Taxonomy

You will learn:

- Why is taxonomy important
- Methods for identification of microorganisms
- Correlation between taxonomy & evolution

Taxon = group

nomy ≈ distribution

Taxa = groups

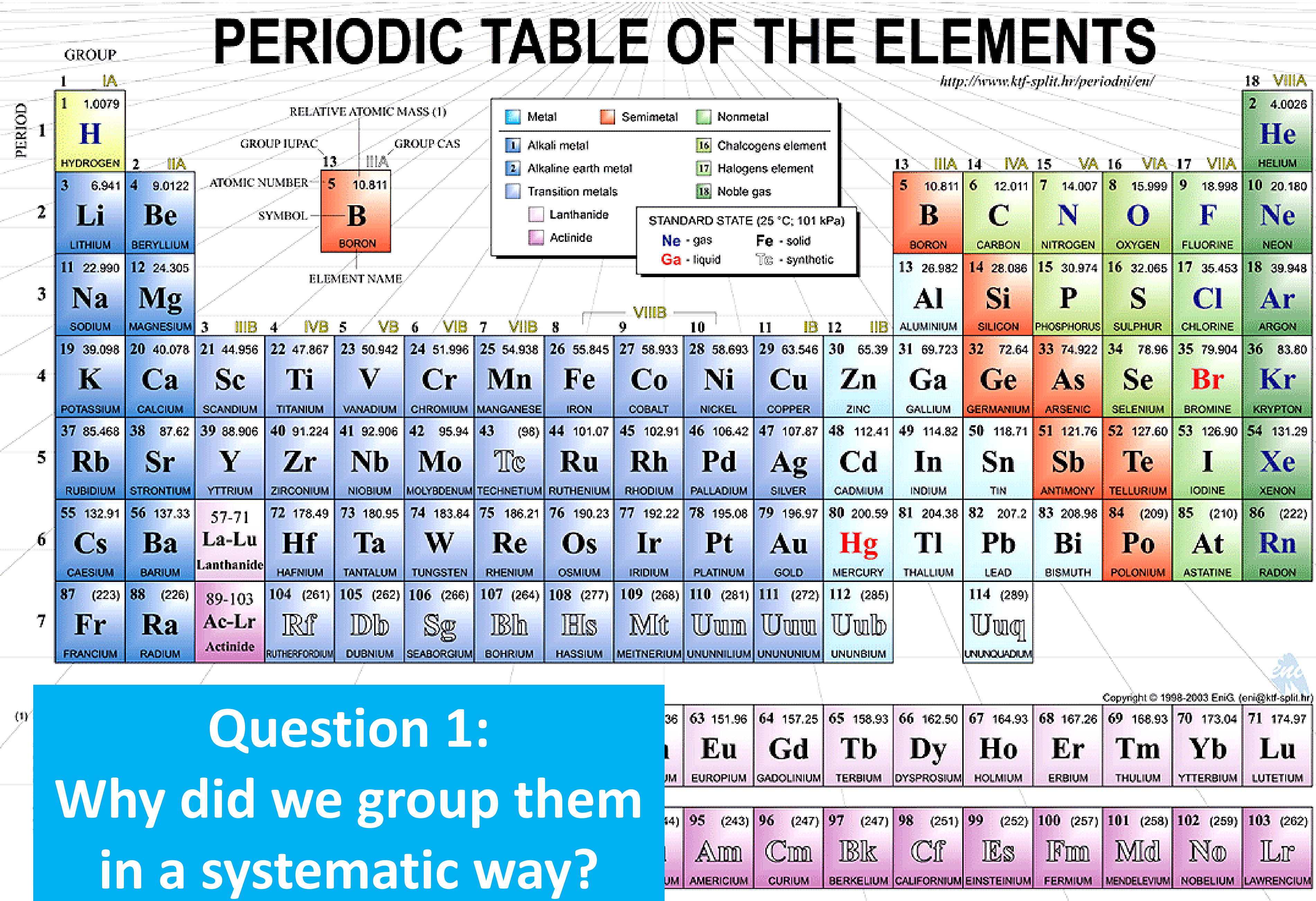
More info: Bergey's Manual:

http://link.springer.com/book/10.1007%2F0-387-28021-9

Why Taxonomy?

- **Classification** - Arrangements in groups (taxa)
- **Nomenclature** - Assigning names to taxa
- **Identification** - Determination of taxon to which an isolate belongs (most practical part of taxonomy)

Making sense of Nature



Question 1:
Why did we group them in a systematic way?

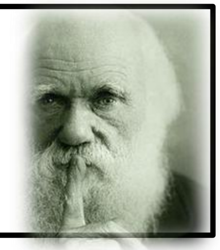
Founding father of taxonomy: Carl von Linné (1707 – 1778)

Born 1707 Stenbrohult, Småland
1727 Un of Lund (medicine)
1728 Un of Uppsala (bottany)
1731 Lapland excursion
1735 Un of Harderwijk (medical degree)
1735 Un of Leiden
Systema Naturae, 1st Edition
1738 Sweden – Stockholm
1741 Professor at Un of Uppsala
Died 1778

Some important developments

Linnaeus
1735

C Darwin
1859



C Woese
1977

Natural classification

Phylogenetic classification

Modern Phylogeny

Plants
Animals

Microorganisms
Macroorganisms

Classification

- **Natural** - anatomical characteristics
- **Phenetic** - phenotypic characteristics
- **Genotypic** - genetic characteristics
- **Phylogenetic** - evolutionary links

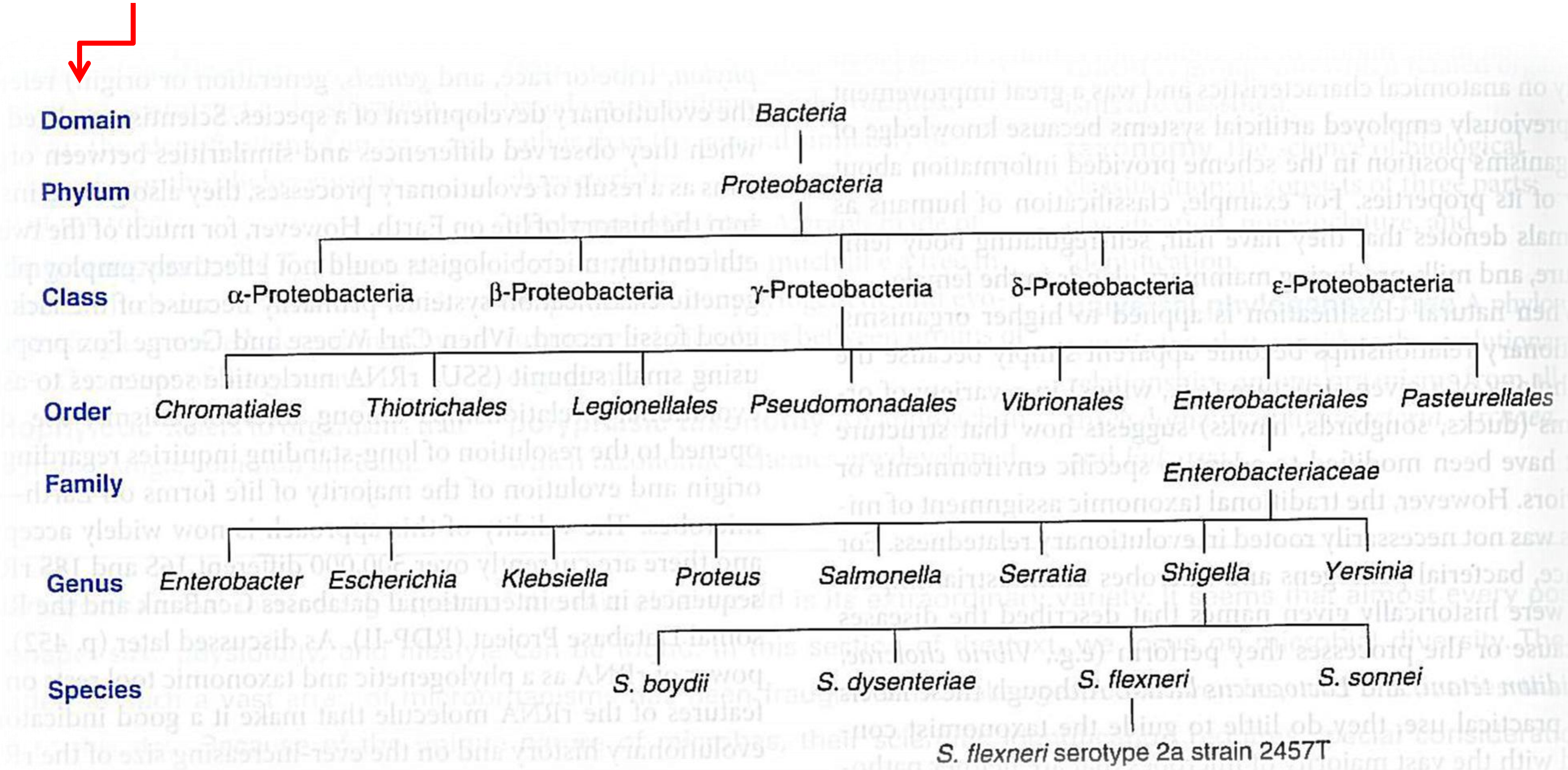
Polyphasic taxonomy

- Incorporates information from genetic, phenotypic and phylogenetic analysis
- Used for determining the genus* and species of a newly discovered (micro-)organism

*genus – well defined group of one or more species that is clearly separate from other genera

Classification: Hierarchical arrangement

Taxonomic ranks



Microbiologist use binomial system of Linné: genus & species

Question 2

Living in the 1970s....

Without molecular techniques

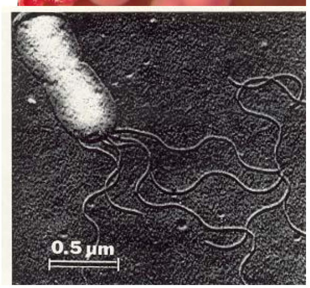
Which techniques would you have used to classify a microorganism?

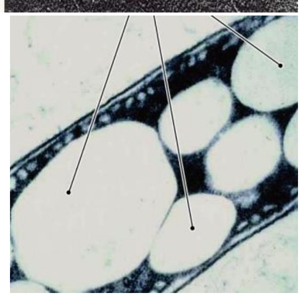
Which properties of the microorgansms were considered then?

Answer: several examples

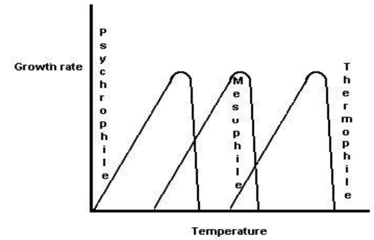
Classical methods!

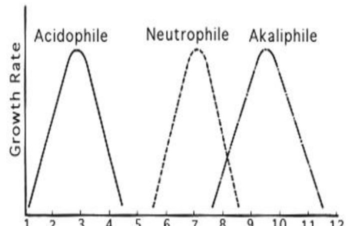
Morphology

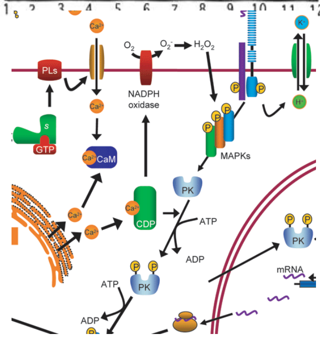


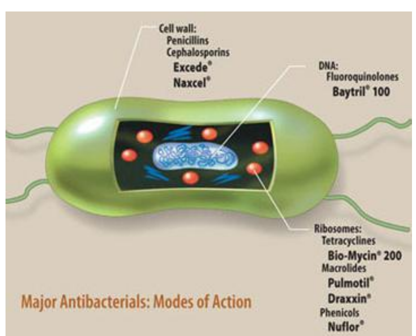


Physiology

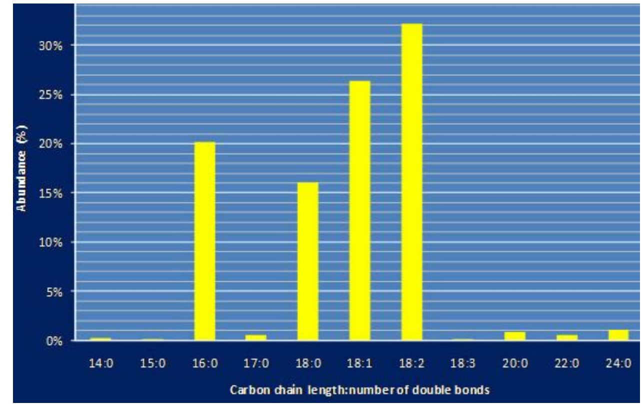








Biochemistry



Ecology

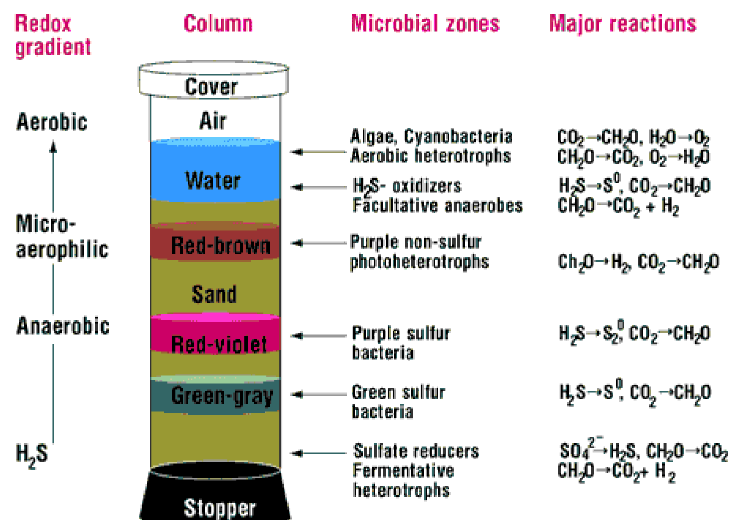


Table 19.10	Some Characteristic Differences between Gram-Negative and Gram-Positive Bacteria		
Property	**Gram-Negative Bacteria**	**Gram-Positive Bacteria**	**Mycoplasmas**
Cell wall	Gram-negative type wall with inner 2–7 nm peptidoglycan layer and outer membrane (7–8 nm thick) of lipid, protein, and lipopolysaccharide. (There may be a third outermost layer of protein.)	Gram-positive type wall with a homogeneous, thick cell wall (20–80 nm) composed mainly of peptidoglycan. Other polysaccharides and teichoic acids may be present.	Lack a cell wall and peptidoglycan precursors; enclosed by a plasma membrane
Cell shape	Spheres, ovals, straight or curved rods, helices or filaments; some have sheaths or capsules.	Spheres, rods, or filaments; may show true branching	Pleomorphic in shape; may be filamentous, can form branches
Reproduction	Binary fission, sometimes budding	Binary fission, filamentous forms grow by tip extension	Budding, fragmentation, and/or binary fission
Metabolism	Phototrophic, chemolithoautotrophic, or chemoorganoheterotrophic	Usually chemoorganoheterotrophic, a few phototrophic	Chemoorganoheterotrophic; most require cholesterol and long-chain fatty acids for growth.
Motility	Motile or nonmotile. Flagella placement can be varied—polar, lophotrichous, peritrichous. Motility may also result from the use of axial filaments (spirochetes) or gliding motility.	Most often nonmotile; have peritrichous flagella when motile	Usually nonmotile
Appendages	Can produce several types of appendages—pili and fimbriae, prosthecae, stalks	Usually lack appendages (may have spores on hyphae)	Lack appendages
Endospores	Cannot form endospores	Some groups	Cannot form endospores

Outcome of Classical methods

Methods for identification

Classical

See previous slides

Molecular

GC content

DNA-DNA hybridization

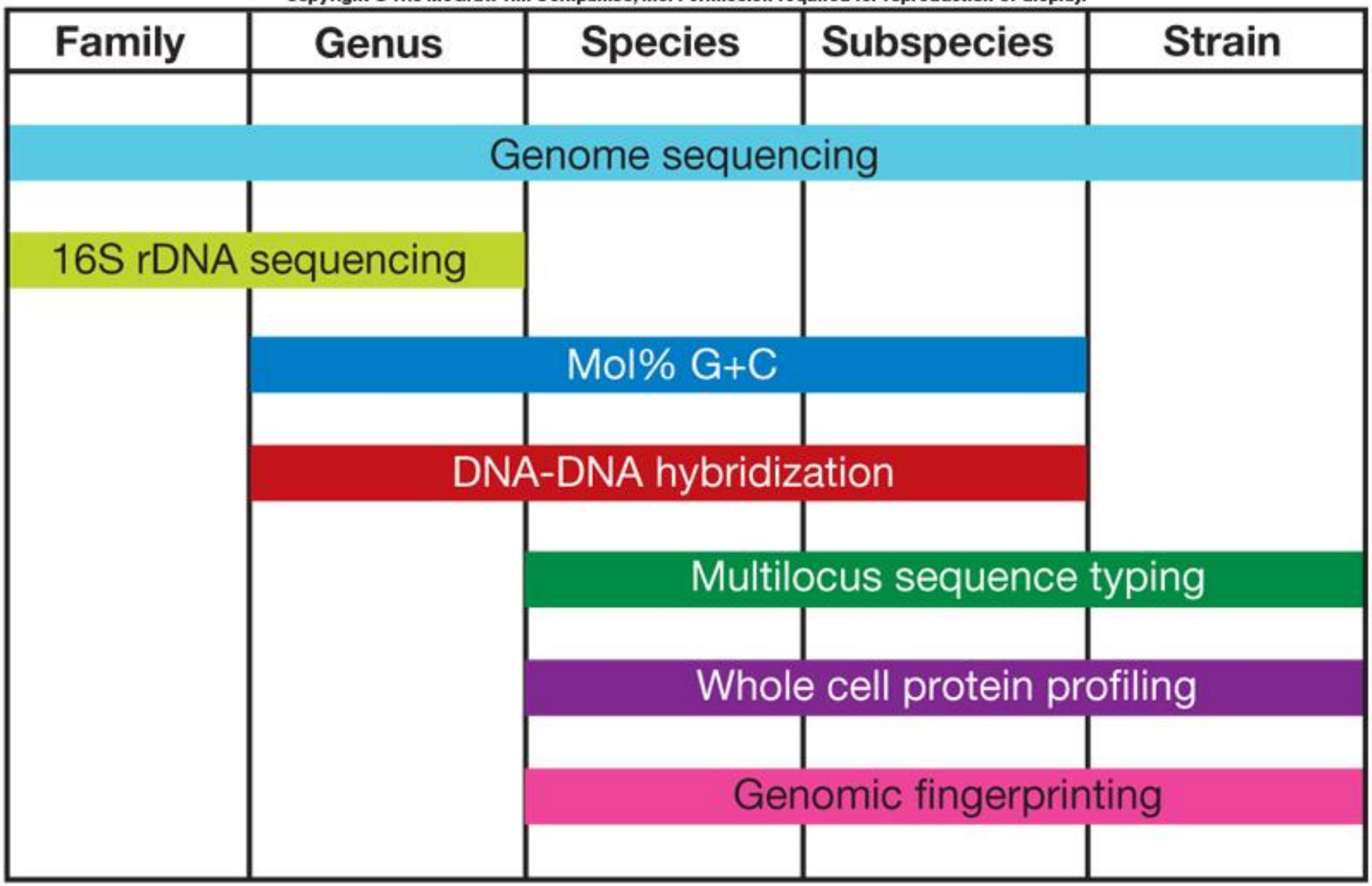
Amino acid sequencing

16R rRNA sequencing

Genomic fingerprinting

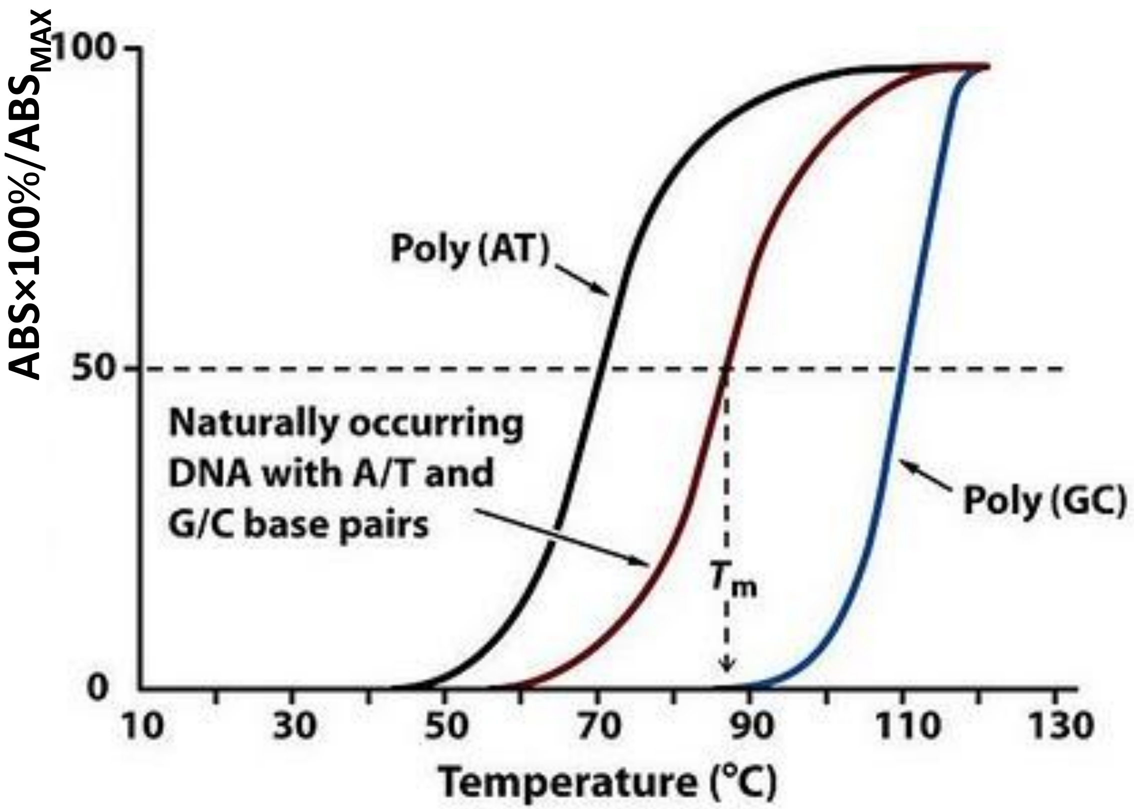
Genome sequencing

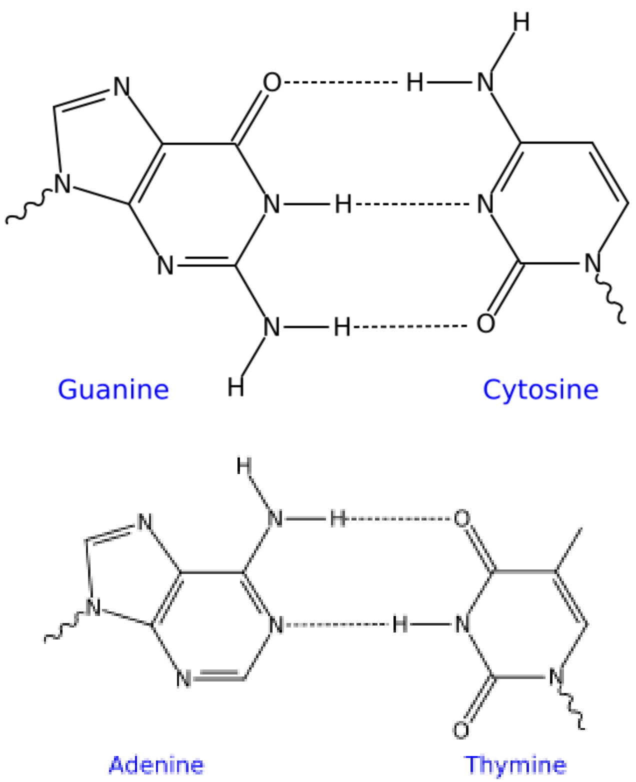
Relative taxonomic resolution



GC Content (nucleic acid base composition)

Question 3: Why T_m Poly(AT) < T_m poly(GC)?





Absorption at 260 nm (UV)

Principle DNA DNA hybridisation

Measure of sequence homology

common procedure:

- **Bind nonradioactive DNA to nitrocellulose filter**
- **Incubate filter with radioactive single-stranded DNA**
- **Measure amount of radioactive DNA attached to filter**

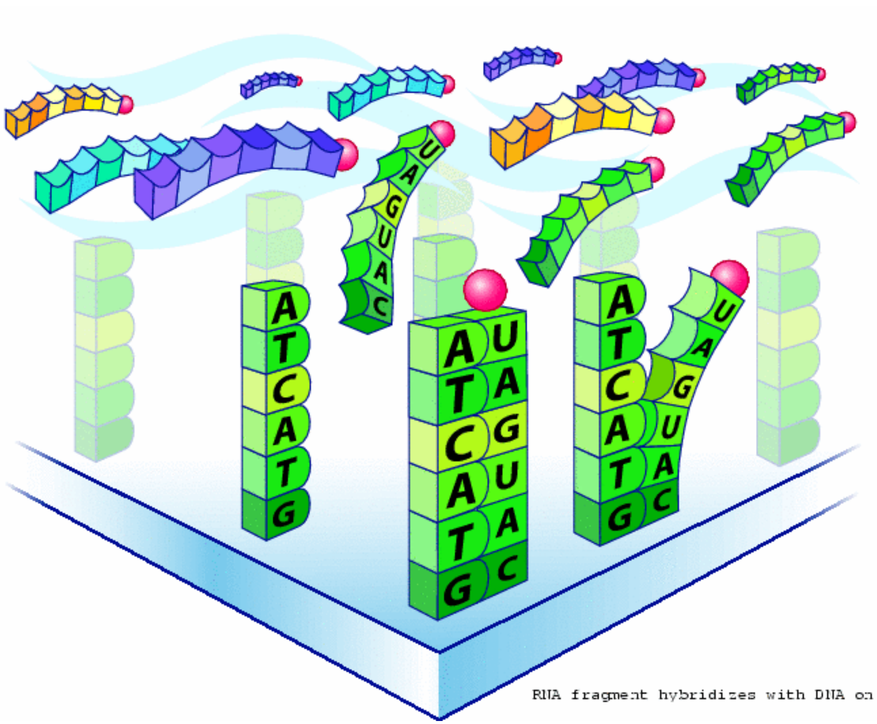
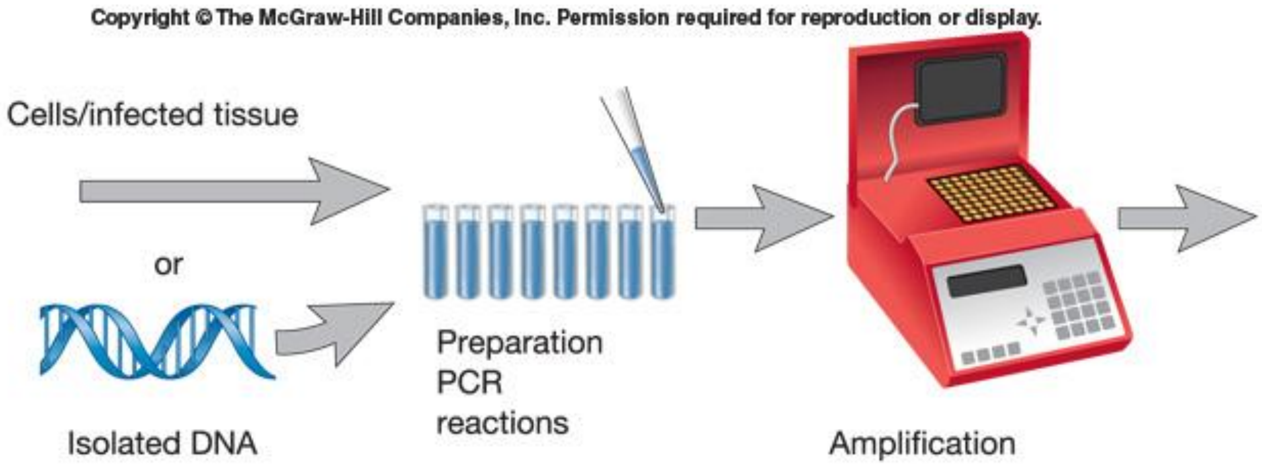


Table 17.4 Comparison of *Neisseria* Species by DNA Hybridization Experiments

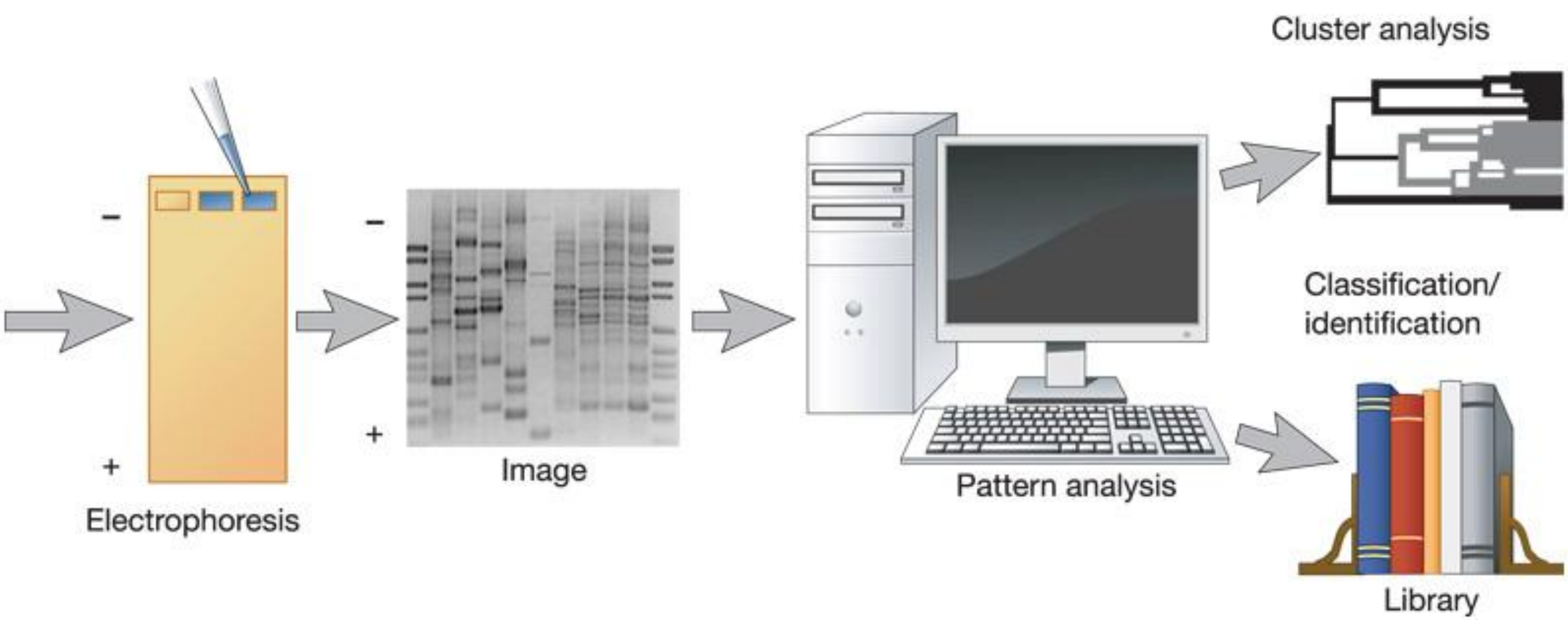
Membrane-Attached DNA[a]	*Percent Homology*[b]
Neisseria meningitidis	100
N. gonorrhoeae	78
N. sicca	45
N. flava	35

http://www.youtube.com/watch?v=76eKnmCtCTU

Principle of genomic fingerprinting



Add Specific primers for Repetitive Nucleotide Sequences



PhD student improves police DNA analysis

29 April 2011

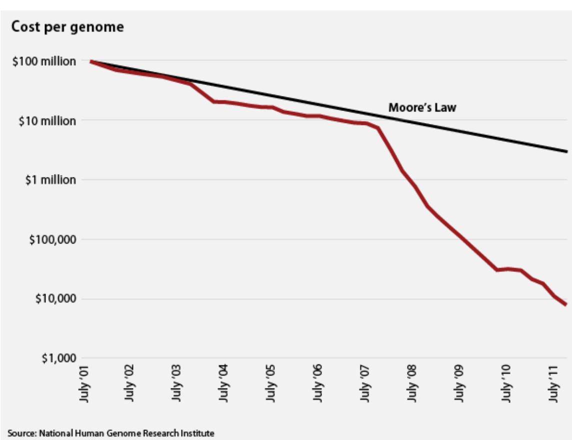
Statens kriminaltekniska laboratorium - SKL

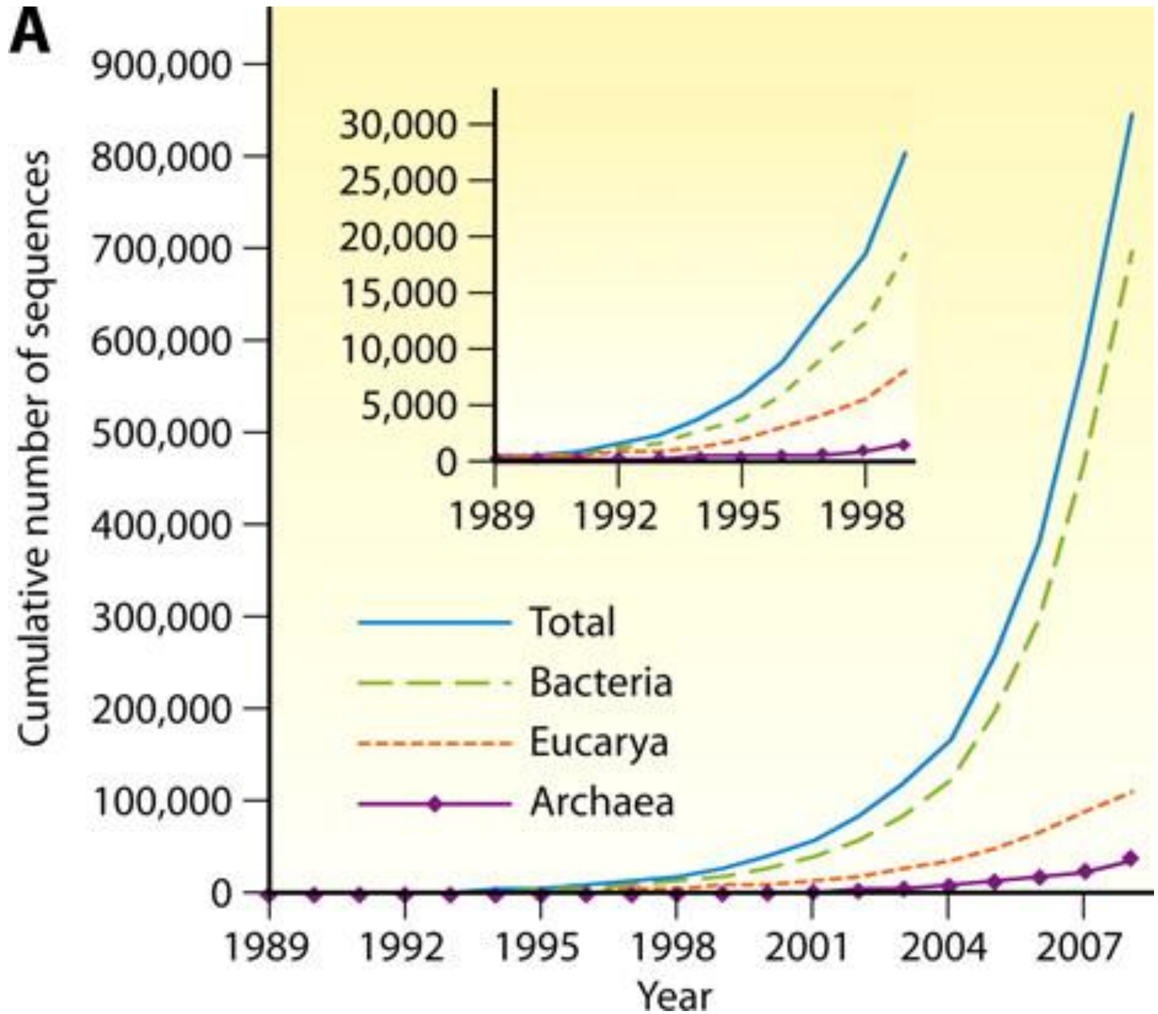
The police have begun using an improved method of DNA analysis, developed by **Johannes Hedman**, a doctoral student at **Applied Microbiology**. Together with the Swedish National Laboratory of Forensic Science, **SKL**, he has created a new enzyme combination that makes DNA profiles from crime scene samples clearer. This raises the chances of linking the perpetrator to the crime when there is little genetic material and the sample is dirty – which is often the case.

Whole genome sequencing

Technique becomes:

-**Cheaper**
- **Easier**
- **Scaled down:**
  -**Lab bench size**

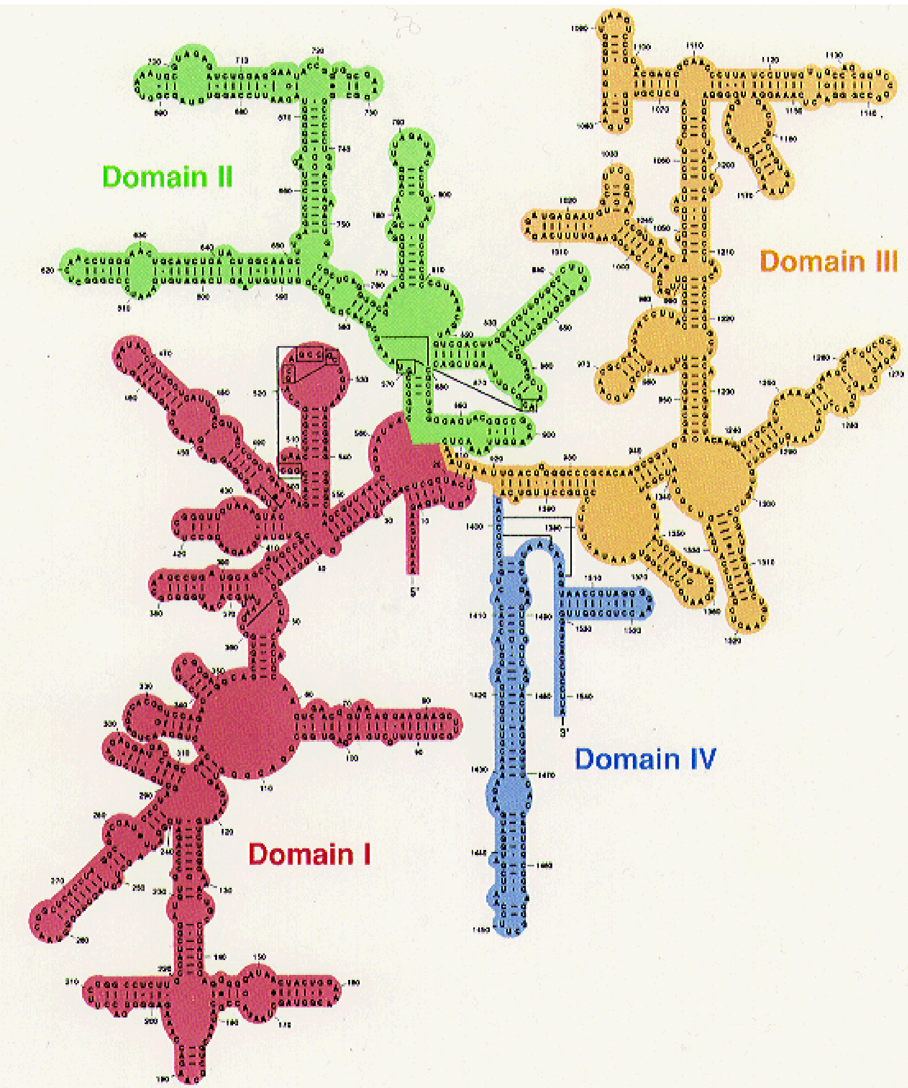


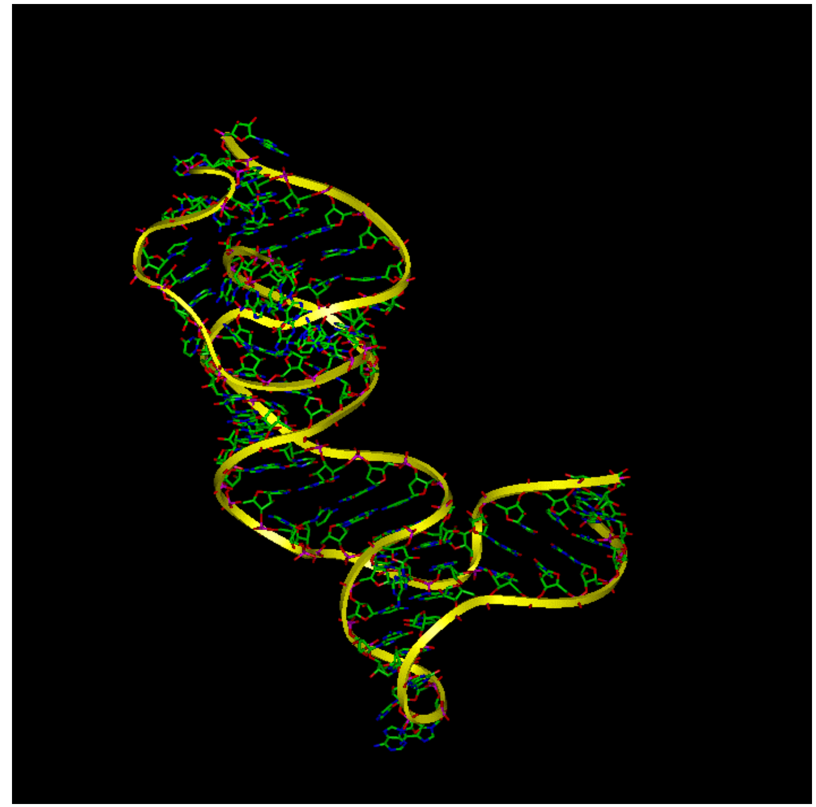


Pace (2009) Microbiol Mol Biol Rev 73:565-576

Sequencing 16S rRNA for prokaryotic phylogenetic tree

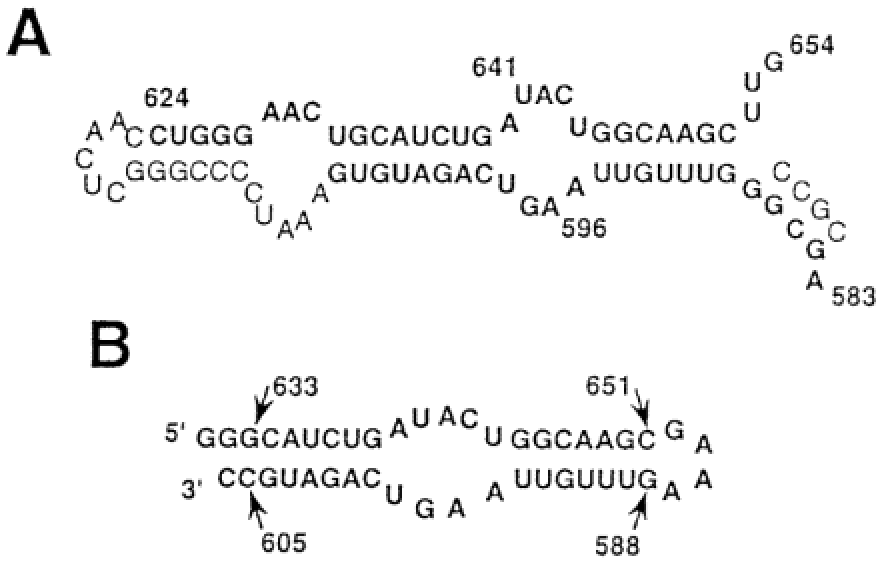
2D & 3D forms



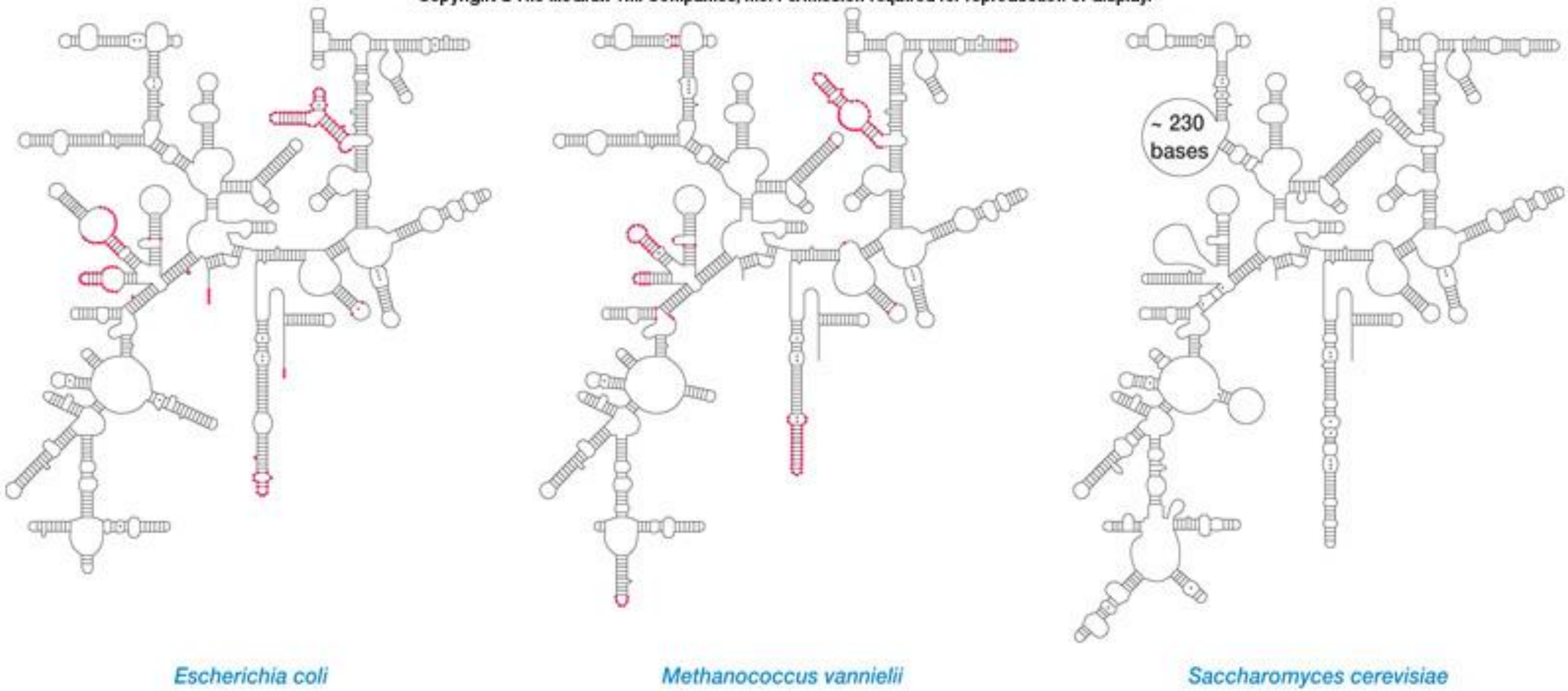


16S rRNA fluctuates during equilibrium as simulated here

Question 4: how does the RNA form stems and loops?



16S rRNA & 18S rRNA



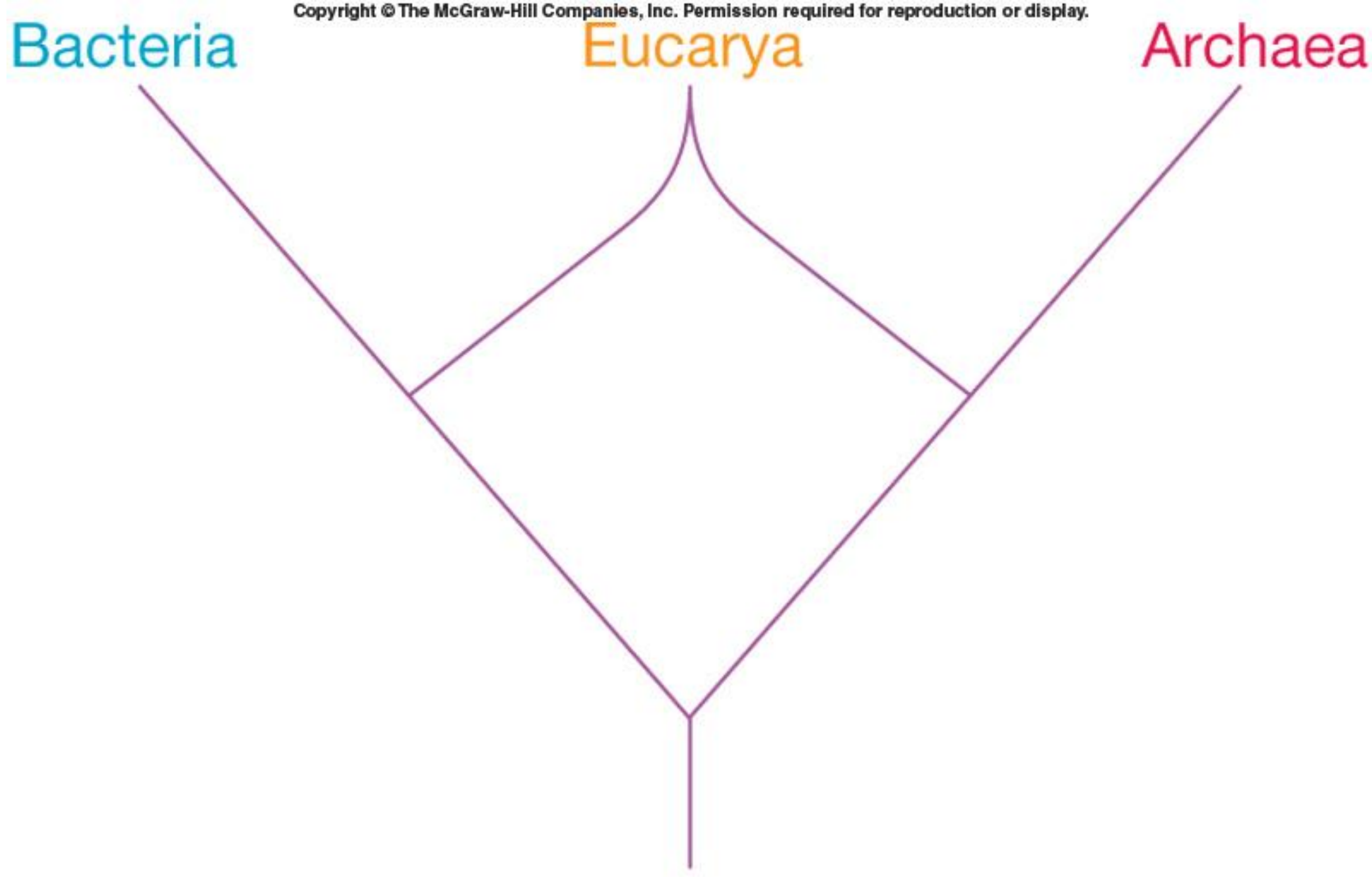
Bacterium **Archea** **Eukaryote**

Table 19.1 Comparison of *Bacteria*, *Archaea*, and *Eucarya*

Property	*Bacteria*	*Archaea*	*Eucarya*
Membrane-Enclosed Nucleus with Nucleolus	Absent	Absent	Present
Complex Internal Membranous Organelles	Absent	Absent	Present
Cell Wall	Almost always have peptidoglycan containing muramic acid	Variety of types, no muramic acid	No muramic acid
Membrane Lipid	Have ester-linked, straight-chained fatty acids	Have ether-linked, branched aliphatic chains	Have ester-linked, straight-chained fatty acids
Gas Vesicles	Present	Present	Absent
Transfer RNA	Thymine present in most tRNAs *N*-formylmethionine carried by initiator tRNA	No thymine in T or TψC arm of tRNA Methionine carried by initiator tRNA	Thymine present Methionine carried by initiator tRNA
Polycistronic mRNA	Present	Present	Absent
mRNA Introns	Absent	Absent	Present
mRNA Splicing, Capping, and Poly A Tailing	Absent	Absent	Present
Ribosomes			
Size	70S	70S	80S (cytoplasmic ribosomes)
Elongation factor 2 reaction with diphtheria toxin	Does not react	Reacts	Reacts
Sensitivity to chloramphenicol and kanamycin	Sensitive	Insensitive	Insensitive
Sensitivity to anisomycin	Insensitive	Sensitive	Sensitive
DNA-Dependent RNA Polymerase			
Number of enzymes	One	One	Three
Structure	Simple subunit pattern (6 subunits)	Complex subunit pattern similar to eucaryotic enzymes (8–12 subunits)	Complex subunit pattern (12–14 subunits)
Rifampicin sensitivity	Sensitive	Insensitive	Insensitive
Polymerase II Type Promoters	Absent	Present	Present
Metabolism			
Similar ATPase	No	Yes	Yes
Methanogenesis	Absent	Present	Absent
Nitrogen fixation	Present	Present	Absent
Chlorophyll-based photosynthesis	Present	Absent	Present[a]
Chemolithotrophy	Present	Present	Absent

[a]Present in chloroplasts (of bacterial origin).

A favoured phylogenetic tree of the 3 Domains

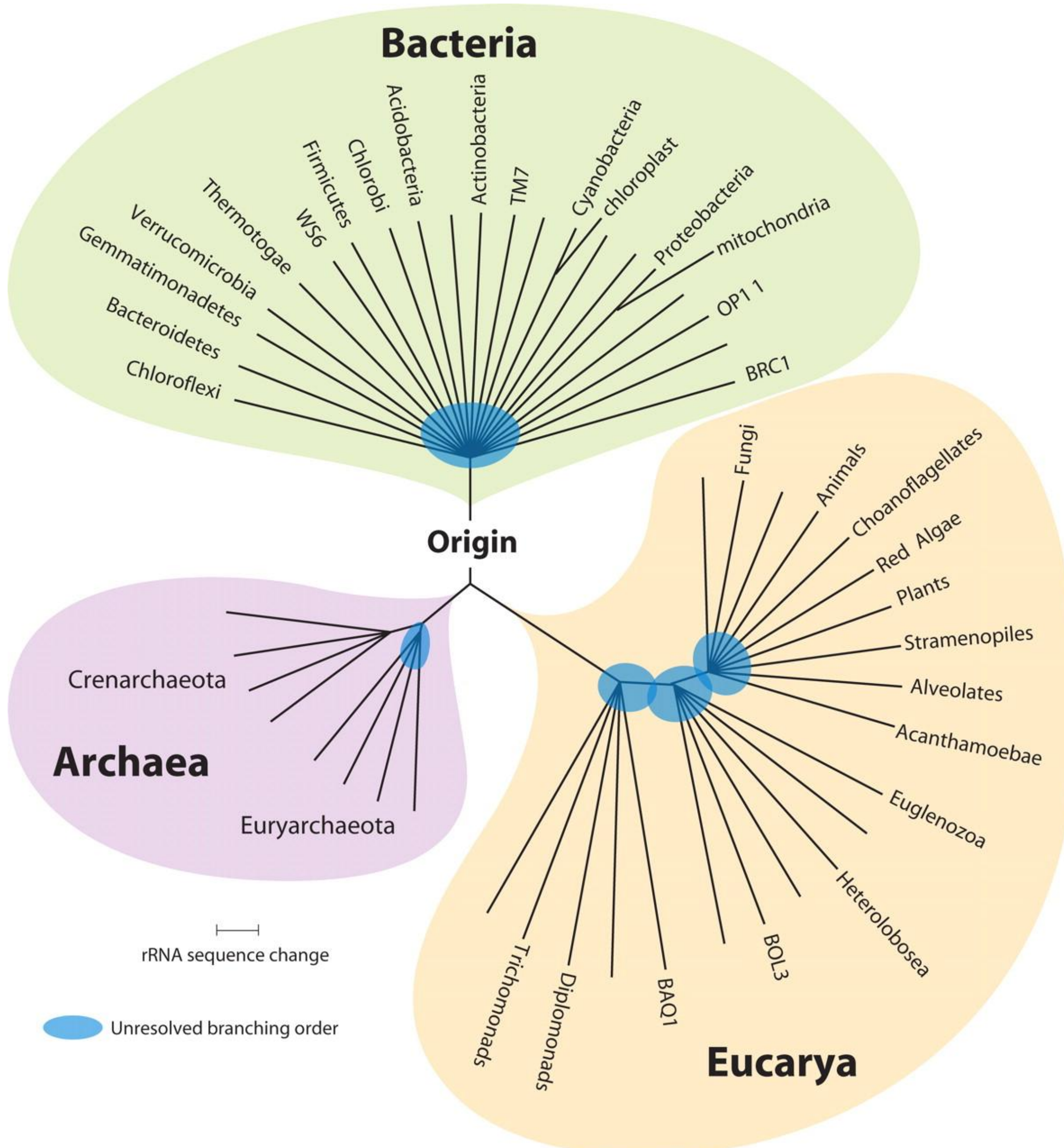


Woese 1977

3 Domains of life

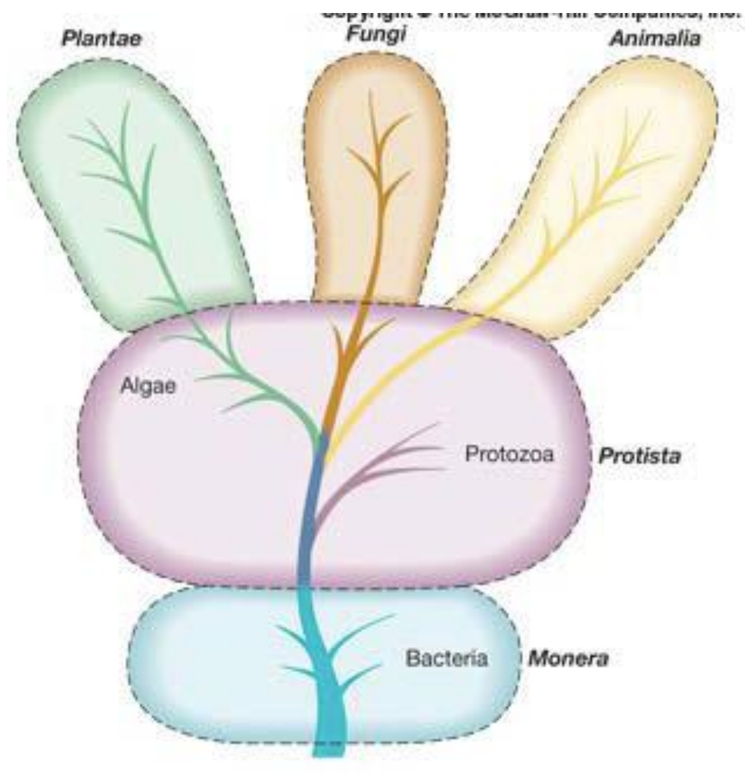
Microbiologist point of view ☺

Question 5:
What does the biologist not like in this picture?



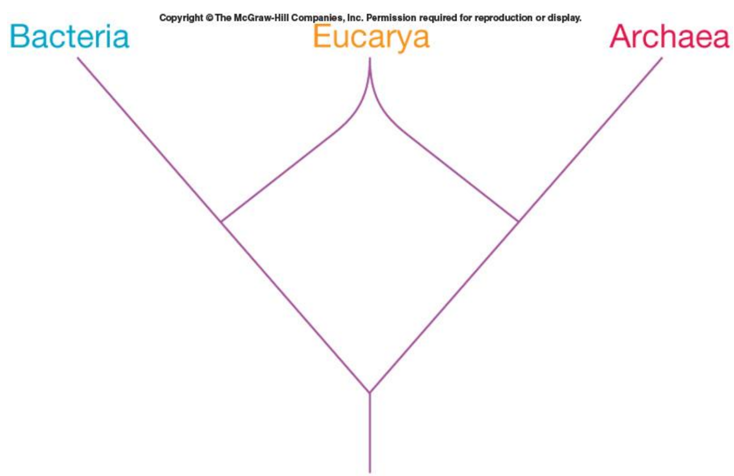
Pace (2009) Microbiol Mol Biol Rev 73:565-576

Suggested: Kingdoms of life

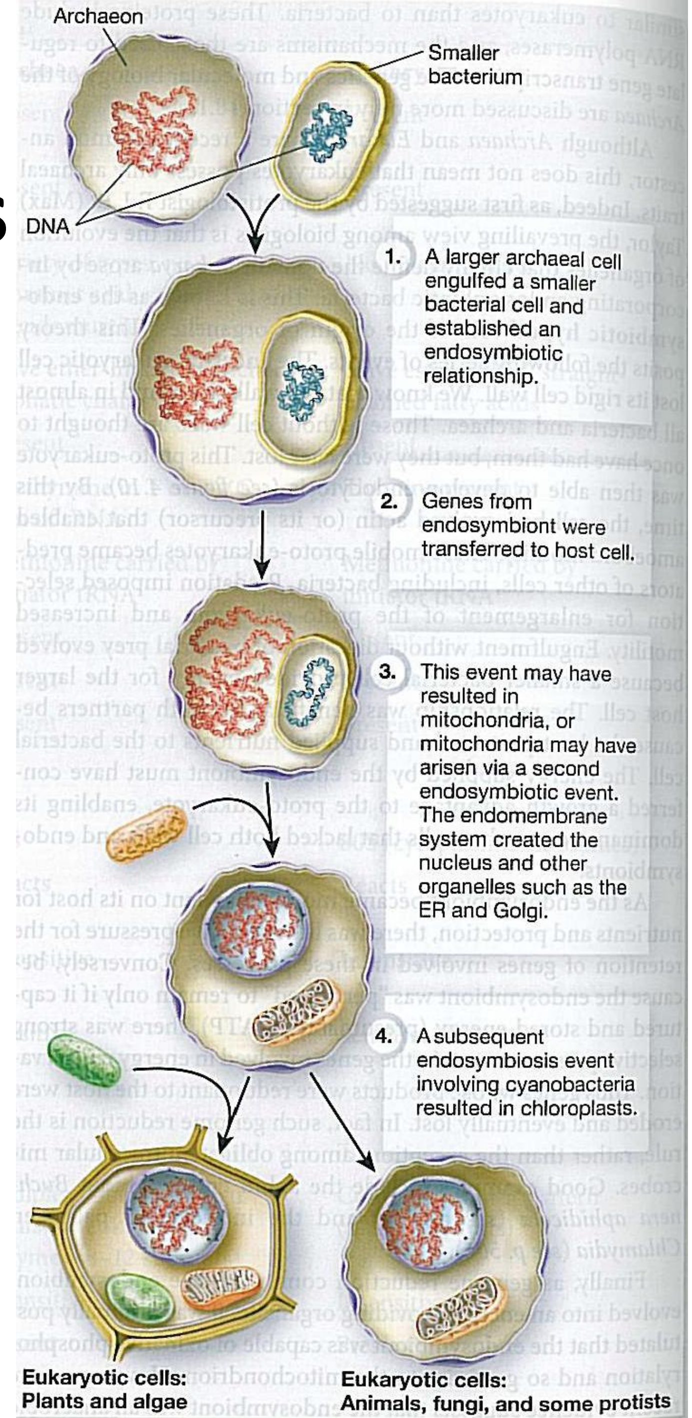


Biologists point of view

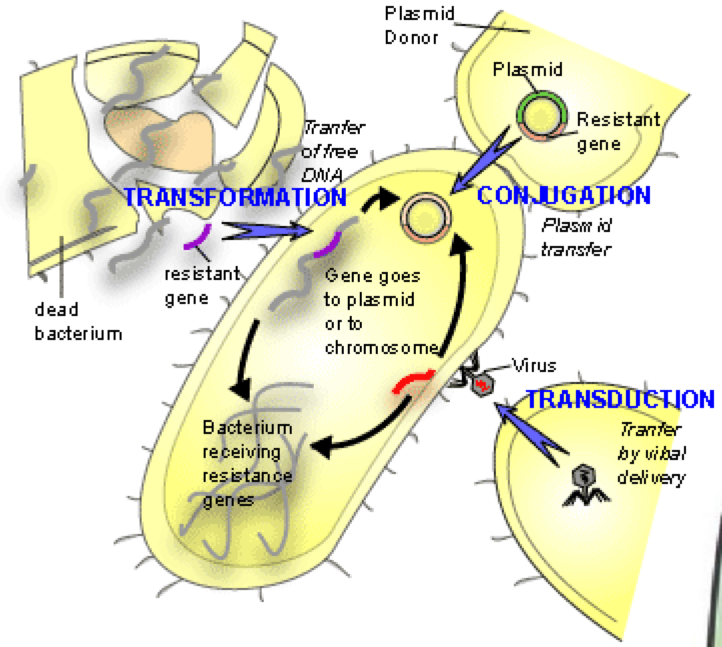
From prokaryotic endosymbionts to eukaryotes

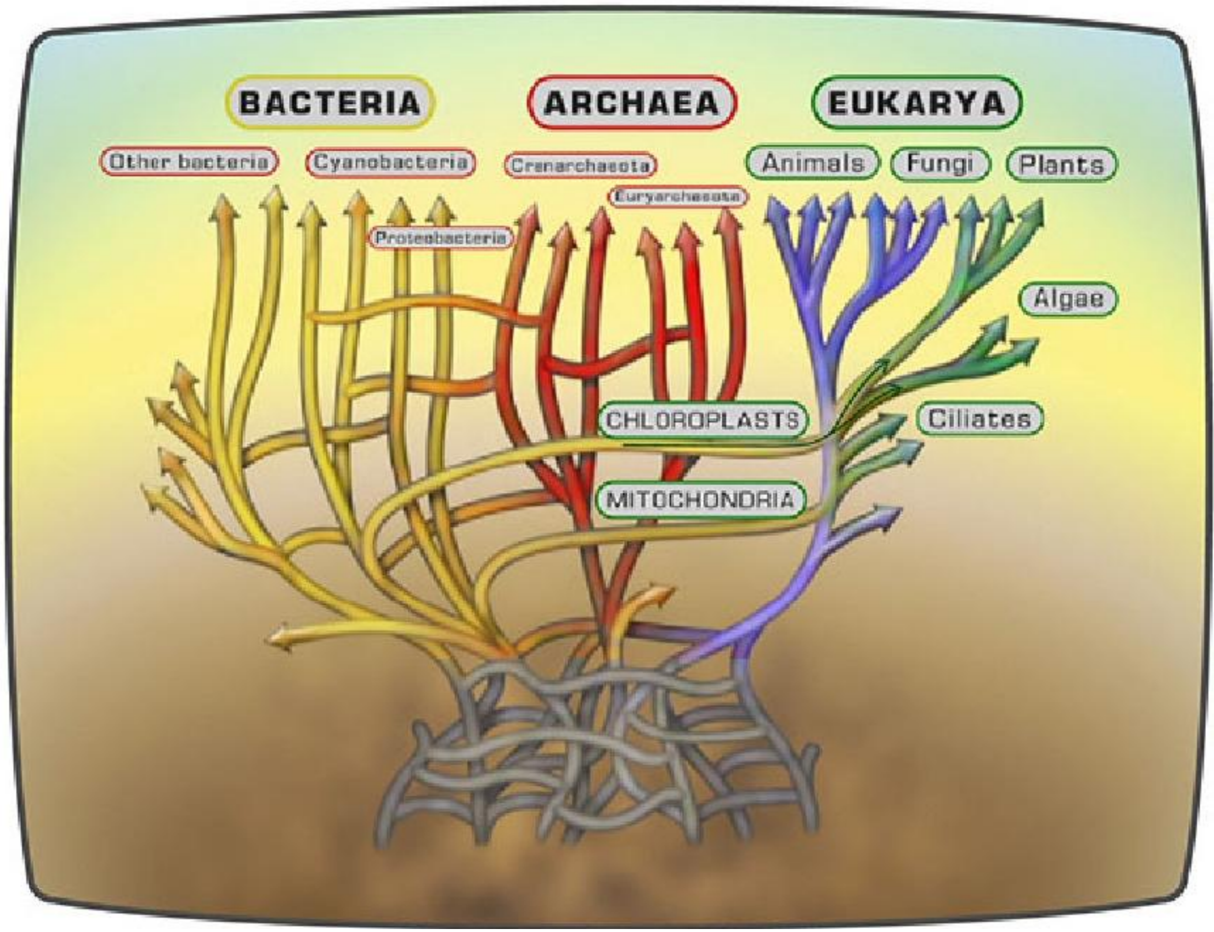


Lynn Margulis 1970
endosymbiosis



Lateral gene transfer





Taxonomy mindmap

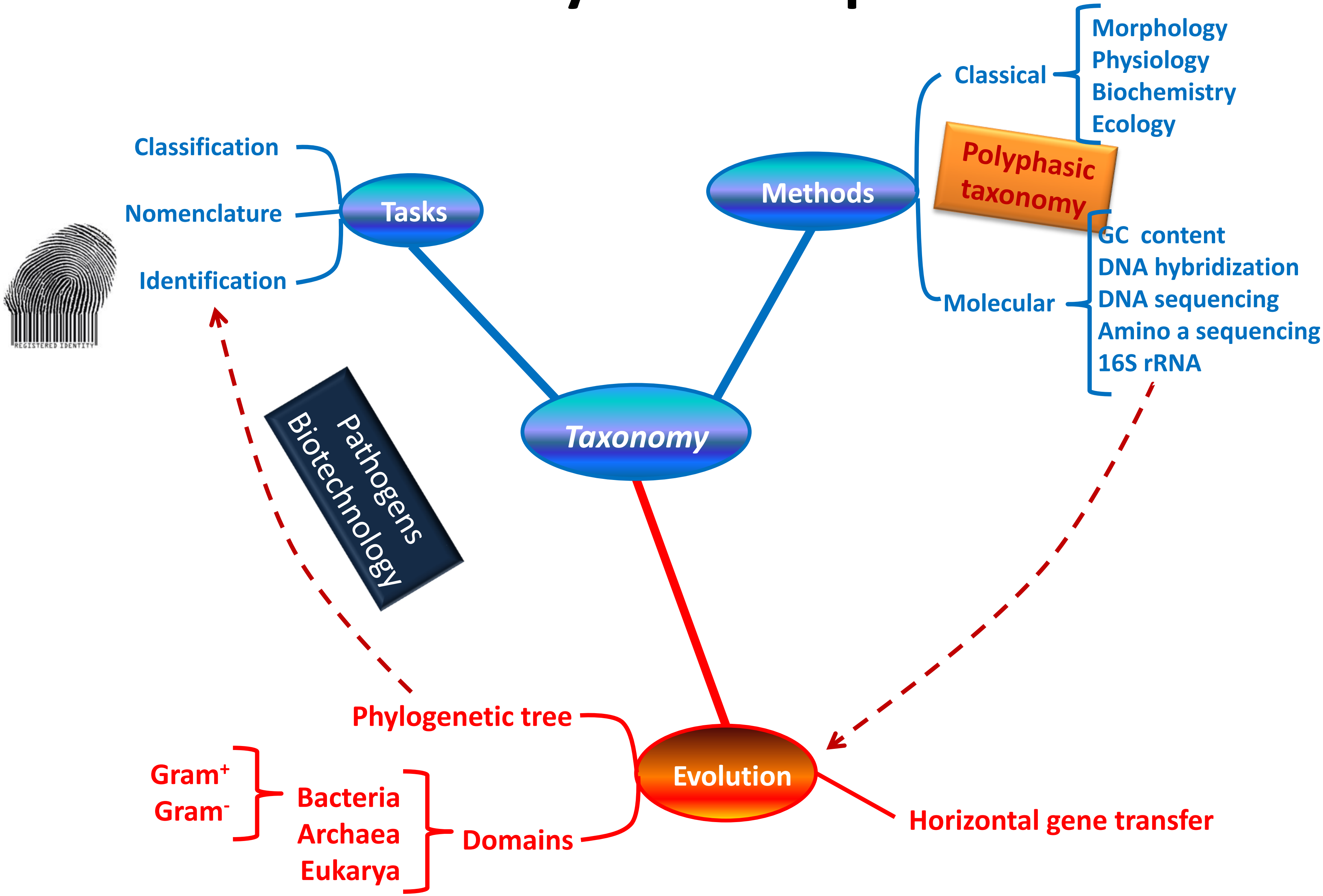
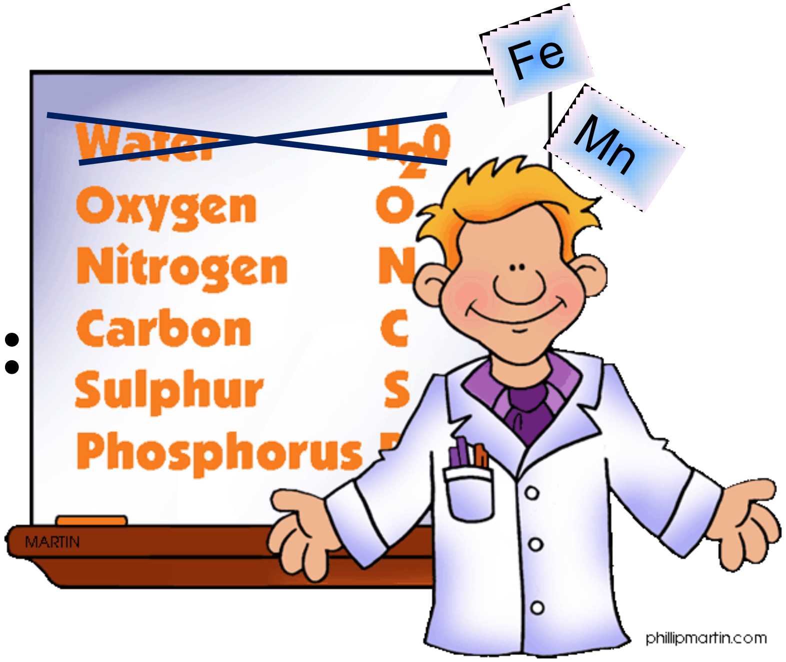


Table 19.6 Representative G + C Content of Microorganisms

Organism	Percent G + C
Bacteria	
Actinomyces	59–73
Anabaena	39–44
Bacillus	32–62
Bacteroides	28–61
Bdellovibrio	49.5–51
Caulobacter	62–65
Chlamydia	41–44
Chlorobium	49–58
Chromatium	48–70
Clostridium	21–54
Cytophaga	33–42
Deinococcus	62–70
Escherichia	48–59
Halobacterium	66–68
Hyphomicrobium	59–67
Methanobacterium	32–50
Micrococcus	64–75
Mycobacterium	62–70
Mycoplasma	23–40
Myxococcus	68–71
Neisseria	48–56
Nitrobacter	59–62
Oscillatoria	40–50
Prochloron	41
Proteus	38–41
Pseudomonas	58–69
Rhodospirillum	62–66
Rickettsia	29–33
Salmonella	50–53
Spirillum	38
Spirochaeta	51–65
Staphylococcus	30–38
Streptococcus	33–44
Streptomyces	69–73
Sulfolobus	31–37
Thermoplasma	46
Thiobacillus	52–68
Treponema	25–53
Protists	
Acanthamoeba castellanii	56–58
Acetabularia mediterranea	37–53
Amoeba proteus	66
Chlamydomonas	60–68
Chlorella	43–79
Cyclotella cryptica	41
Dictyostelium	22–25
Euglena gracilis	46–55
Lycogala	42
Nitella	49
Nitzschia angularis	47
Ochromonas danica	48
Paramecium spp.	29–39
Peridinium triquetrum	53
Physarum polycephalum	38–42
Plasmodium berghei	41
Scenedesmus	52–64
Spirogyra	39
Stentor polymorphus	45
Tetrahymena	19–33
Trichomonas	29–34
Trypanosoma	45–59
Volvox carteri	50
Fungi	
Agaricus bisporus	44
Amanita muscaria	57
Aspergillus niger	52
Blastocladiella emersonii	66
Candida albicans	33–35
Claviceps purpurea	53
Coprinus lagopus	52–53
Fomes fraxineus	56
Mucor rouxii	38
Neurospora crassa	52–54
Penicillium notatum	52
Polyporus palustris	56
Rhizopus nigricans	47
Saccharomyces cerevisiae	36–42
Saprolegnia parasitica	61

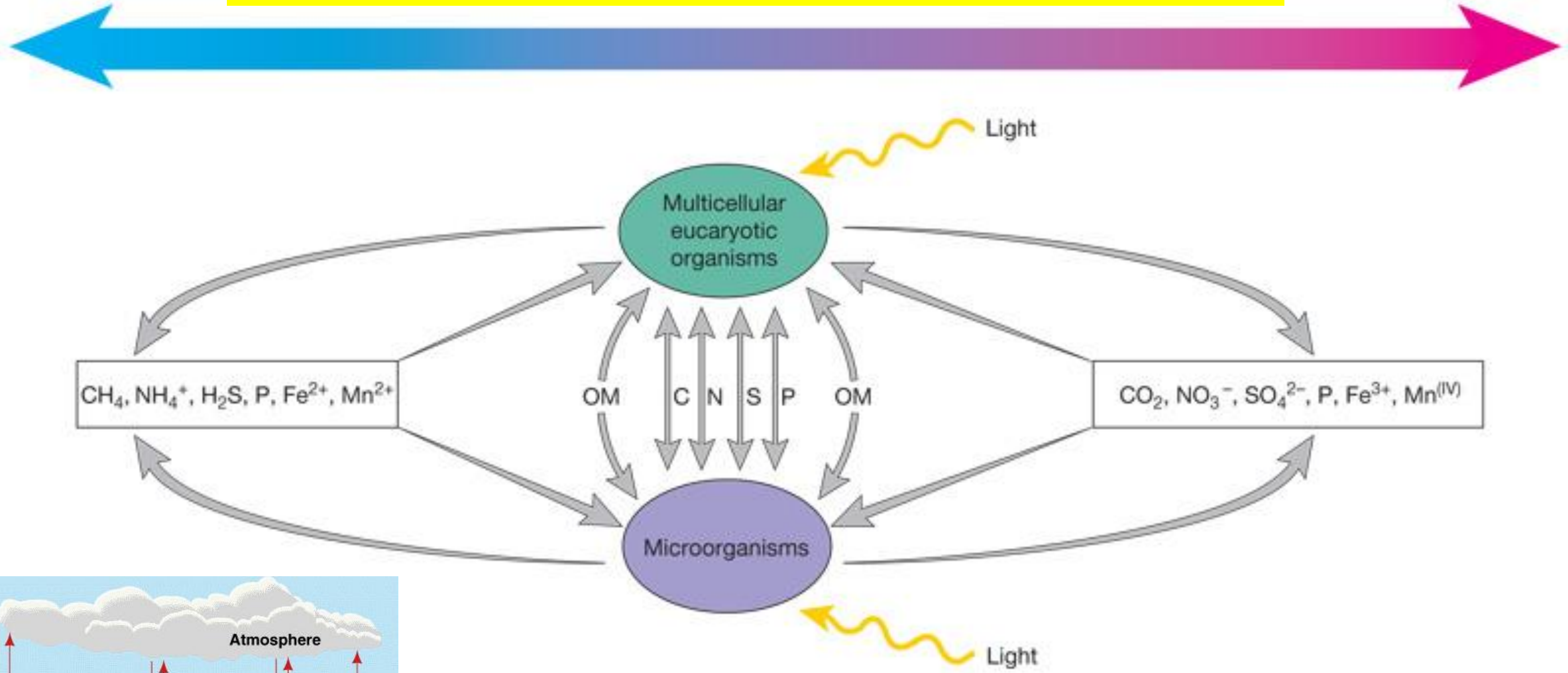
Biogeochemical cycles & Mineralisation

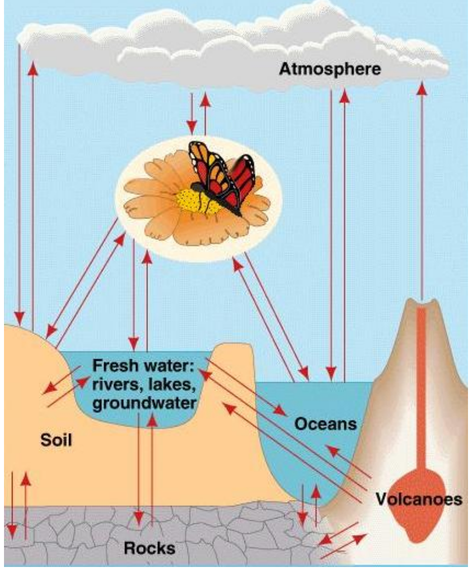
a) Elemental cycles:



Biomass substitute for oil

Macrobiogeochemistry





Question 1: Where do you expect
- The reduced forms to be produced?
- The oxidized forms to be produced?

Fig. 27.3 Influence of oxygen on degradation of organic matter.

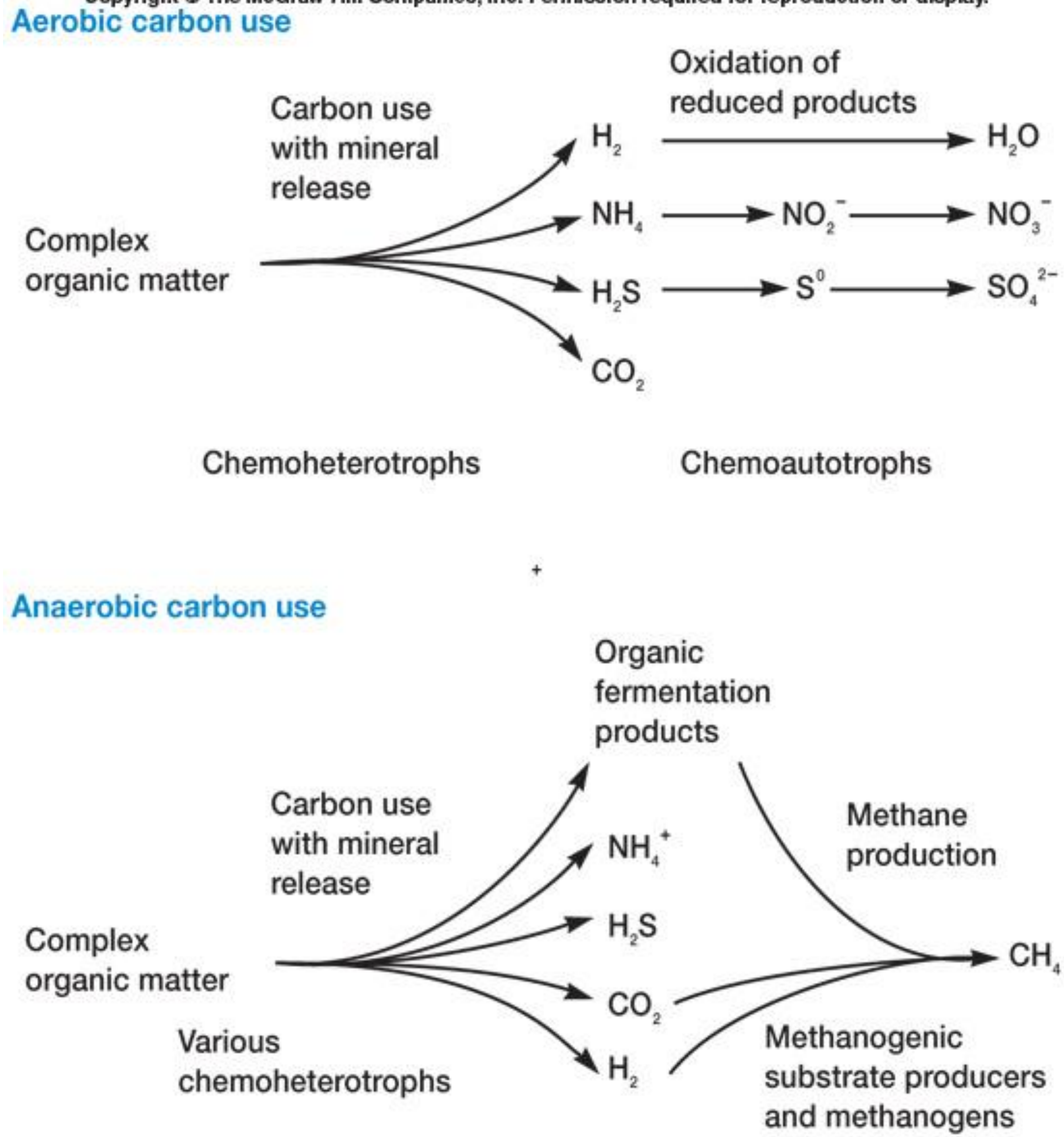


Table 5.1 Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source
Heterotrophs	Reduced, preformed, organic molecules from other organisms
Energy Sources	
Phototrophs	Light
Chemotrophs	Oxidation of organic or inorganic compounds
Electron Sources	
Lithotrophs	Reduced inorganic molecules
Organotrophs	Organic molecules

Question 2: What is a
-Photolithoautotroph? Give an example
- Chemoorganoheterotroph? Give an example

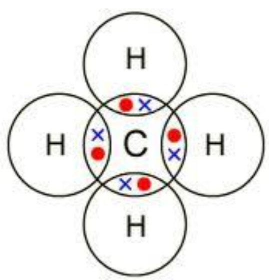
Table 5.2 Major Nutritional Types of Microorganisms

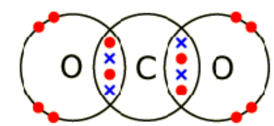
Nutritional Type	Carbon Source	Energy Source	Electron Source	Representative Microorganisms
Photolithoautotrophy (photolithotrophic autotrophy)	CO_2	Light	Inorganic e^- donor	Purple and green sulfur bacteria, cyanobacteria
Photoorganoheterotrophy (photoorganotrophic heterotrophy)	Organic carbon, but CO_2 may also be used	Light	Organic e^- donor	Purple nonsulfur bacteria, green nonsulfur bacteria
Chemolithoautotrophy (chemolithotrophic autotrophy)	CO_2	Inorganic chemicals	Inorganic e^- donor	Sulfur-oxidizing bacteria, hydrogen-oxidizing bacteria, methanogens, nitrifying bacteria, iron-oxidizing bacteria
Chemolithoheterotrophy or mixotrophy (chemolithotrophic heterotrophy)	Organic carbon, but CO_2 may also be used	Inorganic chemicals	Inorganic e^- donor	Some sulfur-oxidizing bacteria (e.g., *Beggiatoa*)
Chemoorganoheterotrophy (chemoorganotrophic heterotrophy)	Organic carbon	Organic chemicals often same as C source	Organic e^- donor, often same as C source	Most nonphotosynthetic microbes, including most pathogens, fungi, many protists, and many archaea

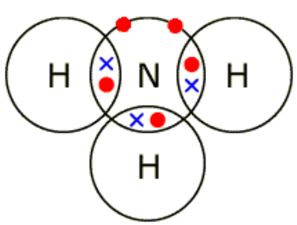
Table 27.1 The Major Forms of Carbon, Nitrogen, Sulfur, and Iron Important in Biogeochemical Cycling

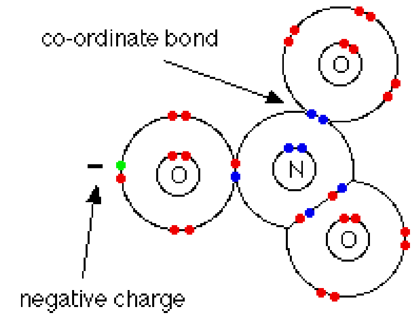
		Major Forms and Valences				
Cycle	Significant Gaseous Component Present?	Reduced Forms	Intermediate Oxidation State Forms			Oxidized Forms
C	Yes	Methane: CH_4 (−4)	Carbon monoxide CO (+2)			CO_2 (+4)
N	Yes	Ammonium: NH_4^+, organic N (−3)	Nitrogen gas: N_2 (0)	Nitrous oxide N_2O (+1)	Nitrite: NO_2^- (+3)	Nitrate: NO_3^- (+5)
S	Yes	Hydrogen sulfide: H_2S, SH groups in organic matter (−2)	Elemental sulfur: S^0 (0)	Thiosulfate: $S_2O_3^{2-}$ (+2)	Sulfite: SO_3^{2-} (+4)	Sulfate: SO_4^{2-} (+6)
Fe	No	Ferrous iron: Fe^{2+} (+2)				Ferric Iron: Fe^{3+} (+3)

Note: The carbon, nitrogen, and sulfur cycles have significant gaseous components, and these are described as gaseous nutrient cycles. The iron cycle does not have a gaseous component, and this is described as a sedimentary nutrient cycle. Major reduced, intermediate oxidation state, and oxidized forms are noted, together with valences.

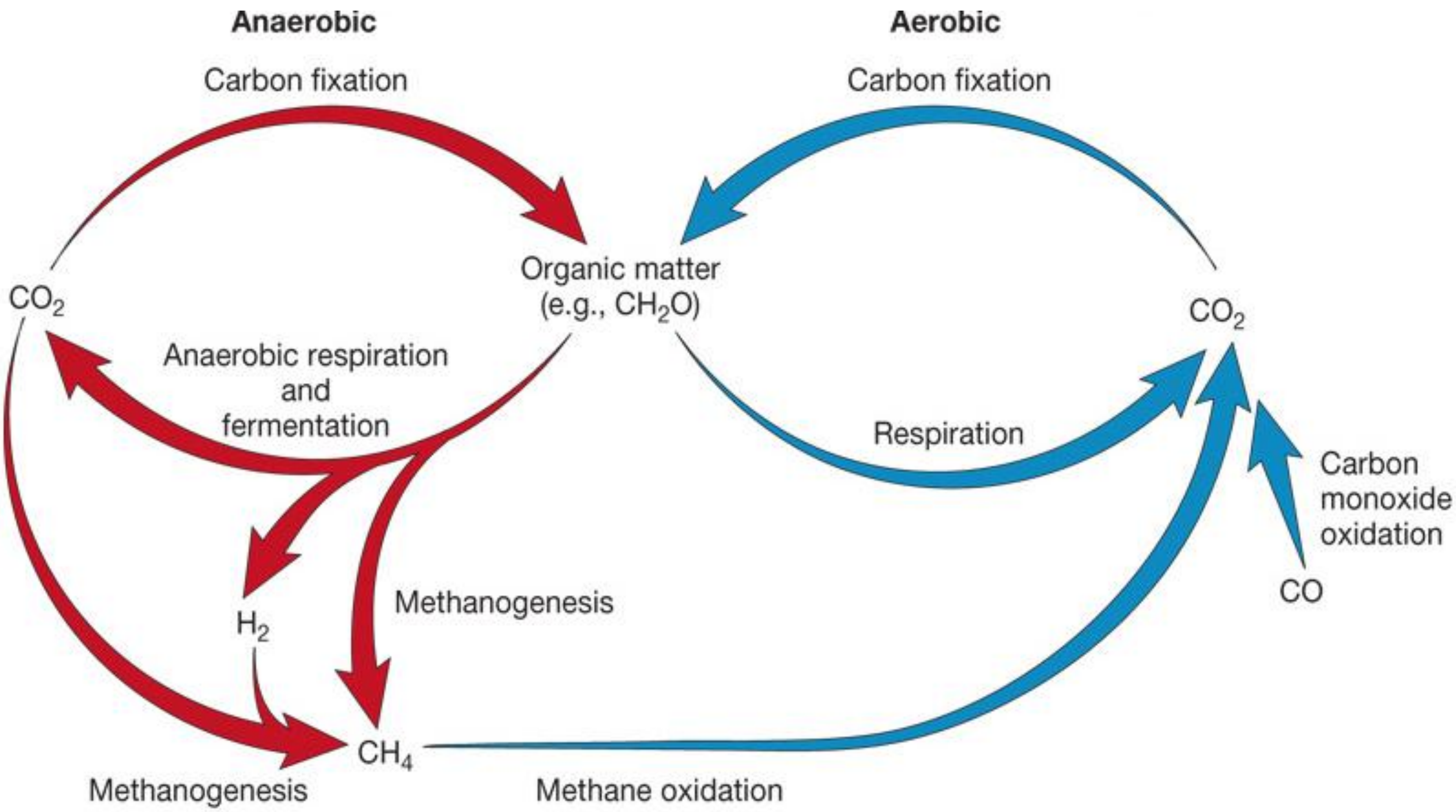


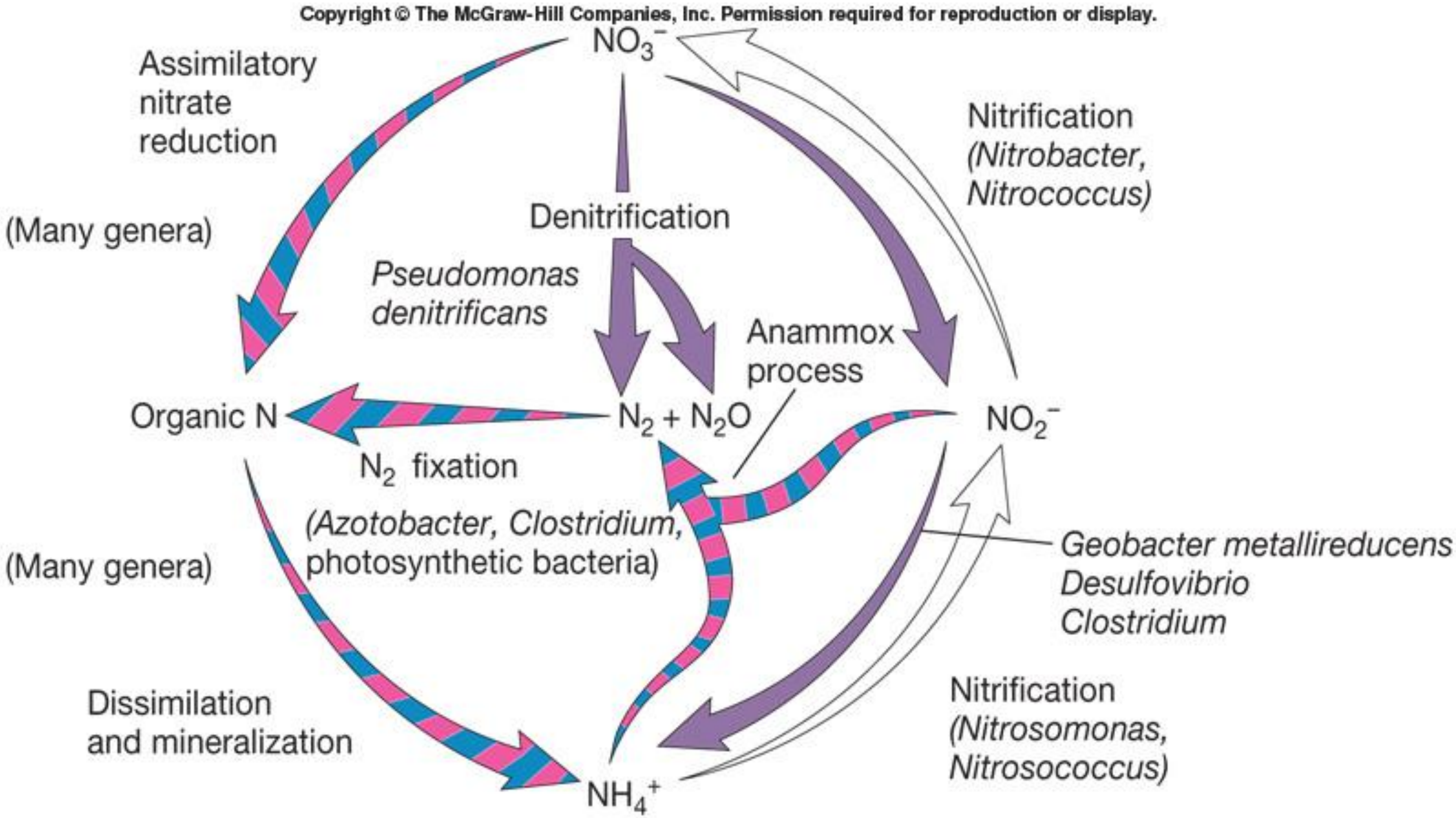






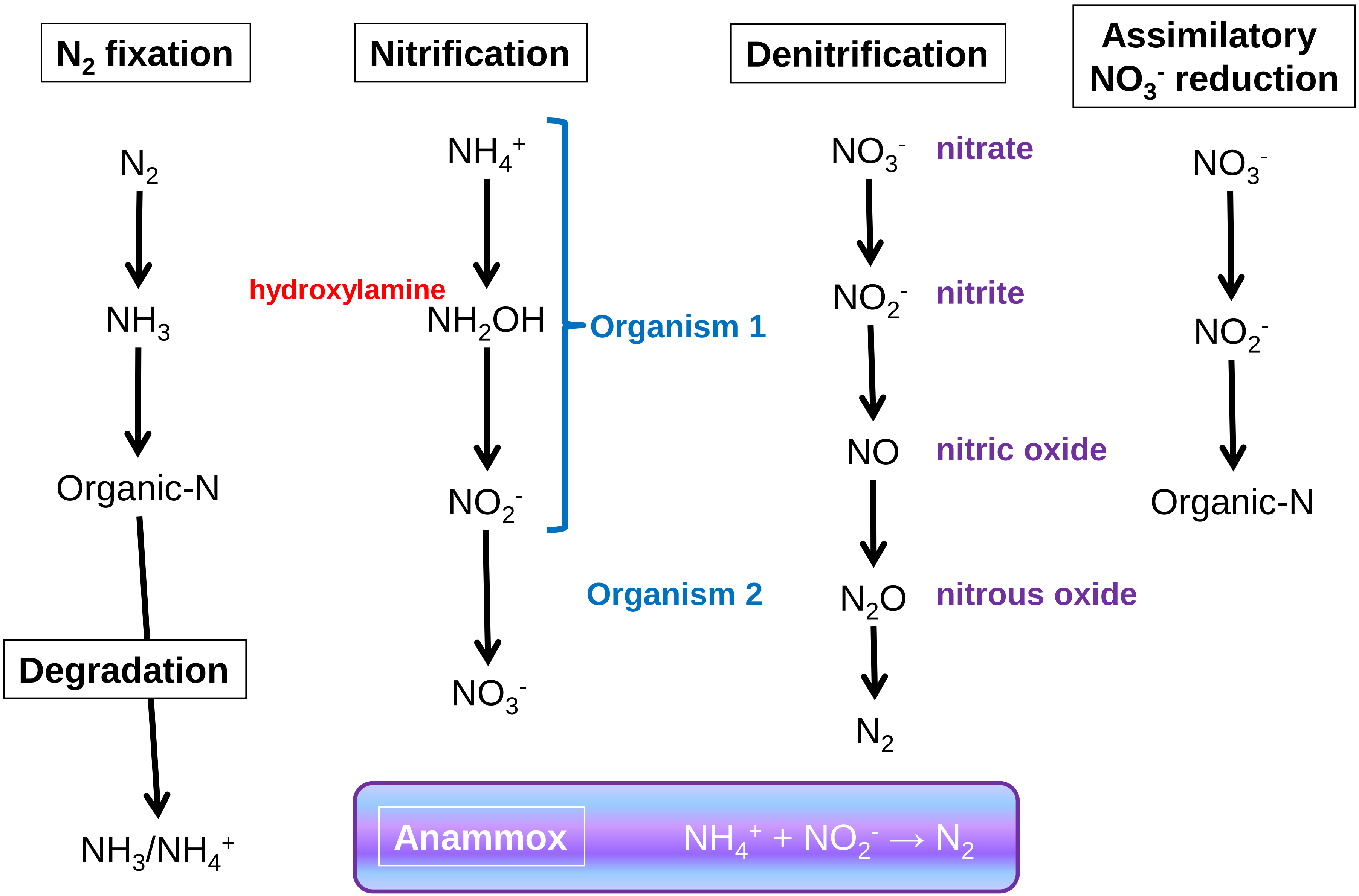
Carbon cycle





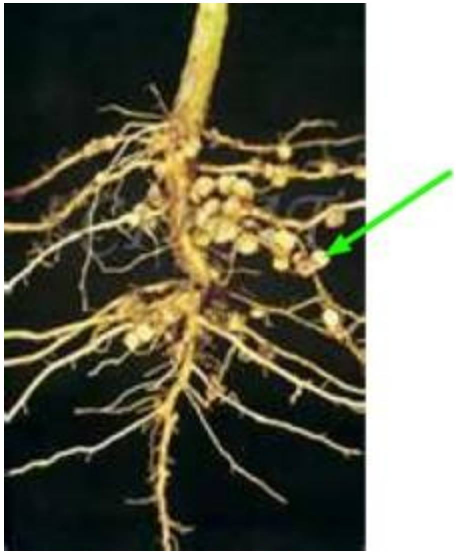
Nitrogen cycle

Nitrogen reactions more detailed



Nodules on the roots of Legume plant

Rhizobium species live in the nodules to fix N_2. Dead rhizobia are N-source for plant to synthesize amino acids.



$$N_2 + 3H_2 \rightarrow 2\ NH_3$$

Nitrogenase, ATP, Mo, Fe

$165 \cdot 10^6$ ton N/yr

Haber-Bosch process:

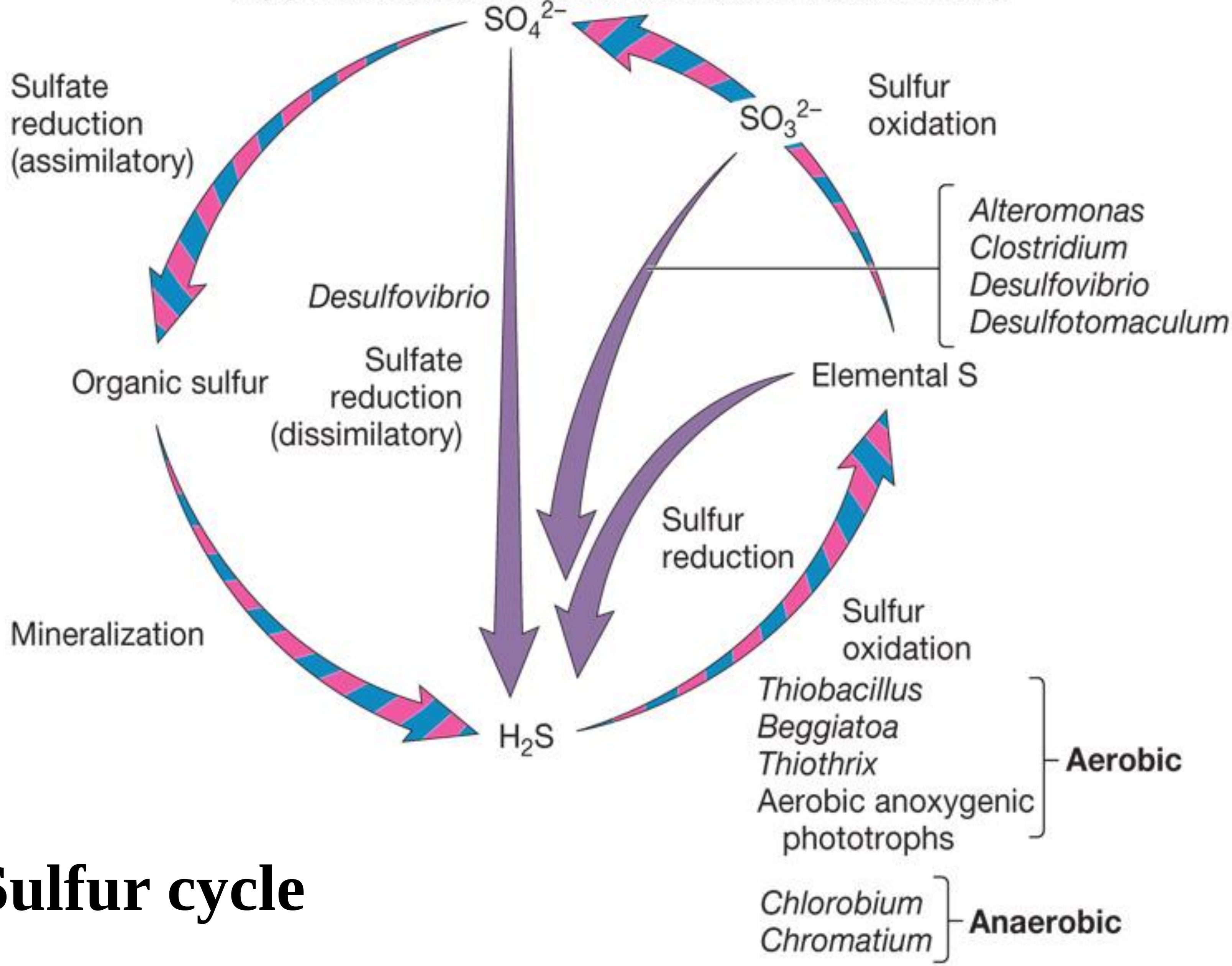
$40 \cdot 10^6$ ton N/yr

(400-450°C, 200 atm)

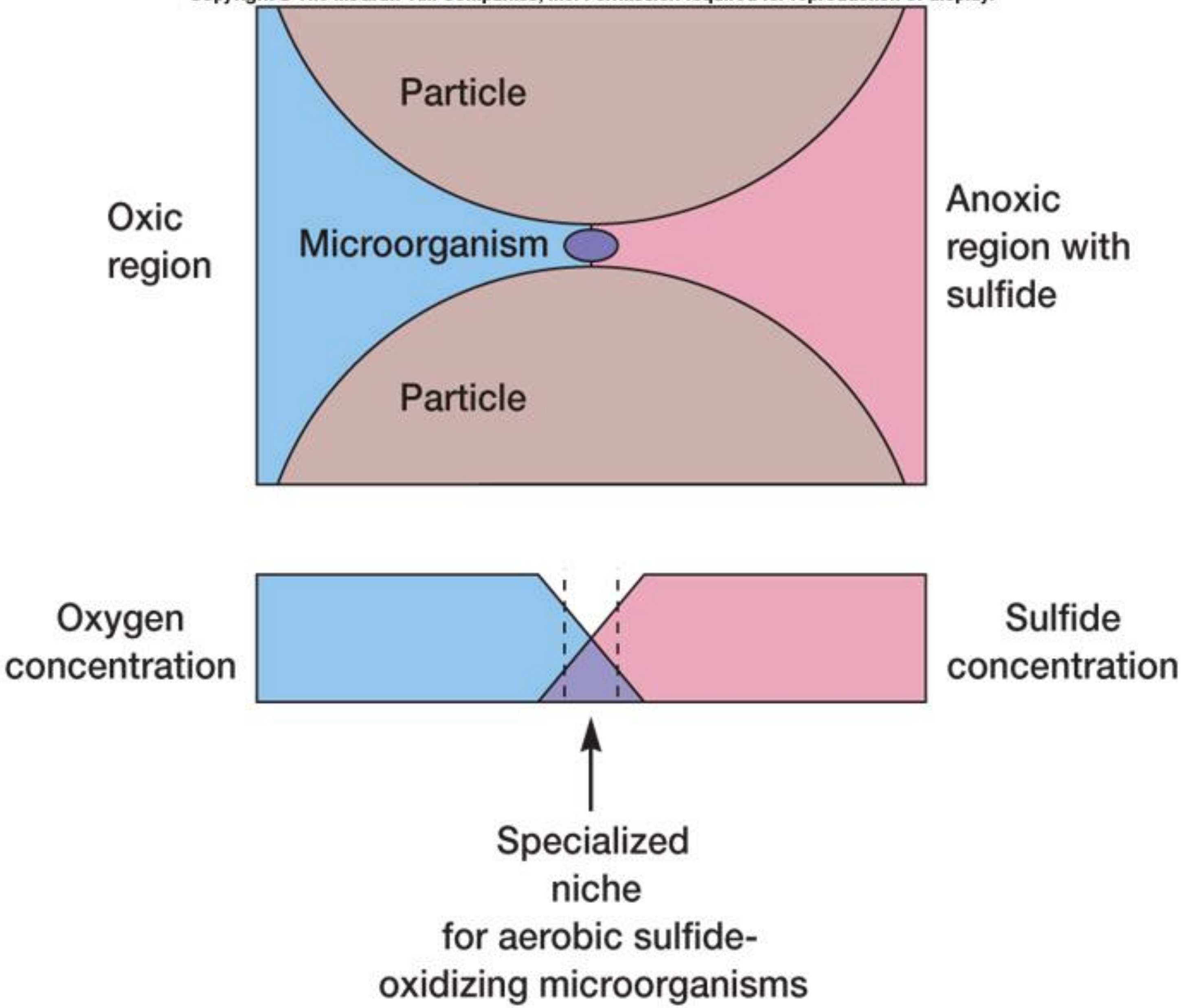
Biological nitrogen fixation

• **Symbiotic**	kg N/ha·yr
Rhizobium	20 – 500
clover, sojabeans, pea, luzern	
Actinomyceter	40 – 500
alder, buckthorn, myrtle, avens	
• **Non-symbiotic**	
Azotobacter, *Clostridium*	0.2
Bluegreen algae	10 - 20

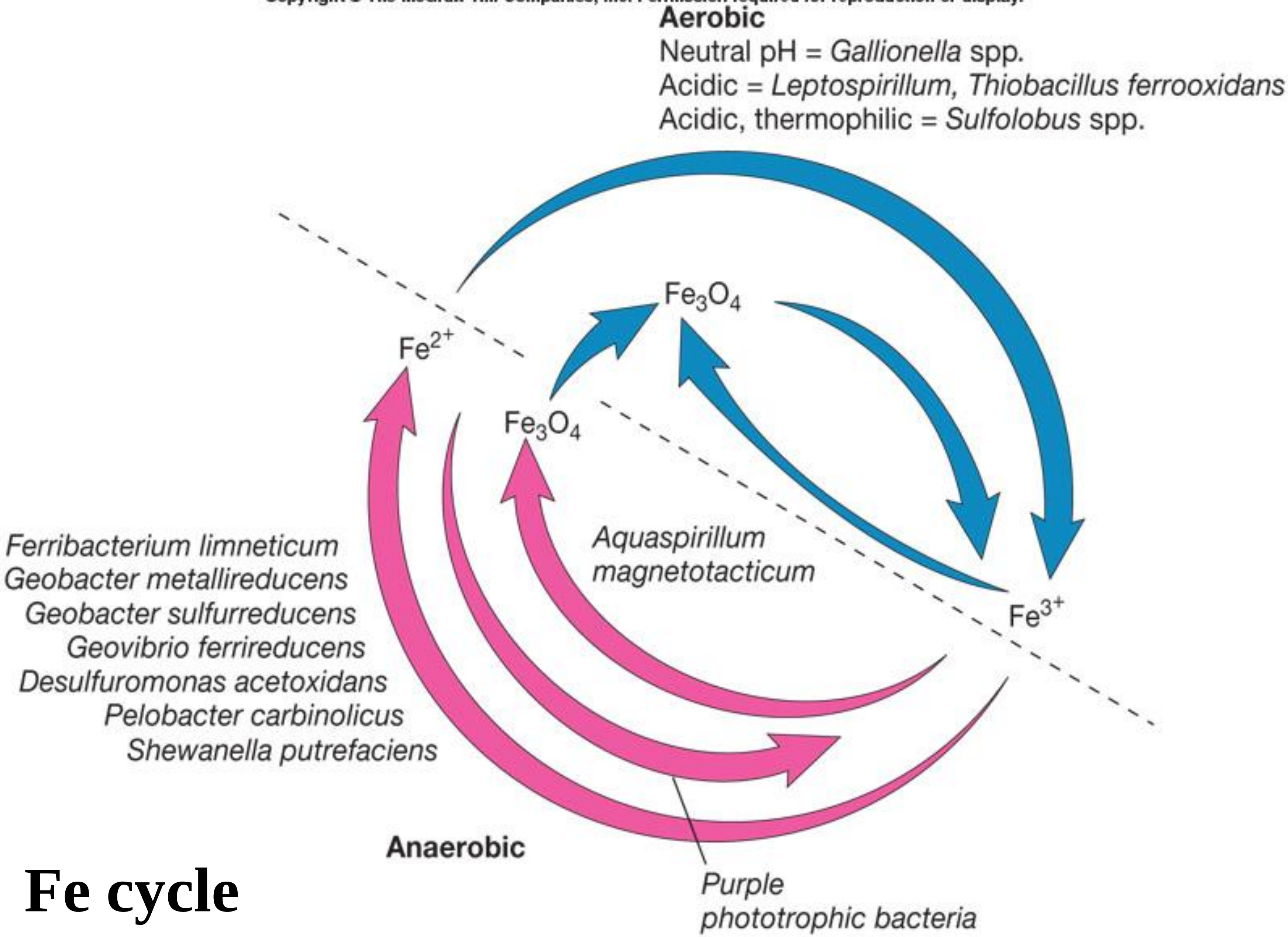
Question 4: How come there is more N fixed via symbiotic relationships?



Sulfur cycle

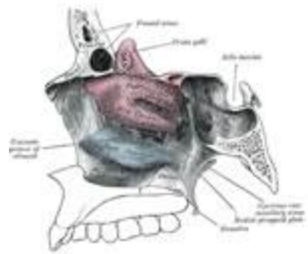


Microenvironments

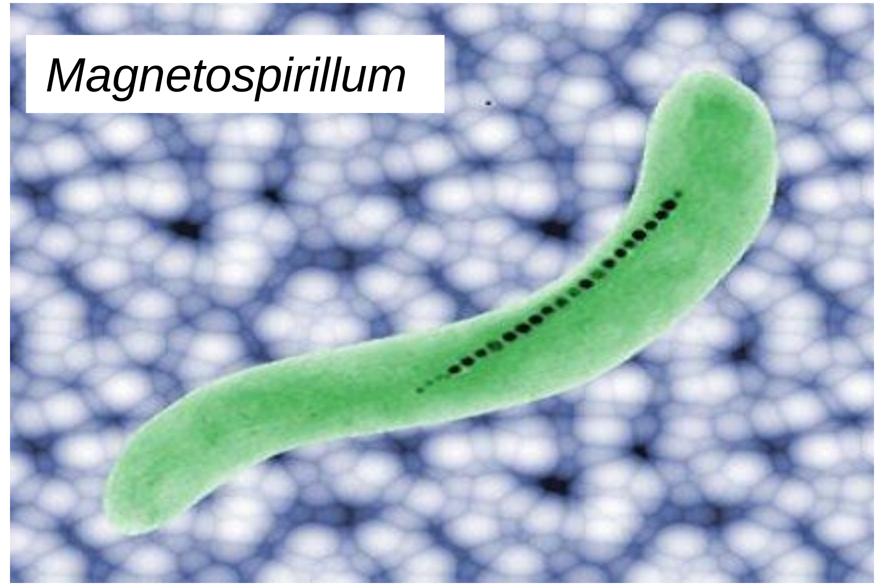

Aerobic
Neutral pH = Gallionella spp.
Acidic = Leptospirillum, Thiobacillus ferrooxidans
Acidic, thermophilic = Sulfolobus spp.
Fe_3O_4
Fe^{2+}
Fe_3O_4
Aquaspirillum magnetotacticum
Fe^{3+}
Ferribacterium limneticum
Geobacter metallireducens
Geobacter sulfurreducens
Geovibrio ferrireducens
Desulfuromonas acetoxidans
Pelobacter carbinolicus
Shewanella putrefaciens
Anaerobic
Purple phototrophic bacteria

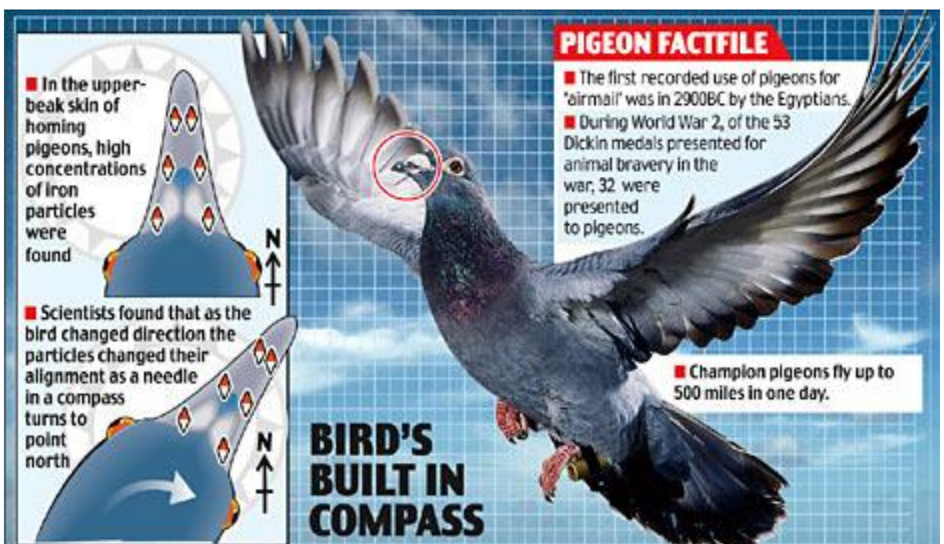
Fe cycle

Magnetite: wonderful stuff!

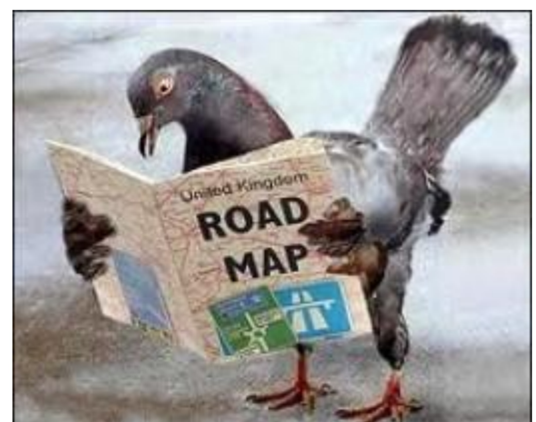


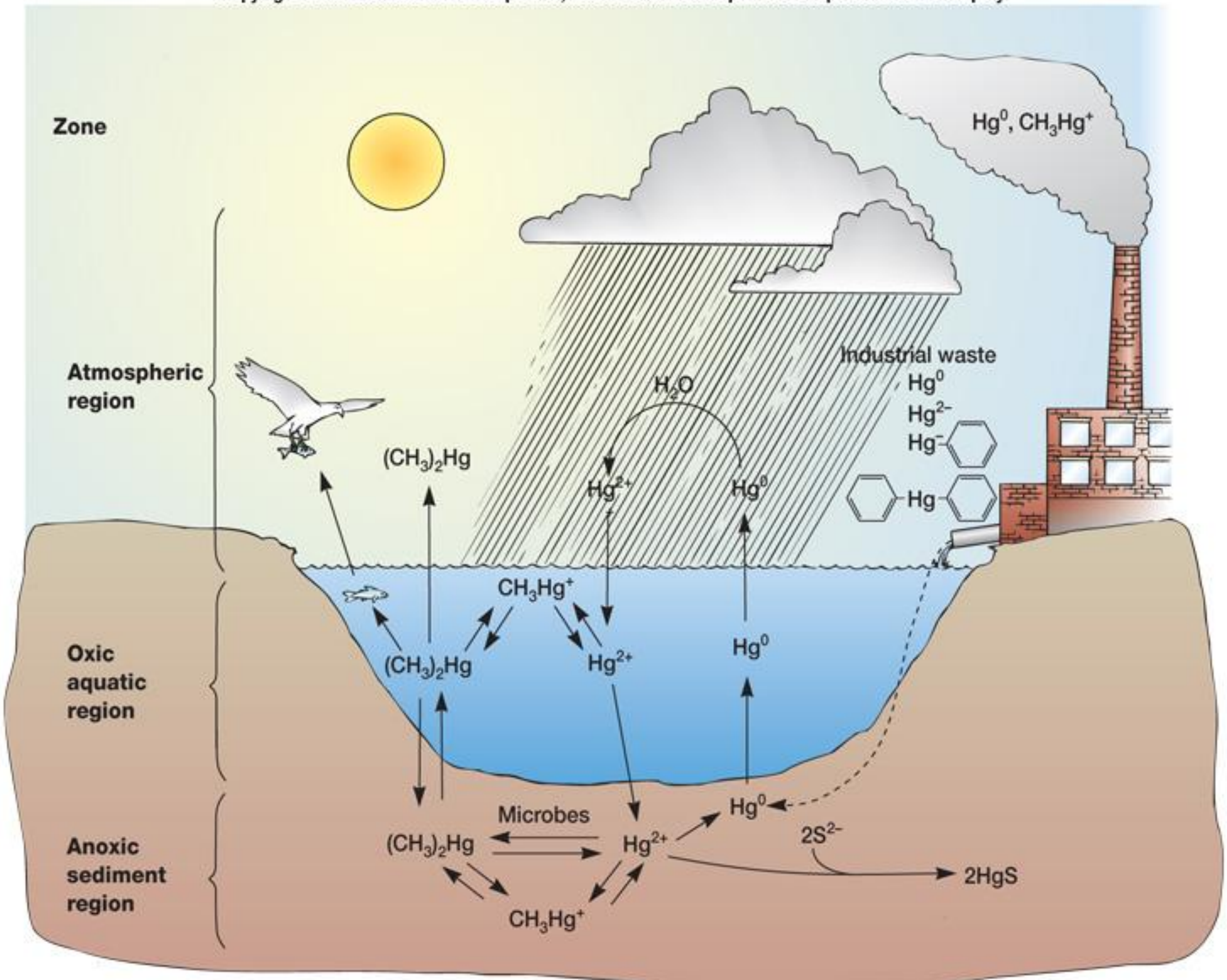
Humans





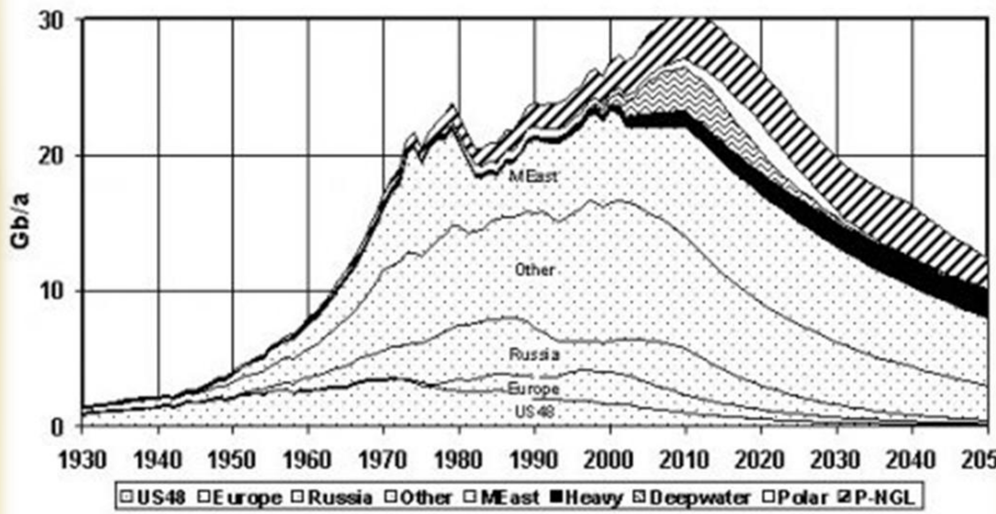
That's why you never see this:





The Mercury cycle

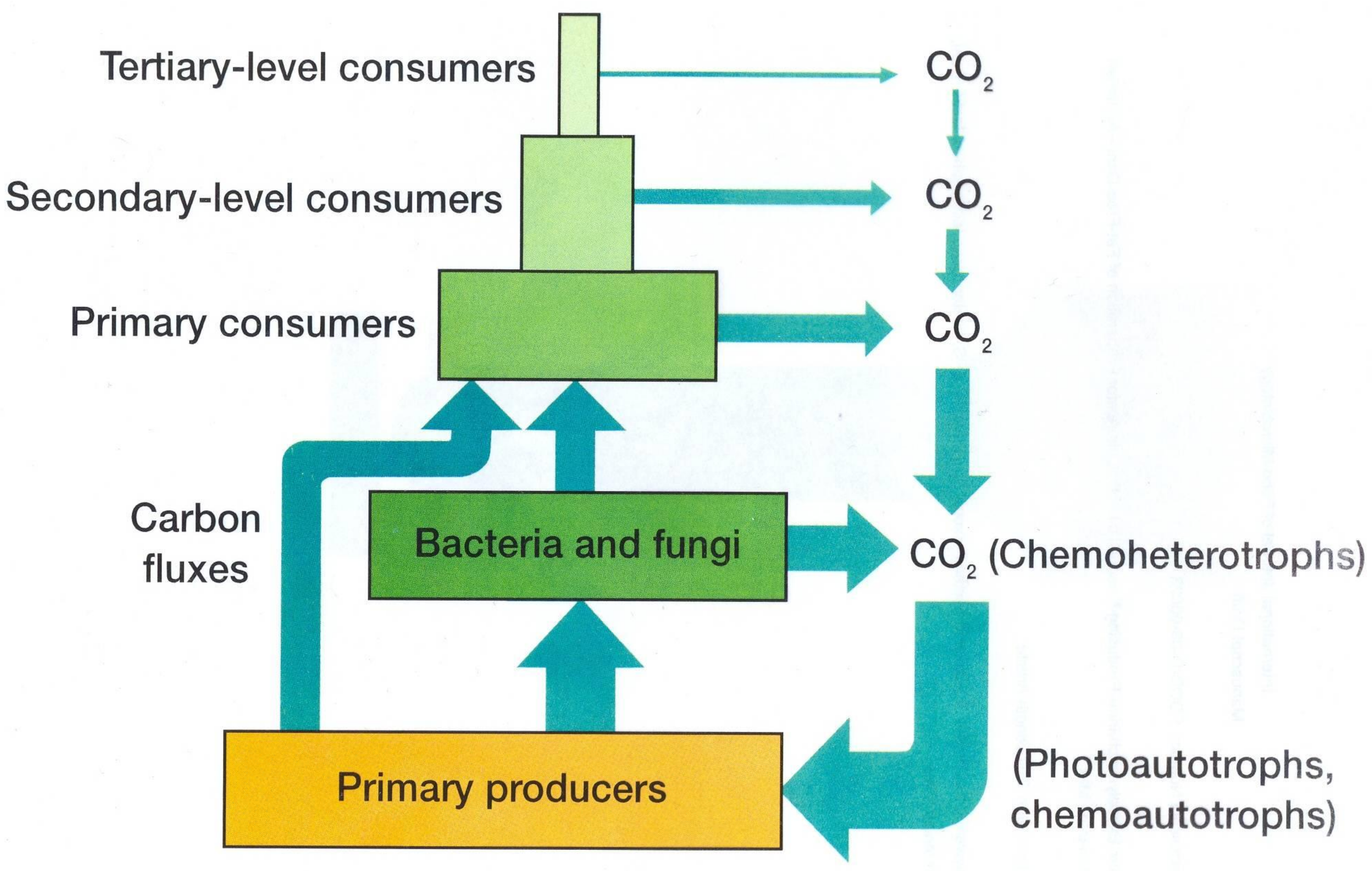
Oil & Natural Gas Liquids
2003 Base Case Scenario



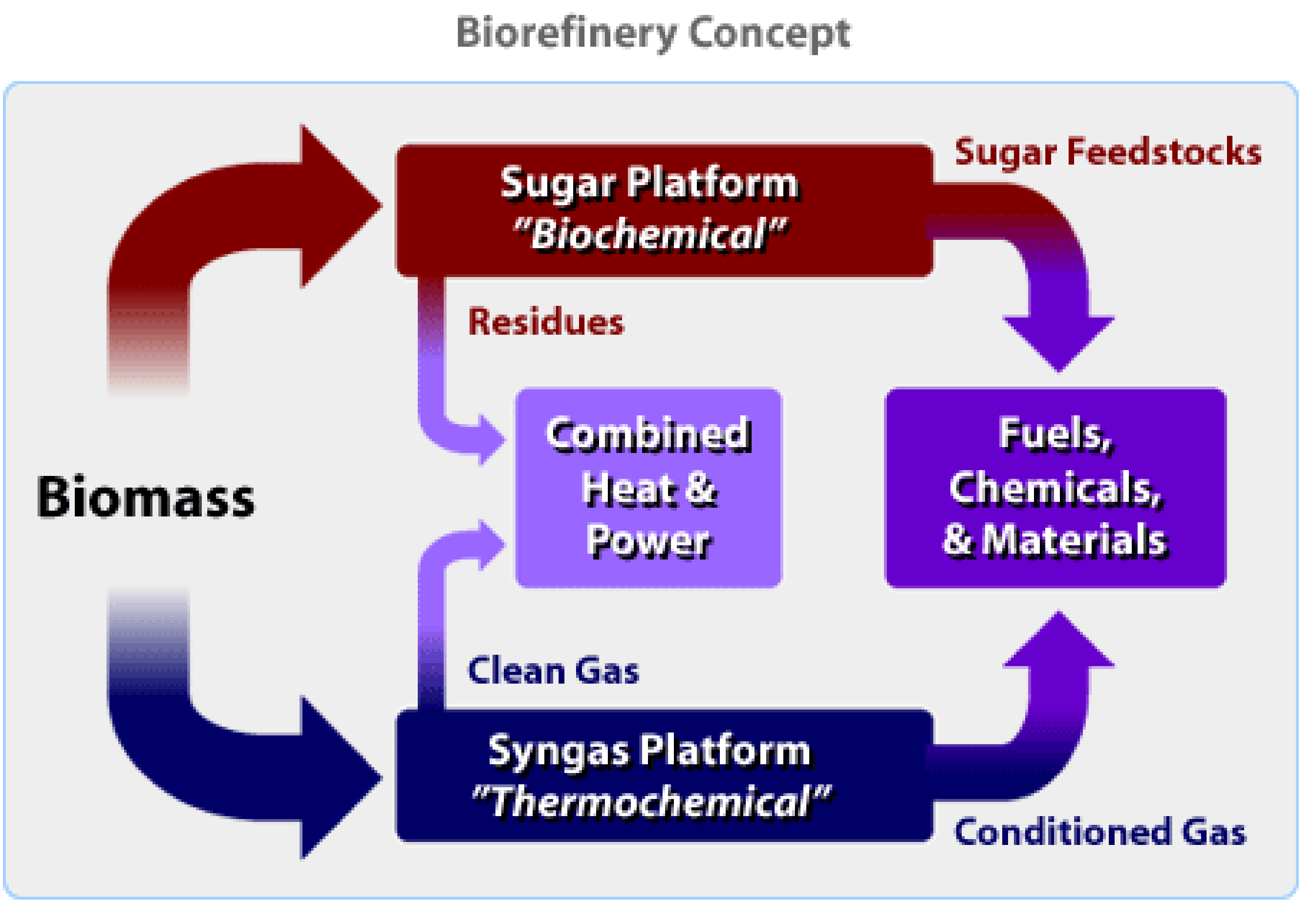
From the ASPO Newsletter; graph developed by Colin Campbell.

http://www.asponews.org/

Role of microorganisms in flow of organic carbon and CO_2



Q 4: Where can we tap organic carbon for replacing oil?


Biorefinery Concept
Sugar Platform
"Biochemical"
Sugar Feedstocks
Residues
Biomass
Combined
Heat &
Power
Fuels,
Chemicals,
& Materials
Clean Gas
Syngas Platform
"Thermochemical"
Conditioned Gas

Biogeochemical cycle mindmap