Environmental Microbiology

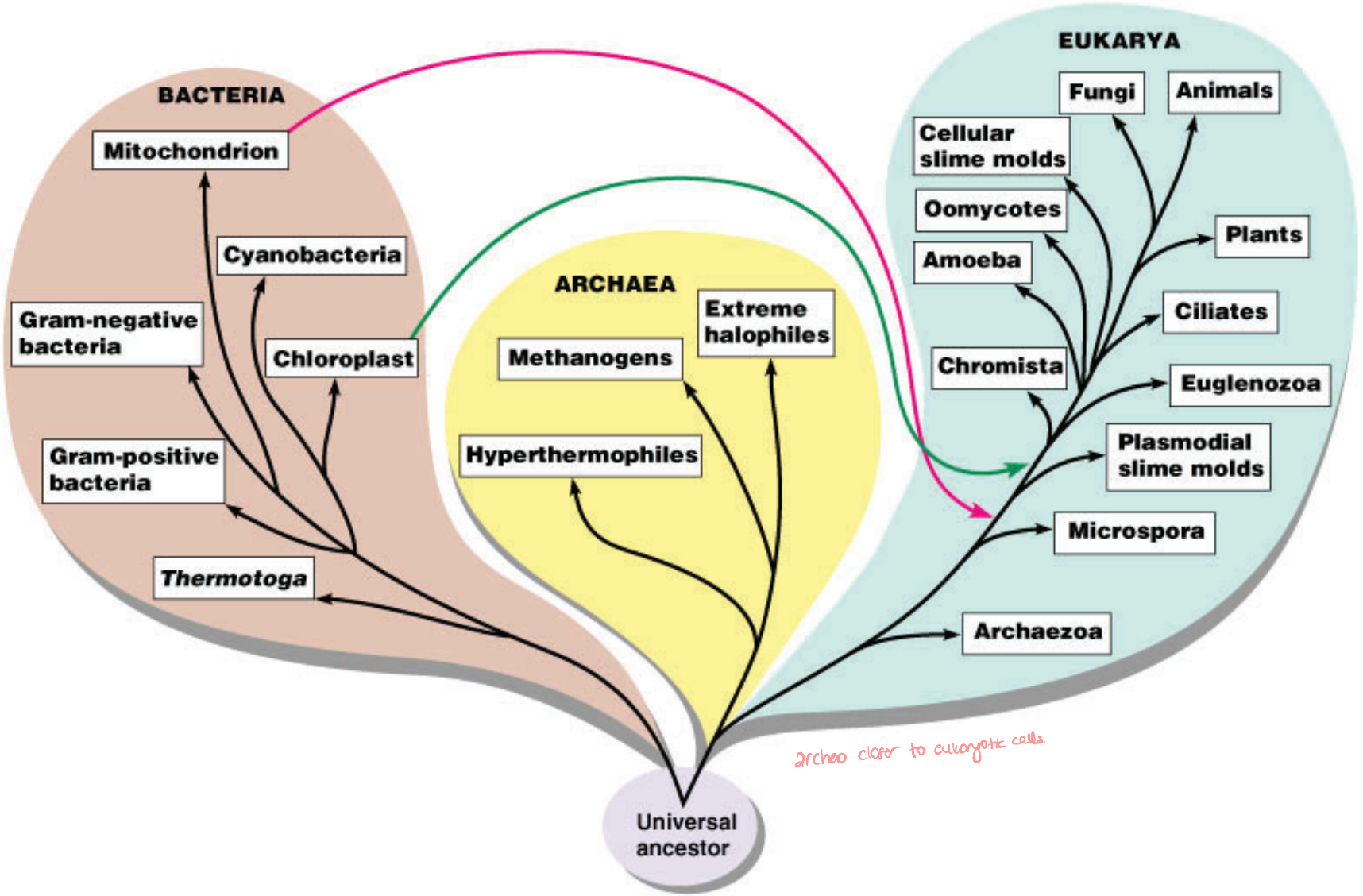
Classification of Microorganisms Taxonomy

prepared by Prof. Bulent Icgen

Taxonomy

- The science of classifying organisms
- Provides universal names for organisms
- Provides a reference for identifying organisms

The Three-Domain System



The Three-Domain System

TABLE 10.1 **Some Characteristics of Archaea, Bacteria, and Eukarya**

Methanosarcina

E. coli

Amoeba

	Archaea	Bacteria	Eukarya
Cell Type	Prokaryotic	Prokaryotic	Eukaryotic
Cell Wall	Varies in composition; contains no peptidoglycan	Contains peptidoglycan	Varies in composition; contains carbohydrates
Membrane Lipids	Composed of branched carbon chains attached to glycerol by ether linkage	Composed of straight carbon chains attached to glycerol by ester linkage	Composed of straight carbon chains attached to glycerol by ester linkage
First Amino Acid in Protein Synthesis	Methionine	Formylmethionine	Methionine
Antibiotic Sensitivity	No	Yes	No
rRNA Loop*	Lacking	Present	Lacking
Common Arm of tRNA†	Lacking	Present	Present

*Binds to ribosomal protein; found in all bacteria.
†A sequence of bases in tRNA found in all eukaryotes and bacteria: guanine-thymine-pseudouridine-cytosine-guanine.

The Three-Domain System

Comparative rRNA sequencing has defined three distinct lineages of cells called domains:

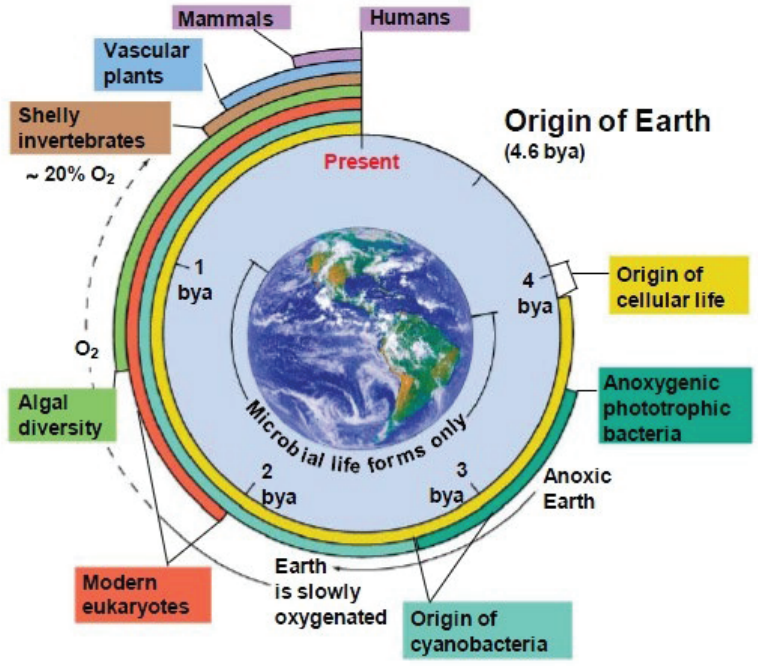
- Bacteria (prokaryotic)
- Archaea (prokaryotic)
- Eukarya (eukaryotic)

- ✓ Archaea and Bacteria are NOT closely related
- ✓ Archaea are more closely related to Eukarya than Bacteria
- ✓ Eukaryotic microorganisms were the ancestors of multicellular organisms

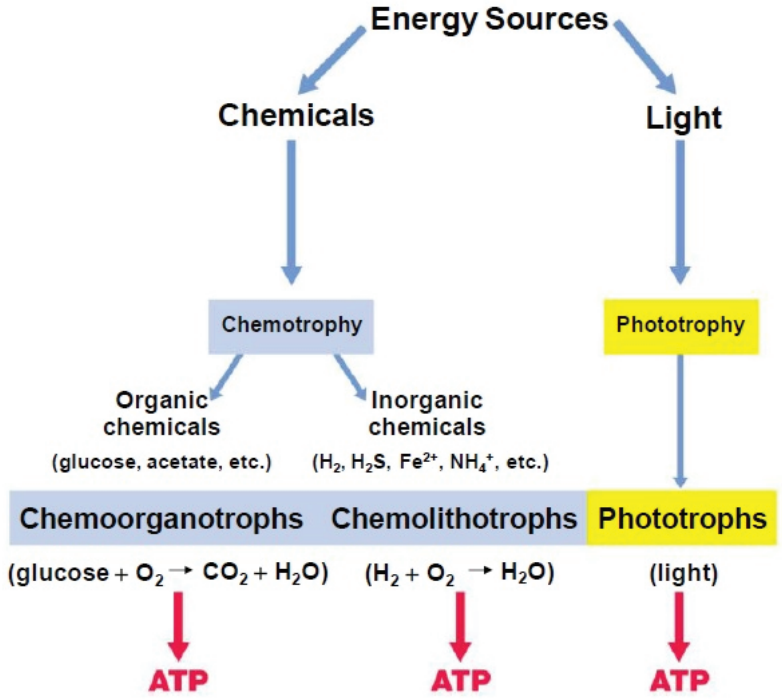
Metabolic Diversity

- The diversity in microbial cells is the product of almost 4 billion years of evolution
- Microorganisms differ in size, shape, motility, physiology, pathogenicity, etc.
- Microorganisms have exploited every conceivable means of obtaining energy from the environment

A summary of life on Earth through time and origin of the cellular domains



Metabolic options for conserving energy



Metabolic Diversity

Chemoorganotrophs

- Obtain their energy from the oxidation of organic molecules
- Aerobes use oxygen to obtain energy
- Anaerobes obtain energy in the absence of oxygen

Chemolithotrophs

- Obtain their energy from the oxidation of inorganic molecules
- Process found only in prokaryotes

Phototrophs

- Contain pigments that allow them to use light as an energy source
- Oxygenic photosynthesis produces oxygen
- Anoxygenic photosynthesis does not produce oxygen

Metabolic Diversity

All cells require carbon as a major nutrient

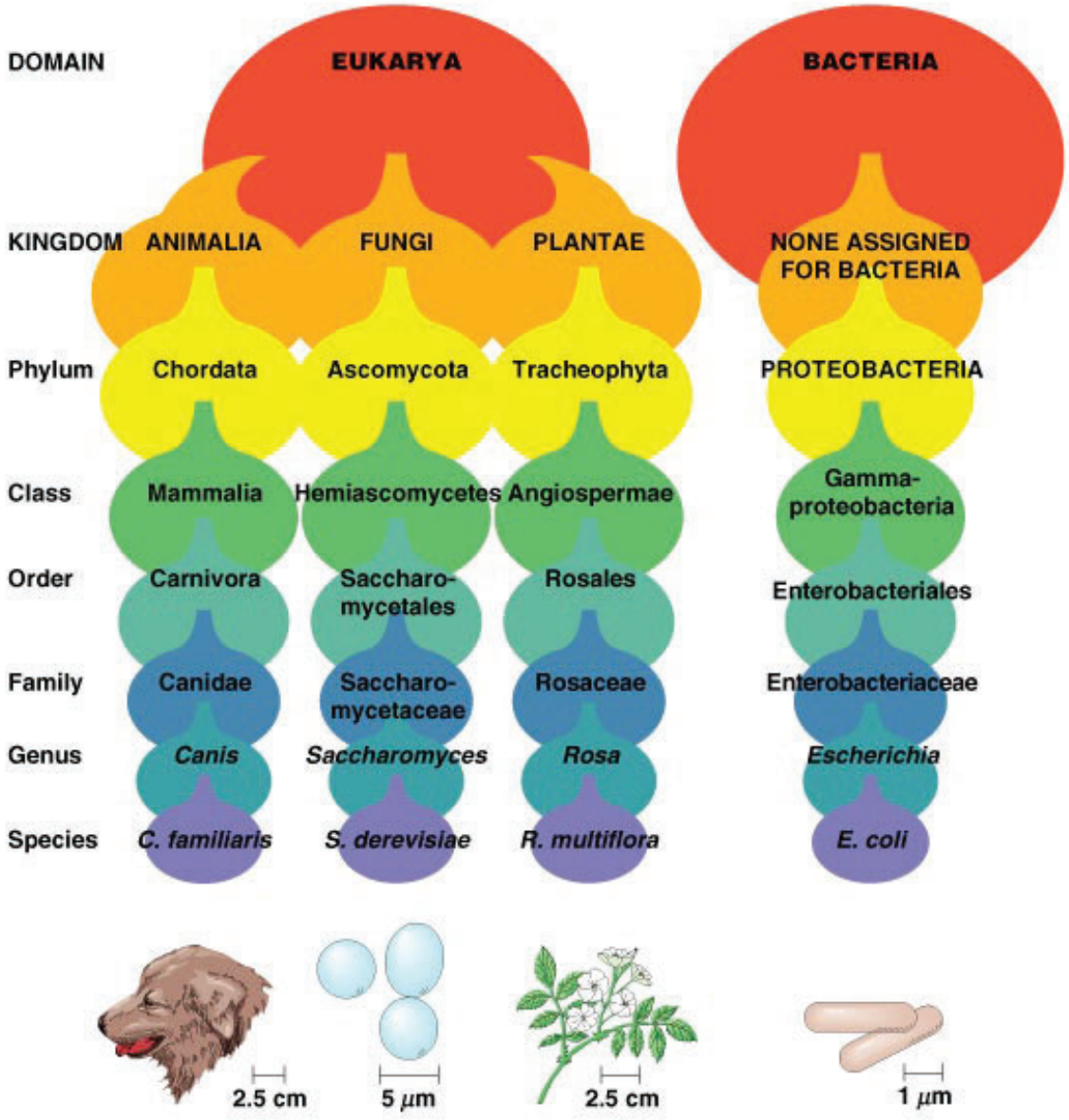
Autotrophs

- Use carbon dioxide as their carbon source
- Sometimes referred to as primary producers

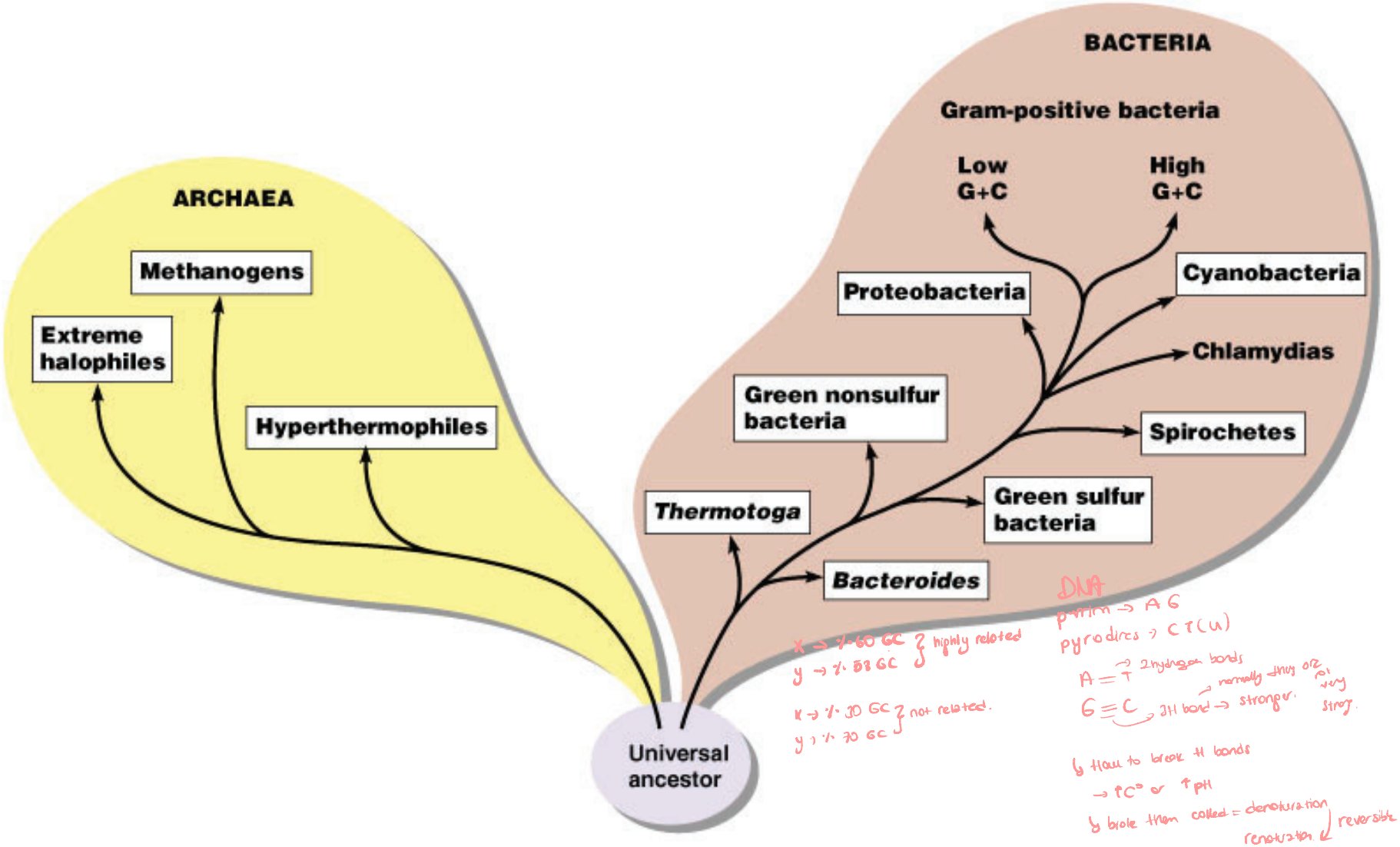
Heterotrophs

- Require one or more organic molecules for their carbon source
- Feed directly on autotrophs or live off products produced by autotrophs

Taxonomic Hierarchy



Prokaryotes



x → %60 GC } highly related
y → %58 GC }

x → %30 GC } not related.
y → %70 GC }

DNA
purine → A G
pyrodines → C T(U)
A = T → 2 hydrogen bonds
G ≡ C → 3H bond → stronger. normally they are very strong.

to break H bonds
→ ↑C° or ↑pH
broke them called = denaturation
renaturation ↓ reversible

Tm = melting temp = the temp you applied to DNA to get single strands

if you have high GC conc → Tm is also high.
vice-versa

Bacteria

The domain *Bacteria* contains an enormous variety of prokaryotes

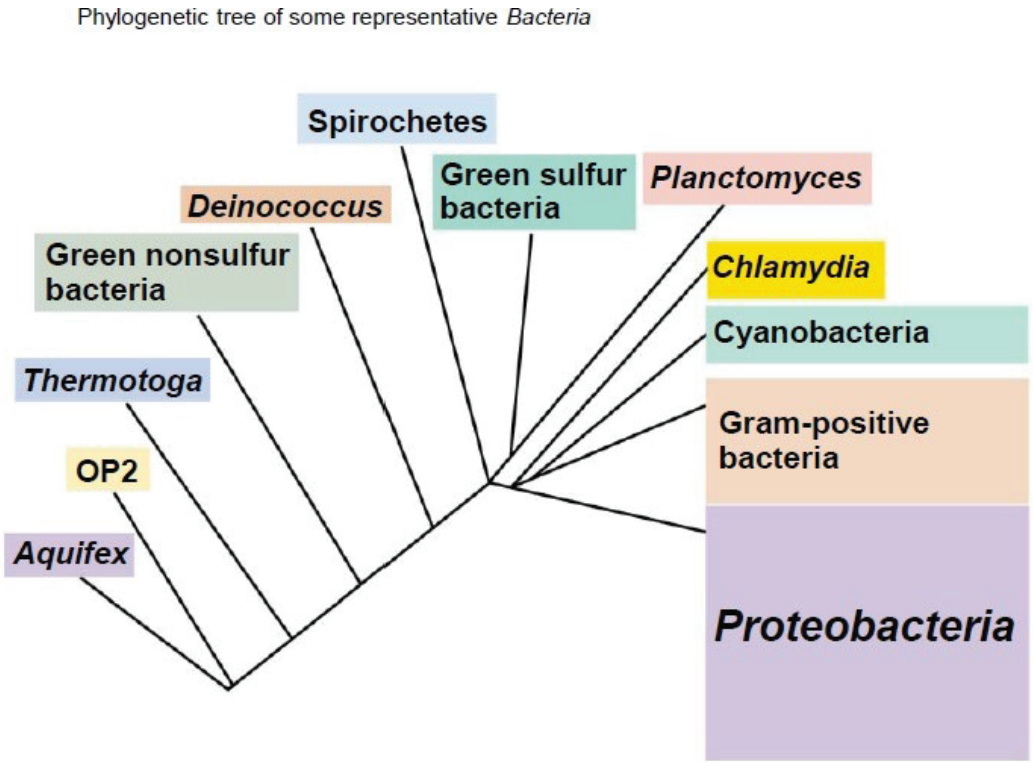
- All known pathogenic prokaryotes are *Bacteria*
- The *Proteobacteria* make up the largest phylum of Bacteria

Gram-negative

Examples: *E. coli, Pseudomonas*, and *Salmonella*

- Gram-positive phylum united by phylogeny and cell wall structure

Cyanobacteria are relatives of gram-positive bacteria



Bacteria

Many Other Phyla of *Bacteria*

- Green sulfur bacteria and green nonsulfur bacteria are photosynthetic
- Deinococcus is extremely resistant to radioactivity
- Chlamydia are obligate intracelluar parasites

Archaea

- Two Phyla of the Domain *Archaea*

Euryarchaeota

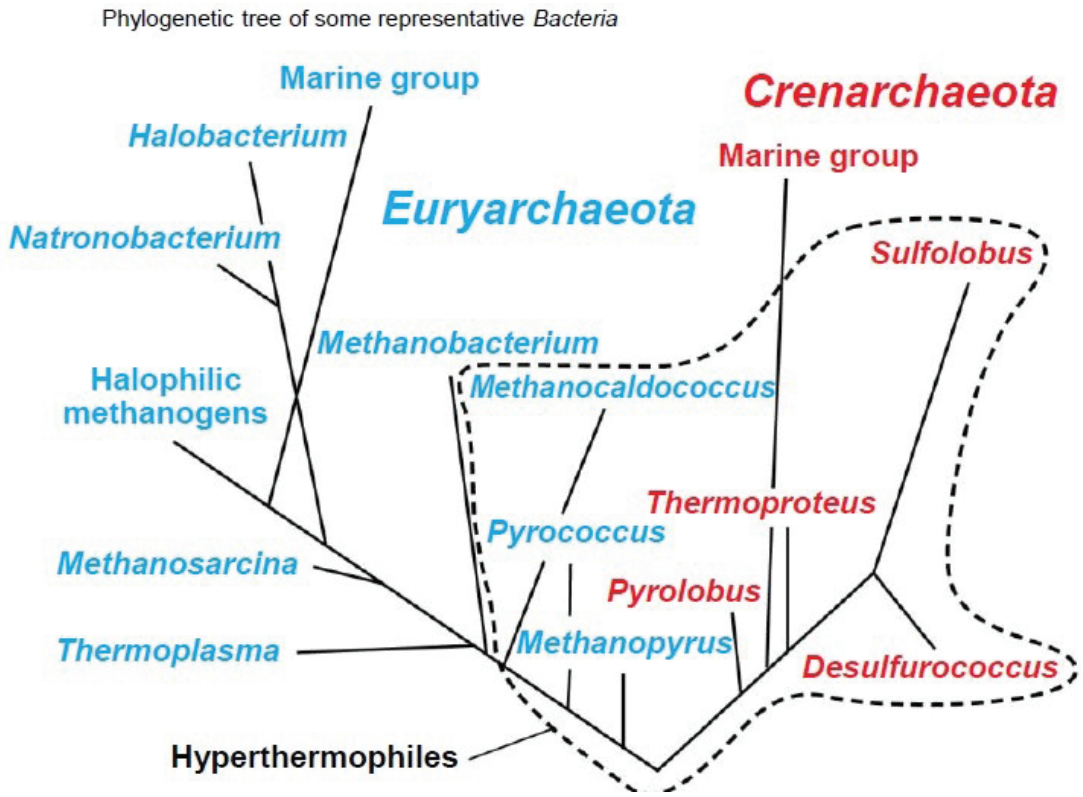
- Methanogens: degrade organic matter anaerobically, produce methane (natural gas)
- Extreme halophiles: require high salt concentrations for metabolism and reproduction
- Thermoacidophiles: grow in moderately high temperatures and low-pH environments

Crenarchaeota

- Vast majority of cultured Crenarchaeota are hyperthermophiles
- Some live in marine, freshwater, and soil systems

Archaea

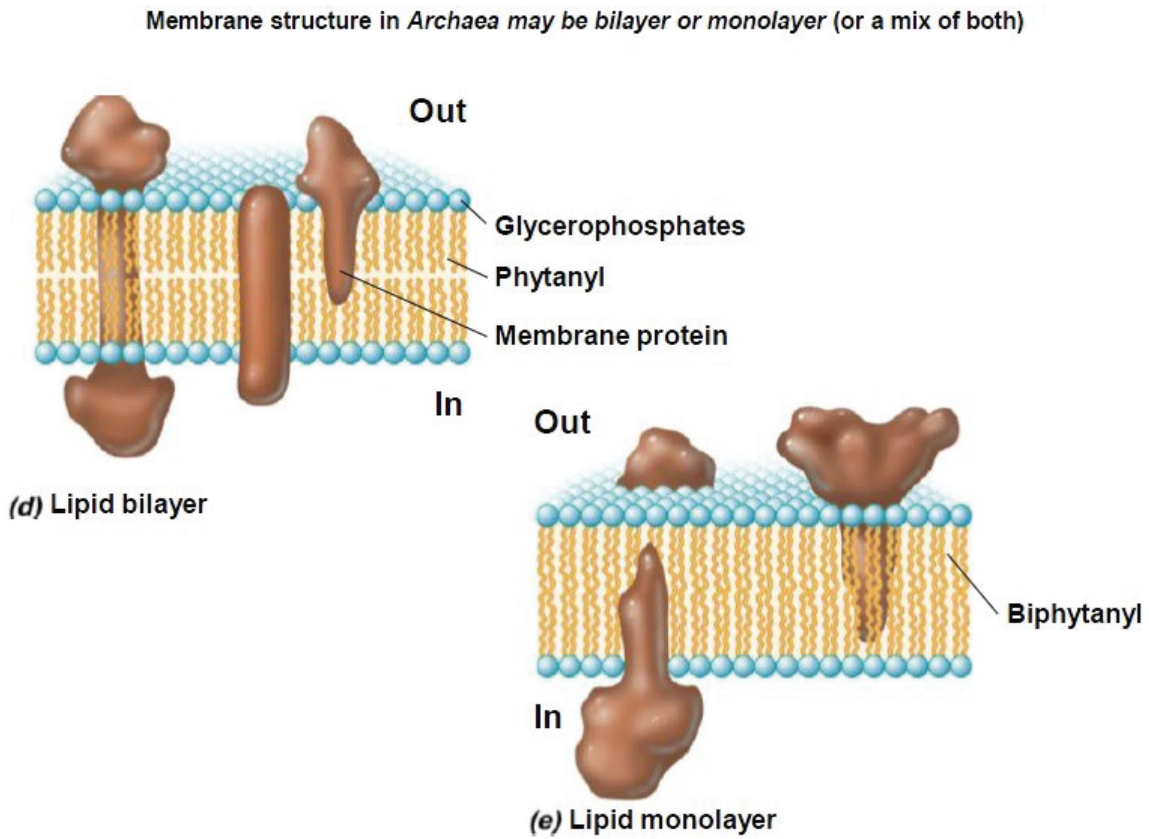
- Microbiologists believe that we have cultured only a small fraction of the *Archaea* and *Bacteria*
- Studies done using methods of molecular microbial ecology, devised by Norman Pace
- Microbial diversity is much greater than laboratory culturing can reveal



Archaea

Archaeal Membranes

- Ether linkages in phospholipids of *Archaea*
- *Bacteria* and Eukarya that have ester linkages in phospholipids
- Can exist as lipid monolayers, bilayers, or mixture



Archaea

Cell Walls of *Archaea*

- No peptidoglycan
- Typically no outer membrane
- Pseudomurein

Polysaccharide similar to peptidoglycan composed of N-acetylglucosamine and N-acetyltalosaminuronic acid

Found in cell walls of certain methanogenic *Archaea*

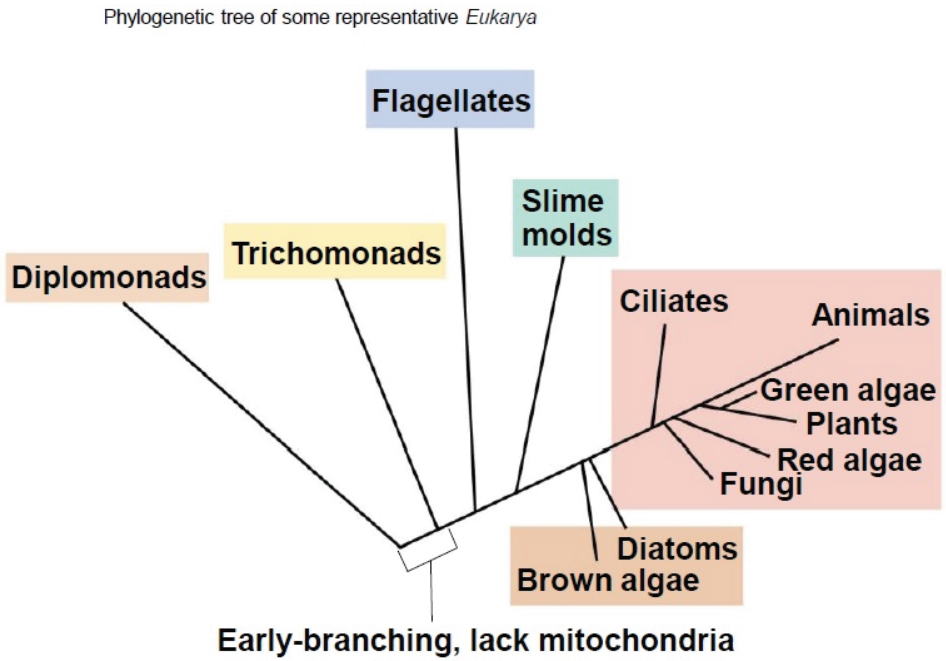
- Cell walls of some *Archaea* lack pseudomurein

Domain Eukarya

- Animalia: Multicellular; no cell walls; chemoheterotrophic
- Plantae: Multicellular; cellulose cell walls; usually photoautotrophic
- Fungi: Chemoheterotrophic; unicellular or multicellular; cell walls of chitin; develop from spores or hyphal fragments
- Protista: A catchall for eukaryotic organisms that do not fit other kingdoms

Eukarya

- Eukaryotic microorganisms include Fungi, Algae, Protozoa, and Helminths
- Protists include algae and protozoa
- The algae are phototrophic
- Protozoa NOT phototrophic
- Fungi are decomposers
- Algae and fungi have cell walls, whereas protozoa and slime molds do not

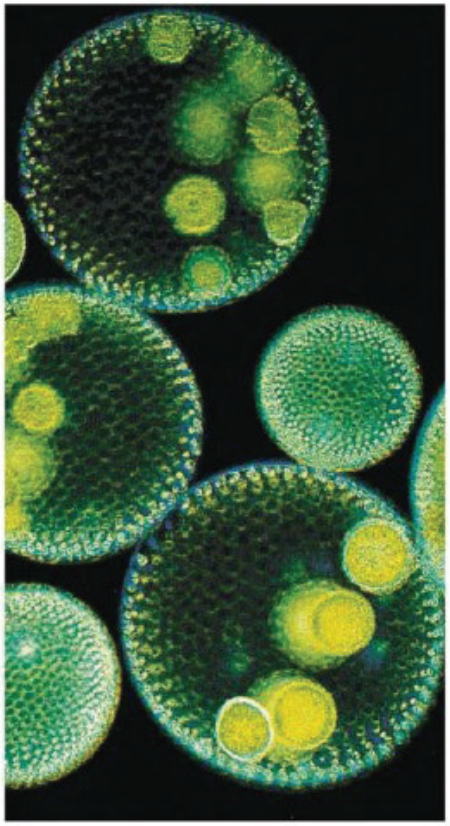


Fungi

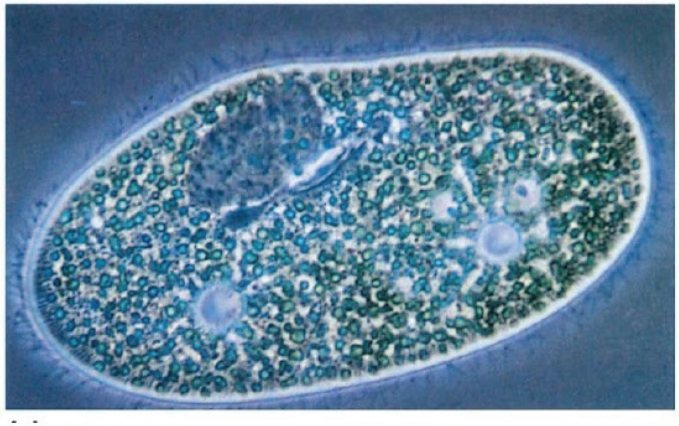
	Fungi	Algae	Protozoa	Helminths
Kingdom	Fungi	Protist	Protist	Animalia
Nutritional type	Chemoheterotroph	Photoautotroph	Chemoheterotroph	Chemoheterotroph
Multicellularity	All, except yeasts	Some	None	All
Cellular arrangement	Unicellular, filamentous, fleshy (such as mushrooms)	Unicellular, colonial, filamentous; tissues	Unicellular	Tissues and organs
Food acquisition method	Absorptive	Absorptive	Absorptive; ingestive (cytostome)	Ingestive (mouth); absorptive
Characteristic features	Sexual and asexual spores	Pigments	Motility; some form cysts	Many have elaborate life cycles, including egg, larva, and adult
Embryo formation	None	None	None	All

Microbial Eukarya

Microbial *Eukarya - Algae*

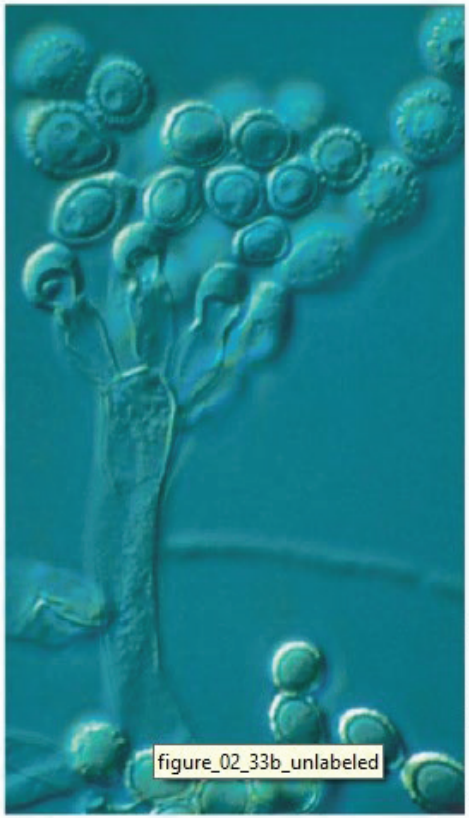


Microbial *Eukarya - Protozoa*



Sydney Tamm

Microbial *Eukarya - Fungi*



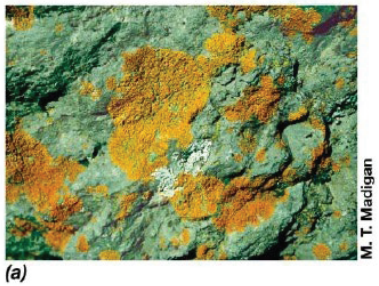
Barry Katz, Mycosearch

Microbial Eukarya

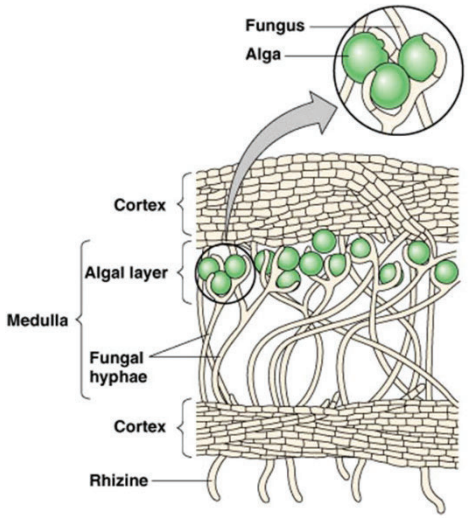
Lichens are a mutualistic relationship of an alga (or cyanobacterium) and fungus

- Alga produces and secretes carbohydrates, fungus provides holdfast

Lichens. (a) An orange-pigmented lichen growing on a rock, and (b) a yellow-pigmented lichen growing on a dead tree stump,



(a) Three types of lichens



(b) Lichen thallus

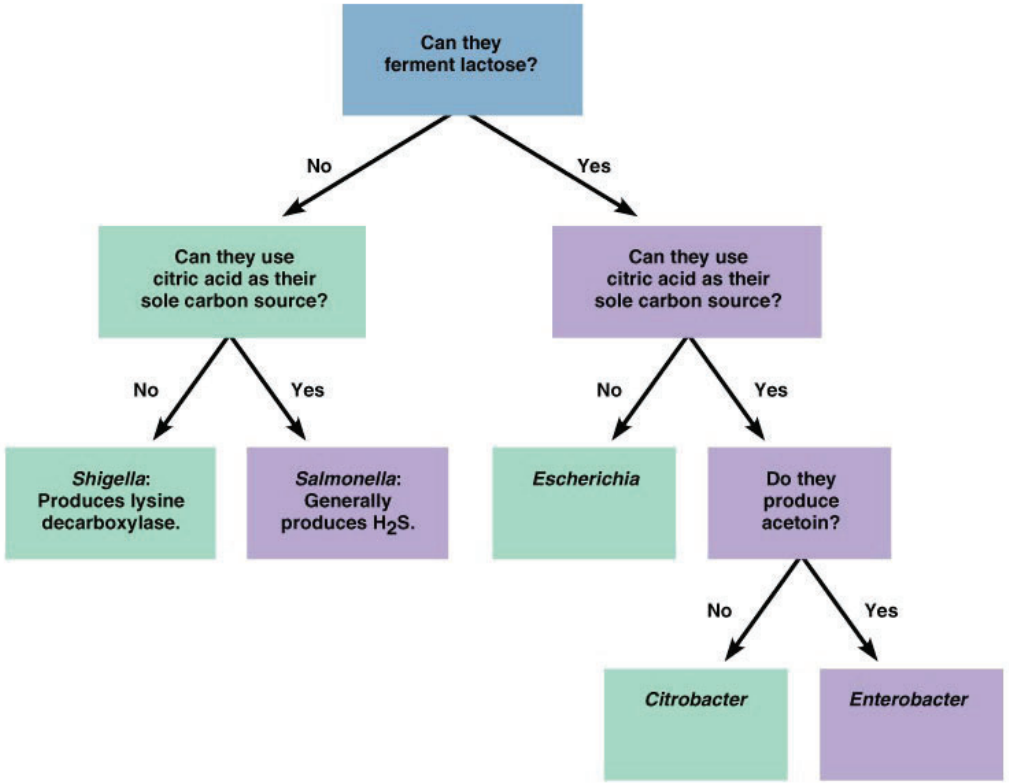
Species Definition

- Eukaryotic species:
  - A group of closely related organisms that breed among themselves
- Prokaryotic species:
  - A population of cells with similar characteristics
  - Clone: Population of cells derived from a single cell
  - Strain: Genetically different cells within a clone
- Viral species:
  - Population of viruses with similar characteristics that occupies a particular ecological niche

Identification Methods

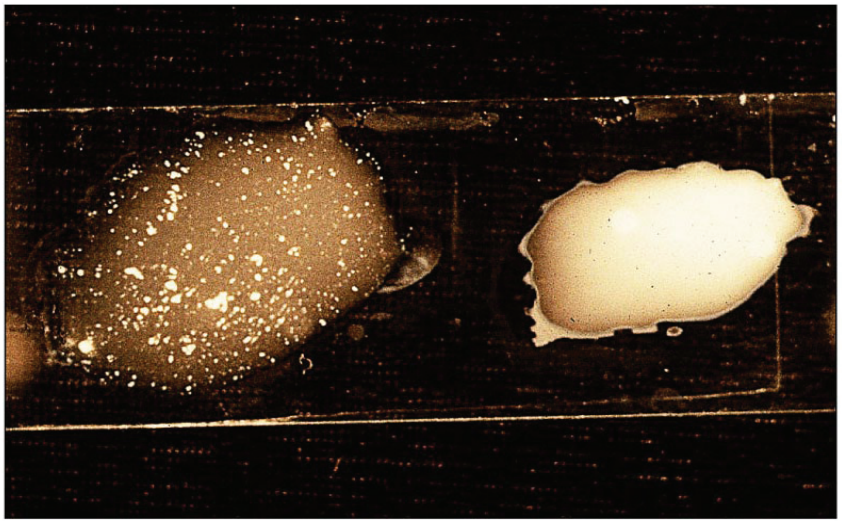
- Morphological characteristics: Useful for identifying eukaryotes
- Differential staining: Gram staining, acid-fast staining
- Biochemical tests: Determines presence of bacterial enzymes
- Fatty acid methyl ester (FAME) analysis by GS-MS

HC # and order HC chains
↓
long chains



Serology

- Combine known antiserum + unknown bacterium → if it works = agglutination (clumps)
- Slide agglutination
- ELISA Enzyme linked Immunosorbent Assay → direct → check for antigen → by using labeled antibodies
  → indirect → check for antigen antibody complex
- Western blot



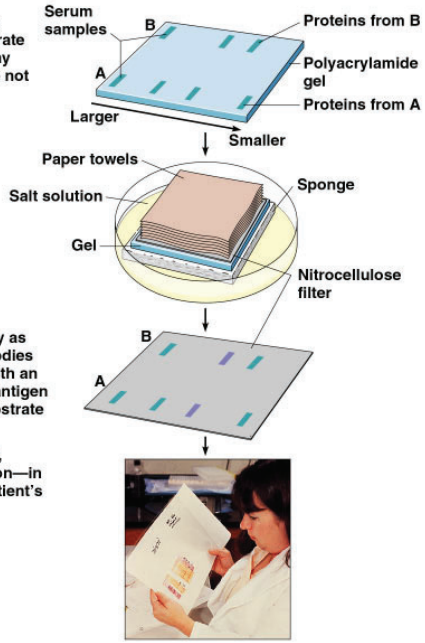
Western Blot & Phage Typing

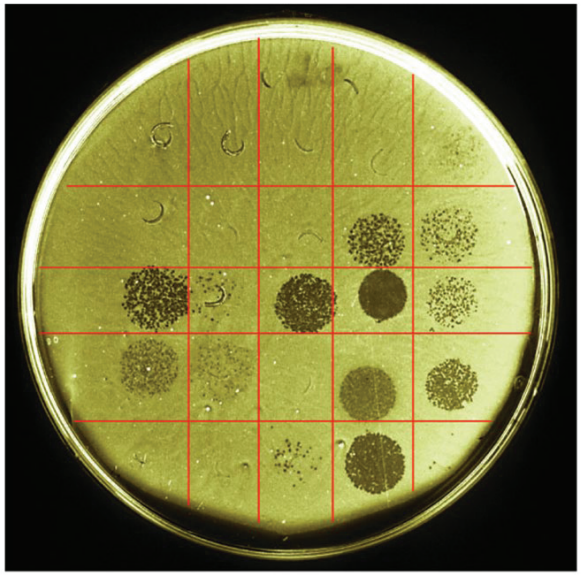
1. If Lyme disease is suspected in a patient, a sample of the patient's serum is taken. Electrophoresis is used to separate proteins in the serum sample. Each band consists of many molecules of a particular protein (antigen). The bands are not visible at this point.

2. The bands are transferred to a nitrocellulose filter by

3. The proteins (antigens) are positioned on the filter exactly as they were on the gel. The filter is then washed with antibodies against a particular antigen. The antibodies are tagged with an enzyme. The antibodies that combine with their specific antigen are visible (shown here in purple) when the enzyme's substrate is added.

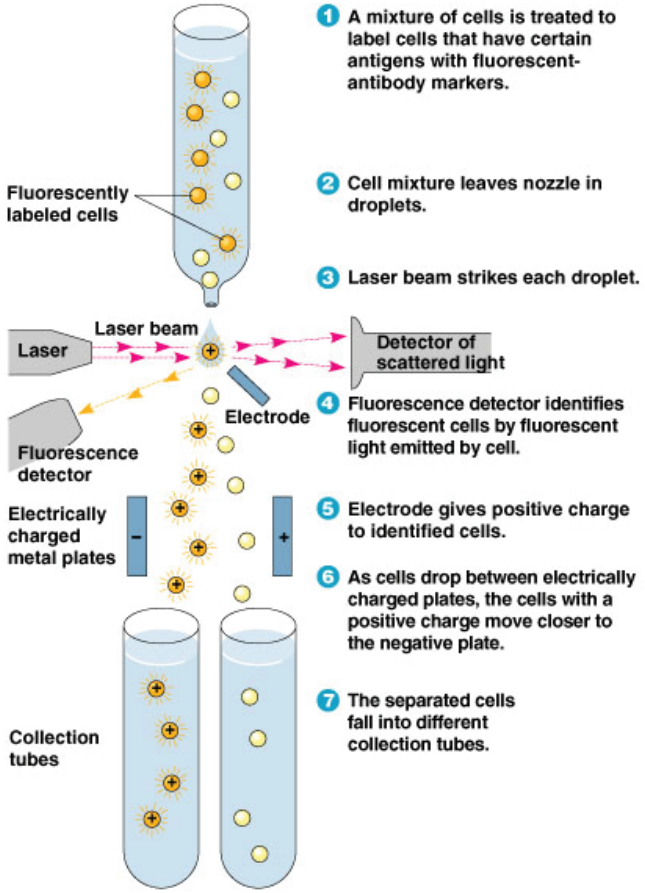
4. The test is read. If the tagged antibodies stick to the filter, evidence of the presence of the microorganism in question—in this case, *Borrelia burgdorferi*—has been found in the patient's serum.





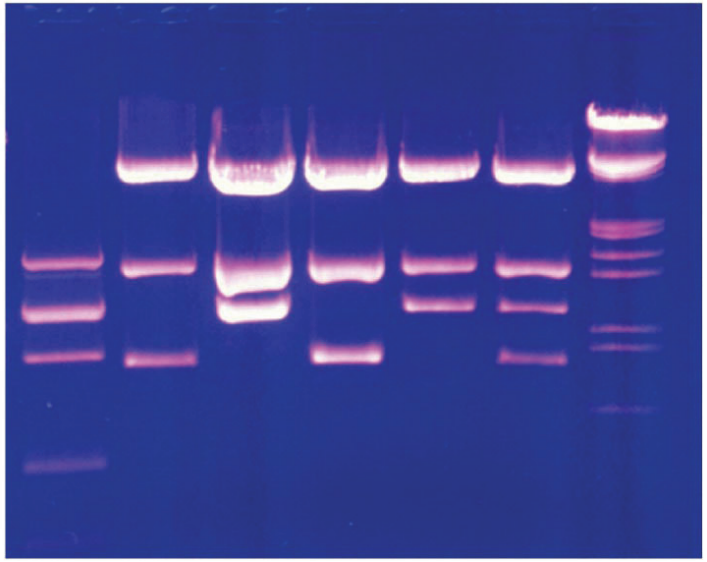
Flow Cytometry Uses

- Differences in electrical conductivity between species
- Fluorescence of some species
- Cells selectively stained with antibody + fluorescent dye

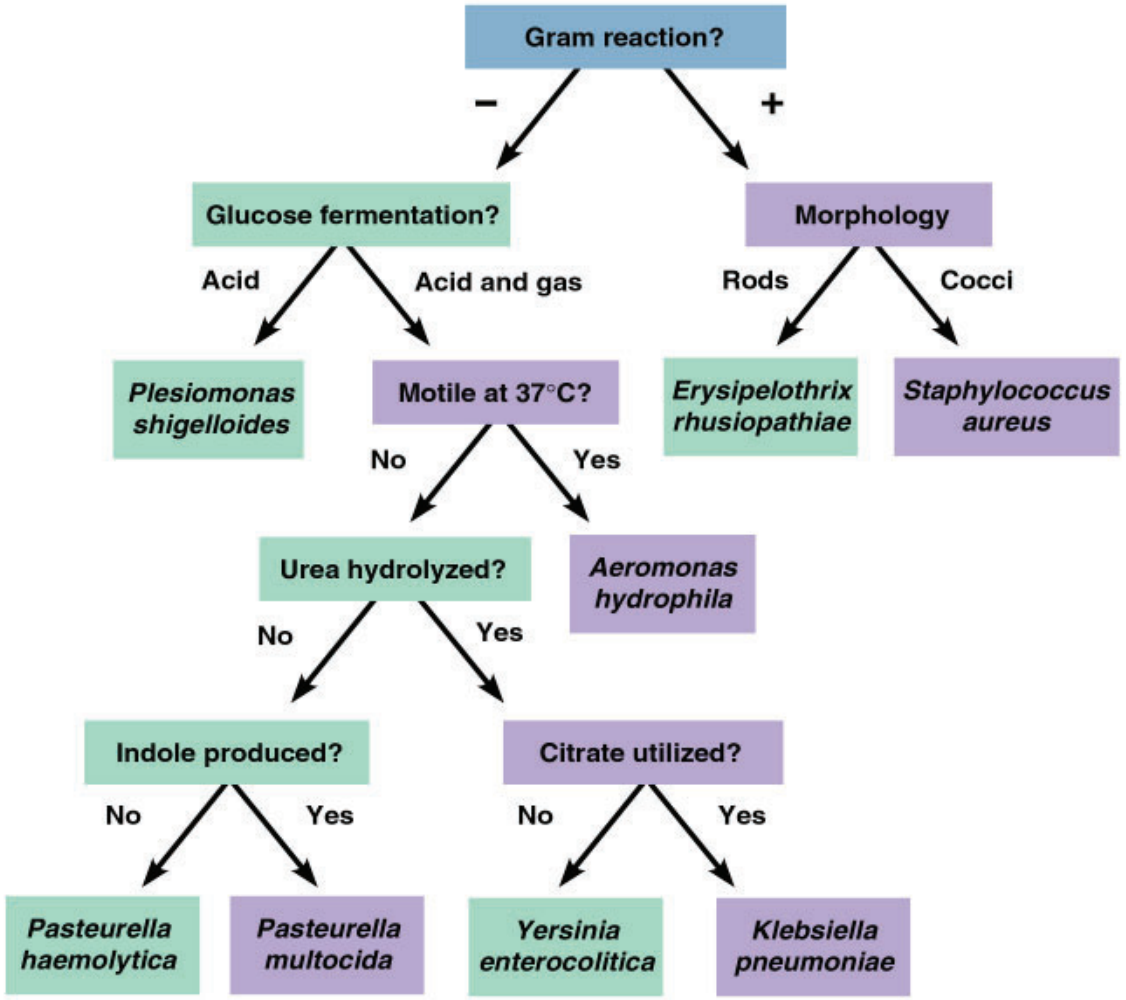


Genetics

- DNA base composition
  - Guanine + cytosine moles% (GC)
- DNA fingerprinting
  - Electrophoresis of restriction enzyme digests
- rRNA sequencing
- Polymerase Chain Reaction (PCR)



Dichotomous Key



rRNA Sequencing and Phylogeny

Figure 2.16 Ribosomal RNA (rRNA) gene sequencing and phylogeny. *(a) DNA is extracted* from cells. *(b) Many identical copies* of a gene encoding rRNA are made by the polymerase chain reaction

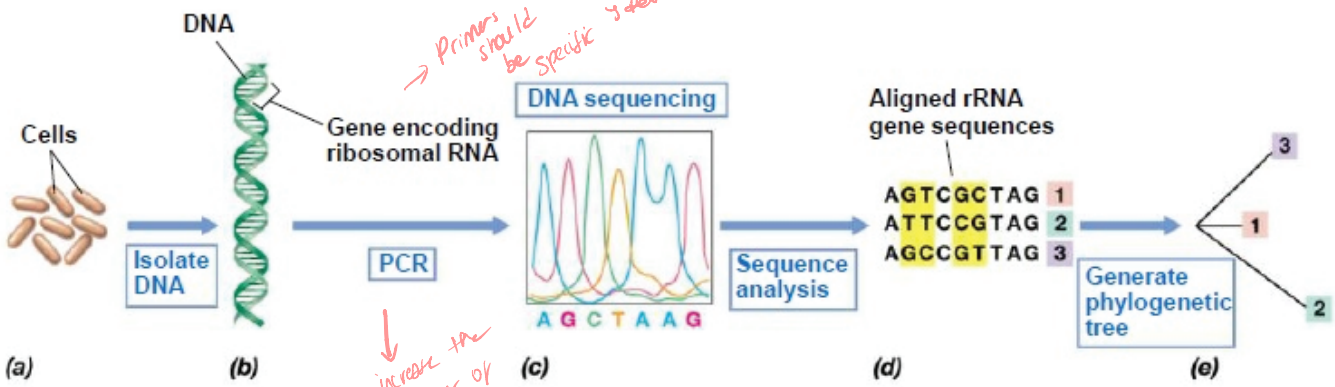
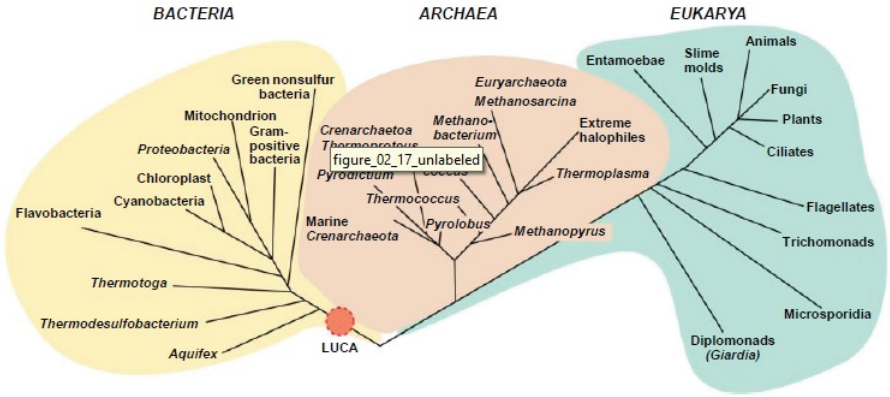


Figure 2.17 The phylogenetic tree of life as defined by comparative rRNA gene sequencing



Cladogram

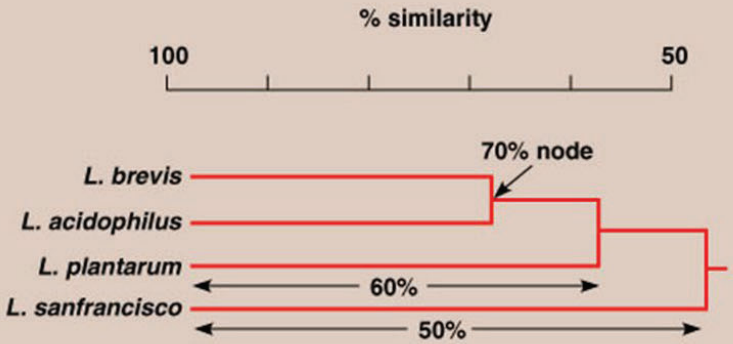
1. Determine the sequence of bases in an rRNA molecule for each organism. Only a short sequence of bases is shown for this example.

Lactobacillus brevis	AGUCCAGAGC
L. sanfrancisco	GUAAAAGAGC
L. acidophilus	AGCGGAGAGC
L. plantarum	ACGUUAGAGC

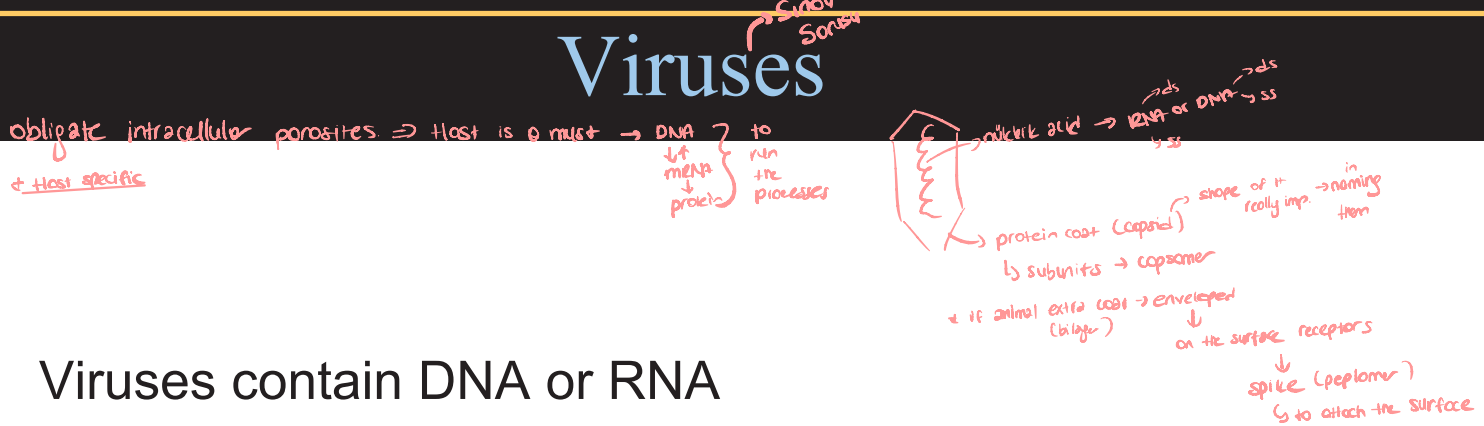
2. Calculate the percentage of similarity in the nucleotide bases between each species. For example, there is a 70% similarity between the sequences for *L. brevis* and *L. acidophilus*.

	Percent similarity
L. brevis → *L. sanfrancisco*	50%
L. brevis → *L. acidophilus*	70%
L. brevis → *L. plantarum*	60%
L. sanfrancisco → *L. acidophilus*	50%
L. sanfrancisco → *L. plantarum*	50%
L. plantarum → *L. acidophilus*	60%

3. Construct a cladogram. The length of the horizontal lines corresponds to the percent similarity values. Each branch point, or node, in the cladogram represents an ancestor common to all species beyond that node. Each node is defined by a similarity in rRNA present in all species beyond that branch point.

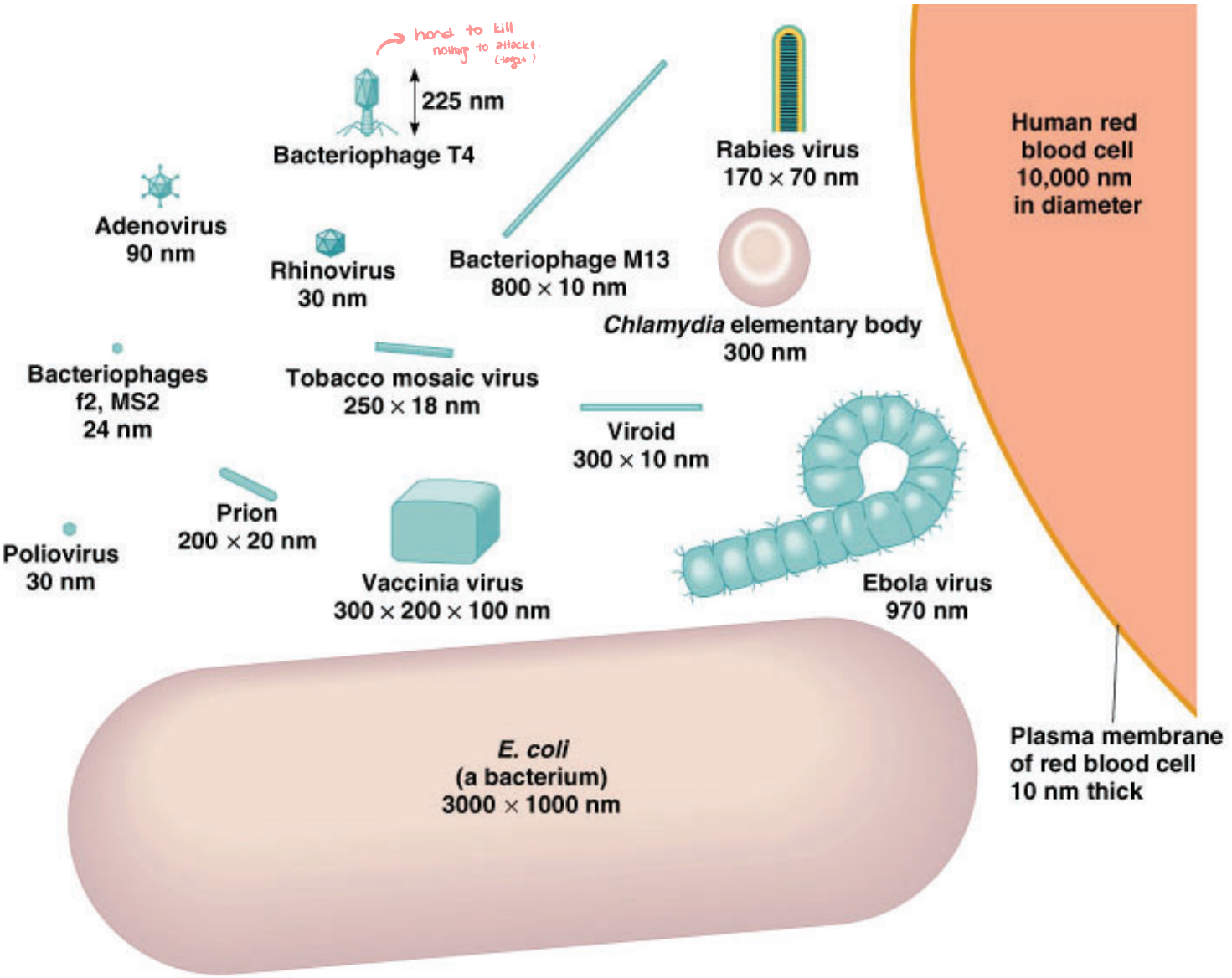


Viruses

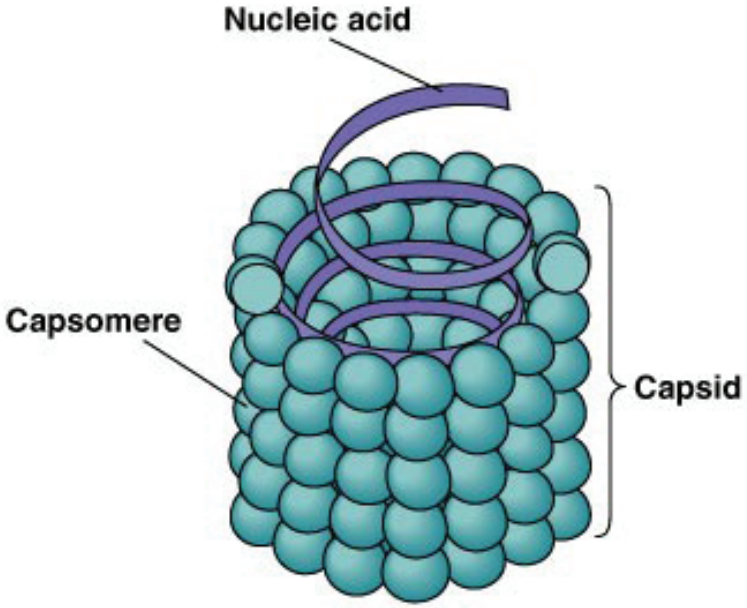


- Viruses contain DNA or RNA
- And a protein coat
- Some are enclosed by an envelope
- Some viruses have spikes
- Most viruses infect only specific types of cells in one host
- Host range is determined by specific host attachment sites and cellular factors

Viruses


hard to kill
nothing to attack.
(target)
225 nm
Bacteriophage T4
Adenovirus
90 nm
Rhinovirus
30 nm
Bacteriophage M13
800 × 10 nm
Rabies virus
170 × 70 nm
Human red
blood cell
10,000 nm
in diameter
Chlamydia elementary body
300 nm
Bacteriophages
f2, MS2
24 nm
Tobacco mosaic virus
250 × 18 nm
Viroid
300 × 10 nm
Prion
200 × 20 nm
Poliovirus
30 nm
Vaccinia virus
300 × 200 × 100 nm
Ebola virus
970 nm
E. coli
(a bacterium)
3000 × 1000 nm
Plasma membrane
of red blood cell
10 nm thick

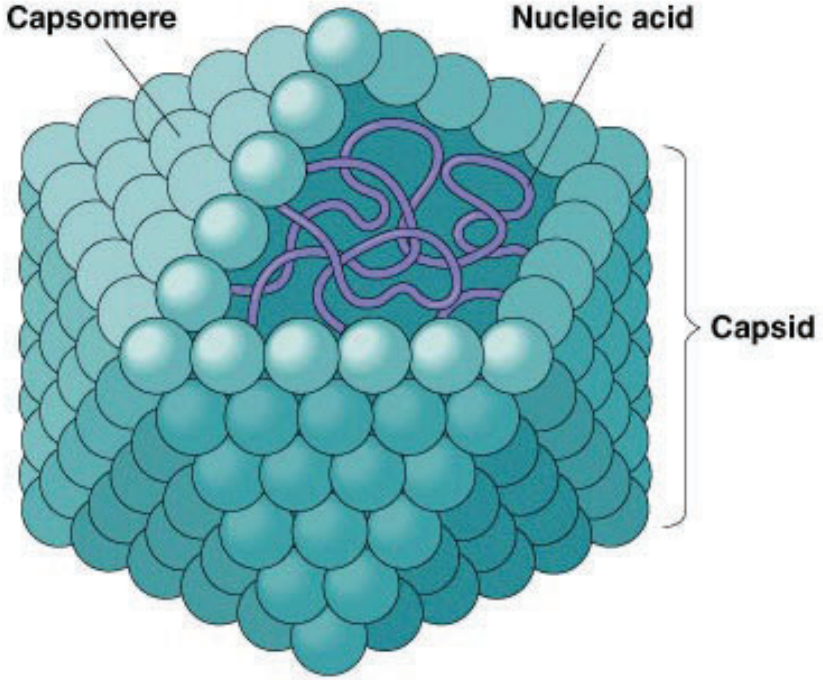
Helical Viruses



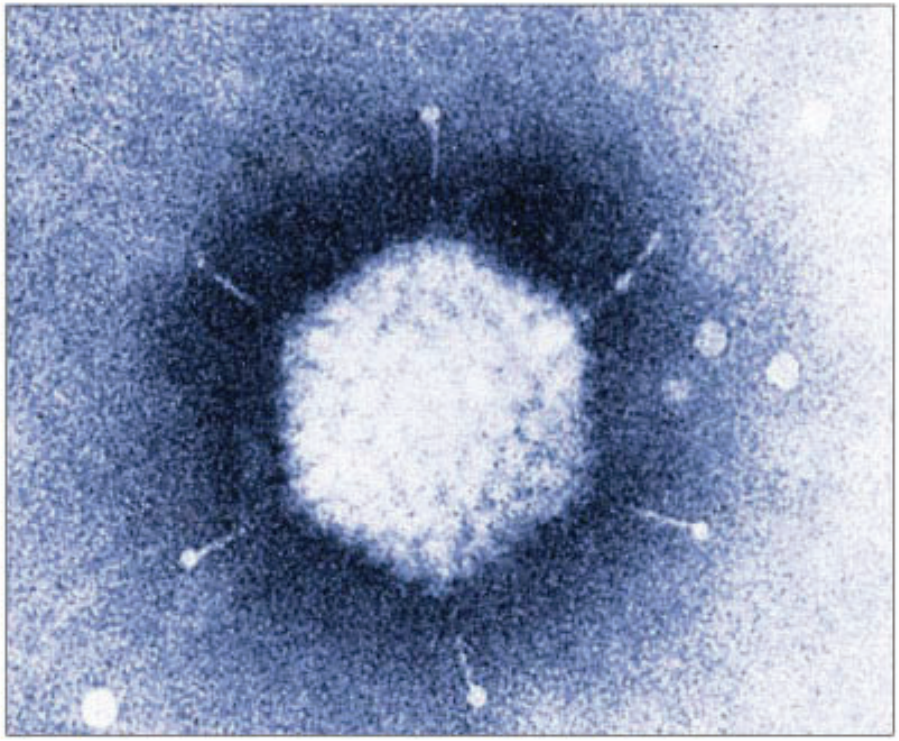
(a) A helical virus

(b) Ebola virus

Polyhedral Viruses

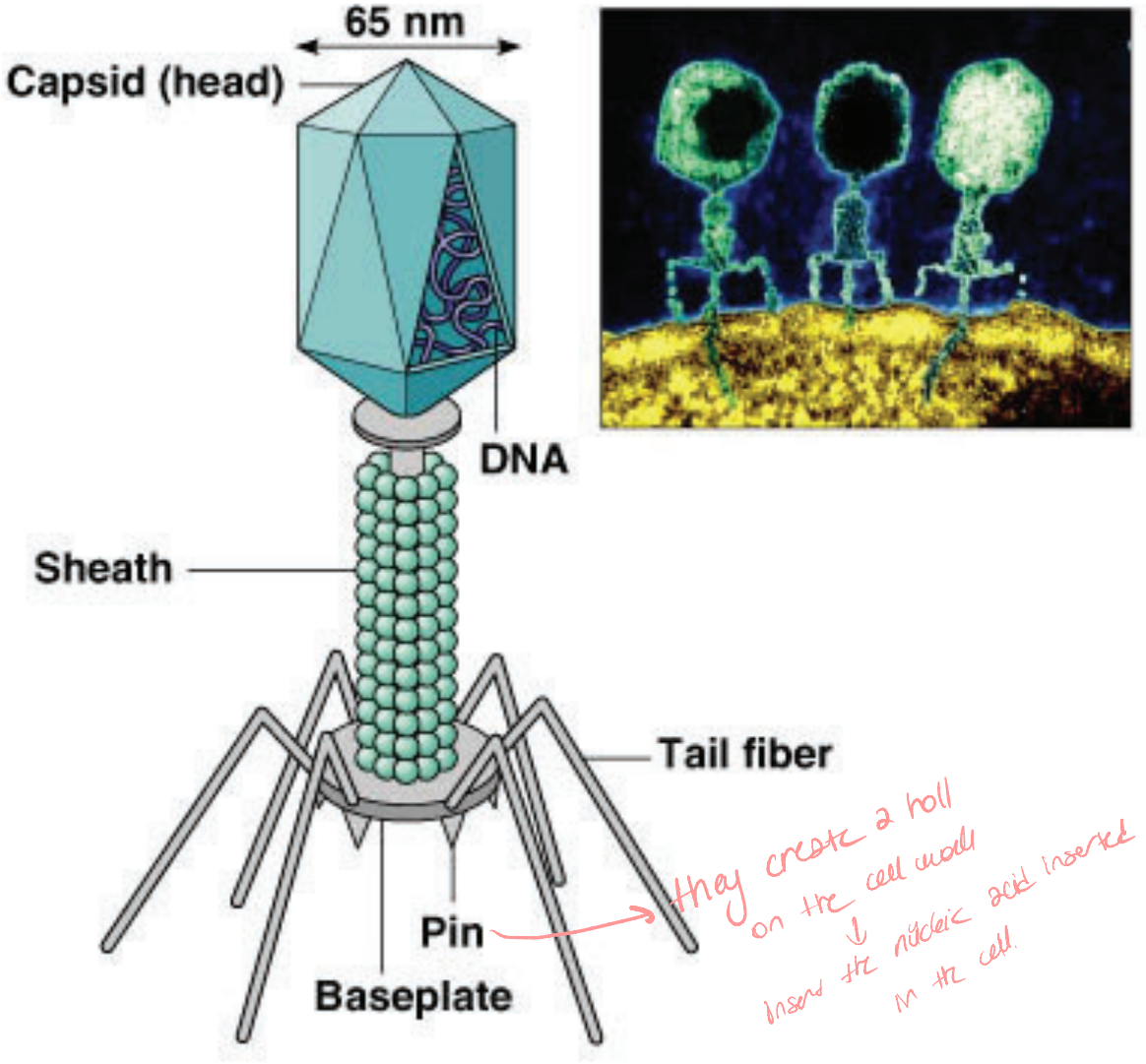


(a) A polyhedral virus



(b) A *Mastadenovirus*

Complex Viruses (phage (bacteria phage))



(a) A T-even bacteriophage

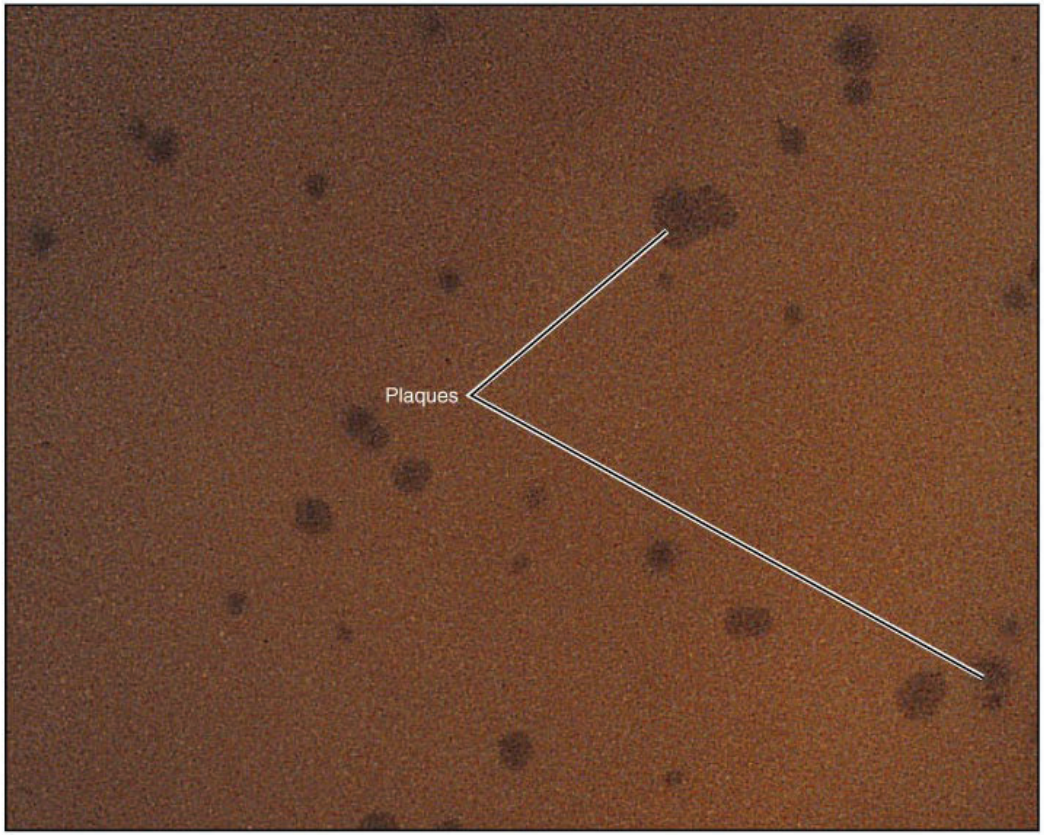
Viral Taxonomy

- Family names end in -viridae
- Genus names end in -virus
- Viral species: A group of viruses sharing the same genetic information and ecological niche (host). Common names are used for species
- Subspecies are designated by a number

Growing Viruses

for phage viruses

- Viruses must be grown in living cells.
  - Bacteriophages form plaques on a lawn of bacteria. → after they lyse the bacteria

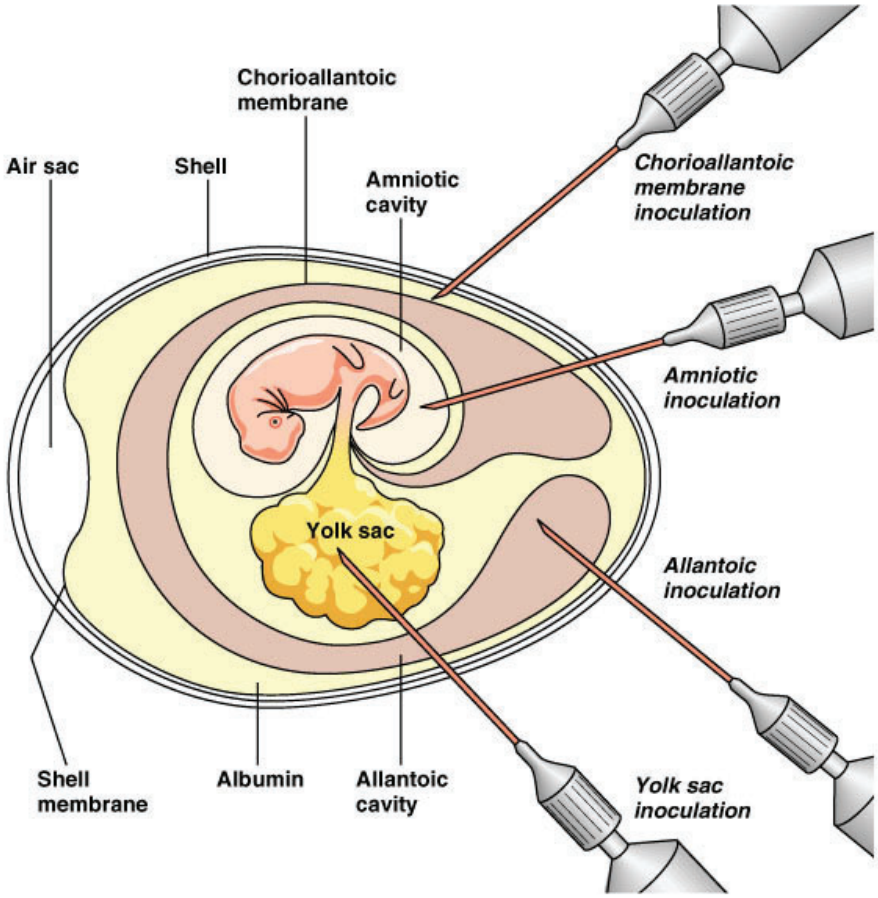


Growing Viruses

For growing bacteria

SINAV SORUSU

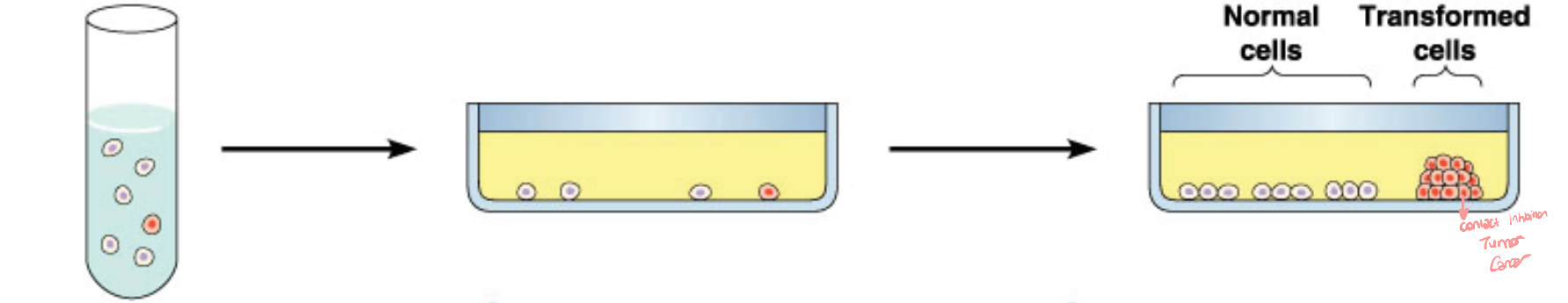
- Animal viruses may be grown in living animals or in embryonated eggs.



Growing Viruses

For growing viruses

- Animal and plants viruses may be grown in cell culture.
  - Continuous cell lines may be maintained indefinitely.



Ready to use cell-lines !
↓
immortal cells (available in the market)

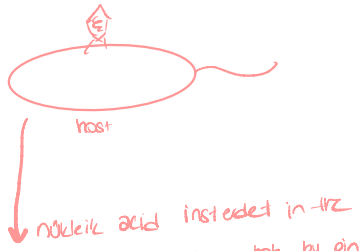
Ex: HeLa cell line

Virus Identification

- Cytopathic effects
- Serological tests
  - Detect antibodies against viruses in a patient
  - Use antibodies to identify viruses in neutralization tests, viral hemagglutination, and Western blot
- Nucleic acids
  - RFLPs
  - PCR

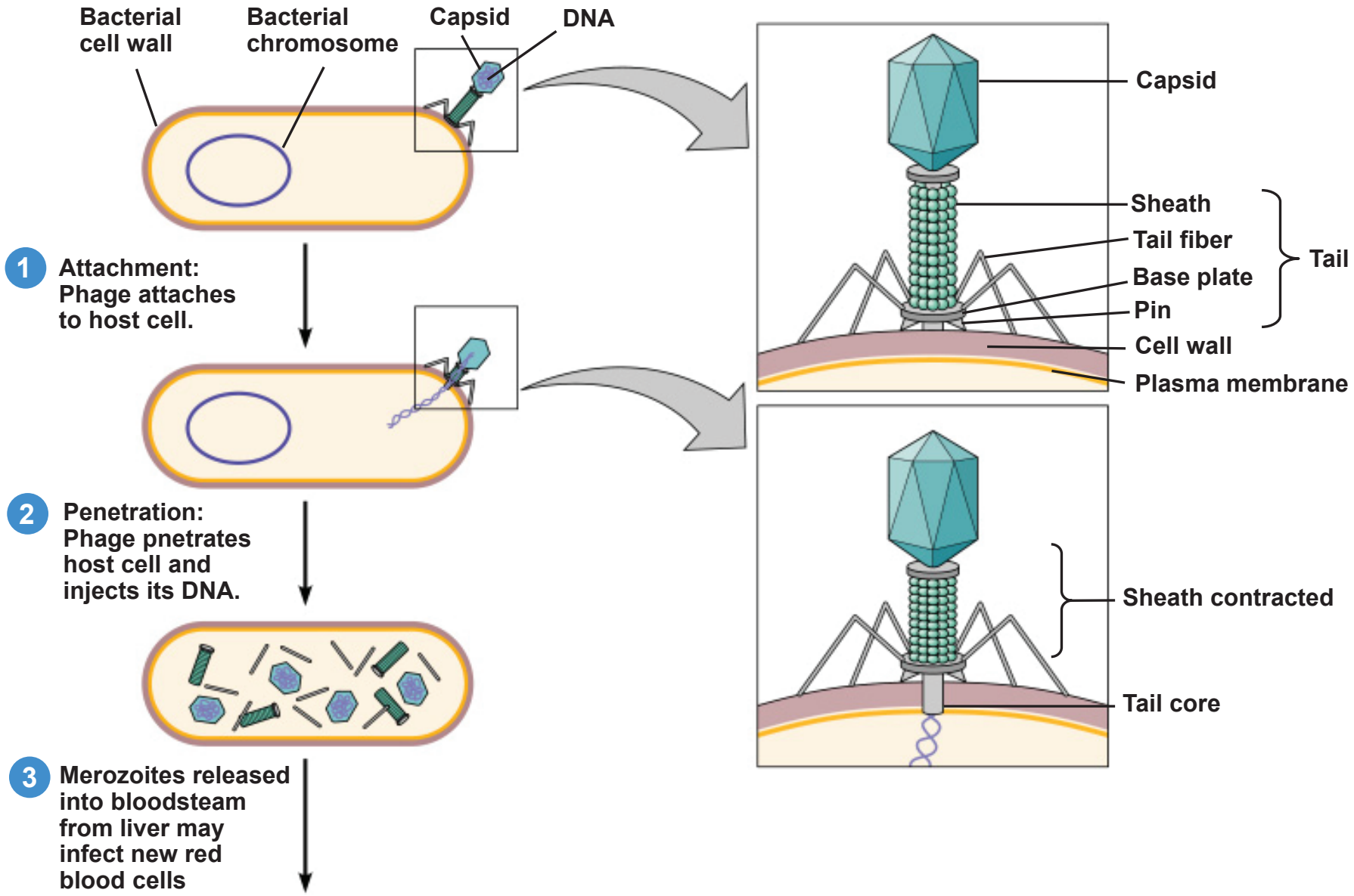
Multiplication of Bacteriophages (Lytic Cycle)

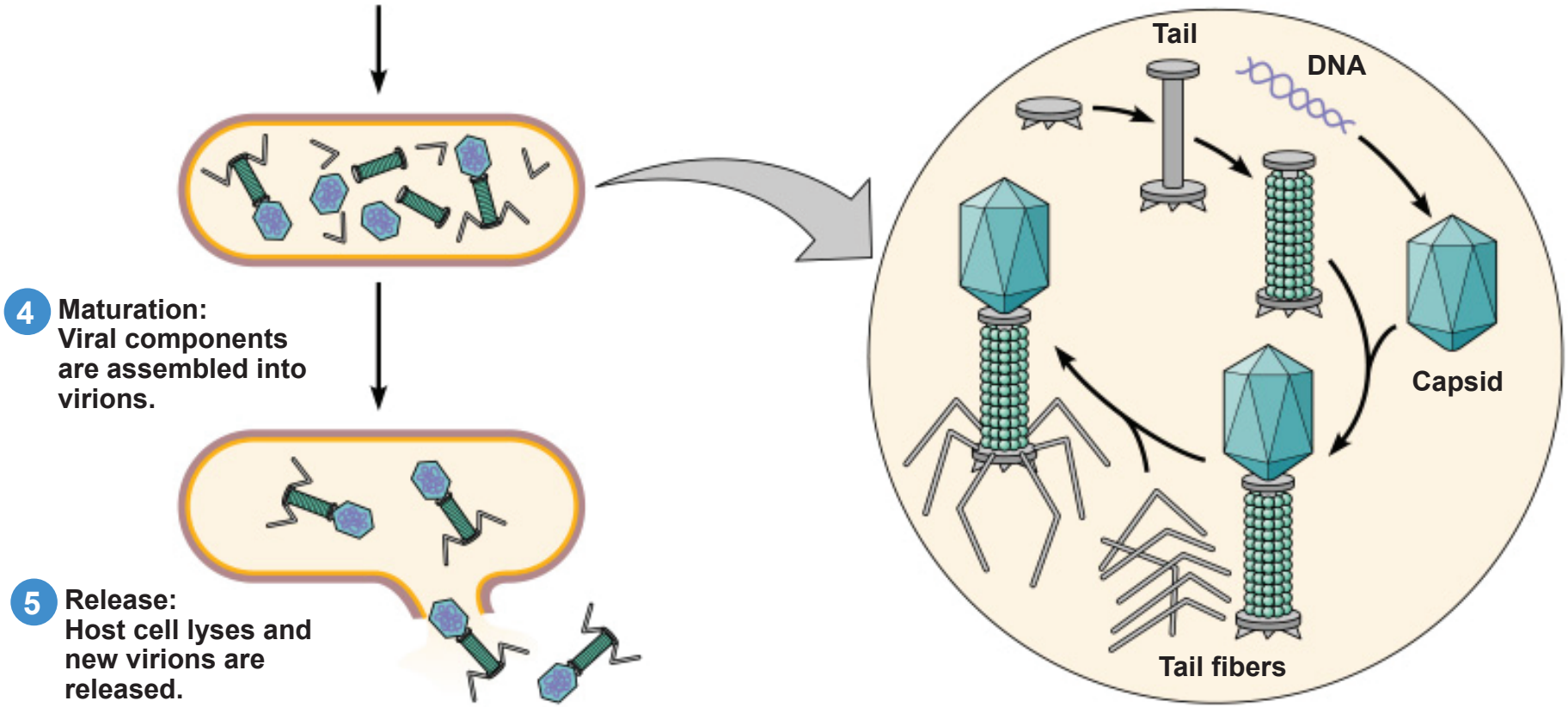
- Attachment — Phage attaches by tail fibers to host cell
- Penetration — Phage lysozyme opens cell wall, tail sheath contracts to force tail core and DNA into cell
- Biosynthesis — Production of phage DNA and proteins
- Maturation — Assembly of phage particles
- Release — Phage lysozyme breaks cell wall



but seperatly.

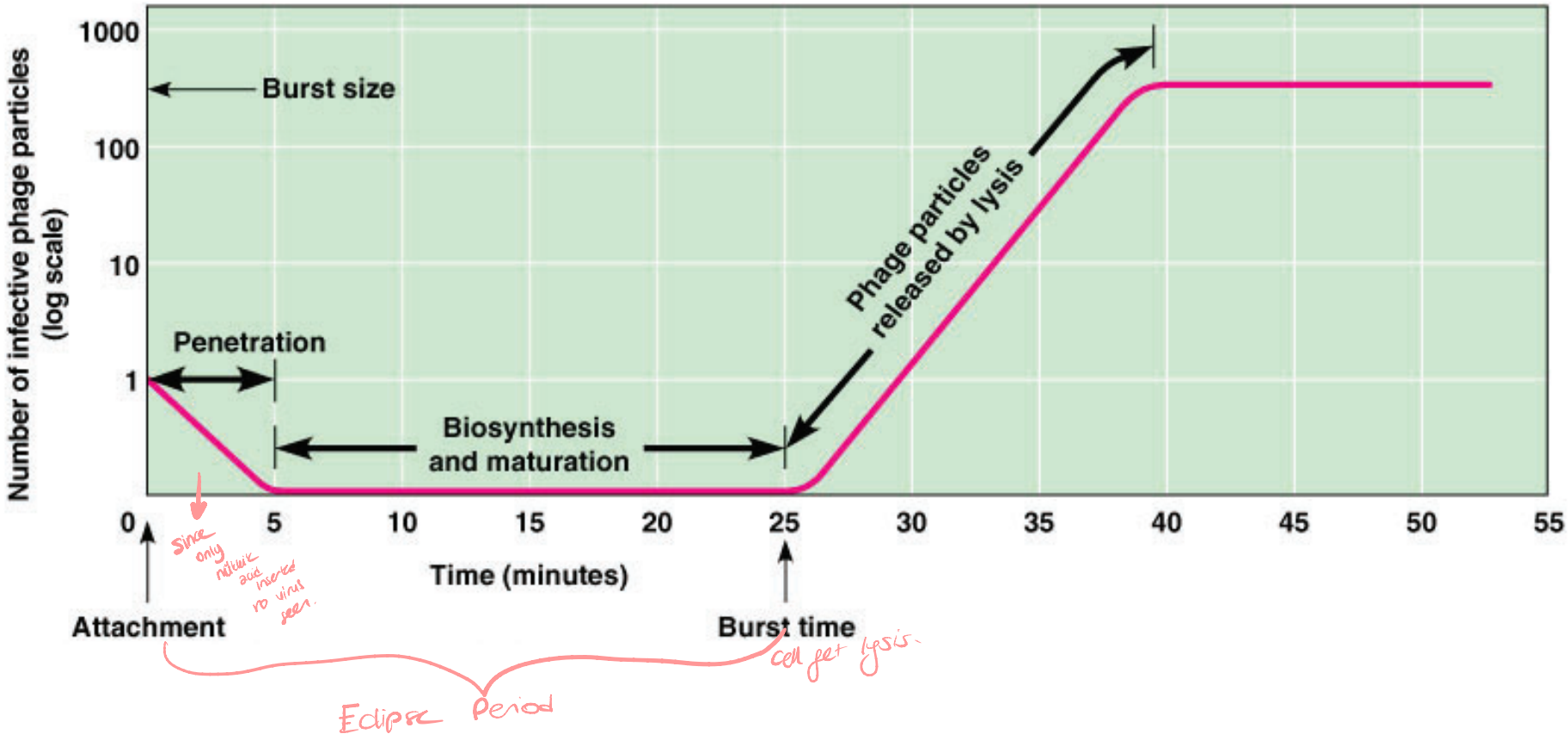
virion
newly synthesized phage.


Bacterial cell wall
Bacterial chromosome
Capsid
DNA
1 Attachment: Phage attaches to host cell.
2 Penetration: Phage pnetrates host cell and injects its DNA.
3 Merozoites released into bloodsteam from liver may infect new red blood cells
Capsid
Sheath
Tail fiber
Base plate
Pin
Tail
Cell wall
Plasma membrane
Sheath contracted
Tail core


4 Maturation:
Viral components
are assembled into
virions.
5 Release:
Host cell lyses and
new virions are
released.
Tail
DNA
Capsid
Tail fibers

SINAV SORUSU

One-step Growth Curve



- Lytic cycle — Phage causes lysis and death of host cell
- Lysogenic cycle — Prophage DNA incorporated in host DNA

↳ host survives.

↳ After time lysogenic cycle can be lytic cycle

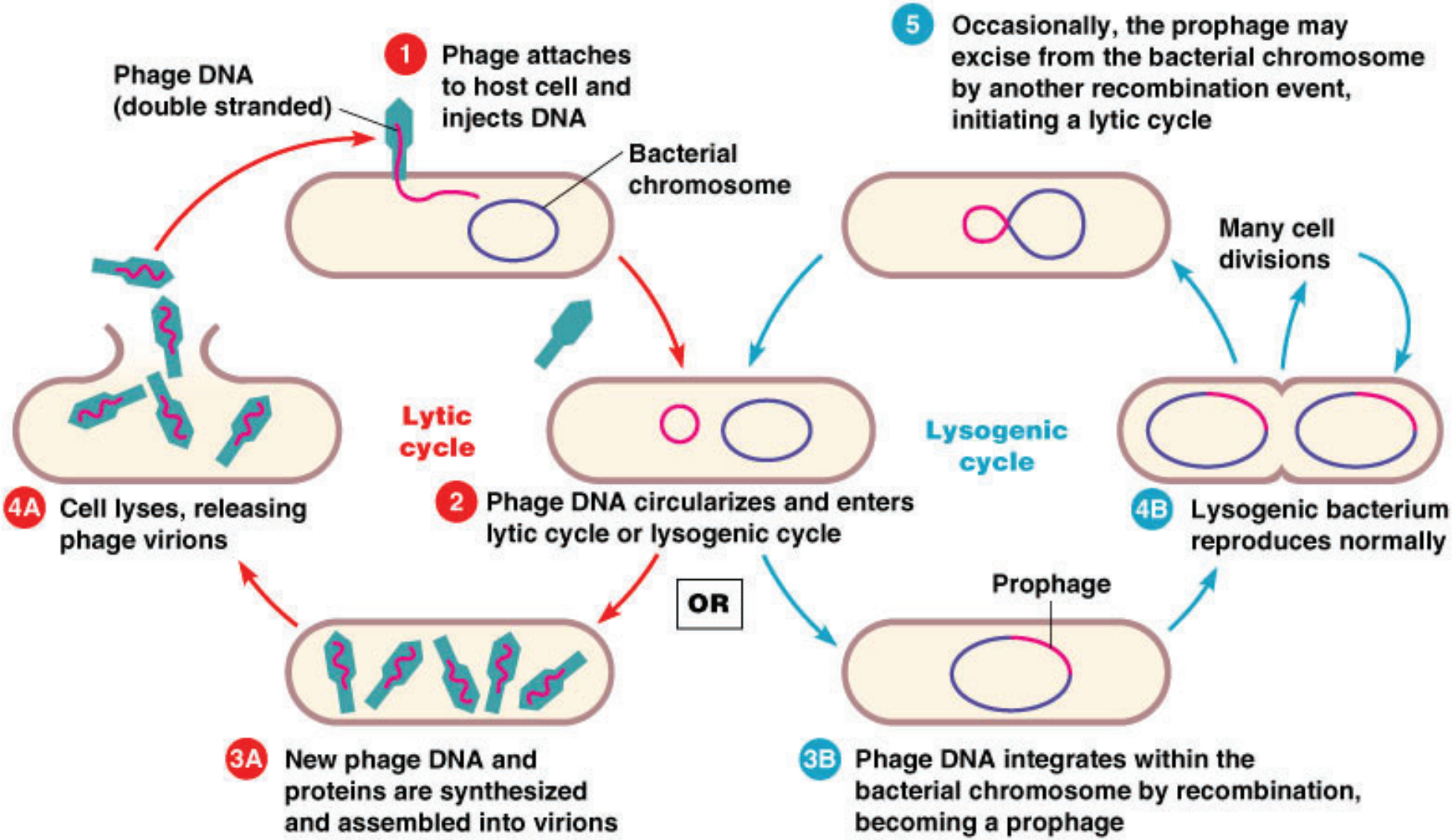
↓

called prophage. (inserted nucleic acid)

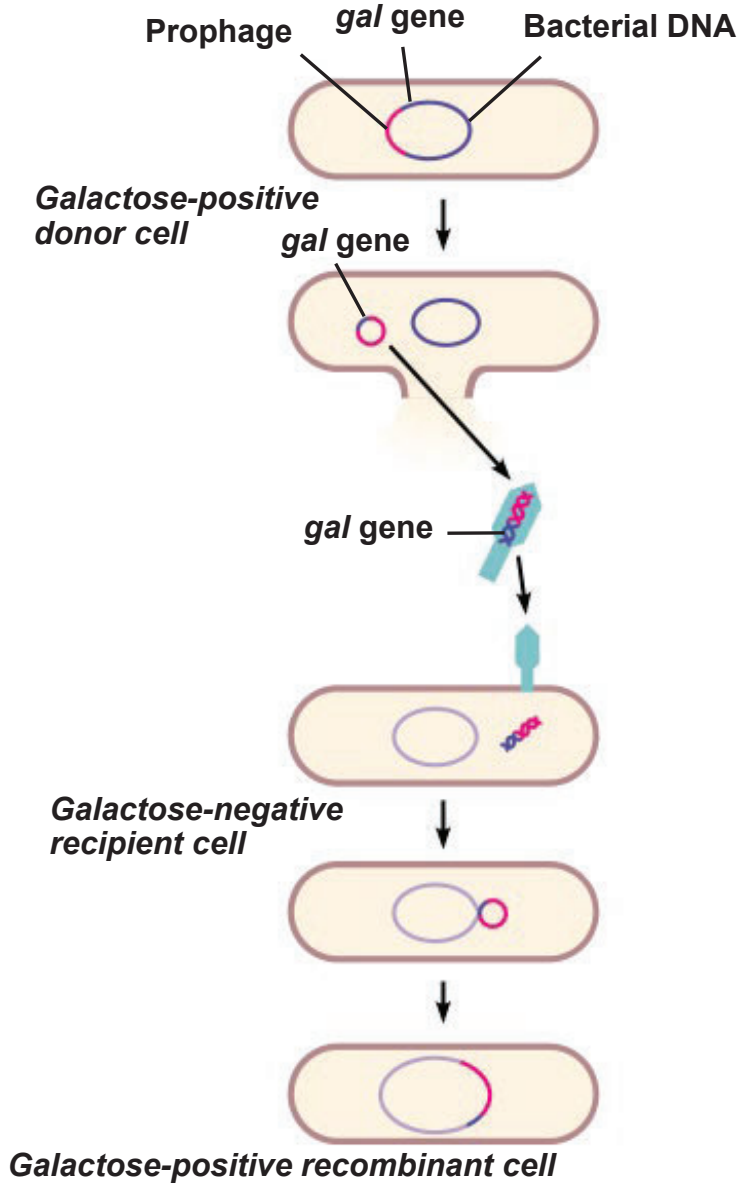
temperate phage => means viruses called can cause lysogenic cycle

"pop out"

The Lysogenic Cycle



Specialized Transduction



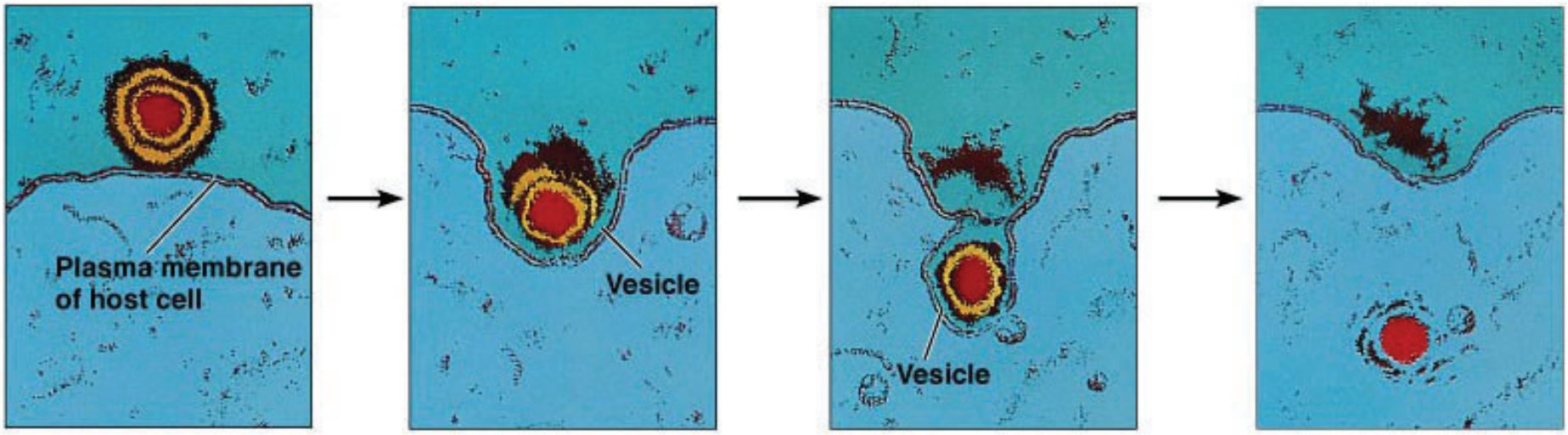
1. Prophage exists in galactose-using host (containing the *gal* gene).
2. Phage genome excises, carrying with it the adjacent *gal* gene from the host.
3. Phage matures and cell lyses, releasing phage carrying *gal* gene.
4. Phage infects a cell that cannot utilize galactose (lacking *gal* gene).
5. Along with the prophage, the bacterial *gal* gene becomes integrated into the new host's DNA.
6. Lysogenic cell can now metabolize galactose.

Multiplication of Animal viruses

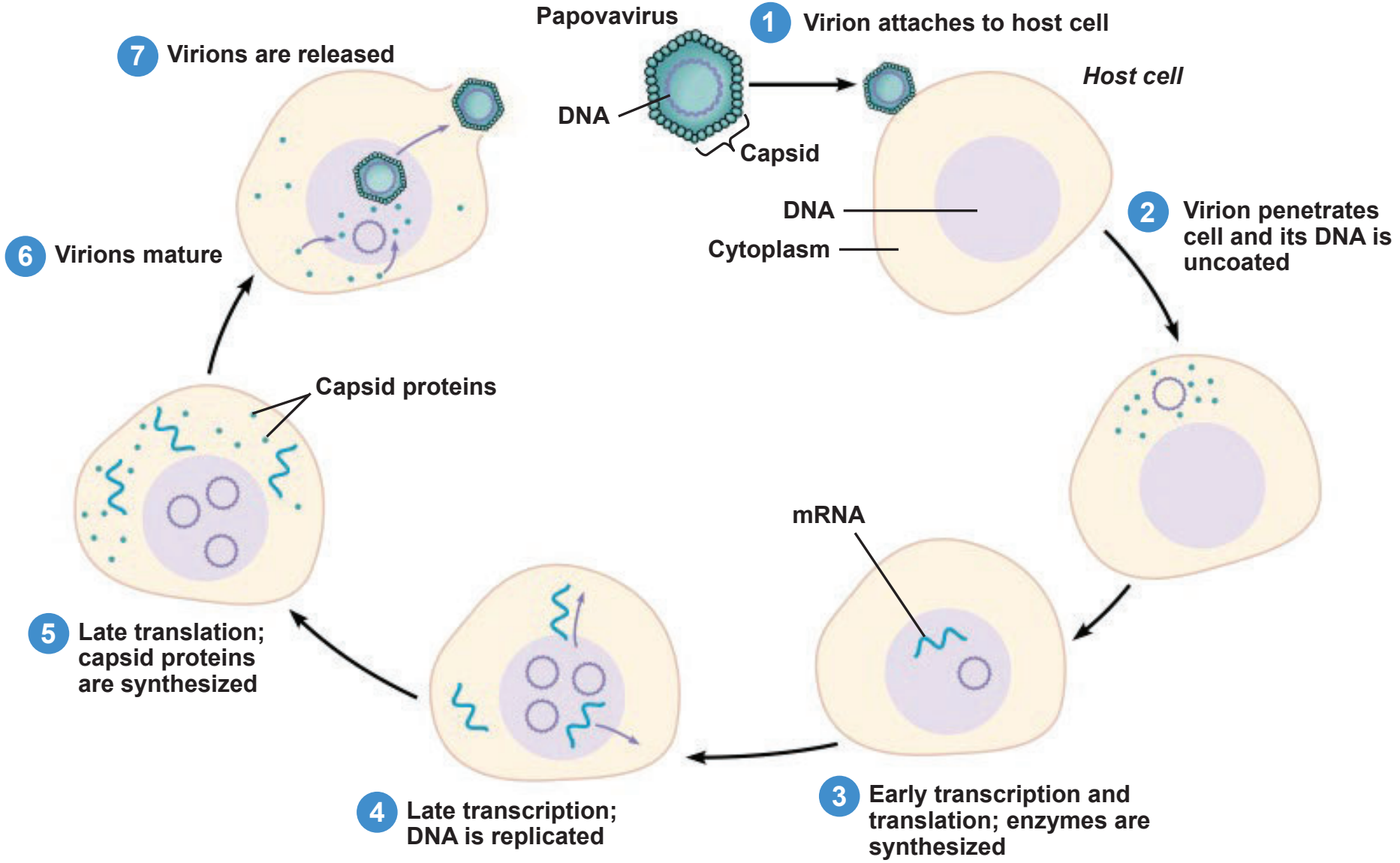
- Attachment Viruses attaches to cell membrane
- Penetration By endocytosis or fusion
- Uncoating By viral or host enzymes → Envelope.
- Biosynthesis Production of nucleic acid and proteins
- Maturation Nucleic acid and capsid proteins assemble
- Release By budding (enveloped viruses) or rupture

Y means cell doesn't die

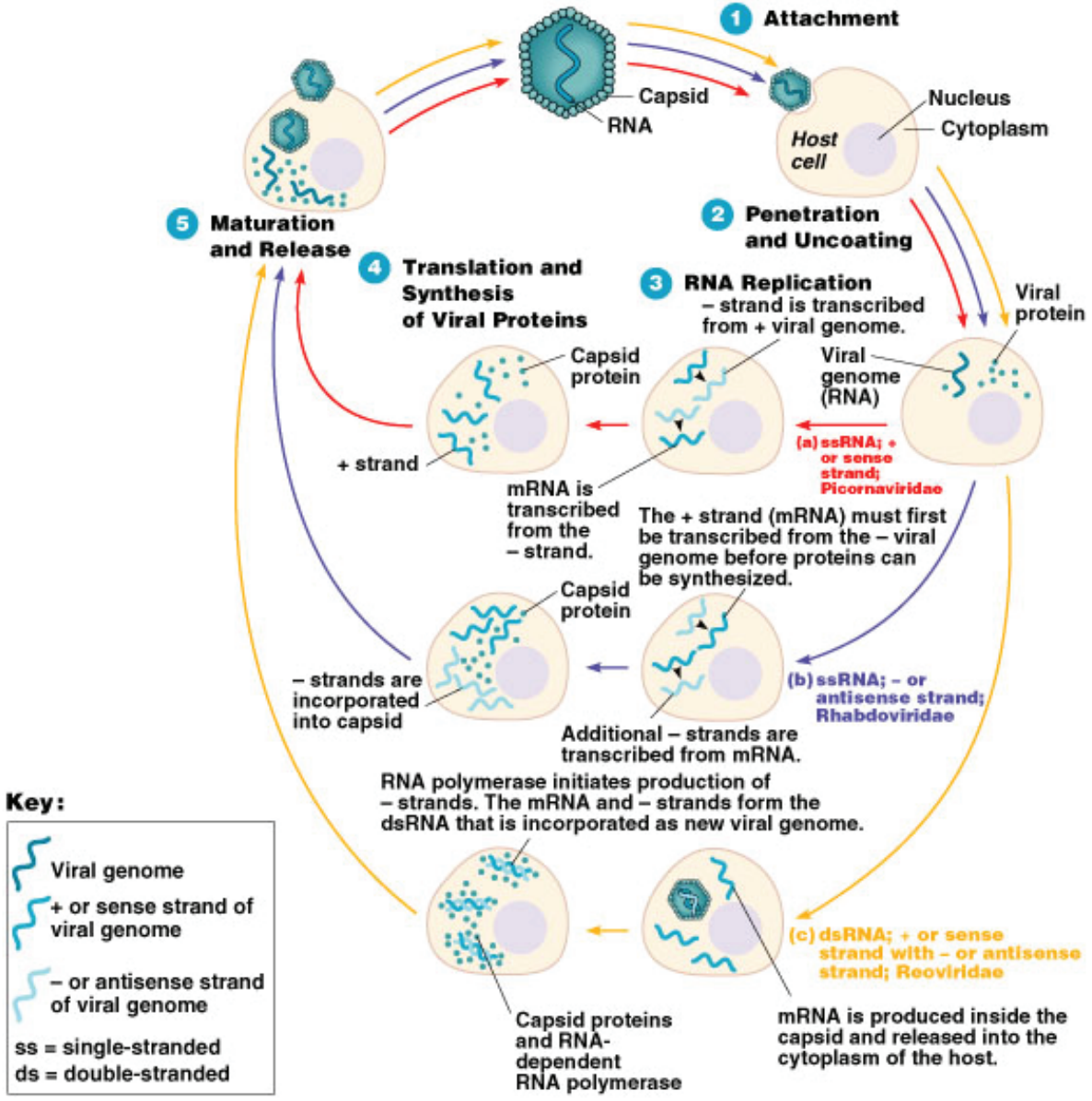
Attachment, Penetration, and Uncoating



Multiplication of DNA Virus



Pathways of Multiplication for RNA-Containing Viruses



Cancer

- Activated oncogenes transform normal cells into cancerous cells.
- Transformed cells have increased growth, loss of contact inhibition, tumor specific transplant and T antigens.
- The genetic material of oncogenic viruses becomes integrated into the host cell's DNA.

Oncogenic Viruses

- Oncogenic DNA Viruses
  - Adenoviridae
  - Heresviridae
  - Poxviridae
  - Papovaviridae
  - Hepadnaviridae
- Oncogenic RNA viruses
  - Retroviridae
    - Viral RNA is transcribed to DNA which can integrate into host DNA
    - HTLV 1
    - HTLV 2

- Latent Viral Infections
  - Virus remains in asymptomatic host cell for long periods
    - Cold sores, shingles
- Persistent Viral Infections
  - Disease processes occurs over a long period, generally fatal
    - Subacute sclerosing panencephalitis (measles virus)

in bacterio phages we dont have fats (only in animal viruses)

Prions

- Infectious proteins → called "prions"
- Inherited and transmissible by ingestion, transplant, & surgical instruments
- Spongiform encephalopathies: Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, mad cow disease
- PrP^{C}, normal cellular prion protein, on cell surface
- PrP^{Sc}, abnormal prion protein, accumulate in brain cells forming plaques

→ Scorpic ! → Deli Dona Hastalığı

- Plant Viruses
  - Plant viruses enter through wounds or via insects
- Viroids → just RNA molecule
  - Viroids are infectious RNA; potato spindle tuber disease