

MICROBIOLOGY OF EXTREME ENVIRONMENTS

MICROBIAL PHYSIOLOGY, EVOLUTION AND ECOLOGY: A BRIEF RECAP

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On today recap

Microbial physiology

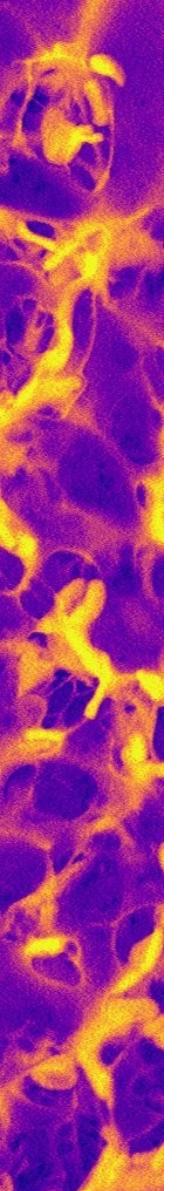
- Prokaryotes
- Viruses
- Size, distribution, growth
- Genes and genomes
- Metabolisms: the basics

Microbial evolution

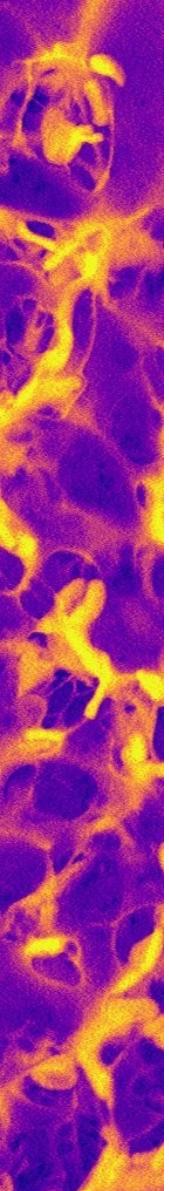
- Species concept
- A microbial world
- The tree of life
- Origin of Life, Endosymbiotic theory and Eukaryogenesis

Microbial Ecology

- Ecosystems
- Community structure
- Community interactions

A vertical strip of a microscopic image showing numerous small, rod-shaped and spherical microorganisms against a dark background.

MICROBIAL PHYSIOLOGY: A VERY BRIEF GUIDE

A vertical strip on the left side of the slide showing a microscopic image of various microorganisms, possibly bacteria or archaea, with different colors like yellow, orange, and purple.

Brief History of Microbiology

- 1665** – Hooke – Discovery of microorganisms
- 1676** – Van Leeuwenhoek – First bacteria observation
- 1768** – Spallanzani – Disproved Spontaneous Generation
- 1866** – Cohn – Beggiatoa sulfur granules
- 1876** – Kock – Koch's postulates
- 1880** – Pasteur – The germ theory and first vaccine
- 1881** – Winogradsky – Chemolithoautotrophy
- 1884** – Gram – Gram Staining
- 1916** – Vernadsky – The Biosphere concept
- 1929** – Fleming – Penicillin
- 1977** – Woese – Discover the Archaea
- 1977** – Ballard – Discovery of Hydrothermal Vent

Prokaryotes

Bacteria: medical and environmental relevance

Not all bacteria are bad!

About $>16,000$ bacterial species are known today ($>150,000$ if we include uncultured).

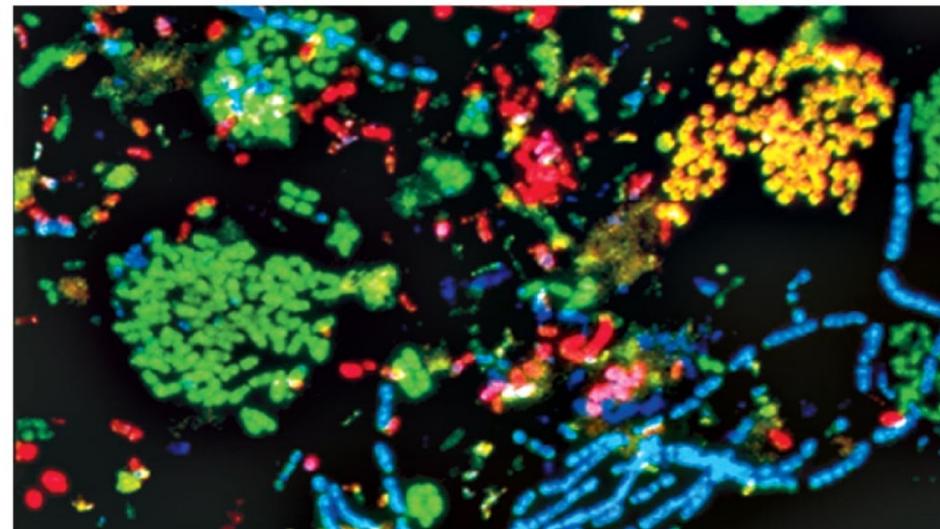
538 are known pathogens or facultative pathogens ($\sim 0.36\%$)

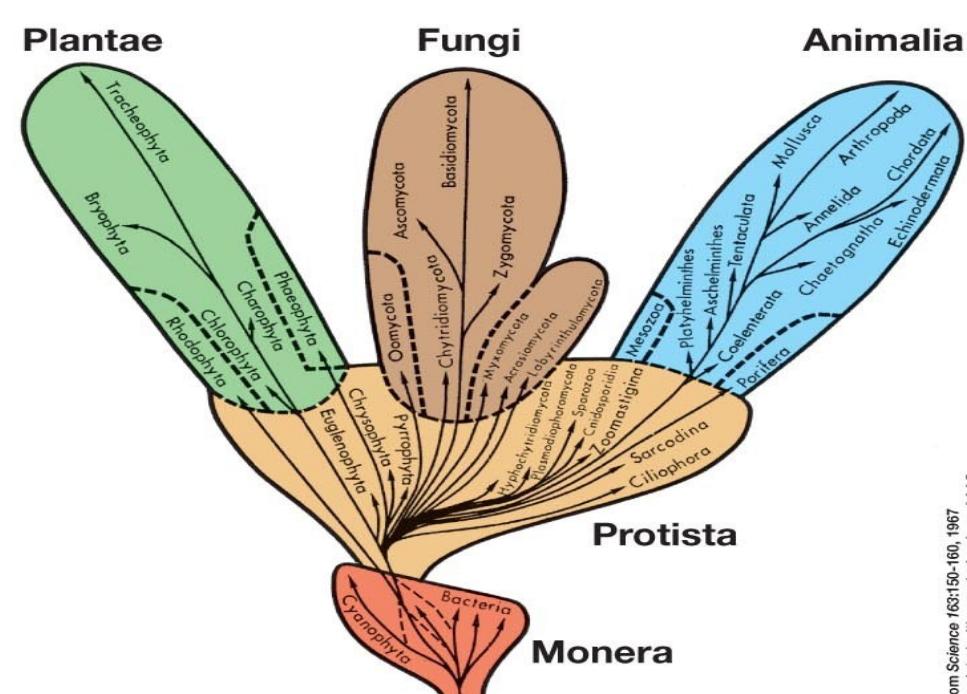
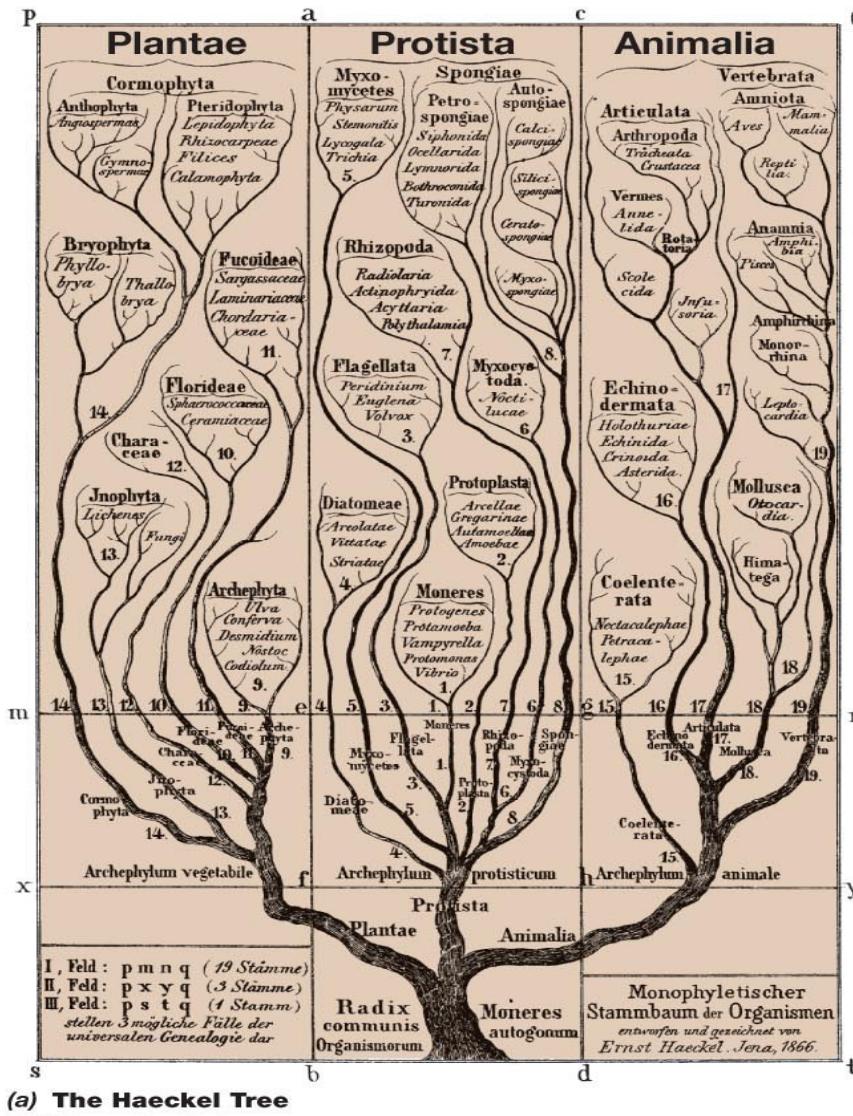
Prokaryotes

Prokaryotes include the Bacteria and the Archaea and consist of small and structurally rather simple cells.

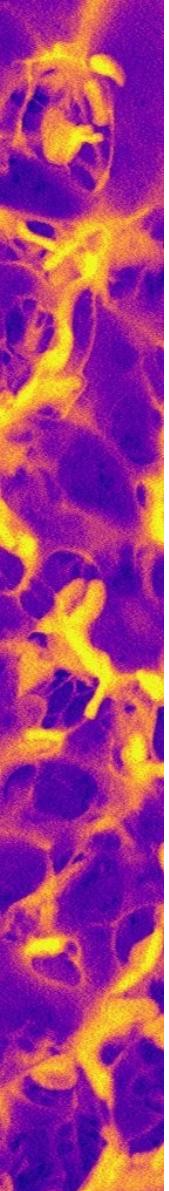
The word “prokaryote” does not imply evolutionary relatedness. Archaea and Bacteria are two distinct domain of life, similarly to Bacteria and Eukarya

(q)





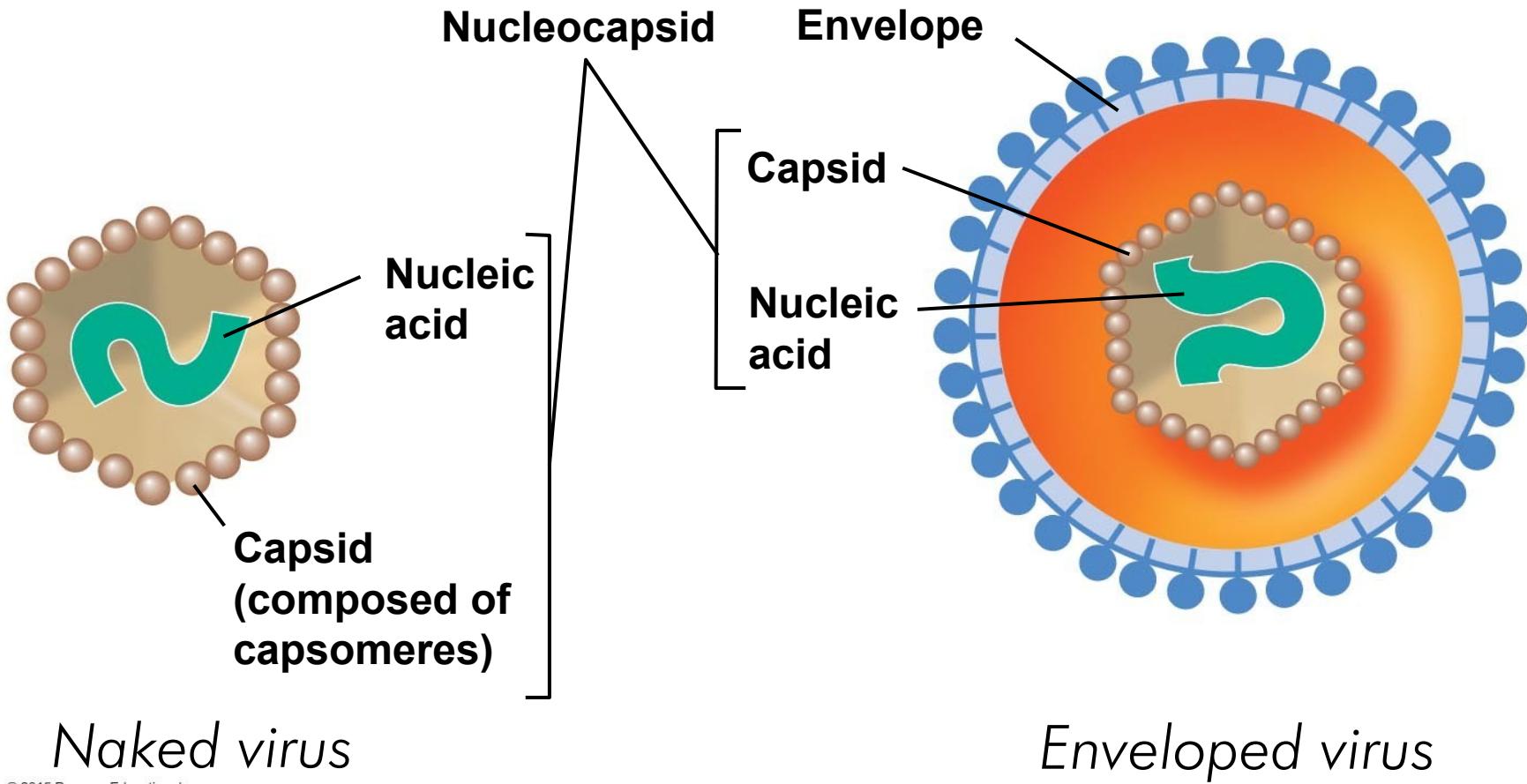
Monera lead the way to Prokaryotes which became Eubacteria and Archeabacteria before becoming Bacteria and Archaea



Viruses

- A **virus** is a genetic element that can replicate only inside a living cell, called the host cell
- The word virus comes from the Latin, poison (syn. Venum)
- Viruses can only replicate themselves by infecting a host cell and therefore cannot reproduce on their own
- At the most basic level, viruses consist of genetic material contained within a **protective protein coat** called a **capsid**. They infect a wide variety of organisms: both eukaryotes and prokaryotes
- A virus that infects bacteria is known as a **bacteriophage**, often shortened to phage
- The study of viruses is known as virology, and those who study viruses are known as virologists
- Simply stated a virus is a “piece of bad news wrapped in a protein case”

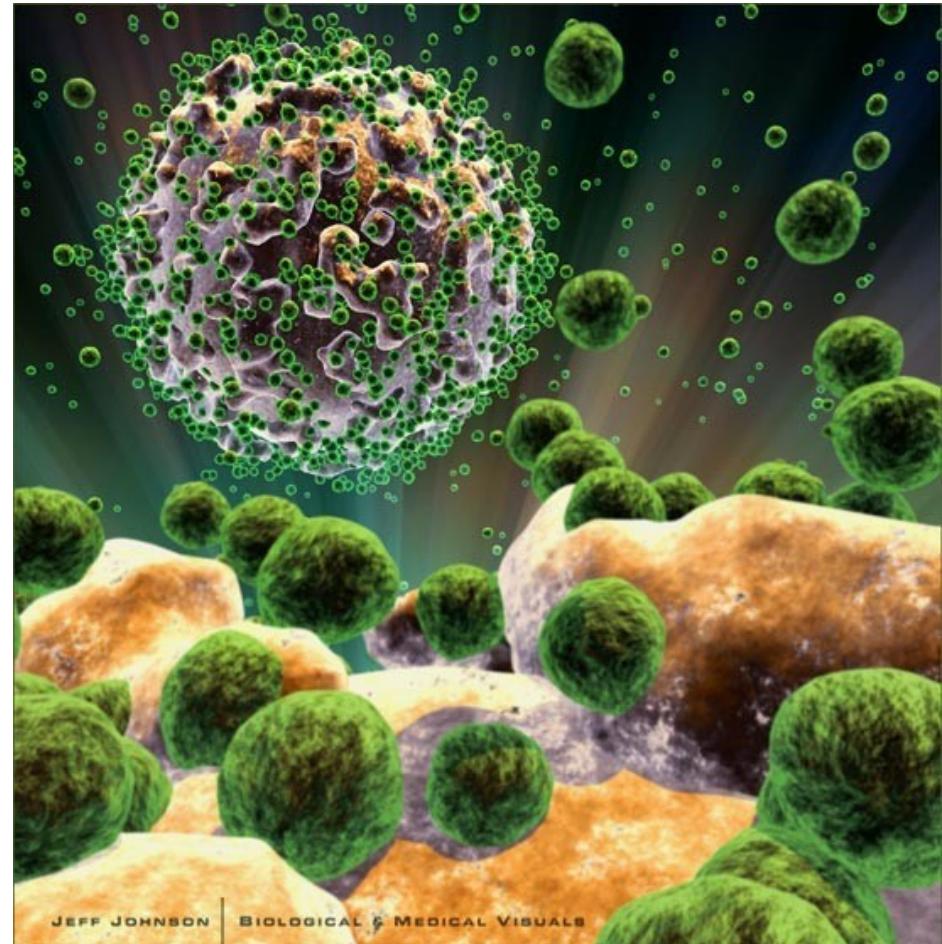
Viruses



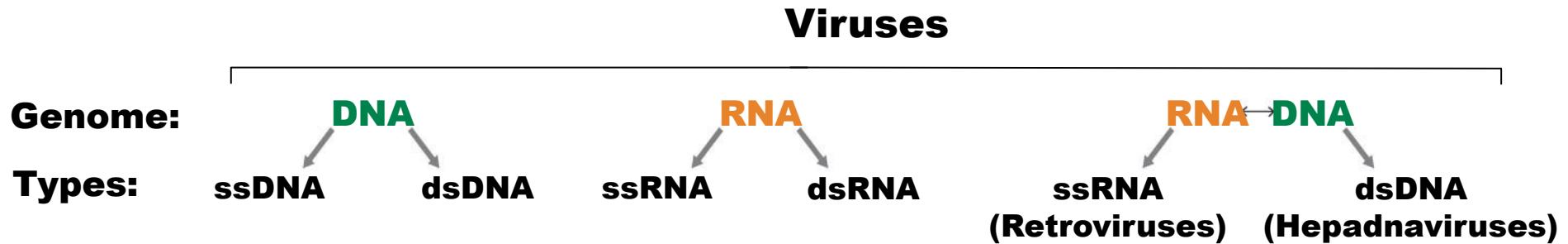
Viruses

There are an estimated 10^{31} viruses on Earth.

The sheer number of viruses and their intimate relationship with microbial life suggest that viruses play a critical role in the planet's biosphere.

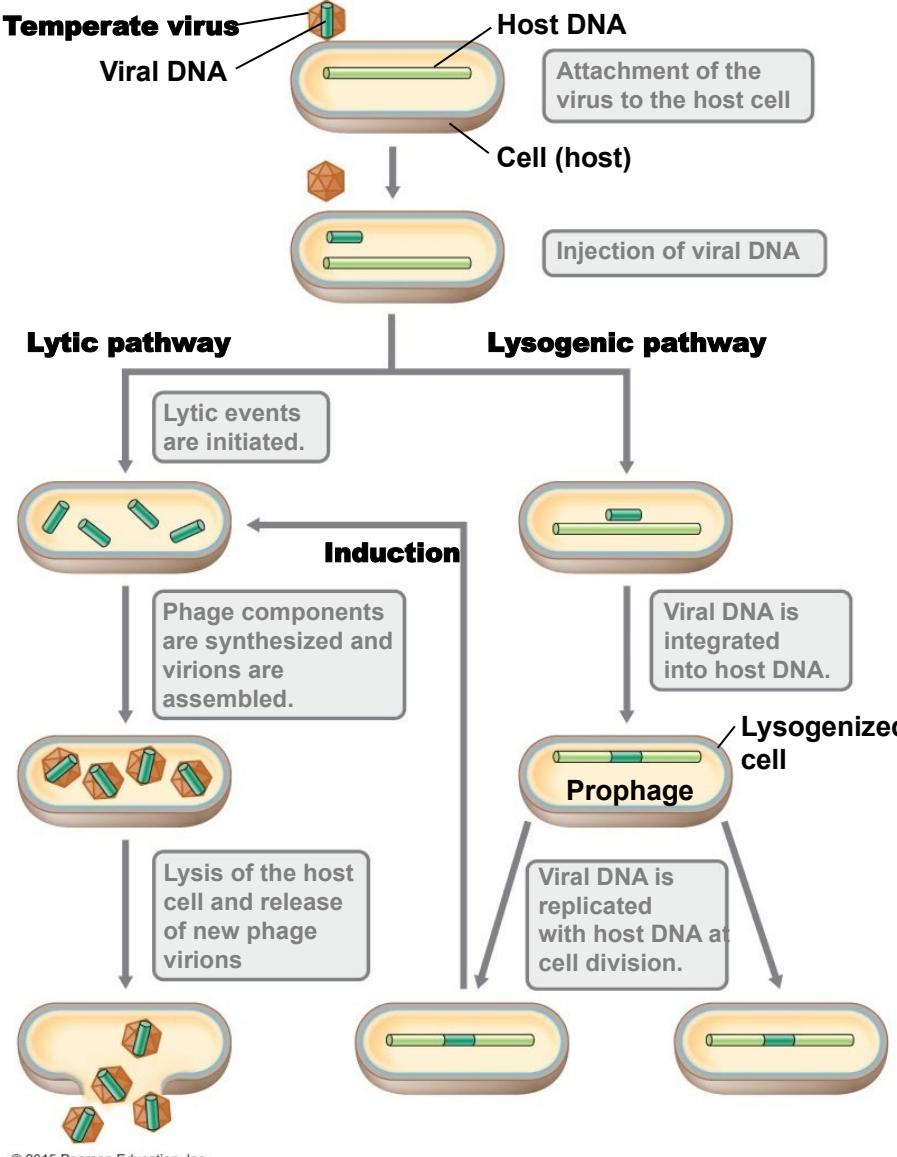


Viruses



All cells contain double-stranded DNA genomes

By contrast, viral genomes consist of either DNA or RNA and are further subdivided based on whether the genome is single-stranded or double-stranded

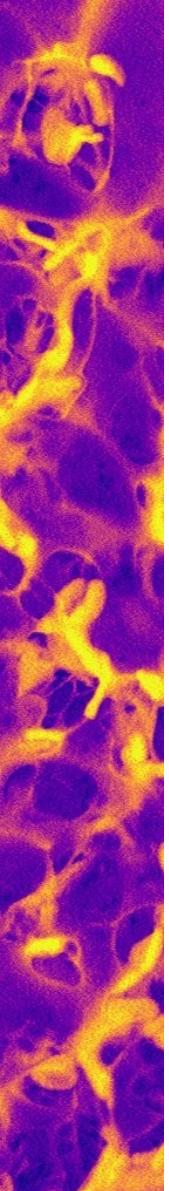


Virus Life Cycle

Lytic pathway. The phage infects and lyse the host cell immediately after reaching burst size

Lysogenic pathway. The phage becomes dormant (i.e. integrated) into the host genome until induced. Integration can become permanent

Chronic pathway. The virus remains inside the host cell releasing viral particle without lysing the cell

A vertical strip on the left side of the slide showing a microscopic view of numerous small, yellowish, rod-shaped viruses against a dark background.

Viral diversity in the environment

- Highest diversity of unknown genes with no matches in databases
- Highest number of unknown viral families
- Lowest number of “cultured” types available, mostly from model organisms
- Huge potential to influence diversity, evolution and biogeochemistry

Size, distribution, growth

Although cell morphology is easily determined, it is a poor predictor of other properties of a cell. For example, under the microscope many rod-shaped Archaea look identical to rod-shaped Bacteria, yet we know they are of different phylogenetic domains.

Prokaryotes vary in size from cells smaller of about $0.2\text{ }\mu\text{m}$ in diameter to those more than $700\text{ }\mu\text{m}$. The vast majority of rod-shaped prokaryotes that have been cultured are between 0.5 and $4\text{ }\mu\text{m}$ wide and less than $15\text{ }\mu\text{m}$ long.

Size, distribution, growth

Table 2.1 Cell size and volume of some cells of *Bacteria*, from the largest to the smallest

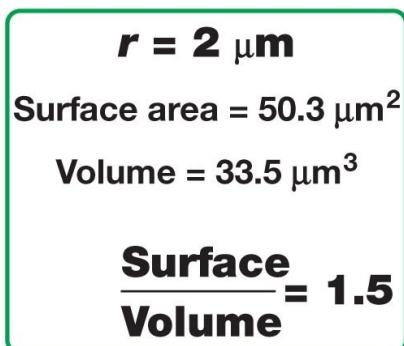
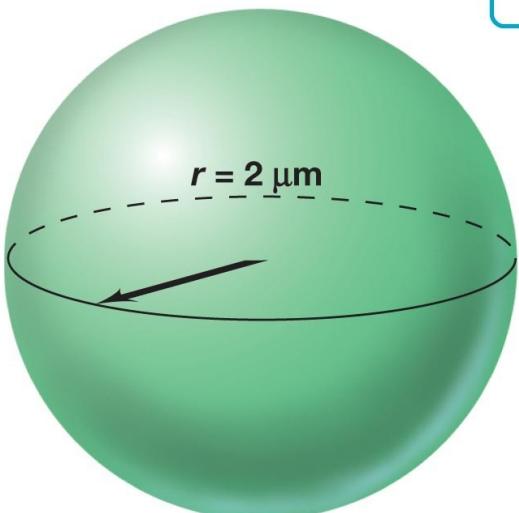
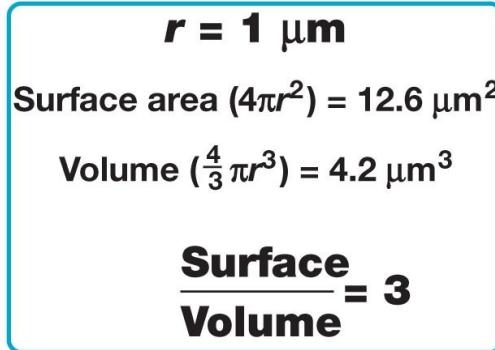
Organism	Characteristics	Morphology	Size ^a (μm)	Cell volume (μm^3)	E. coli volumes
<i>Thiomargarita namibiensis</i>	Sulfur chemolithotroph	Cocci in chains	750	200,000,000	100,000,000
<i>Epulopiscium fishelsoni</i> ^a	Chemoorganotroph	Rods with tapered ends	80 × 600	3,000,000	1,500,000
<i>Beggiatoa</i> species ^a	Sulfur chemolithotroph	Filaments	50 × 160	1,000,000	500,000
<i>Achromatium oxaliferum</i>	Sulfur chemolithotroph	Cocci	35 × 95	80,000	40,000
<i>Lyngbya majuscula</i>	Cyanobacterium	Filaments	8 × 80	40,000	20,000
<i>Thiovulum majus</i>	Sulfur chemolithotroph	Cocci	18	3,000	1,500
<i>Staphylothermus marinus</i> ^a	Hyperthermophile	Cocci in irregular clusters	15	1,800	900
<i>Magnetobacterium bavaricum</i>	Magnetotactic bacterium	Rods	2 × 10	30	15
<i>Escherichia coli</i>	Chemoorganotroph	Rods	1 × 2	2	1
<i>Pelagibacter ubique</i> ^a	Marine chemoorganotroph	Rods	0.2 × 0.5	0.014	0.007
<i>Mycoplasma pneumoniae</i>	Pathogenic bacterium	Pleomorphic ^b	0.2	0.005	0.0025

^aWhere only one number is given, this is the diameter of spherical cells. The values given are for the largest cell size observed in each species. For example, for *T. namibiensis*, an average cell is only about 200 μm in diameter. But on occasion, giant cells of 750 μm are observed. Likewise, an average cell of *S. marinus* is about 1 μm in diameter. The species of *Beggiatoa* here is unclear and *E. fishelsoni*, *Magnetobacterium bavaricum*, and *P. ubique* are not formally recognized names in taxonomy.

^b*Mycoplasma* is a bacterium that lacks a cell wall and can thus take on many shapes (*pleomorphic* means "many shapes").

Source: Data obtained from Schulz, H.N., and B.B. Jørgensen. 2001. *Ann. Rev. Microbiol.* 55: 105–137.

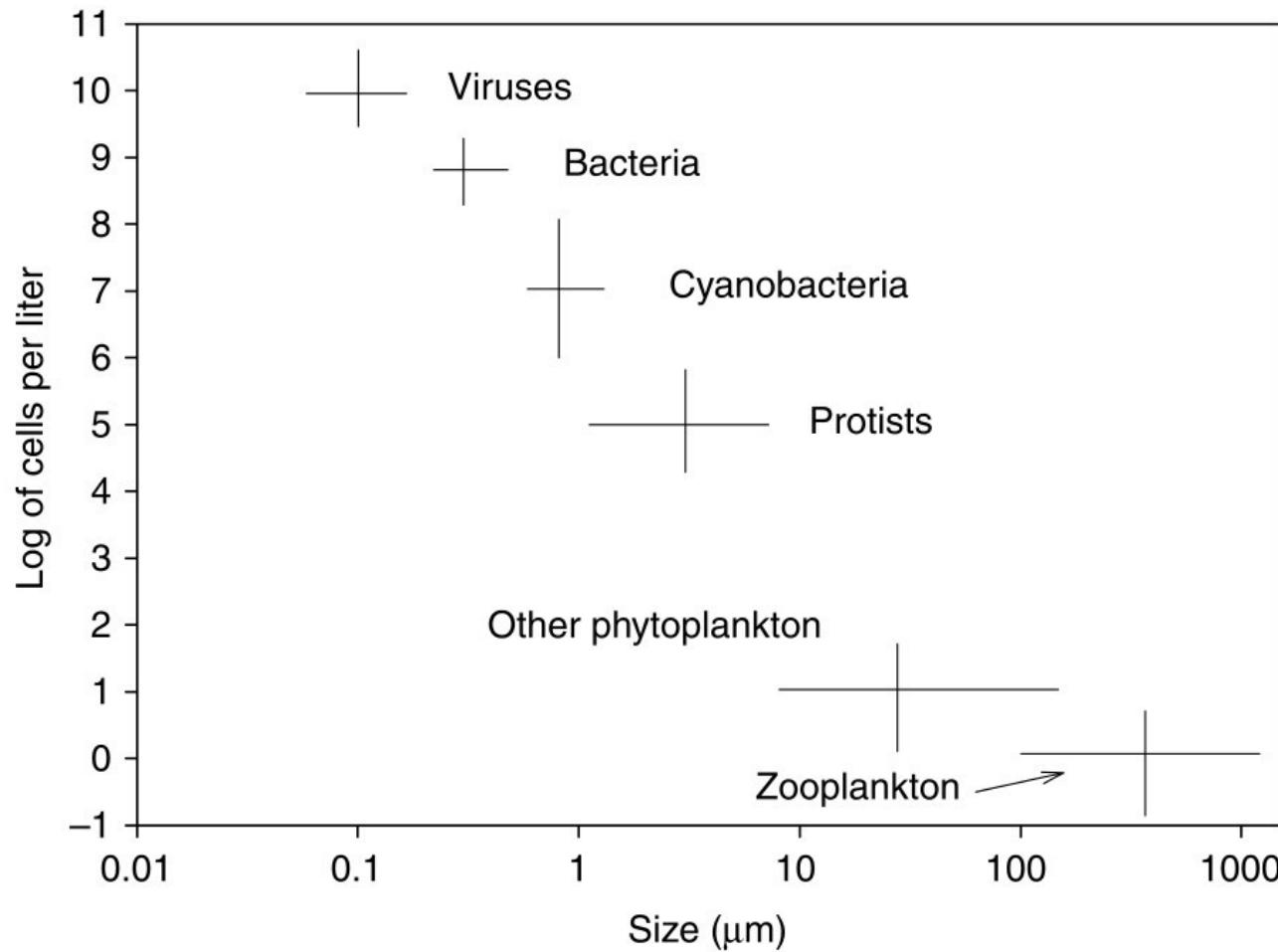
When Size Matters!



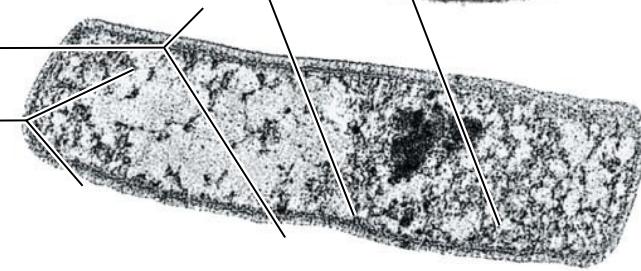
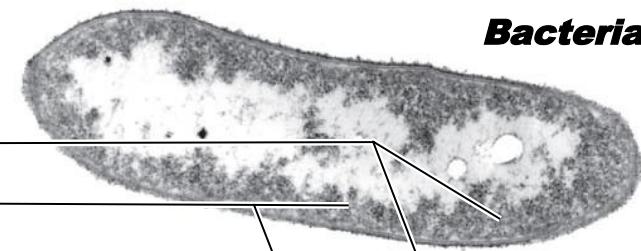
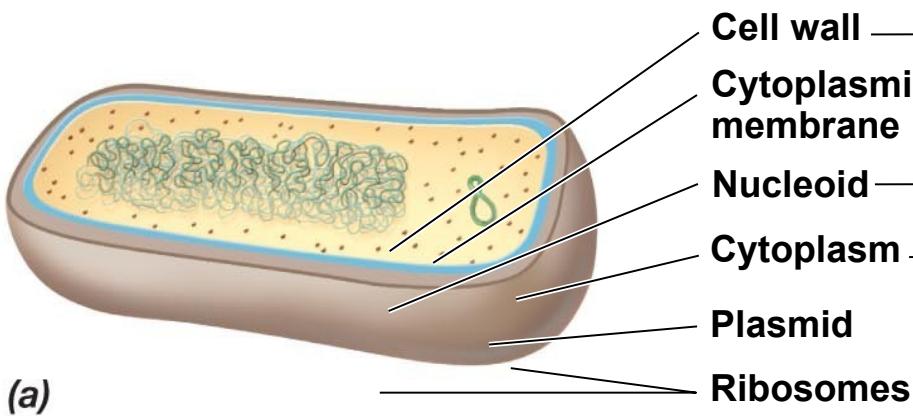
The lower limit is controlled by volume needed to house the essential components of a free-living cell, calculated around $0.1 \mu\text{m}$ in diameter.

The upper limit is controlled by diffusion limitation of nutrients and waste from and to the surrounding environment.

Size, distribution, growth

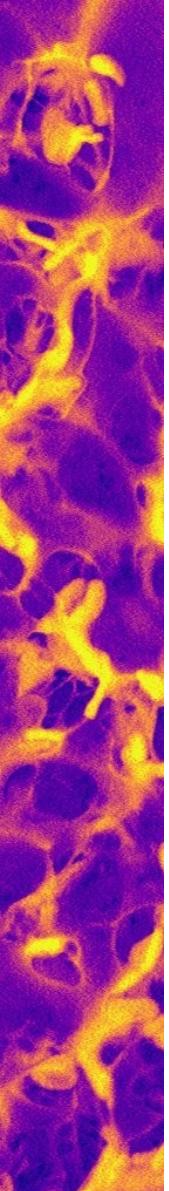


Size, distribution, growth



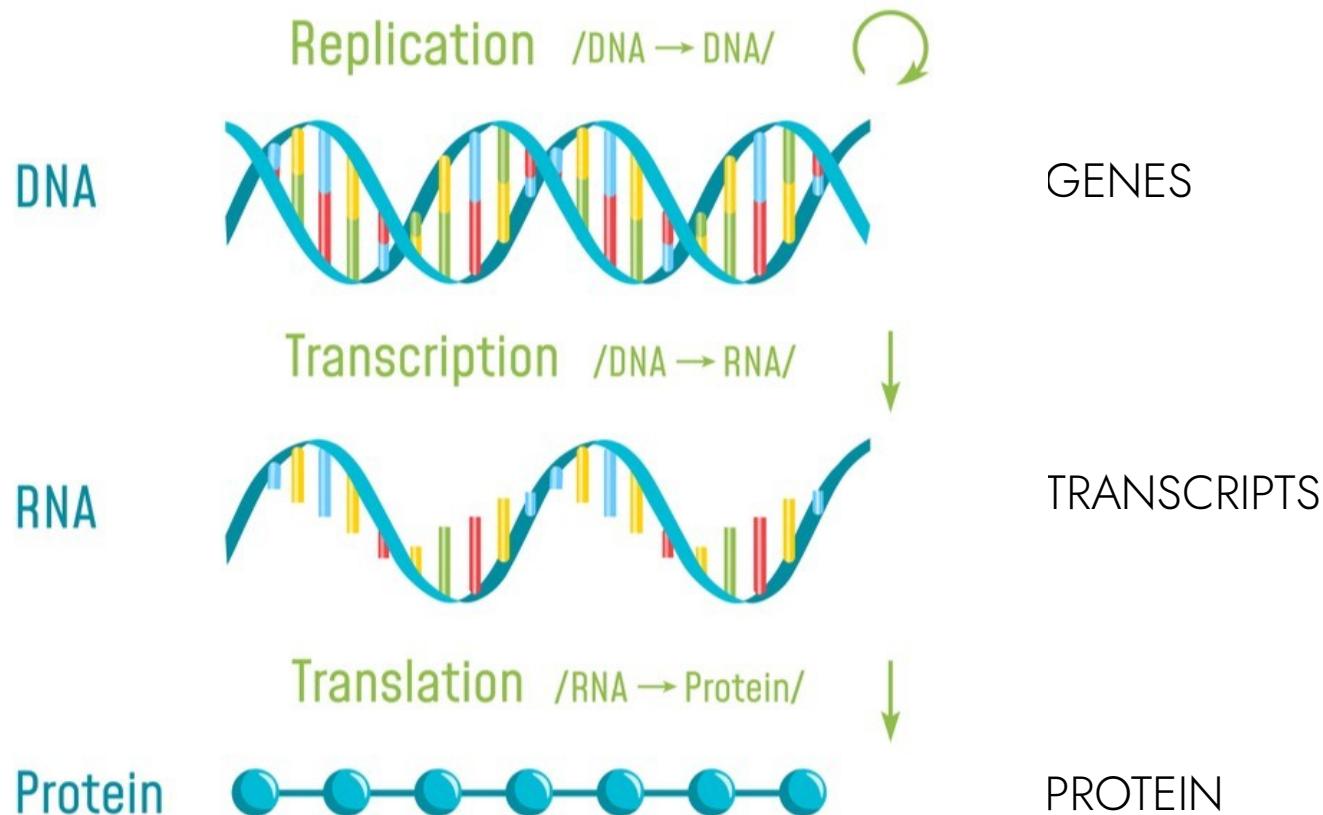
John Bozzola and
M.T. Madigan

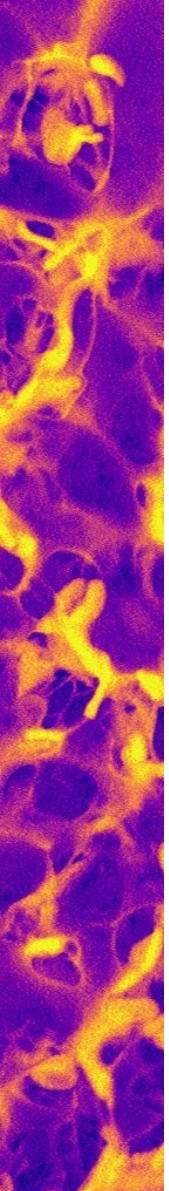
H. König and
K.O. Stetter



Gene and Genomes

Genes and genomes

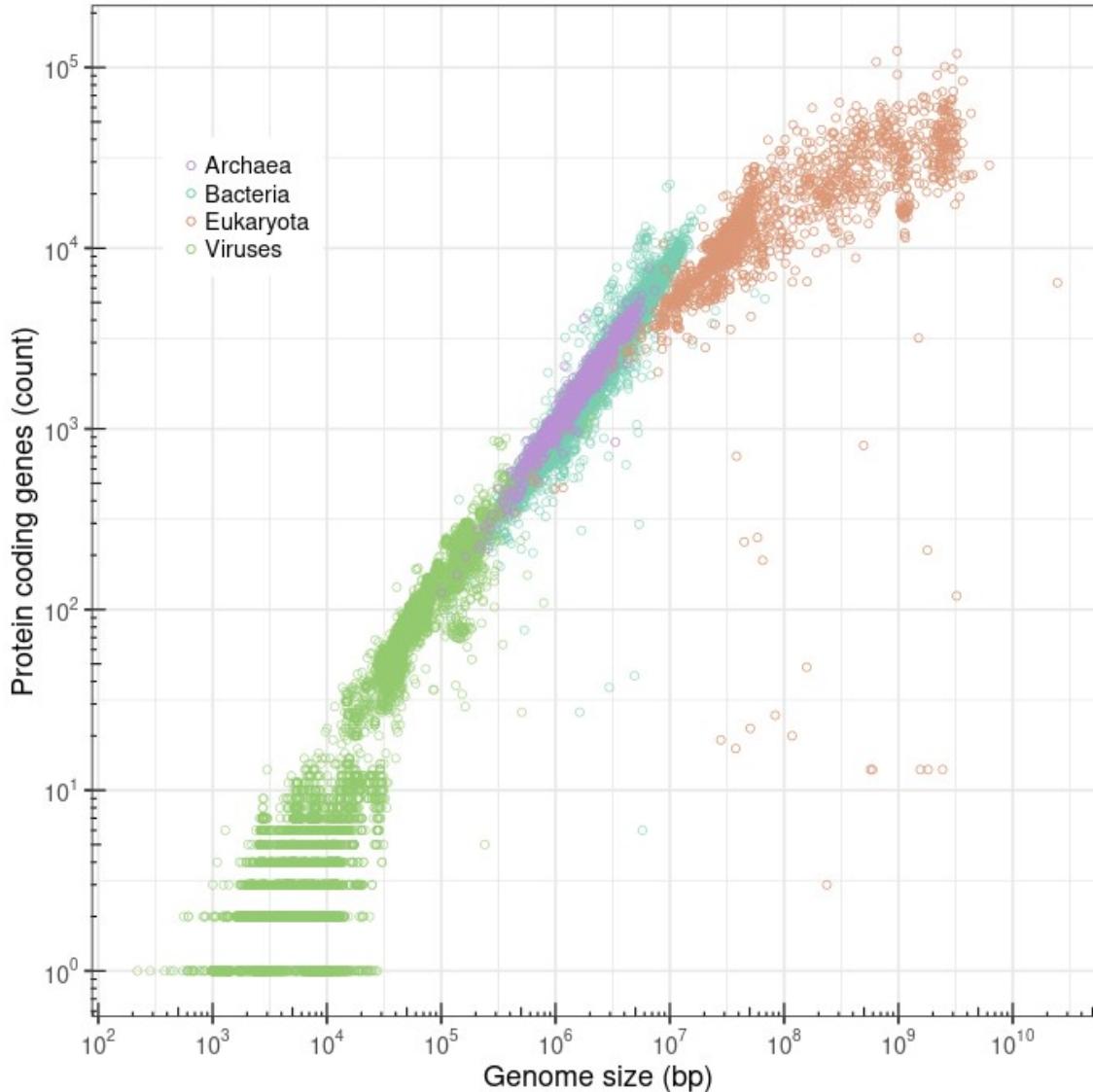


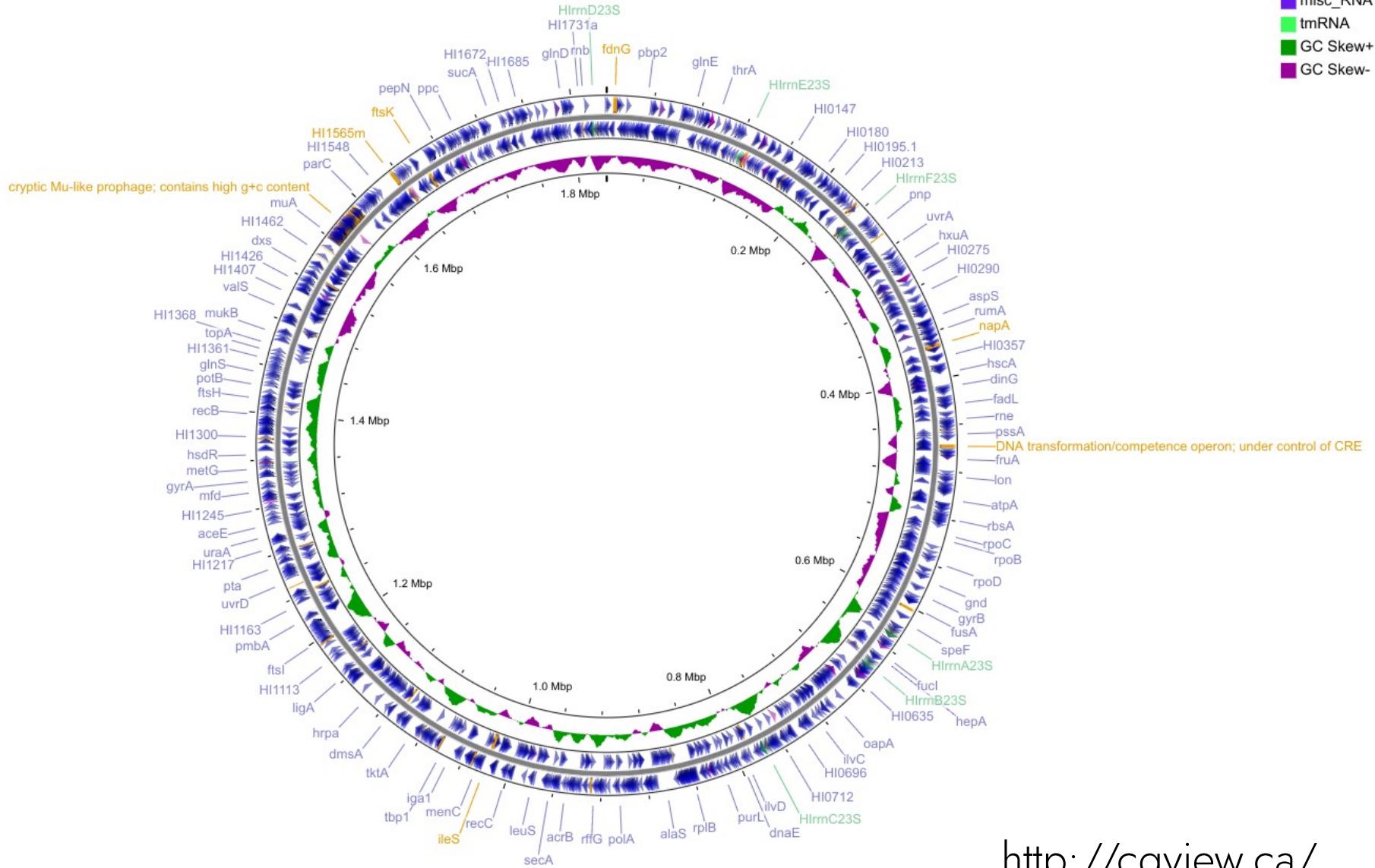
A vertical strip on the left side of the slide showing a microscopic view of various microorganisms, possibly bacteria or yeasts, with different colors like yellow, orange, and purple.

Genes and genomes

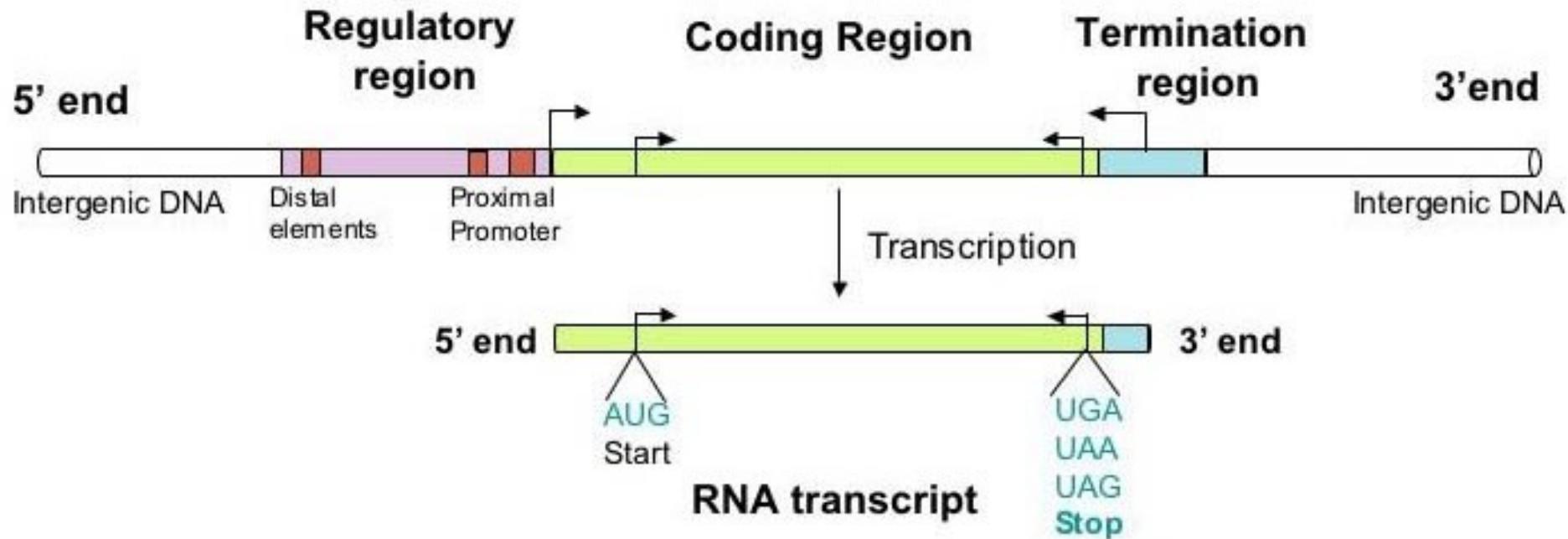
- Prokaryotic **genomes range in size** from those of large viruses to those of eukaryotic microbes
- Prokaryotic genomes are **generally circular**. One or more chromosome may be present
- One or more **plasmid** can be present (**circular or linear**), coding (often) for non essential genes conferring additional characteristics
- The **gene density for 1 kbp is around 1**, and number of genome repeat is small. By comparison simple eukaryotes (*C. elegans*) has a gene density of 0.2, while humans have a gene density of 0.015
- Unlike prokaryotes, eukaryotic genomes contain a large fraction of noncoding DNA

Genome size vs. protein count across NCBI genomes

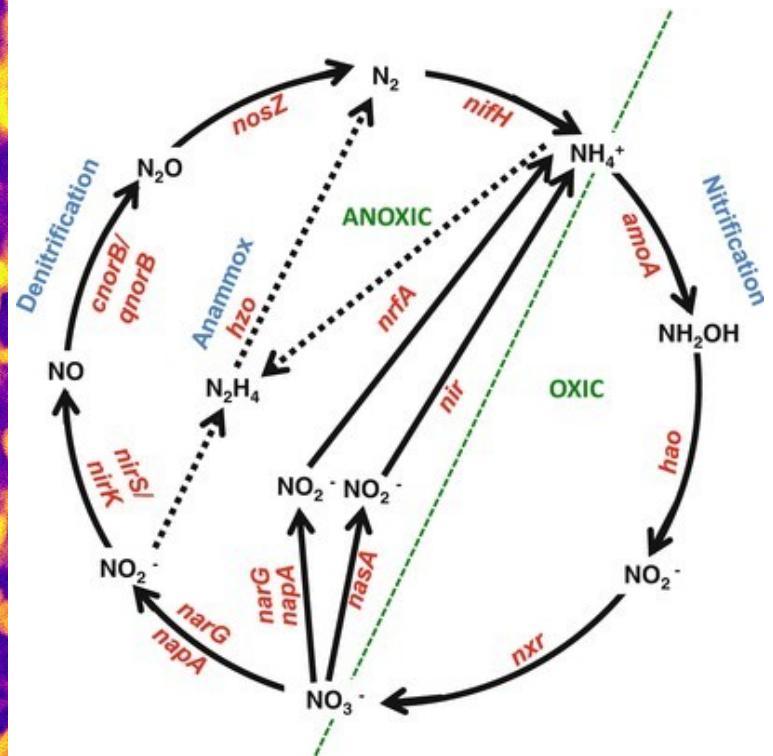




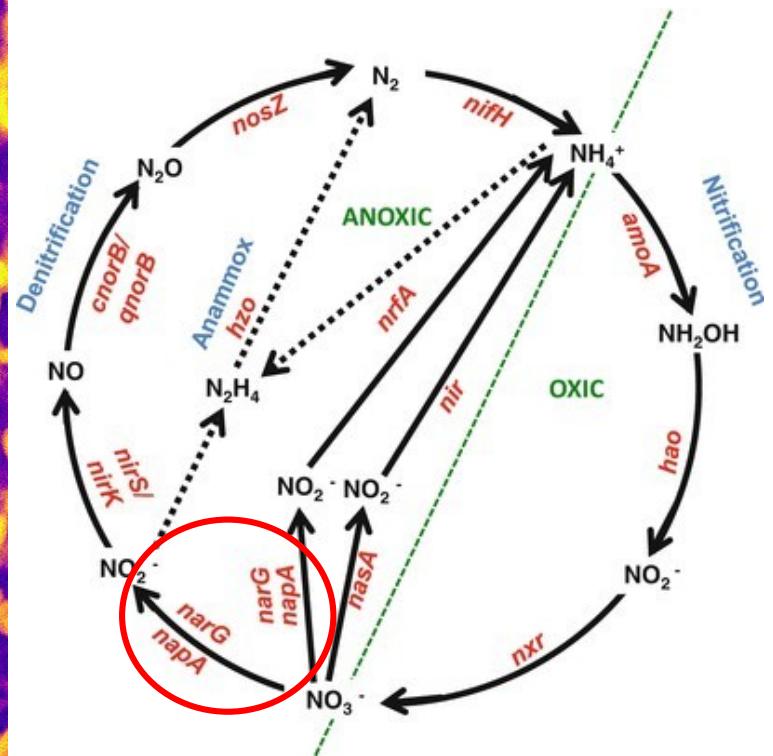
Gene organization in Prokaryotes



Visualizing genes organization



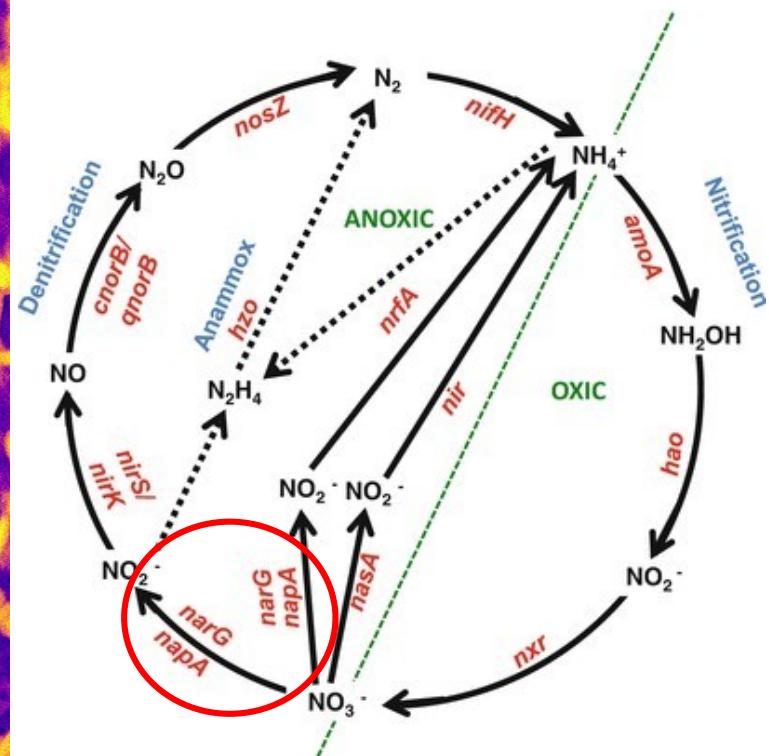
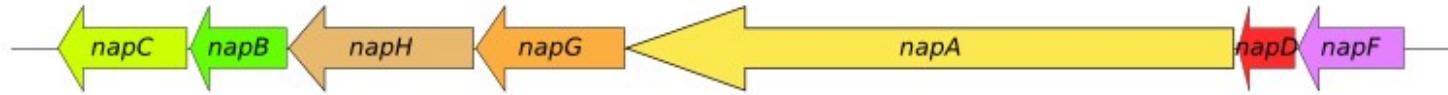
Visualizing genes organization



Visualizing genes organization

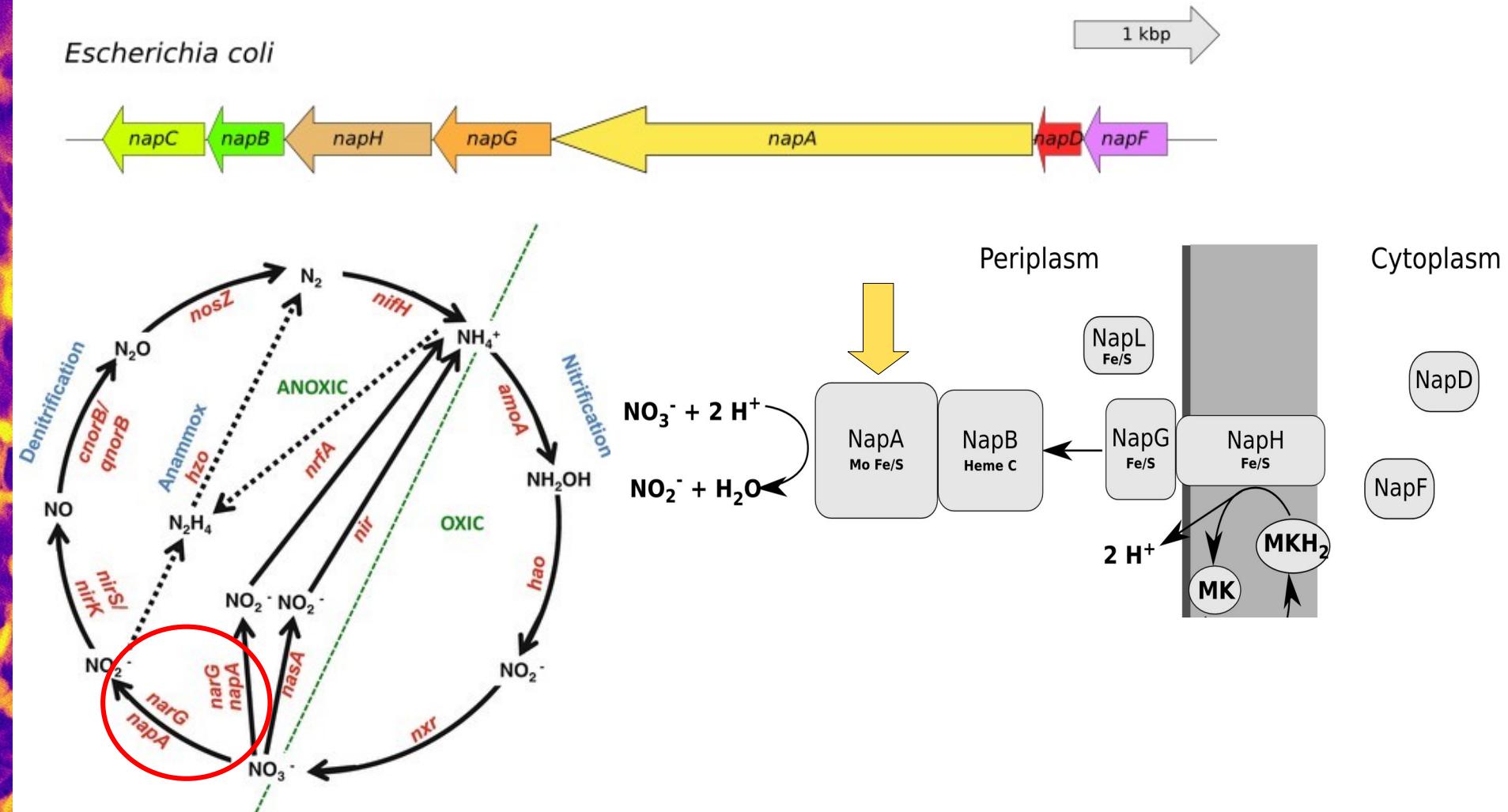
Escherichia coli

1 kbp



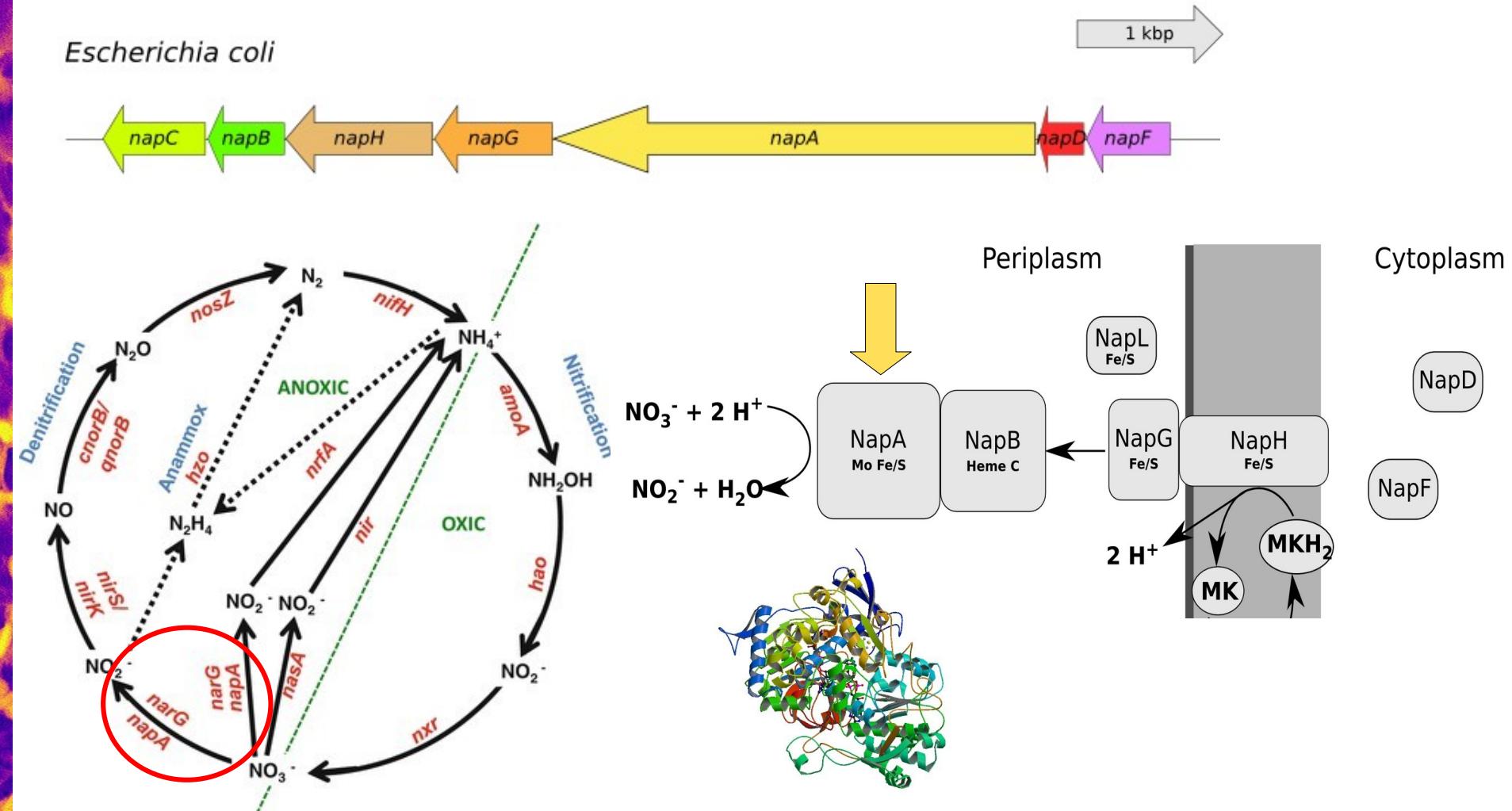
Visualizing genes organization

Escherichia coli



Visualizing genes organization

Escherichia coli



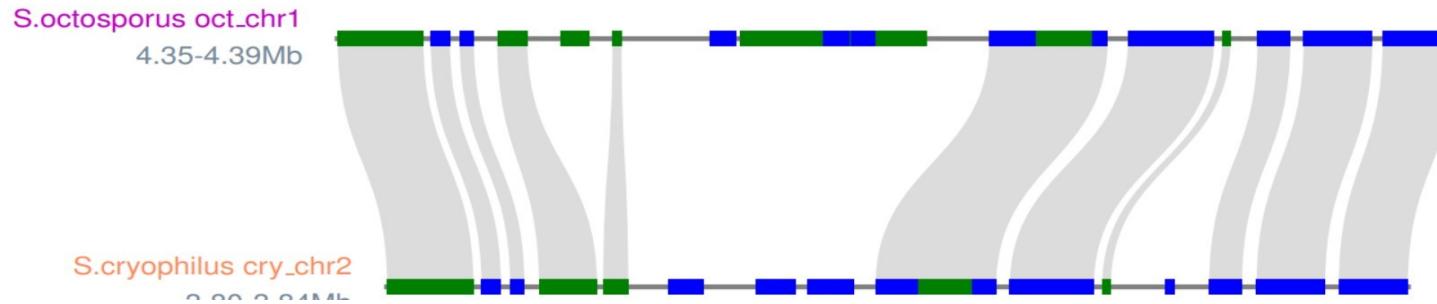
Sequence Homology

Sequence similarity: Defines the degree of similarity between two sequences. Similarity is calculated by different algorithms assigning a different weight to gaps

Sequence homology: assumes that the similarity observed is derived from common ancestry

Conserved regions: portion of a gene that do not change significantly when comparing homologs

Synteny: area of a gene/genome that is conserved in organization across different organisms



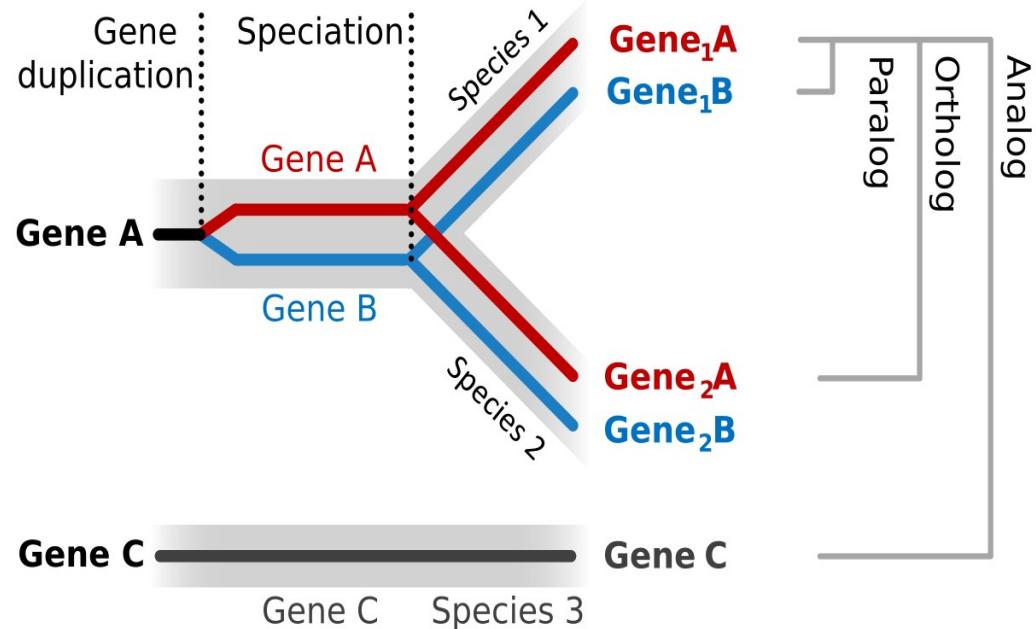
Gene Families, Duplications, and Deletions

Homologs: related sequence that have common genetic ancestry

Paralogs: genes within an organism whose similarity to one or more genes in the same organism is the result of gene duplication

Gene families: groups of homologous genes

Orthologs: genes found in one organism that are similar to those in another organism but differ because of speciation



position 12

↓

helix H0

sheet

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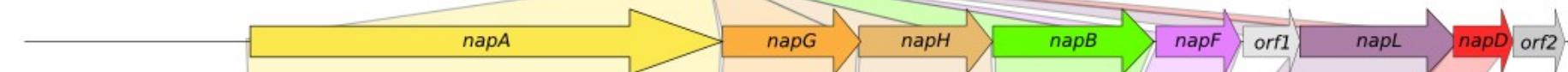
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R	Y	D	S	R	T	T	I	F	S	P	L	R	E	G	R	L	Y	Q	V	E	Y	A	M	E	A	I	S	H	A	.	G	T	C	L	G	I	L	S
R	Y	D	S	R	T	T	I	F	S	P	.	.	E	G	R	L	Y	Q	V	E	Y	A	Q	E	A	I	S	N	A	.	G	T	A	I	G	I	L	S
R	Y	D	S	R	T	T	I	F	S	P	.	.	E	G	R	L	Y	Q	V	E	Y	A	M	E	A	I	S	H	A	.	G	T	C	L	G	I	L	A
R	Y	D	S	R	T	T	I	F	S	P	.	.	E	G	R	L	Y	Q	V	E	Y	A	M	E	A	I	G	H	A	.	G	T	C	L	G	I	L	A
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S	Y	D	S	R	T	T	I	F	S	P	.	.	E	G	R	L	Y	Q	V	E	Y	A	L	E	A	I	N	H	A	.	G	V	A	L	G	I	V	A

C. mediatlanticus

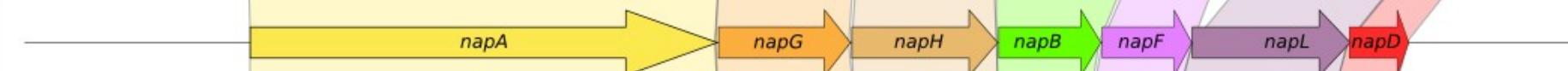
1 kbp



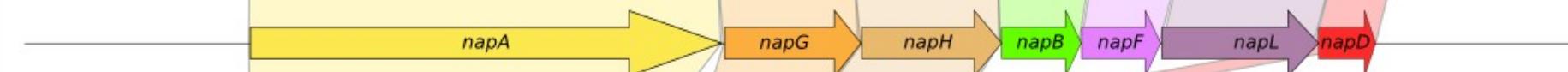
Sulfurovum sp.



Sulfurohydrogenibium sp.



Hydrogenivirga sp.



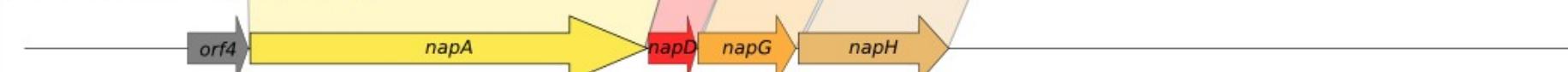
Desulfovibrio desulfuricans



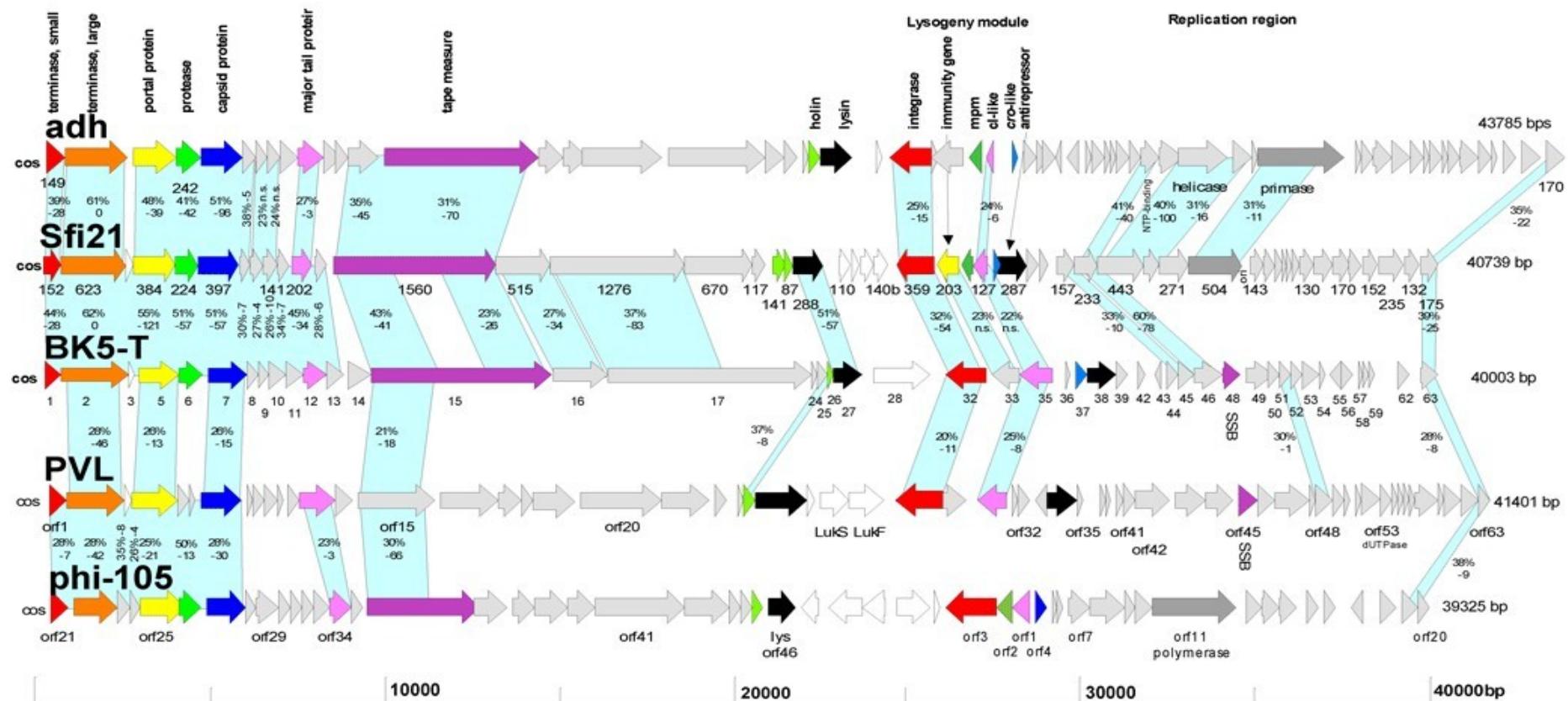
T. ammonificans



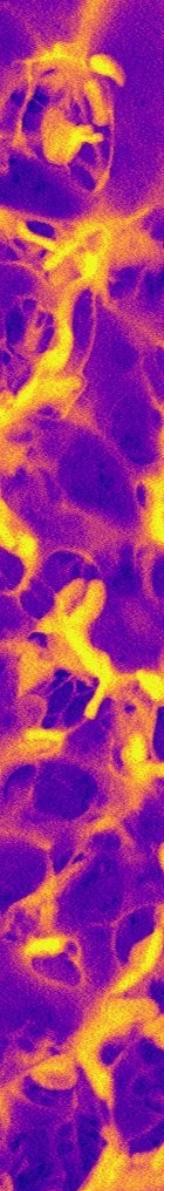
Desulfobulbus propionicicus



Viral Genomes

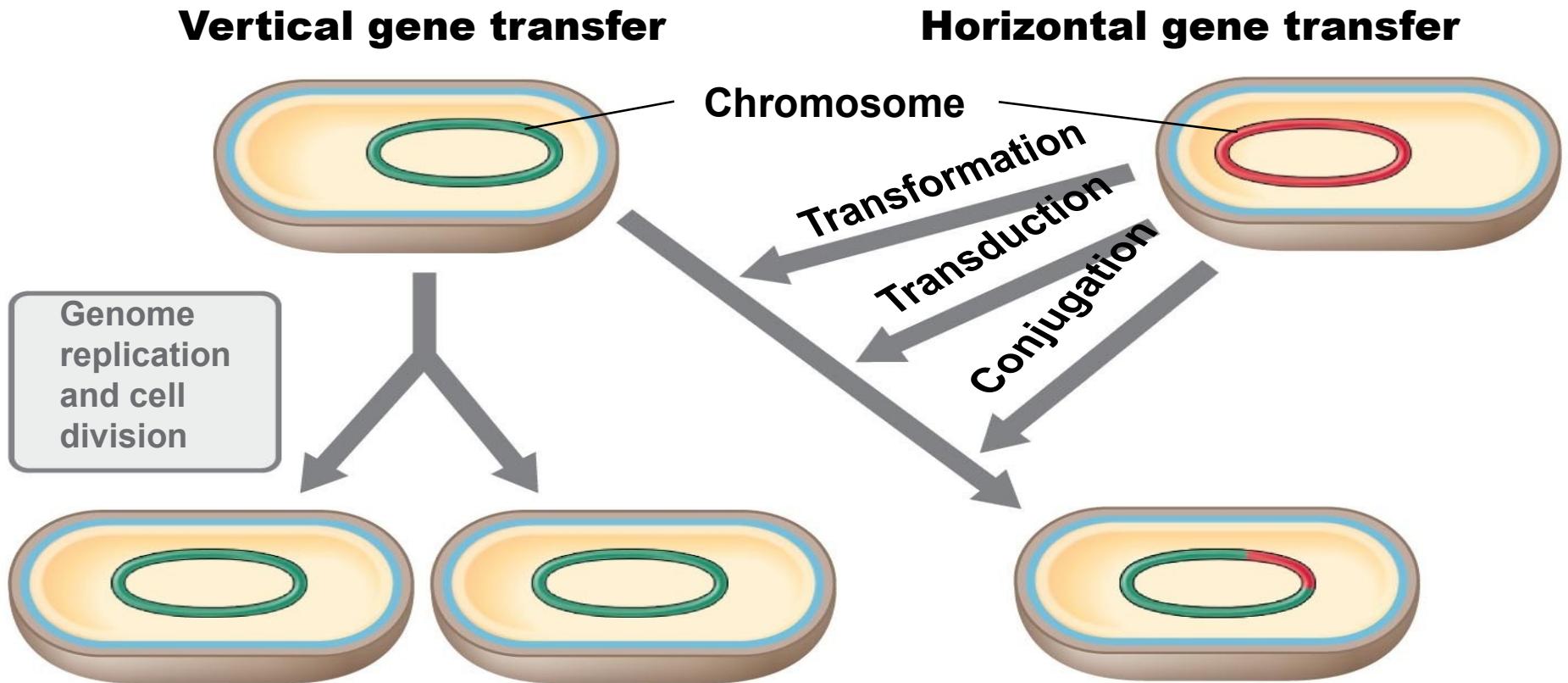


20,000-100,000 bp in size (30-100 genes) on average

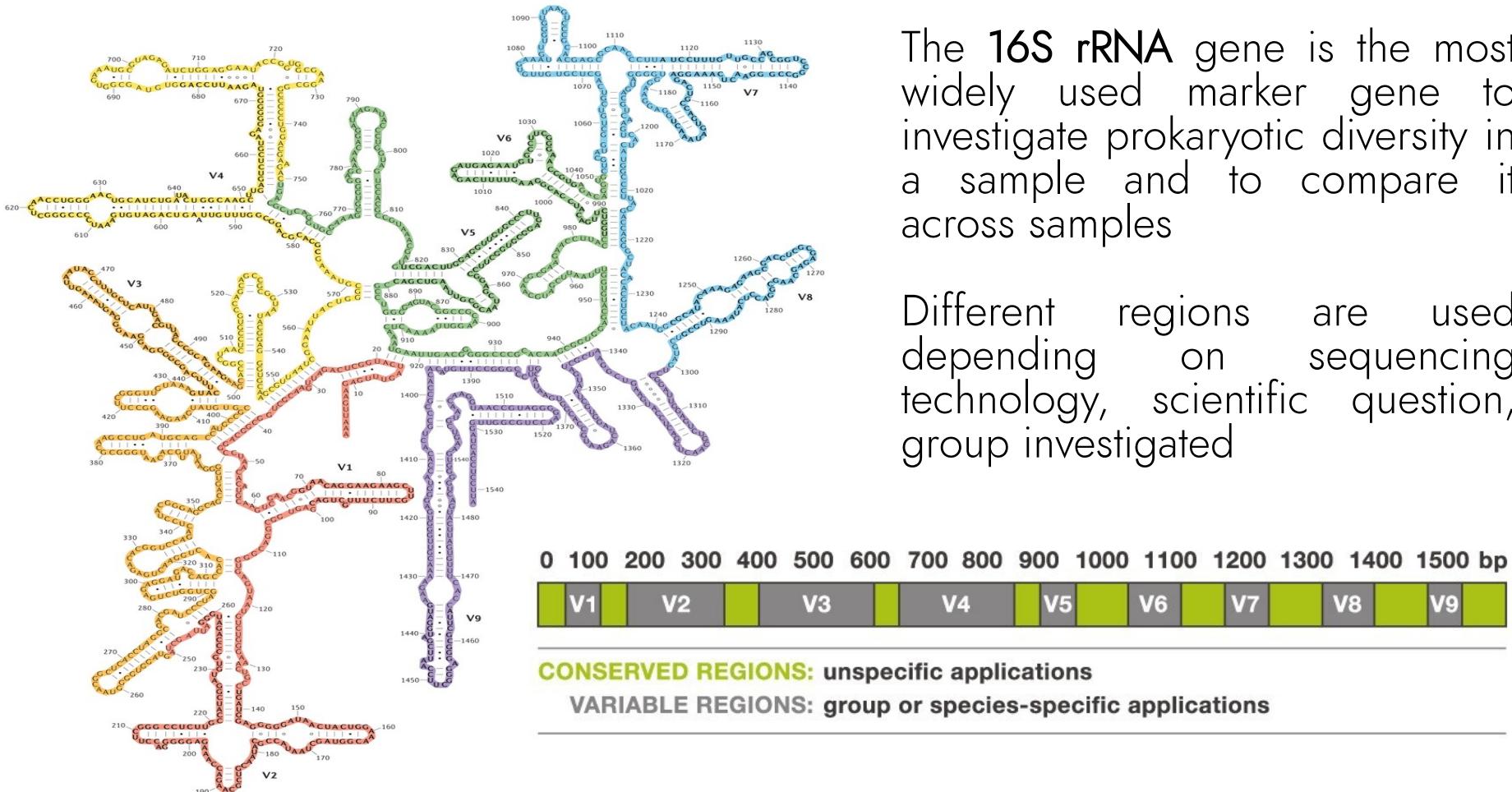


Horizontal Gene Transfer

- Horizontal gene transfer is the transfer of genetic information between organisms, as opposed to vertical inheritance from parental organism(s)
- It is considered one of the **major mode** of prokaryotic evolution
- May be extensive in nature
- It is a major confounding mechanism when studying prokaryotic evolution



Diversity and Marker Genes



The **16S rRNA** gene is the most widely used marker gene to investigate prokaryotic diversity in a sample and to compare it across samples

Different regions are used depending on sequencing technology, scientific question, group investigated

Proc Natl Acad Sci U S A. 2002 Dec 24;99(26):17020-4. Epub 2002 Dec 5.

Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*.

Welch RA¹, Burland V, Plunkett G 3rd, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, Stroud D, Mayhew GF, Rose DJ, Zhou S, Schwartz DC, Perna NT, Mobley HL, Donnenberg MS, Blattner FR.

Author information

Abstract

We present the complete genome sequence of uropathogenic *Escherichia coli*, strain CFT073. A three-way genome comparison of the CFT073, enterohemorrhagic *E. coli* EDL933, and laboratory strain MG1655 reveals that, amazingly, only 39.2% of their combined (nonredundant) set of proteins actually are common to all three strains. The pathogen genomes are as different from each other as each pathogen is from the benign strain. The difference in disease potential between O157:H7 and CFT073 is reflected in the absence of genes for type III secretion system or phage- and plasmid-encoded toxins found in some classes of diarrheagenic *E. coli*. The CFT073 genome is particularly rich in genes that encode potential fimbrial adhesins, autotransporters, iron-sequestration systems, and phase-switch recombinases. Striking differences exist between the large pathogenicity islands of CFT073 and two other well-studied uropathogenic *E. coli* strains, J96 and 536. Comparisons indicate that extraintestinal pathogenic *E. coli* arose independently from multiple clonal lineages. The different *E. coli* pathotypes have maintained a remarkable synteny of common, vertically evolved genes, whereas many islands interrupting this common backbone have been acquired by different horizontal transfer events in each strain.

Basic Metabolisms

Metabolisms: the basics

Life uses energy in two forms, chemical and light. All life uses free energy released from one chemical reaction to drive another. More specifically, life catalyzes redox chemistry (i.e. electron transfer), capturing energy from the resulting flow of electrons to drive further reactions.

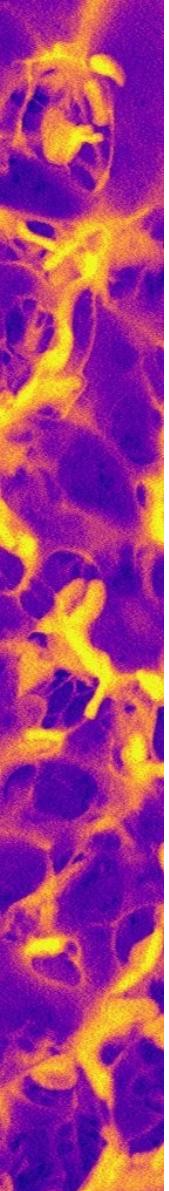
Metabolisms: the basics

Life is based on RedOx reactions

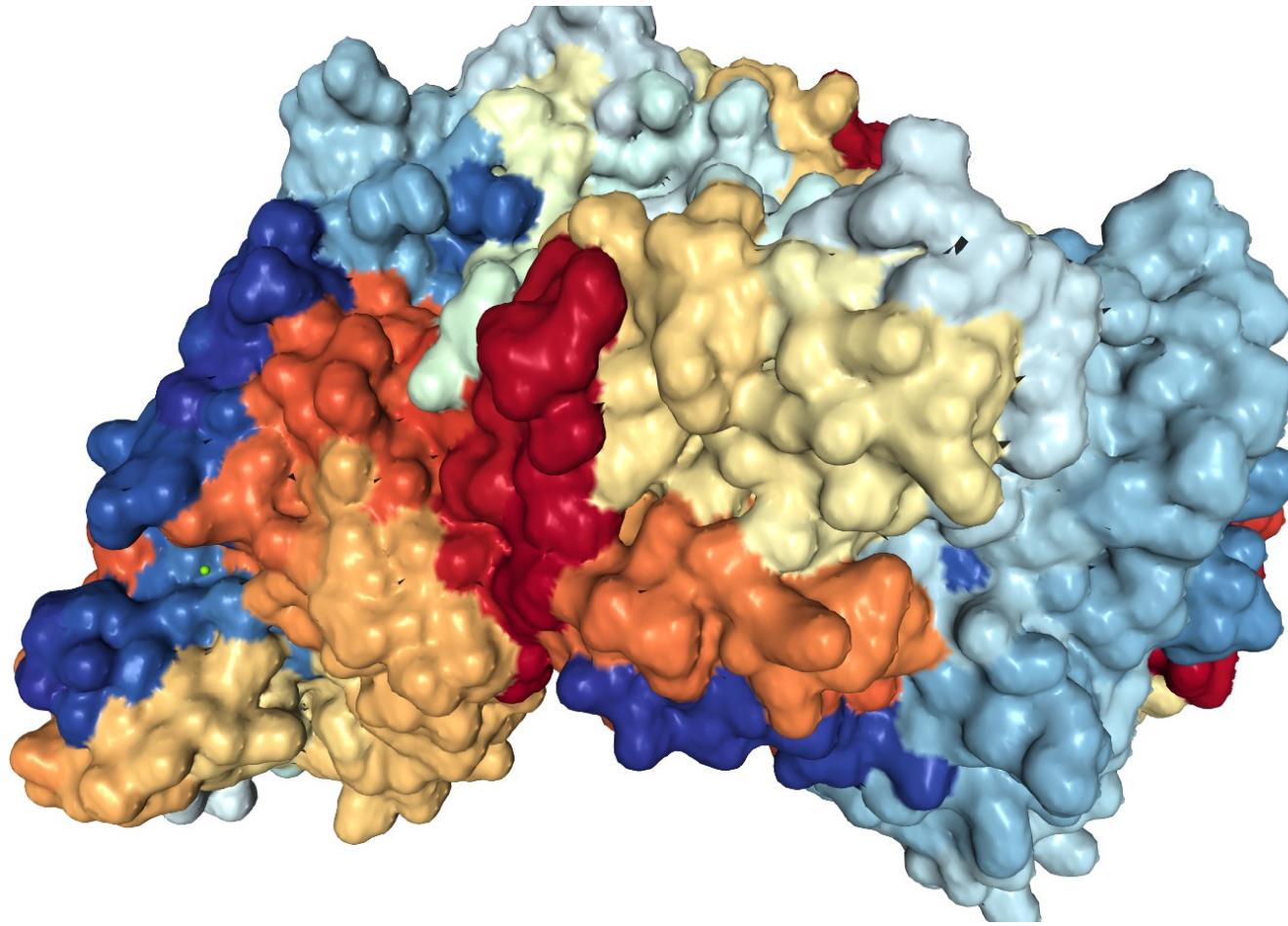
Refresher:

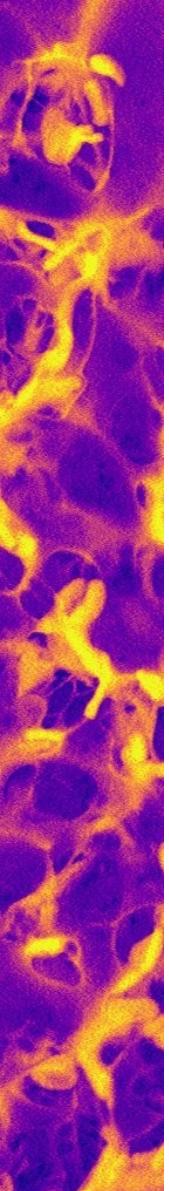
An **oxidation-reduction** (redox) **reaction** is a type of chemical reaction that involves a **transfer of electrons** between two species. An oxidation-reduction reaction is any chemical reaction in which the oxidation number of a molecule, atom, or ion changes by gaining or losing an electron.



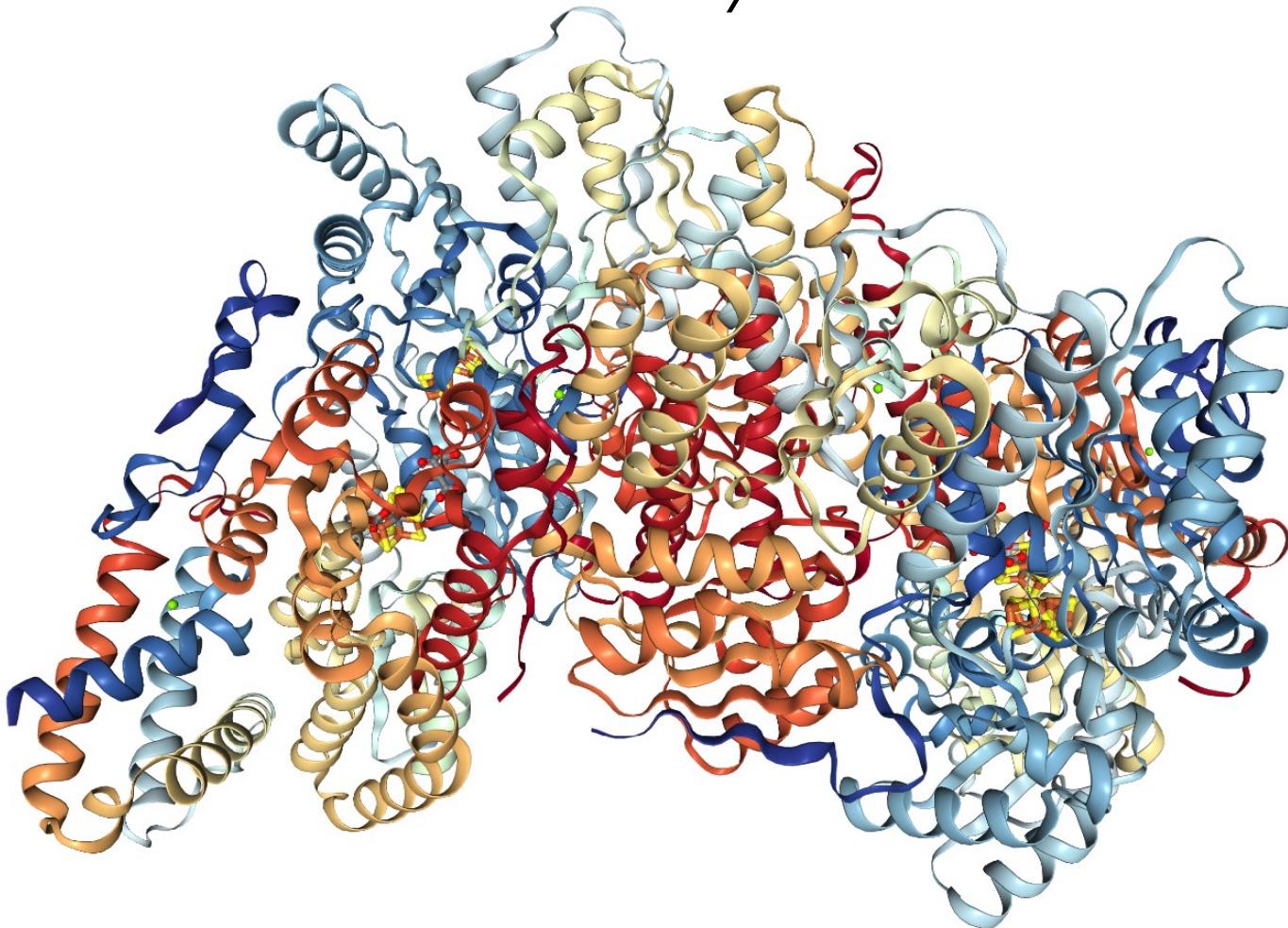


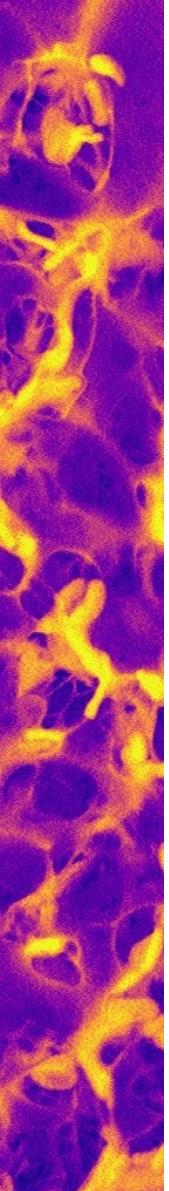
Life is literally electric



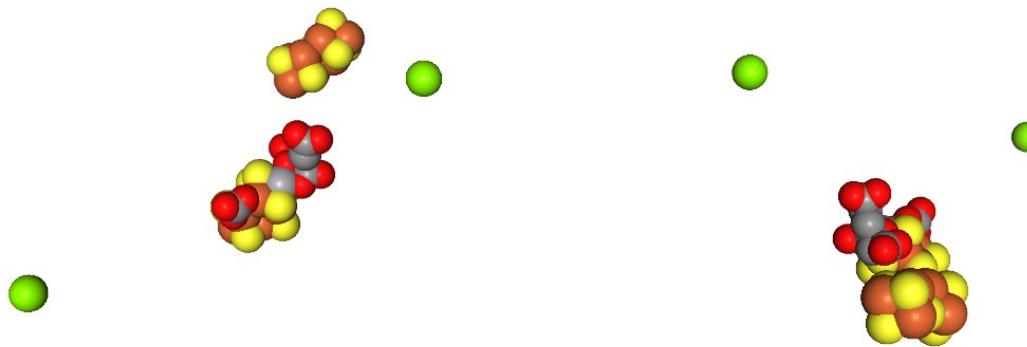


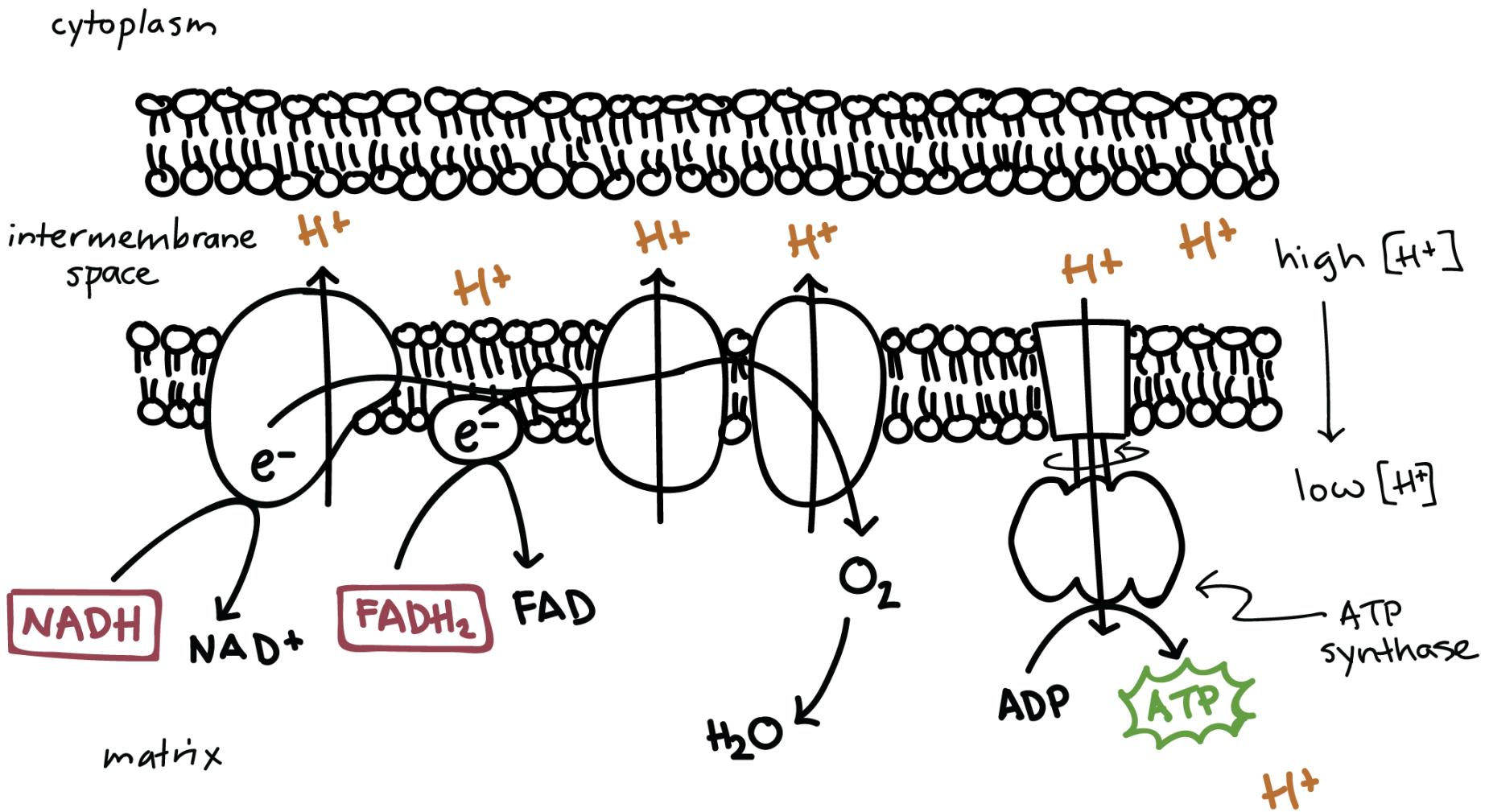
Life is literally electric





Life is literally electric





Free Energy of Formation and Calculating ΔG^0

Table A1.1 Free energies of formation (G_f^0) for some substances (kJ/mol)^a

Carbon compound	Carbon compound	Metal	Nonmetal	Nitrogen compound
CO, -137.34	Glutamine, -529.7	Cu ⁺ , +50.28	H ₂ , 0	N ₂ , 0
CO ₂ , -394.4	Glyceraldehyde, -437.65	Cu ²⁺ , +64.94	H ⁺ , 0 at pH 0; -39.83 at pH 7 (-5.69 per pH unit)	NO, +86.57
CH ₄ , -50.75	Glycerate, -658.1	CuS, -49.02	O ₂ , 0	NO ₂ , +51.95
H ₂ CO ₃ , -623.16	Glycerol, -488.52	Fe ²⁺ , -78.87	OH ⁻ , -157.3 at pH 14; -198.76 at pH 7; -237.57 at pH 0	NO ₂ ⁻ , -37.2
HCO ₃ ⁻ , -586.85	Glycine, -314.96	Fe ³⁺ , -4.6	H ₂ O, -237.17	NO ₃ ⁻ , -111.34
CO ₃ ²⁻ , -527.90	Glycolate, -530.95	FeCO ₃ , -673.23	H ₂ O ₂ , -134.1	NH ₃ , -26.57
Acetaldehyde, -139.9	Glyoxalate, -468.6	FeS ₂ , -150.84	PO ₄ ³⁻ , -1026.55	NH ₄ ⁺ , -79.37
Acetate, -369.41	Guanine, +46.99	FeSO ₄ , -829.62	Se ⁰ , 0	N ₂ O, +104.18
Acetone, -161.17	α -Ketoglutarate, -797.55	PbS, -92.59	H ₂ Se, -77.09	N ₂ H ₄ , +128
Alanine, -371.54	Lactate, -517.81	Mn ²⁺ , -227.93	SeO ₄ ²⁻ , -439.95	
Arginine, -240.2	Lactose, -1515.24	Mn ³⁺ , -82.12	S ⁰ , 0	
Aspartate, -700.4	Malate, -845.08	MnO ₄ ⁻ , -506.57	SO ₃ ²⁻ , -486.6	
Benzene, +124.5	Mannitol, -942.61	MnO ₂ , -456.71	SeO ₄ ²⁻ , -744.6	
Benzoic acid, -245.6	Methanol, -175.39	MnSO ₄ , -955.32	S ₂ O ₃ ²⁻ , -513.4	
n-Butanol, -171.84	Methionine, -502.92	HgS, -49.02	H ₂ S, -27.87	
Butyrate, -352.63	Methylamine, -40.0	MoS ₂ , -225.42	HS ⁻ , +12.05	
Caproate, -335.96	Oxalate, -674.04	ZnS, -198.60	S ²⁻ , +85.8	
Citrate, -1168.34	Palmitic acid, -305			
o-Cresol, -37.1	Phenol, -47.6			
Cratone, -277.4	n-Propanol, -175.81			
Cysteine, -339.8	Propionate, -361.08			
Dimethylamine, -3.3	Pyruvate, -474.63			
Ethanol, -181.75	Ribose, -757.3			
Formaldehyde, -130.54	Succinate, -690.23			
Formate, -351.04	Sucrose, -370.90			
Fructose, -951.38	Toluene, +114.22			
Fumarate, -604.21	Trimethylamine, -37.2			
Glucconate, -1128.3	Tryptophan, -112.6			
Glucose, -917.22	Urea, -203.76			
Glutamate, -699.6	Valerate, -344.34			

^aValues for free energy of formation of various compounds can be found in Dean, J. A. 1973. *Lange's Handbook of Chemistry*, 11th edition. McGraw-Hill, New York; Garrels, R. M., and C. L. Christ. 1965. *Solutions, Minerals, and Equilibria*. Harper & Row, New York; Burton, K. 1957. In Krebs, H. A., and H. L. Komberg. Energy transformation in living matter. *Ergebnisse der Physiologie* (appendix). Springer-Verlag, Berlin; and Thauer, R. K., K. Jungermann, and H. Decker. 1977. Energy conservation in anaerobic chemotrophic bacteria. *Bacteriol. Rev.* 41: 100-180.

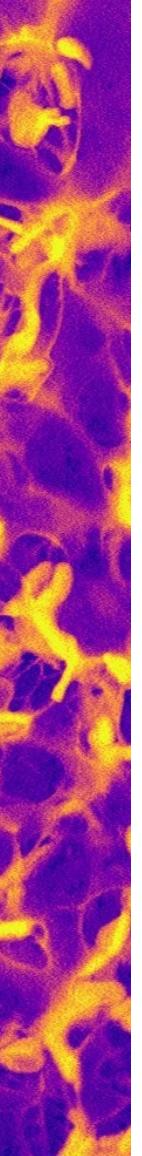
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Free Energy (G): defined as the energy released that is available to do useful work G_f^0 indicates the free energy of formation for a given compound, defined as the energy yielded or required for the formation of a given molecule from its constituent elements

$\Delta G^0'$ is the change in free energy under standard conditions (pH 7, 25°C, 1 atm., [reactants] = 1 M)

$$\Delta G^0 = \Delta G_f^0 (\text{products}) - \Delta G_f^0 (\text{reactants})$$

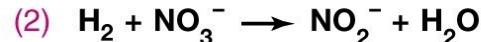
Neg. ΔG^0 / energy release / exergonic reaction
 Pos. ΔG^0 / energy requirement / endergonic reaction



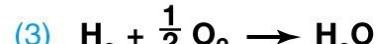
Examples of reactions with H₂ as e⁻ donor



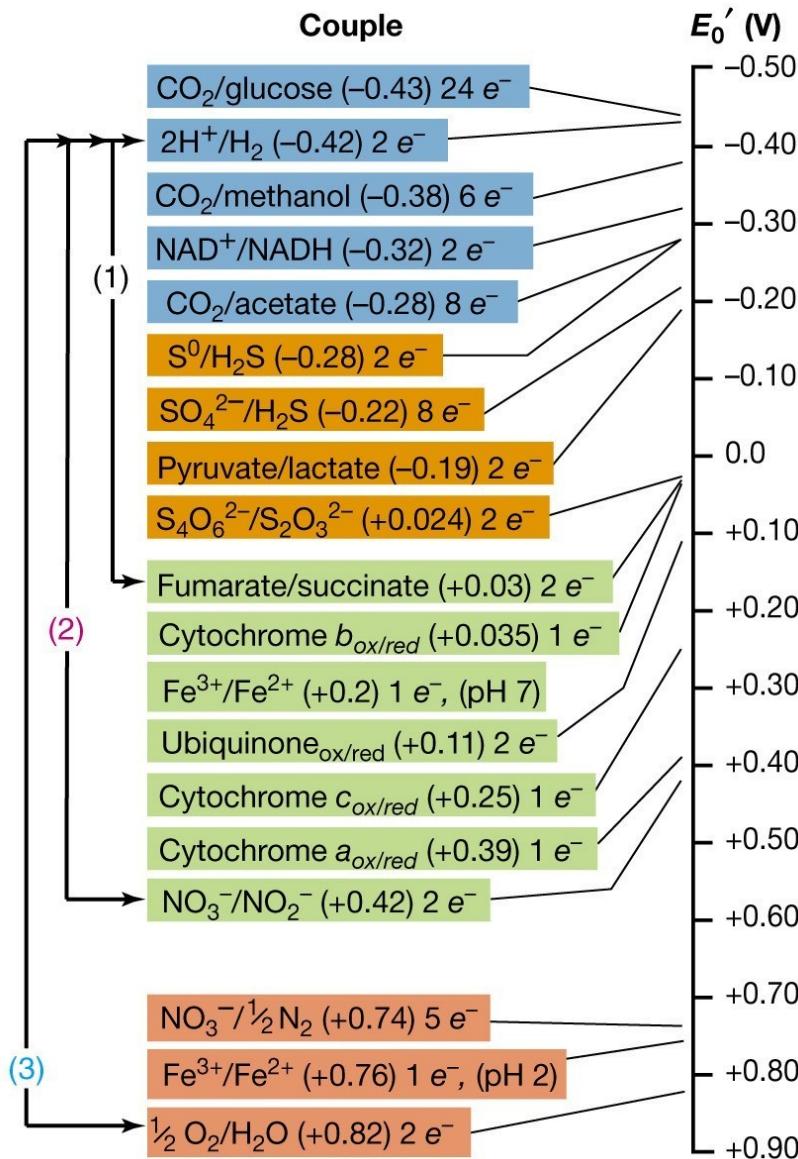
$$\Delta G^{\circ'} = -86 \text{ kJ}$$

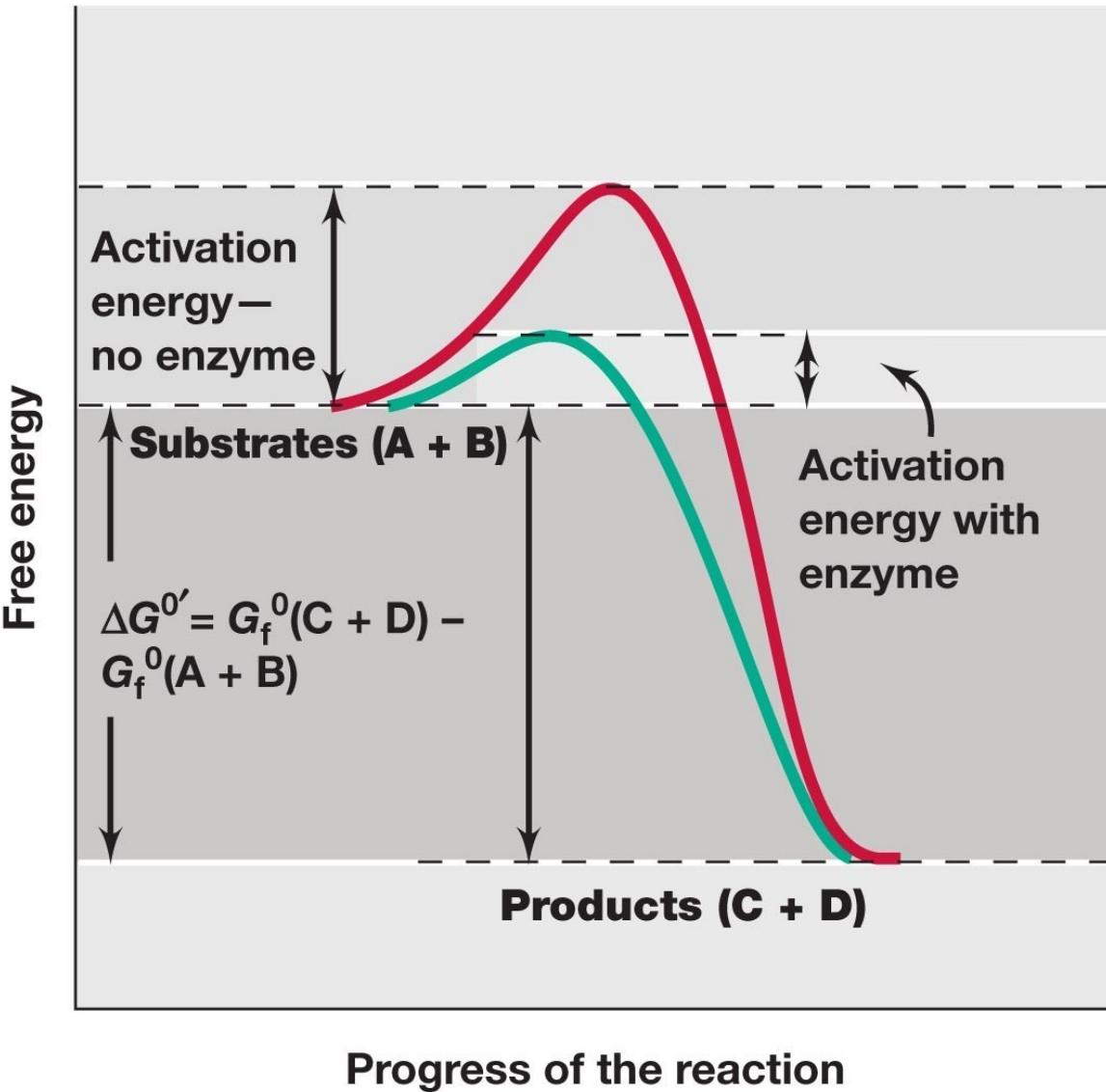


$$\Delta G^{\circ'} = -163 \text{ kJ}$$

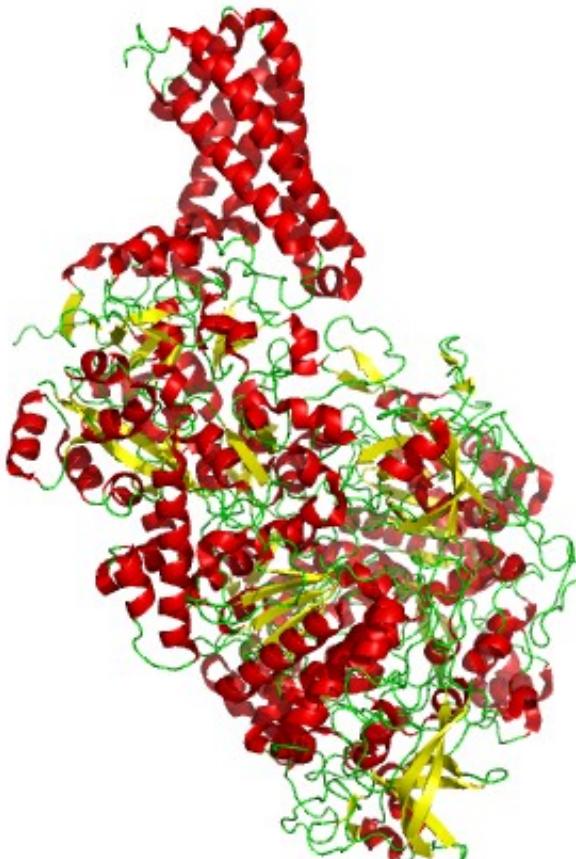


$$\Delta G^{\circ'} = -237 \text{ kJ}$$



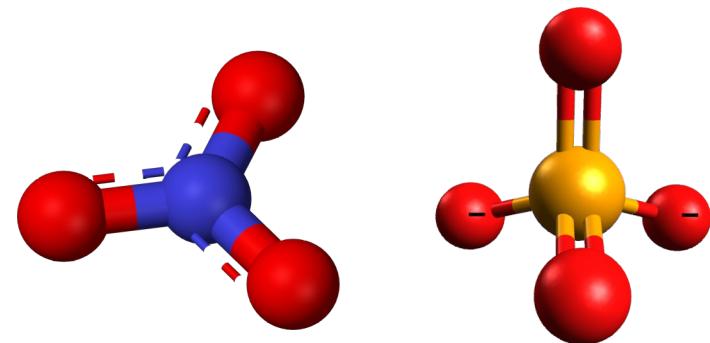


One more thing to keep in mind...



nitrite reductase enzyme $\text{NO}_3^- \rightarrow \text{NO}_2^-$

While working primarily on **Nitrate**, this reductase enzyme can also act on **Selenate**, thus being **promiscuous** with regard to the substrate

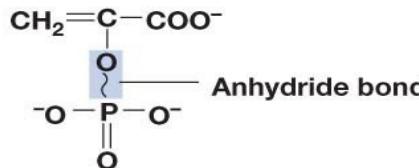


Substrate promiscuity is widespread in nature

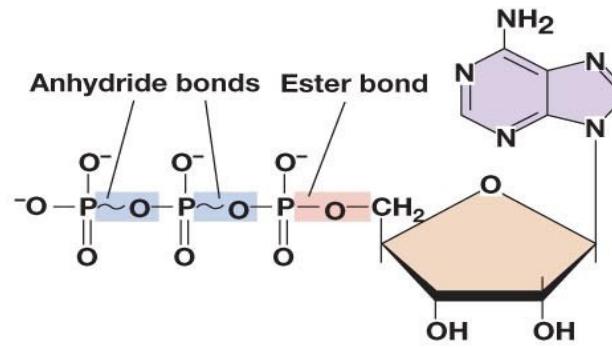
High-energy bonds

The energy released in redox reactions is conserved in the formation of certain compounds that contain energy-rich phosphate or sulfur bonds

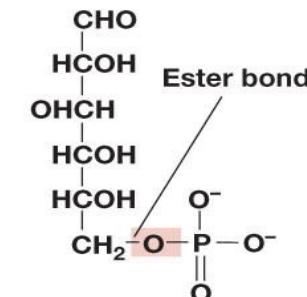
ATP contains three phosphates, but only two of them are high energy (anhydride bonds, shown in blue)



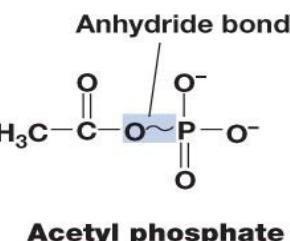
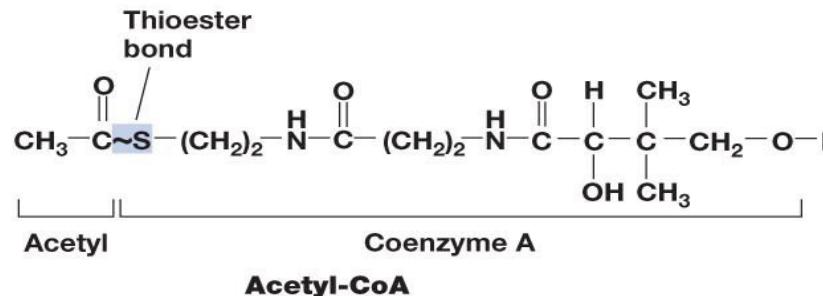
Phosphoenolpyruvate



Adenosine triphosphate (ATP)

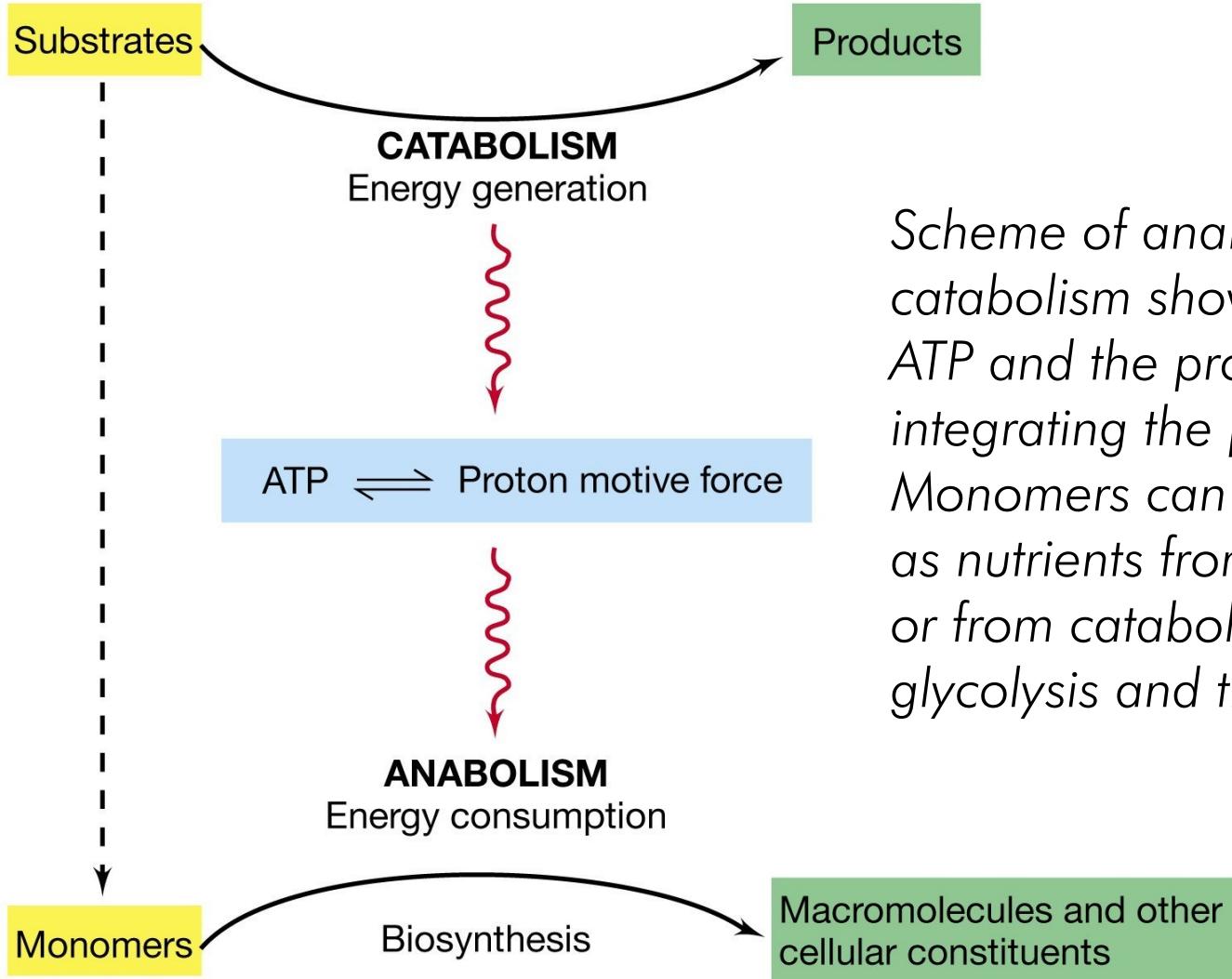


Glucose 6-phosphate



Acetyl phosphate

Compound	$\Delta G^\circ' \text{ kJ/mol}$
$\Delta G^\circ' > 30\text{kJ}$	
Phosphoenolpyruvate	-51.6
1,3-Bisphosphoglycerate	-52.0
Acetyl phosphate	-44.8
ATP	-31.8
ADP	-31.8
Acetyl-CoA	-35.7
$\Delta G^\circ' < 30\text{kJ}$	
AMP	-14.2
Glucose 6-phosphate	-13.8



Scheme of anabolism and catabolism showing the key role of ATP and the proton motive force in integrating the processes. Monomers can come preformed as nutrients from the environment or from catabolic pathways like glycolysis and the citric acid cycle.

Proton Motive Force

With the exception of fermentation, in which substrate-level phosphorylation occurs, all other mechanisms for energy conservation employ the **proton motive force**. Whether electrons come from the oxidation of organic or inorganic chemicals or from light-driven processes, in all forms of respiration and photosynthesis, energy conservation is linked to the establishment of a PMF and its dissipation by ATPase to form ATP.

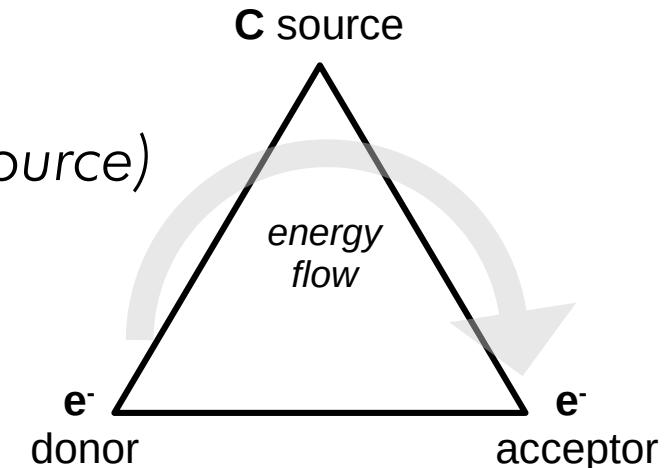
Respiration and anaerobic respiration can thus be viewed as variations on a theme of different electron acceptors. Likewise, **chemoorganotrophy**, **chemolithotrophy**, and **photosynthesis** are variations on a theme of different electron donors. Electron transport and the pmf link all of these processes, bringing these seemingly quite different forms of energy metabolism into a common focus.

Basic Metabolism

An **electron donor** (also known as energy source)

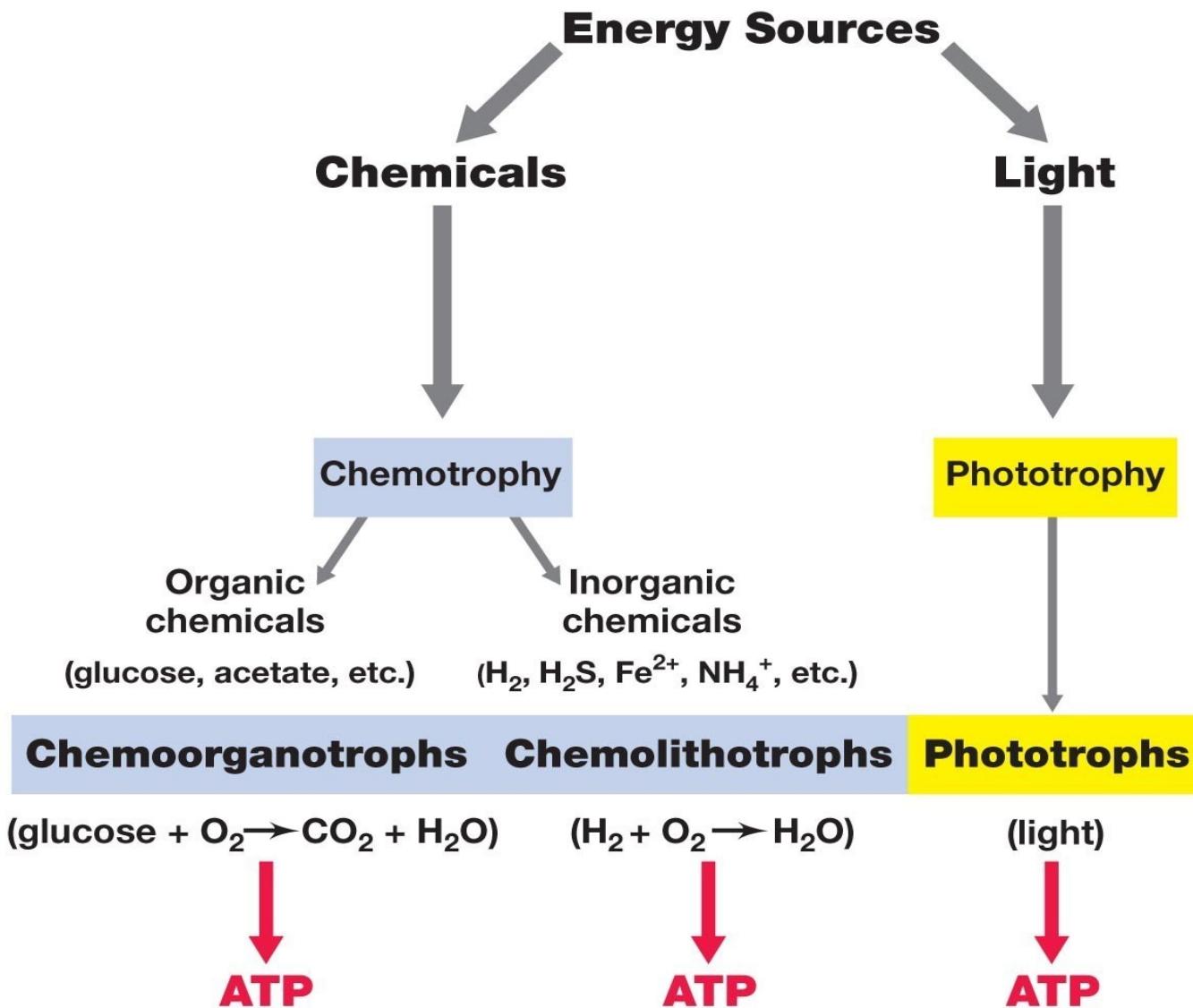
A **carbon source** (for biosynthesis)

An **electron acceptor**



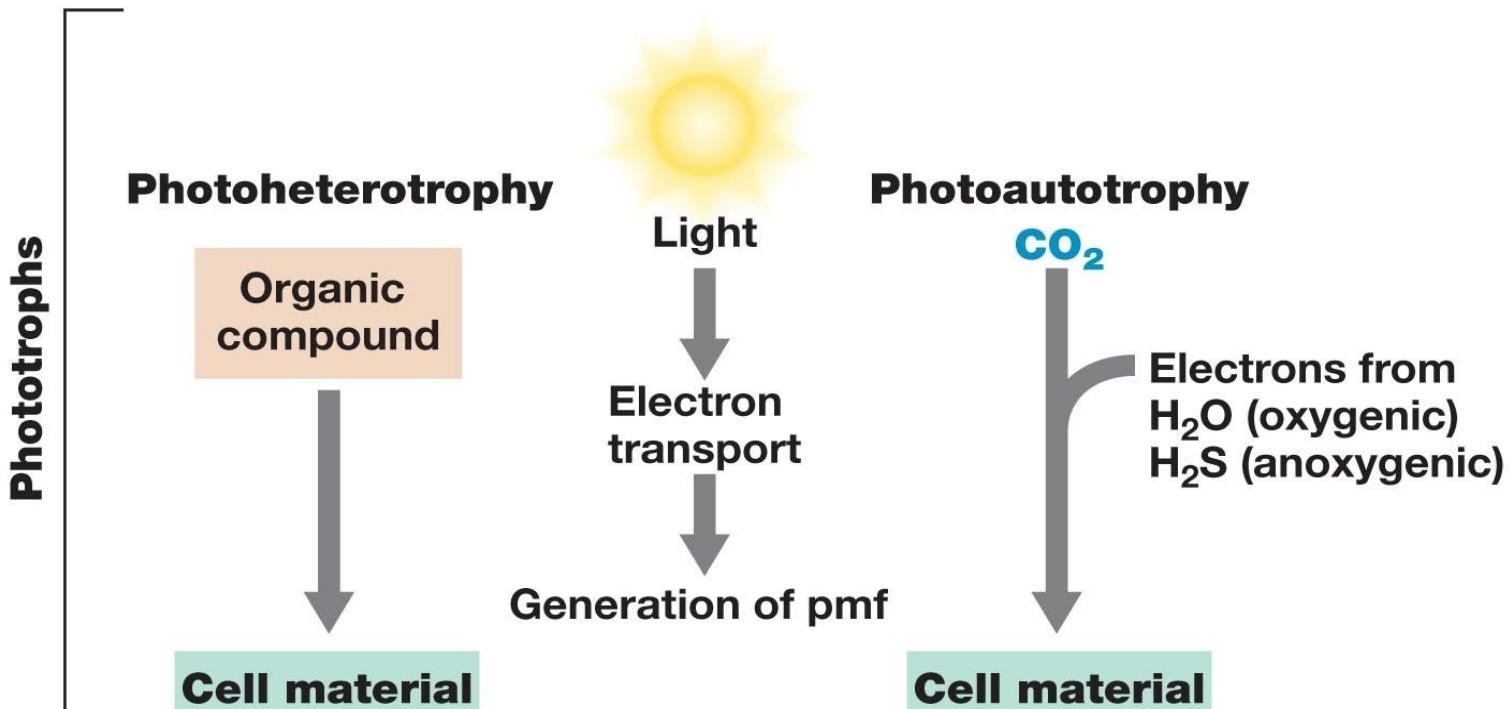
All type of metabolism, requires these three basic elements.

An electron donor (the source of reducing power used to carry out redox reactions), a carbon source used as a donor of carbon for biosynthetic purposes, and an electron acceptor, used to dispose of excess reducing equivalents.



Phototrophy

Energy



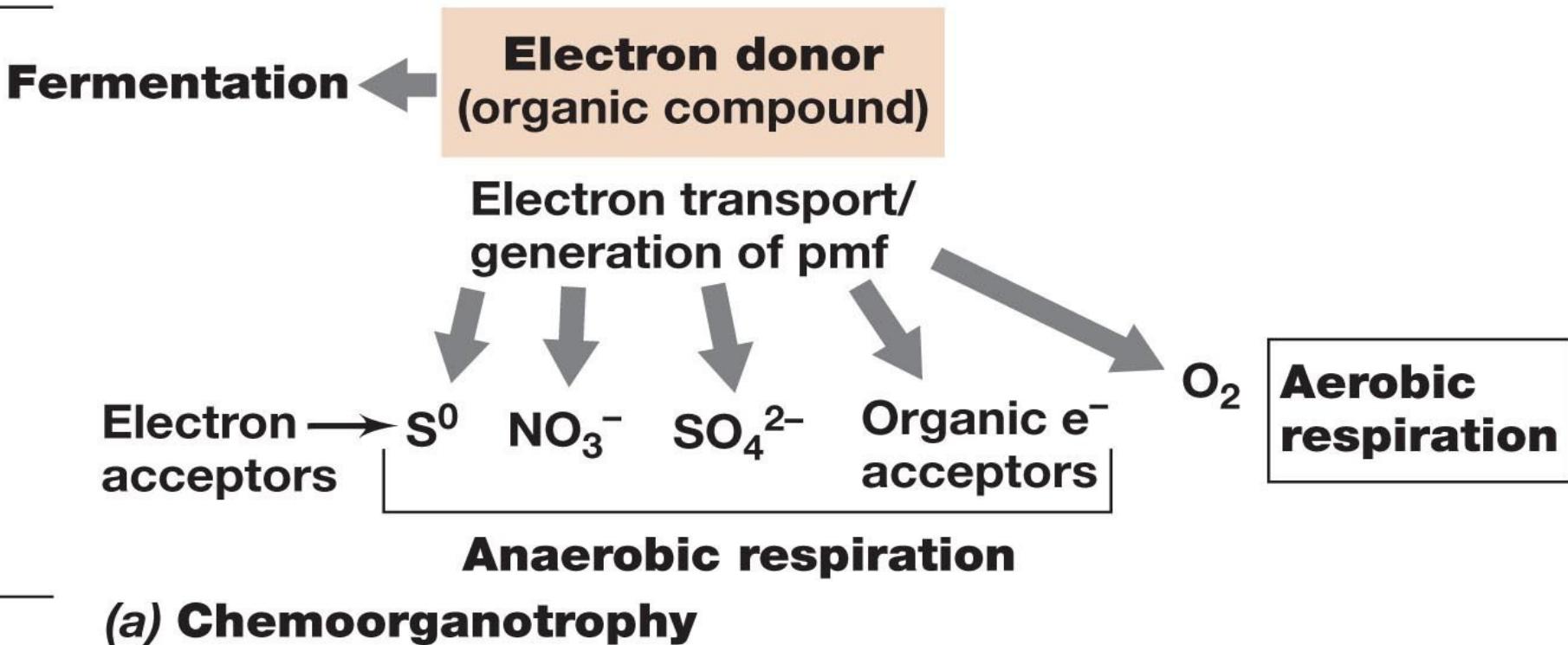
(c) Phototrophy

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Photoheterotrophs, Photoautotrophs: Anoxygenic photoautotrophs and Oxygenic photoautotrophs

Chemoorganotrophy

Chemotrophs



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Chemoorganotrophs can perform **Aerobic Respiration**, **Anaerobic Respiration** and **Fermentation**

Chemolithotrophy

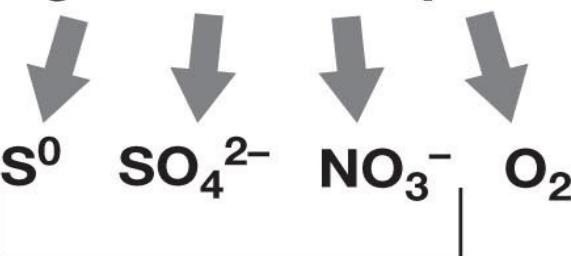
Chemotrophs

Energy Electrons

Electron donor
 $(H_2, H_2S, Fe^{2+}, NH_4^+, \text{etc.})$

Electron transport/
generation of pmf

Electron
acceptors



Aerobic respiration

Anaerobic respiration

(b) Chemolithotrophy

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Chemolithotrophs can perform **Aerobic Respiration** and **Anaerobic Respiration**

Source of:

Energy Carbon Electrons

Photo-	auto-	litho-	
Chemo-	hetero-	organo-	trophy

Photo-litho-auto-trophic

Chemo-litho-auto-trophic

Chemo-litho-hetero-trophic

Special cases:

Methylotrophic

Methanogenic

Syntrophic

Photosynthesis

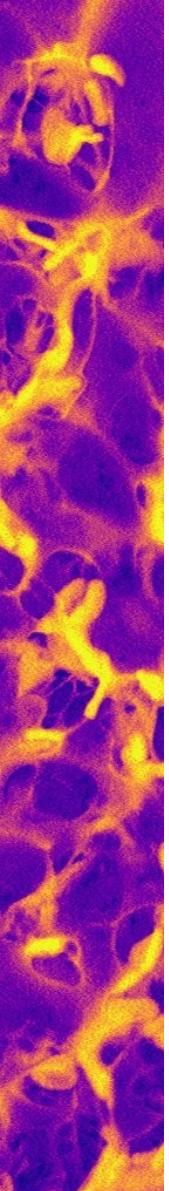
- *Phototrophs* are organisms that carry out photosynthesis
- *Most phototrophs are also autotrophs*
- Photosynthesis requires light-sensitive pigments called *chlorophyll*
- Photoautotrophy requires ATP production and CO₂ reduction
- Oxidation of H₂O produces O₂ (oxygenic photosynthesis)
- Oxygen not produced (anoxygenic photosynthesis), other electron donors used (H₂S, Fe²⁺, H₂)
- *Oxygenic Photosynthesis* is the most important biological process on Earth

Heterotrophy

- *Heterotrophy* is the use of *organic carbon sources* for anabolic purposes
- Major pathways are *Glycolysis* and *Tri-Carboxylic Acid cycle* (TCA cycle)
- Coupled to *aerobic or anaerobic respiration*. Aerobic chemorganoheterotrophy is a *major sink of oxygen*
- Many heterotrophic microorganisms have the “*fermentation option*”

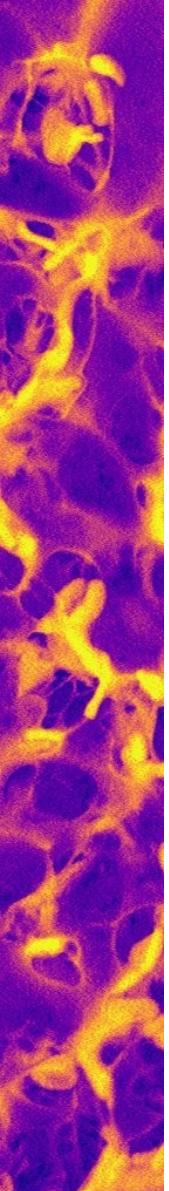
Fermentation

- Fermentation is a major metabolism in *absence of inorganic electron acceptors*. Fermenting organisms can be *obligate* or *facultative*
- In fermentation redox balance is achieved by having the substrate serve as *both electron donor and electron acceptor* and that ATP is synthesized by *substrate-level phosphorylation*. PMF is not involved
- Redox balance is achieved in fermentations by the *excretion* from the cell of fermentation products, reduced substances such as *acids or alcohols* that are produced as end products of the catabolism of the original fermentable substance
- Fermentations are classified by either the substrate fermented or the products formed. A diverse array of products can be produced

A vertical strip on the left side of the slide shows a microscopic image of various microorganisms, including bacteria and protists, against a dark background.

Aerobic and Anaerobic Respiration

- *Respiration* is the process of using an *inorganic terminal electron acceptor* in the electron transport chain
- *Aerobic respiration* is the dominant process in the extant biosphere
- Because the O_2/H_2O couple is most electropositive, more energy is available when O_2 is used as a terminal electron acceptor than when any other acceptor is used
- Other electron acceptors involved in anaerobic respiration are manganic ion (Mn_4^+), ferric iron (Fe_3^+), nitrate (NO_3^-), and nitrite (NO_2^-), sulfate (SO_4^{2-}), elemental sulfur (S^0), and carbon dioxide (CO_2) among others
- Prokaryotes performing aerobic and anaerobic respiration can be both *heterotrophs* and *autotrophs*

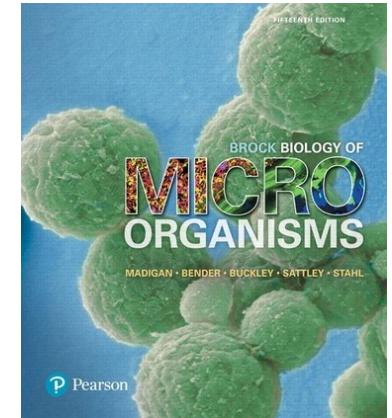


Assimilative and Dissimilative Reductions

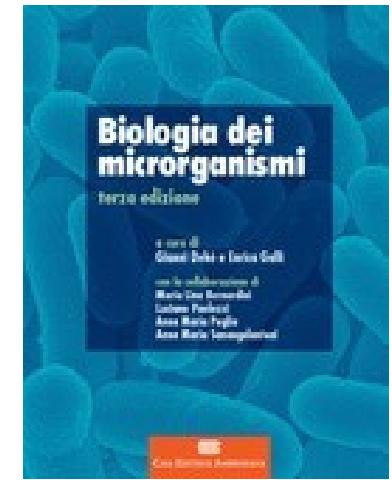
- Inorganic compounds (e.g. NO_3^- , SO_4^{2-} , CO_2) are reduced by many organisms as sources of cellular nitrogen, sulfur, and carbon (e.g. amino groups, sulphydryl groups, organic carbon). This is **assimilative reduction** because the group is assimilated
- This is different from respiration, which is the reduction of inorganic compound for energy conserving reason. This is also known as **dissimilative reduction**, and often the product are excreted from the cell
- Most organisms carry out an assortment of assimilative metabolisms, whereas a more restricted group catalyze dissimilative metabolisms
- Several inorganic compounds can be both electron donor or electron acceptors (e.g. S^0) **depending on the redox couple** involved

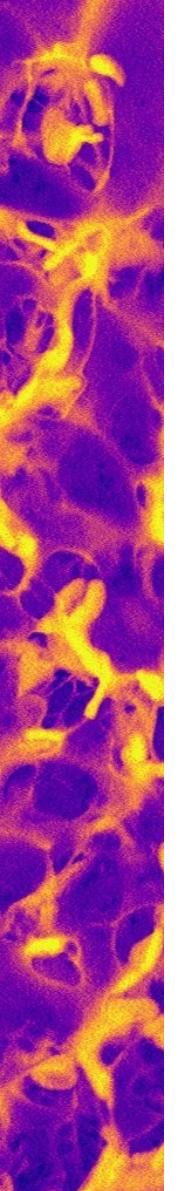
Books suggestion so far:

Brock Biology of Microorganisms (15th Edition)
Chapters 2, 3, 4, 5,

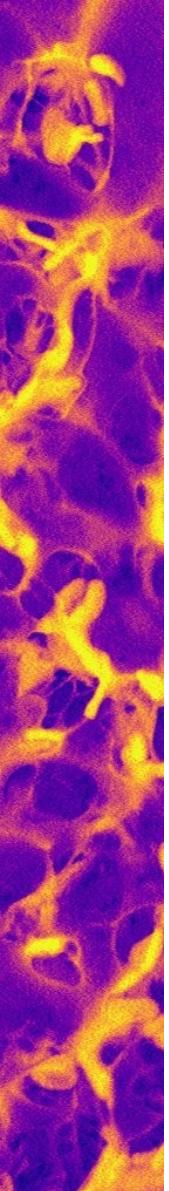


Dehò Galli Biologia dei Microorganismi (2019 Edition)
Chapters 2, 3, 4, 7, 8, 10



A vertical strip on the left side of the slide showing a microscopic view of various microorganisms, possibly bacteria or fungi, with a color palette ranging from purple to yellow.

MICROBIAL EVOLUTION: A VERY BRIEF GUIDE



Microbial Species Concept

Microbial species concept. The species concept is complex when applied to microbiology. In biology species are defined primarily based on their sexual segregation, either by genetic, behavioral or geographical barrier (and even this can be sometimes challenging, as all human-imposed categories).

For microbes the **absence of sexual reproduction, horizontal gene transfer** and a **high genome plasticity** make the classic species concept difficult to apply.

The current golden standard for defining a species is called a **polyphasic approach**, combining **phylogenetics, physiology and chemotaxonomy**.

In more simple terms, microbial species are defined based on their genetic properties, specifically the similarity of the **16S rRNA marker gene** (since it is a conserved universal marker not subject to HGT) or the **whole genome similarity** (also called ANI, Average Nucleotide Identity).

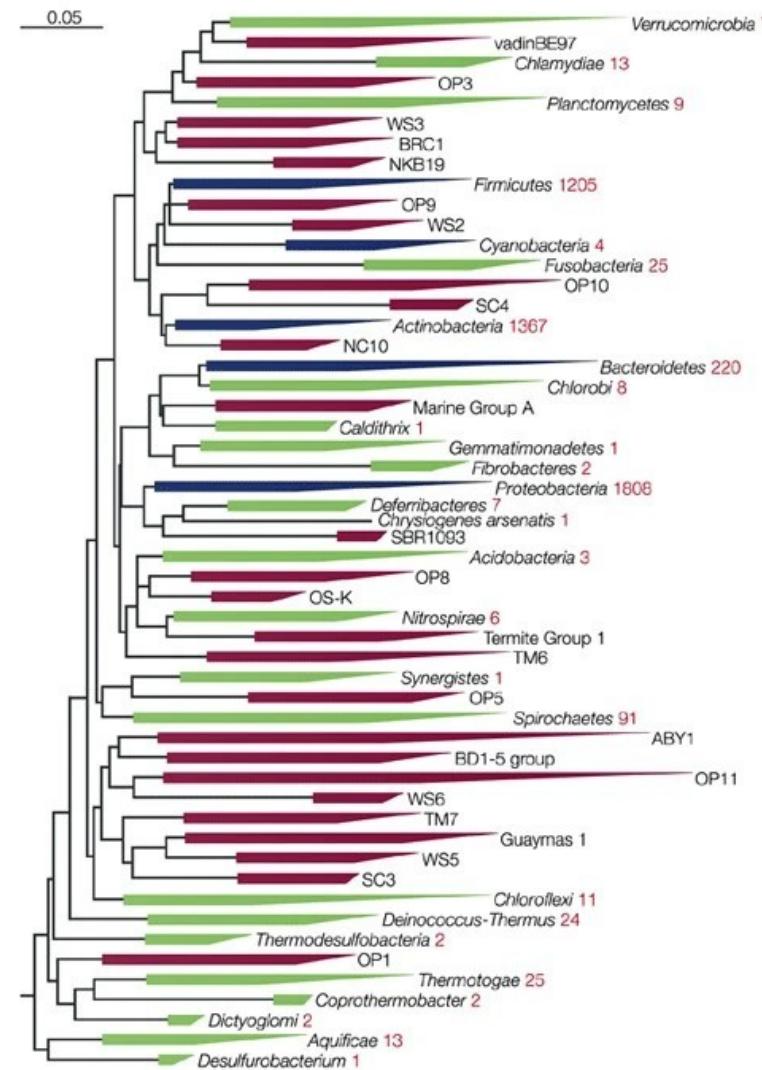
Current standard are **16S rRNA similarity >97%** for the same species and an **whole genome ANI >70%**.

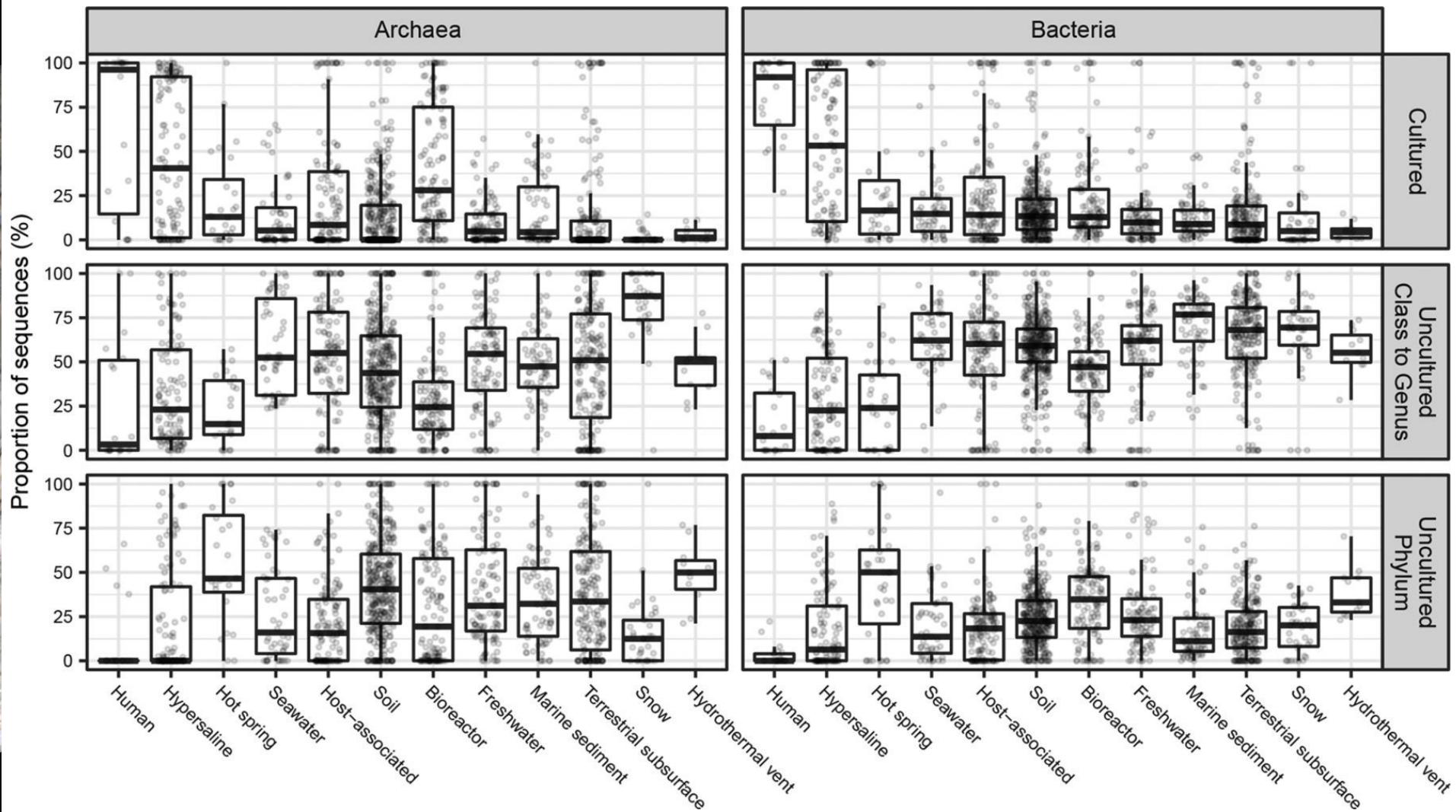
The Uncultured Majority

The uncultured majority refers to our current inability to cultivate the large majority of microorganisms in many environments. The first description is from Staley and Konopka, 1985, and was later dubbed the “Great Plate Count Anomaly”.

The simple observation that counts of growing microbes on plates differed greatly from counts using microscopy suggested that the known diversity was dwarfed by the actual diversity.

Metagenomic has revolutionized the study of microbial diversity in many ways, allowing us to probe into the unknown.

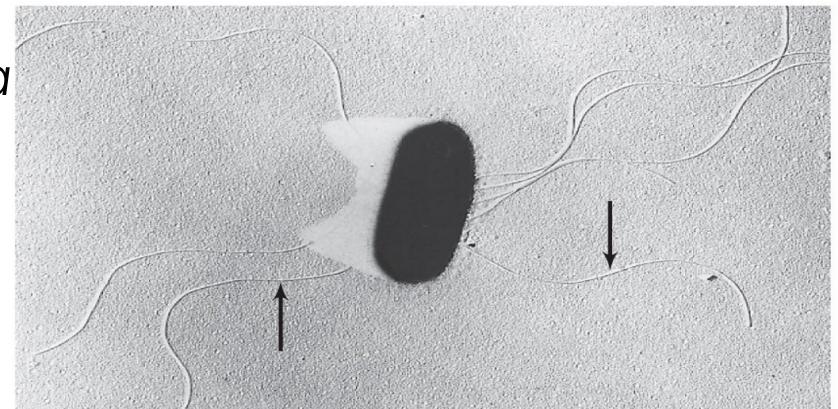




Microbial Species Concept: Taxonomy

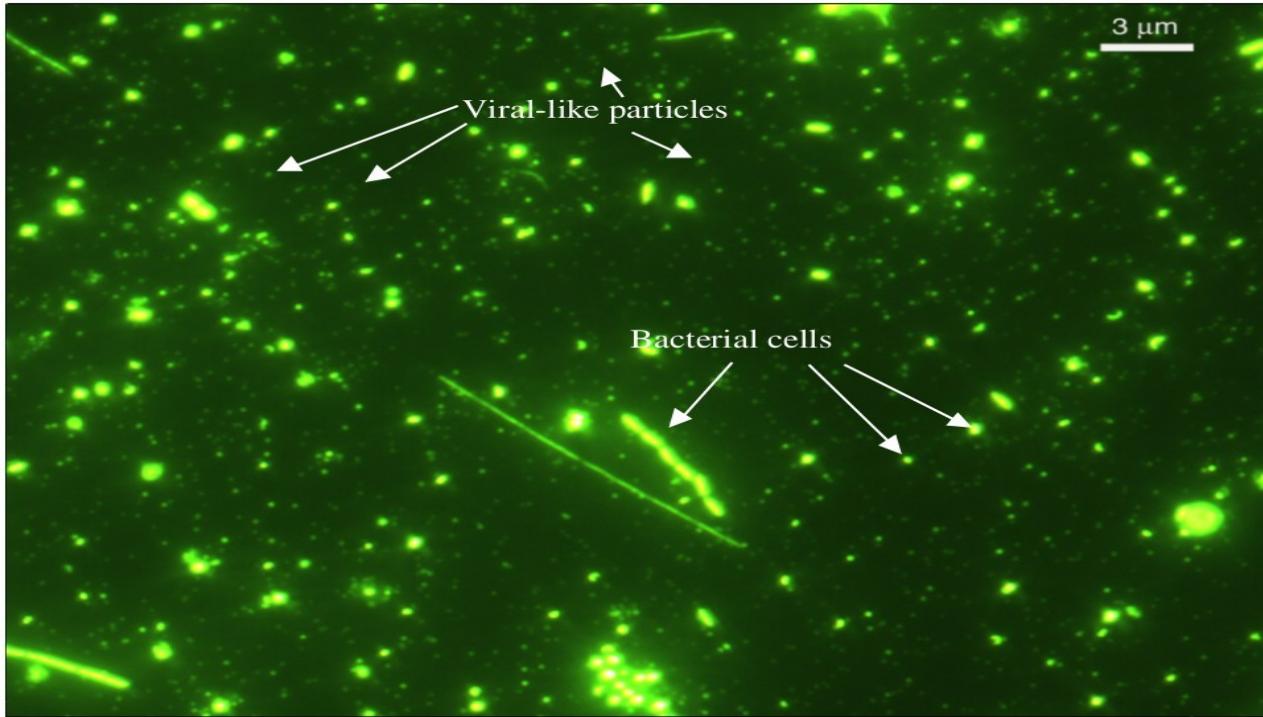
Taxonomic nomenclature follows the binomial systems. There are numerous rules and conventions, all established by the International Committee on the Systematics of Prokaryotes.

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacterales
Family: Enterobacteraceae
Genus: Escherichia
Species: *Escherichia coli*
Strain: K12



Arthur Kelman

A microbial world



Although microorganisms are the smallest forms of life, collectively they constitute the bulk of biomass on Earth and carry out many necessary chemical reactions for higher organisms.

A microbial world

Global biomass: 546 billion tonnes of carbon

Terrestrial

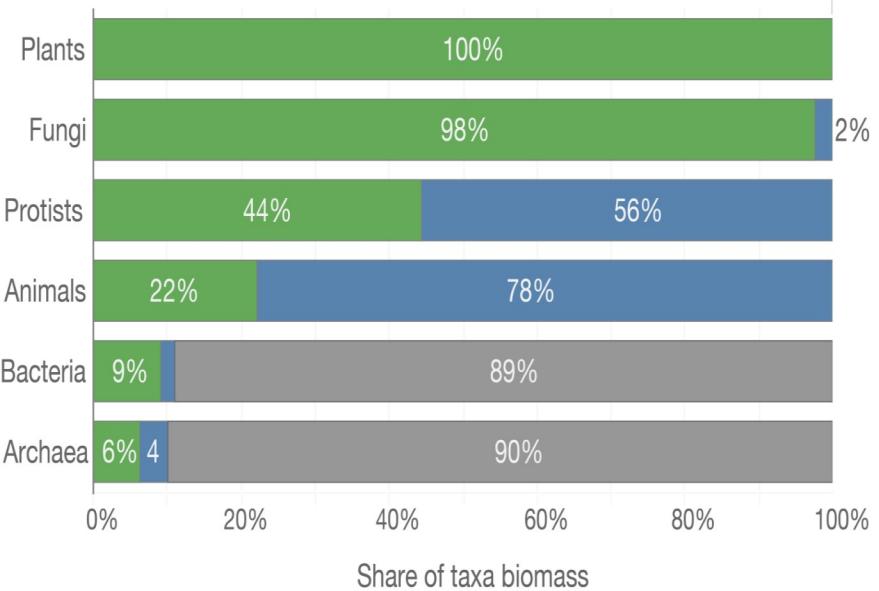
470 billion tonnes of carbon
86% of total biomass

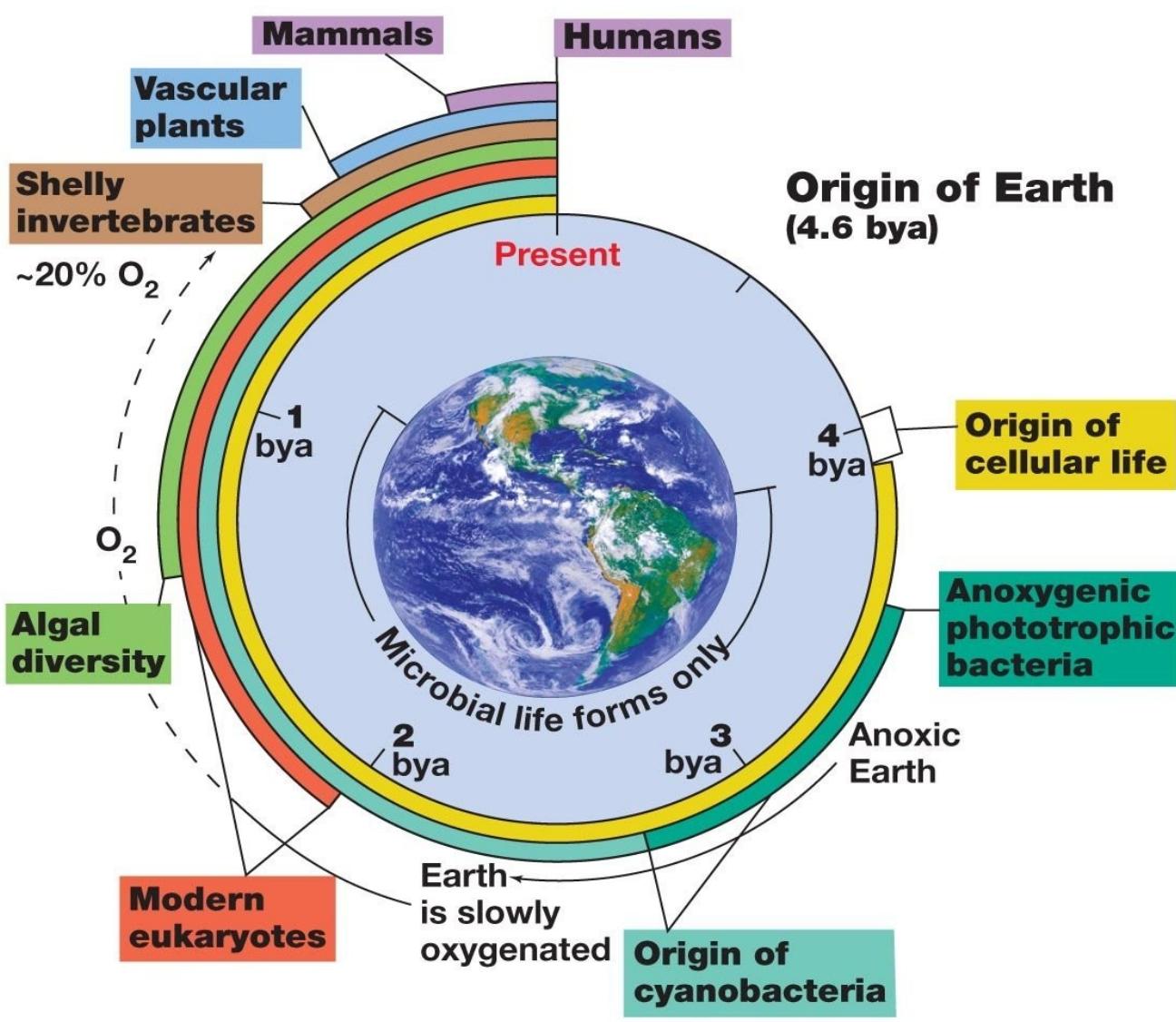
Deep subsurface
70 billion tonnes of carbon
13% of total biomass

Marine

6 billion tonnes of carbon
1% of total biomass

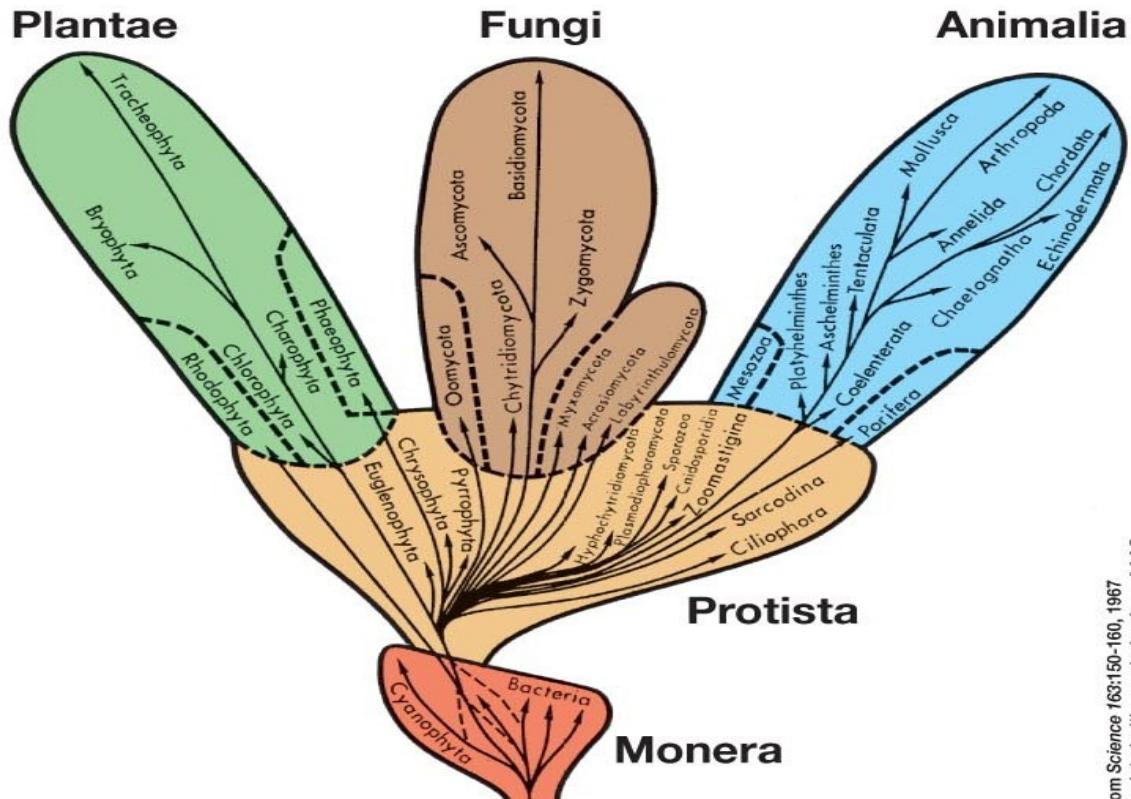
In which environment do taxa live?





(a)

The tree of life: before sequencing



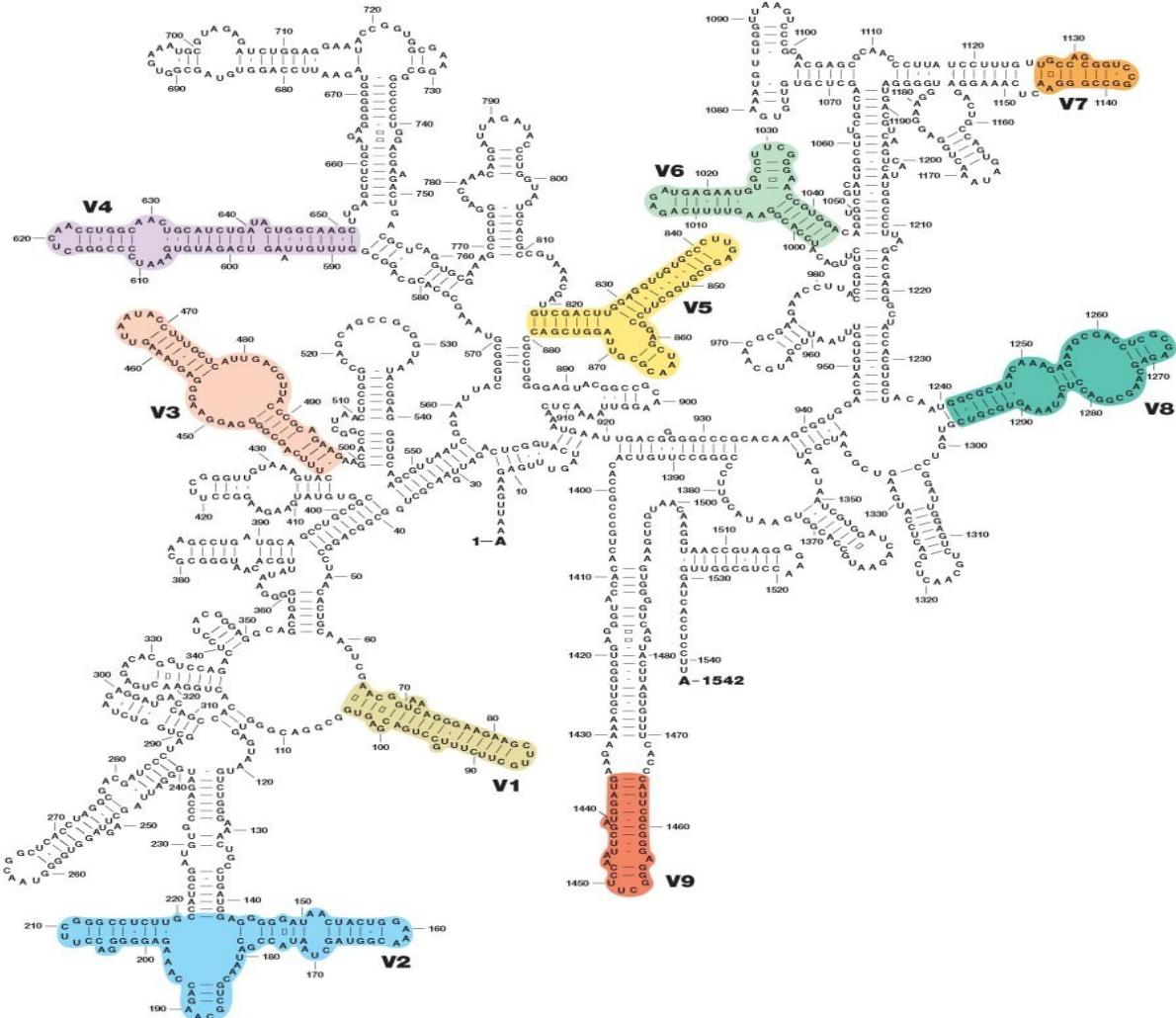
(b) The Whittaker Tree

From Science 163:150-160, 1967
Reprinted with permission from AAAS

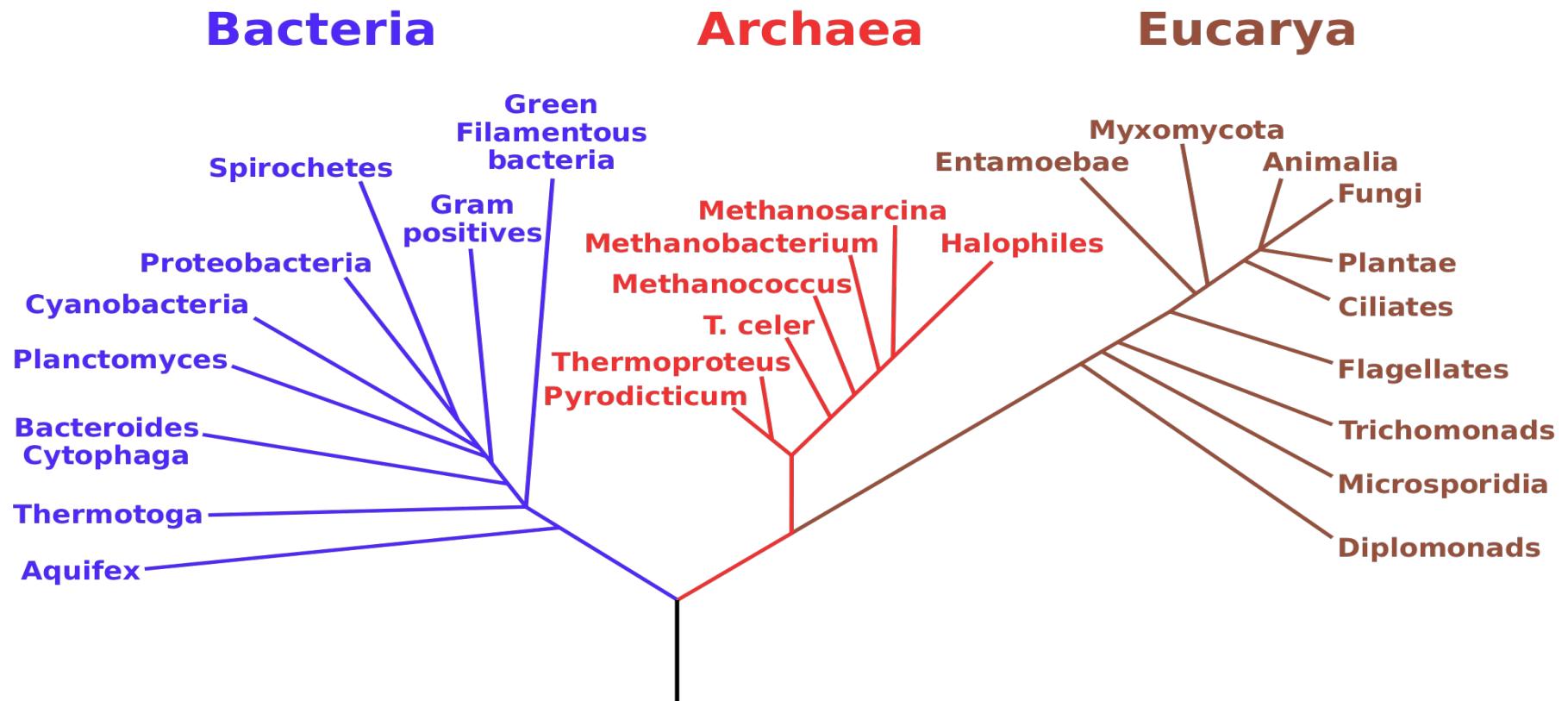
rRNA as universal marker

In 1977 Carl R. Woese reported the discovery of a new group of organisms, that he called Archeabacteria, previously thought to be Bacteria.

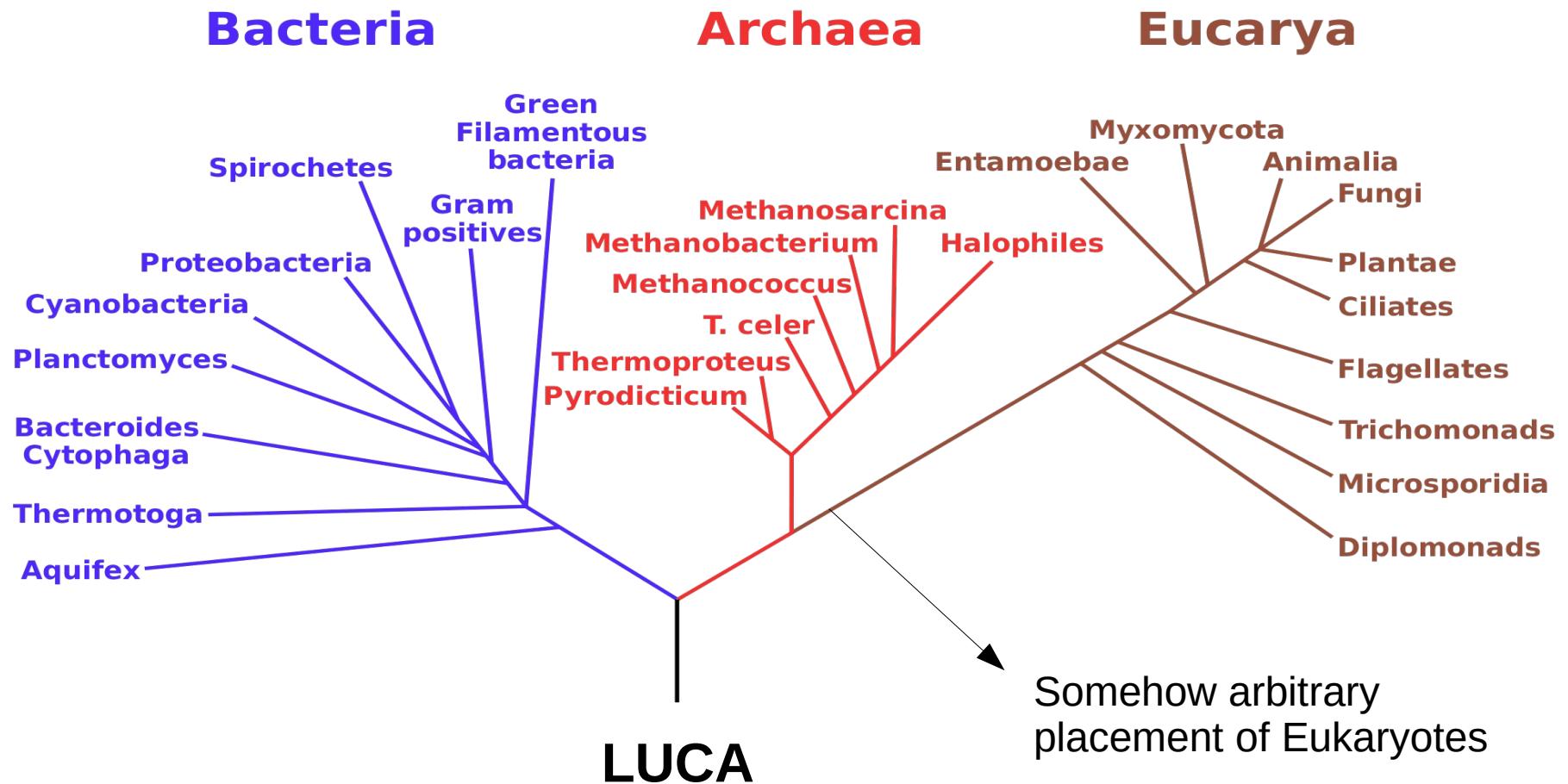
The discovery was possible thanks to sequencing technology and the realization that 16S rRNA could be used as a marker gene for diversity

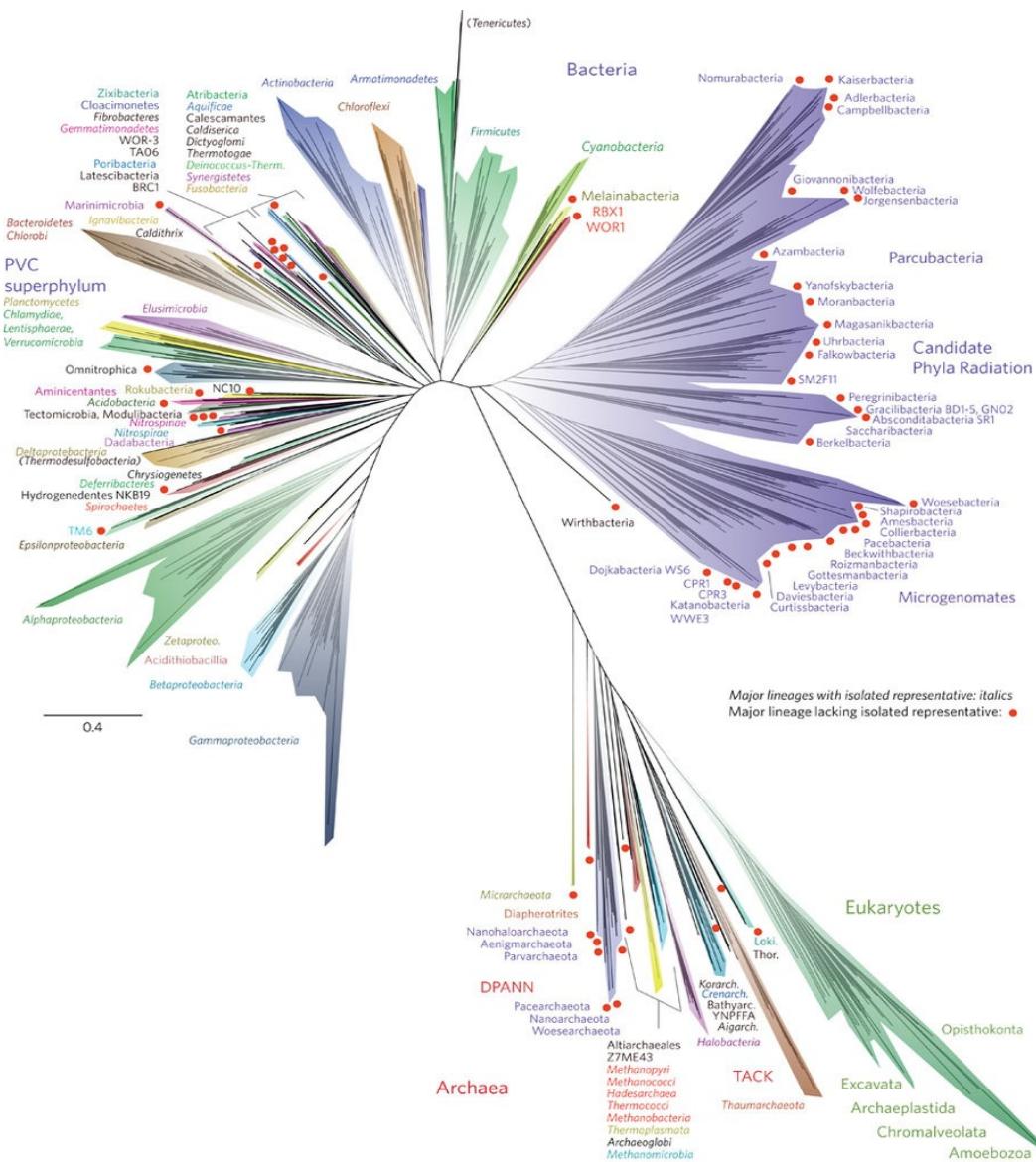


The tree of life: post 1977 (textbook version)



The tree of life: post 1977 (textbook version)





A more accurate view of the tree of life

All the branches with a red dot represent organisms that have no known cultivated representative, and for which their environmental role is often unknown or uncertain.

There are more uncultured phyla of Bacteria and Archaea than there are cultured ones.

Diverse uncultivated ultra-small bacterial cells in groundwater

Birgit Luef, Kyle R. Frischkorn, Kelly C. Wrighton, Hoi-Ying N. Holman, Giovanni Birarda, Brian C. Thomas, Andrea Singh, Kenneth H. Williams, Cristina E. Siegerist, Susannah G. Tringe, Kenneth H. Downing, Luis R. Comolli & Jillian F. Banfield

[Affiliations](#) | [Contributions](#) | [Corresponding authors](#)

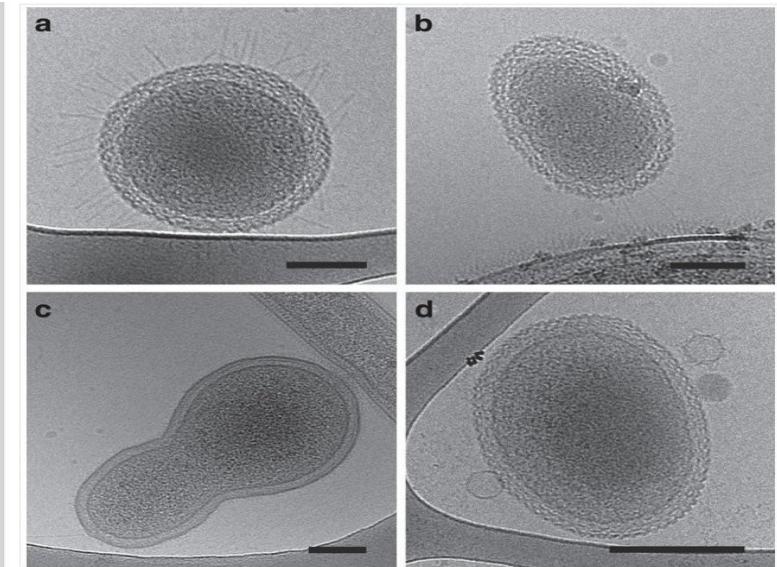
Nature Communications 6, Article number: 6372 | doi:10.1038/ncomms7372

Received 08 August 2014 | Accepted 23 January 2015 | Published 27 February 2015

OP11, DO1 was detected using metagenomic analysis of 0.2 µm filtered water

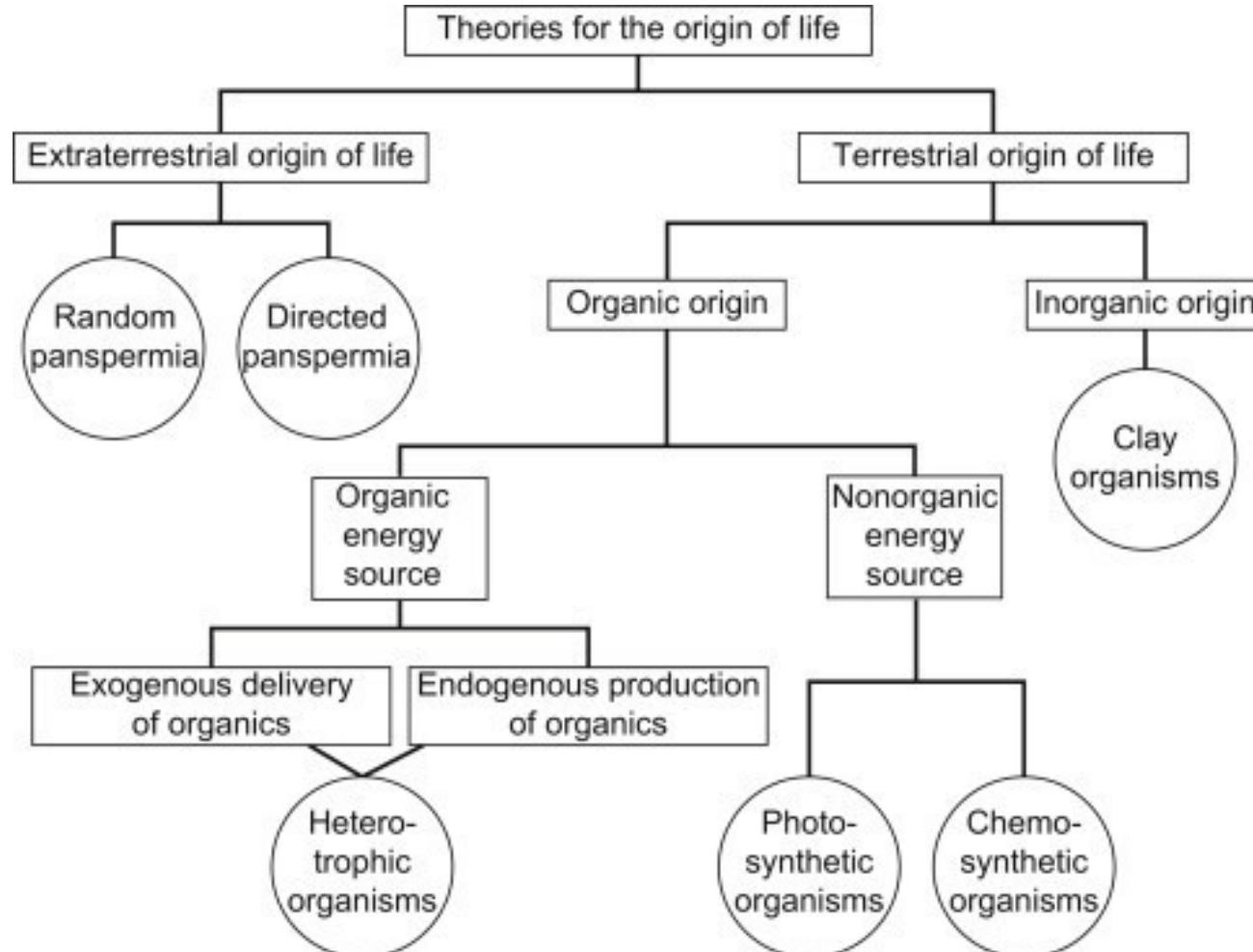
The genome size is < 1 Mbp

These bacteria rely on other community members for basic resources

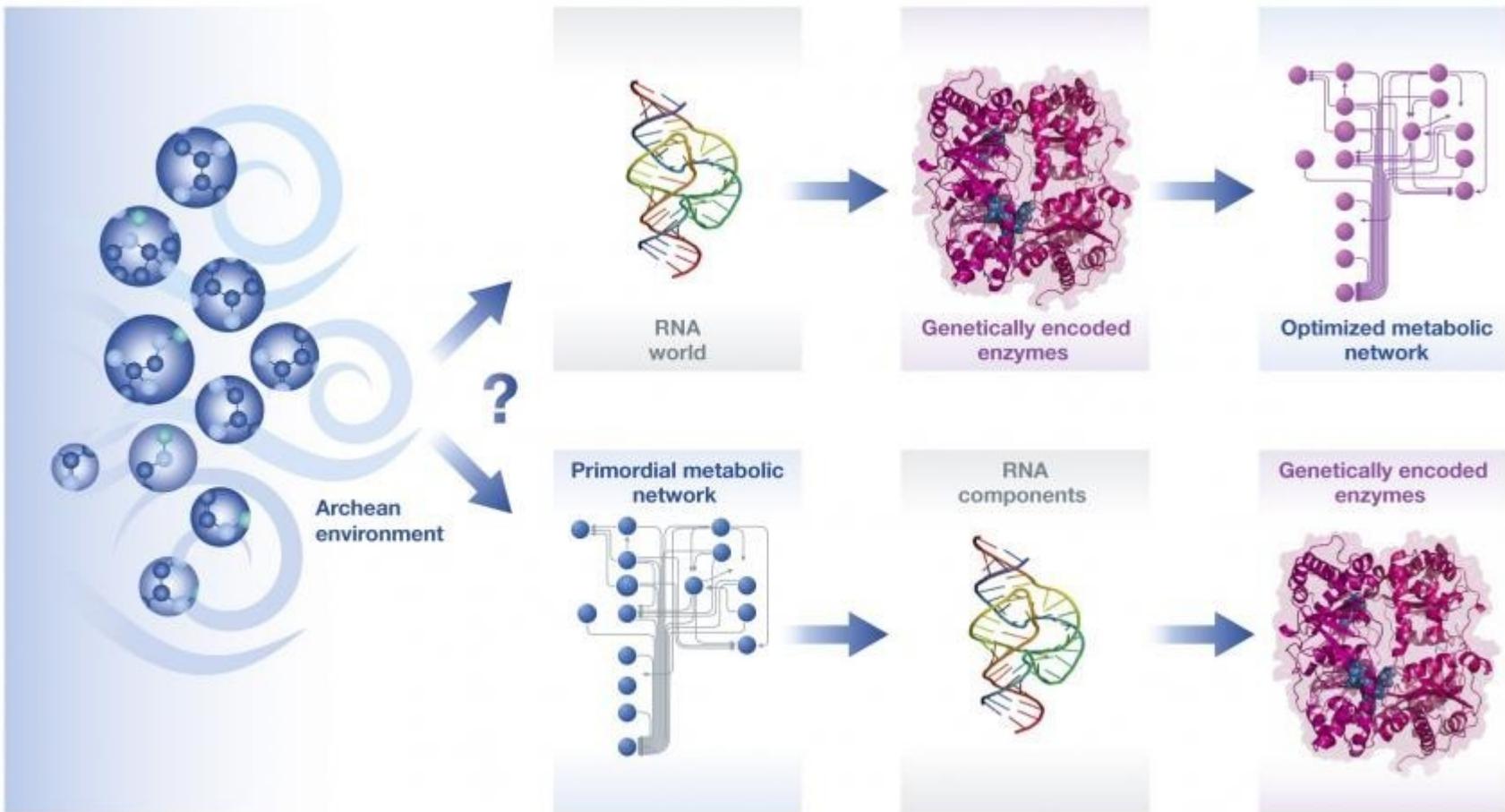


The cell envelope includes a remarkable and distinct S-layer. Pilus-like structures are clearly discernible: numerous radiating pilus-like structures cover the surface of the cell in **a**, whereas polar pilus-like structures occur on the cell in **b**, apparently connecting it to an adjacent bacterium (only part of the bacterium shown). (**c**) A dividing ultra-small bacterium in contact with a Spirochete cell (only small region shown; also see Supplementary Fig. 14). Note the contrast at the interface, suggesting cell-to-cell interaction. Three bacteriophages are associated with the surface of the cell in **d**. Scale bars, 100 nm.

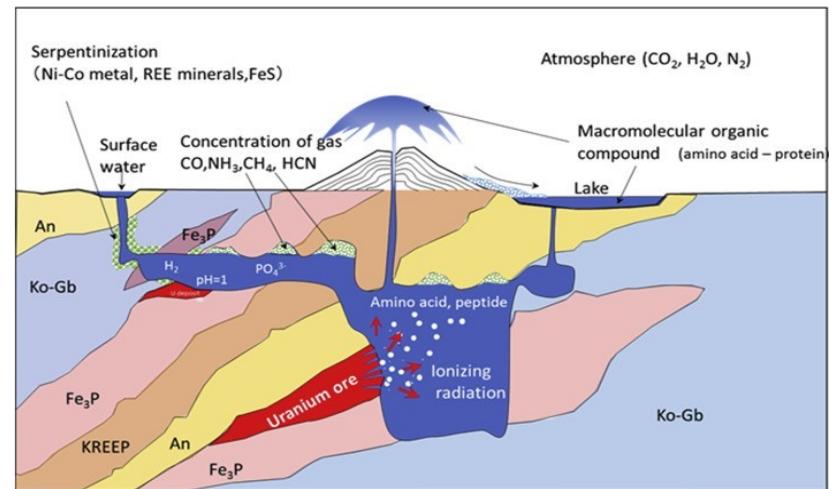
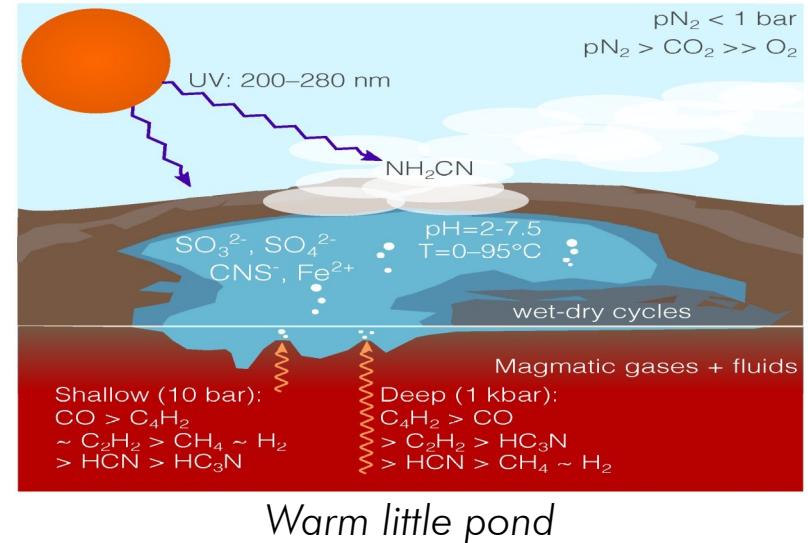
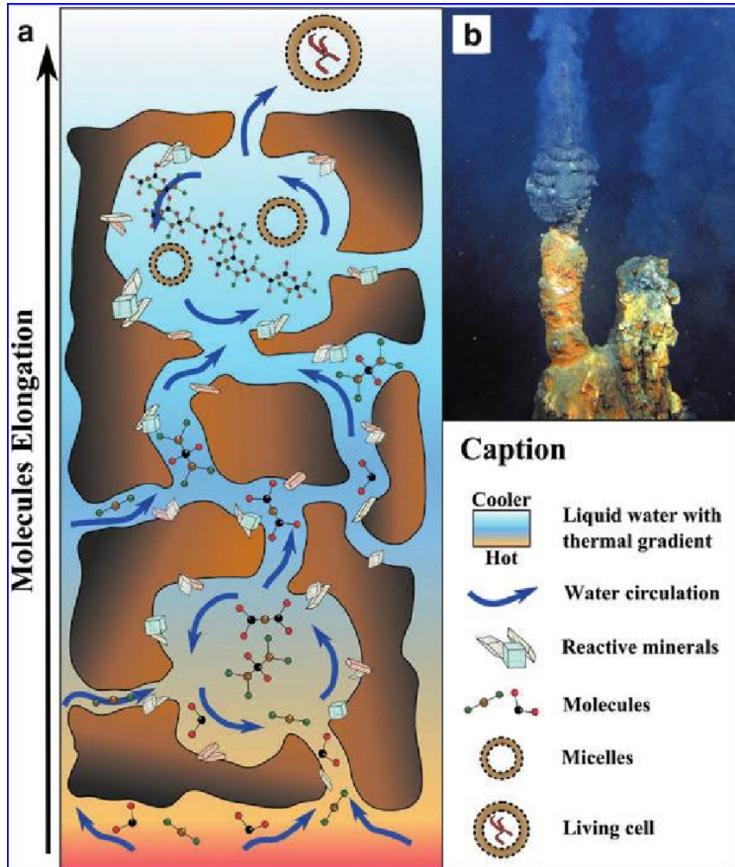
Origin of Life



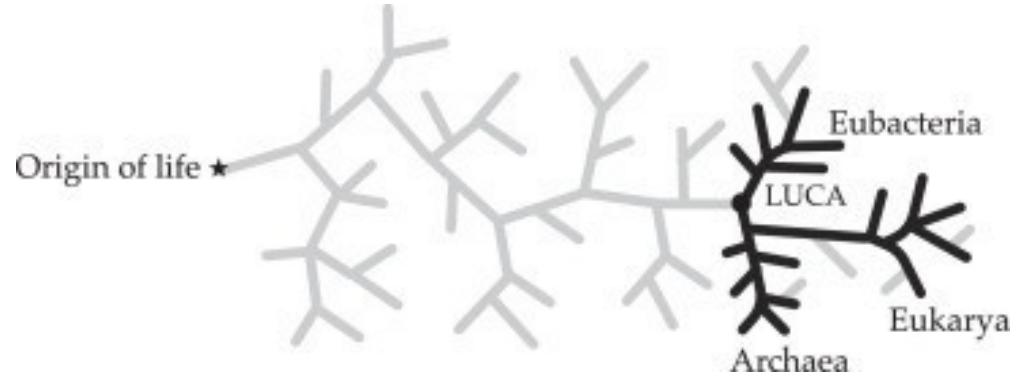
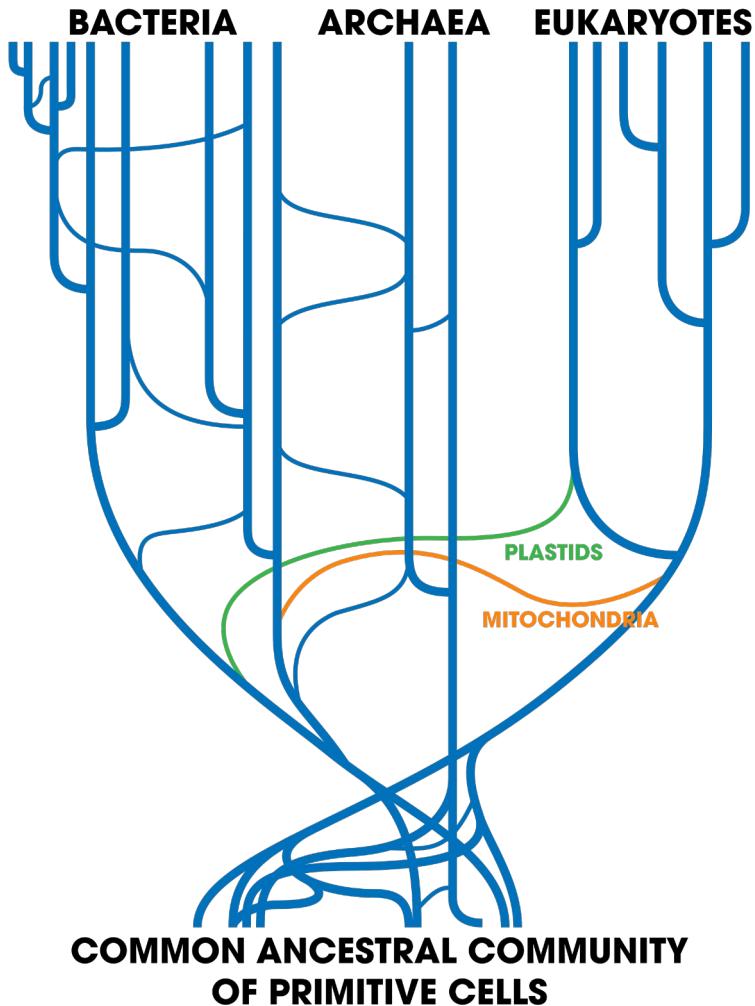
Origin of Life



Origin of Life Scenarios

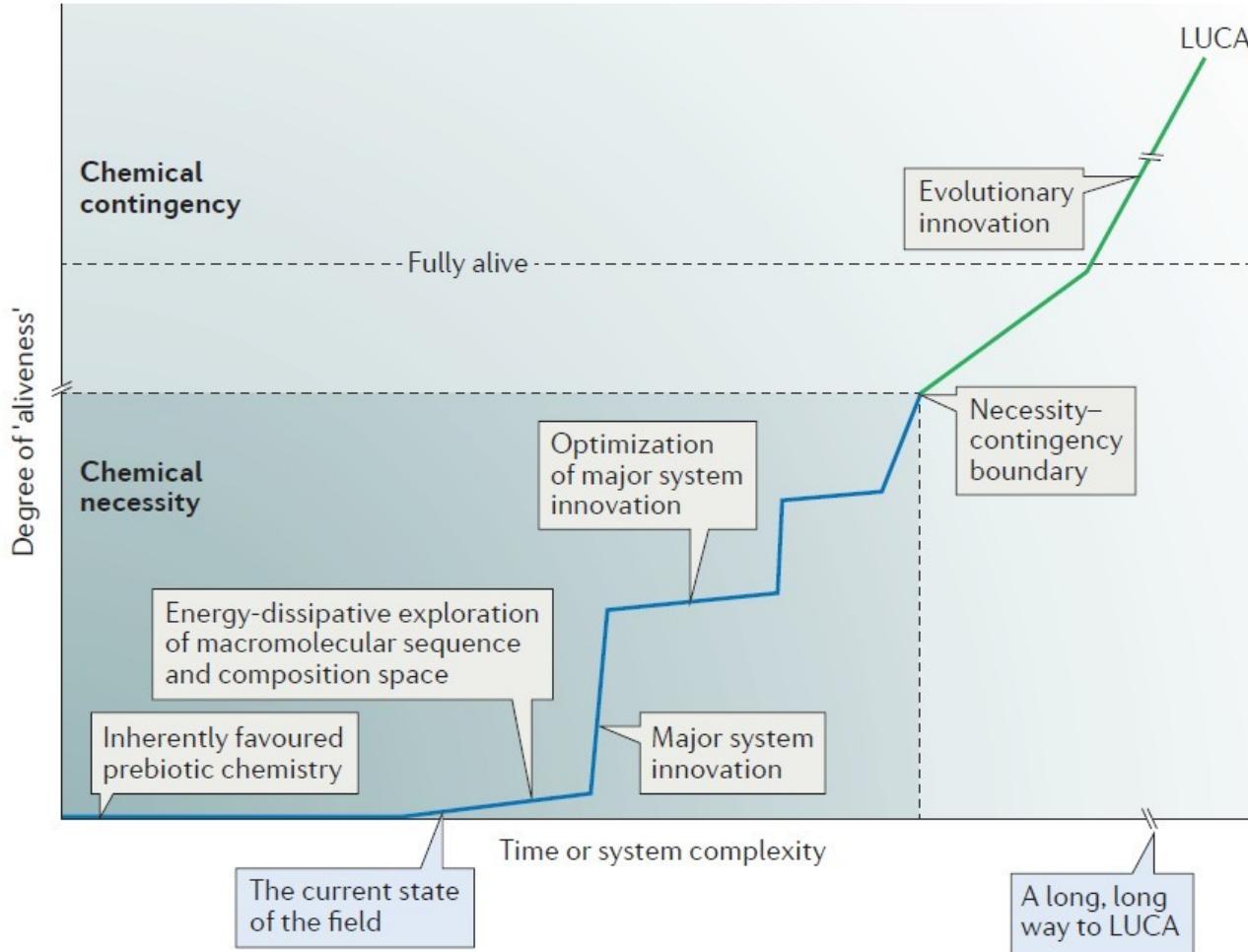


Last Universal Common Ancestor (Survivor?)



LUCA, the Last Universal Common Ancestor, is an operational definition that assumes a single type of organisms of bottleneck for all life. What about Viruses? What about other possible life form? Should we speak about Last Universal Common Survivors (LUCS) instead?

Last Universal Common Ancestor (Survivor?)



Endosymbiotic theory and Eukaryogenesis

Symbiogenesis, or endosymbiotic theory (Mereschowsky 1910 and Lynn Margulis in 1967) is a theory on the evolution of eukaryotes.

Primary endosymbiosis is the process in which a eukaryote engulfs another living prokaryote.

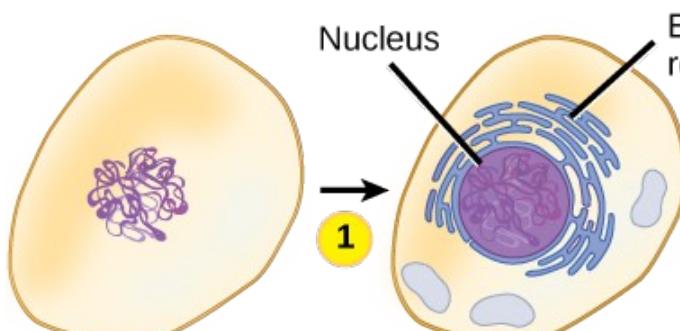
The mitochondria and plastids (for example chloroplasts), and possibly other organelles of eukaryote cells are thought to represent formerly free-living prokaryotes taken one inside the other in endosymbiosis around 1.5 billion years ago.

Molecular and biochemical evidence suggest that mitochondria developed from Proteobacteria (Alphaproteobacteria, like SAR11) and chloroplasts from Cyanobacteria (in particular, nitrogen-fixing filamentous cyanobacteria).

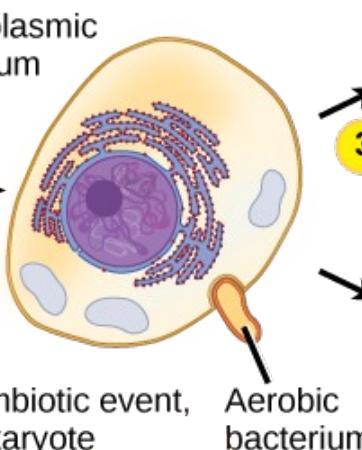
Endosymbiotic theory and Eukaryogenesis

The ENDOSYMBIOTIC THEORY

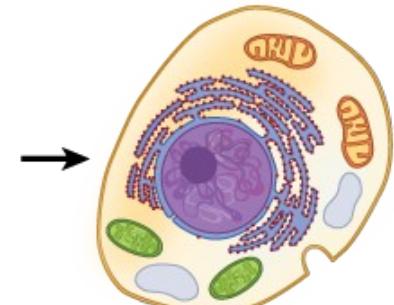
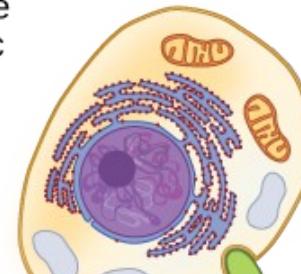
1 Infoldings in the plasma membrane of an ancestral prokaryote gave rise to endomembrane components, including a nucleus and endoplasmic reticulum.



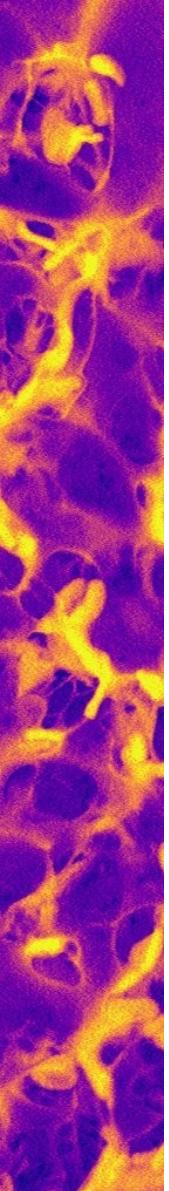
3 In a second endosymbiotic event, the early eukaryote consumed photosynthetic bacteria that evolved into chloroplasts.



2 In a first endosymbiotic event, the ancestral eukaryote consumed aerobic bacteria that evolved into mitochondria.



Modern heterotrophic eukaryote



Endosymbiotic theory: supporting evidences

- Both mitochondria and chloroplasts can arise only from preexisting mitochondria and chloroplasts. They cannot be formed in a cell that lacks them because nuclear genes encode only some of the proteins of which they are made.
- Both mitochondria and chloroplasts have their own genome, and it resembles that of bacteria not that of the nuclear genome.
- Both genomes consist of a single circular molecule of DNA.
- There are no histones associated with the DNA.
- Both mitochondria and chloroplasts have their own protein-synthesizing machinery, and it more closely resembles that of bacteria than that found in the cytoplasm of eukaryotes, and are sensitive to a number of antibiotics (e.g. streptomycin, rifampicin).
- Genes for the functioning of both mitochondria and chloroplast are shared between their own genomes and the nuclear genomes.

Alternative Eukaryogenesis theories

In 1996, Woese and colleagues published in the journal Science the first complete genome structure of an archaeon, *Methanococcus jannaschii*.

Based on this work, they concluded that the Archaea are more closely related to humans than to bacteria. "The Archaea are related to us, to the eukaryotes; they are descendants of the microorganisms that gave rise to the eukaryotic cell billions of years ago" Woese said at the time.

This led to the current idea that the protoeukaryotes and the Archaea had a common ancestor, therefore the placement of the Eukaryotes on the tree of life. New data has changed this view...

Eukaryogenesis: modern view



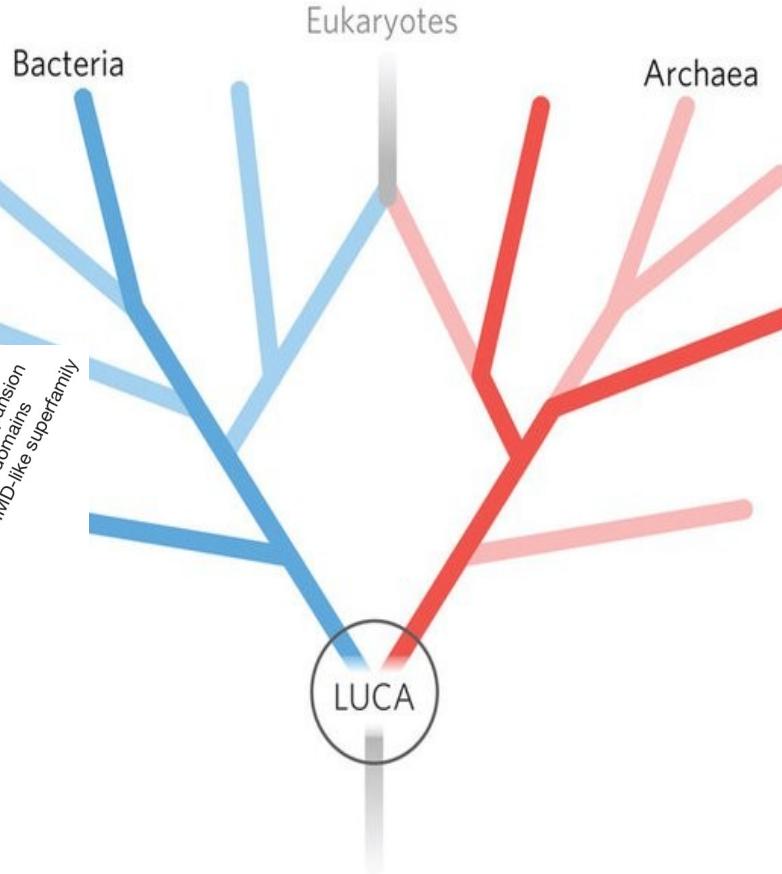
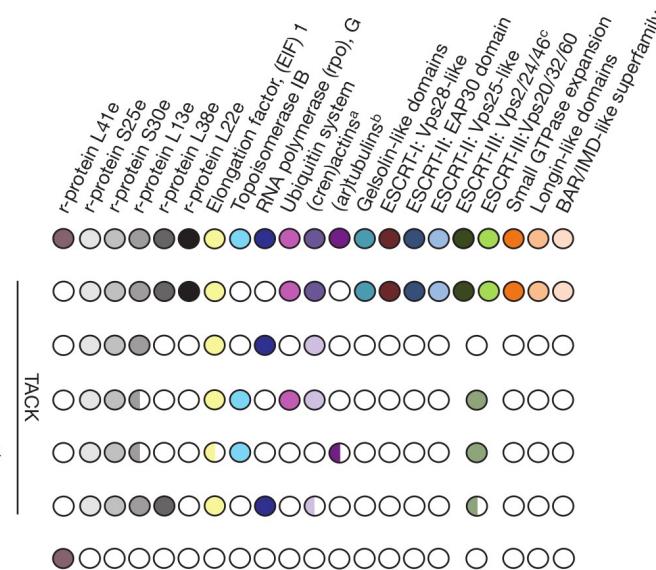
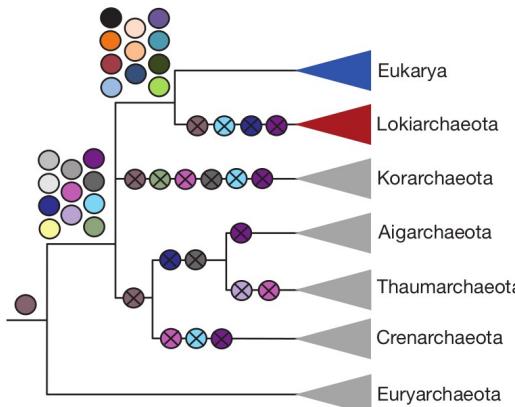
nature
International journal of science

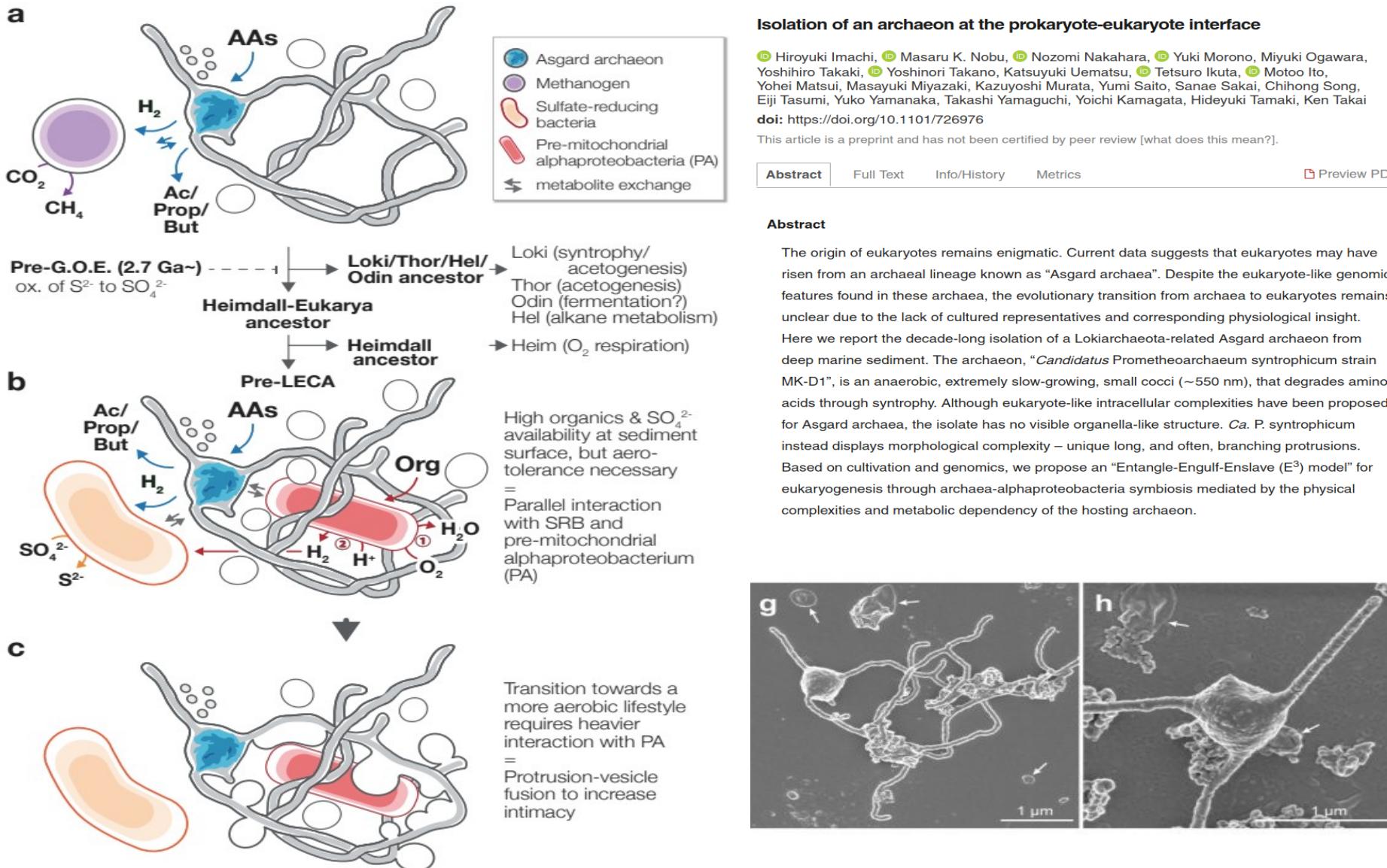
Article | Published: 06 May 2015

Complex archaea that bridge the gap between prokaryotes and eukaryotes

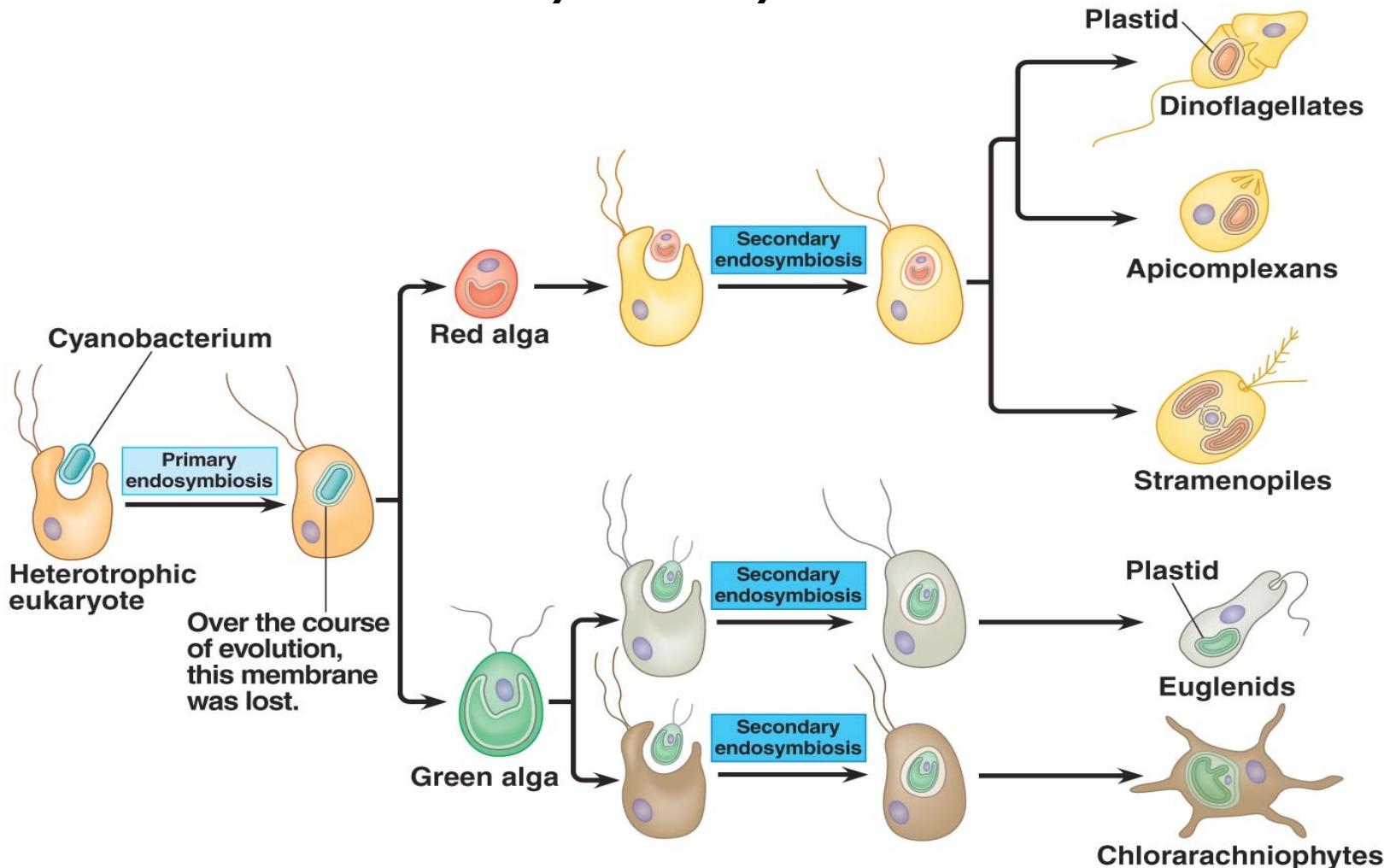
Anja Spang, Jimmy H. Saw, Steffen L. Jørgensen, Katarzyna Zaremba-Niedzwiedzka, Joran Martijn,
Anders E. Lind, Roel van Eijk, Christa Schleper, Lionel Guy & Thijs J. G. Ettema

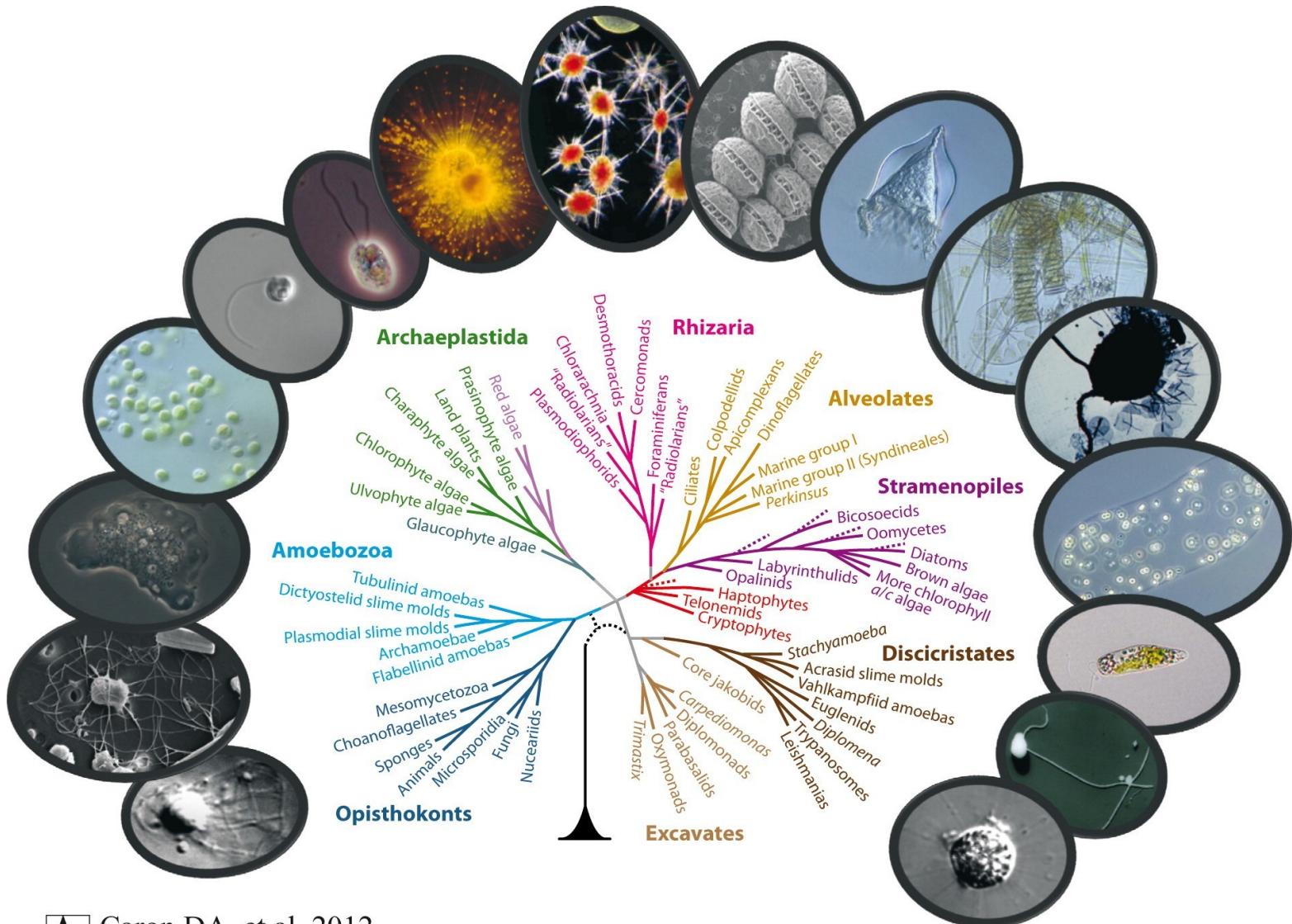
Nature 521, 173–179 (14 May 2015) | Download Citation ↴

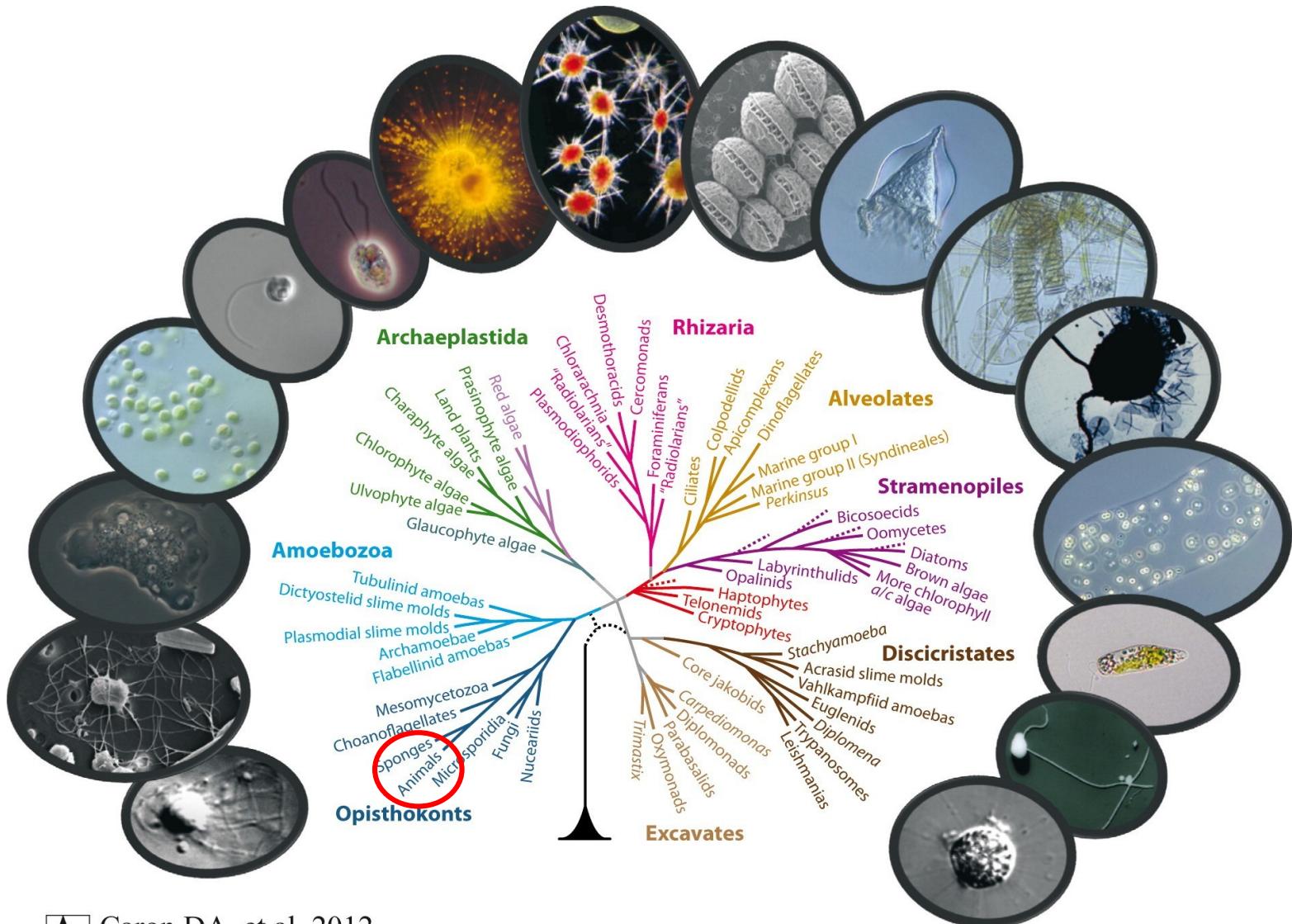




Secondary endosymbiosis

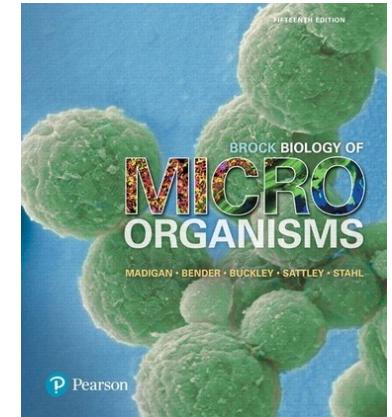




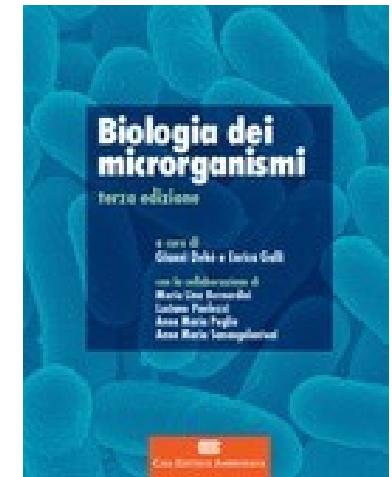


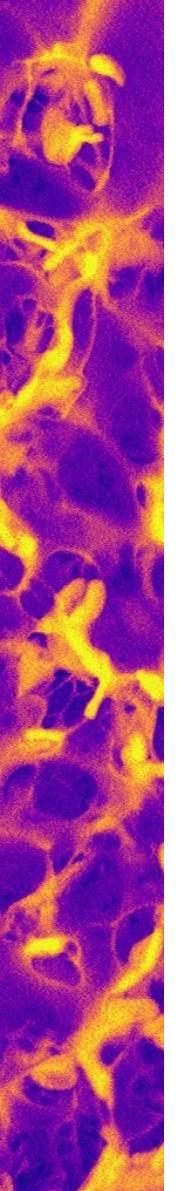
Books suggestion so far:

Brock Biology of Microorganisms (15th Edition)
Chapter 13



Dehò Galli Biologia dei Microorganismi (2019 Edition)
Chapter 16



A vertical strip of a microscopic image showing various microorganisms, possibly bacteria or fungi, with a color palette ranging from purple to yellow.

MICROBIAL ECOLOGY: A VERY BRIEF GUIDE

Ecosystems

ecosystem

/'i:kəʊsɪstəm/

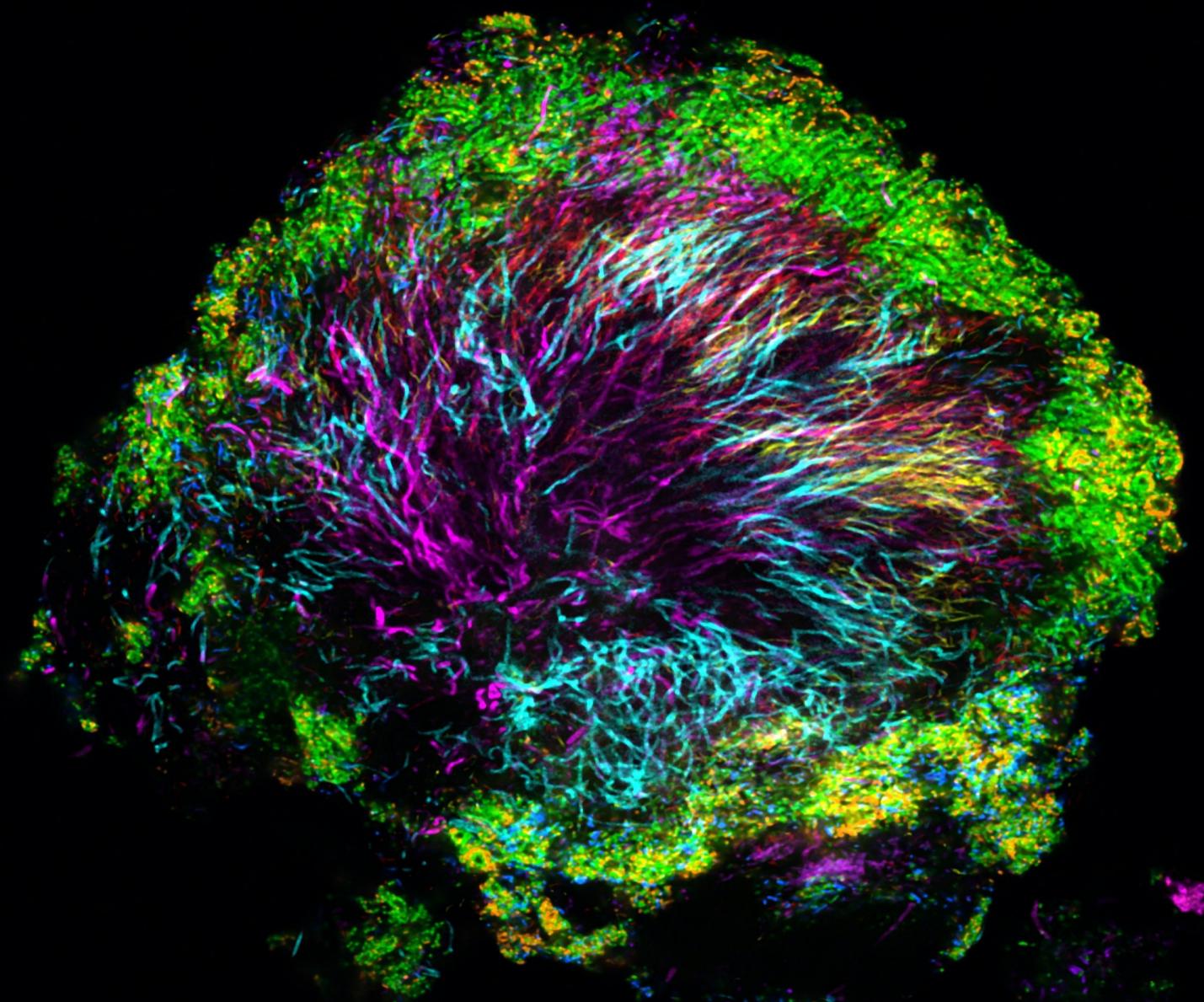
Noun

composite from greek oikos (οἶκος, family, family property, house) and the english system. Oikos was the basic unit of greek society.

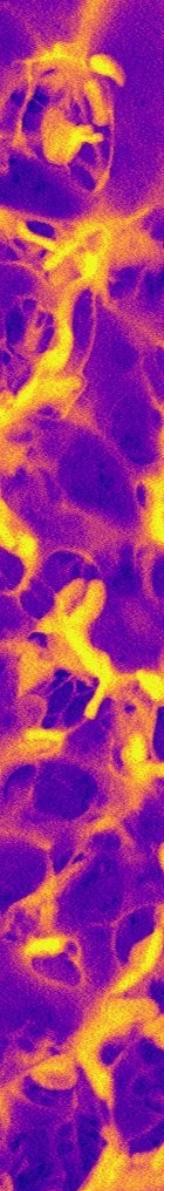
- a biological community of interacting organisms and their physical environment
- (in general use) a complex network or interconnected system

First used in 1935 by Sir Arthur George Tansley, ecosystem







A vertical strip on the left side of the slide showing a microscopic view of microorganisms, possibly bacteria or yeast, with various shapes and colors including yellow, orange, and purple.

Ecosystems

The definition of an ecosystem is arbitrary, and its boundaries can be artificially altered to match the desired research question.

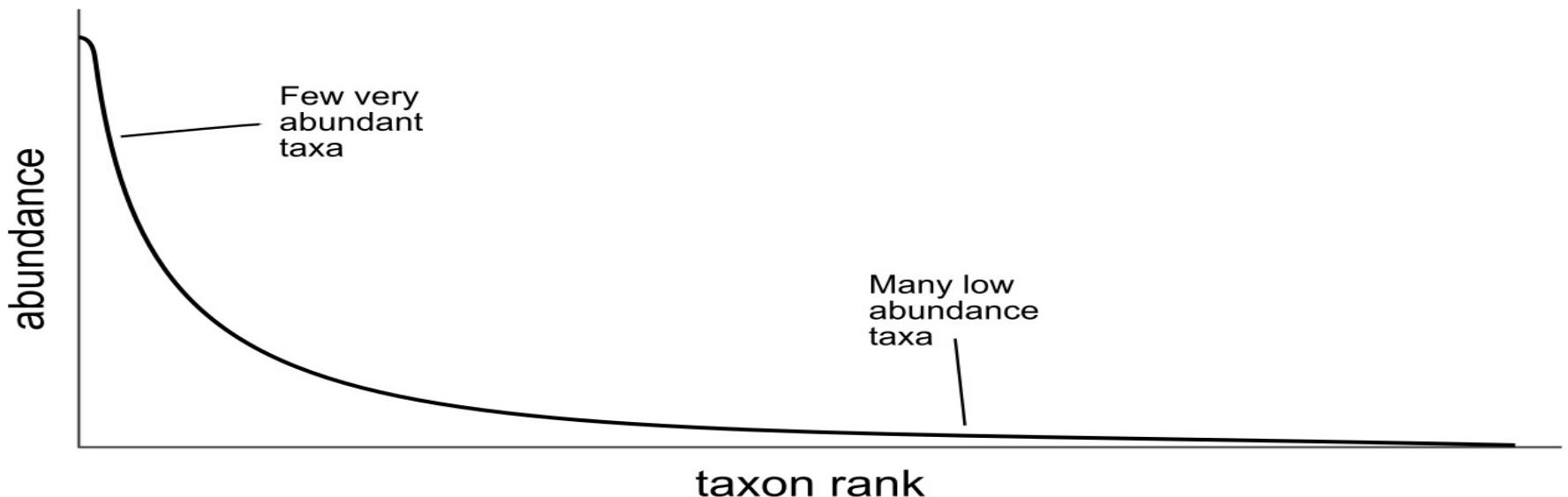
When investigating the microbial world, we need to keep into account what the microbes experience in the environments with respect to our perception of an ecosystem.

Life at “low Reynolds numbers” presents a completely different set of problems and necessary adaptations.

When taking macroscopic and microscopic drivers into account for the definition of the basic characteristics and processes of an ecosystem, the “microscale” becomes an important starting point.

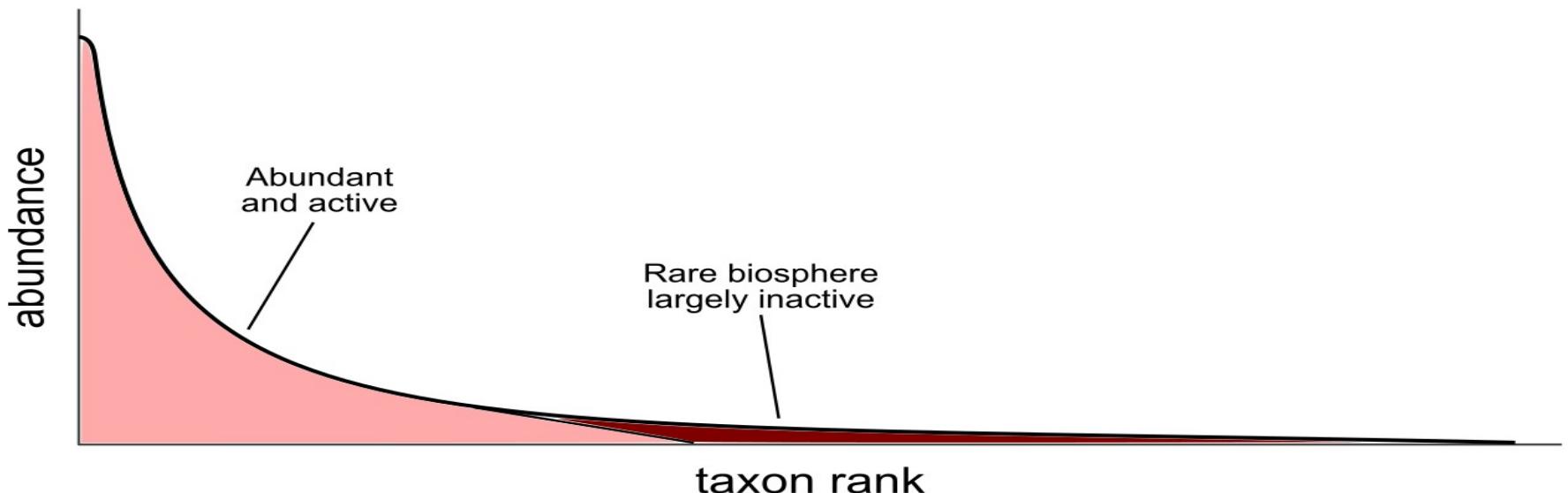
Community structure

Microbial seed-banks theory. Relatively few microbial taxa are abundant and active, while the large majority of the diversity is constituted by a large number of low abundance taxa, often in a dormancy state. Dormancy is a bet-hedging strategy. Dormant microorganisms generate a seed bank capable of being resuscitated following environmental change



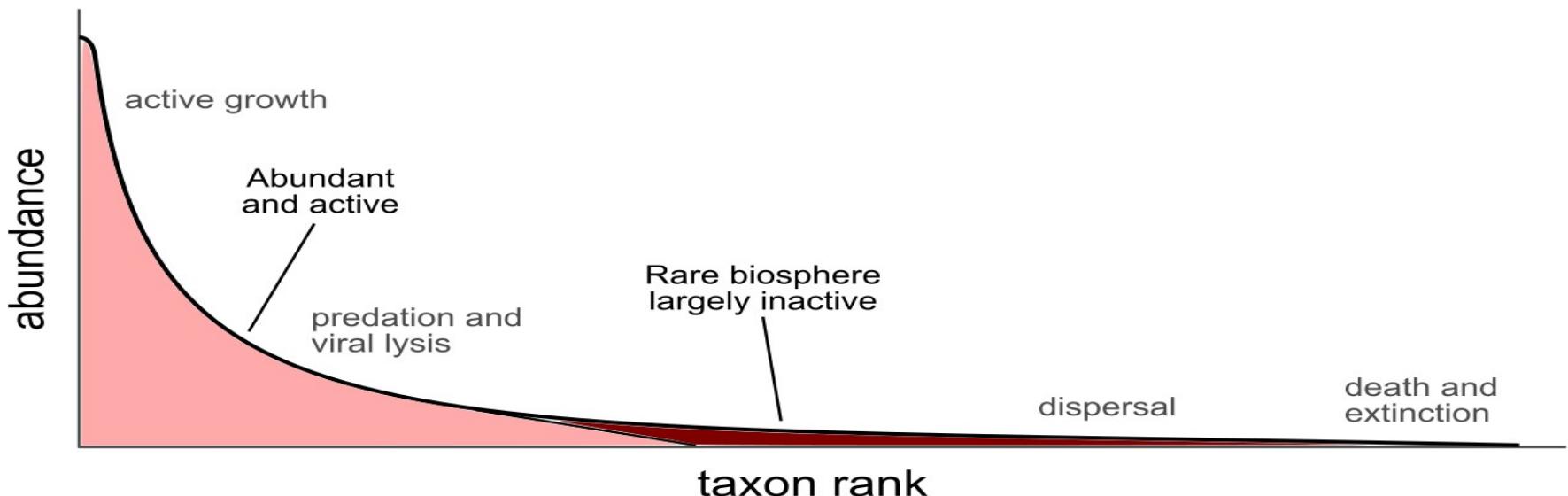
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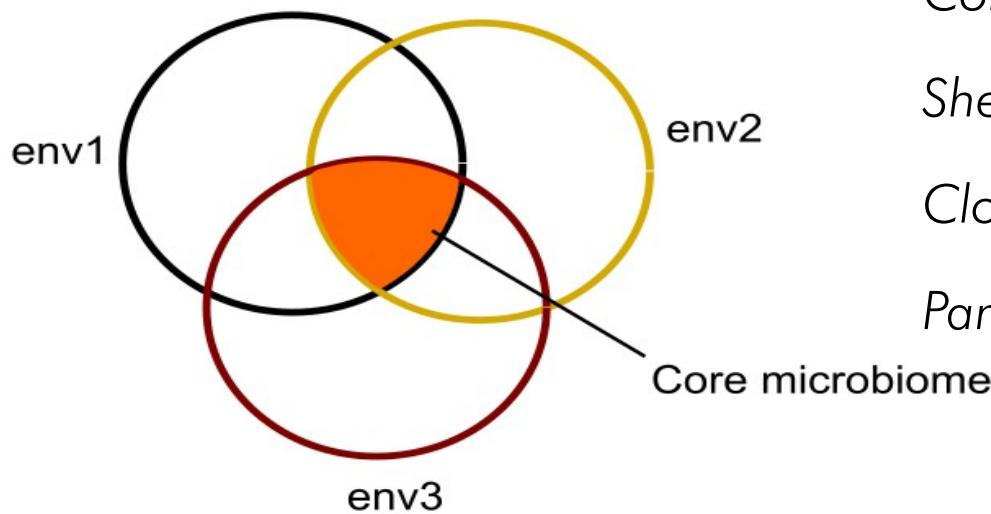


Community structure

Microbial seed-banks theory. Relatively few microbial taxa are abundant and active, while the large majority of the diversity is constituted by a large number of low abundance taxa, often in a dormancy state. Dormancy is a bet-hedging strategy. Dormant microorganisms generate a seed bank capable of being resuscitated following environmental change



Common taxa: the core microbiome



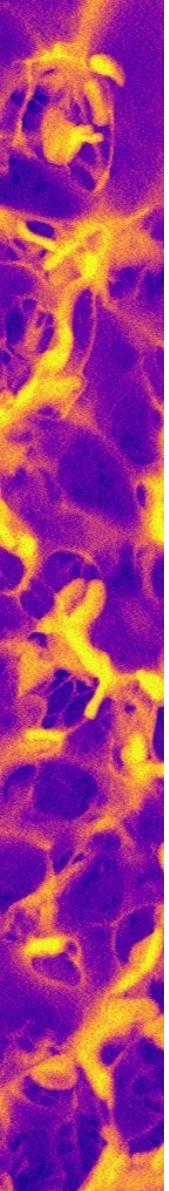
$$\text{Core} = A \cap B \cap C$$

$$\text{Shell} = A \cap B + A \cap C + B \cap C$$

$$\text{Cloud} = (A \cup B + A \cup C + B \cup C) - (A \cap B \cap C)$$

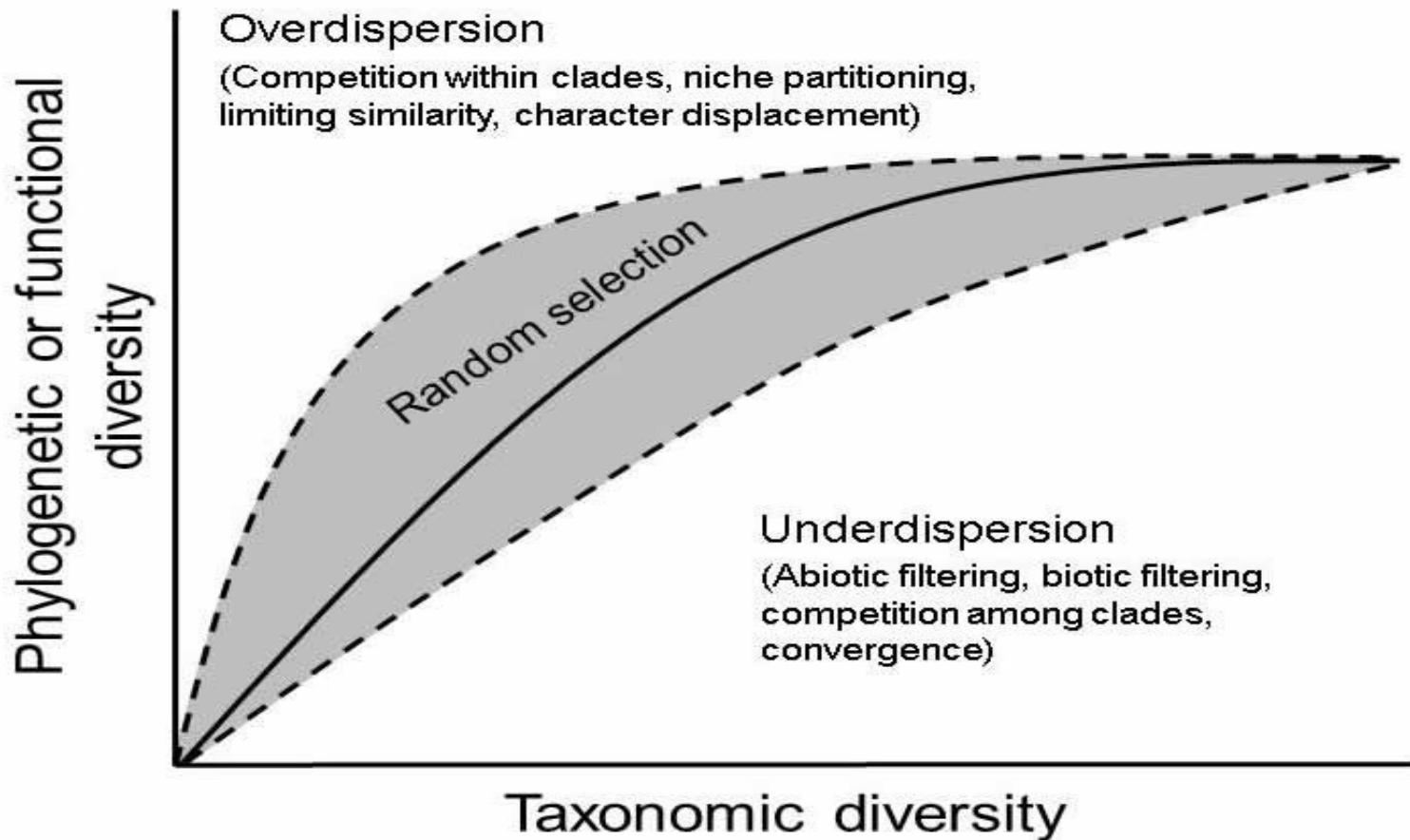
$$\text{Pan} = A \cup B \cup C$$

The **core microbiome** (or microbiota) is the taxa present in all the observed samples. It can be defined **across an environment** (core microbiome in surface sediments), for a **selected area** (the core microbiome of the Antarctic waters) or for a **specific functions** (the core microbiome carrying out nitrogen fixation). A similar concept can be used for functional diversity (the core functions instead of taxa). It can be seen as the deterministic portion of the community.



Ecosystem Functions (microbial)

Functional redundancy



Ecosystem Functions

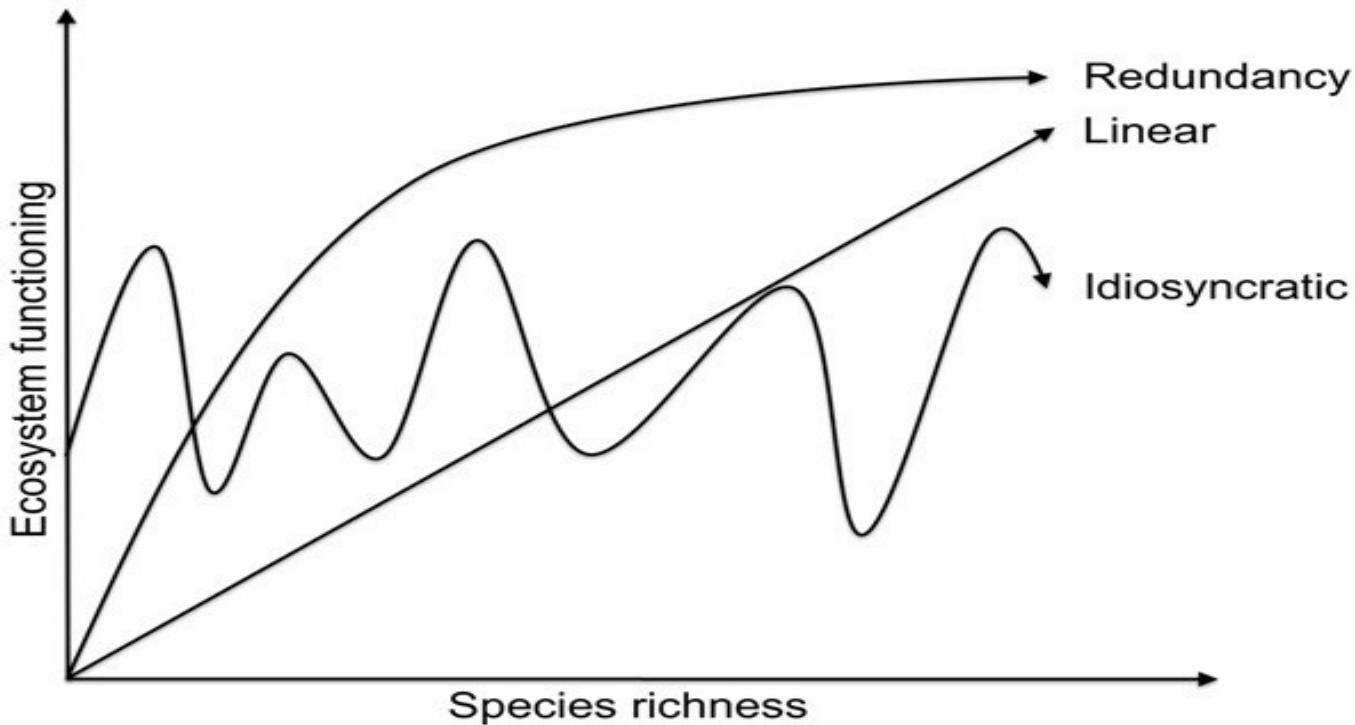
'Ecosystem function' is the technical term used in the framework to define the biological, geochemical and physical processes and components that take place or occur within an ecosystem

Or more simply put, ecosystem functions relate to the structural components of an ecosystem (e.g. vegetation, water, soil, atmosphere and biota) and how they interact with each other, within ecosystems and across ecosystems

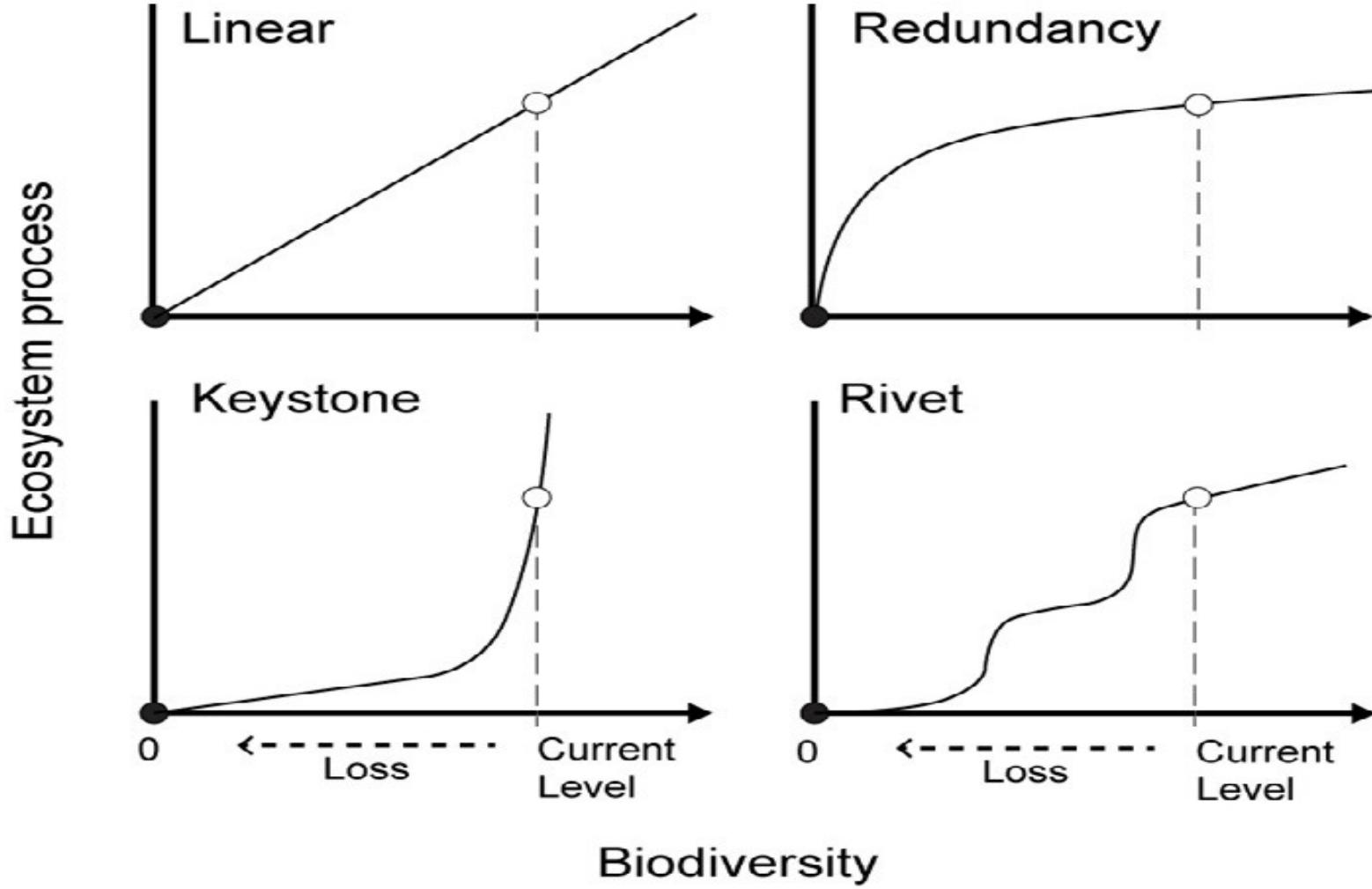
Sometimes, ecosystem functions are called ecological processes. Different ecosystem functions provide ecosystem services to humans and the environment alike.

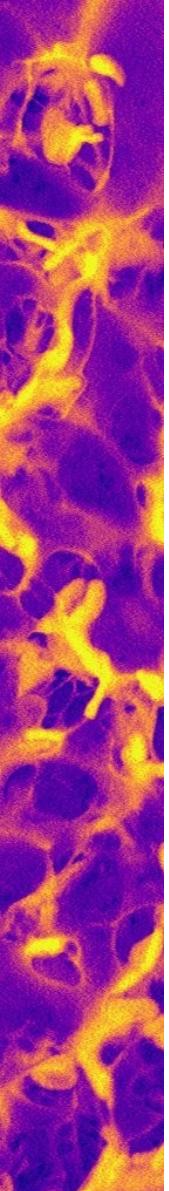
Given the key role of microorganisms in element and nutrient cycling the ecosystem functions provided by the microbial community (and by specific groups of microbes) are fundamental for the functioning of the ecosystem.

Biodiversity-ecosystem function relationship



The relationship between **biodiversity** and **ecosystem functioning** (called **BEF** relation) are widely studied to understand the role of different taxa and the overall functioning and vulnerability of an ecosystem

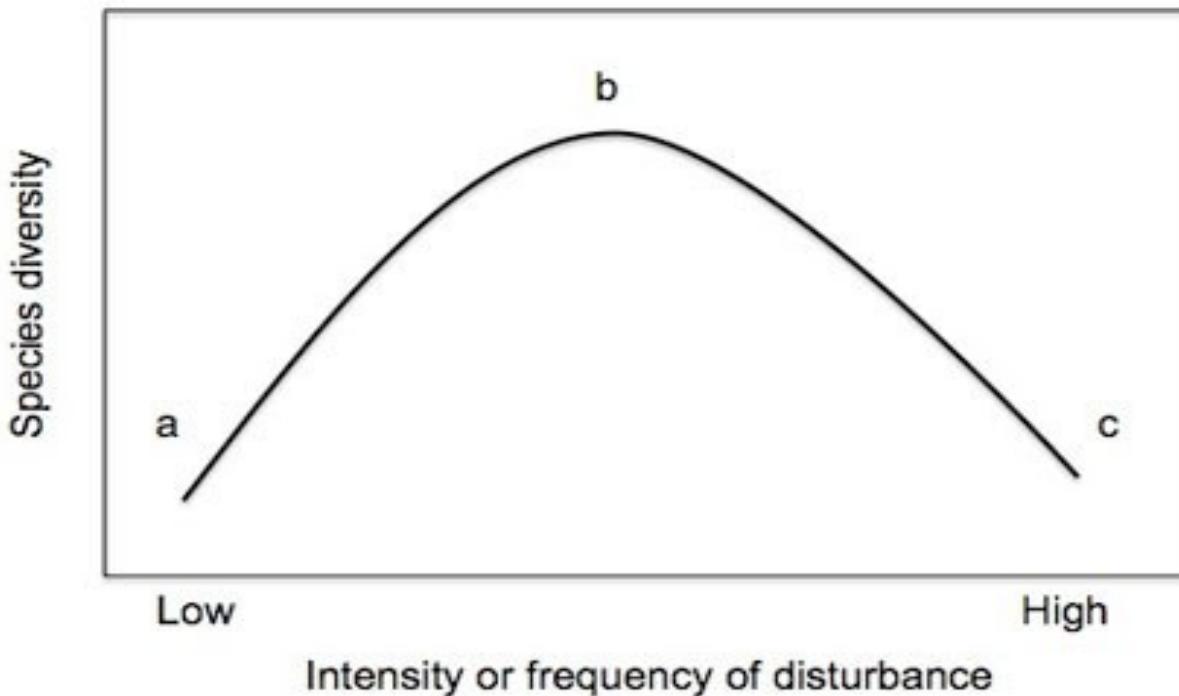


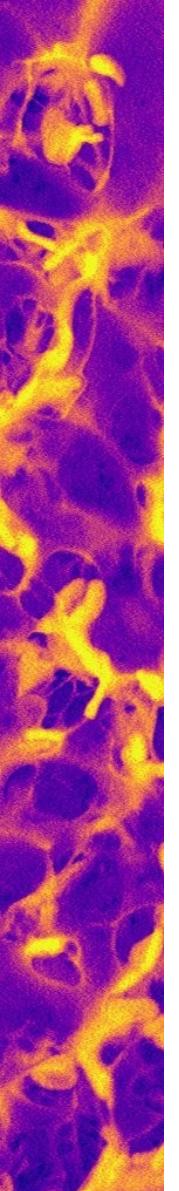


Disturbance theory and ecosystem recovery

Intermediate disturbance hypothesis

The intermediate disturbance hypothesis (IDH) suggests that local species diversity is maximized when ecological disturbance is neither too rare nor too frequent

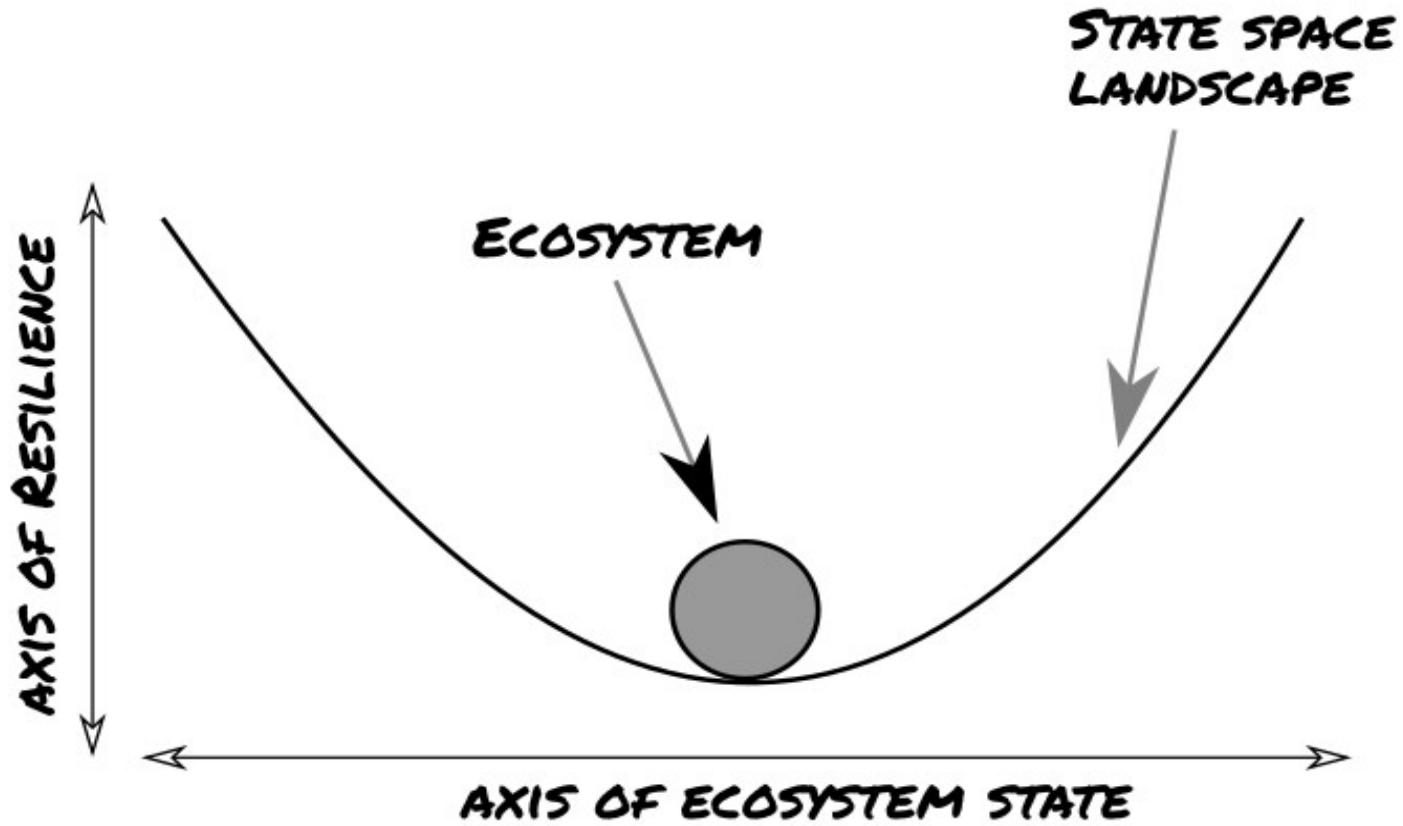


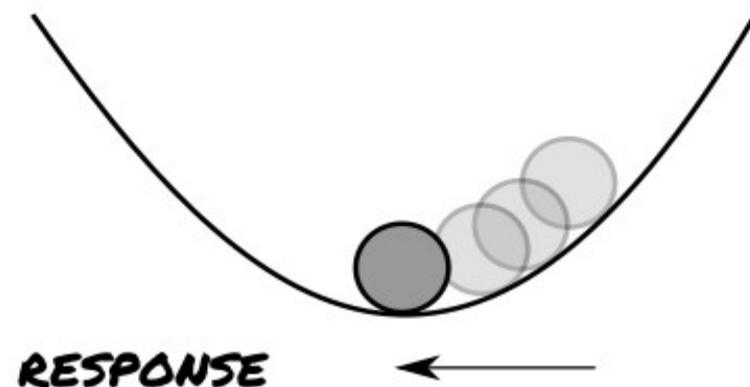
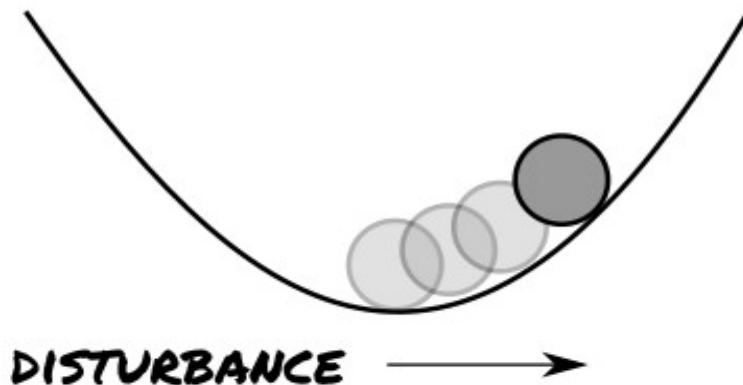


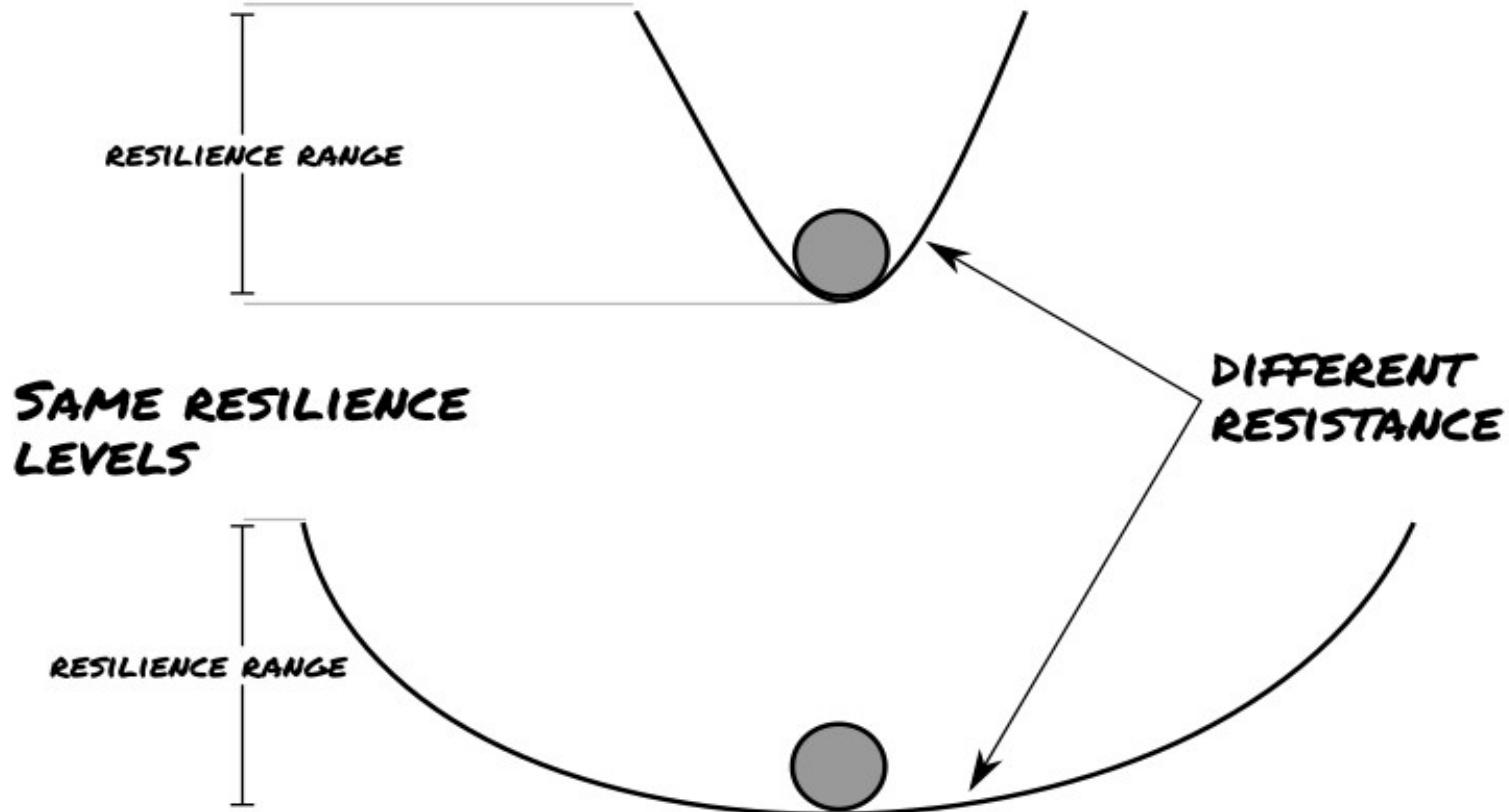
For each ecosystem we can define two basic properties that describe how an ecosystem responds to forcing factors (i.e. change):

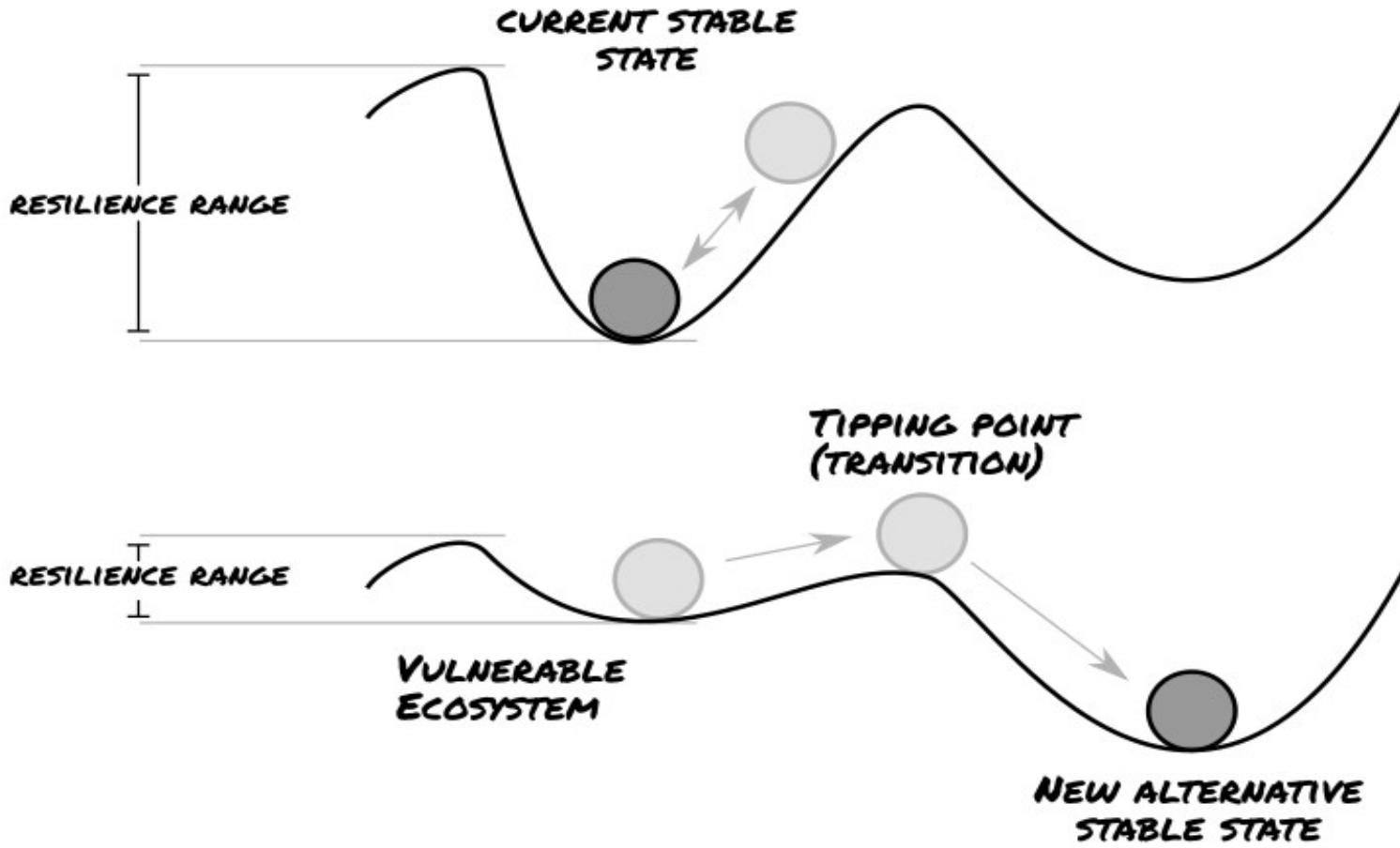
Resistance is the ability for an ecosystem to remain unchanged when being subjected to a disturbance or disturbances. Some ecosystems are better at resisting change than others, and therefore have high resistance

Resilience is the ability and rate of an ecosystem to recover from a disturbance and return to its pre-disturbed state. Some ecosystems can shift greatly from their previous state and still return to pre-disturbance conditions. The measure for how far an ecosystem can be shifted from its previous state and still return to normal is called its amplitude









Most ecosystem state are metastable, and can be pushed to a number of alternative (meta)stable states that depend on the nature of the the ecosystem itself

alternative stable states

Recovering coral habitat



Seaweed habitat



Risk factors for *C. difficile* colonization

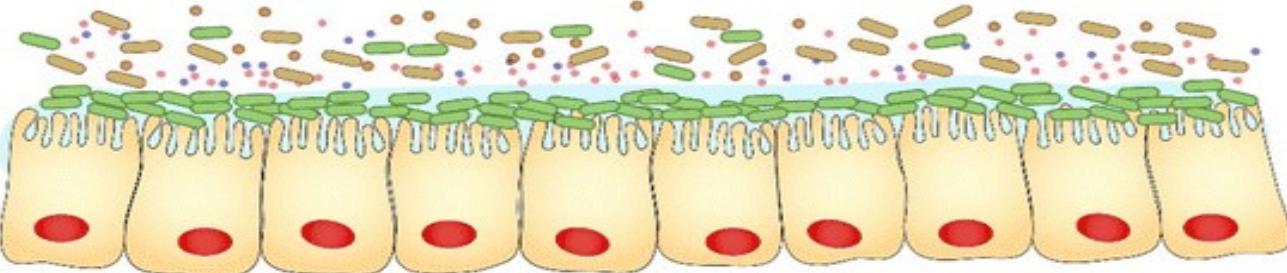
- Previous hospitalization
- Exposure to antibiotics
- Chemotherapy treatment
- Corticosteroid use
- Haemodialysis / renal disease

Exposure to *C. difficile* spores leading to asymptomatic *C. difficile* colonization



Risk factors for symptomatic CDI

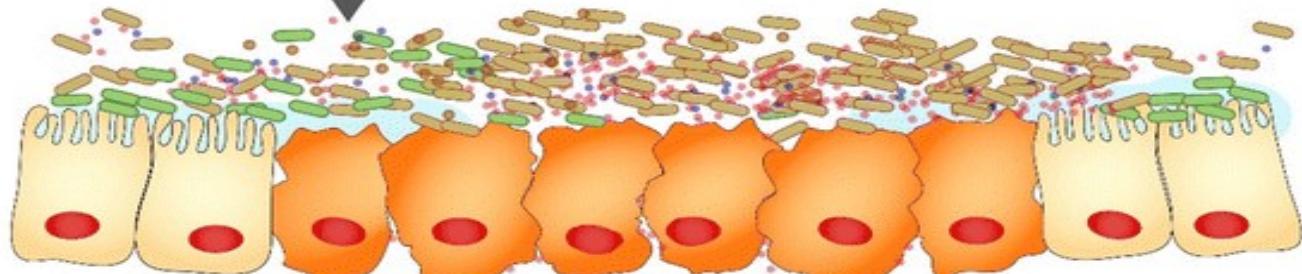
- Increased age
- Exposure and duration of antibiotics
- Presence of nasogastric tube
- Severe underlying disease
- Prolonged hospital admission
- Exposure to drugs that reduce stomach pH



Healthy intestinal epithelial cells with intact microbiome in an individual with asymptomatic *C. difficile* colonization

Factors that protect against progression to symptomatic CDI

- Increased levels of IgG and IgA
- Intact indigenous microbiome
- Colonization by less virulent *C. difficile* strain



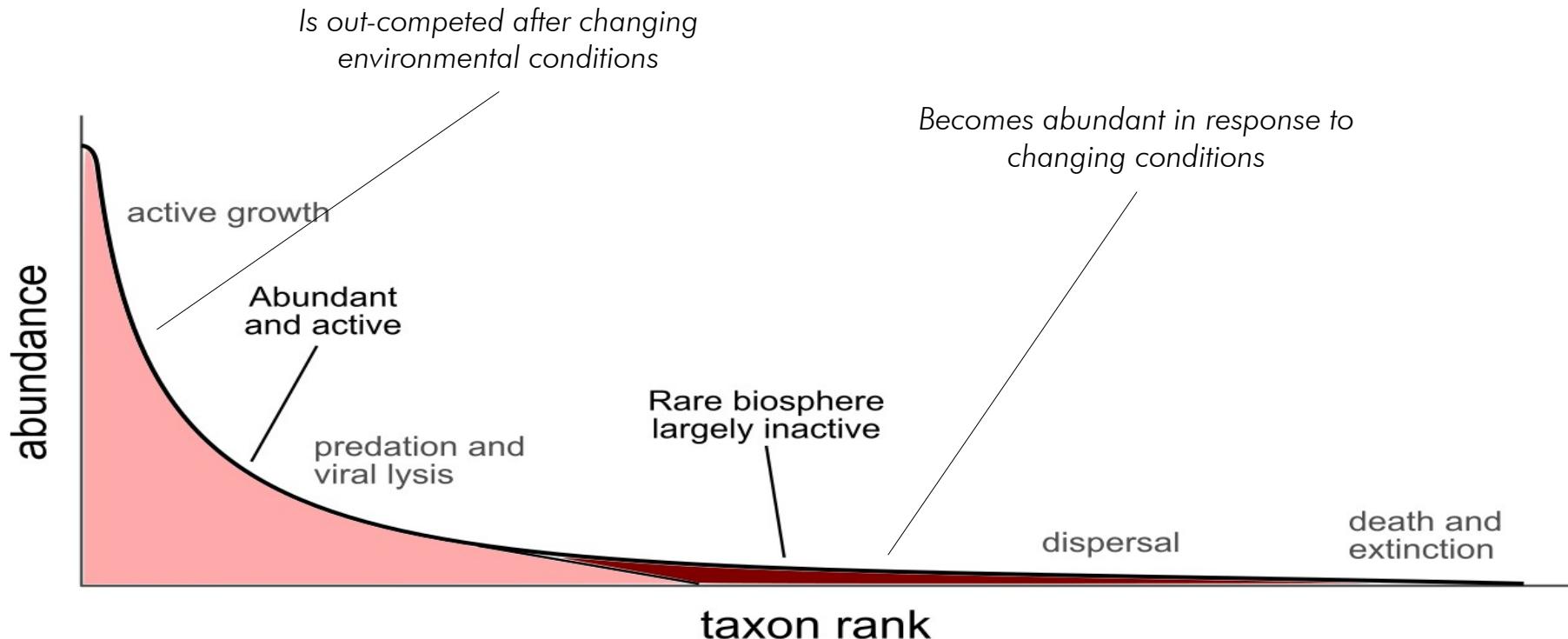
Damaged intestinal epithelial cells in an individual with symptomatic CDI

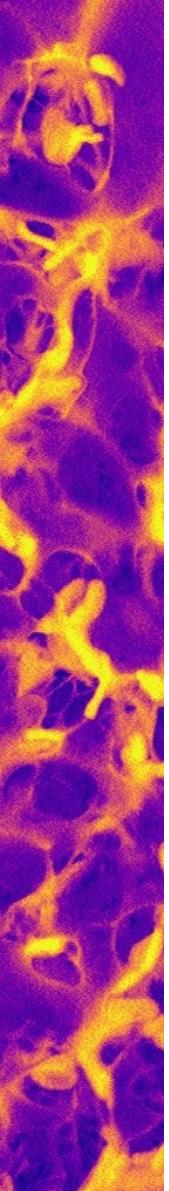
Legend

- Clostridium difficile* cell
- Normal colonic flora
- Clostridium difficile* spore
- Toxin A
- Toxin B



Role of rare and dormant species as a reservoir of functions and adaptations in response to environmental disturbance





Community Controls

Types of Control

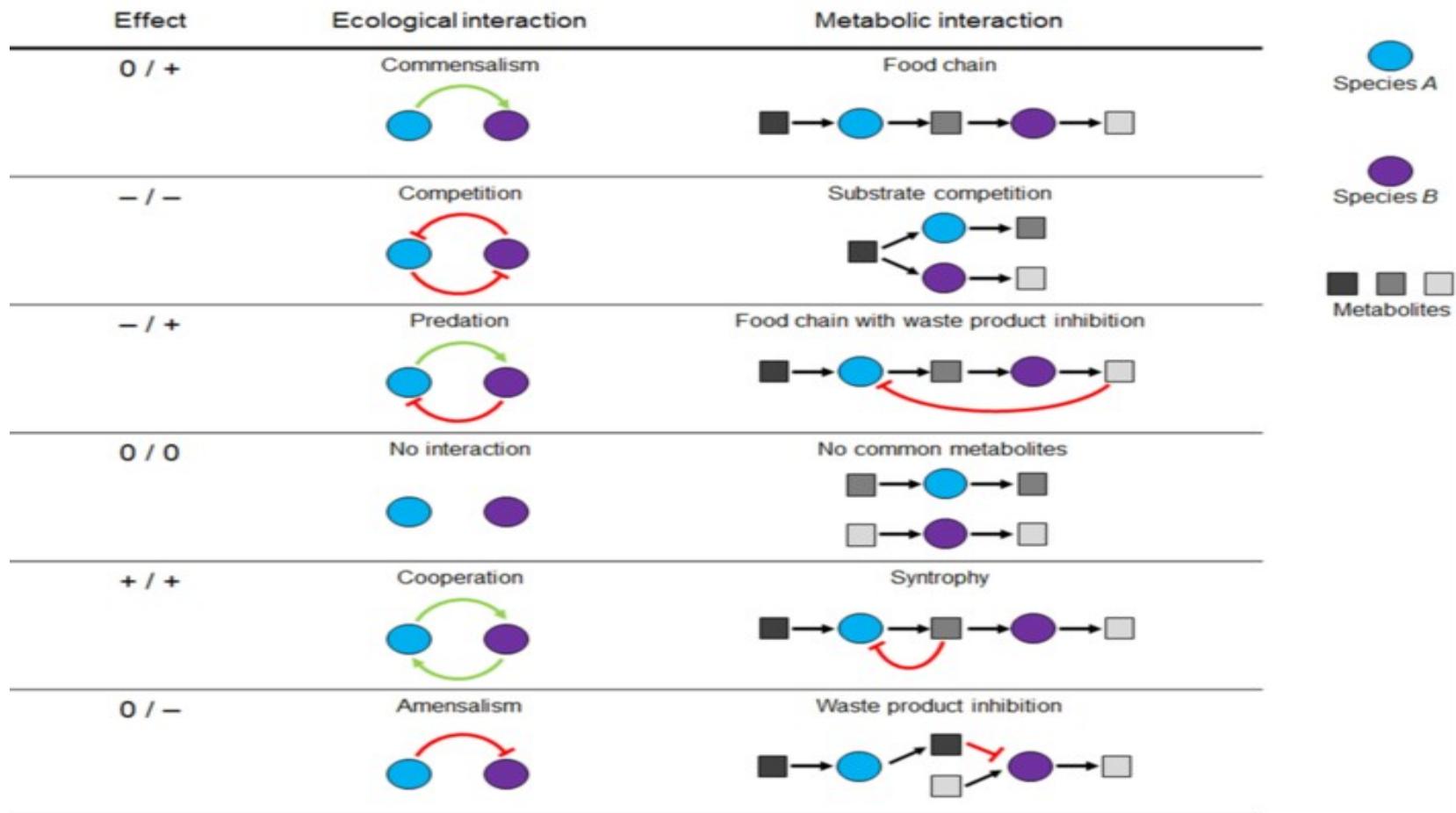
Resource or **bottom-up control** refers to the limitation of a prokaryotic community by **carbon and nutrients** derived from allochthonous inputs, primary production, and heterotrophic production

Top-down control refers to the **predatory limitation** of a prokaryotic community below levels supportable by resources alone. Top-down control refers to situations where the abundance, diversity or biomass of lower trophic levels depends on effects from consumers at higher trophic levels

A **trophic cascade** is a type of top-down interaction that describes the indirect effects of predators. In a trophic cascade, predators induce effects that cascade down food chain and affect biomass of organisms at least two links away

Sideways control refers to within community interaction, such as **competition and chemical warfare**

Community interactions



The microbial loop

The **microbial loop** describes a trophic pathway in the marine microbial food web where dissolved organic carbon (DOC) is returned to higher trophic levels via its incorporation into bacterial biomass, and then coupled with the classic food chain formed by phytoplankton-zooplankton-nekton.

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The Ecological Role of Water-Column Microbes in the Sea*

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The microbial loop

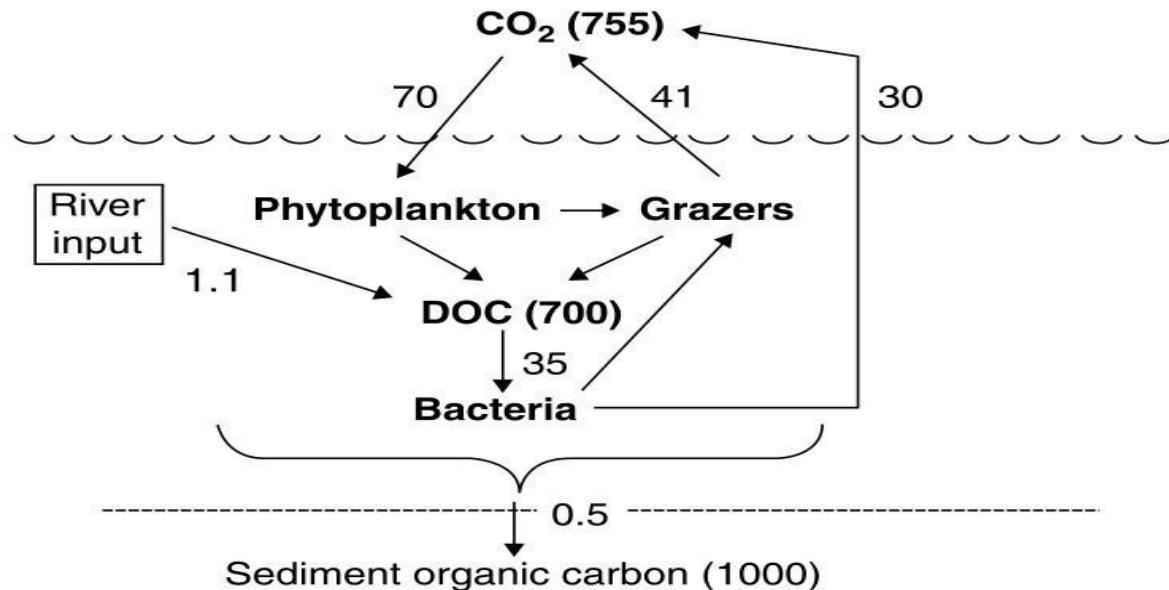
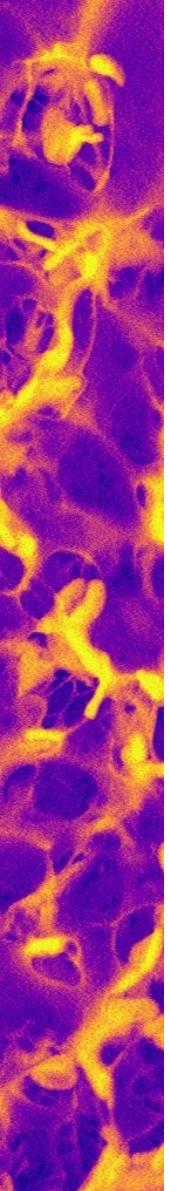


Figure 1.1 The role of microbes in the oceanic carbon cycle. The numbers in parentheses are standing stocks of carbon with units of pgC ($1 \text{ pgC} = 10^{15} \text{ gC}$). The other numbers are rates with units of pgC per year. The numbers are from Hedges and Oades (1997) and from estimates of the average fraction of primary production routed through dissolved organic carbon (DOC) to bacteria (Chapter 9), assuming a growth efficiency of 15 percent.



Tiny players big effects: Viral effects on biogeochemistry

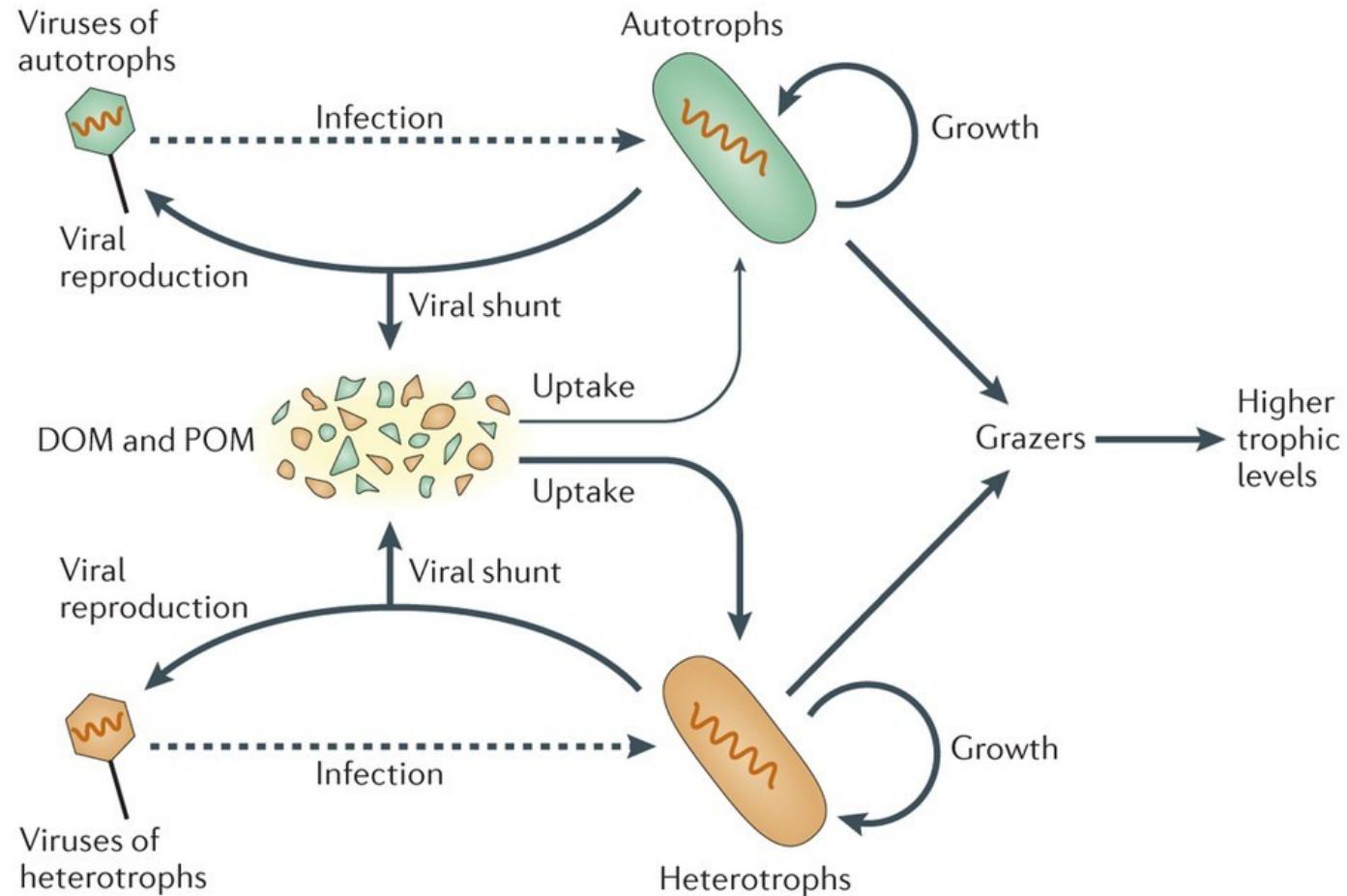
By infecting and lysing prokaryotic cells, viruses are potentially responsible for cycling huge amounts of organic carbon and other nutrients in many ecosystems

For example in the Ocean, viruses may turn over as much as 150 gigatons of carbon per year—more than 30 times the standing abundance of carbon in marine plankton (though **Viral shunt**)

Top-Down control of prokaryotic and phytoplankton populations, and effect on community structure (**Killing the winner hypothesis**)

They are evolutionary drivers through transduction and horizontal gene transfer (**Red queen hypothesis**)

Ecological Role: Viral Shunt



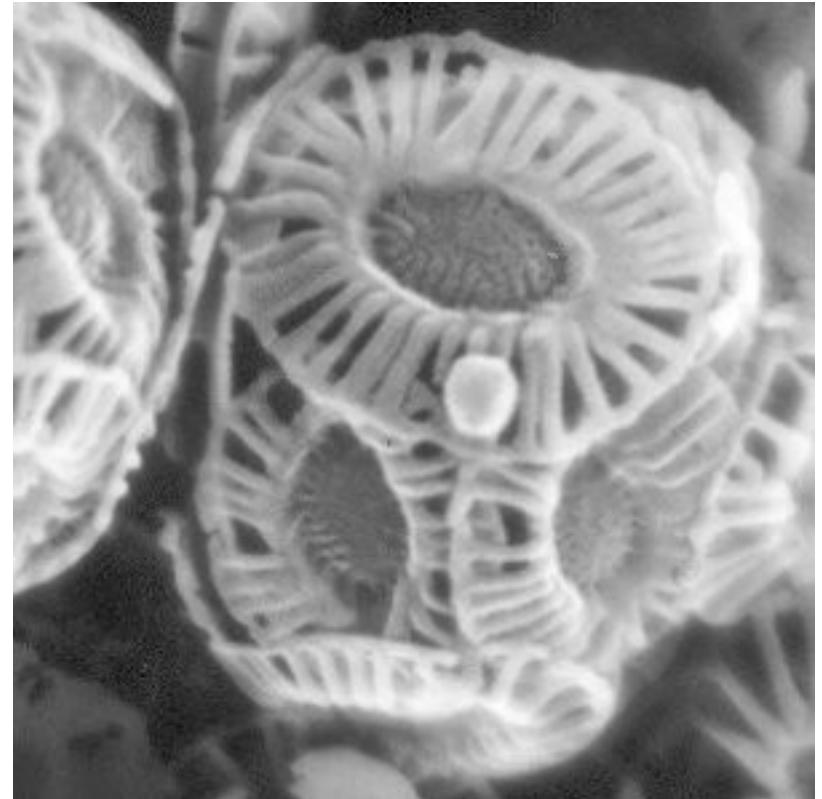
How virus influence climate



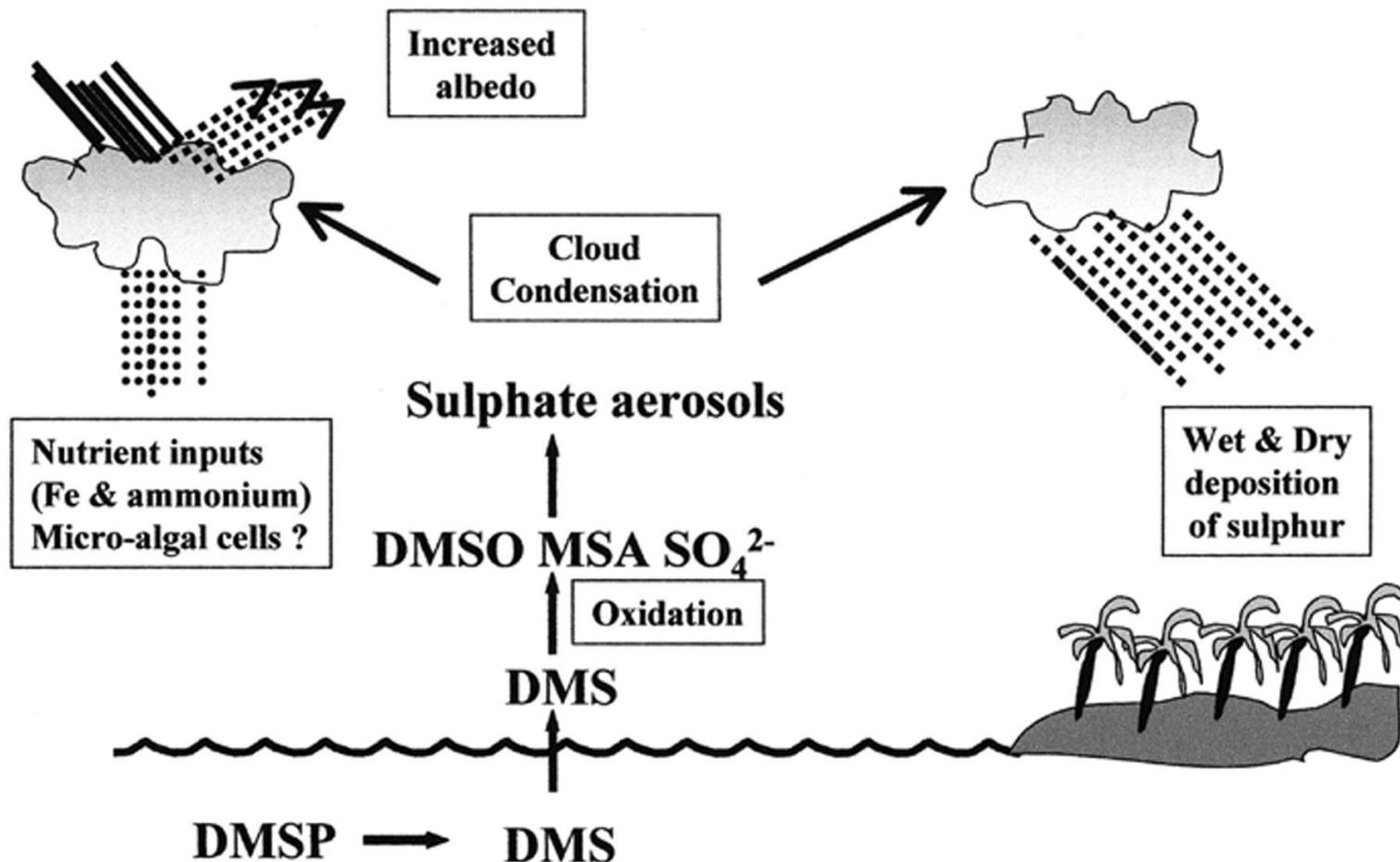
How virus influence climate

- Virus infect phytoplankton
- Since their infection is controlled by random contact (they are not motile), most abundant organisms are more likely to be infected
- This can trigger bloom termination due to viral infection

On the side: EhV1 infecting the coccolithophore *Emiliana heuxleii*



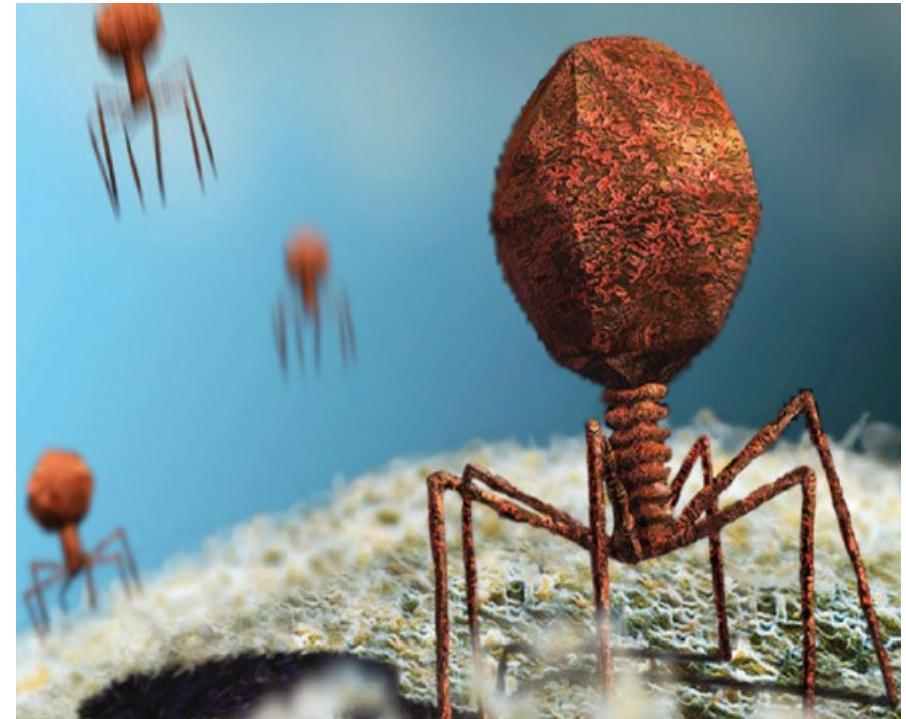
How virus influence climate



Virus affect the amount of released DMSP in the Oceans, thus affecting climate

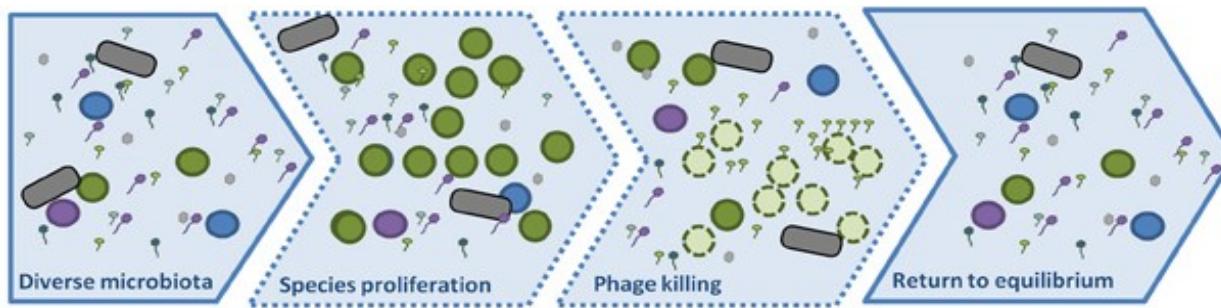
Killing the Winner Hypothesis

- The theory states that virus kill the most abundant organisms in a population (frequency dependent selection)
- Supported by both experimental and modeling data
- Alternative models are possible



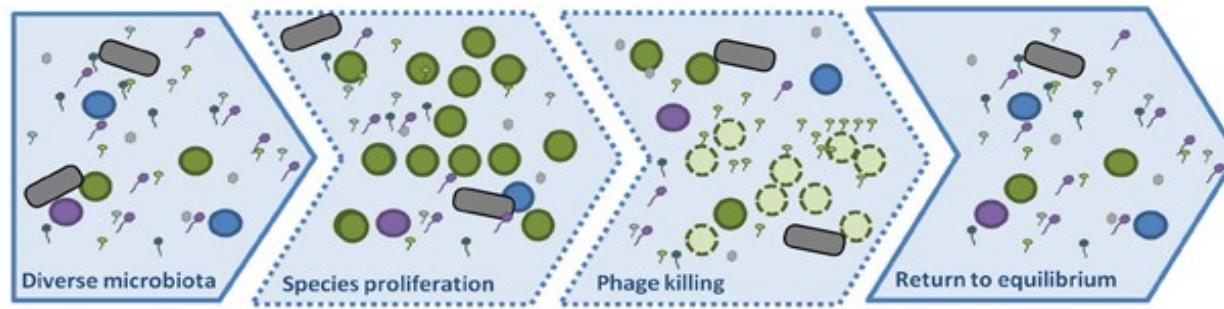
A

Kill the
winner



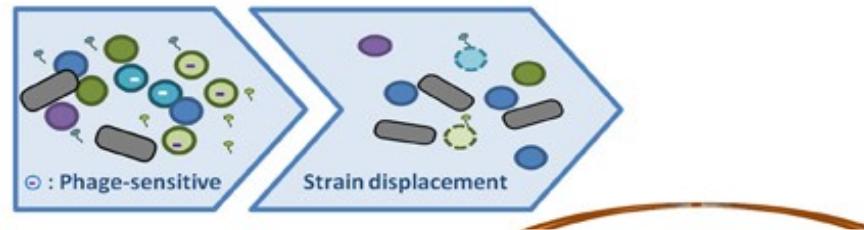
A

Kill the
winner



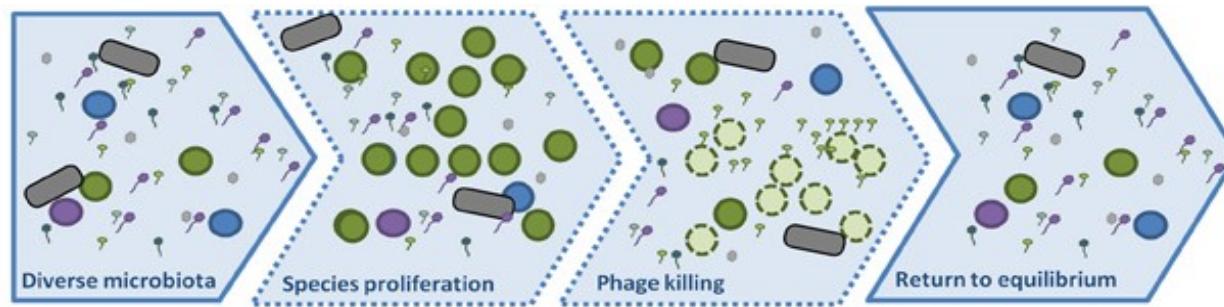
B

Kill the
relative

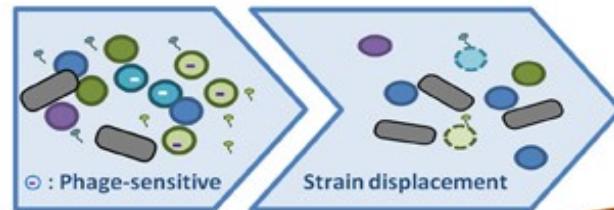


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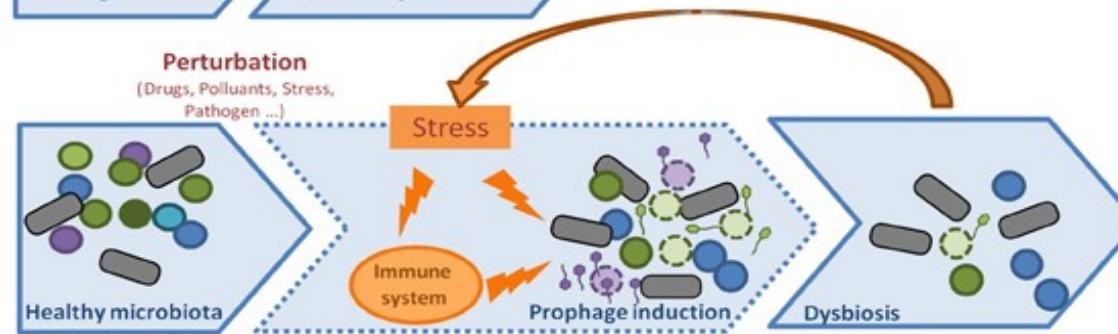
Kill the
winner

**B**

Kill the
relative

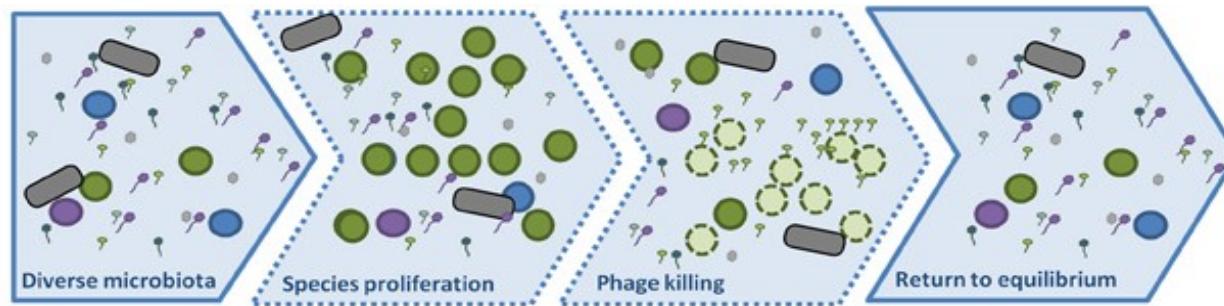
**C**

Community
shuffling

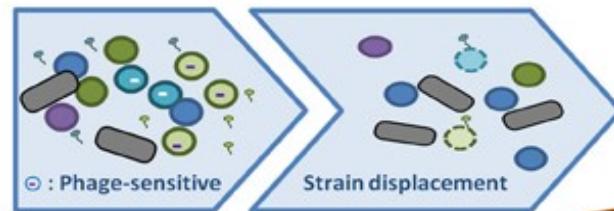


A

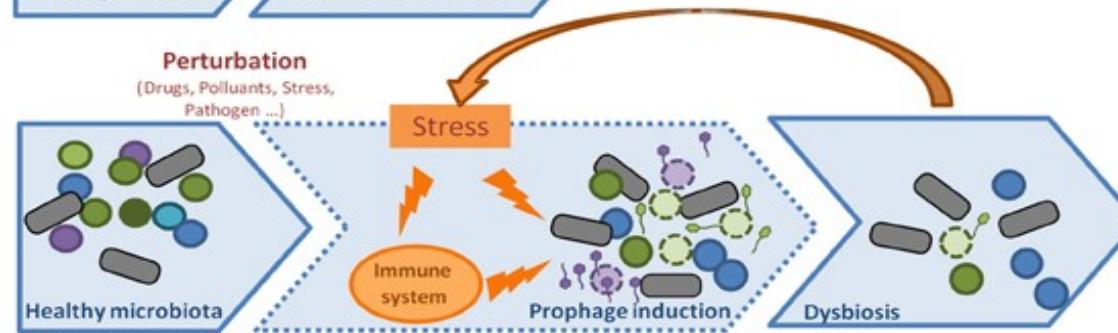
Kill the
winner

**B**

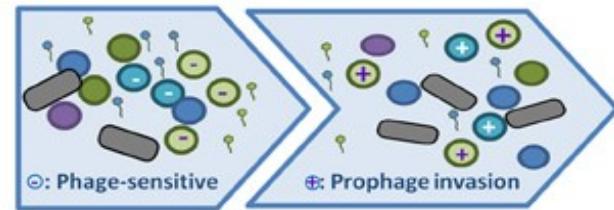
Kill the
relative

**C**

Community
shuffling

**D**

Invoke the
relative



The Red Queen Hypothesis

The Red Queen hypothesis is an evolutionary hypothesis which proposes that organisms must constantly adapt, evolve, and proliferate not merely to gain reproductive advantage, but also simply to survive while pitted against ever-evolving opposing organisms in an ever-changing environment, and intends to explain two different phenomena

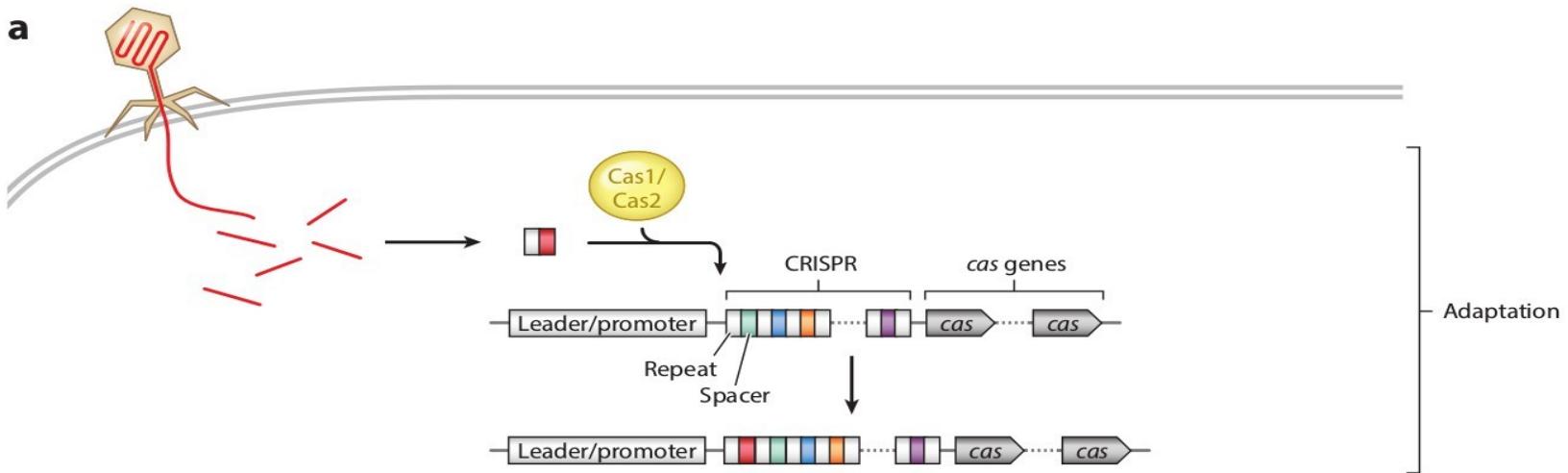


"The Red Queen has to run faster and faster in order to keep still where she is. That is exactly what you all are doing!"

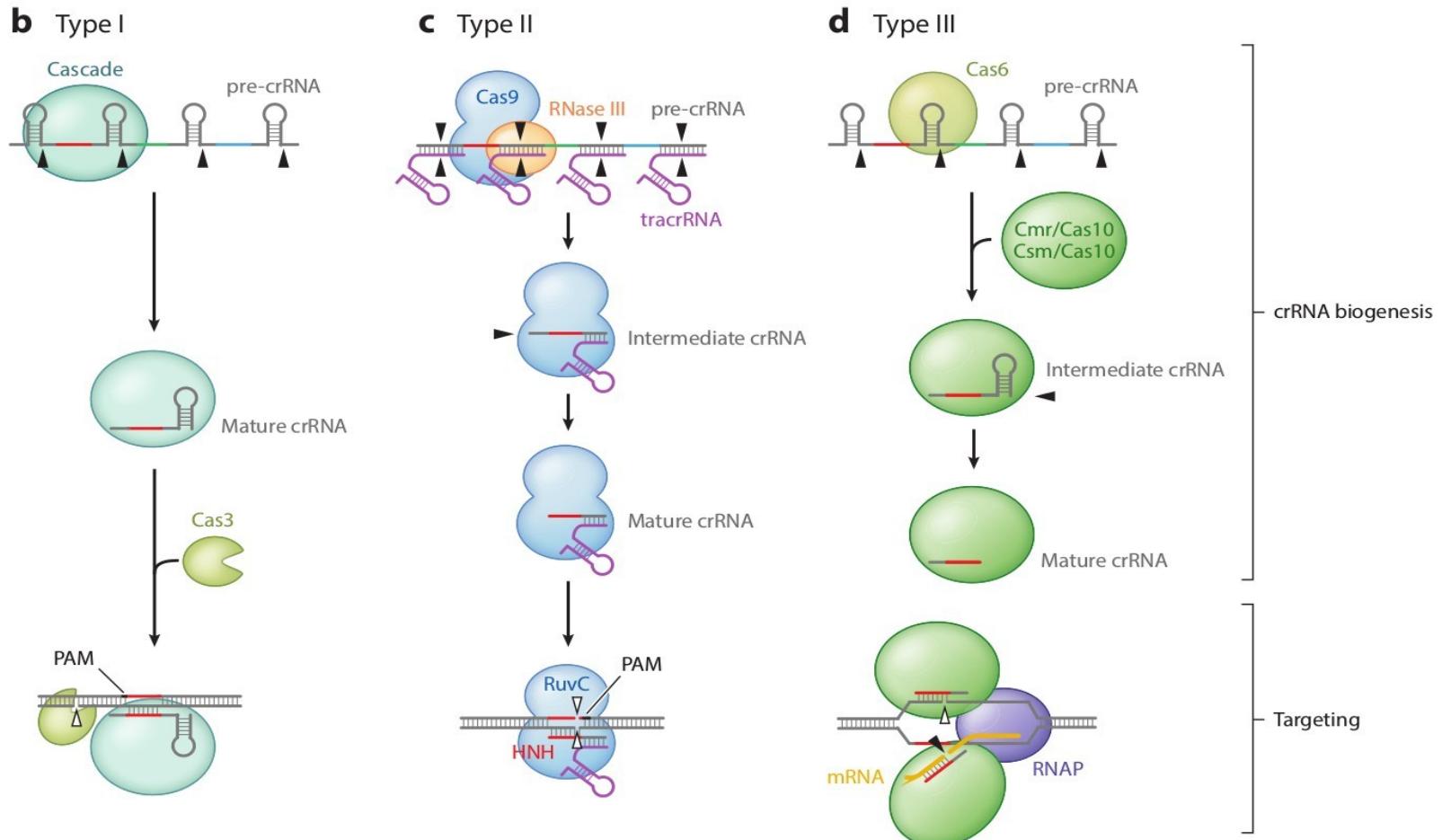
CRISPR-Cas System

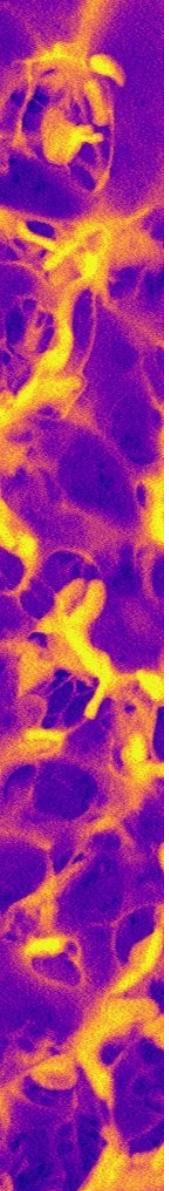
Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated Cas proteins function as an adaptive, small-RNA-based immune system that protects prokaryotes from infectious viruses and plasmids.

CRISPR-Cas immunity develops in three phases:



CRISPR-Cas System





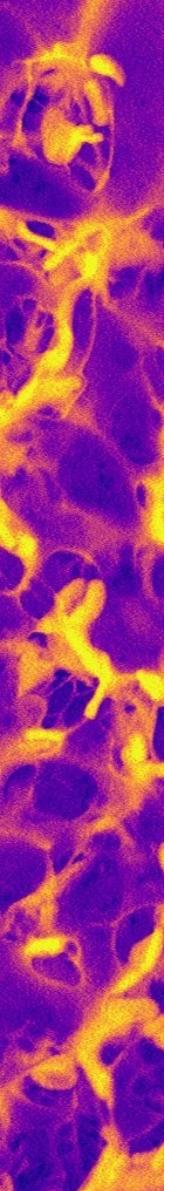
Virus role in controlling metabolism

Infection of microbes can alter host metabolism. Virus-induced changes in host metabolism make it biochemically and metabolically a very different cell.

Examples:

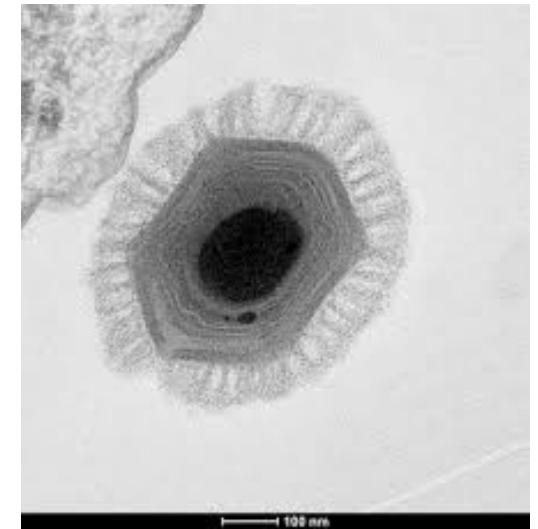
Phage-infected Cyanobacteria exhibit a higher rate of photosynthesis than their noninfected counterparts.

Bacterial cells undergoing active phage infections can also have altered distributions of other major elements, such as nitrogen and phosphorus, making them biochemically unique.



Megavirus, and emerging group

- Capsid cross-sections that can exceed 500 nm, an order of magnitude larger than prototypical viruses
- Giant viruses infect eukaryotic hosts, including the protists and unicellular green algae
- Carry genomes larger than nearly all previously identified viral types, in some cases upwards of 1 million base pairs
- In both marine and non-marine contexts, researchers have even identified viruses that can infect giant viruses, the so-called **virophages**



This week read

Welch, R.A., Burland, V., et al. (2002). Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 99(26), pp. 17020-17024 doi: 10.1073/pnas.252529799

Woese, C. R., and Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *PNAS* 74, 5088–5090. doi:10.1073/pnas.74.11.5088.

Pace, N. R., Sapp, J., and Goldenfeld, N. (2012). Phylogeny and beyond: Scientific, historical, and conceptual significance of the first tree of life. *PNAS* 109, 1011–1018. doi:10.1073/pnas.1109716109.

Spang, A., Saw, J. H., Jørgensen, S. L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A. E., et al. (2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature advance online publication*. doi:10.1038/nature14447.