

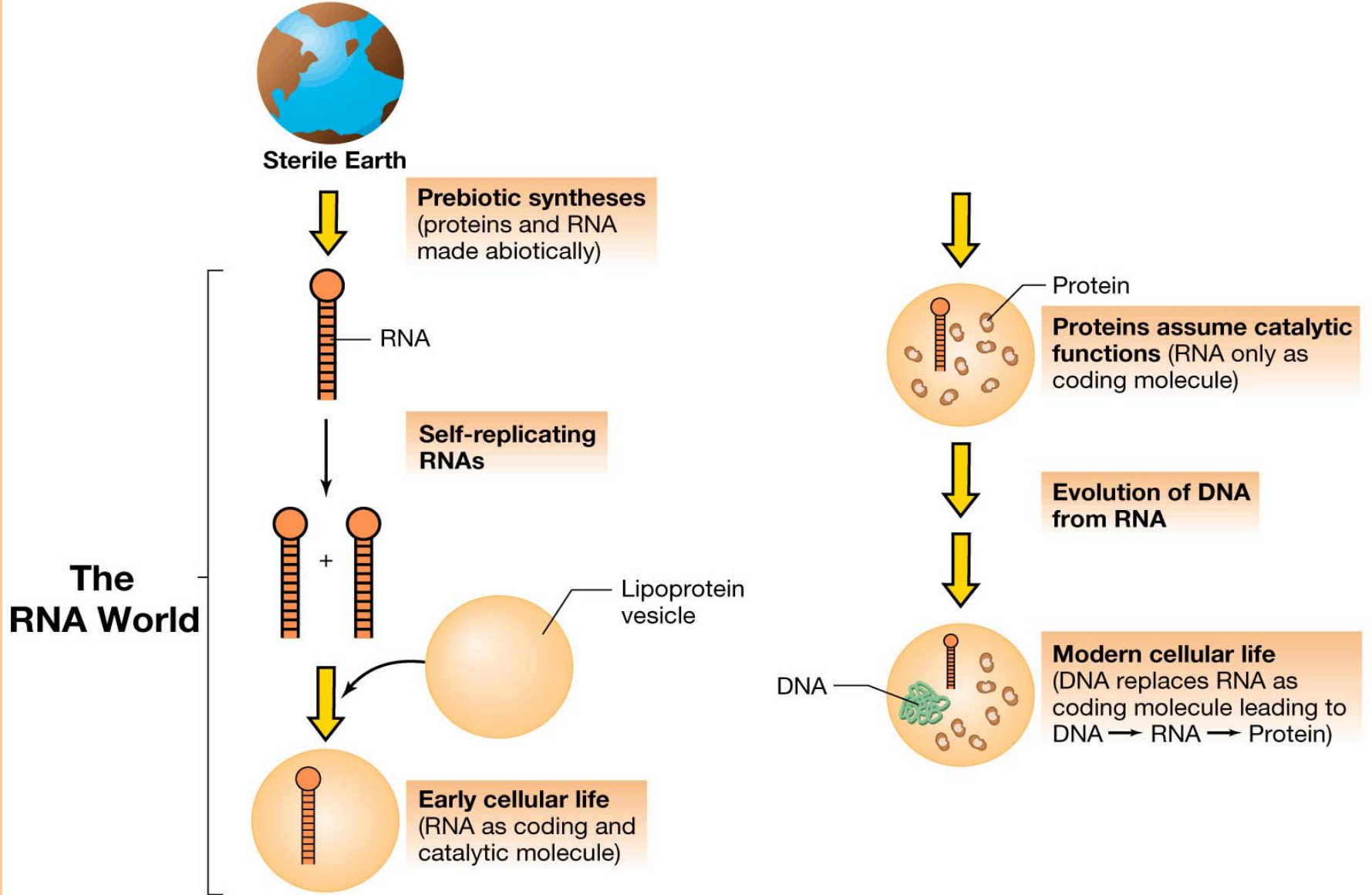
Evolución Microbiana

Dra. Mónica Vásquez

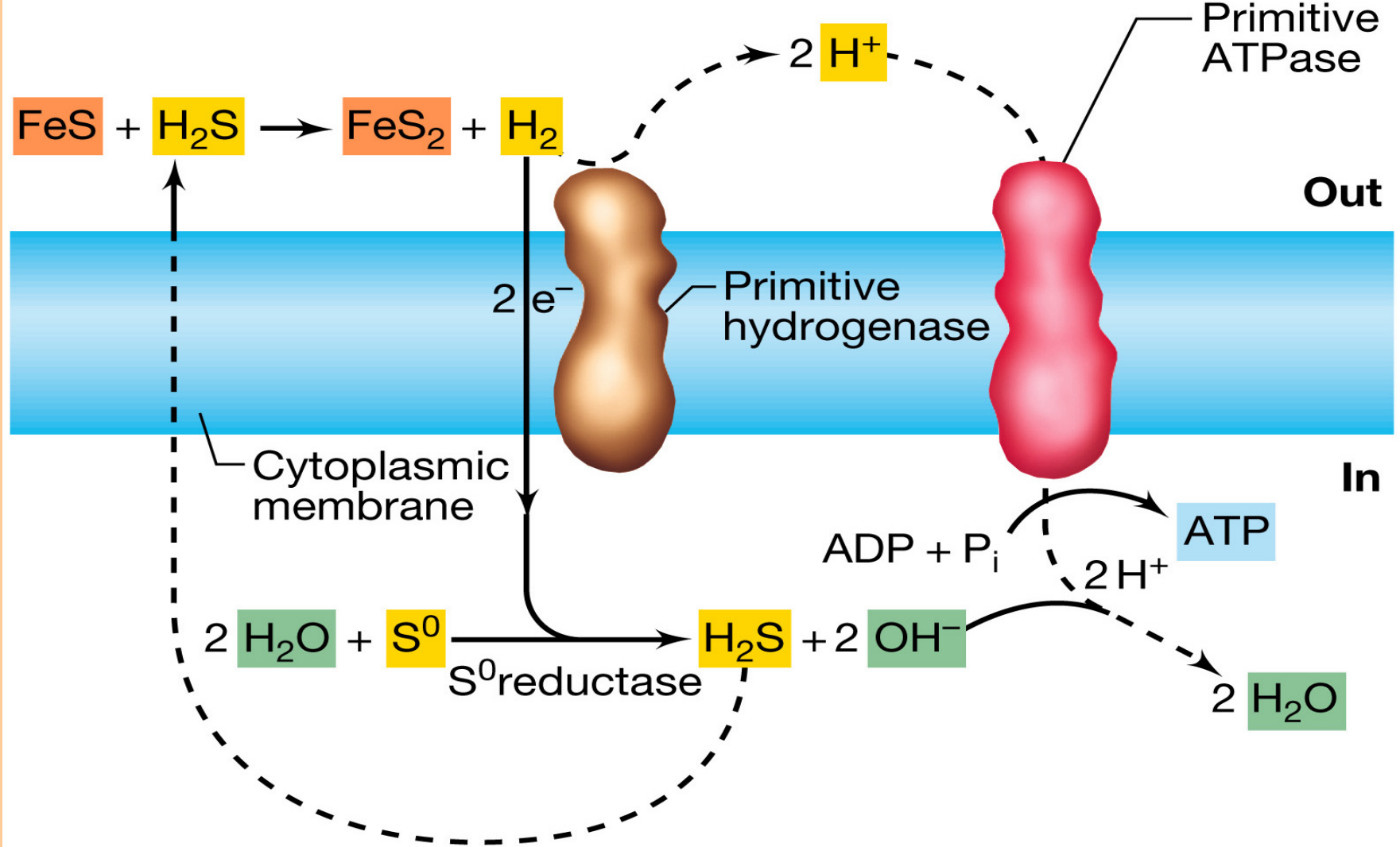
Figures from Brock Biology of Microorganisms



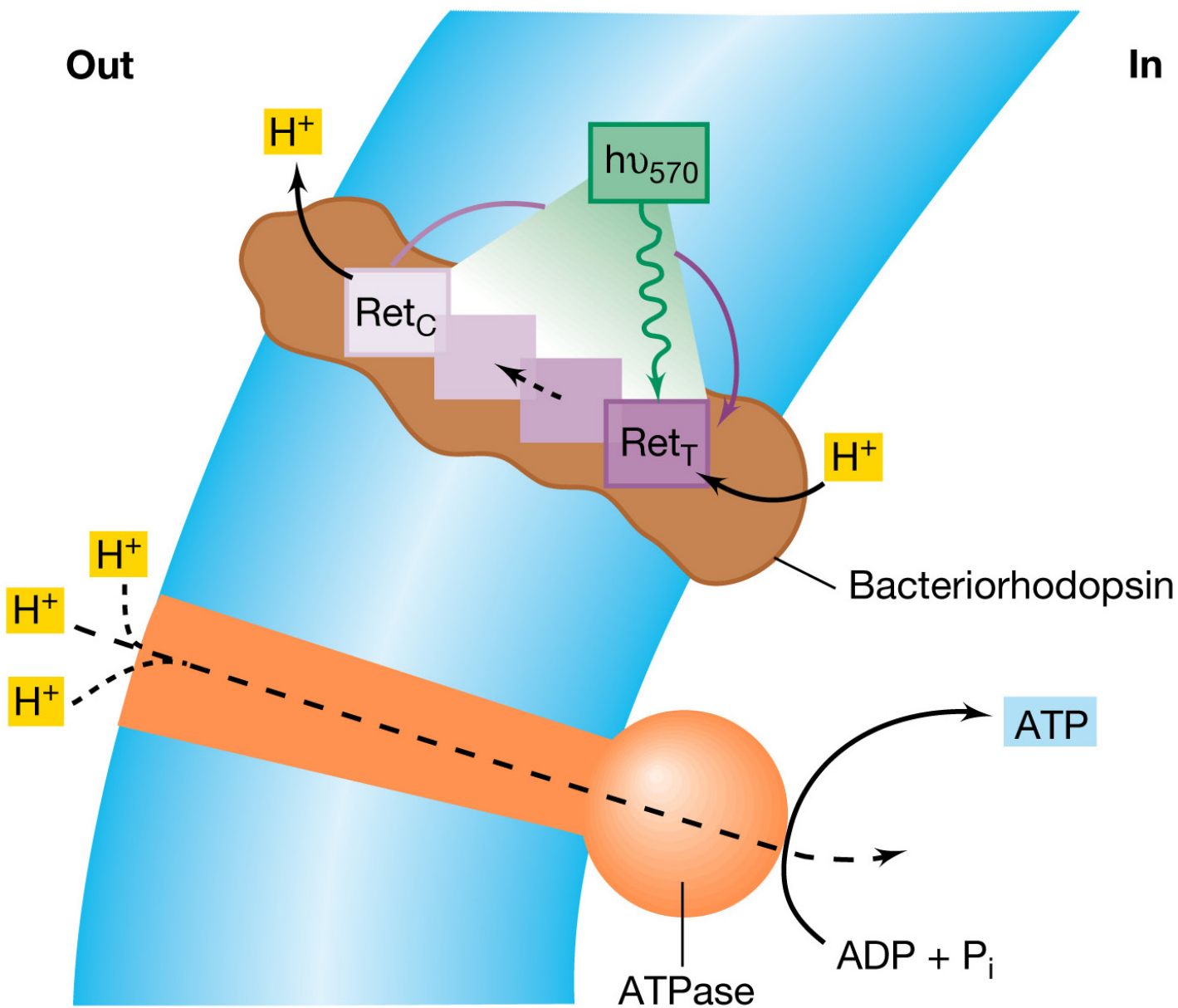
Ancient and modern stromatolites. (f) Modern stromatolites composed of thermophilic cyanobacteria growing in a thermal pool in Yellowstone National Park. Each structure is about 2 cm high.



Possible scenario for the evolution of cellular life forms from RNA life forms. Self-replicating RNAs could have become cellular entities by becoming stably integrated into lipoprotein vesicles. With time, proteins replaced the catalytic functions of RNA and DNA replaced the coding functions of RNA.



A hypothetical energy-generating scheme for primitive cells. Formation of pyrite leads to H_2 production and S^0 reduction, which fuels a primitive ATPase. Note how H_2S plays only a catalytic role; the net substrates would be FeS and S^0 . Also note how few different proteins would be required. The ΔG of the reaction $\text{FeS} + \text{H}_2\text{S} \rightarrow \text{FeS}_2 + \text{H}_2 = -42 \text{ kJ}$.

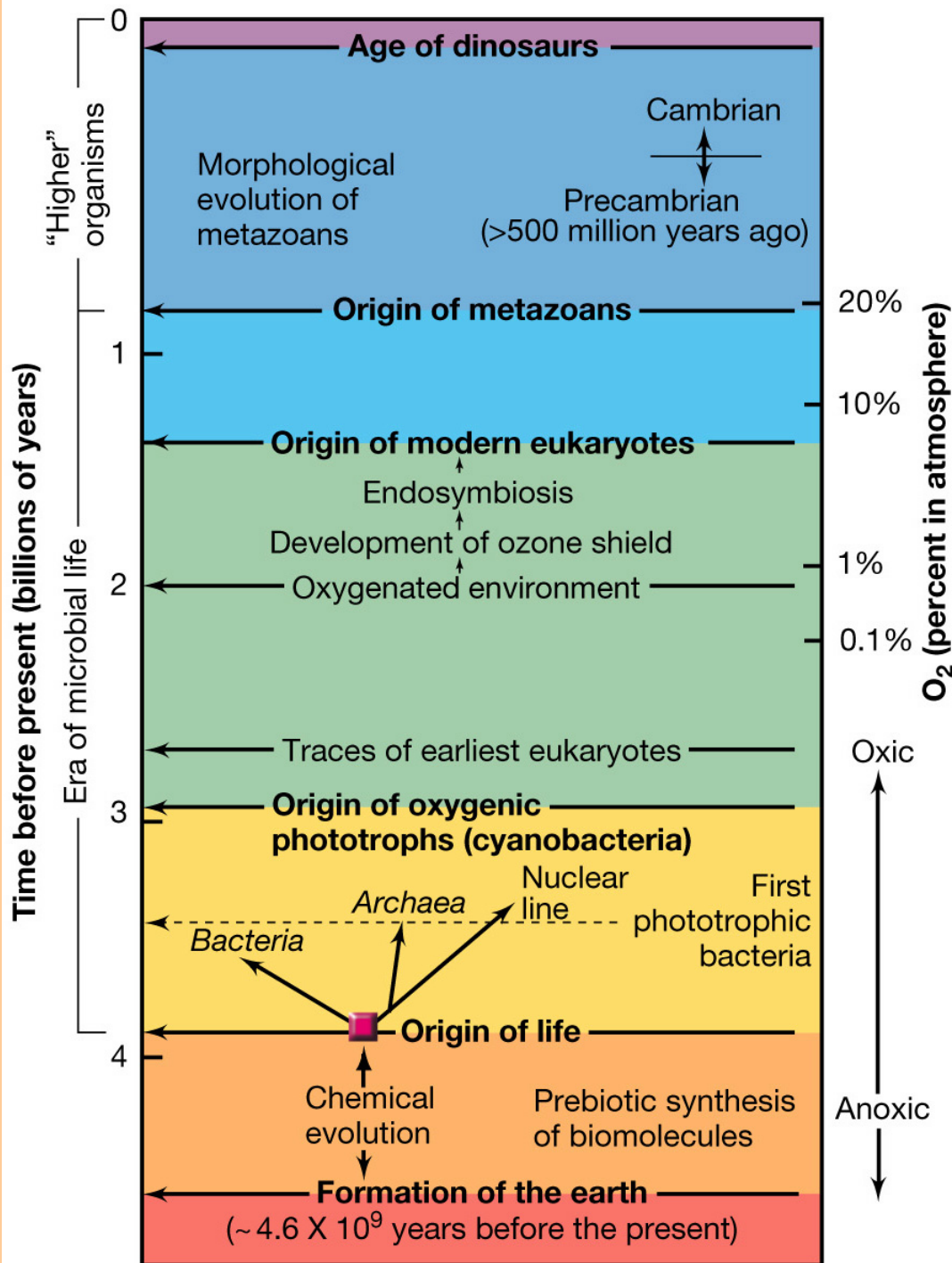


Model for the mechanism of bacteriorhodopsin activity. Light near 570 nm ($h\nu_{570}$) converts the protonated retinal of bacteriorhodopsin from the trans form (Ret_T) to the cis form (Ret₀), along with translocation of a proton to the outer surface of the membrane, thus establishing a proton motive force. ATPase activity (Section 5.12) is driven by the proton motive force.

Microbial Evolution and Systematics

Physical Evolution of Earth

Big Bang leads to generation of our solar system and primitive Earth
as the theory goes, before the big bang, all matter in the universe was concentrated as a point-source of infinite mass 12.5 (+/- 3) billion years ago (Ba), this infinitely massive point-source exploded, very rapidly expanding the matter to ever-enlarging volume, spewing intense radiation and energy (recent evidence indicates that this process may be a recurrent one, with cycles of expansion followed by contraction followed by big bangs) within a few minutes, when the temperature had decreased enough, protons and neutrons formed, then merged to form the nuclei of light elements like hydrogen, helium and lithium these atomic nuclei then captured electrons to form atoms
by 300,000 years, the universe was composed mostly of clouds of hydrogen and helium atoms
at some point, slightly higher densities of hydrogen grew larger and clumpier until they coalesced via collisions to form stars ... and galaxies
these stars gave rise to all the heavy elements via fusion reactions
subsequently, some of these stars disintegrated via novas or supernovas, generating clouds of interstellar matter



Major landmarks in biological evolution. The positions of the stages on the time scale are approximate. Note how the oxygenation of the atmosphere due to cyanobacterial metabolism was a gradual process, occurring over a period of about 2 billion years. Also note that for the bulk of Earth's history, only microbial life forms existed. Although microfossil evidence is lacking, microchemical evidence for eukaryotic cells goes back as far as 2.7 billion years ago.

Patchy condensation of a cloud of interstellar matter from one of these disintegrating stars formed our solar system; most of the matter coalesced near the center to form Sol (our sun) and most of the remaining matter coalesced to form orbiting planetismals, then planetoids, then planets

Planet Earth formed ~4.6 Ba (K40/Ar40 radiodating)

geology

- inner core (0-1227km) - solid center composed of crystalline iron and nickel formed due to immense pressure of collisions during coalescence
- outer core (1227-3486km) - liquid layer composed of molten iron, oxygen and sulfur formed during coalescence, maintained by heat energy released by radioactive decay of heavy elements in inner core
- mantle (3486-6350km) semi-liquid layer composed of oxides of Si, Al, Mg surrounds the outer core
- crust (6350km-surface) - solid layer composed of lighter elements (mainly silicates) plus some lighter mantle layer elements "floats" on the mantle layer formed as Earth cooled by radiating heat into space
 - oceanic crust formed from relatively smooth basalt
 - continental crust formed from irregular granite protocontinents - as cooling continued, the crust contracted and cracked, releasing molten mantle layer rock and gasses by volcanic activity, increasing crust thickness, and generating a primitive atmosphere

primitive atmosphere (alternative ideas)

- reducing - if Earth coalesced slowly, generating little heat, iron exposed on the surface and would capture all molecular oxygen, so the primitive atmosphere would have consisted mostly of water, hydrogen, nitrogen, methane, and ammonia, with little carbon dioxide or monoxide
- mildly oxidizing - if Earth coalesced rapidly, generating much heat, most iron would have melted and flowed to the core, allowing oxygen to combine with carbon, and the primitive atmosphere would have consisted mostly of water, carbon monoxide and carbon dioxide, with some nitrogen, sulfides, methane and ammonia, but very little oxygen

liquid water appeared on Earth's surface ~4 Ba as indicated by discovery of ~3.8 billion year-old sedimentary rocks (which require liquid water for their formation) in Greenland

upon its release from volcanoes, water vapor expanded and cooled, then condensed and fell back towards the surface as rain

because the crust was still very hot, rain evaporated before it reached the surface, resulting in continuing cycles of water expansion, cooling, condensation and rainfall, thus initiating the water cycle

this water cycle slowly cooled the crust to temperatures at which liquid water could form, leading to sequential water accumulation to form droplets, puddles, pools, ponds, lakes, rivers and oceans

because the rain was rather acidic (carbon dioxide, sulfur dioxide, etc. form acid when they dissolve in water) the flowing water (on its way to the lower elevation basaltic surface) dissolved some of the granitic material, generating salt water (the ocean is ~3% salt)

Origin of Life on Earth

Primitive organisms capable of metabolism (ability to accumulate and modify nutrients and energy) and reproduction (ability to generate more organisms like themselves) first appeared ~3.6 Ba, were most likely thermophilic anaerobes, and may have depended on RNA for both enzymatic and genetic activities

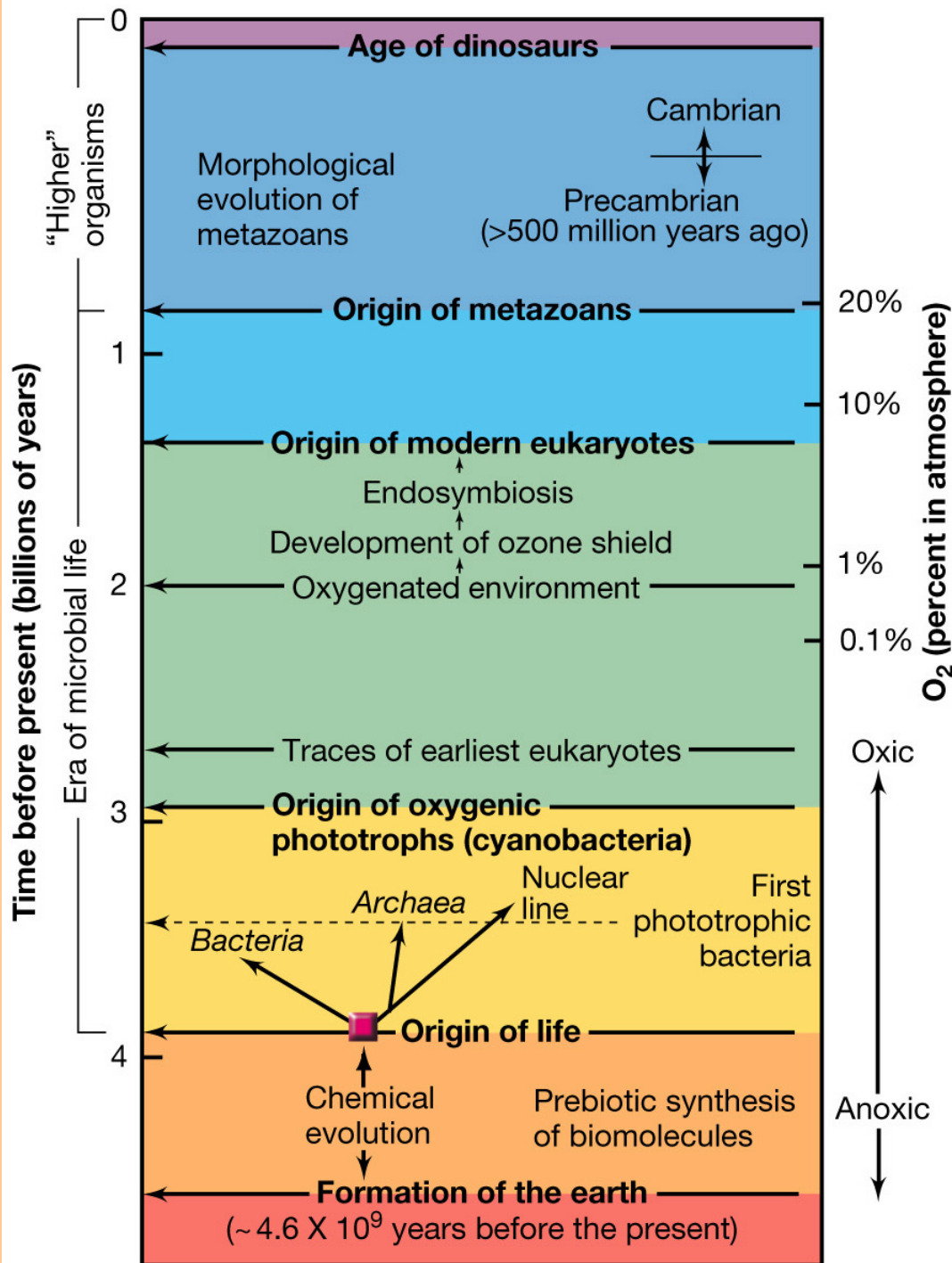
Primordial soup (solution) theories

chemical reactions formed fatty acids, sugars, amino acids, purines, pyrimidines, nucleotides, and polymers of all these ~4.1 Ba when primitive (reducing) atmospheric gases were exposed to lightning and intense UV light while dissolved in the salty acidic water

accumulation of these compounds over time, leading to oceans (puddles?) with very rich organic molecule content (primordial soup)

spontaneous aggregation of lipids and proteins ~3.9 Ba formed primitive membranes, inside of which were accidentally incorporated just the "right" combination of organic (and inorganic) chemical components

Primordial soup theories predict that the original organisms were **thermophilic anaerobic fermenters** (obtained both energy and carbon from organic compounds)



Major landmarks in biological evolution. The positions of the stages on the time scale are approximate. Note how the oxygenation of the atmosphere due to cyanobacterial metabolism was a gradual process, occurring over a period of about 2 billion years. Also note that for the bulk of Earth's history, only microbial life forms existed. Although microfossil evidence is lacking, microchemical evidence for eukaryotic cells goes back as far as 2.7 billion years ago.

Surface theories

pyrite theory - life started as a result of a metabolic process that could occur on iron pyrite surfaces

iron pyrite provides positive charges for bonding of phosphates, etc., thus fostering polymerization reactions

polymerization of lipids formed semi-permeable membranes across which proton gradients could be generated and maintained, thus providing energy for synthetic reactions involving organic compounds generated either inside or outside of these membranes

clay theory - life started on clay surfaces, where polymerization reactions could readily occur (similar to pyrite theory, but lacks some of the metabolic possibilities)

Surface theories predict that the original organisms were **thermophilic anaerobic lithotrophs** (obtained both energy and carbon from inorganic compounds)

Early Evolution of Organisms with Increasing Complexity

Fossils prevalent in rocks formed ~3.5 Ba

individual rod-shaped bacteria

stromatolites - microbial mats comprised of layers of filamentous prokaryotes containing trapped sediment

current-day very simple prokaryotes

Mutations caused by high levels of UV irradiation (and other mutagens) occurred continually, and selection among them allowed adaptations that led to development of more complex microorganisms with cell walls, greater biosynthetic capabilities, more extensive membranes, cytochromes, and chlorophylls, thus giving rise to phototrophs, which derive energy from sunlight and carbon from inorganic compounds

Anoxygenic photosynthesizers

evolved ~0.2 billion years after the first organisms

use photosystem I exclusively - purple or green photosynthetic bacteria probably formed the original stromatolites, since they are anaerobic photosynthesizers and conditions on Earth were still anoxic

Oxygenic photosynthesizers

evolved ~1.2 billion years after the first organisms

use a combination of photosystem I and photosystem II

Atmosphere Evolved as Microbes Produced Oxygen ~2.5 Ba

Oxygen content slowly increased

0.1% oxygen in atmosphere after 0.1 billion years (~2.4 Ba)

1% oxygen in atmosphere after 0.5 billion years (~2 Ba))

10% oxygen in atmosphere after 1 billion years (~1.5 Ba))

21% oxygen in atmosphere after 1.6 billion years (~0.9 Ba))

Ozone layer formed as UV light energy was absorbed by oxygen

lower UV flux on surface - absorption of UV by ozone

lower mutation rate - due to decreasing UV flux

Atmospheric Oxygen Availability Led to Increasing Diversity

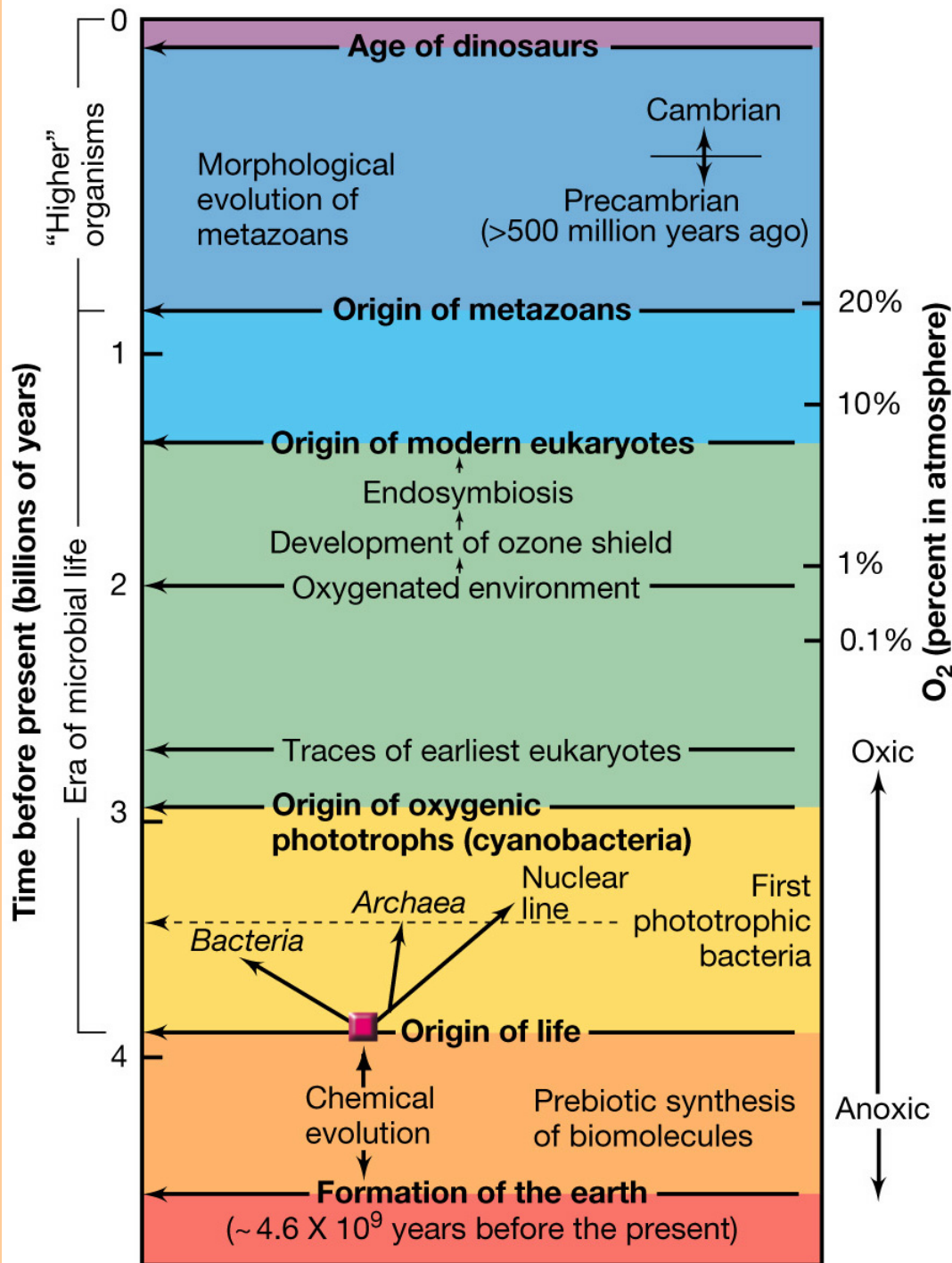
Organisms with oxygen as final electron acceptor evolved as the atmosphere changed from reducing to oxidizing - more energy available with aerobic respiration than with anaerobic metabolism

Microbial diversity increased ~0.5 billion years after oxygen generation began

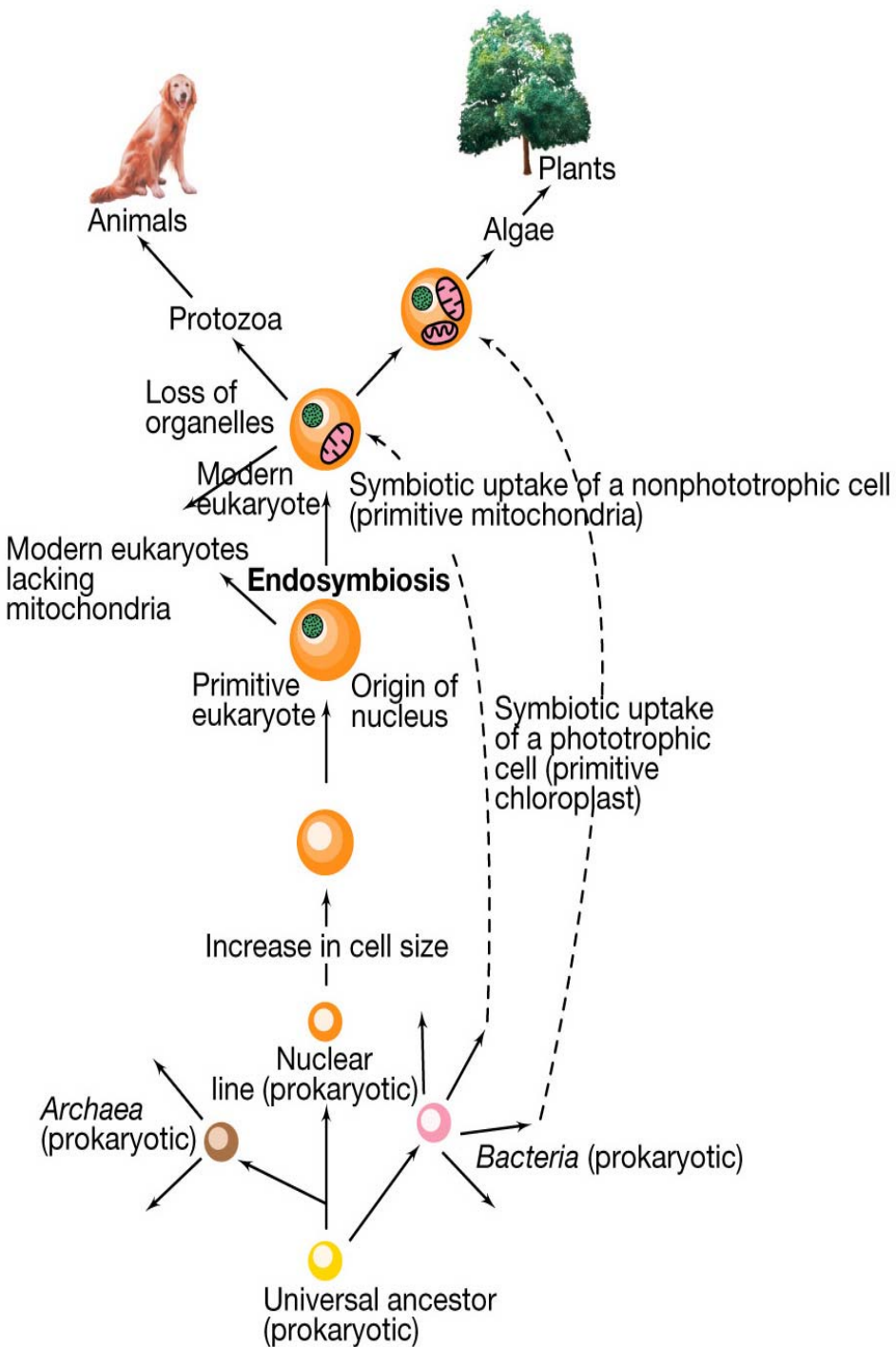
"Modern" eukaryotes evolved ~1.3 Ba (1.2 billion years after oxygen generation began)

eukaryotic diversity increased ~0.1 billion years later (1.2 Ba)

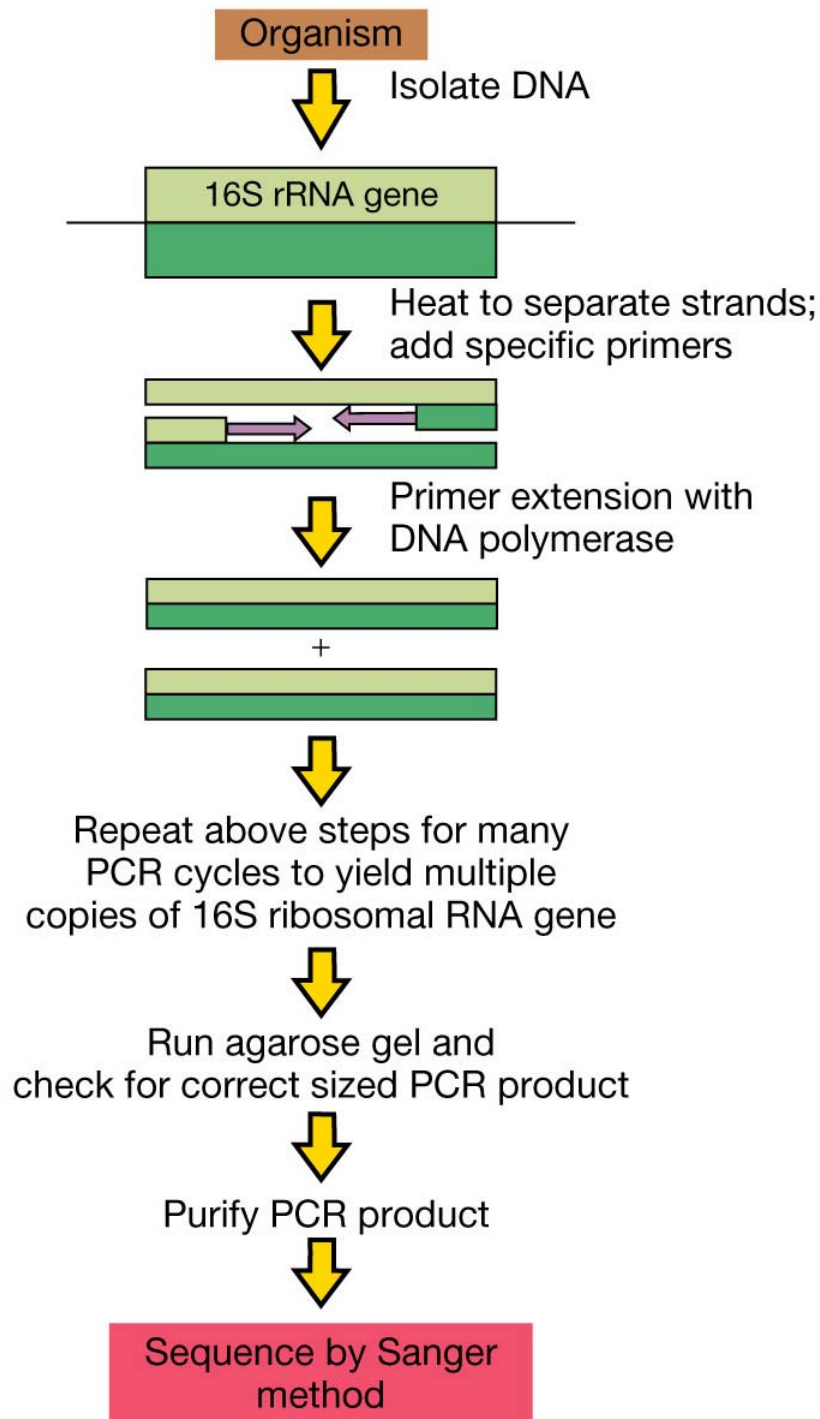
metazoans appeared ~0.3 billion years later (0.9 Ba, when atmospheric oxygen reached ~21%)



Major landmarks in biological evolution. The positions of the stages on the time scale are approximate. Note how the oxygenation of the atmosphere due to cyanobacterial metabolism was a gradual process, occurring over a period of about 2 billion years. Also note that for the bulk of Earth's history, only microbial life forms existed. Although microfossil evidence is lacking, microchemical evidence for eukaryotic cells goes back as far as 2.7 billion years ago.



Origin of modern eukaryotes by endosymbiotic events. Note how organelles originated from Bacteria rather than Archaea. Endosymbiosis was unlikely to have been a one-time event and probably occurred in various types of cells of the nuclear line of descent. Note, however, how some primitive eukaryotes either never underwent endosymbiotic events or permanently lost their symbionts, but otherwise maintained the basic properties of eukaryotic cells. Extant examples of such eukaryotes, all microbial, are known today (Chapter 14). The increase in cell size in the nuclear line of descent allowed for larger genomes to evolve, but also likely led to the evolution of the nucleus to affect the orderly replication and



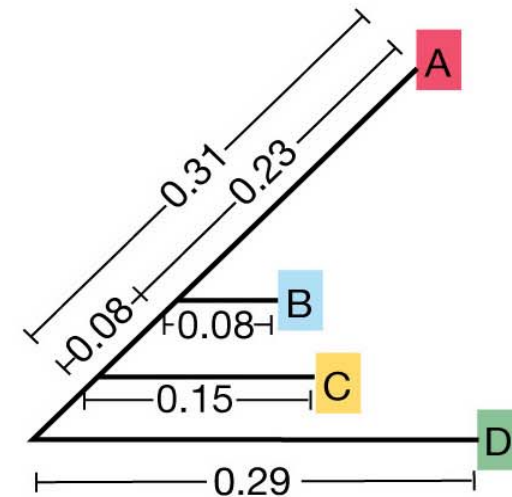
Ribosomal RNA sequencing of a pure culture of a microorganism using the polymerase chain reaction (PCR). The 16S rRNA gene is amplified and then sequenced by the Sanger method (Section 10.13). The primers added are complementary to conserved sequences in one of the domains of 16S rRNA (see Figure 11.8c). A cloning step may also be used in this procedure to clone the DNA encoding the 16S rRNA following its PCR amplification.

Organism	Sequence	Analysis
A	C G U A G A C C U G A C	For A \rightarrow B, three differences occur out of a total of twelve; thus $\frac{3}{12} = 0.25$
B	C C U A G A G C U G G C	
C	C C A A G A C G U G G C	
D	G C U A G A U G U G C C	

(a) Sequence alignment and analysis

Evolutionary distance	Corrected evolutionary distance
E_D A \rightarrow B 0.25	0.30
E_D A \rightarrow C 0.33	0.44
E_D A \rightarrow D 0.42	0.61
E_D B \rightarrow C 0.25	0.30
E_D B \rightarrow D 0.33	0.44
E_D C \rightarrow D 0.33	0.44

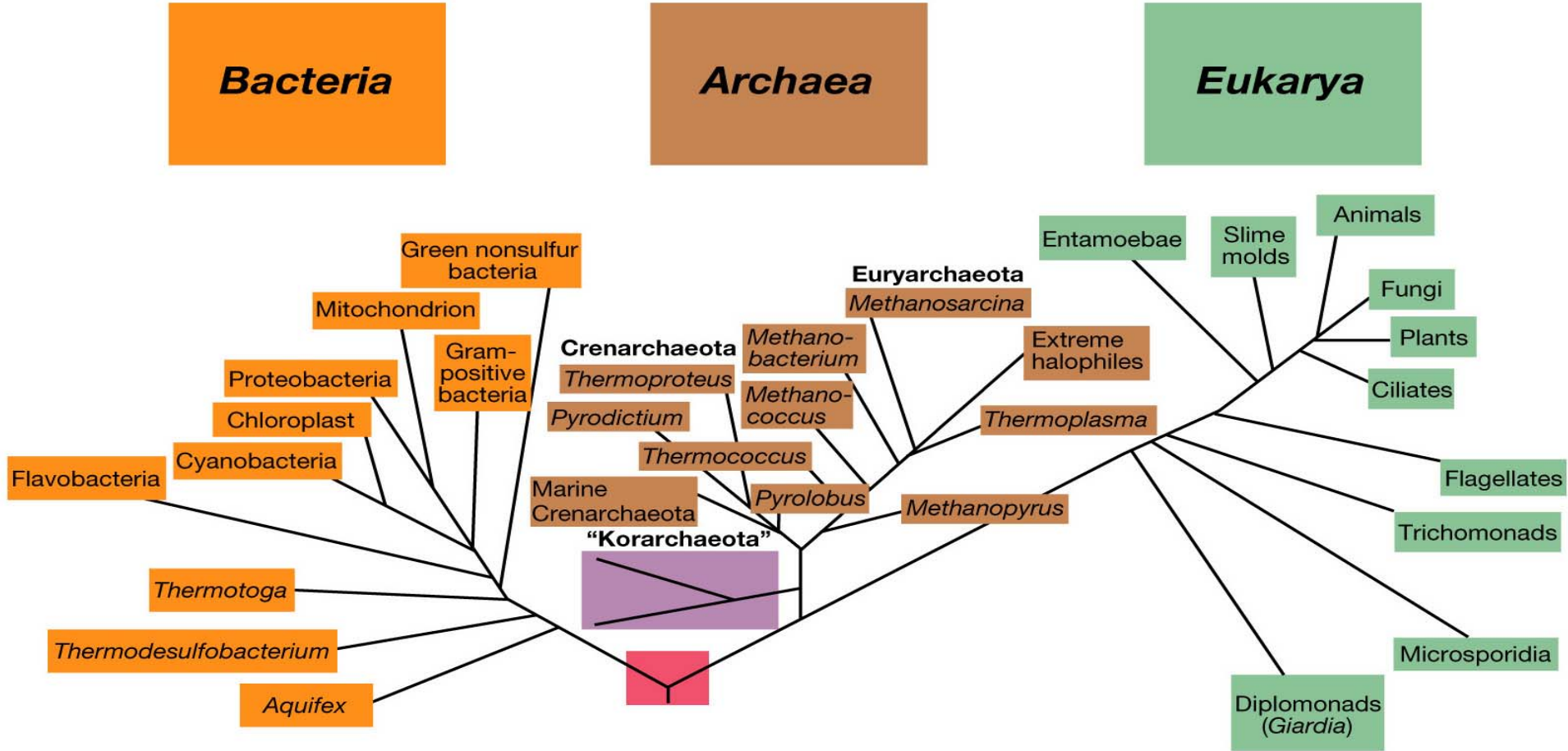
(b) Calculation of evolutionary distance



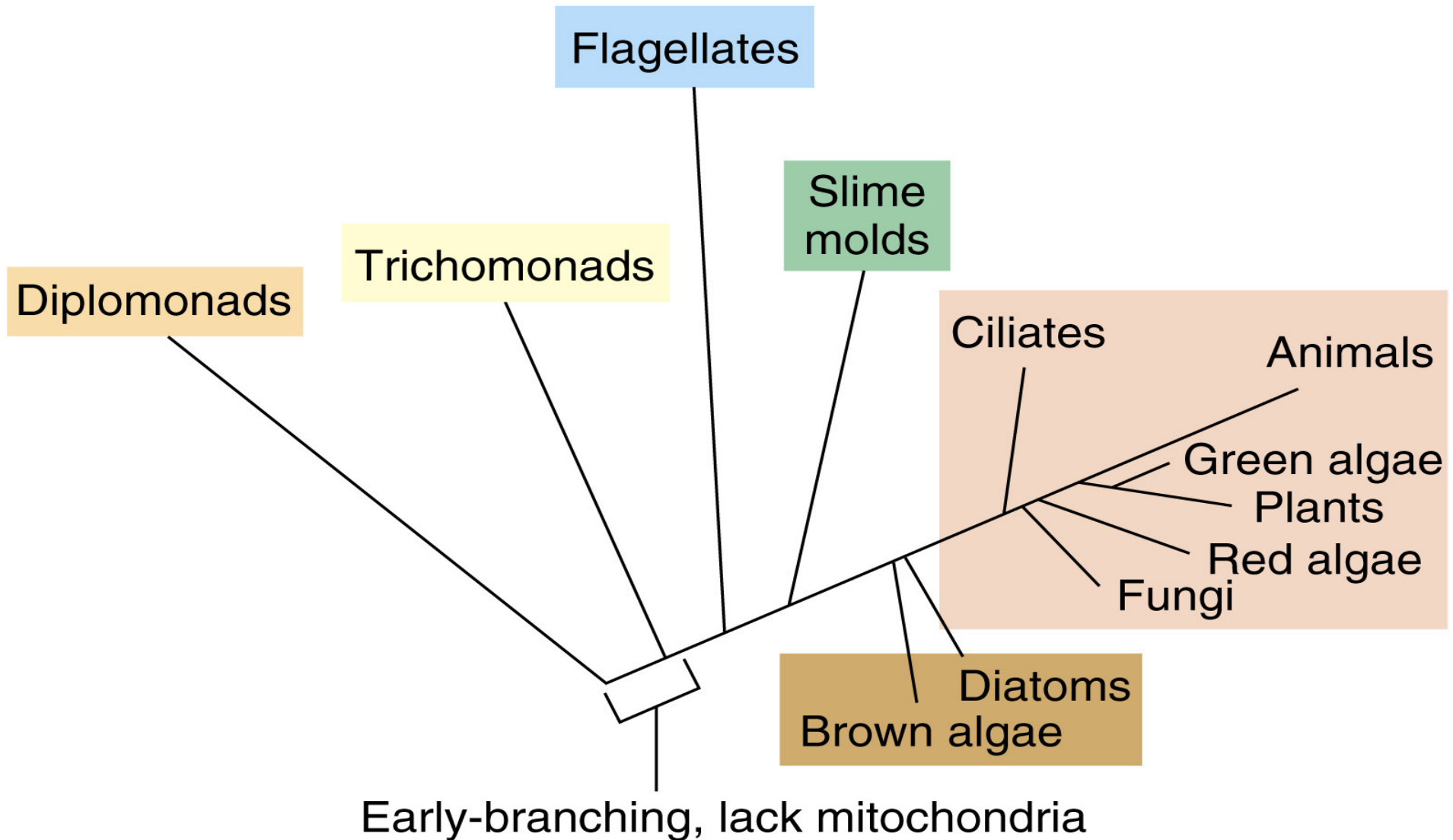
(c) Phylogenetic tree

Preparing a phylogenetic distance tree from 16S ribosomal RNA sequences. For illustrative purposes, only short sequences are shown. The evolutionary distance (ED) in (b) is calculated as the percentage of nonidentical sequences between the RNAs of any two organisms. The corrected ED is a statistical correction necessary to account for either back mutations to the original genotype or additional forward mutations at the same site that could have occurred.

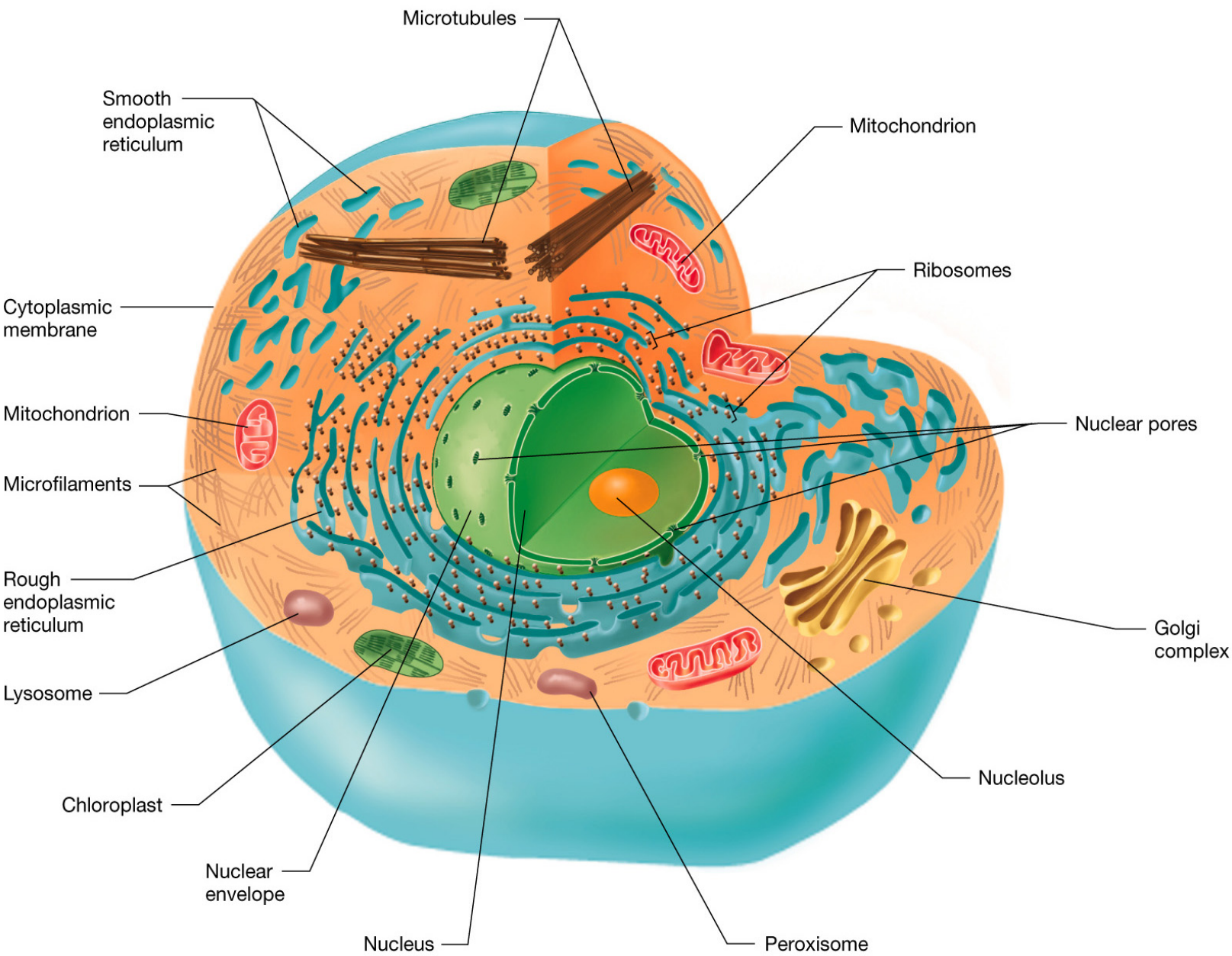
The tree (c) is ultimately generated by computer analysis of the data to give the best fit. The total length of the branches separating any two organisms is proportional to the calculated evolutionary distance between them. In actual analyses a statistical process called bootstrapping is typically used whereby the computer generates hundreds of versions of the tree to confirm that the final tree is indeed the best fit to the data set. In addition, insertions of several nucleotides may separate regions of sequence homology in two organisms' rRNA, and these insertions are "masked" (not considered) in the actual analyses.



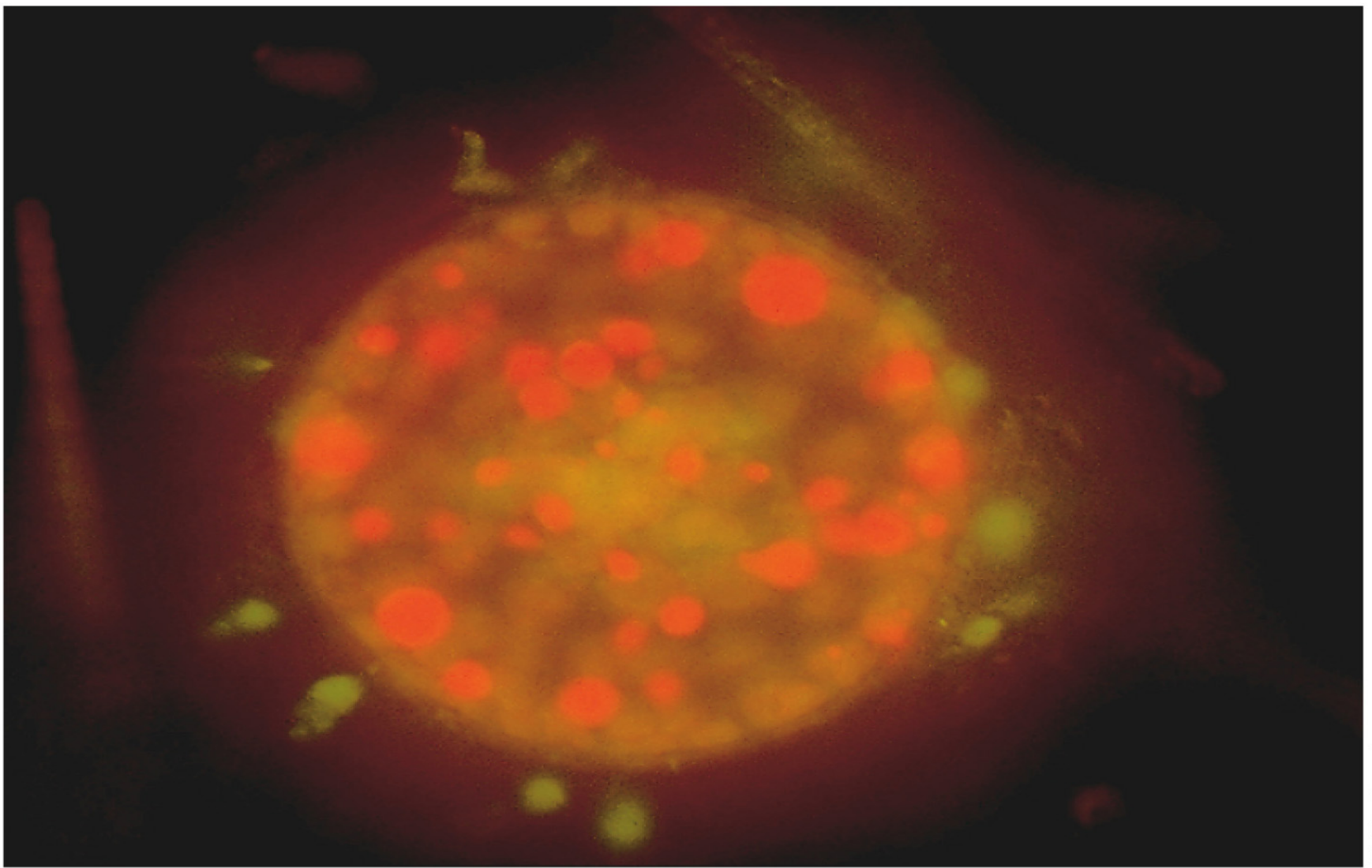
Universal phylogenetic tree as determined from comparative ribosomal RNA sequencing. Only a few key organisms or lineages are shown in each domain. For detailed domain trees refer to Figures 12.1, 13.1, and 14.11. Of the three domains, two (Bacteria and Archaea) contain only prokaryotic representatives. The location highlighted in red is the hypothetical root of the tree, which represents the position of the universal ancestor of all cells.



Detailed tree of *Eukarya*. Not all known lineages of *Eukarya* are depicted. Some early-branching species of *Eukarya* lack organelles other than the nucleus. Note how "higher organisms" (plants and animals) branch near the apex of the tree.



Schematic, cut-away view of a eukaryotic cell. Although all eukaryotic cells contain a nucleus, not all organelles or other structures shown are present in all eukaryotic cells.



T. D. Brock

(a)

Photomicrograph of algal cells showing chloroplasts. (a) Fluorescence photomicrograph of the diatom *Stephanodiscus*. The chlorophyll in the chloroplasts absorbs light and fluoresces red.

Phylogenetic classification of the major sub-groups of eubacteria

Derived by ribosomal RNA analysis this lists the major sub-groups of eubacteria

Aquifex	extremely thermophilic chemolithotrophs
Thermotoga	extremely thermophilic chemoorganotrophs
Chloroflexus	thermophilic photosynthetic bacteria
Deinococcus	radiation resistant bacteria
Thermus	thermophilic chemoheterotrophs
Spirochaetes	helical bacteria with periplasmic flagella
Proteobacteria	Gram-negative and purple photosynthetic bacteria
Cyanobacteria	blue-green photosynthetic bacteria
Gram-positives	Gram-positive bacteria
Bacteroides/Flavobacterium	strict anaerobes/ strict aerobes with gliding motility
Chlorobium	photoautotrophic sulphur-oxidisers
Planctomyces	budding bacteria with no peptidoglycan
Chlamydia	intracellular parasites

In biology , particularly cell biology , the term organelle is used to describe several structures with specialized functions, suspended in the cytoplasm of a eukaryotic cell .
A few large organelles probably originated from endosymbiont bacteria :

- chloroplast
- mitochondrion

Other organelles include:

- centriole
- endoplasmic reticulum
- golgi apparatus
- lysosome
- myofibril
- nucleus
- peroxisome
- ribosome
- vacuole
- vesicle

Other related structures:

- flagellum
- cytosol
- endomembrane system
- nucleosome
- microtubule
- cell membrane

History

Organelles are historically identified through the use of some form of microscopy . Organelles were also identified through the use of cell fractionation .

Chloroplasts are [organelles](#) found in [plant cells](#) and [eukaryotic algae](#) which conduct [photosynthesis](#) .

These are one of the forms a [plastid](#) may take, and are generally considered to have originated as [endosymbiotic cyanobacteria](#) .

In green plants chloroplasts are surrounded by two [lipid bilayer membrane](#) s, now thought to correspond to the outer and inner membranes of the ancestral cyanobacterium.

The fluid within the chloroplast is called the [stroma](#) , corresponding to the cytoplasm of the bacterium, and contains tiny circular [DNA](#) and [ribosomes](#) , though most of their proteins are synthesized by the cell nucleus.

The genome is considerably reduced compared to that of free-living cyanobacteria, but the parts that are still present show clear similarities. Within the stroma are stacks of [thylakoids](#) , the sub-organelle where photosynthesis actually takes place.

A stack of thylakoids is called a granum. A thylakoid looks like a flattened disk, and inside is an empty area called the thylakoid space. The photosynthesis reaction takes place on the surface of the thylakoid. The photosynthetic proteins in the membrane bind [chlorophyll](#) , which is present with various accessory pigments. These give chloroplasts their green colour. Algal chloroplasts may be golden, brown, or red and show variation in the number of membranes and the presence of thylakoids.

A **mitochondrion** is an [organelle](#) found in the [cells](#) of most [eukaryotes](#). Mitochondria are sometimes described as "[cellular power plants](#)" because their primary [function](#) is to [manufacture adenosine triphosphate](#) (ATP), which is used as a source of [energy](#). The number of mitochondria found in different types of cells varies widely. At one end of the spectrum, the [Trypanosome protozoan](#) has one large mitochondrion; by contrast, [human liver](#) cells normally have between one and two thousand each. Mitochondria can occupy up to 25% of cell cytosol.

Structure

Mitochondria are composed of two membranes the inner of which has folds called *cristae*, which give a much increased surface area on which chemical reactions can occur.

The outer membrane encloses the entire [organelle](#) and contains channels made of [protein](#) complexes called porins through which [molecules](#) and [ions](#) can move in and out of the mitochondrion. It is composed of 50% lipids and 50% proteins. Large molecules are excluded from traversing this membrane.

The inner membrane, folded into [cristae](#), encloses the [matrix](#) (the internal fluid of the mitochondrion). It contains several protein complexes, and is 20% lipids and 80% protein. *Stalked particles* are found on the *cristae*: these are the ATP synthase enzyme molecules, which produce ATP.

The *intermembrane space* between the two membranes contains enzymes that use ATP to [phosphorylate](#) other [nucleotides](#) and that catalyze other reactions.

Mitochondrion" literally means 'thread granule', which is what they look like under a light [microscope](#) : tiny rod-like structures present in the [cytoplasm](#) of all cells. The matrix contains [soluble](#) enzymes that [catalyze](#) the oxidation of [pyruvate](#) and other small organic molecules. Parts of the Krebs cycle occur within mitochondria. The matrix also contains several copies of the [mitochondrial DNA](#) (usually 5-10 circular DNA molecules per mitochondrion), as well as special mitochondrial [ribosomes](#) , [tRNAs](#) , and proteins needed for [DNA replication](#) . When the cell divides, mitochondria replicate by [fission](#). They also replicate if the long-term energy demands of a cell increase. For example, [fat](#) storage cells, which require little energy, have very few mitochondria, but energy-demanding [muscle](#) cells tend to have many. Mitochondria are generally theorised to be highly adapted symbiotic [bacteria](#), probably belonging to the alpha-proteo bacteria (with the closest known candidate being *Rickettsia*, the causative agent of [typhus](#)), and are believed to have been incorporated only once (compare [chloroplast](#)).

Proteins

The mitochondrial proteins are found on the outer membrane, the inner membrane, or the intermembrane space.

Outer membrane proteins

Stop-transfer sequences anchor proteins to the outer membrane. Matrix-targeting sequences [target](#) the protein for the mitochondrial matrix.

Energy conversion

Mitochondria convert the potential energy of food molecules into ATP. The production of ATP is achieved by the Krebs cycle (see [citric acid cycle](#)), electron transport and [oxidative phosphorylation](#). Without [oxygen](#), these processes cannot occur.

The energy from [food molecules](#) (e.g., [glucose](#)) is used to produce NADH and FADH₂ molecules, via [glycolysis](#) and the Krebs cycle. This energy is transferred to oxygen (O₂) in several steps. The protein complexes in the inner membrane ([NADH dehydrogenase](#), [cytochrome c reductase](#), cytochrome c oxidase) that perform the transfer use the released energy to pump [protons](#) (H⁺) against a [gradient](#) (the concentration of protons in the intermembrane space is higher than that in the matrix). An [active transport](#) system (energy requiring) pumps the protons against their physical tendency (in the "wrong" direction) from the matrix into the intermembrane space.

As the proton concentration increases in the intermembrane space, a strong *diffusion gradient* is built up. The only exit for these protons is through the [ATP synthase](#) complex. By transporting protons from the intermembrane space back into the matrix, the ATP synthase complex can make [ATP](#) from ADP and inorganic phosphate (Pi). This process is called chemiosmosis and is an example of [facilitated diffusion](#). Part of the 1997 [Nobel Prize](#) in Chemistry was awarded to [Paul D. Boyer](#) and John E. Walker for their clarification of the working mechanism of ATP synthase.

Other functions

Mitochondria have several important functions besides the production of ATP. This variety of functions corresponds to the variety of [mitochondrial diseases](#).

Some mitochondrial functions are performed only in specific types of cells. For example, mitochondria in liver cells contain enzymes that allow them to detoxify ammonia, a waste product of protein metabolism. These enzymes are not made in the mitochondria of cardiac cells.

Mitochondria also play a role in the following:

- [apoptosis](#)
- [glutamate](#)-mediated excitotoxic [neuronal](#) injury
- cellular proliferation
- regulation of the cellular [redox](#) state
- [heme](#) synthesis
- [steroid](#) synthesis
- heat production (enabling the organism to stay warm)

Use in population genetic studies

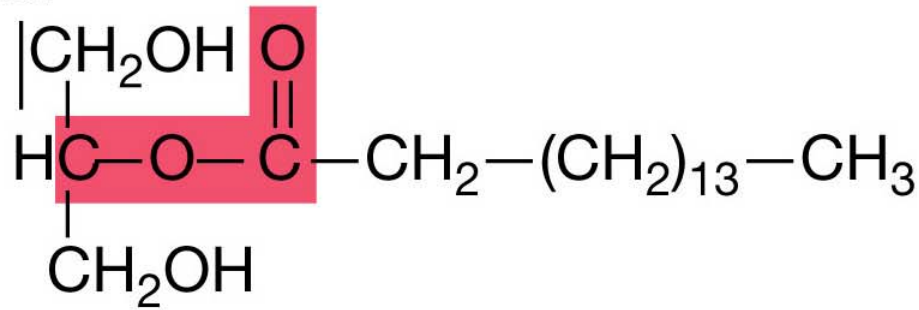
Because [eggs](#) destroy the mitochondria of the [sperm](#) that [fertilize](#) them, the [mitochondrial DNA](#) of an individual derives exclusively from the mother. Individuals inherit the other kinds of genes and DNA from both parents jointly. Because of the unique matrilineal transmission of mitochondrial DNA, scientists in [population genetics](#) and [evolutionary biology](#) often use data from mitochondrial DNA sequences to draw conclusions about [genealogy](#) and [evolution](#). See: [mitochondrial Eve](#).

The endosymbiotic hypothesis

Mitochondria are unusual among organelles in that they contain [ribosomes](#) and their own genetic material. Mitochondrial [DNA](#) is circular and employs characteristic variants of the standard eukaryotic [genetic code](#).

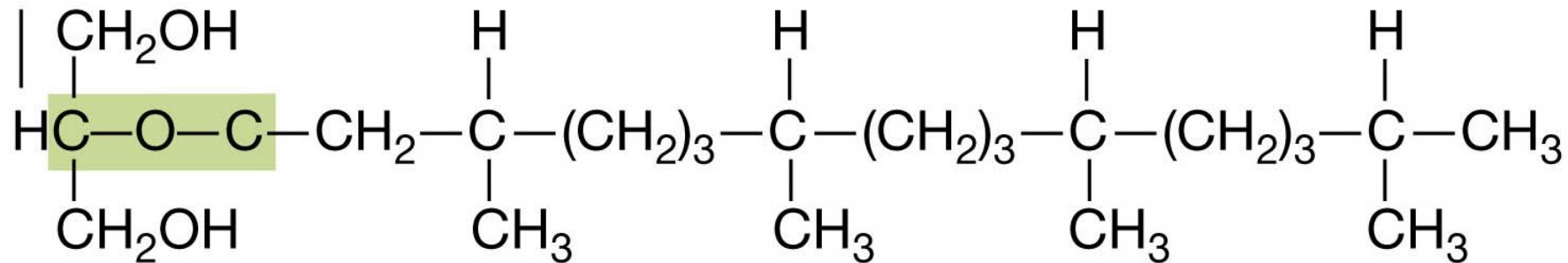
These and similar pieces of evidence motivate the endosymbiotic hypothesis — that mitochondria originated as prokaryotic [endosymbionts](#). Essentially this widely accepted hypothesis postulates that the ancestors of modern mitochondria were independent bacteria that colonized the interior of the ancient [precursor](#) of all eukaryotic life.

Ester



Bacteria, Eukarya

Ether



Archaea

Lipids in *Bacteria*, *Eukarya*, and *Archaea*. In *Bacteria* and *Eukarya*, lipids contain fatty acids (palmitic acid is shown) bonded by ester linkages to glycerol. In *Archaea*, the side chains are branched hydrocarbons (phytanyl, C₂₀, is shown) bonded by ether linkages to glycerol. Phytanyl is synthesized from isoprene (Figure 4.19c).

Fin parte I

Isolation of bacterium from
intestine of warm-blooded animal

Obtain pure culture

Gram reaction

Gram-negative

Rod-shaped

Facultative

Ferments lactose,
producing acids
and gas

Perform biochemical tests:
(Positive: indole, methyl red, mucate;
negative: citrate, Voges-Proskauer, H₂S)

Escherichia coli

Example of methods that would be used for identification of a newly isolated enteric bacterium, using classic microbiological methods (the example given shows the procedures that would be used for identifying *Escherichia coli*). Note that most of the analyses here require that the organisms be grown in pure culture and that solely phenotypic criteria be used in the identification. A description of biochemical tests is presented in Chapter 24 (Section 24.2, Table 24.3, and Figure 24.7).

Organism

Prokaryotes

Bacteria

Archaea

Eukaryotes

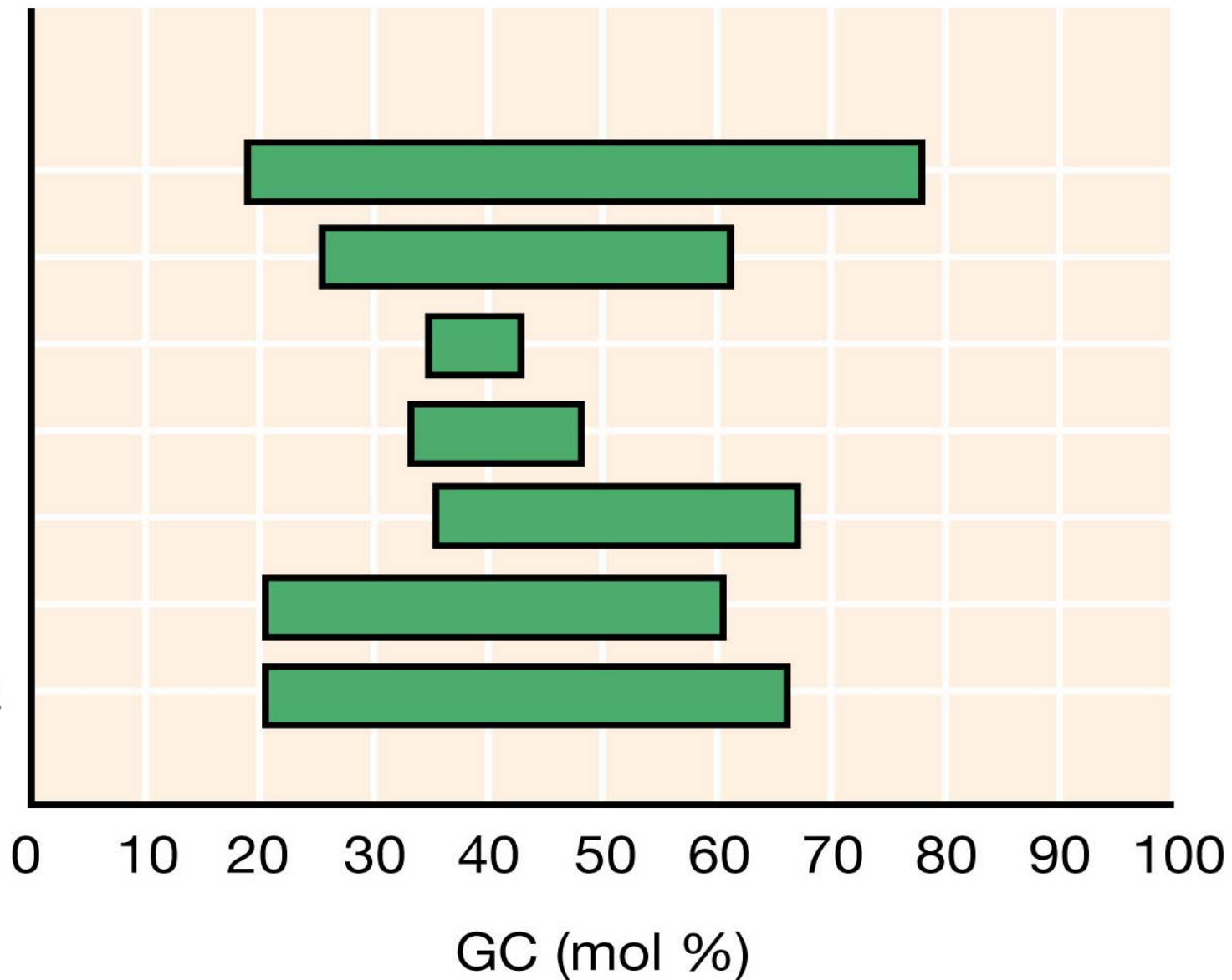
Animals

Plants

Algae

Fungi

Protozoa

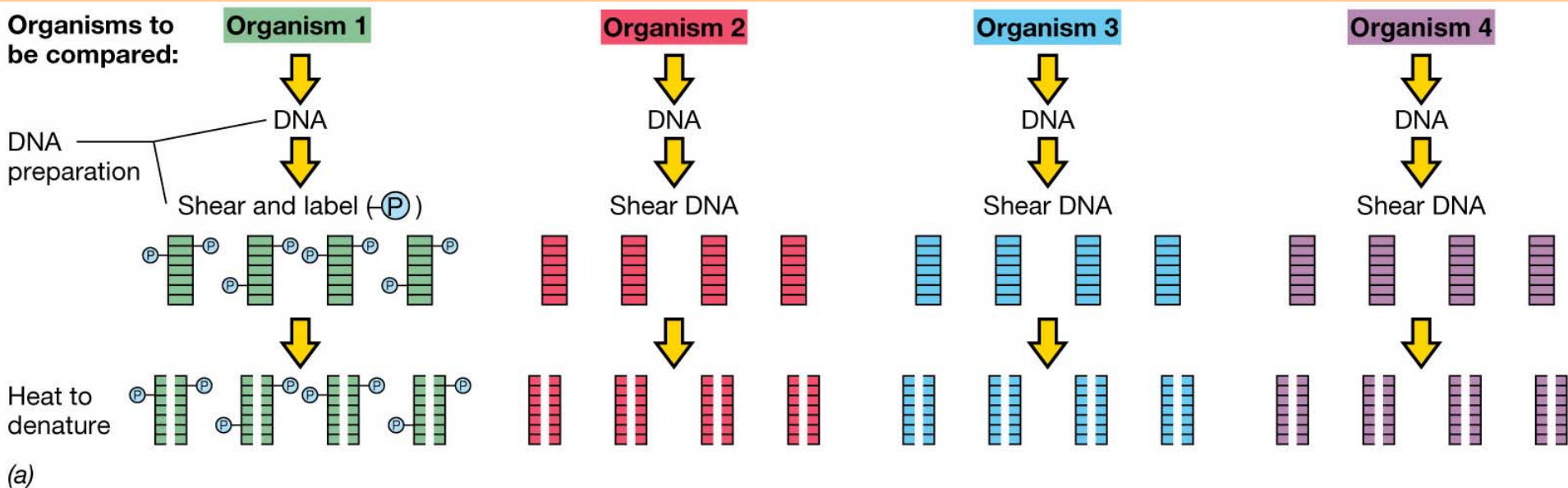


Ranges of DNA base composition of various organisms. Note that the greatest range exists with bacteria.

Domain Bacteria	Domain Archaea	Domain Eukarya
1-10 μm	1-10 μm	10-100 μm
Cell wall of peptidoglycan	Cell wall various	Cell wall of cellulose or chitin
No introns in chromosomes	Some introns present	Introns present
Membranes based on fatty acids	Membranes based on isoprenes	Membranes based on fatty acids
No membrane-bounded orgelles	No membrane-bounded orgelles	Membrane-bounded orgelles
4-subunit RNA polymerase	Many-subunit RNA polymerase	Many-subunit RNA polymerase

Introns: DNA "punctuation" between protein-coding sequences

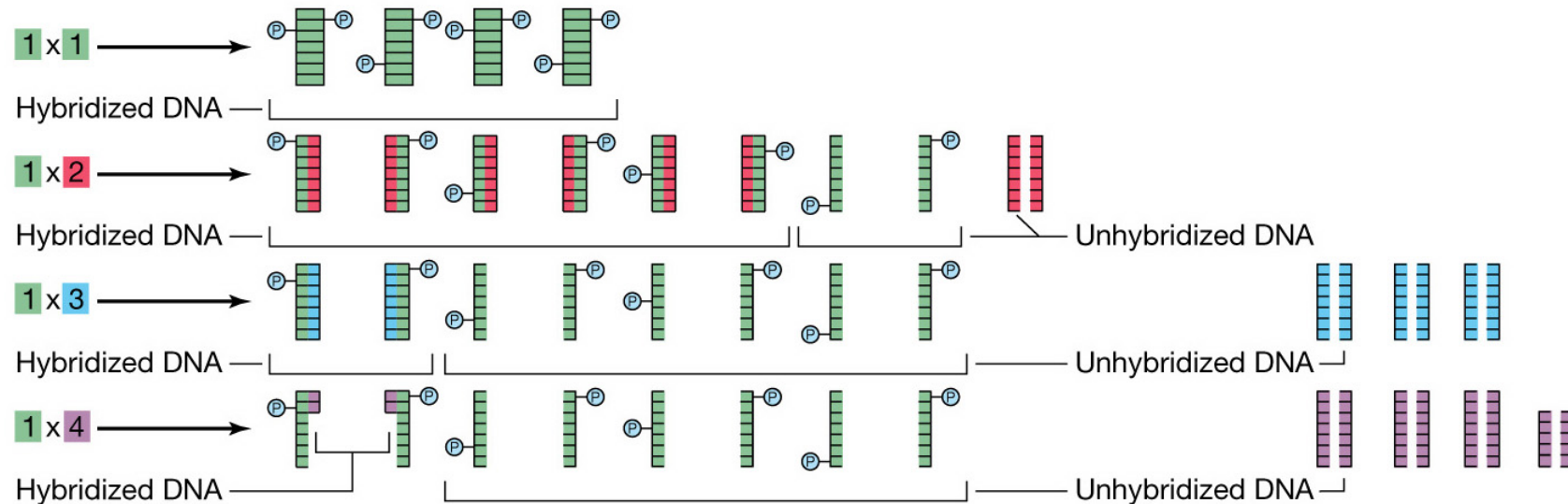
RNA polymerase: Enzyme that transcribes RNA from DNA www.madsci.org/posts/archives/jan2000/946839789.Cb.r.html



Genomic hybridization as a taxonomic tool. (a) DNA is isolated from test organisms. One of the DNAs is labeled (shown here as radioactive phosphate in the DNA of Organism 1).

Hybridization

experiment: Mix DNA from two organisms—unlabeled DNA is added in excess:

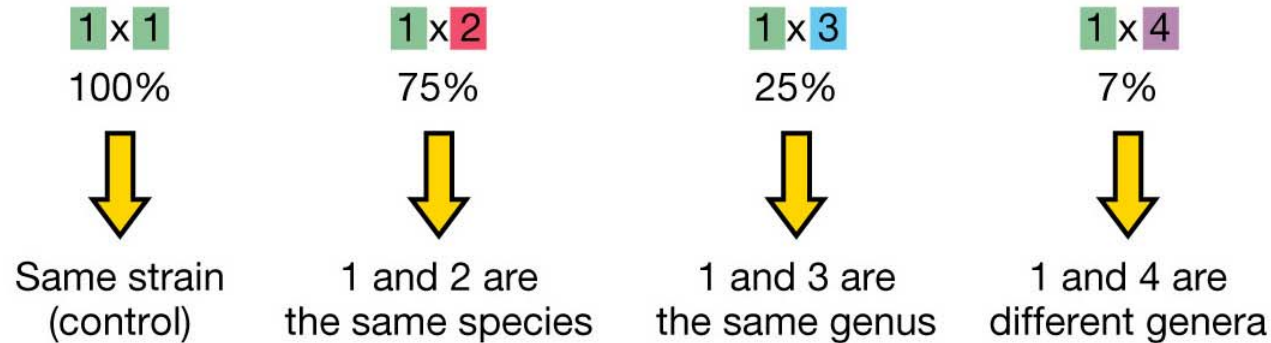


(b)

Genomic hybridization as a taxonomic tool. (b) Actual hybridization experiment. All combinations are tried and excess unlabeled DNA is added in each experiment to prevent labeled DNA from reannealing with itself. Following hybridization, hybridized DNA is separated from unhybridized DNA before measuring radioactivity in the hybridized DNA only.

Results and interpretation:

Percentage Hybridization



(c)

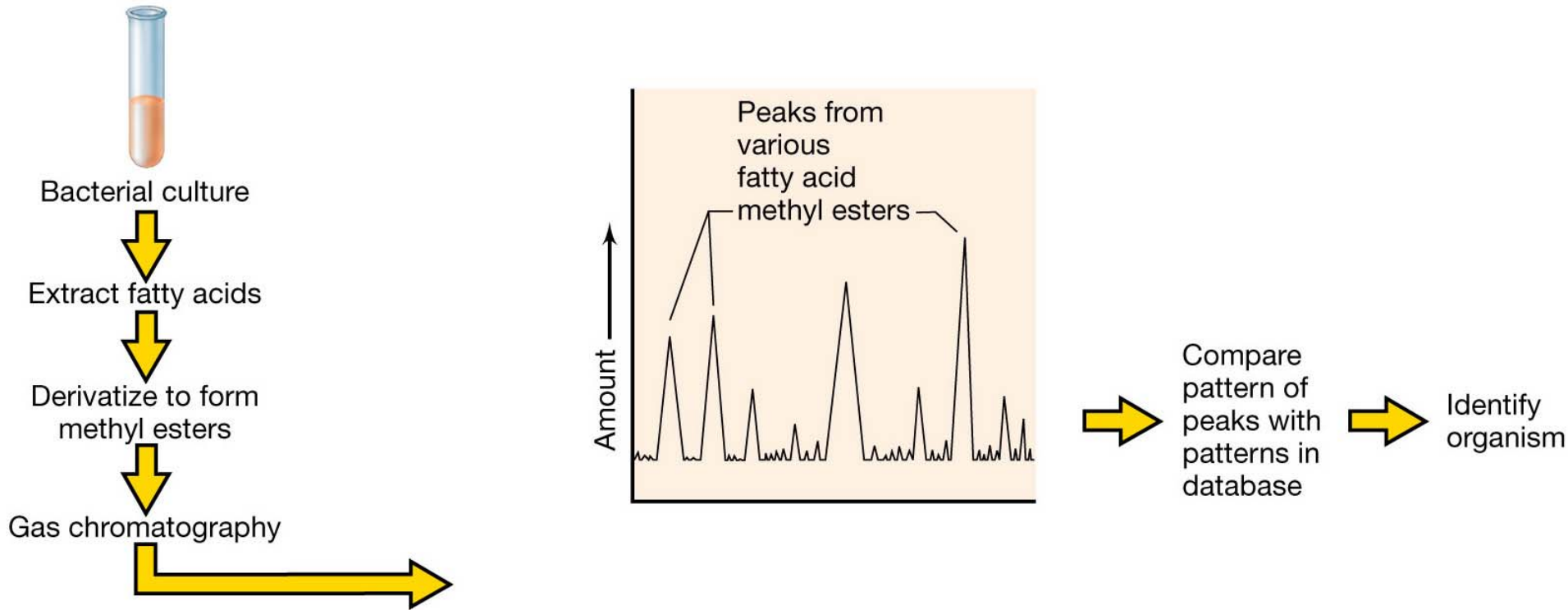
Genomic hybridization as a taxonomic tool. (c) Results. Radioactivity in the control is taken as the 100% hybridization value.

Classes of Fatty Acids in *Bacteria*

Class	Example	Structure of example
Saturated	tetradecanoic acid	$\begin{array}{c} \text{O} \\ \parallel \\ \text{HO}-\text{C}-(\text{CH}_2)_{12}-\text{CH}_3 \end{array}$
Unsaturated	<i>omega</i> -7- <i>cis</i> hexadecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{H} \quad \text{H} \\ \parallel \qquad \qquad \quad \\ \text{HO}-\text{C}-(\text{CH}_2)_6-\text{C}=\text{C}-(\text{CH}_2)_6-\text{CH}_3 \\ \\ \text{H} \end{array}$
Cyclopropane	<i>cis</i> 7-8 methylene hexadecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{H} \quad \text{H} \\ \parallel \qquad \qquad \quad \\ \text{HO}-\text{C}-(\text{CH}_2)_7-\text{C}=\text{C}-(\text{CH}_2)_5-\text{CH}_3 \\ \qquad \qquad \diagup \quad \diagdown \\ \text{H} \qquad \qquad \text{C} \\ \qquad \qquad \diagdown \quad \diagup \\ \qquad \qquad \text{H} \quad \text{H} \end{array}$
Branched	13-methyltetradecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{CH}_3 \\ \parallel \qquad \qquad \\ \text{HO}-\text{C}-(\text{CH}_2)_{10}-\text{C}-\text{CH}_3 \\ \qquad \qquad \\ \text{H} \qquad \qquad \text{H} \end{array}$
Hydroxy	3-hydroxytetradecanoic acid	$\begin{array}{c} \text{O} \qquad \qquad \text{H} \\ \parallel \qquad \qquad \\ \text{HO}-\text{C}-\text{CH}_2-\text{C}-(\text{CH}_2)_{10}-\text{CH}_3 \\ \qquad \qquad \\ \text{H} \qquad \qquad \text{OH} \end{array}$

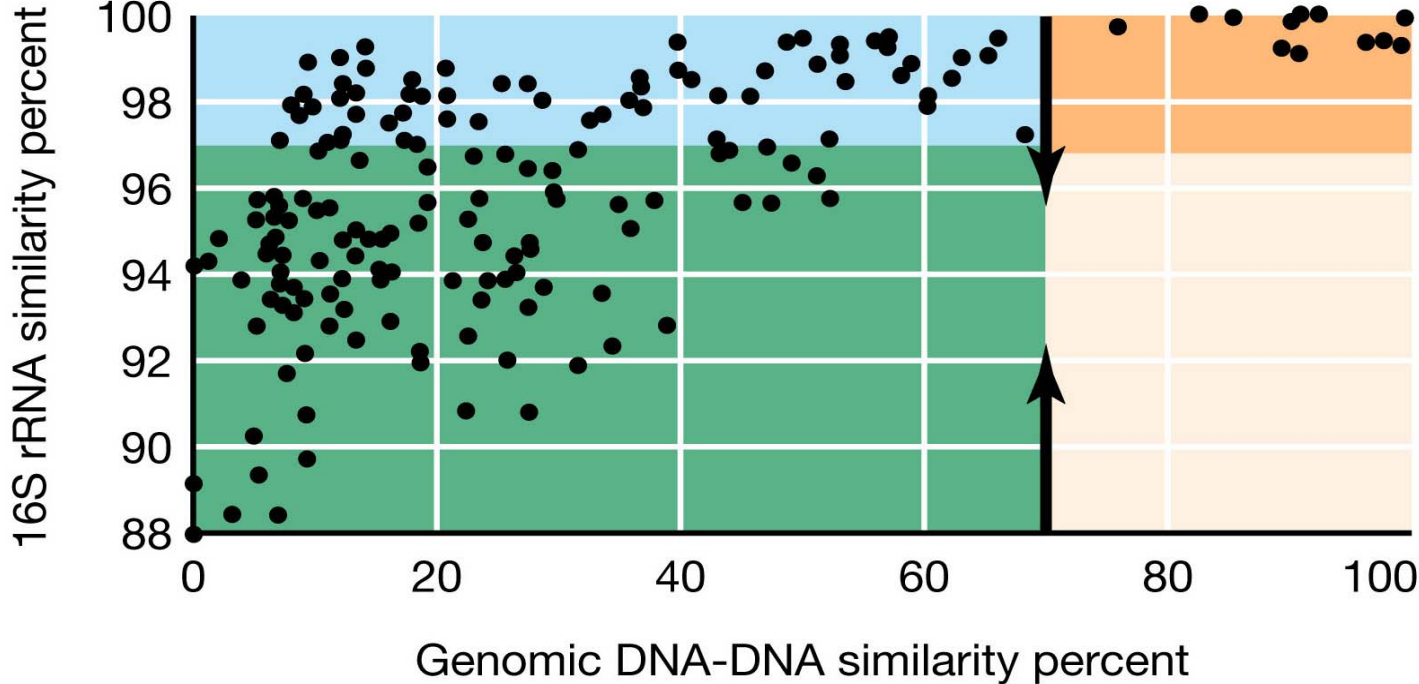
(a)

Fatty acid methyl ester (FAME) analysis in bacterial identification. (a) Classes of fatty acids in *Bacteria*. Only a single example is given of each class, but in actuality, more than 200 different fatty acids have been discovered from bacterial sources. A methyl ester contains a methyl group (CH₃) in place of the proton on the carboxylic acid group (COOH) of the fatty acid.



(b)

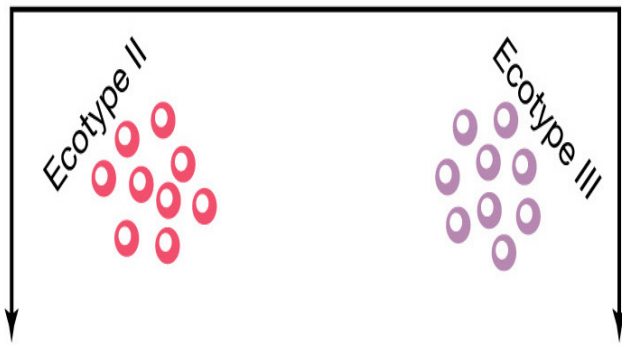
Fatty acid methyl ester (FAME) analysis in bacterial identification. (b) Procedure. Each peak from the gas chromatograph is due to one particular fatty acid methyl ester and the peak height is proportional to the amount.



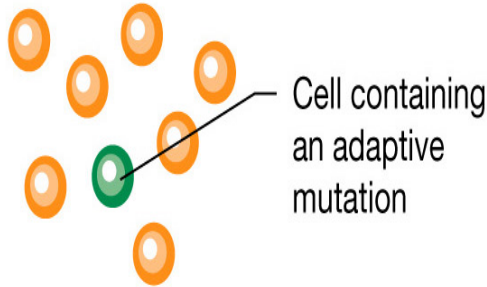
Relationship between 16S ribosomal RNA sequence similarity and genomic DNA hybridization between different pairs of organisms.

These data are the results from several independent experiments with various species of the domain Bacteria. Points in the orange box represent combinations where 16S sequence similarity and genomic hybridization were both very high; thus, in each case, the two organisms tested were clearly the same species. By contrast, points in the green box represent combinations that indicate the two organisms tested were different species, and both methods show this. Points in the blue box indicate that the two test organisms were different species as measured by genomic DNA hybridization but not by 16S sequencing. Note how above 70% DNA hybridization, no 16S similarities were found of less than 97%. Data redrawn from Rosselló-Mora, R., and R. Amann. 2001. *FEMS Microbiol. Rev.* 25:30-67.

One microbial habitat



Ecotype I

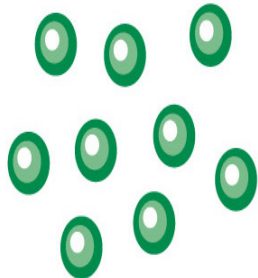


Periodic selection



Adaptive mutant survives. Original Ecotype I wild-type cells out competed

Population of mutant Ecotype I

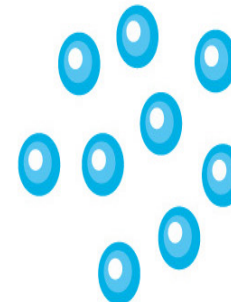


Possible mechanism of bacterial speciation. Several ecotypes can coexist in a single microbial habitat, each occupying their own prime ecological niche. When a beneficial mutation occurs within an ecotype, the cell containing the adaptive mutation will eventually form a population that will replace the original ecotype. As this occurs repeatedly within a given ecotype, a genetically distinct population of cells arises that represents a new species.

Repeat process many times



New species of Ecotype I



Because other ecotypes do not compete for the same resources, they are unaffected by genetic and selection events that occur outside of their ecotype.

Taxonomy and Diversity

Purpose of taxonomy is to provide useful ways for identifying and comparing organisms. Another goal is to assess the extent of diversity of different types of organisms.

Two very different ways to construct a taxonomy: **1. Phenetic system:** groups organisms based on mutual similarity of phenotypic characteristics. May or may not correctly match evolutionary grouping. Example: group (motile) organisms in one group, non-motile organisms in another group. This is useful, but does it reflect underlying evolutionary ancestry?

Numerical Taxonomy: a common approach to phenetic taxonomy

Use a variety of characteristics: e.g., Gram stain, cell shape, motility, size, aerobic/anaerobic capacity, nutritional capabilities, cell wall chemistry, immunological characteristics, etc.

Relies on similarity coefficients

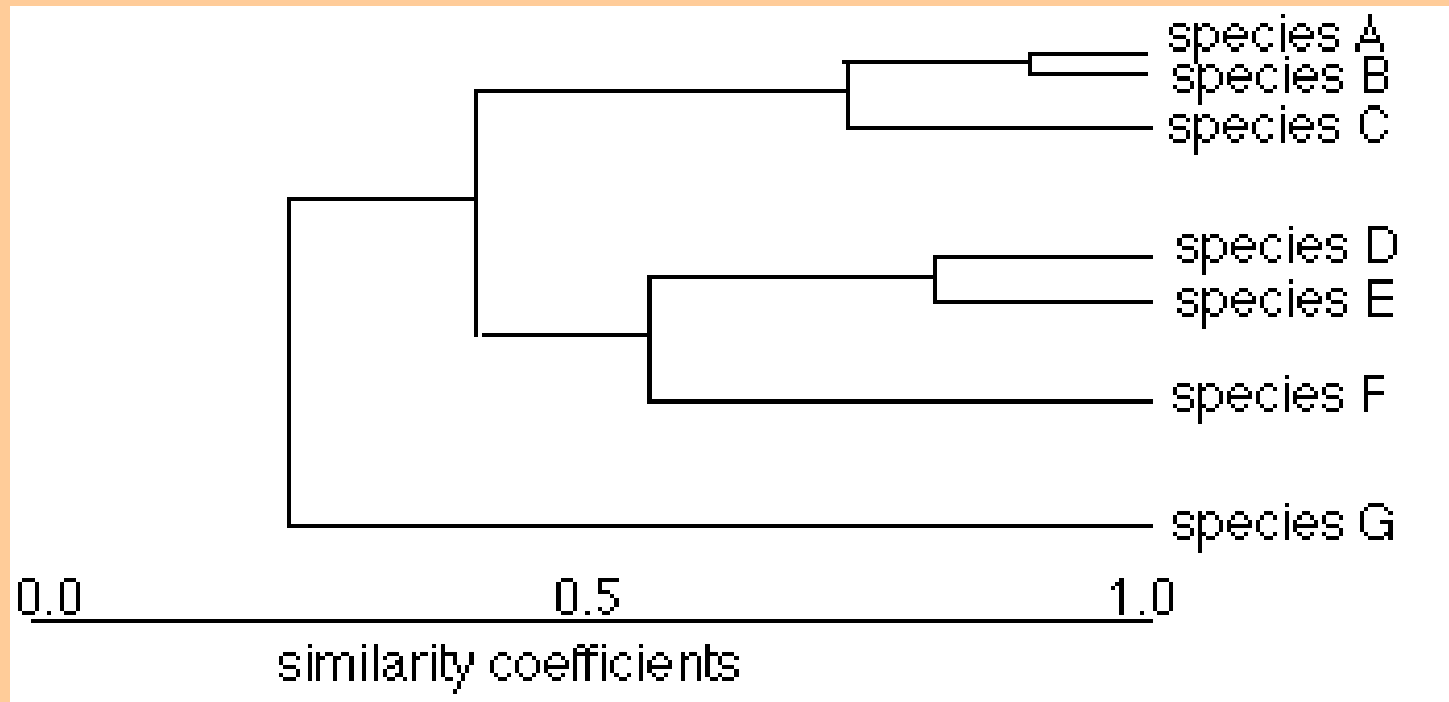
If use 10 characteristics, then match organisms.

Ex. A and B share 8 characters out of 10: similarity coefficient S_{ab} is $8/10 = 0.8$

Can use many such values to establish similarity matrix

Dendrograms help display this information clearly.

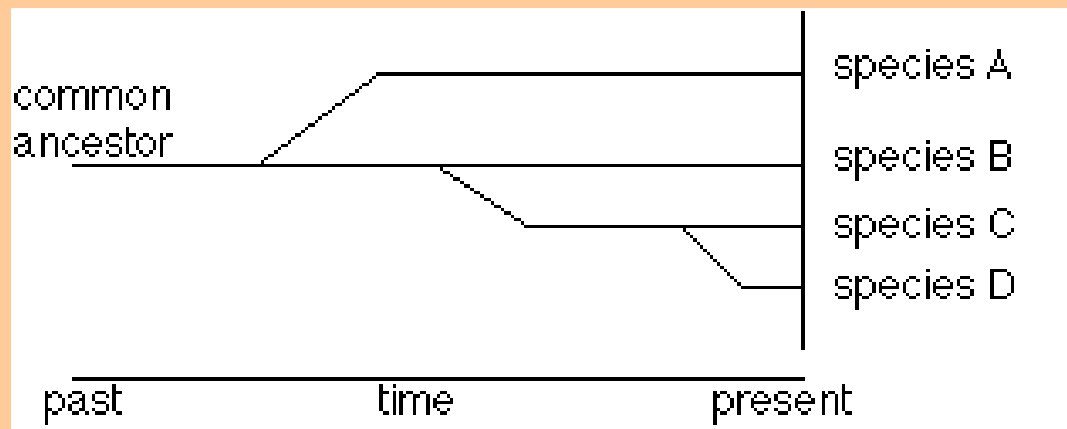
<http://www.sp.uconn.edu/~terry/229sp04/terry/taxonomy.html>



Note: dendrogram is just a graphical display of similarity coefficients; but one often assumes that these are representative of a deeper evolutionary relationship. This may or may not be legitimate conclusion, depending on the traits used.

2. Phylogenetic system: groups organisms based on shared evolutionary heritage. Example: **Mycoplasma** (no wall) and **Bacillus** (walled Gram+ rods) are not obviously similar, would not be grouped together phenetically. But evolutionarily they are similar, more so than either to Gram- organisms.

- The diagram below is a hypothetical evolutionary diagram, superficially similar to a dendrogram but actually quite different, since it seeks to portray an accurate picture of how and when organisms diverged from common ancestors over time.



To get accurate phylogeny, must decide which characteristics give best insight. DNA and RNA sequencing techniques are considered to give the most meaningful phylogenies.

Strain

descended from a single organism

different isolates may be same species but are different strains; often have slight differences. Example: *E. coli* K12, B, etc.

Type strain

often the first strain isolated or best characterized

kept in collections; e.g., ATCC ([American Type Culture Collection](#)) maintains the following frozen or freeze-dried stocks: (number of species in parentheses)

- Algae (~ 120)

- Bacteria (~ 18,000 strains)

- Fungi and Yeasts (~ 27,000 strains)

- Protozoa (~ 1100)

- Cell lines: animal (~ 2300)

- Cell lines: plant (~ 25)

- Viruses: animal (~ 2000)

- Viruses: plant (~ 700)

- Viruses: bacteria (~ 500)

Species

Species concept in eukaryotes is based grouping organisms that can reproduce into species category

Species concept is applied in microbiology as well, but harder to define. In practice, strains that share certain type properties can be called the same species even if they differ by up to 30% in DNA variation.

Linnaean hierarchy

Sequence from smaller to larger levels of organization

strain, species, genus, family, order, class, division, kingdom

Example:

Genus: ***Escherichia***

Species: ***coli***

Family: **Enterobacteriaceae**

Class: **Scotobacteria**

Division: **Gracilicutes**

Kingdom: **Procaryotae**

Note: for bacteria, it is challenging to decide where to draw boundaries such as genus and family.

Bergey's Manual of Systematic Bacteriology: The definitive guide to procaryotic taxonomy

First edition published in 1923, now in 9th edition.

- Uses both morphological and Physiological characteristics

- Very practical system. Use successive "key" features to narrow down identification

- Ex. Gram + or -? Then shape? Then motile or not? etc. Eventually only a few organisms match the process of elimination.

Second edition now being published, a major reorganization

- Primary emphasis is phylogenetic, not phenetic

- Example: pathogens are not grouped together, instead they are scattered in different

- Five volumes have instructive titles:

 - The Archaea, and the Deeply Branching and Phototrophic Bacteria

 - The Proteobacteria

 - The Low G + C Gram-positive Bacteria

 - The High G + C Gram-positive Bacteria

 - The Planctomyces, Spirochaetes, Fibrobacters, Bacterioidetes, and Fusobacteria

See text Chapters 20-24 for a highly abridged but useful overview of some of the information found in Bergey's Manual.

<http://assets.cambridge.org/0521809347/sample/0521809347WS.pdf>

Broad Evolutionary Picture

Original organism replicated and evolved for millions of years, then gave rise to the universal ancestor (common to all later forms of life known to currently exist on earth)

Universal ancestor was the result of considerable evolution of the original life form and gave rise to three domains of living things Bacteria, Archaea and Eukarya, via considerable evolution

Classification

Phylogeny vs. Taxonomy - phylogeny depends upon genotypic analysis as a basis of classification, whereas taxonomy depends upon phenotypic (generally biochemical) analysis

Binomial nomenclature - organisms are always given two names; one designates their species (similar strains), one their genus (similar species)

Molecular Approaches to Phylogeny

Evolutionary distances (ED) between phylogenetic groups can be measured by differences in nucleic acid (amino acid) sequence, if the molecules used are:

- Universally distributed across the group studied

- Functionally homologous - have identical function

- Properly aligned - so regions of homology and heterogeneity can be identified correctly

- Rate of sequence changes - commensurate with the evolutionary distance between members

Molecules used:

- Initial studies used **proteins** with fundamental physiological function, such as cytochrome c

- then **5S ribosomal RNA (rRNA)**, which is small and easy to isolate, but has little complexity

- Some current studies use **ATP synthase** - functionally homologous in species where it is found, properly aligned, changes sequence at rate commensurate with ED, genes are easy to isolate

- Most current studies use **16S rRNA (eukaryotic, 18S rRNA)** isolated from the small subunit of ribosomes because it is highly conserved, meets the precepts outlined above, has the needed level of complexity, and is relatively easy to isolate and work with using current techniques
first, the primary structure is determined using reverse transcriptase to make a DNA copy (cDNA) of rRNA, cDNA is sequenced, then rRNA sequence is inferred from cDNA sequence

rRNA sequences are then aligned and their degree of homology analyzed by one of two methods:

- (cataloging (80%) - uses signature sequences determined from secondary structure of rRNA

- (distance matrix (20%) - ED calculated by recording number of positions at which compared molecules differ; ED matrix then analyzed by computer program that produces phylogenetic trees

- Ef-Tu and Ef-G, protein synthesis elongation factors used in were used to "root" the Three Domain Tree

Bacteria, Archaea and Eukarya

Bacterial Phylogeny - highly diverse (e.g., purple and green bacteria are less related than plants and animals) ... [more info on bacterial phylogeny](#)

Proteobacteria (Phylum 1) - although these five groups of Gram-negative bacteria are quite diverse, it appears that they evolved from a common **phototrophic ancestor**

alpha group contains phototrophs, lithotrophs and organotrophs) - *Rhodobacter*, purple anoxygenic phototrophs; *Rhodopseudomonas*, budding photoorganotrophs; *Rhizobium*, plant endosymbiotic organotrophs; *Agrobacterium*, plant pathogens; *Nitrobacter*, budding nitrogen oxidizing lithotrophs; *Aquaspirillum*, microaerophilic organotrophs; *Hyphomicrobium*, motile, stalked, budding organotroph; *Acetobacter*, aerobic organotroph; *Paracoccus*, hydrogen-oxidizing lithotroph; *Methylococcus*, methanotroph; some *Pseudomonas*, respiratory

beta group contains phototrophs, lithotrophs and organotrophs - *Rhodocyclus*, phototrophs; *Nitrosomonas*, *Spirillum*, *Bordetella*, *Neisseria*, non-fluorescent *Pseudomonas* *Sphaerotilus*, sheathed metal oxidizers; *Thiobacillus*, sulfur- and iron-oxidizing lithotrophs; *Alcaligenes*, hydrogen-oxidizing lithotrophs; *Neisseria*, oxidative organotrophs; some *Pseudomonas*, respiratory organotrophs

gamma group contains phototrophs, lithotrophs and organotrophs - *Chromatium* and *Thiospirillum*, purple sulfide-oxidizing phototrophs; *Beggiatoa*, lithotroph that oxidizes sulfide; *Legionella*, intracellular pathogen; *Azotobacter*, free-living nitrogen fixer; fluorescent *Pseudomonas*, ; enterics (*Escherichia*, *Shigella*, *Erwinia*, *Salmonella*, *Serratia*, *Proteus*, *Providencia*, *Vibrio*, *Yersinia*), frequently associated with large intestine of humans and other animals; *Leucothrix*, sulfur-oxidizing lithotroph

delta group contains organotrophs (only) - *Desulfovibrio*, anaerobic sulfate-reducer; *Bdellovibrio*, parasitizes bacteria; *Myxobacteria*, motile swarming myxospore-forming organotrophs

epsilon group contains lithotrophs and organotrophs - *Thiovulum*, sulfur-oxidizing lithotrophs; *Wolinella*, hydrogen-utilizing fumarate-based fermenter; *Campylobacter* and *Helicobacter*, parasitic

Gram-Positive Bacteria (Phylum 2) - motile (via flagella) or non-motile rods (*Bacillus*, *Clostridium*, *Corynebacterium*, *Lactobacillus*) and cocci (*Staphylococcus*, *Streptococcus*) with thick peptidoglycan cell walls lacking outer membrane; also wall-less forms (*Mycoplasma*); many spore-forming species (*Actinomyces*, *Bacillus*, *Clostridium*, *Sporolactobacillus*); anaerobic (*Clostridium*, *Desulfotomaculum*, *Veillonella*), facultatively aerobic (*Bacillus*, *Streptococcus*) or aerobic (*Sporosarcina*); exoenzymes common

Cyanobacteria, Prochlorophytes and Chloroplasts (Phylum 3) - Gram-negative phototrophs with gliding motility; share last ancestor with proteobacteria; unicellular forms (*Anacystis*, *Synechococcus*, *Pleurocapsa*) or filamentous forms (*Anabena*, *Nostoc*, *Oscillatoria*); heterocystous cyanobacteria such as *Anabena* and *Nostoc* are nitrogen-fixers

Chlamydia (Phylum 4) - Gram-negative obligate intracellular parasites whose cell walls lack peptidoglycan

Planctomyces/Pirella (Phylum 5) - Gram-negative budding, obligate aerobes, lacking peptidoglycan in their cell walls (proteinaceous instead); organotrophic, but require very dilute culture media for growth (oligotrophic); many have holdfasts; only two genera, *Planctomyces* and *Pirella*

Verrucomicrobia (Phylum 6) - Gram-negative prosthecate aerobic to facultatively aerobic sugar fermenters found in freshwater/marine waters and forest/agricultural soils

Bacteroides/Flavobacterium (Phylum 7) - Gram-negative; some are obligate anaerobes (*Bacteroides*, *Fusobacterium*); others are rod-shaped or filamentous respiratory aerobes with gliding motility(*Flavobacteria*)

Cytophaga (Phylum 8) - Gram-negative obligately aerobic organotrophs with gliding motility; *Cytophaga*, *Sporocytophaga* are long, slender rods with pointed ends that digest cellulose and are found on surfaces of the cellulose-containing material (or fish gills); *Flexibacter* is a filamentous soil and freshwater saprophyte with carotenoids that does not digest cellulose

Green Sulfur Bacteria (Phylum 9) - Gram-negative anaerobic photolithotrophs (*Chlorobium*, *Prosthecochloris*)

Spirochetes (Phylum 10) - Gram-negative, spiral (helical) in shape, with axial filaments; organotrophic; many pathogens (*Borrelia*, *Treponema*, *Leptospira*)

Deinococci (Phylum 11) - *Deinococcus*, Gram-positive radiation-resistant organotroph; *Thermus*, Gram-negative thermophilic organotroph whose cell wall contains ornithine instead of diaminopimelic acid

Green Nonsulfur Bacteria (Phylum 12) - Gram-negative, mostly **thermophilic photoorganotrophs** (*Chloroflexus*), but some are chemotrophs (*Thermomicrobium*, which has unusual membrane lipids that contain 1,2-dialcohols (rather than glycerol) with a long hydrocarbon chain (no ester or ether linkages, because these are)

Thermotogae (Phylum 13)

Thermotoga Gram-negative thermophiles from benthic hydrothermal vents and continental hot springs; these anaerobic fermenters have a sheath-like envelope (hence the term "toga" in the name)

Thermodesulfobacterium - Gram-negative anaerobic thermophilic sulfate-reducing organotroph with an optimum growth temperature of 70C, and ether-linked lipids (very Archaea-like!)

Aquifex, Thermocrinus and Relatives (Phylum 14) - *Aquifex* is a submarine volcanic hot spring bacterium that is hyperthermophilic (optimum temperature for growth is 80C, but can grow up to 90C) lithotroph (reverse TCA cycle) that oxidizes hydrogen, sulfur or thiosulfate and uses oxygen (microaerophilic growth) or nitrate (anaerobic growth) as terminal electron acceptors; most ancient branch of the bacterial phylogenetic tree

Nitrospira (Phylum 15) - Gram-negative, aerobic oxidizers (*Nitrospira*, *Leptospirillum*, *Thermodesulfovibrio*)

Deferribacter group (Phylum 16) - Gram-negative anaerobes (*Deferribacter*, *Geovibrio*, *Flexistipes*)

Archaeal Phylogeny - Archaea appear to have evolved more slowly than other organisms, perhaps due to the extreme environments they inhabit; they are **prokaryotic**, nonsporulating, **lack peptidoglycan** (use polysaccharide, glycoprotein, protein, or pseudopeptidoglycan instead) and have **ether** (rather than ester) linkages in membrane lipids; reproduce by binary fission ... [more on archaeal phylogeny](#)

Korarchaeota - newly discovered extreme thermophiles; these are the most primitive life forms discovered to date

Crenarchaeota - most are extreme thermophiles with growth optima at temperatures greater than 80°C and many of these are anaerobic lithotrophs (*Pyrodictium*), organolithotrophs or organotrophs (*Thermococcus*, *Thermoproteus*) that require elemental sulfur for optimal growth (*Desulfurococcus*)

Sulfolobus is an aerobic organolithotroph
there are also a number of as yet unidentified marine crenarchaeotes, including some cryophiles

Euryarchaeota includes:

extreme halophiles includes *Halobacterium*, *Halococcus*, *Haloferax*, *Halorubrum*, *Haloarcula*, *Natronobacterium*, *Natronococcus*; require high salt (all require at least 1.5M salt, and most require 3-4M salt for growth; obligate aerobes; most are organotrophic (via respiration), others (some *Halobacterium*) use photophosphorylation via bacteriorhodopsin

methanogens are Gram-positive or Gram-negative, strictly anaerobic organolithotrophs that can generate methane from carbon dioxide and hydrogen or from formate, acetate or organic methyl groups; includes *Methanopyrus*, *Methanococcus*, *Methanothermus*, *Methanobacterium*, *Methanospirillum*, *Methanosarcina* and some methanogenic halophiles; *Methanobacterium* and *Methanococcus* are also halophilic

Thermoplasmatales includes *Thermoplasma* and *Picrophilus*

Hyperthermophilic euryarchaeota includes Thermococcales and *Methanopyrus*

sulfur utilizers *Archaeoglobus* is a sulfate-reducing, methanogenic, thermophilic anaerobe; many others also utilize sulfur

extreme thermophiles such as *Thermoplasma*, an acidophilic thermophilic aerobic organotrophs that lacks a cell wall

Eukaryal Phylogeny - the nuclear line that led to Eukarya appears to be just as old as the prokaryotic lines that led to Bacteria and Archaea; original "**nucleus**" was very simple with little DNA; as the DNA content and nuclear complexity increased, DNA was compartmentalized into chromosomes, nuclear membranes evolved to segregate them from the cytoplasm, and specialized microtubules evolved as mitotic spindles; eukaryotes evolved rapidly after atmospheric oxygen and ozone levels developed; evolution of **organelles** from endosymbiotic bacteria (mitochondria, related to proteobacteria; chloroplasts, ancestors of Cyanobacteria; cilia, perhaps related to spirochetes?); although very successful, Eukarya did not compete Bacteria and Archaea out of existence because the Eukarya could not survive in habitats that allow Bacteria and Archaea to thrive

Groups of Eukaria

Protozoa - eukaryotic; unicellular; no cell wall; chemoheterotrophic aerobes (some anaerobes) with absorptive or ingestive nutrient uptake; motility via pseudopodia or undilipodia; some form (dormant) cysts; asexual reproduction via binary fission or budding; sexual reproduction via fusion of haploid gametes

Fungi - eukaryotic; generally larger than bacteria; uni- or multicellular; dimorphic (yeast or mold); chitinous cell walls; aerobic heterotrophs, absorptive nutrient uptake; asexual reproduction via spores, binary fission, budding, fragmentation; sexual reproduction via fusion of haploid nuclei leading to formation of spores

Plants - eukaryotic; uni- or multicellular; **cellulosic cell walls**; **chloroplasts** with chlorophyll a; motility (in unicellular forms) via undilipodia; photosynthetic aerobes (some are facultatively organotrophic); asexual reproduction via binary fission, fragmentation or sporulation; sexual reproduction via fertilization of eggs by sperm, leading to zygosporangia which form zygote that matures into adult form

Animals - eukaryotic; multicellular; **lack cell walls**; possess organs and organ systems; sexual (only) reproduction via fertilized eggs that mature into offspring

Endosymbiotic theory of eukaryotic organelle origin and evolution

endosymbiosis has been proposed as the **mechanism underlying the origin and evolution of mitochondria and chloroplasts** in Eukarya based on this evidence and the fact that a number of primitive protozoa clearly exist as consortia of symbiotic microorganisms

endosymbiotic theory proposes that...

prokaryotes and primitive eukaryotes (members of an ancient cell line that possessed a nucleus, but lacked organelles) formed symbiotic associations (probably parasitic at first) that eventually developed into today's nuclear cells with mutually-interdependent remnants of those prokaryotic endosymbionts that we now call organelles

through the millenia during which these associations were established and refined the genes that were originally present in the endosymbiotes were gradually moved to the nucleus (although it is much larger in plant cells than in animal cells, organelle DNA is rather small compared with that of the typical prokaryote)

evidence supporting the endosymbiotic theory includes the facts that mitochondria and chloroplasts:

- are enclosed in **double lipid bilayer membranes**
- **replicate** themselves **independently** of the cell nucleus
- contain a **single**, circular, double-stranded DNA **chromosome** that replicates itself independently of the nucleus of the cell
- contain **70S ribosomes**, and use them for synthesis of proteins not coded for by nuclear genes
 - the 16S rRNA of mitochondria shows a high degree of homology with proteobacteria
 - 16S rRNA of chloroplasts shows a high degree of homology with cyanobacteria

analysis of rRNA sequences of a large number of **mitochondria and chloroplasts** has shown that:

- **endosymbiotic events** leading to generation of **mitochondria** appear to have been quite **restricted** (only one, most likely) and to have occurred earlier than those leading to generation of chloroplasts
- **endosymbiotic events** leading to generation of **chloroplasts** appear to have occurred **several times** (at least 6) and were more recent than those leading to generation of mitochondria
- **proposed, but not well-supported, hypotheses** include the ideas that:
 - spirochetes** which attached themselves to the exterior of primitive eukaryotes and established an ectosymbiosis with those eukaryotes eventually **evolved into undulipodia** (flagella and cilia); examples of modern-day eukaryotes with ectosymbiotic spirochetes that provide motility to the consortium include *Trypanosoma*
 - the **origin of the nucleus** itself **may have involved a series of similar events** that originated from refinement of associations of consortia of mutualistic prokaryotes into the combined reproductive/metabolic units that we now think of as