4. Cell Lysis and Extraction

4.1 The Raw Material

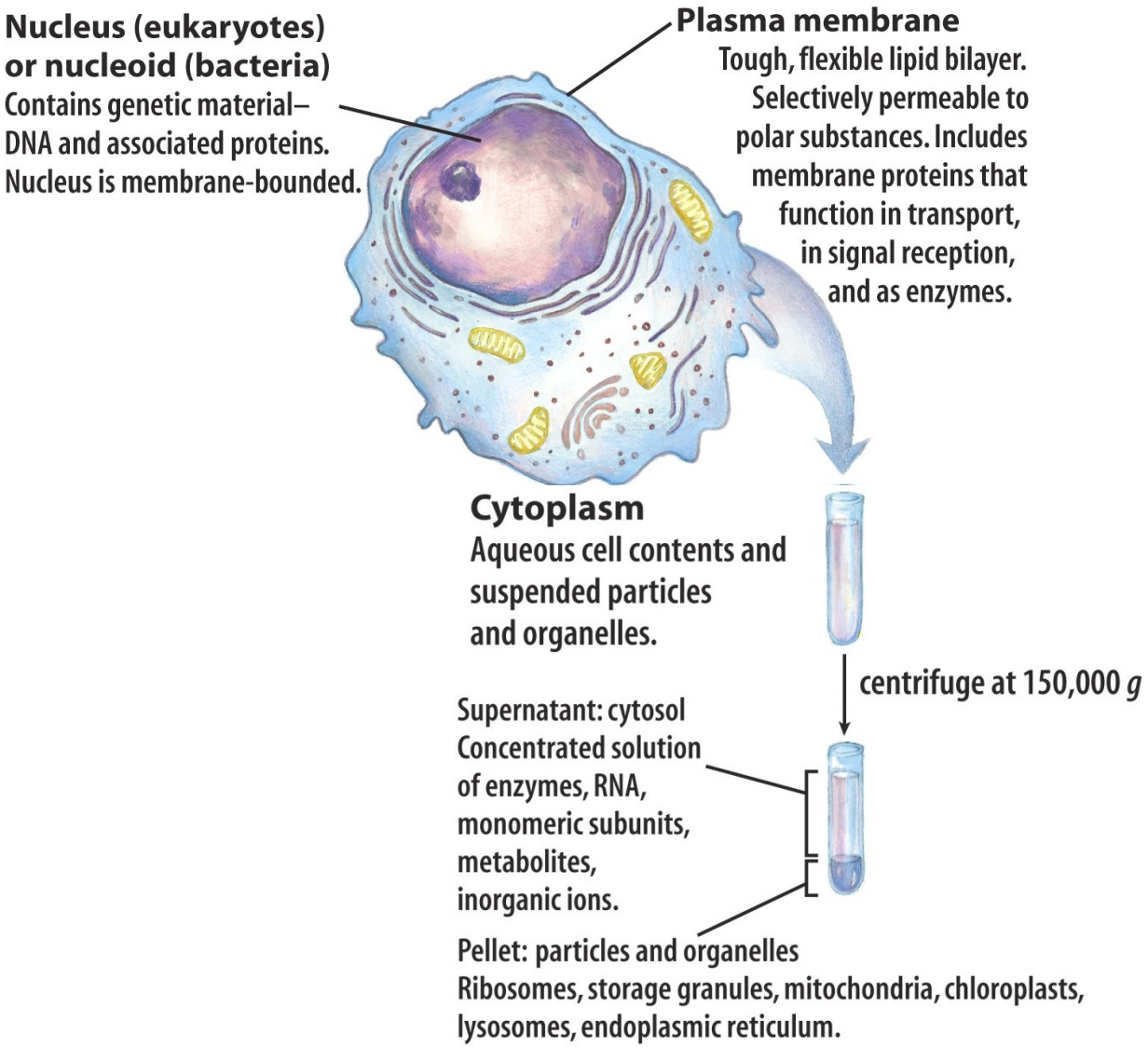
The Raw Material

- **Animals**
  - Rat (liver), rabbit (skeletal muscle), cow and pig (organs)
  - Human (blood, placenta)
  - Animal cell culture
- **Plant**
  - Large volume of vacuole
  - 1~2 % of the total cell volume is cytoplasm
- **Microorganisms**
  - Algae, fungi, yeasts, bacteria
- **Recombinant proteins expressed in diverse host cells**

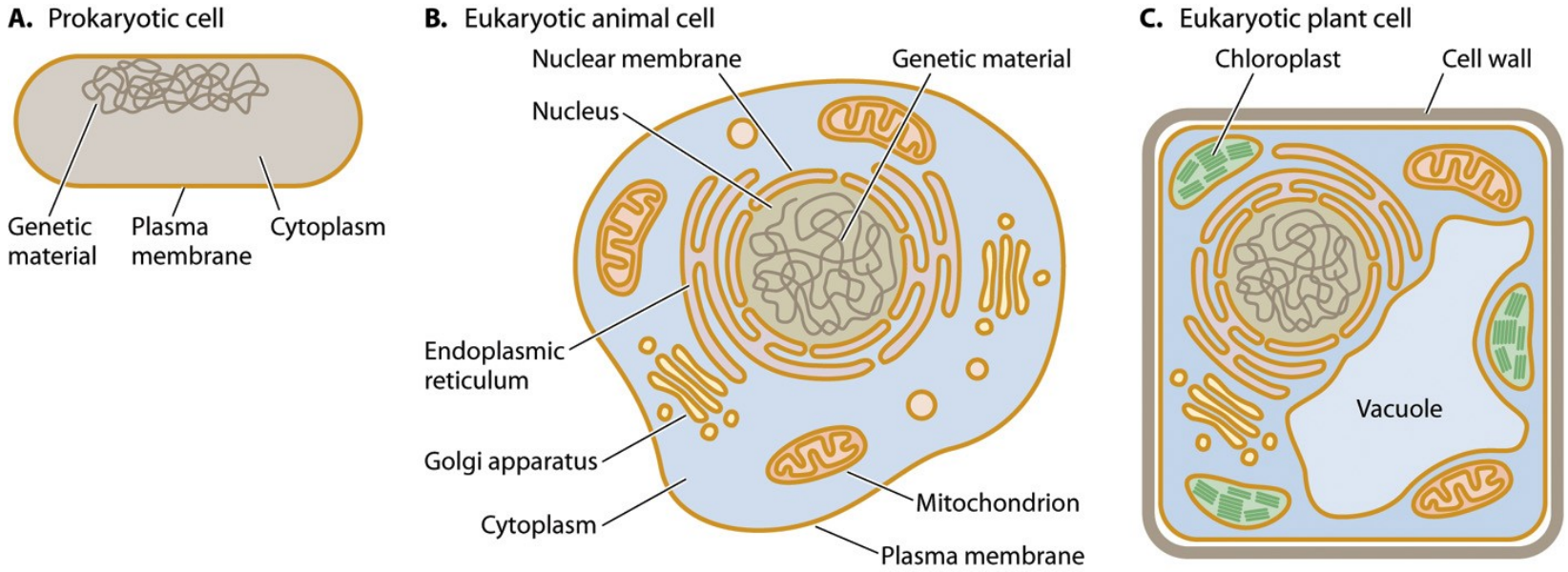
Cells

- **Cells**
  - Structural and functional units of living organisms
- **Structure of cells**
  - Plasma membrane
    - Structural barrier from the surroundings
    - Barrier of molecular transport
    - Composed of lipids and proteins
  - Cytoplasm
    - Internal volume
    - Cytosol: aqueous solution of cytoplasm
      - Enzymes, coenzymes, RNA, building blocks, metabolites, inorganic ions
    - Particles and organelles
      - Ribosome, ER, mitochondria, lysosomes, chloroplasts (plants)
  - Nucleus (eukaryotes) or nucleoid (prokaryotes)
    - Storage of genome and replication

Universal Features of Living Cells

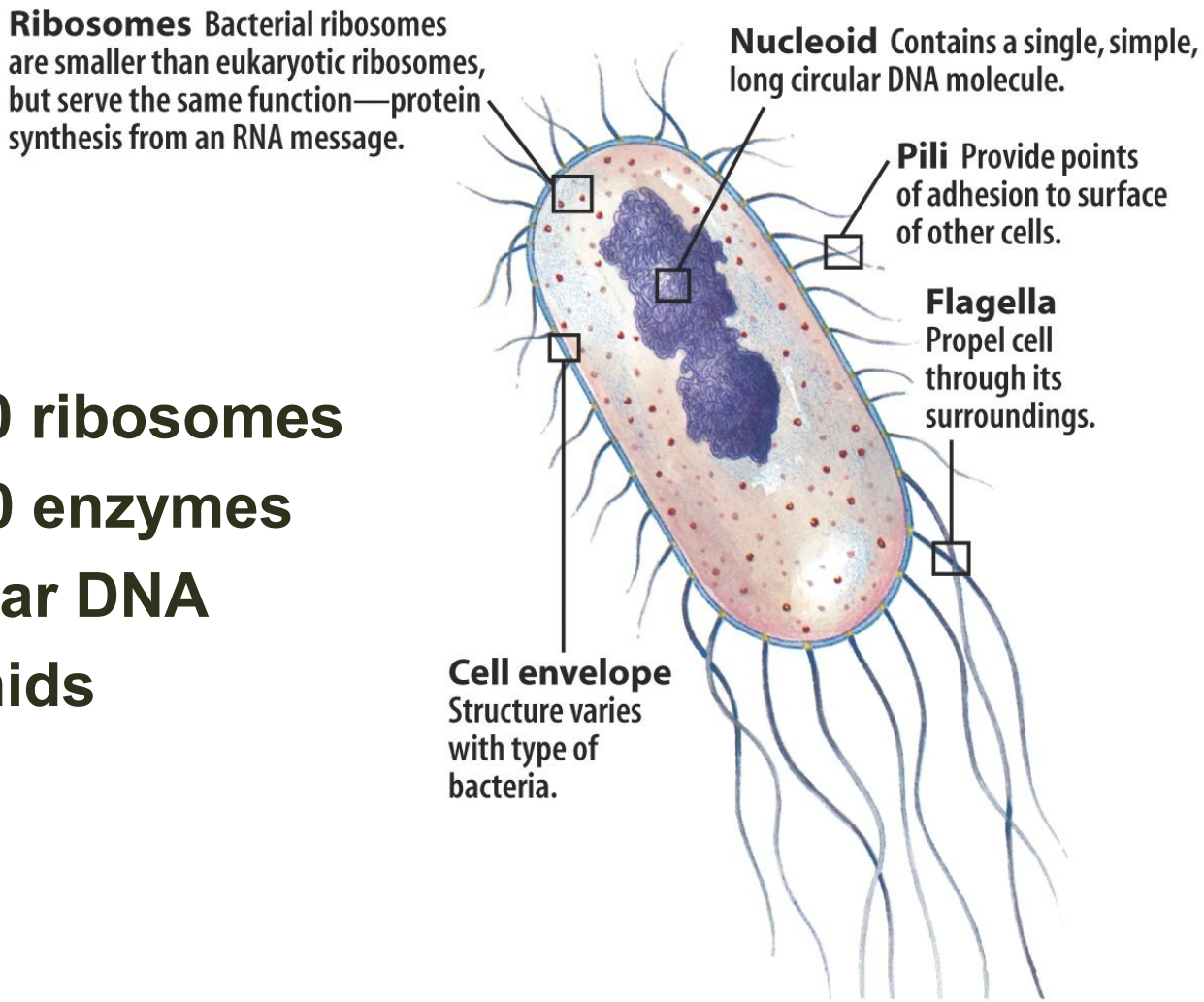


Two Fundamental Cell Types

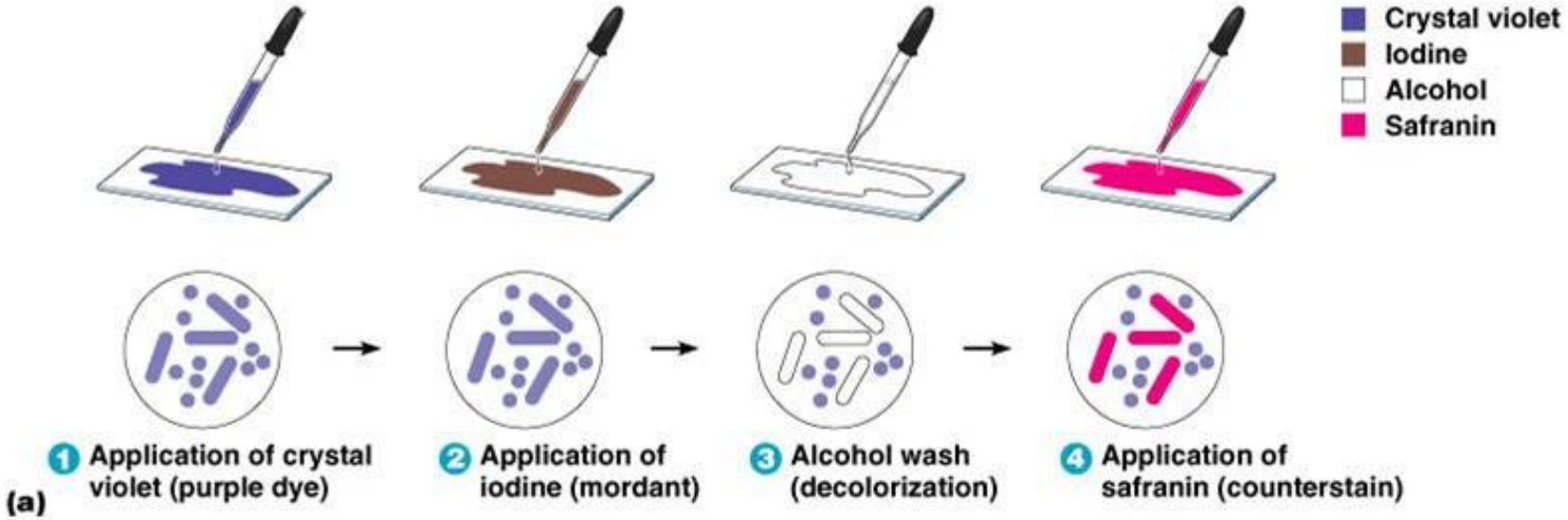


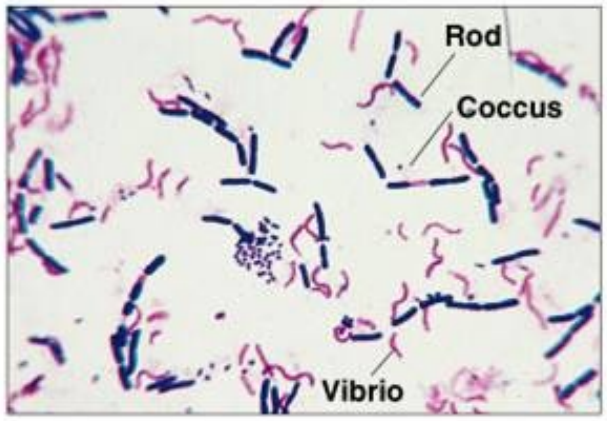
Structure of *E. coli*

- 15,000 ribosomes
- ~1,000 enzymes
- Circular DNA
- Plasmids

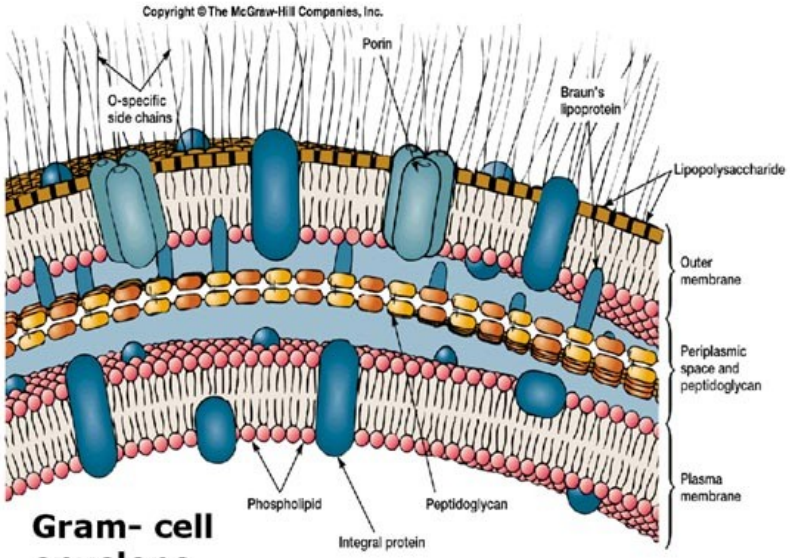


Gram Staining

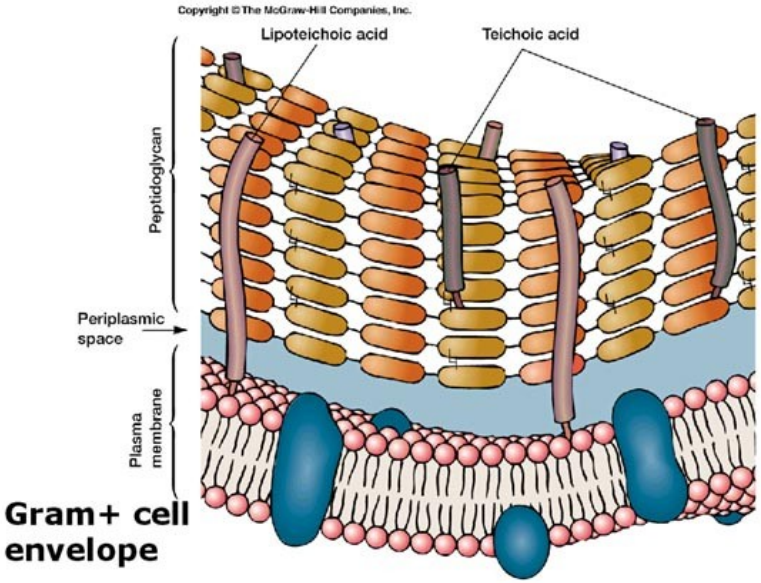




Cell Envelopes of Prokaryotes



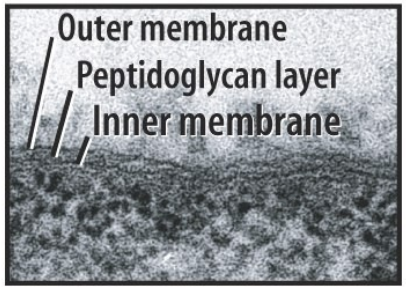
Gram- cell envelope



Gram+ cell envelope

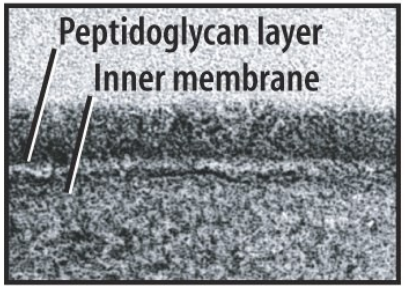
Cell envelope

Structure varies with type of bacteria.



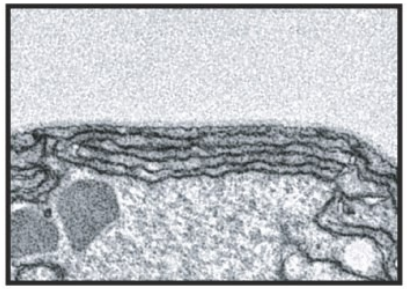
Gram-negative bacteria

Outer membrane; peptidoglycan layer



Gram-positive bacteria

No outer membrane; thicker peptidoglycan layer



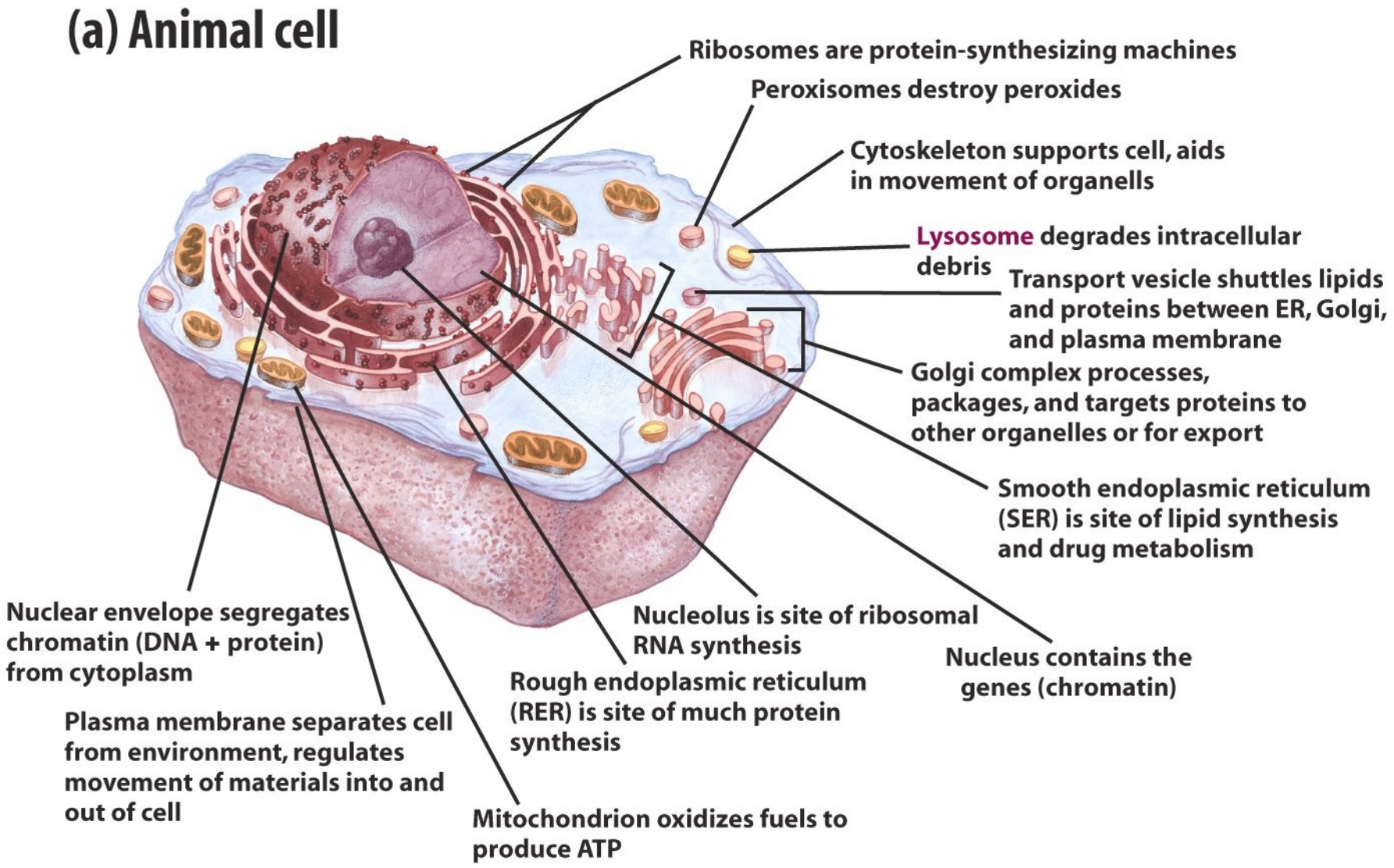
Cyanobacteria

Gram-negative; tougher peptidoglycan layer; extensive internal membrane system with photosynthetic pigments

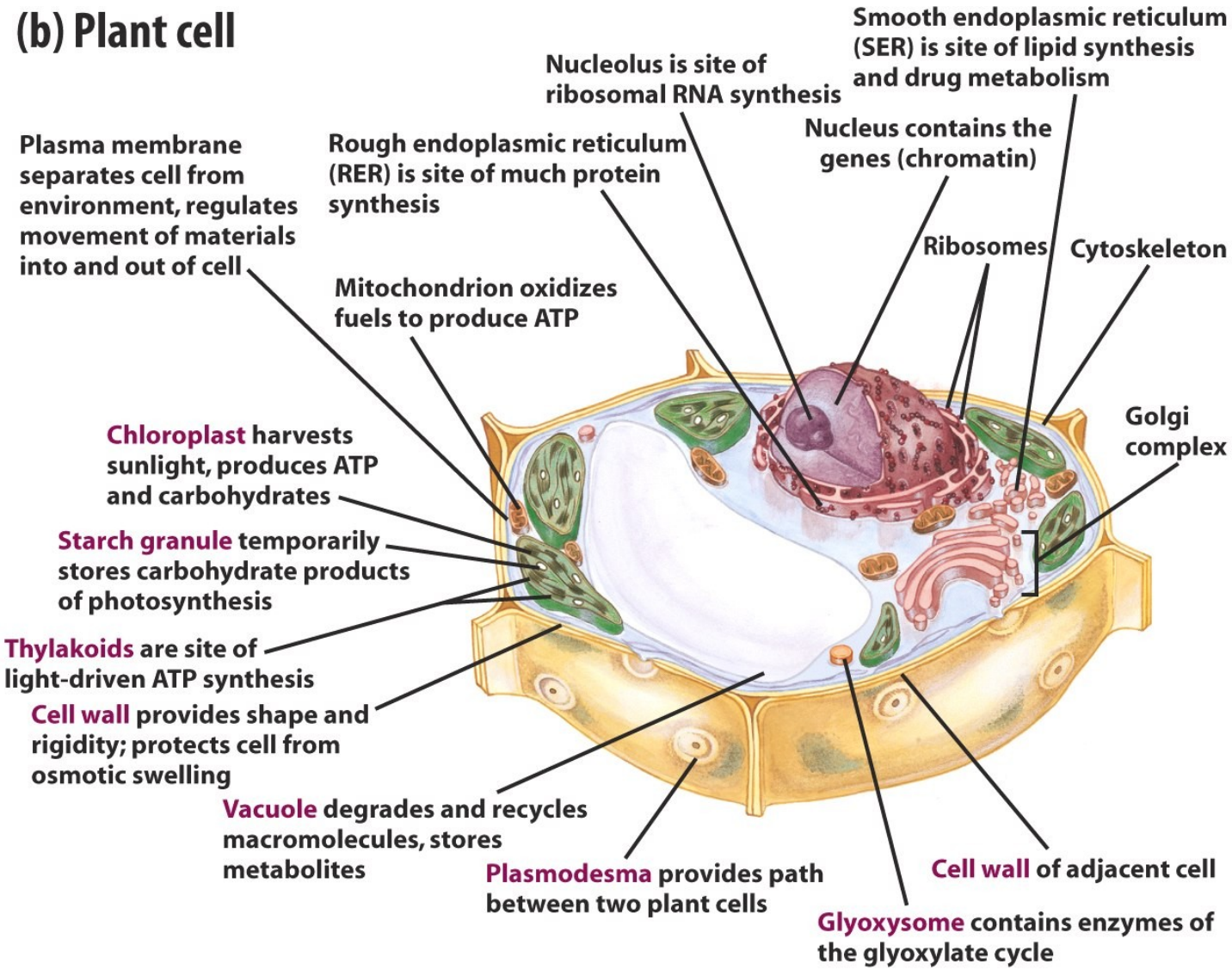
Archaebacteria

No outer membrane; peptidoglycan layer outside plasma membrane

Animal Cell


(a) Animal cell
Ribosomes are protein-synthesizing machines
Peroxisomes destroy peroxides
Cytoskeleton supports cell, aids in movement of organells
Lysosome degrades intracellular debris
Transport vesicle shuttles lipids and proteins between ER, Golgi, and plasma membrane
Golgi complex processes, packages, and targets proteins to other organelles or for export
Smooth endoplasmic reticulum (SER) is site of lipid synthesis and drug metabolism
Nuclear envelope segregates chromatin (DNA + protein) from cytoplasm
Nucleolus is site of ribosomal RNA synthesis
Nucleus contains the genes (chromatin)
Rough endoplasmic reticulum (RER) is site of much protein synthesis
Plasma membrane separates cell from environment, regulates movement of materials into and out of cell
Mitochondrion oxidizes fuels to produce ATP

Plant Cell


(b) Plant cell
Smooth endoplasmic reticulum (SER) is site of lipid synthesis and drug metabolism
Nucleolus is site of ribosomal RNA synthesis
Nucleus contains the genes (chromatin)
Plasma membrane separates cell from environment, regulates movement of materials into and out of cell
Rough endoplasmic reticulum (RER) is site of much protein synthesis
Ribosomes
Cytoskeleton
Mitochondrion oxidizes fuels to produce ATP
Golgi complex
Chloroplast harvests sunlight, produces ATP and carbohydrates
Starch granule temporarily stores carbohydrate products of photosynthesis
Thylakoids are site of light-driven ATP synthesis
Cell wall provides shape and rigidity; protects cell from osmotic swelling
Vacuole degrades and recycles macromolecules, stores metabolites
Plasmodesma provides path between two plant cells
Cell wall of adjacent cell
Glyoxysome contains enzymes of the glyoxylate cycle

Freshness and Storage of Raw Material

- **Using the raw material**
  - The sooner the better
- **Frozen storage**
  - Freezing material
    - Raw material
    - Extracts : can be optimized for freezing conditions
  - Problems
    - Growth of ice crystal
      - Destructive for membranes
    - Concentration of salts and protein
      - pH change
  - Freezing conditions
    - Fast freezing below -25 ^{o}C
    - Storage at even lower temperature (-80^{o}C)
      - Prevention of protease attack
  - Thawing conditions
    - The faster, the better
    - Immerse the container in warm (40-50 ^{o}C) water and agitate frequently

4.2. Cell Disintegration and Extraction

Cell Disintegration and Extraction

- **Localization of proteins**
  - Extracellular
    - Usually small and stable (disulfide bond)
    - No need to disrupt the cells
    - Lysozyme, ribonuclease, chymotrypsin etc.
  - Intracellular
    - Cytosol
    - In specific organelles
    - Insoluble (e.g. membrane proteins)
    - Need to disrupt the cells
- **Breaking cells**
  - Animal cells
    - Soft (erythrocytes) to tough (blood vessels, smooth muscle-containing cells)
  - Plant cells
    - Cellulosic cell walls → hard to disrupt
  - Bacteria
    - Fragile organisms to more resilient species with thick cell wall

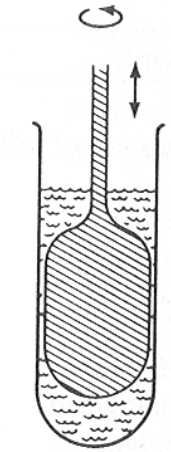
Cell Disintegation and Extraction

Techniques	Example	Principle
Gentle		
Osmotic lysis	Erythrocytes	Osmotic disruption of cell membrane
Enzyme Digestion	Lysozyme treatment of bacteria	Cell wall digested, leading to osmotic disruption
Hand Homogenizer	Liver tissue	Cells forced through narrow gap
Mincing (grinding)	Muscle	Shear force
Moderate		
Blade Homogenizer	Muscle tissue, most animal tissues, plant tissues	Chopping action breaks up large cells, shears apart smaller ones
Grinding with abrasive (Alumina, sand)	Plant tissues Bacteria	Microroughness rips off cell walls
Vigorous		
French Press	Bacteria, plant cells	Cells forced through small orifice at very high pressure; shear force disrupts cells
Ultrasonication	Cell suspensions	Microscale high-pressure sound waves cause disruption by shear forces and cavitation
Bead mill	Cell suspensions	Rapid vibration with glass beads rips cell walls off
Manton-Gaulin homogenizer	Cell suspensions	As for French Press, but on a large scale

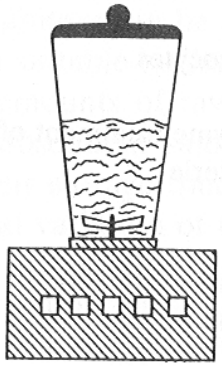
Chemical Cell Lysis

- **Osmotic cell lysis**
  - Cell lysis by drastic reduction in extracellular concentration of solutes (0.15- 0.001 M)
  - van't Hoff law : Osmotic transmembrane pressure
    - $\pi = RT\,(c_i - c_o)$
  - Animal cells: quick osmotic lysis
  - Bacteria and plant cells: need to weaken cell walls
- **Enzyme and antibiotics**
  - Lysozyme (from hen egg) : digestion of bacterial cell wall
  - Antibiotics (e.g. penicillin) : inhibition of prokaryotic cell wall synthesis
- **Detergents**
  - Nonionic detergents
    - Breaking plasma membranes
    - Far less denaturing for proteins and other biological compounds than ionic detergents
      - Triton X-100 : polyoxyethylene [9-10]p-t-octyl phenol , 1~3 %
      - Tween 20 (PEG-20 sorbitan monolaurate)
- **Solvents**
  - Toluen : yeast lysis
  - Acetone : dissolving membrane and excess fat of animal tissue

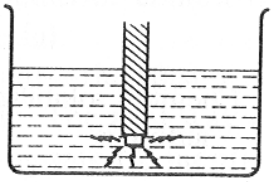
Mechanical Methods



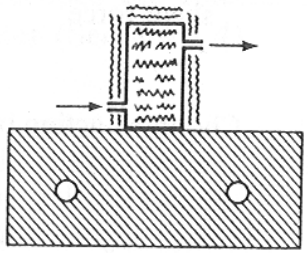
(a) Hand-operated or motor-driven



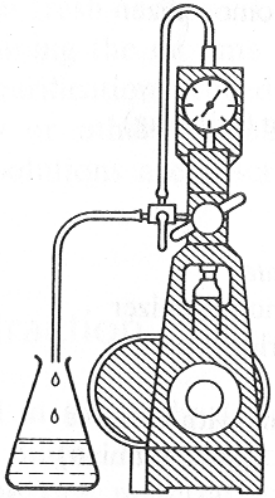
(b) Waring blender



(c) Ultrasound



(d) Vibrating bead mill

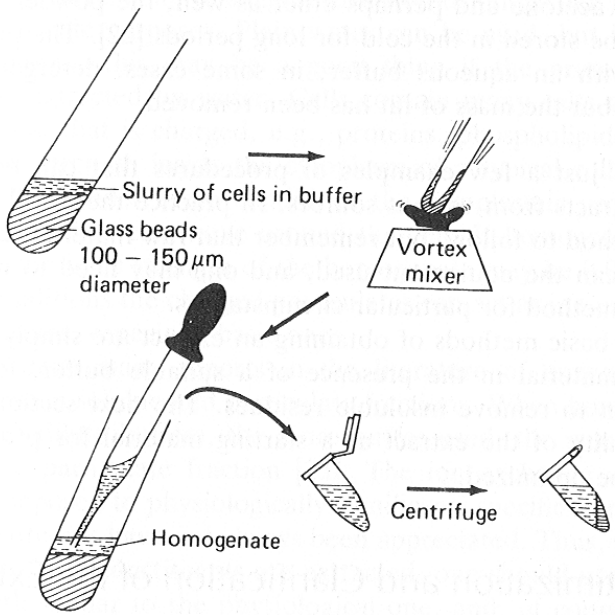


(e) Manton-Gaulin homogenizer

Mechanical Press

Making Cell Extract

- **Animal cells**
  - Add 2 2/1 volume of extraction buffer for tissue homogenates
  - The volume of insoluble cell residues as much as the original tissue after centrifugation of homogenates
- **Plant cells**
  - Large amount of liquid is release from vacuole and intercellular space
    - Require small amount of extraction buffer
  - Insoluble residues around 20-40% of the volume of the original tissue
- **Microorganisms**
  - Similar to animal cells for the volume of residues after centrifugation



Small scale disintegration of bacteria

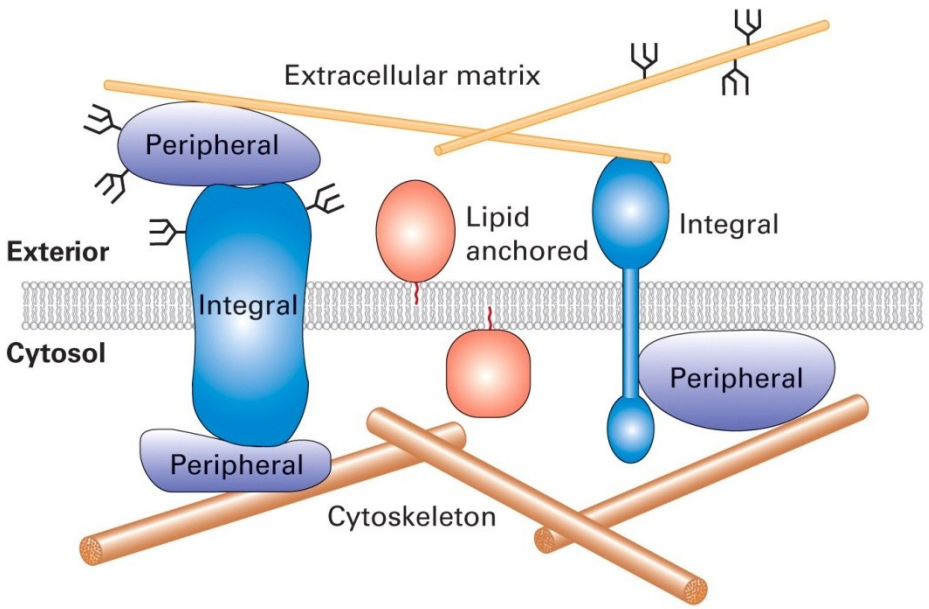
Extraction Buffer

- **Buffer with ionic strength and pH similar to the physiological one (0.1~ 0.2 M)**
  - 20-50 mM phosphate (pH 7-7.5)
  - 0.1 M Tris-HCl (pH 7.5)
  - 0.1 M KCl
- **Buffer for isolation of organelles**
  - Isoosmotic buffer
    - Sucrose, mannitol, sorbitol
- **Other components**
  - EDTA (1-5 mM)
    - Metal chelating agent
  - β-mercaptoethanol or cystein (5-20 mM)
    - Reducing agents
  - Specific stabilizing agents
    - e.g. Zn^{2+} for zinc-containing proteins

4.3. Extraction of Membrane Proteins

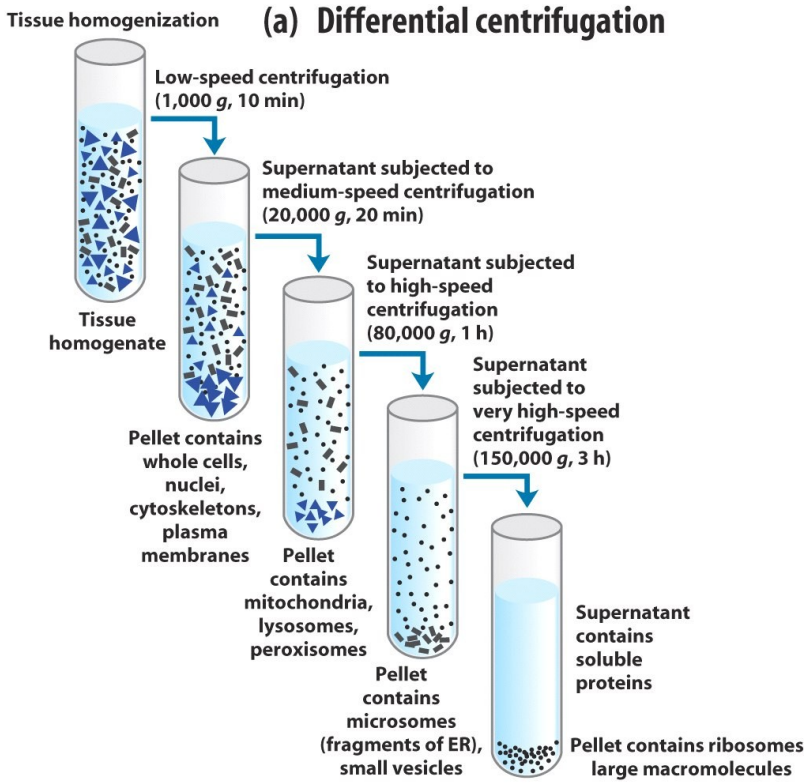
Proteins Interacting with Membrane

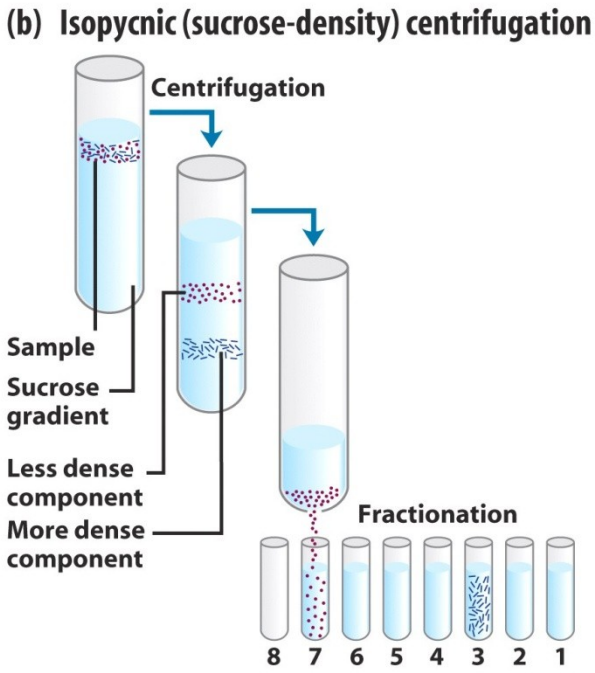
- **Integral membrane proteins (transmembrane proteins)**
  - Cytoplasmic and exoplasmic domain
  - Membrane spanning domain
    - A helices or multiple β strands, glycosylation
- **Lipid-anchored membrane proteins**
  - Covalent binding to lipid molecules
- **Peripheral membrane proteins**
  - Bind to membrane by interacting with integral membrane proteins or with lipid head groups
  - Exoplasmic peripheral proteins often attach to extacellular matrix or to the cell wall of bacteria or plant



Extraction of Membrane Proteins

- **Starting material**
  - Membrane or organelles
    - Differential centrifugation
  - Total cell extract
    - Suitable for large scale production



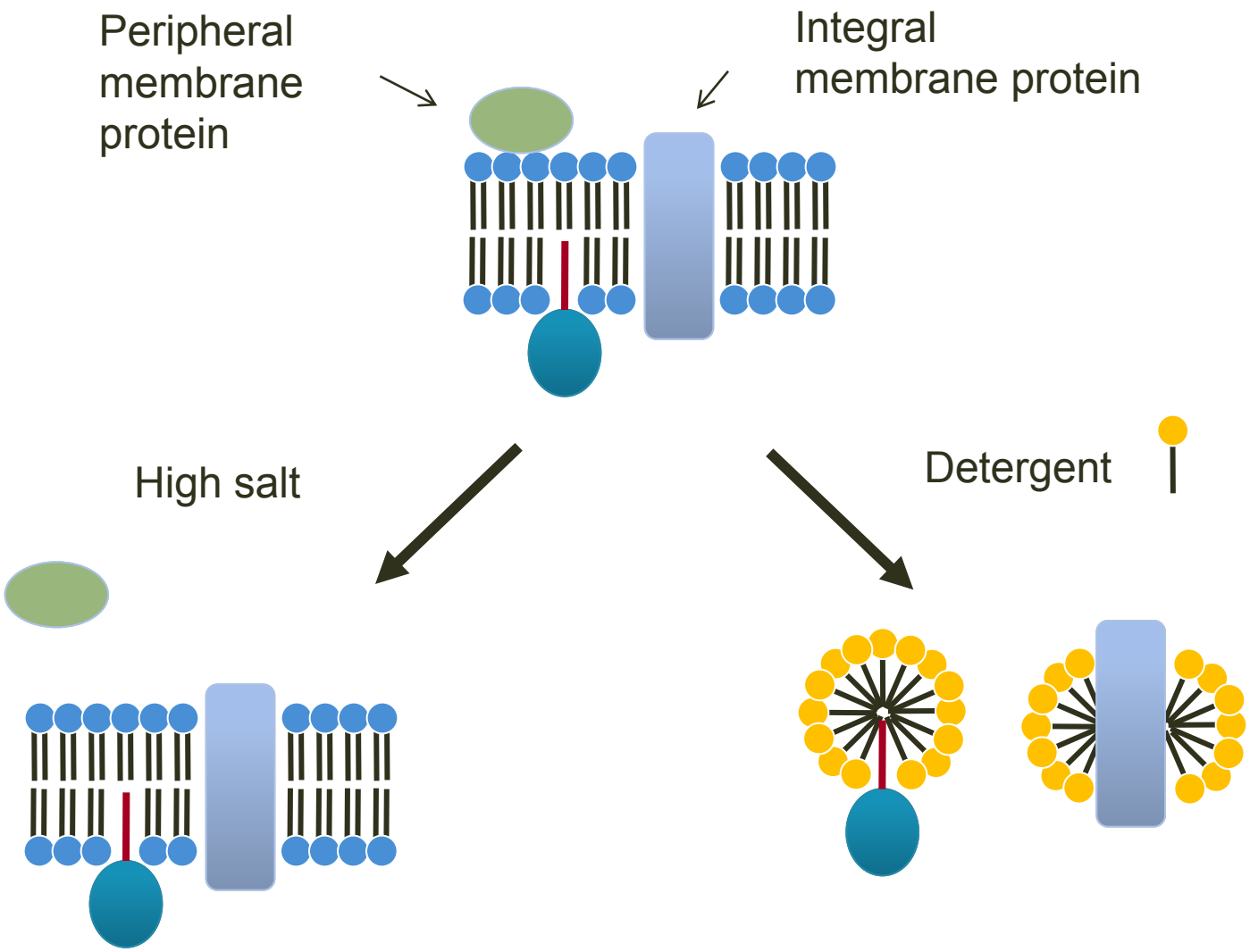


Extraction of Membrane Proteins

- **Peripheral membrane protein**
  - Weak salt or hydrophobic interactions with membrane
  - Mild treatment
    - Sonication
    - Metal chelators: EDTA, EGTA (1-10 mM)
    - Mild alkaline conditions (pH 8-11) at low ionic strength
    - Dilute nonionic detergent
    - Low concentrations of partially miscible organic solvents : n-butanol
    - High ionic strength : 1 M NaCl
    - Phospholipase treatment

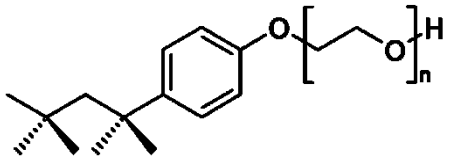
- **Integral membrane protein**
  - Solubilize membrane
  - Retain lipophilic component (detergent) to prevent aggregation of membrane proteins

Extraction of Membrane Proteins



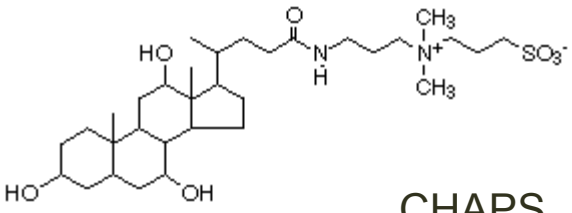
Detergents for Extraction of Membrane Proteins

- **Types of detergents**
  - Nonionic
    - Tween80, Triton X-100



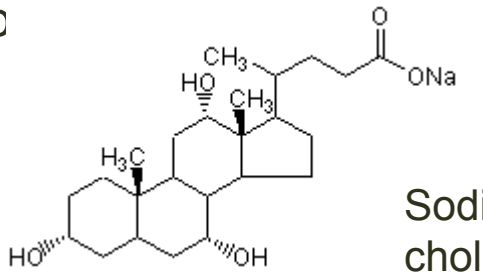
Triton X-100

  - Zwitterionic
    - Lysolecithin, CHAPS



CHAPS

  - Ionic
    - Cholate, deoxycholate, SDS
    - Can cause irreversible denaturatic



Sodium cholate

Detergents for Extraction of Membrane Proteins

- **Factors to be considered**
  - Detergent can cause fragmentation of membrane to vesicles without solubilizing the membrane protein
  - In general, use 2 mg of detergent for 1 mg of membrane (1 mg for protein, 1 mg for lipid)
  - Detergent forms micelle above critical micelle concentration
    - MW 30,000 to 1000,000 : inhibit protein purification procedure

- **Examples**
  - Tightly bound membrane protein
    - Preextraction in milder conditions
    - Extraction with SDS
  - Extraction using Triton X-114
    - Reduced water solubility at high temperature
      - Solubilize membrane protein at around 0^{o}C
      - Warming up the extract during centrifugation to 25^{o}C
        - » Separation of detergent layer containing most integral membrane proteins