



University of Naples "Federico II"  
**Marine Microbial Diversity**

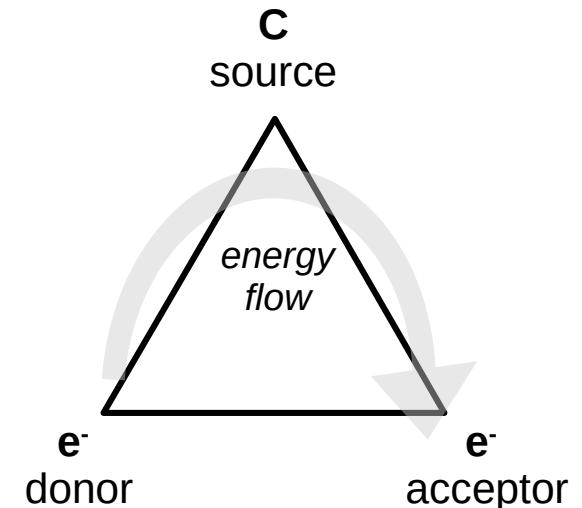
# Nutritional Groups 3

# 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.

# *Types of Chemolithotrophy*

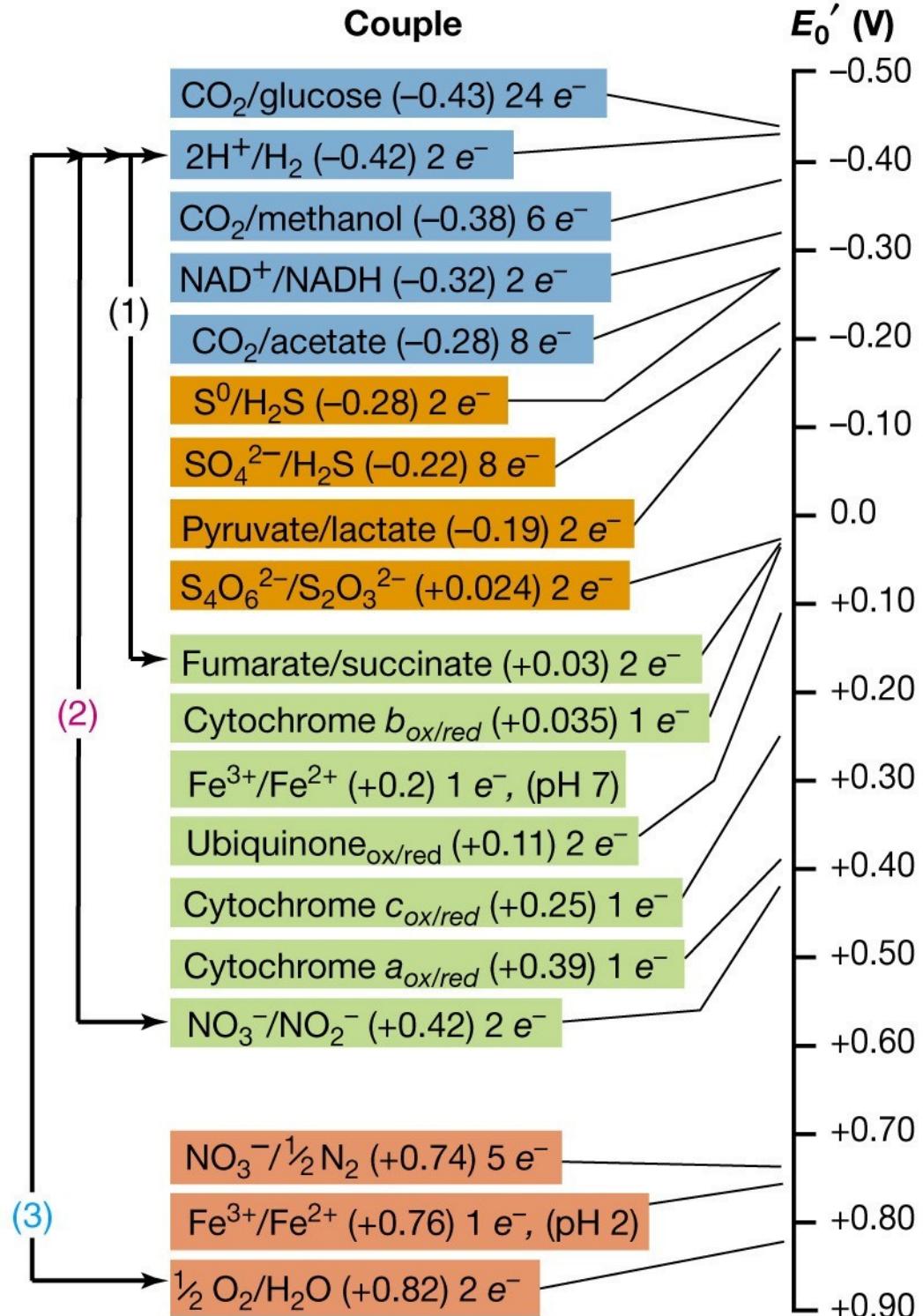
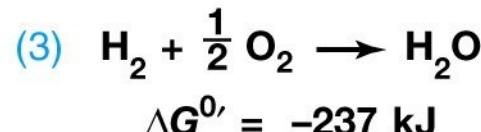
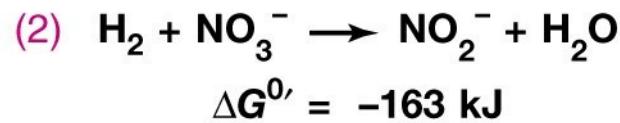
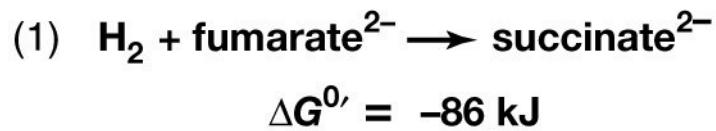
e<sup>-</sup> donors

$H_2$ ,  $NH_4^+$ ,  $H_2S$ ,  $S_{(n)}^-$ ,  $S^0$ ,  $S_2O_3^{2-}$ ,  $S^{2-}$ ,  $CH_4$ ,  $CO$ ,  $Fe^{2+}$ ,  $As^{3+}$ , etc...

e<sup>-</sup> acceptors

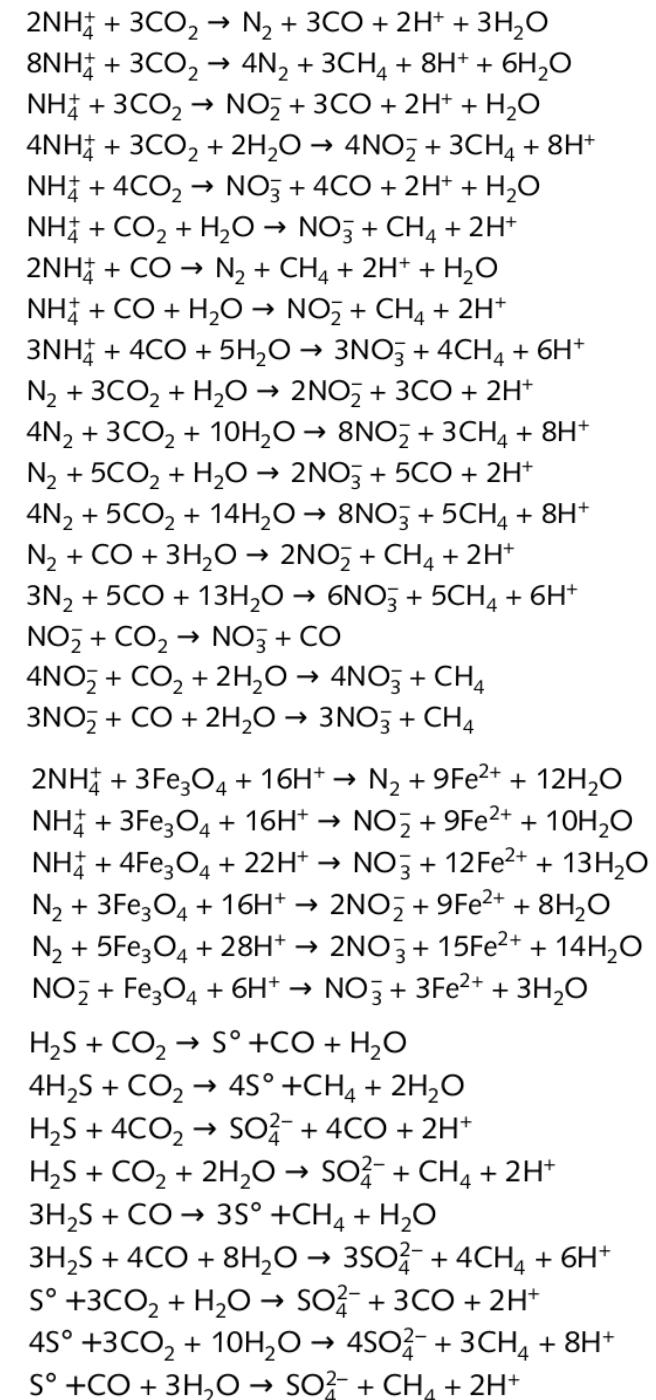
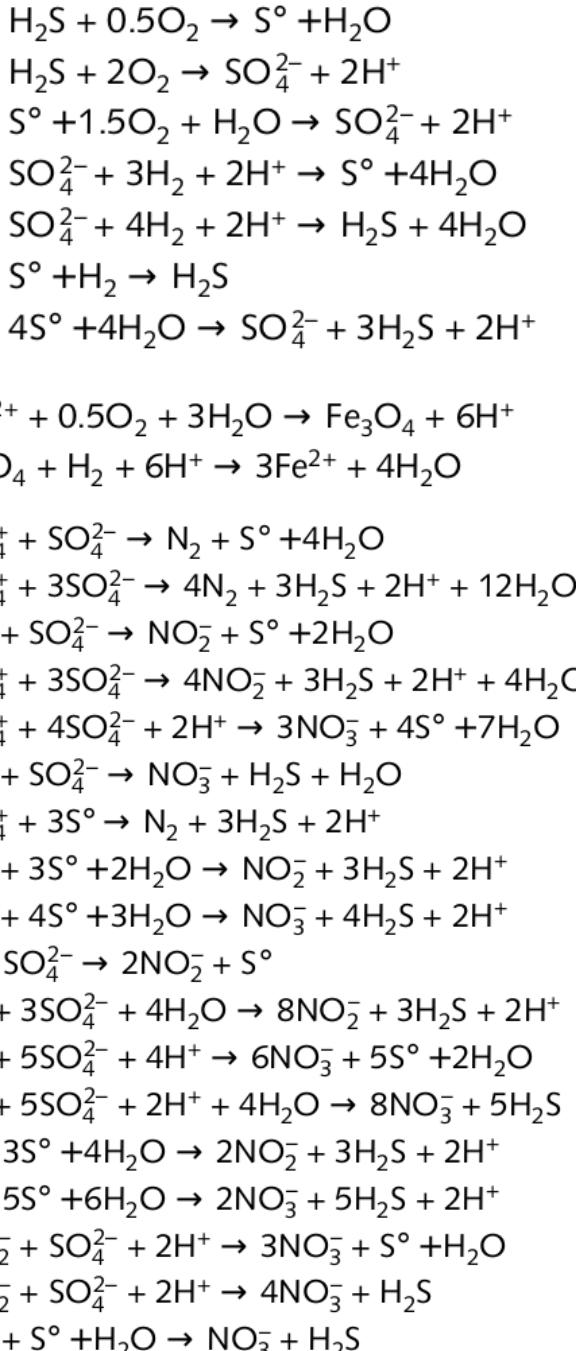
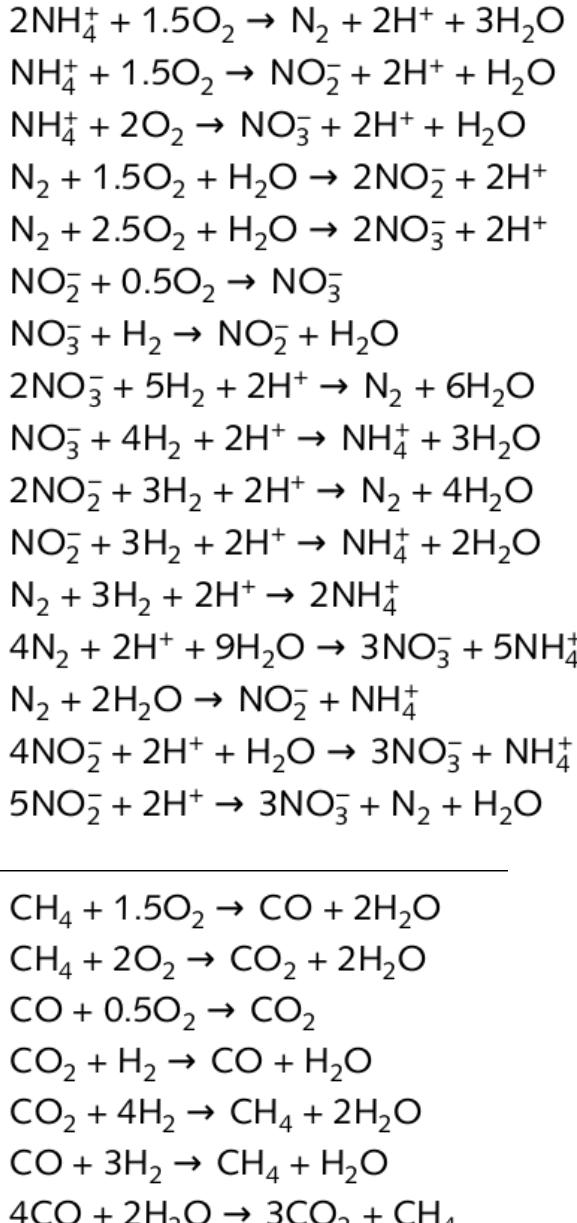
$O_2$ ,  $NO_3^-$ ,  $NO_2^-$ ,  $N_2O$ ,  $NO$ ,  $SO_4^{2-}$ ,  $SO_3^{2-}$ ,  $S^0$ ,  $CO_2$ ,  $Fe^{3+}$ ,  $Mn^{2+}$ ,  $SeO_4^{2-}$ ,  $AsO_4^{3-}$ ,  $UO_3^{2-}$ ,  $TeO_4^{2-}$ , etc...

**Examples of reactions  
with H<sub>2</sub> as e<sup>-</sup> donor**



# Previously on this channel...

## Reaction

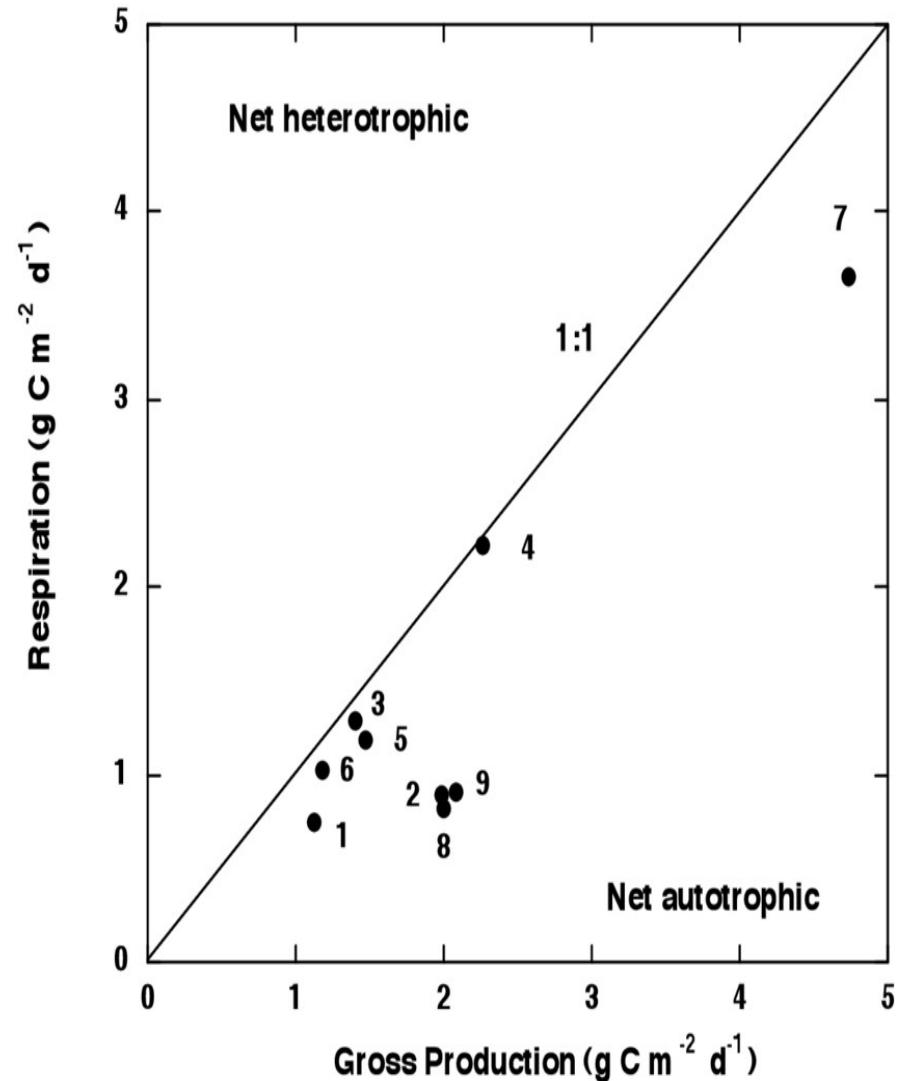


# *Net productivity*

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





*from NASA*

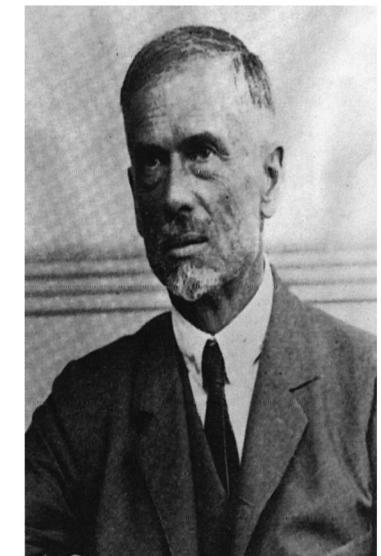
# The history of Chemolithotrophy

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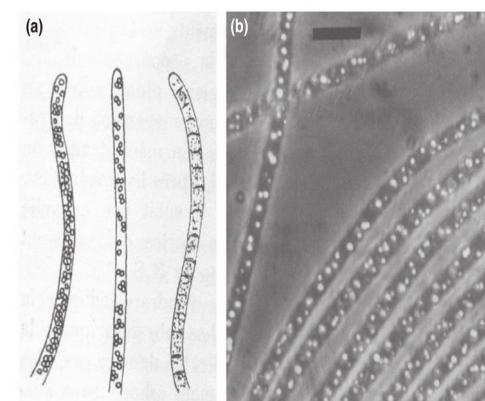
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In his memoirs, in a more informal fashion Winogradsky described the moment that he arrived at this hypothesis. 'Then one day as I was following the Île canal on my way home after a tiring day of chemical work which also involved hydrogen sulfide and 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



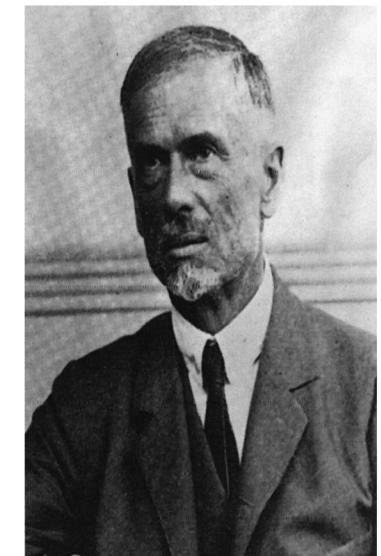
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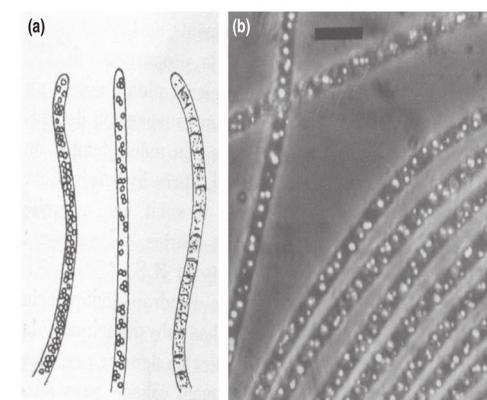
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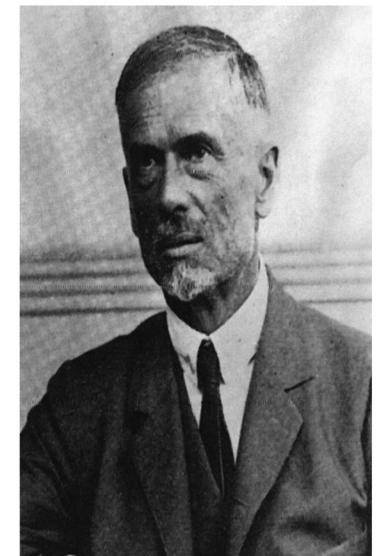
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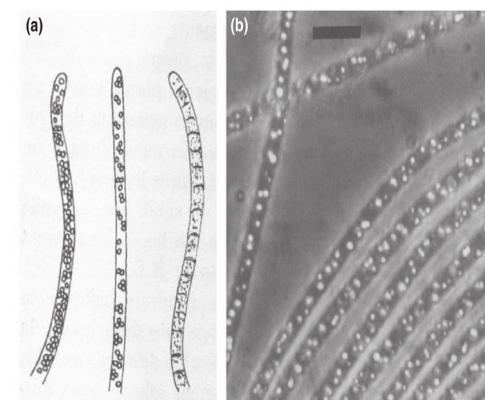
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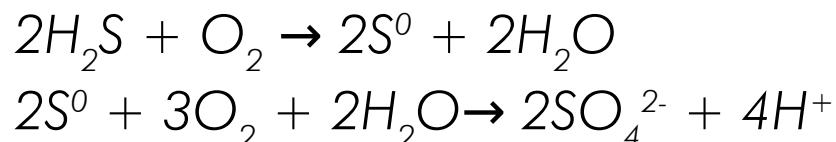


**Beggiatoa** is a genus of bacteria in the class Gammaproteobacteria, in the Proteobacteria phylum.

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.

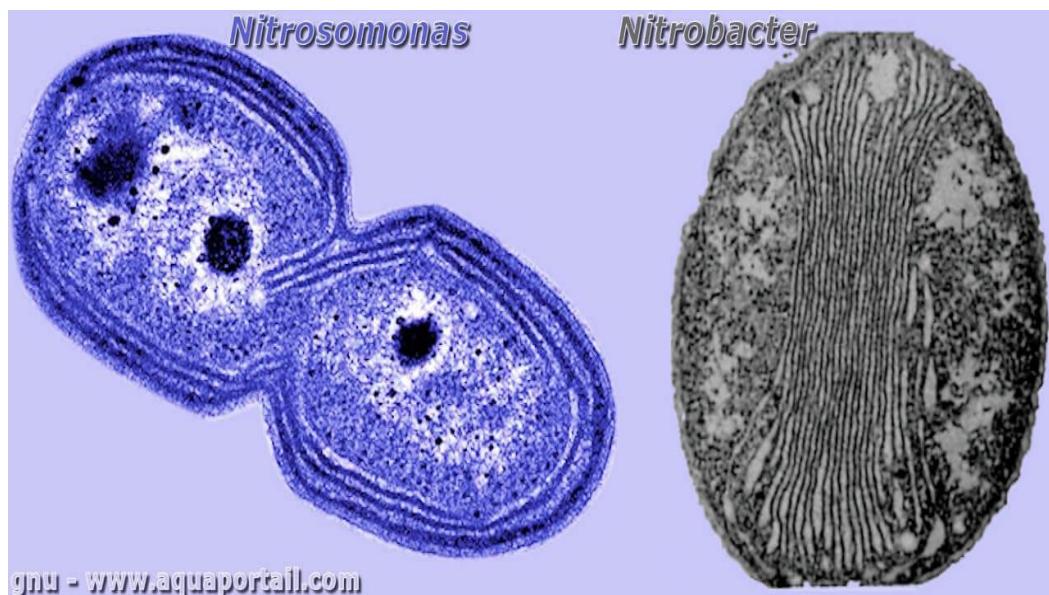
Winogradsky referred to this form of metabolism as *inorgoxidation* (oxidation of inorganic compounds).



# *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



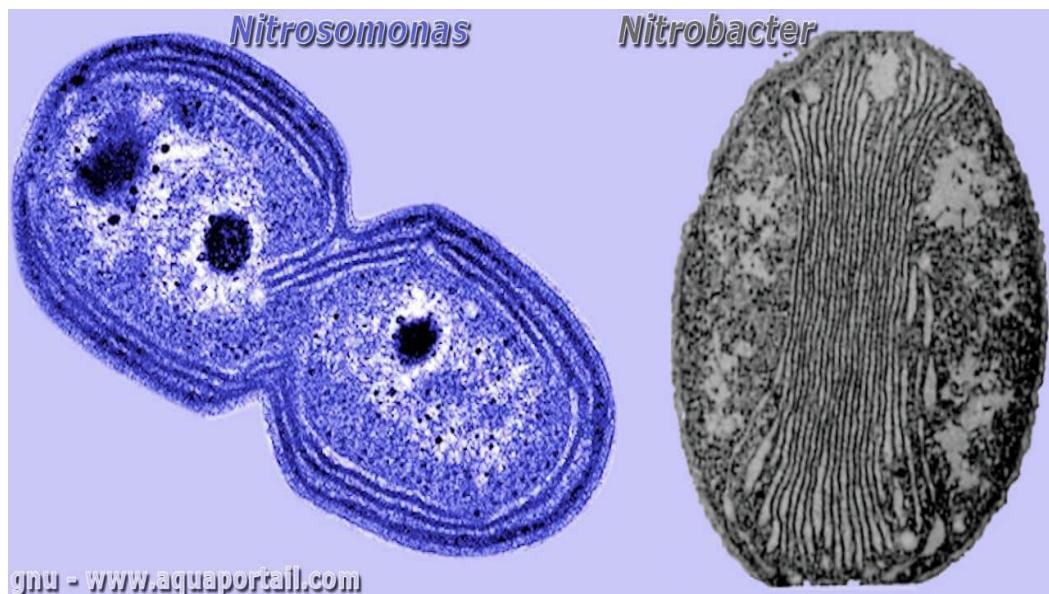
gnu - www.aquaportal.com

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 CO<sub>2</sub> thus generated, subtracted the amount of CO<sub>2</sub> 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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## Winogradsky Column: Microbial Ecology in a Bottle

Sergei N. Winogradsky  
1856 – 1933

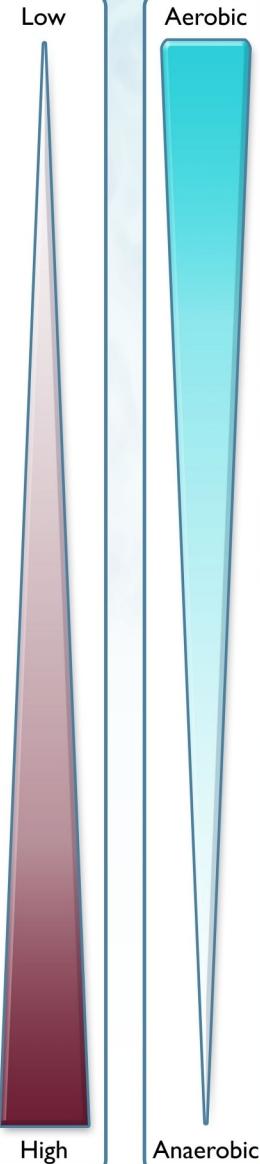
*Life* *Environment*



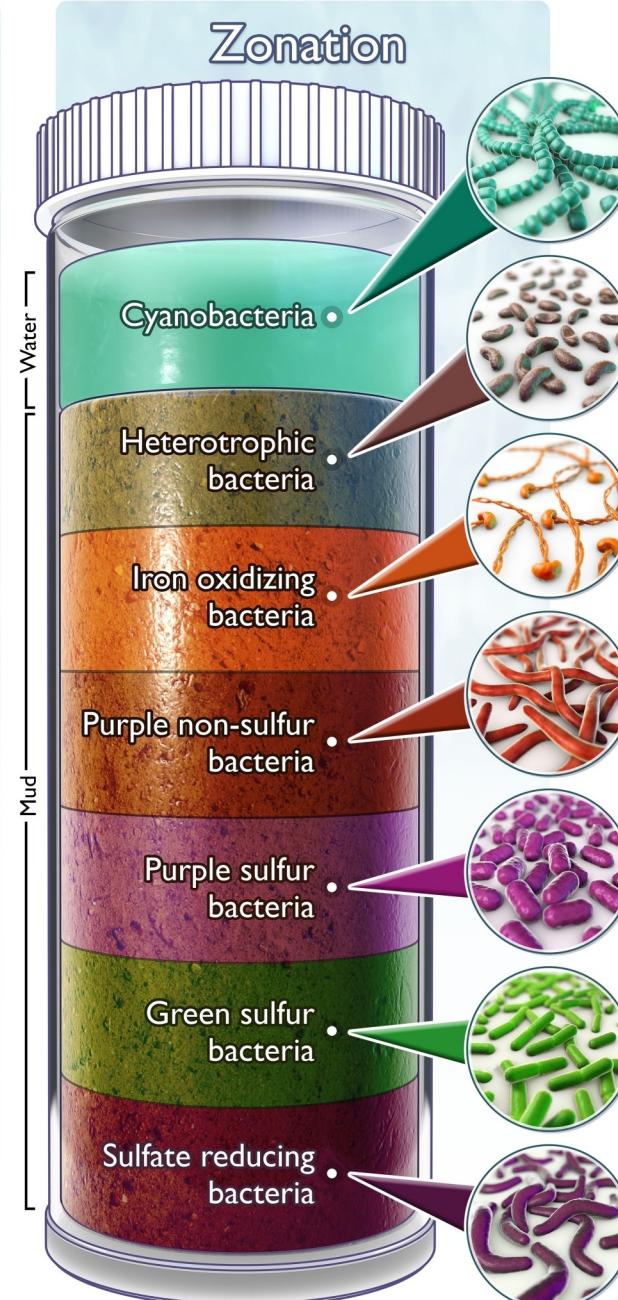
A soil or sediment sample is collected from nearly any source and amended with a variety of compounds such as carbon, sulfur, iron, and/or calcium. The mixture is added to a clear container and topped with water; the container is lightly capped to prevent evaporation. The column is incubated for weeks to months in well-lit conditions, thereby establishing gradients of oxygen, nutrients, and light. Different microbial taxa are adapted to different niches within these overlapping gradients, creating a stratified ecosystem defined by metabolic potential.

## Gradients

### Sulfide      Oxygen

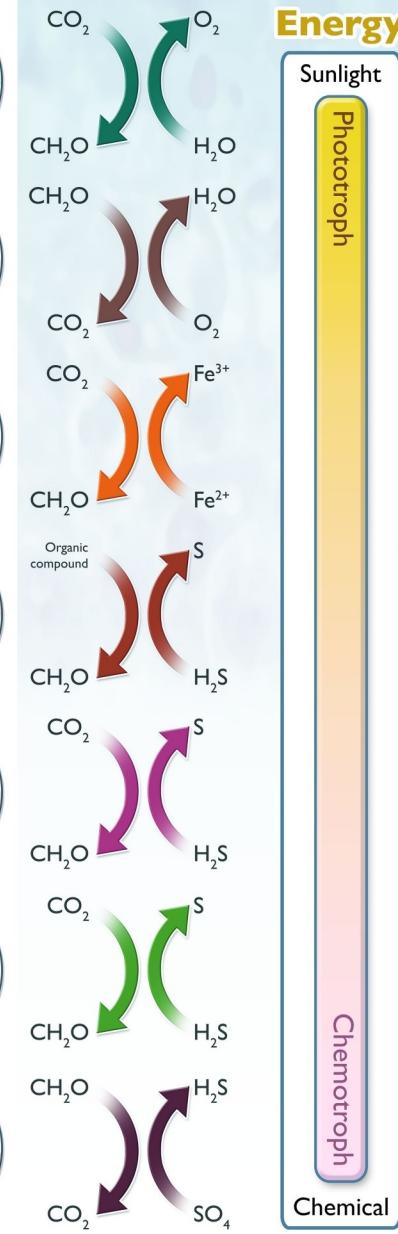


## Zonation



## Metabolic Niches

### Energy



Sunlight

Phototroph

Chemical

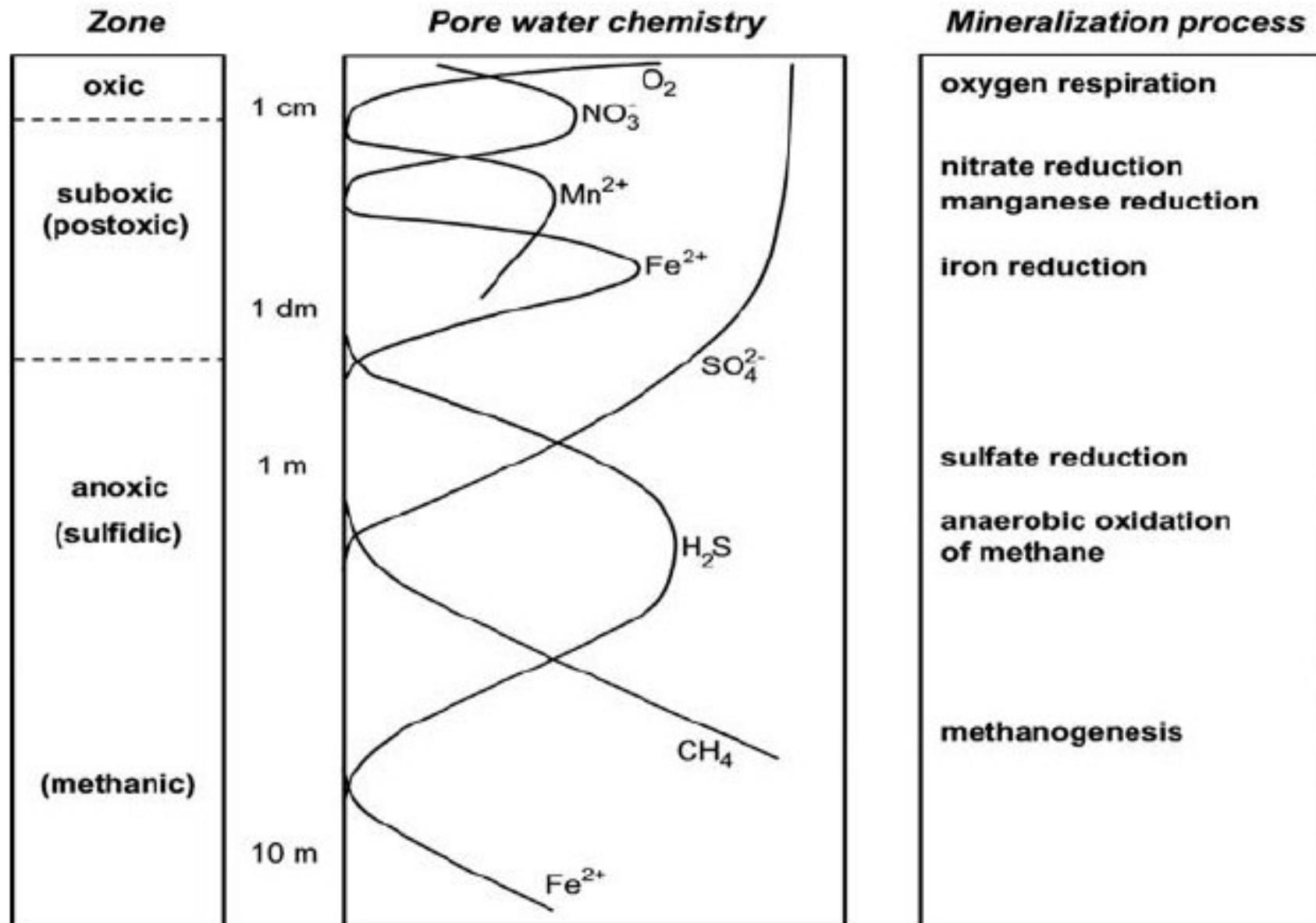
Chemotroph

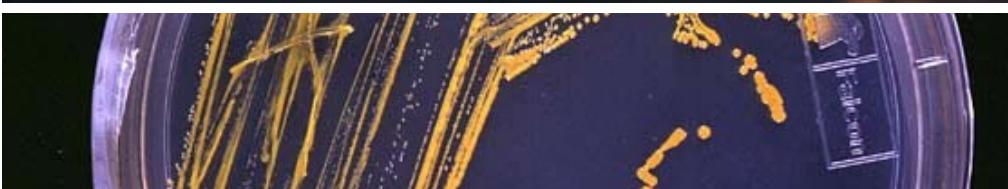
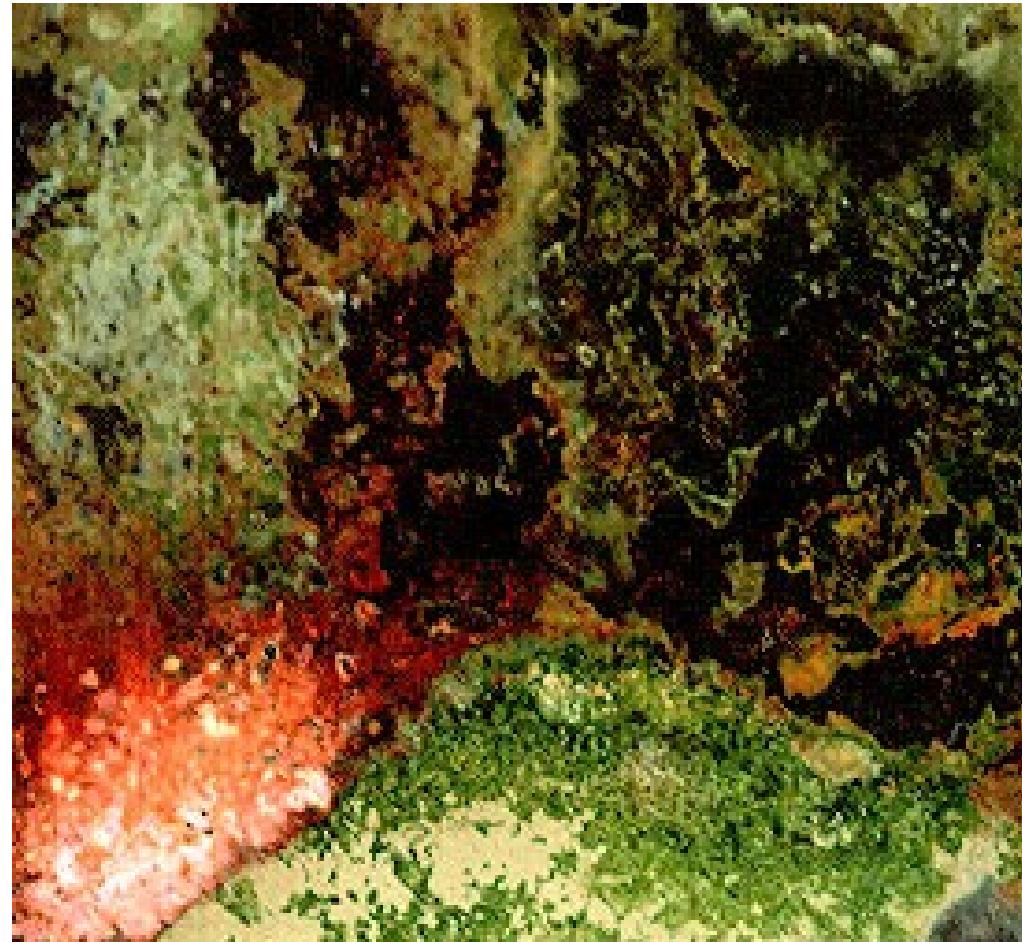
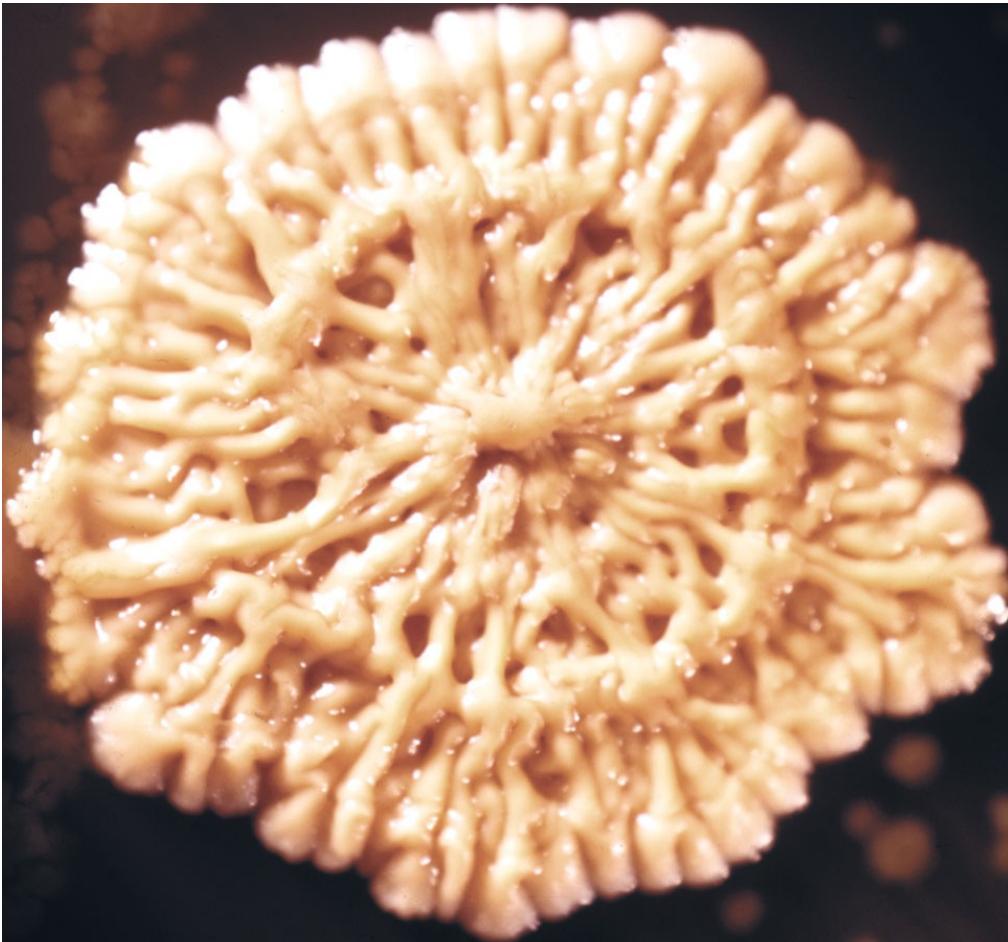
All life on Earth can be categorized according to an organism's carbon and energy source. Energy can be obtained from light reactions (phototroph) or chemical oxidations (chemotroph), and carbon for cellular synthesis can be obtained from carbon dioxide (autotroph) or from preformed organic compounds (heterotroph). These categories combined form the four basic life strategies and can be found among the bacteria within a single Winogradsky column: photoautotrophy, photoheterotrophy, chemoautotrophy, and chemoheterotrophy. Depending on conditions, Winogradsky columns can enrich for many different types of bacteria. The illustration above lists some common examples.



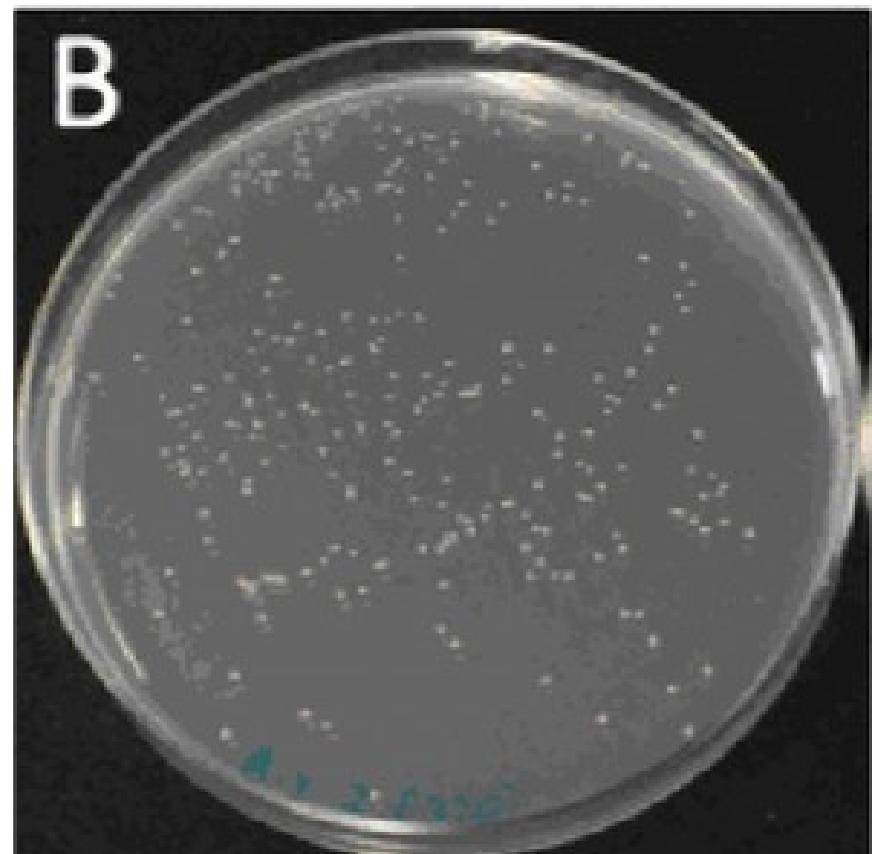
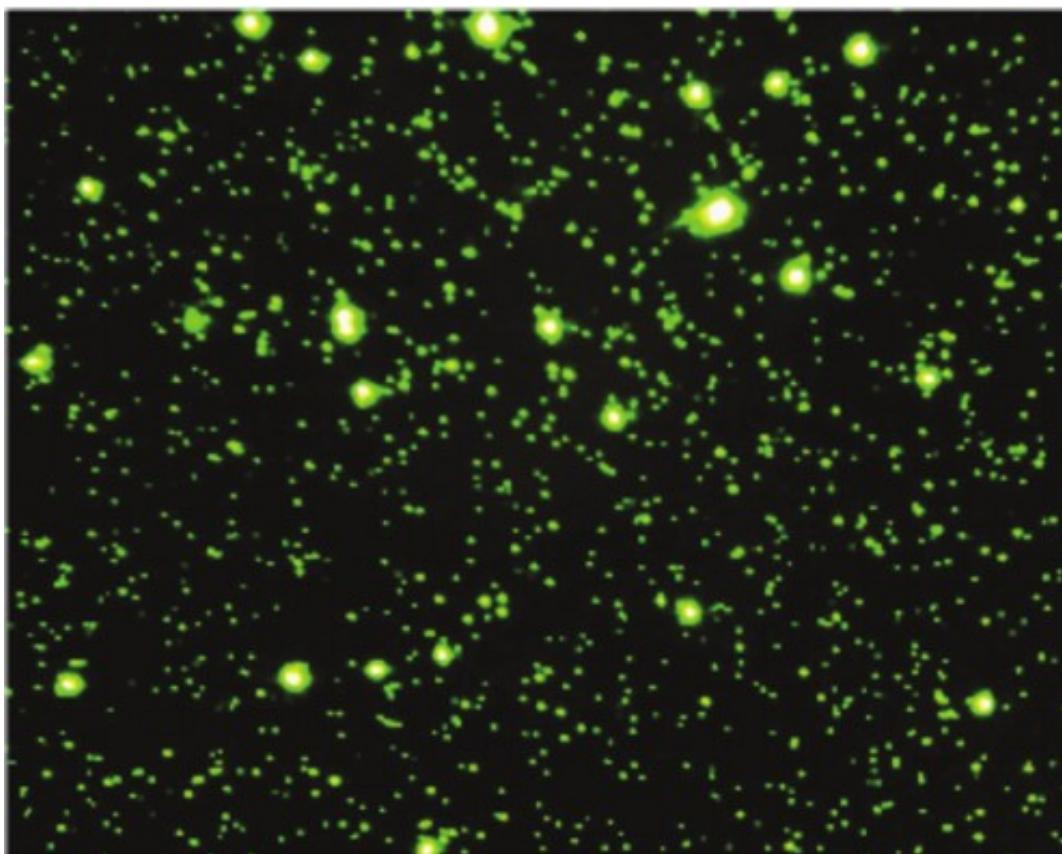
#GiovannelliLab  
<http://dgiovannelli.github.io>

# Down the thermodynamic ladder



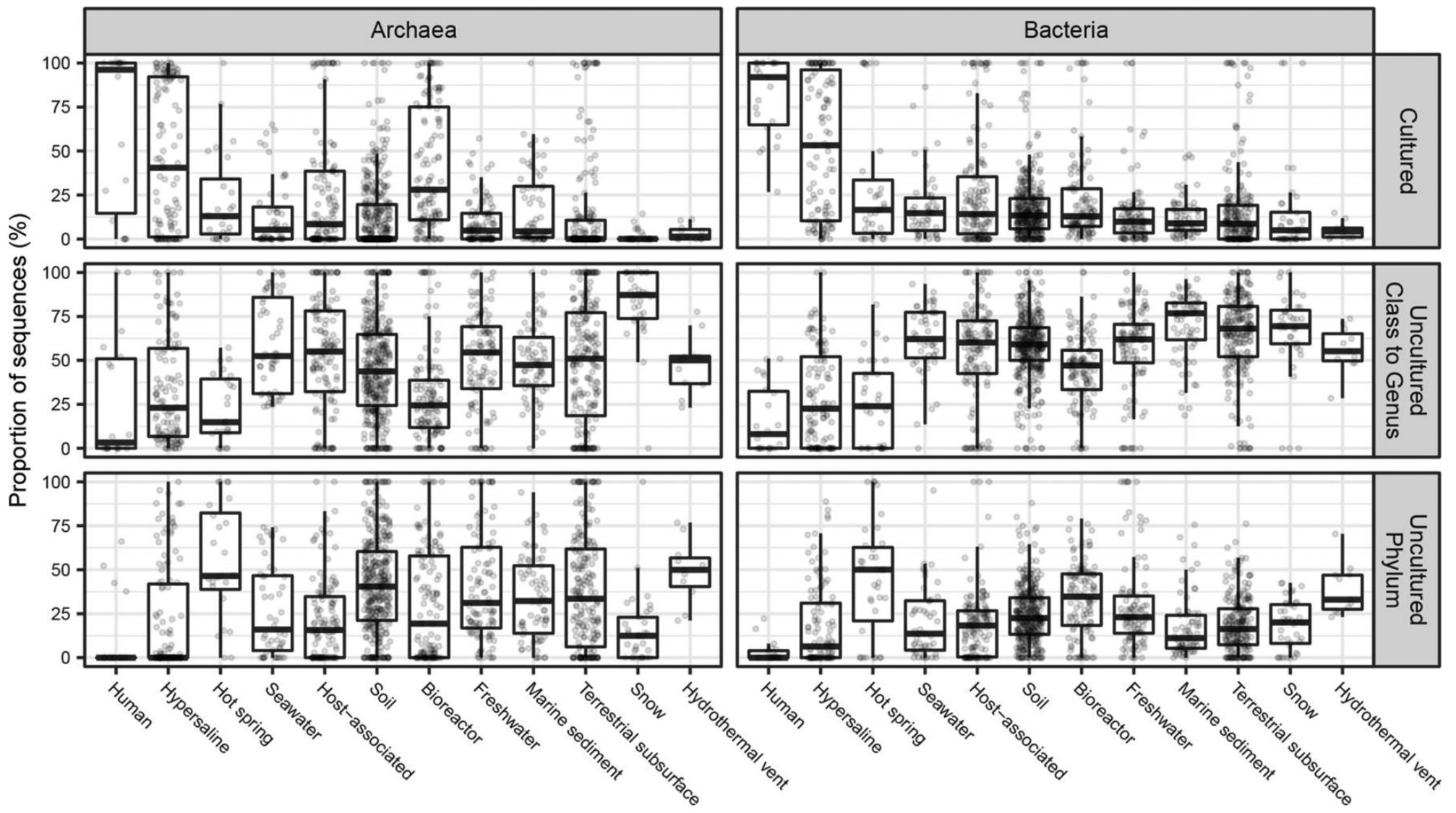


[...] Invented almost exactly 130 years ago by their respective eponyms, the German Julius Richard Petri and the Russian-French Sergei N. Winogradsky, [these two techniques] embody two different, even contrary approaches to study the microbial world – one, Petri's, based on pure cultures, synthetic media and medicine, the other, Winogradsky's inspired by natural environment and ecology."



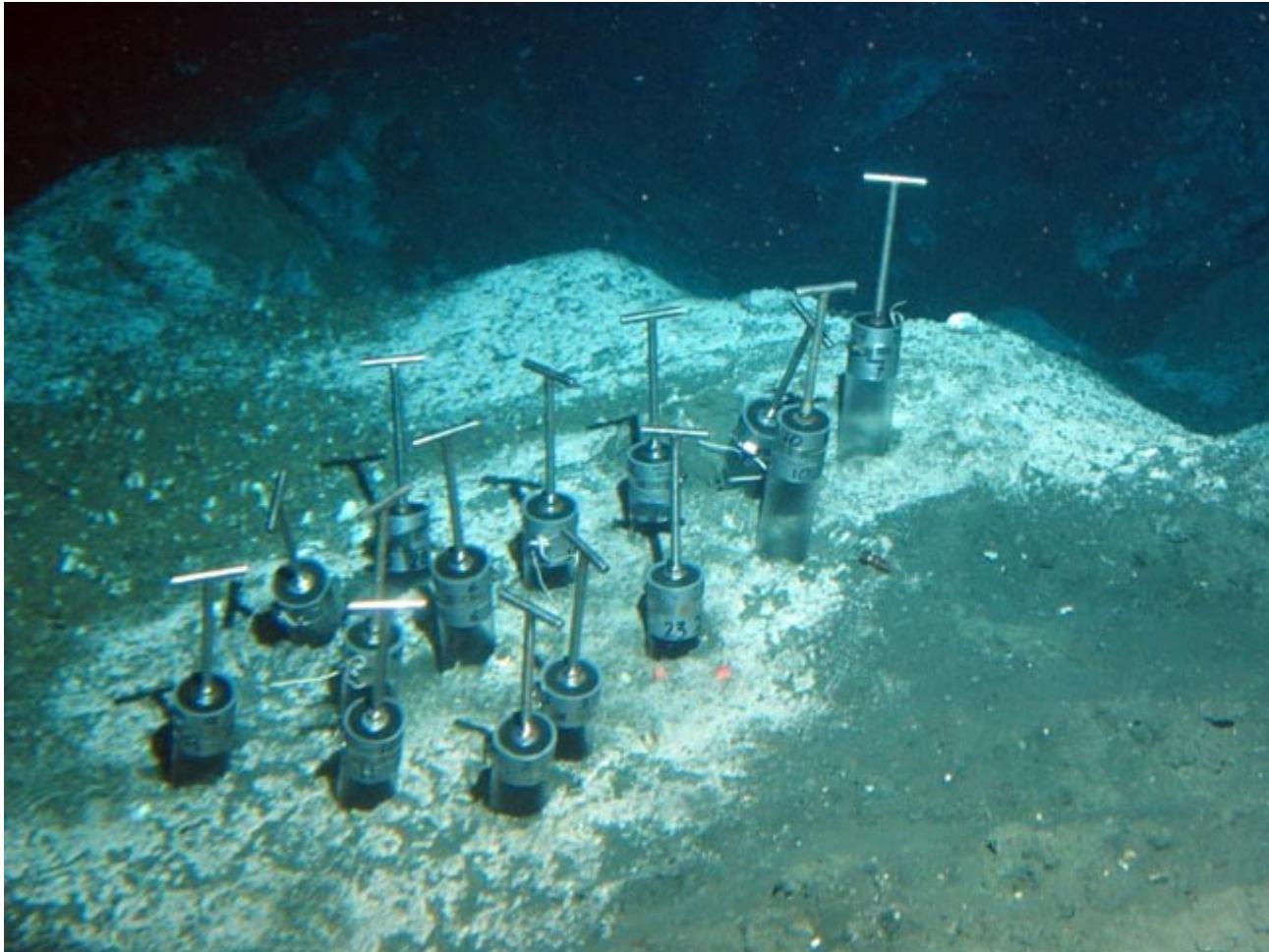
### *The Great Plate Count Anomaly*

The **great plate count anomaly** is the observation that most of the microbes seen in the microscope cannot currently be grown under laboratory conditions, some may actually be nonviable, others are viable but nonculturable (VBNC)



Staley JT, Konopka A. 1985. Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu Rev Microbiol.* 39:321-46.

Lloyd, K. G., Steen, A. D., Ladau, J., Yin, J., and Crosby, L. (2018). Phylogenetically Novel Uncultured Microbial Cells Dominate Earth Microbiomes. *mSystems* 3, e00055-18. doi:10.1128/mSystems.00055-18.

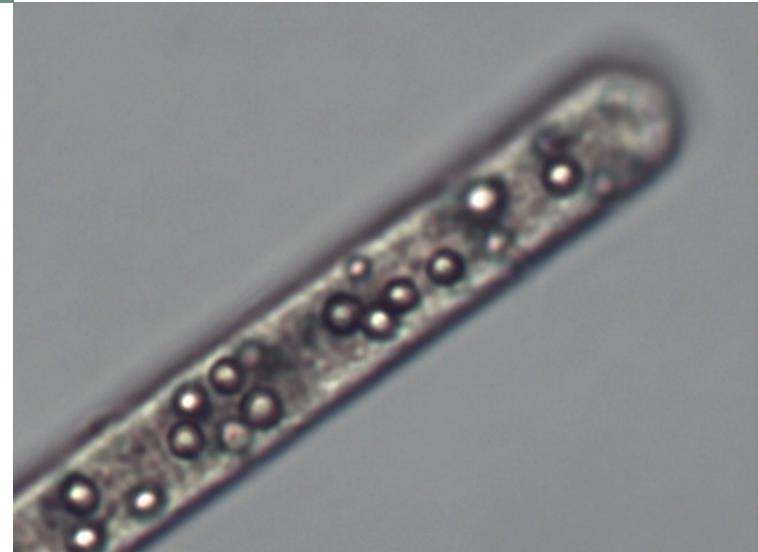


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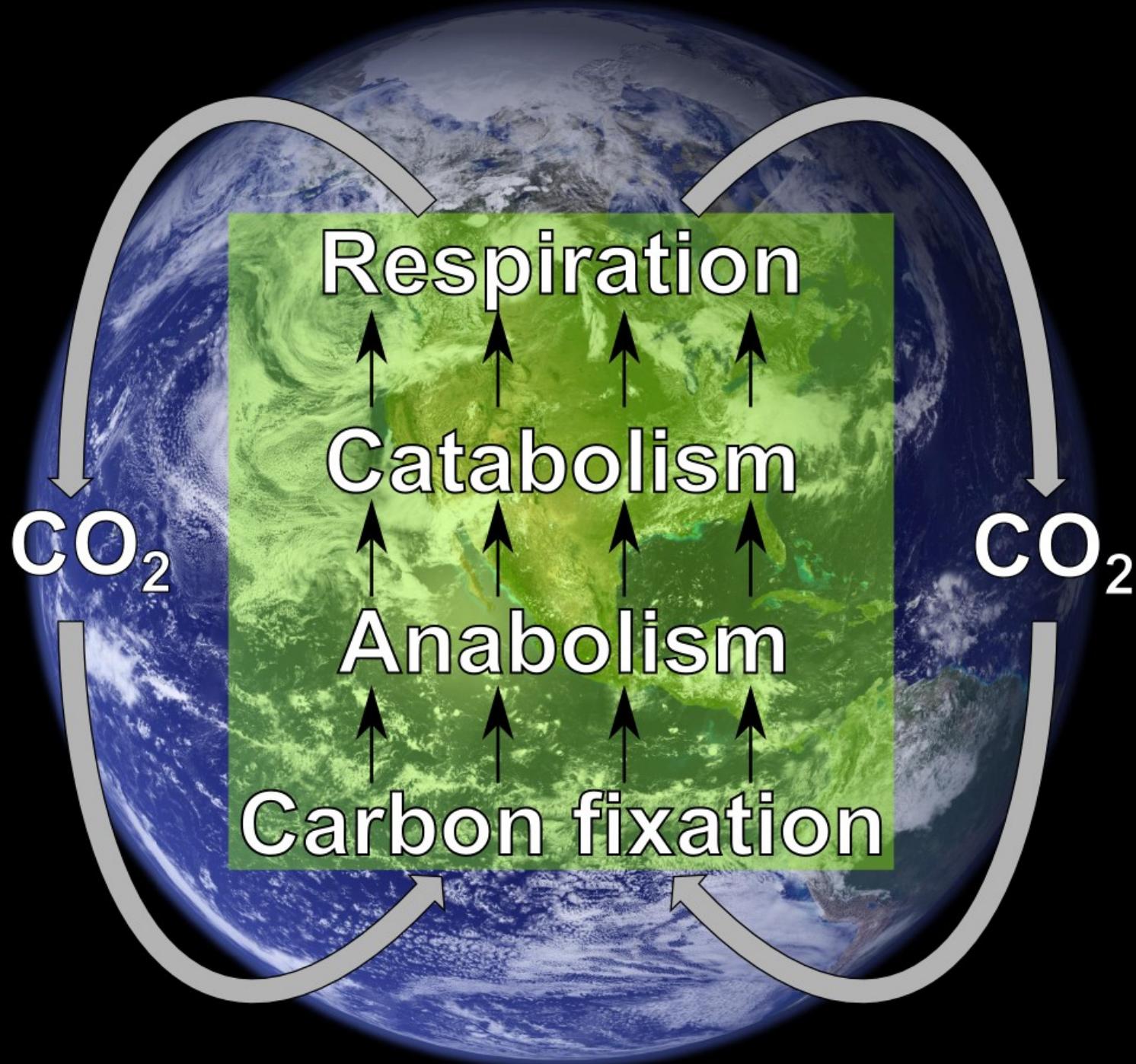
# *Autotrophy*

- *Authotrophy* is the process of building biomass from *inorganic carbon sources* (e.g., CO<sub>2</sub>, CO)
- *Six different major pathways* of carbon fixation are known
- The *Calvin-Benson-Bassam* cycle is the major pathway in the extant biosphere, and is used by *oxygenic photoautotrophs* and several *aerobic chemolithoautotrophs*
- All six pathways partially overlap due to the centrality of certain metabolic intermediate

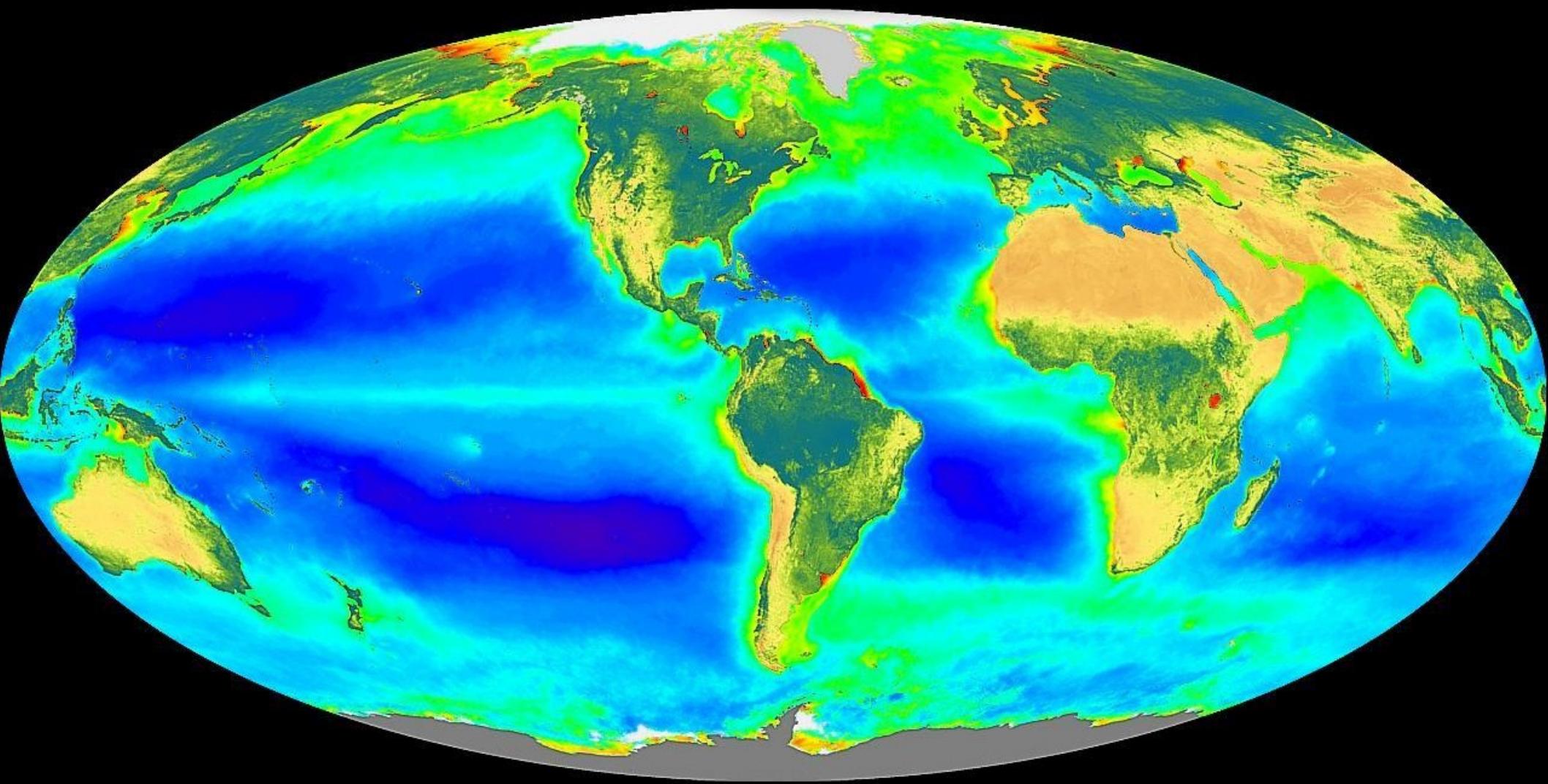


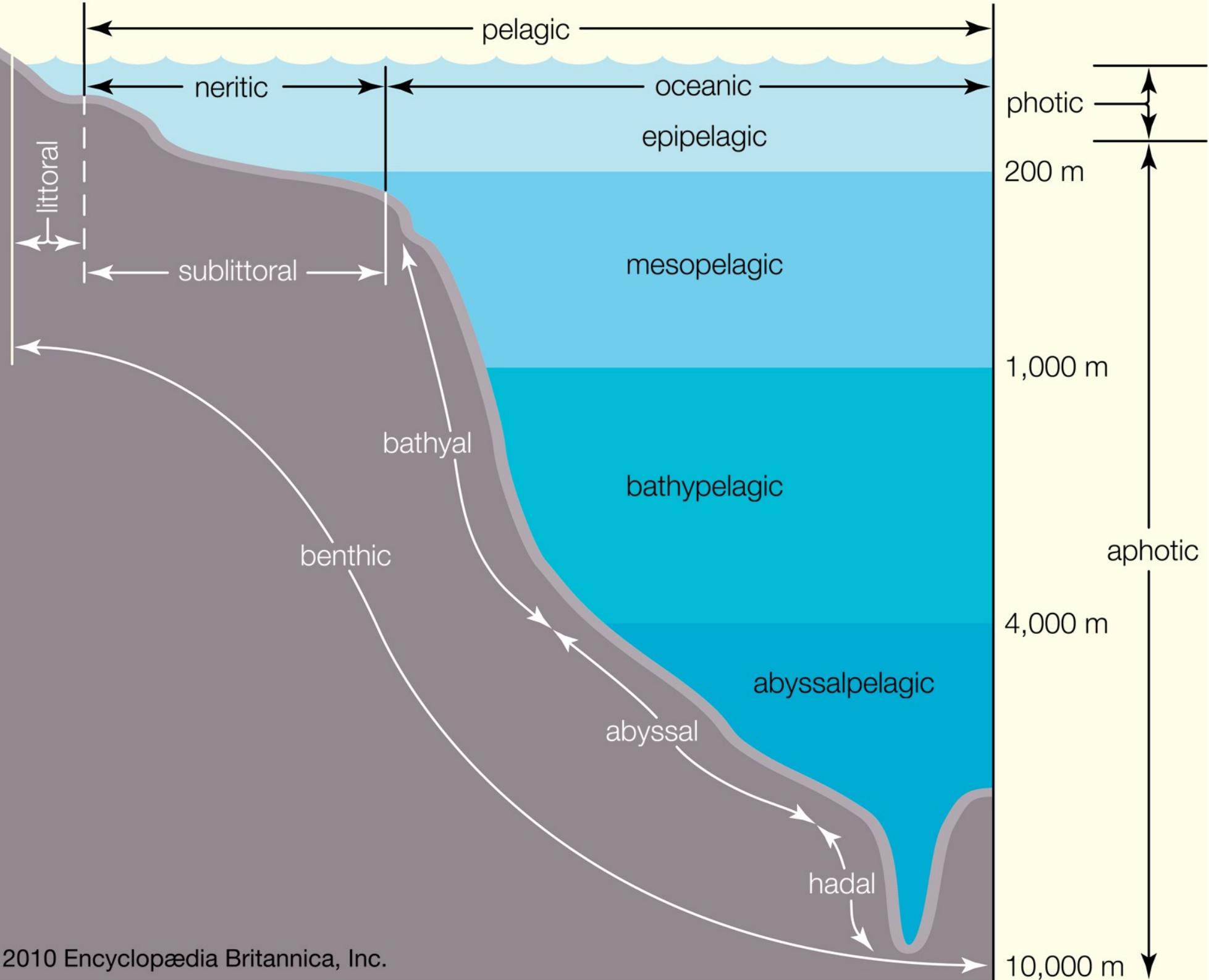
**OUR PLANET IS GLOBALLY  
AUTOTROPHIC**

*from NASA*



*redrawn from Braakman and Smith, 2013*





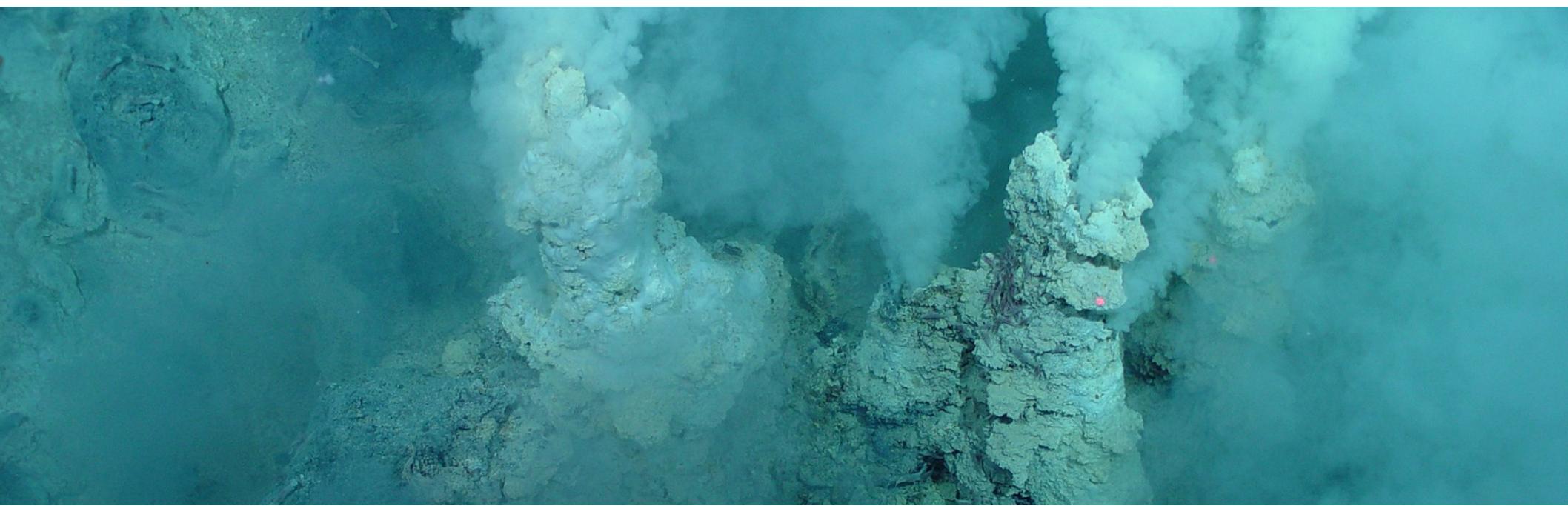
# Chemolithoautotrophy

Carbon fixation in the absence of light

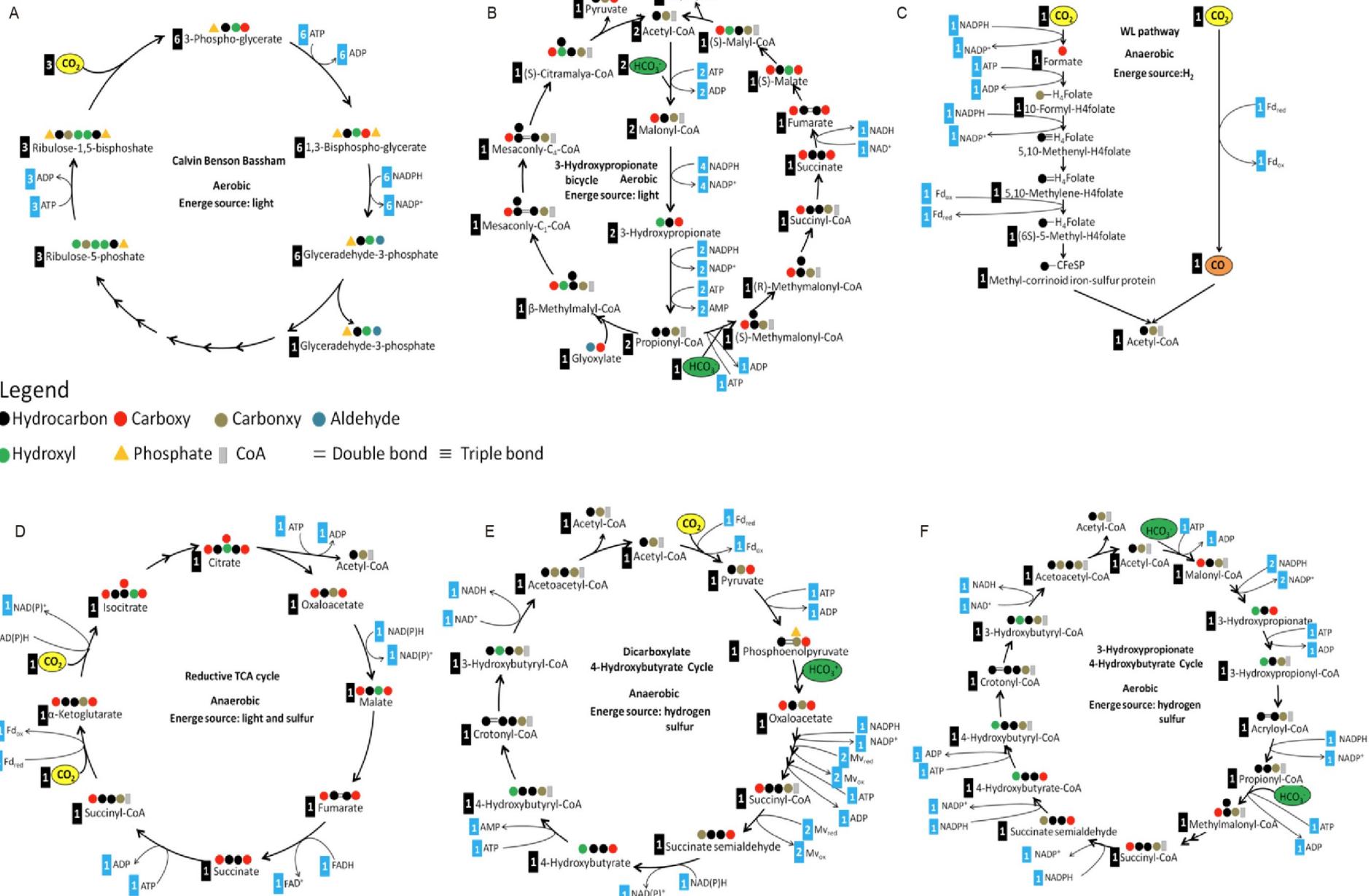
A form of chemotrophic nutrition in which a simple inorganic compound is oxidized in the course of the synthesis of complex organic compounds from carbon dioxide.

Also known as **dark carbon fixation** or **dark primary production**, chemolithoautotrophy is a key metabolic strategy potentially sustaining food webs and higher trophic levels throughout the deep oceans, in the sediments, in extreme environments and in subsurface ecosystems.

It is a diverse metabolic strategy widespread among Bacteria and Archaea.



# Carbon Fixation Pathways



**Figure 1** Six natural CO<sub>2</sub>-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 bicyclic**
- **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 CO<sub>2</sub> (oxidation state of +4) into cellular carbon (average oxidation state of 0, as in carbohydrates) requires four reducing equivalents (electrons)

CO<sub>2</sub> is organized via one of two mechanisms: carboxylation, in which CO<sub>2</sub> is attached to an existing metabolite, or reduction, in which CO<sub>2</sub> is converted to formate or carbon monoxide before further assimilation

Enzymes responsible for CO<sub>2</sub> incorporation are redox enzymes (oxidoreductases) that use cofactors to lower the energy of activation. These cofactors are often metals

# **Forming Carbon-Carbon bonds**

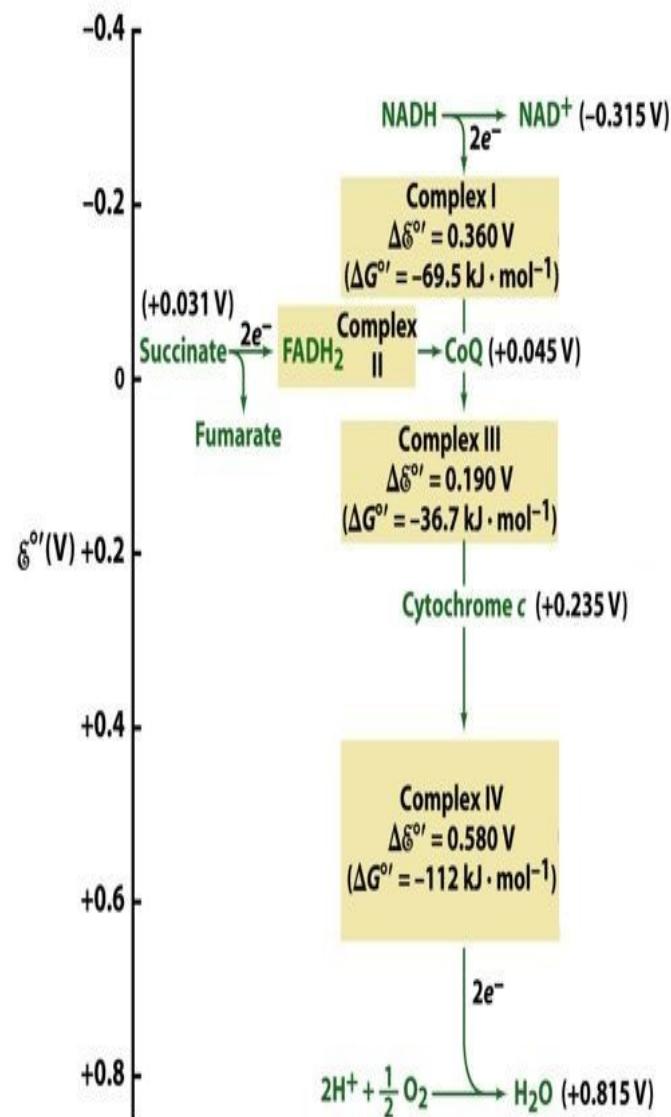
An *input of energy* is also required for the reductive conversion of CO<sub>2</sub> to cell carbon and is provided by ATP hydrolysis

Anaerobes often use low-potential electron donors like reduced ferredoxin for CO<sub>2</sub> fixation, whereas aerobes usually rely on NAD(P)H as a reductant

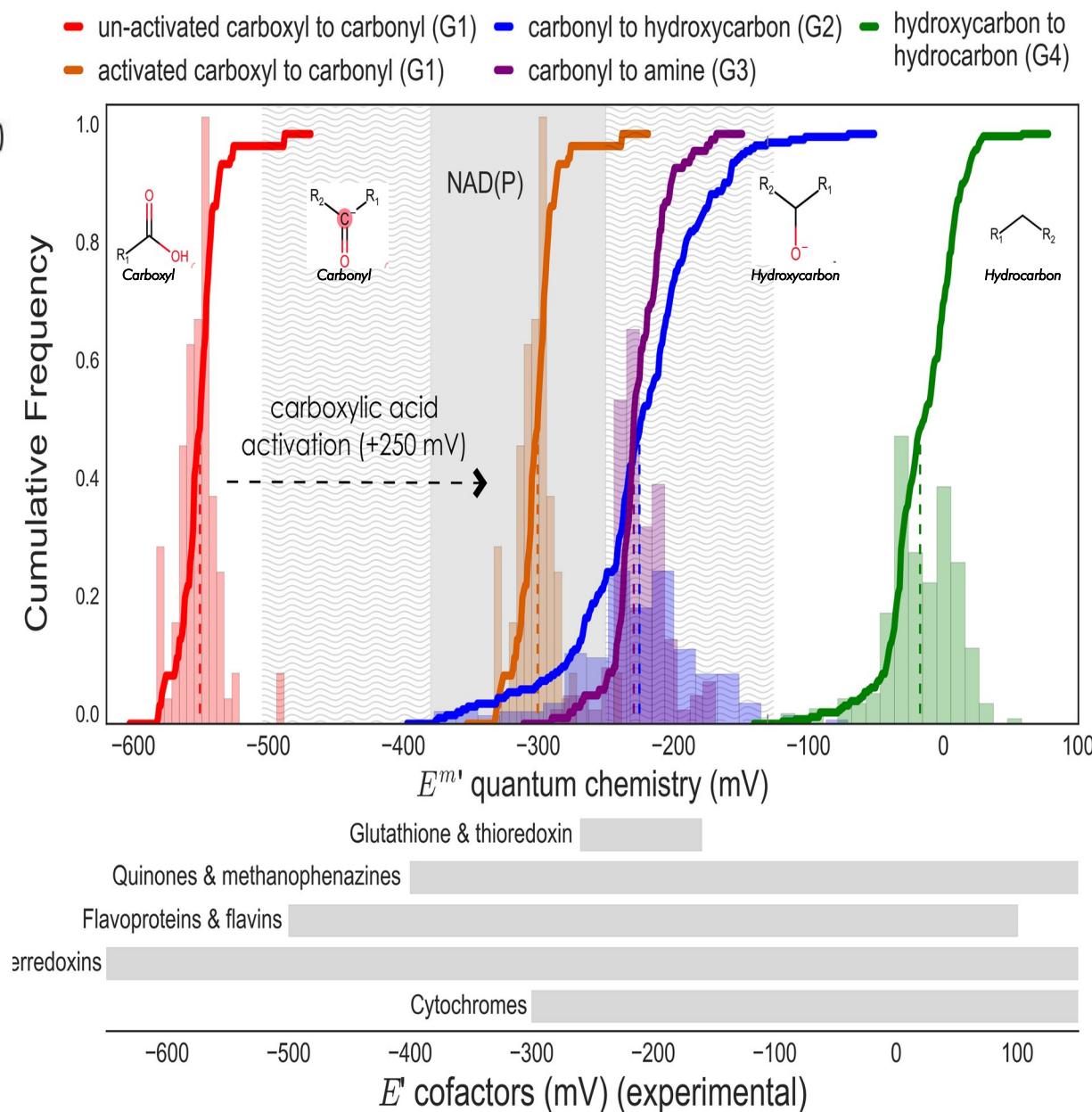
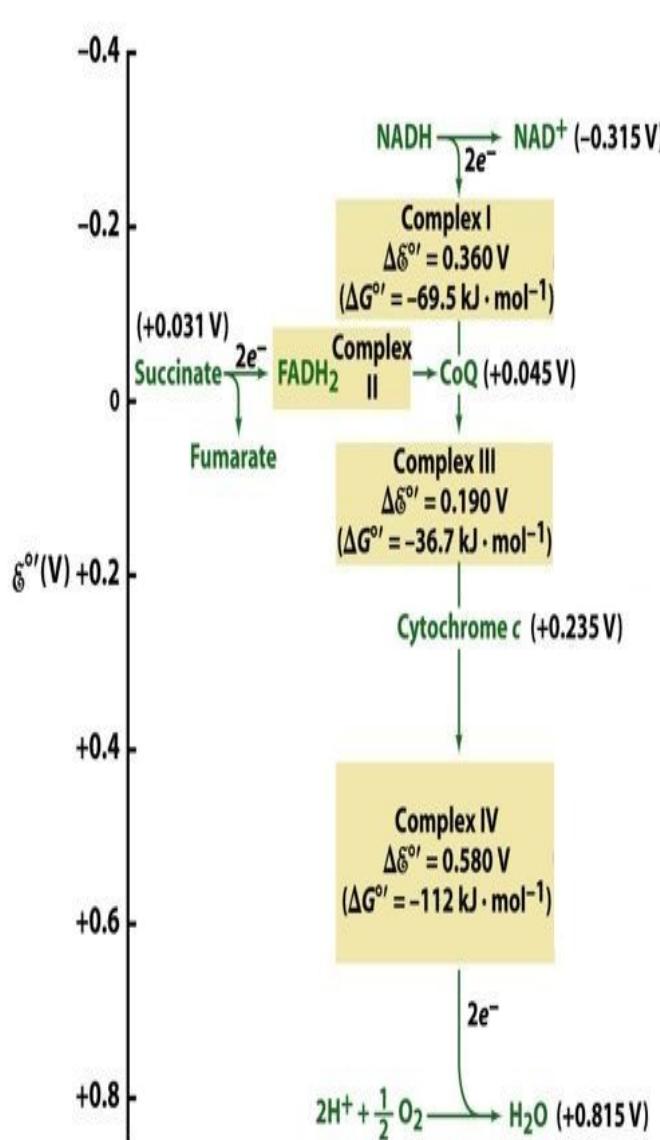
The CO<sub>2</sub> taken up by the organism generates relatively reductive pyruvate or acetyl-CoA, both of which are important intermediate metabolites for cell growth.

Since the carbon atom in a CO<sub>2</sub> molecule is in the highest positive valence state. Consequently, the changes of the Gibbs free energy ( $\Delta G$ ) of these CO<sub>2</sub> reductive reactions are usually positive, thus thermodynamically unfavorable and challenging.

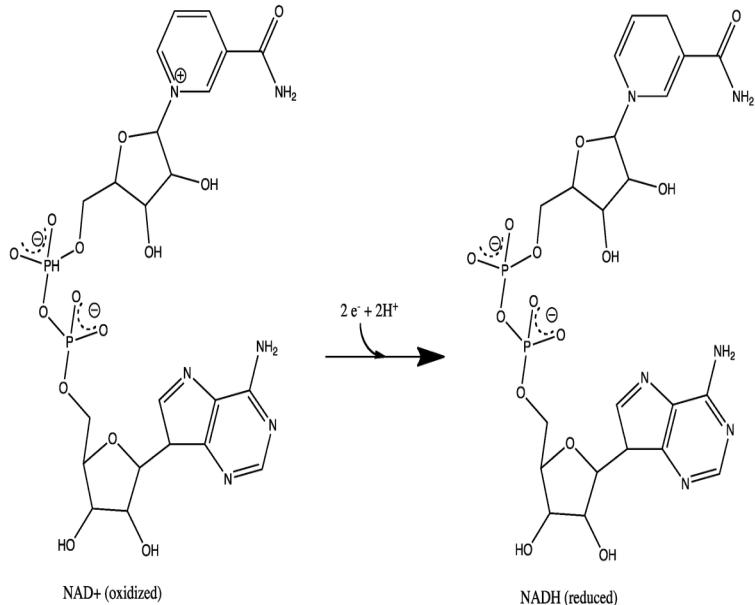
# The need for reduction potential



# The need for reduction potential

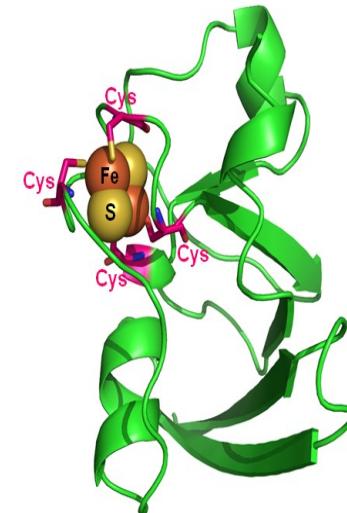


# *The need for reduction potential*



**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

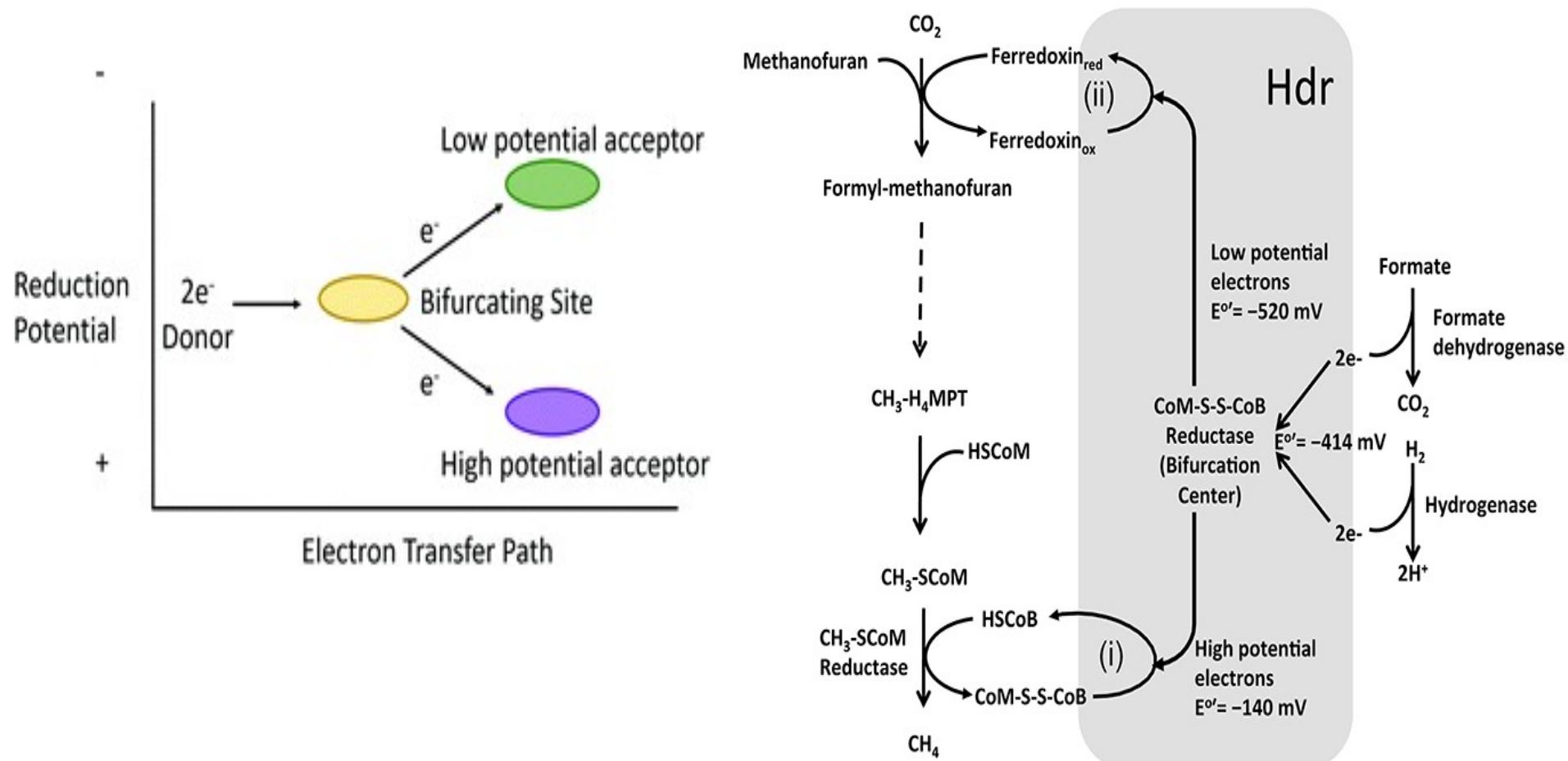
Molecular hydrogen ( $H_2$ ) is often used by anaerobes as preferred electron donor, given its low potential ( $-414$  mV). Despite this, low potential needs to be achieved through a complex mechanism known as **electron bifurcation**

A variety of different mechanisms exist under this name:

Ferredoxin Reduction by NADH in membrane-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.



# PATHWAYS OF CARBON FIXATION

# *Calvin-Benson-Bassham Cycle*

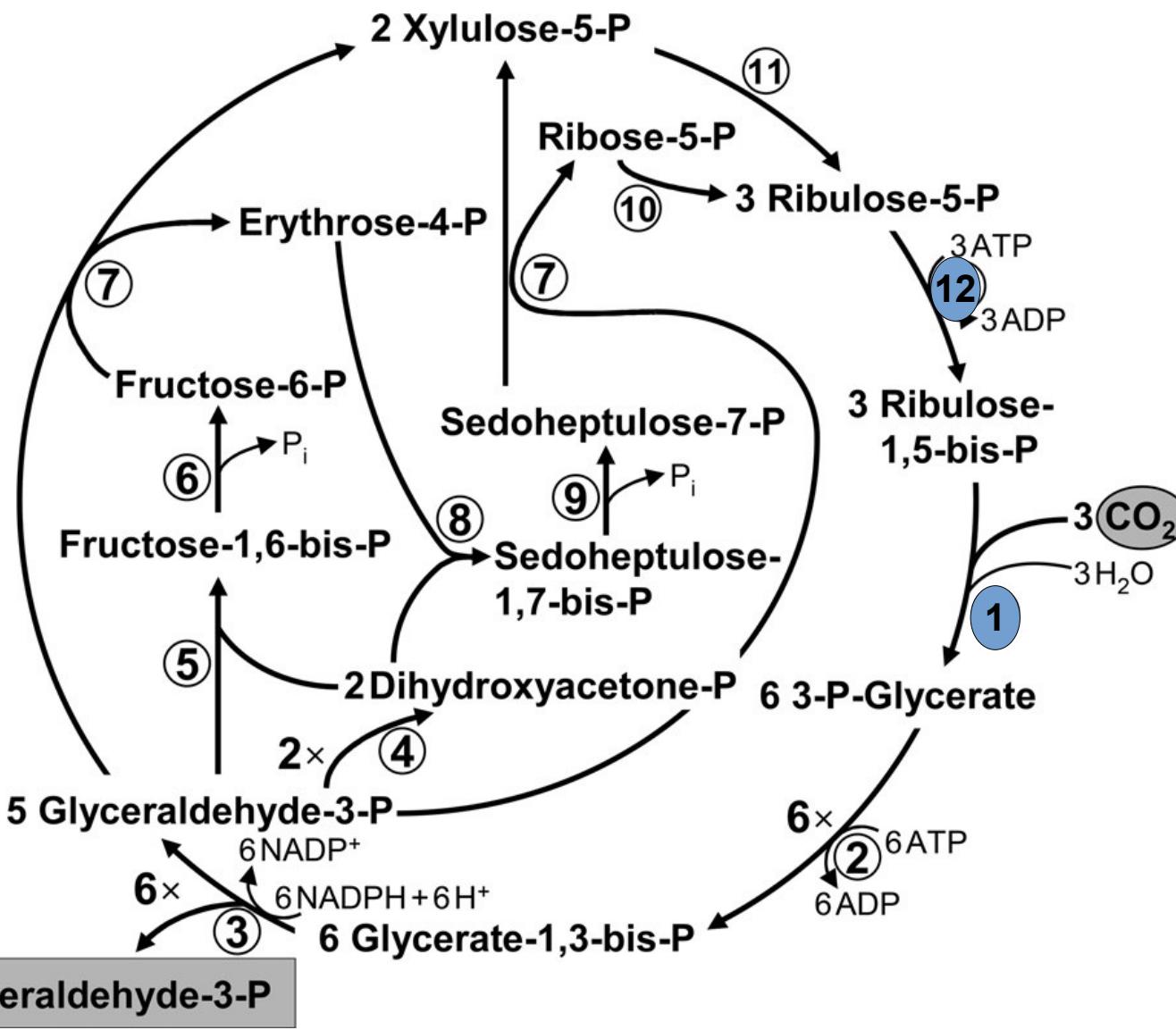
*Discovered in 1950 in Green Algae, the Calvin Cycle is the dominant cycle in the extant biosphere, and dominated autotrophic processes in the surface of our planet*

*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

Glyceraldehyde-3-P

# RuBisCo

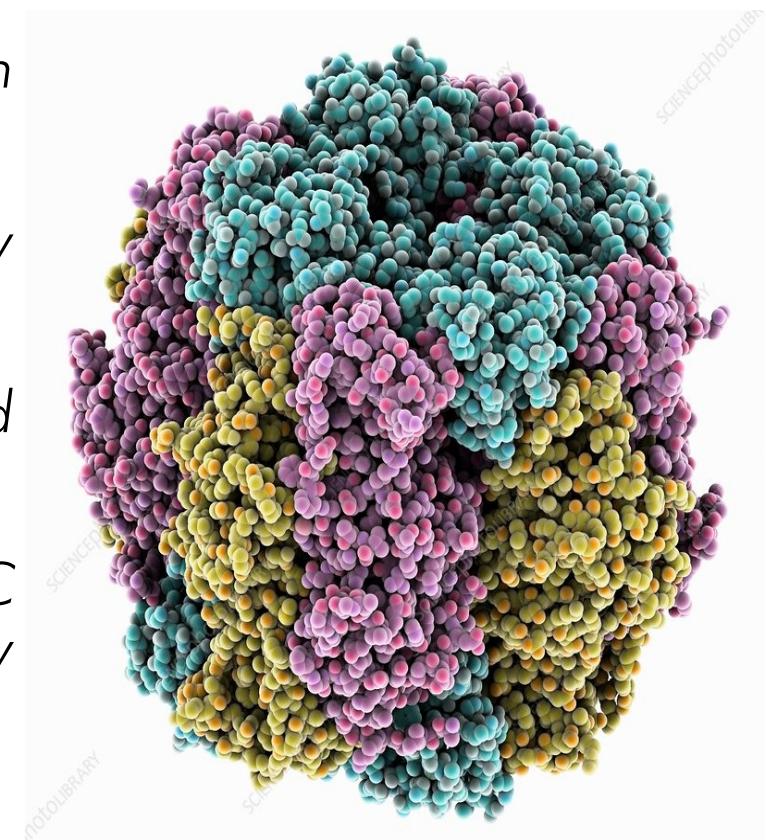
RuBisCo is a large (500 kDa) heterodimeric enzymes formed by a large and a small subunit

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

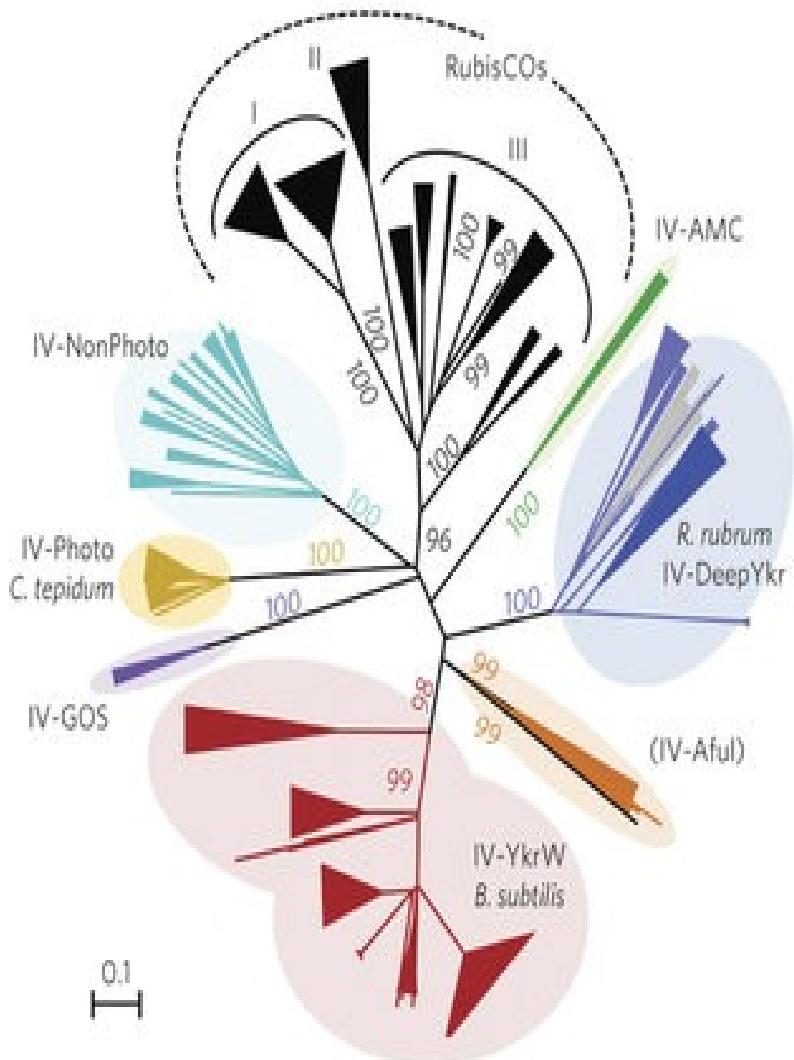
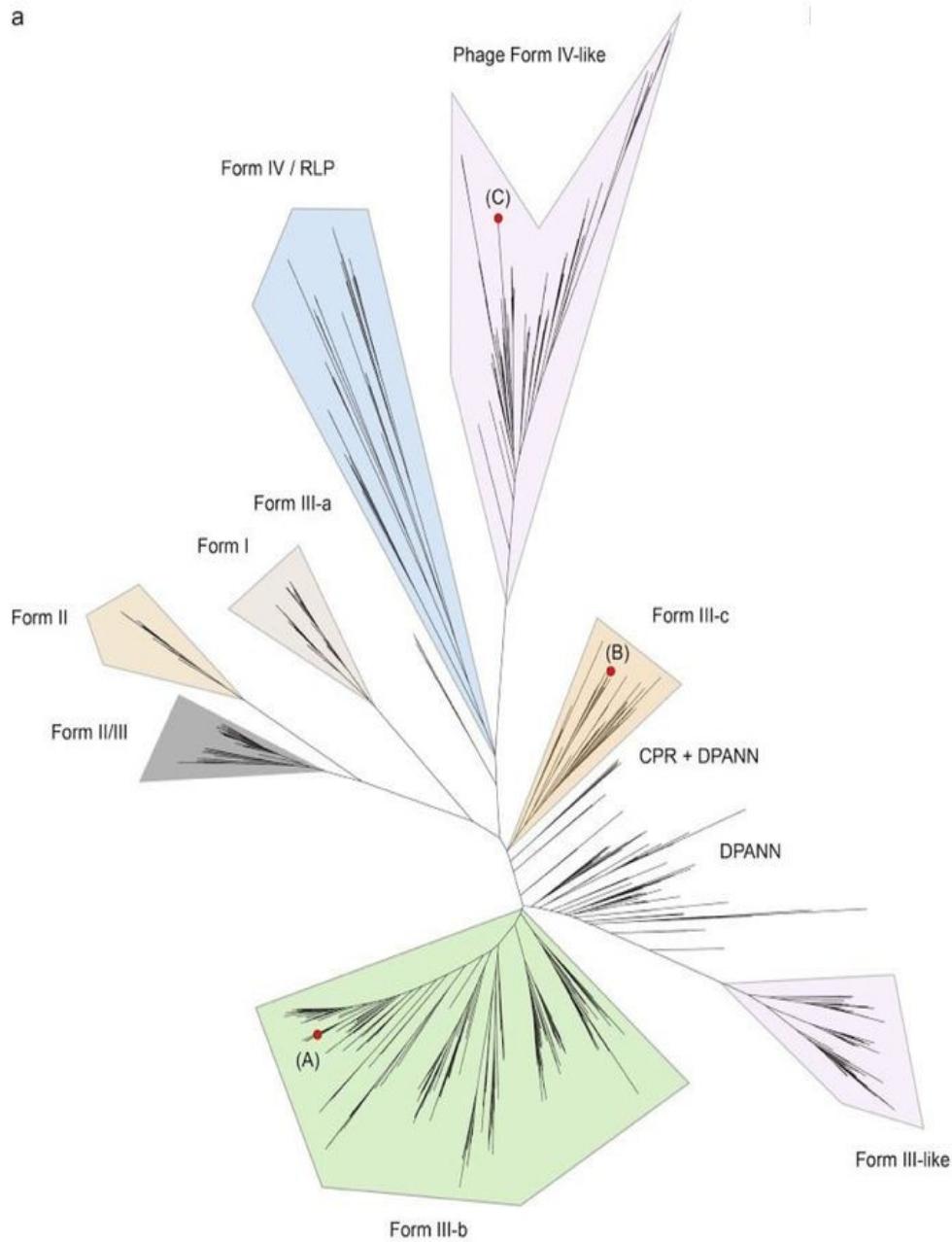
Can make up to 50% of chloroplast protein, and is probably the most abundant protein on Earth

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

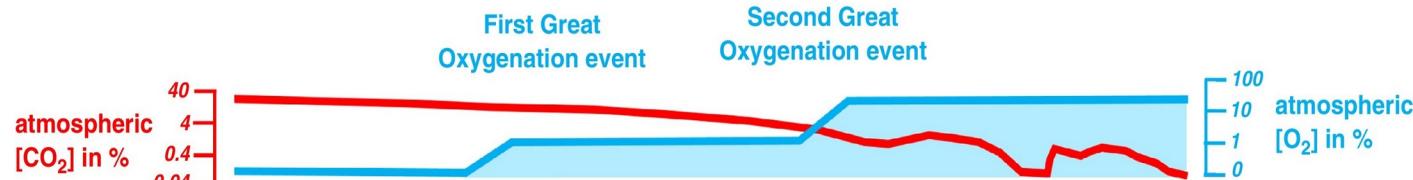
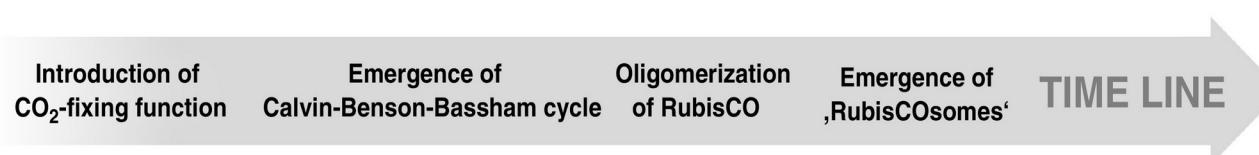
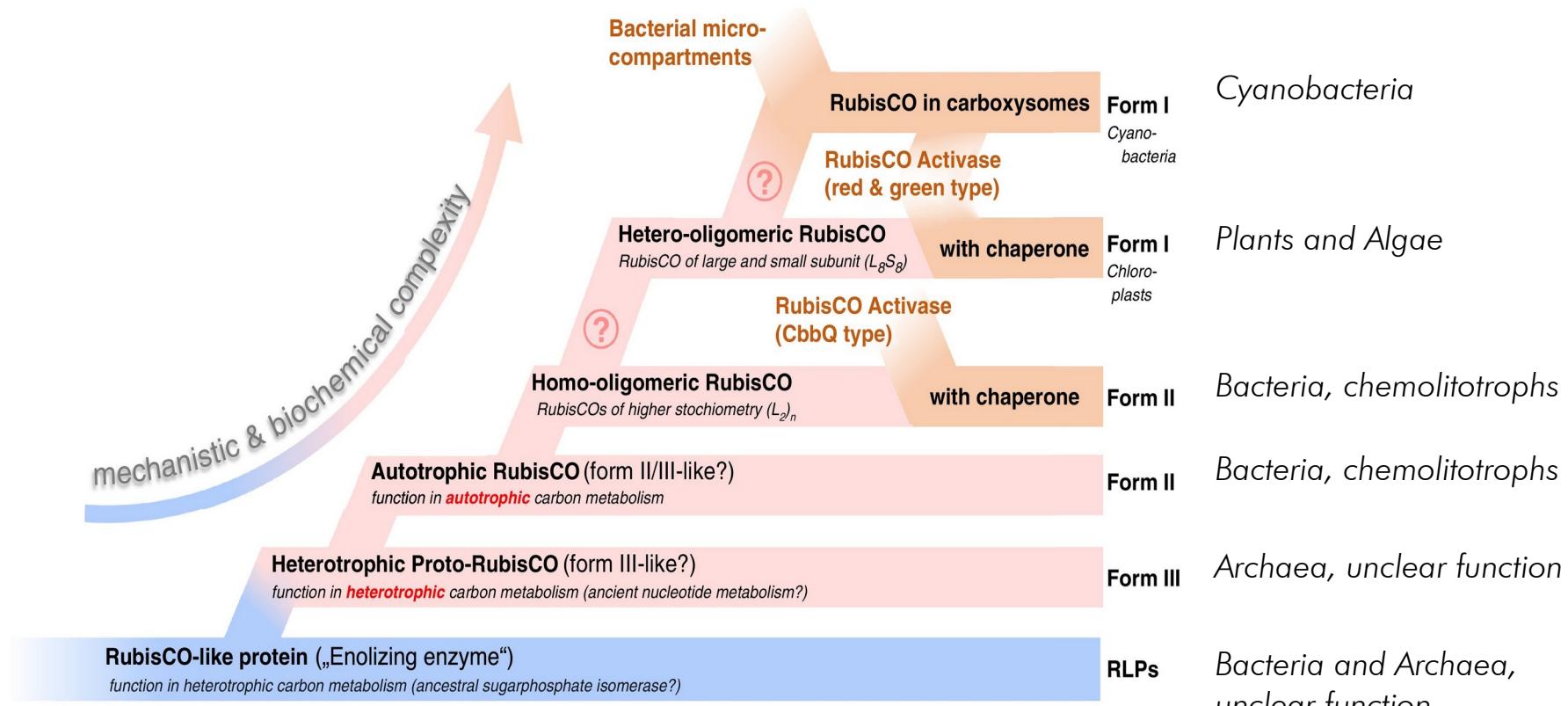


# RuBisCo and RLP phylogeny

a



# Evolution of RuBisCo



Current Opinion in Biotechnology

# **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 CO<sub>2</sub> and 2-3 ATP are used to generate 1 molecule of Pyruvate, making the rTCA cycle a very energy efficient carbon fixation pathway

Δ<sup>13</sup>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

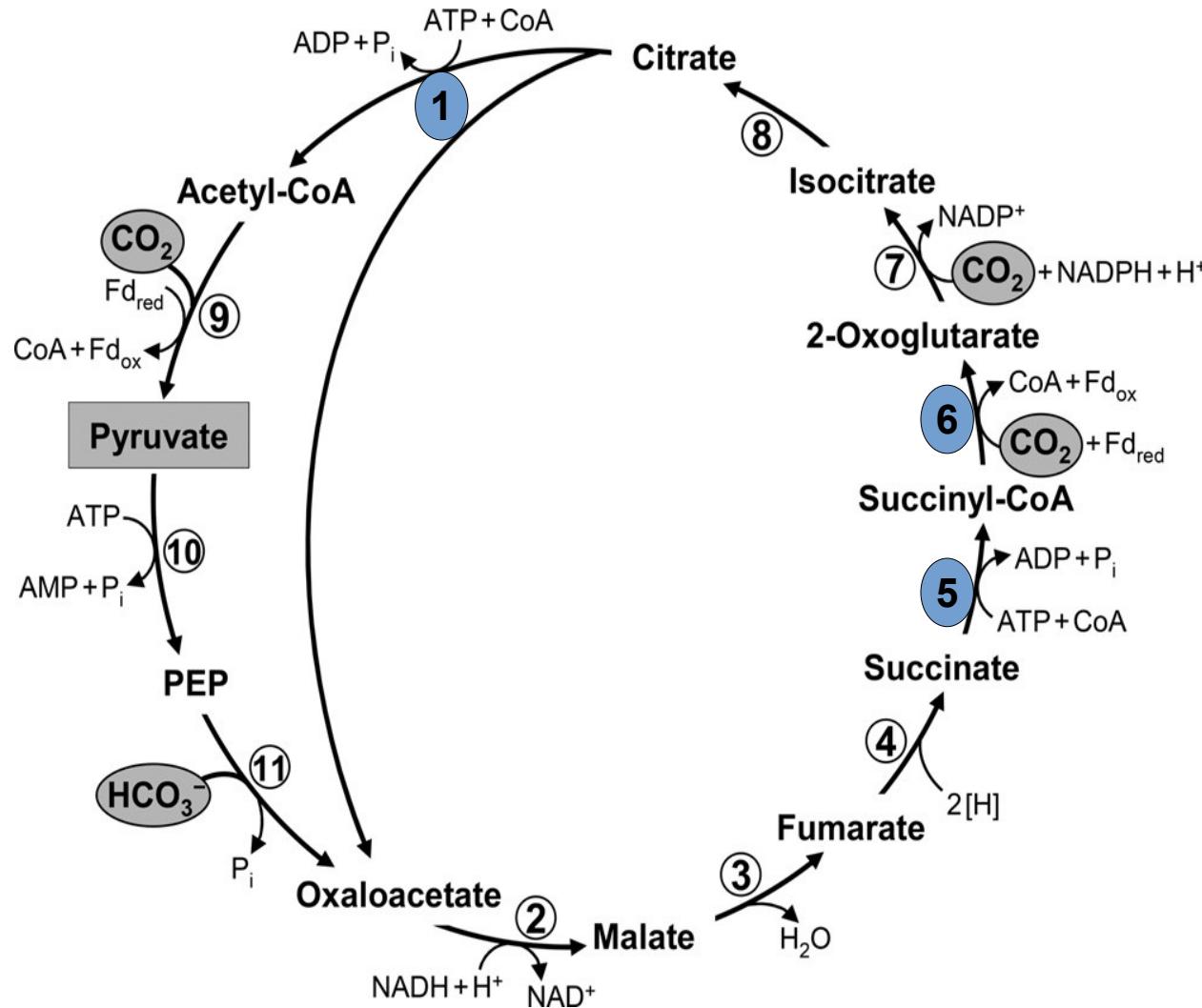
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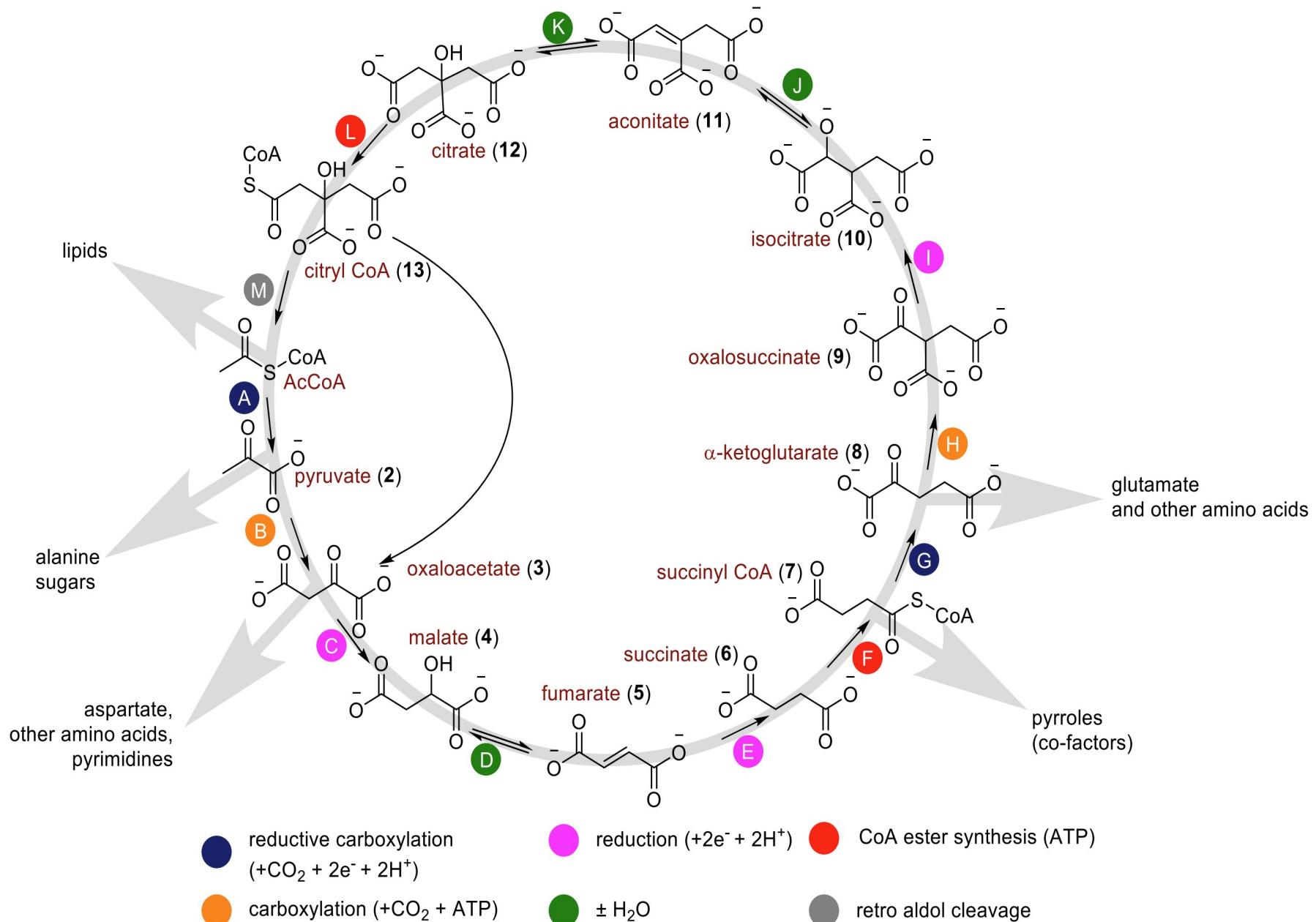
Buchanan

# Arnon-Buchanan Cycle

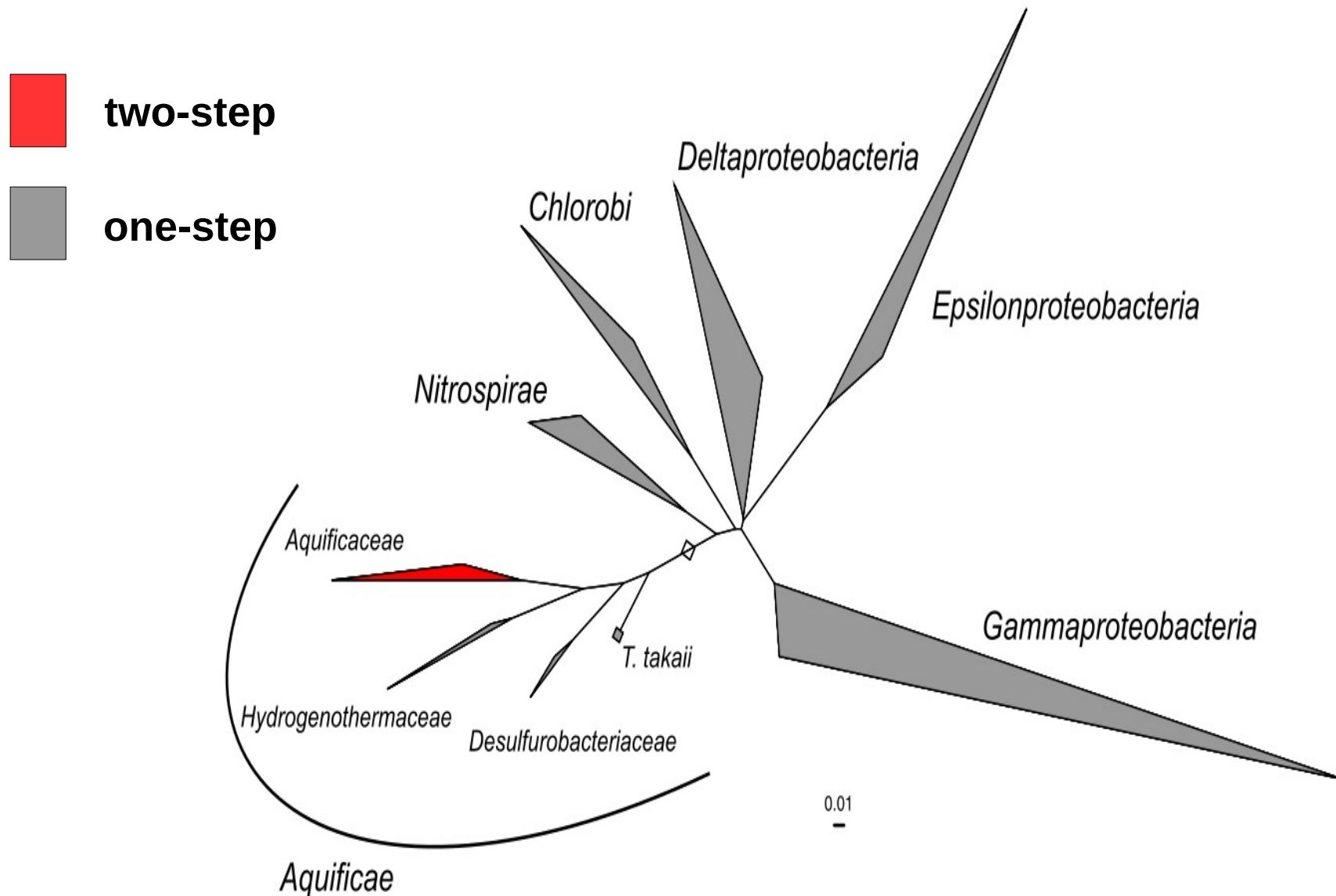


1. ATP-citrate lyase
2. malate dehydrogenase
3. fumarate hydratase
4. fumarate reductase
5. succinyl-CoA synthetase
6. ferredoxin (Fd)-dependent 2-oxoglutarate synthase
7. isocitrate dehydrogenase
8. aconitate hydratase
9. Fd-dependent pyruvate synthase
10. PEP synthase
12. PEP carboxylase

# Arnon-Buchanan Cycle: centrality of intermediates

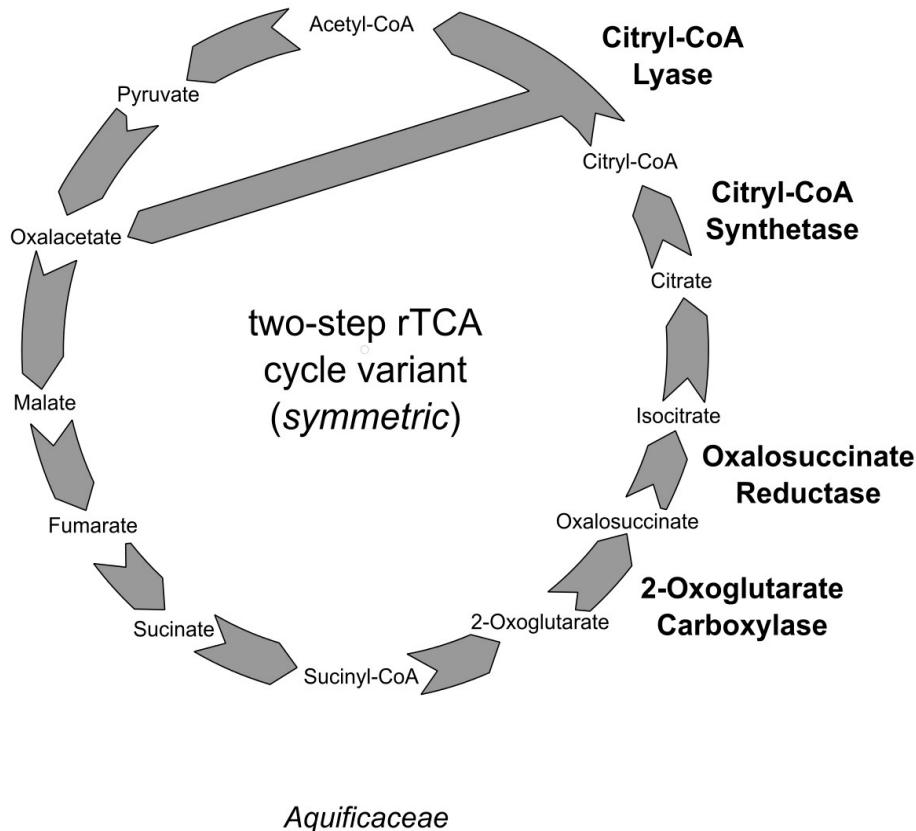


# Arnon-Buchanan Cycle

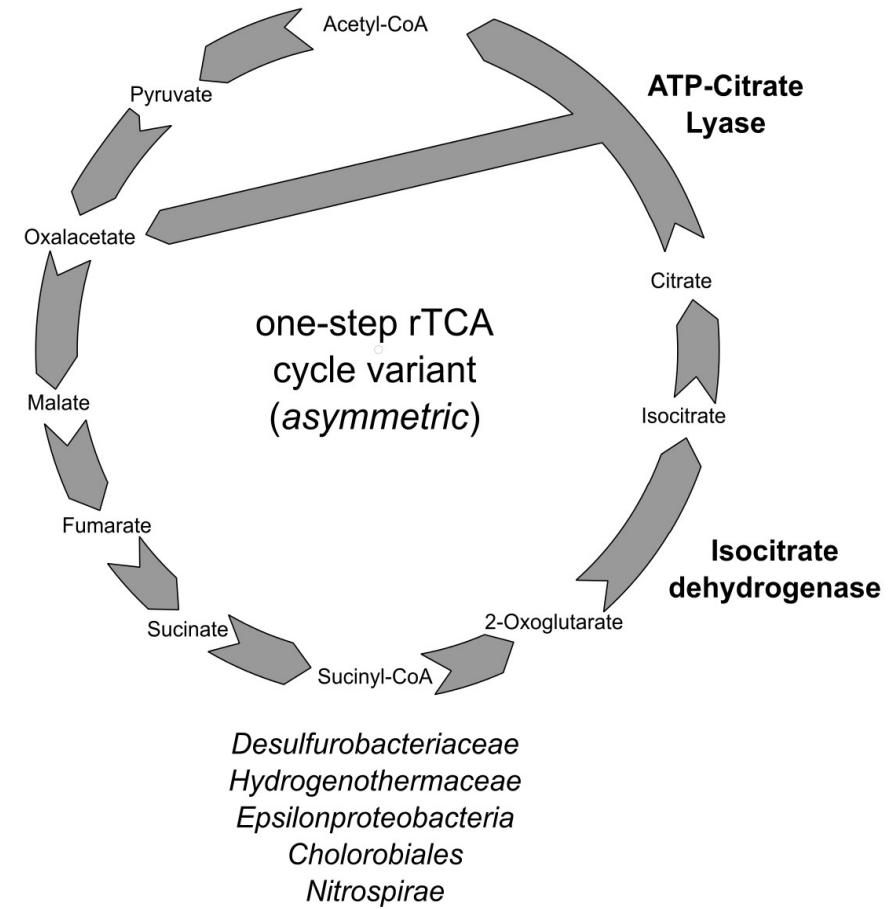


# Arnon-Buchanan Cycle: two different variant

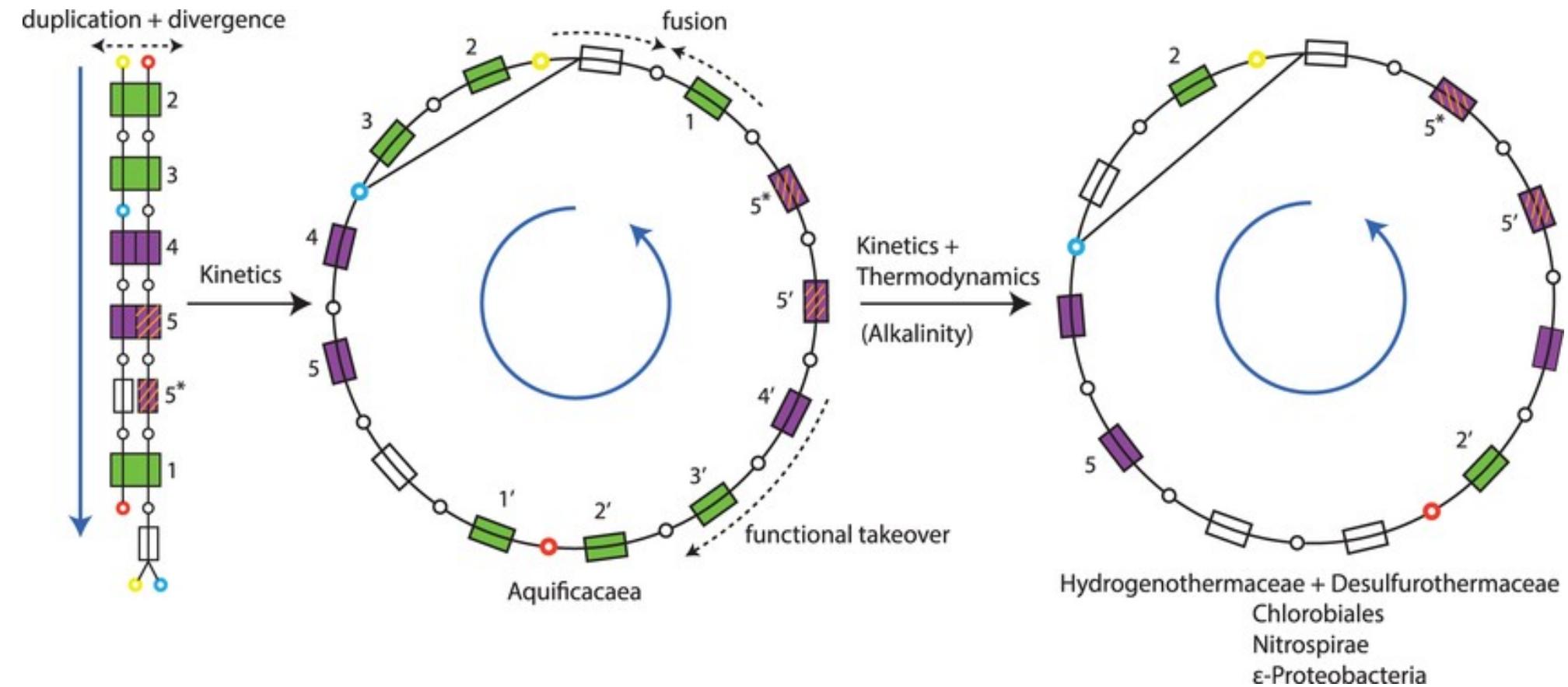
A



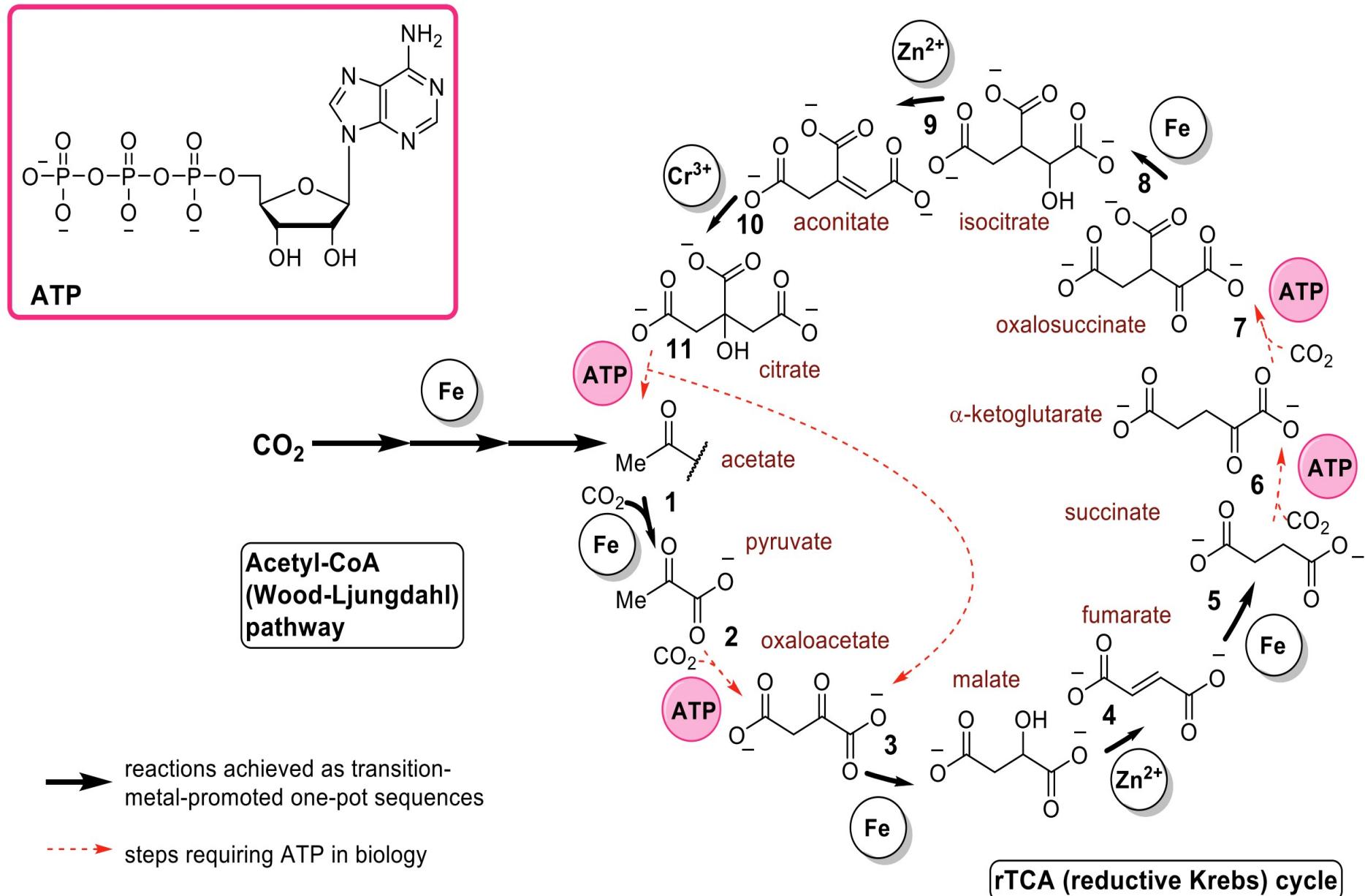
B



# 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*

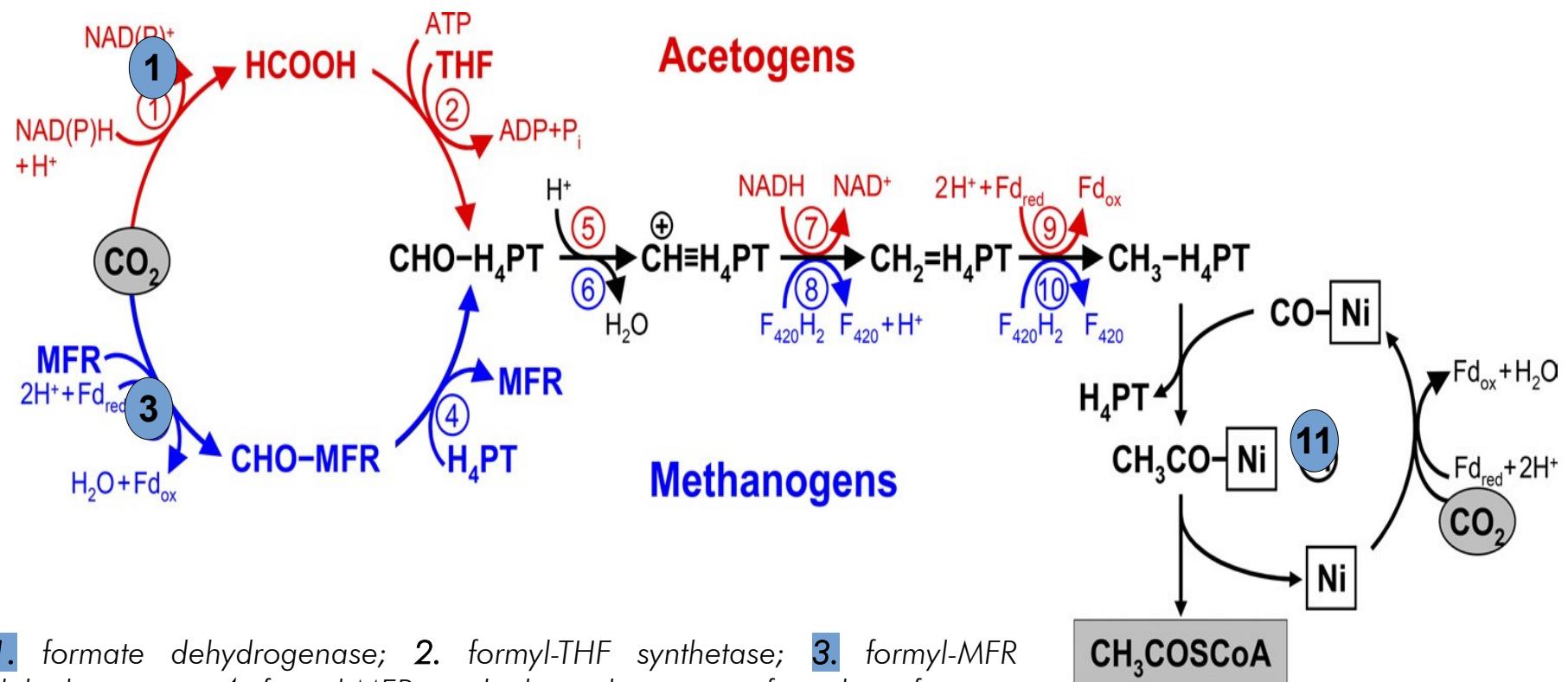
*The methyl branch is functionally similar but not homologous between Bacteria and Archaea, while the carbonyl branch is homologous, this complicates all evolutionary work*

*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*

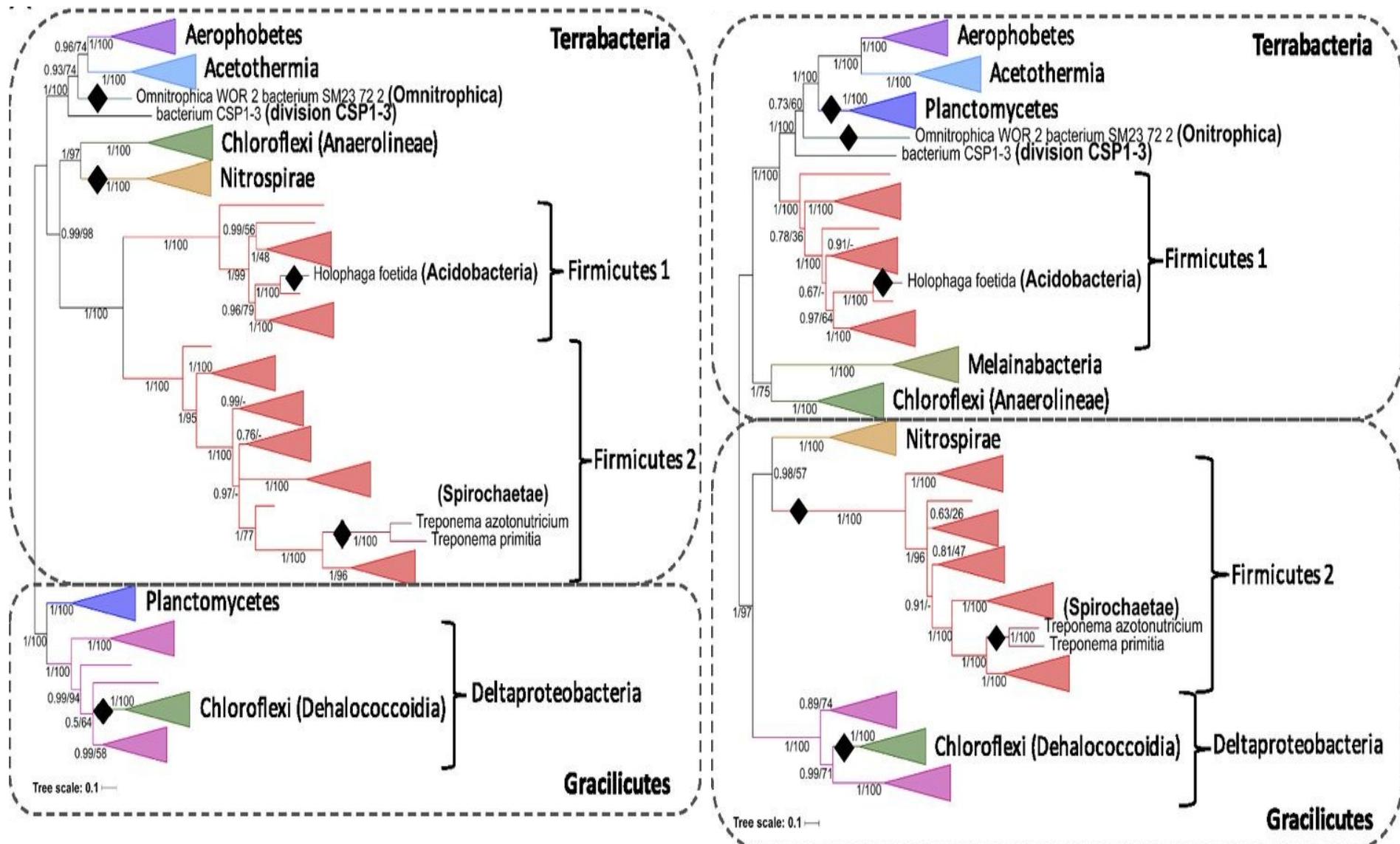
*$\Delta^{13}\text{C}$  isotopic fractionation is variable and between –15 and –80 ‰*

# Wood-Ljungdahl pathway

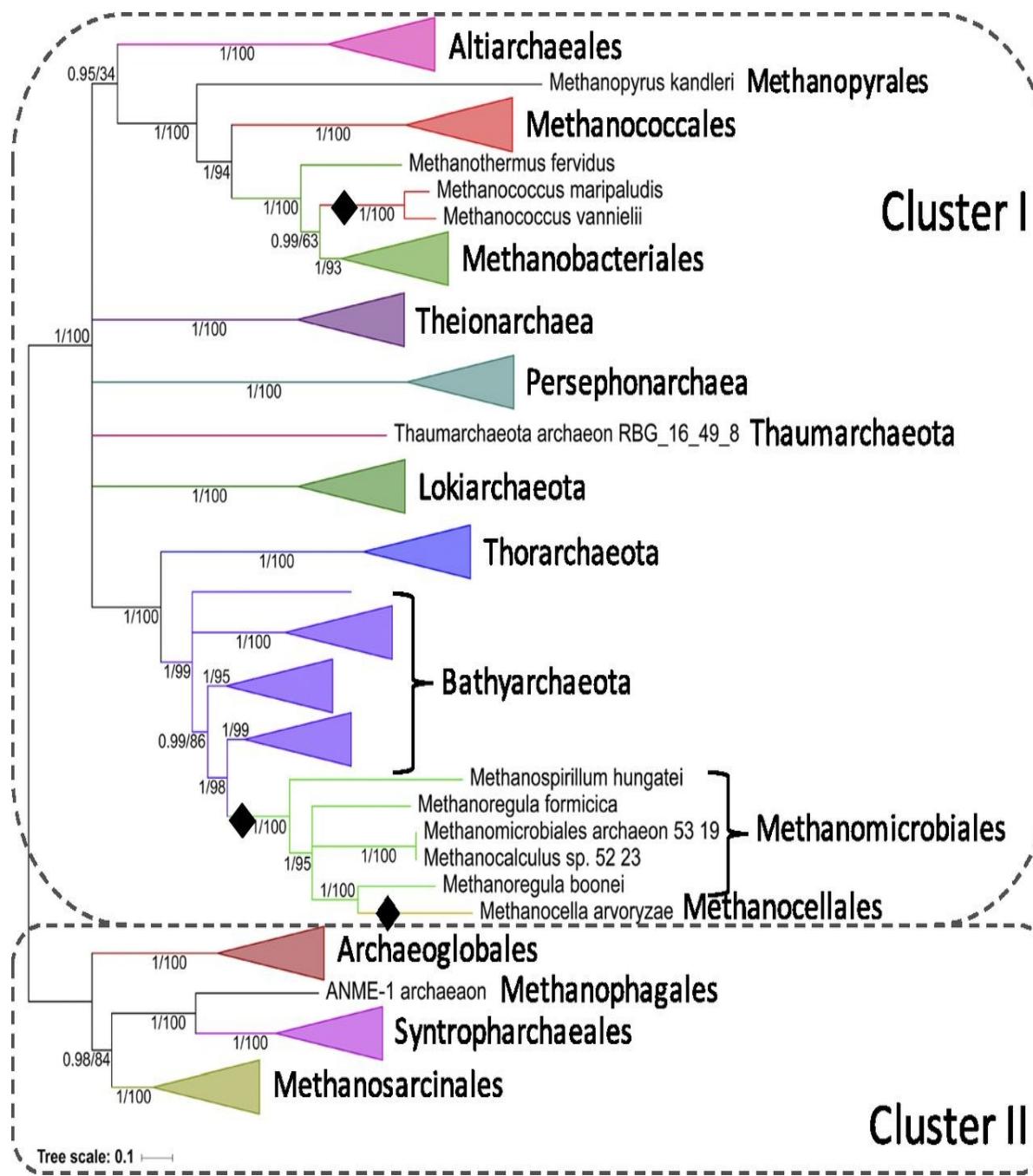


1. formate dehydrogenase; 2. formyl-THF synthetase; 3. formyl-MFR dehydrogenase; 4. formyl-MFR:tetrahydromethanopterin formyltransferase;
5. methenyl-THF cyclohydrolase; 6. methenyl-tetrahydromethanopterin cyclohydrolase; 7. methylene-THF dehydrogenase; 8. methylene-tetrahydromethanopterin dehydrogenase;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

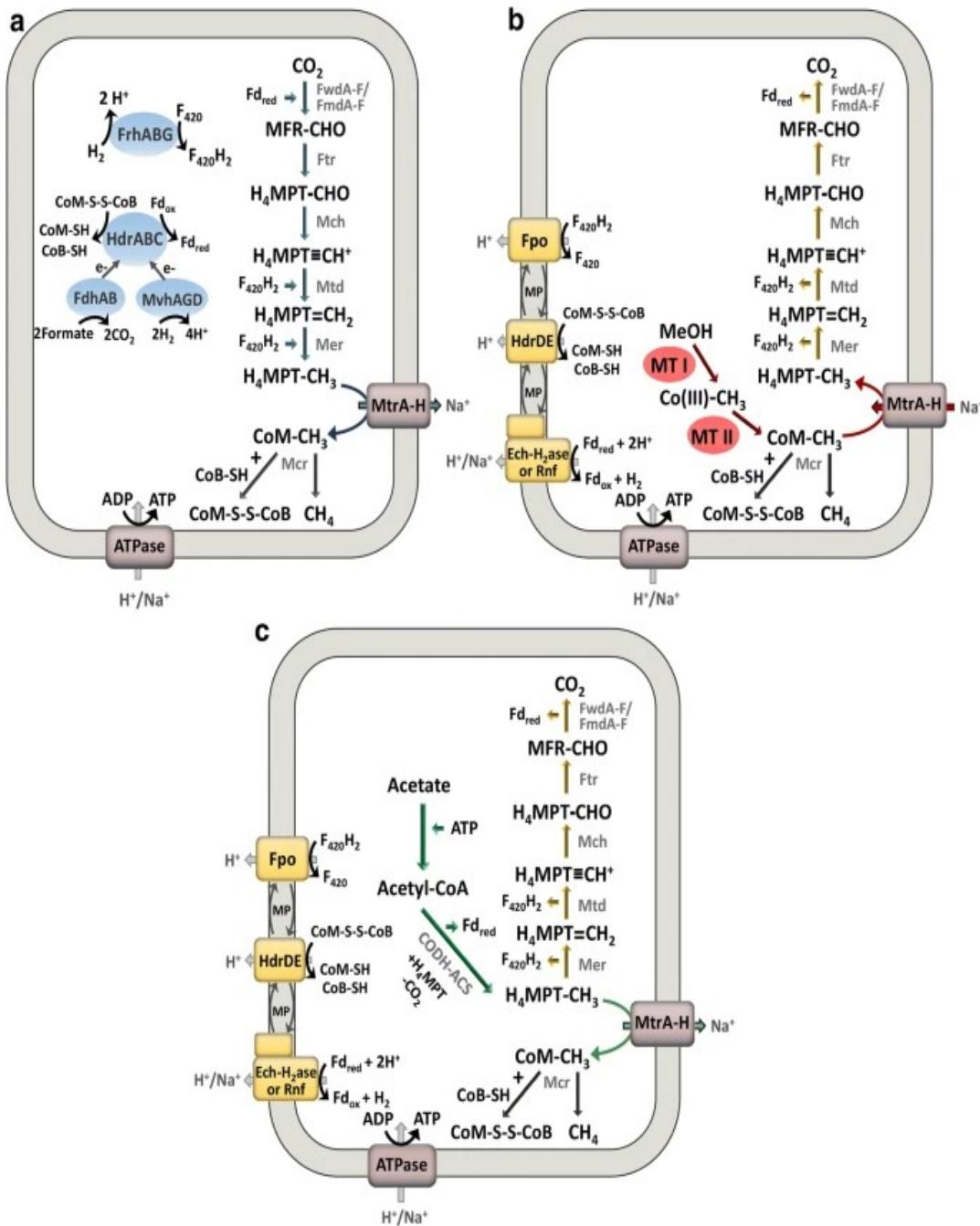
# Wood-Ljungdahl pathway: distribution



# Wood-Ljungdahl pathway: distribution



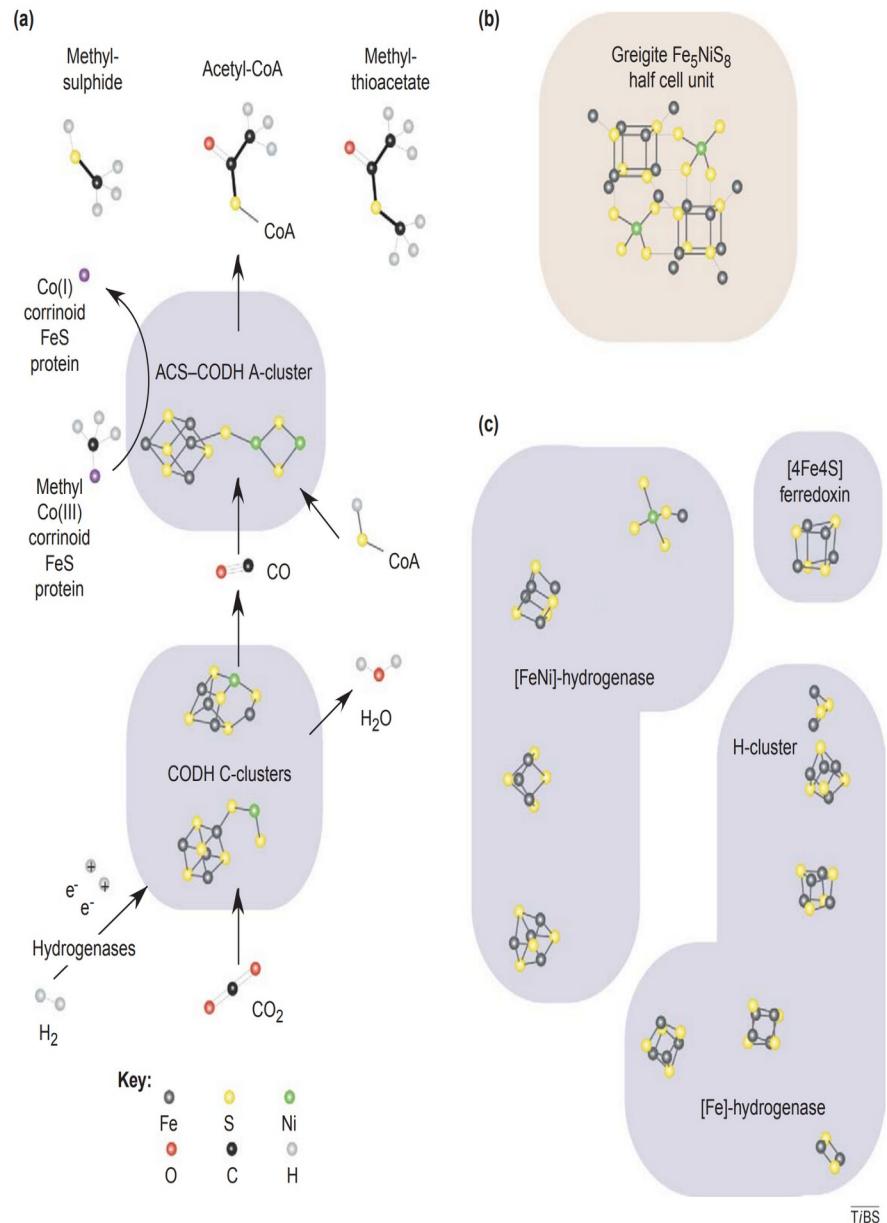
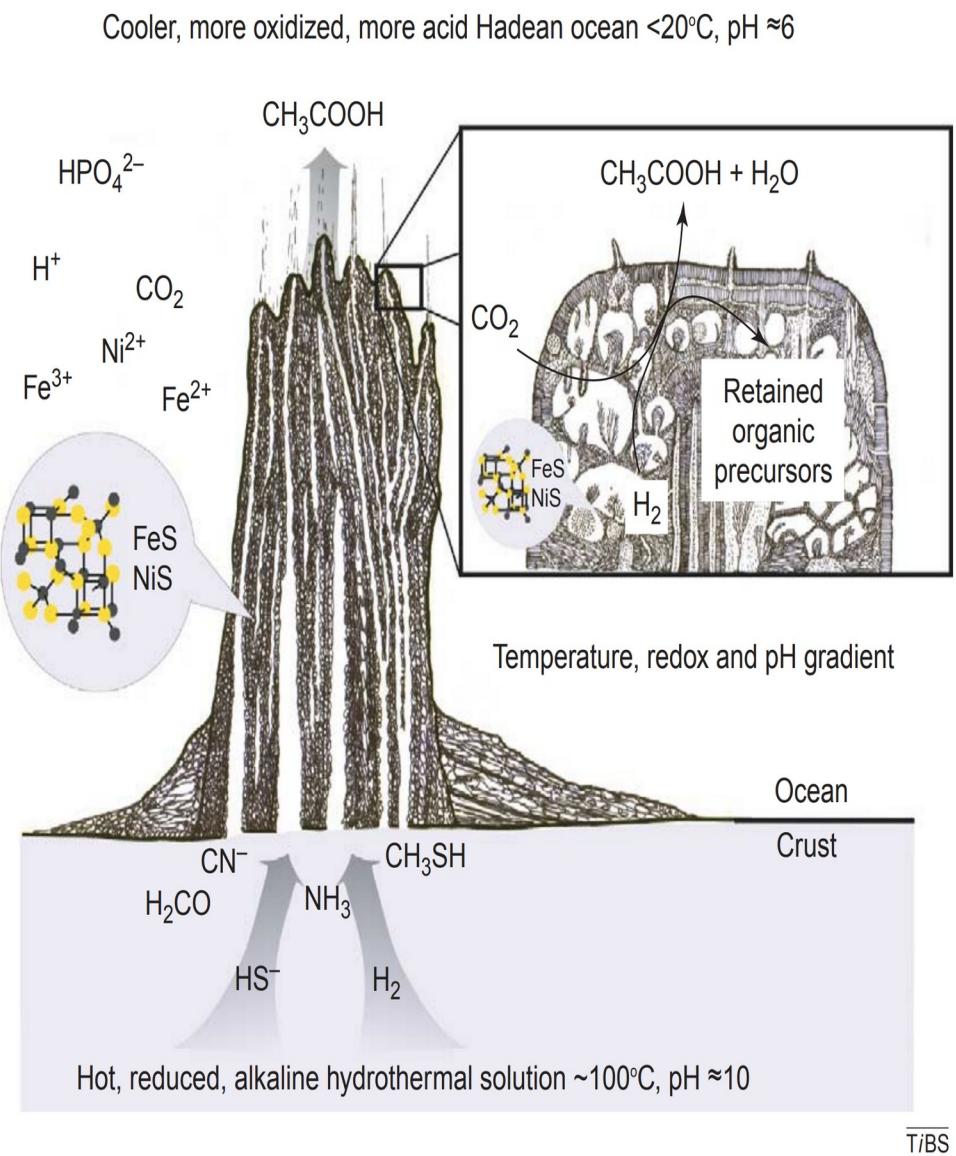
# Wood-Ljungdahl pathway: Methanogens



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

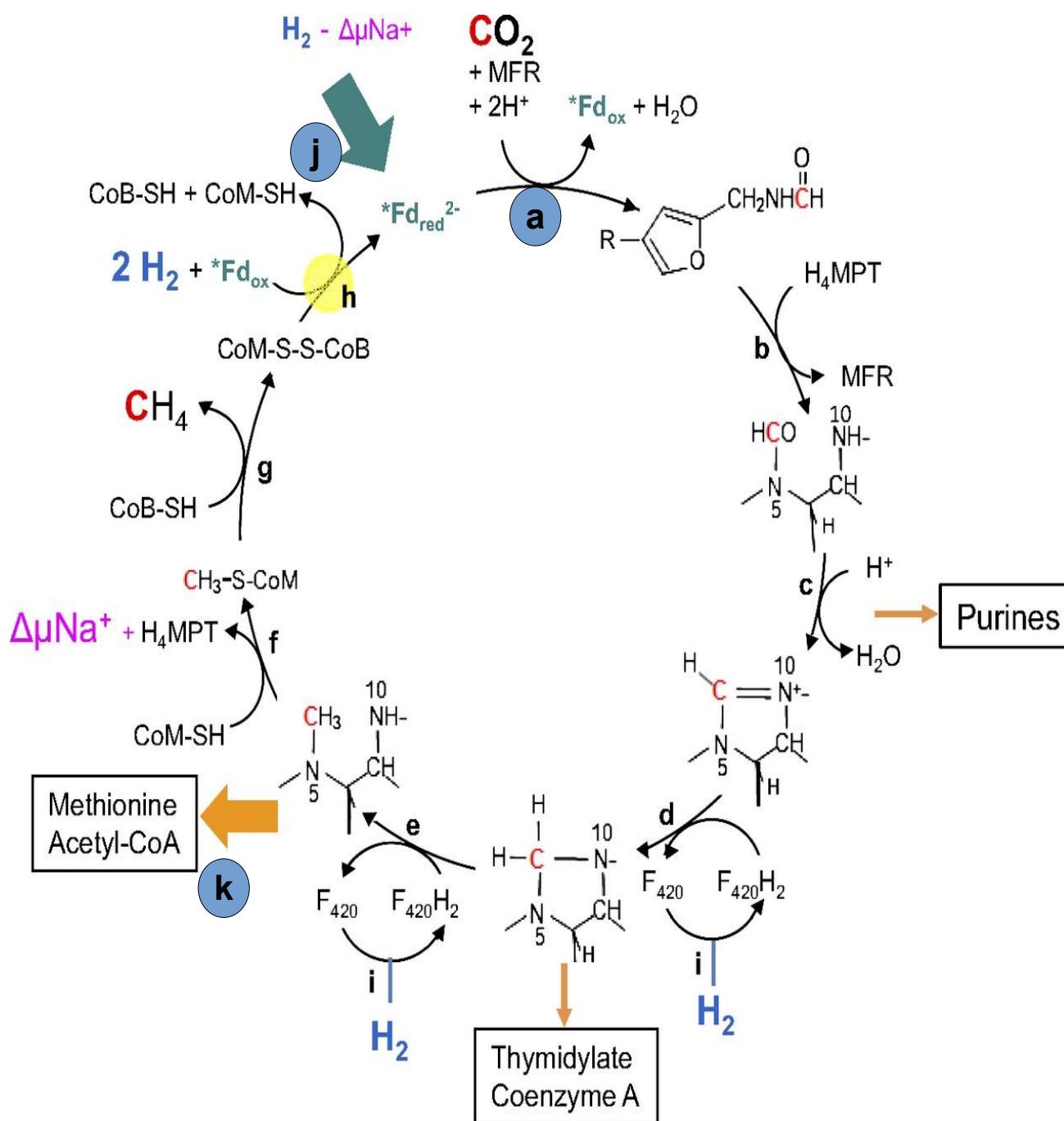
In its essence, it is a cyclization of the reductive Acetyl-CoA pathway. The nature of the cycle was confirmed only in 2012 by Lie and colleagues

Wolfe Cycle reduces CO<sub>2</sub> to methane with 4 H<sub>2</sub> in hydrogenotrophic methanogenic archaea

The reduction of CO<sub>2</sub> 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)

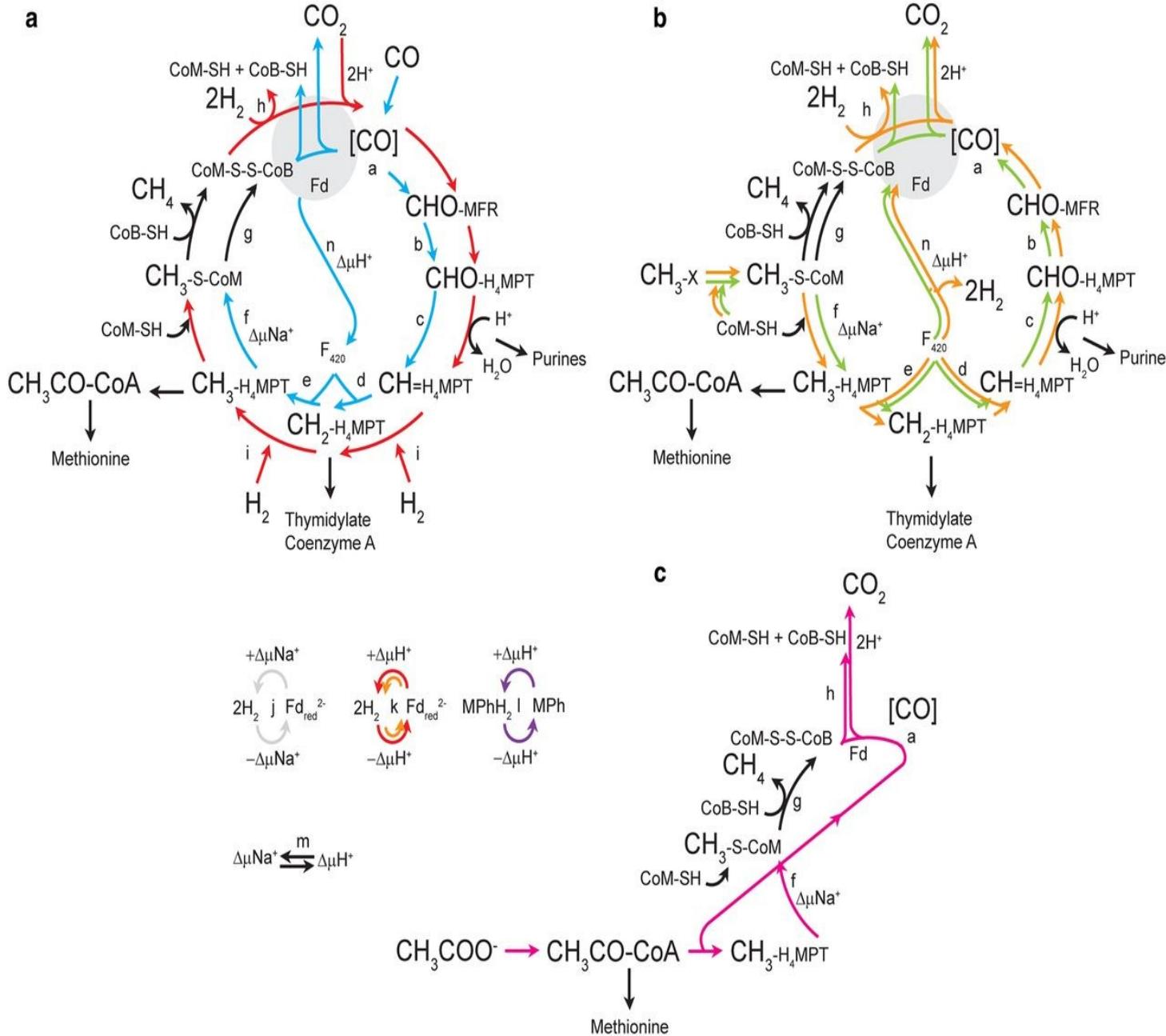
The sodium motive force-driven reduction of ferredoxin with H<sub>2</sub> catalyzed by the energy-converting hydrogenase EhaA-T is used to replenish the lost intermediates

# 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  $\text{H}_2$
- 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<sub>2</sub> 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

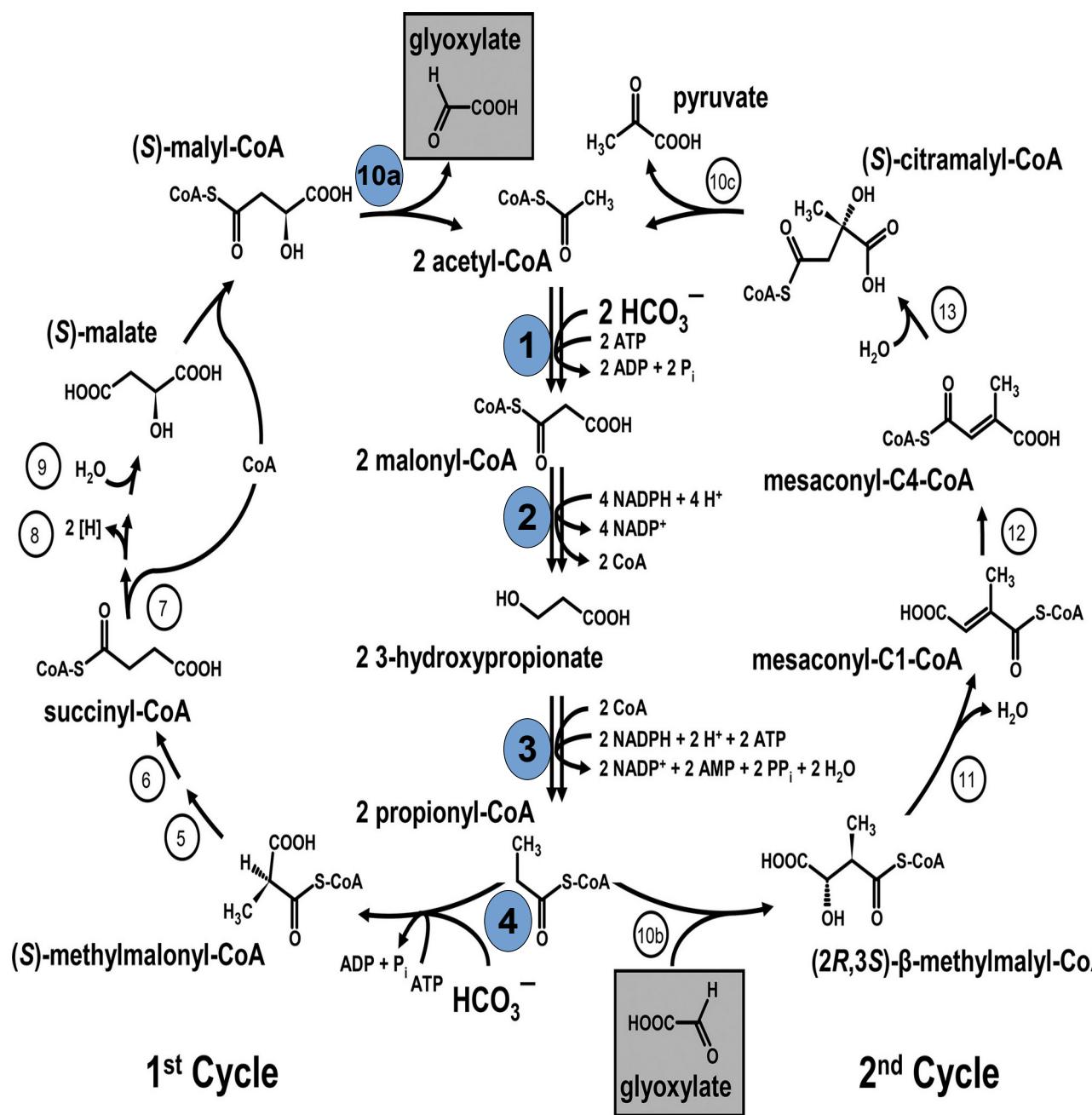
The two connected cycles generate two free characteristic intermediates of this bicycle: 3-hydroxypropionate and (S)-mallyl-CoA

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)-methylmalyl-CoA (a)/<sup>N</sup><sub>L</sub>-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 CO<sub>2</sub> fixation cycles recently described in Crenarchaeota

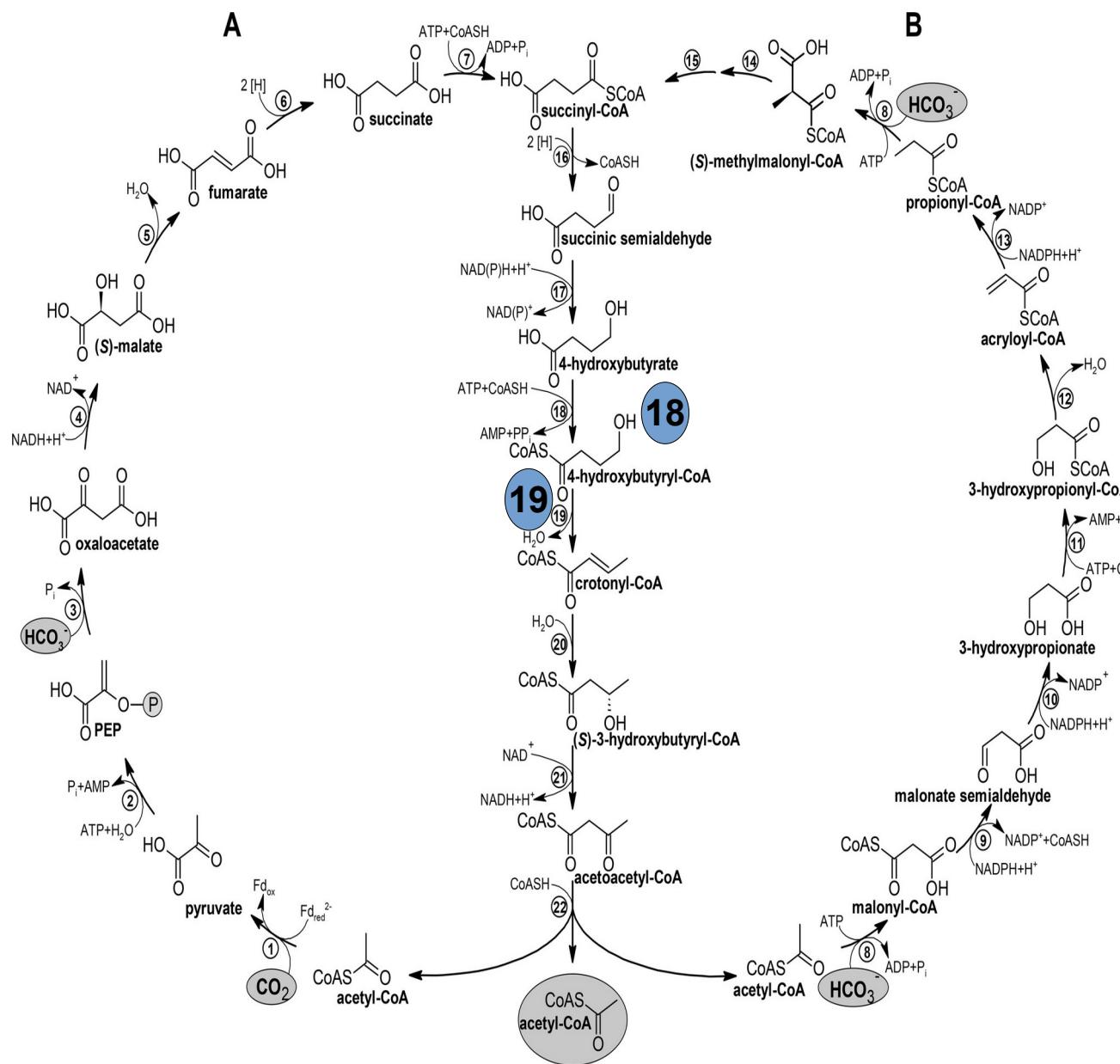
In both cycles (named together as the 4-hydroxybutyrate cycles), acetyl-CoA and two inorganic carbons are converted to succinyl-CoA, although this is accomplished with different carboxylases

The two variants function in Sulfobolales 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<sub>L</sub>-ketothiolase

# Mixotrophy: Reversible TCA cycle

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

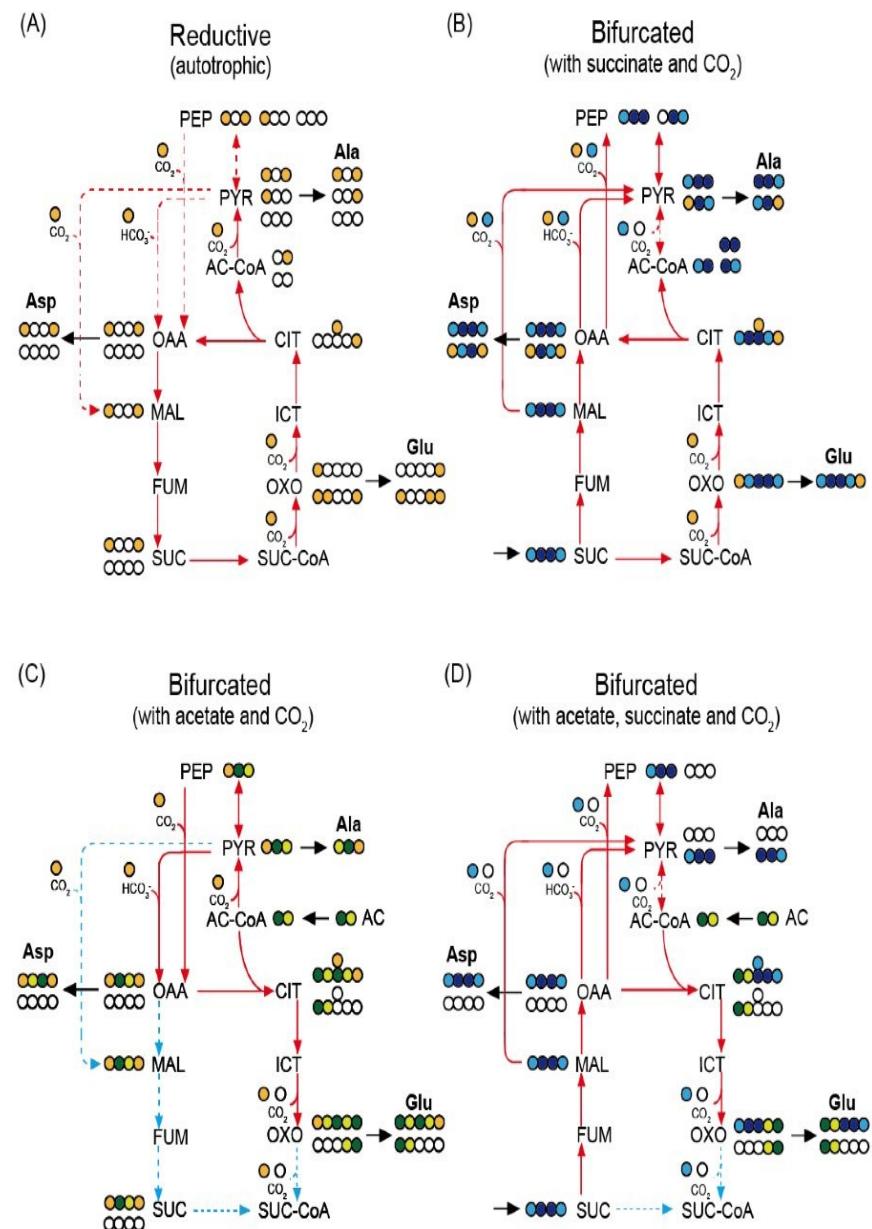
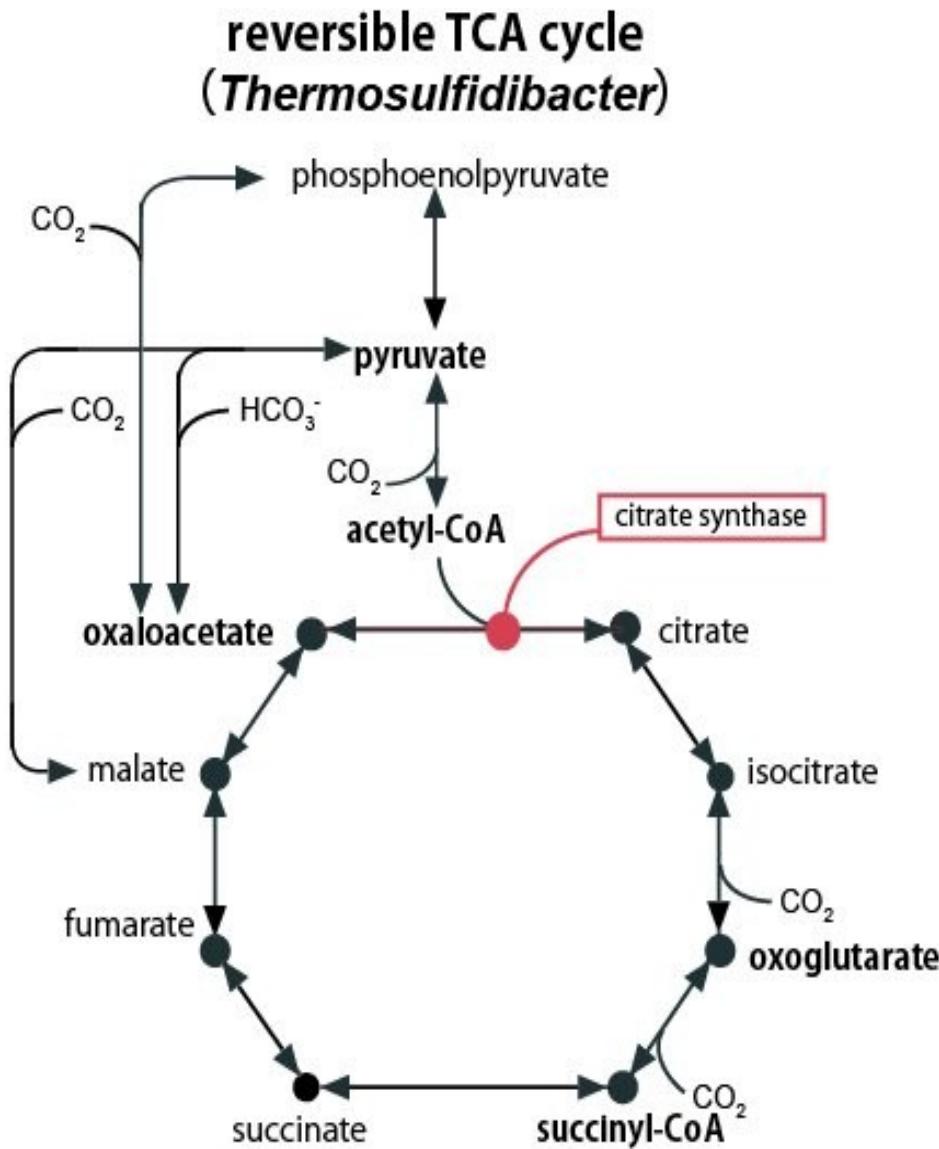
The switch from the oxidative to the reductive version of the TCA cycle had been considered impossible in the absence of specific carboxylases and ATP-consuming reactions (ATP citrate lyase)

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



● bicarbonate

● succinate

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

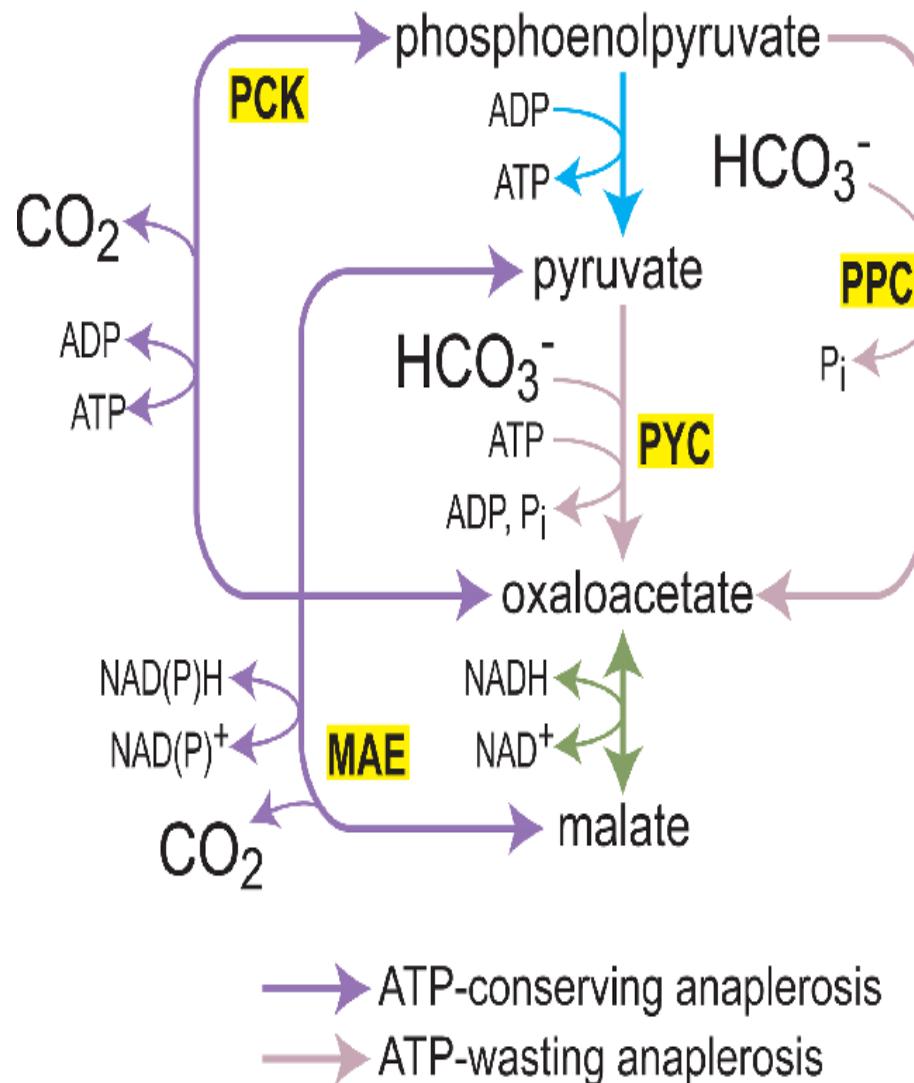
Anaplerotic reactions relevant to carbon fixation are generally carried out by carboxylase enzymes, and are present both in autotrophs and heterotrophs, that form part of their central and peripheral metabolic pathways

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

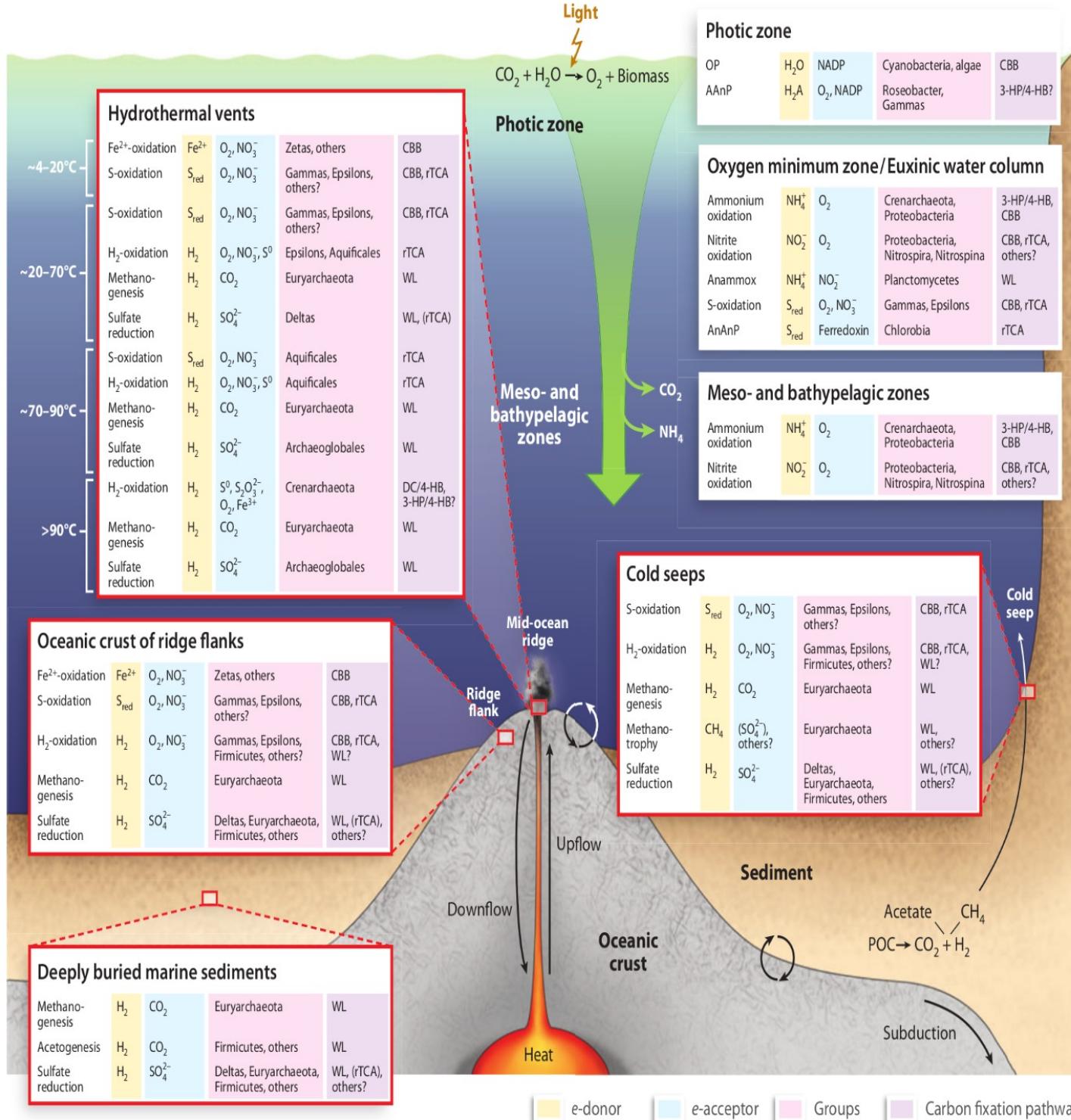


# Carbon Fixation Pathways: Ecology and Distribution

Pathway	Distribution
Calvin Cycle	Plants, algae, Cyanobacteria, most aerobic or facultative aerobic chemolithoautotrophic Bacteria
rTCA Cycle	Chlorobiales, Aquificae, Epsilonproteobacteria, some Deltaproteobacteria, few Alphaproteobacteria ( <i>Magnetococcus</i> ), one Gammaproteobacteria, Nitrospirae
rAcetyl-CoA	Methanogenic and sulfate reducing Euryarchaeota, acetogenic Firmicutes, some Spirochaetes, many Deltaproteobacteria, Annamox 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



# **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*

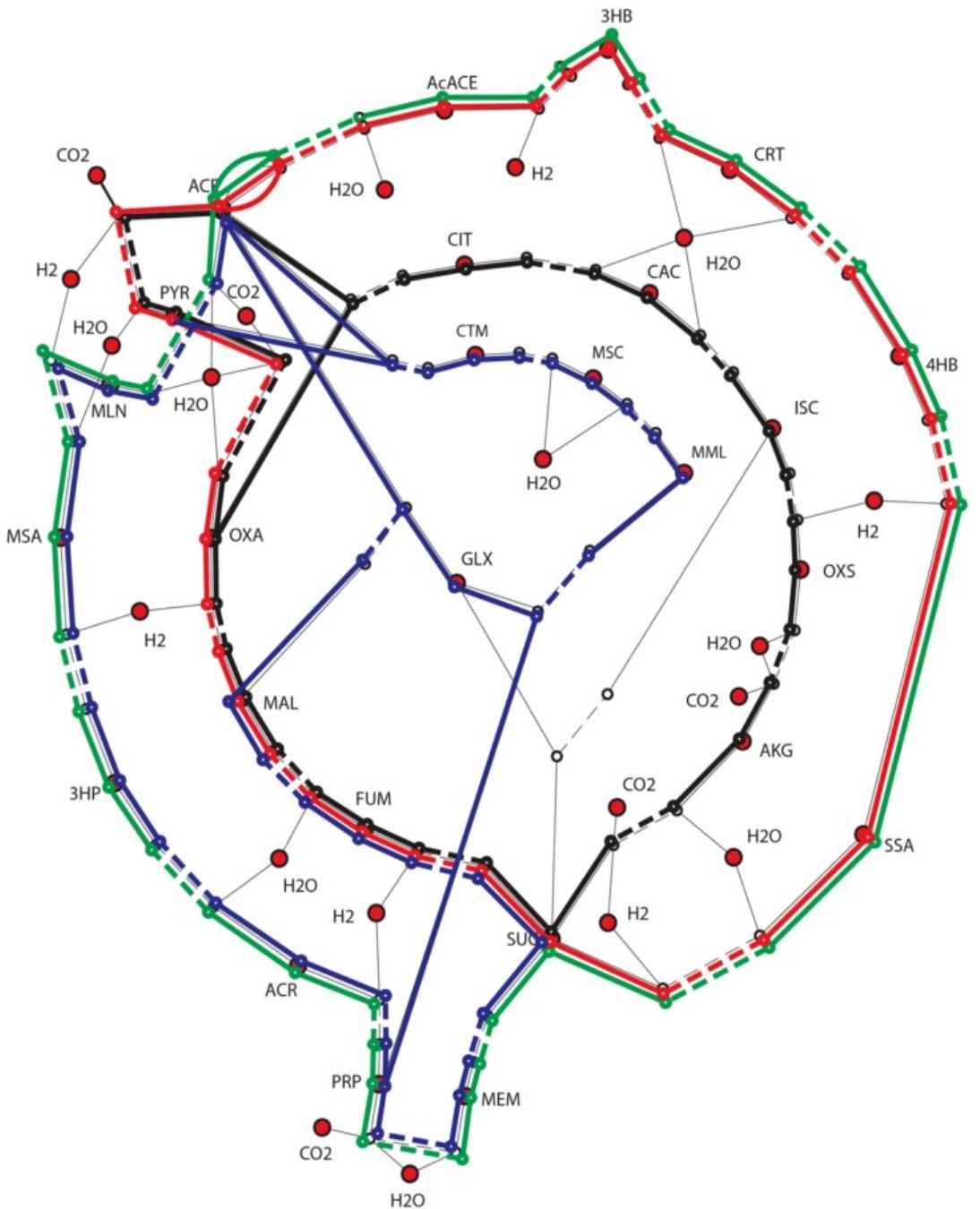
*It is possible to identify modules, composed either of subsets of chemicals and reactions, subsets of functions, with a conserved internal structure*

*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

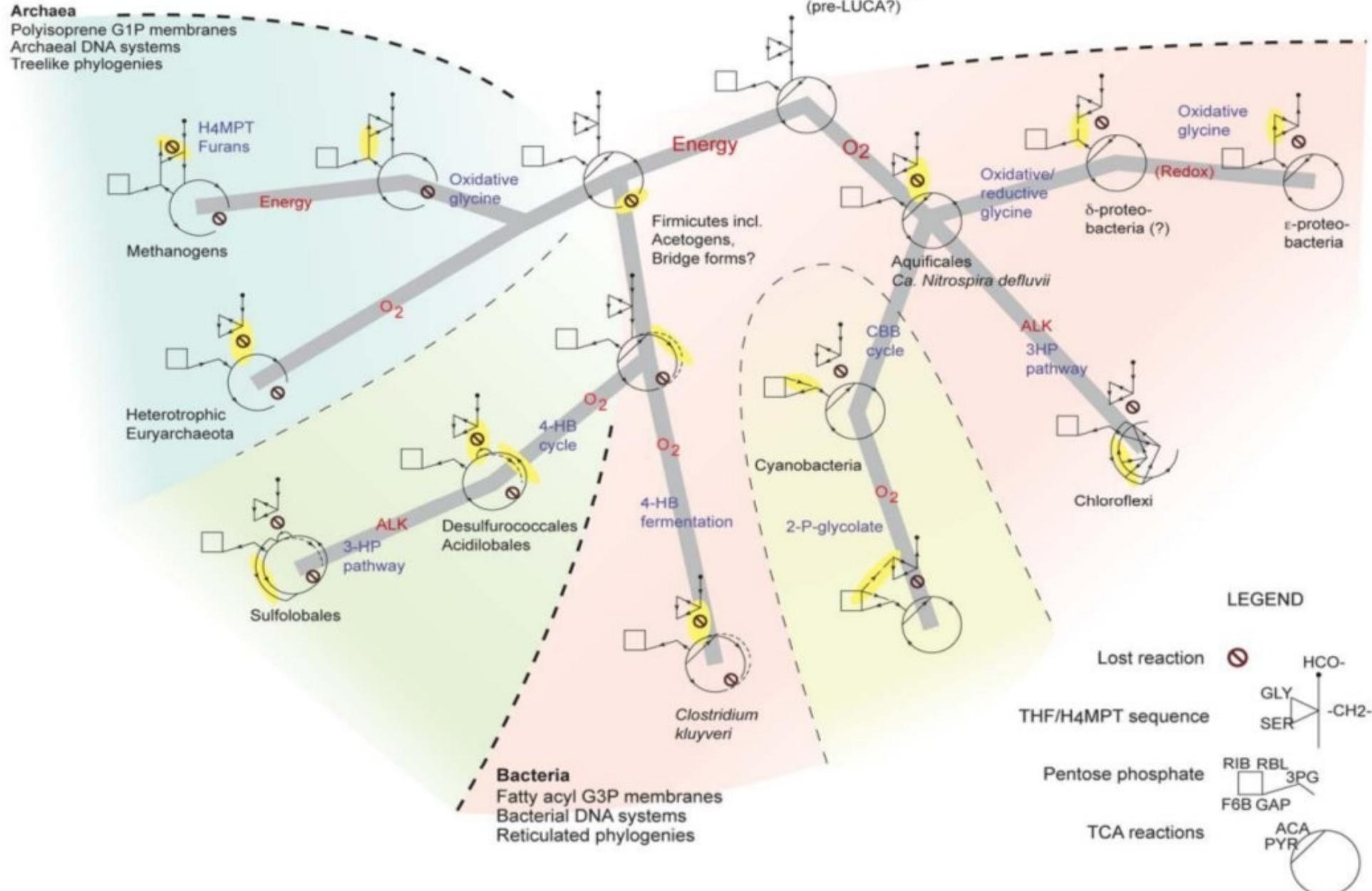
rTCA is black, DC/4HB is red, 3HP-bicycle is blue, and 3HP/4HB is green

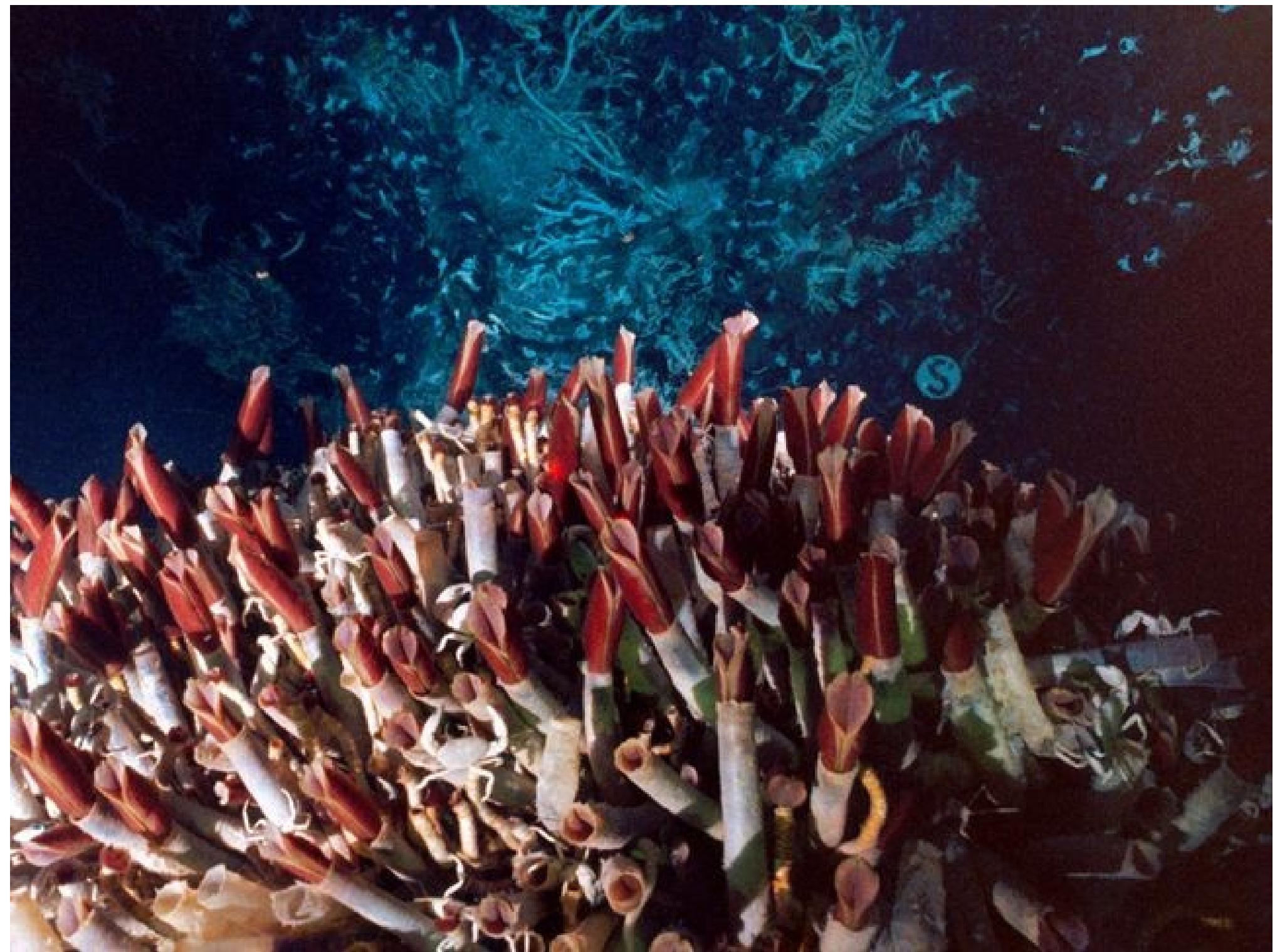
The module-boundary nature of acetate (ACE) and succinate (SUC) is shown by the intersection of multiple paths in these compounds

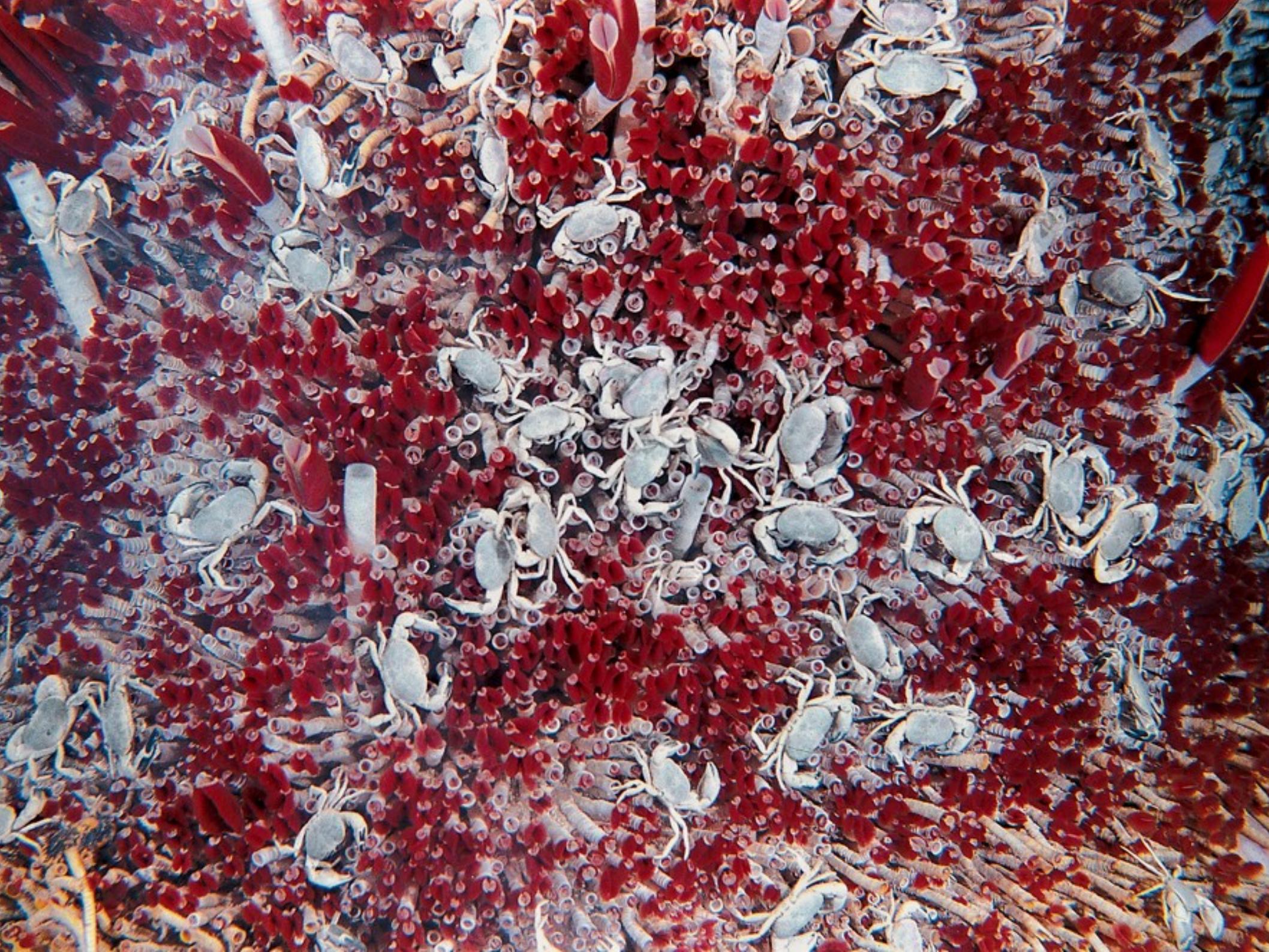
Radially aligned reactions are homologous in local-group chemistry; deviations from strict homology in different pathways appear as excursions

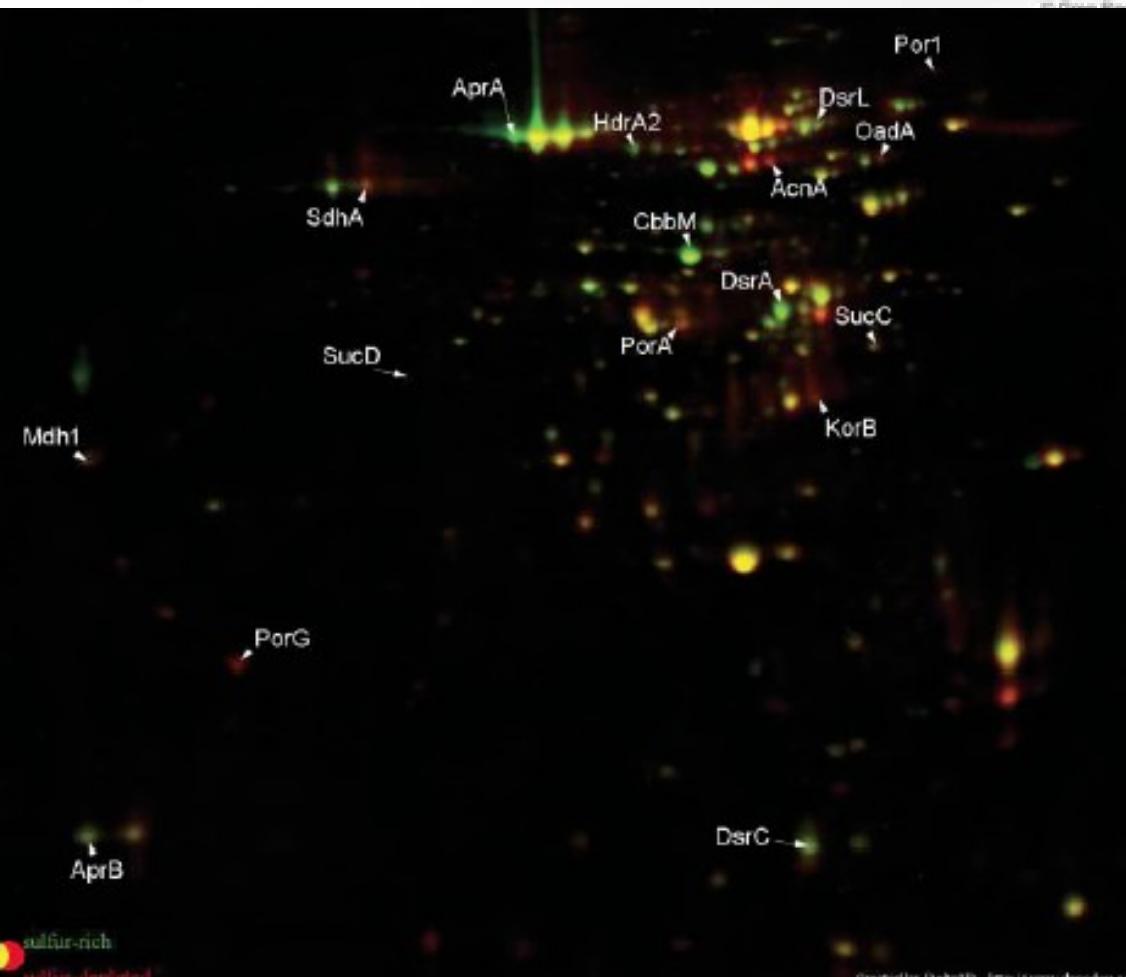
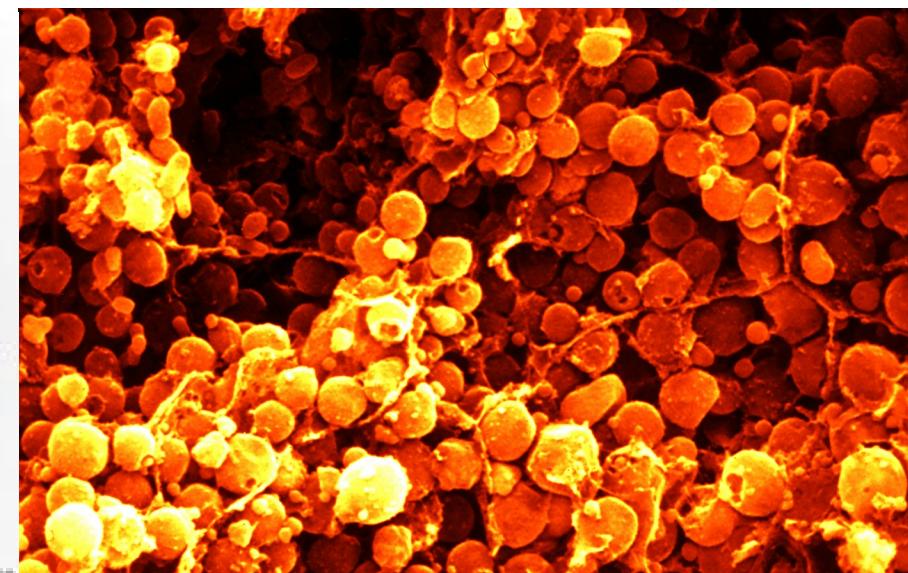
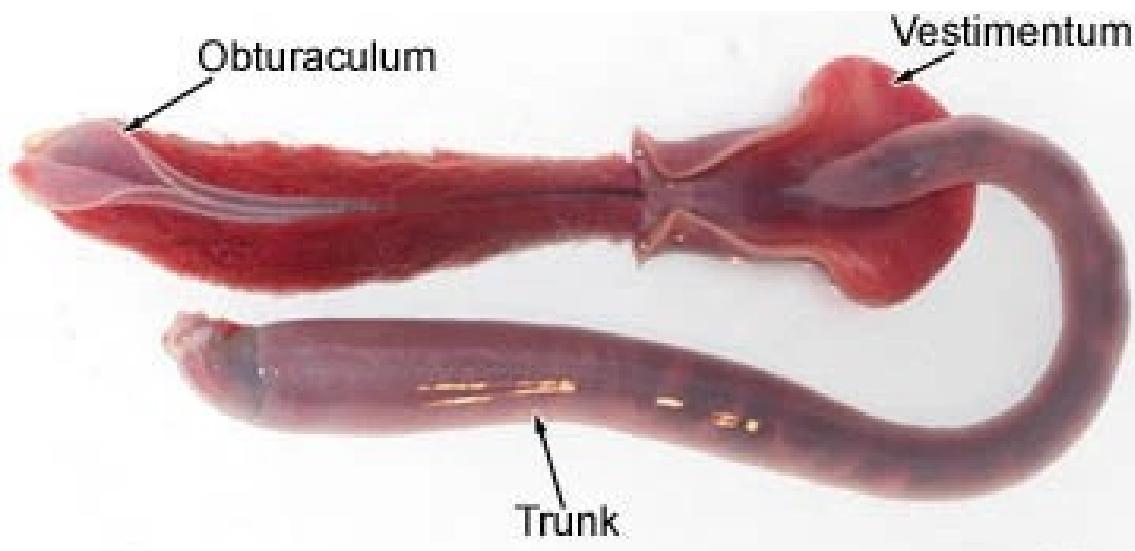
Brackman and Smith 2013 Phys Biol

# Emergence of Carbon Fixation Pathways



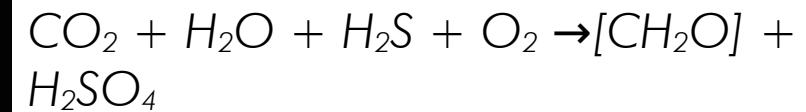






## Riftia pachyptila symbiont

Thiotrophic symbionts



Phylum **Proteobacteria**

Class **Gammaproteobacteria**

Can use either the **Calvin-Benson** cycle and the **rTCA** cycle in response to different sulfide levels in the environment

Markert et al., 2007 Science

# *This week reads*

Berg, I. A. (2011). *Ecological Aspects of the Distribution of Different Autotrophic CO<sub>2</sub> Fixation Pathways*. *Appl. Environ. Microbiol.* 77, 1925–1936. doi:10.1128/AEM.02473-10.

Hügler, M., and Sievert, S. M. (2011). *Beyond the Calvin Cycle: Autotrophic Carbon Fixation in the Ocean*. *Annual Review of Marine Science* 3, 261–289. doi:10.1146/annurev-marine-120709-142712.