O26SV_L01

You belong here !

★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.

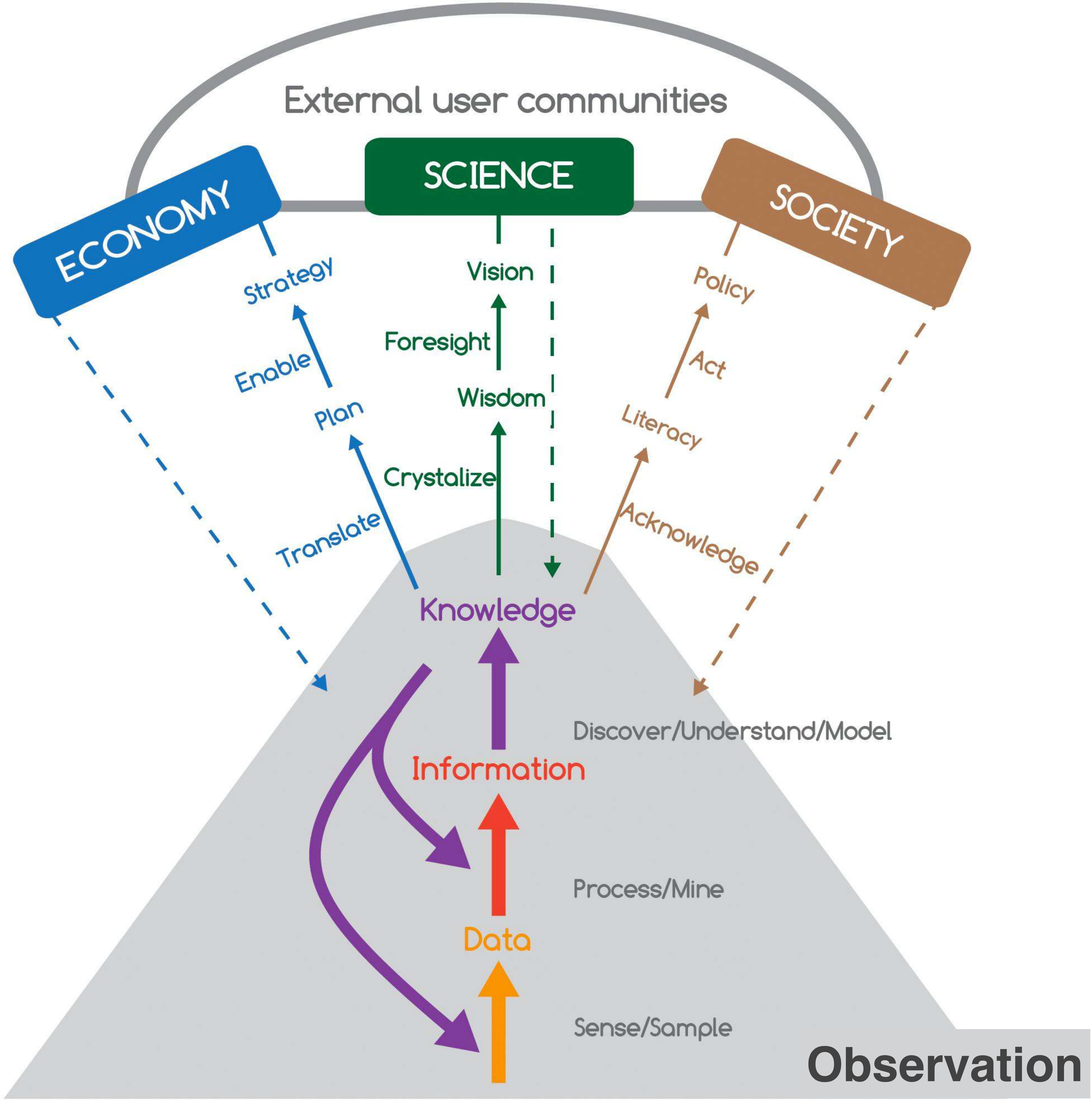
Sviluppo cognitivo e **pensiero critico**: due antidoti ai pregiudizi e ai preconcetti del presente

di Daniele Scarampi

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

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



ENVIRONMENT - MODEL ORGANISM

BEFORE

Microbes not important

Microbes=Disease

AFTER

Microbes are everywhere and will always be…

Microbes as ecosystem engineers

Microbes keep the ecosystem functioning

Humans and biota as microbial ecosystems

Ricevimenti concordati via email: fmalfatti@units.it

Tre report di laboratorio, in forma guidata, di gruppo per un valore totale di 3 punti su 30 punti (i.e. trentesimi). Ogni report puo' esser valutato da 0 a 1 punto in trentesimi.

Esame scritto della durata di 1 ora, per un valore pari a 27 su 30 del voto finale individuale. Esame scritto conterrà 2 domande a risposta aperta, 1 disegno/schema da fare dall'esaminando e 12 domande a vero-falso Le domande aperte e il disegno/schema valgono 5/30 ciascuna. Le domande vero-faslo valgono 1/30.

1. Introduzione al concetto di microbiologia e storia dal XVII secolo fino ad oggi ed **origine della vita sulla terra**

2. Biologia di **Bacteria e Archaea** con particolare attenzione alla **morfologia della cellula** batterica e ad alcuni meccanismi metabolici di base (capsula, parete cellulare, peptidoglicano, spazio periplasmico, membrane, citoplasma, vescicole appendici batteriche con flagelli e pili, endospore, aspetti del **genoma**, **crescita**, **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, 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)

6. **Regolazione dell'espressione genica** in risposta a diversi stimoli ambientali (fattore sigma, regolazione positiva e negativa tramite molecole attivatrici, repressori, sistema a due componenti e punti di controllo a livello tradizionale, stress e motilita', metiloma)

7. **Interazioni tra microrganismi ed essere umano** I. (**Quorum sensing, simbiosi e biofilm**)

8. Interazioni tra microrganismi ed essere umano II. (**Infezioni e patogenicita'** dei microrganismi & **OneHealth**)

9. **Interazioni tra microrganismi ed ambiente** (**Diversita', abbondanza e servizi ecosistemici**)

10. **Metodologie** di isolamento, caratterizzazione e fenotipizzazione di microrganismi ambientali e tecniche di microscopia, nuove metodologie-omiche (genomica, trascrittomica, proteomica, metabolomica, meta-genomica, meta-proteomica, meta-trascrittomica)

Why are the slides written in English?

Brief self-introduction and future career

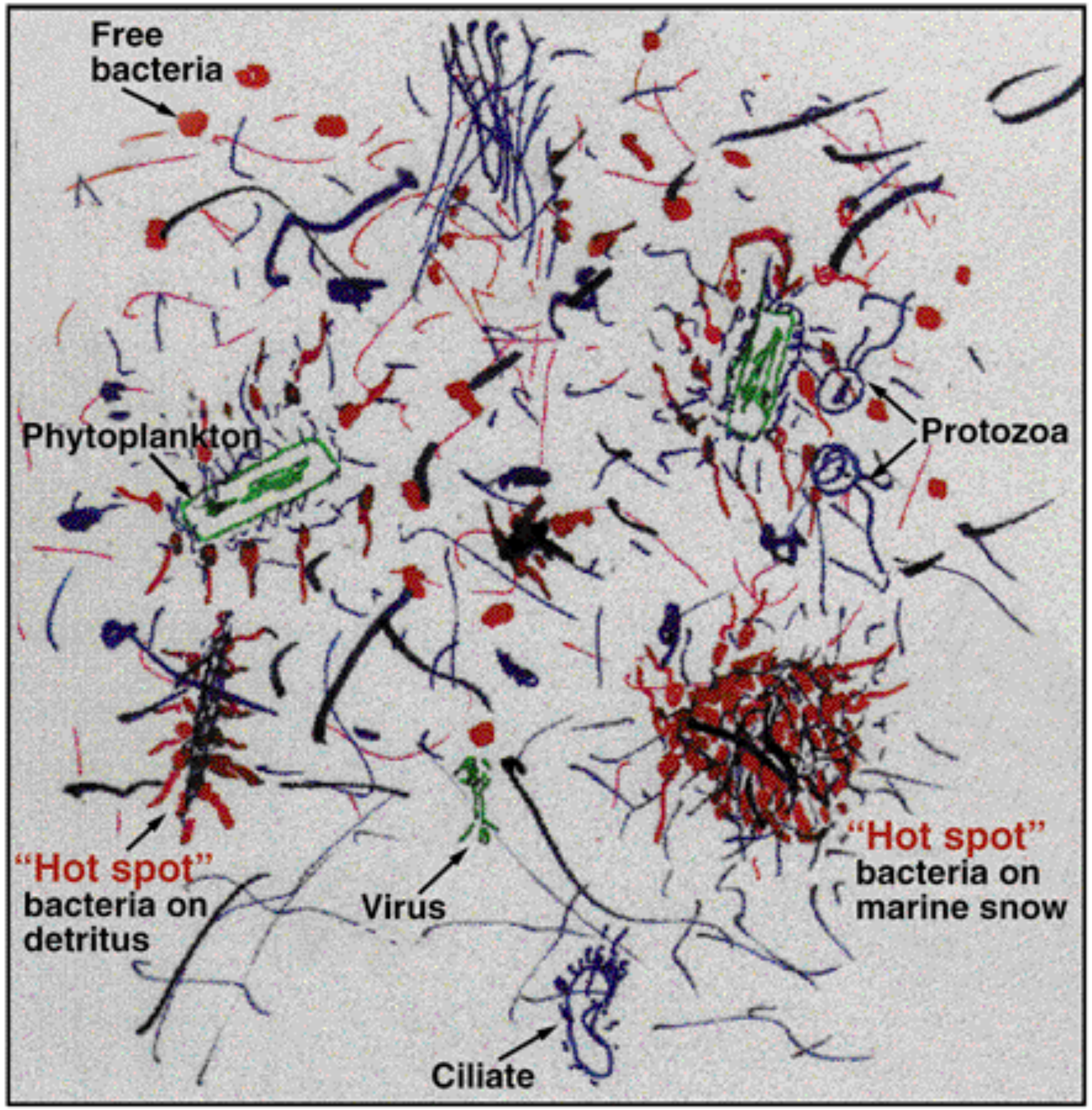
SIO, Scripps Institution of Oceanography, UCSD, USA

Dr. Farooq Azam

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

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

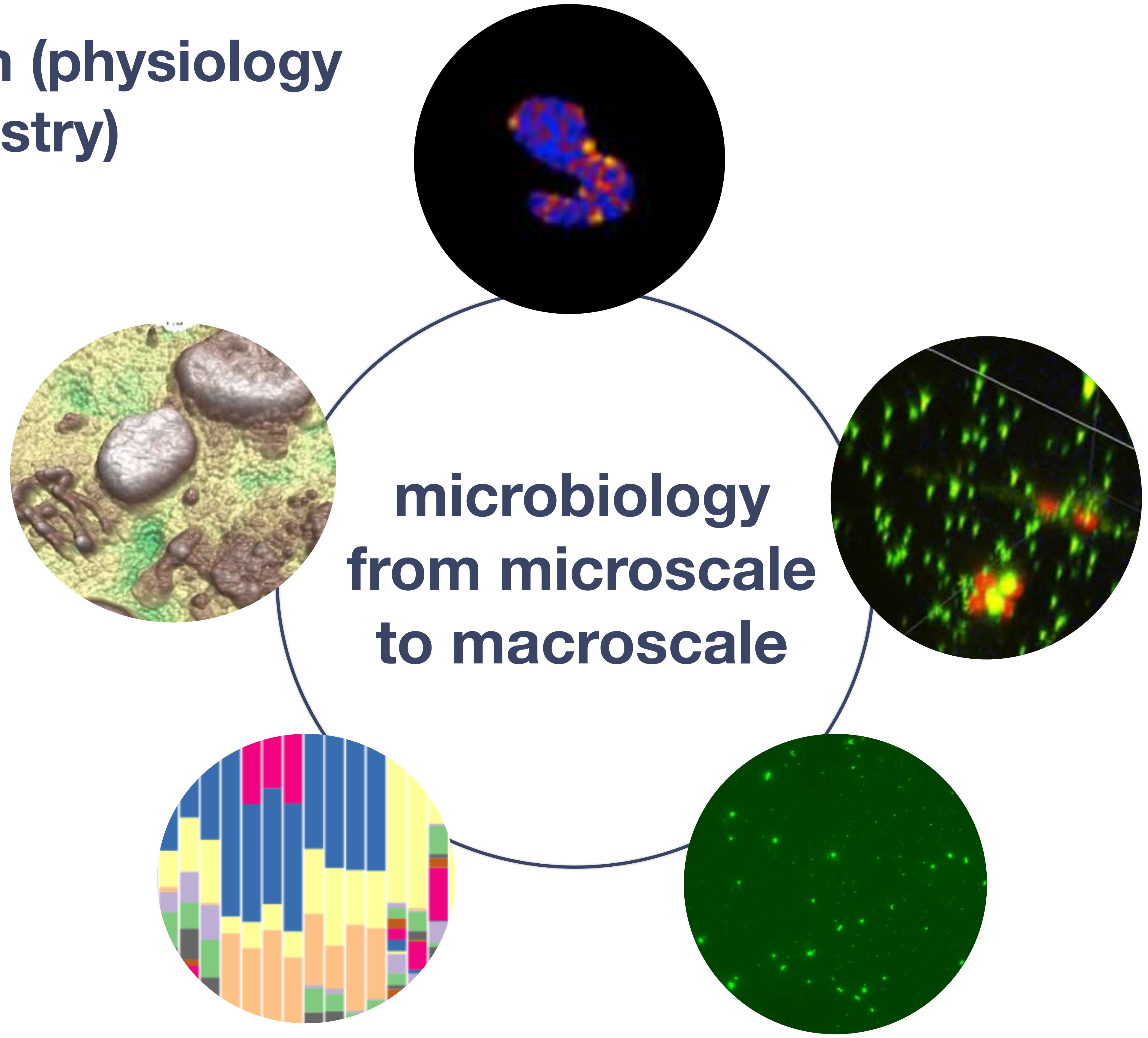
Impressionistic view, F. Azam



Azam, F. 1998 Science 280:694-696

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

Mechanistic integrative approach



Tell me and I will forget,

show me and I may remember,

involve me and I will understand.

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

- History
- Microbiology
- Origin of life

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

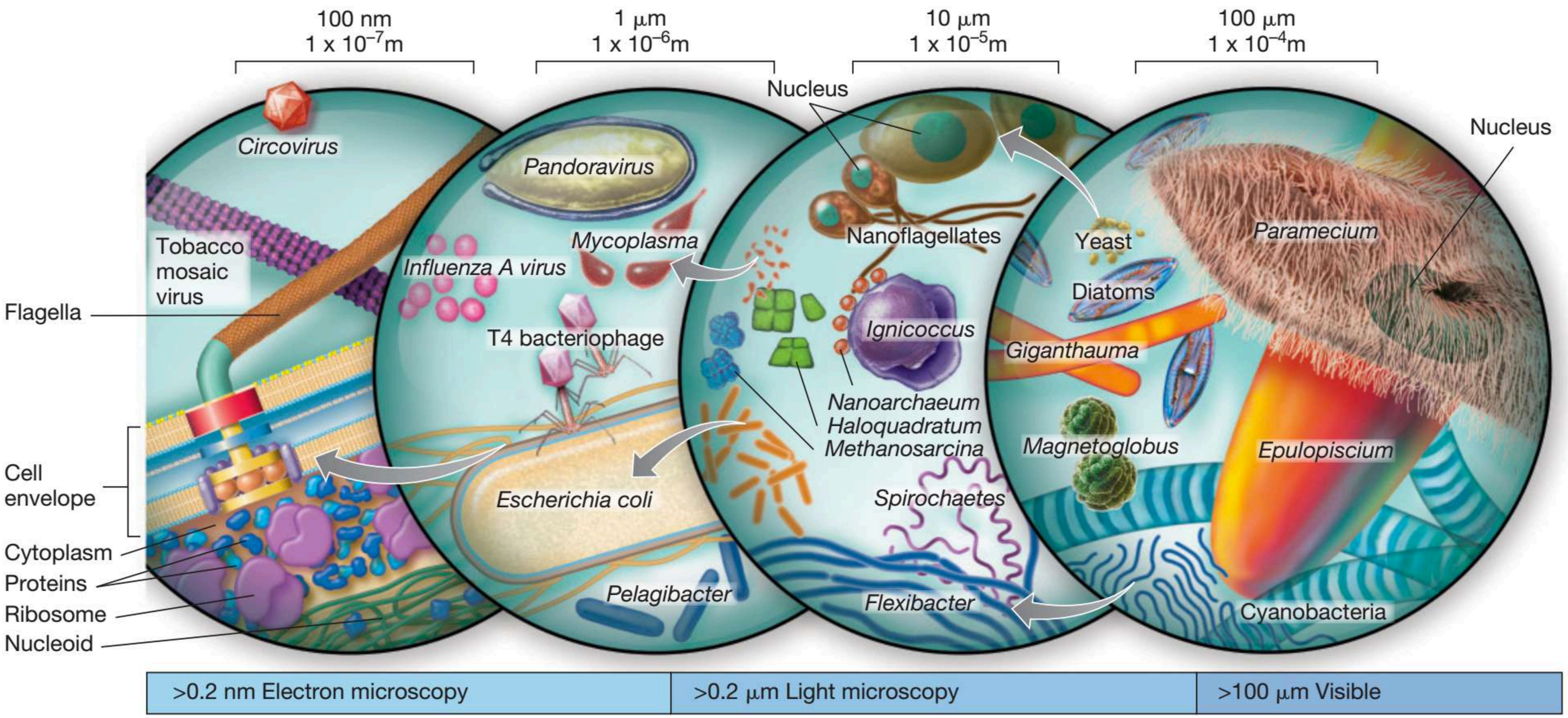
1 μm= 1 micrometer is 1/10^6 meter

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

Bacteria, Archaea, Viruses & small Eukarya

Everywhere on Earth and in/on every organism

Microbial size range



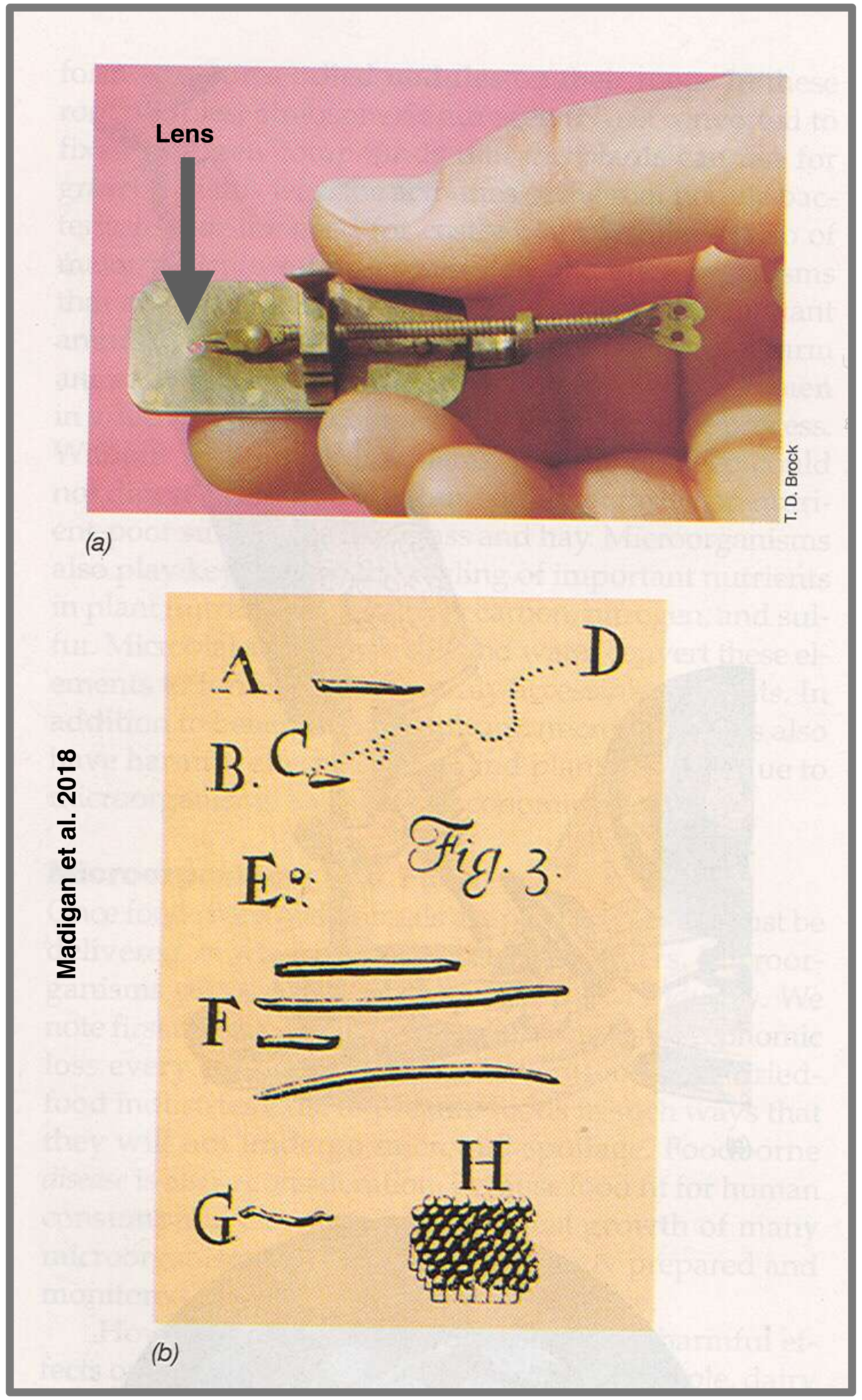
Madigan et al. 2018

Microbiology is the discovery of LIFE as we know it

History

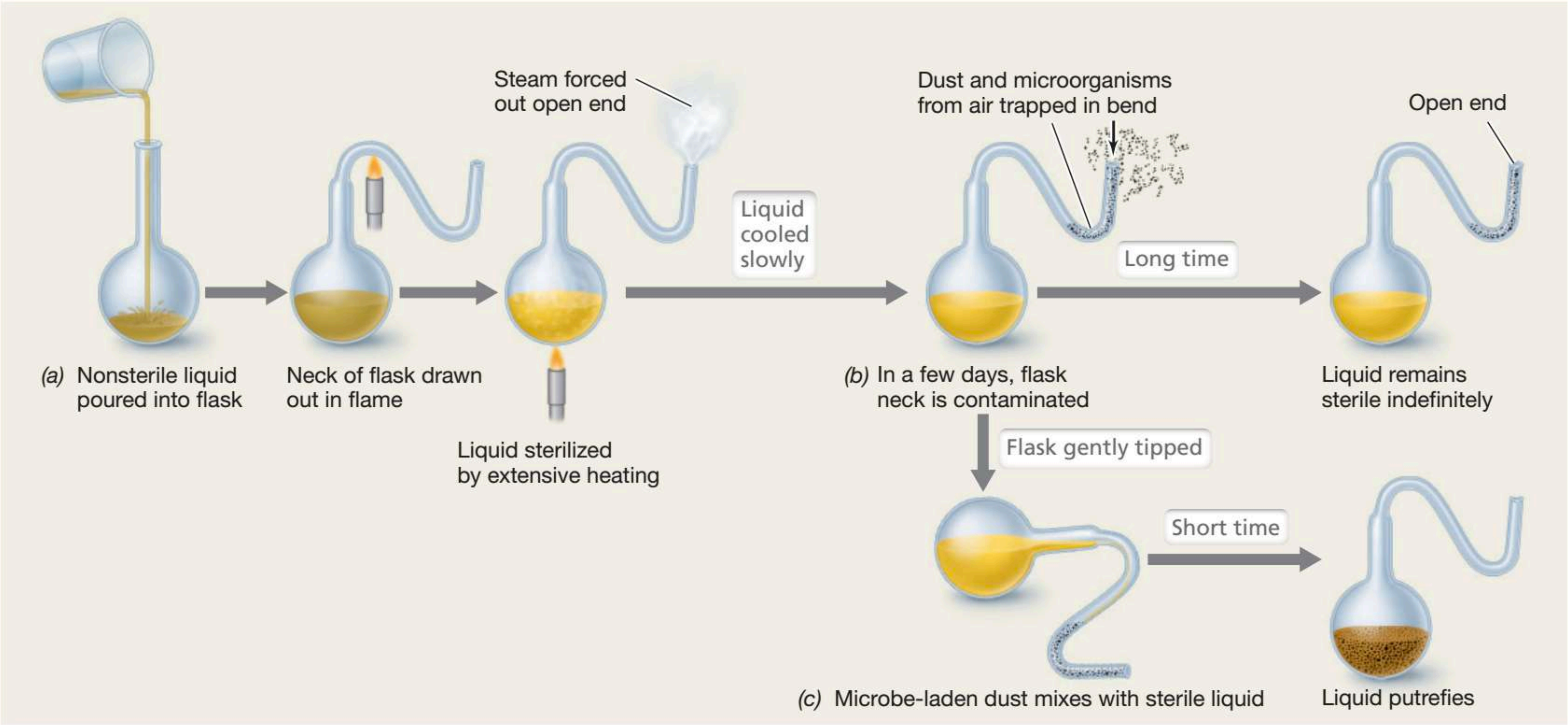
- 1665 Robert Hooke, invention of the microscope
- *1676 van Leeuwenhoek, discovery bacteria at the microscope*
- 1857 Pasteur, microbes cause fermentation & dispelling spontaneous generation of life
- *1881 Koch, Germ theory of disease & use of gelatin plates*
- End 19th century Beijerinck and Winogradsky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

Developing Tools
enabling discovery of the microbial worlds!



1676 van Leeuwenhoek
the first microscope for bacteria

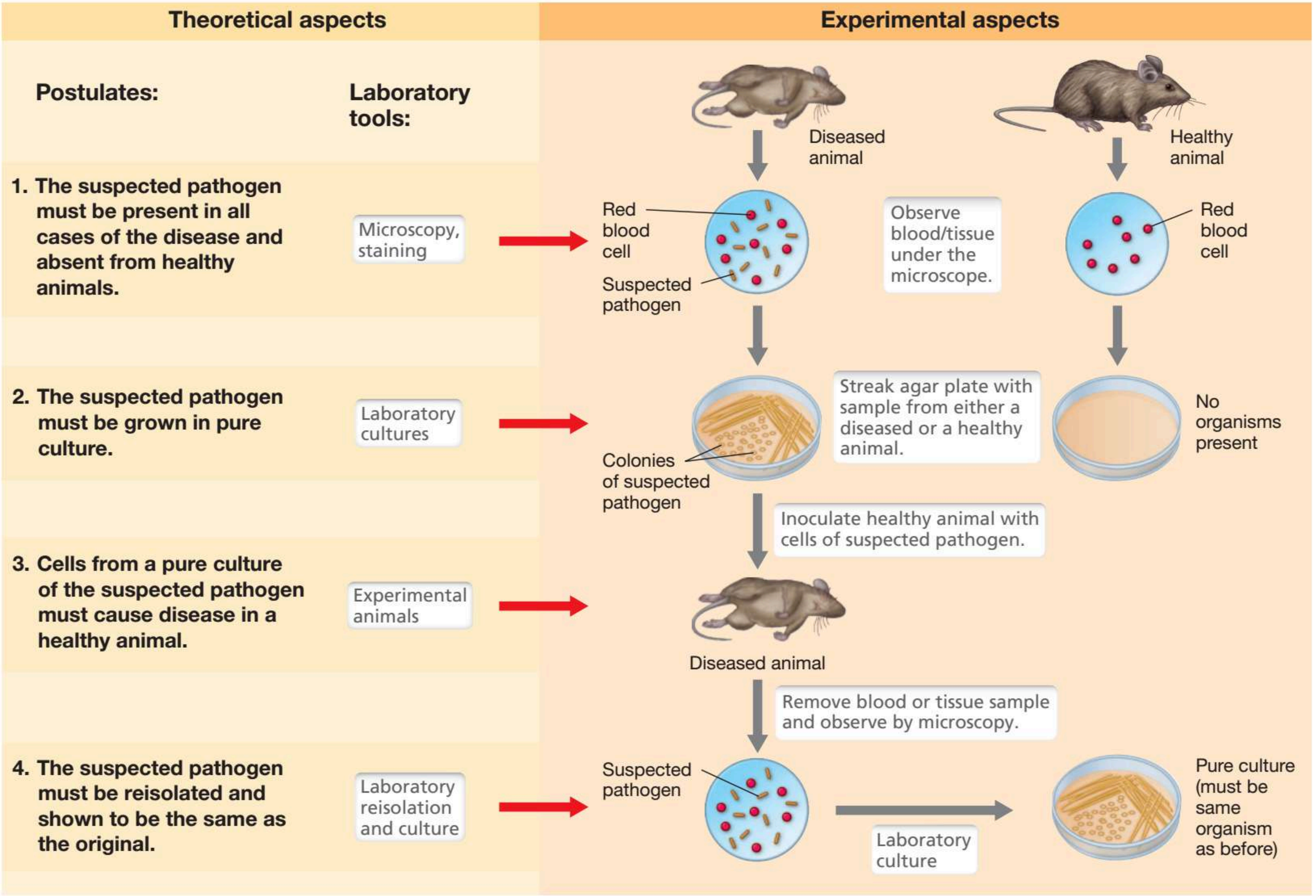
Early focus: Human microbial diseases
Later focus: Biogeochemical role
Today focus: One Health



Madigan et al. 2018

Pasteur: Experiment dispelling the theory of spontaneous generation of life (environmental change is microbial 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

Beijerinck **Winogradsky** **Kluyver**

Environmental microbiology —> *need to understand environment*

1. Bacteria from environment don't live on Koch rich media

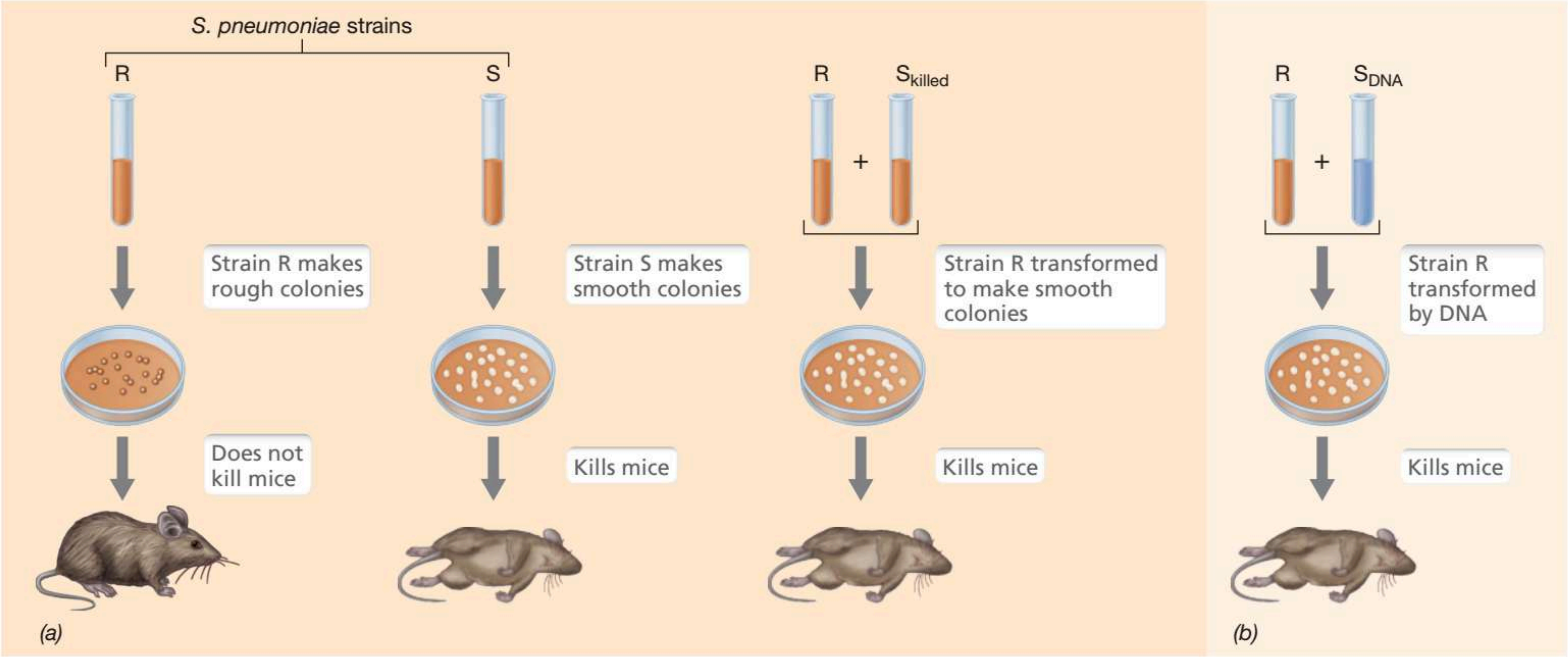
2. Need to create specific enrichment media to imitate the environment

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 constrains are similar for microbes

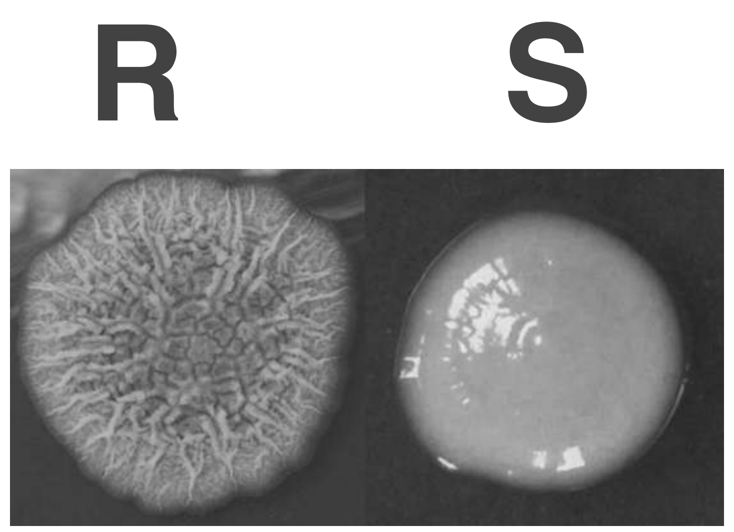
- End 19th century Griffith & 1944 Avery-MacLeod-McCarty, **DNA as a transforming principle**
- Early 20th century Alexander Fleming's **discovery of penicillin as an antiseptic antibiotic** (Howard Florey, Ernst Chain and Norman Heatley) and **lysozyme**
- First half 20th century Watson, Crick & Franklin: **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

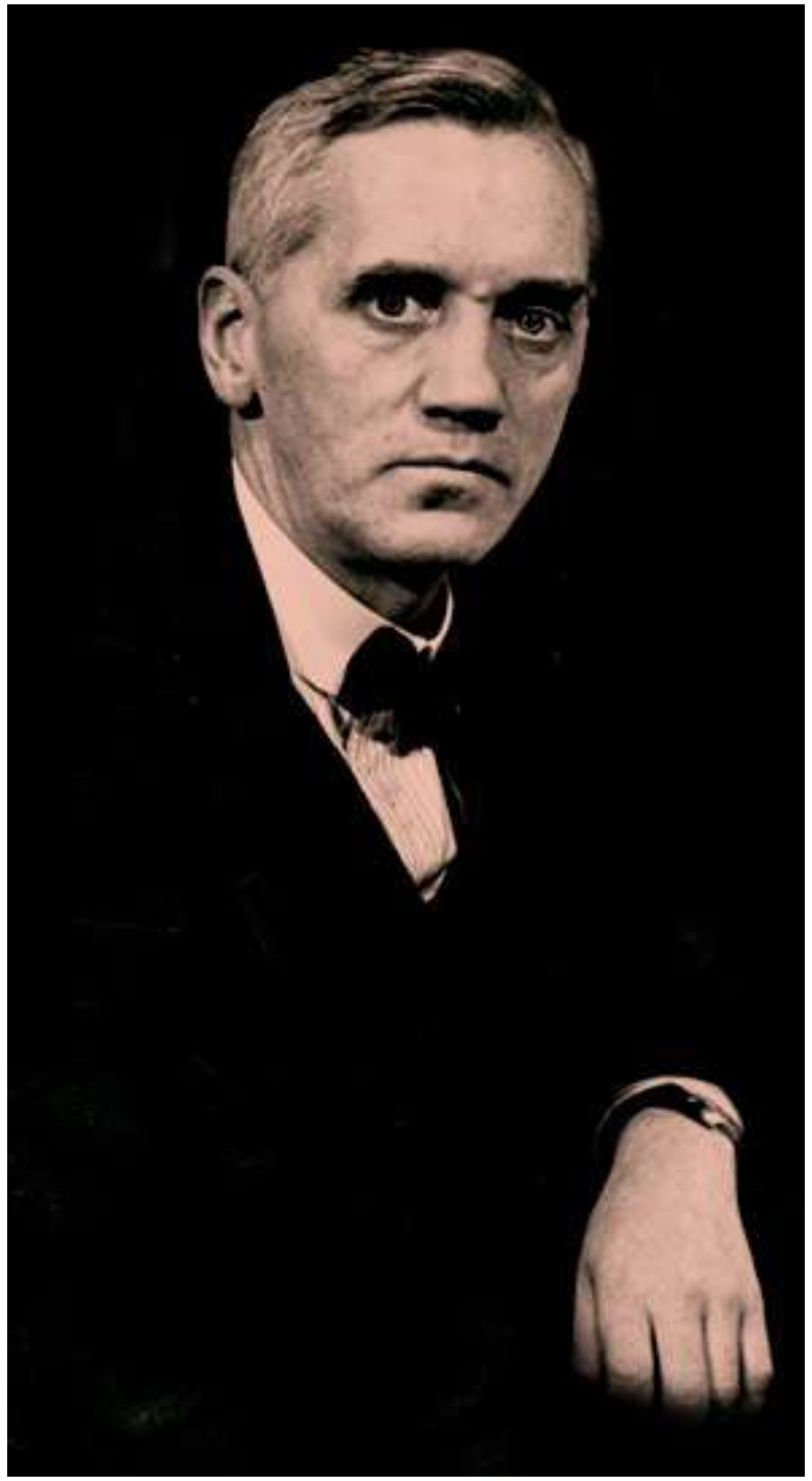
Streptococcus pneumoniae



DNA contains genetic information
DNA is the molecular basis of hereditary



10.1128/JCM.01249-13 Rüger et al.,



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 with the lysozyme (saliva)

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

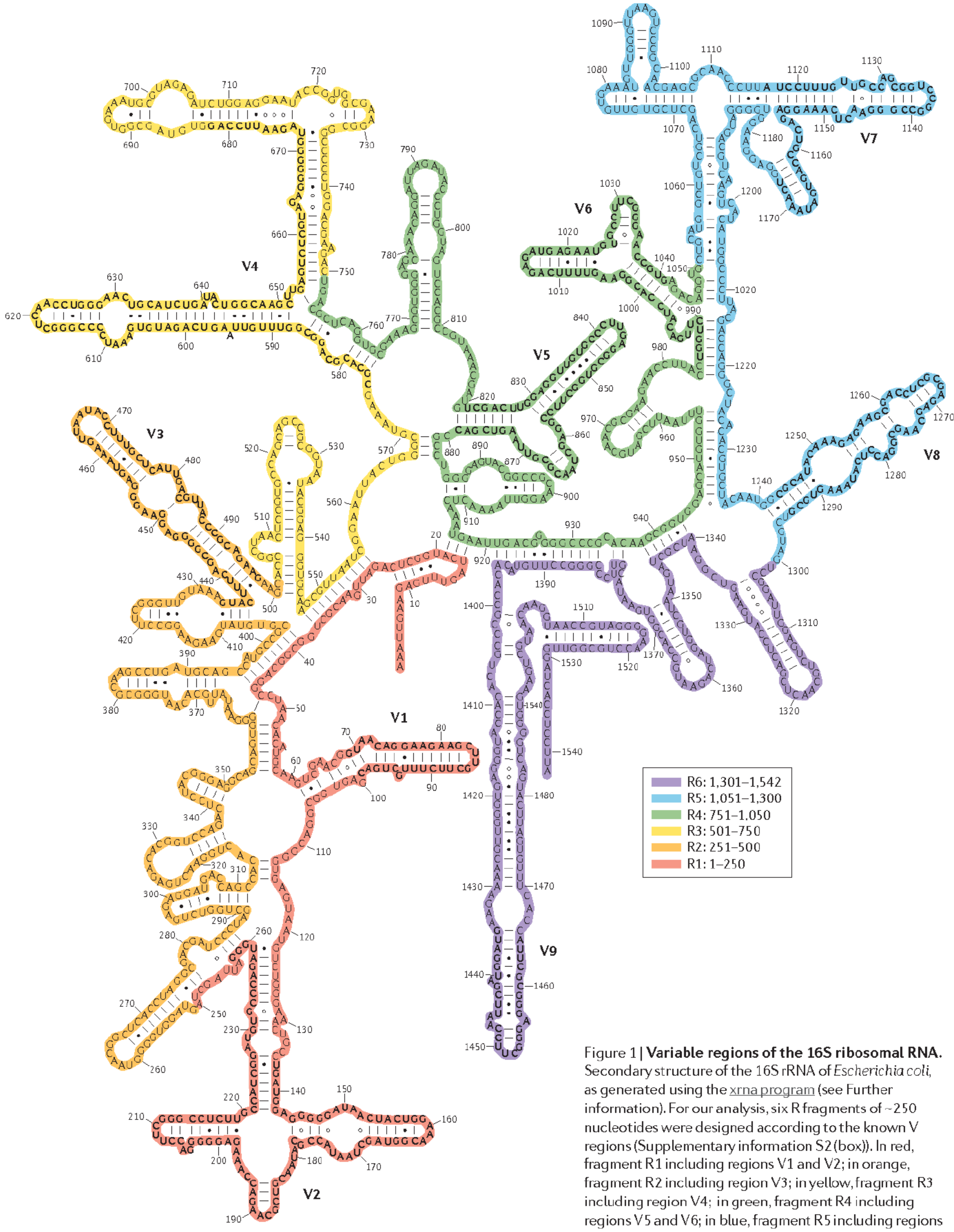


Figure 1 | **Variable regions of the 16S ribosomal RNA.** Secondary structure of the 16S rRNA of *Escherichia coli*, as generated using the xrna program (see Further information). For our analysis, six R fragments of ~250 nucleotides were designed according to the known V regions (Supplementary information 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

Yarza et al. 2014 10.1038/nrmicro3330

Woese

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

(1) universally distributed,

(2) functionally constant,

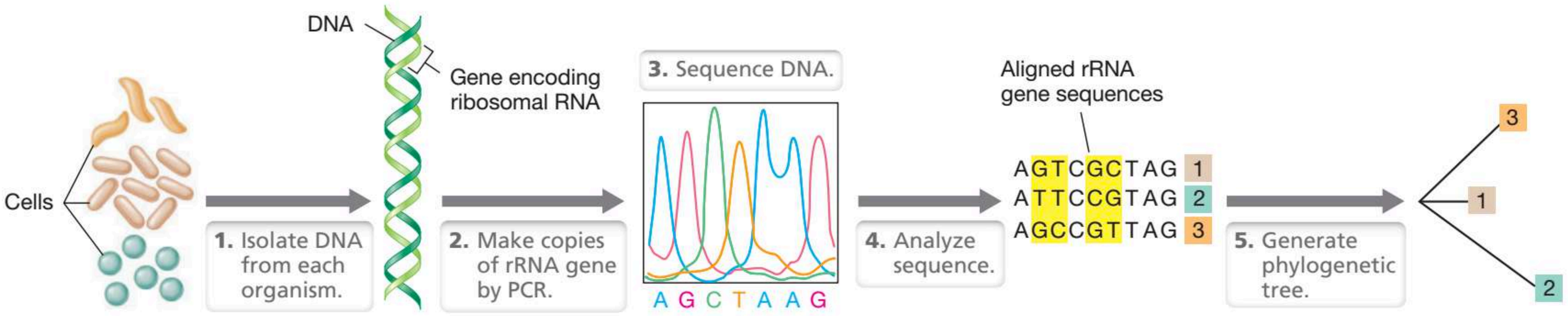
(3) highly conserved (that is, slowly changing),

(4) adequate length to provide a deep view of evolutionary relationships,

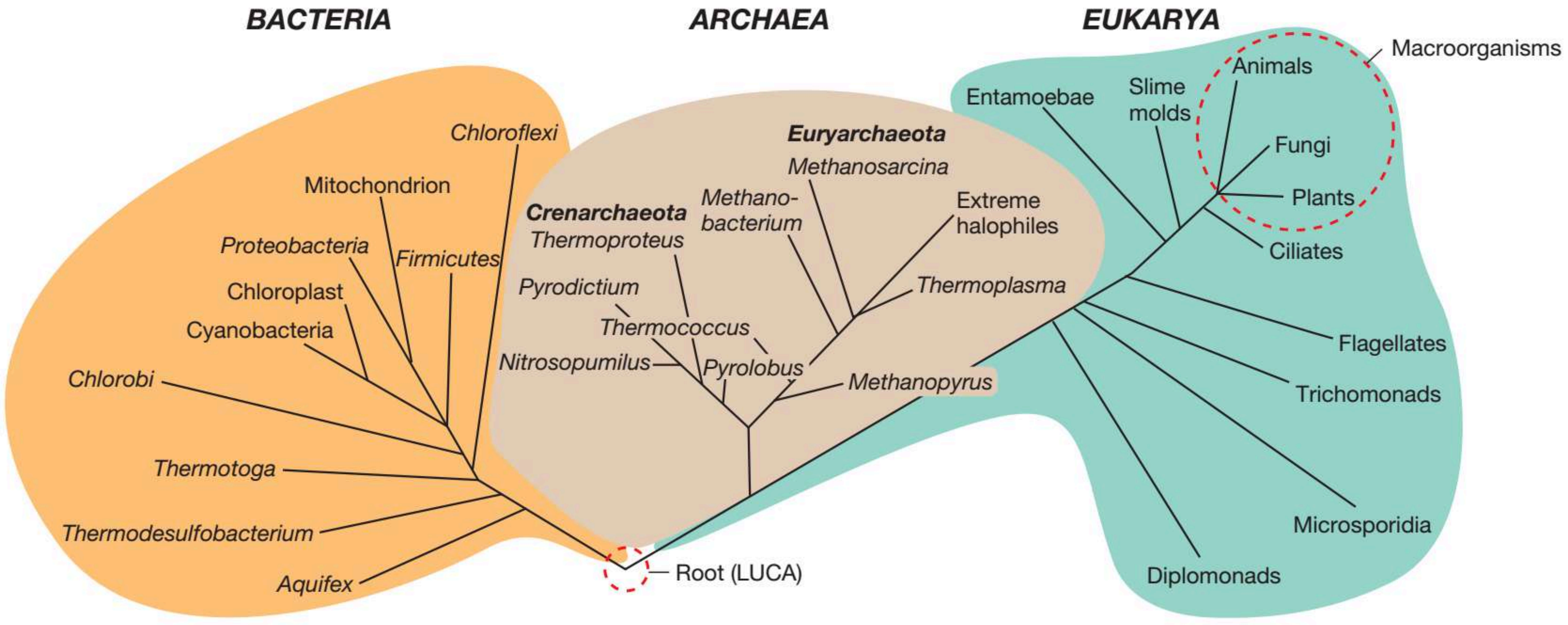
(5) diverse in different ‘species’

Using pure cultures of Bacteria and Archaea

Step-by-step technology for evolutionary classification of microbes



Phylogenetic Tree of Life



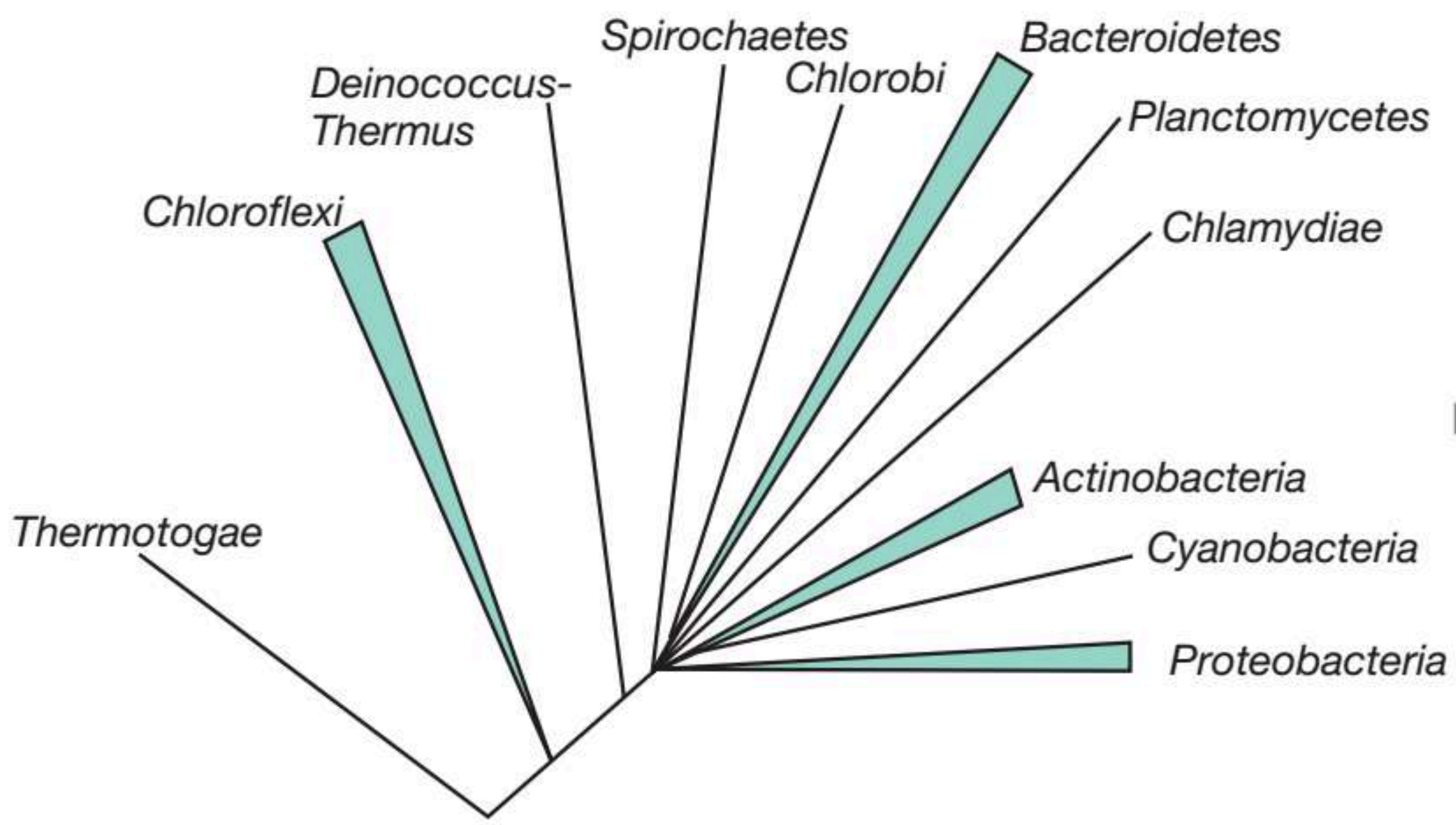
LUCA: Last Universal Common Ancestor

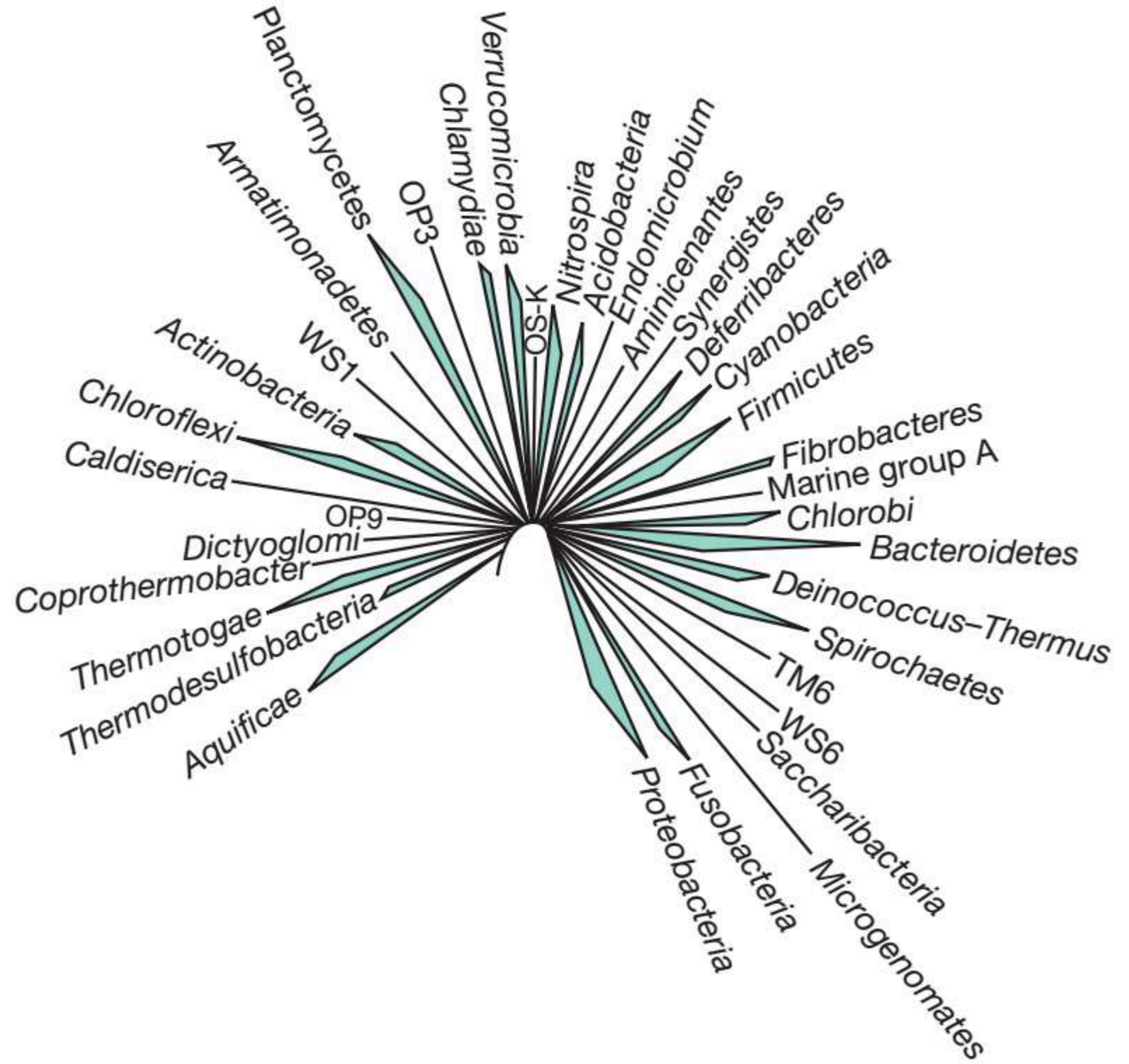
Madigan et al. 2018

Woese

Woese

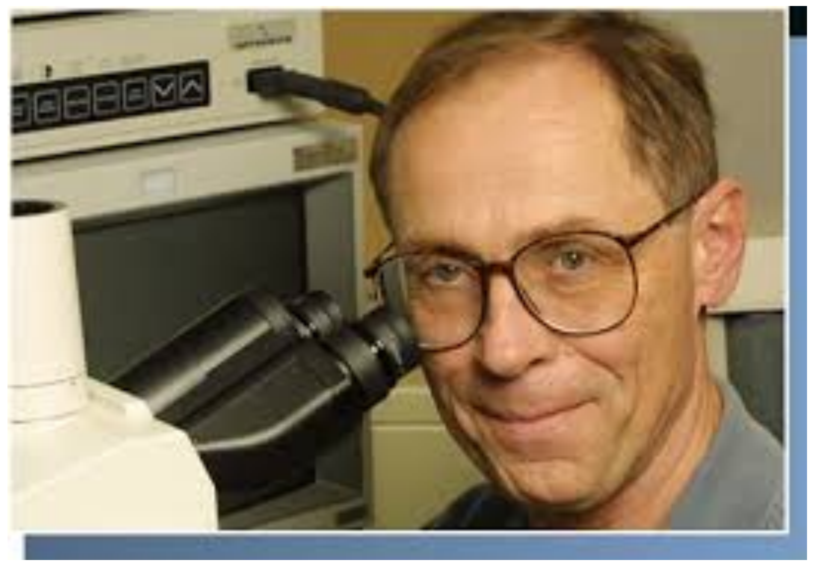
Pace





Madigan et al. 2018

Cultivation dependent



Cultivation independent

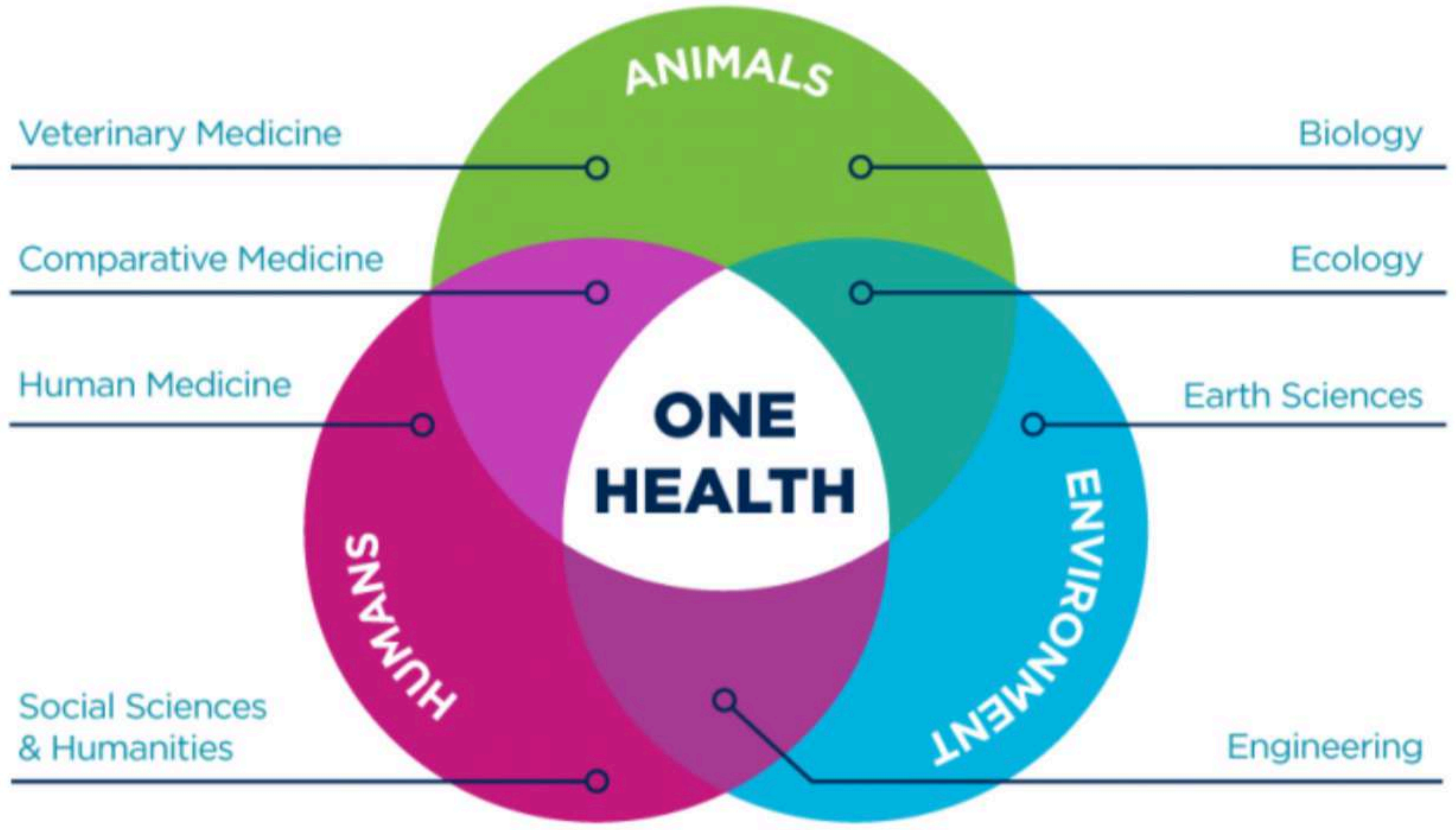
Microbes living in the environment

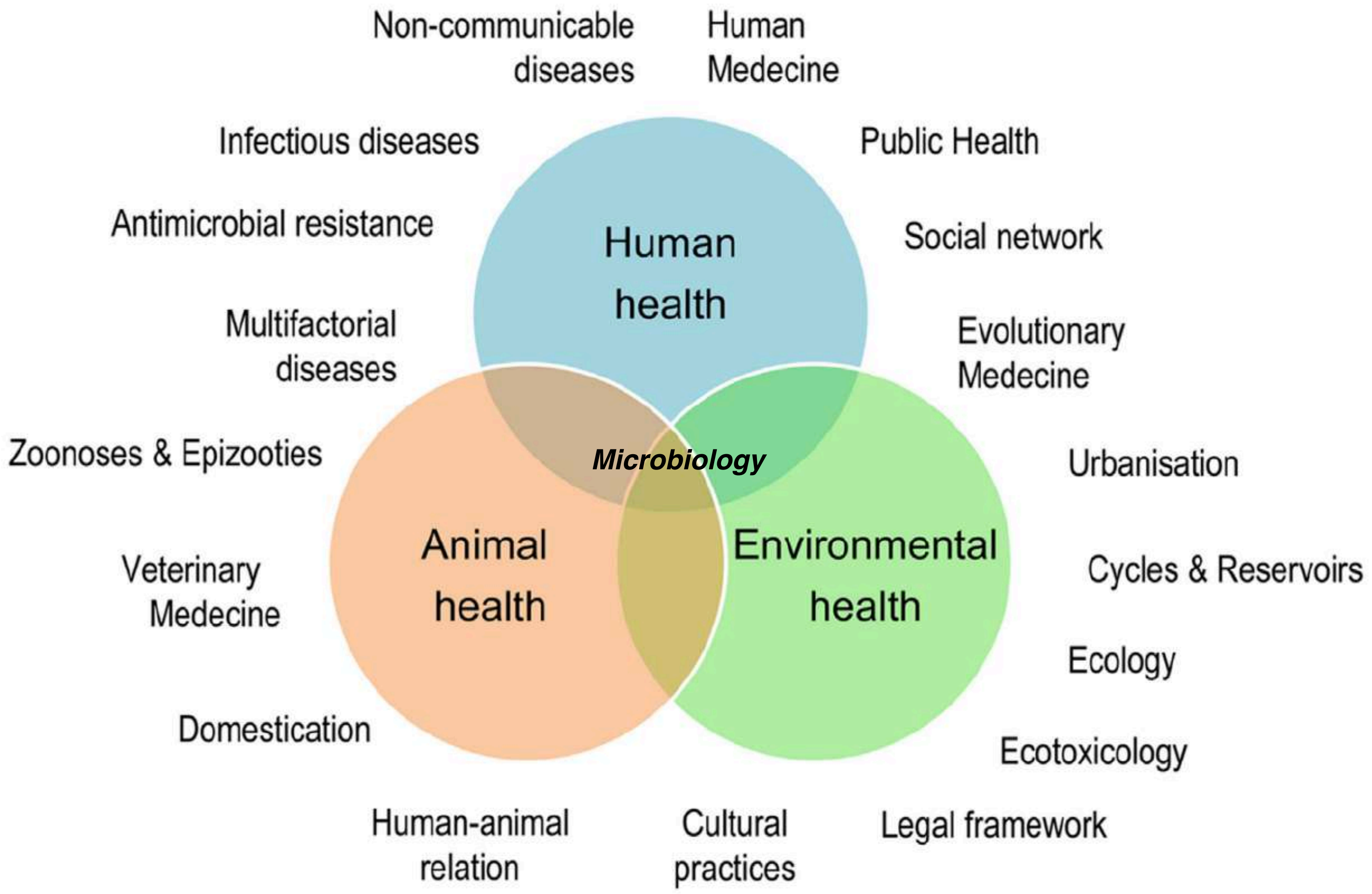
Microbes can or can't be cultivable

- 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

Original author not found





Destoumieux-Garzon et al., 2018

Microbiology:

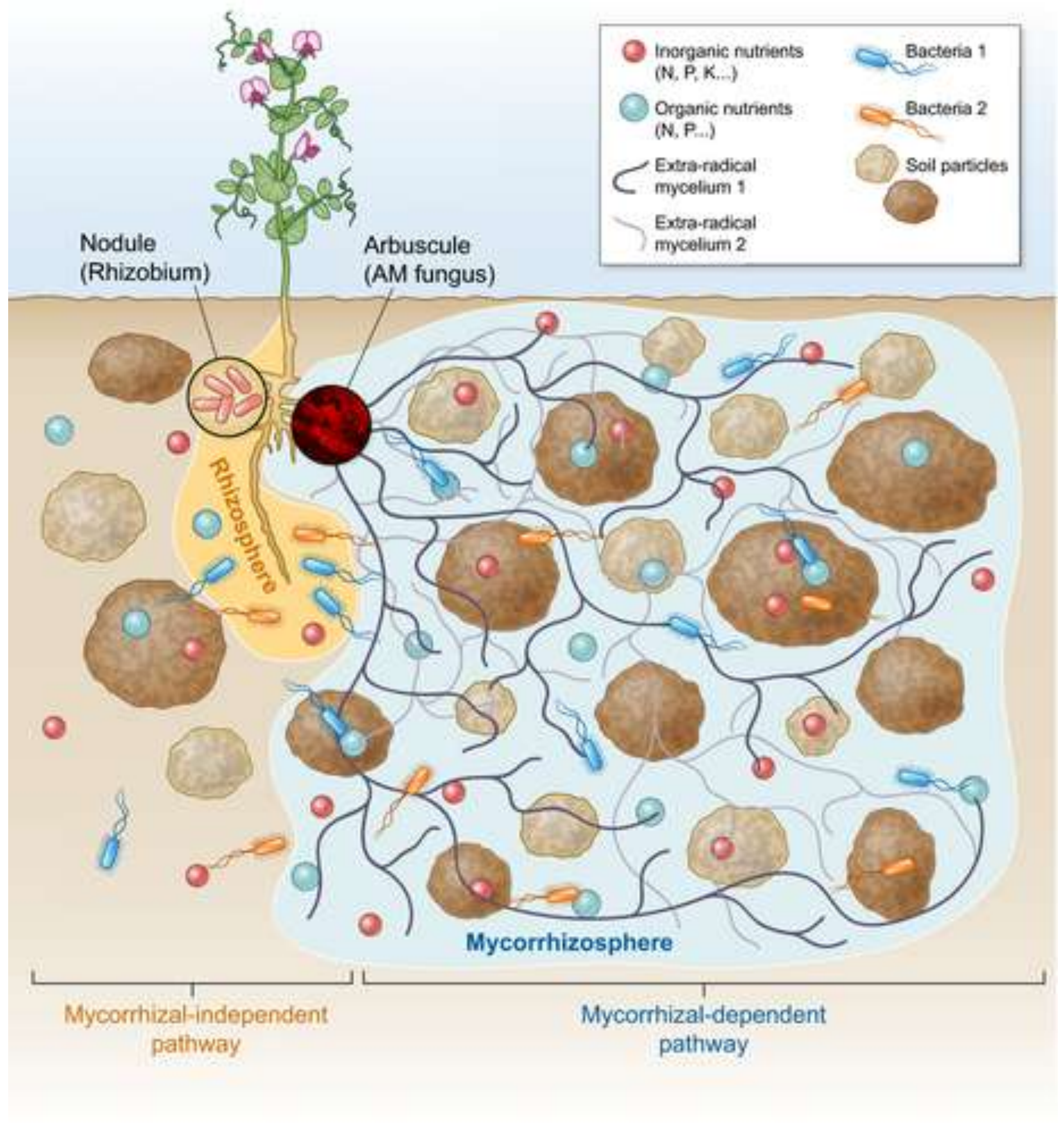
Holistic study of the function of microbial cells and their impact on medicine, industry, environment and technology (Madigan et al. 2018).

Goals of the course:

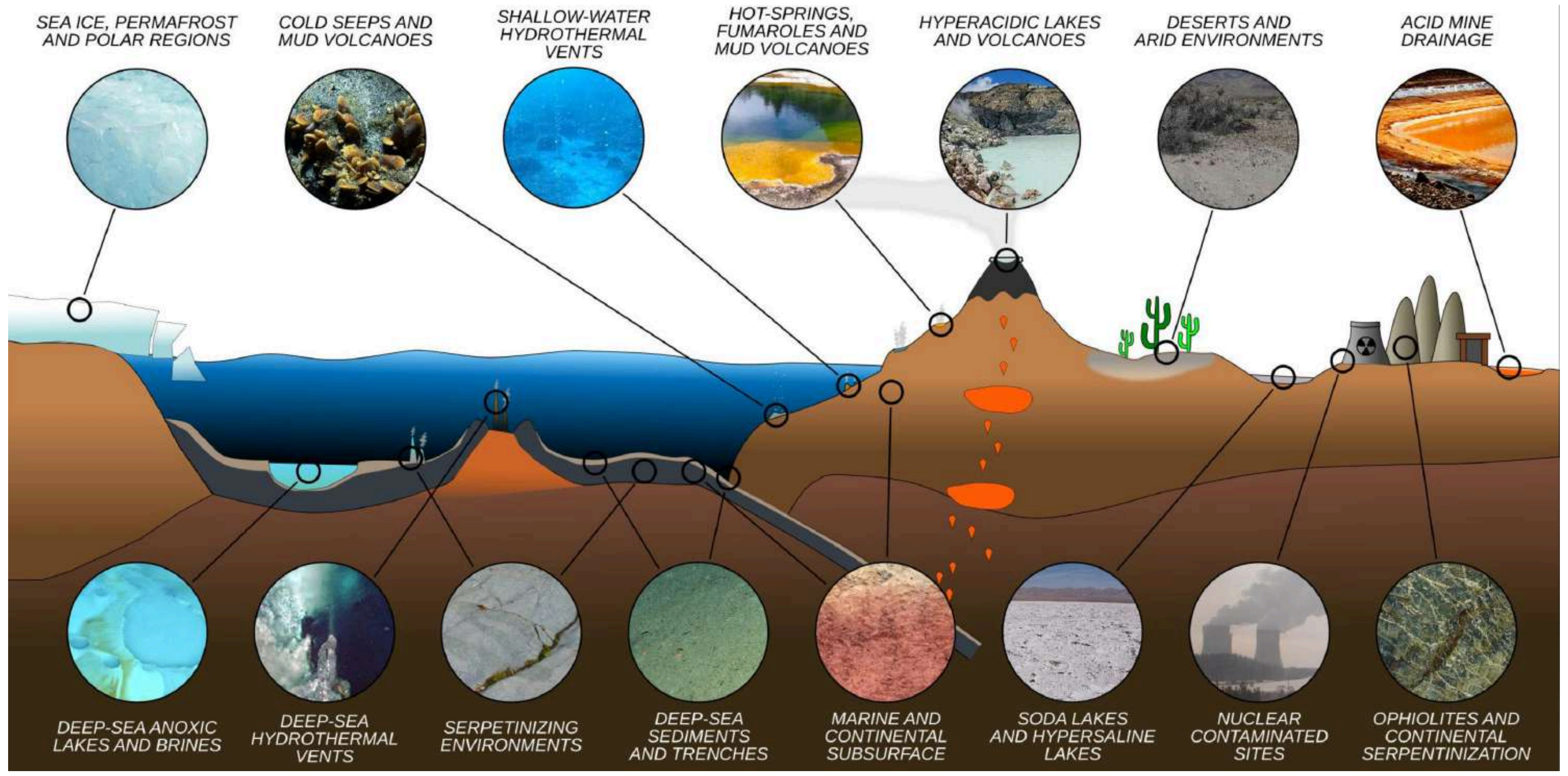
Proficiency in microbial literacy and gaining fundamental understanding of microbes life and their function in the environment, thus included the human beings in health and disease

Microbial environments

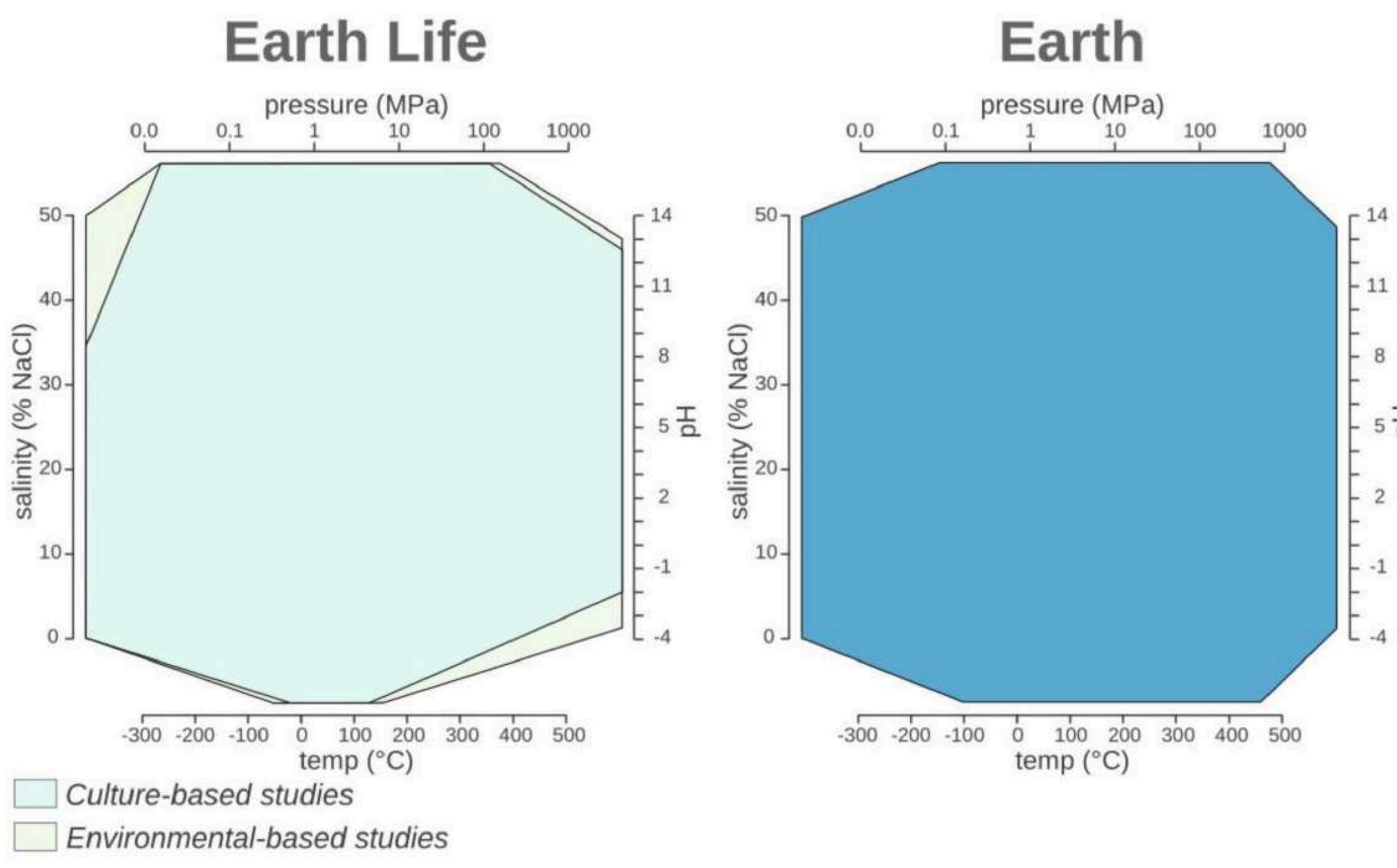
- Temperature
- pH
- Light/Dark
- Humidity
- Pressure
- Radiations (not on Earth)



Wipf et al. 2019



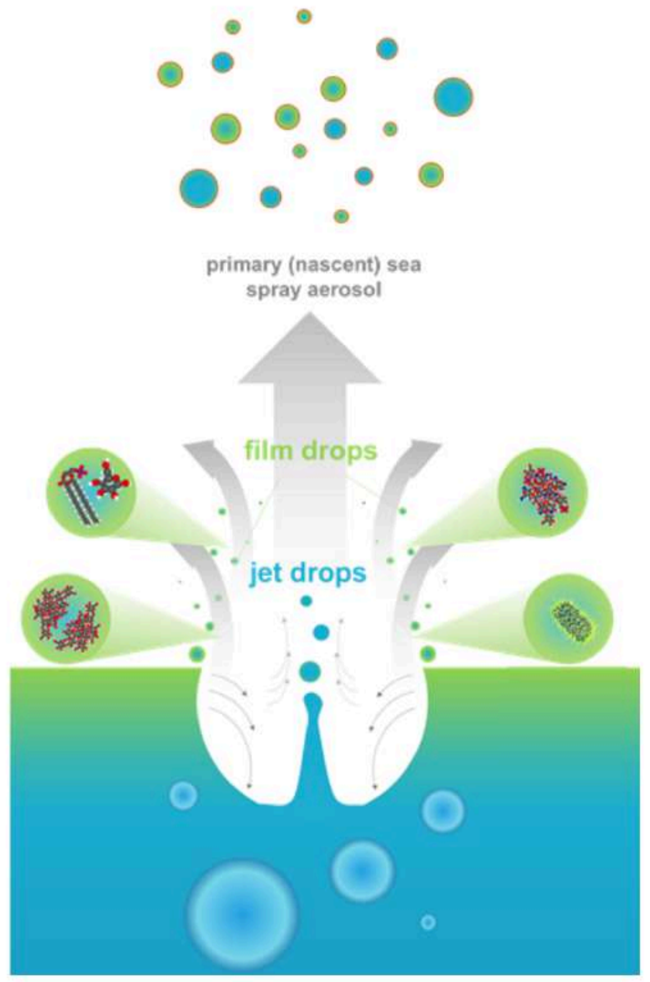
Merino et al. 2019



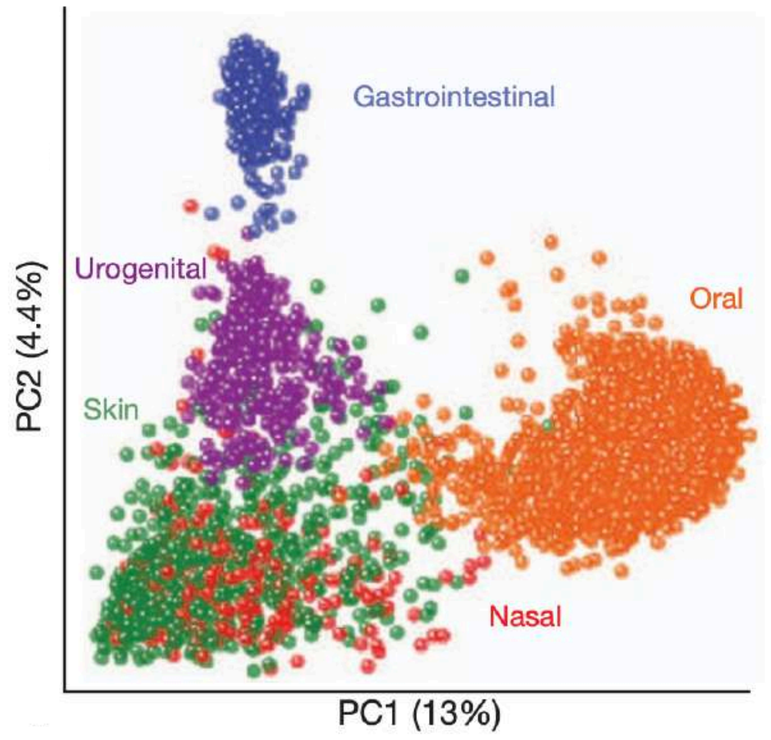
Merino et al. 2019

https://youtu.be/i-icXZ2tMRM

Microbial environments



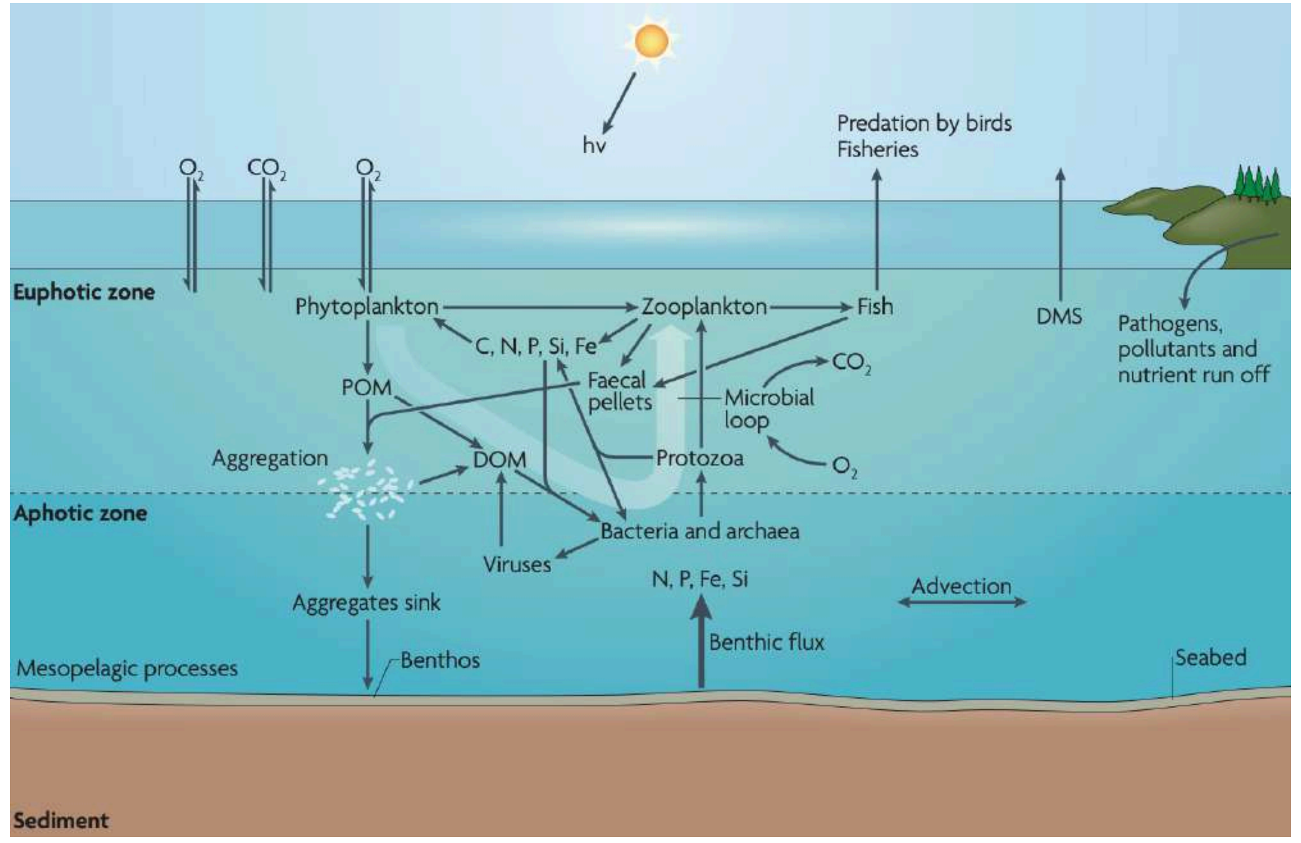
2020 CENTER FOR AEROSOL IMPACTS ON CHEMISTRY OF THE ENVIRONMENT



HMPC 2012

- 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

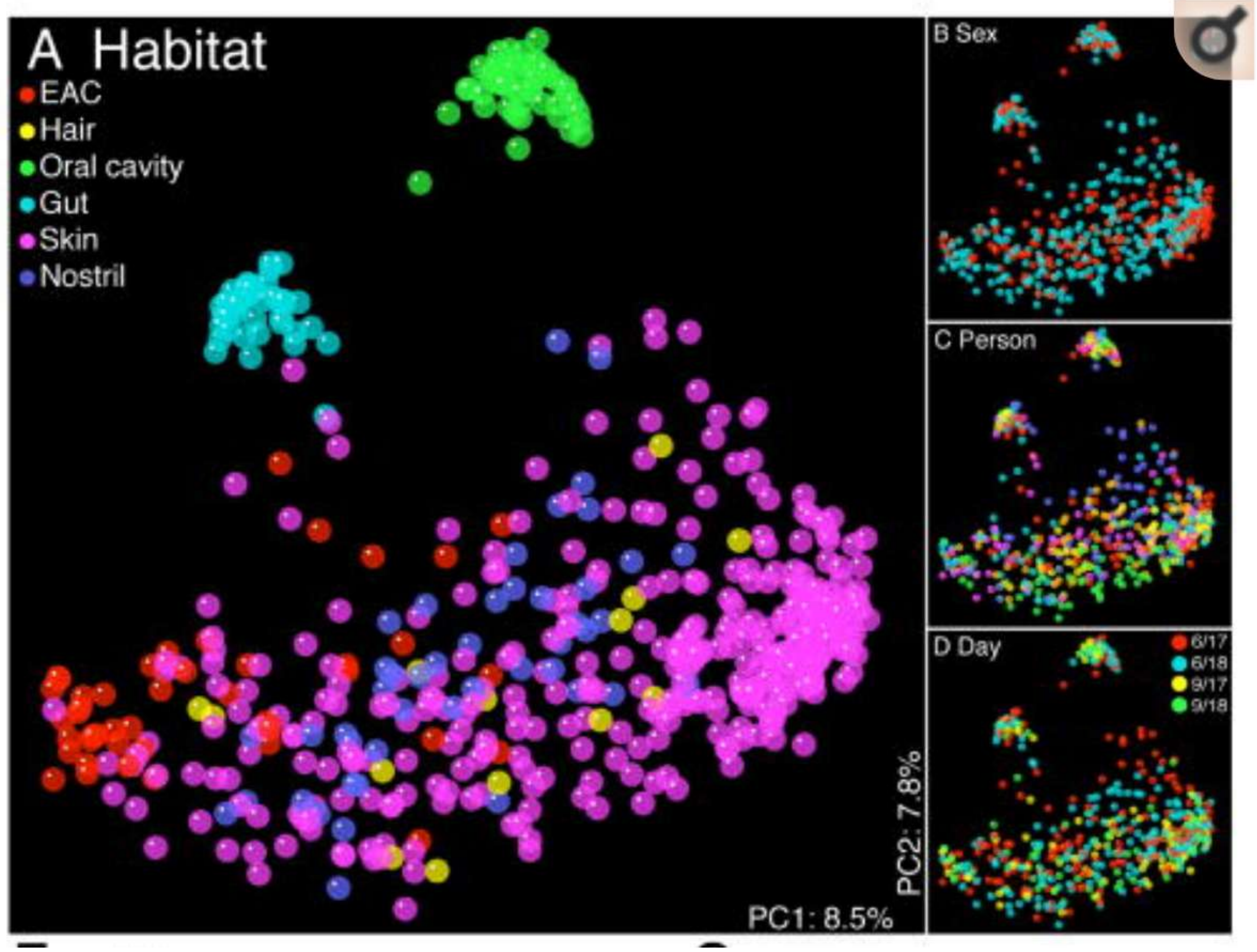
Azam & Malfatti 2007



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 circles
- Eukaryotes and Viruses (ecology, chemistry and physiology)
- pH:
  - ★ skin~5.5
  - ★ blood~7.4
  - ★ mouth~ 6.7-7.3
  - ★ vagina ~3.8-4.5
  - ★ esophagus 5-7
  - ★ stomach 2-5
  - ★ duodenum 6.8



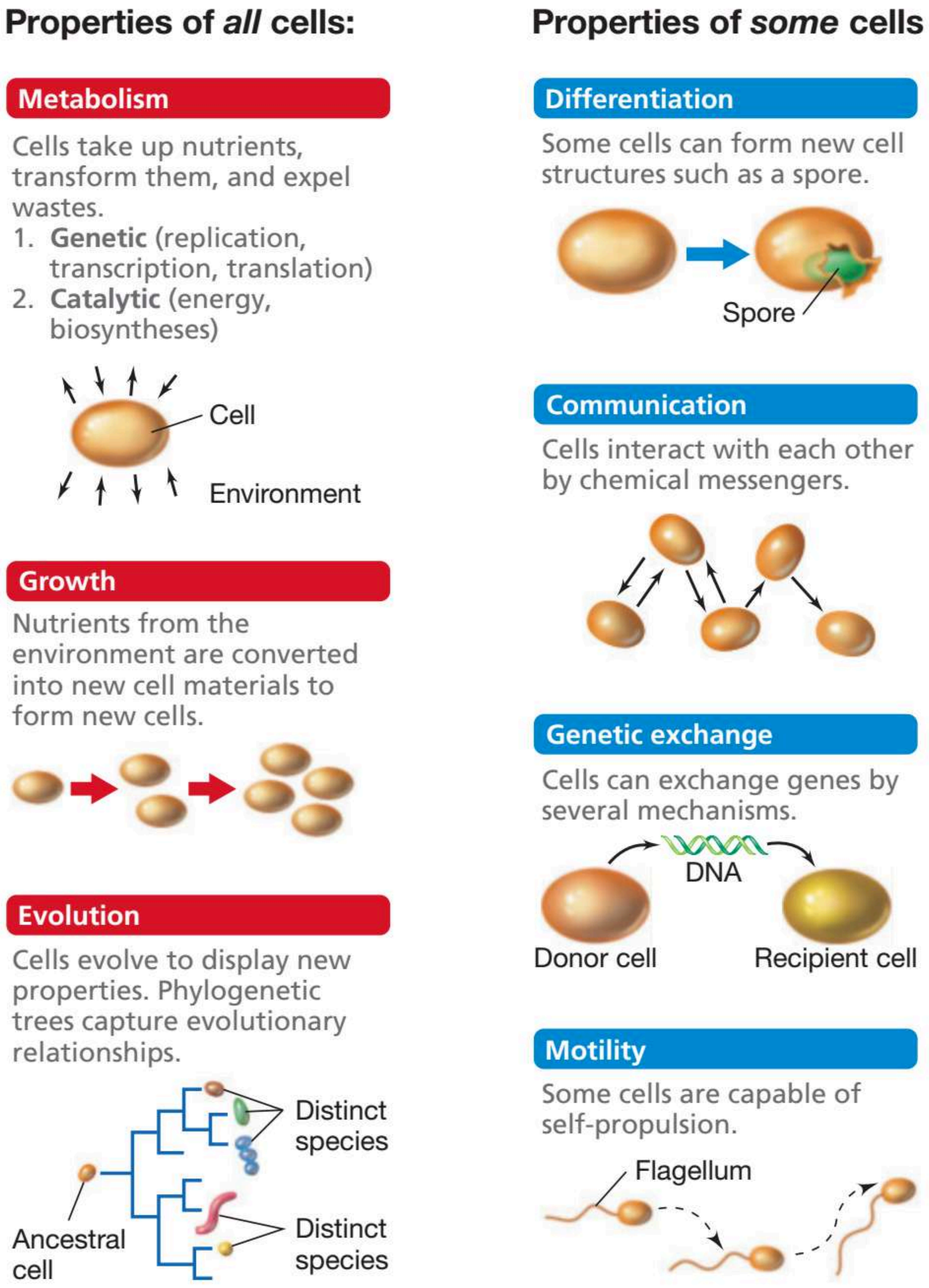
Costello et al., 2005

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

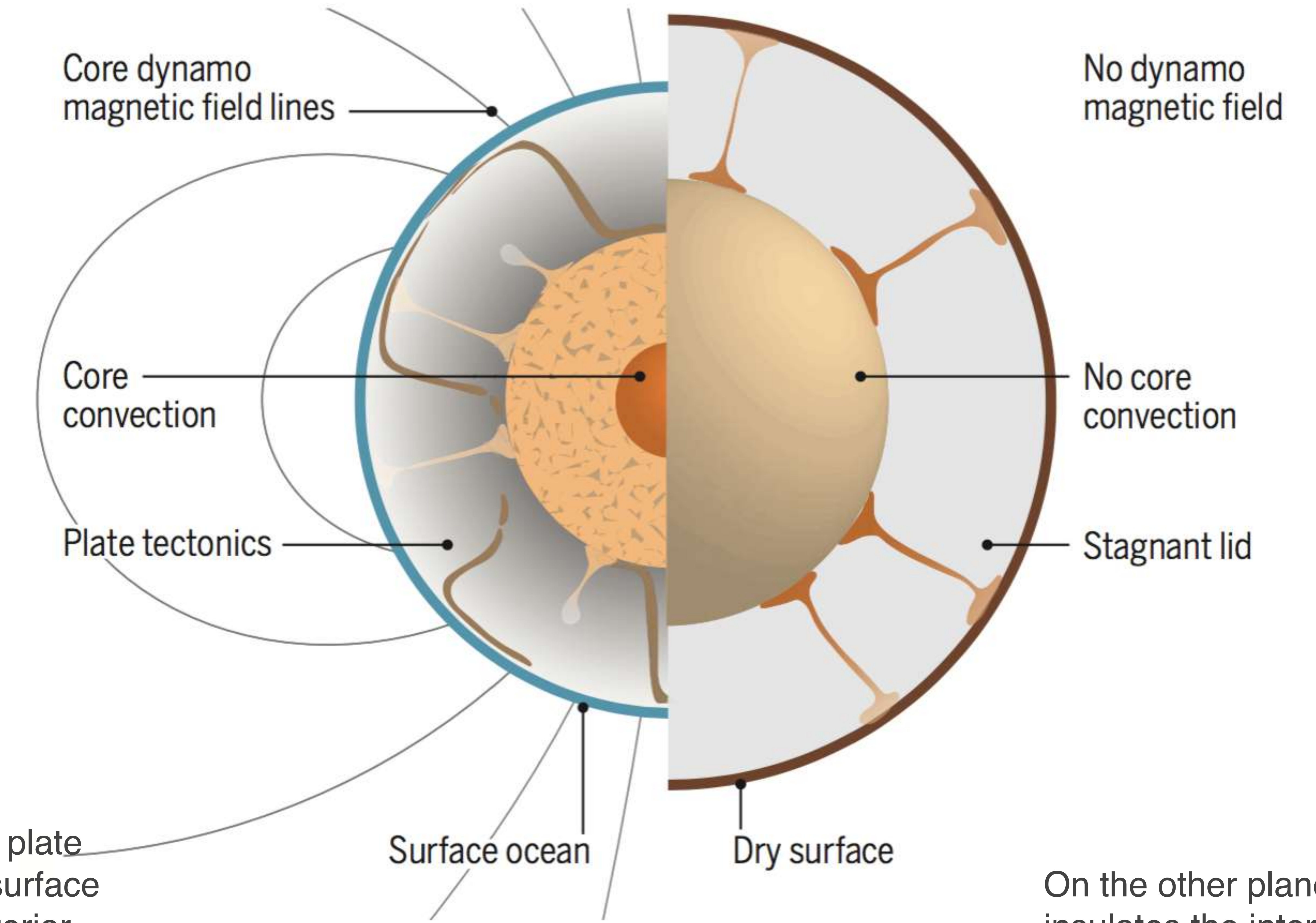
From where do we start? —> *ab initio*

Being a microbe



Madigan et al. 2018

Habitable features of Earth and Exoplanets



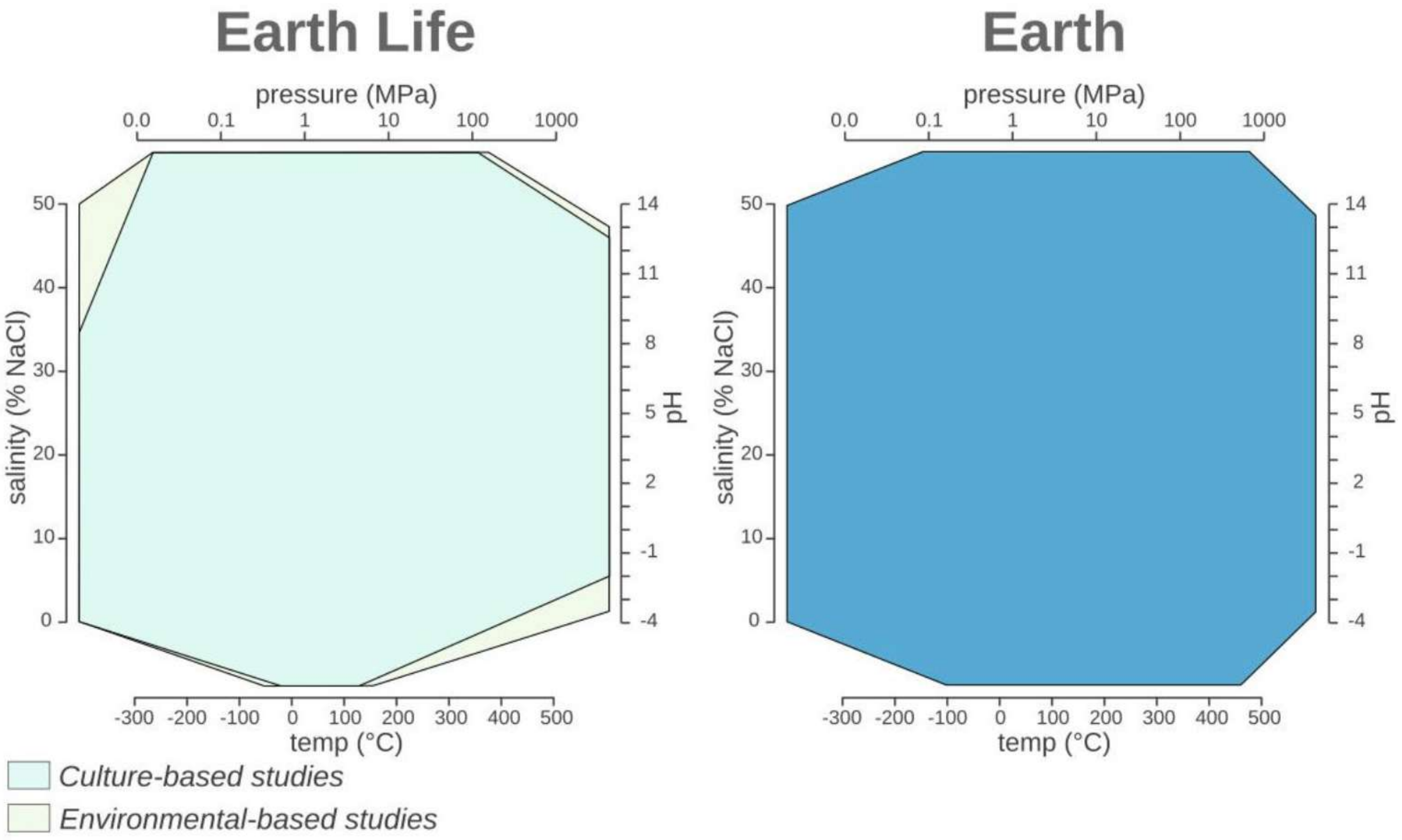
On the habitable planet, plate tectonics stabilizes the surface climate and cools the interior fast enough to generate a magnetic field that in turn shields the surface from water loss and harmful radiation

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

The James Webb Space Telescope, due to launch in 2021, will attempt to detect atmospheres

Microbial Life on Earth

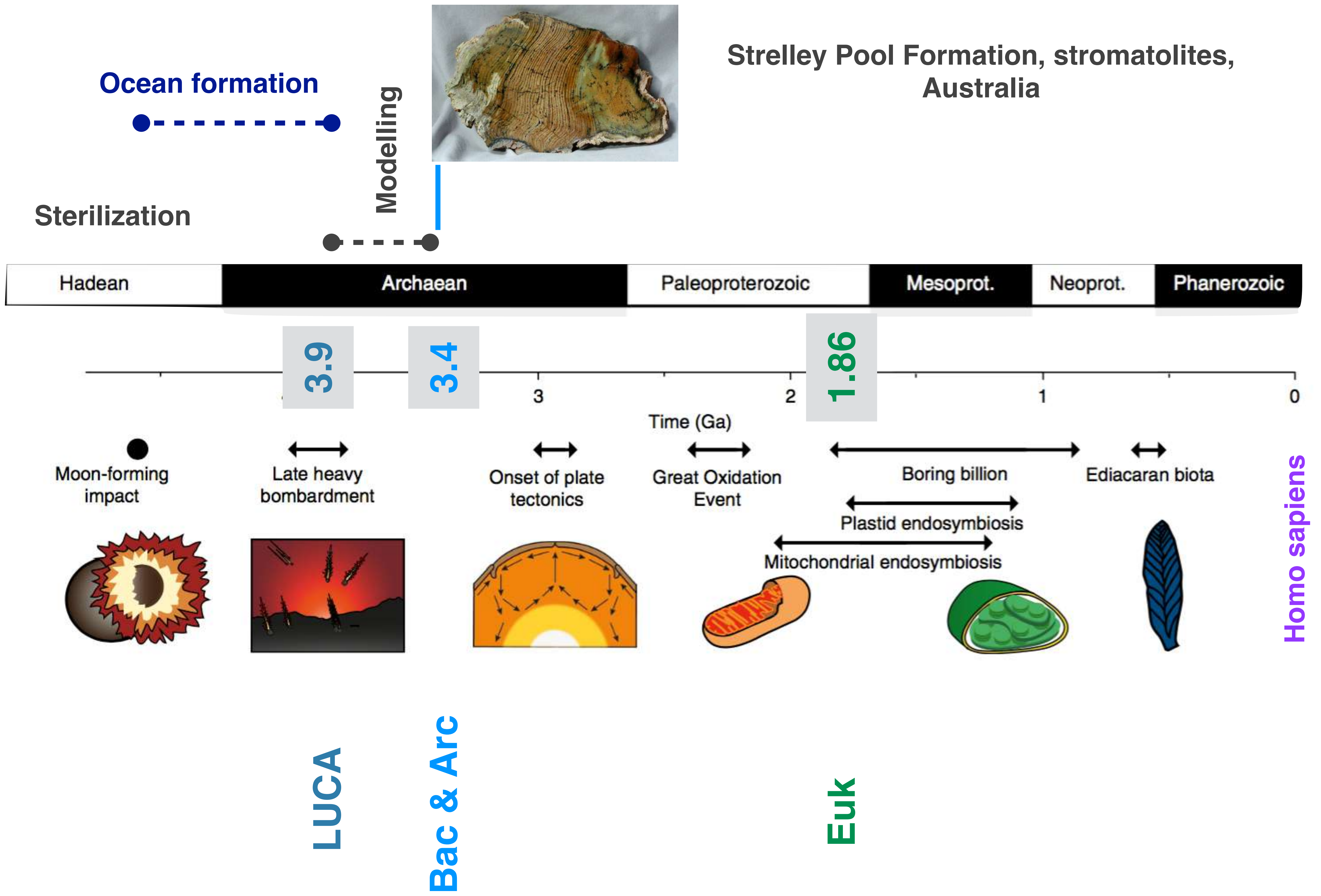
Merino et al., 2019



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

Biospheric capacity equivalent to ~1% of Earth's geosphere and troposphere —> a minimum **biospheric volume** of ~1

Origin of Life: **when**



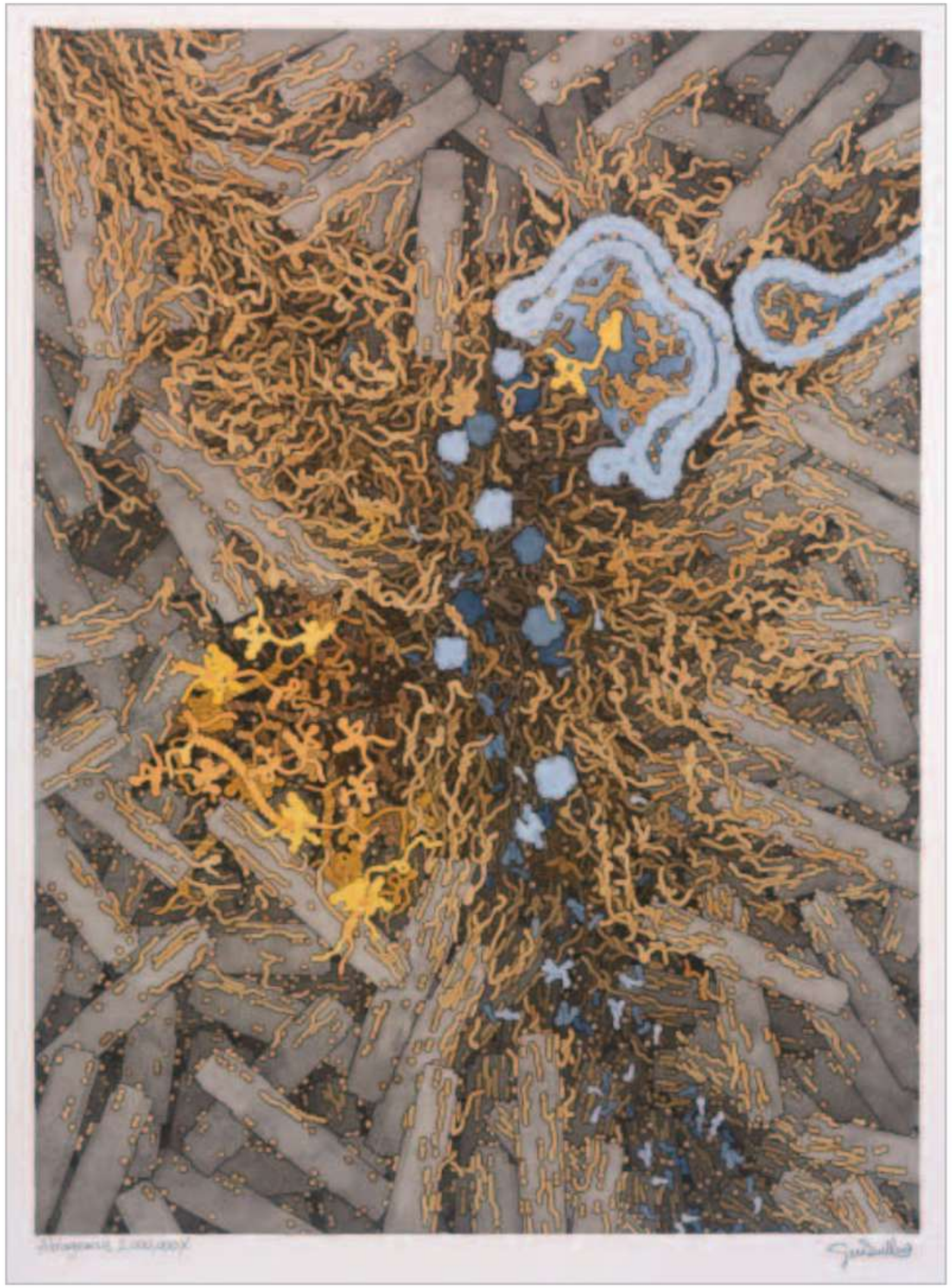
Strelley Pool Formation, stromatolites, Australia

Betts et al., 2018

LUCA: Last Universal Common Ancestor

Origin of Life: **“Abiogenesis”**

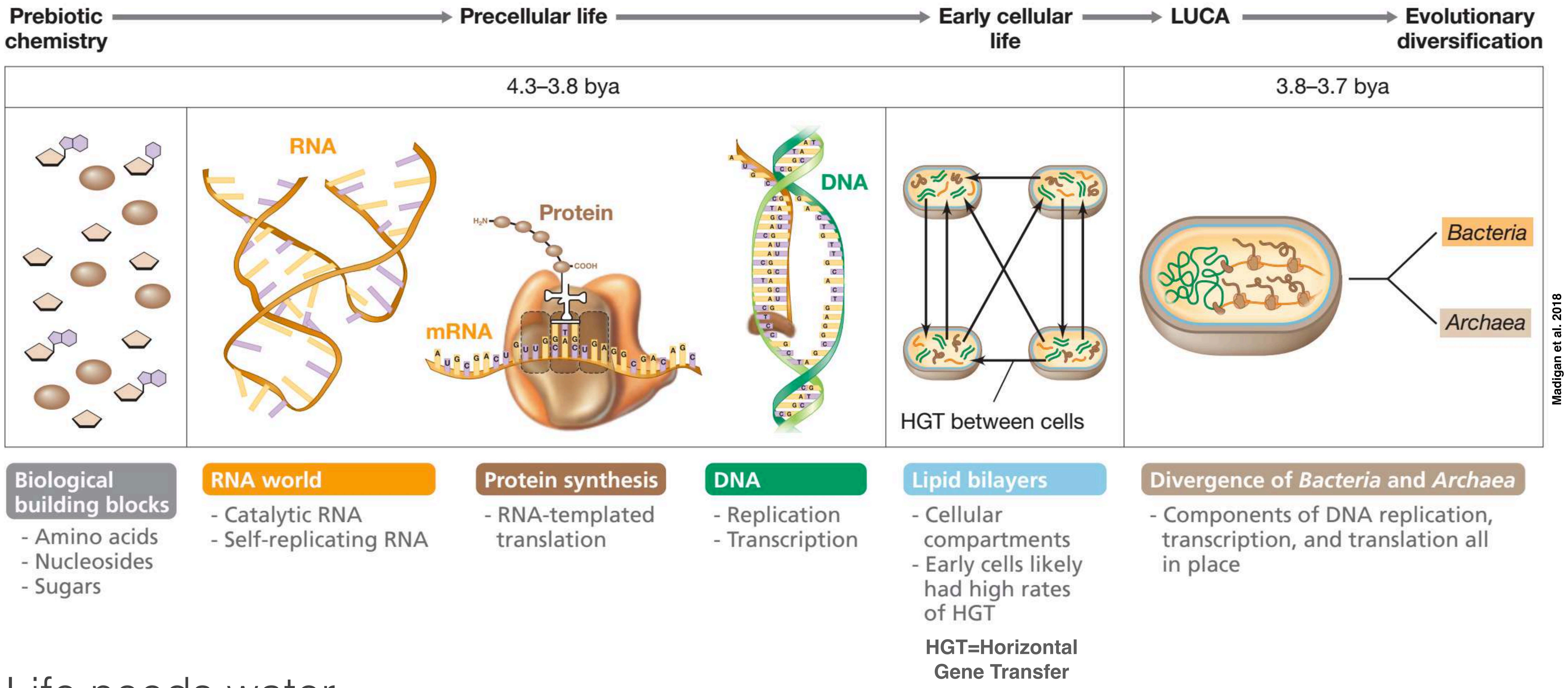
David S. Goodsell



- 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**

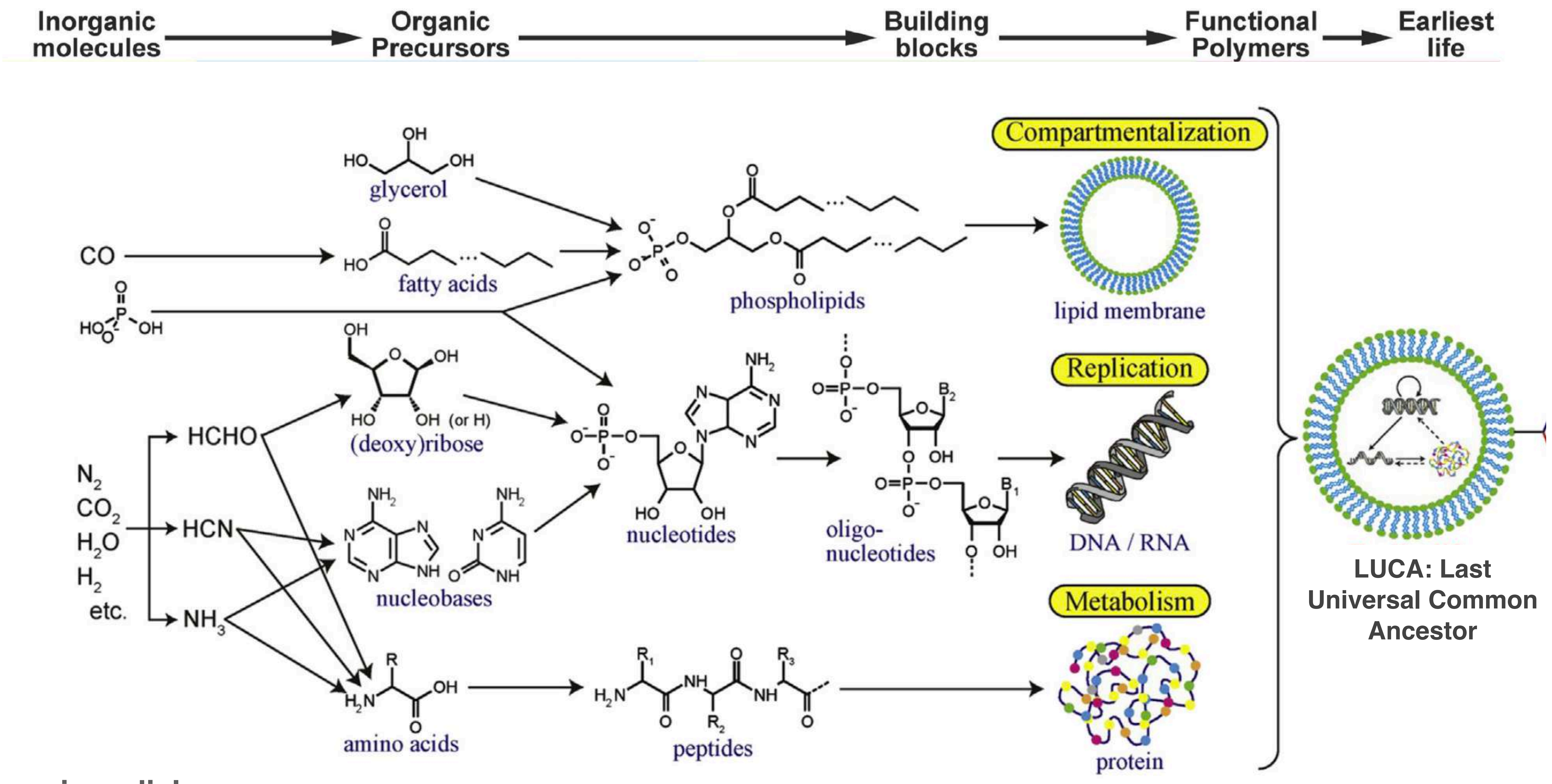
Kitaday & Maruyama, 2018



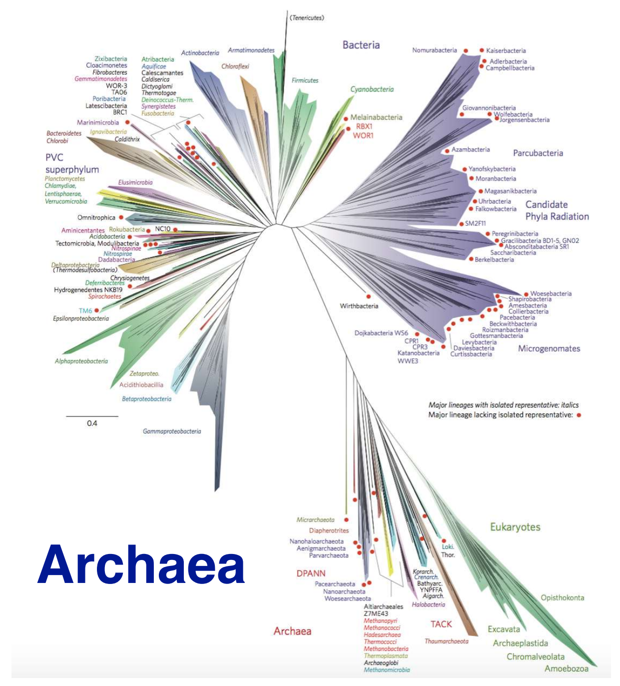
Life needs water

Building complexity to achieve the 3 fundamental functions of Life

Kitaday & Maruyama, 2018



Bacteria



Archaea

Eukarya

Hug et al., 2016

<u>primordial soup</u>

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 (CH4/H2O/NH3/H2S and later H2O, N2, and CH4, CO2, 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**

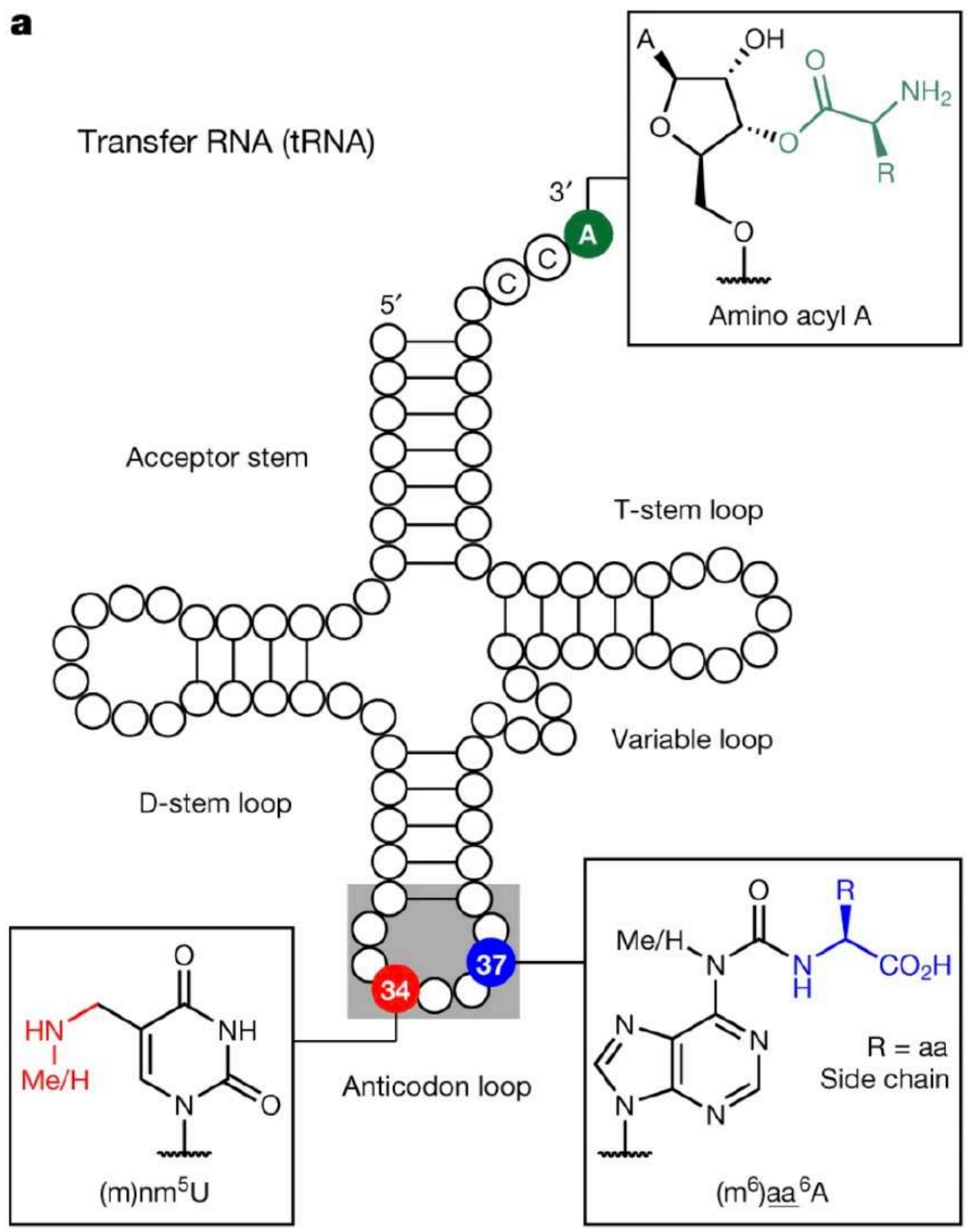
Catalysts made of RNA alone are much less efficient than the protein-based enzymes found in all living cells today

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

Müller et al., 2022

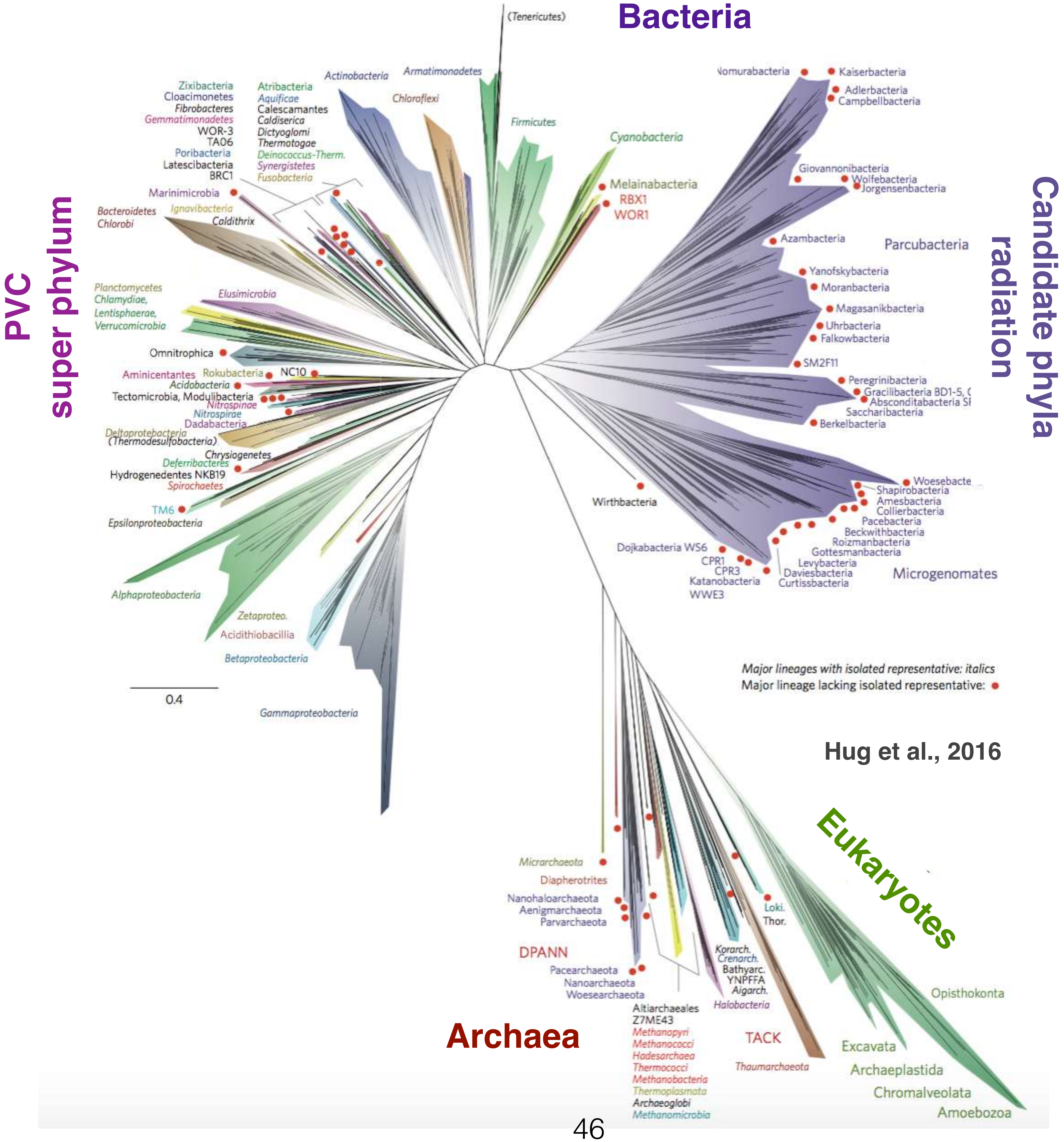


The 3′-amino acid-acylated adenosine is located at the CCA 3′ end in contemporary tRNAs

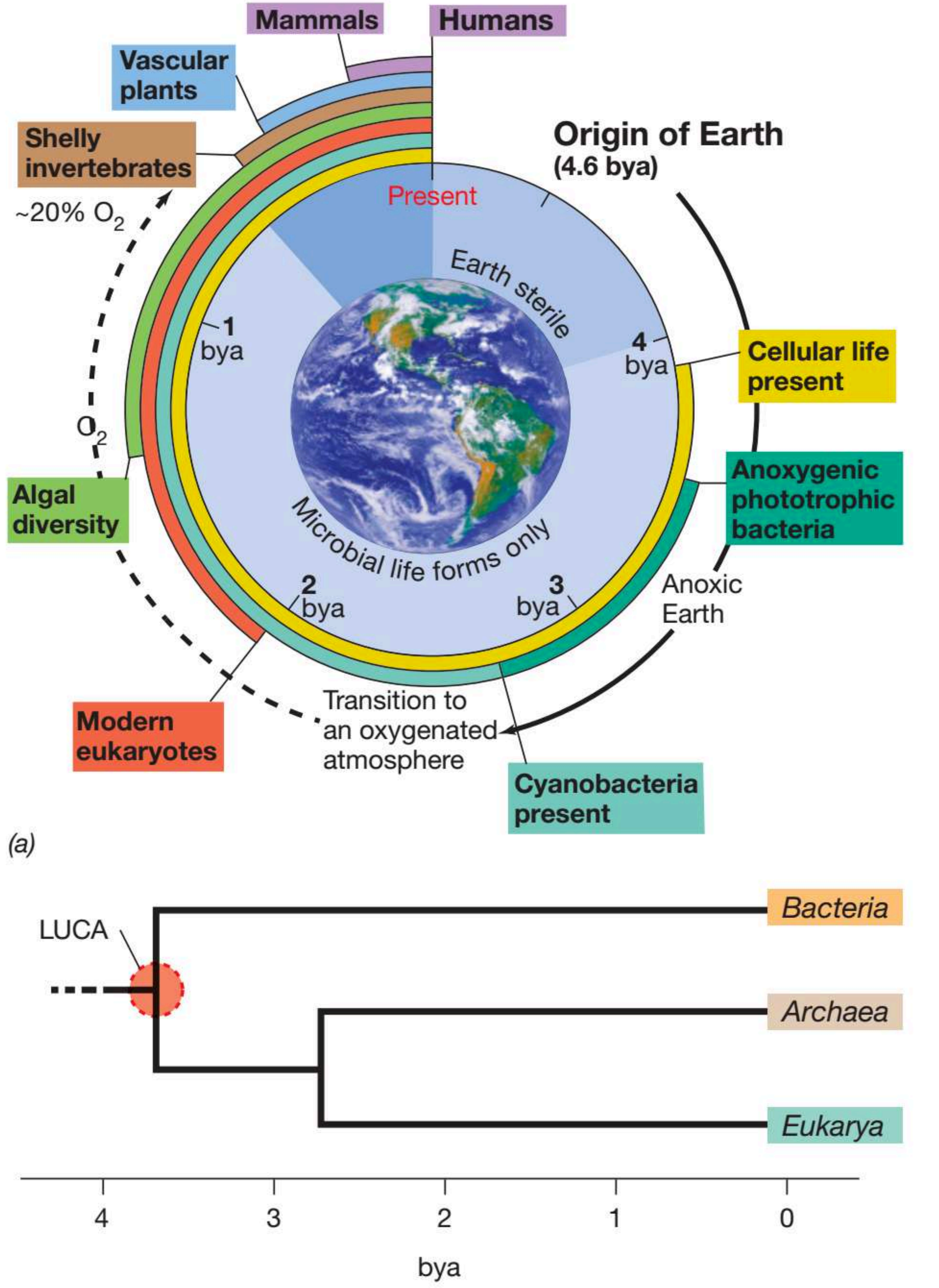
5-Methylaminomethyl uridine, mnm5U, is found in the wobble position 34

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

Microbial diversity on Earth

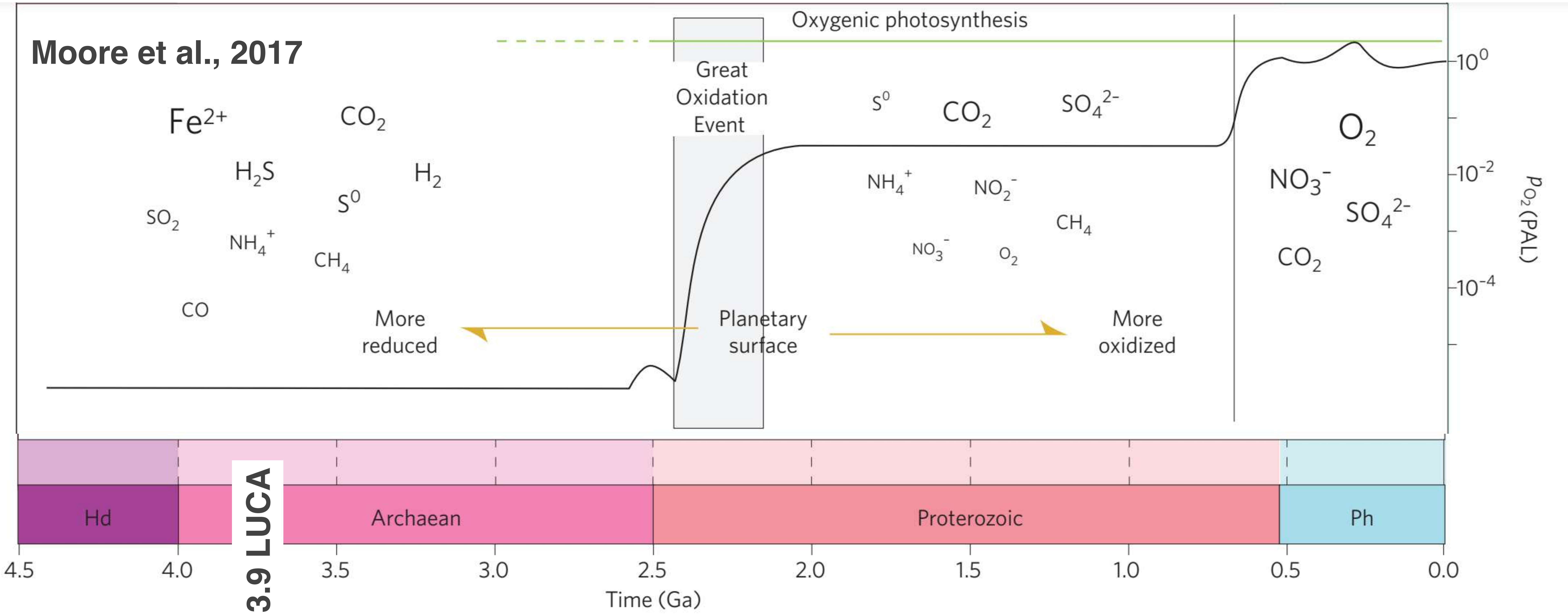


Hug et al., 2016



Microbial Metabolic diversity impacts Earth ecosystem

Earth redox state changes

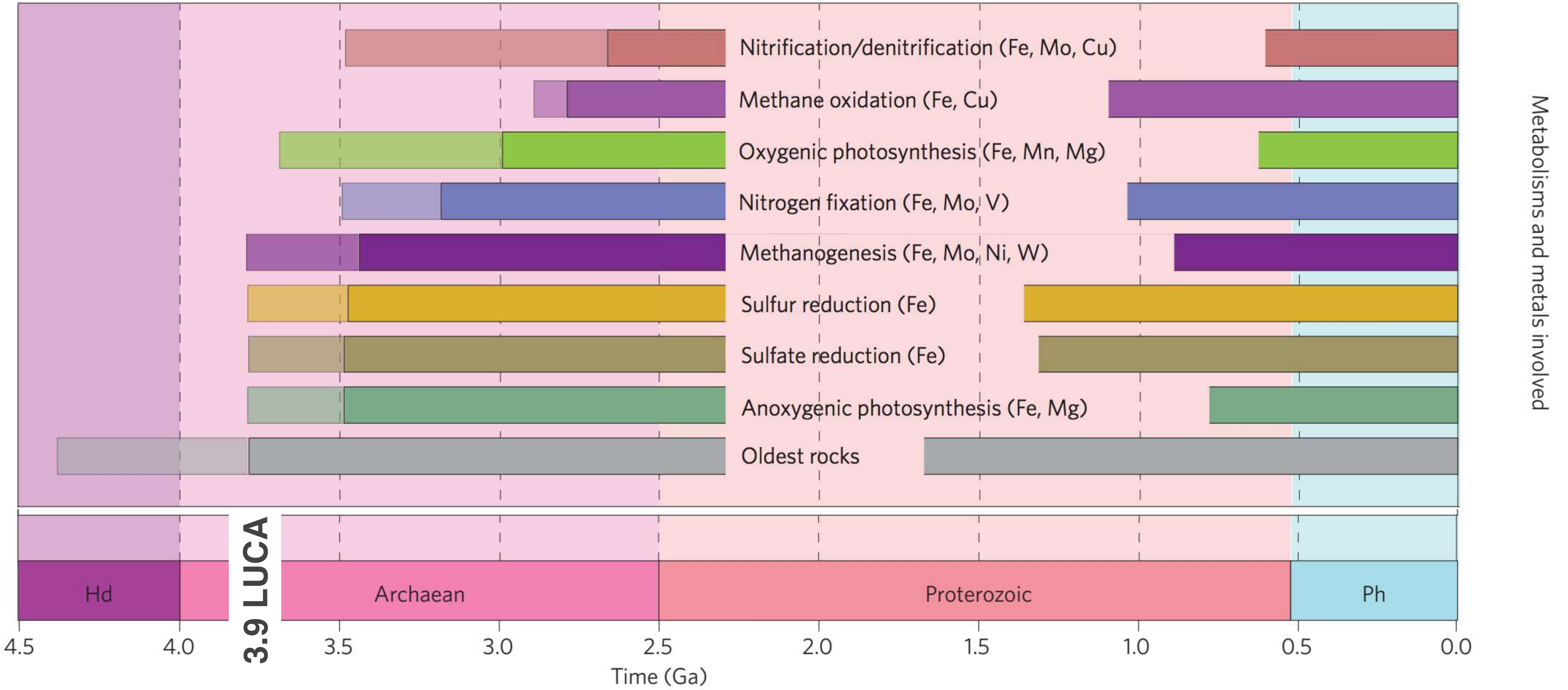


The availability of different metals and substrates has changed over the course of Earth's history as a result of secular changes in redox conditions of the mantel

Solar energy used by early microbes

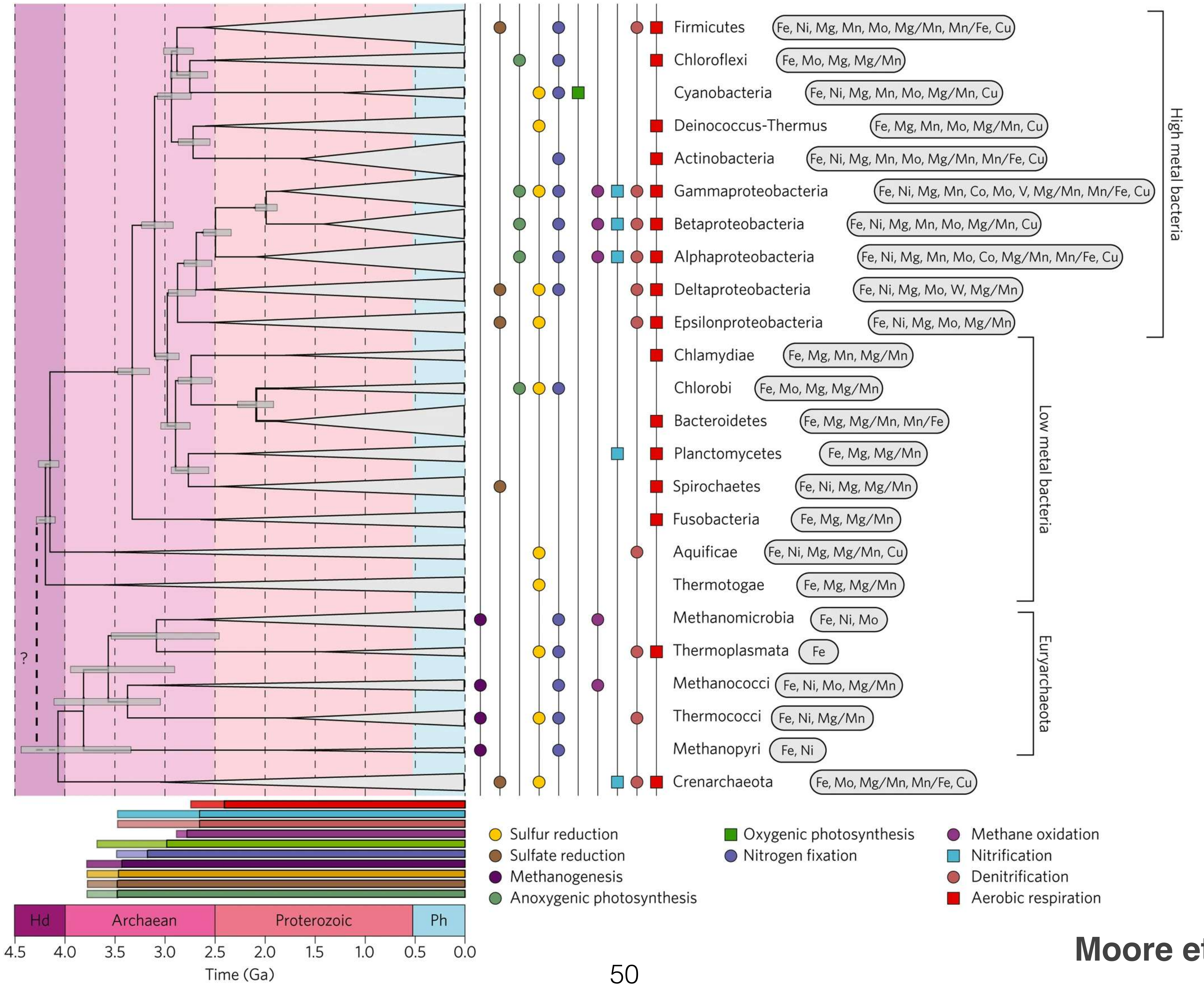
Emerging microbial metabolisms

Moore et al., 2017



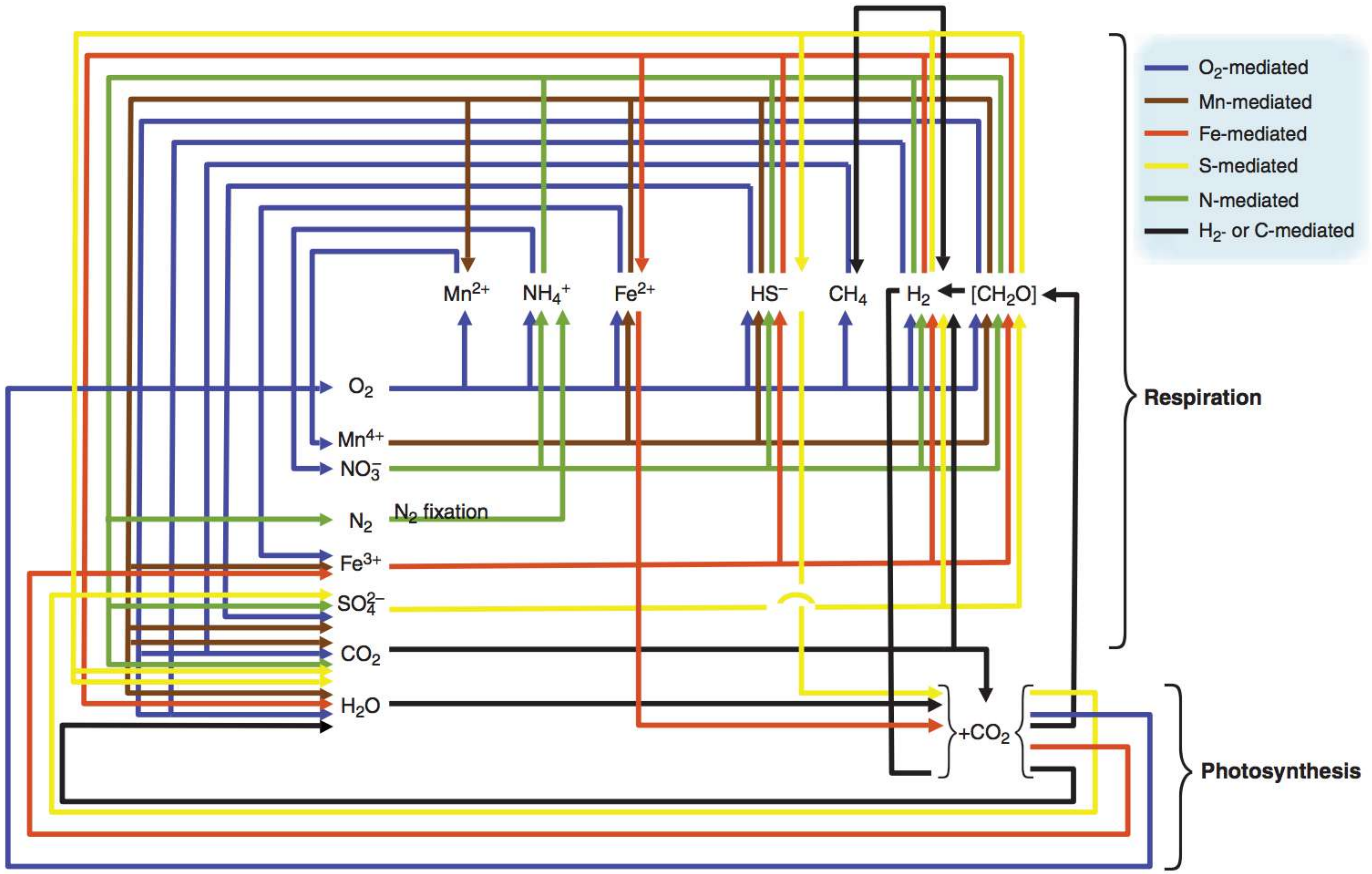
The oxidoreductases responsible for these metabolisms incorporated metals that were readily available in Archaean oceans: iron and iron–sulfur clusters

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



Moore et al., 2017

Present microbial metabolism on Earth



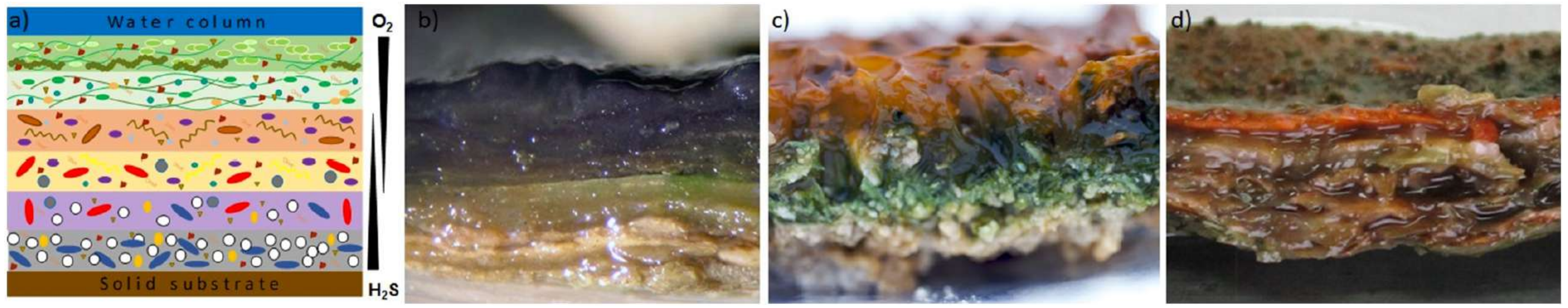
Falkoski et al., 2008

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

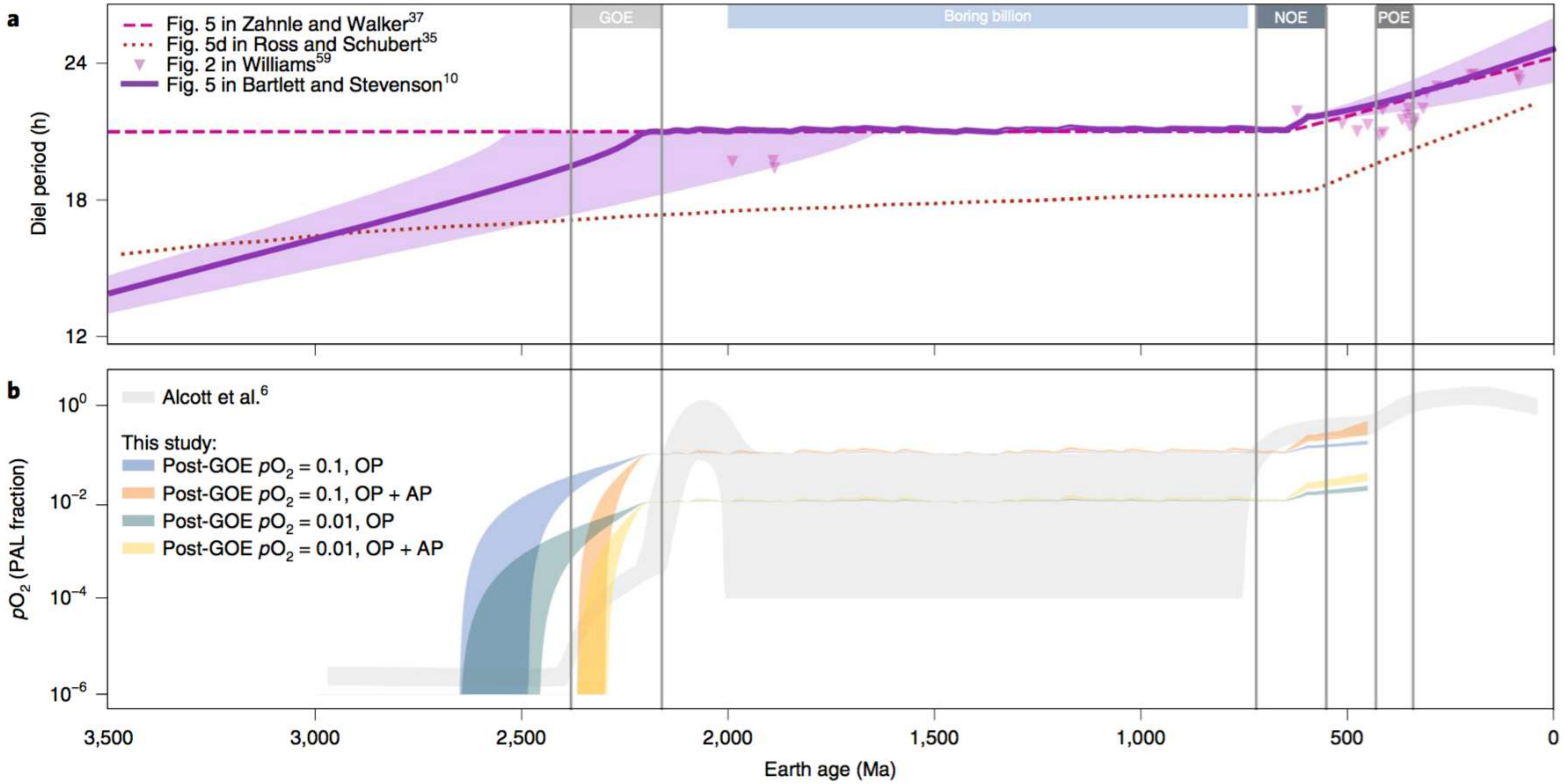
Earth's rotation rate —> day-length—> and oxygenation

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

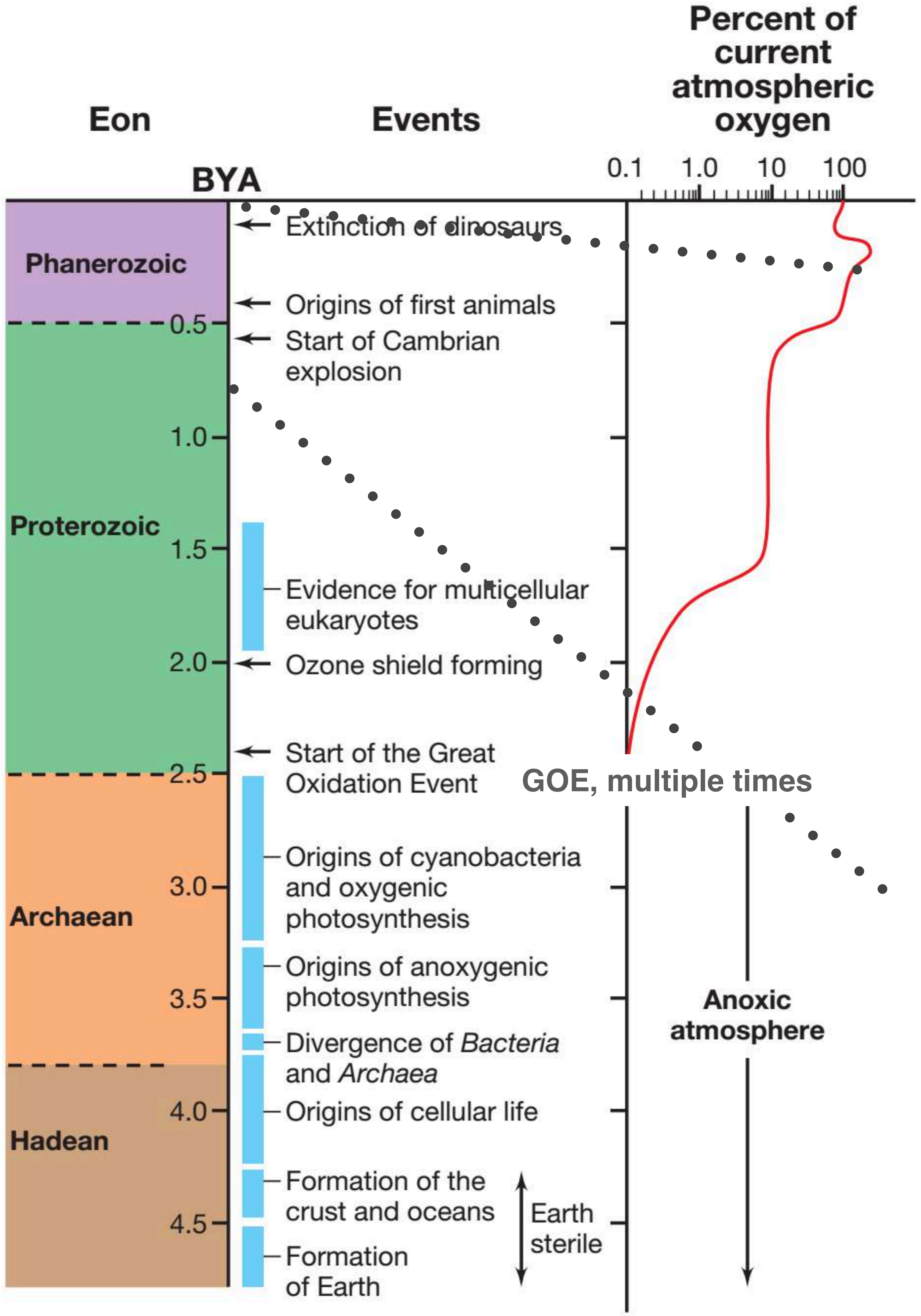


Prieto-Barajas et al. 2018

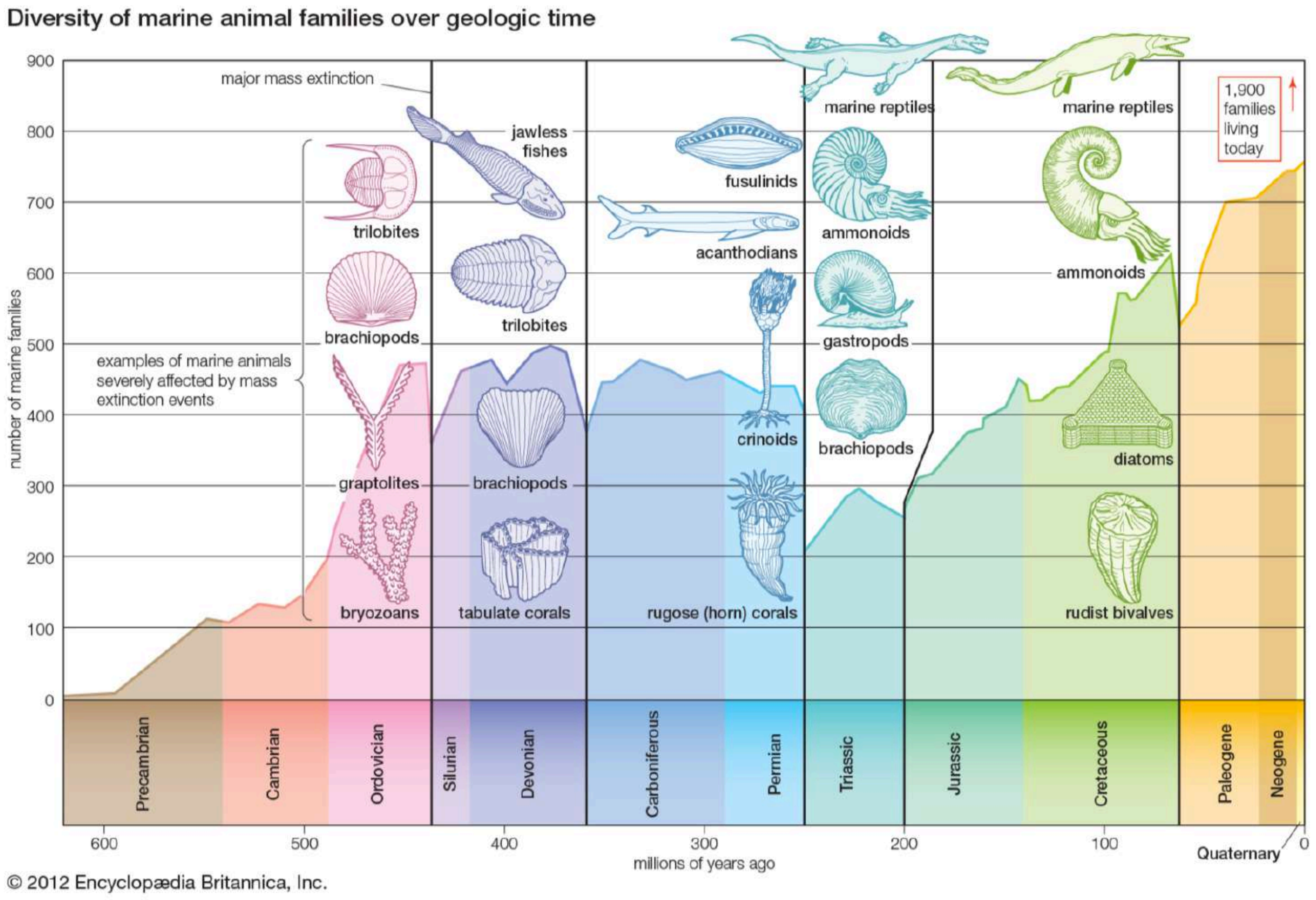


Klatt et al. 2021

Origin of Life: **how**



Madigan et al. 2018



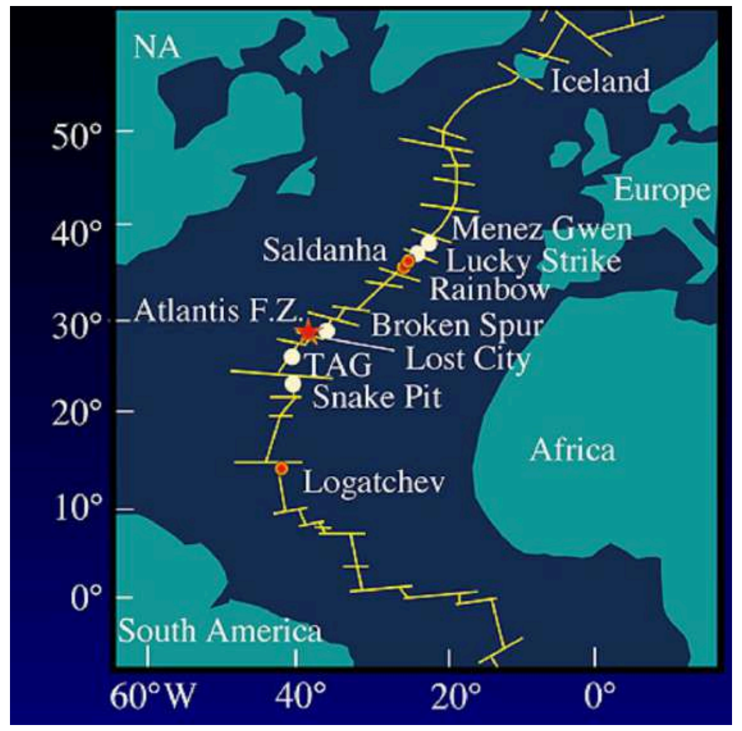
- **GOE: microbial extinction, segregation of anaerobes in microenvironment**
- **O_3 layer protecting UV**
- **Many massive extinctions for megafauna… still today**

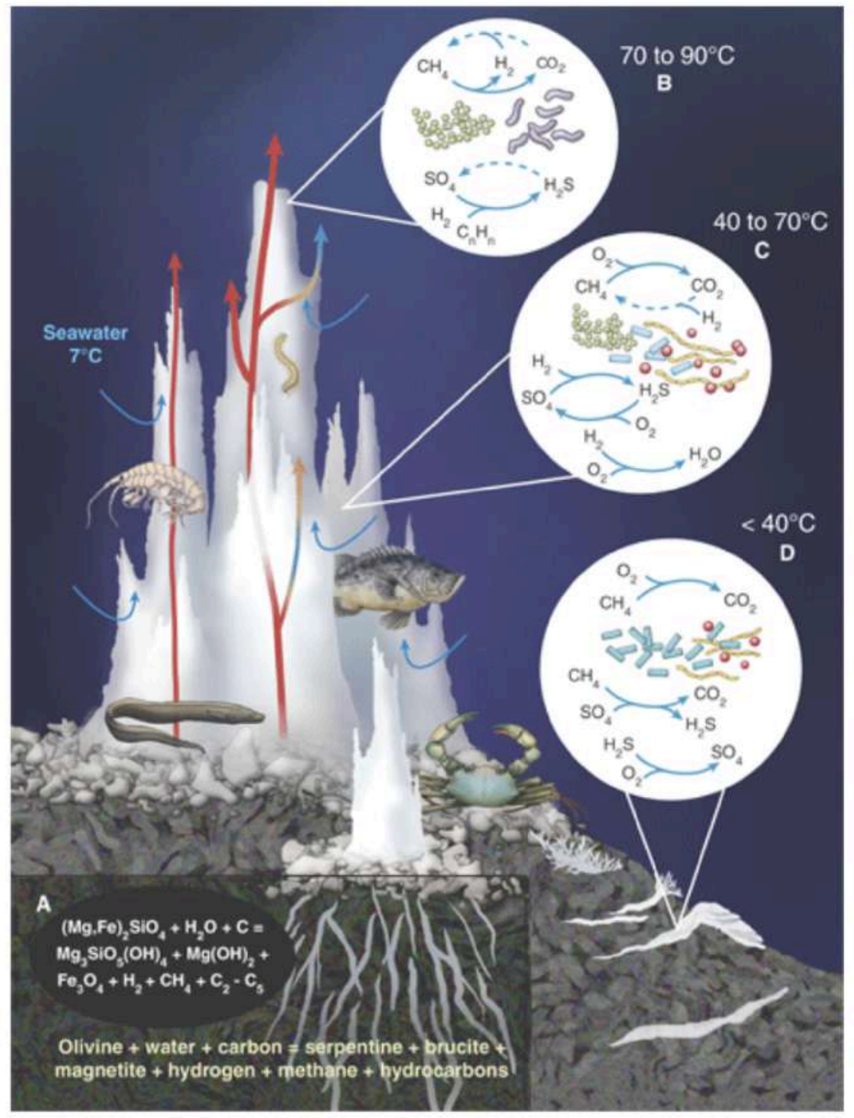
Origin of Life: **where**

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

1. Mineral surfaces on microporous rock (similar at hydrothermal vent, LOST CITY)



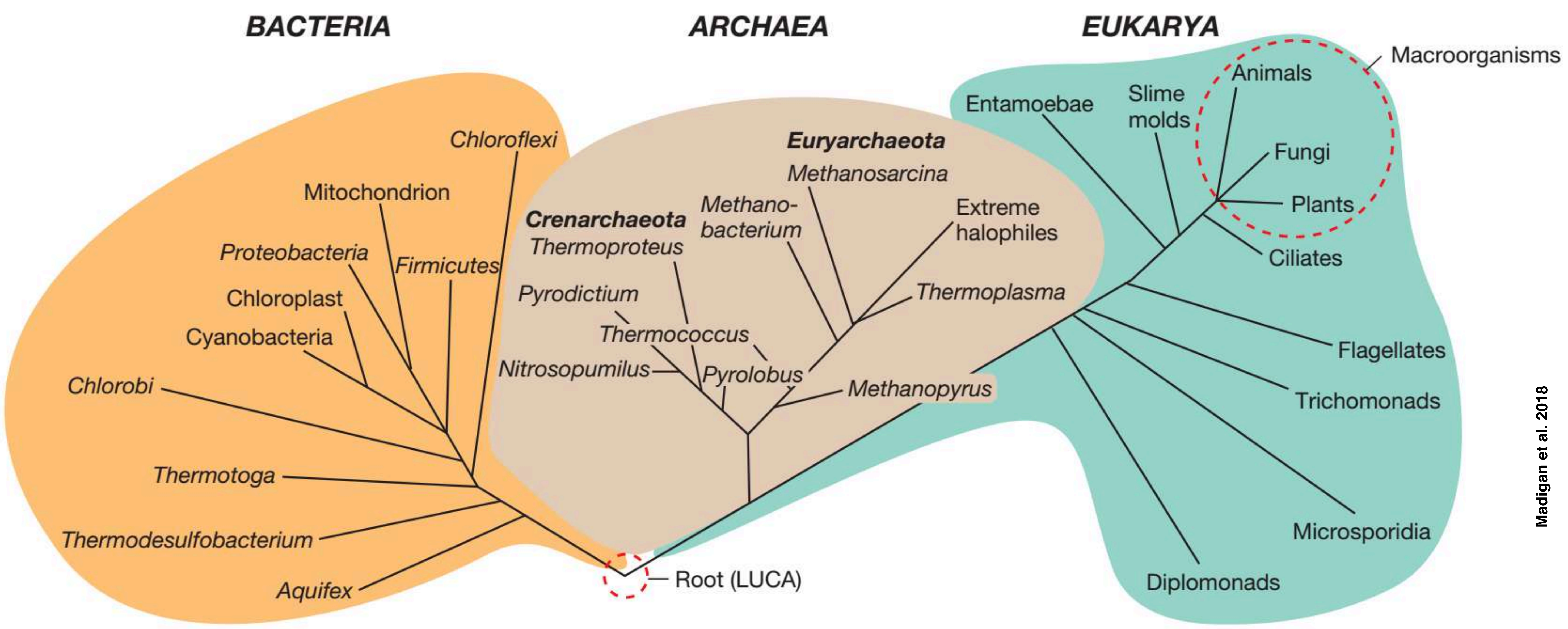


Boetius 2005 and NOAA

2. Shallow terrestrial ponds with geothermal energy

yellowstonepark.org

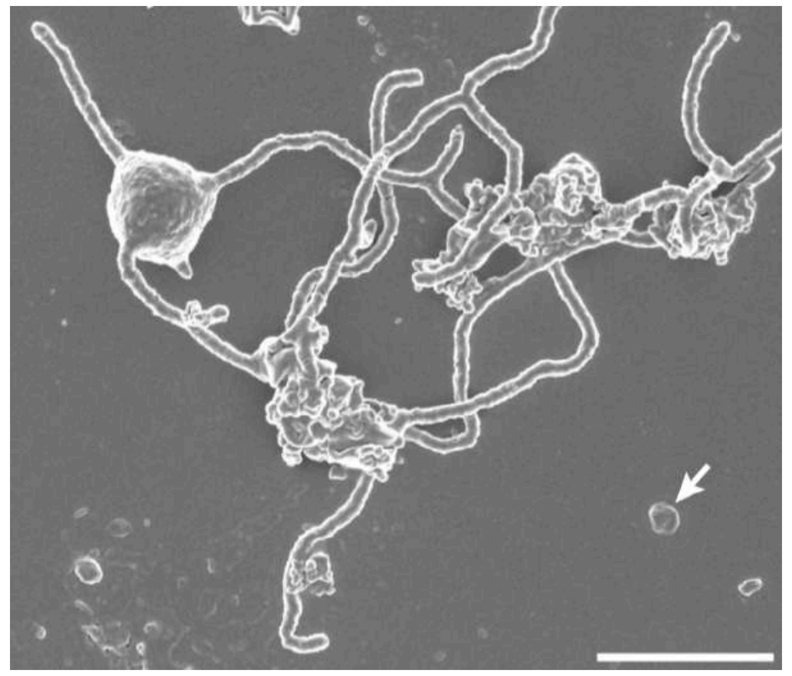
The rise of the Eukarya: eukaryogenesis



Madigan et al. 2018

LUCA: Last Universal Common Ancestor

~1.86 billions



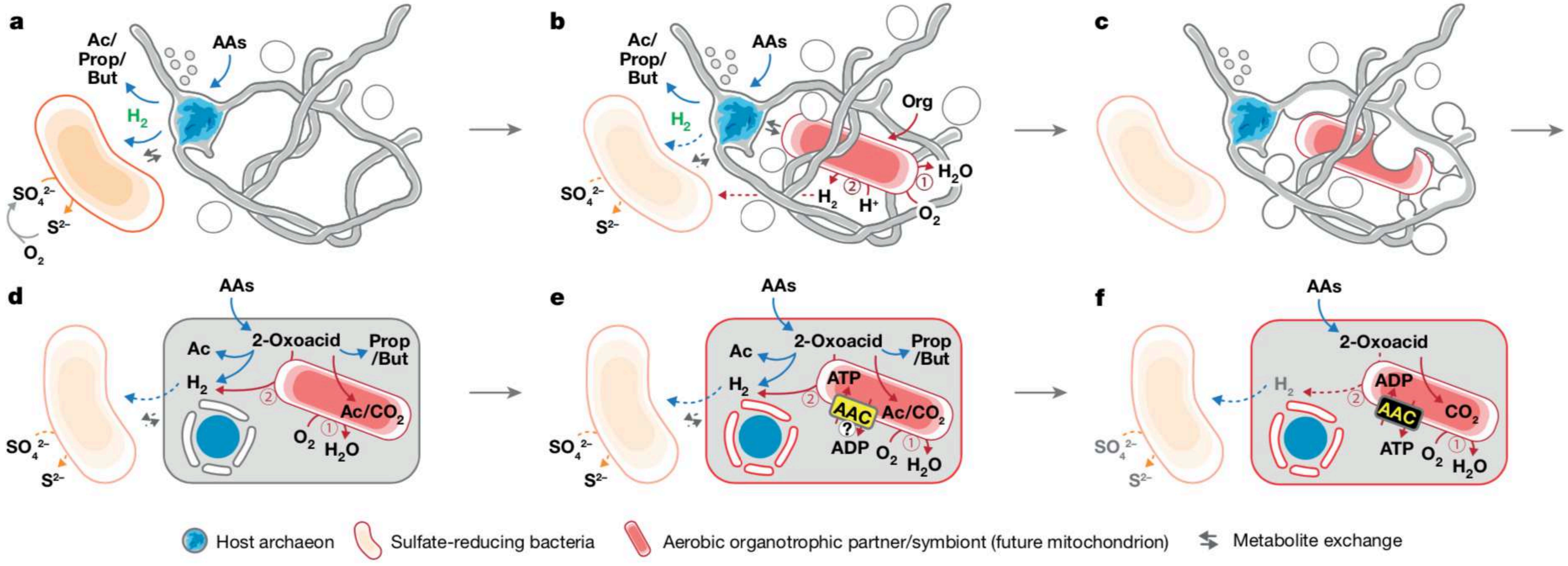
Imachi et al. 2020

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

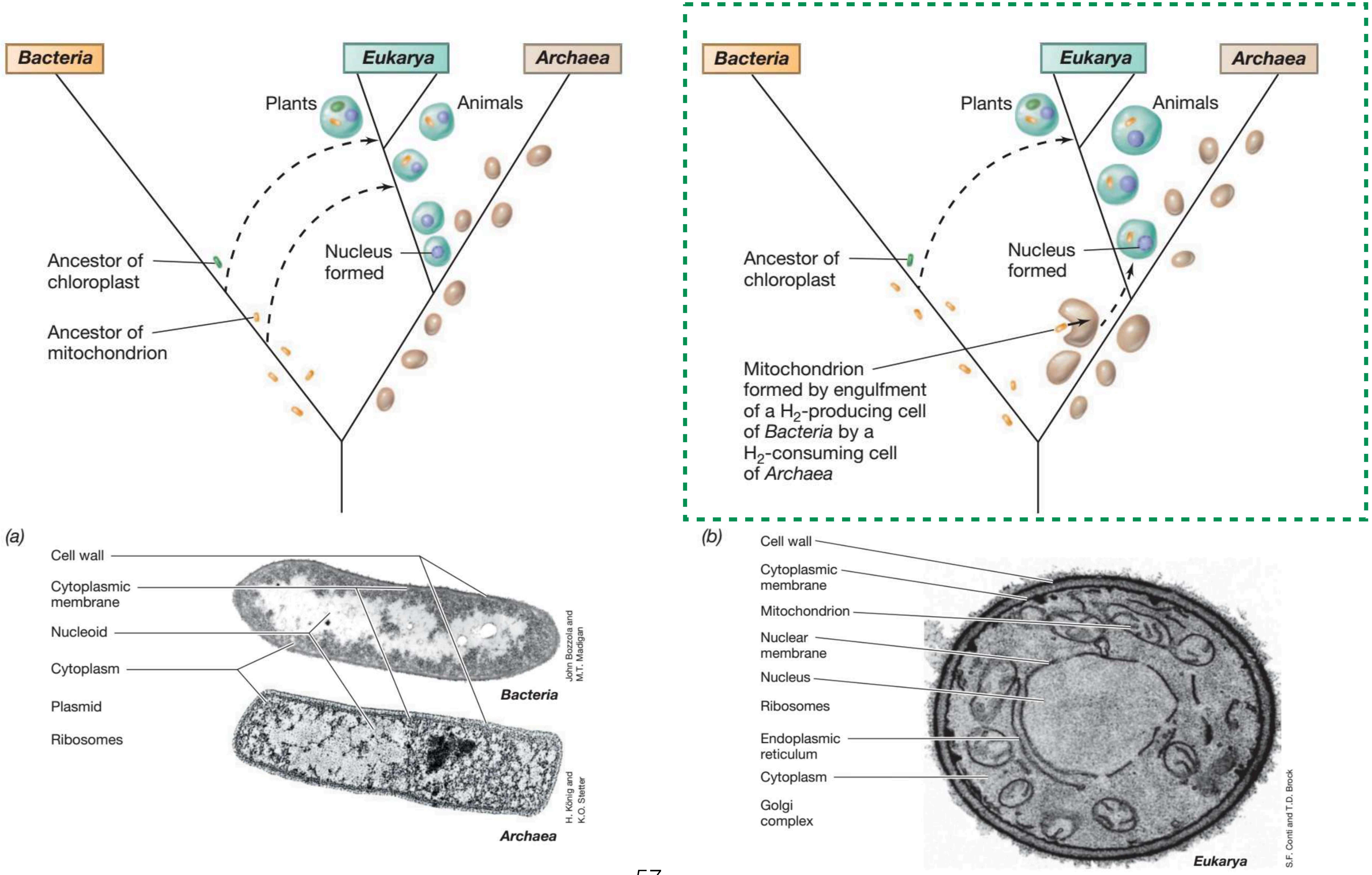
(2) Gain of an O2-respiring and ATP-providing endosymbiont (future mitochondrion, alpha-Protobacterium)

(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

	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