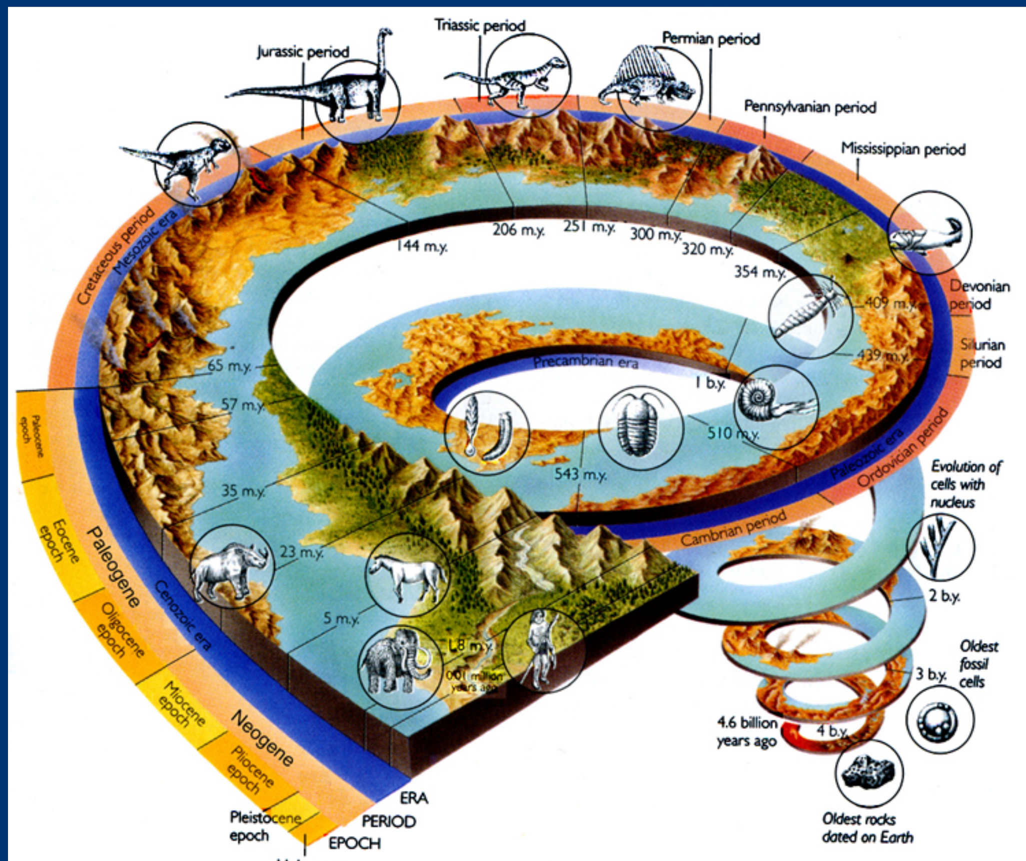
# The Solar Supercharge: Using Systems Biology to Reconstruct how the Invention of Photosynthesis Transformed the Biosphere




Adapted from Press & Siever, 2000, Understanding Earth

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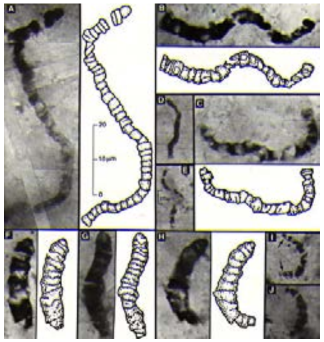
# The importance of being interdisciplinary: integrated knowledge to reconstruct Earth's biogeochemical history

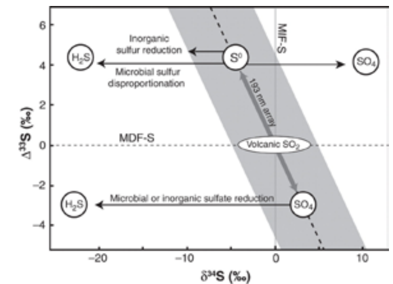
- Geology/geochem: fossil/rock record, isotope analysis

Apex chert microfossils



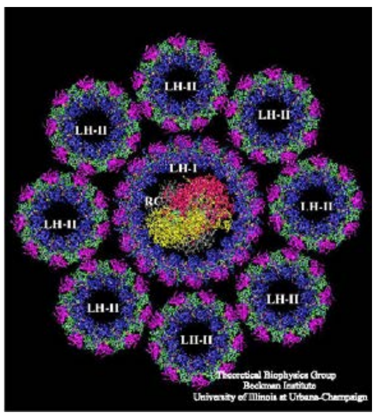
Fossilized stromatolite cross section

Isotope analysis of ancient rocks/minerals

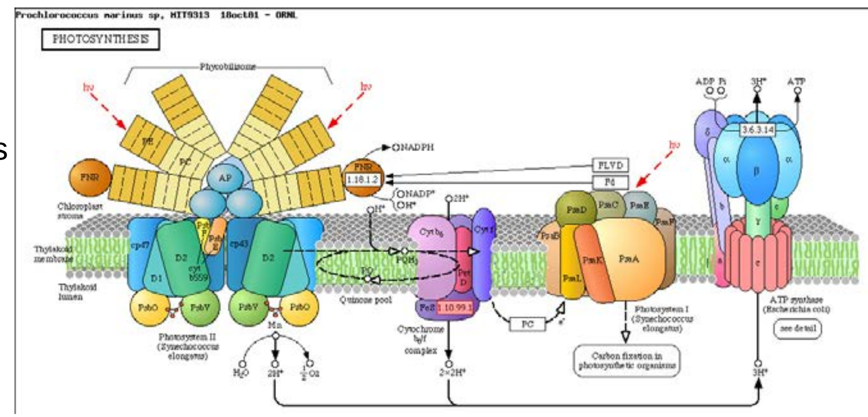



- Biochemistry: protein structure and function, metabolism

3D structures of purple bacterial reaction center/light harvesting complexes

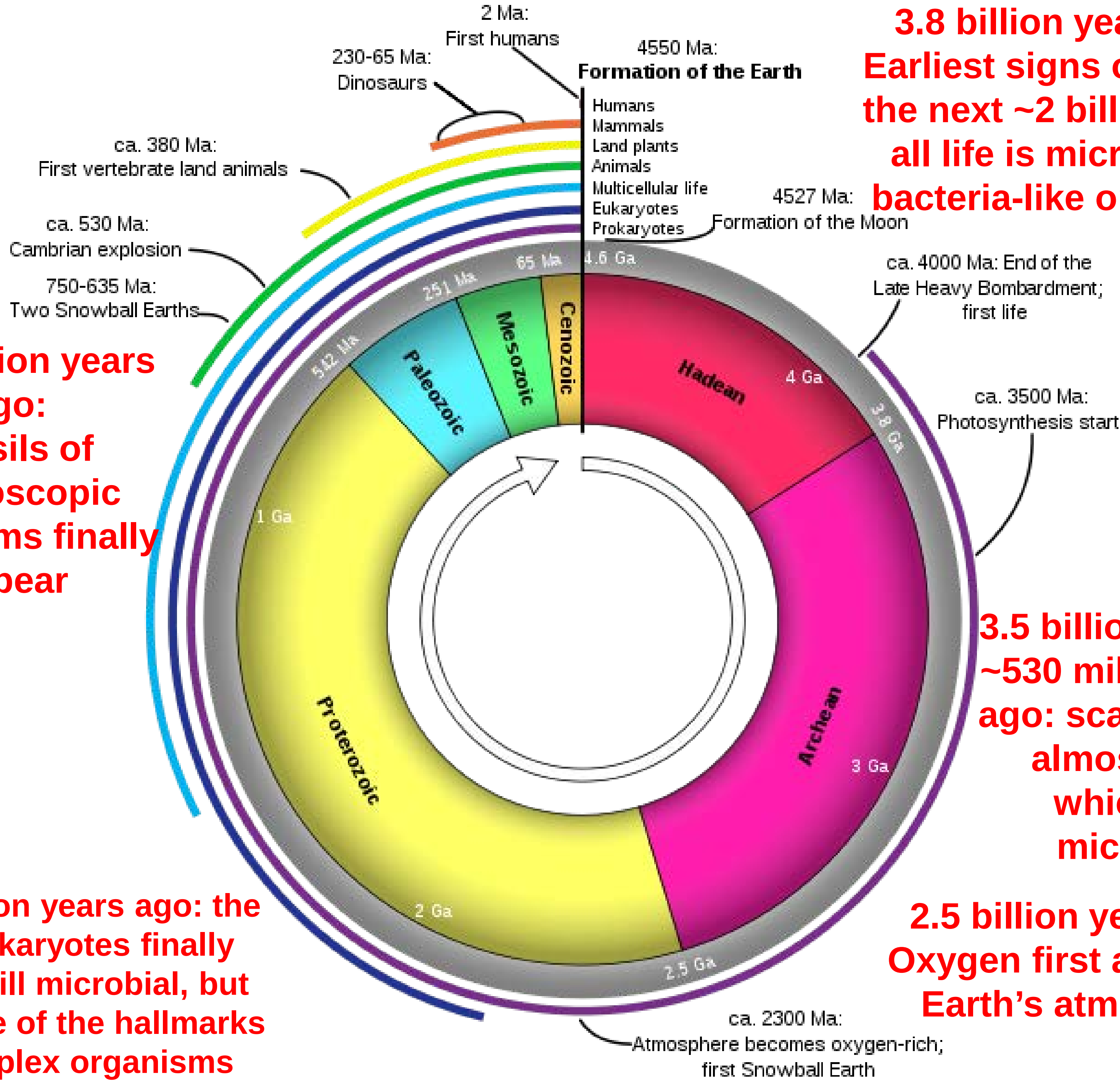



Protein complexes involved in photosynthetic electron transport




- Genomics/evolution: sequence comparisons and tree building, comparative and functional genomics

**3.8 billion years ago: Earliest signs of life. For the next ~2 billion years, all life is microscopic bacteria-like organisms**




**530 million years ago: Fossils of macroscopic organisms finally appear**

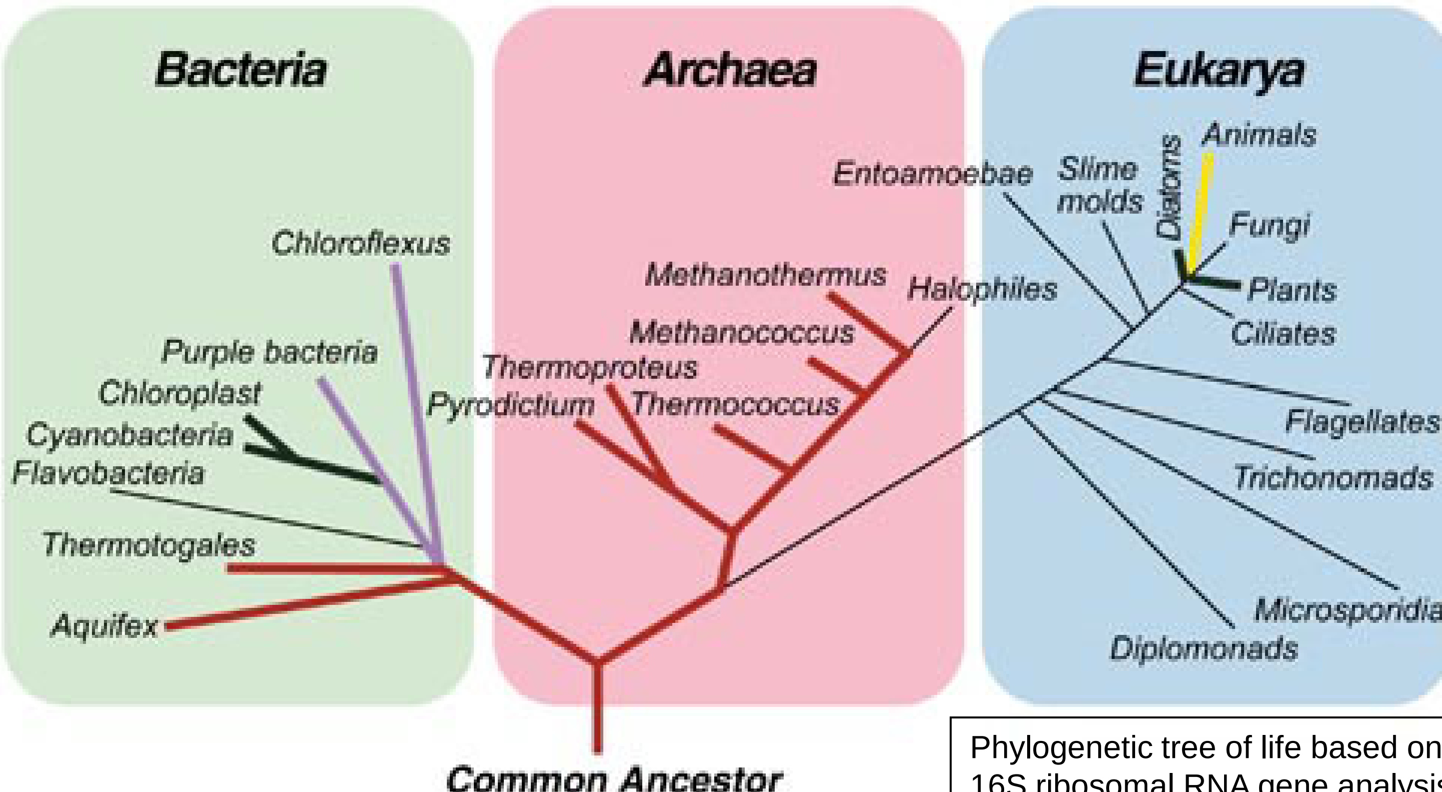
**3.5 billion through ~530 million years ago: scant fossils, almost all of which are microbial**

**~1.9 billion years ago: the first eukaryotes finally arise; still microbial, but has some of the hallmarks of complex organisms**

**2.5 billion years ago: Oxygen first appears in Earth's atmosphere**

# The genealogy of life

- The 'five kingdom' model has been replaced by the three domain (bacteria, archaea, eukarya) tree of life
- This phylogenetic tree is based on models of how single genes (and more recently entire genomes) evolve through time
- ***We're inhabitants of a microbial world!*** Macroscopic, complex life occupies a few remote branches at the tip of domain Eukarya



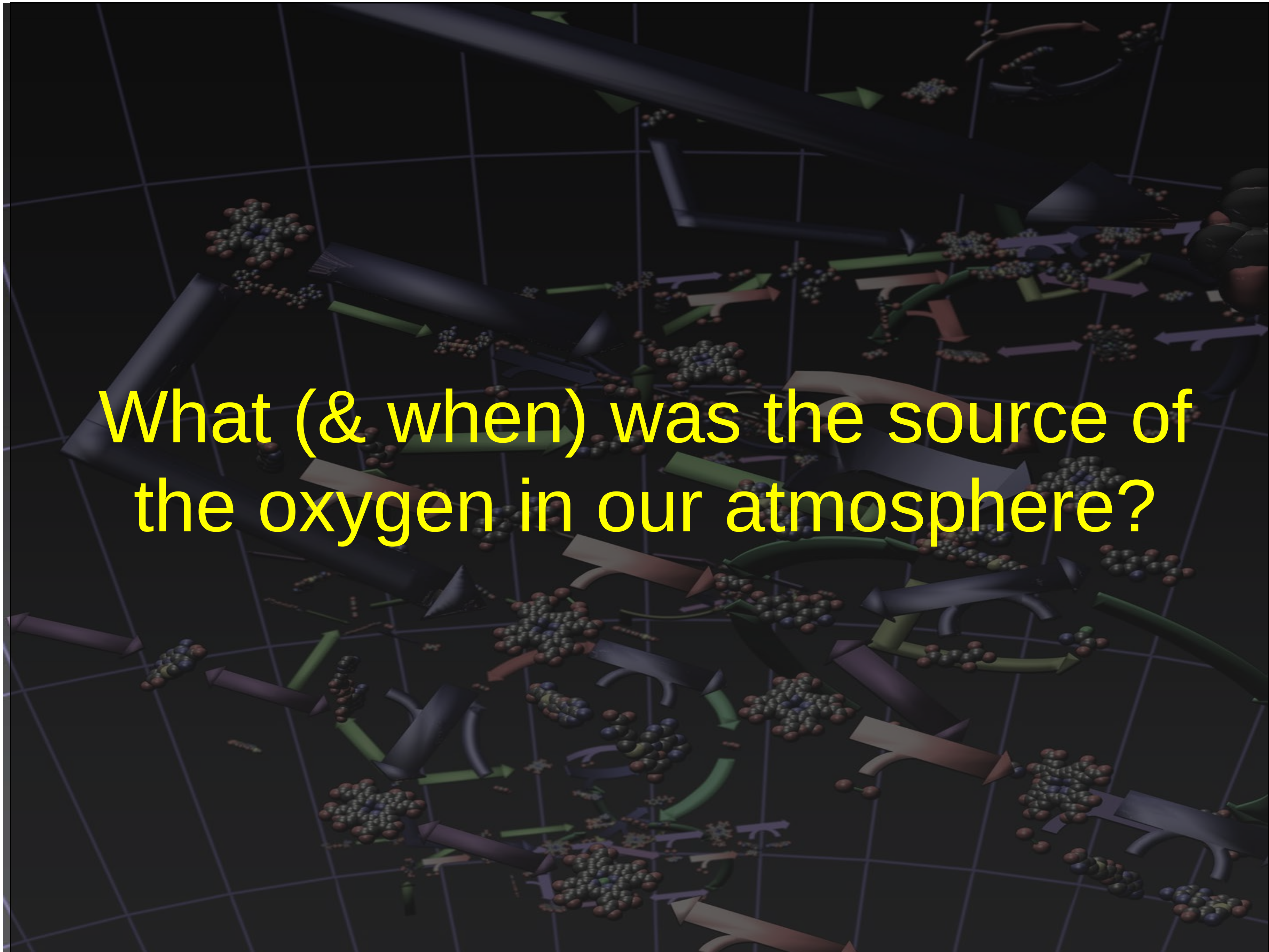

Phylogenetic tree of life based on 16S ribosomal RNA gene analysis

# The key role of oxygen ($O_2$) for (complex) life on our planet

- Complex life as we know it is impossible without an oxygenated atmosphere
- Furthermore, the history of oxygen illustrates that the evolution of life is inextricably linked to the evolution of our planet

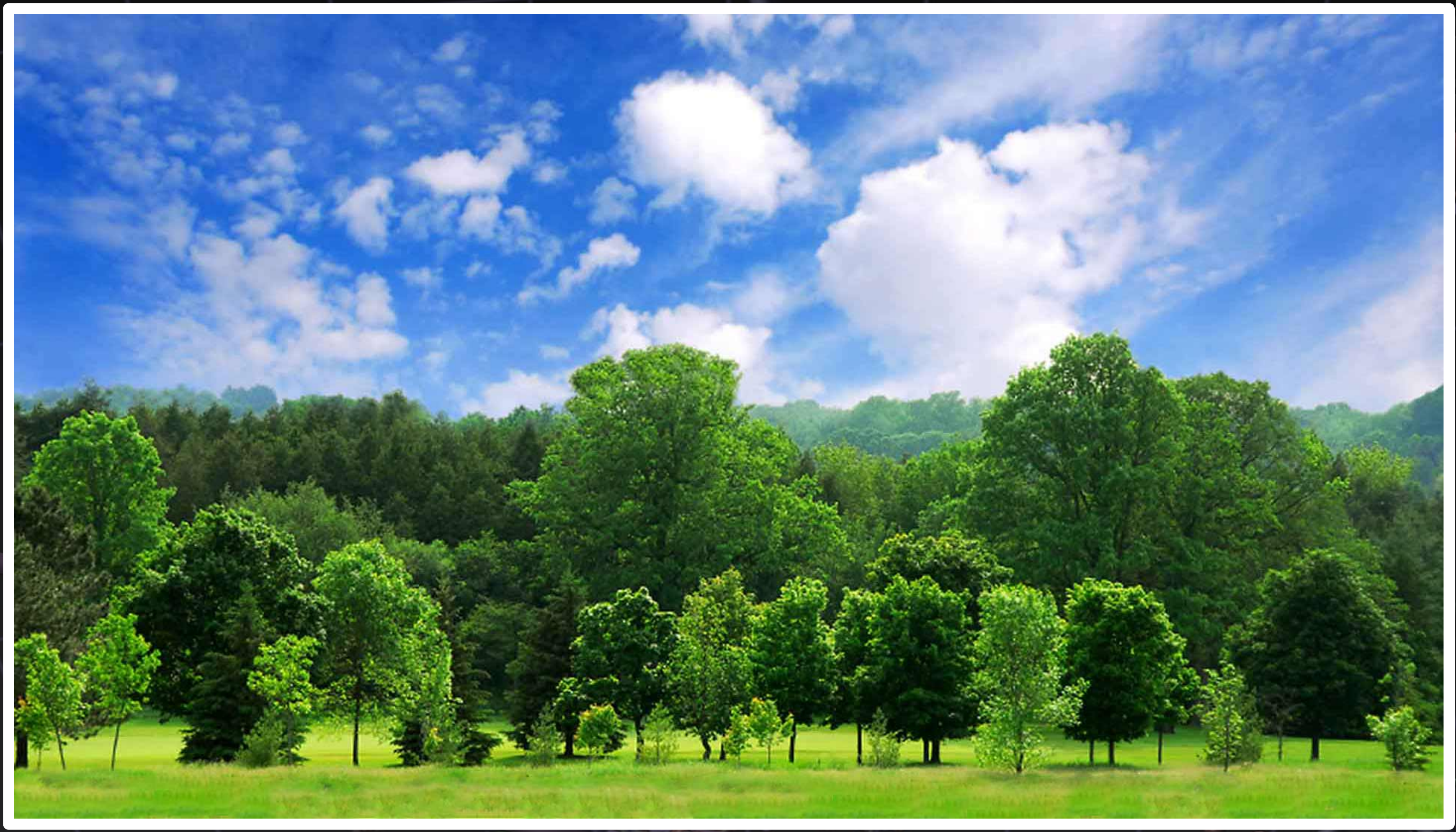
Driving questions:

- What/when was the origin of atmospheric oxygen?
- What was Earth/life like before $O_2$? How did $O_2$ change the biosphere and the planet?
- The oxygen imperative: is oxygen a reliable/necessary proxy for complex life to arise elsewhere in the universe?

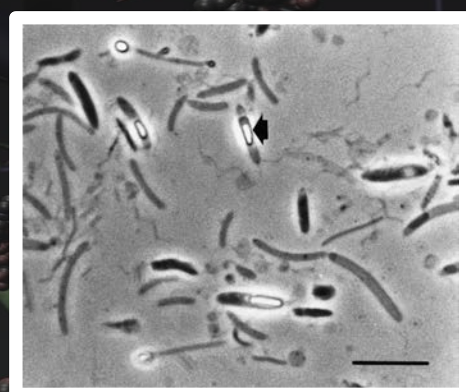


# What (& when) was the source of the oxygen in our atmosphere?

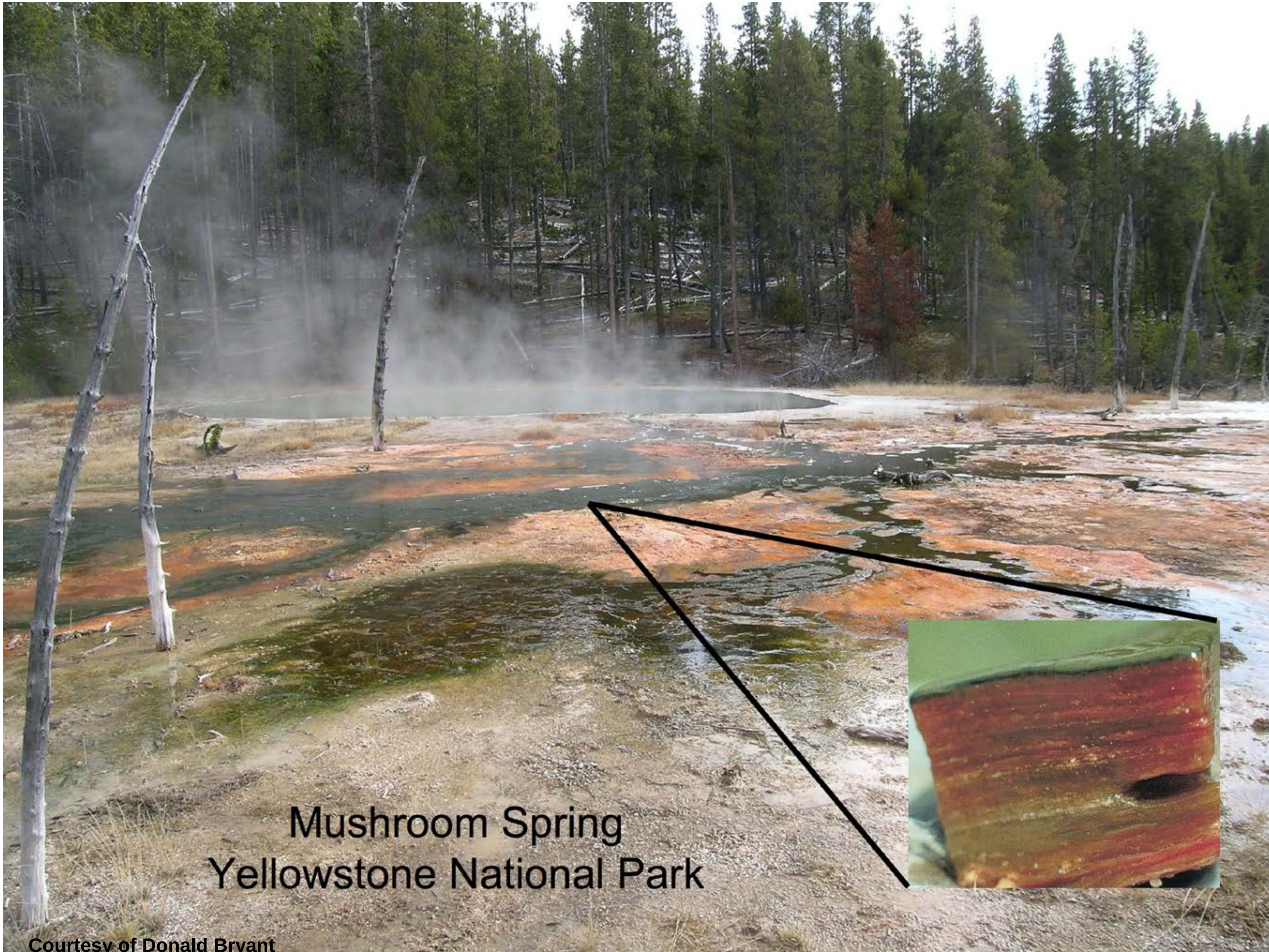
*When we think of photosynthesis and its biodiversity, we tend to think macroscopically: trees, forests, grasslands, algae, corals*





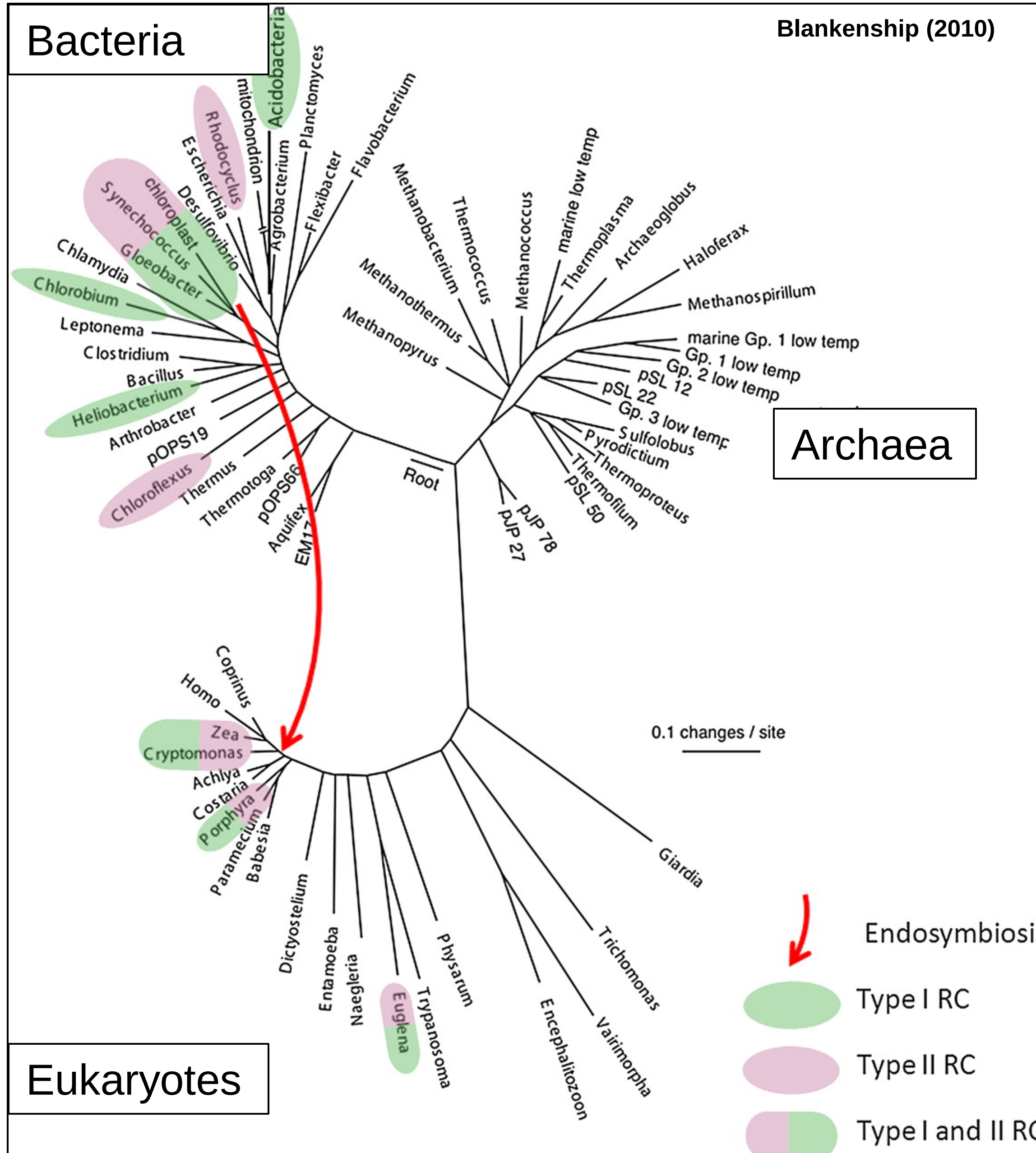


*However, <u>photosynthesis is a bacterial process</u>; not only was it invented in bacteria (and later 'borrowed' by plants/algae) but it is much more diverse in microbes*


Mushroom Spring
Yellowstone National Park
Courtesy of Donald Bryant

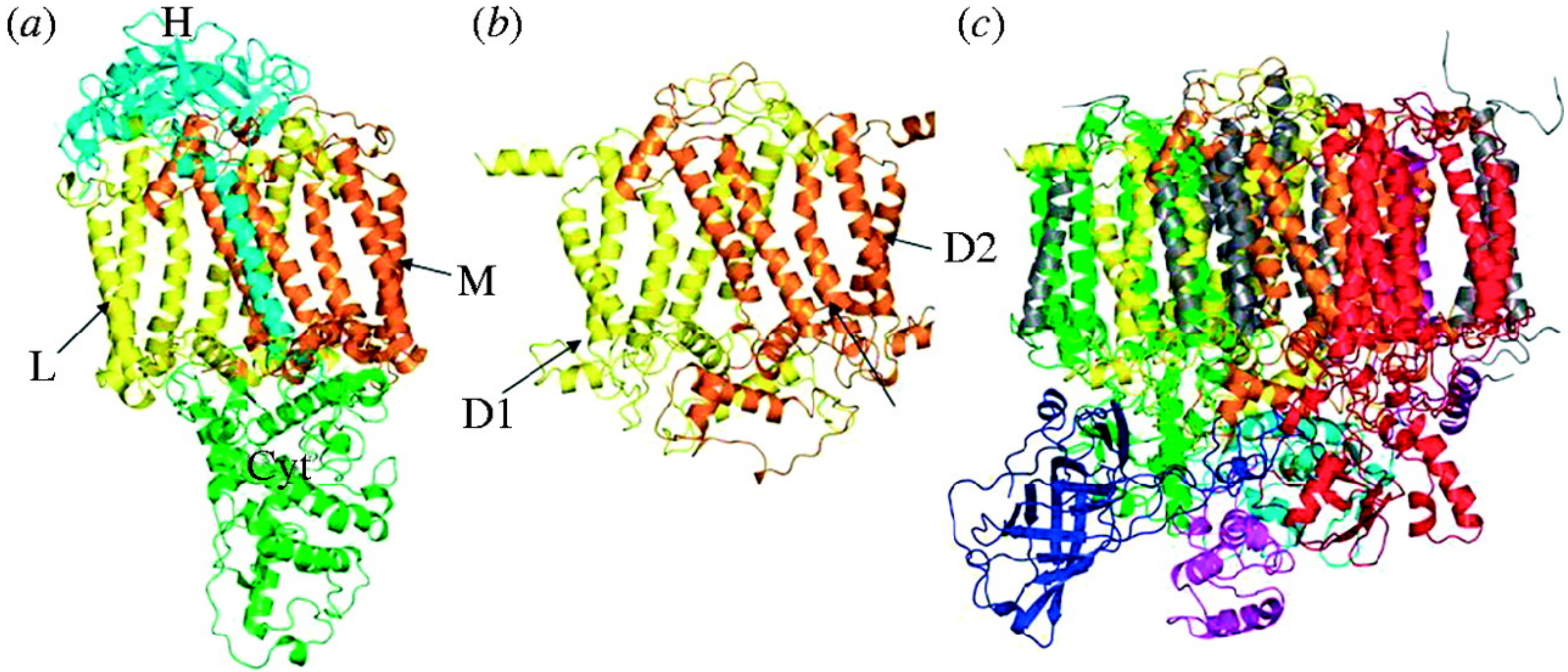
# Photosynthesis is a bacterial process

- Six different phyla of bacteria are known to have the process
- Only one bacterial phylum (Cyanobacteria) has oxygenic photosynthesis
- Eukaryotes (plants and algae) acquired photosynthesis via endosymbiosis
- No reason to suspect that the earliest cellular life was photosynthetic



# *Photosynthesis was invented only once*

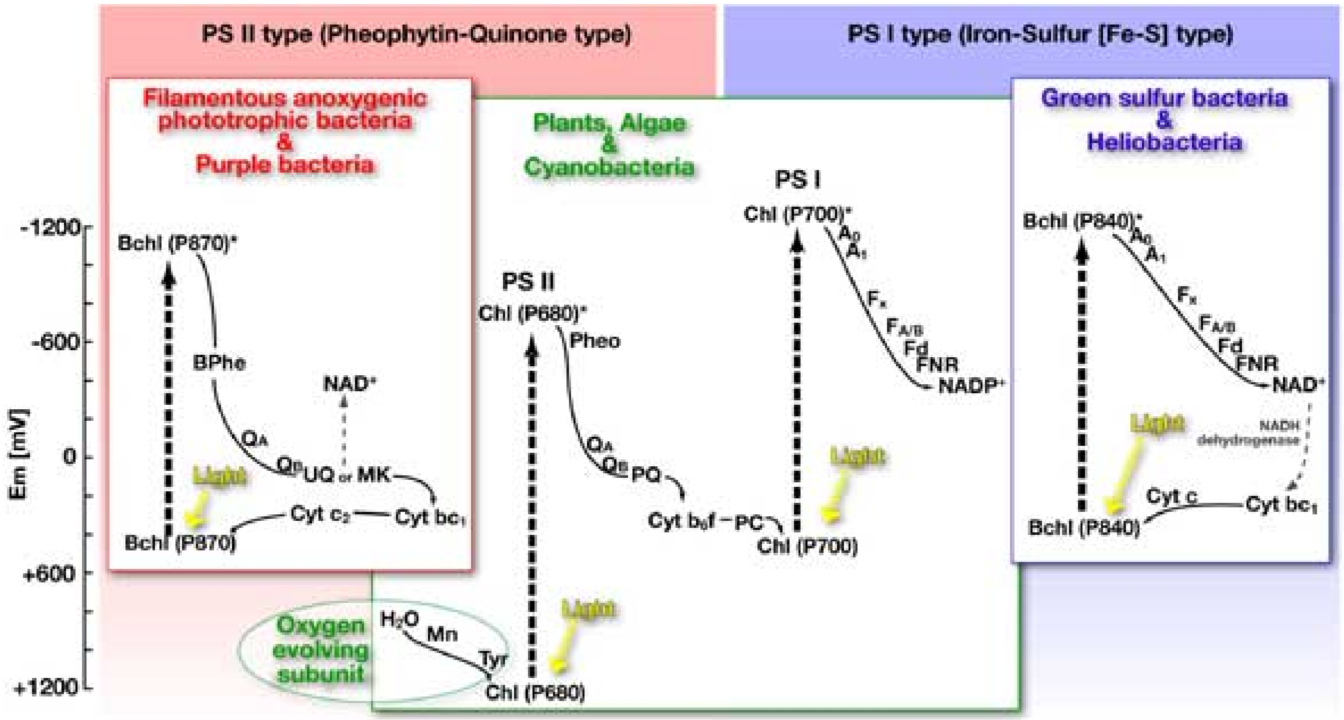
- Though the process is spread all over the tree of life (polyphyletic), all known photosynthetic organisms use variations on the same structural core (the so-called photosynthetic reaction center)



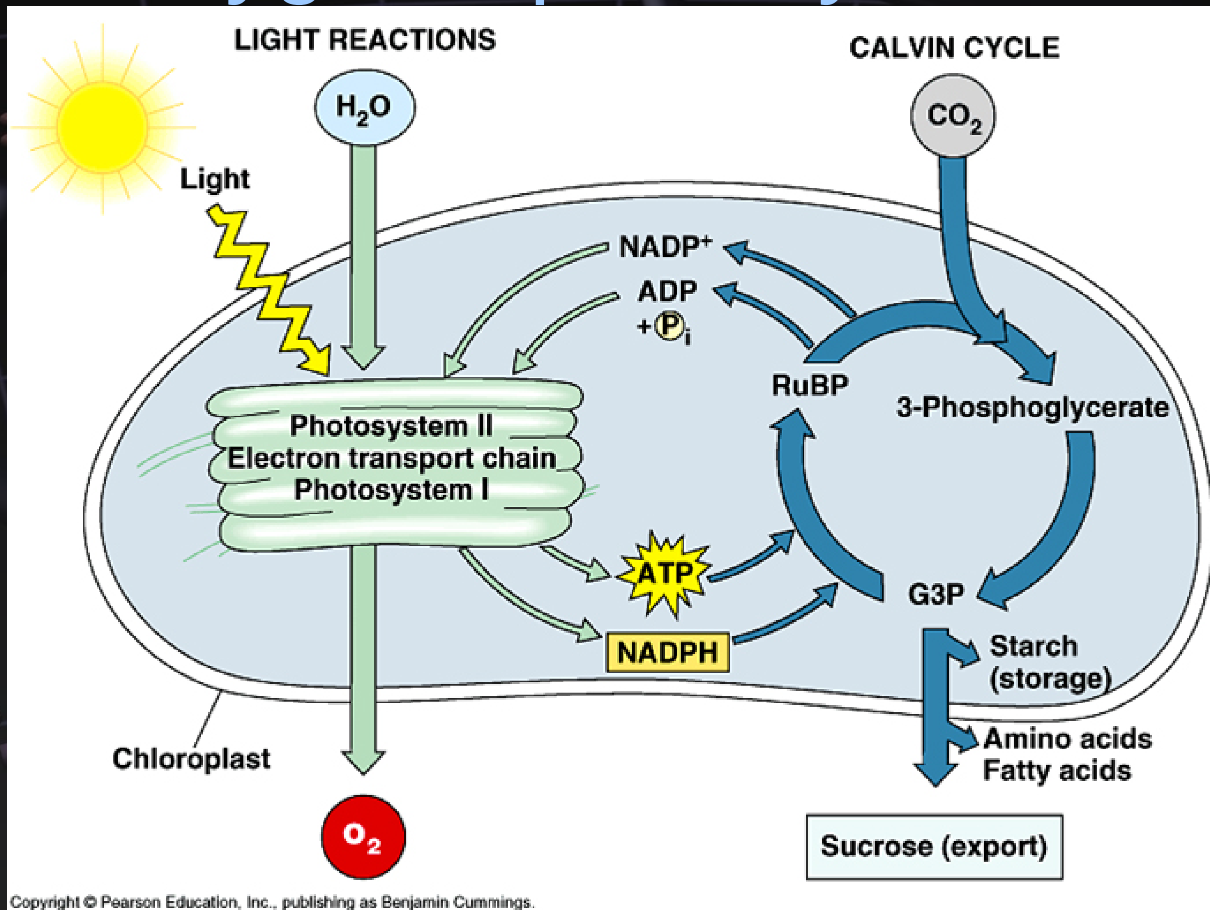

- The organisms that do oxygenic photosynthesis are unique in that they use two variations of this reaction center simultaneously (next slide)

# *Oxygenic photosynthesis evolved from anoxygenic photosynthesis*

- Supported by phylogenetic analysis of the proteins that are part of the photosynthetic machinery (*and there are lots!*)
- O2-photosynthesis is more complex: uses two reaction centers in concert to harvest extra solar energy. This energy is used to strip the electrons off of water
- Oxygen is the by-product of this chemical reaction

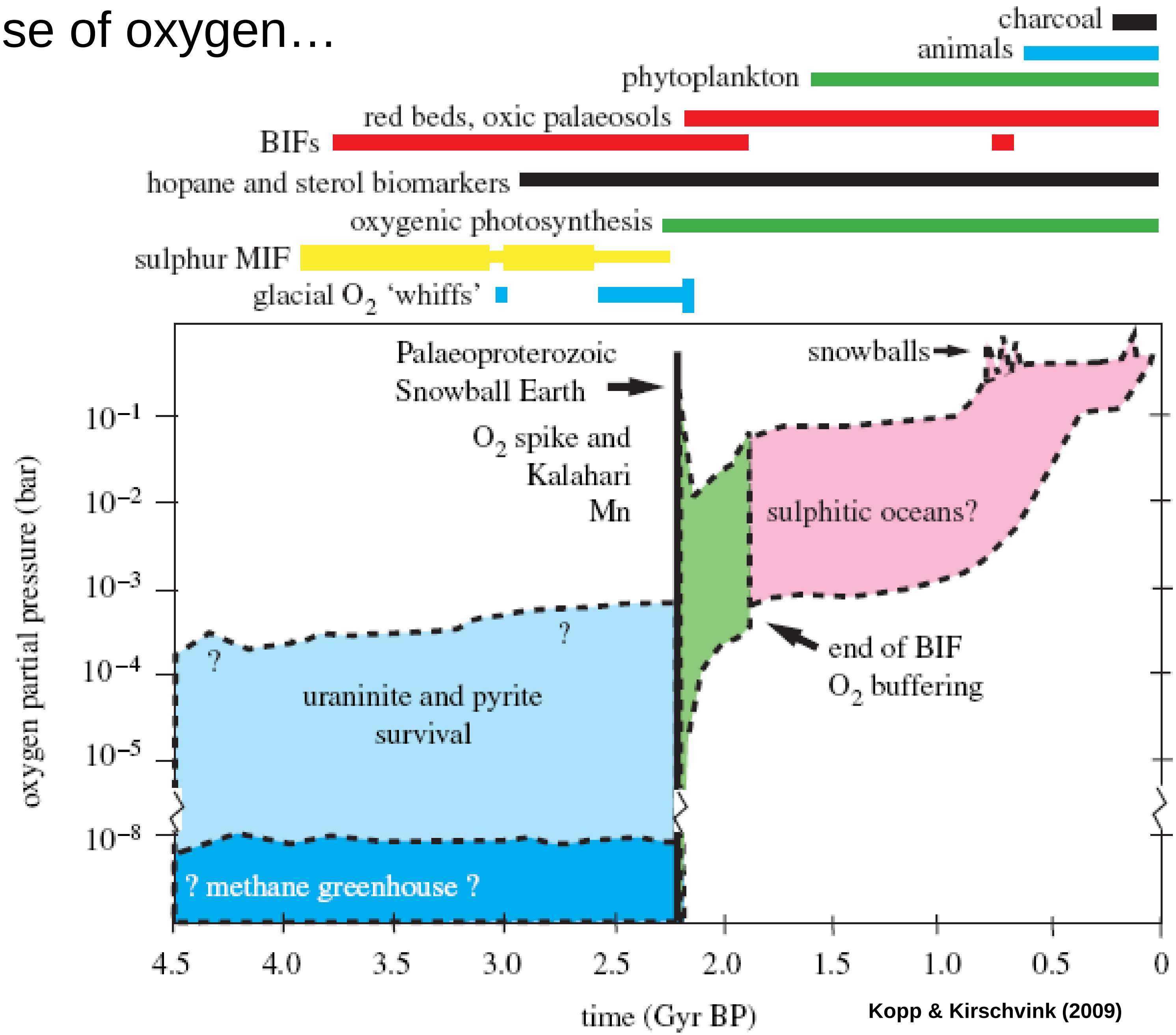


# The origin of atmospheric oxygen: oxygenic photosynthesis




*Oxygen is the by-product of using water as a source of electrons. The Earth's geochemical/geological record suggests this process is at least 2.2-2.5 billion years old*

# The rise of oxygen…

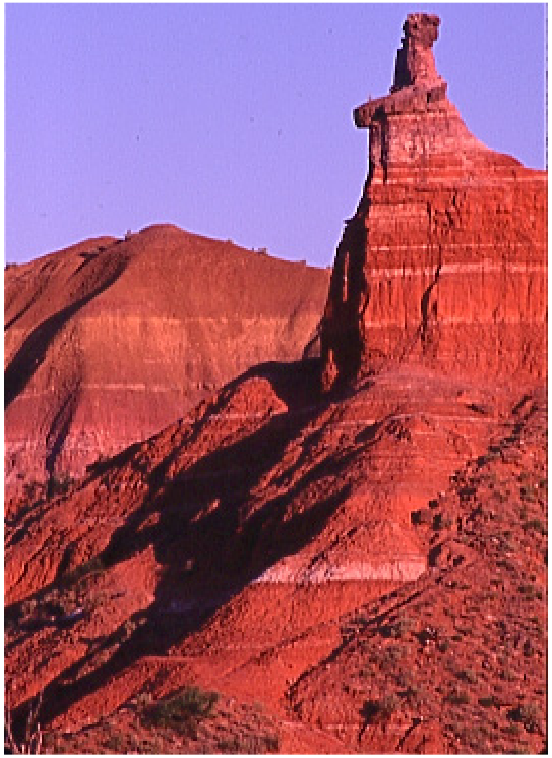



Kopp & Kirschvink (2009)

The Archaean Earth (<2.5 Gya) was essentially anoxic; O2 did not approach modern levels until around 800 million years ago

# The rise of $O_2$ beginning ~2.5 billion years ago left indelible signatures across the Earth

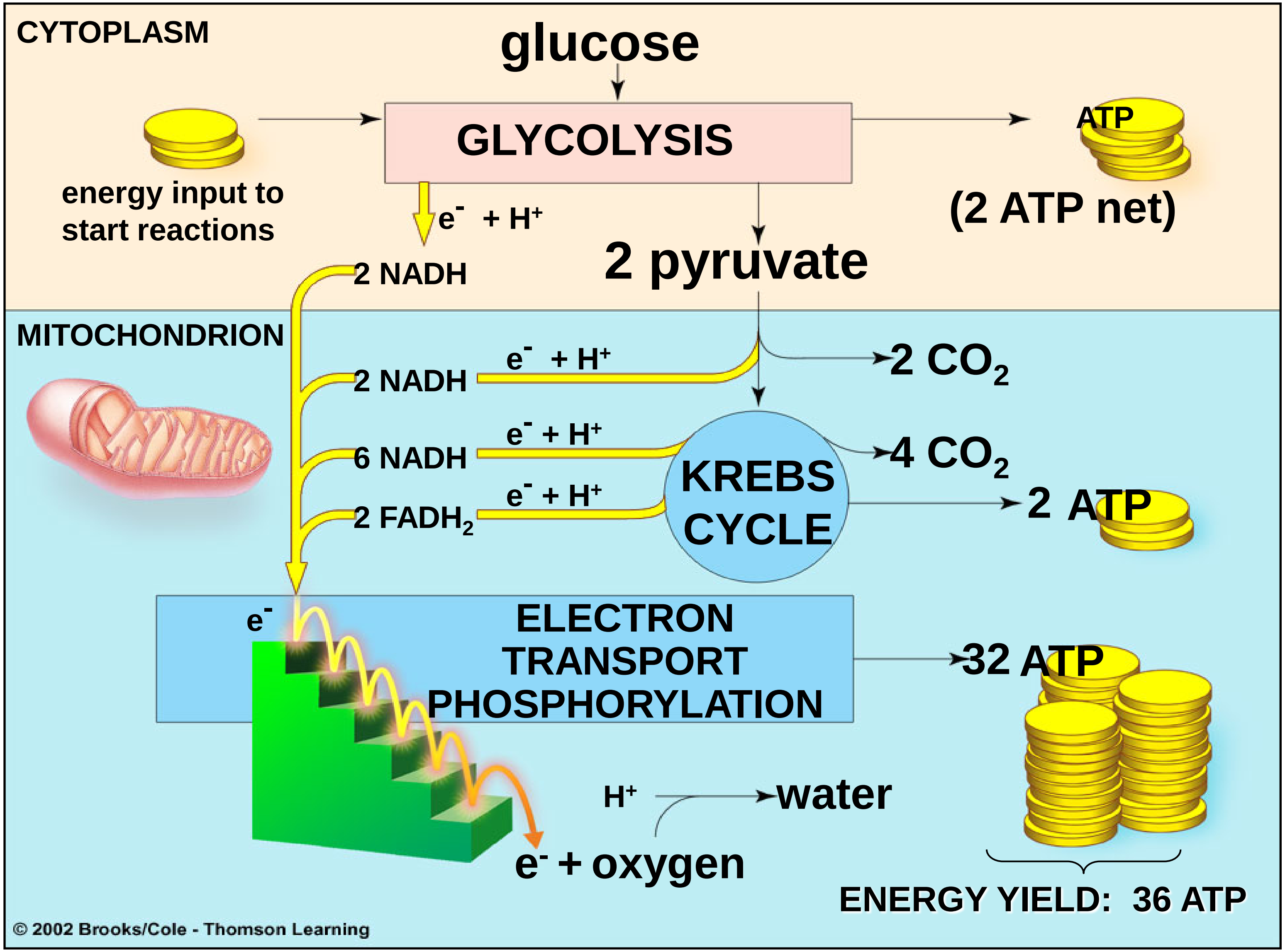
***Records of carbon, sulfur, molybdenum/molybdate, uraninite (oxidized uranium), hematite/magnetite (oxidized iron), pyrite, vanadate, …***



Oklo, Gabon, West Africa

**(At right) The change in solubility of oxidized uranium contributed to a remarkable occurrence 2 billion years ago in Gabon, West Africa: the world's first self-sustaining nuclear reactors**

# What about the effect of oxygen on life?




Aerobic respiration is essential for ATP production in complex life… but none of this existed before oxygen

# Almost all life depends on energy from redox reactions

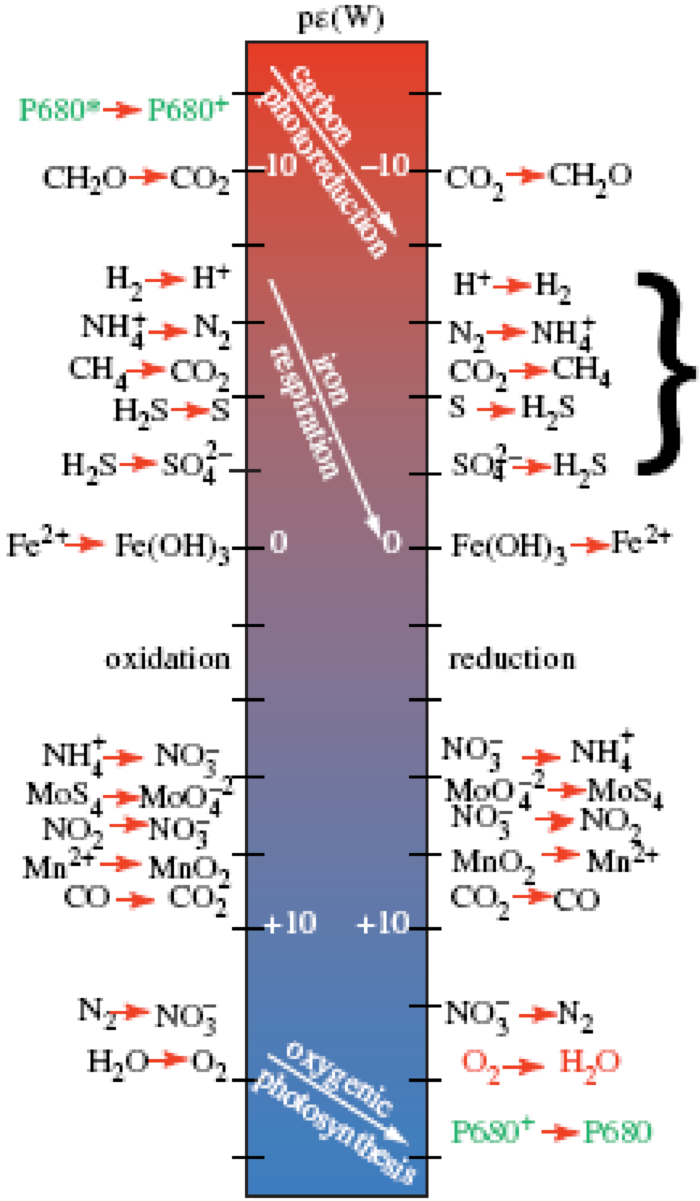



Figure 1. Electron activity (pε) of typical redox couples in water at pH 7 and 25°C (adapted from Gaidos *et al.* 1999).

The redox tower (summarizes the energy available from coupling oxidation of one molecule (pair at left) to reduction of another (right)

The amount of energy is given by the total distance between the oxidant and reductant (pick your reductant on the right and your oxidant on the left)

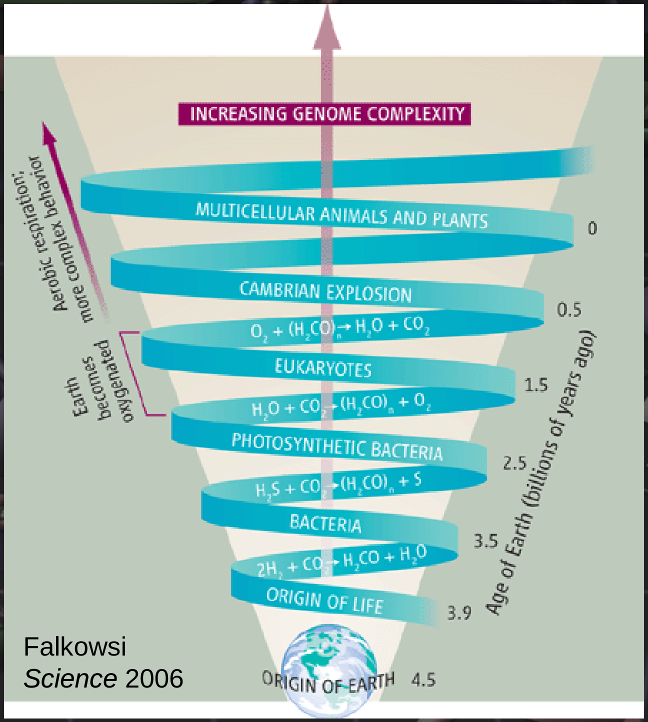
For instance, sulfate reducing bacteria couple the oxidation of organic matter ($CH_2O$), like glucose, to the reduction of sulfate

**All these options available—where would you go to eat?**

Oxygen is the most readily accessible oxidant on the modern Earth… is the extra energy it provides necessary to support complex life?

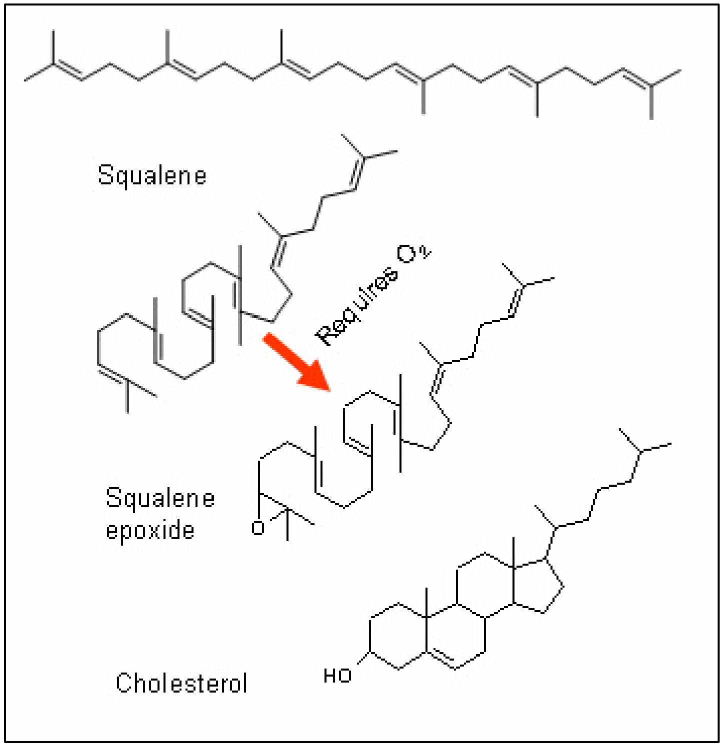
Tantalizing connection between rise of oxygen and the appearance of macroscopic eukaryotic fossils

The “oxygen imperative”: the extra energy available by using oxygen as an electron acceptor dramatically increased the energy available for life—is this required for life elsewhere in the universe?



Falkowsi
*Science* 2006

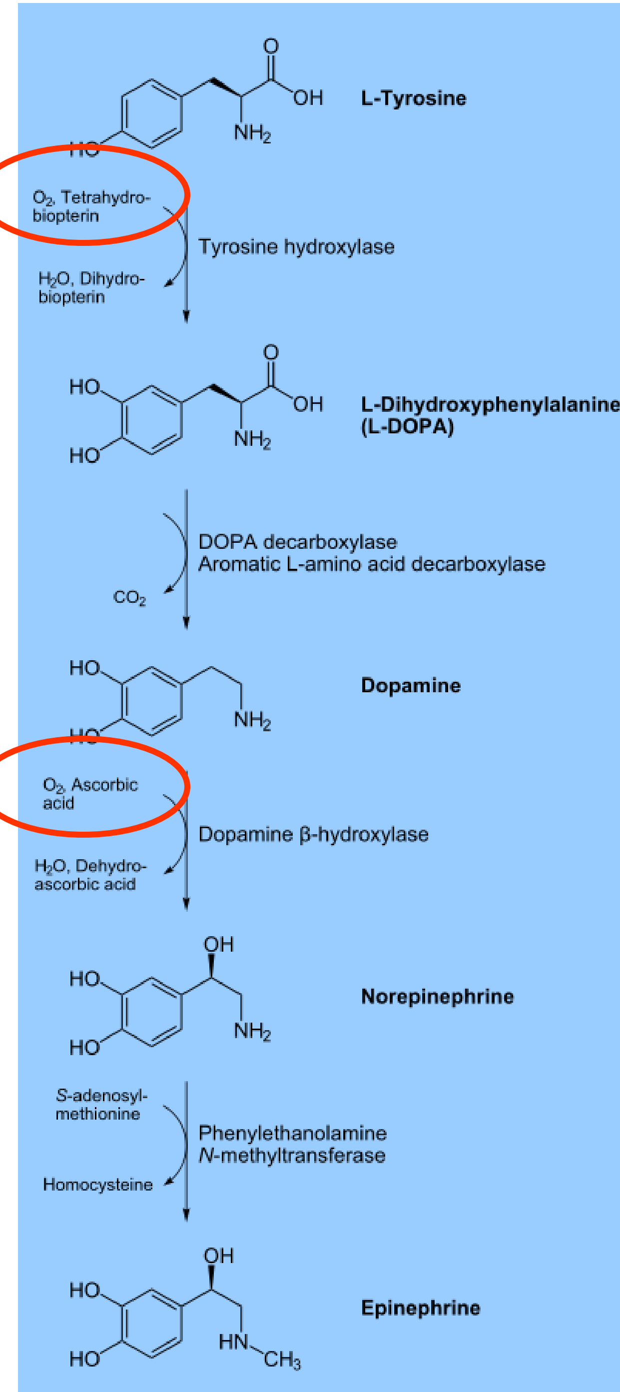
# $O_2$ is not just key in aerobic respiration: many biological pathways have evolved to use oxygen



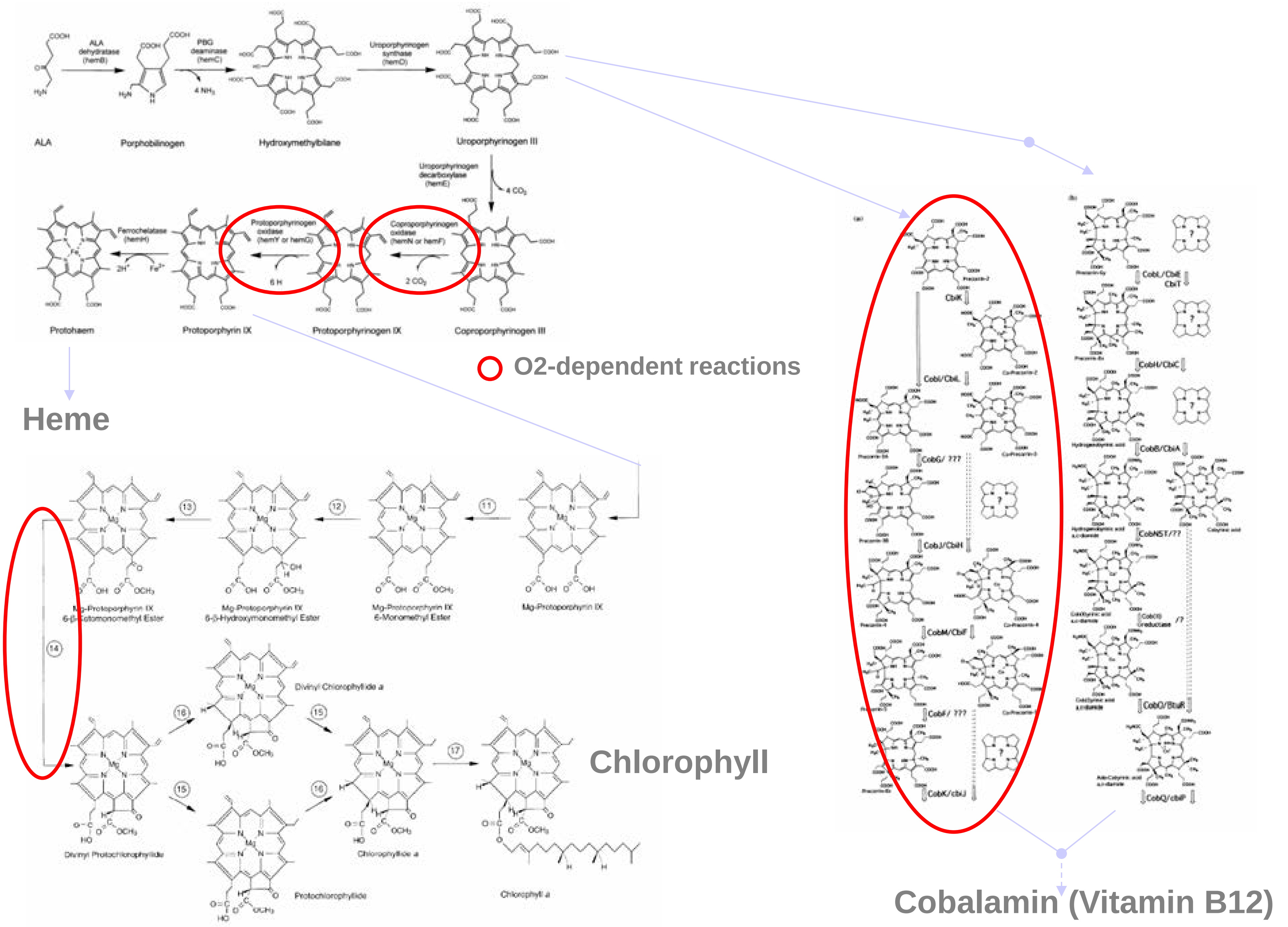

**(left) Squalene/cholesterol biosynthesis. (right) Dopamine and epinephrine biosynthesis**

- Dozens of anabolic pathways require oxygen (synthesis of cholesterol, dopamine/epinephrine, vitamins C and B12, …)

- Most catabolic pathways also use oxygen or one of its derivatives (such as hydrogen peroxide)



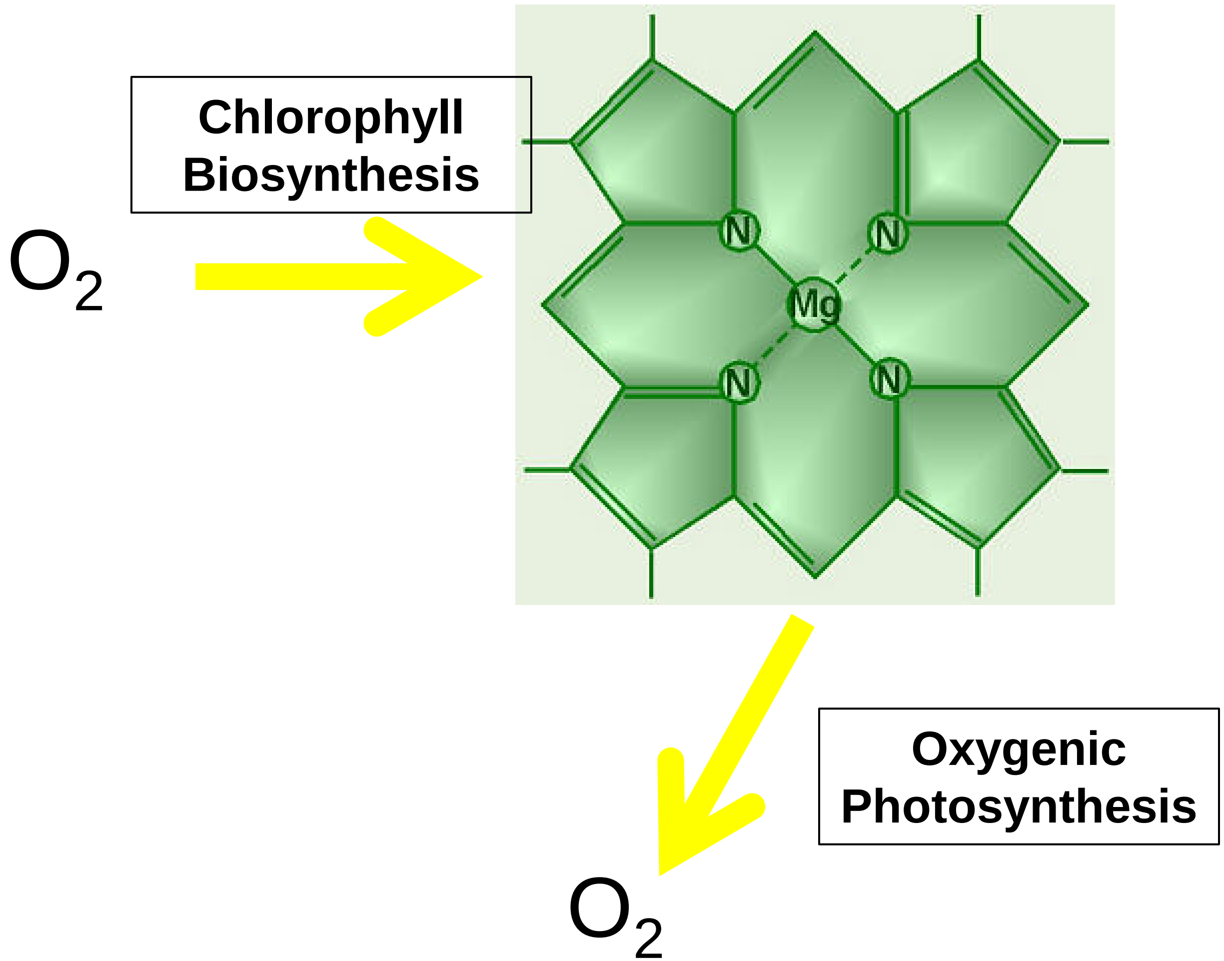
# Tetrapyrrole biosynthesis: shared pathway of biosynthesis for heme, vitamin B12, and chlorophyll



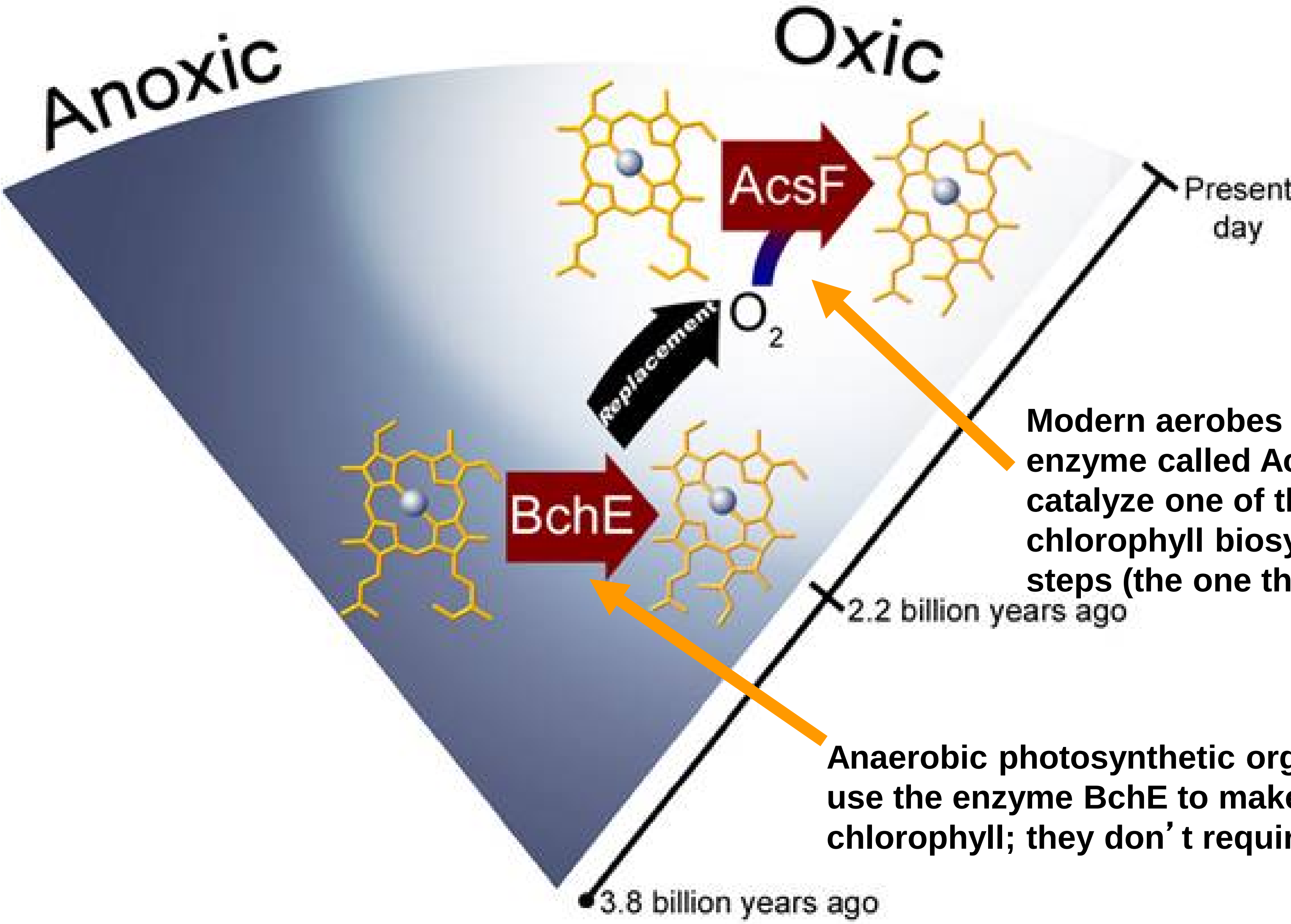
One of these pathways raises an apparent paradox:

$O_2$ is required for chlorophyll biosynthesis

*…but chlorophyll is required for photosynthesis to make oxygen?*



## Solution to this paradox: following the "great oxidation event" 2.2 billion years ago, many new enzymes must have evolved to take advantage of oxygen—many replaced their pre-$O_2$ enzyme counterparts




**Modern aerobes use an enzyme called AcsF to catalyze one of the key chlorophyll biosynthetic steps (the one that uses $O_2$)**

**Anaerobic photosynthetic organisms use the enzyme BchE to make chlorophyll; they don't require oxygen**

# Identical enzymatic reactxions by O2-dependent and O2-independent enzymes

| $O_2$-independent | $O_2$-dependent | Conserved reaction |
|---|---|---|
| L-glutamate dehydrogenase (1.4.1.3) | L-glutamate oxidase (1.4.3.11) | L-glutamate = 2-oxoglutarate + $NH_3$ |
| L-aspartate dehydrogenase (15) | L-aspartate oxidase (1.4.3.16) | L-aspartate = oxaloacetate + $NH_3$ |
| L-amino acid dehydrogenase (1.4.3.5) | L-amino acid oxidase (1.4.3.2) | L-amino acid = 2-oxo acid + $NH_3$ |
| choline dehydrogenase (1.1.99.1) | choline oxidase (1.1.3.17) | choline = betaine aldehyde |
| $O_2$-independent coproporphyrinogen oxidase (1.-.-.-) | $O_2$-dependent coproporphyrinogen oxidase (1.3.3.3) | coproporphyrinogen III = protoporphyrinogen IX |
| cellobiose dehydrogenase (1.1.99.18) | cellobiose oxidase (1.1.3.25) | cellobiose = cellobiose-1,5-lactone |
| dimethylglycine dehydrogenase (1.5.99.2) | dimethylglycine oxidase (1.5.3.10) | N,N-dimethylglycine = sarcosine + formaldehyde |
| N-acetylhexosamine-1-dehydrogenase (1.1.1.240) | N-acylhexosamine oxidase (1.1.3.29) | N-acetyl-D-glucosamine = N-acetyl-D-glucosaminate |
| L-sorbose dehydrogenase (1.1.99.12) | L-sorbose oxidase (1.1.3.11) | L-sorbose = 5-dehydro-D-fructose |
| dihydroorotate dehydrogenase (1.3.99.11) | dihydroorotate oxidase (1.3.3.1) | (S)-dihydroorotoate = orotate |
| dihydrouracil dehydrogenase (1.3.1.1) | dihydrouracil oxidase (1.3.3.7) | 5,6-dihydrouracil = uracil |
| glycerol-3-phosphate dehydrogenase (1.1.1.8) | glycerol-3-phosphate oxidase (1.1.3.21) | SN-glycerol-3-phosphate = glycerone phosphate |
| sarcosine dehydrogenase (1.5.99.1) | sarcosine oxidase (1.5.3.1) | sarcosine = glycine + formaldehyde |
| glucose dehydrogenase (1.1.99.17) | glucose oxidase (1.1.3.4) | D-glucose = D-glucono-1,5-lactone |
| glutathione peroxidase/ dehydrogenase (1.11.1.9/1.8.5.1) | glutathione oxidase (1.8.3.3) | 2 glutathione = oxidized glutathione |
| glycolate dehydrogenase/reductase (1.1.99.14/1.1.1.26) | glycolate oxidase (1.1.3.15) | glycolate = glyoxylate |
| *$O_2$-independent oxidative cyclase* | *$O_2$-dependent oxidative cyclase* | *Mg-protoporphyrin = Mg-protochlorophyllide* |
| *class II/III ribonucleotide reductase* | *$O_2$-dependent (class I) ribonucleotide reductase* | *NTP = dNTP* |
| *anaerobic cobalt chelatase* | *aerobic cobalt chelatase* | *Cobalt insertion into corrin precursor (complete pathway rearrangement)* |

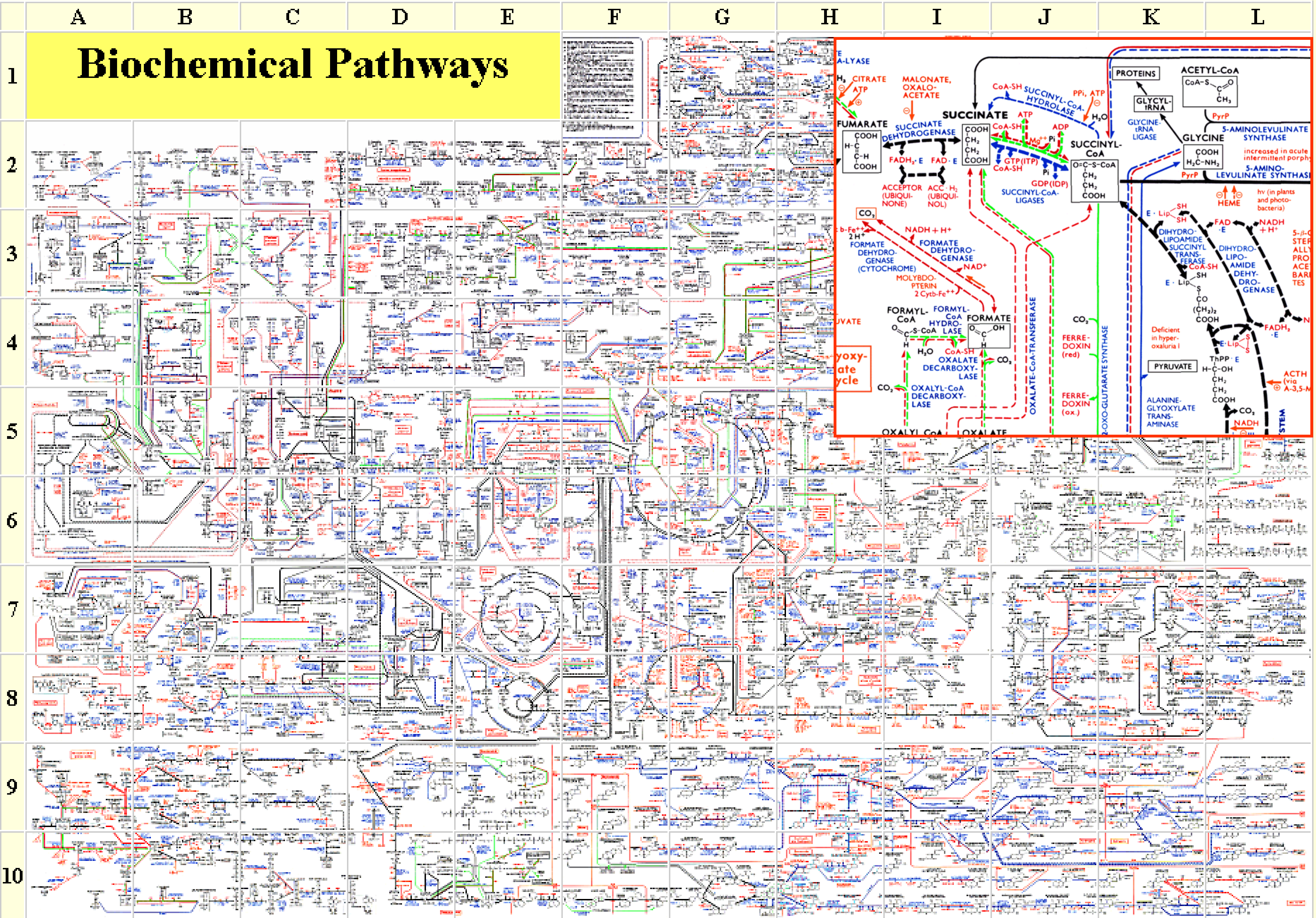
## Take home messages (so far):

- Protein sequences are constantly evolving; sometimes an entire enyzme will be replaced by another… makes evolutionary analysis of genes and proteins challenging!

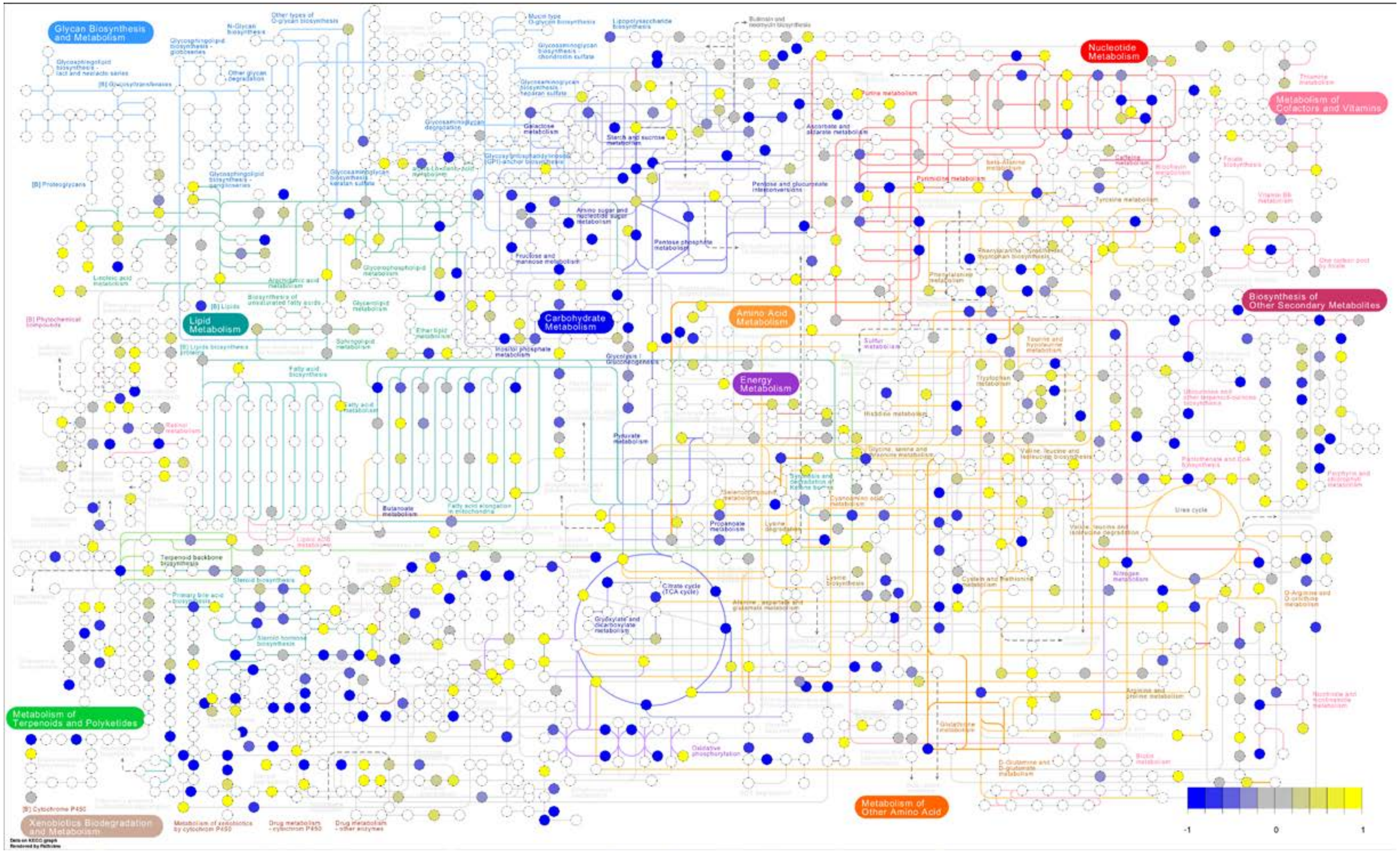
- Importantly, the catalytic steps carried out by enzymes are highly conserved

- We need methods that can interrogate evolution independent of canonical phylogenetic analyses, focusing on the evolution of catalysis and of biochemical pathways

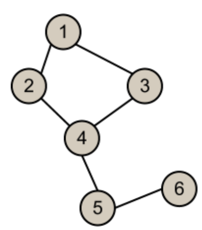
# Biochemical Pathways



# A network-level view of biochemistry




Undirected Graph & Adjacency Matrix

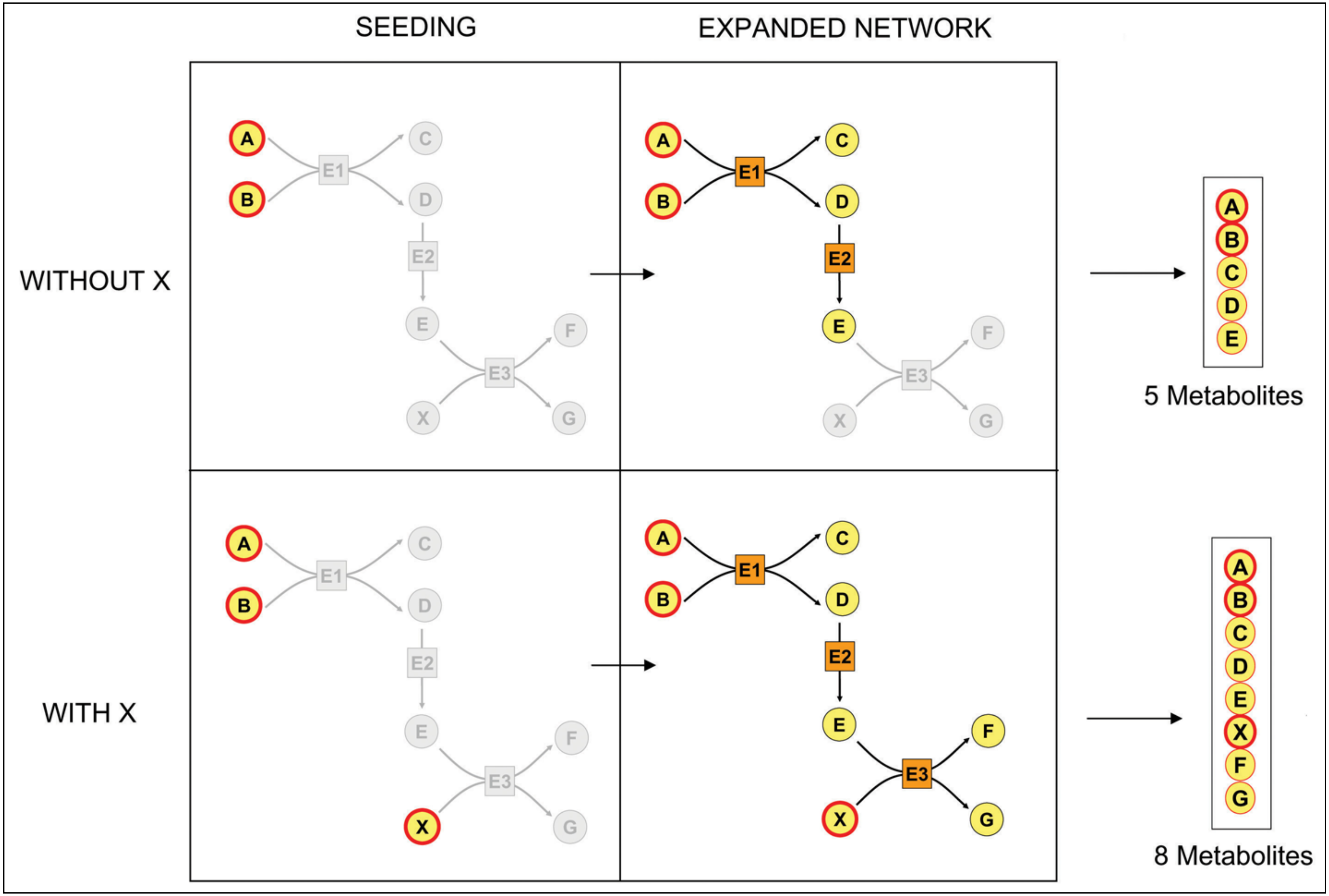



| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| 2 | 1 | 0 | 0 | 1 | 0 | 0 |
| 3 | 1 | 0 | 0 | 1 | 0 | 0 |
| 4 | 0 | 1 | 1 | 0 | 1 | 0 |
| 5 | 0 | 0 | 0 | 1 | 0 | 1 |
| 6 | 0 | 0 | 0 | 0 | 1 | 0 |

Undirected Graph

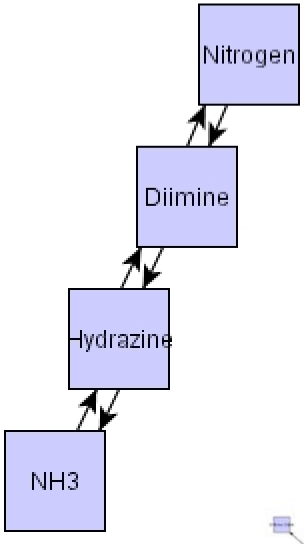
Adjacency Matrix

# Simulating biochemical network growth: network expansion

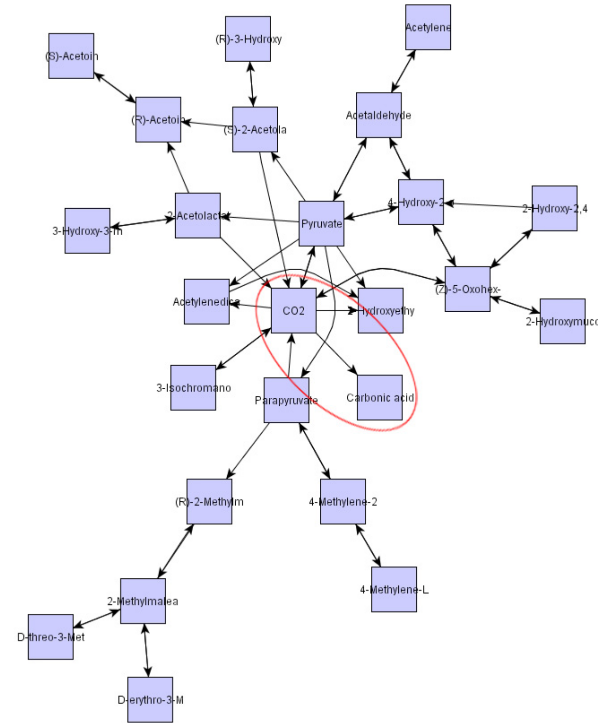


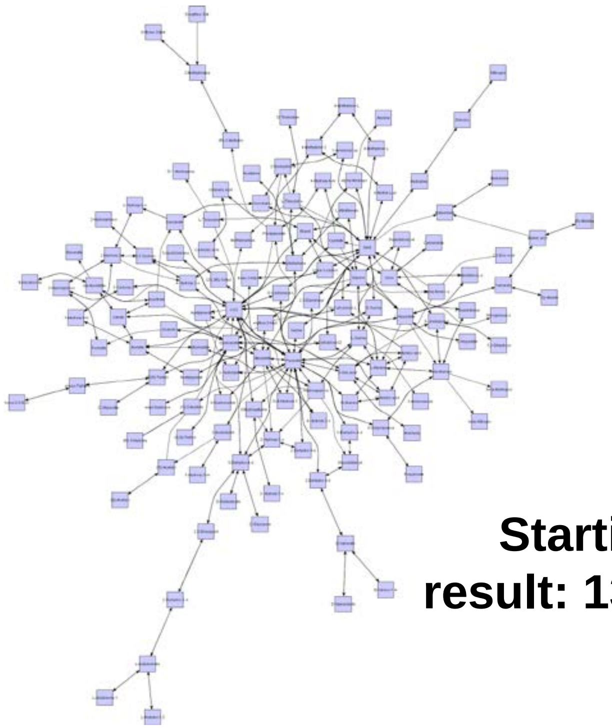
# Network expansion is nonlinear

**Starting w/ $NH_3$**
**result: 4 metabolites, 3 reactions**




**Starting w/ pyruvate**
**result: 25 metabolites, 26 reactions**



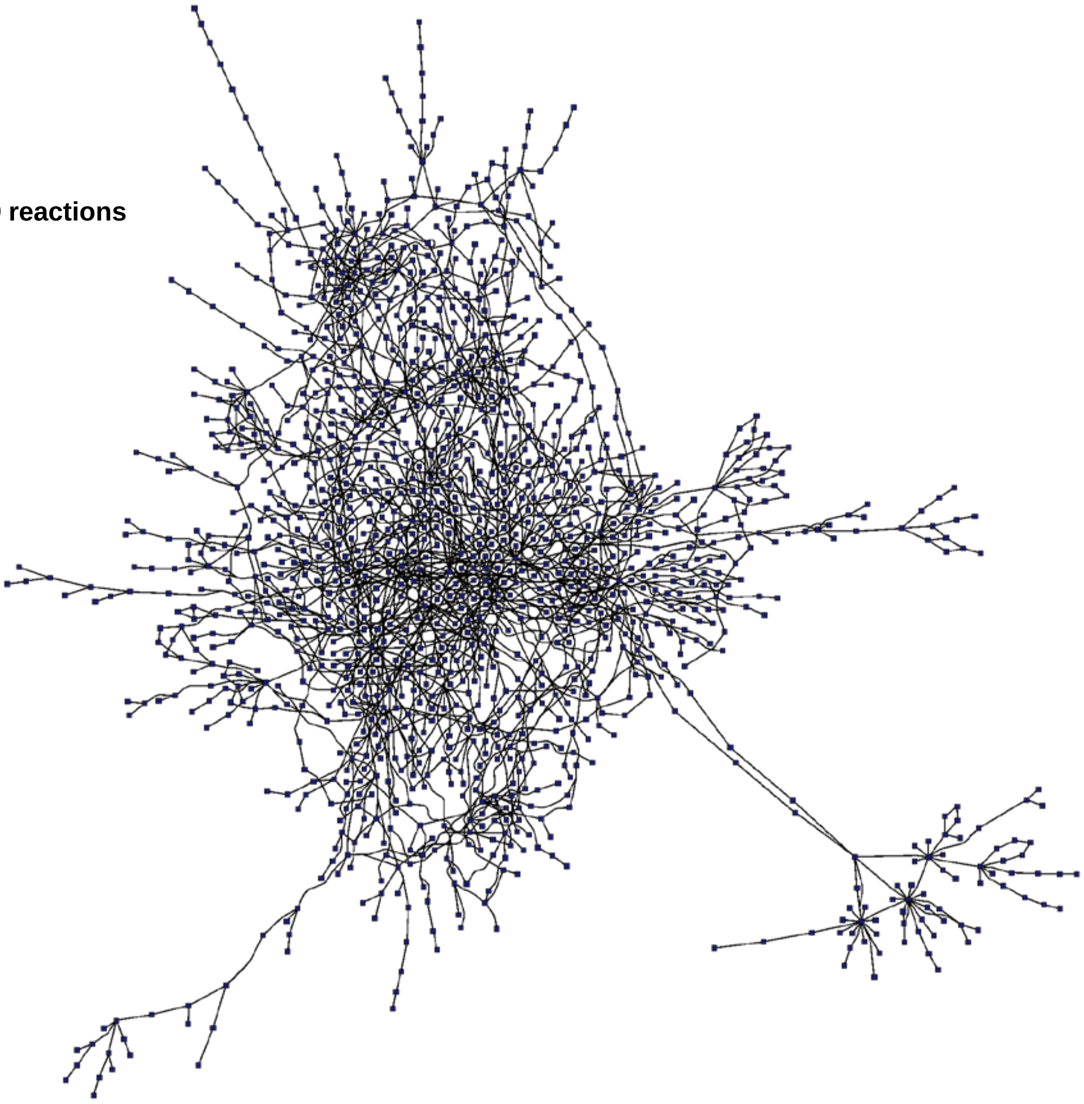



**Starting with $NH_3$ *and* pyruvate**
**result: 133 metabolites, 107 reactions**

# How has oxygen altered biochemical network architecture?

•Seed w/ plausibly prebiotic compounds ($H_2S$, $N_2$, $CO_2$, cofactors + ATP & NADH)

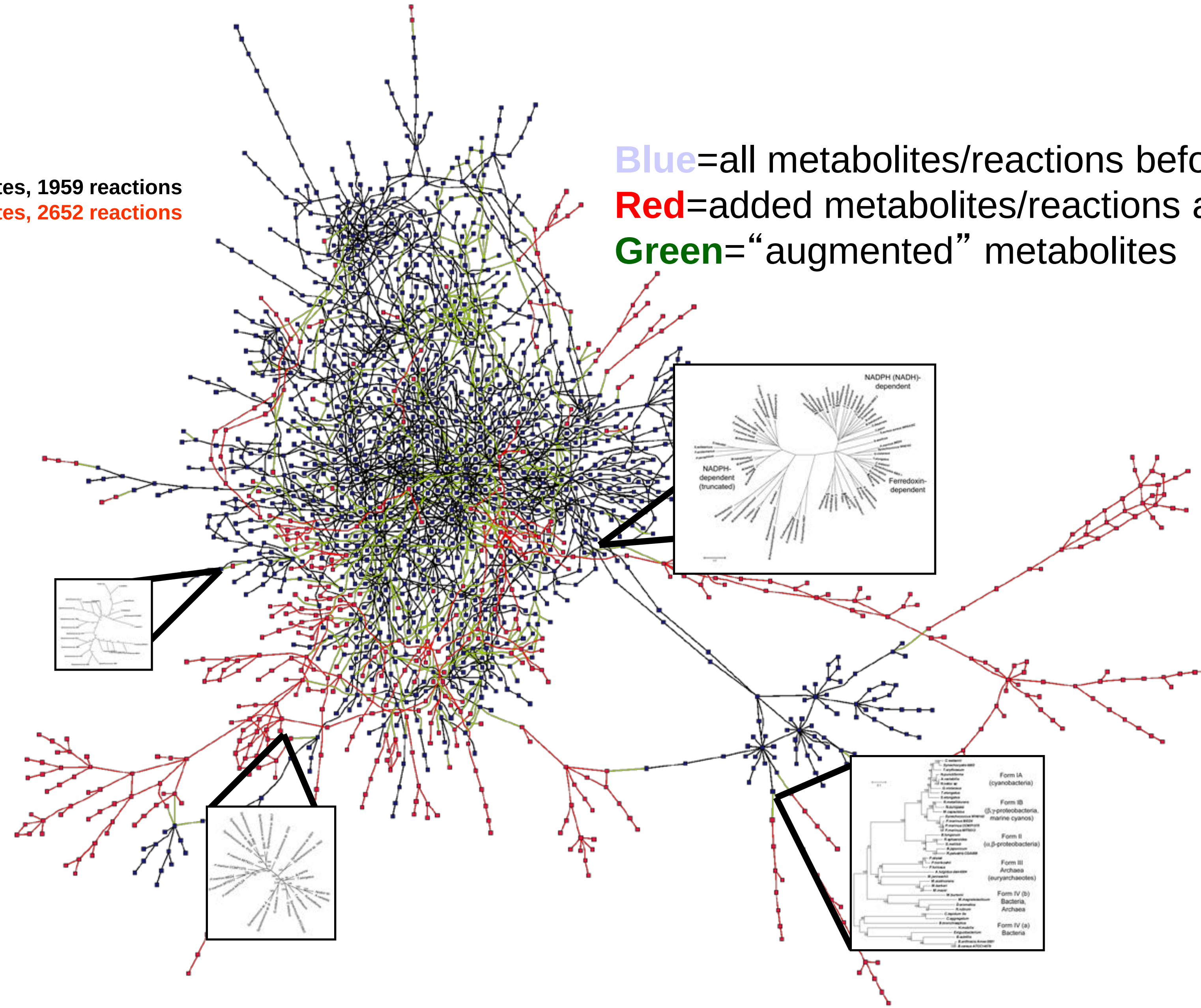
**1323 metabolites, 1959 reactions**



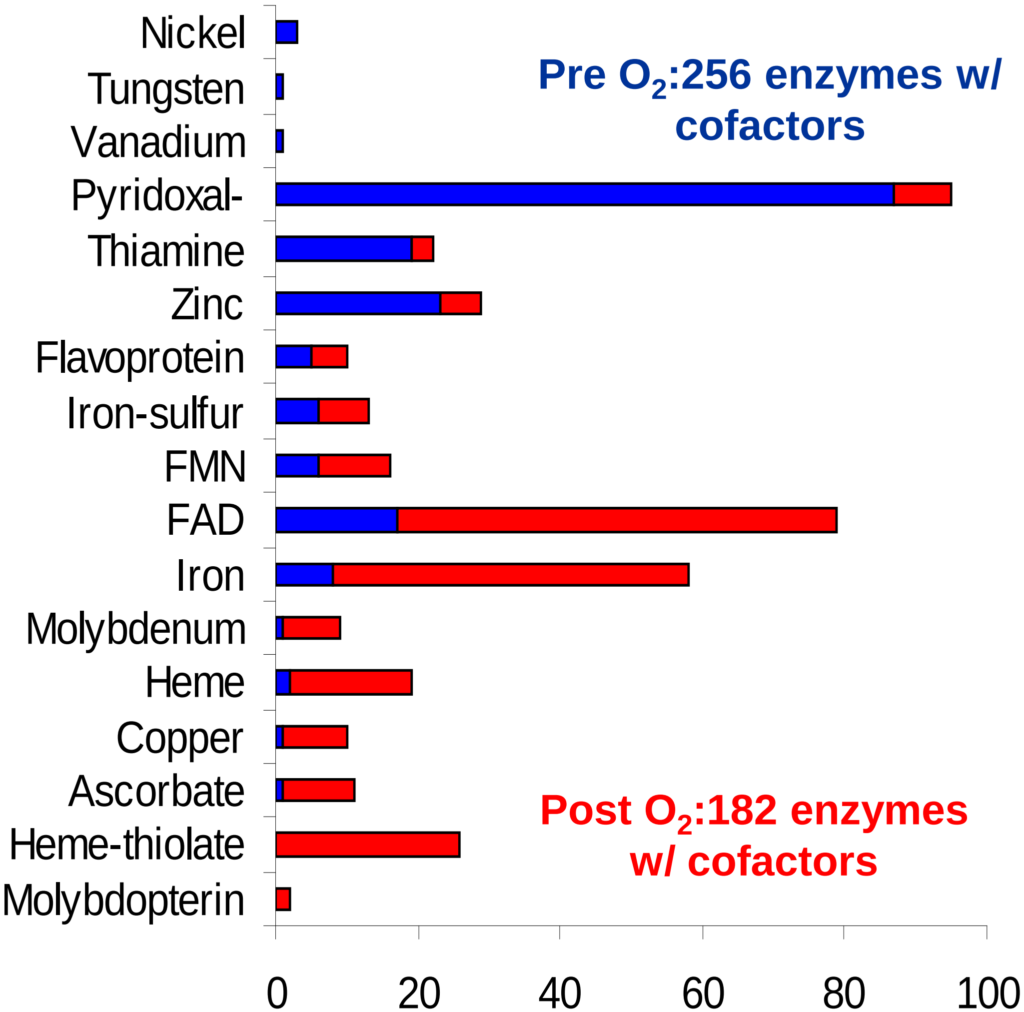
# Adding oxygen to the “seed” set dramatically expands the number of reactions and metabolites in the network—oxygen dependent biochemistries

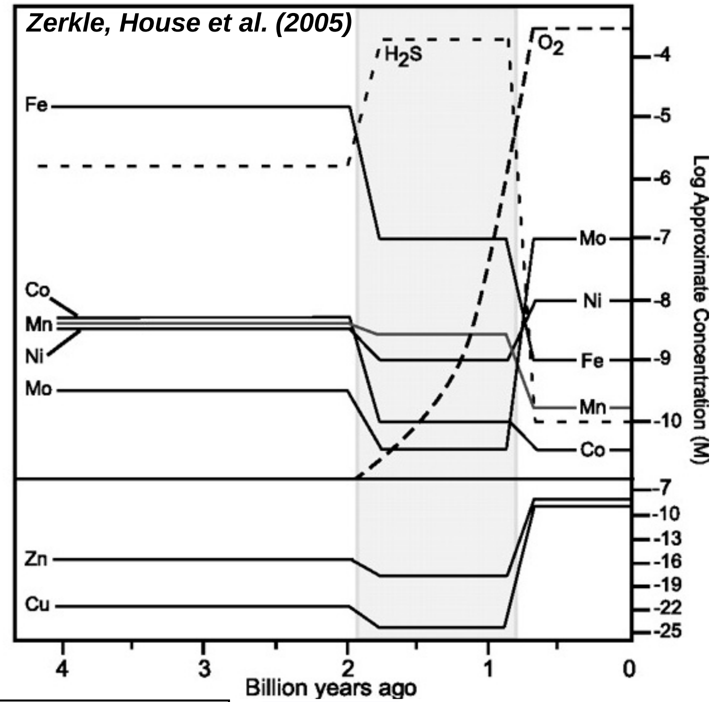
**1323 metabolites, 1959 reactions**
**1861 metabolites, 2652 reactions**

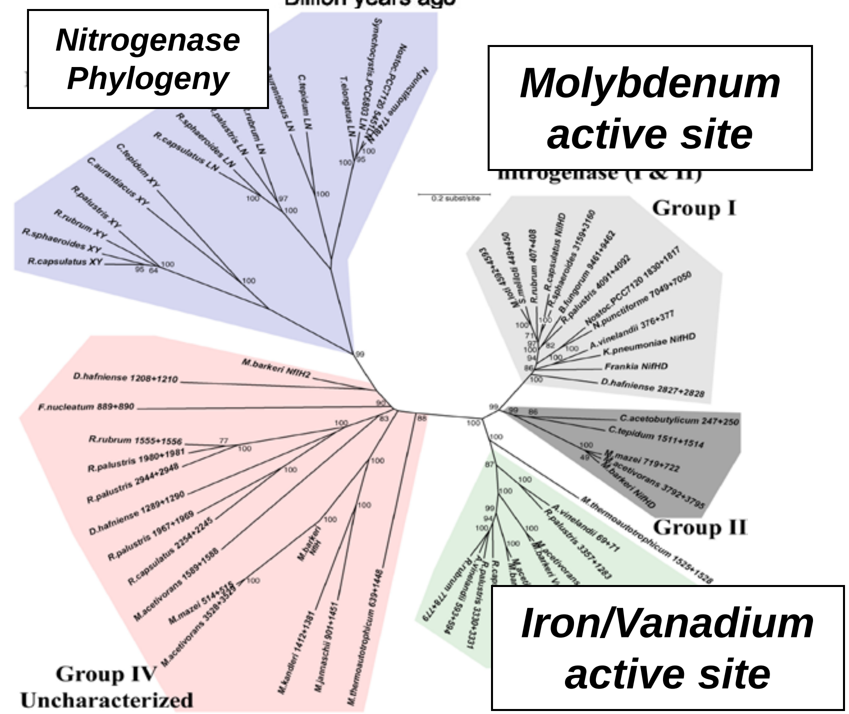
**Blue**=all metabolites/reactions before $O_2$
**Red**=added metabolites/reactions after $O_2$
**Green**=“augmented” metabolites



**Also allows us to probe how the utilization of different metals and cofactors has changed using $O_2$**




Raymond, Siefert, et al. (2004)

**And to integrate phylogenetic analysis of the thousands of sequences that catalyze these reactions**

•Oxygen resulted in a remarkable shift in the energy available to early life—perhaps a 10-fold increase in the amount of ATP

•Moreover, oxygen dramatically changed the repertoire of enzymes and cofactors used in biochemistry, as well as the diversity of chemical compounds that early organisms could synthesis

•It is argued that oxygen might have been a driver for mitochondrial endosymbiosis, and not long after oxygen became available, the earliest eukaryotic fossils appear in the rock record

•Is the “oxygen imperative” Earth-centric?  Are there other similarly energetic redox reactions that might arise on other potentially habitable worlds?

***Grypania spiralis*: possibly your earliest eukaryotic ancestor (2100 million years); Han & Runnegar (1992)**

# Thank you

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Dr. Eric Alsop (former grad student, now joint DOE-Shell postdoc)

Matt Kellom (ASU Ph.D. student)

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Prof. Everett Shock (ASU)

Prof. Eivind Almaas (NTSU/Norway)

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