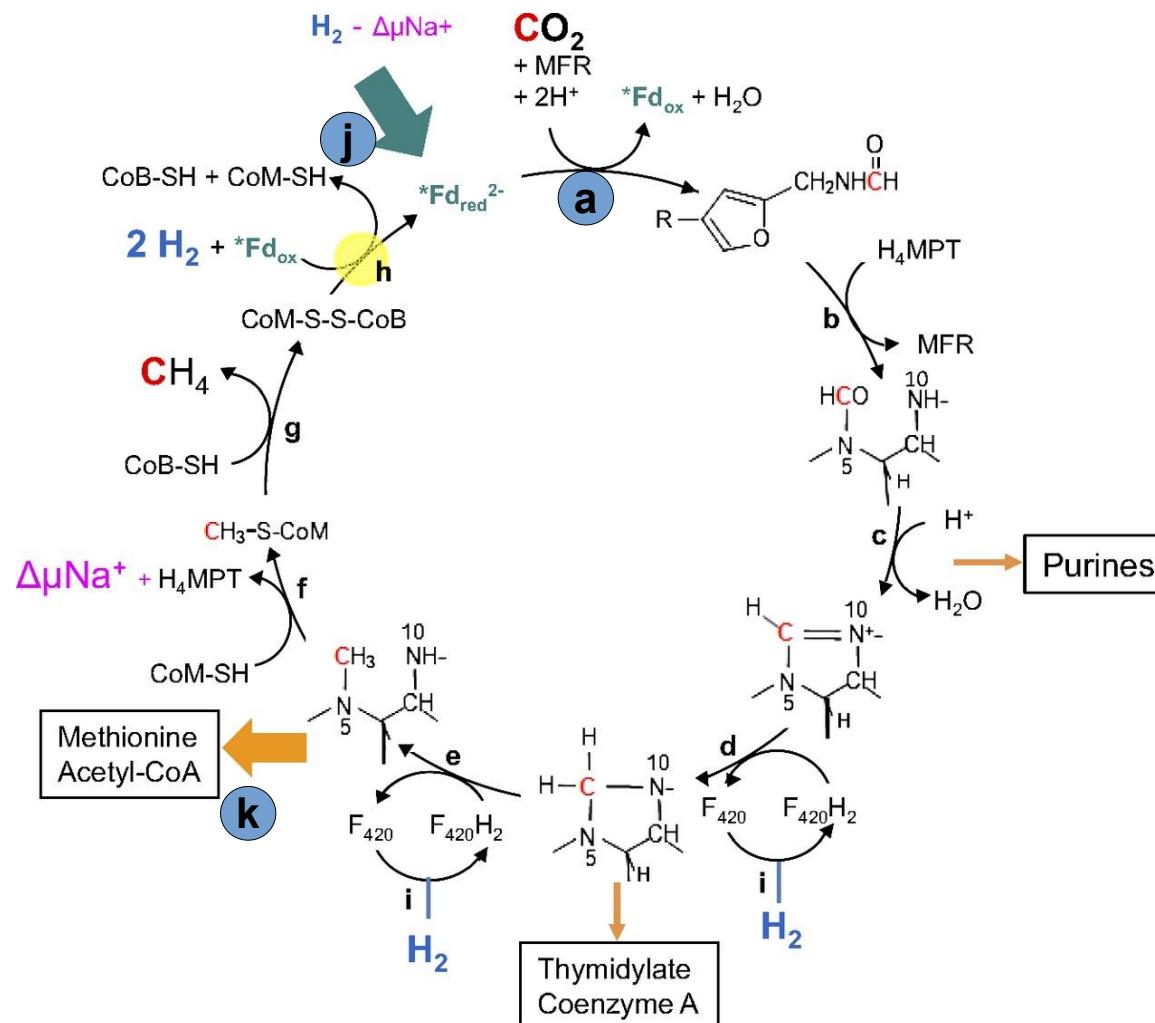
In its essence, it is a cyclization of the reductive Acetyl-CoA pathway. The nature of the cycle was confirmed only in 2012 by Lie and colleagues

Wolfe Cycle reduces CO₂ to methane with 4 H₂ in hydrogenotrophic methanogenic archaea

The reduction of CO₂ to formylmethanofuran is coupled to the last step, the reduction of the heterodisulfide (CoM-S-S-CoB) to coenzyme M (CoM-SH) and coenzyme B (CoB-SH)

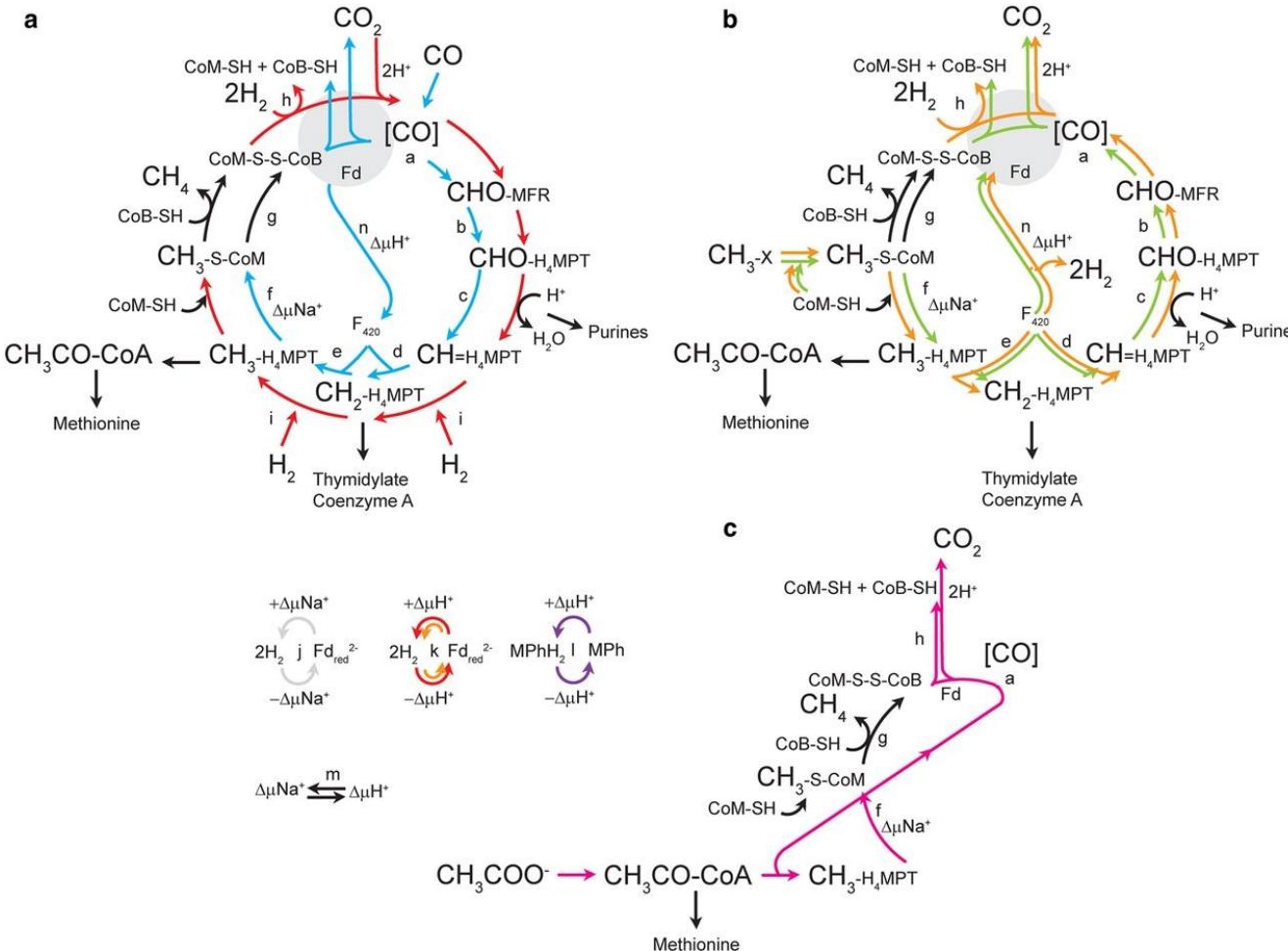
The sodium motive force-driven reduction of ferredoxin with H₂ catalyzed by the energy-converting hydrogenase EhaA-T is used to replenish the lost intermediates

Wolf Cycle



- formylmethanofuran dehydrogenase
- formylmethanofuran/H4MPT formyltransferase
- methenyl-H4MPT cyclohydrolase
- methylene-H4MPT dehydrogenase
- methylene-H4MPT reductase
- methyl-H4MPT/coenzyme M methyltransferase
- methyl-coenzyme M reductase
- electron-bifurcating hydrogenase–heterodisulfide reductase complex
- F420 -reducing hydrogenase
- energy-converting hydrogenase catalyzing the sodium motive force-driven reduction of ferredoxin with H_2
- CO dehydrogenase/acetyl-CoA synthase (not shown)

Wolf Cycle



(a) Hydrogenotrophic (red) and carboxydrotrophic (blue) methanogenesis pathways. Formic acid and primary or secondary alcohols are oxidized to CO_2 and hence methanogens that grow on these substrates use the hydrogenotrophic pathway. (b) Methyl respiration pathway (orange) and methylotrophic pathway (green). (c) Acetoclastic pathway (fuchsia).

3-Hydroxypropionate bicycle

The 3-hydroxypropionate bicycle was discovered in *Chloroflexus aurantiacus* and its also known as the Holo-Fuchs bicycle

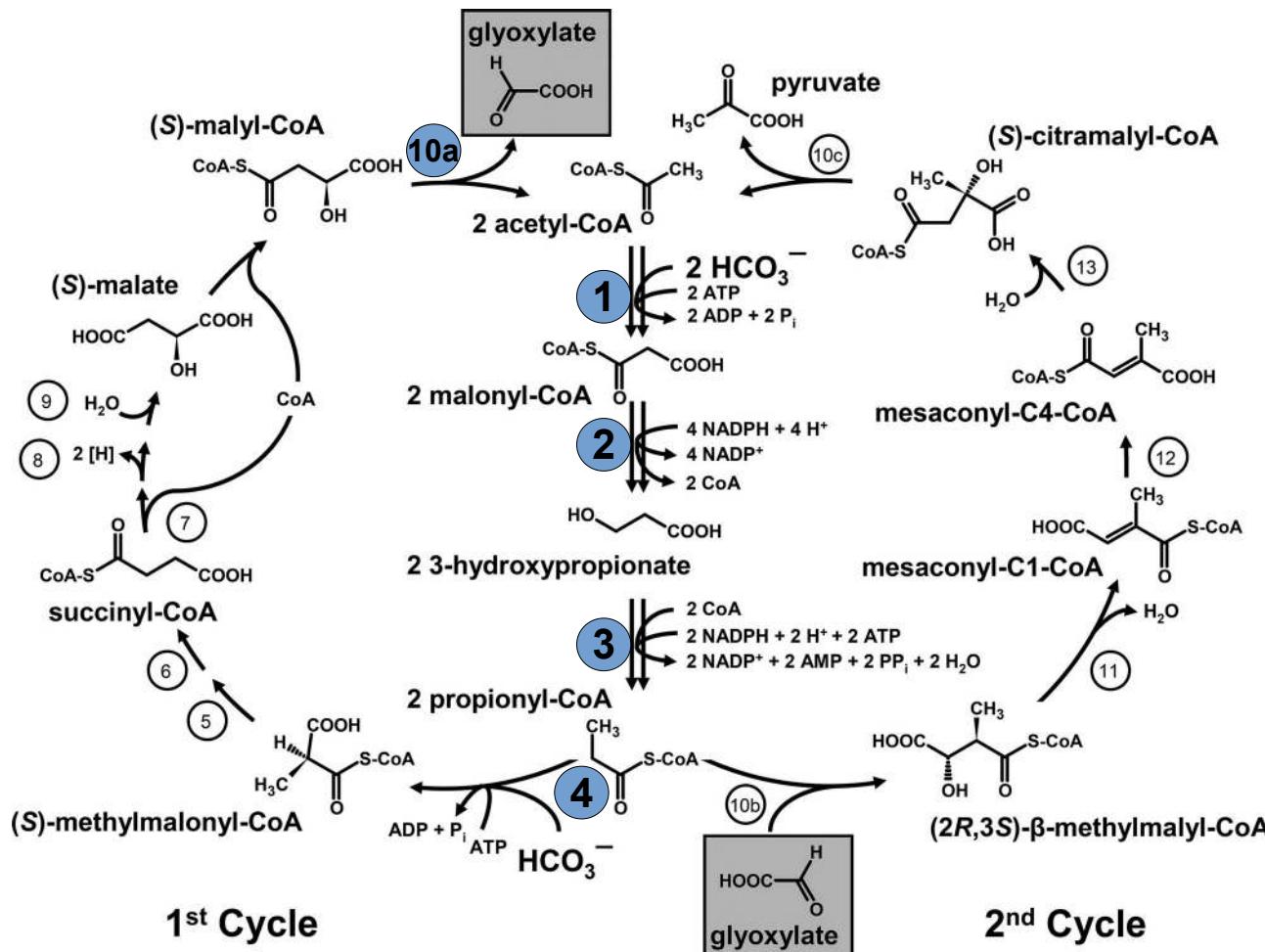
The two connected cycles generate two free characteristic intermediates of this bicycle: 3-hydroxypropionate and (S)-mallyl-CoA

The energy costs of the 3-hydroxypropionate bicycle are high, it requires 7 ATP equivalents for the synthesis of pyruvate and 3 additional ATPs for triose phosphate

Its key carboxylase(s), biotin-dependent acetyl-CoA/propionyl-CoA carboxylase, is virtually irreversible and uses bicarbonate as an active inorganic carbon species

The bicycle allows coassimilation of numerous compounds (e.g., fermentation products like acetate, propionate, and succinate and osmoprotectant dimethylsulfoniopropionate. This makes the pathway best suitable for mixotrophy and photoheterotrophy

3-Hydroxypropionate bicycle



1. acetyl-CoA carboxylase
2. malonyl-CoA reductase
3. propionyl-CoA synthase
4. propionyl-CoA carboxylase
5. methylmalonyl-CoA epimerase
6. methylmalonyl-CoA mutase
7. succinyl-CoA:(S)-malate-CoA transferase
8. succinate dehydrogenase
9. fumarate hydratase
- 10a, -b, -c, trifunctional (S)-methylmalonyl-CoA (a)/ N_L -methylmalyl-CoA (b)/(S)-citramalyl-CoA lyase (c)
11. mesaconyl-C1-CoA hydratase
12. mesaconyl-CoA C1-C4 CoA transferase
13. mesaconyl-C4-CoA hydratase.

4-Hydroxybutyrate cycles

The 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle and the dicarboxylate/4-hydroxybutyrate (DC/HB) cycle are two autotrophic CO₂ fixation cycles recently described in Crenarchaeota

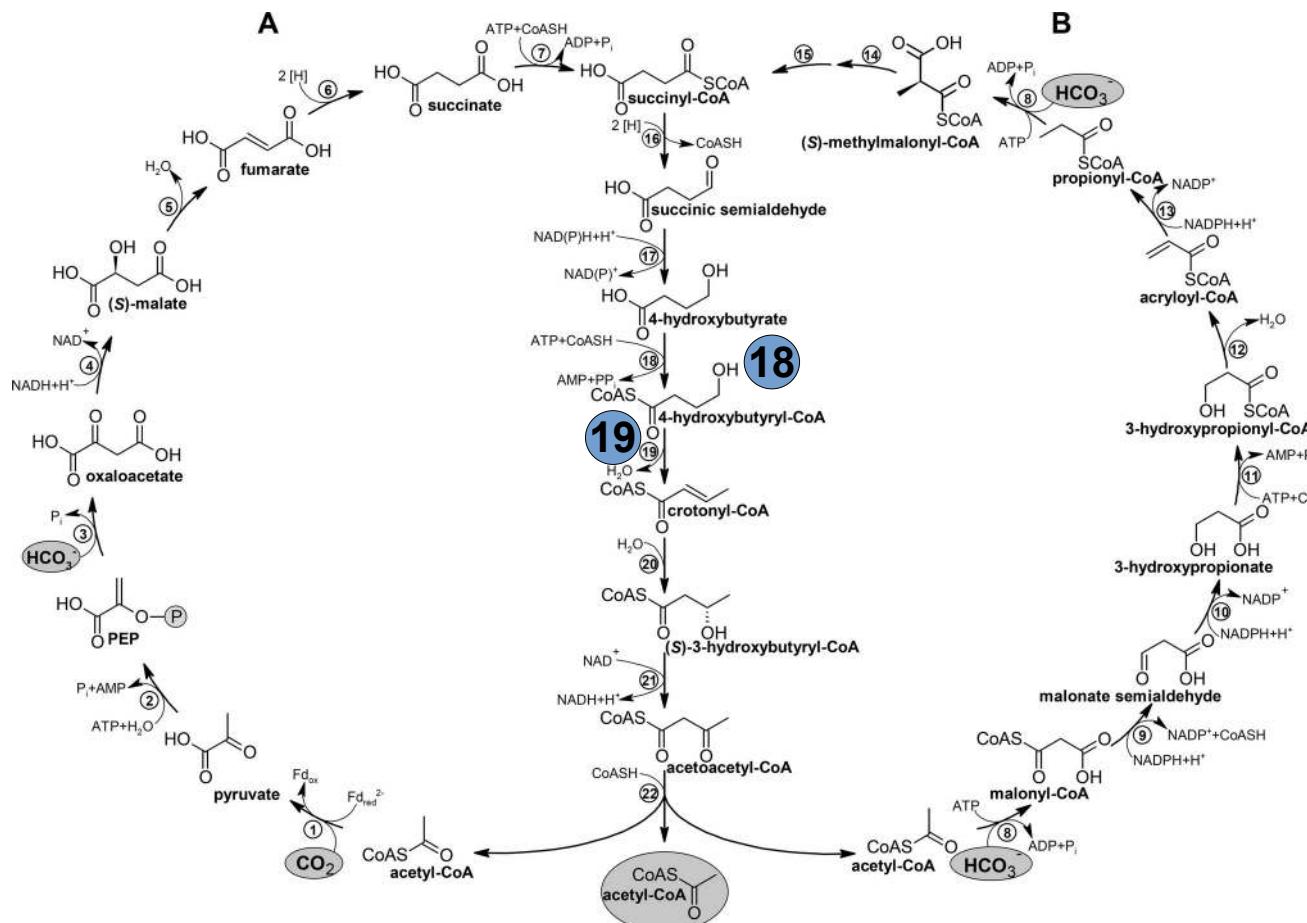
In both cycles (named together as the 4-hydroxybutyrate cycles), acetyl-CoA and two inorganic carbons are converted to succinyl-CoA, although this is accomplished with different carboxylases

The two variants function in Sulfolobales and Thaumarchaeota (A) and in Desulfurococcales and Thermoproteales (B) share the the regeneration of acetyl-CoA from succinyl-CoA proceeds through the same intermediates

The HP/HB cycle (A) is present in aerobic organisms, while the DC/HB cycle (A) is present in anaerobic organisms

Both cycles are expensive, with the DC/HB being cheaper but using low-potential electron acceptors. The synthesis of 1 pyruvate requires 5 ATP equivalents in the DC/HB cycle (1 pyrophosphate is formed) and 9 ATP equivalents in the HP/HB cycle (generating 3 molecules of pyrophosphate)

HP/HB and DC/HB cycles



1. pyruvate synthase; 2. pyruvate:water dikinase; 3. PEP carboxylase; 4. malate dehydrogenase; 5. fumarate hydratase; 6. fumarate reductase (natural electron acceptor is not known); 7. succinyl-CoA synthetase; 8. acetyl-CoA/propionyl-CoA carboxylase; 9. malonyl-CoA reductase; 10. malonic semialdehyde reductase; 11. 3-hydroxypropionate-CoA ligase; 12. 3-hydroxypropionyl-CoA dehydratase; 13. acryloyl-CoA reductase; 14. methylmalonyl-CoA epimerase; 15. methylmalonyl-CoA mutase; 16. succinyl-CoA reductase; 17. succinic semialdehyde reductase; 18. 4-hydroxybutyrate-CoA ligase; 19. 4-hydroxybutyryl-CoA dehydratase; 20. crotonyl-CoA hydratase; 21. (S)-3-hydroxybutyryl-CoA dehydrogenase (NAD+); 22. acetoacetyl-CoA N_L-ketothiolase

Mixotrophy: Reversible TCA cycle

Recently, the possibility of running the Krebs Cycle in reverse without the need for specific enzymes was demonstrated in two distinct bacterial Phyla

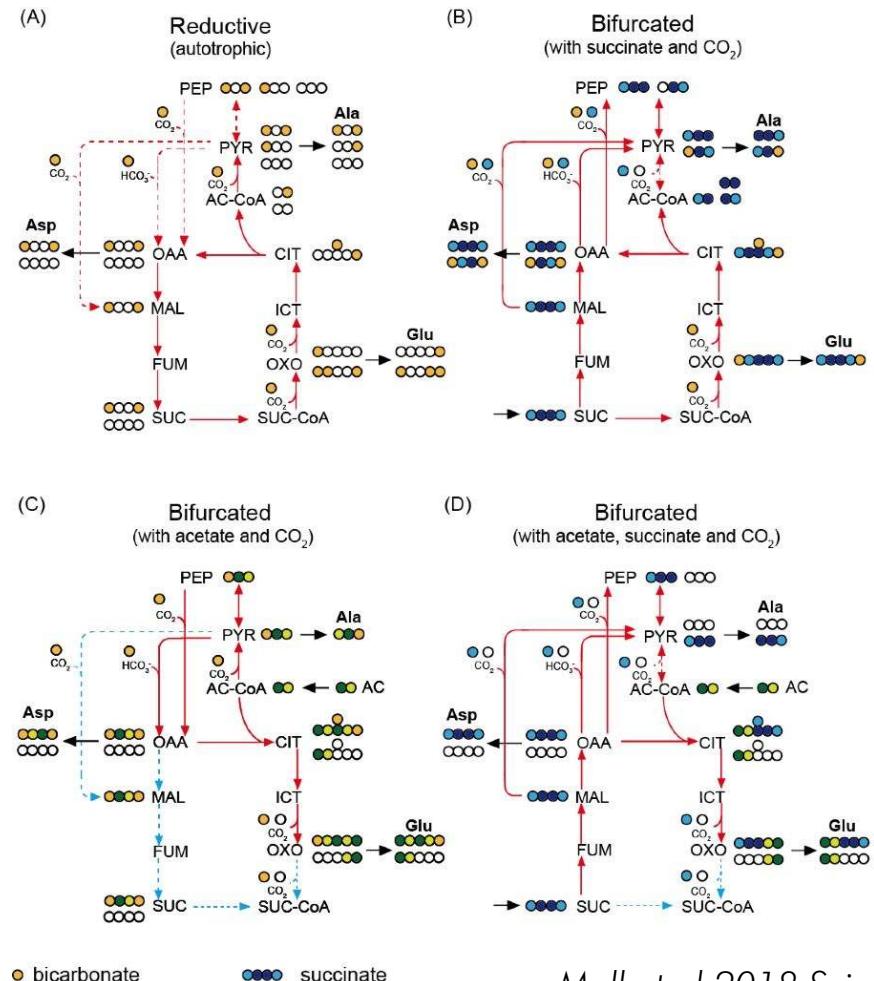
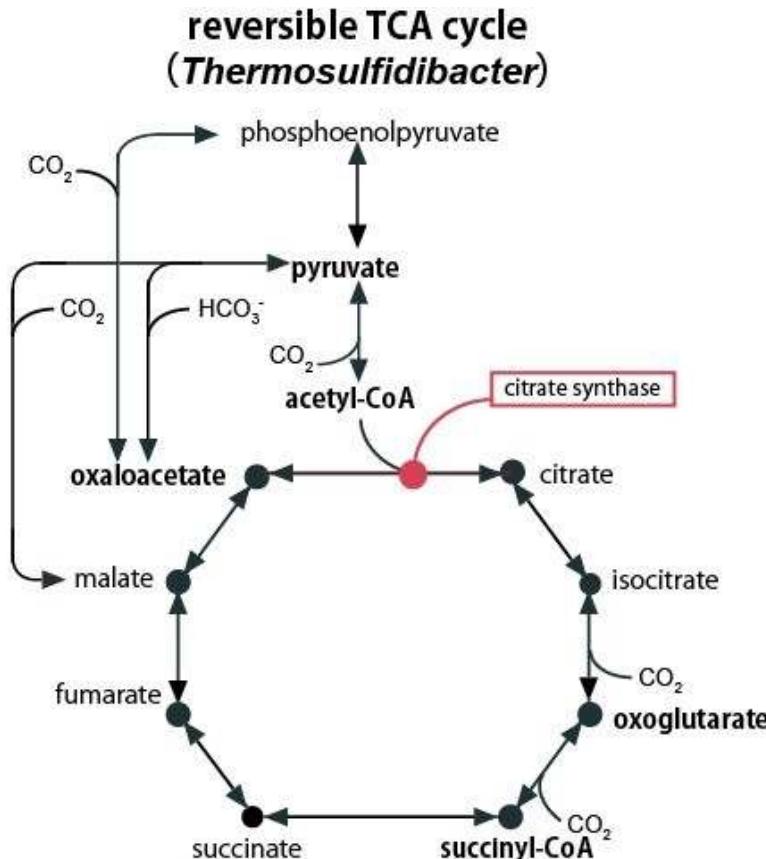
The switch from the oxidative to the reductive version of the TCA cycle had been considered impossible in the absence of specific carboxylases and ATP-consuming reactions (ATP citrate lyase)

The switch was demonstrated under metabolic product overload in the Deltaproteobacteria *Desulfurella acetivorans* (Mall et al 2018 Science) and Aquificae *Thermusulfidibacter takaii* (Nunoura et al 2018 Science)

This opens up the possibility that numerous organisms originally described as strict heterotrophs missing key enzymes might be functional mixotrophs

Raises also concerns about our ability to predict autotrophy from shotgun metagenomic sequences in the absence of *in situ* physiological measurements

Mixotrophy: Reversible TCA cycle



Mall et al 2018 Science
Nunoura et al 2018 Science

Mixotrophy: Anaplerotic Reactions

Anaplerotic reactions are chemical reactions that form intermediates of a metabolic pathway

Not all anaplerotic reactions are relevant to carbon fixation, several are “simply” existing shunts within the central metabolism

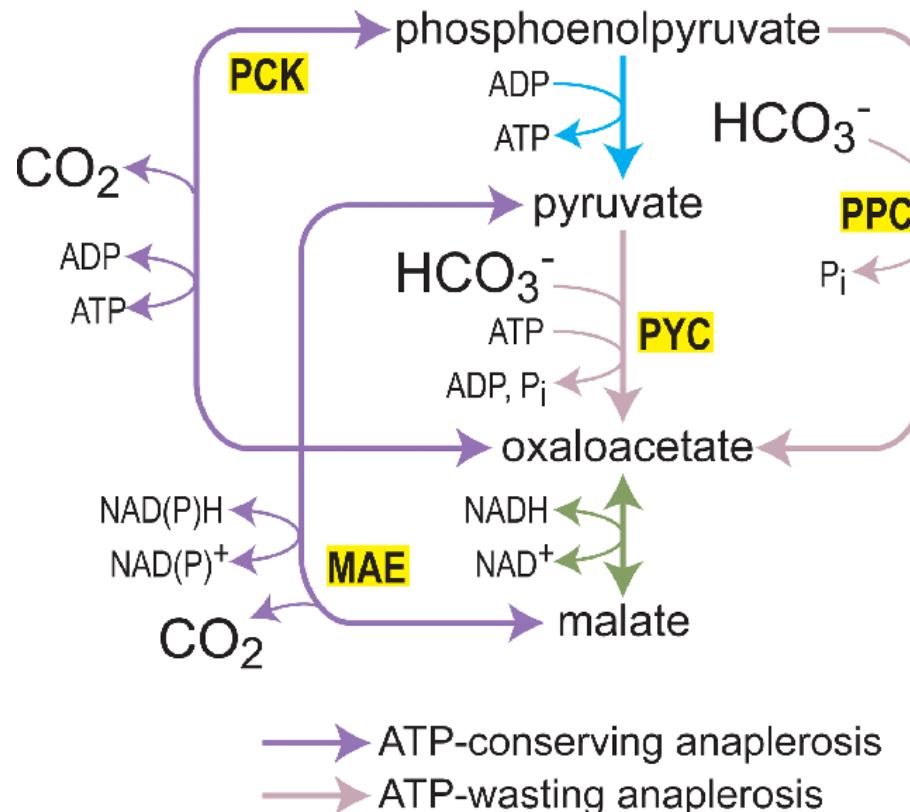
Anaplerotic reactions relevant to carbon fixation are generally carried out by carboxylase enzymes, and are present both in autotrophs and heterotrophs, that form part of their central and peripheral metabolic pathways

Anaplerotic reactions are catalyzed by pyruvate carboxylase, PEP carboxylase, PEP Carboxykinase, Malic Enzyme

Albeit less studied, anaplerotic reactions might contribute up to >30% of the carbon incorporated into biomass under oligotrophic conditions

The presence of Anaplerotic reaction enzymes further complicates the sequence based classification of organisms and ecosystems in trophic groups

Mixotrophy: Anaplerotic Reactions



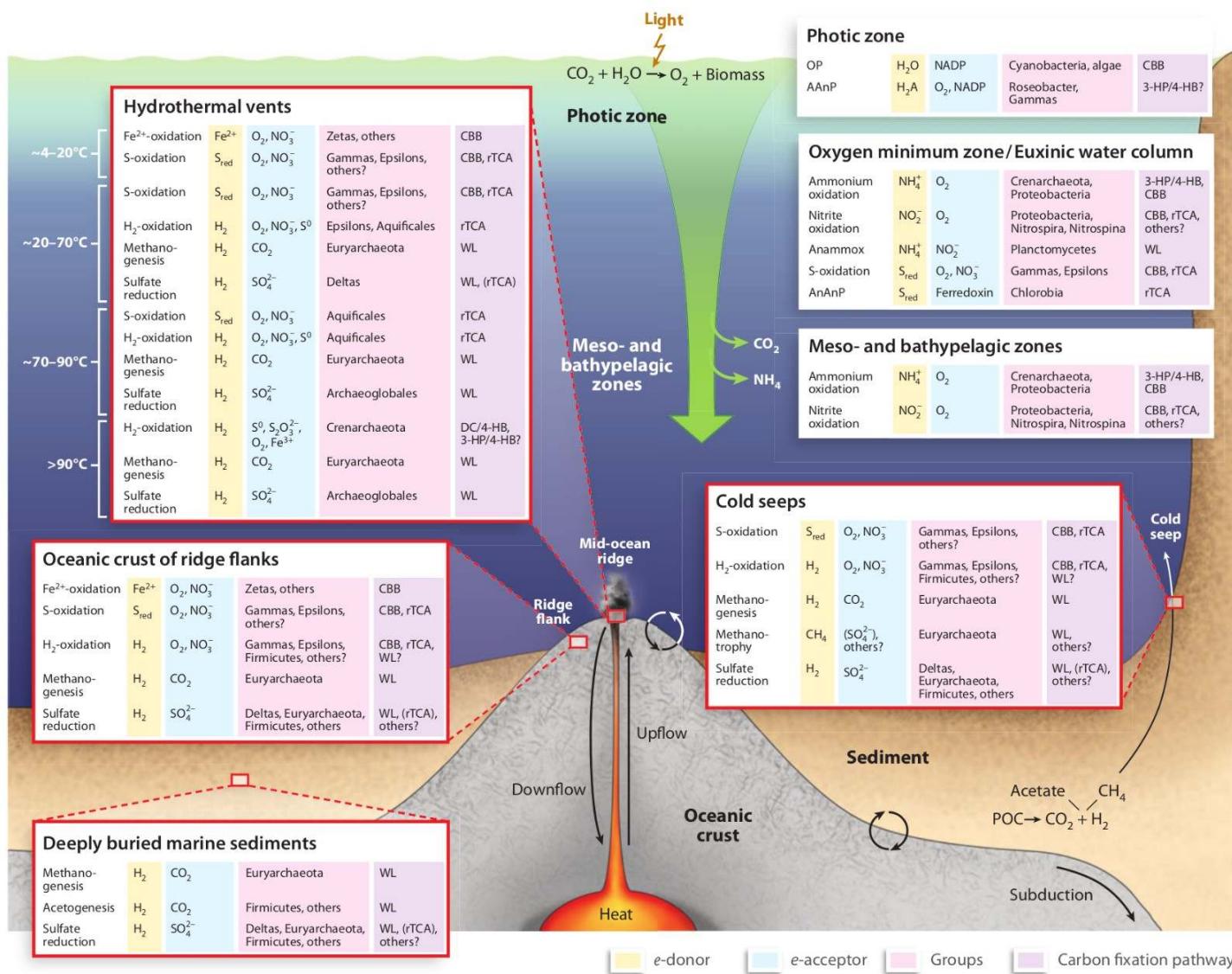
Carbon Fixation Pathways: Ecology and Distribution

Pathway	Distribution
Calvin Cycle	Plants, algae, Cyanobacteria, most aerobic or facultative aerobic chemolithoautotrophic Bacteria
rTCA Cycle	Chlorobia, Aquifae, Epsilonproteobacteria, some Deltaproteobacteria, few Alphaproteobacteria (<i>Magnetococcus</i>), one Gammaproteobacteria, Nitrospirae
rAcetyl-CoA	Methanogenic and sulfate reducing Euryarchaeota, acetogenic Firmicutes, some Spirochaetes, many Deltaproteobacteria, Anammox bacteria of Plancomycetes
3-hydroxypropionate Bycycle	Chloroflexaceae
DC/4HBCycle	Anaerobic Thermoproteales, Desulfurococcales (Crenarchaeota)
3HP/4HB Cycle	Aerobic Sulfolobales (Crenarchaeota), marine and soil ammonia oxidizing Thaumarchaeota

Carbon Fixation Pathways: Ecology and Distribution

Pathway	ATP for 1 Pyruvate	Environment	Key enzymes
Calvin Cycle	7	Aerobic and Microaerophilic	RubisCO; phosphoribulokinase
rTCA Cycle	2-3	Anaerobic and Microaerophilic	2-Oxoglutarate synthase; Succinyl-CoA synthase; ATP-citrate lyase
rAcetyl-CoA	1	Anaerobic	Formate dehydrogenase; F-MTF synthase; Acetyl-CoA synthase/ CO dehydrogenase
3-hydroxypropionate Bycycle	5	Anaerobic	Malonyl-CoA reductase; propionyl-CoA synthase; malyl-CoA lyase
DC/4HBCycle	7	Anaerobic	4-Hydroxybutyryl-CoA dehydratase
3HP/4HB Cycle	9	Aerobic	Acetyl-CoA/propionyl-CoA carboxylase

Vertical distribution of the different carbon fixation pathways and associated energy metabolism in the marine water column and subsurface ecosystems



Carbon Fixation and Central Metabolites

Despite their diversity, known carbon fixation pathway (and central carbon pathways) share a large number of intermediate metabolites, cofactors and to a certain extent enzymes

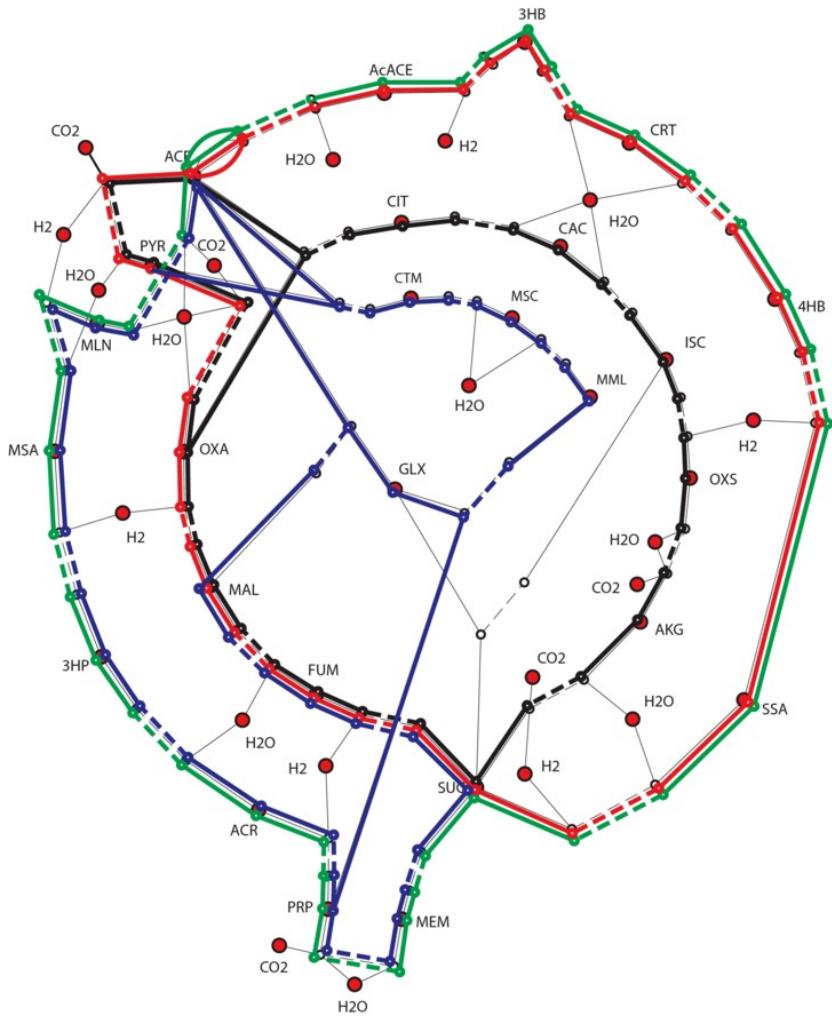
It is possible to identify modules, composed either of subsets of chemicals and reactions, subsets of functions, with a conserved internal structure

Module boundaries are often associated with the most complex reaction mechanisms, catalyzed by highly conserved enzymes

Cofactors form a biosynthetically and functionally distinctive control layer over the small-molecule substrate, with the most complex cofactors often associated with the reactions at module boundaries in the substrate networks

This suggests that early evolution of core carbon-fixation, appears to have required very few innovations to produce adaptations to simple chemical or energetic differences of environment without diverse solutions and without historical contingency

Carbon Fixation and Central Metabolites



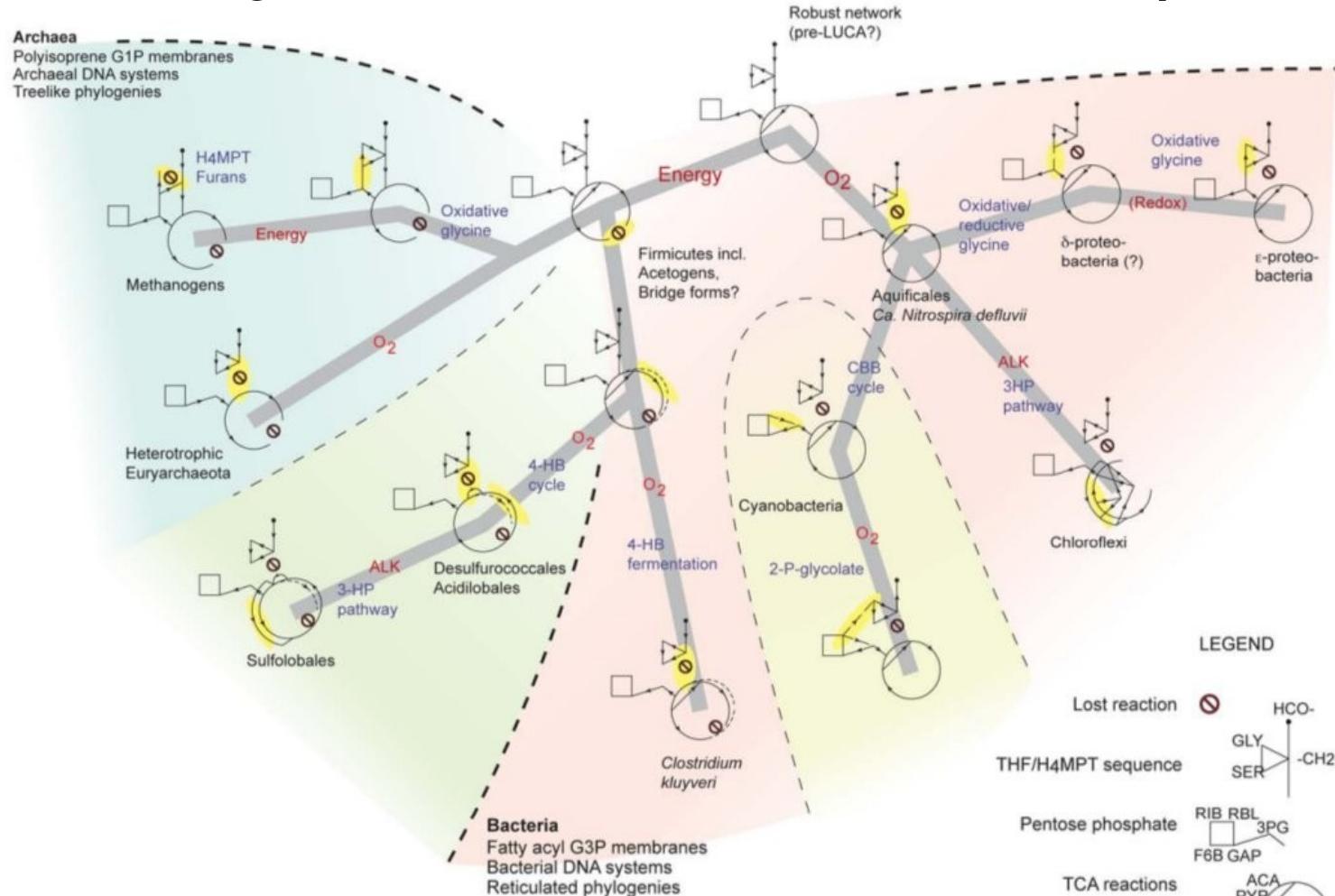
The four loop carbon-fixation pathways that pass through some or all of the universal biosynthetic precursors

rTCA is black, DC/4HB is red, 3HP-bicycle is blue, and 3HP/4HB is green

The module-boundary nature of acetate (ACE) and succinate (SUC) is shown by the intersection of multiple paths in these compounds

Radially aligned reactions are homologous in local-group chemistry; deviations from strict homology in different pathways appear as excursions from concentric circles

Emergence of Carbon Fixation Pathways



This week Readings

Dworkin, M., and Gutnick, D. (2012). Sergei Winogradsky: a founder of modern microbiology and the first microbial ecologist. *FEMS Microbiol Rev* 36, 364–379. doi:10.1111/j.1574-6976.2011.00299.x.

Berg, I. A. (2011). Ecological Aspects of the Distribution of Different Autotrophic CO₂ Fixation Pathways. *Appl. Environ. Microbiol.* 77, 1925–1936. doi:10.1128/AEM.02473-10.

Russell, M. J., and Martin, W. (2004). The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Sciences* 29, 358–363. doi:10.1016/j.tibs.2004.05.007.

Nunoura, T., Chikaraishi, Y., Izaki, R., Suwa, T., Sato, T., Harada, T., et al. (2018). A primordial and reversible TCA cycle in a facultatively chemolithoautotrophic thermophile. *Science* 359, 559–563. doi:10.1126/science.aoa3407.

Braakman, R., and Smith, E. (2013). The compositional and evolutionary logic of metabolism. *Phys. Biol.* 10, 011001. doi:10.1088/1478-3975/10/1/011001.