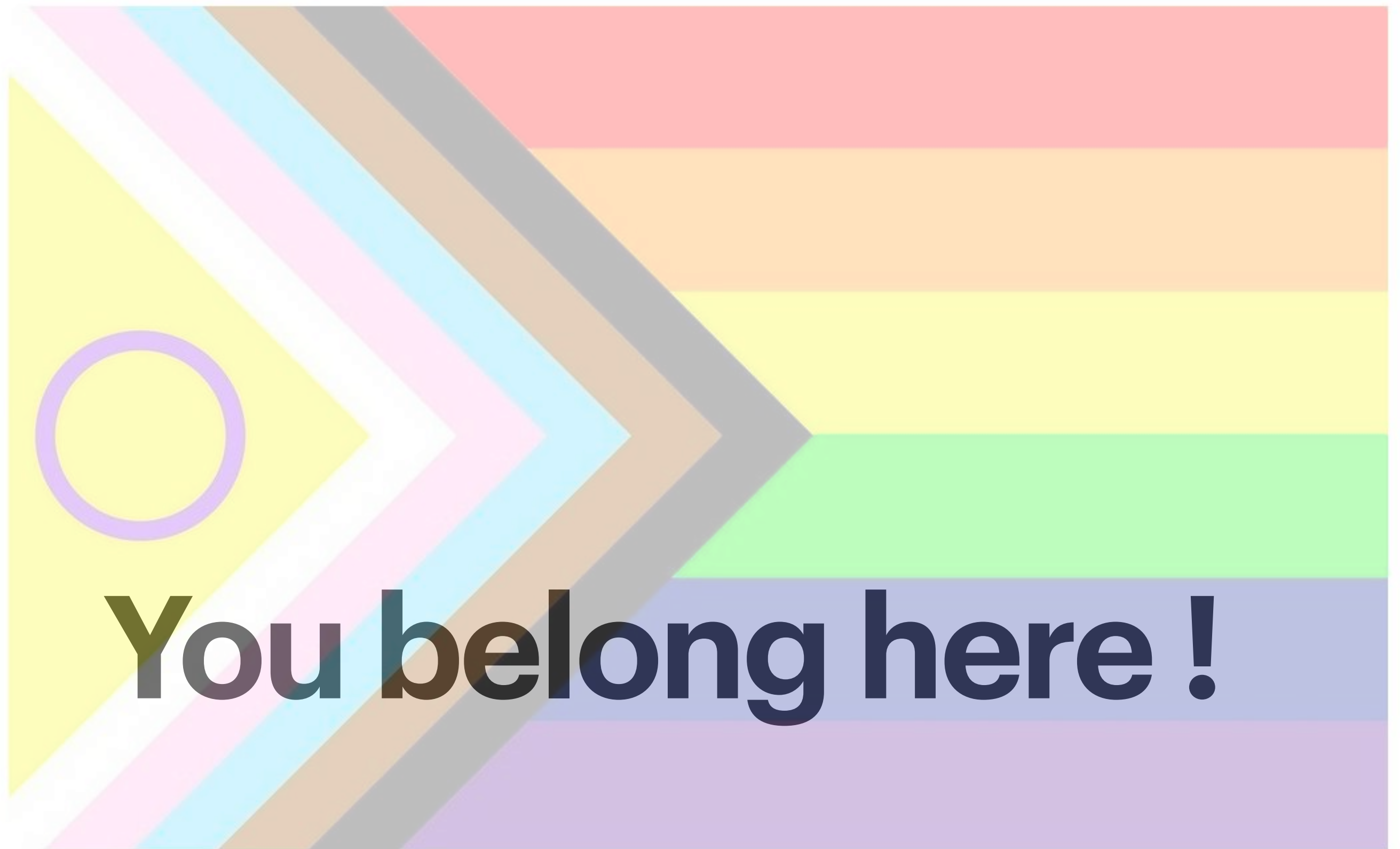


212 SM

Environmental microbiology



★ Inclusivity statement:

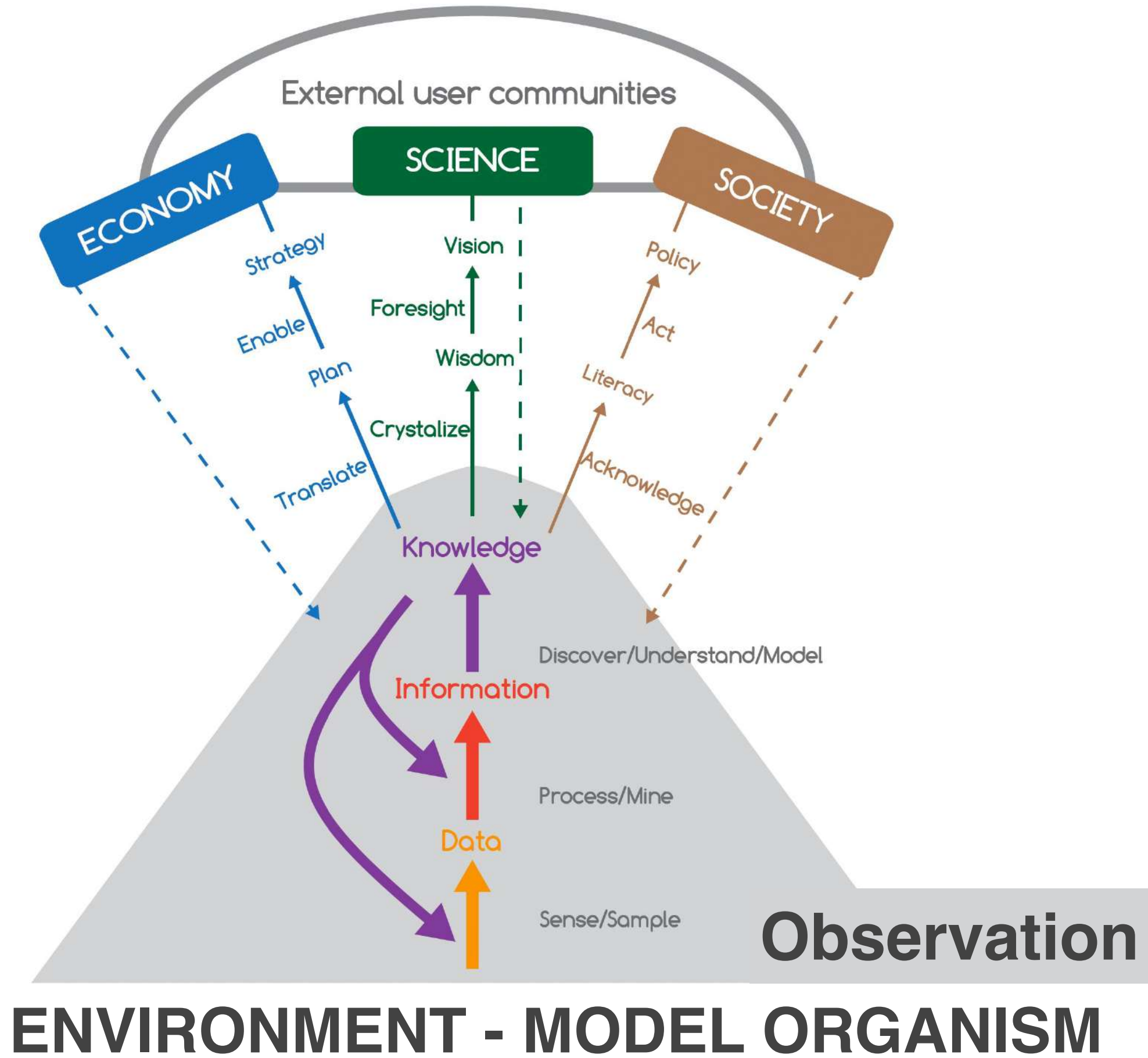
My laboratory and my philosophy aim to create an inclusive environment in which students of all backgrounds, cultures and orientations can feel safe learning, growing, and participating within their community.

From my experience and working with many people in my field, I have found this to be an important topic to bring up and present myself as a resource for those who find they might need it.

**My personal goal as a
teacher/educator**

Crise A, Ribera d'Alcalà M, Mariani P, Petihakis G, Robidart J, Iudicone D, Bachmayer R and Malfatti F

(2018) A Conceptual Framework for Developing the Next Generation of Marine OBservatories (MOBs) for Science and Society. Front. Mar. Sci. 5:318. doi: 10.3389/fmars.2018.00318



Sviluppo cognitivo e pensiero critico: due antidoti ai pregiudizi e ai preconconcetti del presente

di Daniele Scarampi

https://www.treccani.it/magazine/lingua_italiana/articoli/scritto_e_parlato/pensiero_critico.html

Logistics, I

- Instructor: Francesca Malfatti (fmalfatti@units.it; FC, 3rd floor left)
★ **Office hour TBD via email;**
- Schedule
 - *Tuesday 8-10 and Thursday 11-13*
 - *Lab on Friday 8-12*
- Course structure
 - **Lectures: 10 topics**
- Books
 - **Madigan et al. (year 2018-2020; 15th, 16th Edition). Brock Biology of Microorganisms**
 - **Madigan et al. (anno 2016: 14 Edizione). Brock Biologia dei Microrganismi**

Logistics, II

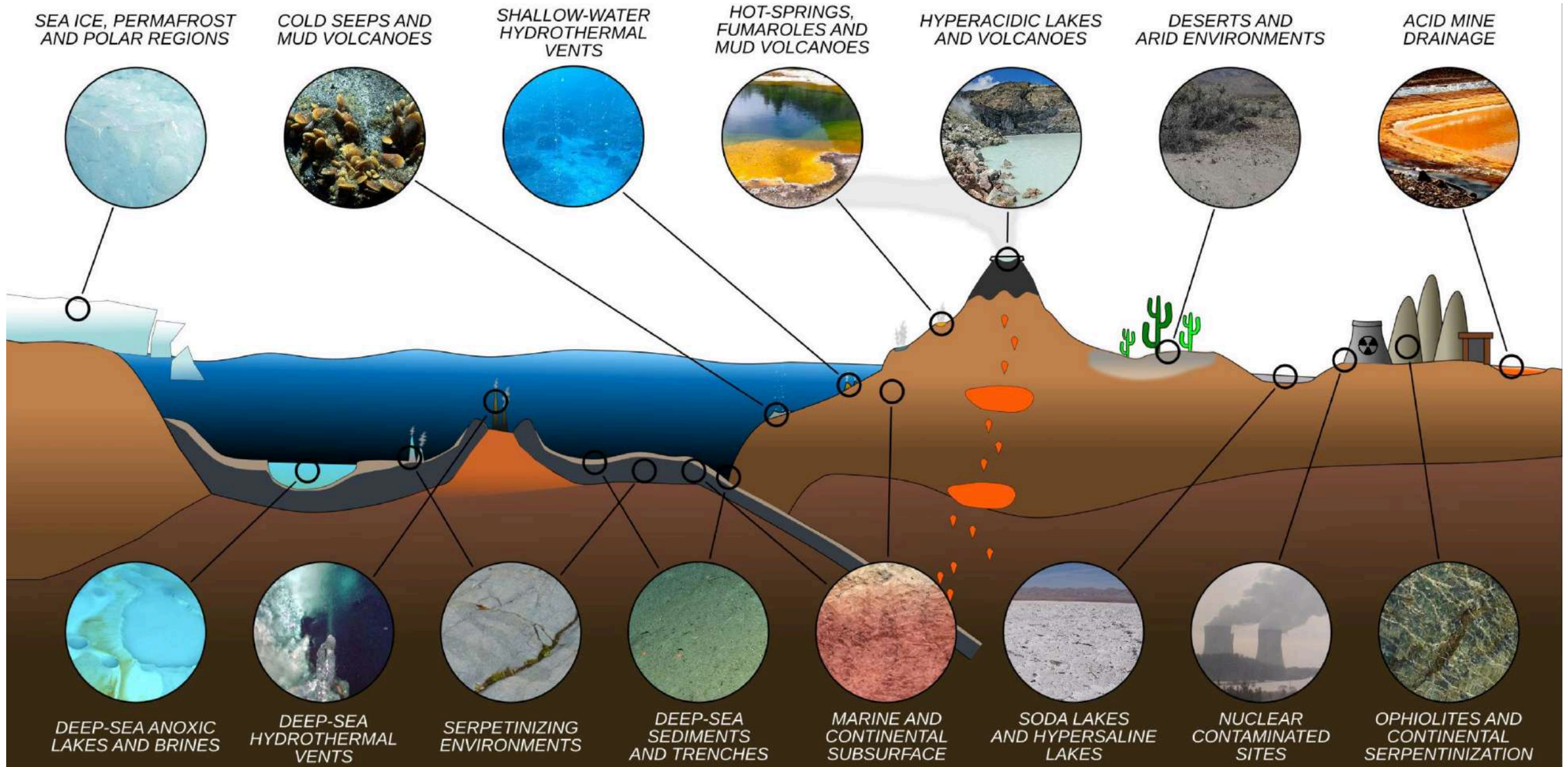
Sistema misto di valutazione dell'apprendimento:

1. Presentazione orale di gruppo pari a punti 3/30 del voto finale individuale su un argomento microbico trattato nel programma.
2. Esame scritto della durata di un'ora, per un valore di punti 27/30 del voto finale individuale.

Esame scritto conterrà 2 domande a risposta aperta, 1 fotografia da commentare e descrivere e 12 domande a risposta vero-falso. Le domande a risposta aperta e la fotografia valgono ognuna 5/30. Le risposte vero-falso valgono 1/30.

Extreme microbial environments on Earth

Merino et al. 2019



Logistics, III

Gli studenti con certificati (i.e., DSA) devono informare il docente all'inizio del corso per poter meglio pianificare i metodi di valutazione dell'apprendimento

Syllabus, I

Il corso è organizzato in 10 argomenti diversi volti ad analizzare aspetti importanti e attuali della microbiologia ambientale:

1-Introduzione al concetto di microbiologia, microbiologia ambientale e la loro storia dal XVII secolo fino ad oggi ed origine della vita sulla terra.

2- Biologia di Batteri ed Archaea con particolare attenzione alla morfologia della cellula batterica e ad alcuni meccanismi metabolici di base (capsula, parete cellulare, peptidoglicano, membrana, appendici batteriche con flagelli e pili, endospore, aspetti del genoma batterico, crescita batterica, diffusione attiva e passiva delle molecole attraverso la membrana).

3- Metabolismo batterico e nutrizione microbica (macro- e micronutrienti, diversi tipi di metabolici in base alla fonte di energia utilizzata: fototrofi e chemiotrofi, oppure in base alla fonte di carbonio: autotrofi o eterotrofi; differenti strategie metaboliche come la fermentazione, la respirazione aerobica ed anaerobica) e crescita.

4-Virus (Bacteria, Archaea e Eukarya).

5-DNA-RNA-Proteine (antibiotici) e meccanismi di movimento di DNA tra microorganismi (trasposizione, trasformazione, coniugazione e trasduzione).

Syllabus, II

6. Regolazione dell'espressione genica in risposta a diversi stimoli ambientali (fattore sigma, regolazione positiva e negativa tramite molecole attivatrici, repressori, sistema a due component e punti di controllo a livello tradizionale, stress e motilità).
7. Ecologia microbica: concetto di specie ed evoluzione, ecosistemi microbici (suolo, sedimento marino, oceano, lago-fiume, atmosfera) cicli biogeochimici degli elementi e microrganismi-clima.
8. Comportamento: quorum sensing, biofilm dall'ambiente all'essere umano e simbiosi.
9. Tecniche di biorisanamento (*in situ- ex situ*, fito-risanamento, biomining e bioleaching) e microbial factories.
10. Metodologie di isolamento, caratterizzazione e fenotipizzazione di microrganismi ambientali e tecniche di microscopia. Accenni sulle nuove metodologie-omiche e la loro importanza rivoluzionaria nel campo della microbiologia (genomica, trascrittomica, proteomica, metabolomica, meta-genomica, meta-proteomica, metatrascrittomica).

**Tell me and I will forget, show me and I may remember;
involve me and I will understand**

6 h, C1 basement

**Esperienze di laboratorio per imparare le tecniche di sterilità, coltivazione
e fisiologia dei microorganismi**

Questions

**Why are the slides
written in English?**

Brief self-introduction

LAB Dimensions



**UNIVERSITÀ DEGLI STUDI
DI TRIESTE**



NSF center for aerosol impacts
on chemistry of the environment



 **SCRIPPS INSTITUTE OF
OCEANOGRAPHY**
UC San Diego



 **Università
di Genova**

 **OGS** Istituto Nazionale
di Oceanografia
e di Geofisica
Sperimentale

**Microscale interactions
(Human & Ocean)**

**Ocean - Atmosphere microbiology &
biogeochemistry**

**Ocean - Sediment microbiology
(Human and Climate impact)**

Scientific Milieu

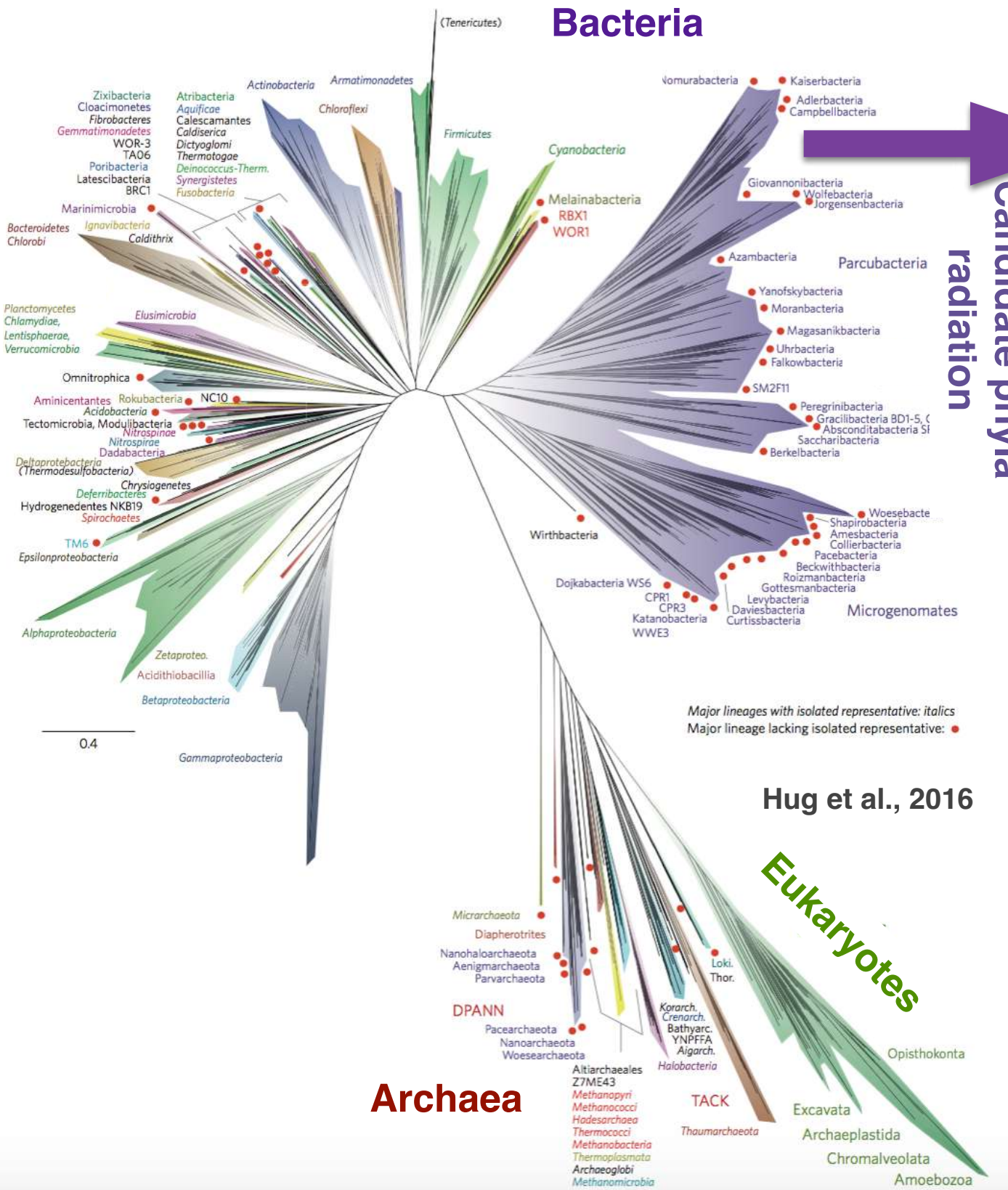


Dr. Farooq Azam

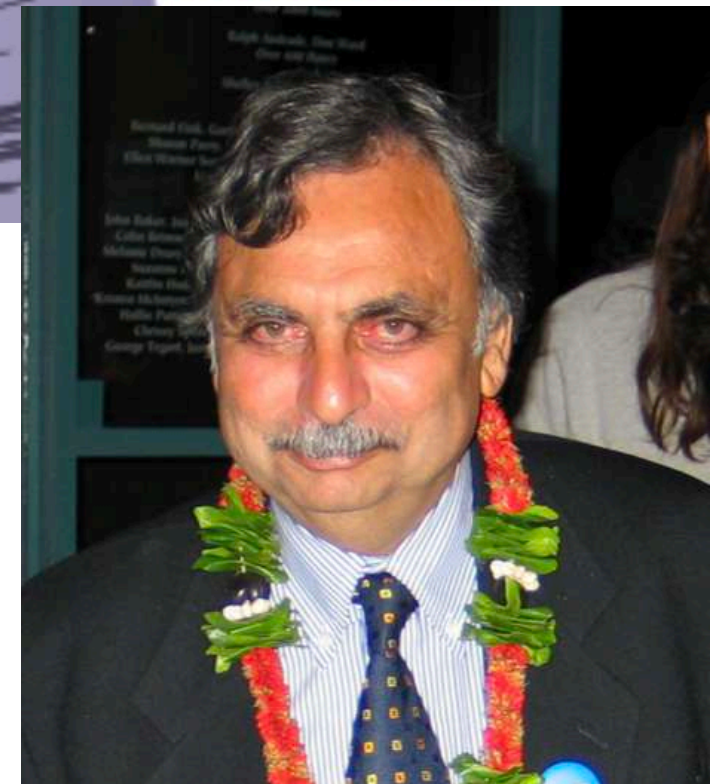
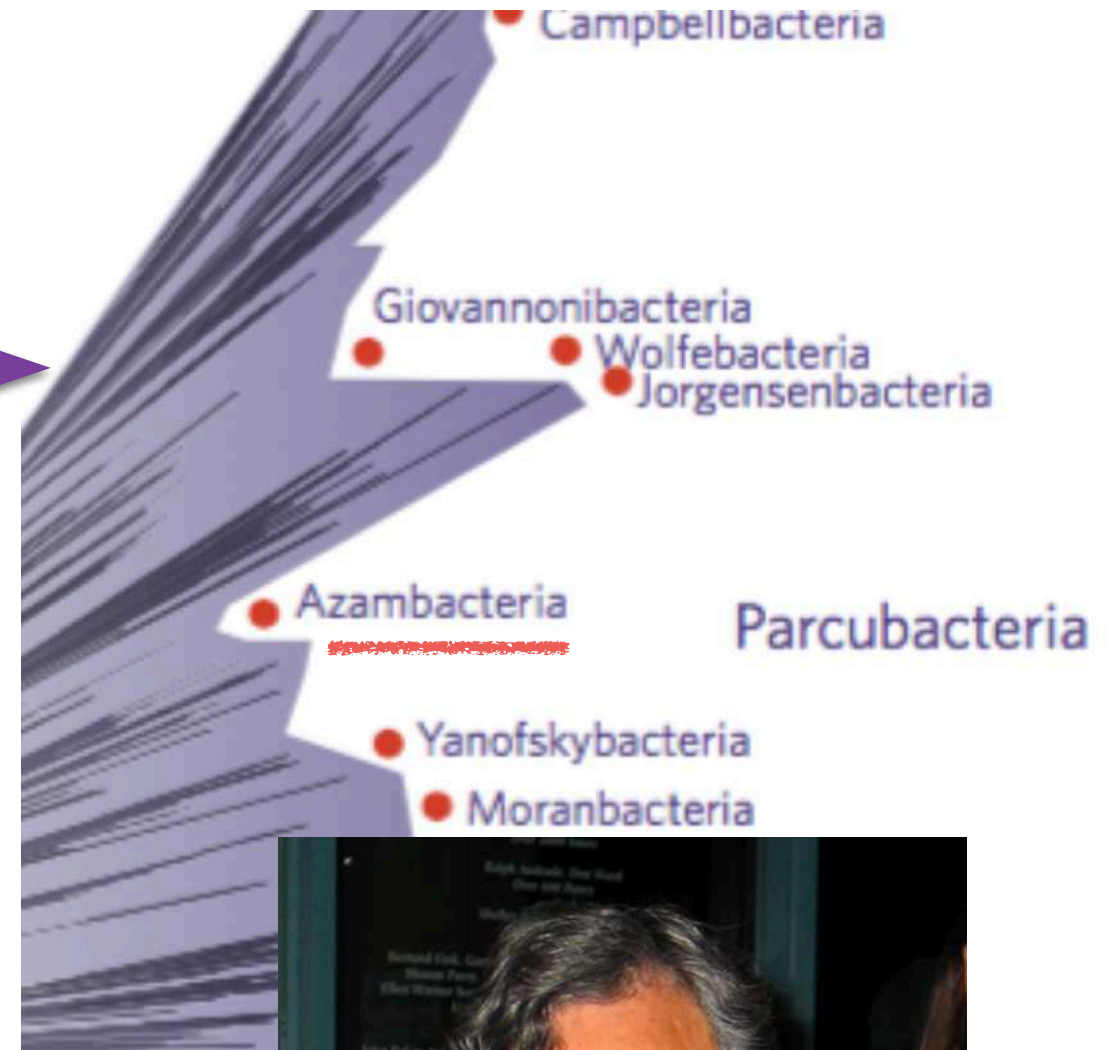
Dr. Andrew Benson (Calvin-Benson-Bashar: Photosynthesis)

Ms. Judith Munn (wife of Dr. Walter Munn, Normandy landing, 2 WW)

PVC
super phylum

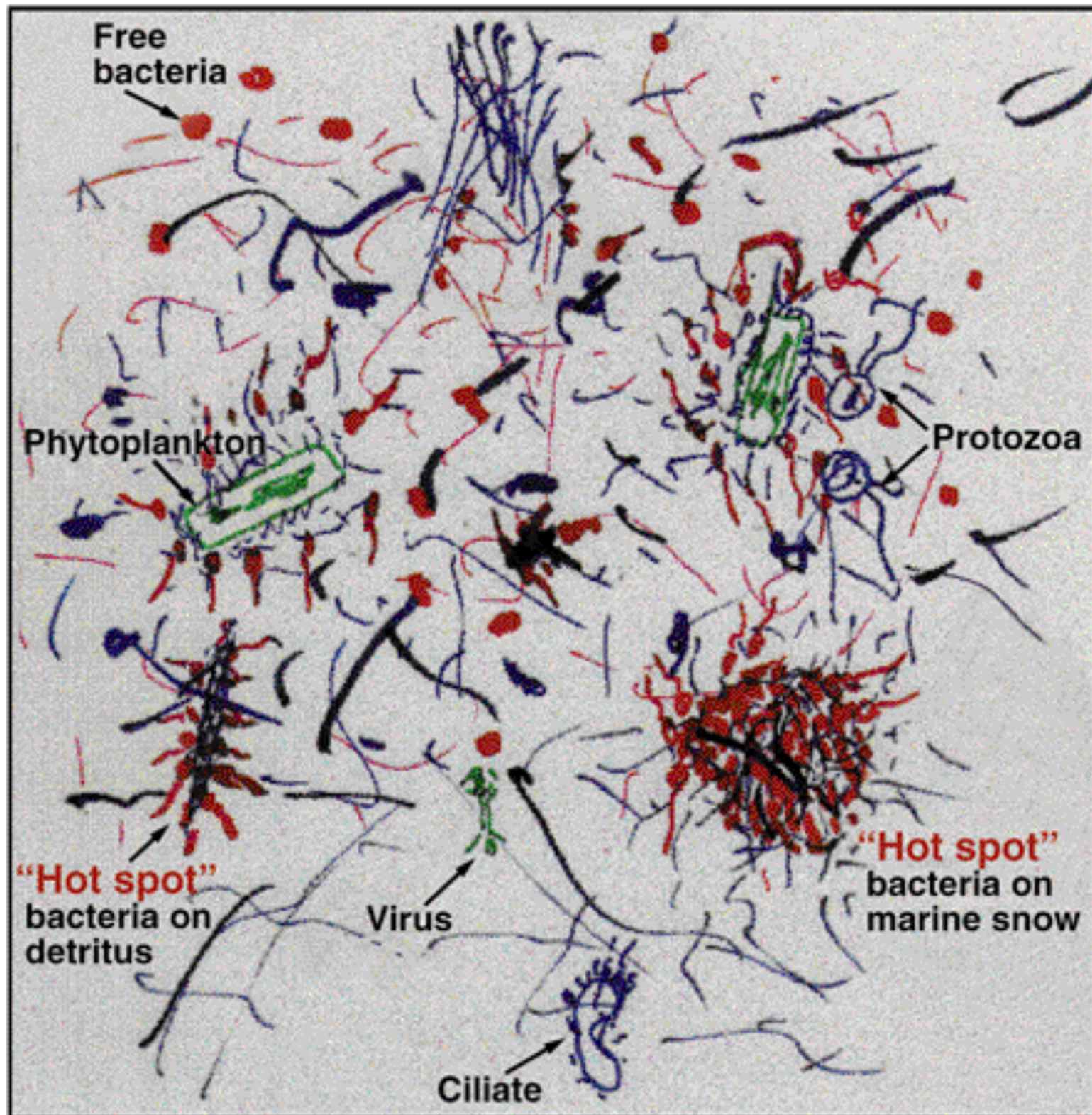


Candidate phyla
radiation



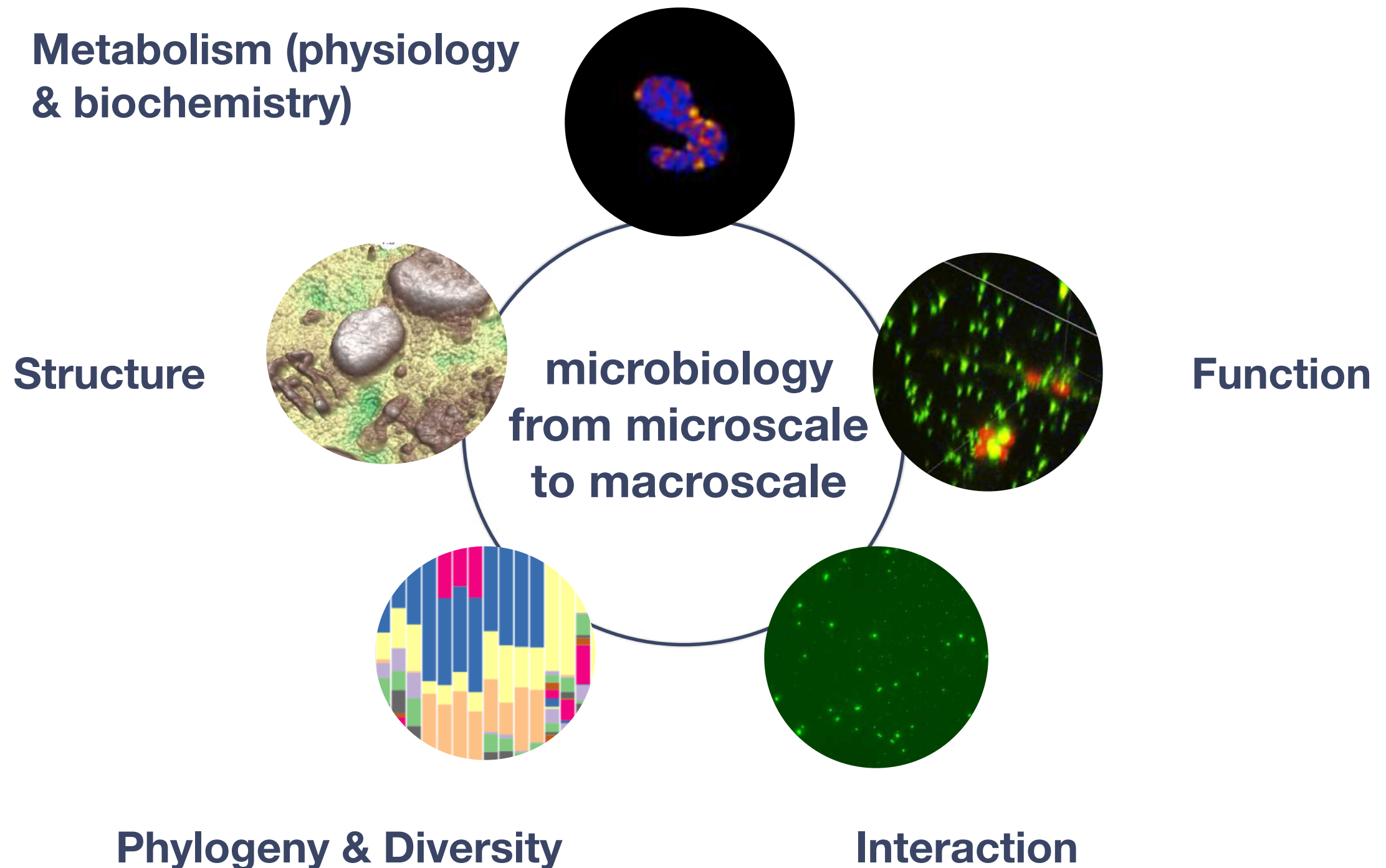
Dr. Farooq Azam

Impressionistic view, F. Azam



The microscale structure, chemistry and physics of the microbial environment dictate microbial life

Mechanistic integrative approach

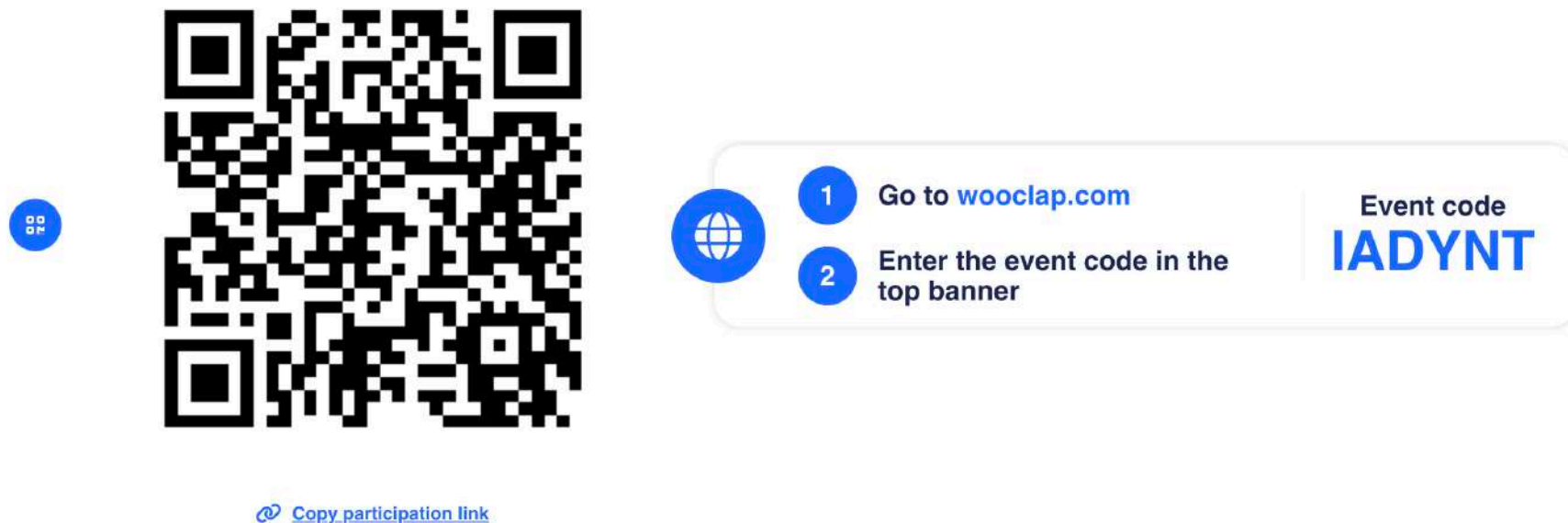


Questions

Che domande avete sui microbes?

**Che cosa volete ottenere da
questo corso?**

How to participate?



Scrivete una frase "falsa" sui microrganismi (aka fake news)

Scrivete cosa volete sapere sui microrganismi

What is LIFE?

What is LIFE?

- Natural**
- Synthetic**
- Mirror**

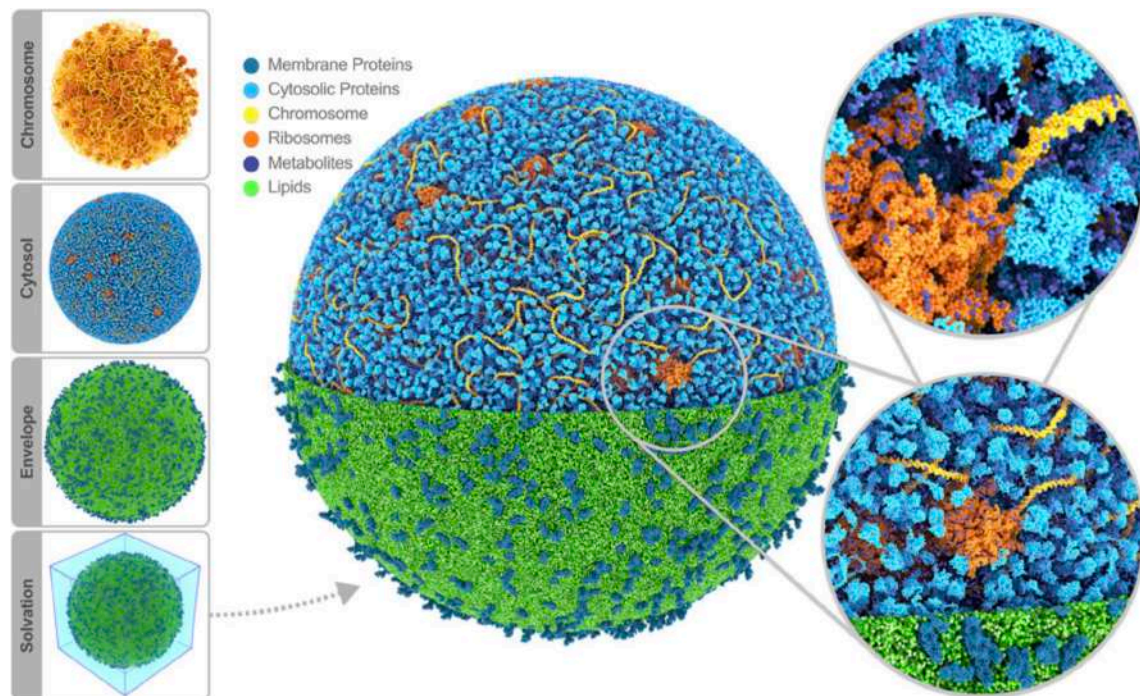
What is LIFE?

- Natural
- Synthetic
- Mirror

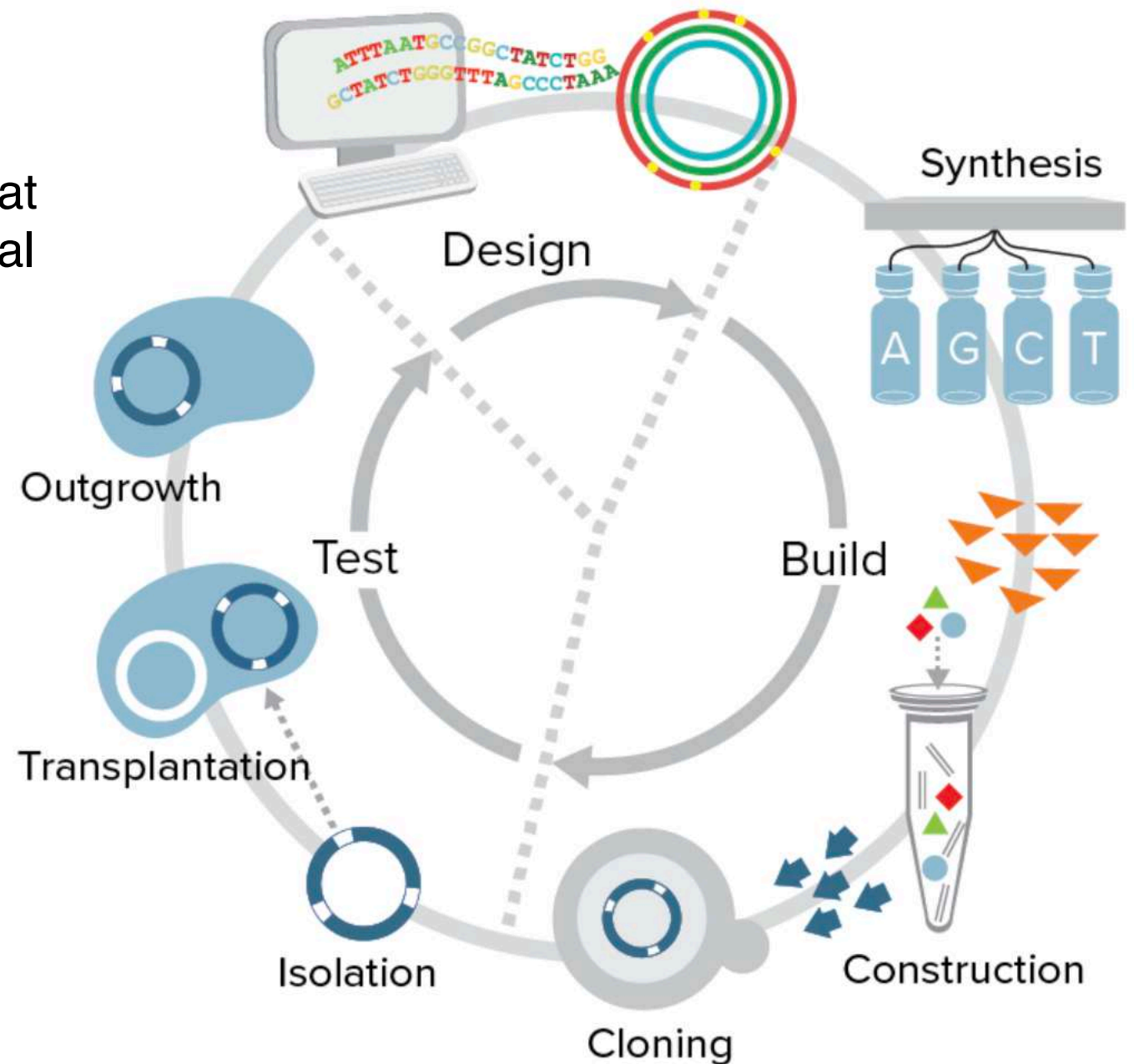
SYNTHETIC LIFE

A major goal in synthetic biology is to have the capacity to predictably design and build DNA that produces a cell with new and improved biological functions that do not already exist in nature

Stevens et al. 2023



The new minimal synthetic cell contains only 531,000 base pairs and just 473 genes making it the smallest genome of any self-replicating organism (from *Mycoplasma mycoides* JCVI-syn1.0)



<https://www.jcvi.org/research/first-minimal-synthetic-bacterial-cell#overview>

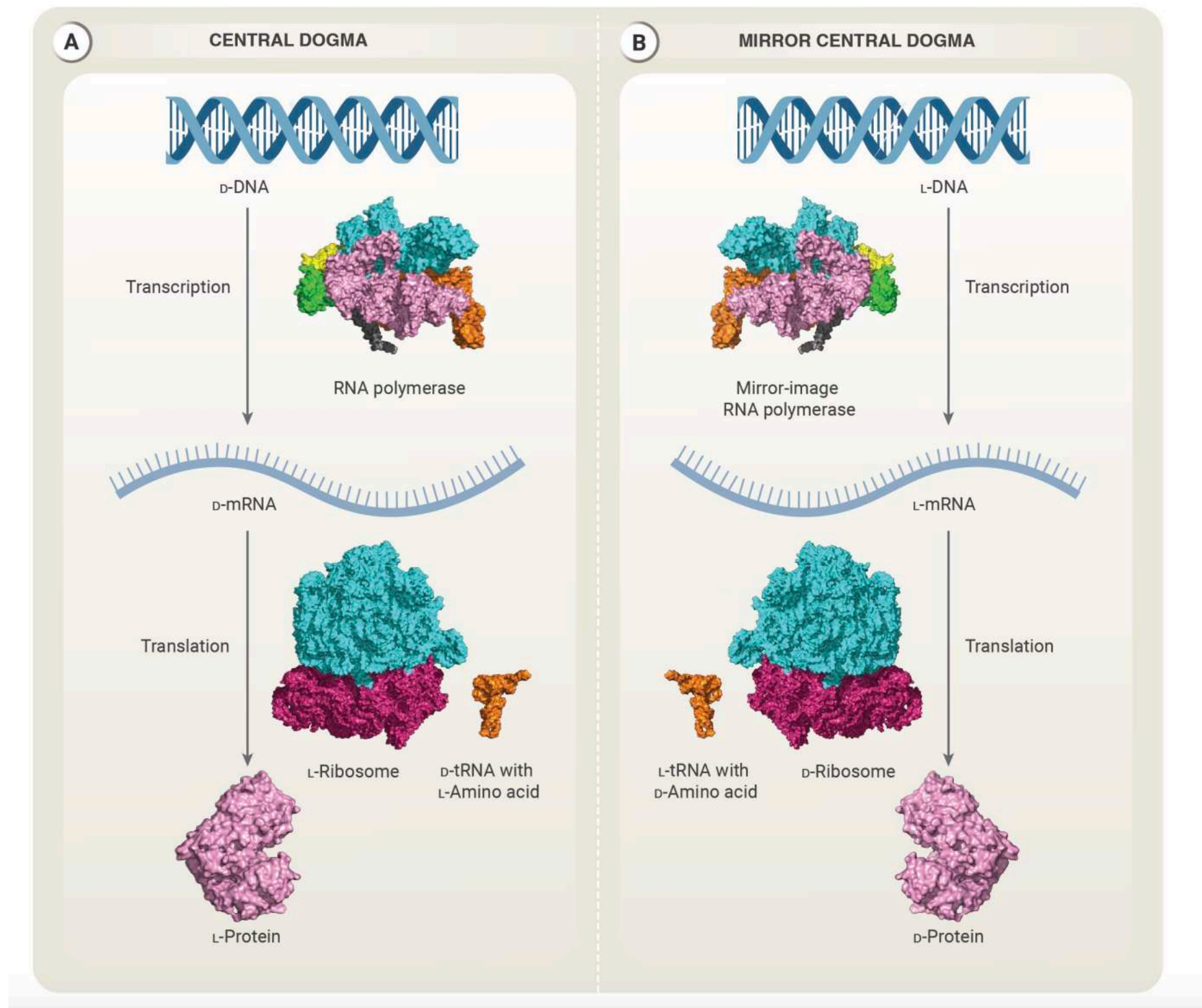
Mirror Bacteria

All known life on Earth shares a common set of chiral molecules. Proteins, sugars, lipids, and nucleic acids can all physically exist in either of two mirror-image configurations, but living organisms overwhelmingly use only one of these two configurations.

By convention, one member of the pair is designated to be the L, or “left-handed” form, while the other is designated the D, or “right-handed” form. **Their crystalline structures are mirror images of each other and melt at the same temperatures.** They have **identical solubilities in water and identical acid-base dissociation constants**, but they *rotate polarized light in opposite directions*.

Virtually all proteins are constructed solely from the 20 canonical amino acids, of which all but achiral glycine are **L-amino acids**. Their mirror images are D-amino acids, which play only marginal roles in existing life.

The ribose in RNA is always in the form of **D-ribose**; its mirror image L-ribose is essentially absent from nature. Pairs of molecules that are mirror images of each other are known as enantiomers, which have almost exactly mirrored physical and chemical properties.



Confronting risks of mirror life

- No natural predator
- No natural antibiotic or chemical warfare
- Potentially share same habitat and niche of “natural” microbes
- Potentially might outcompete “natural” microbes

BEFORE

Microbes not important

Microbes=Disease

Microbiologists stay only in
the lab

AFTER

**Microbes are everywhere and will
always be...**

Microbes as ecosystem engineers

**Microbes keep the ecosystem
functioning**

**Humans and biota as microbial
ecosystems**

**Microbiologists go sampling in the
field**

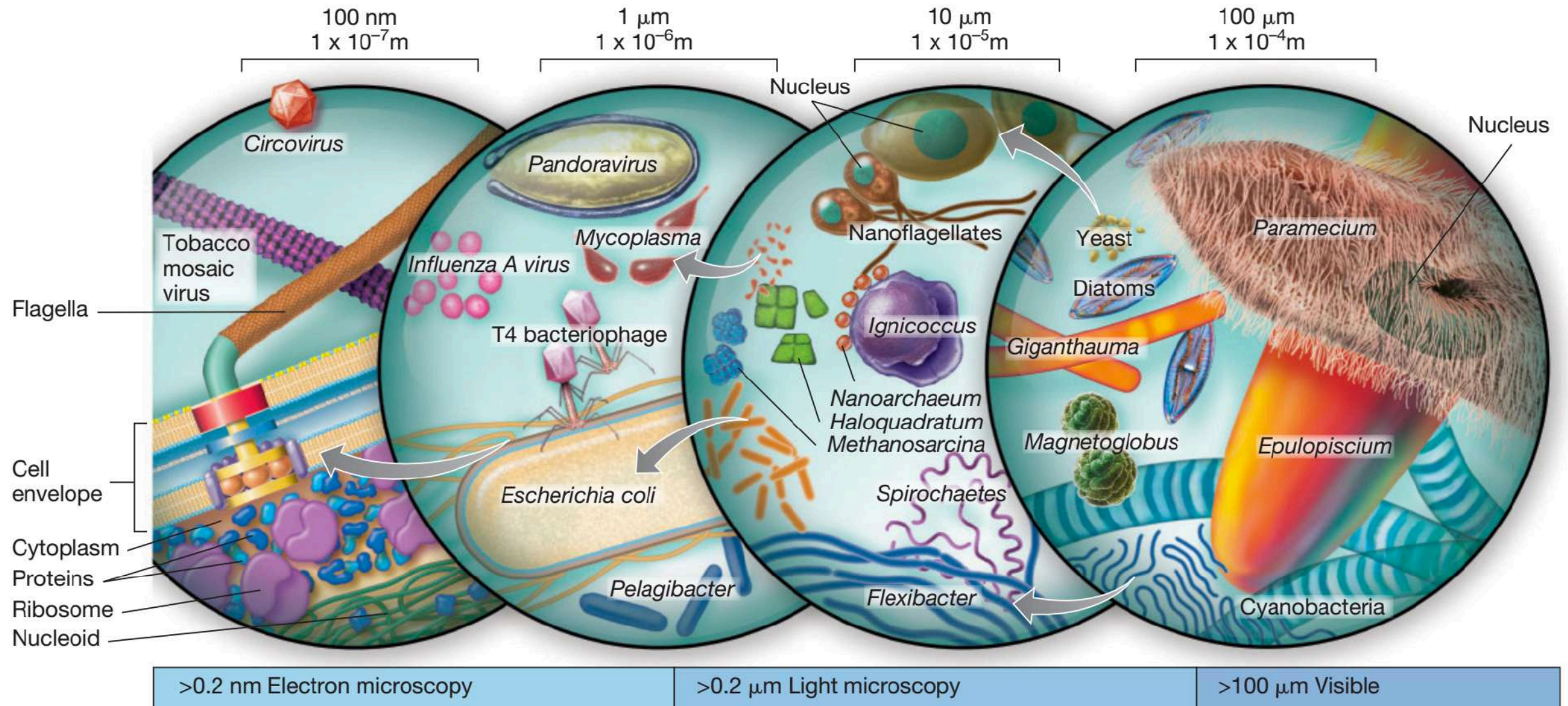
**Microbes produce antibiotics as
weapons**

Topic 01: Course Overview & Introduction to the Microbial World, History of Microbiology and Origin of Life

Lecture 01: **Introduction, History & Origin of Life**

- History
- Microbiology
- Goals of the course
- Origin of life

Microbial size range





- How big are microbes? - How small are microbes?
- Who are the microbes?
- Where do microbes live?

1 μm = 1 micrometer is $1/10^6$ meter

1 nm = 1 nanometer is $1/10^9$ meter

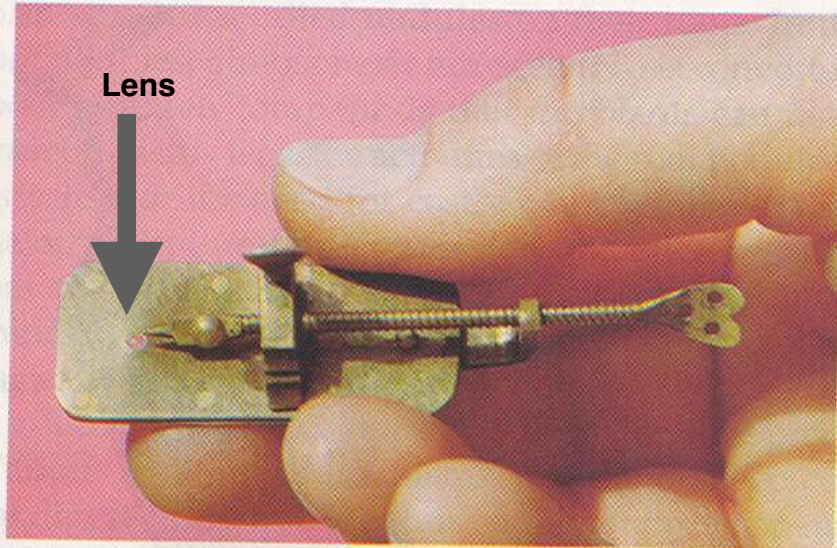
Bacteria, Archaea, Viruses & small Eukarya

Everywhere on Earth and in/on every organism

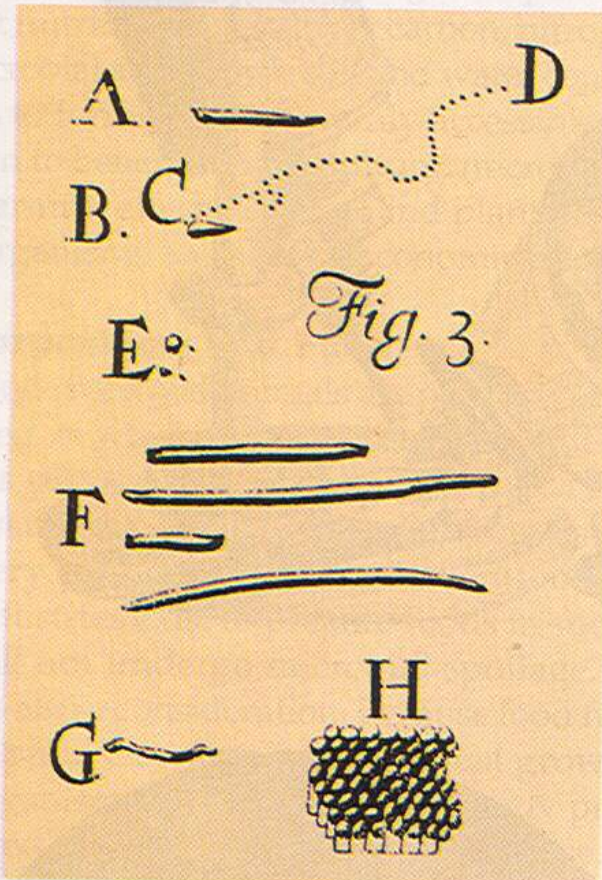
History

- 1665 Robert Hooke, invention of the microscope
- 1676 van Leeuwenhoek, discovery bacteria at the microscope
- 1857 Pasteur, microbes cause fermentation & dispelling spontaneous generation of life
- 1881 Koch, Germ theory of disease & use of gelatin plates
- End 19th century Beijerinck and Winogradsky —>
environmental microbiology
- End 20th **One Health and Human being as a microbial world**

Developing Tools enabling discovery of the microbial worlds!



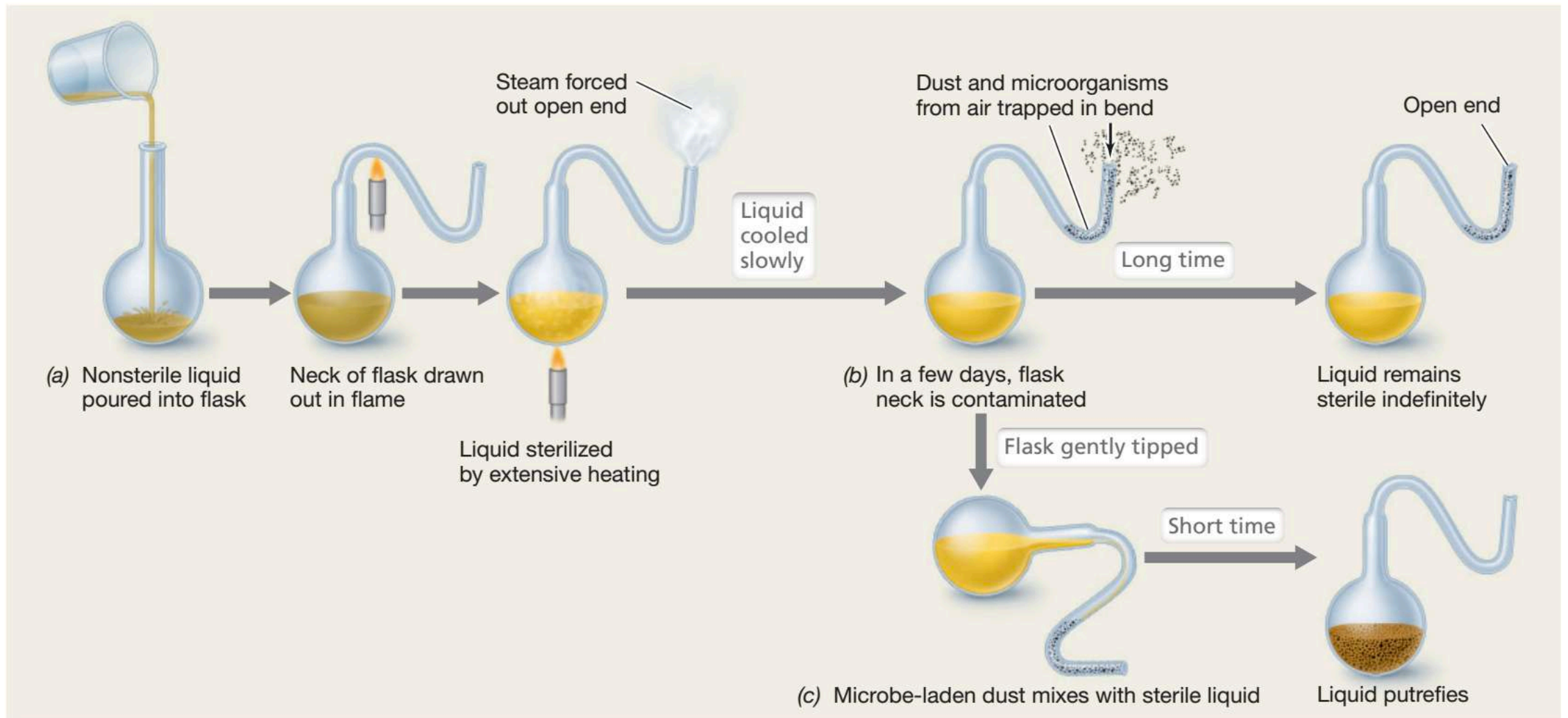
(a)



(b)

1676 van Leeuwenhoek
the first microscope for bacteria

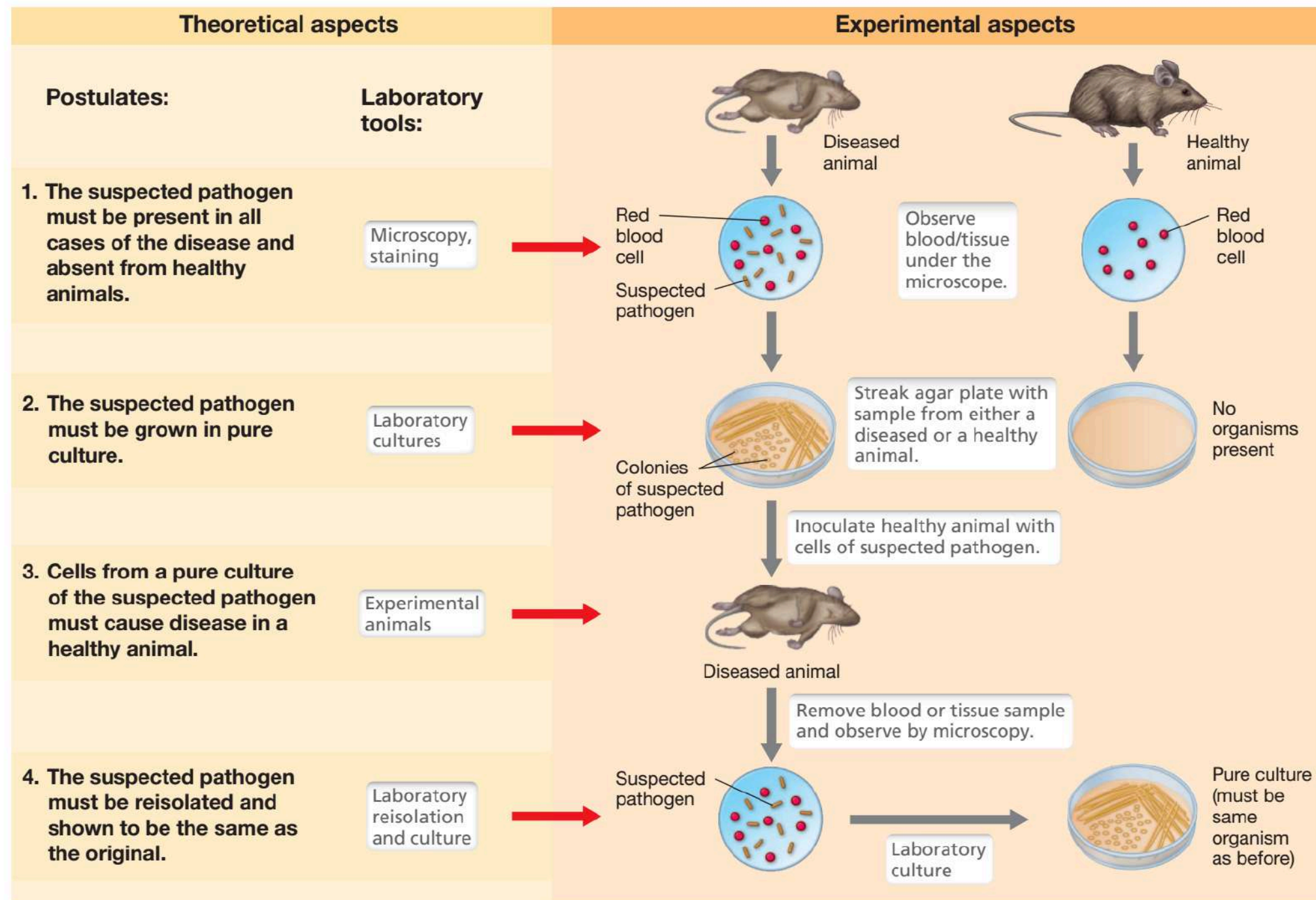
Early focus: Human microbial diseases
Later focus: Biogeochemical role
Today focus: One Health



Madigan et al. 2018

Pasteur: Experiment dispelling the theory of spontaneous generation of life (environmental change is microbe driven)

Koch → infectious diseases: Koch's postulates



Madigan et al. 2018

Vibrio cholerae and *Mycobacterium tuberculosis*
...what is missing?

Environmental microbiology —> *need to understand environment*

- 1. Bacteria from environment don't live on Koch rich media*
- 2. Need to create specific enrichment media to imitate the environment*

Delft School of Microbiology, Holland



Figure 2.11: Martinus Beijerinck
Martinus Beijerinck (1851–1931), a major contributor to our understanding of the role of microbes in nature. From *Martinus Willem Beijerinck: His Life and His Work*, by G. van Boven (A. L. J. den Driessche de Jong), and A. J. Kluyver, Martinus Nijhoff, The Hague, 1942.



Figure 2.12: Sergei Winogradsky
Sergei Winogradsky (1856–1953), a Russian-born microbiologist. Winogradsky was the father of autotrophy. He lived from the days of Pasteur and Koch to the modern era of microbiology. From *Sergei N. Winogradsky: His Life and Work*, by S. A. Waksman, © 1953 by the Trustees of Rutgers-Camden. Reprinted by permission of Rutgers University Press.



Beijerinck

Winogradsky

Kluyver

Environmental microbiology —> *need to understand environment*

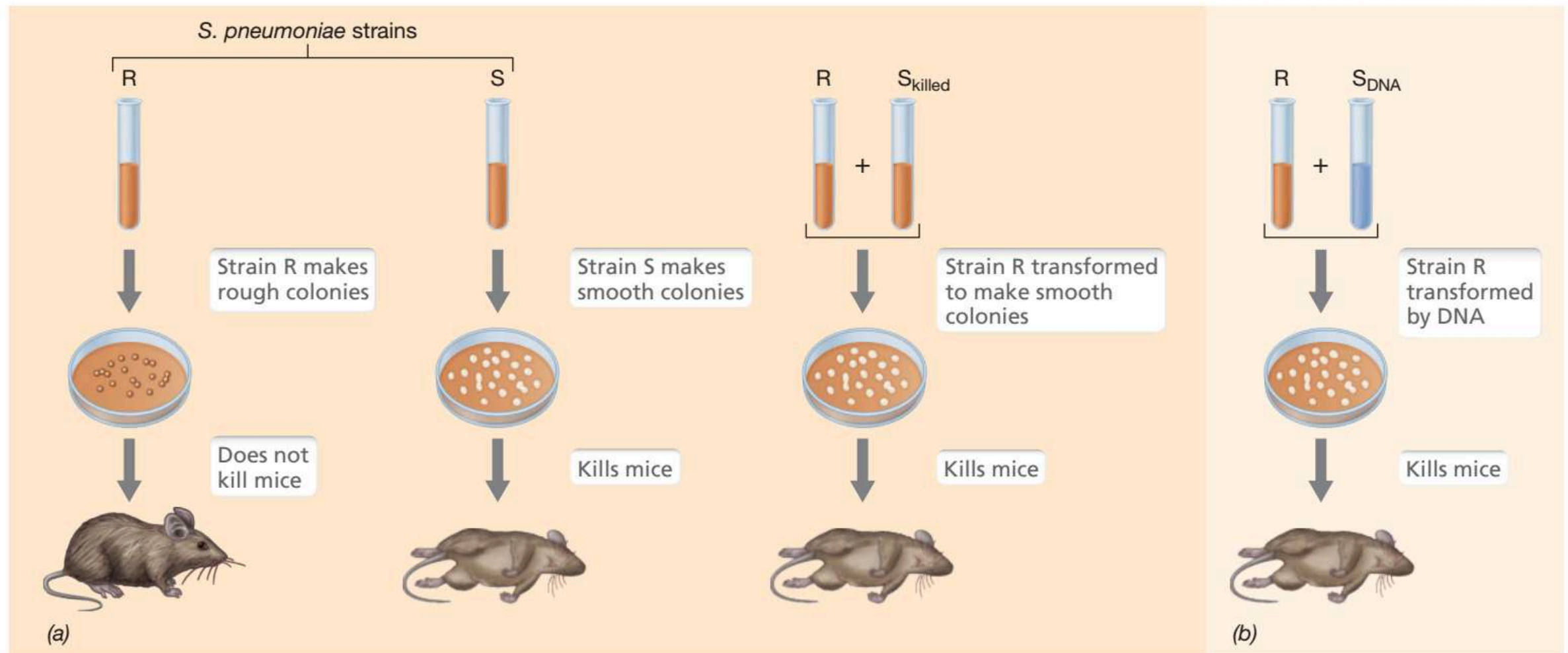
- 1. Bacteria from environment don't live on Koch rich media*
- 2. Need to create specific enrichment media to imitate the environment*

1. **Beijerinck**: Enrichment culture: Isolated pure culture of soil and aquatic microbes (aerobic nitrogen fixing bacteria, sulfur reducing and sulfur oxidizing bacteria);
2. **Winogradsky**: Diversity and environmental functions of microbes: Discovered nitrifying & sulfur oxidizing bacteria; chemolithotrophy;
3. **Kluyver**: unity of the biochemistry, stating that same biochemical pathways and thermodynamic constraints are similar for microbes

- End 19th century Griffith & 1944 Avery-MacLeod-McCarty, **DNA as a transforming principle**
- Early 20th century Alexander Fleming's **discovery of penicillin as an antiseptic antibiotic** (Howard Florey, Ernst Chain and Norman Heatley) and **lysozyme**
- First half 20th century Watson, Crick & Franklin: **DNA structure**
- 20th century Zuckerkandl & Pauling: **molecular sequences for evolutionary reconstruction relationship**
- 20th century Woese: **ribosomal RNA (rRNA) genes** for studying evolution in microbes (**cultivation dependent**)
- 20th century Pace: **ribosomal RNA (rRNA) genes** for assessing diversity of microbes (**cultivation independent**)

Griffith & Avery-MacLeod-McCarty

Streptococcus pneumoniae



DNA contains genetic information
DNA is the molecular basis of heredity





Alexander Fleming (1881-1955)

The discovery of antibiotics is a great milestone in the history of medicine

Many doctors believe that penicillin is one of the greatest medical advances

Penicillin can treat most forms of killer diseases such as meningitis, pneumonia and diphtheria, blood poisoning and septic wounds

In 1922, Fleming discovered a way of destroying bacteria, lysozyme

Alexander Fleming Laboratory Museum (Imperial College Healthcare NHS Trust).

Woese



Ribosomal RNAs are components of ribosomes, the structures that synthesize new proteins in the process of translation.



16S ribosomal RNA gene

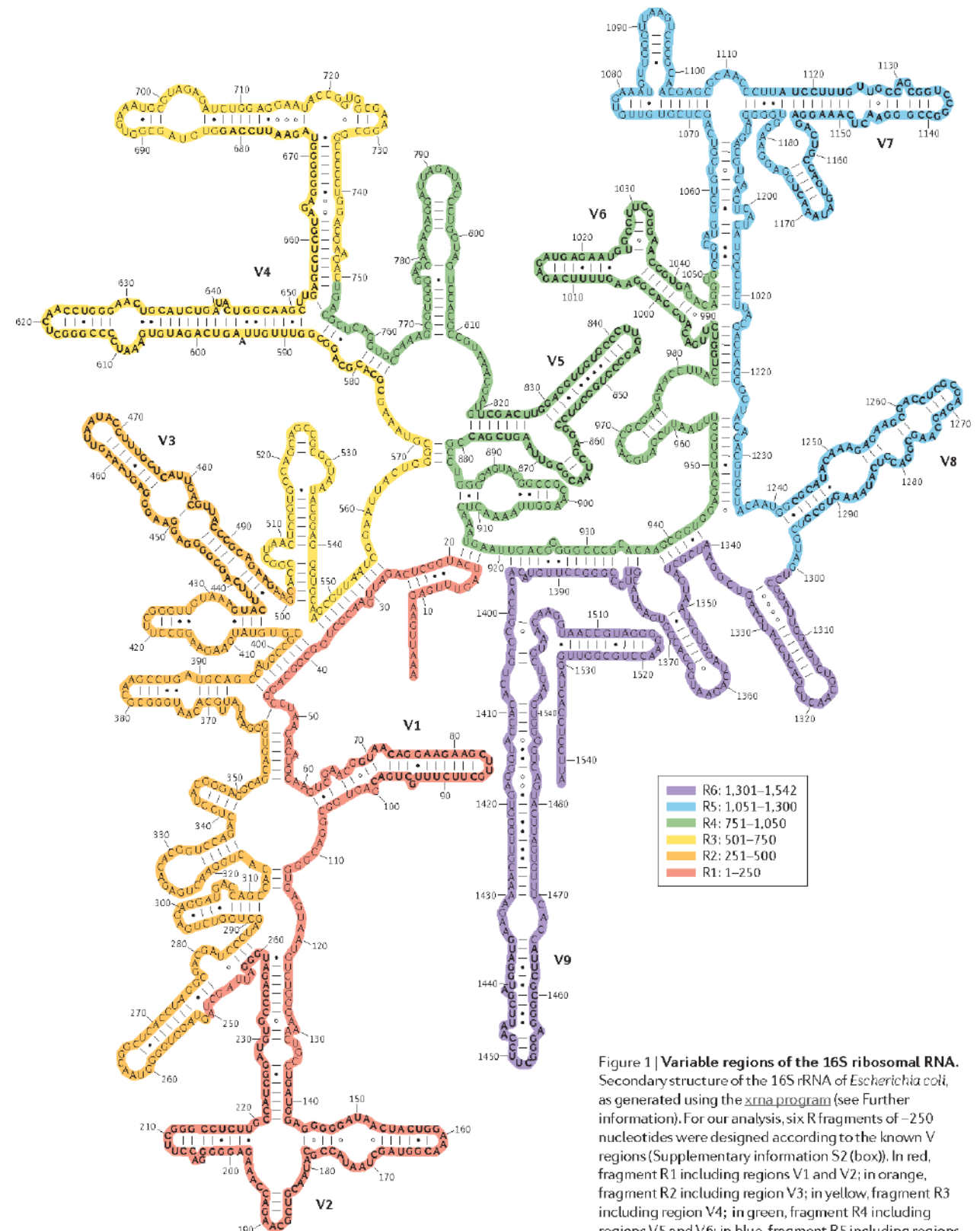


Figure 1 | **Variable regions of the 16S ribosomal RNA.** Secondary structure of the 16S rRNA of *Escherichia coli*, as generated using the [xrna program](#) (see Further information). For our analysis, six R fragments of ~250 nucleotides were designed according to the known V regions (Supplementary information S2 (box)). In red, fragment R1 including regions V1 and V2; in orange, fragment R2 including region V3; in yellow, fragment R3 including region V4; in green, fragment R4 including regions V5 and V6; in blue, fragment R5 including regions

Woese

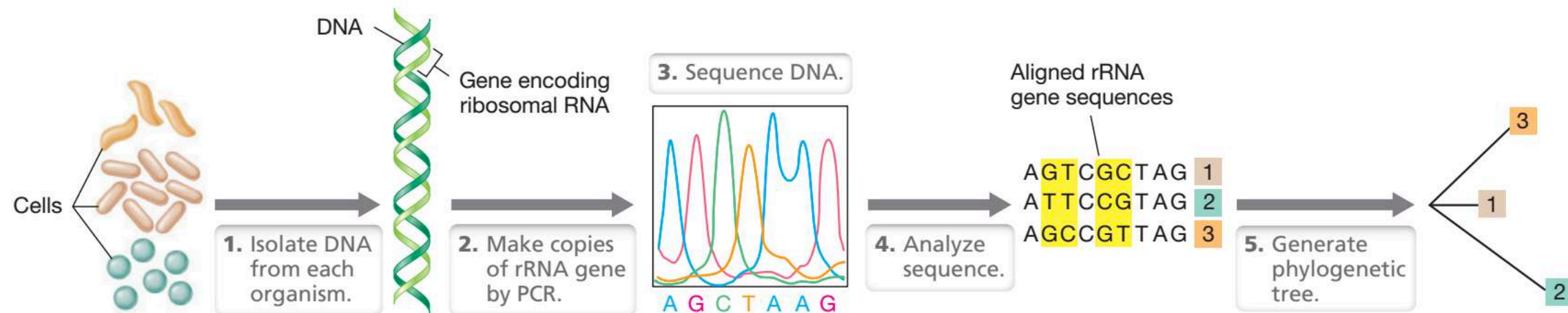
Genes encoding rRNAs are excellent candidates for **phylogenetic analysis** because they are:

- (1) universally distributed,
- (2) functionally constant,
- (3) highly conserved (that is, slowly changing),
- (4) adequate length to provide a deep view of evolutionary relationships,
- (5) diverse in different 'species'

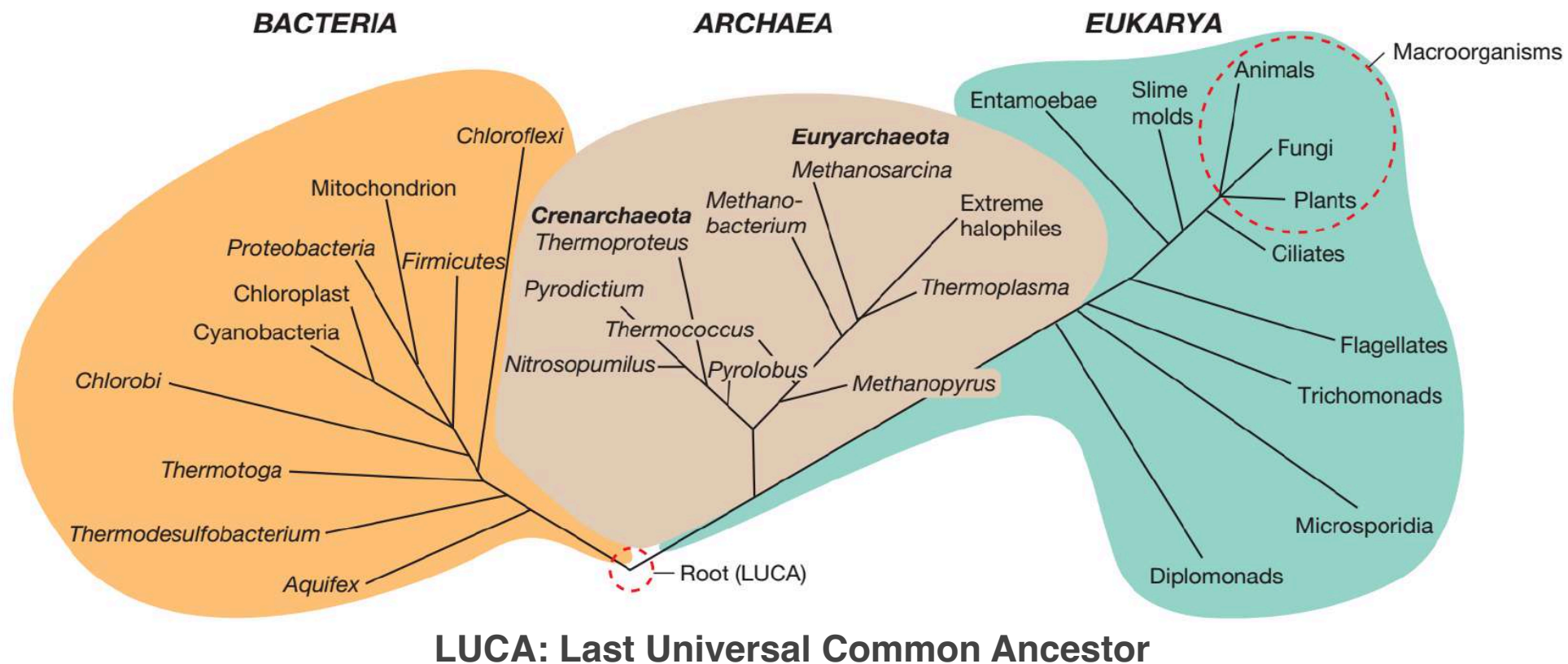
Using pure cultures of bacteria and Archaea



Step-by-step technology for evolutionary classification of microbes



Phylogenetic Tree of Life

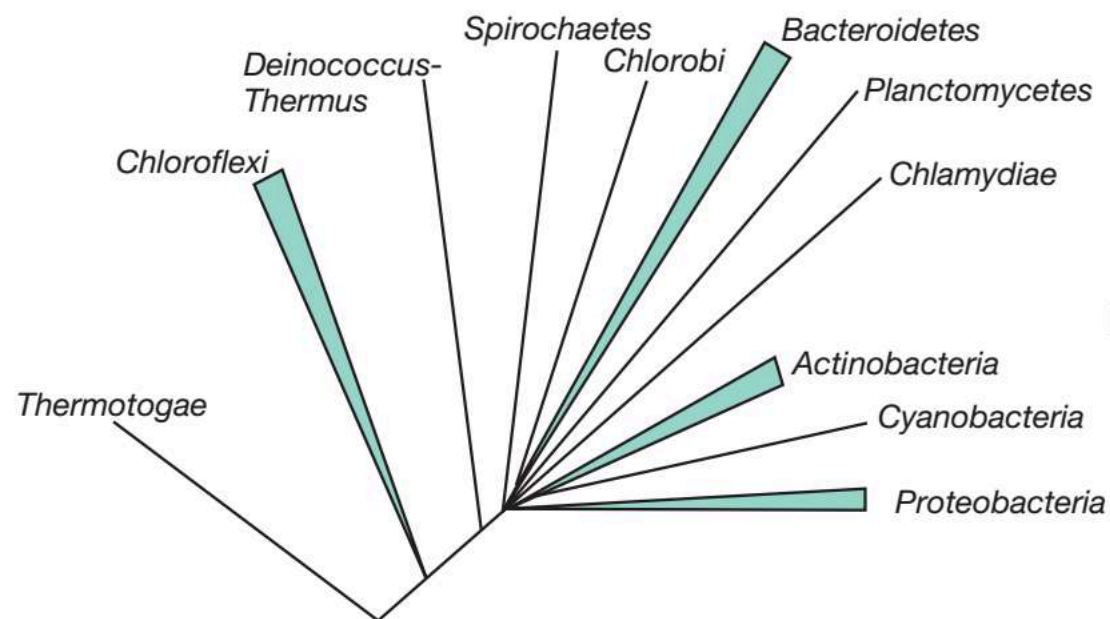


Woese

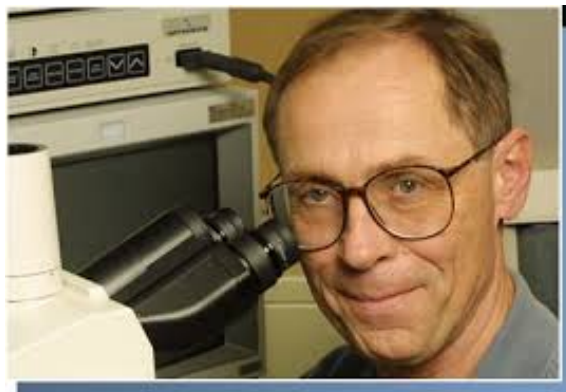


16S ribosomal RNA gene

Woese

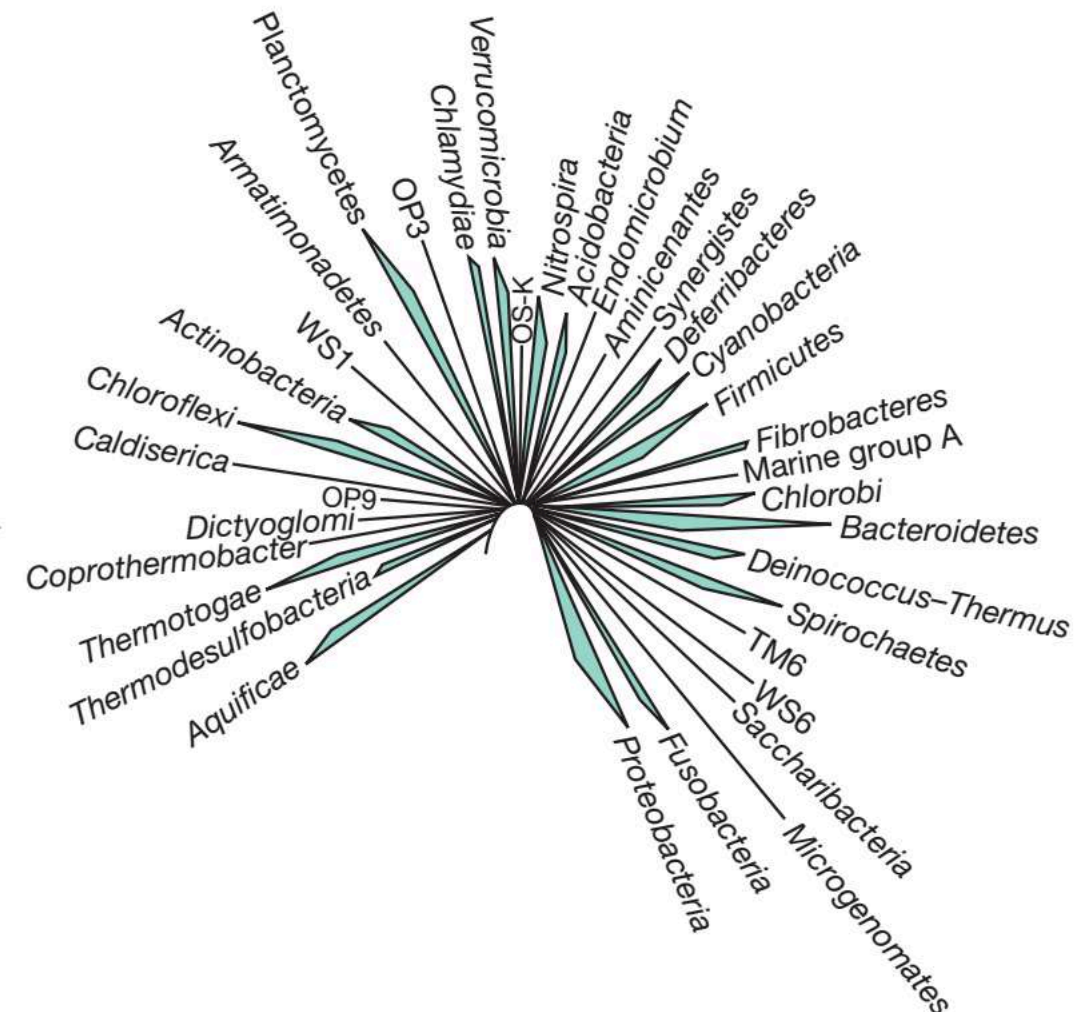


Cultivation dependent



Pace

Pace



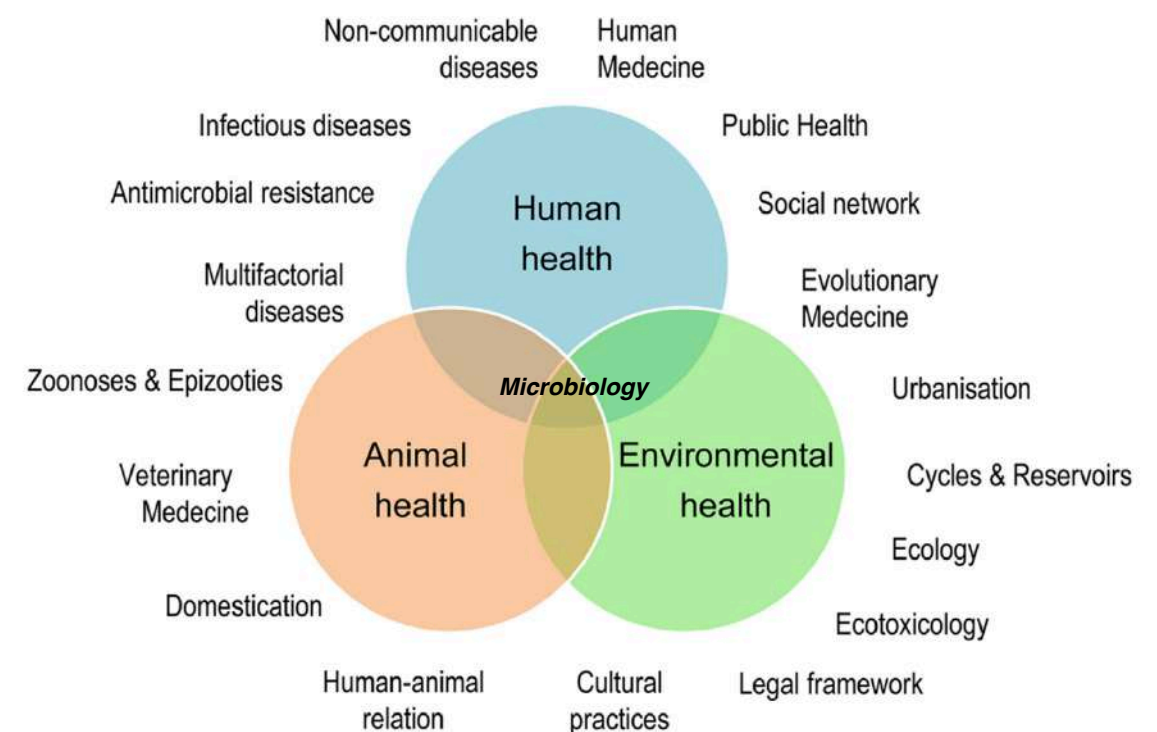
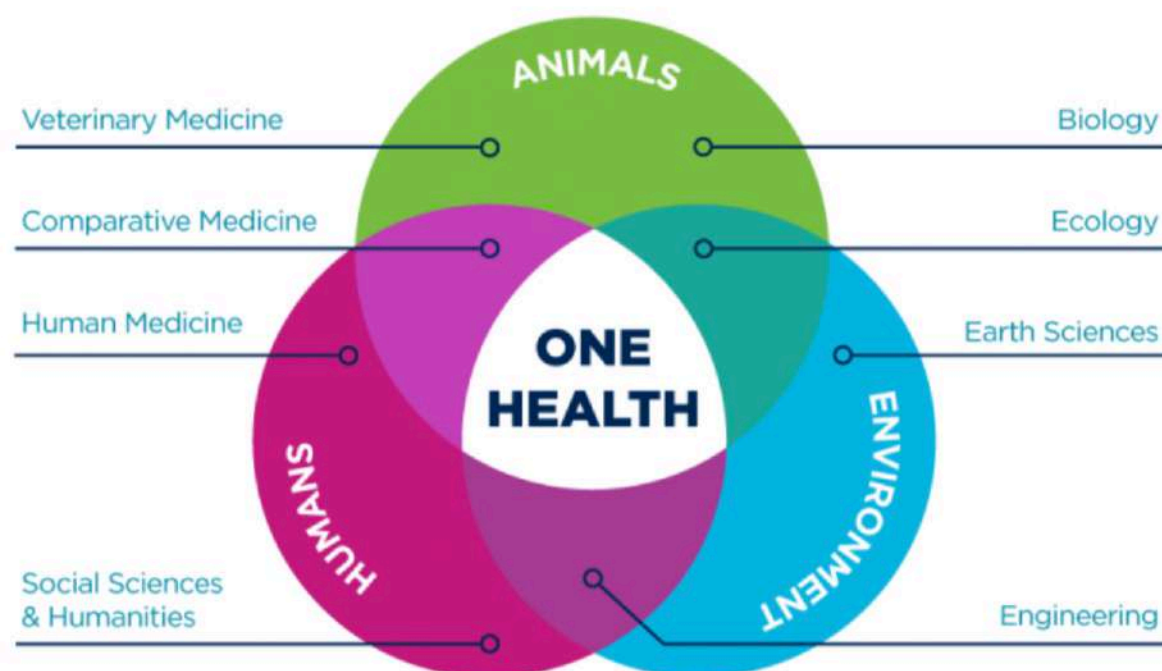
Cultivation independent

Microbes living in the environment

Microbes can or can't be cultivable

- Now and in the near FUTURE: **One Health**: approach to designing and implementing programs, policies, legislation and research in which multiple sectors communicate and work together **to achieve better public health outcomes**

Holistic approach where interactions matter



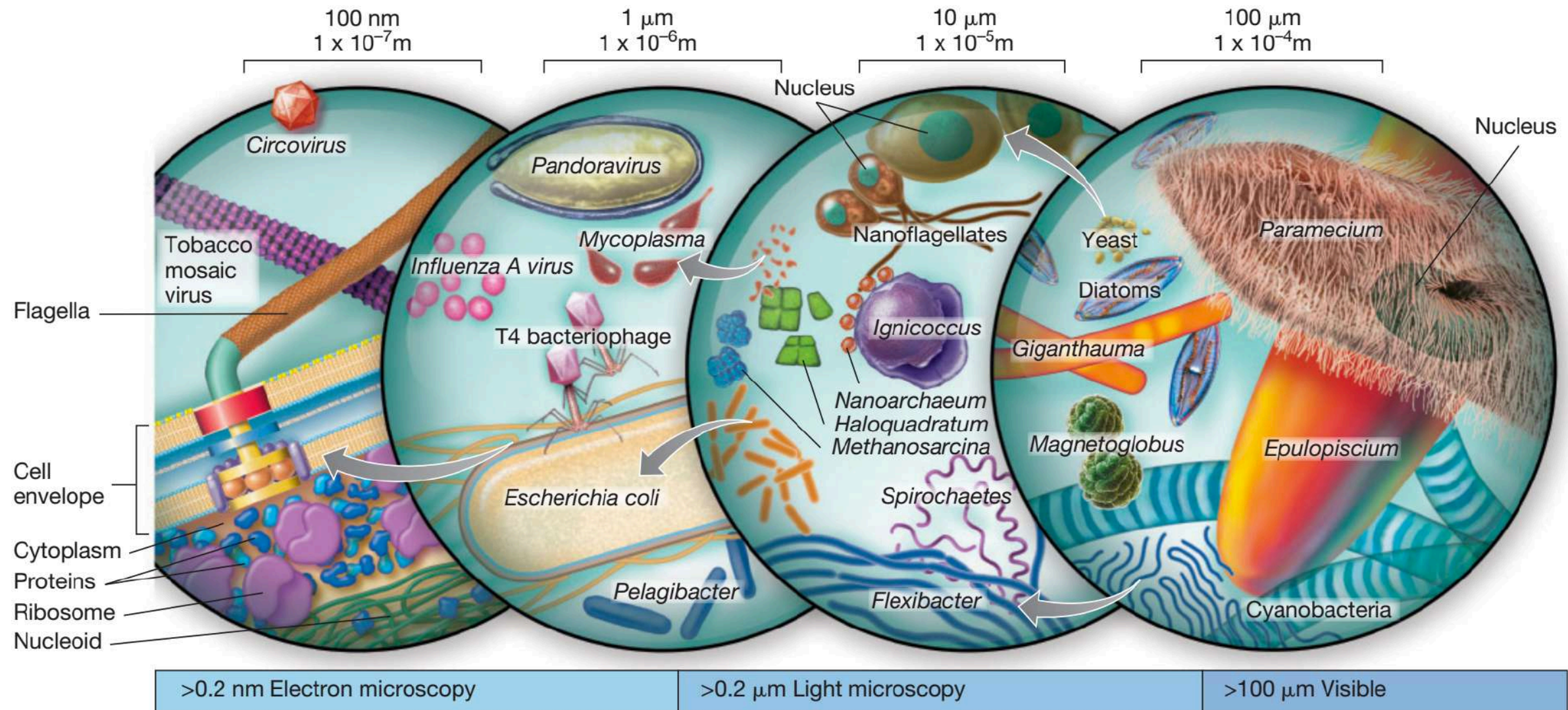
Microbiology:

Holistic study of the function of microbial cells and their impact on medicine, industry, environment and technology (Madigan et al. 2018).

Goals of the course

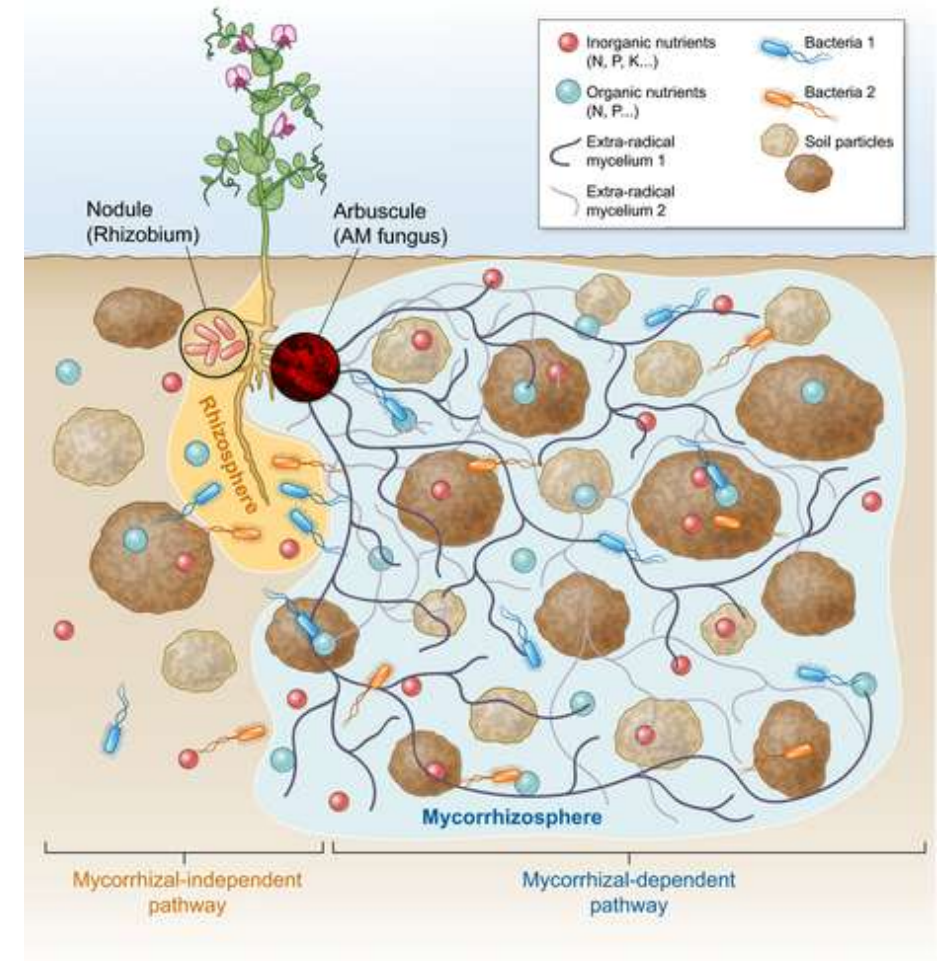
Microbial literacy and fundamental understanding of microbes life and their function in the environment, thus included the human beings.

Microbial size range

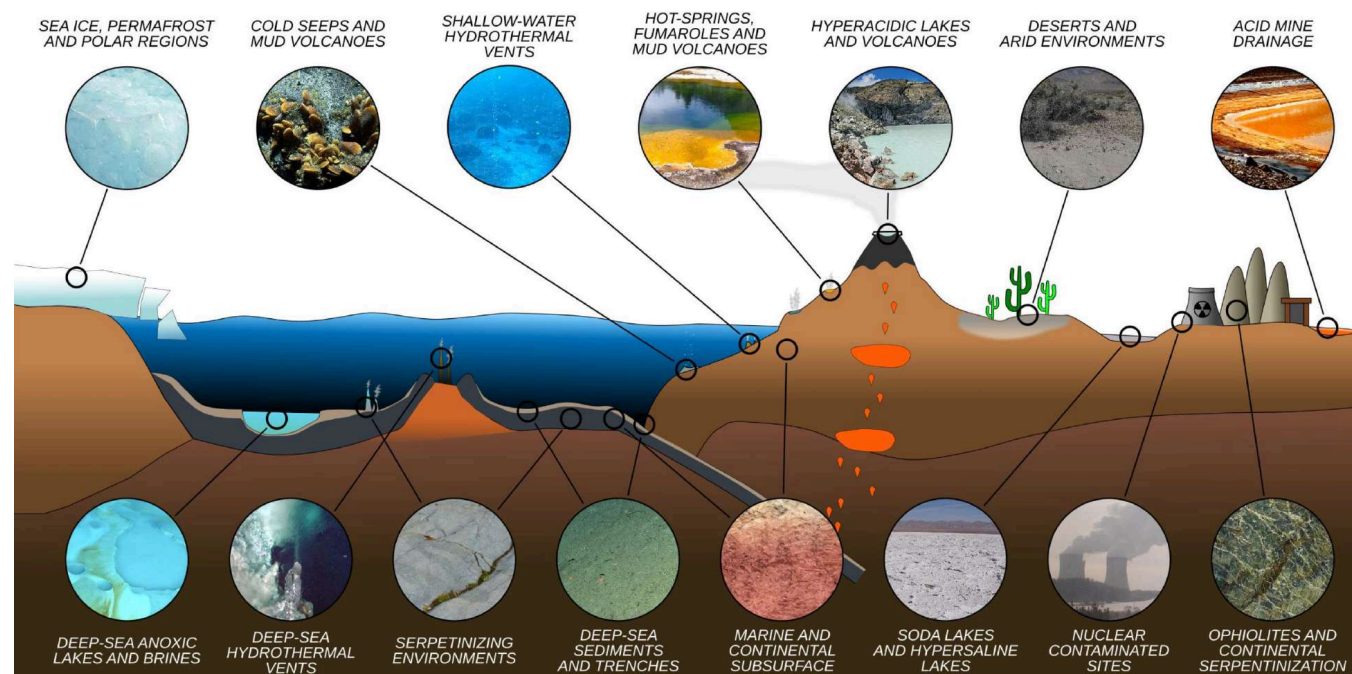


Microbial environments

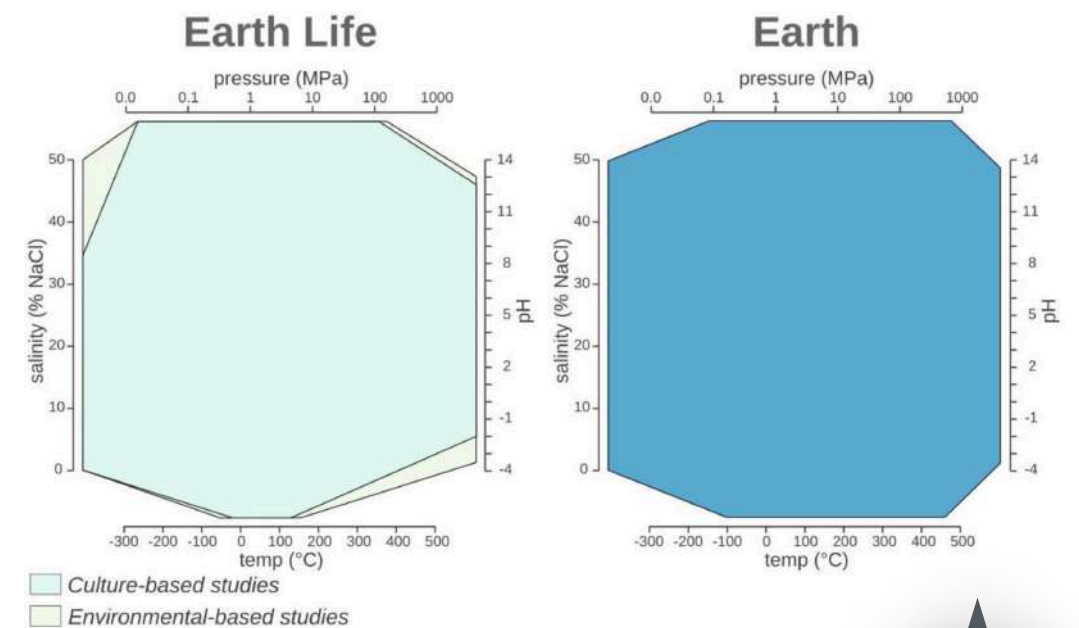
- Temperature
- pH
- Light/Dark
- Humidity
- Pressure
- Radiations (not on Earth)



Wipf et al. 2019



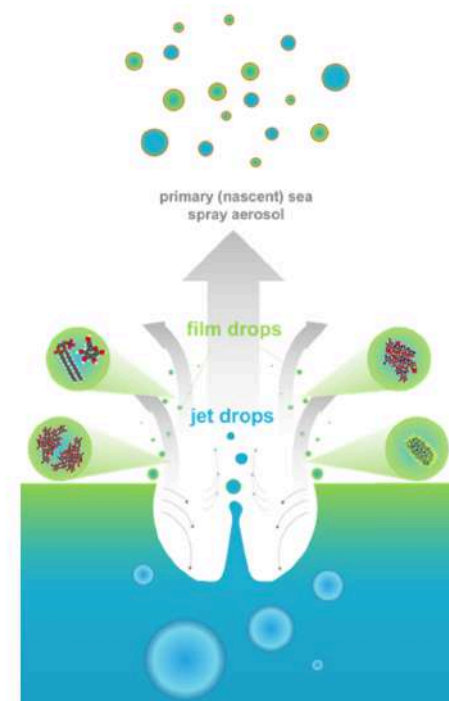
Merino et al. 2019



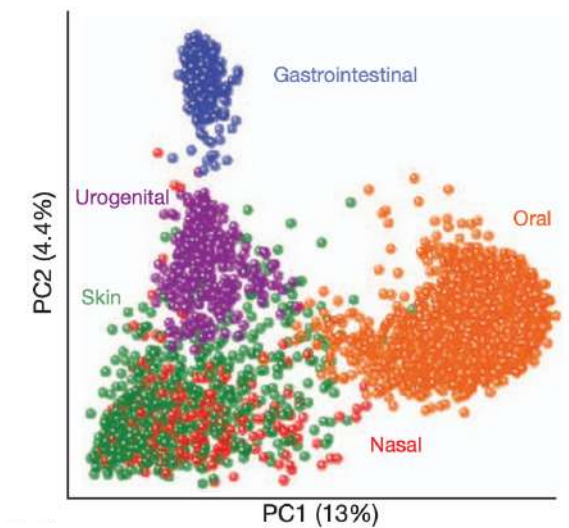
Merino et al. 2019

Microbial environments

- Ionic strength/Salinity
- State of water
- Organic matter concentration
- Oxygen and other redox active molecules
- 3D structure in space and time
- Other microorganisms and their biology
- Humans

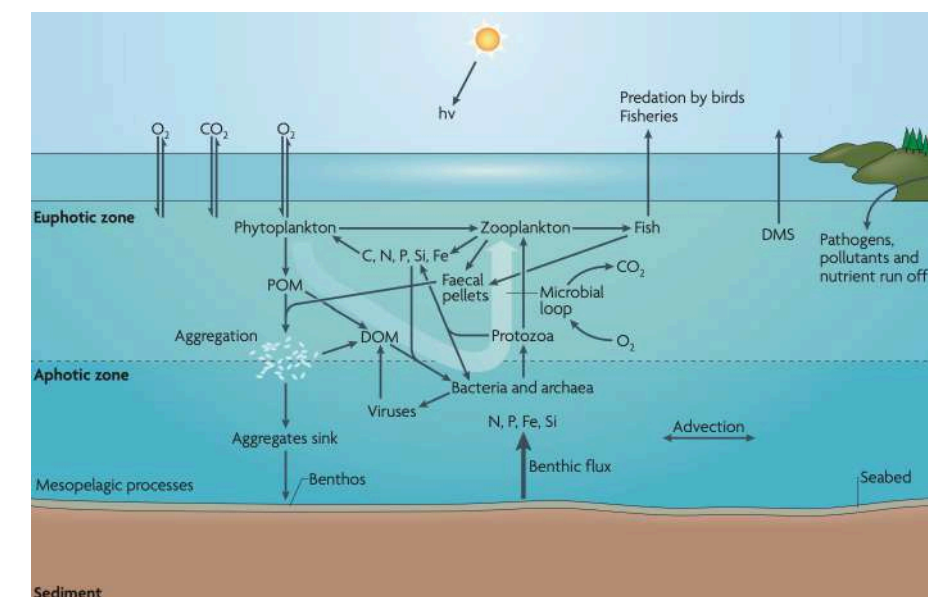


HMPC 2012



2020 CENTER FOR AEROSOL
IMPACTS ON CHEMISTRY OF
THE ENVIRONMENT

Azam & Malfatti 2007

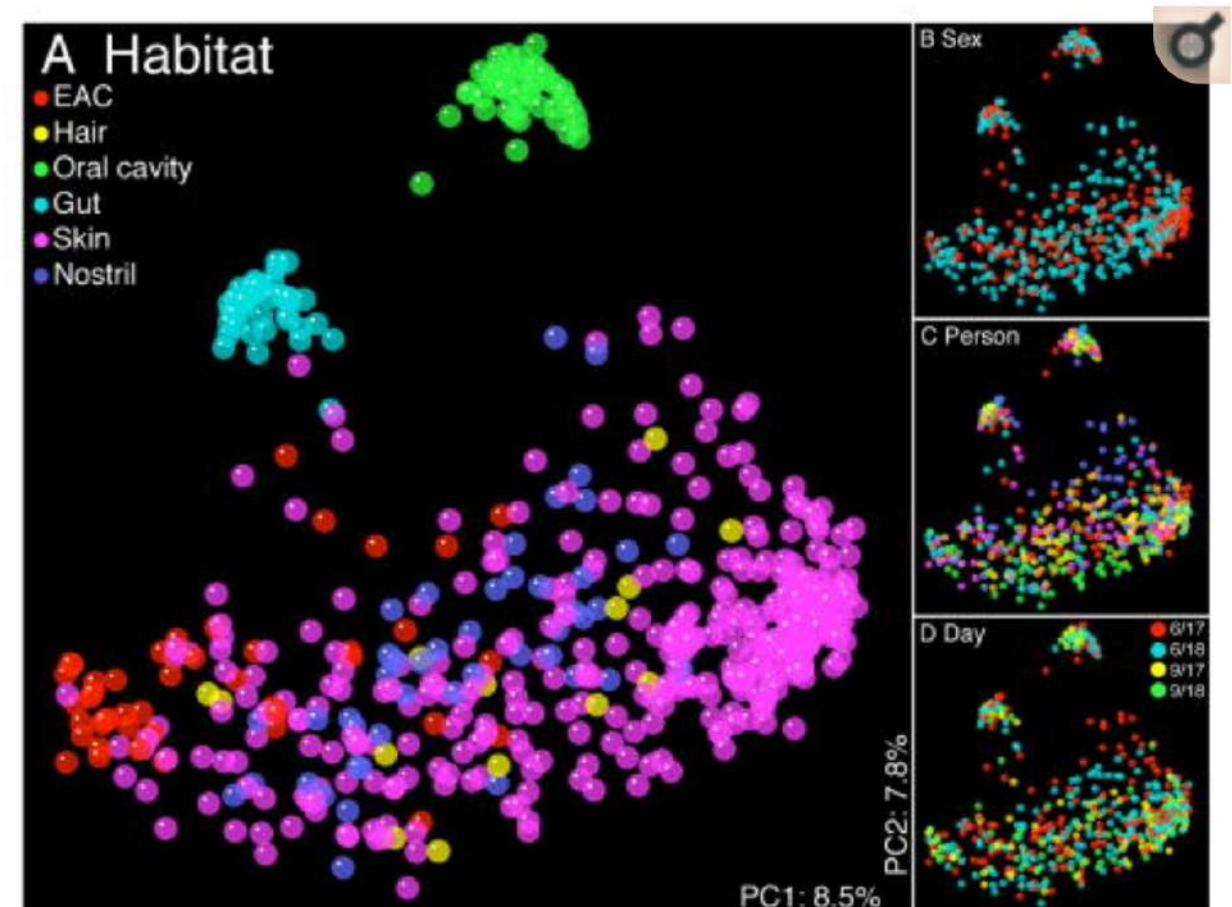


Specific adaptation to grow in the microenvironment



Human as a microbial environment

- 36-37°C and > 38°C
- Rich environment: proteins, sugar and lipids
- Oxygen 5-20 mL/dL (in the blood) to 0 in the stomach
- Oxygen changes with altitude 100 mmHg = 20 mL/dL (148-43 mmHg from London to Mt. Everest)
- Light/Dark cycles
- Immune system
- Eukaryotes and Viruses (ecology, chemistry and physiology)
- pH:
 - ★ skin~5.5
 - ★ blood~7.4
 - ★ mouth~ 6.7-7.3
 - ★ vagina ~3.8-4.5
 - ★ oesophagus 5-7
 - ★ stomach 2-5
 - ★ duodenum 6.8



Why are we studying microbes?

- Microbes have been profoundly shaping the Earth's environment
- Microbes have invented biochemistry (unifying concept, Kluyver, 1956)
- Microbes are very diverse and productive despite size
- Microbes are everywhere
- Microbes have made Earth habitable
- Humans have evolved from them
- Microbes have changed Humans and still changing them

From where do we start? —> *ab initio*



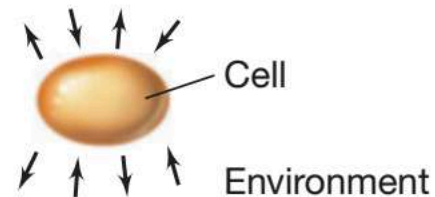
Being a microbe

Properties of *all* cells:

Metabolism

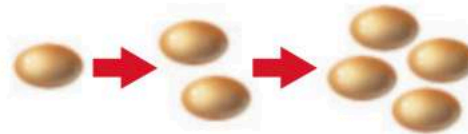
Cells take up nutrients, transform them, and expel wastes.

1. **Genetic** (replication, transcription, translation)
2. **Catalytic** (energy, biosyntheses)



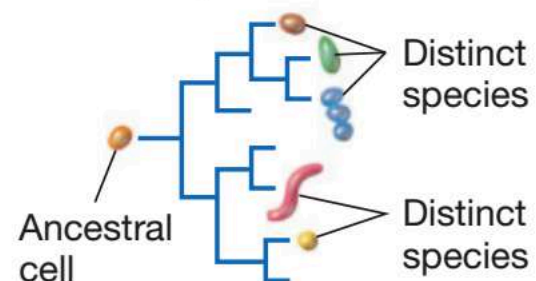
Growth

Nutrients from the environment are converted into new cell materials to form new cells.



Evolution

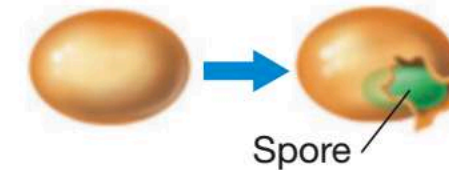
Cells evolve to display new properties. Phylogenetic trees capture evolutionary relationships.



Properties of *some* cells

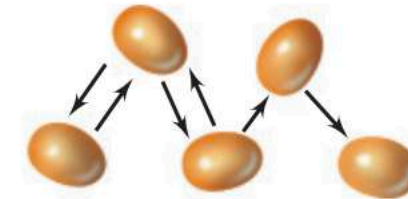
Differentiation

Some cells can form new cell structures such as a spore.



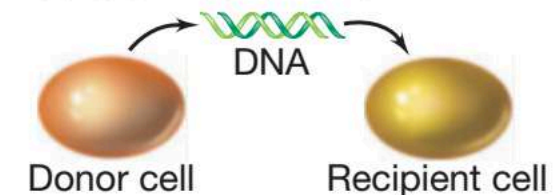
Communication

Cells interact with each other by chemical messengers.



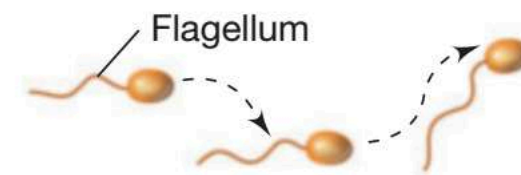
Genetic exchange

Cells can exchange genes by several mechanisms.



Motility

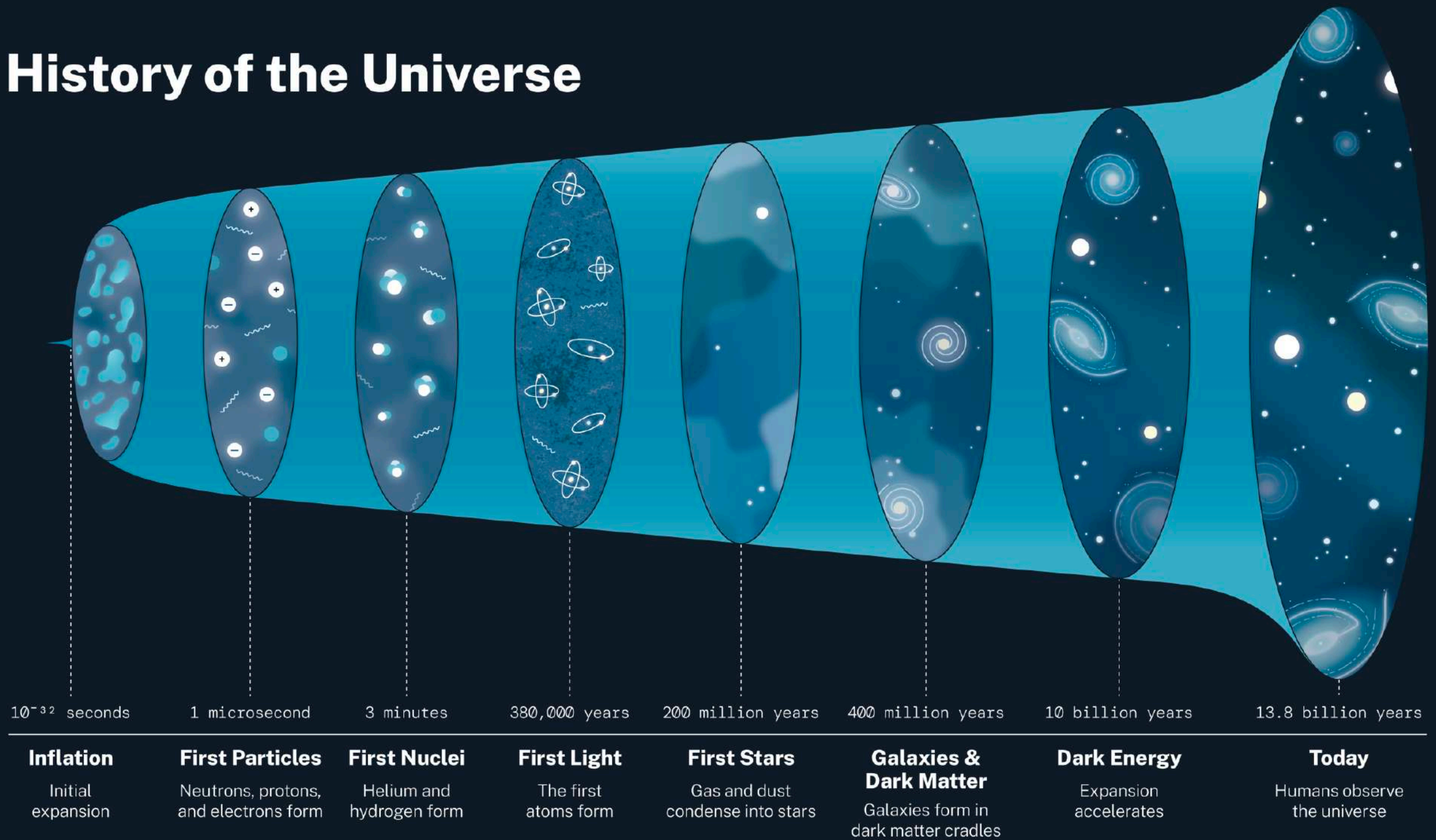
Some cells are capable of self-propulsion.



Ab initio

BIG BANG

History of the Universe



<https://science.nasa.gov/universe/overview/>

GALAXIES

10 billion and 13.6 billion years old



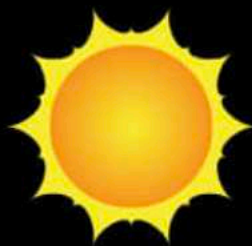
How old is it?



The Universe
~13.8 billion years



The Milky Way
~13.6 billion years



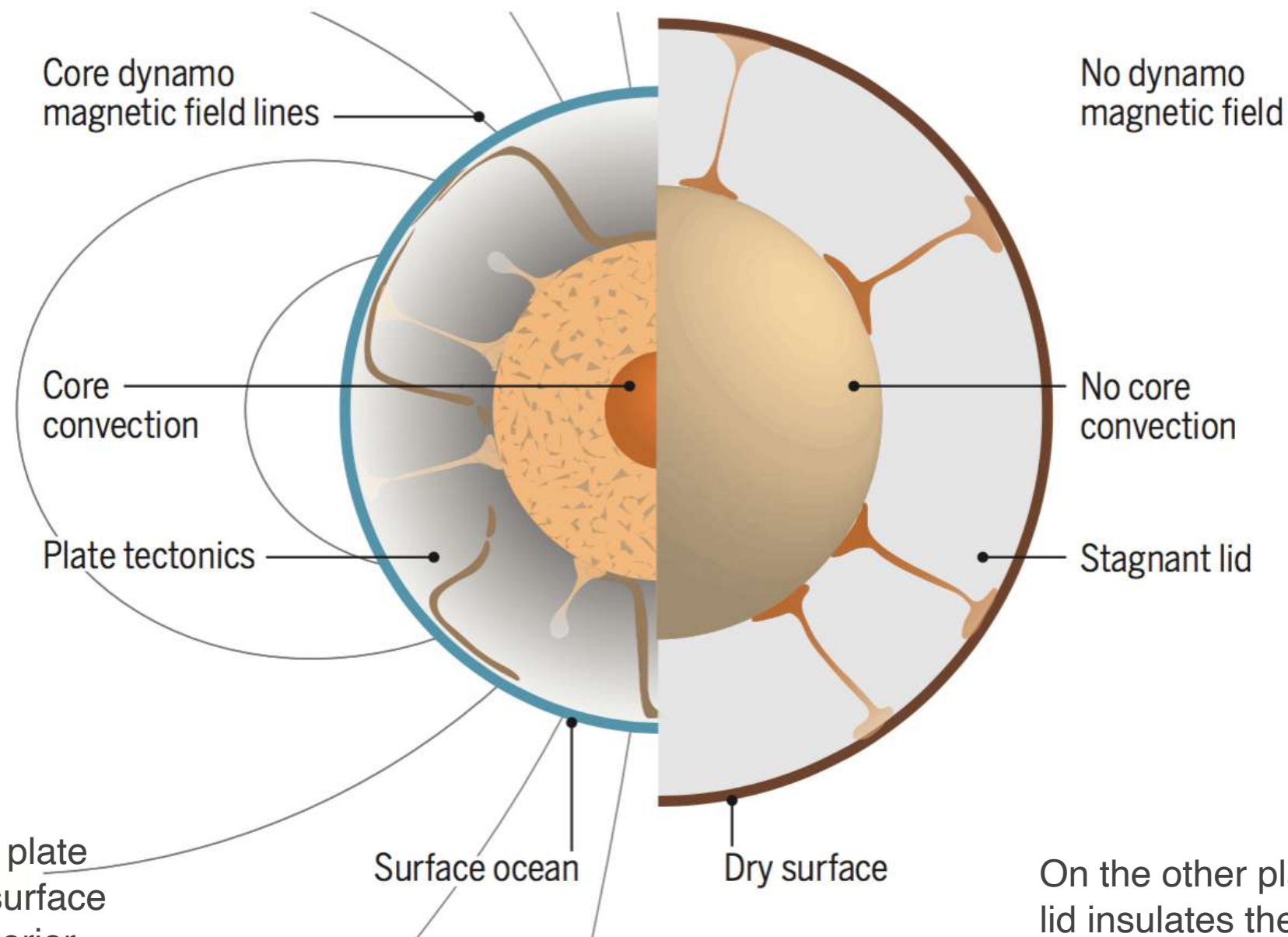
Our Sun
~4.5 billion years



Earth
~4.5 billion years

<https://spaceplace.nasa.gov/galaxies-age/en/#:~:text=Astronomers%20believe%20that%20our%20own,approximately%2013.6%20billion%20years%20old.>

Habitable features of Earth and Exoplanets



On the habitable planet, plate tectonics stabilizes the surface climate and cools the interior fast enough to generate a magnetic field that in turn shields the surface from water loss and harmful radiation

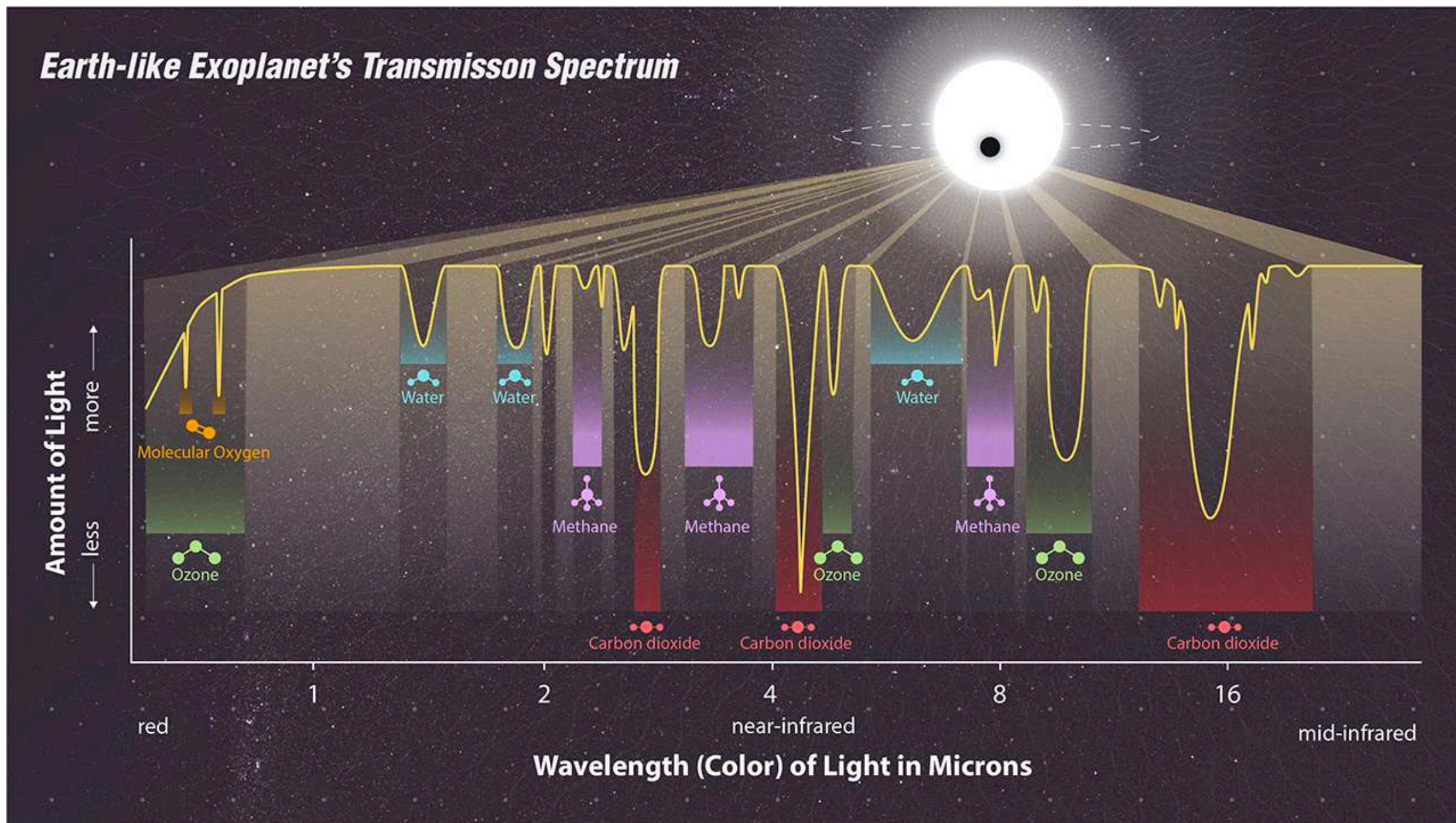
On the other planet, the stagnant lid insulates the interior, inhibiting magnetic field generation, allowing water loss to space, and rendering the surface too hot and dry for life

Volume of planet Earth

$$1.08321 \times 10^{12} \text{ km}^3$$

The Biosphere occupies about 0.00008 % of the mass of the Earth, $M_{\oplus} = 5.9722 \times 10^{24} \text{ kg}$ (Knight and Schlager 2002) and or 0.00007 % of Earth volume

Prokaryote volume $1 \mu\text{m}^3$

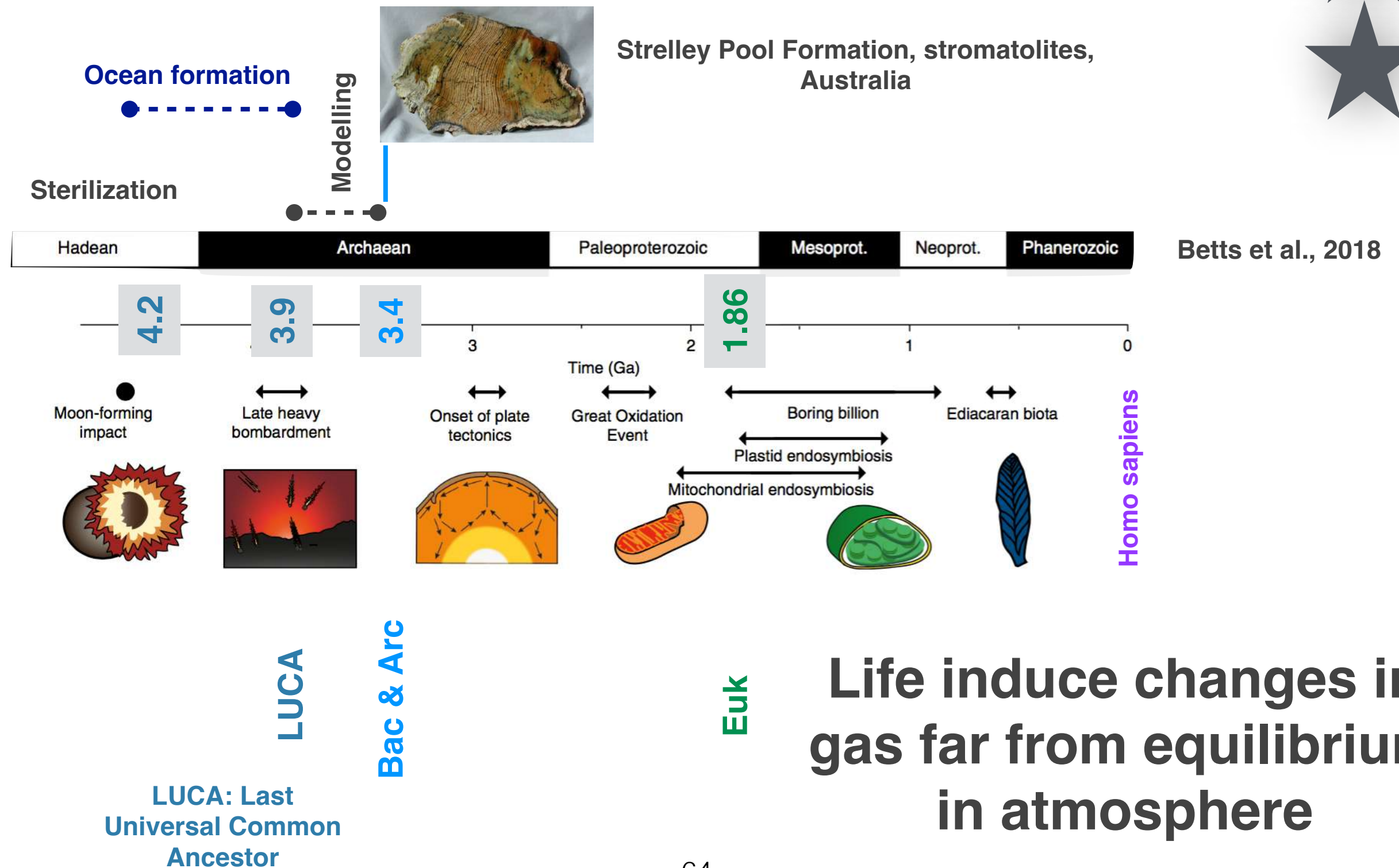


This is a transmission spectrum of an Earth-like exoplanet. The graph, based on a simulation, shows what starlight looks like as it passes through the atmosphere of an Earth-like exoplanet. As the exoplanet moves in front of the star, some of the starlight is absorbed by the gas in that exoplanet's atmosphere and some is transmitted through it. Each element or molecule in the atmosphere's gas absorbs light at a very specific pattern of wavelengths. This creates a spectrum with dips that show where the wavelengths of light are absorbed, as seen in the graph. Each dip is like a "signature" of that element or molecule.

Just saying atmospheres around us....

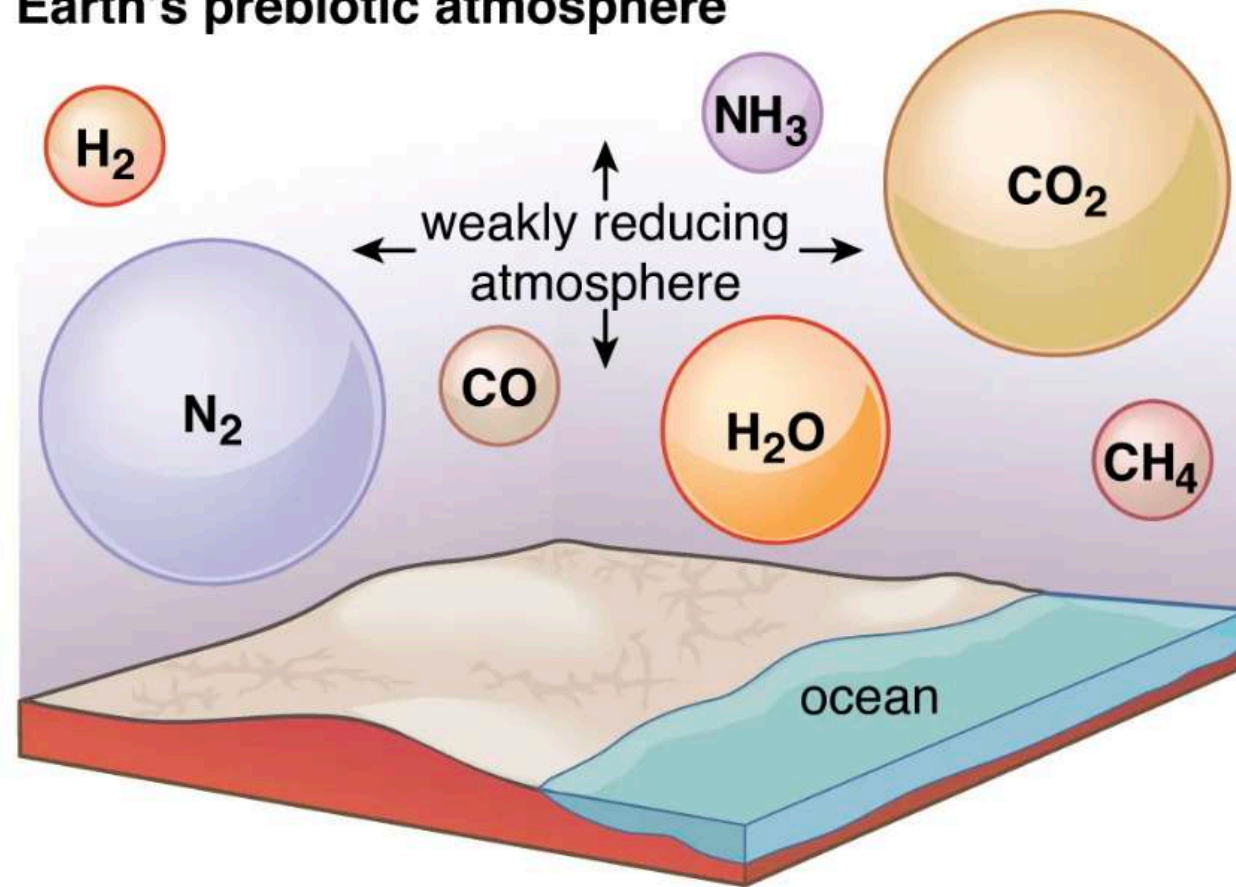
Object	Mass (kilograms)	Carbon Dioxide	Nitrogen	Oxygen	Argon	Methane	Sodium	Hydrogen	Helium	Other
Sun	3.0×10^{30}							71%	26%	3%
Mercury	1000			42%			22%	22%	6%	8%
Venus	4.8×10^{20}	96%	4%							
Earth	1.4×10^{21}		78%	21%	1%					<1%
Moon	100,000				70%		1%		29%	
Mars	2.5×10^{16}	95%	2.7%		1.6%					0.7%
Jupiter	1.9×10^{27}							89.8%	10.2%	
Saturn	5.4×10^{26}							96.3%	3.2%	0.5%
Titan	9.1×10^{18}		97%			2%				1%
Uranus	8.6×10^{25}					2.3%		82.5%	15.2%	
Neptune	1.0×10^{26}					1.0%		80%	19%	
Pluto	1.3×10^{14}	8%	90%			2%				

Origin of Life: **when**

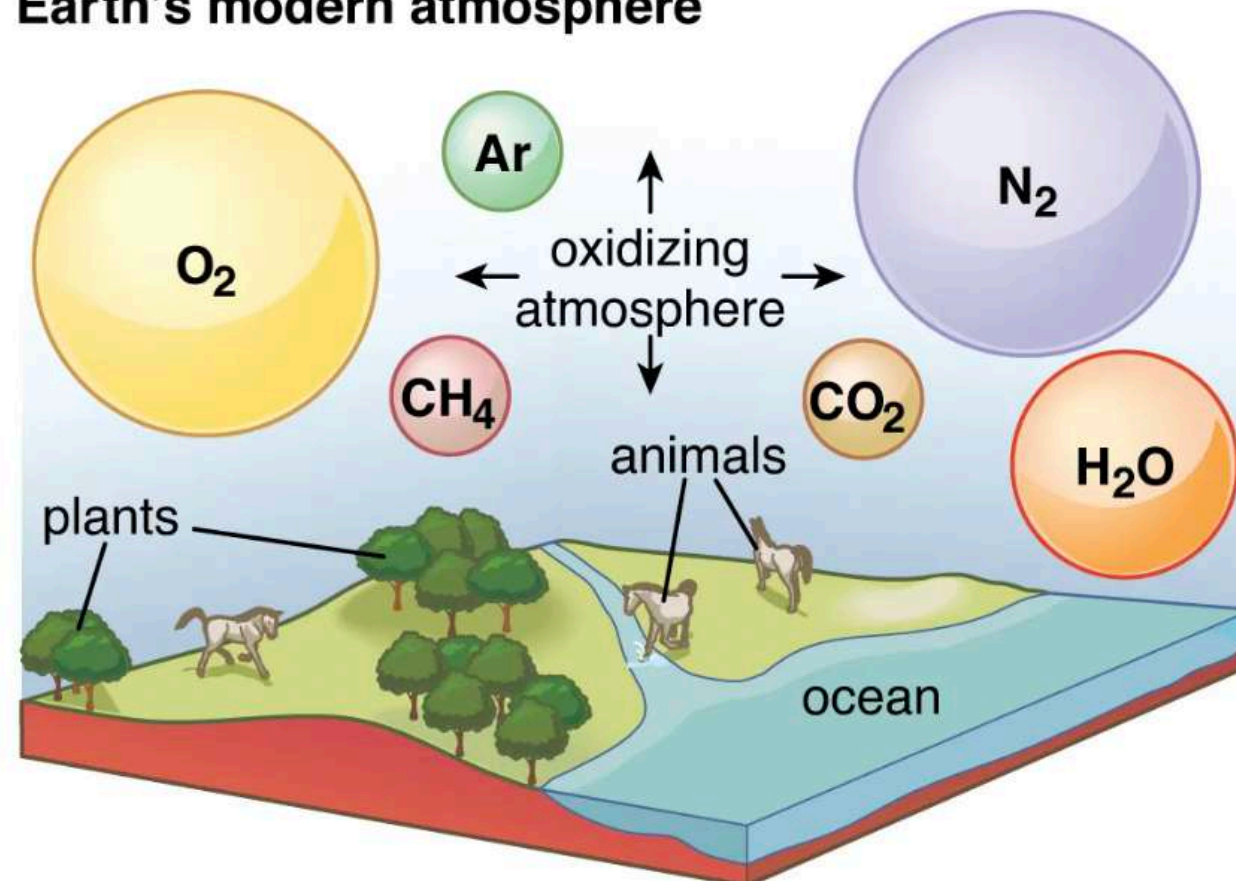


EARTH'S ATMOSPHERE

Earth's prebiotic atmosphere

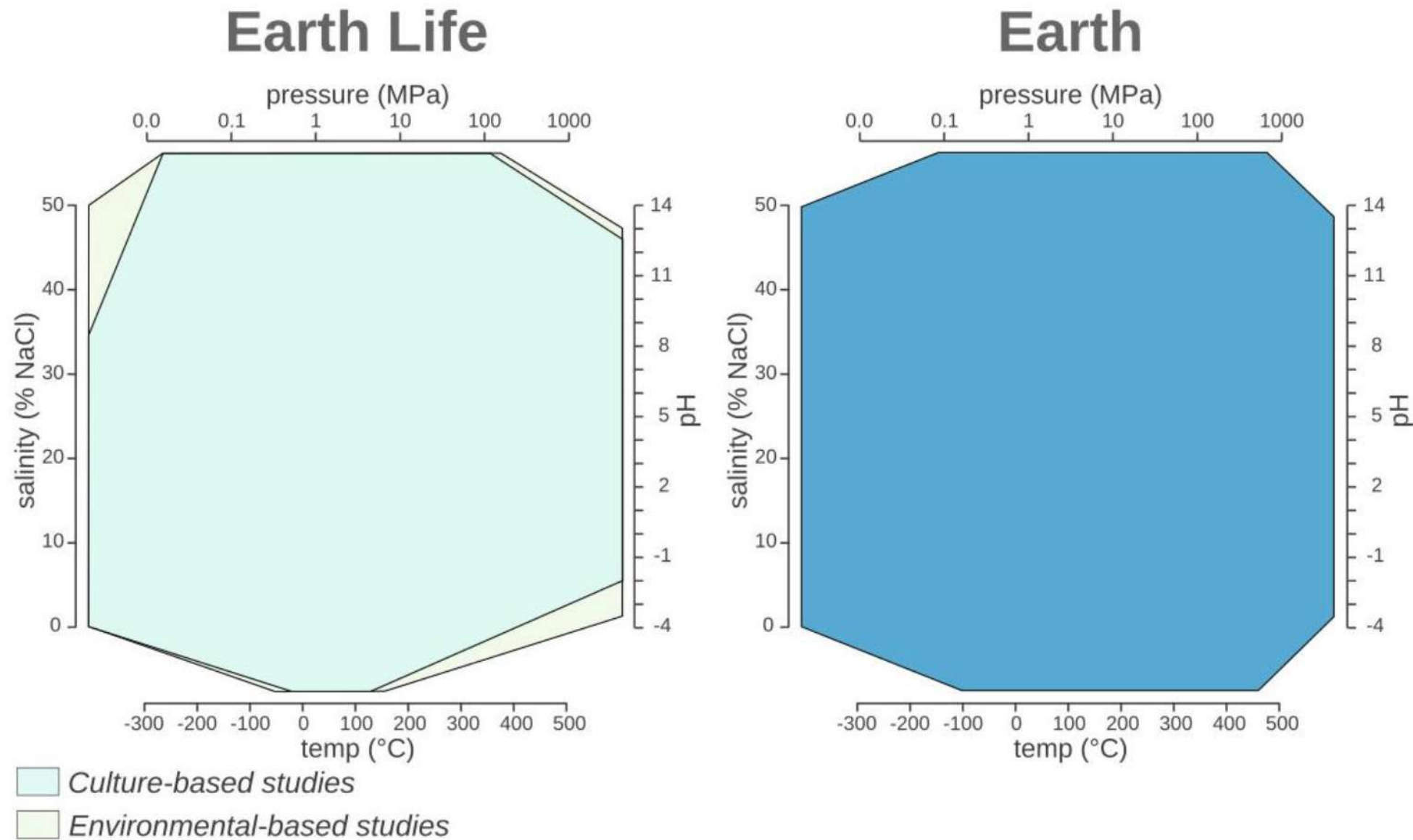


Earth's modern atmosphere



Microbial Life on Earth

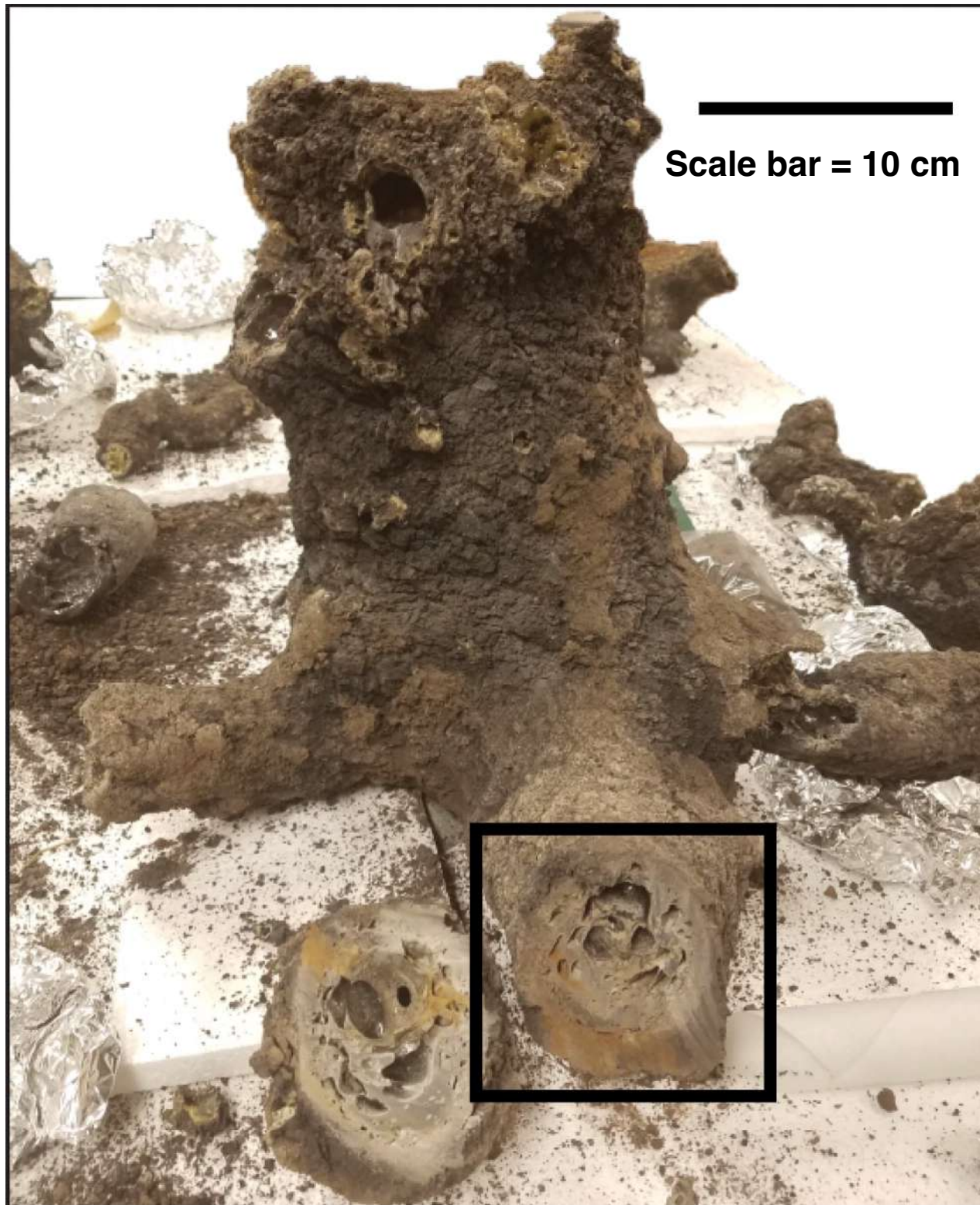
Merino et al., 2019



Earth is bursting with life, and its **biosphere** extends from ~10 km altitude to ~10 km into the oceans and oceanic crust as well as ~5 km into the continental crust

Biospheric capacity equivalent to ~1% of Earth's geosphere and troposphere → a minimum biospheric volume of ~10¹⁰ km³

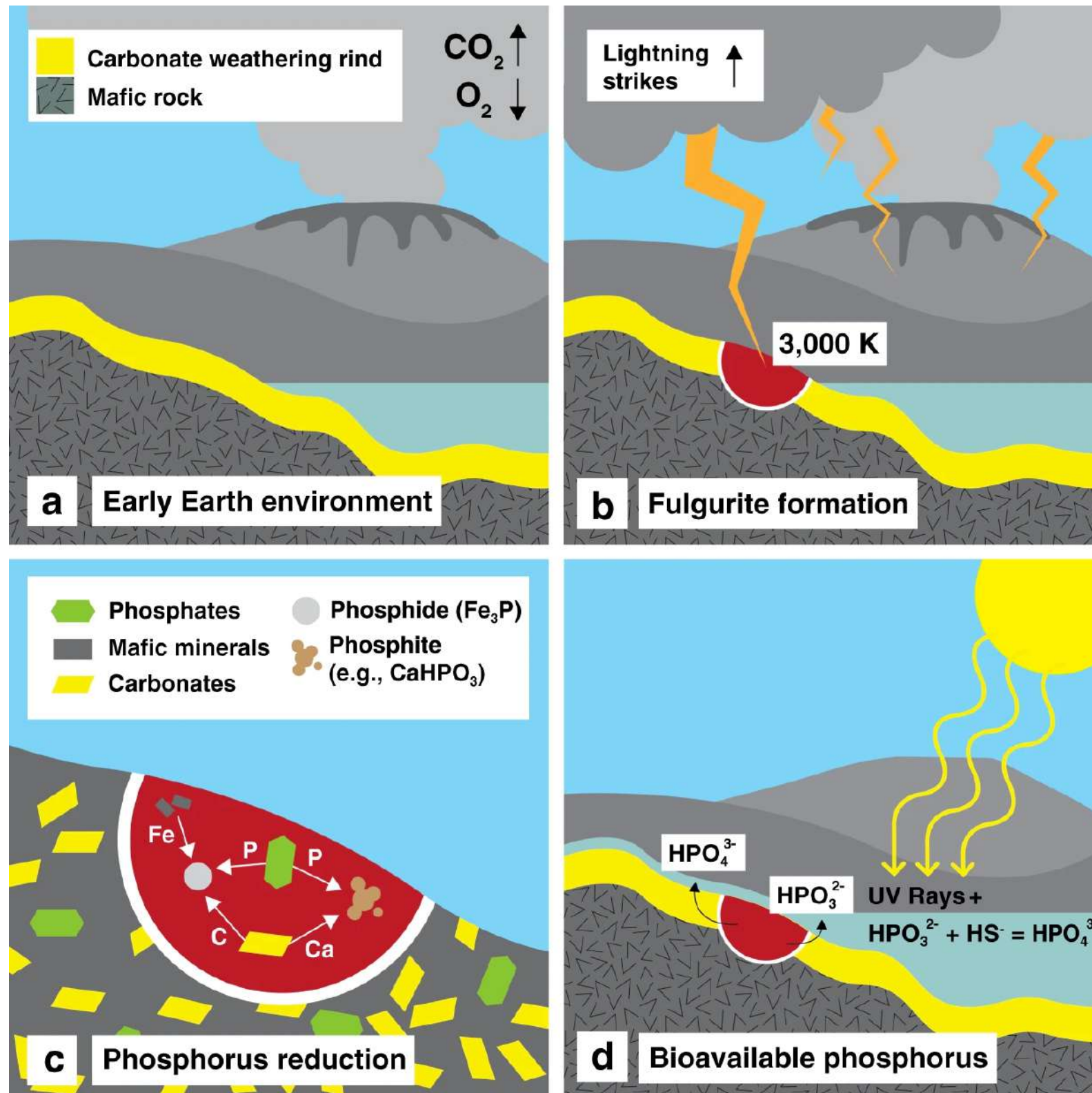
Lightning strikes as a major facilitator of prebiotic phosphorus reduction on early Earth



- Reduced phosphorus such as phosphide (P_0)
- Present in meteorite
- Present in fulgurite
- Significant source of prebiotic, reactive phosphorus which would have been concentrated on landmasses in tropical regions



Phosphorus reduction by lightning on early Earth



Intermediate phosphorus species react with UV rays and volcanically sourced HS^- to form additional phosphates available for prebiotic chemistry

LIFE

The origin of life is an extended continuum from the prebiotic chemistry to the first reproducing cells

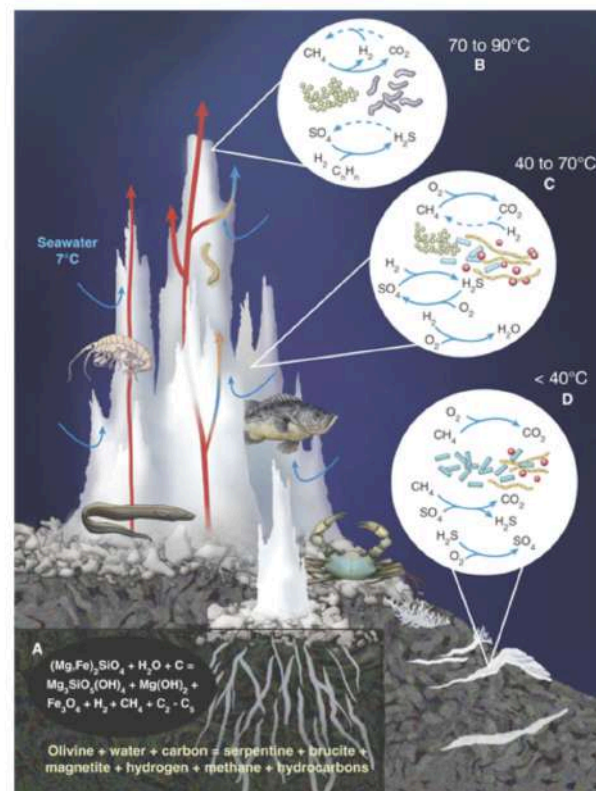
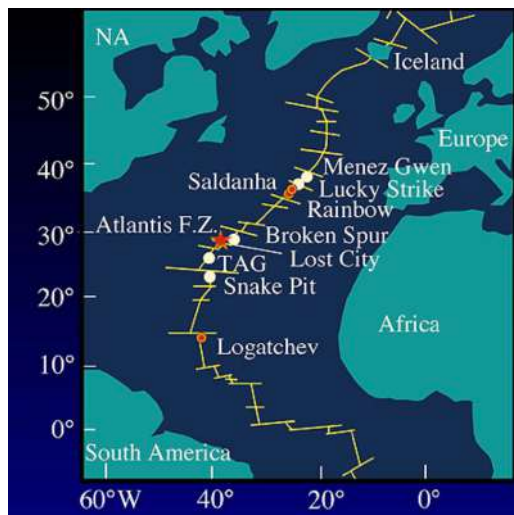
Origin of Life: **where**

At the interface:

- a. Diffusion limited surfaces
- b. Hydrophobic surfaces
- c. Adsorption of organic pre-biotic molecules
- d. Fe, S, other minerals acting as catalyst

- **Mineral surfaces on microporous rock (similar at hydrothermal vent, LOST CITY)**

- **Shallow terrestrial ponds with geothermal energy**



**The need of testable predictions according to where the
cradle of life has been**

PREBIOTIC SOUP

The synthesis of organic molecules begins with derivatives of cyanide, energised by ultraviolet radiations

- 1. Where does cyanide come from?**
- 2. How these reservoir of materials come to life when condition changed?**
- 3. Nucleotides are concentrated in small ponds that alternate dry and wet periods to polymerise and form RNA**

It implies: RNA act as a catalyst and a template —> favouring strands that are simpler, lacking metabolic capabilities.....

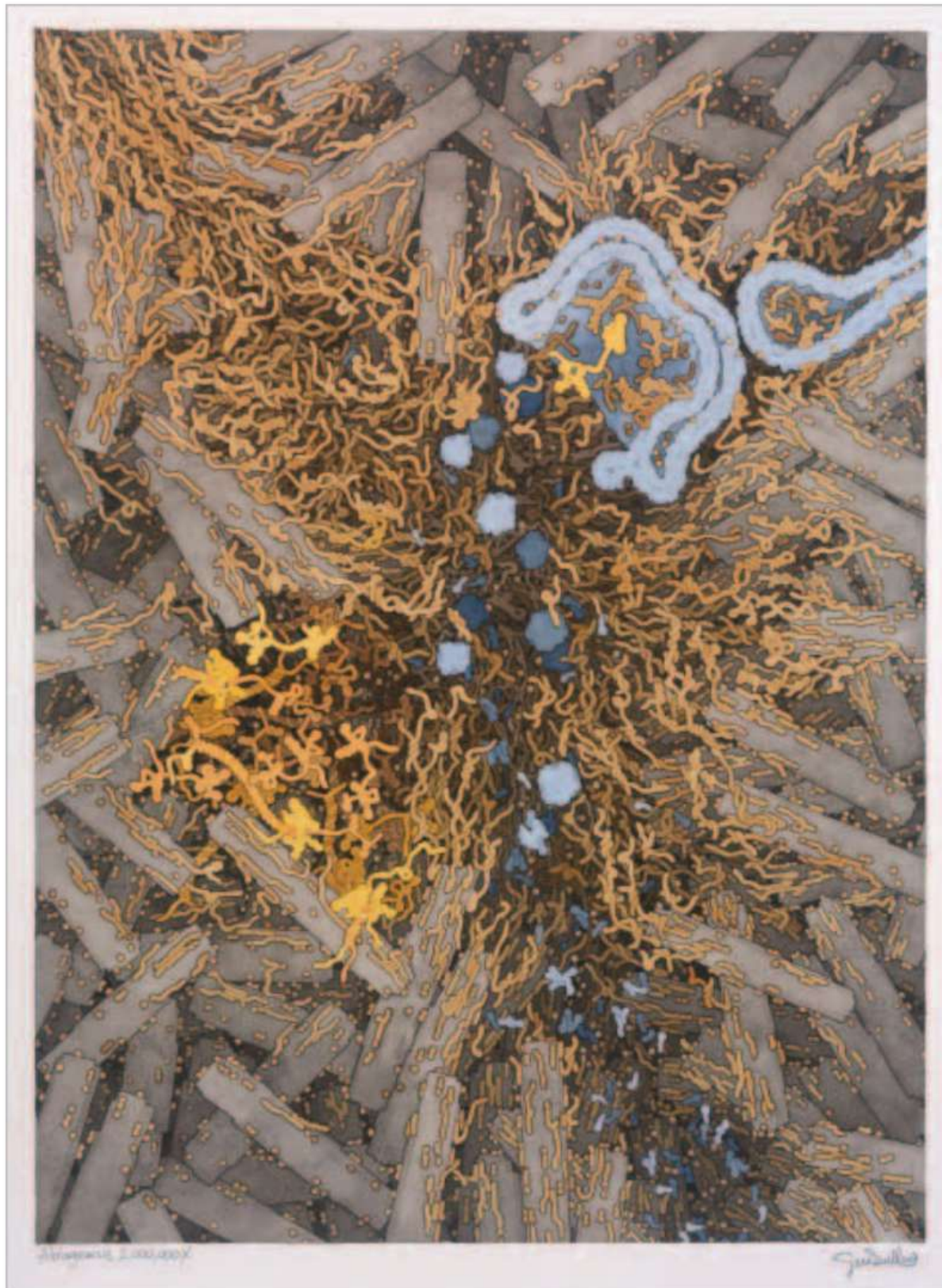
HYDROTHERMAL SYSTEMS

Carbon dioxide and hydrogen feed a network of reactions with a topology resembling metabolism

- 1. Carbon dioxide and hydrogen are not very reactive**
- 2. Deep-sea vents are labyrinths of interconnected pores with resembling cells, acids outsides and alkaline inside**
- 3. Flow of protons that promotes synthesis of carboxylic acids and long-chain fatty acids —> assemble in cell-like structures (i.e., vesicles)**
- 4. Enzymes are missing**
- 5. Polymerization happening at the water mineral surface interface**

Origin of Life: “**Abiogenesis**”

David S. Goodsell

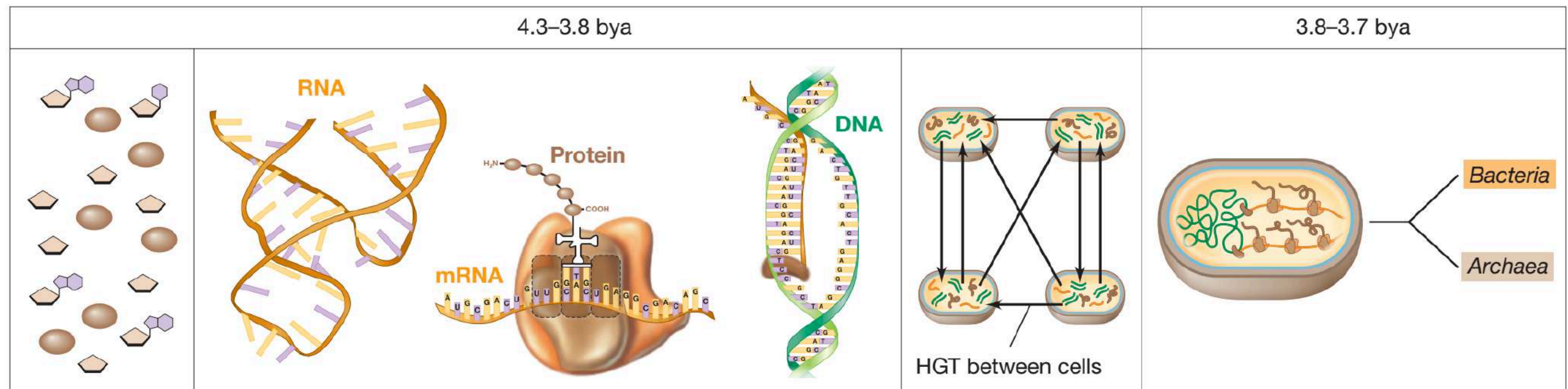


- Dawn of life, when **molecules gained the ability to replicate**
- **Cavity** in a mineral deposit at an alkaline hydrothermal vent
- **Nucleotide and lipid** building blocks are flowing in from lower right
- The nucleotides **interact** with the **mineral** crystals, catalyzing the formation of **RNA strands (brightest yellow)** —> ability to replicate other RNA strands (many copies of itself)
- The molecules in blue are simple lipids that have a useful property: they **assemble into membranes** that allow the nucleotides, but not RNA, to cross
- **If a closed vesicle is formed with a replicator inside** (like the autophagy-type vesicle forming at top right), nucleotides can enter and the RNA products will be retained inside, forming the **first protocell**

Origin of Life: **how**



Kitaday & Maruyama, 2018



Biological building blocks

- Amino acids
- Nucleosides
- Sugars

RNA world

- Catalytic RNA
- Self-replicating RNA

Protein synthesis

- RNA-templated translation

DNA

- Replication
- Transcription

Lipid bilayers

- Cellular compartments
- Early cells likely had high rates of HGT

HGT=Horizontal Gene Transfer

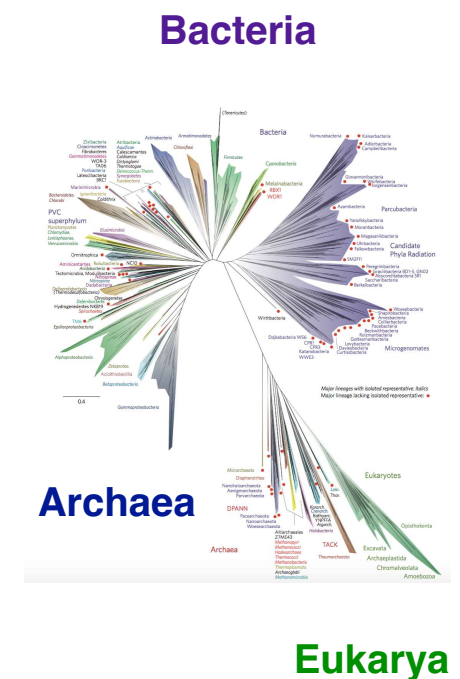
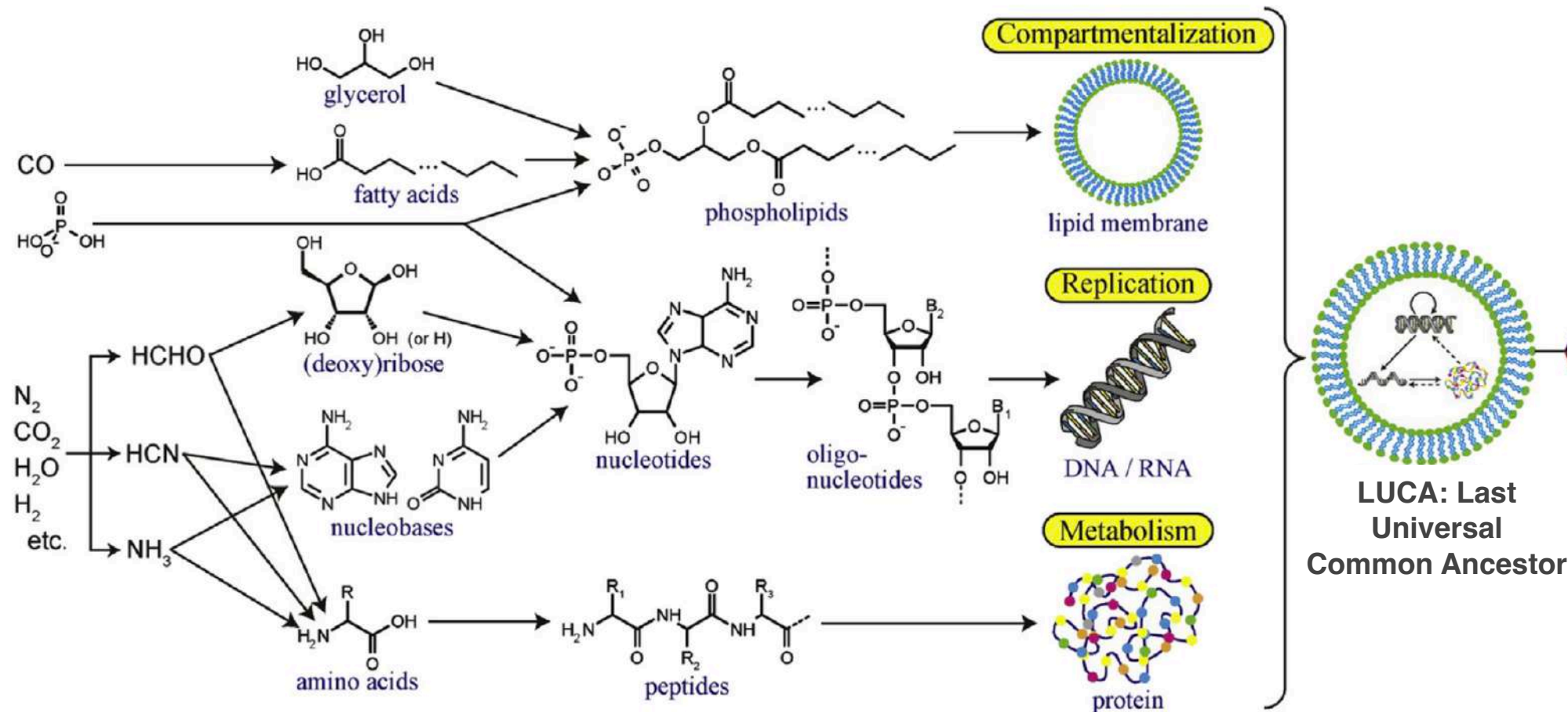
Divergence of *Bacteria* and *Archaea*

- Components of DNA replication, transcription, and translation all in place

Life needs water

Building complexity to achieve the 3 fundamental functions of Life

Inorganic molecules → Organic Precursors → Building blocks → Functional Polymers → Earliest life



primordial soup

Oparin AI. The Origin of Life. Izd. Moskovshii Rabochii; 1924

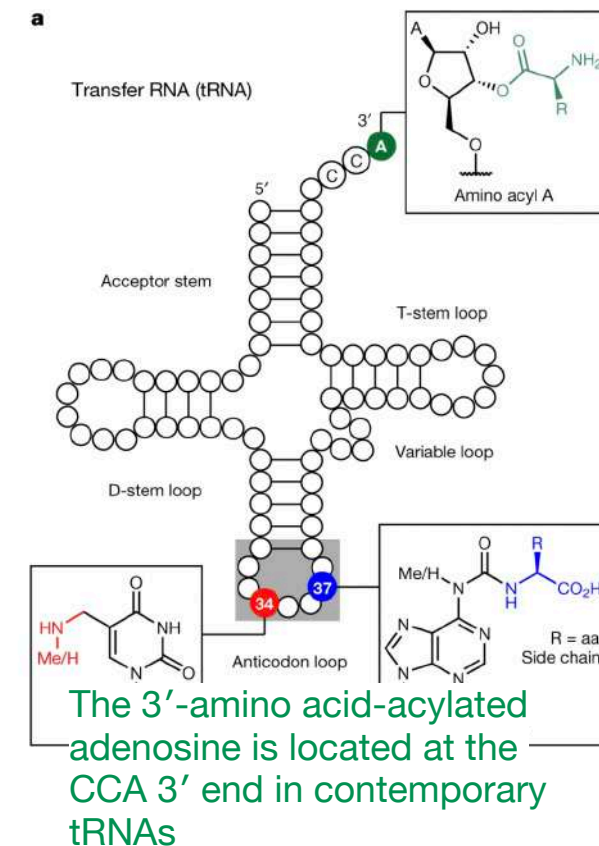
Haldane JB. The origin of life. Rationalist Annu. 1929

Miller-Urey's experiment mimicked lightning by the action of an electric discharge on a mixture of gases representing the early atmosphere ($\text{CH}_4/\text{H}_2\text{O}/\text{NH}_3/\text{H}_2\text{S}$ and later H_2O , N_2 , and CH_4 , CO_2 , or CO), in the presence of a liquid water reservoir, representing the early oceans → hydrogen cyanide, formaldehyde, and amino acids

Parker et al. 2014

RNA-peptide world

- RNA world concept: life evolved from increasingly **complex self-replicating RNA molecules**
- In RNA world: complex proto-**RNA** strands were **able** to both **copy themselves** and **compete with other strands**
- Later, these ‘**RNA enzymes**’ could have evolved the **ability to build proteins** and ultimately to **transfer** their **genetic information into more-stable DNA**
- **Catalysts made of RNA alone are much less efficient** than the protein-based enzymes found in all living cells today
- How this RNA world then advanced to the next stage, in which **proteins became the catalysts of life** and **RNA reduced its function** predominantly to **information storage**
- **Non-canonical RNA bases** are considered to be **relics of the RNA world** and are able to establish peptide synthesis directly on RNA (transfer and ribosomal RNAs)
- Complex peptide-decorated RNA chimeric molecules, which suggests the early existence of an RNA-peptide world → ribosomal peptide synthesis may have emerged



5-Methylaminomethyl uridine, mnm5U, is found in the wobble position 34

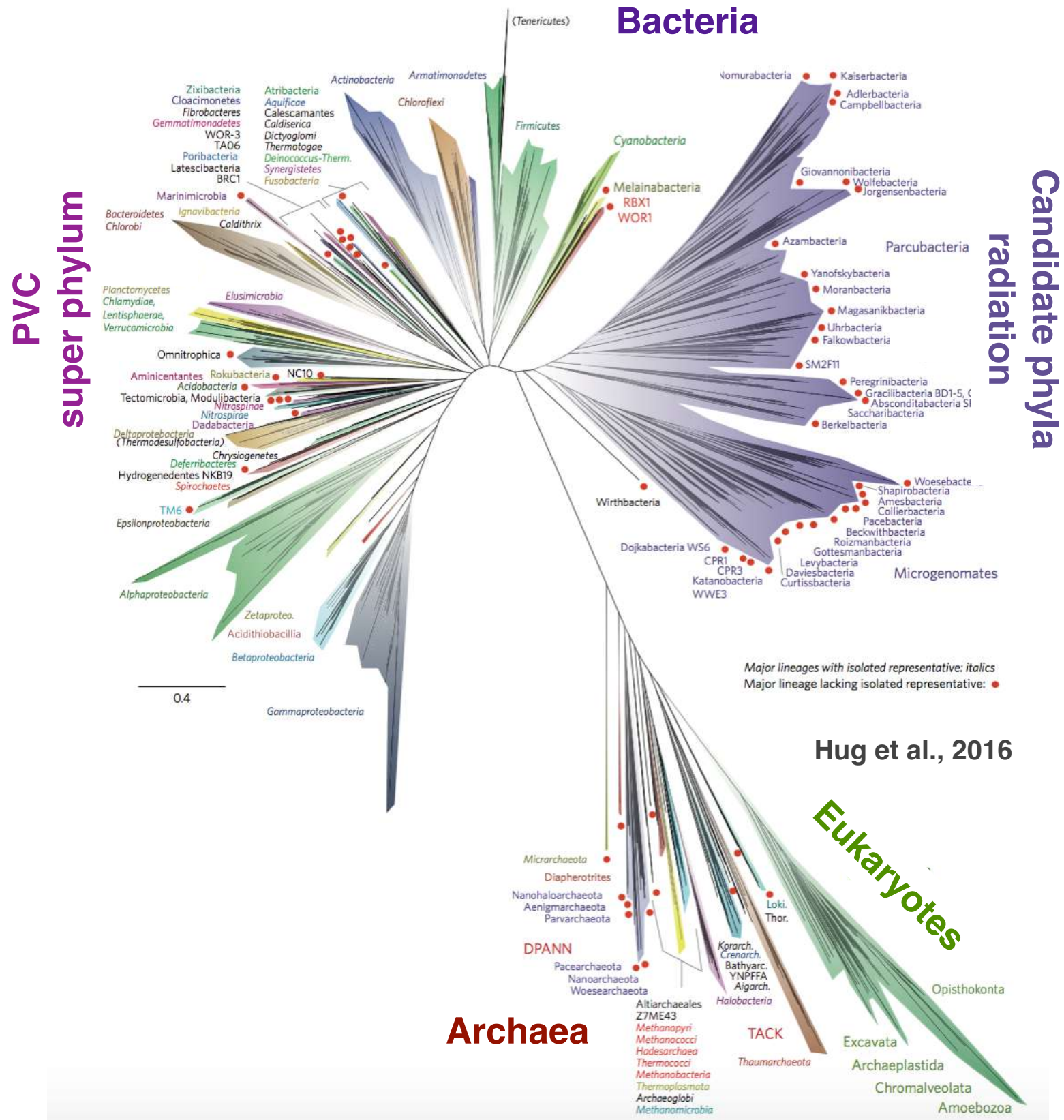
The amino acid-modified carbamoyl adenosine, (m6)aa6A (aa, amino acid), is present at position 37 in certain tRNAs

Müller et al., 2022

LUCA code

- **Smaller amino acids were added to the code earlier**, with no additional predictive power in the previous consensus order
- **Metal-binding (cysteine and histidine) and sulfur-containing (cysteine and methionine) amino acids were added** to the genetic code much earlier than previously thought.
- Methionine and histidine were added to the code earlier than expected from their molecular weights and glutamine later.
- Early methionine availability is compatible with inferred early use of S-adenosylmethionine
- and early histidine with its purine-like structure and the demand for metal binding.
- Even more ancient protein sequences—those that had already diversified into multiple distinct copies prior to LUCA—have significantly **higher frequencies of aromatic amino acids (tryptophan, tyrosine, phenylalanine, and histidine) and lower frequencies of valine and glutamic acid than single-copy LUCA sequences.**
- If at least some of these sequences predate the current code, then their distinct enrichment patterns provide hints about earlier, alternative genetic codes.

Microbial diversity on Earth

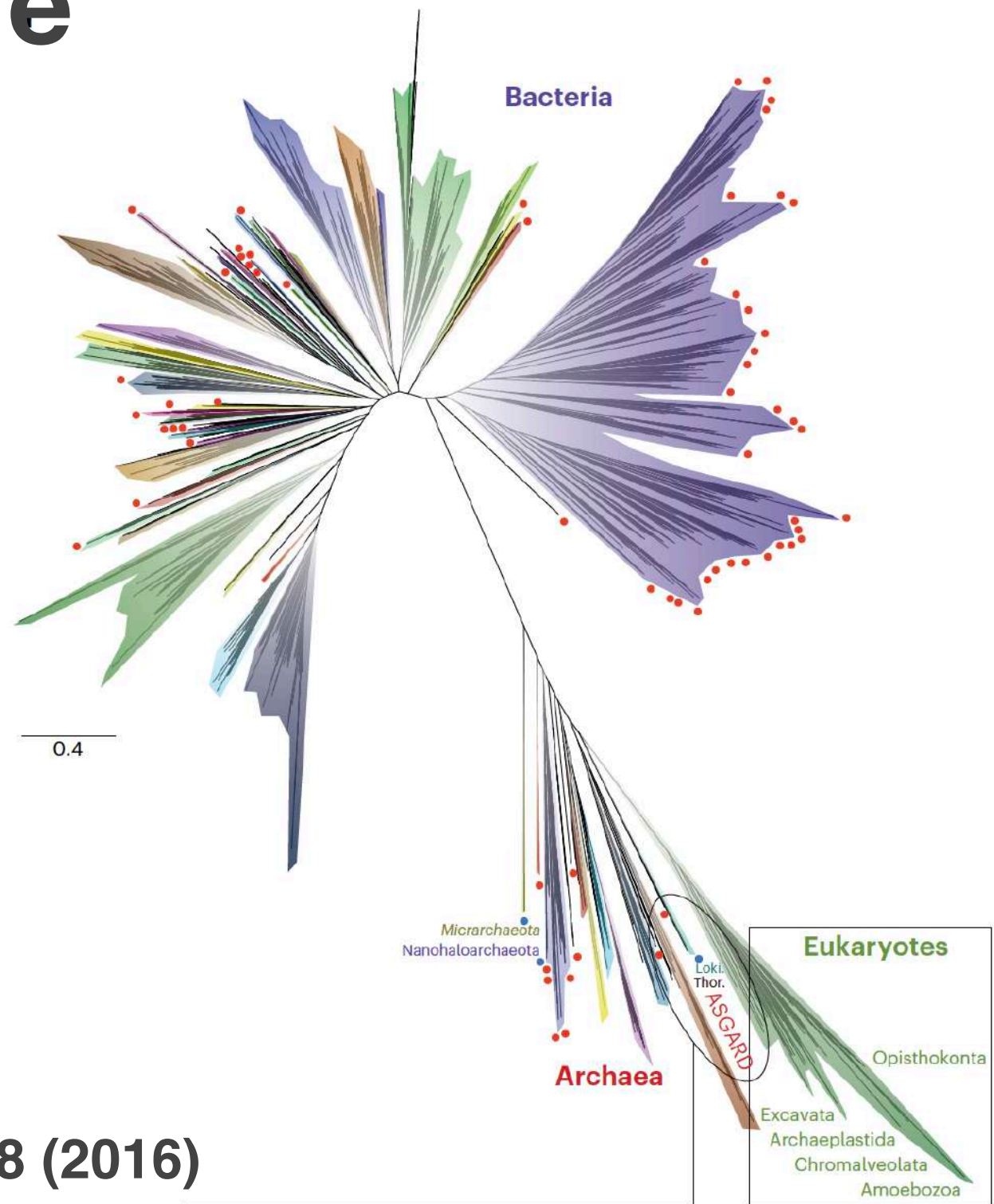


The Tree of Life

Concatenated 16-ribosomal protein tree with phyla highlighted with coloured wedges

Lineages with **no cultivated** representatives identified by **red dots**

Blue dots: lineages that have **gained cultivated** representatives since 2016

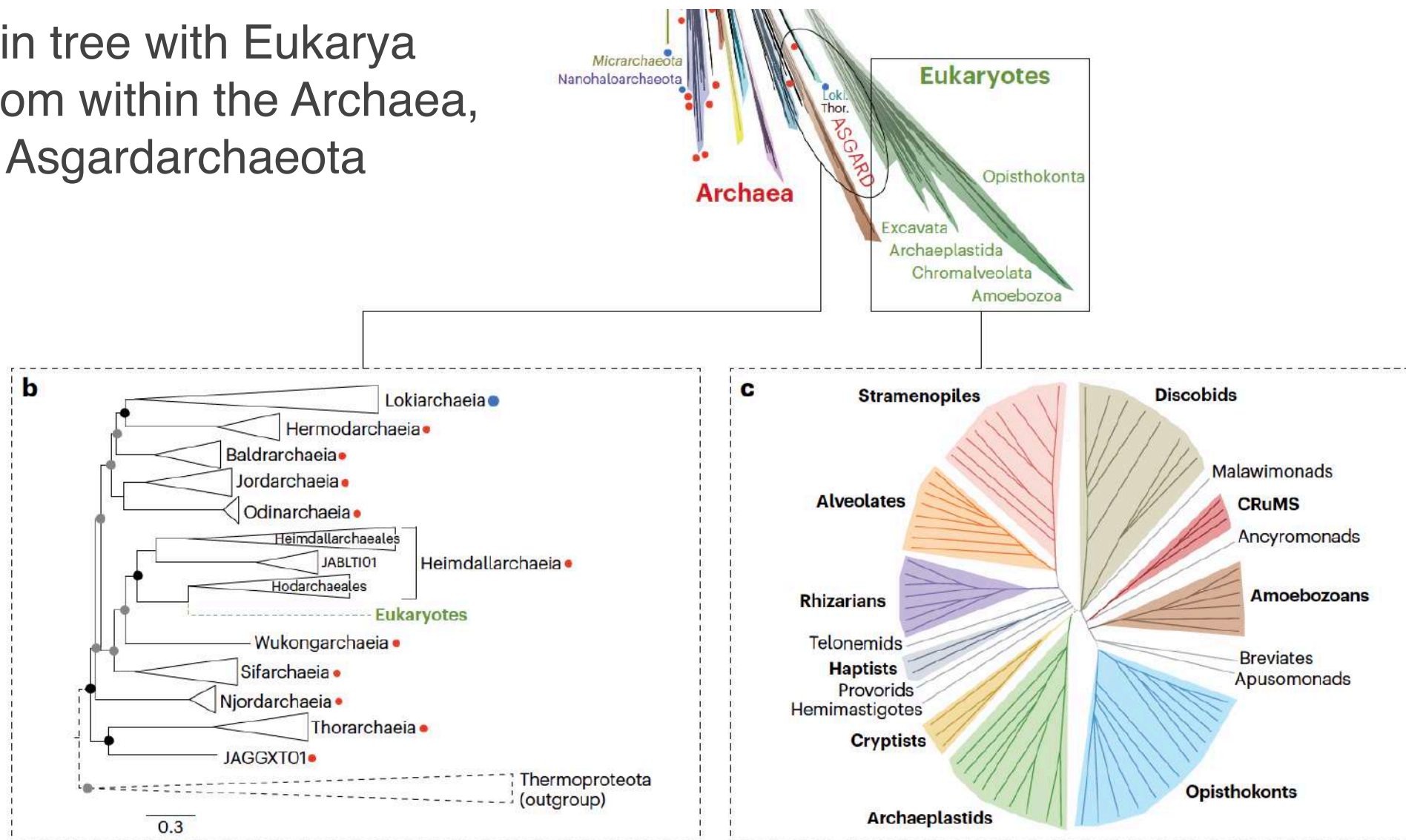


Hug et al. Nat. Microbiol. 1, 16048 (2016)

Hug 2024 <https://doi.org/10.1038/s41564-024-01768-w>

Archaea and Eukaryotes

A two-domain tree with Eukarya branching from within the Archaea, sister to the Asgardarchaeota

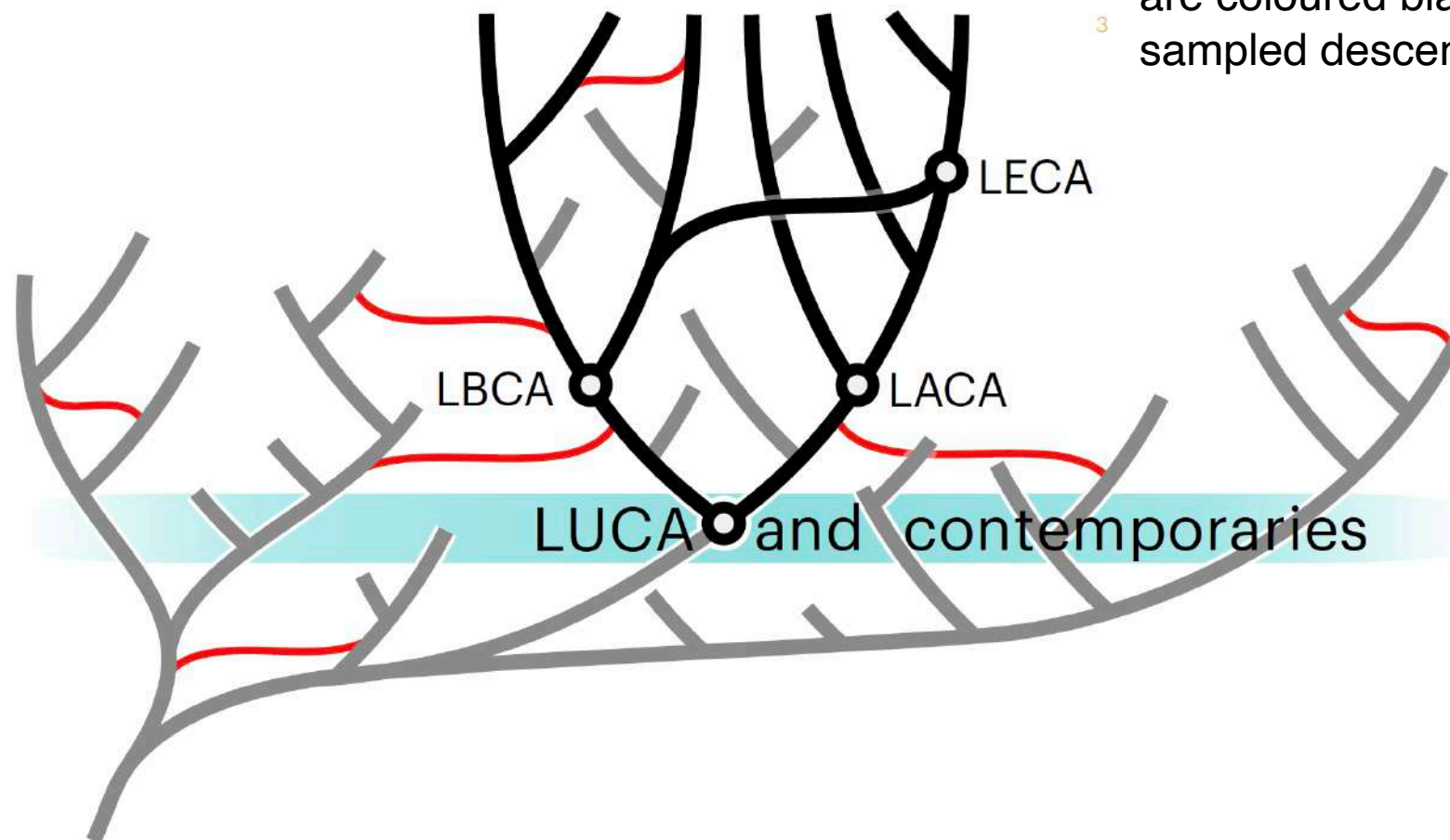


Microbial/Life evolution on Earth

Many different and diverse microbial communities rose and got extinct

Only LUCA survived

Branches on the tree of life that have left sampled descendants today are coloured black, those that have left no sampled descendants are in grey

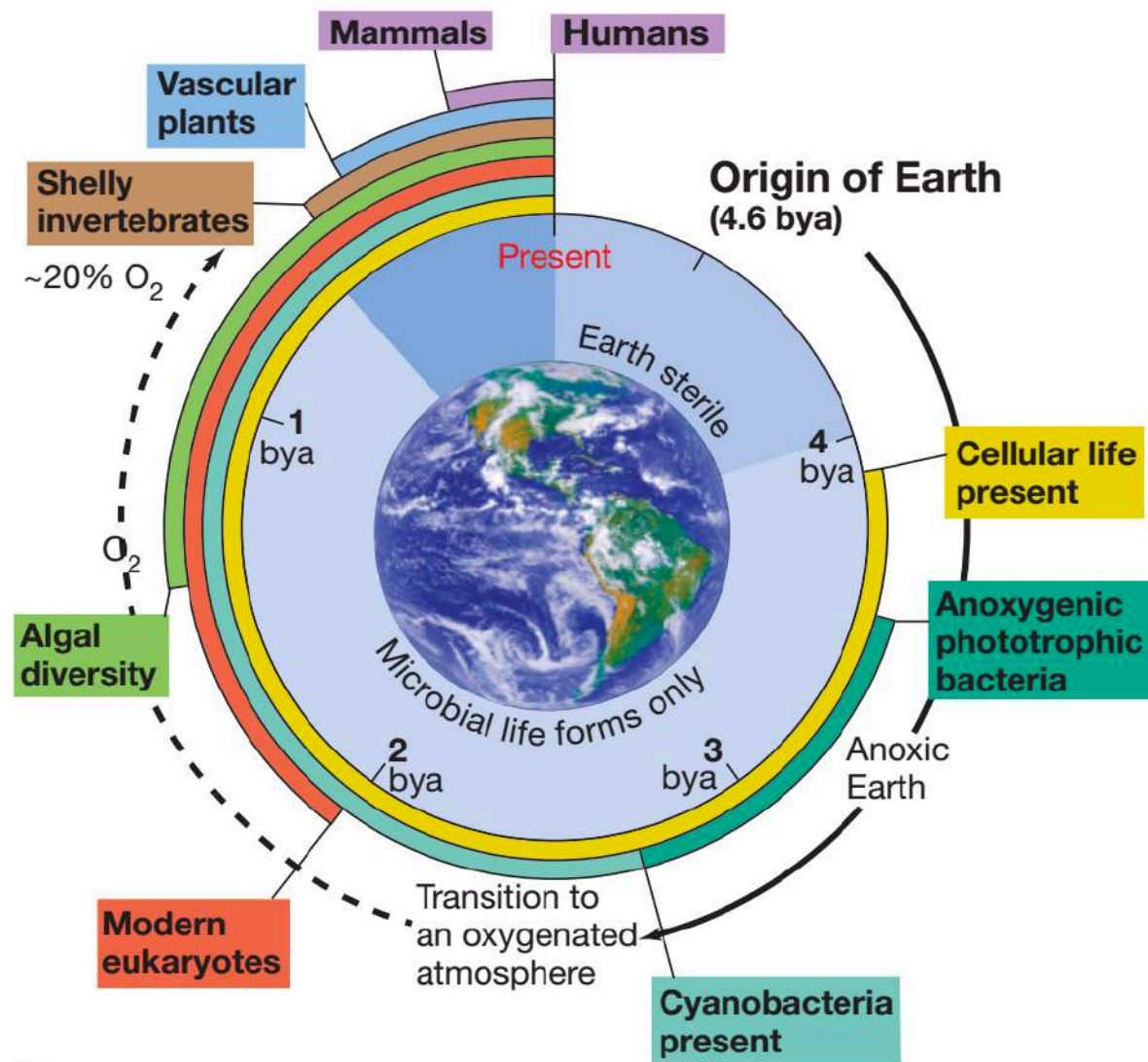


Moody et al., 2024

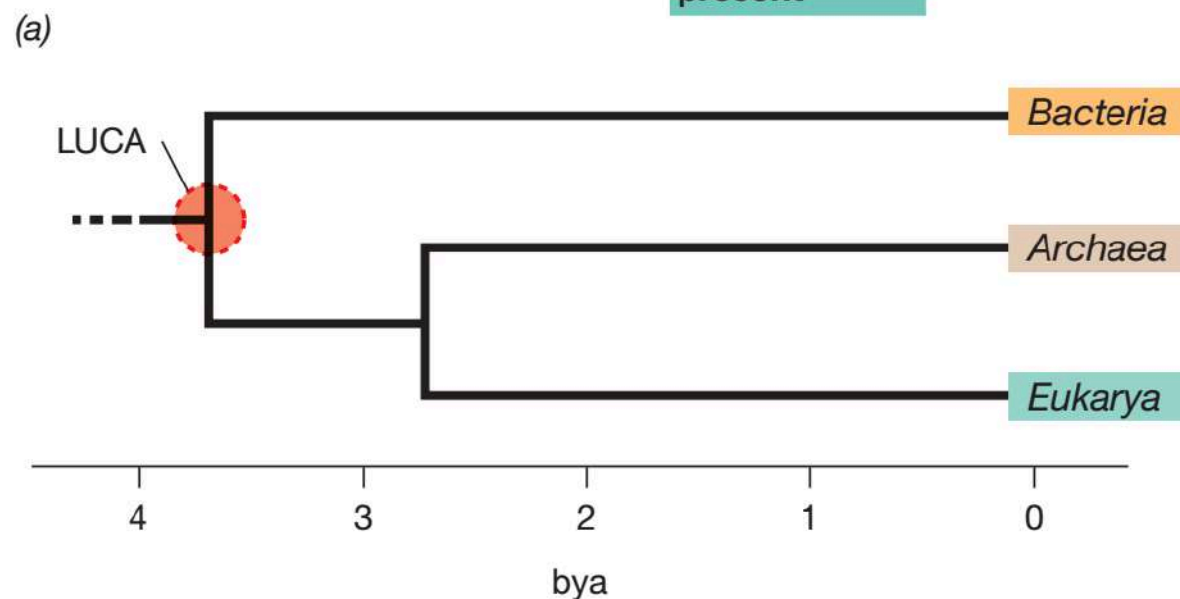
Last universal, archaeal, bacterial and eukaryotic common ancestors (LUCA, LACA, LBCA and LECA, respectively); the last common ancestor of the mitochondrial lineage (Mito-LECA); and the last plastid-bearing common ancestor (LPCA)



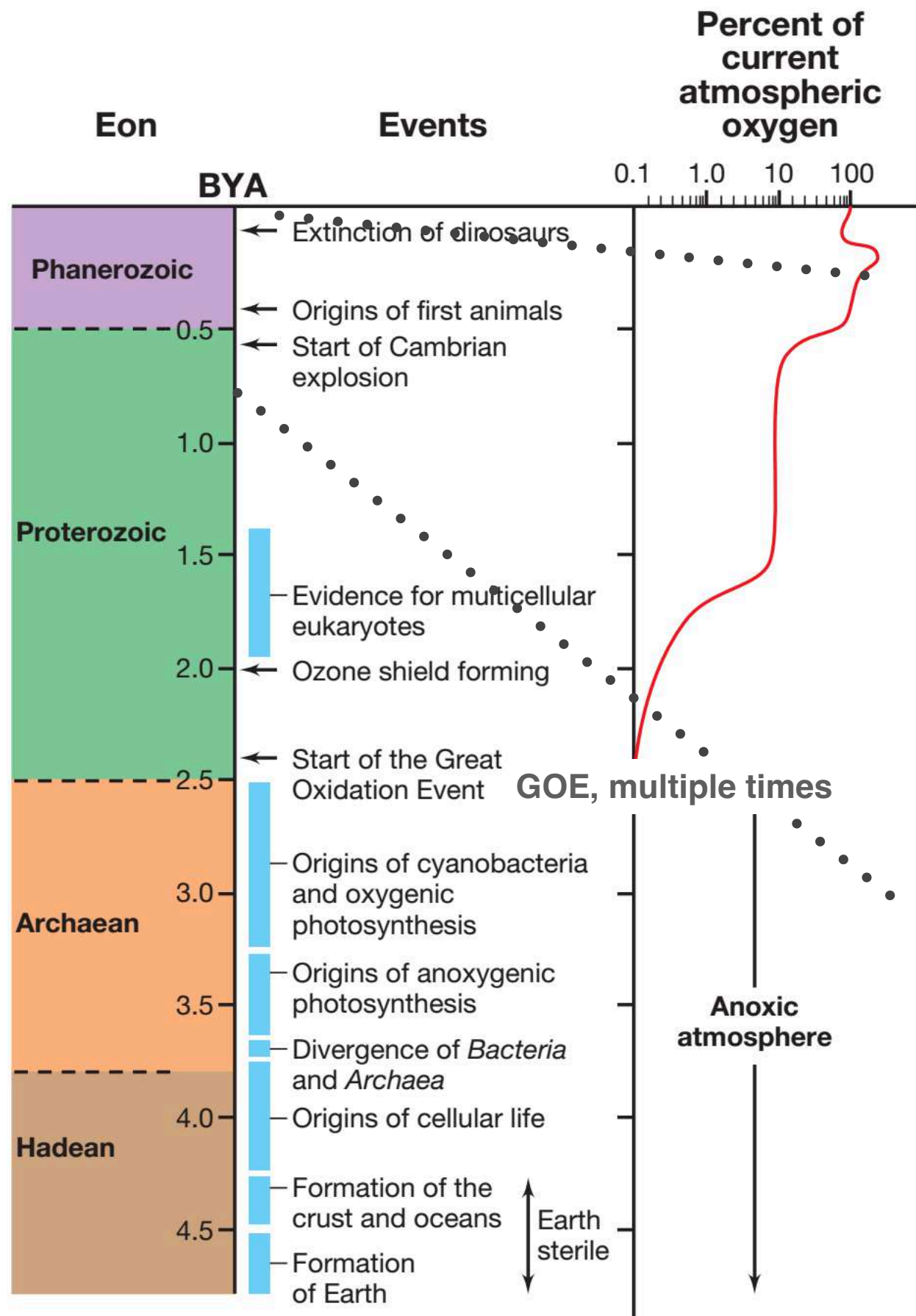
Microbial metabolic diversity impacts Earth



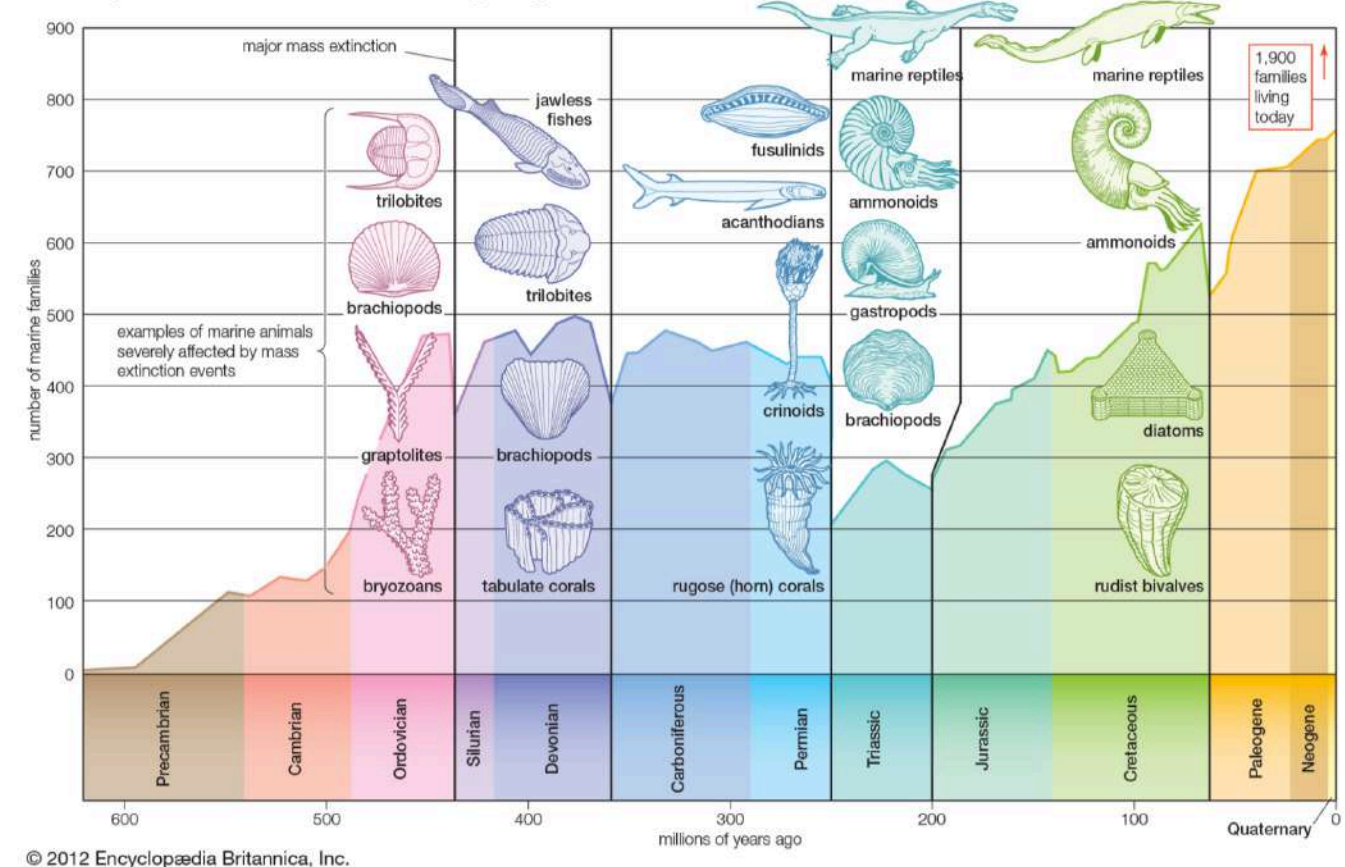
- Sulfur and Fe based-metabolism
- H₂ as e⁻ donor
- CO₂
- Acetate
- H₂S → H₂O: Oxygenic photosynthesis



Origin of Life: **how**



Diversity of marine animal families over geologic time

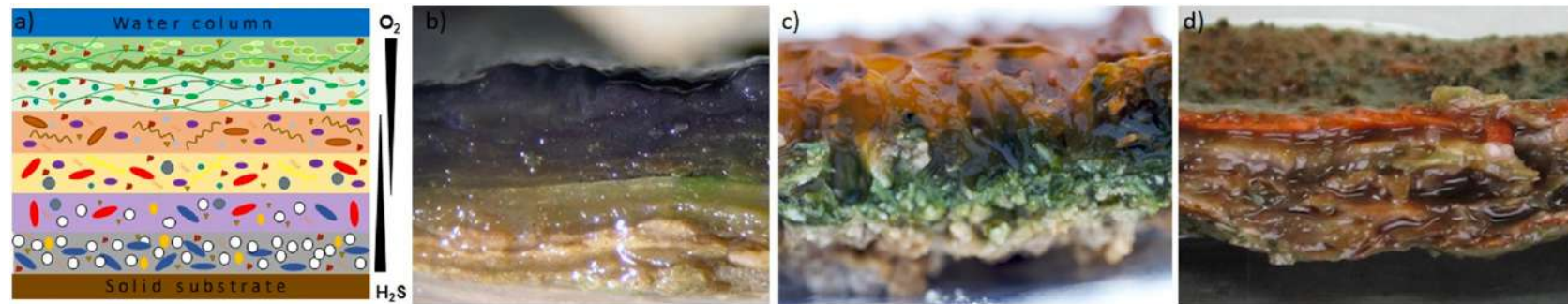


- **GOE: microbial extinction, segregation of anaerobes in microenvironment**
- **O₃ layer protecting UV**
- **Many massive extinctions for megafauna...still today**

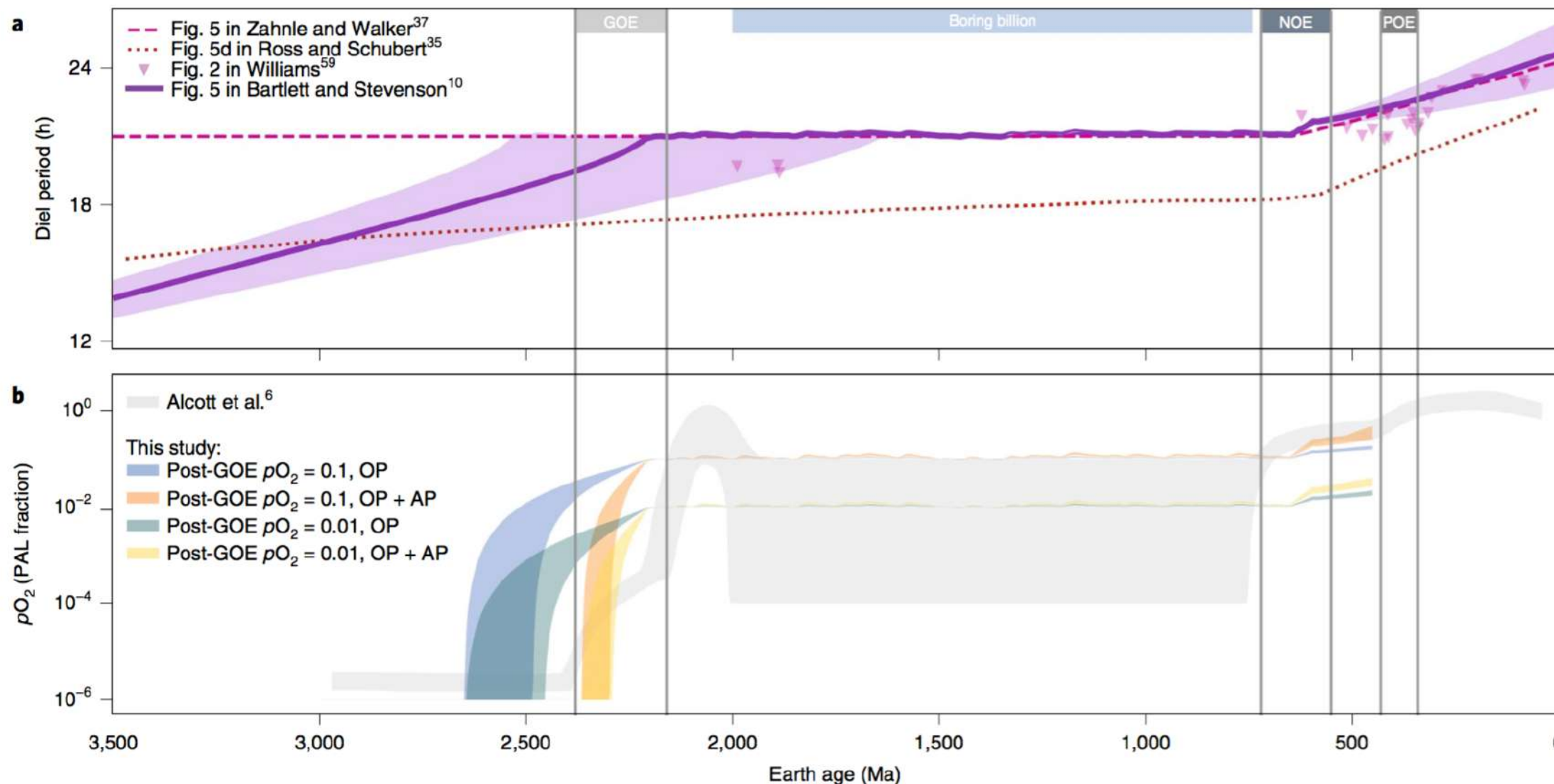
Earth's rotation rate → day-length → and oxygenation

Oxygenic photosynthesis (OP) in microbial mats was a substantial source of O_2 for the Great Oxidation Event (GOE) ~2.4 billion years ago (Ga), during the stable low- O_2 conditions that followed and for the Neoproterozoic Oxygenation Event (NOE) ~600Ma

Day-length, which has increased through geological time due to Earth's rotational deceleration caused by tidal friction

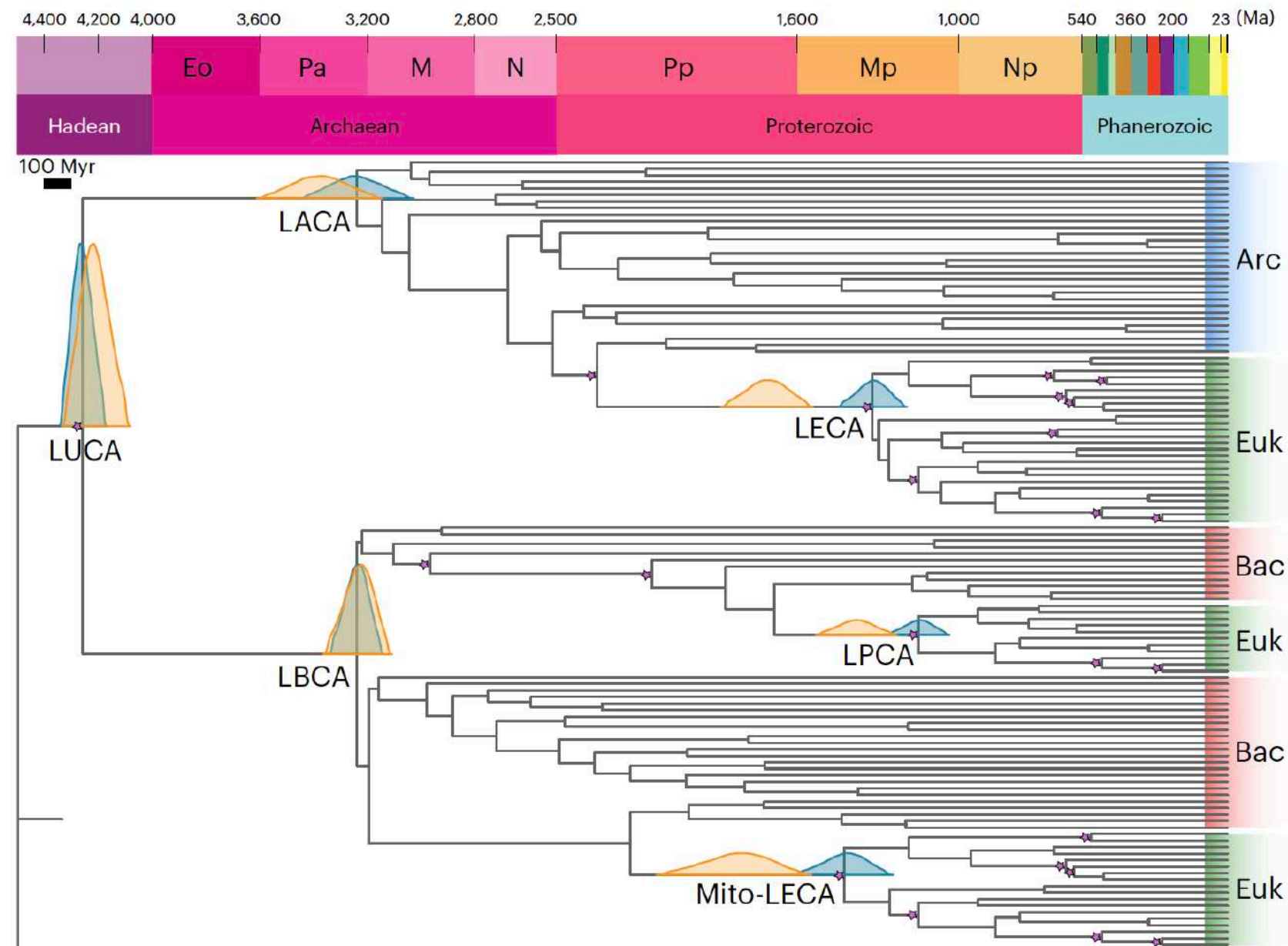


Prieto-Barajas et al. 2018



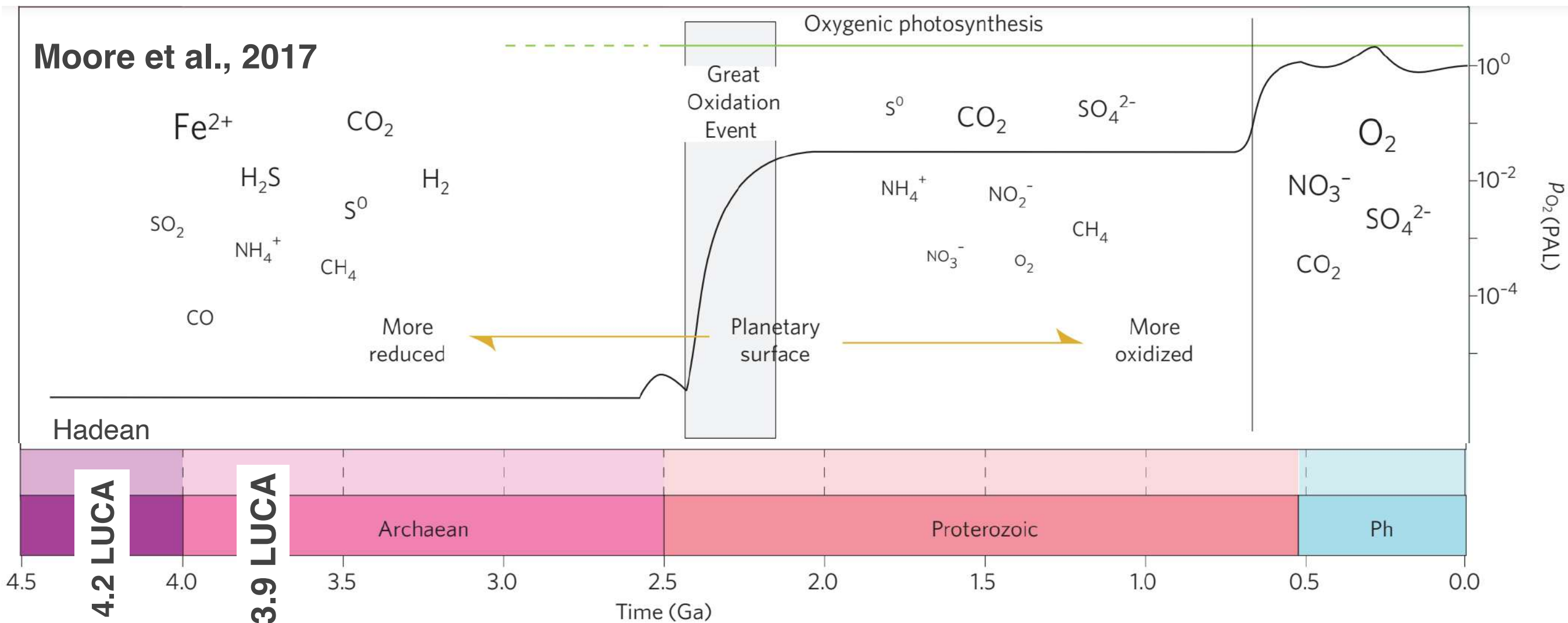
Klatt et al. 2021

Inference: LUCA lived ~4.2 Ga (4.09–4.33 Ga) through divergence time analysis of pre-LUCA gene duplicates, calibrated using microbial fossils and isotope records under a new cross-bracing implementation



Moody et al., 2024

Earth redox state changes



The availability of different metals and substrates has changed over the course of Earth's history as a result of secular changes in redox conditions of the mantle

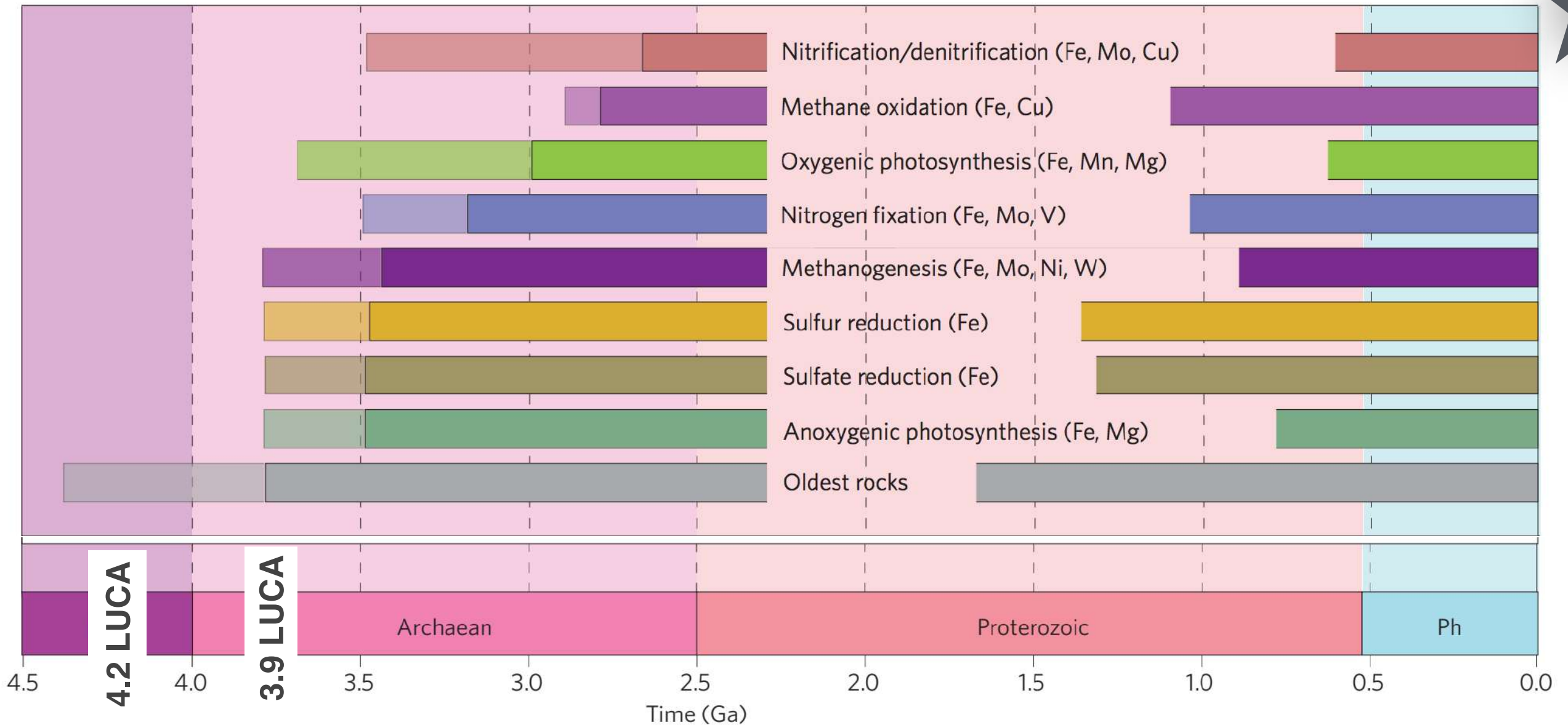
Solar energy used by early microbes

Emerging microbial metabolisms

Moore et al., 2017

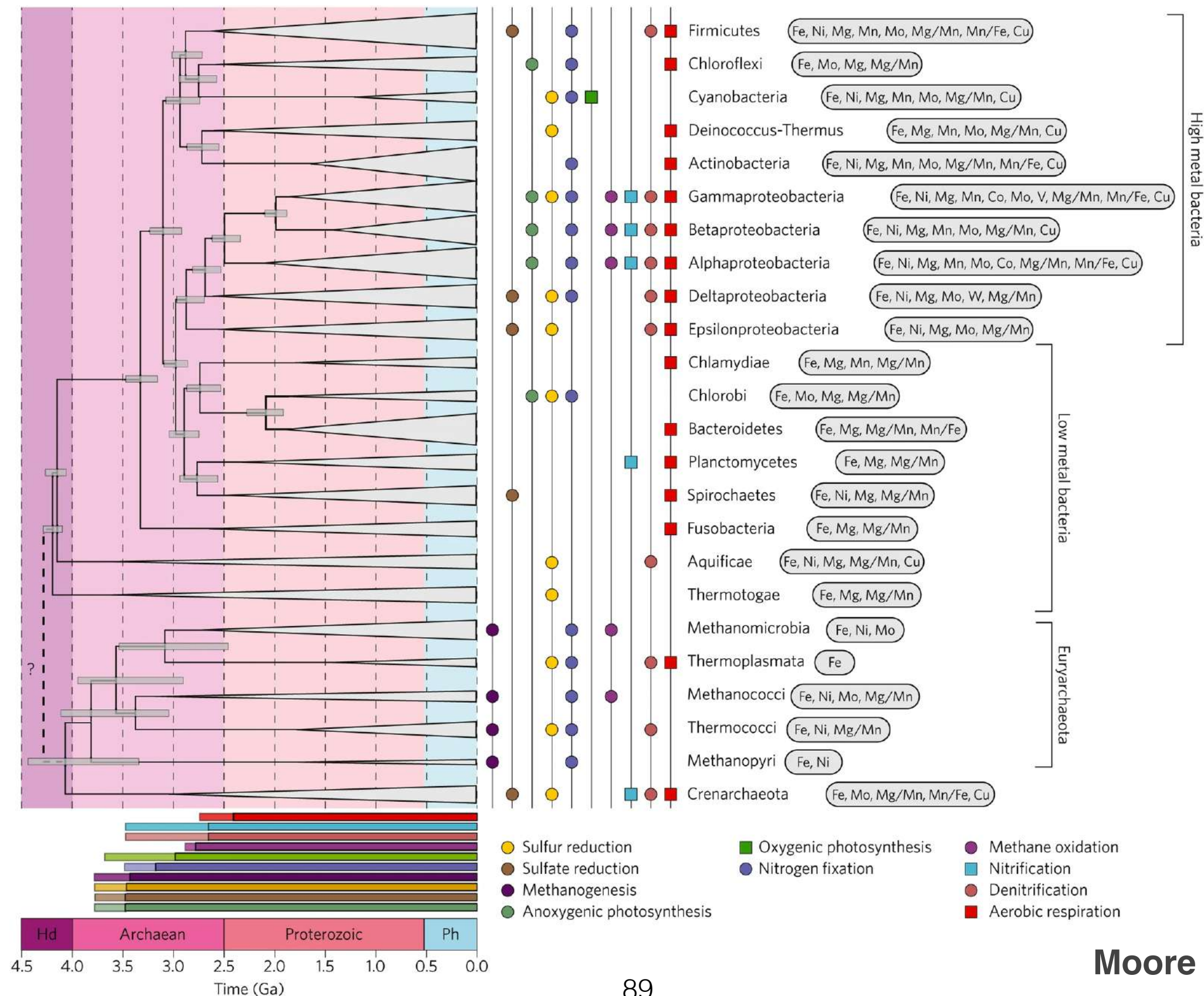


Metabolisms and metals involved



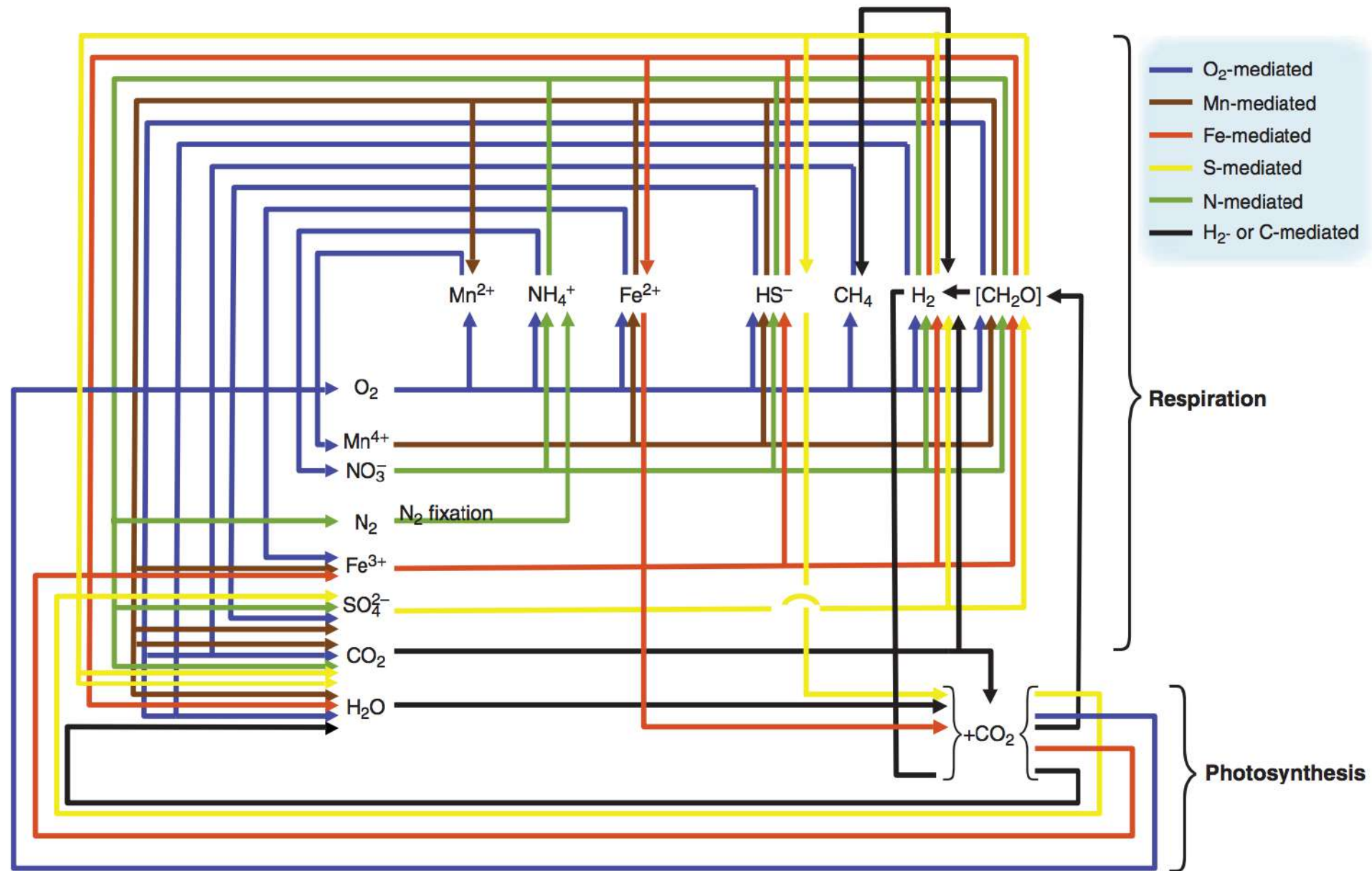
The oxidoreductases responsible for these metabolisms incorporated metals that were readily available in Archaean oceans: iron and iron–sulfur clusters

Phylogenetic tree of the main lineages of Bacteria and Archaea and their putative divergence times



Present microbial metabolism on Earth

Falkowski et al., 2008



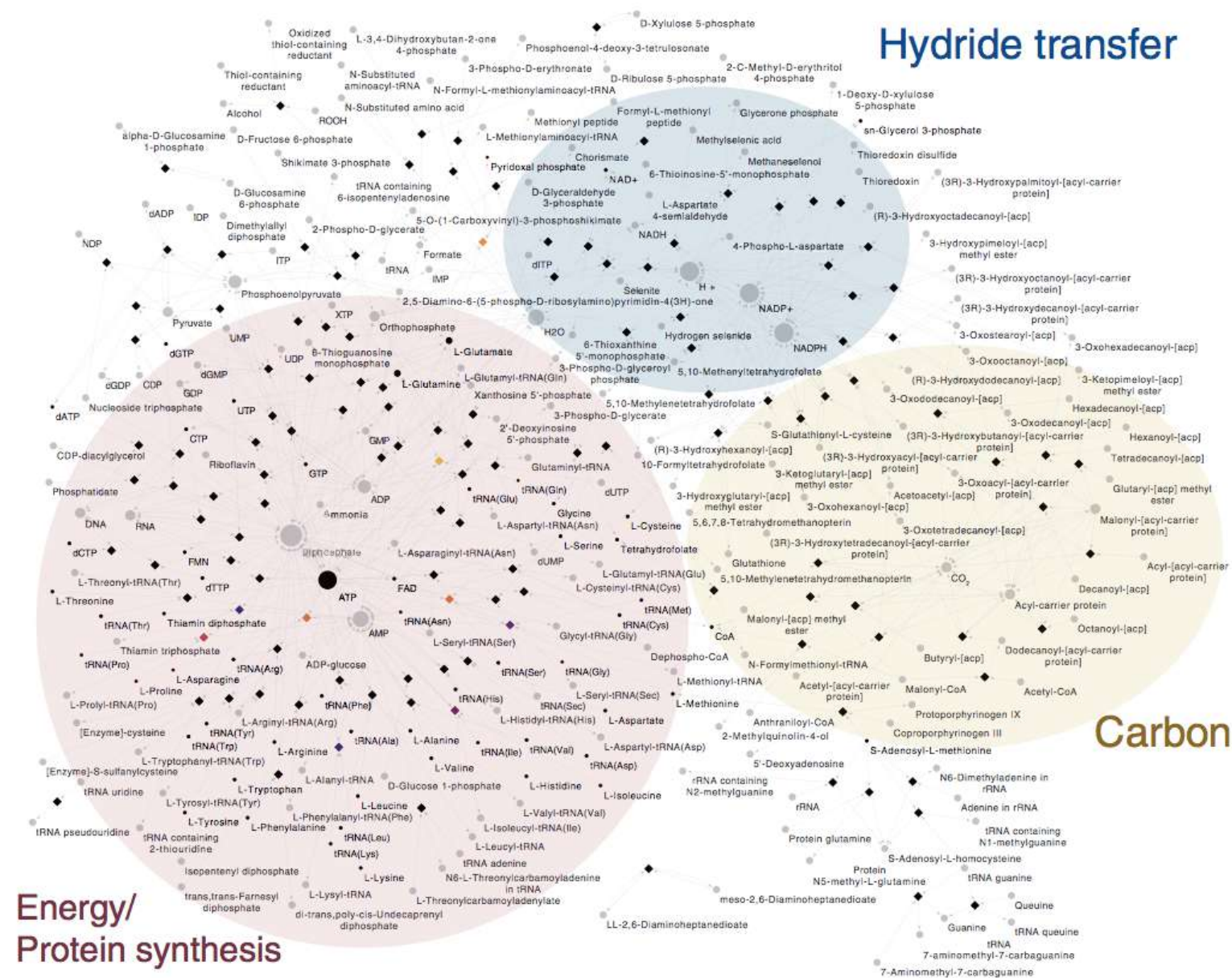
A global, interconnected network of the biologically mediated cycles for hydrogen, carbon, nitrogen, oxygen, sulfur, and iron

A large portion of these microbially mediated processes are associated only with anaerobic habitats

Phylogenomic reconstructions:

- **5443 reference genomes from bacteria** and selected those **1089** classified as **anaerobic** by virtue of **lacking oxygen reductases** and having **>1000 protein sequences**—> **manually annotated** in families
- LUCA was a thermophilic anaerobe that lived from gasses in a hydrothermal setting
- LACA was a methanogen, or a similar anaerobic autotroph that fixed carbon via the Wood–Ljungdahl (also known as acetylCoA) pathway
- Like LUCA and LACA, LBCA must have been an anaerobe
- The **most important difference** between anaerobes and aerobes is related to **energy**
- **Anaerobic pathways** such as fermentation, sulfate reduction, acetogenesis, and methanogenesis **yield only a fraction of the energy** when compared to aerobic pathways, but this is **compensated** by the circumstance that the **synthesis of biomass costs 13 times more energy per cell** in the presence of **O₂** than under anoxic conditions

The three major metabolic hubs of LBCA



LBCA's network is highly structured around three major metabolic hubs:

- (i) ATP/diphosphate,
- (ii) NADP(H)/H⁺, and
- (iii) CO₂/ACP/malonyl-ACP.

These represent the cores of (i) **energy**, (ii) **hydride transfer**, and (iii) **carbon metabolism** of LBCA

Oxygenic Photosynthesis Invention by CYANOBACTERIA

Ocean formation



Modelling

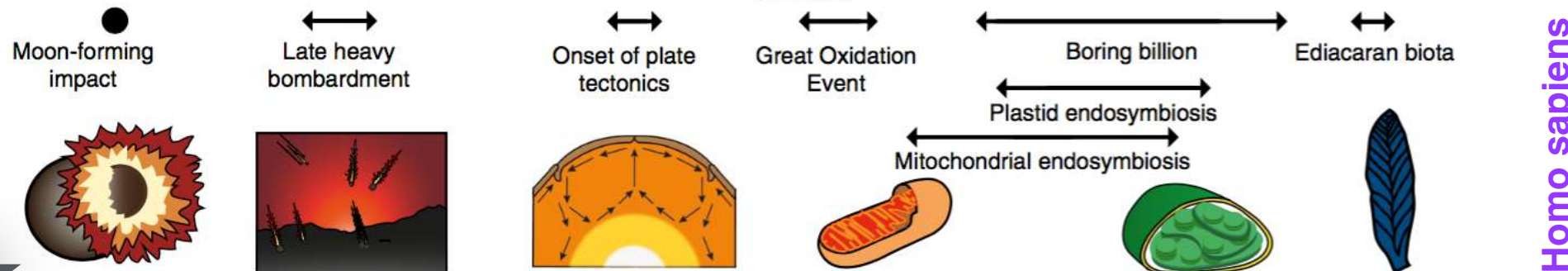


Strelley Pool Formation, stromatolites,
Australia

Sterilization



Betts et al., 2018



LUCA: Last
Universal Common
Ancestor

LUCA

Bac & Arc

Euk

Life induce changes in gas far from
equilibrium in atmosphere
The Great Oxygenation Event (GOE)
spurred the global-scale relevance of
the full oxidative suite of metabolisms

Rewiring of exhibiting membrane-associated micromachies

Electron transport chains as a window into the earliest stages of evolution

Signatures of early evolution across different types of chemiosmotic energy conservation.

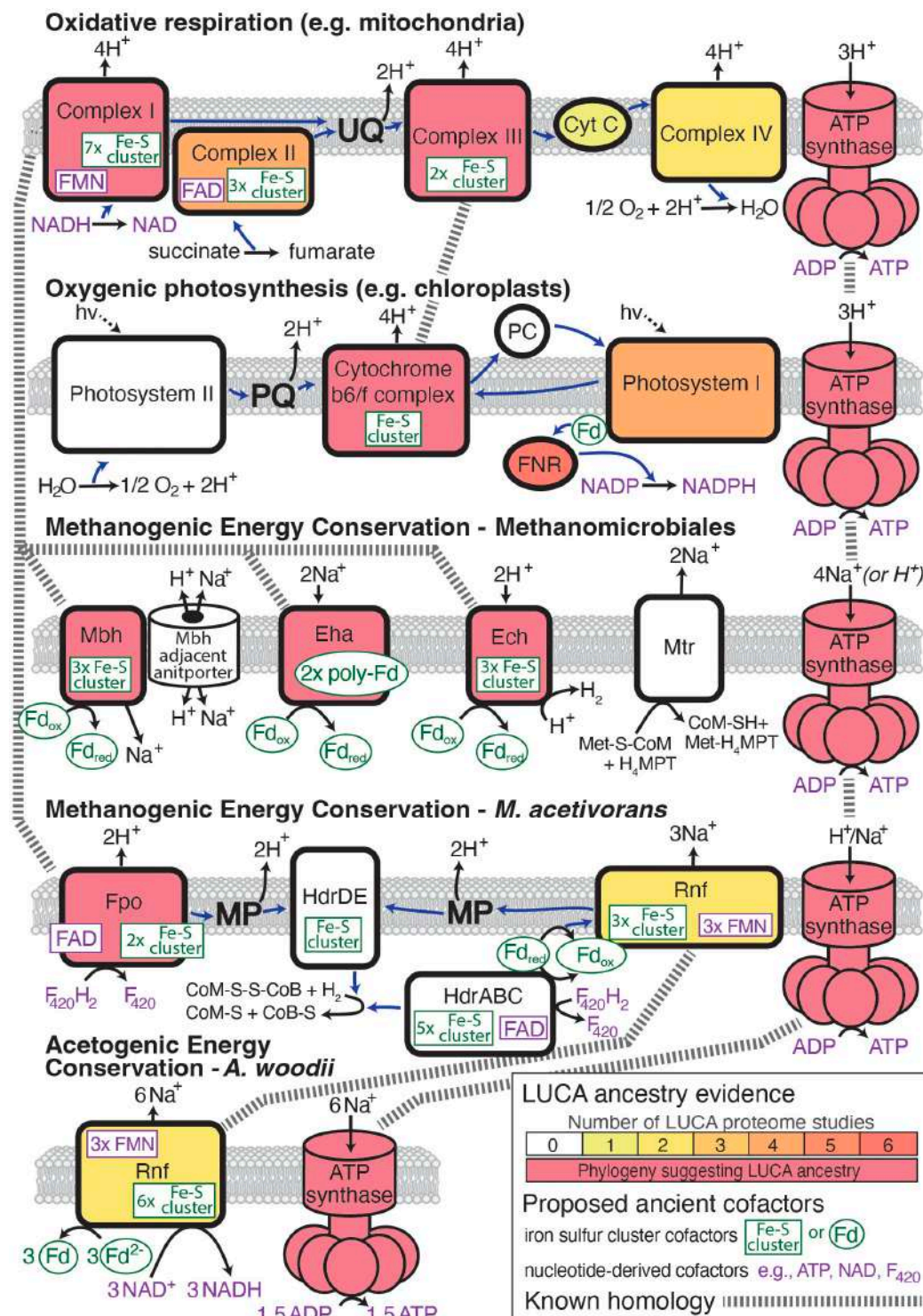
Electron flow is shown as blue arrows.

Likely ancestry from the LUCA is reflected by either direct phylogenetic evidence or the number of different LUCA proteome studies (out of eight total) that predict a component of the complex to be descended from the LUCA.

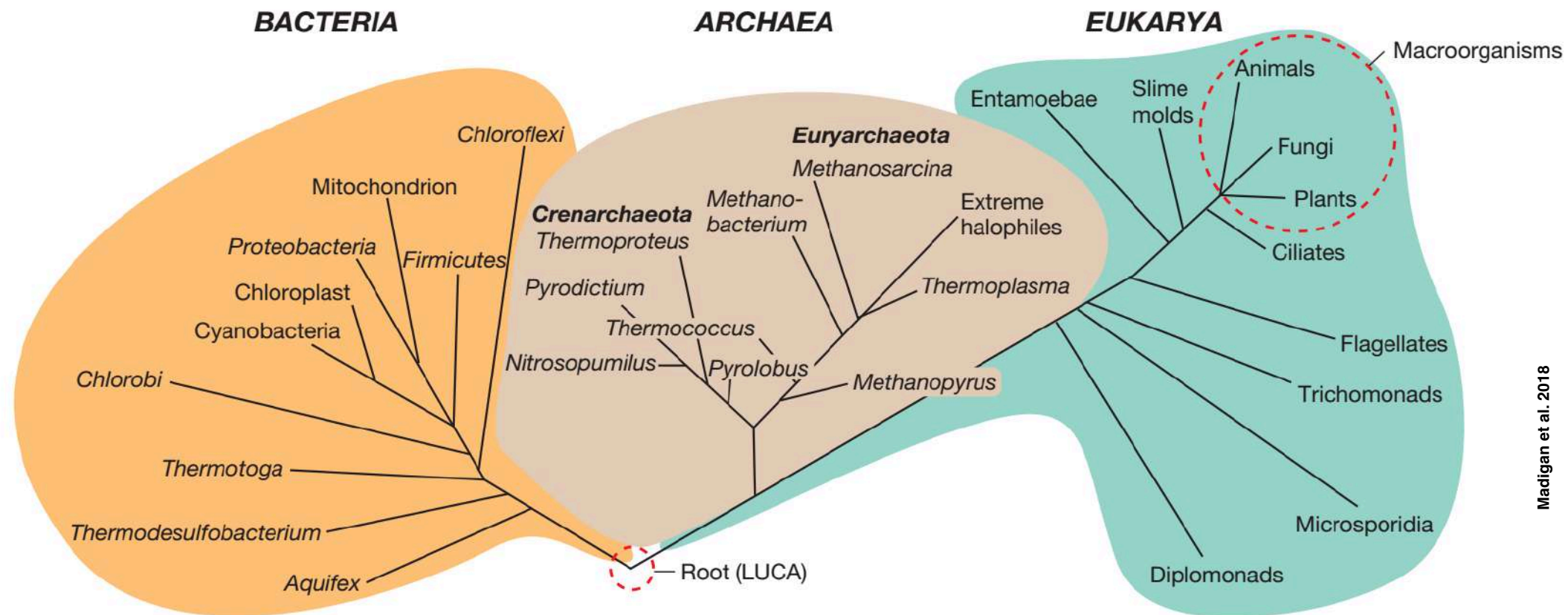
Protein cofactors that are potential relics of prebiotic mineral catalysis or ribozyme catalysts are highlighted in green and purple, respectively.

Homology across different ETC components is indicated by a dashed line.

Electron carrier proteins that are components of ETC complexes such as cytochrome B are not shown.



The rise of the Eukarya: eukaryogenesis

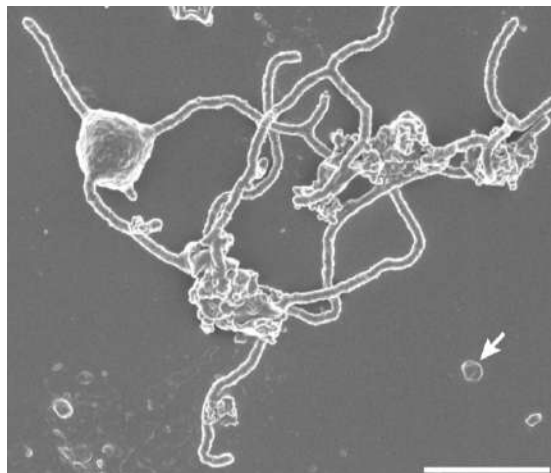


Madigan et al. 2018

LUCA: Last Universal Common Ancestor

~1.86 billions

Imachi et al. 2020

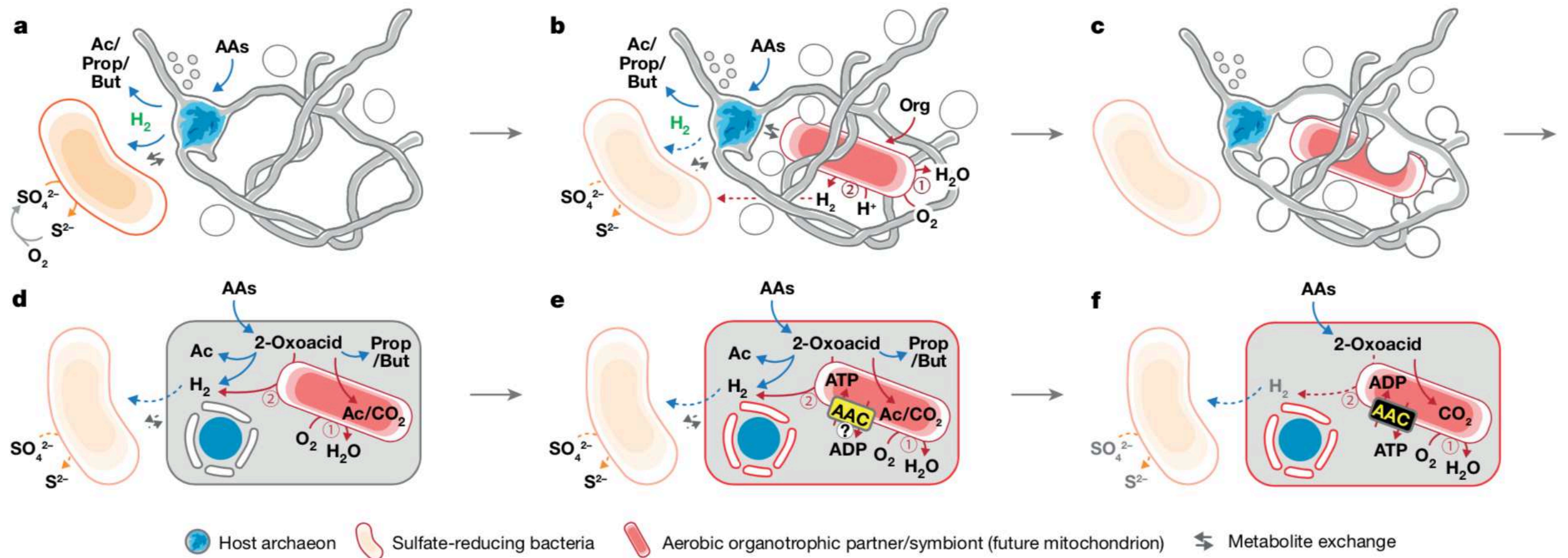


2,000 days to enrich such organisms from anaerobic marine methane-seep sediments

Entangle–Engulf–Endogenize (also known as E³) model

Entangle–Engulf–Endogenize, E3 model

Imachi et al. 2020

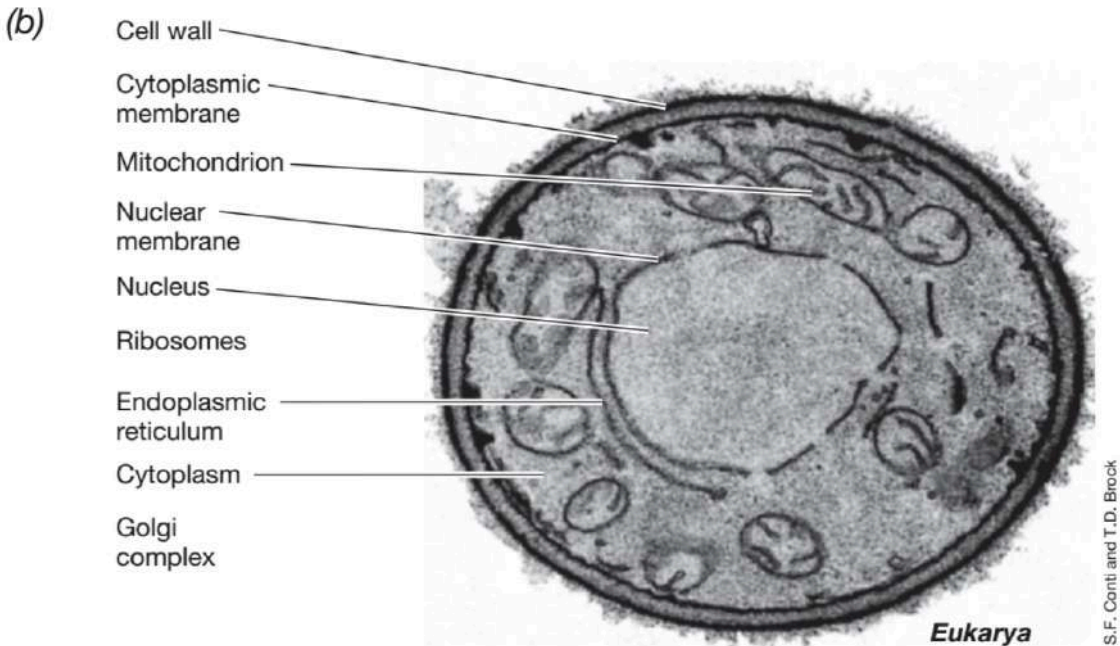
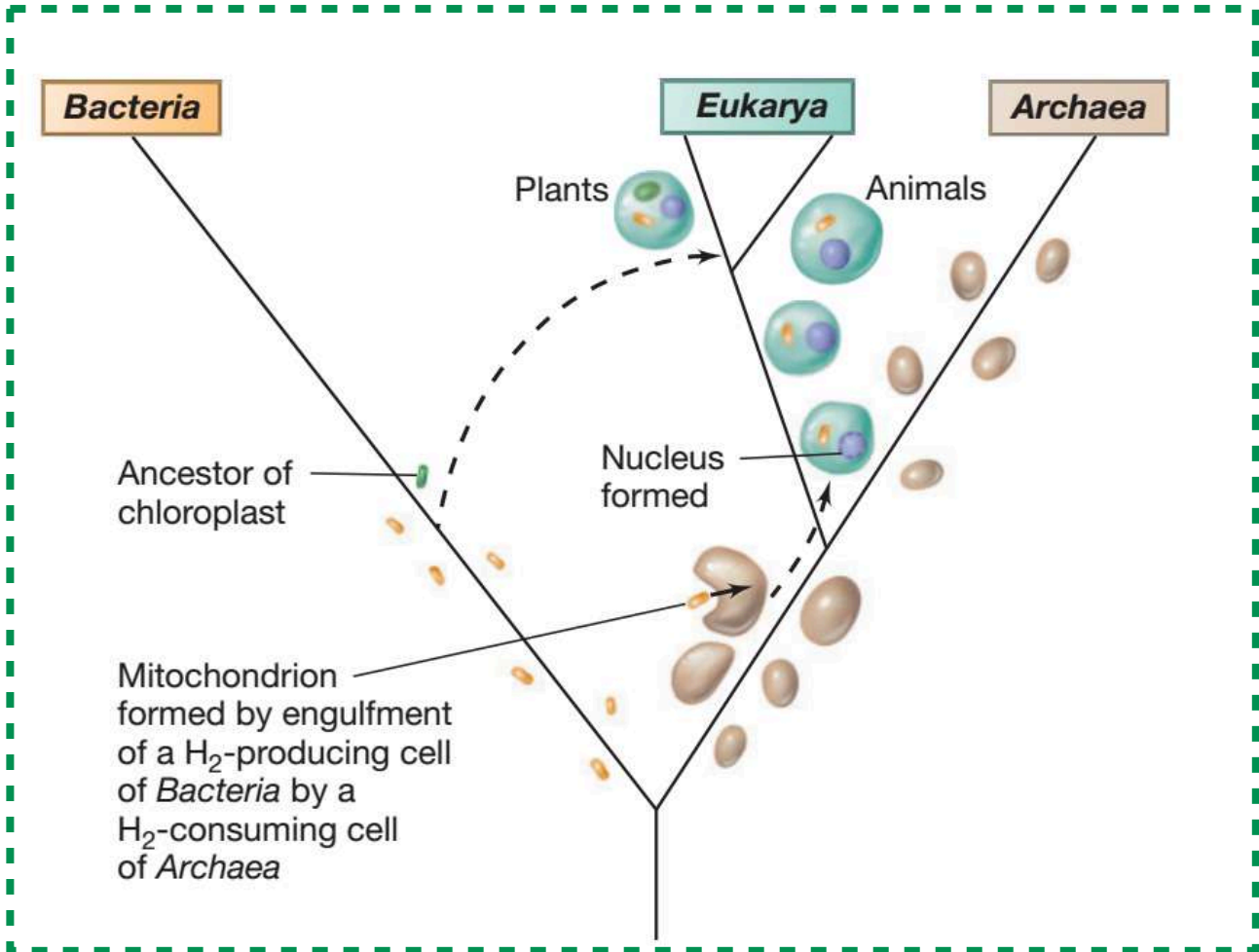
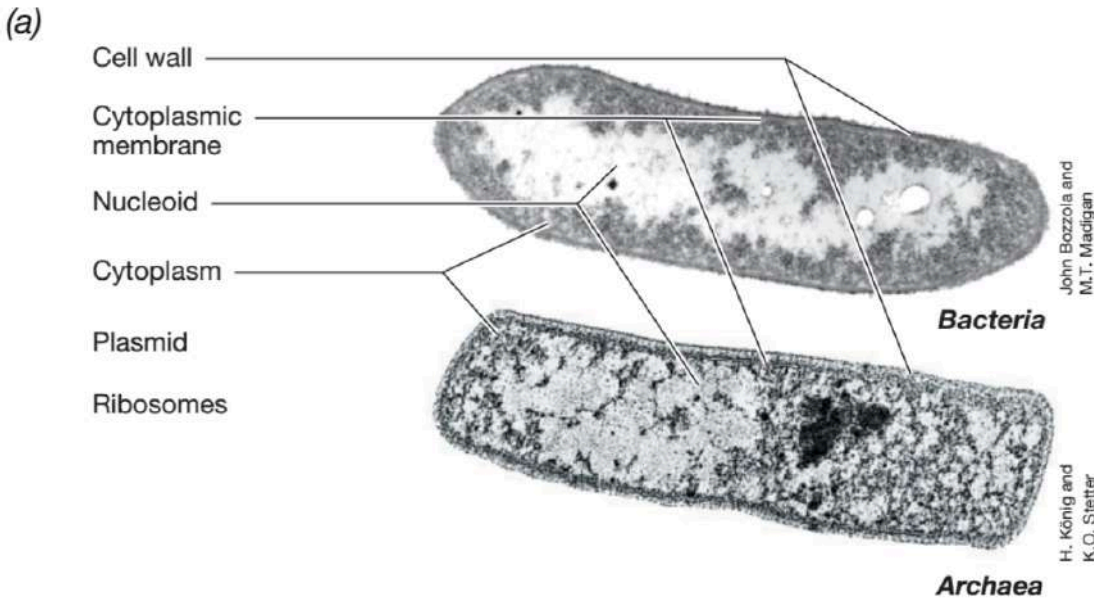
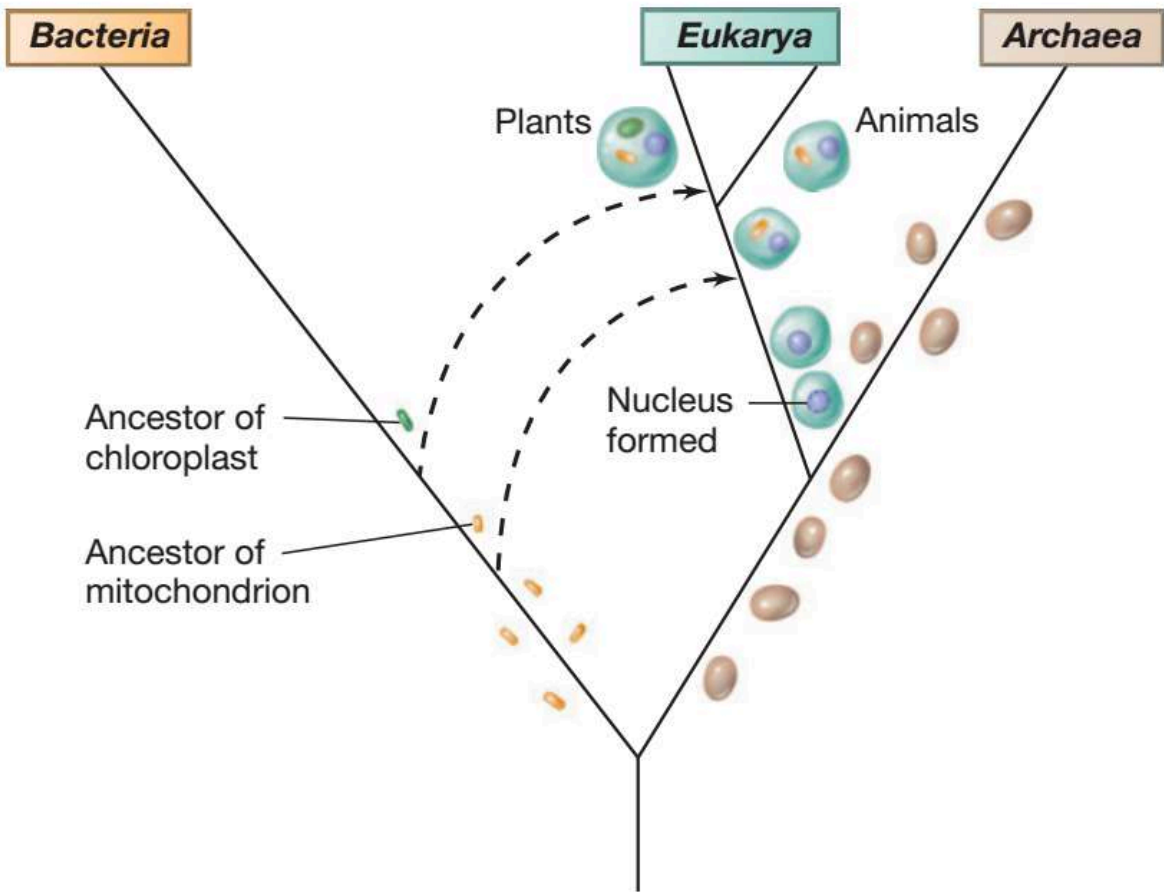


- (1) Transition from anaerobiosis to aerobiosis
- (2) Gain of an O_2 -respiring and ATP-providing endosymbiont (future mitochondrion, alpha-Proteobacterium)
- (3) Development of intracellular structures
- (4) Later stage Gain of a Cyanobacteria (future chloroplast)

Host archaeon (isolated over 2000 days of culture from deep-sea methane-seep sediment, basalt medium + antibiotics) engulfed the metabolic partner using extracellular structures and simultaneously formed a primitive chromosome surrounding structure similar to the nuclear membrane

Entangle–Engulf–Endogenize model to solve the structural and metabolic puzzle

Madigan et al. 2018



Bacteria-Archaea-Eukarya Comparison



	16S rRNA gene		18S rRNA gene
	Bacteria	Archaea	Eukarya
Prokaryotic cell structure	+	+	-
Chromosomal DNA in closed circle	+	+	-
Histone proteins with DNA	-	+	+
Nucleus	-	-	+
Mitochondria/chloroplast organelles	-	-	+
Cell wall with muramic acid	+	-	-
Membrane lipids	Ester-linked	Ether-linked	Ester-linked
Ribosome mass	70S	70S	80S
Intons	-	-	+
Initiator tRNA	FormylMet	Met	Met
RNA polymerase	One	Several	Three
Genes as operons	+	+	-
mRNA tailed polyA	-	-	+
Sensitivity to antibiotics	+	-	-
Growth above 70°C	+	+	-
Growth above 100°C	-	+	-
Chemolithotrophy	+	+	-
N ₂ -fixation	+	+	-
Nitrogen fixation	+	+	-
Denitrification	+	+	-
Dissimilatory reduction	+	+	-
Methanogenesis	-	+	-

...and still evolving

Core Concept

01: Evolution, Thermodynamics, Habitat diversity, Ecology, Physiology their integration define Microbiology

02: Unique goal of microbial life: survival, maintenance, generation of ATP, growth of new cells

03: Planet's habitat diversity results in genetic, molecular, metabolic and physiological microbial diversity

FYI:

<https://climate.nasa.gov/news/2914/the-atmosphere-earths-security-blanket/>

https://forces.si.edu/atmosphere/02_02_01.html

<https://www.britannica.com/topic/evolution-of-the-atmosphere-1703862>