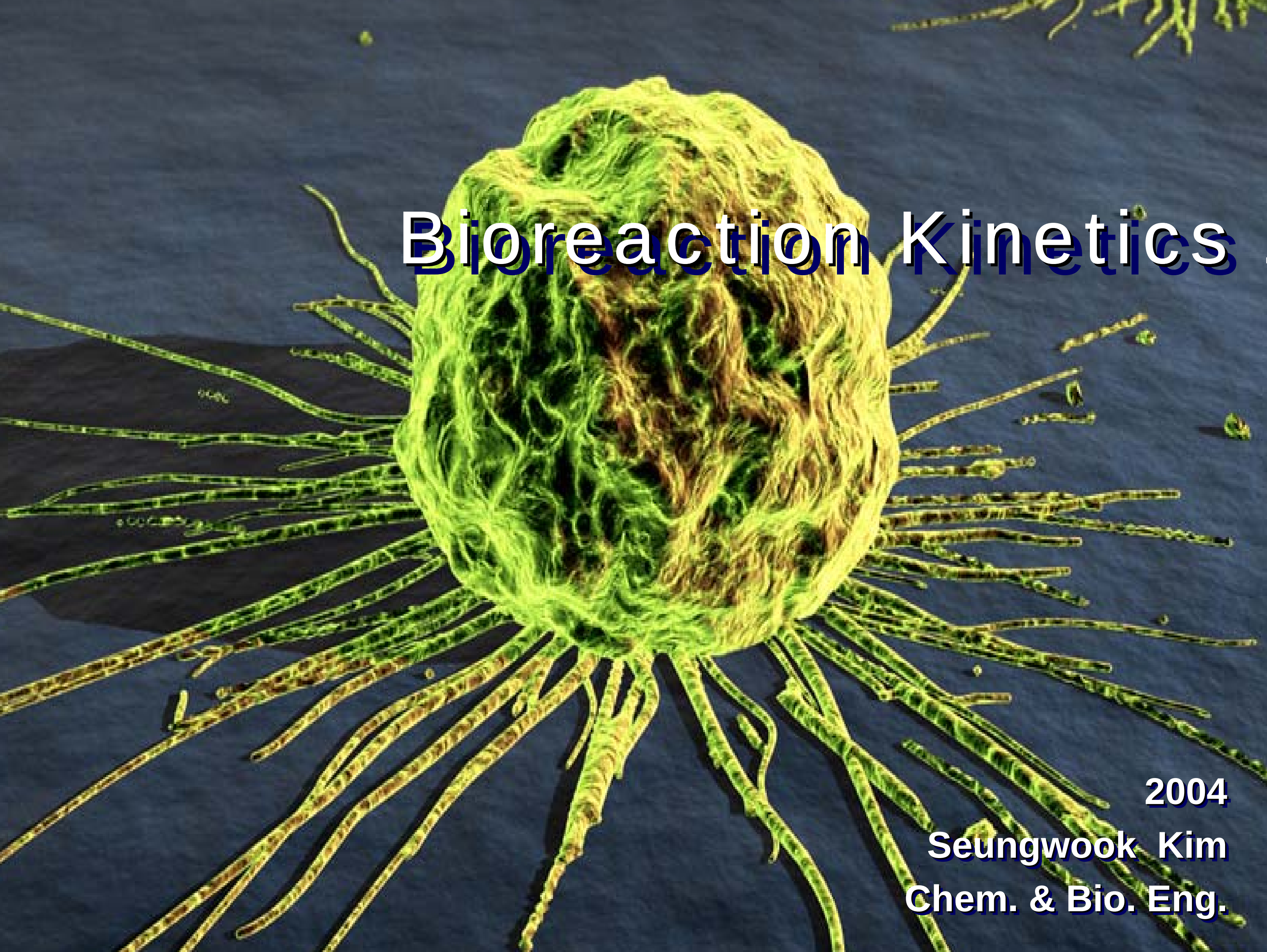


Bioreaction Kinetics

2004

Seungwook Kim
Chem. & Bio. Eng.

Reference

- **Bioprocess Engineering Basic Concepts,**

  2nd edition,

  M.L. Shuler and F. Kargi,

  Prentice Hall.

Microbial Diversity

- **Psychrophiles :** Cells that grow best at low temperatures (below 20°C).
- **Mesophiles :** temperature optima (20°C ~ 50°C).
- **Thermophiles :** temperatures greater than 50°C.

- **Aerobic organisms :** Some cells require oxygen for growth and metabolism.
- **Anaerobic organisms :** Some organisms are inhibited by the presence of oxygen and grow only anaerobically.
- **Facultative organisms :** Some organisms can switch the metabolic pathway to allow them to grow under either circumstances.

Microbial Diversity

- **Cyanobacteria (Blue-green algae) :** These organisms can grow in an environment with only a little moisture and a few dissolved minerals. These bacteria are photosynthetic and can convert CO_2 from the atmosphere into the organic compounds necessary for life. They can also convert N_2 into NH3 for use in making the essential building blocks of life.

- **Extremophiles :** Some cells can grow at extreme environments.

Naming Cells

- Dual name (binary nomenclature) are given in Latin.
- Genus: a group of related species
- Species: organisms that are substantially alike.
- Ex) *Escherichia coli* K12

Classification of Cell Type

- **Procaryotes:**
  - **Simple structure with single chromosome**
  - **No nuclear membrane and no organelles such as the mitochondria and endoplasmic reticulum.**
- **Eucaryotes**
  - **More complex internal structure, with more than one chromosome(DNA molecule) in the nucleus.**
  - **A true nuclear membrane, mitochondria, endoplasmic reticulum, golgi apparatus and specialized organelles.**

A Comparison of Procaryotes with Eucaryotes

Characteristic	Procaryotes	Eucaryotes
Genome		
No. of DNA molecules	One	More than one
DNA in organelles	No	Yes
DNA observed as chromosomes	No	Yes
Nuclear membrane	No	Yes
Mitotic and meiotic division of the nucleus	No	Yes
Formation of partial diploid	Yes	No
Organelles		
Mitochondria	No	Yes
Endoplasmic reticulum	No	Yes
Golgi apparatus	No	Yes
Photosynthetic apparatus	Chlorosomes	Chloroplasts
Flagella	Single protein, simple structure	Complex structure, with microtubules
Spores	Endospores	Endo- and exospores
Heat resistance	High	Low

Primary Subdivisions of Cellular Organisms That Are Now Recognized

Group	Cell structure	Properties	Constituent groups
Eucaryotes	Eucaryotic	Multicellular; extensive differentiation of cells and tissues	Plants (seed plants, ferns, mosses) Animals (vertebrates, invertebrates)
		Unicellular, coenocytic or mycelial; little or no tissue differentiation	Protists (algae, fungi, protozoa)
Eubacteria	Procaryotic	Cell chemistry similar to eucaryotes	Most bacteria
Archaebacteria	Procaryotic	Distinctive cell chemistry	Methanogens, halophiles, thermoacidophiles

With permission, from R. Y. Stanier et al., *The Microbial World*, 5th ed., Pearson Education, Upper Saddle River, NJ, 1986.

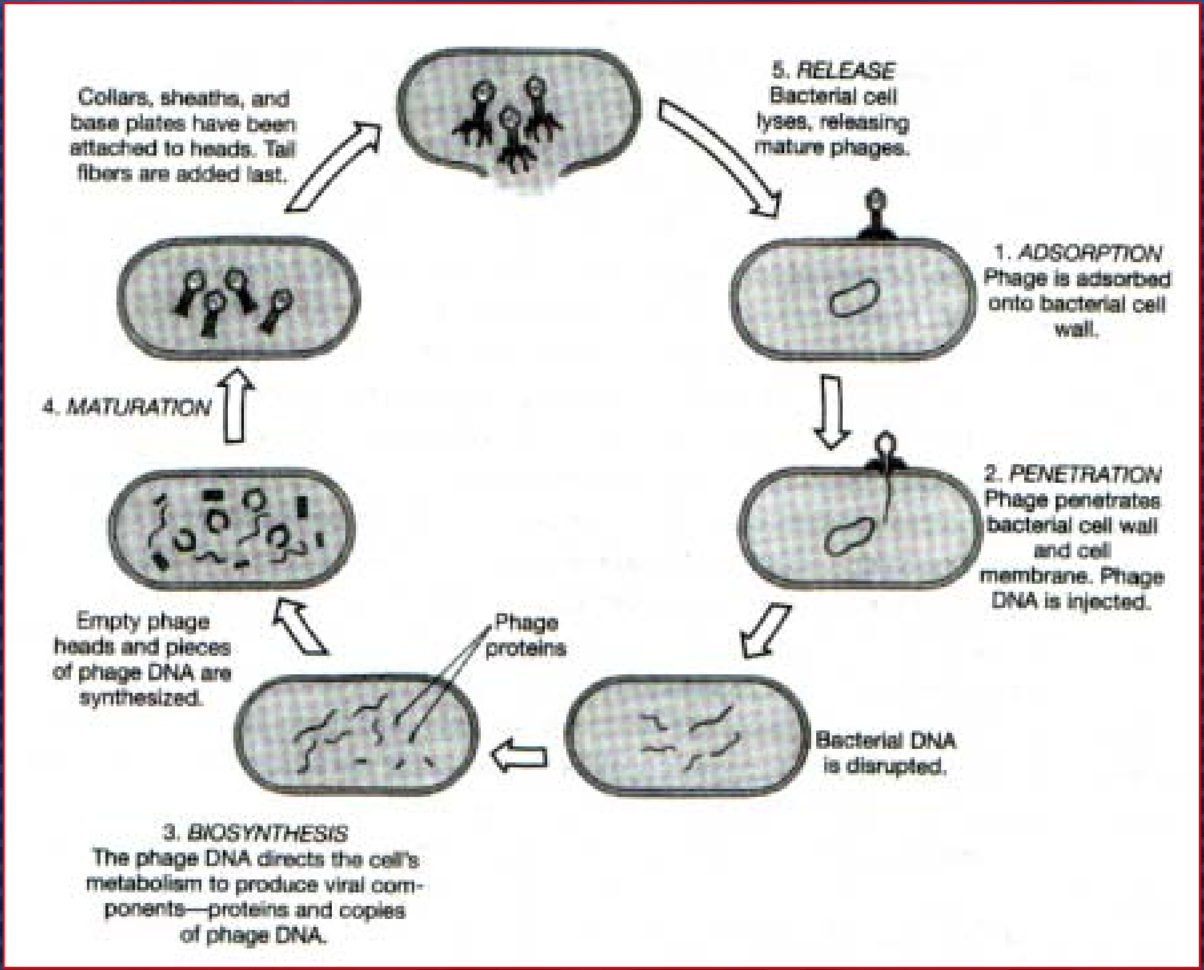
Viruses

- are obligate parasites of other cells, such as bacterial, yeast, plant, and animal cells.
- Viruses are functionally active when inside their host cells.
- Size : 30 ~ 200 nm
- Type : DNA viruses or RNA viruses
- The nuclear material is covered by a protein called a capsid.

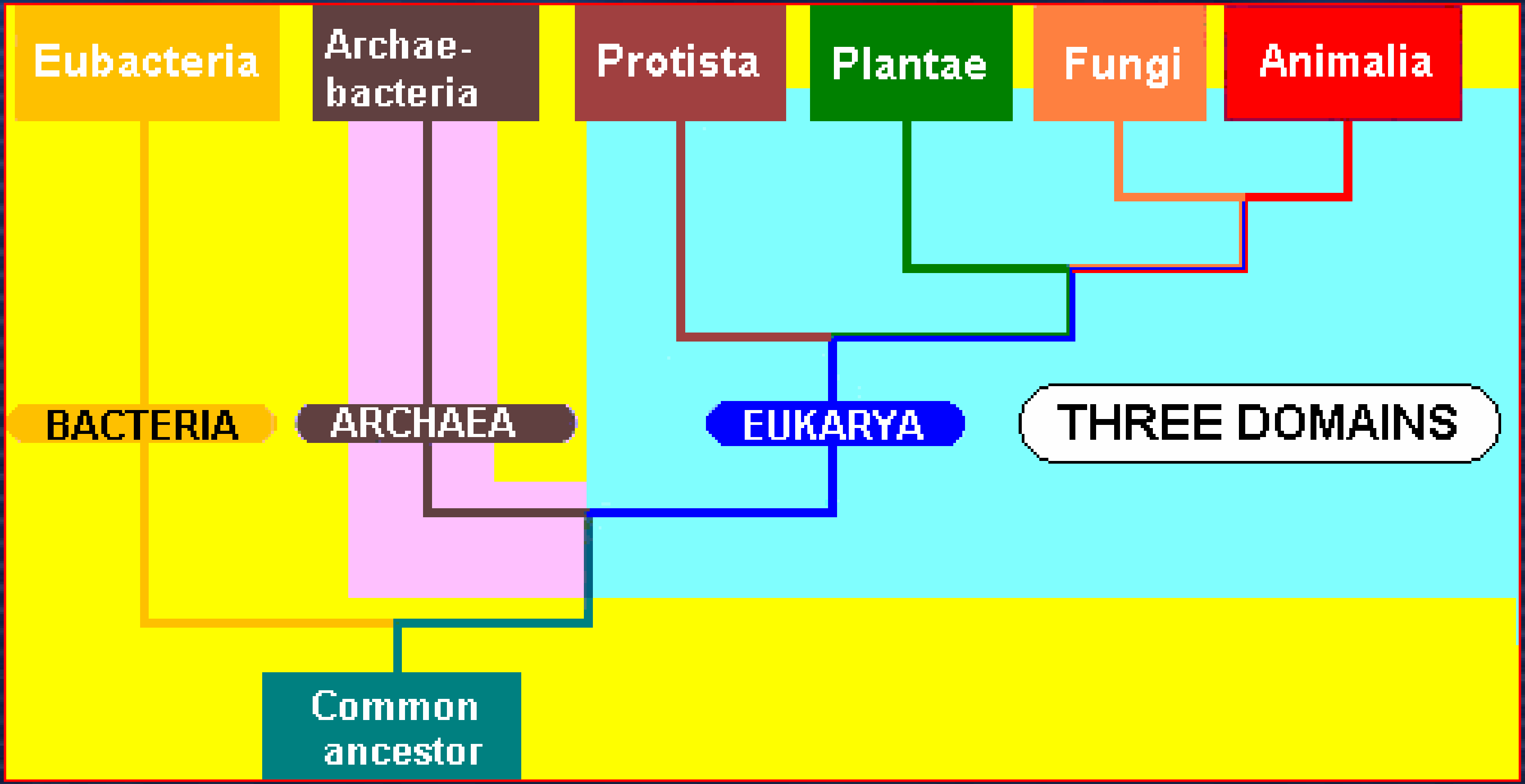
Bacteriophages

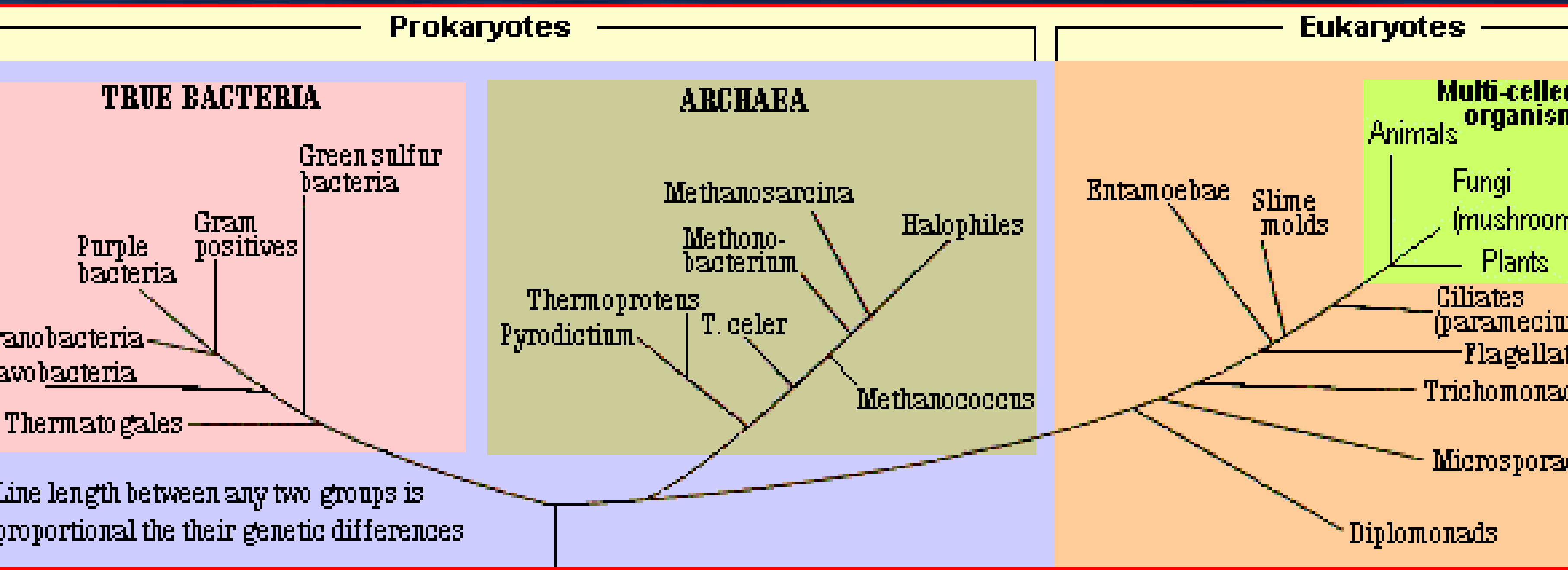
- viruses infecting bacteria.
- Lytic cycle : reproduction mode of viruses.
- Lysogenic cycle: phage DNA may be incorporated into the host DNA and the host may continue to multiply in this state.

Replication of a virulent bacteriophage



Six Kingdoms

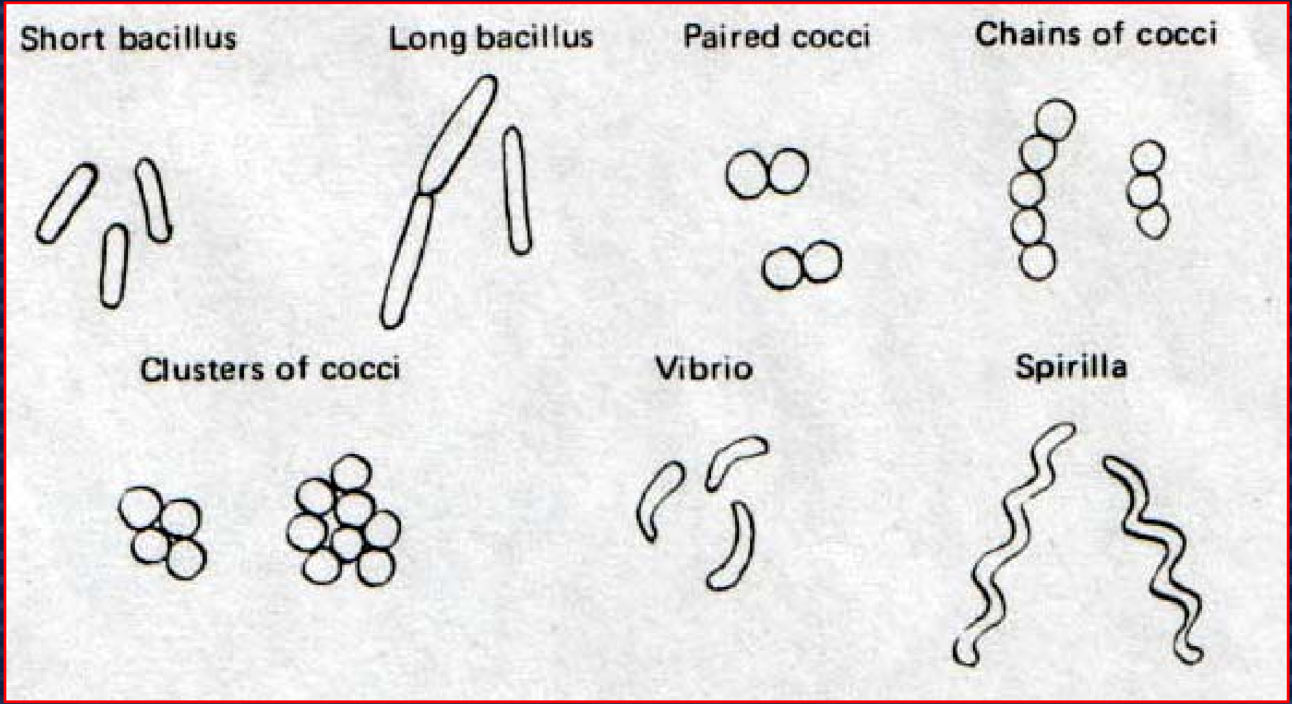




Prokaryotes
Eukaryotes
TRUE BACTERIA
Green sulfur bacteria
Gram positives
Purple bacteria
anobacteria
avobacteria
Thermatogales
ARCHAEA
Methanosarcina
Methono-bacterium
Halophiles
Thermoproteus
T. celer
Pyrodictium
Methanococcus
Multi-celled organism
Animals
Fungi (mushroom
Plants
Entamoebae
Slime molds
Ciliates (paramecin
Flagellat
Trichomona
Microspora
Diplomonads
Line length between any two groups is proportional the their genetic differences

Procaryotes

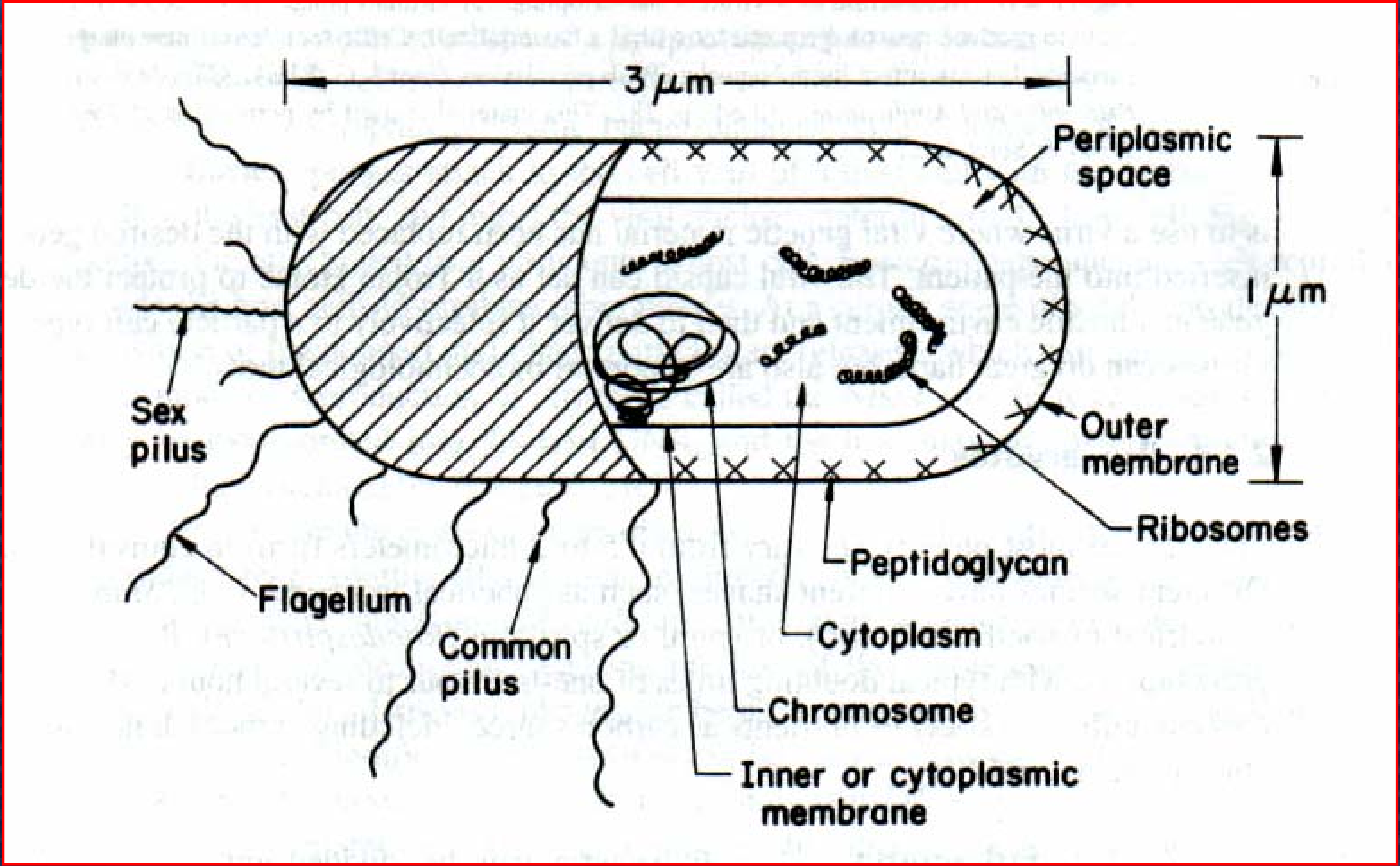
- Size : 0.5 ~ 3 µm
- Shape: various
- Doubling time: 1/2 ~ several hours
- Common bacterial shapes



Eubacteri

- Gram-negative cells : *Escherchia coli*
  - Outer membrane is supported by a thin peptidoglycan layer.
  - Peptidoglycan ia a complex polysaccharide with amino acids.
  - Inner membrane(Cytoplasmic membrane) is separated from the outer membrane by the periplasmic space. It contains about 50% protein, 30% lipids, and 20% carbohydrates.
  - Loss of membrane integrity leads to cell lysis(cells breaking open) and cell death.
  - The cell envelop is crucial to the transport of selected material in and out of the cell.

Schematic of a Typical Gram-Negative bacterium (*E. coli*)



Eubacteria

- Gram-positive cells : *Bacillus subtilis*
  - Gram-positive cell do not have an outer membrane. They have a very thick, rigid cell wall with multiple layers of peptidoglycan and also contain teichoic acids covalently bonded to the peptidoglycan.
  - Because they have only a cytoplasmic membrane, they are often much better suited to excretion of proteins.

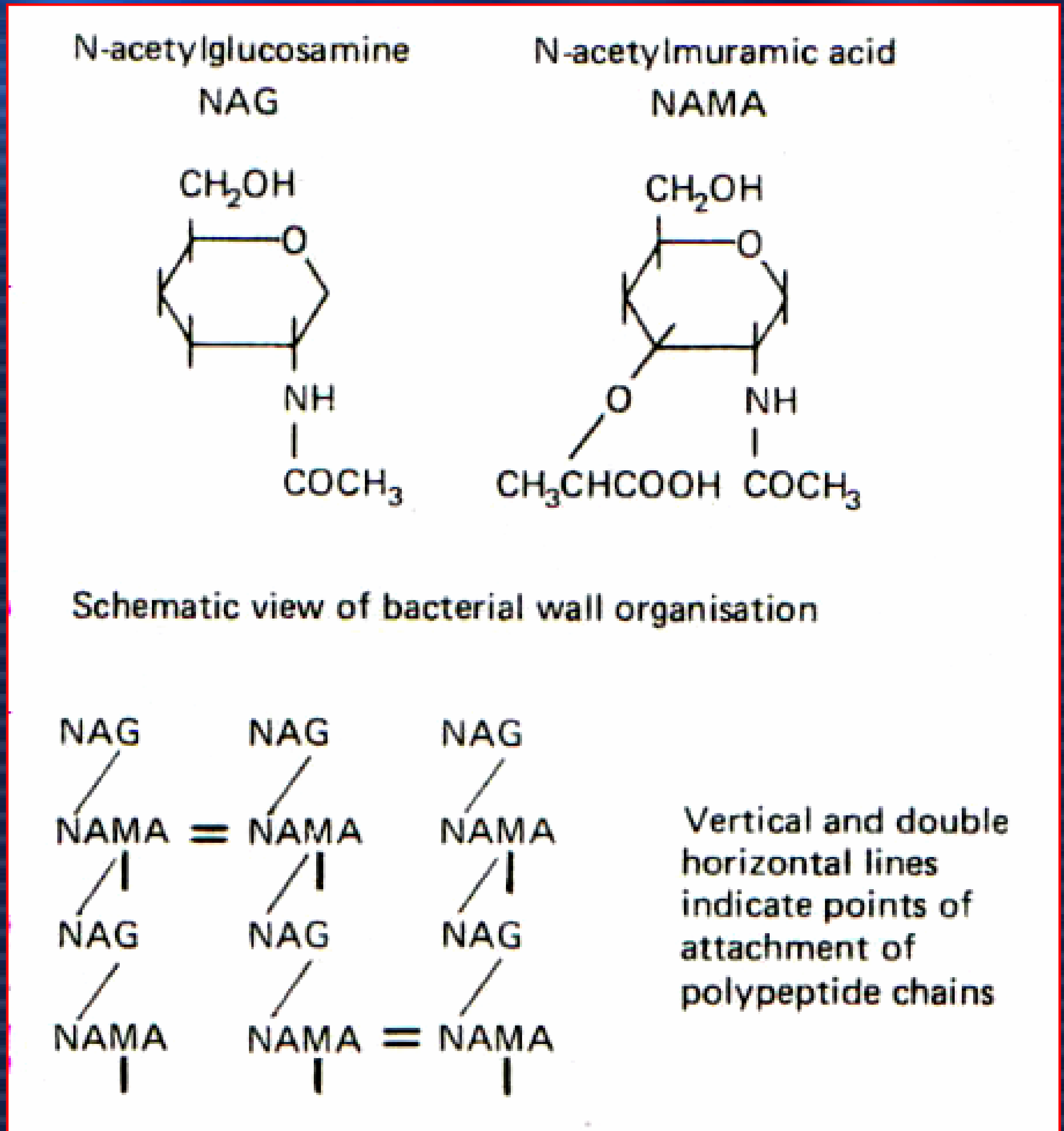
Staining Properties

- It is based on specific morphological characters. Gram's method is the most widely used and is developed by Christian Gram in 1884. It enables one to subdivide bacteria into two groups : Gram positive and Gram negative. This may be due to the differences in cell wall composition.
- In general, all sporing bacteria are Gram positive, all bacteria with polar flagella are Gram negative.
- Some bacteria that are not Gram-positive or Gram-negative : Mycoplasma
  - It has no cell walls. These bacteria are important not only clinically, but also because they commonly contaminate media used industrially for animal cell culture.

- **Comparisons of the composition of walls of Gram positive and Gram negative bacteria. (Ref. J.I. Williams and M. Shaw, Micro-organisms, 2nd Ed., 1982).**

Components	Gram Positive	Gram Negative
Mucopeptide	Up to 95%	As little as 5%
Lipid	Up to 2%	Up to 20%
Range of amino acids	Up to 4%	Up to 18%
Teichoic acid	Present	Absent

Chemical Composition of Mucopeptid



Actinomycetes

- Actinomyces, Thermomonospora, Streptomyces.
- They are bacteria, but morphologically, actinomycetes resemble molds with their long and highly branched hyphae.
- The lack of a nuclear membrane and the composition of the cell wall require classification as bacteria.
- Actinimycetes are important sources of antibiotics.

Archaebacteria

- In many ways the archaebacteria are as similar to the eucaryotes as they are to the eubacteria.
- Differences between archaebacteria and eubacteria
  1. Archaebacteria have no peptidoglycan.
  2. The nuclotide sequences in the ribosomal RNA are similar within the archaebacteria but distinctly different from eubacteria.
  3. The lipid composition of the cytoplasmic membrane is very different for the two groups.

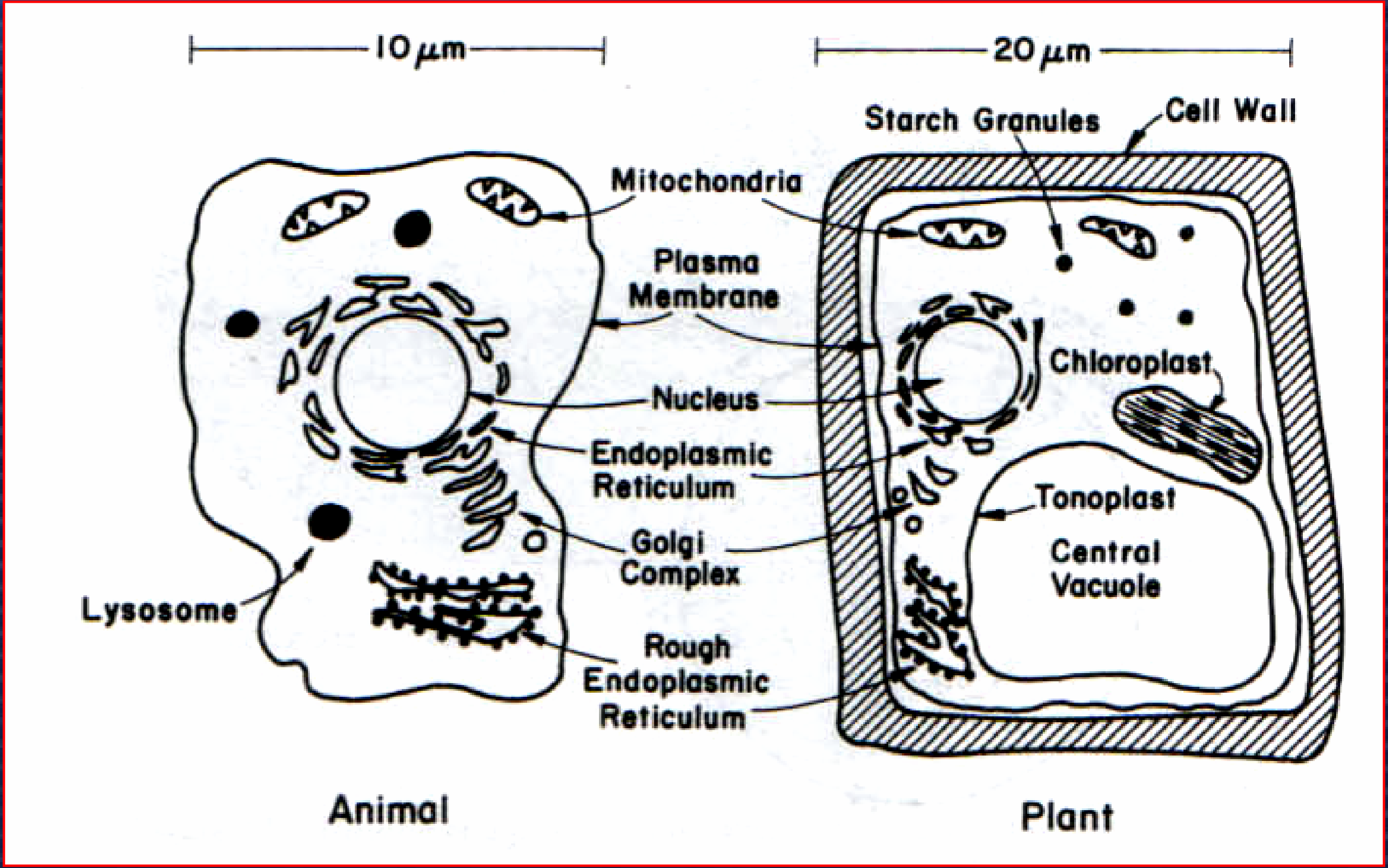
Archaebacteria

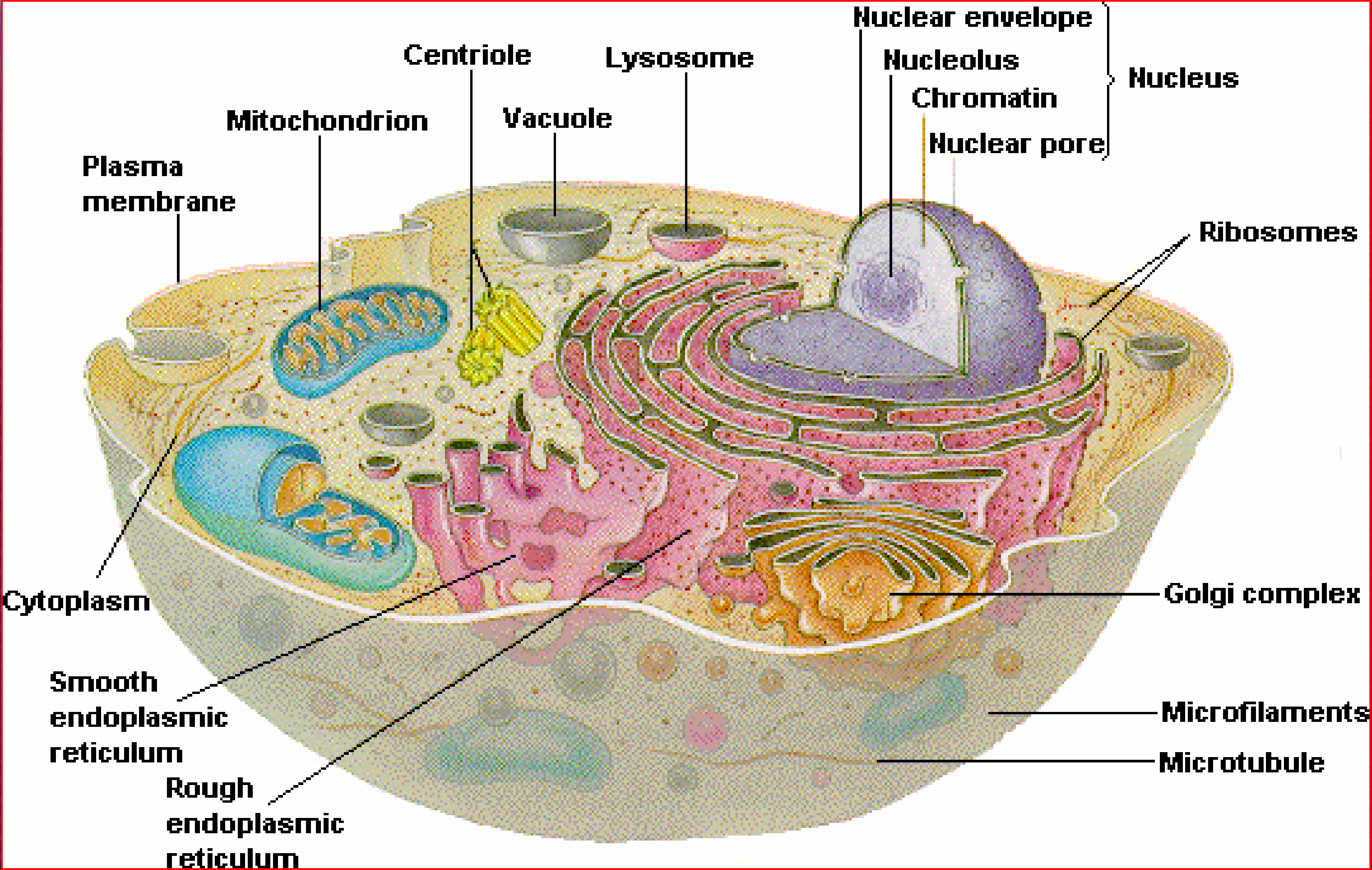
- They usually live in extreme environment and possess unusual metabolism.
- Examples
  - Methanogens: methane-producing bacteria
  - Thermoacidophiles : they can grow at high temperatures and low pH values.
  - Halobacteria : they can live only in very strong salt solutions.

Eucaryotes

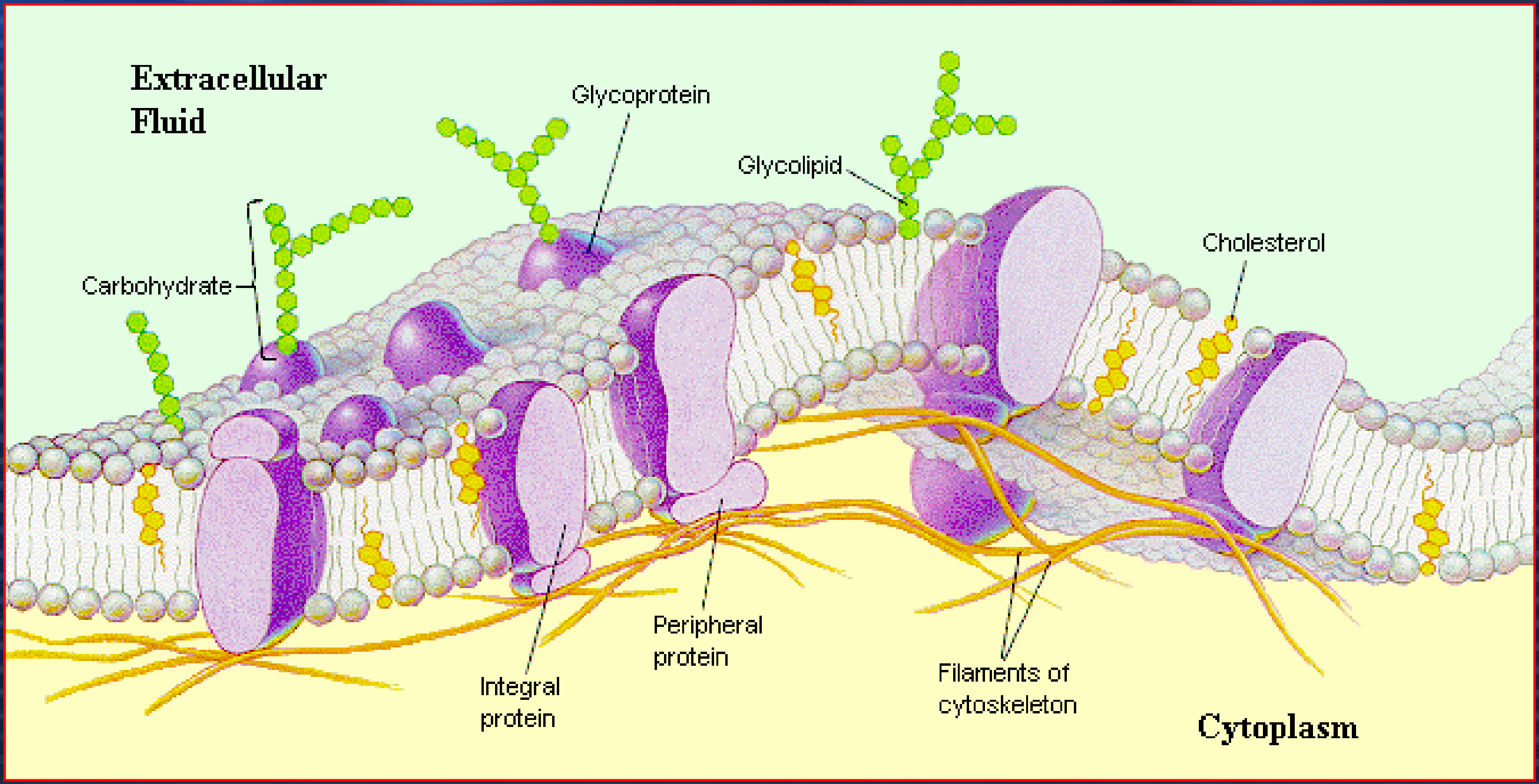
- Fungi (yeasts and molds), algae, protozoa, and animal and plant cells
- Eucaryotes are five to ten times larger than procaryotes in diameter.
  - yeast: about 5 µm, animal cells: about 10 µm, plant cells: about 20 µm
- Eucaryotes have a true nucleus and a number of cellular organelles inside the cytoplasma.
- Cell wall and cell membrane structure : These are similar to procaryotes.

Two Primary Types of Eucaryotic Cells





Nuclear envelope
Nucleolus
Chromatin
Nuclear pore
Nucleus
Centriole
Lysosome
Vacuole
Mitochondrion
Plasma membrane
Ribosomes
Cytoplasm
Golgi complex
Smooth endoplasmic reticulum
Rough endoplasmic reticulum
Microfilaments
Microtubule



Extracellular Fluid
Glycoprotein
Glycolipid
Carbohydrate
Cholesterol
Peripheral protein
Integral protein
Filaments of cytoskeleton
Cytoplasm

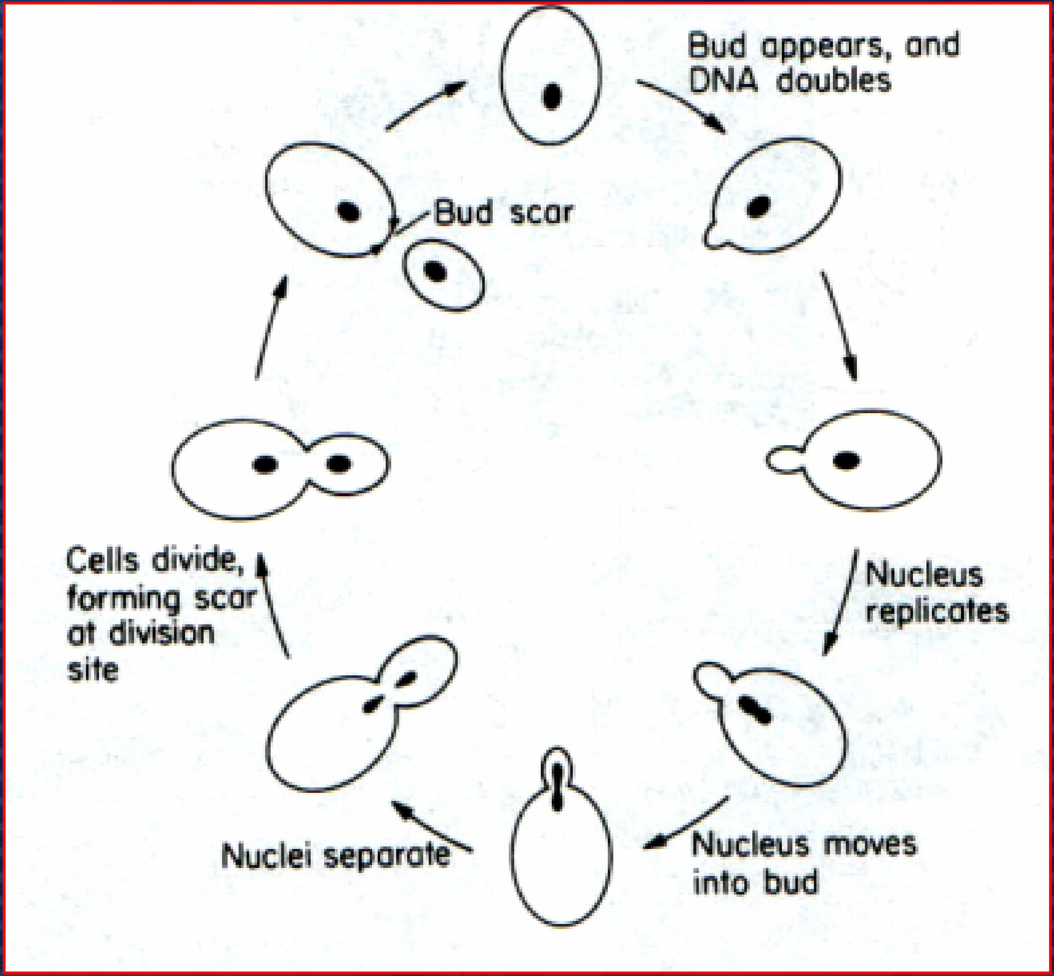
Fung

- Heterotroph
  - A cell or an organism that requires a variety of carbon-containing compounds as its source of carbon, and that synthesizes all of its carbon-containing bimolecules from these compounds and from small inorganic molecules.
- Fungal cells are larger than bacterial cells.
- Their internal structures(nucleus, vacuoles) can be seen easily with a light microscope.
- Two major groups of fungi are yeasts and molds.

Yeast

- Size : single small cells of 5~ to 10~µm
- Shape : spherical, cylindrical, or oval
- Reproduction : asexual or sexual means
  - Asexual reproduction: budding or fission
    - Budding : a small bud cell forms on the cell, which gradually enlarges and separates from the mother cell.
    - Fission : it is similar to that of bacteria. Only a few species of yeast can reproduce by fission. Cells grow to a certain size and divide into two equal cells.
  - Sexual reproduction: formation of a zygote(a diploid cell) from fusion of two haploid cells, each having a single set of chromosomes. The nucleus of the diploid cells divides several times to form ascospores. Each ascospore eventually becomes a new haploid cell and may reproduce by budding and fission.
- Ex) Baker's yeast, Brewer's yeast

Cell-Division Cycle of a Typical Yeast
Saccharomyces cerevisiae

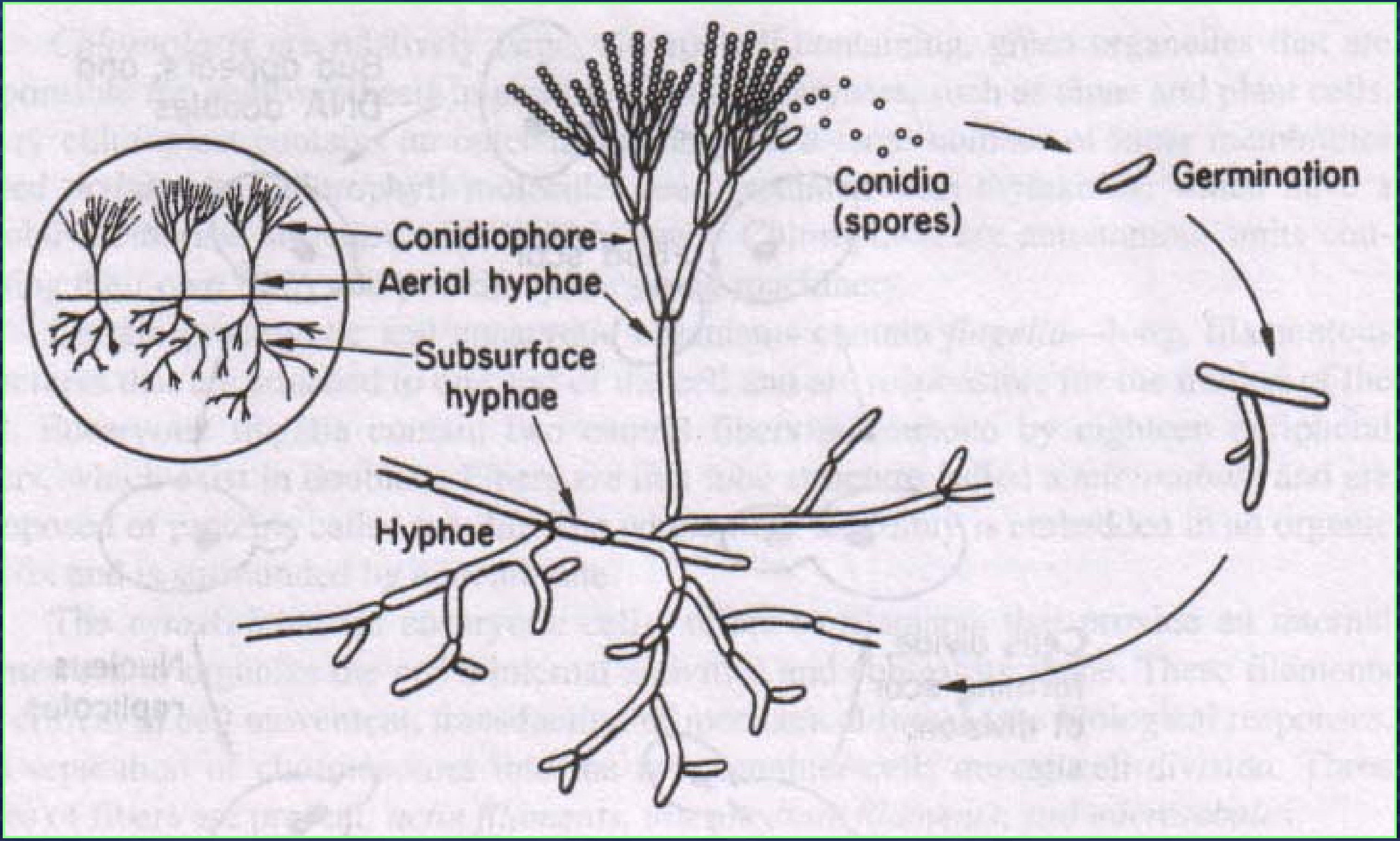


Molds

- Filamentous fungi having a mycelial structure.
- Mycelium : A highly branched system of tubes that contains mobil cytoplasm with many nuclei.
- Hyphae : Long, thin filaments on the mycelium
- Conidia
  - Asexual spores; these are formed on aerial branches.
  - Nearly spherical in structure and often pigmented.
  - Some molds reproduce by sexual means and form sexual spores.
  - These spores provide resistance against heat, freezing, drying, and some chemical agents.
  - Both sexual and asexual spores of molds can germinate and form new hyphae.
- Size : Filamentous form - 5 ~ 20 μm

  Pellets (Cell aggregates) - 50 μm ~ 1mm

Structure and Asexual Reproduction of Molds



Classification of Fung
by mode of sexual reproductio

1. The phycomycetes : algalike fungi
   - They do not possess chlorophyll and cannot photosynthesize.
   - Ex) Aquatic and terrestrial molds
2. The ascomycetes
   - They form sexual spores(ascospores), which are contained within a sac(a capsule structure).
   - Ex) *Neurospora, Aspergillus*, some yeasts

Classification of Fung
by mode of sexual reproductio

3. The basidiomycetes
   - They reproduce by basidiospores, which are extended from the stalks of specialized cells(basidia).
   - Ex) Mushrooms

4. The deuteromycete(Fungi imperfecti)
   - They cannot reproduce by sexual means.
   - Only asexually reproducing molds.
   - Ex) Trichophyton(causes athelete's foot)

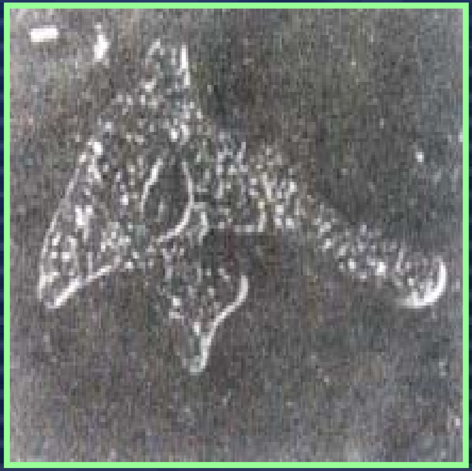
Algae

- Usually unicellular organisms
- Some plantlike multicellular structures are present in marine waters.
- All algae are photosynthetic and contain chloroplasts(sites of chlorophyll pigments).
- Size : 10 ~ 30 μm
- Multicellular algae sometimes form a branched or unbranched filamentous structure.
- Some algae contain silica or calcium carbonate in their cell wall.
- Ex) *Chlorella, Scenedesmus, Spirullina, Dunaliella*

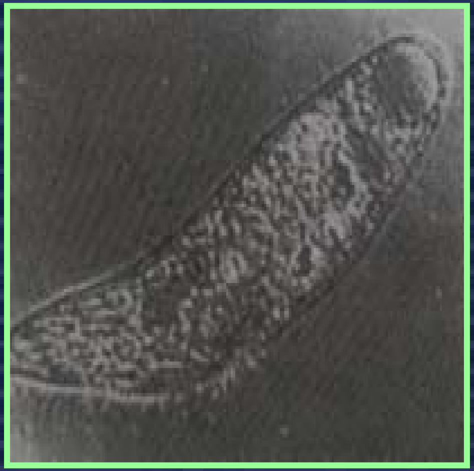
Protozoa

- Unicellular, motile, lack in cell wall
- Size: 1 ~ 50 mm
- They ingest other orgainisms such as bacteria.
- They are classified on the basis of their motion.
  - Amoebae : ameboid motion, whereby the cytoplasm of the cell flows forward to form a pseudopodium(false foot), and the rest of the cell flows towards this lobe.
  - Flagellates : move by their flagella.
  - Ciliates : move by cilia
  - Sporozoans : nonmotile and contain members that are human and animal parasites.

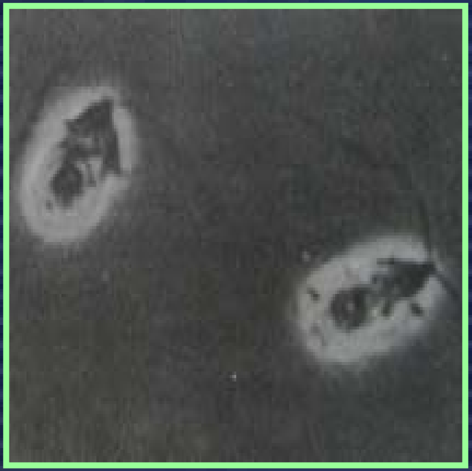
Protozoa



Amoeba proteus



Blepharisma



Dunaliella

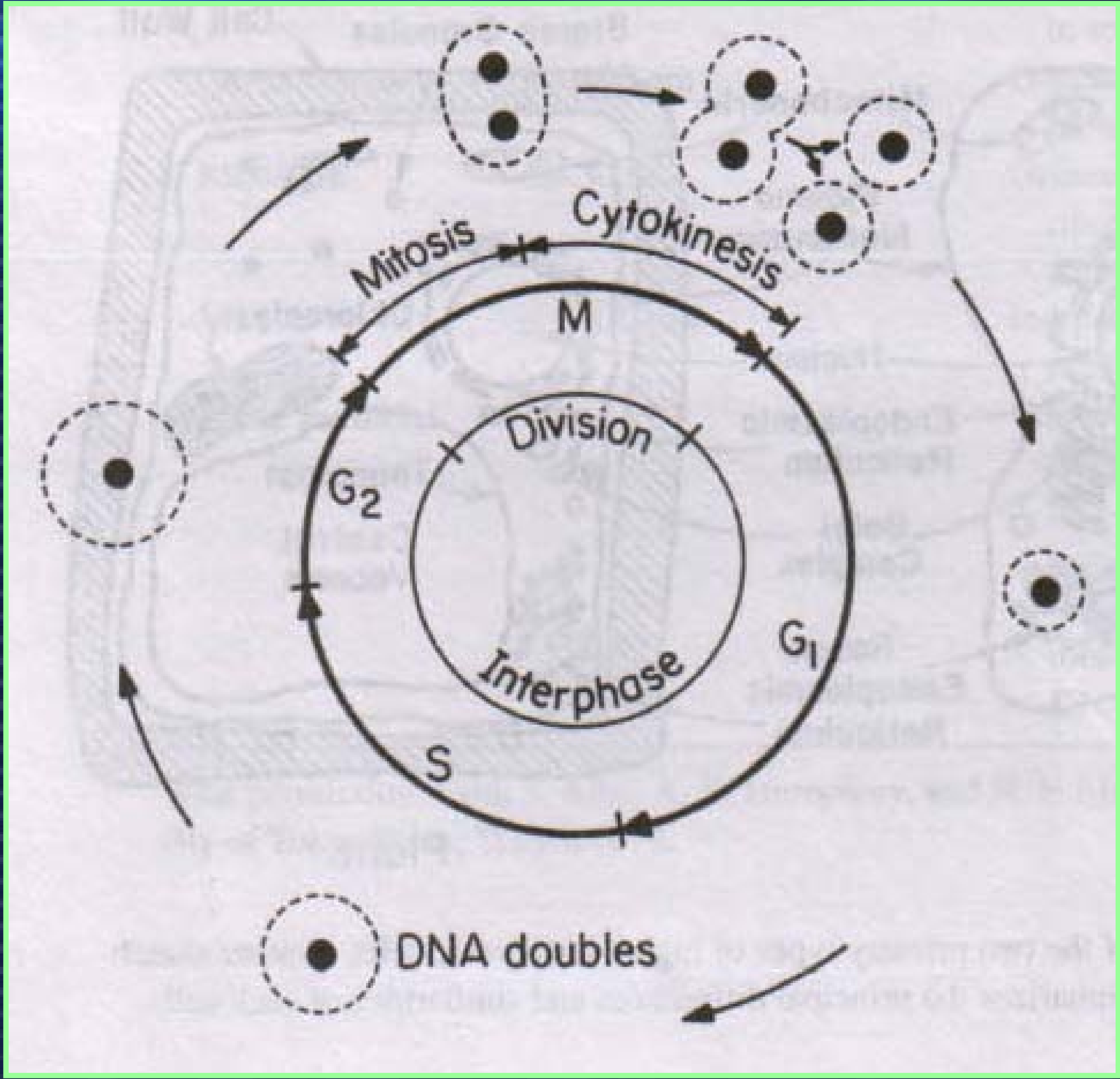
Cell Division

- Cell Division (asexual) in eucaryotes involves several major steps : **DNA synthesis, nuclear division, cell division, and cell separation.**
- Sexual reproduction in eucaryotic cells involves the conjugation of two cells (egg and sperm cells - Gametes).

  Zygotes : **The single cell formed from the conjugation of gametes.**
- Zygotes has twice as many chromosomes as does the gamete
- Gametes: **haploid cells**

  Zygotes: **diploid cells**
- For humans, a haploid cell contains 23 chromosomes, and diploid cells have 46.

Cell Division Cycle



Cell-Division Cycle (4 Phases)

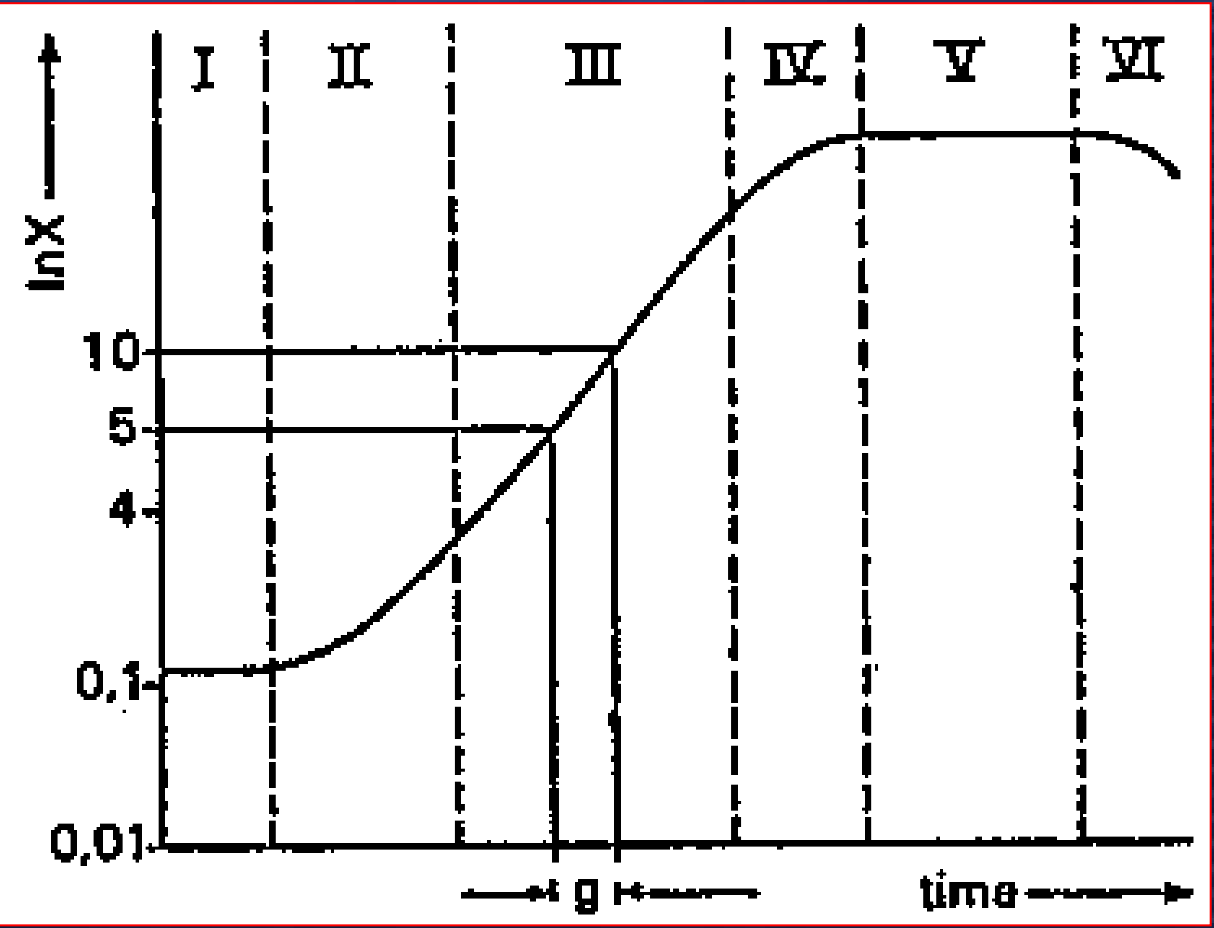
1. M phase
   - consists of mitosis (where the nucleous divides) and cytokinesis (where the cell splits into separate daughter cells).
   - Interphase : The rest of 3 phases(G1, S, G2). The cell increases in size during the interphase period.
2. G1 phase : Growth period following mitosis.
3. S phase : The cell replicates its nuclear DNA for mitosis in M phase.
4. G2 phase : Second growth period following S (Protein synthesis for mitosis).

- There are key checkpoints in the cycle when the cell machinery must commit to entry to the next phase. Checkpoints exist for entry into the S and M phases and exit from M phase.
- Cell may also be in a Go state, which is a resting state where there is no growth.

Cell Kinetics

Reference :
Stanbury & Whitaker,
Principles of Fermentation Technolog

Growth Phases of a Simple Batch Culture



1. Lag phase
2. Transient acceleration
3. Exponential growth phase

   (Log phase)
4. Deceleration phase
5. Stationary phase
6. Decline phase3

Growth Phases of a Simple Batch Culture

1. **Lag phase**
   - **A period of no observable growth**
   - **A time of adaptation (the cells are adapting to new environment)**
   - **The growth will begin**
   - **Synthesis of mRNA and enzymes**
   - **Zero net growth** $(\mu = 0)$

μ : Specific growth rate (hour^{-1})

$$= \frac{\text{the rate of increase of conc. of organism (growth rate)}}{\text{unit of organism concentration}} = \frac{1}{x}\frac{dx}{dt}$$

Growth Phases of a Simple Batch Culture

2. **Transient acceleration phase**

3. **Exponential growth phase (Log phase)**
   - **The cells grow at a constant, maximum rate**
   - **Nutrients are in excess and inhibitors absent** $(\mu = \mu_{max})$

Maximum specific growth rate

$$dx/dt = \mu x ---(1)$$

B.C. : t=0, $x=x_0$

t=t, $x=x_t$

$$x_t = x_0 e^{\mu t} ---(2)$$

$$\ln x_t = \ln x_0 + \mu t$$

4. **Deceleration phase**
   - **As nutrients are used up by the growing cells population, unlimited growth will be replaced by limited growth and although the cell population is still increasing the specific growth rate.** $(\mu < \mu_{max})$

Growth Phases of a Simple Batch Culture

5. **Stationary phase**
   - The cessation of growth may be due to depletion of some essential nutrient in the medium (substrate limitation), the accumulation of some autotoxic product of the organism in the medium (toxin limitation) or a combination of two.
   - No more overall growth $(\mu = 0)$
   - Biomass behaves, rate of growth = rate of death
   - Most important biotechnological products are optimally formed

6. **Decline phase (Death phase)**
   - Decreasing metabolism and cell lysis $(\mu < 0)$

Definitions of Various Kinetics term

- **Biomass doubling time (t_d)**
  - **The period of time required for the doubling in weight of biomass.**

$$\ln(x_t / x_0) = \mu t,\ x_t = 2x_0 \text{ and } t = t_d$$

$$\therefore\ t_d = 0.693 / \mu$$

- **Degree of multiplication :** $x_t / x_0 = e^{\mu t}$
- **If the biomass undergoes n doublings or generations,**

$$x_t / x_0 = 2^n, \quad n = 3.32 \log(x_t / x_0)$$

  - **Often in culture experiments it is useful to use an inoculum size which is 10% of the final biomass, n will then be 3.32.**
- **Growth yield**

$$\Delta x / \Delta s = Y, \quad \lim_{\Delta s \to 0} \Delta x / \Delta s = dx / ds = Y, \quad x - x_0 = Y(S_R - s)$$

Monod (in 1942

- Mathematical equations
  - Essential features of microbial growth in bioreactors.
  - Relationship between growth and utilisation of substrate.
  - The decrease in growth rate and the cessation of growth, due to the depletion of substrate, may be described by the relationship between μ and the residual growth-limiting substrate, represented by equation

$$\mu = \frac{\mu_{\max} S}{K_s + S}$$

Where,
S : residual substrate conc.
K_s : substrate utilisation constant
(saturation constant)

Pirt (in 1975

- Kinetics of product formation by microbial cultures in terms of growth-linked products(Primary metabolites) and non-growth-linked products(Secondary metabolites).
- The formation of a growth-linked product,
  - $$\frac{dp}{dt} = q_p x - -(1)$$ where p : Conc. of product
    q_p : Specific rate of product formation
- The product formation is related to biomass production by the eq.,
  - $$\frac{dp}{dx} = Y_{p/x} - -(2)$$

  where $Y_{p/x}$: Yield of product in terms of substrate consumed
- Multiply eq.(2) by dx / dt
  - $$dp/dx = Y_{p/x} dx/dt, \quad dp/dt = Y_{p/x} \mu x$$
- Combining eq. (1) and (3),
  - $$q_p = Y_{p/x} \mu$$