



# Foundational concepts of biology

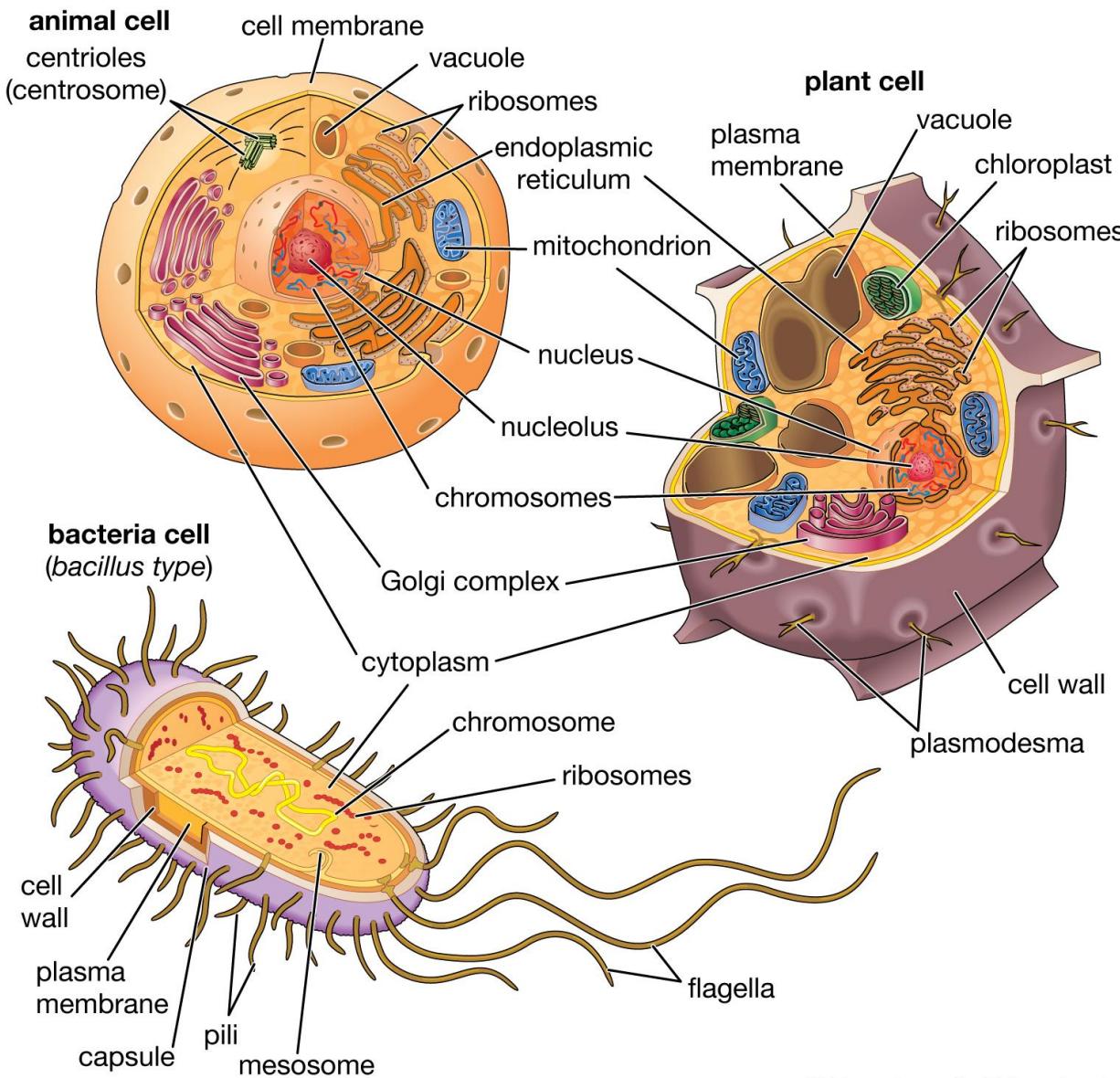
## BIOLOGICAL FOUNDATIONS OF BIOINFORMATICS

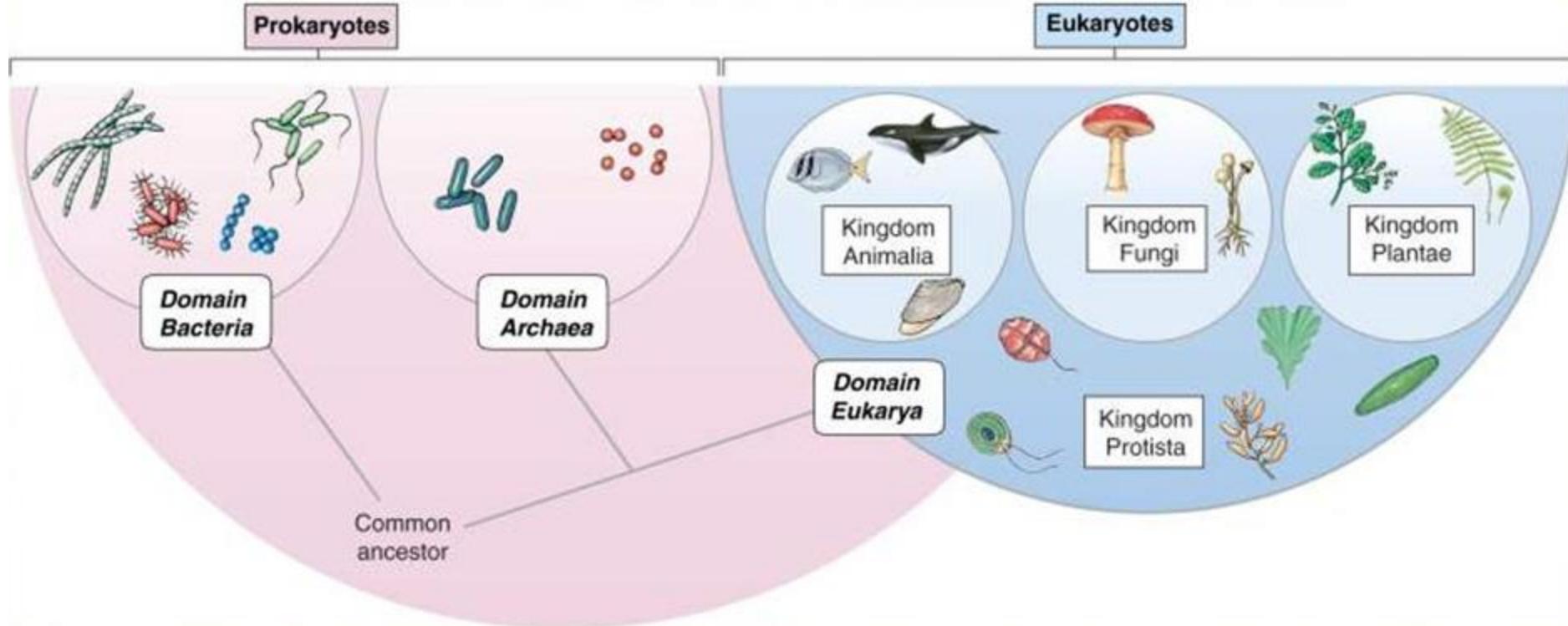


MAG. RER. NAT. ALEXANDRA HUBER  
SS 2024

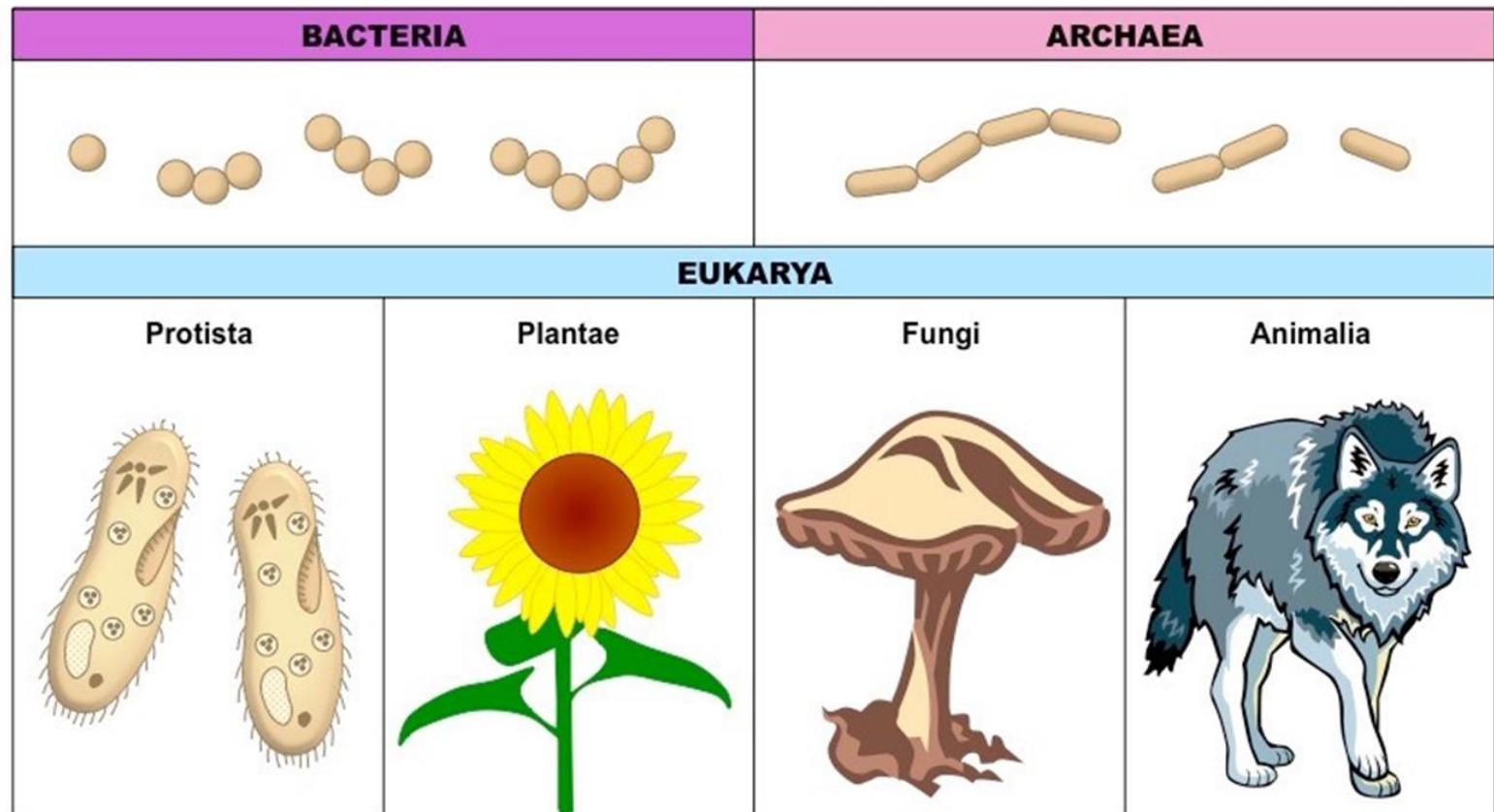
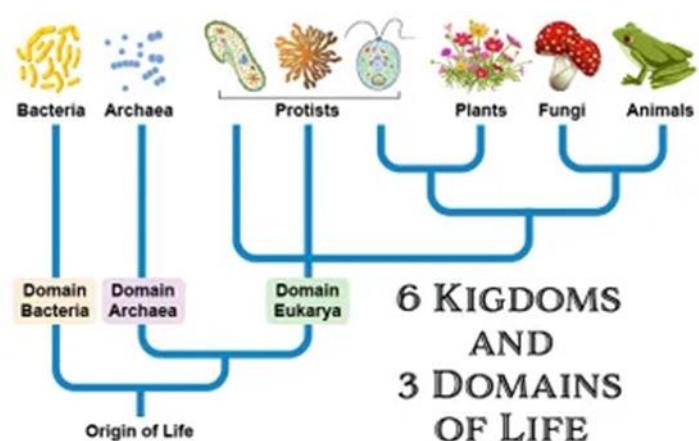
# OVERVIEW

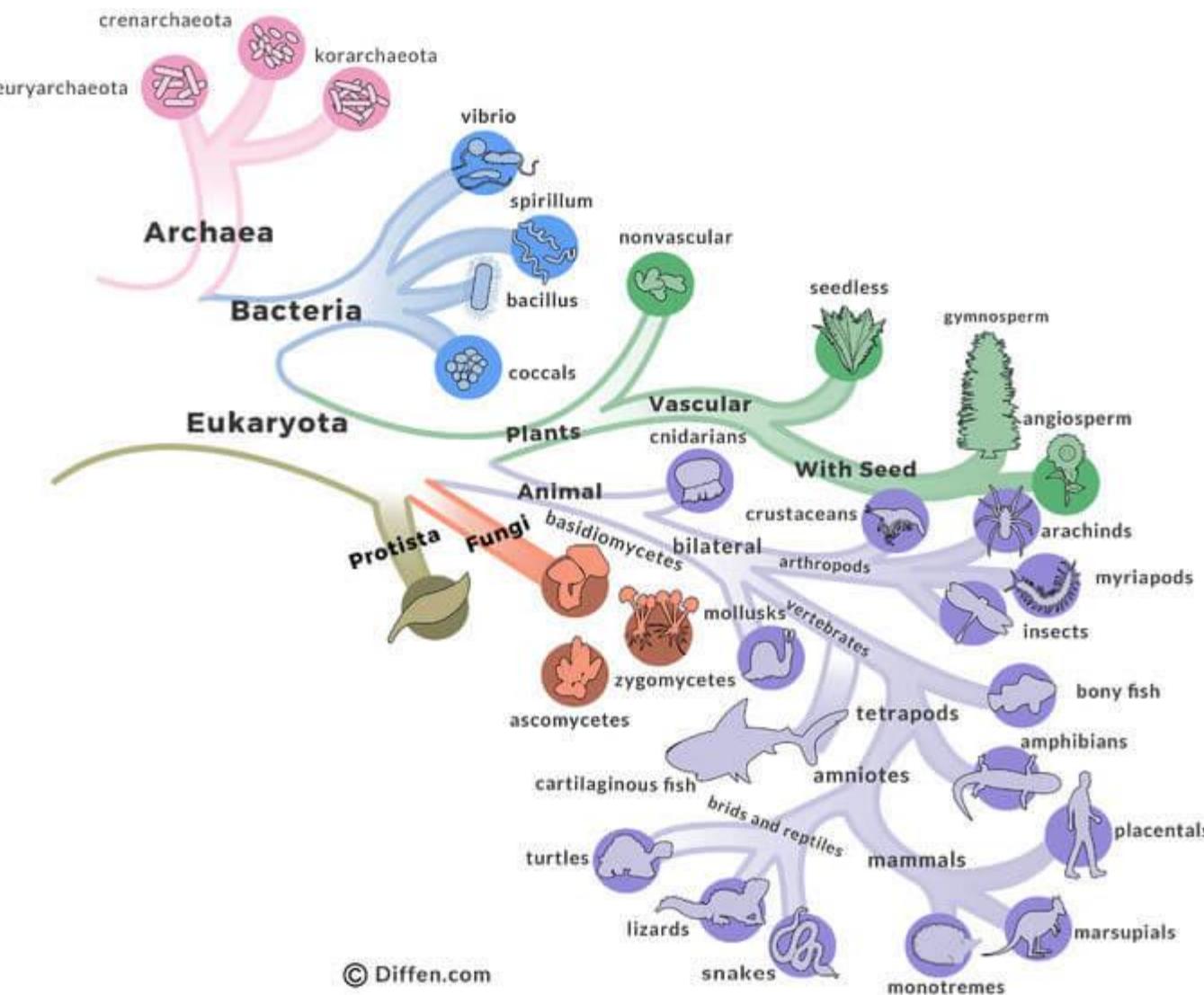
- Domains of life
- Archaea
- Bacteria
- Cell division
- Cell metabolism
- Viruses
- Mutations
- PCR





# DOMAINS OF LIFE







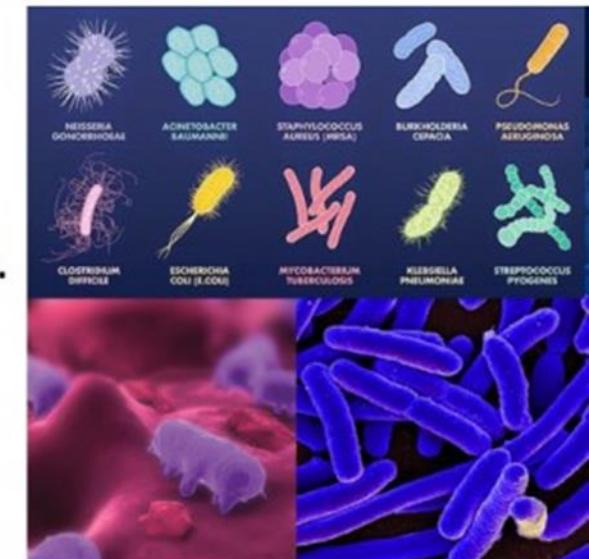
# ■ Achaea

# ARCHEAE VS BACTERIA

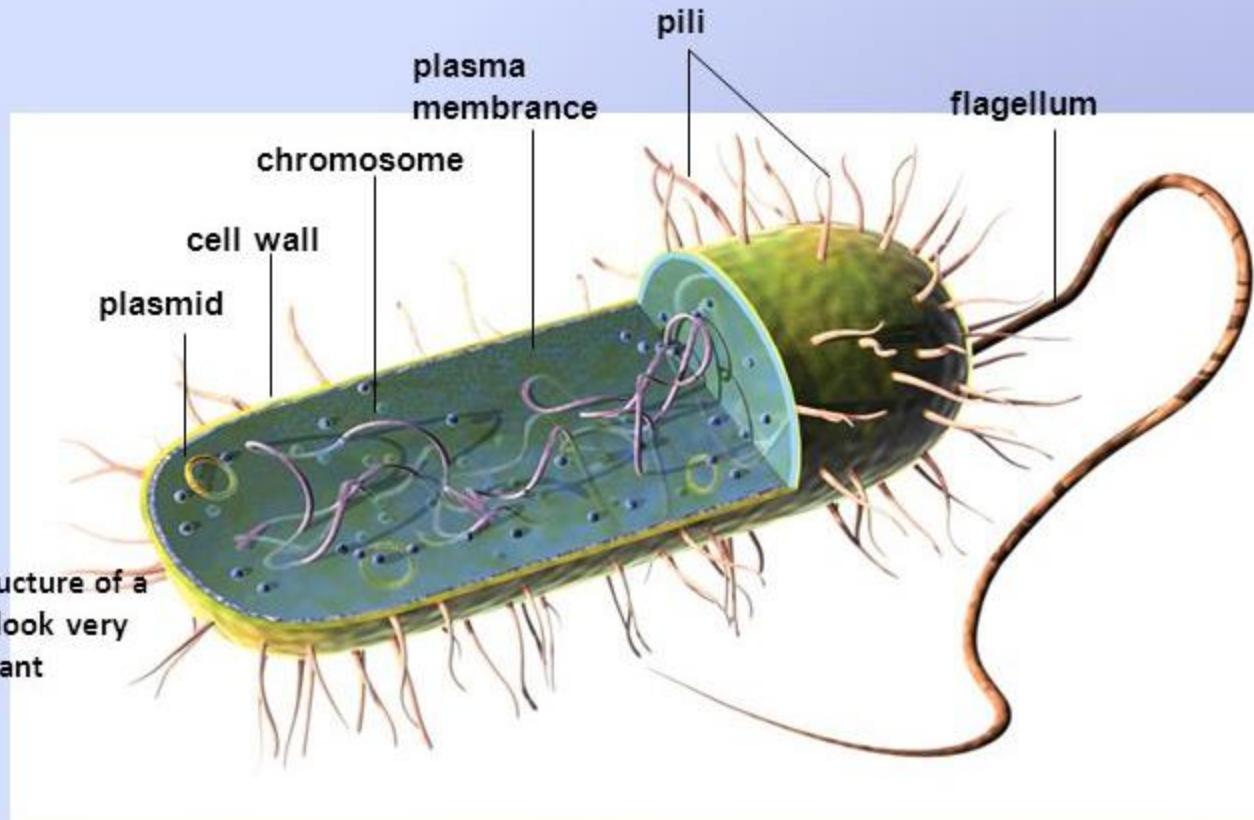
- Both share a prokaryotic cell type and metabolism and are unicellular, but archaea show different cell walls and histons (which are absent in bacteria)
- Bacteria are the most diverse and widespread group of living organisms
- Archaea can live in extreme and harsh conditions!



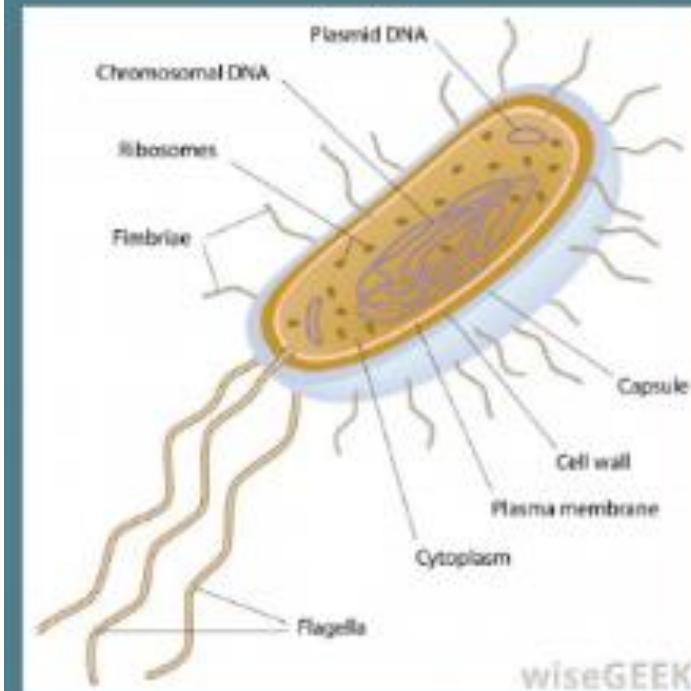
Vs.



- Eubacteria and Archaeabacteria are structurally similar but have different molecular characteristics.

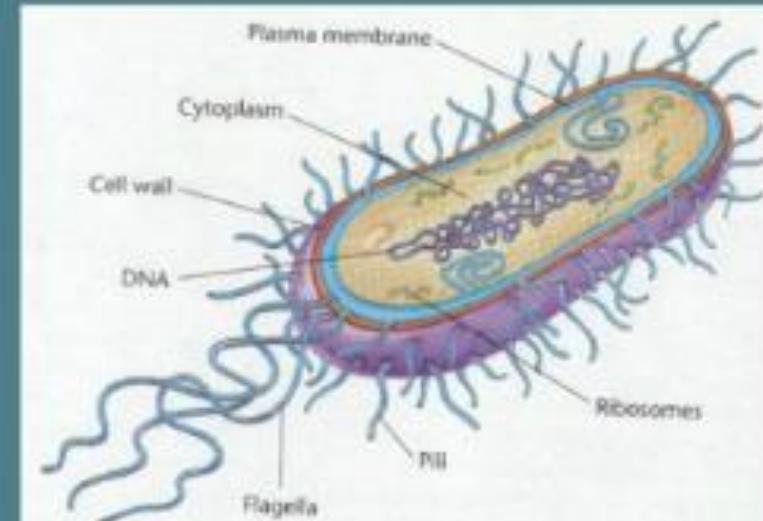


# Archaeabacteria



wiseGEEK

# Eubacteria



## ARCHAEA AND BACTERIA

- Both Bacteria and Archaea are microorganisms that live in a wide range of habitats, including the human body.
- They look very similar to one another, even under a microscope.
- Before the discovery of archaea, scientists believed that all prokaryotes were a single type of organism called bacteria.
- Their chemical makeup, however, is quite different from one another.
- Archaeal and bacterial DNA and RNA are quite different from one another.

# ARCHAEA AND BACTERIA

- In the late 1970s experiments on organisms believed to be bacteria showed startling results: One group of so-called bacteria were radically different from the rest.
- This unique group of microorganisms lived in extremely high temperatures and produced methane.
- Their genetic makeup was so different from the bacteria that a third domain of life was proposed. Instead of organizing life into two domains (prokaryotes and eukaryotes), there were now three domains: eukaryotes, bacteria, and archaea.
- Archaea, like bacteria, exist in a huge range of environments, including the human body.
- Like bacteria, Archaea play an important role in many biological processes.



## ARCHAEA

- the most fascinating aspect of Archaea is their ability to live in incredibly extreme environments.
- They are capable of thriving where no other organism can survive.
- **As archaea inhabit extreme environments they have distinct metabolic pathways (and genes) that support their survival.**

# ARCHAEA ARE EXTREMOPHILES

- the archaeal *Methanopyrus kandleri* strain can grow at above 100 degrees Centigrade
- *Picrophilus torridus* can thrive at the incredibly acidic pH of 0.06
- hydrothermal vents at the bottom of the ocean
- deep underground in petroleum deposits
- archaea may be able to survive in toxic waste

## Sources:

- Nkamga, Vanessa Demonfort, et al. "Archea: Essential Inhabitants of the Human Digestive Microbiota." *Human Microbiome Journal*, vol. 3, 2017, pp. 1-8., doi:10.1016/j.humic.2016.11.005
- Jarrell, Ken F., et al. "Major Players on the Microbial Stage: Why Archaea Are Important." *Microbiology*, vol. 157, no. 4, 2011, pp. 919-936., doi:10.1099/mic.0.047837-0
- Rampelotto, Pabulo Henrique. "Extremophiles and Extreme Environments." *Life*, vol. 3, no. 3, 2013, pp. 482-485., doi:10.3390/life3030482)

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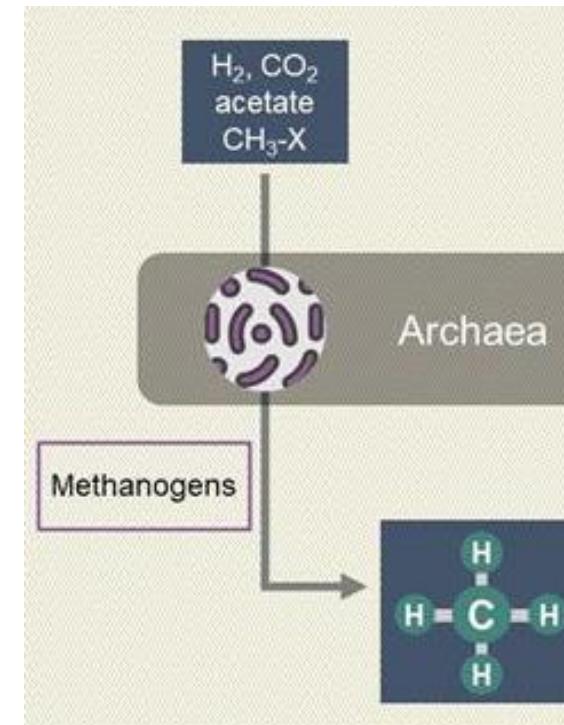
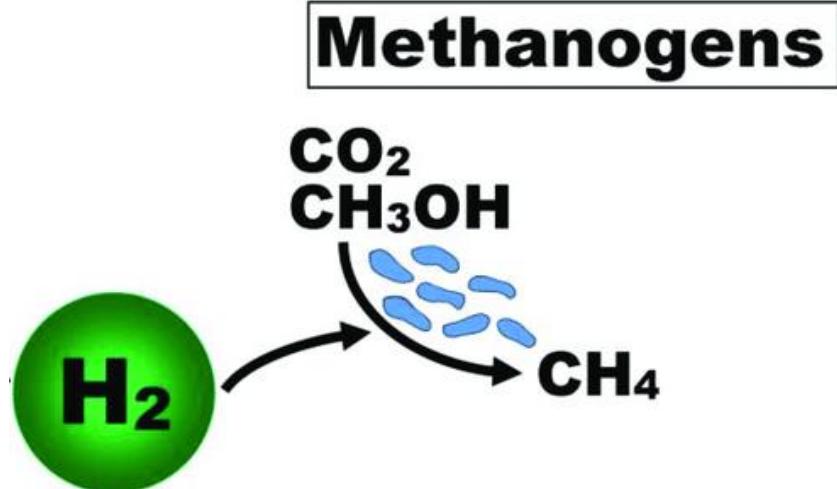
Archaea are extremophiles, capable of surviving in extreme conditions (like boiling hot springs).



# ARCHAEA

- Archaea is a group of prokaryotes that based on their distinct characteristics form a separate domain from bacteria and eukaryotes.
- Organisms in this domain share some characteristics with both bacteria and eukaryotes (e. g. no nucleus like bacteria, but enzymes that are also observed in eukaryotes).
- However, these organisms also have some unique characteristics, e. g. a unique membrane structure.
- The term ‘Archaea’ is derived from a Greek word, ‘archaios’ which means primitive or ancient.
- These organisms **usually inhabit extreme environments** like deep-sea vents, saline waters or hot springs.
- Archaea are mostly anaerobic or live in low-oxygen environments. Most of them cannot be cultured in laboratories and thus, have to be identified through culture-independent techniques.
- Many archaea are methanogens that utilize **anaerobic cellular respiration** to produce methane as a by-product.

# METHANOGENS

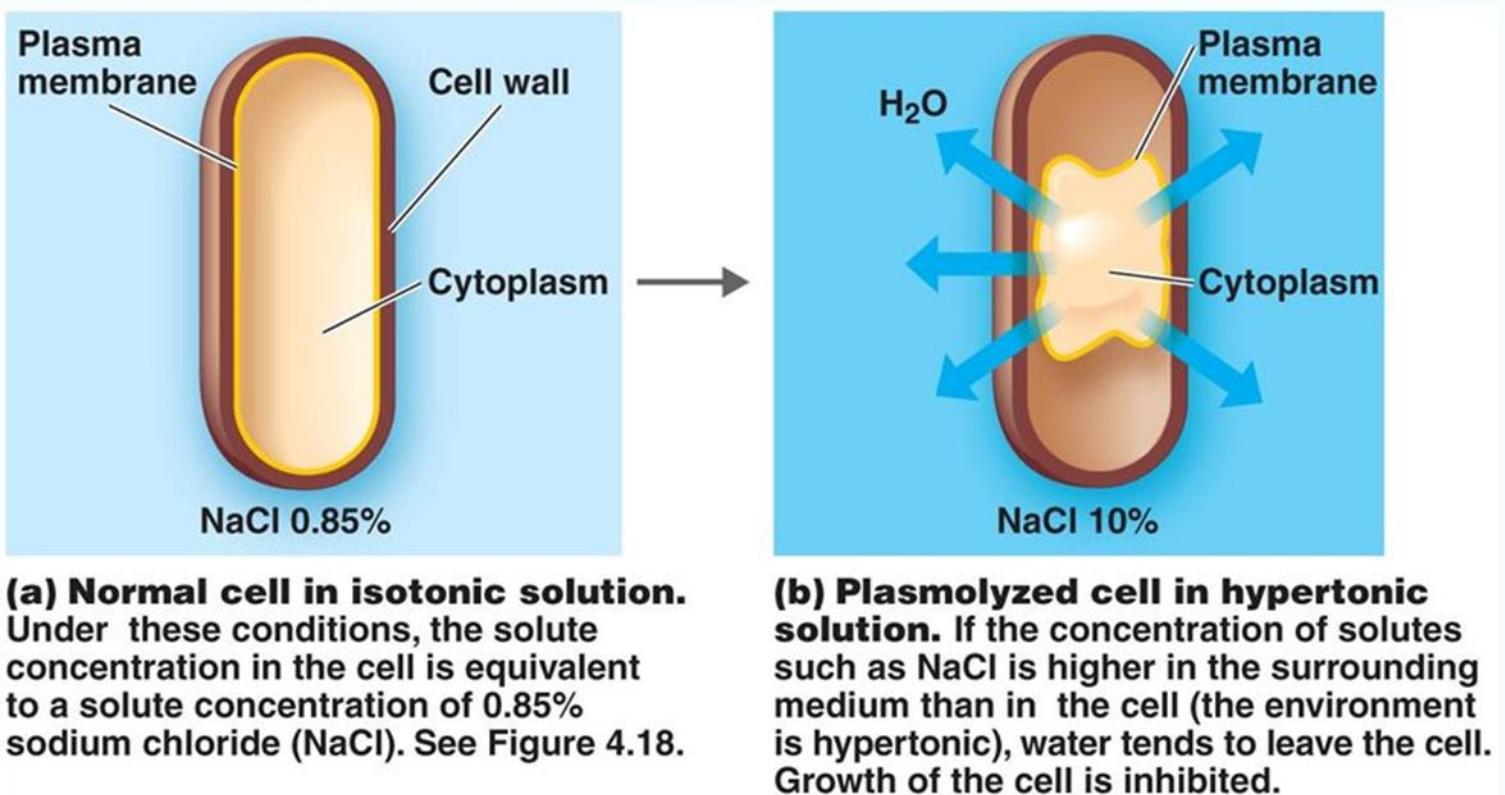


$\Delta G_0' \text{ (kJ/mol CH}_4)$

$\text{CO}_2 + 4 \text{ H}_2$	$\rightarrow \text{CH}_4 + 2 \text{ H}_2\text{O}$	-130
4 $\text{HCOOH}$	$\rightarrow \text{CH}_4 + 3 \text{ CO}_2 + 2 \text{ H}_2\text{O}$	-120
$\text{CO}_2 + 2 \text{ Ethanol}$	$\rightarrow \text{CH}_4 + 2 \text{ Acetate}$	-116
4 $\text{CO} + 5 \text{ H}_2\text{O}$	$\rightarrow \text{CH}_4 + 3 \text{ HCO}_3^- + 3 \text{ H}^+$	-196
4 $\text{CH}_3\text{OH}$	$\rightarrow 3 \text{ CH}_4 + \text{CO}_2 + 2 \text{ H}_2\text{O}$	-103
$\text{CH}_3\text{OH} + \text{H}_2$	$\rightarrow \text{CH}_4 + \text{H}_2\text{O}$	-113

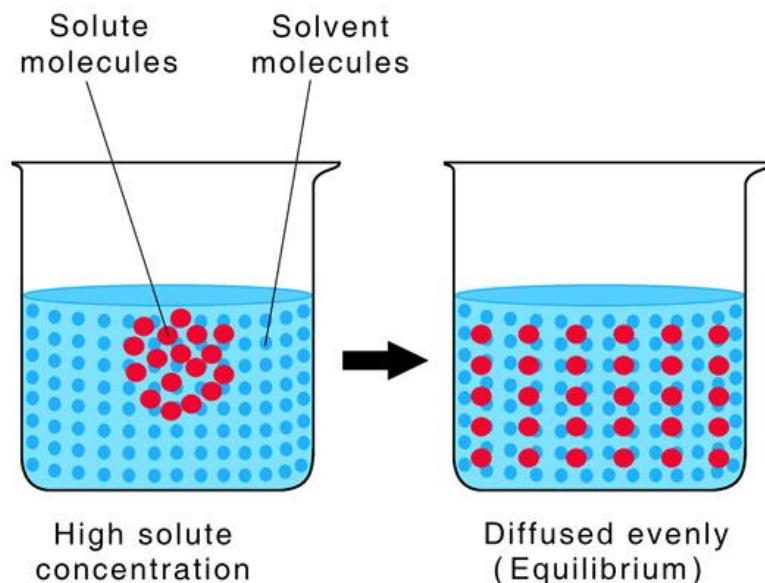
# HALOPHILIC ARCHAEA

- halophilic archaea have a unique set of genes that limit the extent of osmosis, facilitating their survival.



# Diffusion

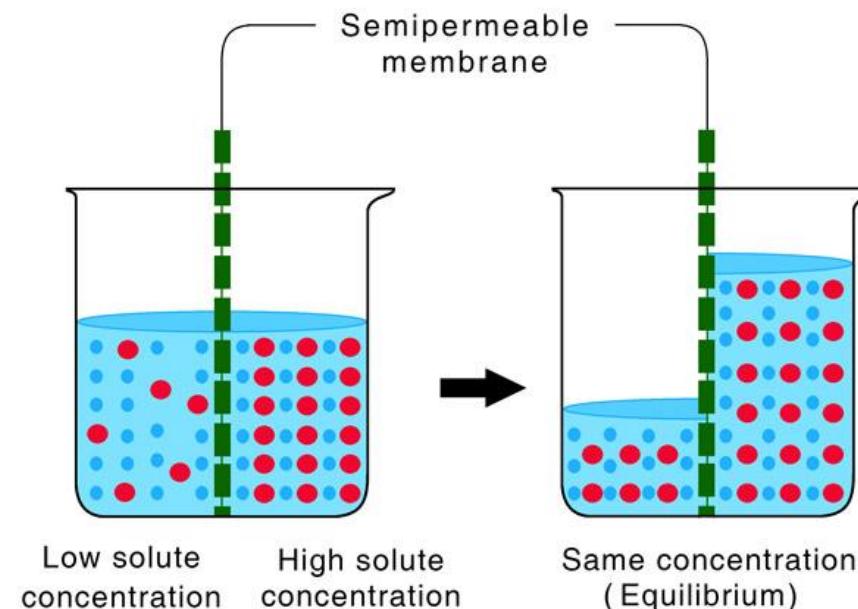
Solute molecules move from high to low concentration



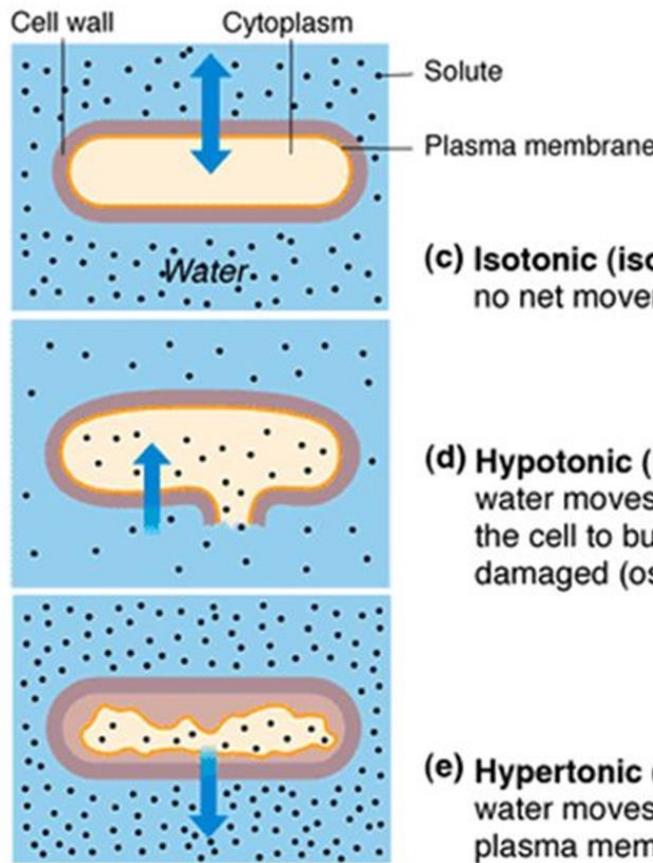
vs

# Osmosis

Solvent molecules move from low to high solute concentration



# EFFECTS OF OSMOSIS ON CELLS



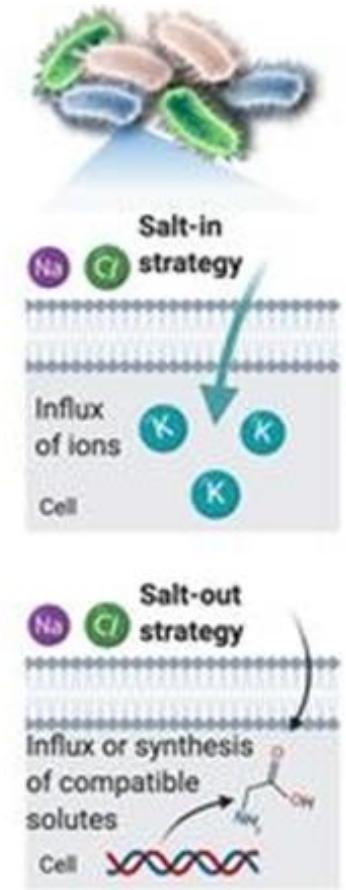
(c) Isotonic (isosmotic) solution—  
no net movement of water

(d) Hypotonic (hypoosmotic) solution—  
water moves into the cell and may cause  
the cell to burst if the wall is weak or  
damaged (osmotic lysis)

(e) Hypertonic (hyperosmotic) solution—  
water moves out of the cell, causing its  
plasma membrane to shrink (plasmolysis)

# MECHANISMS FOR SALINE TOLERANCE

- Halophilic bacteria utilize two mechanisms for halotolerance known as the salt-out and the salt-in strategies.
- The salt-in strategy used by species such as *Salinibacter ruber*, involves the **accumulation of ions in the cytoplasm** through an influx of potassium ions until the internal osmotic potential equals the external environment. The mechanism requires structural differences in biomolecules for functioning in high ion concentrations.
- The salt-out strategy involves the uptake or synthesis of osmoprotectants. **Osmoprotectants** include small molecules, such as ectoines, amino acids, sugars, and betaines, and accumulate in the cytoplasm to protect the cells from lysis caused by an imbalance of osmotic pressure.

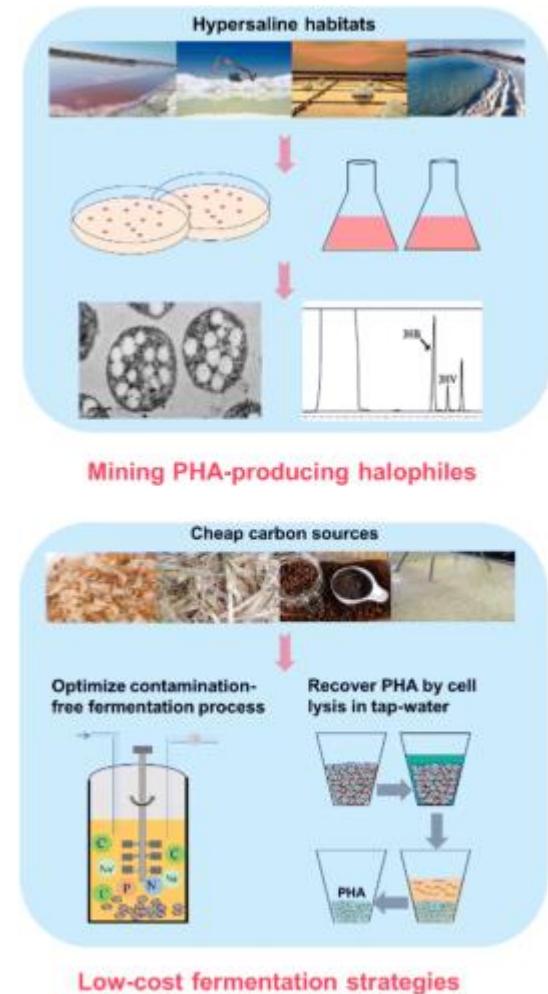


# HALOPHILES IN BIOTECHNOLOGY

Due to the adaptations to the extreme environments, metabolites produced by haloarchaea are of high interest in biotechnology.

Several biocompounds such as enzymes or PHAs, have focused the attention of many researchers around the world.

- Halophilic enzymes present **thermophilic** character too; consequently, they are stable in a broad range of temperatures. Haloarchaea may endure high temperatures in their natural environment, and halophilic protein need to be not only soluble at high salt concentrations but thermostable as well. These unique characteristics make halophilic enzymes very attractive for biotechnological applications. They are also active and **stable** in media with low water activity.
- Polyhydroxyalkanoates (PHAs) are polyesters (**bioplastics**) composed of hydroxy fatty acids, synthesized and stored in the cytoplasm. They serve as intracellular storage material. The advantages of using haloarchaea to produce PHAs are numerous: first, these microorganisms have simple growth requirements; second, the presence of high salt concentrations in their growth media prevents any kind of contamination from other organisms, so the requirements for sterile conditions can be reduced.



# HYPERTHERMOPHILES I

Hyperthermophiles and their enzymes have been intensively investigated for implementation in various high-temperature biotechnological processes.

- Biocatalysts of hyperthermophiles have proven to show extremely high thermo-activities and thermo-stabilities and are identified as suitable candidates for numerous industrial processes with harsh conditions, including the process of an efficient plant **biomass pretreatment** and conversion.
- Archaea-originated glycoside hydrolases (GHs) have shown highly impressive features and numerous enzyme characterizations indicated that these **biocatalysts show maximum activities at a higher temperature range compared to bacterial ones**.
- However, compared to bacterial biomass-degrading enzymes, the number of characterized archaeal ones remains low. To discover new promising archaeal GH candidates, it is necessary to study in detail the microbiology and enzymology of extremely high-temperature habitats, ranging from terrestrial to marine hydrothermal systems.
- State-of-the art technologies such as sequencing of genomes and metagenomes combined with classical microbiological culture-dependent approaches, have been successfully performed to detect novel promising biomass-degrading **hyperthermozymes**.

## HYPERTHERMOPHILES II

- The **first generation of biofuels** uses plant biomass from sugarcane, sugar beet, wheat and crops. Hence, first-generation biofuels, including bioethanol and biodiesel, are mainly produced from starch and vegetable oils.
- Since biomass for first-generation biofuels consists of **potentially edible plant material** and, further, requires large areas of agriculture fields, **other sources of biomass** had to be considered.
- This led to the development of the **second generation of biofuels**, which is **based on lignocellulosic biomass**. Lignocellulose consists of valuable polysaccharides, is abundant in agricultural residues and wood materials, and can be obtained from non-food feedstocks.
- Despite these advantages, a major challenge is formed by the character of lignocellulose, which necessitates a **pretreatment** of this substrate for fractionation, for example, by combining physical and chemical pretreatment methods.

## HYPERTHERMOPHILES III

- Efficient pretreatment results in the cleavage of lignocellulose enabling the enzymatic accessibility of its components: cellulose, hemicellulose and lignin.
- The first two components can be enzymatically hydrolyzed to yield hexose and pentose monomers, which can subsequently be fermented to ethanol or other alcohols and chemicals by anaerobic bacteria and fungi.
- To save energy and avoid expensive cooling steps, combinatorial approaches for physicochemical biomass pretreatment with simultaneous enzymatic hydrolysis were developed.
- For this purpose, extremely heat-active and heat-stable glycoside hydrolases are needed. Since **archaea** have been significantly less studied than bacteria and eukaryotes, they present a so-far underexploited **source of novel hyperthermozymes** particularly useful for biorefineries.

To develop a sustainable economy without the use of fossil resources, governments worldwide initiated research and development strategies for the transition from an oil-based to a circular bio-based economy.

A central element of this bioeconomy is the **development of sustainable biorefineries**, which use renewable resources as feedstock, such as plant biomass, instead of oil.

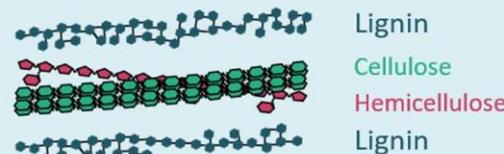
### Biomass

- Residues from agriculture and forestry
- Non-food crops
- Biological waste



### Pretreatment

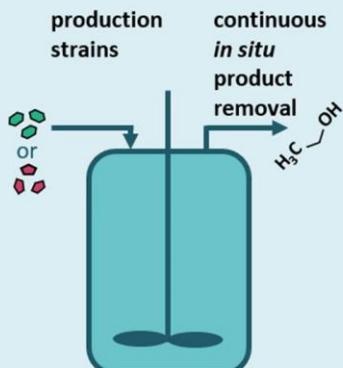
- Physical, chemical, biological or combined methods



T > 100°C, pH 3

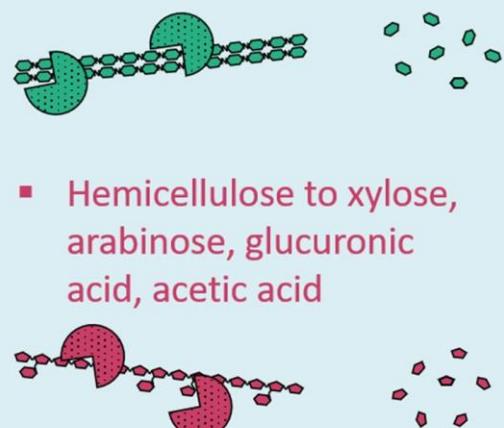
### Fermentation

- Bioethanol production



### Enzymatic hydrolysis

- Cellulose to glucose
- Hemicellulose to xylose, arabinose, glucuronic acid, acetic acid



Application of plant-degrading hyperthermophilic enzymes in biorefineries

# PROTEIN ENGINEERING TO TAILOR PLANT-DEGRADING ENZYMES FOR INDUSTRIAL PROCESSES

- While the implementation of plant-degrading hyperthermophilic enzymes in integrated biorefineries is a straightforward application of these robust biocatalysts, even these naturally already thermo-active and thermo-stable enzymes need further improvement before being subjected to the biorefinery process.
- In general, wild-type enzymes are not directly suitable for industrial application but have to be modified prior to usage in biotechnological processes due to oxidation sensitivity and generally low activities of the native enzymes.
- The replacement of oxidation-sensitive methionine residues is performed by site-directed mutagenesis, whereas improvements of substrate specificity and activity are gained by various rounds of protein-engineering coupled with screening for desired activities and/or stabilities.
- With the advance of more thorough analyses of archaea, including the Archaeal Proteome Project (ArcPP), further insights into the mechanisms and beneficial properties of archaeal enzymes are expected in the near future.

# ARCHAEA - COMPONENTS OF COMPLEX MICROBIOMES

Archaea interact closely with viruses, microorganisms, and holobionts (a collection of different species of organisms) such as plants, animals and humans.

- In holobionts, the archaeome reveals biogeographic patterns, indicating various functions.
- Methanogens, in particular, support bacterial fermentation processes.
- No archaeal pathogen has been identified thus far.
- Recent findings have shaken our picture of the biology of the archaea and revealed novel traits beyond archaeal extremophily and supposed ‘primitiveness’. The **archaea constitute a considerable fraction of the Earth’s ecosystems**, and their potential to shape their surroundings by a profound interaction with their biotic and abiotic environment has been recognized.
- Moreover, archaea have been identified as a substantial component, or even as keystone species, in complex microbiomes. They coexist in plant, animal, and human microbiomes, where syntrophy (one species uses the metabolic products of another species) allows them to thrive.
- Due to methodological limitations, the archaeome remains mysterious, and many questions with respect to function and interactions with their host and other microorganisms remain.

# ARCHAEA - COMPONENTS OF PLANT MICROBIOMES

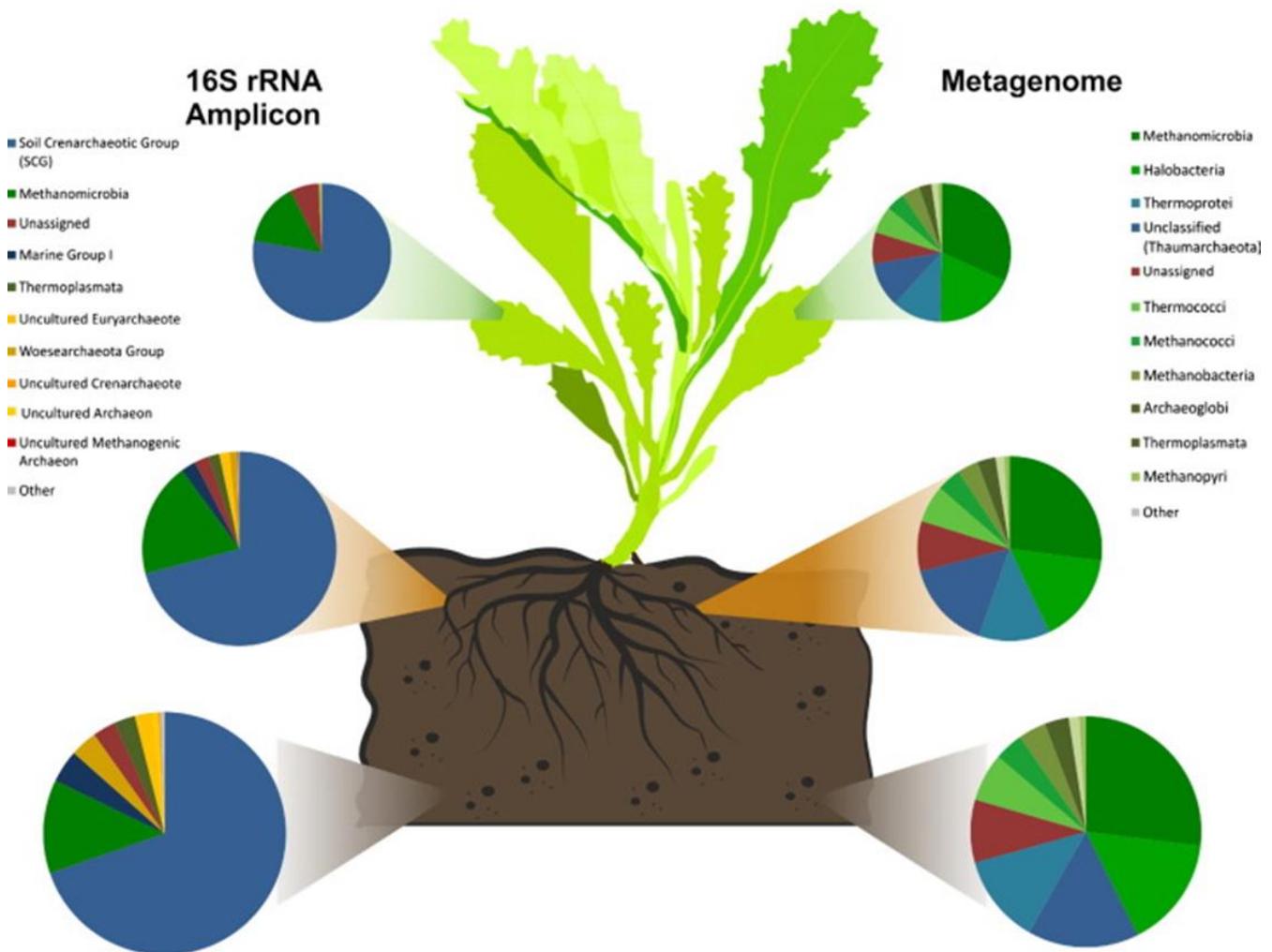
Taxonomic composition of archaeal communities of arugula (*Eruca sativa*) revealed by 16S rRNA amplicon and sequencing-based metagenomics analysis.

The archaeal community is described at the class level for each habitat: soil, rhizosphere and phyllosphere.

The abundances of archaeal genera are displayed relative to all sequences assigned to Archaea in the metagenomics dataset as well as relative to all sequences assigned to the 16S rRNA gene fragment dataset.

Overall, the metagenomics sequencing approach revealed a more diverse taxonomy due to differences in database entries and errors during PCR amplification and amplicon sequencing.

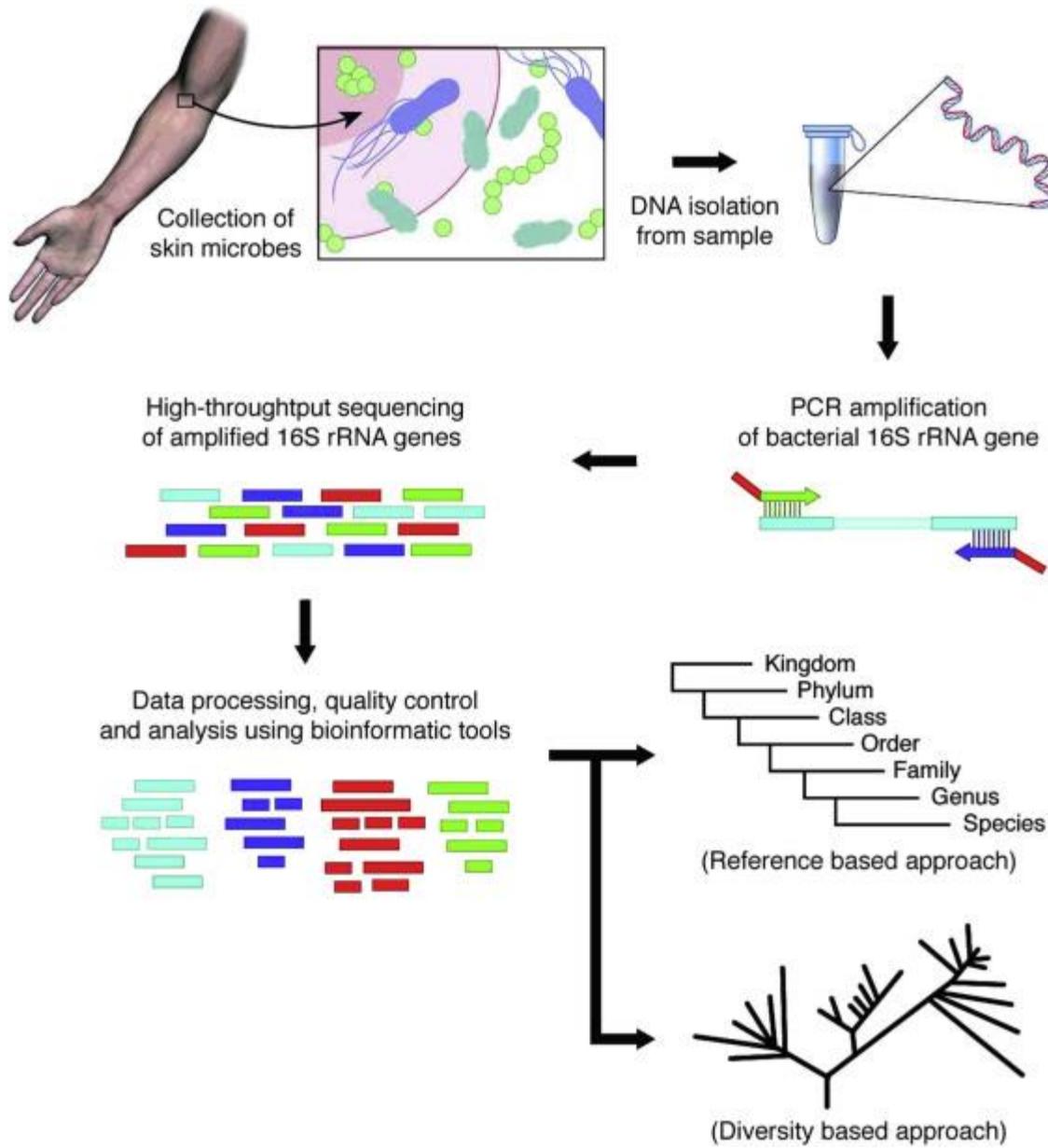
Source: Julian Taffner, Institute of Environmental Biotechnology, 8010 Graz

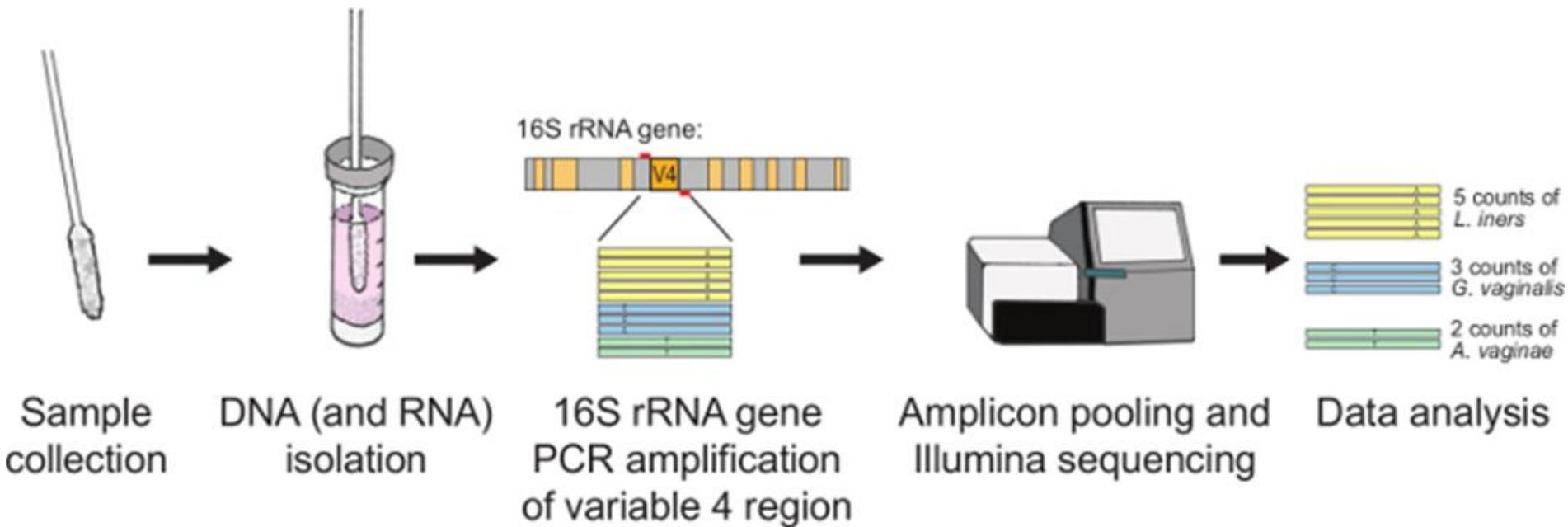


# 16S RIBOSOMAL DATABASES

- 16S\* ribosomal RNA (or 16S rRNA) is the RNA component of the 30S subunit (the smaller one) of a prokaryotic ribosome.
- 16S rRNA gene sequencing is commonly used for identification, classification and quantitation of microbes within complex biological mixtures such as environmental samples (ex marine water) and gut samples (ex human gut microbiome).
- The **16S rRNA gene** is used as the standard, because it is **present in most microbes and shows proper changes**.
- Type strains of 16S rRNA gene sequences for most bacteria and archaea are available on **public databases**, such as NCBI (National Center for Biotechnology Information).
- However, the quality of the sequences found on these databases is often not validated. Therefore, secondary databases that collect only 16S rRNA sequences are widely used. A frequently used database is e. g. :
- The Ribosomal Database Project (RDP) is a curated database that offers ribosome data along with related programs and services. The offerings include phylogenetically ordered alignments of ribosomal RNA (rRNA) sequences, derived phylogenetic trees, rRNA secondary structure diagrams and various software packages for handling, analyzing and displaying alignments and trees. Due to its large size the RDP database is often used as the basis for bioinformatic tool development.

\*(a Svedberg unit is a measure of a particle's size indirectly based on its sedimentation rate under acceleration in a centrifuge tube)





# ARCHAEA AND THE ORIGINS AND FUTURE OF LIFE

- Archaea, particularly those that thrive in extreme heat, are genetically close to the “universal ancestor” of all organisms on Earth. This finding suggests that Archaea may be the key to understanding the evolutionary origins of life on Earth.

(Source: Eme, Laura, et al. "Archaea and the Origin of Eukaryotes." *Nature Reviews Microbiology*, vol. 15, 2017, pp. 711-723., doi:10.1038/nrmicro.2017.133)

- Archaea's ability to survive in extraordinarily harsh environments could provide insight into **extraterrestrial life**. The nature of extremophiles makes them a natural focus for researchers exploring the question of what, if anything, can survive in interstellar space or on planets where typical Earth-based plants and animals would quickly die. One study subjected Archaea to temperature, UV radiation, humidity, and pressure resembling conditions on Mars and on the moon Europa; not surprisingly, the microorganisms lived and thrived.

(source: Mastascusa, V., et al. "Extremophiles Survival to Simulated Space Conditions: An Astrobiology Model Study." *Origins of Life and Evolution of the Biosphere*, vol. 44, no. 3, 2014, pp. 231-237., doi:10.1007/s11084-014-9397-y)

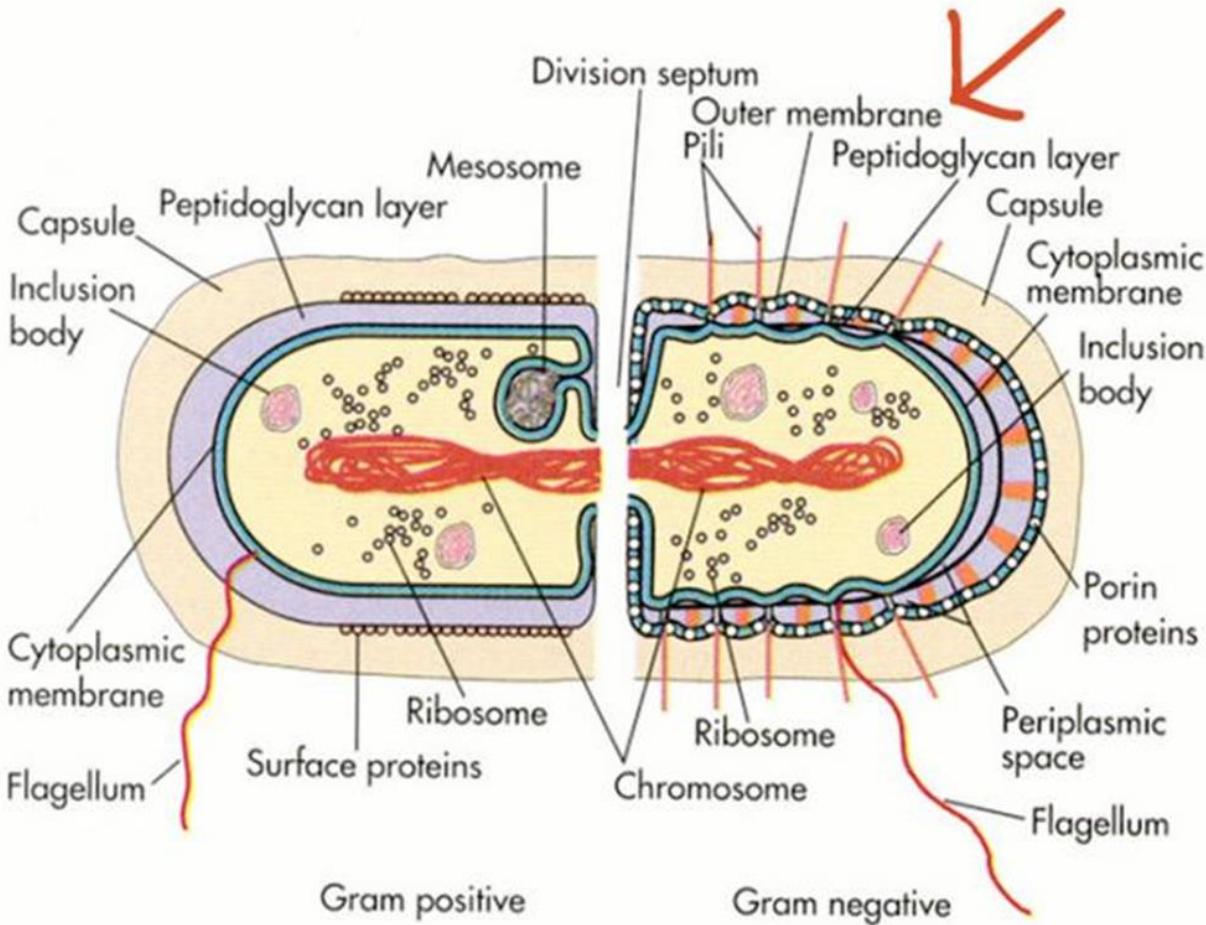


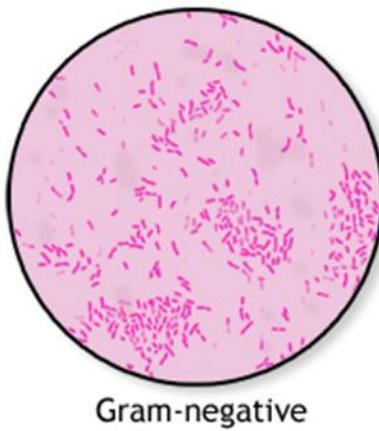
# ■ Bacteria

# BACTERIA

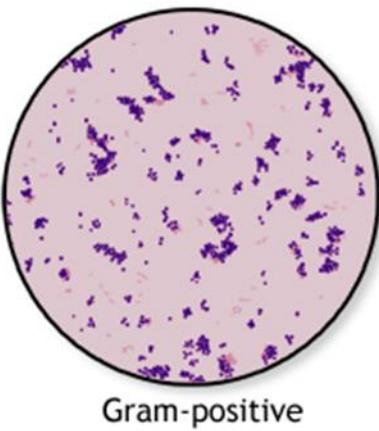
- Being able to differentiate bacterial species is important for many reasons, from diagnosing infection or checking food safety, to identifying which species it is that gives a cheese its fantastic character.
- Bacterial species, and even specific strains can be differentiated using a number of molecular techniques such as PCR, genome sequencing and mass spectrometry.
- But there are also phenotypic differences between groups of bacteria that can be used to differentiate them. This includes characteristics like their shape (bacilli vs cocci for example), growth in particular nutrients and preference for high or low oxygen environments.
- Depending on the characteristic being studied, bacterial species may be broken down into broad groups. One such useful classification – if a bacterium is Gram positive or Gram negative - is based on the structure of bacterial cell walls.
- Gram-positive bacteria are bacteria classified by the color they turn in the staining method. Hans Christian Gram developed the staining method in 1884. The staining method uses crystal violet dye, which is retained by the thick peptidoglycan cell wall found in gram-positive organisms. If the peptidoglycan layer is thin, bacteria are classified as gram negative.

# GRAM POSITIVE AND GRAM NEGATIVE BACTERIA





Gram-negative



Gram-positive

Gram positive bacteria	Gram negative bacteria
Distinctive purple appearance after gram staining	Pale reddish color after gram staining
Bacteria include all staphylococci, all streptococci and some listeria species	Bacteria include enterococci, salmonella species and pseudomonas species
Thick peptidoglycan layer	Thin peptidoglycan layer
No outer lipid membrane	Outer lipid membrane present

# Bacteria

## Gram Negative bacteria

*Xanthomonas* (xanthan gum)  
*Acetobacteraceae* (vinegar)

*Escherichia coli*  
*Klebsiella pneumoniae*  
*Salmonella*  
*Pseudomonas aeruginosa*

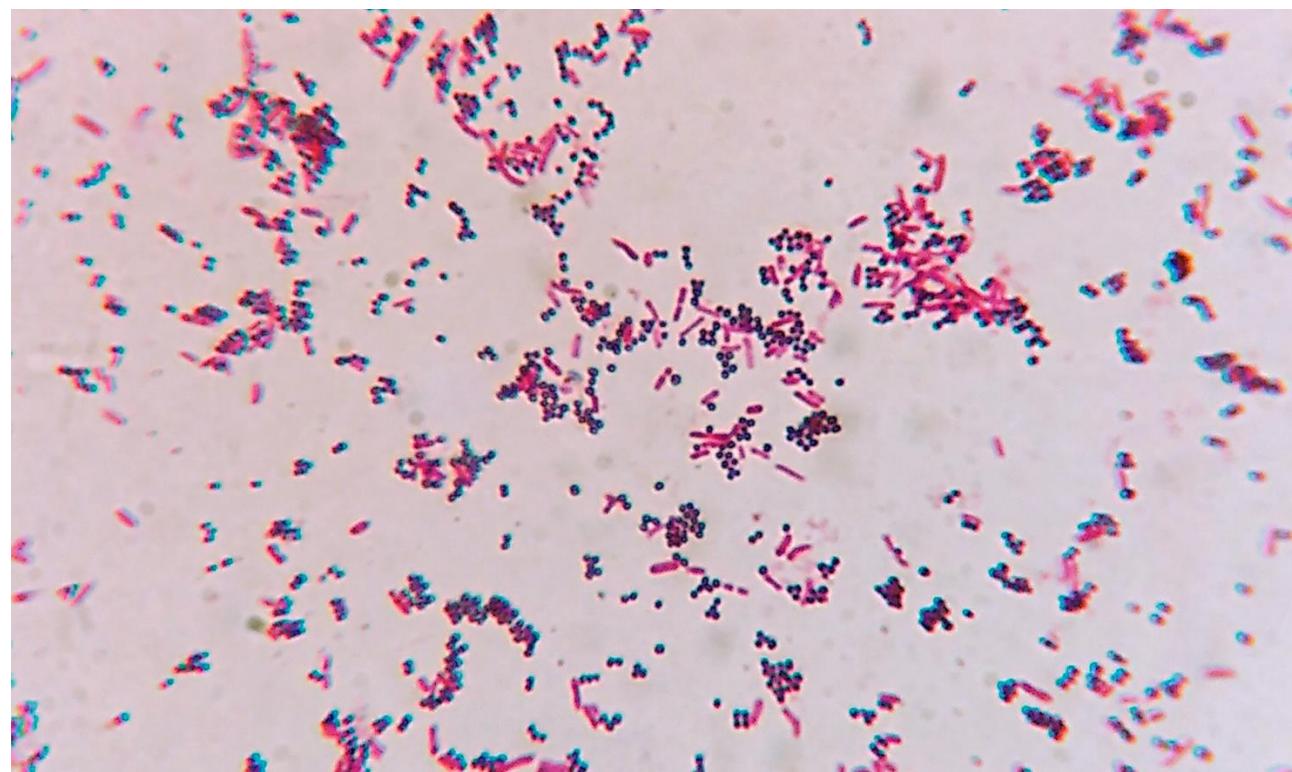
## Gram Positive bacteria

*Lactobacillus acidophilus* (tempeh, yogurt, miso)  
*Propionibacterium freudenreichii* (cheese)  
*Bifidobacterium*

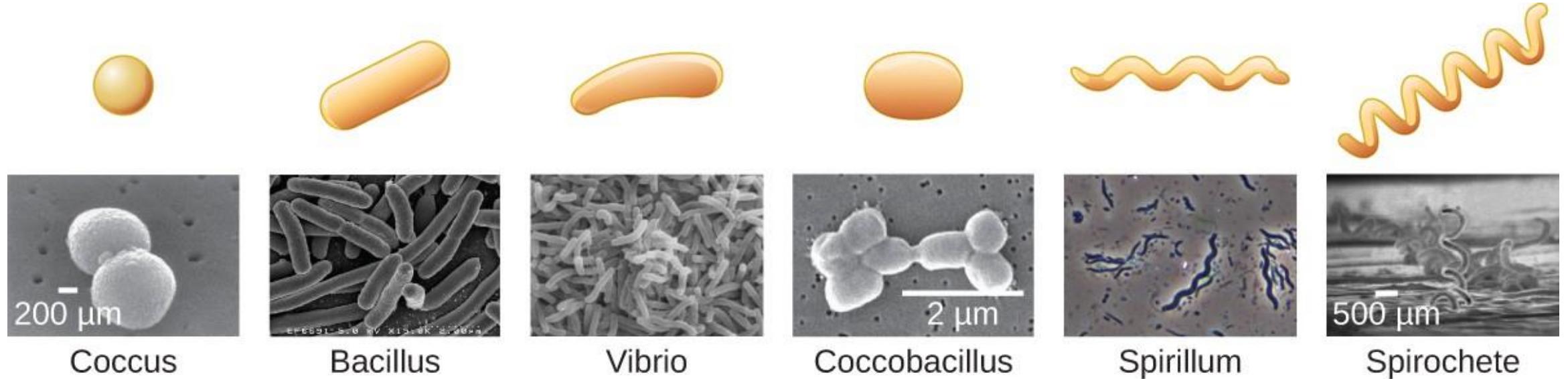
*Staphylococcus aureus*  
*Mycobacterium tuberculosis*  
*Anthrax*

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## GRAM POSITIVE COCCI AND GRAM NEGATIVE BACILLI



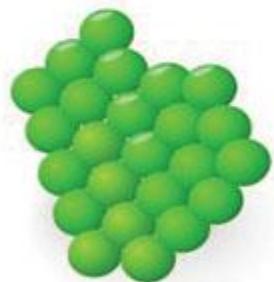
# BACTERIA SHAPES



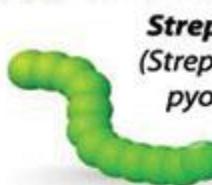
## SPHERES (COCCI)



**Diplococci**  
(*Streptococcus pneumoniae*)



**Staphylococci**  
(*Staphylococcus aureus*)



**Tetrad**



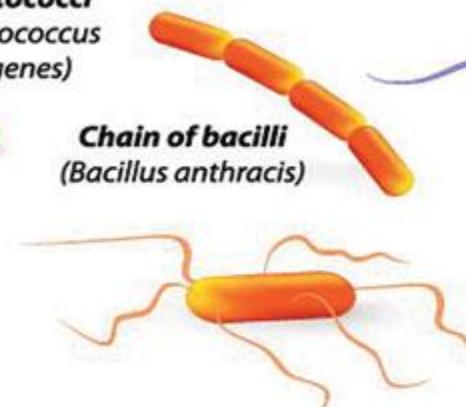
**Sarcina**  
(*Sarcina ventriculi*)



## RODS (BACILLI)

**Streptococci**  
(*Streptococcus pyogenes*)

**Chain of bacilli**  
(*Bacillus anthracis*)



**Flagellate rods**  
(*Salmonella typhi*)



**Spore-former**  
(*Clostridium botulinum*)

## SPIRALS

**Vibrios**  
(*Vibrio cholerae*)



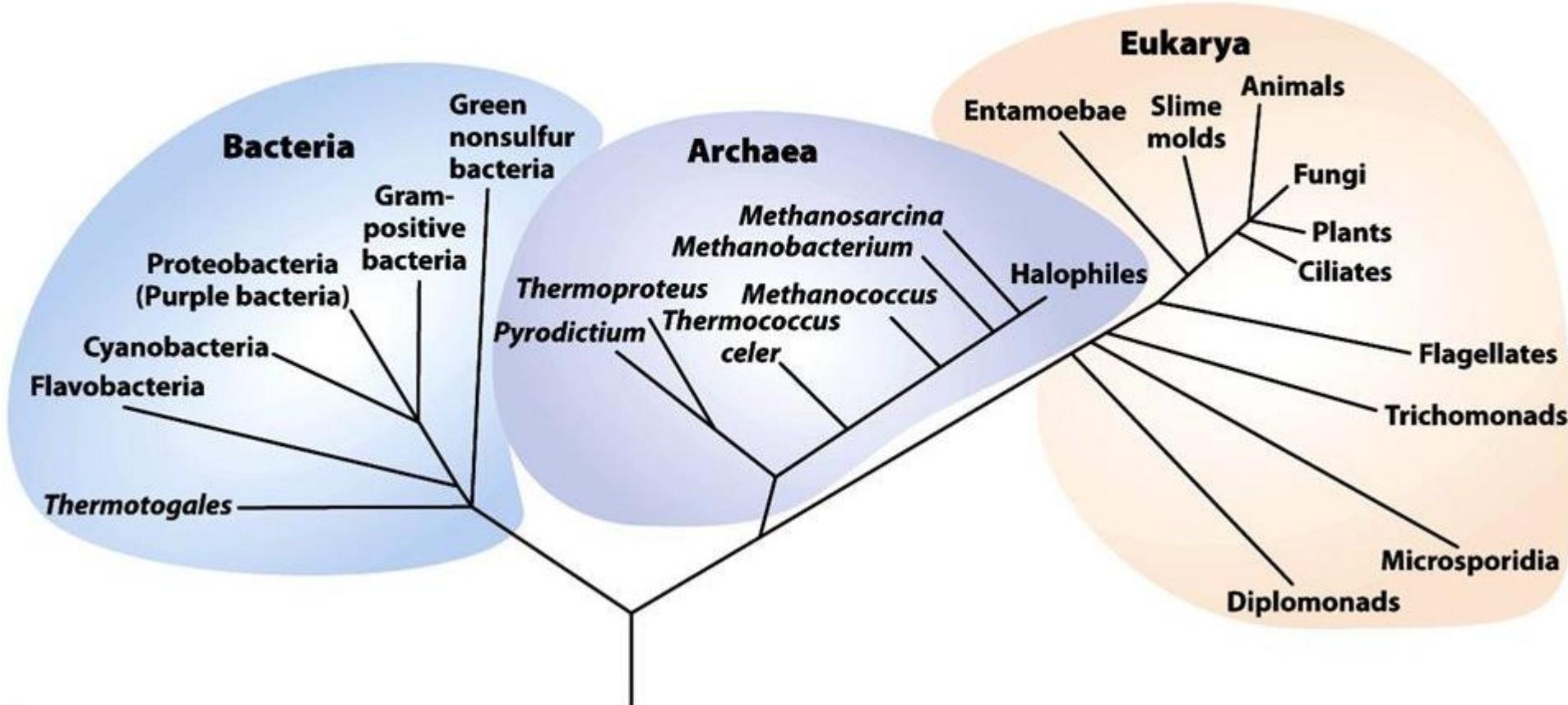
**Spirilla**  
(*Helicobacter pylori*)



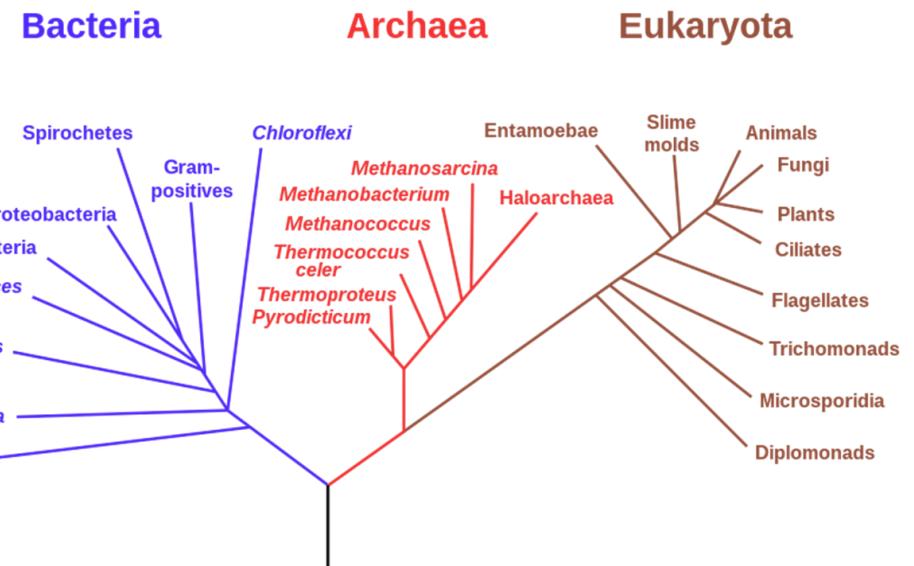
**Spirochaetes**  
(*Treponema pallidum*)

## Three Domains of Life

- Differences in cellular and molecular level define three distinct domains of life

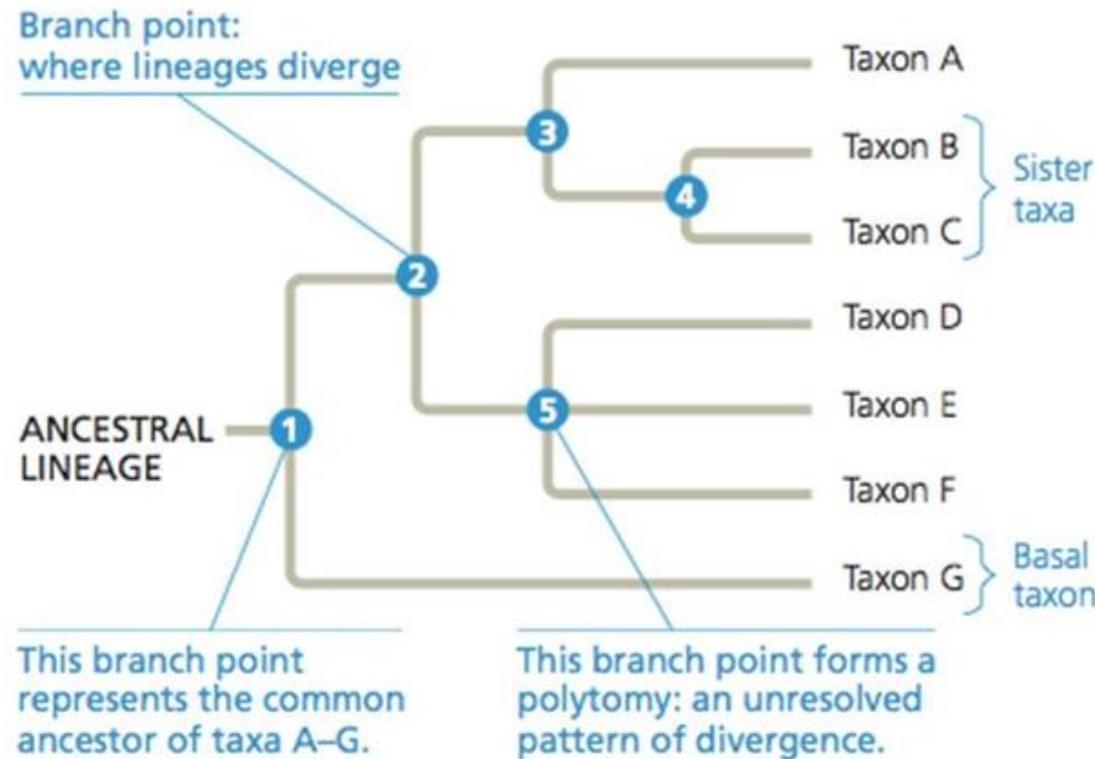


# PHYLOGENETIC TREES



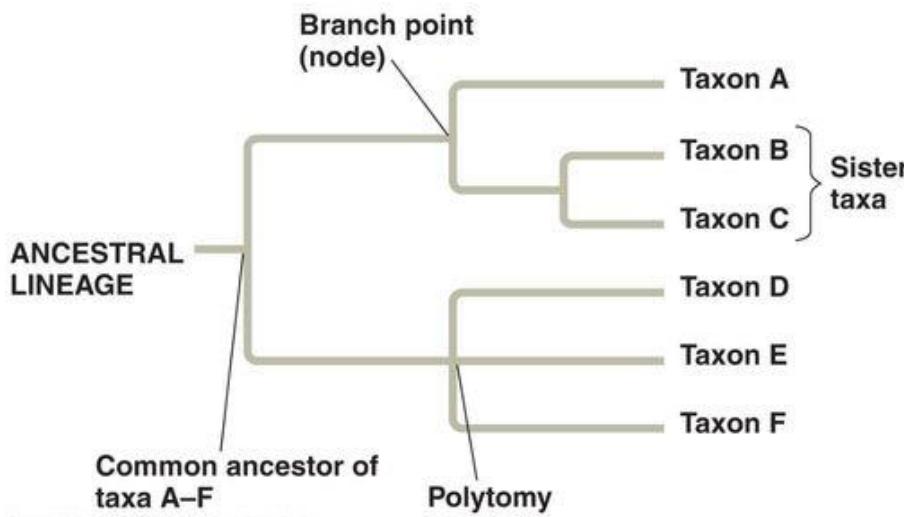
- The diagram shows a phylogenetic tree based on rRNA genes, showing the three life domains. The black branch at the bottom of the phylogenetic tree connects the three branches of living organisms to the last universal common ancestor.
- Phylogeny is the evolutionary history of a species or group of species.
- A **phylogenetic tree** is a branching diagram showing the **evolutionary relationships** among various biological species based upon similarities and differences in their physical or genetic characteristics. All life on Earth is part of a single phylogenetic tree, indicating common ancestry.
- In a rooted phylogenetic tree, each node with descendants represents the inferred most recent common ancestor of those descendants, and the edge lengths in some trees may be interpreted as time estimates. Each node is called a taxonomic unit.

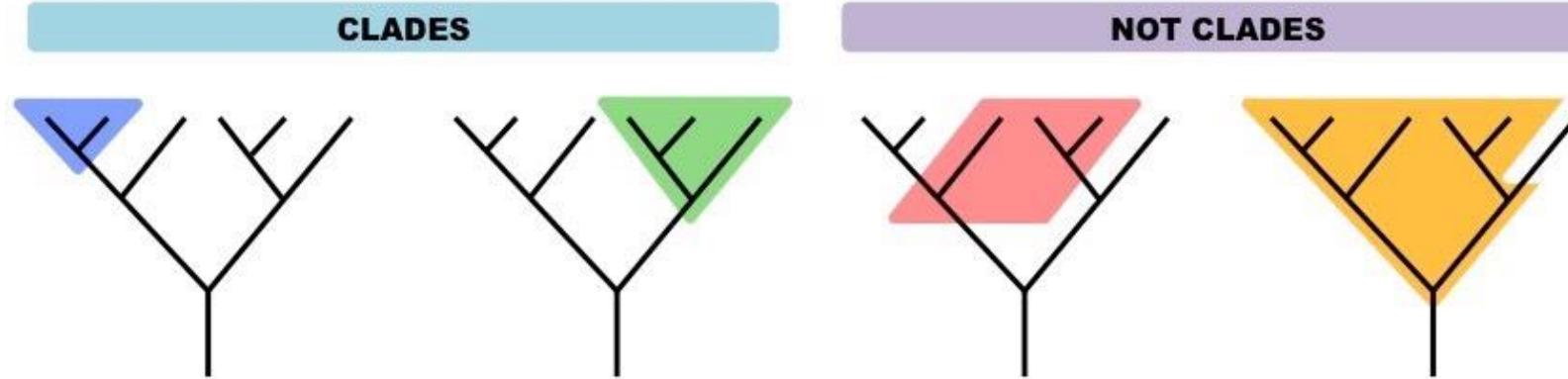
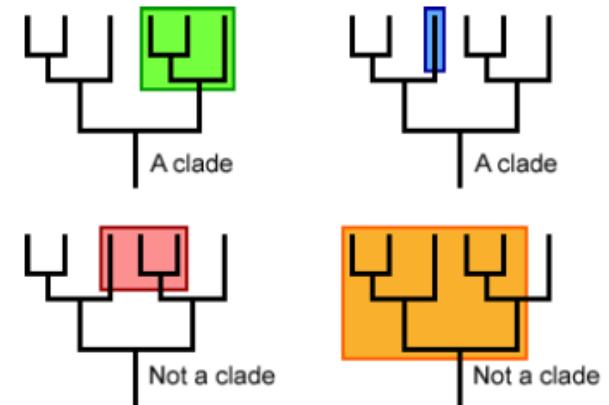
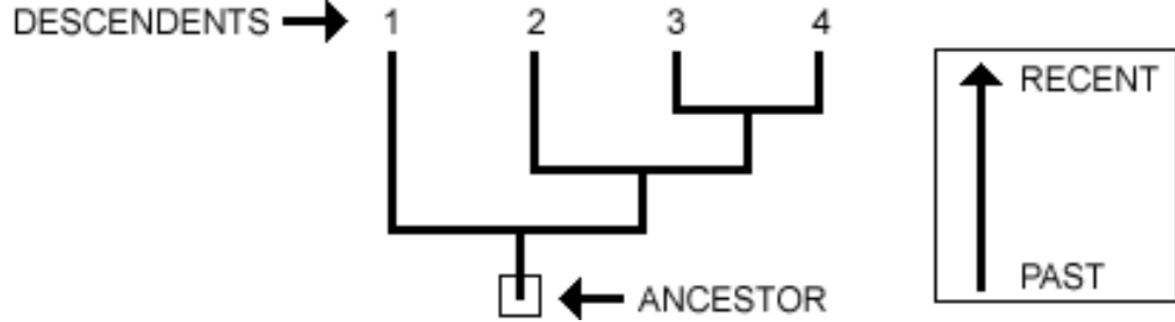
# How to read a phylogenetic tree



# Phylogenetic Trees and Cladograms

- Keep in mind phylogenetic trees and cladograms represent a hypothesis about evolutionary relationships and are **ever-changing** based on new evidence
- Each **branch point** represents the divergence of two species
- **Sister taxa** are groups that share an immediate common ancestor
- A **rooted** tree includes a branch to represent the last common ancestor of all taxa in the tree
- A **polytomy** is a branch from which more than two groups emerge





A clade is a group of organisms that are all descended from a common ancestor; thus a clade includes an ancestor and all descendants of that ancestor.

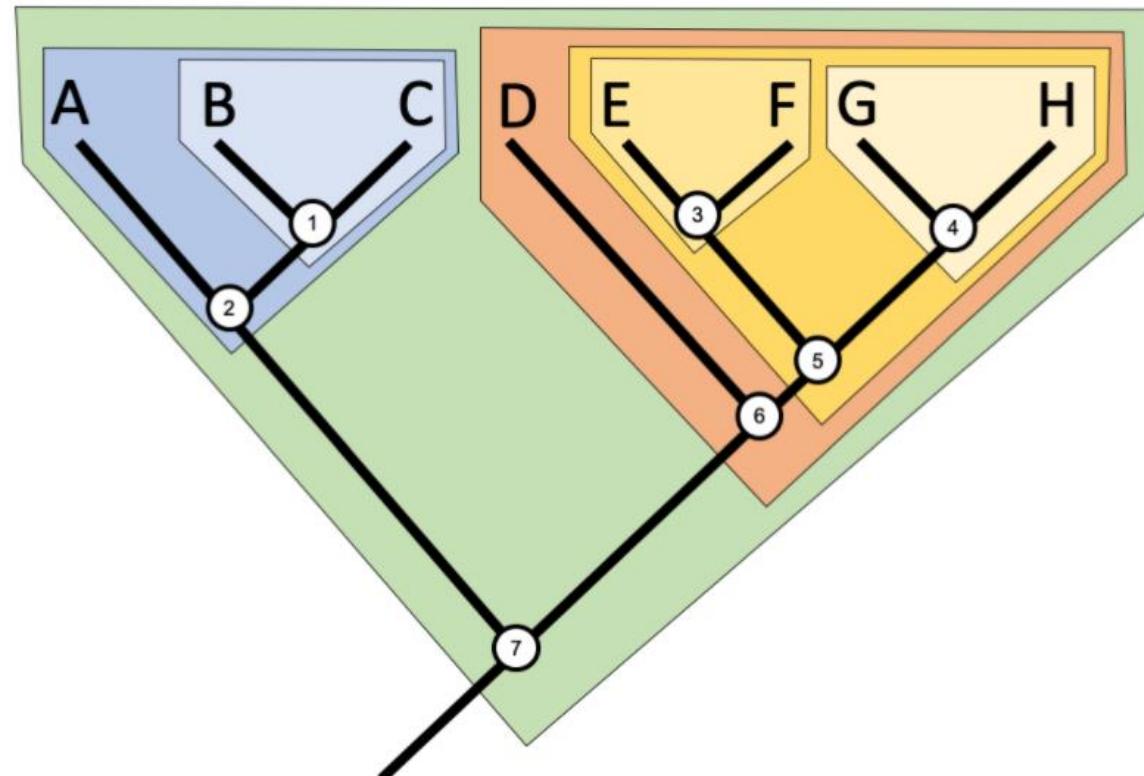
You can think of a clade as a branch on the tree of life.

Clades are not mutually exclusive, but rather form nested sets on a tree. Thus, any given taxon can belong to many clades.

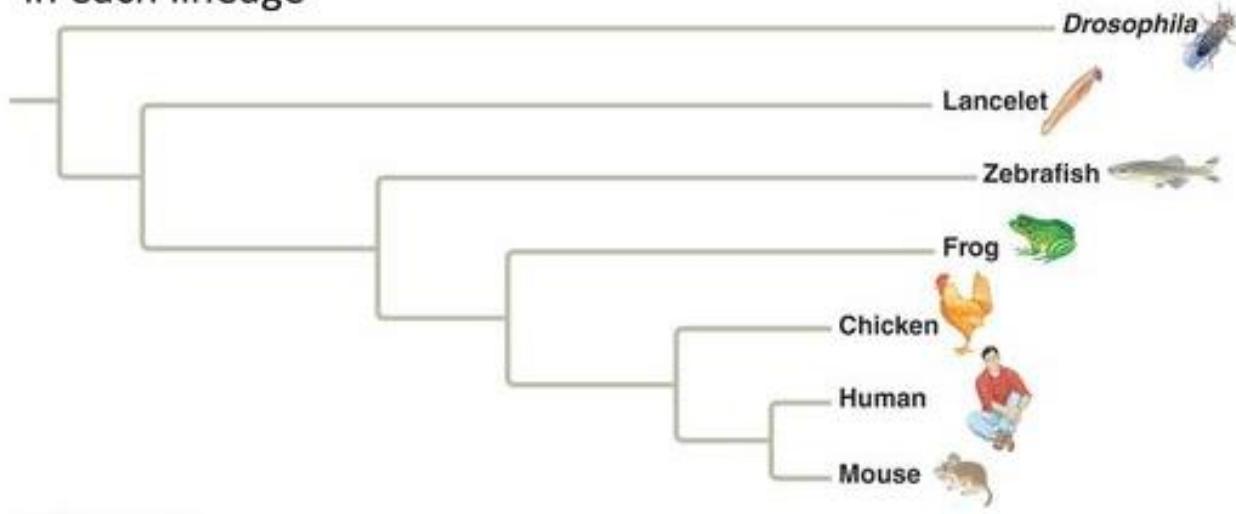
For example, in the tree above, Taxon B belongs to three clades, a clade defined by Node 1, a more inclusive clade defined by Node 2, and an even more inclusive clade defined by Node 7. Taxon E belongs to four clades defined by Nodes 3, 5, 6, and 7, respectively.

Sister taxa or sister groups are pairs of terminal taxa and/or clades that branch from a common node and are often considered closely related.

Pairs of sister terminal taxa in the figure above include: B and C, E and F, and G and H. The clade defined by Node 3 (Node 3 + Taxon E + Taxon F) is sister to the clade defined by Node 4 (Node 4 + Taxon G + Taxon H). Terminal taxon A is sister to the clade defined by Node 1 (Node 1 + Taxon B + Taxon C).



**Phylogenetic tree – branch length based on relative genetic change in each lineage**



### The Meaning of Branch Lengths

Deeper nodes are older than the shallower nodes to which they are connected.

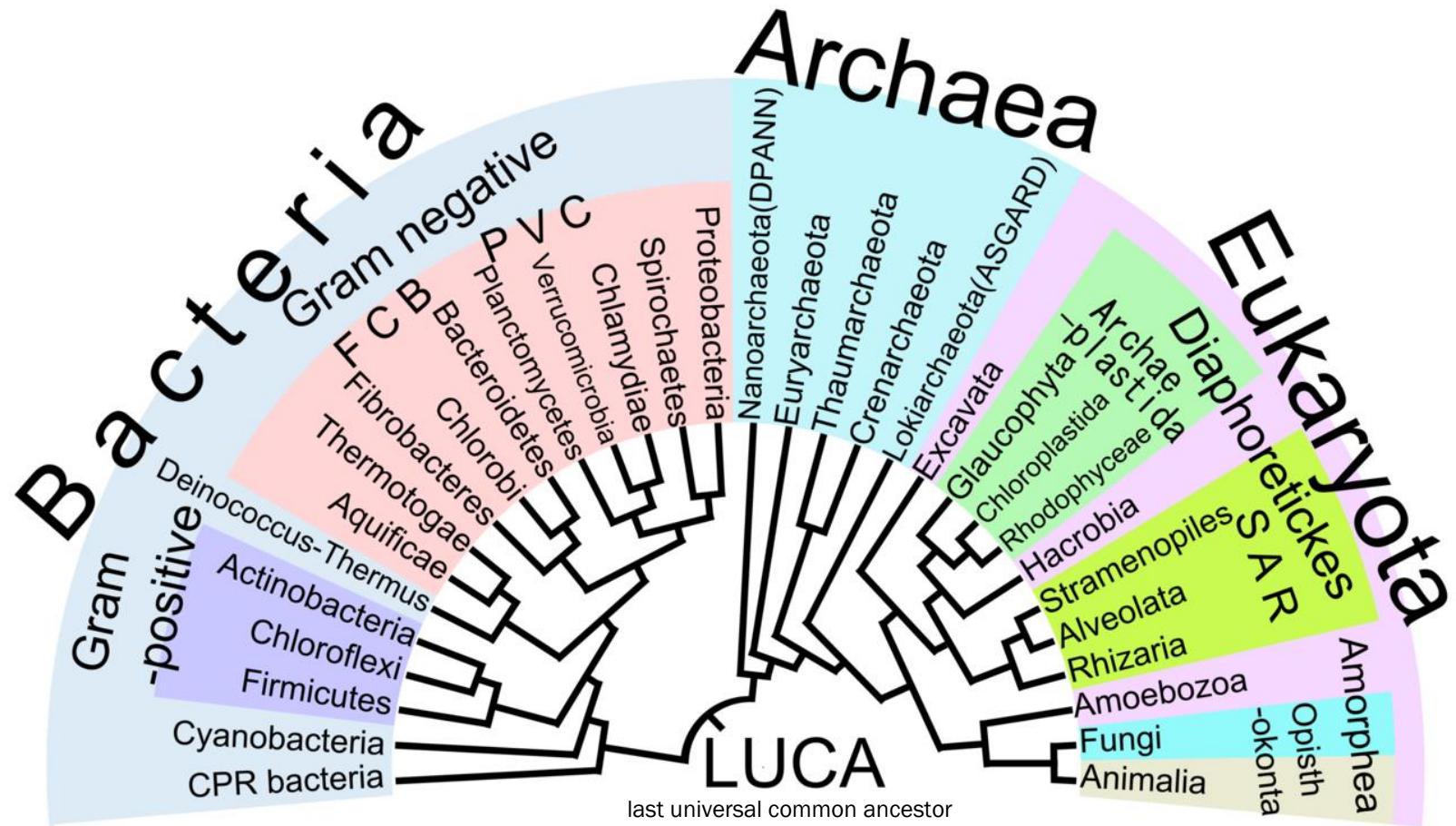
Thus, deeper nodes indicate both more distant relationships among the terminal taxa that they connect, as well as a greater age for the most recent common ancestor of those taxa.

The second thing is evolutionary modification, or the accumulation of hereditary genetic and/or structural changes along branches. While these changes are often not shown (mapped) directly on the branches, it is these inferred changes that underpin the construction and interpretation of a phylogenetic tree. When systematists talk about "branch lengths," they are typically referring to the number of these changes.

# TREE OF LIFE

As evolutionary science continued evolving, the tree of life grew more complicated.

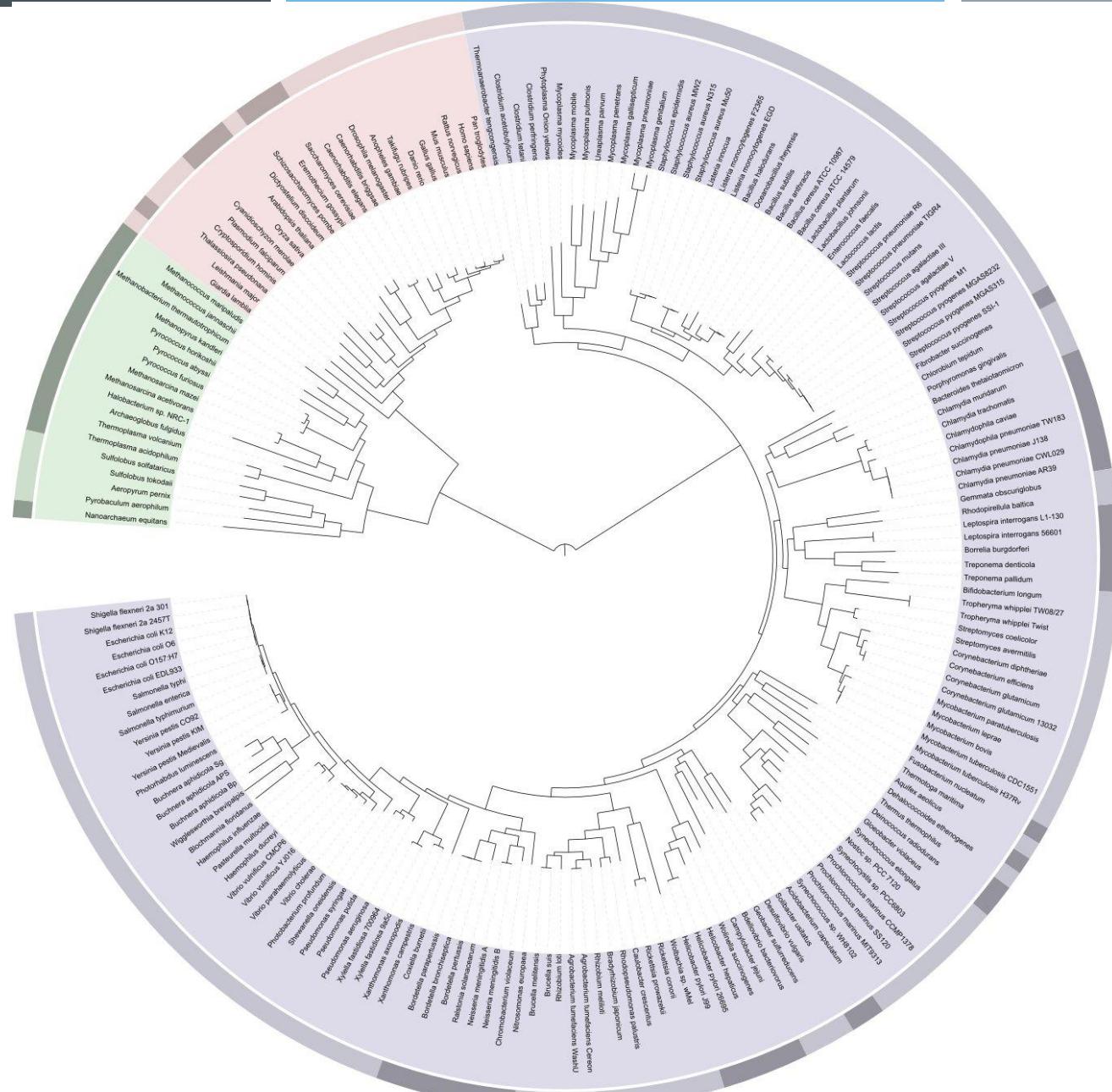
It began to emphasize molecular methods over the observation of physical traits, and to focus more closely on less obvious life forms like bacteria and archaea.



## WE CLEARLY STILL HAVE A LOT TO LEARN ABOUT LIFE ON EARTH

- The 2.3 million species known to science so far may only represent 20 percent of Earth's total biodiversity. We're still fumbling around in the dark, trying to describe and categorize a biosphere we can barely see.
- Our vision is improving, though, with new ways to study tiny life forms.
- Many of the newfound microbes couldn't be studied in a lab because they rely on other organisms to survive, either as parasites or symbiotic partners. Scientists can only detect them by searching for their genomes directly in the wild, rather than trying to grow them in a lab dish. As there is no laboratory access to them; we have only their blueprints and their metabolic potential from their genome sequences.
- These "uncultivable bacteria" are not only common, but seem to represent about a third of all biodiversity on Earth. Other bacteria account for another third, leaving "a bit less than one-third" for archaea and eukaryotes, the latter of which contains all multicellular life — including plants, fungi and animals.
- This incredible diversity means that **there are a mind-boggling number of organisms that we are just beginning to explore** the inner workings of that could change our understanding of biology,

**Tree of life**,  
based on completely  
sequenced genomes  
(bluegrey – bacteria;  
pink- Archaea; green –  
Eukaryota)



Sources: Letunic, Ivica; Bork. "Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation" (PDF). Bioinformatics. 23 (1): 127–128. doi:10.1093/bioinformatics/btl529. ISSN 1367-4803. PMID 17050570. Archived (PDF) from the original on November 29, 2015.

# PHYLOGENETIC ANALYSIS

- Computational phylogenetics is the application of computational algorithms, methods, and programs to phylogenetic analyses. The goal is to assemble a phylogenetic tree representing a hypothesis about the evolutionary ancestry of a set of genes, species, or other taxa. For example, these techniques have been used to explore the relationships between specific genes shared by many types of organisms.
- Traditional phylogenetics relies on morphological data obtained by measuring and quantifying the phenotypic properties of representative organisms, while the more recent field of molecular phylogenetics uses nucleotide sequences encoding genes or amino acid sequences encoding proteins as the basis for classification.
- Many forms of molecular phylogenetics are closely related to and make extensive use of **sequence alignment** in constructing and refining phylogenetic trees, which are used to classify the evolutionary relationships between homologous genes represented in the genomes of divergent species. The phylogenetic trees constructed by computational methods are unlikely to perfectly reproduce the evolutionary tree that represents the historical relationships between the species being analyzed.
- Although phylogenetic trees produced on the basis of sequenced genes or genomic data in different species can provide evolutionary insight, these analyses have important limitations. The trees do not necessarily accurately represent the evolutionary history of the included taxa. The analysis can be confounded by genetic recombination, horizontal gene transfer, or hybridisation between species.

# SEQUENCE ALIGNMENT

- In bioinformatics, a sequence alignment is a way of arranging the sequences of DNA, RNA, or protein to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences. Aligned sequences of nucleotide or amino acid residues are typically represented as rows within a matrix. Gaps are inserted between the residues so that identical or similar characters are aligned in successive columns.
- If two sequences in an alignment share a common ancestor, mismatches can be interpreted as point mutations and gaps as indels (that is, insertion or deletion mutations) introduced in one or both lineages in the time since they diverged from one another.
- In sequence alignments of proteins, the degree of similarity between amino acids occupying a particular position in the sequence can be interpreted as a rough measure of how conserved a particular region or sequence motif is among lineages.
- The **absence of substitutions**, or the presence of only very conservative substitutions (that is, the substitution of amino acids whose side chains have similar biochemical properties) in a particular region of the sequence, suggest that this region has **structural or functional importance**. Although DNA and RNA nucleotide bases are more similar to each other than are amino acids, the conservation of base pairs can indicate a similar functional or structural role.

# SEQUENCE ALIGNMENT

**Histone H1 (residues 120-180)**

HUMAN	KKASKPKKAASKAPTKKPATPVKKAKKKLAATPKKAKKPKTVKAKPVKASKPKKAKPVK
CHIMP	KKASKPKKAASKAPTKKPATPVKKAKKKLAATPKKAKKPKTVKAKPVKASKPKKAKPVK
MOUSE	KKAAPKKAASKAPSKKPKATPVKKAKKKPAATPKKAKKPKVVVKPVKASKPKKAKTVK
RAT	KKAAPKKAASKAPSKKPKATPVKKAKKKPAATPKKAKKPKIVKVVPVKAASKPKKAKPVK
COW	KKAAPKKAASKAPSKKPKATPVKKAKKKPAATPKKTPKPTVKAKPVKASKPKKTPVK

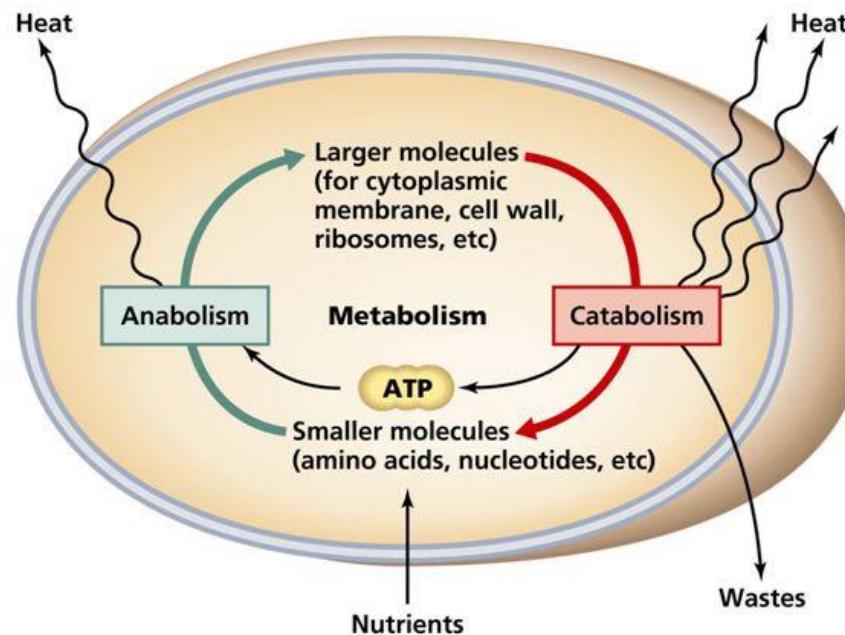
A sequence alignment of mammalian histone proteins.

- Conserved proteins across all sequences are highlighted in grey.

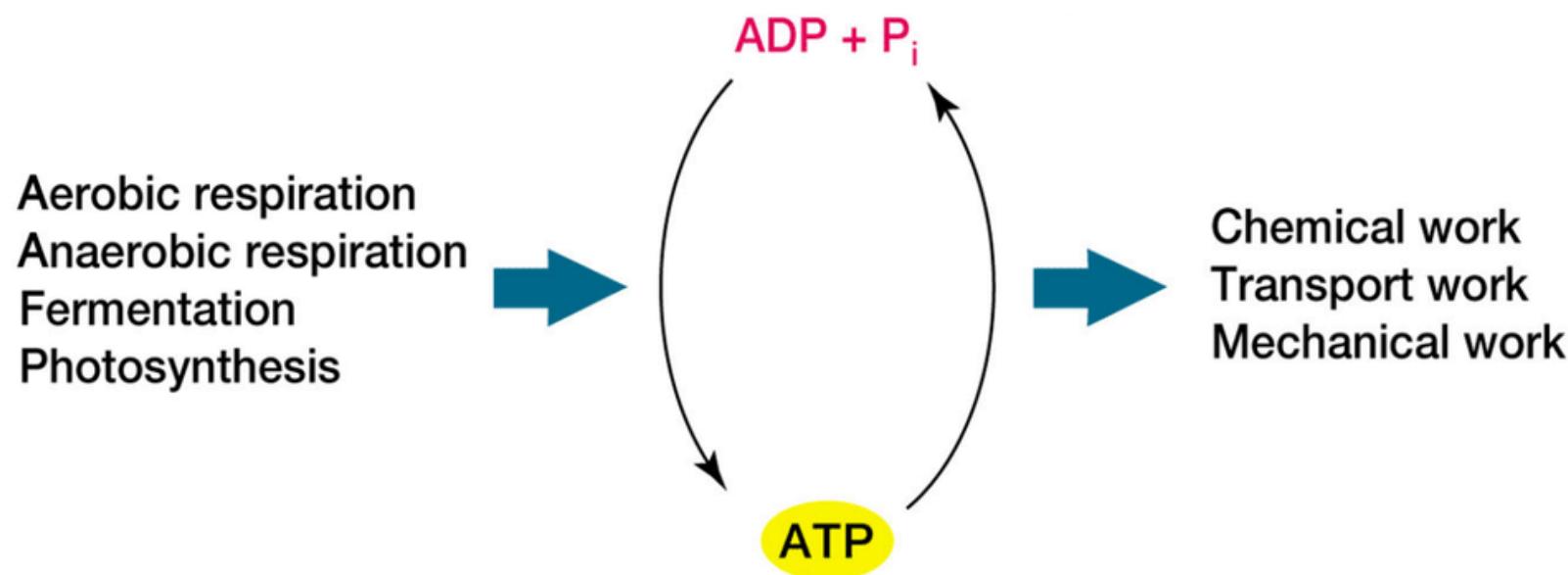
# BACTERIAL METABOLISM

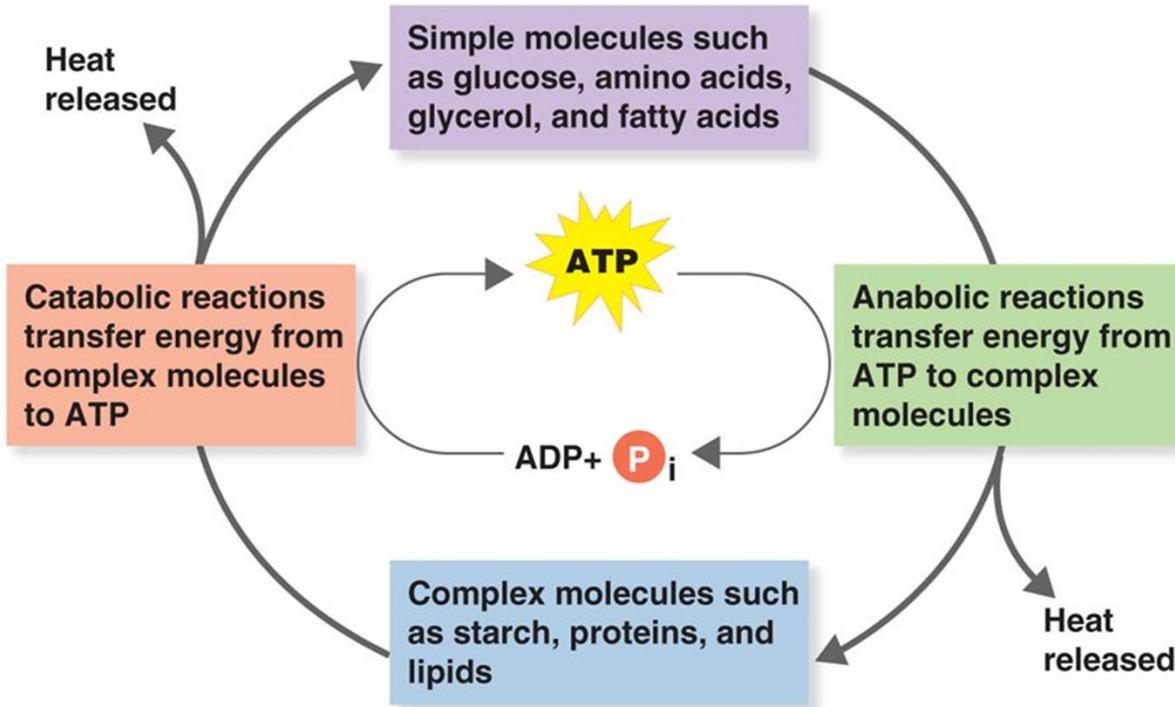
## Bacterial metabolism

- Catabolism + anabolism → Metabolism



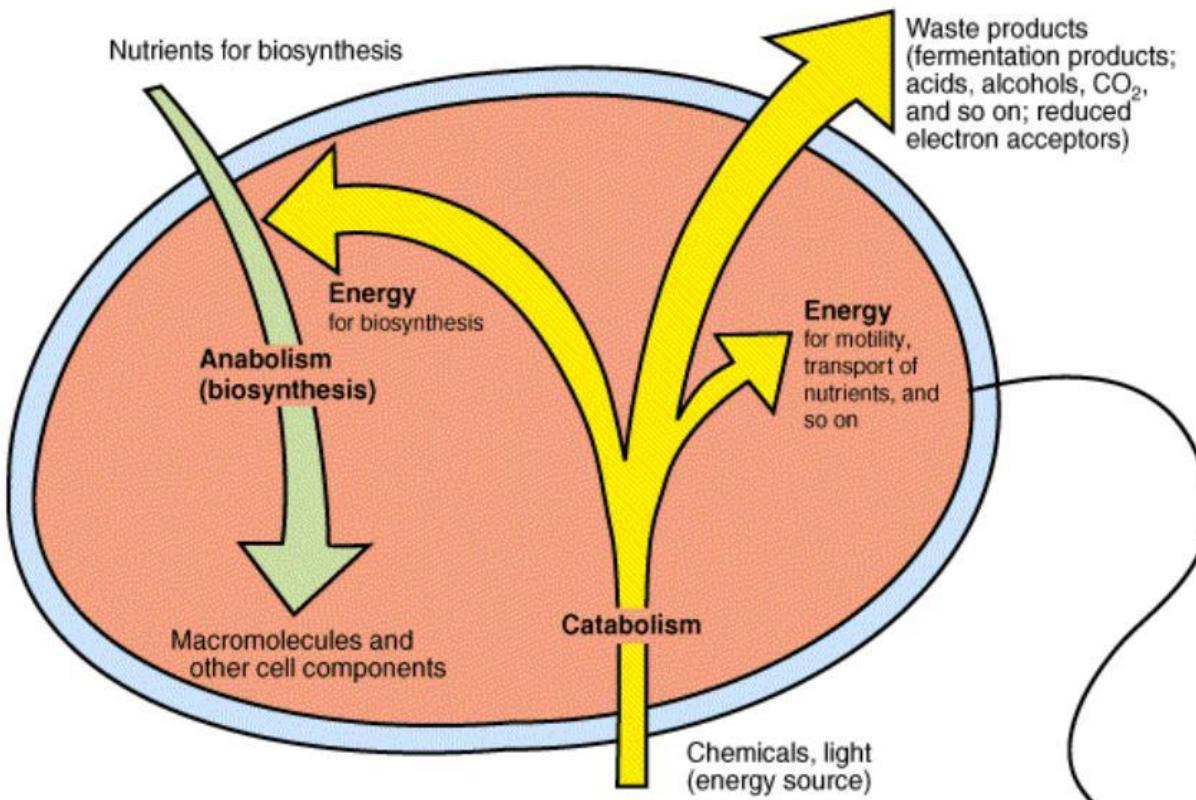
# Microbial Metabolism



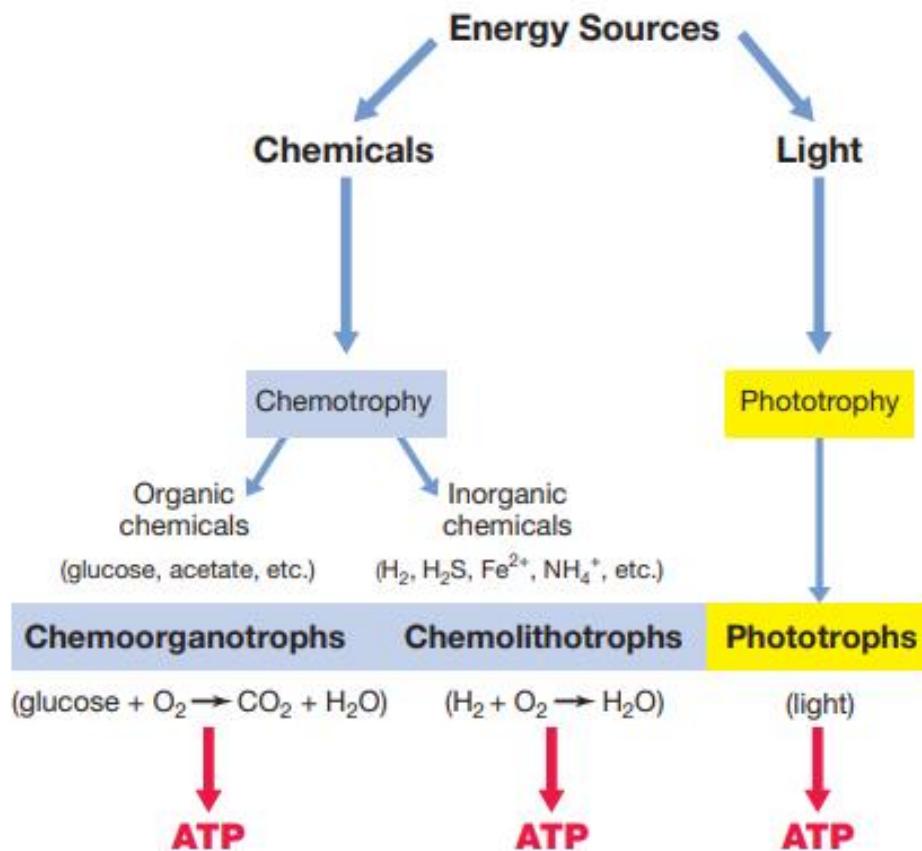


# Microbial Nutrition

## Cell metabolism



# ENERGY SOURCES



# METABOLIC OPTIONS OF OBTAINING ENERGY

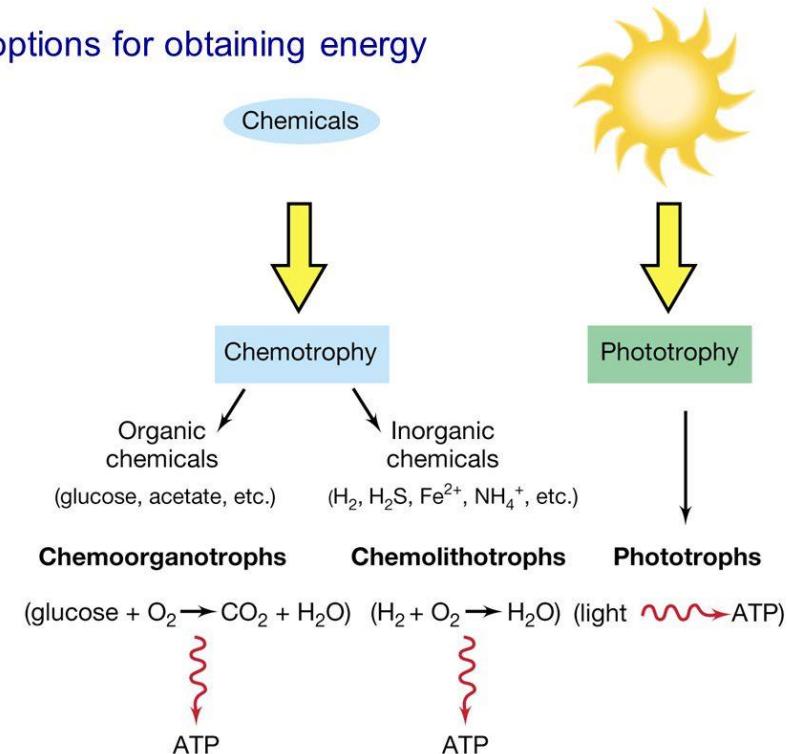
## BACTERIAL ENERGY SOURCES

Phototroph	Light
Chemotroph	Chemicals
Chemoorganotroph	Organic compounds
Chemolithotroph	Inorganic compounds

## BACTERIAL CARBON SOURCES

Autotroph	Carbon dioxide
Heterotroph	Organic compounds

## Metabolic options for obtaining energy



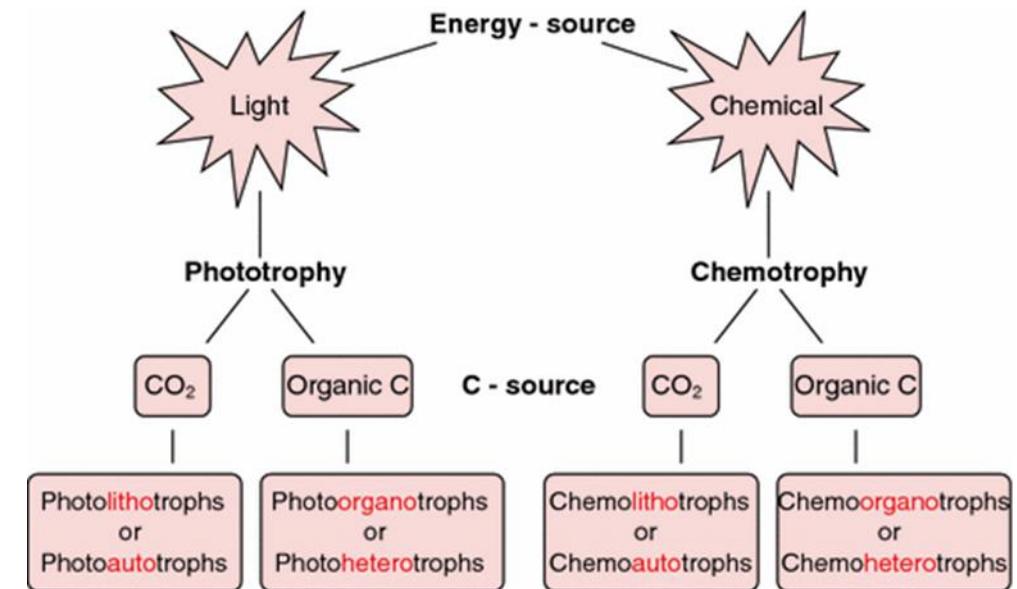
# METABOLIC OPTIONS FOR OBTAINING ENERGY

All organisms require an energy source to drive energy-consuming life processes.

Energy can be tapped from light or chemicals (organic and inorganic chemicals).

Organisms that can utilize radiant energy (sunlight) are called phototrophs.

Chemotrophs are organisms that can harvest energy from chemicals.



# CHEMOTROPHS

## Chemoorganotrophs

- Organisms that **conserve energy from organic chemicals** are called chemoorganotrophs. Thousands of different organic chemicals can be used by one or another microorganism. Indeed, all-natural and even most synthetic organic compounds can be metabolized. Energy is conserved from the oxidation of the compound and is stored in the cell in the energy-rich bonds of the compound adenosine triphosphate (ATP).
- Aerobes obtain energy from an organic compound in the presence of oxygen, anaerobes obtain energy in the absence of oxygen and facultative anaerobes can break down organic compounds in both aerobic and anaerobic conditions.

## Chemolithotrophs

- The **oxidation of inorganic compounds to yield energy is known as chemolithotrophy**. Like phototrophic organisms, chemolithotrophic bacteria are also autotrophs.
- Chemolithotrophy occurs only in prokaryotes and is widely distributed among species of Bacteria and Archaea. Several inorganic compounds can be oxidized; for example,  $H_2$ ,  $H_2S$  (hydrogen sulfide),  $NH_3$  (ammonia), and  $Fe_2$  (ferrous iron), and thus we have “sulfur” bacteria, “iron” bacteria, and so on.
- The capacity to conserve energy from the oxidation of inorganic chemicals is a good metabolic strategy because competition from chemoorganotrophs, is not an issue. In addition, many of the inorganic compounds oxidized by chemolithotrophs, for example,  $H_2$  and  $H_2S$ , are actually the waste products of chemoorganotrophs. Thus, chemolithotrophs have evolved strategies for exploiting resources that chemoorganotrophs are unable to use, so it is common for species of these two physiological groups to live in close association with one another.

# PHOTOTROPHS

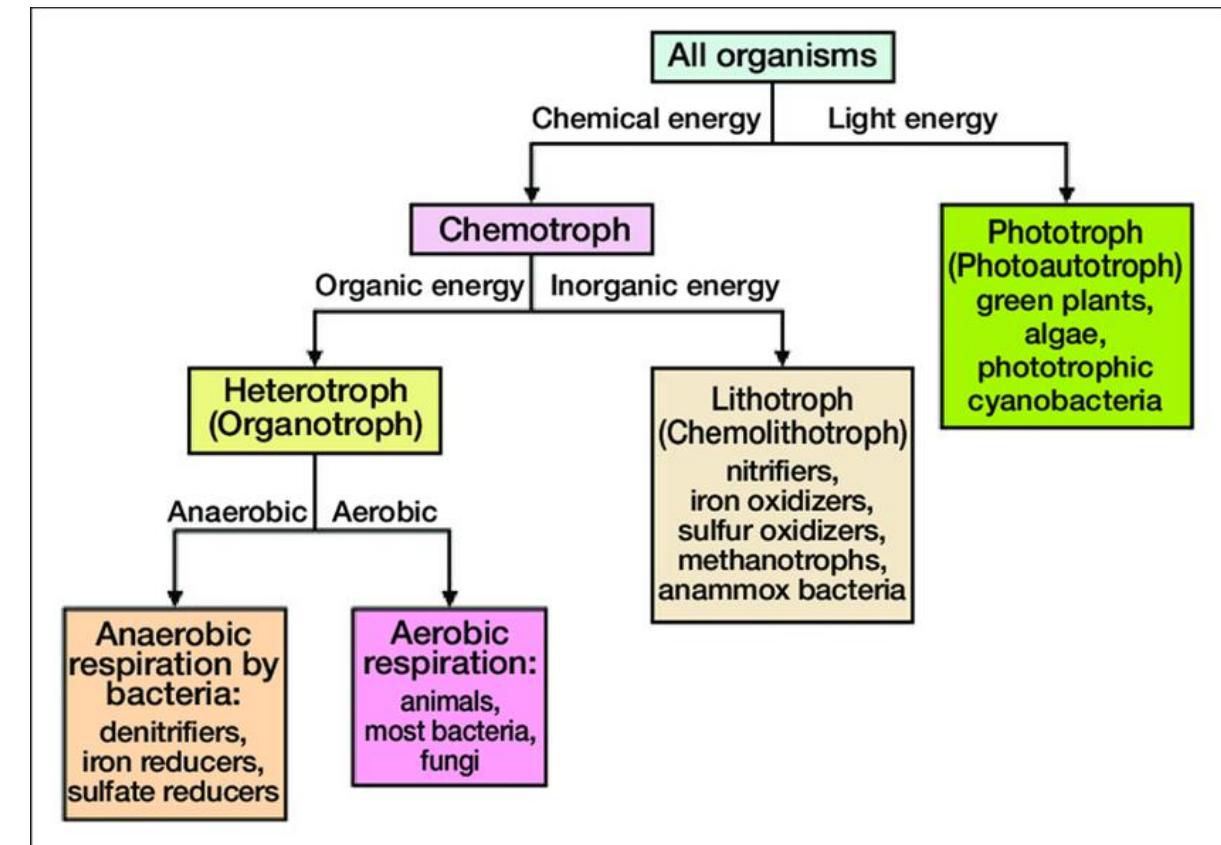


- Sunlight is available in many microbial habitats on Earth, phototrophic microorganisms living in those areas harvest energy from sunlight. They contain pigments that allow them to convert light energy into chemical energy, and thus their cells appear colored.
- Purple bacteria appeared on Earth long before oxygenic phototrophs evolved. Green sulfur bacteria were some of the first phototrophs to evolve on Earth.
- Two major forms of phototrophy are known in prokaryotes.
- Oxygenic photosynthesis: oxygen ( $O_2$ ) is produced. Among microorganisms, oxygenic photosynthesis is characteristic of cyanobacteria and algae (oxygenic phototrophs).
- Anoxygenic photosynthesis: does not yield  $O_2$ . Purple sulfur bacteria, green bacteria, and heliobacteria are anoxygenic phototrophs.

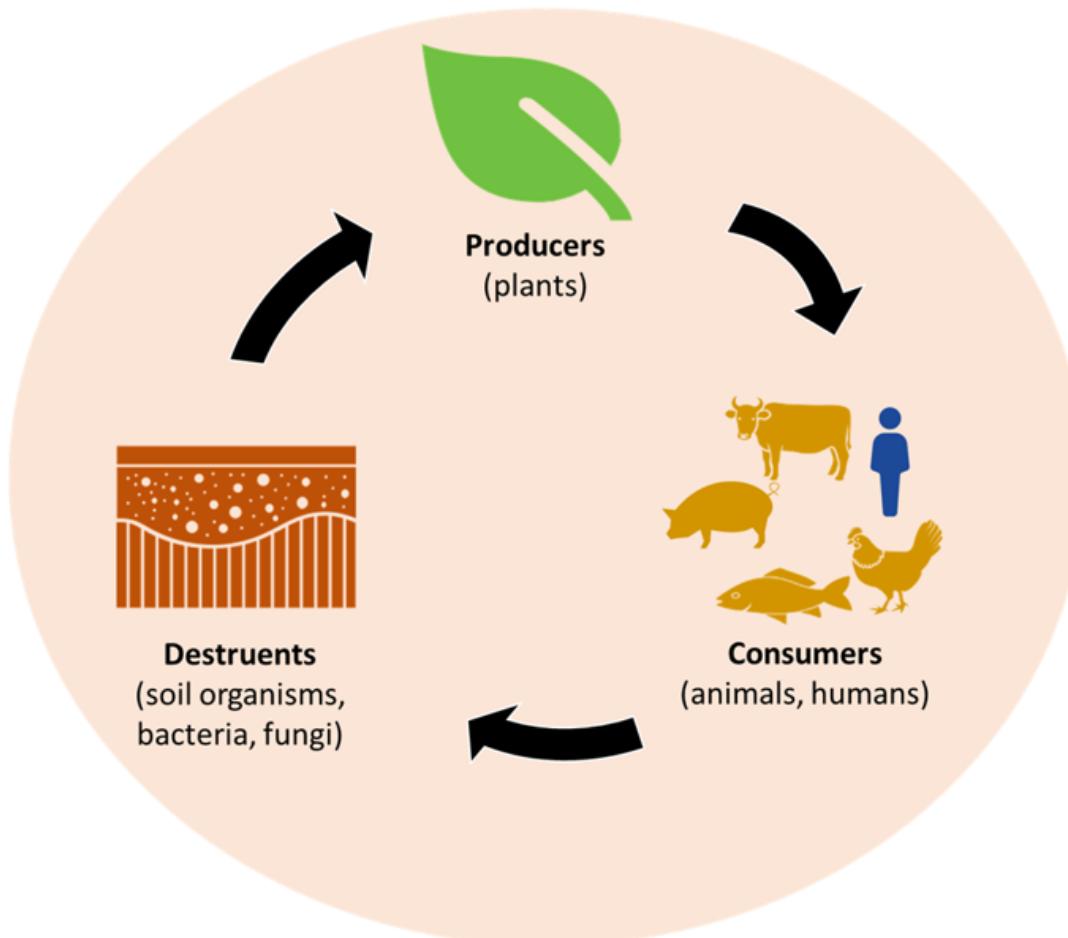
# AUTOTROPHES AND HETEROTROPHES

Autotrophs are primary producers because they synthesize new organic matter from  $\text{CO}_2$ . Autotrophs can transform inorganic compounds into carbohydrates, proteins, nucleic acids, lipids, vitamins, and other complex organic substances required for the cells.

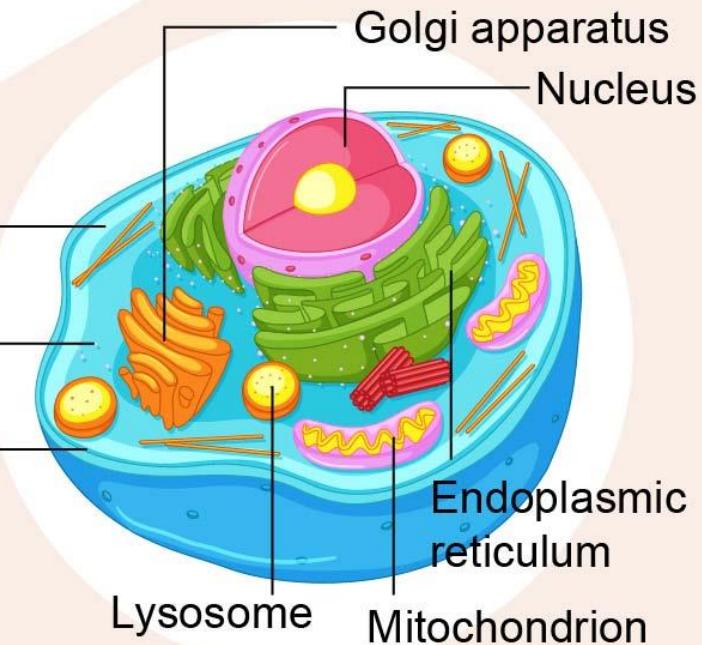
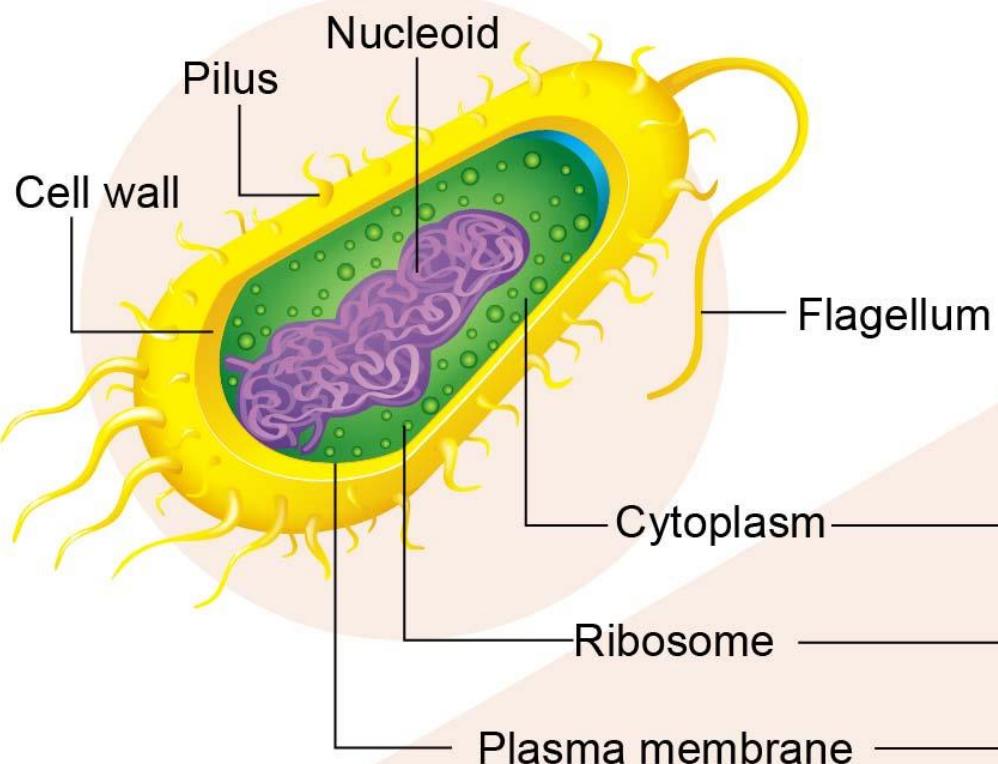
Heterotrophs either feed directly on the cells of primary producers or live off products they excrete. Virtually all organic matter on Earth has been synthesized by primary producers. Heterotrophs rely on autotrophs for their foods and are also called consumers of the food chains.



## Closed biogeochemical nutrient cycle



# PROKARYOTE CELL

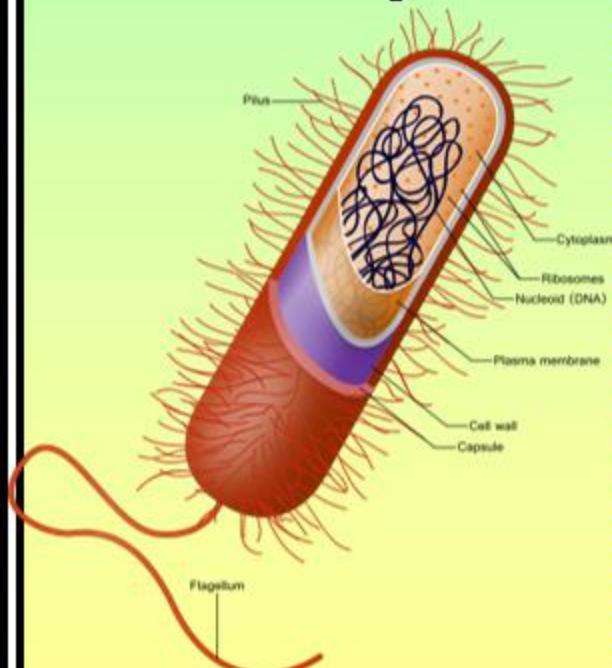


# EUKARYOTE CELL

Prokaryotic cells	Eukaryotic cells
Believed to have existed millions of years ago	Believed to have evolved from prokaryotic cells through evolution
Bacteria, cyanobacteria, algae, etc.	Animal and plant cells
Lack of nuclear membrane; chromatin is free in the cytoplasm	Presence of a nuclear membrane; chromatin is bound by it
Absence of cellular organelles (mitochondria, endoplasmic reticulum, Golgi body, etc.)	Presence of cellular organelles
Considerably primitive and simplistic	Complex, efficient, and more organized

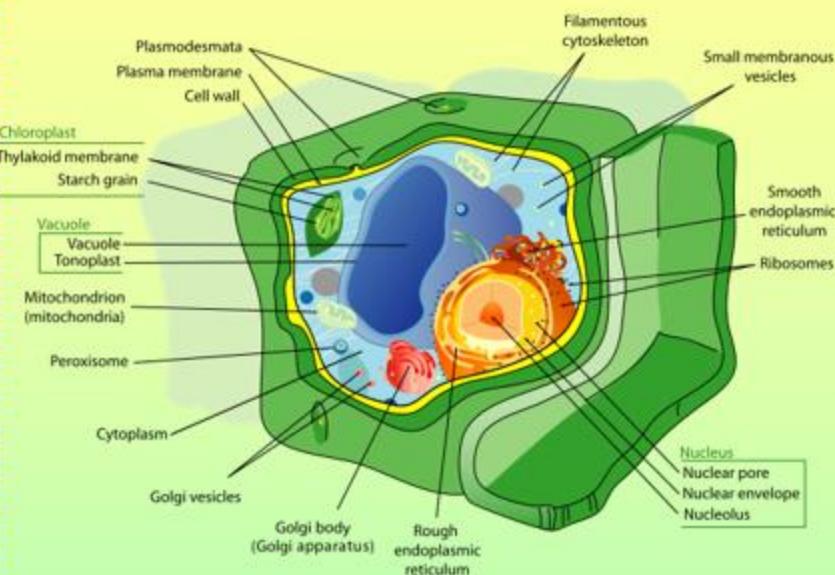
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# Prokaryote

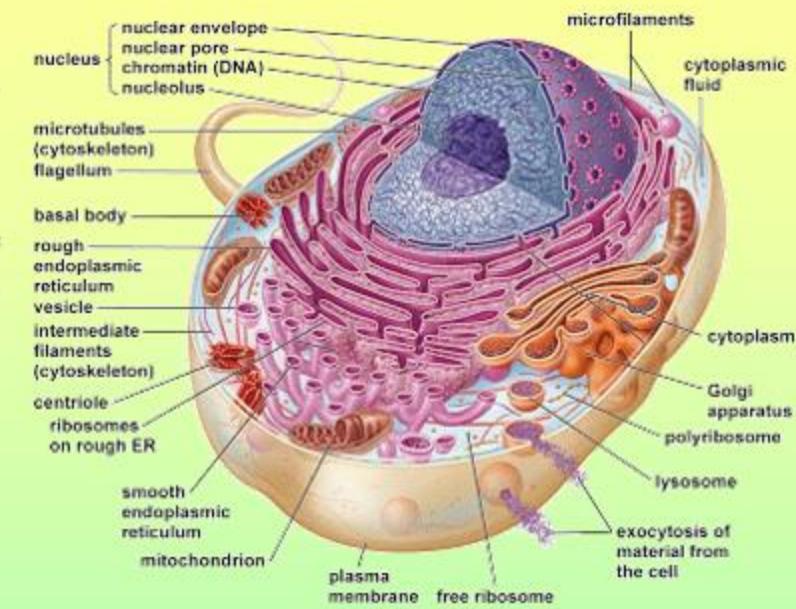


Bacterium

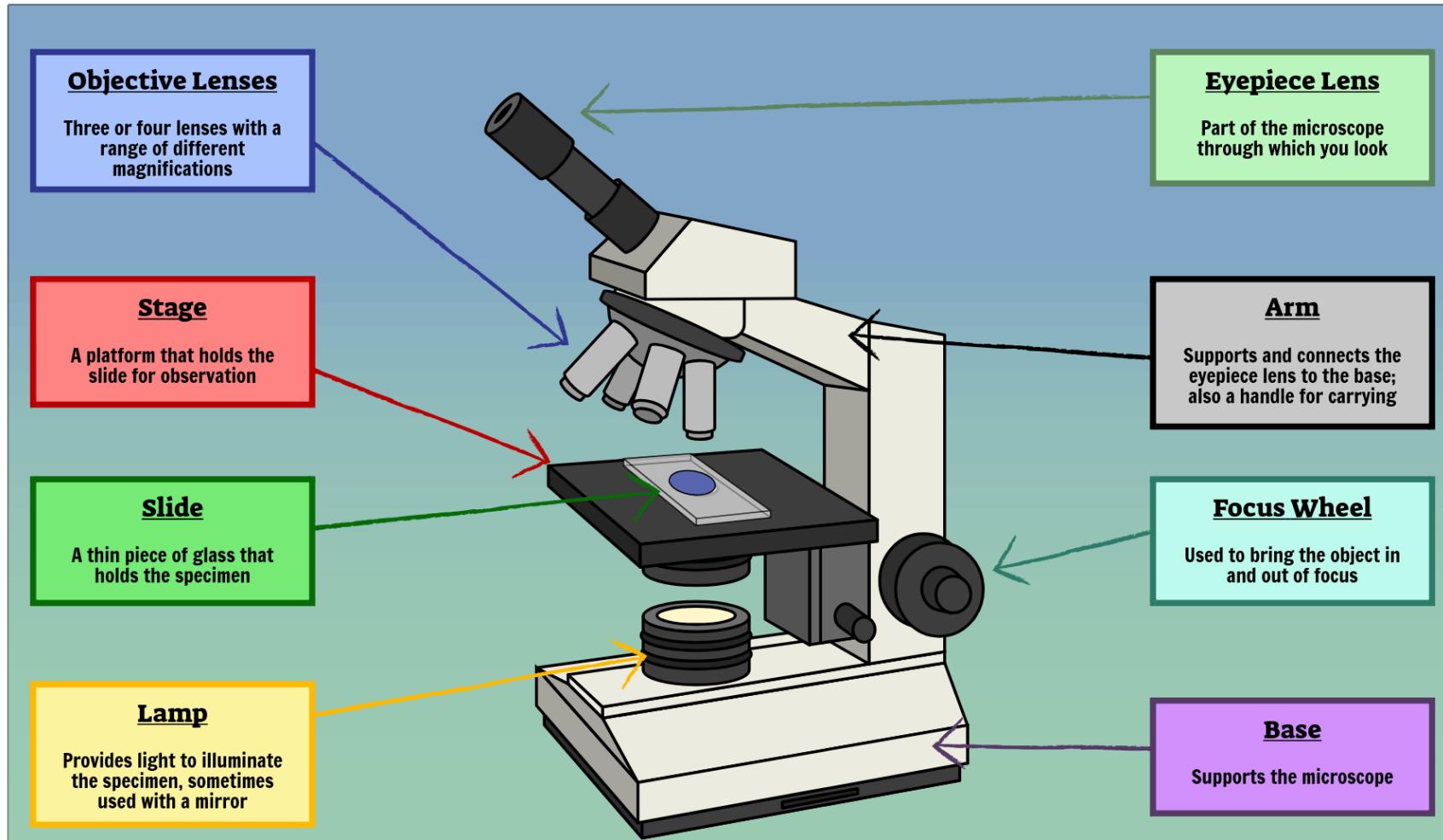
# Eukaryotes

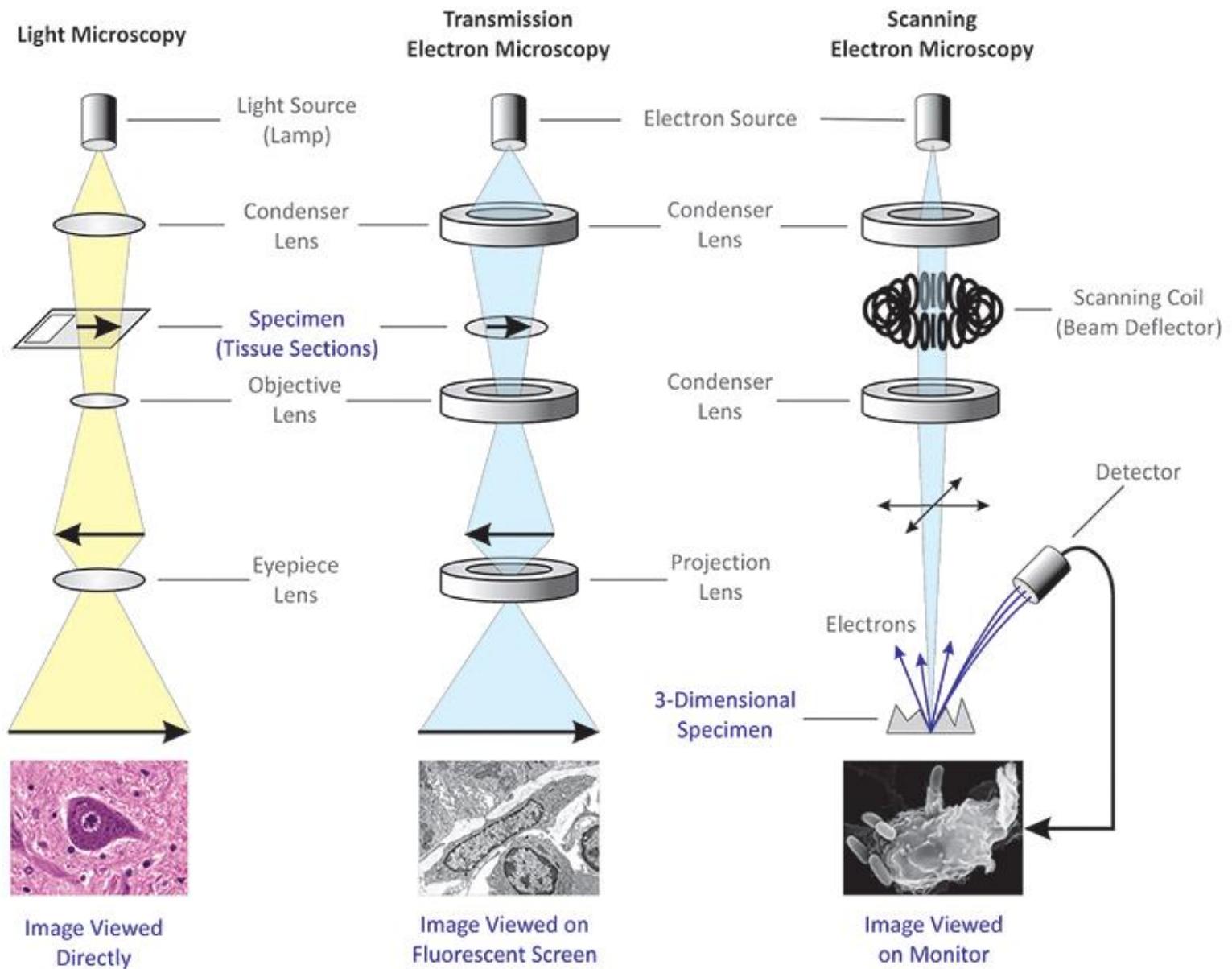


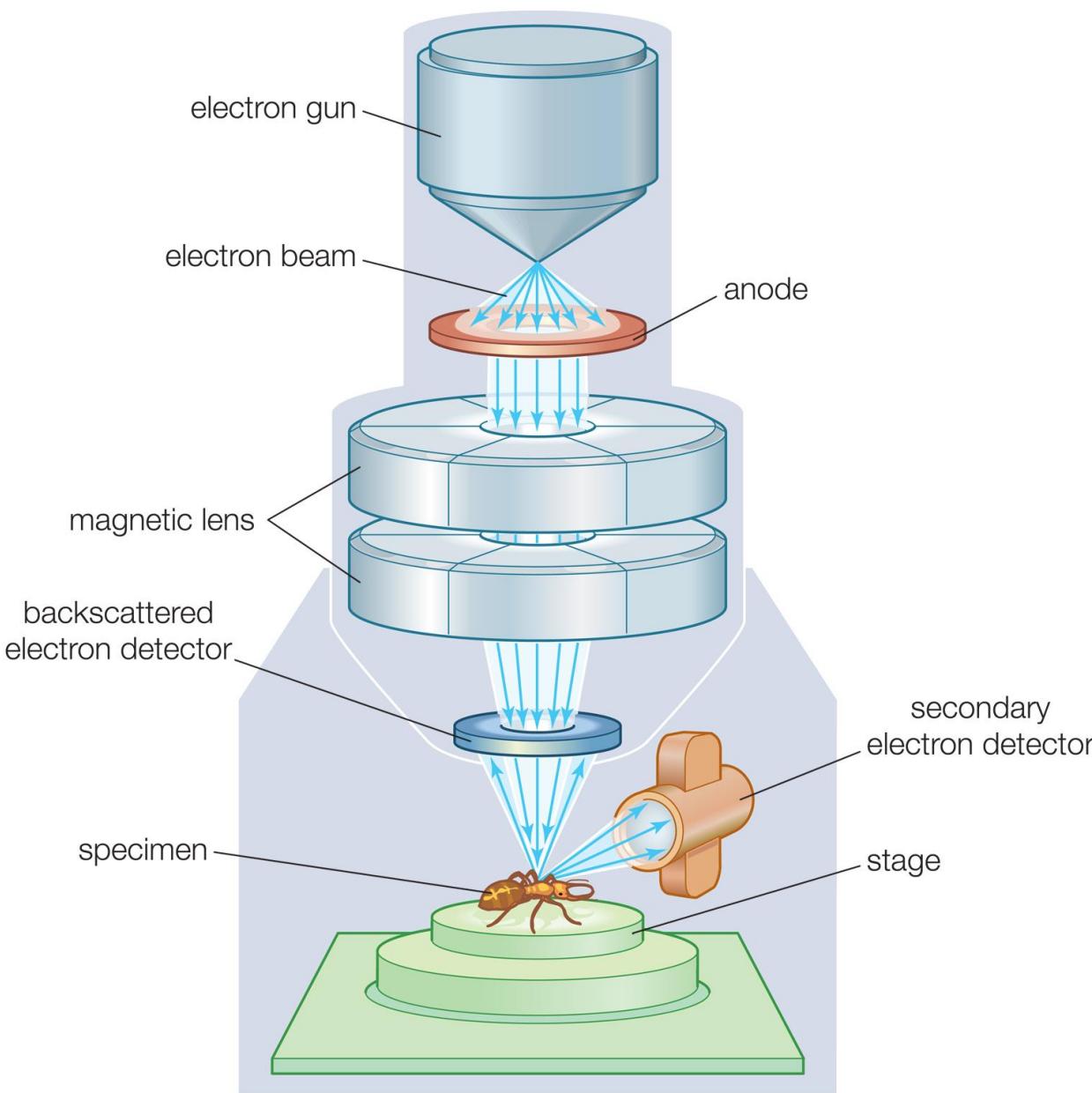
Plant Cell

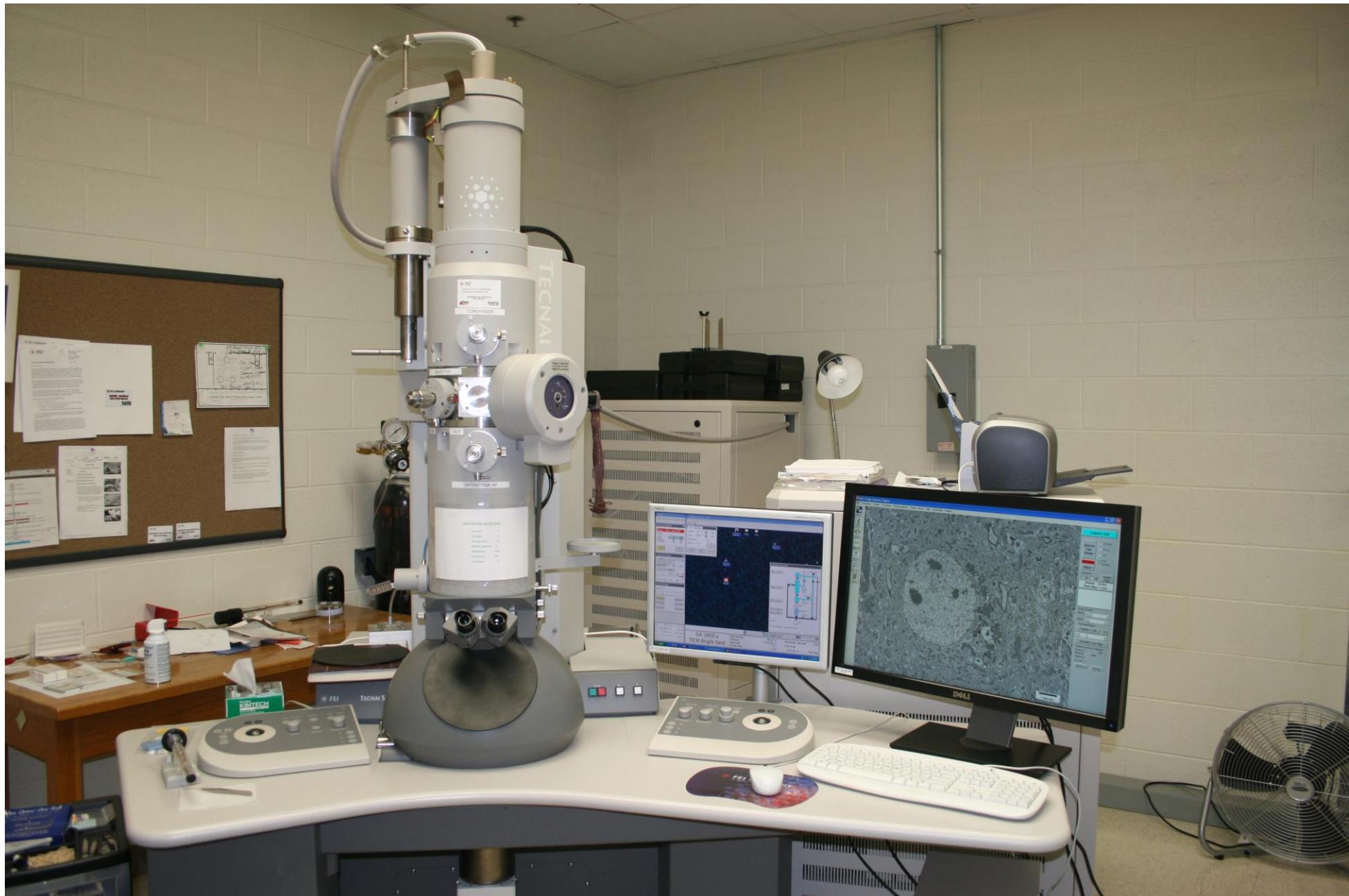


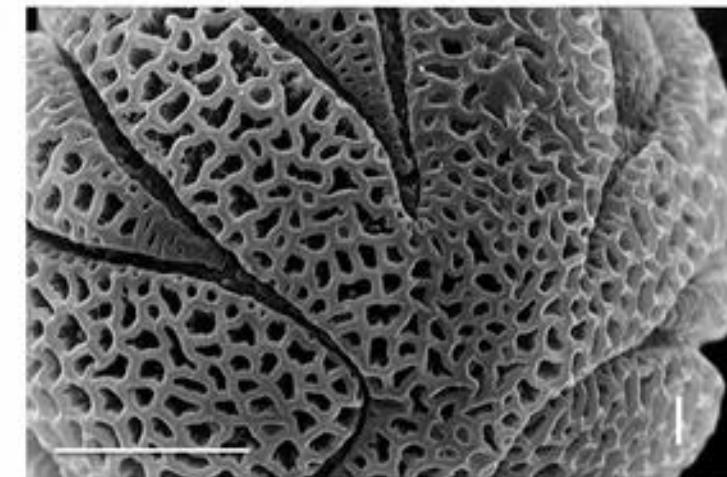
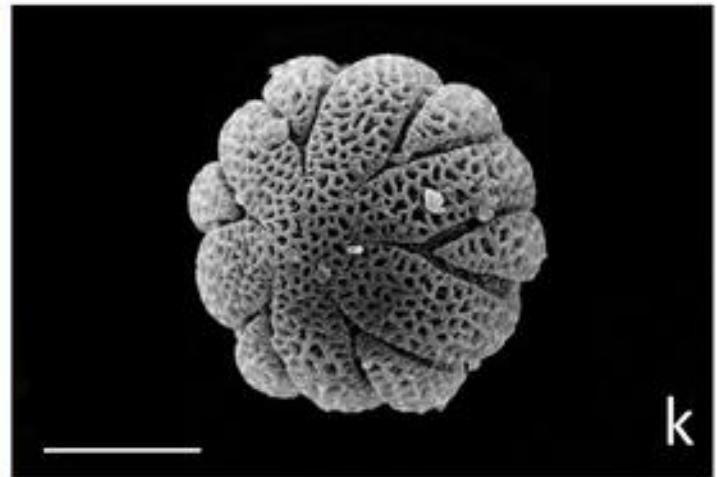
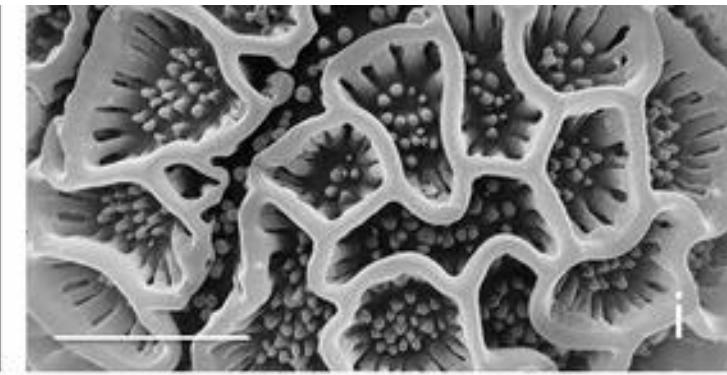
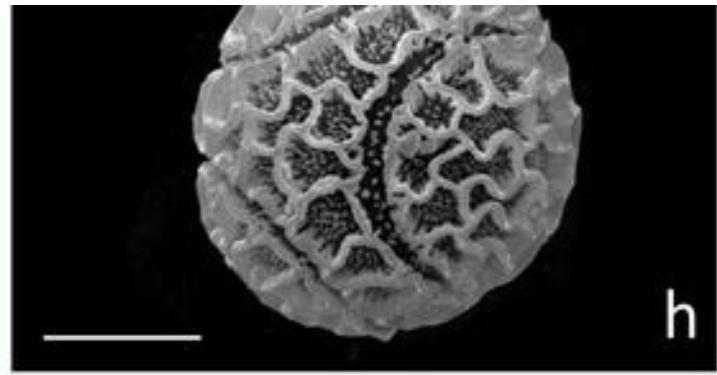
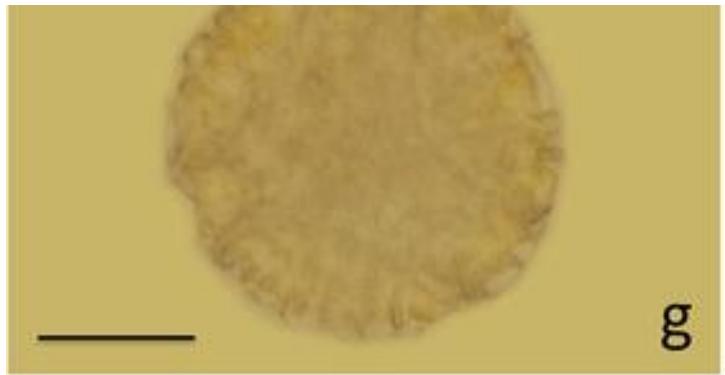
Animal Cell

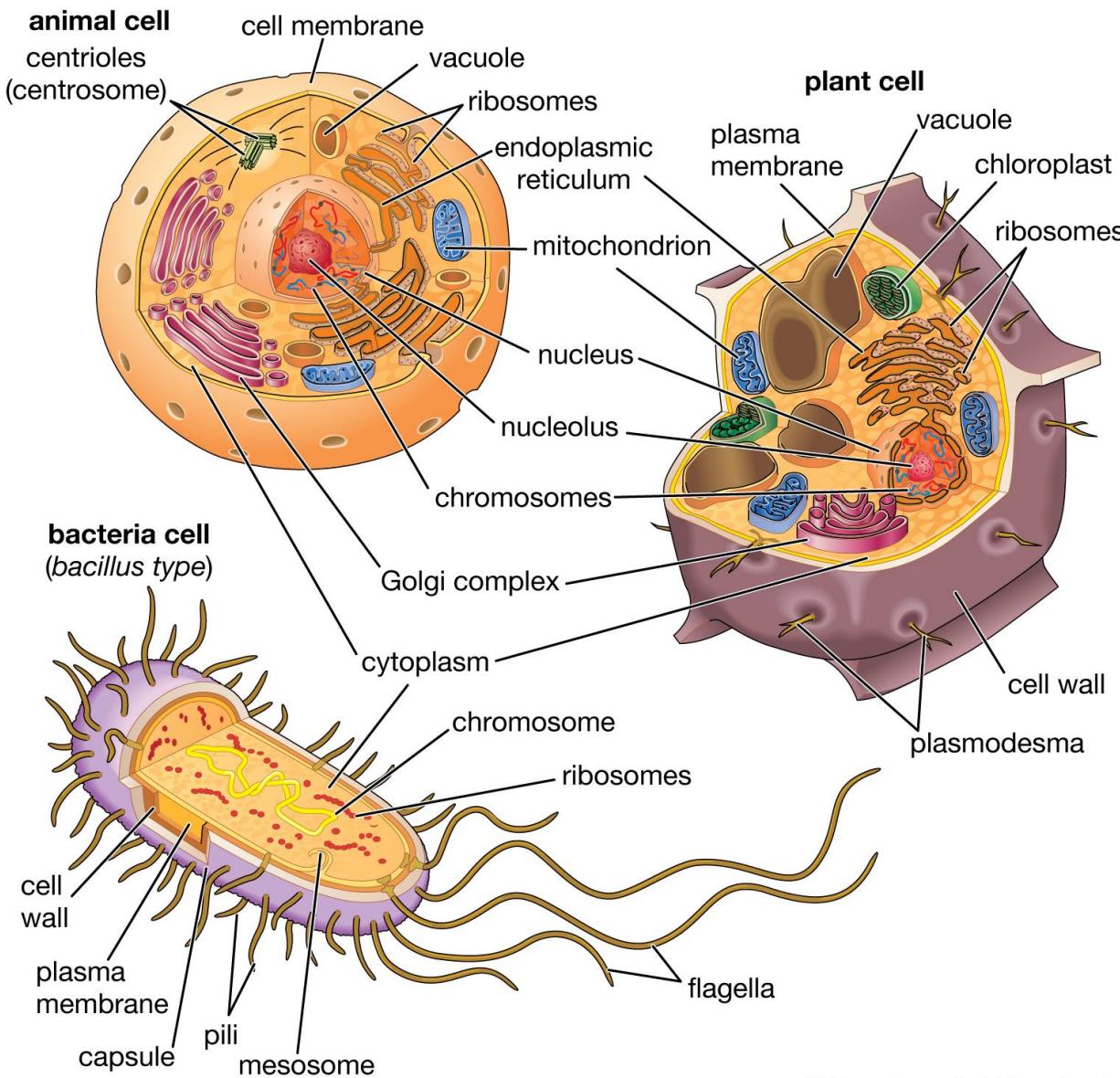


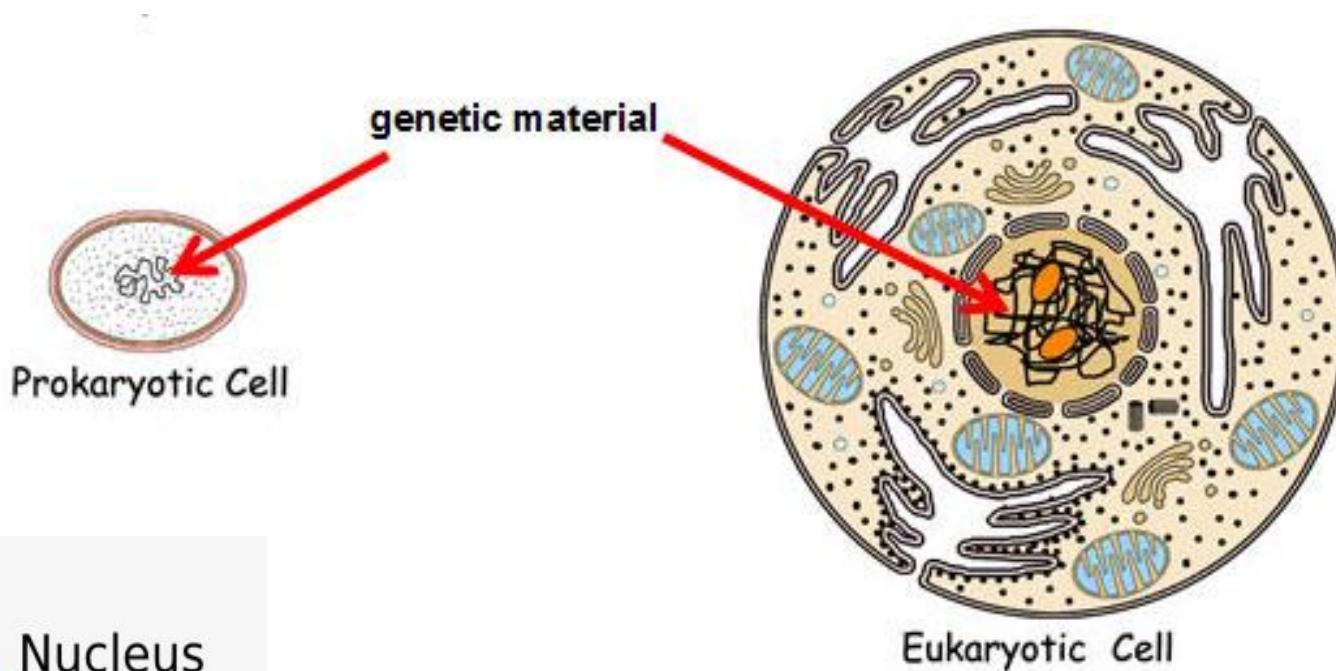
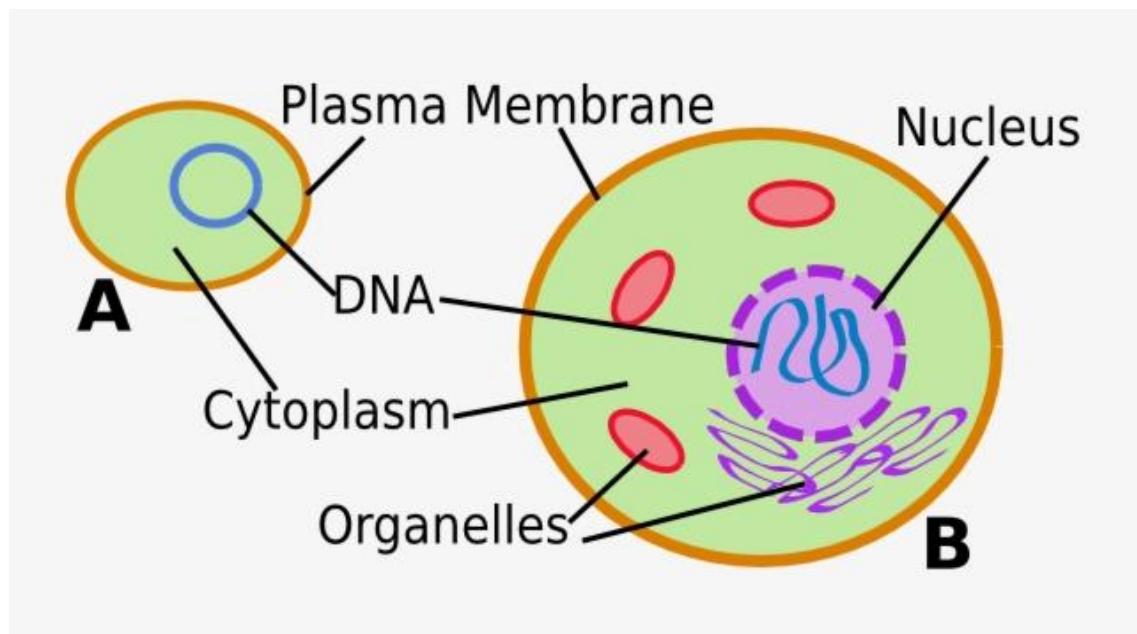




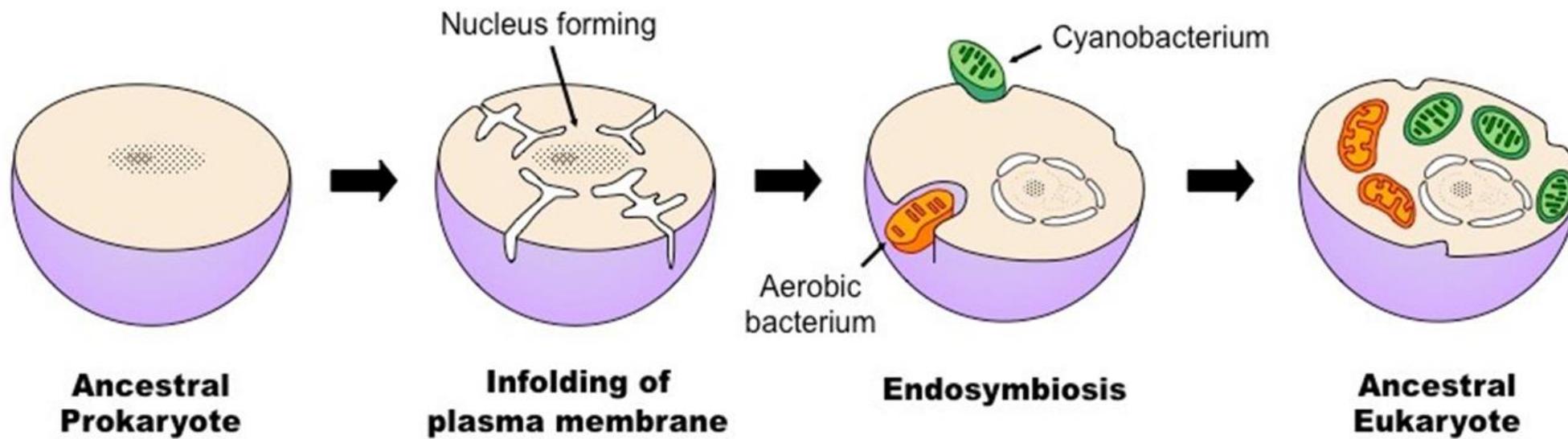




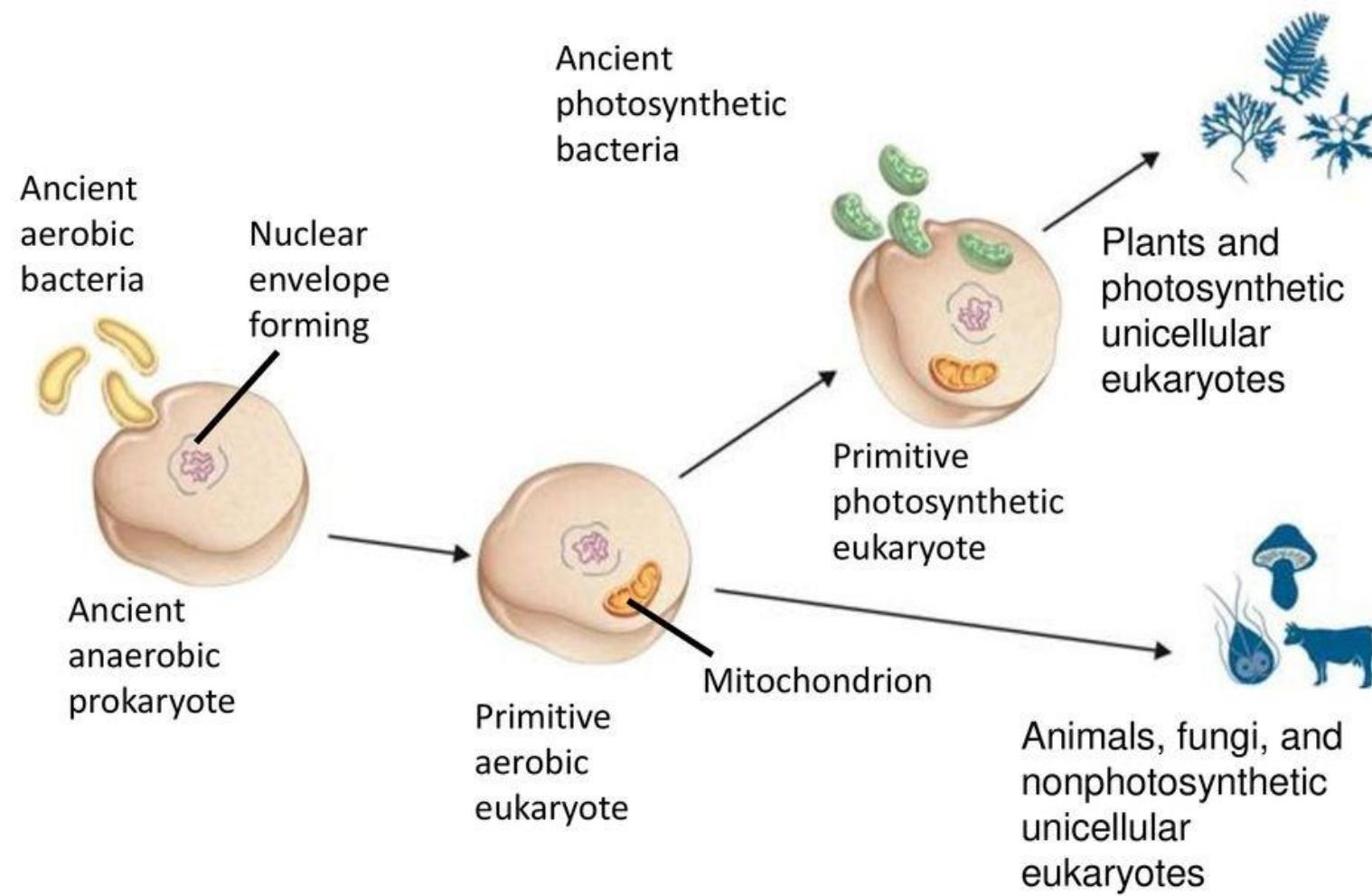




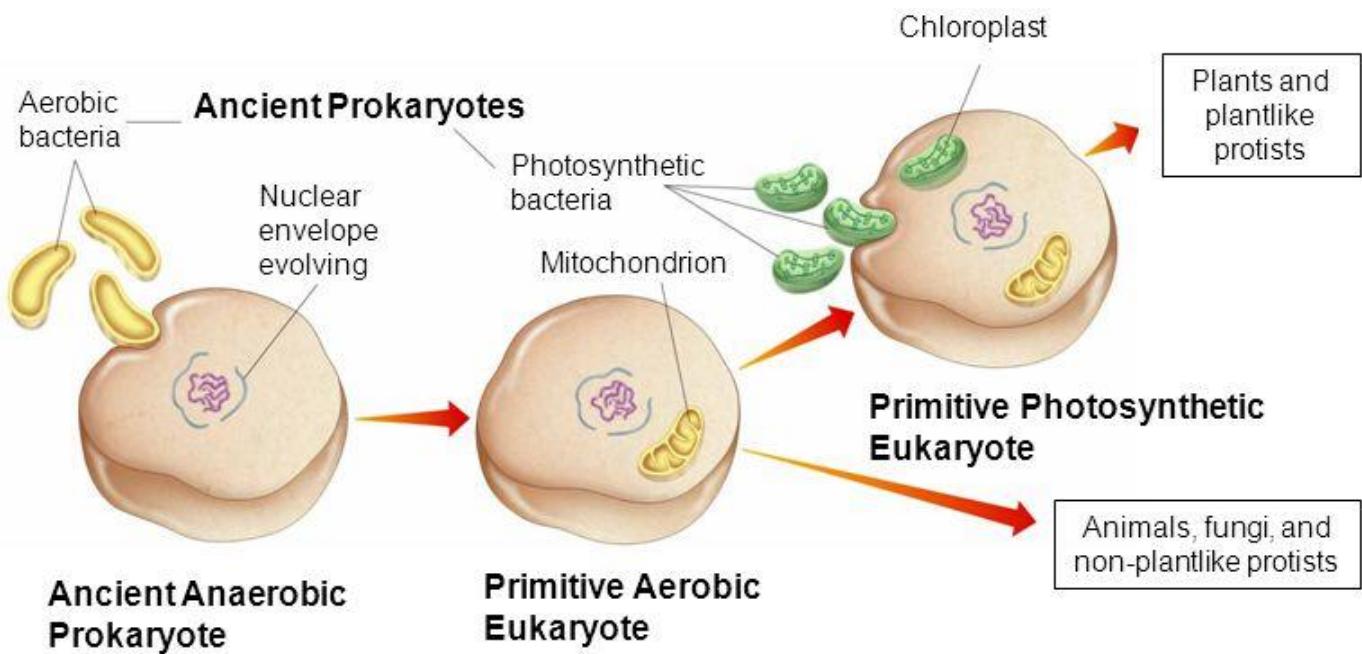
# ENDOSYMBIOSIS

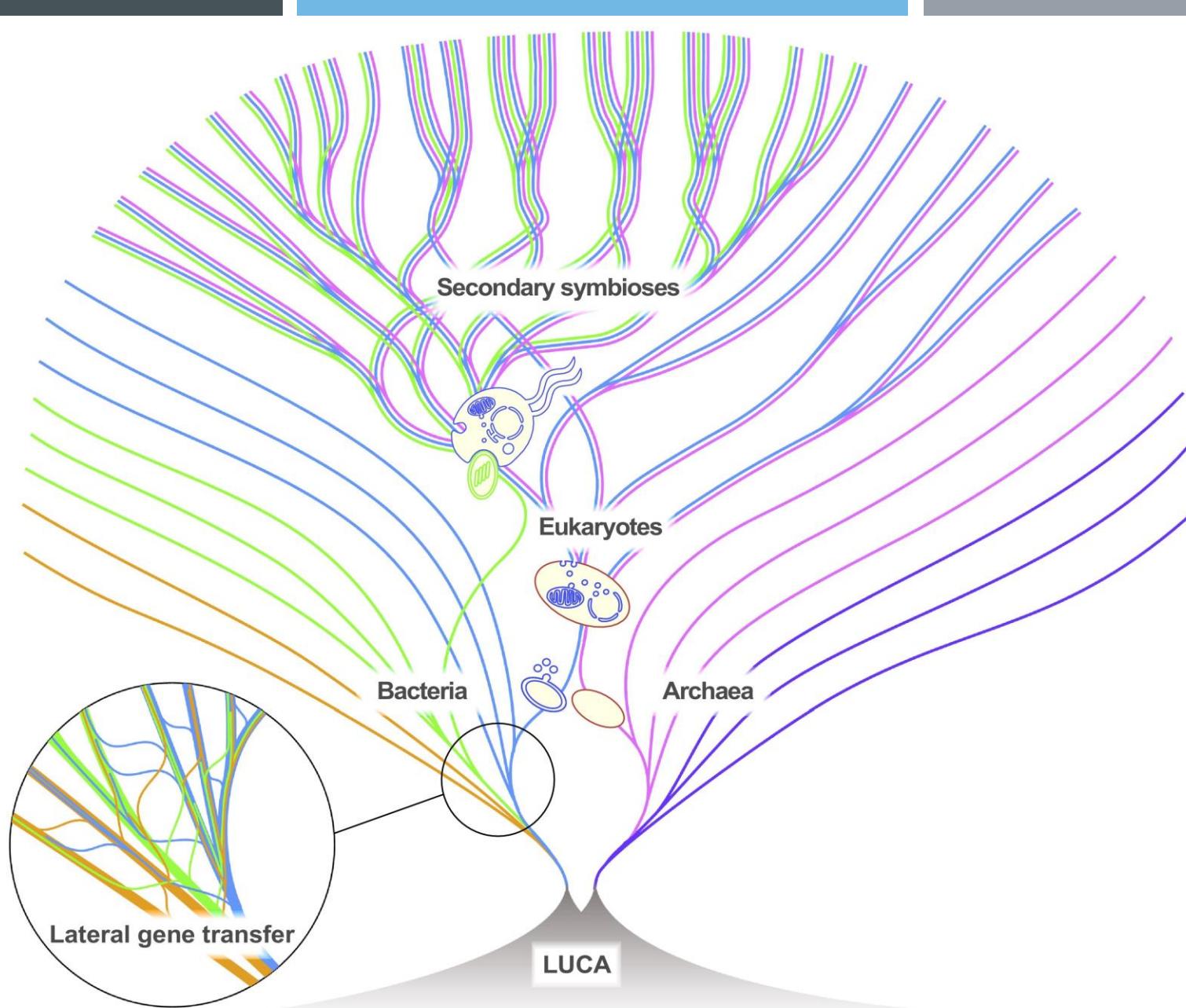


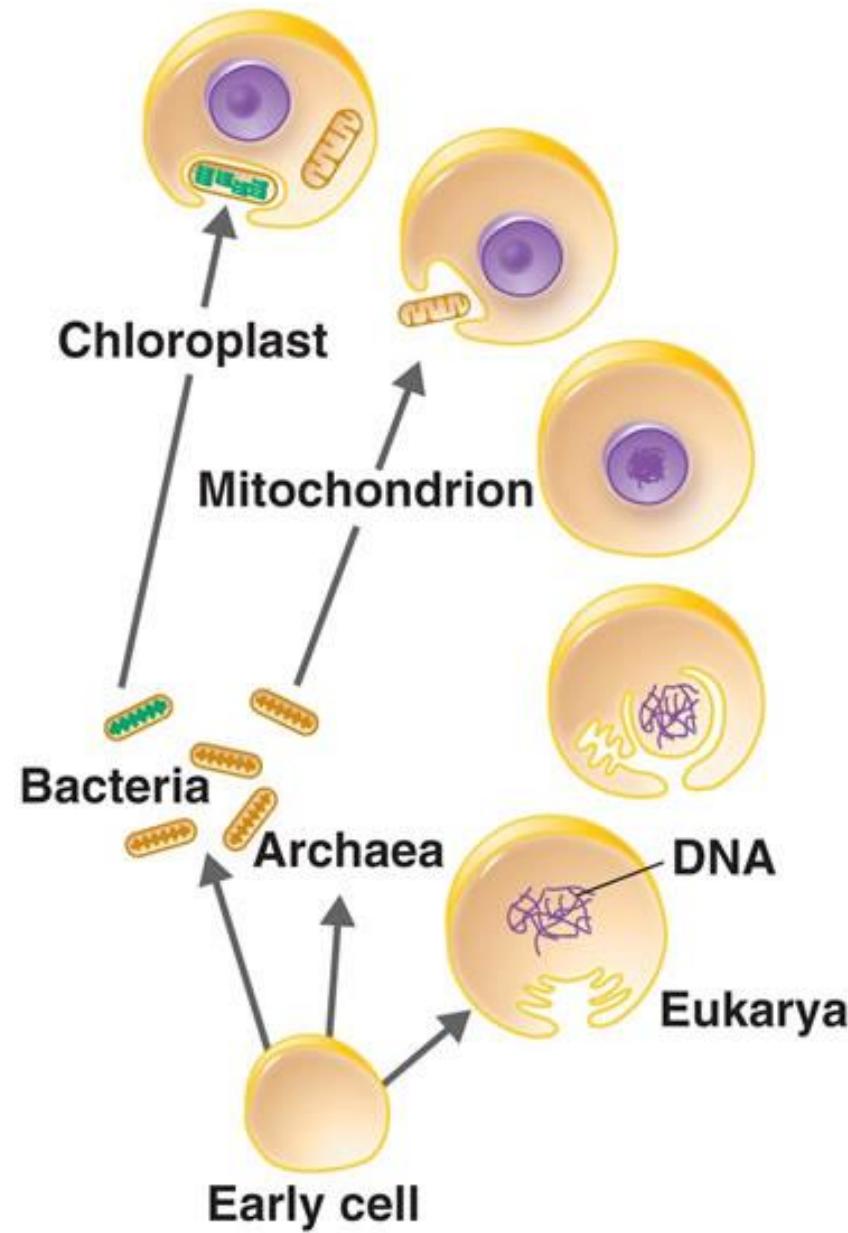
# Origin of Eukaryotic Cells

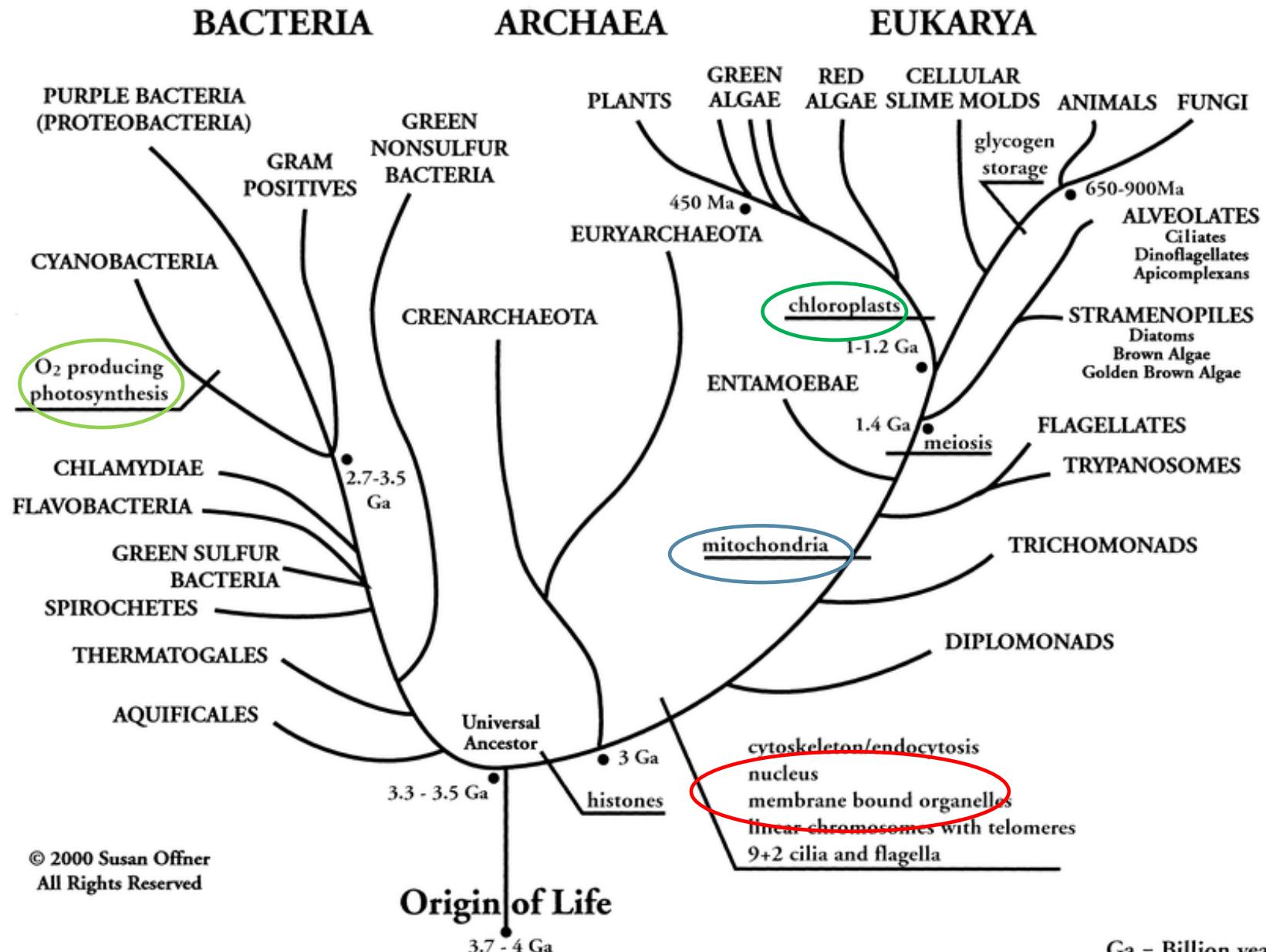


# Endosymbiotic Theory



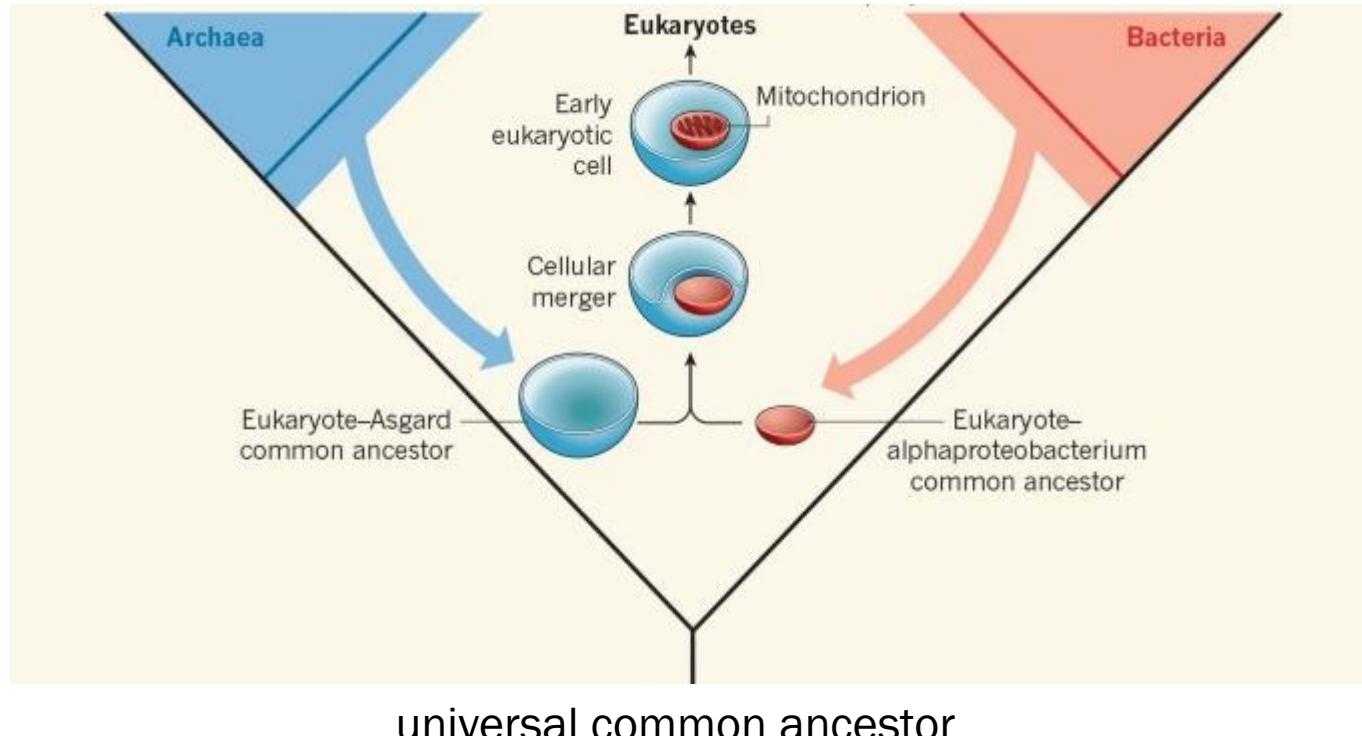






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Ga = Billion years ago  
Ma = Million years ago

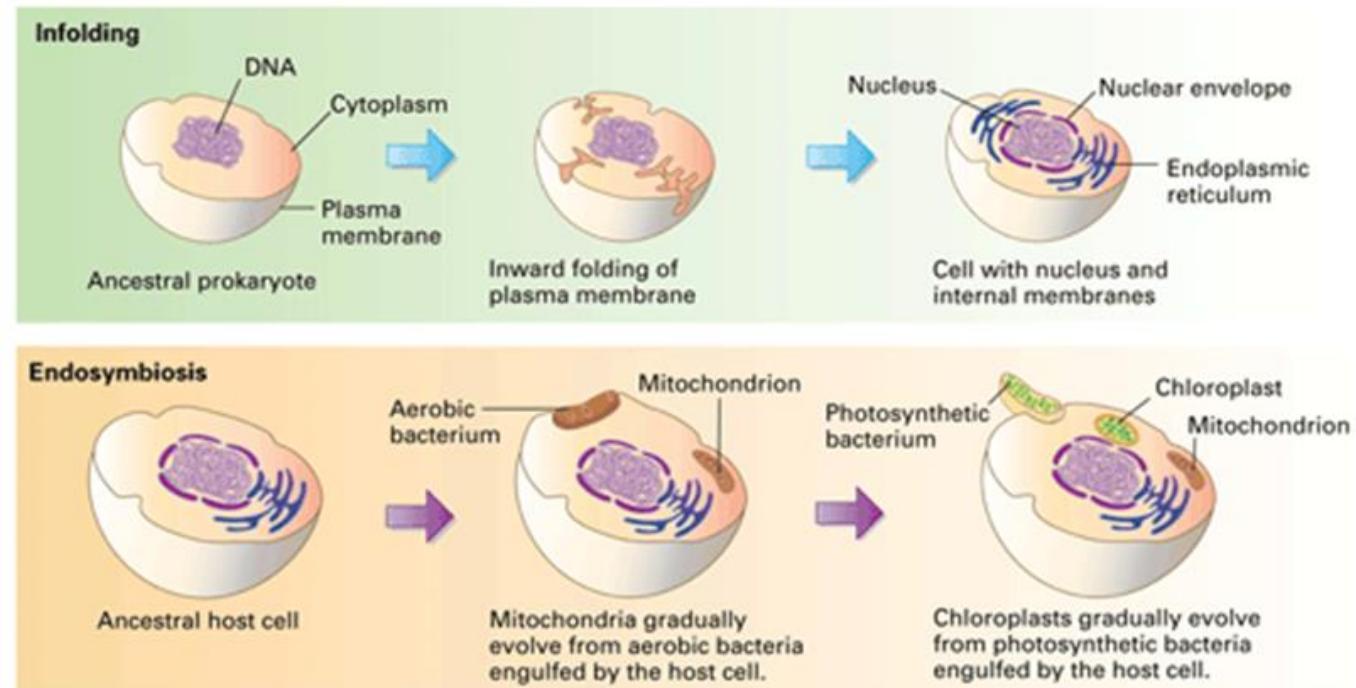


# THE ORIGIN OF EUKARYOTES

Among the most fundamental questions in biology is how the complex eukaryotic cell evolved from much simpler prokaryotic cells.

Eukaryotic cell organelles may have evolved through a combination of two processes.

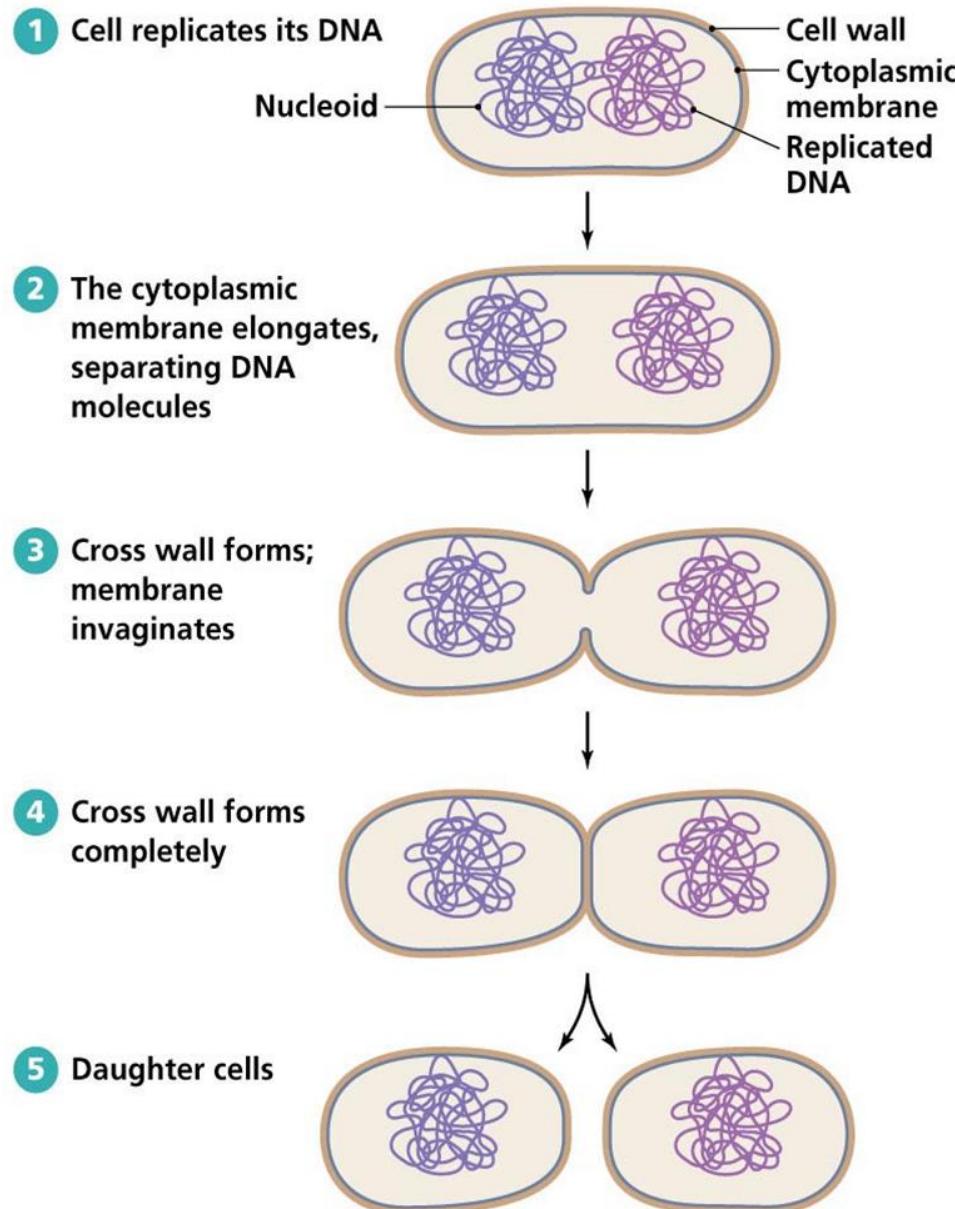
- Infolding of the plasma membrane could have produced internal membranes.
- Certain prokaryotes could have become residents within larger host cells, eventually evolving into mitochondria and chloroplasts.



# ENDOSYMBIOSIS THEORY

- Scientists hypothesize that chloroplasts and mitochondria evolved from small symbiotic prokaryotes that lived within other, larger host prokaryotes. (Symbiosis is a relationship between two organisms of different species that live in close contact with one another.)
- The symbiotic ancestors of mitochondria may have been aerobic bacteria that were able to use oxygen in cellular respiration. An ancestral host cell may have ingested some of these aerobic cells for food. Instead of being digested, some of these bacterial cells might have remained alive and continued to perform respiration within the host cell. In a similar way, the ancestors of chloroplasts could have been photosynthetic bacteria that lived inside a larger host cell.
- The endosymbiosis theory is supported by a variety of evidence. For instance:
  - present-day mitochondria and chloroplasts are similar to prokaryotic cells in a number of ways.
  - mitochondria and chloroplasts contain DNA, RNA, and ribosomes, all of which resemble their counterparts in prokaryotes more than those in eukaryotes.
  - both types of organelles copy their own DNA and reproduce within the host cell by a process resembling that of prokaryotes.

# CELL DIVISION IN BACTERIA



# ENDOSYMBIOTIC THEORY - MITOCHONDRIA

- Billions of years ago, a prokaryotic organism called archaea captured a bacterial endosymbiont
- Archaea and bacteria that have been around from 2 billion years ago have given biologists a clue as to how mitochondria became an inseparable part of animal and plant cells.
- Today, mitochondria are well known to be integral parts of the eukaryotic cell.
- They are dubbed the ‘power houses’ of the cell, because they help in generating energy in the form of ATP within the cell, powering it.
- But they were not always part of the animal and plant cells. Once, about two billion years ago, a prokaryotic organism (without a nucleus) called archaea captured a bacterial cell. The bacterial cell learnt to live within the archaea as an endosymbiont.
- How this happened has been an important question among biologists. “In the late 19th century, microscopists observed that organelles like chloroplast and mitochondria undergo division inside eukaryotic cells that resembles bacterial division, which led them to suspect that these organelles might have arisen from bacterial endosymbionts.

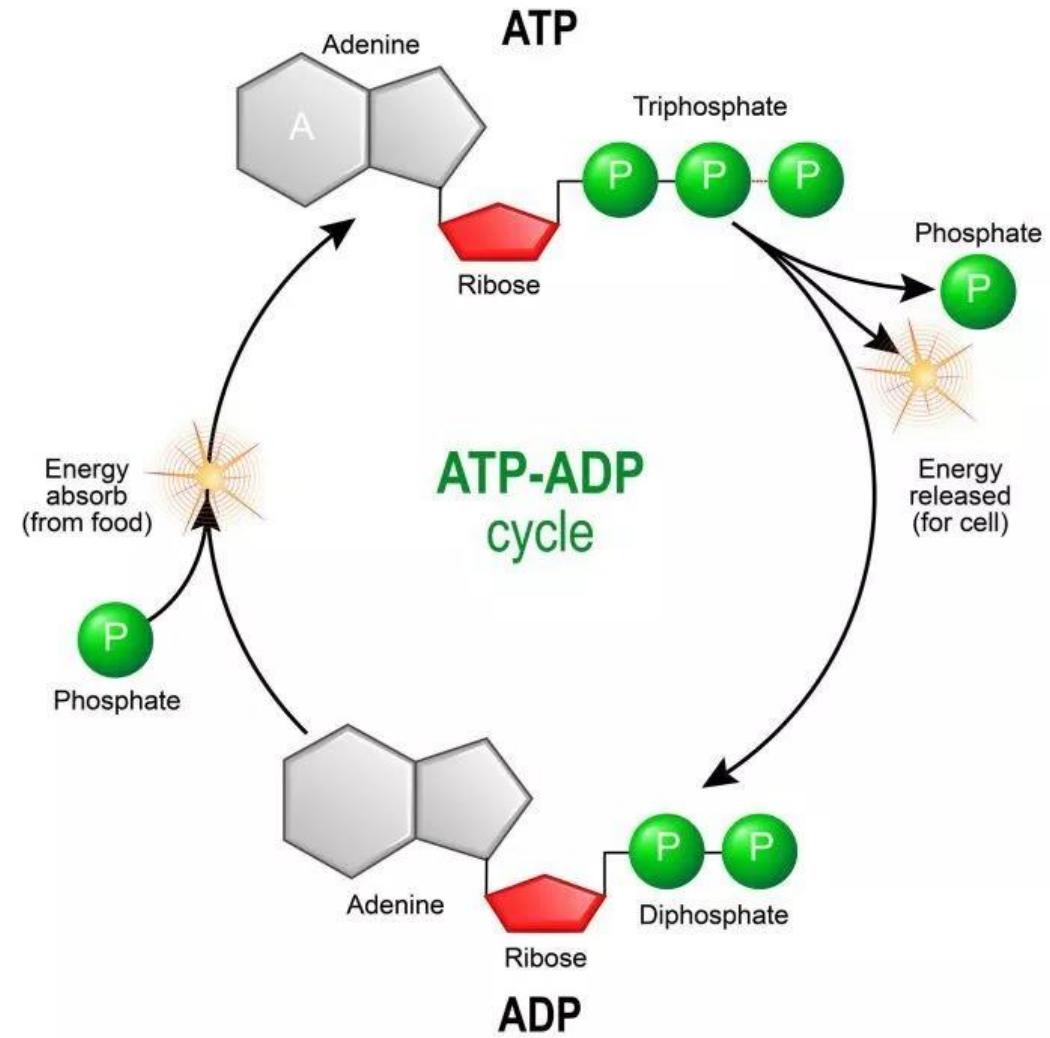
# CELLULAR RESPIRATION

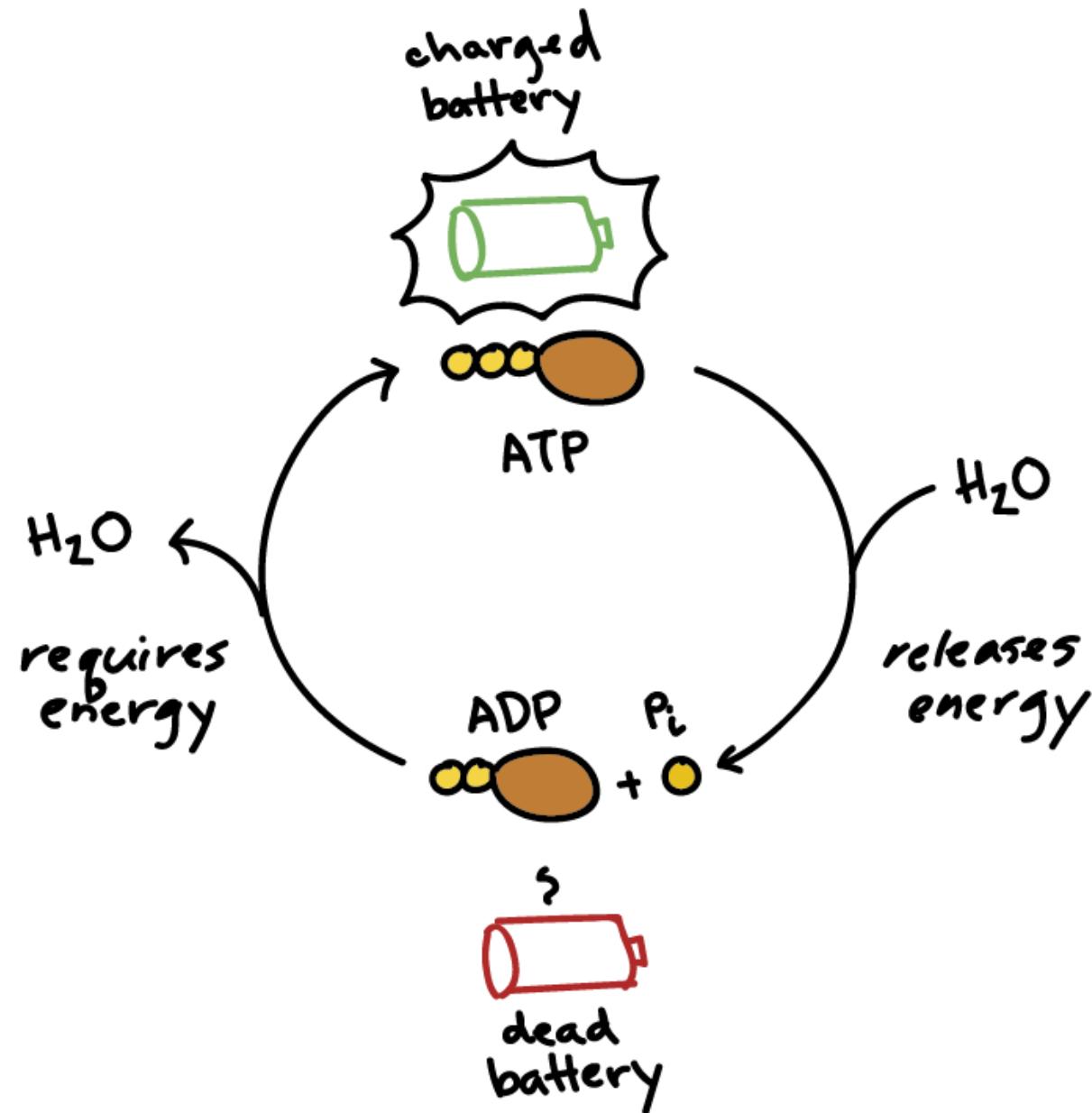
- metabolic reactions and processes that take place in the cells of organisms to convert chemical energy from nutrients into adenosine triphosphate (ATP), and then release waste products.
- Aerobic organisms combine oxygen with foodstuff molecules, diverting the chemical energy in these substances into life-sustaining activities and discarding, as waste products, carbon dioxide and water.
- Organisms that do not depend on oxygen degrade foodstuffs in a process called fermentation.

## ATP-ADP CYCLE

One objective of the degradation of foodstuffs is to convert the energy contained in chemical bonds into the energy-rich compound adenosine triphosphate (ATP), which captures the chemical energy obtained from the breakdown of food molecules and releases it to fuel other cellular processes.

In eukaryotic cells the enzymes that catalyze the individual steps involved in respiration and energy conservation are located in highly organized rod-shaped compartments called mitochondria; e. g. a liver cell has about 1,000 mitochondria. In prokaryotic cells the enzymes occur as components of the cell membrane.

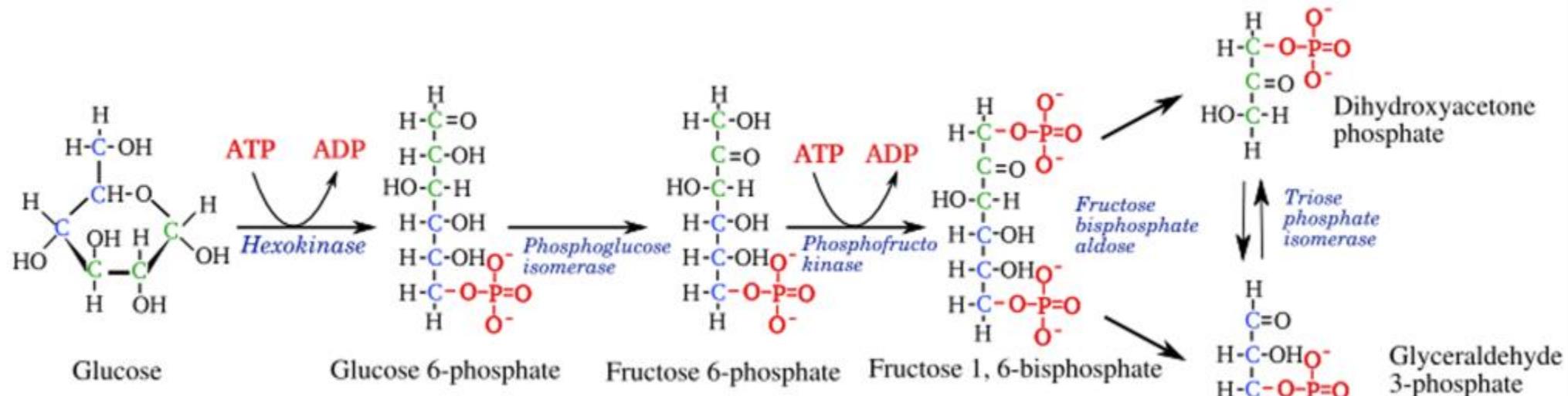




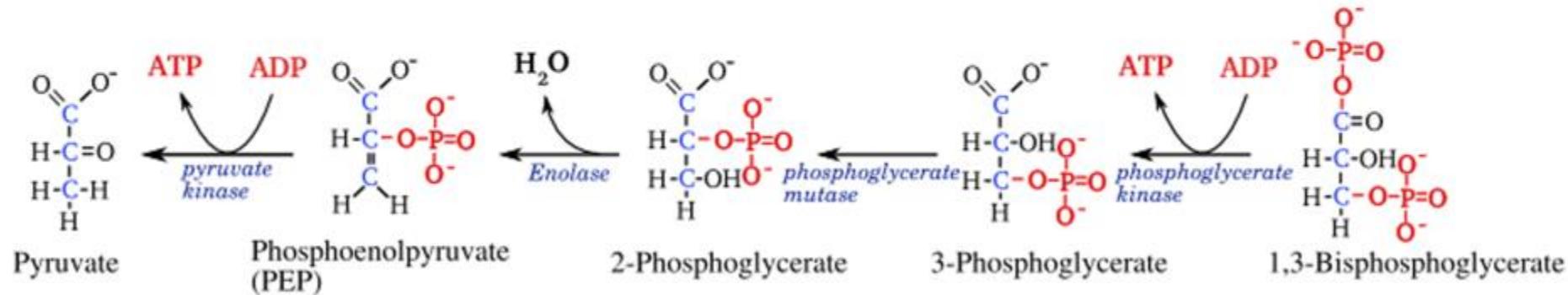
# ATP PRODUCTION

The three processes of ATP production include glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation. In eukaryotic cells the latter two processes occur within mitochondria. Electrons that are passed through the electron transport chain ultimately generate free energy capable of driving the phosphorylation of ADP.

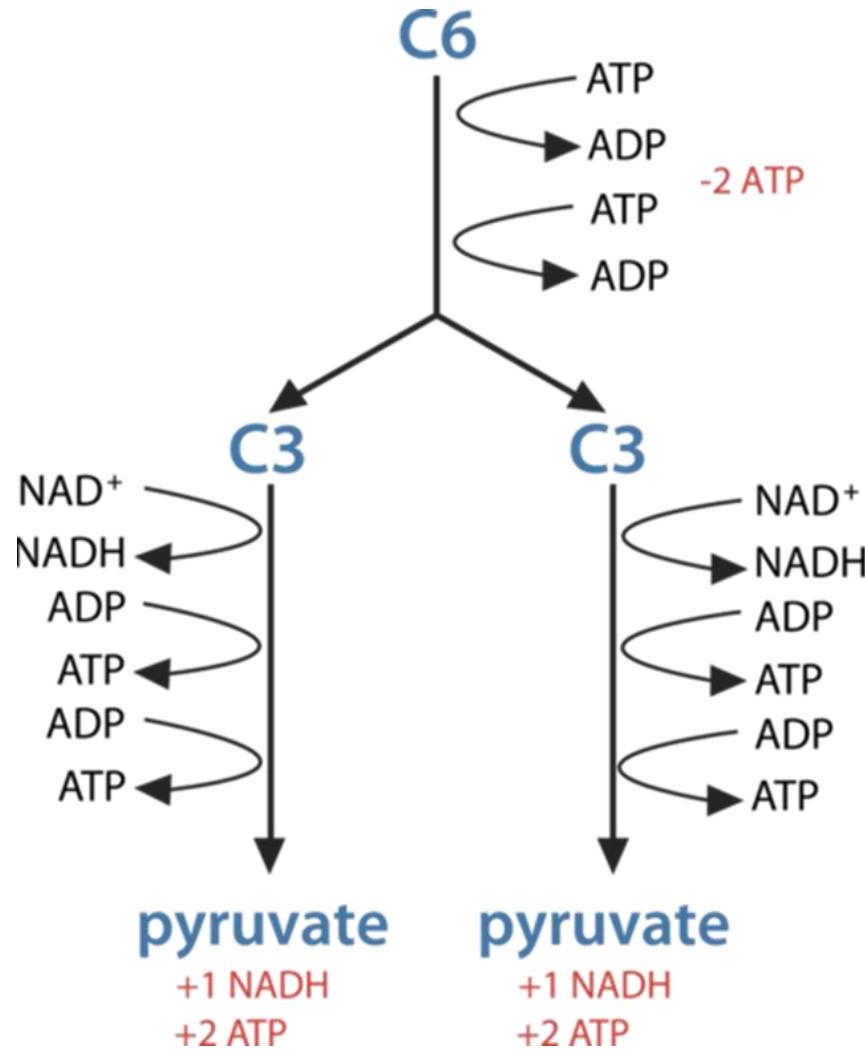
- Energy is released in three phases.
  - In the first, **large molecules**, such as those of proteins, polysaccharides, and lipids, are **broken down (glycolysis)**; small amounts of energy are released in the form of heat in these processes.
  - In the second phase, the **small molecules are oxidized**, liberating chemical energy to form ATP as well as heat energy, to form one of the three compounds: acetate, oxaloacetate, or  $\alpha$ -oxoglutarate. These are oxidized to **carbon dioxide** during the third phase, a cyclic reaction sequence called the tricarboxylic acid (or Krebs) cycle.
  - **Hydrogen atoms** or electrons from the intermediate compounds **formed during the cycle** are **transferred** (through a succession of carrier molecules) ultimately **to oxygen**, **forming water**. These events, the most important means for generating ATP in cells, are known as terminal respiration and oxidative phosphorylation.



## GLYCOLYSIS

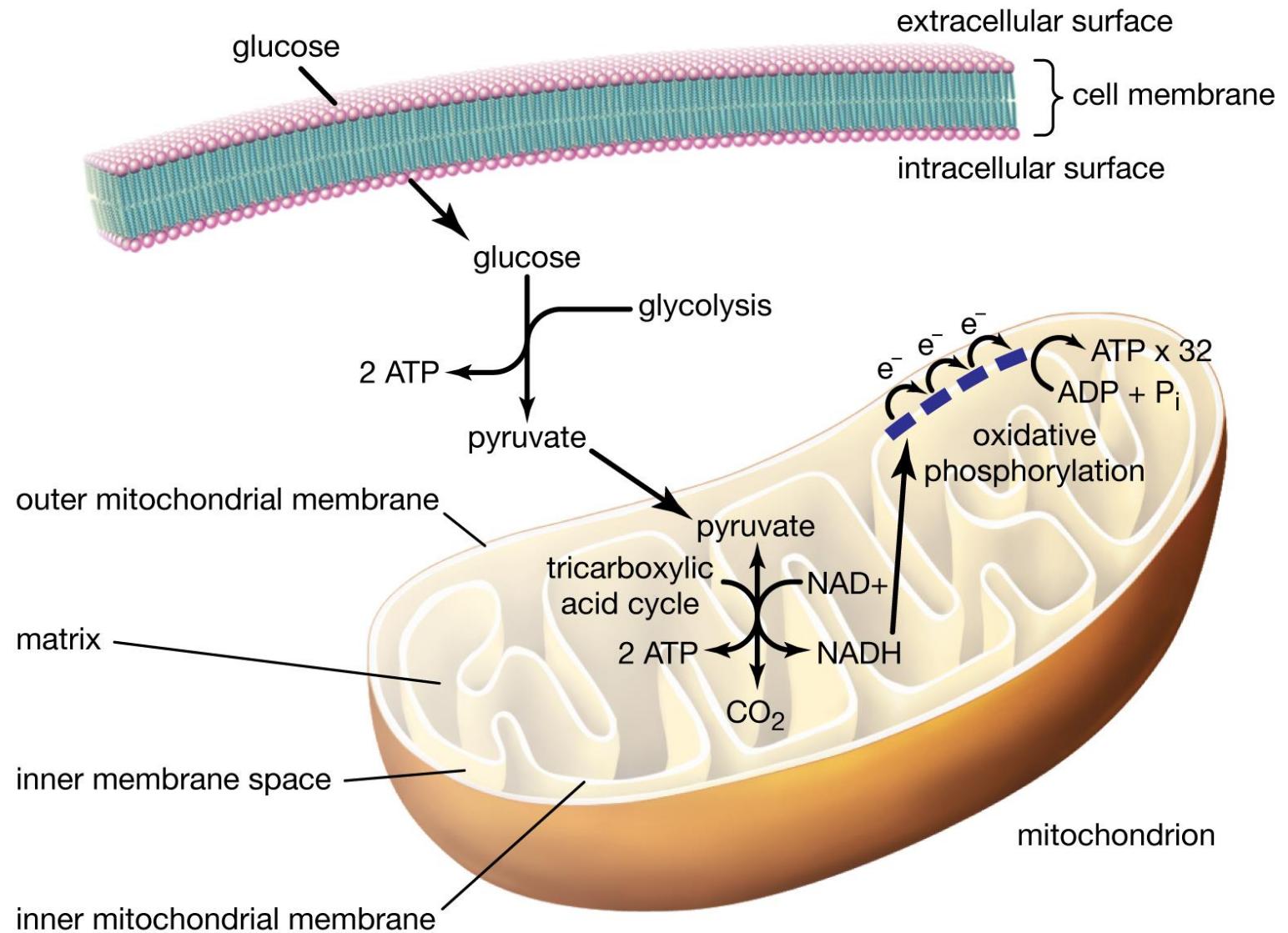


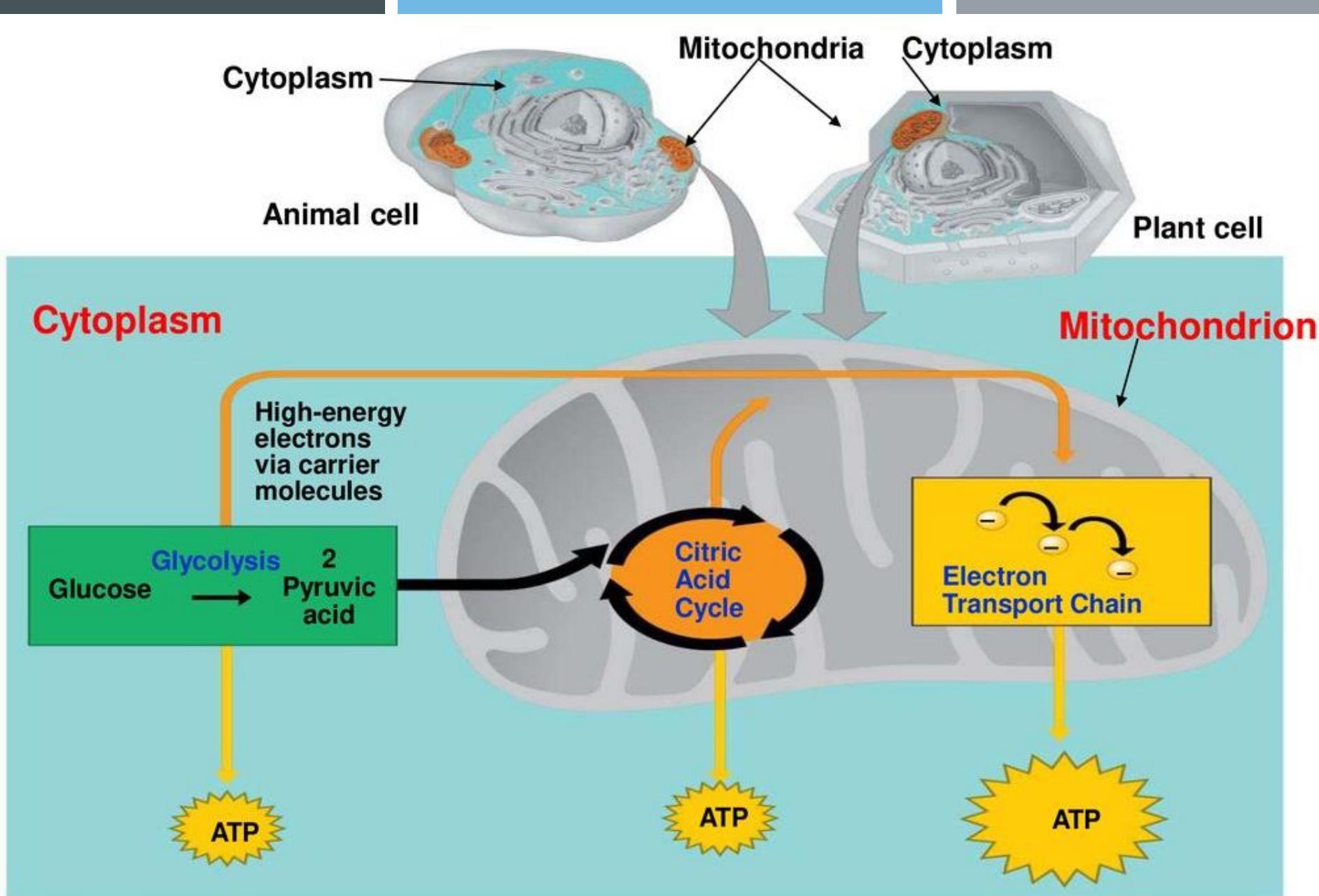
# GLYCOLYSIS



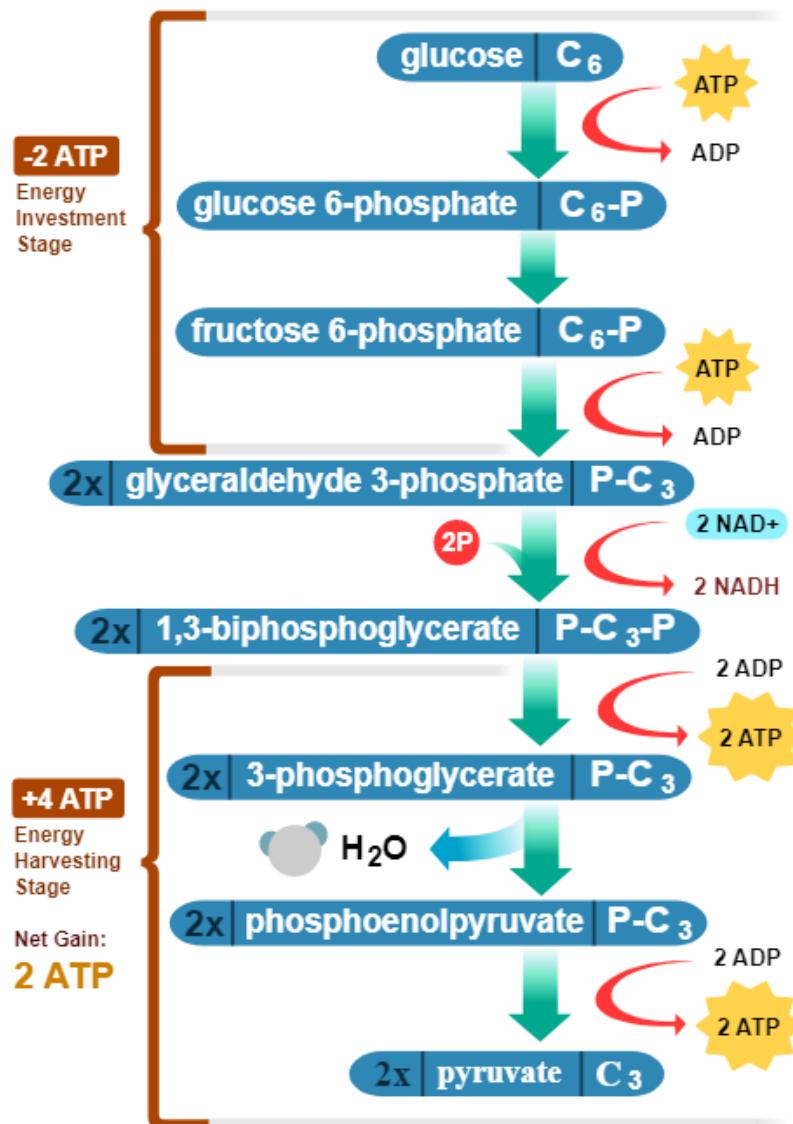
# ATP PRODUCTION

## Basic overview of processes of ATP production

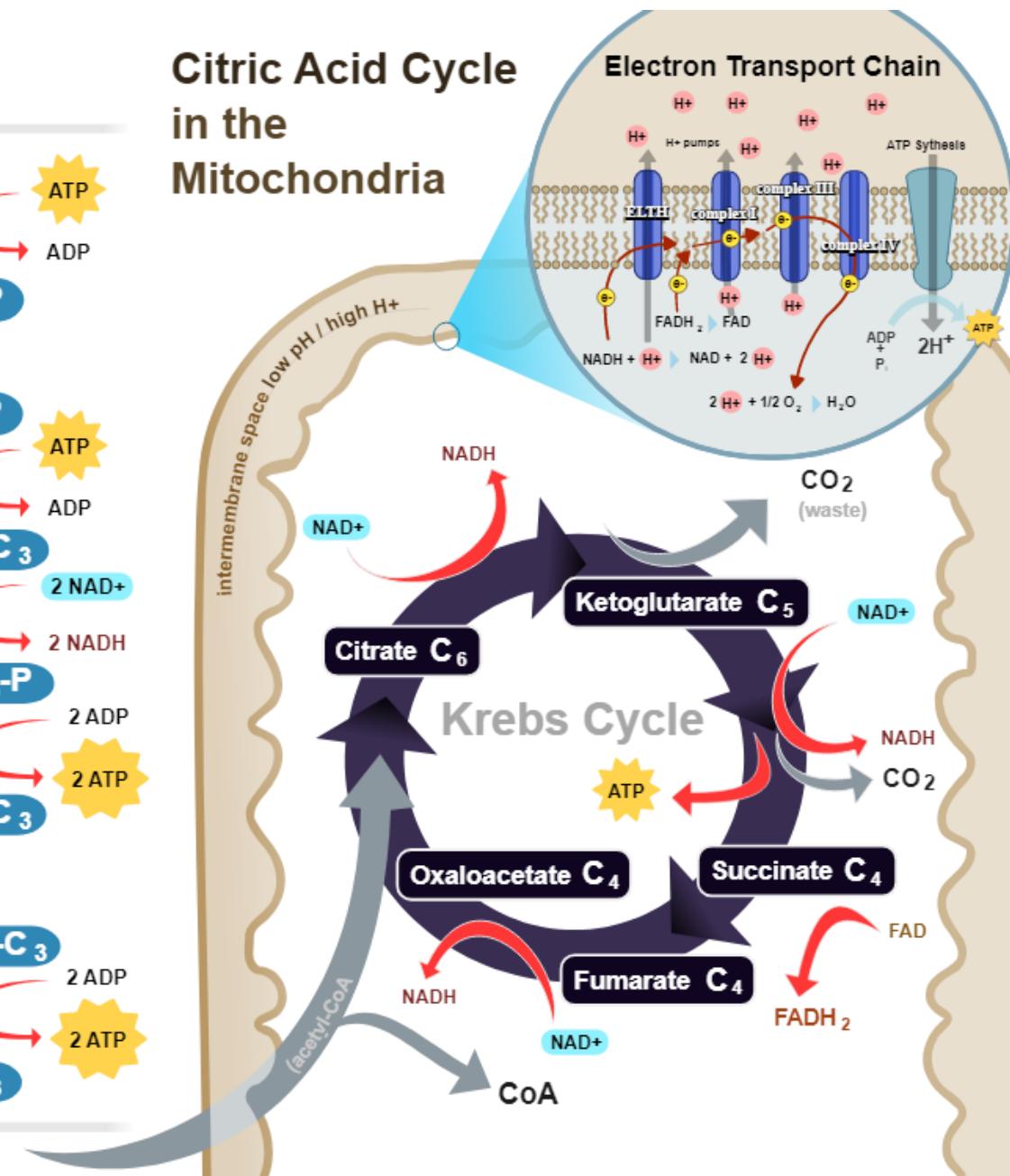




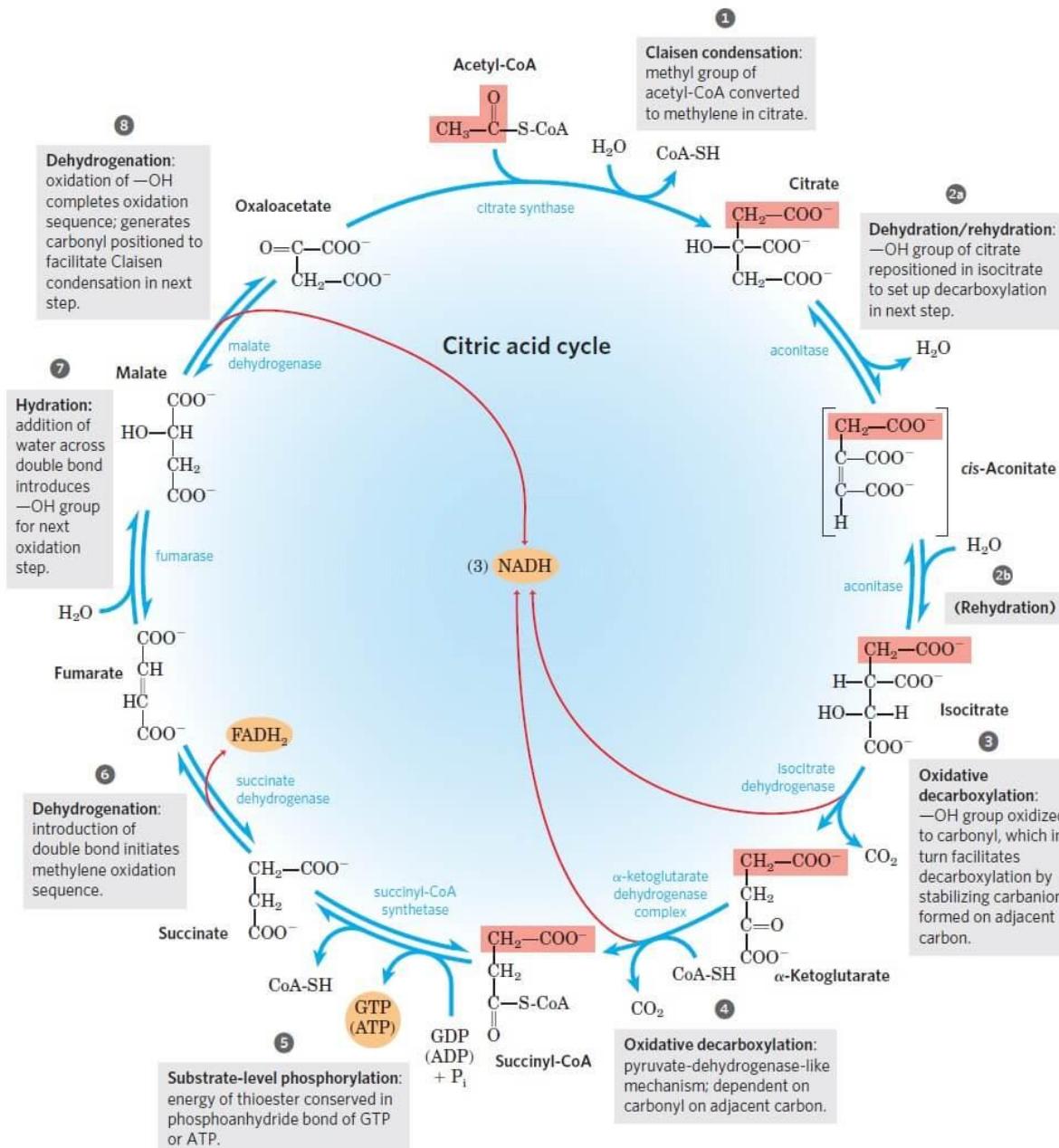
## Glycolysis in the Cytoplasm



## Citric Acid Cycle in the Mitochondria



The TCA cycle consists of eight steps catalyzed by eight different enzymes. The cycle is initiated (1) when acetyl CoA reacts with the compound oxaloacetate to form citrate and to release coenzyme A (CoA-SH). Then, in a succession of reactions, (2) citrate is rearranged to form isocitrate; (3) isocitrate loses a molecule of carbon dioxide and then undergoes oxidation to form alpha-ketoglutarate; (4) alpha-ketoglutarate loses a molecule of carbon dioxide and is oxidized to form succinyl CoA; (5) succinyl CoA is enzymatically converted to succinate; (6) succinate is oxidized to fumarate; (7) fumarate is hydrated to produce malate; and, to end the cycle, (8) malate is oxidized to oxaloacetate. Each complete turn of the cycle results in the regeneration of oxaloacetate and the formation of two molecules of carbon dioxide.

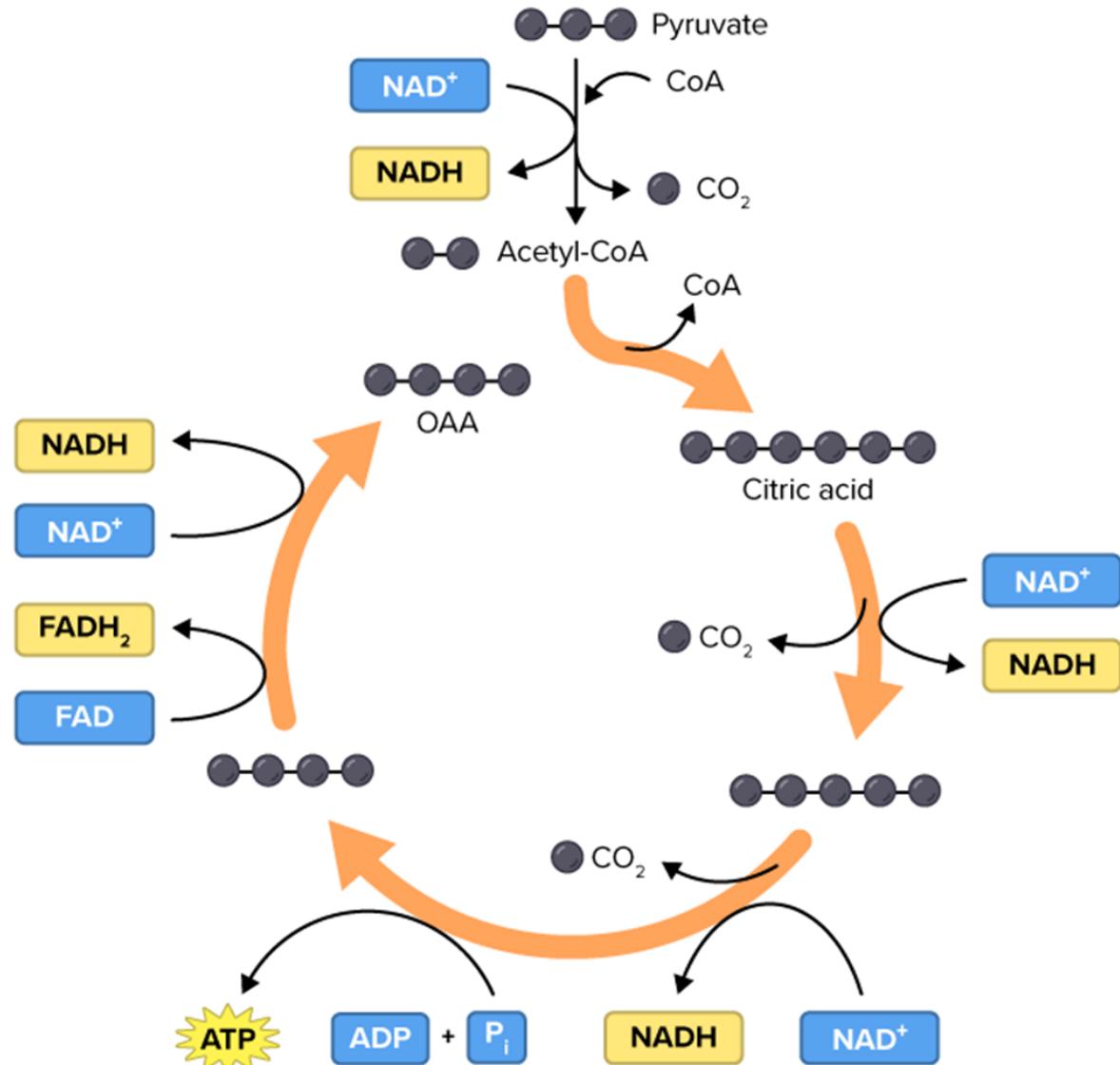


## KREBS CYCLE

- Energy is produced in a number of steps in this cycle of reactions. In step 5, one molecule of adenosine triphosphate (ATP), the molecule that powers most cellular functions, is produced. Most of the energy obtained from the TCA cycle, however, is captured by the compounds nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) and flavin adenine dinucleotide (FAD) and converted later to ATP.
- Energy transfers occur through the relay of electrons from one substance to another, a process carried out through the chemical reactions known as oxidation and reduction, or redox reactions. (Oxidation involves the loss of electrons from a substance and **reduction** the **addition of electrons**.) For each turn of the TCA cycle, three molecules of  $\text{NAD}^+$  are **reduced** to  $\text{NADH}$  and one molecule of FAD is reduced to  $\text{FADH}_2$ . These molecules then transfer their energy to the electron transport chain, a pathway that is part of the third stage of cellular respiration. The electron transport chain in turn releases energy so that it can be converted to ATP through the process of oxidative phosphorylation.

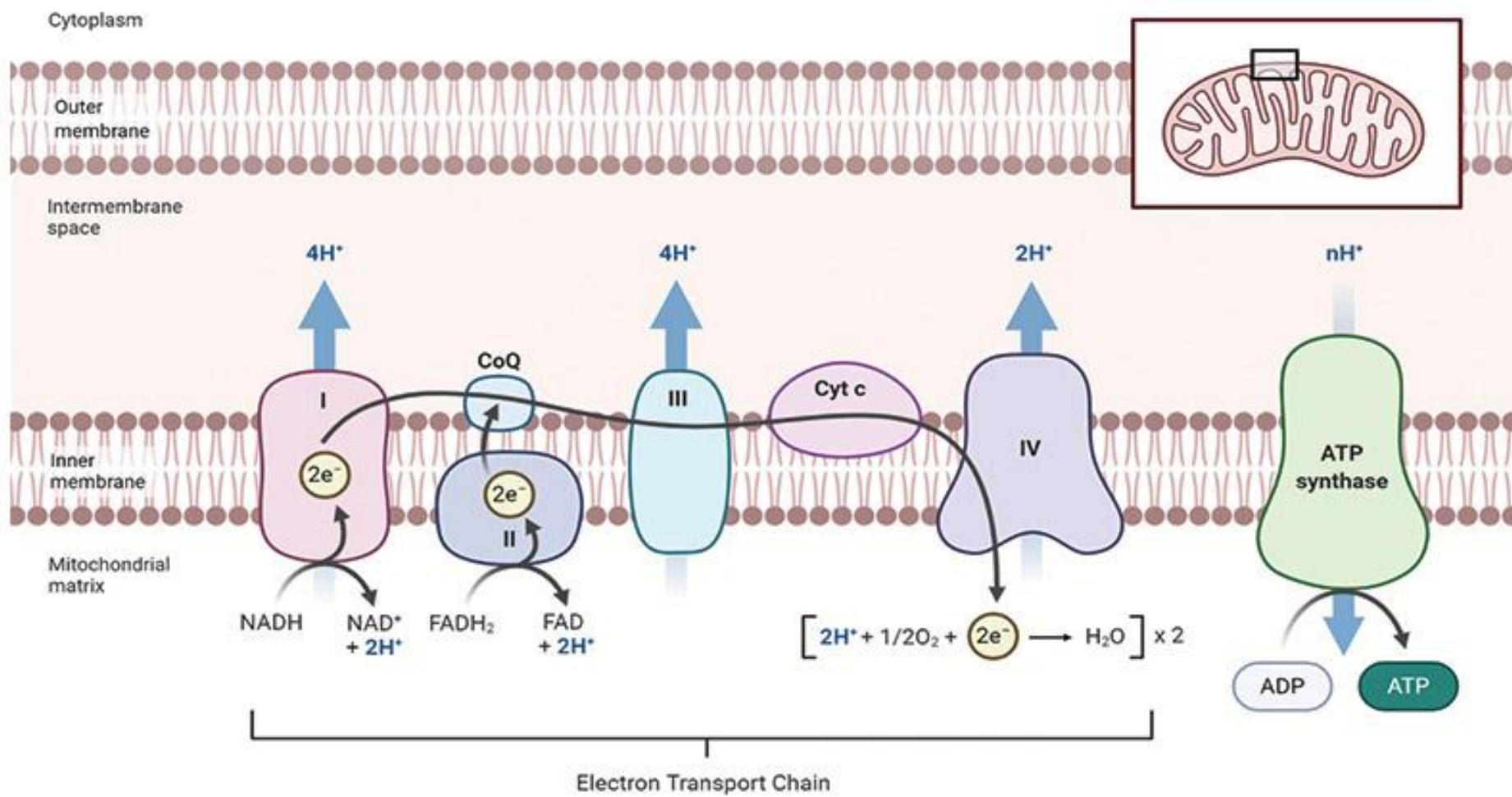
# KREBS CYCLE

- Also called tricarboxylic acid cycle (TCA cycle) and citric acid cycle.
- The TCA cycle plays a central role in the breakdown, or catabolism, of glucose.
- Before the rather large molecule can enter the TCA cycle it must be degraded into a two-carbon compound called acetyl coenzyme A (acetyl CoA).
- Once fed into the TCA cycle, acetyl CoA is converted into carbon dioxide and energy.

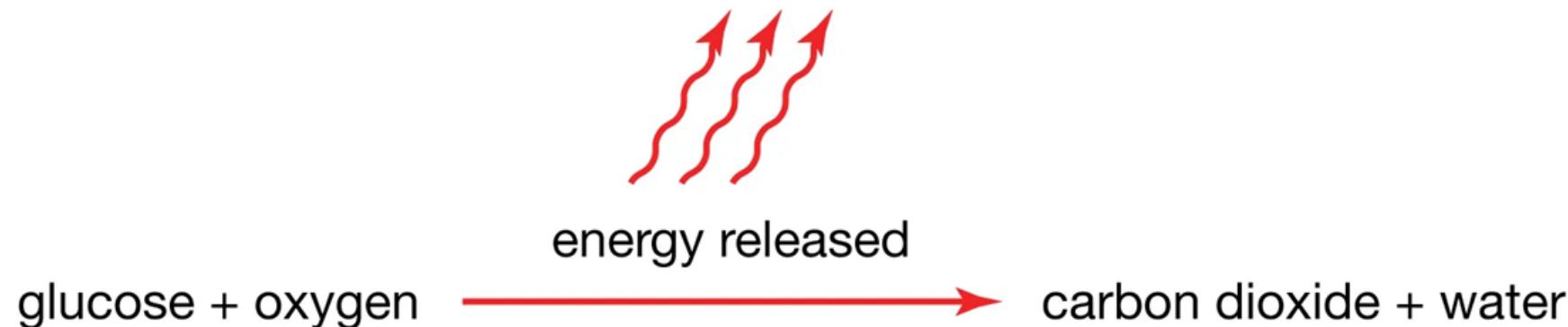


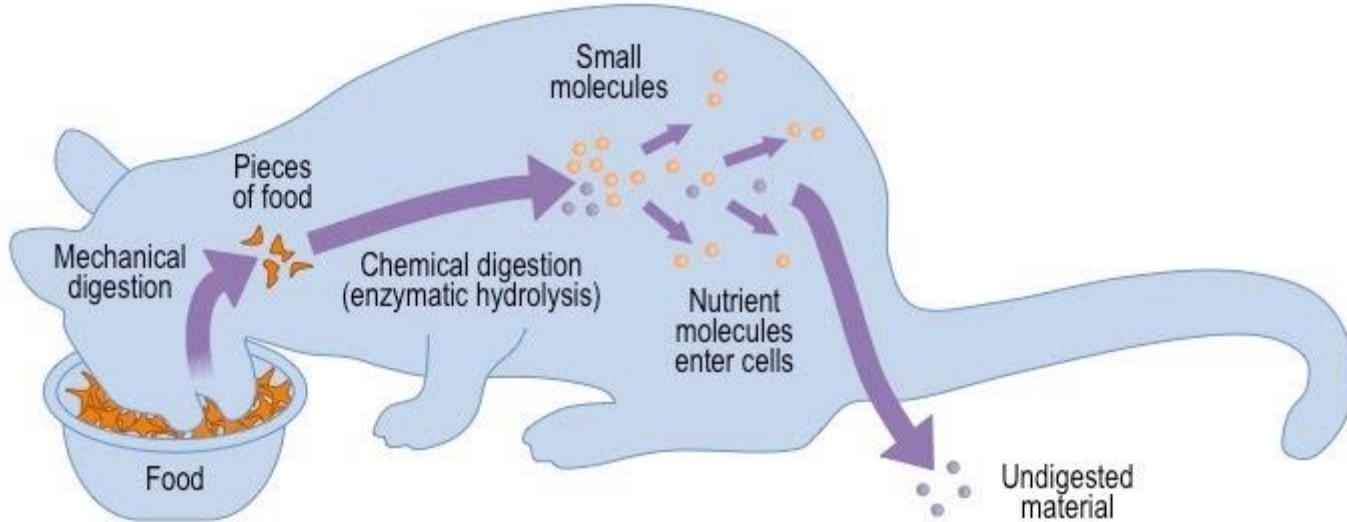
# OXIDATIVE PHOSPHORYLATION

- In oxidative phosphorylation the oxidation of catabolic intermediates by molecular oxygen occurs via a highly ordered series of substances that act as hydrogen and electron carriers. They constitute the electron transfer system, or respiratory chain.
- In most animals, plants, and fungi, the electron transfer system is fixed in the membranes of mitochondria; in bacteria (which have no mitochondria) this system is incorporated into the plasma membrane.
- Sufficient free energy is released to allow the synthesis of ATP.



## The release of energy during cellular respiration

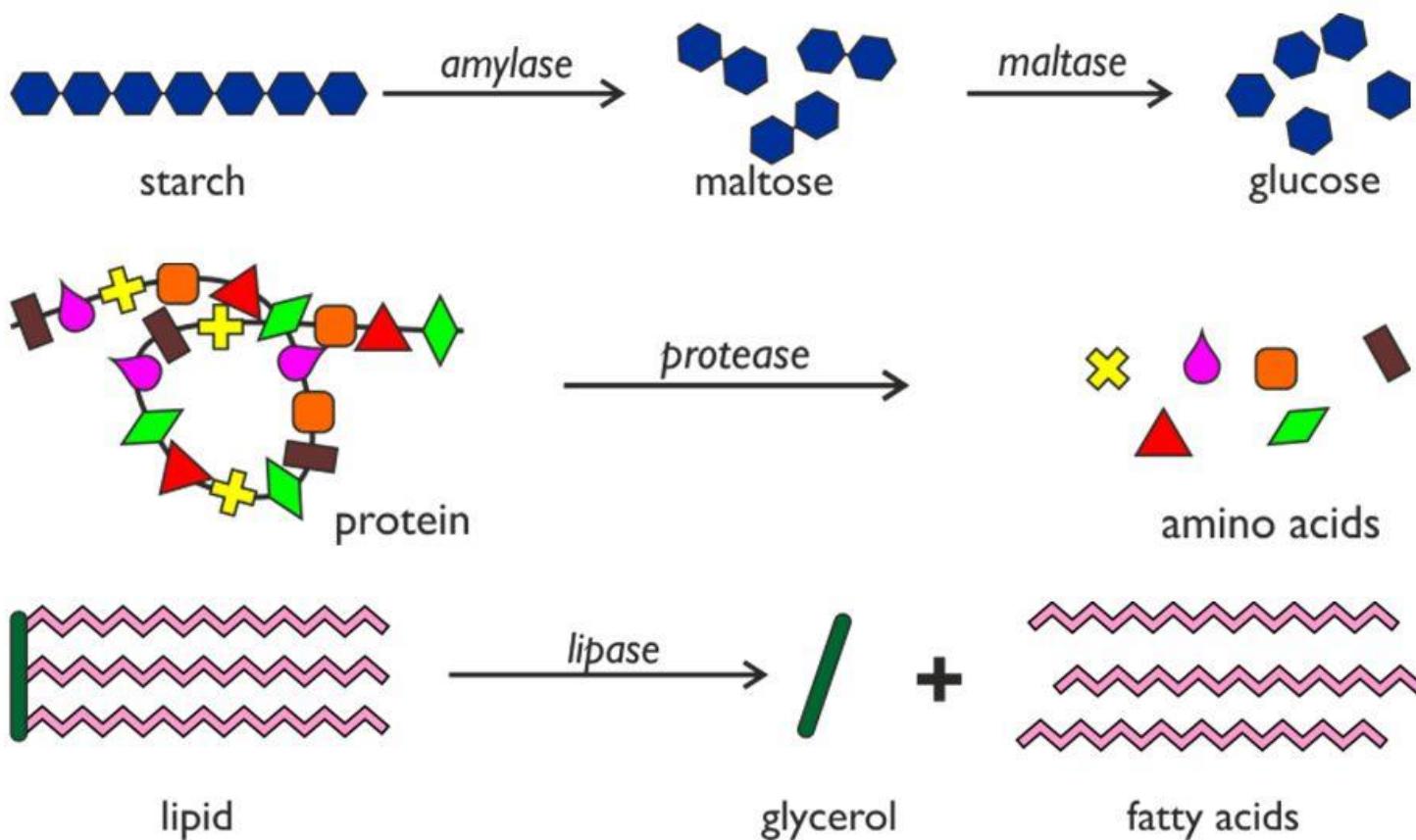




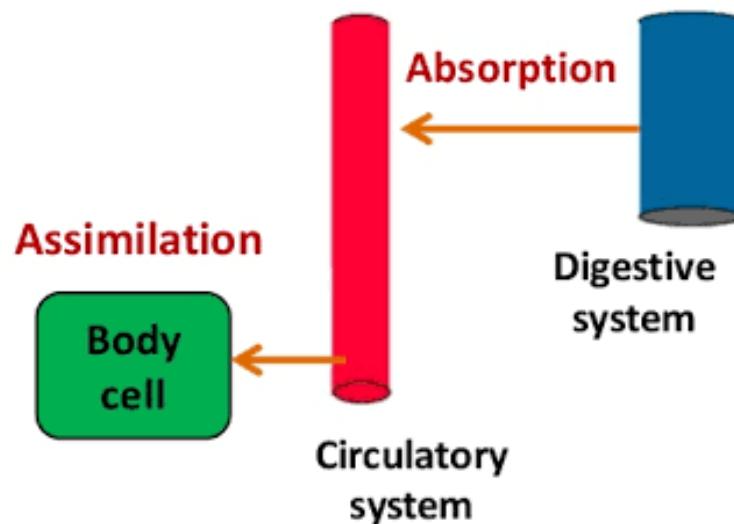
INGESTION	DIGESTION	ABSORPTION	ASSIMILATION	ELIMINATION
<i>Taking food into body</i>	<i>Breaking down food</i>	<i>Moving food into cells</i>	<i>Making food part of cell</i>	<i>Removing unused food</i>

# Digestive Enzymes

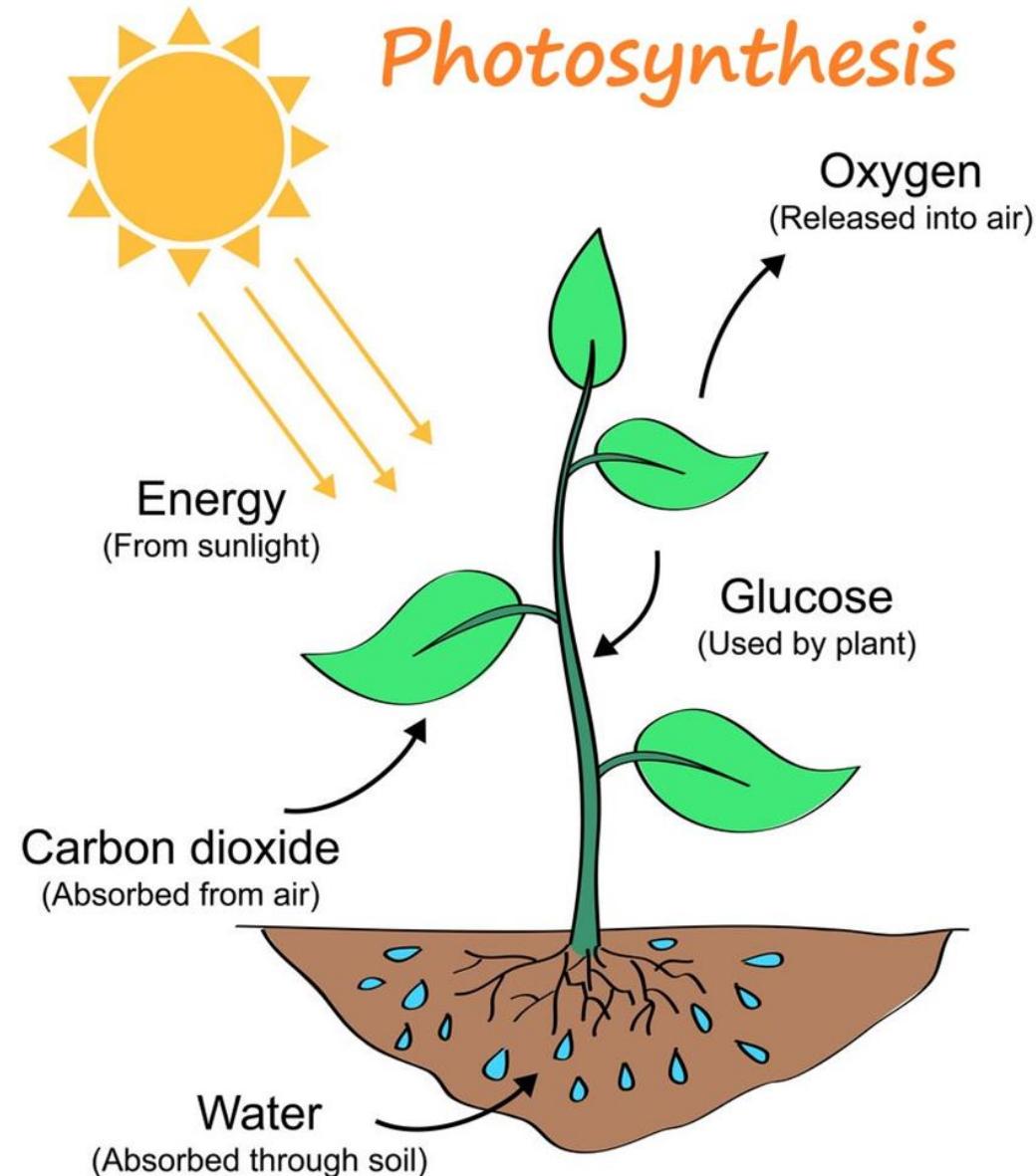
Large molecules are digested to small molecules by enzymes. Enzymes are specific, so there are different enzymes for different substrate molecules.



Digested food is absorbed & then  
assimilated by the cells

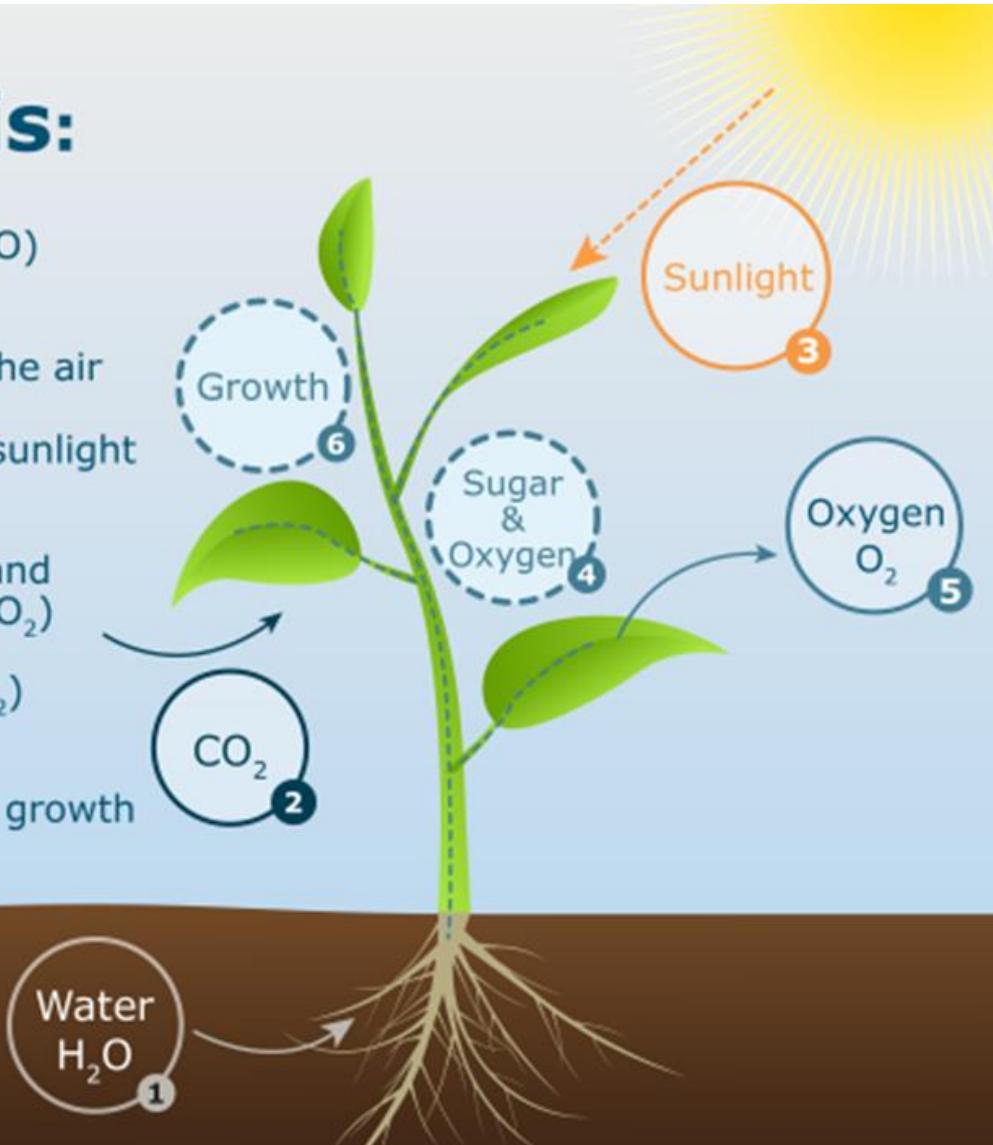


# PHOTOSYNTHESIS



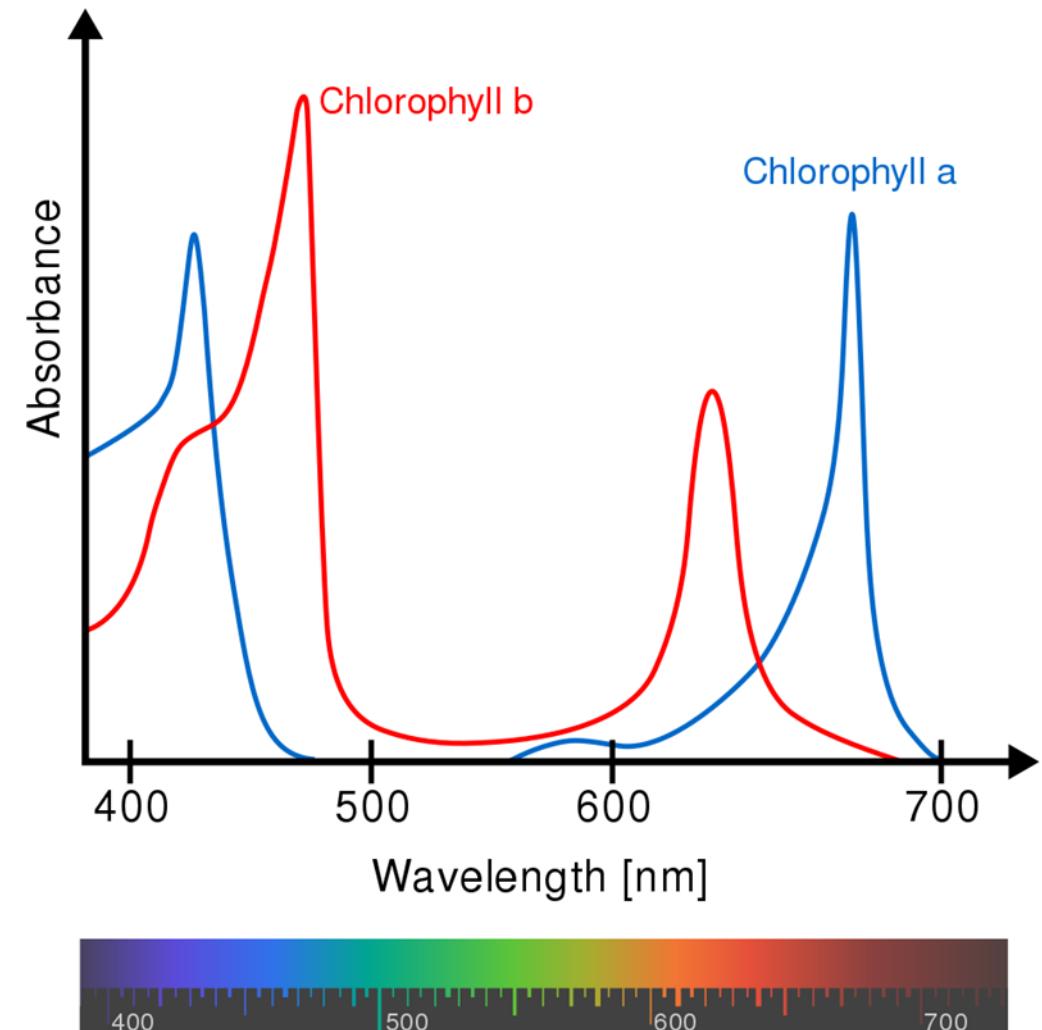
# Photosynthesis:

1. The plant draws up water ( $H_2O$ ) through its roots
2. The leaves take in  $CO_2$  from the air
3. The leaves trap energy from sunlight
4. The plant uses the energy of sunlight to turn water ( $H_2O$ ) and  $CO_2$  into sugars and oxygen ( $O_2$ )
5. The plant releases oxygen ( $O_2$ ) into the air
6. The plant uses the sugars for growth

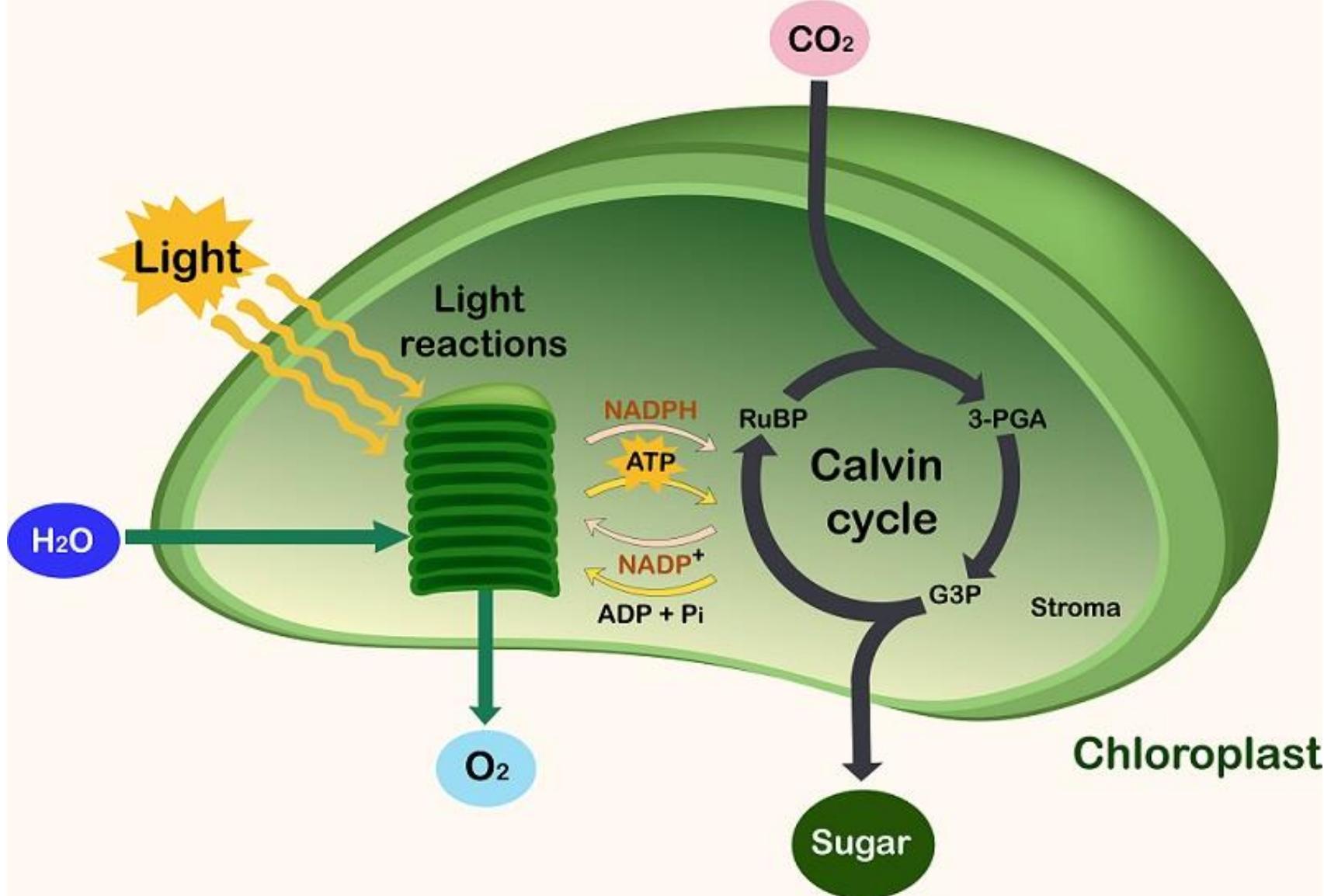


# CHLOROPHYLL

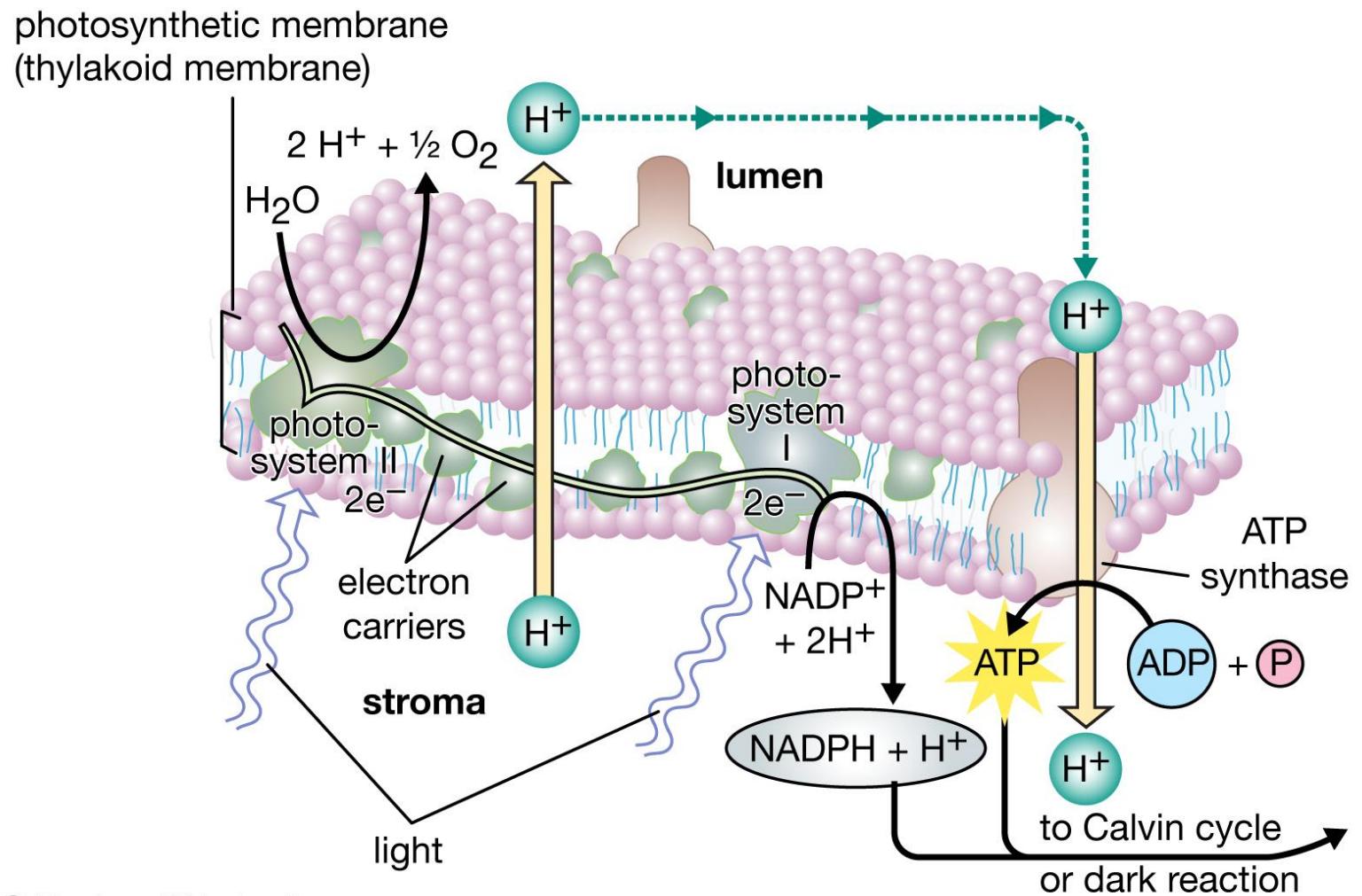
- green pigment found in cyanobacteria and in the chloroplasts of algae and plants. Chlorophyll allow plants to absorb energy from light.
- Chlorophylls absorb light most strongly in the blue and red portion of the electromagnetic spectrum. Conversely, it is a poor absorber of green. Hence chlorophyll-containing tissues appear green because green light is reflected by the cell.
- Two types of chlorophyll exist in the photosystems of green plants: chlorophyll a and b



# Photosynthesis in plant



# PHOTOSYNTHESIS-LIGHT REACTION



# PHOTOSYNTHESIS - CALVIN CYCLE

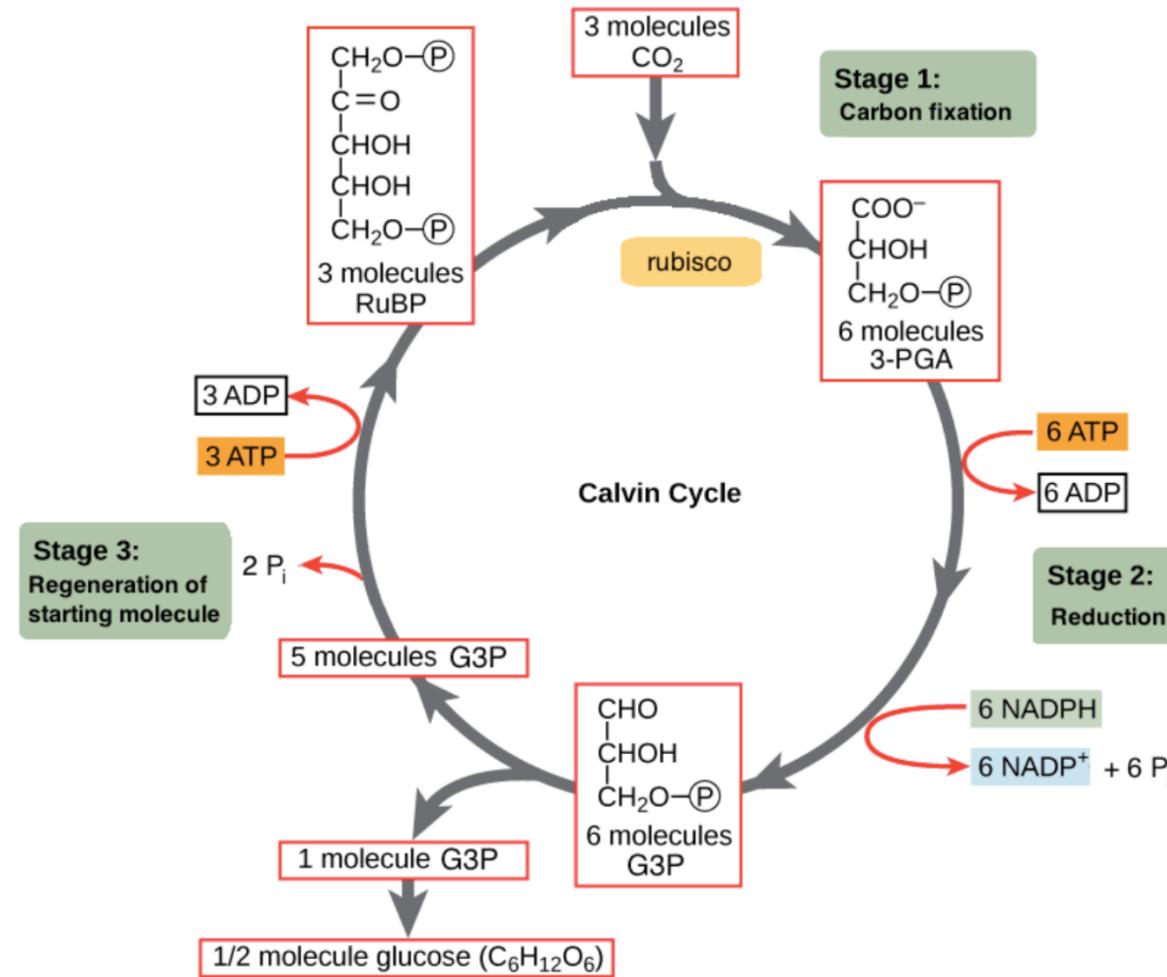


Image modified from "Using light energy to make organic molecules:  
Figure 1," by OpenStax College, CC BY 4.0

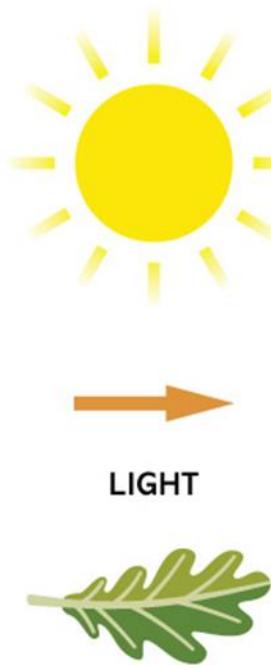
## PHOTOSYNTHESIS



CARBON  
DIOXIDE



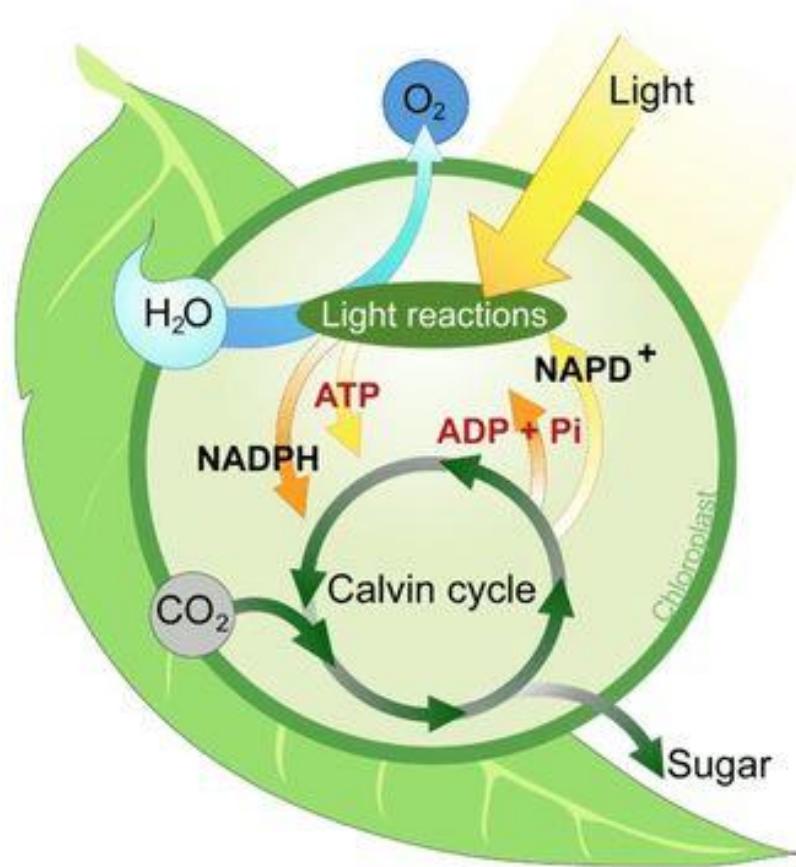
WATER

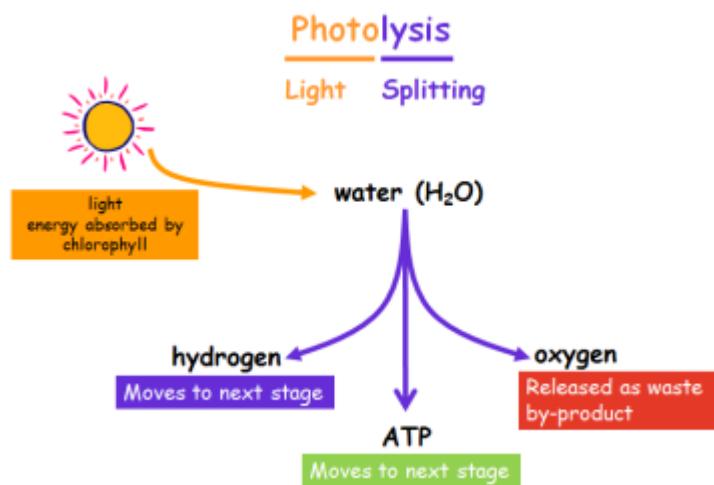


GLUCOSE



OXYGEN

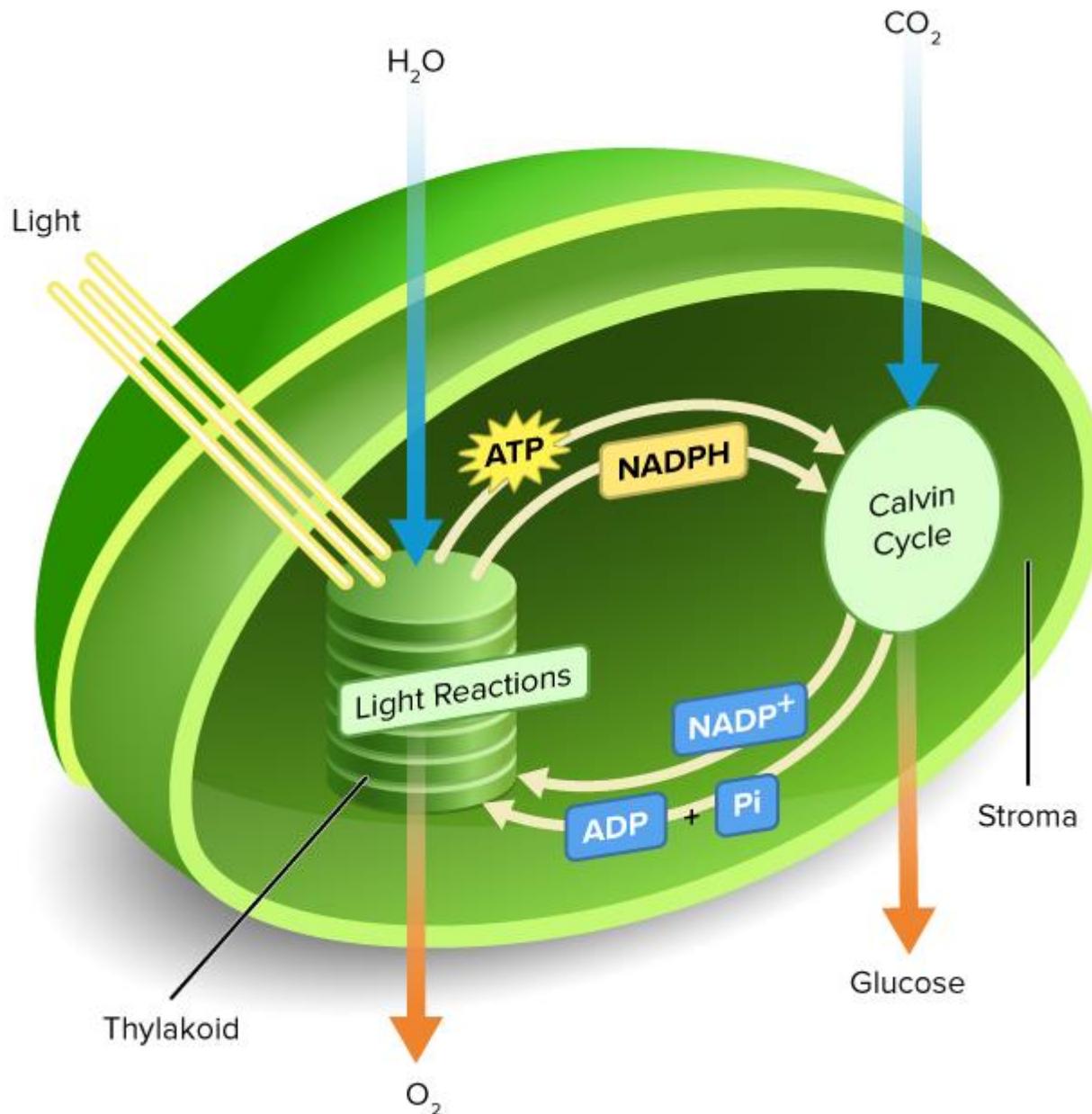
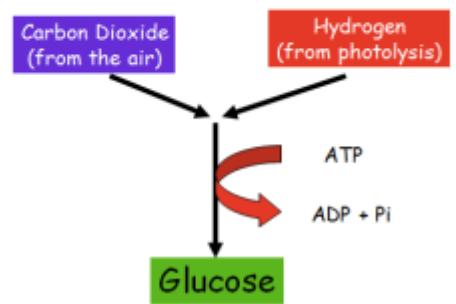


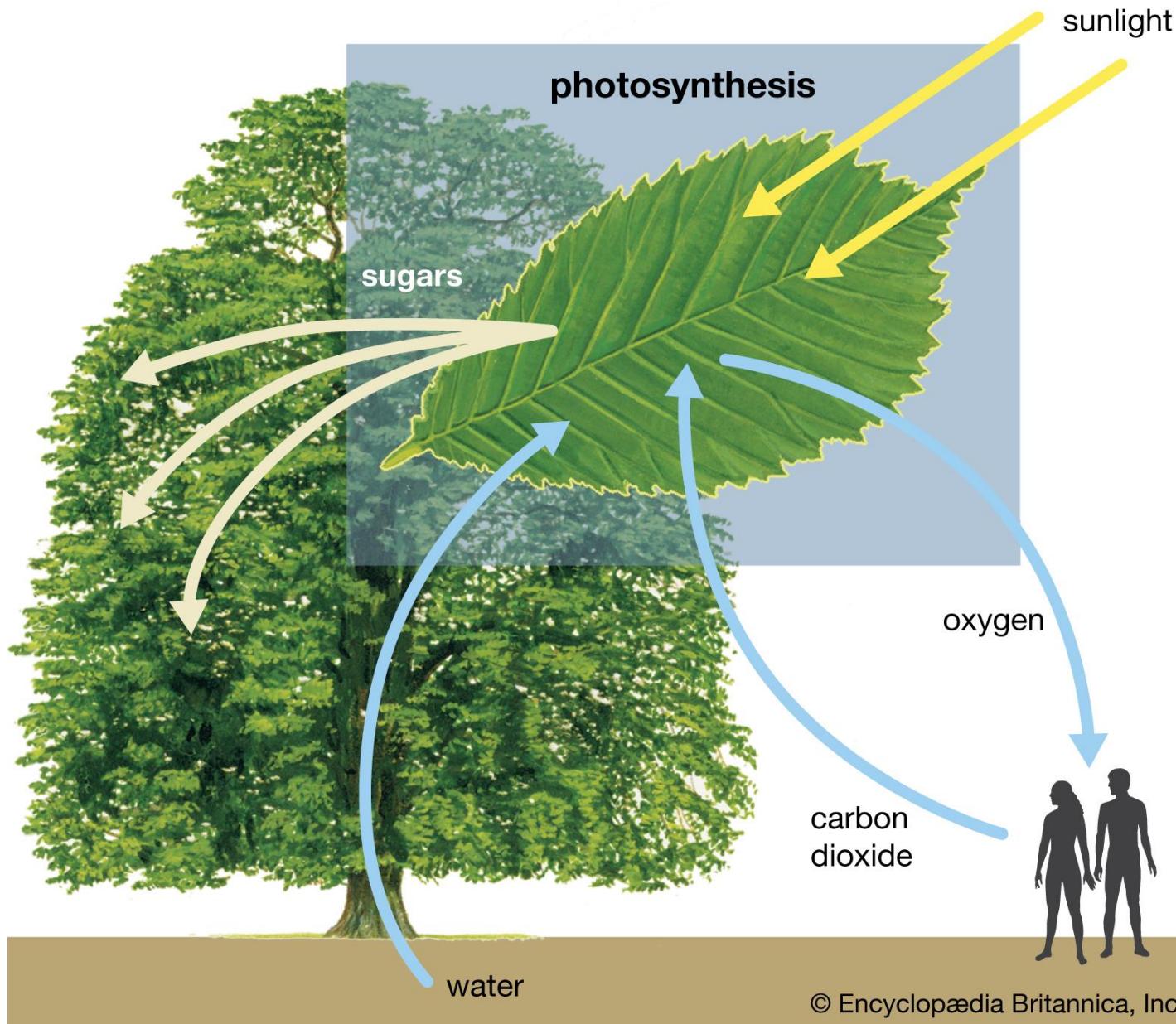


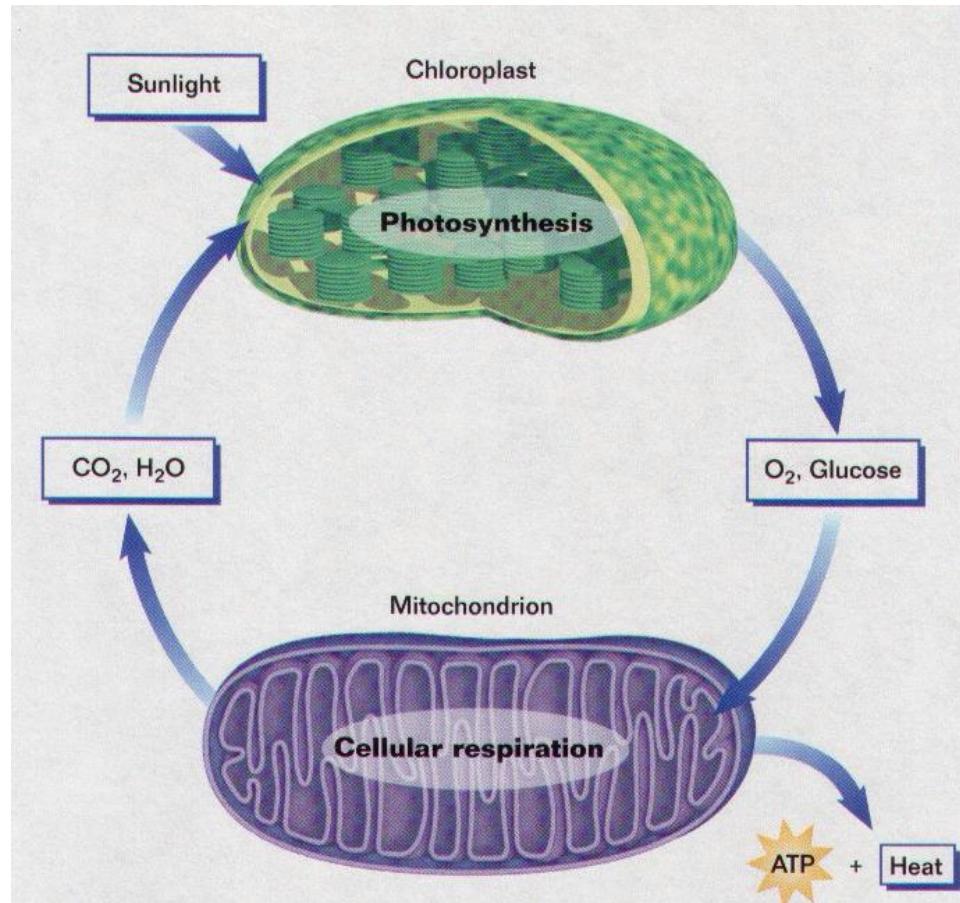
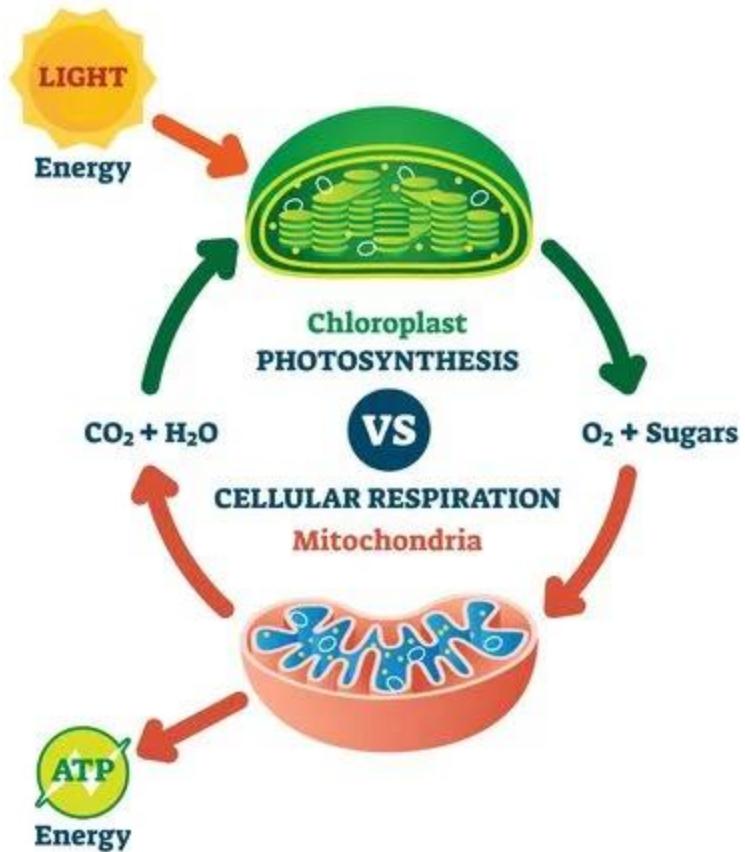
### Carbon Fixation Stage

Carbon dioxide joins with hydrogen to make glucose.

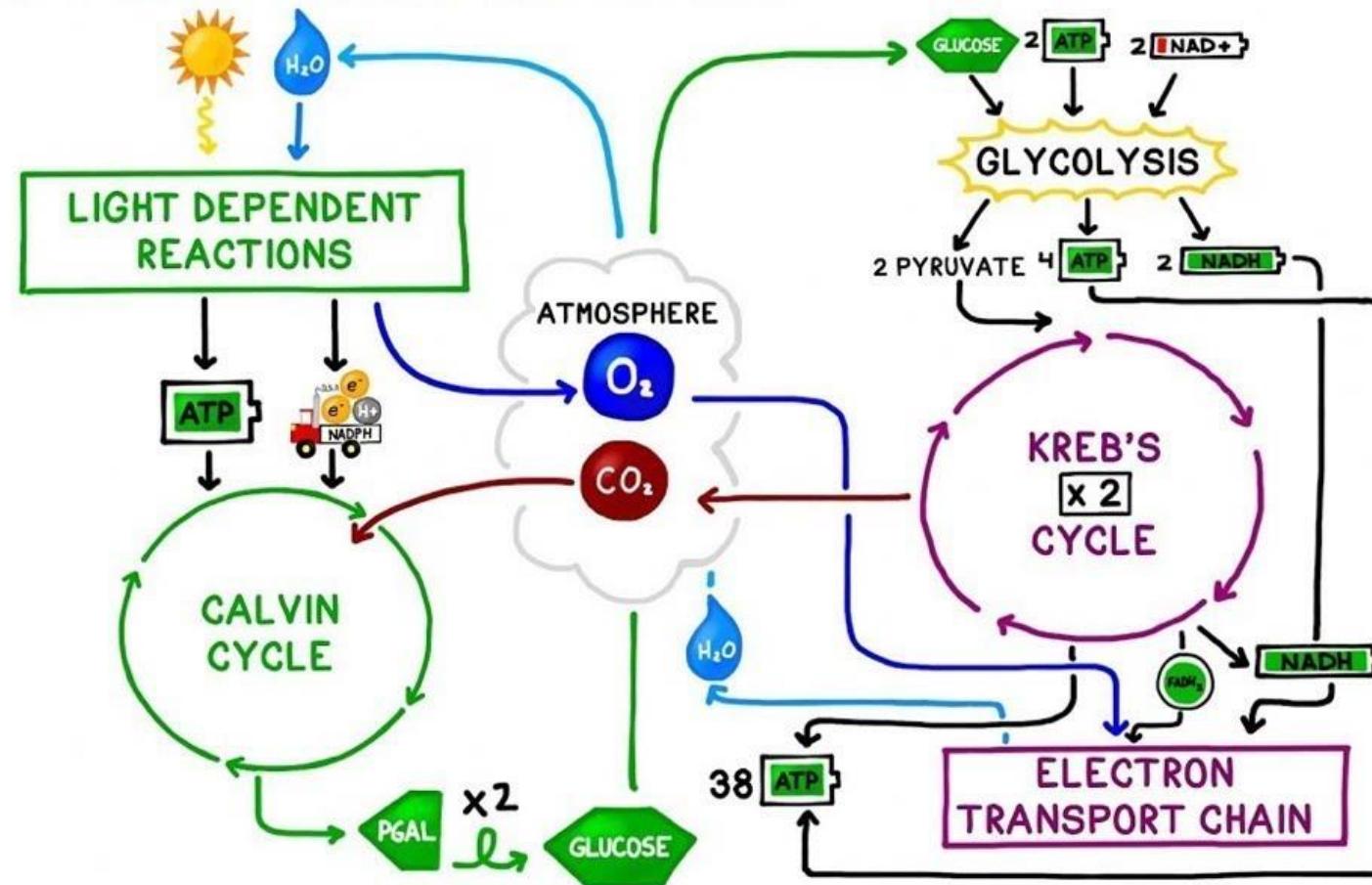
ATP energy is needed to do this:



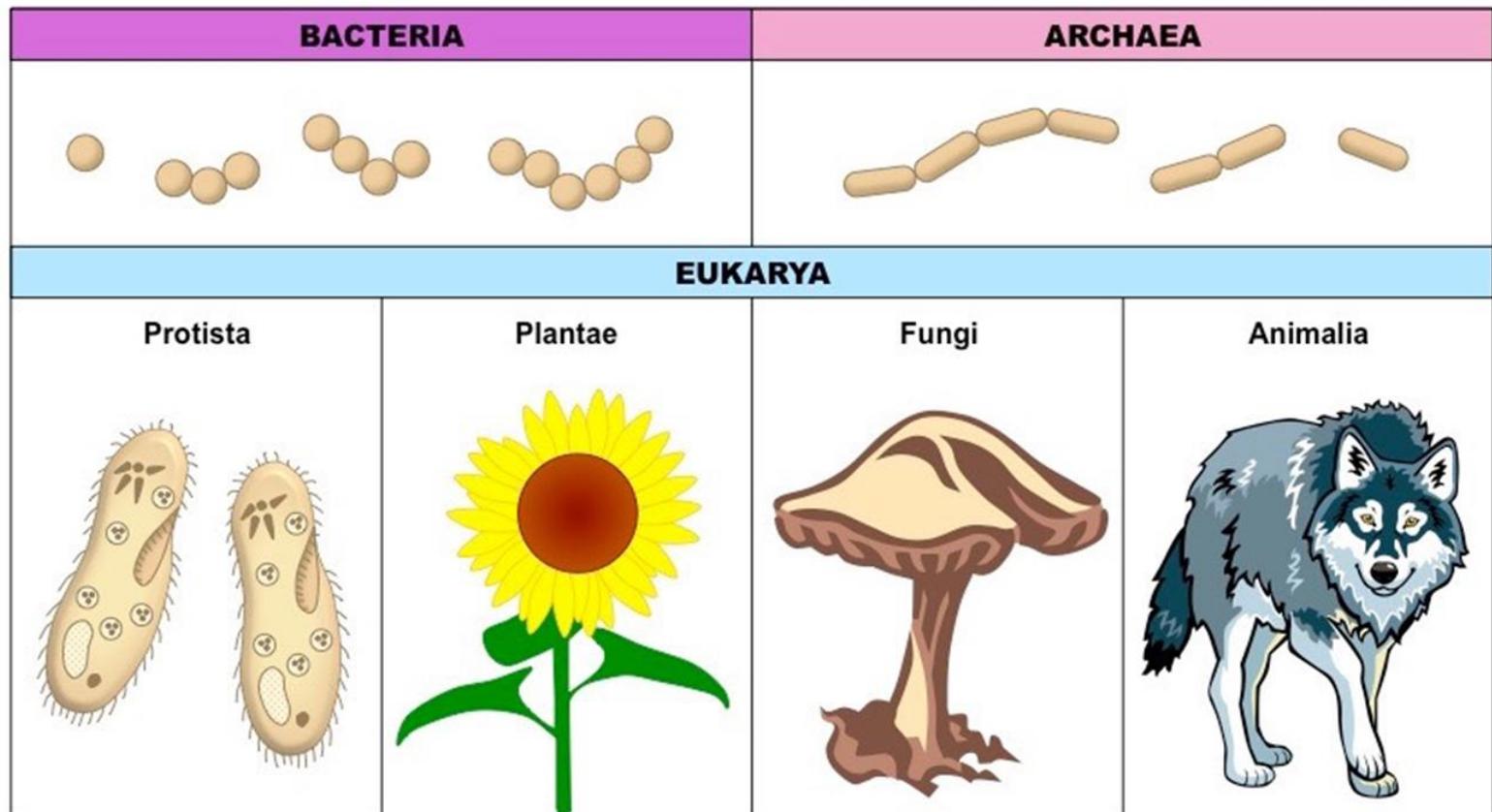
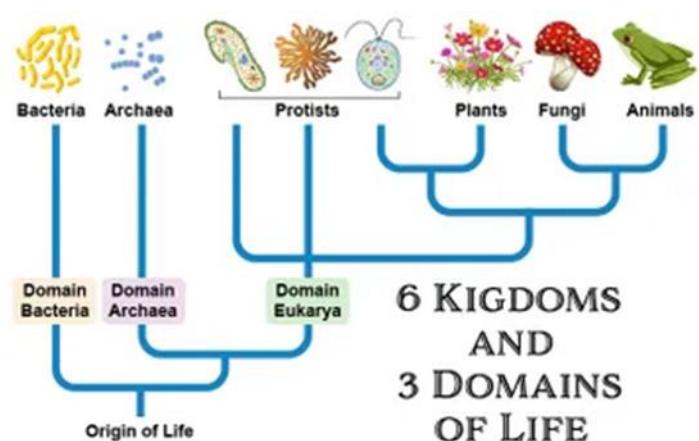




# PHOTOSYNTHESIS      RESPIRATION



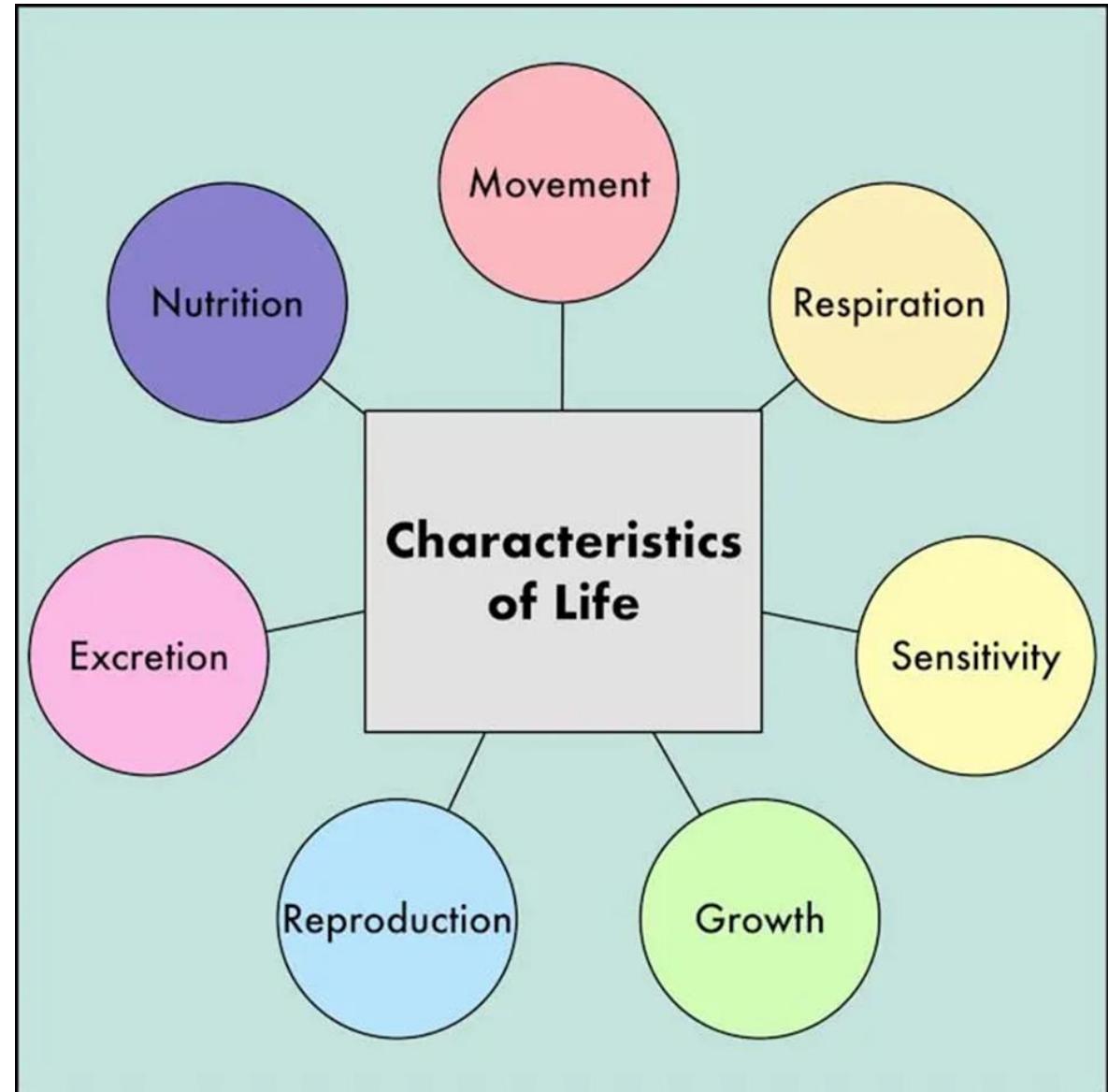
# DOMAINS OF LIFE



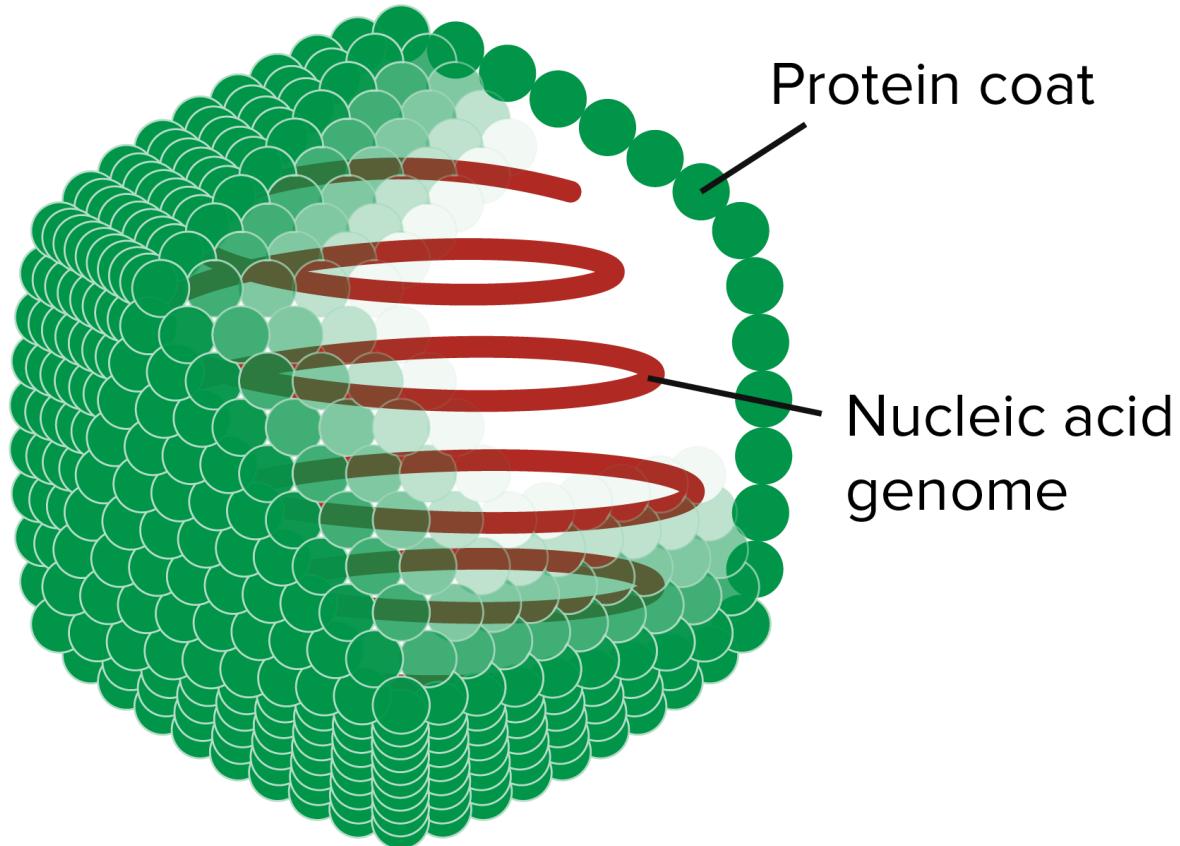
# VIRUSES – LIVING ORGANISMS?

## CHARACTERISTICS OF LIFE

- 1. cellular organization**
- 2. reproduction**
- 3. metabolism**
- 4. homeostasis**
- 5. heredity**
- 6. response to stimuli**
- 7. growth and development**
- 8. adaptation through evolution**



## Structure of a virus



Most biologists consider viruses to be **nonliving** because they don't exhibit all the criteria for life.

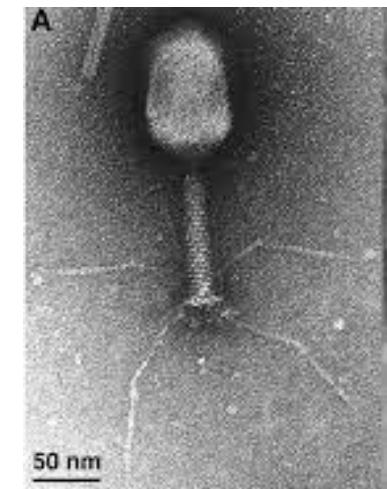
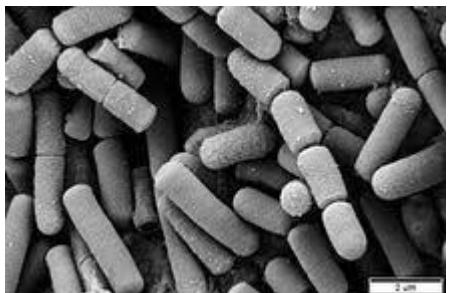
They don't carry out respiration, grow, or develop.

All viruses can do is replicate—make copies of themselves—and they can't even do that without the help of living cells.

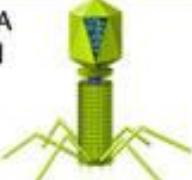
Host cell: a cell in which a virus replicates

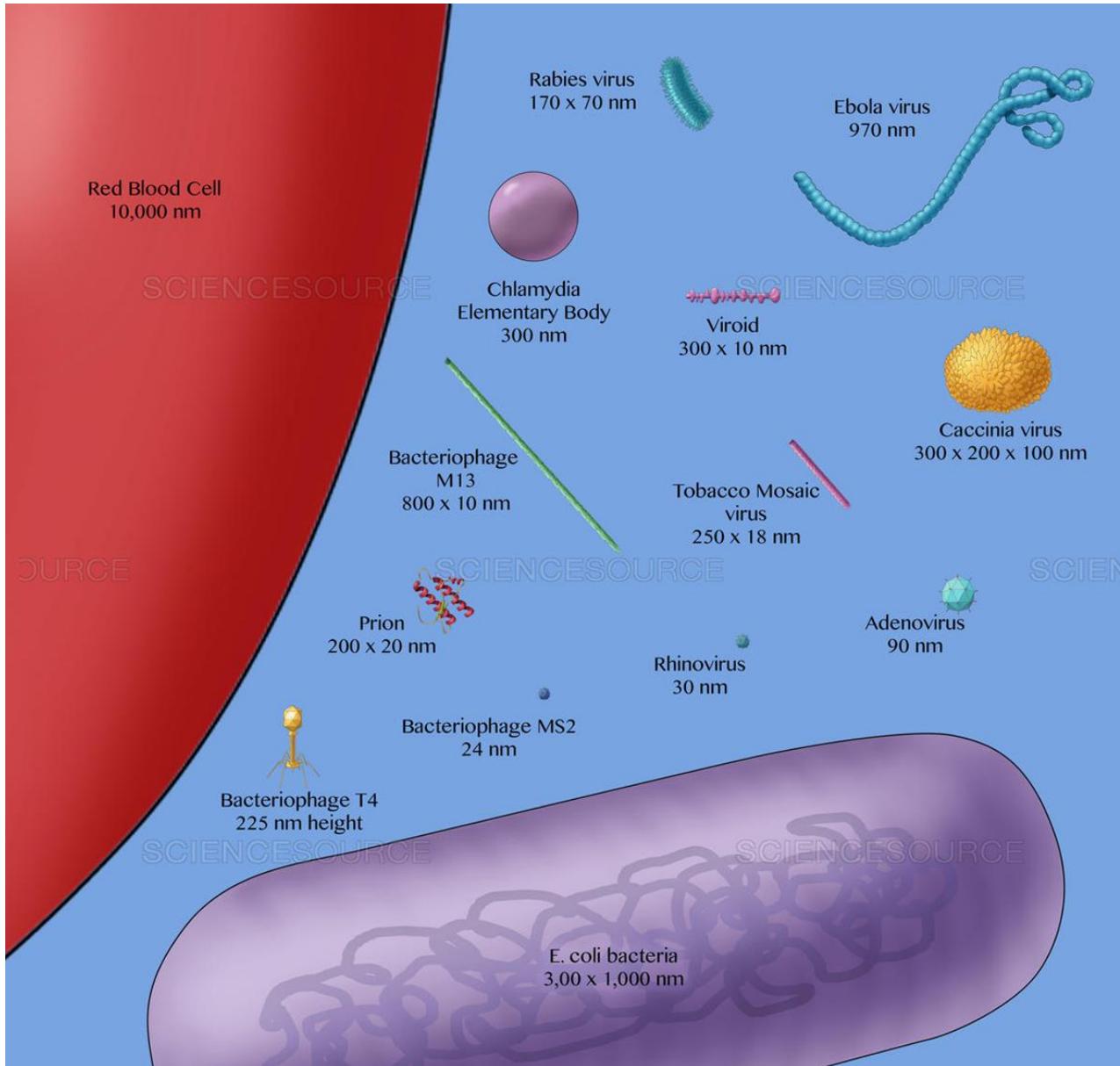
# Characteristics of Living Organisms

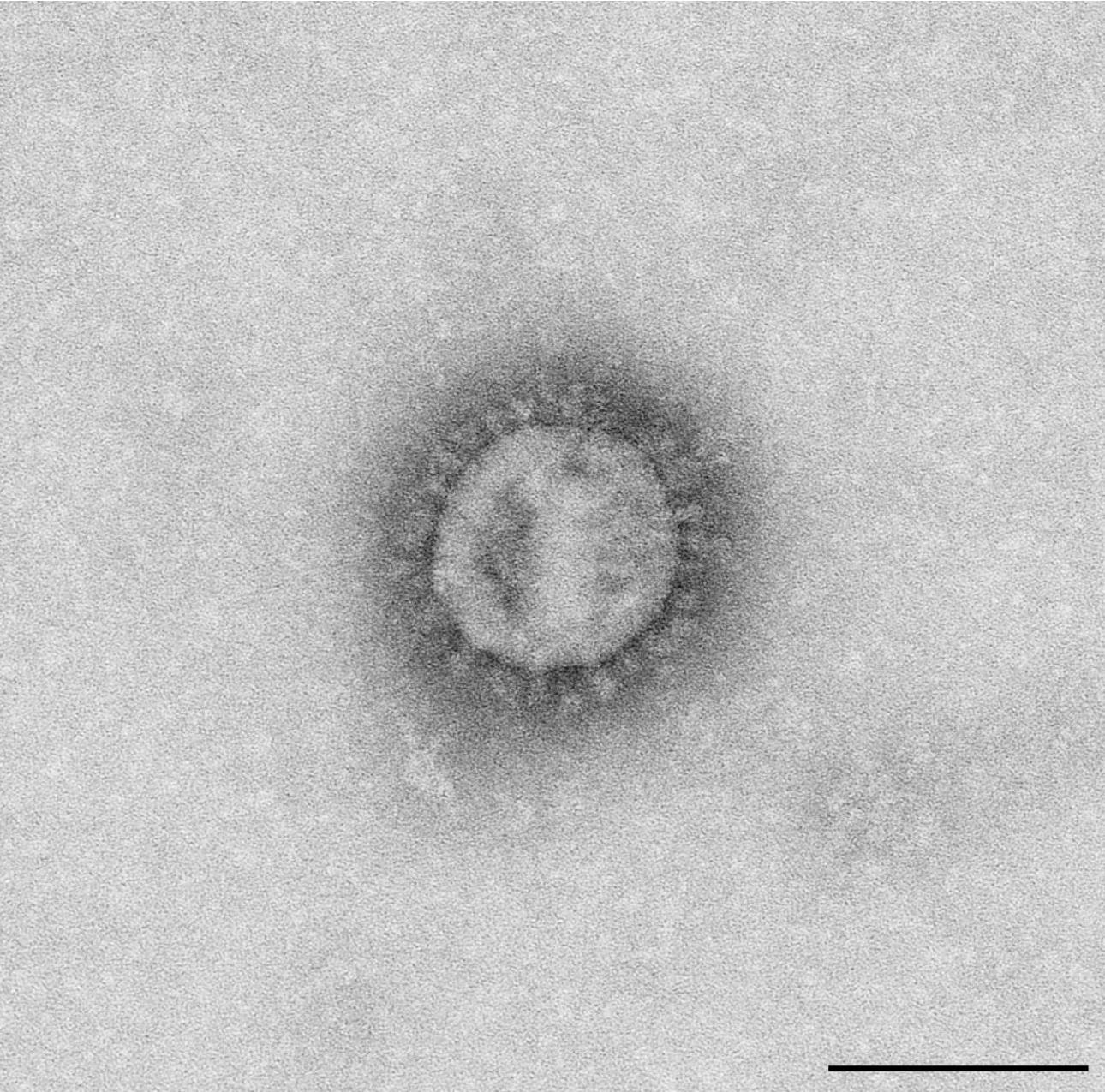
- **Metabolism** – enzyme-catalyzed chemical reactions  
*Bacillus cereus* – Yes                      Bacteriophage T4 – No
- **Reproduction** – progeny formed sexually or asexually  
*Bacillus cereus* – Yes                      Bacteriophage T4 – Yes
- **Differentiation** – different cell types can occur  
*Bacillus cereus* – Yes                      Bacteriophage T4 – No
- **Communication** – signaling within and between cells  
*Bacillus cereus* – Yes                      Bacteriophage T4 – No
- **Locomotion** – relative movement of cell or organism  
*Bacillus cereus* – Yes                      Bacteriophage T4 – No
- **Evolution** – genetic change over time  
*Bacillus cereus* – Yes                      Bacteriophage T4 – Yes



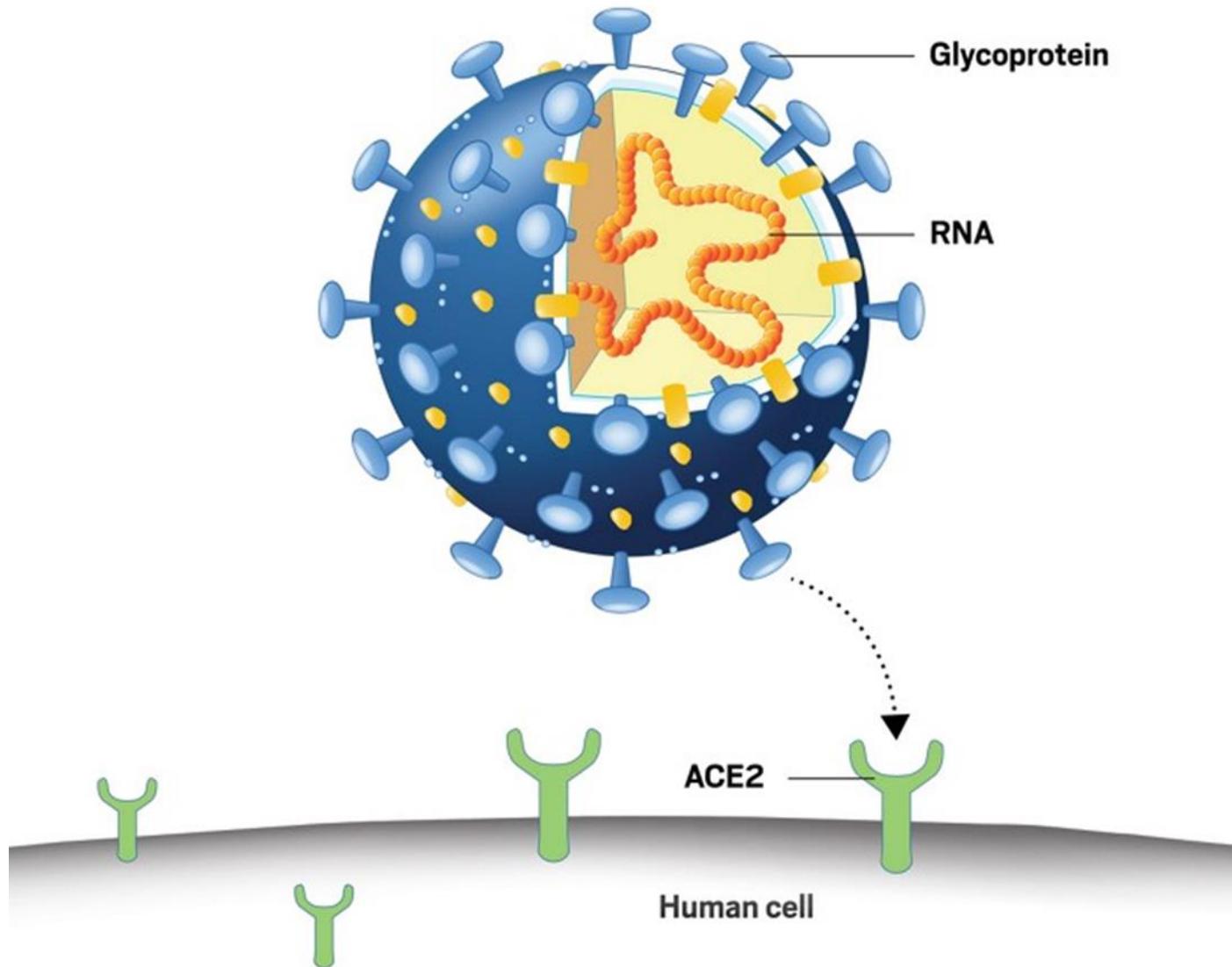
# VIRUSES VS CELLS

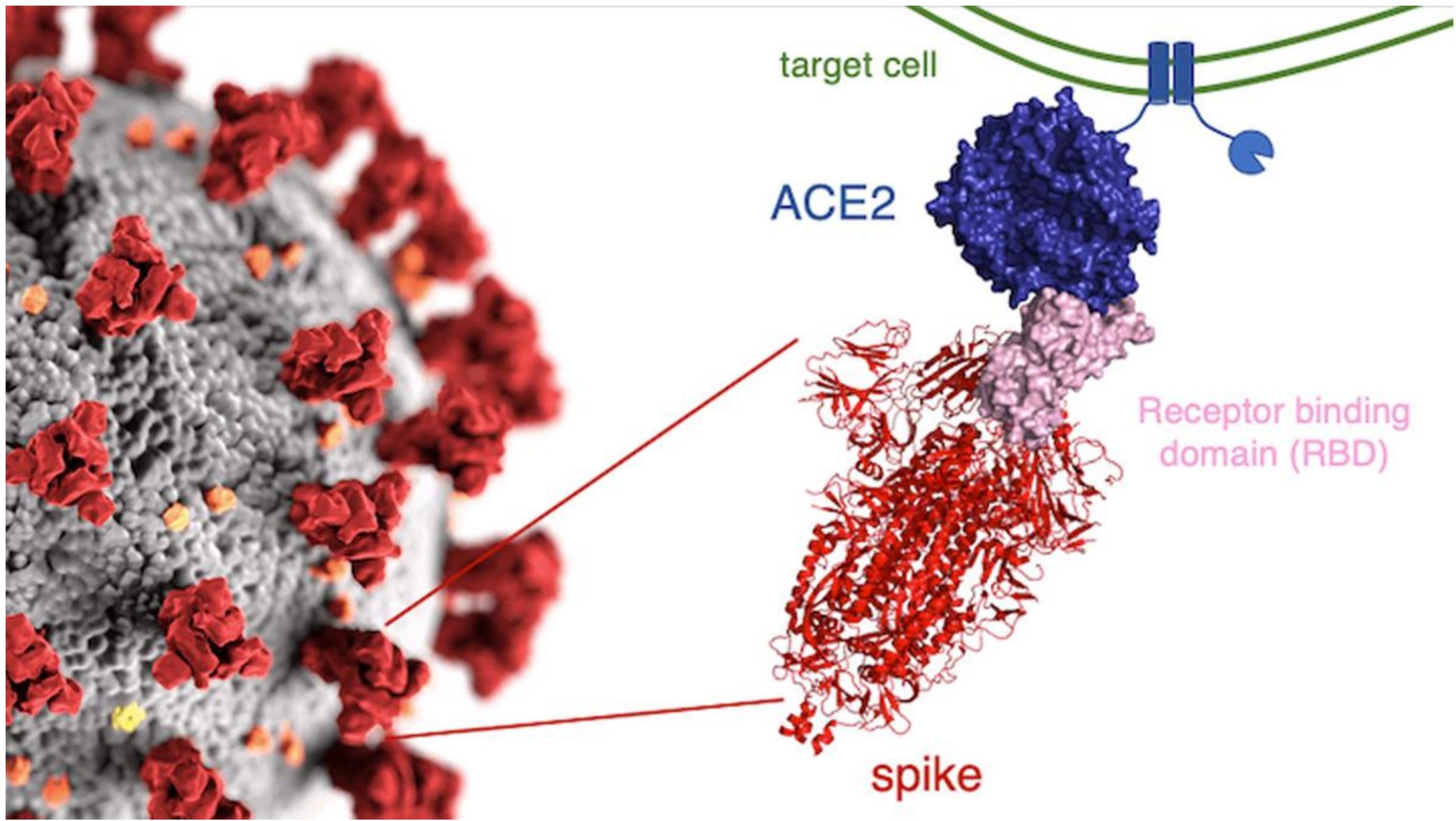
Viruses and Cells		
Characteristic	Virus	Cell
Structure	DNA or RNA core, capsid 	Cell membrane, cytoplasm; eukaryotes also contain nucleus and organelles 
Reproduction	only within a host cell	independent cell division either asexually or sexually
Genetic Code	DNA or RNA	DNA
Growth and Development	no	yes; in multicellular organisms, cells increase in number and differentiate
Obtain and Use Energy	no	yes
Response to Environment	no	yes
Change Over Time	yes	yes



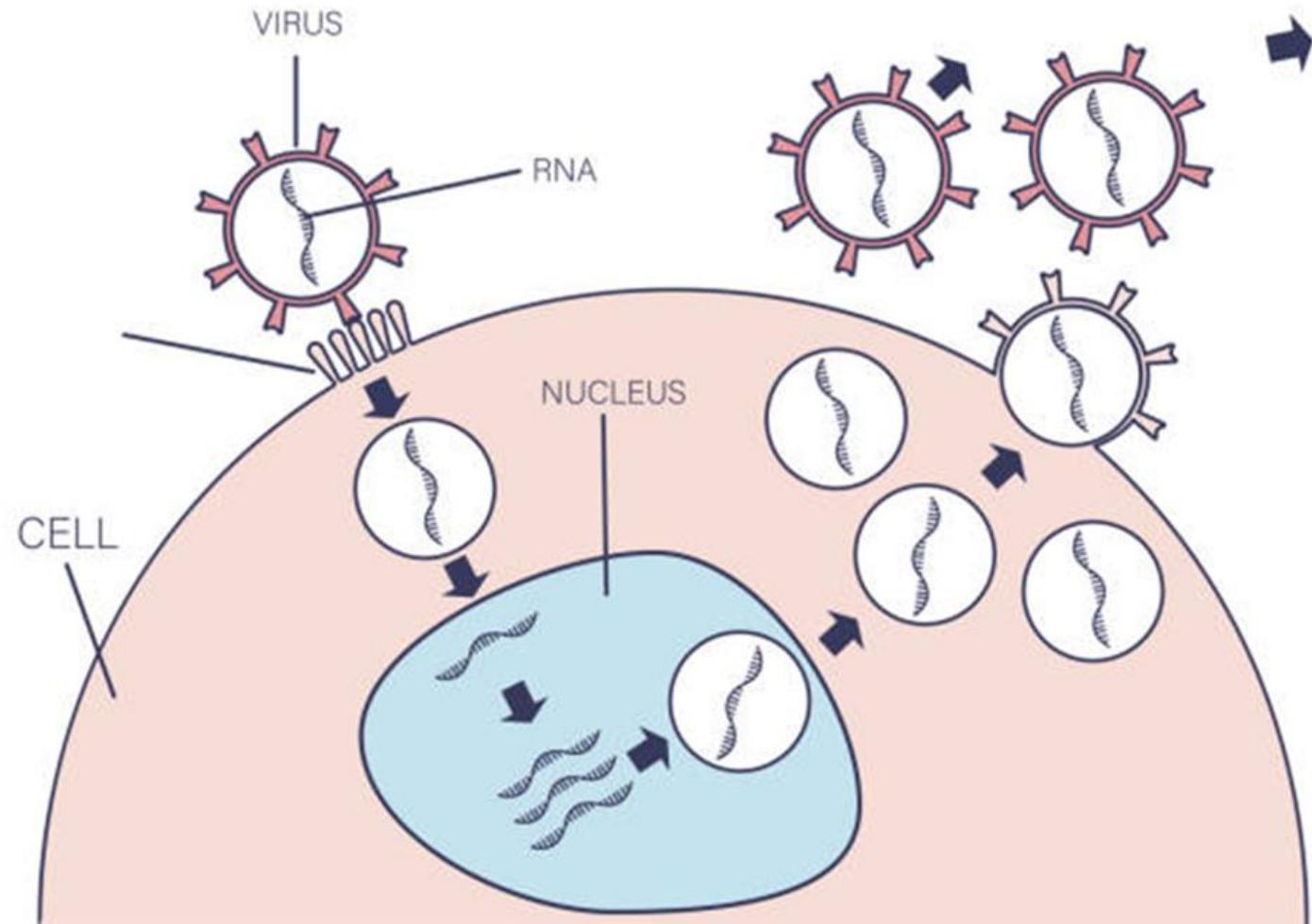


SARS-  
Coronavirus-2  
(SARS-CoV-2,  
Isolat SARS-CoV-  
2/Italy-INMI1).  
EM 100 nm.  
source: Robert  
Koch-Institute



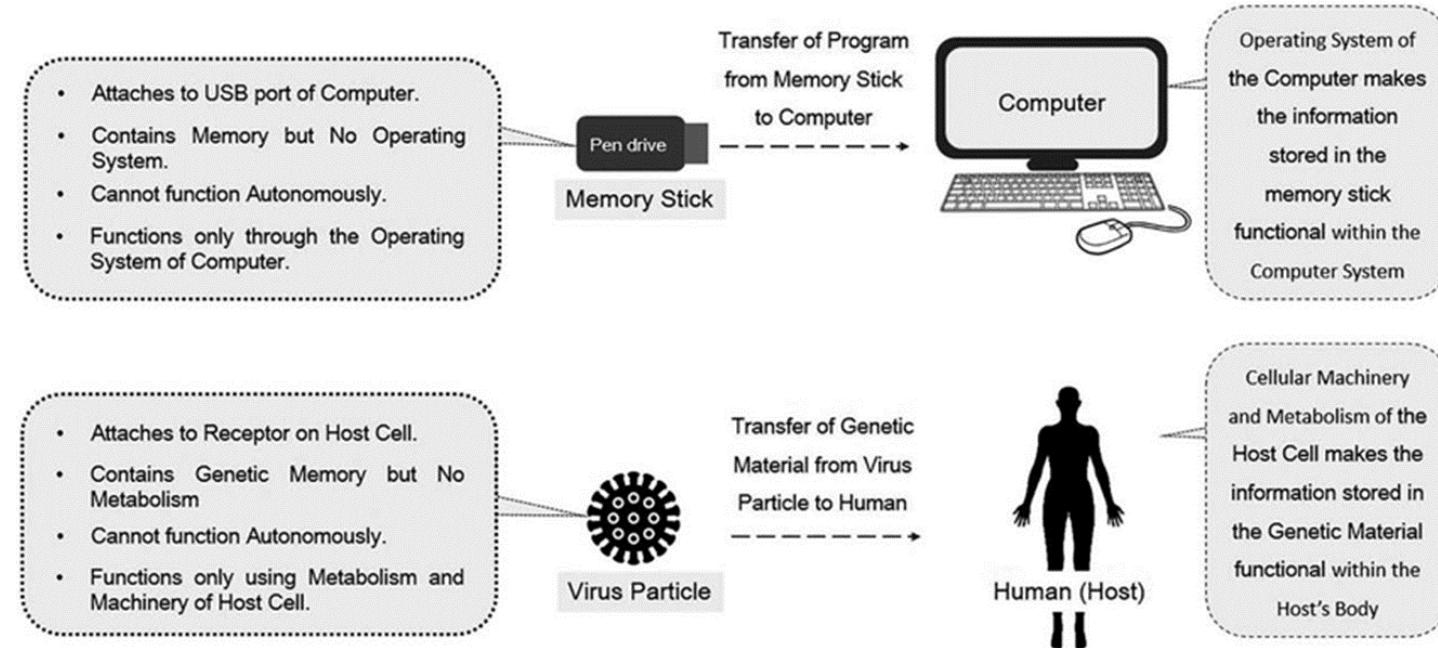


# VIRUS REPLICATION CYCLE



# The biological analogy

## BIOLOGICAL VS COMPUTER VIRUS



- **Biological Virus**

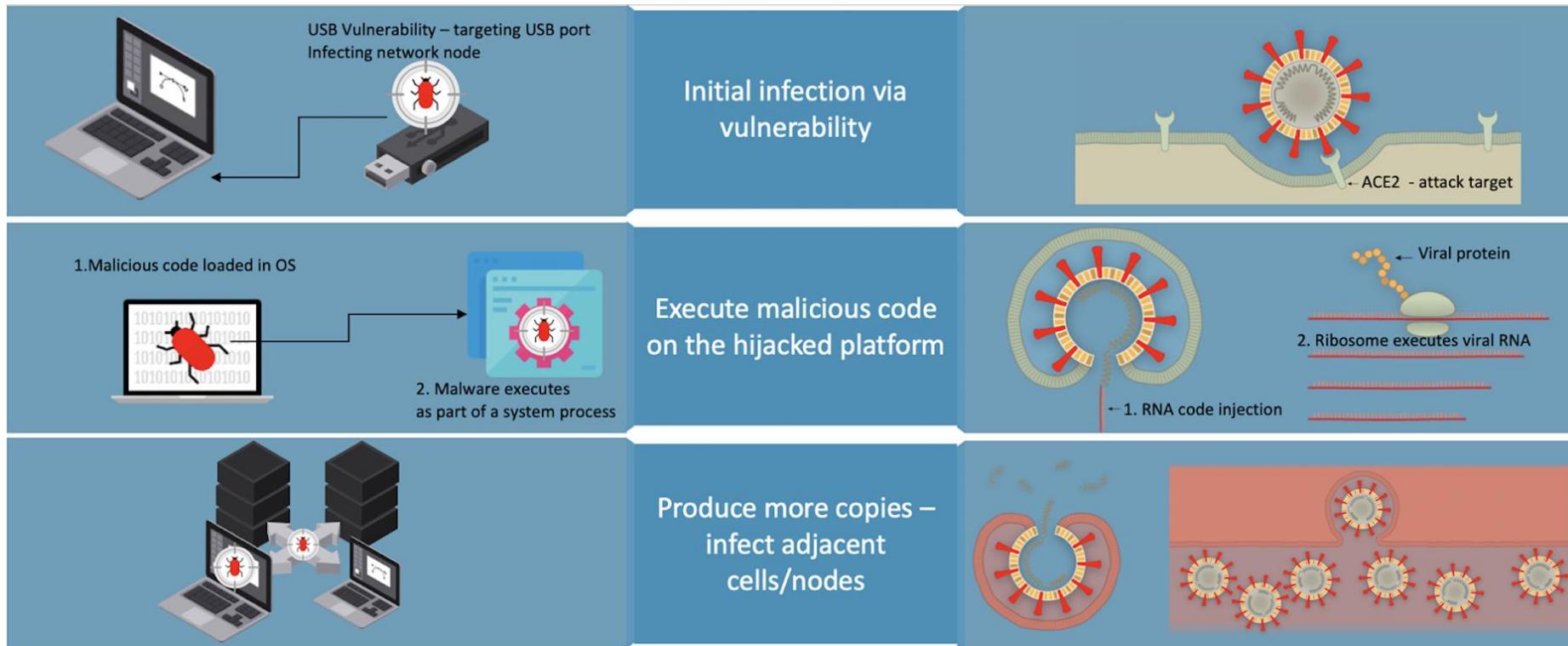
- Cells
- Gene code
- Spread via replication
- Able to do harm
- Able to evolve

- **Computer Virus**

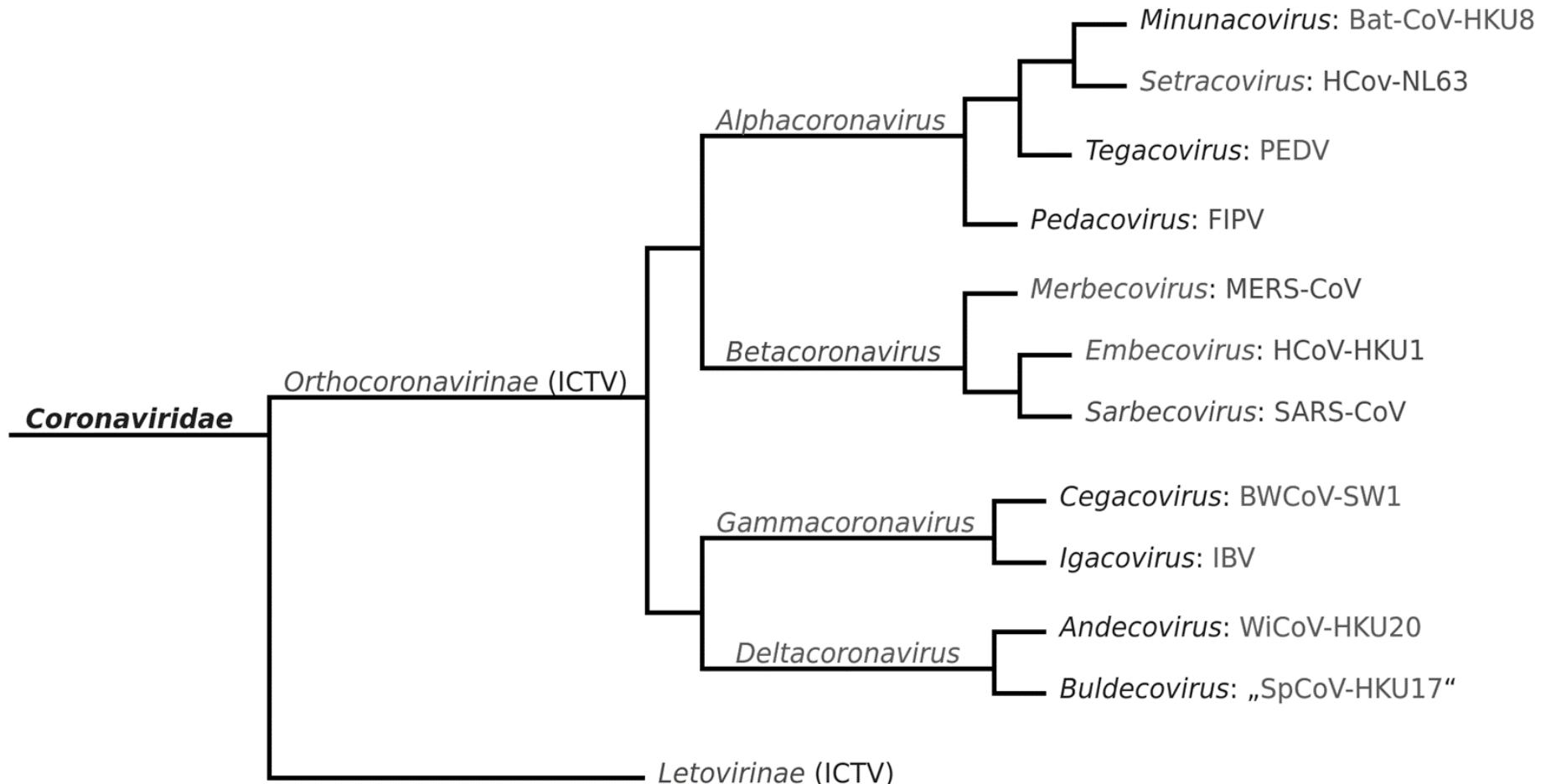
- Computers
- Binary code
- Spread via replication
- Able to do harm
- Able to evolve

	<b>Biological Virus</b>	<b>Computer Virus</b>
<b>Parasitism</b>	Replicates only inside the cells of a living organism using their resources.	Replicates by copying itself into other computer programs.
<b>Invisible Phase</b>	Replicates inside an organism for a while without any symptoms; known as the incubation period.	Can be programmed to cause harm only after a certain event, such as the launch of an app.
<b>Mutation</b>	Can produce copies with mutations that may become resistant to antibodies and drugs.	Can change the code in its copies to avoid detection by antivirus software.
<b>Self-Defense</b>	Can attack and/or compromise the immune system to protect itself.	Can block antivirus software to protect itself.

# BIOLOGICAL VS COMPUTER VIRUS

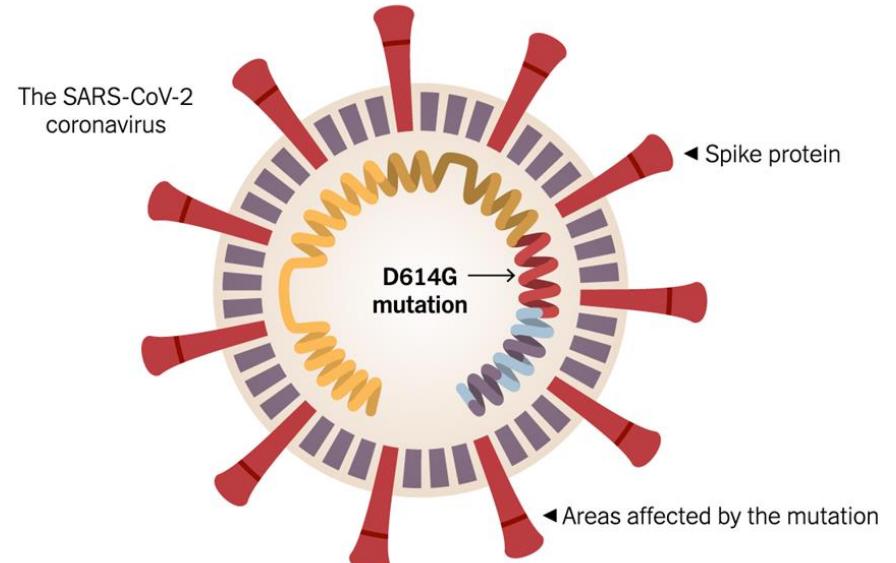


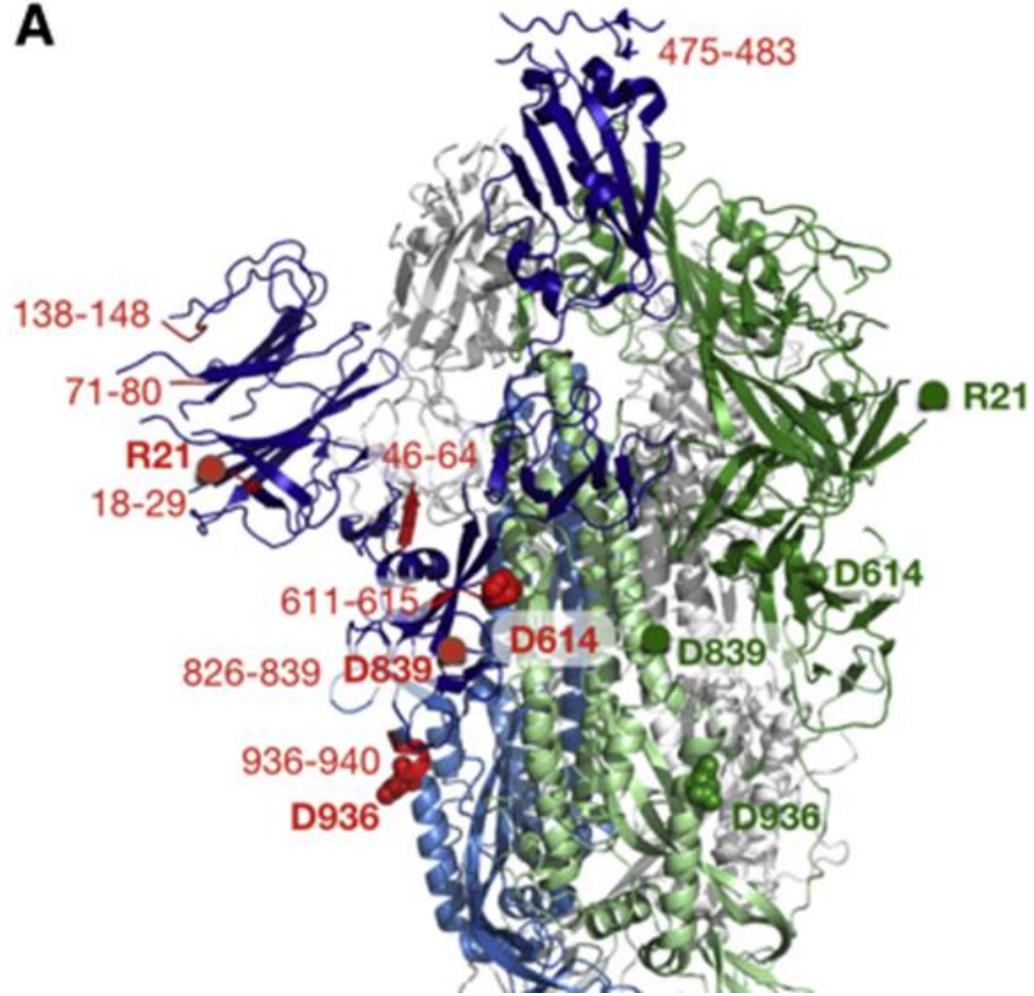
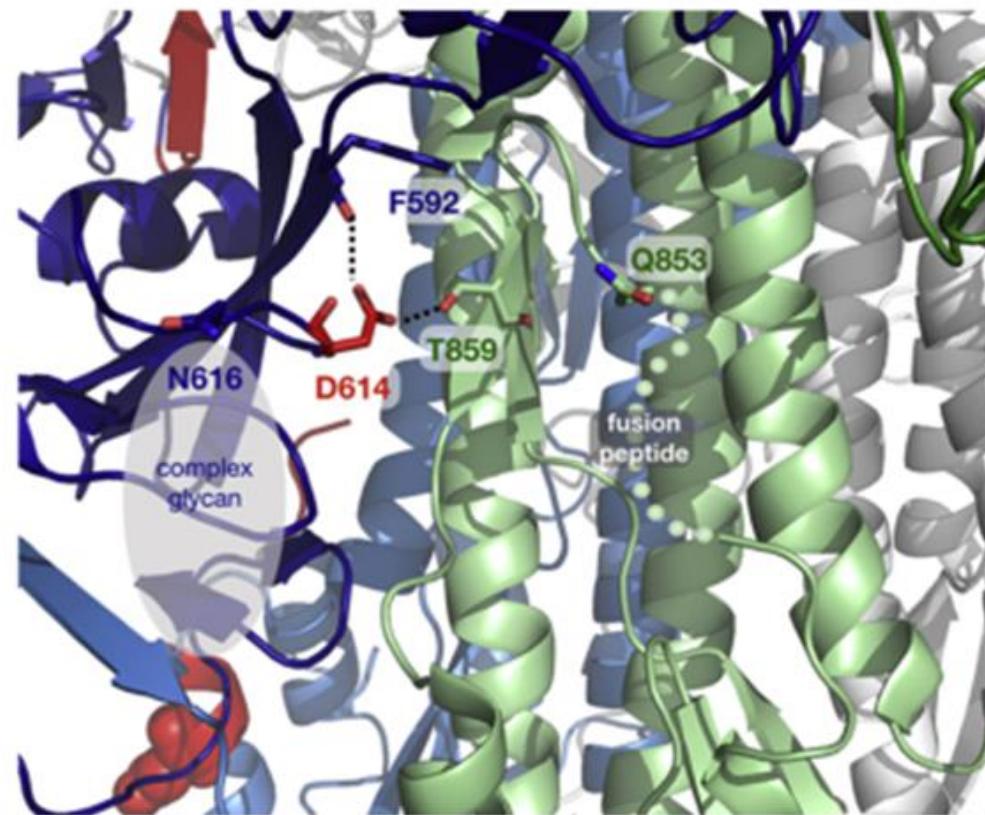
# CLADOGRAM CORONAVIRIDAE



# SARS COV 2 - MUTATION

- In late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan and rapidly developed into the COVID-19 pandemic. By the end of September 2020, the worldwide death toll had passed one million people with more than 37 million infections.
- Cell entry of SARS-CoV-2 is dependent on the interaction of the spike glycoprotein (S) and the host cell surface receptor angiotensin-converting enzyme 2 (ACE2)
- **S-D614G** is a protein variant containing a substitution in the S protein outside of the RBD and is thought to cause a conformational change. It weakens the 'latch' in the S protein between the S1 and S2 domains and **causes a more "open" conformation** that **improves ACE2 binding** and increases the probability of infection.
- Over the course of the pandemic, the SARS-CoV-2 S-614G variant rapidly superseded the parental S-614D variant in frequency to become globally dominant.

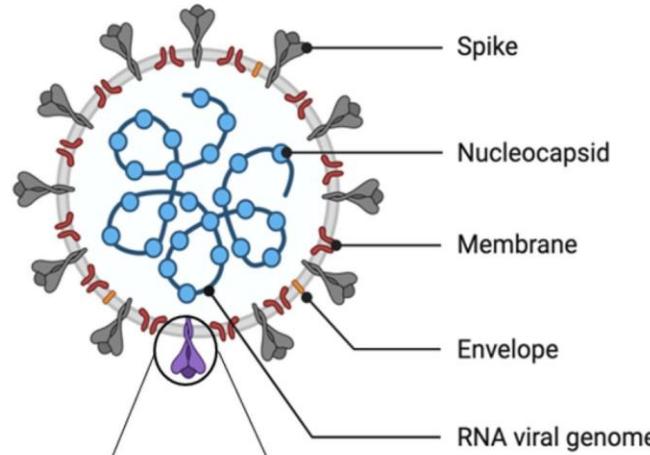


**A****B**

## SARS COV 2 - MUTATION

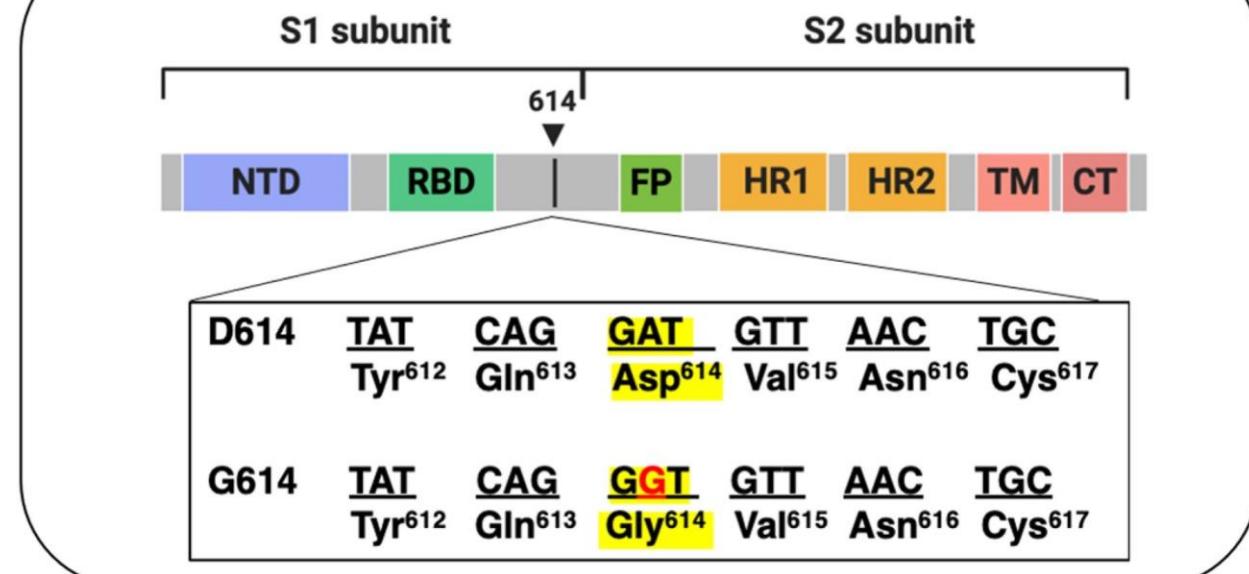
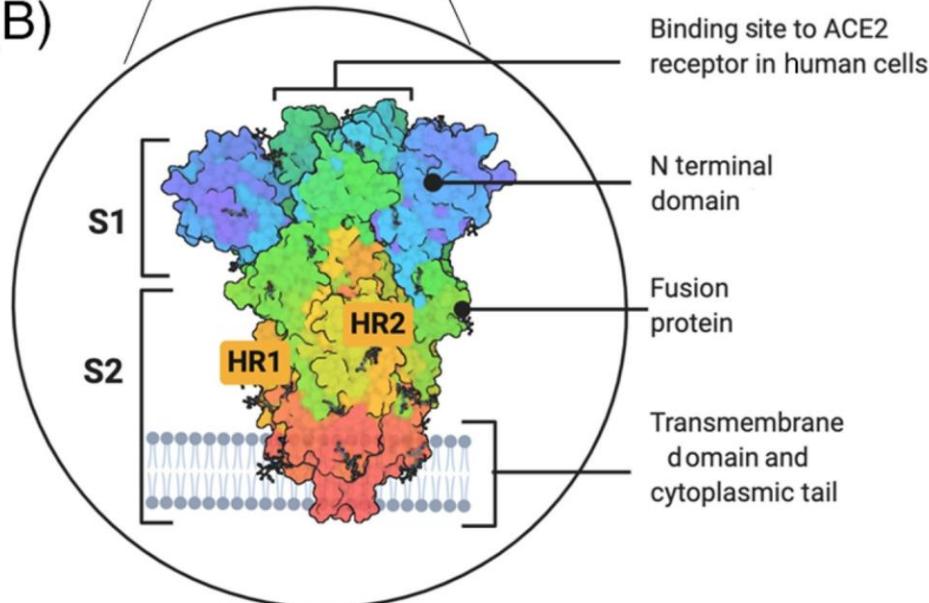
- The Spike D614G amino acid change is caused by an A-to-G nucleotide mutation at position 23,403 in the Wuhan reference strain. First the G614 form was rare globally but it soon gained prominence in Europe.
- Structural implications of the Spike D614G change
  - D614 is located on the surface of the Spike protein.
  - the side chains of D614 and the T859 of the neighboring protomer (Figure B) form a hydrogen bond, bringing together the S1 unit of one protomer and the S2 unit of the other protomer. The **change to G614** would **eliminate** this side-chain **hydrogen bond**, possibly **increasing** main-chain **flexibility** and altering between-protomer interactions.

(A)

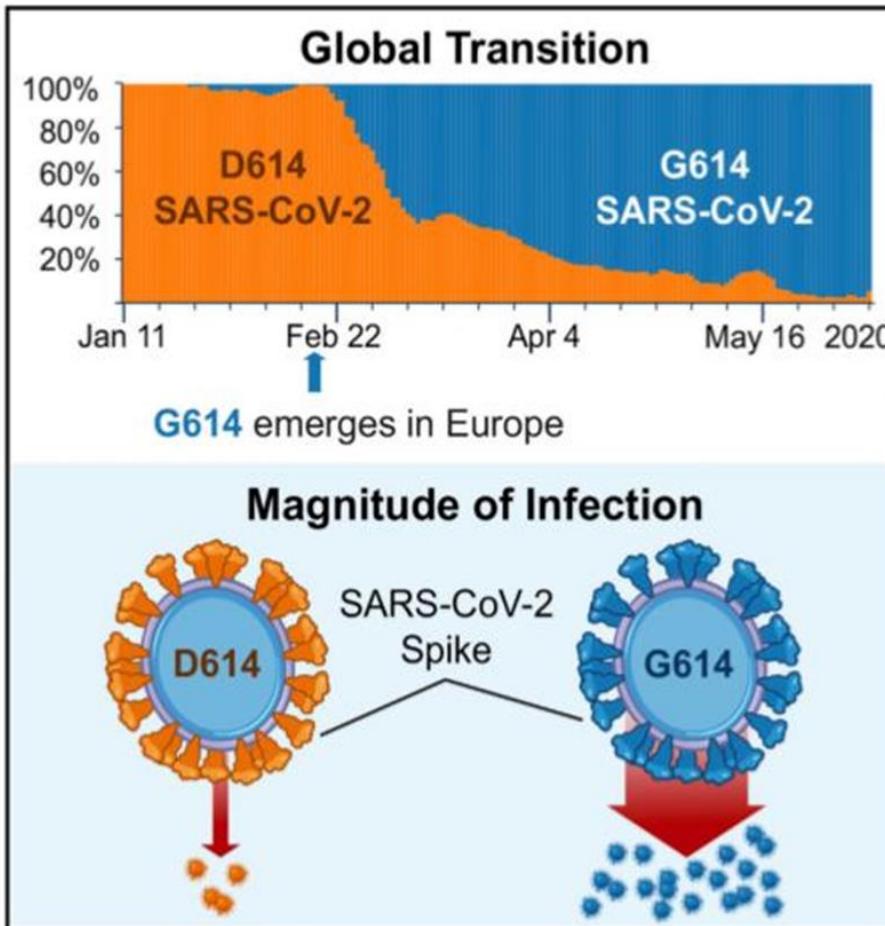


(C)

(B)



# SARS COV 2 - MUTATIONS



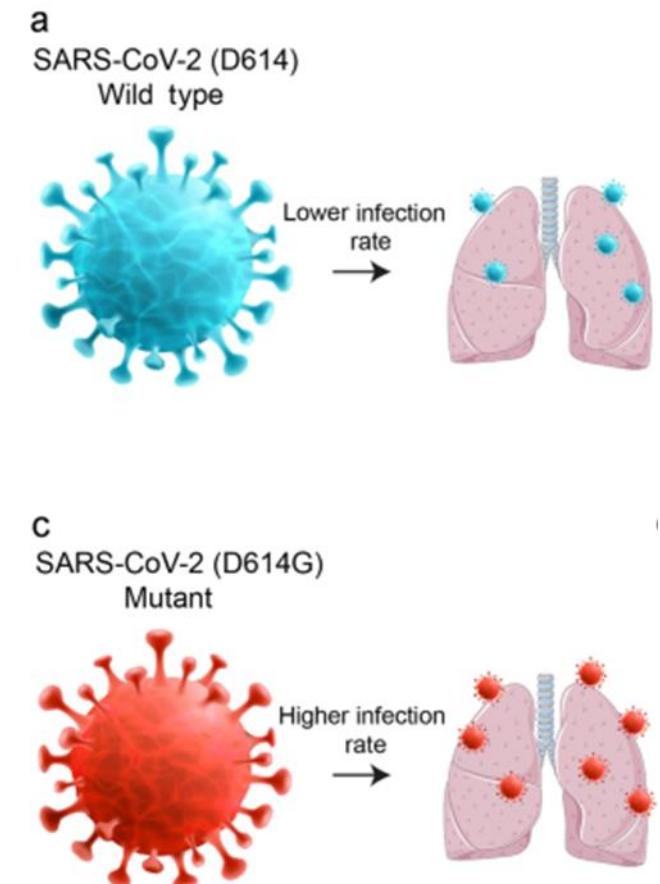
- The D614G variation took place in the important spike protein where the key amino acid changed from aspartic acid (D) to glycine (G) at position 614<sup>1</sup>.
- The “G clade” mutation was rare in January, but gained global prominence beginning in Europe around late February, early March<sup>2</sup>.

1. Zhang L, Jackson CB, Mou H, et al. The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity. Preprint. bioRxiv. 2020;2020.06.12.148726. Published 2020 Jun 12. doi:10.1101/2020.06.12.148726

2. Korber B, Fischer WM, Gnanakaran S, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 2020;S0092-8674(20)30820-5. doi:10.1016/j.cell.2020.06.043

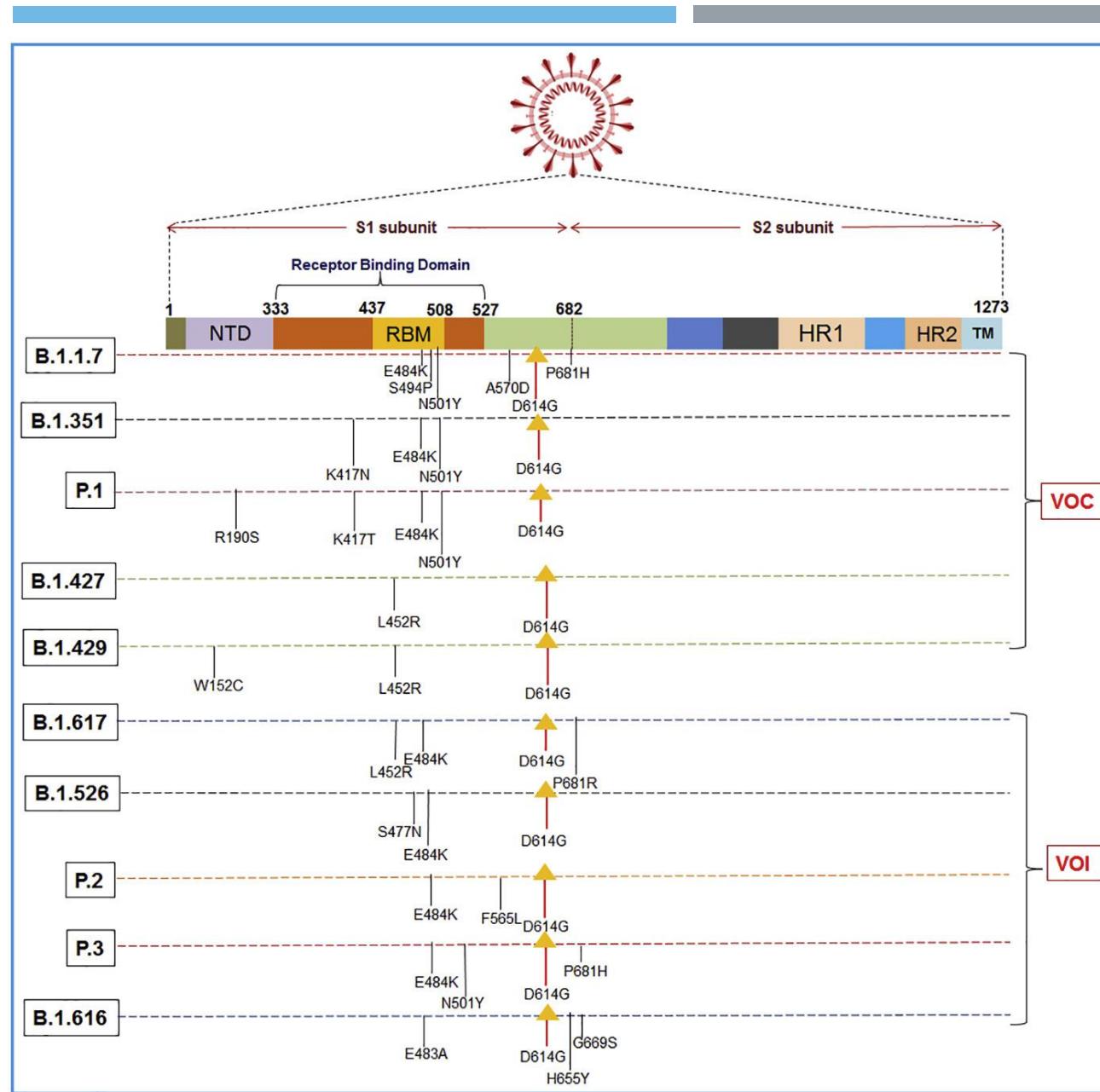
# D614G MUTATION EVENTUATES IN ALL VOI AND VOC IN SARS-COV-2

- Several emerging variants of SARS-CoV-2 have originated from the Wuhan strain and spread throughout the globe. One mutation, D614G, is prominent in all VOI and VOC in SARS-CoV-2. This mutation might help to increase the viral fitness in all emerging variants where the mutation is present. With the help of this mutation (D614G), the SARS-CoV-2 variants have gained viral fitness to enhance viral replication and increase transmission.
- The RNA viruses such as the common cold, influenza, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and COVID-19 are inherently prone to mutations.
- The evolution of SARS-CoV-2's genome (by means of random mutations) led to mutant specimens of the virus (i.e., genetic variants), observed to be more transmissible. Notably, both the Alpha and the Delta variants were observed to be more transmissible than previously identified viral strains.



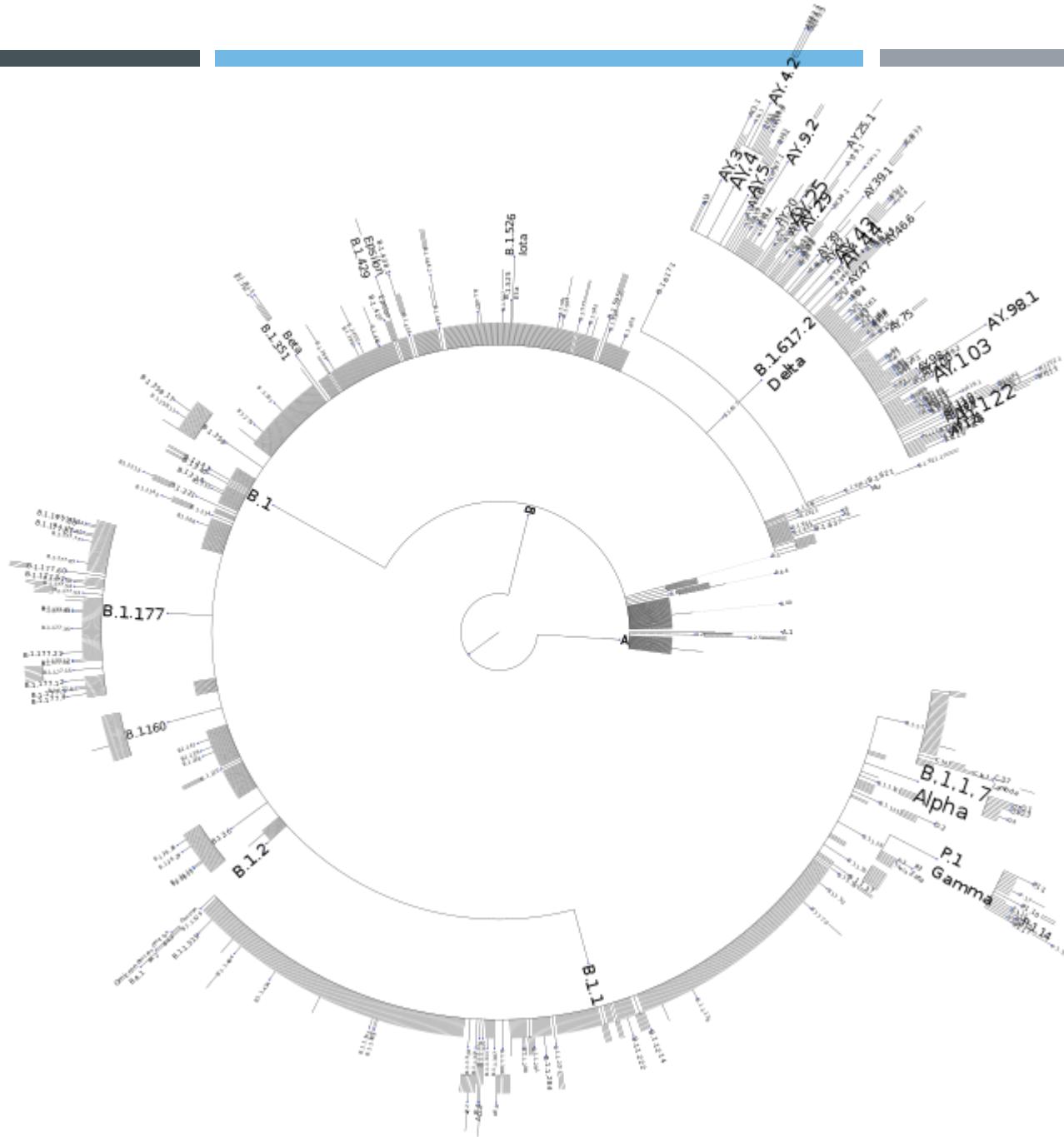
All of the emerging VOC and VOI have gained the mutation, which is found in all the emerging variants. D614G mutation occurs in the highest frequency compared to other mutations. (A)

The schematic diagram shows the D614G mutation in S-glycoprotein of all emerging variants of concern (VOC) and interest (VOI) of SARS-CoV-2.



# FROM ALPHA TOOMICRON: SARS-COV-2 VARIANTS OF CONCERN

- In May 2021, the WHO began assigning variants of interest and variants of concern letters of the Greek alphabet. This was both to make them easier to remember, and to remove the stigma associated with referring to them by the country where they were first detected, which isn't necessarily reflective of where they first emerged and may disincentivise countries from reporting their existence.
- Alpha (B.1.1.7)
- First detected in the United Kingdom, and designated a variant of concern in December 2020, Alpha had then been verified in 192 locations worldwide (Dec 2021). It contains **several key mutations in the spike protein** that mark it out from the original Wuhan strain. One is a N501Y mutation, which improves spike protein binding to cellular receptors making the virus more contagious. It also contains a D614G mutation, thought to enhance viral replication.
- Alpha is estimated to be around 50% more transmissible (contagious) than the original Wuhan strain. It is also thought to be associated with increased disease severity. Fortunately, COVID-19 vaccines and monoclonal antibody treatments remain highly effective against it.
- Omicron has a large number of mutations. These include N501Y, D614G, K417N and T478K mutations, which are also found in other variants of concern.



# WHY ARE THERE SO FEW ANTIVIRALS?

- The differences between bacterial and human cells are what make antibiotics possible.
- For example, penicillin is effective because it interferes with the construction of the bacterial cell wall. Cell walls are made of a polymer called peptidoglycan. Human cells don't have a cell wall or any peptidoglycan. So antibiotics that prevent bacteria from making peptidoglycan can inhibit bacteria without harming the human taking the medicine. This principle is known as selective toxicity.
- Viruses use our own cells to replicate. Unlike bacteria, viruses cannot replicate independently outside a host cell.
- To replicate, viruses enter a host cell and hijack its machinery. Once inside, some viruses lie dormant, some replicate slowly and leak from cells over a prolonged period, and others make so many copies that the host cell bursts and dies. The newly replicated virus particles then disperse and infect new host cells.
- An antiviral treatment that intervenes in the viral “life” cycle during these events could be successful. The problem is that if it targets a replication process that is also important to the host cell, it is likely to be toxic to the human host as well.
- **Killing viruses is easy. Keeping host cells alive while you do it is the hard part.**



## WHY ARE THERE SO FEW ANTIVIRALS?

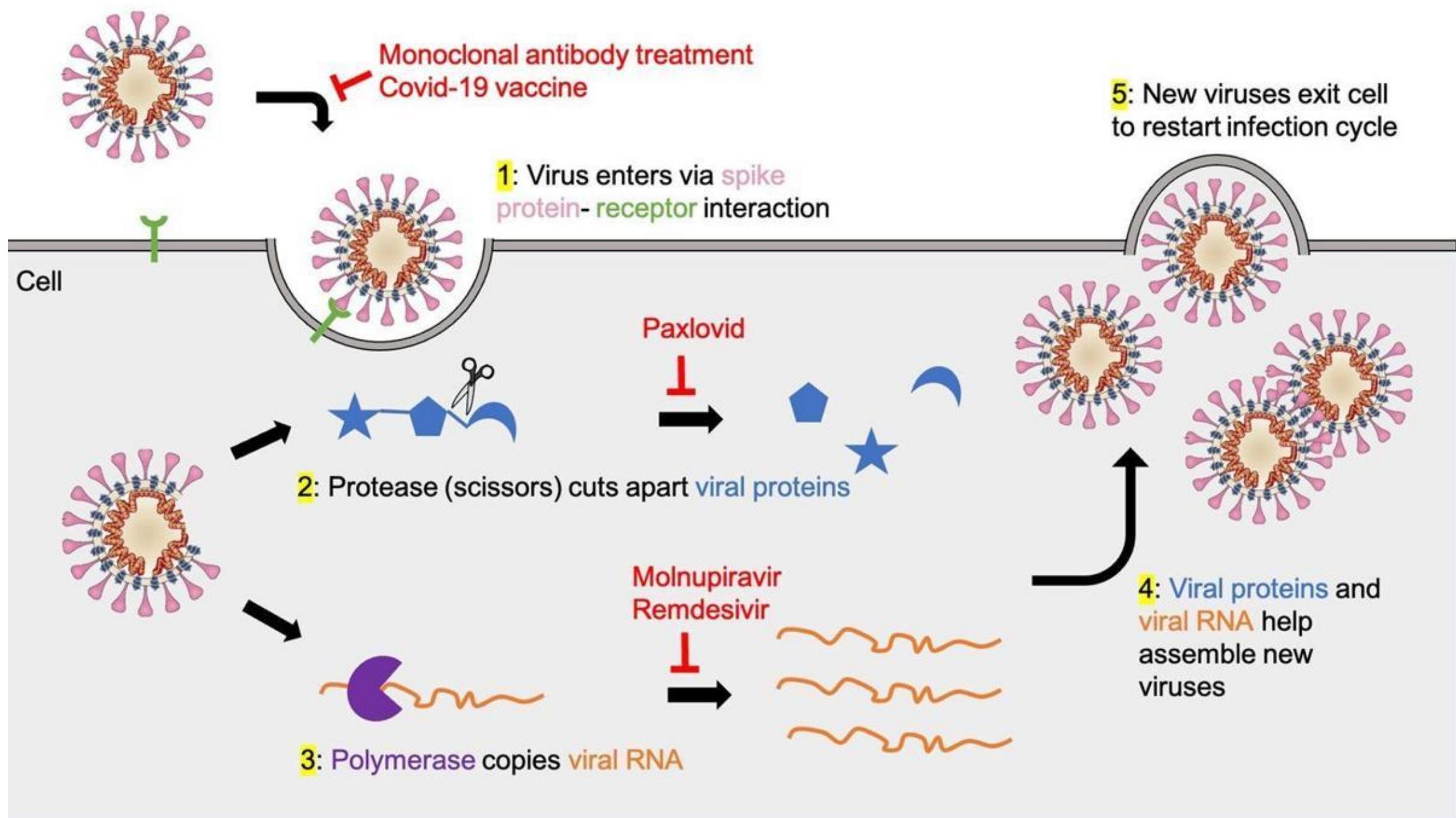
- Another complication is that different viruses vary from each other much more than different bacteria do. Bacteria all have double-stranded DNA genomes and replicate independently by growing larger and then splitting into two, similar to human cells.
- But there is extreme diversity between different viruses. Some have DNA genomes while others have RNA genomes, and some are single-stranded while others are double-stranded. This makes it practically impossible to create a broad spectrum antiviral drug that will work across different virus types.

## ANTIVIRAL SUCCESS STORIES

- Nevertheless, points of difference between humans and viruses do exist, and their exploitation has led to some success. One example is influenza A, which is one form of the flu.
- Once inside our cells, the virus needs to remove its outer coat to release its RNA into the cell.
- A viral protein called matrix-2 protein is key to this process, facilitating a series of events that releases the viral RNA from the virus particle. Once the viral RNA is released inside the host cell, it is transported to the cell nucleus to start viral replication.
- But if a drug jams the matrix-2 protein, the viral RNA can't exit the virus particle to get to the cell nucleus, where it needs to be replicated.
- Zanamivir (Relenza) and oseltamivir (Tamiflu) are drugs that have also had success in treating patients infected with influenza A or B. They work by **blocking a key viral enzyme**, obstructing virus release from the cell, slowing the spread of infection within the body, and minimising the damage the infection causes.

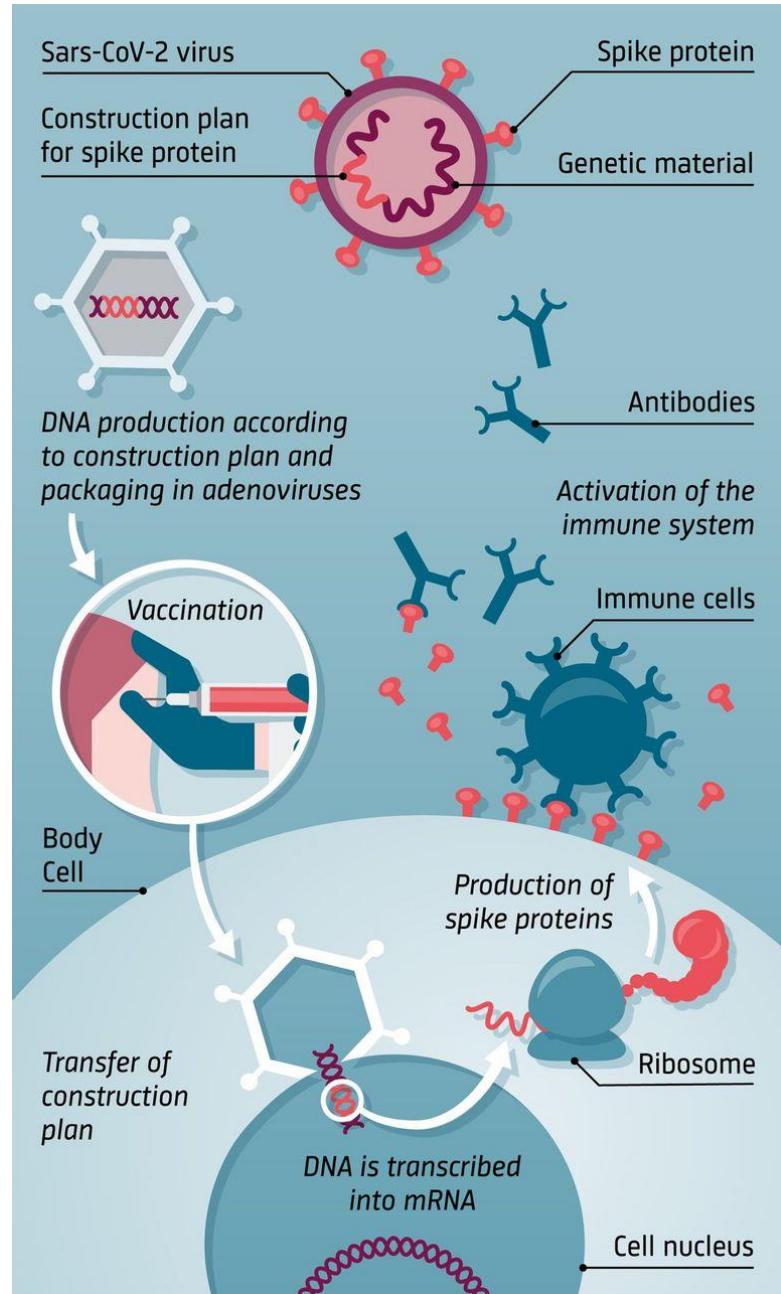
# ANTIVIRALS COVID

- Blocking production of viral machinery: Paxlovid Once a virus gets into your cells, it starts making more copies of itself. While viruses use many of your cellular machines (called proteins) for this process, they also come prepared: the genetic information inside of the virus contains instructions to make some viral proteins needed for replication. In viruses, this genetic information is made up of RNA (we humans have DNA instead).
- Viruses are small: they can't hold much RNA. This long stretch of RNA produces the viral proteins needed for replication, but they're all connected to each other. The viral proteins aren't functional until they are cut apart by a **viral protease**: a machine that acts like **scissors** to selectively cut the connected protein machinery into individual functional units.
- The Paxlovid drug **inhibits this protease** and therefore treats Covid-19 by cutting off viral replication: without protease, the virus cannot activate the protein machinery it needs to replicate. Therefore, Paxlovid is a type of drug called a protease inhibitor. Many other viral diseases, including HIV and hepatitis C, are also treated with protease inhibitors.

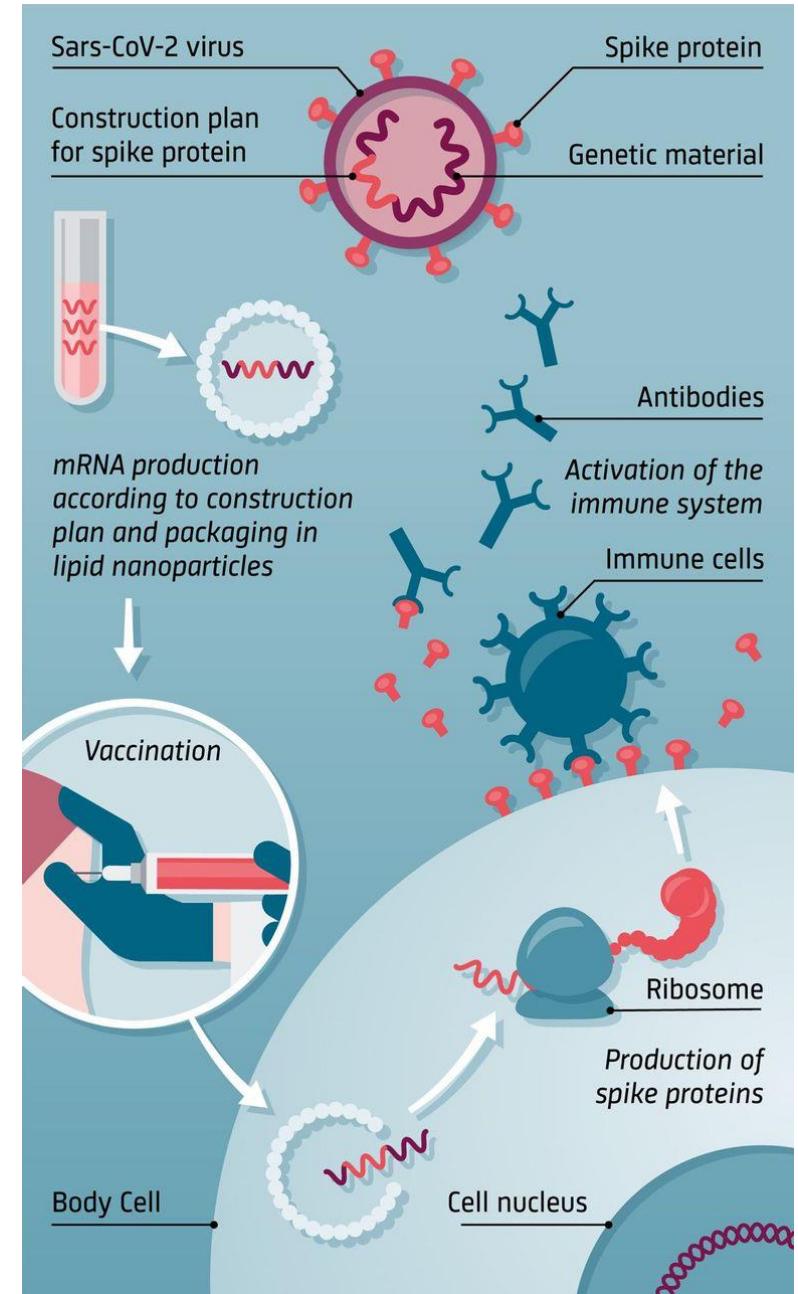


# ANTIVIRALS COVID

- Interfering with viral replication: Molnupiravir and Remdesivir.
- Once viruses inside of your cells cut apart (and therefore activate) the proteins they need, they start making more copies of themselves.
- To do this, viruses use a protein called polymerase, which basically acts as a copy machine to replicate the viral RNA.
- Remdesivir, works by **inhibiting this polymerase** so the SARS-CoV-2 viruses already in the body cannot make more copies of themselves.
- Molnupiravir also interferes with viral RNA replication, but in a different way. When Molnupiravir is metabolized in your cells, it becomes a small molecule that the viral polymerase can accidentally **add into the RNA during replication**. This leads to accumulating errors in the genetic information of the virus, eventually disrupting the function of many viral proteins, and therefore shutting off viral replication.
- Each of the drugs have pros and cons. The oral medications (Molnupiravir, Remdesivir, and Paxlovid) appear to work well against variants but can cause negative interactions when taken with other medications, can be damaging to fetuses and growing children, and cannot prevent Covid-19 infection.



# VACCINES

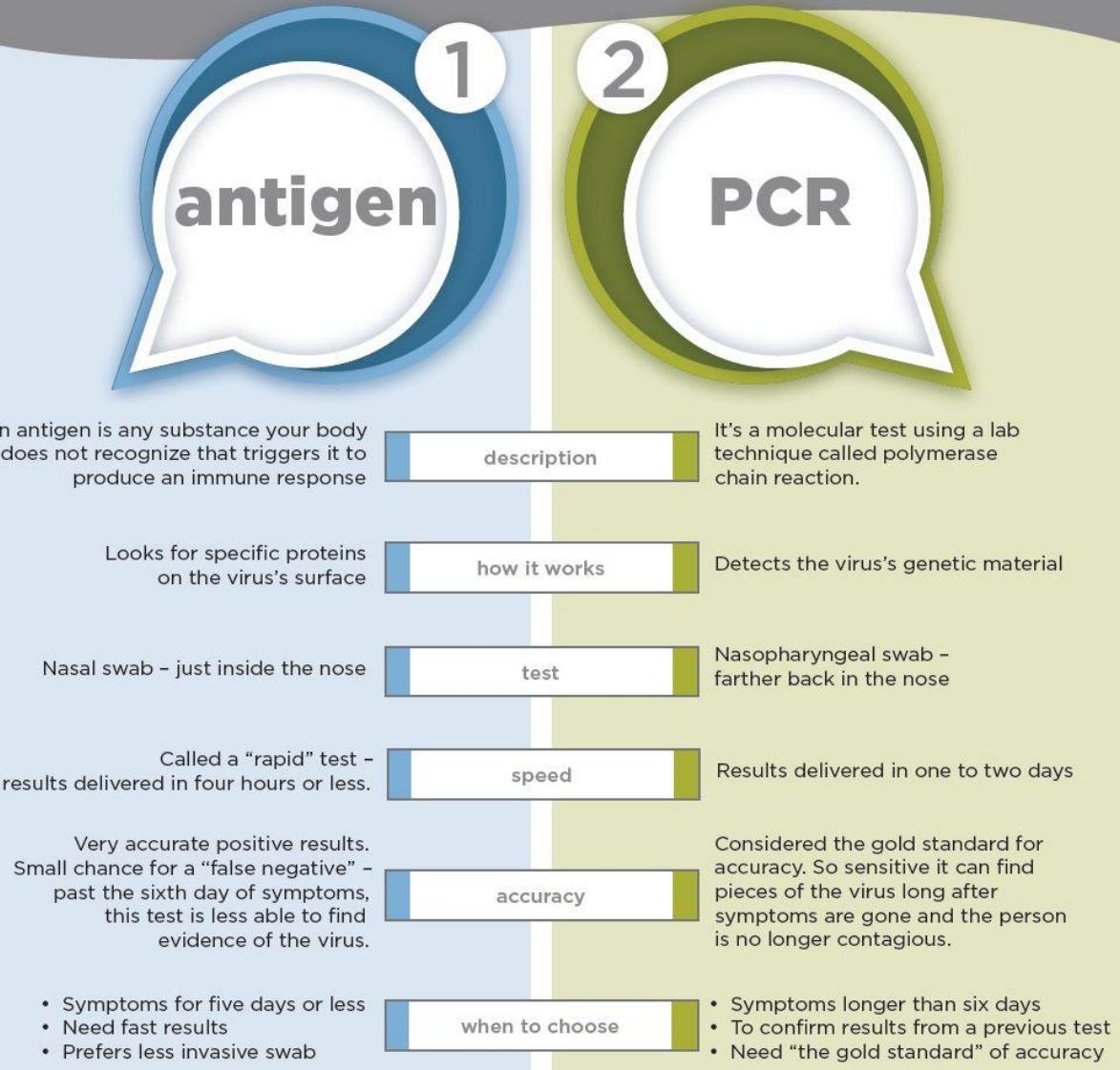


Vector based vaccine left  
m-RNA vaccine right

# CORONA TESTING

## 2 types of COVID-19 tests

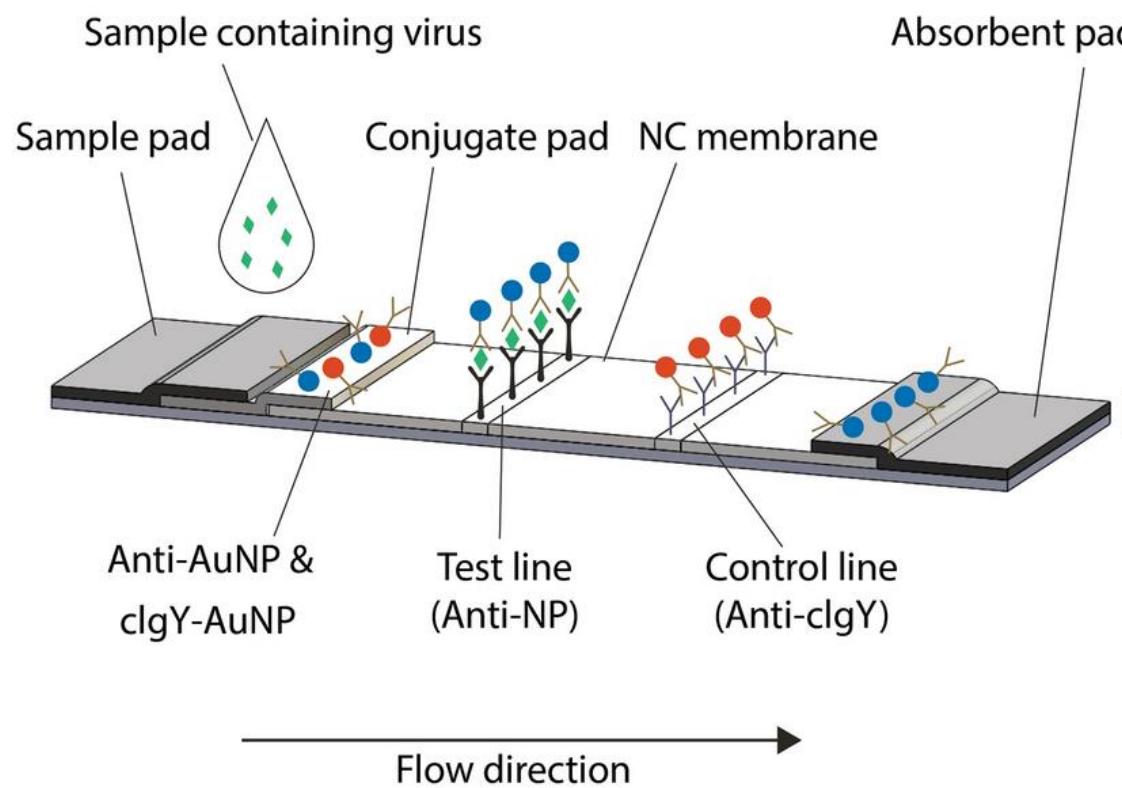
both are looking for the active virus



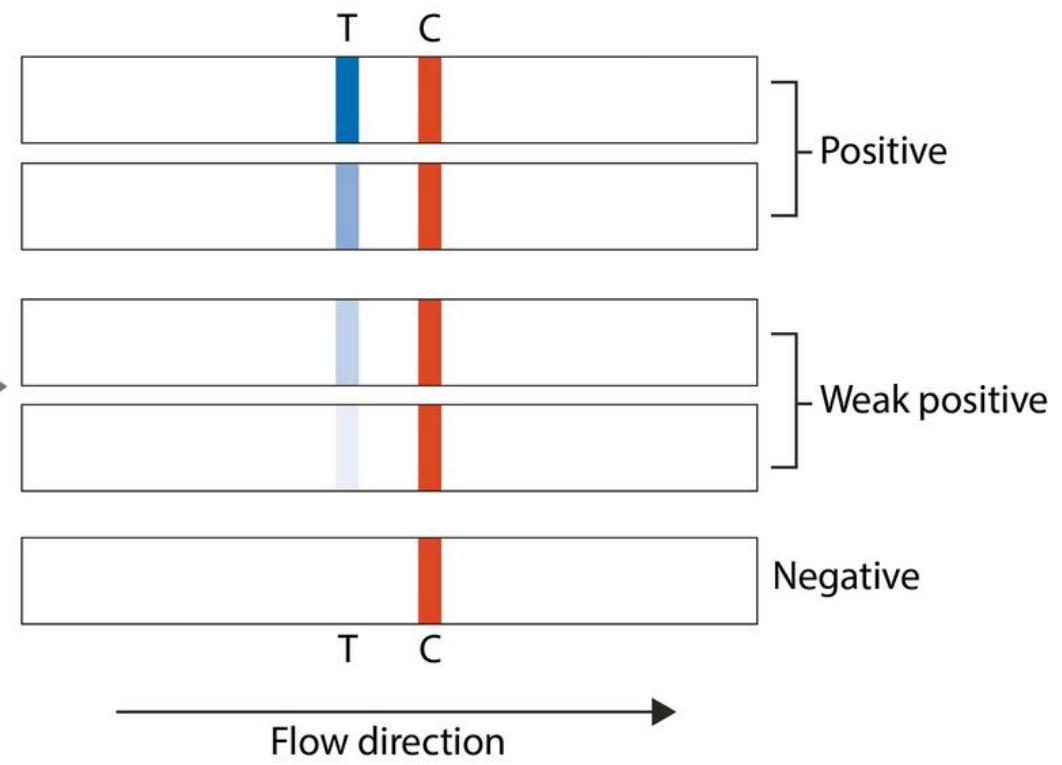
# QUICK GUIDE TO COVID-19 TESTING

	MOLECULAR	ANTIGEN	ANTIBODY
ALSO KNOWN AS	PCR, NAAT	Rapid	Serological
SAMPLE NEED	Swab of nasopharynx, nose, or throat; or saliva	Swab of nasopharynx, nose, or throat; or saliva	Blood
WHAT IT TESTS FOR	Presence of virus's genetic material (RNA)	Presence of one or more proteins that are part of the virus	Antibodies produced in response to an infection
WHY YOU WOULD GET THIS TEST	To accurately diagnose or rule out active coronavirus infection	To rapidly diagnose active coronavirus infection, with results in as soon as two hours	To see if you've had coronavirus infection in the past
ACCURACY OF RESULTS	The "gold standard" test that usually doesn't need to be repeated	Positive results are generally accurate. A molecular test may be recommended to confirm a negative result. If an infection is thought to be very unlikely (e.g., no symptoms and no known exposure), a molecular test may also be recommended to confirm a positive.	An antibody test may be negative in the early phases of infection. Additionally, the sensitivity and specificity of antibody tests vary.

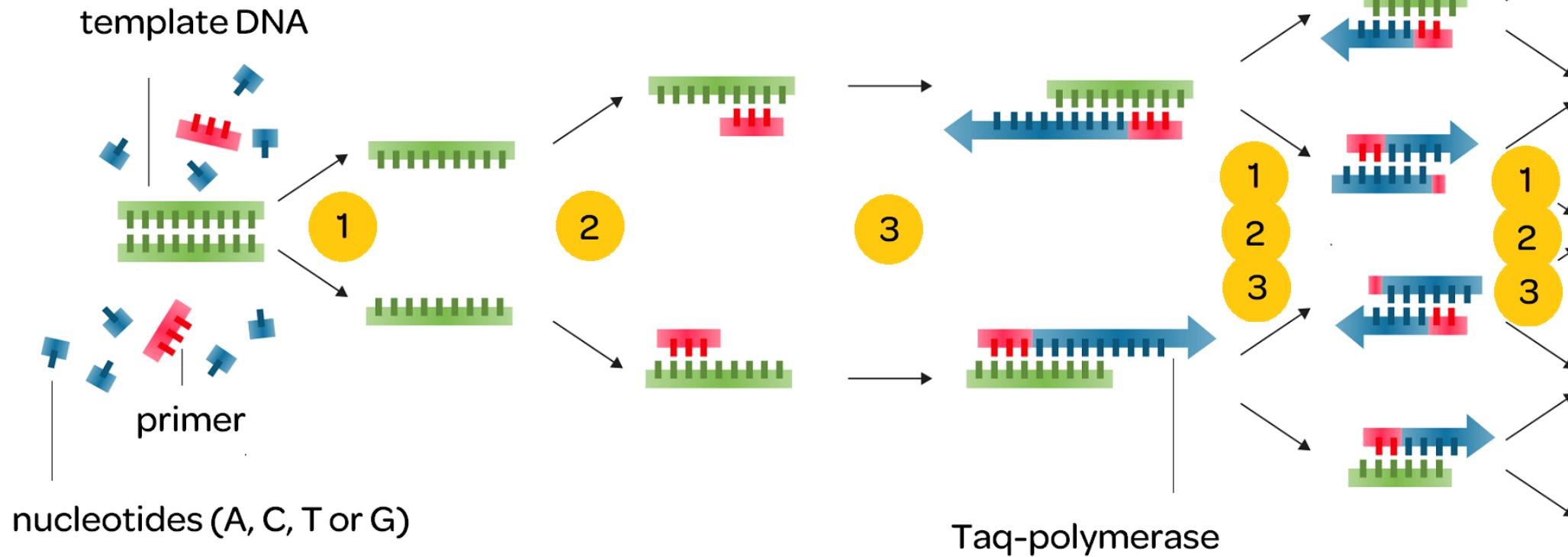
## A Test strip illustration



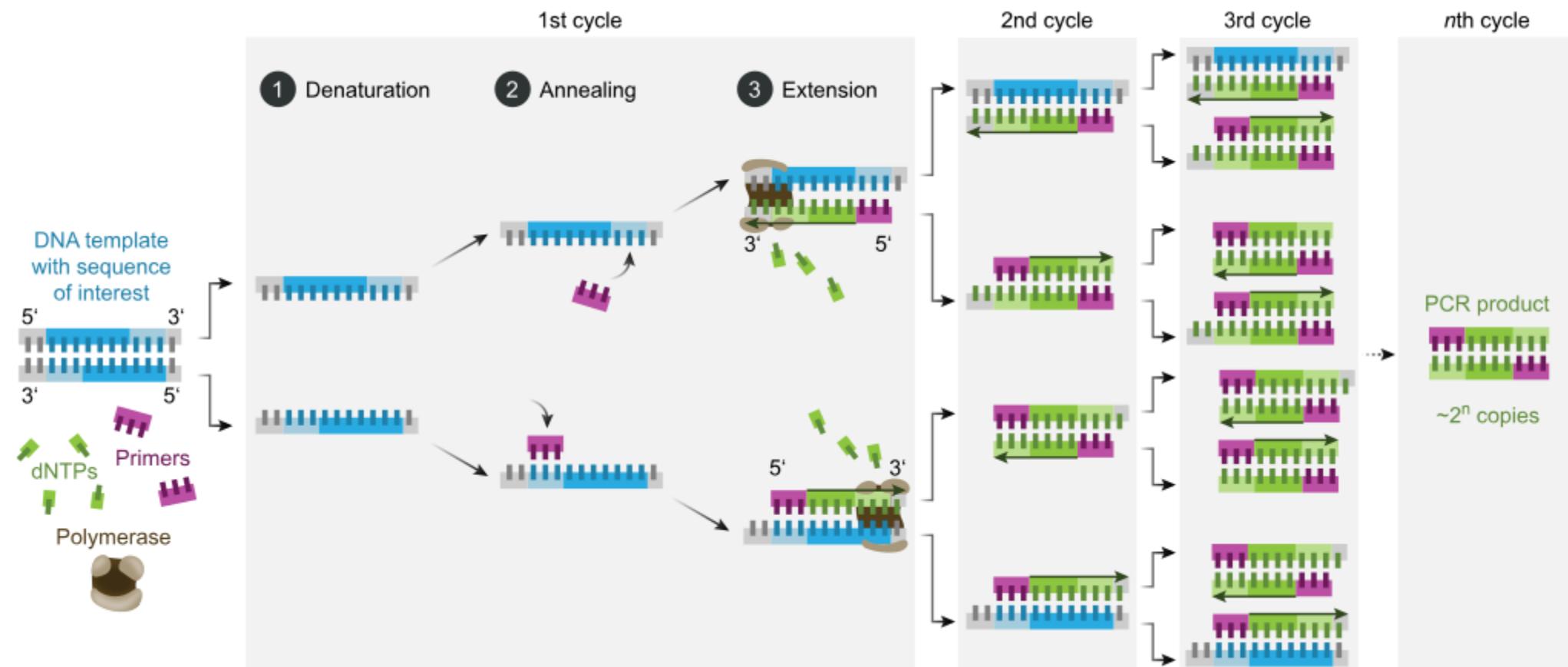
## B Result interpretation



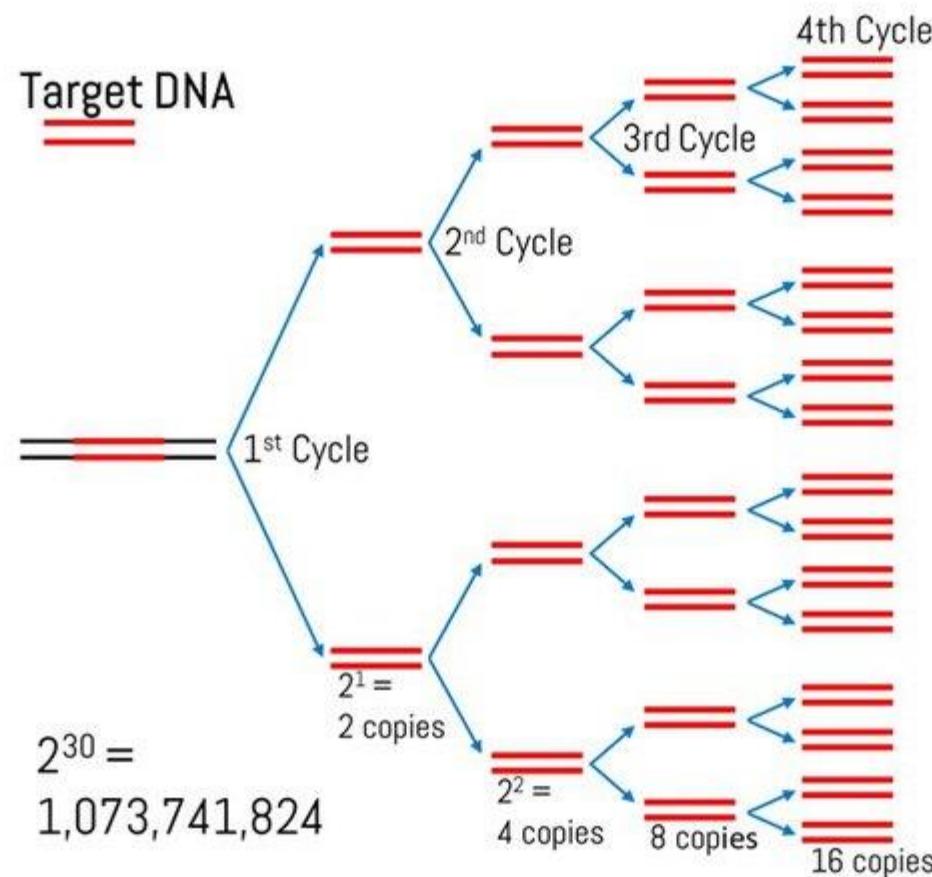
# PCR - Polymerase Chain Reaction



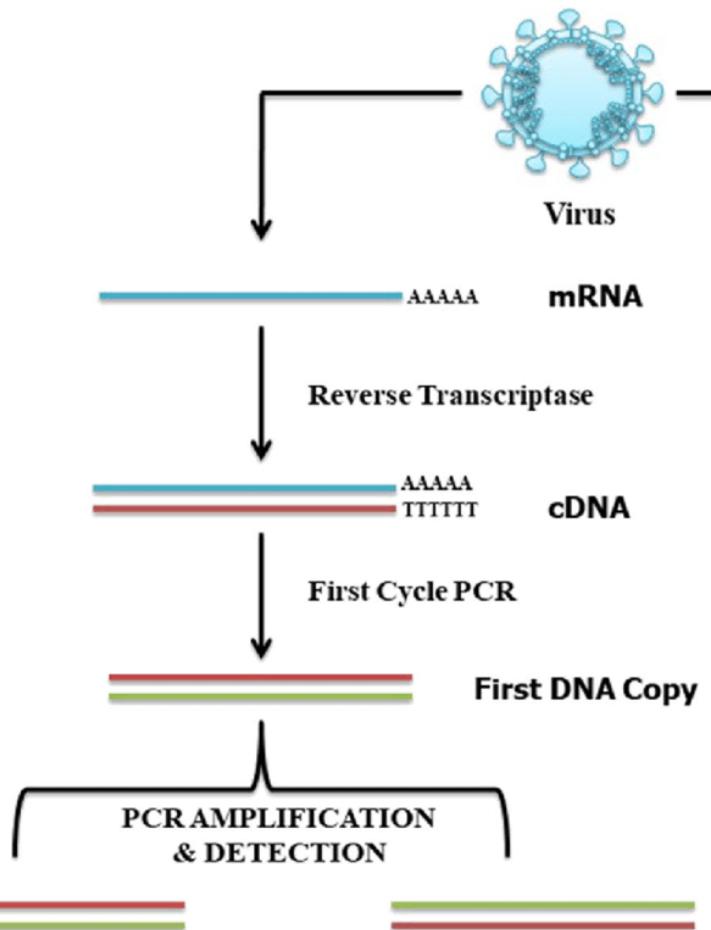
- 1 **Denaturation:** 90 - 95°C. The two strands of the template DNA split
- 2 **Hybridization:** 50 - 60°C. The primer attaches to the template DNA
- 3 **Elongation:** ~72°C. Taq-polymerase places new nucleotides



# Amplify DNA exponentially



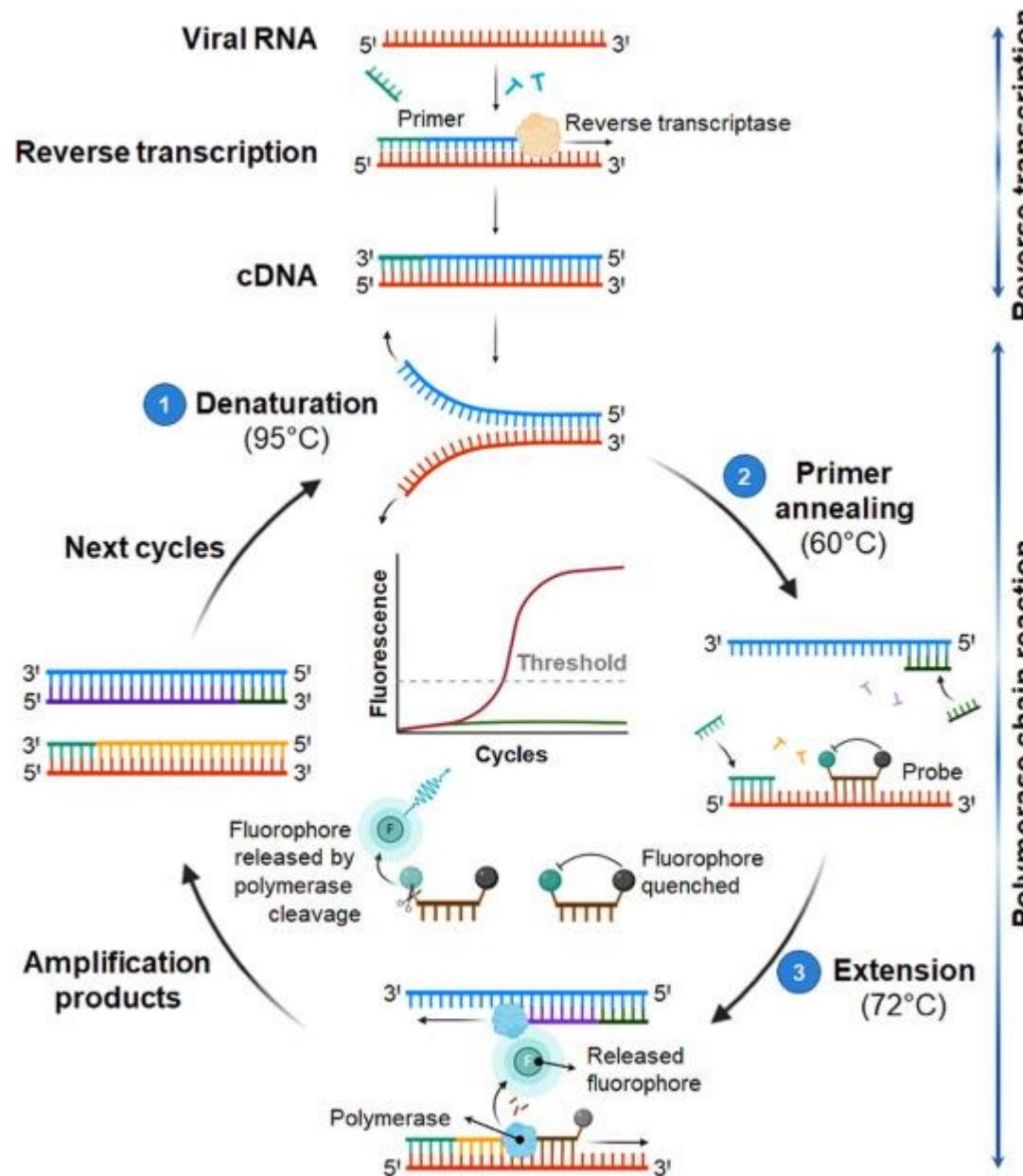
# RT-PCR



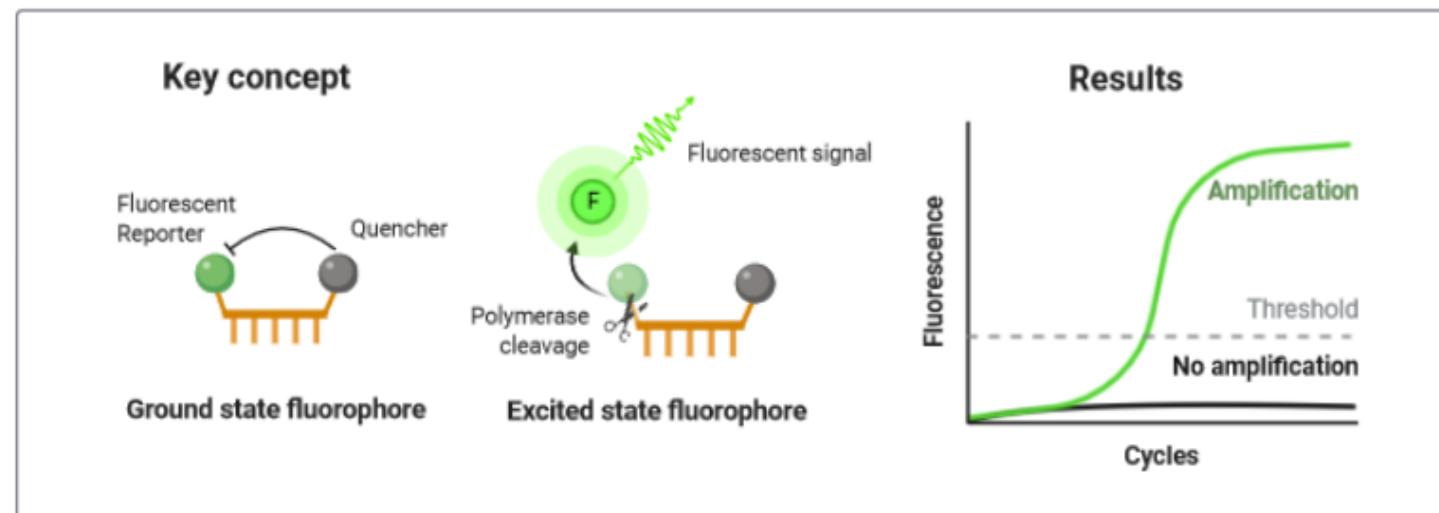
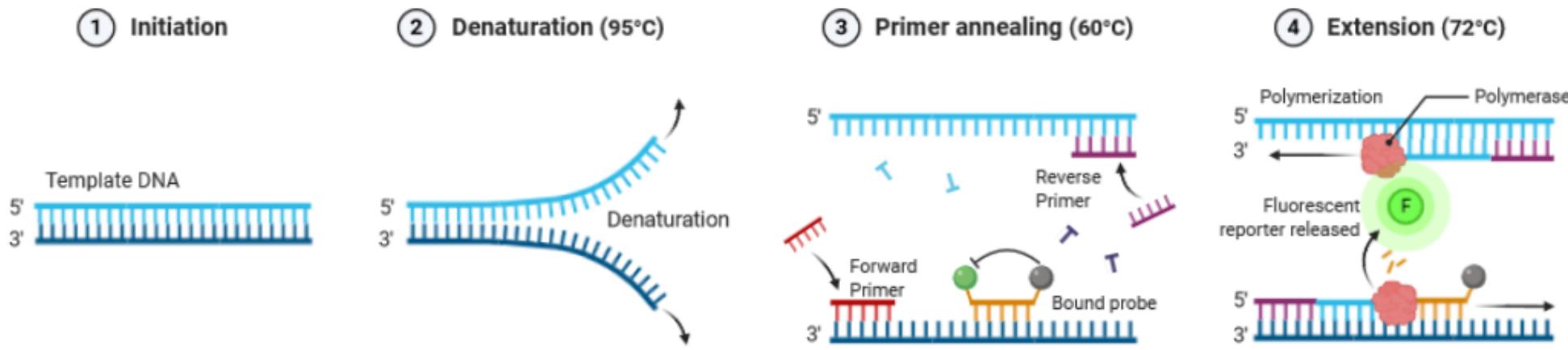
- In reverse transcription, RNA is used as a template to produce DNA. The enzyme reverse transcriptase transcribes RNA to generate a single strand of complementary DNA (cDNA). The enzyme DNA polymerase converts the single-stranded cDNA into a double-stranded molecule as it does in DNA replication. Special viruses known as retroviruses use reverse transcription to replicate their viral genomes. Scientists also use reverse transcriptase processes to detect retroviruses.
- Eukaryotic cells also use reverse transcription to extend the end sections of chromosomes known as telomeres. The enzyme telomerase reverse transcriptase is responsible for this process. The extension of telomeres produces cells that are resistant to apoptosis, or programmed cell death, and become cancerous. The molecular biology technique known as reverse transcription-polymerase chain reaction (RT-PCR) is used to amplify and measure RNA. Since RT-PCR detects gene expression, it can also be used to detect cancer and aid genetic disease diagnosis.

probe and primers anneal to the DNA target.

Polymerisation of a new DNA strand is initiated from the primers, and once the polymerase reaches the probe, it separates the fluorescent reporter from the quencher, resulting in an increase in fluorescence.



# Fluorescent Probe-Based Real Time PCR (qPCR)



The tests use **primers** that are specific to regions of the SARS-CoV-2 genome



Different tests look for different regions, but what matters is that the primers bind a sequence that's only in SARS-CoV-2's genome, not in our DNA, or other viruses or bacteria, etc.

in addition to primers specific to SARS-CoV-2 genes, the tests use fluorescent **probes** that bind inside the copied region



on one end is a **fluorophore** and on the other end is a **quencher**



if you shine the right wavelength light at it, it lets off light of a different wavelength



on its own it's pretty boring...



but get the quencher near the fluorophore & it can prevent light from being given off



The polymerase used (Taq Pol) cleaves the probe when it runs into it, so a fluorescent signal is seen, indicating that a copy was made, telling you that the sequence was present to be able to copy

**fluorophore & quencher close**

FRET (non-radiative energy transfer)



**fluorophore & quencher FAR**

