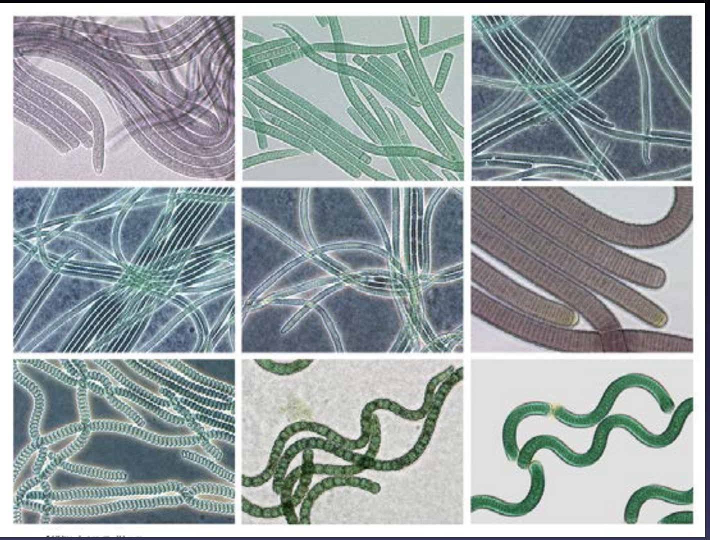
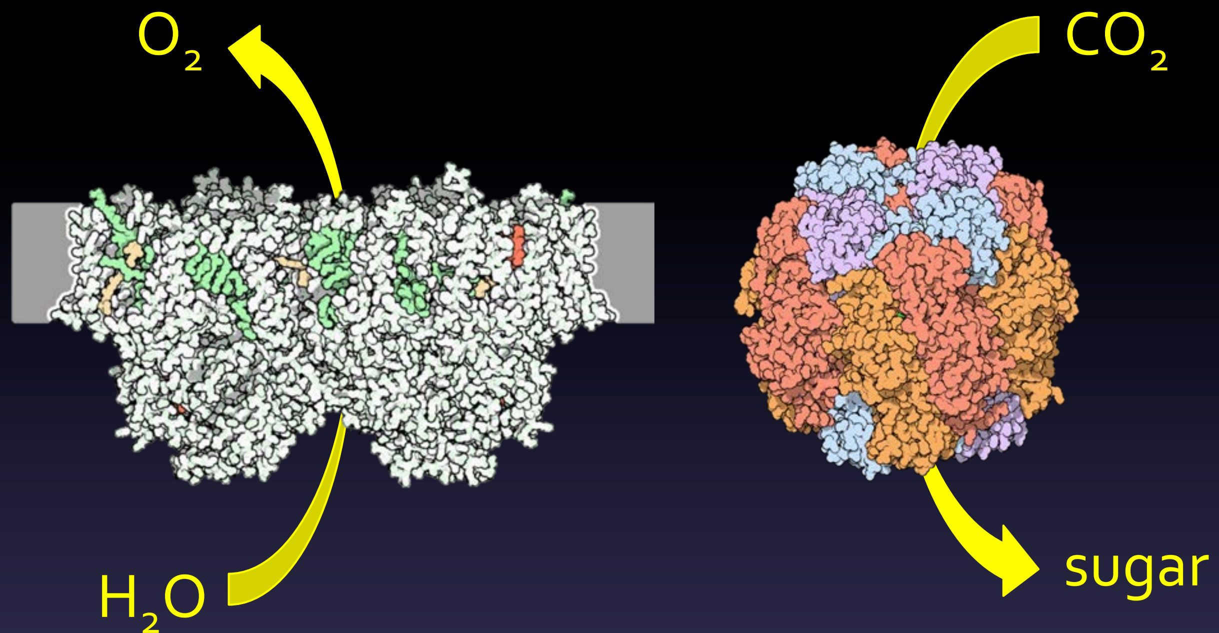
Reconstructing the early evolution of photosynthesis

Patrick Shih
Joint BioEnergy Institute
Lawrence Berkeley National Lab



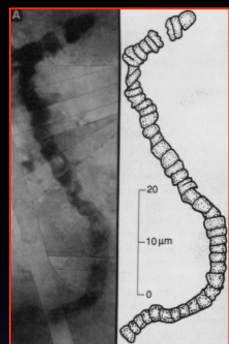
Oxygenic photosynthesis is the most important biological innovation in the history of life

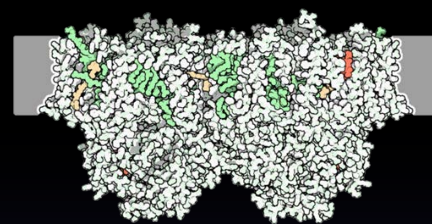


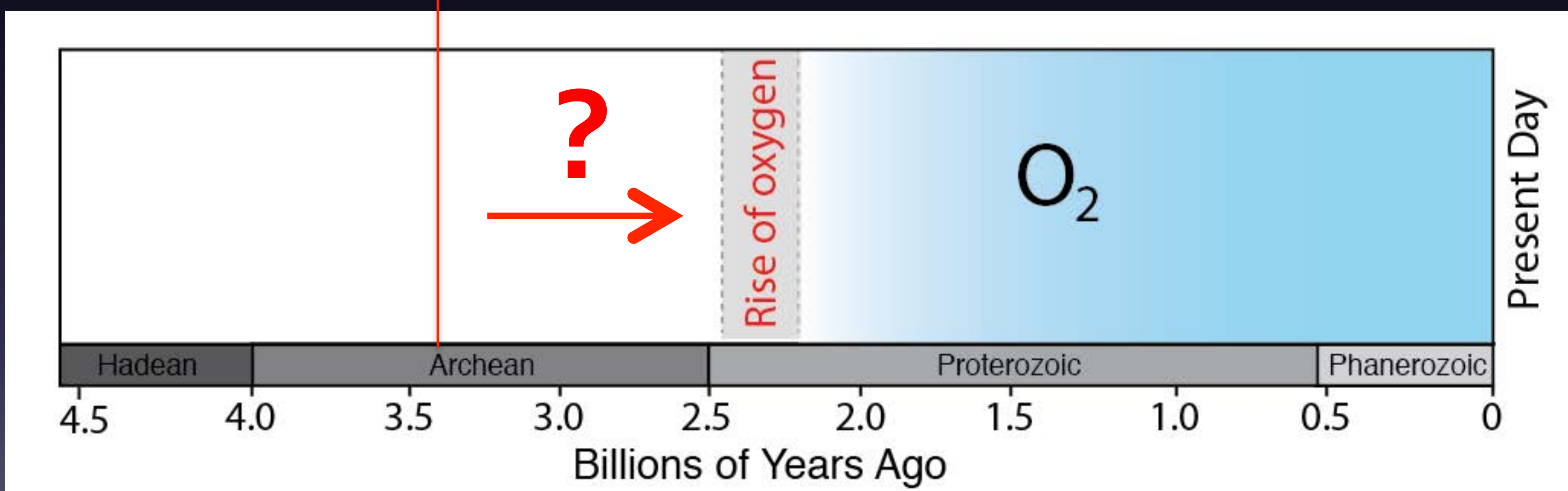
Photosystem II **RuBisCO**

http://www.pdb.org

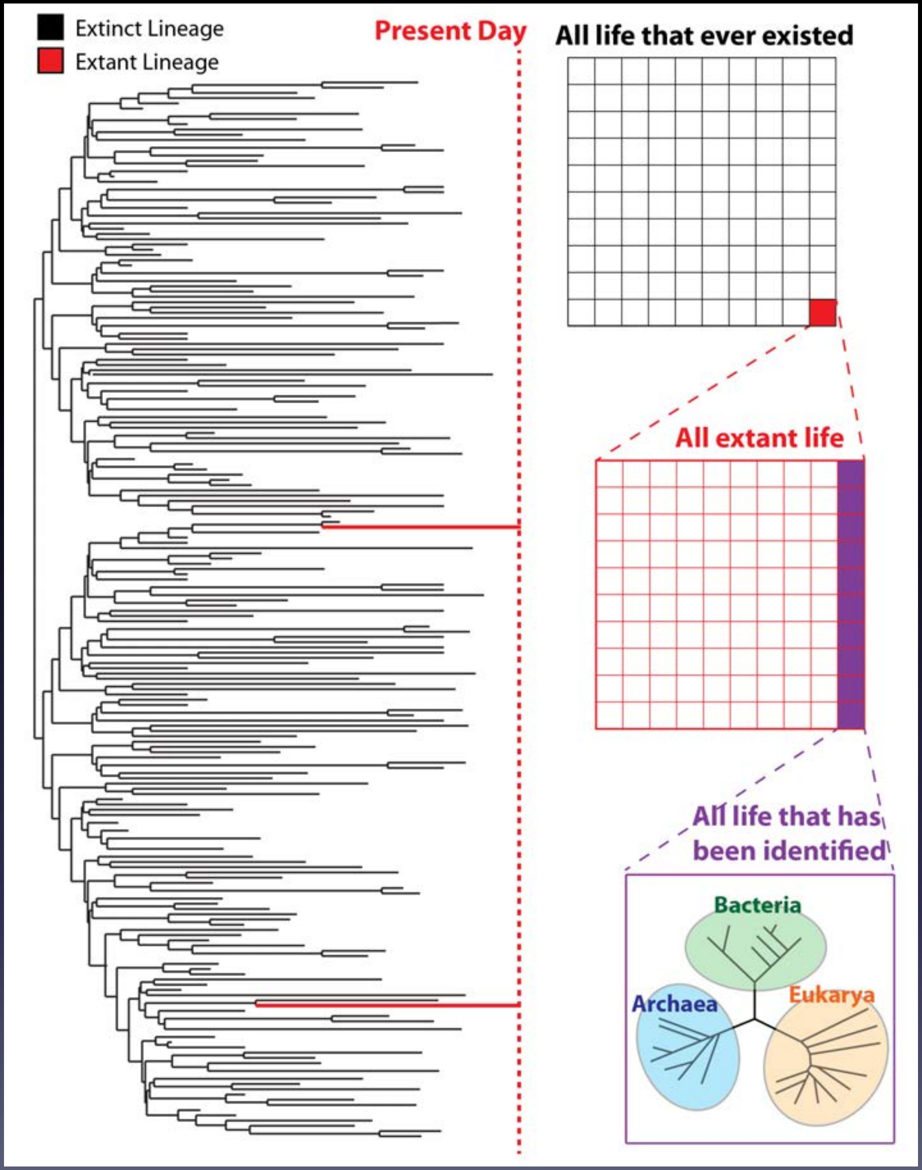
The Rise of Oxygen is the most fundamental environmental change in Earth's history







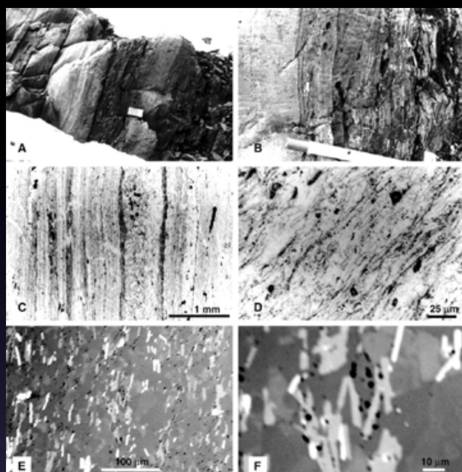
We know very little about the history of life



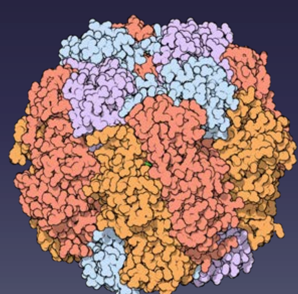
Shih, Curr Biol 2016

We have traditionally been limited in our tools to study the past

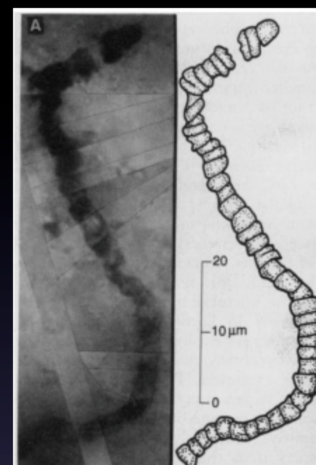
Isotope geochemistry



Rosing 1999, Science



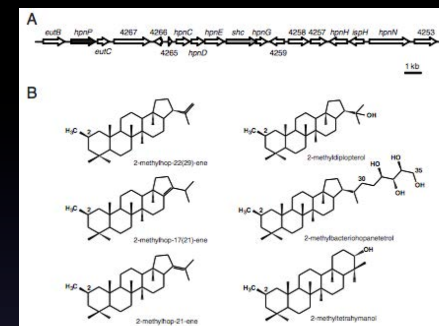
Fossil record

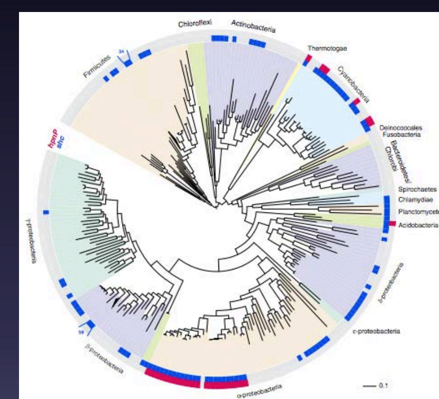


Schopf et al 1993, Science

We need new approaches to study early life!

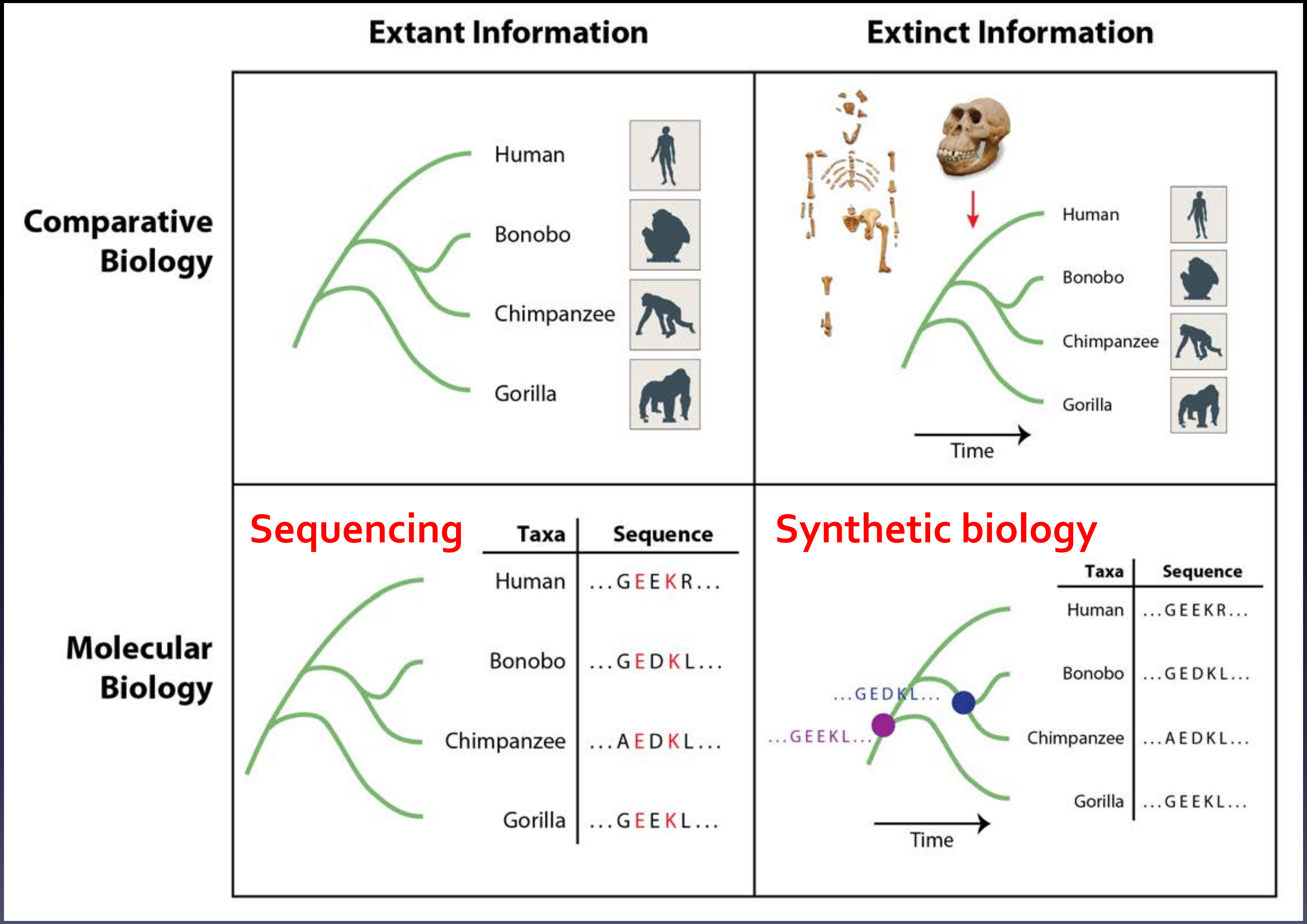
Biomarkers

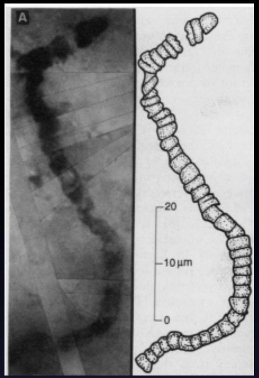




Rashby et al 2007, PNAS
Welander et al 2010, PNAS

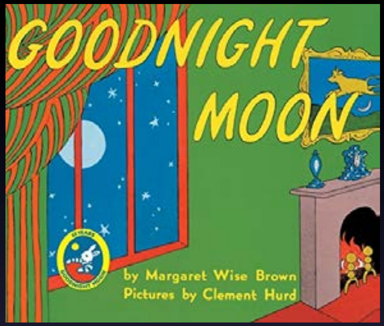
Major approaches to studying evolution

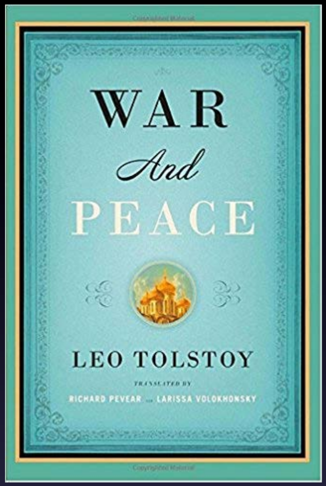




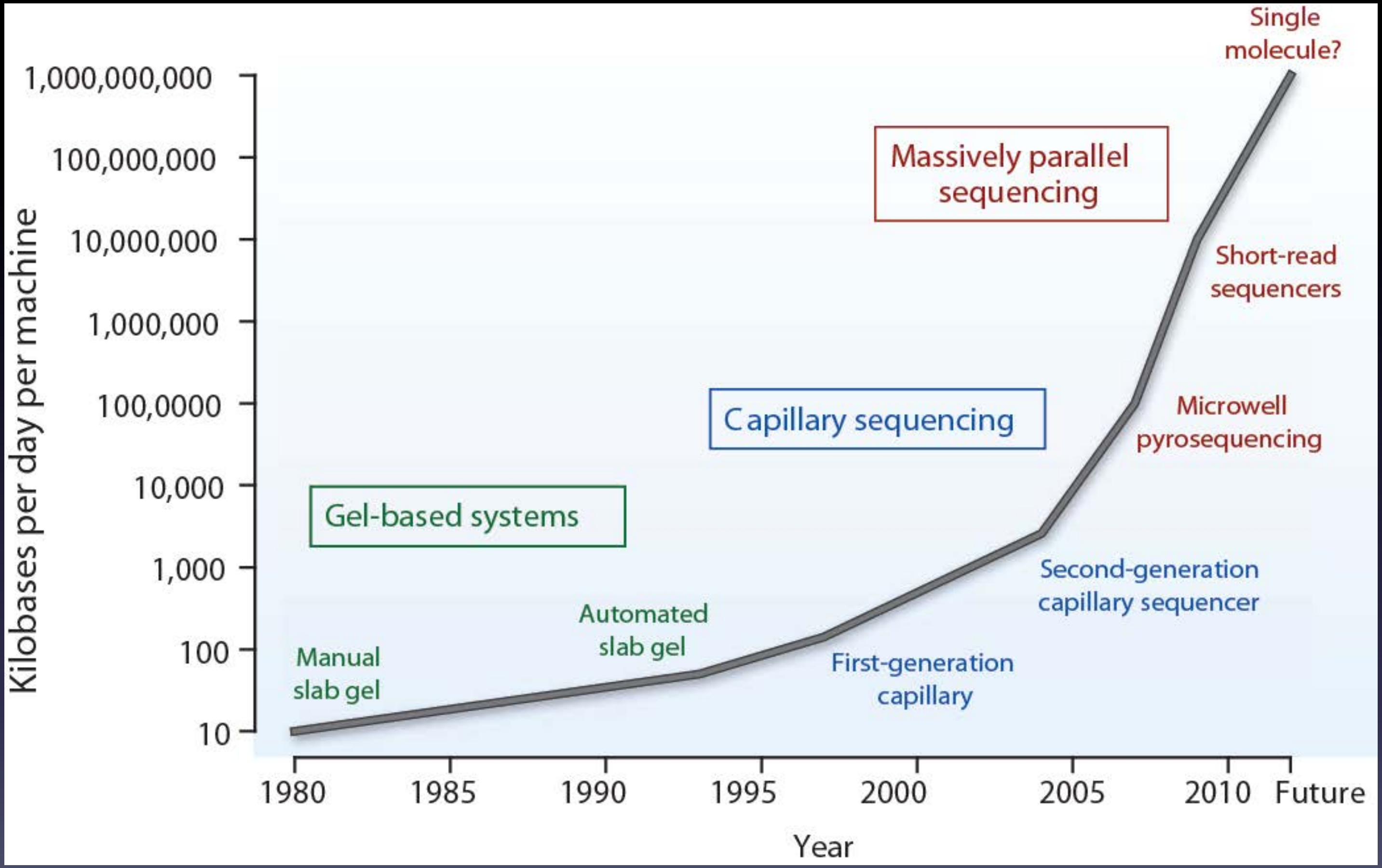
Fundamental advances in molecular biology have improved our understanding of life

1. Sequencing – reading
2. Synthesis – writing



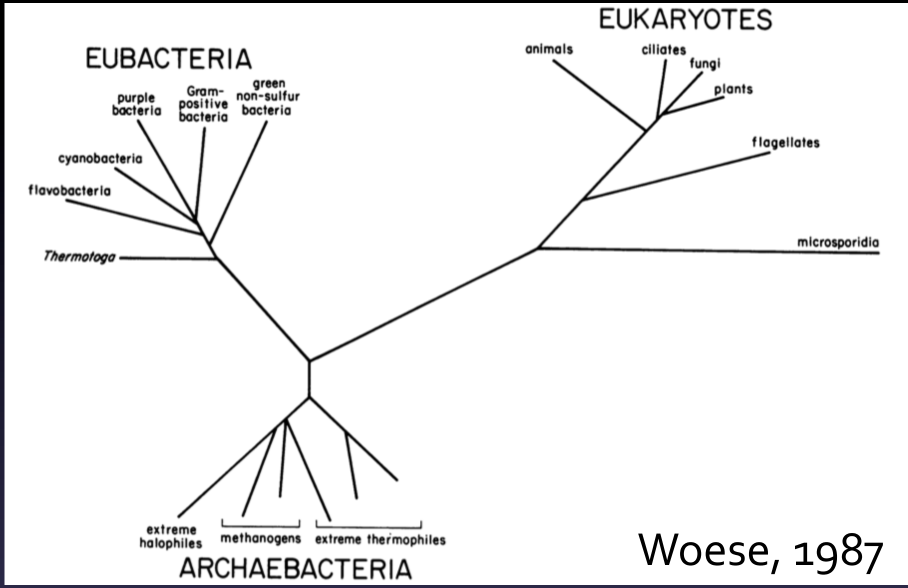


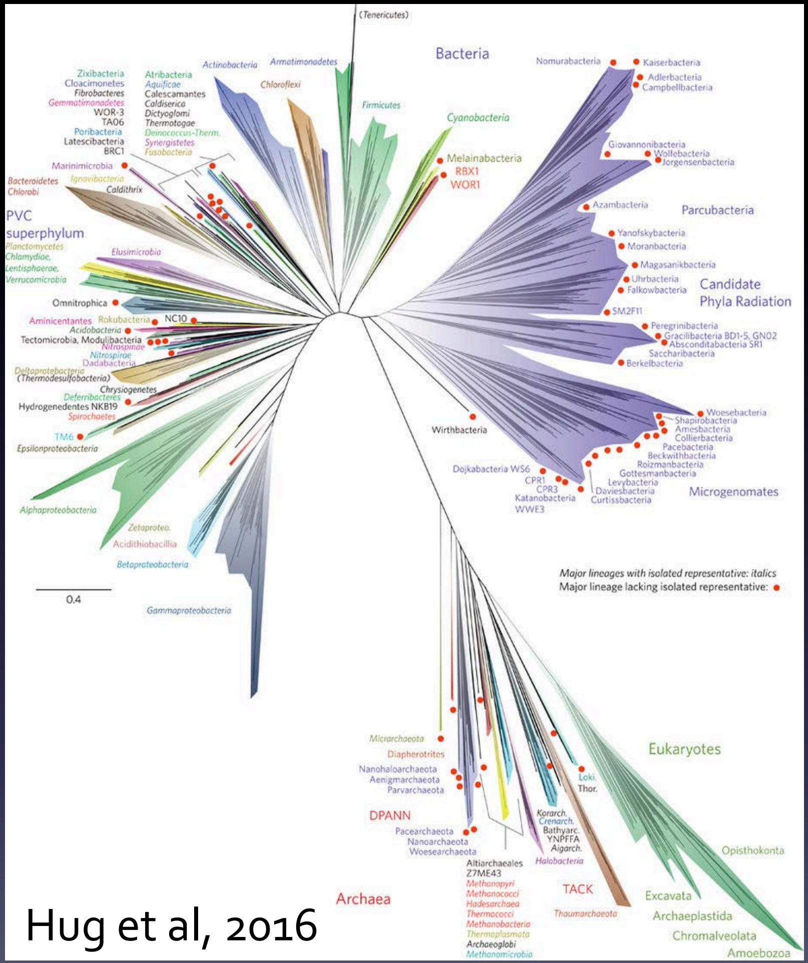
New approaches leverage the exponentially growing amount of sequence data



Stratton et al, Nature 2009

Increased sequence diversity improves phylogenetic resolution





Outline

I. When did photosynthesis evolve?

II. How did photosynthesis evolve at molecular level?

III. Can we reconstruct and redesign photosynthesis?

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II. How did photosynthesis evolve at molecular level?
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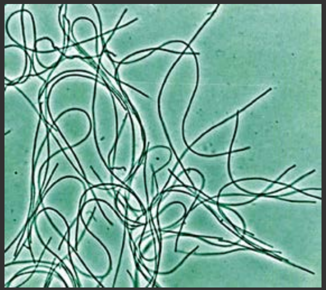
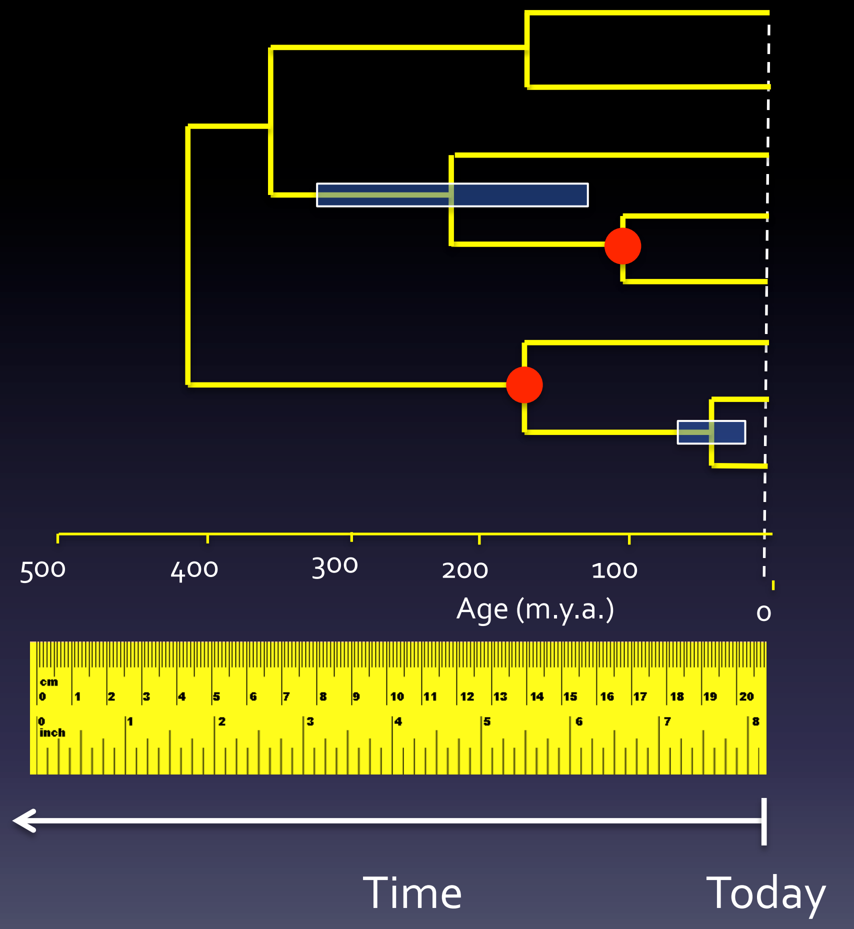
Improving molecular clocks

Origins of oxygenic photosynthesis

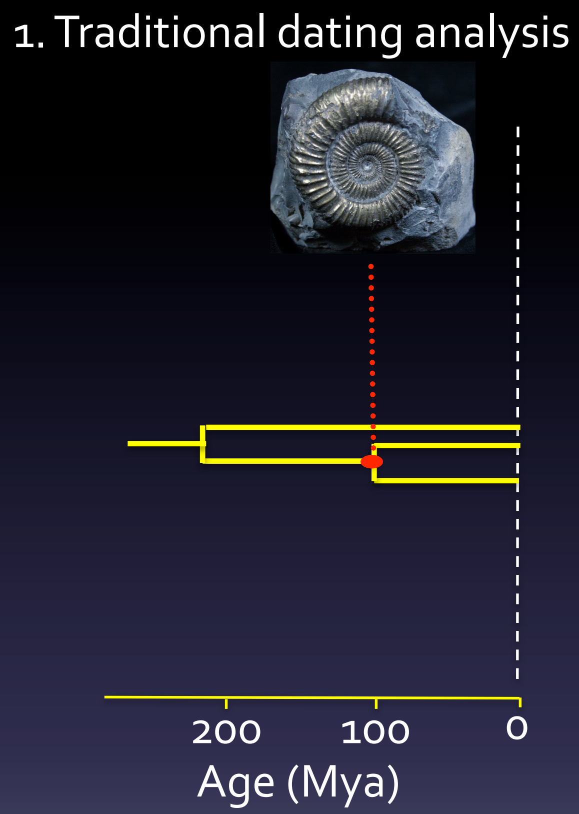
Origins of anoxygenic photosynthesis

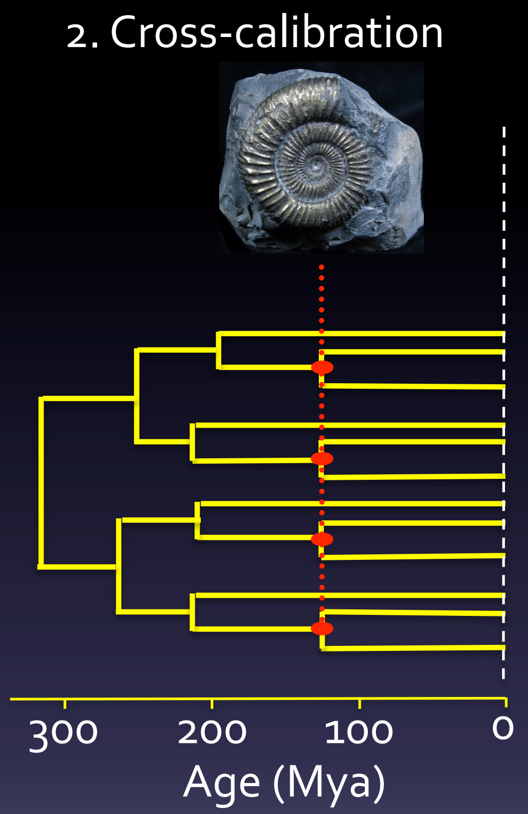


Molecular clocks incorporate dating information to reconstruct evolutionary histories

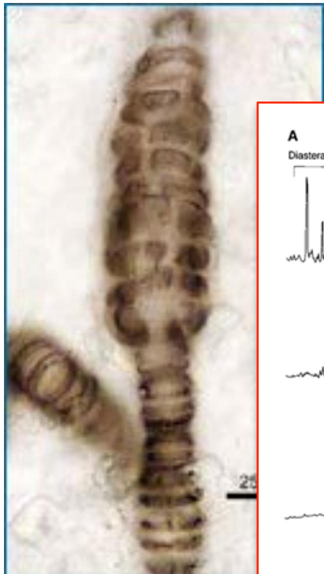


Increasing dating information with cross calibrations

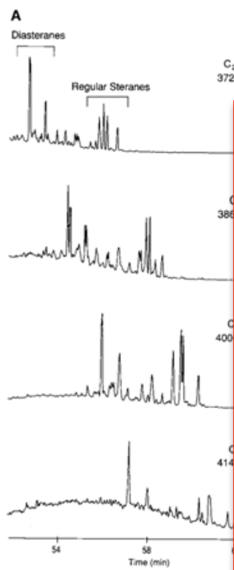




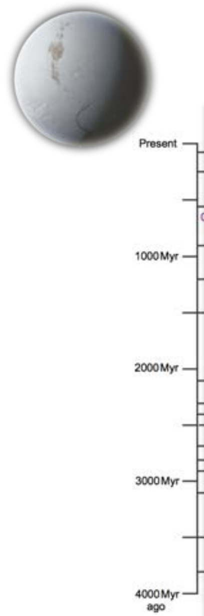
How old are eukaryotes?



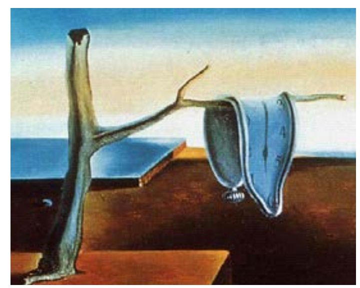
Red algal microfossils
1200 Mya



Sterane biomarkers
2700 Mya



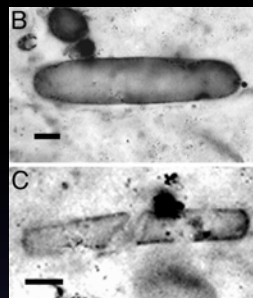
Snowball Earth glaciation event
900 Mya

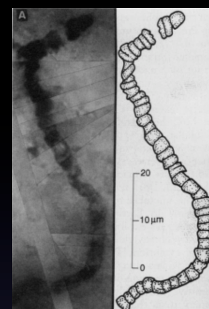


Molecular clock analysis
850-2300 Mya

Molecular Clock Study	Eukaryotic origin
Berney and Pawlowski, 2006	850 Mya
Douzery et al, 2004	1100 Mya
Parfrey et al 2011	1800 Mya
Hedges et al 2004	2300 Mya

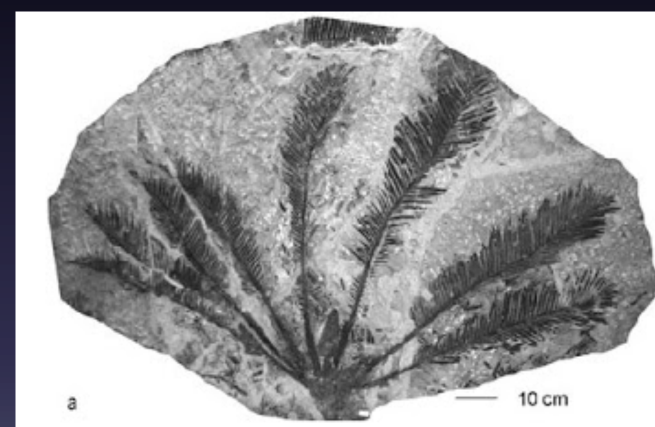
Molecular clock analyses are dependent on reliable calibration points





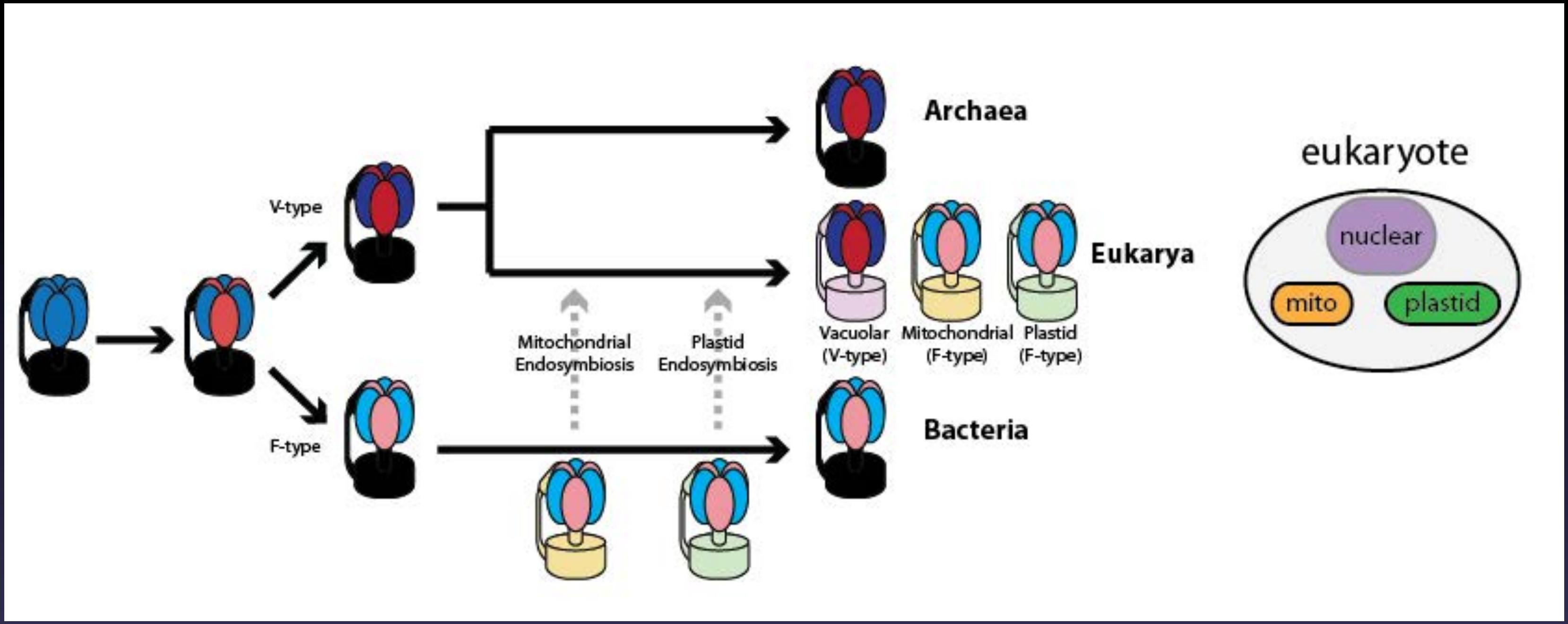
Taxon	Constraint in Mya (±std dev)
Monocotyledoneae†	156(±14).
Angiospermae†	217(±40)
Gymnospermatophyta†	327(±30)
Tracheophyta†	432(±30).
Land Plants†	477(±70)
Human/Chicken§	300(±30)
Fly/Mosquito§	235(±24)

†Adapted from SA Smith et al (2009)
§Adapted from ML Berbee and JW Taylor (2010)

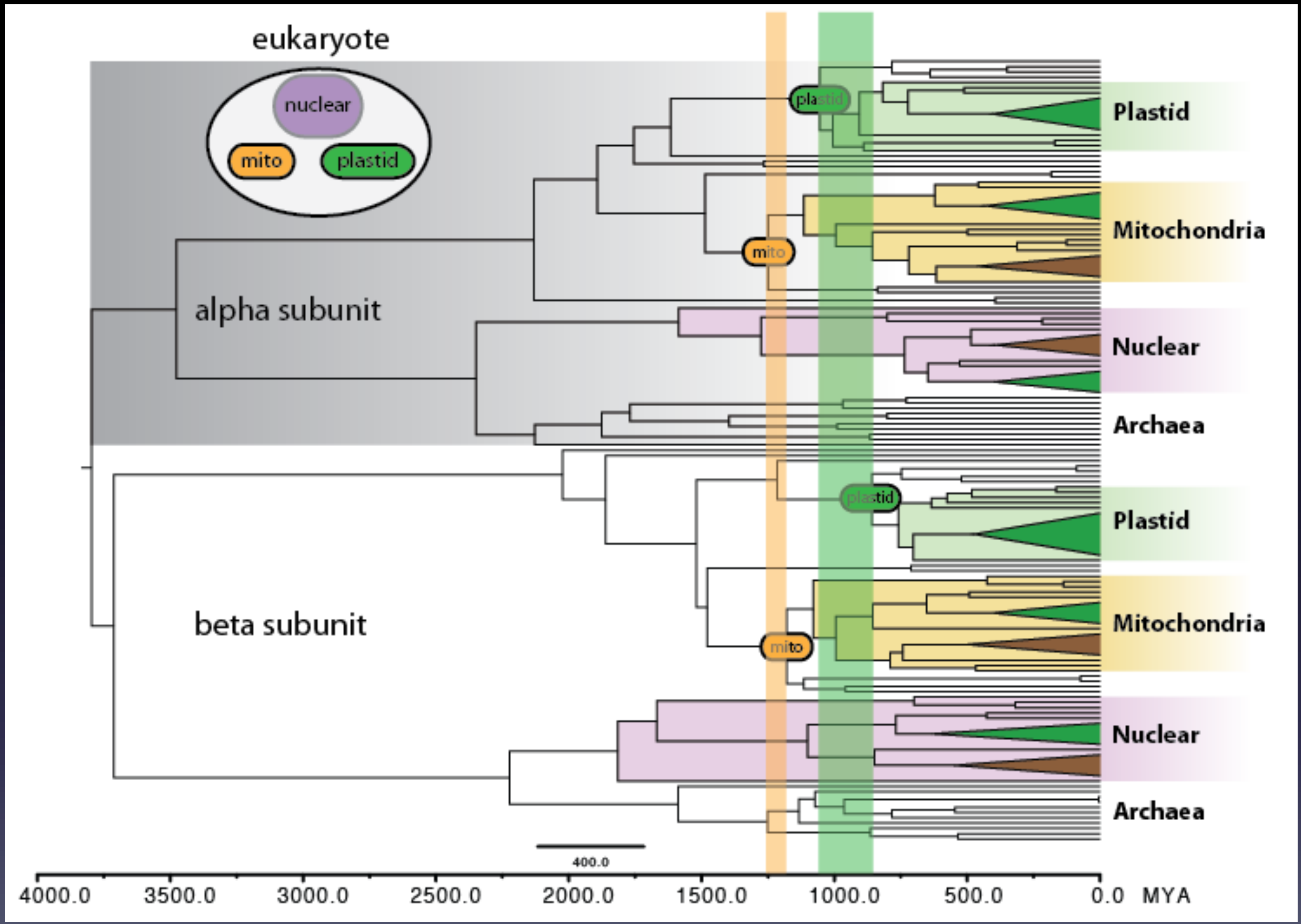


Tomitani et al, PNAS 2006
Schopf et al 1993, Science
Wang et al, Sci Bull 2009
Crane et al, Am J Bot 2004

ATPase has gone through a unique evolutionary history

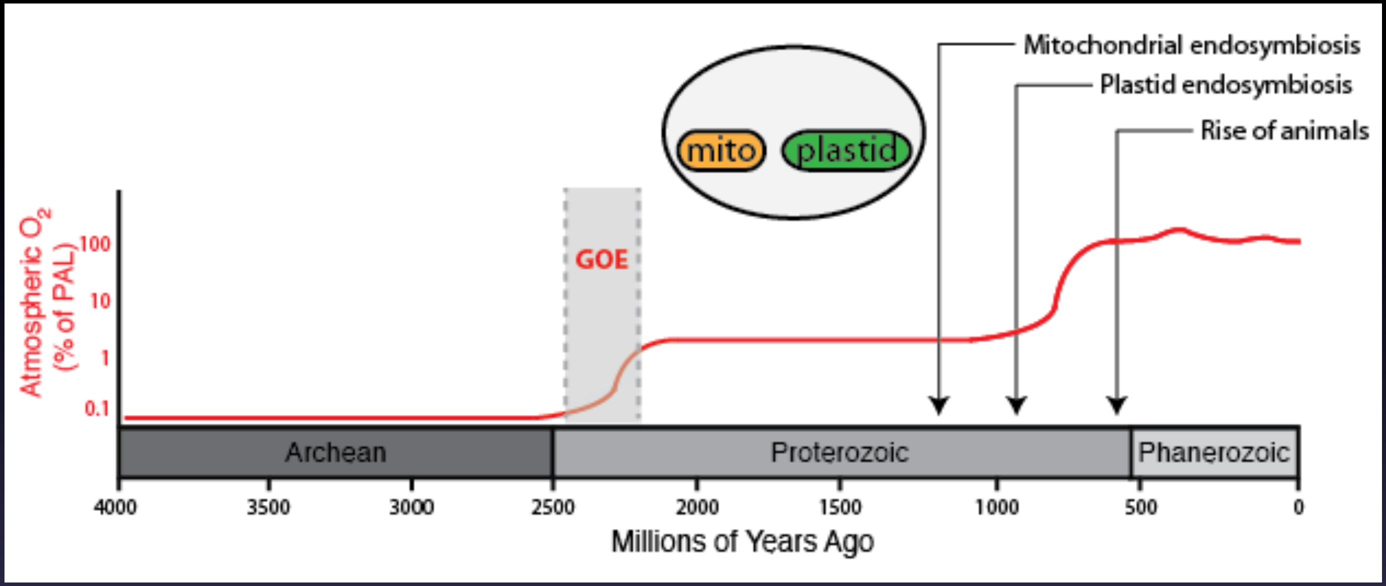


ATPase duplication and endosymbiosis events add valuable information for dating the Tree of Life

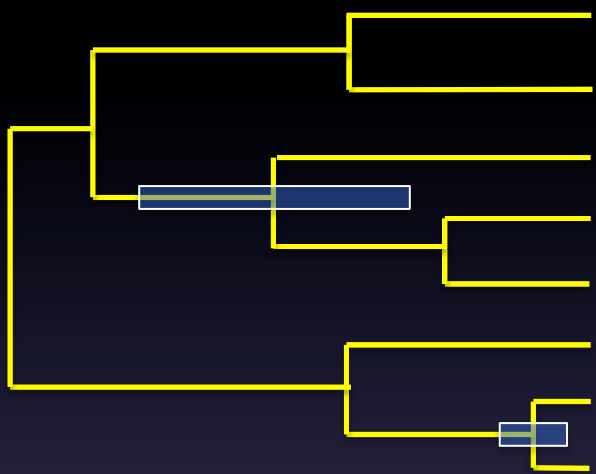


Shih and Matzke, 2013

Molecular clock analyses are consistent with the oxygen record

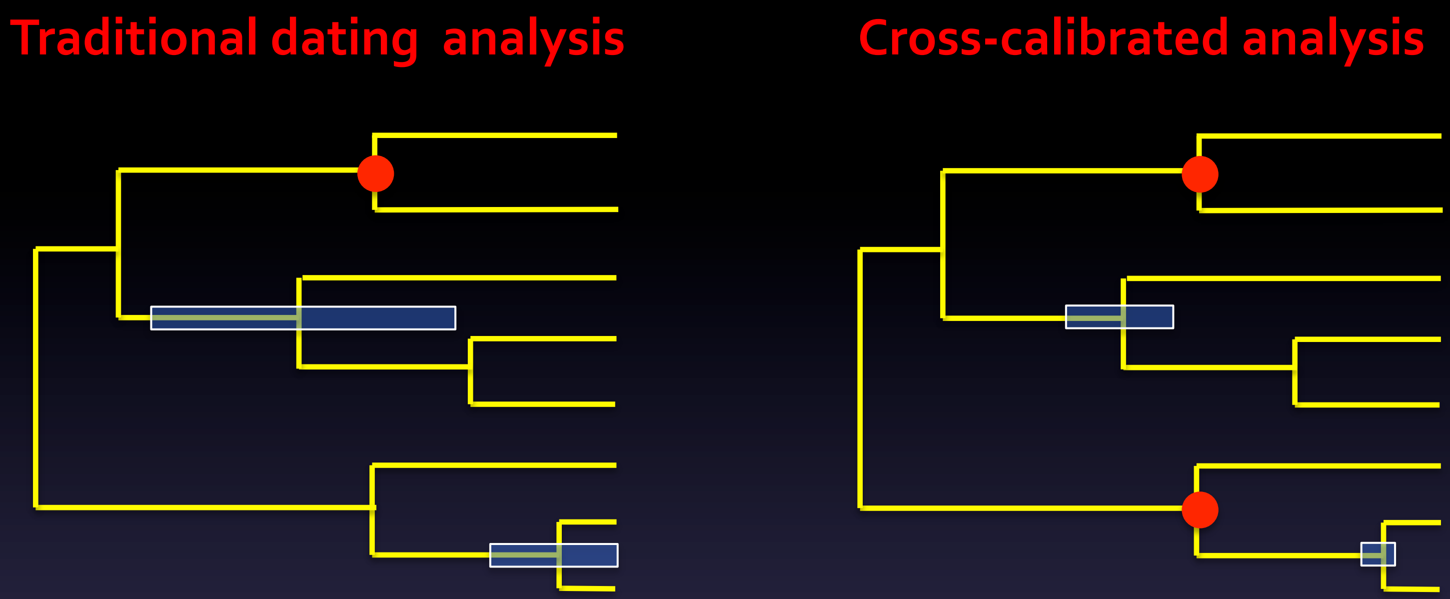


Cross-calibration decreases date uncertainty



Amount of Uncertainty
(width of Confidence Interval)

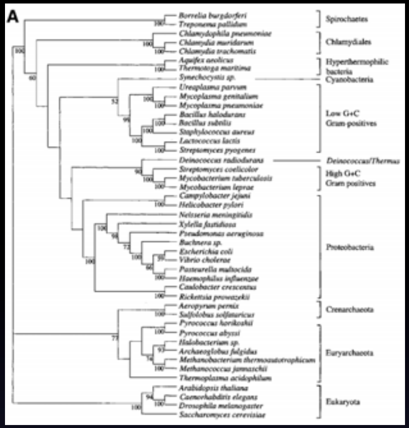
Cross-calibration decreases date uncertainty



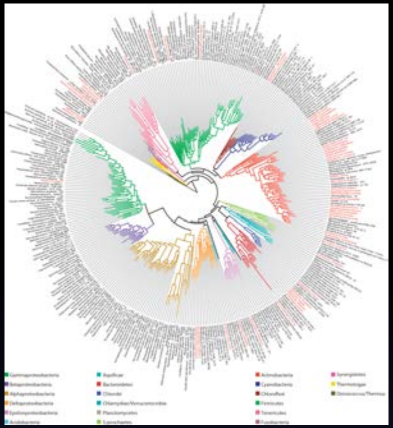
When did oxygenic photosynthesis evolve?

Origins of oxygenic photosynthesis

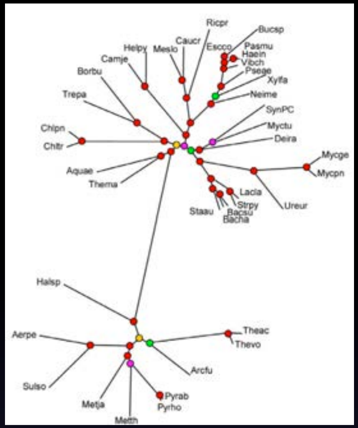
Who is the closest relative to Cyanobacteria?



Firmicutes
Daubin et al 2002

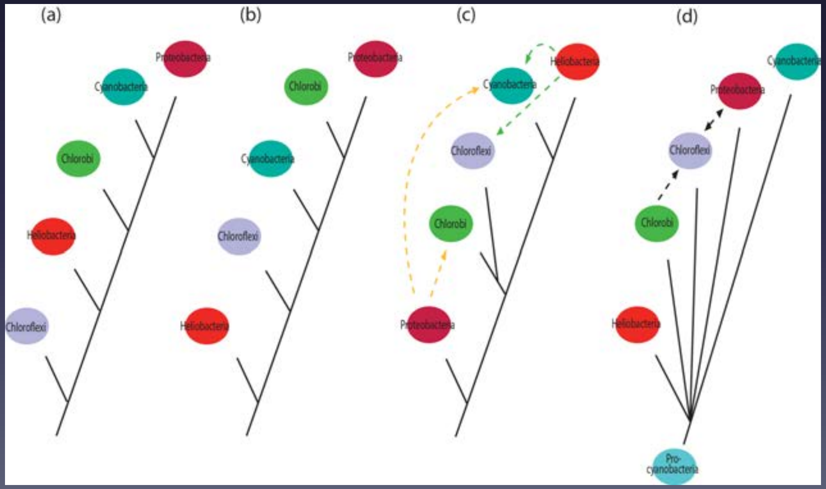


Chloroflexi
Wu et al, 2009



Mycobacteria
Wolf et al 2001

Thermus-Deinococcus
Bustard et al, 1997

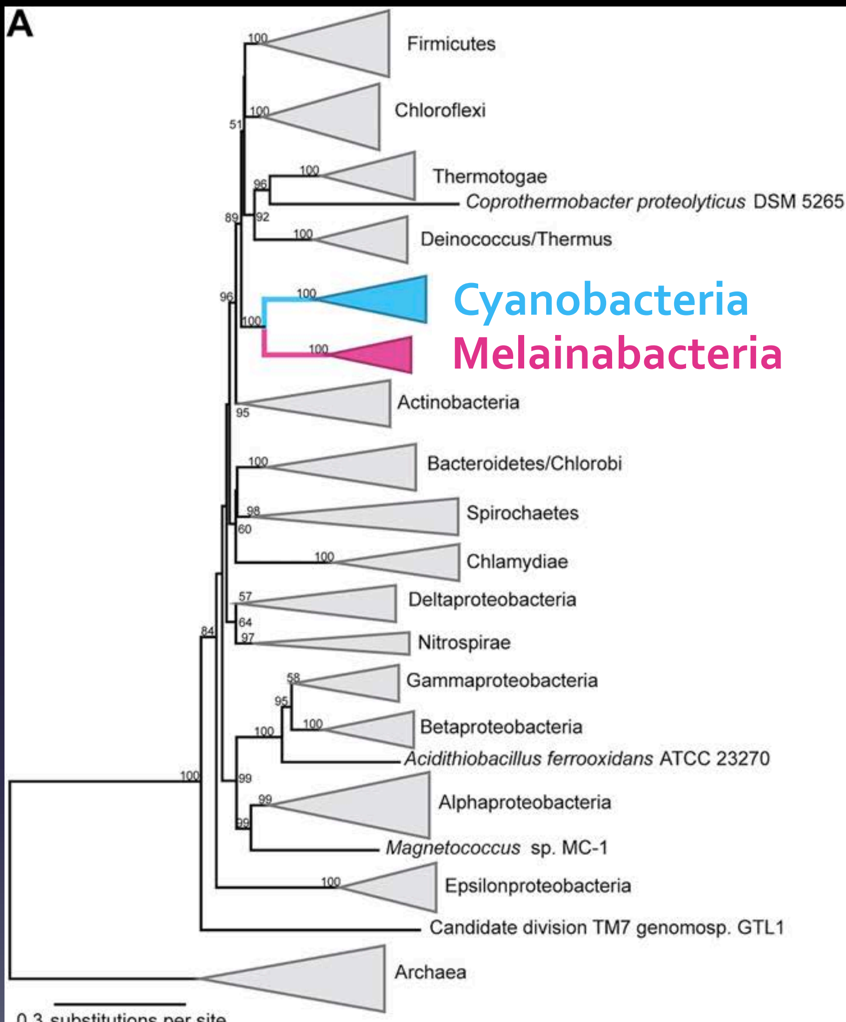


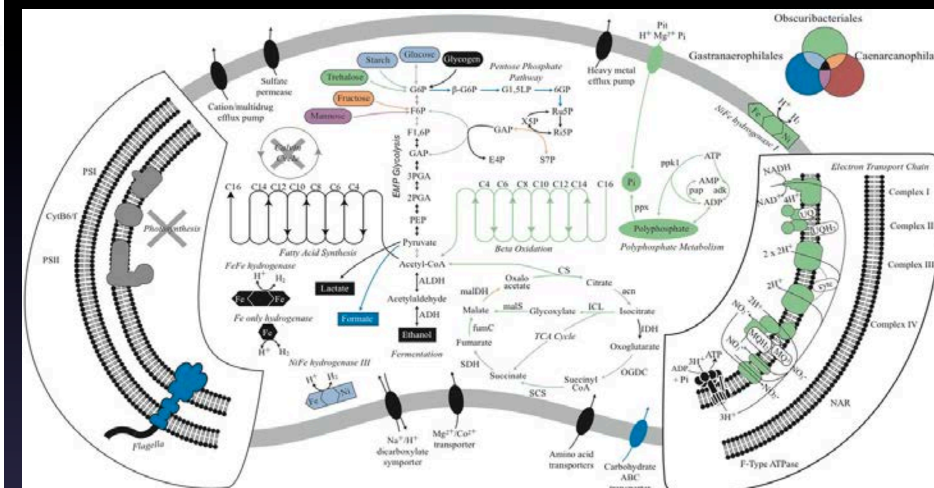
Proteobacteria?
Chlorobi?
Heliobacteria?
Xiong et al, 2006

Perspective can come from unexpected places

Shih et al,
PNAS 2013

The closest living relatives to Cyanobacteria are non-photosynthetic

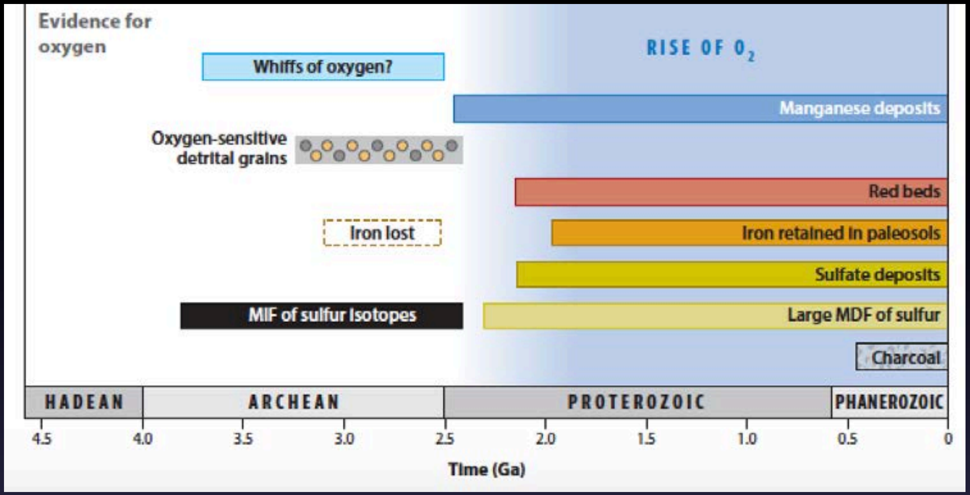




Di Rienzi et al, eLife 2013
Soo et al, Genome Biol Evol 2014

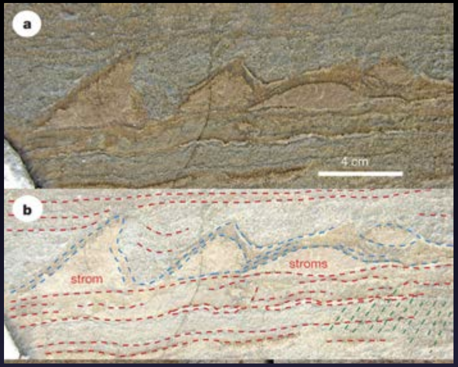
How old are cyanobacteria?

Geological proxies for oxygen

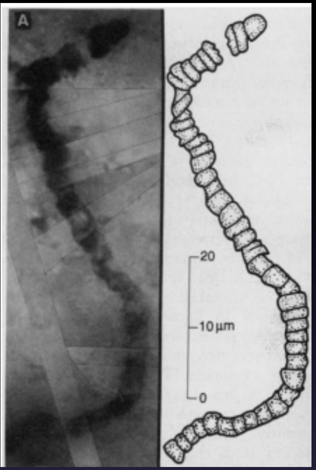


Fischer et al 2016, AREPS

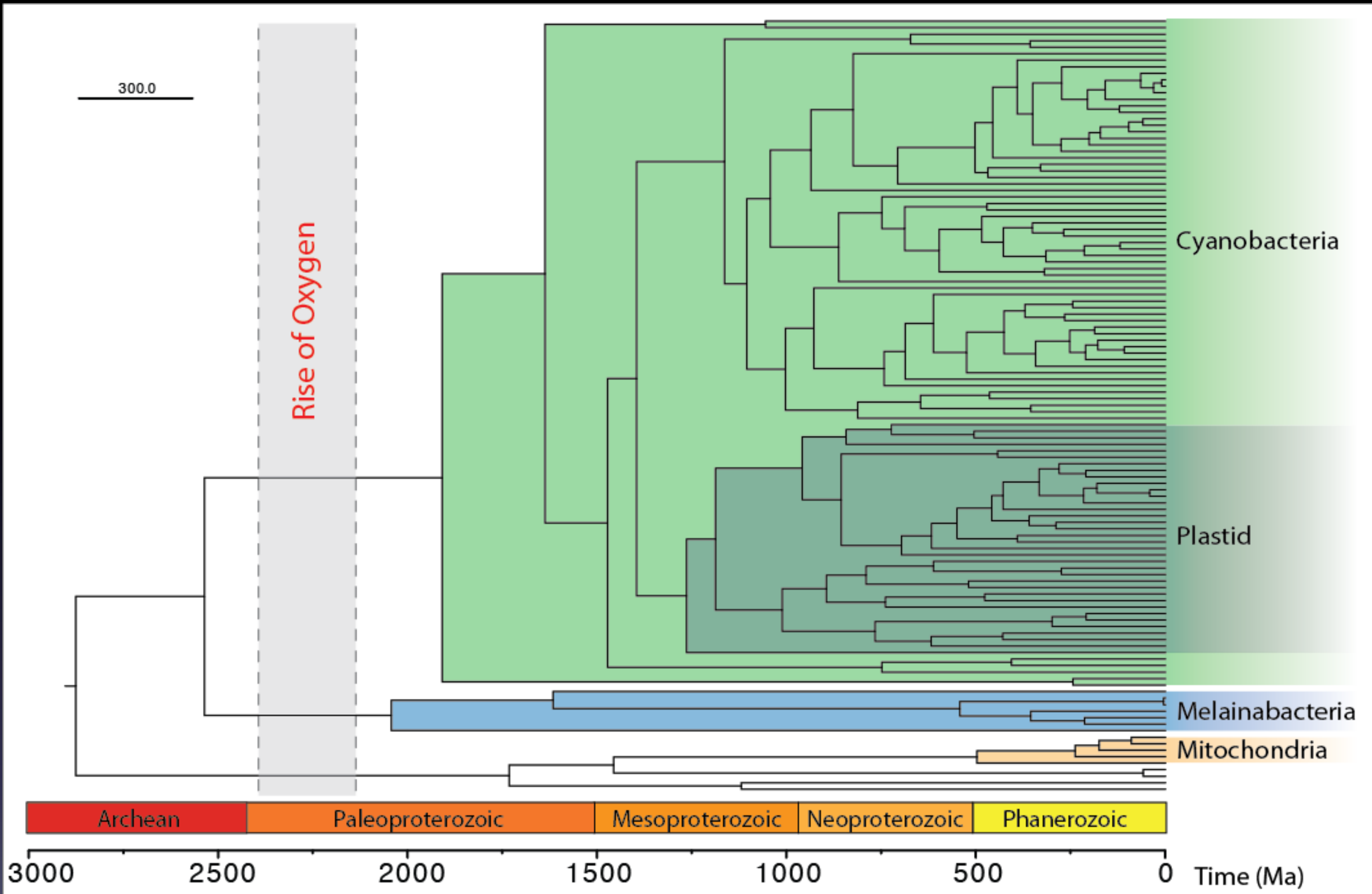
Putative fossil evidence



Nutman et al. 2016 Science

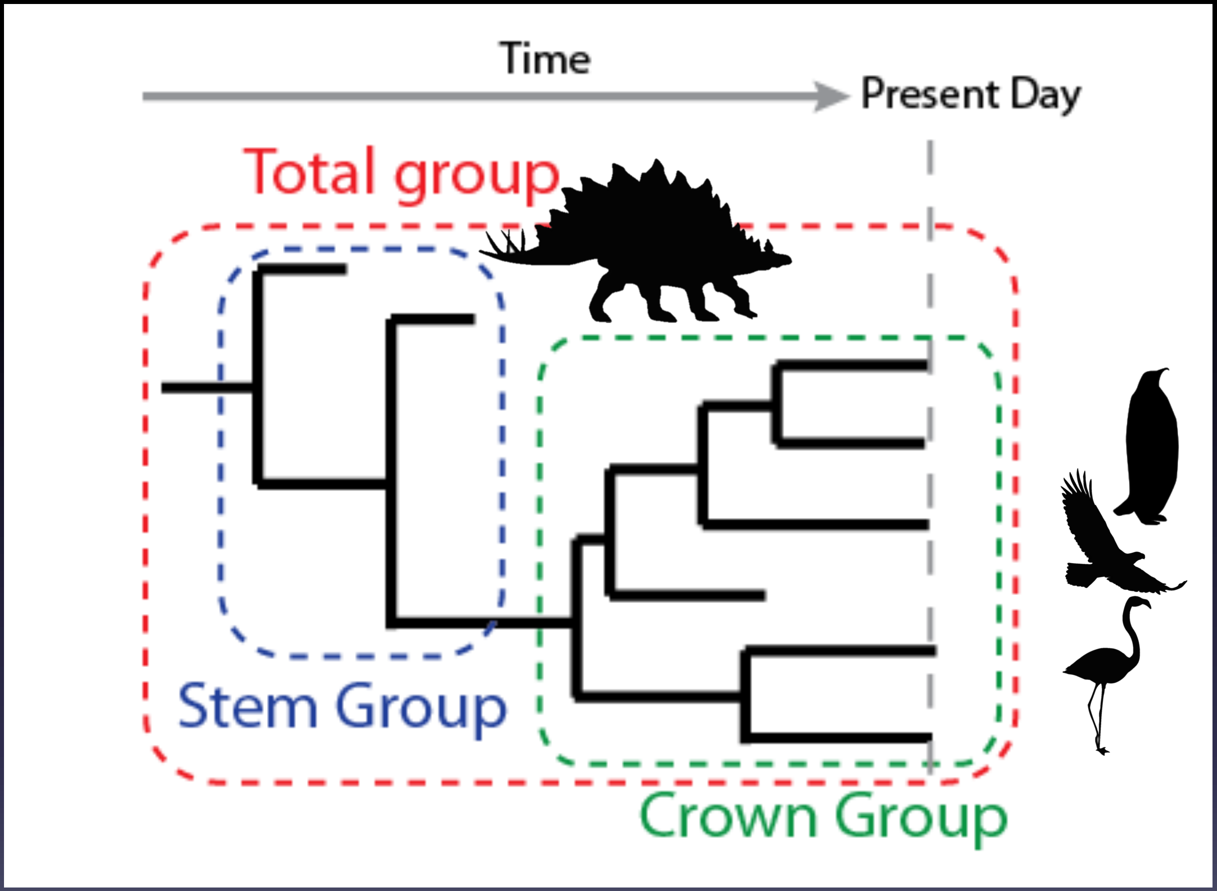


Schopf et al 1993, Science

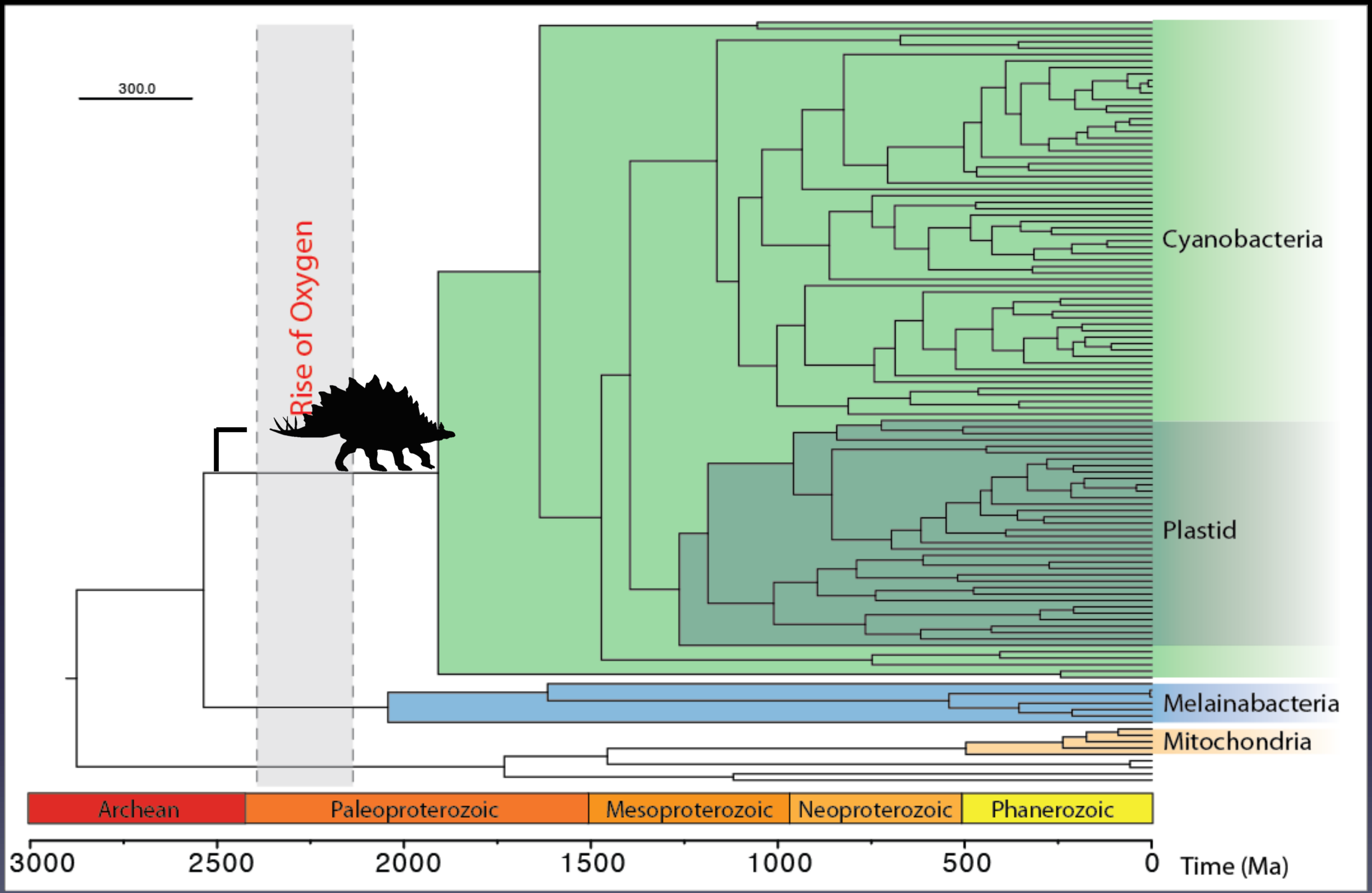


Shih et al, 2017

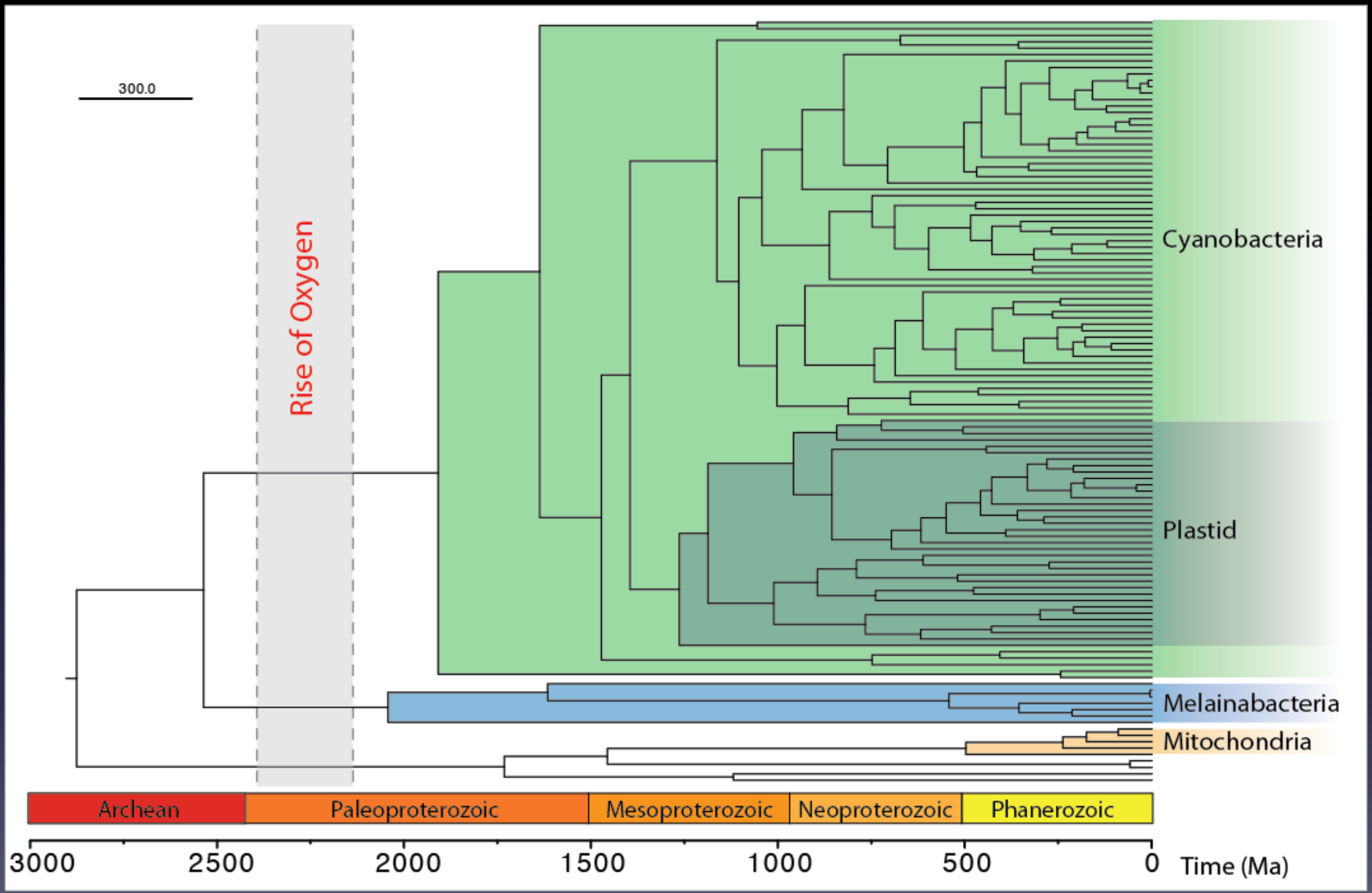
Crown group cyanobacteria postdate the Rise of Oxygen



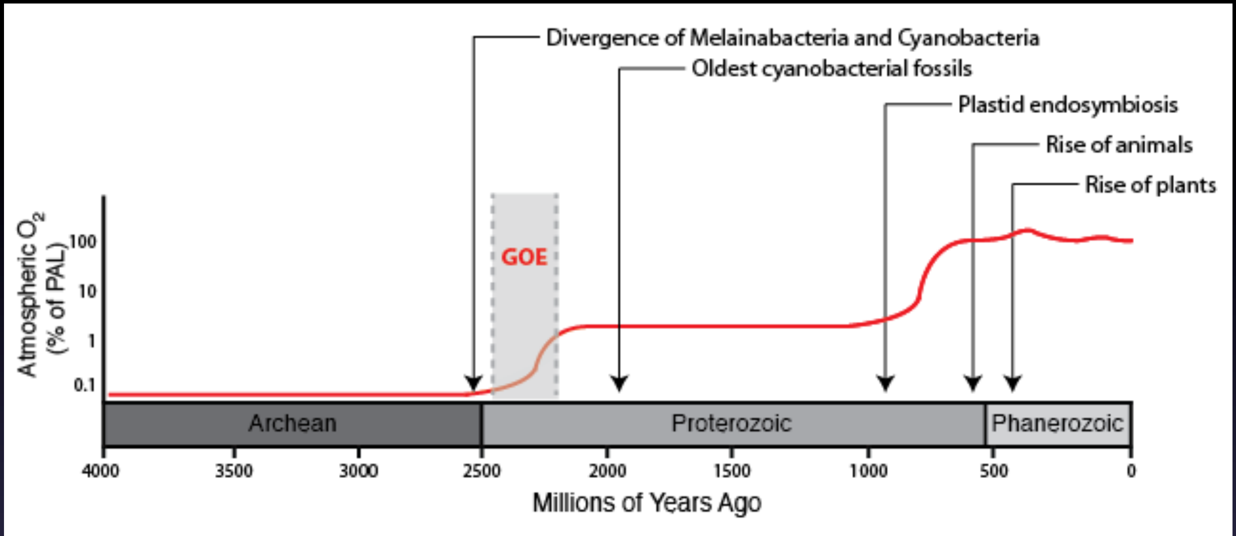
Crown group cyanobacteria postdate the Rise of Oxygen

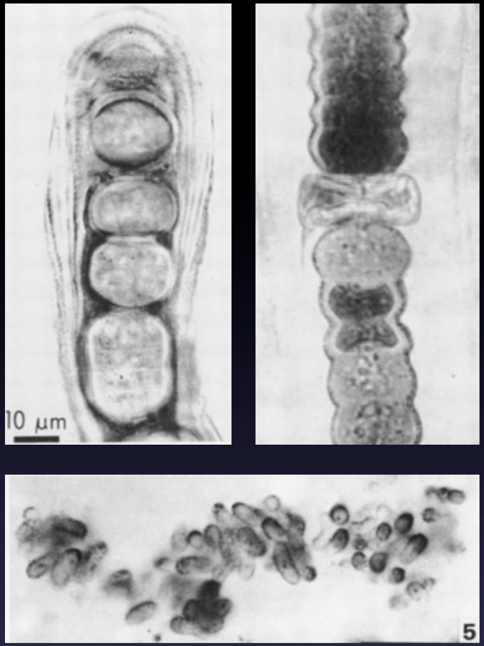


Melainabacteria and Cyanobacteria diverged prior to the Rise of Oxygen



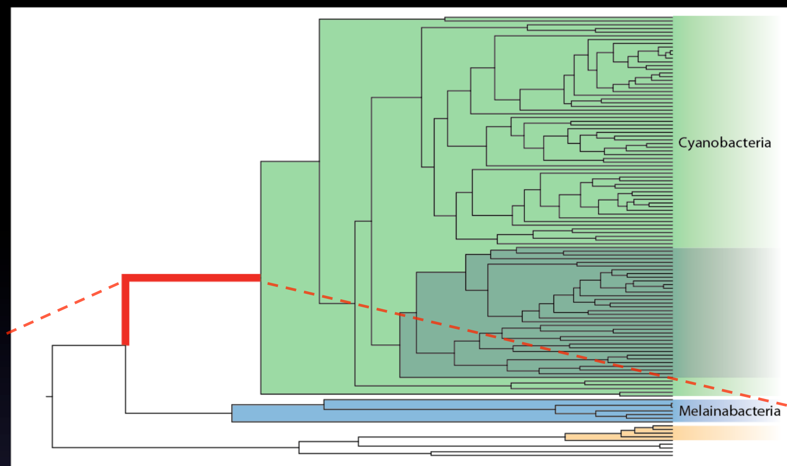
Molecular clock analyses support the fossil record

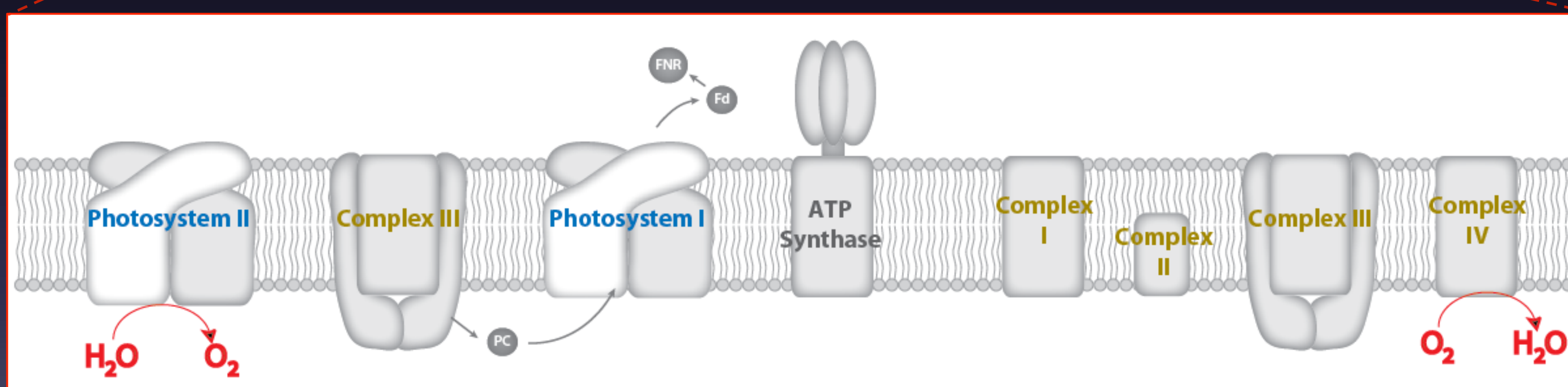




Hoffman, Chem Geol 1976

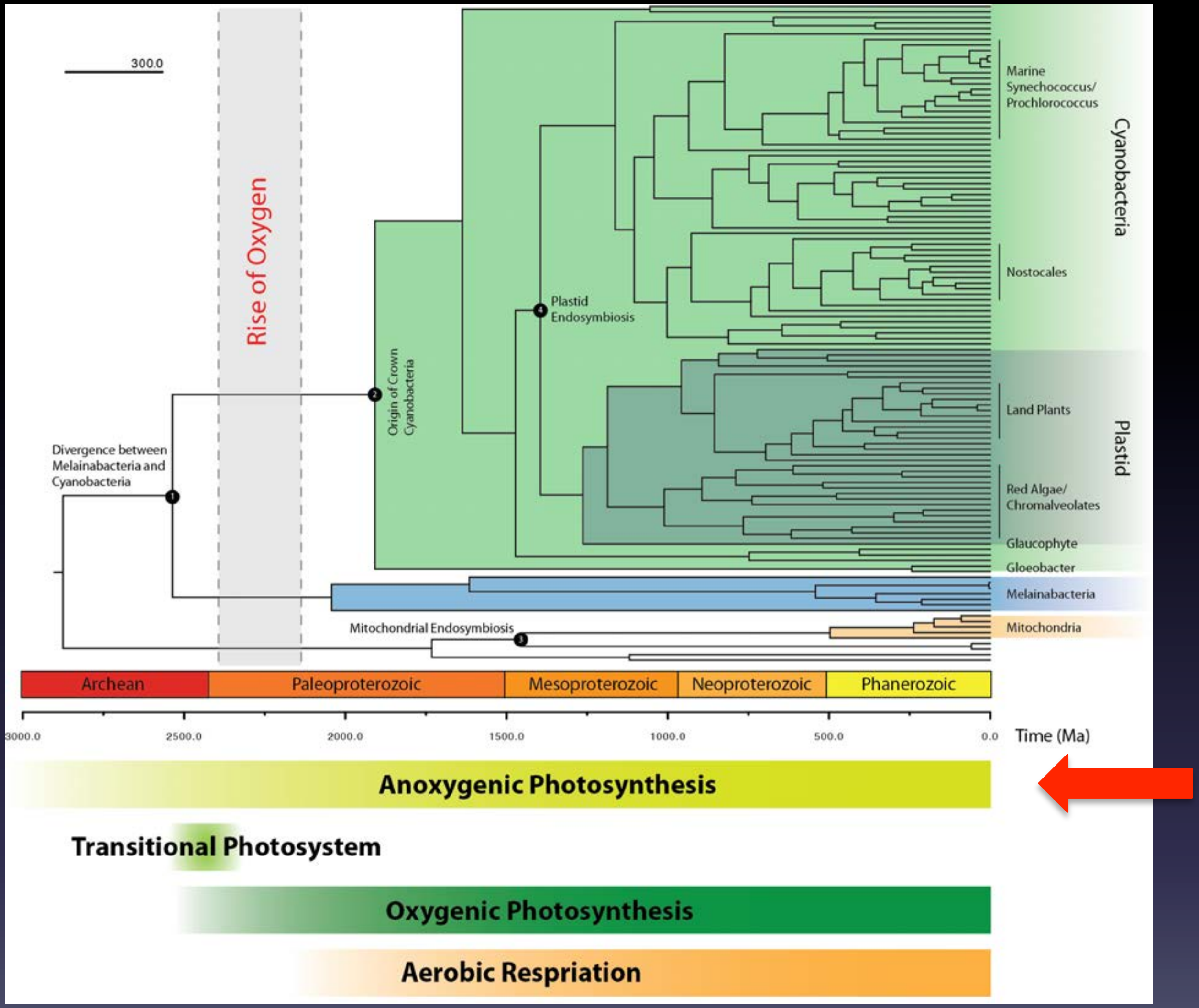
Modern photosynthesis requires more than just photosystem II





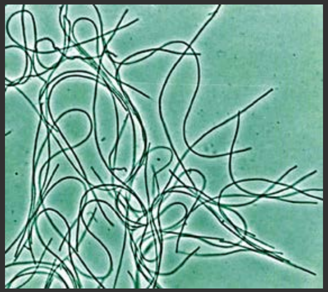
Adapted from Fischer et al, AREPS 2016

A best guess on the evolution of photosynthesis

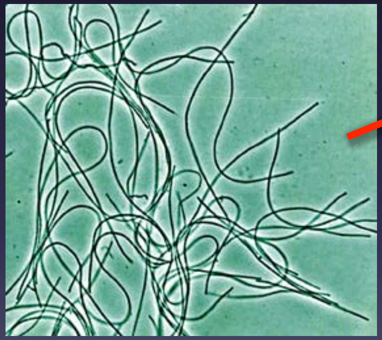


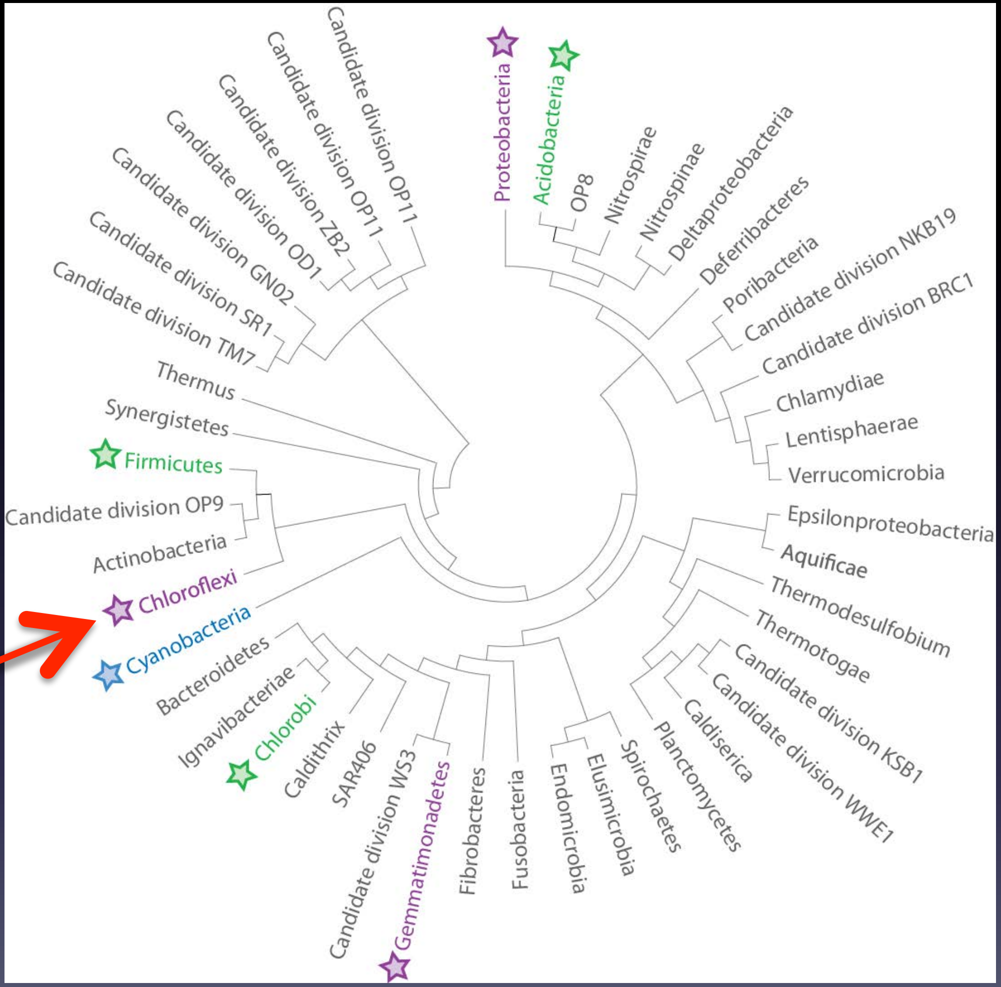
When did anoxygenic photosynthesis evolve?

Origins of anoxygenic photosynthesis



It is unclear which phyla anoxygenic photosynthesis arose from

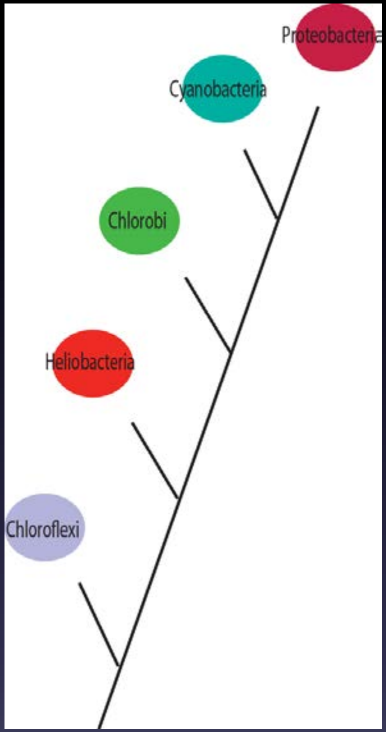




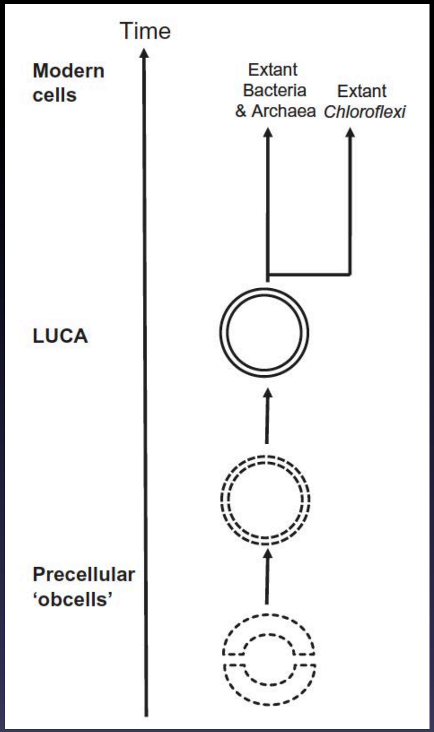
Fischer et al, AREPS 2016

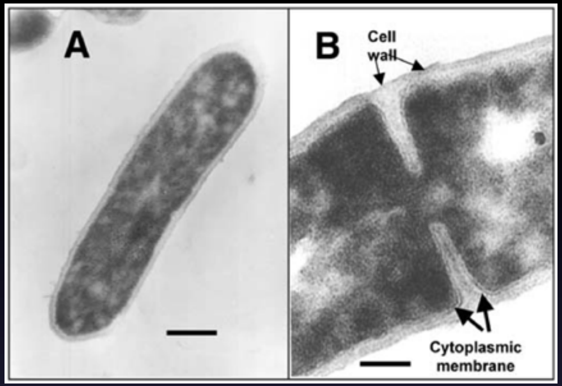
The Chloroflexi have traditionally been suggested to be one of the oldest bacterial lineages

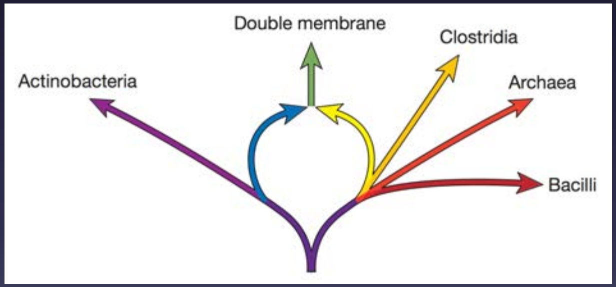
16S phylogeny



Membrane architecture

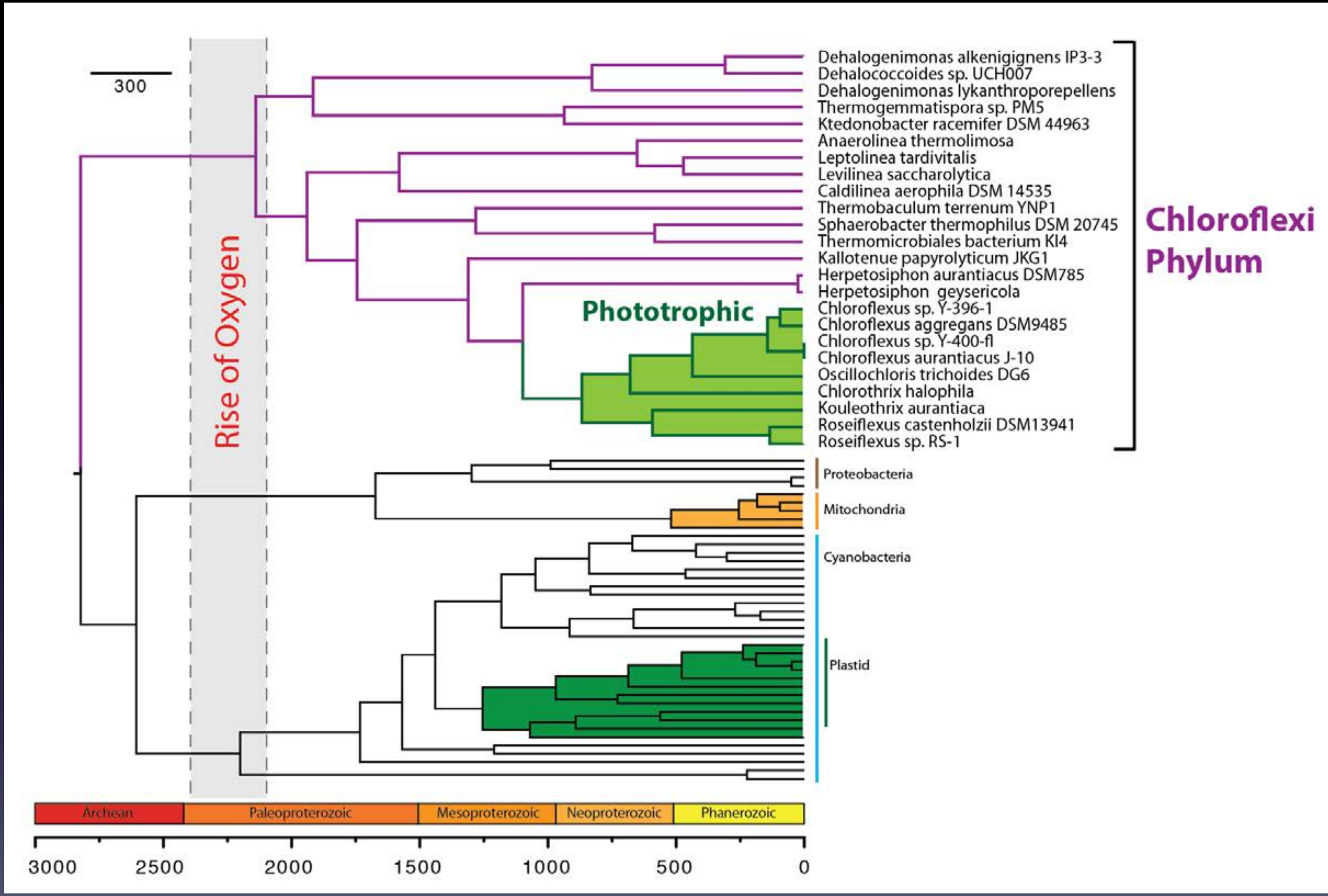






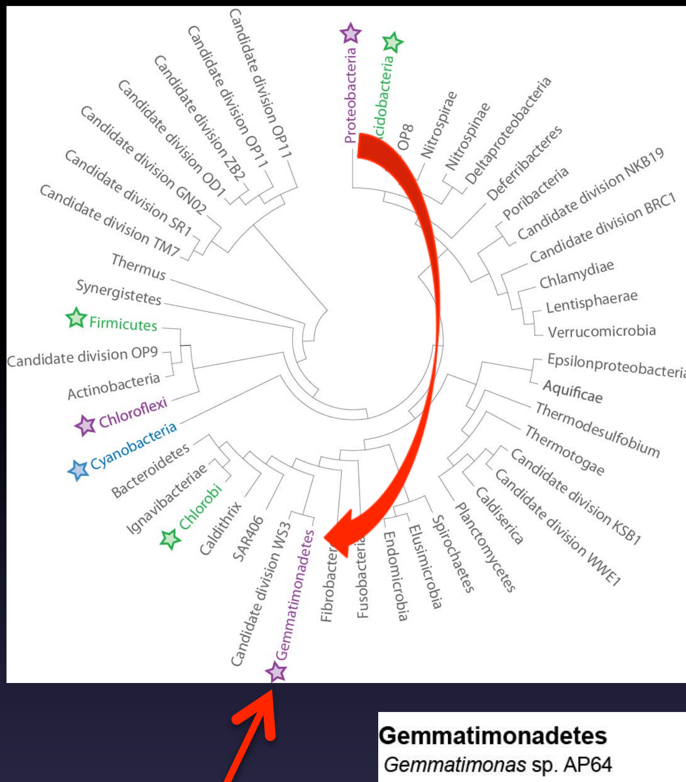
Xiong et al, Genome Biol 2006
Sutcliffe, Environ Microbiol 2011
Lake, Nature 2009
Botero et al, Arch Microbiol 2004

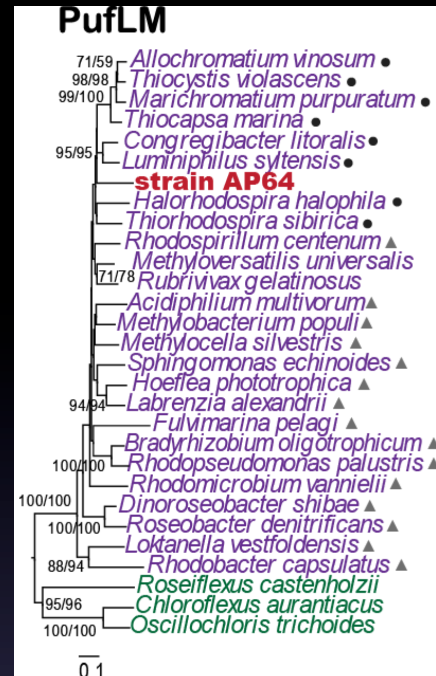
Late evolution of phototrophy in the Chloroflexi



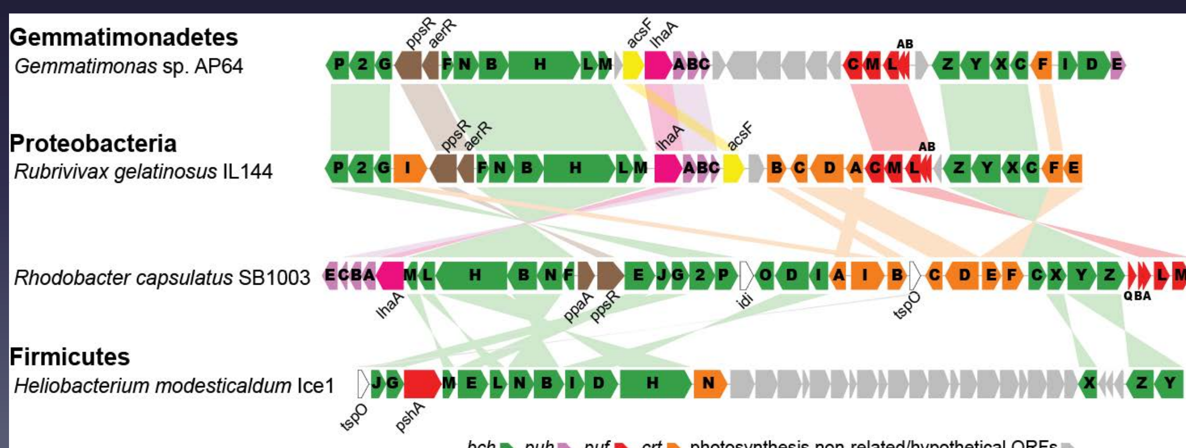
Shih et al, PNAS 2017

Phototrophy can be horizontally transferred



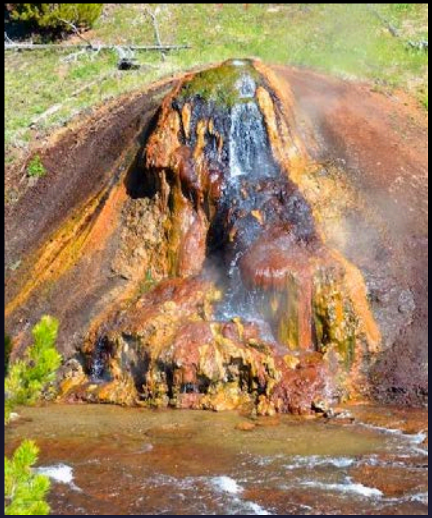


What about carbon fixation?

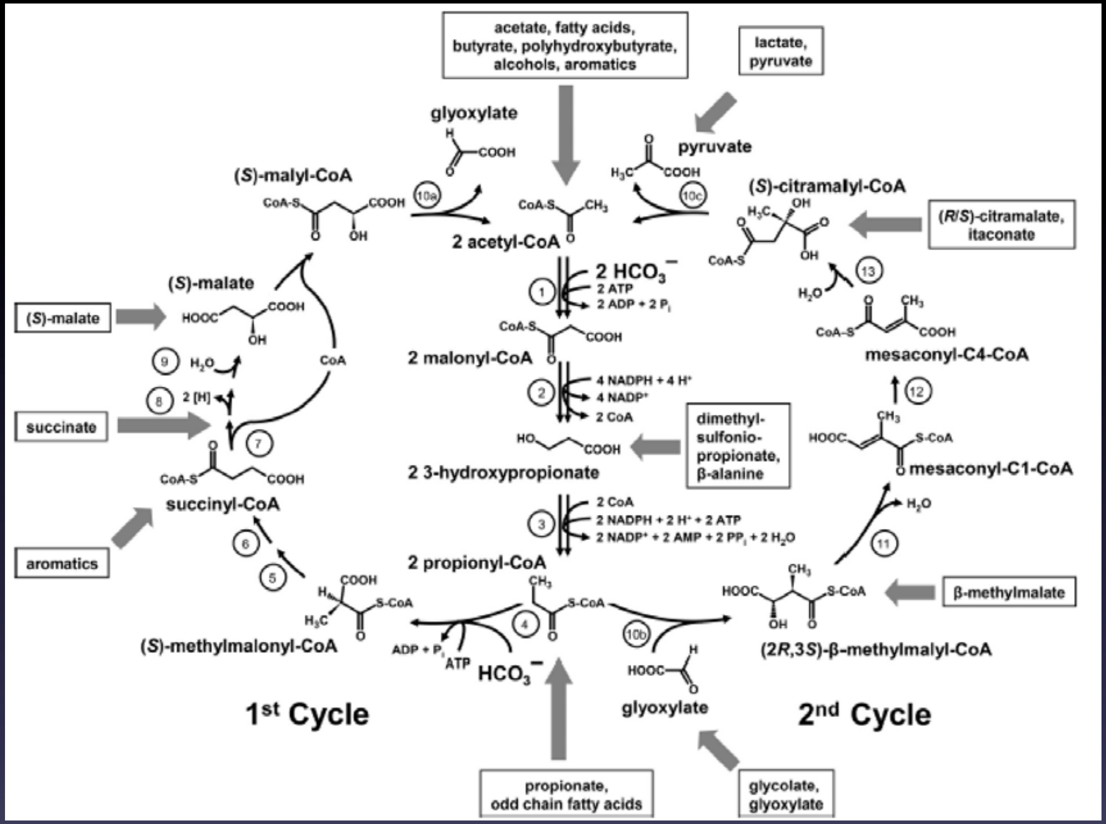


Zeng et al, 2014

Plasticity of central carbon metabolism in Chloroflexi enabled evolution of 3HP

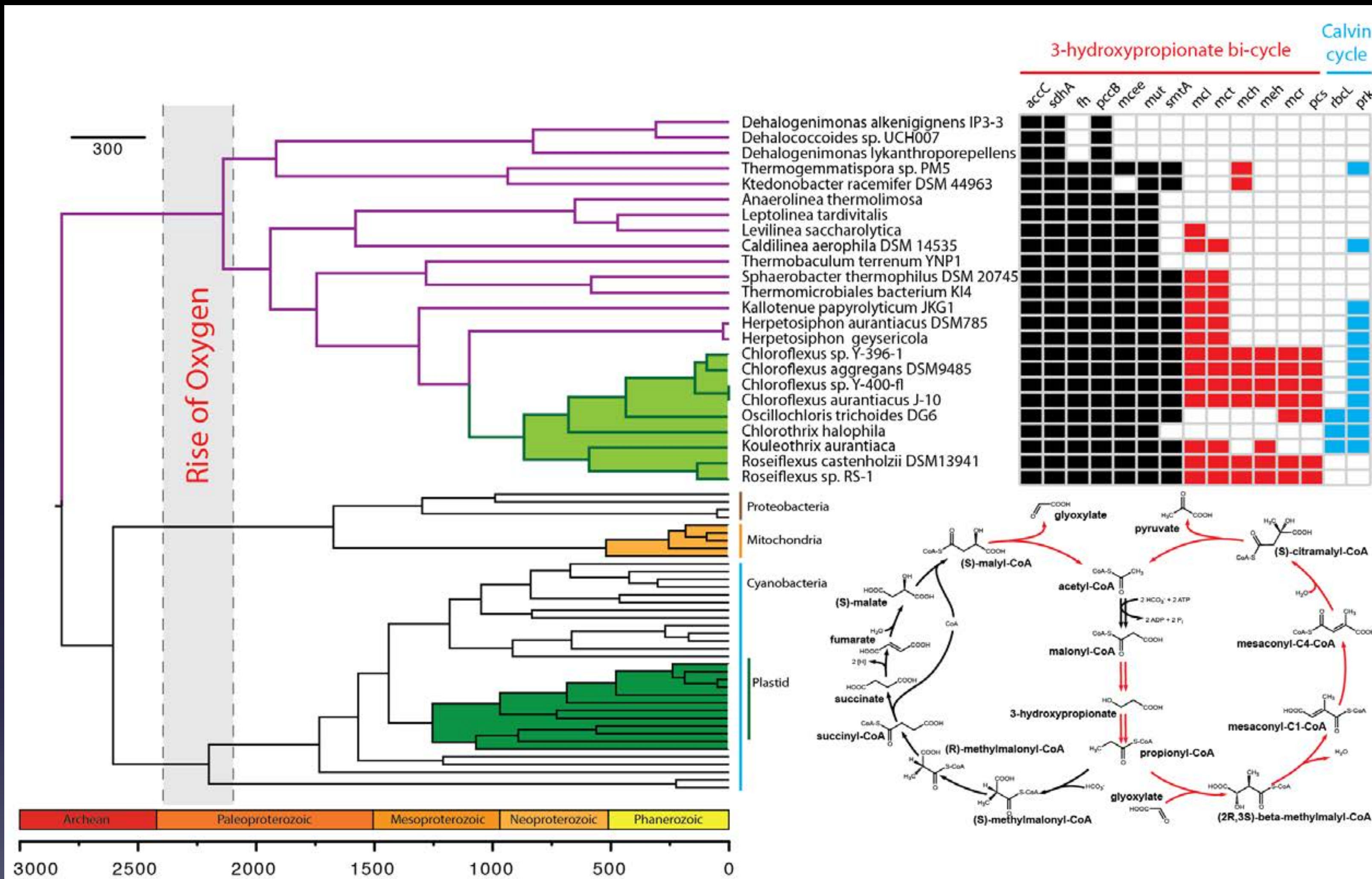


Chocolate Pots, Yellowstone

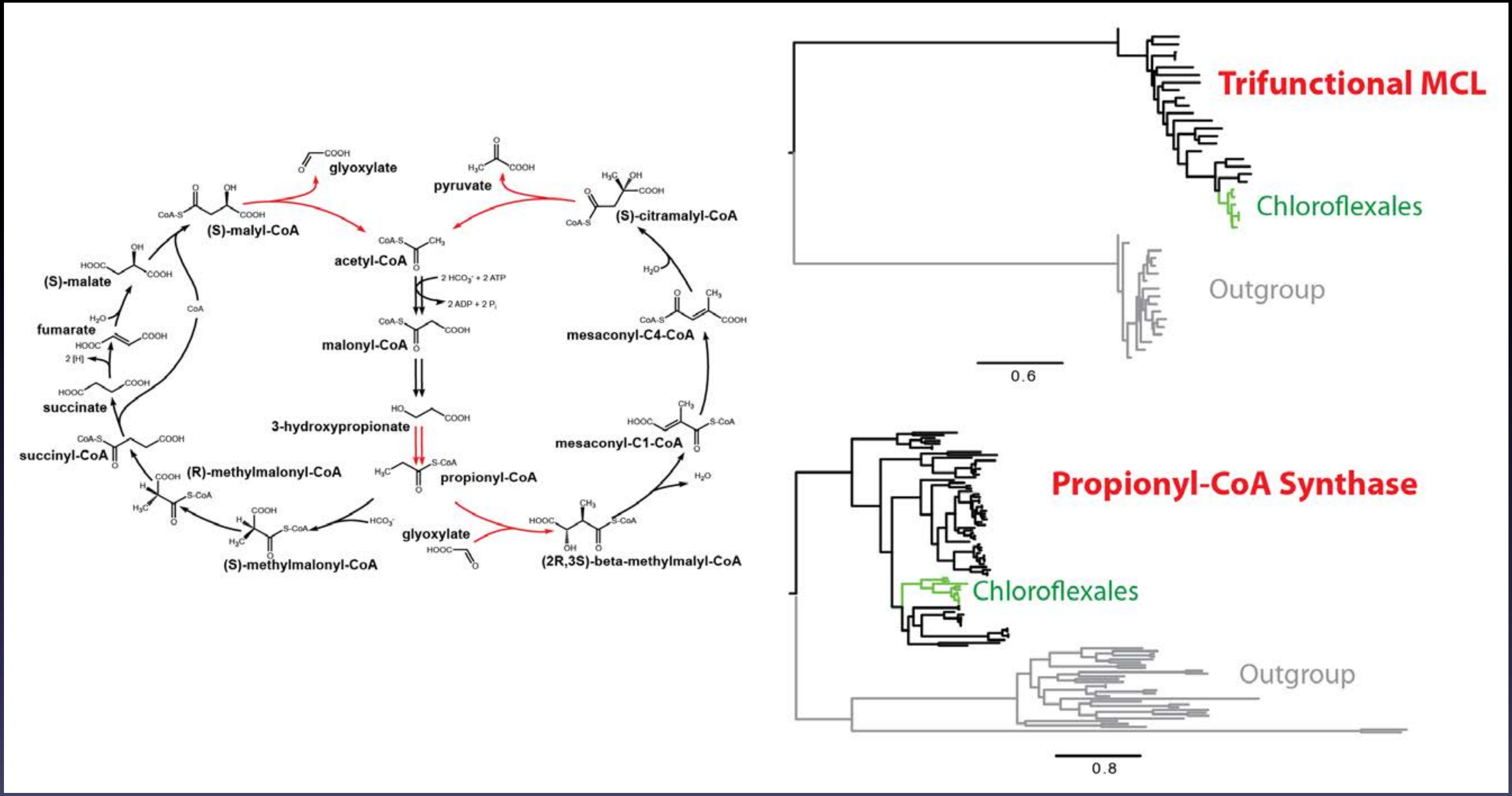


Zarzycki and Fuchs, AEM 2011

Comparative genomics reveals piecemeal evolution of the 3HP bicycle



Horizontal gene transfer of key enzymes enabled late evolution of the 3HP bicycle



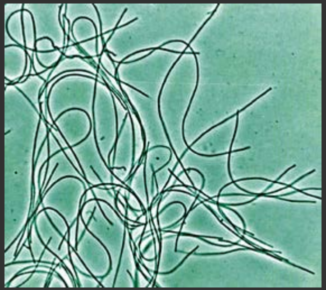
Conclusions

- Advances in sequencing technologies and metagenomics will help discover more missing links to fill in major evolutionary gaps
- Improvements in molecular clocks will enable us to leverage sequence data to interrogate the timing of major events in early life

Improving molecular clocks

Origins of oxygenic photosynthesis

Origins of anoxygenic photosynthesis

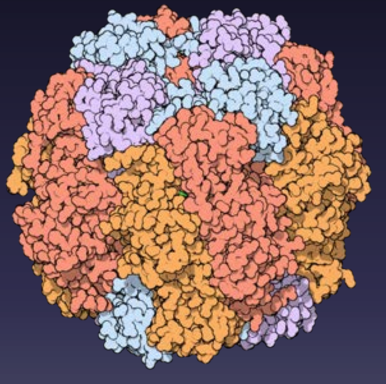


Outline

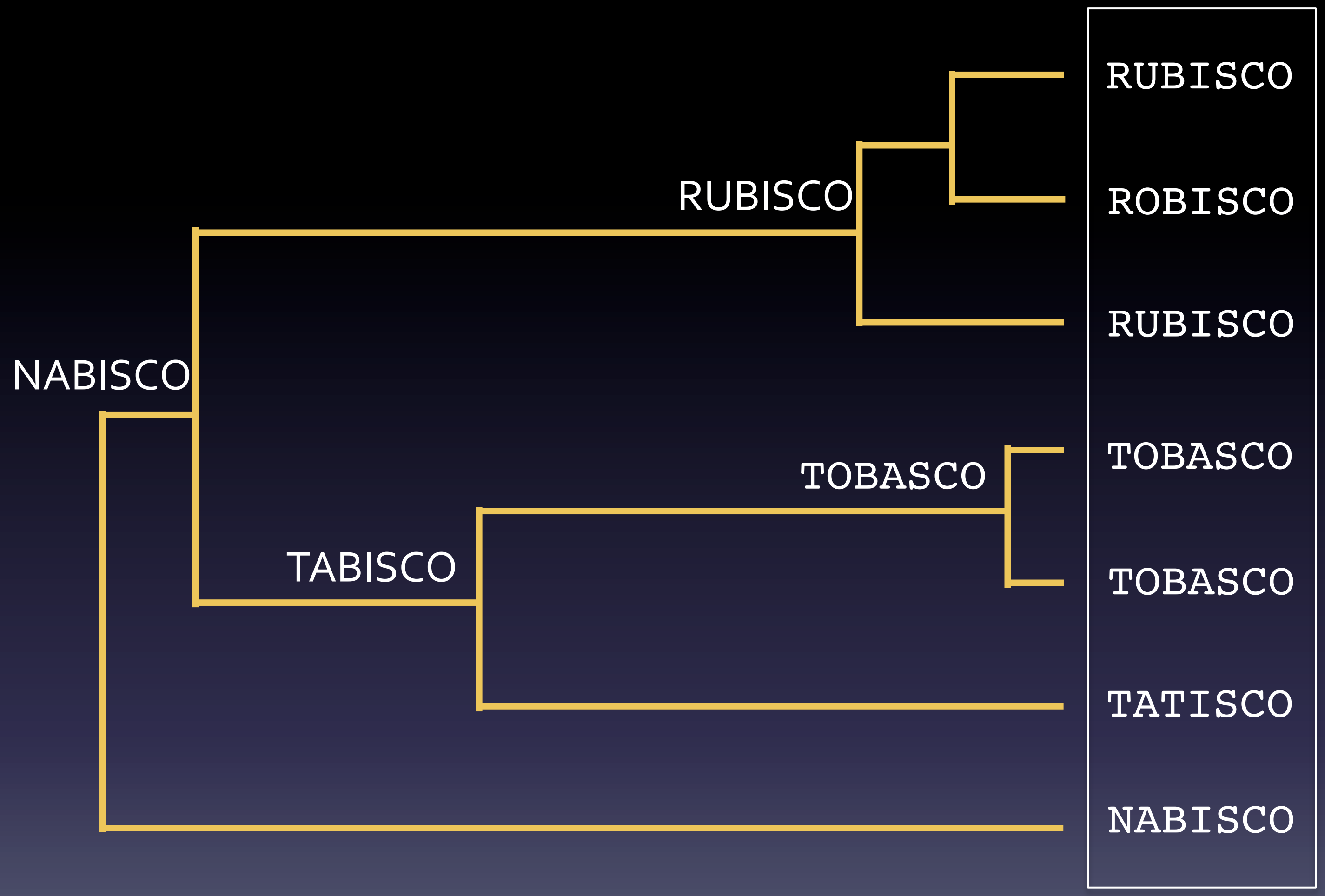
I. When did photosynthesis evolve?

II. How did photosynthesis evolve at molecular level?

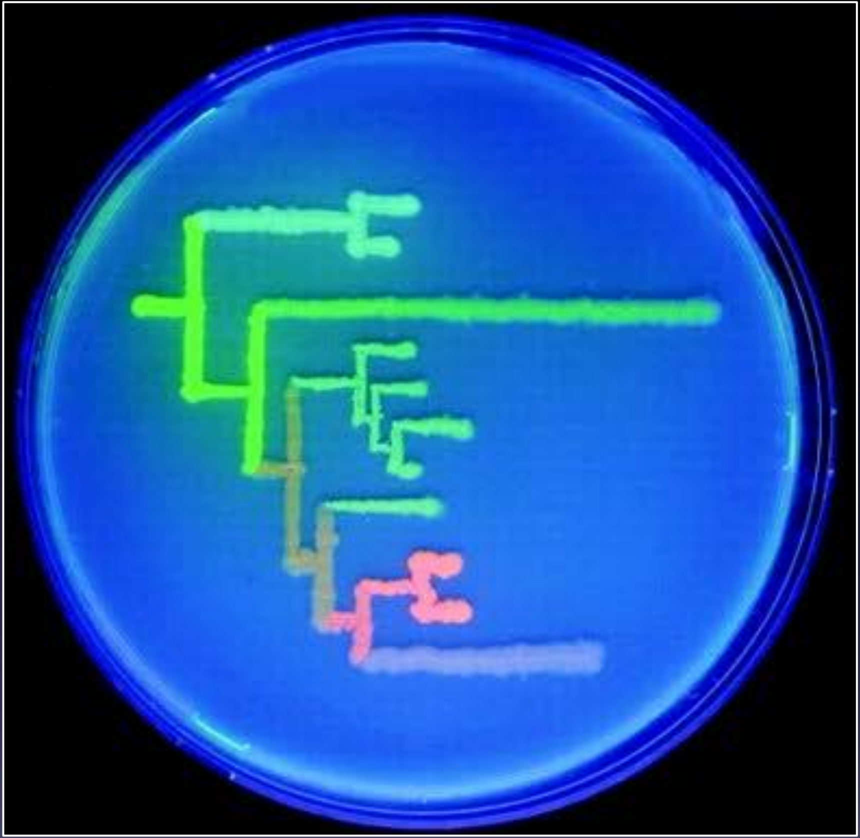
III. Can we reconstruct and redesign photosynthesis?



Ancestral Sequence Reconstruction

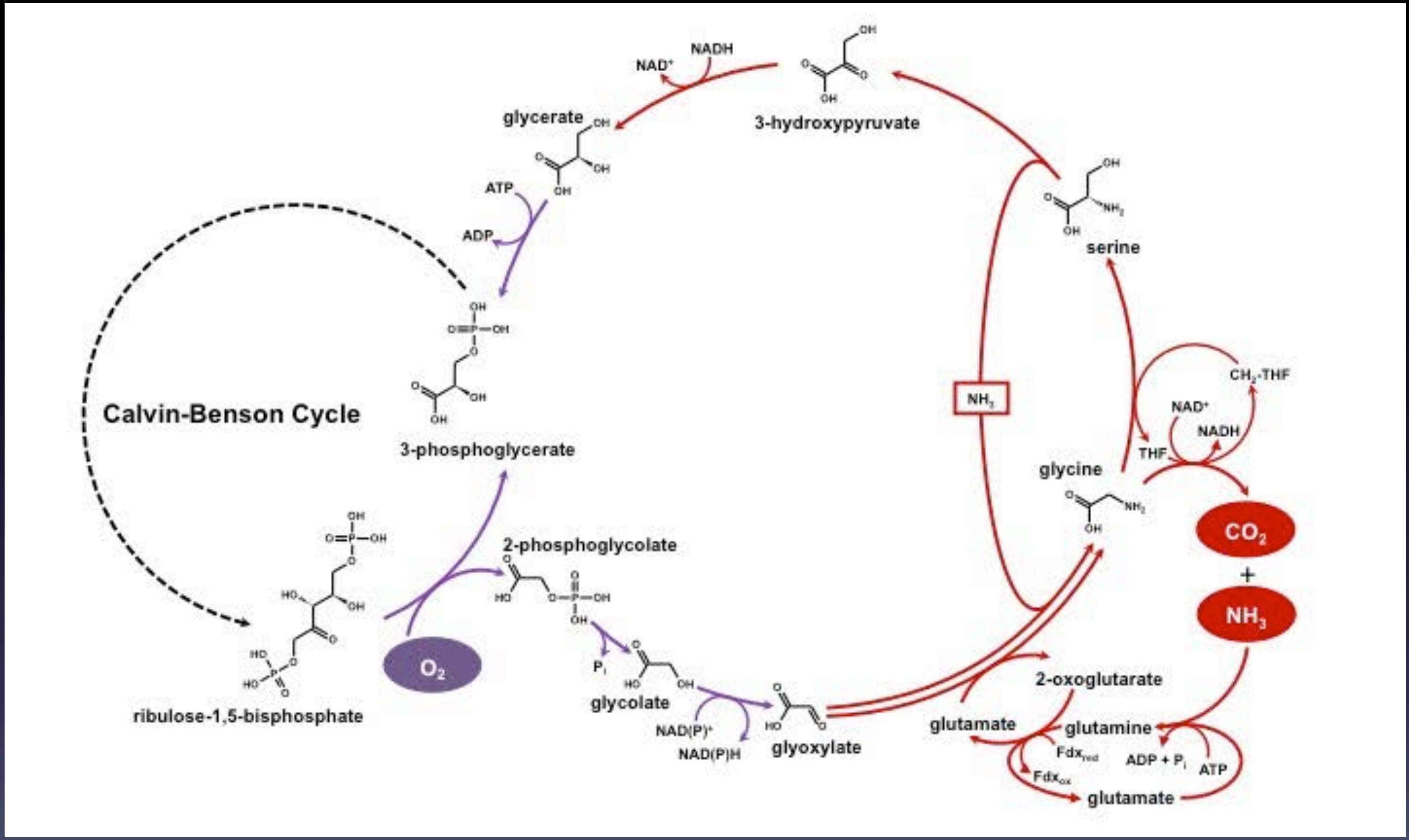


Ancestral Sequence Reconstruction enables us to study the properties of prehistoric proteins

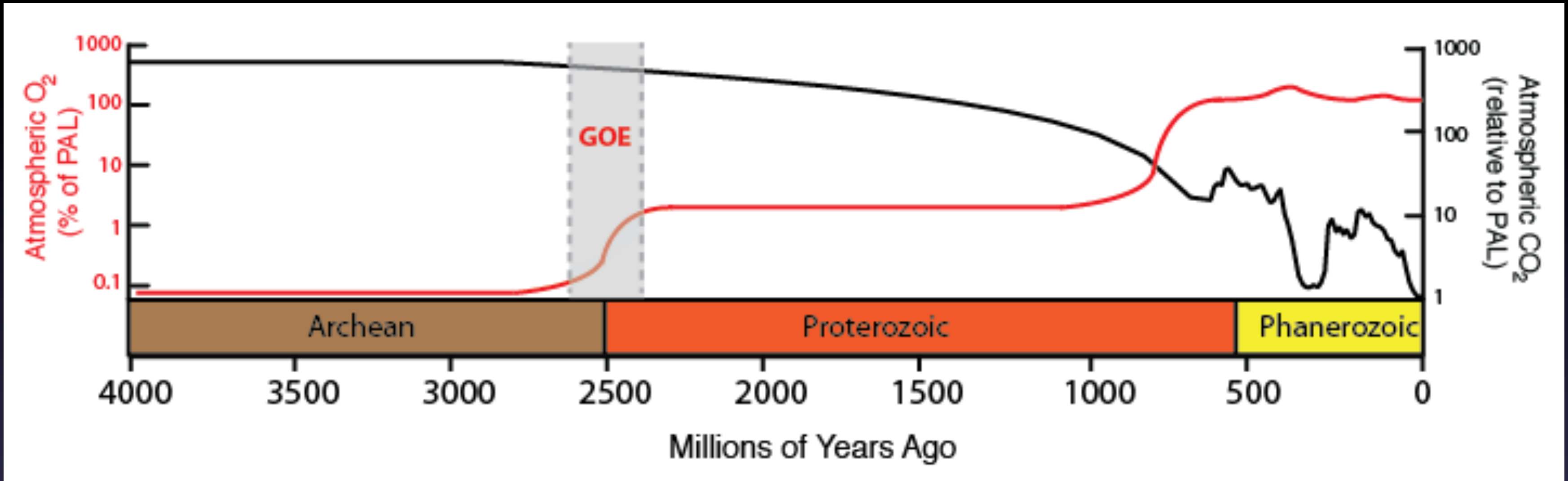


Ugalde et al, Science 2004

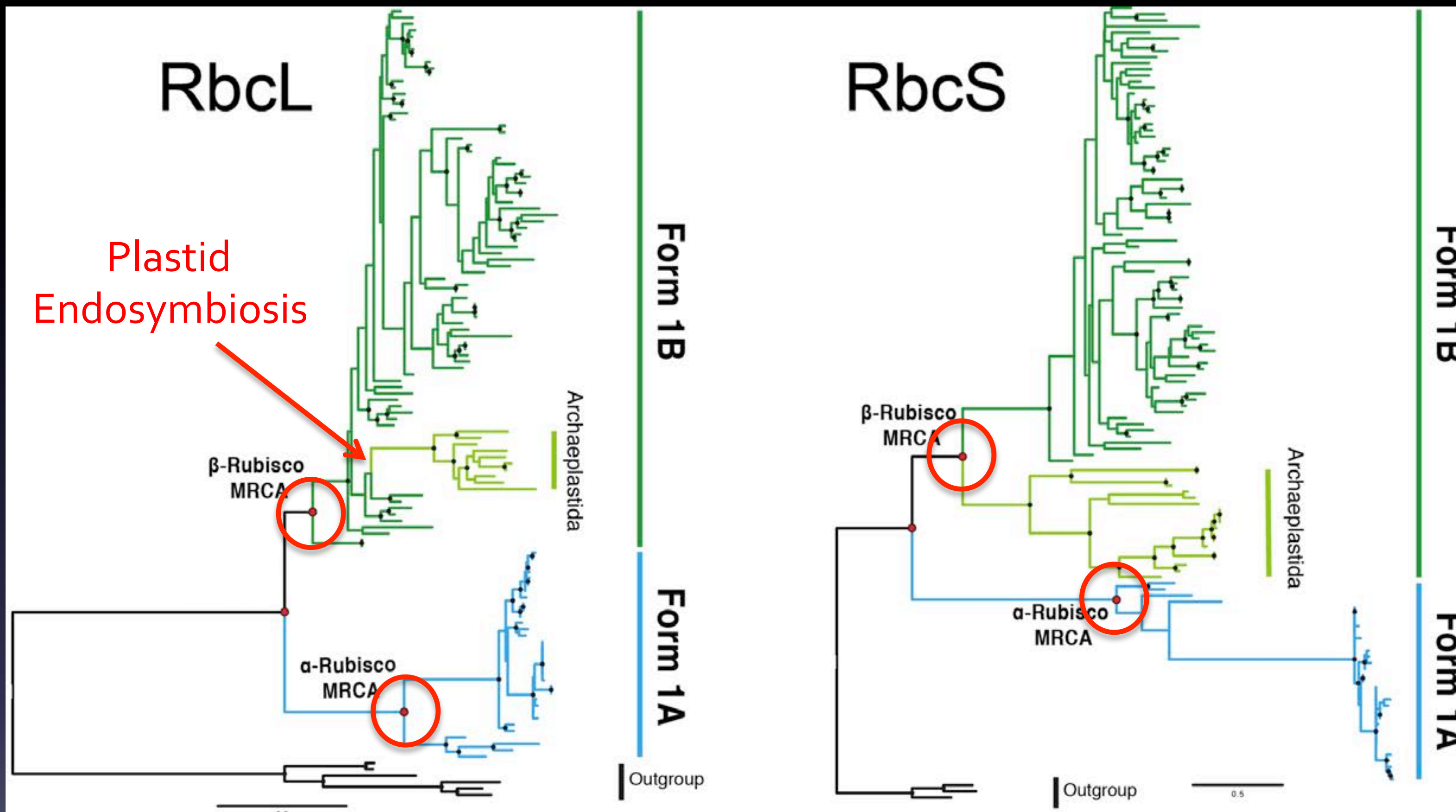
RuBisCO catalyzes two different reactions: Carboxylase and Oxygenase activities



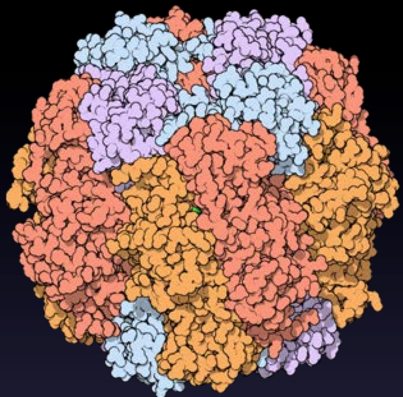
The Proterozoic atmosphere was substantially different from today's atmosphere

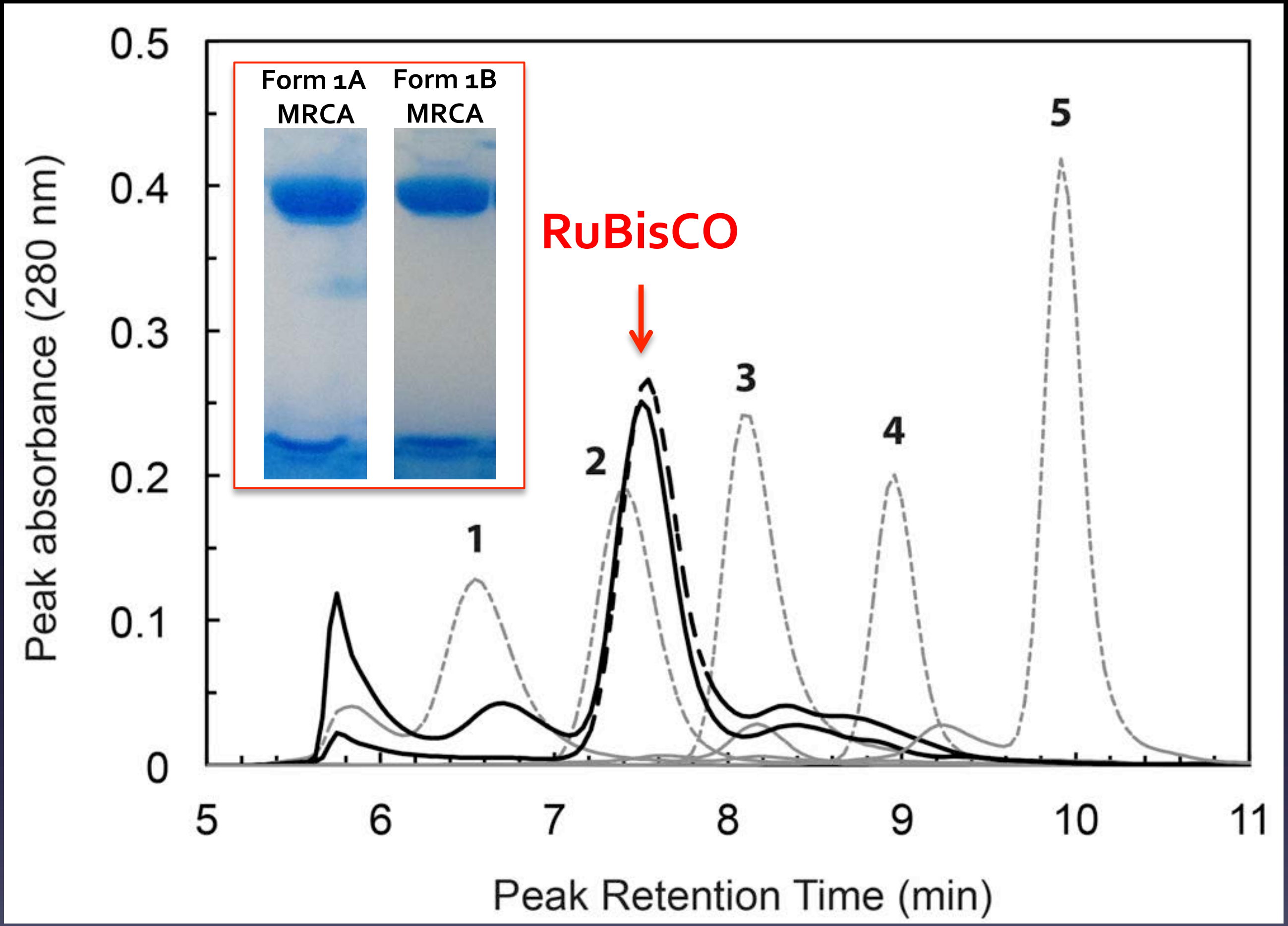


Using ancestral sequence reconstruction to investigate RuBisCO evolution



Ancestral RuBisCOs form L_8S_8 holocomplex

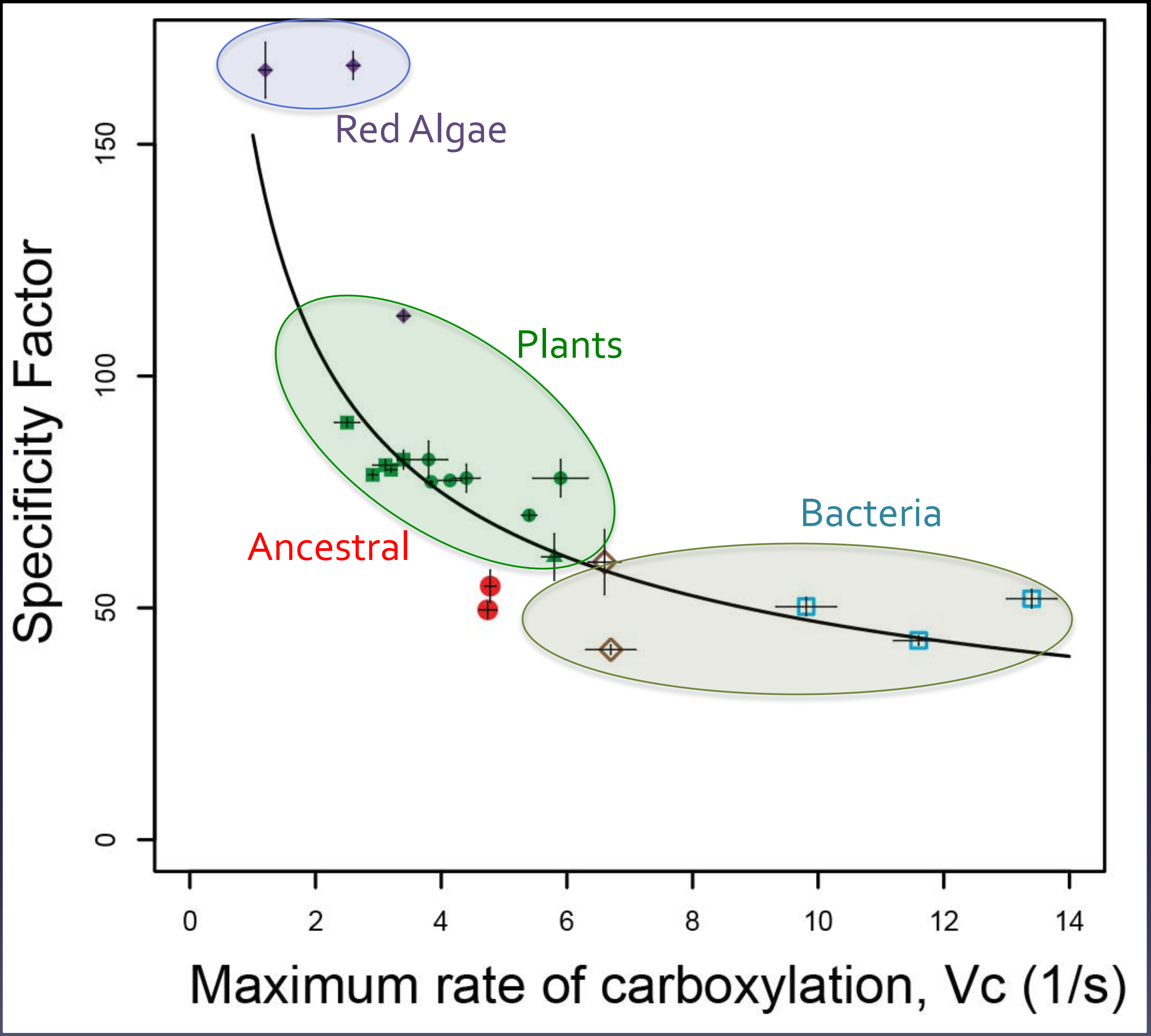




Ancestral RuBisCO kinetic properties correspond to the Proterozoic atmosphere

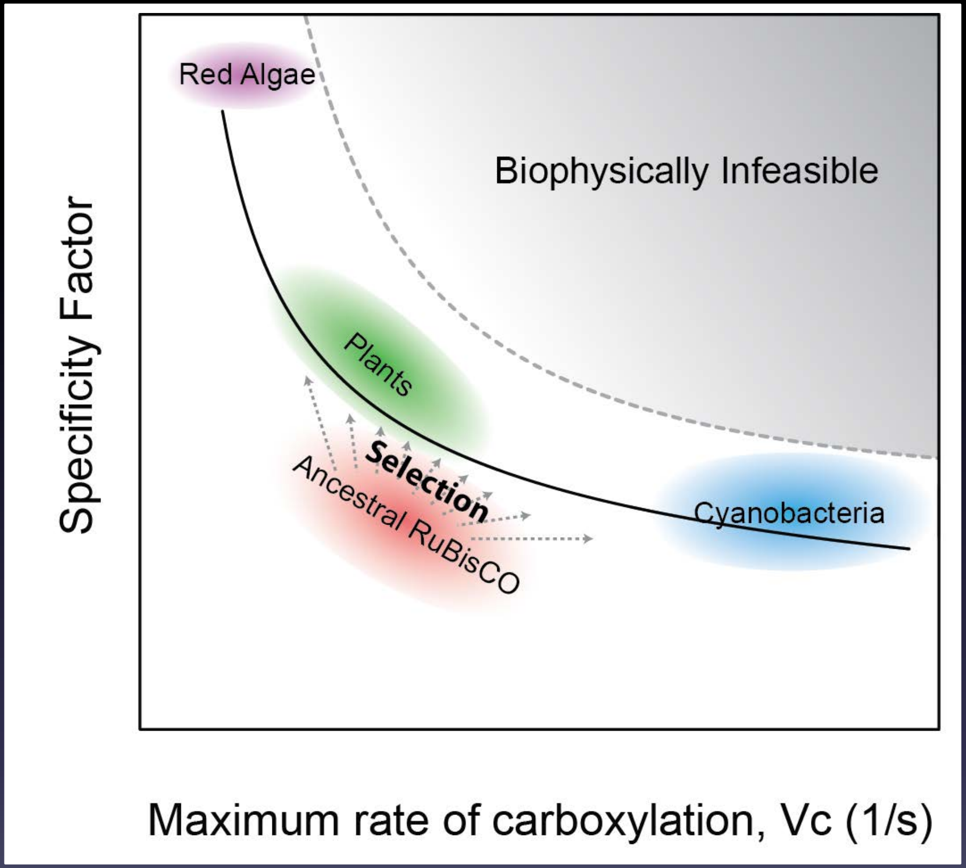
RuBisCO	Maximum rate of carboxylation (s^{-1})	Specificity factor
Ancestral form 1A	4.77 ± 0.09	54.7 ± 3.5
Ancestral form 1B	4.72 ± 0.14	49.6 ± 1.8
Prochlorococcus marinus MIT9313	6.58 ± 0.25	59.9 ± 7.0
Synechococcus sp. PCC 6301	9.78 ± 0.48	50.3 ± 2.0
Triticum aestivum	2.92 ± 0.08	100.0 ± 3.8

Shih et al, 2016



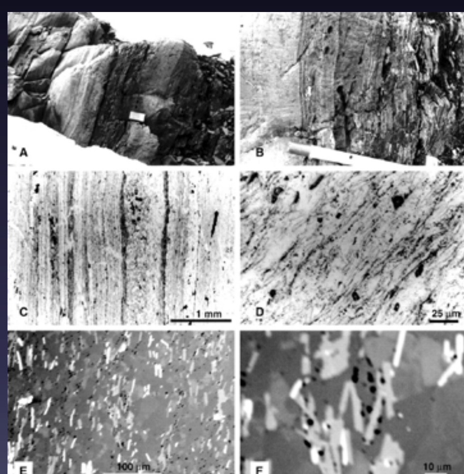
Red Algae
Plants
Ancestral
Bacteria
Specificity Factor
0
50
100
150
0
2
4
6
8
10
12
14
Maximum rate of carboxylation, Vc (1/s)

Atmospheric selective pressures guided divergent evolutionary paths of ancestral RuBisCO

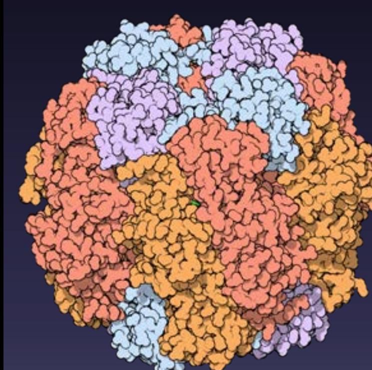


Conclusions

- Synthetic biology provides a means to resurrect extinct life
- Synthetic biology will allow us to link molecular sequences to the rock record



Rosing 1999, Science



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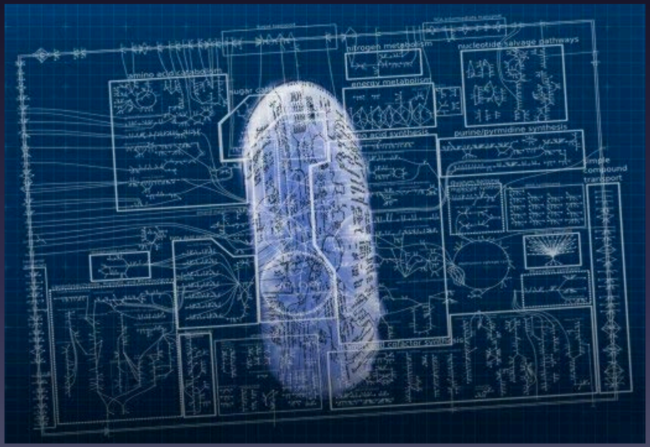
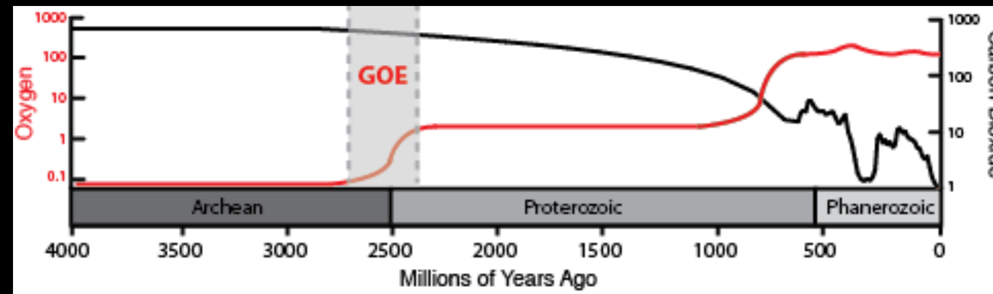
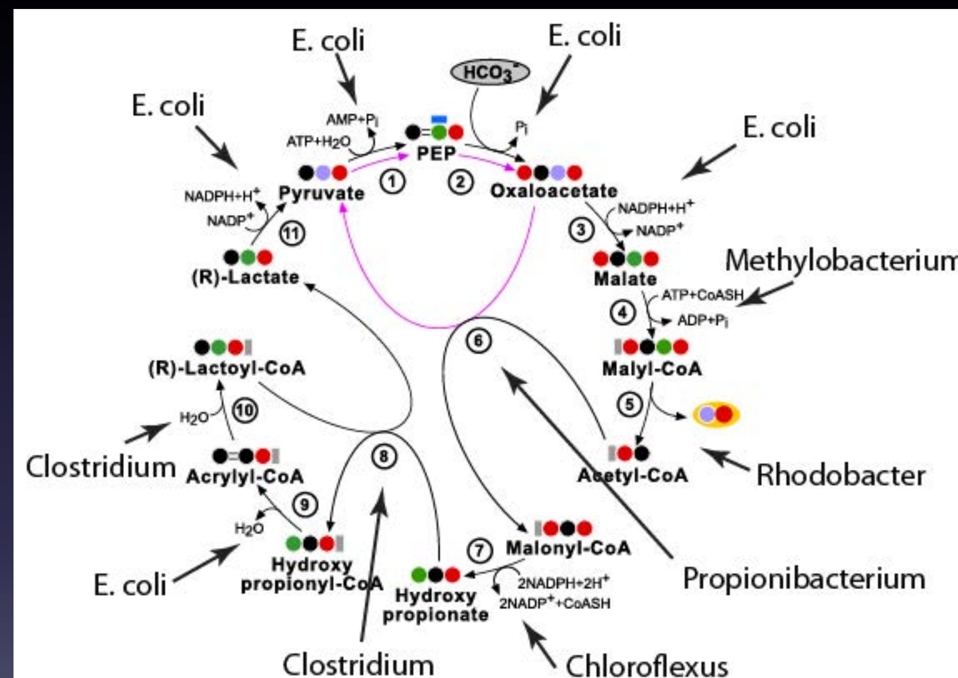


Rethinking carbon fixation

Pathway	CO_2-fixing enzymes	Carbon isotope fractionation
Reductive pentose phosphate cycle (Calvin–Benson–Bassham cycle)	RubisCO	−20 to −30 ‰
Reductive citric acid cycle (Arnon–Buchanan cycle)	2-Oxoglutarate synthase	−2 to −12 ‰
	Isocitrate dehydrogenase	
	Pyruvate synthase	
	PEP carboxylase	
Reductive acetyl-CoA pathway (Wood–Ljungdahl pathway)	Acetyl-CoA synthase–CO dehydrogenase	< −30 ‰
	Formylmethanofuran dehydrogenase (in methanogens)	
	Pyruvate synthase	
3-Hydroxypropionate bicycle	Acetyl-CoA and propionyl-CoA carboxylase	−12.5 to −13.7 ‰
3-Hydroxypropionate–4-hydroxybutyrate cycle	Acetyl-CoA and propionyl-CoA carboxylase	−0.2 to −3.8
Dicarboxylate–4-hydroxybutyrate cycle	Pyruvate synthase	−0.2 to −3.8
	PEP carboxylase	

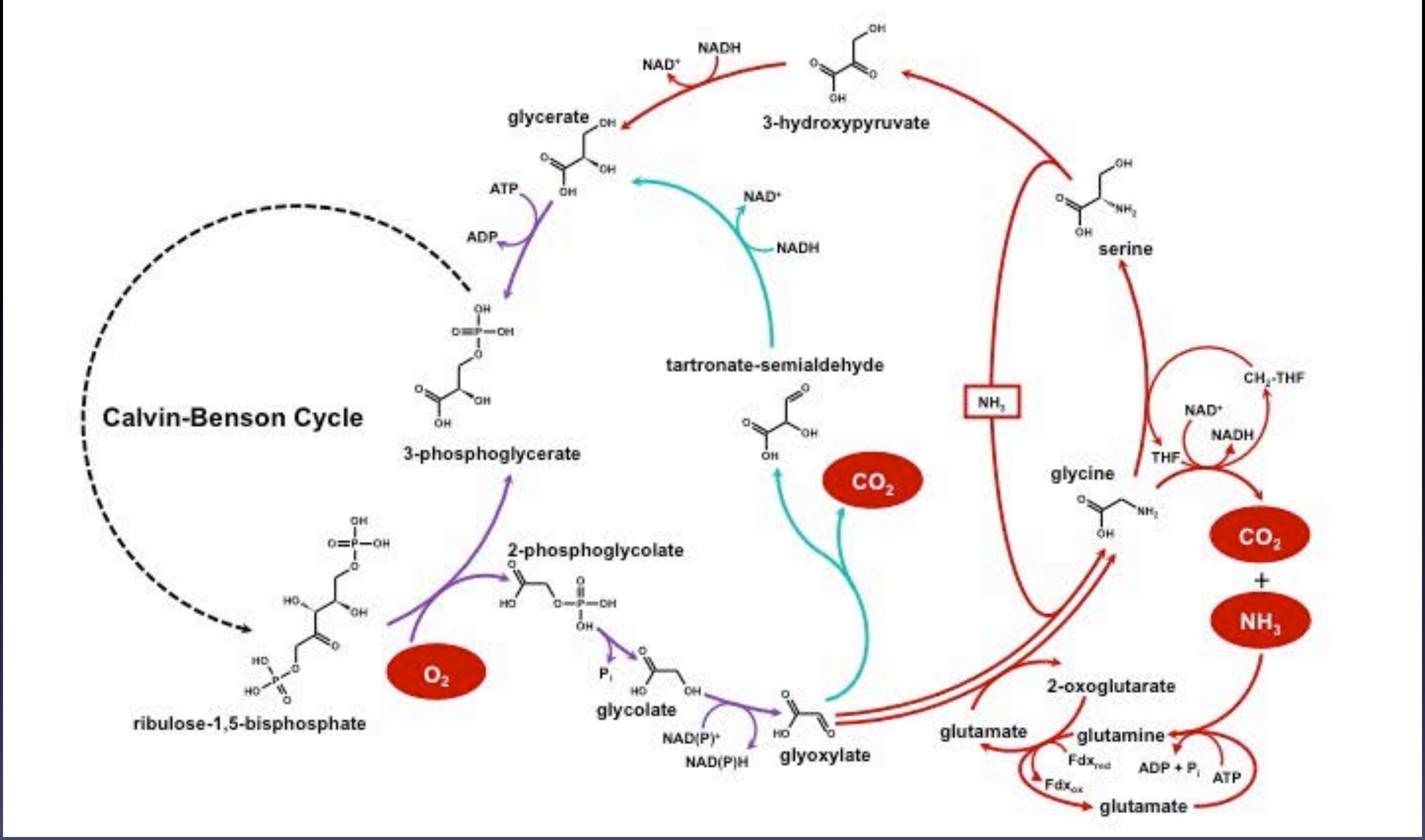




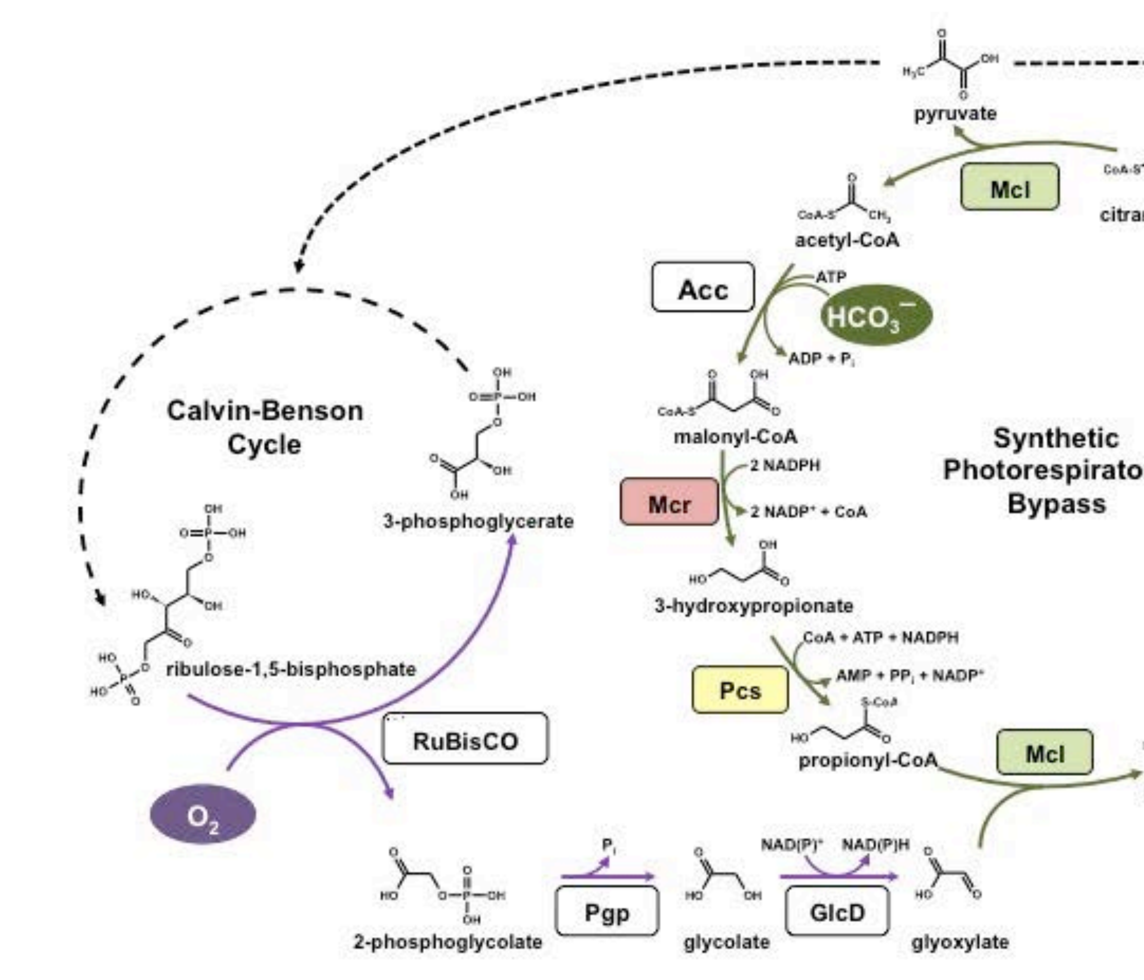
Berg et al, Nat Rev Microbiol 2010

Bar-Even et al, PNAS 2010

Testing our understanding of photorespiration with new pathways

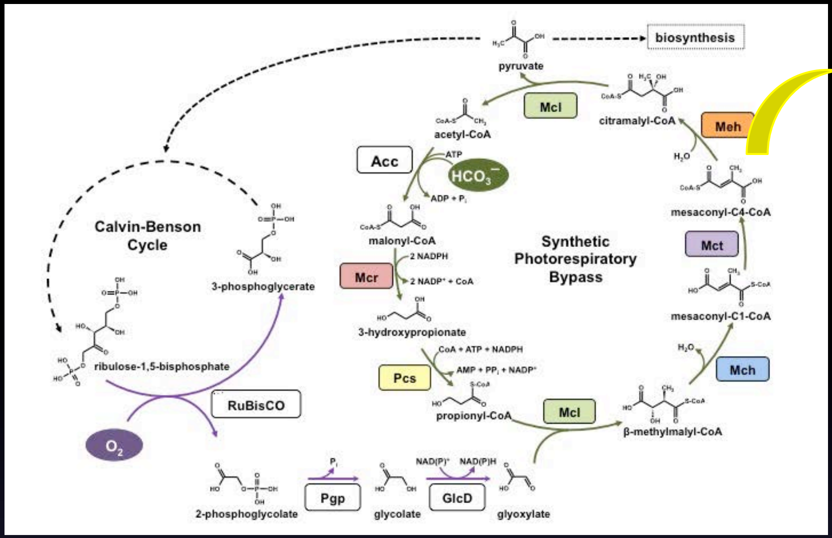


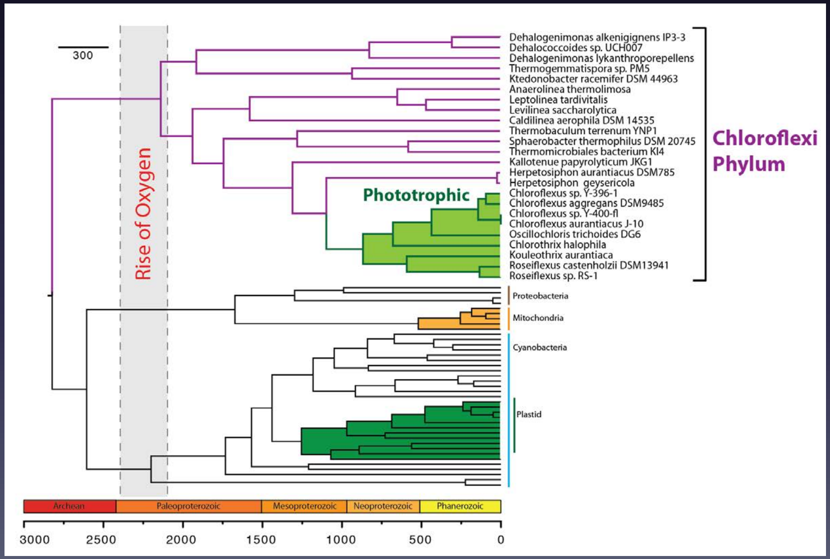
Engineering the 3HP bicycle as a synthetic CO2-fixing photorespiratory pathway



No loss of N
Gain in C

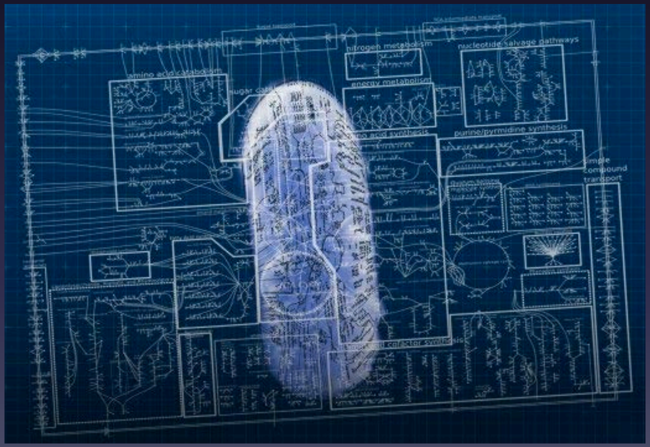
Can we improve photosynthesis in plants?



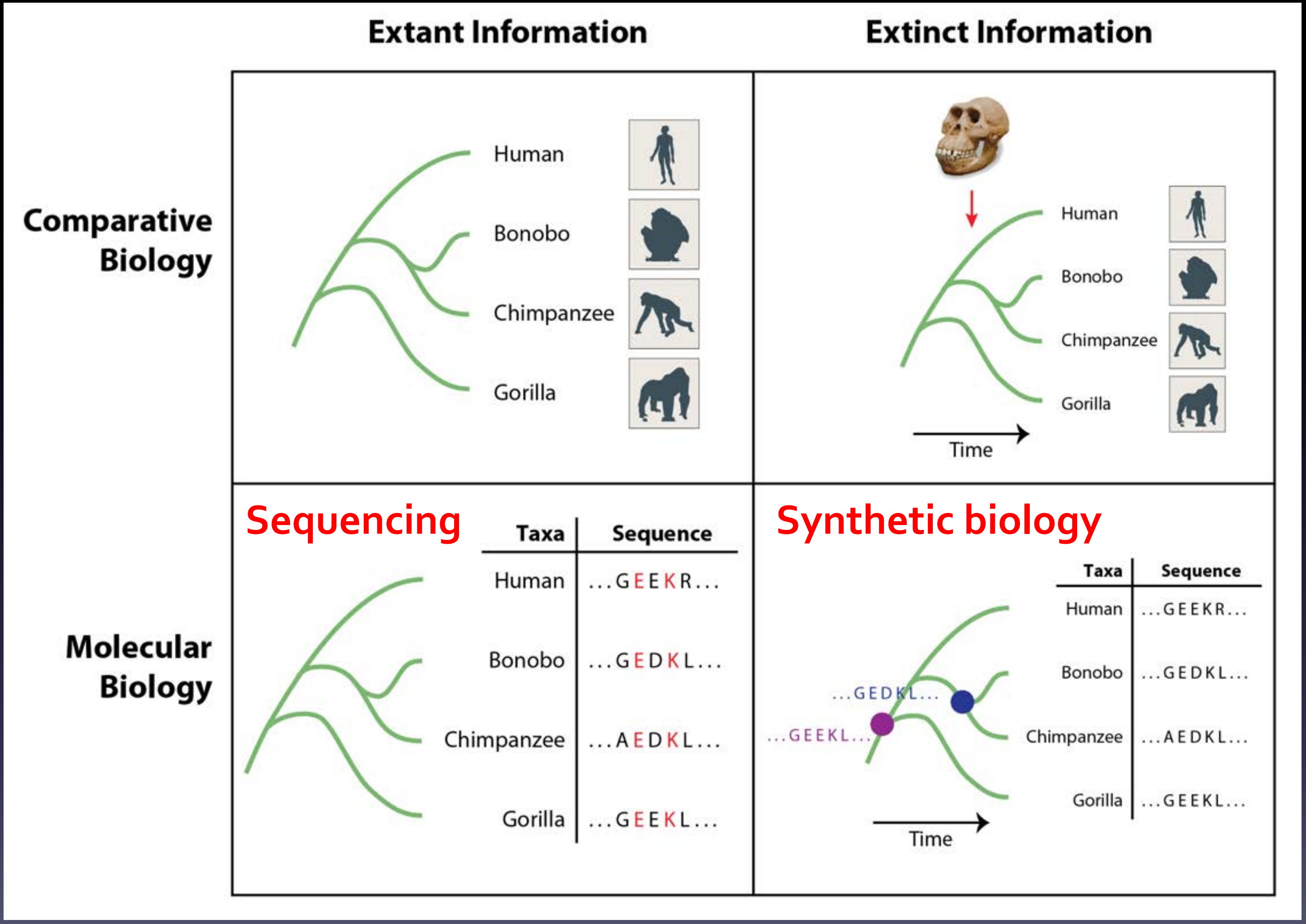


Conclusions

- We are in the early days of synthetic biology
- Synthetic biology may enable us to reconstruct more than just single genes but whole pathways and systems to interrogate early life
- We may be able to utilize synthetic biology to engineer and improve biological processes for useful applications



Final thoughts



Acknowledgements

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Dominique Loque