University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

● Slides ○ Sheet ○ Handout ○ other.....

Lecture # 1
Title: Introduction
Dr.- Dr. Mamoun Ahram
Done By:
Date:
Price:

Designed by Nadeen Al-Freihat

Lecture 1: Introduction

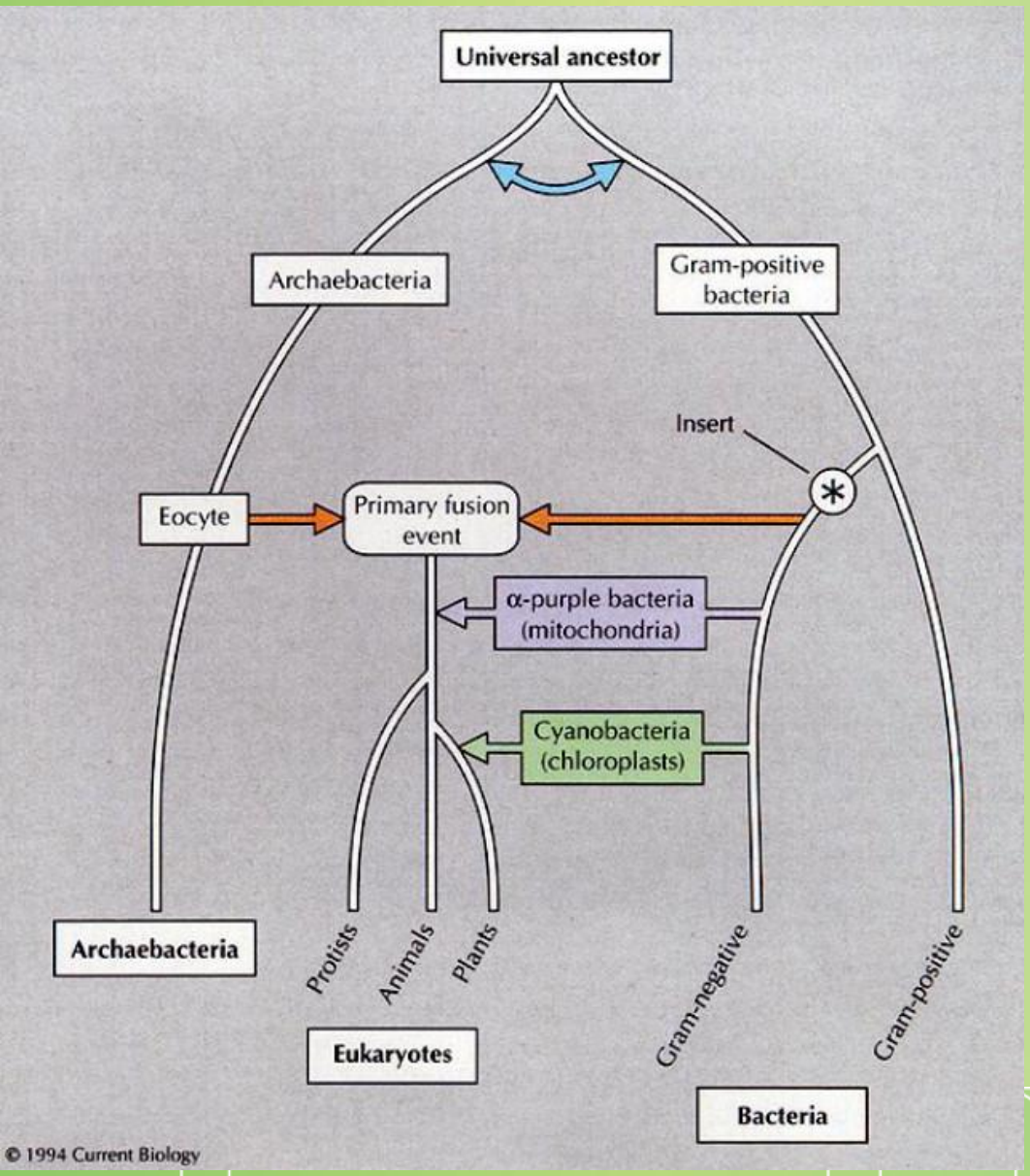
Dr. Mamoun Ahram
Faculty of Medicine
Second year, Second semester, 2014-2014

Principles of Genetics and Molecular Biology

Syllabus and references

- **Part I: Cell biology (Dr. Mamoun Ahram)**
  - **The Cell: A Molecular Approach, Geoffrey M. Cooper and Robert E. Hausmann, 6th edition, Sinauer Associates, 2013.**
- **Part II: Molecular biology (Prof. Said Ismail)**
  - **Mark's Basic Medical Biochemistry, Smith, Marks and Lieberman, Lippincott, Williams and Wilkins, 2009.**
- **Part III: Genetics (Prof. Mohammad El-Khateeb**
  - **Emery's Elements of Medical Genetics, Muller & Young, Churchil Livingstone, 13th edition, 2011.**

The origin of life

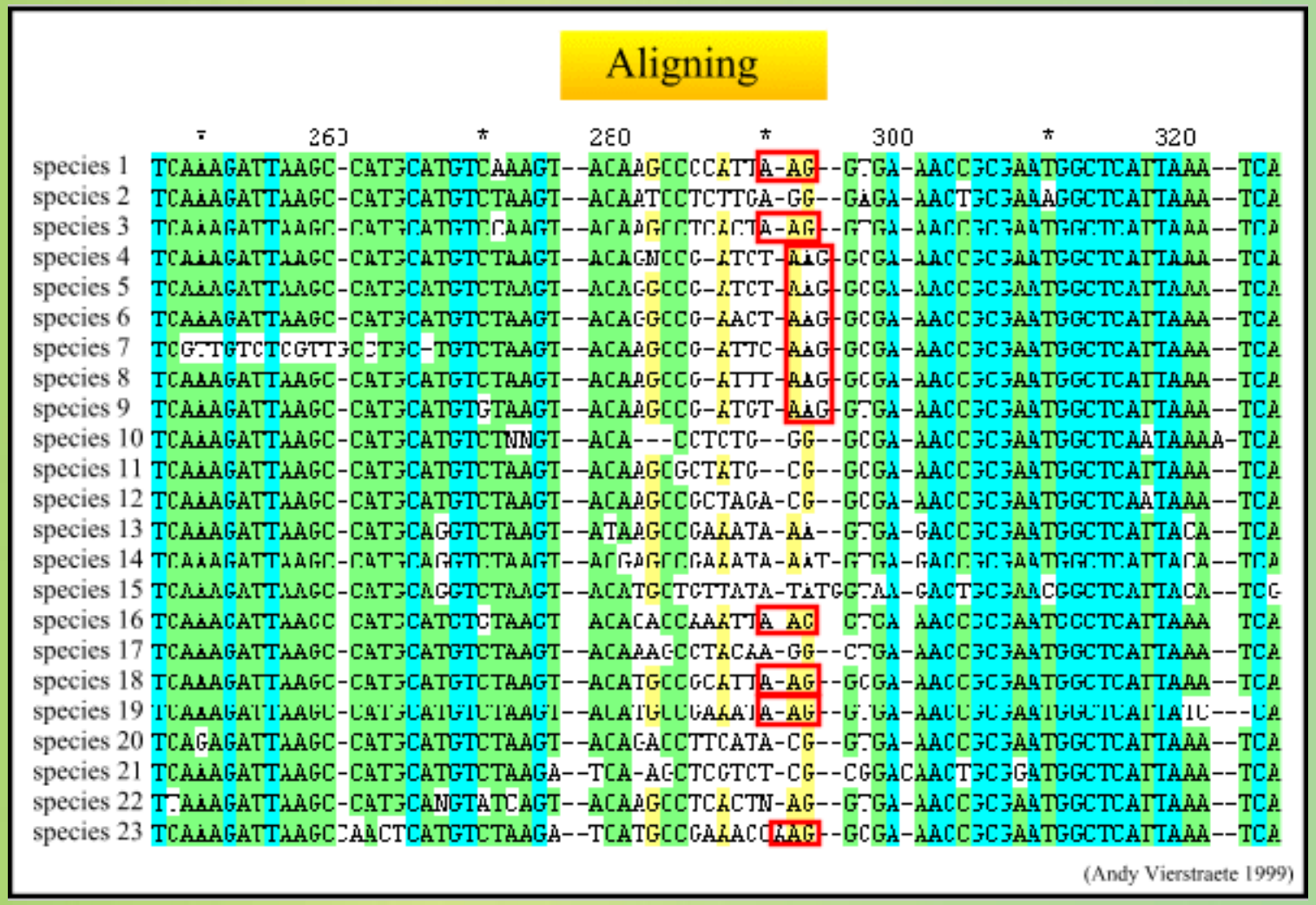


قَالَ رَبُّنَا ٱلَّذِىٓ أَعۡطَىٰ كُلَّ شَىۡءٍ خَلۡقَهُۥ ثُمَّ هَدَىٰ

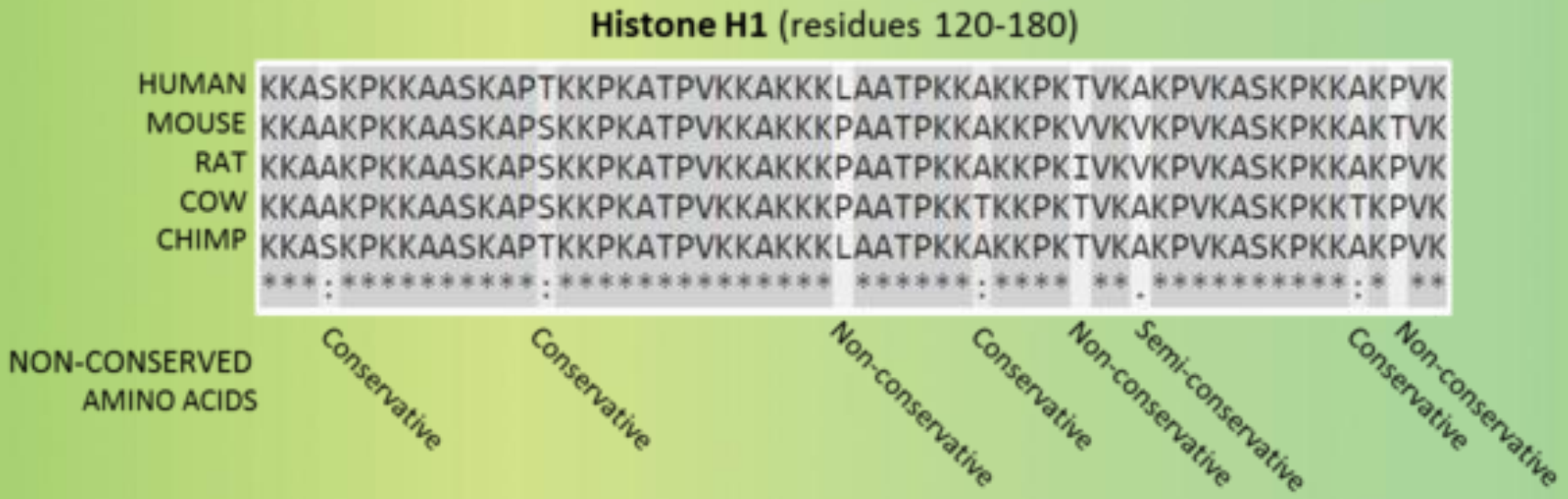
{ وَجَعَلۡنَا مِنَ ٱلۡمَآءِ كُلَّ شَيۡءٍ حَيٍّ

وَبَدَأَ خَلۡقَ ٱلۡإِنسَٰنِ مِن طِينٍ

DNA homology



Humans and others



"We are all Arabs"!!

The Arabian Cradle: Mitochondrial Relicts of the First Steps along the Southern Route out of Africa

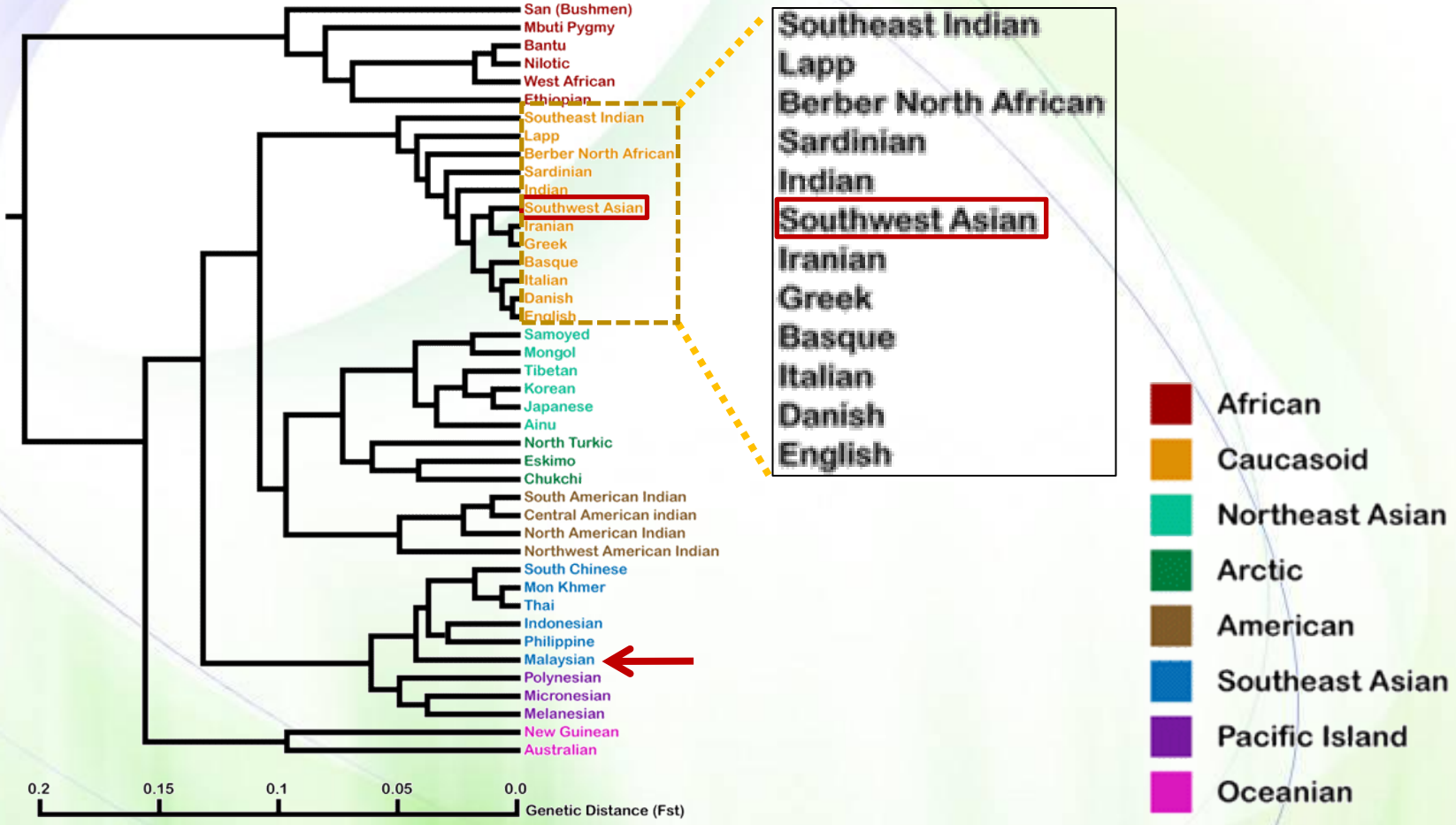
Verónica Fernandes,[1,2] Farida Alshamali,[3] Marco Alves,[1] Marta D. Costa,[1,2] Joana B. Pereira,[1,2] Nuno M. Silva,[1] Lotfi Cherni,[4,5] Nourdin Harich,[6] Viktor Cerny,[7,8] Pedro Soares,[1] Martin B. Richards,[2,9,11] and Luísa Pereira[1,10,11,*]

A major unanswered question regarding the dispersal of modern humans around the world concerns the geographical site of the first human steps outside of Africa. The "southern coastal route" model predicts that the early stages of the dispersal took place when people crossed the Red Sea to southern Arabia, but genetic evidence has hitherto been tenuous. We have addressed this question by analyzing the three minor west-Eurasian haplogroups, N1, N2, and X. These lineages branch directly from the first non-African founder node, the root of haplogroup N, and coalesce to the time of the first successful movement of modern humans out of Africa, ~60 thousand years (ka) ago. We sequenced complete mtDNA genomes from 85 Southwest Asian samples carrying these haplogroups and compared them with a database of 300 European examples. The results show that these minor haplogroups have a relict distribution that suggests an ancient ancestry within the Arabian Peninsula, and they most likely spread from the Gulf Oasis region toward the Near East and Europe during the pluvial period 55–24 ka ago. This pattern suggests that Arabia was indeed the first staging post in the spread of modern humans around the world.

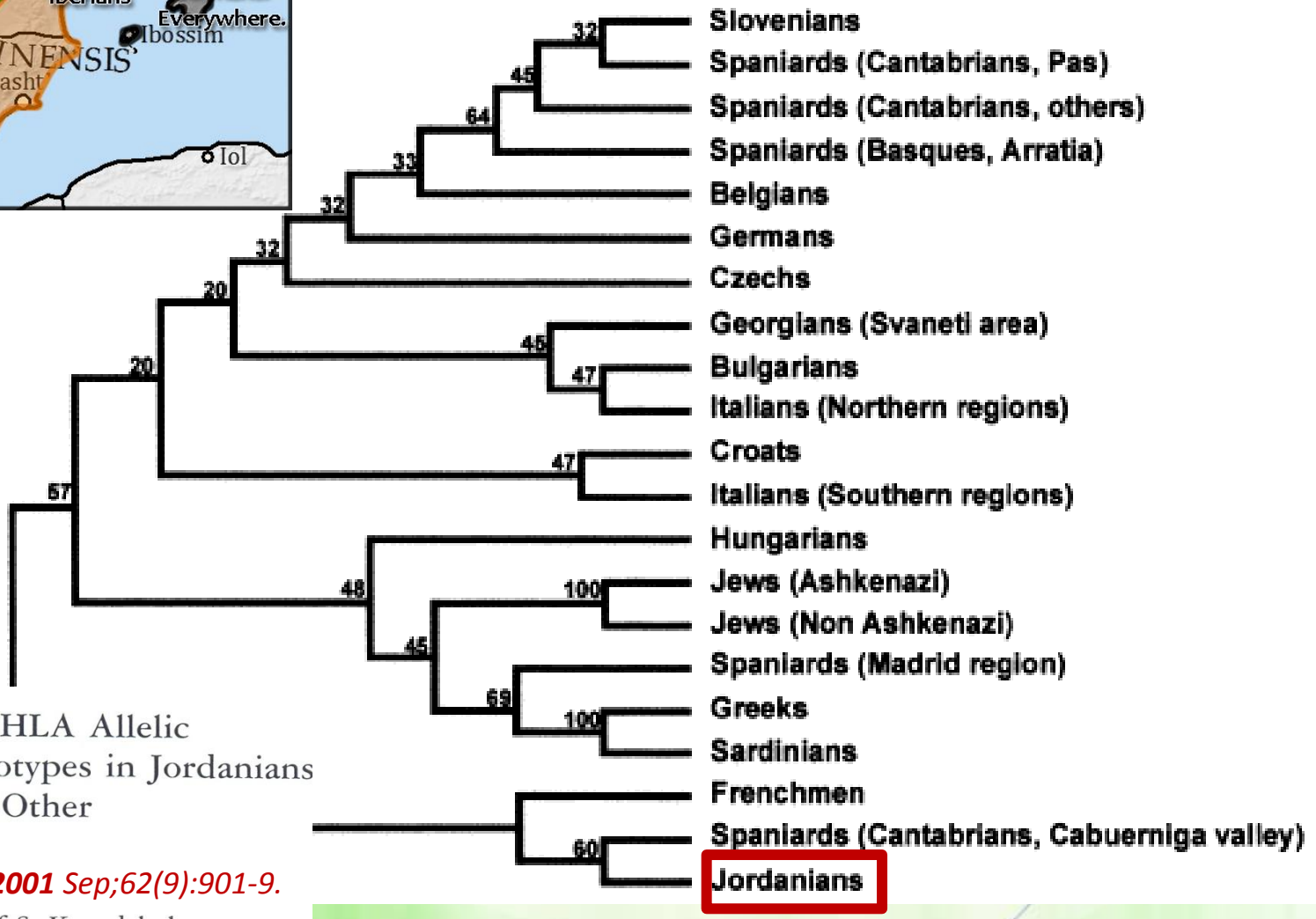
Arabia was indeed the first staging post in the spread of modern humans around the world.

The American Journal of Human Genetics 90, 1–9, February 10, 2012

Genetic relatedness of world populations



Cavalli-sforza et al., 1988



Molecular Analysis of HLA Allelic Frequencies and Haplotypes in Jordanians and Comparison with Other Related Populations

Hum Immunol. ***2001*** *Sep;62(9):901-9.*

Pablo Sánchez-Velasco, Naif S. Karadsheh, Alfredo García-Martín, Carlos Ruíz de Alegría, and Francisco Leyva-Cobián


The island of Rockall is not shown.
Lerwick
SHETLAND ISLANDS
ORKNEY ISLANDS
Scapa
HEBRIDES
Peterhead
Aberdeen
Scotland
Ben Nevis
Dundee
North Sea
Grangemouth
Edinburgh
Glasgow
Newcastle upon Tyne
Londonderry
Belfast
Middlesbrough
Kingston upon Hull
Isle of Man (U.K.)
Manchester
Irish Sea
IRELAND
Liverpool
England
The Fens
Birmingham
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Felixstowe
Cardiff
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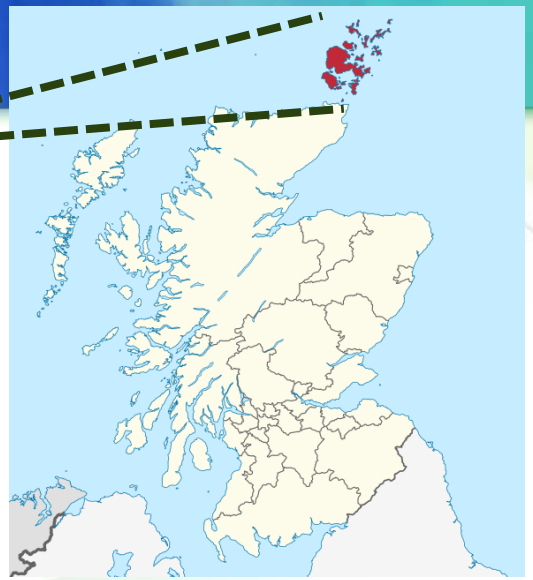
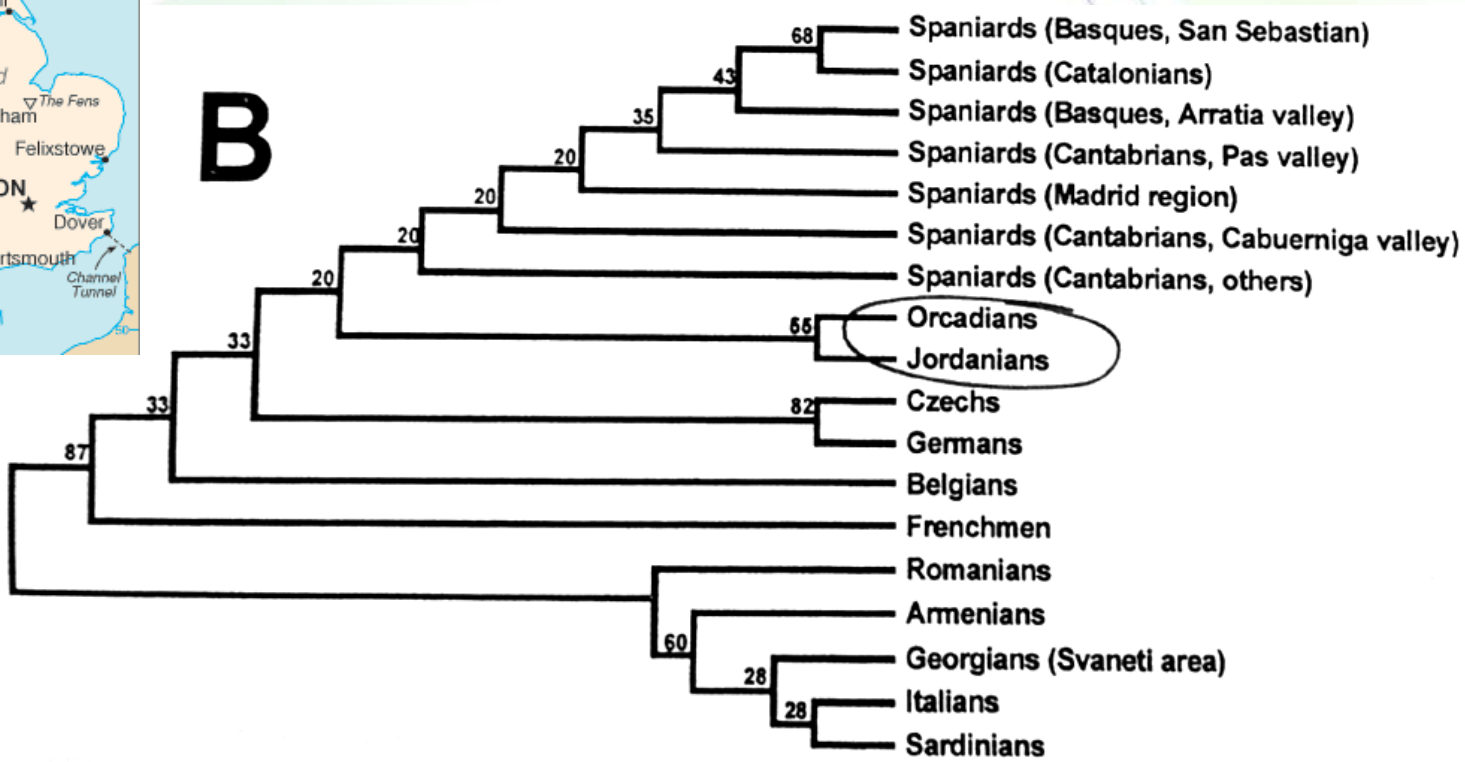
Bristol
Dover
Southampton
Portsmouth
Channel Tunnel
Plymouth
Celtic Sea
Falmouth

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Spaniards (Basques, San Sebastian)
Spaniards (Catalonians)
Spaniards (Basques, Arratia valley)
Spaniards (Cantabrians, Pas valley)
Spaniards (Madrid region)
Spaniards (Cantabrians, Cabuerniga valley)
Spaniards (Cantabrians, others)
Orcadians
Jordanians
Czechs
Germans
Belgians
Frenchmen
Romanians
Armenians
Georgians (Svaneti area)
Italians
Sardinians

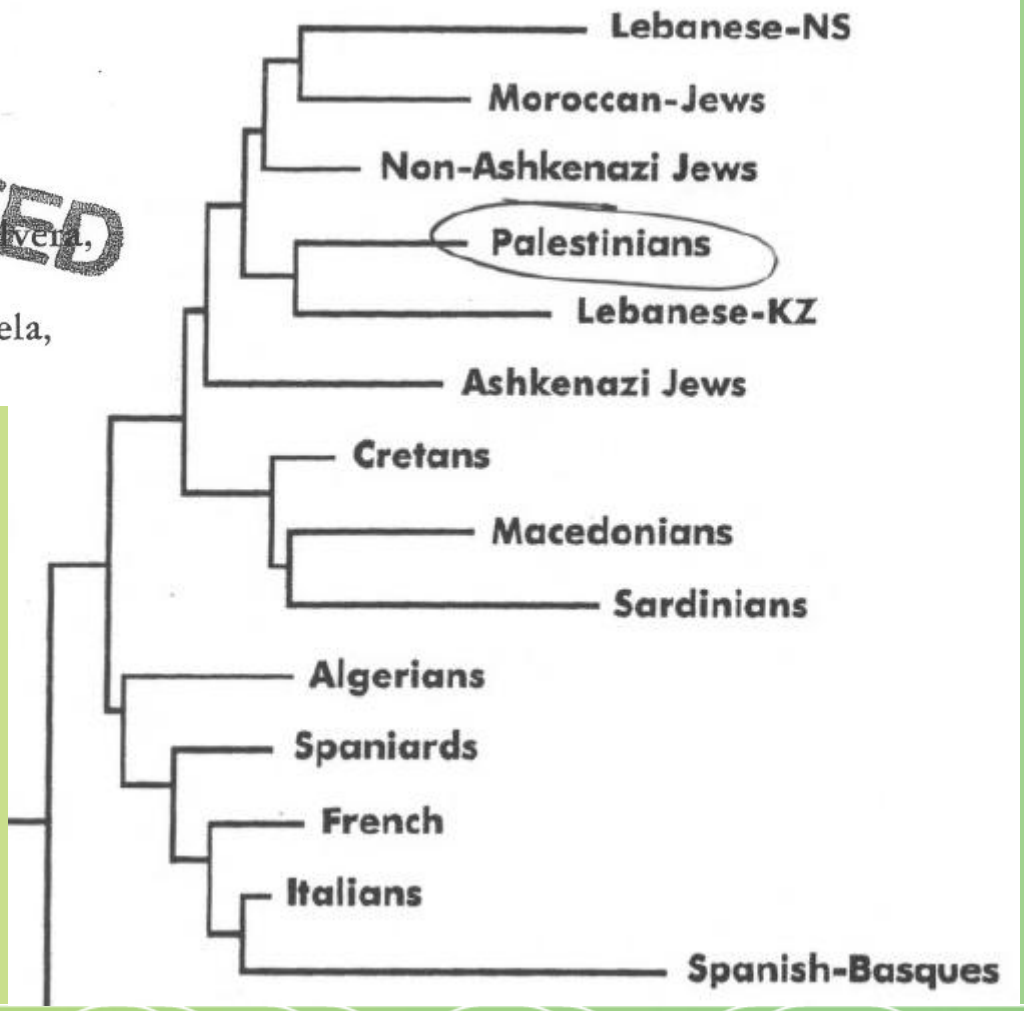
Human Immunology 62, 889–900 (2001)

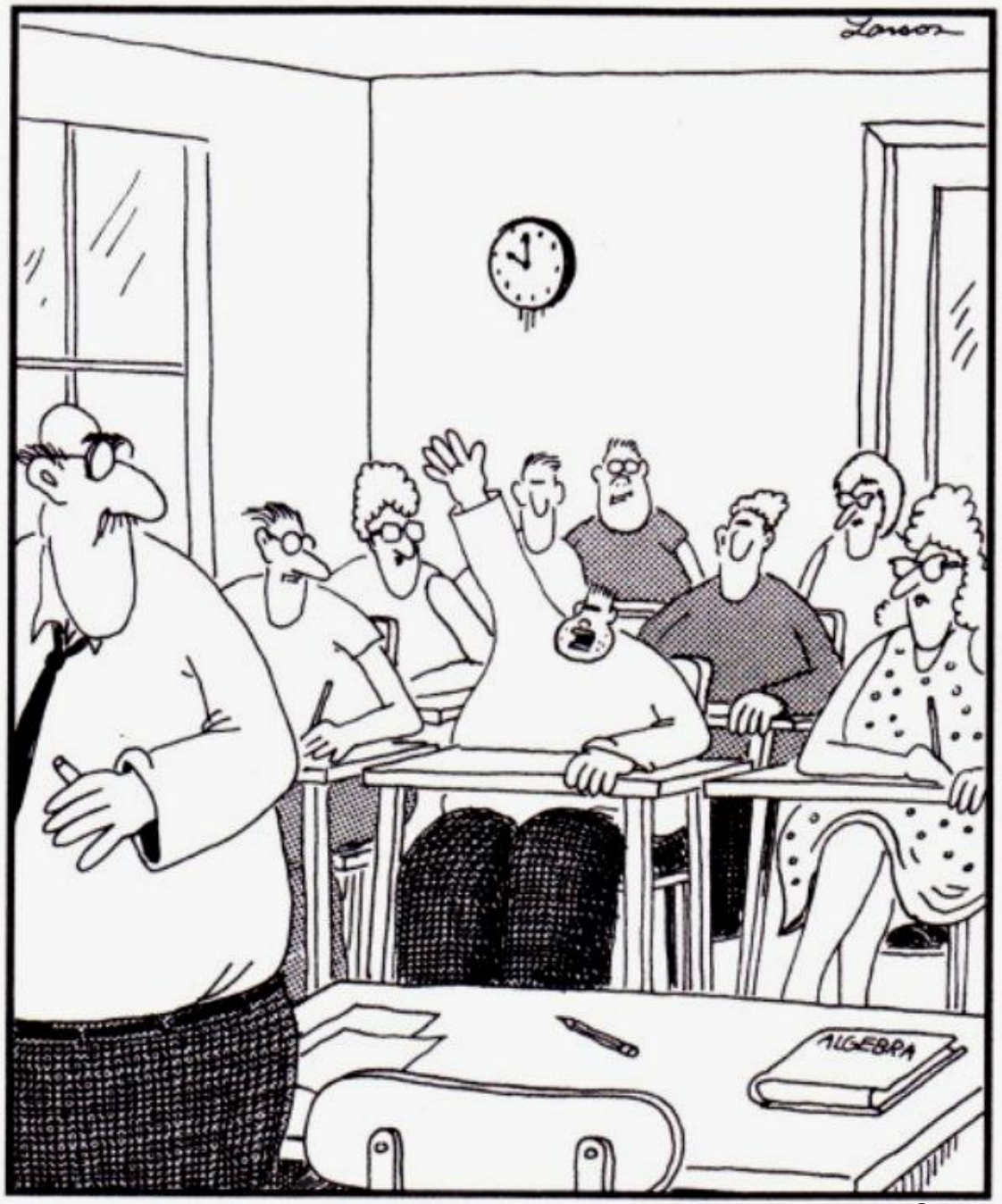
Published by Elsevier Science Inc.

The Origin of Palestinians and Their Genetic Relatedness With Other Mediterranean Populations

RETRACTED

Antonio Arnaiz-Villena, Nagah Elaiwa, Carlos Silvera, Ahmed Rostom, Juan Moscoso, Eduardo Gómez-Casado, Luis Allende, Pilar Varela, and Jorge Martínez-Laso



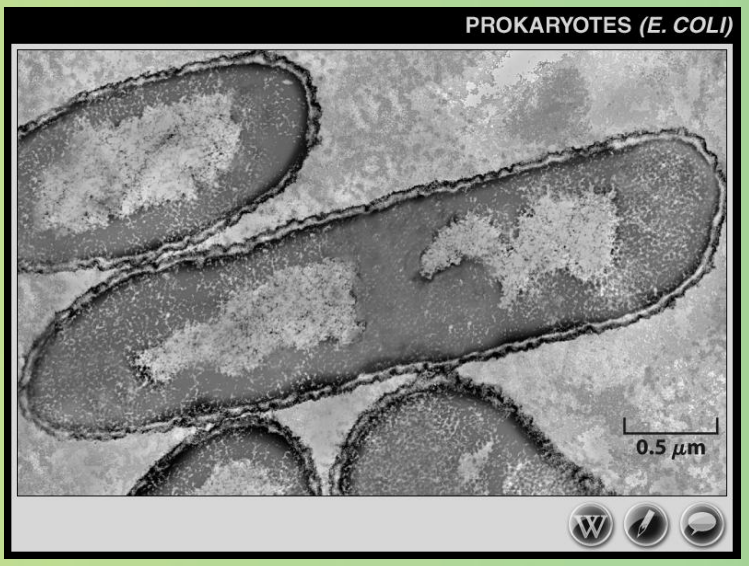


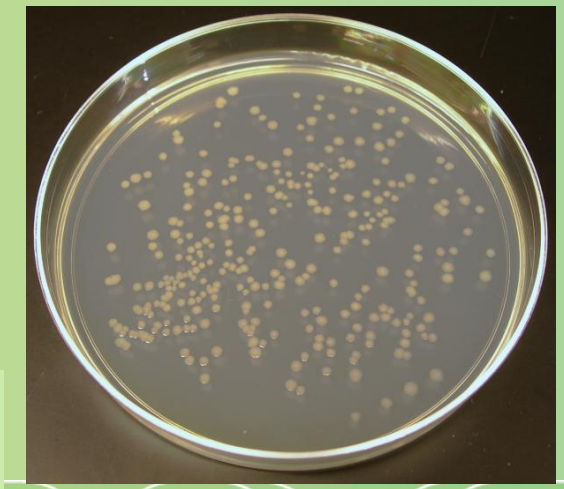
"Dr. Ahram may I be excused? My brain is full."

Model systems

Prokaryotic cells (E. coli)

- Simple, rapid growth, small genome.
- They are ideal models to study basics of biochemistry and molecular biology.
  - *E. coli* is used to understand basic mechanisms of molecular genetics.
  - It has ~4.6m base pairs/4300 genes/1 chromosome.

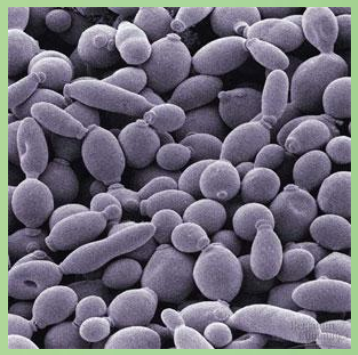


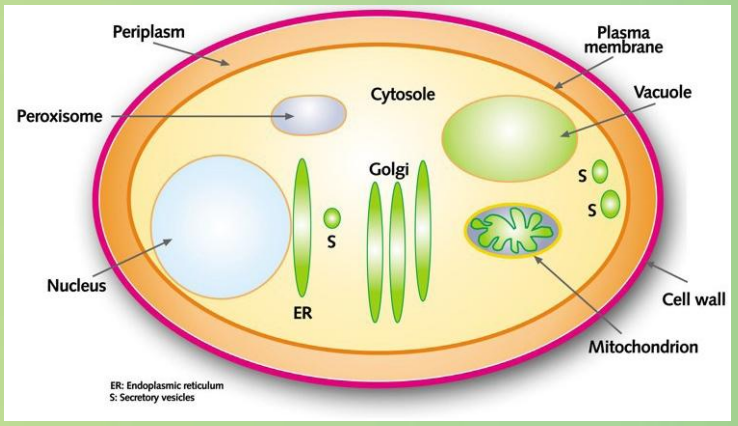


They cannot be used to study aspects of eukaryotic cell structure and function.

Yeast

- The simplest eukaryotes with features of eukaryotic cells.
  - A distinct nucleus surrounded by a nuclear membrane
  - A cytoskeleton and subcellular organelles
- Growth is rapid and as colonies.
- 12 million base pairs of DNA/6000 genes/ 16 chromosomes

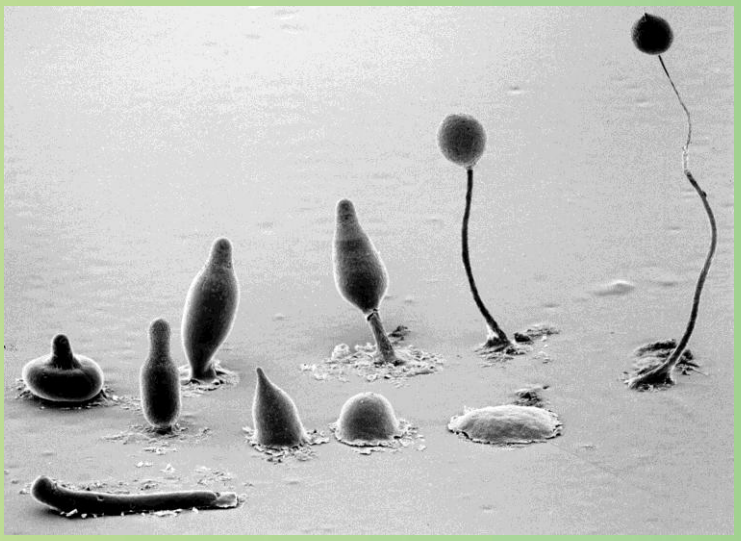




Used to understand DNA replication, transcription, RNA processing, protein sorting, and regulation of cell division.

Dictyostelium discoideum

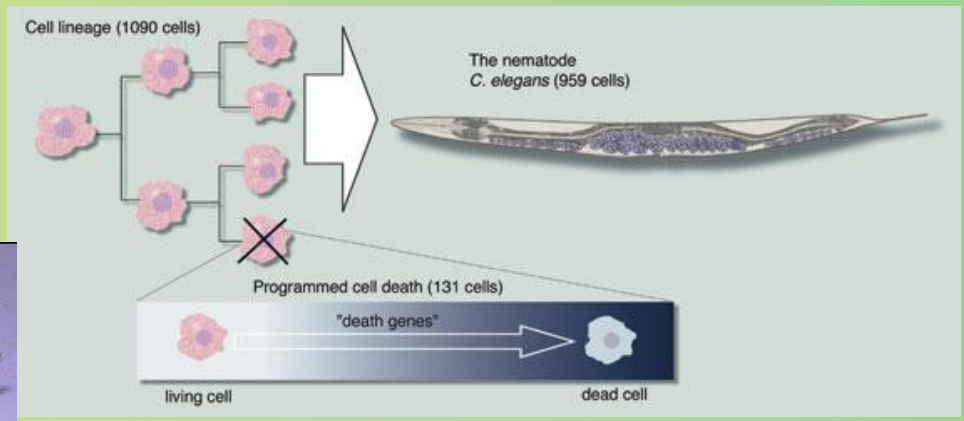
- A cellular slime mold
- More complex genome than yeast's, but simpler than that of higher eukaryotes'.
- can be readily grown in the laboratory and undergo genetic manipulations
- Highly mobile cells used to study molecular mechanisms of animal cell movements.

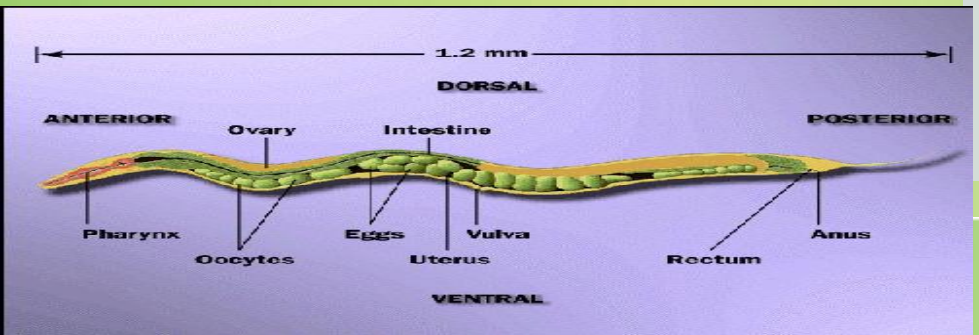


Single cells can aggregate into multicellular structures

Caenorhabditis elegans

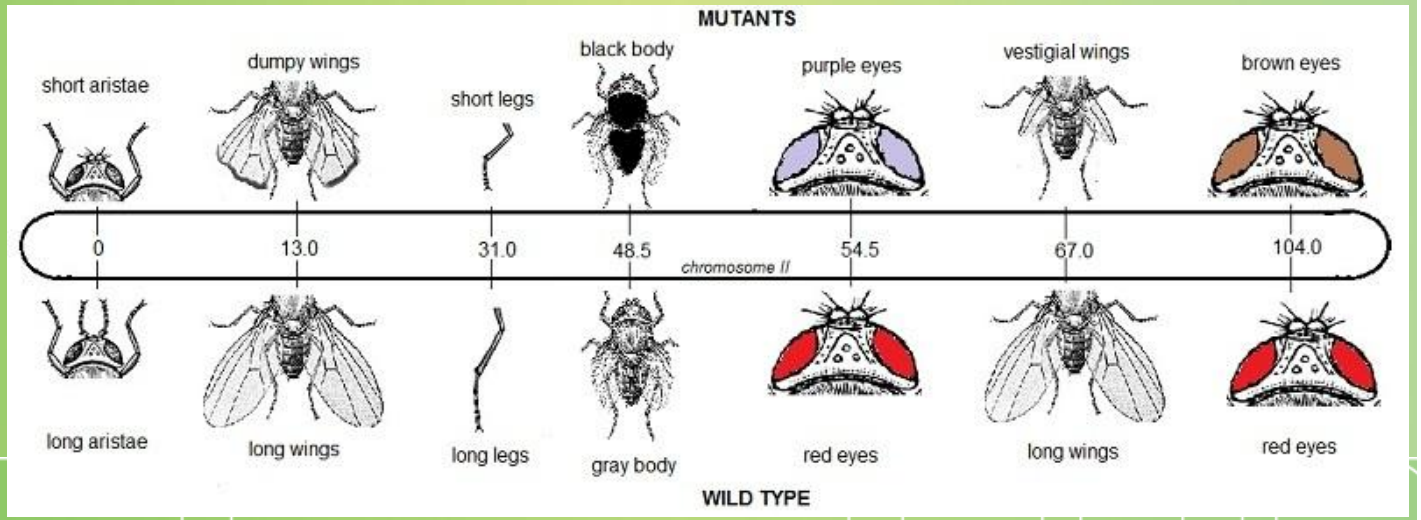
- More complex eukaryotes
- 97m base pairs of DNA/~19,000 genes/6 chromosomes
- easily grown in lab and genetically manipulated
- Adult worms consist of only 959 somatic cells starting from 1090.
- used for studies of animal development and cell differentiation and apoptosis

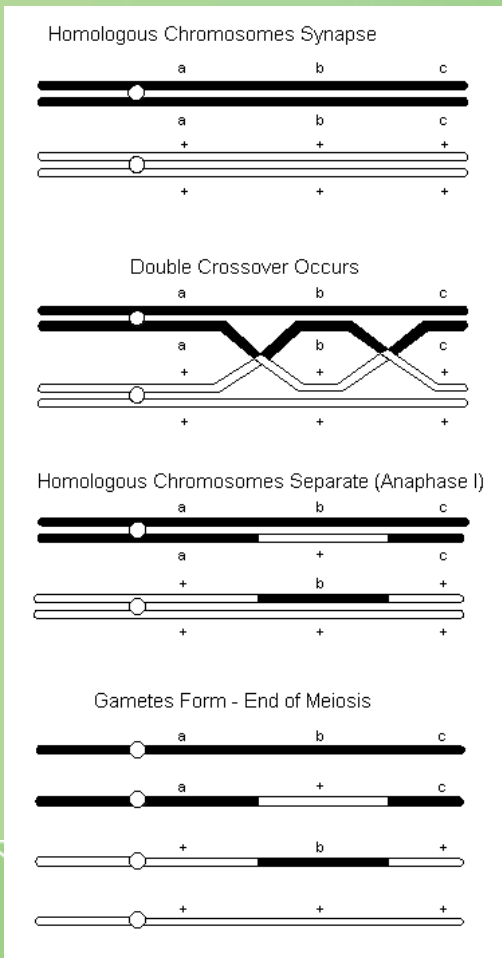


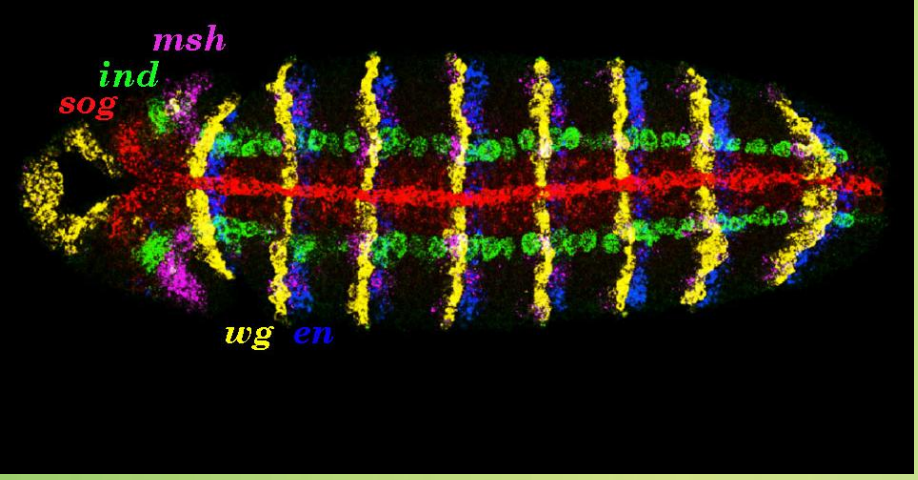


Drosophila melanogaster
(The fruit fly)

- 180m base pairs, four chromosomes, 14,000 genes
- Easily maintained and bred in the laboratory with short reproductive cycle (~2 weeks)
- unravel genes involved in development and differentiation
- Determine the relationship between genes and chromosomes



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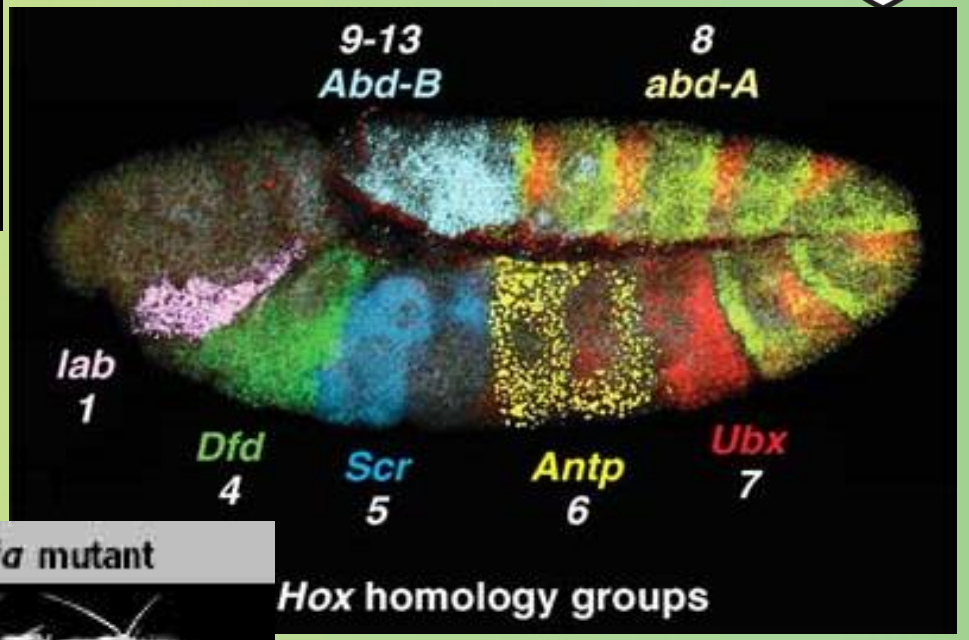
9-13
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abd-A
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Dfd
4
Scr
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Antp
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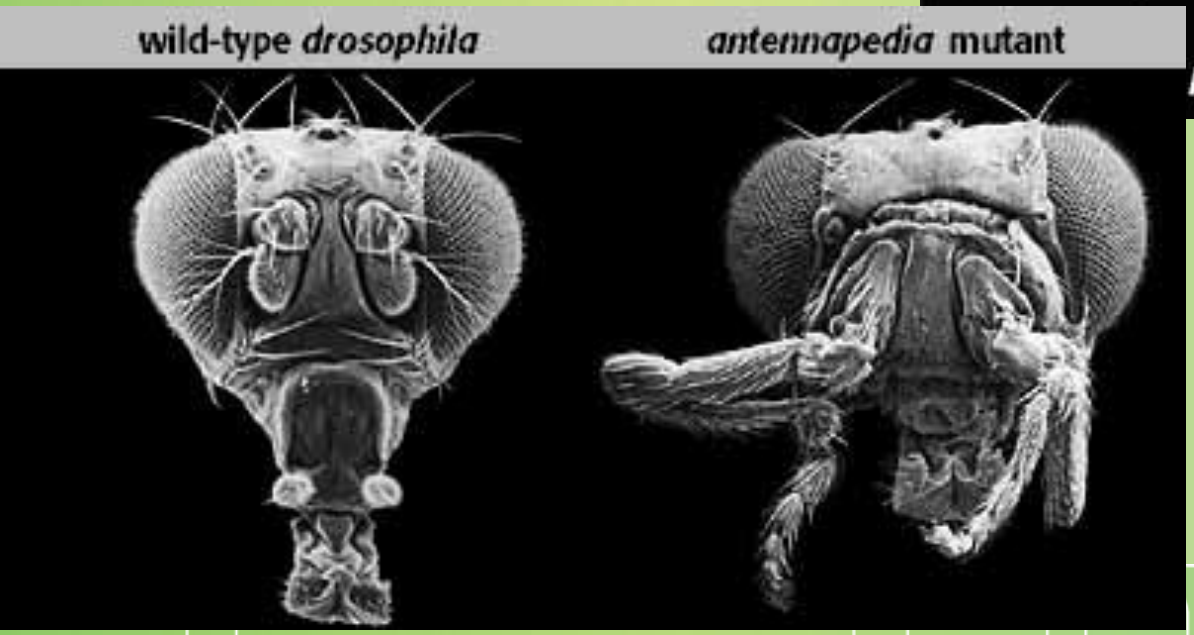
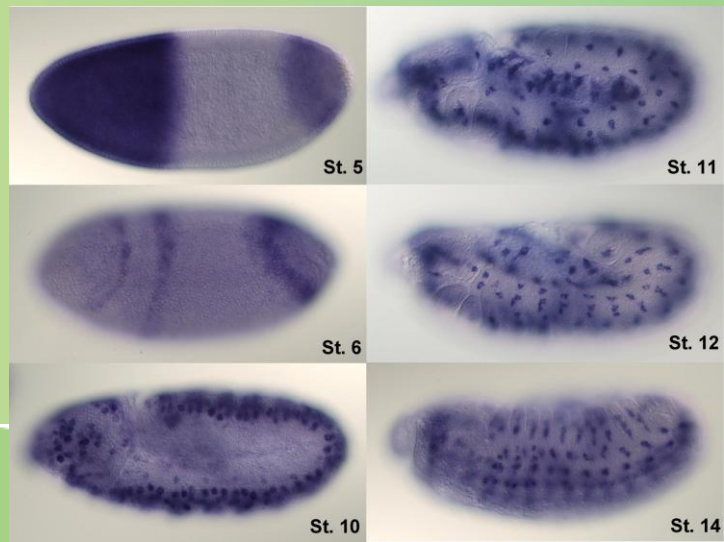
Ubx
7
Hox homology groups


wild-type drosophila
antennapedia mutant

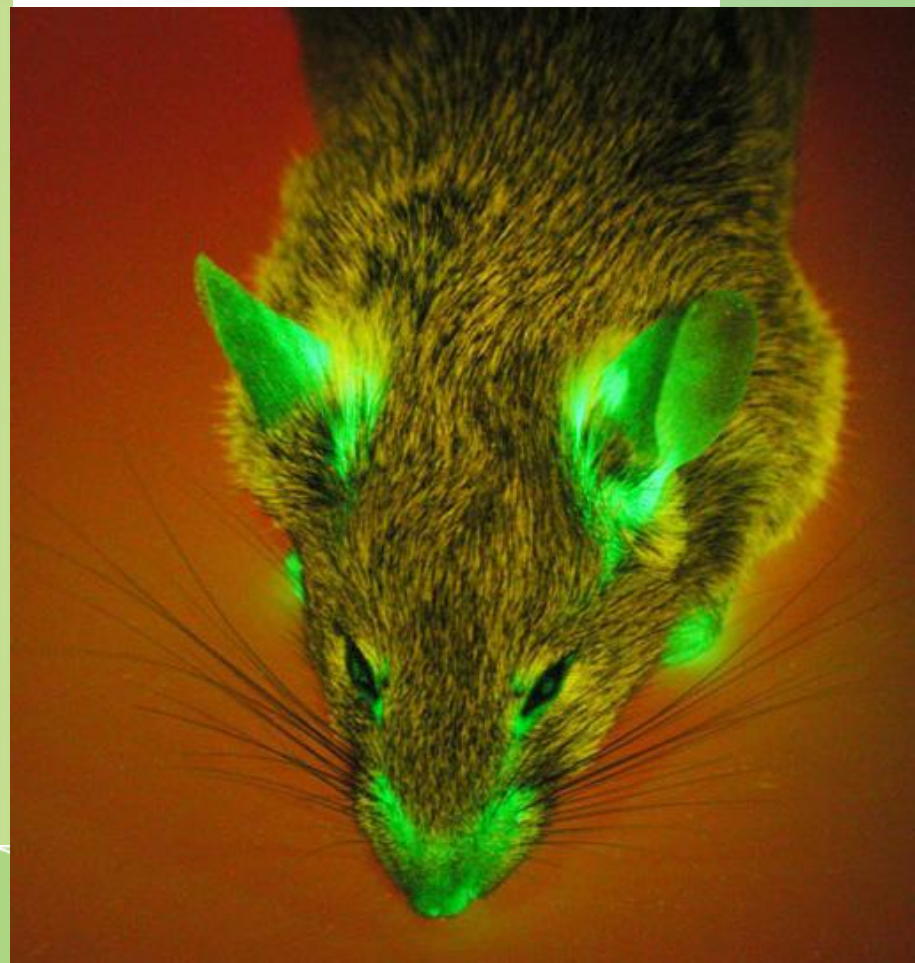

St. 5
St. 11
St. 6
St. 12
St. 10
St. 14

Xenopus laevis (a frog)

- used in studies of early vertebrate development.
- *Xenopus* eggs are large (a diameter of ~ 1 mm) and develop outside of the mother
  - stages of development can be studied in the laboratory.
- Used in studying development, differentiation, and embryonic cell division.

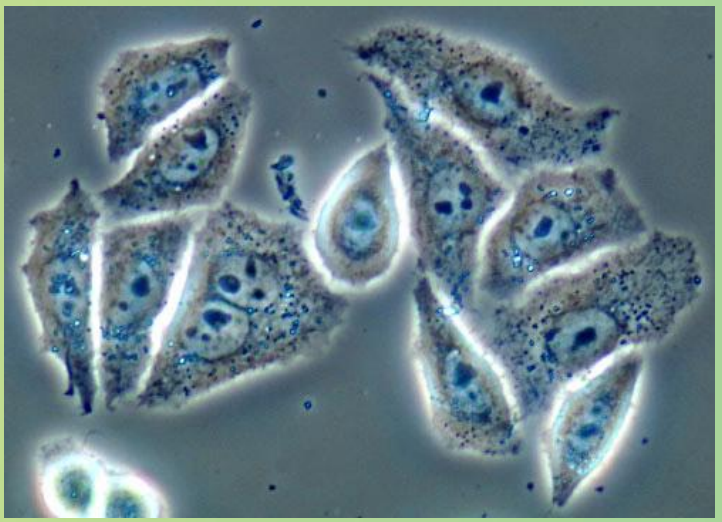
Mice

- 3000m base pairs, 20,000-25,000 genes/20 chromosomes
- Transgenic mice with specific mutant genes introduced into the mouse germ line



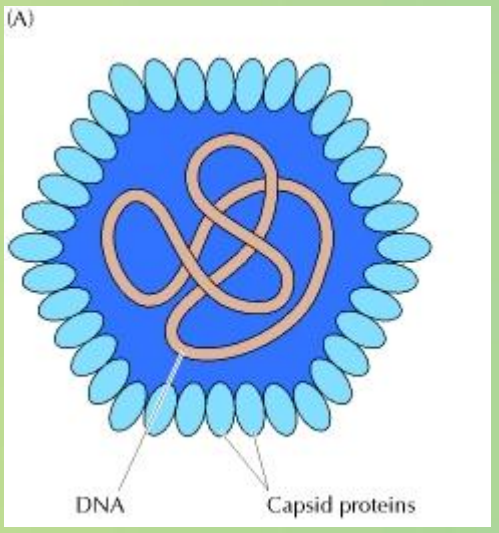
Cultured mammalian cells

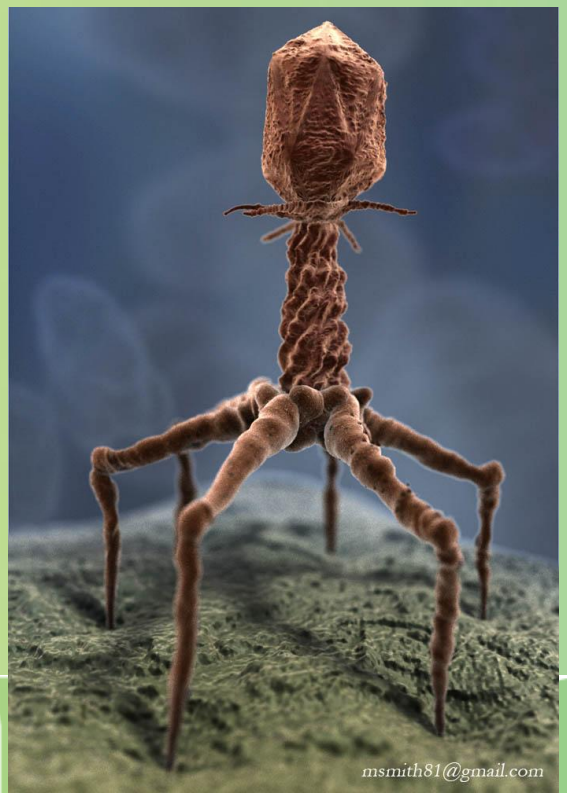
- Mammalian cells are isolated, get immortalized, and grown in dishes
- can be manipulated under controlled laboratory conditions.
- Uses: many aspects of mammalian cell biology, (DNA replication, gene expression, protein synthesis and processing, and cell division, etc.)



Viruses

- intracellular parasites
- They reproduce by infecting host cells and taking over the cellular machinery.
- In their simplest forms, viruses consist of genomic nucleic acid surrounded by a protein coat.
- Bacteriophages (bacterial viruses) allowed understanding basic molecular genetics.





Organelles

TABLE 4-2 *Organelles*

Organelle	Function
Mitochondrion	transfers energy from organic compounds to ATP
Ribosome	organizes the synthesis of proteins
Endoplasmic reticulum (ER)	prepares proteins for export (rough ER); synthesizes steroids, regulates calcium levels, breaks down toxic substances (smooth ER)
Golgi apparatus	processes and packages substances produced by the cell
Lysosome	digests molecules, old organelles, and foreign substances
Microfilaments and microtubules	contribute to the support, movement, and division of cells
Cilia and flagella	propel cells through the environment; move materials over the cell surface
Nucleus	stores hereditary information in DNA; synthesizes RNA and ribosomes
Cell wall*	supports and protects the cell
Vacuole*	stores enzymes and waste products
Plastid*	stores food or pigments; one type (chloroplast) transfers energy from light to organic compounds

*Cell walls, large vacuoles, and plastids are found in the cells of plants and some other eukaryotes, but not in the cells of animals.

Major components of cells

- Nucleic acids
- Carbohydrates
- Proteins
- Lipids (50% of mass of plasma membranes, 30% of mitochondrial membranes)

Membrane proteins

Composition and properties of membranes

Cholesterol is an essential component of animal plasma membrane.
It is not resent in bacteria and plant cells, but latter cells contain sterols.

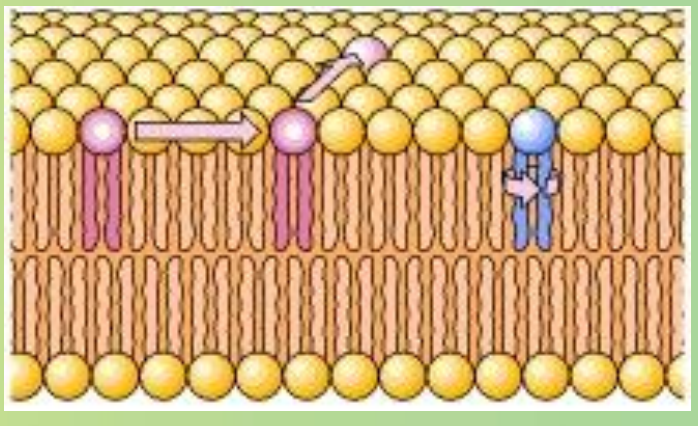
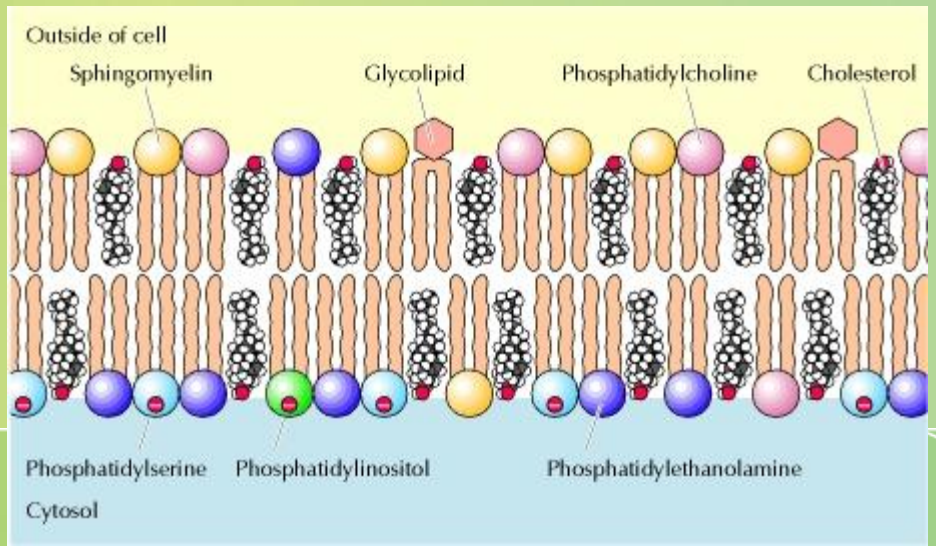


Table 8.18. Mass % Biochemical Composition of Cell and Organelle Membranes[531,939,996,997]

Type of Membrane Molecule	Liver Cell Plasma Membrane	Red Cell Plasma Membrane	Myelin Sheath	Mitochondrion Inner/Outer Membranes	Endoplasmic Reticulum Membrane	*E.coli* (Bacterial Membrane)
Lipid	--	40%	~81%	~24%/~48%	--	--
Protein	~50%	52%	~19%	~76%/~52%	~50%	~50%
Carbohydrate	--	8%	--	--	--	--
Lipid Class:						
Cholesterol	17%	23%	22%	3%	6%	0%
Phospholipids						
Phosphatidylethanolamine	7%	18%	15%	35%	17%	70%
Phosphatidylserine	4%	7%	9%	2%	5%	trace
Phosphatidylcholine	24%	17%	10%	39%	40%	0%
Sphingomyelin	19%	18%	8%	0%	5%	0%
Glycolipids	7%	3%	28%	trace	trace	0%
Other lipids	22%	13%	8%	21%	27%	30%

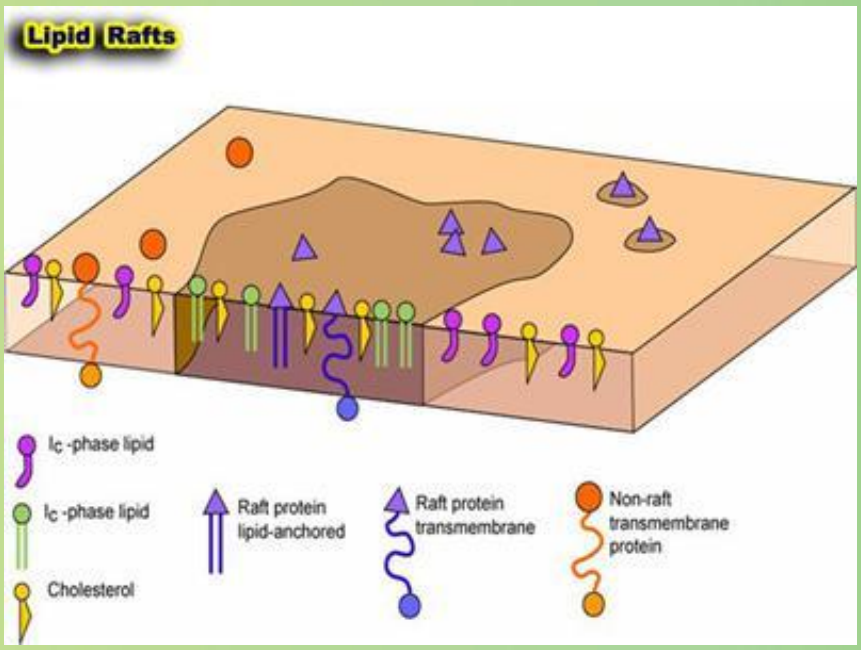
Composition and properties of plasma membranes

- **The phospholipids are asymmetrically distributed between the two halves of the membrane bilayer.**
  - **The outer leaflet: Ⓟcholine, sphingomyelin**
  - **The inner leaflet:Ⓟethanolamine, Ⓟserine, Ⓟinositol (minor)**
  - **Ⓟinositol has a role in cell signaling.**
  - **The head groups of both Ⓟserine and Ⓟinositol are negatively charged making the cytosolic face of the plasma membrane having a net negative charge.**



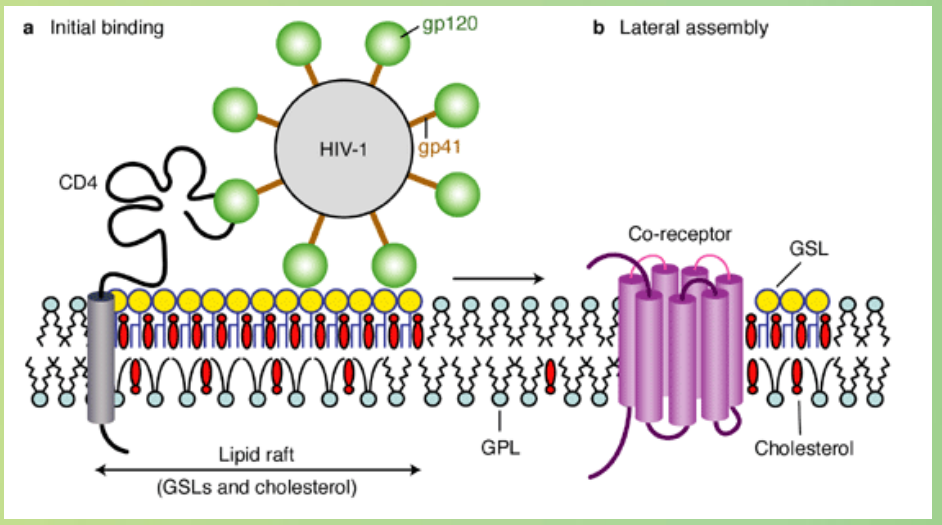
Lipid rafts

- These are clusters of cholesterol and the sphingolipids (sphingomyelin and glycolipids).
- Sphingolipids provide an ordered lipid environment.
- Rafts are enriched in glycosylphosphatidylinositol (GPI)-anchored proteins, as well as proteins involved in signal transduction and intracellular trafficking.

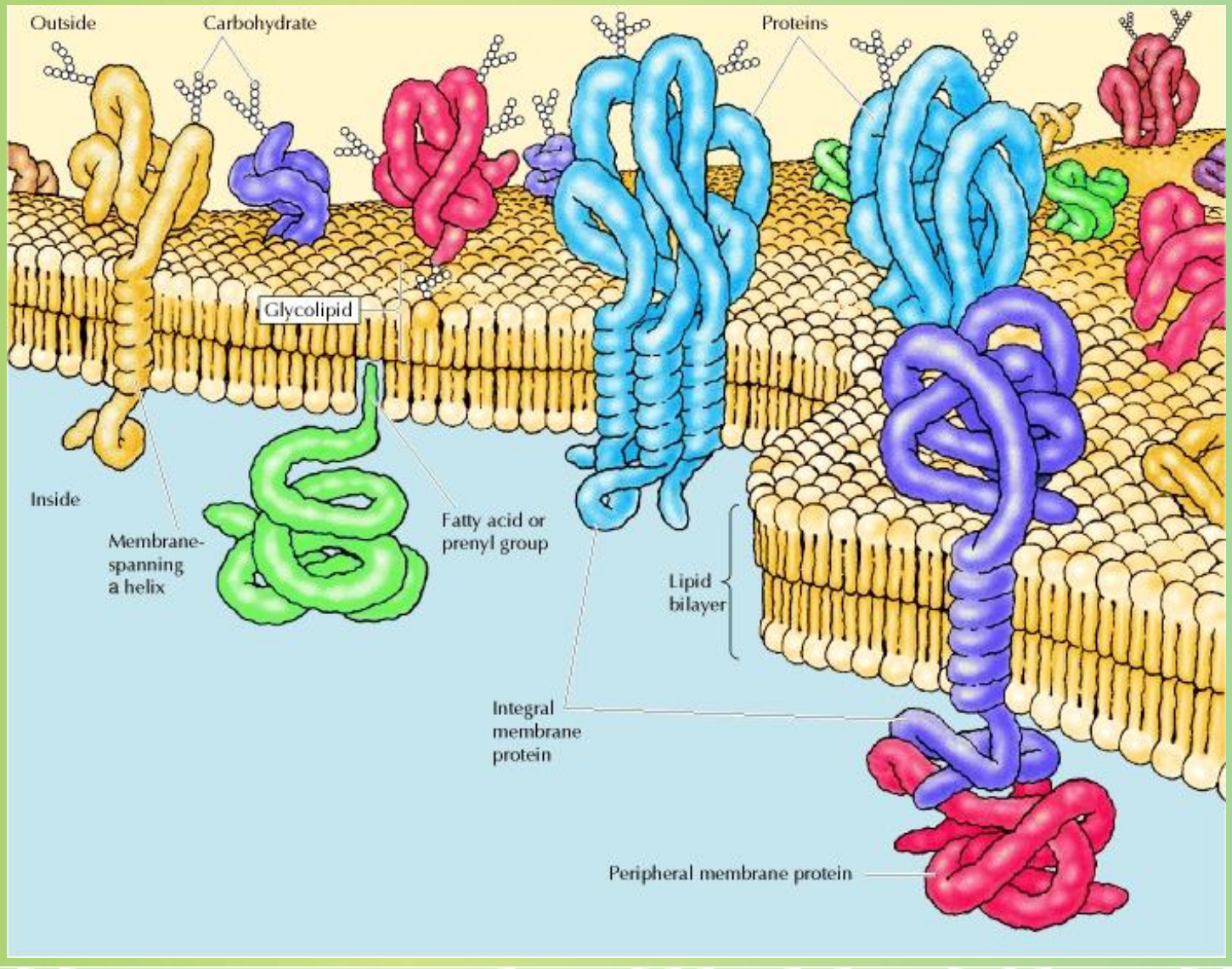


Lipid rafts and diseases

- **HIV virus**
  - Budding may occur from lipid rafts
- **Influenza virus**
  - Raft-associated glycoproteins in envelope
- **Prion disorder**
  - Normal prion protein (PrPc) is converted to abnormal proteins (PrPsc) in lipid rafts .

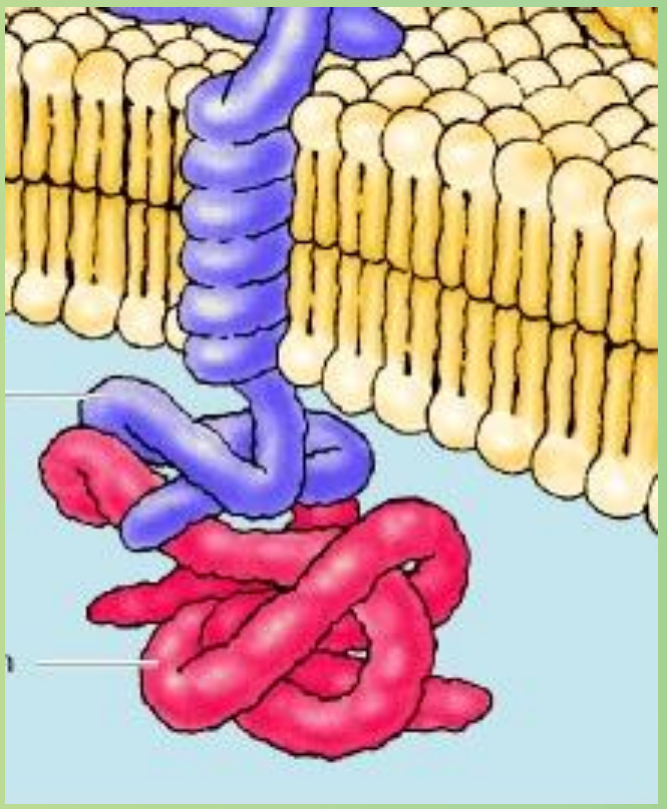


Membrane proteins



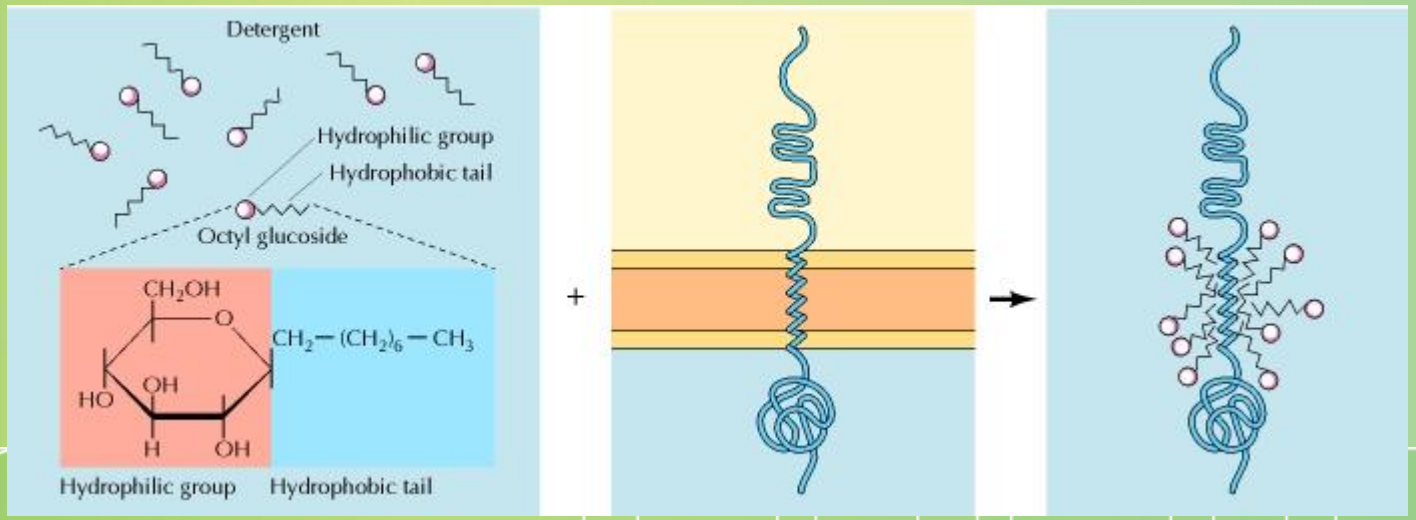
Peripheral membrane proteins

- They are proteins that dissociate from the membrane following treatments with polar reagents (solutions of extreme pH or high salt concentration) that do not disrupt the phospholipid bilayer.
- Once dissociated, they are soluble in aqueous buffers.
- They are indirectly associated with membranes through protein-protein interactions, mainly ionic bonds.



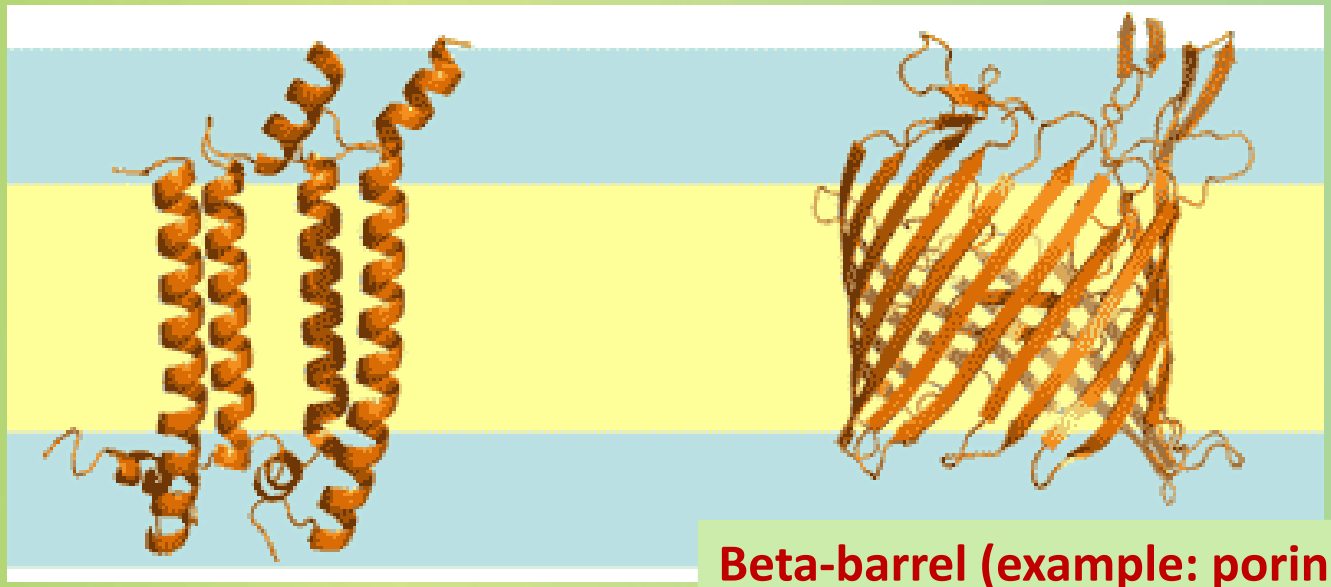
Integral membrane proteins

- **Portions of these integral membrane proteins are inserted into the lipid bilayer.**
- **They are dissociated by reagents of small amphipathic molecules.**
  - **The hydrophobic portions of detergents disrupt hydrophobic interactions.**
  - **The hydrophilic part makes the detergent-protein complexes soluble in aqueous solutions.**



α-helices vs. β-sheets

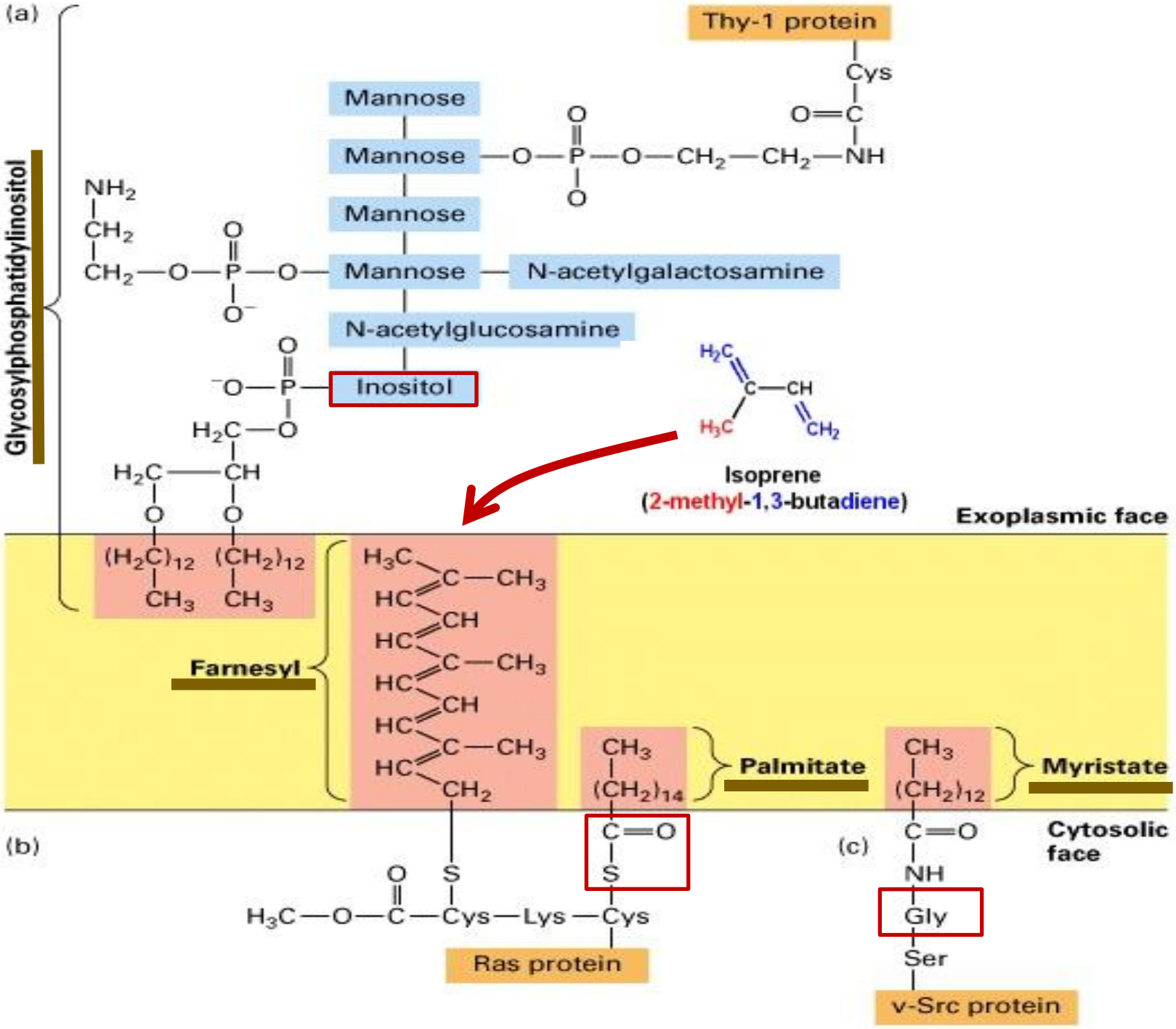
- The membrane-spanning portions of transmembrane proteins are usually α-helices of 20-25 hydrophobic amino acids.
- They are usually glycosylated with the oligosaccharides exposed on the surface of the cell.



Beta-barrel (example: porins)
(bacteria, chloroplast, mitochondria)

Lipid-anchored membrane proteins

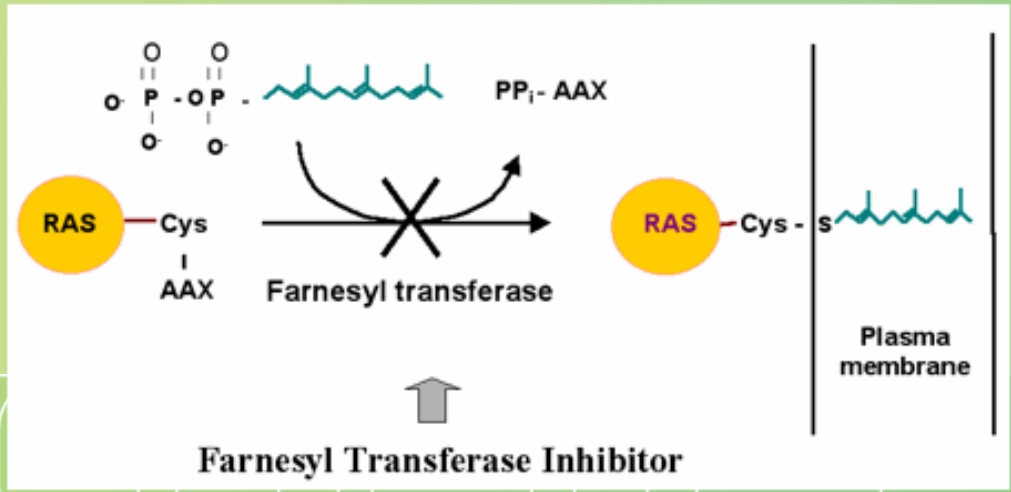
- **Four types have been found:**
  - Myristoylation
    - Myristate attached to glycine at inner face of membrane
  - Palmitoylation
    - Palmitate is added to sulfur atoms of the side chains of internal cysteine residues.
  - Prenylation
    - It refers to linking of "isoprene"-based groups
    - Attached to cysteine near C-terminus of proteins
  - Glycolipid (glycosyl phosphatidylinositol) anchors
    - The carbohydrate bridges the protein with the fatty acid chains of the phospholipid (usually ethanolamine)


(a)
Thy-1 protein
Cys
Mannose
Mannose
Mannose
Mannose
N-acetylgalactosamine
N-acetylglucosamine
Inositol
Glycosylphosphatidylinositol
Isoprene
(2-methyl-1,3-butadiene)
Exoplasmic face
Farnesyl
Palmitate
Myristate
Cytosolic face
(b)
Ras protein
(c)
Gly
Ser
v-Src protein

Example: farnesylation of Ras

- Ras is an oncogene that farnesylation is important for its function and oncogenic activity.
- Farnesyltransferase inhibitors (FITs) had impressive anti-tumor activity in preclinical cell culture and mouse models, but they failed in human clinical trials because:
  - FTIs did not block prenylation of other Ras isoforms (N-Ras and K-Ras) and their tumorigenic activity.
  - There are other farnesylated proteins with important roles in the cell including growth regulation.

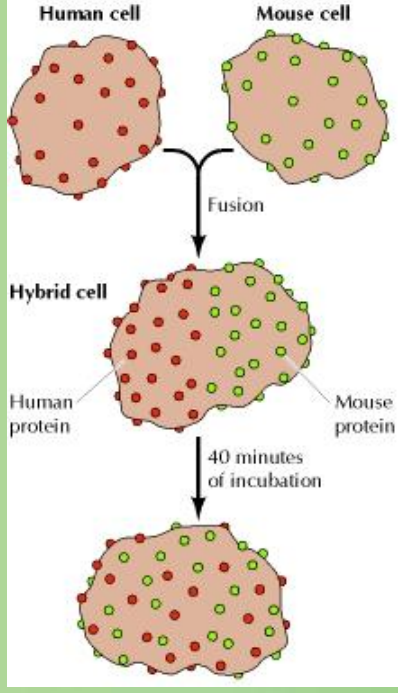
FTIs are considered for the treatment of other diseases such as Hutchinson-Gilford Progeria Syndrome (AKA progeria), which is caused by mutated gene encoding lamin A, a farnesylated protein.

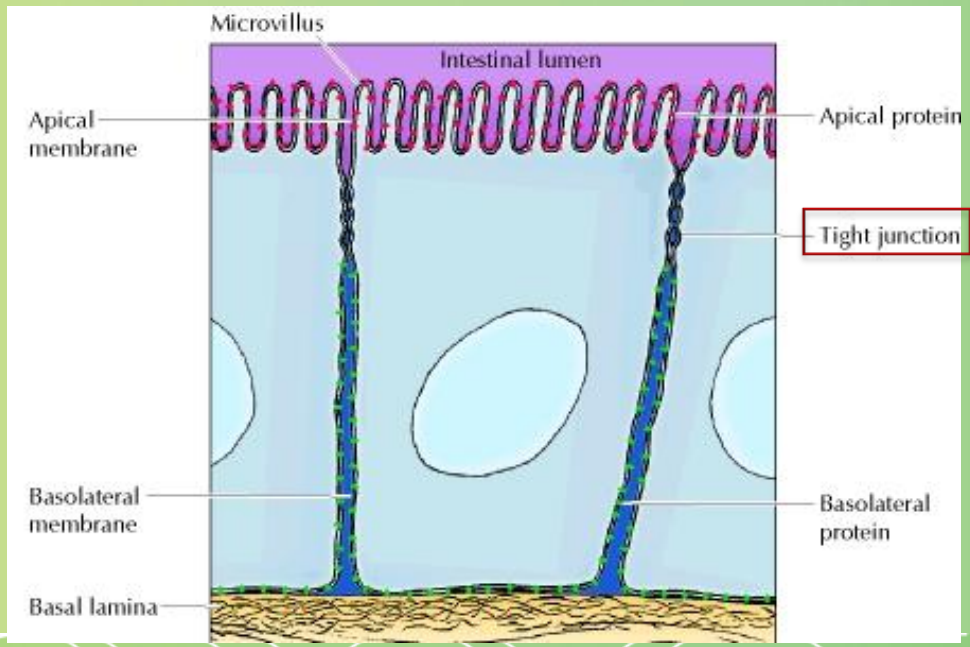


Protein mobility

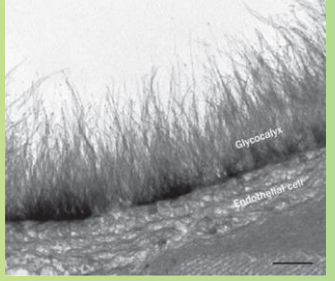
- **Both proteins and lipids are able to diffuse laterally through the membrane.**

- The mobility of membrane proteins is restricted by
  - Their association with the cytoskeleton
  - Specific membrane domains such as tight junctions, which maintain the specific distribution of apical and basolateral proteins,
  - Lipid composition restrict protein mobility (e.g. lipid rafts).





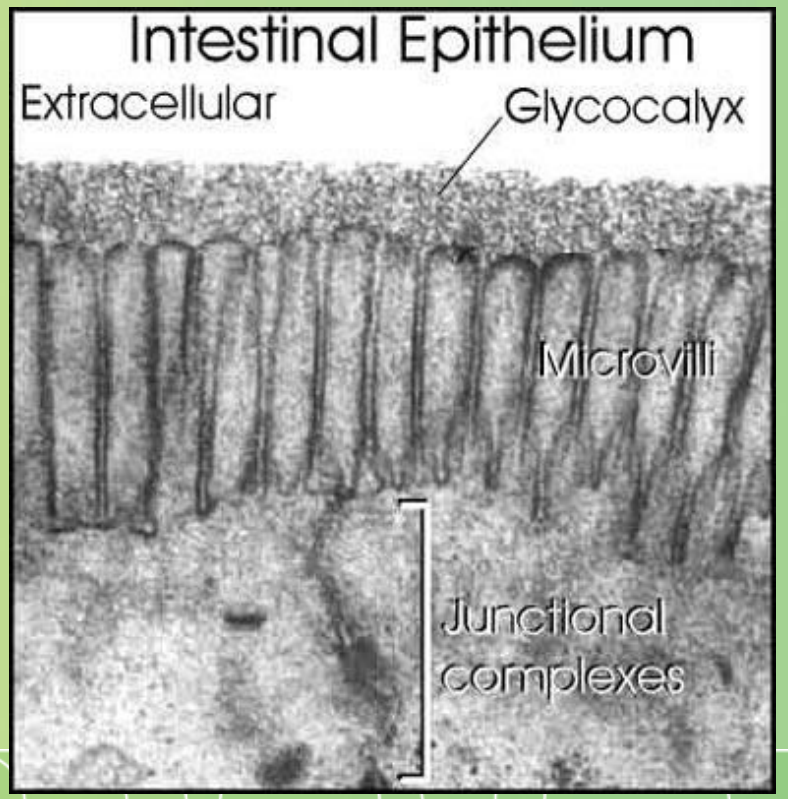
Glycocalyx



- **The surface of the cell is covered by a carbohydrate coat, known as the glycocalyx, formed by the oligosaccharides of glycolipids and transmembrane glycoproteins.**

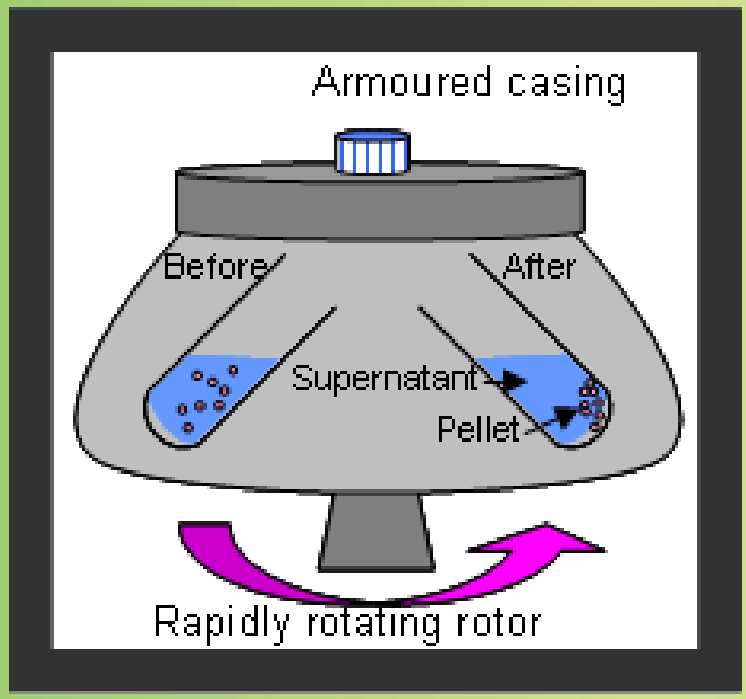
Functions:

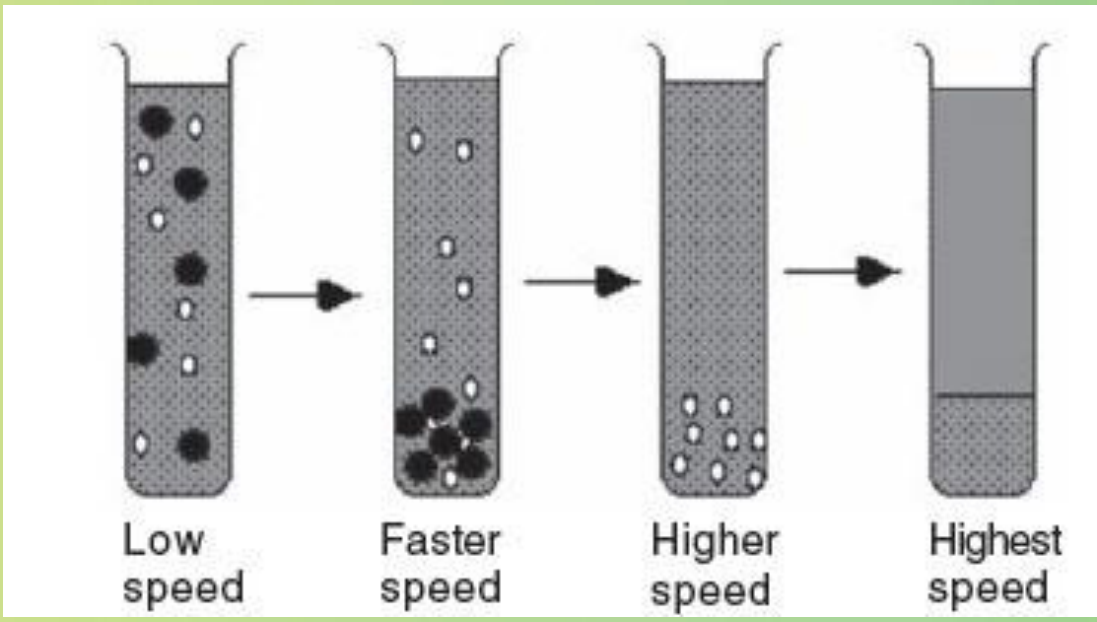
- **Cell-cell interactions (e.g, leukocytes)**
- **Protection of cell surface from ionic and mechanical stress**
- **Formation of a barrier for microorganisms**



Techniques

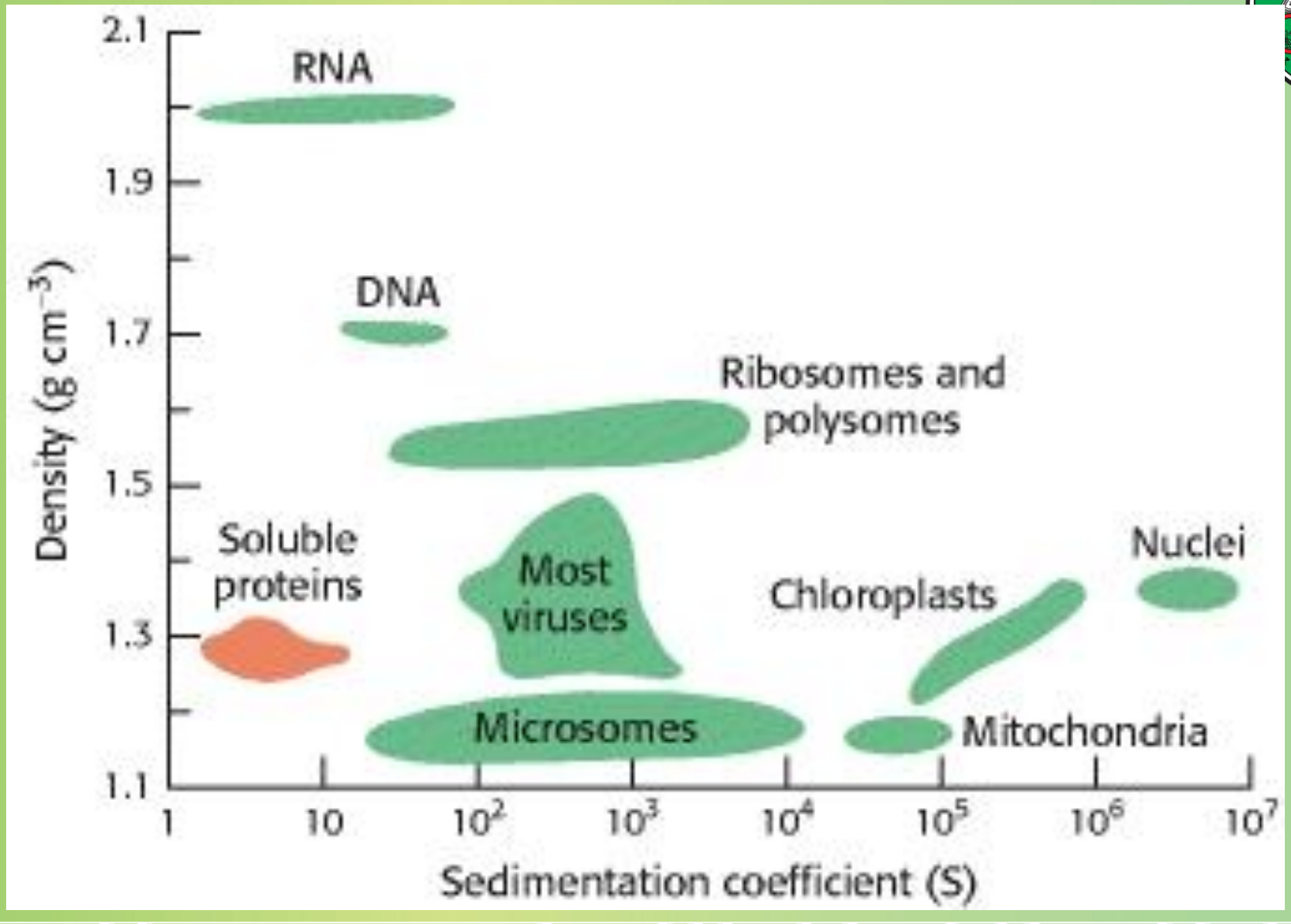
Centrifugation (ultra-)



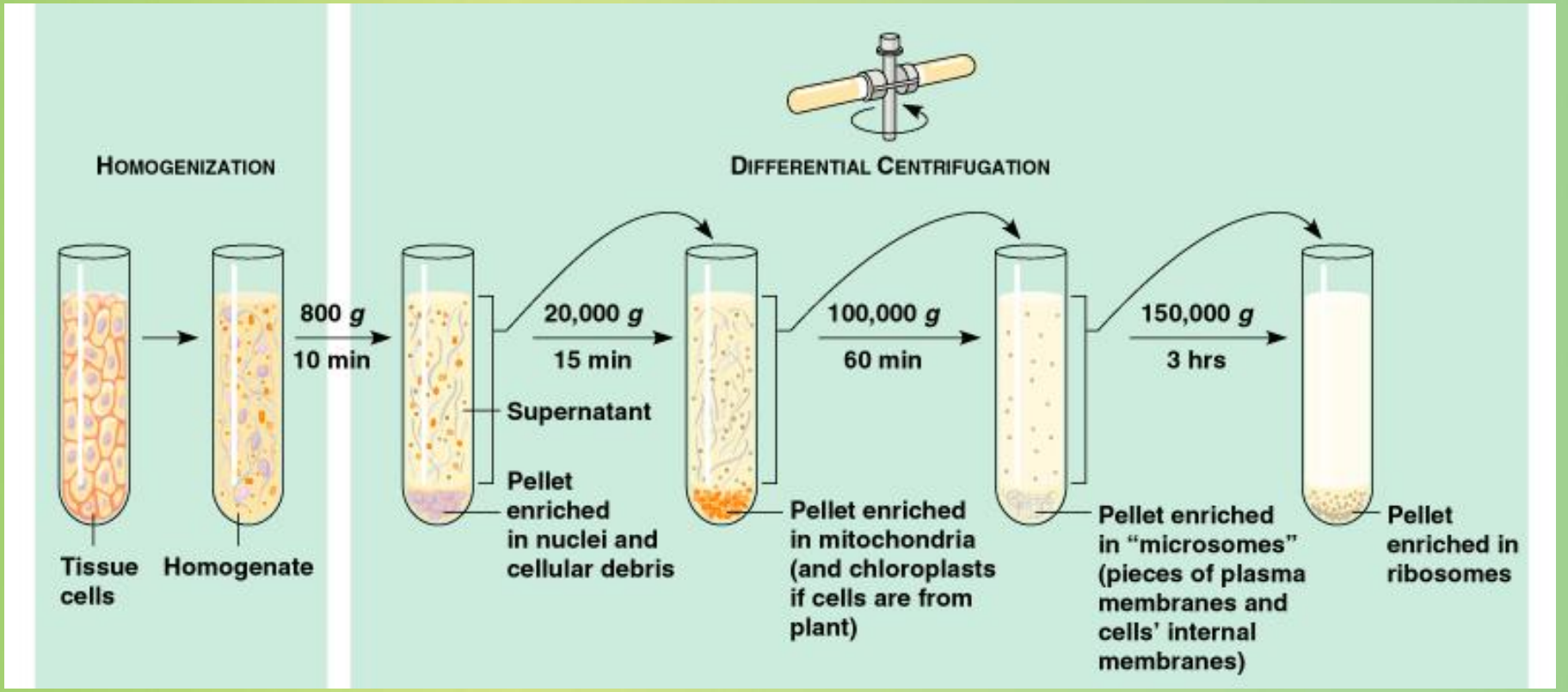


Sedimentation

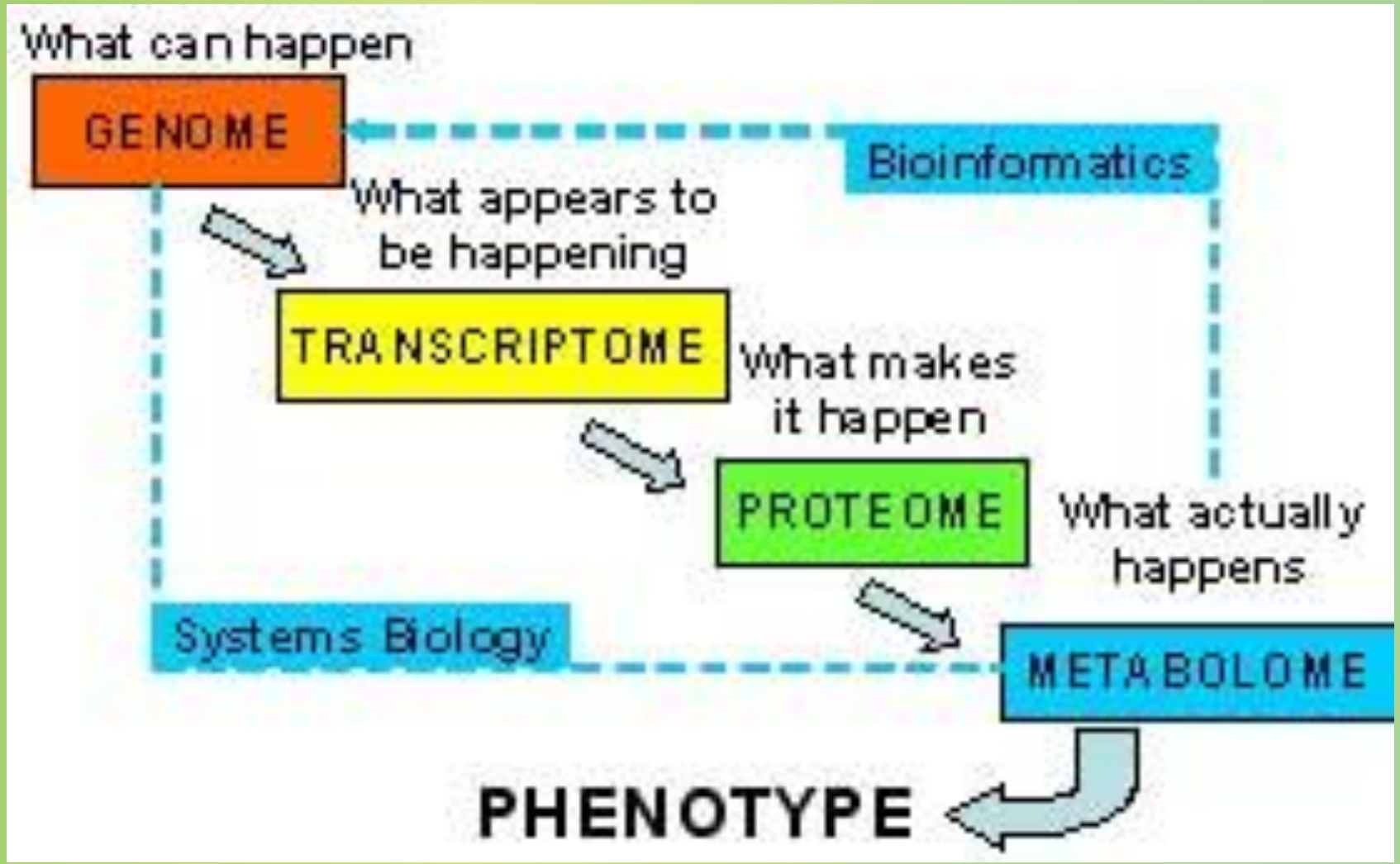
- A particle sediments by centrifugation
- Sedimentation depends on its mass and shape
- The sedimentation of a particle is constant and can be defined as a sedimentation coefficient
  - Sedimentation coefficient = 10^{-13} S Svedberg
- The sedimentation of a particle depends on its
  - Mass (direct correlation)
  - Density (direct correlation)
  - Shape (inverse correlation)
  - The density of the solution (inverse correlation)


RNA
DNA
Ribosomes and polysomes
Soluble proteins
Most viruses
Nuclei
Chloroplasts
Microsomes
Mitochondria
Density (g cm^{-3})
2.1
1.9
1.7
1.5
1.3
1.1
1
10
10^2
10^3
10^4
10^5
10^6
10^7
Sedimentation coefficient (S)

Example: cell fractionation

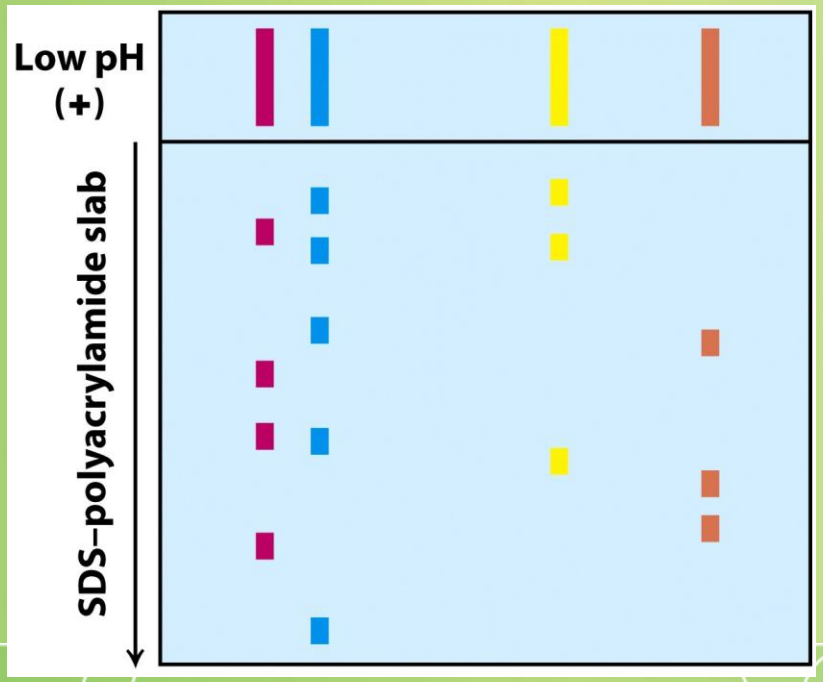


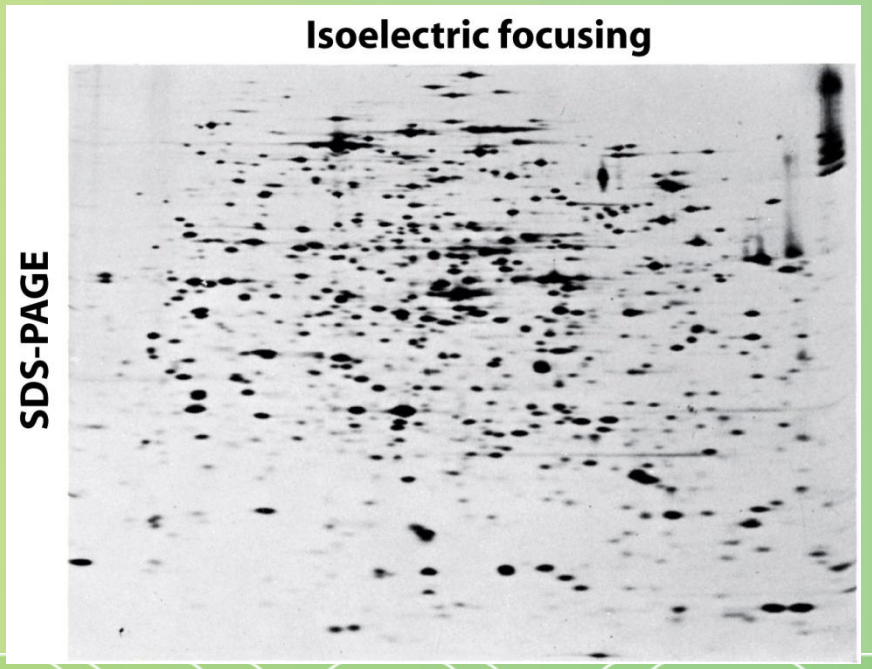
The science of -omics



Two-dimensional gele electrophoresis (2D-PAGE)

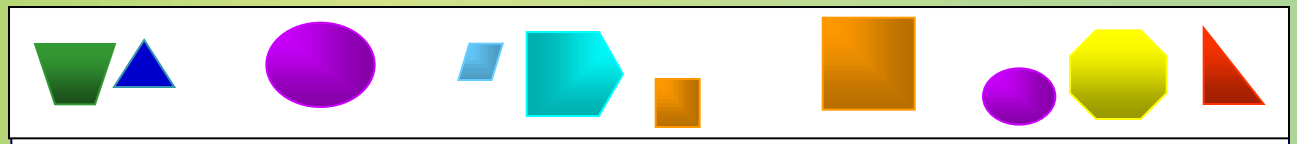
- In 2D-PAGE, proteins are separated by first, isoelectric focusing, then through an SDS-PAGE
- Thus, it allows protein separation based on both charge and size





pH 3 → 10

\+



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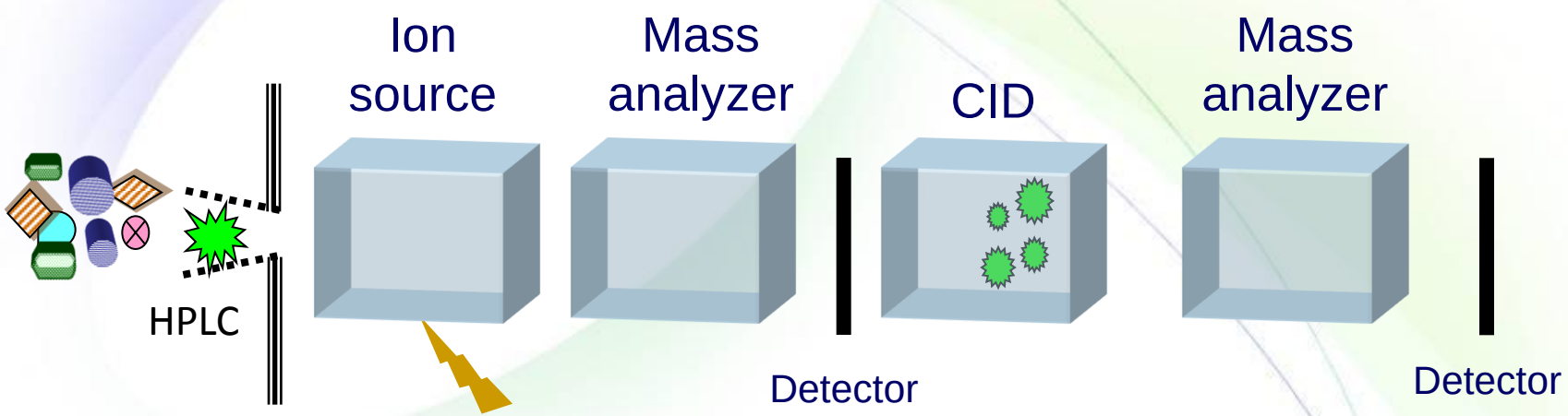
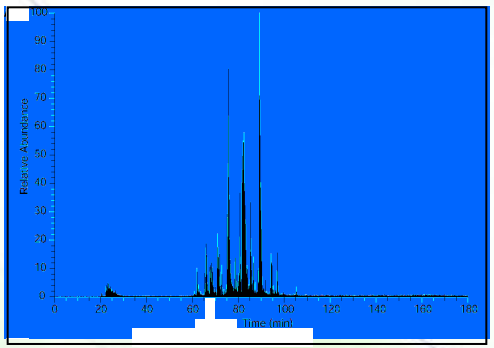
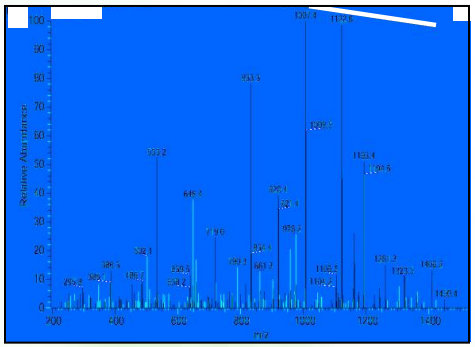
High MW

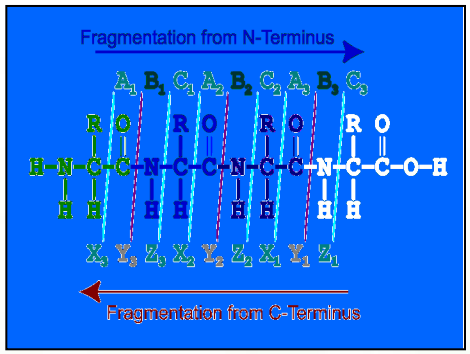
Low MW

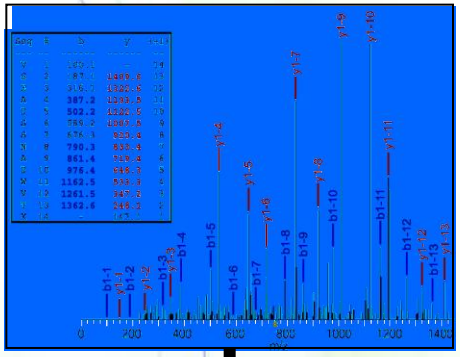
Basics of tandem mass spectrometry







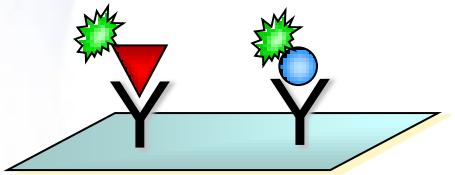




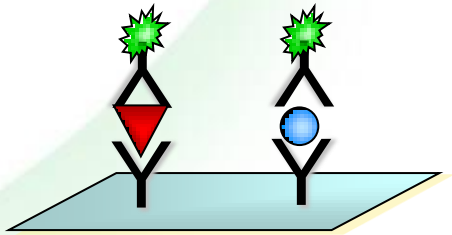
Protein identification

Types of protein microarrays

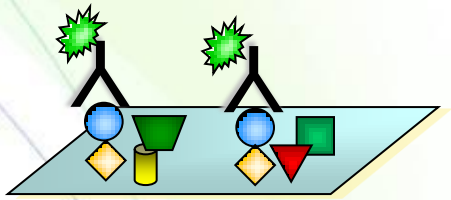
Expression microarrays



Direct labeling
Forward-phase microarray

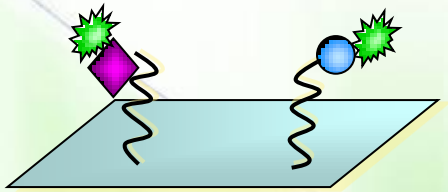


Indirect labeling
Forward-phase microarray

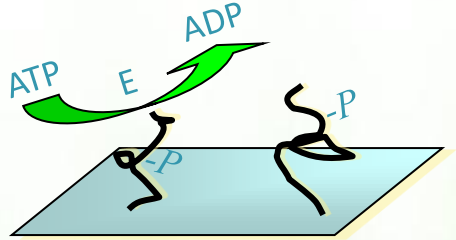


Reverse-phase microarray

Functional microarrays



Interaction microarray



Enzymatic microarray

Interactome