

BT1010 Introduction to Life Sciences



Lecture 1: Introduction to Life Sciences, Biomolecules: Carbohydrates and Lipids

13/06/2022

Course Instructor:

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Course Structure

BT1010

3 hours of lectures/week

1 credit

Total registered students: 317

Slot A

Monday: 9:00 - 9:55 am

Wednesday: 11:00 - 11:55 am

Thursday: 10:00 - 10:55 am

Slot B

Monday: 10:00 - 10:55 am

Wednesday: 9:00 - 9:55 am

Thursday: 11:00 - 11:55 am

Venue: Auditorium (Academic Block A, Ground Floor)

Course Schedule – BT1010-Apr-May 2022

Date	Topic
13/06	Introduction to Life Sciences, Biomolecules: Carbohydrates and Lipids
15/06	Biomolecules: Proteins and Nucleic Acids
16/06	continued....
20/06	DNA-Based Information Technologies
22/06	Microscopy to Visualize Cellular Processes
23/06	Cell Organelles and Nuclear Organization
27/06	Cellular Metabolism
29/06	Enzyme Kinetics
30/06	Evaluation: Quiz (20 points)

Instructor's Biodata

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2003-2006 B.Sc. Biochemistry, St. Xavier's College, Ahmedabad, Gujarat, India

2007-2009 M.Sc. Microbiology, M.S. University of Baroda, Vadodara, Gujarat, India

2009-2015 Ph.D. Biosciences and Bioengineering, IIT Bombay, Mumbai, Maharashtra, India

Professional Experience:

2015-2020 Post-Doctoral Fellow at the National Institutes of Health (NIH), MD, USA

Research Interests:

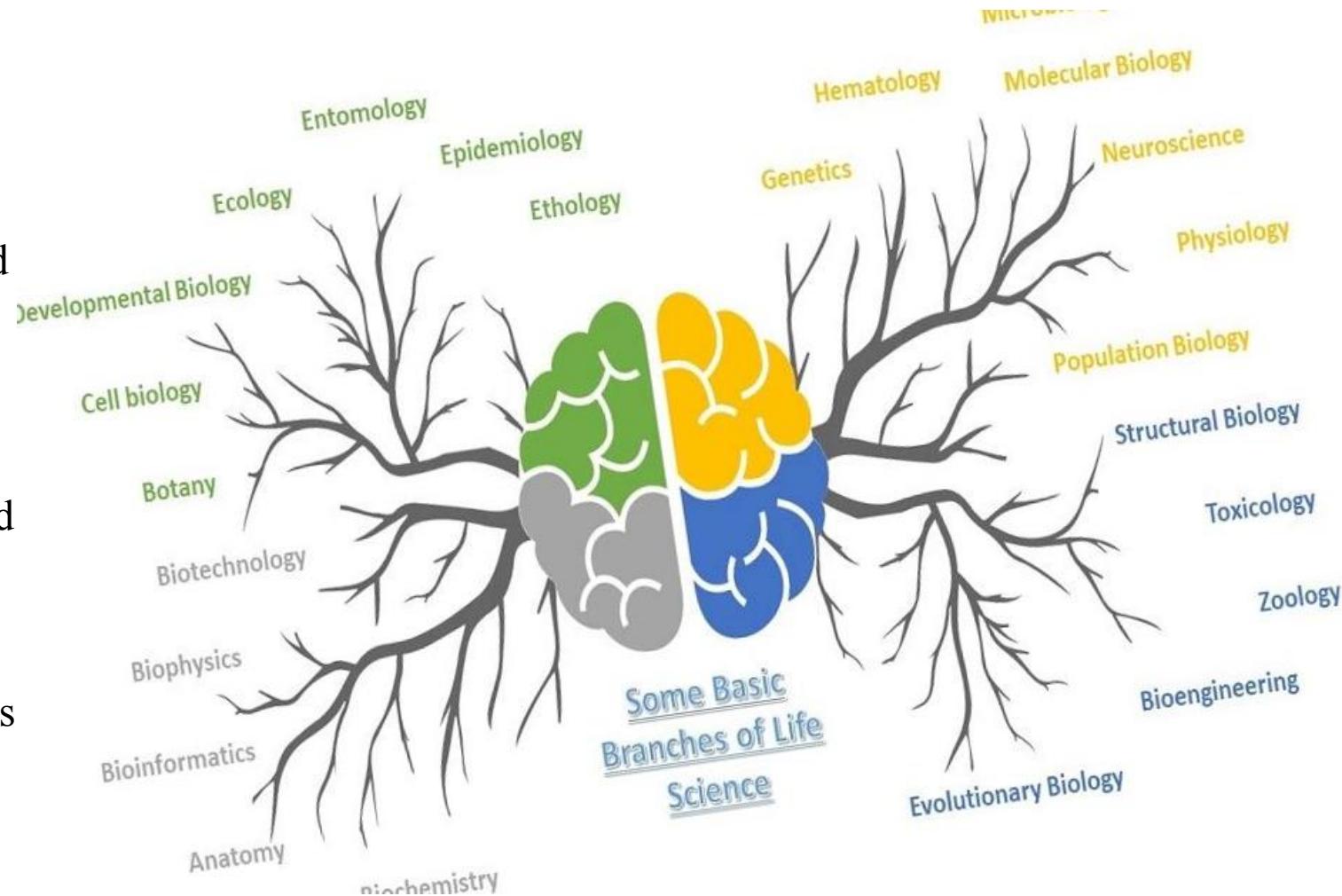
Chromosome Dynamics, Gene Regulation, Single-Molecule Imaging, Genetic Disorders, Cancer

Instructions

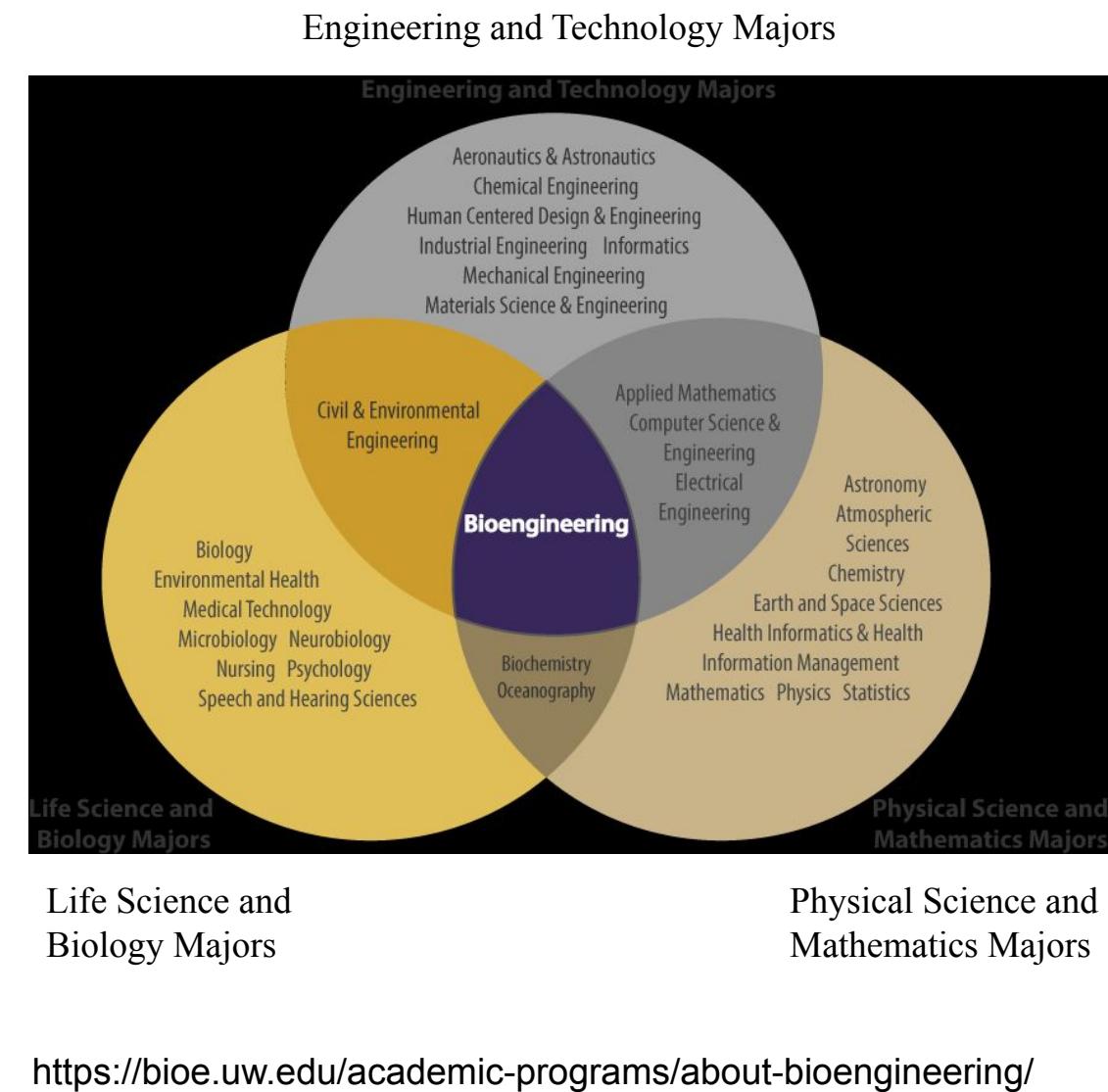
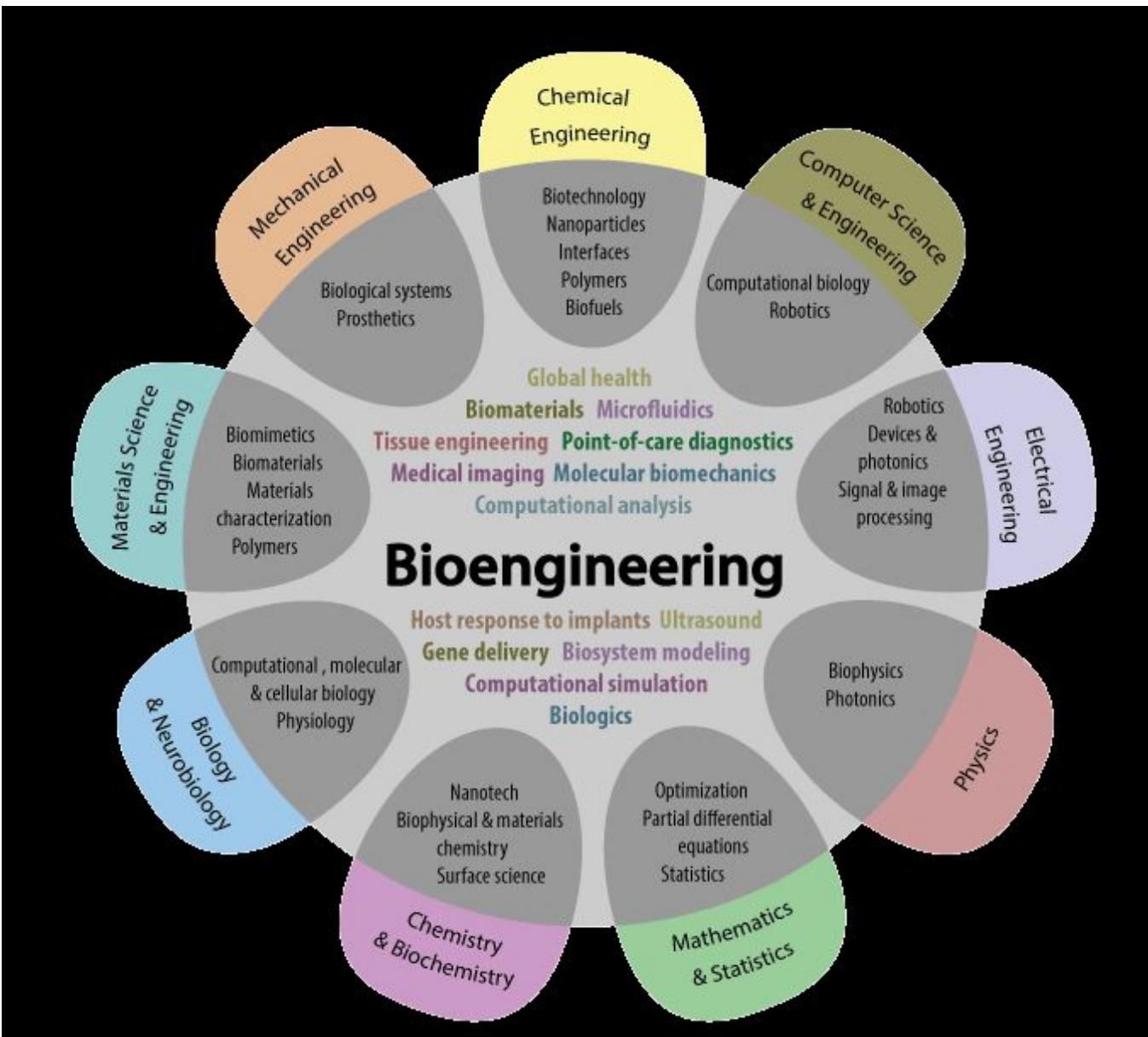
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Introduction to Life Sciences

- Life science studies life in all its forms, past and present.
- This can include plants, animals, viruses and bacteria, single-celled organisms, and even cells.
- Life sciences study the biology of how these organisms live, which is why you may hear this group of specialties referred to as biology.
- The simplest way to define life sciences is the study of living organisms and life processes.

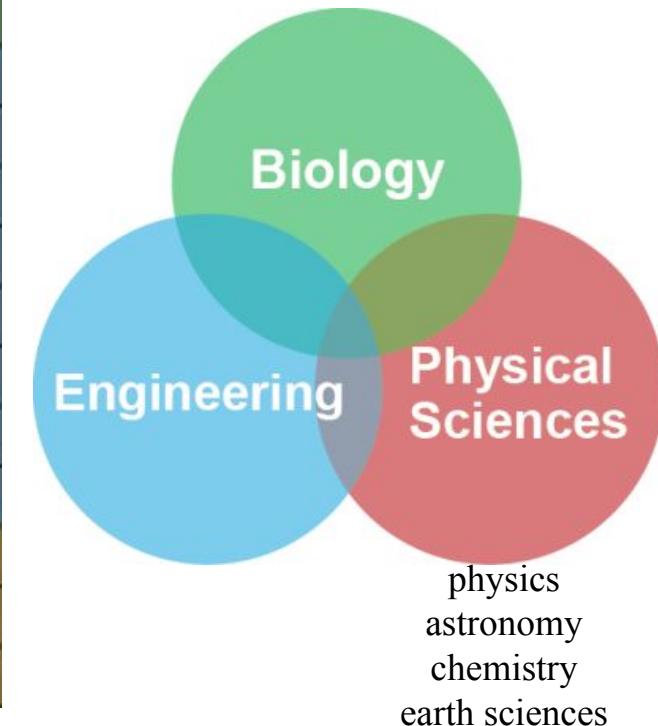


Why engineers need to study Biology/Life Sciences?

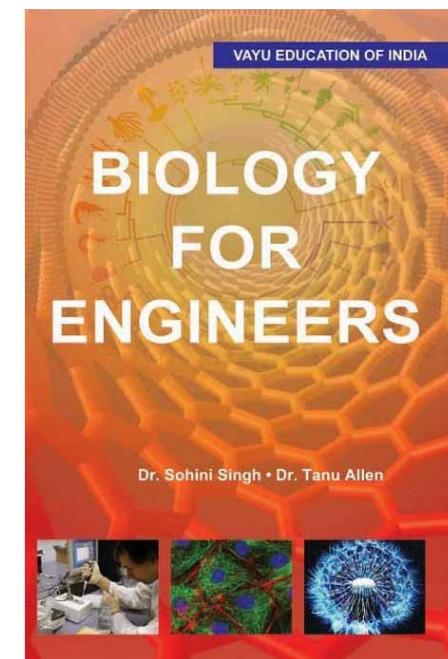
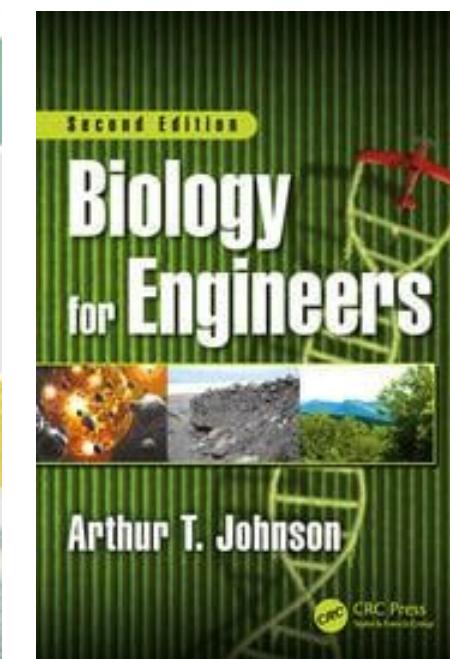
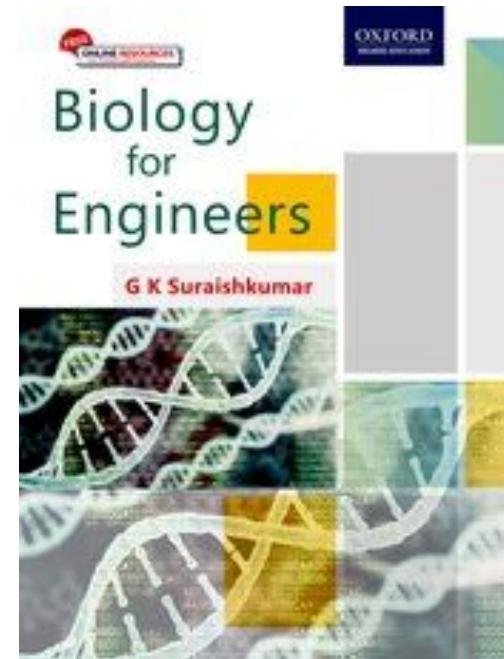
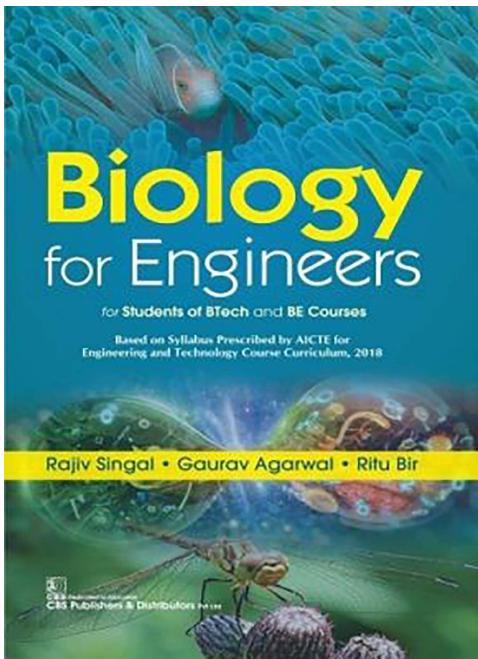
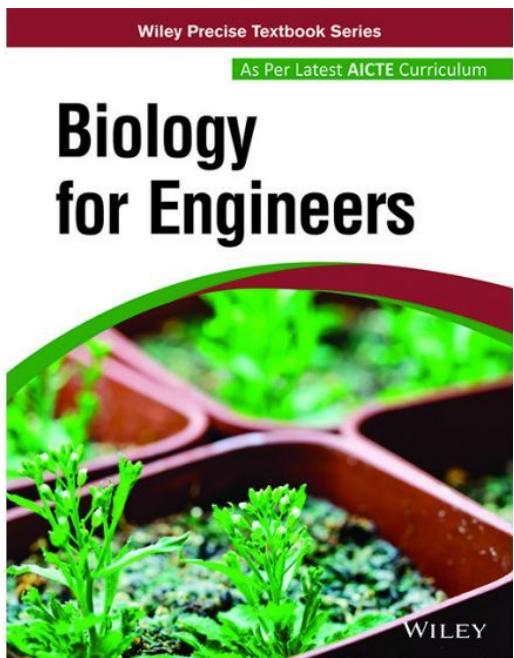


How Biology/Biomedical Engineering is linked to other disciplines of Engineering?

Biomedical Engineering	<i>Mechanical Engineering</i>	Biomechanics
		Biofluid
		Bionics
		Rehabilitation Engineering
		Orthopaedic Bioengineering
	<i>Electrical and Computer Engineering</i>	Biomedical Electronics
		Biomechatronics
		Bioinstrumentation
		Medical Imaging
		Systems Physiology
	<i>Biological, Chemical, Material Sciences</i>	Neural Engineering
		Bioinformatics
		Clinical Engineering
		Biomaterials
		Cellular and Tissue Engineering
		Genetic Engineering

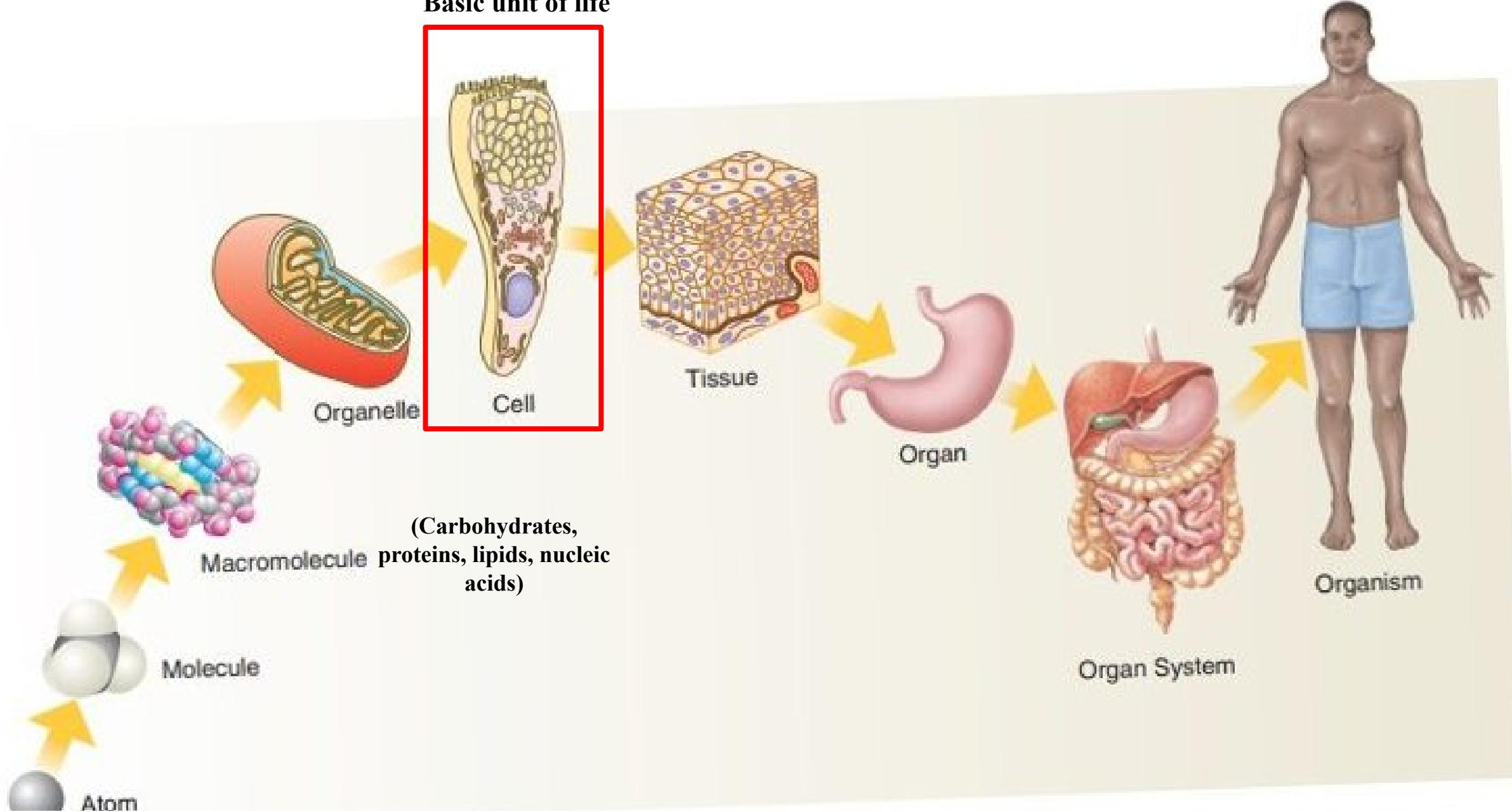


- So, Biology/Life Sciences is an important subject that you should study with full attention to succeed in your journey as an engineer!
- So many books on “Biology for Engineers”



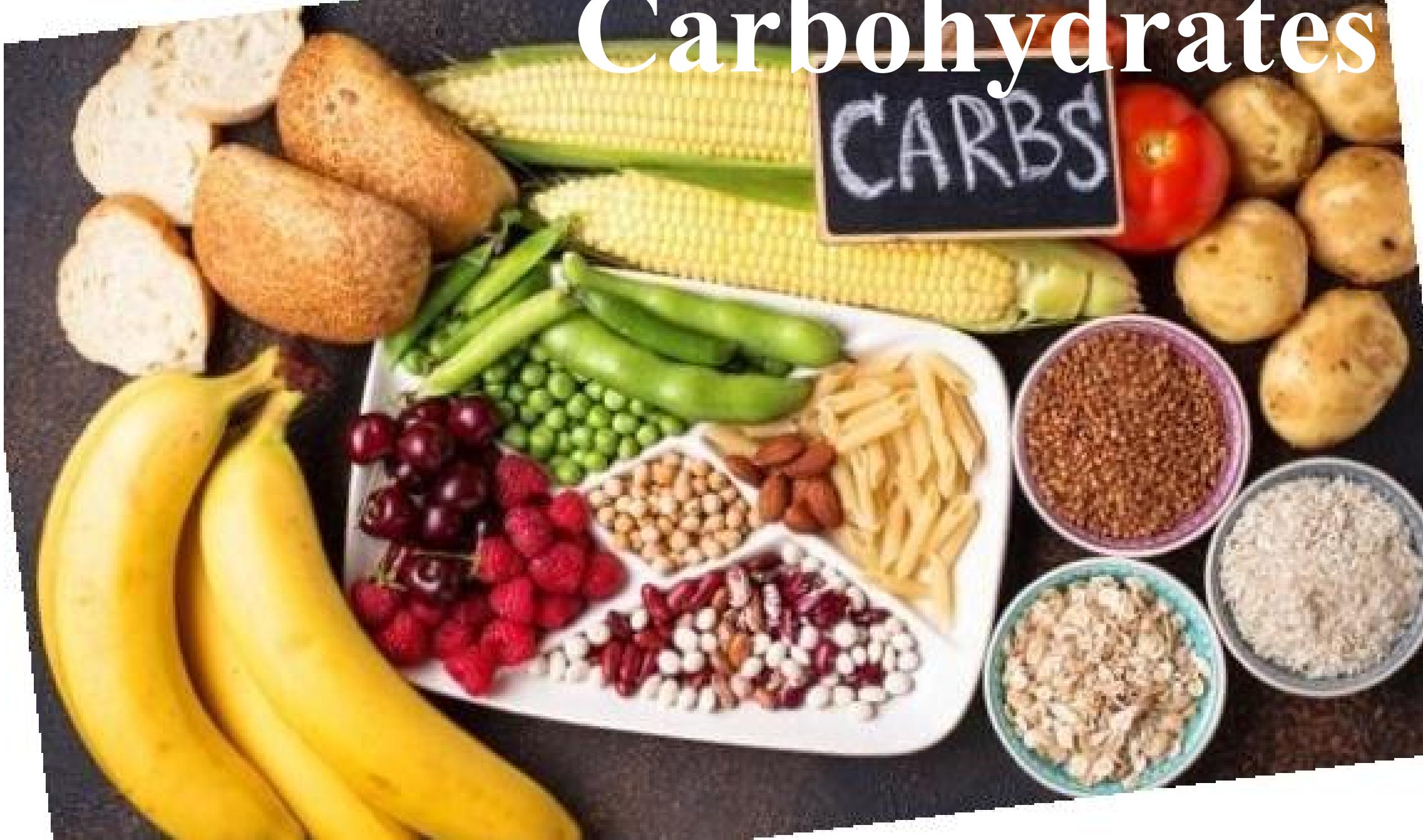
Organization level of the body

Basic unit of life



Carbohydrates

CARBS



What are carbohydrates?



NUTRITIONAL INFORMATION	
(TYPICAL VALUES PER 100ml)	
Energy	65kcal
Protein	0g
Carbohydrate	16.2g
Natural Fruit Sugars	2.5g
Added Sugar	13.3g
Fat	0g
Vitamin A	120mcg
Calcium	8mg

$$16.2 \text{ gm} \times 4 = 65 \text{ cal}$$



* Nutritional Information per 100g	
1. Energy	562 kcal
2. Carbohydrate	46.10 g
Sugar	0.25 g
3. Total Fat	38.12 g
Saturated Fatty Acids	16.50 g
Monounsaturated Fatty Acids	13.64 g
Polyunsaturated Fatty Acids	3.10 g
Trans Fatty Acids	0 g
4. Protein	8.68 g
5. Fiber	0.83 g
6. Sodium	615 mg
7. Cholesterol	0 mg

*These are approximate values

$$46.10 \text{ gm} \times 4 = 184.4 \text{ cal}$$

$$8.68 \text{ gm} \times 4 = 34.72 \text{ cal}$$

$$38.12 \text{ gm} \times 9 = 343.08 \text{ cal}$$

$$\text{Total} = 562 \text{ cal}$$

1 gm of carbohydrates provide 4 calories.

1 gm of proteins provide 4 calories

1 gm of fats/lipids provide 9 calories

Note: Nutritional calorie is the equivalent of the kilocalorie used by chemists and biochemists. So, 65 calorie diet is the equivalent of a 65 kcal diet.

What are carbohydrates?

- Carbohydrates, or carbs, are sugar molecules. Along with proteins and fats, carbohydrates are one of three main nutrients found in foods and drinks.
- Carbohydrates are an essential macronutrient the body requires in large amounts to run smoothly.
- Your body (digestive system) breaks down carbohydrates into glucose. Glucose, or [blood sugar](#), is the main source of energy for your body's cells, tissues, and organs. Glucose can be used immediately or stored in the liver and muscles for later use.

Which foods have carbohydrates?

- Grains, such as bread, noodles, pasta, crackers, cereals, and rice
- Fruits, such as apples, bananas, berries, mangoes, melons, and oranges
- Dairy products, such as milk and yogurt
- Legumes, including dried beans, lentils, and peas
- Snack foods and sweets, such as cakes, cookies, candy, and other desserts
- Juices, regular sodas, fruit drinks, sports drinks, and energy drinks that contain sugar
- Starchy vegetables, such as potatoes, corn, and peas

Some foods don't have a lot of carbohydrates, such as meat, fish, poultry, some types of cheese, nuts, and oils.

What are different types of carbohydrates?

- **Sugars:** simple carbohydrates. They can be added to foods, such as the sugar in candy, desserts, processed foods, and regular soda. They also include the kinds of sugar that are found naturally in fruits, vegetables, and milk.
- **Starches:** complex carbohydrates. Your body needs to break starches down into sugars to use them for energy. Starches include bread, cereal, and pasta. They also include certain vegetables, like potatoes, peas, and corn.
- **Fiber:** complex carbohydrate. Your body cannot break down most fibers, so eating foods with fiber can help you feel full and make you less likely to overeat. Diets high in fiber have other health benefits. They may help prevent stomach or intestinal problems, such as constipation. They may also help lower cholesterol and blood sugar. Fiber is found in many foods that come from plants, including fruits, vegetables, nuts, seeds, beans, and whole grains.

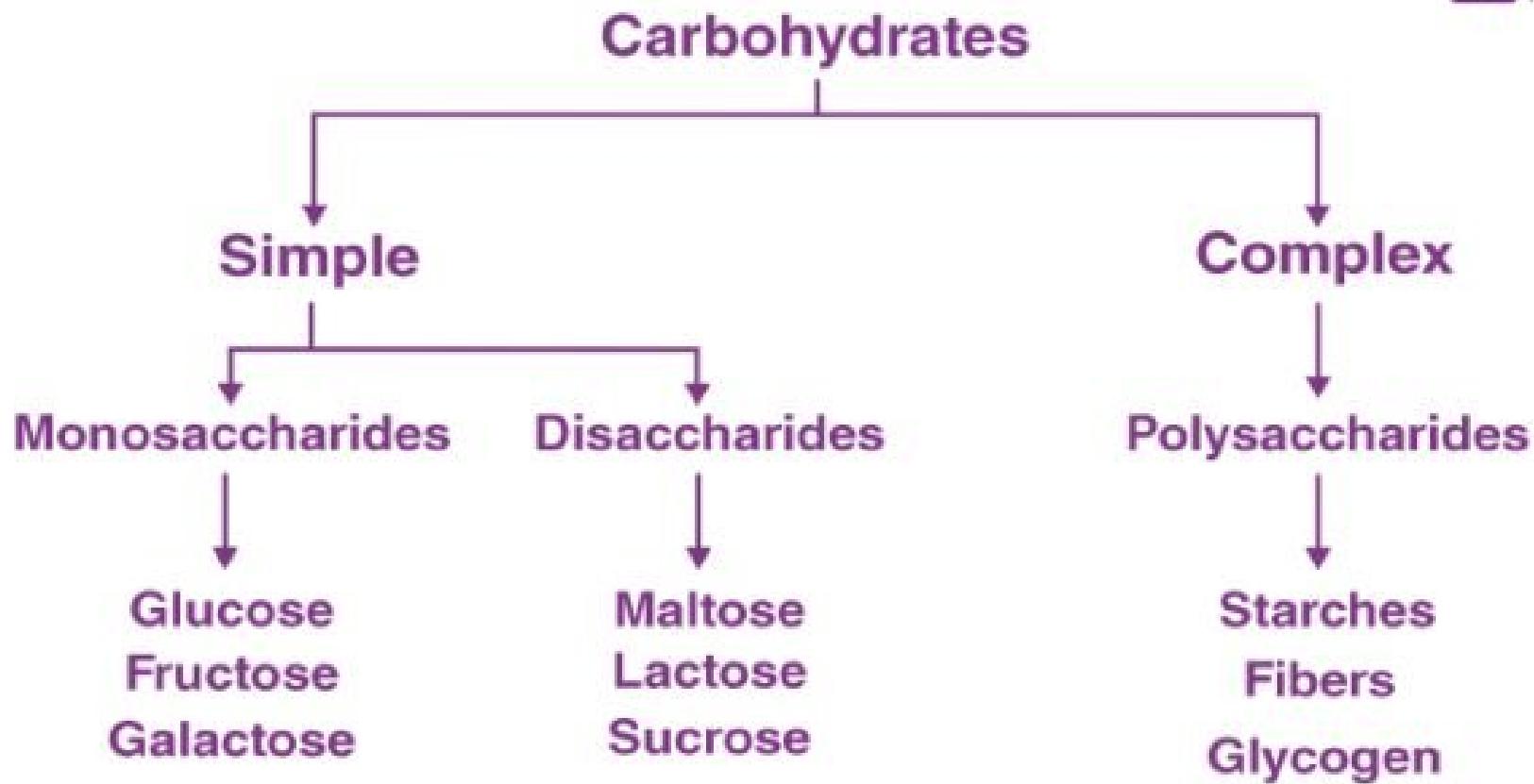
Which types of carbohydrates you should eat?

You do need to eat some carbohydrates to give your body energy. But it's important to eat the right kinds of carbohydrates for your health:

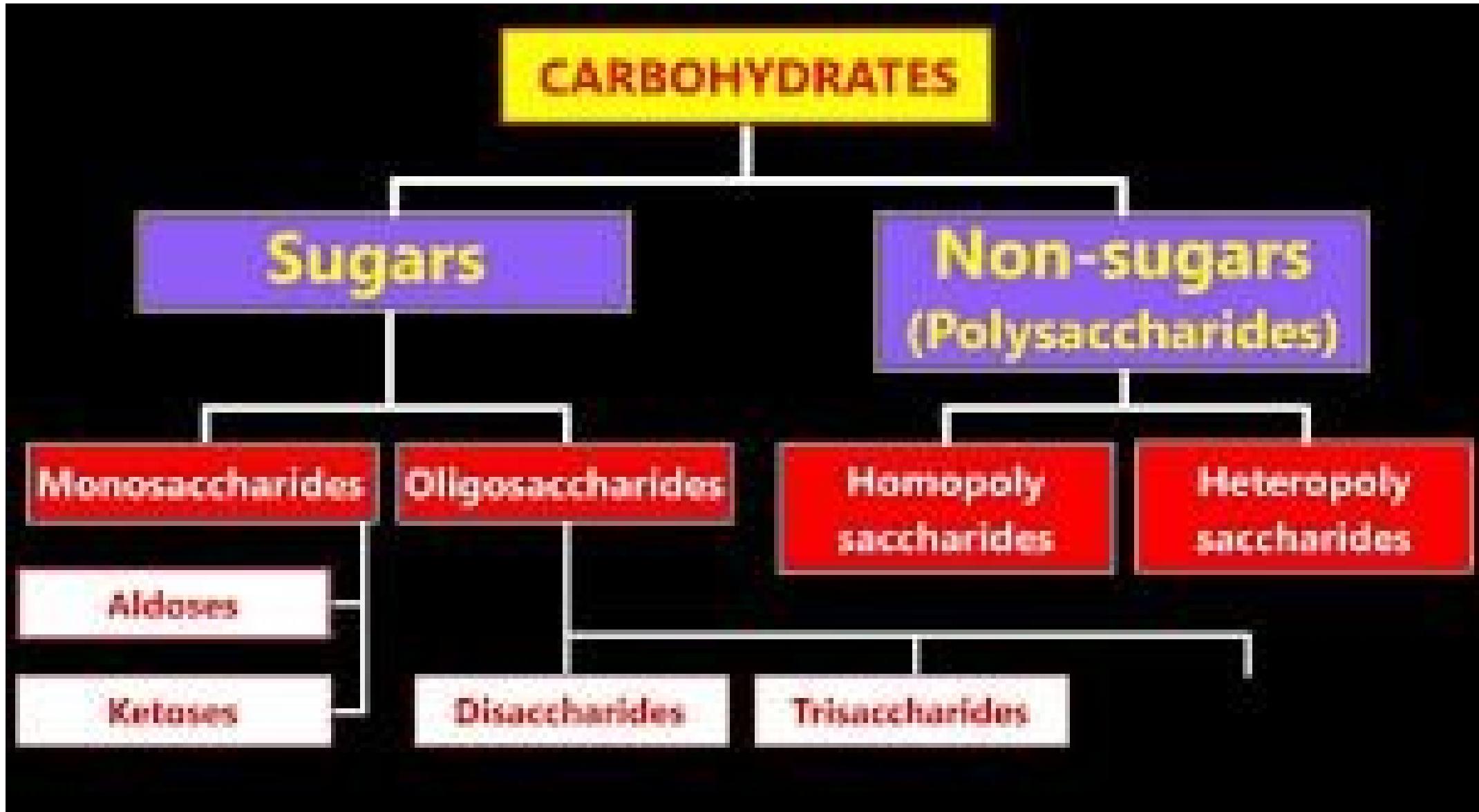
- When eating grains, choose mostly **whole grains and not refined grains**:
 - Whole grains are foods like whole wheat bread, brown rice, whole cornmeal, and oatmeal. They offer lots of nutrients that your body needs, like vitamins, minerals, and fiber.
 - Refined grains are foods that have had some parts of the grains removed. This also removes some of the nutrients that are good for your health.
- Eat foods with **lots of fiber**. The Nutrition Facts labels on the back of food packages tells you how much fiber a product has.
- Try to avoid foods that have a lot of **added sugar**. These foods can have many calories but not much nutrition. **Eating too much added sugar raises your blood sugar and can make you gain weight.**

Classification of carbohydrates (Types of carbohydrates)

Carbohydrates were named from their chemical composition: **carbon, hydrogen, and oxygen**.

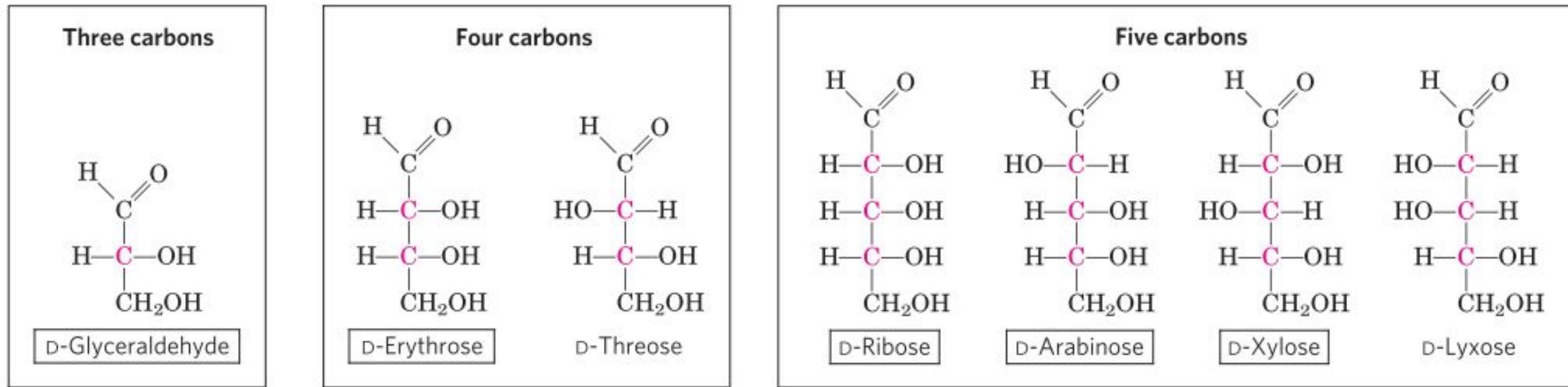


Classification of carbohydrates (Types of carbohydrates)

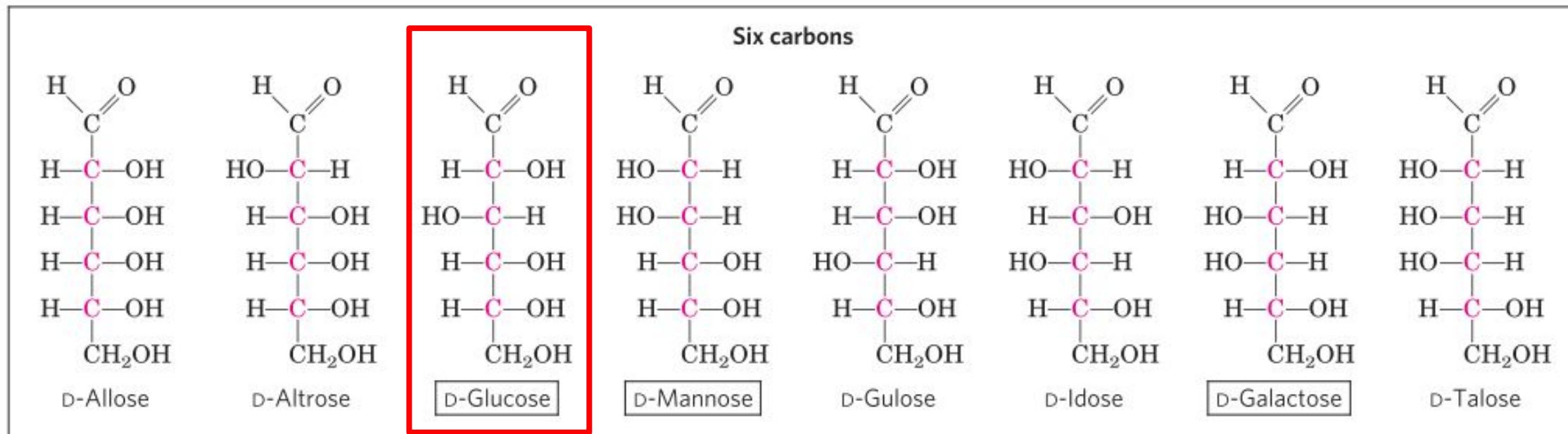


Structures of carbohydrates_Aldoses

(a) D-Aldoses



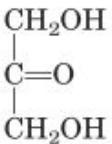
Chiral carbon atoms are shown in pink.



Structures of carbohydrates_Ketoses

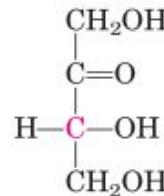
(b) D-Ketoses

Three carbons



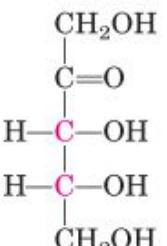
Dihydroxyacetone

Four carbons



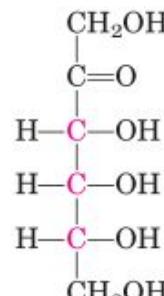
D-Erythrulose

Five carbons

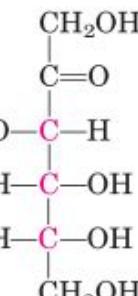


D-Ribulose

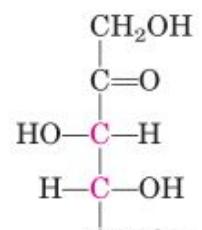
Six carbons



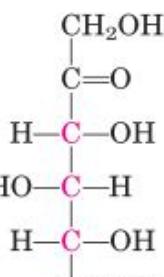
D-Psicose



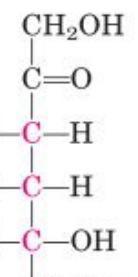
D-Fructose



D-Xylulose

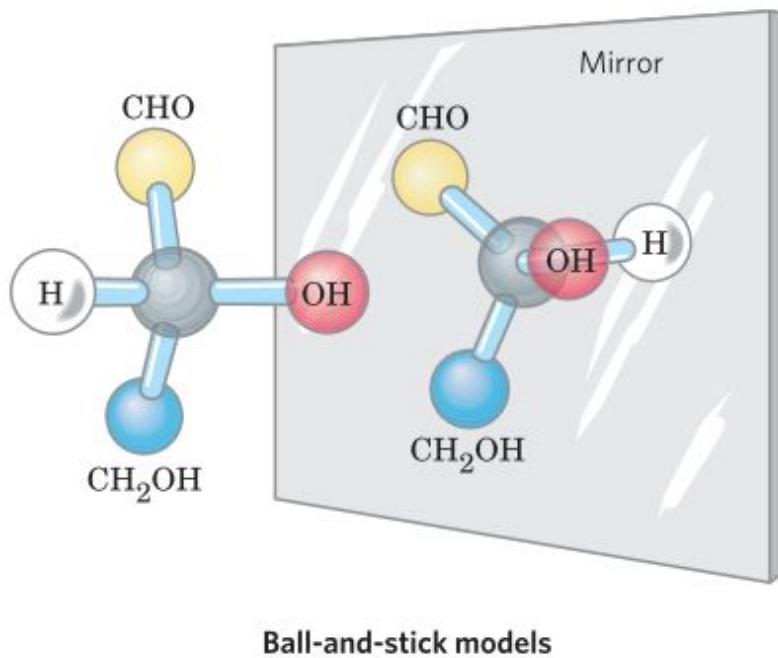


D-Sorbose

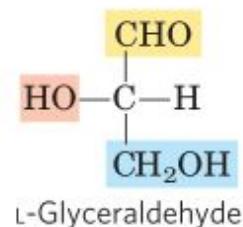
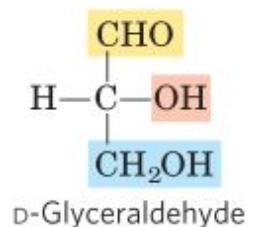


D-Tagatose

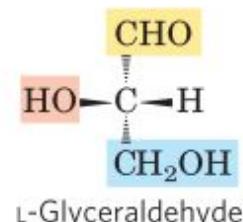
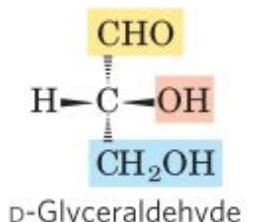
Stereoisomers of monosaccharides



Ball-and-stick models



Fischer projection formulas



Enantiomers are the mirror images of each other.

Enantiomers of Glyceraldehyde

A molecule with n chiral centers can have 2^n stereoisomers.

Glyceraldehyde has $2^1 = 2$,
Aldohexoses with 4 chiral carbons have $2^4 = 16$.

FIGURE 7–2 Three ways to represent the two enantiomers of glyceraldehyde. The enantiomers are mirror images of each other. Ball-and-stick models show the actual configuration of molecules. Recall (see Fig. 1–18) that in perspective formulas, the wide end of a solid wedge projects out of the plane of the paper, toward the reader; a dashed wedge extends behind.

Epimers of glucose

Monosaccharides generally contain several chiral carbons and exist in a variety of stereochemical forms.

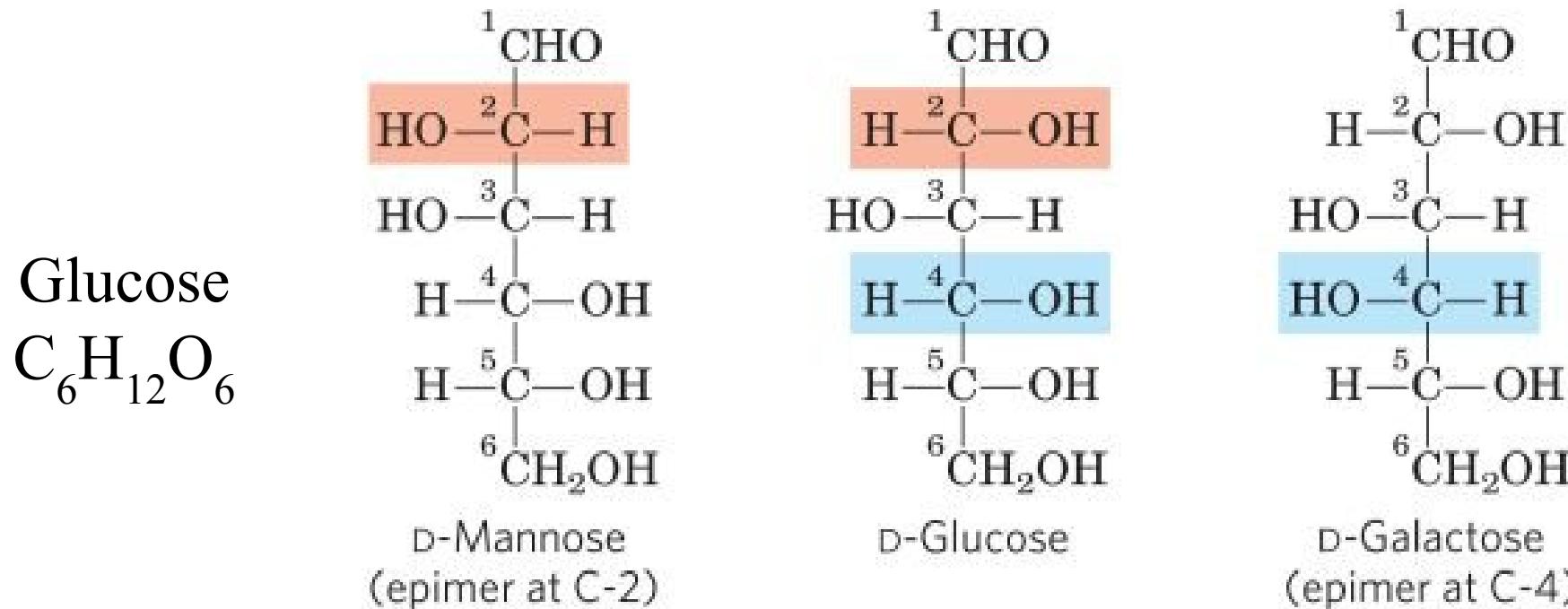
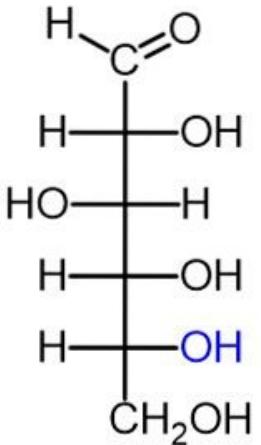


FIGURE 7-4 Epimers. D-Glucose and two of its epimers are shown as projection formulas. Each epimer differs from D-glucose in the configuration at one chiral center (shaded light red or blue).

Chiral carbon atoms are the carbon atoms that are attached to four substituents/groups.

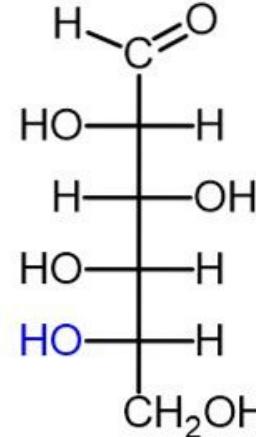
Enantiomer and Epimer of D-glucose



D-glucose

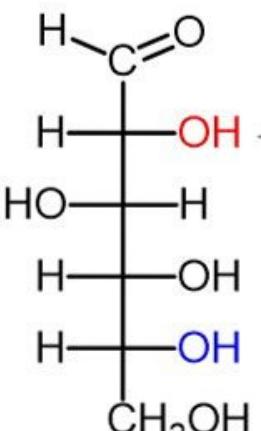


Enantiomers



L-glucose

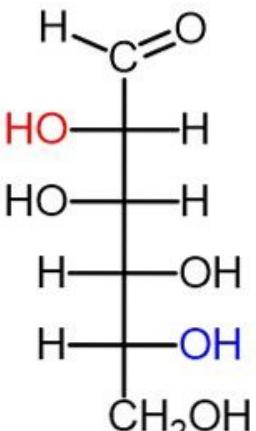
All the chiral centers have
different configuration



D-Glucose

Only one chiral
center with
different
configuration

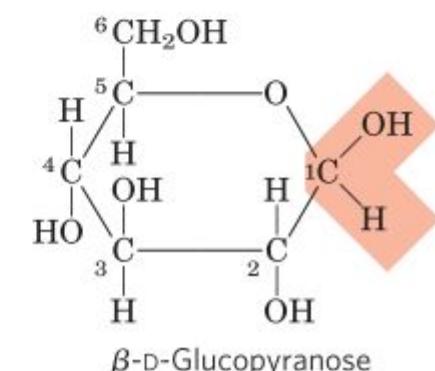
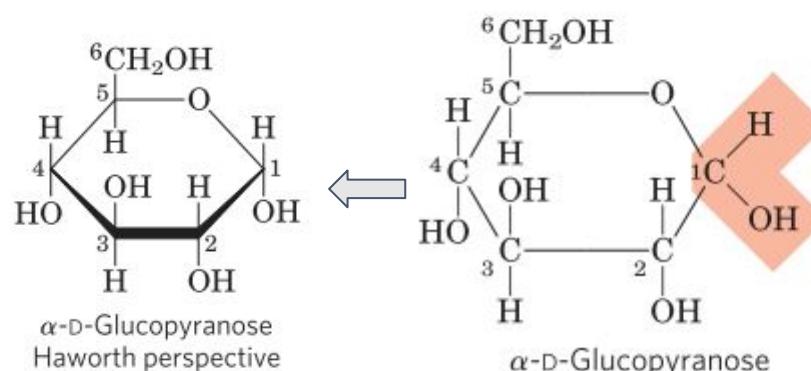
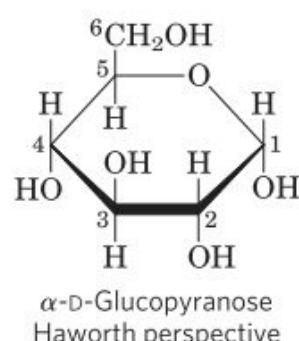
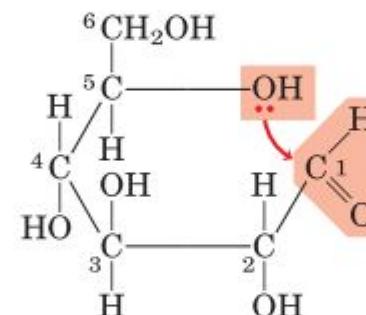
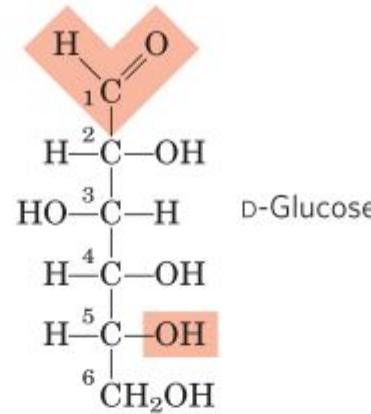
Epimers



D-Mannose

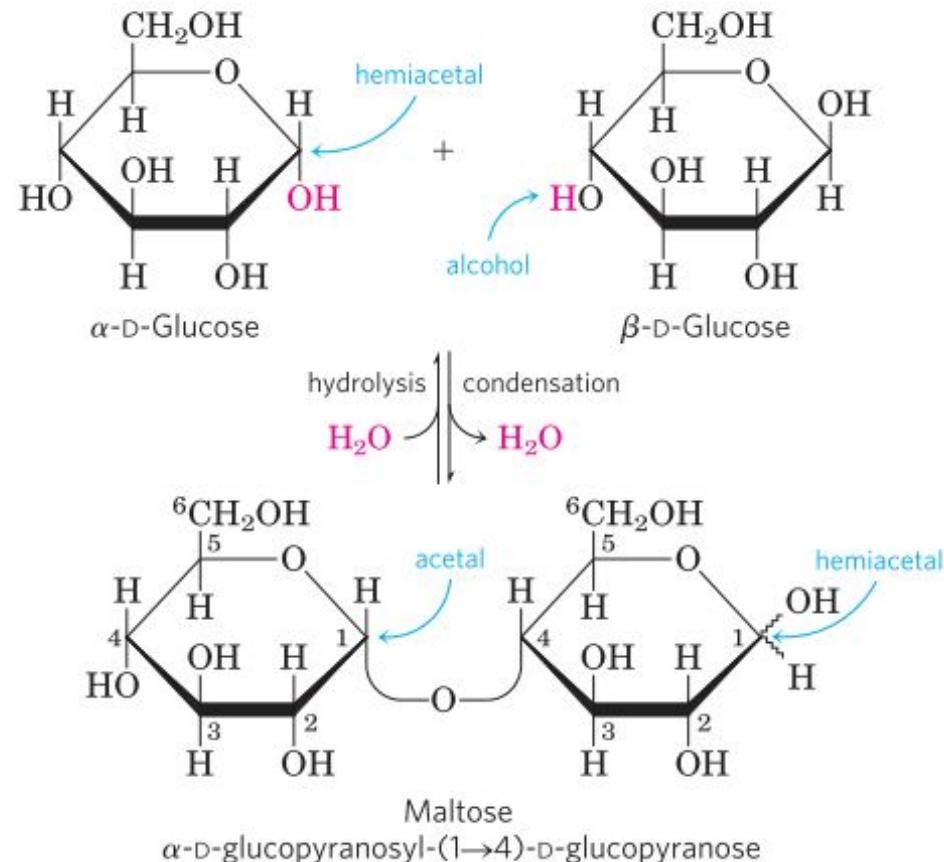
Cyclic (ring) structures of monosaccharides

- In aqueous solution, monosaccharides with 5 or more carbons occur predominantly as cyclic (ring) structures.
- Carbonyl group forms a covalent bond with the oxygen of a hydroxyl group along the chain.
- Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called **anomers**, and the carbonyl carbon is called the **anomeric carbon**.
- Mutarotation:** The interconversion of α - and β - anomers is called mutarotation. This is a spontaneous process.

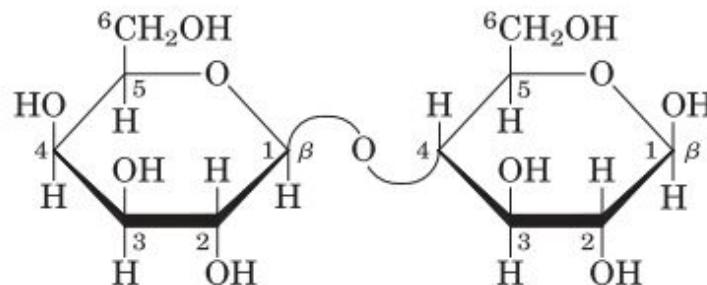


Disaccharides contain a glycosidic bond

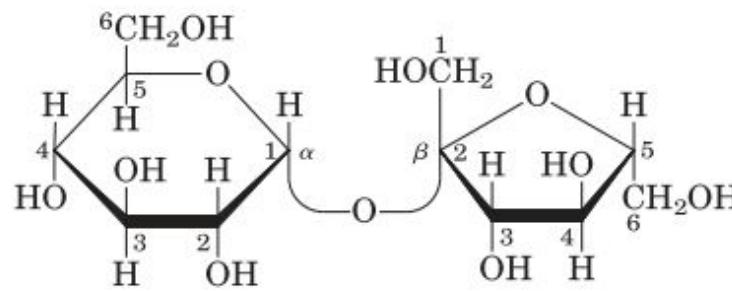
- Disaccharides (such as maltose, lactose and sucrose) consist of two monosaccharides joined covalently by an O-glycosidic bond, which is formed when a hydroxyl group of one sugar reacts with the anomeric carbon of the other.
- Anomeric carbon is involved in a glycosidic bond, hence the easy interconversion of linear and cyclic forms is prevented.



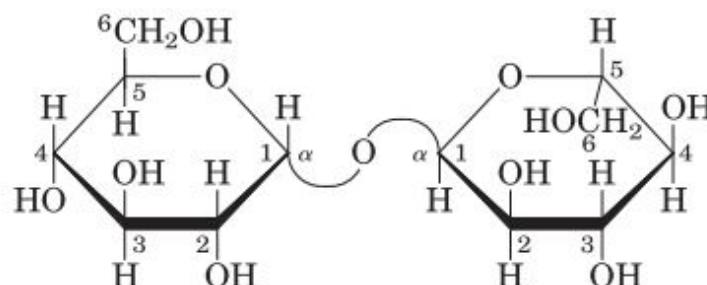
Disaccharides and their structures



Lactose (β form)
 β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-glucopyranose
Gal(β 1 \rightarrow 4)Glc



Sucrose
 β -D-fructofuranosyl α -D-glucopyranoside
Fru(2 β ↔α1)Glc ≡ Glc(α1↔2 β)Fru



Trehalose
 α -D-glucopyranosyl α -D-glucopyranoside
Glc(α1↔1α)Glc

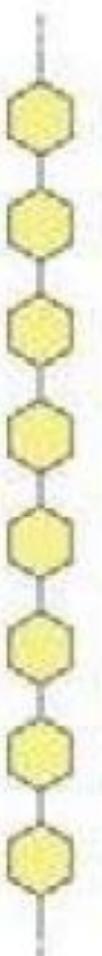
Polysaccharides

- Most carbohydrates found in nature occur as polysaccharides, polymer of medium to high molecular weight.
- Polysaccharides are also known as glycans.
- They serve as stored fuel (e.g. starch, glycogen) and as structural components of cell walls (cellulose, chitin, dextran) and extracellular matrix.
- Homopolysaccharides contain only a single monomeric species. e.g. starch, glycogen, cellulose, chitin, dextran.
- Heteropolysaccharides contain two or more different kinds. e.g. peptidoglycan,

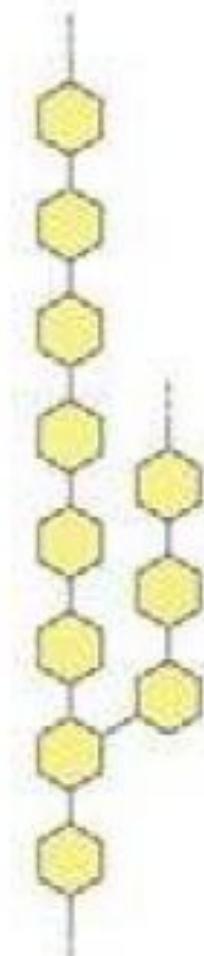
Homo- and Hetero- polysaccharides

Homopolysaccharides

Unbranched



Branched

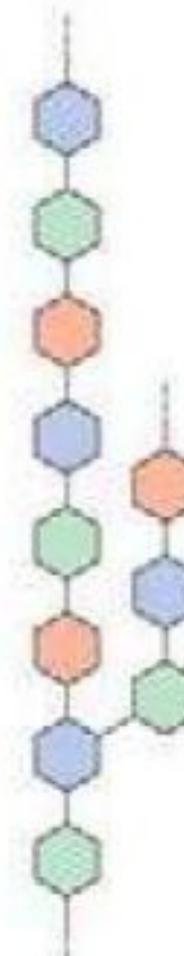


Heteropolysaccharides

Two monomer types, unbranched

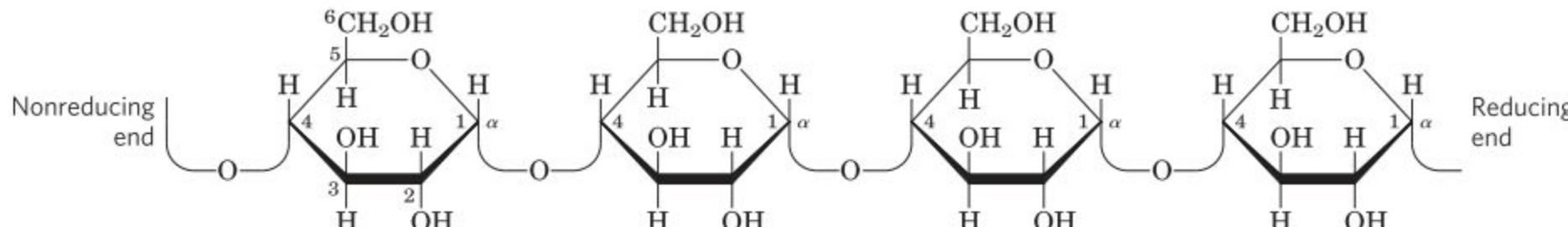


Multiple monomer types, branched



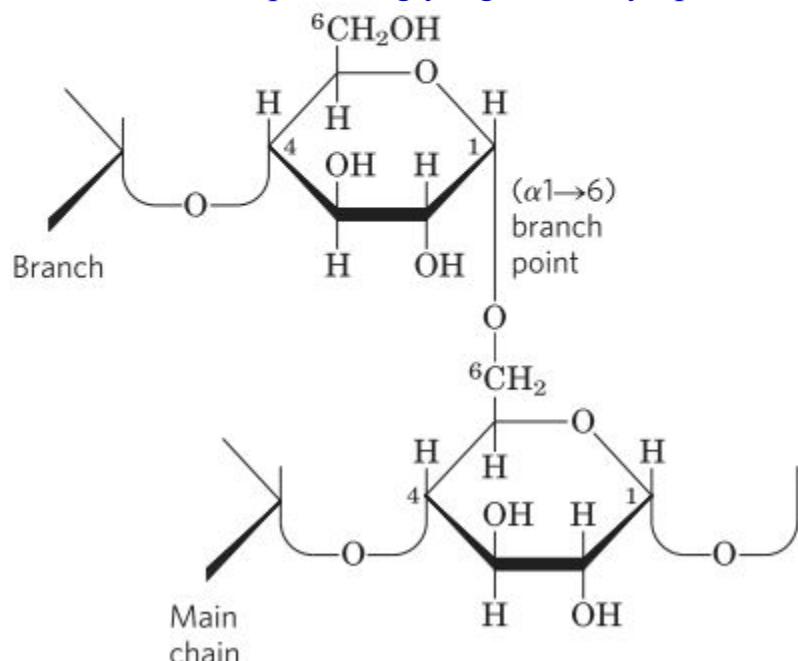
Homo- and Hetero- polysaccharides

A short segment of Amylose, a linear polymer of D-glucose residues in $\alpha 1 \rightarrow 4$ linkage.



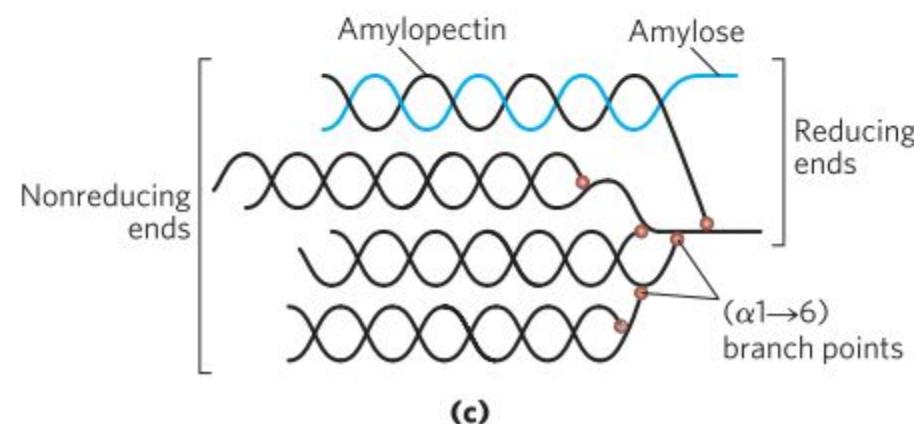
An $\alpha 1 \rightarrow 6$ branch point of glycogen or amylopectin.

(a) Amylose



(b)

A cluster of amylose and amylopectin in starch granules.

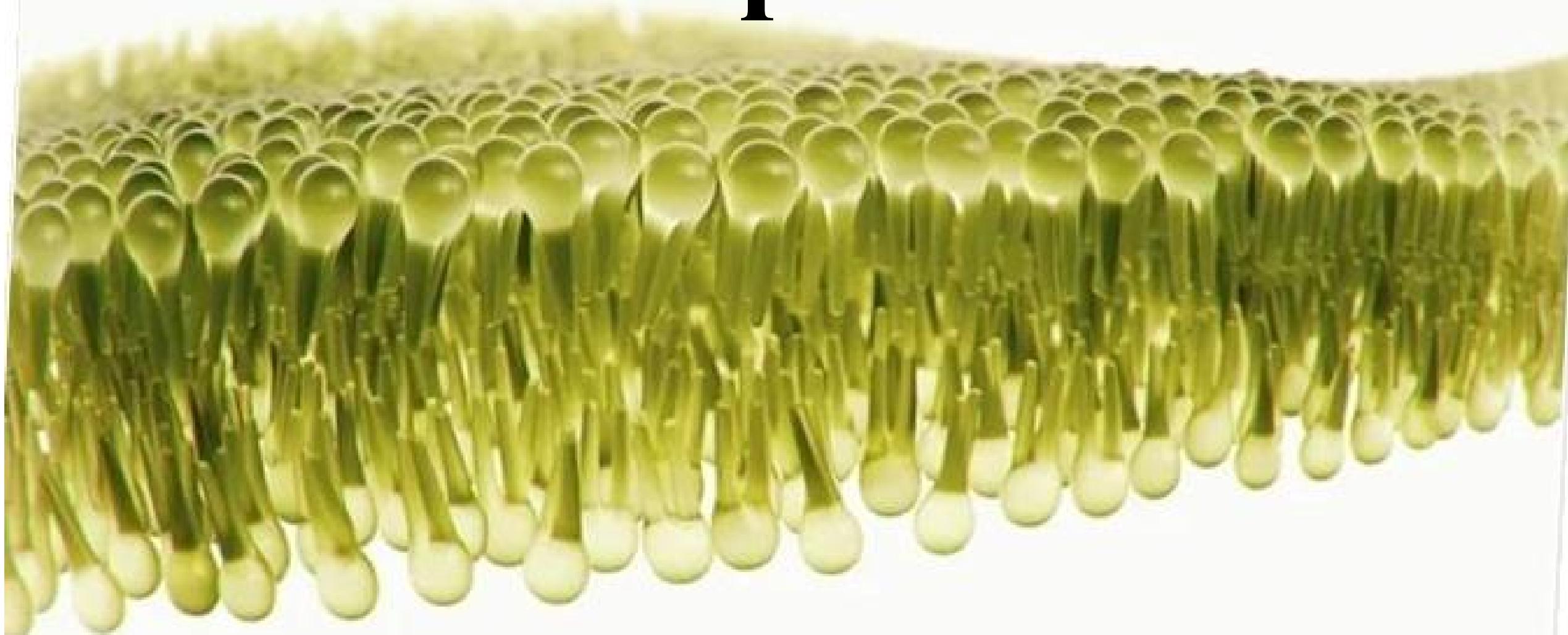


(c)

TABLE 7–2 Structures and Roles of Some Polysaccharides

Polymer	Type*	Repeating unit [†]	Size (number of monosaccharide units)	Roles/significance
Starch				Energy storage: in plants
Amylose	Homo-	(α 1→4)Glc, linear	50–5,000	
Amylopectin	Homo-	(α 1→4)Glc, with (α 1→6)Glc branches every 24–30 residues	Up to 10^6	
Glycogen	Homo-	(α 1→4)Glc, with (α 1→6)Glc branches every 8–12 residues	Up to 50,000	Energy storage: in bacteria and animal cells
Cellulose	Homo-	(β 1→4)Glc	Up to 15,000	Structural: in plants, gives rigidity and strength to cell walls
Chitin	Homo-	(β 1→4)GlcNAc	Very large	Structural: in insects, spiders, crustaceans, gives rigidity and strength to exoskeletons
Dextran	Homo-	(α 1→6)Glc, with (α 1→3) branches	Wide range	Structural: in bacteria, extracellular adhesive
Peptidoglycan	Hetero-; peptides attached	4)Mur2Ac(β 1→4) GlcNAc(β 1	Very large	Structural: in bacteria, gives rigidity and strength to cell envelope
Agarose	Hetero-	3) D -Gal(β 1→4)3,6-anhydro-L-Gal(α 1	1,000	Structural: in algae, cell wall material
Hyaluronan (a glycosaminoglycan)	Hetero-; acidic	4)GlcA(β 1→3) GlcNAc(β 1	Up to 100,000	Structural: in vertebrates, extracellular matrix of skin and connective tissue; viscosity and lubrication in joints

Lipids



What are Lipids?

- Biological lipids are chemically diverse group of compounds, which are insoluble in water.
- Fats and oils are the stored form of energy.
- Phospholipids and sterols are major structural elements of biological membranes.
- Other lipids are hormones, enzyme cofactors, light absorbing pigments, electron carriers, intracellular messengers.

Common types of storage and membrane lipids

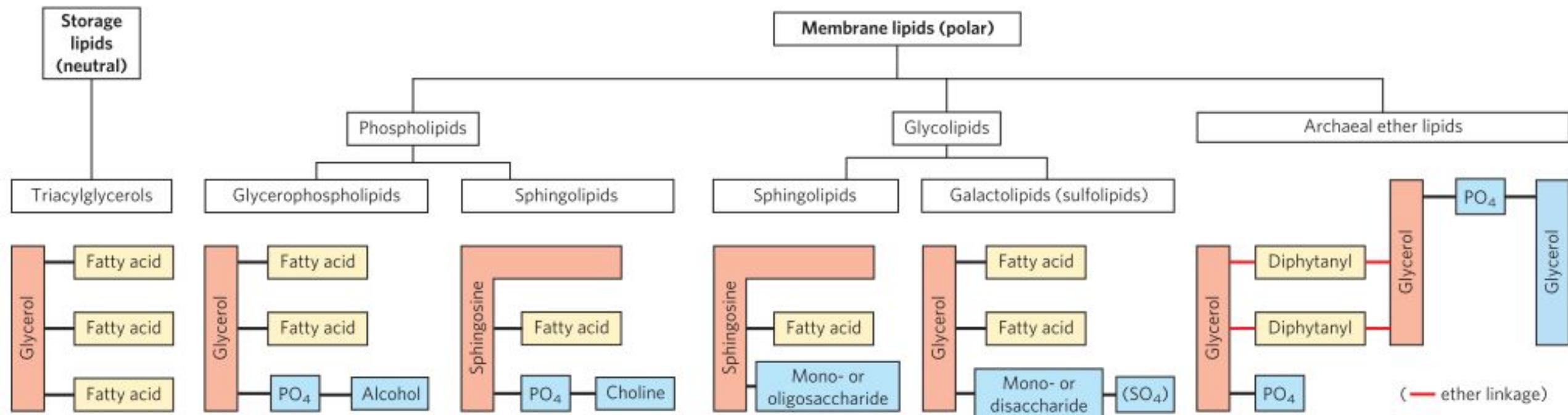
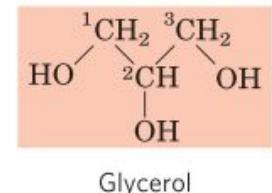


TABLE 10-3 Eight Major Categories of Biological Lipids

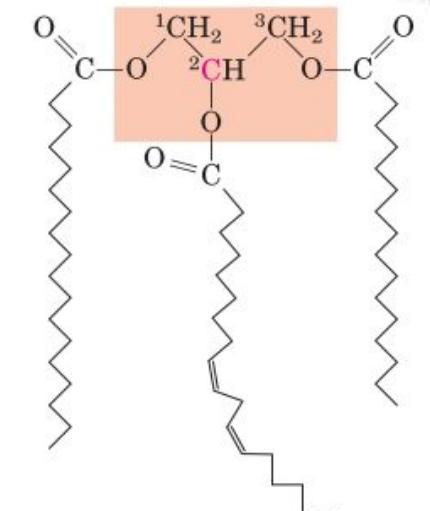
Category	Category code	Examples
Fatty acids	FA	Oleate, stearoyl-CoA, palmitoylcarnitine
Glycerolipids	GL	Di- and triacylglycerols
Glycerophospholipids	GP	Phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine
Sphingolipids	SP	Sphingomyelin, ganglioside GM2
Sterol lipids	ST	Cholesterol, progesterone, bile acids
Prenol lipids	PR	Farnesol, geraniol, retinol, ubiquinone
Saccharolipids	SL	Lipopolysaccharide
Polyketides	PK	Tetracycline, erythromycin, aflatoxin B ₁

Triacylglycerols are fatty acid esters of glycerol and they serve as stored energy

- The simplest lipids constructed from fatty acids are the triacylglycerols, also referred to as triglycerides, fats or neutral fats.
- Triacylglycerols are composed of three fatty acids each in ester linkage with a single glycerol.
- Those containing same fatty acids in all three positions are called simple triacylglycerols.
16:0, 18:0 and 18:1 are tripalmitin, tristearin and triolein.
- Triacylglycerols provide stored energy and they are stored in adipocytes (fat cells). They are also stored as oils in the seed of many plants, provides energy during seed germination.
- Humans have fat tissue (adipocytes) under the skin, in the abdominal cavity and in the mammary glands. Moderately obese people with 15-20 kg of triacylglycerols deposited in their adipocytes could meet their energy needs for months. In contrast, the human body can store less than a day's energy supply in the form of glycogen.



Glycerol



1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol,
a mixed triacylglycerol

Advantages of storing energy as triacylglycerols compared to polysaccharides

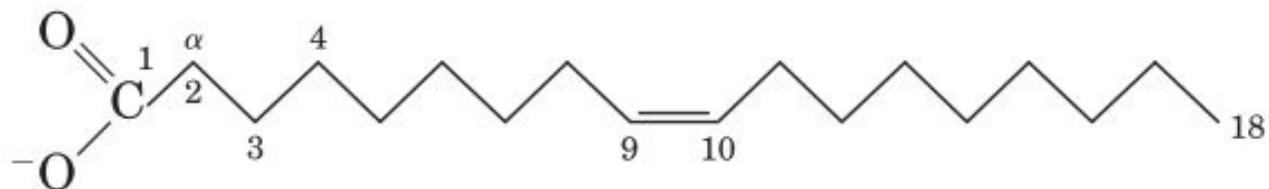
- 1) Oxidation of triacylglycerols provides more than twice the energy compared to polysaccharides:
 - 1 gm of carbohydrates provides 4 calories.
 - 1 gm of triacylglycerols provides 9 calories.
- 2) As triacylglycerols are hydrophobic (unhydrated), the organism that carries fat as fuel doesn't have to carry the extra weight of water of hydration that is associated with stored polysaccharides (2 g per gram of polysaccharide).

Storage Lipids

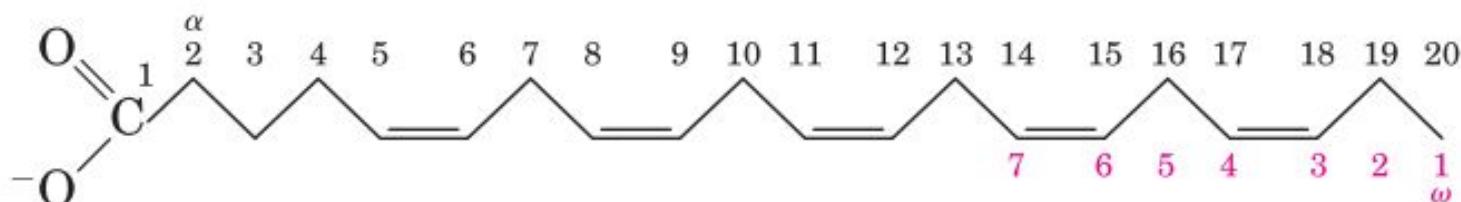
- The fats and oils used as stored forms of energy are derivatives of fatty acids.
- Fatty acids are hydrocarbon derivatives. They are carboxylic acids with hydrocarbon chains ranging from 4 to 36 carbons long (C4 to C36).
- In some fatty acids, this chain is unbranched and fully saturated (no double bonds).

16-carbon saturated palmitic acid is abbreviated 16:0

18-carbon oleic acid, with one double bond is abbreviated 18:1.



(a) 18:1(Δ^9) *cis*-9-Octadecenoic acid



(b) 20:5($\Delta^{5,8,11,14,17}$) Eicosapentaenoic acid (EPA),
an omega-3 fatty acid

PUFA: Polyunsaturated fatty acid

KEY CONVENTION: The family of **polyunsaturated fatty acids (PUFAs)** with a double bond between the third and fourth carbon from the methyl end of the chain are of special importance in human nutrition. Because the physiological role of PUFAs is related more to the position of the first double bond near the *methyl* end of the chain than to the carboxyl end, an alternative nomenclature is sometimes used for these fatty acids. The carbon of the methyl group—that is, the carbon most distant from the carboxyl group—is called the *ω* (omega) carbon and is given the number 1 (Fig. 10-1b). In this convention, PUFAs with a double bond between C-3 and C-4 are called **omega-3 (ω-3) fatty acids**, and those with a double bond between C-6 and C-7 are **omega-6 (ω-6) fatty acids**. ■

TABLE 10-1 Some Naturally Occurring Fatty Acids: Structure, Properties, and Nomenclature

Carbon skeleton	Structure*	Systematic name [†]	Common name (derivation)	Melting point (°C)	Solubility at 30 °C (mg/g solvent)	
					Water	Benzene
12:0	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	<i>n</i> -Dodecanoic acid	Lauric acid (Latin <i>laurus</i> , "laurel plant")	44.2	0.063	2,600
14:0	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	<i>n</i> -Tetradecanoic acid	Myristic acid (Latin <i>Myristica</i> , nutmeg genus)	53.9	0.024	874
16:0	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	<i>n</i> -Hexadecanoic acid	Palmitic acid (Latin <i>palma</i> , "palm tree")	63.1	0.0083	348
18:0	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	<i>n</i> -Octadecanoic acid	Stearic acid (Greek <i>stear</i> , "hard fat")	69.6	0.0034	124
20:0	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$	<i>n</i> -Eicosanoic acid	Arachidic acid (Latin <i>Arachis</i> , legume genus)	76.5		
24:0	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$	<i>n</i> -Tetracosanoic acid	Lignoceric acid (Latin <i>lignum</i> , "wood" + <i>cera</i> , "wax")	86.0		
16:1(Δ^9)	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis</i> -9-Hexadecenoic acid	Palmitoleic acid	1 to -0.5		
18:1(Δ^9)	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis</i> -9-Octadecenoic acid	Oleic acid (Latin <i>oleum</i> , "oil")	13.4		
18:2($\Delta^{9,12}$)	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis,cis</i> -9,12-Octadecadienoic acid	Linoleic acid (Greek <i>linon</i> , "flax")	1–5		
18:3($\Delta^{9,12,15}$)	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis,cis,cis</i> -9,12,15-Octadecatrienoic acid	α -Linolenic acid	-11		
20:4($\Delta^{5,8,11,14}$)	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}$	<i>cis,cis,cis</i> , <i>cis</i> -5,8,11,14-Icosatetraenoic acid	Arachidonic acid	-49.5		

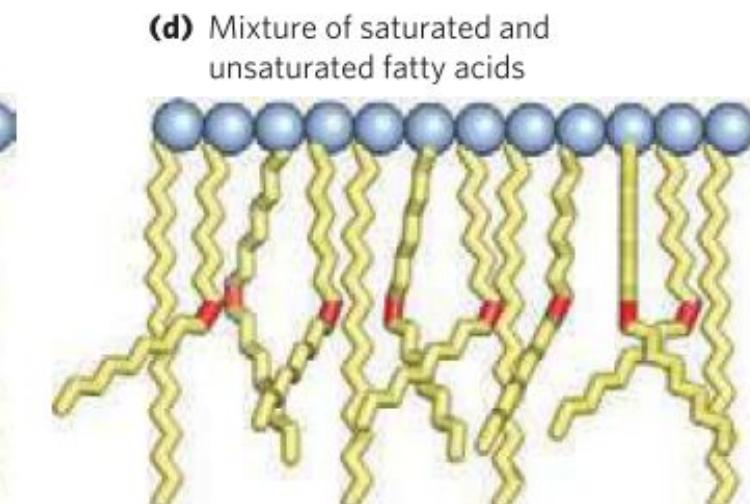
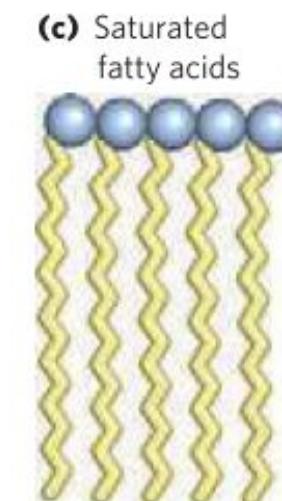
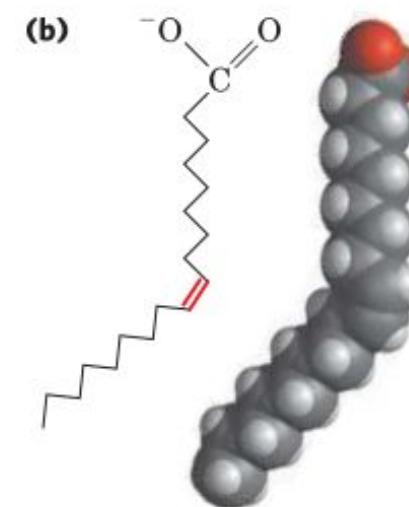
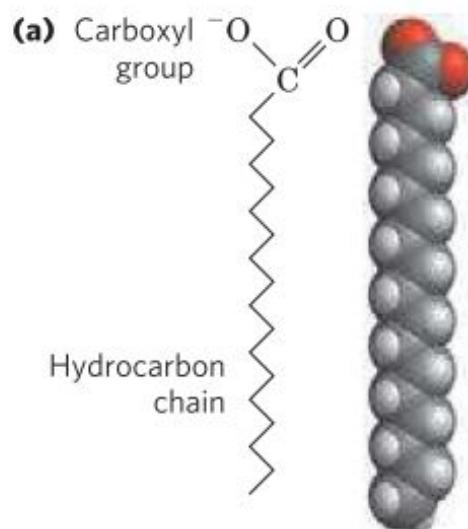
Why omega-3 PUFA are required in your diet?



Humans require but do not have the enzymatic capacity to synthesize the omega-3 PUFA α -linolenic acid (ALA; 18:3($\Delta^{9,12,15}$), in the standard convention), and must therefore obtain it in the diet. From ALA, humans can synthesize two other omega-3 PUFAs important in cellular function: eicosapentaenoic acid (EPA; 20:5($\Delta^{5,8,11,14,17}$), shown in Fig. 10–1b) and docosahexaenoic acid (DHA; 22:6($\Delta^{4,7,10,13,16,19}$)). An imbalance of omega-6 and omega-3 PUFAs in the diet is associated with an increased risk of cardiovascular disease. The optimal dietary ratio of omega-6 to omega-3 PUFAs is between 1:1 and 4:1, but the ratio in the diets of most North Americans is closer to 10:1 to 30:1. The “Mediterranean diet,” which has been associated with lowered cardiovascular risk, is richer in omega-3 PUFAs, obtained in leafy vegetables (salads) and fish oils. The latter oils are especially rich in EPA and DHA, and fish oil supplements are often prescribed for individuals with a history of cardiovascular disease. ■

Physical properties of the fatty acids

- The physical properties of the fatty acids are determined by the length and degree of unsaturation of the hydrocarbon chain.
- The nonpolar hydrocarbon chain accounts for the poor solubility of fatty acids in water.
- **The longer the fatty acyl chain and the fewer the double bonds, the lower is the solubility in water.**
- The carboxylic acid group is polar and accounts for the slight solubility of short-chain fatty acids in water.
- Melting points are also influenced by the length and degree of unsaturation of hydrocarbon chain.
- At room temperature, the saturated fatty acids from 12:0 to 24:0 have a waxy consistency, whereas unsaturated fatty acids are oily liquids.
- In unsaturated fatty acids, the double bond forces a kink in the hydrocarbon chain, hence they cannot pack together as tightly as fully saturated fatty acids.



Partial Hydrogenation of cooking oils produces trans fatty acids

- When oily foods are exposed too long to the oxygen in air, they may spoil and become **rancid**.
- The unpleasant smell and taste associated with rancidity result from the oxidative cleavage of double bonds in unsaturated fatty acids, which produces aldehydes and carboxylic acids of shorter chain length.
- To improve the shelflife of vegetable oils used in cooking, partial hydrogenation is used which converts cis double bonds of fatty acids to single bonds.
- Partial hydrogenation has another undesirable effect: some cis double bonds are converted to **trans double bonds**.
- Dietary intake of trans fats leads to higher incidences of cardiovascular disease.
- Dietary trans fats raise the level of triacylglycerols and of LDL (“bad”) cholesterol in the blood and lower the level of HDL (“good”) cholesterol.
- Many fast foods are deep-fried in partially hydrogenated vegetable oils and therefore contain high levels of trans fatty acids. Several countries have restricted the use of partially hydrogenated oils in restaurants.

TABLE 10–2 Trans Fatty Acids in Some Typical Fast Foods and Snacks

	Trans fatty acid content	
	In a typical serving (g)	As % of total fatty acids
French fries	4.7–6.1	28–36
Breaded fish burger	5.6	28
Breaded chicken nuggets	5.0	25
Pizza	1.1	9
Corn tortilla chips	1.6	22
Doughnut	2.7	25
Muffin	0.7	14
Chocolate bar	0.2	2

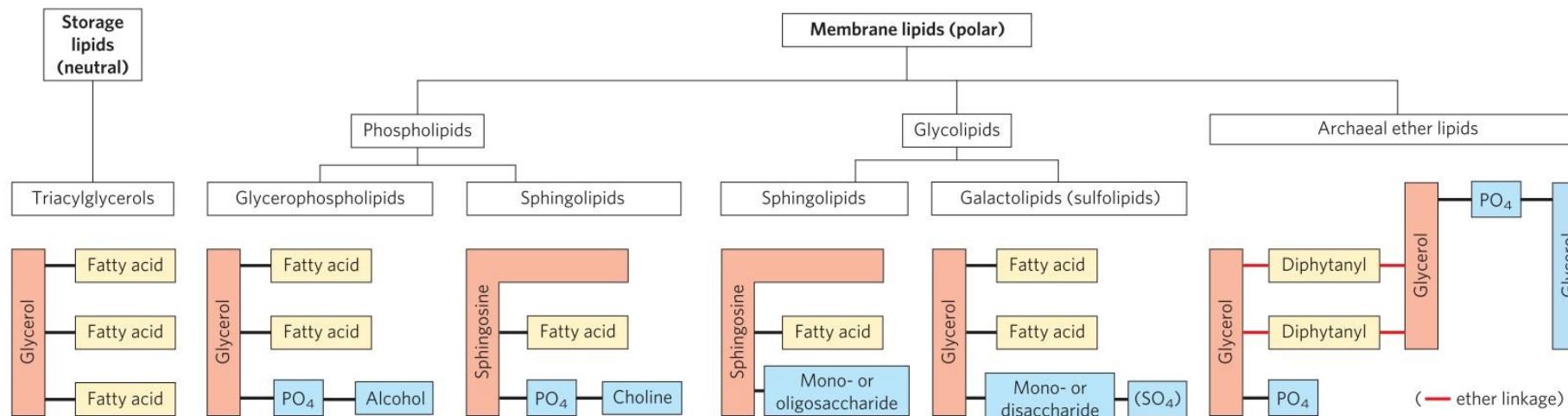
Structural Lipids in Membranes

Biological membranes (cell membrane, nuclear membrane) are made up of lipid bilayers.

Membrane lipids are amphipathic: one end is hydrophobic and the other end is hydrophilic.

Five types of membrane lipids:

- 1) glycerophospholipids: two fatty acids joined to glycerol.
- 2) galactolipids and sulfolipids: two fatty acids esterified to glycerol, but lack the phosphate of phospholipids.
- 3) tetraether lipids: two very long alkyl chains are ether-linked to glycerols at both ends
- 4) sphingolipids: a single fatty acid is joined to fatty amine, sphingosine
- 5) sterols: rigid system of four fused hydrocarbon rings.



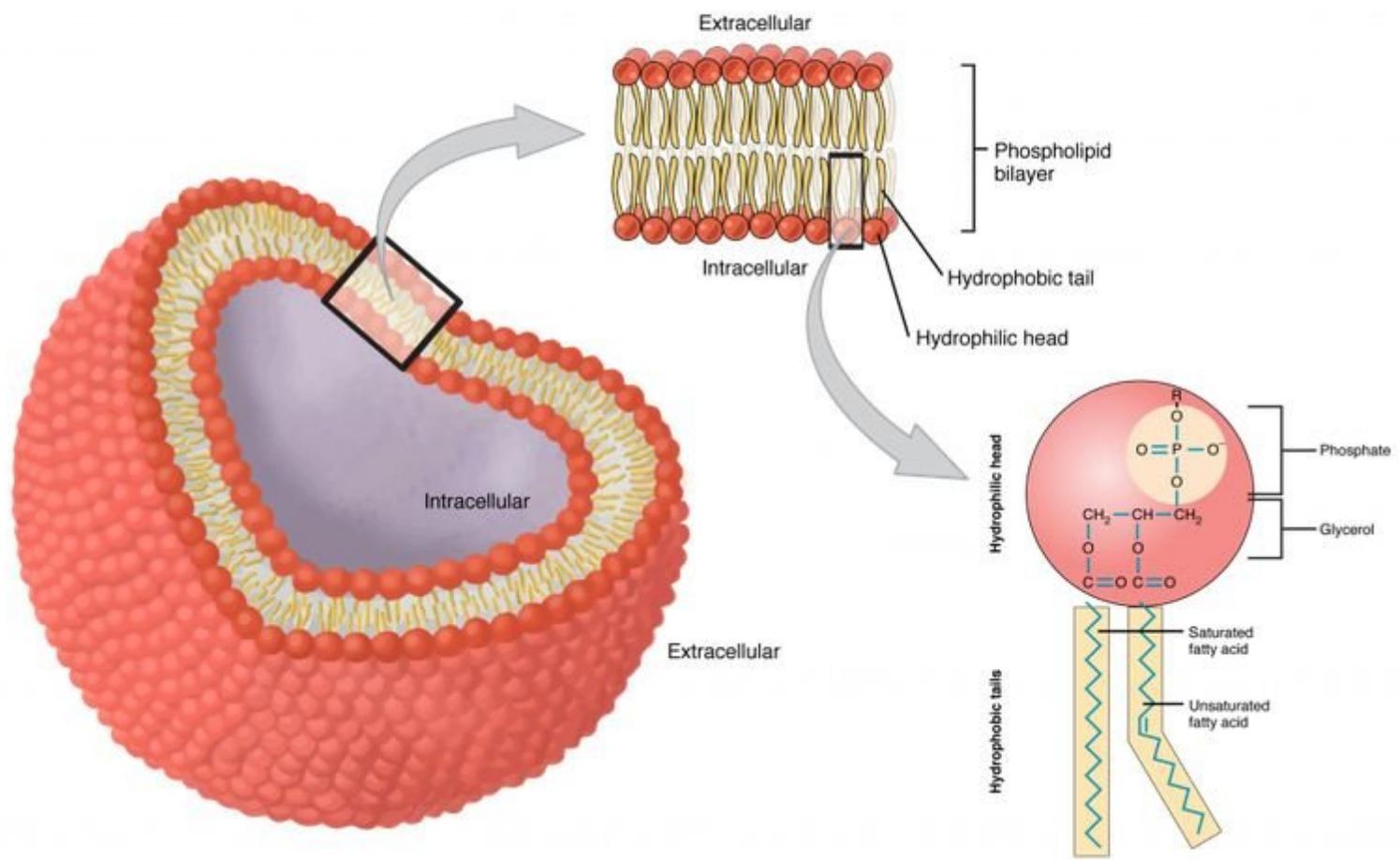
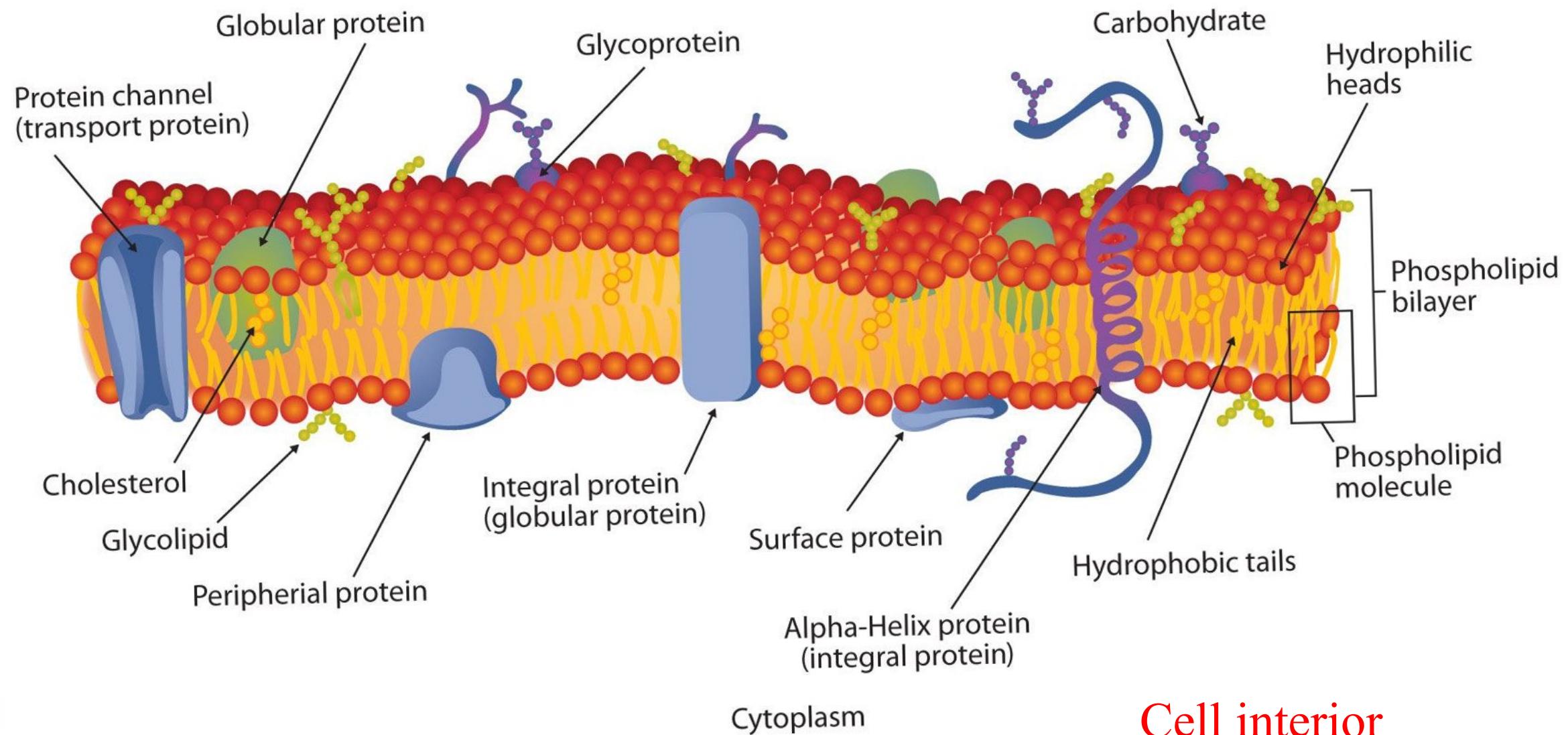


Figure 3.1.1 – Phospholipid Structure and Bilayer: A phospholipid molecule consists of a polar phosphate “head,” which is hydrophilic and a non-polar lipid “tail,” which is hydrophobic. Unsaturated fatty acids result in kinks in the hydrophobic tails. The phospholipid bilayer consists of two adjacent sheets of phospholipids, arranged tail to tail. The hydrophobic tails associate with one another, forming the interior of the membrane. The polar heads contact the fluid inside and outside the cell.

Extracellular Fluid

Cell Exterior



Glycosphingolipids as determinants of blood groups

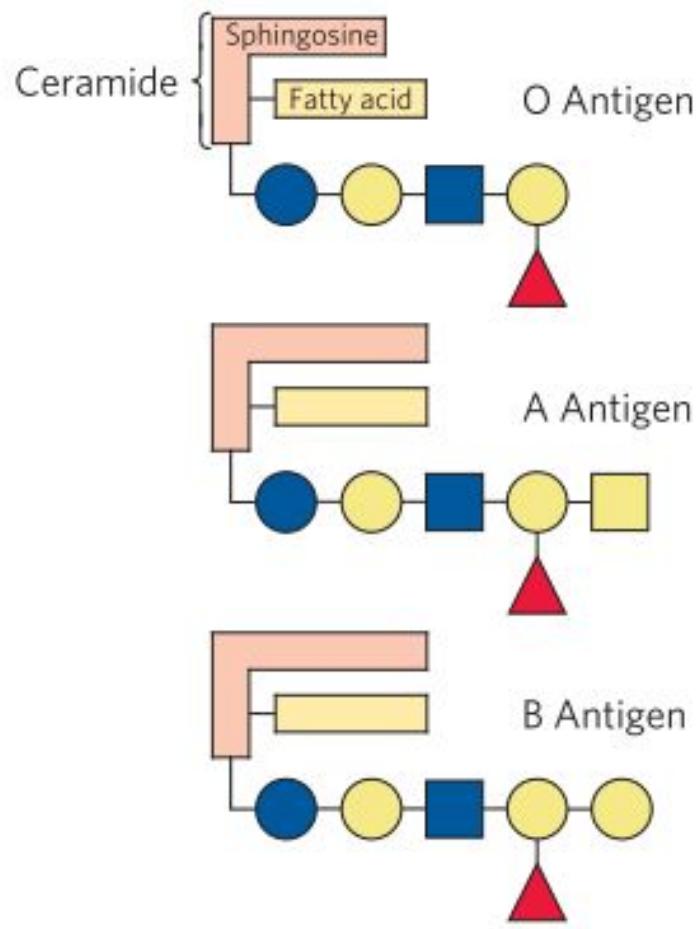


FIGURE 10–15 Glycosphingolipids as determinants of blood groups.

The human blood groups (O, A, B) are determined in part by the oligosaccharide head groups of these glycosphingolipids. The same three oligosaccharides are also found attached to certain blood proteins of individuals of blood types O, A, and B, respectively. Standard symbols for sugars are used here (see Table 7–1).

Glycosphingolipids are present on the outer surface of the plasma membrane.

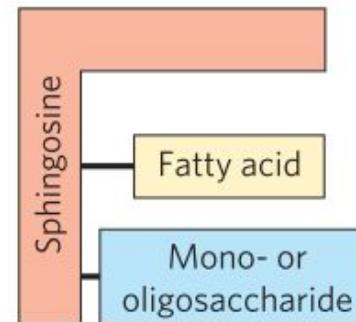


TABLE 7–1 Symbols and Abbreviations for Common Monosaccharides and Some of Their Derivatives

Abequose	Abe	Glucuronic acid	◆ GlcA
Arabinose	Ara	Galactosamine	■ GalN
Fructose	Fru	Glucosamine	■ GlcN
Fucose	▲ Fuc	<i>N</i> -Acetylgalactosamine	■ GalNAc
Galactose	● Gal	<i>N</i> -Acetylglucosamine	■ GlcNAc
Glucose	● Glc	Iduronic acid	◆ IdoA
Mannose	● Man	Muramic acid	Mur
Rhamnose	Rha	<i>N</i> -Acetylmuramic acid	Mur2Ac
Ribose	Rib	<i>N</i> -Acetylneurameric acid (a sialic acid)	◆ Neu5Ac
Xylose	★ Xyl		◆ Neu5Gc

Further reading

Book: Lehninger: Principles of Biochemistry, 6th edition. ISBN: 1464109621.

Chapter 7: Carbohydrates and Glycobiology, Page 243-280.

Chapter 10: Lipids, Page 357-383.

Next class on 15/06/2022 (Wednesday)

BT1010 Introduction to Life Sciences



Lecture 2: Biomolecules: Proteins and Nucleic Acids
16/06/2022

Course Instructor:

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Proteins



TOP VEGETARIAN PROTEIN SOURCES



Greek Yogurt
23 grams of protein per cup



Lentils
4 grams of protein per 1/4 cup (cooked)



Beans (chickpeas, black beans, etc.)
4 grams of protein per 1/4 cup



Cottage Cheese
14 grams of protein per 1/2 cup



Hemp Seeds
4 grams of protein per 1 tablespoon



Chia Seeds
3 grams of protein per 1 tablespoon



Edamame
5 grams of protein per 1/4 cup (shelled)



Green Peas
8 grams of protein per cup



Quinoa
8 grams of protein per cup (cooked)



Peanut Butter
3.5 grams of protein per 1 tablespoon



Almonds
3 grams per 1/2 ounce



Eggs
6 grams of protein per large egg

How much protein?



2 eggs (size 6), boiled
13.2g



95g can of tuna, drained
25.1g



100g salmon, pan-fried
20g



100g steak, braised
32.1g



100g chicken breast, grilled
31.2g



1 cup (150g) canned chickpeas, drained
10.8g



150g Tofu, stir-fried
26.9g



½ cup peas (85g), boiled
4.1g

Source: New Zealand FOODfiles™ 2018 Version 01²

Types of biomolecules that we eat.



NUTRITIONAL INFORMATION	
(TYPICAL VALUES PER 100ml)	
Energy	65kcal
Protein	0g
Carbohydrate	16.2g
Natural Fruit Sugars	2.5g
Added Sugar	13.3g
Fat	0g
Vitamin A	120mcg
Calcium	8mg

$$16.2 \text{ gm} \times 4 = 65 \text{ cal}$$



* Nutritional Information per 100g	
1. Energy	562 kcal
2. Carbohydrate	46.10 g
Sugar	0.25 g
3. Total Fat	38.12 g
Saturated Fatty Acids	16.50 g
Monounsaturated Fatty Acids	13.64 g
Polyunsaturated Fatty Acids	3.10 g
Trans Fatty Acids	0 g
4. Protein	8.68 g
5. Fiber	0.83 g
6. Sodium	615 mg
7. Cholesterol	0 mg

*These are approximate values

$$46.10 \text{ gm} \times 4 = 184.4 \text{ cal}$$

$$8.68 \text{ gm} \times 4 = 34.72 \text{ cal}$$

$$38.12 \text{ gm} \times 9 = 343.08 \text{ cal}$$

$$\text{Total} = 562 \text{ cal}$$

1 gm of carbohydrates provide 4 calories.

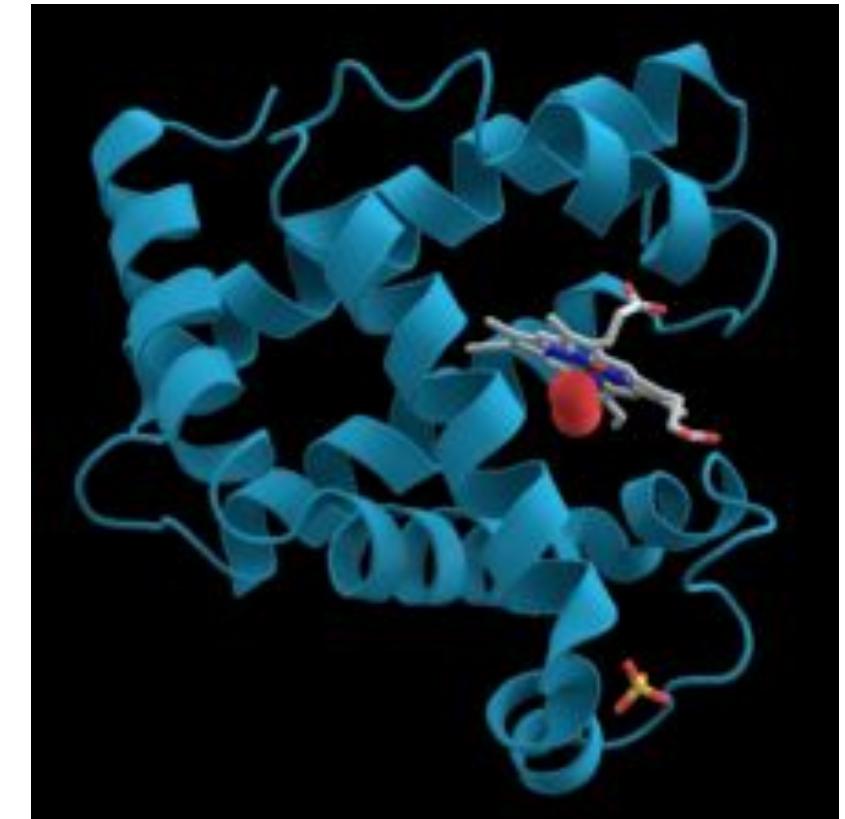
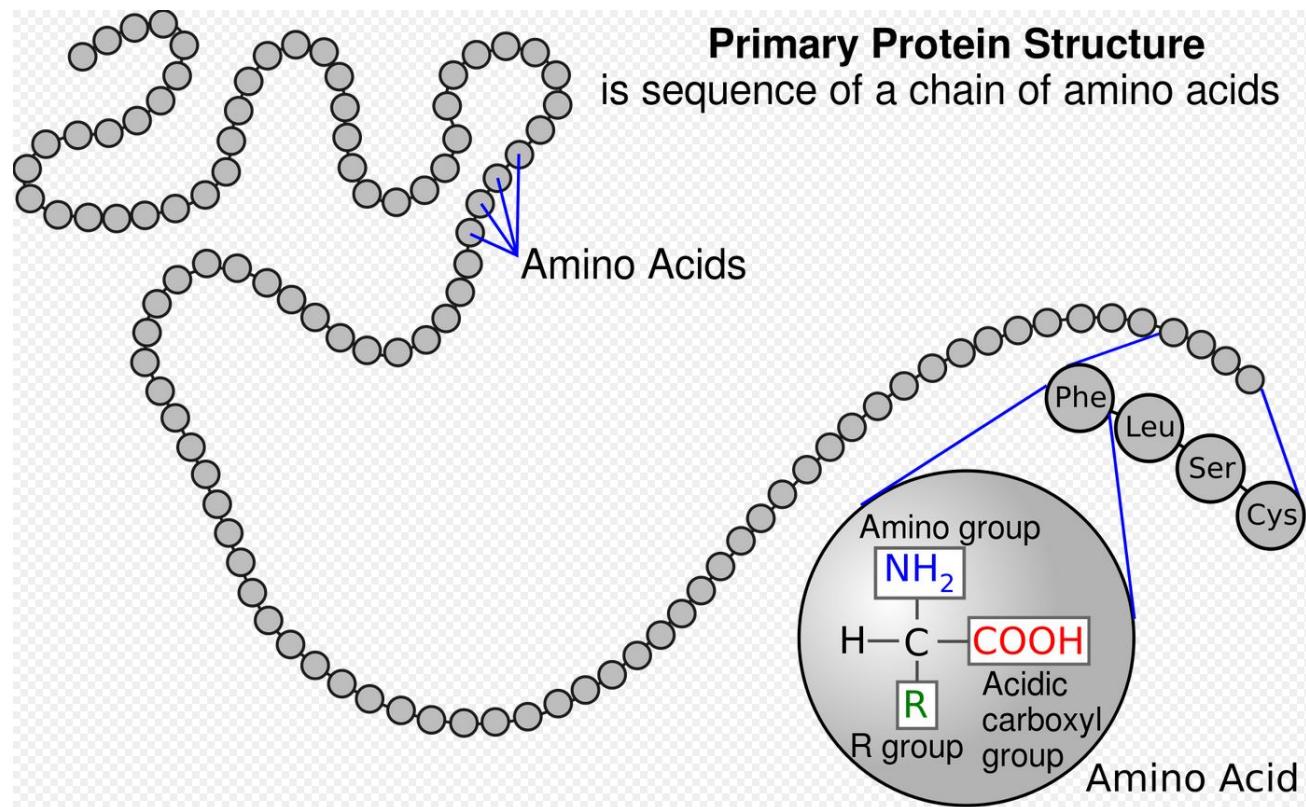
1 gm of proteins provide 4 calories

1 gm of fats/lipids provide 9 calories

Note: Nutritional calorie is the equivalent of the kilocalorie used by chemists and biochemists. So, 65 calorie diet is the equivalent of a 65 kcal diet.

What are proteins?

- Proteins are large biomolecules and macromolecules that comprise long chains of amino acids, linked by the peptide bond.
- Proteins perform a vast number of functions in our body such as catalysing metabolic reactions, DNA replication, providing structural support to the cells, transporting molecules.
- Proteins are nanomachines of the cells.



The 20 common amino acids of proteins

- Amino acids** are organic compounds that contain amino ($-\text{NH}_3^+$) and carboxyl ($-\text{COO}^-$) functional groups, along with a side chain (R group) specific to each amino acid.
- They are classified based on their polarity, ionization and side chain group type (aliphatic, acyclic, aromatic).
- Natural amino acids have the L configuration.
- There are several **Uncommon amino acids** such as 4-hydroxyproline, 5-hydroxylysine, 6-N-Methyllysine, Selenocysteine. See page no. 82 in Lehninger.

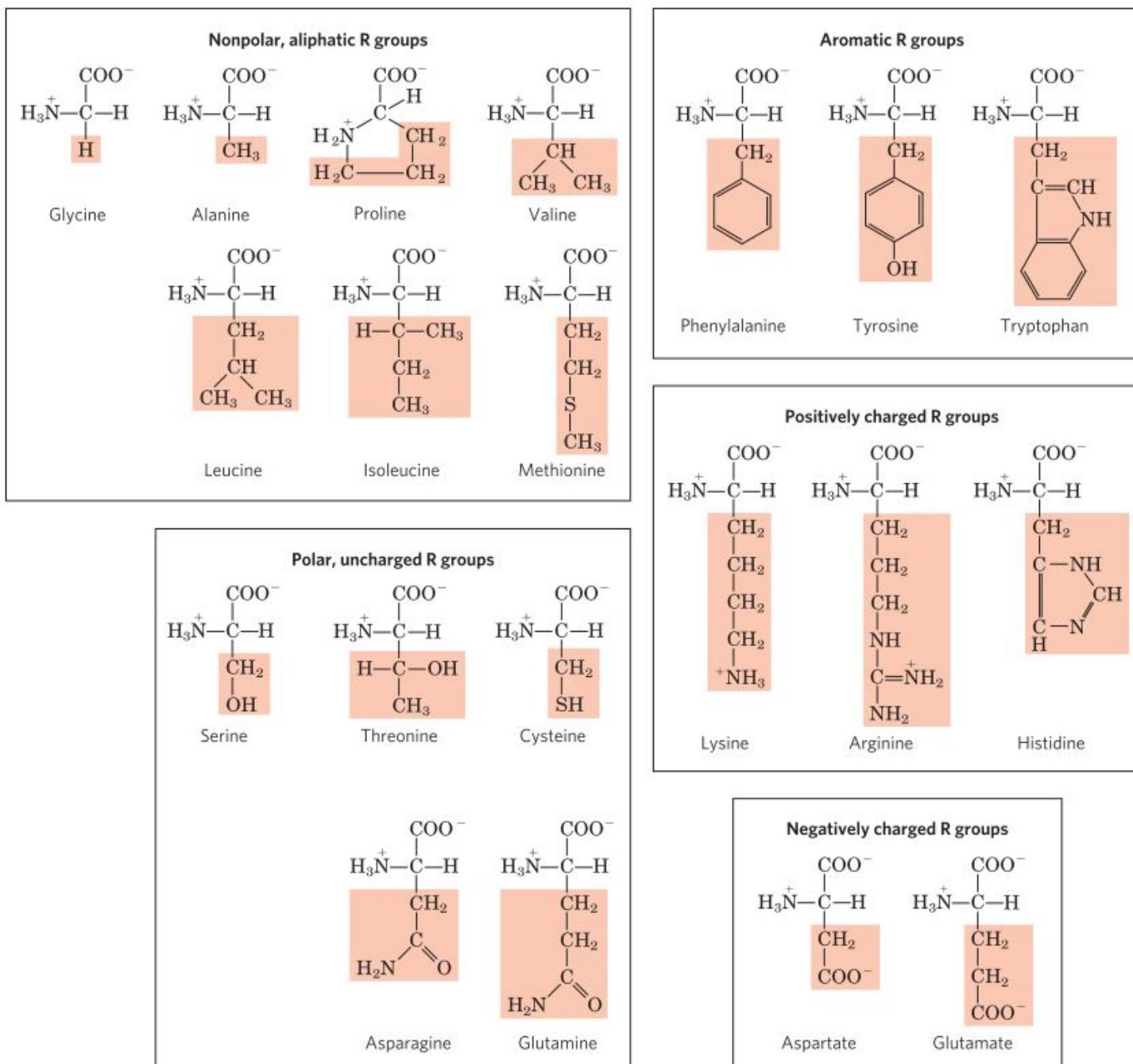
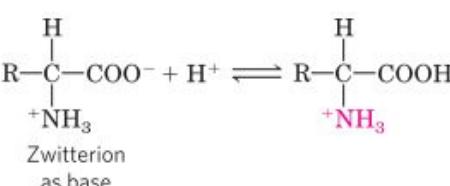
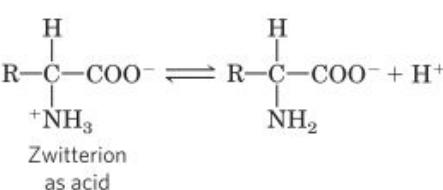
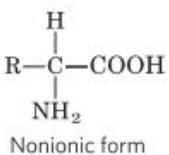
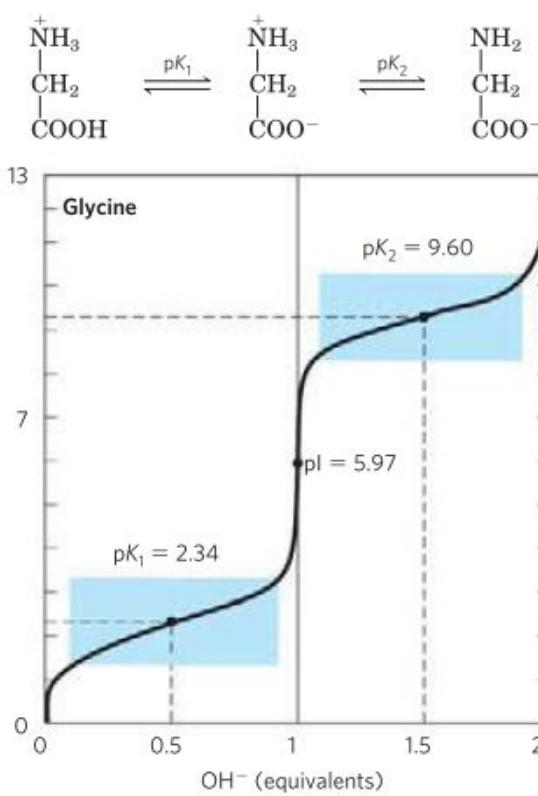


Table of standard amino acid abbreviations and properties

Amino acid ↗	3- and 1-letter symbols		Side chain			Hydropathy index ^[34]	Molar absorptivity ^[35]		Molecular mass ↗	Abundance in proteins (%) ^[36]	Standard genetic coding, IUPAC notation
	3 ↗	1 ↗	Class ↗	Polarity ^[37]	Net charge at pH 7.4 ^[37]		Wavelength, λ_{\max} (nm)	Coefficient ϵ ($\text{mM}^{-1}\cdot\text{cm}^{-1}$)			
Alanine	Ala	A	Aliphatic	Nonpolar	Neutral	1.8			89.094	8.76	GCN
Arginine	Arg	R	Fixed cation	Basic polar	Positive	-4.5			174.203	5.78	MGR, CGY ^[38]
Asparagine	Asn	N	Amide	Polar	Neutral	-3.5			132.119	3.93	AAY
Aspartate	Asp	D	Anion	Brønsted base	Negative	-3.5			133.104	5.49	GAY
Cysteine	Cys	C	Thiol	Brønsted acid	Neutral	2.5	250	0.3	121.154	1.38	UGY
Glutamine	Gln	Q	Amide	Polar	Neutral	-3.5			146.146	3.9	CAR
Glutamate	Glu	E	Anion	Brønsted base	Negative	-3.5			147.131	6.32	GAR
Glycine	Gly	G	Aliphatic	Nonpolar	Neutral	-0.4			75.067	7.03	GGN
Histidine	His	H	Aromatic cation	Brønsted acid and base	Positive, 10% Neutral, 90%	-3.2	211	5.9	155.156	2.26	CAY
Isoleucine	Ile	I	Aliphatic	Nonpolar	Neutral	4.5			131.175	5.49	AUH
Leucine	Leu	L	Aliphatic	Nonpolar	Neutral	3.8			131.175	9.68	YUR, CUY ^[39]
Lysine	Lys	K	Cation	Brønsted acid	Positive	-3.9			146.189	5.19	AAR
Methionine	Met	M	Thioether	Nonpolar	Neutral	1.9			149.208	2.32	AUG
Phenylalanine	Phe	F	Aromatic	Nonpolar	Neutral	2.8	257, 206, 188	0.2, 9.3, 60.0	165.192	3.87	UUY
Proline	Pro	P	Cyclic	Nonpolar	Neutral	-1.6			115.132	5.02	CCN
Serine	Ser	S	Hydroxylic	Polar	Neutral	-0.8			105.093	7.14	UCN, AGY
Threonine	Thr	T	Hydroxylic	Polar	Neutral	-0.7			119.119	5.53	ACN
Tryptophan	Trp	W	Aromatic	Nonpolar	Neutral	-0.9	280, 219	5.6, 47.0	204.228	1.25	UGG
Tyrosine	Tyr	Y	Aromatic	Brønsted acid	Neutral	-1.3	274, 222, 193	1.4, 8.0, 48.0	181.191	2.91	UAY
Valine	Val	V	Aliphatic	Nonpolar	Neutral	4.2			117.148	6.73	GUN

Amino Acids have characteristic titration curves

- Amino acids have two ionizable groups, the carboxyl group and the amino group.
- When you titrate with a strong base (NaOH), the plot shows two distinct stages, corresponding to deprotonation of two different groups of the amino acids.
- The $\text{p}K_a$ is a measure of the tendency of a group to give up a proton, with that tendency decreases tenfold as the $\text{p}K_a$ increases by one unit.
- For Glycine, $\text{p}K_a$ of COOH group is 2.34, whereas $\text{p}K_a$ of NH_2 group is 9.6.
- The carboxyl group of glycine is over 100 times more acidic (more easily ionized) than the carboxyl group of acetic acid ($\text{p}K_a = 4.76$).
- The titration curve of glycine indicates that this amino acid has two regions of buffering power. One of these is the relatively flat portion of the curve, 1 pH unit on either side of the $\text{p}K_1$ of 2.34 and the second one is near $\text{p}K_2$ of 9.60.



Titration curve predict the electric charge of amino acids

- At pH 5.97, glycine is present predominantly as its dipolar form, fully ionized but with no net electrical charge.
- The characteristic pH at which the net electric charge is zero is called the **isoelectric point** or **isoelectric pH**, designated **pI**. At this pH, the amino acid exists in its zwitterionic form.

$$pI = \frac{1}{2}(pK_1 + pK_2) = \frac{1}{2}(2.34 + 9.60) = 5.97$$

- However, amino acids differ in their acid-base properties, based on the ionization capacity of the R group. The titration curve of all the amino acids are not the same. See page no. 84-85 in Lehninger. Titration curves for glutamate and histidine.

Peptides and Proteins

- Amino acids are linked together by the peptide bond to form a **peptide** (two/three/four/five amino acids)/**oligopeptide** (a few amino acids)/**polypeptide** (many amino acids)/**protein** (thousands of amino acids).

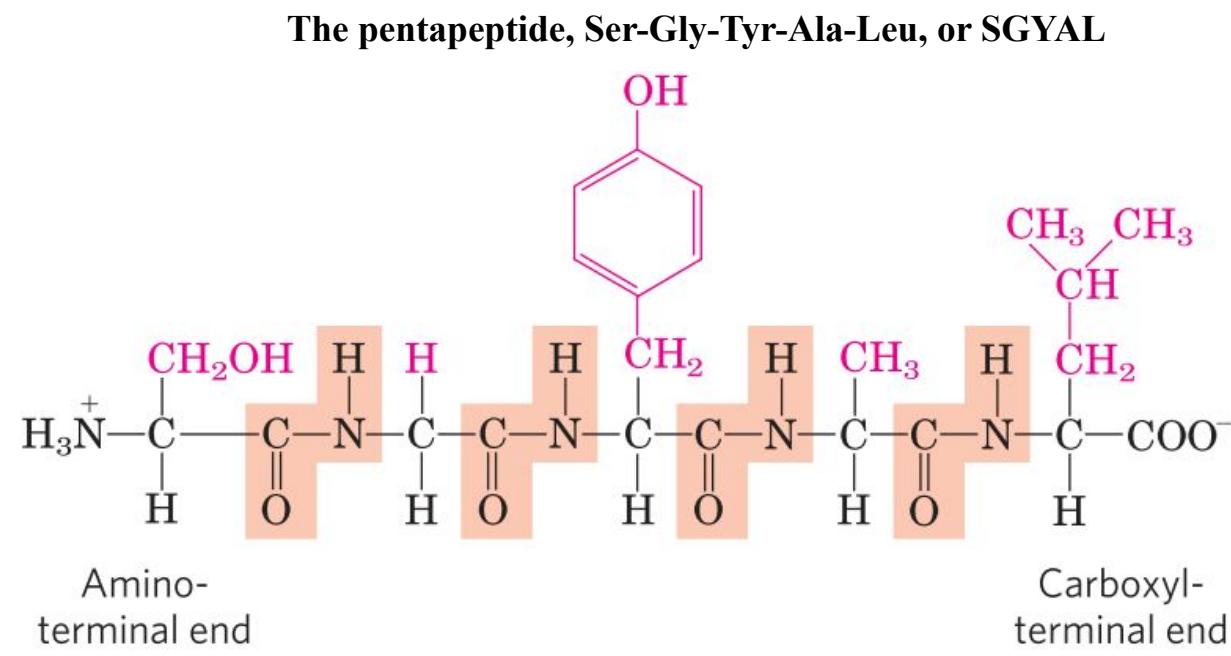
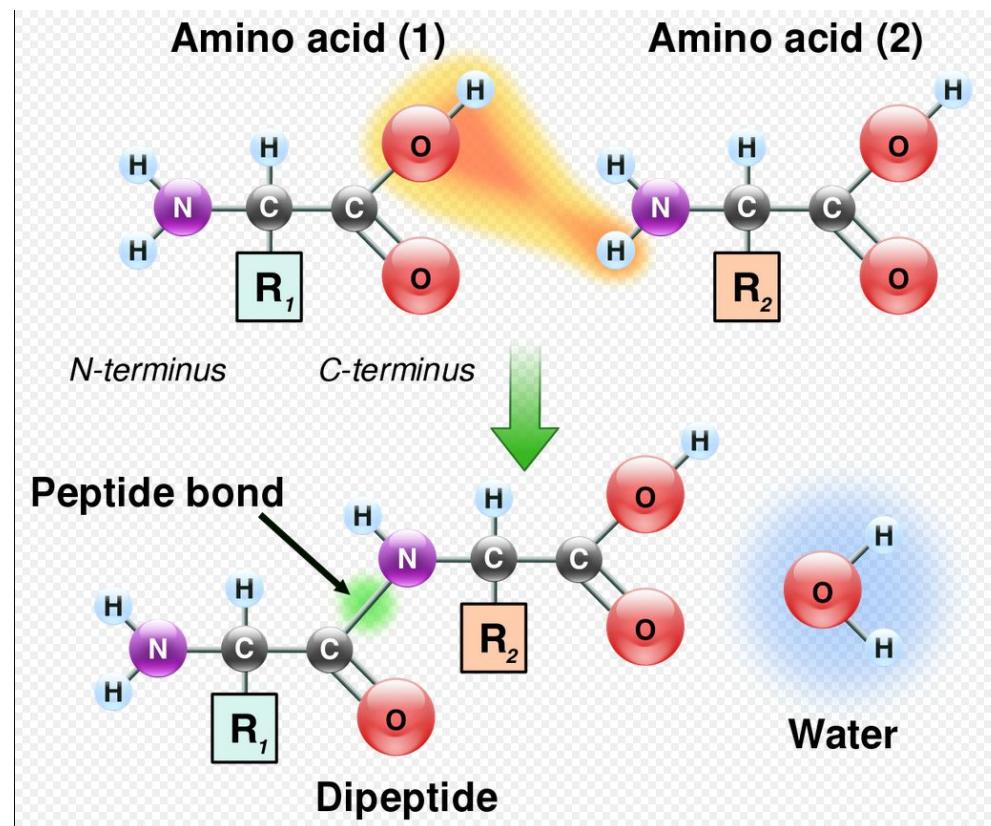


TABLE 3–2 Molecular Data on Some Proteins

	Molecular weight	Number of residues	Number of polypeptide chains
Cytochrome <i>c</i> (human)	12,400	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	14,300	129	1
Myoglobin (equine heart)	16,700	153	1
Chymotrypsin (bovine pancreas)	25,200	241	3
Chymotrypsinogen (bovine)	25,700	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	66,000	609	1
Hexokinase (yeast)	107,900	972	2
RNA polymerase (<i>E. coli</i>)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (<i>E. coli</i>)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

Prosthetic group

- Some proteins contain chemical groups other than amino acids. This chemical group is known as **prosthetic group**.

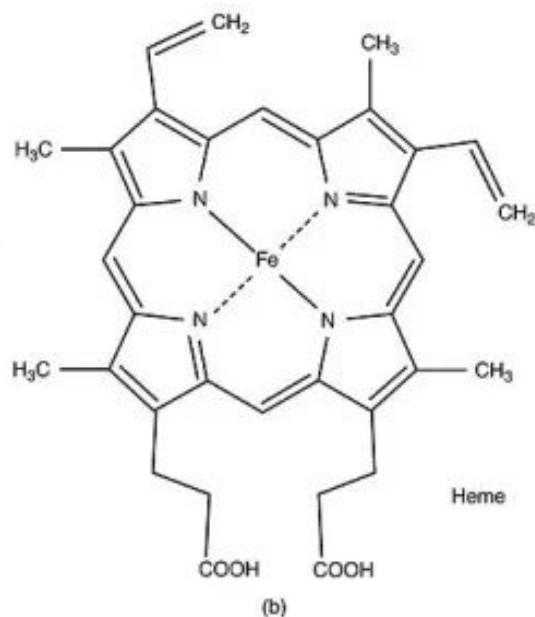
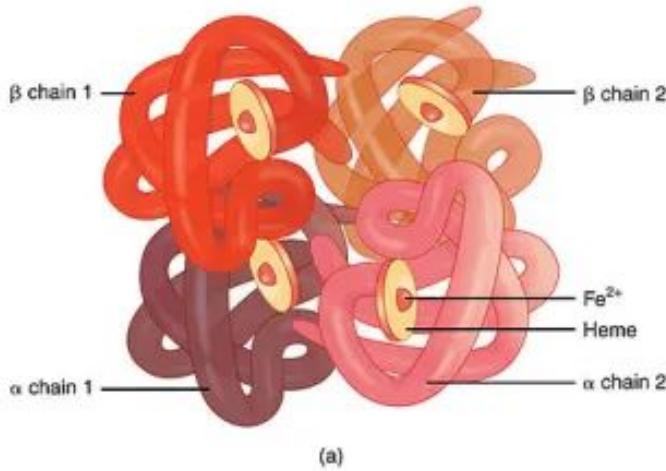


TABLE 3–4 Conjugated Proteins

Class	Prosthetic group	Example
Lipoproteins	Lipids	β_1 -Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron Zinc Calcium Molybdenum Copper	Ferritin Alcohol dehydrogenase Calmodulin Dinitrogenase Plastocyanin

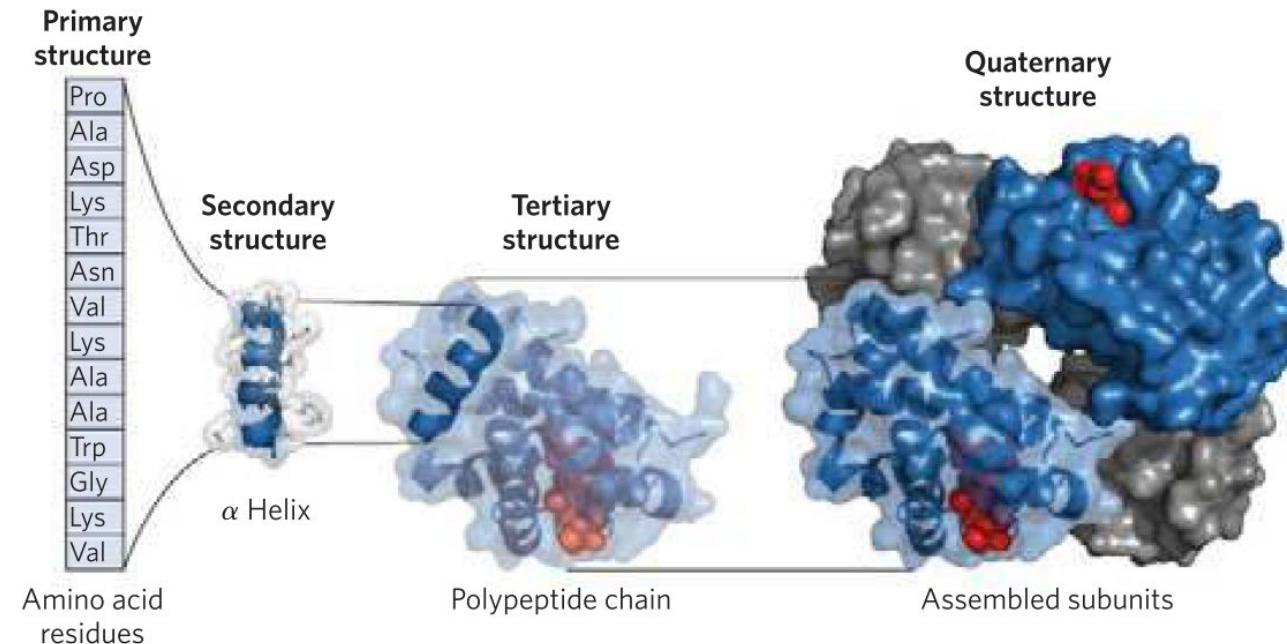
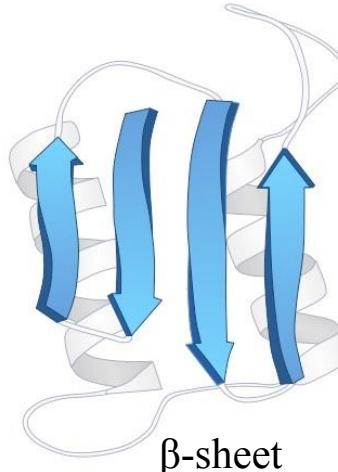
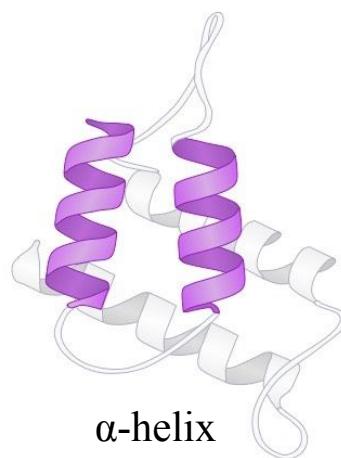
The structure of proteins

Primary structure: the linear sequence of amino acids: Gly-Ala-Lys-Trp

Secondary structure: stable arrangement of amino acids giving rise to recurring structural patterns, such as α -helix and β -sheet.

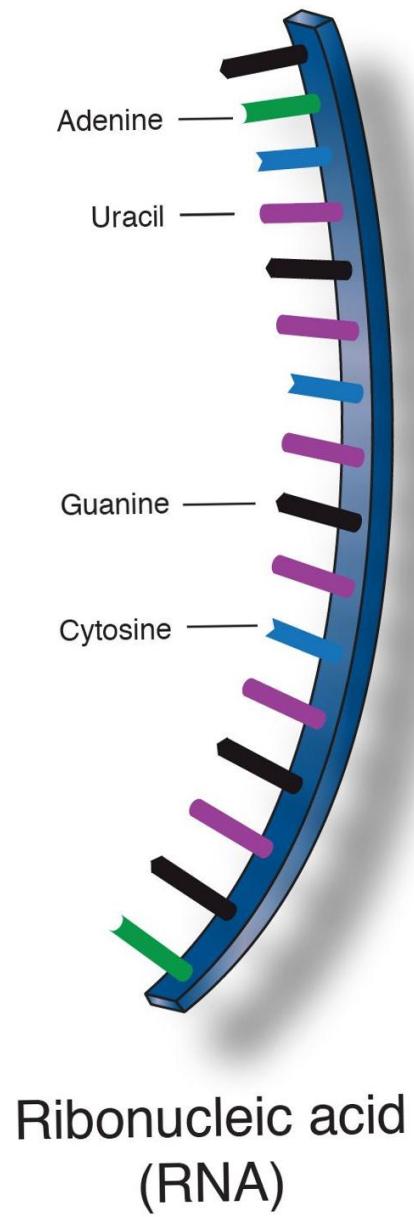
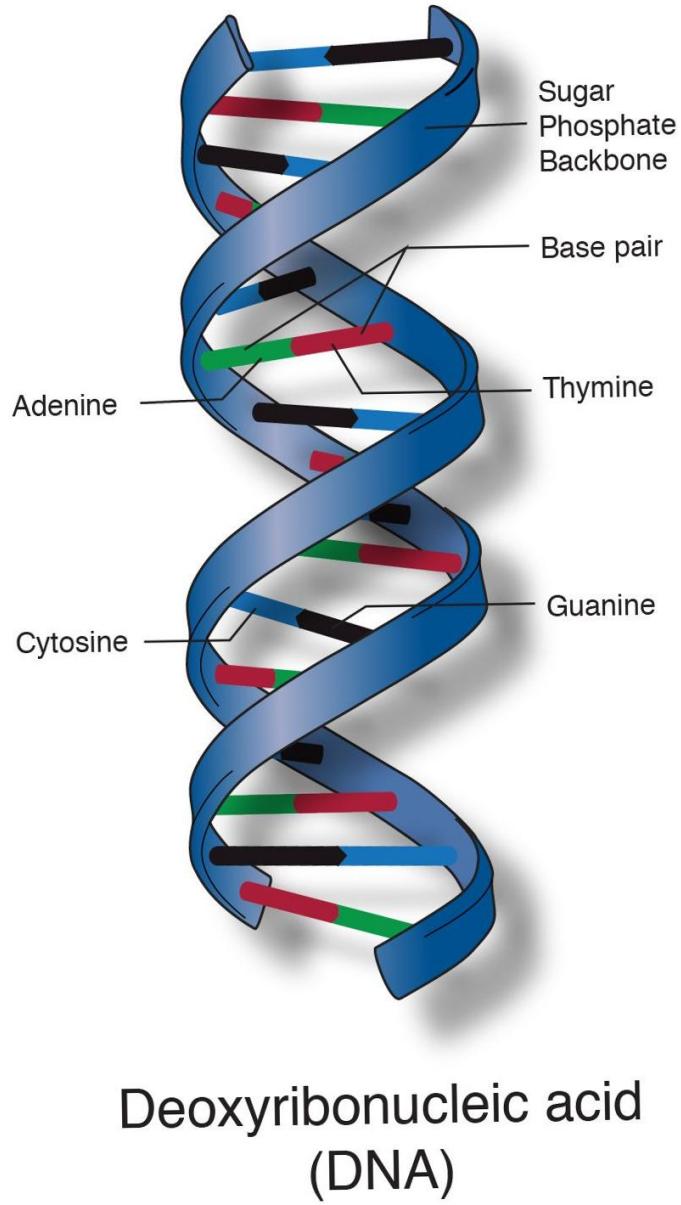
Tertiary structure: three-dimensional folding of a polypeptide chain.

Quaternary structure: when a protein has two or more polypeptide chains, their arrangement in space.



Our Hairs and Nails are proteins

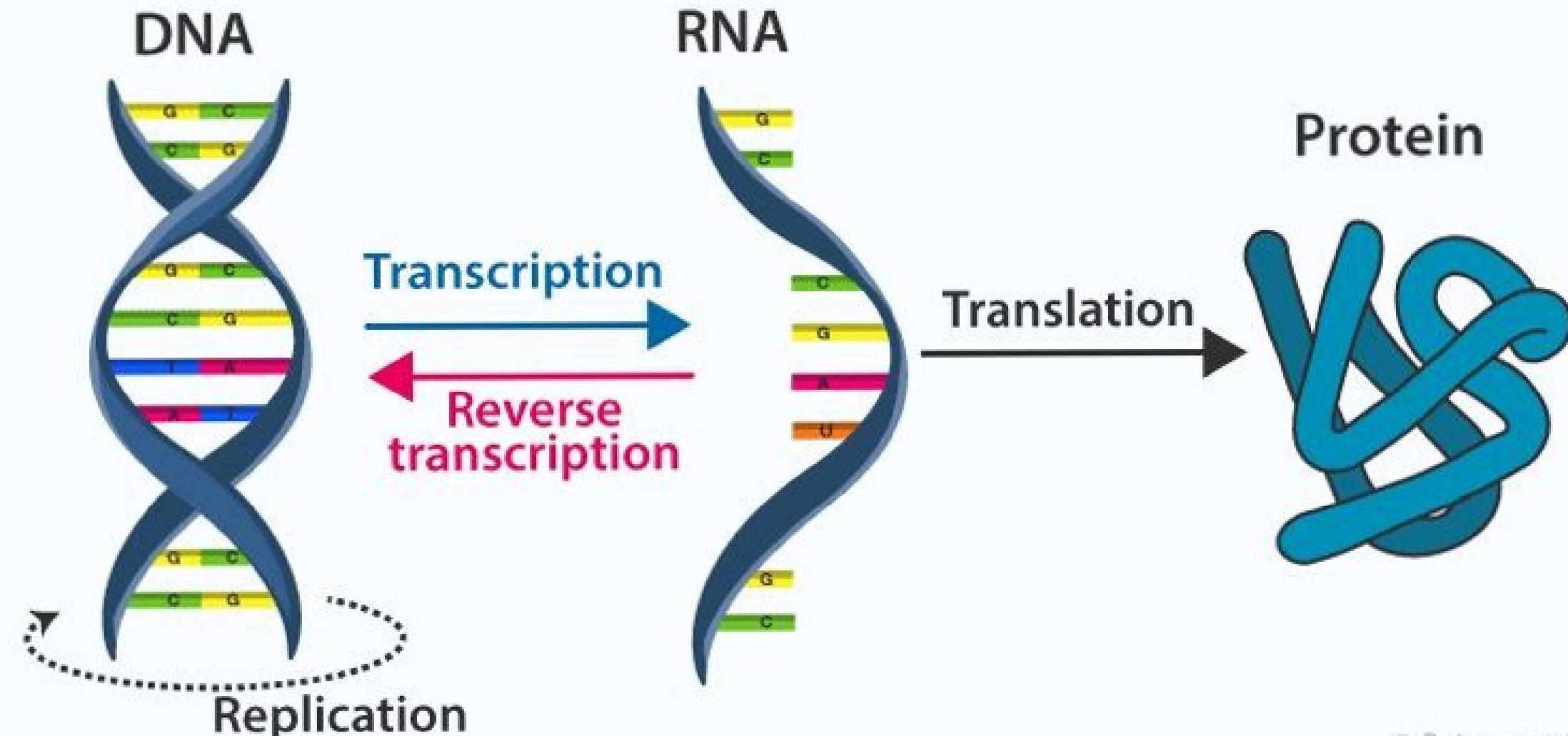
- Our hairs and nails are made up of a protein, known as α -Keratin. Hair grows at a rate of 15-20 cm/year, whereas nails grow 3.5 mm each month.
- The fundamental structural element of α -Keratin is the α -helix.



Nucleic Acids

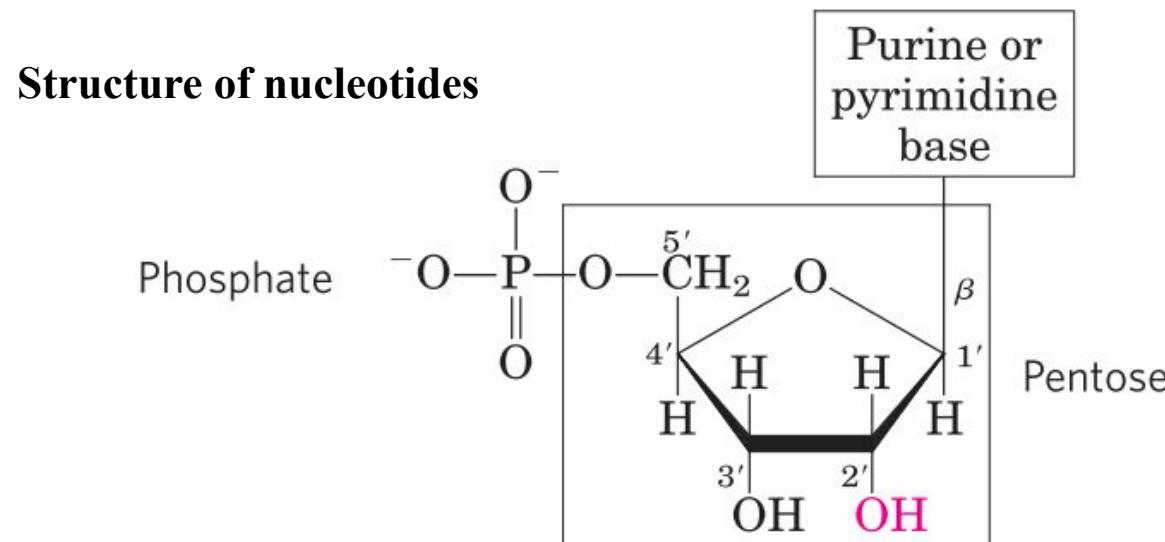
- Genetic material responsible for inheritance.
- Nucleotides are the building blocks of nucleic acids.
- The amino acid sequences of proteins are specified by a nucleotide sequence in the cell's DNA.

CENTRAL DOGMA : DNA TO RNA TO PROTEIN



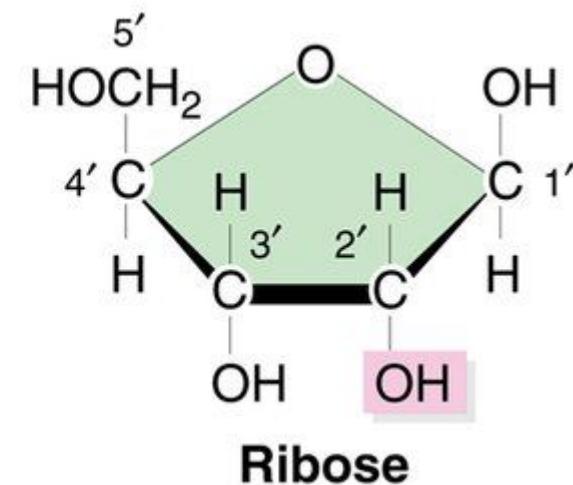
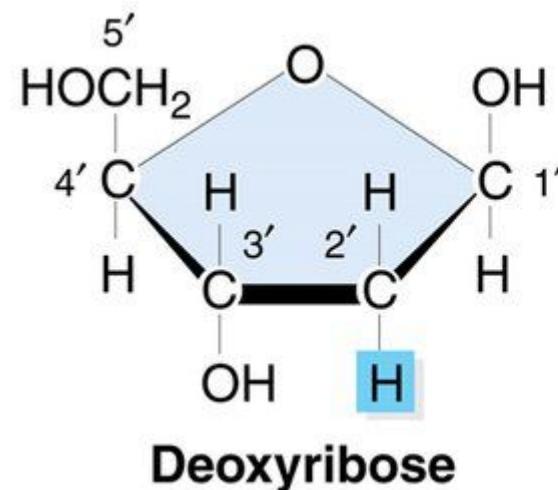
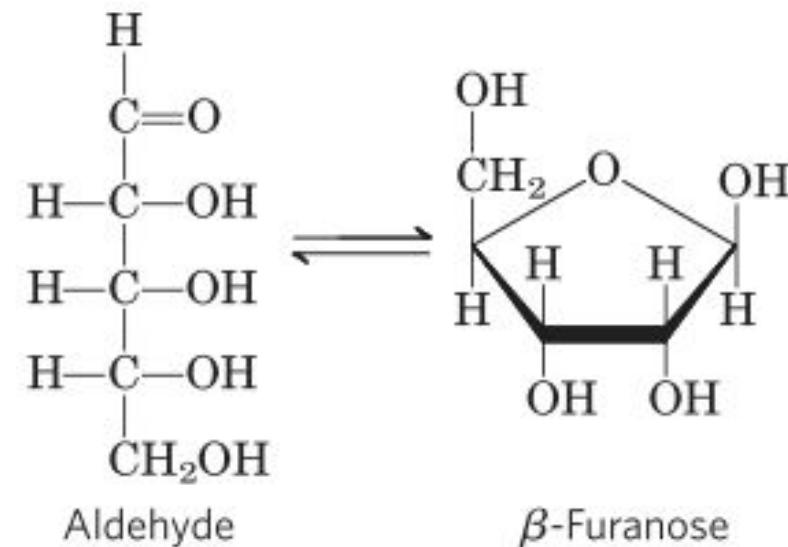
Building blocks of the nucleic acids (DNA and RNA)

- Nucleotides have three characteristic components: 1) a nitrogenous base, 2) a pentose sugar, 3) one or more phosphates.
- The molecule without a phosphate group is called a nucleoside.
- The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine.

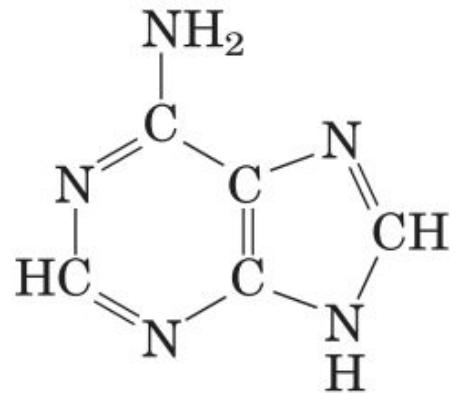


Types of pentose sugars

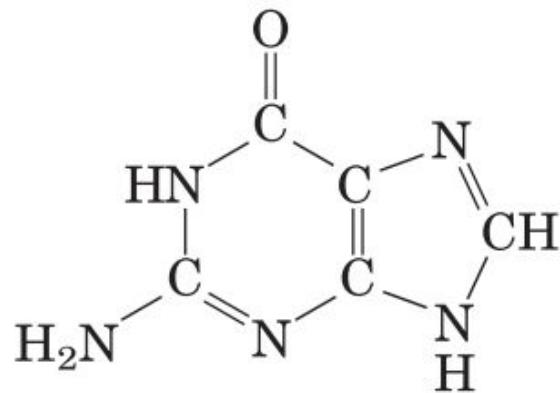
- Nucleic acids have two kinds of pentoses.
- DNA contains 2'-deoxy-D-ribose
- RNA contains D-ribose



Major purine and pyrimidine bases of nucleic acids

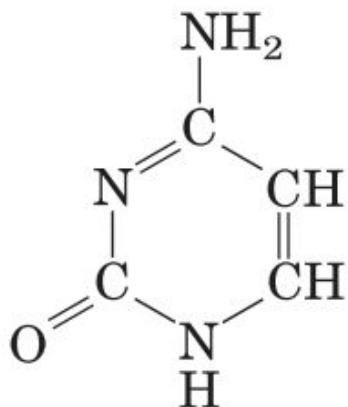


Adenine

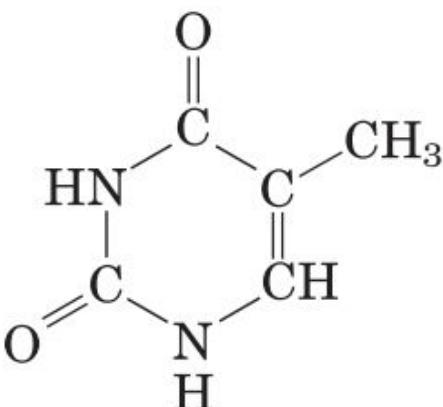


Guanine

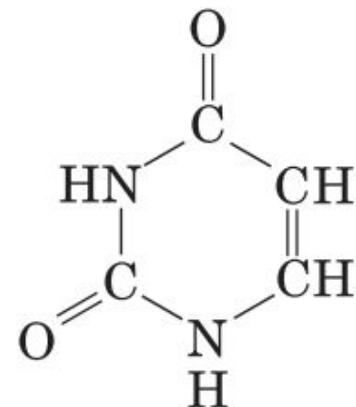
Purines



Cytosine



Thymine
(DNA)



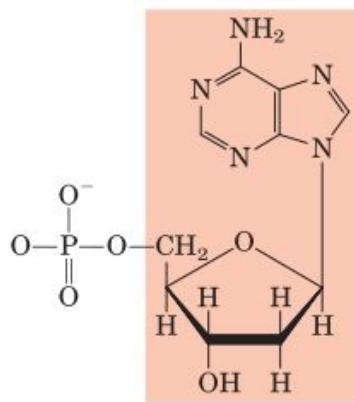
Uracil
(RNA)

Pyrimidines

TABLE 8–1 Nucleotide and Nucleic Acid Nomenclature

Base	Nucleoside	Nucleotide	Nucleic acid
Purines			
Adenine	Adenosine	Adenylate	RNA
	Deoxyadenosine	Deoxyadenylate	DNA
Guanine			
Guanine	Guanosine	Guanylate	RNA
	Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines			
Cytosine	Cytidine	Cytidylate	RNA
	Deoxycytidine	Deoxycytidylate	DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

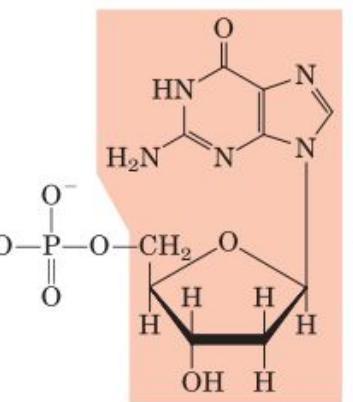
Deoxyribonucleotides and ribonucleotides of nucleic acids



Nucleotide: Deoxyadenylate
(deoxyadenosine 5'-monophosphate)

Symbols: A, dA, dAMP

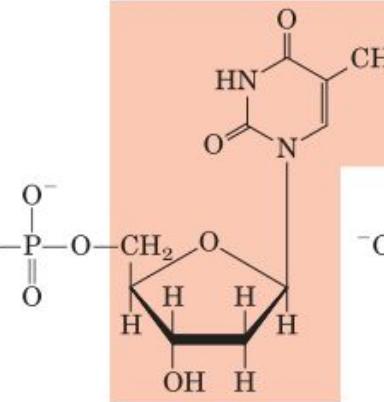
Nucleoside: Deoxyadenosine



Nucleotide: Deoxyguanylate
(deoxyguanosine 5'-monophosphate)

Symbols: G, dG, dGMP

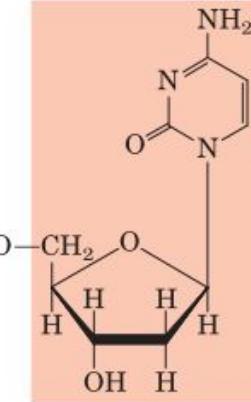
Nucleoside: Deoxyguanosine



Nucleotide: Deoxythymidylate
(deoxythymidine 5'-monophosphate)

Symbols: T, dT, dTMP

Nucleoside: Deoxythymidine

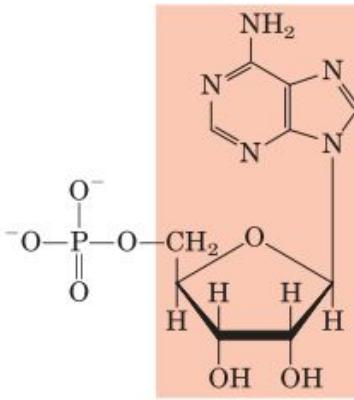


Nucleotide: Deoxycytidylate
(deoxycytidine 5'-monophosphate)

Symbols: C, dC, dCMP

Nucleoside: Deoxycytidine

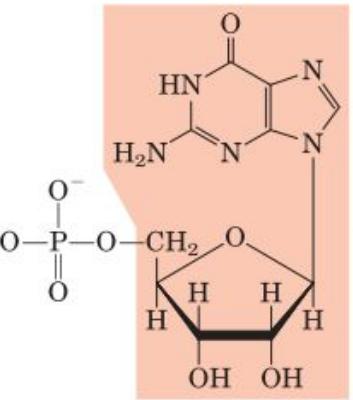
(a) Deoxyribonucleotides



Nucleotide: Adenylate (adenosine 5'-monophosphate)

Symbols: A, AMP

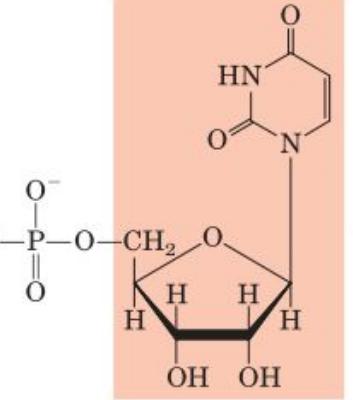
Nucleoside: Adenosine



Nucleotide: Guanylate (guanosine 5'-monophosphate)

Symbols: G, GMP

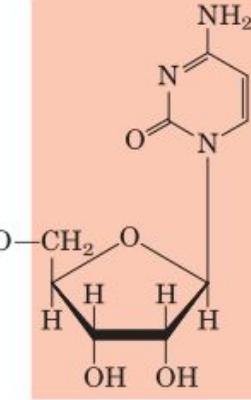
Nucleoside: Guanosine



Nucleotide: Uridylate (uridine 5'-monophosphate)

Symbols: U, UMP

Nucleoside: Uridine



Nucleotide: Cytidylate (cytidine 5'-monophosphate)

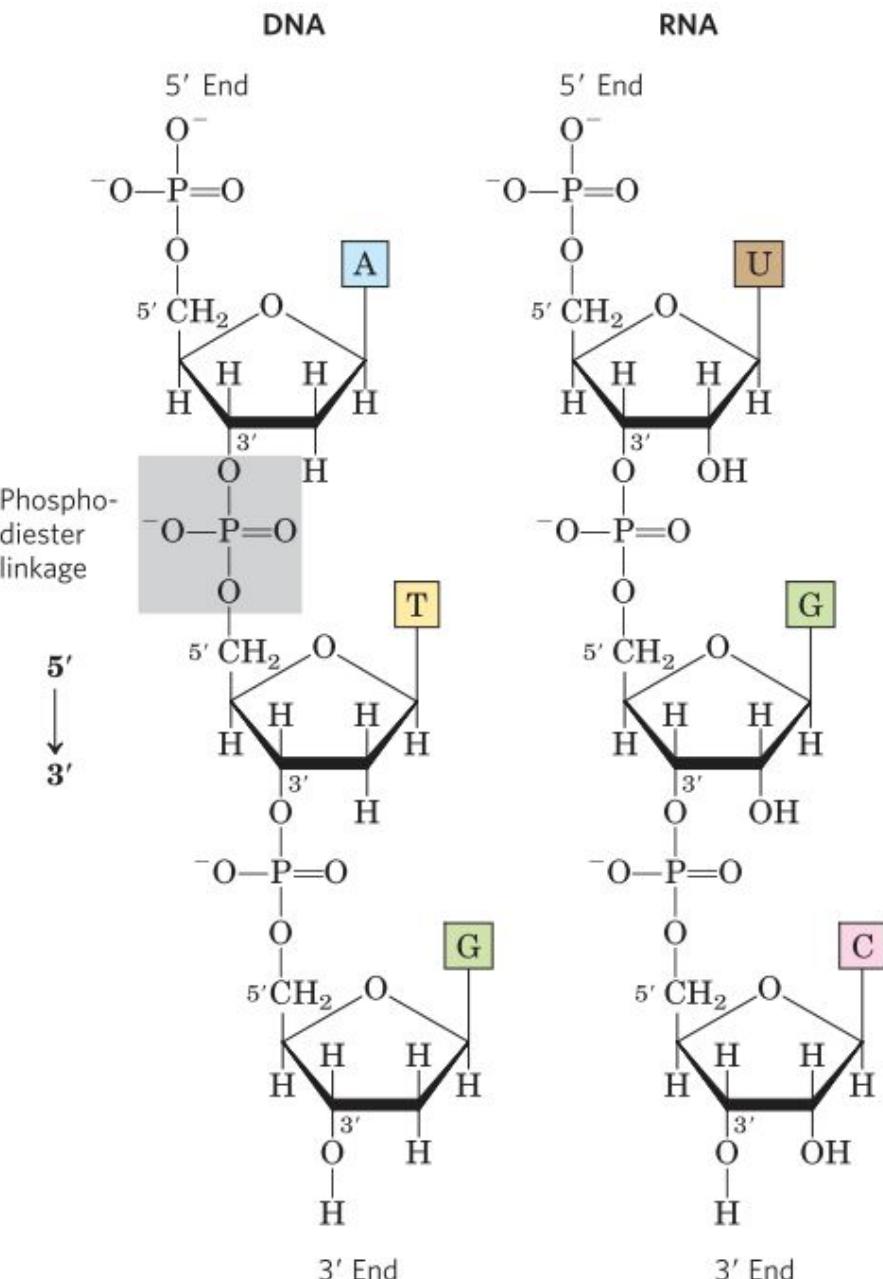
Symbols: C, CMP

Nucleoside: Cytidine

(b) Ribonucleotides

Phosphodiester bonds link successive nucleotides in nucleic acids

- The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group “bridges”, in which the 5'-phosphate group of one nucleotide is joined to the 3'-hydroxyl group of the next nucleotide, creating a **phosphodiester linkage**.



Hydrogen-bonding patterns in the base pairs

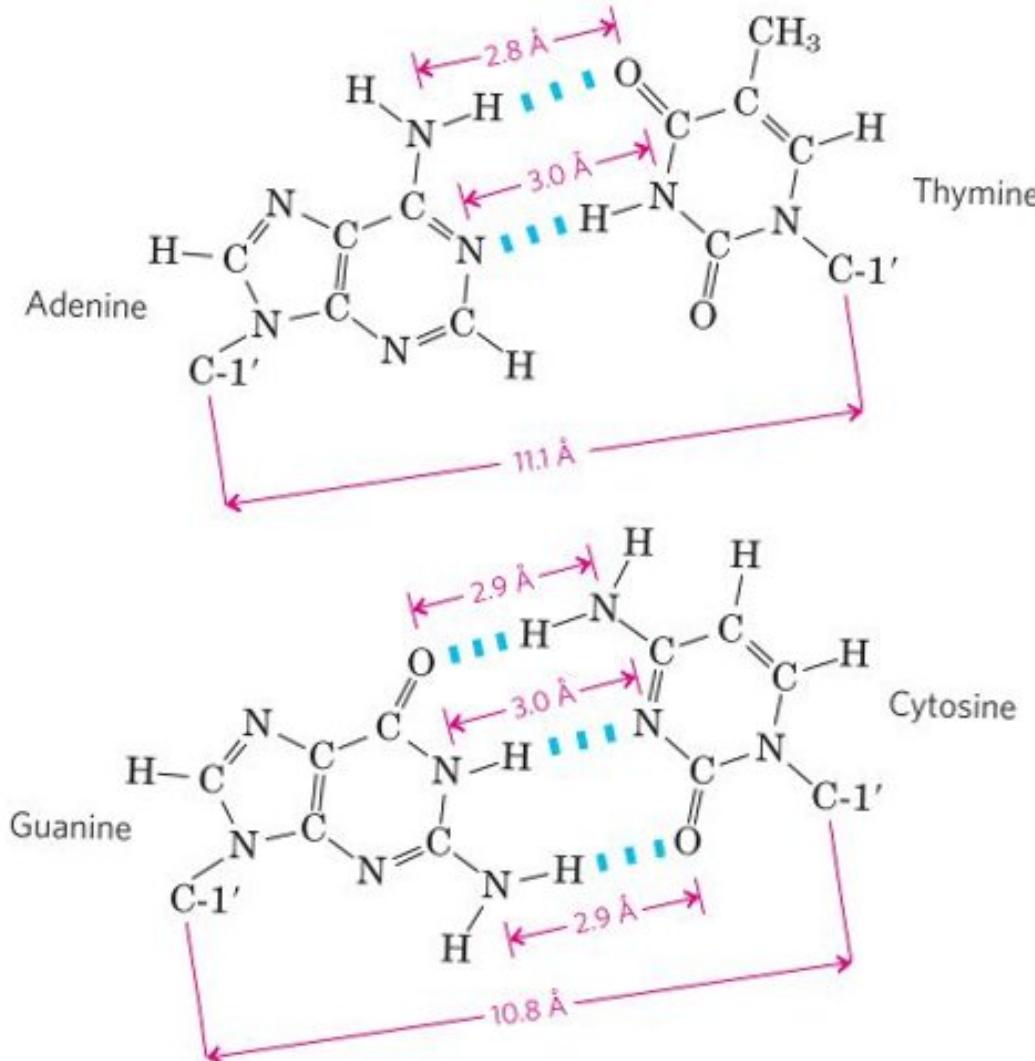
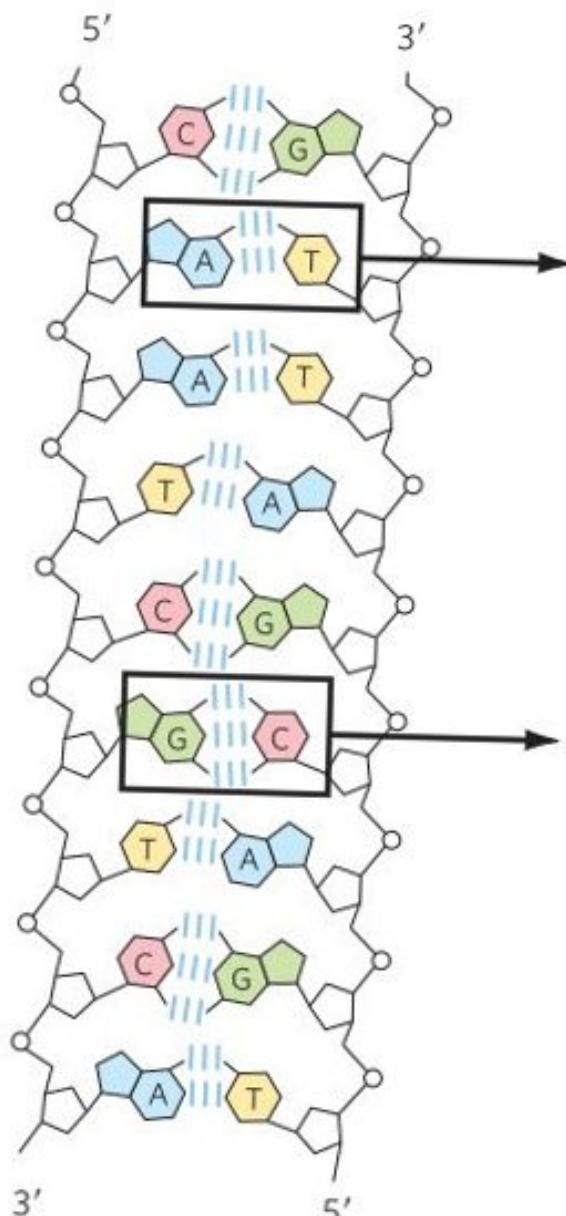


FIGURE 8-11 Hydrogen-bonding patterns in the base pairs defined by Watson and Crick. Here as elsewhere, hydrogen bonds are represented by three blue lines.

History and Discovery of DNA

- DNA was first isolated and characterized by Friedrich Miescher in [1868](#). He called the phosphorous-containing substance “nuclein”.
- In [1940](#), Avery, MacLeod and McCarty found that the DNA extracted from a virulent (disease-causing) strain of the bacterium *Streptococcus pneumoniae* and injected into a nonvirulent strain transformed the nonvirulent strain into a virulent strain.
- In [1952](#), Hershey and Chase studied the infection of bacterial cells by a virus (bacteriophage) with radioactively labeled DNA or protein confirmed that DNA, not protein, is the genetic material.
- In 1940, Erwin Chargaff provided following conclusions: ([Chargaff's Rules](#))
 - 1) The base composition of DNA generally varies from one species to another.
 - 2) DNA specimens isolated from different tissues of the same species have the same base composition.
 - 3) The base composition of DNA in a given species does not change with an organism's age, nutritional status, or changing environment.
 - 4) In all cellular DNA, A=T and G=C. ($A+G = T+C$)

History and Discovery of DNA

- In 1950s, Rosalind Franklin and Maurice Wilkins used X-Ray diffraction to analyze DNA fiber.
- In 1953, James Watson and Francis Crick relied on this accumulated information about DNA and postulated a 3D structure of DNA.
- In 1962, Watson, Crick and Wilkins were awarded the Nobel Prize in Physiology or Medicine.

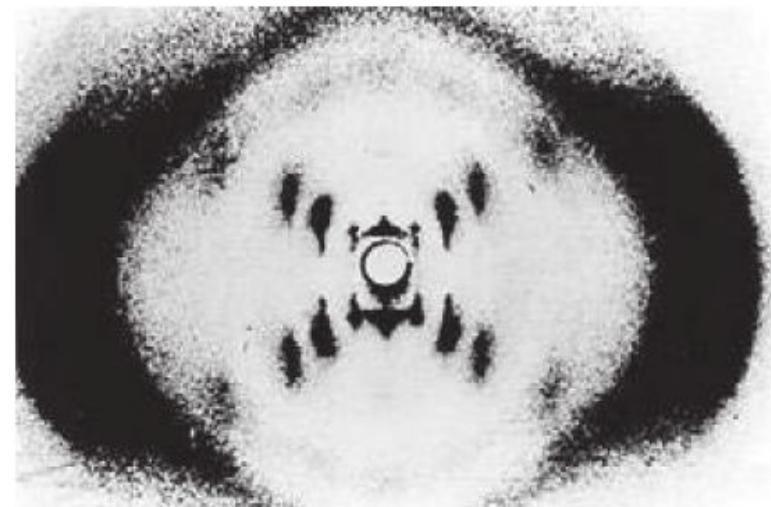


Rosalind Franklin,
1920-1958



Maurice Wilkins,
1916-2004

X-Ray diffraction pattern of DNA fibers



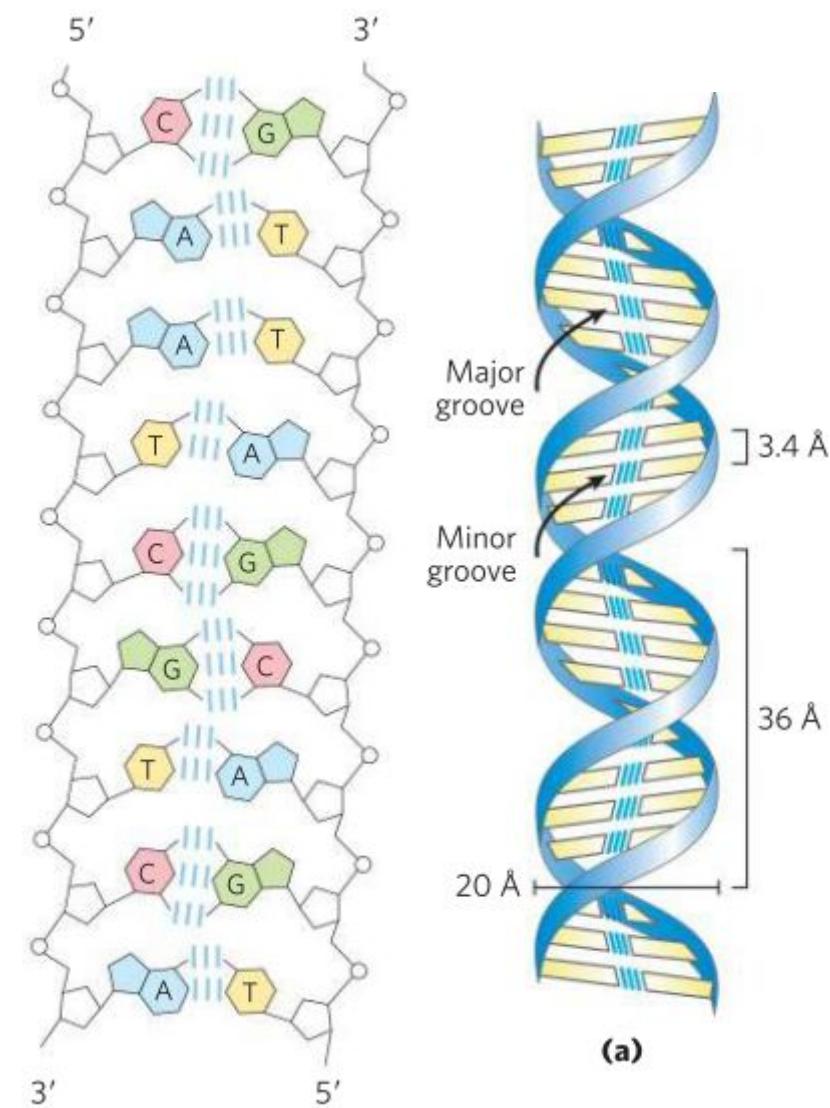
James D. Watson



Francis Crick, 1916-2004

Structure of DNA

- It consists of two helical DNA chains wound around the same axis to form a right-handed double helix.
- The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the outside of the double helix, facing the surrounding water.
- The offset pairing of the two strands creates a major groove and minor groove on the surface of the duplex.
- Three hydrogen bonds form between G and C, whereas only two hydrogen bonds form between A and T.
- Both strands of DNA are in antiparallel orientation.
- Vertically stacked bases are 3.4 Å apart.



DNA can occur in different three-dimensional forms

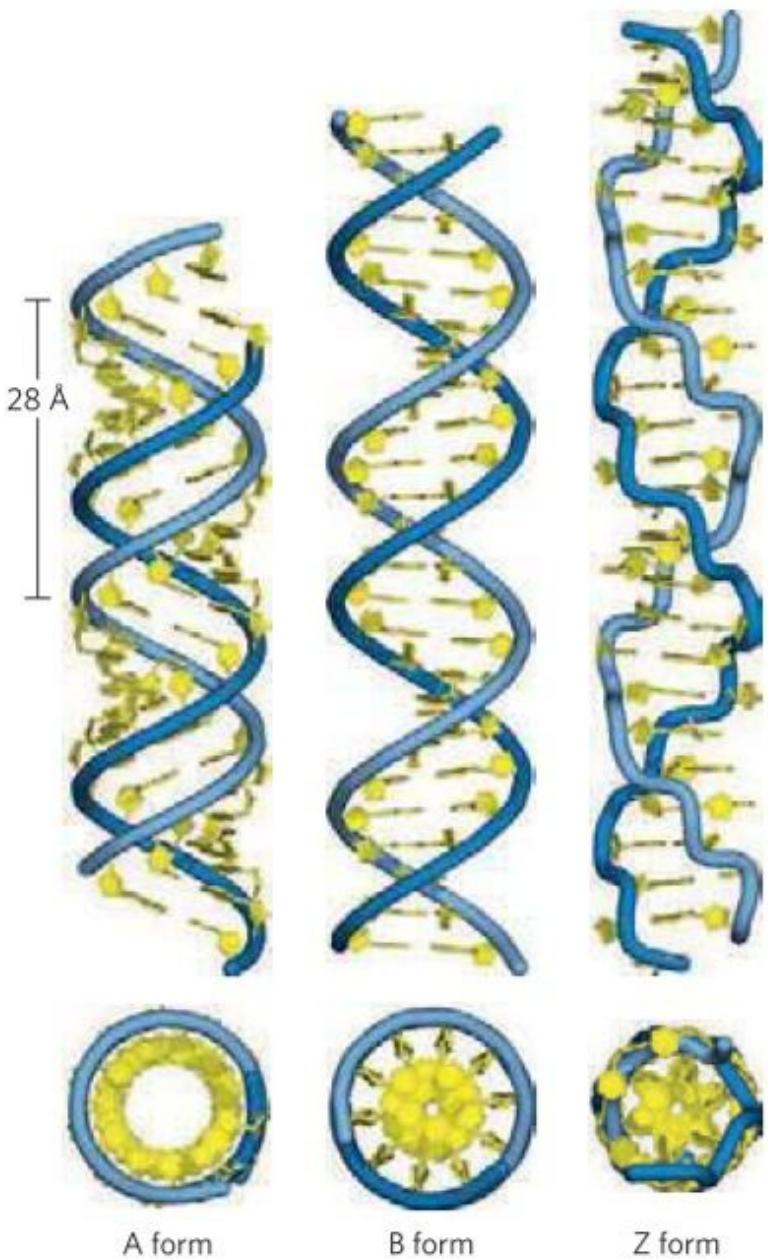


FIGURE 8-17 Comparison of A, B, and Z forms of DNA. Each structure shown here has 36 base pairs. The riboses and bases are shown in yellow. The phosphodiester backbone is represented as a blue rope. Blue is the color used to represent DNA strands in later chapters. The table summarizes some properties of the three forms of DNA.

The Watson and Crick's structure is also referred to as the B form and it is the most stable structure under physiological conditions.

A form is preferred in many solutions which are devoid of water.

	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3' endo	C-2' endo	C-2' endo for pyrimidines; C-3' endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

RNA (Ribonucleic Acid)

- RNA is the second major form of nucleic acid in cells.
- RNA carries genetic information from DNA to the protein biosynthetic machinery of the ribosome.
- By the process of transcription, RNA is synthesized from the DNA template.
- Three types of RNAs involved in protein synthesis:
 - 1) mRNA: Messenger RNA
 - 2) tRNA: Transfer RNA
 - 3) rRNA: Ribosomal RNA

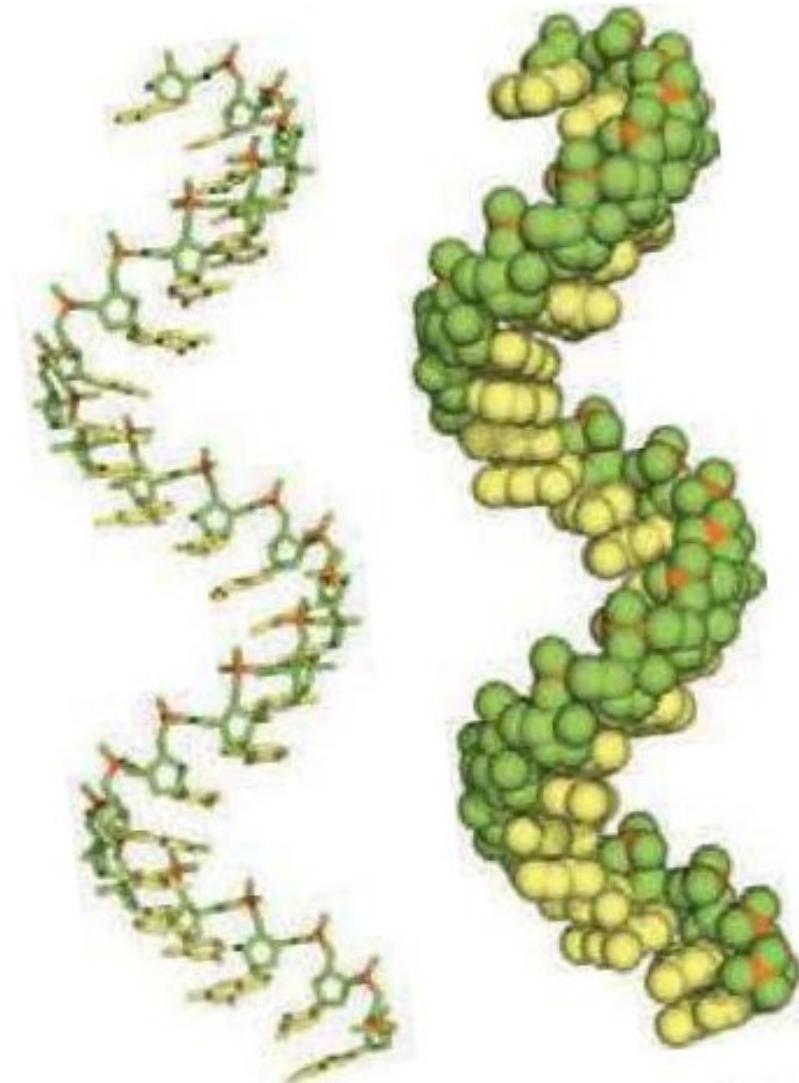
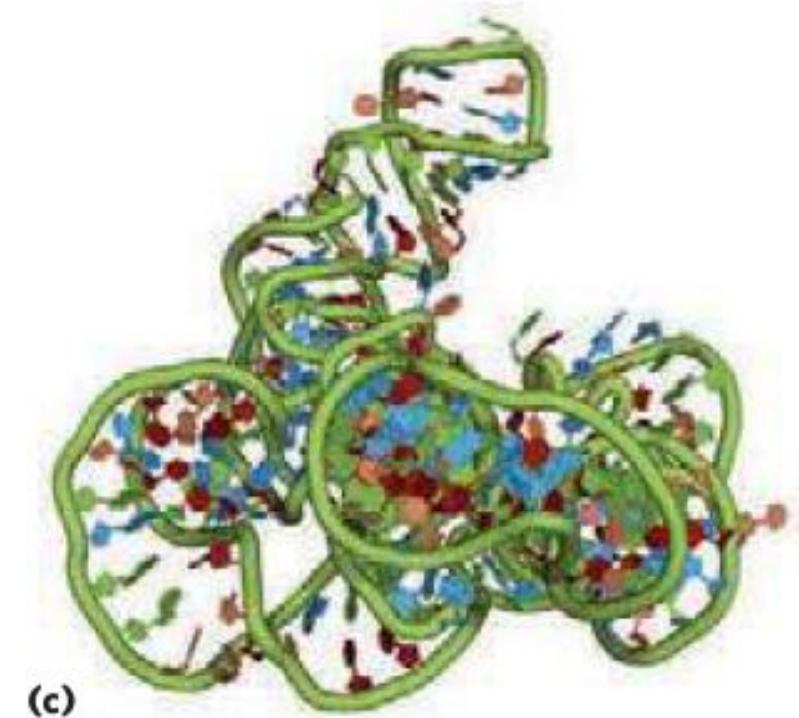
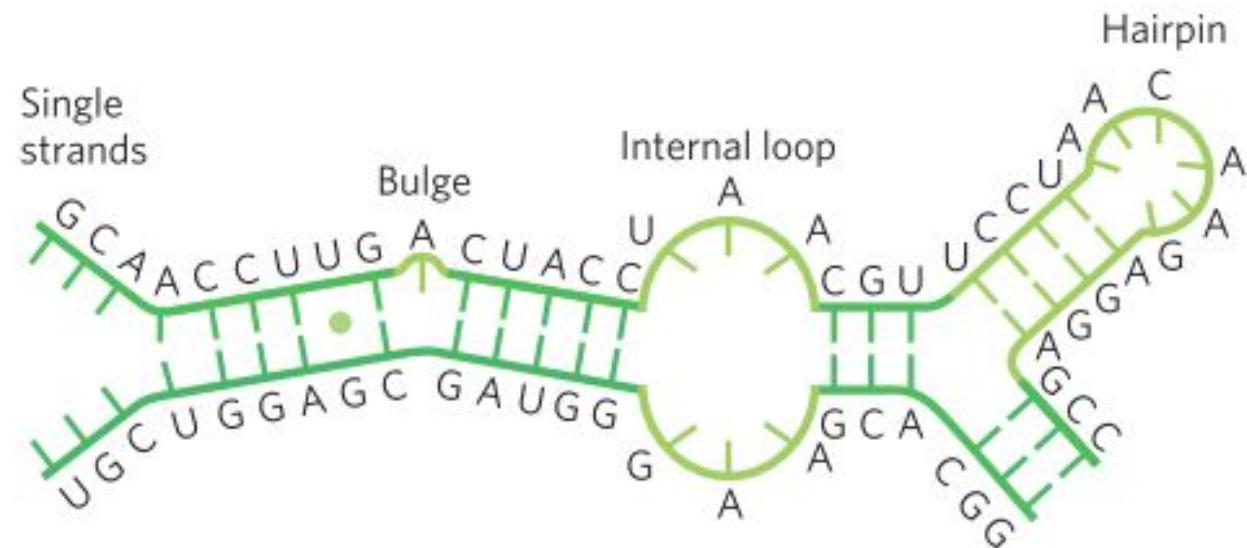
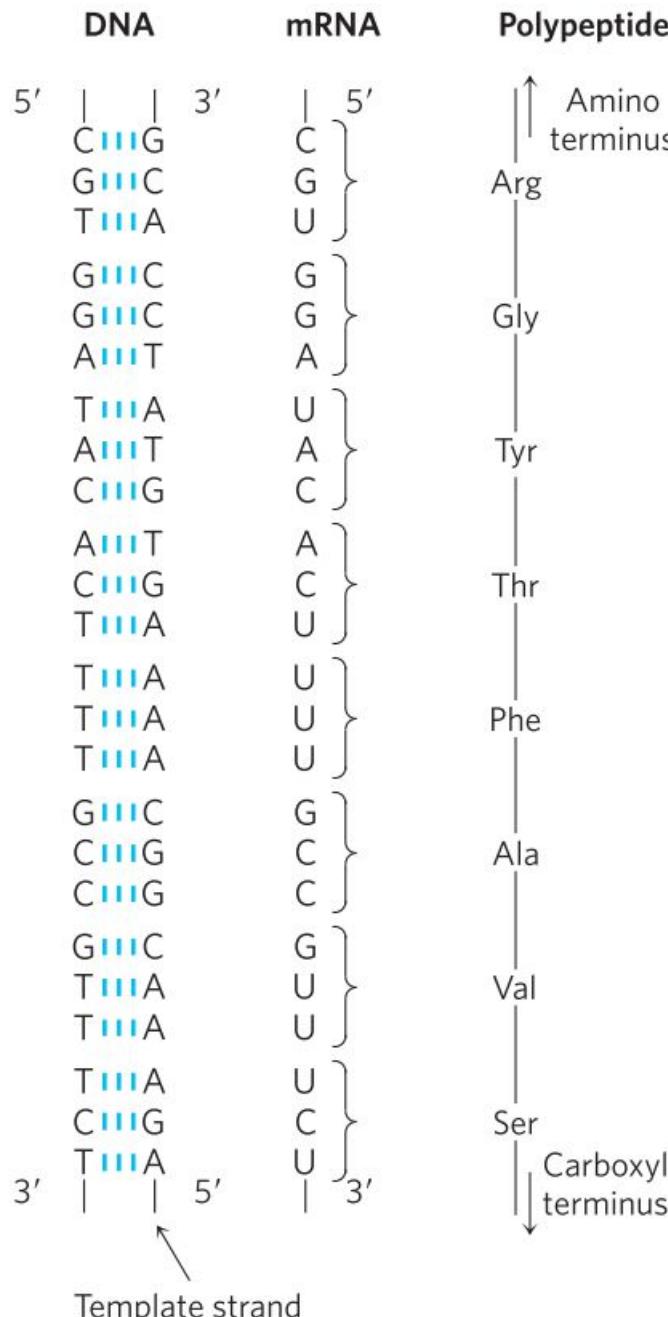


FIGURE 8-22 Typical right-handed stacking pattern of single-stranded RNA. The bases are shown in yellow, the phosphorus atoms in orange, and the riboses and phosphate oxygens in green. Green is used to represent DNA strands in succeeding chapters, just as blue is used for DNA.

Secondary and Tertiary Structures of RNA



Central Dogma of the Molecular Biology



Further reading

Book: Lehninger: Principles of Biochemistry, 6th edition. ISBN: 1464109621.

Chapter 3: Amino Acids, Peptides, and Proteins, Page 75-114.

Chapter 8: Nucleotides and Nucleic Acids, Page 281-312.

Next class on 20/06/2022 (Monday)

BT1010 Introduction to Life Sciences



Lecture 3: DNA-Based Information Technologies

20/06/2022

Course Instructor:

Dr. Gunjan Mehta, Ph.D.

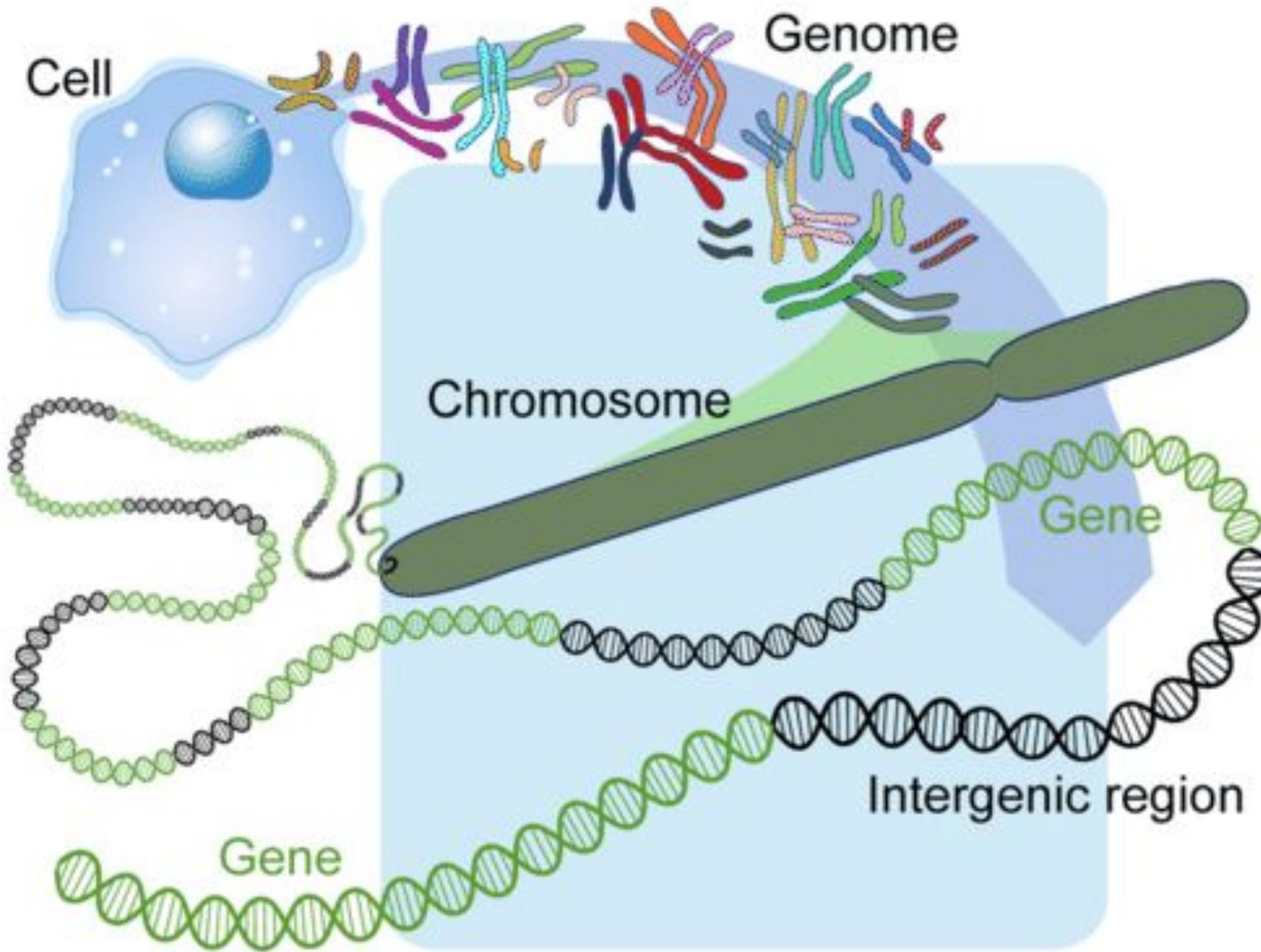
Assistant Professor

Department of Biotechnology

IIT Hyderabad

(M.) +91 70168 96886 Email: gunjanmehta@bt.iith.ac.in

What is the difference between a Gene, a Chromosome and a Genome?



Human Genome:

2 meter long DNA

20000-25000 genes

23 chromosomes

Gene (DNA)

↓
Transcription

RNA

↓
Translation

Protein

Cloning and its types

- Cloning is a technique scientists use to make exact genetic copies of living organisms, cells, tissue, DNA.
- Three types of cloning:
 - 1) Reproductive cloning/organism cloning (**legally restricted**)
 - 2) Therapeutic cloning/stem cell cloning
 - 3) Molecular cloning/DNA cloning

Dolly the sheep: was the first mammal to have been successfully cloned from an adult somatic cell.

Reproductive cloning/ Organism cloning



Dolly's taxidermied remains at the National Museum of Scotland.

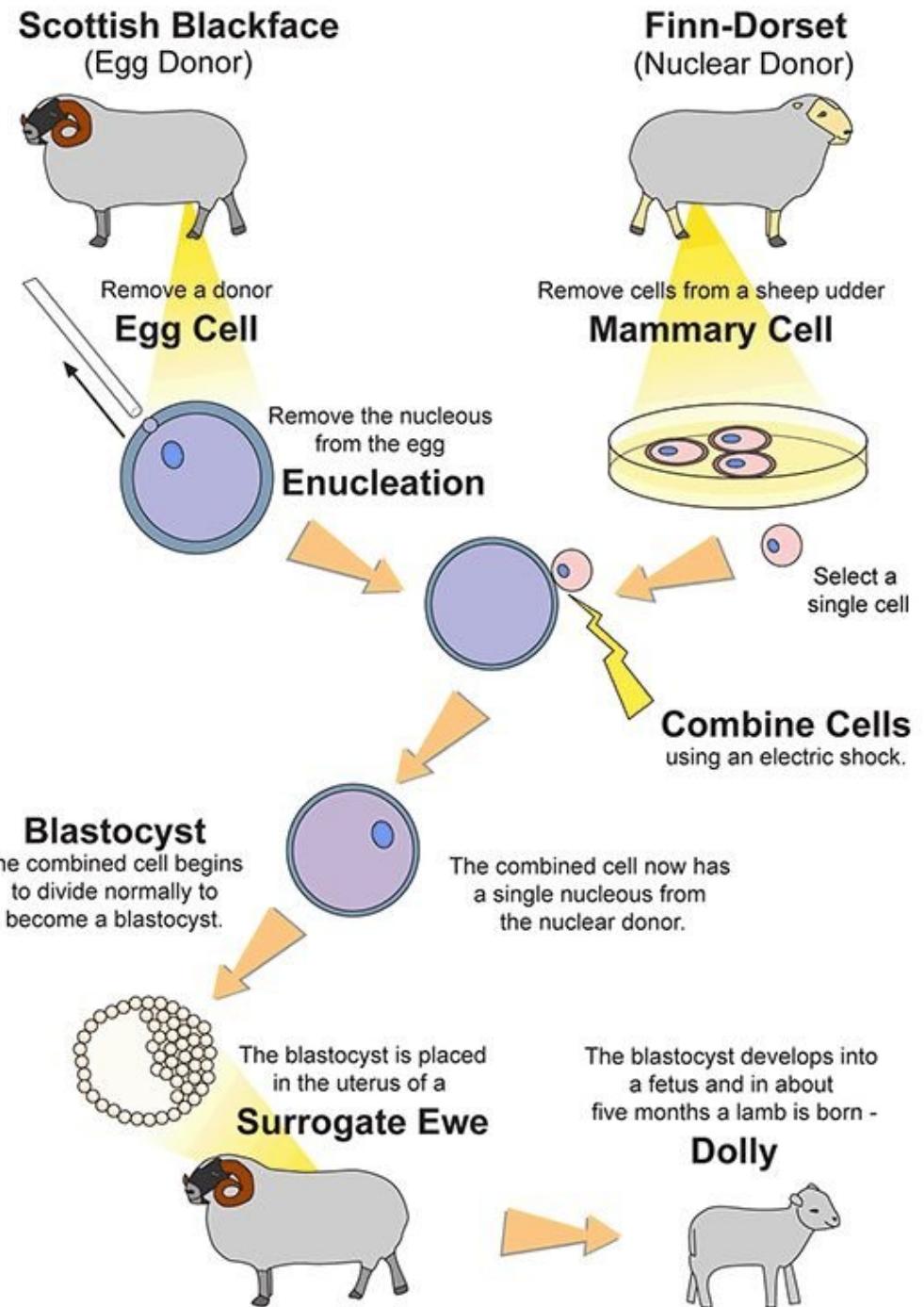


Professor Ian Wilmut cloned Dolly in 1996 at the Roslin Institute of Scotland

Reproductive cloning:

How Dolly was cloned?

- Dolly had three mothers — a DNA donor, an egg donor, and a surrogate.



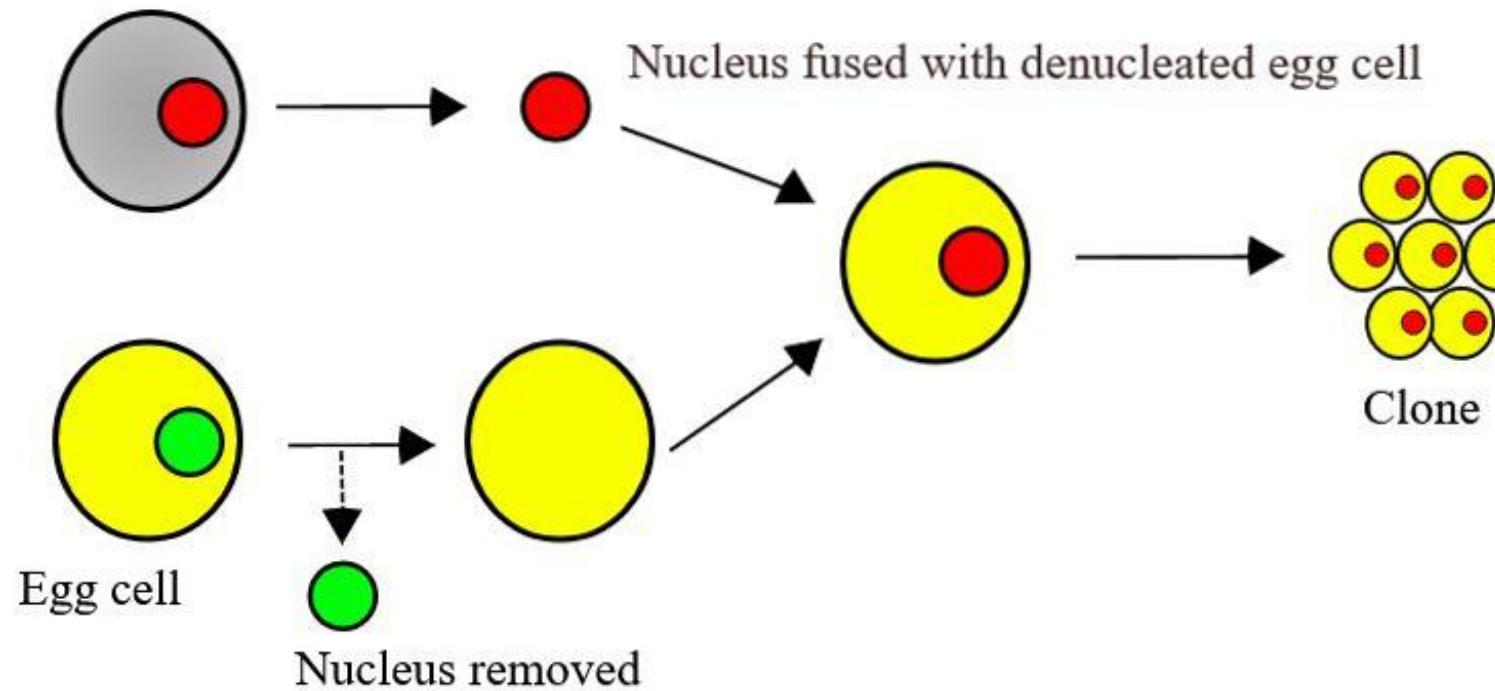
Ethical Issues of Cloning Animals

- There are a variety of ethical concerns regarding the possibilities of cloning, especially human cloning.
- While many views are religious in origin, there are also secular perspectives.
- Advocates support development of therapeutic cloning to generate tissues and whole organs to treat patients.
- Advocates for reproductive cloning believe that patients who cannot reproduce should have access to the technology.
- Opponents of cloning have concerns that the technology can be prone to abuse and how cloned individuals could integrate with families and with society at large.
- Religious groups believes that the technology can be misused as usurping “God’s place”. Also this technology uses embryos, so it destroys human life. Other religious groups support therapeutic cloning’s potential life-saving benefits.
- Cloning of animals is opposed by animal-groups due to the number of cloned animals that suffer from malformations before they die.

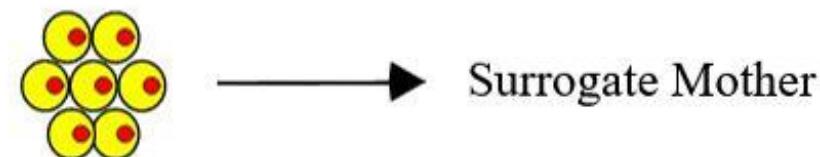
Therapeutic cloning/Stem cell cloning

- In multicellular organisms, **stem cells** are undifferentiated cells that can differentiate into various types of cells and proliferate indefinitely to produce more of the same stem cell.

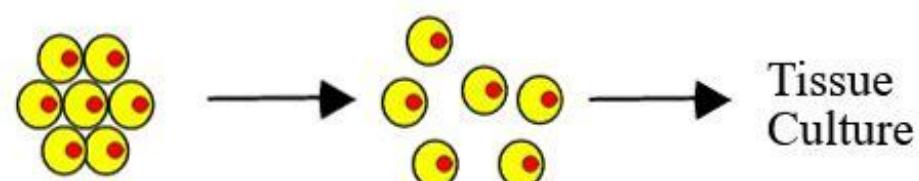
Somatic body cell with desired genes



REPRODUCTIVE CLONING

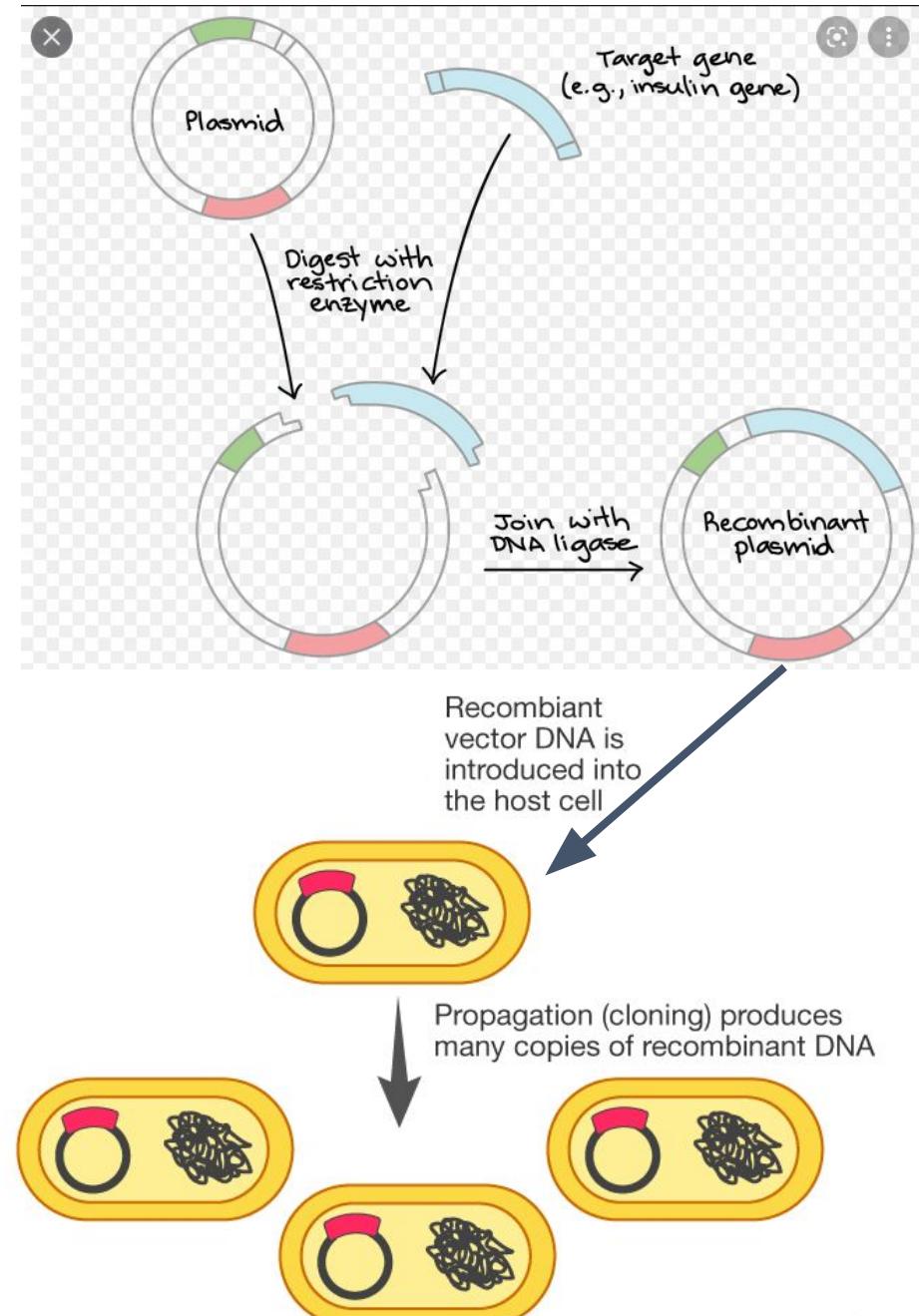


THERAPEUTIC CLONING



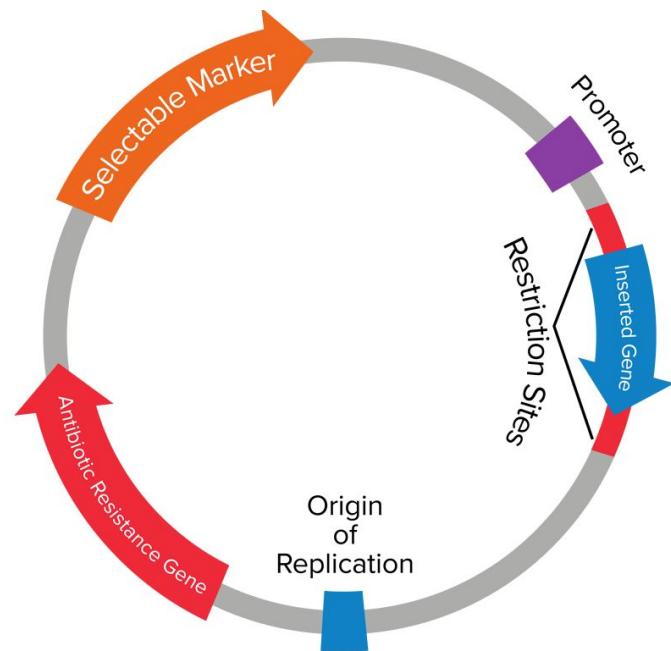
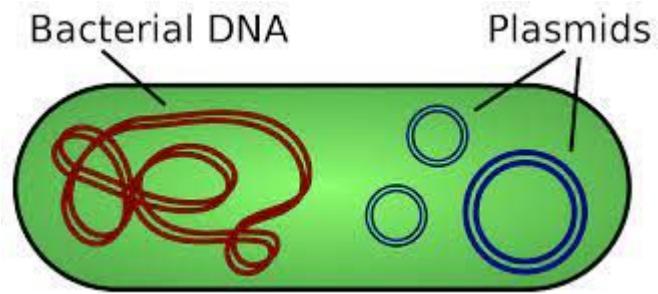
DNA Cloning / Molecular Cloning / Recombinant DNA Technology / Genetic Engineering

- DNA cloning is a molecular biology technique that makes many identical copies of a piece of DNA, such as a gene (e.g. Insulin gene).
- In a typical DNA cloning procedure, the gene (perhaps a gene for a medically important human protein) is first inserted into a circular piece of DNA called a **plasmid**. The insertion is done using enzymes that “cut and paste” DNA, and it produces a molecule of **recombinant DNA**.
- Cloning of DNA entails five general procedures:
 - 1) **Cutting target DNA at precise locations.** (Sequence-specific endonucleases) provide the necessary molecular scissors.
 - 2) **Selecting a small carrier DNA capable of self-replication.** These DNAs are called cloning vectors or plasmids.
 - 3) **Joining two DNA fragments covalently.** DNA ligase is an enzyme that links cloning vector and the DNA to be cloned. The resultant DNA molecule is known as recombinant DNA.
 - 4) **Moving recombinant DNA from the test tube to a host cell** that will provide enzymatic machinery for DNA replication.
 - 5) **Selecting host cells** that contain recombinant DNA.
- The methods used to accomplish these and related tasks are collectively referred to as **recombinant DNA technology** or **Genetic engineering**.



Plasmid (Vector)

- A plasmid is a small, extrachromosomal DNA within a cell that is physically separated from chromosomal DNA and can replicate independently.
- In nature, plasmids often carry genes that benefit the survival of the organism and confer selective advantage such as antibiotic resistance.
- Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA within host organism.



Vector Element	Description
Origin of Replication (ORI)	DNA sequence which allows initiation of replication within a plasmid by recruiting replication machinery proteins
Antibiotic Resistance Gene	Allows for selection of plasmid-containing bacteria.
Multiple Cloning Site (MCS)	Short segment of DNA which contains several restriction sites allowing for the easy insertion of DNA. In expression plasmids, the MCS is often downstream from a promoter.
Insert	Gene, promoter or other DNA fragment cloned into the MCS for further study.
Promoter Region	Drives transcription of the target gene. Vital component for expression vectors: determines which cell types the gene is expressed in and amount of recombinant protein obtained.
Selectable Marker	The antibiotic resistance gene allows for selection in bacteria. However, many plasmids also have selectable markers for use in other cell types.
Primer Binding Site	A short single-stranded DNA sequence used as an initiation point for PCR amplification or sequencing. Primers can be exploited for sequence verification of plasmids.

Cloning vectors (plasmids) allow amplification of inserted DNA segments

- Plasmids: A plasmid is a circular DNA molecule that replicates separately from the host chromosome.
- Their size ranges from 5000 to 400000 bp.
- To survive in the host cell, plasmids incorporate several specialized sequences that enable them to make use of the cell's resources for their own replication and gene expression.
- pBR322, constructed in 1977, is a good example of a plasmid with features useful in almost all cloning vectors.
 - 1) Origin of replication (ori), a sequence where replication is initiated by cellular enzymes. An associated regulatory system is present that limits replication to maintain pBR322 at a level of 10 to 20 copies per cell.
(Two different plasmids cannot function in the same cell if they use the same ori.)
 - 1) Antibiotic resistance genes Tet^R (Tetracycline Resistance) and Amp^R (Ampicillin Resistance) allow selection of cells that contain the intact plasmid or a recombinant version of the plasmid.
 - 1) RE sites to be cut and insert foreign DNA.
 - 2) Small size of the plasmid (4361 bp) facilitates its entry into cells and the biochemical manipulation of the DNA.

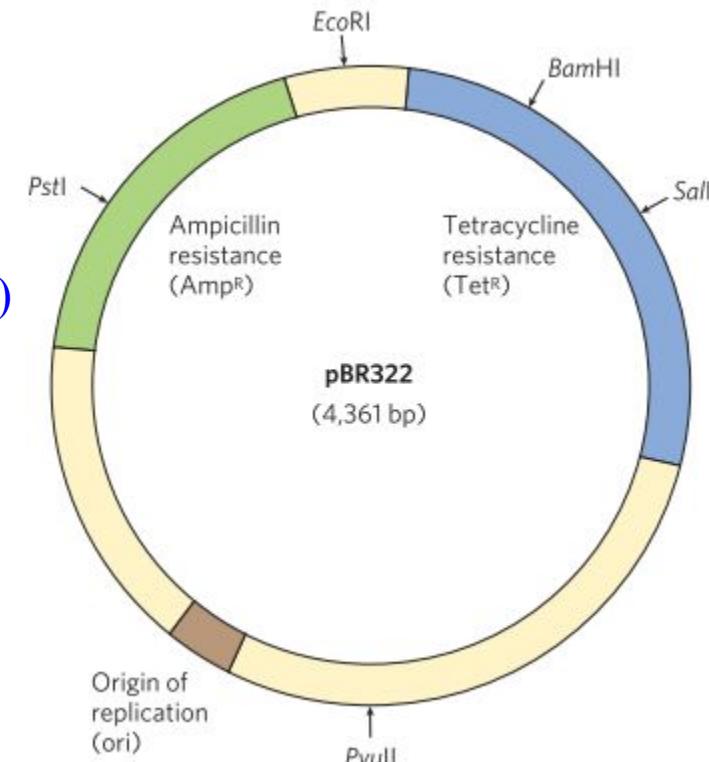


TABLE 9–1 Some Enzymes Used in Recombinant DNA Technology

Enzyme(s)	Function
Type II restriction endonucleases	Cleave DNAs at specific base sequences
DNA ligase	Joins two DNA molecules or fragments
DNA polymerase I (<i>E. coli</i>)	Fills gaps in duplexes by stepwise addition of nucleotides to 3' ends
Reverse transcriptase	Makes a DNA copy of an RNA molecule
Polynucleotide kinase	Adds a phosphate to the 5'-OH end of a polynucleotide to label it or permit ligation
Terminal transferase	Adds homopolymer tails to the 3'-OH ends of a linear duplex
Exonuclease III	Removes nucleotide residues from the 3' ends of a DNA strand
Bacteriophage λ exonuclease	Removes nucleotides from the 5' ends of a duplex to expose single-stranded 3' ends
Alkaline phosphatase	Removes terminal phosphates from either the 5' or 3' end (or both)

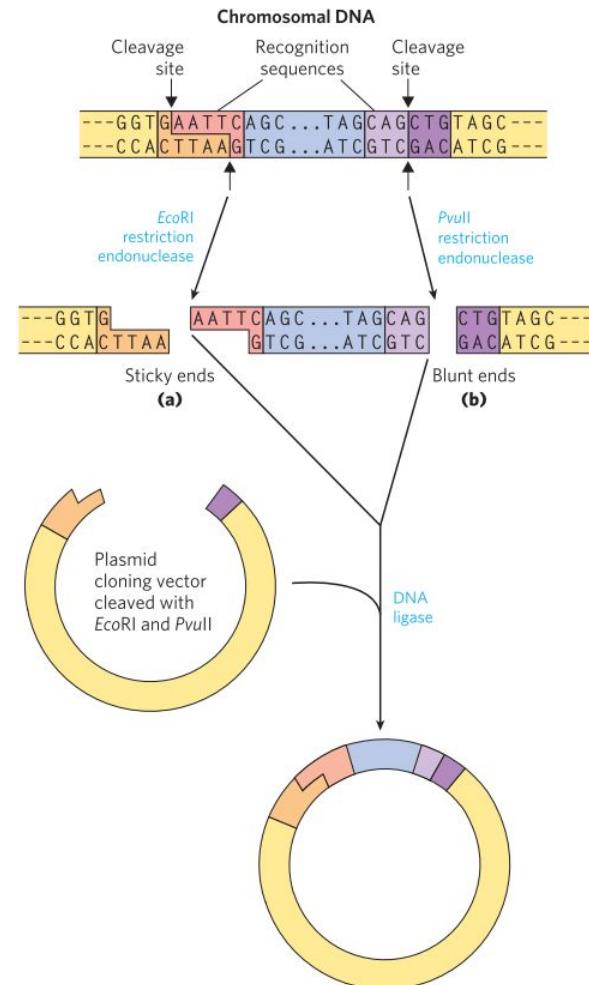
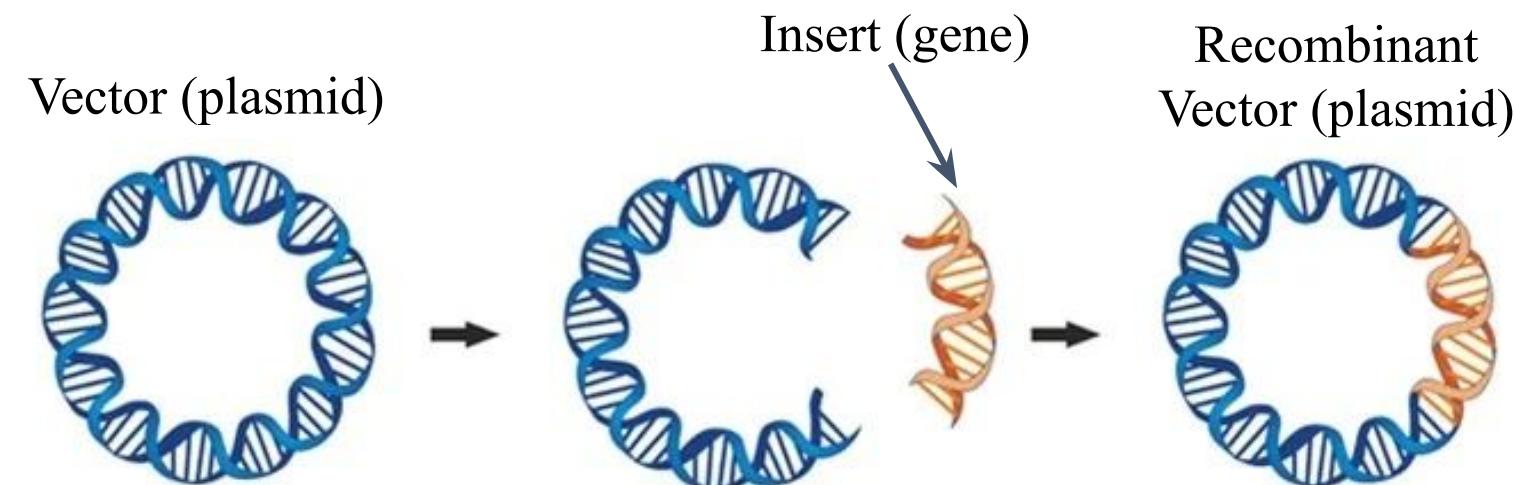
Restriction Endonucleases

- **Restriction endonucleases (RE)**, also called **restriction enzymes**, recognize and cleave DNA at specific sequences (**recognition sequences** or **restriction sites**) to generate a set of smaller fragments.
- REs are found in a wide range of bacterial species. However, in its host cells, REs can't digest its DNA because of methylation of the DNA bases. This mechanism is known as **Restriction Modification System**.
- Three types of REs: Type I, Type II and Type III
- Type I and III are generally large, multisubunit complexes containing both the endonuclease and methylase activities. They require ATP for energy.
- Type I REs cleave DNA at random sites that can be more than 1000 bp from the recognition sequences.
- Type III REs cleave the DNA about 25 bp from the recognition sequences.
- Type II REs cleave the DNA within the recognition sequence itself. They don't need ATP. The recognition sequences are usually 4 to 6 bp long and they are palindromic.

Some of the Type II REs make staggered cuts on the two DNA strands, leaving two to four nucleotides of one strand unpaired at each resulting end. These unpaired strands are referred to as sticky ends.

DNA ligase joins the DNA molecule

- DNA ligase is used to join two DNA molecules. It forms phosphodiester bonds between the DNA molecules.
- A vector (plasmid) digested by *Eco*RI can be ligated to an insert digested by the same RE. It can not be ligated to an insert digested by *Bam*HI.
- Blunt ends can also be ligated, albeit less efficiently.



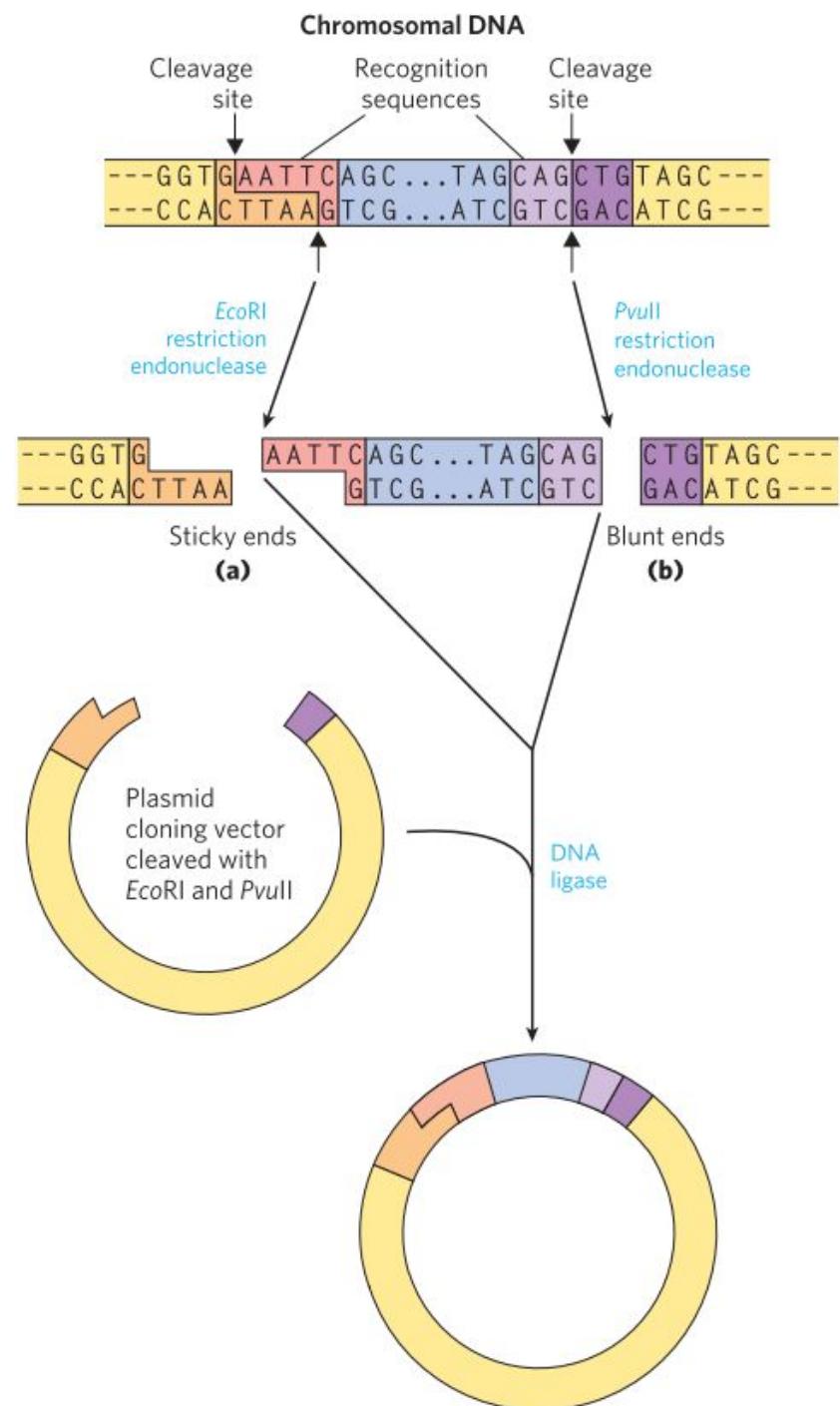


TABLE 9–2 Recognition Sequences for Some Type II Restriction Endonucleases

BamHI	(5') \downarrow G G A T C C (3') C C T A G G *↑	HindIII	(5') \downarrow A A G C T T (3') T T C G A A ↑
Clal	(5') \downarrow A T C G A T (3') T A G C T A *↑	NotI	(5') \downarrow G C G G C C G C (3') C G C C G G C G ↑
EcoRI	(5') \downarrow G A A T T C (3') C T T A A G *↑	PstI	(5') \downarrow C T G C A G (3') G A C G T C ↑
EcoRV	(5') \downarrow G A T A T C (3') C T A T A G ↑	PvuII	(5') \downarrow C A G C T G (3') G T C G A C ↑
HaeIII	(5') \downarrow G G C C (3') C C G G *↑	Tth11I	(5') \downarrow G A C N N N G T C (3') C T G N N N C A G ↑

In general, a 6 bp sequence recognized by a RE (such as BamHI) would occur on average once every 4^6 (4096) bp.

An enzyme that recognize a 4 bp sequence would occur on an average once every 4^4 (256) bp.

How to introduce plasmids into bacterial cells?

There are two ways:

1) Transformation:

The cells (often *E. coli*) and plasmid DNA are incubated together at 0 °C in a calcium chloride solution, then subjected to heat shock by rapid shifting the temperature to between 37 °C and 43 °C.

1) Electroporation:

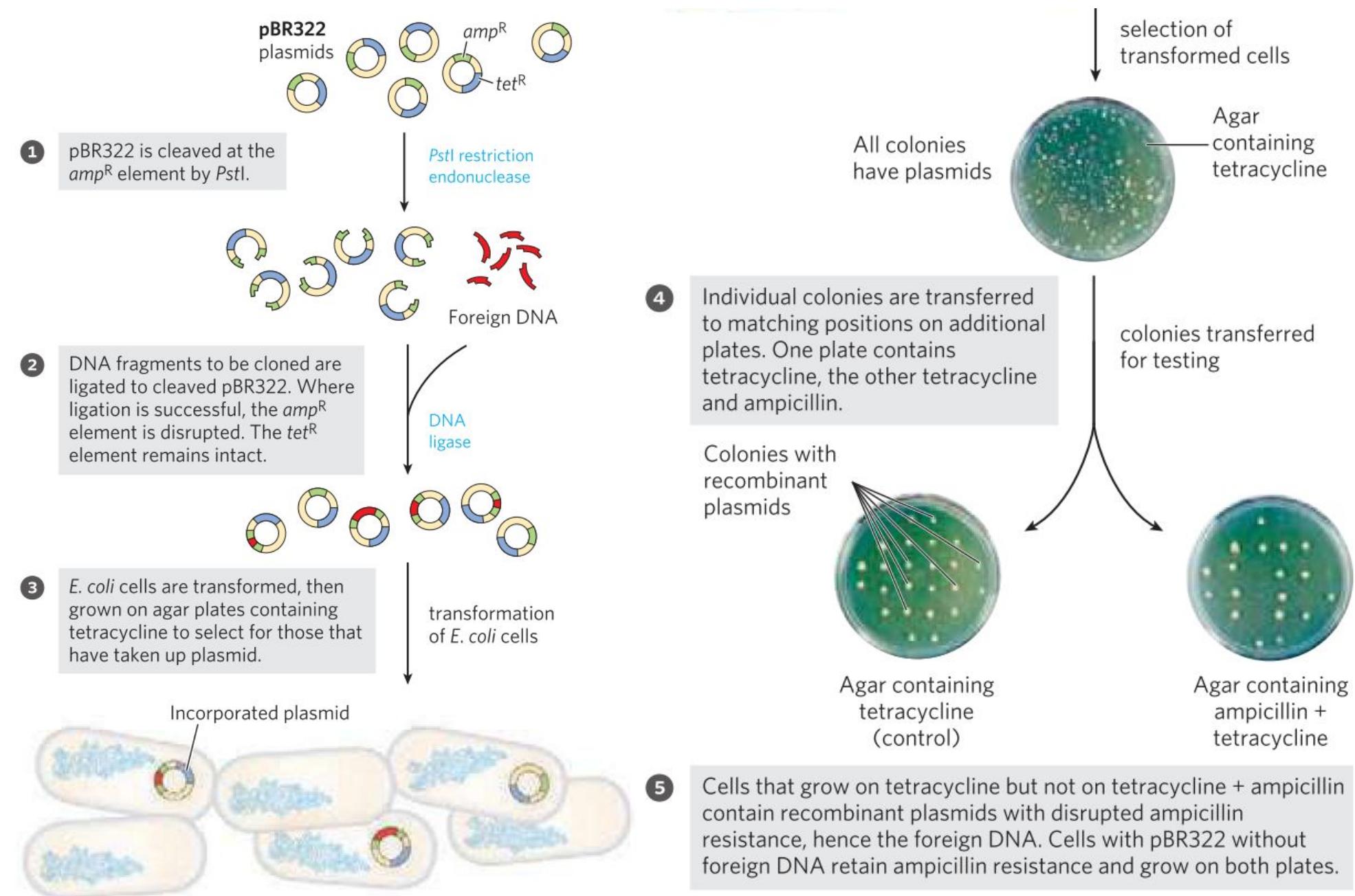
The cells (often *E. coli*) are incubated with plasmid DNA and subjected to high-voltage electric pulse. This approach transiently renders the bacterial membrane permeable to large molecules.

Regardless of the approach, relatively few cells take up the plasmid DNA, so a method is needed to identify those cells which contain the plasmid.

Plasmids usually contains one or two selectable markers (such as Tet^R, Amp^R).

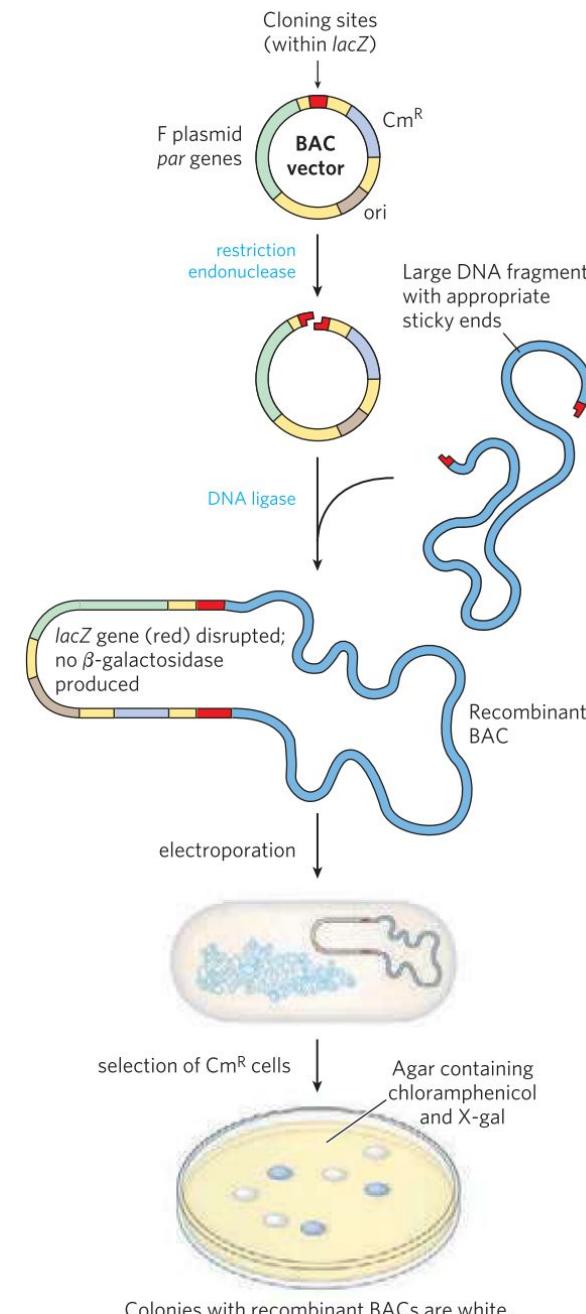
Use of pBR322 to clone foreign DNA in *E. coli* and identify cells containing it.

A cloning strategy with pBR322



Bacterial Artificial Chromosomes (BAC)

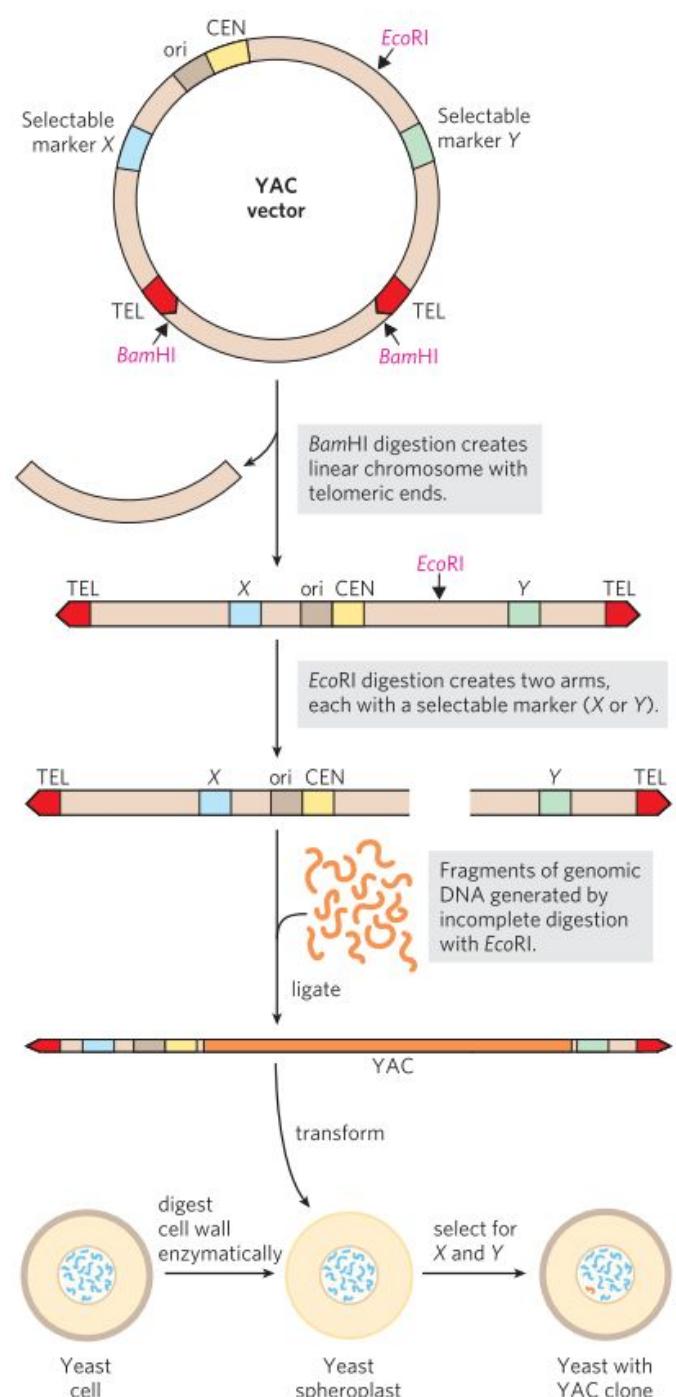
- Bacterial plasmids can clone up to 10 Kb DNA fragment, but to clone a large DNA fragment (typically 100 Kb to 300 Kb), a specialized plasmids are used which are known as Bacterial Artificial Chromosomes (BACs).
- BACs have stable ori, that maintain the plasmid at one or two copies per cell.
- The low copy number is useful in cloning large segments of DNA, because it limits the opportunities for unwanted recombination reactions that can unpredictably alter large cloned DNAs overtime.
- BACs contain *par* genes, which encode proteins that direct the reliable distribution of the recombinant chromosomes to daughter cells at cell division, so that each daughter cell carry one copy.
- BACs also contain a selection marker (Cm^R) and a screenable marker (LacZ).
- LacZ codes for β -galactosidase catalyze the conversion of the colorless molecule 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) to a blue product, which helps in differentiating cells containing BACs from the cells without BACs.
- If the gene (LacZ) is intact, the colonies will be blue. If the gene is disrupted by the introduction of a cloned DNA segment, the colony will be white.



Yeast Artificial Chromosomes (YACs)

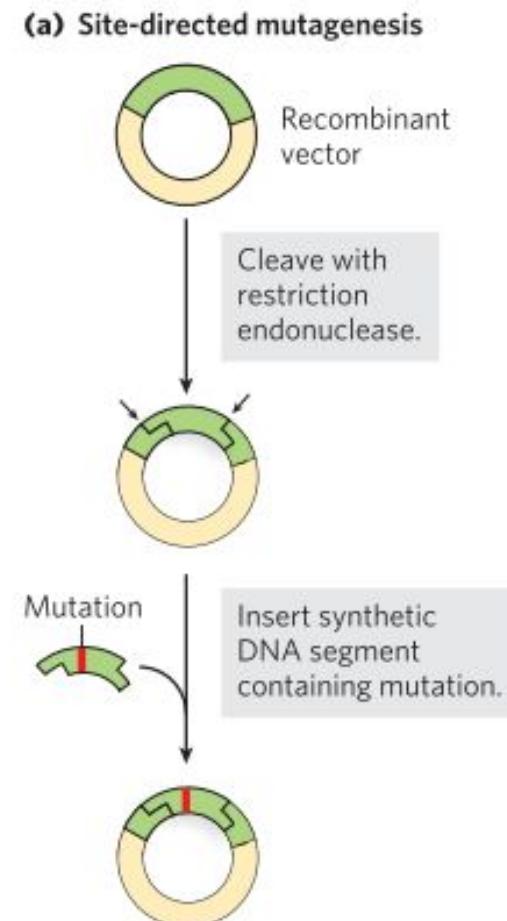
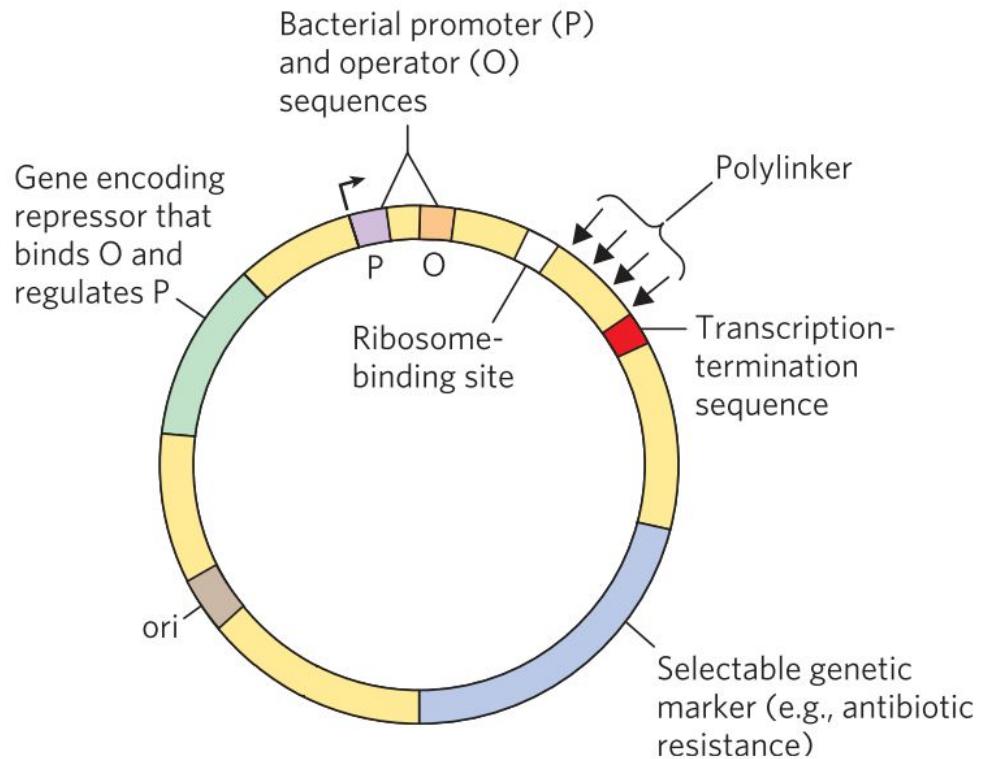
- Yeast *Saccharomyces cerevisiae* (baker's yeast) can be easily maintained and propagated in the laboratory.
- Some plasmids contain multiple Ori and other elements that allow them to be used in more than one species (e.g. in yeast and in *E. coli*). Such vectors are called shuttle vectors.
- YAC vectors contain all the elements needed to maintain a eukaryotic chromosome in the yeast nucleus: a yeast Ori, two selectable markers, and specialized sequences (derived from centromeres and telomeres) needed for stability and proper segregation of chromosomes at cell division.

- For its use in cloning, the vector (YAC) is propagated as a circular bacterial plasmid and then isolated and purified.
- Cleavage with *Bam*HI removes a length of DNA between two telomeres (TEL), leaving the telomeres at the ends of the linearized DNA.
- Cleavage at another internal site by *Eco*RI divides the vector into two DNA segments, each with a different selection marker.
- A DNA fragment (up to about 2×10^6 bp) are mixed with the prepared vector and ligated.
- The ligated mixture is then used to transform yeast cells. The cloned YAC now have structure and size to be considered yeast chromosomes.
- Based on the selection markers and the appropriate selection strategies, the yeast cells bearing YACs with large foreign DNA are selected.
- YACs with inserts of more than 150 Kb are nearly as stable as chromosomes, whereas inserts with less than 100 Kb are gradually lost during mitosis.



Cloned genes can be expressed to amplify protein production

- Frequently, the product of the cloned gene, rather than the gene itself, is of primary interest—particularly when the protein has commercial, therapeutic or research value.
- Cloning vectors with the transcription and translation signals needed for the regulated expression of a cloned gene are called **expression vectors**.
- Site-directed mutagenesis can be used to produce a mutant version of the protein.



Further reading

Book: Lehninger: Principles of Biochemistry, 6th edition. ISBN: 1464109621.

Chapter 9: DNA-Based Information Technologies, Page 313-355.

Next class on 22/06/2022 (Wednesday)

BT1010 Introduction to Life Sciences



Lecture 4: Microscopy to visualize cellular processes
22/06/2022

Course Instructor:

Dr. Gunjan Mehta, Ph.D.

Assistant Professor

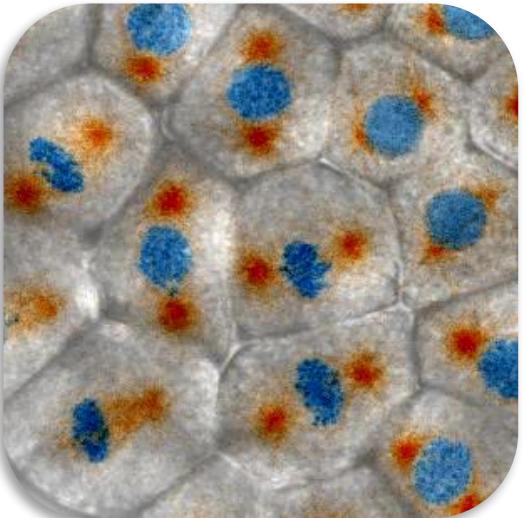
Department of Biotechnology

IIT Hyderabad

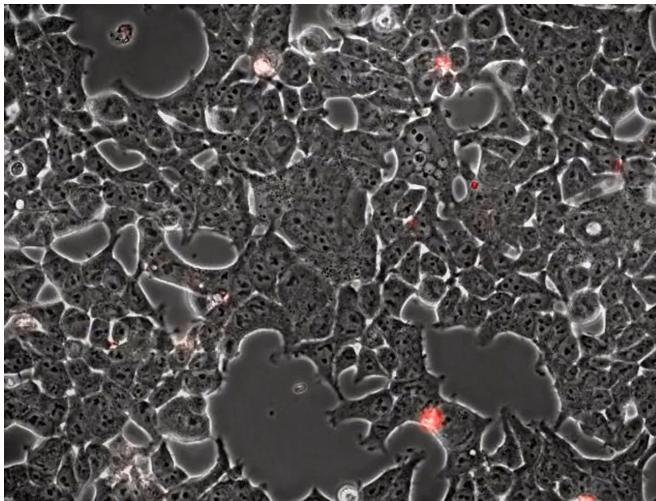
(M.) +91 70168 96886 Email: gunjanmehta@bt.iith.ac.in

Applications of Light Microscopy

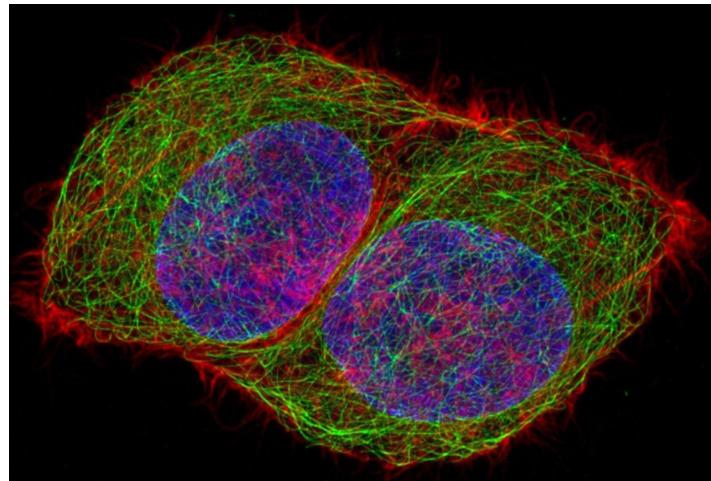
Seeing is believing



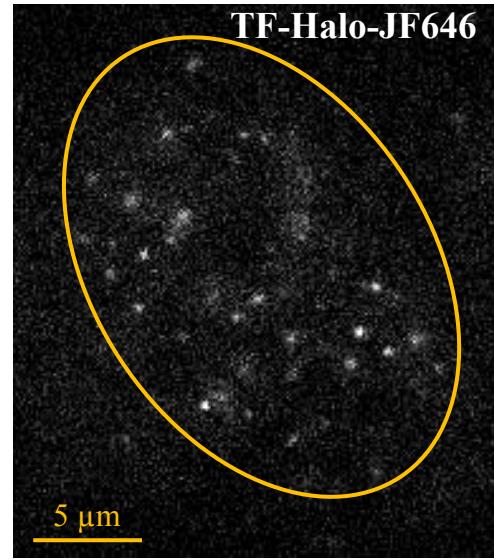
How cell divides and chromosomes Segregate?



How SARS-CoV-2 causes cell fusion and death in bat brain cells?



HeLa cells stained for DNA (blue), microtubules (green) and F-actin (red)



Single-Molecule Imaging of transcription factor (TF) in human cell nucleus

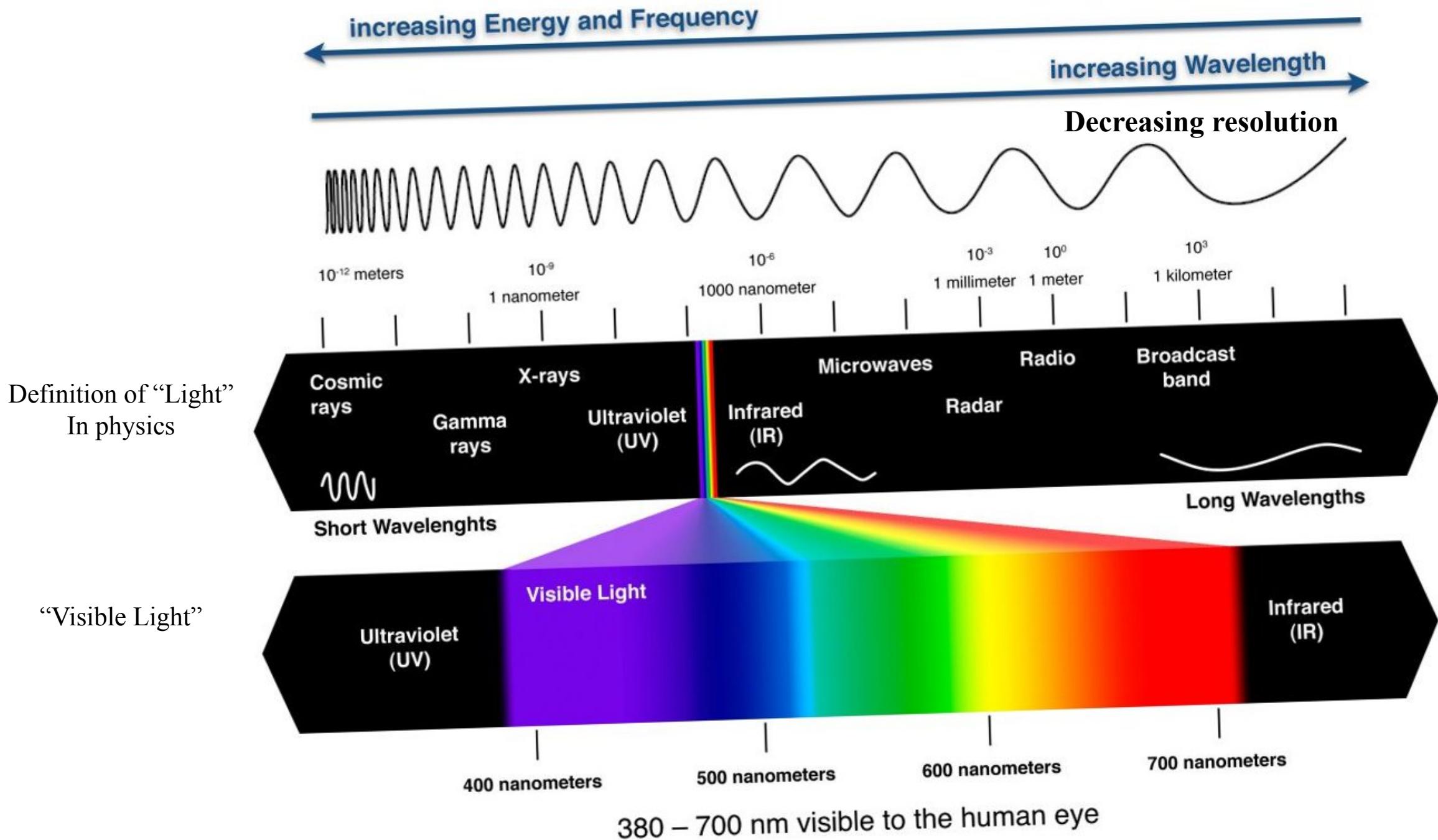
Mouse retina, confocal 3D microscopy: https://youtu.be/Jo7MvM_IXa8

3D imaging of collagen fibre network: <https://youtu.be/pof6ywsTK6Y>

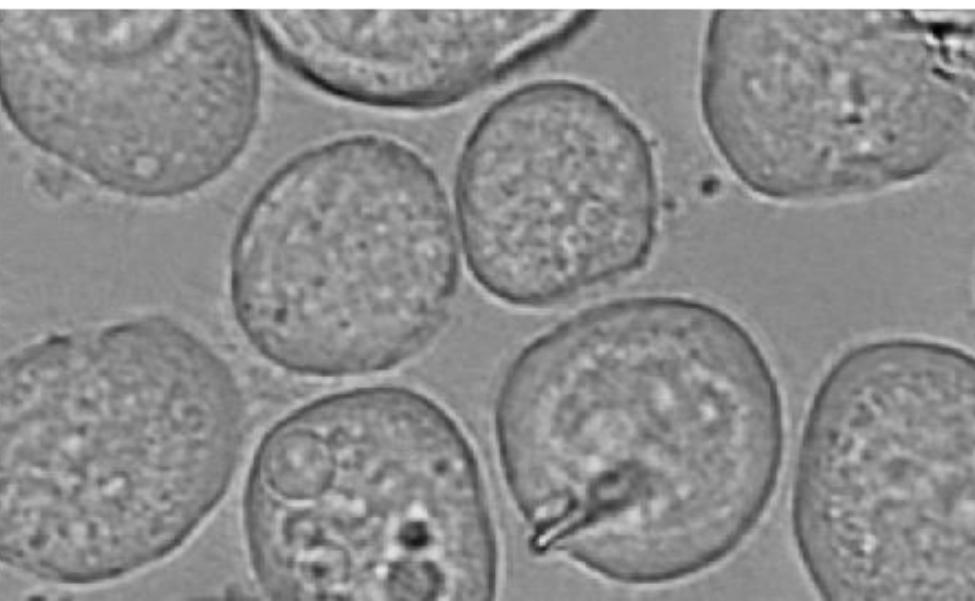
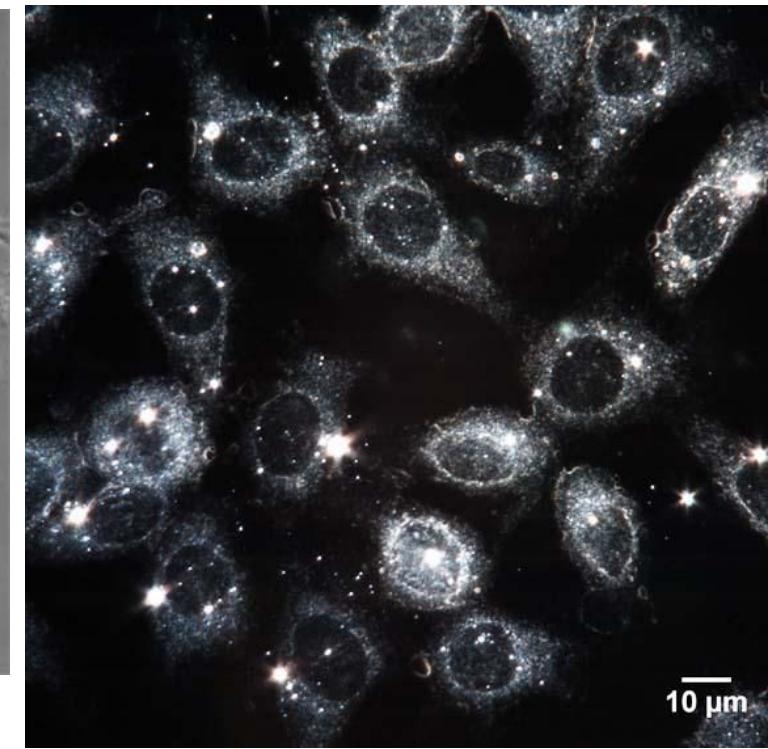
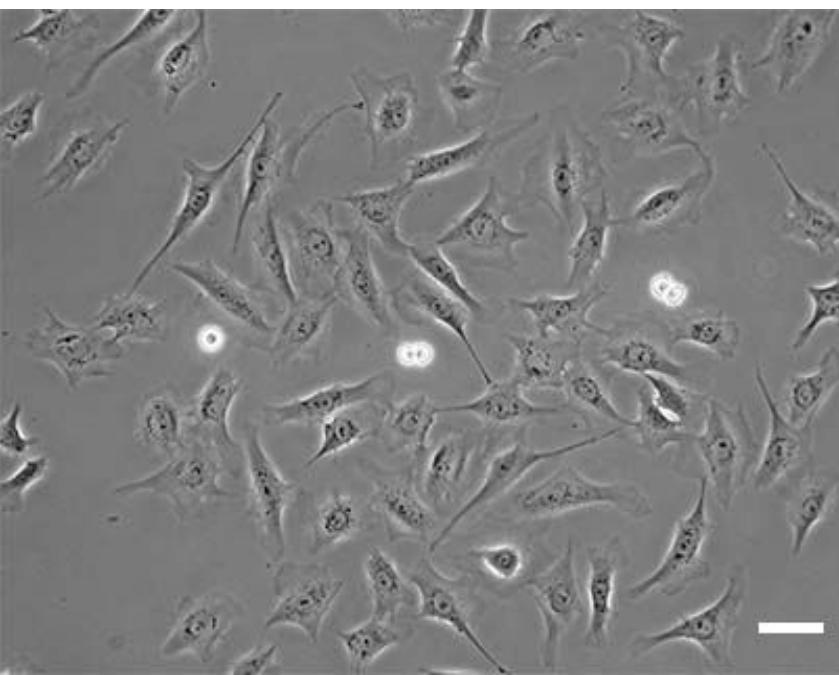
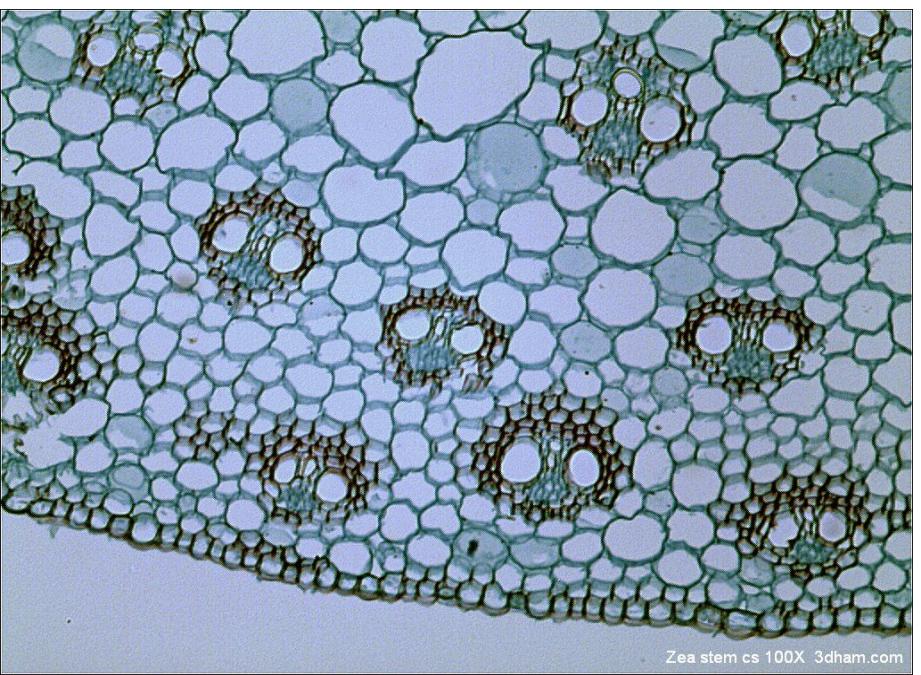
Kidney cells mitosis: <https://youtu.be/ed6OkXi6M5Q>

Zebrafish embryonic development at single-cell resolution: https://youtu.be/RQ6vkDr_Dec

Light: the electromagnetic spectrum



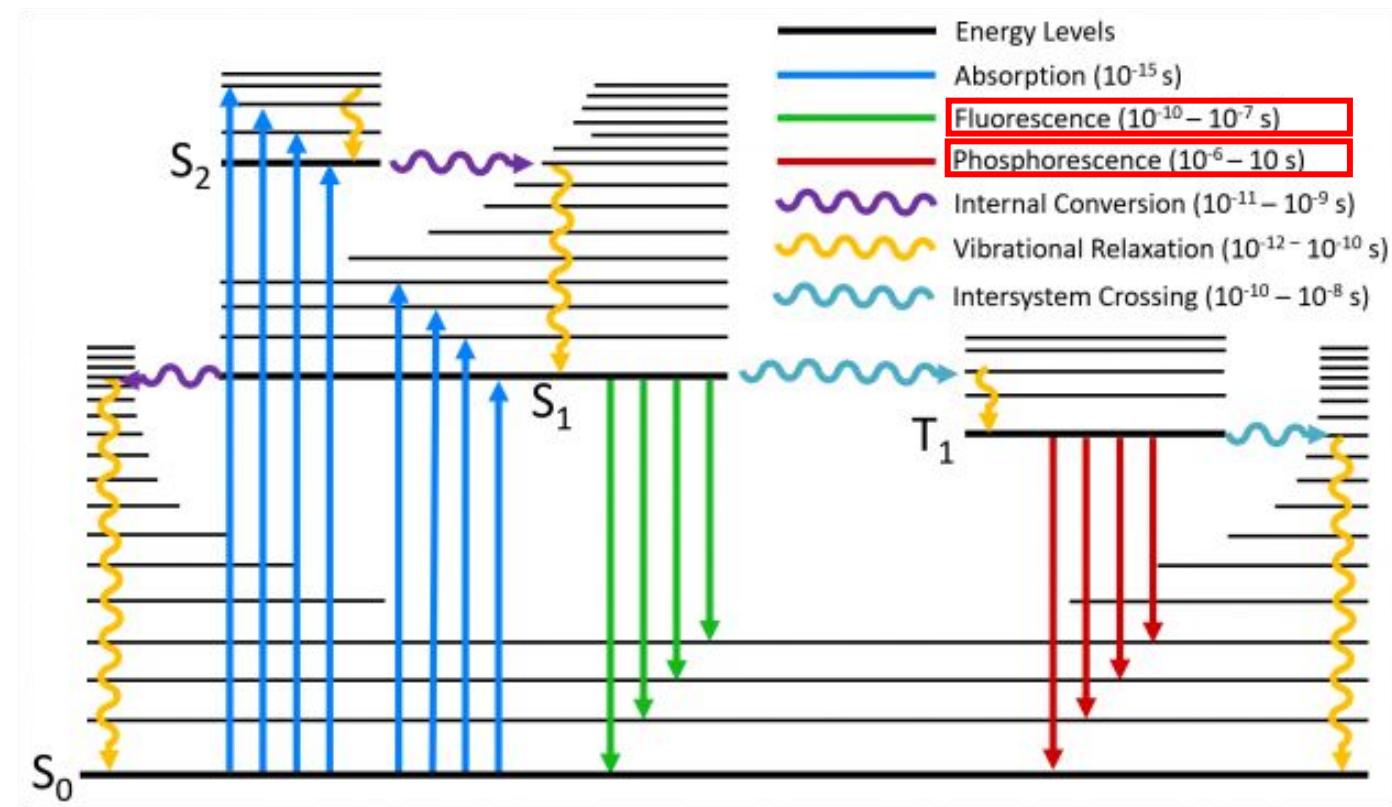
Bright field / Dark Field / Phase Contrast Microscopy



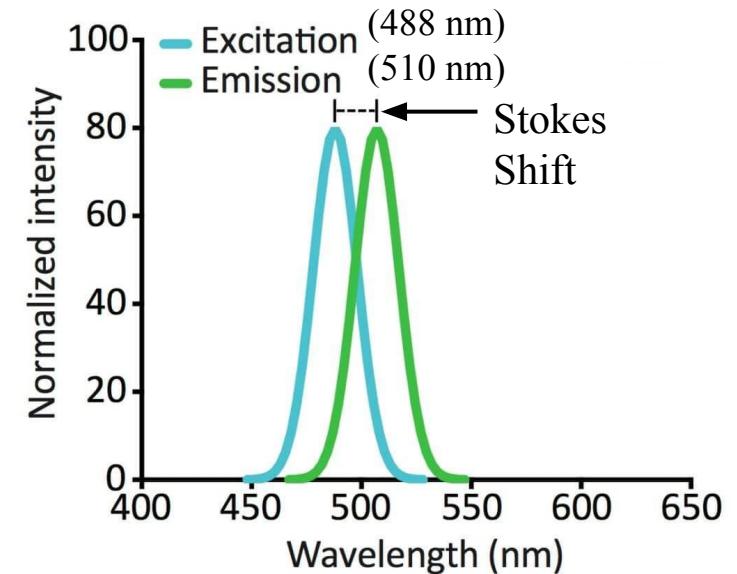
What is fluorescence?

- Fluorescence is the emission of light by a molecule that has absorbed light.
- Fluorescence has higher wavelength than absorbed light.

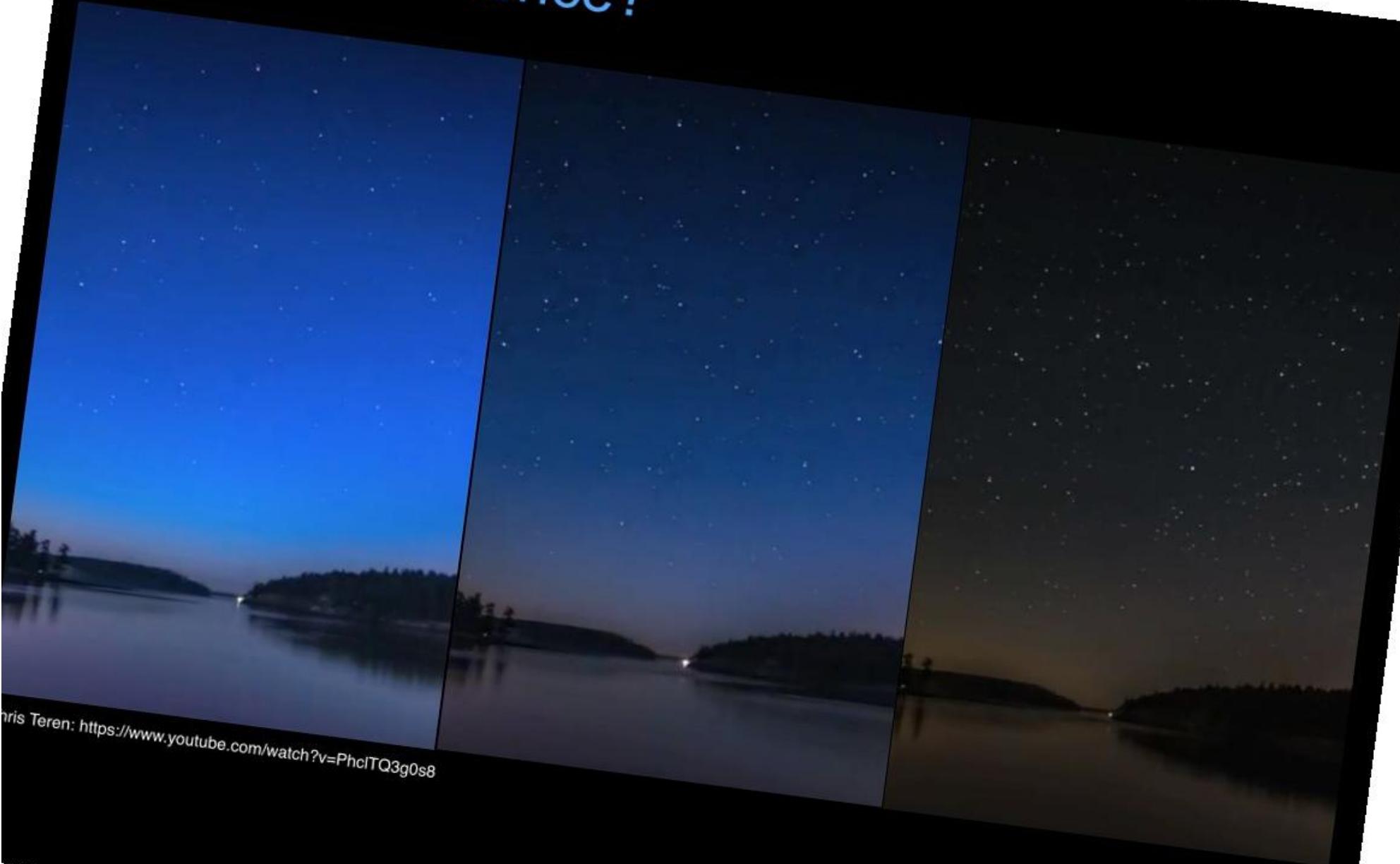
Jablonski diagram



Green Fluorescence Protein (GFP)



Why Fluorescence?

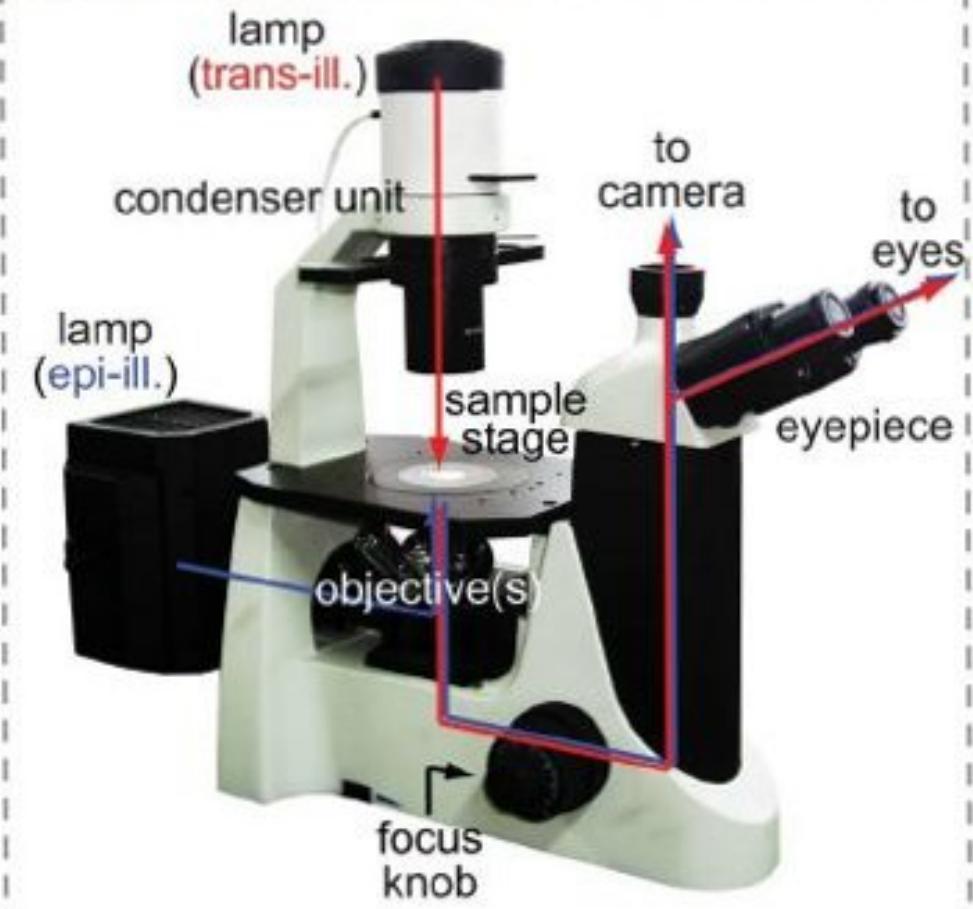


Chris Teren: <https://www.youtube.com/watch?v=PhcITQ3g0s8>

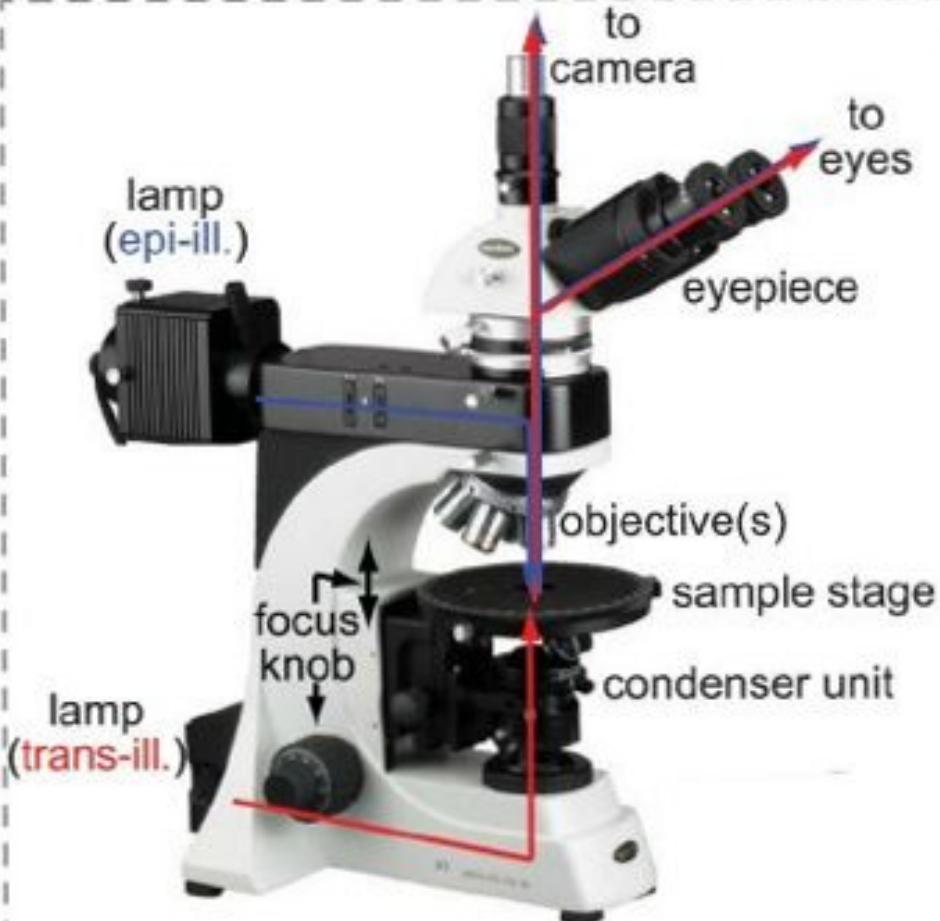
CONTRAST

Upright Vs. Inverted Microscope

INVERTED MICROSCOPE



UPRIGHT MICROSCOPE



Trans-
illumination
light path:



Epi-
illumination
light path:



Anatomy of the Fluorescence Microscope

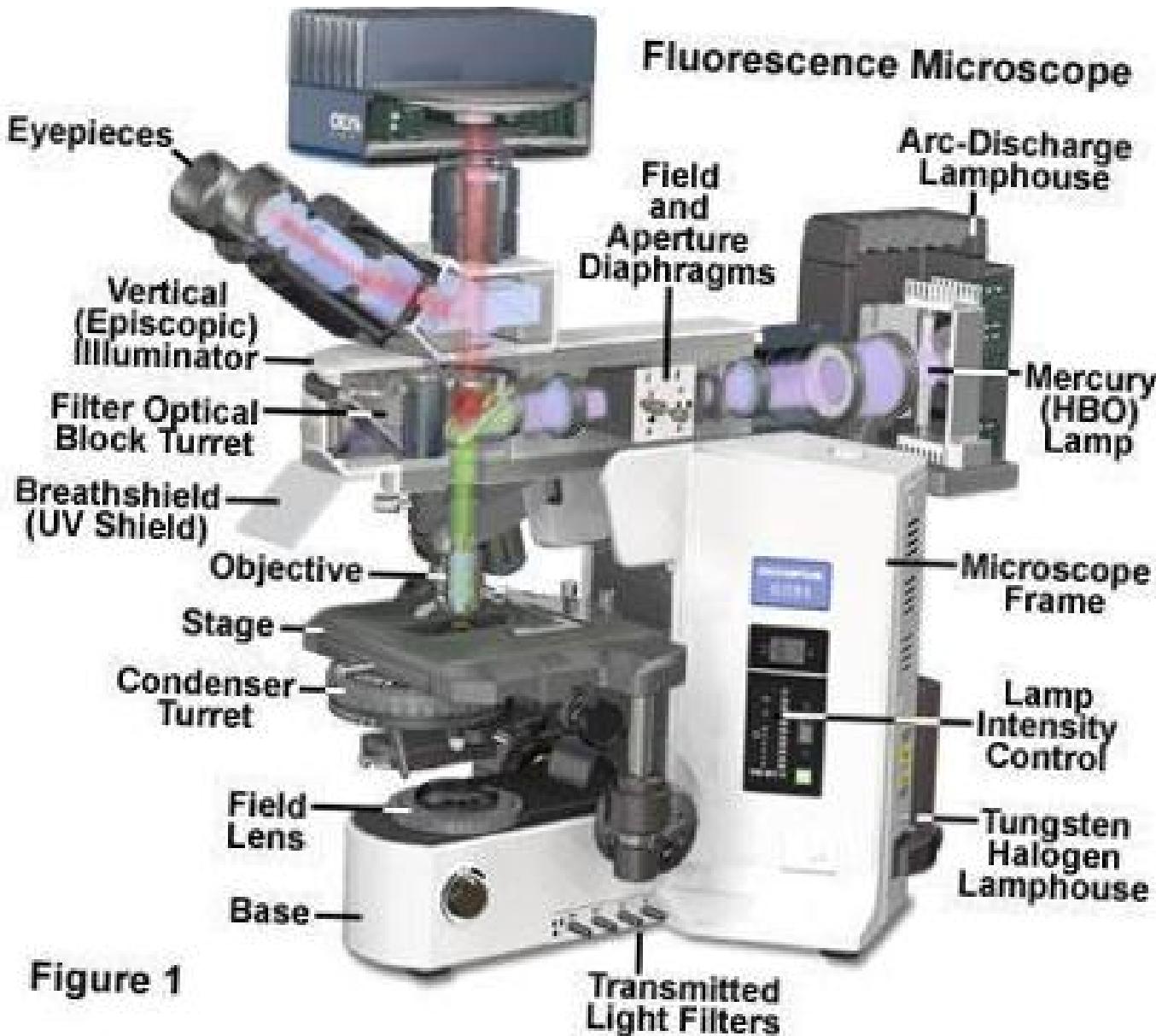


Figure 1

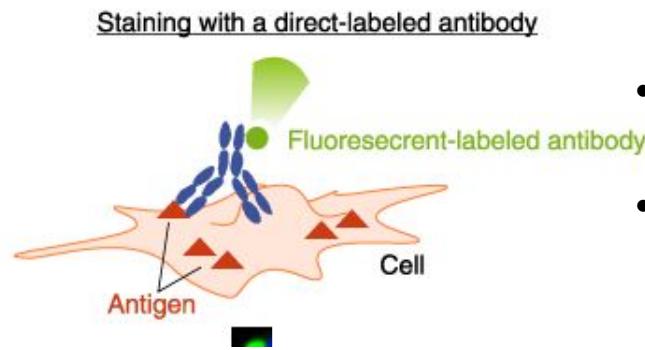
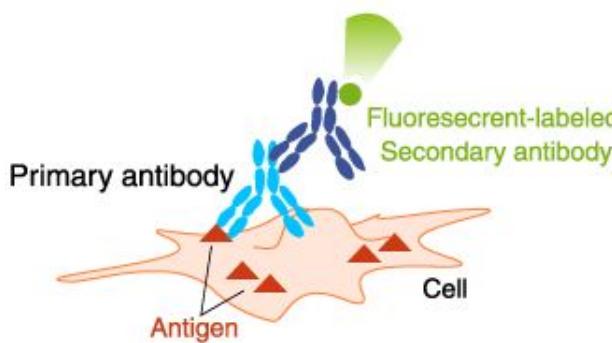
Protein Labelling Strategies

- Genetic encoding: C-terminal or N-terminal fusion of a gene of interest with a gene of fluorescent protein.



- Pros: Specificity of labeling, essential for live cell imaging
- Cons: Time required for genetic engineering

- Antibody based detection: (Immunofluorescence, immunohistochemistry)



- Pros: No genetic engineering is required if antibody is available commercially. So, less time consuming.
- Cons: suitable for fixed cells (not for live cells)
Non-specific binding may occur.

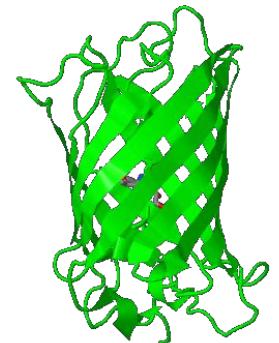
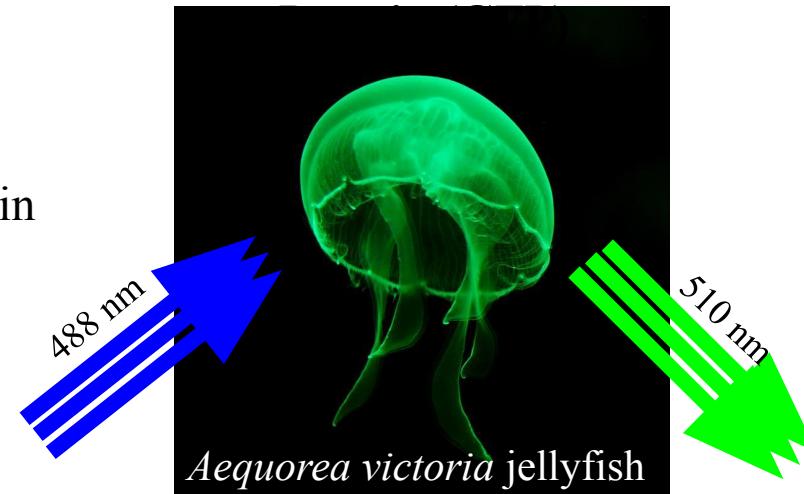
- Covalent modifications: (How to attach a fluorophore to the protein of interest?)

1. Self-labeling enzyme derivatives/tags: HaloTag (34 kD), SNAP-Tag (19 kD), CLIP-Tag (19 kD)
2. Chemical Labeling: fluorophore binds to the target protein through chemical modification (covalent or non-covalent binding). Pros: robust, easy to perform, efficient labelling. Cysteine and lysine are the nucleophilic amino acids which can be covalently linked to fluorescent dyes/probes. Suitable for *in-vitro* studies only.
3. Enzymatic Labeling: Enzymatic reaction allow fast, highly efficient and selective labeling *in-vivo* and *in-vitro* and can be used to target proteins or whole cells.

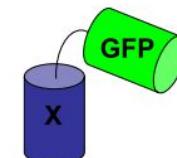
Discovery of Green Fluorescent Protein (GFP) leads to revolution in fluorescence microscopy

- The prospects of fluorescence microscopy changed dramatically with the discovery of fluorescent proteins in the 1950s.
- GFP was first discovered in the jellyfish *Aequorea victoria*.
- Nobel prize awarded to Roger Tsien, Osamu Shimomura and Martin Chalfie in 2008 for their discovery of GFP.
- Fluorescence due to three amino acids at positions S65, T66, G67.
- Major excitation peak at 395 nm
Minor excitation peak at 475 nm
Emission peak is at 509 nm
Fluorescence quantum yield (QY) is 0.79
Molecular weight ~27 kD
Amino Acids: 238
- Quantum yield (ϕ) is the ratio of the photons emitted to the number of photons absorbed.

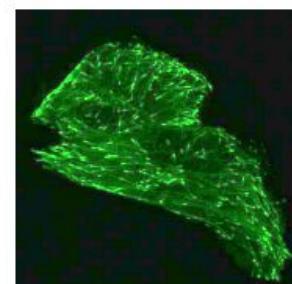
Green Fluorescence



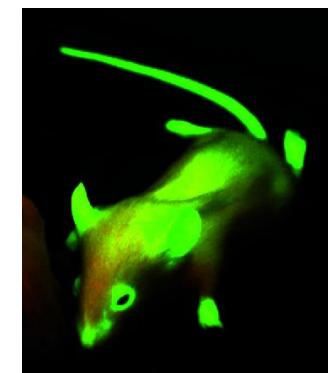
Link GFP sequence to gene of your favourite protein



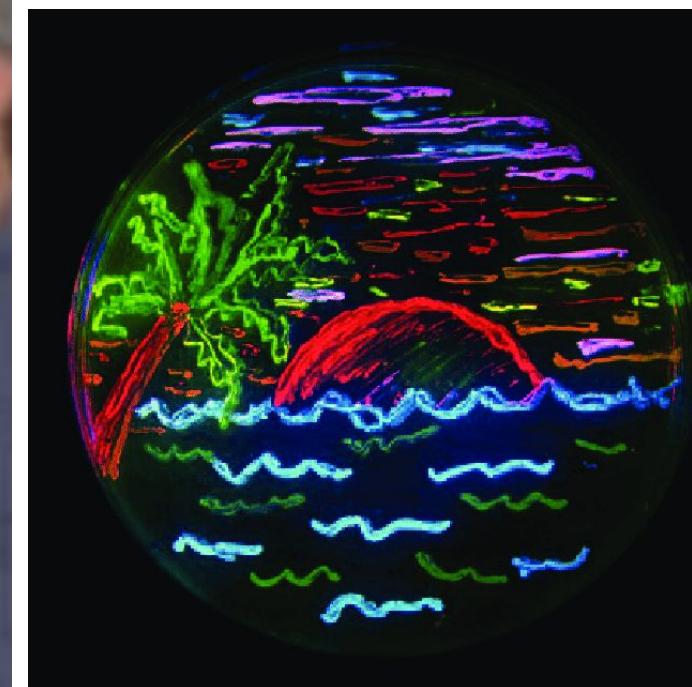
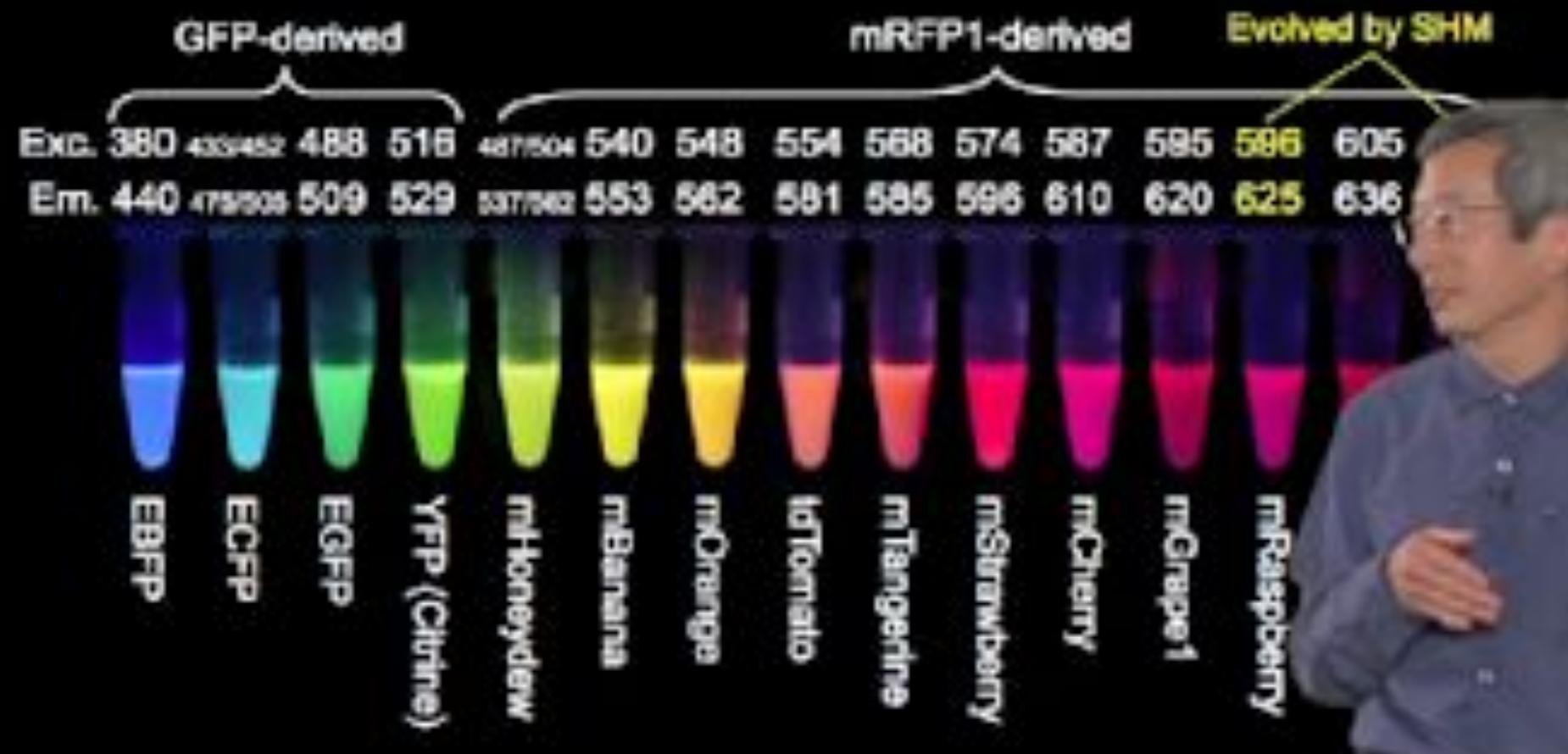
GFP folds and becomes fluorescent



GFP lights up your favourite protein in cell



The 2004 palette of nonoligomerizing fluorescent proteins



Agar plate of bacterial colonies expressing various colours of fluorescent proteins.

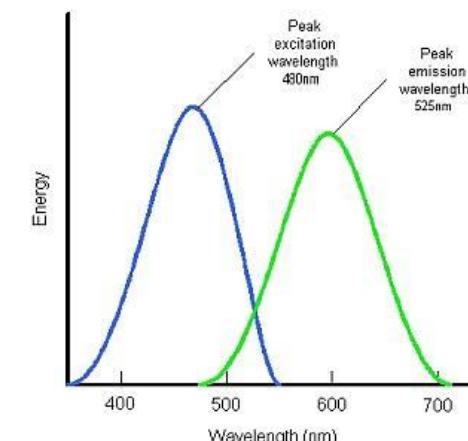
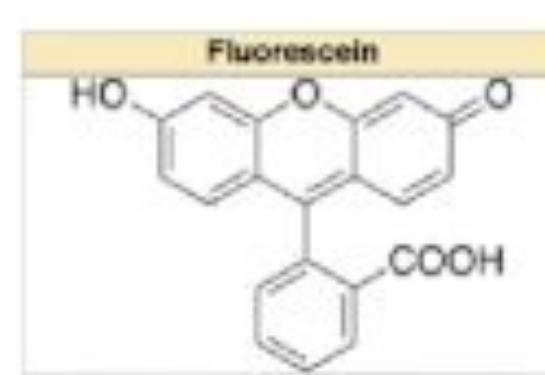
Shaner et al (2004) *Nature Biotech.* 22: 1567-1572

Wang et al (2004) *Proc. Natl. Acad. Sci. USA* 101: 16745-16749

Takem (2006) *FEBS Lett.* 579: 927-932

Fluorescent Dyes

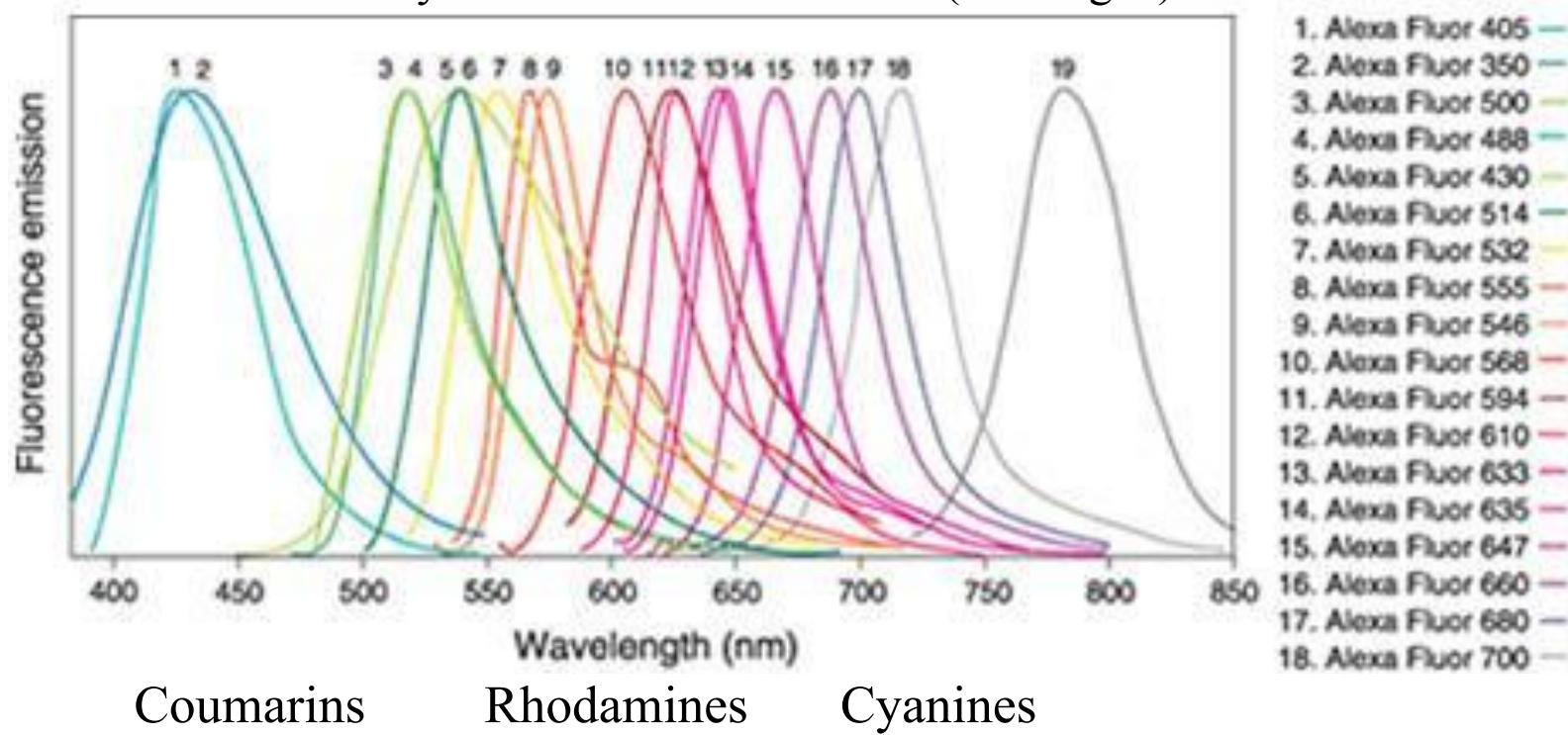
- Fluorescent proteins can only be used when genetic engineering is possible with the cells, however, there are certain instances, when genetic engineering is not possible, such as histological samples, immunofluorescence staining are used to visualize the protein of interest.
- Disadvantages of fluorescent proteins (FPs) over fluorescent dyes:
 - Use of FPs requires genetic engineering, a time consuming process
 - Fusion of protein of interest with FP increases its mol. weight by ~27 kD, which may affect protein function.
 - Fast photobleaching compared to fluorescent dyes
 - Limited spectral range compared to fluorescent dyes
 - Less brightness compared to fluorescent dyes
 - Most FPs tend to oligomerize
- First fluorescent probe was discovered in 1871, which was named Fluorescein.



List of fluorescent dyes: <https://www.leica-microsystems.com/science-lab/fluorescent-dyes/>

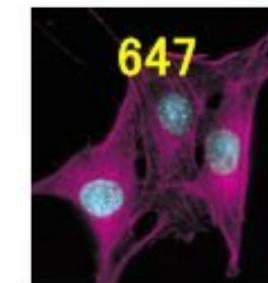
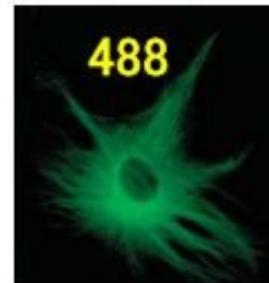
Modern probes (dyes) cover the entire visible spectrum

Alexa Fluor Dyes from Molecular Probes (Invitrogen)



Multiplexing– four main colours

Excitation
wavelengths:



Emission
wavelengths:

Blue

green

orange/red

far red

350

400

450

500

550

600

650

700

DAPI/UV

FITC

TRITC

FAR RED

Alexa Fluor® 350

Coumarin, AMCA

Alexa Fluor® 488

Fluorescein (FITC)

Cy2

Alexa Fluor® 555

Rhodamine,

TAMRA, TRITC

Cy3

Alexa Fluor® 647

Cy5, APC

Alexa Fluor® 594

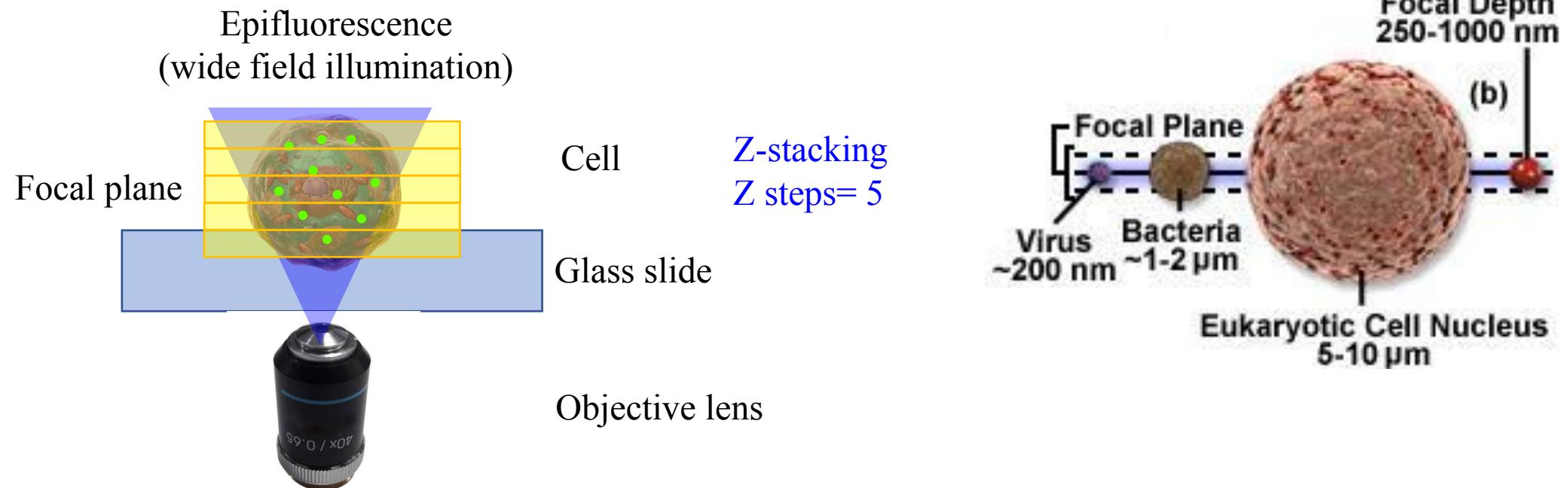
Texas Red, Cy3.5

Colour Selection

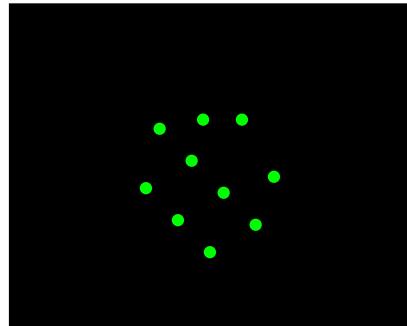
Brightness

Photostability

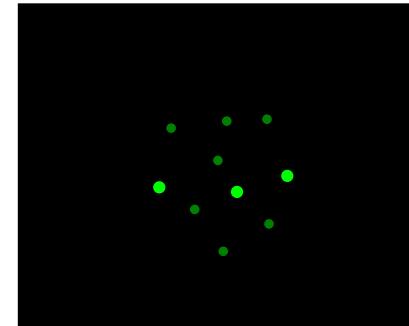
3D imaging (Epifluorescence)



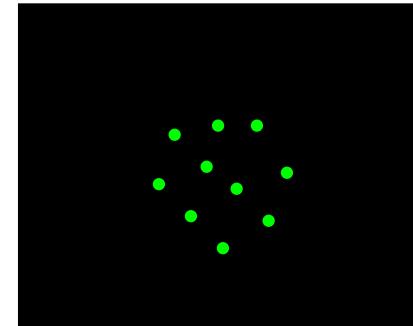
Ideal Image



Actual 2D Image



MIP 3D Image

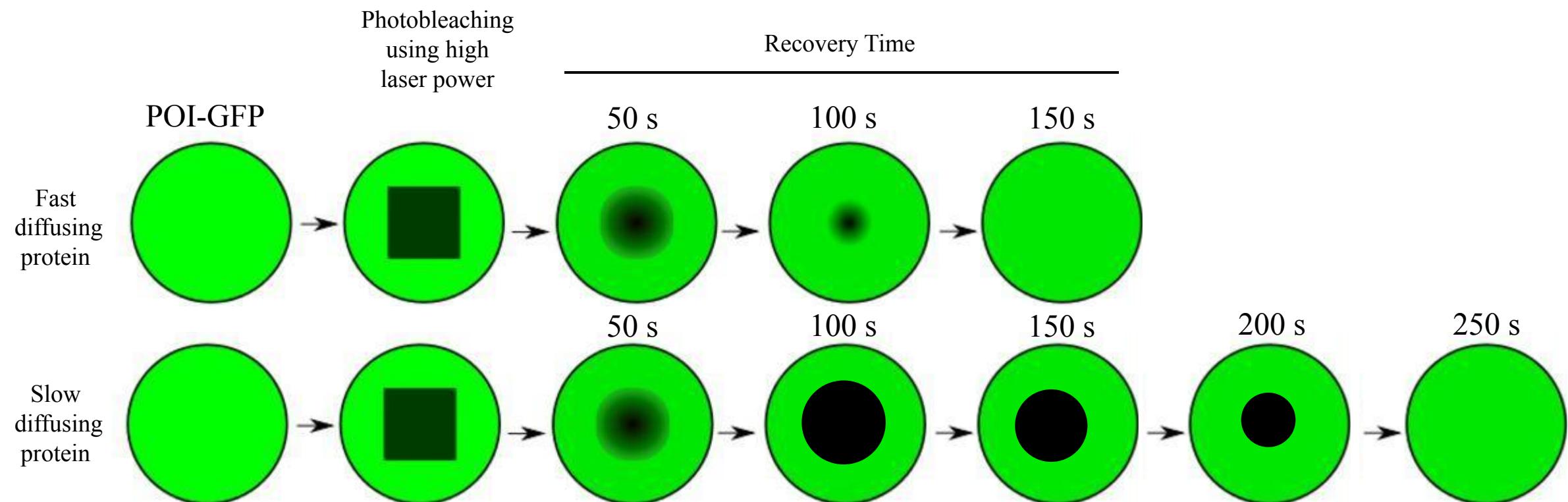


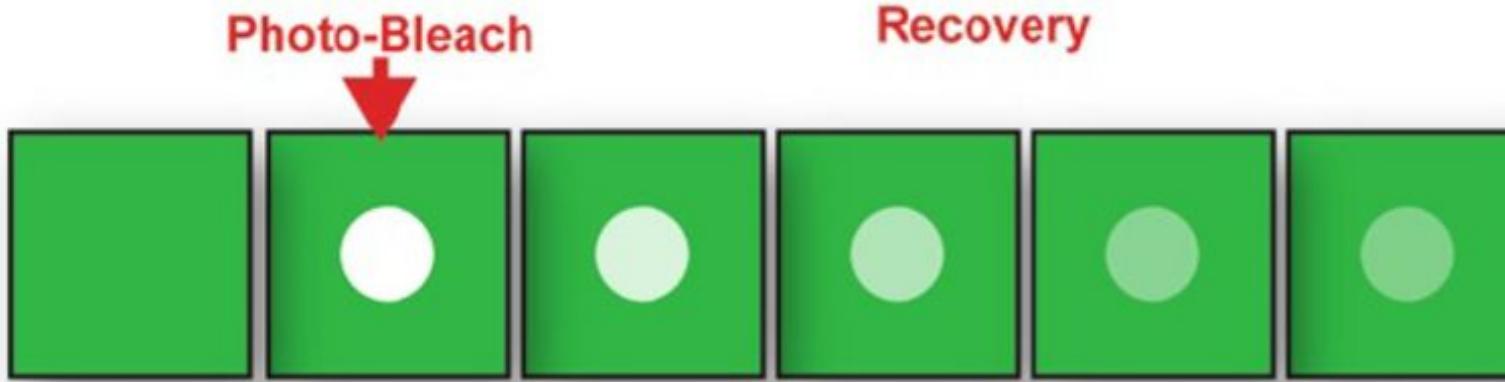
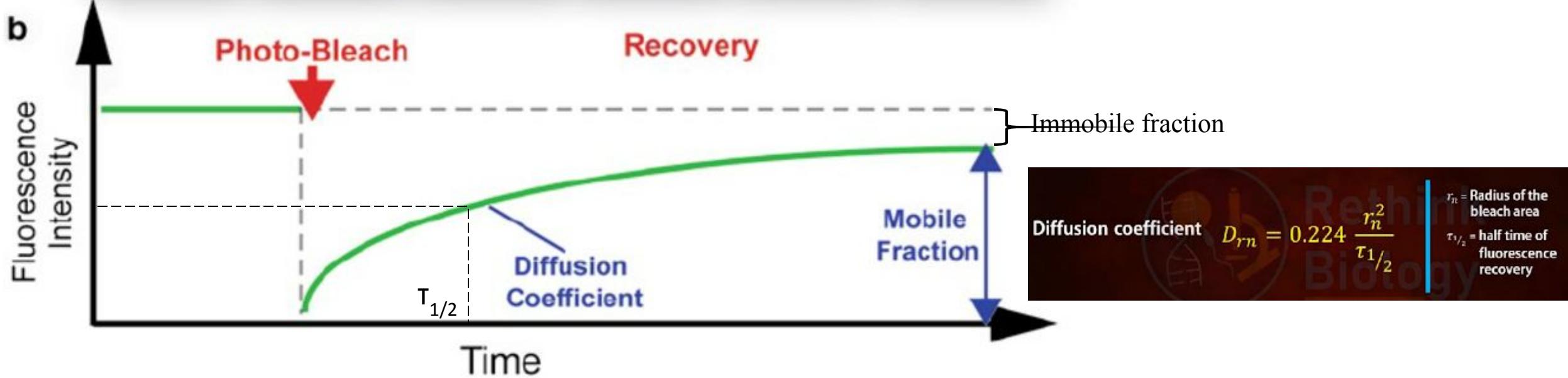
MIP=Maximum Intensity Projection

Fluorescence Recovery After Photobleaching (FRAP) to study protein dynamics

- FRAP was established by Axelrod et al. (1976) to study protein mobility/dynamics in living cells.
- It can be used to measure the diffusion dynamics of proteins in live cells.

POI=Protein of Interest



a**b**

Mobile fraction: fraction of fluorescent protein diffusing into bleached region during the time course of the experiment.

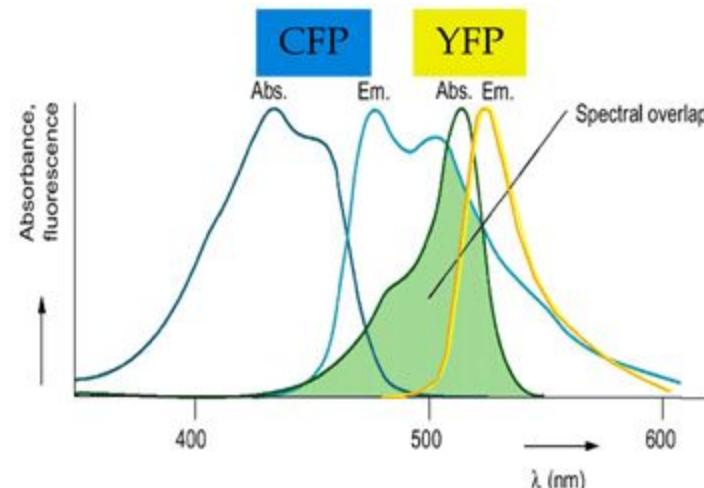
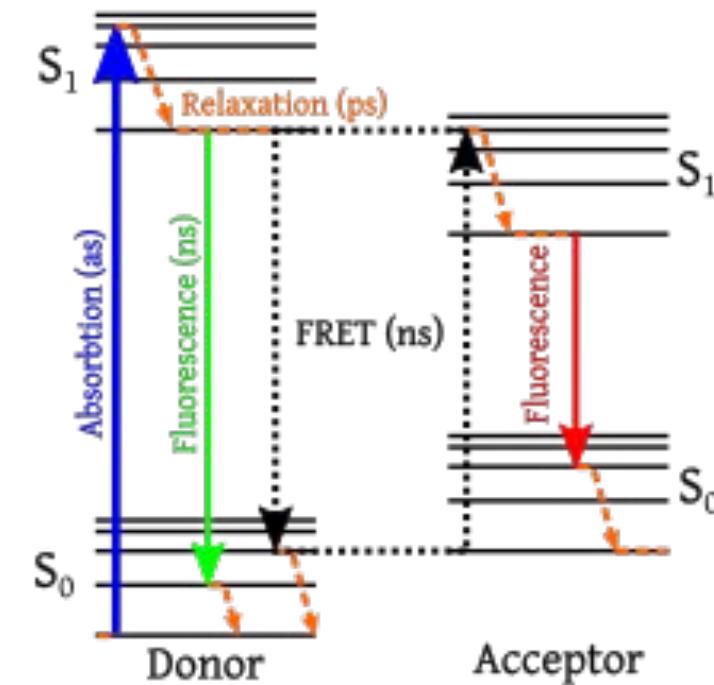
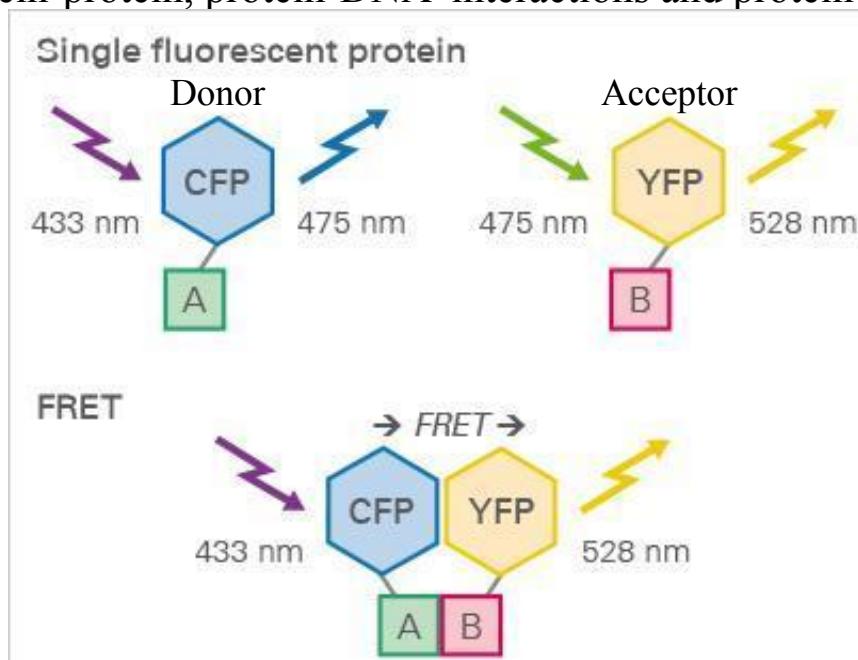
Immobile fraction: fraction of immobile bleached molecules within the bleach spot that don't allow fluorescent molecules to occupy their space.

Aggregation of proteins, binding and diffusional barriers reduces mobile fraction.

Diffusion coefficient: Rate of movement of proteins

Fluorescence Resonance Energy Transfer (FRET) to study protein-protein interactions

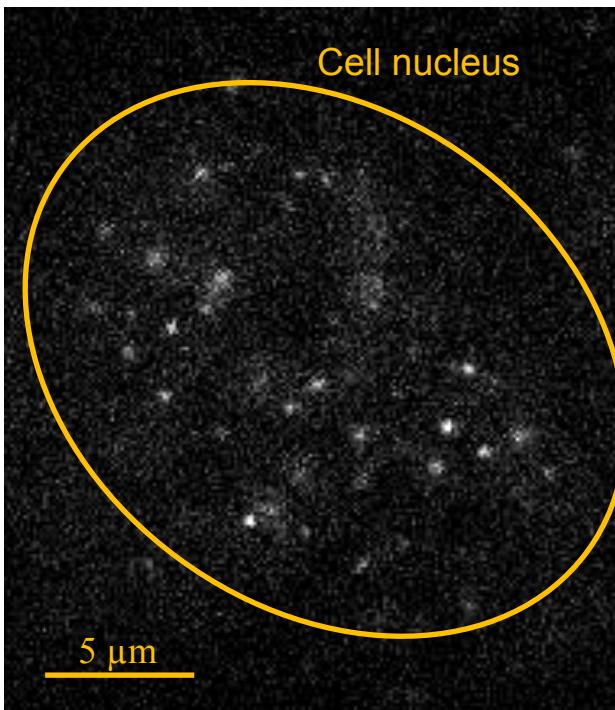
- Forster or Fluorescence Resonance Energy Transfer (FRET) is a mechanism describing energy transfer between two light-sensitive molecules (chromophores).
- A donor chromophore in its excited state, transfer energy to an acceptor chromophore through nonradiative dipole-dipole coupling.
- The efficiency of this energy transfer is inversely proportional to the 6th power of the distance between donor and acceptor, making FRET extremely sensitive to small change in distance (within 1-10 nm).
- FRET is used to check protein-protein, protein-DNA interactions and protein conformation dynamics.



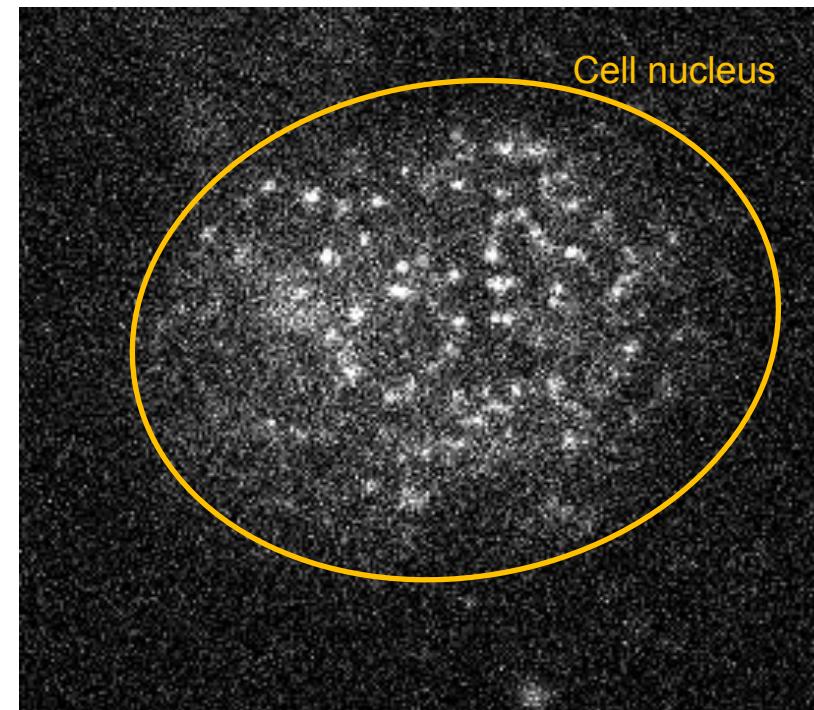
Single-Molecule Imaging to study protein dynamics

- Single-Molecule Imaging (SMI) is a method of visualizing and quantifying the dynamics of the biomolecules at the single-molecule level, either *in-vivo* or *in-vitro*.
- Classical biochemical/microscopy methods (such as IF, Western Blot, ChIP, Proteomics, FRAP etc.) describe the average behavior of biomolecules from 1000s of cells/1000s of molecules within a cell.
- Such methods don't allow the identification of subpopulations of biomolecules.
- SMI visualizes the activity of single-molecules and record it with high spatiotemporal resolution.

Transcription factor binding to chromatin



Histone H2B binding to chromatin



Biological processes are highly dynamic.
e.g. phosphorylation, transcription factor binding

Western blot
(phospho-x)

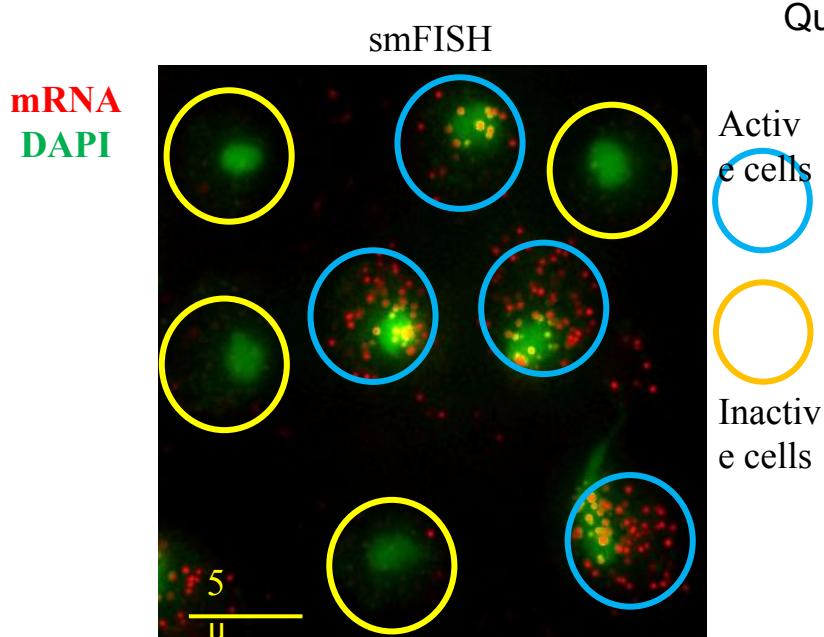
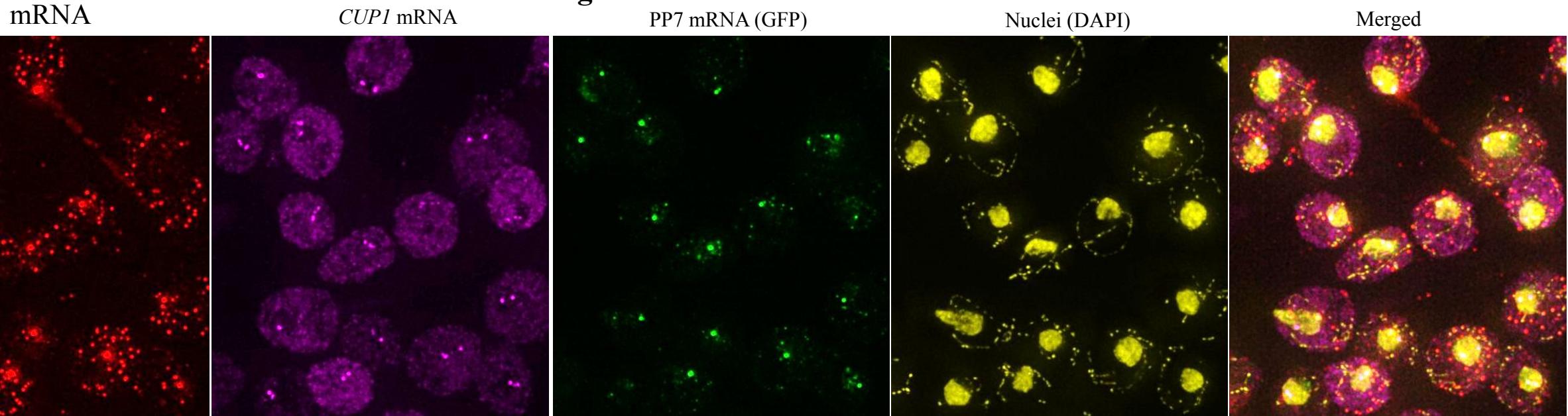
Mass spec
(stoichiometry)



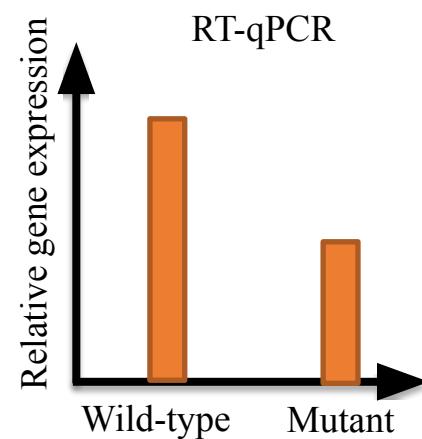
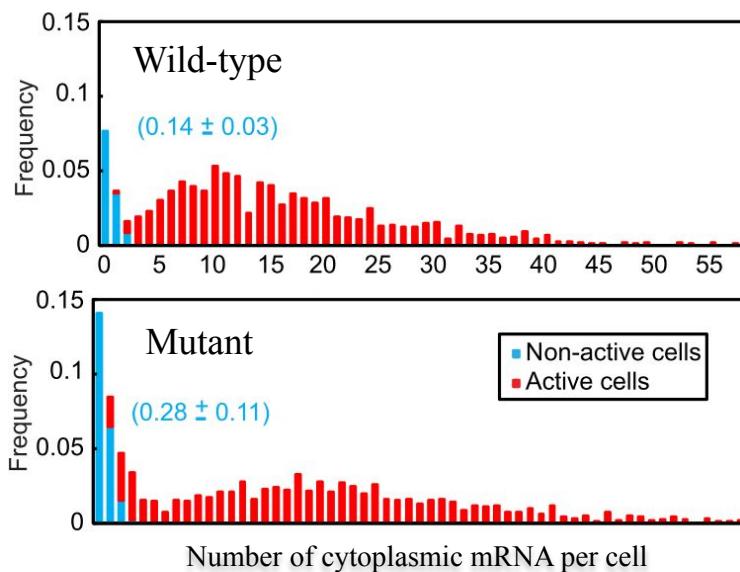
50% 50%
phosphorylated

Single-Molecule FISH to quantify number of mRNA/cell

To visualize single-molecules of mRNA in fixed cells



Quantification of transcription by smFISH and RT-qPCR



Optical Tweezers to measure biological forces

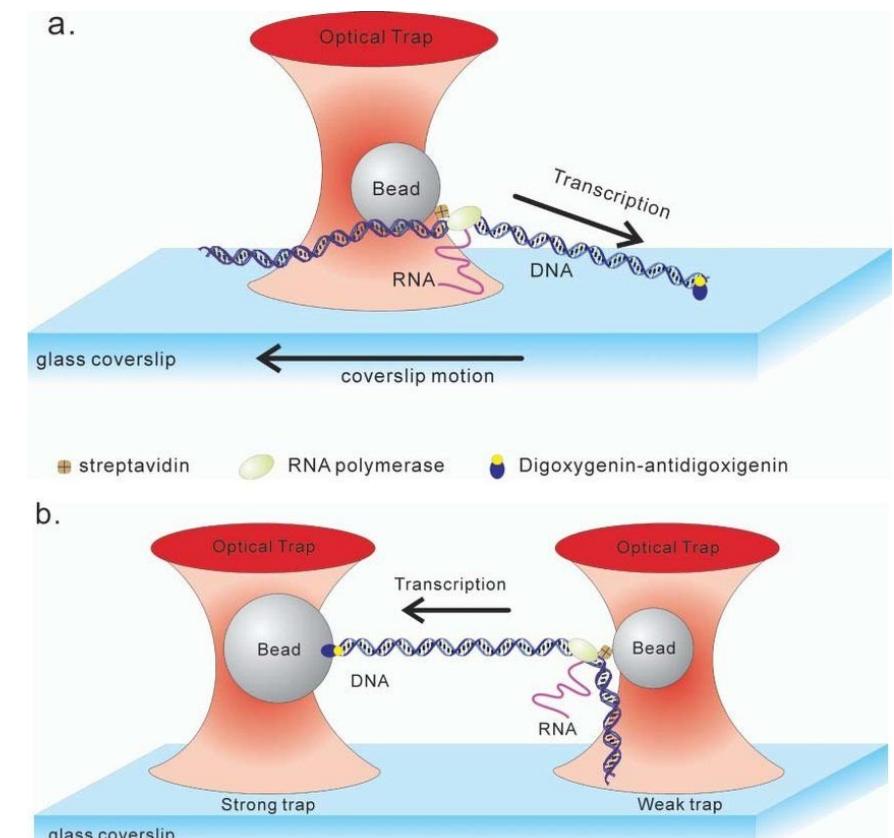
Optical tweezers use tightly focussed light beam (**IR laser**) to hold and manipulate microscopically small objects.

Microscope objective lens (high NA) is used for tight focussing of the IR laser beam. This region becomes an optical trap that can hold small objects in 3D.

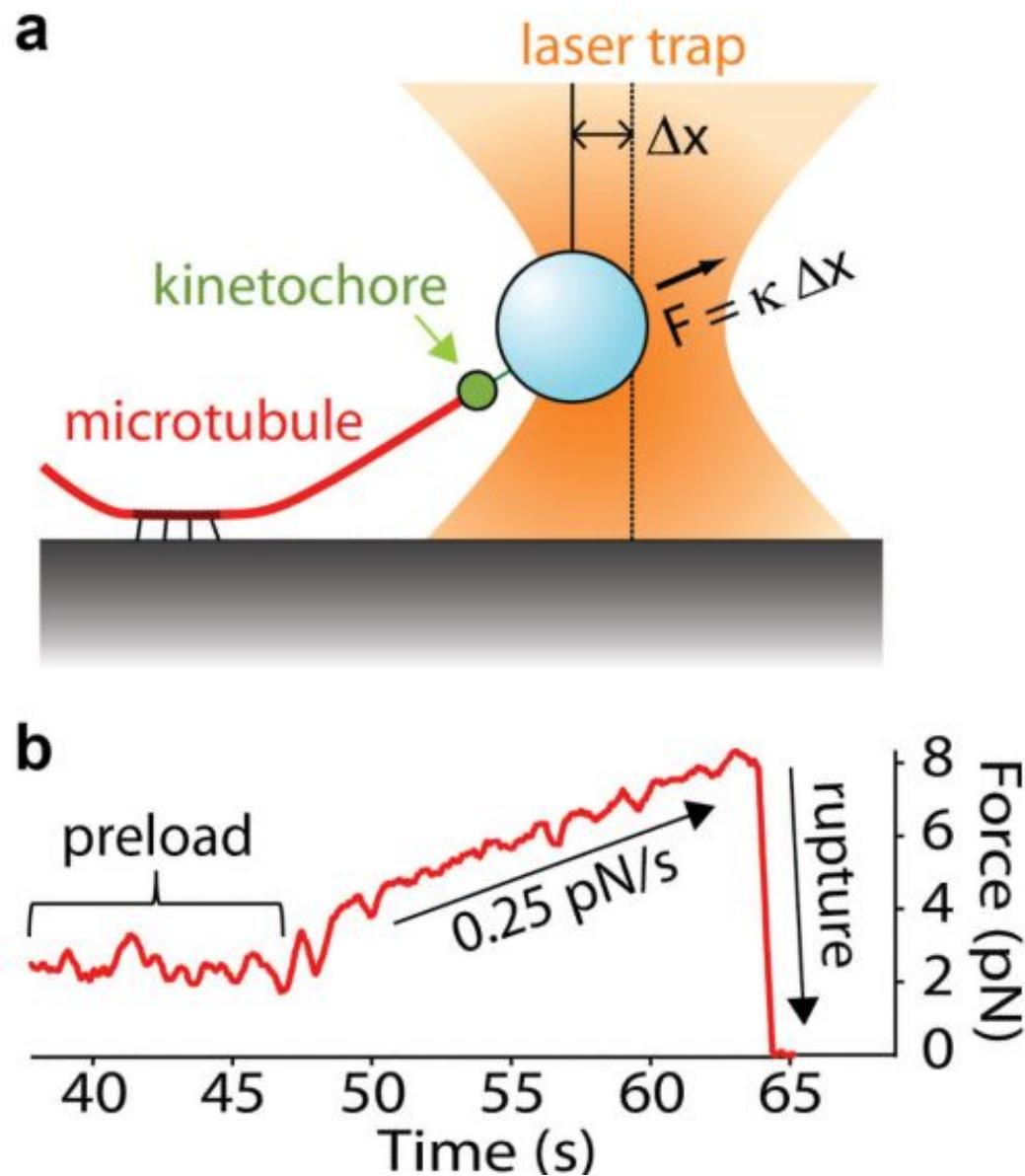
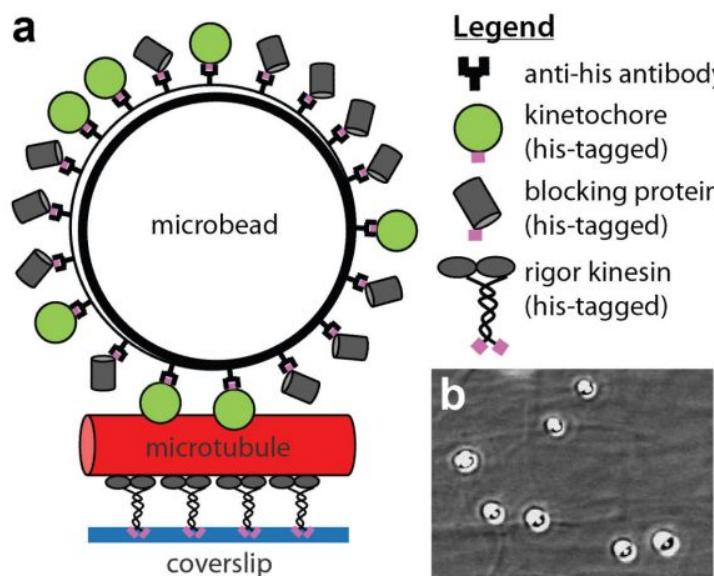
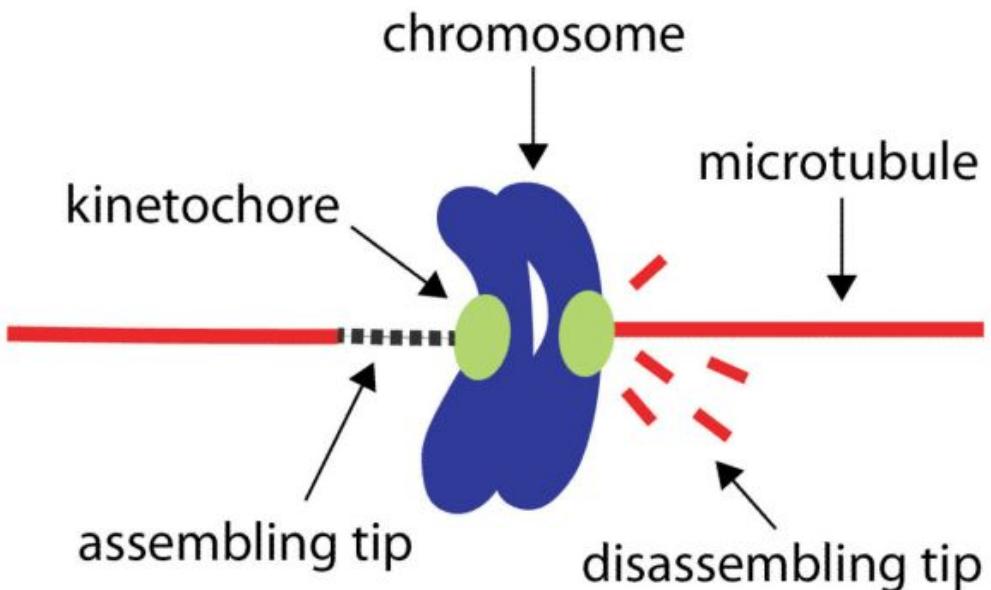
Optical tweezers can also make accurate measurements of the sub-picoNewton forces exerted on the trapped objects and the small displacements (nanometer).

Since the diameter of biomolecules is generally between 1 and 10 nanometers, the optical tweezer cannot directly observe and manipulate. Hence, it is necessary to connect the molecule to the microspheres (beads) and indirectly manipulate and measure by using the beads as the “handle” of the manipulation. For example, the two ends of the DNA are connected to two beads, and the beads are manipulated by a double-beam tweezers to stretch the DNA and measure its elastic properties.

Optical tweezers have been used with great success in the field of single-molecule biophysics. For example, they have helped researchers unravel the complex elasticity and folding dynamics of DNA, RNA, proteins (especially motor protein dynamics) and other long-chain “biopolymers”.



Strength of Kinetochore-Microtubule attachments



Further reading

- Book: Fundamentals of Light Microscopy and Electronic Imaging by Douglas Murphy and Michael Davidson, 2nd edition, Wiley-Blackwell publisher, **eBook ISBN** 9781118382912
- Book: Fluorescence Microscopy: From Principles to Biological Applications by Ulrich Kubitscheck, 2nd edition, John Wiley & Sons publisher, **eBook ISBN** 9783527687725
- e-resource: <https://www.microscopyu.com/>
- e-resource: <https://www.ibiology.org/online-biology-courses/microscopy-series/>
- e-resource: <http://zeiss-campus.magnet.fsu.edu/index.html>
- e-resource: <https://www.leica-microsystems.com/science-lab/topics/basics-in-microscopy/>
- e-resource: <https://www.olympus-lifescience.com/en/microscope-resource/>
- e-resource: <https://imagej.nih.gov/ij/docs/examples/index.html>
- e-resource: <https://www.youtube.com/playlist?list=PL5ESQNFM5lc7SAMstEu082ivW4BDMvd0U>

BT1010 Introduction to Life Sciences

Lecture 5: Cell Organelles and Nuclear Architecture
23/06/2022



Course Instructor:

Dr. Gunjan Mehta, Ph.D.

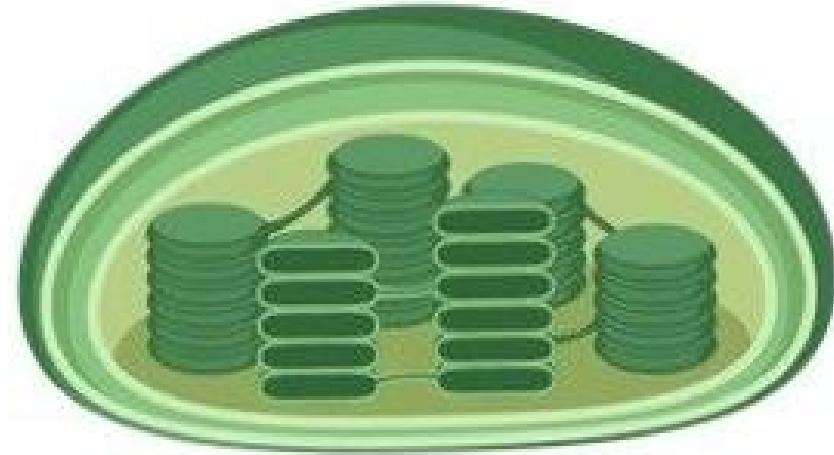
Assistant Professor

Department of Biotechnology

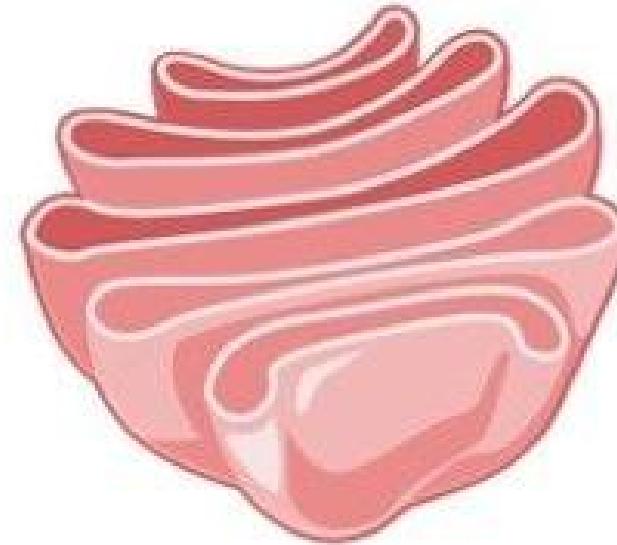
IIT Hyderabad

(M.) +91 70168 96886 Email: gunjanmehta@bt.iith.ac.in

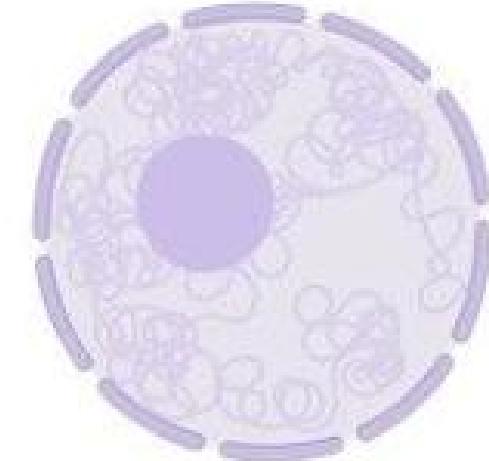
Chloroplast



Golgi Apparatus

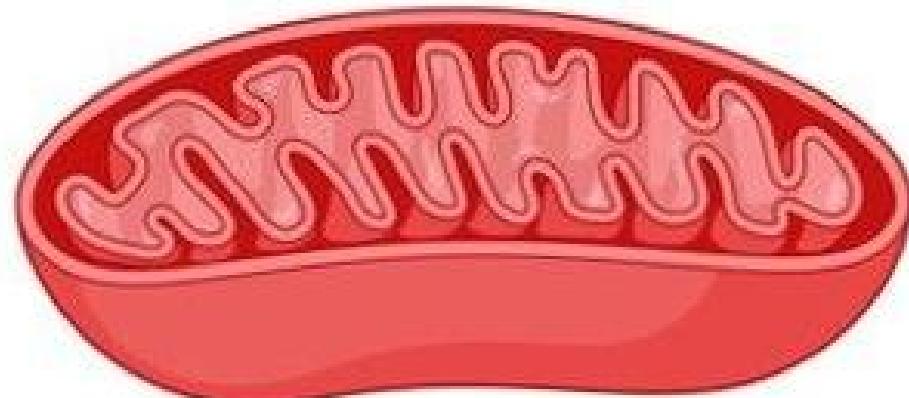


Nucleus

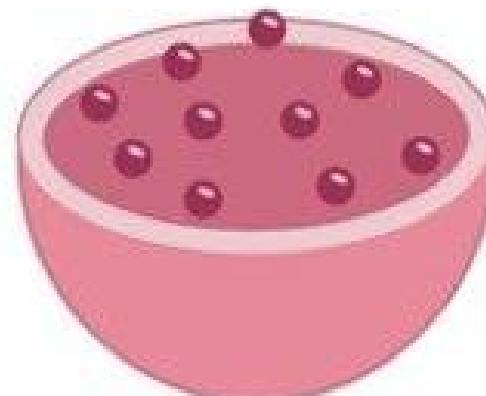


Cell Organelles

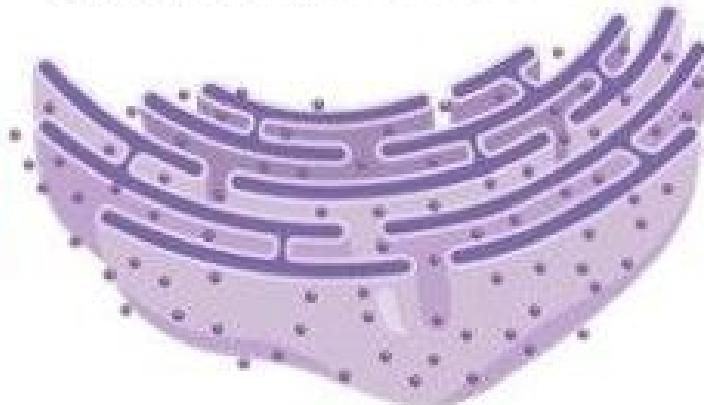
Mitochondria



Lysosome



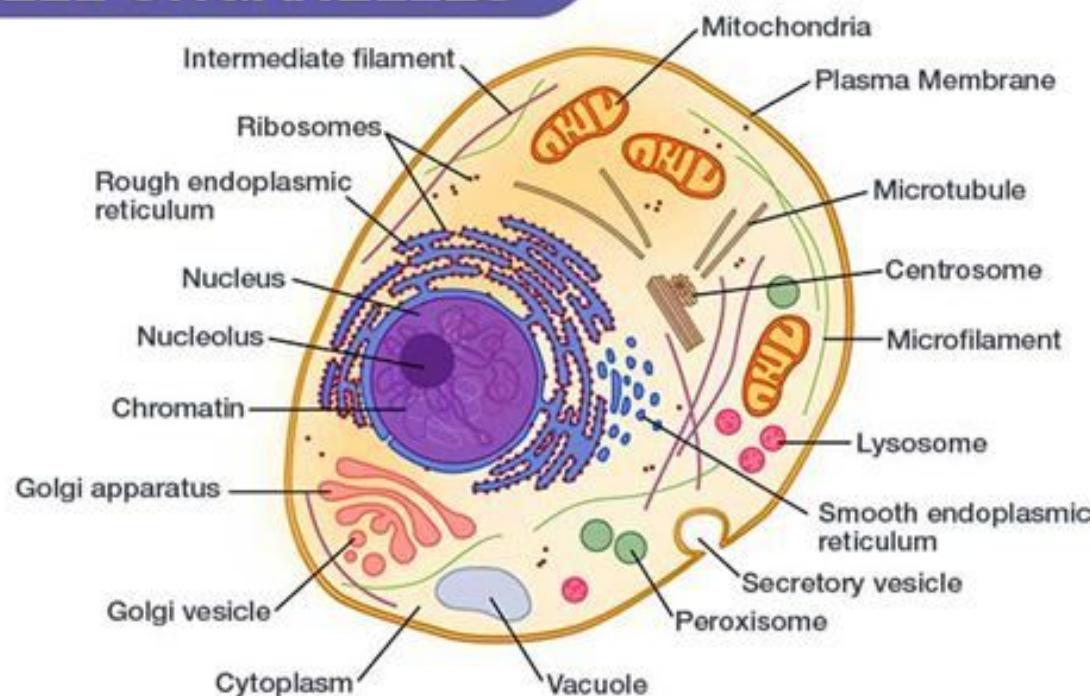
Endoplasmic Reticulum



Cell Organelles

- Cell organelles are specialized entities present inside a particular type of cell that performs a specific function.

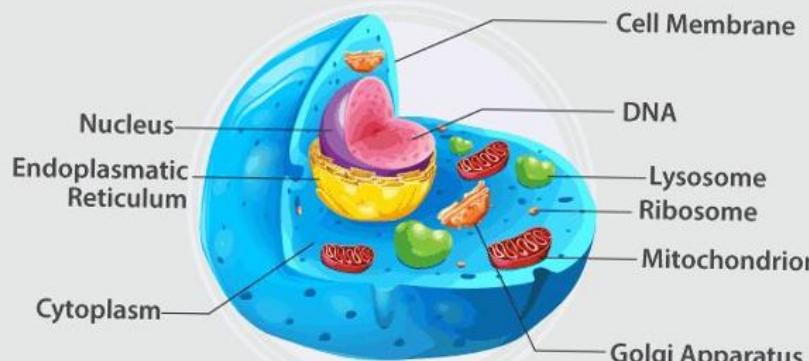
CELL ORGANELLES



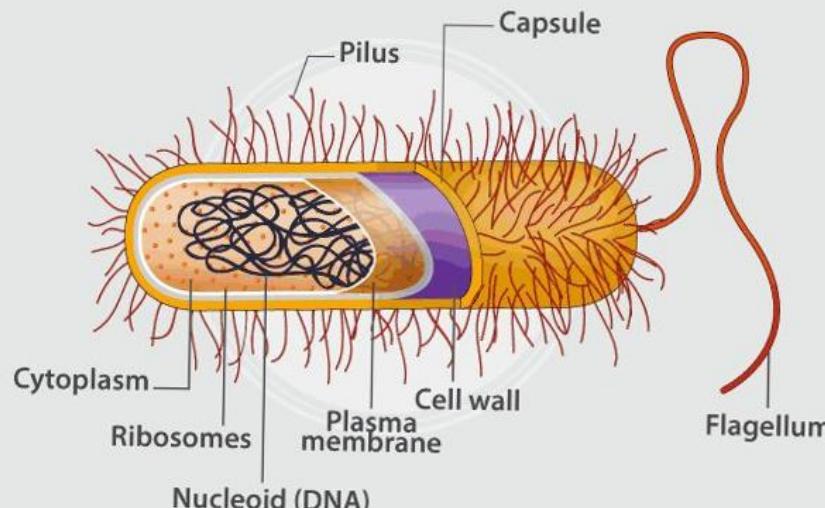
Organelle	Responsible for
Nucleus	Housing DNA, 'brain' of the cell
Mitochondria	Energy production, 'power house' of the cell
Golgi apparatus	Sorting, packaging and transport of proteins
Endoplasmic reticulum	Synthesis and processing of proteins, lipid expression
Chloroplast	Photosynthesis, only present in plants
Flagellum	Locomotion and sensory functions
Vacuole	Storage and maintaining homeostasis
Lysosome	Digestions of larger molecules
Peroxisome	Degradation of hydrogen peroxide
Ribosome	Synthesis of proteins
Proteasome	Break down of proteins with expired function

Prokaryotic and Eukaryotic cells

PROKARYOTIC CELL VS EUKARYOTIC CELL



Eukaryotic Cell



Prokaryotic Cell

Prokaryotes

Type of Cell	Always unicellular	Unicellular and multi-cellular
Cell size	0.2 μm – 2.0 μm in diameter	10 μm – 100 μm in diameter
Cell wall	Present	Present/Absent
Nucleus	Absent	Present
Ribosomes	Present. Smaller in size and spherical in shape	Present. Comparatively larger in size
DNA	Circular	Linear
Mitochondria	Absent	Present
Cytoplasm	Present, but cell organelles absent	Present, cell organelles present
Endoplasmic reticulum	Absent	Present
Plasmids	Present	Very rarely found in eukaryotes
Ribosome	Small ribosomes	Large ribosomes
Lysosome	Lysosomes and centrosomes are absent	Lysosomes and centrosomes are present
Cell division	Through binary fission	Through mitosis
Flagella	The flagella are smaller in size	The flagella are larger in size
Reproduction	Asexual	Both asexual and sexual
Example	Bacteria and Archaea	Plant and Animal cell

Difference between plant cells and animal cells

Plant Cell Structure

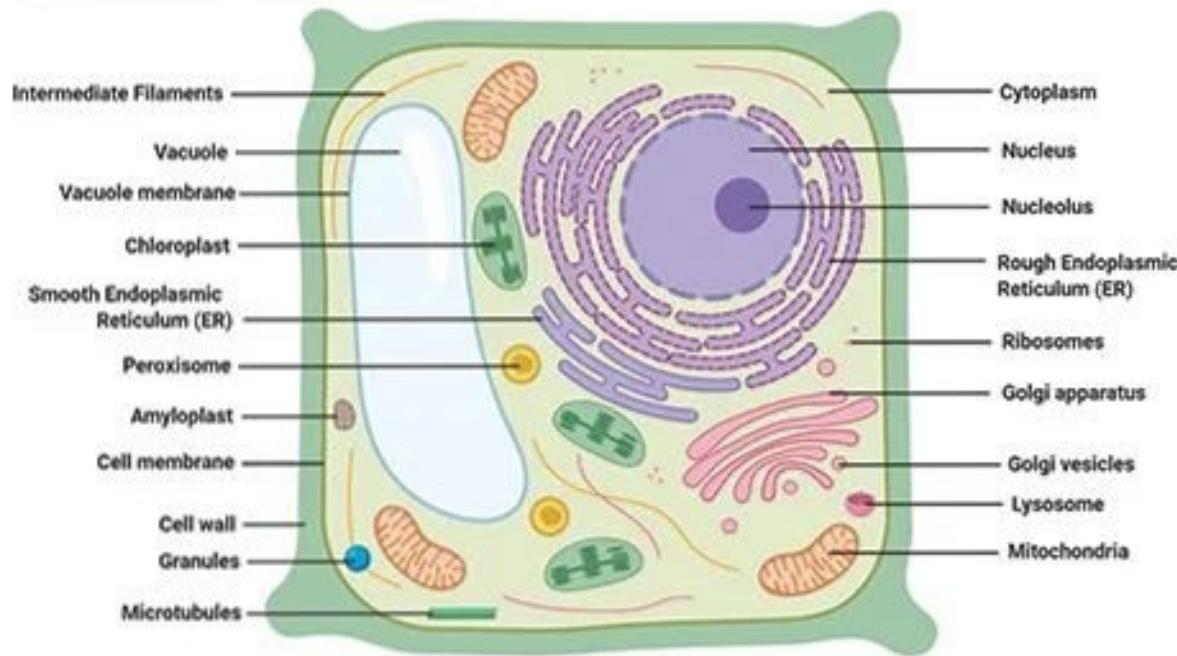


Figure: Plant Cell Structure, Image Copyright © Sagar Aryal, www.microbenotes.com

Animal Cell Structure

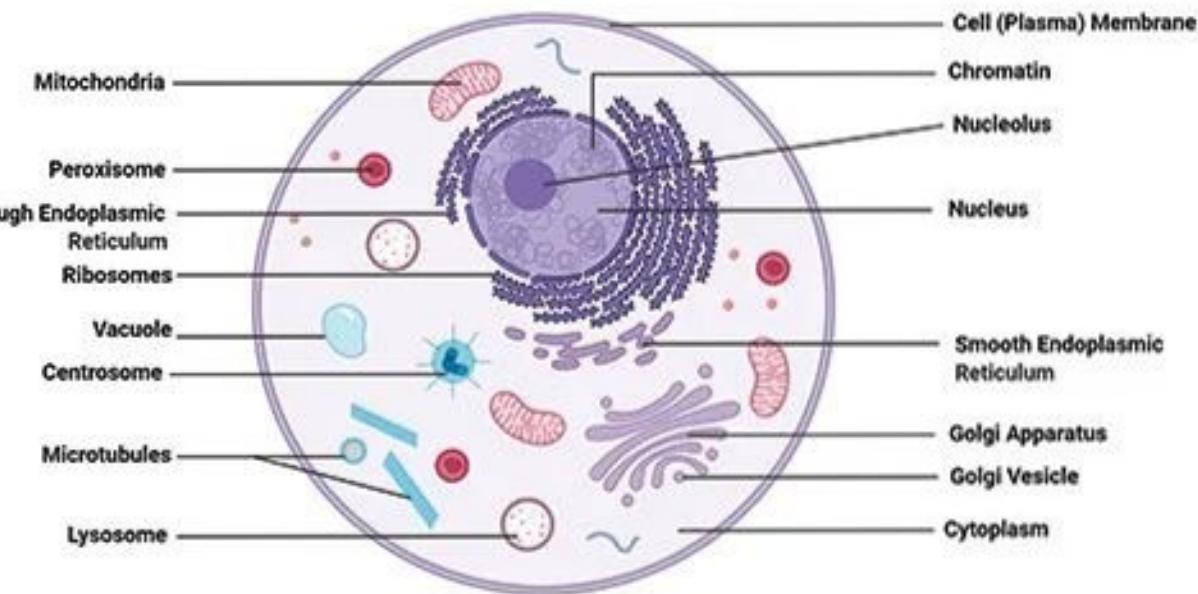
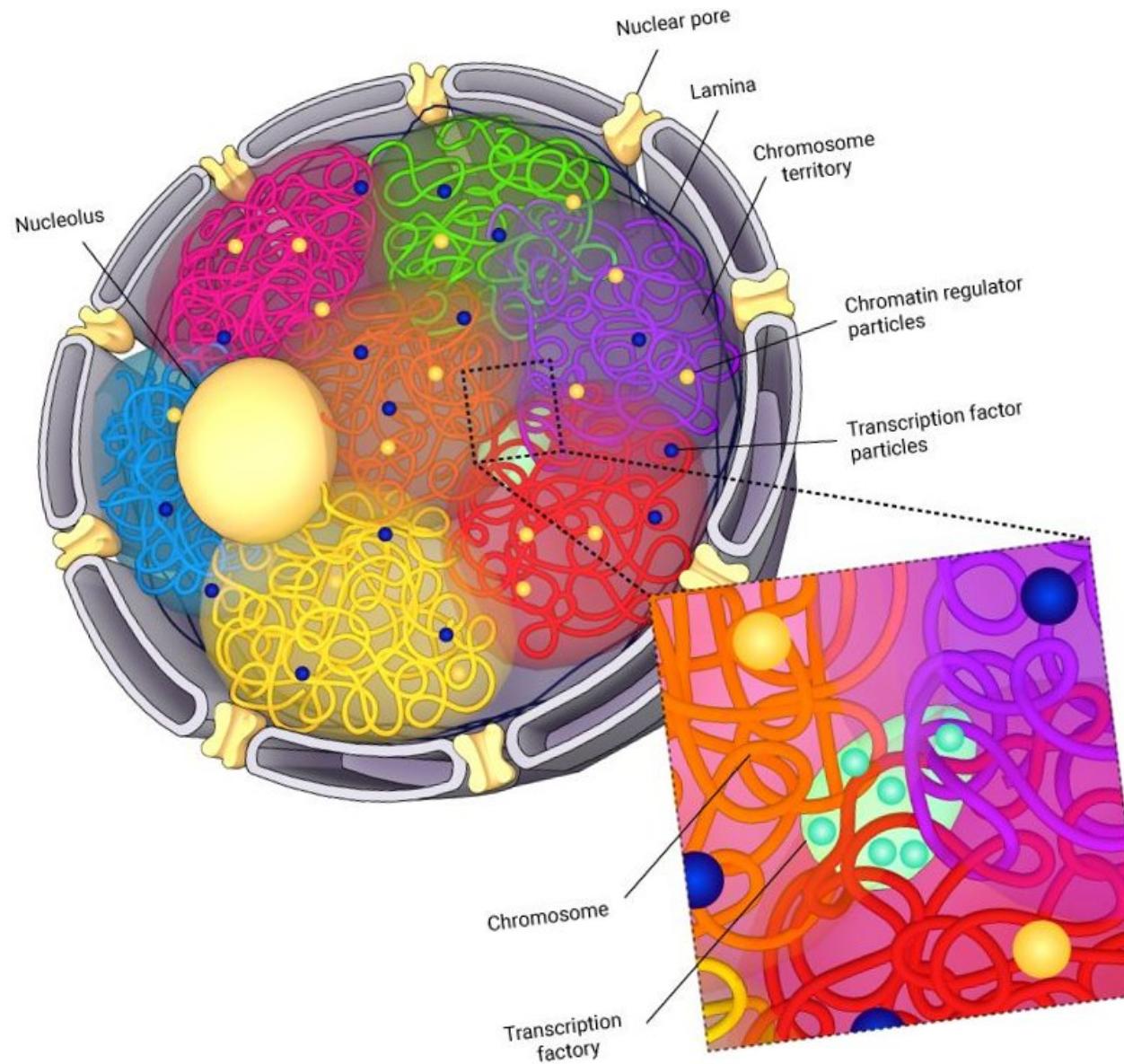


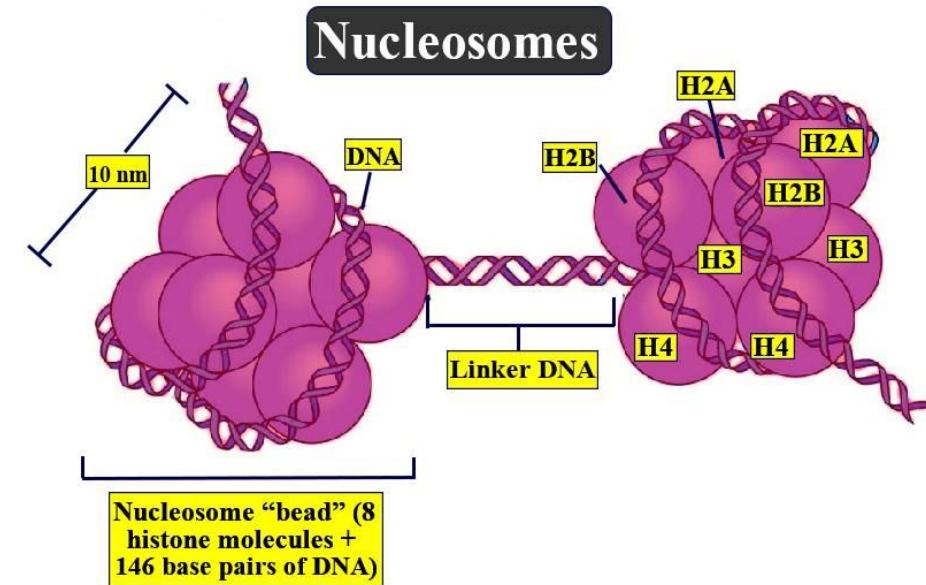
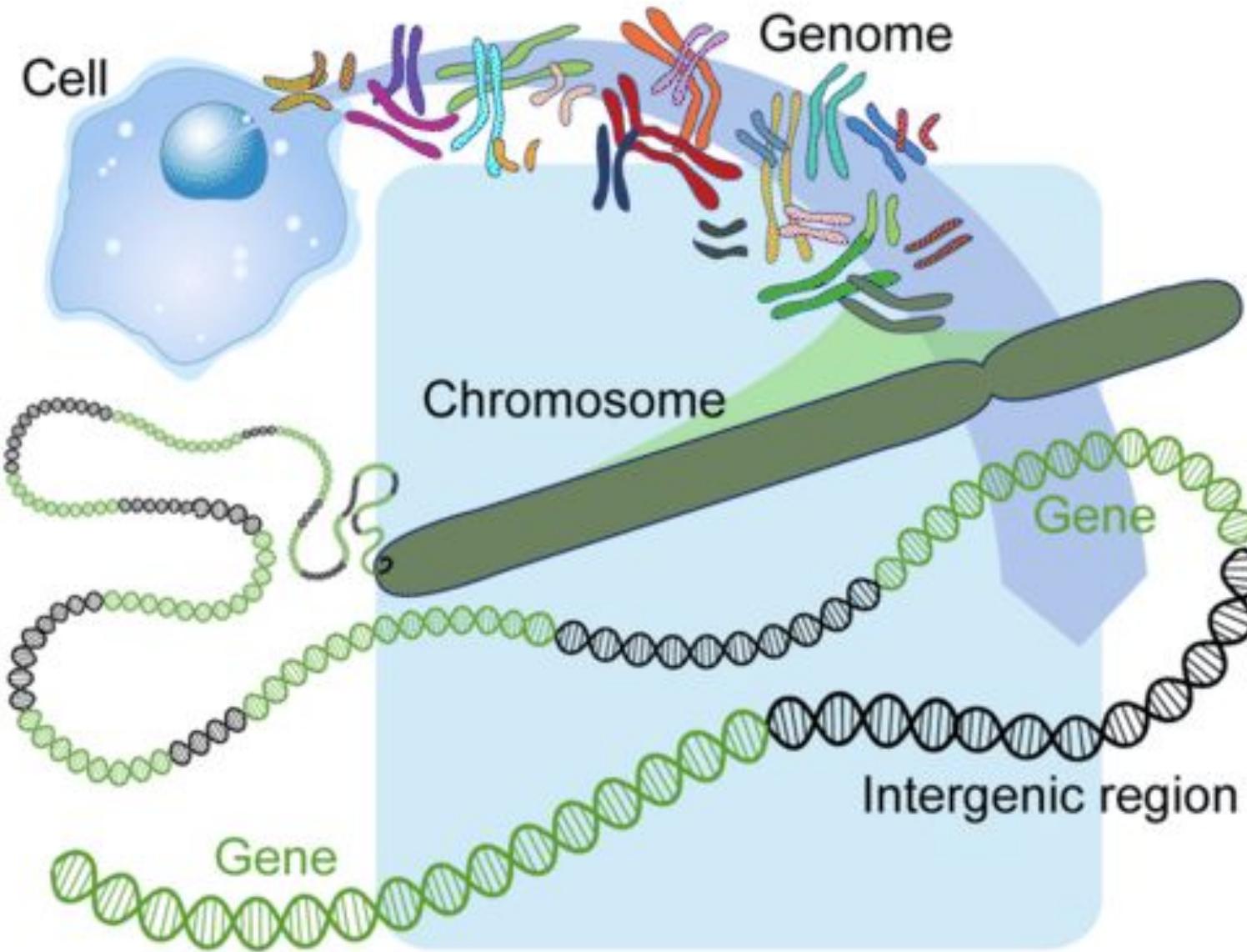
Figure: Animal Cell Structure, Image Copyright © Sagar Aryal, www.microbenotes.com

- Structures that are specific to plants are the cell wall and chloroplasts.
- Structures that are common to plant and animal cells are the cell membrane, nucleus, mitochondria, and vacuoles.
- Plant cells have a cell wall, but animal cells do not. Cell walls provide support and give shape to plants.
- Plant cells have chloroplasts, but animal cells do not. Chloroplasts enable plants to perform photosynthesis to make food.
- Plant cells usually have one or more large vacuole(s), while animal cells have smaller vacuoles, if any are present. Large vacuoles help provide shape and allow the plant to store water and food for future use.

Genome Organization and Nuclear Architecture



What is the difference between a Gene, a Chromosome and a Genome?

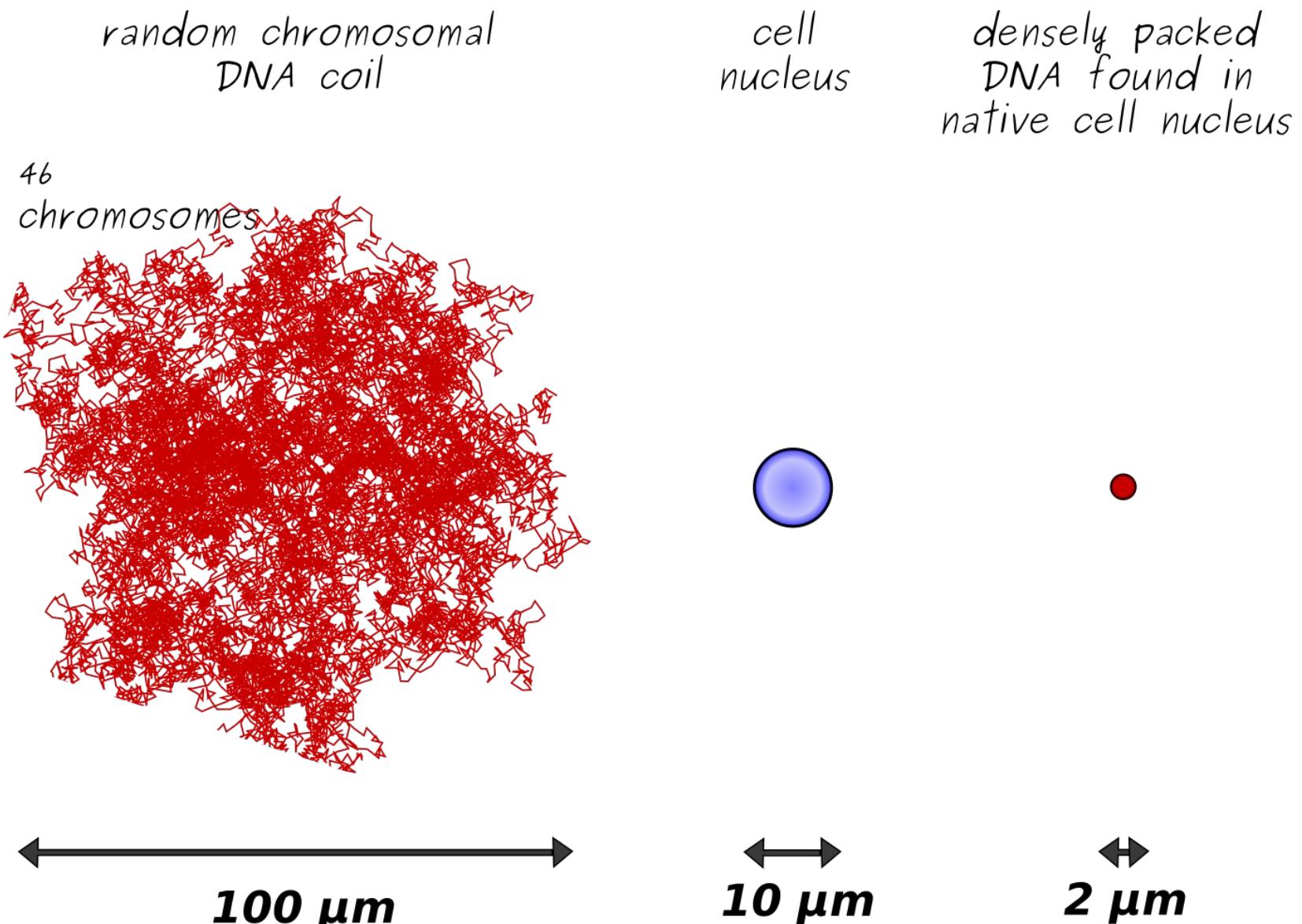


Length of the human genome: 2 meter

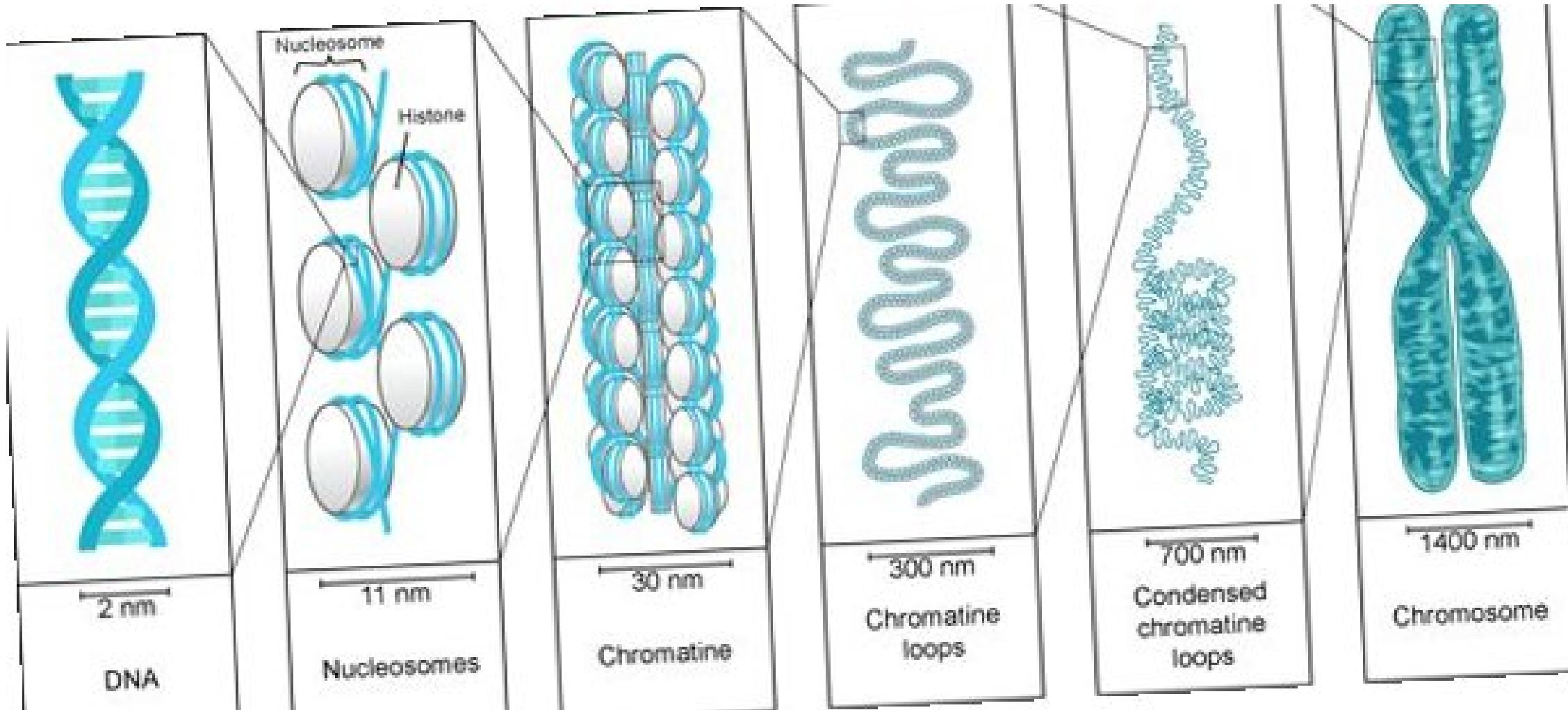
Bibliographic Entry	Result (w/surrounding text)	Standardized Result
Mitchel, Campbell Reece. <i>Biology Concept and Connections</i> . California, 1997.	"At actual size, a human cell's DNA totals about 3 meters in length."	3.0 m
<i>McGraw Hill Encyclopedia of Science and Technology</i> . New York: McGraw Hill, 1997.	"If stretched out, would form very thin thread, about 6 feet (2 meters) long."	2.0 m
Matthews, Harry R. <i>DNA Structure Prerequisite Information</i> . 1997.	"The length is (length of 1 bp)(number of bp per cell) which is (0.34 nm)(6 × 10 ⁹)"	2.0 m
Leltinger, Albert L. <i>Biochemistry</i> . New York: Worth, 1975.	"Chromosome 13 contains a DNA molecule about 3.2 cm long."	1.5 m
"Cell." <i>The World Book Encyclopedia</i> . Chicago: Field Enterprises, 1996.	"On the average, a single human chromosome consists of DNA molecule that is about 2 inches long."	2.3 m

- The haploid human genome contains approximately **3 billion base pairs of DNA** packaged into 23 chromosomes.
- Most cells in the body are diploid, that makes a **total of 6 billion base pairs of DNA** per cell.
- Because each base pair is around 0.34 nanometers long (a nanometer is one-billionth of a meter), **each diploid cell** therefore contains about **2 meters of DNA** $[(0.34 \times 10^{-9}) \times (6 \times 10^9)]$.

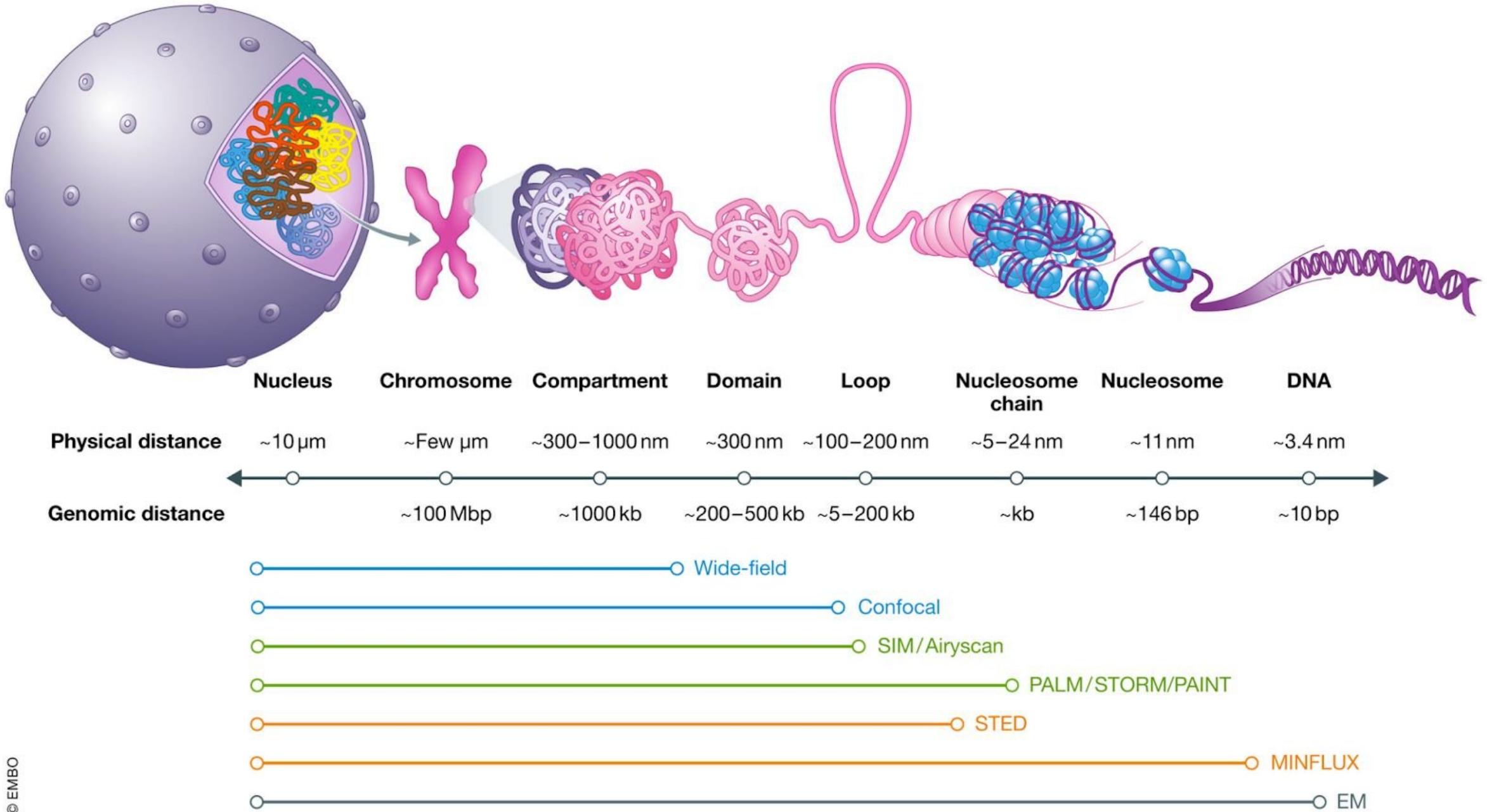
DNA in the interphase nuclei



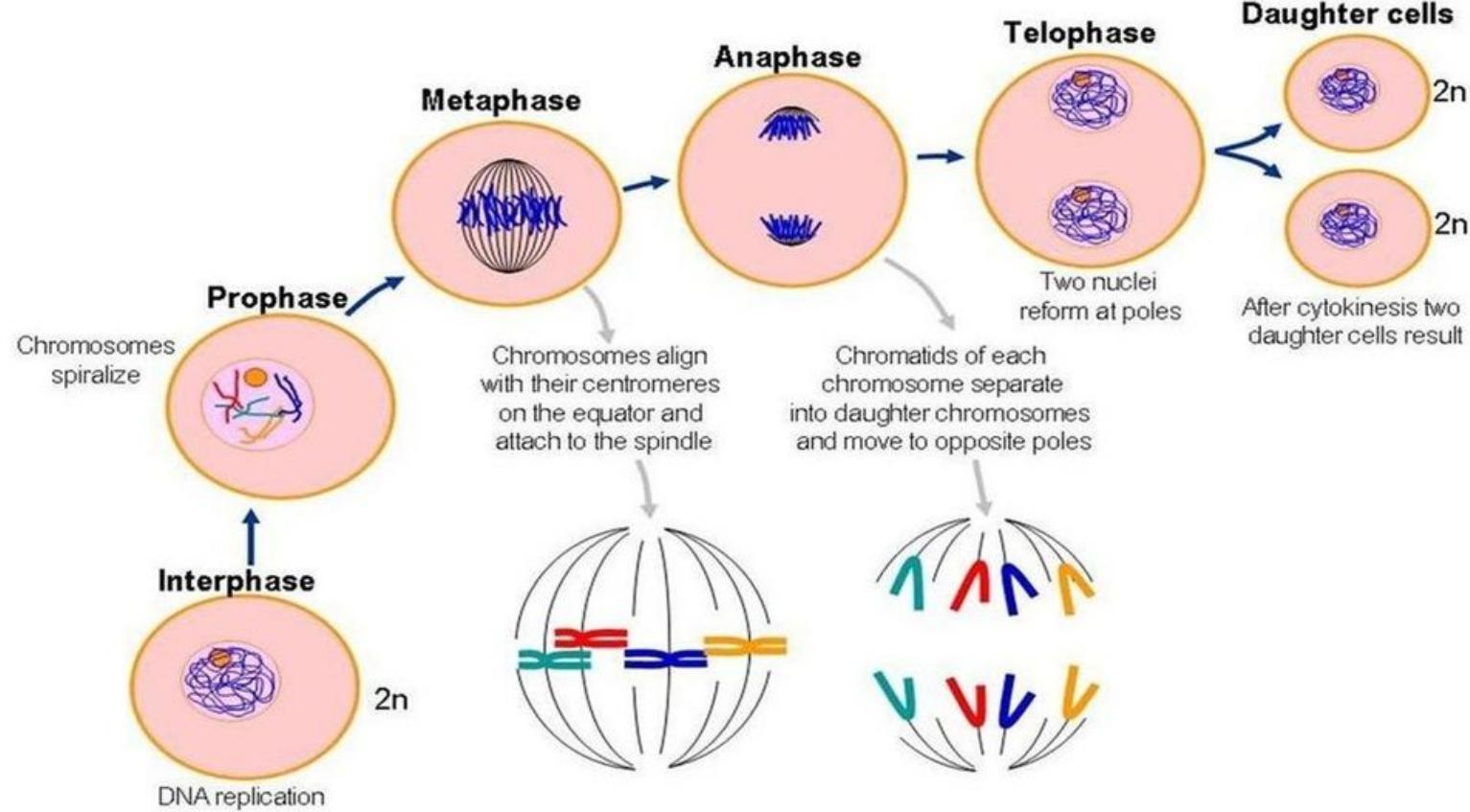
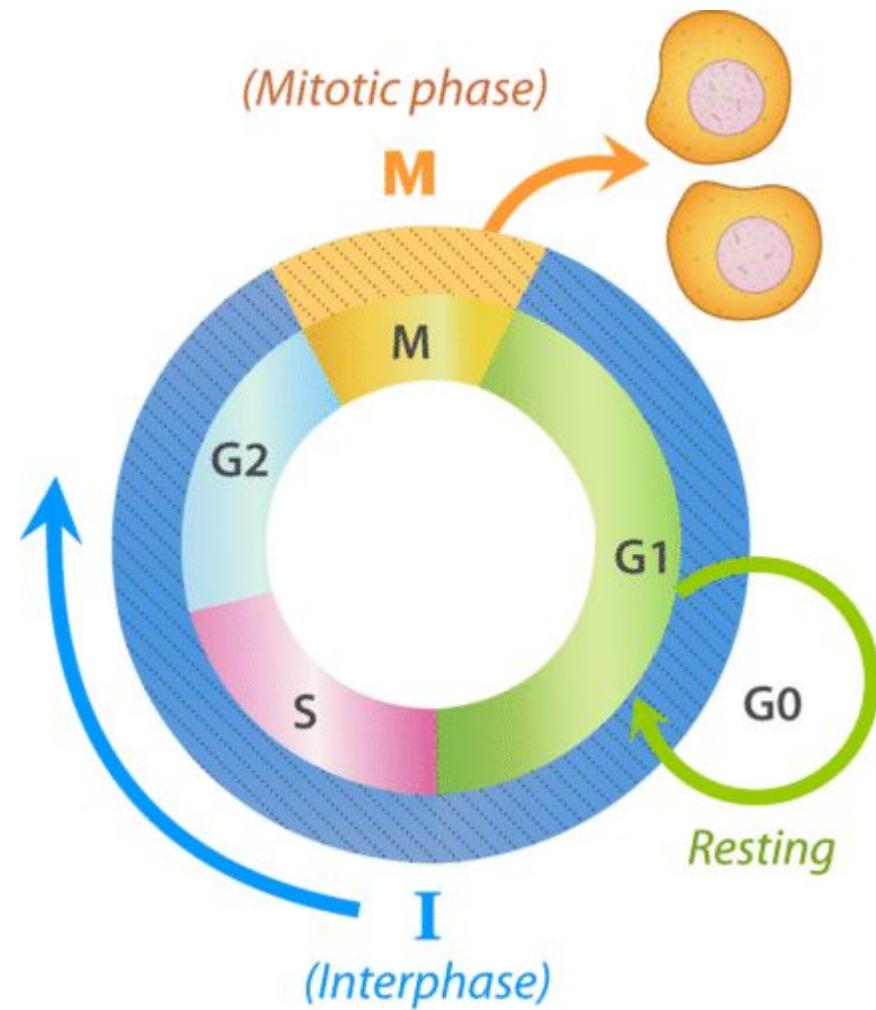
How DNA is packaged into chromosomes during mitosis (cell division)?



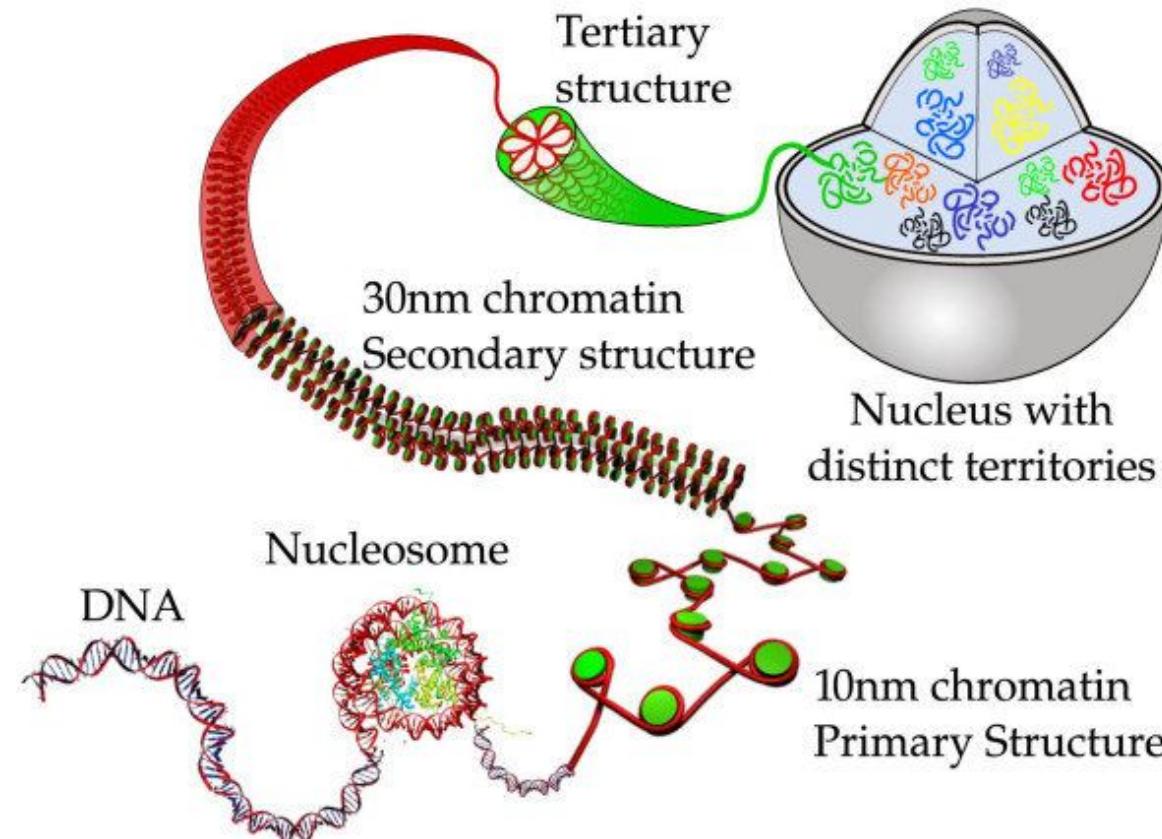
Relative sizes: from DNA to Cell Nucleus



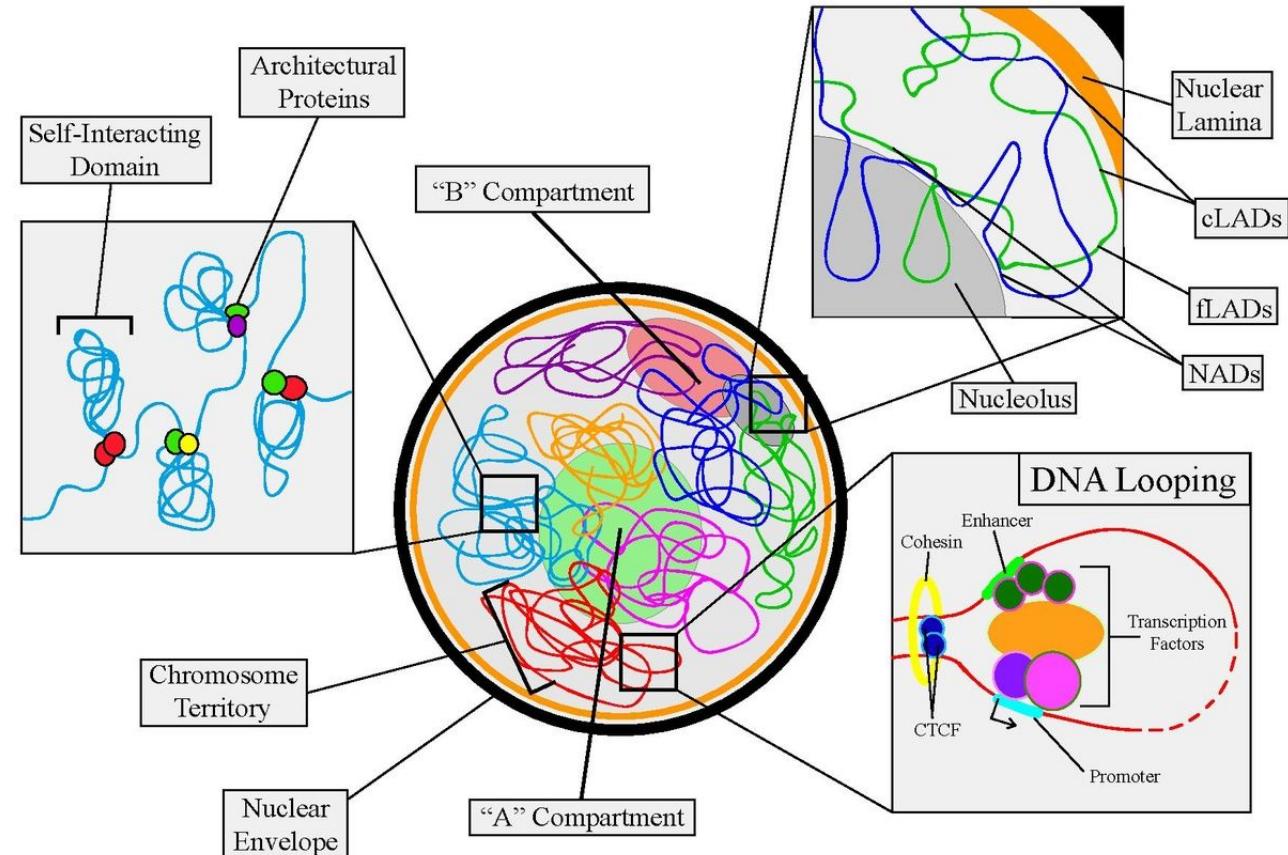
Cell Cycle: Interphase and Mitosis



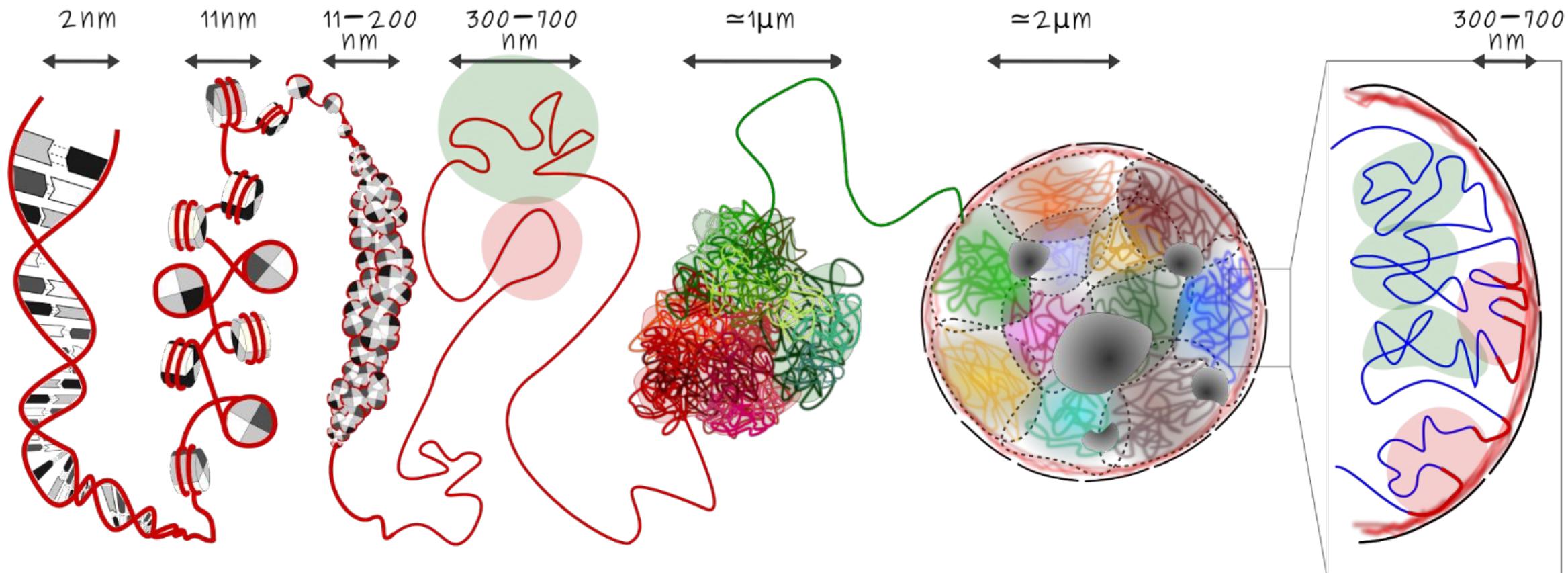
How 2 meter long DNA is organized inside the cell nucleus (~10 um) during interphase?



Chromosome Territories

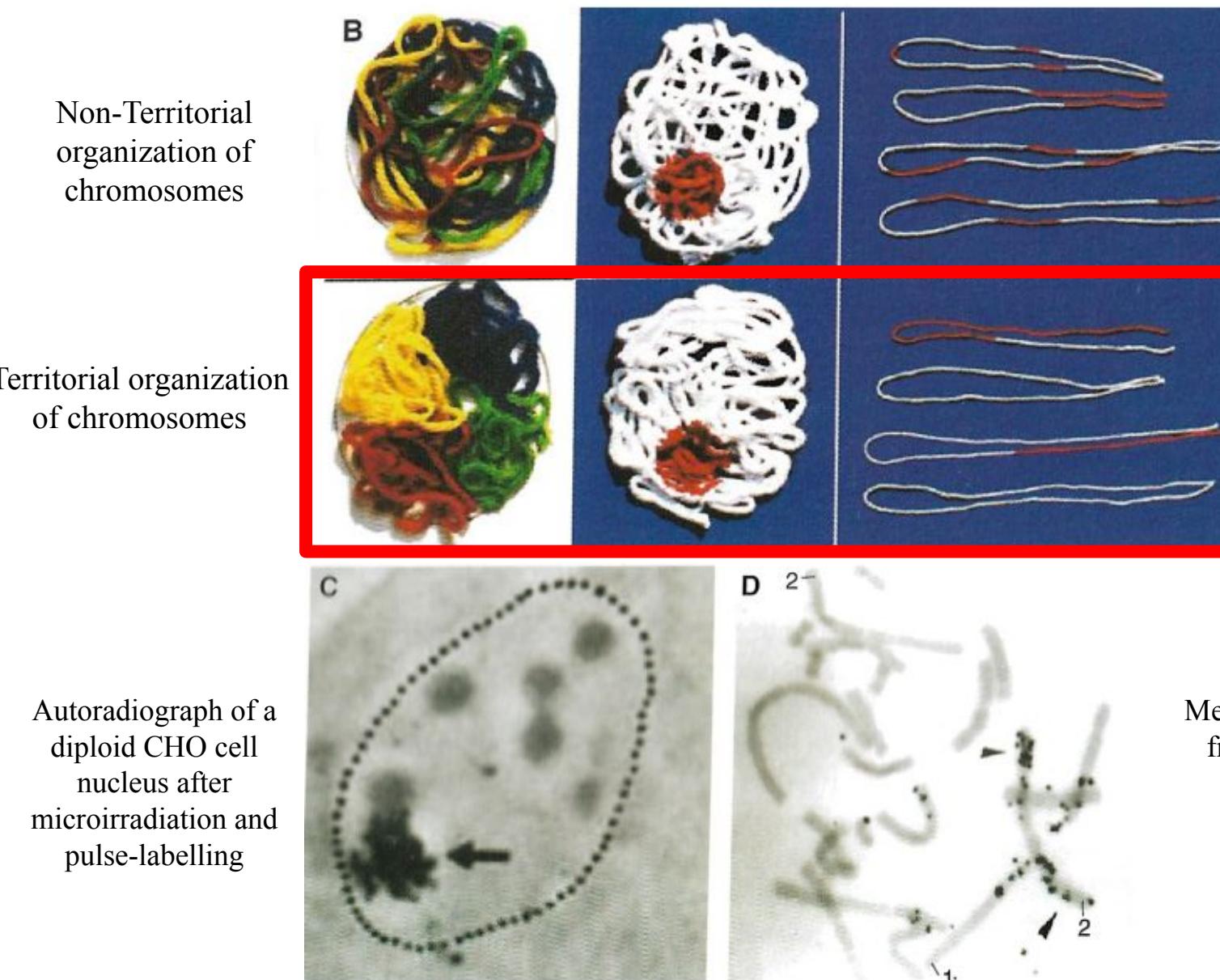


DNA Chromatin fiber DNA loop & Compartment, Chromatin/TAD domains Chromosome territory inside cell nucleus Lamina-associated domain(LAD)



- Nucleobases
- Nucleosome, composed of 8 histones protéines
- Repressive chromatin domain, mainly heterochromatin
- Active chromatin domain, mainly euchromatin
- Chromosome territory
- Nucleolus
- Nuclear speckles
- Nuclear membrane, including schematic nuclear pores
- Nuclear lamina
- LAD

Laser-UV-microirradiation experiment to answer whether the chromosome organization is non-territorial or territorial.

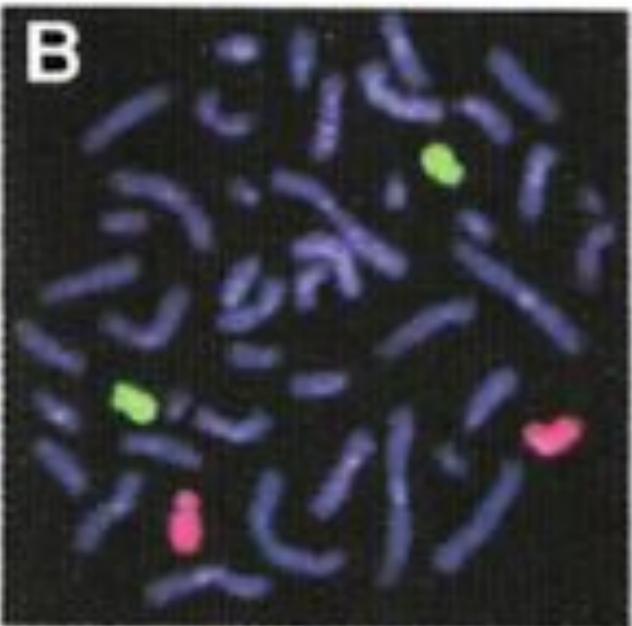


Steps of the Laser-UV-microirradiation experiment:

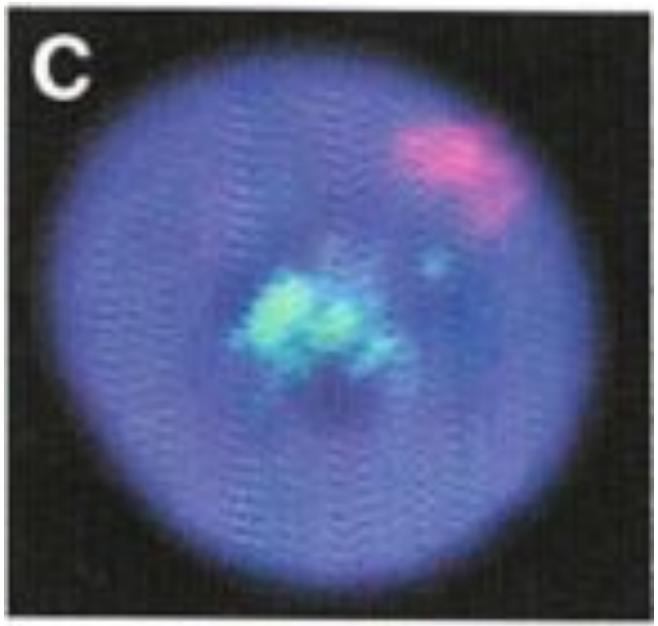
- 1) The nucleus of the live cell was microirradiated in G1
- 2) Pulse-labeled with $^{3\text{H}}$ thymidine
- 3) Fixed immediately
- 4) Autoradiography

Zorn et al. 1976
Zorn et al. 1979
Cremer et al. 1982
Cremer et al. 1982

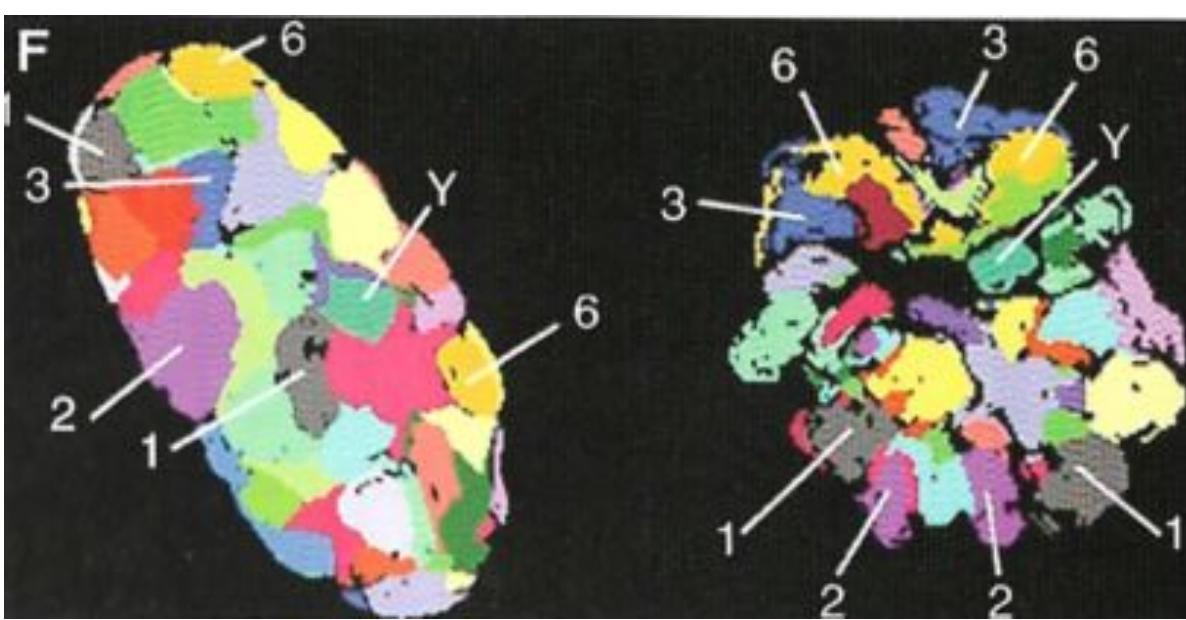
Direct visualization of CTs using Fluorescence In Situ Hybridization (FISH): 1980 onwards



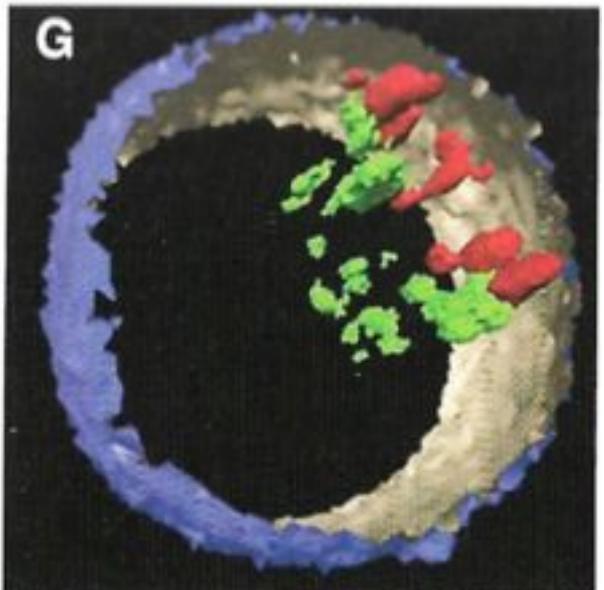
Chr 18 in red, Chr 19 in green by FISH
in a human metaphase plate



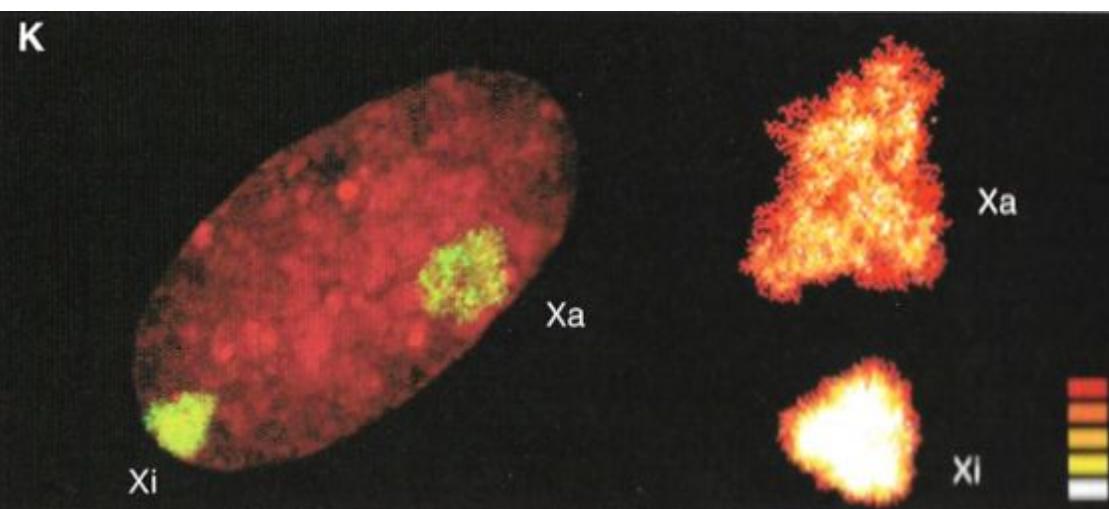
Nucleus of human lymphoblastoid cell
after 3D FISH, Chr 18 in red, Chr 19 in
green



Simultaneous delineation of all chromosomes in a human fibroblast nucleus
(left) and a prometaphase rosette (right) by multi-color FISH.

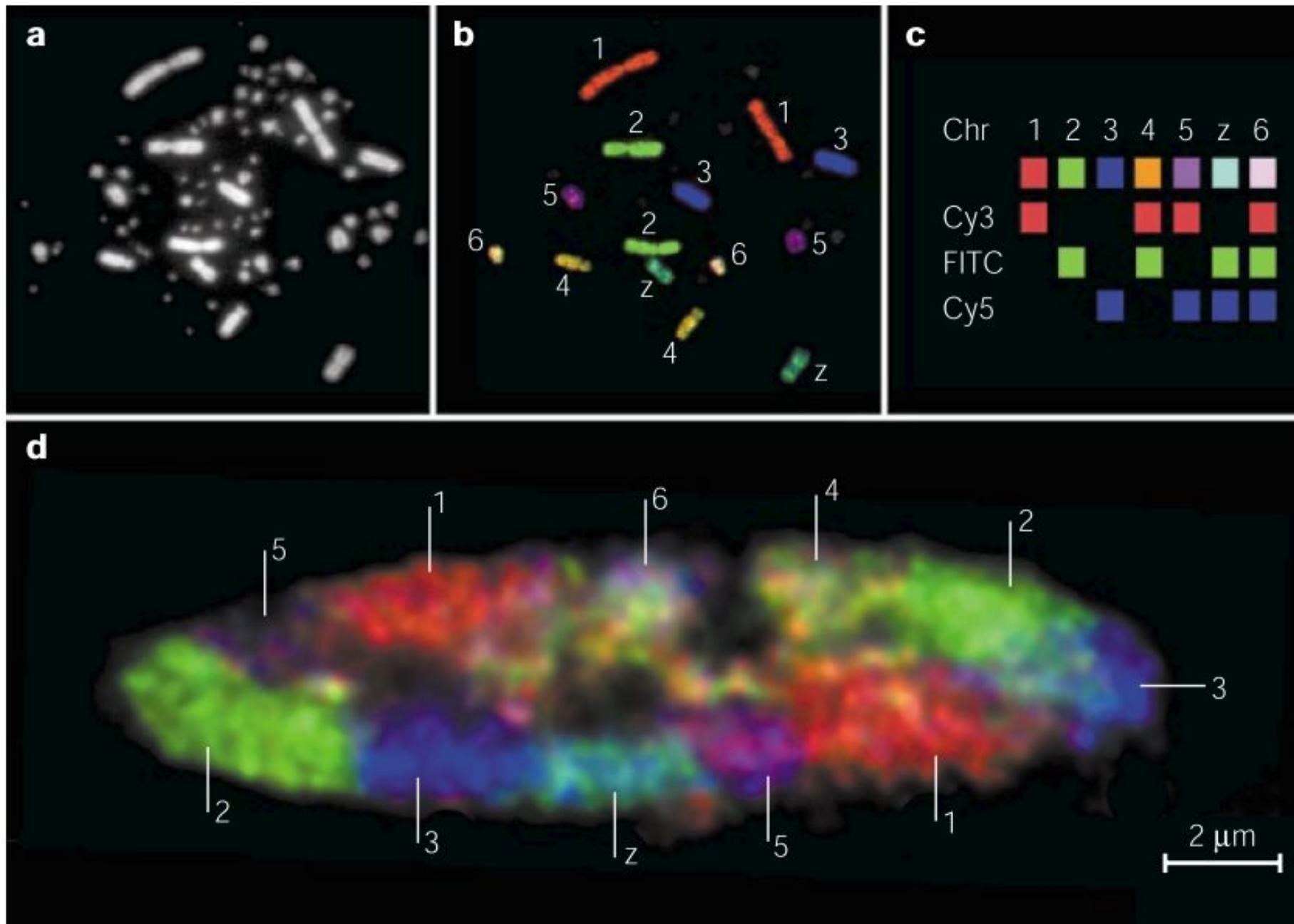


3D-FISH of human lymphocyte
nucleus, Chr 12: gene dense regions
(green), gene poor regions (red)

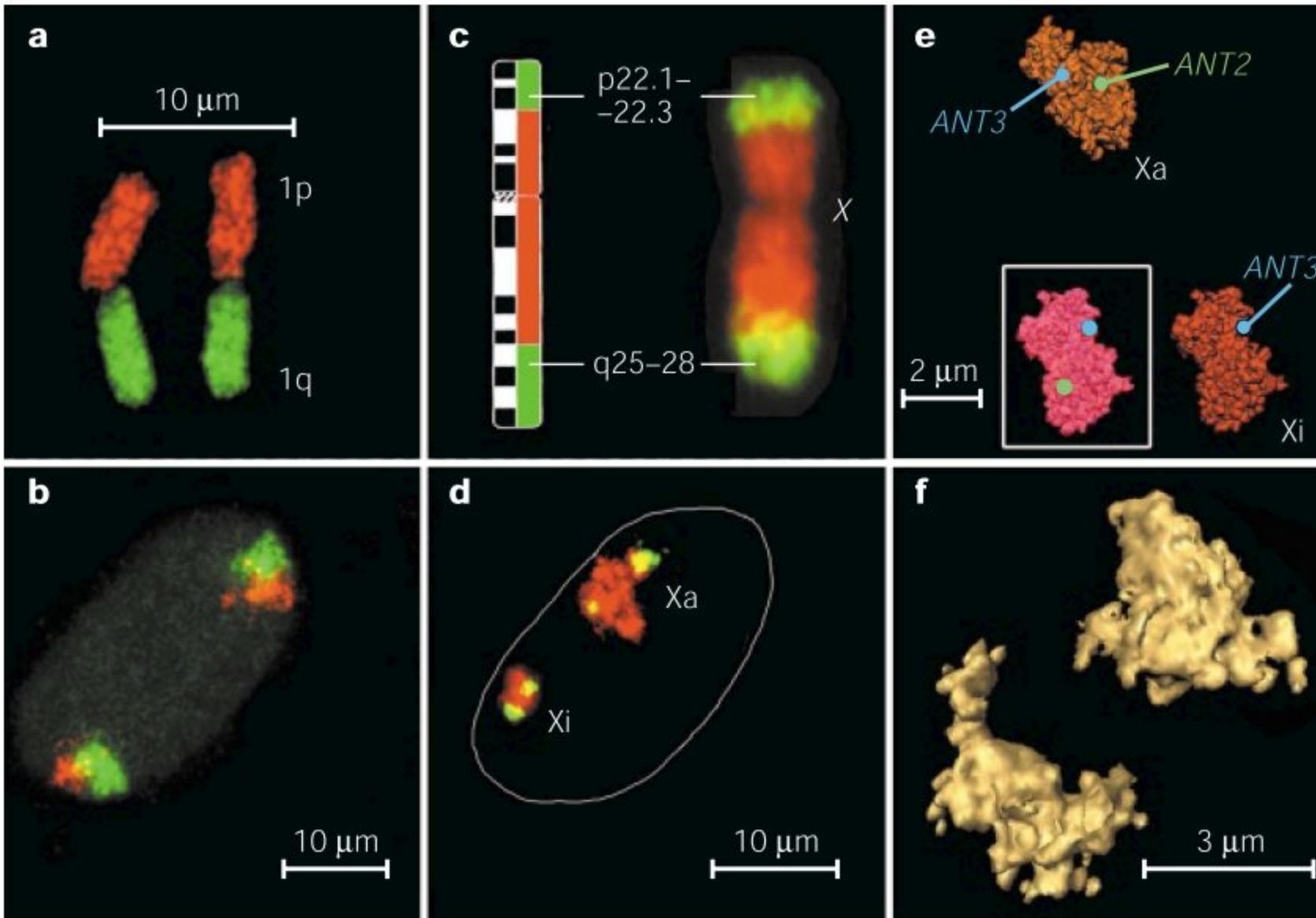


Xa and Xi territory in a female human fibroblast nucleus (46, XX).
Right: intensity profiles of enlarged images of Xi- and Xa- territory.

Chromosome territories in the chicken cell nucleus visualized by chromosome paint

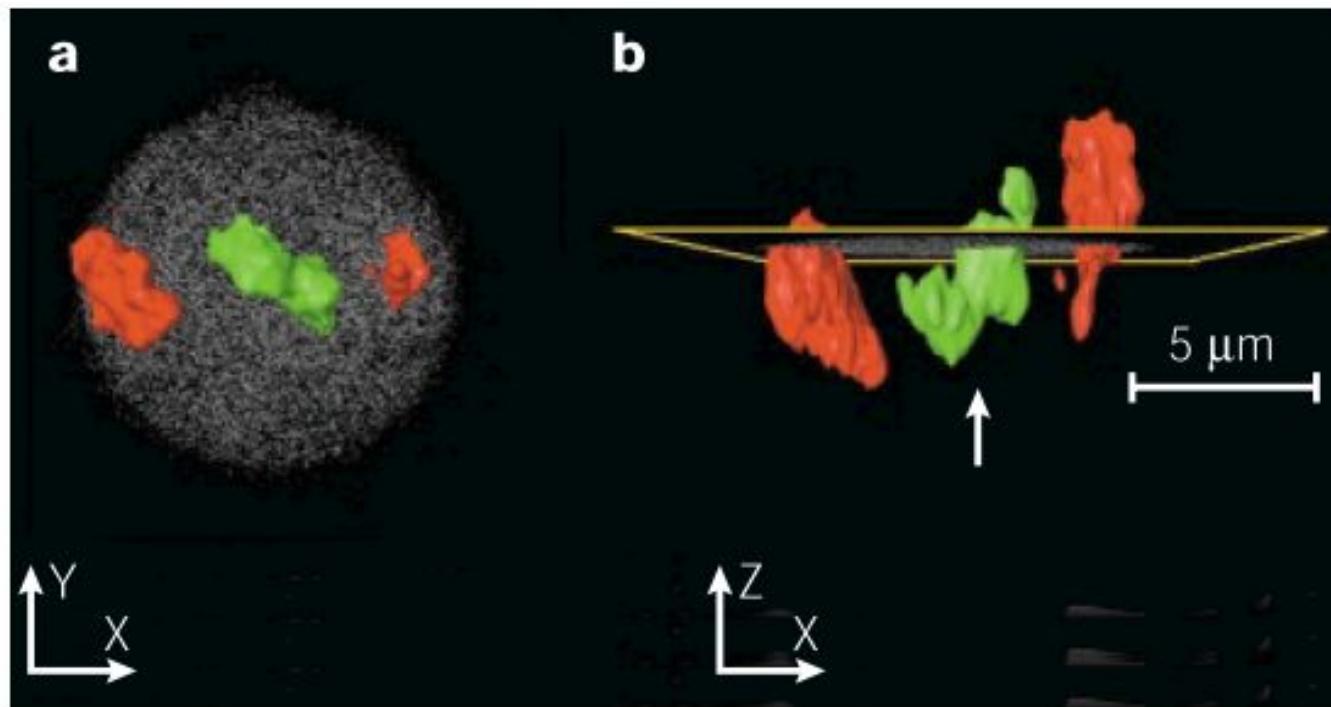


Features of human chromosome territories



How the chromosome territories are organized? Is it a random organization or has some preference?

Chromosome 18 Gene Poor CTs: Red
Chromosome 19 Gene Rich CTs: Green

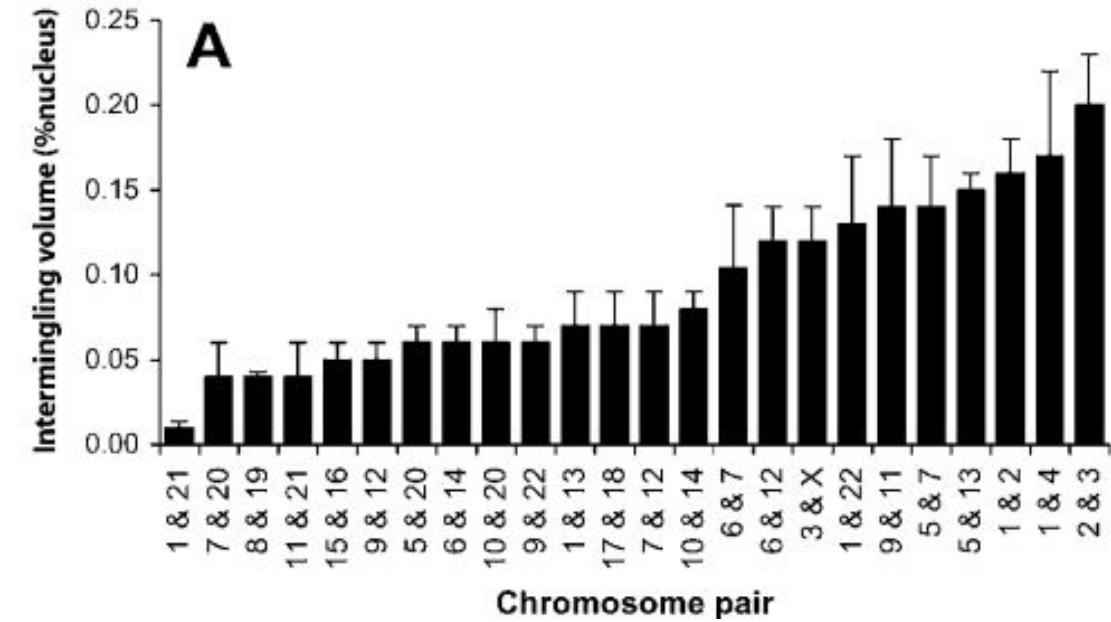
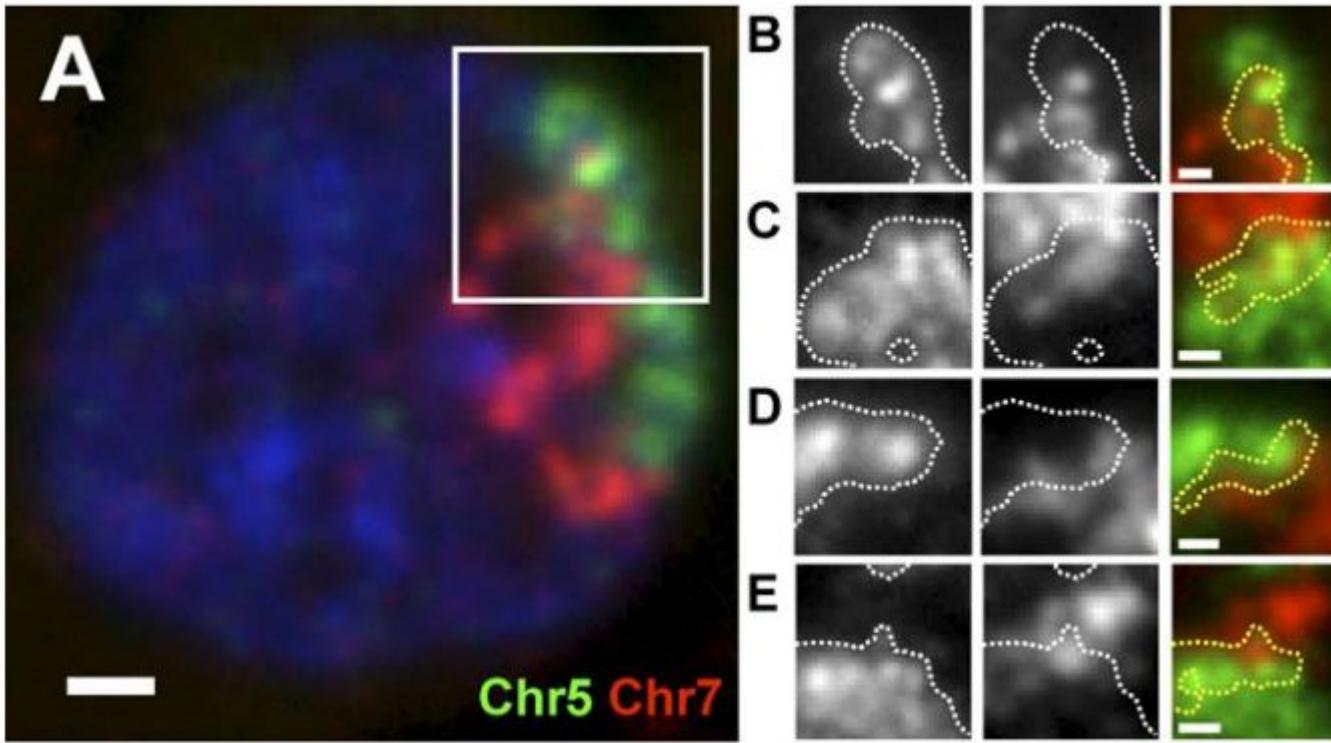


Both chromosomes have same DNA content, still different preference for nuclear localization.

Figure 4 | Gene-rich and gene-poor chromosome territories. Three-dimensional reconstructions of chromosome 18 (red; gene-poor) and 19 (green; gene-rich) territories painted in the nucleus of a non-stimulated human lymphocyte. (Image courtesy of Marion Cremer and Irina Solovei.) Chromosome 18 territories were typically found at the nuclear periphery, whereas chromosome 19 territories were located in the nuclear interior⁴². **a** | X,Y view: a mid-plane section of the nucleus is shown as a grey shade. Only the parts of the territories below this section can be seen. **b** | X,Z view: the arrow marks the side from which the section in part **a** is viewed.

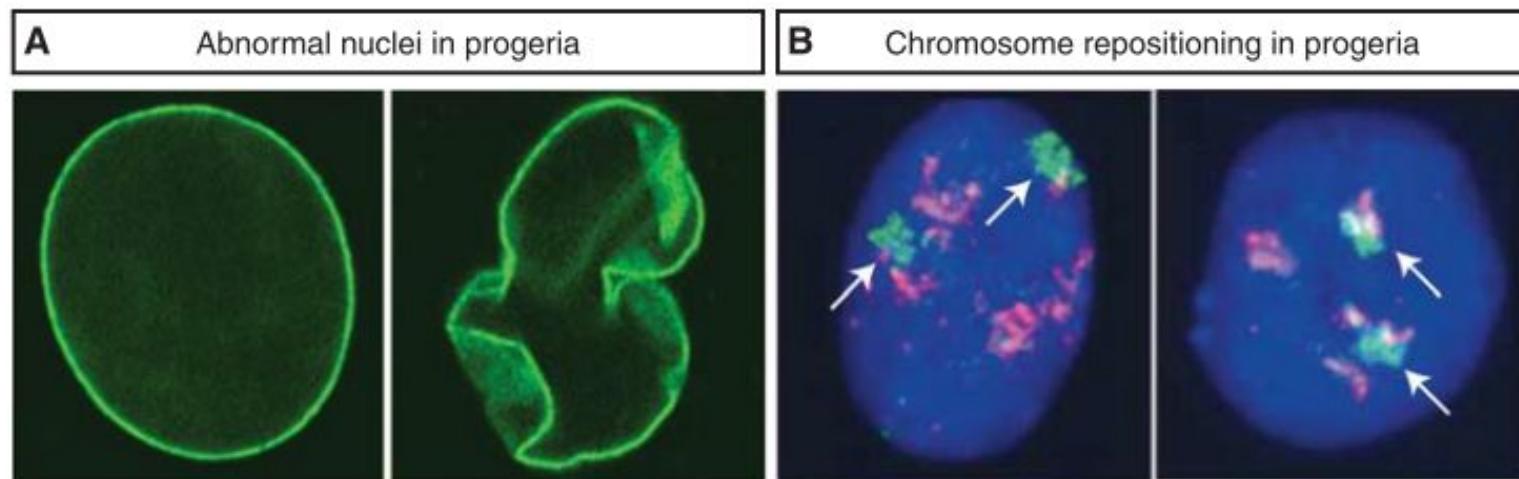
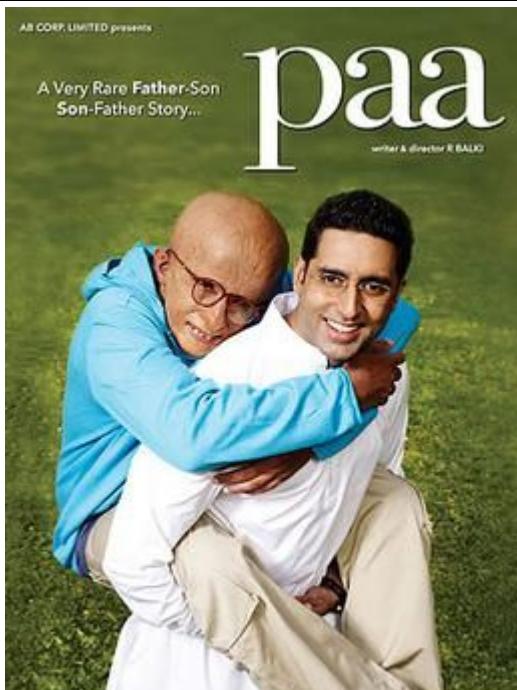
Intermingling of chromosome territories revealed by Cryo-FISH

Intermingling of chromosome territories



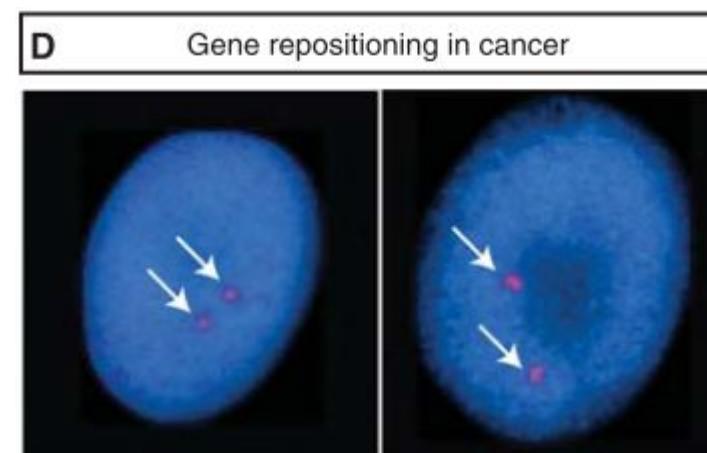
Altered gene positioning in diseases

- Lamins and emerin (EMD) are integral proteins of the nuclear envelope and are linked to a group of rare genetic disorders called nuclear envelopathies. (Webster et al. 2009)
- Well known nuclear envelopathies such as Hutchinson-Gilford progeria syndrome (HGPS, premature aging) and Emery-Dreifuss muscular dystrophy (EDMD) are associated with mutations of the lamin A/C or emerin genes and are often characterized by alterations in nuclear morphology and may compromise gene expression.
- Studies in primary fibroblasts of several nuclear envelopathies (including HGPS and EDMD) show repositioning of chromosomes 13 and 18 toward the nuclear interior. (Meaburn et al. 2007)

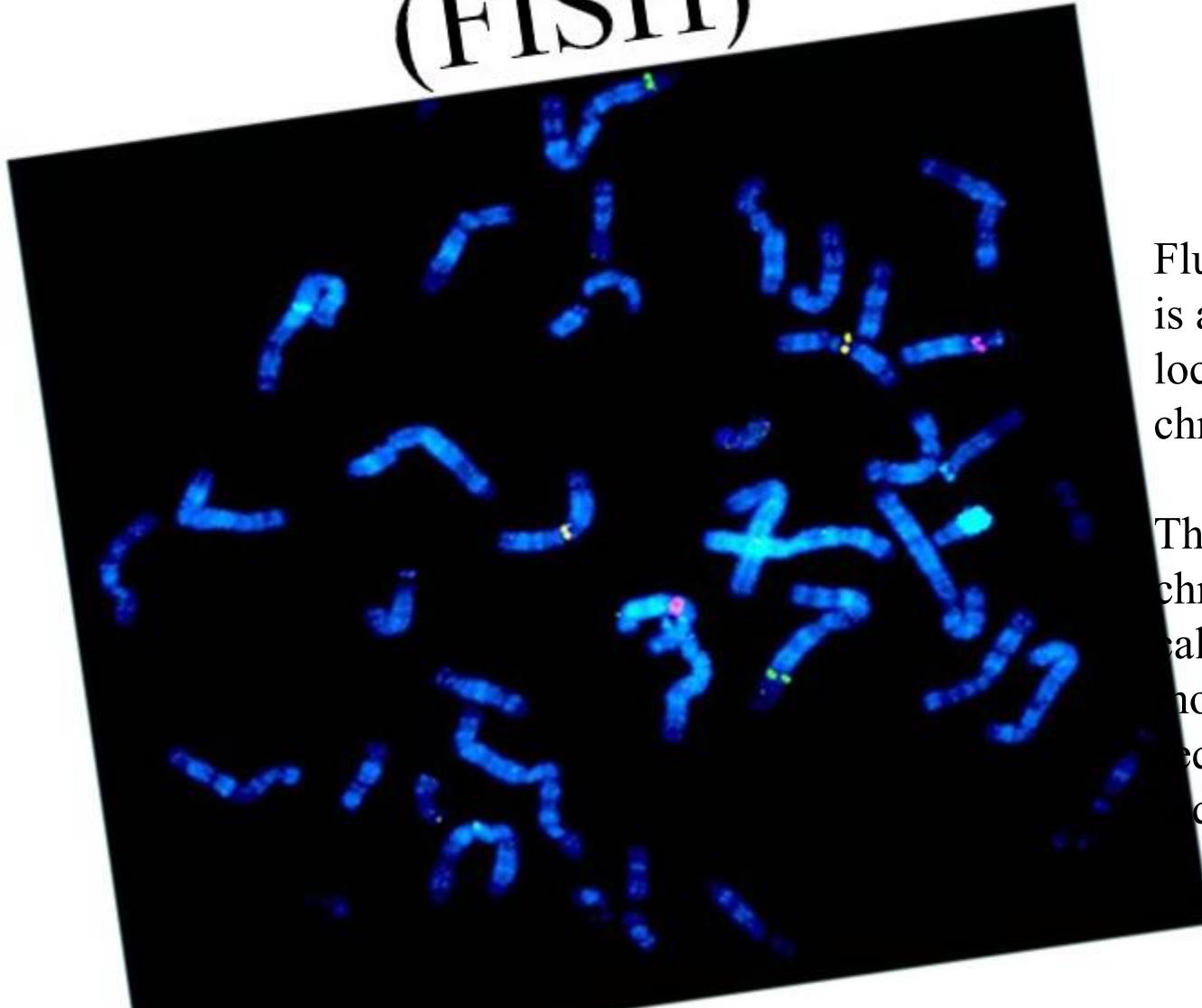


Altered gene positioning in diseases

- Altered chromosome positioning has also been associated with numerous types of cancer in which altered gene expression patterns lead to malignant transformation of the cell.
- Chronic leukemias are associated with enlarged nuclei in lymphoid cells.
- Abnormal relocation of chromosome 18 from the nuclear periphery to the interior has been documented in several types of tumor cell lines, including melanoma-derived line, cervix carcinoma, colon carcinoma, Hodgkin-derived cell line and colon carcinoma metastatic cells.
- A radial shift of chromosome 8 and a significant change in its shape was observed in pancreatic neoplastic tissue as well as a radial shift of centromeric regions of chromosome 17 and HER2 domains in tumor breast tissues.
- In vitro induction of tumorigenesis in early breast cancer showed altered positioning of a set of cancer-associated genes such as AKT1, BCL2, ERB2, VEGF, although no correlation was found between radial redistribution and gene activity level.

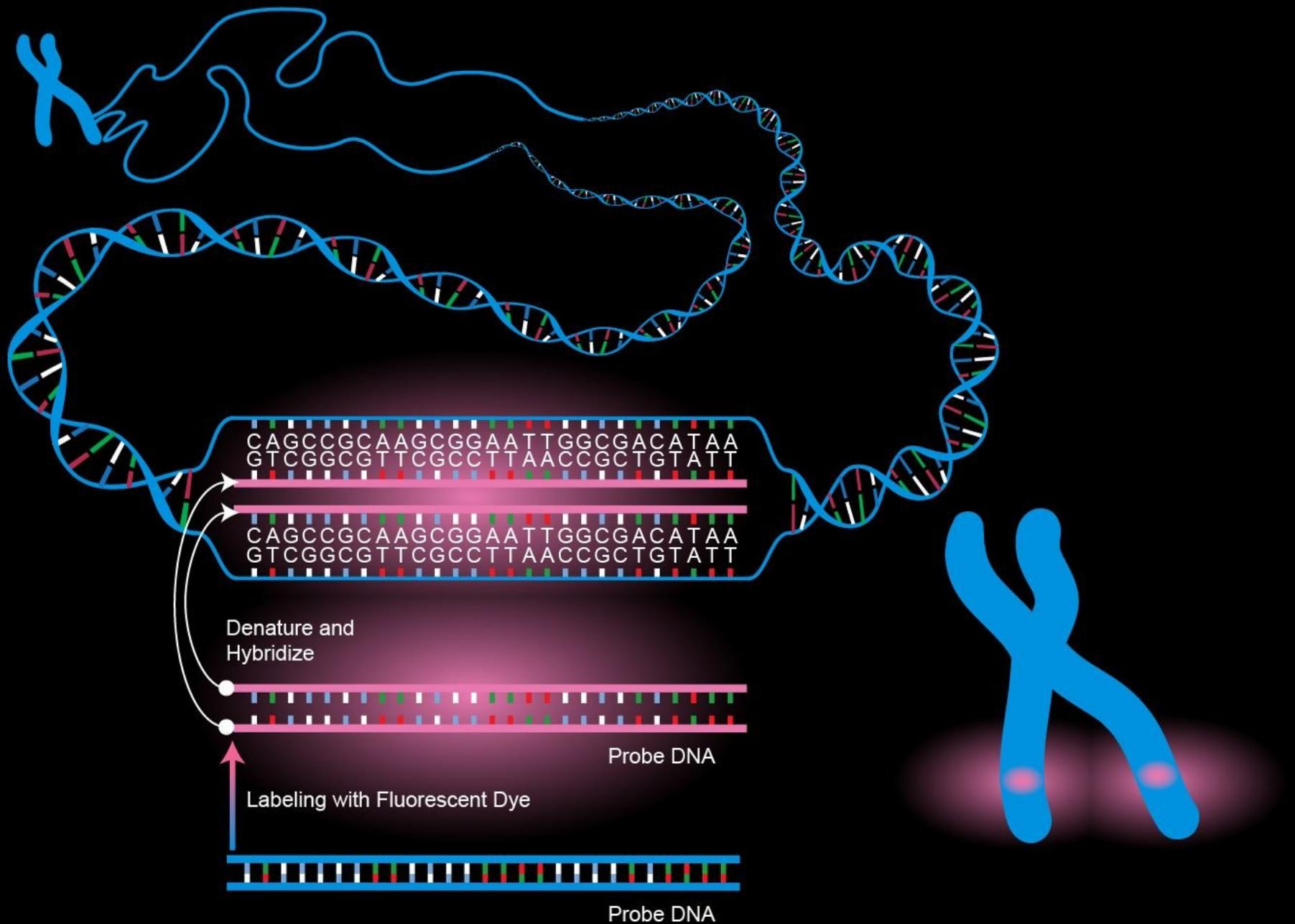


Fluorescence *In-situ* Hybridization (FISH)

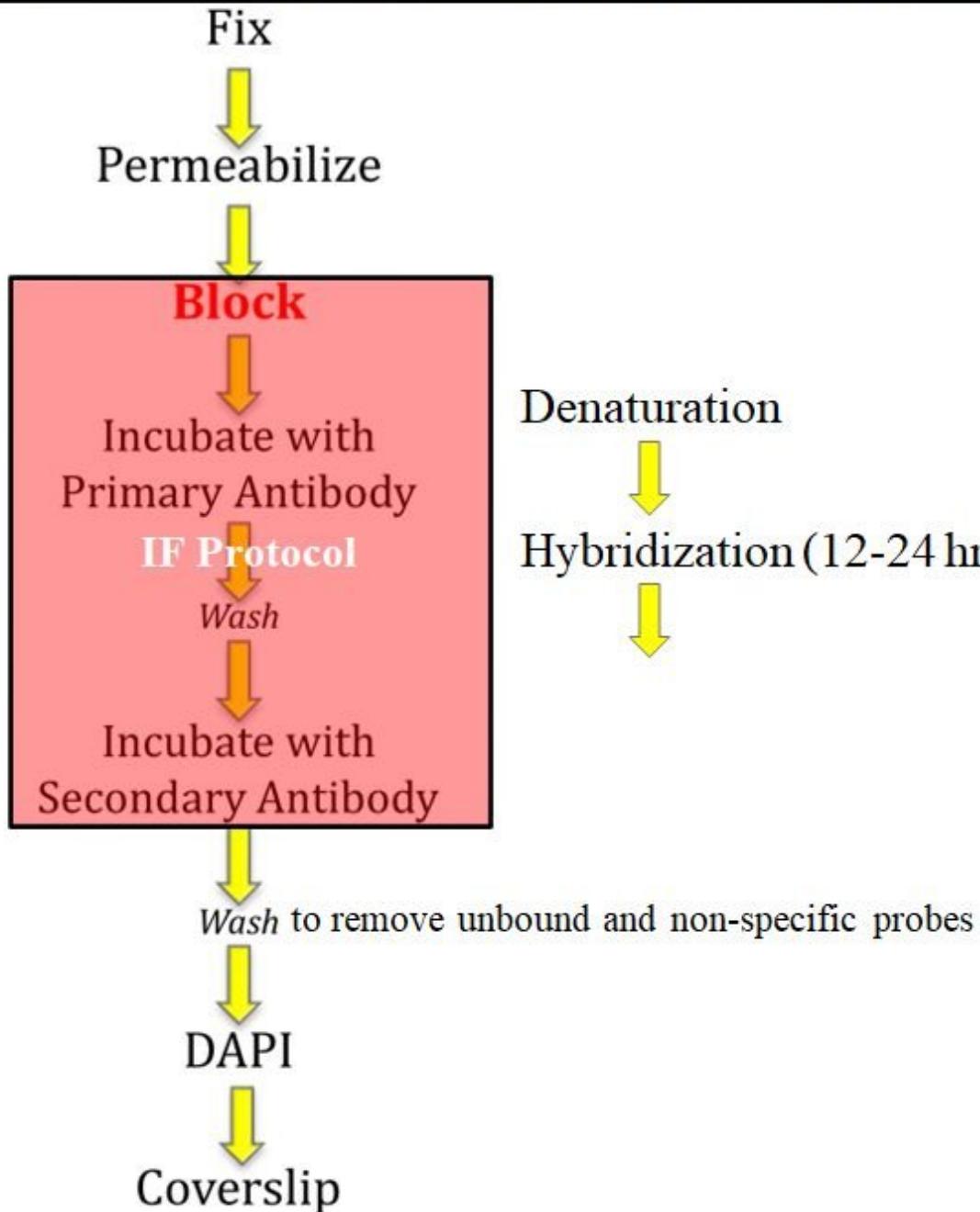


Fluorescence in situ hybridization (FISH) is a laboratory technique for detecting and locating a specific DNA sequence on a chromosome.

The technique relies on exposing chromosomes to a small DNA sequence called a probe that has a fluorescent molecule attached to it. The probe sequence binds to its corresponding sequence on the chromosome.



In-Situ Hybridization (ISH) or Fluorescence In Situ Hybridization (FISH)



ISH or FISH is a technique that is used for localization and detection of **specific DNA** and **RNA** sequences in fixed cells and tissues by hybridizing the complementary strand of a nucleotide probe to a particular sequence.

Fluorescent probes (~20 bp DNA/RNA conjugated with fluorescent dye) bind to the target DNA/RNA by complementary base pairing.

Applications:

- Karyotyping: Chromosomal aberrations
- Gene positioning in the nucleus
- Gene expression profiling

Types of probes for DNA FISH

Centromere probes

- Alpha and Satellite III probes
- Generated from repetitive sequences found in centromeres
- Centromere regions are stained brighter

Whole chromosome

- Collection of probes that bind to the whole length of chromosome
 - Multiple probe labels are used
 - Hybridize along the length of the chromosome

Probes

Telomere

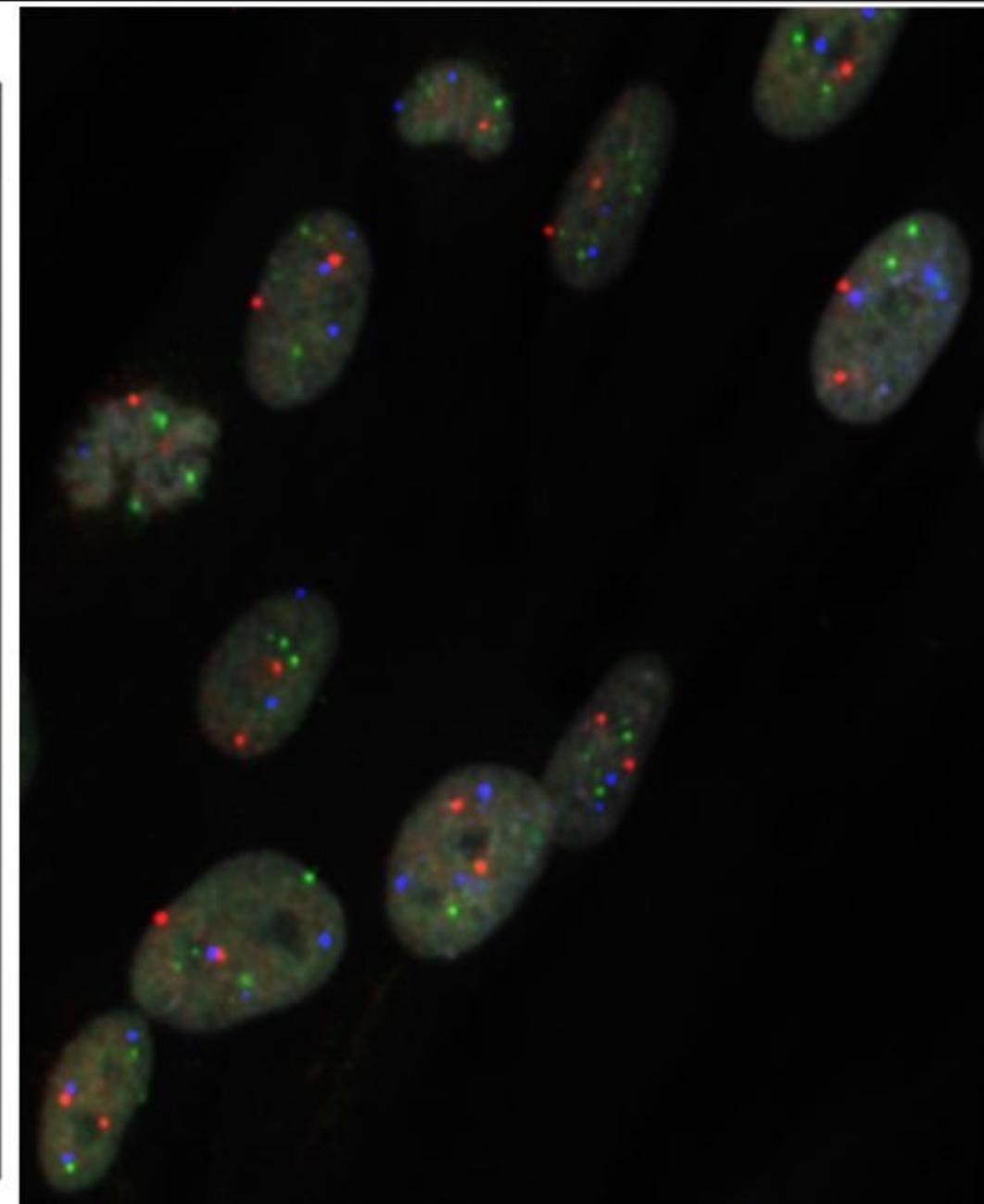
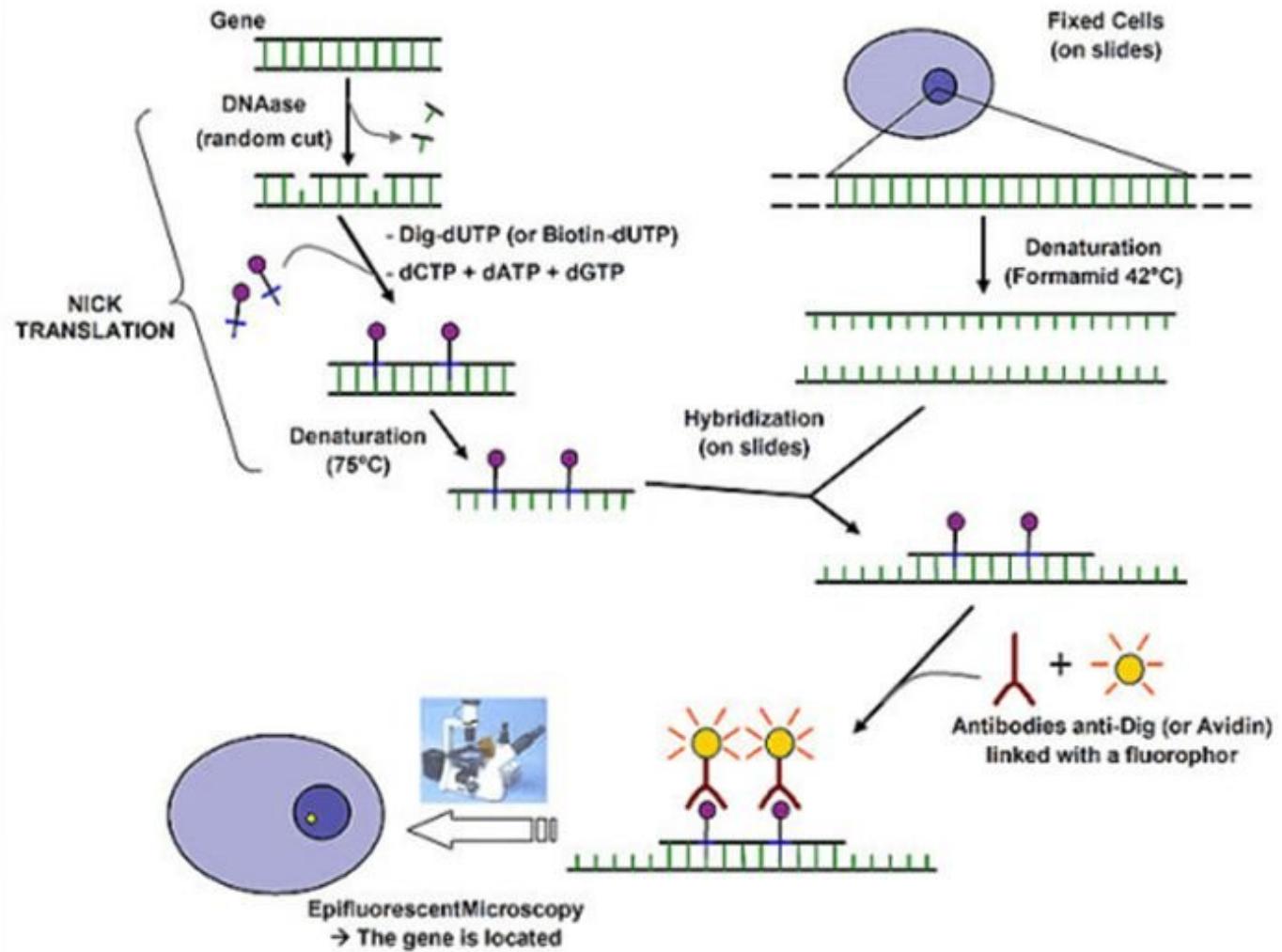
- Specific for telomeres
- Specific to the 300 kb locus at the end of specific chromosome

Locus

- Deletion
 - Translocation probes
- Gene detection & localization probes
 - Gene amplification probes

Multiplexed DNA FISH

FISH (Fluorescent In Situ Hybridization)



Further reading

Review Articles:

- 1) Branco MR & Pombo A. Intermingling of chromosome territories in interphase suggests role in translocation and transcription-dependent associations. PLoS Biology 2006; 4(5):e138. DOI: [10.1371/journal.pbio.0040138](https://doi.org/10.1371/journal.pbio.0040138)
- 2) Cremer T & Cremer C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nature 2001;2:292-301. DOI: [10.1038/35066075](https://doi.org/10.1038/35066075)
- 3) Meaburn KJ & Misteli T. Cell Biology: Chromosome Territories. Nature 2007;445(7126):379-789. DOI: [10.1038/445379a](https://doi.org/10.1038/445379a)
- 4) Cremer T & Cremer M. Chromosome Territories. Cold Spring Harbor Perspective in Biology 2010;2:a003889. DOI: [10.1101/cshperspect.a003889](https://doi.org/10.1101/cshperspect.a003889).

Next class on 27/06/2022 (Monday)

BT1010 Introduction to Life Sciences



Lecture 6: Metabolism
29/06/2022

Course Instructor:

Dr. Gunjan Mehta, Ph.D.

Assistant Professor

Department of Biotechnology

IIT Hyderabad

(M.) +91 70168 96886 Email: gunjanmehta@bt.iith.ac.in

What is Metabolism?

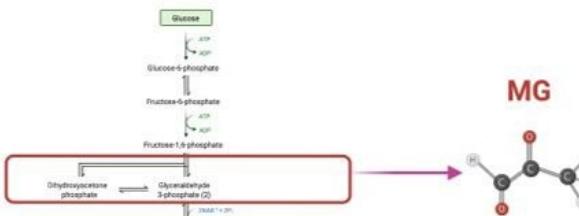
- Metabolism is the set of life-sustaining chemical reactions in organisms.
- The three main purpose of metabolism are:
 - 1) the conversion of food energy (carbohydrates, proteins, fats) to cellular energy (ATP);
 - 2) the conversion of food to building blocks for protein, lipids, nucleic acids, carbohydrates;
 - 3) the elimination of metabolic wastes.
- Metabolic reactions may be categorized as **catabolic** (the breaking down of compounds, e.g. glucose to pyruvate for cellular respiration) or **anabolic** (the synthesis of compounds such as proteins, carbohydrates, lipids, nucleic acids).
- Usually, catabolism releases energy and anabolism consumes energy.

Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway

Catabolic pathways for breaking down carbohydrates to produce energy (ATP)

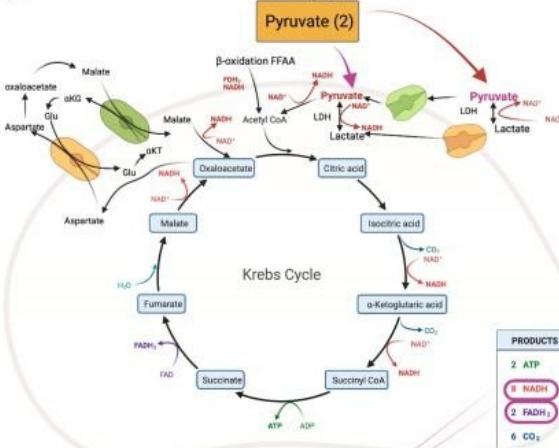
Complete digestion (catabolism) of glucose is a three step process

1.- Glycolysis



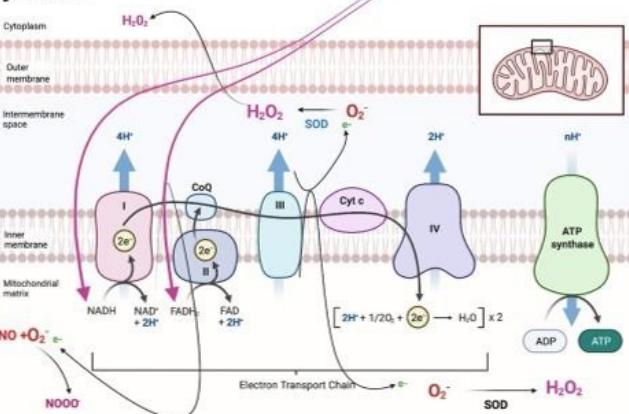
Cytoplasm

2.- TCA cycle



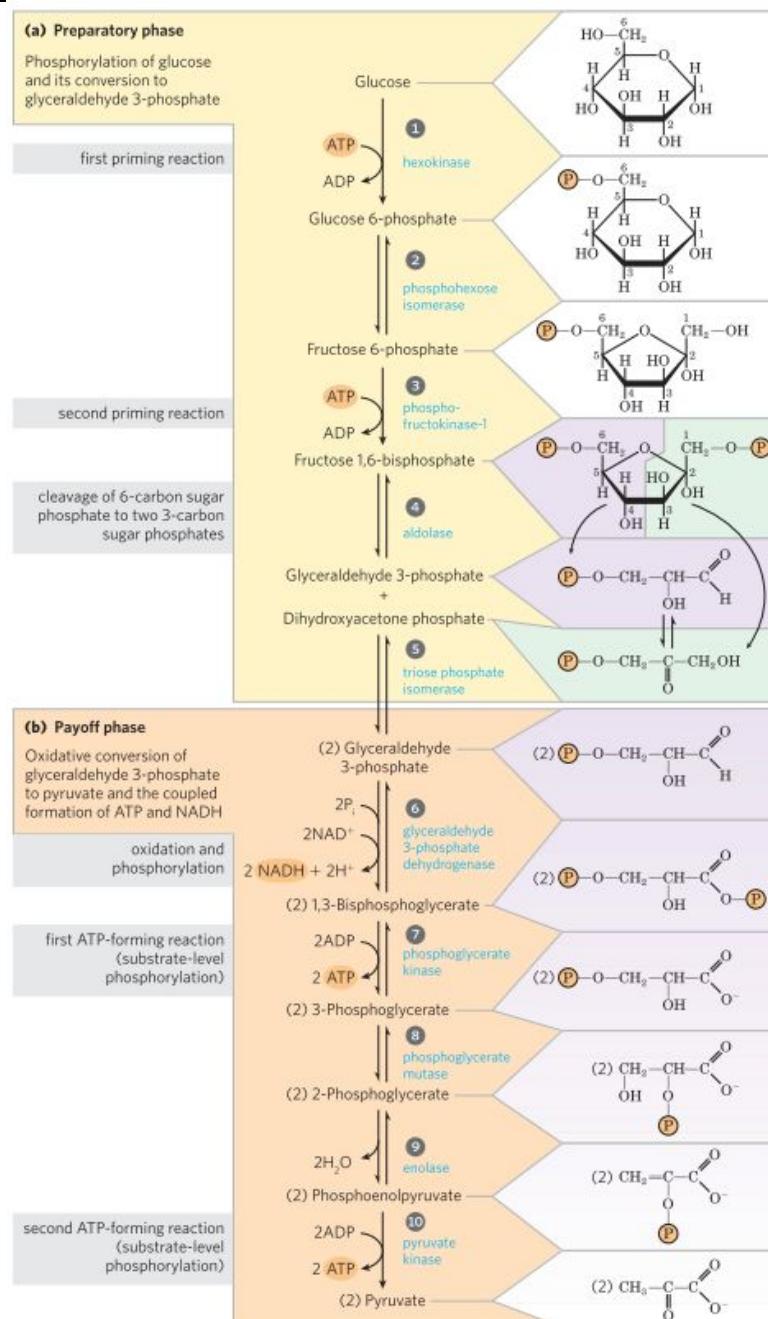
Mitochondria

3.- Oxidative phosphorylation



Mitochondria

Step 1: Glycolysis (in cytoplasm)

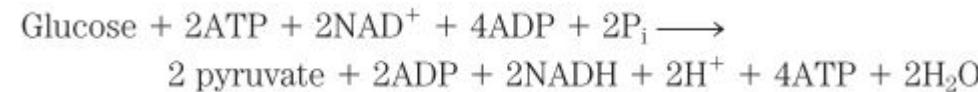


Glycolysis is a near-universal pathway by which a glucose molecule is oxidized to two molecules of pyruvate, with energy conserved as ATP and NADH.

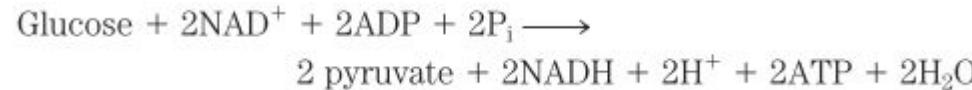
In the preparatory phase, ATP is invested to convert glucose to Fructose 1,6-bisphosphate and eventually to two molecules of Glyceraldehyde-3-phosphate.

In the payoff phase, each of the two molecules of glyceraldehyde-3-phosphate undergoes oxidation at C-1, the energy of this oxidation reaction is conserved in the form of one NADH and two ATP.

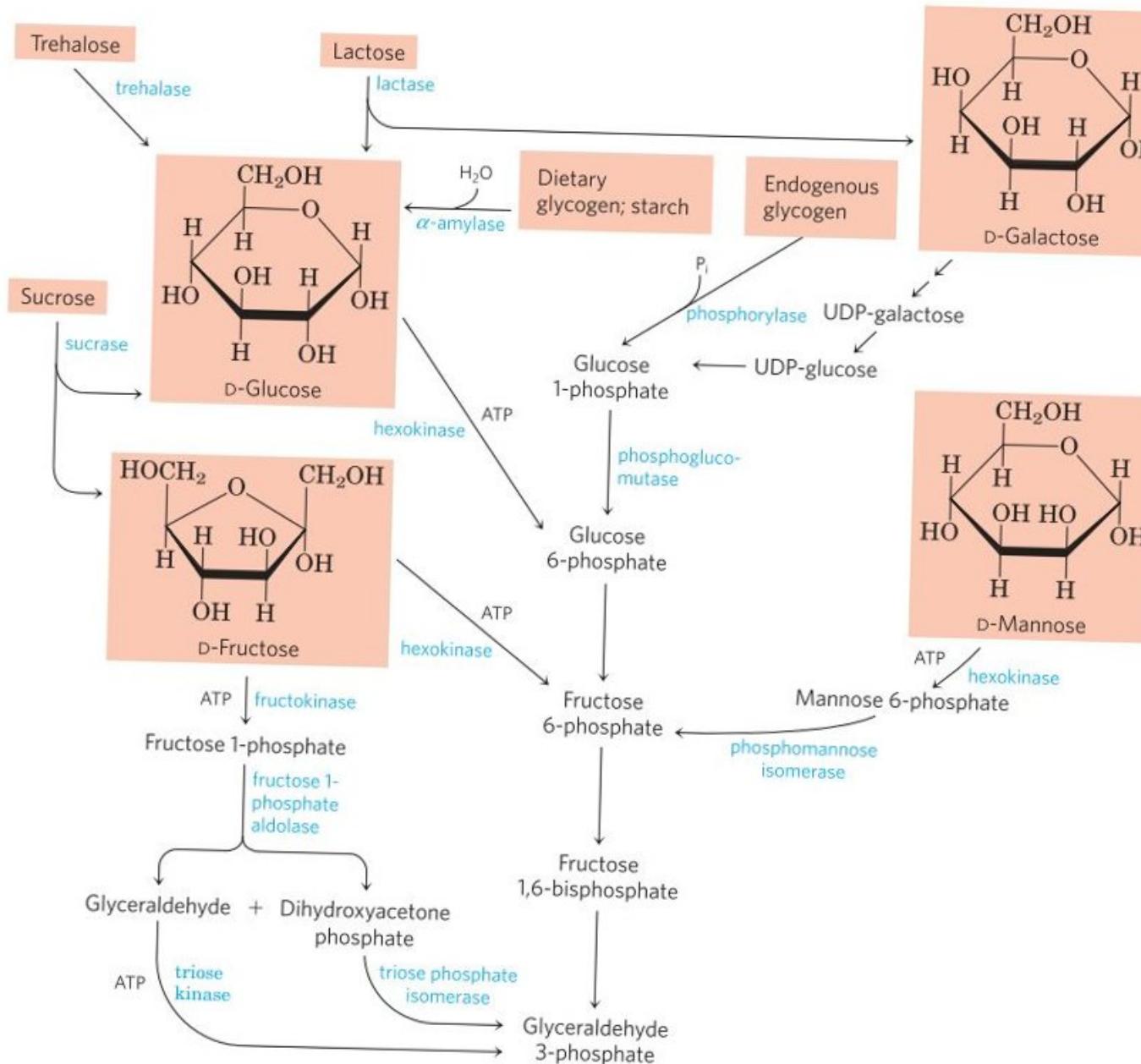
The overall balance sheet shows a net gain of ATP



Cancelling out common terms on both sides,

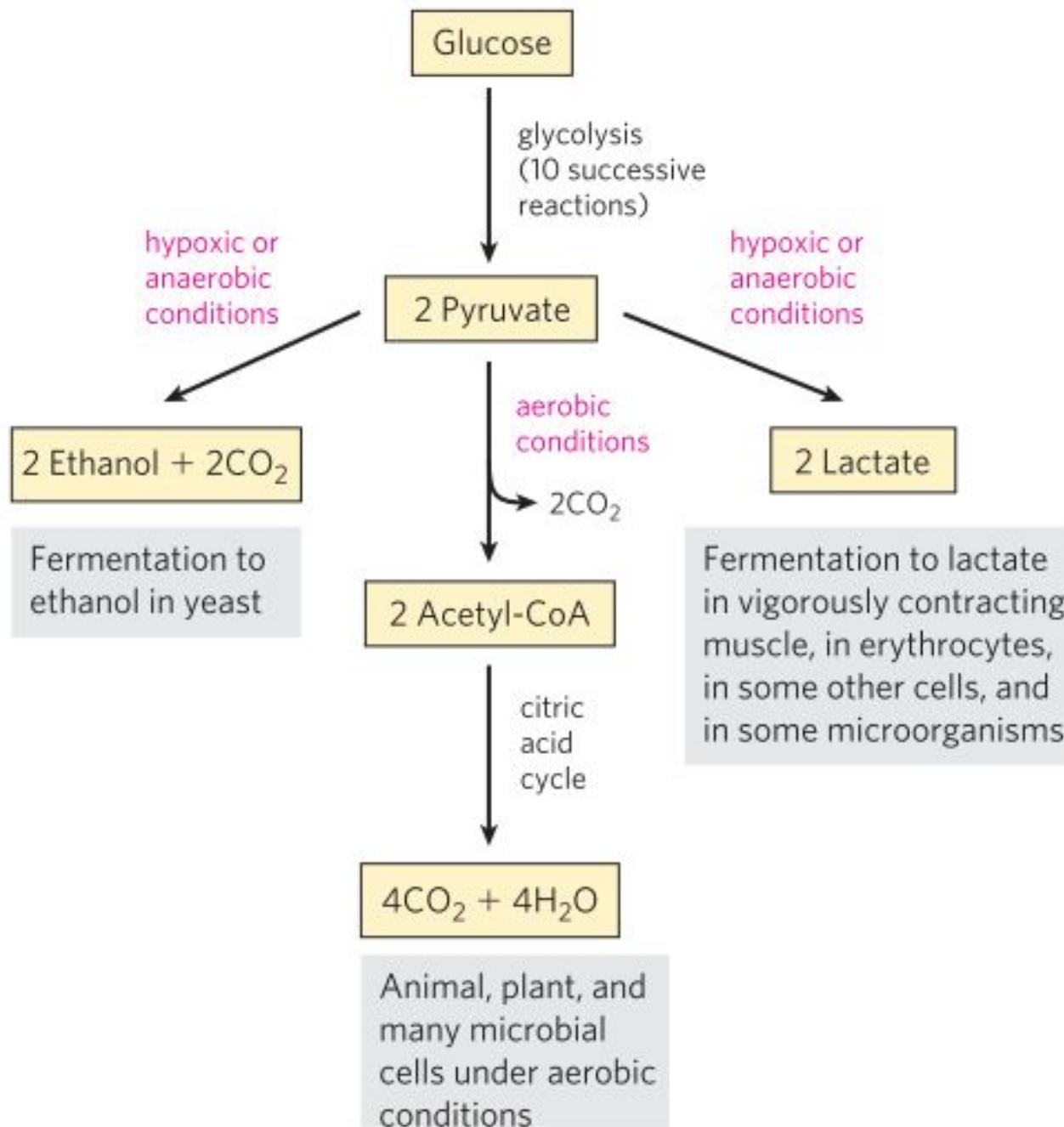


Entry of dietary glycogen, starch, disaccharides and hexoses in to the glycolysis



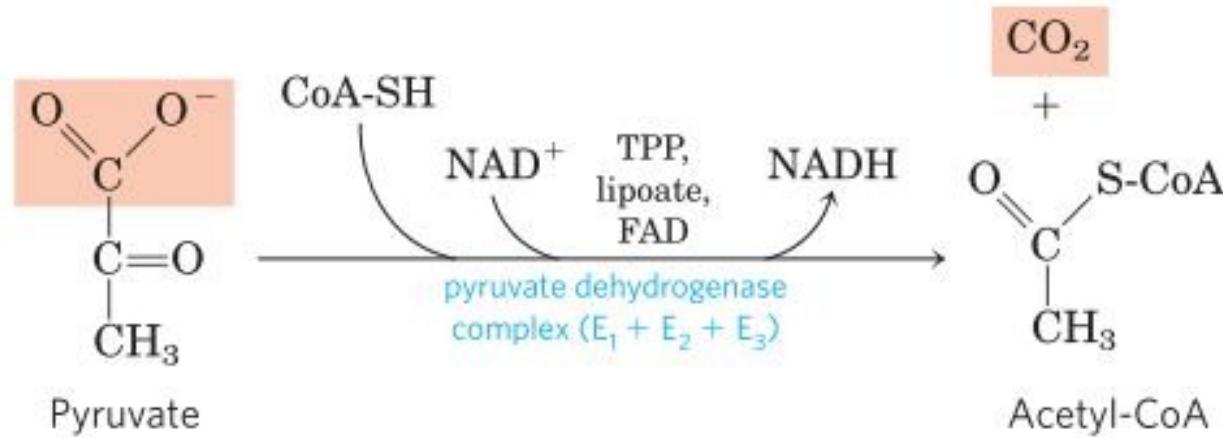
All different types of carbohydrates (starch, glycogen, polysaccharides, glucose, fructose, sucrose etc.) that we eat in our diet, are eventually utilized by glycolysis for the energy (ATP) production.

Fates of pyruvate



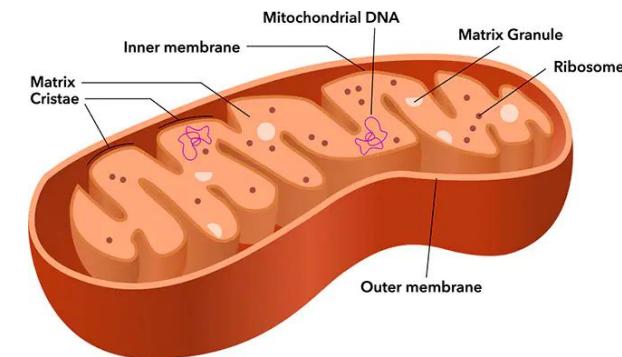
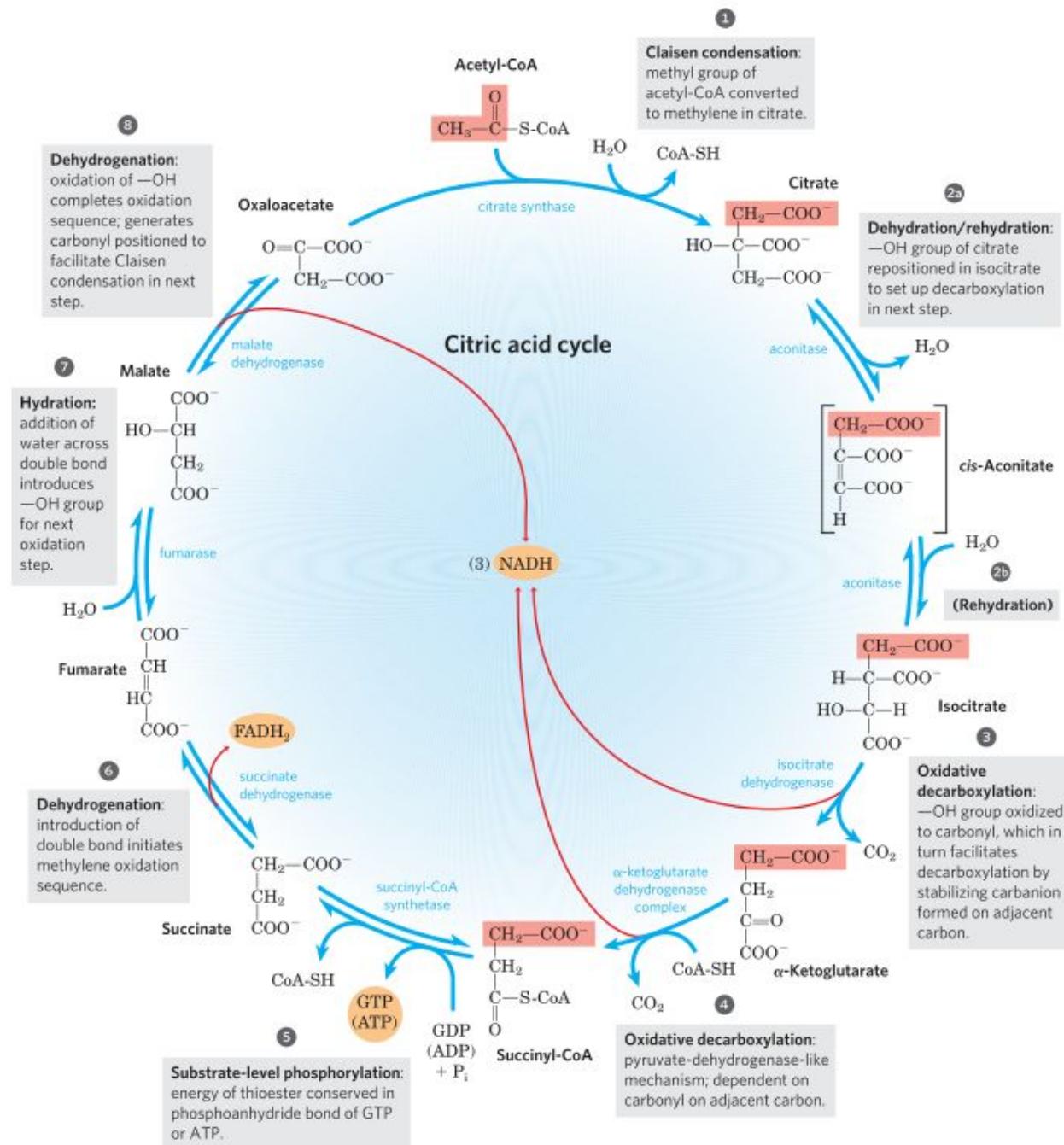
Step 2: TCA Cycle (Tricarboxylic Acid Cycle, Citric Acid Cycle, Krebs cycle)

- In aerobic organism, glucose and other sugars, fatty acids and most amino acids are ultimately oxidized to CO_2 and H_2O via the citric acid cycle and the respiratory chain.
- Before entering the TCA cycle, the carbon skeleton is degraded to the **acetyl-CoA**.
- Pyruvate dehydrogenase is the enzyme which converts pyruvate to Acetyl CoA.



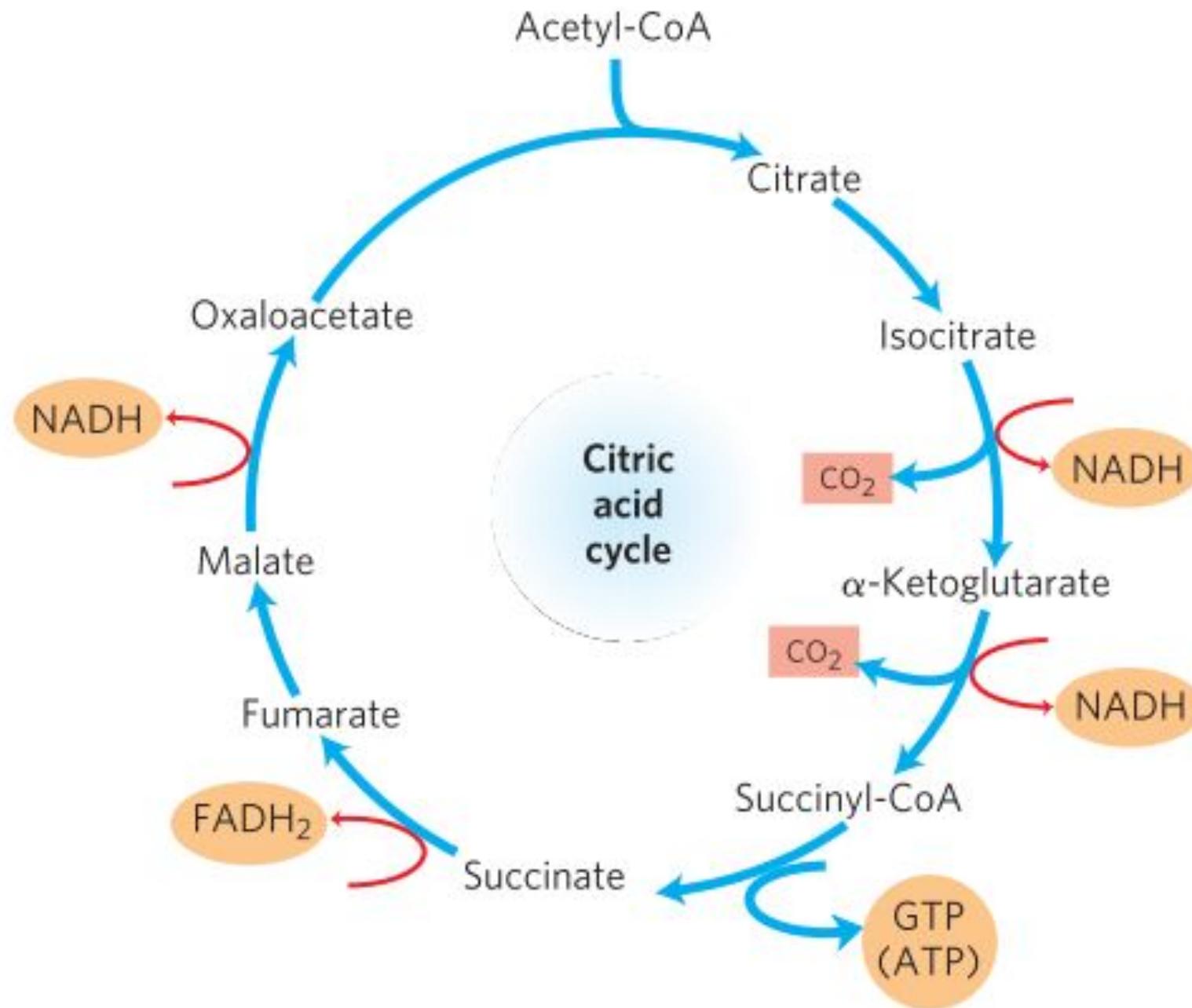
- Acetyl CoA enters the TCA cycle for further oxidation.

Step 2: TCA Cycle (The Citric Acid Cycle)



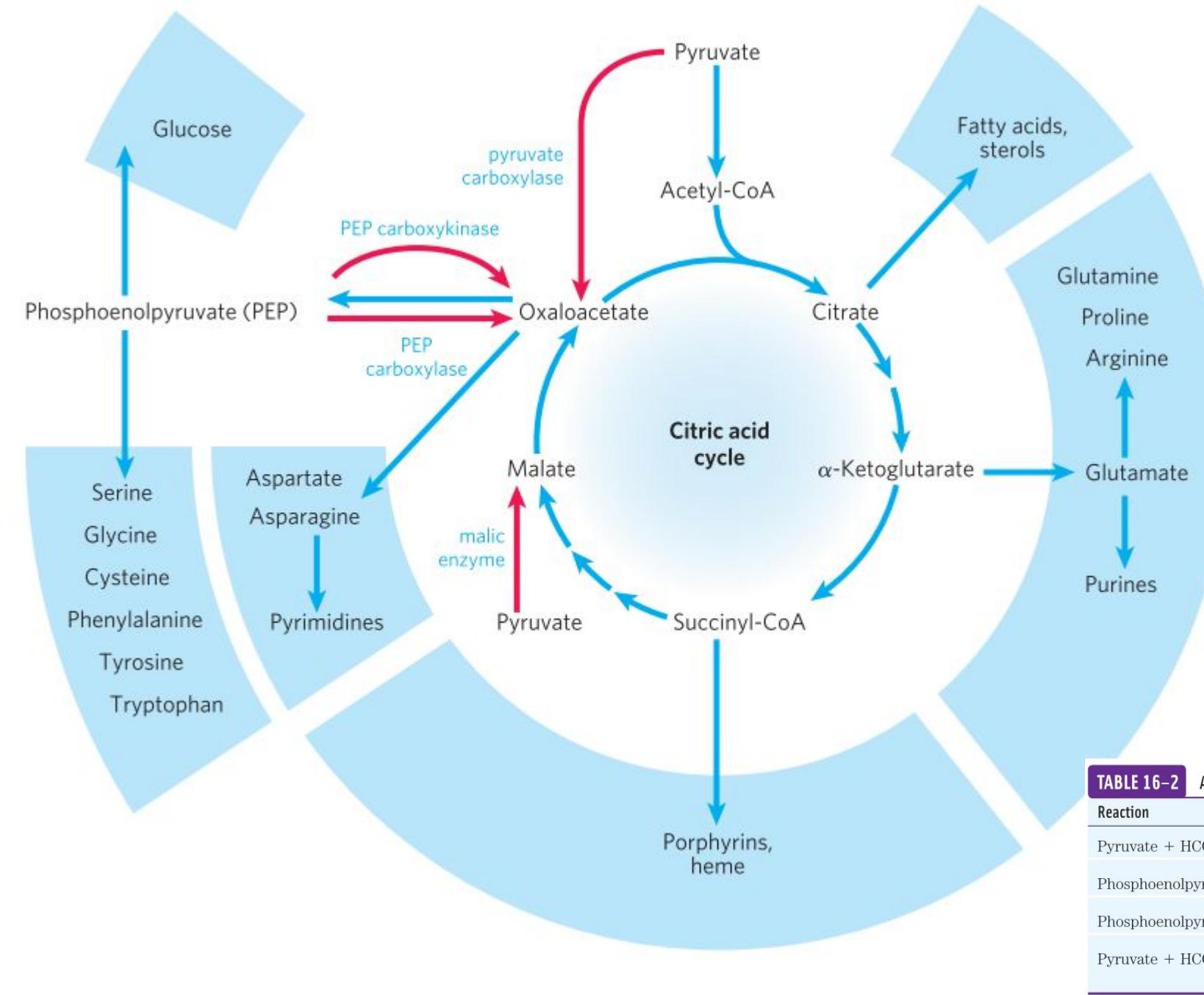
TCA cycle takes place in Mitochondria.

Products of one turn of the TCA cycle



- NADH, FADH₂ are electron carriers.
- They carry electrons from TCA cycle to the electron transport chain for oxidative phosphorylation.
- Oxidative phosphorylation produces 2.5 ATP from NADH and 1.5 ATP from FADH₂.

Role of the citric acid cycle in anabolism



Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways.

Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates.

TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + \text{HCO}_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + \text{P}_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + \text{CO}_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + \text{HCO}_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + \text{P}_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + \text{HCO}_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and bacteria

STEP3: Oxidative Phosphorylation (in mitochondria)

Flow of electrons and protons through the four complexes of the respiratory chain

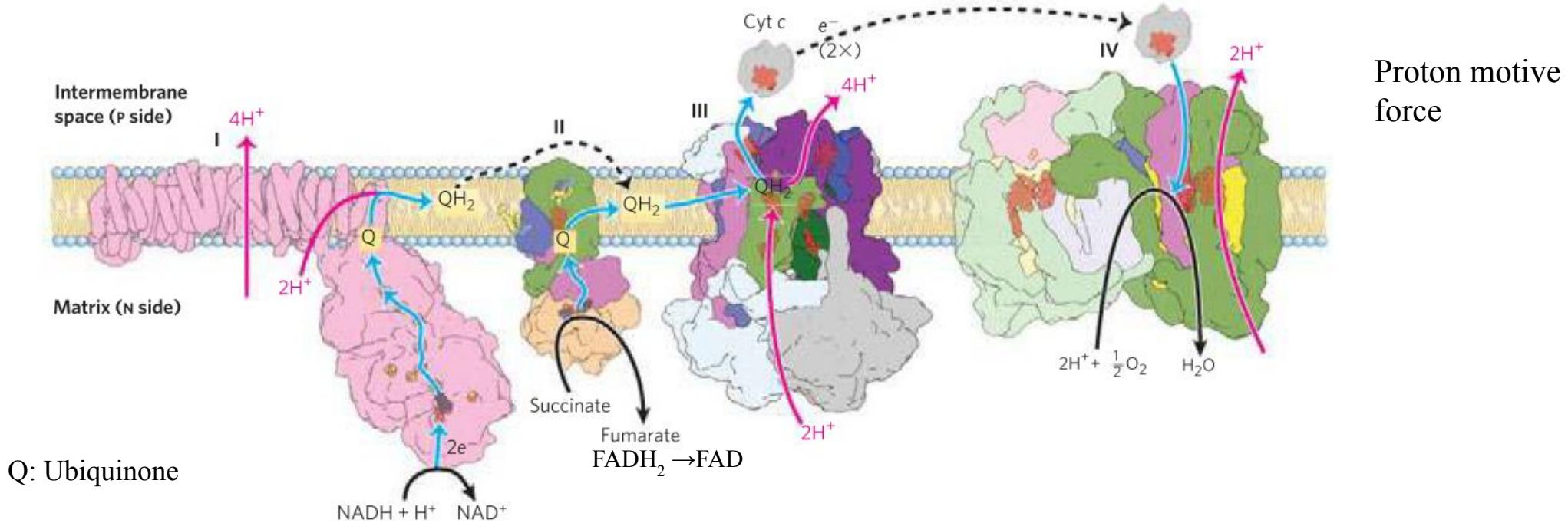


TABLE 19–6 Respiratory Proteins Encoded by Mitochondrial Genes in Humans

Complex	Number of subunits	Number of subunits encoded by mitochondrial DNA
I NADH dehydrogenase	43	7
II Succinate dehydrogenase	4	0
III Ubiquinone:cytochrome <i>c</i> oxidoreductase	11	1
IV Cytochrome oxidase	13	3
V ATP synthase	8	2

Chemiosmotic model: ATP synthesis by proton-motive force

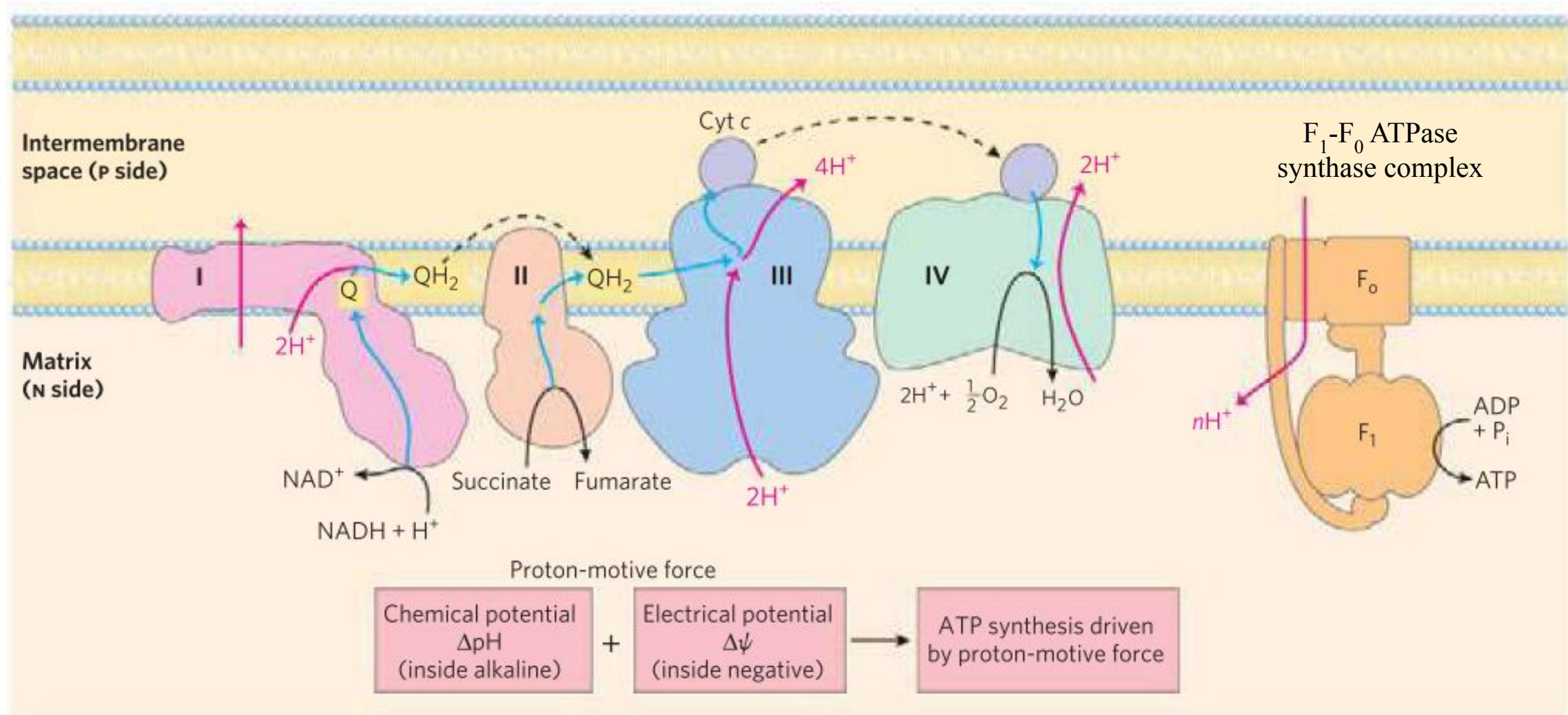


TABLE 16-1

Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed*
Glucose → glucose 6-phosphate	-1 ATP	-1
Fructose 6-phosphate → fructose 1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate	2 NADH	3 or 5 [†]
2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	5
2 Isocitrate → 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate → 2 fumarate	2 FADH ₂	3
2 Malate → 2 oxaloacetate	2 NADH	5
Total		30–32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per FADH₂. A negative value indicates consumption.

[†]This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–30 and 19–31.

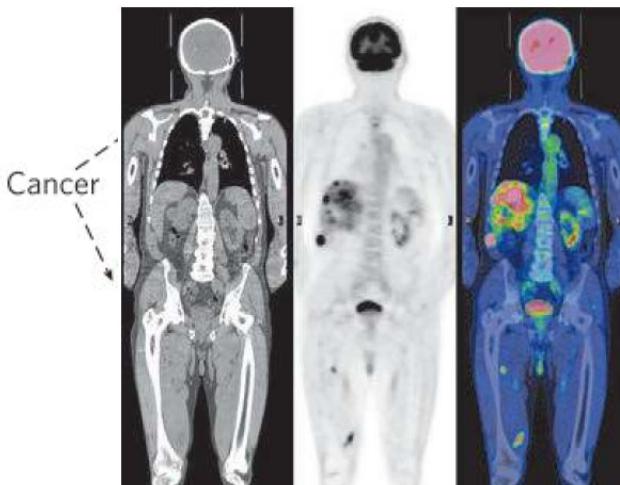
BOX 14-1**MEDICINE**

High Rate of Glycolysis in Tumors Suggests Targets for Chemotherapy and Facilitates Diagnosis

In many types of tumors found in humans and other animals, glucose uptake and glycolysis proceed about 10 times faster than in normal, noncancerous tissues. Most tumor cells grow under hypoxic conditions (i.e., with limited oxygen supply) because, at least initially, they lack the capillary network to supply sufficient oxygen. Cancer cells located more than 100 to 200 μm from the nearest capillaries must depend on glycolysis alone (without further oxidation of pyruvate) for much of their ATP production. The energy yield (2 ATP per glucose) is far lower than can be obtained by the complete oxidation of pyruvate to CO_2 in mitochondria (about 30 ATP per glucose; Chapter 19). So, to make the same amount of ATP, tumor cells must take up much more glucose than do normal cells, converting it to pyruvate and then to lactate as they recycle NADH. It is likely that two early steps in the transformation of a normal cell into a tumor cell are (1) the change to dependence on glycolysis for ATP production, and (2) the development of tolerance to a low pH in the extracellular fluid (caused by release of the end product of glycolysis, lactic acid). In general, the more aggressive the tumor, the greater is its rate of glycolysis.

There is also evidence that the tumor suppressor protein p53, which is mutated in most types of cancer (see Section 12.12), controls the synthesis and assembly of mitochondrial proteins essential to the passage of electrons to O_2 . Cells with mutant p53 are defective in mitochondrial electron transport and are forced to rely more heavily on glycolysis for ATP production (Fig. 1).

This heavier reliance of tumors than of normal tissue on glycolysis suggests a possibility for anticancer therapy: inhibitors of glycolysis might target and kill tumors by depleting their supply of ATP. Three inhibitors of hexokinase have shown promise as chemotherapeutic agents: 2-deoxyglucose, lonidamine, and 3-bromopyruvate. By preventing the formation

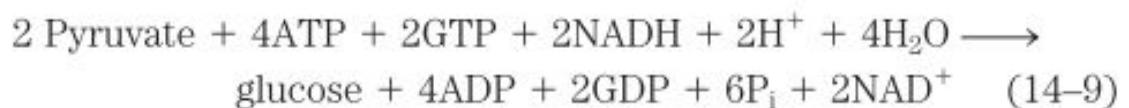


PET scan
(Positron Emission Tomography)

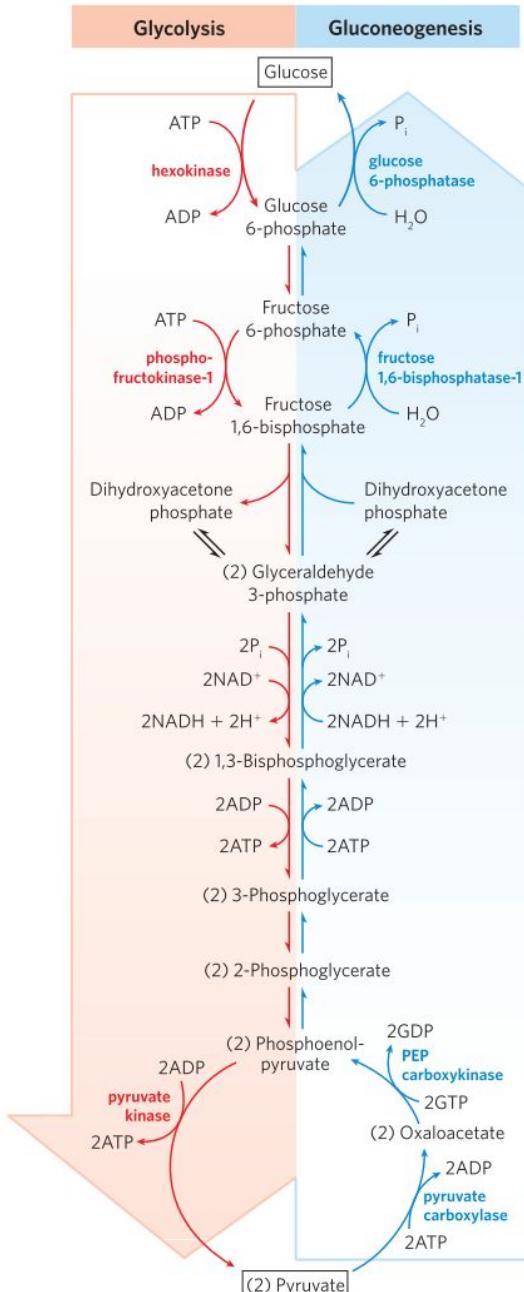
FdG: ^{18}F -labeled
2-fluoro-2-deoxyglucose

Gluconeogenesis (opposite to Glycolysis)

- The supply of glucose from the stores is not always sufficient.
 - Between meals and during longer fasts, or after vigorous exercise, glycogen is depleted.
 - For these times, organisms need a method for synthesizing glucose from noncarbohydrate precursors (lactate, pyruvate, glycerol, citrate, isocitrate etc).
 - This is accomplished by a pathway called gluconeogenesis, which converts pyruvate to glucose.
 - Gluconeogenesis is energetically expensive, but essential:



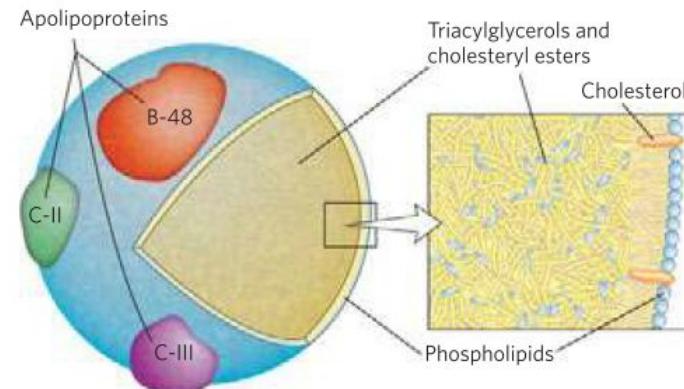
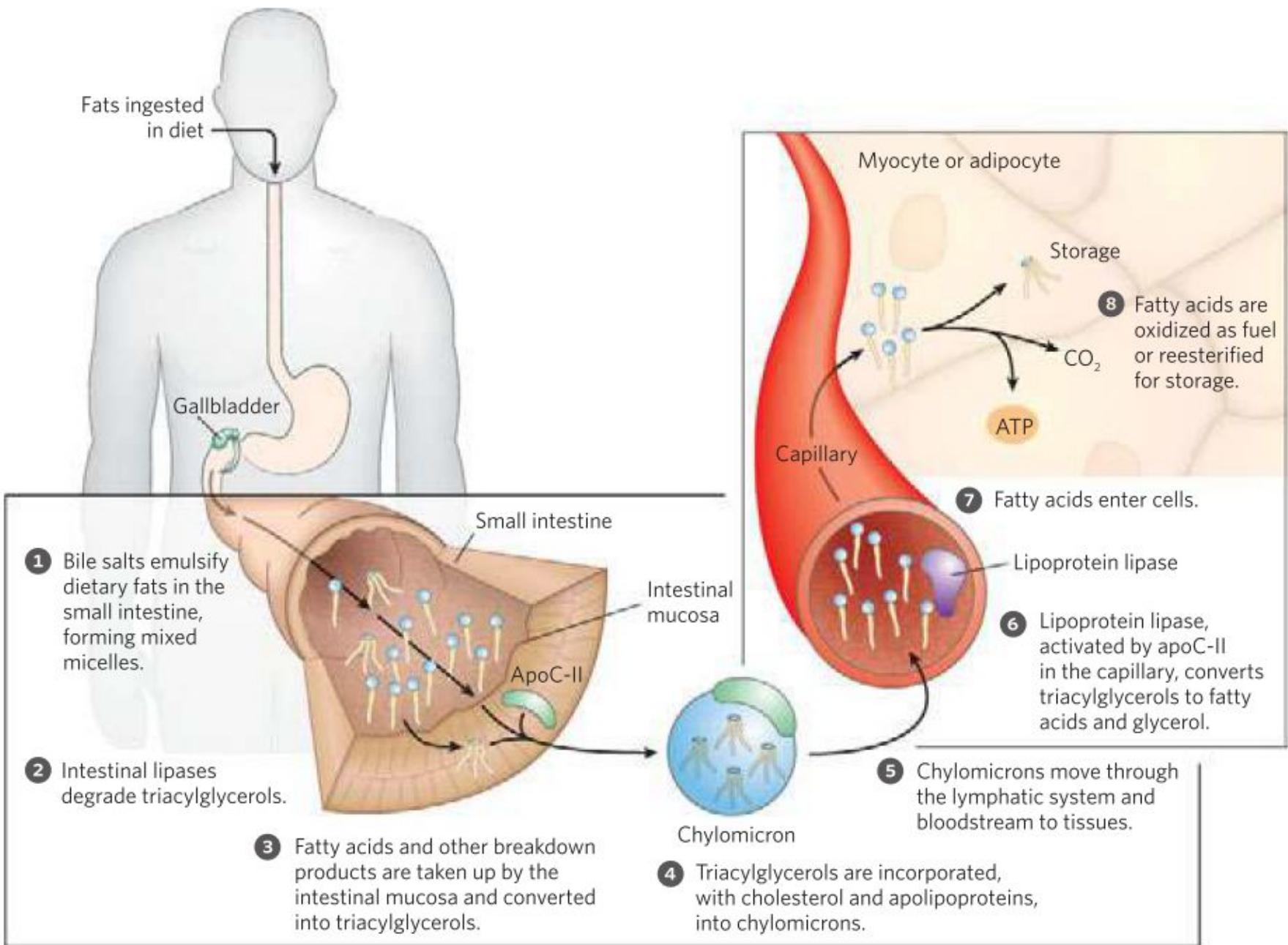
- In mammals, gluconeogenesis in the liver, kidney and small intestine provides energy glucose for use by the brain, muscle and erythrocytes.



17

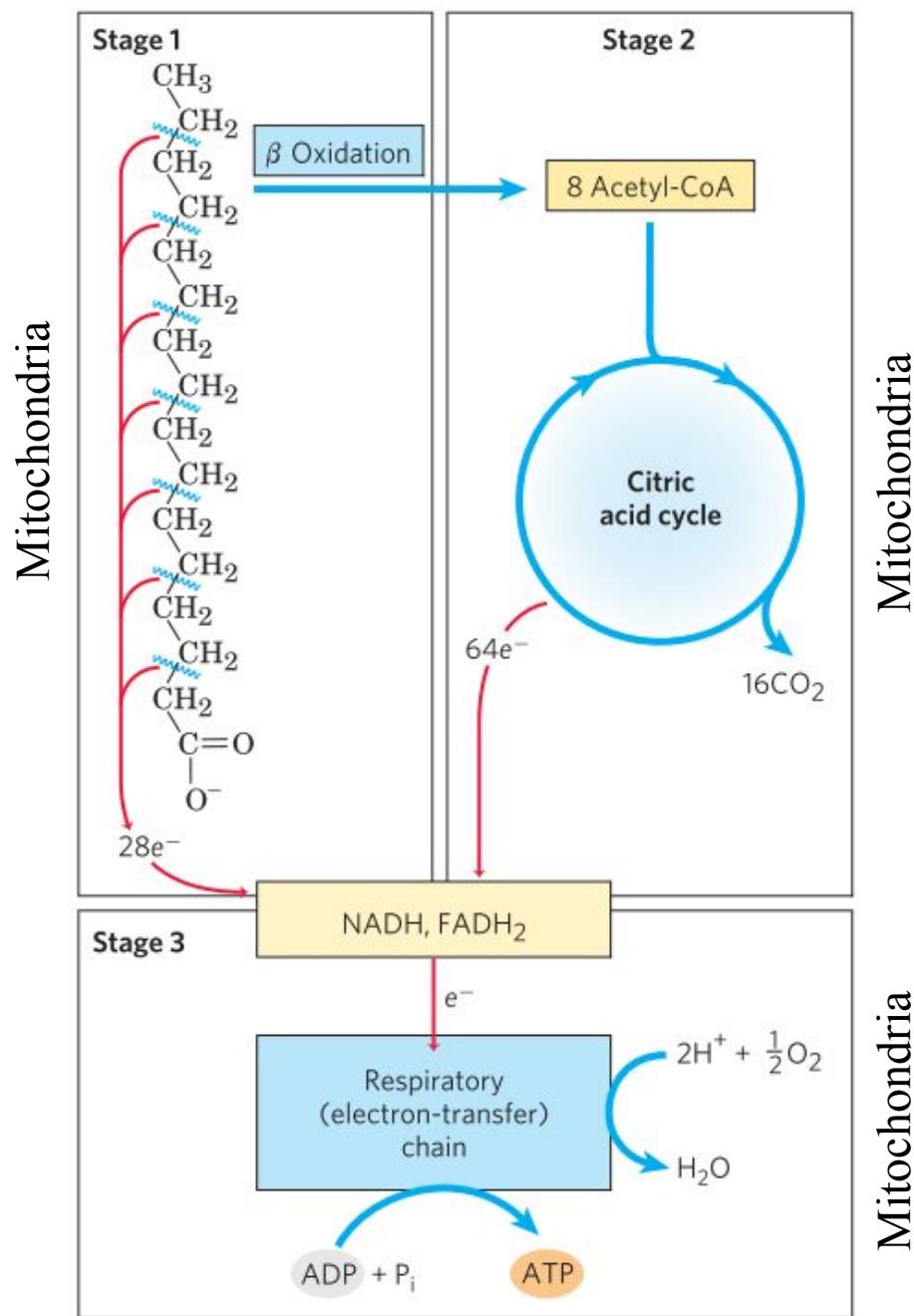
Fatty Acid Catabolism

Processing of dietary lipids in vertebrates



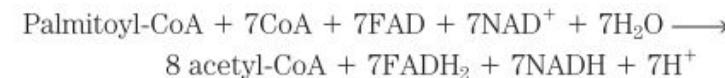
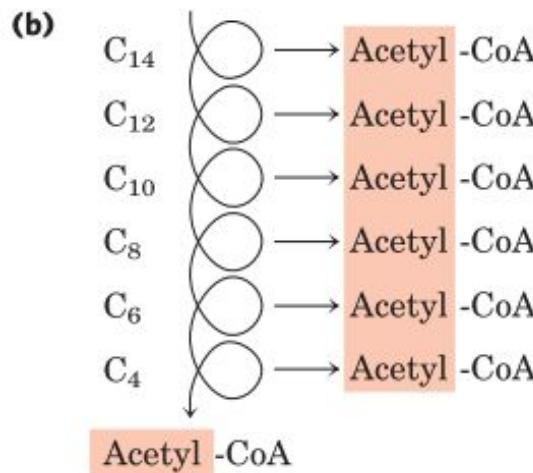
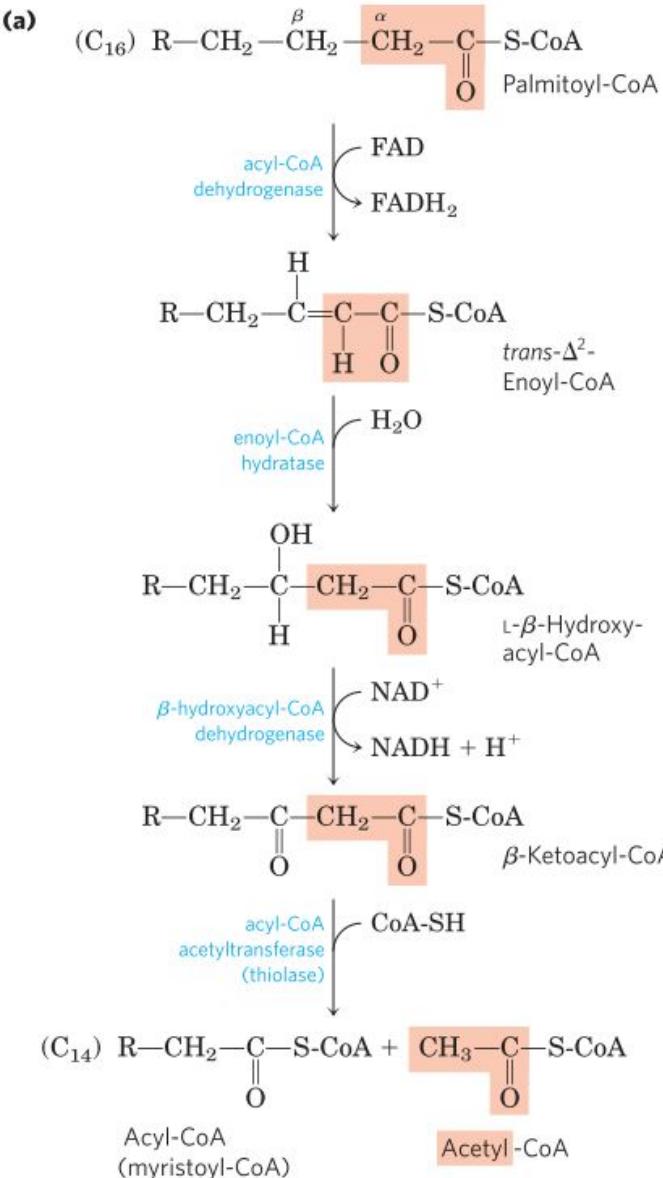
- Dietary triacylglycerols are emulsified in the small intestine by bile salts, hydrolyzed by intestinal lipases, absorbed by intestinal epithelial cells, reconverted into triacylglycerols, then formed into chylomicrons by combination with specific apolipoproteins.
- Chylomicrons deliver triacylglycerols to tissue, where lipoprotein lipase releases free fatty acids for entry into cells.

Stages of fatty acid oxidation

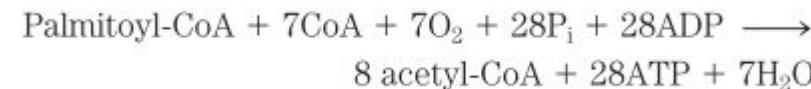


- Long chain fatty acid is oxidized to yield acetyl residues in the form of acetyl-CoA. This process is called β -oxidation.
- The acyl groups are oxidized to CO₂ via the citric acid cycle.
- Electrons derived from the oxidation of stages 1 and 2 pass to O₂ via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

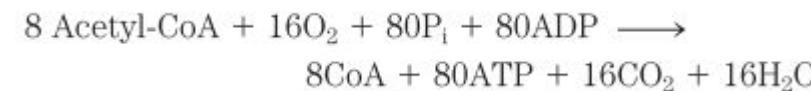
The β -oxidation Pathway



Overall equation for oxidation of palmitoyl-CoA to eight molecules of acetyl-CoA, including the electron transfers and oxidative phosphorylation is



Acetyl-CoA can be further oxidized in the TCA cycle



Combining both these equations,

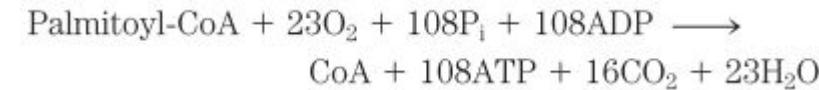


TABLE 17-1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO₂ and H₂O

Enzyme catalyzing the oxidation step	Number of NADH or FADH ₂ formed	Number of ATP ultimately formed*
Acyl-CoA dehydrogenase	7 FADH ₂	10.5
β -Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α -Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 FADH ₂	12
Malate dehydrogenase	8 NADH	20
Total		108

*These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per FADH₂ oxidized and 2.5 ATP per NADH oxidized.

†GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 526).

Amino Acid Oxidation and the Production of Urea

In which conditions cells produce energy from amino acids?

- Amino acids are not the major source of energy for the cell, however, cells can obtain energy from the amino acids under specific conditions:
 - when a diet is rich in protein and the ingested amino acids exceed the body's need for protein synthesis, the surplus is catabolized, as amino acids can not be stored.
 - During starvation or in uncontrolled diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, cellular proteins are used as fuel.
 - during normal synthesis and degradation of cellular proteins, some amino acids that are released from protein breakdown and are not needed for new protein synthesis undergo oxidative degradation.
- During amino acid catabolism, the amino group is separated from the carbon skeleton and shunted into the pathways of amino group metabolism and the carbon skeleton is shunted into the TCA cycle.

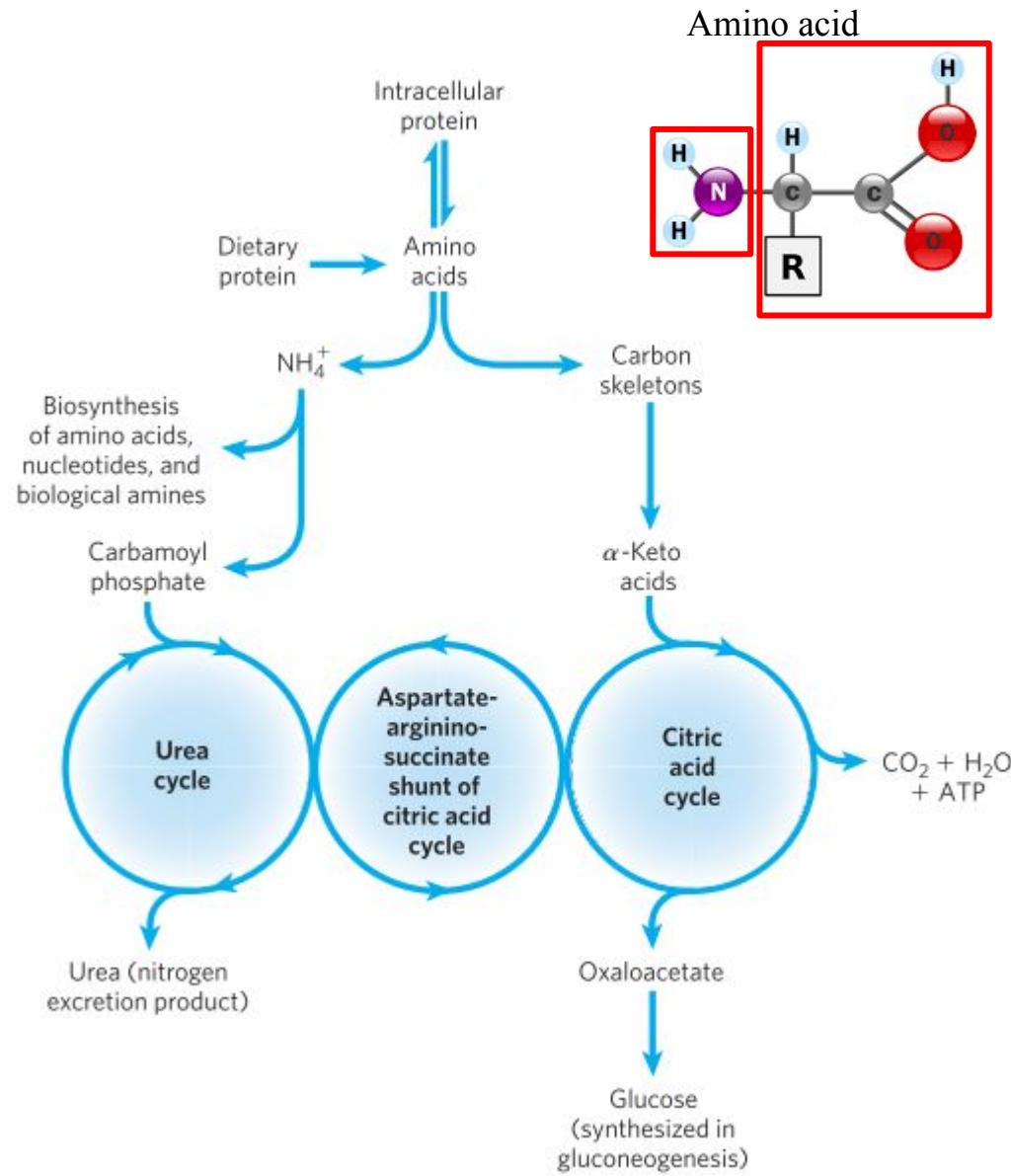


FIGURE 18-1 Overview of amino acid catabolism in mammals. The amino groups and the carbon skeleton take separate but interconnected pathways.

Amino group catabolism

Amino acids from
ingested protein

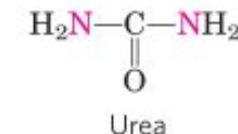
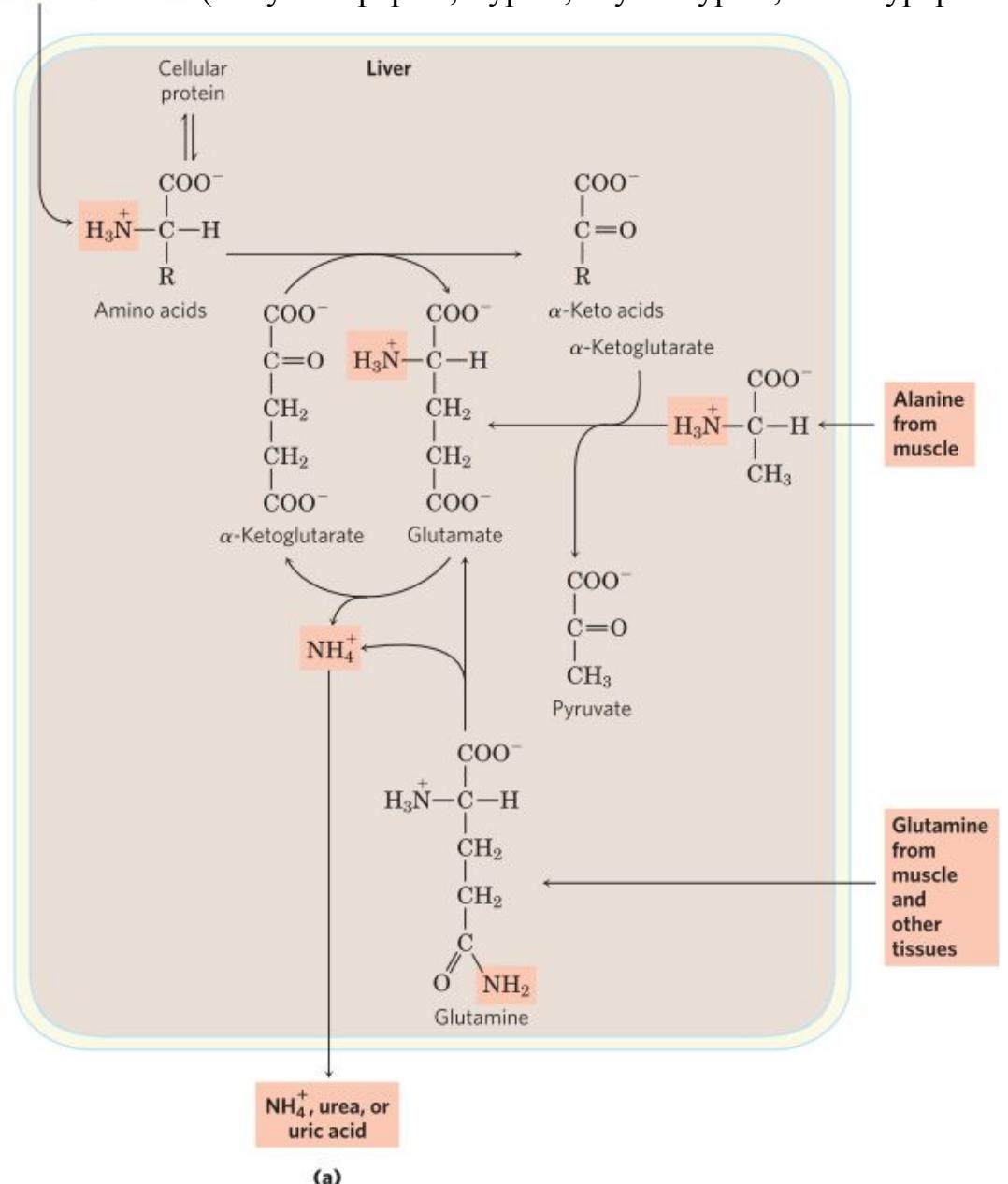
Dietary proteins are enzymatically degraded to amino acids in GI tract.
(Enzymes: pepsin, trypsin, chymotrypsin, carboxypeptidase A/B etc.)

Excretory forms of nitrogen

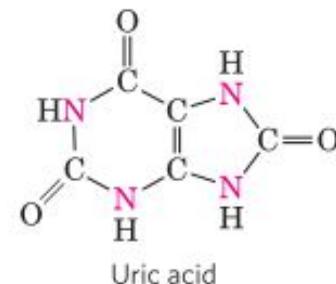


Ammonia (as
ammonium ion)

Ammonia is
toxic to animals



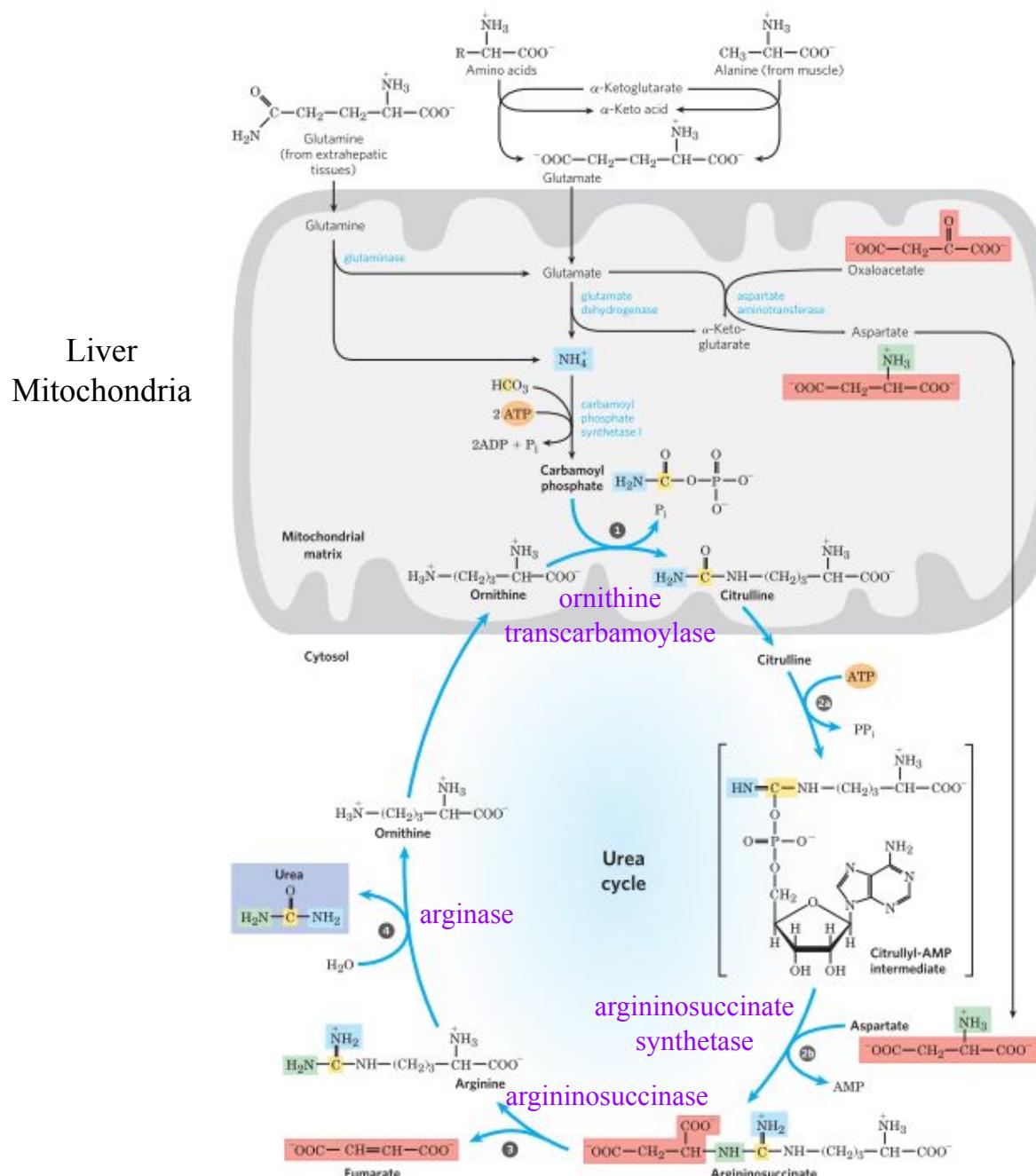
Ureotelic animals:
many terrestrial
vertebrates; also sharks



Uricotelic animals:
birds, reptiles

(b)

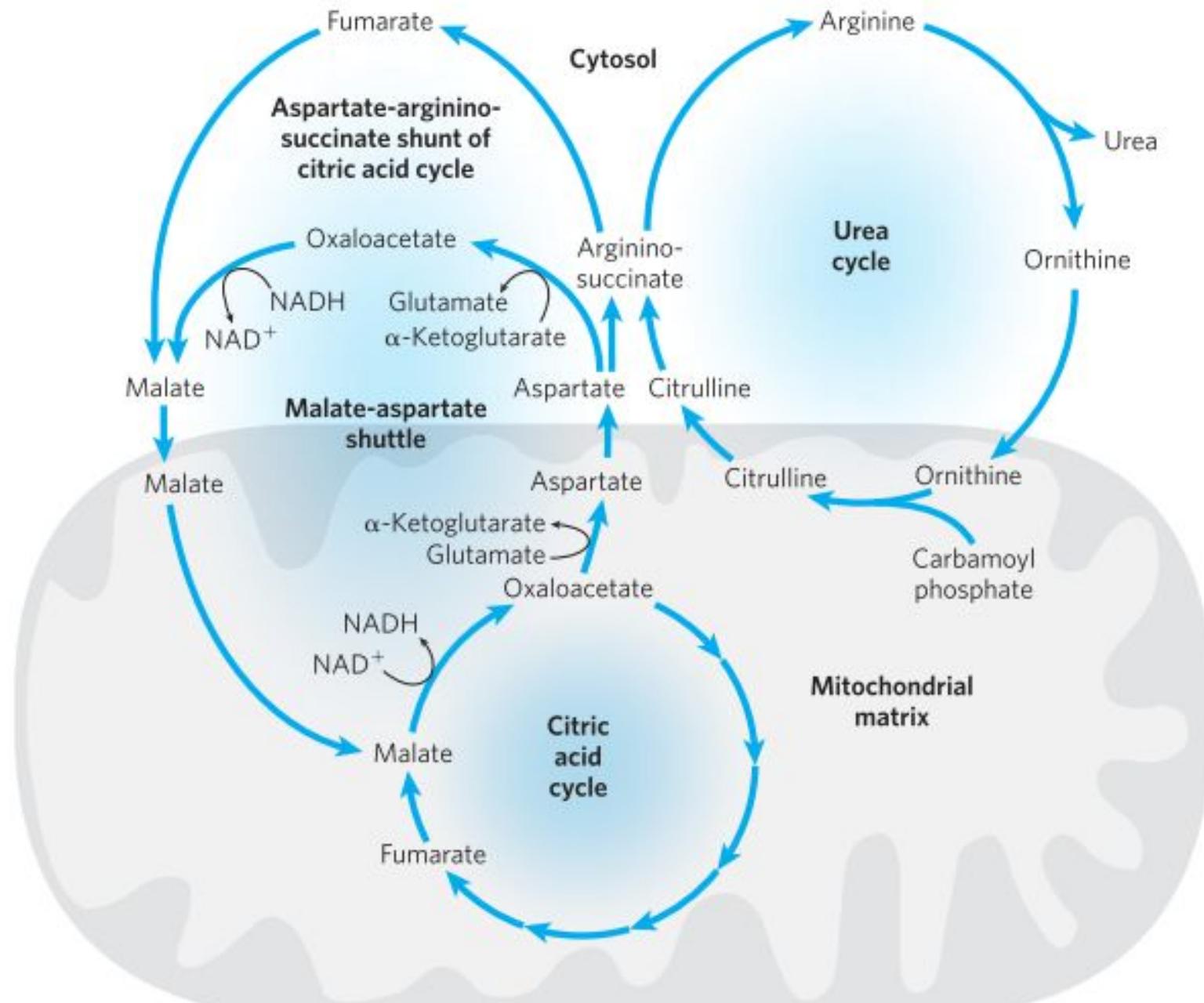
Urea Cycle



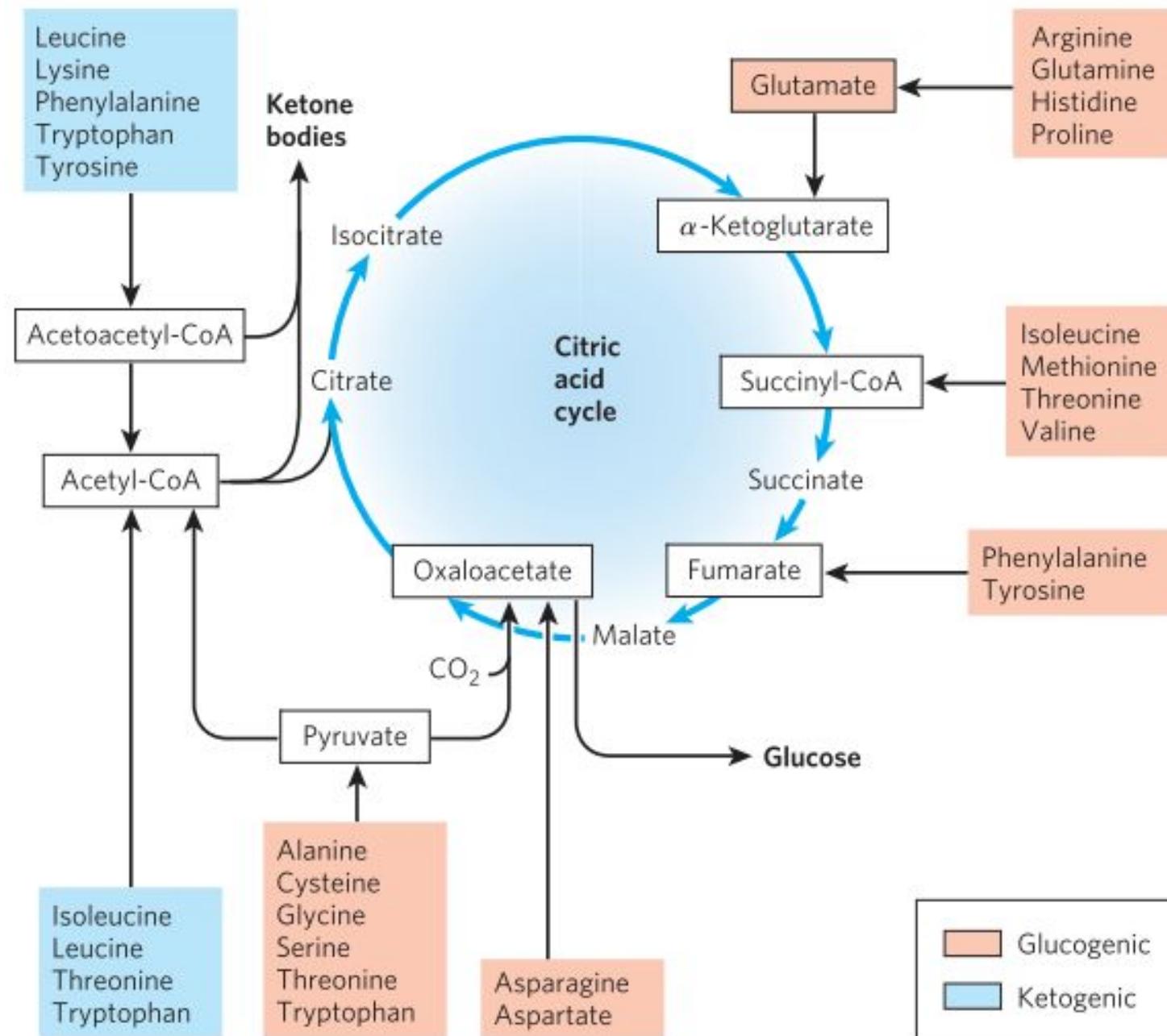
Urea is produced from ammonia in five enzymatic steps.

FIGURE 18–10 Urea cycle and reactions that feed amino groups into the cycle. The enzymes catalyzing these reactions (named in the text) are distributed between the mitochondrial matrix and the cytosol. One amino group enters the urea cycle as carbamoyl phosphate, formed in the matrix; the other enters as aspartate, formed in the matrix by transamination of oxaloacetate and glutamate, catalyzed by aspartate aminotransferase. The urea cycle consists of four steps. ① Formation of citrulline from ornithine and carbamoyl phosphate (entry of the first amino group); the citrulline passes into the cytosol. ② Formation of argininosuccinate through a citrullyl-AMP intermediate (entry of the second amino group). ③ Formation of arginine from argininosuccinate; this reaction releases fumarate, which enters the citric acid cycle. ④ Formation of urea; this reaction also regenerates ornithine. The pathways by which NH_4^+ arrives in the mitochondrial matrix of hepatocytes were discussed in Section 18.1.

Links between the Urea cycle and TCA cycle



Summary of amino acid catabolism



Further reading

Book: Lehninger: Principles of Biochemistry, 6th edition. ISBN: 1464109621.

Chapter 14: Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway, Page 543-580

Chapter 17: Fatty Acid Catabolism, Page 667-688.

Chapter 18: Amino Acid Oxidation and Production of Urea, Page 695-724.

Next class on 29/06/2022 (Wednesday)

BT1010 Introduction to Life Sciences



Lecture 7: Enzymes and protein/enzyme engineering
27/06/2022

Course Instructor:

Dr. Gunjan Mehta, Ph.D.

Assistant Professor

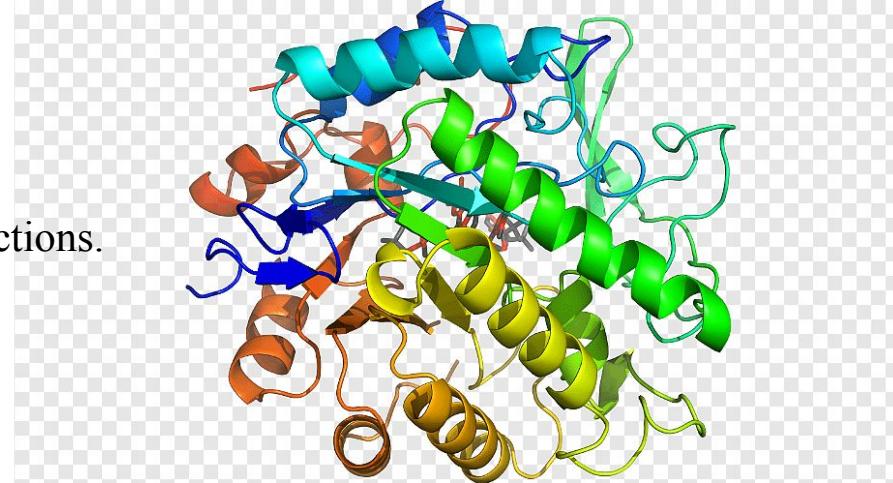
Department of Biotechnology

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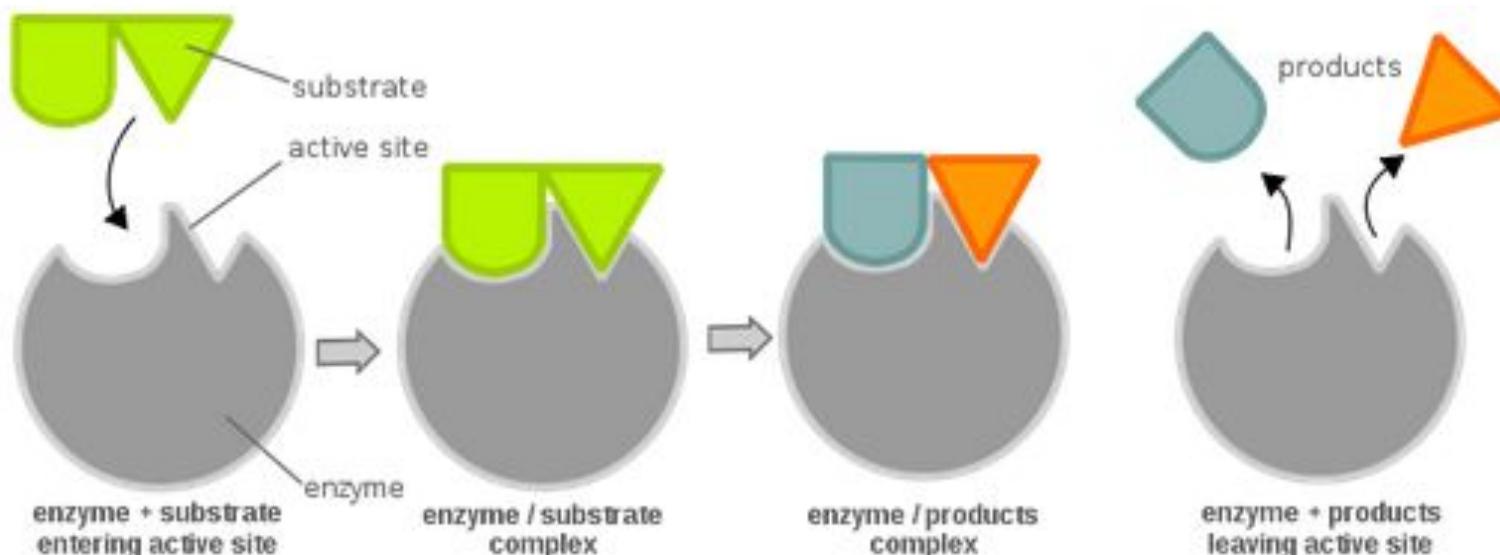
What are Enzymes?

- Enzymes are proteins.
- Enzymes are bio-catalysts.
- Catalysts accelerate chemical reactions. Similarly, enzymes accelerate biochemical reactions.
- Like all catalysts, enzymes increase the reaction rate by lowering its activation energy.

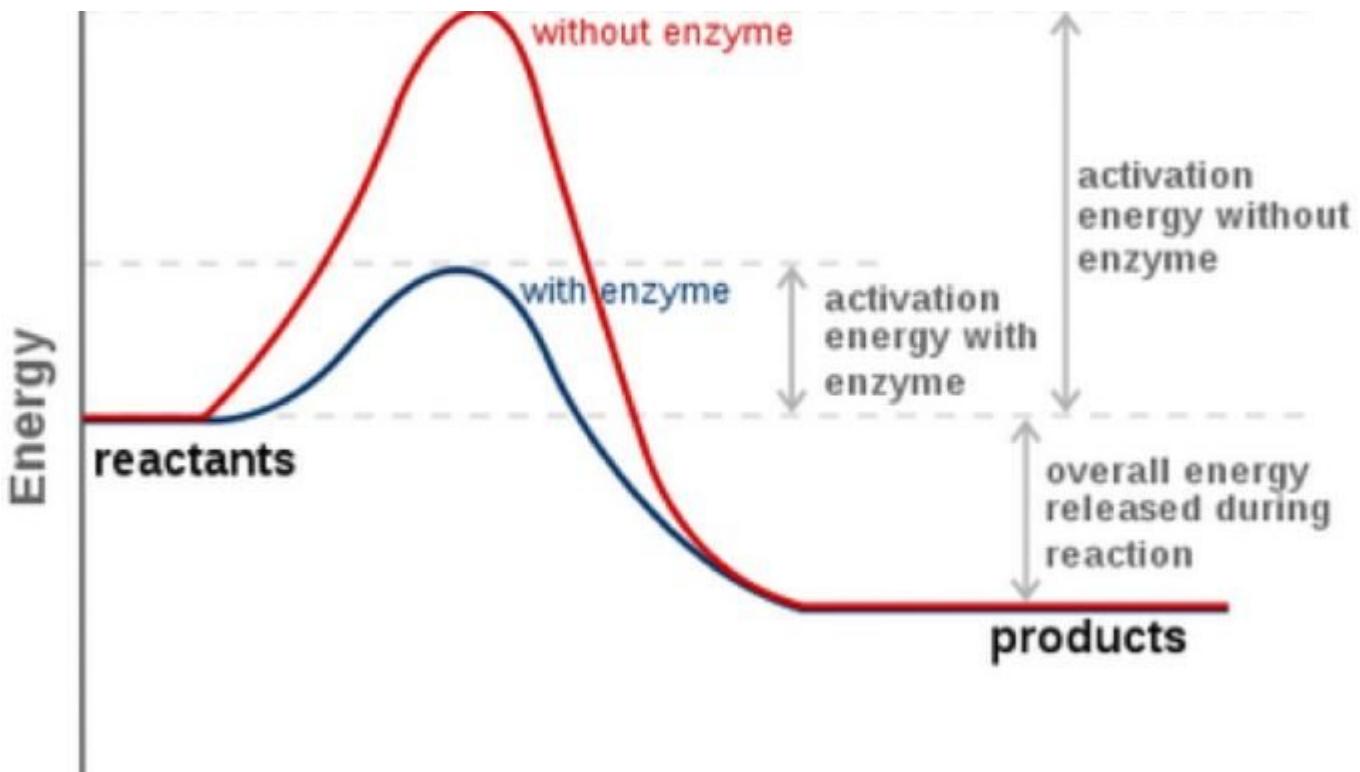


Enzymes Affect Reaction Rates, Not Equilibria

A simple enzymatic reaction might be written



Activation Energy



Activation Energy: the difference between the energy levels of the ground state and the transition state is known as the activation energy (ΔG).

Enzymes enhance the reaction rate by lowering activation energy.

International Classification of Enzymes

Enzymes are classified according to the type of reaction they catalyze.

TABLE 6–3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Enzyme Kinetics

Substrate concentration [S] affects the rate of enzyme-catalyzed reactions.

However, the substrate concentration [S] keeps changing in the enzymatic reaction as products are formed.

So, the initial velocity (V_0) is measured.

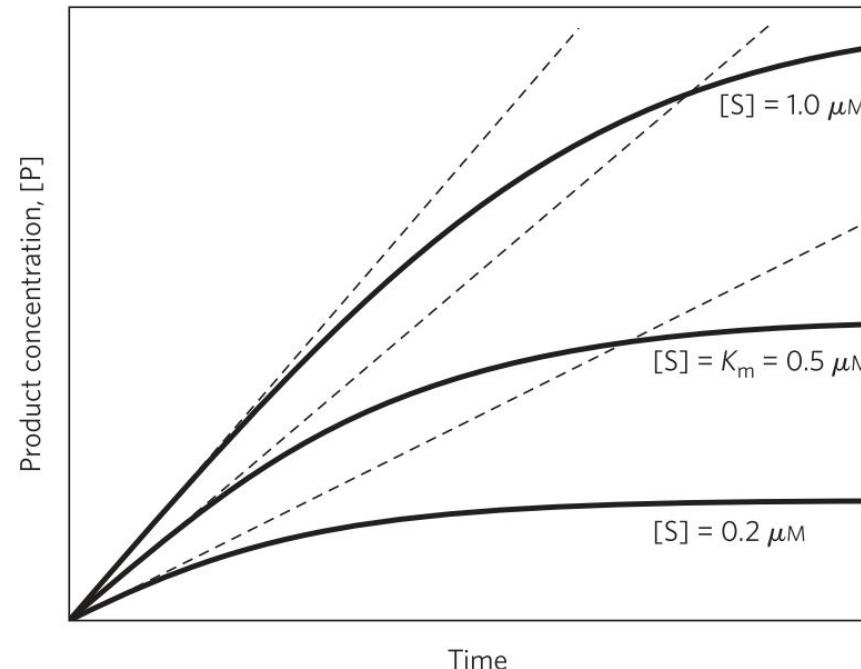
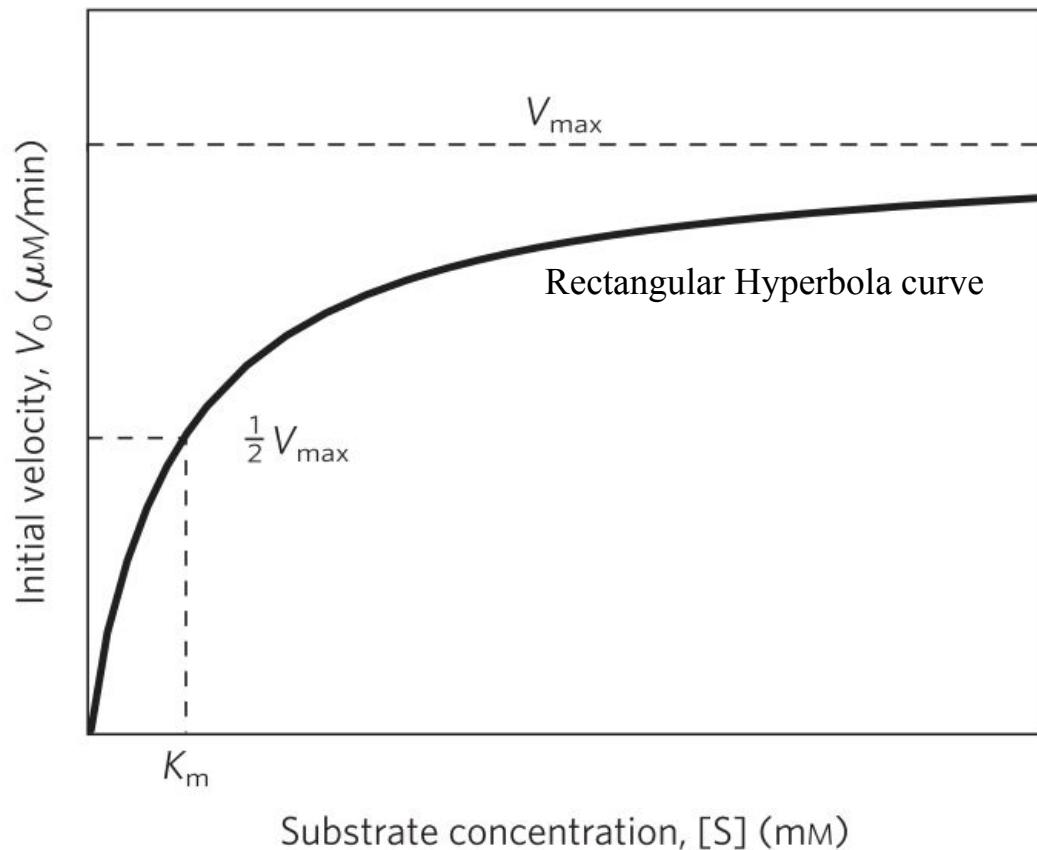


FIGURE 6–10 Initial velocities of enzyme-catalyzed reactions.

Kinetic parameters for an enzymatic reaction

The effect on V_0 of varying $[S]$ when the enzyme concentration is held constant.



V_{\max} = maximum velocity (unit $\mu\text{M}/\text{min}$)

K_m = The substrate concentration at which the velocity is $V_{\max}/2$. (unit mM) $K_m = [S]$ when $V_0 = \frac{1}{2}V_{\max}$

K_{cat} = turnover number (the number of substrate molecules converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate. (unit s^{-1})

TABLE 6-6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
	Glycyltyrosinylglycine	108
β -Galactosidase	<i>N</i> -Benzoyltyrosinamide	2.5
	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

TABLE 6-7 Turnover Number, k_{cat} , of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β -Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

The relationship between Substrate Concentration [S] and Reaction Rate (V_0)

The rectangular hyperbola curve can be expressed algebraically by the [Michaelis-Menten Equation](#).

$$V_0 = \frac{V_{\max}[S]}{K_m + [S]}$$

where, K_m is the Michaelis constant.

The value of K_m is inversely related to the affinity of the enzyme for its substrate. **High values of K_m correspond to low enzyme affinity for substrate** (it takes more substrate to get to V_{\max}).

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	<i>N</i> -Benzoyltyrosinamide	2.5
β -Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Lineweaver-Burk equation/plot for calculating Km and Vmax

Michaelis-Menten equation

$$V_0 = \frac{V_{\max}[S]}{K_m + [S]}$$

Transformed

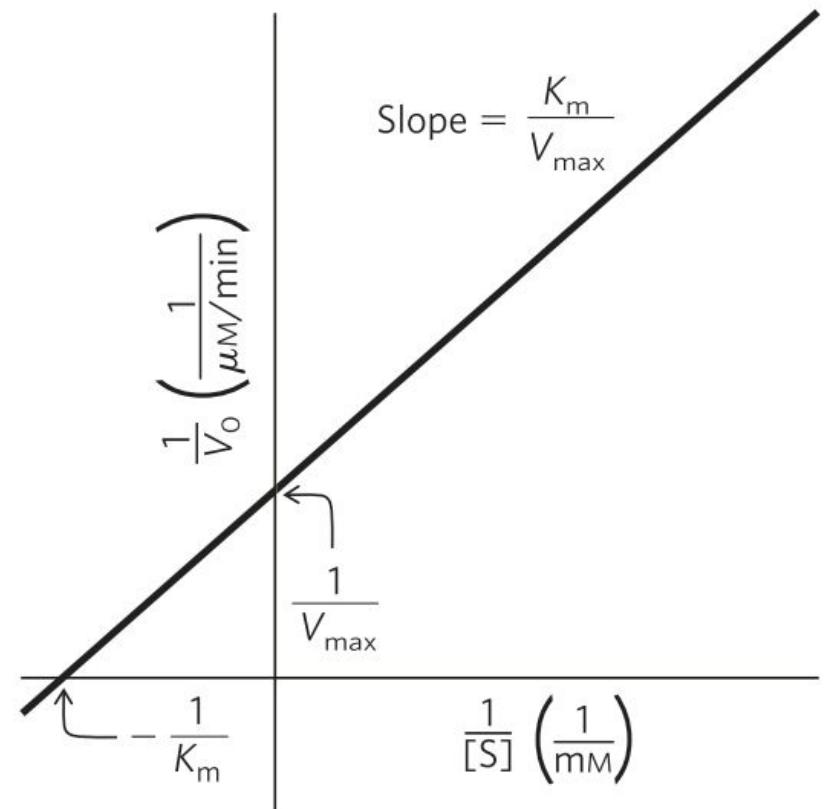
Lineweaver-Burk equation

$$\frac{1}{V_0} = \frac{K_m}{V_{\max}[S]} + \frac{1}{V_{\max}}$$

Plot of $1/V_0$ versus $1/[S]$ (double reciprocal) yields a straight line.

This line has a slope of K_m/V_{\max}

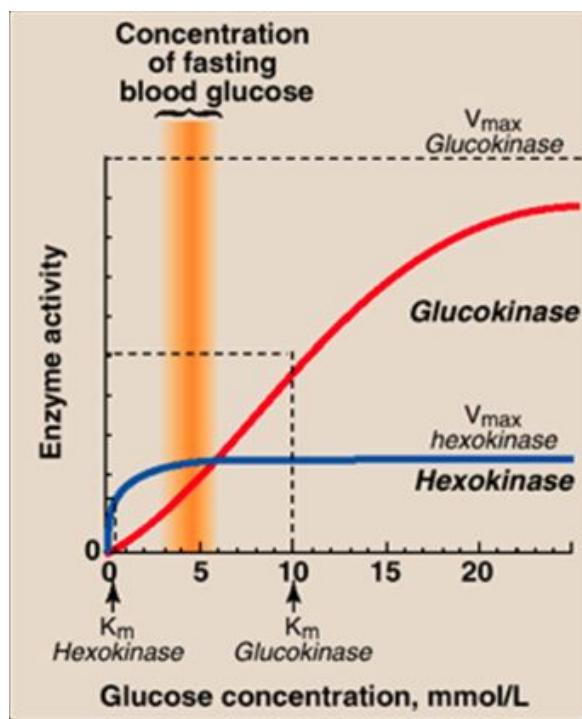
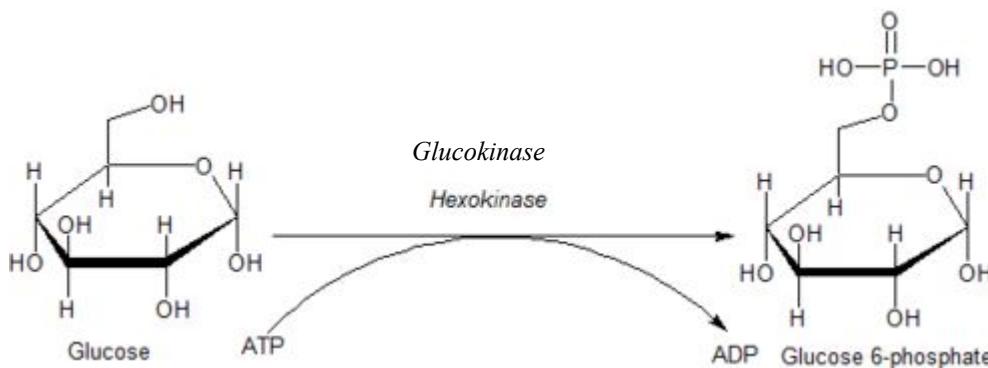
Accurate determination of V_{\max}



A double-reciprocal or Lineweaver-Burk plot.

Enzymes with same function but different kinetics

Hexokinase and Glucokinase, both are enzymes that phosphorylates glucose.



Difference between Hexokinase and Glucokinase

	Hexokinase	Glucokinase
Substrate specificity	All hexoses	Mainly Glucose
K_m	Low (high affinity) <i>Works at normal glucose concentration</i>	High (low affinity) <i>works only when glucose levels are elevated</i>
Location	Universal	Mainly liver and Beta cells of pancreas
V_{max} (rate of reaction)	Low	High
Glucose-6-PO ₄ (Allosteric inhibition)	Inhibits the enzyme	No inhibition
Insulin	No regulation	Positive regulation

Comparing catalytic mechanisms and efficiencies of two different enzymes

- The kinetic parameters K_{cat} and K_m are useful for comparing different enzymes.
- The best way to compare the catalytic efficiencies of different enzymes or the turnover of different substrates by the same enzyme is to compare the ratio of K_{cat}/K_m for the two reactions.
- This ratio is known as **Specificity Constant**.
- The upper limit to K_{cat}/K_m , imposed by the rate at which E and S can diffuse together in an aqueous solution. This diffusion controlled limit is 10^8 to $10^9 \text{ M}^{-1}\text{s}^{-1}$.

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to $10^9 \text{ M}^{-1}\text{s}^{-1}$)

Enzyme	Substrate	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m ($\text{M}^{-1}\text{s}^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β -Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

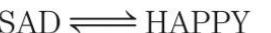
Catalytic perfection

Defination of Enzyme Unit (International Unit)

- The **enzyme unit**, or **international unit** for enzyme (symbol **U**, sometimes also **IU**) is a unit of enzyme's catalytic activity.
- 1 U ($\mu\text{mol}/\text{min}$) is defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.
- One **katal** is the enzyme activity that converts one mole of substrate per second under specified assay conditions, so $1 \text{ U} = 1 \mu\text{mol}/\text{min} = 1/60 \mu\text{mol}/\text{s} \approx 16.67 \text{ nmol}/\text{s}$;
 $16.67 \text{ nkat} = 16.67 \text{ nmol}/\text{s}$; Therefore, $1 \text{ U} = 16.67 \text{ nkat}$

WORKED EXAMPLE 6–1 Determination of K_m

An enzyme is discovered that catalyzes the chemical reaction



A team of motivated researchers sets out to study the enzyme, which they call happyase. They find that the k_{cat} for happyase is 600 s^{-1} . They carry out several experiments.

When $[E_t] = 20 \text{ nM}$ and $[\text{SAD}] = 40 \mu\text{M}$, the reaction velocity, V_0 , is $9.6 \mu\text{M s}^{-1}$. Calculate K_m for the substrate SAD.

Solution: We know k_{cat} , $[E_t]$, $[\text{S}]$, and V_0 . We want to solve for K_m . Equation 6–26, in which we substitute $k_{\text{cat}}[E_t]$ for V_{max} in the Michaelis-Menten equation, is most useful here. Substituting our known values in Equation 6–26 allows us to solve for K_m .

$$V_0 = \frac{k_{\text{cat}}[E_t][S]}{K_m + [S]}$$

$$9.6 \mu\text{M s}^{-1} = \frac{(600 \text{ s}^{-1})(0.020 \mu\text{M})(40 \mu\text{M})}{K_m + 40 \mu\text{M}}$$

$$9.6 \mu\text{M s}^{-1} = \frac{480 \mu\text{M}^2 \text{s}^{-1}}{K_m + 40 \mu\text{M}}$$

$$9.6 \mu\text{M s}^{-1}(K_m + 40 \mu\text{M}) = 480 \mu\text{M}^2 \text{s}^{-1}$$

$$K_m + 40 \mu\text{M} = \frac{480 \mu\text{M}^2 \text{s}^{-1}}{9.6 \mu\text{M s}^{-1}}$$

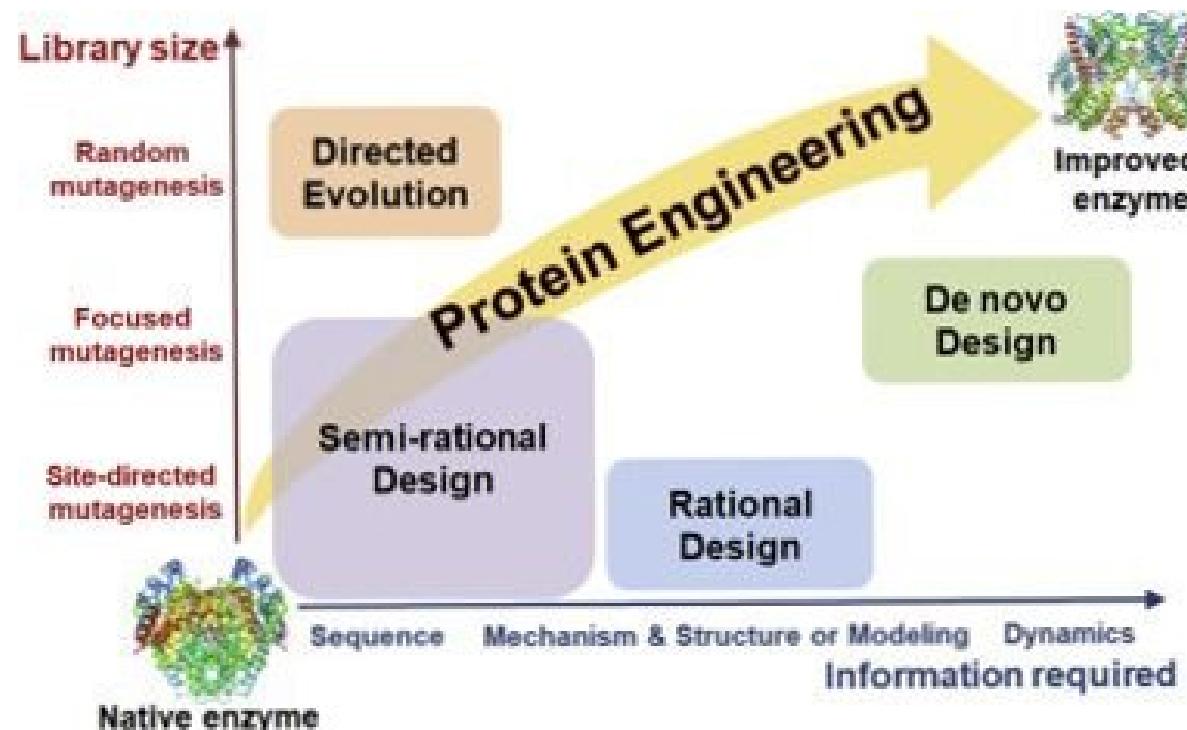
$$K_m + 40 \mu\text{M} = 50 \mu\text{M}$$

$$K_m = 50 \mu\text{M} - 40 \mu\text{M}$$

$$K_m = 10 \mu\text{M}$$

Enzyme Engineering / Protein Engineering

- Several enzymes are industrially important or they are required for the research purpose.
- To improve their catalytic performance, several genetic manipulations are performed, which is known as enzyme engineering.
- Enzyme engineering can be defined as the process through which the sequence of amino acids is changed by recombinant DNA technology to design enzymes with desired function/activity.



Enzyme engineering

- Directed evolution
- Rational design
- *De novo* enzyme engineering
- Site-directed mutagenesis
- Random mutagenesis and DNA shuffling
- Phage display and mRNA display
- Incorporation of unnatural amino acids

Engineered enzymes for application in biofuel industry

Enzyme	Source	Application	Goal	Method	Modification
Lipase	<i>Geobacillus stearothermophilus</i> T6	Biodiesel production	Improve enzyme stability and methanol tolerance	Random mutagenesis (error-prone PCR) and structure-guided consensus	Gln185Leu; His86Tyr/Ala269Thr
Endo- β -1,4-glucanase	<i>Streptomyces</i> sp. G12	Lignocellulose conversion	Improve hydrolytic activity	Directed evolution (error-prone PCR)	epCS_1: Gly263Cys, and Arg307His epCS_2: Gly145Asp and Asn207Lys epCS_3: Pro228Arg epCS_4: Thr67Asn, Asp142Glu Ser218Asn, Val242Asp, and Asp330Glu epCS_5: Thr157Ile, Gly251Asp, and Val259Asp
Endoglucanase	<i>Penicillium verruculosum</i>	Lignocellulose conversion	Improve thermostability	Structure-based disulfide bond (DSB) engineering and site-directed mutagenesis	DSB2: Ser127Cys/Ala165Cys DSB3: Tyr171Cys/Leu201Cys
Cellobiohydrolase	<i>Chaetomium thermophilum</i>	Saccharification of lignocellulosic biomass	Improve catalytic activity and thermostability	Site-directed mutagenesis	Tyr119Phe and Ser131Trp
β -Glucosidases	<i>Aspergillus niger</i>	Production of ethanol from cellulosic biomass	Increase hydrolysis activity	Directed evolution (error-prone PCR)	Tyr305Cys(most relevant)
Endo-xylanase	<i>Thermoascus aurantiacus</i> CBMAI 756	Xylan saccharification, applied to many industrial sectors, including production of biofuels	Improve thermostability	Site-directed mutagenesis	Gln158Arg, His209Asn, and Asn257Asp (most relevant)

Further reading

Book: Lehninger: Principles of Biochemistry, 6th edition. ISBN: 1464109621.

Chapter 6: Enzymes, Page 189-242.

Try understanding some of the worked problems and try solving problems provided at the end of the chapter.

Next class on 29/06/2022 (Wednesday)

BT1010 Introduction to Life Sciences



Lecture 8: Methods for life science research
29/06/2022

Course Instructor:

Dr. Gunjan Mehta, Ph.D.

Assistant Professor

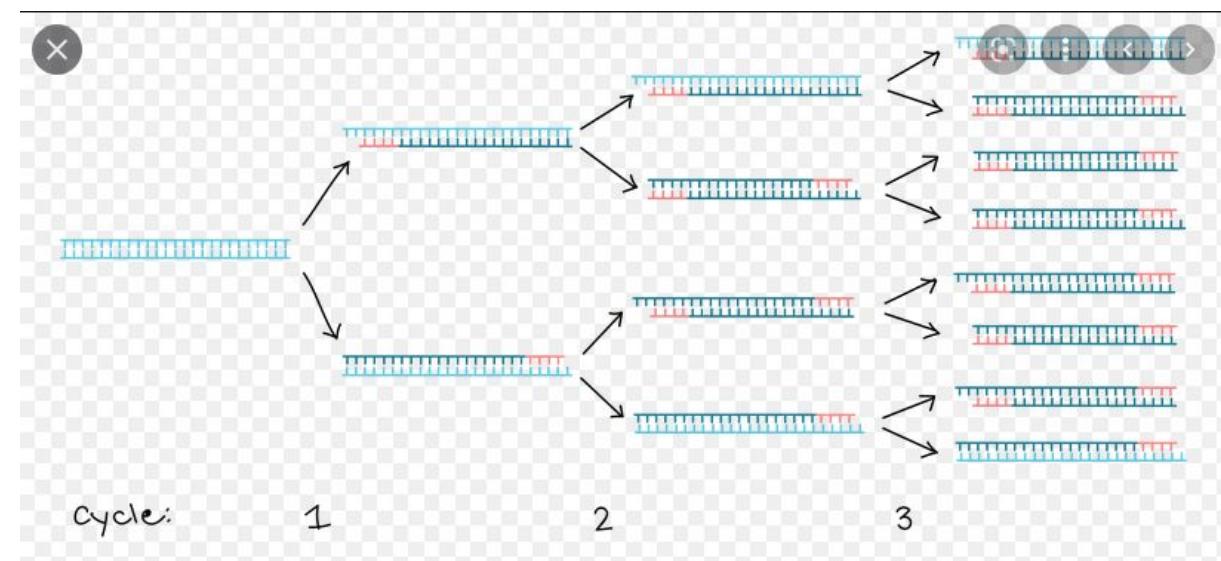
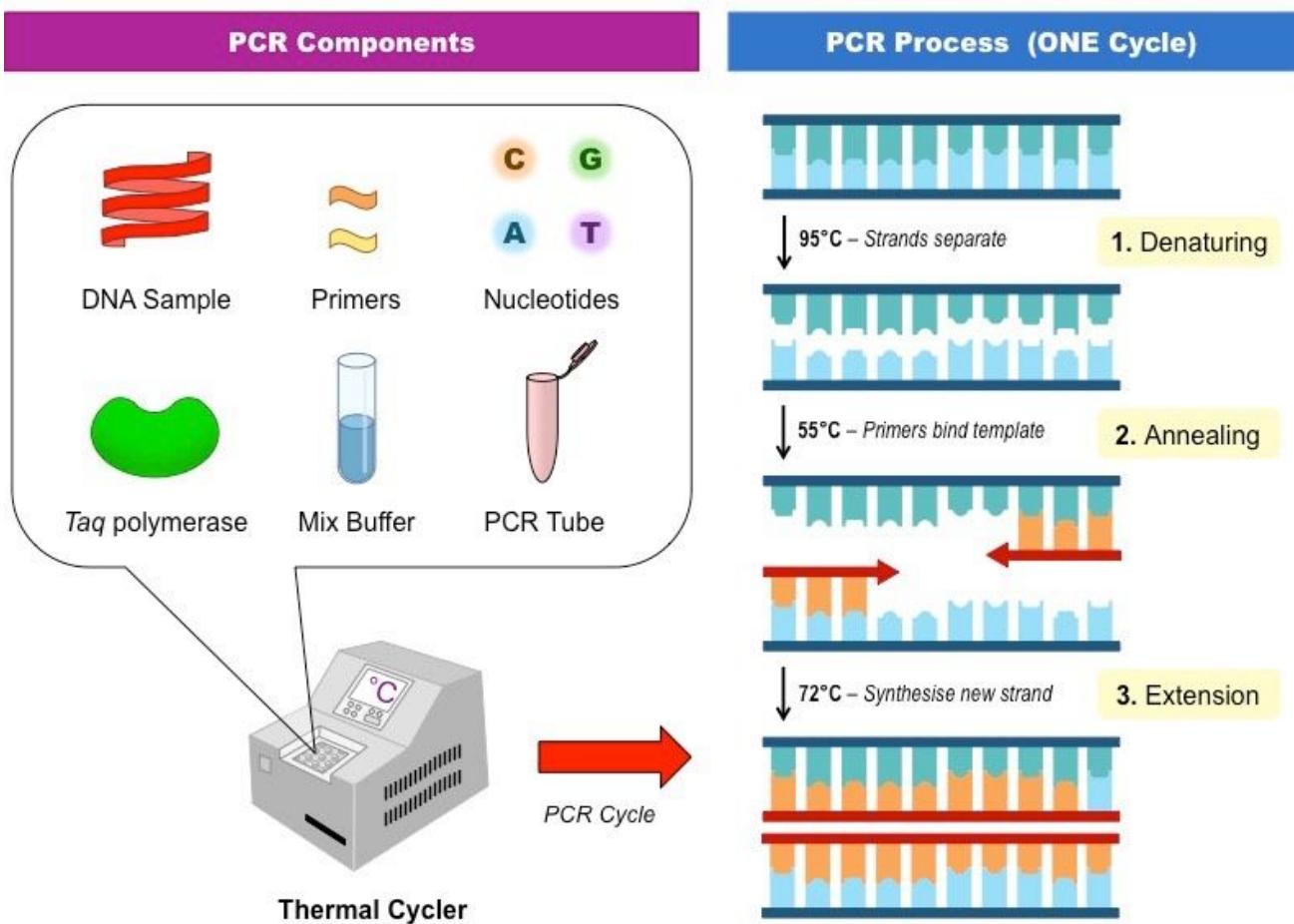
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Polymerase Chain Reaction (PCR)

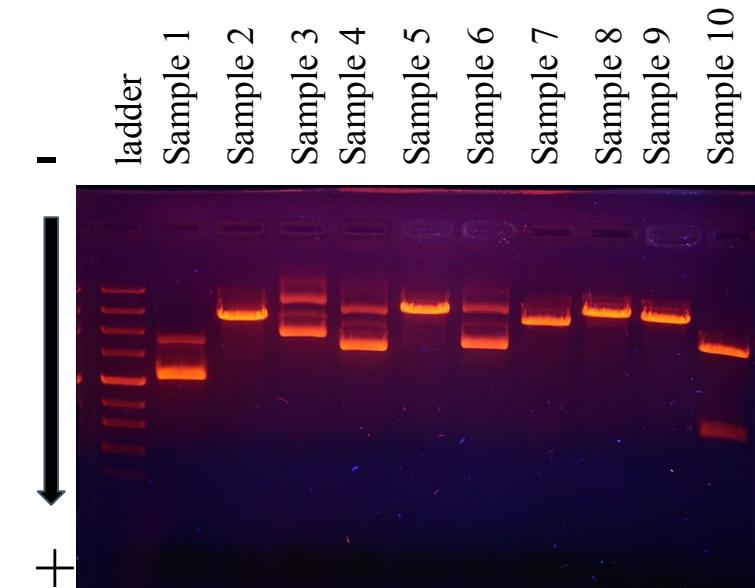
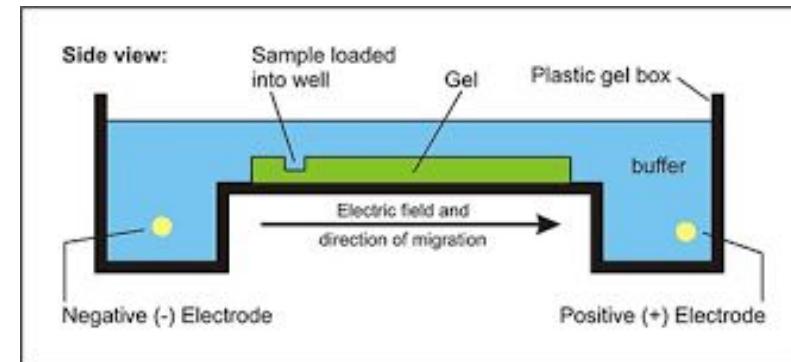
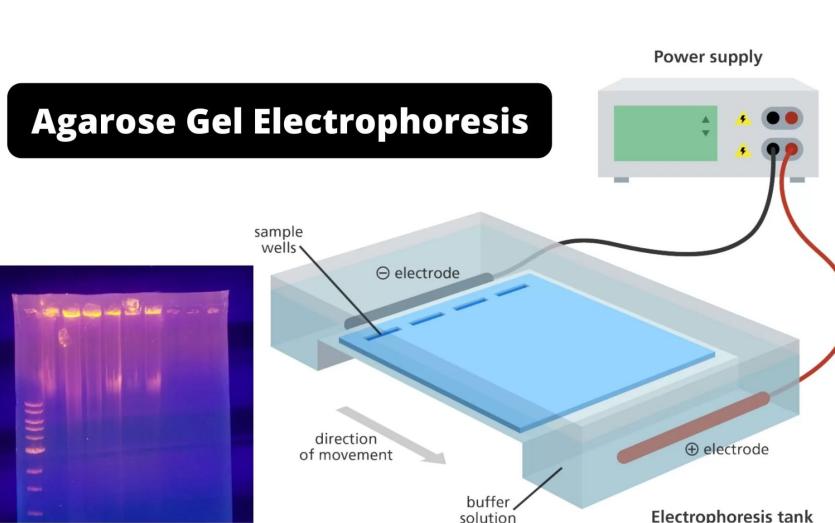
- PCR is used to amplify DNA fragment.
- Thermostable Taq DNA polymerase enzyme is used to amplify the DNA.



Watch this video on youtube:
<https://youtu.be/KlczxSr6lcE>

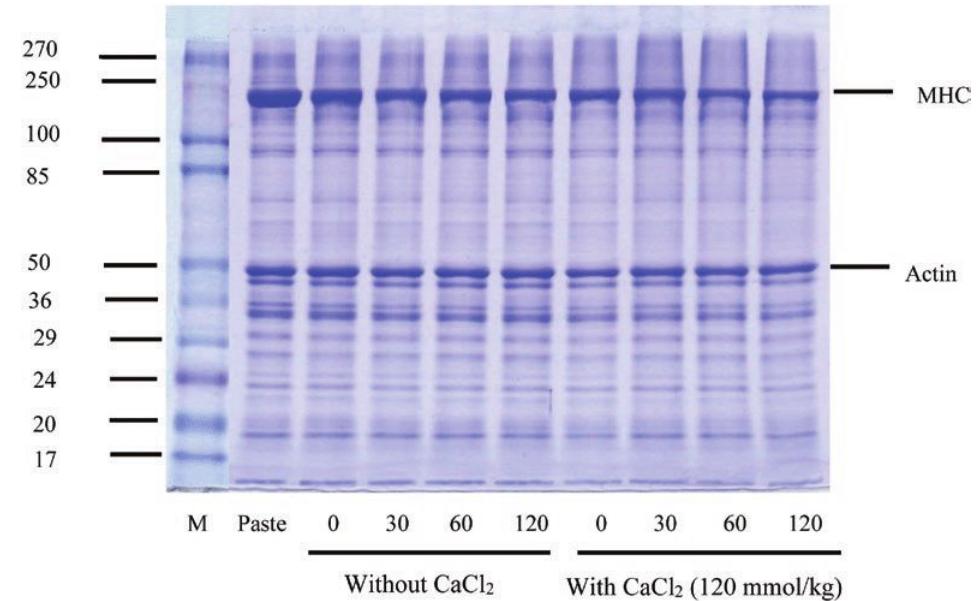
Agarose Gel Electrophoresis to separate DNA

- Agarose gel electrophoresis is a method to separate a mixed population of DNA, based on their sizes (molecular weight).
- Agarose gel is a 3D matrix, in which the mixture of DNA is loaded and the electric field is applied.
- DNA migrates from negative to positive electrode and the distance it migrates depends on the size of the DNA. Small DNA molecules (e.g. 300 bp) moves faster, compared to large DNA molecules (i.e. 1000 bp).
- The agarose gel is stained with ethidium bromide (EtBr) and observed under the Ultraviolet light to see the bands of DNA.
- DNA molecular weight marker (DNA ladder) is used as a reference of molecular weight.



Polyacrylamide Gel Electrophoresis to separate proteins (SDS-PAGE)

- SDS-PAGE or Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis is a method used for separation of proteins based on their molecular weight.
- SDS imparts negative charge to the proteins.
- Gel is made up of Polyacrylamide and protein mixture is loaded on the top.
- Electric field is applied, so the proteins migrate from negative to positive direction. The smaller proteins migrate faster compared to the larger proteins.



Further reading

Book: Lehninger: Principles of Biochemistry, 6th edition. ISBN: 1464109621.

Next class on 30/06/2022 (Thursday)