Fall | 10

BIOLOGY 102

QUEEN'S STUDENTS OFFERING SUPPORT

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Chapter 2: Chemical Basis of Life

Atoms

- Atoms consist of electrons, protons, and neutrons
    - Electrons are found around the nucleus and carry a negative charge
    - Protons and neutrons exist within the nucleus and have much more mass than electrons
        - Protons carry a positive charge
        - Neutrons have no charge
- Atoms are mostly empty space
    - You can think of electrons, protons, and neutrons as marbles in football fields

Electrons

- Electrons travel within regions surrounding the nucleus called orbitals
    - S orbitals are spherical
    - P orbitals are propeller shaped
- Each orbital can only hold 2 electrons
- Valence electrons are the unpaired electrons in the outermost shell (the furthest layer of orbitals)
    - These are the most likely electrons to be involved in chemical reactions (**RXN**s)
- The further away an electron is from the nucleus, the more potential energy it has

Atomic mass

- Mass is a quantity of matter
- Weight is the gravitational pull on a given mass
    - Therefore depending on the strength of the gravitational pull, the weight of an object can change even though its mass remains the same
        - A mass of 154 pounds weighs 25 pounds on the moon but 21 trillion pounds on a neutron star
- Atomic mass is defined as the total number of protons and neutrons in an atom
    - Measured in Daltons (**d**) or atomic mass units (**AMU**s)
- Daltons are equal to $1/12^{th}$ the mass of a carbon atom
    - Carbon has an atomic mass of 1 d
    - The most common form of carbon (^{12}C) also has 6 protons and 6 neutrons
        - Therefore the mass of one proton or one neutron is 1 d
- Protons and neutron are about equal in mass (about 1800 times greater than an electron)
- One mole (**mol**) of an element is 6.022×10^{23} number of atoms of that element
    - 6.022×10^{23} is Avogadro's number
    - 1 mole of a substance would be 6.022×10^{23} number of molecules of that substance
- In biology, 1 mol concentrations of substances is very rare so we often use millimoles (mmol), micromoles (µmol), or nanomoles (nmol)

Isotopes

- Isotopes refer to multiple forms of an element that differ in the number of neutrons
    - Superscript before an element tells you which isotope it is
        - ^{14}C is carbon 14
    - Also tells you that isotopes atomic mass

- May different uses in biology
    - Biological processes within organisms rarely use isotopes
        - Some enzymes can recognize them, but not many
    - Mostly used in technical applications
        - Eg. tissue imaging, tracking metabolites, DNA gels, etc.

HONC

- **HONC** = Hydrogen, Oxygen, Nitrogen, Carbon
- Typically make up about 95% of the atoms in living organisms
    - H & O occur primarily in water
    - N found in proteins/nucleic acids, C is the building block of all living matter

Mineral and trace elements

- Mineral elements constitute less than 1%
- Trace elements less than 0.01%
- Both are essential for normal growth and function
    - Think of iron in hemoglobin

Molecules and compounds

- Molecules are 2 or more atoms bonded together: O_2
- Compounds are composed of 2 or more elements: H_2O, CO_2
- Molecular formula contains chemical symbols of elements found in a molecule
    - Subscript indicates how many of each atom are present: $C_6H_{12}O_6$

3 Types of Bonds

1. Covalent
    a. Polar covalent
2. Hydrogen
3. Ionic

Octet Rule

- Atoms stable when their outer shell (valence shell) is full
    - For many atoms, the outer shell fills with 8 electrons
- One exception is hydrogen, which fills its outer shell with 2 electrons
- A full valence shell (with 8 or 2 electrons) is called a Noble Gas Configuration

Covalent Bonds

- Covalent bonds occur when atoms share a pair of electrons
    - Occurs between atoms whose outer electron shells are not full
- Covalent bonds are often the strongest of all chemical bonds, because the shared electrons behave as if they belong to each atom
- Can share:
    - 1 pair of electrons - single bond H-F
    - 2 pairs of electrons - double bond O=O
    - 3 pairs of electrons - triple bond N ≡ N

- Generally, the more number of bonds the harder it will be to separate those two atoms

Polar Covalent Bonds

- Polar covalent bonds are a type of covalent bonds where the electrons are shared unequally
    - This means that the electrons are closer to the nucleus of one atom most of the time
    - Since electrons are negative this makes that atom (or region that atom occupies in the molecule) more negative and the atom (or region) where the electron is not more positive
    - This is often shown as δ^+ (slightly positive) or δ^- (slightly negative) in diagrams
- This unequal sharing is governed by the electronegativity of the atoms
    - The more electronegative the atom is the greater "pull" it exerts on the electrons in the bond
    - In H_2O the electronegativity of O draws electrons away from H resulting in a polar H_2O compound

Hydrogen Bonds

- Hydrogen bonds (H bonds) are important in biology, they are the most abundant and flexible
- H bonds do not involve the sharing of electrons
    - Instead they involve magnetic forces
        - A compound that has a region (or atom) that is δ^- attracts the δ^+ region that is near the H atom in another compound
- Most often this occurs between compounds, like two molecules of H_2O, and is called intermolecular H bonding
    - Intramolecular (within the same compound) H bonding can occur as well
        - This is important in maintaining tertiary protein structure

Flexibility

- Rotation of elements within compounds is possible but only around single bonds
- This is important in small and large compounds

Ionic Bonds

- Ion = atom or molecule that has gained/lost one or more electrons (net electric charge)
    - Cations = net positive charge
    - Anions = net negative charge
        - Remember that A Negative ion is an ANion
- Ionic bond occurs when a cation binds to an anion

Chemical Reactions

- Occurs when one or more substances are changed into other substances
    - When reactants (initial starting materials) are changed into products (end result)
- Most biological chemical reactions share many properties
    - All require a source of energy
    - Reactions in living organisms often require a catalyst (enzymes)
    - Tend to proceed in a particular direction but will eventually reach equilibrium
    - Occur in liquid environment, usually water

        - Some occur in lipid environments as well, like those that occur in cellular membranes
- In biological organisms/cells chemical reactions also need to occur in specific places and at controlled rates

Reduction and Oxidation

- Reduction is the gain of an electron
- Oxidation is the loss of an electron
- LEO the lion says GER
    - Loss of Electrons is Oxidation (LEO), Gain of Electrons is Reduction (GER)
- Reduction/oxidation (redox) reactions involve transfer of electrons

Hydrophilic: Water loving

- Readily dissolve in water
- Occurs in ions (molecules or compounds that carry a charge) and molecules that contain polar covalent bonds

Hydrophobic: Water fearing

- Does not readily dissolve in water
- Nonpolar molecules like lipids (membranes)
- Hydrophobic molecules like other hydrophobic molecules

Amphipathic Molecules

- Have both polar or ionized regions at one or more sites and nonpolar regions at other sites
- May form micelles in water, e.g. phospholipids
- Important in shielding otherwise hydrophobic regions of some molecules as well
    - Some help to preserve the tertiary structure of proteins

Water, Not Just a Solvent

- Water has many critical functions in living organisms
    - Participates in chemical reactions
        - Hydrolysis or dehydration
    - Provides force or support
        - In some plants it is critical to the maintenance of their stucture
    - Remove toxic waste components
    - Evaporative cooling
        - The specific heat capacity of water is high
        - This means that it takes a lot of energy for it to evapourate
        - When you sweat it takes the heat from your body and uses that to evapourate, cooling you down in the process
    - Cohesion and adhesion
        - Cohesion is water binding to itself and adhesion is when it interacts with other things
        - The cohesive strength between water molecules is very strong, without it large trees would not be able to pull water up to their highest branches

- Lubrication

Colligative Properties of Water

- Addition of solutes to H2O lowers its freezing point below 0°C and raises its boiling point above 100°C
  - Does this by disrupting water-water interactions (disrupts H bonding)
- Depend strictly on the concentration of dissolved solute particles and not on the specific type of particle
  - Often cells concentrate compatible solutes
    - This refers to solutes that do not interfere with cellular processes
- Some animals + plants produce antifreeze molecules

Properties of Acids/Bases

- Acid (proton donor): dissociates in solution to yield H^+ and an anion
- Base (proton acceptor): dissociates to yield a OH^- ion and a cation when dissolved
- pH = $-\log_{10}[H^+]$
  - Remember that pH is a LOG SCALE
  - This means that a pH of 6 is 10 times more acidic than pH of 5
- pH control is critical to function of biomolecules
  - Many molecules/cellular processes can only occur (or maximally occur) at a particular pH (the pH optimum)
  - This results in cells putting a lot of energy into maintaining a proper pH
    - Often this means sequestering H^+ or actively pumping H^+ in or out of the cell

Chapter 3: Organic Molecules

Carbon

- Carbon has 4 electrons in its outer shell
- Needs 4 more electrons to fill the shell and adopt a stable configuration
- It can make up to 4 bonds
    - Usually single or double bonds, occasionally triple bonds
- Carbon can form nonpolar and polar bonds
    - Molecules with nonpolar bonds (like hydrocarbons) are poorly water soluble
        - Hydrocarbons are made up of carbon-carbon bonds
        - C-C bonds are electrically neutral and nonpolar
    - Molecules with polar bonds are more water soluble
        - Carbon is not particularly electronegative so more electronegative atoms can often pull electrons away to create polar bonds

Functional Groups (FG)

- Groups of atoms with special chemical features that are functionally important
- Each type of FG exhibits the same properties in all molecules in which it occurs
    - FGs change the physical and chemical properties of molecules their attached to
- Compounds may have more than one FG
    - Amino acids have a carboxylate group and an amine group

Isomers

- Identical molecular formula but different structures and characteristics
    - Same molecular formula but different arrangements of the atoms in the molecule
    - This gives the compound different characteristics
        - Isopropyl alcohol and propyl alcohol (both C_3H_7OH) have different boiling points
- Structural isomers - contain the same atoms but in different bonding relationships
- Stereoisomers - identical bonding relationships, but the spatial positioning of the atoms differs in the two isomers
    - Geometric isomers - positioning around double bond
    - Enantiomers - mirror image of another molecule
        - Non-superimposible, think of your two hands
        - Classified into L or D enantiomers
- Many enzymes have evolved to recognize only one isomer
    - For example L amino acids and D sugars are used predominantly over their counterparts in biological systems

Carbohydrates

- Composed of C, H, and O
- General formula of a carbohydrate is $C_n(H_2O)_n$
    - This is a GENERAL formula, it is still possible to find different functional groups on them
- Most of the carbon atoms in a carbohydrate are linked to a hydrogen atom and a hydroxyl group

Monosaccharides

- Simplest sugars
- Most common are 5 or 6 carbons
    - Pentoses - ribose ($C_5H_{10}O_5$), deoxyribose ($C_5H_{10}O_4$)
        - 5 carbon
        - Deoxyribose is very important in DNA
    - Hexose - glucose ($C_6H_{12}O_6$)
        - 6 carbon
        - Glucose is a very important energy source
- Different ways to depict structures
    - Ring or linear (ring forms in aqueous solution)

Disaccharides

- Composed of two monosaccharides via dehydration (condensation) reaction
    - Condensation reactions release water
- Broken apart by hydrolysis
    - Hydrolysis reactions use water to break bonds
- Examples - sucrose, maltose, lactose

Polysaccharides

- Many monosaccharides linked together to form long polymers
    - Chains of monosaccharides
- Examples
    - Energy storage - starch, glycogen
    - Structural role - cellulose, chitin, glycosaminoglycans
        - Cellulose is most abundant organic compound on Earth (present in most plants)
        - Chitin is used in exoskeletons
        - Glycosaminoglycans are involved in cartilage tissues
- Starch is an amazing energy storage molecule
    - It is a very long, moderately branching chain
    - Moderate branching makes it essentially insoluble
    - This insolubility means that it doesn't create an osmotic problem for the cell
        - This means it is not a colligative molecule
- Glycogen has too many H areas for water to bind so often animals use fats to store energy instead
- Cellulose is not branched, allowing them to be packed densely together
    - This limits how much water can interact with the strands
    - It also makes it very difficult to break down

Lipids

- Composed predominantly of H & C
- Nonpolar bonds therefore very insoluble in water
- Examples: fats, phospholipids, steroids

Fats

- Mixture of triglycerides (3 fatty acids + 1 glycerol)
    - Also known as triacylglycerols

- Formed by bonding glycerol to three fatty acids
- Joined by dehydration, broken via hydrolysis
- Important for energy storage
    - 1 gram of fat stores twice as much energy as 1 gram of glycogen or starch
- Fats can also be structural in providing cushioning and insulation
- Fats are very highly reduced
    - When they are oxidised they provide more energy than more reduced compounds (like sugars)

Fatty acids

- Saturated - all carbons are linked by single covalent bonds
    - Tend to be solid at room temperature
        - This is because they are linear molecules so they can pack together tightly
        - Remember hydrophobic likes hydrophobic
    - Very highly reduced form (high energy)
    - Each carbon is saturated with hydrogens
- Unsaturated - contain one or more double bonds
    - 1 double bond - monounsaturated
    - 2 or more – polyunsaturated
    - When you add double bonds the carbons are NOT saturated with hydrogens
    - Tend to be liquids at room temperature (oils)
        - This is because double bonds create kinks in the chain so that they can't pack together as tightly
        - Unsaturated fats in the plasma membrane also helps to makes it more fluid and flexible (helps in freeze tolerance of the membrane)

Phospholipids

- Glycerol, 2 fatty acids and a phosphate group
- Amphipathic molecule
    - Head region - polar, hydrophilic
    - Fatty acid chains - nonpolar, hydrophobic
- Important in membrane structure, signalling, etc.

Steroids

- Four interconnected rings of carbon atoms
- Usually not very water soluble
- Many are used as signalling molecules (eg. hormones)
- Cholesterol is a steroid
- Estrogen and testosterone differ only slightly but biologically they produce very different results
    - Receptors must be able to tell the differences between the two with very high accuracy

Proteins

- Composed of C, O, H, and N
    - Sometimes S
    - Often they are modified
- Proteins fulfill many functions, transportation, signalling, motor, defence, etc.

- Machines of the cell
    - DNA is the blueprint for these machines
- Amino acids are the monomers
    - Common structure with variable R-group
    - 20 L-amino acids that are used in proteins
        - Some D-amino acids, but not too many
    - Side-chain determines the structure and function
        - Often designated as the "R" groups
- L amino acids come in three groups
    - Nonpolar
    - Polar uncharged
        - Polar covalent bonds but none carry a charge
    - Polar charged
        - Come in basic and acid forms
        - Polar covalent bonds and they carry a charge
- Joined by dehydration reaction
    - Peptide bond
    - Forms polypeptides
    - Proteins are made up of 1 or more polypeptides
        - Proteins can be just a few amino acids or thousands
- Broken apart by hydrolysis
- Numbering starts at the N-terminus(H_3N+) towards C-terminus (COO-)
    - N-terminus = amino terminus
    - C-terminus = carboxy terminus

Protein Structures

- Primary: sequence of amino acids determined by the gene (sequence of amino acids in the polypeptide chain)
    - This ultimately determines the secondary and tertiary structures
        - This means that any alterations to the primary structure has the potential to create a butterfly effect on higher structures
    - When identifying a specific amino acid in the polypeptide chain they are referred to as residues
        - Residues are numbered starting at the N-terminus
    - Can think of this as beads on the change
- Secondary: α-helices and β-sheets
    - α means alpha, β means beta
    - α-helices, think about it like a phone cord
        - You can bunch these together into different shapes (this would be tertiary structure)
    - You can also have random coils that don't fall into the other two category
- Tertiary: 3-D shape of the protein
- Quaternary: Refers to the shape of multimeric complexes
    - Multimeric complexes are structures that are made up of more than one protein
    - The proteins that make up these complexes are referred to as subunits
    - A multimeric complex is made up of 2 or more subunits
    - Some enzymes are multimeric

    - If the subunits are the same you would refer to the complex as monomeric
    - If the subunits are different you would refer to the complex as heteromeric
    - Depending on the number of subunits you would refer to the complex as dimeric (2 subunits), trimeric (3 subunits), etc.
    - So a complex made up of 3 different subunits would be heterotrimeric, 3 same subunits would be monotrimeric, etc.
- If you think of Lego, an individual block would be the tertiary structure and something made out of multiple blocks would be a multi-meric complex (quaternary structure)

5 Factors Promoting Protein Folding and Stability with examples

1. Hydrogen bonds
    a. Bonds form between atoms in the polypeptide backbone and between atoms in different side chains
2. Ionic bonds
    a. Form between oppositely charged side chains
3. Hydrophobic effects
    a. Nonpolar amino acids in the center of the protein avoid contact with water
4. Van der Waals forces
    a. Attractive forces occur between atoms that are optimal distances apart
5. Disulfide bridges
    a. A covalent bond forming between two cysteine side chains (S-S)

Protein-protein interactions

- Many cellular processes involve steps in which two or more different proteins interact with each other
    - Specific binding at surface
        - High degree of accuracy (fidelity) and high affinity for one another
    - Use the first 4 factors
    - Almost all proteins interact with another protein at some point (eg. post-translational modification, phosphorylation, forming multimeric complexes, etc.)
- Proteins can fold back into their original shape (reform their tertiary structure) on their own (Ribonuclease experiment, Nobel Prize 1972: Christian Anfinsen)
    - See Fig. 3.19 in your text

Proteins contain functional domains within their structures

- Module or domains in proteins have distinct structures and function
    - Domains in proteins would be a particular sequence in a polypeptide that serves a specific function
    - These domains can be found (exactly the same) in many different proteins and serve the same function in every protein
    - Think of them as sort of the same to proteins as functional groups are to molecules
- Signal transducer domain and activator of transcription (STAT) domain are examples
- Each domain of this protein is involved in a distinct biological function
- Proteins that share one of these domains also share that function
- You can use the domains that you find in the primary structure to determine functions of unknown proteins

Nucleic Acids

- Responsible for the storage, expression, and transmission of genetic information
- Two classes
    - Deoxyribonucleic acid (DNA)
        - Use the deoxy sugar because it is more stable
        - Store genetic information coded in the sequence of their monomer building blocks
    - Ribonucleic acid (RNA)
        - Involved in decoding this information into instructions for linking together a specific sequence of amino acids to form a polypeptide chain
    - They differ in their sugars and one base (uracil)
- Made up of phosphate group, a five carbon sugar (either ribose or deoxyribose), and a single or double ring of carbon and nitrogen atoms known as a base
    - Monomer is a nucleotide
    - Sugar-phosphate backbone
        - Phosphodiester bonds
    - Base + sugar = nucleoside, Base + sugar + phosphate(s) = nucleotide
    - C≡G and A=T, Purines: 2 rings
    - Pyrimidines and Uracil: 1 ring
        - Remember pyrimidines with "You CUT a Py)
        - T is pretty much methylated uracil
    - Read a DNA sequence from the 5' to the 3' end
- Uracil is used instead in RNA and not DNA because:
    - Methylation group
        - Slightly hydrophobic and allow thymine to base pair very specifically with adenine (helps it fit into the major groove), uracil is more promiscuous
        - Protects your DNA because in viruses that chop up DNA they have difficulties with methylated sites
    - Cytosine can be transformed into uracil as a mutation
        - If there was a C→U mutation in DNA the enzyme that repairs it "knows" that U should not be in DNA and changes it back to a C
        - If U was found in DNA however, it would never know that a mutation had occurred
        - C→U mutations in RNA aren't that big of a deal because you can make the transcript again, DNA is the master copy and must be maintained in perfect condition

DNA vs. RNA

DNA	**RNA**
Deoxyribonucleic acid	Ribonucleic acid
Deoxyribose	Ribose
Thymine (T)	Uracil (U)
Adenine (A), guanine (G), cytosine (C), used in both	
2 strands forming a double helix	Single stranded
1 form	Several forms

Chapter 4: Cell Theory

Microscopy

- Magnification
  - Ratio between the size of an image produced by a microscope and its actual size
- Resolution (clarity)
  - Ability to observe two adjacent objects as distinct from one another
- Contrast
  - Ability to distinguish different structures
  - Can be enhanced using dyes
    - Dyes and proteins can also be used to target things of interest and make them easy to identify
- Check out figure 4.1 in Brooker text for relative scale of size of biological structures
- Light microscope
  - Uses light for illumination
  - Examine things at the cellular level (organelles, whole cells, etc.)
  - Resolution 0.2 µm
  - Contrast can be enhanced by using dyes, florescent proteins, differences in light refraction, etc.
    - Can help you target and identify different tissues or objects of interest
- Electron microscope
  - Uses an electron beam
  - Resolution 2 nm

Electron Microscopes

- Transmission electron microscopy (TEM)
  - Thin slices stained with heavy metals
    - Must be a dense material (often gold is used)
  - Some electrons are scattered while others pass through to form an image
- Scanning electron microscopy (SEM)
  - Sample coated with heavy metal
    - The sample is whole and has not been sliced, the outer surface is coated with heavy metal
  - Beam scans surface to make 3D image
- Samples are dead when they are scanned
- See fig 4.3 in Brooker text for example

Prokaryotic cells

- Simple cell structure
- Lack a membrane-enclosed nucleus and membrane enclosed-organelles
- 2 categories - bacteria and archaea
  - Both small
  - Bacteria - abundant, most not harmful
  - Archaea - less common, often found in extreme environments

Typical Bacterial Cell

- Plasma membrane-barrier
- Cytoplasm – everything contained inside plasma membrane
- Nucleoid - region where genetic material found (DNA)
- Ribosomes - involved in protein synthesis
- Many structures are located outside the plasma membrane
    - Cell wall - support and protection
    - Glycocalyx - traps water, protection
        - Mucus like secretion (proteins with sugar moieties) helps to keep the cell hydrated
        - Protects some bacterial pathogens from immune systems
    - Appendages - pilli (attachment), flagella (locomotion)
        - Pilli can attach to surfaces (substrates) and other bacteria
        - When they attach to other bacteria sometimes genetic information can be transferred between them
        - Cilia are eukaryotic flagella, different than pilli
        - Pilli are also not used for locomotion

Eukaryotic Cells

- DNA housed inside nucleus
- Eukaryotic cells exhibit compartmentalization
- Organelle: membrane-bound compartment with its own unique structure and function
- Shape, size, and organization of cells vary considerably among different species and even among different cell types of the same species
- Plant cells have chloroplasts and cell walls, animal cells do not
    - Plant cells also have large vacuoles, much larger than in animal cells
    - Cell wall is a rigid structure
- See examples in Brooker text fig 4.6

The Proteome Determines the Characteristics of a Cell

- How does a single organism produce different types of cells?
- Identical DNA in different cells but different proteomes
- The proteome of a cell determines its structure and function
- Gene regulation, amount of protein, amino acid sequence of a particular protein and protein
- Proteomes in healthy cells are different from the proteomes of cancerous cells
- Genome is the information contained in DNA
    - Animal cells have 2 genomes (mitochondrial and nuclear)
    - Plant cells have 3 genomes (mitochondrial, nuclear, and chloroplast)
- Proteome is the suite of proteins in a specific cell, tissue, organelle, etc.
    - Each organelle has a different proteome in comparison to genomes
- Proteome is probably larger and more complex than the proteome
    - From a single gene you can ultimately get a lot of different proteins
        - Different exons, how the protein is modified after it's made, etc.

Cytosol

- Surrounds organelles inside the plasma membrane
- Cytoplasm includes everything inside the plasma membrane (including organelles)

- Cytosol = the milieu surrounding organelles
  - This is where glycolysis occurs

Metabolism

- Cytosol is central coordinating region for many metabolic activities of eukaryotic cells
- Catabolism - breakdown of a molecule into smaller components
- Anabolism - synthesis of cellular molecules and macromolecules

Translation

- Process of polypeptide synthesis
- Information within a gene is ultimately translated into the sequence of amino acids in a polypeptide
- Ribosome - site of synthesis
  - Transfer RNA (tRNA) - brings amino acids
  - Messenger RNA (mRNA) - information to make a polypeptide

Cytoskeleton

- CS proteins are critical for cell function: mutations often lead to disease
- Scaffolding system that gives it structural integrity and shape
- Also important in moving things around in the cell and organizing things
  - Helps with compartmentalization, without organization it would be nothing
- Network of three different types of protein filaments
- Microtubules (tubulin)
  - Dynamic instability
  - Centrosome or microtubule-organizing center (MTOC) in animals
- Intermediate filaments
  - Tend to be more stable than microtubules and actin filaments, which readily polymerize and depolymerise
- Actin filaments
  - Also known as microfilaments
- See table 4.1 in the Brooker text

Motor Proteins

- Use ATP to promote movement
- Three domains called the head, hinge, and tail
- Walking analogy
  - Ground is a cytoskeletal filament, your leg is the head of the motor protein, and your hip is the hinge
  - Head is where most of the conformational change will take place and ATP hydrolysis
  - Hinge is where bending occurs
  - Tail binds to other components
- Three different kinds of movements
  - Motor protein moves the cargo along cytoskeleton
  - Motor protein can remain in place and move filament
  - Motor protein attempting to walk (both the motor protein and filament restricted in their movement) exerts a force that causes the filament to bend

- Examples of motor proteins include myosins, kinesins, and dyneins
- The job of motor proteins is to move things around in the cell using the energy supplied by the catalysis of ATP
    - Moves vesicles for exocytosis, organelles around within the cell, physically change the shape of the cell, move proteins, etc.

Flagella and Cilia

- Flagella usually longer than cilia and present singly or in pairs
- Cilia are often shorter than flagella and tend to cover all or part of the surface of a cell
- Typically both are used in movement
- Share the same internal structure
    - Consists of microtubules, dynein, and axoneme
    - Axoneme describes the structure itself
    - 9 + 2 arrangement of microtubules
        - 9 doublets of microtubules around a pair of microtubules
        - Held together by linker proteins
        - Dynein provides the driving force for the bending
    - Basal body is the bottom of the flagella/cilia
    - Firing on one side and then the other (asynchronous firing) that creates the wavelike action
- In prokarya flagella move in a rotary movement while in eukarya they move in a back and forth motion
- Movement involves the propagation of a bend, which begins at the base of the structure and proceeds toward the tip
    - An asynchronous bending to produce the wavelike oscillations
    - Requires a coordination of the motor protein activities
- Cilia can be used for motion but are not always
    - Think of cilia in our lungs or noses
- See Brooker text fig 4.11 examples

Sheetz & Spudich Showing Myosin Walks Along Actin Filaments

- Early researchers proposed the sliding-filament model based on work with living cells in vivo 1983, Michael Sheetz and James Spudich devised an approach to study myosin in vitro
- Nitella cells were used as a source of actin filaments
- Myosin was purified and attached to a fluorescently labelled bead
- Confirmed that myosin is a motor protein that uses ATP to walk along actin filaments
    - Whenever they added ATP they could see the beads fluoresce

Endomembrane System

- Consists of a network of membranes enclosing the nucleus, ER, Golgi, lysosomes, peroxisomes and vacuoles
    - Peroxisomes were just recently added to this system
    - Some of them are physically connected to each other (eg. nuclear membrane and ER)
    - They all move things between each other
        - This occurs via vesicles
- Also includes plasma membrane

- May be directly connected to each other or pass materials via vesicles

Nuclear Envelope

- Double-membrane structure enclosing nucleus
  - Lipid bilayer
- Nuclear pores provide passageways
  - Holes that selectively let things in and out of the nucleus
  - Multimeric complexes
- Outer membrane is continuous with the ER membrane
- Materials within the nucleus are not part of the endomembrane system
- Its primary function is to organize genetic materials (DNA)

Nucleus

- Chromosomes
  - Composed of DNA and protein
  - Chromatin
- Primary function involves the protection, organization, and expression of the genetic material
- Ribosome assembly occurs in the nucleolus
- Contains DNA and its own proteome

Endoplasmic Reticulum

- Network of membranes that form flattened, fluid filled tubules called cisternae
- ER membrane encloses a single compartment called the ER lumen
- Rough endoplasmic reticulum (rough ER)
  - Studded with ribosomes
  - Involved in protein synthesis and sorting
    - Only if these proteins are destined for somewhere in the endomembrane system or excreted from the cell
- Smooth endoplasmic reticulum (smooth ER)
  - Lacks ribosomes
  - Detoxification, carbohydrate metabolism, calcium balance, synthesis and modification of lipids
- ER has a large surface area

Golgi Apparatus

- Also called the Golgi body, Golgi complex, or simply Golgi
- Stack of flattened, membrane-bounded compartments which are not continuous with the ER
- Vesicles transport materials between stacks
- Three overlapping functions
  - Secretion, processing, and protein sorting
  - Often vesicles come to the Golgi from the ER
- The cis face faces the ER and the trans face faces the plasma membrane

Lysosomes

- Contain acid hydrolases that perform hydrolysis

    - Perform a lot of the digestion in the cell
    - Sort of like the garbage can of the cell
    - All of these enzymes that break things down are contained within the lysosomes because you don't want them running amok in the cell
        - It contains them
- Various acid hydrolases to break down proteins, carbohydrates, nucleic acids, and lipids
- Autophagy: Recycling of worn-out organelles through endocytosis
    - Specialized vesicles engulf the organelles (autophagosome) and then merge with a lysosome resulting in the digestion of the organelle
    - Need the autophagosome or else lysosome would be able to merge with organelles directly and controlling that would be very difficult
- Not present in plant cells

Tay-Sachs Disease

- Abnormal lipid breakdown in brain cells, accumulation of fatty acid derivatives in the lysosomes
- Retardation and death in infants and young children

Vacuoles

- Functions are extremely varied, and differ among cell types and even environmental conditions
- Central vacuoles in plants for storage and support
    - Provide structural rigidity, Turgor support
- Contractile vacuoles in protists for expelling excess water
    - Sometimes used as a form of jet propulsion
- Phagocytic vacuoles in protists and white blood cells for degradation
- In plants they perform a similar function that lysosomes fulfill

Peroxisomes

- Relatively small, found in all eukaryotic cells
- Origin remains controversial (semiautonomous?)
    - Seem to be able to undergo binary fission even though they don't have their own genome
    - Their main origin (ER) is from the endomembrane system so they are designated within this system
- General function to catalyze certain chemical reactions, typically those that break down molecules by removing hydrogen or adding oxygen
    - Lots of oxidation reactions (eg. oxidation of fatty acids)
- Reaction by-product is hydrogen peroxide (H_2O_2)
- Catalase breaks down H_2O_2 without forming dangerous free radicals
    - Peroxisomes keeps oxidation reactions contained in a certain area within the cell
- Plants have specialized peroxisomes in seeds
    - Called glyoxisomes, they liberate energy from seed food stores
- Zellweger (cerebro-hepab-renal) syndrome
    - Inability to beta oxidize very long chain fatty acids in the peroxisomes
    - Facial dysmorphism of Zellweger syndrome

Plasma Membrane

- Boundary between the cell and the extracellular environment
    - Ultimate form of compartmentalization, without this there would be no cell
    - Every cell requires a plasma membrane, all subsequent compartmentalization is within the plasma membrane
- Membrane transport in and out of cell
    - Selectively permeable, not anything can pass through the membrane
    - Some molecules are able to freely pass through the membrane however many must go through protein channels or gates
- Important in cell signalling using receptors
    - Eg. hormone receptors
- Important in cell-cell adhesion
    - Accomplished mainly via transmembrane proteins (proteins that go through the plasma membrane)
    - Can also occur via linker proteins
    - Adhesion is almost always facilitated by proteins anchored in the plasma membrane
- See Fig 5.10 and 5.11 in Brooker text

Semiautonomous Organelles

- Not completely autonomous
    - Mitochondria, chloroplasts
- Can grow and divide to reproduce (binary fission)
- Mito and chloro have small, circular genomes like bacteria
    - Mito and chloro each have their own genome
    - The genomes contain the information for SOME (not all) of the proteins that they require
        - Still reliant on the nuclear genome for most of the proteins that they require
- Endosymbiotic theory
    - Mitochondria are ancestrally derived from purple bacteria, chloro from cyanobacteria
    - Thought that they were phagocytosed and eventually evolved to live entirely within cells

Mitochondria

- Outer and inner membrane (double membrane structure)
    - Intermembrane space and mitochondrial matrix
    - Intermembrane space is where the driving force is to make ATP (through concentration gradients)
    - Mito matrix is where the Krebs cycle (TCA) takes place
        - This is the space inside the inner membrane
- Primary role is to make ATP
- Also involved in the synthesis, modification, and breakdown of several types of cellular molecules
- Can also generate heat in brown fat cells

Chloroplasts

- Photosynthesis
    - Capture light energy to synthesize organic molecules such as glucose
- Double membrane structure

- Found in nearly all species of plants and algae
- Outer and inner membrane with an intermembrane space
- Third membrane, the thylakoid membrane, forms flattened tubules that stack to form a granum (plural, grana)
    - Thylakoid membrane is where photosynthesis (PS) takes place
- Other plastids include:
    - Chromoplasts: found in fruit, they give them colour and help to attract fruitivores for seed dispersal
    - Amyloplasts: Found in root tissue, they are starch storing (energy storage)

Chapter 5: Systems Biology

Genomes, Proteomes & Cell Structure/Function & Organization

- Proteome is responsible for the structure and function of cells
- Gene and protein regulation causes the proteome to be dynamic
- Proteins have sorting signals
- Cells require pre-existing organization
    - Necessary before every cell division
- Structure determines function
- Protein-protein interactions critical
    - Has to have high affinity (strength of the attraction between two proteins)
    - Think of the hand-glove analogy
        - Both your hand and glove change shape a little when they interact
- Continual synthesis new molecules and breakdown unwanted components
- See table 5.1 and Figure 5.1 in Brooker text
- Heat shock proteins help fold and unfold proteins under different thermal conditions

Molecular Machines

- A machine is an object that has moving parts and does useful work
- Provide structure and organization to cells
- ATP synthase is a molecular machine
    - Molecular recognition allows for complex assembly
        - Subunits recognize each other and bind in a specific way (high specificity and affinity)
- Other molecular machines include motor proteins, ribosomes, etc.

Cytoskeleton

- Maintains shapes (eg. gives red blood cells the biconcave shape, cytoskeleton connects to red blood cell membrane)
- Transports cellular cargos (eg. a membrane vesicle carries cargo such as a neurotransmitter, which is released from the end of the axon)

Four systems work together

1. Interior of the nucleus
    a. Activating the genes that are needed for the proteins that are needed at that particular time under those particular conditions in that particular cell
    b. Houses the information (kind of like the brain of the cell)
    c. Fig. 5.5 in the Brooker text
2. Cytosol
    a. Site of most of the biosynthetic and catabolic metabolic pathways
3. Endomembrane system
4. Semiautonomous organelles
    a. Mitochondria and chloroplasts
    b. Figure 5.8 in Brooker text
    c. Have their own genome but largely they rely on the cytoplasm
    d. Have dual origin proteome

i. Some comes from the nucleus and some they make themselves

Protein Localization

- Sorting (transit) signals are short amino acid sequences that direct protein to cellular location
    - Mark them like an address on postage
    - These signals cannot have any ambiguity, requires high fidelity and specificity
- Most eukaryotic proteins begin translation on ribosomes in the cytosol
    - Example is plant NADHK
        - NADHK is found only in the peroxisomes
        - Used fluorescent

Palade Demonstrated that Secreted Proteins Move Sequentially Through Organelles of the Endomembrane System

- Thousands of different proteins must be sorted to the correct locations
- George Palade's team used pulse-chase experiments to determine where radioactive proteins were produced and the pathways they took
    - Pulse is adding a short pulse of radioactive label (amino acid) and then chased it with cold (non radioactive) label
    - They can then follow where that radioactive label goes through the cell sequentially
- Studied pancreatic cells secreting proteins
- Followed radioactive proteins from synthesis in the rough ER and movements through cellular compartments
- Proteins that stay in the cytosol lack sorting signals
- Post-translational sorting
    - Sorting to nucleus, mitochondria, chloroplasts, and peroxisomes (some) occur after the protein is made
        - Semiautonomous system
- Co-translational sorting
    - As the protein is being translated from the mRNA it is simultaneously entering the ER, or Golgi, or vacuole, etc.
    - Synthesis of other proteins destined for ER, Golgi, lysosome, vacuole, plasma membrane, or secretion halts until the ribosome is bound to the ER
        - Endomembranous system
    - 1st stage: co-translational protein localization: sorting
        - If protein has TM, it will be incorporated into membrane of ER (Fig 5.15 in Brooker text)
        - Starts with mRNA beginning to be translated, first sequence of amina acids is the ER signal sequence
        - This signal sequence is recognized by SRP (signal recognition particle) which pauses translation and brings it to a channel protein
        - Once the ribosome-SRP complex docks to the channel protein, the SRP leaves, and synthesis continues and the protein is made within the ER
            - Or within its membrane
        - See Fig 5.15 in Brooker text
        - There is specificity at each stage
    - 2nd stage: if no ER retention, then vesicle transport
        - Recognition because v-snares and t-snares ensure specificity

        - Proteins within the ER bind to receptors in the ER inner membrane
        - A vesicle starts to form around these proteins
        - The membrane surrounding this vesicle is pinched off of the ER
        - V-snares on the outer surface of the vesicle bind with high specificity to t-snares on the outer membrane of the vesicles destination (plasma membrane, golgi membrane, etc.)
        - Vesicle then fuses with the target membrane and delivers its cargo
- Proteins that stay in the ER have ER retention signals (in addition to ER signal)
- Other proteins must be sorted
    - Transported by vesicle
    - Vesicles incorporate coat proteins (specificity)
    - Also incorporates v-snare indicative of cargo
    - T-snare on target recognizes v-snare and vesicle fuses with target membrane

Glycosylation in ER

- Attachment of a carbohydrate to a protein
    - Adding a sugar to a protein, making it a glycoprotein
- May aid in protein folding, extracellular protection, and protein sorting (recognition)

Post-translational Sorting

- Most proteins for mitochondria, chloroplasts, and all (?) proteins for peroxisomes sorted post-translationally
- Must have a sorting signal
    - Sorting signal of the protein determines where the proteins will go (where it will be sorted)
- Mitochondrial example
    - Protein is translated in the cytosol
    - Chaperone proteins help keep the protein linear (in an unfolded state)
    - Targeting sequence (sorting signal) binds to the right receptor on the mitochondrial outer membrane surface
    - Chaperone proteins are releases, protein is transferred to the inner mitochondrial matrix
    - Chaperones may bind again within the matrix and the protein may be cleaved in order for it to become active
- See figure 5.18 in Brooker text for overview

Molecular Recycling

- Large molecules, except DNA, have finite lifetimes
    - Half-life varies from 5 minutes for mRNA in prokaryotes to 30 minutes to several days for mRNA in eukaryotes
- Includes the continual degradation of faulty or non-functional proteins and synthesis of new ones
- Turnover
    - What the proteins biological half-life is
    - Helps to regulate protein activity
        - Once that proteins job is done, it is quickly disposed of

Proteasome

- Molecular machine for protein degradation
- Ubiquitin directs unwanted proteins to proteasomes in eukaryotes
    - Actually polyubiquinilation (addition of multiple ubiquitin to the protein)
    - Think of it like the sorting signal that destines the protein for destruction
    - Accomplished by ubiquitin-ligases
        - Enzymes that add ubiquitin to proteins
- Proteases degrade the unwanted protein into peptides and amino acids
- Also targets misfolded proteins
- Critical for environmental responses
- DO NOT CONFUSE WITH PROTEOME
    - Proteasome degrades proteins
    - Proteome is the collection of proteins

Chapter 6: Membrane Structure and Transport

Biological Membranes

- Membranes have 3 main functions
  - Regulation of transport
  - Compartmentalization
  - Communication/signal transduction
- Basic framework is phospholipid bilayer
- Phospholipids are amphipathic
  - Hydrophobic region faces in
  - Hydrophilic region faces out
- Also contain proteins & carbohydrates
  - Relative amount of each vary
- Fluid-mosaic model
  - Membranes are semi-fluid
    - Not a solid barrier, it is fluid like
    - Proteins can diffuse/move around within the membrane
  - Mosaic because it is made of many different things (proteins, phospholipids, etc.)

Proteins Bound to Membranes

- Integral membrane proteins
  - Physically anchored to the membrane
    - Very difficult to knock them off (requires strong detergents)
  - Transmembrane (TM) proteins
    - Serpentine through the membrane multiple times
  - Lipid anchors
- Peripheral membrane proteins
  - Indirectly or loosely associated to the membrane
    - Indirectly would be like being associated to a transmembrane protein (this would be called a peripheral membrane protein)
  - Noncovalently bound to integral membrane proteins or to the polar head groups of phospholipids (PLs)
- Proteins are a critical aspect of membrane structure and function

Approximately 25% of All Genes Encode Membrane Proteins

- Membranes are important biologically and medically (70% drugs target membrane proteins)
- Can predict TMs via algorithms
  - Flu shots
- Around 20-30% of all genes predicted
- Function of many proteins unknown

Factors Affecting Fluidity

- Length of fatty acyl tails
  - Shorter acyl tails are less likely to interact, which makes the membrane more fluid
    - Less surface area to interact with other acyl tails
- Presence of double bonds in the acyl tails

    - DB creates kink tail, making it more difficult for neighbouring tails to interact = more fluid
        - Prevents physical packing of tails
- Presence of cholesterol (animals)
    - Cholesterol tends to stabilize membranes
    - Effects depend on temperature

Experiments on Lateral Transport

- Larry Frye and Michael Edidin conducted an experiment that verified the lateral movement of membrane proteins
- Involved the fusion of mouse and human cells
- Mouse membrane proteins were H-2 fluorescently labelled
    - These are mouse-specific antibodies that only fuse to the mouse membrane proteins
- They then observed where the labelled proteins were under two temperature treatments - 0°C or 37°C
    - 0°C cells-label stays on mouse side
        - Same as right after they add the label
    - 37°C cells-label moves over entire cell
- This experiment looked only at proteins in the membranes that were not anchored to the cytoskeleton

Glycosylation

- Covalently attach carbohydrate to a protein or lipid
    - Glycolipid: carbohydrate to lipid
    - Glycoprotein: carbohydrate to protein
        - Often done post-translationally
- Can serve as recognition signals for other cellular proteins
- Often play a role in cell surface recognition
- Protective effects
    - Cell coat or glycocalyx - carbohydrate rich zone on the cell surface shielding cell from immune systems

Electron Microscopy

- Transmission electron microscopy (TEM), uses a biological sample that is thin sectioned and stained with heavy-metal dyes
- Dye binds to the polar head groups of phospholipids, but it doesn't bind well to the fatty acyl chains

FFEM

- Freeze fracture electron microscopy, specialized form of TEM, can be used to analyze the interiors of phospholipid bilayers
    - Sample is frozen in liquid nitrogen and fractured with a knife
    - Due to the weakness of the central membrane region, the leaflets separate into a P face (the protoplasmic face that was next to the cytosol) and the E face (the extracellular face)
    - Can provide significant 3-dimensional detail about membrane protein form and shape

Phospholipid Bilayer is a Barrier

- Hydrophobic interior makes formidable barrier
- Selectively-permeable
    - Some things can freely pass, others need to go through gates or channels
- Diffusion
    - Movement of solute from an area of higher concentration to an area of lower concentration
    - Passive diffusion - without transport protein
    - Solutes vary in their rates of penetration

Permeability of the membrane

- High permeability
    - Gases
    - Very small uncharged polar molecules
- Moderately permeable
    - Water
    - Urea
    - Both of these are uncharged but quite polar
- Low permeability
    - Polar organic molecules (eg. glucose)
- Very low permeability
    - Ions
    - Charged polar molecules and macromolecules
        - Macromolecules include proteins, DNA, polysaccharides, etc.
- Permeability decreases with polarization, charge, and size

Cells Maintain Gradients

- Transmembrane gradient
    - Concentration of a solute is higher on one side of a membrane than the other
- Ion electrochemical gradient
    - Both an electrical gradient AND a chemical gradient (eg. via proton pump)

Passive Transport

- Passive transport does not require an input of energy
- 2 types
    - Passive diffusion
        - Diffusion of a solute through a membrane without transport protein
    - Facilitated diffusion
        - Diffusion of a solute through a membrane with the aid of a transport protein (specificity!)
        - Occurs through a channel, not simply a pore (or hole)

Tonicity – Effect on cell volume (shape)

- Refers to the concentration of solutes
- Water balance is critical to all cells

    - Too much and it bursts, too little and it cannot function
- Tonicity is the shape of the cell and movement of water
- Isotonic
    - Equal water and solute concentrations on either side of the membrane (no difference)
- Hypertonic
    - Solute concentration is higher (and water concentration lower) on one side of the membrane (more stuff outside the cell, higher concentration outside the cell)
    - If the extracellular environment is hypertonic relative to the inside of the cell, the cell will shrink
- Hypotonic
    - Solute concentration is lower (and water concentration higher) on one side of the membrane (more stuff inside the cell, higher concentration inside the cell)
    - If the extracellular environment is hypotonic relative to the inside of the cell, the cell will swell
- Shrinkage/swelling is due to the movement of water down its chemical potential
    - Movement is from high potential to low
    - Movement is passive (no energy used)
    - Chemical potential will always be lower wherever there are more solutes
- Osmolarity is the total concentration of solutes from the point of view of the cell
- Take a cell and put it into a medium of 10 mmol urea
    - The solution is hyperosmotic but not hypertonic (ultimately)
        - Remember permeability; urea can move easily across the membrane to balance the hyperosmotic pressure so water will not move out of the cell

Osmosis

- Water diffuses through a membrane to balance solute concentration
- Osmotic pressure can result if solutes are impermeable (cell shrinking, swelling)
- Animal cells must maintain a balance between extracellular and intracellular solute concentrations to maintain their size and shape
- Crenation: shrinking in a hypertonic solution
- Plants cell wall prevents major changes in cell size
    - Due to wall they cannot explode
- Turgor pressure
    - Maintains shape and size via vacuole
    - Loss of turgor can result in wilting, eventually plasmolysis and death
- If you think about photosynthesis, how can plant cells make so much sugar without causing osmotic problems?
    - Change the glucose that it makes into starch which is insoluble (therefore doesn't affect osmolarity

Agre Discovered Osmosis Occurs Faster in Cells with Transport Proteins That Allow the Facilitated Diffusion of Water

- Water passively diffuses across plasma membranes
- Certain cell types allow water to move across the plasma membrane at a much faster rate than would be predicted by passive diffusion
- Peter Agre and his colleagues first identified a protein that was abundant in red blood cells and kidney cells, but not found in many other cell types

    - CHIP28 protein
- Striking difference was observed between frog oocytes that expressed CHIP28 versus the control
    - Found that when they put both the control and the CHIP28 oocytes into a hypotonic solution the CHIP28 ones swelled much faster than could have been predicted by the without some sort of facilitated movement of water
- Aquaporins
    - Specialized pores in the plasma membrane that act as water channels
- Agre was awarded the Nobel Prize in 2003 for this work

Transport proteins

- Transport proteins enable biological membranes to be selectively permeable
- 2 main classes
    - Channels
    - Transporters

Channels

- Conduit for the direct diffusion of ions or molecules across the membrane
    - Eg. Aquaporins
    - State of open or closed channels is regulated
- These channels also have high specificity
    - Aquaporins will only move water, not anything
- Most channels are gated open or closed, can be opened or closed in the following ways:
    - Ligand-gated
        - Change triggered by the binding of a ligand (eg. hormone, neurotransmitter, etc.)
    - Intracellular regulatory proteins
        - Change triggered by binding of a regulatory protein on its intracellular surface
    - Phosphorylation
        - Change triggered by the phosphorylation of the protein
    - Voltage-gated
        - Change triggered by a change in the electrical gradient across the membrane
    - Mechanosensitive channels
        - Physically opened or closed (eg. sound)

Transporters

- Also known as carriers
- Different than channels because there is a physical binding in some stage of the transport
- Conformational change transports the solute
    - When the solute binds to the transporter it causes it to physically change shape
    - This change in shape makes it have a lower affinity for the solute and it disassociates
    - When the solute is gone the transporter returns to its original shape
- Principal pathway for uptake of organic molecules, such as sugars, amino acids, nucleotides
- Key role in export
- Transporter types
    - Uniporter
        - Moves one solute in a single direction

- Symporter/cotransporter
  - Moves two solutes in a single direction
  - In general one binds first and makes the transporter have a higher affinity for the other
- Antiporter
  - Moves two things in opposite directions
  - Again this usually happens sequentially

Cystic Fibrosis: case study

- CFTR normally a Cl^- transporter
- Its role is to help with ion homeostasis
  - Important in mucus formation
- Mutations impair (or destroy) its function
  - Leads to a build up of viscous mucus → infections, etc. in lungs
- Serves as an excellent example for the following principles
  - Small mutation in the primary structure leading to a change in the tertiary structure which leads to a disease state
  - Membrane function (transport)
    - Cl^- transport
  - Protein function domains, protein folding, protein-protein interactions
    - ATP binding domains, transmembrane domains, etc.
  - Ion homeostasis, tonicity effects
  - Endomembrane system (subcellular targeting of proteins)

Pump

- Couples conformational changes to an energy source, such as:
- ATP-driven pumps (use ATP to work)
  - ATP hydrolysis provides the energy for the work
  - Can be uniporters, symporters, or antiporters
  - Active transport (requires energy)

Active Transport

- Movement of a solute across a membrane against its gradient
- Energetically unfavorable: requires energy input
- Primary active transport
  - Directly use energy to transport solute
    - Eg. uses ATP to pump the solute
- Secondary active transport
  - Use pre-existing gradient to drive transport of solute
    - Uses an existing gradient to move other solutes against their gradient
    - Commonly exploits H^+ and K^+ gradiants

ATP-Driven Ion Pumps Generate Ion Electrochemical Gradients

- Na^+/K^+-ATPase
  - Actively transport Na+ and K+ against their gradients by using the energy from ATP hydrolysis

- 3 Na^+ exported for 2 K^+ imported into cell
  - Antiporter (Na^+ out and K^+ in)
  - Electrogenic pump - export 1 net positive charge
  - Moves more positive charges out then in

Exocytosis/Endocytosis

- Transport larger molecules such as proteins and polysaccharides, and even very large particles
- Exocytosis
  - Material inside the cell, which is packaged into vesicles, is excreted into the extracellular medium
  - Starts in the endomembrane system
- Endocytosis
  - Plasma membrane invaginates, or folds inward, to form a vesicle that brings substances into the cell
  - Receptor-mediated endocytosis
  - Pinocytosis
    - Solutes in solution (cell drinking)
  - Phagocytosis
    - Solutes not in solution (cell eating)
- Both endo and exocytosis require high fidelity of the receptors for their cargo
  - You don't want to bring in or excrete anything that you don't mean to
  - Some pathogens try to take advantage of this
    - They form glycoproteins on their Glycocalyx that are similar to cargoes and trick the receptors

Chapter 7: Enzymes and Metabolism

- Sum total of all chemical reactions that occur within an organism
- Also refers to specific chemical reactions at the cellular level
- Enzymes, metabolism, and cellular respiration

Chemical Reactions

- 2 factors govern fate of a chemical RXN
    - Direction
        - Many biological reactions wouldn't happen spontaneously therefore they need ATP to drive the RXN in the way you want
        - Many cells use ATP to drive reactions in 1 direction
    - Rate
        - Catalysts called enzyme can speed the reaction rate

Energy

- Ability to promote change
- 2 forms
    - Kinetic: associated with movement
    - Potential: due to structure or location
        - Chemical energy: energy in molecular bonds

2 Laws of Thermodynamics

1. First law
    a. Called the law of conservation of energy
    b. Energy cannot be created or destroyed
2. Second law
    a. Transfer or transformation of energy from one form to another increases entropy or degree of disorder of a system

Change in free energy determines direction

- Entropy is a measure of the disorder that cannot be harnessed to do work

H = G + TS

- H= enthalpy or total energy
- G= free energy or amount of energy for work
    - Gibb's Free energy
- S= entropy or unusable energy
- T= absolute temperature in Kelvin (K)

Spontaneous Reactions

- Thermodynamically favoured
- Not necessarily fast
- Key factor is the free energy change
- $\Delta G = \Delta H - T\Delta S$

- Biochemistry uses $\Delta G^{'0}$: "standard" free energy change
    - ΔG is not a constant
    - $\Delta G^{'0}$ is a constant
- Specific conditions and concentrations

A + B → C + D

- Exergonic
    - $\Delta G^{'0}$<0 or negative free energy change
    - Spontaneous
        - Does not require the input of energy
        - Spontaneous does not mean that this will happen at any time (not the literal meaning of spontaneous)
- Endergonic
    - $\Delta G^{'0}$>0 or positive free energy change
    - Requires addition of free energy
    - Not spontaneous

Why ATP?

- Hydrolysis of ATP $\Delta G^{'0}$ = -7.3 kcal/mole
    - This reaction releases energy (negative $\Delta G^{'0}$)
    - Covalent bonds between the phosphate groups are broken
        - There exists all this repulsion between the negative phosphate groups and by breaking the covalent bond holding them together you release that energy
    - ATP is used because ATP and phosphate are stable and at a much lower energy state
        - This gives it the negative $\Delta G^{'0}$
- Favours formation of products
- Energy liberated can drive a variety of cellular processes
- Link exergonic RXN to drive endergonic

Example:

- Glucose + phosphate → glucose-phosphate + H_2O
    - Endergonic: $\Delta G^{'0}$ = +3.3 Kcal/mol
- ATP + H_2O → ADP + P_i
    - Exergonic: $\Delta G^{'0}$ = -7.3 Kcal/mol
    - P_i is phosphate
    - This is ATP hydrolysis
- Coupled reaction:
- Glucose + ATP → Glucose-phosphate +ADP
    - $\Delta G^{'0}$ = -4.0 Kcal/mol
    - Overall (sum) RXN is exergonic
- There is a physical transfer of P_i to glucose
    - In most ATP hydrolysis the P_i transfer is temporary and eventually falls off
    - As such it often appears on the right side of the reaction
- ATP hydrolysis is also used because it is kept well displaced from equilibrium

Equilibrium constant (K_{eq})

- $aA + bB \leftrightarrow cC + dD$
- $K'_{eq} = [C]^c[D]^d/[A]^a[B]^b$
    - Square brackets [] indicate concentration
- K'_{eq} is determined for each RXN
    - It tells you whether the RXN will occur towards the left or the right (exergonic or endergonic)
    - You can use this value in $\Delta G'^0 = -RT\ln(K_{èq})$
        - R is the gas constant and T is temperature
        - If $\Delta G'^0$ is positive it is endergonic, negative is exergonic
- In the real version of the cell $\Delta G = \Delta G'^0 + RT\ln(Q)$
    - $Q = K'_{eq}$
- Remember that ΔG is not a constant because Q is determined by what is happening in the cell (because of K'_{eq})
- There is two ways to drive endergonic reactions in a cell
    1. Manipulate the ratios of [C] +[D] to [A]+[B]
        - Manipulating the concentrations of reactants and products
        - So if you remove the products right away you can drive this reaction
    2. Couple the reaction with an exergonic reaction

Enzymes

- A spontaneous reaction is not necessarily a fast reaction
- Catalyst - speeds up the rate of reaction without being consumed
- Enzymes - protein catalysts in living cells
- May be multimeric complexes
- Often highly regulated (various levels)
- Decreases activation energy, Brings substrate closer together

Activation Energy

- Initial input of energy to start reaction
    - Even if a RXN is exergonic (favourable) doesn't mean it is just going to happen
    - Some energy needs to be put into it
- Allows molecules to get close enough to cause bond rearrangement
- Can now achieve transition state where bonds are stretched
- Enzymes can lower the activation energy of a reaction
    - Often occurs by bringing the reactant molecules physically closer together
- Enzymes cannot change exergonic reactions in endergonic (vice versa) but it can change the rate of reaction

Overcoming Activation Energy

- 2 common ways
    - Large amounts of heat
    - Using enzymes to lower activation energy
        - Small amount of heat can now push reactants to transition state catalase

Mass action ratio (MAR) and K_{eq}

- $A + B \leftrightarrow C + D$

- K_{eq} = [C] x [D] / [A] x [B]
- MAR = [C] x [D] / [A] x [B]
- The displacement from equilibrium is the "driving force" for the reaction
- MAR is K_{eq} at a specific time at those specific concentrations
    - Not necessarily the concentrations at equilibrium
    - At real cell conditions
- K_{eq} is for concentrations at equilibrium for that RXN
    - At standard conditions
- MAR = K_{eq}
    - The reaction is at equilibrium and there is no net flux in either forward or reverse direction
- MAR <<< K_{eq}
    - The forward reaction is favoured
- MAR >>> K_{eq}
    - The reverse reaction is favoured

Lowering Activation Energy

- Straining bonds in reactants to make it easier to achieve transition state
- Positioning reactants together to facilitate bonding
- Changing local environment
    - Direct participation through very temporary bonding
- Active site = where reaction takes place
    - Binding site for the substrates
    - Possesses a great deal of specificity and affinity for its substrates
        - Induced fit; hand and glove analogy
    - Once substrates are bound to the enzyme the reaction is catalyzed by a conformational change in the enzyme
        - Sometimes a transition state is achieved during the catalysation
    - Once substrates have been converted to products they have a low affinity for the active site and are released
        - Undergoes another conformational change back to original structure
- Substrate(s) = reactant(s) that bind to active site
- Enzyme-substrate complex formed when enzyme and substrate bind
- Optimal temp, pH, etc. affect the physical form of the substrate and also the physical state of the enzymes
    - Outside of these windows their efficiency drops in both accuracy and rate

Substrate Binding

- Enzymes have a high affinity and/or high degree of specificity for a substrate
- Induced fit-interaction also involves conformational changes
- Concepts exploited in pharmacology
    - Eg. drugs designed that look like substrates but are not substrates, often these have higher affinities and will take the place of the real substrate

HIGH AFFINITY = LOW K_m (Michaelis constant)

- Expressed in mM, µM, nM, etc.

- K_m is substrate concentration at half-max "speed"
    - Substrate concentration at half V_{max}
    - This is a description of the affinity of the enzyme for its substrate
    - Low K_m means a very high affinity (tight binding)
        - Needs a very low concentration of substrate to be at half max "speed"

Other Requirements for Enzymes

- Prosthetic groups-small molecules (ion or organic) permanently attached to the enzyme
- Cofactor-usually inorganic ion that temporarily binds to enzyme (eg. Cu^{2+}in cytochromeoxidase, Zn^{2+}in ADH)
    - Usually metals
- Coenzyme-organic molecule that participates in reaction but left unchanged afterward (eg. NADH, vitamins)
    - Enzymes use these to help in catalysis, they are left unchanged afterwards however

Overview of Metabolism

- Chemical reactions occur in metabolic pathways
- Each step is coordinated by a specific enzyme
- Catabolic pathways: breakdown, exergonic
    - Often coupled with anabolic pathways
- Anabolic pathways: synthesis, endergonic
    - Must be coupled to exergonic reaction

Catabolic Reactions

- Breakdown of reactants (e.g. starch, glycogen)
    - These examples are energy stores
- Used for recycling
- Used to obtain energy for endergonic reactions
    - Energy stored in energy intermediates
        - ATP, NADH
- 2 ways to make ATP
    - Substrate-level phosphorylation
    - Chemiosmosis
        - Where most of the ATP synthesis takes place

Energy Intermediates

- Electrons removed by oxidation are used to create energy intermediates like NADH
- NAD^+ Nicotinamideadenine dinucleotide
- NADH, NADPH, $FADH_2$, etc.
    - Oxidized to make ATP
    - Can donate electrons during synthesis reactions
    - Coenzymes
    - Recycling of these energy intermediates is critical for ATP synthesis

Anabolic Reactions

- Biosynthetic reactions
- Powered by catabolism
- Make large macromolecules, tissues, organs, etc or smaller molecules not available from food
- Each ATP undergoes 10,000 cycles of hydrolysis and resynthesis every day

Regulation of Metabolic Pathways

1. Gene regulation
    - Controlling the proteome
2. Biochemical regulation
    - Changing the properties of an enzyme (eg. K_m)

Biochemical regulation of metabolic pathways

- Fine controls
    - This is tweaking the speed or efficiency of the enzyme
    - Coarse control would be controlling the amount of the enzymes
- Competitive inhibitors
    - Something that competes with the substrate for the active site
    - Does not affect V_{max} but makes K_m larger (larger concentration of substrate needed)
    - Can be overcome by increasing the amount of substrate
- Noncompetitive inhibitors
    - Do not compete with the substrate for access to the active site (eg. allosteric inhibitors)
    - Does not affect V_{max} but makes K_m larger (larger concentration of substrate needed)
- Allosteric site-binding causes conformational change in enzyme active site inhibiting enzyme function
    - Feedback inhibition-product of pathway inhibits early steps (earlier enzymes) to prevent over accumulation of product
    - Typically occurs to an enzyme early on in the pathway
    - Sometimes these enzymes are called pacemaker enzymes (control the pace of the pathway)
    - Allosteric inhibitors (or activators) only bind with the allosteric site
    - Sigmoidal kinetics are a result of positive cooperativity
        - Basically when a substrate binds to a subunit of a multimeric enzyme it changes the entire enzymes conformation and enhances its affinity
- Post-translational Modification
    - Modifying an enzyme covalently, usually by other proteins
    - Includes phosphorylation, redox of disulfide bridges, glycosylation, etc.
- Turnover
    - Comparison of ratio of product made vs rate of product degradation
    - For example if you slow down the products destruction than its levels are going to rise
- Activators
    - Activators increase the catalytic rate of enzymes (often by increasing affinity)
    - Opposite of inhibitors
- Substrate levels
    - More or less substrates
    - Providing more substrate can overcome competitive inhibition

Cellular Respiration

- Process by which living cells obtain energy from organic molecules
- Primary aim to make ATP and NADH
- Secondary role is anapleurotic
    - Anapleurotic refers to the formation of intermediates in metabolic pathways
    - These intermediates are often starter blocks for compounds that the cell needs
- Aerobic respiration uses oxygen
    - O_2 consumed and CO_2 released
- Organic molecules + O_2 → CO_2+ H_2O + Energy
    - Ultimately oxygen is reduced (pulls electrons off of organic molecules)

4 Stages of Aerobic Respiration

1. Glycolysis
2. Formation of acetyl CoA
3. Citric acid cycle
4. Electron transport and chemiosmosis

- For each pathway know where it occurs, why it occurs, how its regulated, and what goes into/out of it
- Glycolysis occurs in the cytosol, the rest occur in the mitochondria
    - 2 and 3 occur in the matrix
    - 4 occurs on the inner membrane/inner membrane space

Stage 1: Glycolysis

- Glycolysis can occur with or without O_2
    - Occurs within the cytosol
- Steps nearly identical across species
- 3 phases
    1. Energy investment (invest some ATP)
    2. Cleavage
        - Of the hexoses into trioses (6C into 3C)
    3. Energy liberation (substrate-level phosphorylation)
        - ADP to ATP with the phosphate coming from an organic molecule
        - Different than how ATP is made in chemiosmosis

3 phases of glycolysis

1. Energy investment
    a. Steps 1-3 (rearrangement and phosphorylation of sugar)
    b. 2 ATP hydrolyzed to create fructose-1,6 bisphosphate
2. Cleavage
    a. Steps 4-5
    b. 6 carbon molecule broken into two 3 carbon molecules of glyceraldehyde-3-phosphate
3. Energy liberation
    a. Steps 6-10
    b. Two glyceraldehyde-3-phosphate molecules broken down into two pyruvate molecules producing 2 NADH and 4 ATP

- Net yield of glycolysis: 2 ATP, 2 NADH, 2 pyruvate
- Key regulation point: feedback inhibition

- PFK (phosphofrucktokinase) has allosteric site for inhibition by ATP, citrate, activation by F2,6BP (fructose-2,6-bisphosphate)
    - PFK considered rate-limiting step (in animals)
    - PFK rxn is irreversible
    - F2,6BP is a regulatory metabolite
        - Doesn't undergo metabolism like F1,6BP
        - Its level is manipulated as a regulator of PFK
- Put glucose (6C) in and pyruvate (3C) out
    - Pyruvate forms acetyl CoA
    - Some ATP is also produced
- In review:
    - It happens in the cytosol
    - It occurs to make pyruvate
    - Regulated by the PFK
        - PFK is regulated by ATP, citrate (both inhibitors), and F2,6BP (activator)
    - Glucose goes in, pyruvate, 2 NADH and 2 ATP comes out

Stage 2: Breakdown of pyruvate to an acetyl group (Formation of Acetyl-CoA)

- In eukaryotes, pyruvate transported to the mitochondrial matrix
- Broken down by pyruvate dehydrogenase (PDH)
- Molecule of CO_2 removed from each pyruvate
- Remaining acetyl group attached to CoA
    - Creates acetyl CoA
    - This is a high energy compound that will eventually donate the 2 carbons into the Kreb's cycle for further oxidation
        - Gets more electrons for the electron transport chain
- 1 NADH is made for each pyruvate
- Regulation: PDH allosteric inhib/activation
- In review
    - Happens in mitochondrial matrix
    - It occurs to make acetyl-CoA
    - Regulated by PDH allosteric inhibition and activation
    - Pyruvate goes in, 2 acetyl-CoA and 2 NADH comes out

Stage 3: Citric acid cycle

- Metabolic cycle (regenerative)
- AKA Kreb's cycle, TCA cycle
- Acetyl is removed from Acetyl-CoA and attached to oxaloacetate to form citrate
- Series of steps releases $2CO_2$, 1NTP, 3NADH, and 1 $FADH_2$
    - NADH and $FADH_2$ provide reducing power in stage 4
    - NTP is a nucleotide triphosphate
        - Can be ATP, GTP, etc.
- Oxaloacetate is regenerated to start the cycle again
- In review:
    - Happens in the mitochondrial matrix
    - It occurs to make more NADH and $FADH_2$, regenerate oxaloacetate, further reduce pyruvate (now pyruvate is in the form of acetyl-CoA)

        - Also has anapleurotic roles
        - Intermediates are siphoned off for other reactions in the cell (like making cytoskeletons, protein biosynthesis, etc.)
    - Regulated by mostly by PDH, but oxaloacetate and acetyl-CoA levels as well
        - Oxaloacetate is also competitively inhibited by an intermediate later in the cycle
    - Acetyl-CoA and oxaloacetate goes in, $2CO_2$, 1NTP, 3NADH, and 1 $FADH_2$ come out
        - Also know that oxaloacetate turns into citrate

Stage 4: Oxidative phosphorylation (ETC)

- High energy electrons removed from NADH and $FADH_2$ to make ATP
    - This is where you get the energy back
- Typically requires oxygen
    - O ultimately receives the electrons from NADH and $FADH_2$
- Oxidative process involves electron transport chain
- Phosphorylation occurs by ATP synthase
    - Phosphorylation of ADP into ATP
- ETC is made up of transmembrane proteins and carriers
    - All redox
    - Electrons move from protein to protein
        - Electrons come from NADH and $FADH_2$
    - This provides the energy that moves H^+ into the inner membrane space
        - Against their electrochemical gradient
        - With NADH you're moving 3 H^+ while with $FADH_2$ you only move 2, more energy recovery with NADH
    - This produces the H^+ electrochemical gradient that powers ATP synthase
        - ATP synthase is a turbine that spins as the H^+ moves down its electrochemical gradient and that kinetic energy is used to phosphorylate ADP into ATP
    - Cytochrome oxidase is the last one that reduces oxygen
- In some plants electron transport can become uncoupled from H^+ movement and the energy that that would normally move the protons instead is dissipated as heat
    - In some animals this happens in brown fat
- In review:
    - Occurs in the inner membrane and inner membrane space
    - It occurs to make the H^+ gradient that powers ATP synthase, ultimately to make ATP
    - Regulated by the amount of NADH and $FADH_2$ and the maintenance of the H^+ gradient
    - NADH and $FADH_2$ in, large quantities of ATP and water out

Free Energy Change

- Movement from NADH to O_2 is a very negative free energy change
    - Spontaneous in forward direction
    - Very few electrons move backwards
- Highly exergonic
- Some energy used to pump H^+ across inner mitochondrial membrane and create H^+ electrochemical gradient

ATP Synthase

- An enzyme that harnesses free energy as H^+ flow through membrane embedded region
- Energy conversion: H^+ electrochemical gradient or proton motive force converted to chemical bond energy in ATP
- Racker and Stoeckenius confirmed used of an H^+ electrochemical gradient
- Rotary machine that makes ATP as it spins
    - Watch http://www.youtube.com/watch?v=uOoHKCMAUMc

Yoshida & Kinosita Demonstrate that the y-subunit of the ATP Synthase Spins

- Masasuke Yoshida, Kazuhiko Kinosita, and colleagues set out to experimentally visualize the rotary nature of the ATP synthase
- Released membrane embedded portion and adhered it to a slide
- Visualize y-subunit using fluorescence
    - Made fluorescent actin filaments that attached to the y-subunit
- Added ATP to make reaction run backward
- Rotated counterclockwise to hydrolyze ATP
    - Rotate clockwise to synthesize ATP
- Many organisms depend on nutrients other than glucose
    - Products of protein and lipid catabolism enter same metabolic pathways as glucose (efficient)
    - Amino acids are deaminated
    - The above enter the pathway as intermediates

Anaerobic Metabolism

- 2 strategies
    - Use substance other than O_2 as final electron acceptor in electron transport chain
    - If confined to not using O_2, carry out glycolysis only (doesn't need O_2 to proceed yet still produces ATP)
        - Pyruvate converted to lactate (muscles) or ethanol (yeast, plants)
        - Fermentation "produces" far less ATP
- Pyruvate can turn into lactate or ethanol
    - Lactate is not lactic acid
    - The real point of this is to create NAD^+ to feed back into glycolysis
        - Necessary or glycolysis will shut down
- Acedaldehyde is what gives you a hangover
    - AcDH breaks it down
    - Many Asians lack this mutation and it leads to flushing when drinking
- Aerobic → around 35 ATP via glycolysis + ETC about 50% of total energy recovered
- Anaerobic → around 2 ATP via glycolysis only about 4% of total energy recovered
- Anaerobic is FAR less efficient

Secondary Metabolism

- Primary: essential for cell structure and function
- Synthesis of metabolites that are not necessary for cell structure and growth
- Secondary metabolites unique to a species/group
- Roles in defence, attraction, protection, competition
    - Antibiotics, antimicrobials, pheromones, colour compounds, etc.

4 Categories of secondary metabolites

- Phenolics (Antioxidants with intense flavors and smells)
    - Some are linked to increased longevity in some vertebrate species
    - Resveratrol may be involved in this, also has some antioxidant capabilities
- Alkaloids (Bitter-tasting molecules for defense)
    - Also spicy, capsaicin molecules
    - Atropine is a nerve gase
    - Cocaine, nicotine, morphine, and caffeine are also alkaloids
- Terpenoids (Intense smells and colors)
    - Phytoecdysones
        - Structurally similar to insect molting hormones
        - Disrupts molting and development, often lethal
- Polyketides (Chemical weapons)
    - Antibiotics

Chapter 8: Photosynthesis

Trophic Organization

- Heterotroph
  - Must consume organic molecules from their environment to sustain life
- Autotroph
  - Make organic molecules from inorganic sources
    - Photoautotroph
      - Use light as a source of energy
      - Changes this energy into potential energy by making chemical bonds
      - Green plants, algae, cyanobacteria

Photosynthesis

- Energy within light is captured and used to synthesize carbohydrates
- CO_2+ H_2O + light energy $\rightarrow C_6H_{12}O_6 + O_2$
  - Oxygen is just a by-product
  - It's important to us, but not from the plants point of view
- CO_2 is reduced
- H_2O is oxidized
- Energy from light drives this endergonic reaction

Chloroplast Anatomy

- Outer and inner membrane
  - Intermembrane space
- Thylakoid membrane contains pigment molecules
  - This is where photosynthesis is taking place
  - Some of them are connected to the inner membrane and some of them are only connected to other granal stacks
    - Probably isolated from the inner membrane but there is no concrete evidence either way
- Enclose thylakoid lumen
  - In the thylakoid lumen is where the proton motive force is maintained to drive ATP synthesis
- Granum: stack of thylakoids
- Stroma: fluid filled region between thylakoid membrane and inner membrane
  - Where the Calvin cycle takes place

2 Stages of Photosynthesis

- Light reactions
  - Take place in thylakoid membranes
    - Membrane integrity is absolutely crucial (things like fluidity, scavenging of free radicals, etc.)
  - Produce ATP, NADPH and O_2
- Calvin cycle (carbon fixation)
  - "Dark" reactions

        - This is a misnomer, the light reactions take place in the light because they NEED it
        - The Calvin cycle still occurs in the light
    - Occurs in stroma
    - Uses ATP and NADPH to incorporate CO_2 into organic molecules

Light Energy

- In the visible spectrum
    - Red and blue
- Higher light energy = shorter wavelength
- Absorption boosts electrons to higher energy levels
- Photosynthetic pigments absorb some light energy and reflect others
    - Leaves are green because they reflect green wavelengths
- Wavelength of light that a pigment absorbs depends on the amount of energy needed to boost an electron (from ground state)to a higher orbital (excited state)

Excited States Unstable

- Can release energy: heat or light
- Can transfer energy (captured)
- Cause reaction (photochemistry)

Pigments Responsible for Absorbing Light

- Chlorophyll a
- Chlorophyll b
    - Both chlorophylls have Mg in them
    - Therefore low Mg and mutations to enzymes that incorporate Mg into chlorophylls are bad for plants
- Carotenoids
    - Help absorb light and channel them to chlorophylls
- Hydrophobicity helps to anchor them in the membrane
- Structure of them is what facilitates light absorption

Absorption vs. Action Spectrum

- Absorption spectrum
    - Wavelengths that are absorbed by different pigments in the plant
- Action spectrum
    - Rate of photosynthesis by whole plant at specific wavelengths

Photosystems

- Mechanisms of the light reactions that are responsible for energy capture and providing electrons for electron transport chain
- Figure 8.11 in Brooker text

Photosystem II (PSII)

- 2 main components

- Light-harvesting complex or antenna complex
    - Directly absorbs photons
    - Energy transferred via resonance energy transfer
- Reaction center
    - P680 → $P680^*$
    - Relatively unstable
    - Transfers its electron to primary electron acceptor
    - Removes electrons from water to replace oxidized P680
        - Oxidation of water yields oxygen gas
        - Also creates 2 H^+ in the thylakoid lumen

PhotosystemI (PSI)

- Key role to make NADPH
- Light striking light-harvesting complex of PSI transfers energy to a reaction center (P700)
- High energy electron removed from P700 and transferred to a primary electron acceptor ($NADP^+$ reductase)
    - Electron originally from PSII
- $NADP^+$ reductase
    - $NADP^+$ + 2 electrons + H^+ → NADPH
    - This occurs on the thylakoid membrane but on the side facing the stroma
        - This means that H^+ is used here lowering overall [H^+] in the stroma
- $P700^+$ replaces its electrons from plastocyanin
    - No splitting water, no oxygen gas formed
- All this movement of H^+ into the lumen and using H^+ in the stroma created the proton gradient necessary to drive ATP synthase
    - H^+ moves out of the thylakoid lumen (down its electrochemical gradient) into the stroma
    - Movement through ATP synthase drives ATP production

Cyclic and noncyclic electron flow

- Noncyclic
    - Electrons begin at PSII (water) and eventually transfer to NADPH
    - Linear process produces ATP and NADPH in equal amounts
- Cyclic photophosphorylation
    - Electron cycling releases energy to transport H^+ into lumen driving synthesis of ATP
    - When P700 gets excited it doesn't send electrons to $NADP^+$ reductase
    - Instead it cycles it back in the pathway to pump more electrons into the thylakoid lumen
        - Electrons go to the electron transport protein that feeds electrons to cytochrome complex
    - This creates more ATP (no NADH)

Calvin-Bensen Cycle

- ATP and NADPH used to make carbohydrates
- CO_2 incorporated into carbohydrates
    - Stomata are where CO_2 enters the plant
        - Stomata are also a major source of water loss in plants

    - Precursors to all organic molecules, storage
    - Very endergonic reaction which is why you need ATP or NADPH
- Need 18 ATP + 12 NADPH for every 6 CO_2
- Glucose is not directly made

3 Phases

1. Carbon fixation
    a. CO_2 incorporated in RuBP using rubisco
        i. Via covalent bonds
        ii. Rubisco is an enzyme and catalyzes CO_2 incorporation into RuBP
    b. 6 carbon intermediate splits into two 3PG
2. Reduction and carbohydrate production
    a. ATP is used to convert 3PG into 1,3-bisphosphoglycerate
    b. NADPH electrons reduce 1,3-bisphosphoglycerate to G3P
    c. 6 CO_2 → 12 G3P
        i. 2 for carbohydrates
        ii. 10 for regeneration
3. Regeneration of RuBP
    a. 10 G3P converted into 6 RuBP using 6 ATP

- Regulated mostly by the availability of ATP and NADPH
    - Also regulated by CO_2 levels
    - ATP and NADPH production is regulated by light so mostly regulated by light

Variations in Calvin Cycle

- Certain environmental conditions can influence both the efficiency and way the Calvin cycle works
    - Light intensity
        - Impacts carbon fixation reactions because it drives the production of ATP and NADPH
    - Temperature
        - Increased temperature generally increases rates of reactions, can also affect the properties of the enzymes and the fluidity of the thylakoid membrane
        - Also increases the levels of free radical scavengers
    - Water availability
        - Plants are forced to close stomatal pores, means less CO_2 gets in

Photorespiration

- RuBP+ CO_2 → 2 3PG
    - Rubisco functions as a carboxylase
        - Attaches CO_2 to an organic molecule
    - C_3 plants make 3PG
- Rubisco can also be an oxygenase
    - Adds O_2 to RuBP eventually releasing CO_2
        - The product of this is not as useful, cannot go into the Calvin Cycle
- Photorespiration

- Products of photorespiration needs to go through many organelles to be able to go into the Calvin cycle (energy is also necessary)
- This energy comes from photosynthesis
    - Using O_2 and liberating CO_2 is wasteful
- Not really sure what the role of photorespiration is but there are three hypothesis
    - Artefact of evolution
        - Rubisco probably evolved in an oxygen poor environment so there wasn't a huge selective pressure to be very selective between O_2 and CO_2
    - May be a safety valve
        - Gets rid of excess energy coming into the system
    - May play a role in nitrogen assimilation
        - Not too much evidence to support this
- More likely in hot and dry environment
- Favoured when CO_2 low and O_2 high

C_4 Plants

- C_4 plants make a 4-carbon compound in the first step of carbon fixation
- Hatch-Slack pathway: spatial segregation
    - More energy required for carbon fixation
    - As such they need to grow in higher light, higher water, higher temp, environments
- Leaves have 2-cell layer organization
    - Fixation and Calvin cycle happen in two different compartments
    - Called Krantz anatomy
- Mesophyll cells
    - Carbon fixation
    - CO_2 enters via stomata and 4-C compound (oxaloacetate) formed via PEP carboxylase(no affinity for O_2 = does not promote photorespiration)
        - Only C_3 plants use rubisco for initial carbon fixation
        - PEP carboxylase is much better (doesn't have a problem distinguishing CO_2 and O_2)
        - Oxaloacetate is turned into malate before going to BSC
- Bundle-sheath cells (BSC)
    - Calvin cycle
    - Malate transferred to BSC and is turned into pyruvate
        - Releases CO_2
    - This occurs right in front of rubisco
        - So rubisco never really gets a chance to bind O_2 instead of CO_2
- In warm, dry climates C_4 plants have the advantage in conserving water and preventing photorespiration
    - Have lower CO_2 demands
- In cooler climates, C_3 plants use less energy to fix CO_2
    - 90% of plants are C_3

CAM Plants

- Some C_4 plants separate processes using time
- Crassulacean Acid Metabolism
- CAM plants open their stomata at night

    - CO_2 is only available at night, goes through C_4 pathway of carbon fixation
        - PEP carboxylase is shut down during the day and activated at night to avoid futile cycling
        - Done at a post-translational level
    - This also prevents a lot of water loss (stomata are closed during the hottest time of day)
- CO_2 enters and is converted to malate(C4)
    - Stored as malate
- Stomata close during the day to conserve water
- Malate broken down into CO_2 to drive Calvin cycle
- Temporal segregation

Chapter 9: Communication

Efficient Communication is Vital

- Cells need to communicate with each other, coordinate responses (e.g. development)
- Need to respond to changing environment
- Communication within cells

Regulation and Orchestration

- Cell communication or cell signalling involves incoming and outgoing signals
- Signals are agents that influence the properties of cells,
- Perception of signal required for response
    - Eg. yeast must have glucose receptors so that it knows to start creating metabolic enzymes, create glucose transporters,e tc.
    - Eg. phototropism, the phenomenon whereby plants grow towards light
        - Plants need to be able to sense the signal (light) and respond to it (bend towards it)
        - Done by phototropins (respond predominantly to blue light) which triggers an enzymatic cascade that causes asymmetric growth on the shaded side to bend the plant

Signals Relayed Between Cells

1. Direct intercellular signalling
    a. Cell junctions allow signalling molecules to pass from one cell to another (heart muscle)
    b. Physical connections between two cells with a passageway connecting them
    c. This is a rapid method of signalling
        i. In animals they are called gap junctions
        ii. In plant cells they are plasmodesmata
2. Contact-dependent signalling
    a. Some molecules are bound to the surface of cells and serve as signals to cell coming in contact with them (e.g. nerve-muscle contact)
    b. Not physically connected by a portal, rather the two surfaces are interacting with each other and that signals processes within each respective cell
3. Autocrine signalling
    a. Cells secrete signalling molecules that bind to their own cell surface or neighbouring cells of the same type (e.g. monocytes in immune system, growth factors)
    b. Auto means that it also stimulates itself
4. Paracrine signalling
    a. Signal does not affect cell secreting the signal but influences cells in close proximity(eg. synaptic signalling)
    b. Synaptic signalling would be like one neuron signalling another
        i. The neuron that is producing the signal is not responding to that signal
5. Endocrine signalling
    a. Signals (hormones) travel long distances and are usually longer lasting

- Most signalling molecules are held at low concentrations
    - This allows for large responses to relatively small changes in signalling molecules

Stages of Cell Signalling

1. Receptor activation
    a. Signalling molecule (ligand) binds to receptor
        i. Causes a conformational change that activates it
2. Signal transduction
    a. Activated receptor stimulates sequence of changes - signal transduction pathway
        i. This is a cascade of events that eventually leads to the cellular response
        ii. There has to be physical interaction between the elements in the cascade (at least to detect the conformational change in receptor)
3. Cellular response
    a. Several different responses
        i. Alter activity of 1 or more enzymes
        ii. Alter structural protein function
        iii. Change gene expression

Ligand

- Signaling molecule
- Binds noncovalently to receptor with high degree of specificity/affinity
    - Reversible binding
    - K_d is a measure of the affinity of the ligand to its receptor (dissociation constant)
        - Low K_d means high affinity (same concept as K_m)
- Binding and release between receptor and ligand relatively rapid
- Ligands alter receptor structure-conformational change (activation/inhibition)
- Catalytic receptors have both a K_d (ligand binding to extracellular domain) and a K_m (intracellular, catalytic domain)

Cell Surface Receptors

1. Enzyme-linked receptors (catalytic receptors)
    - Found in all living species
    - Extracellular domain binds signal
    - Causes intracellular domain to become a functional catalyst
    - Most are protein kinases
        - Usually phosphorylate proteins to initiate the cascade
2. G-protein coupled receptors (GPCR)
    - Common in eukaryotes
    - Protein contains 7 transmembrane segments
        - Loop through the membrane
    - Activated receptor binds to G protein
    - The G protein releases GDP and binds GTP instead
        - Replacing GDP with GTP causes the two G protein subunits (active α subunit and β/y dimer) to dissociate from one another
    - Active α subunit and β/y dimer interact with other proteins in a signalling pathway
    - Once the signalling molecule disassociates with the receptor, the active α subunit hydrolyzes GTP back into GDP and the two subunits reassociate to reform the G protein
    - The G protein itself is not catalytic
        - When the two subunits disassociate however, the α subunit becomes catalytic

3. Ligand-gated ion channels
    - Plant and animal cells
    - Ligand binding causes ion channels to open and ions to flow through the ion channels through the membrane
    - Animals- signals between nerve and muscle cells, between 2 nerve cells, Ca^{2+} uptake

Intracellular receptors

- Most receptors on the plasma membrane but some are inside the cell
- Estrogen example (mammalian hormone)
    - Passes through membrane (small, lipophillic molecule) and binds to receptor in nucleus
    - Dimer of estrogen-receptor complexes binds to DNA to activate transcription of specific genes
        - Transcription factors regulate transcription of specific genes
- Auxin example (plant hormone)
    - Able to diffuse into the cell but not out of it
        - It becomes charged when it enters the cell
    - Binding of auxin in the cytosol activates TIR1
    - Gene regulation not direct
        - Genes are being inhibited by inhibitory proteins (negative regulators)
        - TIR1 causes breakdown of inhibitory proteins
    - With gene inhibition relieved, genes are transcribed

Cellular response

- Two-component regulatory system
    - Found in bacteria, fungi and plants (not animals)
- Sensor kinase
    - Enzyme-linked receptor
    - Hydrolyzes ATP and phosphorylates
    - Phosphate group transferred to response regulator
- Response regulator
    - Regulates expression of many genes
- Example is NarQ/ NarL
    - Two-component regulatory system
    - Senses presence of nitrate (NO_3^-) and nitrite(NO_2^-)
        - Does this by binding to NarQ (catalytic receptor) which phosphorylates itself
        - To phosphorylate itself it uses ATP
    - NarQ then transfers its phosphate to NarL (response regulator) via physical contact
        - Once phosphorylated, NarL is in its active state
    - NarL activates genes involved in nitrate and nitrite metabolism and transport
    - You can shut this system down if the nitrate or nitrite disassociates from NarQ (K_d) or you could also dephosphorylate NarL (via phosphatases)
- Look at Fig. 9.11 in Brooker text about Epidermal Growth Factor (EGF)
    - This is a more complex system and has a greater potential for regulation
    - EGF can be endocrine or autocrine, it helps to trigger cell division
    - Starts with the EGF receptor (EGFR) binding EGF
        - Phosphorlyates itself
    - This initiates a signal cascade that eventually activates the G protein Ras

        - Done by exchanging GDP for GTP
    - This initiates a phosphor-relay of protein kinases
        - Goes from one kinase to the next, each phosphorylating the next one
    - Eventually the signal reaches the nucleus and affects transcription factors

Second messengers

- Signals binding to cell surface are first messenger
- Many signal transduction pathways lead to production of second messengers
    - These allow for the amplification and transduction of the signal
- Examples
    - cAMP
    - Ca^{2+}
    - Diacylglycerol and inositol triphosphate (IP3)

Signal transduction via cAMP

- Cyclic adenosine monophosphate
- Signal binding to GPCR activates G protein to bind GTP causing dissociation
- One type of subunit binds to adenylyl cyclase stimulating synthesis of cAMP
    - This is the α subunit and it binds to adenylyl cyclase to promote the synthesis of cAMP from ATP
- One effect of cAMP is to activate protein kinase A (PKA)
    - Binds to the regulatory subunits of the tetrameric PKA
    - When cAMP binds to the regulatory subunits they disassociate from the catalytic subunits and PKA catalytic subunits becomes active
- Activated catalytic PKA subunits phosphorylates specific cellular proteins
- When signalling molecules no longer produced, eventually effects of PKA reversed
    - There is also an enzyme called PDE (phospohodiesterase) that breaks down cAMP, so cAMP levels can be regulated this way as well
    - Protein phosphatases could also be activated to inactivate the final stages

cAMP has 2 Advantages

1. Signal amplification - binding of signal to single receptor can cause the synthesis of many cAMP that activate PKA, each PKA can phosphorylate many proteins
2. Speed - in one experiment a substantial amount of cAMP was made within 20 seconds after addition of signal

Signal transduction via Ca^{2+}

- Cells maintain a very large Ca^{2+} gradient
- 2 types of calcium pumps
- When calcium channels open, influx of Ca^{2+} acts as a second messenger
- Plants - phototropism, opening and closing of stomata, gravitropism, stress response
- Animals - nerve transmission, muscle contraction, secretion of digestive enzymes
- See fig. 9.17 in Brooker text
- Ca^{2+} is sequestered in the ER
    - In order for it to become active in the cytosol it has to go through channels in the ER membrane

    - Often these channels are ligand gated with the ligand being other second messengers (eg. IP_3)
        - Triggered in a G protein coupled receptor cascade
        - A subunit dissociates, activates phospholipase C
        - This releases IP_3 and DAG
        - DAG activates PKC (protein kinase C)
    - Once channels are open Ca^{2+} activates different enzymes (eg. calmodulin, PKC, etc.)
- Ca^{2+} signatures
    - Where do you get specificity if you just release Ca^{2+} as if it was a switch?
    - There is a spatial and temporal context (and magnitude context) for Ca^{2+} release
    - Spatial
        - Ca^{2+} channels only open on a certain area of the ER
    - Pulses of Ca^{2+} release can act like a sort of Morse code to Ca^{2+} sensors

Epinephrine (adrenaline)

- Different effects throughout the body
- Heart muscle cells
    - Contraction controlled by Ca^{2+}
    - Ca^{2+} stored in sarcoplasmic reticulum (specialized ER)
    - Ca^{2+} binds to troponin leading to muscle contraction
    - Dropping Ca^{2+} causes muscle to relax
        - Phospholamban activates Ca^{2+} pump that moves it back into the sarcoplasmic reticulum
- By changing the proteome in different cells/tissues you can get different effects from the same second messenger
- Skeletal muscle cells
    - Epinephrine activates GPCR which activates adenylyl cyclase
    - cAMP levels rise and activates PKA
    - PKA activates phosphorylase kinase and inactivates glycogen synthase
    - Phosphorylase kinase activates glycogen phosphorylase
        - This stimulates glycogen breakdown
    - Glycogen synthase becomes inactive
        - Glycogen synthesis is inhibited
- Epinephrine increases heart rate
- Activates adenylyl cyclase which produces cAMP
- cAMP activates PKA to phosphorylate troponin and phospholamban
- Enhances troponin Ca^{2+} binding and increases rate of contraction
- Enhances Ca^{2+} pump function and increases rate of relaxation

A cell's response to signalling molecules depends on the proteins it makes (proteome)

- One hormone causes different effects in different cell types
- Differential gene expression - all cells contain the same genome but only express particular parts
- Can affect cellular response in a variety of ways
    - Receptor not expressed, different receptors for same signal, different affinities for signal, signal transduction pathways different, protein expression different

Apoptosis

- Programmed cell death (PCD)
    - Old cells, infected cells, unnecessary cells
- Cell shrinks and forms rounder shape
    - Due to destruction of nucleus and cytoskeleton
- Plasma membrane forms blebs
    - Blebs are irregular extensions that break away

Kerr, Wyllie, and Currie found that hormone signals can initiate apoptosis

- ACTH known to increase number of adrenal cortex cells
- Prednisone suppresses ACTH synthesis and decrease number of adrenal cortex cells
- Even in control samples, researchers observed apoptosis
- In prednisolone-treated rates, the rate was higher
- Results indicate tissues decrease cell number and that cell death was induced, in this case, by a hormone
- Coined the term apoptosis

Chapter 10: Nucleic Acid Structure and DNA Replication

Genetic material

- Must be able to replicate itself
  - Must be able to do this at high fidelity (high accuracy)
- Must be able to control living processes

HISTORY OF DNA

- Meitscher 1869 – isolated a phosphate containing acid from the nucleus (which they could see). He called it nuclein.
- Griffith 1928 – used bacteria and mice to demonstrate presence of DNA
  - First mouse – injected with pneumococcus bacteria with a polysaccharide coating (smooth). Mouse died because the sugar coating prevented immune system from killing it.
  - Second mouse – injected with same bacteria without sugar coating (rough). The mouse lived because the immune system killed the bacteria.
  - Third mouse – injected with heat treated smooth bacteria. The mouse lived because the bacteria were killed by the heat.
  - Fourth mouse – injected with heat treated smooth and regular rough. The mouse died even though both alone didn't kill the mouse.
    - Somehow (they didn't know at that point) the bacteria was able to transform from the rough type into the smooth type
  - CONCLUSION: We now know that **DNA** survived the heat treating, allowing it to enter the rough bacteria, encode for the polysaccharide coating, and thrive in the mouse, killing it.
- Hershey-Chase 1952 – used viruses to determine DNA components
  - At this point they knew that DNA existed, but they didn't know what its purpose was
  - They hypothesized that it was for information transfer so they did two experiments
  - In the first, labelled viral casing with sulfur isotope
  - In the second, labelled DNA with a phosphorus isotope
  - In both experiments they:
    - Allowed the virus to infiltrate the bacteria
    - Blended the bacteria to stop further reaction
    - Used a centrifuge to separate the solution (supernatant) from the bacteria (pellet)
  - In the first experiment they found that only the supernatant had the radioactive sulfur
    - Therefore no sulfur was transferred from the virus to the bacteria
    - This meant that the casing of the virus was not being transferred
  - In the second experiment they found that the pellet contained the radioactive phosphorus and the supernatant did not
    - Therefore the phosphorus was being transferred from the virus to the bacteria
    - This meant that the DNA from the virus was being transferred to the bacteria

    - Transforming principal (genetic material) was the DNA
- Chargaff 1930s/40s – showed that adenine is in the same quantity as thymine and that guanine was in the same quantity as cytosine.
    - Discovered Chargaff's Rules
        - Adenine binds with thymine
        - Cytosine binds with guanine
- Franklin and Wilkins 1940-1953 – showed through x-ray diffraction that DNA is linear and in a helical formation.
    - Knew it was helical because the x ray diffraction pattern was an X
- Watson and Crick showed that it is a **double** helix.

DNA STRUCTURE – like a ladder

- Ladder uprights – alternating sugar (deoxyribose) and phosphates. Phosphodiester bonds hold these componants together.
- Ladder rungs – nitrogenous bases. A paired with T, C paired with G. the two base pairs are held together with hydrogen bonds.
    - A and G have double cyclic structure (purines)
    - C and T have single cyclic structure (pyrimidines)
        - Uracil replaces T in RNA
- Two sides of the ladder are antiparallel. One side goes from 5' – 3', the other goes from 3' – 5'. (determined by the numbering of the carbons in the sugar).
- Figure 10.8, 10.11, and 10.12 in the Brooker text
    - From figure 10.12 note the minor and major grooves

DNA REPLICATION

- Central dogma of molecular biology involves
    - Replication
        - DNA replicating itself
    - Transcription
        - Process of transcribing DNA into RNA (mRNA)
    - Translation
        - Translating the mRNA transcript into a protein
- Three types of DNA replication were initially hypothesized:
    - Semiconservative – each daughter strand contains a parent strand and new strand.
    - Conservative – produces a brand new daughter strand containing no parental material.
    - Dispersive – daughter strands contain parental material dispersed throughout it.
    - See figure 10.13 in Brooker text
- Meselsohn and Stahl found that DNA uses **SEMICONSERVATIVE** replication
    - Bacteria take up any isotope of N to make DNA
    - Used ^{15}N (radioactive N) instead of ^{14}N (normal N) to make heavy (radioactive) DNA in bacteria for several generations

        - Did this by growing cells in ^{15}N environment for several generations
    - They then provided the bacteria with ^{14}N containing medium in later generations of growth
        - Grew them for less than 1 generation, 2, 3, etc.
    - DNA was centrifuged with CsCl
        - During centrifugation CsCl makes a density gradient
    - The DNA moves to a region of same density
        - This means that you can tell the density of DNA based on its position in the tube
        - Called isopycnic (same density) centrifugation
    - Found that as generations progressed the DNA had different densities (different isotopes of N were being incorporated)
    - The particular pattern of density change over generations could only be explained by semiconservative replication hypothesis
    - See figure 10.14 in Brooker text
- See figure 10.16 in Brooker text
    - Bidirectional replication, single origin replication, multiple origin replication
- Replication starts at a predetermined place on the DNA (at a particular sequence)
    - This location is called the origin of replication
- Helicase unwinds the DNA (moving 5' to 3')
    - When it is being unwound it is held open by single strand binding proteins
        - This ensures that the strands do not come back together and form a double helix
    - Replication is then free to begin at the replication fork
    - Sometimes there is the problem of excessive coiling
        - Opening double helix causes increased coiling of DNA further downstream
        - Topoisomerases cuts the sugar backbone of DNA to allow uncoiling and then rejoins that region of the DNA
    - See figure 10.18 in Brooker text
- DNA primase adds an RNA primer
- DNA polymerase III makes DNA from RNA primers and then adds nucleotides to the 3' end, according to the parental strand template.
    - DNA polymerase can only begin working on a double strand so that is why the RNA primers are added first
    - Remember A-T, C-G
- Replication occurs from 5' to 3'
    - Because of the antiparallel quality of the strands, one strand is elongates towards the fork (the leading strand), and the other elongates away from the fork (lagging strand).
- Because the fork is opening in one direction, the lagging strand has to replicate in small fragments (Okazaki fragments)
    - This takes more time because the lagging strand grows discontinuously (hence the name lagging).

    - When the DNA polymerase III reaches the RNA primer on the lagging strand it DNA polymerase I comes and replaces the RNA with DNA
        - DNA ligase then attaches and forms phosphodiester bonds
- See figure 10.20 in Brooker text
- Telomeres are stretches of repeated DNA at the end of chromosomes that don't code for anything. They prevent shortening of the chromosome by giving a site for the RNA primer required to close up the zipper.
- There is a section on the 3' end that has no complimentary strand and therefore cannot have a RNA primer attach upstream.
    - See figure 10.23 in Brooker text
- Telomerase attaches these repeated sequences to the ends of chromosomes.
    - Prevents chromosome shortening
- Telomerase lengthens the overhang with the same repeating sequence.
    - RNA primer can now be attached and fill in some of the overhang.
- See figure 10.24 in Brooker text
- Body cells have a predetermined life span
    - Skin sample grown in a dish will double a finite number of times
        - Infants about 80 times
        - Older person 10-20 times
    - Senescent cells have lost the capacity to divide
- Cancerous cells tend to have a lot of telomerase allowing a prolonged cell life, and increased replication capabilities.
    - Prevents telomere shortening and may play a role in continued growth of cancer cells

Chapter 11 – Genetic Expression at the Molecular level

- Genes specify protein structure
- Garrod came up with the idea that enzyme damage or failure was the reason for the failure of metabolic processes
    - The underlying cause of this is often genetic
    - He showed this with alkaptonuria (black urine) caused by a buildup of homogentisic acid
    - Demonstrated that when homogentisic acid oxidase was lost or damaged, alkaptonuria resulted
- Beadle and Tatum, working off of Garrods ideas, came up with the one gene- one enzyme hypothesis
- Using neurospora (bread mold) as a model organism, they broke down the pathway to the mold producing arginine
- They did this by plating UV irradiated mold with minimal medium and then sequentially different growth media, and noted the growth of each plate
- UV irradiation produced mutants that needed certain precursor molecules to grow (wild-type didn't)
    - The question was wan an enzyme missing?
- Analysis of metabolic mutants revealed multiple enzymes that lead to arginine synthesis in a serial manner
    - Different stages of loss of function could be identified
        - Each associated with only one enzyme
- One gene – one enzyme hypothesis is that one gene coded for one unique enzyme
- See figure 11.2 in Brooker text

TRANSCRIPTION

- Transcription and translation occurs in the cytoplasm of prokaryotes. (figure 11.3 in Brooker text)
    - This means that they can produce the protein very quickly in response for the need for that protein
    - May provide a mechanism to explain the fast growth of prokaryotic cells
- In eukaryotes transcription occurs in the nucleus
- Transcription takes DNA, and turns it into messenger RNA (mRNA)
    - Very similar process to replication
        - Moves in a 5'-3' direction (nucleotides being added onto the 3' end),
        - Phosphodiester bonds still bond the nucleotides together
- Some differences between replication and transcription:
    - Thymine isn't added to adenine. It is replaced by Uracil. The other pairs are the same
        - So A-U while G-C
        - A-U is 2 H bonds
        - G-C is 3 H bonds

    - RNA polymerase reads the DNA strand instead of DNA polymerase (intuitive – we want an RNA strand, NOT DNA).
        - RNA strand that is made is complementary to the DNA strand it's created from

Language of Genes

- 4 nucleiotides in DNA and 4 in RNA (U replaces T)
    - All of these need to be able to account for the 20 known amino acids
- There must be a triplet code system
    - 3 bases/amino acid then 64 (4^3) amino acids could be coded
    - Any more would be a waste
- The coding unit is called a codon (3 nucleotides)

Flow of Coding information

- String of amino acids has a direct relationship to nucleotide bases in RNA and DNA
    - Each sequence of 3 nucleotides encodes a specific amino acid (codons)
    - Each of the 64 possible codons are used
        - Some amino acids are encoded by multiple different codons, some codons are start codons, some are stop codons, etc.

Process of transcription

- The sigma factor is a protein that recognizes the promoter region, then binds to the RNA polymerase so it knows where to start transcribing
    - Promotor region is where transcription starts
    - Sigma factor recognizes this region and RNA polymerase binds to the sigma factor
    - After binding the DNA is unwound to form the open complex
- After the open complex is formed the sigma factor is release and RNA polymerase begins to create the mRNA
    - NO RNA PRIMER necessary
    - As the RNA polymerase moves down the DNA (5'-3') the two strands re-associate with each other behind it
- When it reaches the terminal sequence , the new mRNA and RNA polymerase simply dissociates.
- See figure 11.5 in Brooker text
- Nucleotide triphosphates are added to the growing strand at the 3' end
- Phosphodiester bonds are made by DNA dependent RNA polymerases
- The two strands of DNA are referred to as the template strand and the coding strand
    - Template strand is noncoding strand; the other is the coding strand
    - Template strand is what is complementary to the mRNA
    - The other is the coding strand it has the DNA sequence equivalent to that of the mRNA (same nucleotide sequence)
    - Note that both DNA strands can be template strands depending on what protein or mRNA you are referring to

        - See figure 11.6 in Brooker text
- Note the antiparallel, complementary strand

Gene

- A GENE is a nucleotide sequence that carries the information needed to produce a specific RNA or protein product
- Genes consists of:
    - Introns - non-coding regions of the gene
    - Exons - protein coding regions.
- Gilbert 1980s - By splicing out different combinations of exons, you can code for different protein domains, which makes different proteins
    - Allows for very quick evolution of proteins
    - This process is called "exon shuffling".
- During transcription, a cap of 7-methylguanosine is added to the 5' end of the growing mRNA
    - Recognized by cap binding proteins that enable it to be translated
- After transcription, a poly-A tail (100-200 adenines) is added, and the RNA transcript is moved out of the nucleus and into the cytoplasm.
    - Not coded, added enzymatically
    - In eukaryotes, causes mRNA to be more stable
        - In bacteria it causes it to be more unstable, the reason for this is unknown
    - See figure 11.10 in Brooker text
- **Spliceosomes –** small nuclear RNA and proteins (snRNPs) that bind to specific sites on the intron, bind to eachother, and cut out the intron, all the while connecting the 2 exons together.
    - Results in a mRNA transcript that has no introns
    - See figure 11.9 in Brooker text

TRANSLATION

- Translation is the production of protein from a mRNA template
- Occurs in the ribosomes
    - mRNA and tRNA
        - tRNAs bring the individual amino acids (AAs)
        - mRNA is the code for the string of AAs
- Ribosomes are proteins with 2 subunits (small and large), creating a groove. This groove is where the mRNA moves through. Within this groove there are 3 sites:
    - A site **a**ccepts an aminoacyl tRNA. This tRNA has an amino acid attached that is not yet attached to the rest of the growing protein.
    - P site receives the **p**olypeptide bearing tRNA. The protein is connected to the rest of the protein (polypeptide chain).
    - E site is where the tRNA **e**xits the ribosome
- See figure 11.17 in Brooker text
- There are 4 steps in translation

- Activation of the tRNAs
    - Formation of the aminoacyl-tRNA
        - Simple refers to attaching the appropriate AA to the right tRNA
- Initiation of the translation process
    - Starts process of protein production
    - Binding of large and small ribosomal subunits to the mRNA
    - In eukaryotes there is no ribosomal binding sequence
        - Use cap binding proteins to facilitate ribosome binding
    - See figure 11.20 in Brooker text
- Elongation
    - The continued addition of amino acids to the growing polypeptide chain
    - Aminoacyl tRNA enters the A site and binds to codon
        - Complementary anticodon
        - Elongation factors use GTP to facilitate this
    - Peptidyl transfer reaction
        - Aminoacyl tRNA moves from A to P
        - Amino acid is added to the polypeptide chain
            - tRNA is now called peptidyl RNA
        - Carried out by peptidyl transferase
            - Catalytic rRNA (ribosomal RNA)
    - RNA translocates one codon towards the 3’ end
        - Releases uncharged tRNA from E site
- Termination
    - The end of translation; release of protein
    - Occurs when the ribosome encounters a stop codon
    - Ribosome comes to a stop codon
        - No tRNA, release factor binds to the A site instead
    - Bond between peptidyl tRNA and polypeptide is hydrolyzed
        - Causes release of tRNA and polypeptide from ribosome
    - Ribosomal subunits, mRNA, and release factor dissociate

Chapter 12 - GENE REGULATION

Eukaryotic Gene Regulation

- Complex regulation
  - Genes encode single proteins
- Gene expression can change under different conditions
- Gene expression can change depending on stage of development (temporal) and tissue as well
- Genes that code for proteins that are always required and thus readily available in the cell at all times are called **constitutive genes**.
  - Always transcribed (eg. glycolytic enzymes)
- For most genes this is not the case therefore many genes are regulated
  - This means that they are turned on or off depending on the needs of the cell

Lactose metabolism in *E. coli*

- Jacob and Monod 1961 – discovered the regulation of genes using the *lac* operon in *E. coli*
- Lac → Glu + Gal
  - Accomplished using the enzyme β-galactosidase
- After the above, Gal is converted to more Glu for glycolysis
- Jacob and Monod grew colonies of *E. coli* on different media and looked at β-galactosidase concentrations
  - Found that when grown on glucose there was little β-galactosidase
  - When grown on lactose however there was a lot of β-galactosidase
    - Also an increase in galactose permease and galactoside transacetylase
- Operons are collections of genes on the same chromosome that are controlled by one set of regulatory sequences.
- Jacob and Monod described the *lac* operon
  - Gene system that regulates lactose metabolism

Lac operon

- The lac operon controls the breakdown of lactose.
  - There are 6 genes, 3 of which code for different proteins involved in lactose metabolism and one of which codes for a repressor protein
    - LacI makes the lac repressor
    - LacP is the promoter region
    - LacO is the operator region
    - LacZ makes beta-galactosidase
    - LacY makes galactose permease
    - LacA makes galactoside transacetylase
  - When lactose is absent from the environment allolactose is not made and the lac repressor binds to the lac operator which inhibits transcription
  - LacZ, lacY, and lacA are the structural genes

    - The operator is a sequence of nucleotides that provides a binding site for a repressor protein
    - The CAP site is a DNA sequence recognized by an activator protein
    - ipromotor is the promotor region for the lacl gene only
        - Lac promotor is the promotor for the *lac* operon
    - See figure 12.7 in Brooker text
- Lac operon enzymes
- B-galactosidase converts lactose into galactose and glucose
    - Side reaction that produces allolactose
- Lactose permease allows lactose entry into the cell
- Galactosidase transacetylase modifies lactose and analogues
    - May prevent toxic build up
- Regulation of the lac operon
- Controlled by the *lac* repressor
    - *lac* repressor is composed of 4 subunits
- Each possesses an allosteric site for allolactose
    - When allolactose is bound to all 4 subunits the repressor can bind to the operator
- This prevents RNA polymerase from transcribing the *lac* operon
- Trp operon is negative control
    - Brooker text p. 274 and 275
- <u>Positive regulation</u> – activator promotes gene expression.
    - As glucose concentration decreases in the E coli, there is an increase in cyclic AMP because of decreased metabolic activity to restore it. The cAMP binds to CAP proteins (catabolite gene activator protein). The now activated CAP can bind to DNA and increase transcription of the lac operon
        - It would want to activate the lac operon because the metabolism of lactose creates glucose
        - Binds to the cap site to increase transcription
        - See fig 12.10 in Brooker text

Multiple genes compensate for dosage problems

- 1 gene may not be enough so you would need multiple copies of genes
- Tandemly repeated gene sequences occur one after another on chromosomes
    - Also selective gene replication in some cells
    - Gene amplification
        - Gene sequences are repeated on the chromosome so there can be multiple transcriptions occurring simultaneously, increasing efficiency.
- Gene amplification in tumours
    - Her2 in tumours of breast cancer

Parts of a Gene

- Structural genes code for enzymes and related proteins
- Promoters are transcriptional start site – self explanatory – the site on the gene where transcription begins.
    - Often is a sequence of Py_2CAPy_5
    - Py_2 means 2 pyrimidines, C is cytosine, A is adenine, Py_5 means 5 pyrimidines
- TATA box is a site within the transcriptional start site that determines the exact point of transcription initiation.
    - Guides the RNA polymerase to the right spot
    - TATA box and transcriptional starte site form the core promoter
    - Core promotor by itself results in low level basal transcription
    - About 25 base pairs upstream
- Response elements (specific DNA sequences) are sites that affect the initiation of transcription. The number of these determines the relative strength of promotion.
    - Commonly these are 50-300 base paires (b.p.) upstream (towards the 5' end) of the transcriptional start site
    - They are recognized by regulatory proteins that control the initiation of transcription
    - Also known as regulatory elements
        - Enhancer and silencers
- See figure 12.13 in Brooker text

Preinitiation Complex

- Comprised of:
    - RNA polymerase
    - 5 different general transcription factors (GTFs)
    - GTFs and RNA polymerase come together at the core promotor
- GTFs must combine with RNA polymerase for transcription to begin
    - This occurs at the core promotor
    - GTFs and RNA polymerase together at the TATA box are called the **basal transcription apparatus**
    - Activators can bind to GTFs
    - Repressors can bind to it to inhibit the same function.
- Mediators (composed of multiple proteins) partially wrap around the basal transcription apparatus
    - Mediates interactions with activators or repressors of gene activity
        - Activators increase gene activity, repressors the opposite
    - Controls the rate at which RNA polymerase can begin transcription
    - Most do not bind directly to RNA polymerase
        - GTFs DO but these others do not

Activators, Repressors, and Transcription Factors

- Control of RNA polymerase can be accomplished via activators and/or repressors

    - Activators enhance RNA polymerase activity
    - Repressors suppress RNA polymerase activity
    - Often these bind to and influence the function of GTFs
- An example of an activator:
    - Activator binds to the response element
        - Activator must come in first and is the trigger for the rest
    - This binding enhances the ability of the GTF transcription factor II D (TFIID) to bind to the TATA box
        - TFIID helps to initiate the assembly of the preinitiation complex
    - With the activator helping TFIID bind to the TATA box, preinitiation complex formation is enhanced
        - This in turn enhances transcription of that particular gene
    - Repressors can inhibit TFIID
- The opposite of the above would be a repressor

Mediators

- This is another way to control RNA polymerase
- Mediators bind to the preinitiation complex
- When the mediator is bound different activators or repressors can act on it to influence RNA polymerase activity
    - Activators stimulate the function of a mediator by allowing faster initiation
    - Repressors inhibit the mediator so RNA polymerase cannot progress to elongation (delay initiation)
- Activators and repressors bind first to regulatory elements away from the gene and the DNA bends so that the activator/repressor can interact with the mediator

Gene accessibility

- DNA is associated with proteins in the nucleus to form compact chromatin
    - When DNA is arranged in this fashion it is in a closed conformation
- Chromatin packing affects gene expression
    - When the DNA is tightly packed in the closed conformation (as chromatin) it is difficult or impossible for transcription to occur
    - This is because the RNA polymerase simply cannot access it
- Access to the DNA is allowed in the loosely packed open conformation
- At any given time, certain portions of chromosomes are in the open or closed conformation
- Some activators can help decrease the amount of DNA compaction near a gene
    - Activator proteins are recruited
    - Histone acetyltransferase attaches acetyl groups to histone proteins so they don't bind DNA as tightly
        - Histones are proteins that DNA is wrapped around (and bound to) to help compact it

    - ATP dependent chromatin remodelling enzymes also help loosen DNA compaction
    - Some activators bind to enhancer regions that help to decrease compaction
- Therefore the third way to control RNA polymerase is to recruit proteins that influence DNA packing

Example – Steroid hormone

- Some transcription factors respond to steroid hormones
    - Activators are actually steroid receptors
    - These steroid (lipid) hormones are examples of small effector molecules
    - Travel through plasma membrane and bind to receptors
        - Exposes nuclear localization signals
    - Once in the nucleus they dimerize and bind to an enhancer region to activate transcription

Example continued – Glucocorticoid

- Can increase transcription of specific genes
- It is released into the bloodstream after eating a meal
- It is then transported into the cell by a transporter protein and binds to glucocoticoid receptors
- This binding releases proteins called chaperones and exposes nuclear localization signals (NLSs) on the receptors
    - Chaperones keep the receptors in the right conformation
    - These NLSs direct the receptors to the nucleus where they travel through the nuclear pore
        - Two of these receptors together form a dimer that travels through the pore
- The dimer then binds to two adjacent glucocorticoid response elements (GREs) next to particular genes
    - GREs are enhancers therefore they increase transcription of the adjacent gene

Transcription Factor Motifs

- Transcription factor proteins contain domains with specific functions
    - They can bind specific sequences of DNA
- Motifs are domains or portions of domains with similar structures in different proteins
    - Alpha helix – a double helix recognizing feature.
    - Zinc fingers – recognize DNA sequences in the major groove.
- See figure 12.18 in Brooker text
    - Helix-turn helix motif
    - Helix-loop-helix motif
    - Zinc finger motif
    - Leucine zipper motif

Translational control - example

- See figure 12.20 in Brooker text
- Double stranded region of pre-miRNA (micro RNA) or pre-siRNA (silencing RNA) is cut by a dicer protein and released as a 22 b.p. long RNA
- A single stranded miRNA/siRNA binds to a protein called the RISC (RNA induced silencing complex)
    - Binds to a way so that the nucleotide bases are exposed to solution
- RISC binds to a mRNA
    - Binding is due to complementarity with miRNA/siRNA within the RISC
    - If siRNA the mRNA is degraded due to high complementarity
    - If miRNA transcription is only inhibited (due to low complementarity)
    - Complementarity refers to the matching of the base pairs
- The above was termed RNA interference (RNAi) by Fire and Mello

DNA methylation

- Methylation – addition of a methyl group to a carbon to prevent transcription
- Accomplished by DNA methylase which attaches methyl groups (CH_3) to cytosine
- Usually inhibits transcription
- Islands of CpG (cytosines and guanines with phosphodiester bonds) found near promotors determine whether or not the gene is activated or repressed by methylation
    - If the islands are methylated then the gene is repressed
    - If the islands are unmethylated then the gene is active
- Methylation inhibits expression in two ways
    - Methylation prevents binding of activators to UPEs and enhancers
    - It also functions to tighten up the chromatin packing into the closed formation by recruiting proteins that condense the chromatin (pg. 281 in Brooker text)

Control of development

- Developmental Genes known as **Homeotic Genes** control formation of structures during development
- Within these genes are homeoboxes which are 180 base pair sequences
- Homeoboxes code for a protein region called a homeodomain that binds to DNA and affects transcription.
    - Homeodomain is 60 amino acids that form 4 helices
- Homeotic genes such as Antennapedia and bithorax are often called Hox genes.
    - Hox genes are normally clustered together
    - Mutating these genes can cause abnormal development (like legs instead of antennae in *Drosophila*).
    - Spatial arrangement of hox genes correlates to the body layout of the organism.
- Homeotic mutations can result in one set of cells encoding for an entirely different set
    - Eg. legs expressed where antennae should be

    - So cells that would normally turn into one type of tissue are instead turned into something different
- The order of arrangement of hox genes reflects spatial organization of the animal and the order of activation during development
- Hox genes are seen in many different types of organisms both segmented and nonsegmented
    - May have started off in early organisms as just a single set of hox genes
    - Over evolutionary time the hox genes were duplicated and underwent mutations to form the diversity of structure that we see in living organisms today
        - More complex organisms have more sets of hox genes (eg. mice have more than flies) but this is not always the case
        - All hox genes share many characteristics and this is why we think they all come from some sort of progenitor species (like a sponge)
- Evolution of the regulation of genes is just as important as the sequence of the genes themselves
- See pg. 418-420 in Brooker text

Translational controls

- Regulate translation of mRNA into protein
    - Usually relate to ribosomal affinity for the mRNA
- ENZYMES ARE AFFECTED DIRECTLY
- Feedback (end-product) inhibition
    - Product of the pathway feeds back onto elements earlier in the pathway to inhibit them
- Phosphorylation
- Other enzyme modifications (eg. glycosylation)
    - Phosphorylation and other modifications can activate or deactivate proteins
- Proteolytic processing
    - Chopping up proteins to make them into mature forms
    - Eg. proinsulin is produced from the gene (end product of transcription/translation) and its active form (insulin) is created when a portion of it is cleaved off
- Selective degradation
    - Some proteins are stable and some are broken down
        - Relates to turning processes on/off

Biological complexity and the sizes of genomes and proteomes

- Alternative splicing of the pre-mRNA can increase the proteome size without increasing the total number of genes
    - Pre-mRNA is the RNA transcript that is created directly from transcription (contains both introns and exons)
    - See figure 12.19 and figure 19.10 in Brooker text
- For organisms to become more complex (like higher plants and animals) evolution has produced more complex proteomes

- General trend is that less complex organisms tend to have fewer genes
- Frequency of alternative splicing increases with increasing biological complexity

Chapter 13 – Mutation, DNA Repair, and Cancer

- Mutations are any heritable change in genetic material (DNA)
    - Can be brought about by mistakes during replication and cell division
        - Also by chemical treatments (mutagens)
        - Mutagens that cause cancer are called carcinogens
- Essential to the continuity of life
    - Mutations that cause changes that can be selected for/against have the potential to drive natural selection in a species, eventually causing speciation.
    - As such they are the source of variation for natural selection
    - New mutations are usually more harmful than helpful.
- There are three classes of mutations: chromosomal, point mutations, and frameshift
- Chromosomal Mutations
    - Deletion or addition of a DNA segment
    - Breakage
    - Effects:
        - One to multiple proteins lost
        - Often these mutations are very serious and fatal
    - Example is Fragile X syndrome
        - Mutation of addition of CGG base repeats causes methylation which organizes the X chromosome into a closed conformation
        - Large pieces of DNA have changed
    - Example is Klinefelter Syndrome
        - Two X chromosomes
        - Individual looks like a male, can be treated with testosterone patches
        - Normally these individuals are sterile and develop breasts
- Point Mutations – one nucleotide is incorrect – changes up to one amino acid within the protein. Usually less serious.
    - Silent/neutral – no effect because of redundancy in the genetic code (3^{rd} position wobble)
        - Eg. AAG (lysine) to AAA (also lysine)
    - Missence – amino acid changes
        - Eg. AAG (lysine) to AAC (asparagine)
    - Nonsence – stops amino acid change by changing amino acid to a stop codon
        - Eg. AAG (lysine) to UAG (stop codon)
    - See Table 13.1 in Brooker text
    - Example is Sickle cell anemia
        - Missense mutation causes red blood cells to be concave rather than circular
            - Results in RBCs being unable to carry as much O_2 and makes it harder for them to fit through blood vessels
        - Gives them a heterozygous advantage against malaria

- If they have one of each type of gene they have an advantage for battling malaria

- Frameshift Mutation – a shift in the reading frame away from the original alignment
  - Where the gene begins to be read (starting point) is very important because the data in genes are read in groups of three bases at a time (codons)
  - Removal or addition of 1-2 bases substantially changes the output
  - Example sequence (not DNA) THEMANCANRUNNOW
    - The codons would be read THE MAN CAN RUN NOW
    - Imagine an addition of one nucleotide THE**A**MANCANRUNNOW
      - Now reads THE AMA NCA NRU NNO W
    - Imagine a deletion of one nucleotide (deleted in red) THEM**A**NCANRUNNOW
      - Now reads THE MNC ANR UNN OW

Gene mutations outside the coding sequences

- Within the promoter – changes the strength of promotion, affecting transcription levels.
  - Up and down promoter mutations
- Transcriptional response element/operator site – changes the regulation in transcription.
- Within splice junctions – affect proper splicing of introns.
  - Mutations at the boundaries between introns and exons can prevent proper splicing
- Within Translational Response Elements – change the regulation of translation

Genetics of Hemophilia

- Hemophilia are X linked (on the X chromosome)
  - This means that if you a male (XY) you automatically display the disease state
- Restriction enzymes
  - Bacterial origin but they have been isolated from bacteria
  - They are used to cut DNA at very specific sites
  - You can use them to cut normal DNA right where you want
    - If it doesn't cut that means that that sequence of DNA isn't there
- Single nucleotide polymorphisms (SNPs)
  - Refers to one nucleotide that is different in a certain proportion of a population
- Biallelic (2 forms of alleles or gene types)
- DNA repeats – Microsatillite repeats
  - Sequences of DNA that repeat over and over
- SNPs, biallelic, and DNA repeat mutations all lead to introns mutations or incorrect splicing in hemophilia
- Two types of haemophilia
  - Type A is missing factor 8
  - Type B is missing factor 9
- Understand from the pedigree that there must be 2 different point mutations

Mutations

- How do mutations occur?
    - Pre-existing circumstances? (caused by selection pressure)
        - Eg. Does exposure to a certain set of circumstances trigger mutation?
    - Unplanned random events
- Ester and Joshua Lederberg tested this (Figure 13.2 in Brooker text)
    - Only rare mutated cells survived selection
        - The same pattern of colonies survived on each plate
    - Therefore **mutations are random events**

Germ-line or somatic cell mutations

- Timing and location of a mutation are critical to the:
    - Severity of effect
    - Ability to pass on the mutation
        - Mutations that occur in the germ line (gamete producing cells), or the gametes themselves are transmitted across generations
            - Mutation is a part of the entire zygote
        - Mutations in the somatic line are not passed on
- Germ line cells give rise to gametes
    - Mutations can occur in a sperm or egg cell or in the cells that give rise to sperm or eggs
- Somatic cells are all other body cells
    - Mutations can occur early or late in development
        - Genetic mosaic results from patches of mutated tissue
            - Only a certain segment of your body possesses the mutation
- See figure 13.3 in Brooker text

Spontaneous or induced mutations

- Spontaneous mutations are random occurrences
    - Occur once in a million genes.
    - Varies between species and between genes
- Induced mutations are brought on by exposure to environmental agents
    - Rate of mutation is higher than the spontaneous mutation rate
    - Environmental agents include chemicals (benzopyrene found in cigarettes) or physical agents (UV for example) increasing the rate of mutation (mutagens)
- See Table 13.3 in Brooker text
- X and Gamma rays
    - Can cause base deletions, breaks in one or both DNA strands
- Ultraviolet (UV) rays can cause formation of thymine dimer causing gaps or incorporation of incorrect bases
    - This is when adjacent thymines bind to each other

- Figure 13.6 in Brooker text

Ames test

- Bruce Ames developed a test for determining if a chemical is a mutagen
- Uses *Salmonella typhimurium* strain that cannot synthesize histidine due to a point mutation
- One of two things could happen
  - Bacteria could die because they need histidine
  - Mutation occurs allowing synthesis of histidine
- The test monitors the rate at which reverting mutation occurs (back to wild type that can produce histidine)
  - Compares the growth of two plates; one that is a control and the other that contains the mutagen
  - More colonies surviving means that that substance is a mutagen
- See figure 13.7 in Brooker text

DNA REPAIR

- Direct Repair
  - Enzyme physically removes the modification (for example an alkyl group)
- Altered DNA strand is removed and new segment is synthesized
  - More common than direct repair
  - Nucleotide Excision Repair
    - Remove the damaged DNA; use the remaining template to form the removed complimentary strand. This if found in all eukaryotes and prokaryotes.
  - Methyl-directed mismatch repair
    - Corrects non-methylated daughter strand

Nucleotide excision repair (NER)

- Most common DNA repair system
- Region encompassing several nucleotides in the damaged strand is removed from the DNA
  - Region around the mutation is removed as well
- Intact undamaged strand is used as a template for resynthesis of a normal complementary strand
- Found in all eukaryotes and prokaryotes
- See figure 13.8 in Brooker text

NER and human genetic disease (Xeroderma pigmentosum)

- Photosensitivity because of an inability to repair UV induced lesions

CANCER

- Disease of multicellular organisms – uncontrolled cell division
  - Proliferation of cells or tissue where they shouldn't be proliferating
- Cancers originate from a single cell

    - That cell mutates so that the cell grows abnormally
    - Soon forms a tumour (an overgrowth of cells with no purpose)
        - Tumour may be benign or pre-cencerous
- Cancerous stages:
    - Malignant – lost normal growth regulation
    - Invasive – can invade healthy tissue
    - Metastatic – can migrate from its original location to other parts of the body
- Untreated malignant cells will cause the death of the organism
    - Get in the way of normally functioning cells
- See figure 13.16 in Brooker text

Cancer causes

- Most often caused by mutagens
    - Either physical (eg. UV light) or chemical
- A few viruses are known to cause cancer in plants, animals, and humans
    - An example is the Human papilloma virus
    - Some viruses may cause canver by modifying genes in the host cell
    - Others may carry oncogenes in the viral geneome

Cancer causing genes

- Oncogenes
    - When a mutation causes this gene to be overactive, uncontrolled cell growth occurs
- Tumour suppressing genes
    - Normally these genes encode proteins that prevent cancer
    - If a mutation eliminates this function then cancer can occur

Checkpoint protein (tumour suppressor)

- p53 is a G1 checkpoint protein (please refer to cell cycle notes) *p53* gene is induced when DNA is damaged
- When damaged the cell cannot progress from G1 to the S (DNA synthesis/replication) phase of the cell cycle
    - If DNA is severely damaged p53 prevents the cell from entering the S phase (in a normal cell)
    - When p53 is mutated or absent then the cell is allowed to progress to mitosis despite damage to the DNA
- About 50% of all human cancers are associated with defects in *p53* gene

Mutations in approximately 300 Human genes may promote cancer

- Not all of these mutant genes found in cancers directly affect the growth of cells

- They can provide some type of growth advantage for the cell population from which the cancer developed
    - For example, if a gene that makes the cell is able to acquire glucose better than other cells
- Over 1% of our genes have the potential to promote cancer if their function is altered by a mutation

Abnormalities in chromosome structure and number

- Common genetic change associated with cancer
    - If you think about it there are only a few genes that a cell needs to grow and proliferate so if only these ones are allowed to be transcribed there can be a lot more energy put towards them
- If you compare cells in normal and tumour tissues you find bizarre chromosomal abnormalities including:
    - Loss of tumour-suppressing genes
        - Possibly due to DNA compaction
    - Over expression because of extra chromosomes
    - Chimeric (mixed) genes; by translocations

Chapter 14 – Eukaryotic chromosomes, mitosis, and meiosis

Chromosome structure

- Chromosomes come in pairs – found by Sutton in 1903.
- Cells coil around histones so to be compact
    - 8 histones with attached DNA is called a nucleosome
    - Between nucleosomes are linker regions
        - Connected by another histone called H1
    - Nucleosomes form a zig-zag patterm
- The zig zag pattern of nucleosomes is arranged in radial loop domains
    - These are organized into heterochromatin
- The folding of heterochromatin gives chromosomes their characteristic structure
- See figure 10.11, figure 14.1, and figure 14.6 in Brooker text

Cell division

- In prokaryotes, there is simple fission, which creates identical daughter cells.
    - This is asexual reproduction
    - No mitotic spindle
    - Circular DNA molecules separate as the cell wall forms

Cell division in eukaryotes

- Eukaryotes use mitosis to produce identical daughter cells
    - Essentially this is asexual reproduction
- Complex processes involving cytoskeletal systems
- Linear DNA strands become separated by complex motor systems

Human chromosomes

- Somatic (body) cells have 2 sets of chromosomes that are homologous (identical)
    - The diploid chromosome number (number of chromosomes in the 2 sets) is 46 in humans
- In the sex cells (gametes) there is only one set of chromosomes
    - If a cell possesses only one set of chromosomes it is haploid
    - Haploid chromosome number in humans is 23 (half of the diploid 46)

Karyotyping

- Karyotyping is looking at stained chromosomes
    - Uses Giemsa stain
- Sample of blood is collected and treated with drugs that stimulate cell division
- Sample with drugs is then centrifuged
    - Supernatant is discarded

- Pellet is suspended in a hypotonic solution
    - Causes the cells to swell
- The sample is centrifuged a second time
    - This concentrates the cells
- Cells are suspended in a fixative and placed on a slide
    - This is when they are stained as well
- Slide is viewed with a light microscope
    - Often the microscope is attached to a computer so that the chromosomes can be photographed and arranged electronically on the screen
- Note for humans, 2 complete sets of chromosomes from a single cell make a karyotype of that cell
- See figure 14.9 in Brooker text

Cell cycle (briefly)

- See figure 14.10

Eukaryotic cell cycle

- G1 phase
    - Prior to cell division
    - Chromosomes exist as a single chromatid
- S phase
    - Chromosome replication
- G2 phase
    - Cell prepares to divide
    - Replicated chromosomes condense in preparation
    - Chromosomes exist as 2 chromatids joined together
- M phase
    - Mitosis
    - Chromosomes separate and 2 cells are formed during cytokinesis
    - After mitosis each daughter cell has one chromatid each
- End product is 2 daughter cells that have the same genetic complement as each other and as the mother cell
- See figure 14.11 in Brooker text

Mitosis: Early stages

- Interphase
    - Replication has already occurred
    - Centrosomes form from centrioles pairs
    - G1, S, and G2 phases
- Prophase
    - Sister chromatids condense

    - Spindle fibers start to form from the centrosomes, which are moving towards the pores
    - The nuclear membrane begins to breaks apart into vesicles
        - Doesn't disintegrate, these vesicles will eventually reform the nuclear membrane in daughter cells
- Prometaphase
    - Nuclear membrane is gone
    - The spindle fibers are fully formed
    - Sister chromatids attach to spindle via kinetochore microtubules
        - Spindle is the beginning of the mitotic spindle
- Metaphase
    - The chromatids align on the metaphase plate
        - Usually in the center of the cell
    - The centrioles have associated microtubules
        - Also has astral structure that anchors it the centrioles to the poles of the cell
- Mitotic spindle
    - Composed of astral microtubules, kinetochore microtubules (connect to chromosomes), and polar microtubules which start at the poles but don't connect to the chromosomes
    - Also composed of centrosomes
    - Centrioles are not found in plants
        - Other proteins make the poles function for separating DNA
- See figure 14.13 in Brooker text

Mitosis – Formation of daughter cells

- Anaphase
    - Sister chromatids split and move to each pole by the shortening of the kinetochore microtubules
    - The lengthening of the polar microtubules pushes the two poles of the cell apart
- Telophase and cytokinesis
    - Chromosomes decondense, and nuclear membranes reform (from vesicles)
    - Cell cleavage occurs, pinching the two cells apart
    - Actual separation of the cytoplasm into the two daughter cells is called cytokinesis

Animal Cell cytokinesis

- Cytokinesis is actin and myosin dependant
    - Actin and myosin are protein filaments
- Actin/myosin fibres pull like purse strings to nip the cells apart

Daughter cell production in plants

- There are no centrioles in the mitotic spindle
- Vesicles form out of the Golgi over the equatorial plate (metaphase plate)
    - They form a large vesicle and produce the new cell walls

  - Enzymes form the substances necessary for the cell walls
  - Remember that 2 cell walls form (one for each daughter cell)

Regulating cell division

- Cyclin dependant protein kinase regulation of cell division
  - It is first a G1 cyclin and cyclin dependant kinase (cdk) but the G1 cyclin eventually degrades when the cell enters the S phase
    - It was active when bound to G1 cyclin
    - Phosphorylated proteins that helped it get to S phase
  - Once it enters the M phase a mitotic cyclin binds and it becomes the active mitotic cyclin/cdk complex
    - Helps to initiate mitosis by phosphorylating different proteins
  - Eventually the M cyclin degrades as well
- See figure 13.13 in Brooker text
- Anaphase promoting complex (APC) is a regulatory enzyme
  - The APC cleaves bonds which hold the sister chromatids together
  - APC is held off until all the kinetochores are attached.

Sex and division

- Progeny are made when two parental DNA come together. In order to prepare for this, the DNA must go through **meiosis.**

Sex and diploidy (2n)

- Gametes = Sex cells (sperm and egg)
  - They have 1 set of chromosomes and are therefore haploid (1n)
- Gametes combine to form the zygote
  - One gamete is from the mother, the other from the father
  - This fusion creates the diploid (2n) zygote
    - In a 2n organism there are 2 homologous chromosomes of each chromosome type
    - Homologous chromosomes refers to matching chromosomes from the mother and the father

Sex provides greater variability

- Sex promotes diversity and variation allowing better adaptation to an environment
  - This is due to the mixing of the genes from the mother and father
  - Some of these mixes are more favourable than others
  - Even though asexual reproduction is energetically cheaper, the mixing gives you a greater advantage over evolutionary time
- 2n organisms do more than just separate their homologs (homologous chromosomes) during preparation of the sex cells

    - The homologs come together (synapsis)
    - They then form the synaptonemal complex in which there is breakage and crossover of genetic material between chromatids of the two homologs
- The resulting homologs (after breakage and crossover) are not the same as the parental homologs
    - They have a mixture of both parents genes
- It produces a sex cell that is somewhat different in its genetic complement from either parent
    - Not the same as the mother's homolog or the father's homolog
- So when two gametes join during fertilization the resulting zygote has a different genetic complement from either parent

First Meiotic Division

- There are two divisions to mitosis however the second is exactly the same as mitosis
- Prophase I
    - Occurs after pre-meiotic interphase
    - Homologous chromosomes synapse to form bivalents
        - This is when crossover occurs
    - Chromosomes condense and the nuclear membrane begins to break apart as vesicles
- The synaptonemal complex
    - Synaptonemal complex is two pairs of homologous chromosomes held together by various proteins
    - DNA is precisely cut and recombined while part of the synaptonemal complex
    - The synaptonemal complex holds the chromatids of the homologs close together until crossover is complete
        - The two homologs held together in this way are called a bivalent
    - See figure 15.16 in Brooker text
- Crossover and chiasmata
    - The region of crossover forms an X shaped structure
        - DNA is cut and then crosses over with the other chromosome
        - So the maternal and paternal chromosomes become attached and mix
    - This X shape is called a chiasma
- Prometaphase I
    - Nuclear membrane is gone
    - Bivalents become attached to kinetochore microtubules
- Metaphase I
    - Bivalents align along the metaphase plate
    - Before this phase the cell has 2 sets of DNA
- Anaphase I
    - Homologous chromosomes separate and are pulled towards poles.
- Telophase I
    - Nuclear membranes reforms

    - Chromosomes decondense
    - Cytokinesis occurs and results in two cells
- Meiosis II
    - Just like mitosis but with the chromosomes that were created in the first meiotic division
    - Also results in haploid (1n) sex cells called gametes
        - Mitosis results in the formation of two diploid cells
- See figure 14.17 in Brooker text
- Females make **ova** or eggs by oogenesis
    - In ova meiosis results in only 1 cell
    - This is because all the cytoplasmic reserves are put into one cell after the first meiotic division
        - The same thing happens after the second meiotic division
    - The end result is one large haploid egg cell
        - It also has huge amounts of nutrients init
- Males make sperm by spermatogenesis
    - In sperm meiosis creates 4 sperm
- Cells are now ready for fertilization
    - Fertilization is the fusion of the 1n gametes
    - Always results in a 2n zygote that develops into the embryo

Chapter 15 – Inheritance and Phenotype Determination

- Mendel's principles of inheritance
    - Unit characteristics are genes
    - Segregation is that different genes go into the gametes
    - Independent assortment means that genes assort independently of one another during meiosis
    - Dominance means that if a certain type of gene is present it dominates the other one

Why Study Mendel?

- Gregor Mendel was the first to demonstrate the principles of inheritance.
- Systematic scheme based upon an understanding of mathematics and statistics
    - He used excellent scientific method

Blending of characteristics

- Prior to Mendel people thought of inheritance like the concept of blending
    - So children would just be the average of their two parents
    - Breeders combined desired characteristics in crosses of domestic animals
    - Thought that characteristics added together like blending to form the new generation
    - If this were true, after a bunch of generations, we would be identical.

Gregor Mendel's discoveries

- Mendel applied the scientific method and mathematical analysis to show:
- Unit characteristics
    - Segregation of those characteristics
    - Dominance of some characteristics
    - Independent assortment of characteristics

Carefully chosen organism

- Worked with the garden pea *Pisum sativum*
- Great model organism
    - Lots of progeny (very productive)
        - Large sample sizes lead to strong statistics
    - Short life cycle
        - Don't have to wait years to get the progeny
    - Easily cross pollinated
        - Although typically self pollinating
        - Easy to go into the flower and snip off the anthers (male parts)
- It also has distinct phenotypic characteristics
    - Colour and shape of seeds
    - Colour of seeds

- Colour of pods
- Height

True Breeding Stock

- Mendel needed stocks of plants that bread in a reliable manner
  - Developed true breeding stock
    - These are plants that produce progeny that are just like the parents in regards to characteristics (so tall plants produce more tall plants, always)
- True breeding stocks were developed by inbreeding for several years
  - Inbreeding is when you breed the parent to progeny or sibling to sibling
- In other words parental type crossed with parental type creates parental type

Selected Crosses Produce Specific Progeny types

- Definitions
  - P is the parental generation of true breeders
  - F_1 is the 1^{st} filial generation
    - The progeny of crossing true-breeding P generation individuals that had varied in one character
  - F_2 represents the second filial generation
    - Derived from crossing the F_1 generation with itself

One of Mendel's Crosses

- Mendel compared what we call the phenotype
  - It is the outward expression of the genes
- Alleles are different varieties of genes
- Dominant alleles are shown with capital letters
  - In the following example T is dominant over t
- Recessive alleles are lower case letters
  - In the following example t is recessive to T
- Homozygous and heterozygous
  - Homozygous means the same alleles, heterozygous means different alleles
    - TT is homozygous dominant (two of the same dominant allele)
    - tt is homozygous recessive (two of the same recessive allele)
    - Tt is heterozygous (it's phenotype would be dominant or T)
- Crossed tall with dwarf and obtained all tall progeny in the F_1. Self crossing those to create the F_2, he obtained a ratio of 3:1 tall to dwarf.
  - In the F_1 he crossed true tall (TT) with true short (tt) and obtained all tall (Tt) progeny.
    - Because they all had a tall **phenotype**, it can be determined that the T (tall) genotype is dominant.
    - The tall genes overrided the short therefore that gene must be dominant
    - This is evidence that blending doesn't occur

    - Self crossing the F_1, the F_2 were 3:1 tall to short
        - This demonstrated that the F_1 did not breed true (did not create ONLY other tall plants)
            - From the P generation to the F_2 the parental phenotype disappeared and then reappeared
            - This again disproves blending
        - Mendel showed that the short plant bread true (created more short plants when breed with other short plants)
        - He then introduced the concept of unit characteristics (genes as we now know them)
- See Figure 15.5 in Brooker text

Chromosomal basis of allele segregation

- Allele segregation means that characteristics must separate into the gametes and are seen in the subsequent generation
- Characteristics must segregate (separate) into the gametes
- Segregation occurs in meiosis
    - Both in meiosis I and meiosis II
- Ova and sperm give different homolgous chromosomes to progeny
- See figure 15.10 in Brooker text

Mendel's concepts and modern terminology

- Mendel had NO IDEA of the nature of the genetic material
- What he called "characters" are the products of what we now call genes
- The collection of genes in an organism is the genotype or genome
- Genes are positioned at different loci (singular = locus)
    - This is the physical location of a gene on a chromosome

Alleles

- Alleles are varieties of genes
- In a 2n organism, there are two possible alleles for each gene locus
    - If those genes are the same the organism is **homozygous** at that locus
    - If different – **heterozygous.**

Punnett Squares

- Punnett Squares predict the genotypes and phenotypes of the next generation
- The allelic composition of the next generation (progeny) is obtained by adding the alleles of the two parents together
- Sometimes you're not sure if the dominant phenotype is genotypically homozygous dominant (TT) or heterozygous dominant (Tt)

    - So what you do is perform a testcross
    - This is when you cross the plant with the unknown genotype (but dominant phenotype) with a plant with a recessive phenotype
        - Recessive phenotype must be homozygous recessive (tt)
    - Depending on the phenotypic ratio of the progeny you can then determine what the parental genotype must have been
        - If all phenotypically tall then the parent must have been homozygous dominant
            - Subsequently all the progeny are heterozygous dominant
        - If half are tall and half short than the parent must have been heterozygous dominant
            - Subsequently the tall progeny are heterozygous dominant and the short are homozygous recessive
    - See figure 15.7 in Brooker text
- Two factor cross
    - When you are looking at more than one trait
    - Must take into account independent assortment!
        - Each gene assorts into the gametes independently of each other (not physically connected to each other)
        - See figure 15.8 in Brooker text

Product rule

- The probability that two or more independent events (phenotype of one does not affect phenotypes of others) will occur equals the product of their individual probabilities
- For example if you toss a coin twice what is the probability that you will get heads both times
    - Using the product rule you multiple the probability of each individual coin toss
        - ½ x ½ = ¼
        - Probability of each coin toss being heads is ½
- For two heterozygous parents, what is the probability that 3 children will be homozygous recessive
    - Using a Punnet Square you know that the probability of getting a homozygous recessive progeny is ¼
    - Using the product rule you multiply those probabilities
        - ¼ x ¼ x ¼ = 1/64

Sum Rule

- The probability that 1 of 2 or more mutually exclusive outcomes (“either/or” question) will occur is the sum of the probabilities of the possible outcomes
- In a cross between two heterozygous (Tt) pea plants, we may want to know the probability of a particular offspring being a homozygote
    - ¼ of the offspring will be homozygous dominant (TT) and ¼ will be homozygous recessive (Punnett Square)

  - So ¼ + ¼ = ½, half the offspring will be homozygotes (TT or tt)
- See pages 351-353 in Brooker text

Linkage

- Genes can be linked; eg. they are on the same chromosome or on the same piece of DNA
  - They are physically very close to one another on the DNA strand so when crossover occurs during meiosis it is unlikely that the genes become separated
  - This would mean that they would not follow independent assortment
  - Interferes with Mendelian frequencies
  - Linked genes are therefore easily detected
    - If the frequency distribution of genes in the progeny of a dihybrid cross is not Mendelian then the genes are linked
      - Dihybrid cross is a cross involving two traits (two factor cross)

Two-point testcross

- Sometimes a "+" sign will denote dominance
  - For example b^+ is dominant over b (b^+b is heterozygous dominant)
- Similar to a testcross, but it is used to determine the genotype of a parent (offspring from an F_1 generation) with two different genes (eg. b^+bc^+c)
  - It is accomplished the same way by crossing it with a homozygous recessive individual (also from the F_1 generation) at both genes (eg. bbcc)
- If unlinked (no linkage) then you would expect a ratio of 1:1:1:1
- If the ratio is not what you anticipate (eg. 2.7:2.7:1:1) the overabundance may be explained if the genes are linked
  - They are linked because you get an enrichment of the parental types (P generation, NOT F_1)

Crossover determines the relative location of genetic loci

- Crossover is assumed at equal frequency for each loci along a chromosome
- The number of crossovers is proportional to the distance between 2 loci
  - The further apart two loci are, the larger number of crossovers occur between them
  - The closer they are the smaller, this can lead to linkage
- Distances between loci are related to the rate of crossover
  - One percent cross over = one map unit
- See figure 15.18 in Brooker text for some examples of distances between loci on the fruit fly genes

Incomplete dominance

- Incomplete dominance is when the heterozygote has a 3rd, intermediate phenotype
  - So in our T/t example, Tt genotype would not produce a tall phenotype

    - Rather it would produce a medium phenotype
- Occurs when neither allele is dominant
- An example is the plant pink four o;clocks
    - 50% of normal protein does not give enough red colour
    - So individuals that are heterozygous are not red or white, they are pink
    - See figure 15.20 in Brooker text
- Human phenylketonuria (PKU)
    - Heterozygotes appear phenotypically normal but have double the normal levels of phenylalanine
        - This is because they have half the amount of phenylalanine hydroxylase (breaks down phenylalanine)

Multiple Alleles

- 3 or more variants in a population
- Phenotype depends on which 2 alleles are inherited
- An example is ABO blood types in humans
    - Blood type determines what antigens are expressed on the outside of your blood cells
    - Type AB is codominant (both alleles are expressed equally, you express both antigens equally)
        - See table 15.3 in Brooker text

Sex influenced inheritance

- Occurs when an allele is dominant in one sex but recessive in the other
- Increase in 5 alpha-dihydrotestosterone (by 5 alpha-reductase)
- Pattern baldness
    - Baldness allele dominant in men but not in women
    - Only a woman homozygous for baldness allele would be bald (or an adrenal gland tumor causing high testosterone in females)
    - NOT X LINKED

Role of environment

- “Norm of reaction”
    - Effects of environmental variation on a phenotype
- An example is genetically identical plants that grow to different heights in different temperatures
- People with phenylketonuria can develop normally if given a diet free of phenylalanine
    - If the diet has phenylalanine it accumulates and becomes toxic
    - They then suffer from the toxic effects
    - See figure 15.23 in Brooker text

Gene interactions

- A single trait is controlled by 2 or more genes, each of which has 2 or more alleles
- Epistatsis
    - Alleles of one gene mask the expression of the alleles of another
    - Often arises because 2 or more different proteins are involved in a single cellular function
- Polygenic inheritance
    - In humans, 3 unlinked loci
        - All three loci effect the phenotype
        - Different genotypes produce a wide range of phenotypes
    - Follows expected frequency distribution for a Mendelian trait
    - Height is also polygenic (in a normal population)
    - See figure 15.25 in Brooker text

Extranuclear inheritance

- Organelles (mitochondria and chloroplasts) contain their own genomes and can impart different phenotypes

Epigenetic inheritance

- Modification of either a gene or chromosome during egg/sperm formation or the early stages of embryo growth that alters gene expression in a way that is fixed during an individual's lifetime
    - For example if one of the parents gene is inactive during the entire life of the individual
- It permanently affects the phenotype of the individual, but the effect is not permanent over the course of many generations
    - This is because it doesn't actually change the DNA sequence
- X inactivation and Genomic imprinting

X inactivation

- One X chromosome in the somatic cells of female mammals is inactivated
    - If you have both of them functional there are too many gene products
- 2 lines of evidence
    - Barr bodies are found in female but not male cat cells
        - Barr bodies are the closed conformation of the X chromosome
    - Calico cat coat colour pattern
- Calico cat coat colour pattern
    - During embryonic development cells count their X inactivation centers (Xic)
    - The extra X is converted into a Barr body
    - In the early embryo the Xic may inactivate different X chromosomes in different cells
        - So X chromosome 1 might be active in some cells but in other X chromosome 2 is active
        - Depends on which X chromosome is condensed into the Barr Body by Xic

        - As development proceeds the pattern of X inactivation is maintained during subsequent cell divisions
        - This creates regions of the cat that are orange or black
    - If the cat is missing the Xic than both X chromosomes are active
        - This is lethal

Genomic imprinting

- Segment of DNA is imprinted
    - Occurs in numerous species of insects, plants, and mammals
- Imprinting can occur to single genes, parts of chromosomes, entire chromosomes, or all the chromosomes from one parent
    - Offspring distinguishes between maternally and paternally inherited chromosomes (or genes, parts of chromosomes, etc.)
    - **Offspring express either the maternal or paternal allele, but not both**
- Igf-2 gene example
    - IgF-2 gene is silenced (via methylation) than the offspring is normal
    - If is it not silenced than it is a dwarf
    - See figure 15.32 in Brooker text

Chapter 18 – Genetic Technology

Revolution in biological science

- Started in the mid 1970s with recombinant DNA technology
  - This is technology that redirects the genetic activity of organisms
- Techniques and approaches include
  - DNA cloning: isolating and amplifying specific DNA sequences, both *in vivo* (inside) and *in vitro* (out of) the cell
  - Genomics and Proteomics: analysis of entire genomes and proteomes of organisms
  - Biotechnology: addition of new genes to create transgenic or genetically modified organisms

Restriction enzymes

- Discovered by Hamilton Smith, Daniel Nathans, and Werner Arber
- Restriction enzymes are molecular scissors
- They are normally found in bacteria and function to destroy non-host (bacteriophage) DNA
  - A bacteriophage is a virus
- What they do is cut DNA at specific base pair sequences
  - These sequences are usually (not always) palindromes
    - Palindromes are the same sequence forward and backwards (eg. ABBA or RACECAR)
    - So in DNA the sequence would read the same 5' to 3' as it would 3' to 5'
    - Some examples of restriction enzymes are on table 18.1 in Brooker text
- Scientists purify them and use them to cut DNA at specific sequences so that they can then insert genes that they created into the DNA
- When they cut they leave sticky ends
  - These are the ends of the cut DNA
  - The restriction enzyme leaves little sequences of single stranded DNA when it cuts
    - Called 3' or 5' overhangs depending on what strand is single stranded
- Example of using a restriction enzyme (EcoRI) to insert a gene
  - EcoRI is added to vector and chromosomal DNA
    - Vector is a piece of DNA that is used to carry other DNA into a cell
    - Plasmids are circular self replicating DNA in bacteria
    - EcoRI recognizes the sequence GAATTC (complementary is CTTAAG) and cuts it
  - When it cuts it opens up the vector DNA and cuts the chromosomal DNA into many fragments
    - At all the sites that were cut there are sticky ends
  - You can then take your gene of interest from the fragments of chromosomal DNA and introduce it into the open vector
    - When they are introduced the bases of the sticky ends match up and hydrogen bond with each other
  - The gaps in the DNA backbone are then closed when DNA ligase is introduced
    - It closes the gaps by catalyzing the formation of covalent bonds in the DNA backbone
  - See figure 18.2 in Brooker text

Recombinant DNA technology

- Use of laboratory techniques to isolate and manipulate fragments of DNA
- Recombinant DNA contains DNA from 2 or more sources
    - Sources may both be from the same organism, just two different genes
- Once inside a host cell, recombinant molecules are replicated to produce identical copies or clones
- Why create gene clones?
    - Want copies of a gene for study or use
        - It creates large amounts of DNA, enough for DNA sequencing
        - It can also be used as a probe to ID the same or similar genes
    - Obtain lots of gene product (mRNA or protein)
        - Creates large amounts of the protein so it can be studied
        - Can make pharmaceutical products
        - Can be introduced into plants to alter their characteristics
        - Can be used to treat diseases (gene therapy)

Gene cloning

- Vector DNA is a carrier for the DNA segment to be cloned
- When a vector is introduced into a living cell, it can replicate making many copies
- Common vectors are plasmid or viral
    - Plasmids are circular DNA from bacteria
- Also need the gene of interest from chromosomal DNA digested with restriction enzymes

Vectors

- A vector is a carrier that delivers foreign DNA into a host cell
    - Once it gets in there it becomes function (genes work)
- Bacteria and yeast
    - Plasmids are small rings of double stranded DNA
        - Since they are double stranded you can use restriction enzymes on them
    - Viruses
- Mammalian cells
    - Engineered mammalian viruses
        - Viruses deliver genes into the cells
    - When engineering a virus you take out the deleterious things and use it just as a delivery system for your gene
- Plant cells
    - *Agrobacterium* Ti plasmid
        - Ti plasmid is very large
        - Has the ability to transfer DNA into plant genomes (only trans-kingdom gene transfer system known)

Steps to gene cloning

- Isolate vector DNA from the bacterial cell and the gene of interest from the chromosomal DNA
- Insert the gene of interest into the vector
    - This creates the recombinant vector
- Introduce the recombinant vector into a host cell that doesn't already have a vector
    - This can be done via viruses, restriction enzymes +ligases, etc.

- The host cell will copy the vector and divide to produce many cells
    - This increases the translation of the gene of interest
- See figure 18.1 in Brooker text
- The goal for the recombinant bacteria is to be taken up by the bacteria
    - You have to treat the bacteria to make them receptive
        - If you immerse the cell in a solution that is high in Ca than it makes the plasma membrane more permeable to large macromolecules like DNA
        - Electroporation is when you give the cells a short electric shock (also makes the membrane more permeable)
- Transformation
    - Plasmids with the desired genes are inserted into bacteria
    - The bacteria multiply, and when doing so, replicate the recombinant vector along with their normal DNA
    - You must first make the bacteria receptive
- Transfection
    - Viral vectors involve a transfection of DNA via a macrophage into the bacteria
- Once the DNA is in the bacteria, only the "infected" ones will grow in cultures containing ampicillin
    - This is because only the ones that have the vector with the gene for ampicillin resistance (*amp^R)* will be able to survive the ampicillin treatments
- By inserting the DNA into an area of the chromosome that codes for the lacZ gene (a coding gene for beta-galactosidase) it can be seen if the DNA was taken in or not
    - If the lacZ gene is cut up, the cell can't break down galactose
    - By adding X-Gal to the plate, the ones that form blue colonies are the ones coding for beta-galactosidase (showing that they didn't take up the DNA), and the white colonies broke up the lacZ gene (by adding the new DNA)
    - See figure 18.3 in Brooker text
- The lacZ mechanism works cooperatively with the *amp^R* marker because sometimes plasmids will recirculate and still have ampicillin resistance but not have the recombinant DNA
    - Acts as another selectable marker

DNA libraries

- DNA libraries are collections of small DNA fragments (recombinant vectors) obtained by cleavage via restriction enzymes.
    - Genomic libraries – fragments derived from chromosomal DNA
        - Put recombinant plasmid vectors into bacteria, plate colonies on ampicillin plate
        - Those that survive have the recombinant vector
        - See figure 18.4 in Brooker text
    - cDNA – obtained from mRNA using reverse transcriptase, forming the complimentary DNA.
        - Complementary DNA lacks introns so it is simpler to use

Blotting methods can identify gene families

- Southern blotting can detect the presence of a particular gene within a mixture of many chromosomal fragments separated on a gel
- Another common use of Southern blotting is to identify gene families
    - Two or more genes are derived from the same ancestral gene
- The members of a gene family are homologous having similar but not identical DNA sequences
    - Distinguish homologous members of a gene family within a single species or identify homologous genes among different species

Southern Blotting

- Start by performing a gel electrophoresis
    - Start by using restriction enzymes to cut DNA up into fragments then put it into an electrophoresis gel
        - The gel is made of agar or agarose
        - It is roughly rectangular and has wells (small holes) on one side
        - You place you DNA into the wells
        - Connect the gel to an anode (negative) at the well end and a cathode (positive) at the other end
    - When placed in the gel and you charge the gel it separates macromolecules based on charge and size
        - DNA is negative therefore it moves towards the positive electrode
        - Smaller fragments move faster and farther than larger fragments
    - Creates a gel that has distinct bands
        - Bands can be stained or probed after being transferred to a nitrocellulose filter
        - This filter acts like a negative of the gel
    - See figure 18.6 and 18.7 in Brooker text
- Southern blotting of a gel
    - Locations (bands) where the probe binds appear dark on X-ray film
    - High stringency Southern blotting
        - Detects close match between the probe and the chromosomal DNA
        - The sequences between the probe and what it's going to attach to will be almost identical
        - High temperature and/or low salt conditions
    - Low stringency Southern blotting
        - Detects homolgous genes
        - Low temperature and/or high salt conditions

Polymerase Chain Reaction (PCR)

- Copy DNA without vectors and host cells
- Goal is to make many copies of a defined DNA region
- Uses two primers complementary to sequences at the ends of the DNA region to be amplified, deoxynucleotide triphosphates (dNTPs), and heat stable form of DNA polymerase (*Taq* polymerase)

    - dNTPs are the building blocks of DNA and the *Taq* polymerase puts it all together
- *Taq* polymerase was isolated from DNA found in *Thermus aquaticus*
    - This is a bacteria that lives in geothermal vents (very high temperatures)
    - This means that *Taq* polymerase can survive in high temperatures without denaturing
- *Taq* polymerase is also very effective
    - 30 cycles produces a billion times the original amount of DNA
    - This is enough amplification to reveal the presence of a single copy of a specific target sequence

PCR steps

- Need to start with template DNA, the 4 nucleotides, and *Taq* polymerase
    - Also need the 2 different sequence primers that bind to the specific site that is to be amplified
- Start by adding all the ingredients and heating them up to split the DNA template into single strands
- Primer annealing
    - You must lower the temperature for the primer to bind to each strand of template DNA
    - Remember that the primers are necessary because polymerases (like *Taq* polymerase) need double stranded DNA to start working
- Incubate the sample at a lower temperature to allow the synthesis of the complementary strand
- Repeat the cycle to create more and more complementary strands
    - Because you have to reheat the DNA it is important that *Taq* polymerase can survive the heat of DNA denaturation so that you don't have to keep adding more
- See figure 18.8 in Brooker text for step by step of PCR

Uses of PCR

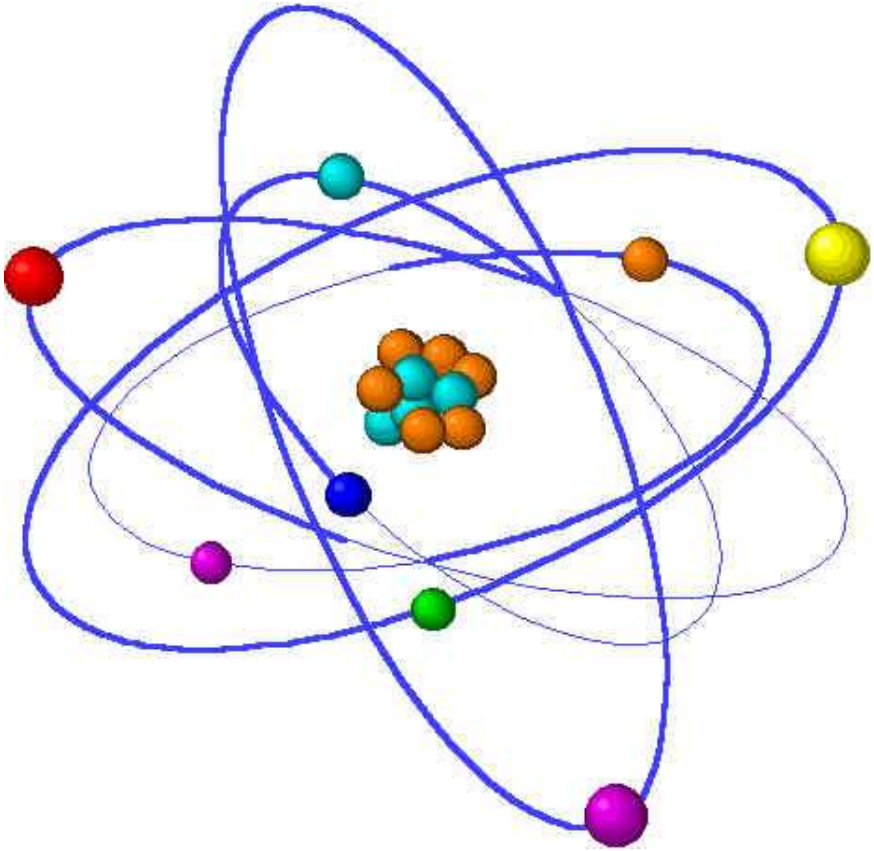
- Examine historical figure and extinct species
- Criminal investigations (DNA fingerprints) from blood or hair
    - Uses primers that amplify several fragments simultaneously
    - Each individuals DNA would prime at distinct locations giving them their own unique DNA fingerprint

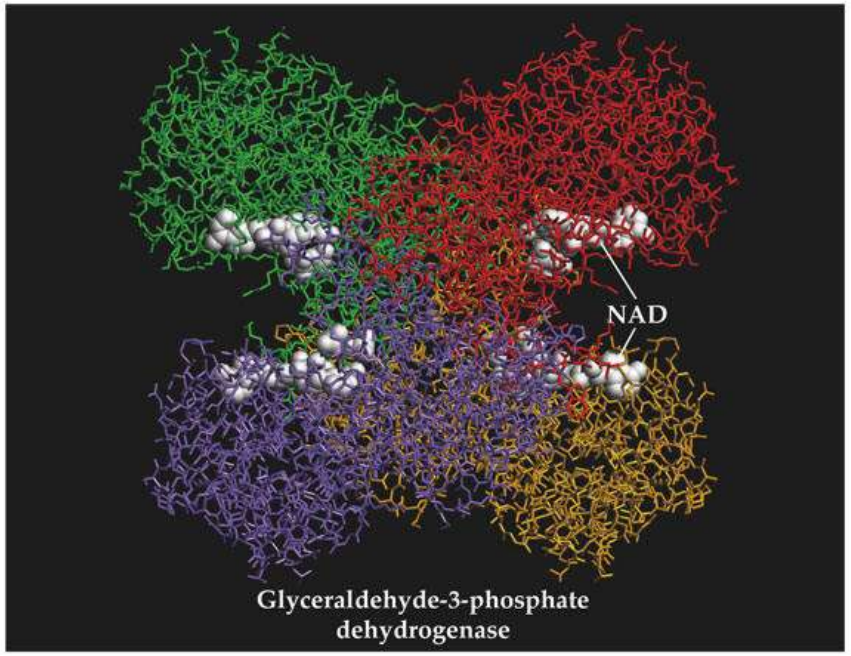
- Genomics and Proteomics – analyze the entire genome and proteome.
- Biotechnology – addition of genes, GMO's…
- DNA fingerprinting/DNA profiling – everyone's DNA is different, so they can use the individualistic properties to identify people, species, and PATERNITY.
- DNA Sequencing – Dideoxynucleoside triphosphates.
    - These are missing the the 3' OH group, which terminates the addition of any more adding on.
    - Take 4 test tubes each with a different ddNTP (A, T, G, C) and the rest of the dNTPs. This allowed the chains to grow to different lengths, but always ending with a predetermined nucleotide.

    - After they have grown, you run an electrophoresis and can determine the sequence of the DNA.
- Proteomics – the study of the proteins generated by the DNA. At any given time, a genome will only produce a fraction of the potential proteins.
    - 2D gel electrophoresis can separate proteins first by pH, then by size.
- Genetic Engineering – alter DNA of organisms to improve function of a system
    - Milk in goats by injecting DNA into fertilized egg cells.
- Molecular Pharming – introduce proteins to improve systems like milk production in cattle.
- Transgenic Plants are plants that have genes put into their somatic tissue, and with hormone therapy, alter the host chromosomes. This can make them resistant to various environmental factors.

BIOL 102

Chapter 2 – Chemical Basis of Life

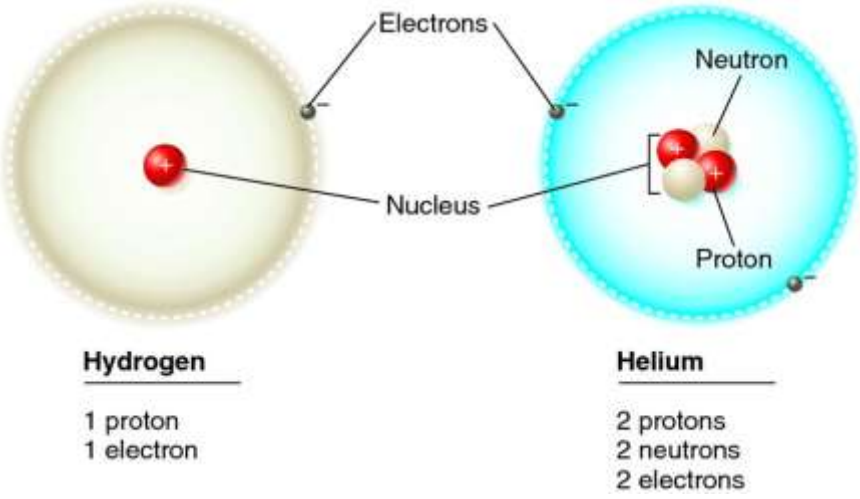




LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 6.15 An Enzyme with a Coenzyme

Atoms

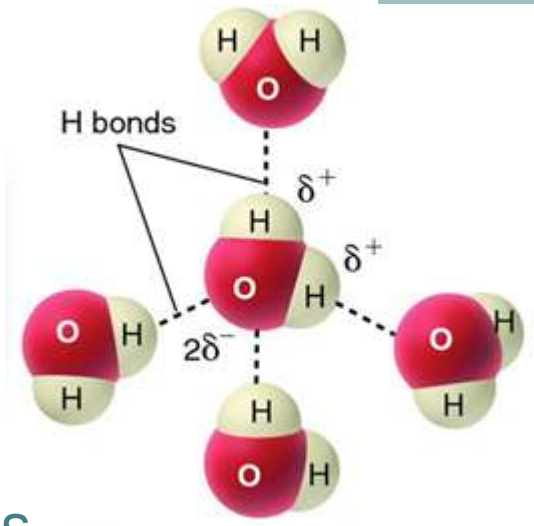
- Consist of electrons, protons, and neutrons
- Electron orbitals
  - S and P orbitals
  - Shells
- Atomic mass
  - Different than weight
  - Daltons of AMUs
- Isotopes
  - Differ in the number of neutrons

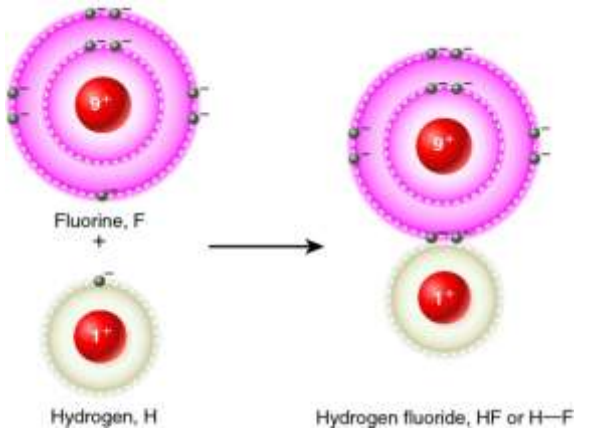


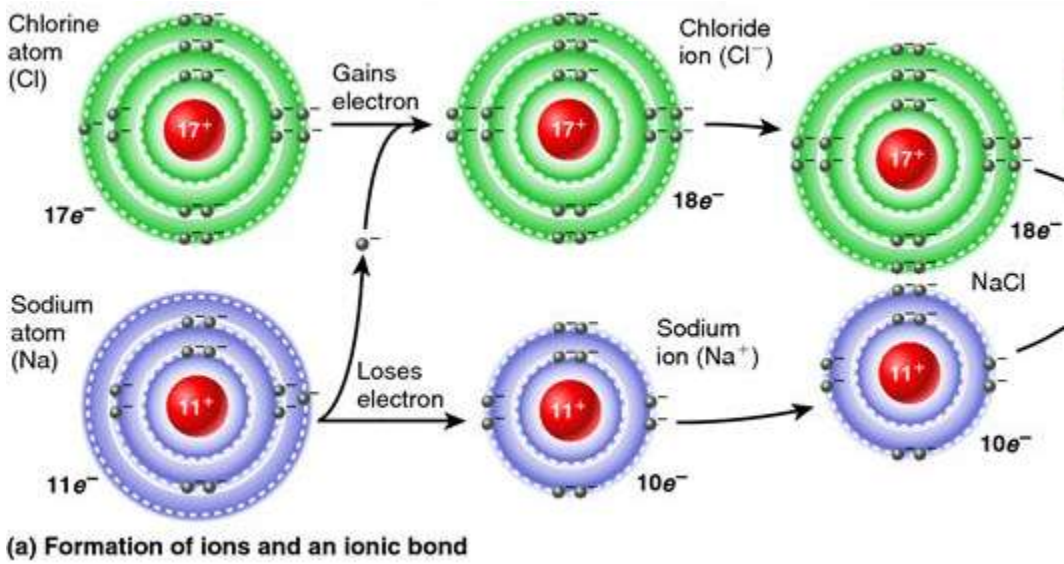
	Shell 1	Shell 2	
Orbital designation	**1*s***	**2*s***	**2*p***
Orbital shape	Spherical	Spherical	Dumbbell
Maximum number of electrons per energy shell	2	8 [2*s* + (3 × 2*p*)]	

Bonds

- Covalent bonds
  - Sharing electrons
  - Polar covalent bonds
    - Unequal sharing
- Hydrogen bonds
  - Magnetic forces
- Ionic bonds
  - Attraction between ions







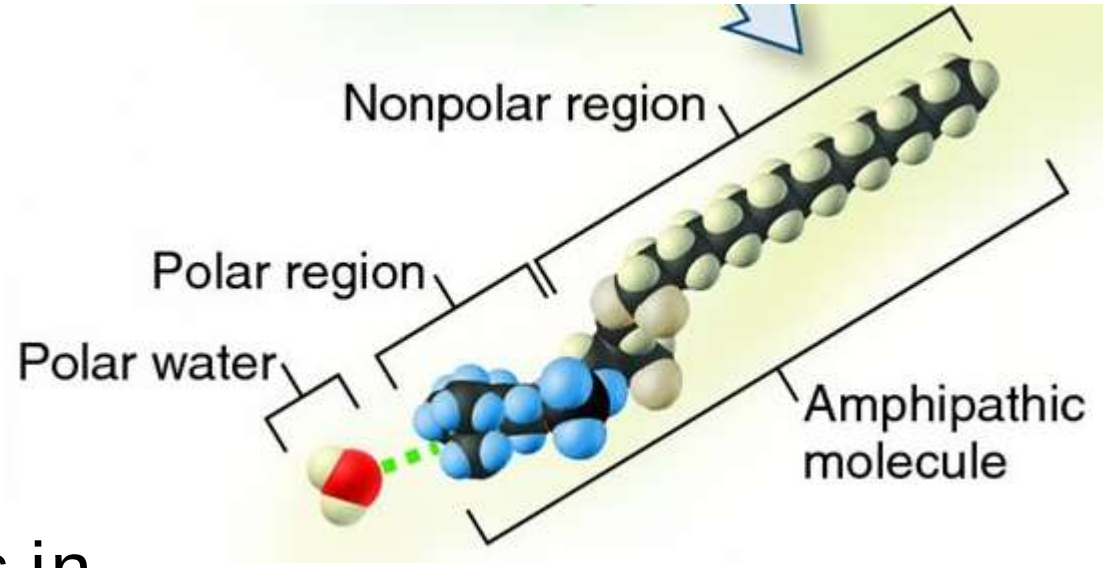
(a) Formation of ions and an ionic bond

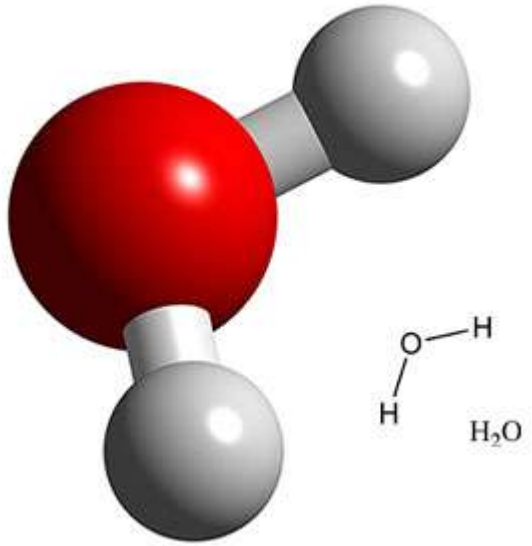
Chemical Reactions

- Changing reactants into products
  - Require energy (E)
  - Directionality
  - Catalysts
- Reduction/Oxidation reactions (REDOX)
  - LEO the lion says GER

Water – H_2O

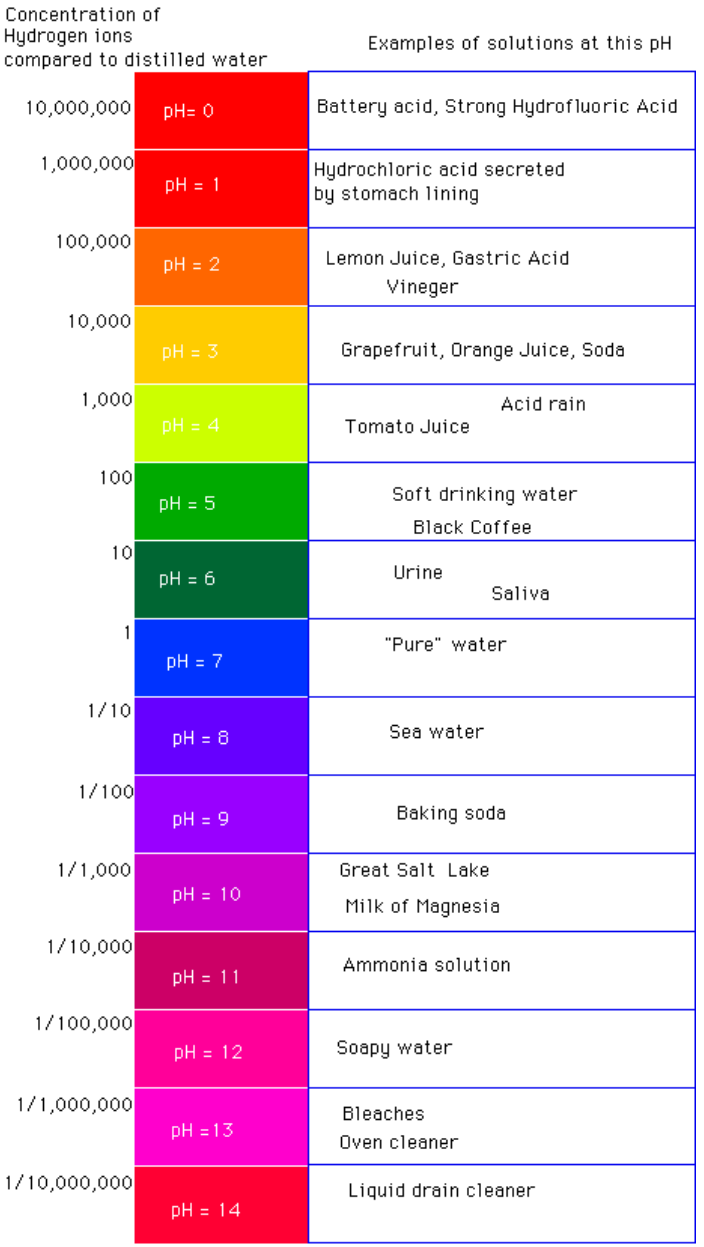
- Hydrophillic, hydrophobic, amphipathic
- Many functions in living organisms
  - Chemical RXNs
  - Support
  - Waste
  - Cooling
  - Cohesion/adhesion





Acids/Bases

- Acids are proton donors
- Bases are proton acceptors
- $pH = -\log_{10}[H^+]$
- pH control is critical to the function of many biomolecules

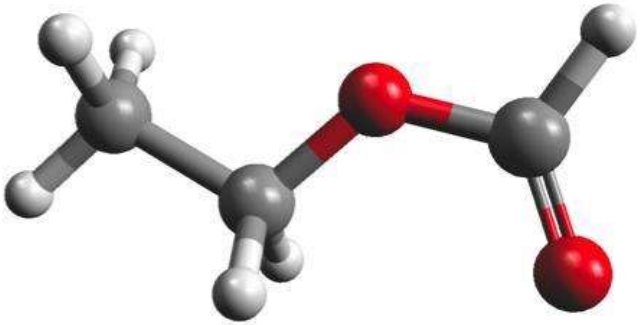


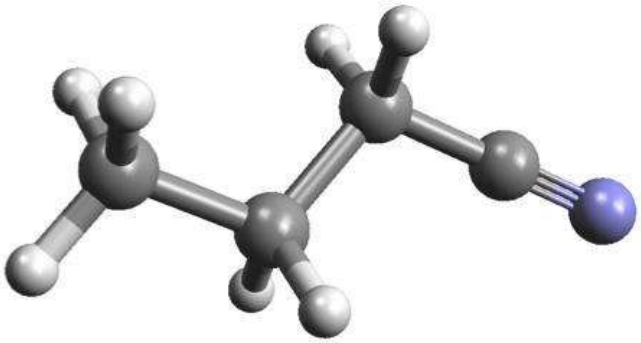
Example Question

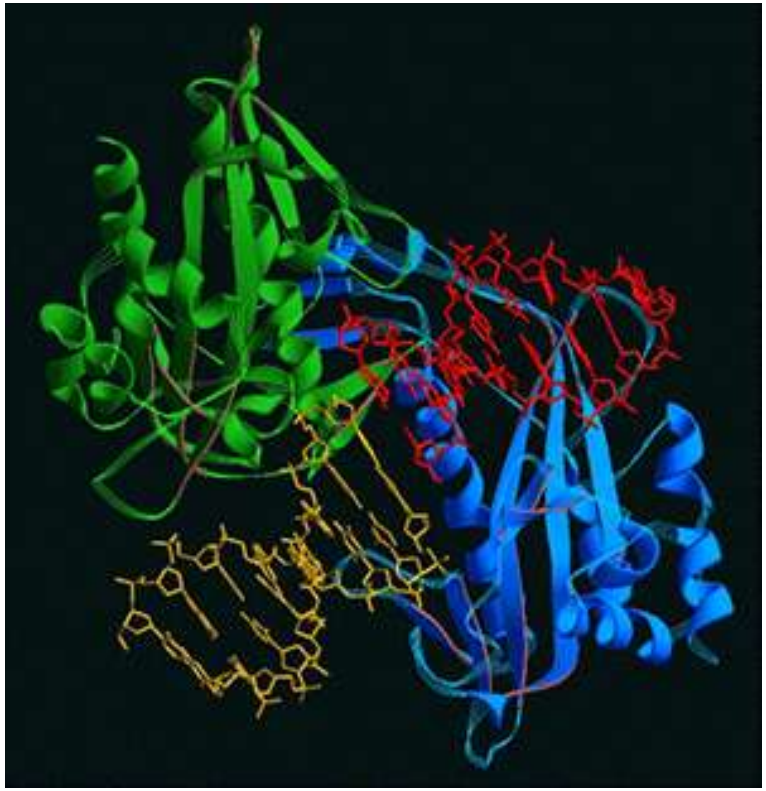
Which of the following statements is TRUE?

a) Atomic mass is the same as weight
b) In polar covalent bonds, electrons are shared equally between the two atoms
c) In a reduction reaction, the molecule becomes more negative
d) A pH of 5 is 50 times greater than a pH of 4
e) Hydrophillic molecules do not dissolve in water

Chapter 3 – Organic Molecules

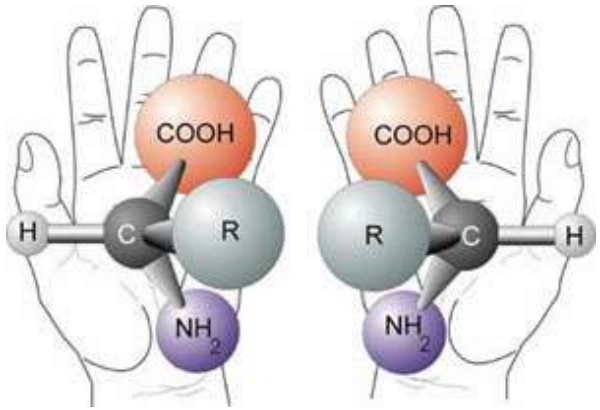


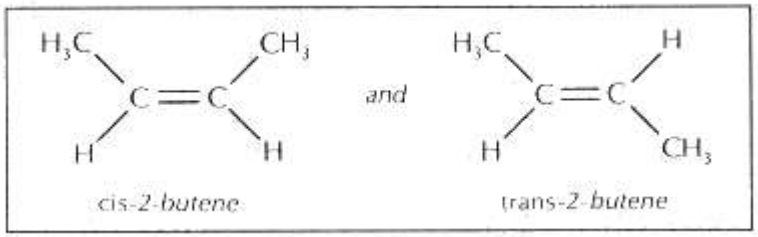


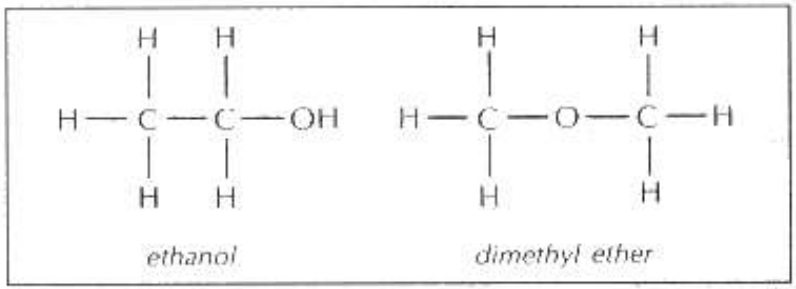


Functional Groups and Isomers

- Groups of atoms with special chemical features
  - Change properties
- Isomers have identical chemical formulas
  - Different characteristics
- Isomer types
  - Structural isomers
  - Steroisomers
    - Enantiomers
    - Geometric isomers

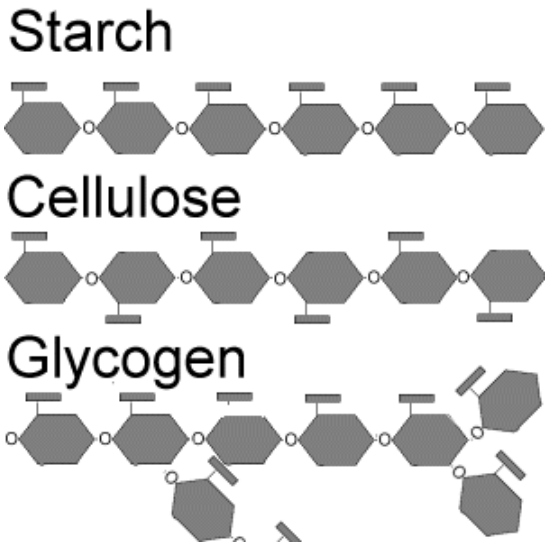




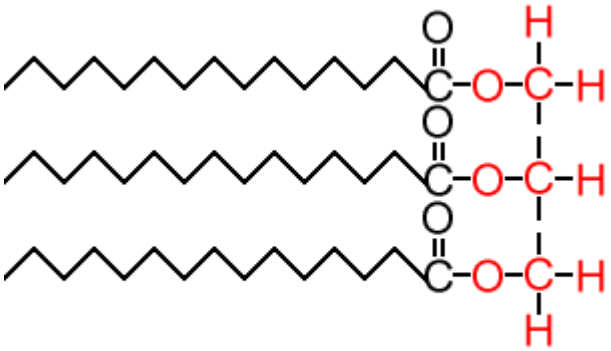


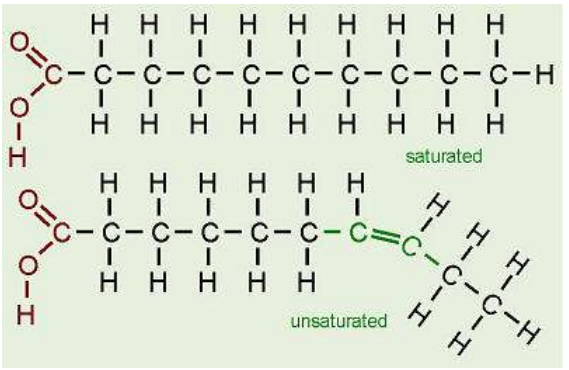
Carbohydrates

- Composed of C, H, and O
- Monosaccharides
  - Simple, small sugars
- Disaccharides
  - Two monosaccharides
  - Condensation reaction
- Polysaccharides
  - Many monosaccharides

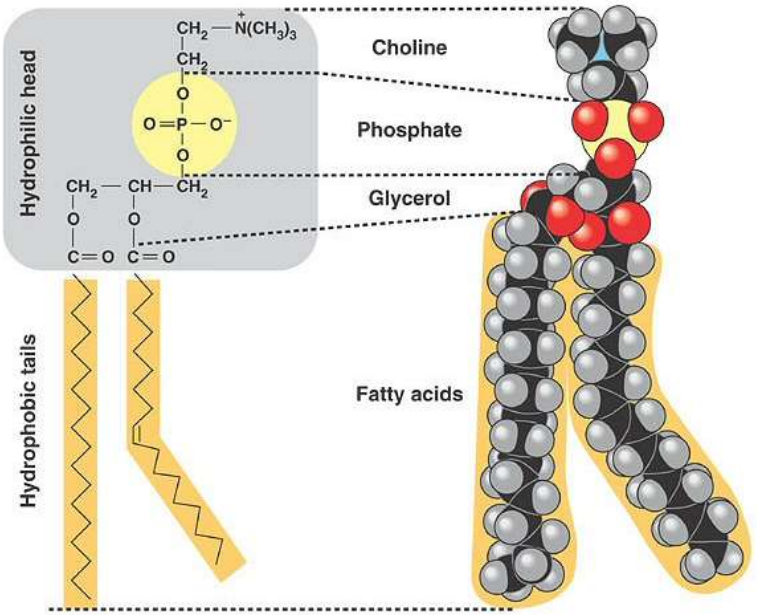


Lipids





- Mostly C and H
  - Non-polar
- Fats are a mixture of triglycerides
  - Energy storage, insulation, cushioning
- Saturated fatty acids
  - Highly reduced form (high E)
- Unsaturated fatty acids
  - Mono vs. poly unsaturated
  - Liquid
- Phospholipids
  - Amphipathic
  - Membranes
- Steroids
  - C rings
  - Hormones



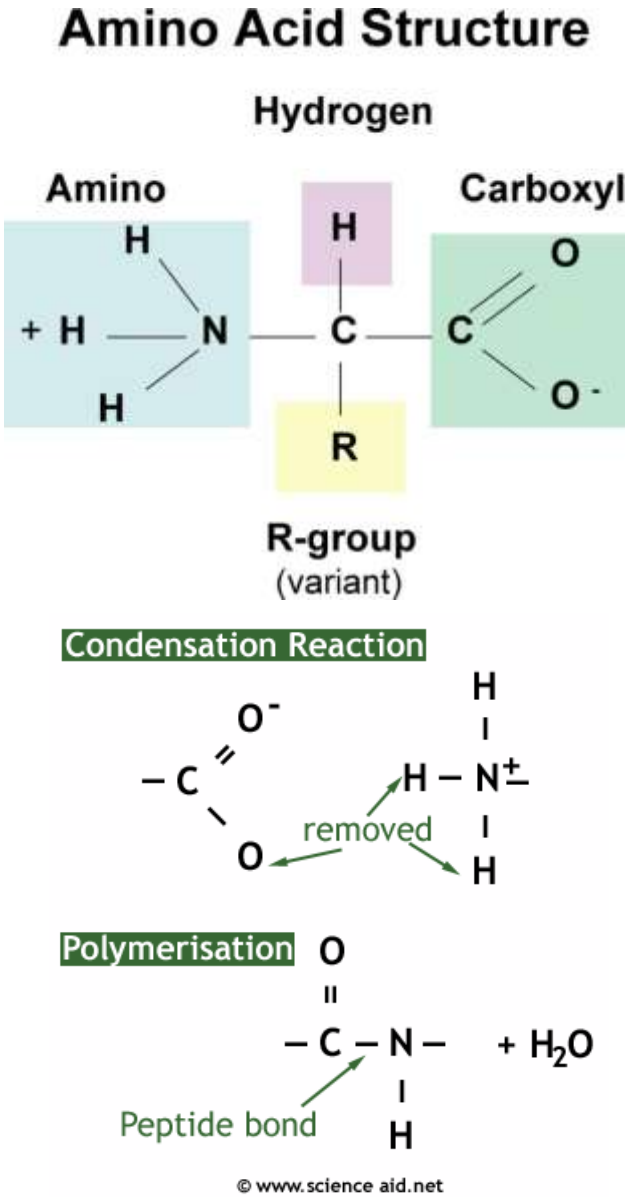
Example Question

Polysaccharides are:

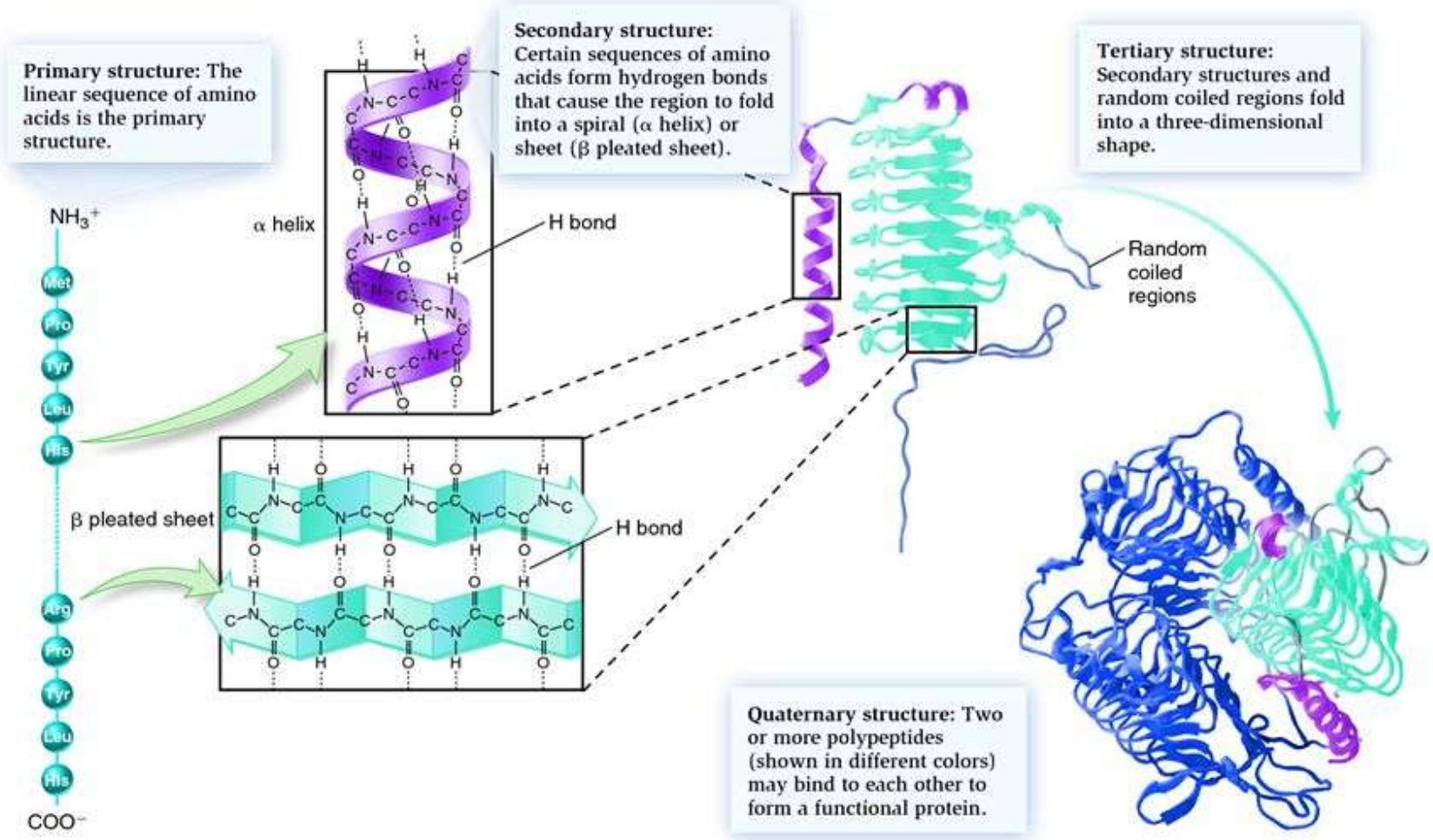
a) A hydrophobic functional group
b) Small, simple sugars
c) Composed of only two monosaccharides
d) Composed of 3 fatty acids and a glycerol
e) Composed of 3 or more monosaccharides

Proteins

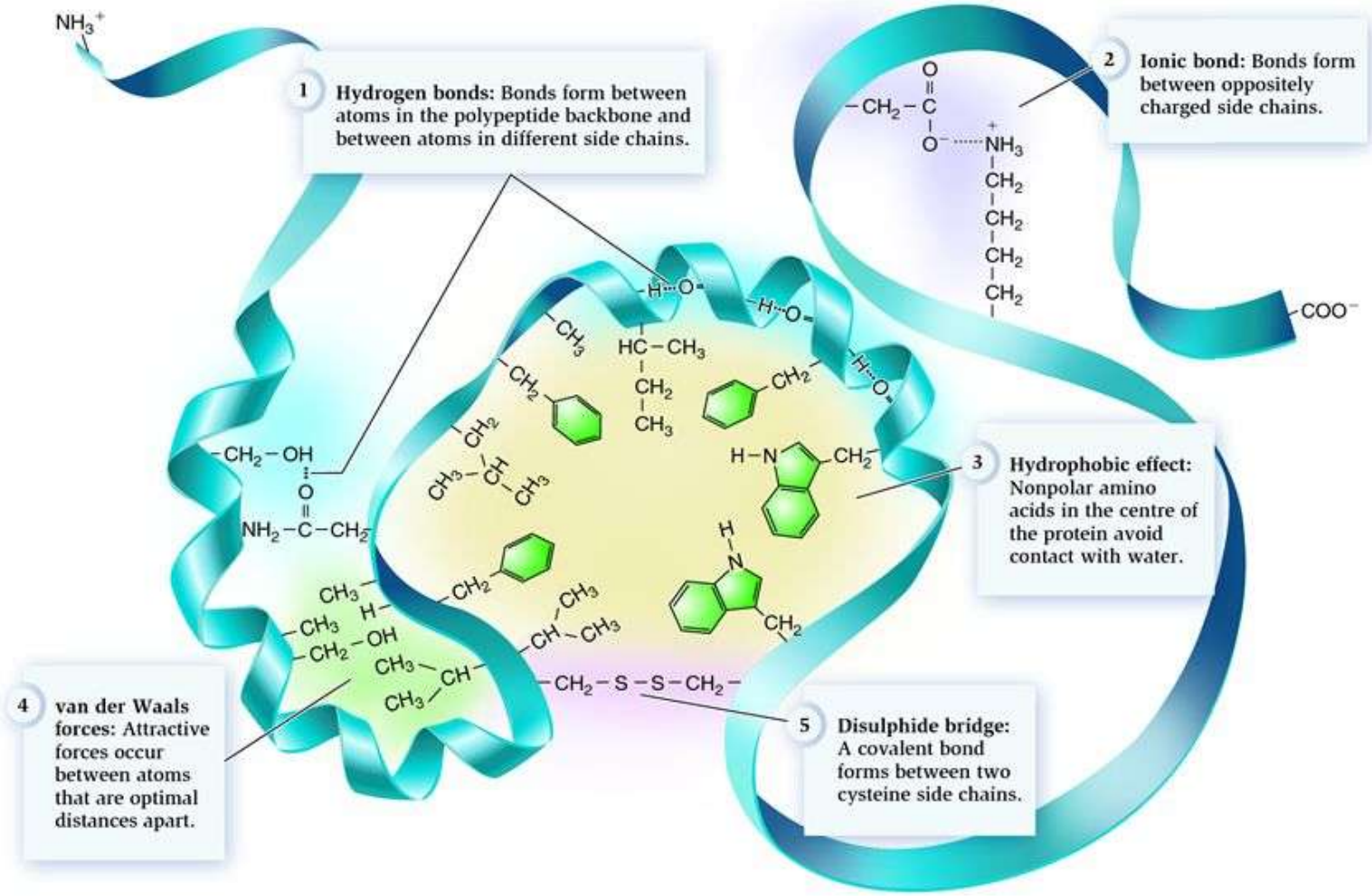
- Composed of C, O, H, and N
- Machines of the cell
- Consist of amino acids
  - 20 L-amino acids
  - Side-chain determines structure and function
  - Peptide bonds
- Numbering
  - Starts at N-terminus towards C-terminus



Protein Structure

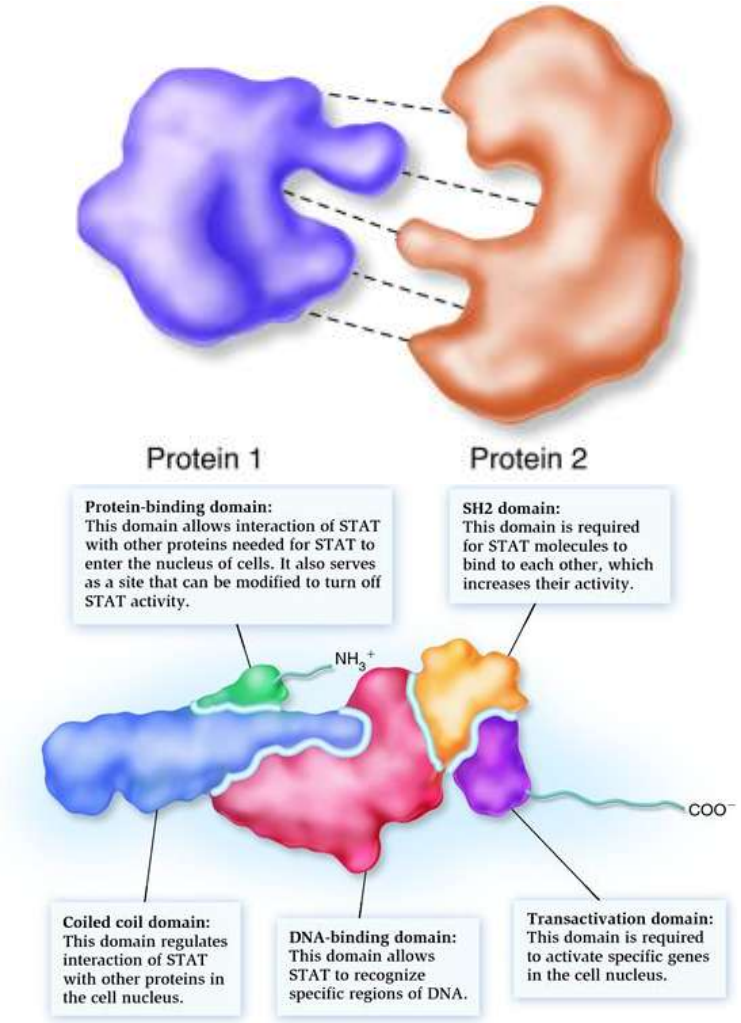


Protein Folding and Stability



Protein Interactions and Functional Domains

- High fidelity and affinity
- Functional domains are found in the 1º structure
  - Sequence of amino acids
  - Functional similarity
  - ID protein function



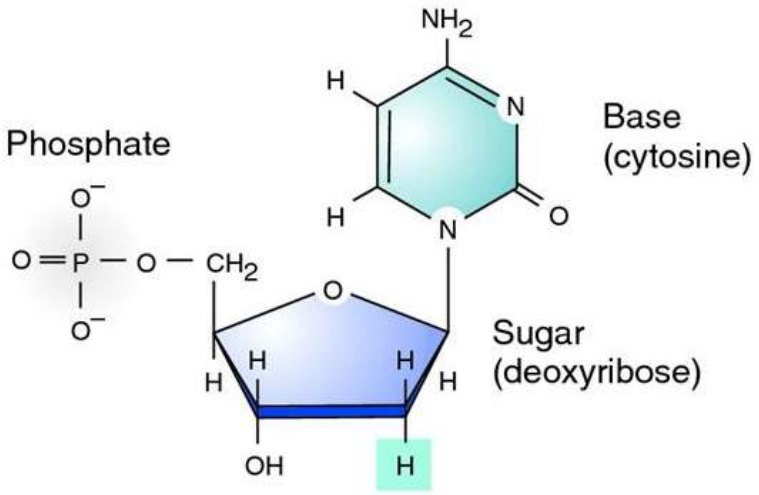
Example Question

A heterotrophic trimeric enzyme is:

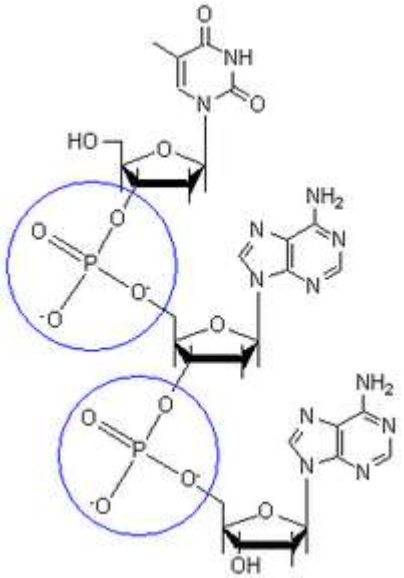
a) Composed of α-helices and β-pleated sheets
b) A string of amino acids
c) An enzyme complex composed of 3 different subunits
d) An enzyme complex composed of 3 of the same subunits
e) The tertiary structure of an enzyme

Nucleic Acids

- Responsible for the storage expression and transmission of genetic information
- Nucleotides are made up of a phosphate, 5 C sugar, and a base
  - Phosphodiester bonds
- 4 nucleotides come in two classes
  - Pyrimidines
  - Purines
- Read from 5´ to 3´



Example of a deoxyribonucleotide



DNA and RNA

- Deoxyribonucleic acid
  - Deoxy sugar
  - Stores genetic info
- Ribonucleic acid
  - Decodes DNA to help make proteins
- Uracil
  - Pyrimidine
  - Methylation group
  - Mutations

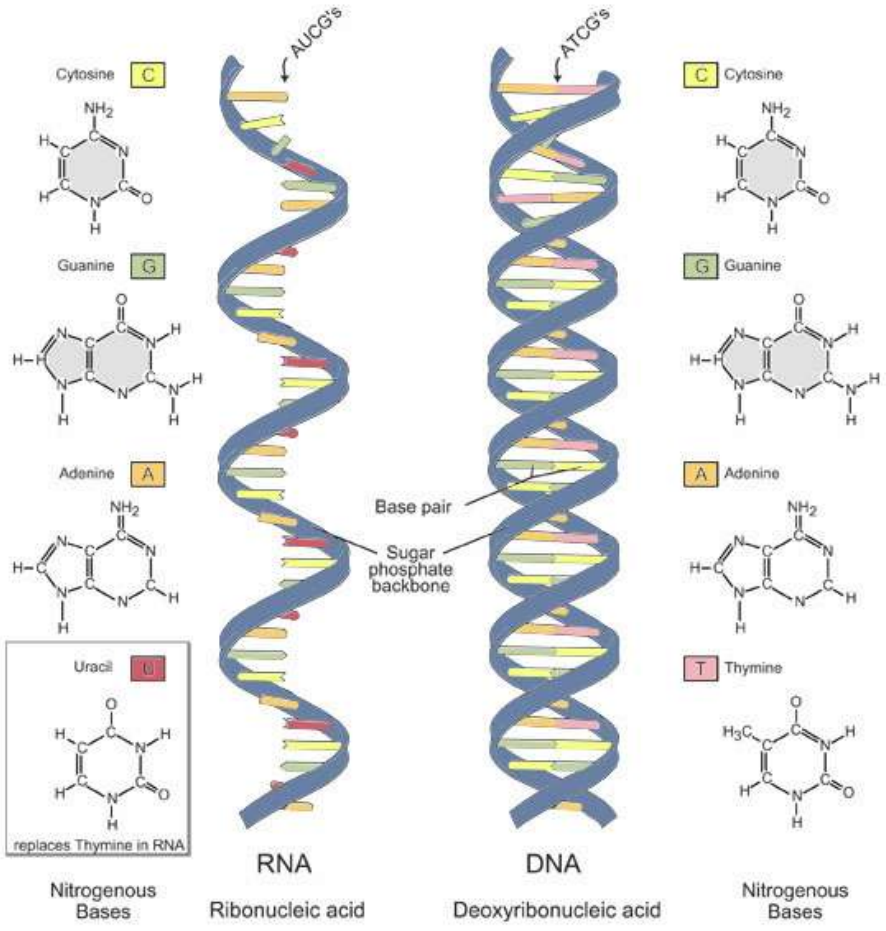


Image adapted from: National Human Genome Research Institute. Talking Glossary of Genetic Terms. Available at: www.genome.gov/Pages/Hyperion//DIR/VIP/Glossary/Illustration/rna.shtml.

DNA and RNA

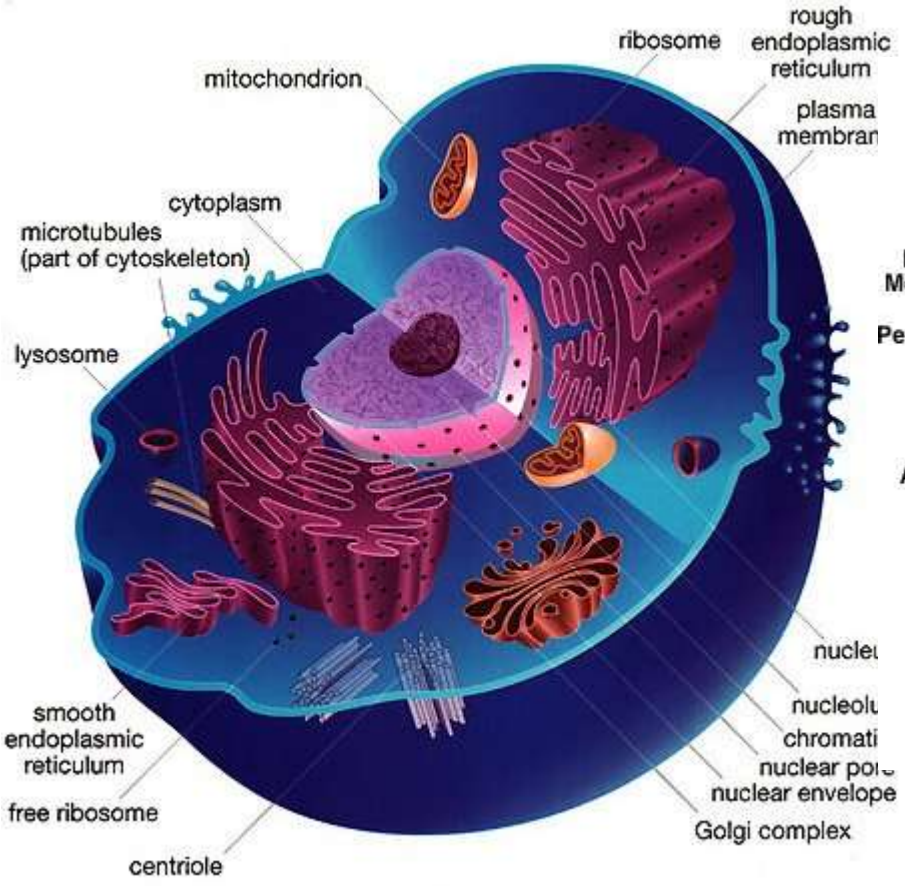
DNA	RNA
Deoxyribonucleic acid	Ribonucleic acid
Deoxyribose	Ribose
Thymine (T)	Uracil (U)
Adenine (A), guanine (G), cytosine (C), used in both	
2 strands forming a double helix	Single stranded
1 form	Several forms

Example Question

Which of the following is correct:

a) Purines are double ringed
b) DNA is read from 3´ to 5´
c) Thymine is methylated uracil
d) A and B are correct
e) A and C are correct

Chapter 4 – Cell Theory



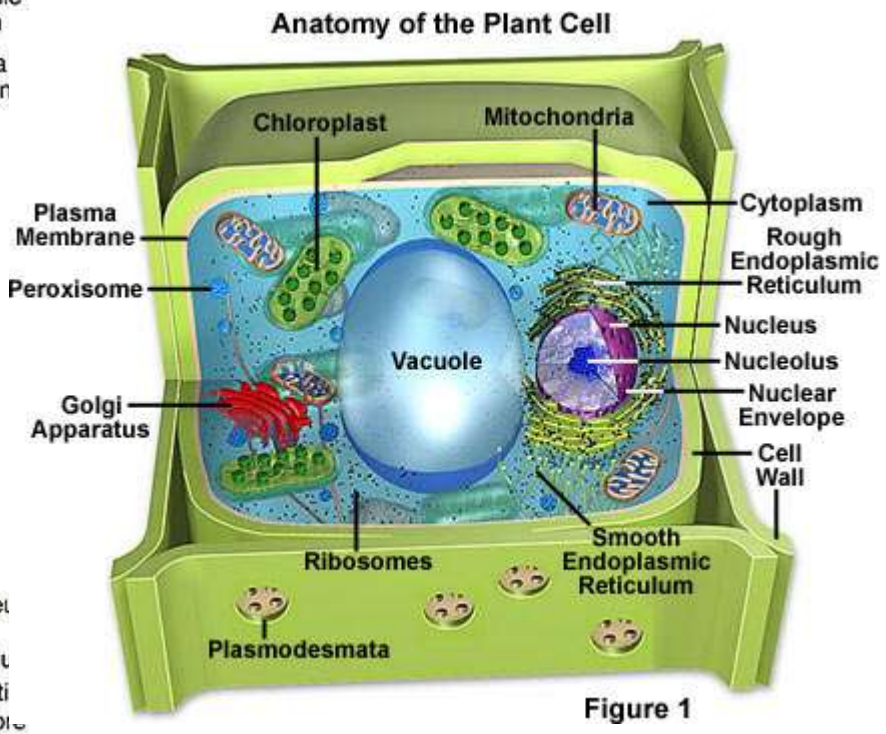
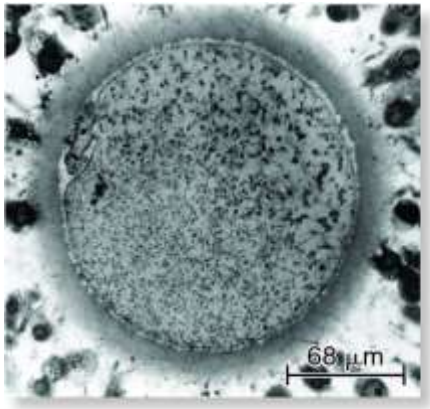


Figure 1

Microscopy

- Magnification
  - Size of the image to actual size
- Resolution
  - Objects as distinct
- Clarity
  - Distinguish structures
- Light microscope
  - Cellular level
- Electron microscopes
  - Electron beam
- Transmission electron microscope
  - Thin slices stained with heavy metal
- Scanning electron microscope
  - Sample coated with heavy metal

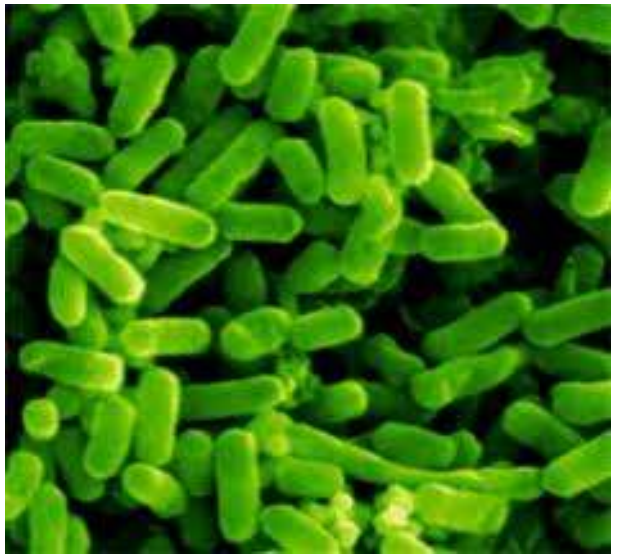


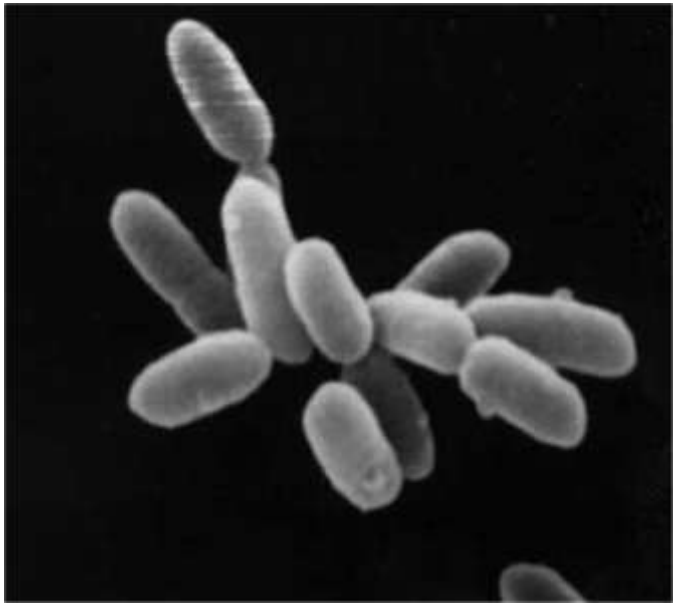
(a) Transmission electron micrograph

(b) Scanning electron micrograph

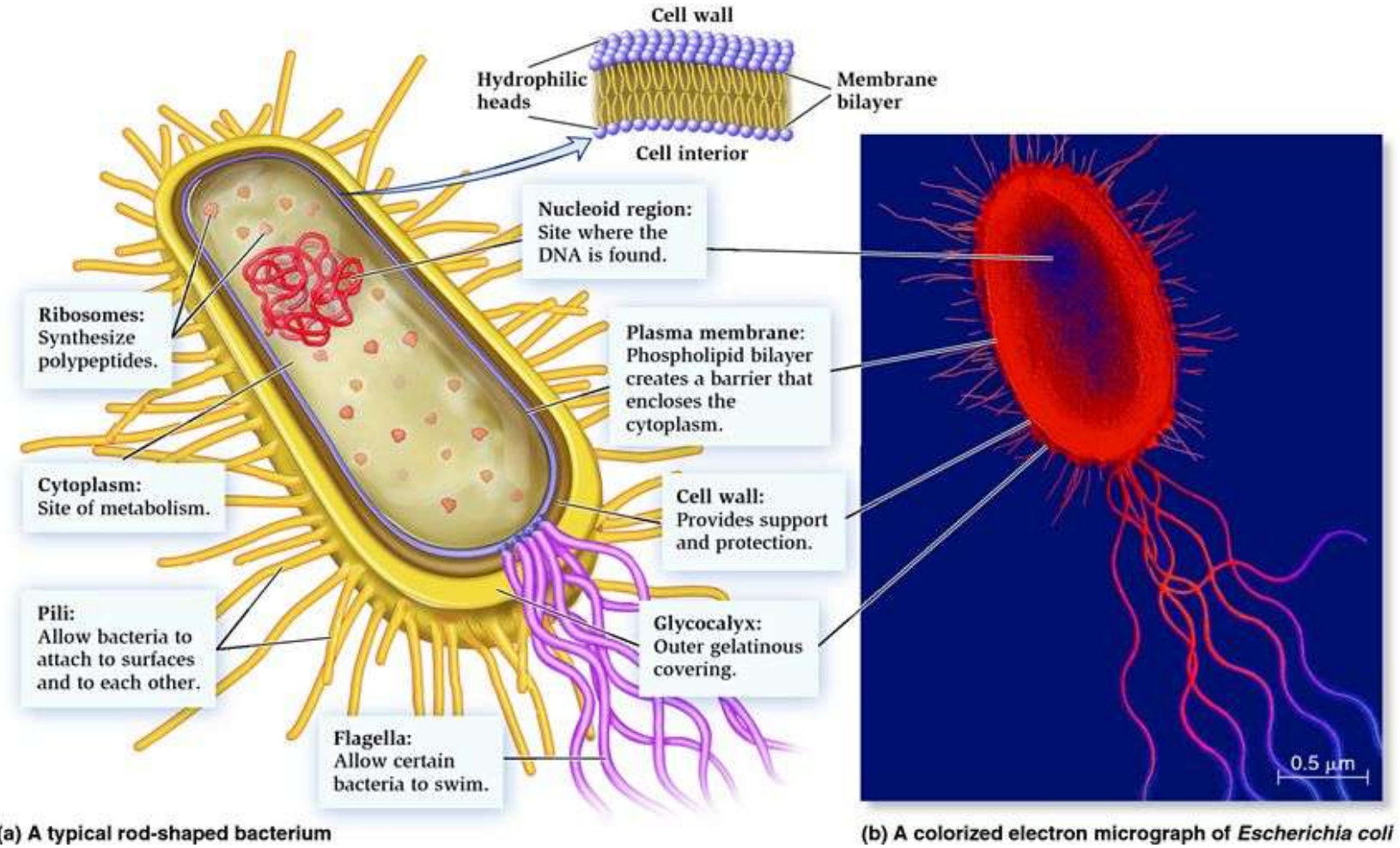
Prokaryotic Cells

- Lack membrane enclosed nucleus and membrane enclosed organelles
- Bacteria and Archaea
  - Small
  - Bacteria are abundant
  - Archaea are found in extreme envionments





Bacterial Cell



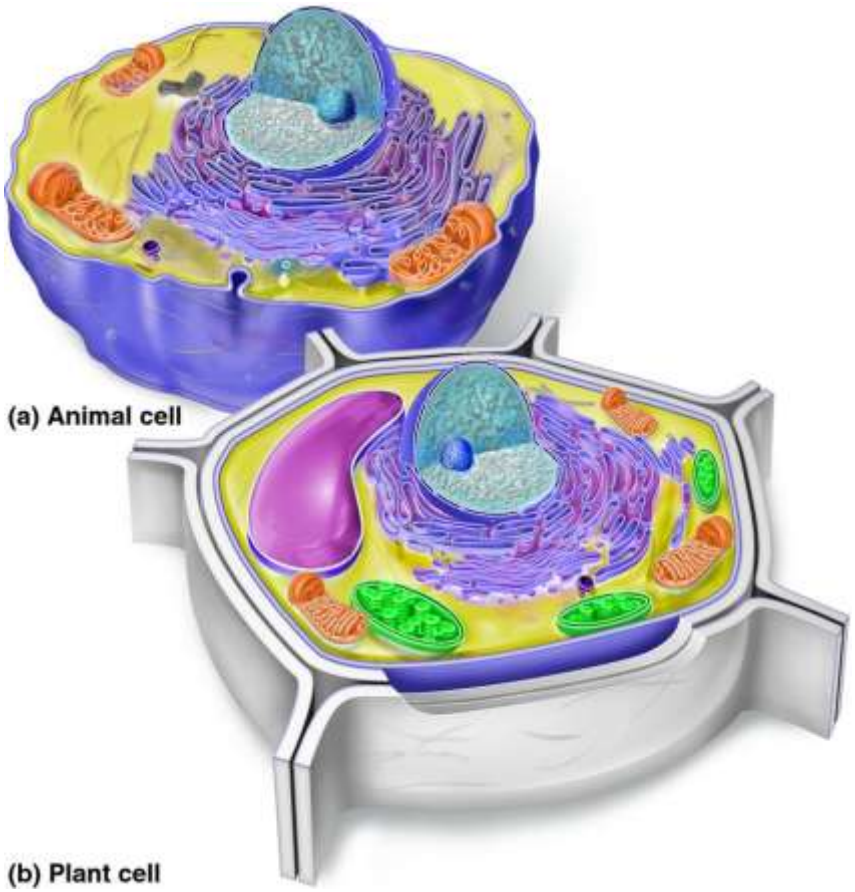
Example Question

Which of the following structures in bacteria provides locomotion?

a) Flagella
b) Pilli
c) Ribosomes
d) Glycocalyx
e) DNA

Eukaryotic Cells

- DNA housed within nucleus
- Compartmentalization
- Membrane bound organelles
- Plant vs. Animal
  - Plants have chloroplasts and cell walls



Cytosol

- Surrounds organelles inside the plasma membrane
- Cytoplasm is everything inside the membrane
- Metabolism
  - Site of glycolysis
  - Catabolism vs. anabolism
- Translation
  - Protein synthesis
  - Ribosomes

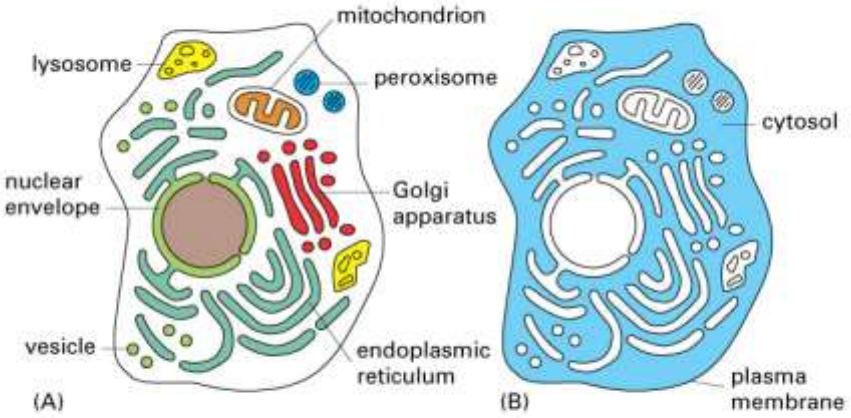
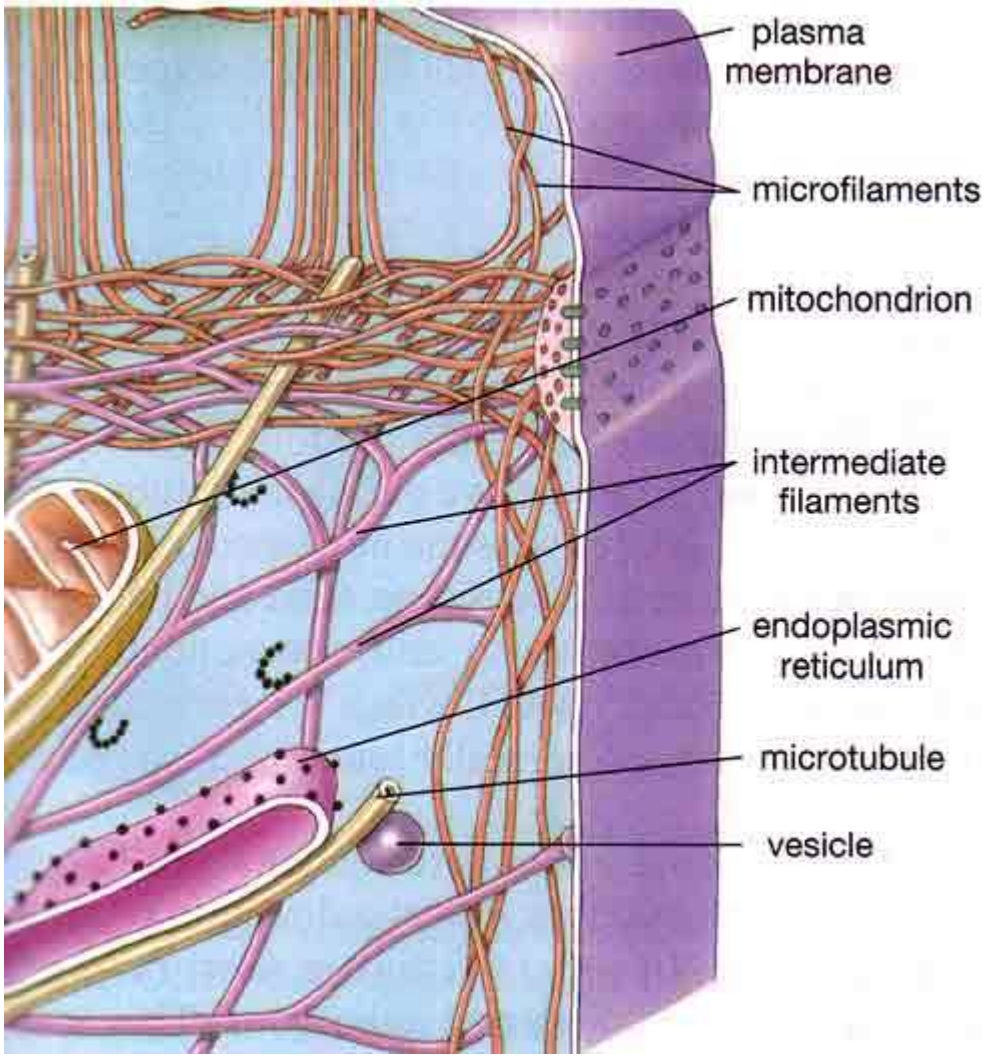


Figure 1-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

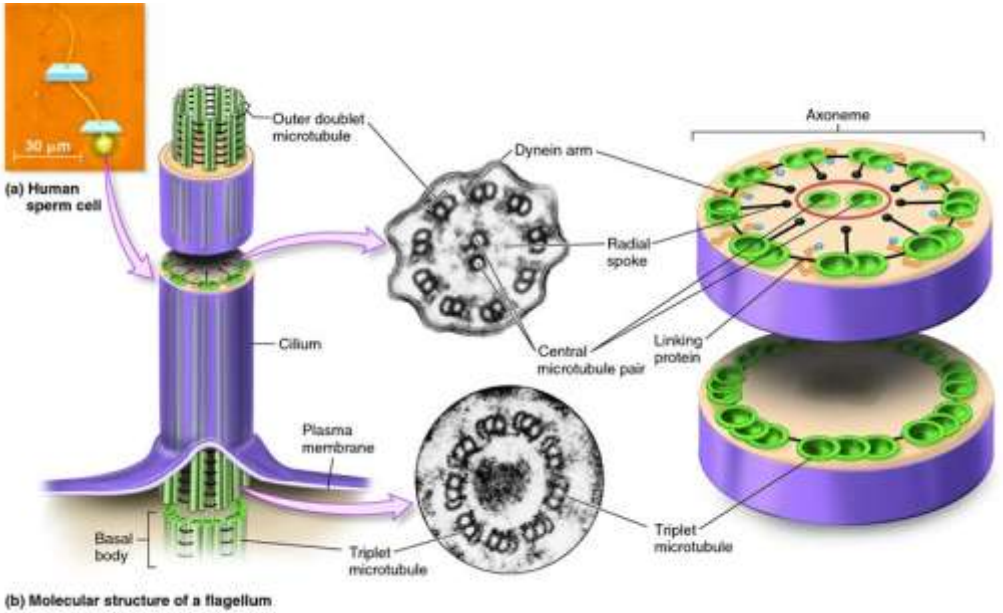
Cytoskeleton

- Scaffolding system
- Microtubules
  - Tubulin
  - Not stable
- Intermediate filaments
  - More stable
- Actin filaments
  - AKA microfilaments
- Intracellular transport
- Motor proteins
  - Use ATP
  - "Walk" along filaments
- Moves in 3 ways
  - Cargo along cytoskeleton
  - Pull filament
  - Attempt to walk → bending



Flagella and Cilia

- Movement
  - Flagella are longer and occur singly or in pairs
- Same internal structure
  - 9 + 2 microtubules
  - Dynein provides driving force



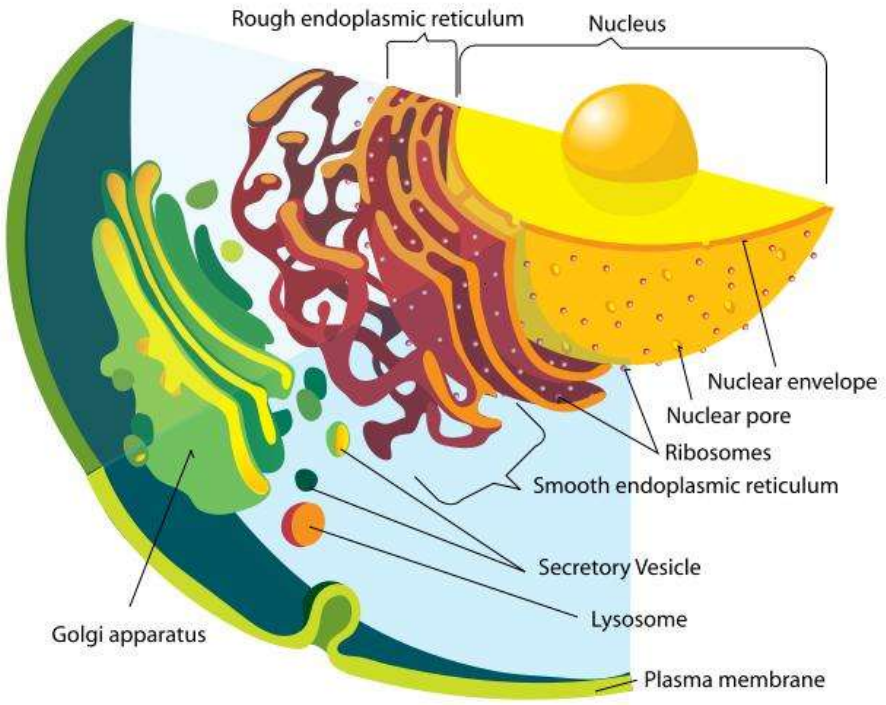
Example Question

The cytoskeleton is composed of:

a) Microtubules
b) Intermediate filaments
c) Actin filaments
d) All of the above

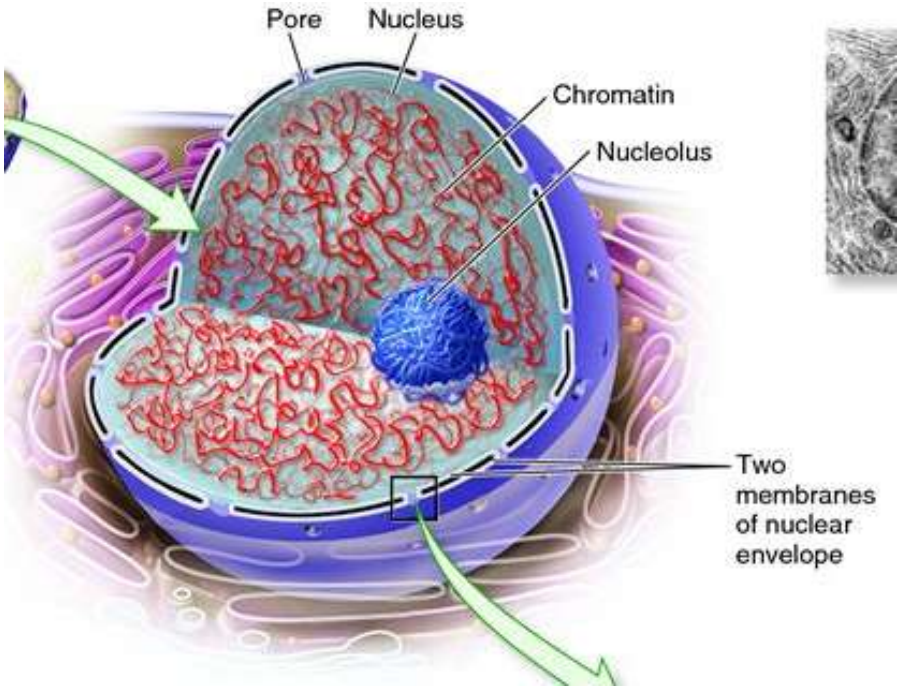
Endomembrane system

- Consists of a network of membranes
  - Nucleus, endoplasmic reticulum (ER), Golgi apparatus, lysosomes, peroxisomes, vacuoles, plasma membrane
- May be directly connected or pass material via vesicles
  - ER and nucleus



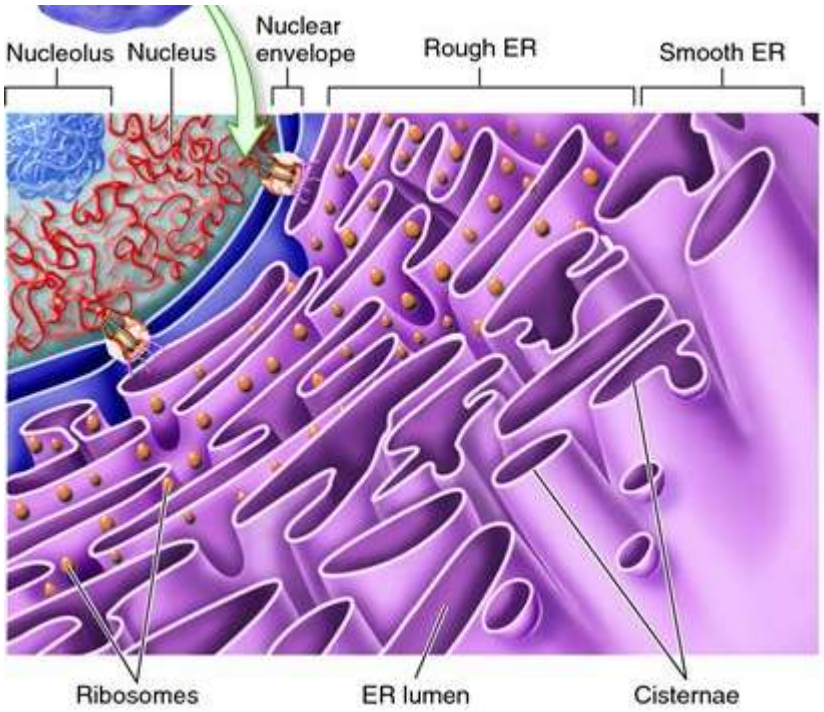
Nucleus

- Surrounded by nuclear envelope
  - Lipid bilayer
  - Nuclear pores
- Organizes, protects, and expresses genetic material (DNA)
  - Contains chromosomes in the nucleolus
    - Central region



Endoplasmic Reticulum (ER)

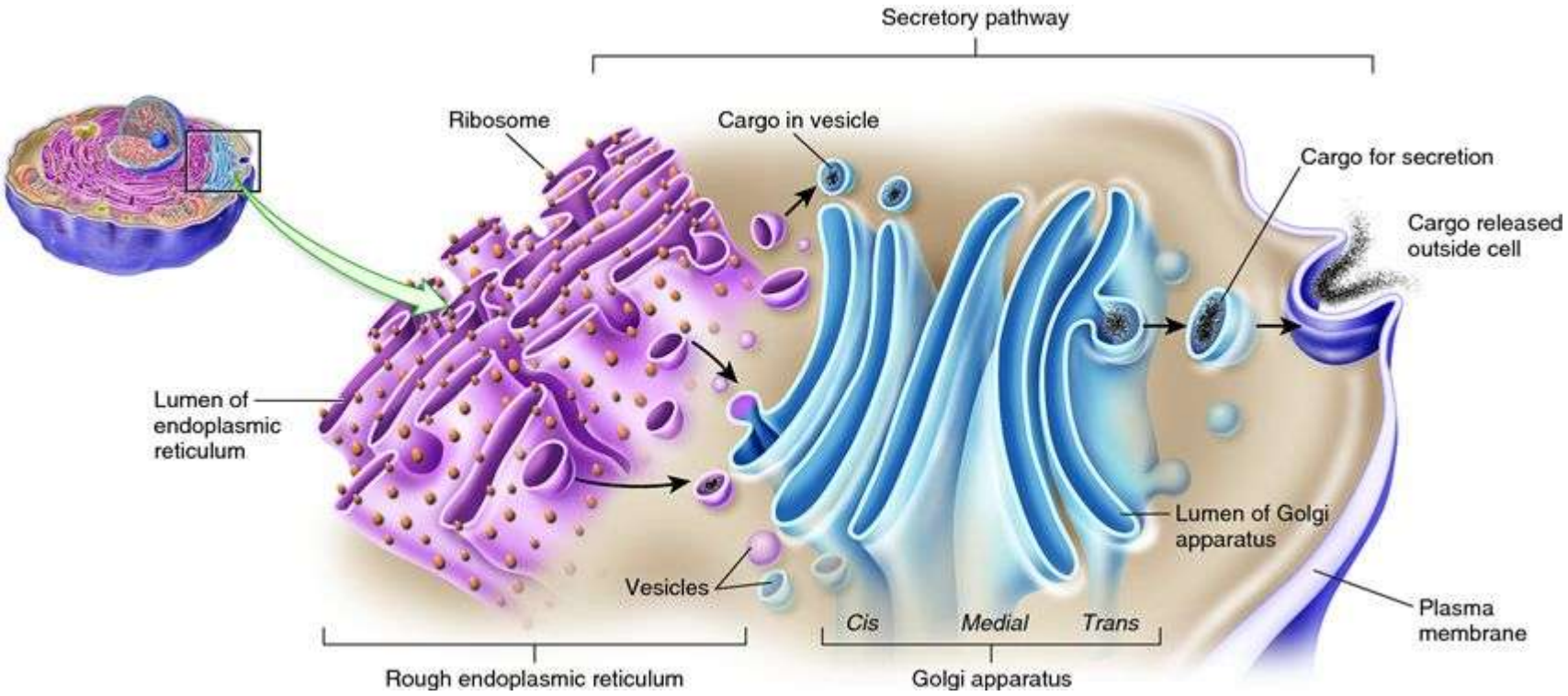
- Network of membranes that form flattened, fluid filled tubules called cisternae
- ER membrane encloses a single compartment called the ER lumen
- Rough endoplasmic reticulum (rough ER)
  - Studded with ribosomes
  - Involved in protein synthesis and sorting
- Smooth endoplasmic reticulum (smooth ER)
  - Lacks ribosomes
  - Detoxification, carbohydrate metabolism, calcium balance, synthesis and modification of lipids



Golgi Apparatus

- Stack of flattened, membrane-bounded compartments which are not continuous with the ER
- Vesicles transport materials between stacks
- Three overlapping functions
  - Secretion, processing, and protein sorting
  - Often vesicles come to the Golgi from the ER
- The cis face faces the ER and the trans face faces the plasma membrane

Golgi Apparatus



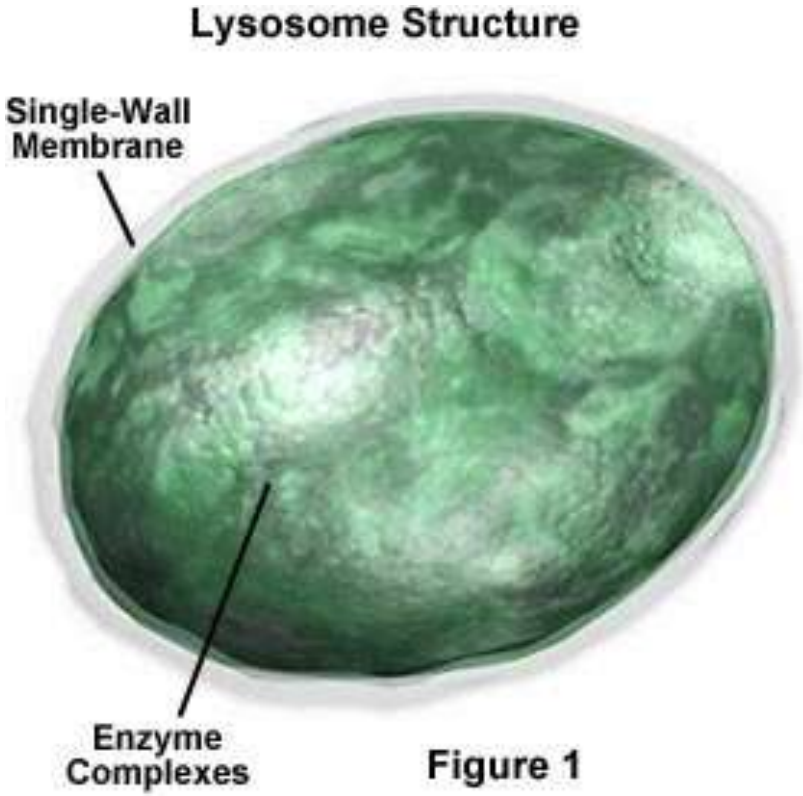
Example Question

Which of the following is TRUE:

a) The ER is continuous with the nuclear envelope
b) The Golgi apparatus is continuous with the nuclear envelope
c) Nuclear pores allow materials to pass in and out of the nucleus
d) A and C are true

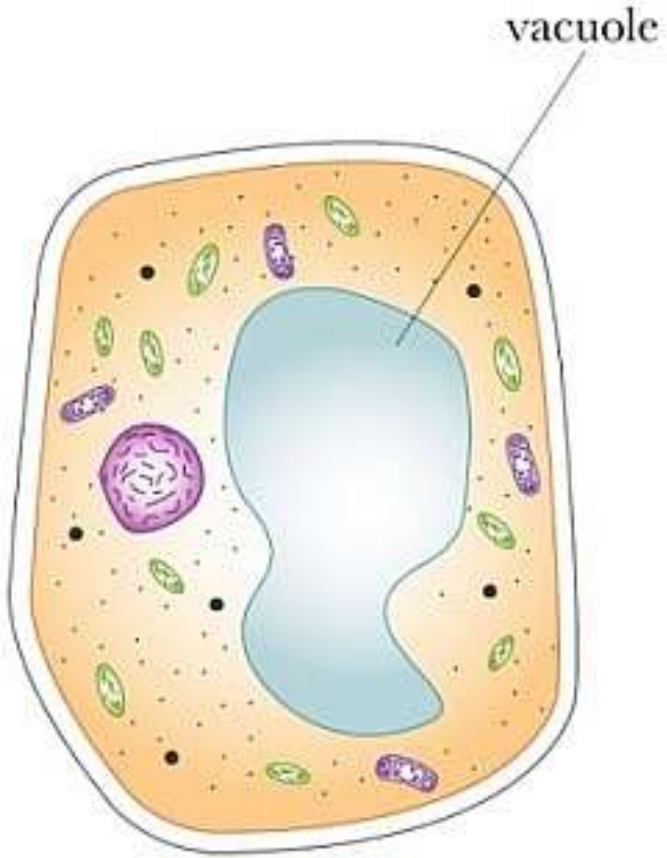
Lysosomes

- "Garbage can" of the cell
  - Contains hydrolases that perform digestion
- Autophagy: Recycling of worn-out organelles through endocytosis
  - Autophagosome → lysosome
- Not present in plant cells



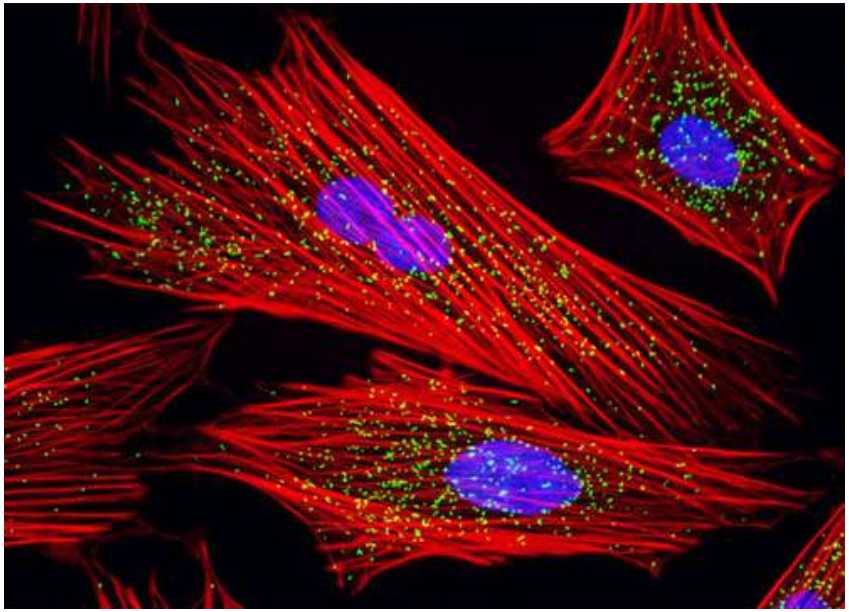
Vacuoles

- Functions are extremely varied
- Differ among cell types and even environmental conditions
  - Central vacuoles in plants for storage and support
  - Contractile vacuoles in protists for expelling excess water
  - Phagocytic vacuoles in protists and white blood cells for degradation
- In plants they perform a similar function that lysosomes fulfill



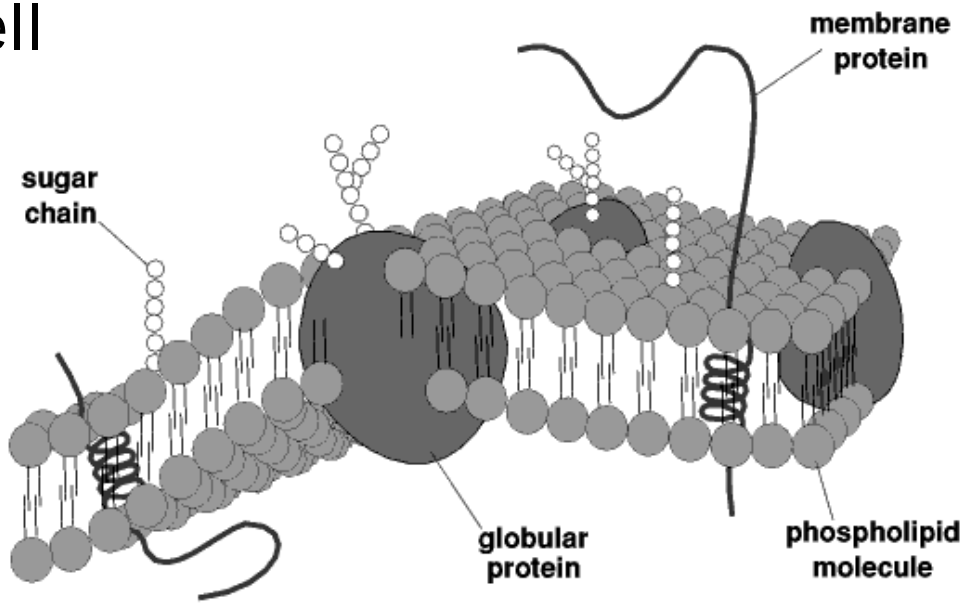
Peroxisomes

- Catalyze certain chemical reactions
- Typically those that break down molecules by removing hydrogen or adding oxygen
  - Oxidation of fatty acids
- Origin remains controversial (semiautonomous?)
  - Binary fission even without a genome
  - Their main origin is the ER



Plasma Membrane

- Boundary between the cell and the extracellular environment
- Membrane transport in and out of cell
- Important in cell signalling
  - Receptors
- Important in cell-cell adhesion
  - Transmembrane proteins



Fluid-mosaic model of membrane structure

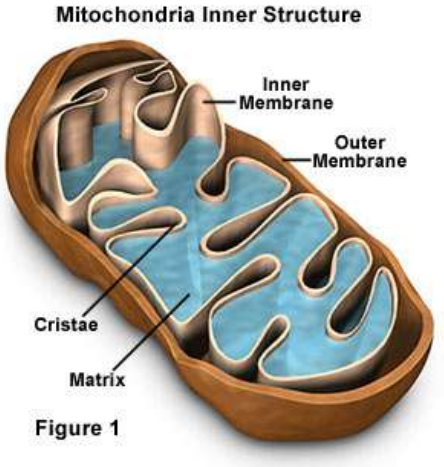
Example Question

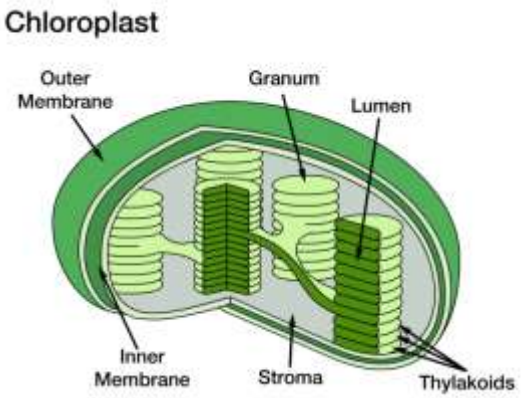
Peroxisomes are remain a controversial member of the endomembranous system because:

a) Their origin is the ER
b) They catalyze many oxidation reactions
c) They function in protein transport
d) They undergo binary fission

Semiautonomous Organelles

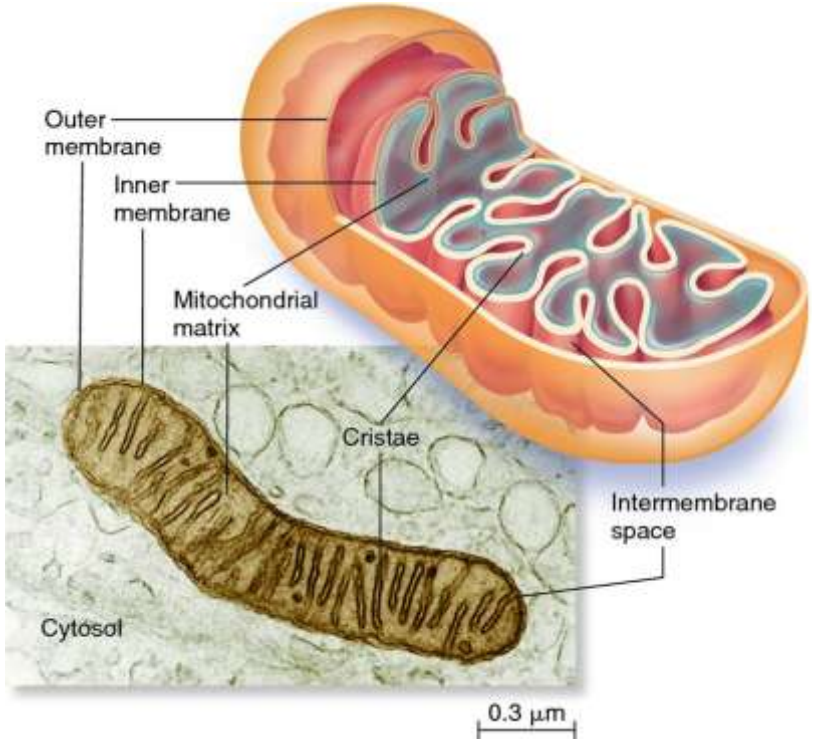
- Can grow and divide to reproduce (binary fission)
- Mitochondria and chloroplasts have small, circular genomes like bacteria
  - Each has their own genome
- The genomes contain the information for SOME (not all) of the proteins that they require
- Endosymbiotic theory





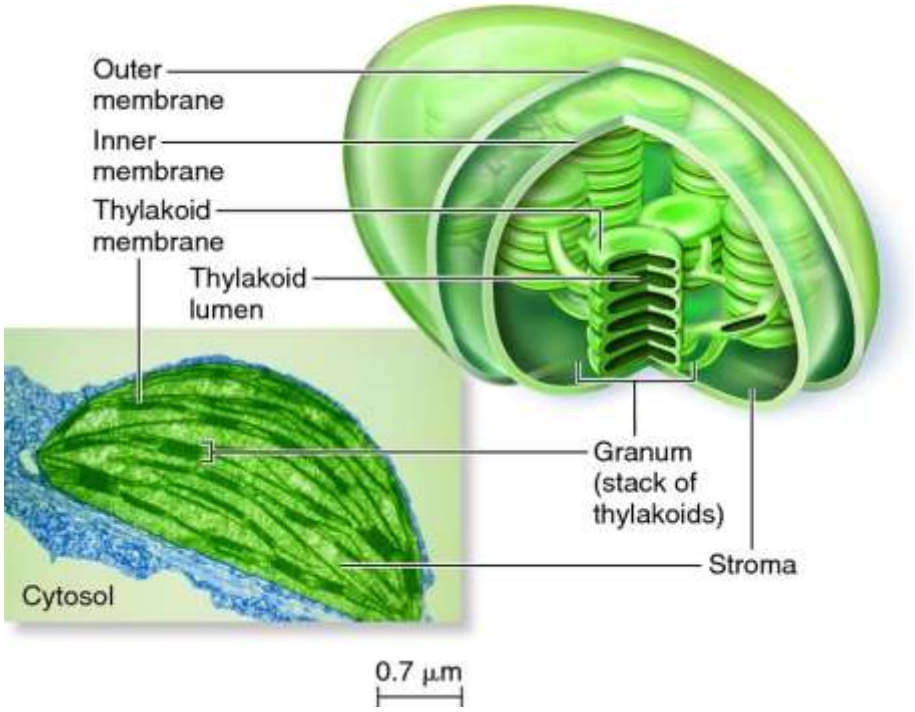
Mitochondria

- Double membrane structure
  - Intermembrane space and mitochondrial matrix
  - Intermembrane space is where the driving force is to make ATP
  - Mito matrix is where the Krebs cycle (TCA) takes place
- Primary role is to make ATP
- Also involved in the synthesis, modification, and breakdown of several types of cellular molecules



Chloroplast

- Double membrane structure
- Third membrane, the thylakoid membrane, forms flattened tubules that stack to form a granum (plural, grana)
  - Thylakoid membrane is where photosynthesis (PS) takes place
- Not found in animal cells

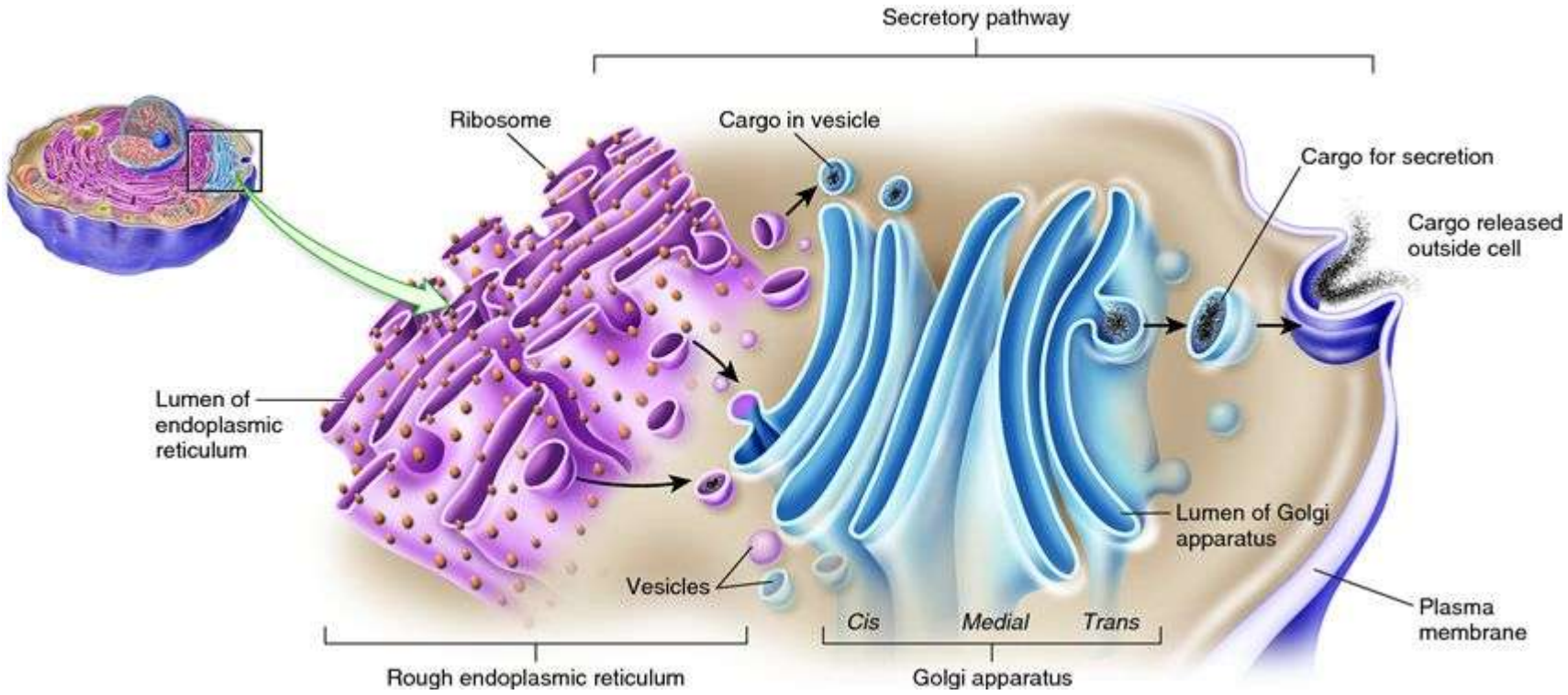


Example Question

Which statement(s) describe semiautonomous organelles.

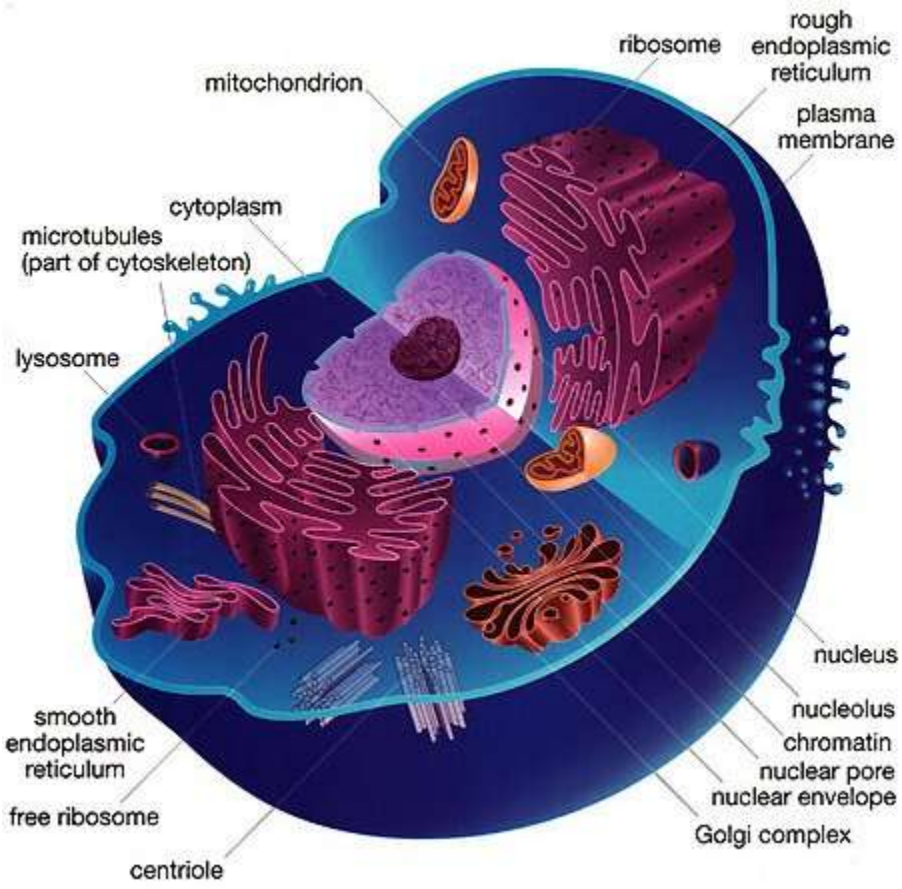
a) Reproduce via binary fission
b) Have circular DNA
c) Can produce some of their necessary proteins
d) All of the above

Chapter 5 – Systems Biology



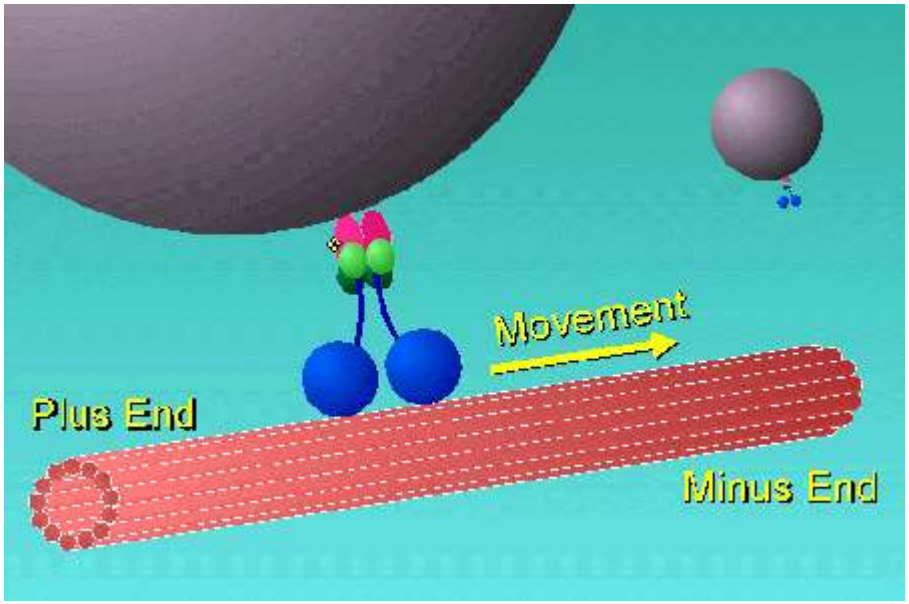
Genomes, Proteomes, and the Environment (cell)

- Genome is the genetic material
  - Codes for proteome
- Proteome is the suite of proteins
- Structure of a cell is critical
  - Compartments for specific cellular processes
- Structure is regulated by proteins



Molecular Machines

- Objects that have moving parts and do work
- They provide critical structure and organization to cells
  - Cytoskeleton
- Include
  - Motor proteins
  - Ribosomes
  - ATP synthase

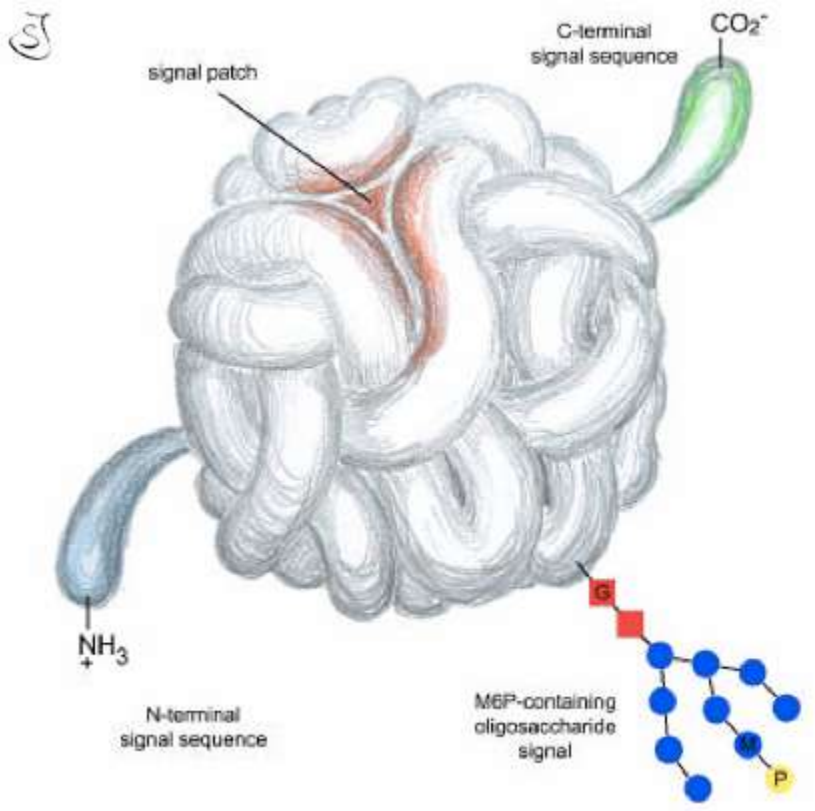


4 Systems Work Together

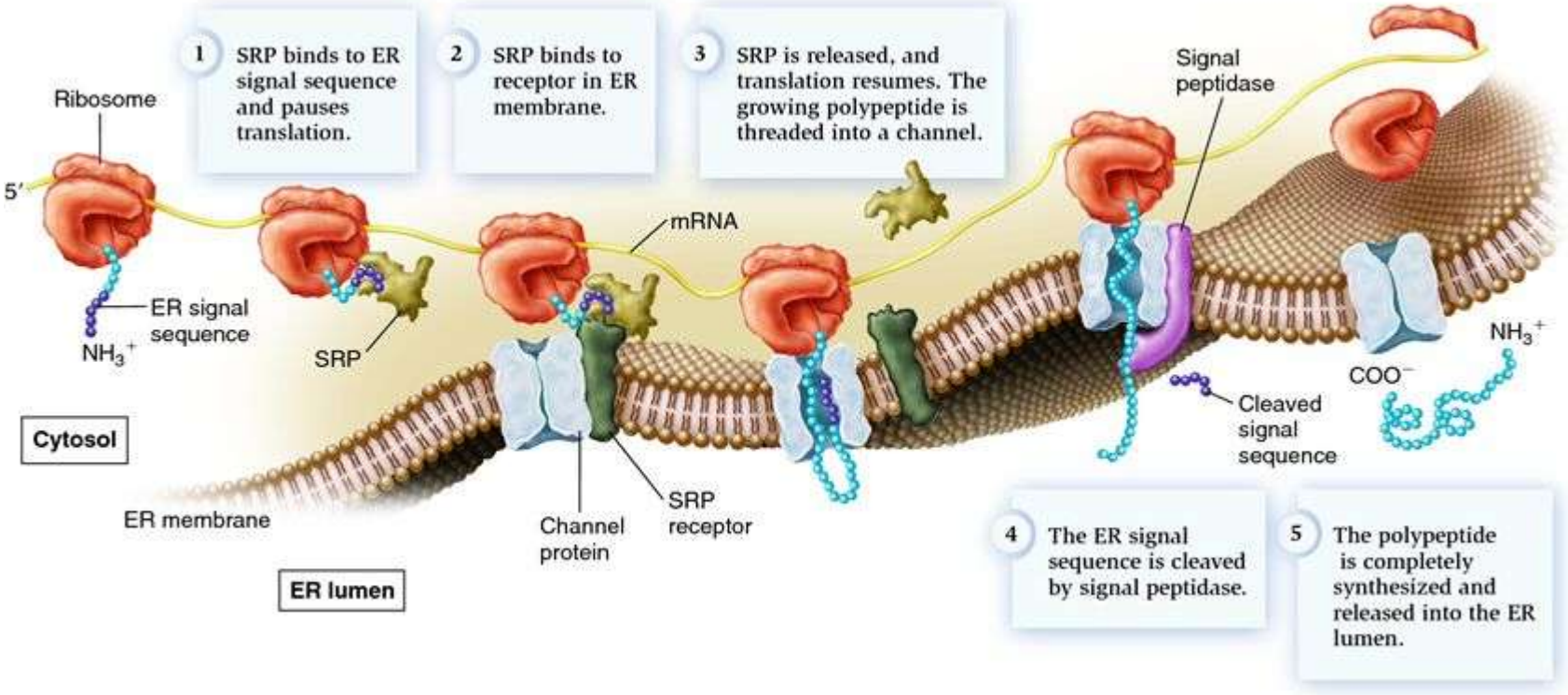
1. Interior of the nucleus
   - Activating the genes
   - Houses the information (kind of like the brain of the cell)
2. Cytosol
   - Site of most of the biosynthetic and catabolic metabolic pathways
3. Endomembrane system
4. Semiautonomous organelles
   - Mitochondria and chloroplasts
   - Have their own genome but largely they rely on the cytoplasm
   - Have dual origin proteome
     - Some comes from the nucleus and some they make themselves

Protein Localization

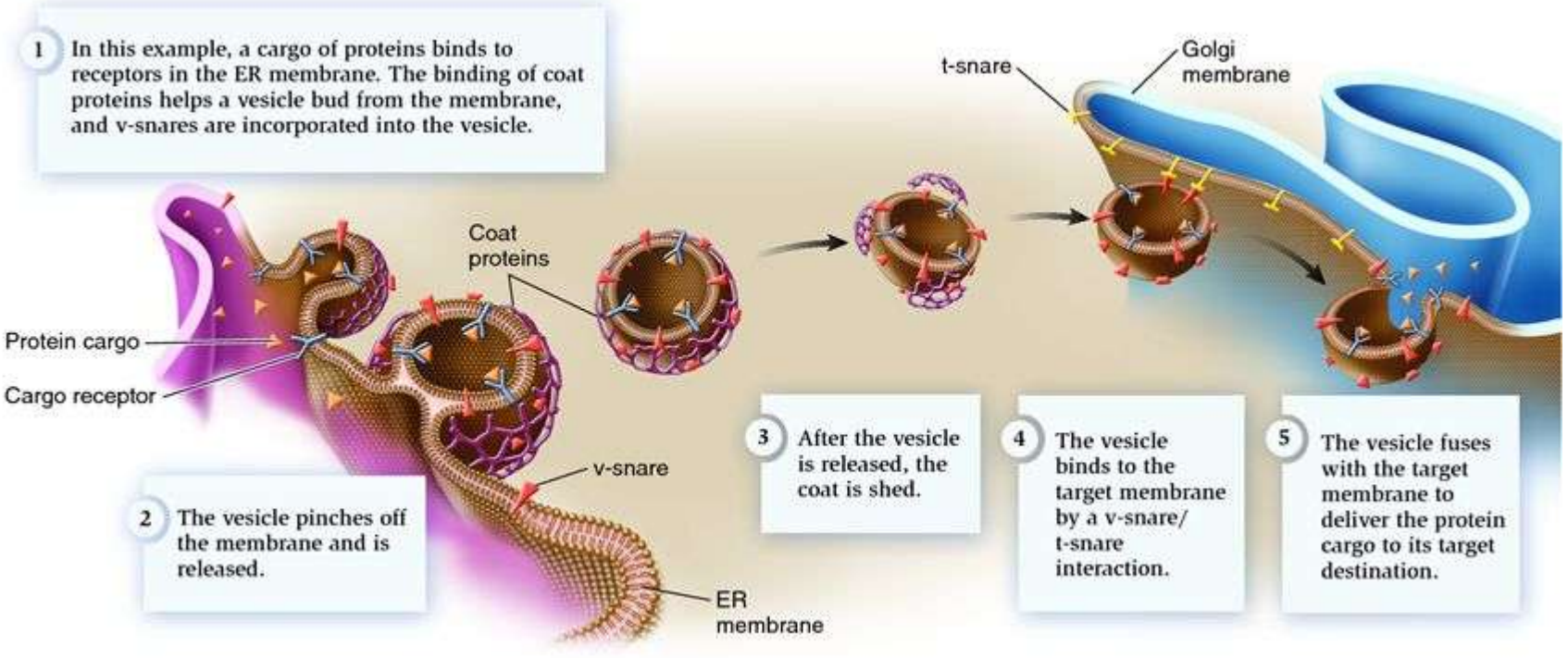
- Getting proteins to where they need to be
- Sorting (transit) signals are short amino acid sequences
  - Postage stamps



Co-translational Sorting



Co-translational Sorting

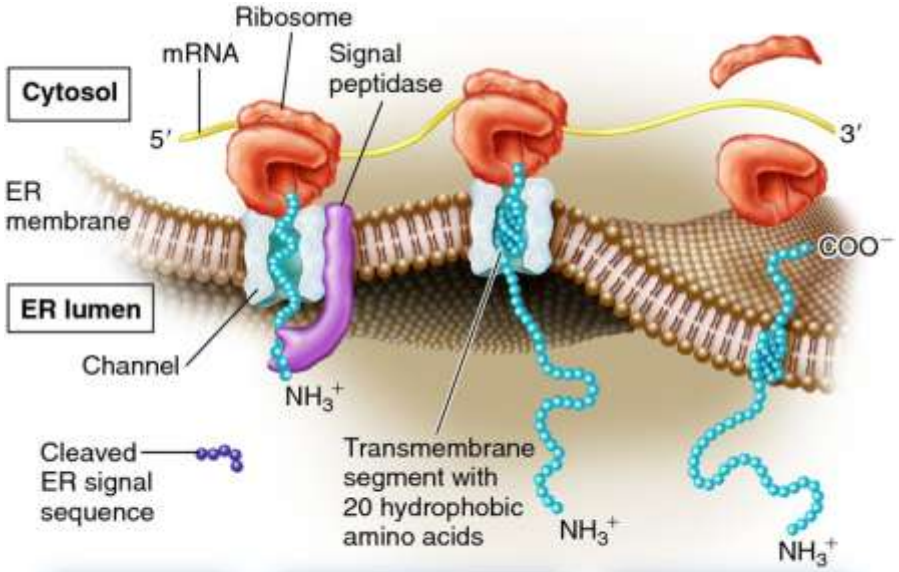


Example Question

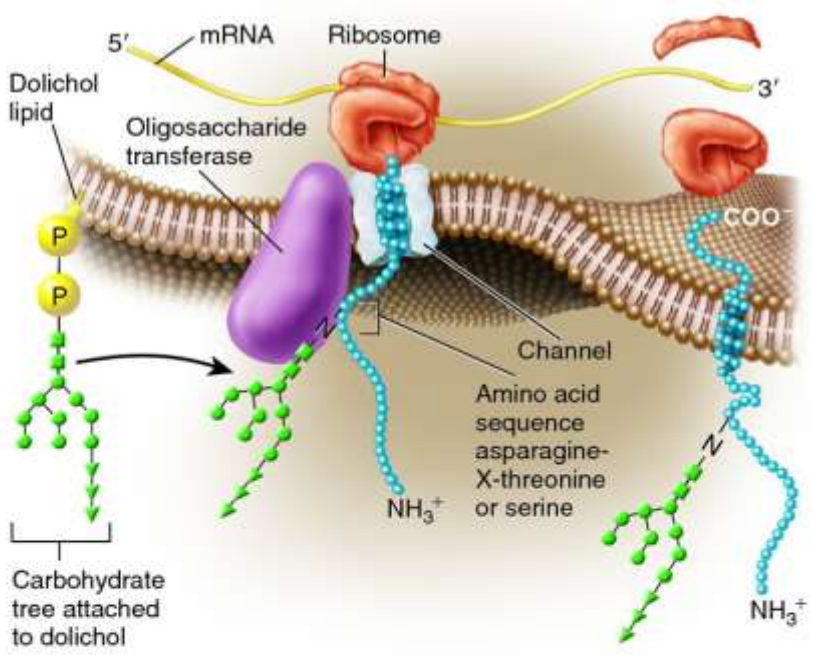
V-snare/t-snare interactions are important for:

a) Guiding ribosomes to channel proteins in the ER
b) Cleaving proteins in the ER lumen
c) Guiding vesicles containing proteins from the ER to the Golgi
d) All of the above

Inserting Transmembrane Proteins

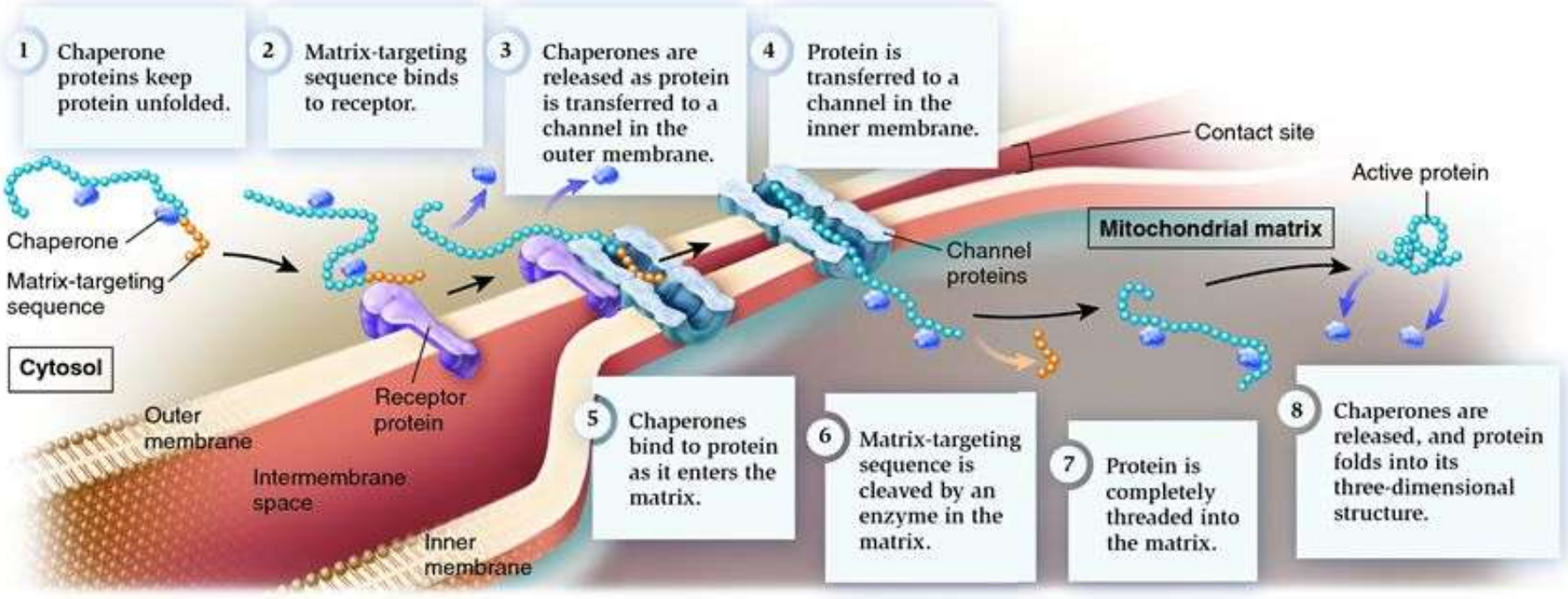


Glycosylation of Proteins



1. Prior to glycosylation of a protein, a group of 14 sugars is built onto the lipid dolichol in the ER membrane.
2. Oligosaccharide transferase removes the carbohydrate tree from dolichol and transfers it to an asparagine in the polypeptide.
3. Polypeptide synthesis is completed.

Post-translational Sorting

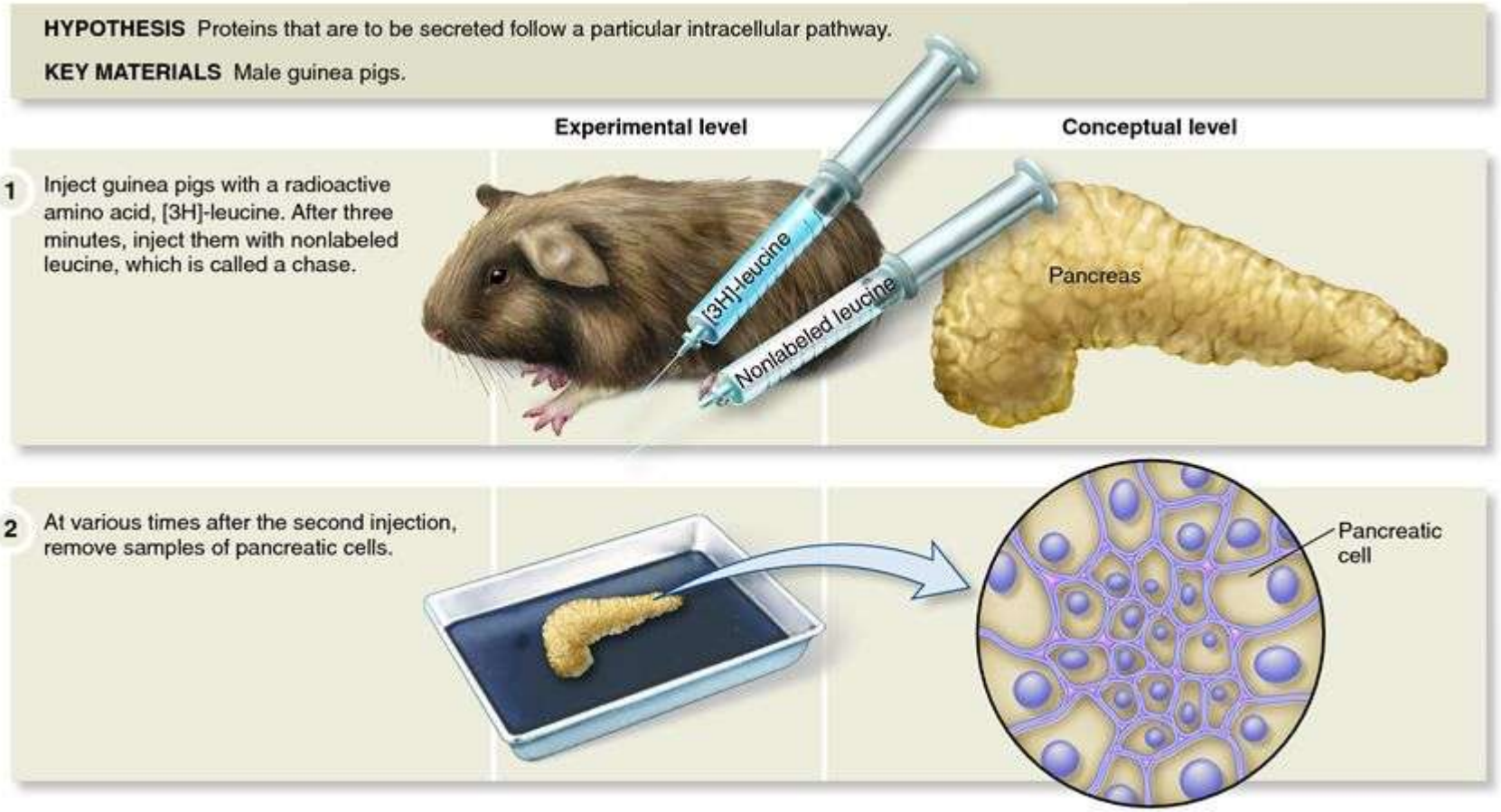


Example Question

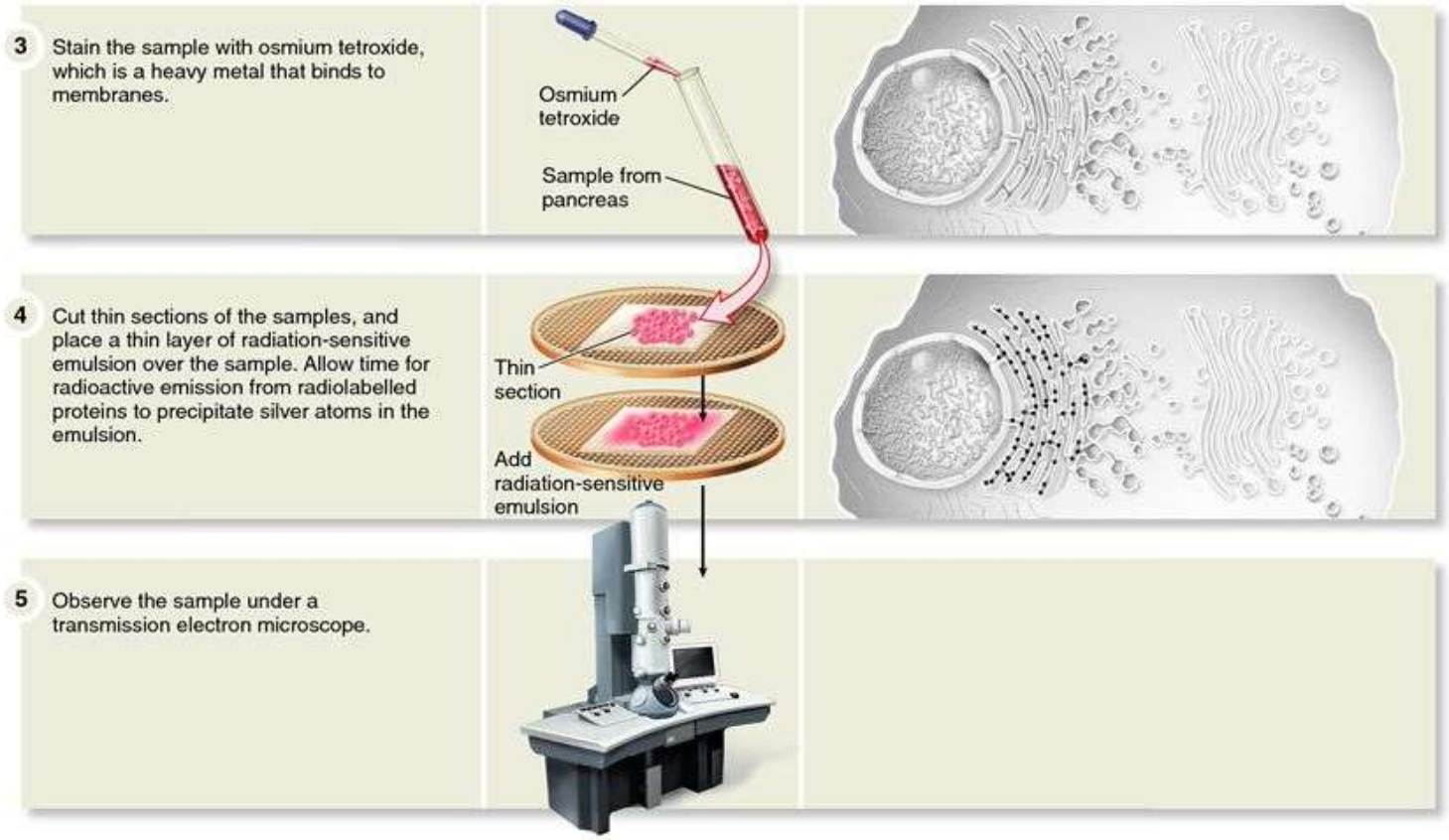
Which of the following is incorrect:

a) Glycosylation plays a role in protein folding
b) All proteins for mitochondria and chloroplasts are post-translationally sorted
c) Hydrophobic transmembrane regions anchor transmembrane proteins
d) Chaperones keep proteins unfolded

Palade's Experiment

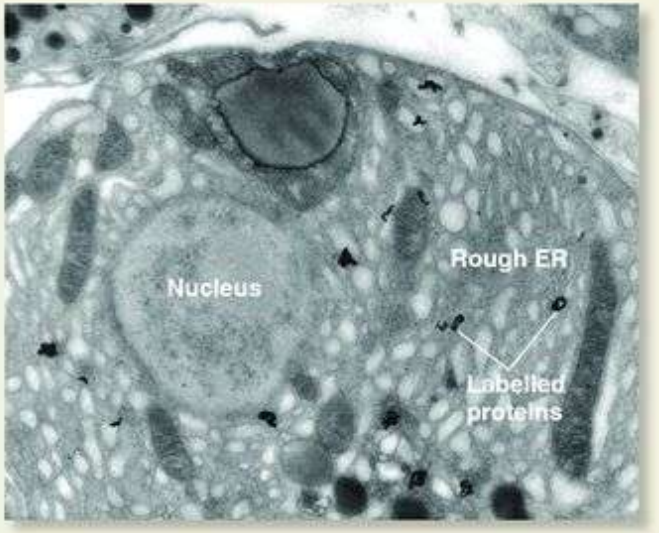


Palade's Experiment

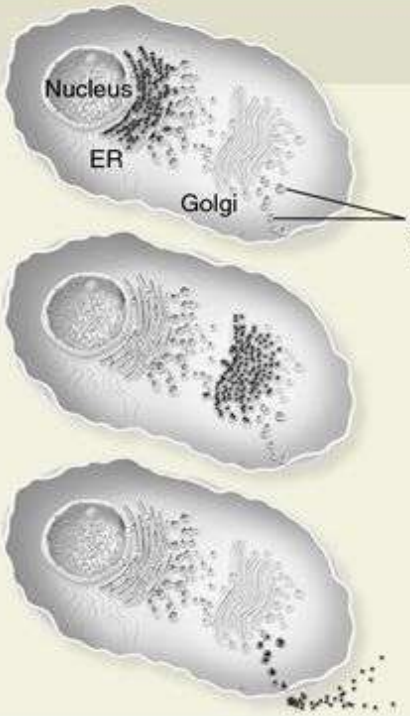


Palade's Experiment

6 THE DATA



Five minutes after chase



Time after chase
5 min
15 min
>30 min

7 CONCLUSION To be secreted, proteins move from the ER to the Golgi to secretory vesicles and then to the plasma membrane, where they are released to the outside of the cell.

8 SOURCE Caro, L.G., and Palade, G.E (1964) Protein synthesis, storage, and discharge in the pancreatic exocrine cell. An autoradiographic study. *Journal of Cell Biology* 20:473–495.

Proteasome

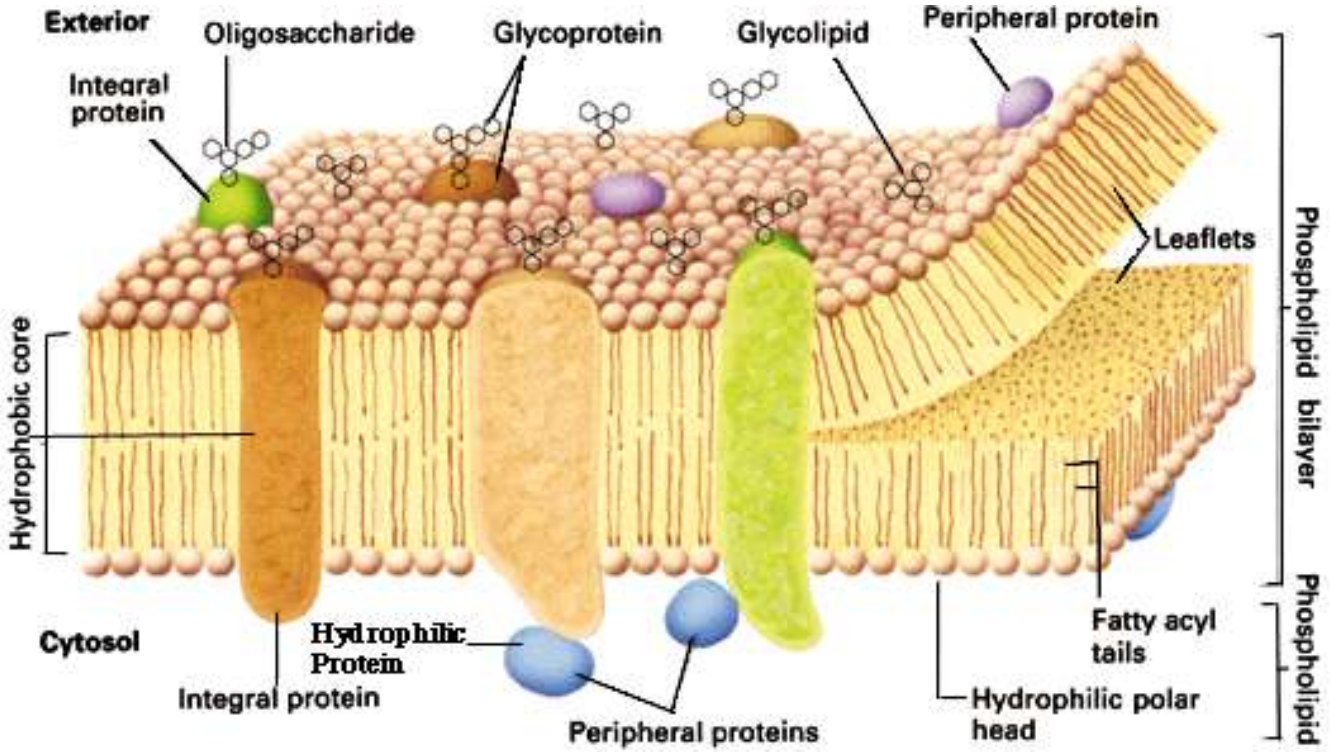
- Faulty or non-functional proteins must be degraded
- Proteasome is the molecular machine for protein degradation
- Ubiquitin directs unwanted proteins to proteasomes in eukaryote
  - Polyubiquinilation
- Proteases degrade the unwanted protein into peptides and amino acids
  - Provides materials for synthesis
- Also targets misfolded proteins
- Critical for environmental responses

Example Question:

Proteasomes are:

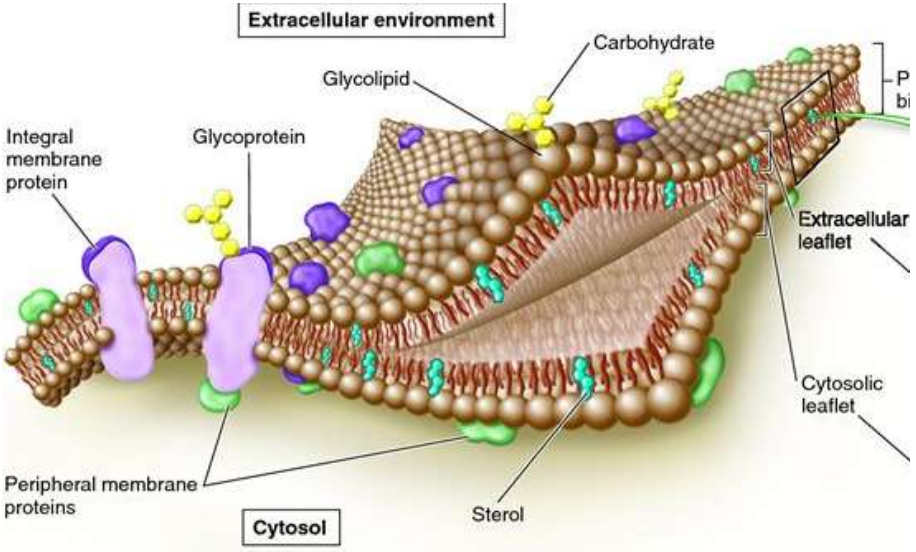
a) The same as proteomes
b) Used in Palade's pulse-chase experiment
c) Sites for protein degradation
d) Polyubiquinilated proteomes

Chapter 6 – Membrane Structure and Transport



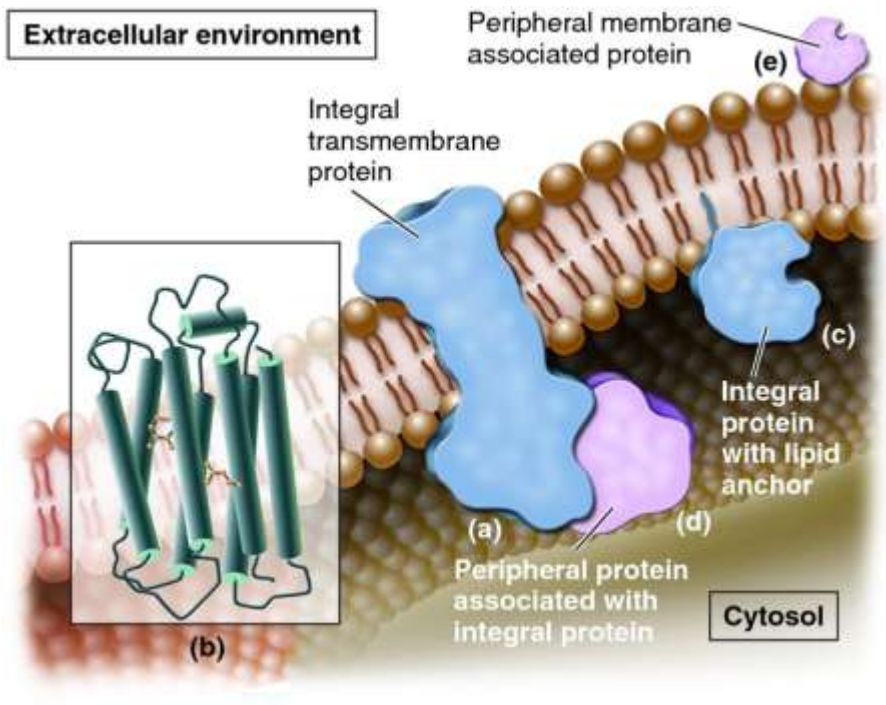
Biological Membranes

- 3 main functions
  - Regulation of transport
  - Compartmentalization
  - Communication/signal transduction
- Phospholipid bilayer
  - Phospholipids are amphipathic
- Also contain proteins & carbohydrates
  - Relative amount of each vary
- Fluid-mosaic model



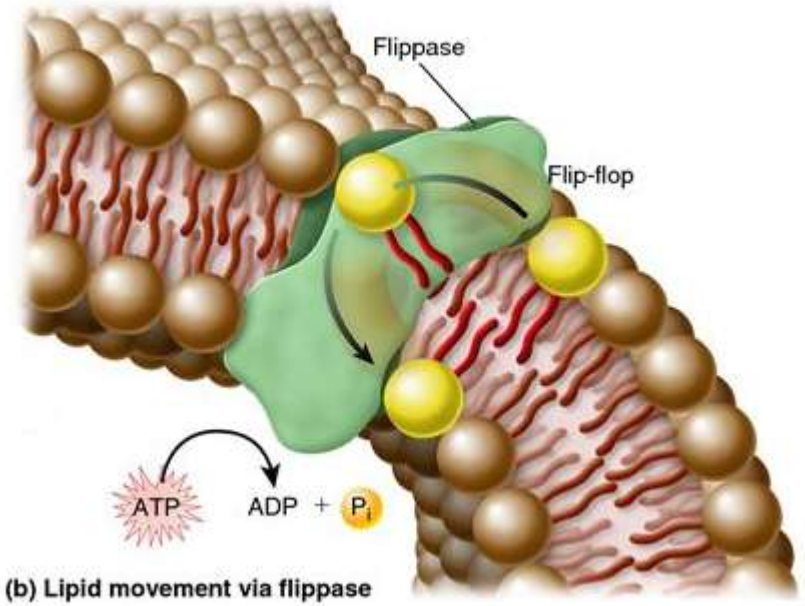
Membrane Proteins

- Proteins are a critical aspect of membrane structure and function
- Integral membrane proteins
  - Physically anchored to the membrane
  - Transmembrane (TM) proteins
  - Lipid anchors
- Peripheral membrane proteins
  - Indirectly or loosely associated to the membrane
  - Noncovalently bound to integral membrane proteins or to the polar head groups of phospholipids (PLs)



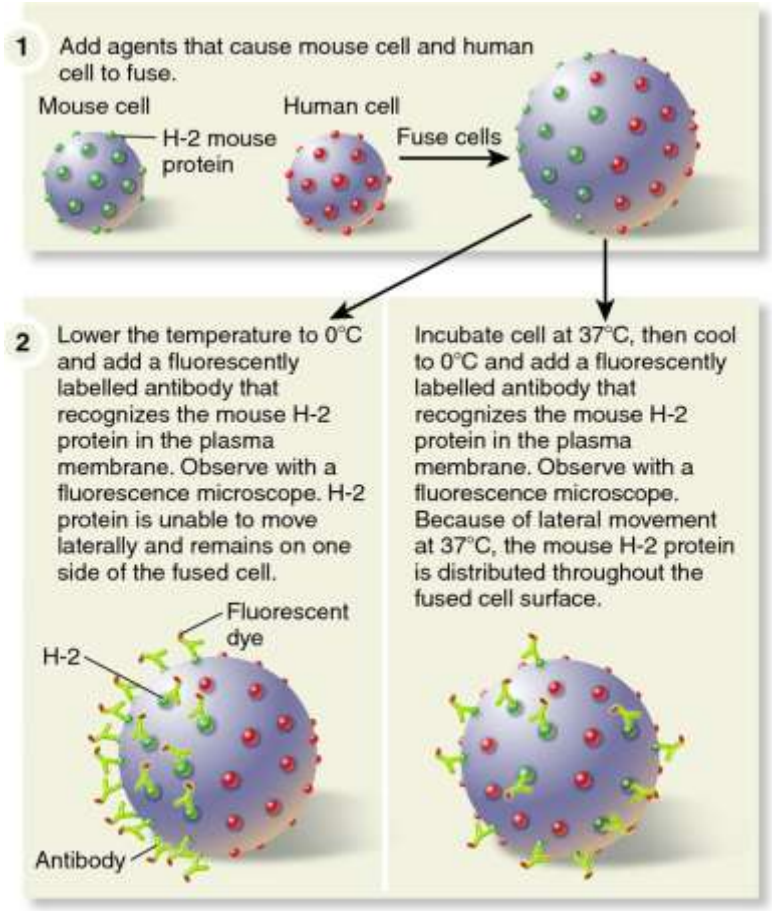
Factors Affecting Fluidity

- Length of fatty acyl tails
  - Shorter acyl tails are less likely to interact, which makes the membrane more fluid
- Presence of double bonds in the acyl tails
  - Kink tail, more difficult for neighbouring tails to interact = more fluid
- Presence of cholesterol (animals)
  - Cholesterol tends to stabilize membranes
- Effects depend on temperature



(b) Lipid movement via flippase

Lateral Transport

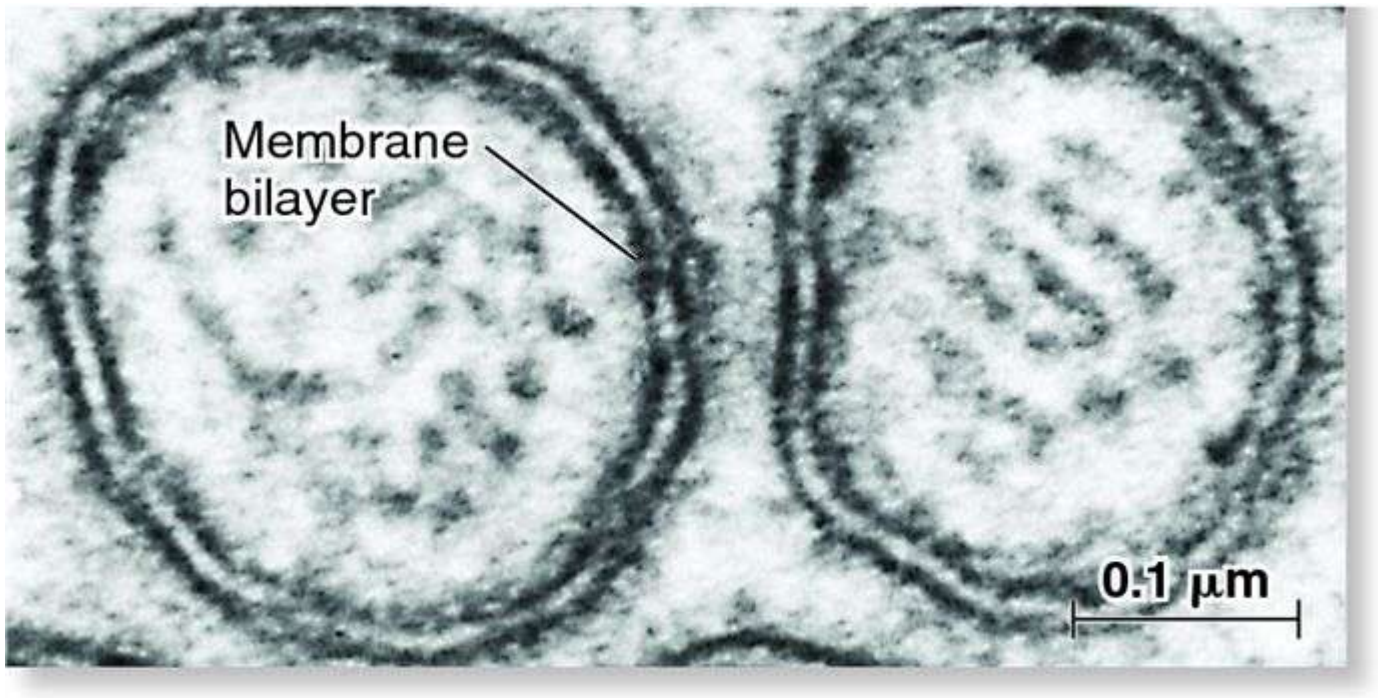


Example Question

What is/are the function(s) of biological membranes?

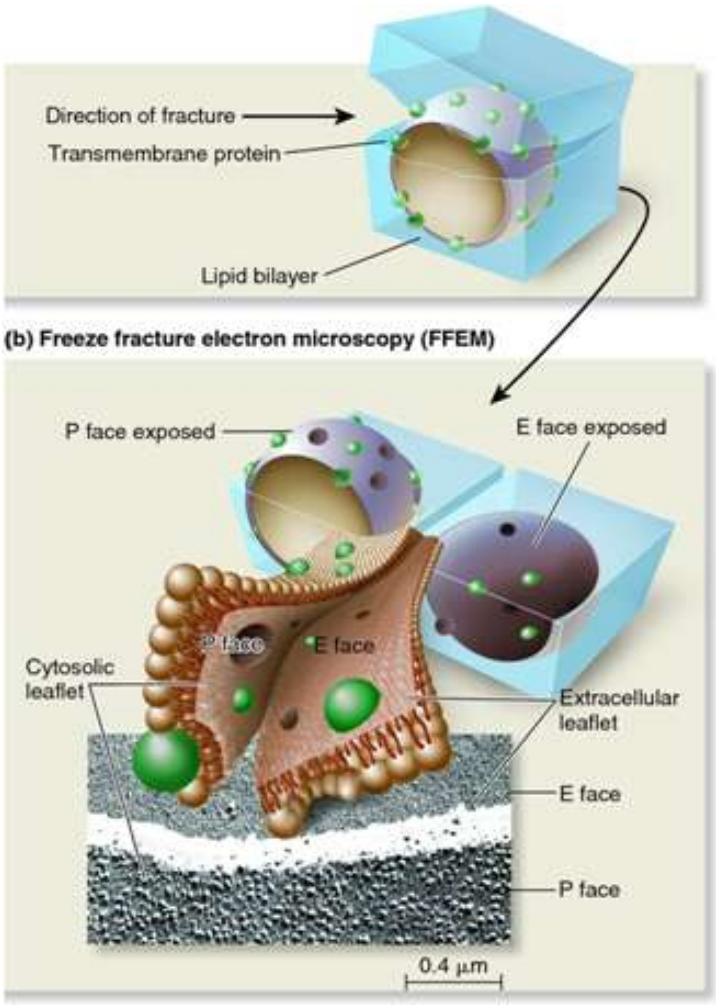
a) Compartmentalization
b) Regulation of transport
c) Communication/signal transduction
d) All of the above

Looking at Plasma Membranes



(a) Transmission electron microscopy (TEM)

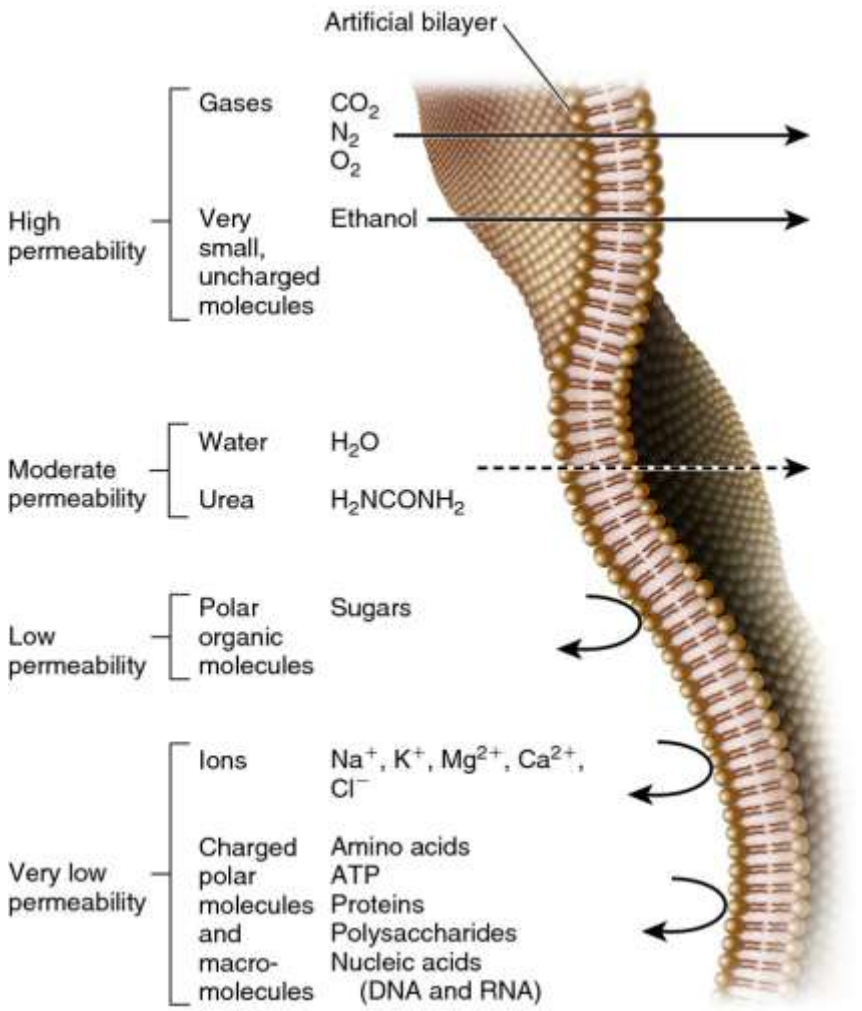
Looking at Plasma Membranes



Phospholipid Bilayer is a Barrier

- Hydrophobic interior makes formidable barrier
- Selectively-permeable
  - Some things can freely pass, others need to go through gates or channels
- Diffusion
  - Movement of solute from an area of higher concentration to an area of lower concentration
  - Passive diffusion - without transport protein
  - Solutes vary in their rates of penetration
- Permeability decreases with polarization, charge, and size

Permeability of the Membrane



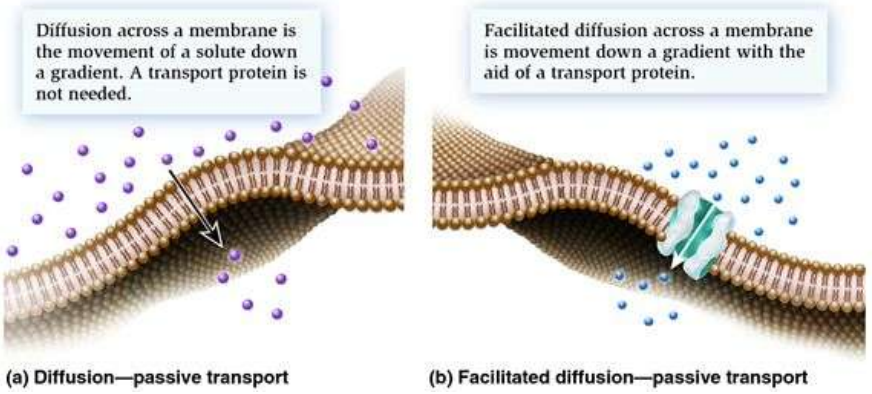
Example Question

A highly permeable molecule would be:

a) Charged
b) Small
c) Non-polar
d) A and B
e) B and C

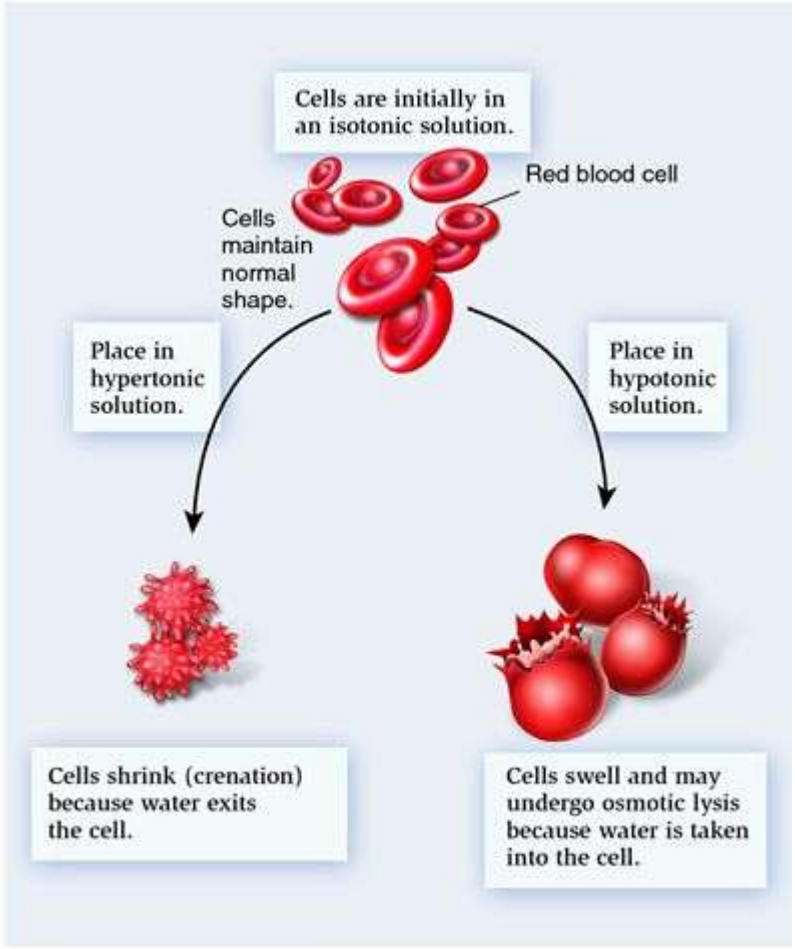
Gradients and Passive Transport

- Can be chemical and/or electrical
- Passive transport
  - No energy needed
- 2 types
  - Passive diffusion
    - Notransport protein
  - Facilitated diffusion
    - Aided by a transport protein (specificity!)
    - Occurs through a channel, not simply a pore (or hole)



Tonicity – Effect on cell volume (shape)

- Refers to the concentration of solutes
- Water balance is critical to all cells
- Isotonic
  - Equal concentrations
- Hypertonic
  - Solute concentration is higher on one side of the membrane
  - Cells shrink
- Hypotonic
  - Opposite of hypertonic
- Shrinkage/swelling is due to the movement of water down its chemical potential
  - Movement is passive (no energy used)
  - Chemical potential will always be lower wherever there are more solutes

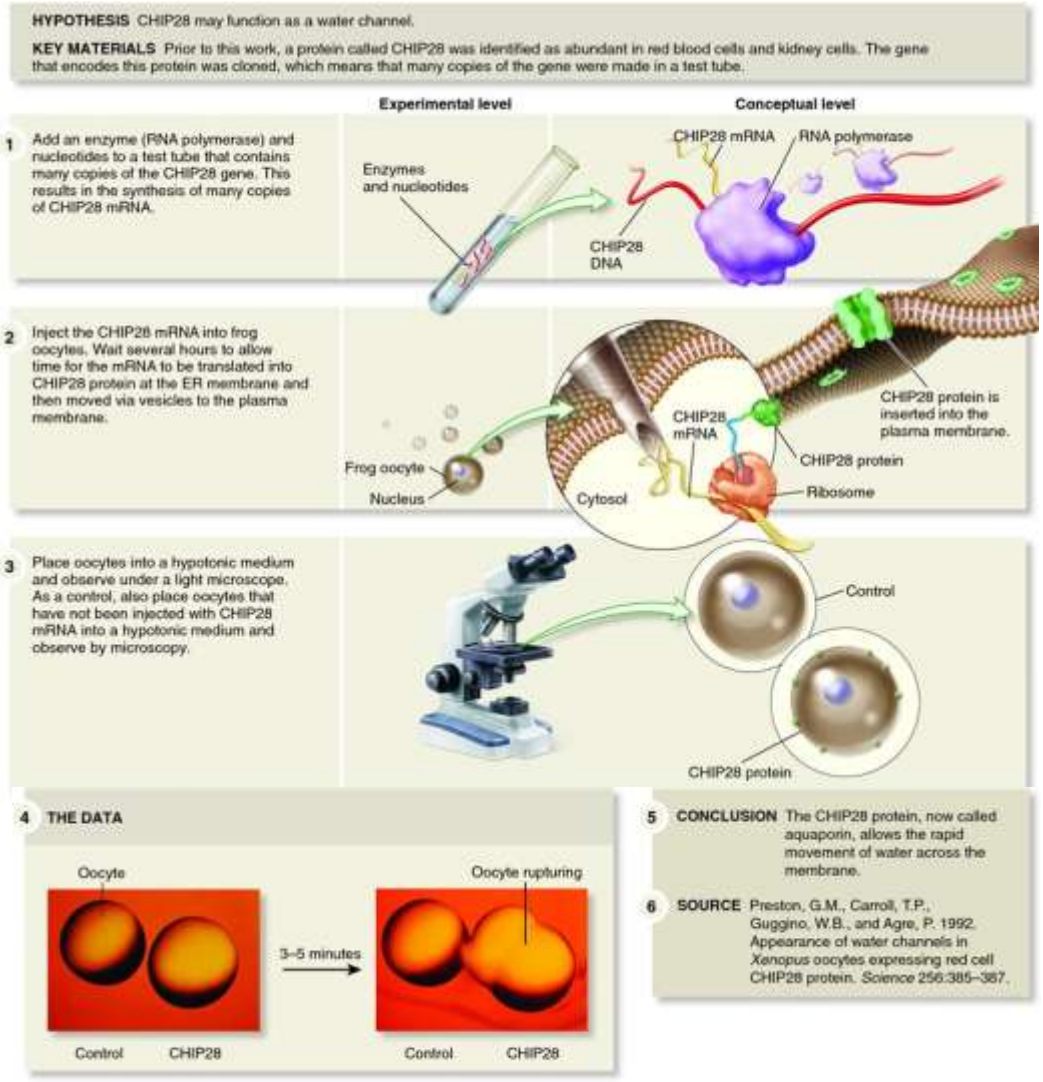


(a) Osmosis in animal cells

Osmolarity

- Osmolarity is the total concentration of solutes from the point of view of the cell
- Take a cell and put it into a medium of 10 mmol urea
  - The solution is hyperosmotic but not hypertonic (ultimately)
- Remember permeability!
  - Urea is permeable

Water Movement



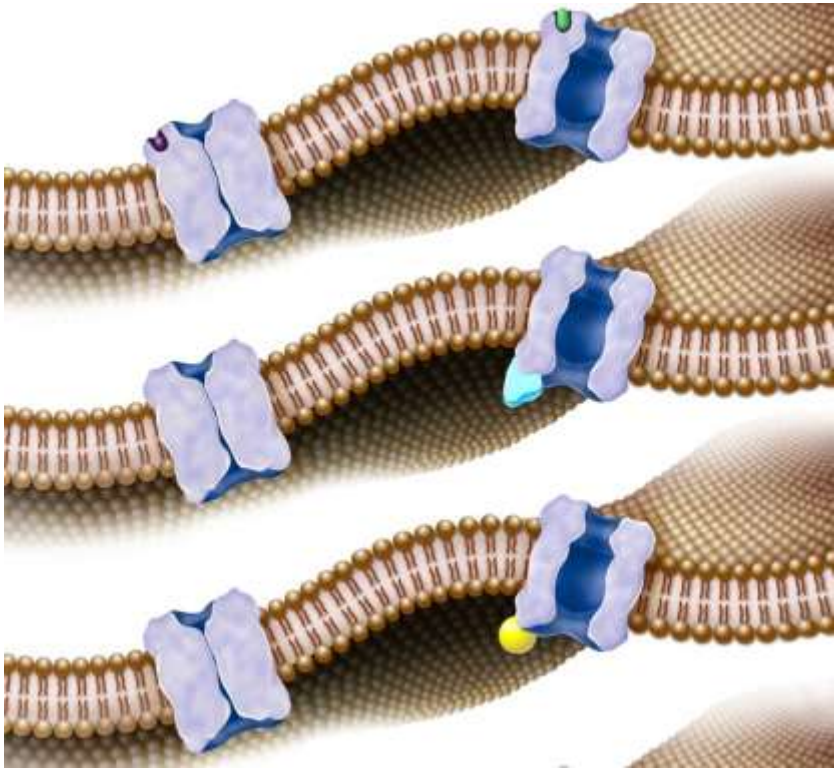
Example Question

An isotonic solution...

a) Causes cells to shrink
b) Causes cells to swell
c) Is caused by unequal concentration gradients
d) None of the above

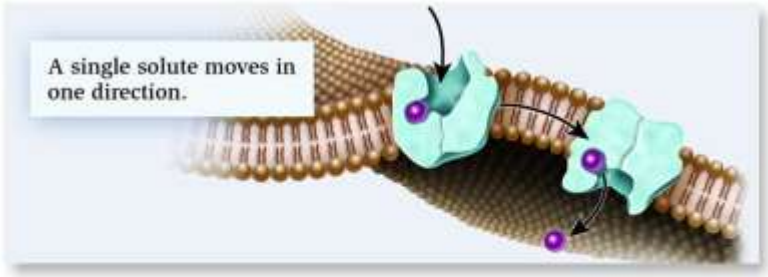
Channel Proteins

- Conduit for the direct diffusion of ions or molecules across the membrane
- These channels also have high specificity
- Most channels are gated open or closed, can be opened or closed in the following ways:
  - Ligand-gated
  - Intracellular regulatory proteins
    - Change triggered by binding of a regulatory protein on its intracellular surface
  - Phosphorylation
  - Voltage-gated
  - Mechanosensitive channels
    - Physically opened or closed (eg. sound)

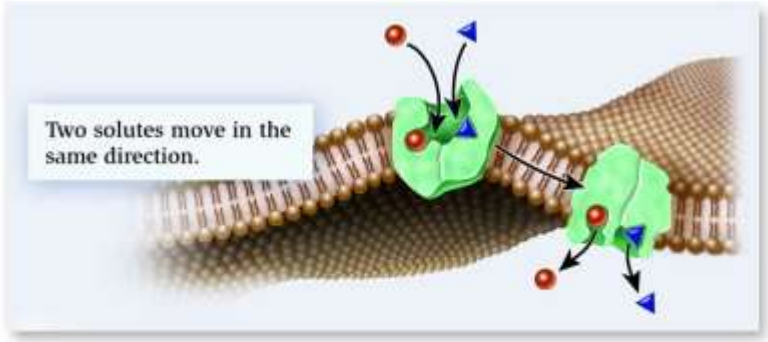


Transporters

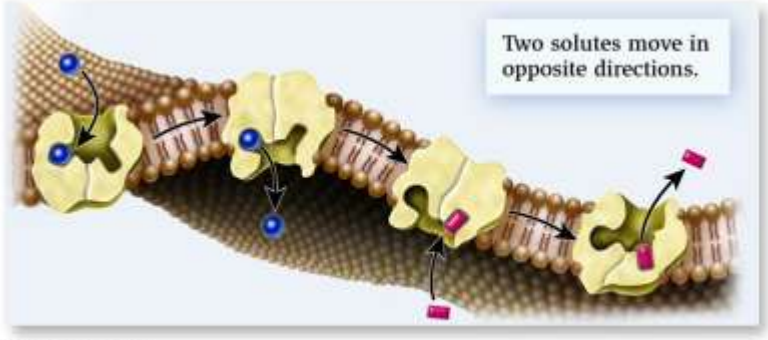
- Different than channels because there is a physical binding of the substance
- Conformational change transports the solute
- Principal pathway for uptake of organic molecules, such as sugars, amino acids, nucleotides
- Key role in export
- Transporter types
  - Uniporter
  - Symporter/cotransporter
    - In general one binds first and makes the transporter have a higher affinity for the other
  - Antiporter
    - Again this usually happens sequentially



(a) Uniporter



(b) Symporter



(c) Antiporter

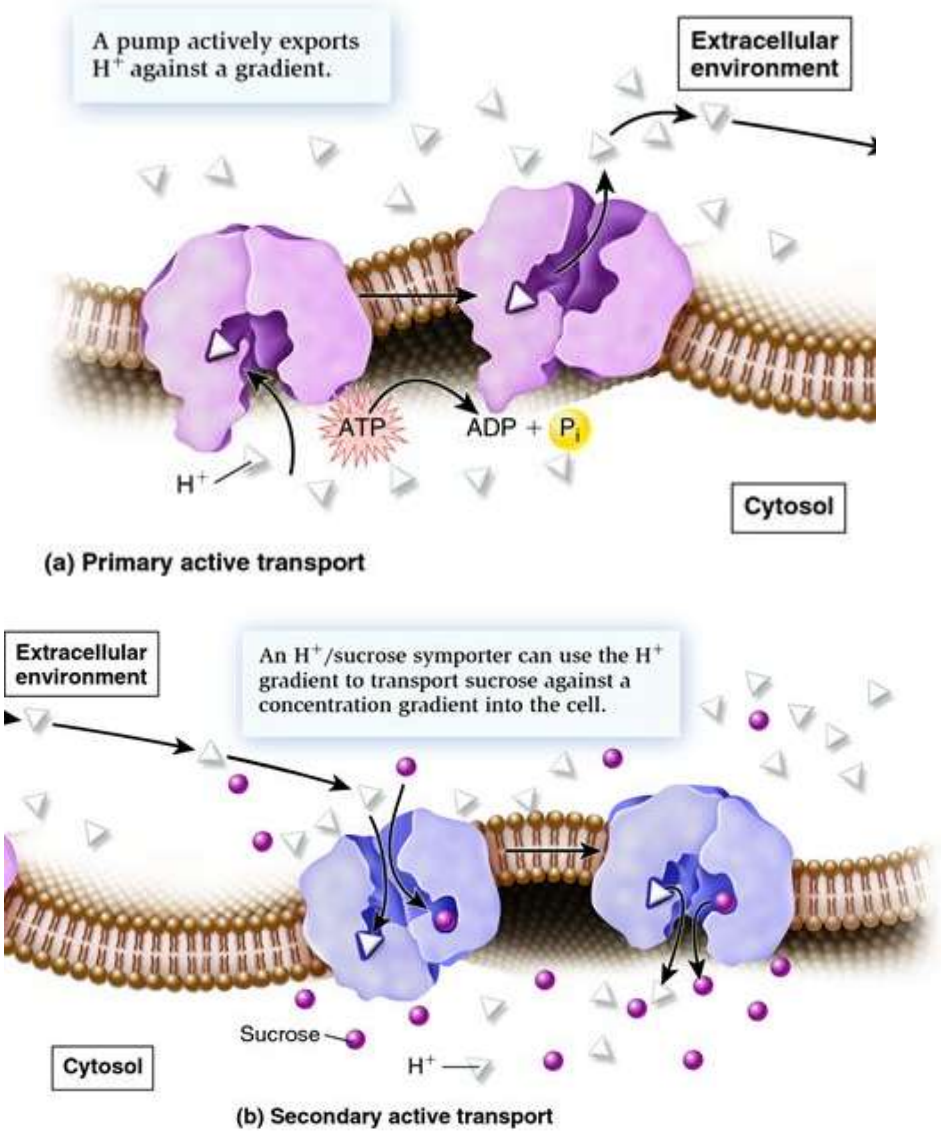
Example Question

Which of the following is a type of channel protein?

a) Symporter
b) Voltage-gated
c) Antiporter
d) A and C
e) None of the above

Active Transport

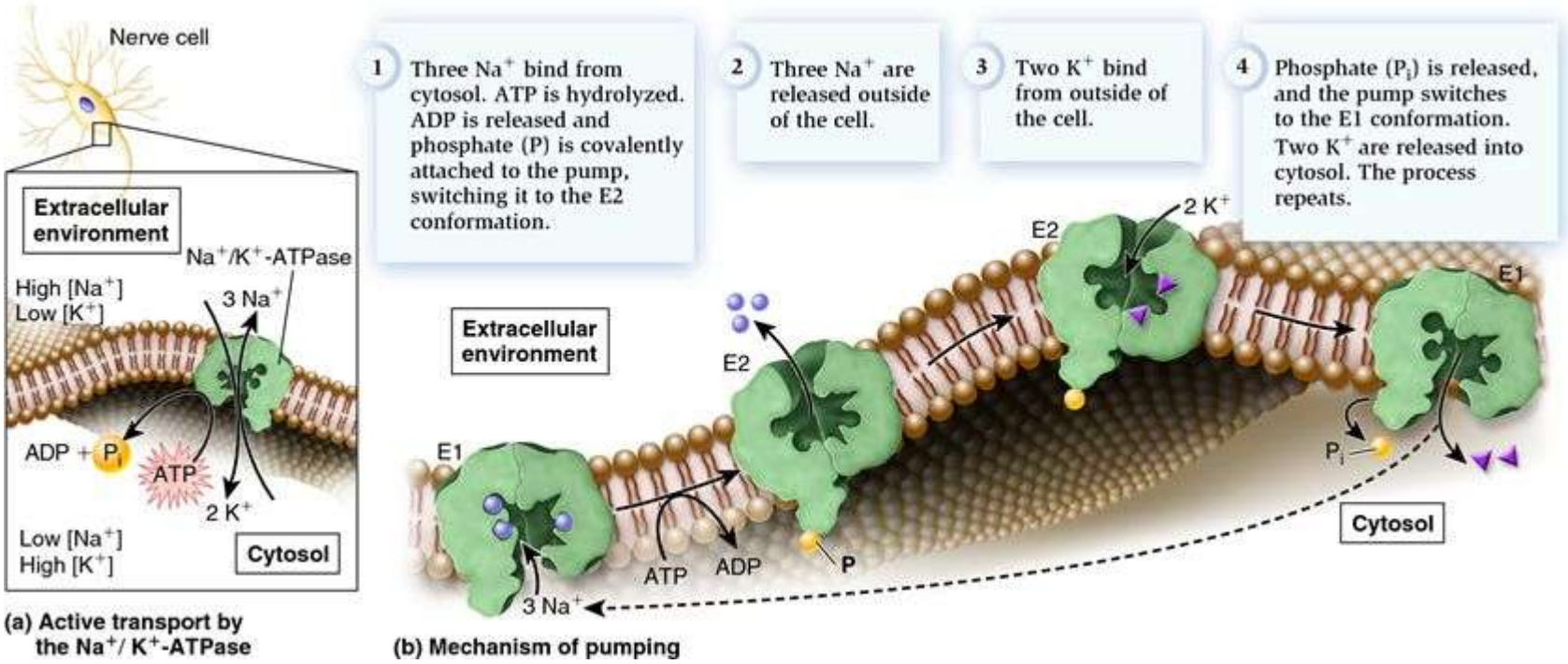
- Movement of a solute across a membrane against its gradient
- Energetically unfavorable: requires energy input
- Primary active transport
  - Directly use energy to transport solute
    - Eg. uses ATP to pump the solute
- Secondary active transport
  - Use pre-existing gradient to drive transport of solute
    - Uses an existing gradient to move other solutes against their gradient
    - Commonly exploits H^+ and K^+ gradients



Pumps

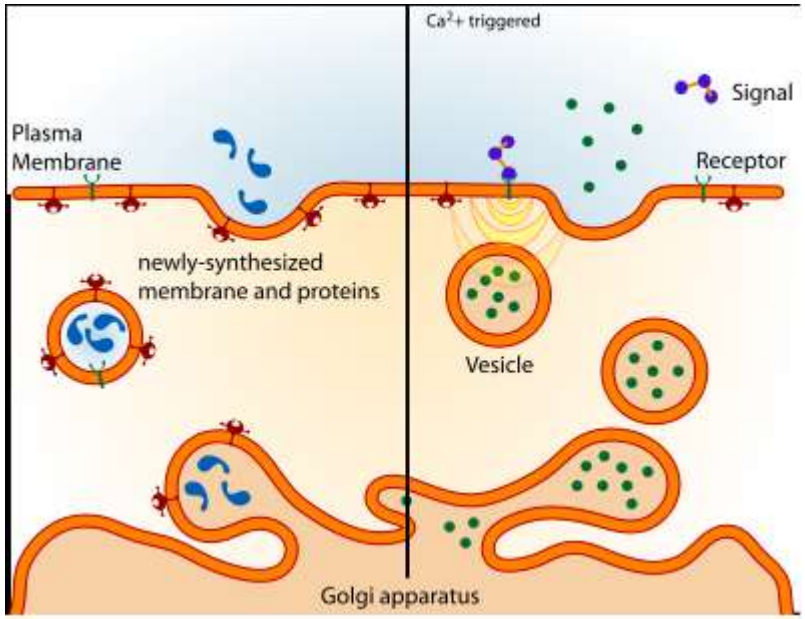
- ATP-driven ion pumps generate electrochemical gradients
- Na^+/K^+-ATPase
  - Actively transport Na+ and K+ against their gradients by using the energy from ATP hydrolysis
  - 3 Na^+ exported for 2 K^+ imported into cell
    - Antiporter (Na^+ out and K^+ in)
    - Electrogenic pump - export 1 net positive charge
    - Moves more positive charges out then in

Pumps


Nerve cell
Extracellular environment
Na+/K+-ATPase
High [Na+]
Low [K+]
3 Na+
ADP + Pi
ATP
2 K+
Low [Na+]
High [K+]
Cytosol
(a) Active transport by the Na+/ K+-ATPase
1 Three Na+ bind from cytosol. ATP is hydrolyzed. ADP is released and phosphate (P) is covalently attached to the pump, switching it to the E2 conformation.
2 Three Na+ are released outside of the cell.
3 Two K+ bind from outside of the cell.
4 Phosphate (Pi) is released, and the pump switches to the E1 conformation. Two K+ are released into cytosol. The process repeats.
Extracellular environment
E1
E2
ATP
ADP
P
3 Na+
2 K+
Pi
Cytosol
(b) Mechanism of pumping

Endocytosis/Exocytosis

- Exocytosis
  - Material inside the cell → outside via vesicles
  - Starts in the endomembrane system
- Endocytosis
  - Plasma membrane invaginates, or folds inward, to form a vesicle that brings substances into the cell
  - Receptor-mediated endocytosis
  - Pinocytosis
    - Solutes in solution (cell drinking)
  - Phagocytosis
    - Solutes not in solution (cell eating)
- Both endo and exocytosis require high fidelity of the receptors for their cargo

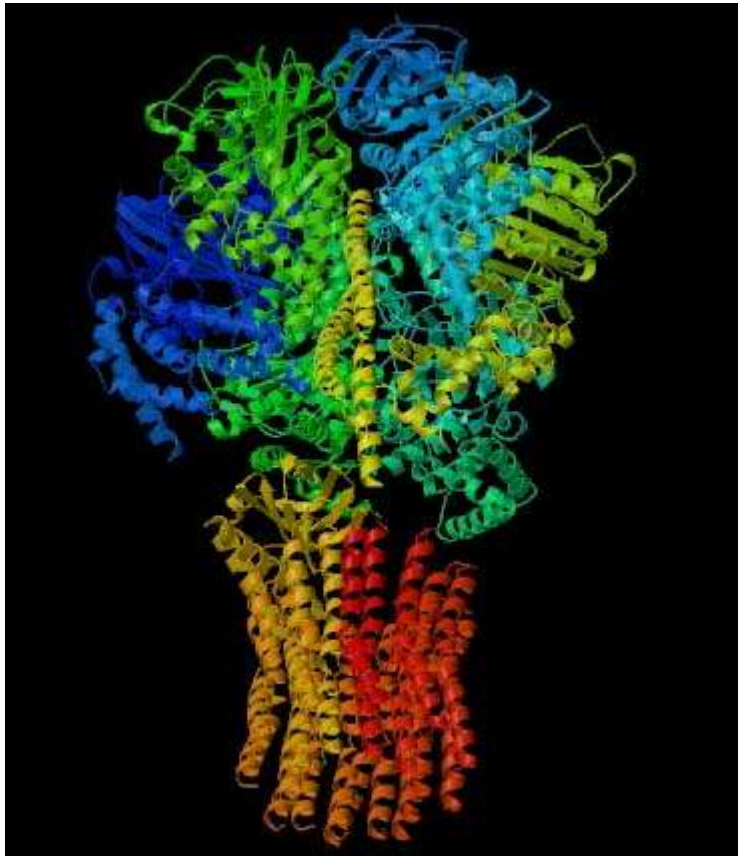


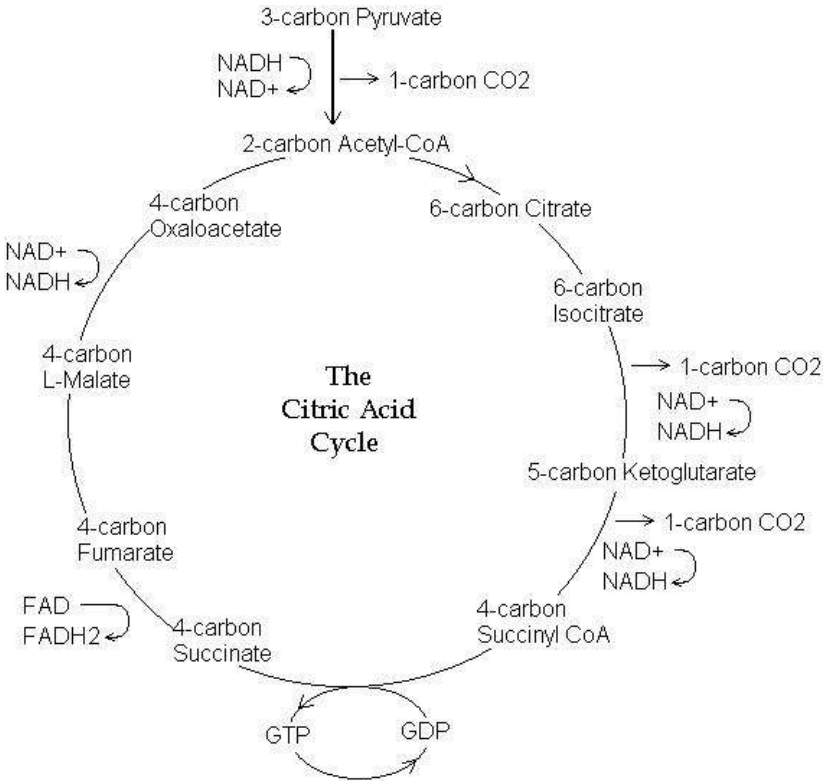
Example Question

Active transport...

a) Directly uses energy to transport a solute
b) Uses a pre-existing gradient to transfer a solute
c) Does not need energy to transfer a solute
d) A and B
e) None of the above

Chapter 7 – Enzymes and Metabolism



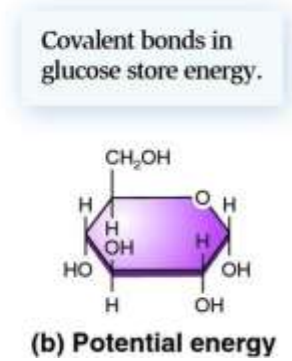


Chemical Reactions and Energy

- 2 factors govern fate of a chemical RXN
  - Direction
    - Need energy to drive it in the right direction
    - ATP
  - Rate
    - Catalysts called enzyme can speed the reaction rate
- Energy
  - Ability to promote change
- 2 forms that we're interested in
  - Kinetic: associated with movement
  - Potential: due to structure or location
    - Chemical energy: energy in molecular bonds

$$aA + bB \rightleftharpoons cC + dD$$

(a) Kinetic energy



(b) Potential energy

Laws of Thermodynamics

- First law
  - Called the law of conservation of energy
  - Energy cannot be created or destroyed
- Second law
  - Transfer or transformation of energy from one form to another increases entropy or degree of disorder of a system

Total energy = usable energy + unusable energy

- H= enthalpy or total energy
- G= free energy or amount of energy for work
  - Gibb's Free energy
- S= entropy or unusable energy
- T= absolute temperature in Kelvin (K)
- Changes in free energy (G) determine whether or not a chemical reaction is spontaneous
  - No additional input of energy necessary

$$H = G + TS$$

$$G = H - TS$$

Spontaneous Reactions

- Thermodynamically favoured
- Not necessarily fast
- Key factor is the free energy change
  - $\Delta G = \Delta H - T\Delta S$
- Biochemistry uses $\Delta G'^{o}$: "standard" free energy change
  - ΔG is not a constant
  - $\Delta G'^{o}$ is a constant
- Specific conditions and concentrations

$$\Delta G = \Delta H - T\Delta S$$

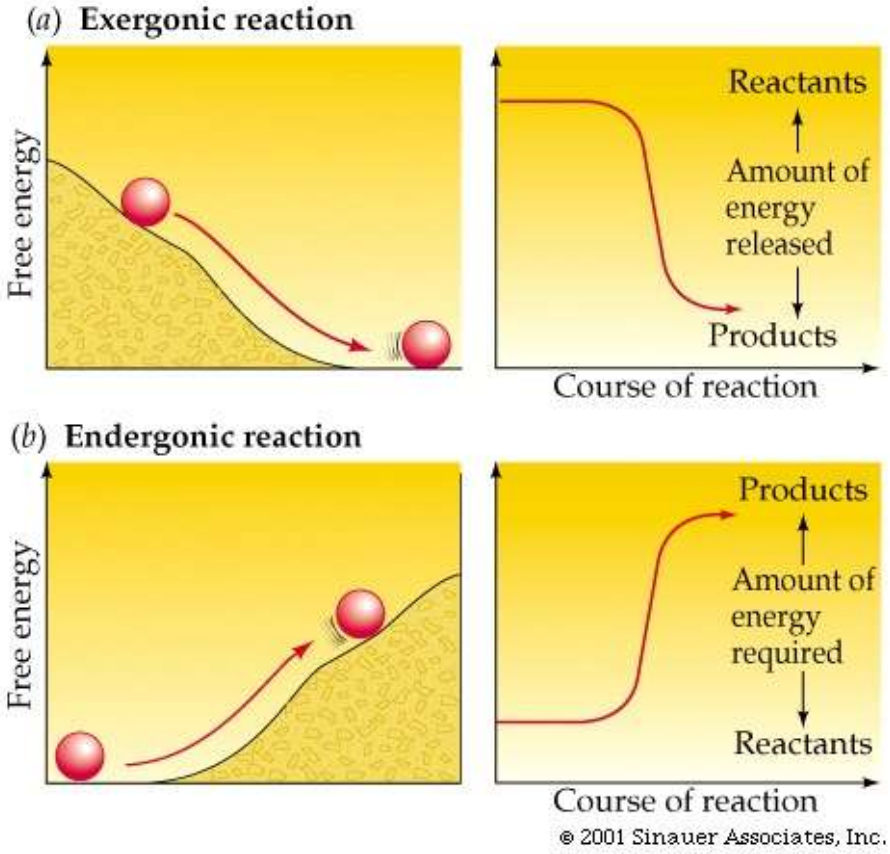
Example Question

The Law of Conservation of Energy states:

a) Transformation of energy from one form to another increases entropy
b) Energy can be destroyed but not created
c) Energy can be created but not destroyed
d) Energy cannot be created or destroyed

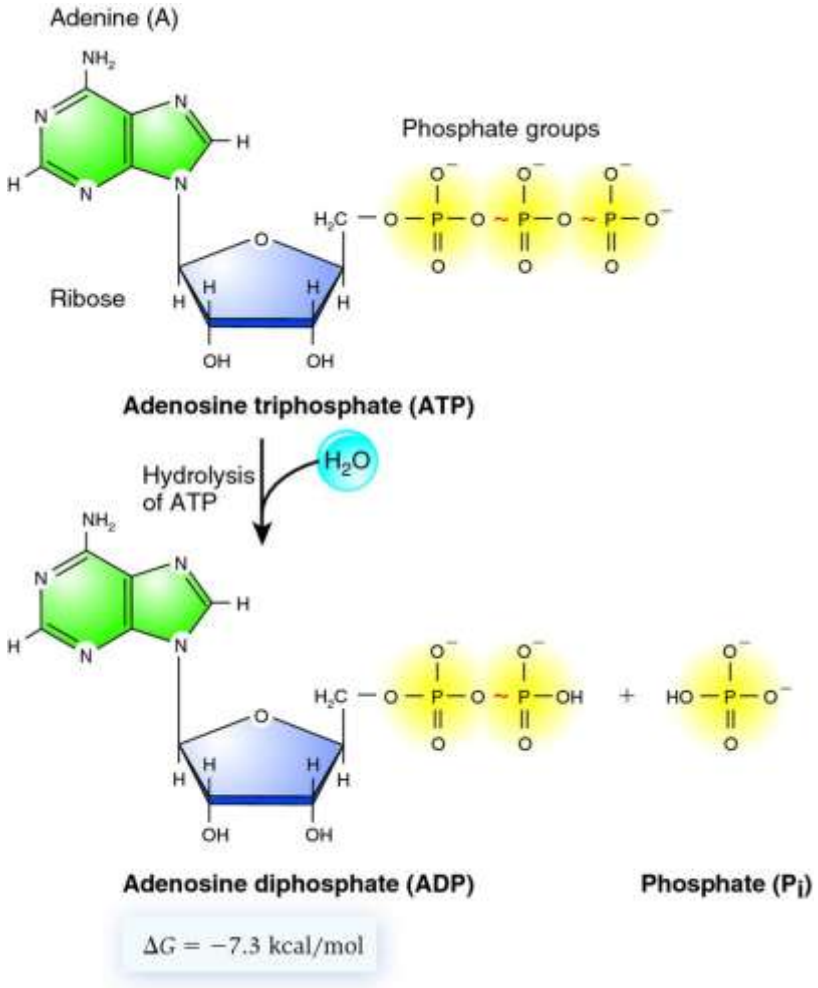
Endergonic vs. Exergonic

- Exergonic
  - $\Delta G'^{o}<0$ or negative free energy change
  - Spontaneous
- Endergonic
  - $\Delta G'^{o}>0$ or positive free energy change
  - Requires addition of free energy
    - ATP



Why use ATP?

- Hydrolysis of ATP $\Delta G'^{o}$ = -7.3 kcal/mole
  - This reaction releases energy
  - Covalent bonds between the phosphate groups are broken
  - ATP is used because ADP and phosphate are stable and at a much lower energy state
- Favours formation of products
- Energy liberated can drive a variety of cellular processes
- Link exergonic RXN to drive endergonic



Coupled Reaction

$$\text{glucose} + \text{phosphate}^{2-} \rightarrow \text{glucose phosphate}^{2-} + H_2O \qquad \Delta G = +3.3 \text{ kcal/mol}$$

$$ATP^{4-} + H_2O \rightarrow ATP^{2-} + P_i^{2-} \qquad \Delta G = -7.3 \text{kcal/mole}$$

$$\text{glucose} + ATP^{4-} \rightarrow \text{glucose phosphate}^{2-} + ADP^{2-} \qquad \Delta G = -4.0 \text{ kcal/mol}$$

- By itself, the first reaction would not be spontaneous
- If the two reactions are coupled the net free energy change for both reactions combined is exergonic

Equilibrium constant (K_{eq})

- Chemical equilibrium is when the rate of formation of products equals the rate of formation of reactants
- K_{eq} can be used to help determine ΔG for a reaction
- The mass action ratio is K_{eq} under a specific set of conditions

$$a\,\mathrm{A} + b\,\mathrm{B} \rightleftharpoons c\,\mathrm{C} + d\,\mathrm{D}$$

$$K_{eq} = \frac{[\mathrm{C}]^c\,[\mathrm{D}]^d}{[\mathrm{A}]^a\,[\mathrm{B}]^b}$$

Equilibrium constant (K_{eq})

- You can use this value in $\Delta G'^{o} = -RT\ln(K_{èq})$
  - R is the gas constant and T is temperature
  - If $\Delta G'^{o}$ is positive it is endergonic, negative is exergonic
- In the real version of the cell $\Delta G = \Delta G'^{o} + RT\ln(Q)$
  - $Q = K'_{eq}$
- Remember that ΔG is not a constant because Q is determined by what is happening in the cell (because of K'_{eq})

$$\Delta G'^{o} = -RT\ln(K_{eq})$$

There are 2 ways to drive endergonic reactions

1. Manipulate the ratios of [C] +[D] to [A]+[B]
   - Manipulating the concentrations of reactants and products
   - So if you remove the products right away you can drive this reaction
2. Couple the reaction with an exergonic reaction

$$K_{eq} = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

$$\text{glucose} + \text{ATP}^{4-} \rightarrow \text{glucose phosphate}^{2-} + \text{ADP}^{2-}$$

$$\Delta G = -4.0 \text{ kcal/mol}$$

Example Question

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{2-} + P_i^{2-}$$

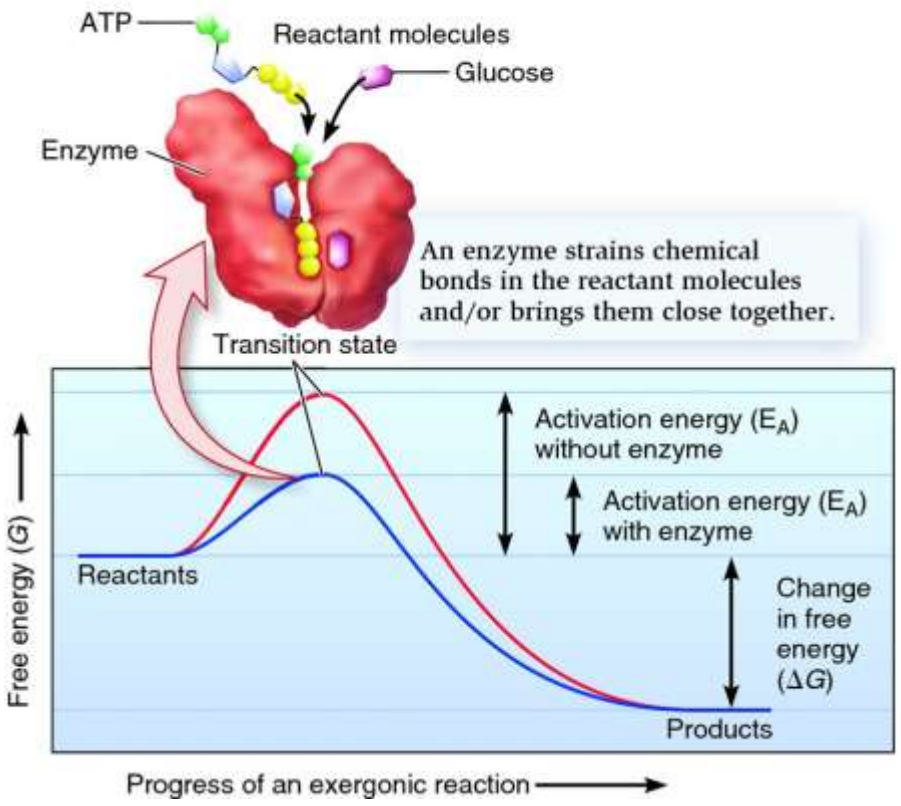
$$K_{eq}' = \frac{[ADP]\,[P_i]}{[ATP]}$$

Using the above equation for K_{eq} and the following concentrations ([ADP] = 10 mmol, the [P_i] = 2 mmol, and [ATP] = 10 mmol) if the reaction occurs at 25 ^{o}C then is it:

a) Exergonic
b) Endergonic
c) Hypergonic
d) Hypogonic

Activation Energy

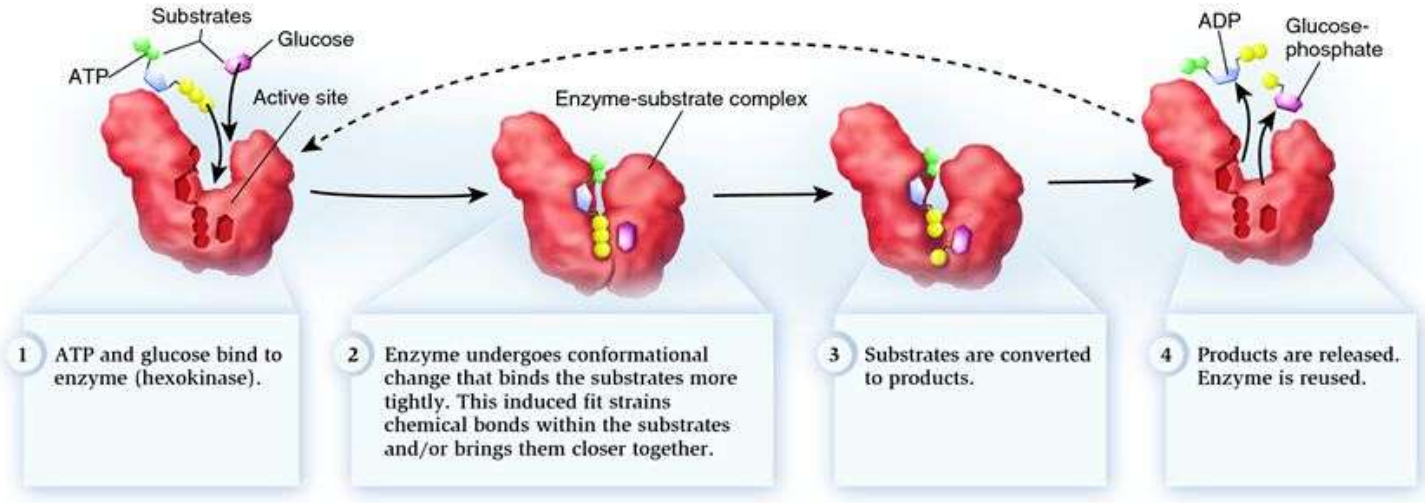
- Initial input of energy to start reaction
  - Even if a RXN is exergonic (favourable) doesn't mean it is just going to happen
  - Some energy needs to be put into it
- Allows molecules to get close enough to cause bond rearrangement
- Enzymes can lower the activation energy of a reaction
  - Often occurs by bringing the reactant molecules physically closer together
- Activation energy can also be overcome with heat



Enzymes

- Catalyst
  - Speeds up the rate of reaction without being consumed
- Enzymes
  - Protein catalysts in living cells
- May be multimeric complexes
- Often highly regulated (various levels)
- Decreases activation energy

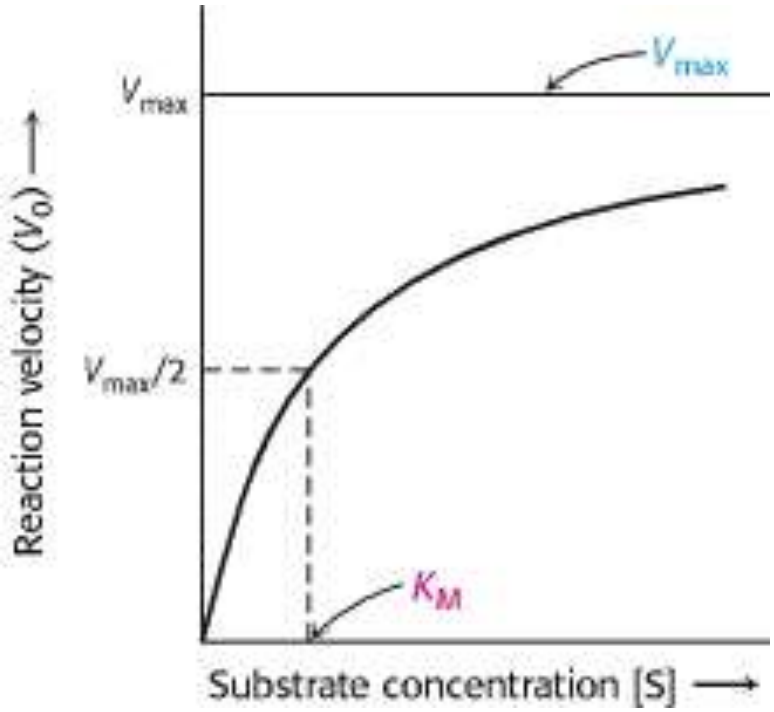
Enzymes Lowering Activation Energy



- Straining bonds in reactants to make it easier to achieve transition state
- Positioning reactants together to facilitate bonding
- Changing local environment of reactants
- Active site = where reaction takes place
  - Possesses a great deal of specificity and affinity for its substrates
  - Reaction is catalyzed by a conformational change in the enzyme
  - Once substrates have been converted to products they have a low affinity for the active site and are released
- Optimal temp, pH, etc. affect the enzymes and substrates

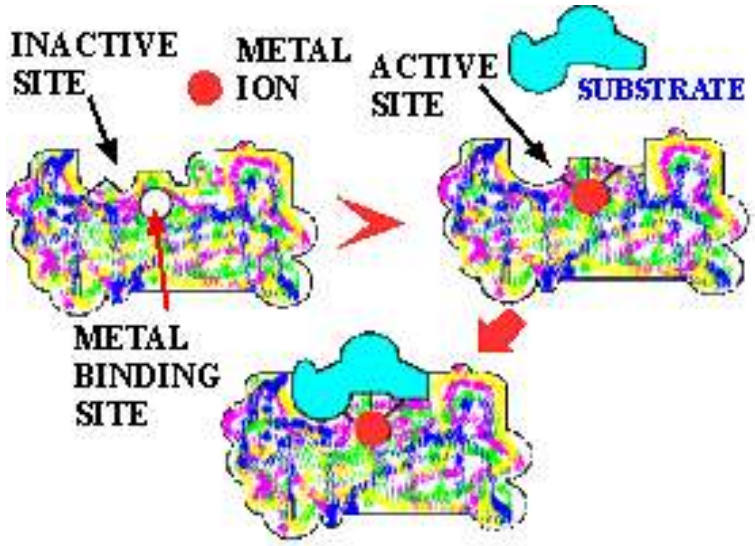
K_m (Michaelis constant)

- The affinity of an enzyme for its substrate is very important
- K_m is substrate concentration at half-max "speed" (V_{max})
  - This is a description of the affinity of the enzyme for its substrate
  - Low K_m means a very high affinity (tight binding)
- Expressed in mM, µM, nM, etc.



Other Enzyme Requirements

- Prosthetic groups
  - Small molecules (ion or organic) permanently attached to the enzyme
- Cofactor
  - Usually inorganic ion that temporarily binds to enzyme
  - Usually metals
- Coenzyme
  - Organic molecule that participates in reaction but left unchanged afterward (eg. NADH, vitamins)
- Enzymes use these to help in catalysis, they are left unchanged afterwards however



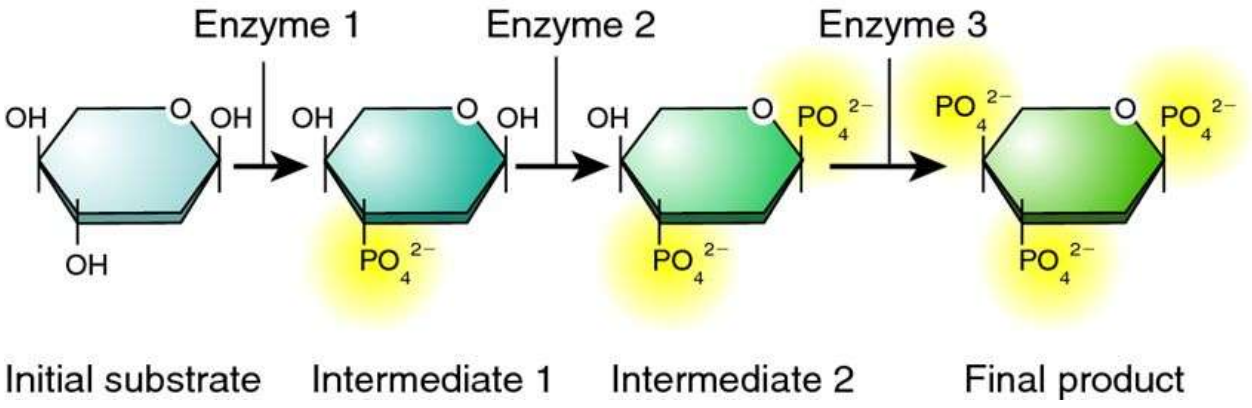
Example Question

Enzymes lower activation energy by

a) Straining bonds in reactants to make it easier to achieve transition state
b) Positioning reactants together to facilitate bonding
c) Changing local environment of reactants
d) All of the above

Metabolism

- Chemical reactions occur in metabolic pathways
- Each step is coordinated by a specific enzyme
- Catabolic pathways: breakdown, exergonic
  - Often coupled with anabolic pathways
- Anabolic pathways: synthesis, endergonic
  - Must be coupled to exergonic reaction

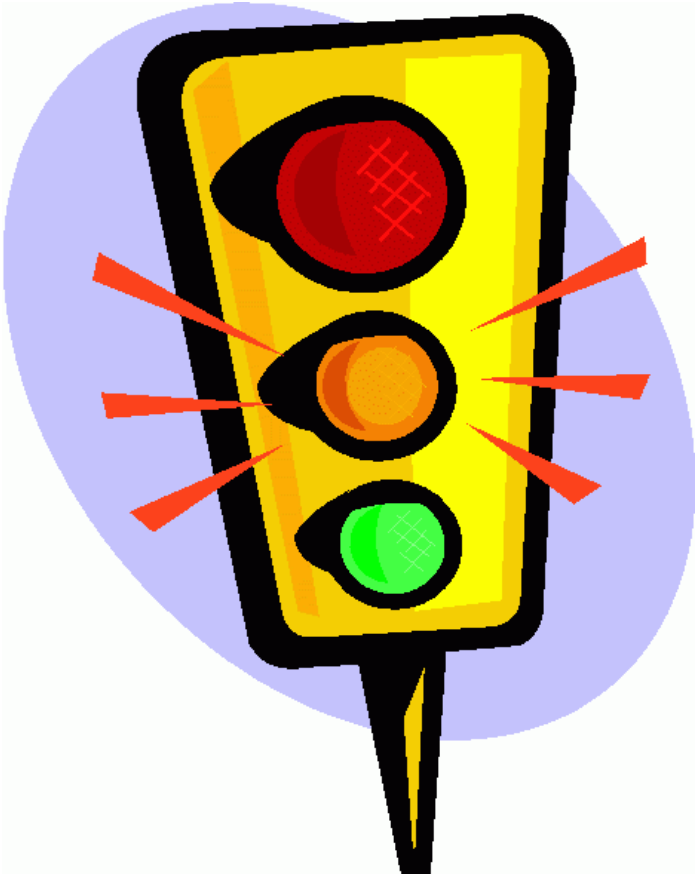


Catabolic Reactions

- Breakdown of reactants (e.g. starch, glycogen)
  - These examples are energy stores
- Used to obtain energy for endergonic reactions
  - Energy stored in energy intermediates
    - ATP, NADH
- Energy intermediates transfer energy by transferring electrons
  - REDOX reactions
- Power anabolic reactions (coupling)

Regulation of Metabolic Pathways

1. Gene regulation
   - Controlling the proteome
2. Biochemical regulation
   - Changing the properties of an enzyme (eg. K_m)

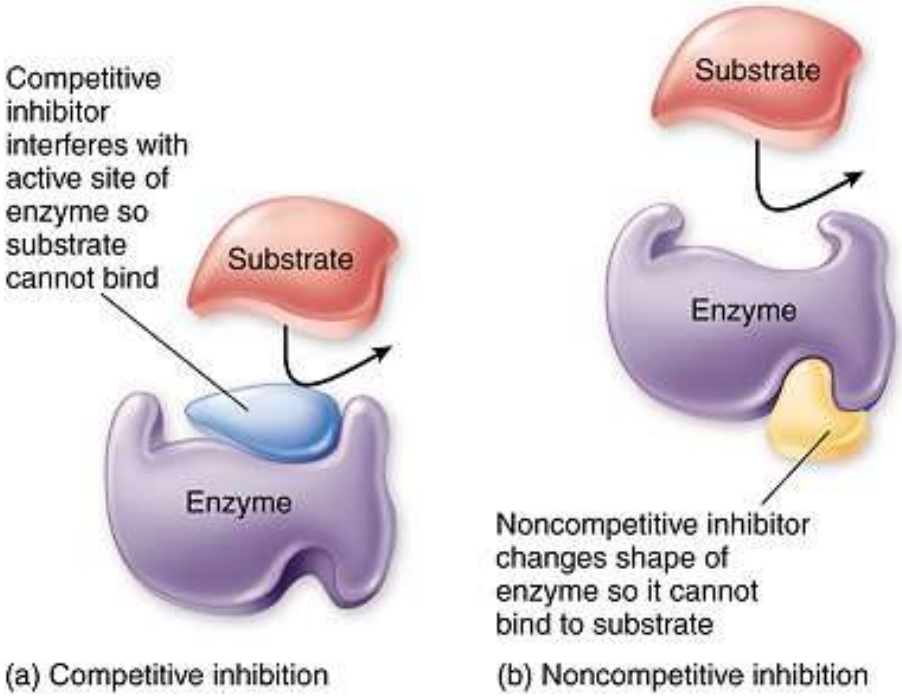


Fine Controls

- This is tweaking the speed or efficiency of the enzyme
  - Phosphorylating enzyme, glycosylation, etc.
- Coarse control would be controlling the amount of the enzymes

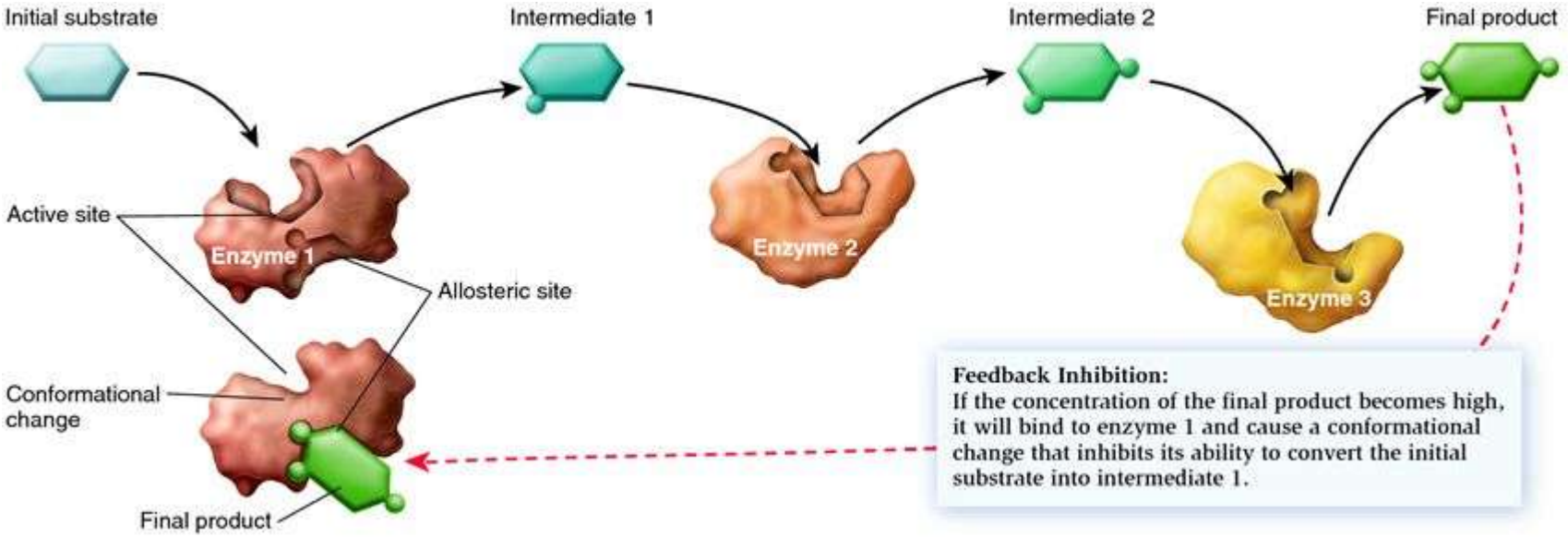
Fine Controls

- Competitive inhibitors
  - Something that competes with the substrate for the active site
  - Can be overcome by increasing the amount of substrate
- Noncompetitive inhibitors
  - Do not compete with the substrate for access to the active site (eg. allosteric inhibitors)
- Both of these types of inhibition do not affect V_{max} but makes K_m larger



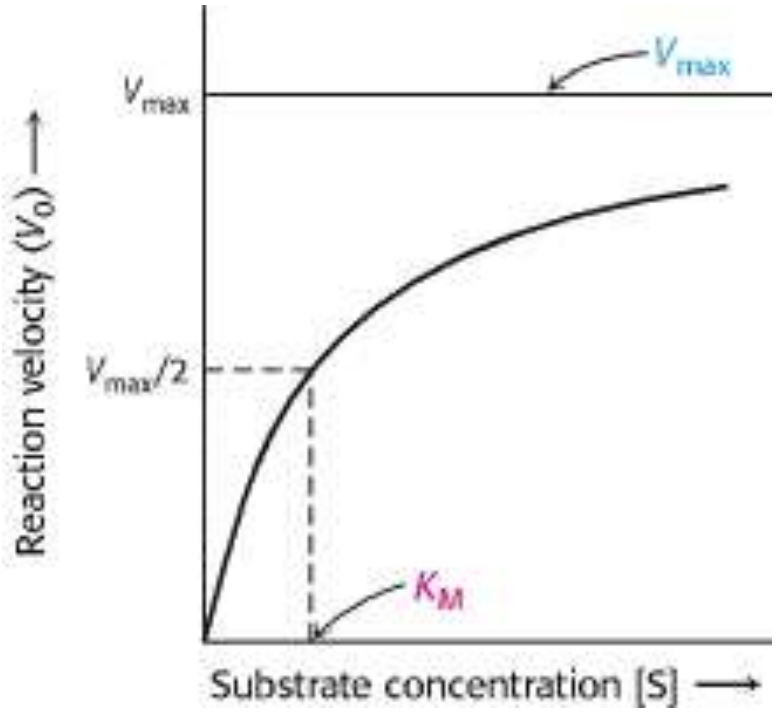
Feedback inhibition

- Feedback inhibition
  - Product of pathway inhibits early steps to prevent over accumulation of product
- Typically occurs to an enzyme early on in the pathway
  - Pacemaker enzymes
- Sigmoidal kinetics are a result of positive cooperativity
  - Basically when a substrate binds to a subunit of a multimeric enzyme it changes the entire enzyme



Coarse Control

- Turnover
  - Ratio of product made vs rate of product degradation
  - For example if you slow down the products destruction than its levels are going to rise
- Substrate levels
  - More or less substrates
- Providing more substrate can overcome competitive inhibition



Example Question

Competitive inhibition…

a) Inhibitor competes with substrate for the active site
b) Inhibitor competes with substrate for the allosteric site
c) Does not affect V_{max}, only K_m
d) A and C
e) None of the above

Cellular Respiration

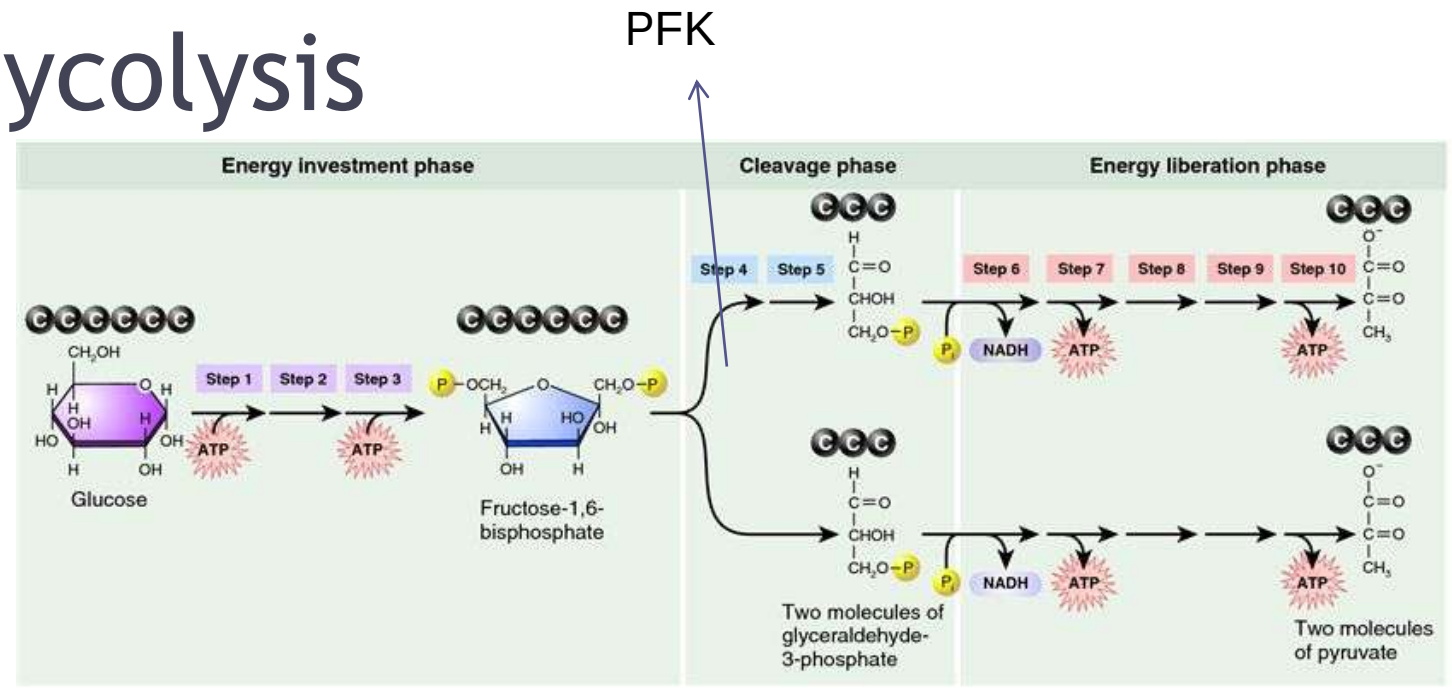
- Process by which living cells obtain energy from organic molecules
- Primary aim to make ATP and NADH
- Secondary role is anapleurotic
  - Anapleurotic refers to the formation of intermediates in metabolic pathways
  - These intermediates are often starter blocks for compounds that the cell needs
- Aerobic respiration uses oxygen
  - O_2 consumed and CO_2 released

$$\text{organic molecules} + O_2 \rightarrow CO_2 + H_2O + \text{energy}$$

4 Stages of Aerobic Respiration

1. Glycolysis
2. Formation of acetyl CoA
3. Citric acid cycle
4. Electron transport and chemiosmosis

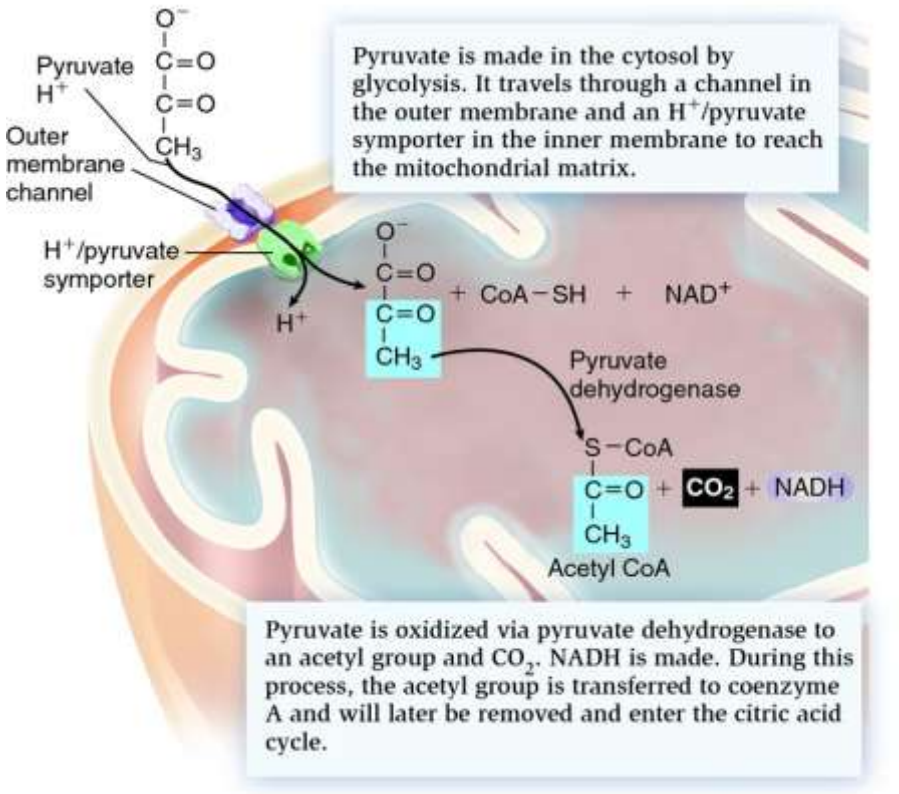
Glycolysis



- It happens in the cytosol
- It occurs to make pyruvate
- Regulated by the PFK
  - PFK is regulated by ATP, citrate (both inhibitors), and F2,6BP (activator)
- Glucose goes in, pyruvate, 2 NADH and 2 ATP comes out

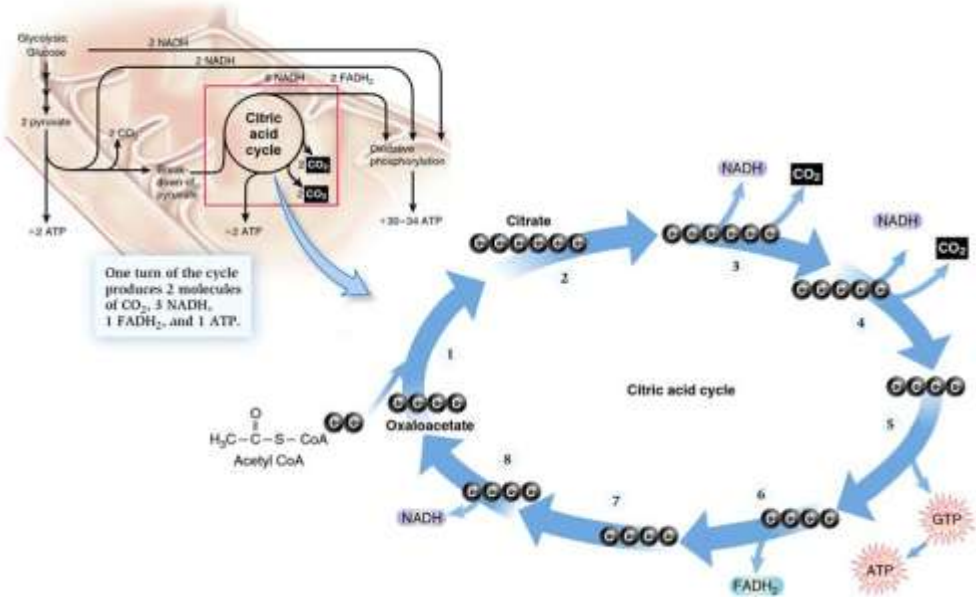
Formation of Acetyl CoA

- Happens in mitochondrial matrix
- It occurs to make acetyl-CoA
- Regulated by pyruvate dehydrogenase (PDH) allosteric inhibition and activation
- Pyruvate goes in, 2 acetyl-CoA and 2 NADH comes out

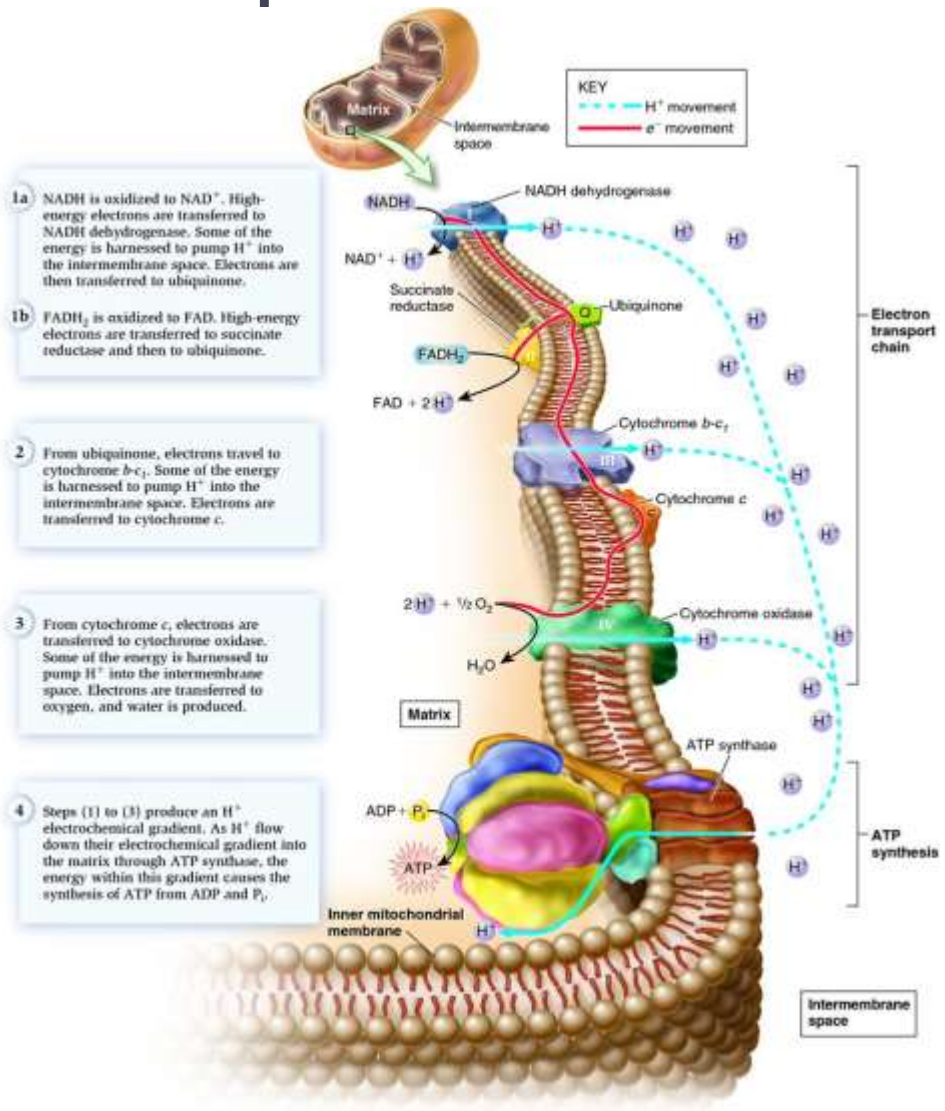


Citric Acid Cycle

- Happens in the mitochondrial matrix
- It occurs to make more NADH and $FADH_2$, regenerate oxaloacetate, further reduce pyruvate (now pyruvate is in the form of acetyl-CoA)
  - Also has anapleurotic roles
- Regulated by mostly by PDH, but oxaloacetate and acetyl-CoA levels as well
  - Oxaloacetate is also competitively inhibited by an intermediate later in the cycle
- Acetyl-CoA and oxaloacetate goes in, $2CO_2$, 1NTP, 3NADH, and 1 $FADH_2$ come out
  - Also oxaloacetate turns into citrate



Electron Transport and Chemiosmosis



Electron Transport and Chemiosmosis

- Occurs in the inner membrane and inner membrane space
- It occurs to make the H^+ gradient that powers ATP synthase, ultimately to make ATP
- Regulated by the amount of NADH and $FADH_2$ and the maintenance of the H^+ gradient
- NADH and $FADH_2$ in, large quantities of ATP and water out

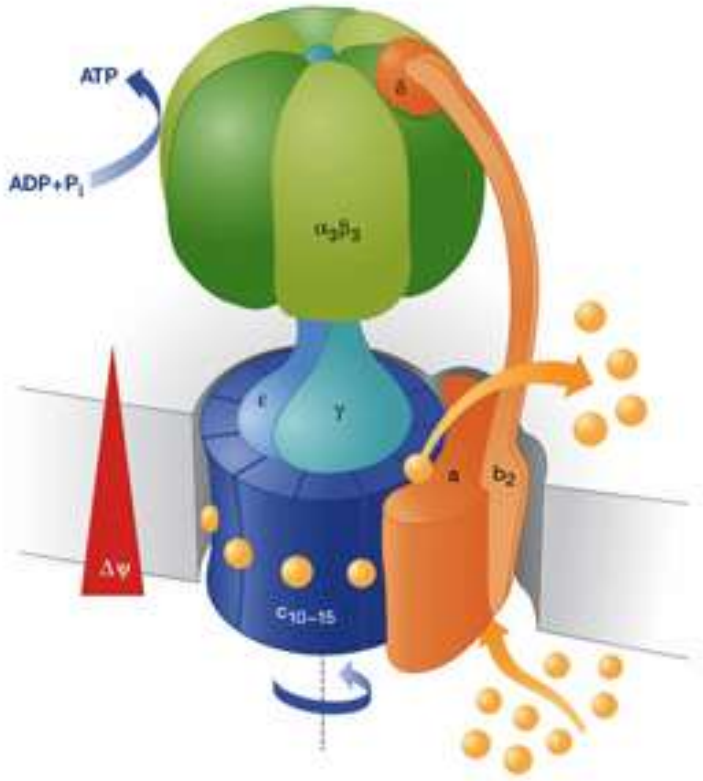
Example question

Which of the four stages of aerobic respiration occurs in the cytosol?

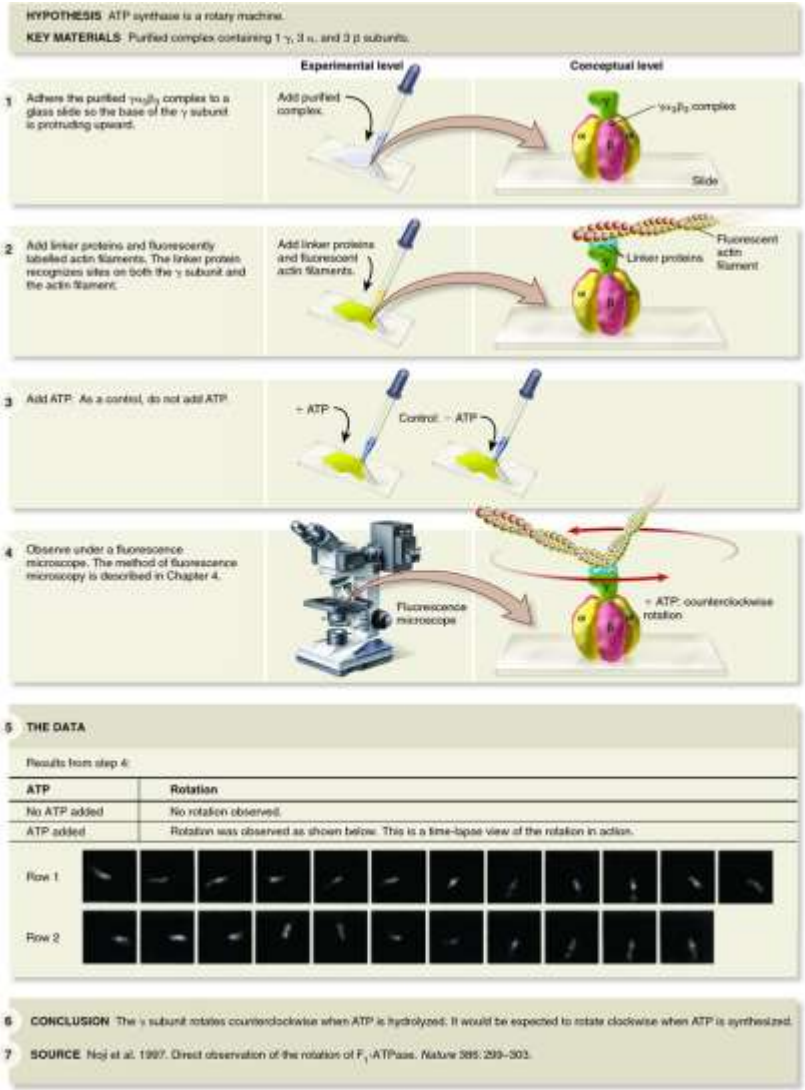
a) Glycolysis
b) Formation of acetyl CoA
c) Citric acid cycle
d) Electron transport and chemiosmosis

ATP Synthase

- Rotary machine that makes ATP as it spins
- Energy conversion
  - H^+ electrochemical gradient (proton motive force) converted to chemical bond energy in ATP
- Movie http://www.youtube.com/watch?v=uOoHKCMAUMc

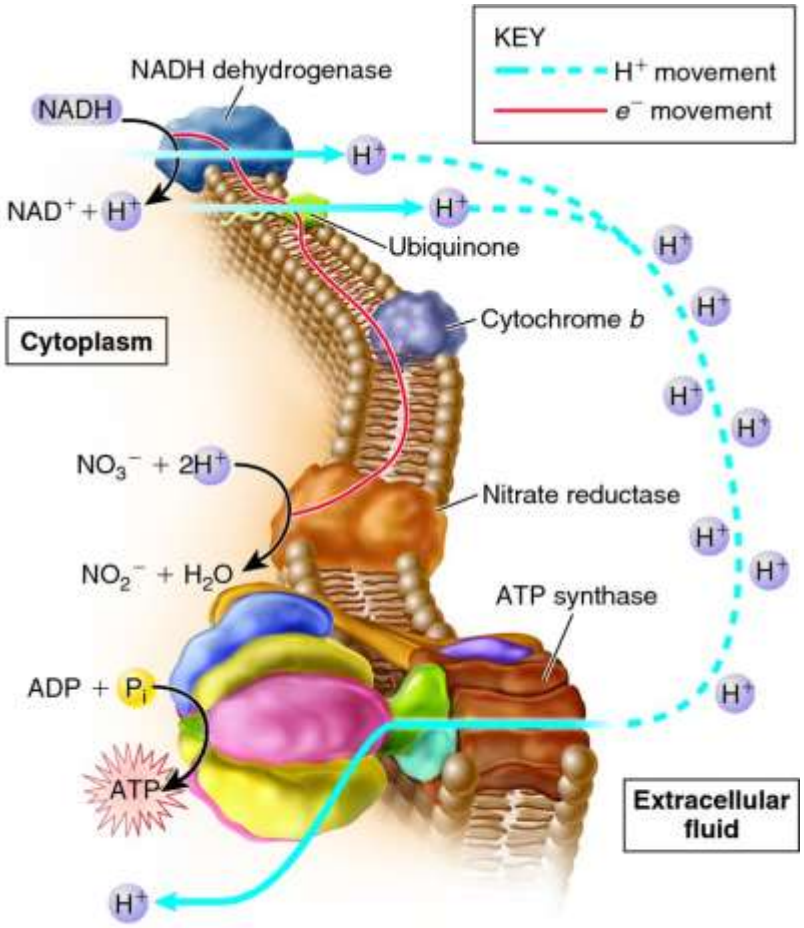


Yoshida & Kinosita Demonstrate that ATP Synthase Spins



Anaerobic Metabolism

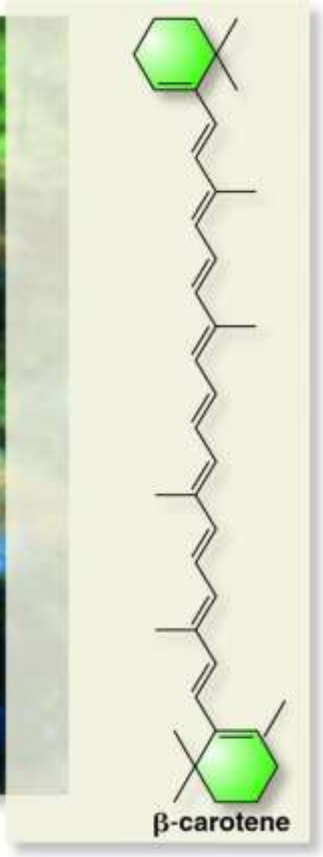
- 2 strategies
  - Use substance other than O_2 as final electron acceptor in electron transport chain
  - If confined to not using O_2, carry out glycolysis only (doesn't need O_2 to proceed yet still produces ATP)
- Aerobic → around 35 ATP via glycolysis + ETC about 50% of total energy recovered
- Anaerobic → around 2 ATP via glycolysis only about 4% of total energy recovered
- Anaerobic is FAR less efficient



Secondary Metabolism

- Primary
  - Essential for cell structure and function
- Secondary
  - Synthesis of metabolites that are not necessary for cell structure and growth
- Secondary metabolites unique to a species/group
- 4 types (TAPP)
  - Phenolics (Antioxidants with intense flavors and smells)
  - Alkaloids (Bitter-tasting molecules for defense)
  - Terpenoids (Intense smells and colors)
  - Polyketides (Chemical weapons)

Flamingo (*Phoenicopterus ruber*)



Which of the following is FALSE?

a) Aerobic respiration produces more ATP than anaerobic respiration
b) ATP synthase harnesses the proton motive force to create ATP
c) Secondary metabolites are essential for growth
d) By attaching florescent actin to ATP synthase, Yoshida and Kinosita showed that ATP synthase spins

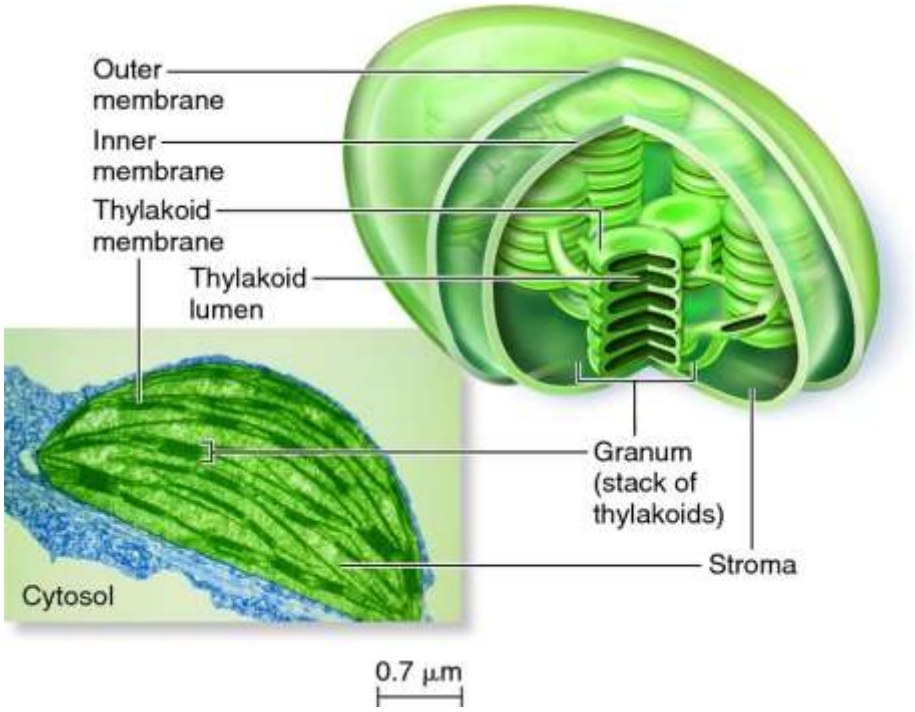
Chapter 8 - Photosynthesis

Photosynthesis

- Energy within light is captured and used to synthesize carbohydrates
- CO_2+ H_2O + light energy $\rightarrow C_6H_{12}O_6 + O_2$
  - Oxygen is just a by-product
- CO_2 is reduced
- H_2O is oxidized
- Energy from light drives this endergonic reaction

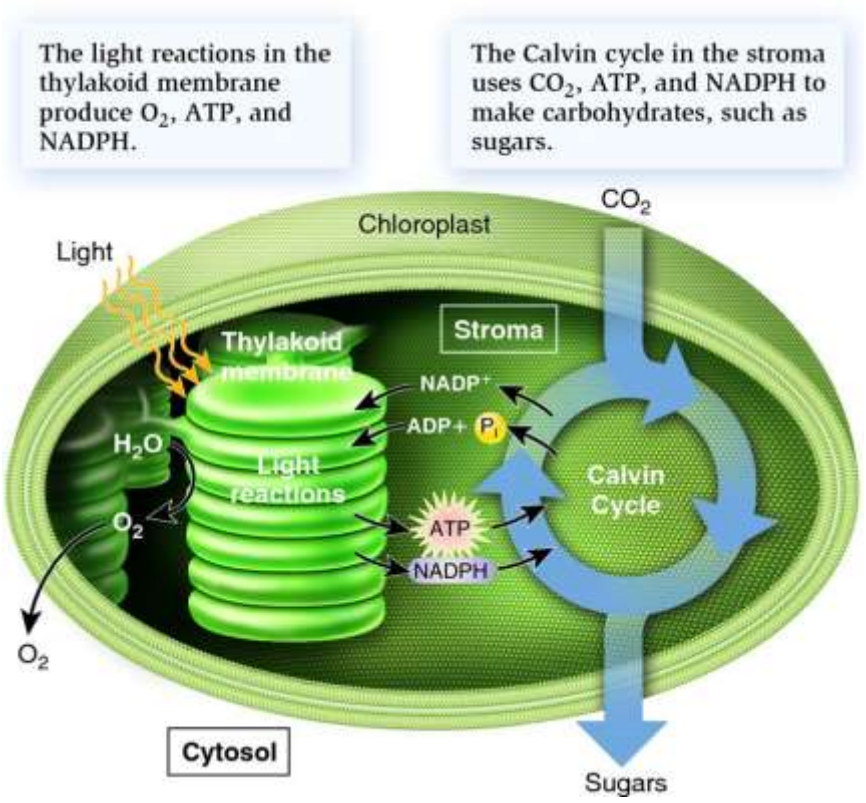
Chloroplasts

- Thylakoid membrane is where photosynthesis occurs
- Enclose thylakoid lumen
  - In the thylakoid lumen is where the proton motive force is maintained to drive ATP synthesis
- Granum: stack of thylakoids
- Stroma: fluid filled region between thylakoid membrane and inner membrane
  - Where the Calvin cycle takes place



2 Stages of Photosynthesis

- Light reactions
  - Take place in thylakoid membranes
    - Membrane integrity is absolutely crucial (things like fluidity, scavenging of free radicals, etc.)
  - Produce ATP, NADPH and O_2
- Calvin cycle (carbon fixation)
  - "Dark" reactions
    - This is a misnomer, the light reactions take place in the light because they NEED it
    - The Calvin cycle still occurs in the light
  - Occurs in stroma
  - Uses ATP and NADPH to incorporate CO_2 into organic molecules



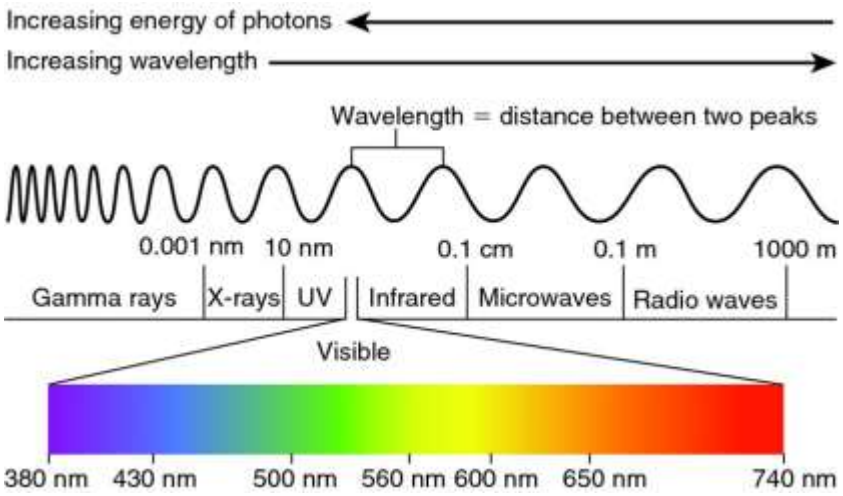
Example Question

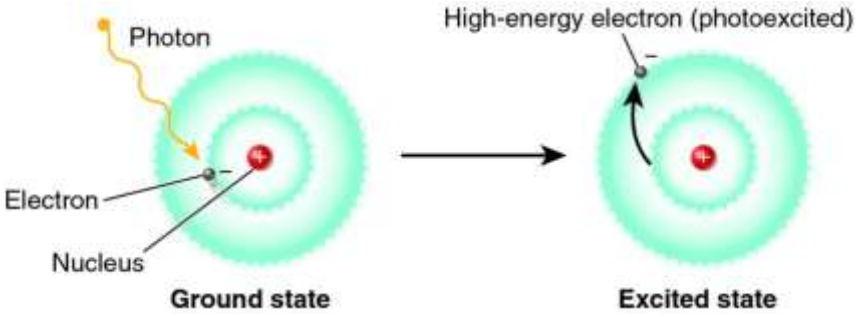
Photosynthesis occurs in the...

a) Chloroplasts
b) Stroma
c) Grana
d) Thylakoid membrane

Light Energy

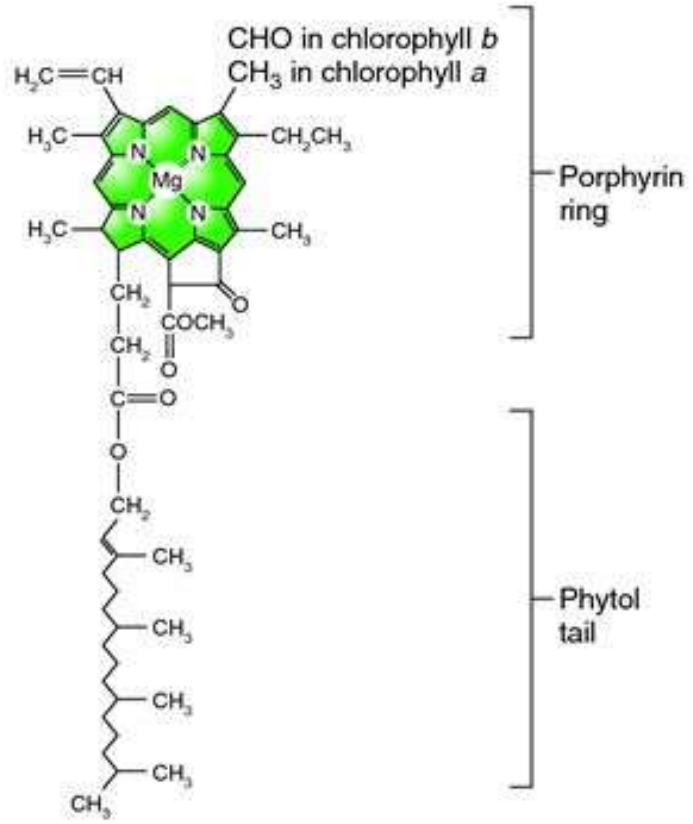
- In the visible spectrum
  - Red and blue
- Higher energy = shorter wavelength
- Photosynthetic pigments absorb some light energy and reflect others
  - Leaves are green because they reflect green wavelengths
  - Absorption boosts electrons to higher energy levels





Pigments

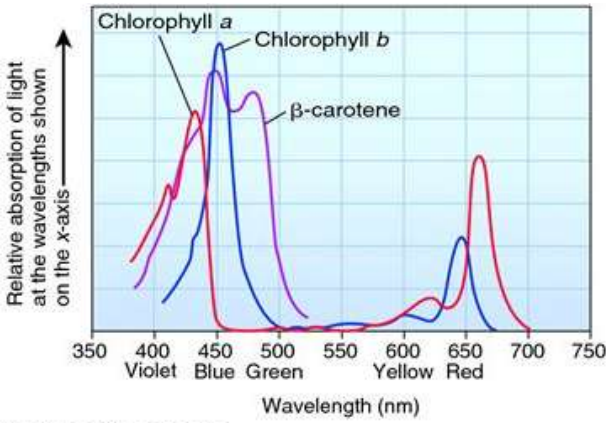
- Found in the thylakoid membrane
- Chlorophyll a and b
  - Both contain Mg
- Carotenoids
  - Help absorb light and channel them to chlorophylls
- Structure is what facilitates light absorption
  - Ring structure where an electron can follow a path around different atoms



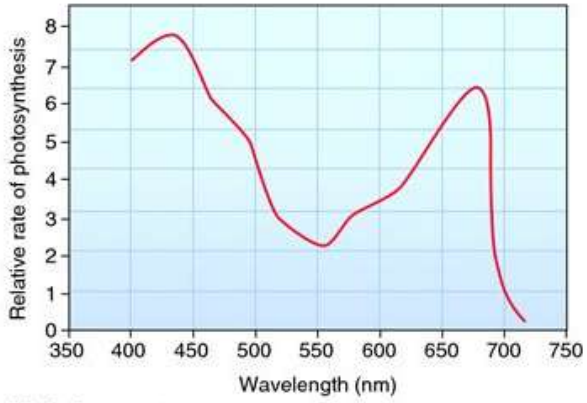
(a) Chlorophylls *a* and *b*

Absorption vs. Action Spectrum

- Absorption spectrum
  - Wavelengths that are absorbed by different pigments in the plant
- Action spectrum
  - Rate of photosynthesis by whole plant at specific wavelengths



(c) Absorption spectra

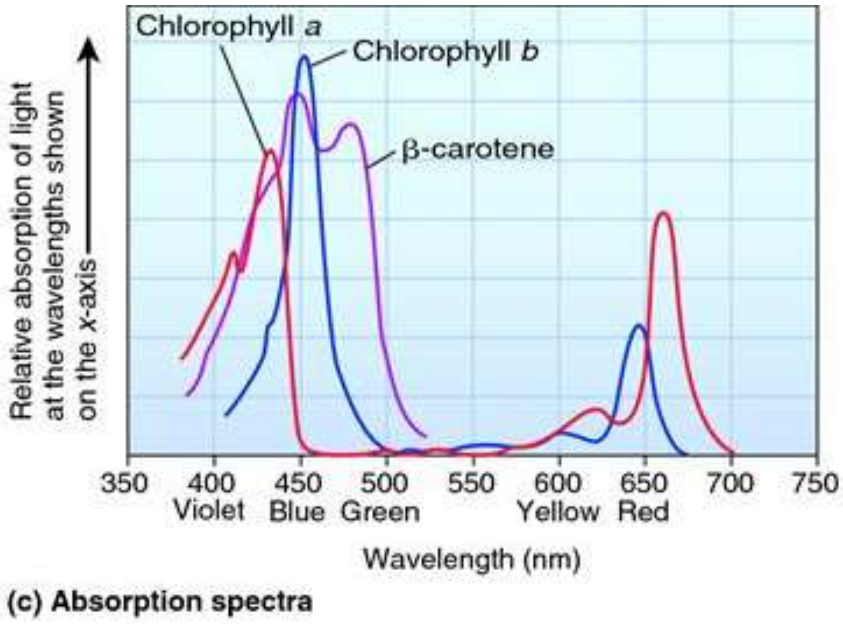


(d) Action spectrum

Example Question

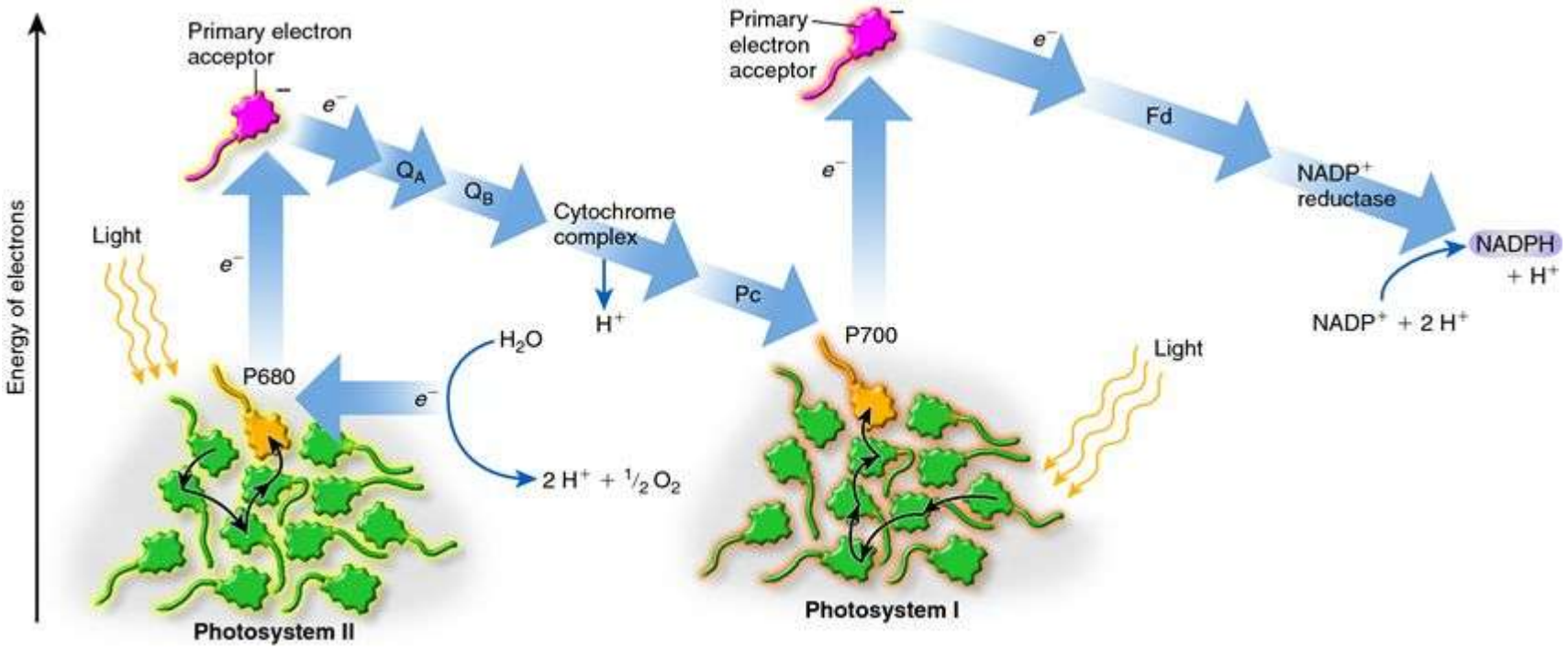
Based on the absorption spectra, which colour of light would boost the most electrons in chlorophyll b to higher levels?

a) Red
b) Yellow
c) Green
d) Blue
e) Violet



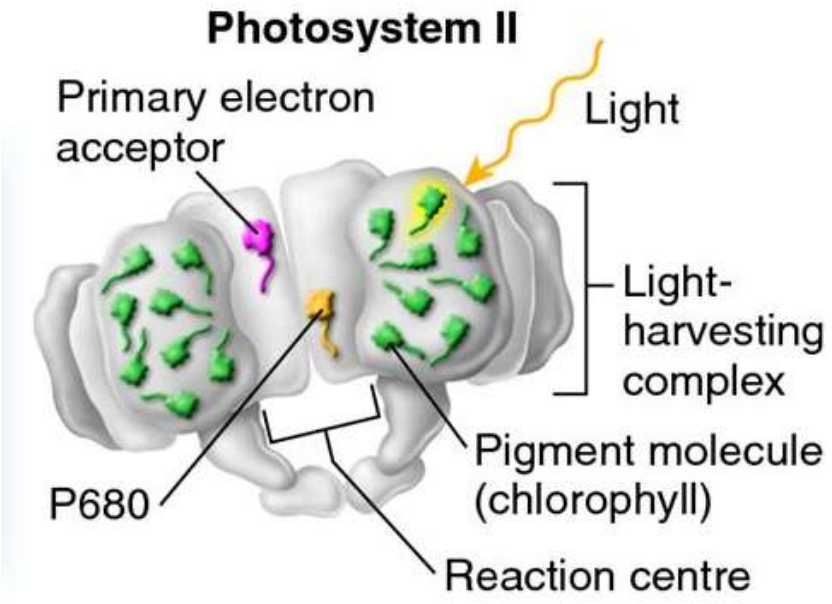
(c) Absorption spectra

Photosystems



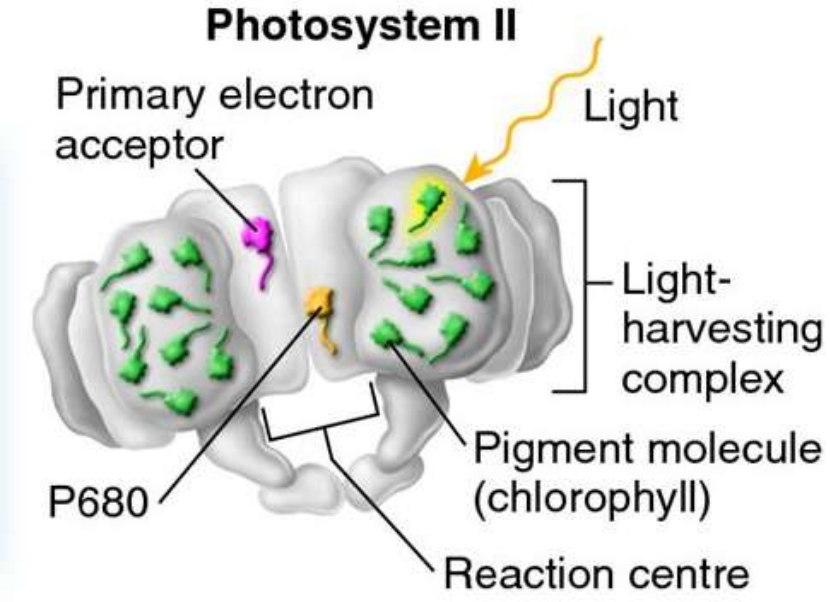
Photosystem II (PSII)

- Light-harvesting complex or antenna complex
  - Directly absorbs photons
  - Energy transferred via resonance energy transfer
- Reaction center
  - P680 → P680*
  - Transfers electron to primary electron acceptor
  - Removes electrons from water to replace oxidized P680
    - Oxidation of water yields oxygen gas
- Also creates 2 H^+ in the thylakoid lumen
- When electrons move from PSII to PSI they go to cytochrome C
  - Pumps more H^+ in



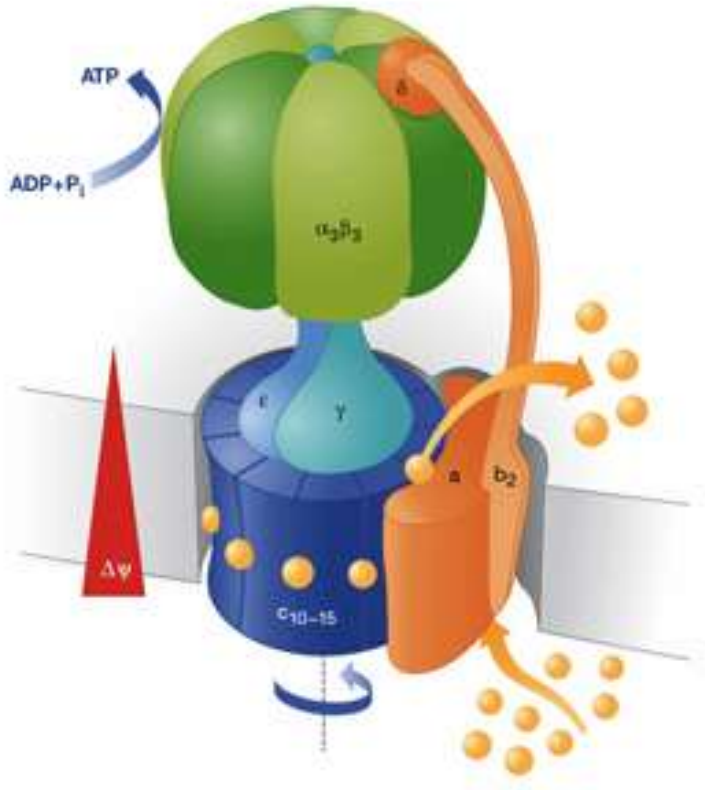
Photosystem I (PSI)

- Primary function is to make NADPH
- Light striking light-harvesting complex of PSI transfers energy to a reaction center (P700)
- High energy electron removed from P700 and transferred to a primary electron acceptor ($NADP^+$ reductase)
  - Electron originally from PSII
- $NADP^+$ reductase
  - $NADP^+ + 2$ electrons + $H^+ \rightarrow$ NADPH
  - This occurs on the thylakoid membrane but on the side facing the stroma
- $P700^+$ replaces its electrons from plastocyanin
  - No splitting water, no oxygen gas formed

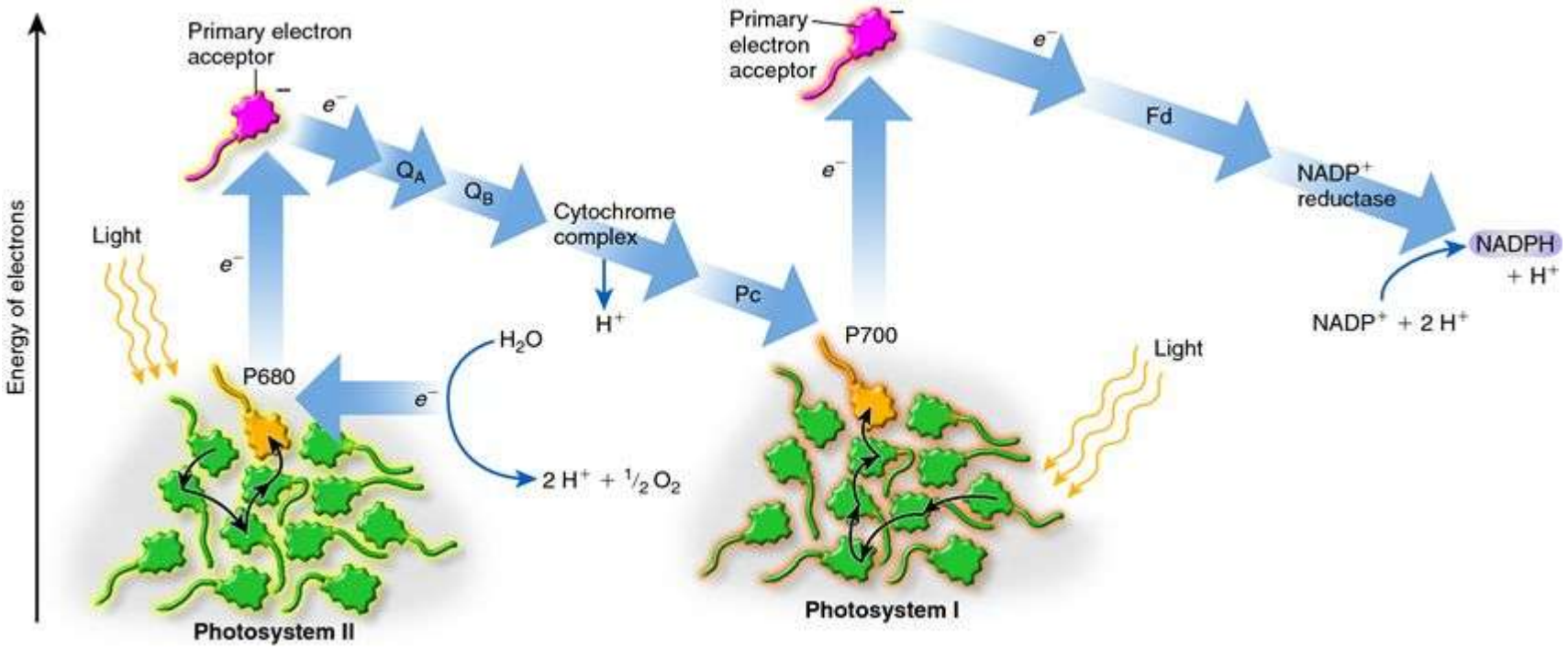


Photosystems Create Electrochemical Gradient

- All this movement of H^+ into the lumen and using H^+ in the stroma created the proton gradient necessary to drive ATP synthase
  - H^+ moves out of the thylakoid lumen (down its electrochemical gradient) into the stroma
  - Movement through ATP synthase drives ATP production

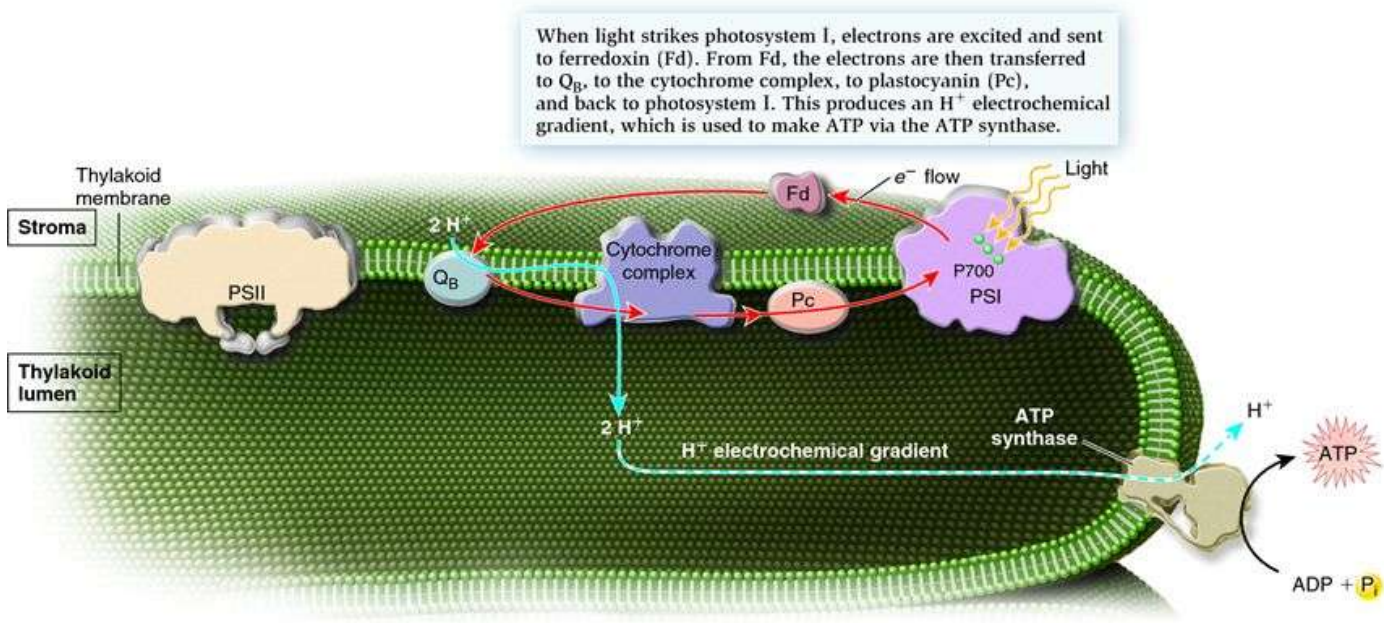


Photosystems



Cyclic vs noncyclic electron flow

- Noncyclic
  - Electrons begin at PSII (water) and eventually transfer to NADPH
  - Linear process produces ATP and NADPH in equal amounts
- Cyclic photophosphorylation
  - Electron cycling releases energy to transport H^+ into lumen driving synthesis of ATP
  - When P700 gets excited it doesn't send electrons to $NADP^+$ reductase
  - Instead it cycles it back in the pathway to pump more electrons into the thylakoid lumen
  - Electrons go to the electron transport protein that feeds electrons to cytochrome complex



Example Question

The electrons that travel through the photosystems originate from...

a) Pyruvate
b) NADH
c) ATP
d) Water

Calvin-Bensen Cycle

- ATP and NADPH used to make carbohydrates
- CO_2 used to make carbohydrates
  - Stomata are where CO_2 enters the plant
    - Stomata are also a major source of water loss in plants
  - Precursors to all organic molecules
  - Very endergonic reaction which is why you need ATP or NADPH
- Need 18 ATP + 12 NADPH for every 6 CO_2
- Glucose is not directly made

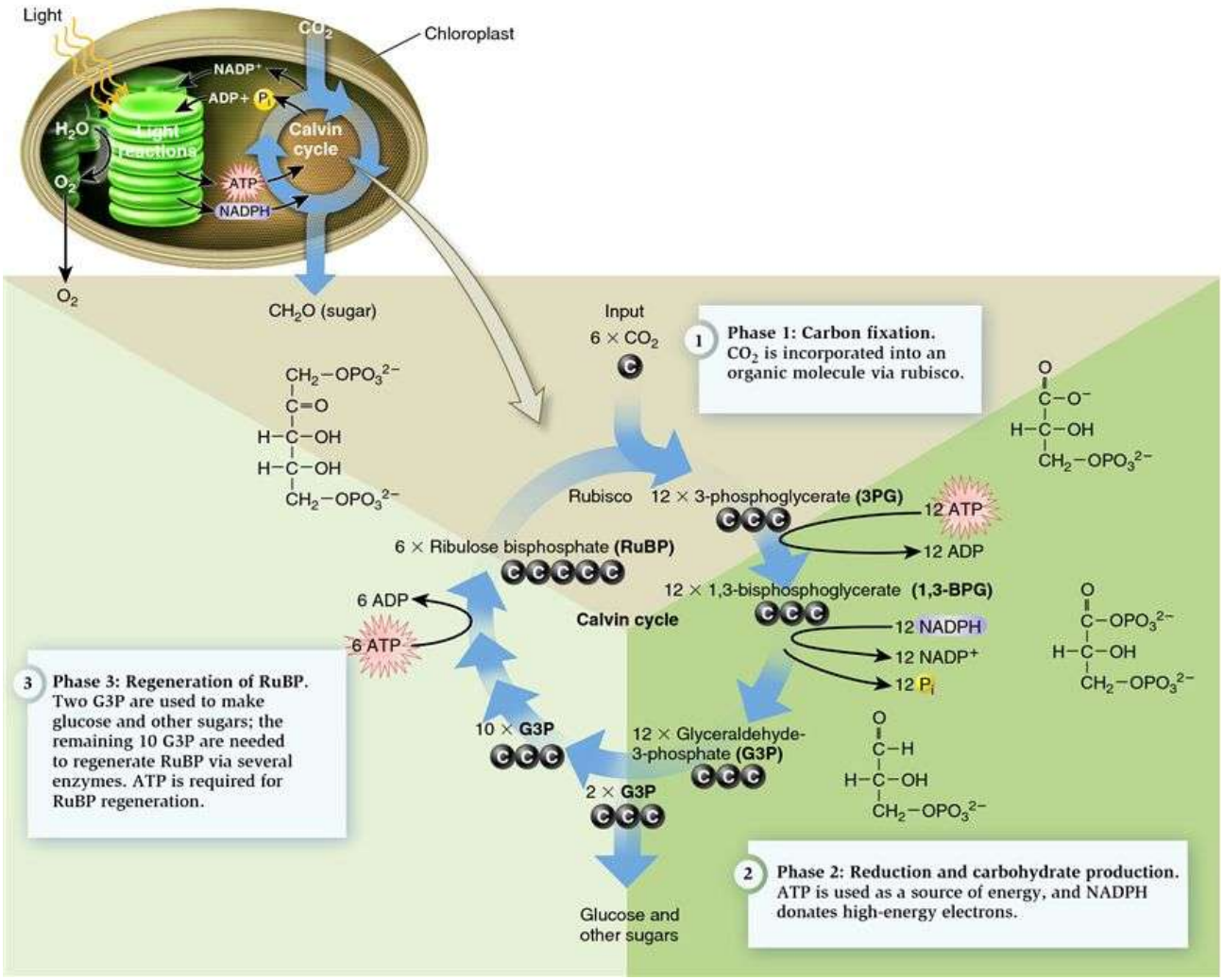
$$6\ CO_2 + 12\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$$

glucose

$$18\ ATP + 18\ H_2O \rightarrow 18\ ADP + 18\ P_i$$

$$12\ NADPH \rightarrow 12\ NADP^+ + 12\ H^+ + 24\ e^-$$

Calvin-Bensen Cycle Phases



Example Question

Converting 3PG into G3P uses...

a) CO_2, ATP, and NADH
b) Water, ATP, and NADH
c) CO_2, Water, and ATP
d) NADH, Pyruvate, and Water

Variations

- Certain environmental conditions can influence both the efficiency and way the Calvin cycle works
  - Light intensity
    - Impacts because it drives the production of ATP and NADPH (regulators)
  - Temperature
    - Can affect thylakoid membrane and therefore photosynthesis
  - Water availability
    - Plants are forced to close stomatal pores, means less CO_2 gets in

Photorespiration

- Photorespiration
  - Rubisco uses O_2
  - Glycolate → CO_2 requires lots of ATP
  - This energy comes from photosynthesis
- Not really sure what the role of photorespiration is but there are three hypothesis
  - Artefact of evolution
    - No pressure to be very selective between O_2 and CO_2
  - May be a safety valve
    - Gets rid of excess energy coming into the system
  - May play a role in nitrogen assimilation
- More likely in hot and dry environment
- Favoured when CO_2 low

$$RuBP + CO_2 \rightarrow 2\ 3PG$$

$$RuBP + O_2 \rightarrow 3\text{-phosphoglycerate} + phosphoglycolate$$

$$phosphoglycolate \rightarrow glycolate \rightarrow \rightarrow \rightarrow CO_2$$

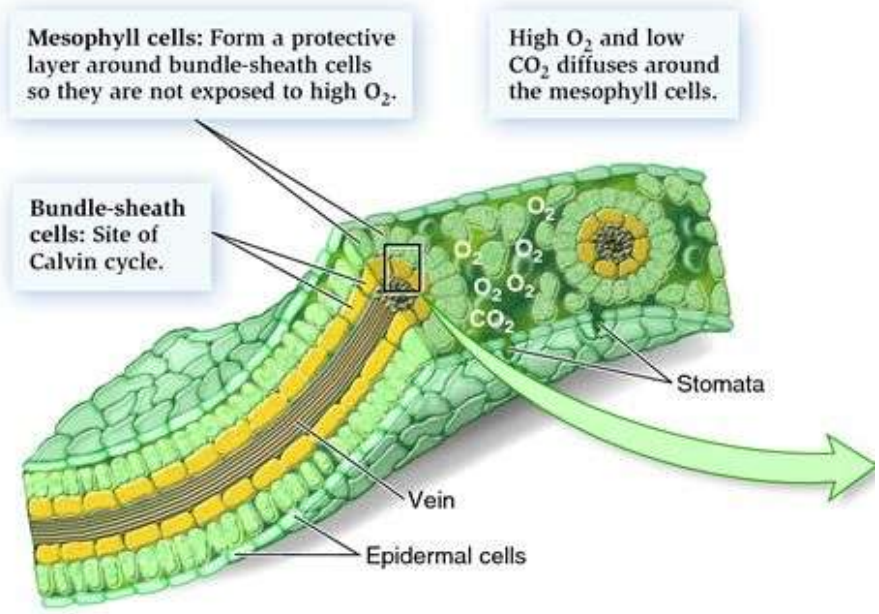
Example Question

Which of the following is not a hypothesis for the existence of photorespiration

a) Artefact of evolution
b) Safety valve
c) Nitrogen assimilation
d) Feed 3PG back into Calvin cycle

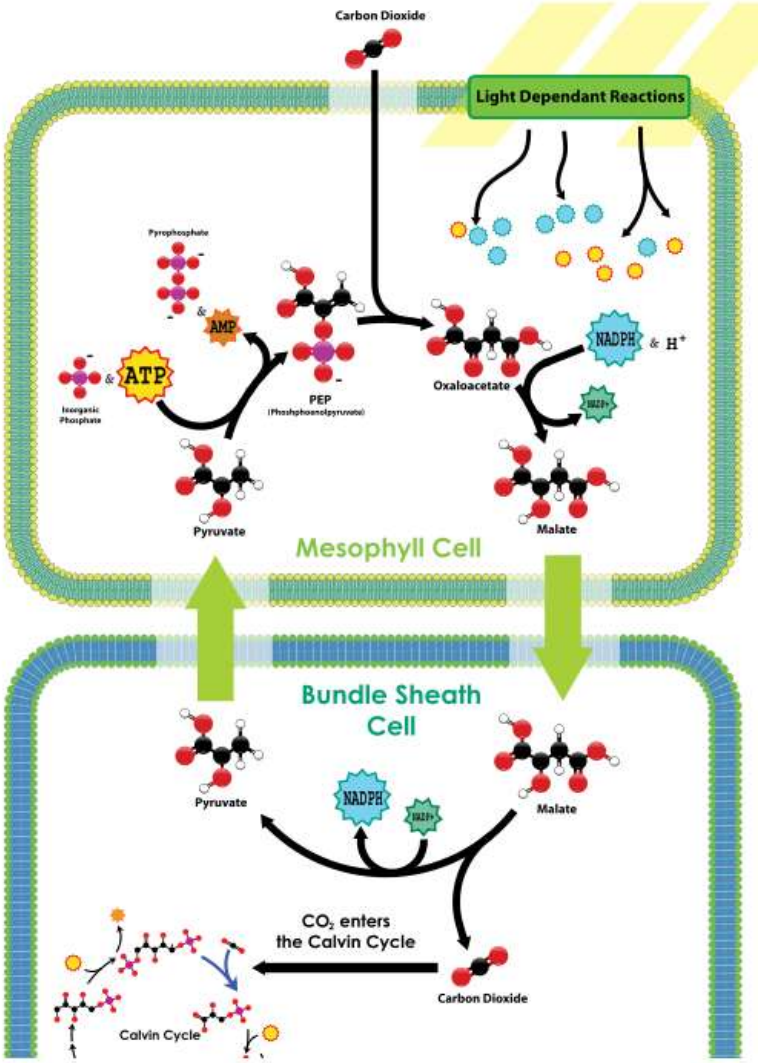
C_4 Plants

- C_4 plants make a 4-carbon compound in the first step of carbon fixation
- Hatch-Slack pathway: spatial segregation
- Leaves have 2-cell layer organization
  - Fixation and Calvin cycle happen in two different compartments
  - Called Krantz anatomy



C_4 Plants

- Mesophyll cells
  - Carbon fixation
  - CO_2 enters via stomata and 4-C compound (oxaloacetate) formed via PEP carboxylase(no affinity for O_2 = does not promote photorespiration)
- Bundle-sheath cells (BSC)
  - Calvin cycle
  - Malate transferred to BSC and is turned into pyruvate
    - Releases CO_2
  - This occurs right in front of rubisco
    - So rubisco never really gets a chance to bind O_2 instead of CO_2



C_4 Plants

- In warm, dry climates C_4 plants have the advantage in conserving water and preventing photorespiration
  - Have lower CO_2 demands
- In cooler climates, C_3 plants use less energy to fix CO_2
  - 90% of plants are C_3

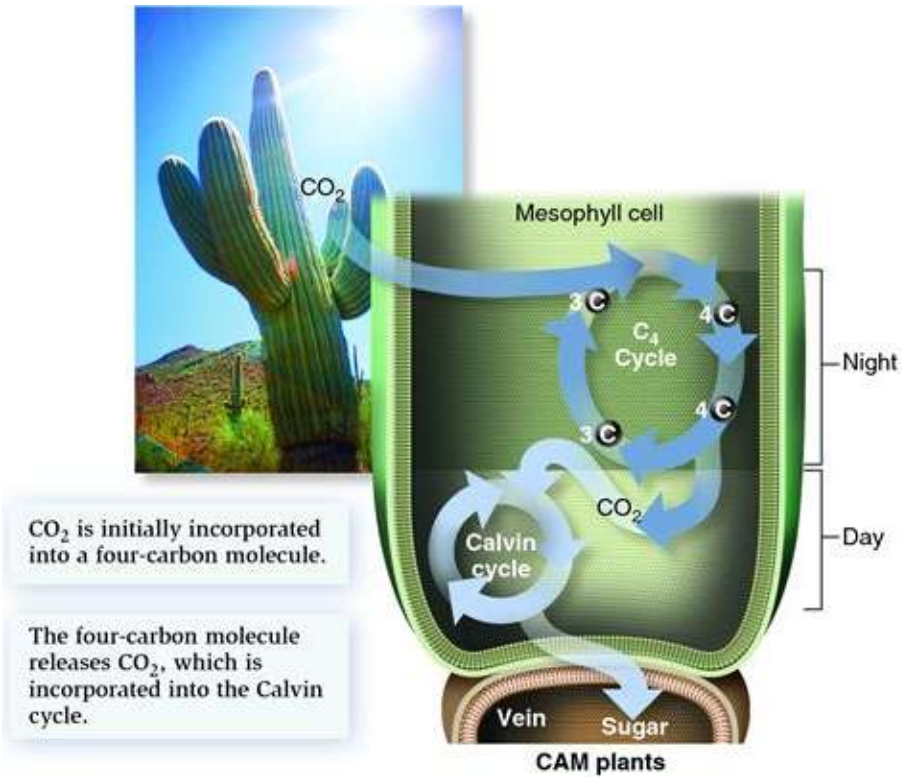
Example Question

Why does photorespiration occur less in C_4 plants?

a) Rubisco has a high affinity for CO2
b) PEP carboxylase has a low affinity for O2
c) CO2 is "delivered" to rubisco
d) B and C

Crassulacean Acid Metabolism (CAM)

- C_4 plants that use temporal segregation
- CAM plants open their stomata at night
  - CO_2 is only available at night, goes through C_4 pathway of carbon fixation
    - PEP carboxylase is shut down during the day and activated at night to avoid futile cycling
    - Done at a post-translational level
  - This also prevents a lot of water loss (stomata are closed during the hottest time of day)
- Malate is stored until the day



Example Question

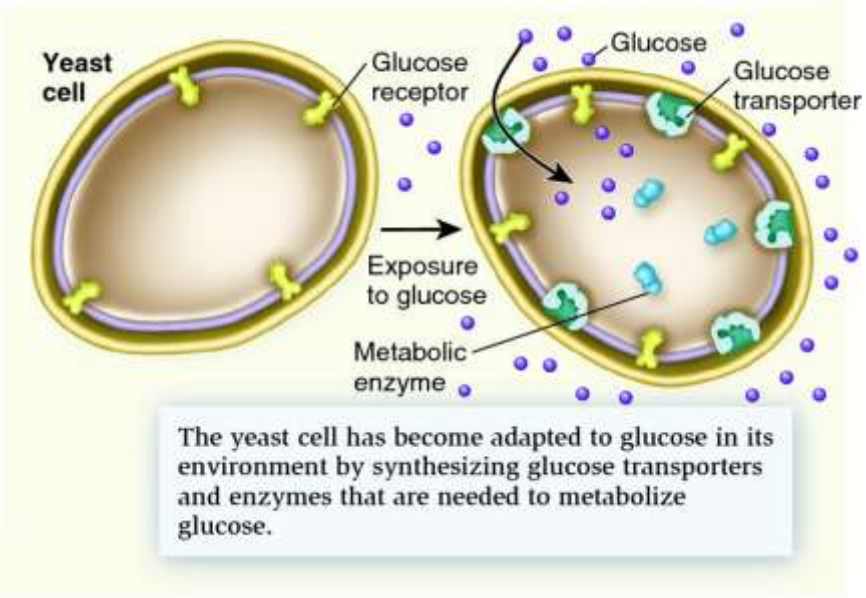
CAM plants differ from C4 plants because...

a) They use temporal segregation
b) They use spatial segregation
c) They store malate
d) A and C

Chapter 9 - Communication

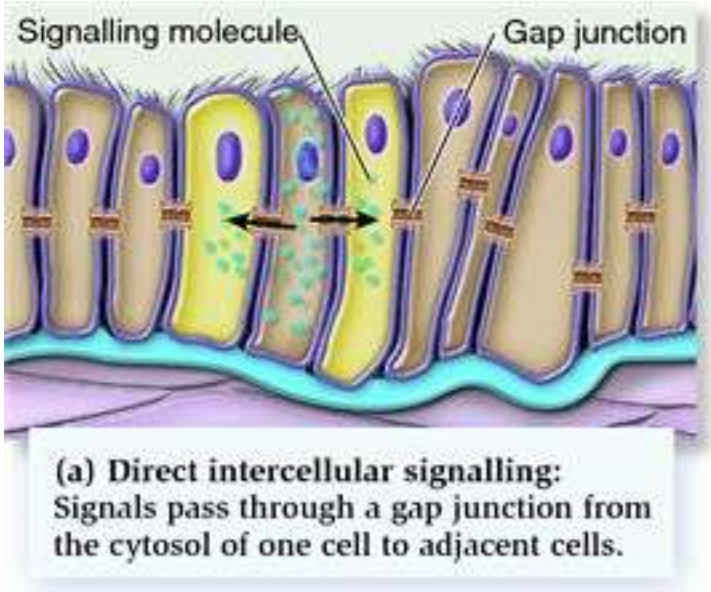
Communication is Vital

- Cells need to communicate with each other, coordinate responses (e.g. development)
  - Communication within cells is important too
- Need to respond to changing environment
- Cell communication or cell signalling involves incoming and outgoing signals
- Signals are agents that influence the properties of cells
- Perception of signal required for response
  - Signals can be things like glucose (yeast) or light (phototropism)



Types of Signalling

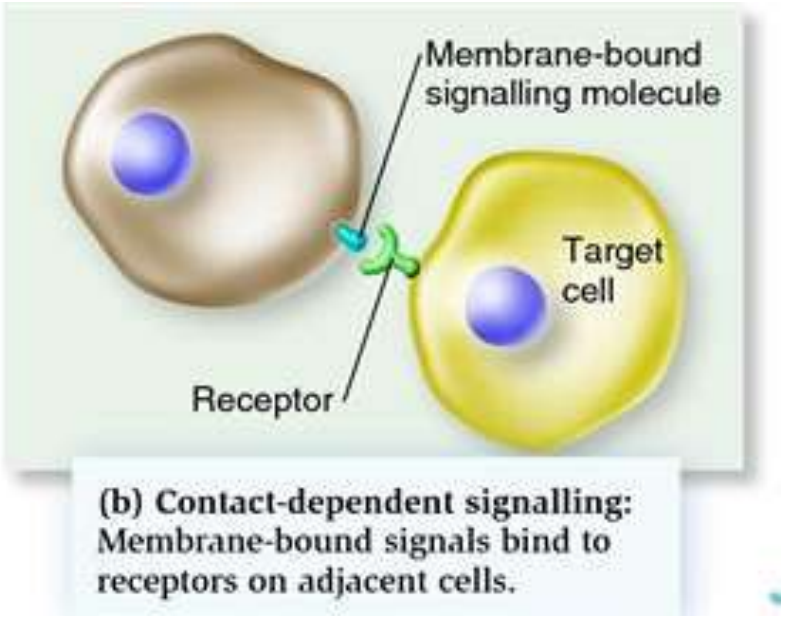
- Direct intercellular signalling
  - Cell junctions allow signalling molecules to pass from one cell to another
  - Physical connections between two cells with a passageway connecting them
  - This is a rapid method of signalling
    - In animals they are called gap junctions
    - In plant cells they are plasmodesmata



(a) Direct intercellular signalling: Signals pass through a gap junction from the cytosol of one cell to adjacent cells.

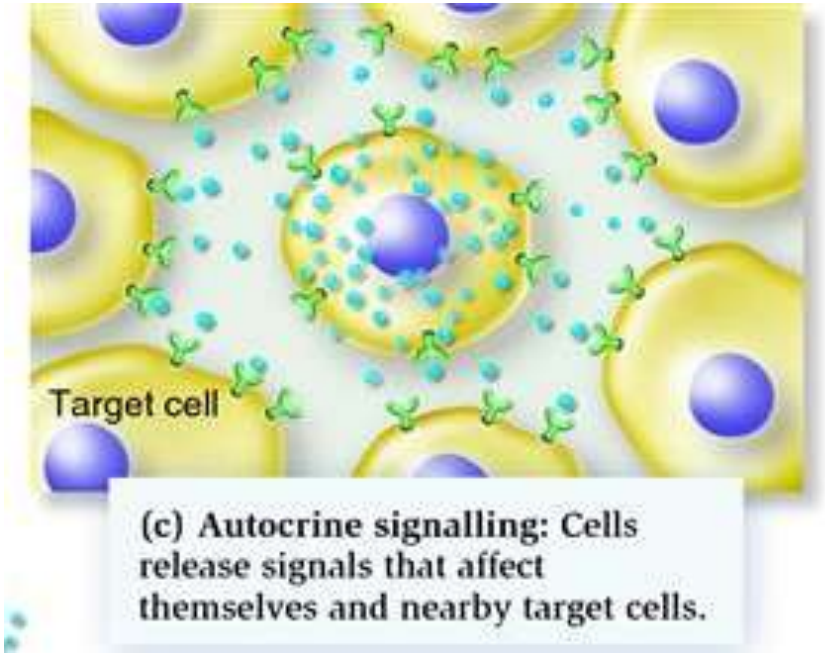
Types of Signalling

- Contact-dependent signalling
  - Some molecules are bound to the surface of cells and serve as signals to cell coming in contact with them
  - Not physically connected by a portal, rather the two surfaces are interacting with each other and that signals processes within each respective cell



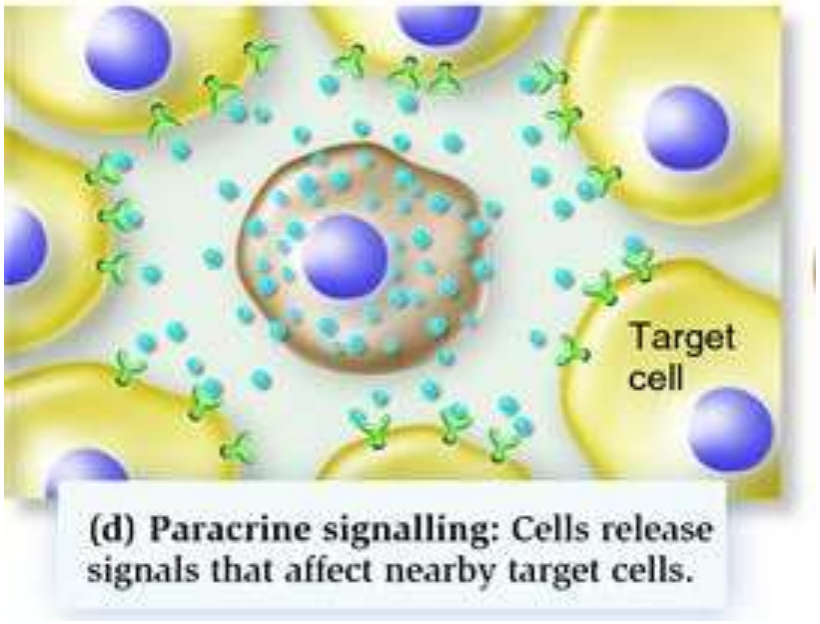
Types of Signalling

- Autocrine signalling
  - Cells secrete signalling molecules that bind to their own cell surface or neighbouring cells of the same type
  - Auto means that it also stimulates itself



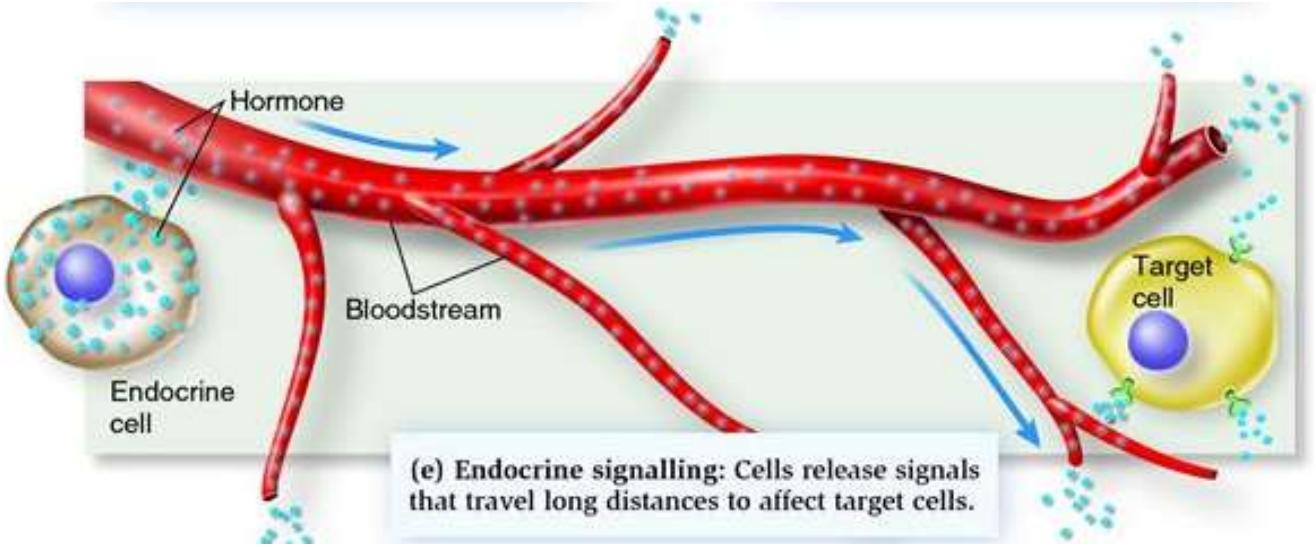
Types of Signalling

- Paracrine signalling
  - Signal does not affect cell secreting the signal but influences cells in close proximity



Types of Signalling

- Endocrine signalling
  - Signals (hormones) travel long distances and are usually longer lasting

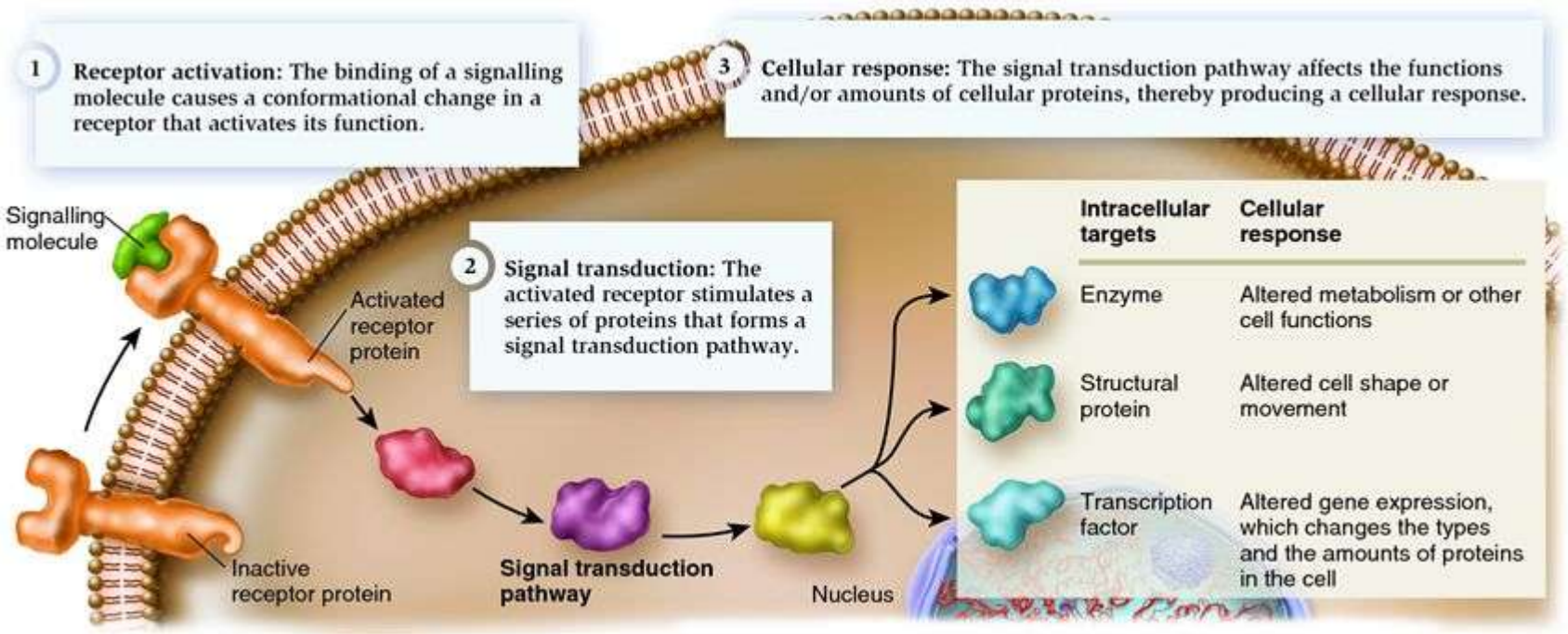


Example Question

Autocrine signalling differs from paracrine signalling because...

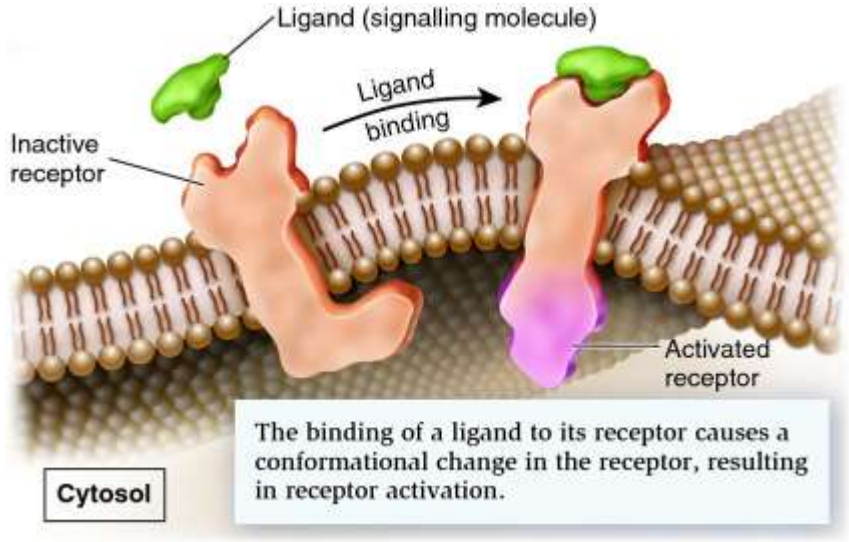
a) Cells secrete molecules that stimulate other nearby cells
b) Cells secrete molecules that stimulate other nearby cells and itself
c) Cells secrete molecules that travel long distances
d) Cells need to be in contact for the signal to be tranduced

Stages of Cell Signalling



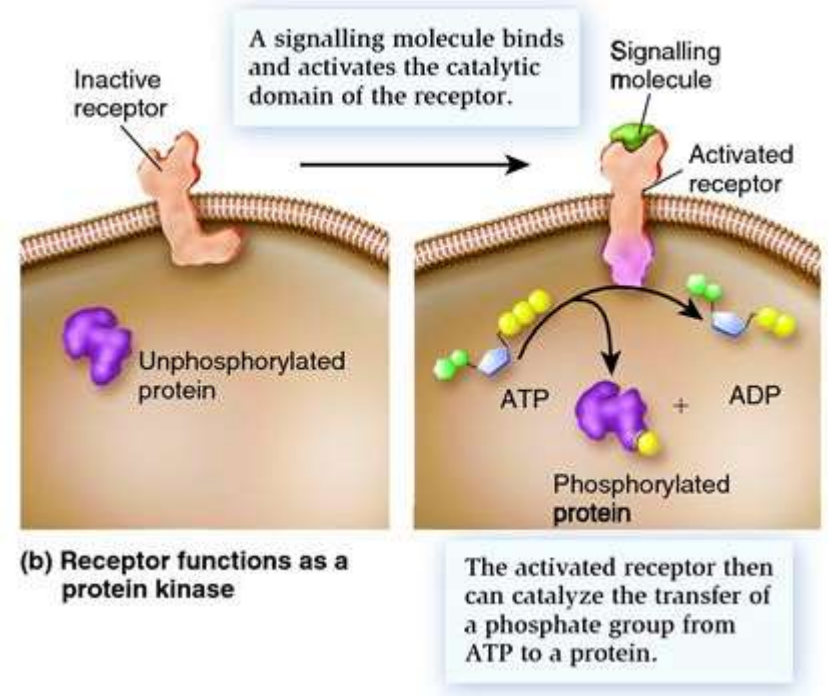
Ligand

- Signaling molecule
- Binds noncovalently to receptor with high degree of specificity/affinity
  - Reversible binding
  - K_d is a measure of the affinity of the ligand to its receptor (dissociation constant)
- Binding and release between receptor and ligand relatively rapid
- Ligands alter receptor structure-conformational change



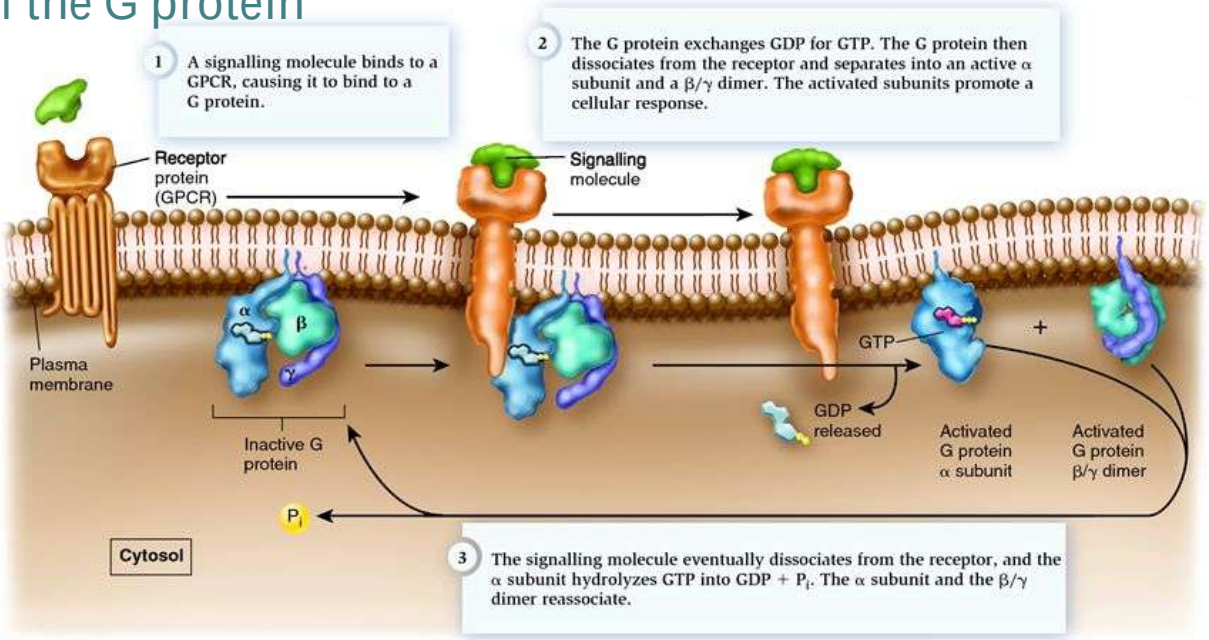
Types of Receptors

- Enzyme-linked receptors (catalytic receptors)
  - Found in all living species
  - Extracellular domain binds signal
  - Causes intracellular domain to become a functional catalyst
  - Most are protein kinases
    - Usually phosphorylate proteins to initiate the cascade
- Catalytic receptors have both a K_d (ligand binding to extracellular domain) and a K_m (intracellular, catalytic domain)



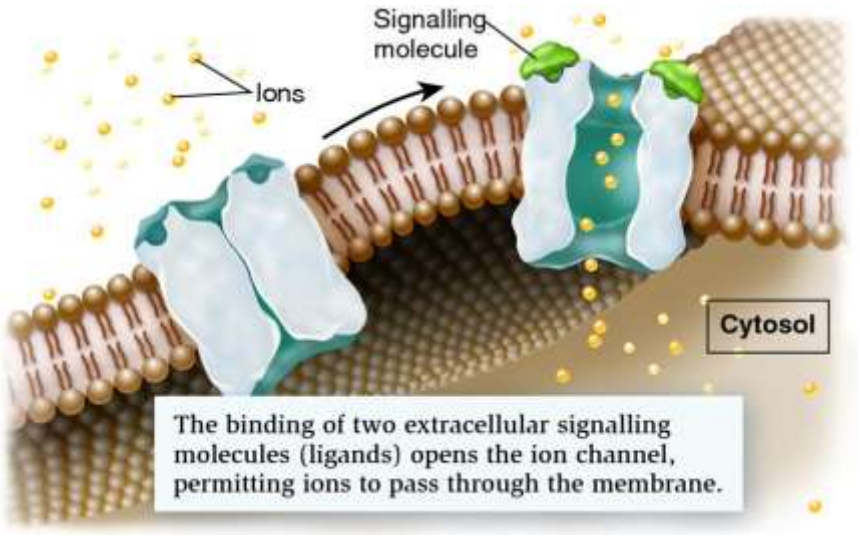
Types of Receptors

- G-protein coupled receptors (GPCR)
  - Common in eukaryotes
  - Transmembrane protein
  - The G protein itself is not catalytic
    - When the two subunits disassociate however, the α subunit becomes catalytic
  - Active α subunit and β/y dimer interact with other proteins in a signalling pathway
  - Once the signalling molecule disassociates with the receptor, the active α subunit hydrolyzes GTP back into GDP and the two subunits reassociate to reform the G protein



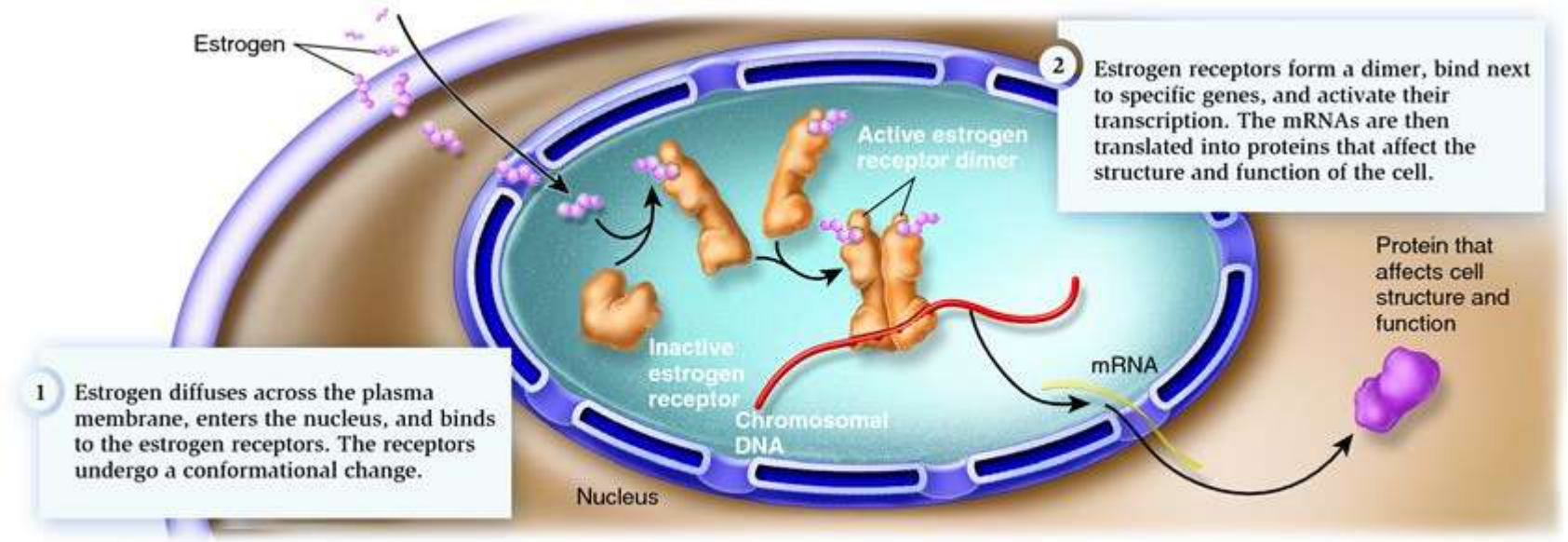
Types of Receptors

- Ligand-gated ion channels
  - Plant and animal cells
  - Ligand binding causes ion channels to open and ions to flow through the ion channels through the membrane



Intracellular Receptors

- Most receptors on the plasma membrane but some are inside the cell
- Estrogen example (mammalian hormone)
  - Passes through membrane (small, lipophillic molecule) and binds to receptor in nucleus
  - Dimer of estrogen-receptor complexes binds to DNA to activate transcription of specific genes
    - Transcription factors regulate transcription of specific genes



(a) Estrogen receptor

Example Question

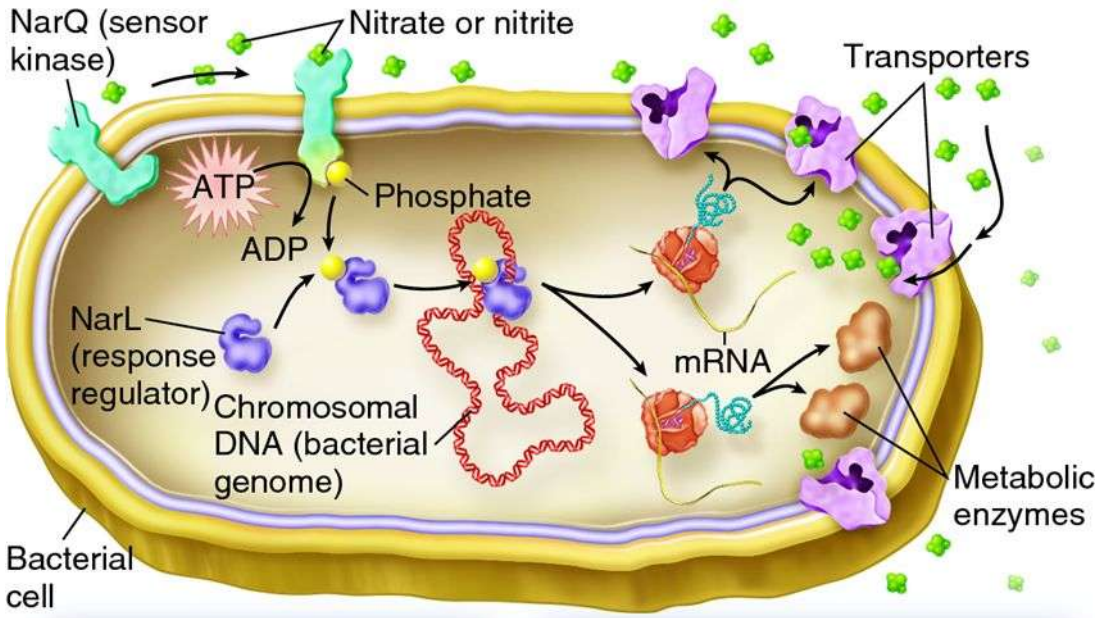
Estrogen receptors...

a) Have both a K_d and a K_m
b) Bind ligands
c) Catalyze reactions
d) All of the above

Cellular Response

- Two-component regulatory system
  - Found in bacteria, fungi and plants (not animals)
- Sensor kinase
  - Enzyme-linked receptor
  - Hydrolyzes ATP and phosphorylates
  - Phosphate group transferred to response regulator
- Response regulator
  - Regulates expression of many genes

NarQ/NarL

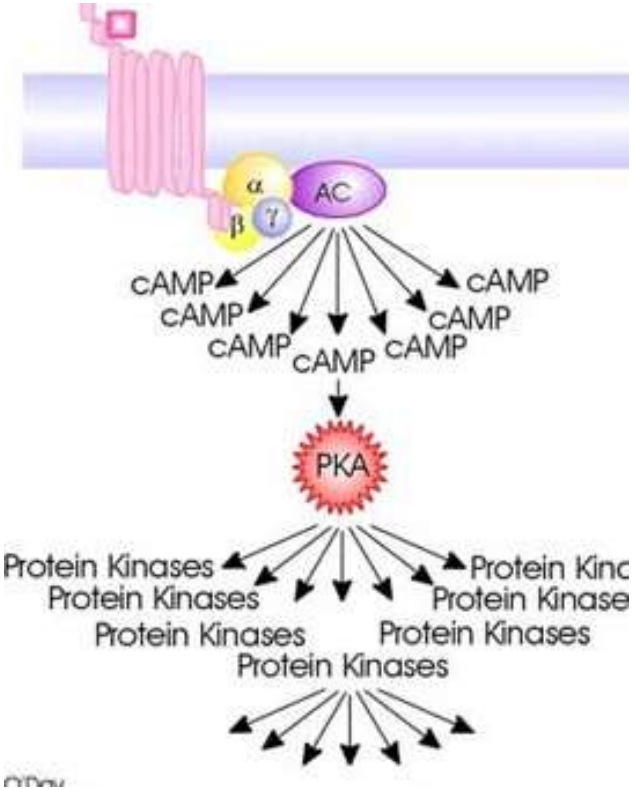


1 Nitrate or nitrite binds to NarQ, which uses ATP to phosphorylates itself, and then transfers the phosphate to NarL.

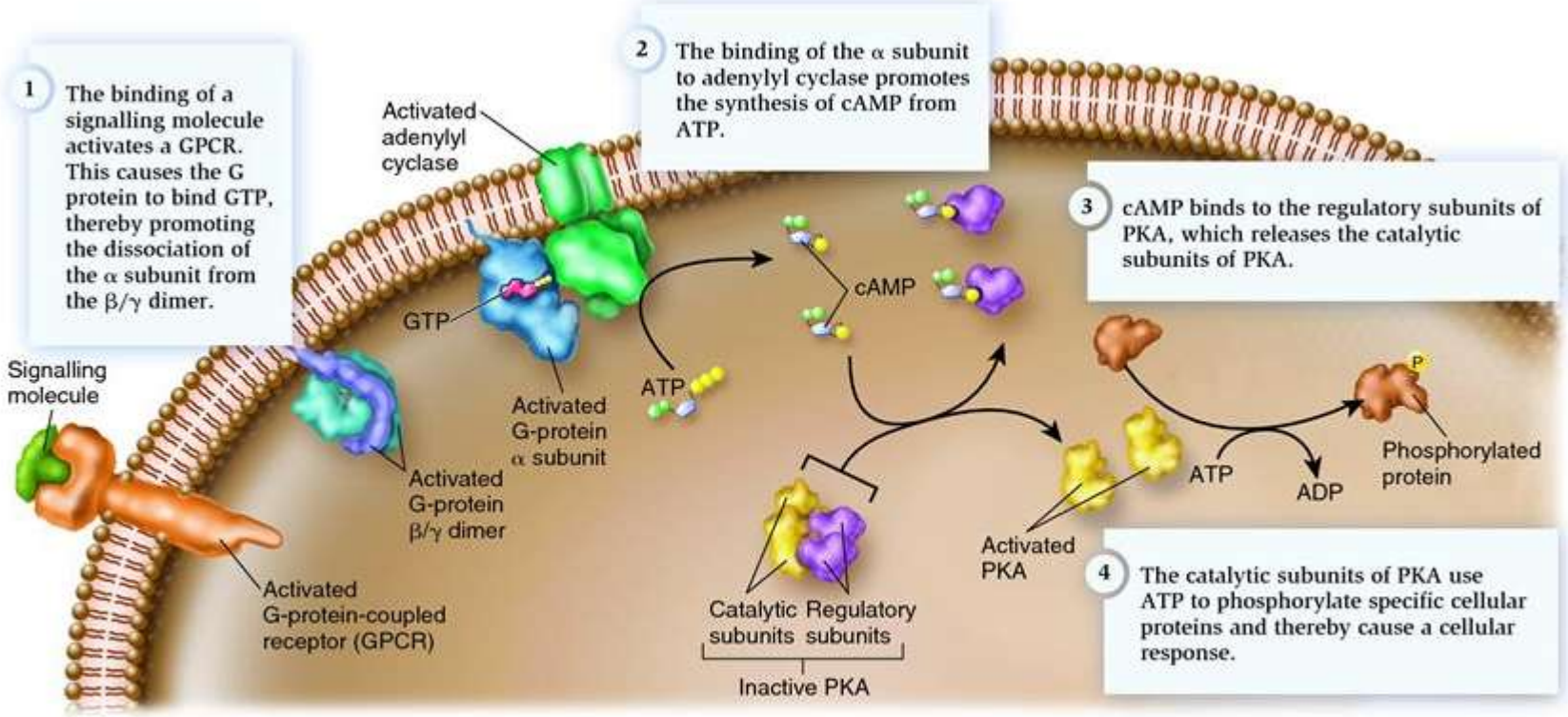
2 The phosphorylated form of NarL activates many genes that encode proteins involved with nitrate and nitrite uptake and metabolism.

Second Messengers

- Signals binding to cell surface are first messenger
- Many signal transduction pathways lead to production of second messengers
  - These allow for the amplification and transduction of the signal
- Examples
  - cAMP
  - Ca^{2+}
  - Diacylglycerol and inositol triphosphate (IP3)

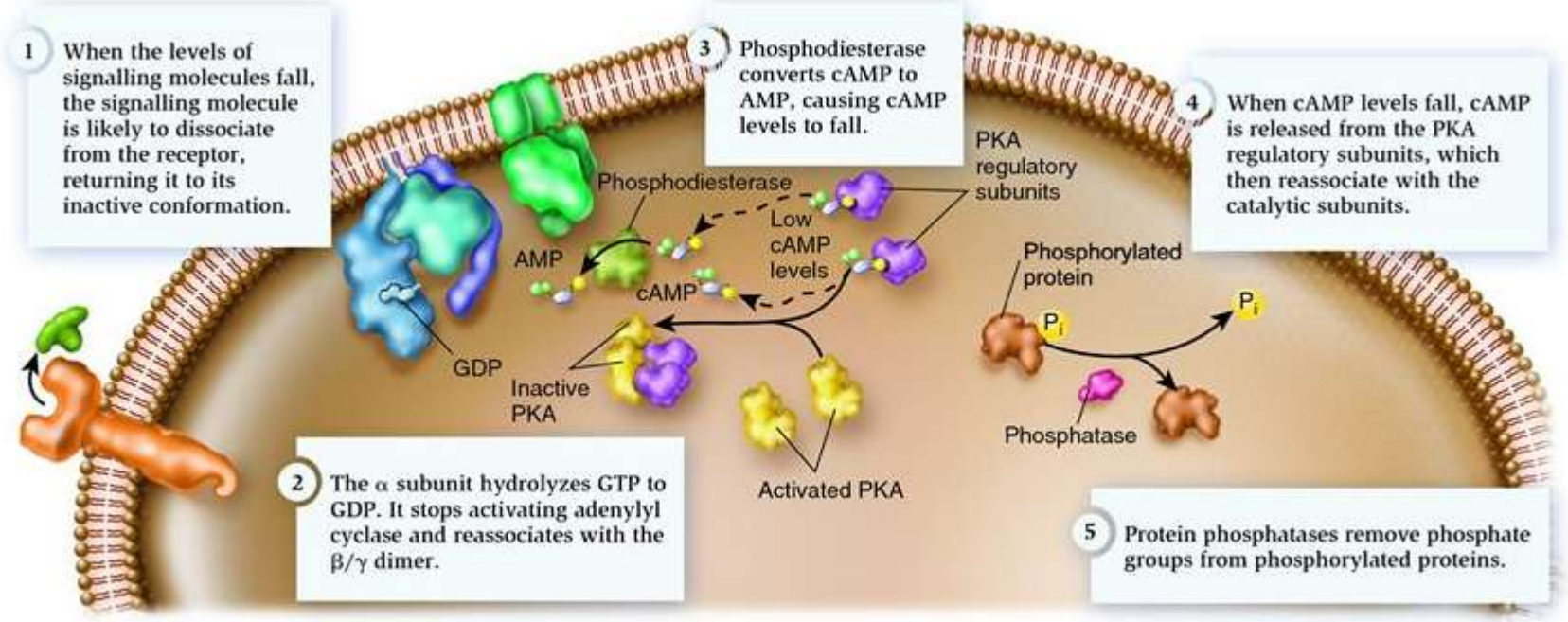


cAMP pathway



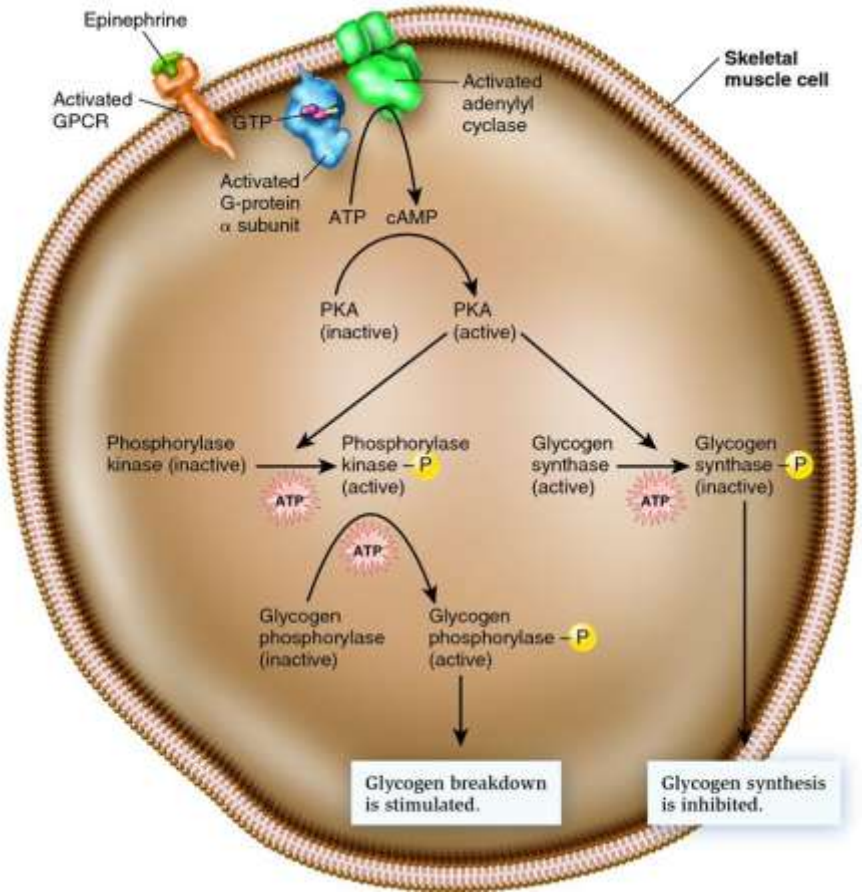
(a) Turning the pathway on.

cAMP pathway



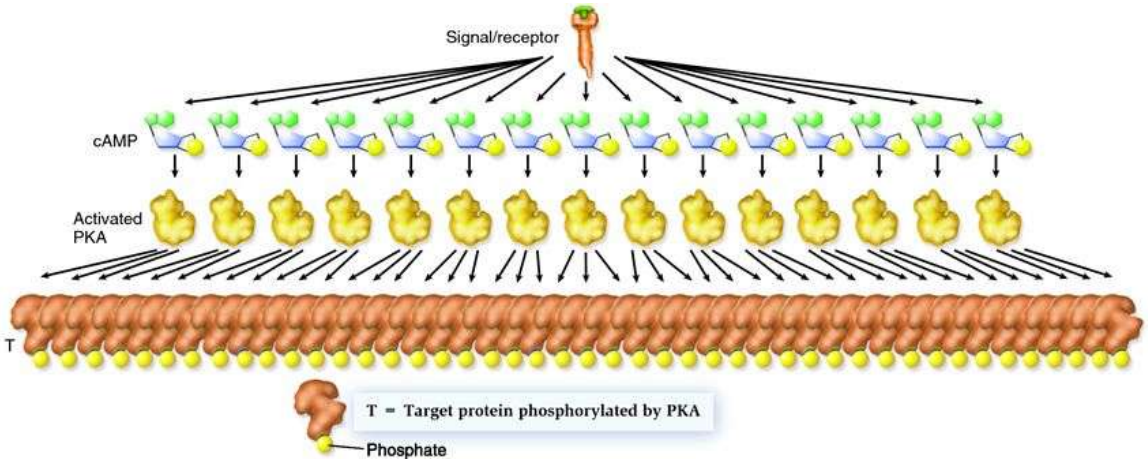
(b) Turning the pathway off

How does PKA work?



Advantages of cAMP pathway

- Signal amplification
- Speed
  - In one experiment a substantial amount of cAMP was made within 20 seconds after addition of signal

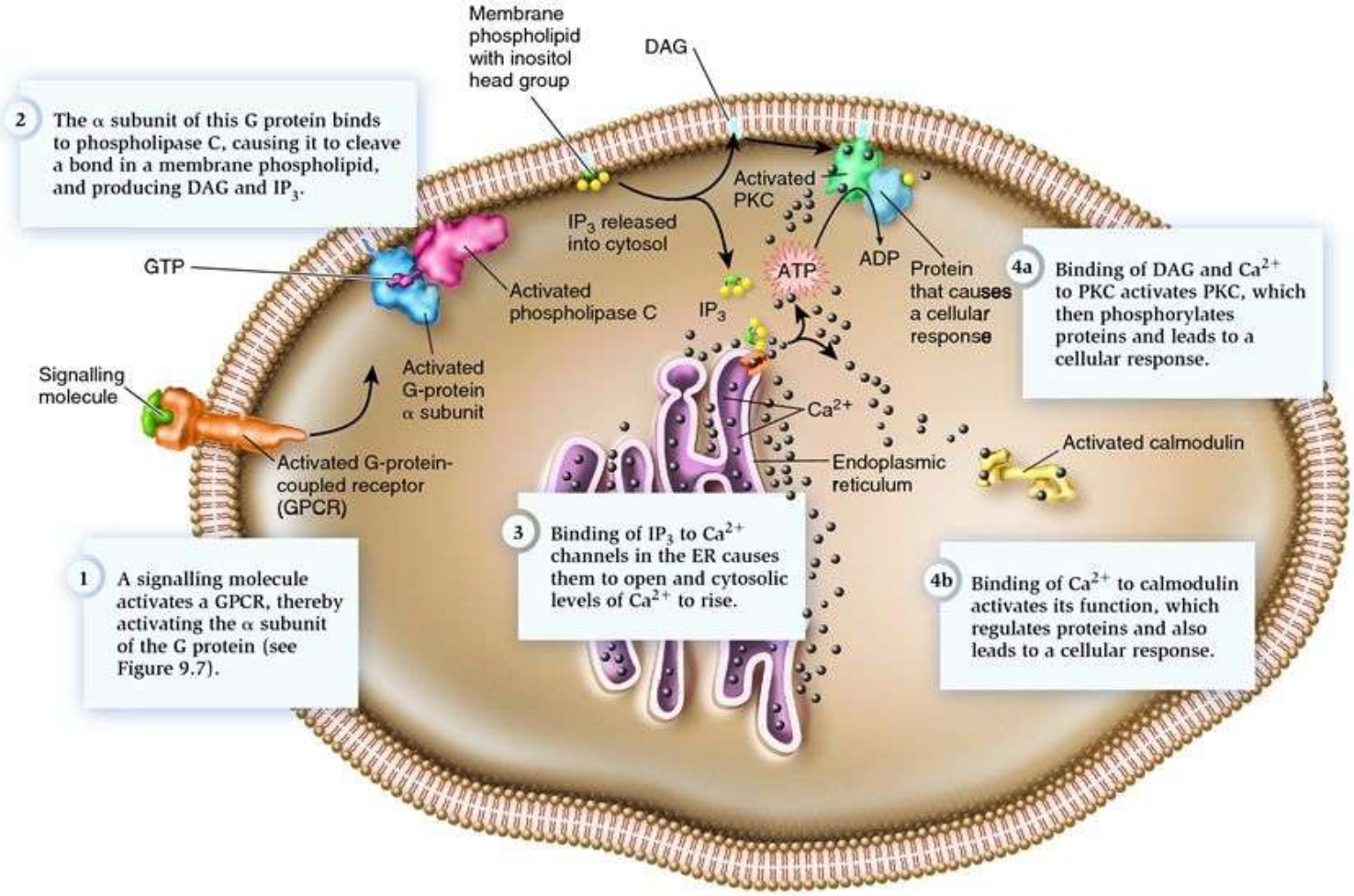


Example Question

cAMP pathway is turned off by...

a) G-protein reassembly
b) PKA reassembly
c) Phosphodiesterase
d) Phosphatase
e) All of the above

Ca^{2+} signalling



Ca^{2+} signatures

- Where do you get specificity if you just release Ca^{2+} as if it was a switch?
- There is a spatial and temporal context (and magnitude context) for Ca^{2+} release
- Spatial
  - Ca^{2+} channels only open on a certain area of the ER
- Pulses of Ca^{2+} release can act like a sort of Morse code to Ca^{2+} sensors

Responses to the same signal can differ

- One hormone causes different effects in different cell types
  - Epi causes contraction in muscle but relaxation in lungs
- Different proteome
- Can affect cellular response in a variety of ways
  - Receptor not expressed
  - Different receptors for same signal
  - Different affinities for signal
  - Signal transduction pathways different
  - Protein expression different

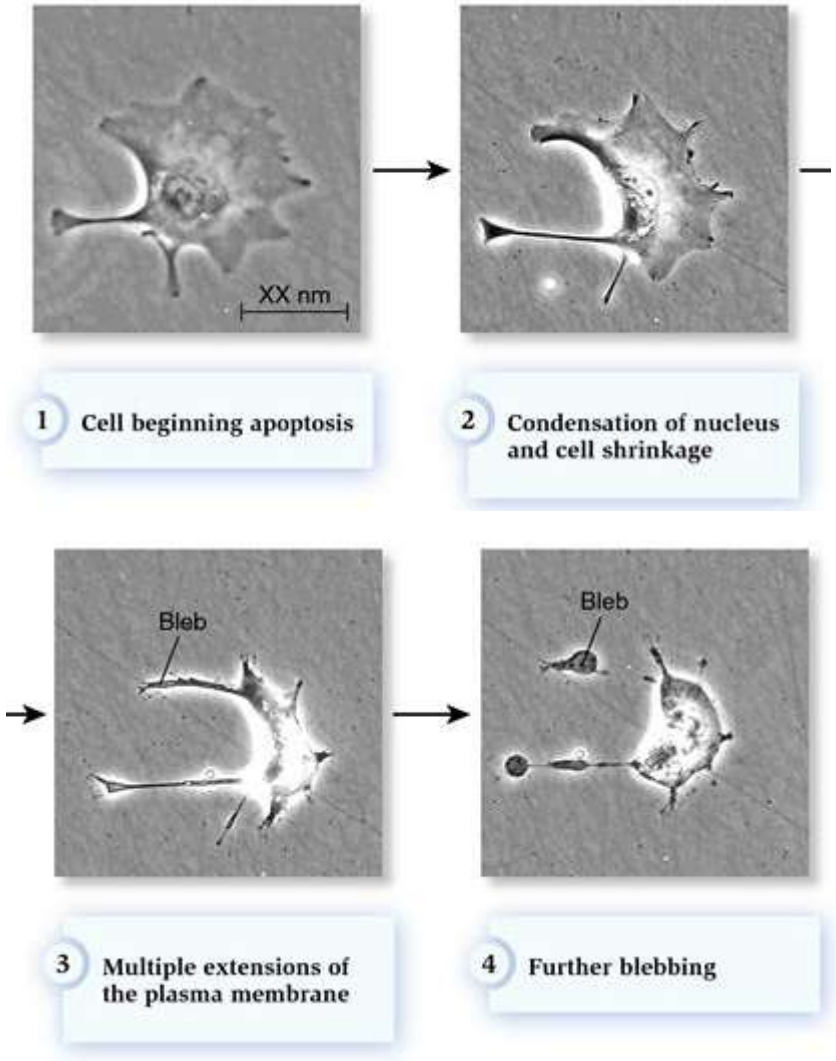
Example question

Why can the same signal have different effects in different cells?

a) Different proteome
b) Different receptors
c) Different signal pathway
d) All of the above

Apoptosis

- Programmed cell death (PCD)
  - Old cells, infected cells, unnecessary cells
- Cell shrinks and forms rounder shape
  - Due to destruction of nucleus and cytoskeleton
- Plasma membrane forms blebs
  - Blebs are irregular extensions that break away



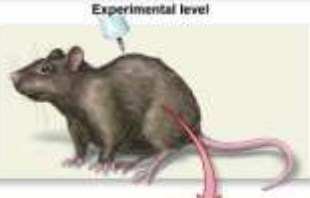
Hormones can initiate apoptosis

HYPOTHESIS Hormones may affect cell number in the adrenal gland by controlling the rate of apoptosis.

KEY MATERIALS Laboratory rats, purified prednisolone, and ACTH.

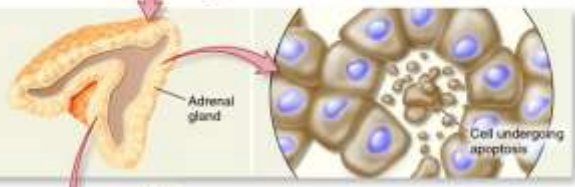
Experimental level | **Conceptual level**

1 As a control, inject rats with saline. Also inject rats with prednisolone alone, prednisolone plus ACTH, or ACTH alone. Note: Five rats were injected in each case.



Previous studies predicted that prednisolone alone may promote apoptosis by lowering ACTH levels.

2 After two days, obtain samples of adrenal tissue from all 20 rats.



3 Observe the samples via light microscopy, which is described in Chapter 4.

4 **THE DATA**

Micrograph of adrenal tissue showing occasional cells undergoing apoptosis (see arrow)

Treatment	Number of animals	Glands with enhanced apoptosis*/ Total number of animals
Saline	5	0/10
Prednisolone	5	9/10
Prednisolone + ACTH	5	0/10
ACTH	5	0/10

*Samples from two adrenal glands were removed from each animal. Enhanced apoptosis means that cells undergoing apoptosis were observed in every sample under the light microscope.

5 **CONCLUSION** Prednisolone alone, which lowers ACTH levels, causes some cells to undergo apoptosis. During this process, the cells shrink and form blebs as they kill themselves. Apoptosis is controlled by hormones.

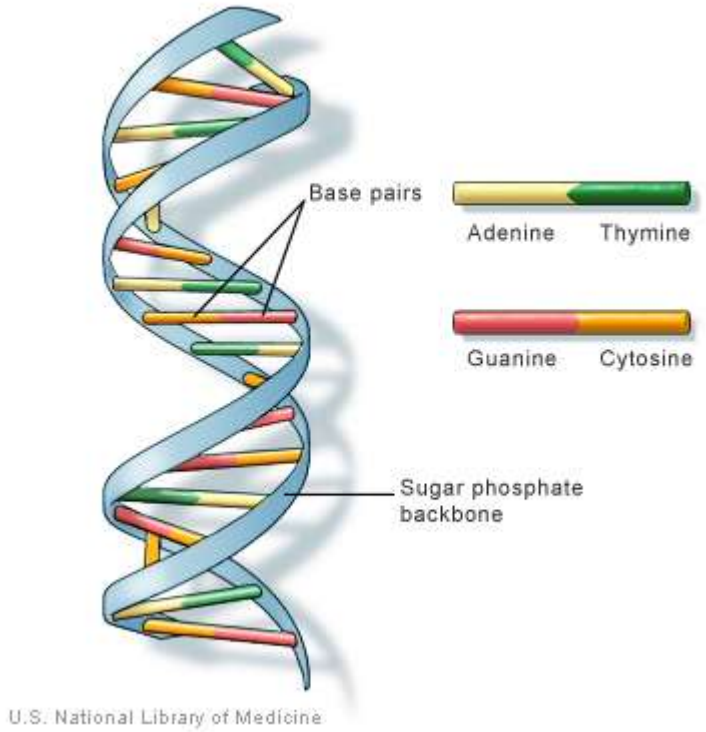
6 **SOURCE** Wyllie, A.H., Kerr, J.F.R., Macaskill, I.A.M., and Currie, A.R. (1973) Adrenocortical cell Deletion: The Role of ACTH. *Journal of Pathology* 111, 85–94.

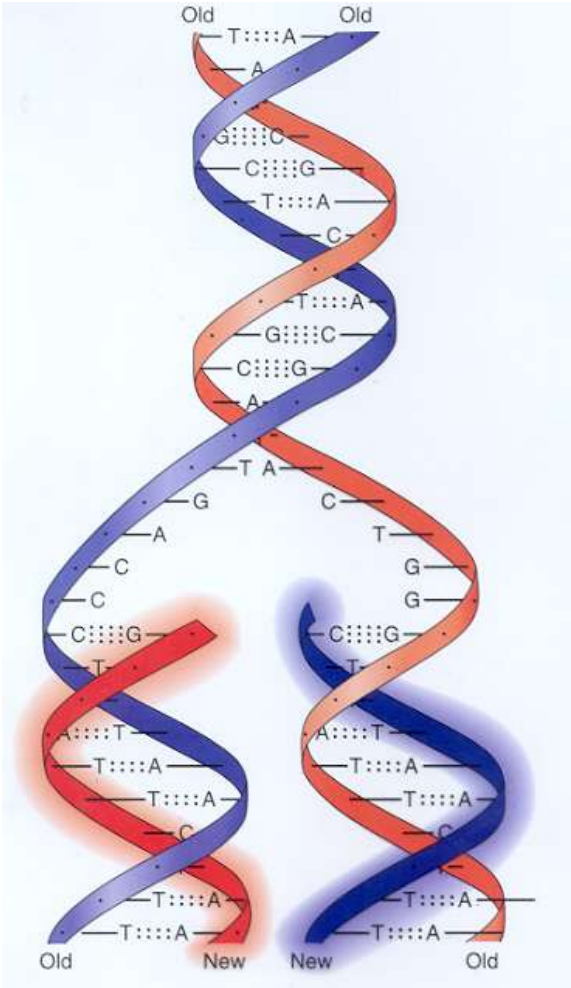
Example Question

Which of the following is not a feature of apoptosis?

a) Blebbing
b) Cell shrinkage
c) Cell splitting
d) Round shape

Chapter 10 – Nucleic Acid Structure and DNA Replication





Genetic Material

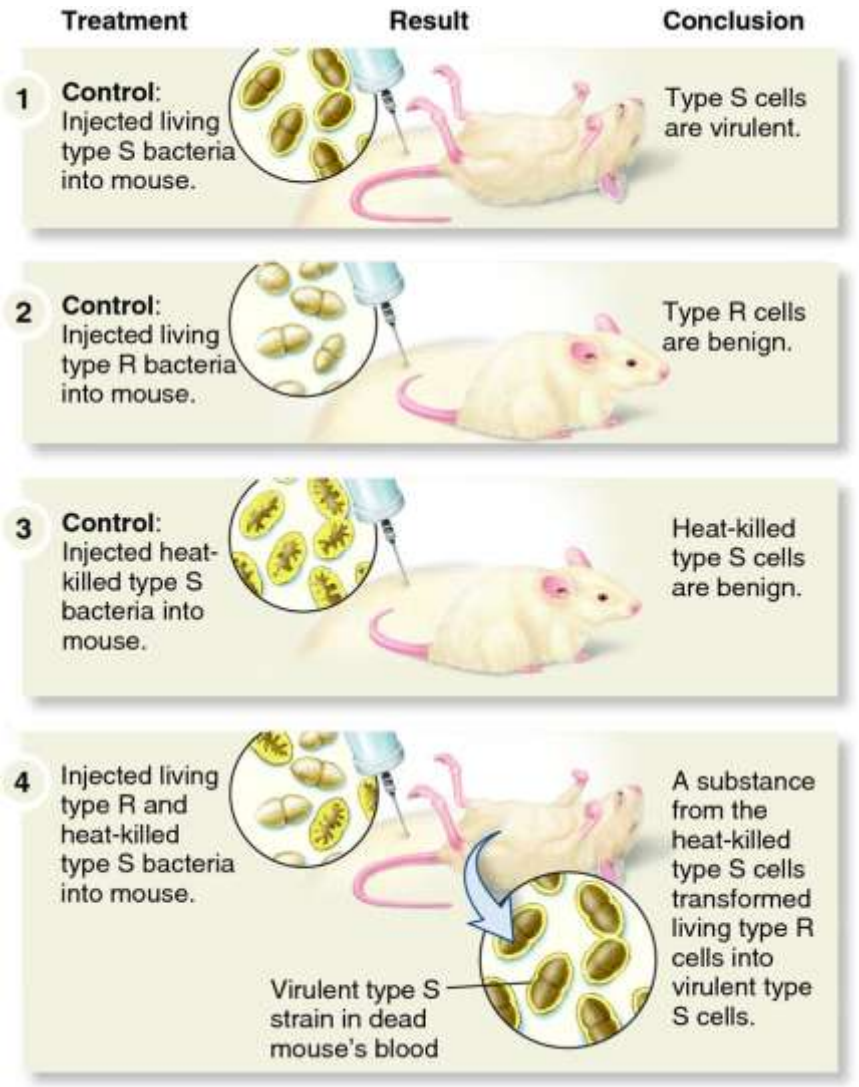
- Must be able to replicate itself
  - Must be able to do this at high fidelity (high accuracy)
- Must be able to control living processes

History of DNA

- Meitscher 1869 – isolated a phosphate containing acid from the nucleus (which they could see)
- He called it nuclein

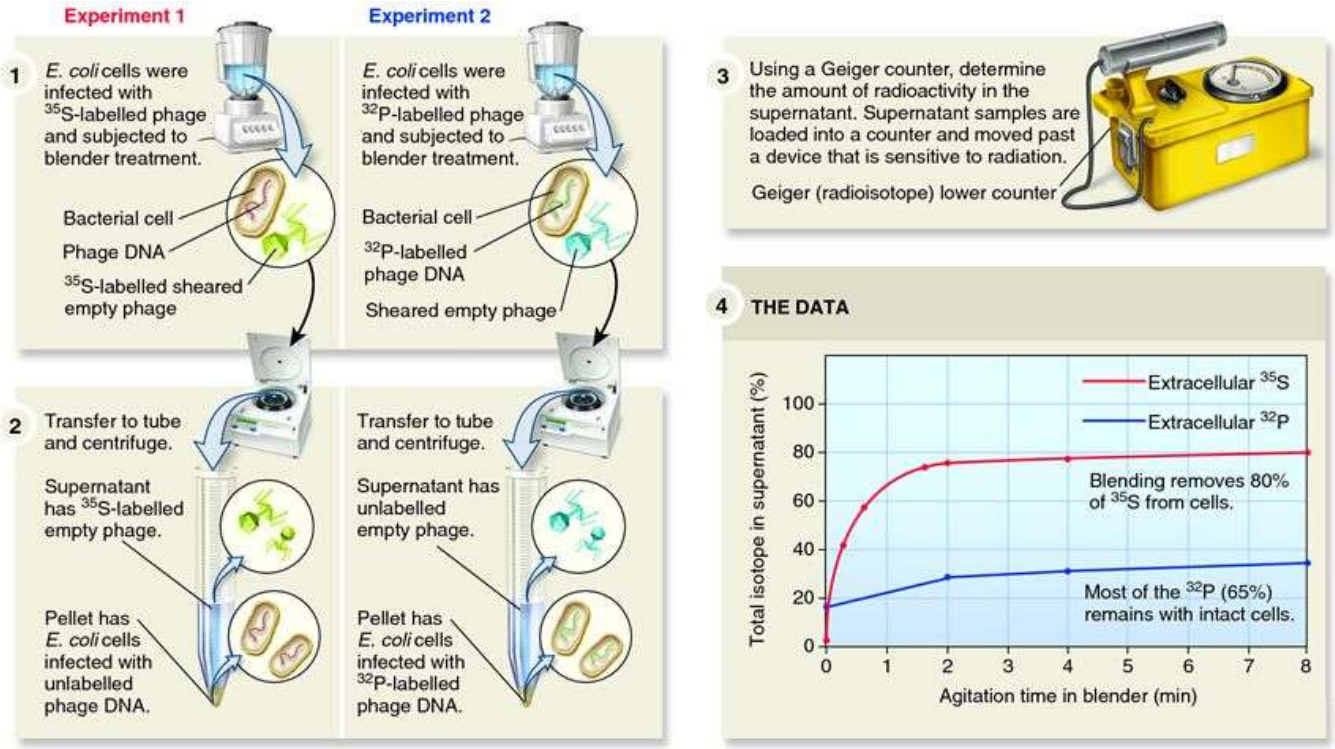
History of DNA (Griffith 1928)

- Showed that something was able to transform the bacteria
- This material was also able to replicate itself between generations



History of DNA (Hershey-Chase 1952)

- Thought it might have been the viral casing
- Discovered that the transforming principle was DNA

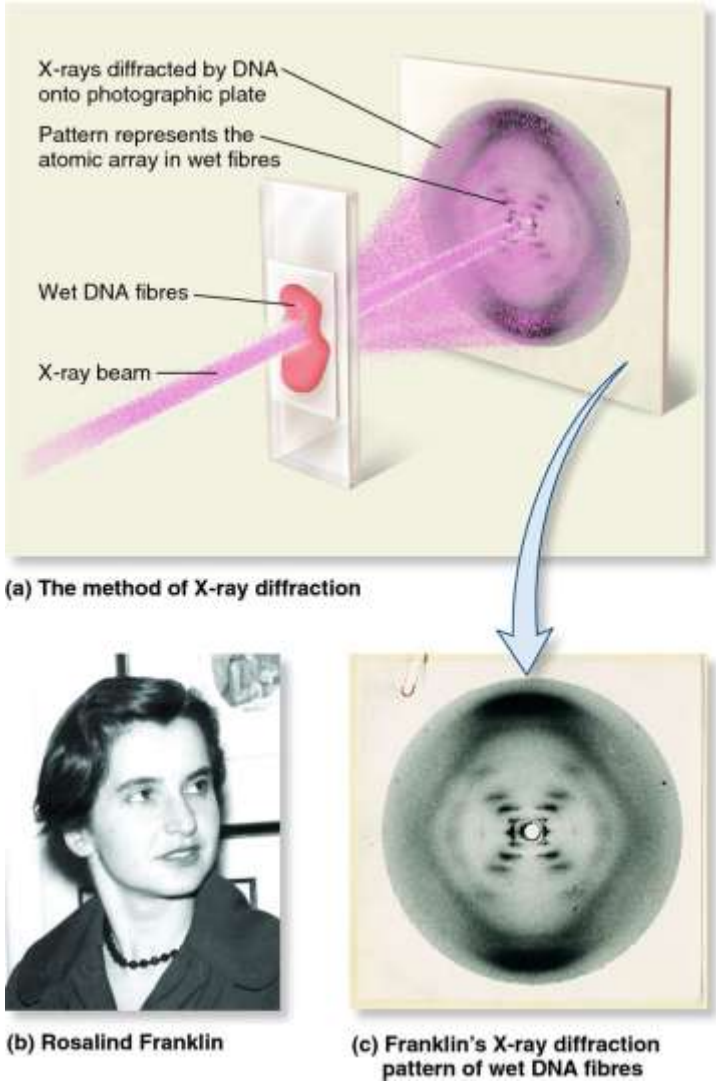


History of DNA (Chargaff 1930s/40s)

- Analyzed DNA of several species and found that:
  - [A] = [T]
  - [C] = [G]
- We now know that A binds with T and C with G
  - Called Chargaff's Rules

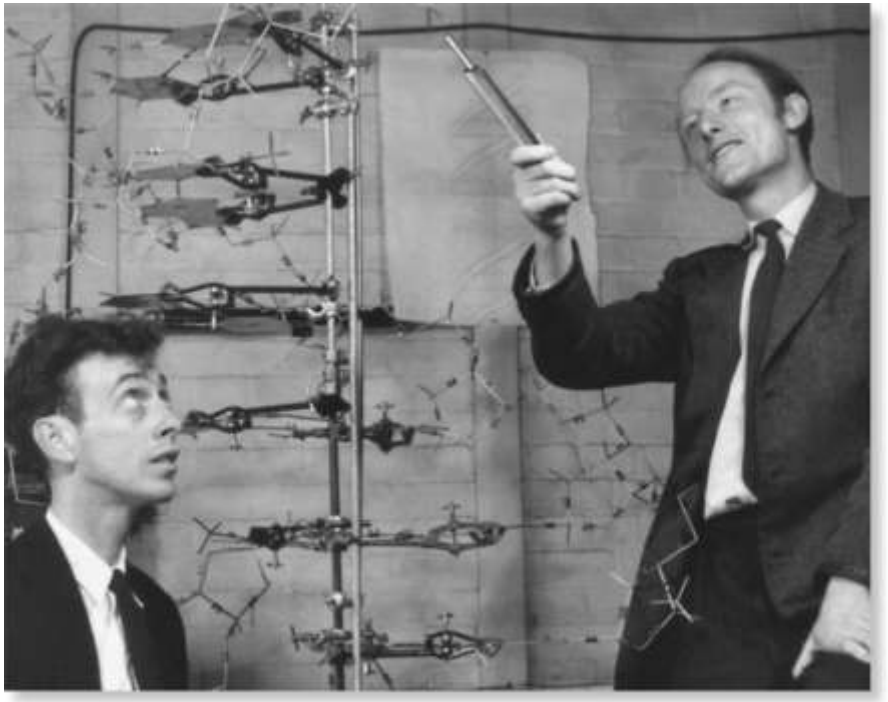
History of DNA (Franklin and Wilkins 1940-1953)

- Discovered that DNA was linear and arranged in a helix



History of DNA (Watson and Crick 1953)

- Modeled the structure of DNA and discovered that it was a double helix



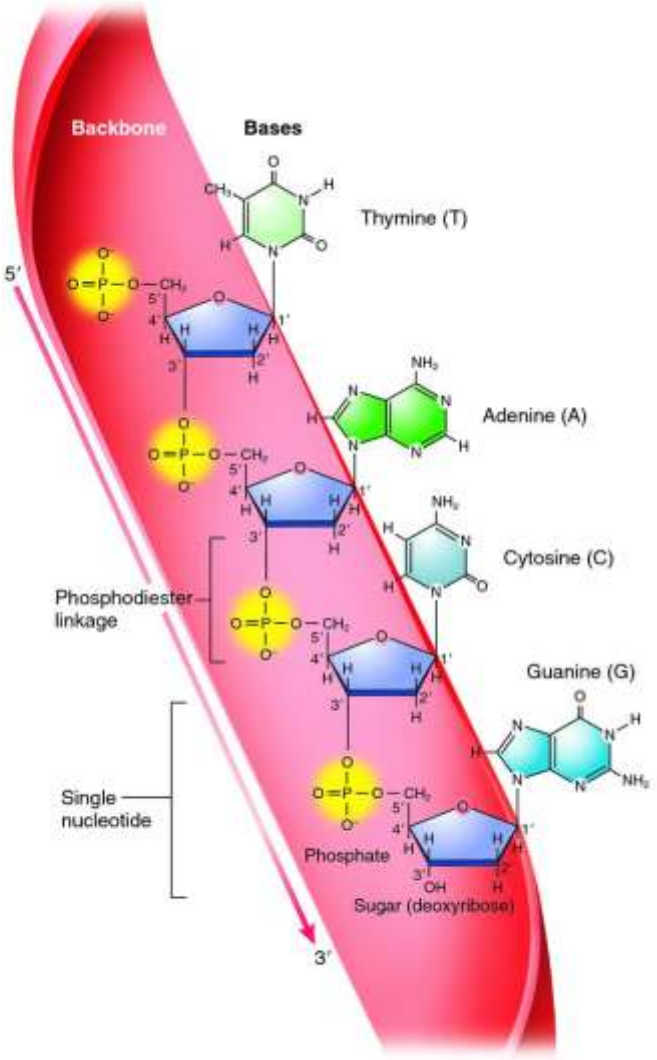
Example Question

Which of the following is FALSE?

a) Hersey and Chase discovered that DNA was the genetic material
b) Griffith discovered that there must have been some sort of transforming principle
c) Franklin and Wilkins discovered that DNA was a double helix
d) Chargaff discovered the ratios of nucleotides

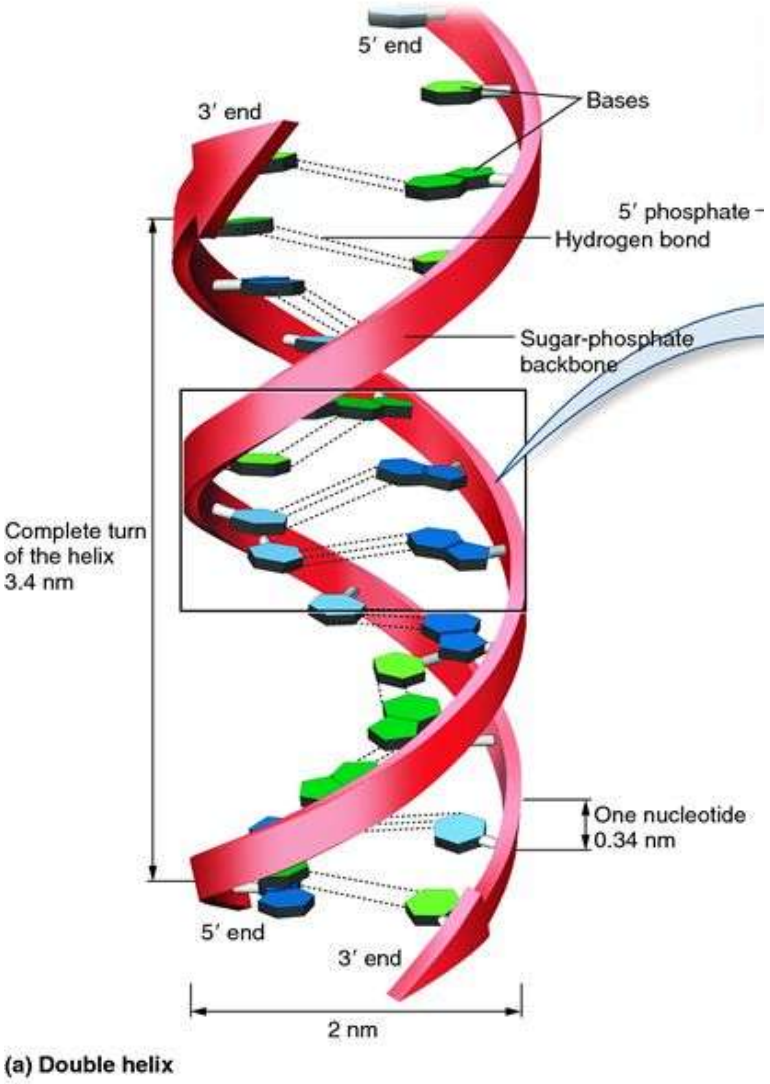
DNA Structure

- Ladder uprights
  - Alternating sugar (deoxyribose) and phosphates
- Phosphodiester bonds hold these components together



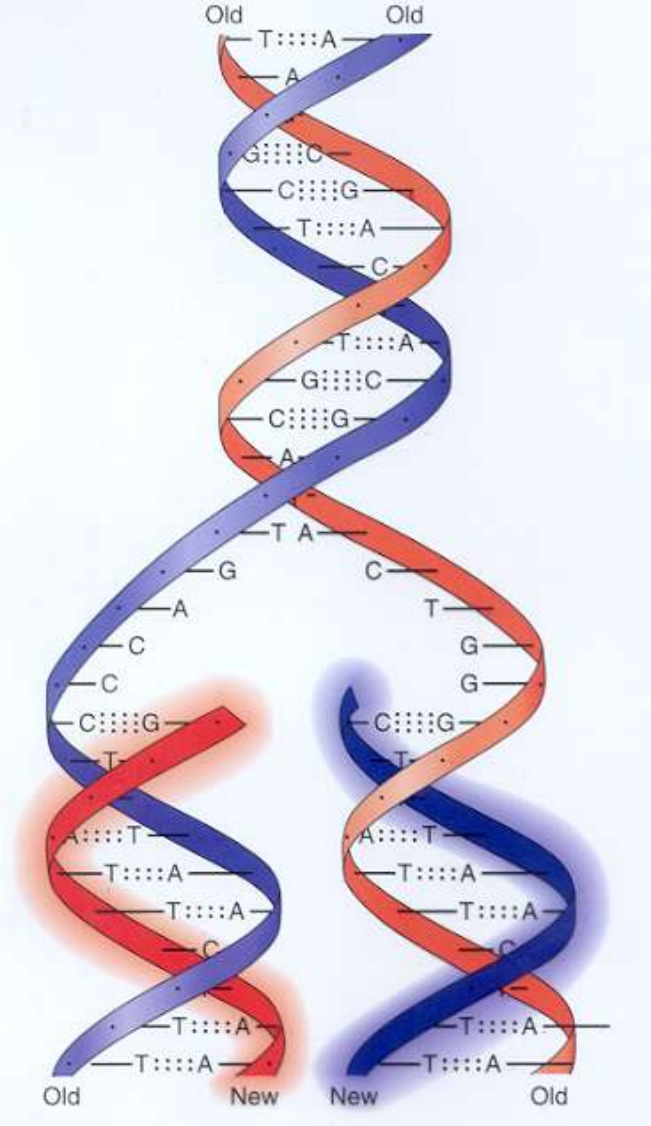
DNA Structure

- Ladder rungs – nitrogenous bases.
  - The two base pairs are held together with hydrogen bonds.
  - A and G have double cyclic structure (purines)
  - C and T have single cyclic structure (pyrimidines)
    - Uracil replaces T in RNA
- Two sides of the ladder are antiparallel.
  - One side goes from 5' – 3', the other goes from 3' – 5'. (determined by the numbering of the carbons in the sugar)
- Major and minor groove

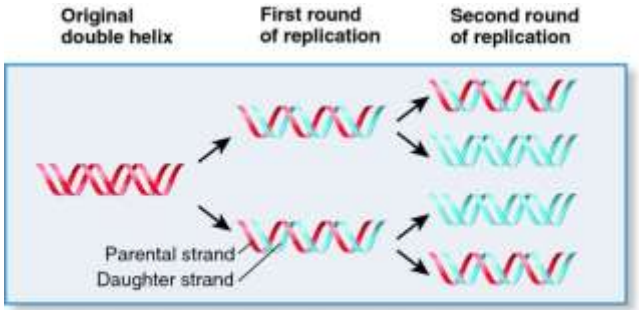


Central Dogma

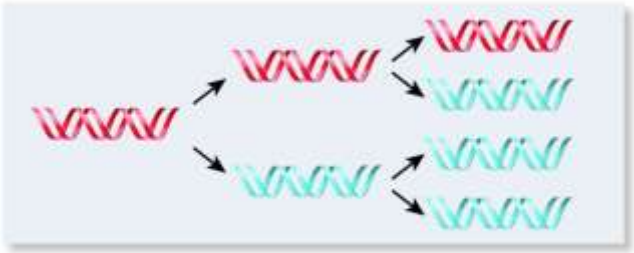
- Central dogma of molecular biology involves
  - Replication
    - DNA replicating itself
  - Transcription
    - Process of transcribing DNA into RNA (mRNA)
  - Translation
    - Translating the mRNA transcript into a protein



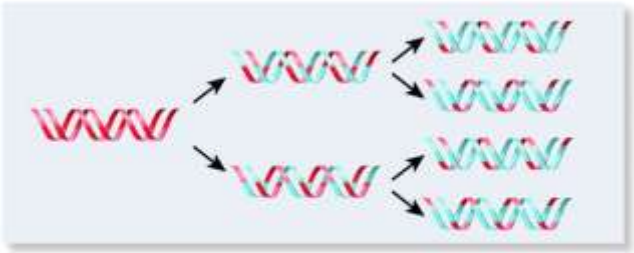
Initial hypotheses for DNA Replication



(a) Semiconservative mechanism. DNA replication produces DNA molecules with one parental strand and one newly made daughter strand. This model fits Meselson and Stahl data.



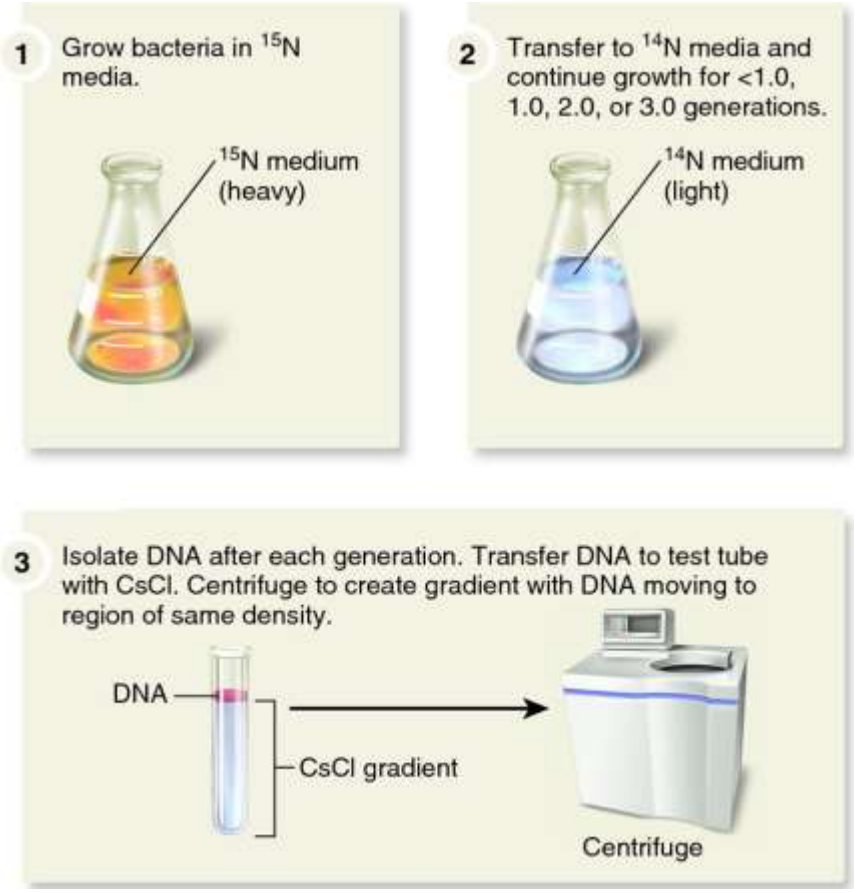
(b) Conservative mechanism. DNA replication produces one double helix with both parental strands and the other with two new daughter strands.



(c) Dispersive mechanism. DNA replication produces DNA strands in which segments of new DNA are interspersed with the parental DNA.

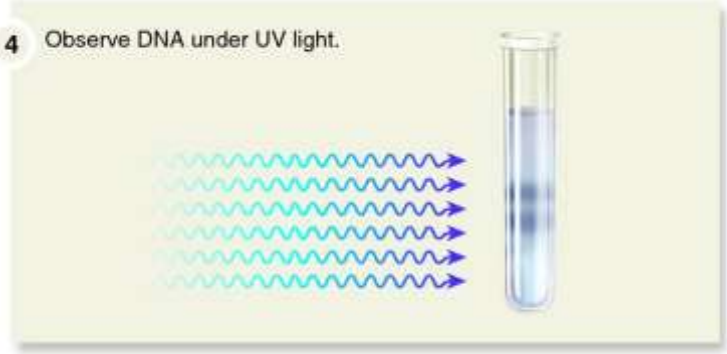
Meselsohn and Stahl

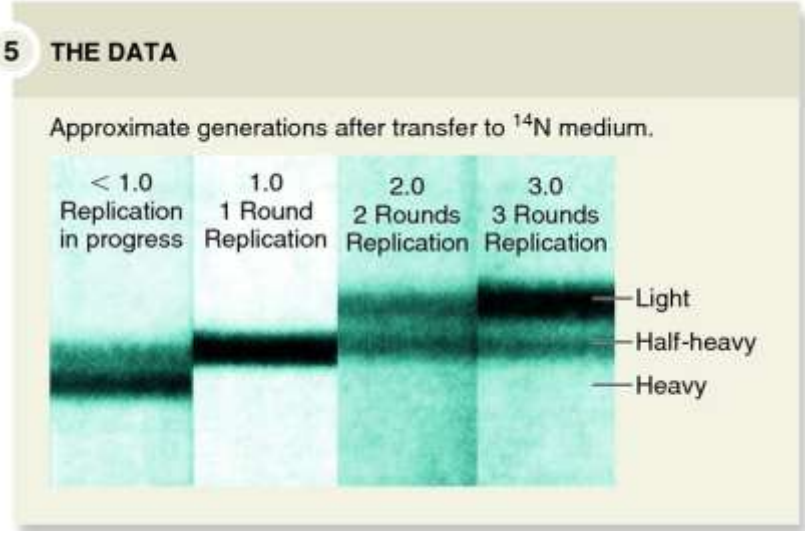
- Bacteria take up any isotope of N to make DNA
- Used ^{15}N (radioactive N) instead of ^{14}N (normal N) to make heavy (radioactive) DNA in bacteria for several generations
  - Did this by growing cells in ^{15}N environment for several generations
- They then provided the bacteria with ^{14}N containing medium in later generations of growth
  - Grew them for less than 1 generation, 2, 3, etc.
- DNA was centrifuged with CsCl
  - During centrifugation CsCl makes a density gradient



Meselsohn and Stahl

- The DNA moves to a region of same density
  - This means that you can tell the density of DNA based on its position in the tube
  - Called isopycnic (same density) centrifugation
- Found that as generations progressed the DNA had different densities
- The particular pattern of density change over generations could only be explained by semiconservative replication hypothesis





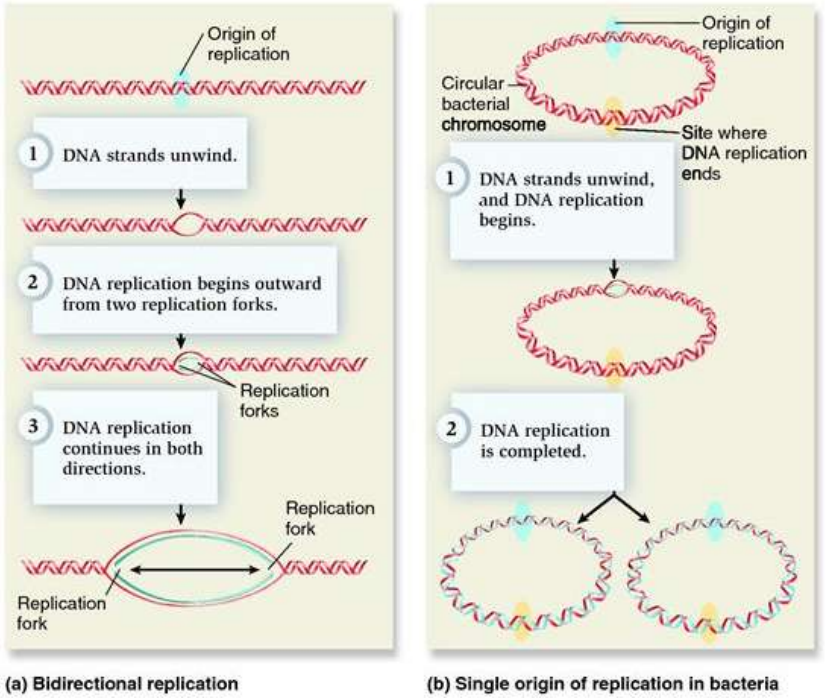
Example Question

Meselsohn and Stahl found that DNA replication via a _____ mechanism

a) Semiconservative
b) Conservative
c) Dispersive
d) Compressive

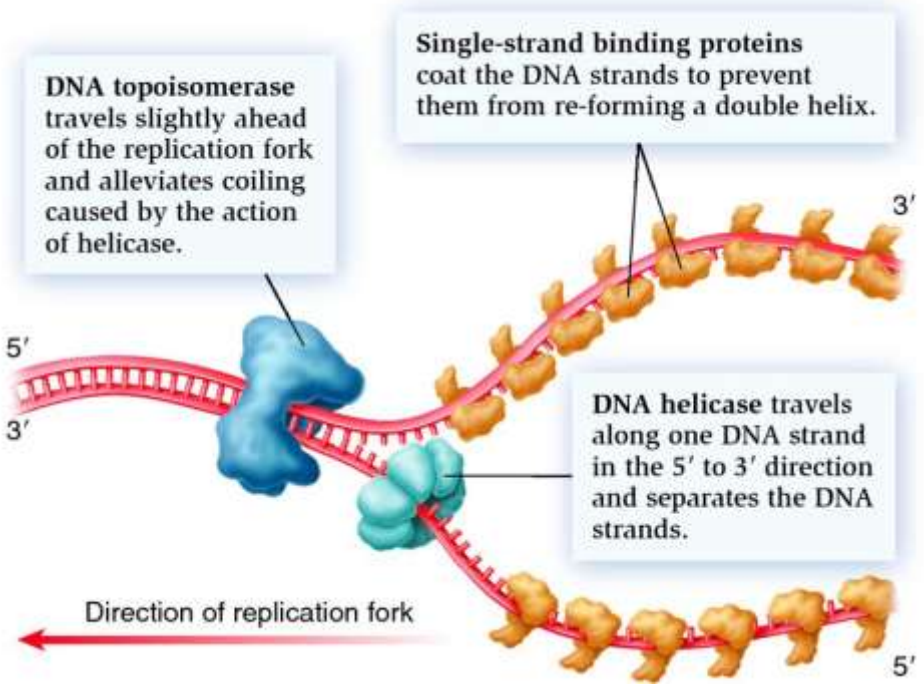
Origin of Replication

- Replication starts at a predetermined place on the DNA (at a particular sequence)
  - This location is called the origin of replication
- Bidirectional replication, single origin replication, multiple origin replication



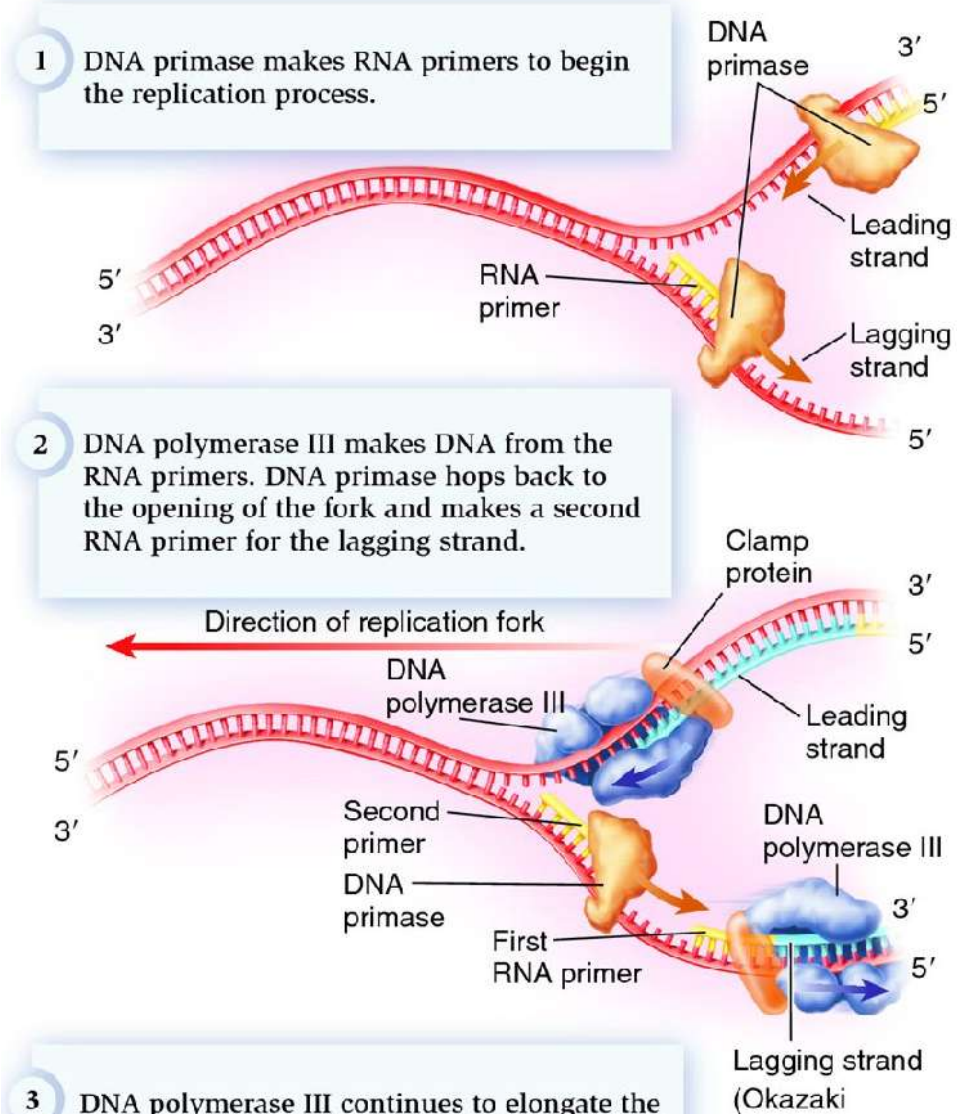
DNA Replication

- Helicase unwinds the DNA (moving 5' to 3')
  - Held open by single strand binding proteins
    - This ensures that the strands do not come back together
  - Replication is then free to begin at the replication fork
  - Excessive coiling
    - Opening double helix causes increased coiling of DNA further downstream
    - Topoisomerases cuts the sugar backbone of DNA to allow uncoiling and then rejoins that region of the DNA



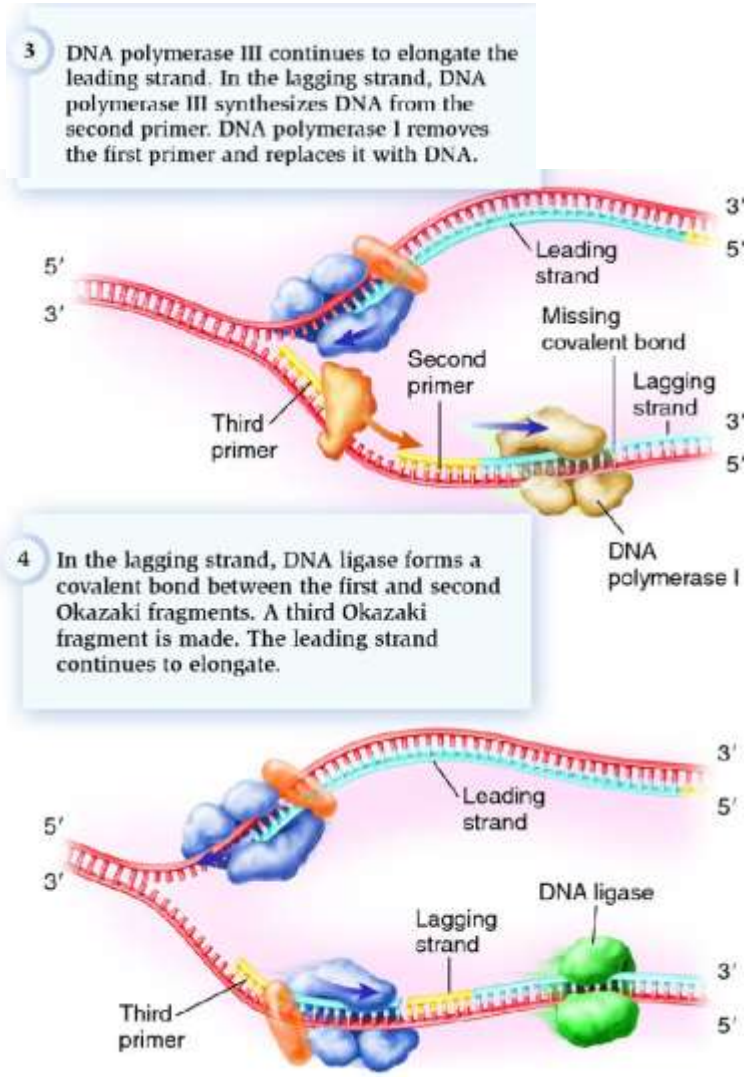
DNA Replication

- DNA primase adds an RNA primer
- DNA polymerase III makes DNA from RNA primers and then adds nucleotides to the 3' end, according to the parental strand template.
  - DNA polymerase can only begin working on a double strand so that is why the RNA primers are added first
- Replication occurs from 5' to 3'
  - Because of the antiparallel quality of the strands, one strand is elongates towards the fork (the leading strand), and the other elongates away from the fork (lagging strand).



DNA Replication

- Because the fork is opening in one direction, the lagging strand has to replicate in small fragments (Okazaki fragments)
  - This takes more time because the lagging strand grows discontinuously (hence the name lagging).
  - When the DNA polymerase III reaches the RNA primer on the lagging strand it DNA polymerase I comes and replaces the RNA with DNA
    - DNA ligase then attaches and forms phosphodiester bonds



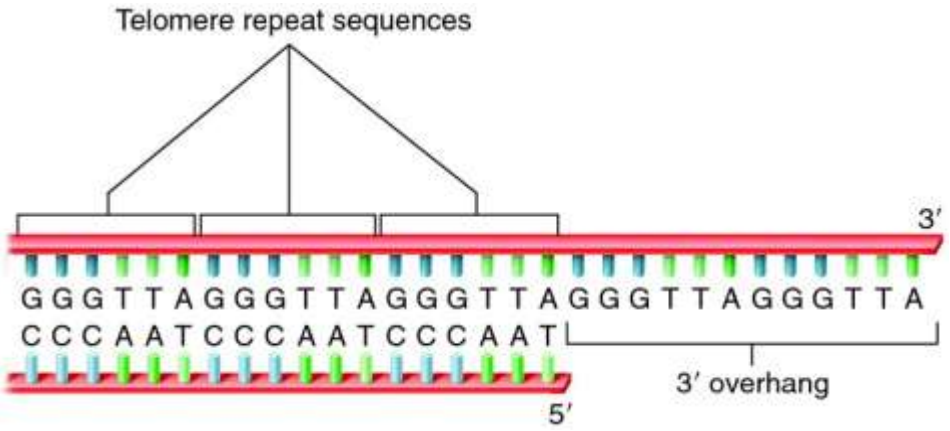
Example Question

Which of the following are not involved in DNA replication?

a) DNA Polymerase III
b) DNA Polymerase II
c) DNA Polymerase I
d) DNA Helicase

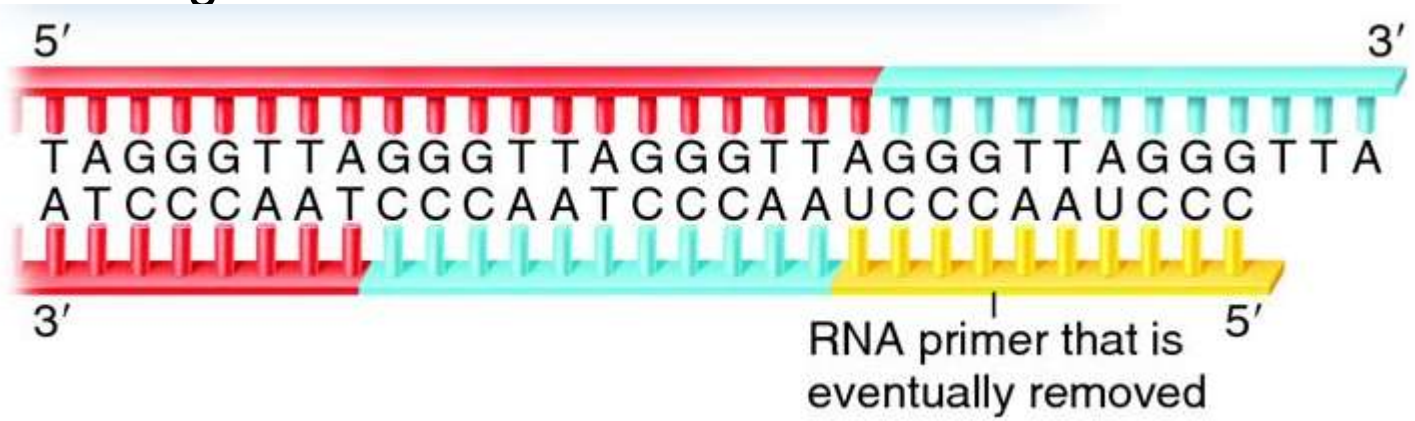
Telomeres

- Stretches of repeated DNA at the end of the chromosome that do not code for anything
- Prevent shortening of the DNA
- 3’ overhang created because of the Okazaki fragments
  - In order to change RNA to DNA, there must be another DNA strand in front of the RNA primer



Telomerase

- Telomerase attaches these repeated sequences to the ends of chromosomes.
  - Prevents chromosome shortening
- Telomerase lengthens the overhang with the same repeating sequence.
- RNA primer can now be attached and fill in some of the overhang.



Senescence and Cancer

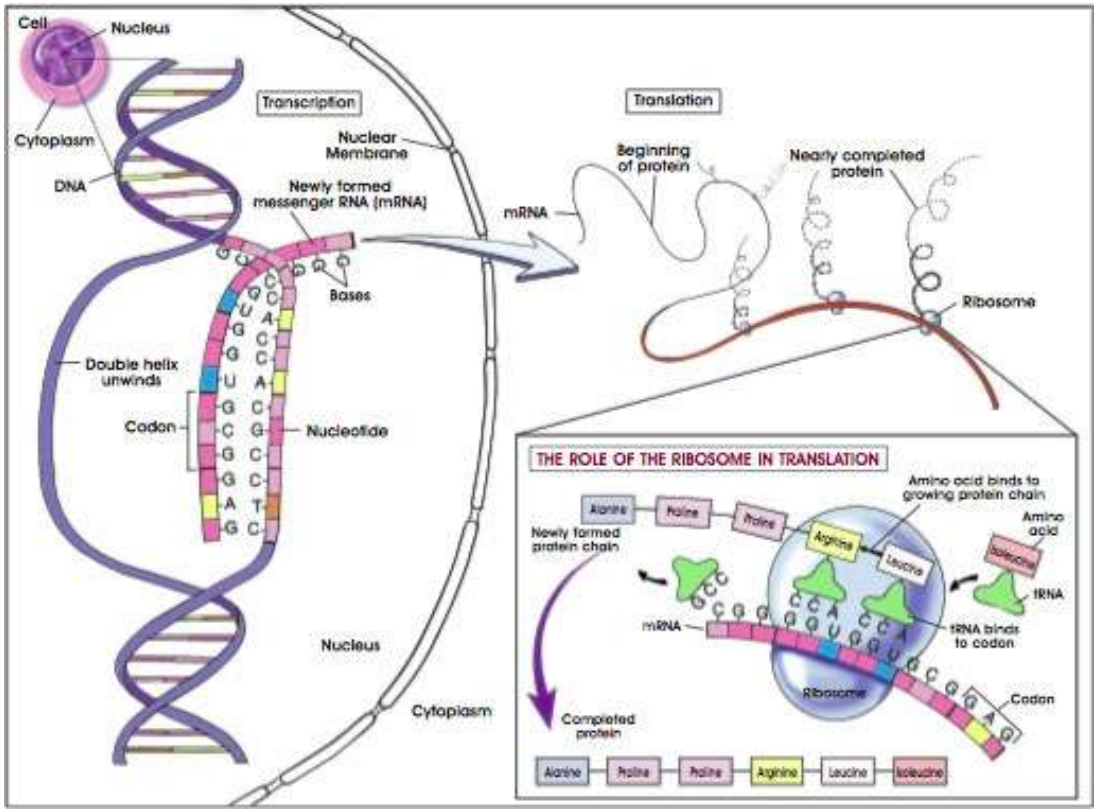
- Decline in telomerase function has been found in cells of old individuals
  - May contribute to aging
- Cancer cells have high telomerase activity
  - May contribute to proliferation

Example Question

Telomerases…

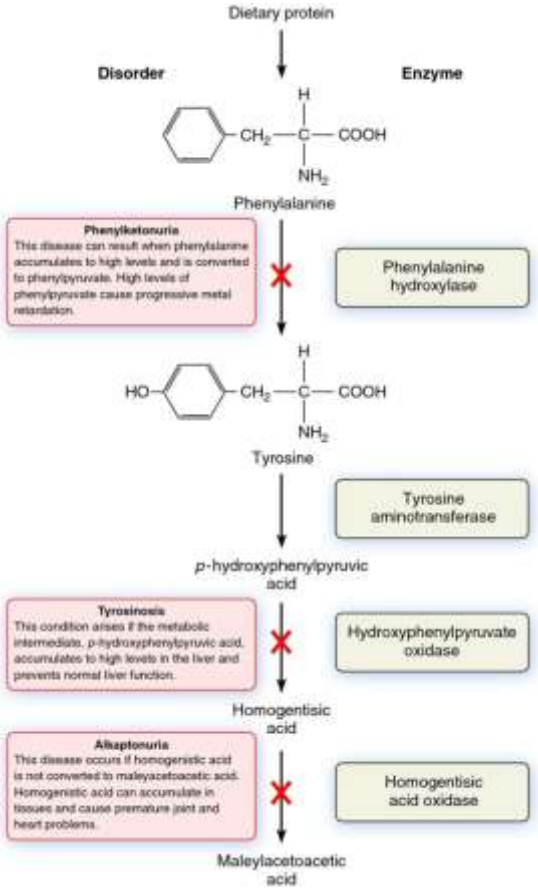
a) Are stretches of repeated DNA at the end of chromosomes
b) Do not code for anything
c) Prevent shortening of chromosomes
d) All of the above
e) None of the above

Chapter 11 – Genetic Expression at the Molecular Level



Genes Specify Protein Structure

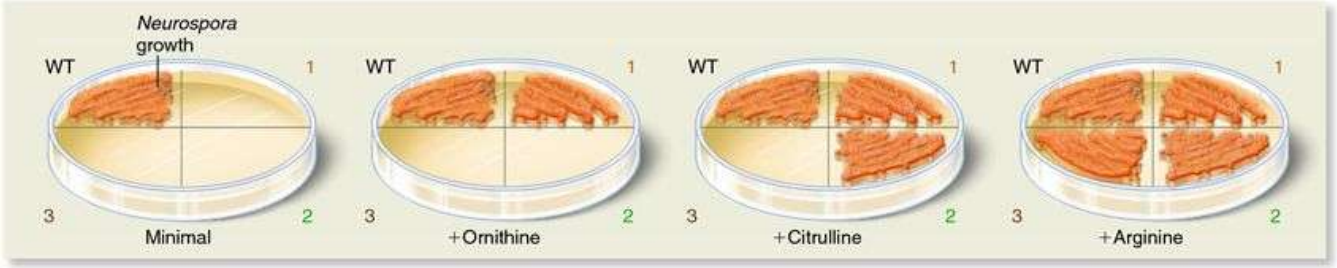
- Garrod thought that enzyme damage or failure was the reason for the failure of metabolic processes
  - The underlying cause of this is often genetic
  - He showed this with alkaptonuria (black urine) caused by a buildup of homogentisic acid
  - Demonstrated that when homogentisic acid oxidase was lost or damaged, alkaptonuria resulted



Beadle and Tatum

- Beadle and Tatum, working off of Garrods ideas, came up with the one gene-one enzyme hypothesis
- Using neurospora (bread mold) as a model organism, they broke down the pathway to the mold producing arginine
- They did this by plating UV irradiated mold with minimal medium and then sequentially different growth media, and noted the growth of each plate

(a) Simplified pathway for arginine synthesis

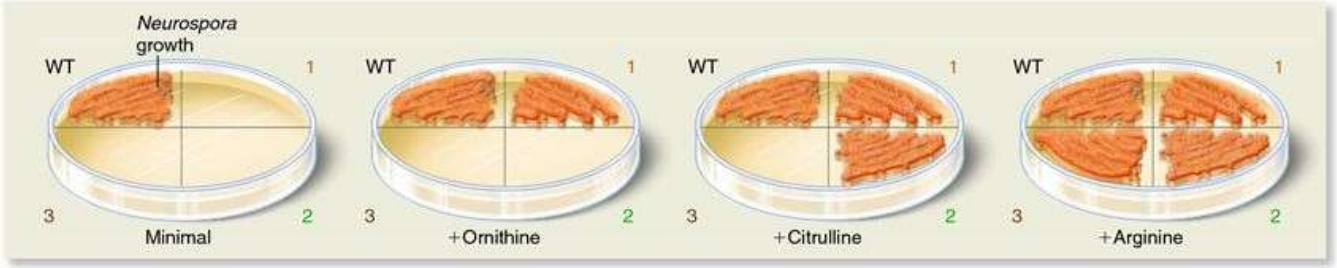


(b) Growth of strains on minimal and supplemented growth media

Beadle and Tatum

- UV irradiation produced mutants that needed certain precursor molecules
  - The question was wan an enzyme missing?
- Analysis of metabolic mutants revealed multiple enzymes that lead to arginine synthesis in a serial manner
  - Different stages of loss of function could be identified
    - Each associated with only one enzyme

(a) Simplified pathway for arginine synthesis



(b) Growth of strains on minimal and supplemented growth media

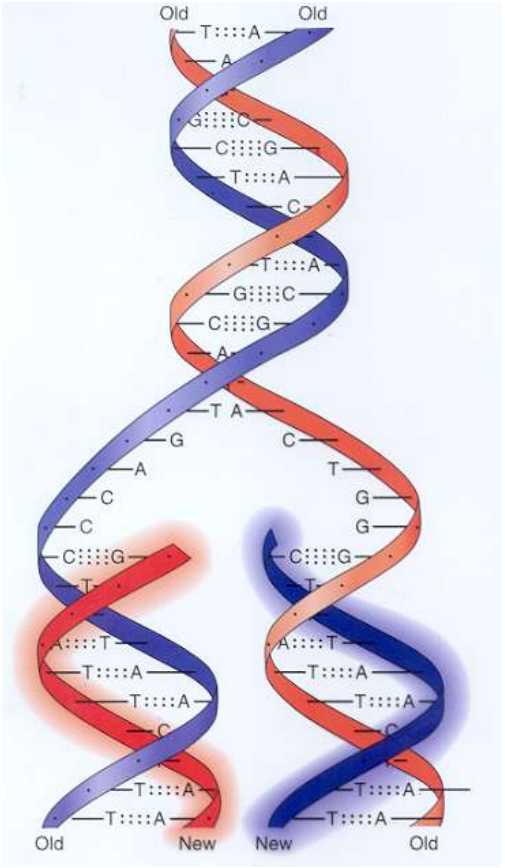
Example Question

The one gene-one enzyme hypothesis states that:

a) Single genes encode single enzymes
b) Single genes encode multiple enzymes
c) Single genes are one enzyme
d) Single genes are multiple enzymes

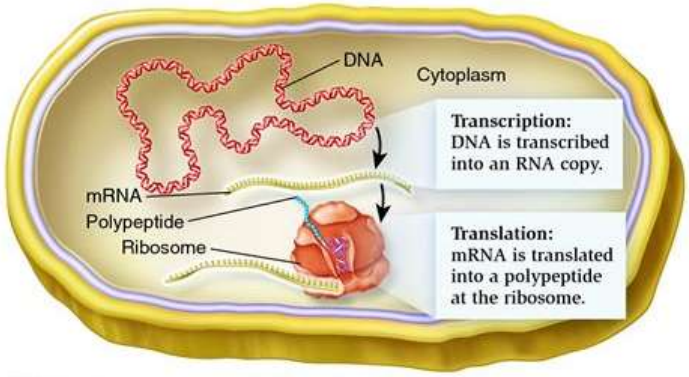
Transcription vs. Replication

- Transcription takes DNA, and turns it into messenger RNA (mRNA)
  - Very similar process to replication
    - Moves in a 5'-3' direction
    - Phosphodiester bonds still bond the nucleotides together
- Some differences between replication and transcription:
  - RNA so A-U instead of A-T
  - RNA polymerase reads the DNA strand instead of DNA polymerase (we want an RNA strand, NOT DNA).
    - RNA strand that is made is complementary to the DNA strand it's created from

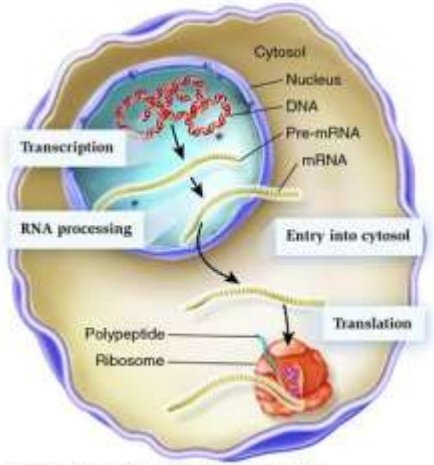


Transcription in Eukaryotes vs. Prokaryotes

- Transcription and translation occurs in the cytoplasm of prokaryotes
  - This means that they can produce the protein very quickly in response for the need for that protein
  - May provide a mechanism to explain the fast growth of prokaryotic cells
- In eukaryotes transcription occurs in the nucleus
  - Translation occurs in the cytoplasm



(a) Molecular gene expression in prokaryotes

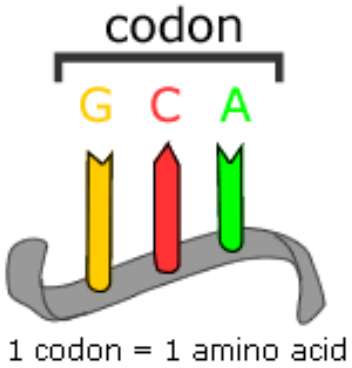


(b) Molecular gene expression in eukaryotes

The Language of Genes

- 4 nucleiotides in DNA and 4 in RNA (U replaces T)
  - All of these need to be able to account for the 20 known amino acids
- There must be a triplet code system
  - 3 bases/amino acid then 64 (4^3) amino acids could be coded
  - Any more would be a waste
- The coding unit is called a codon (3 nucleotides)

First Position	Seconed Position: U code	U Amino Acid	C code	C Amino Acid	A code	A Amino Acid	G code	G Amino Acid	Third Position
U	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	U
	UUC		UCC		UAC		UGC		C
	UUA	leu	UCA		UAA	STOP	UGA	STOP	A
	UUG		UCG		UAG	STOP	UGG	trp	G
C	CUU	leu	CCU	pro	CAU	his	CGU	arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	ile	ACU	thr	AAU	asn	AGU	ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	lys	AGA	arg	A
	AUG	met	ACG		AAG		AGG		G
G	GUU	val	GCU	ala	GAU	asp	GGU	gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	glu	GGA		A
	GUG		GCG		GAG		GGG		G

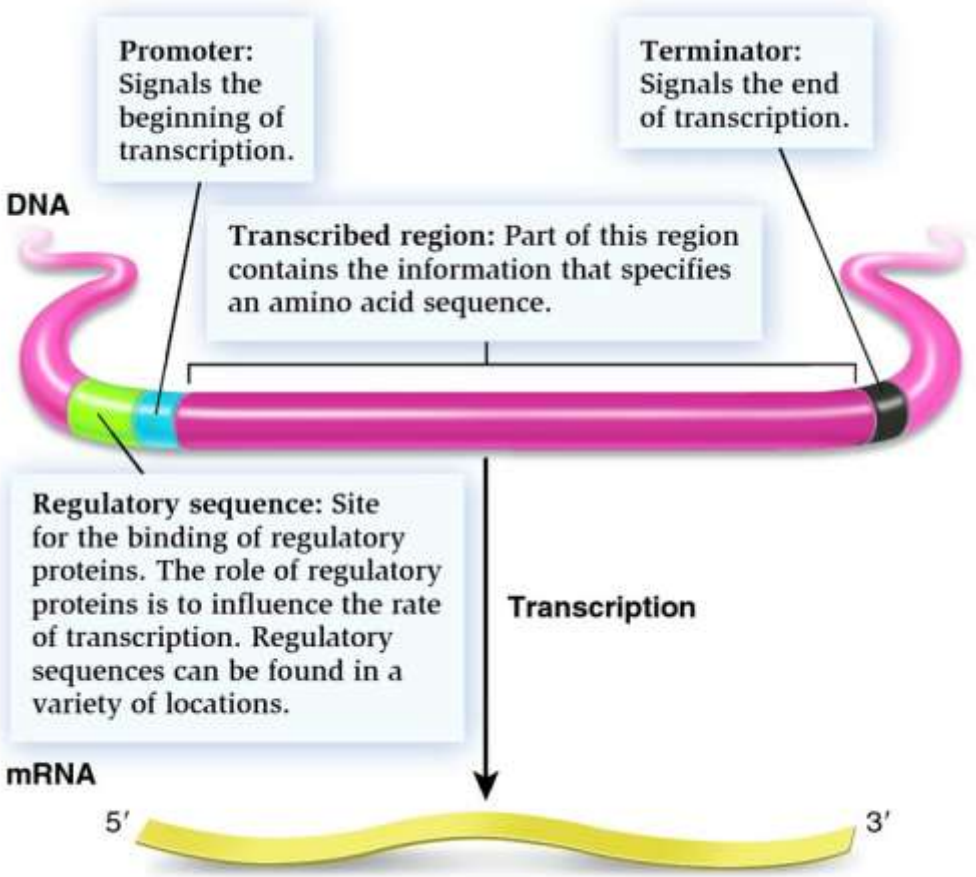


Example Question

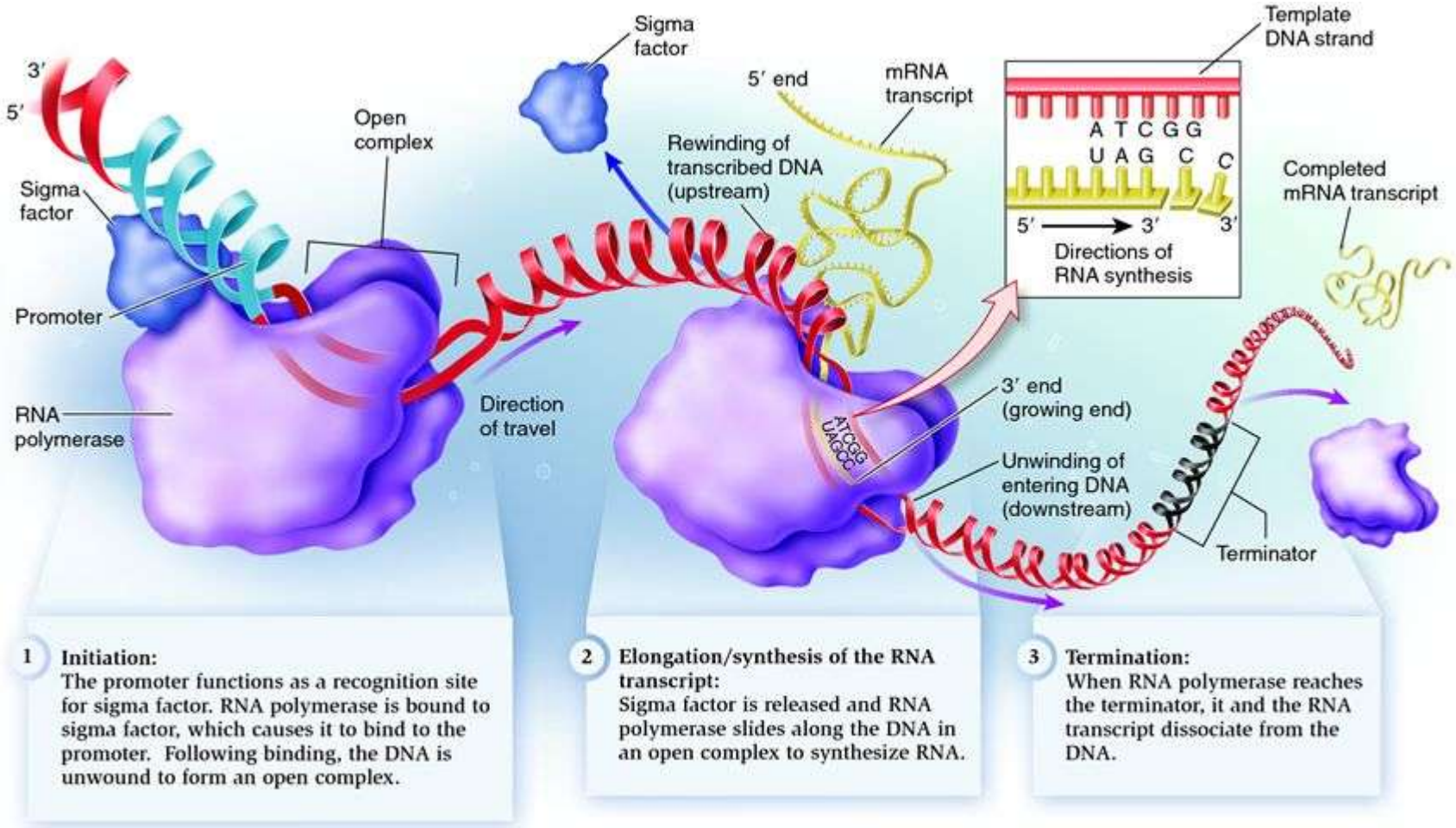
How many possible codons are there?

a) 4
b) 4^2
c) 4^3
d) 4^4

Basic Gene Structure

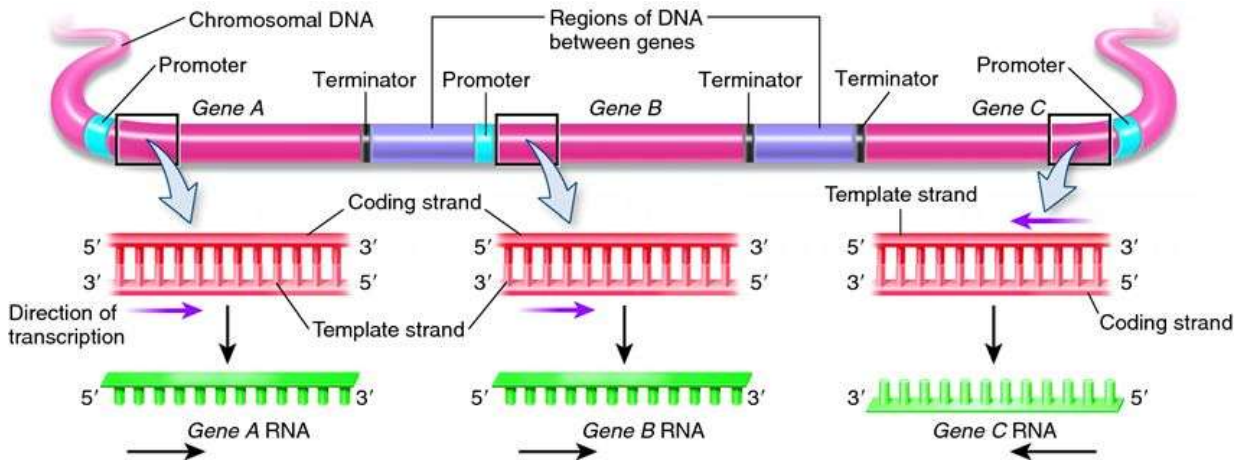


Transcription



Transcription

- The two strands of DNA are referred to as the template strand and the coding strand
  - Template strand is noncoding strand; the other is the coding strand
  - Template strand is what is complementary to the mRNA
  - The other is the coding strand it has the DNA sequence equivalent to that of the mRNA (same nucleotide sequence)
  - Both DNA strands can be template strands depending on what protein or mRNA you are referring to
- Note the antiparallel, complementary strand



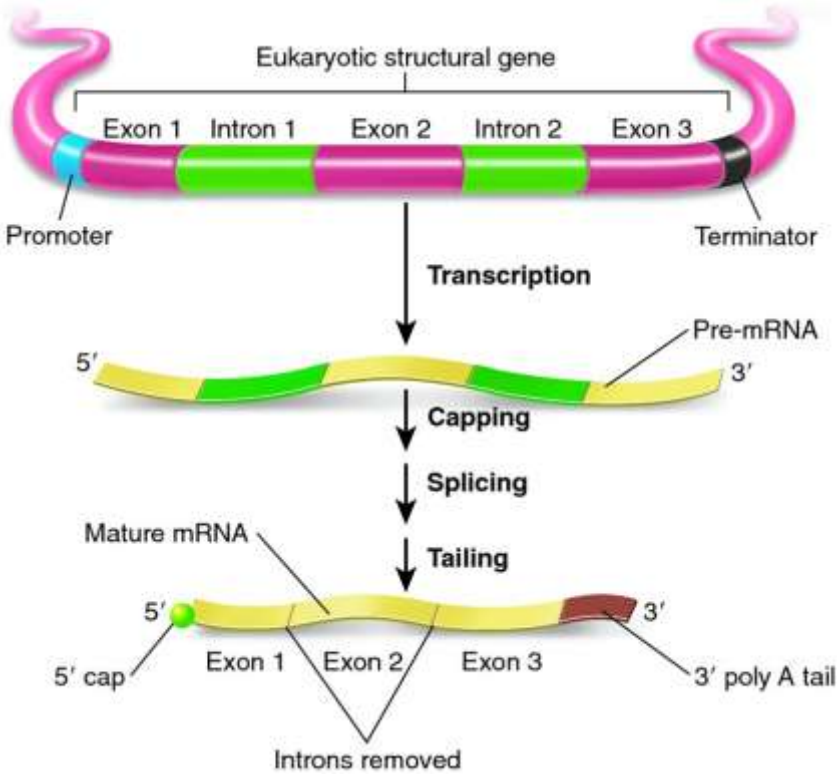
Example Question

RNA Polymerase reads the _____ strand in a 5'to 3' direction until it reaches the _____ sequence.

a) Coding, promotor
b) Coding, termination
c) Template, promotor
d) Template, termination

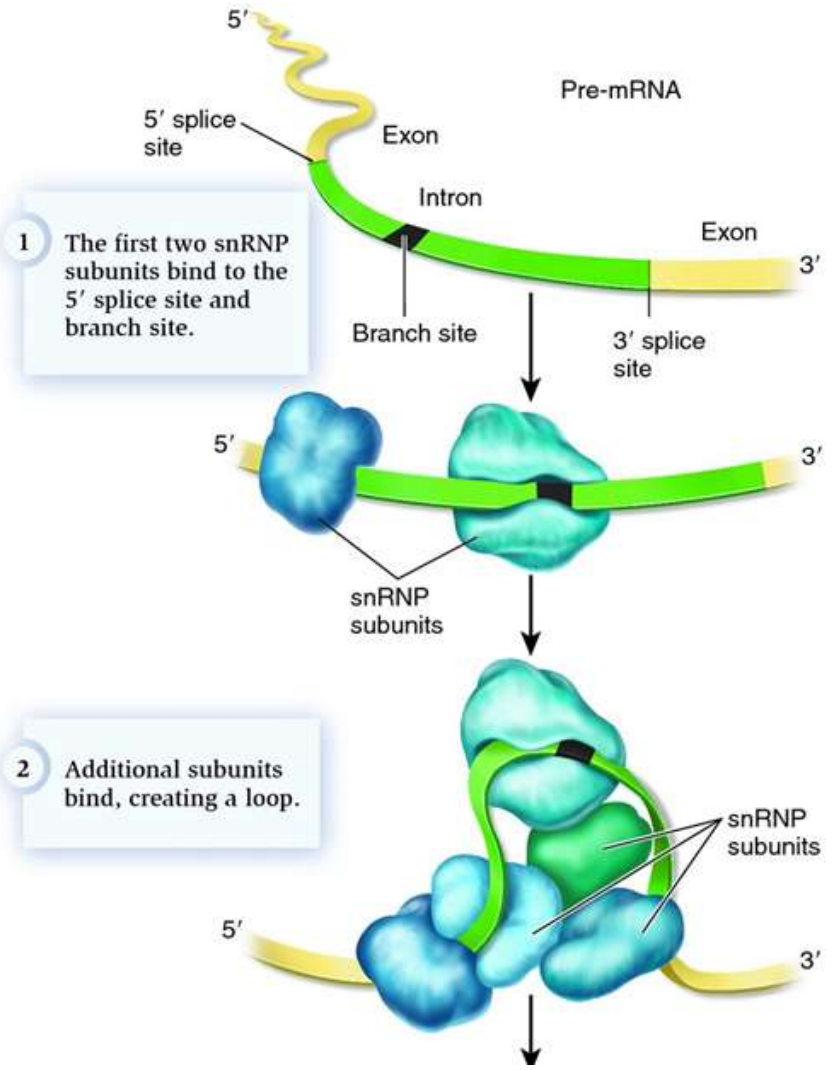
Genes

- A GENE is a nucleotide sequence that carries the information needed to produce a specific RNA or protein product
- Genes consists of:
  - Introns - non-coding regions of the gene
  - Exons - protein coding regions



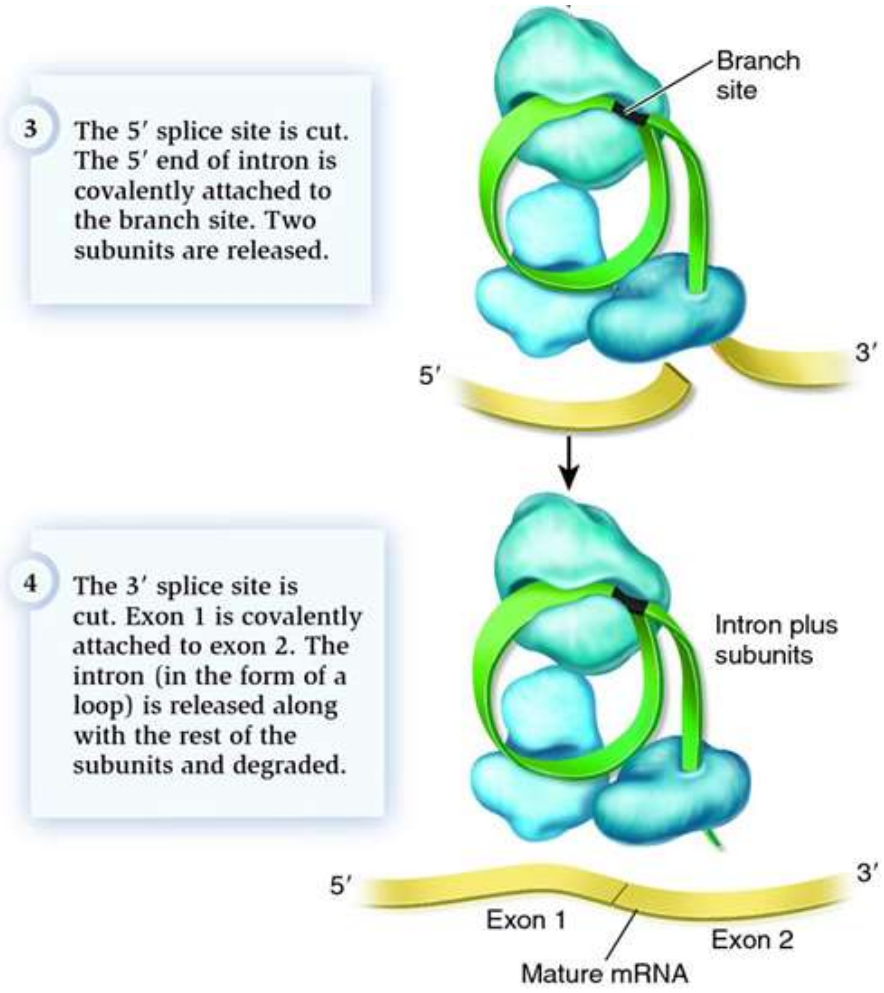
Spliceosomes

- Small nuclear RNA and proteins (snRNPs) that bind to specific sites on the intron
  - Branch sites
  - 5’ splice site
  - 3’ splice site
- snRNPs bind to each other
  - Create a multimeric complex



Spliceosomes

- Cut out the intron
  - 5’ splice site first then 3’
- Also connect the 2 exons together
  - Results in a mRNA transcript that has no introns
- Intron is eventually degraded

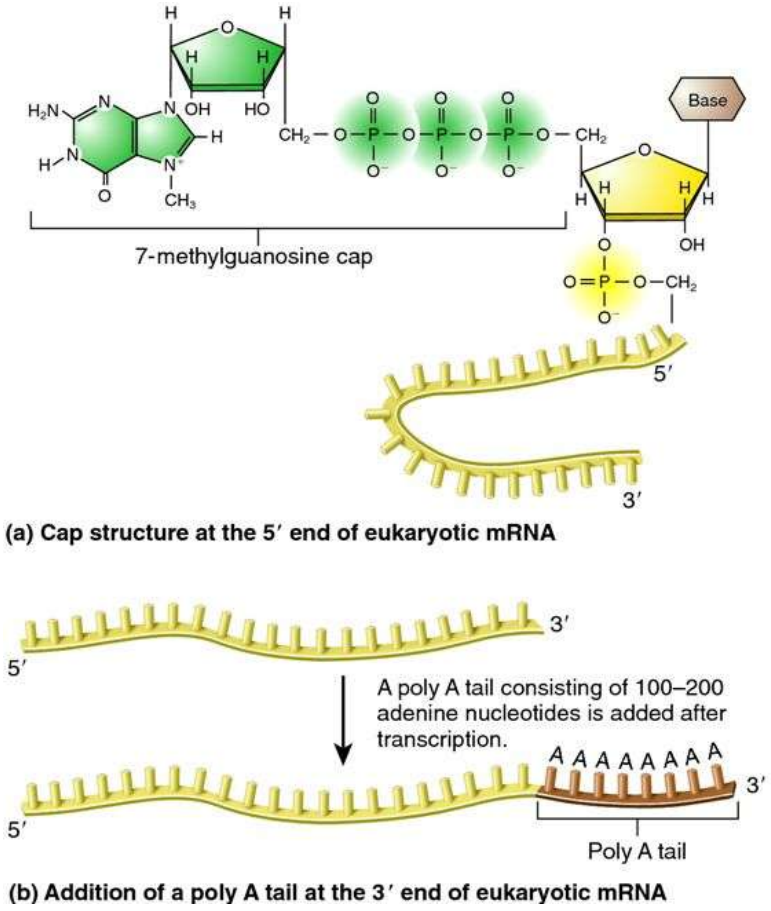


Exon Shuffling

- Proposed by Gilbert in 1977
- Suggested that exons may code for single protein domains
  - Functional units
- This means that you could mix and match exons to form new proteins
  - Shuffle exons
- This allows for the very quick evolution of new proteins

Capping and Poly-A Tail

- During transcription, a cap of 7-methylguanosine is added to the 5' end of the growing mRNA
  - Recognized by cap binding proteins that enable it to be translated
- After transcription, a poly-A tail (100-200 adenines) is added
  - Not coded, added enzymatically
  - In eukaryotes, causes mRNA to be more stable



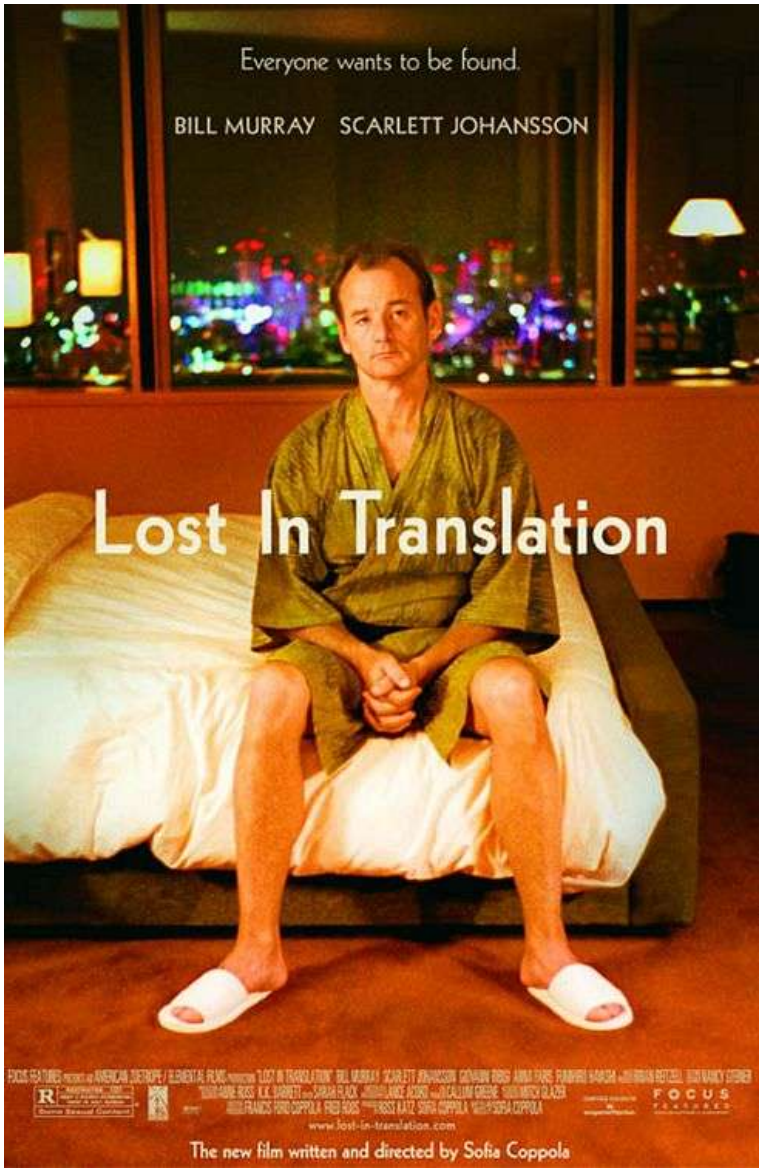
Example Question

_____ are regions of mRNA that do not code for protein and are cut out by _____.

a) Introns, spliceosomes
b) Introns, RNA ligase
c) Exons, spliceosomes
d) Exons, RNA ligase

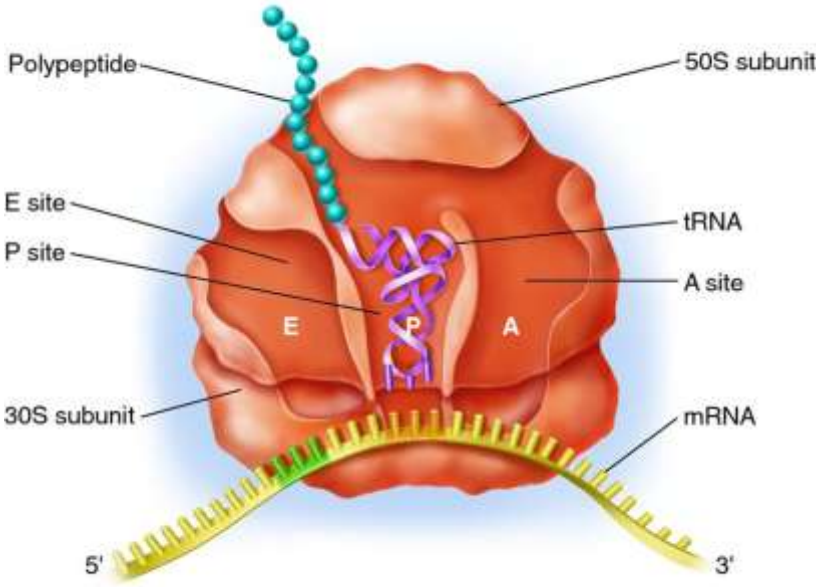
Translation

- Translation is the production of protein from a mRNA template
- Occurs in the ribosomes
  - mRNA and tRNA
    - tRNAs bring the individual amino acids (AAs)
    - mRNA is the code for the string of AAs



Ribosomes

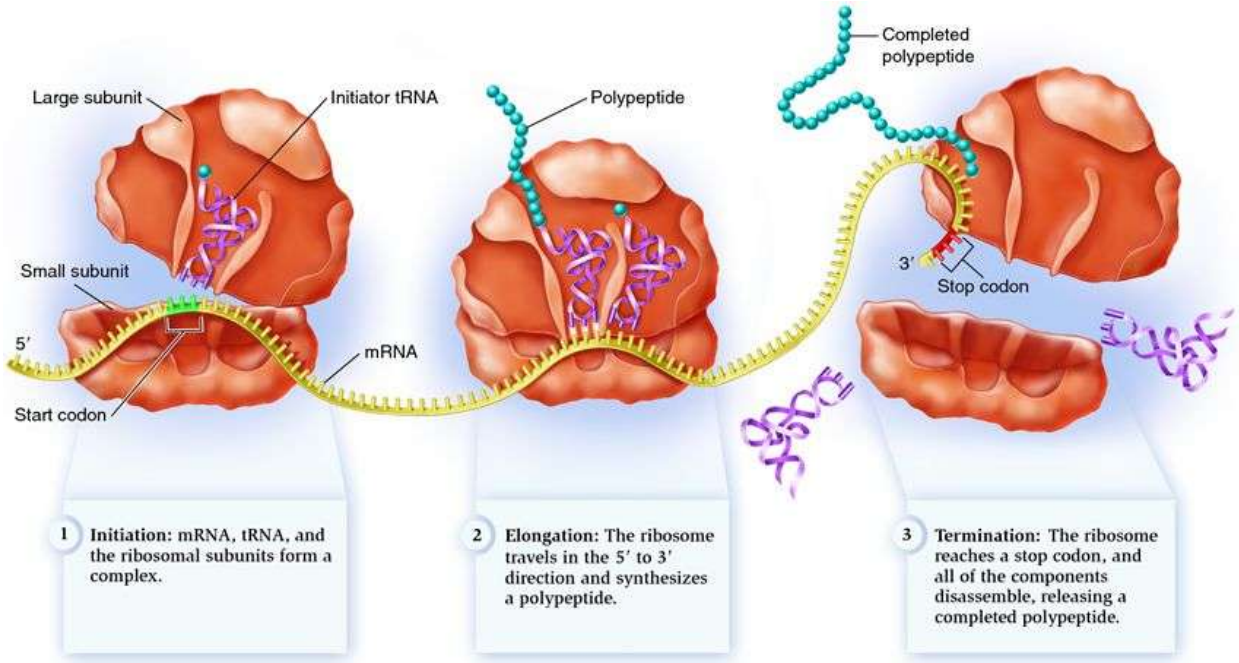
- Ribosomes are proteins with 2 subunits (small and large)
- Between them is a
  - This is where the mRNA moves through
- Within this groove there are 3 sites:
  - A site **a**ccepts an aminoacyl tRNA.
  - P site receives the **p**olypeptide bearing tRNA
    - The protein is connected to the rest of the protein (polypeptide chain).
  - E site is where the tRNA **e**xits the ribosome



(b) Schematic model for ribosome structure

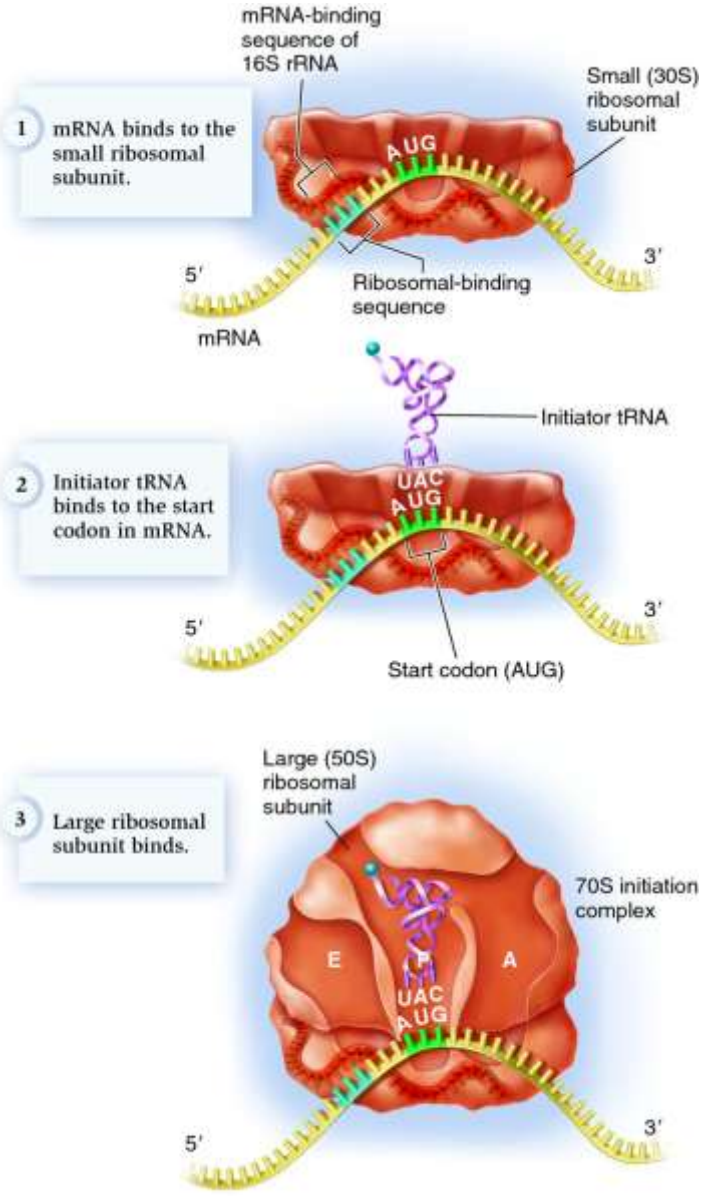
Stages of Translation

- There are 4 steps in translation
- Activation of the tRNAs
  - Formation of the aminoacyl-tRNA
    - Simple refers to attaching the appropriate AA to the right tRNA



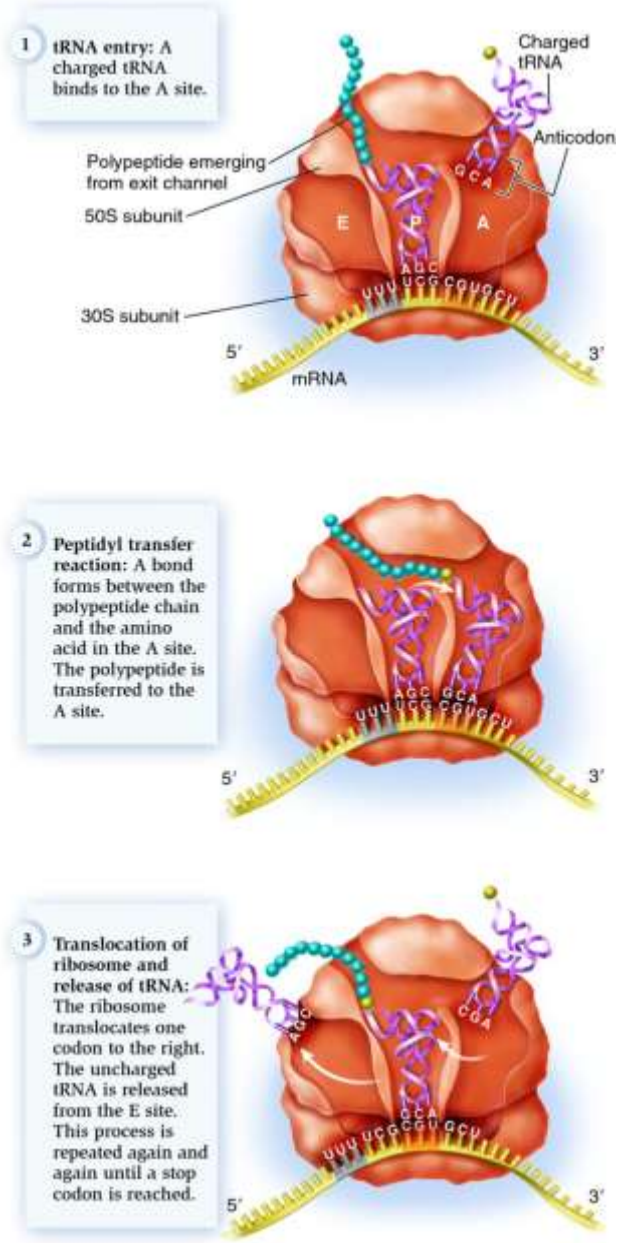
Initiation

- Starts process of protein production
- Binding of large and small ribosomal subunits to the mRNA
- In eukaryotes there is no ribosomal binding sequence
  - Use cap binding proteins to facilitate ribosome binding



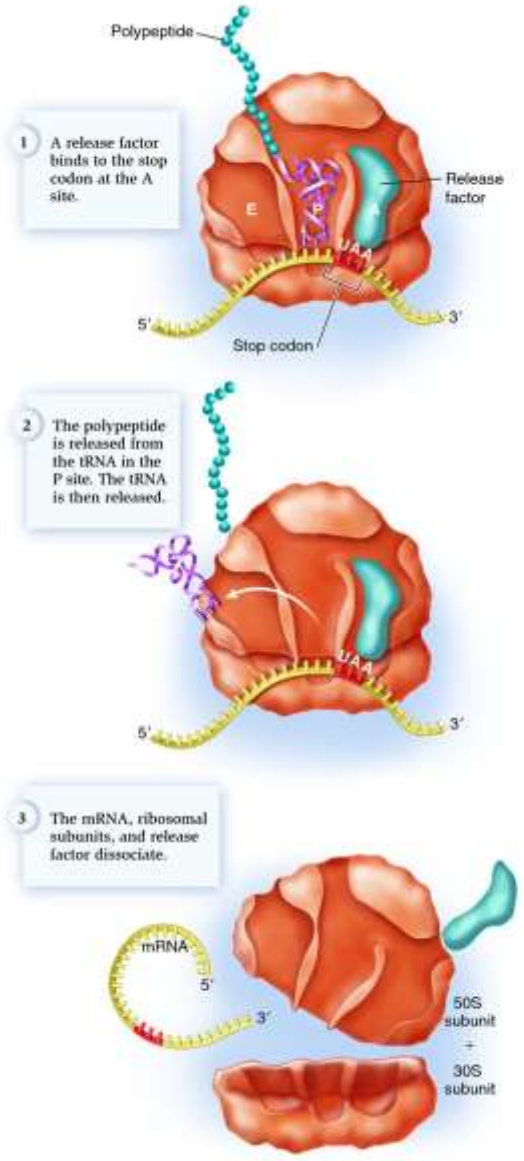
Elongation

- Aminoacyl tRNA enters the A site and binds to codon
  - Complementary anticodon
  - Elongation factors use GTP to facilitate this
- Peptidyl transfer reaction
  - Aminoacyl tRNA moves from A to P
  - Amino acid is added to the polypeptide chain
    - tRNA is now called peptidyl RNA
  - Carried out by peptidyl transferase
    - Catalytic rRNA (ribosomal RNA)
- RNA translocates one codon towards the 3' end
  - Releases uncharged tRNA from E site



Termination

- Ribosome comes to a stop codon
  - No tRNA, release factor binds to the A site instead
- Bond between peptidyl tRNA and polypeptide is hydrolyzed
  - Causes release of tRNA and polypeptide from ribosome
- Ribosomal subunits, mRNA, and release factor dissociate

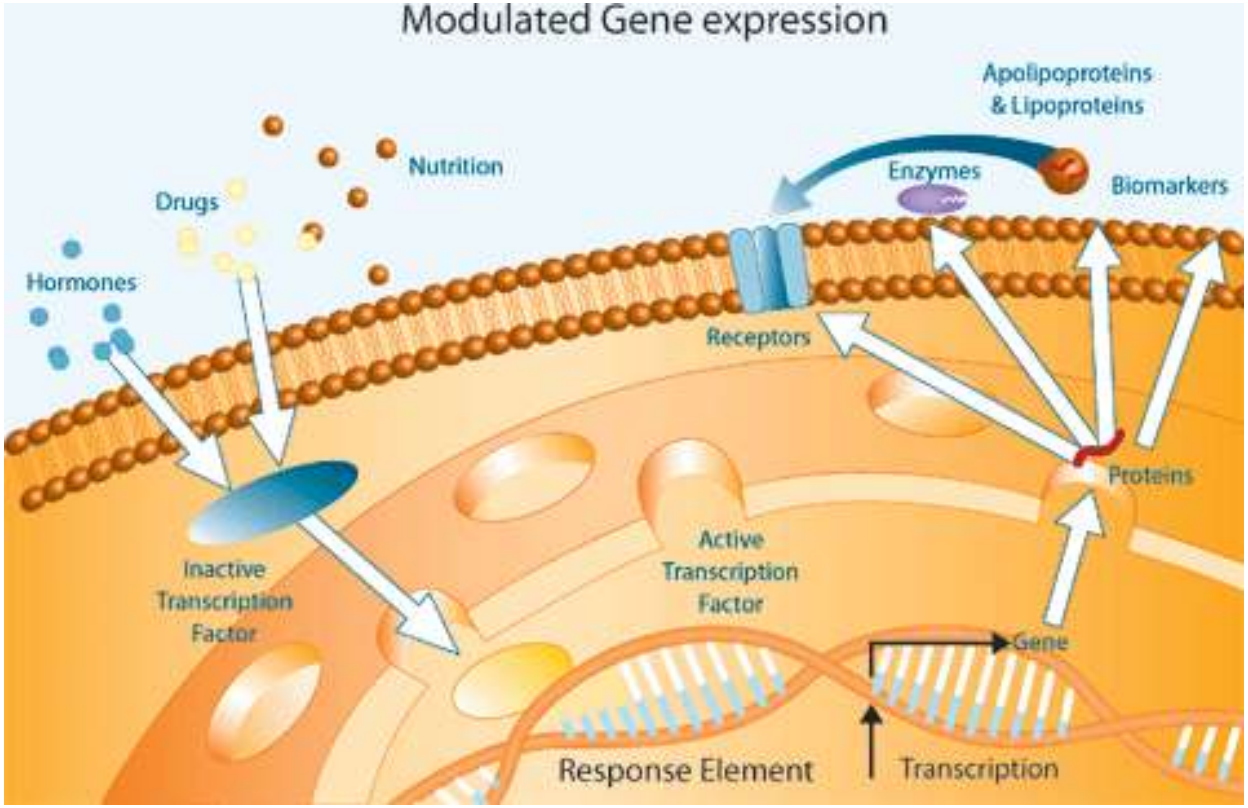


Example Question

Bill Murray is lost studying translation. Help Bill by telling him which of the following is true.

a) Anticodons are not complementary to codons
b) Peptidyl tRNA is found in the E site
c) Eukaryotes use ribosomal binding sequences
d) Formation of aminoacyl tRNA is the first step of translation

Chapter 12 – Gene Regulation

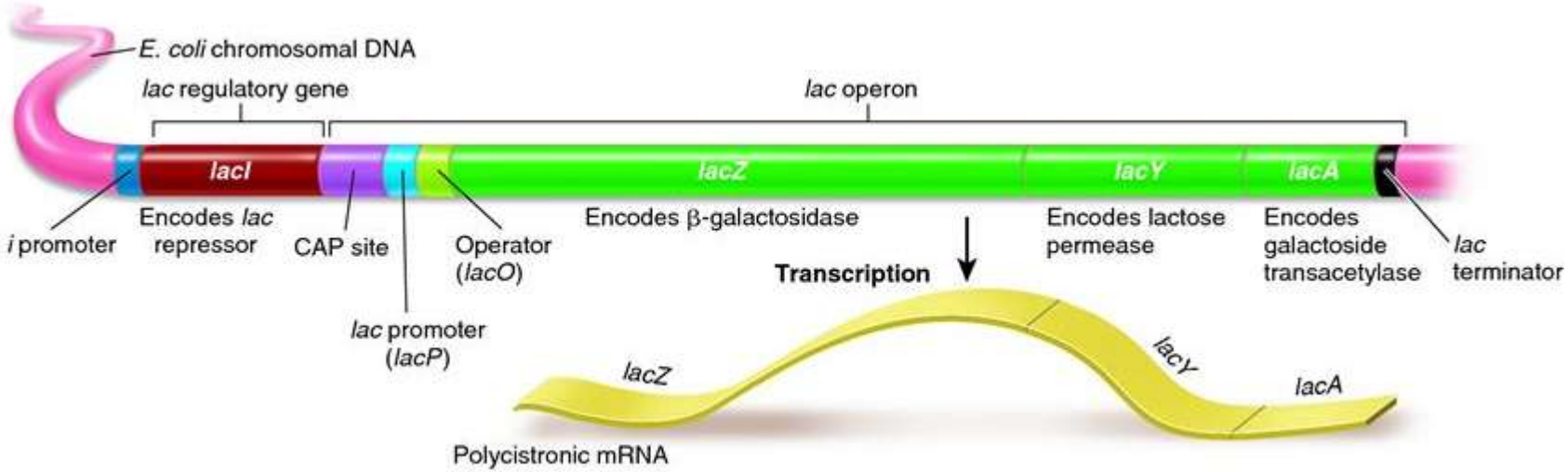


Gene Regulation

- Complex regulation
  - Genes encode single proteins
- Gene expression can change under different conditions
- Gene expression can change depending on stage of development (temporal) and tissue as well
- Genes that code for proteins that are always required and thus readily available in the cell at all times are called constitutive genes.
  - Always transcribed (eg. glycolytic enzymes)
- For most genes this is not the case therefore many genes are regulated

Lac operon

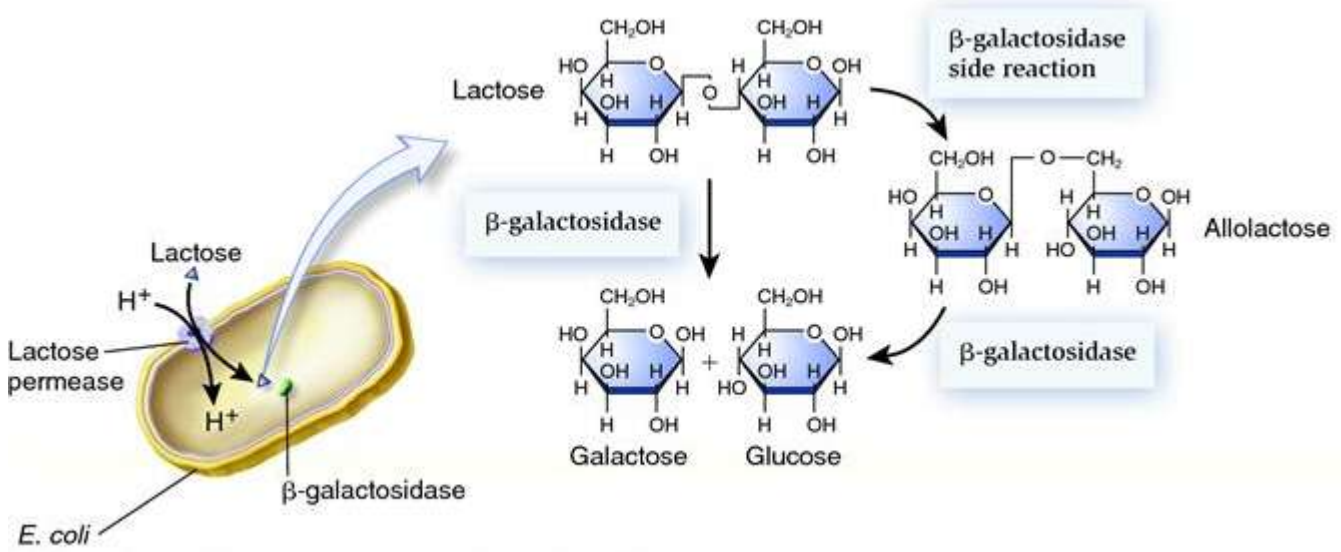
- The lac operon controls the breakdown of lactose.
  - When lactose is absent from the environment allolactose is not made and the lac repressor binds to the lac operator which inhibits transcription
  - LacZ, lacY, and lacA are the structural genes
  - The operator is a sequence of nucleotides that provides a binding site for a repressor protein
  - The CAP site is a DNA sequence recognized by an activator protein
  - ipromotor is the promotor region for the lacI gene only
    - Lac promotor is the promotor for the *lac* operon



(a) Organization of DNA sequences in the *lac* region of the *E. coli* chromosome

Lac operon enzymes

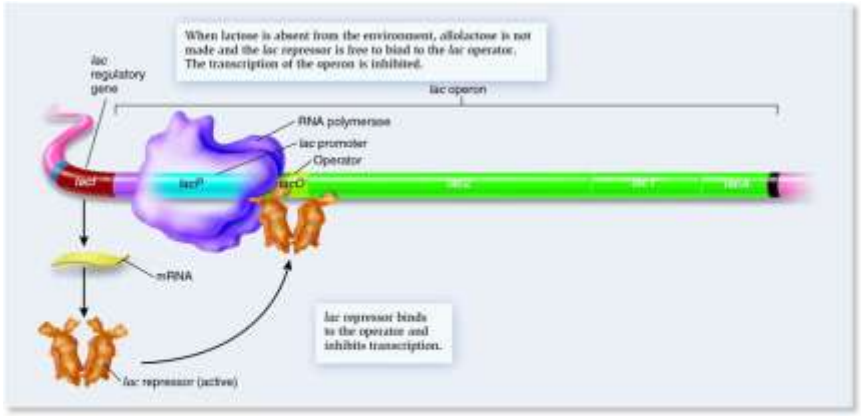
- B-galactosidase converts lactose into galactose and glucose
  - Side reaction that produces allolactose
- Lactose permease allows lactose entry into the cell
- Galactosidase transacetylase modifies lactose and analogues
  - May prevent toxic build up



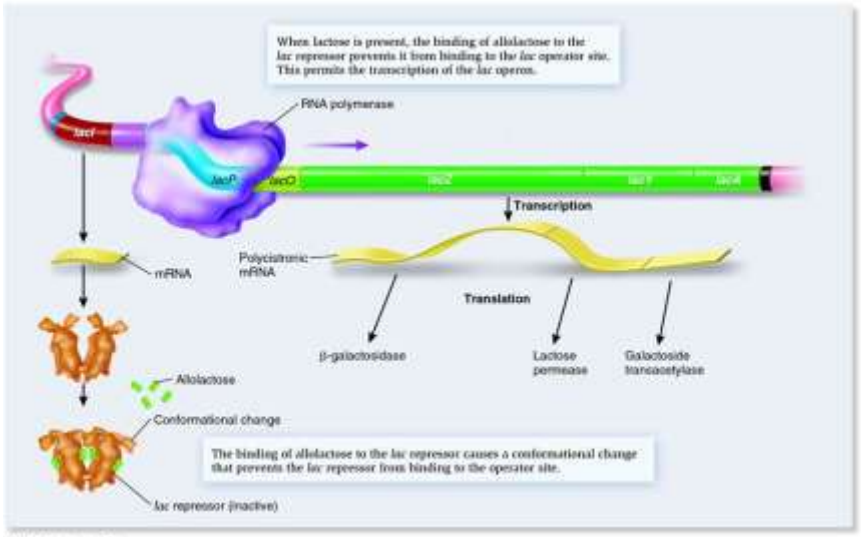
(b) Functions of lactose permease and β-galactosidase

Inihibition of the *lac* operon

- Controlled by the *lac* repressor
- *lac* repressor is composed of 4 subunits
  - Each possesses an allosteric site for allolactose
- When allolactose is bound to all 4 subunits the repressor cannot bind to the operator
  - This allows RNA polymerase to transcribe the *lac* operon
- This is called negative regulation



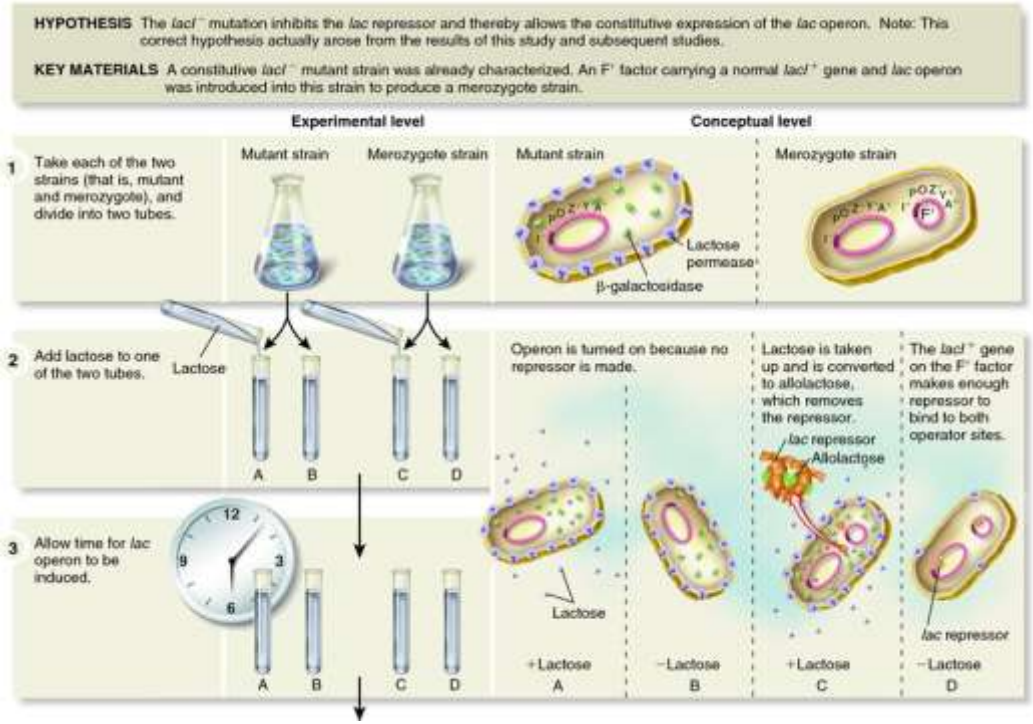
(a) Lactose absent from the environment



(b) Lactose present

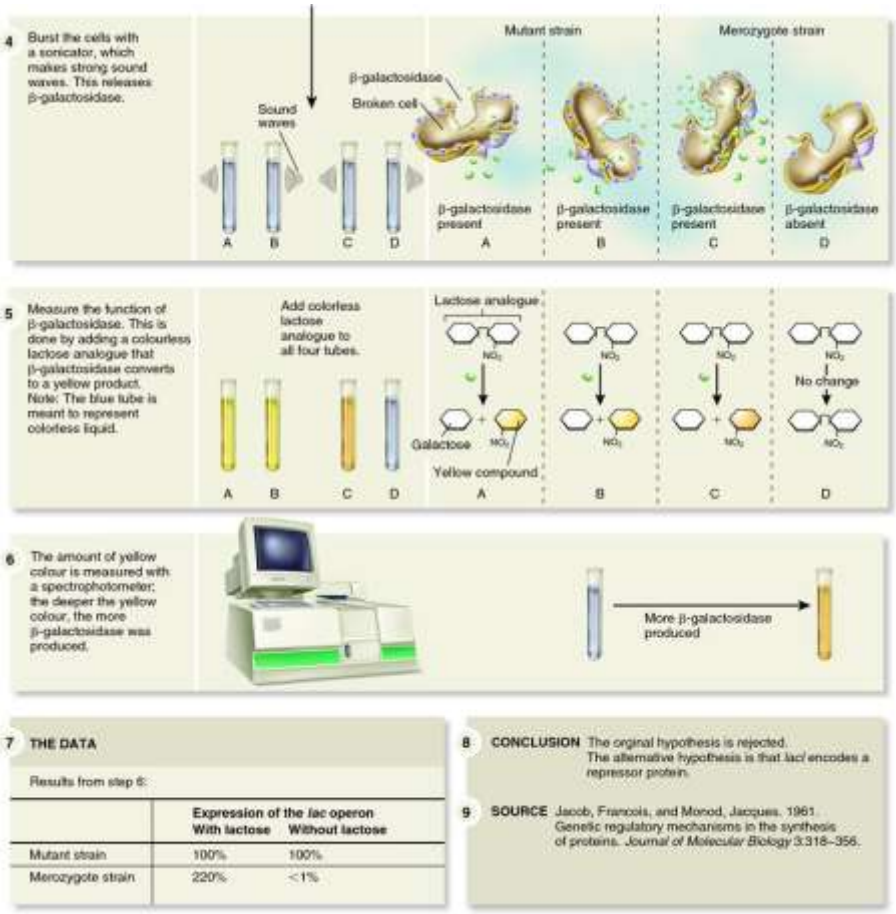
Jacob and Monod

- Knew that the mutant strain *lacI*$^-$ allowed for constitutive expression of the operon
- Wanted to know what happened when they added *lacI*$^+$ (active gene) plus operon to those cells
  - These are the merozygote strain



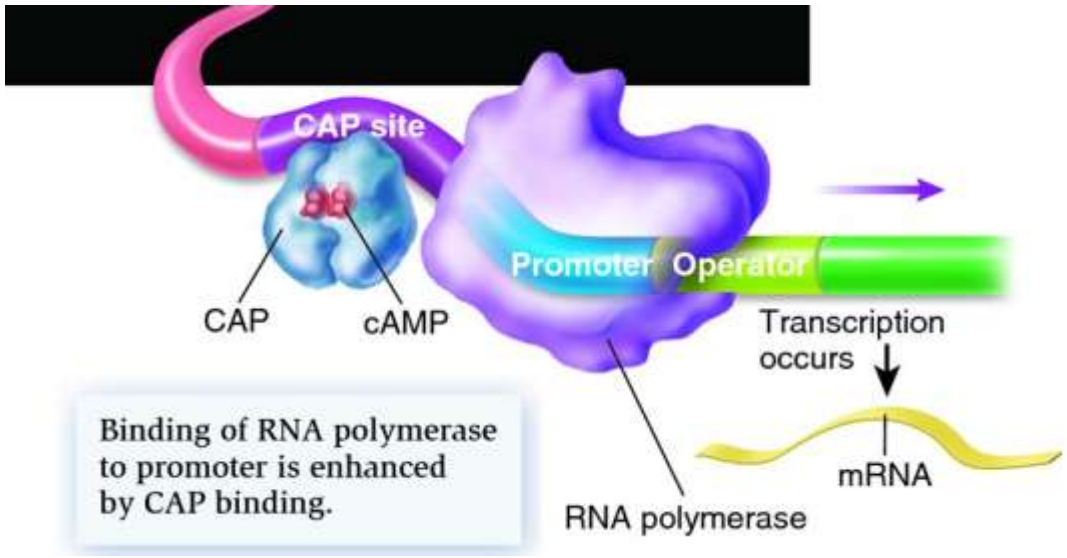
Jacod and Monod

- Found that mutant strains produced β-galactosidase under both lactose treatment
- Merozygote strains produced it only when lactose was present
- Deduced that the *lacI*$^+$ was able to control the regulation of the *lac* operon



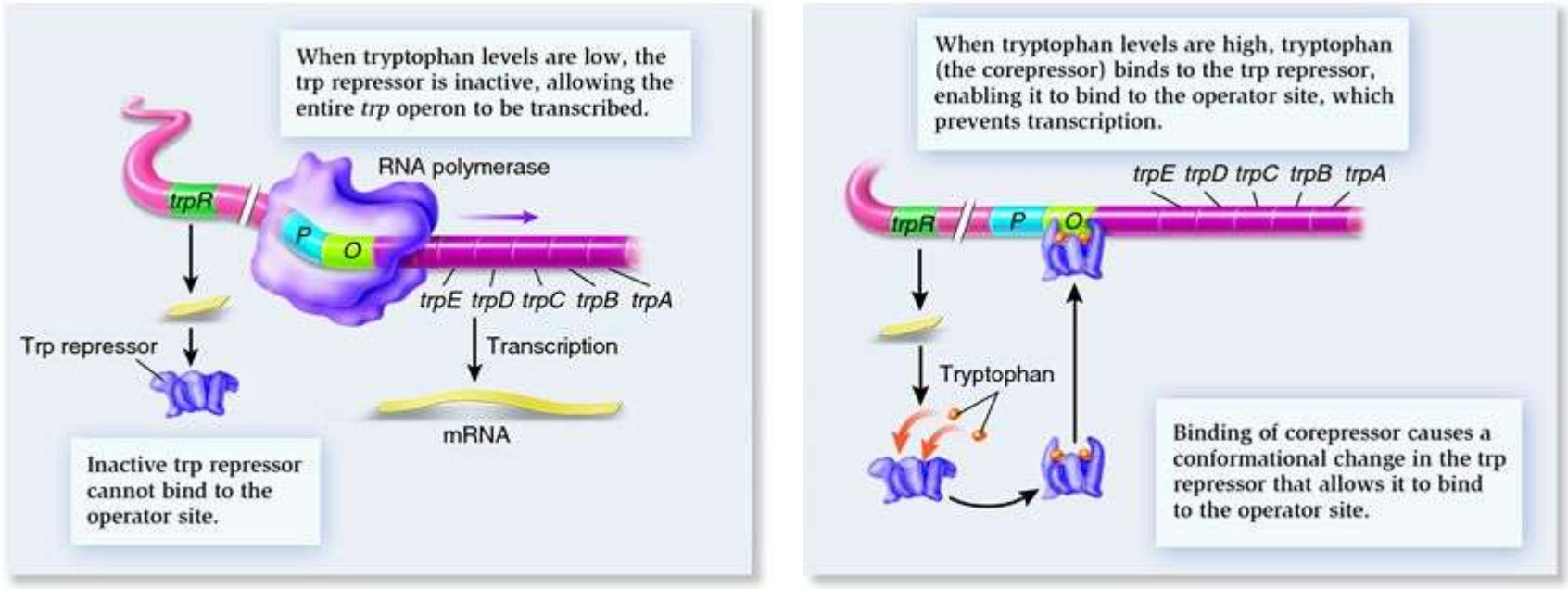
Activation of the *lac* operon

- cAMP binds to CAP to form a CAP dimer
- CAP dimer binds to the CAP site (activator site)
- Causes a bend in the DNA that facilitates the binding of RNA polymerase
  - This activates the gene allowing for transcription
- cAMP levels are regulated by glucose
  - Low when glucose is high
- This is positive regulation



trp operon

- Also negative regulation
- Different from *lac* because when trptophan binds to the repressor it causes it to become active



(a) Low tryptophan

(b) High tryptophan

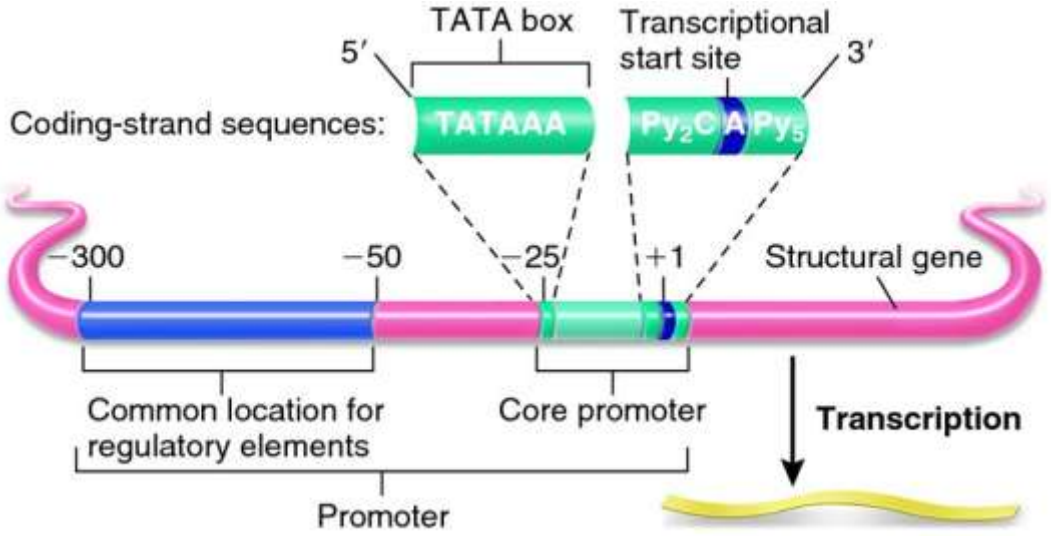
Example Question

In the *lac* operon, negative regulation is controlled by _____. It causes the repressor to _____ to/from the operator.

a) cAMP, bind
b) cAMP, dissociate
c) Allolactose, bind
d) Allolactose, dissociate

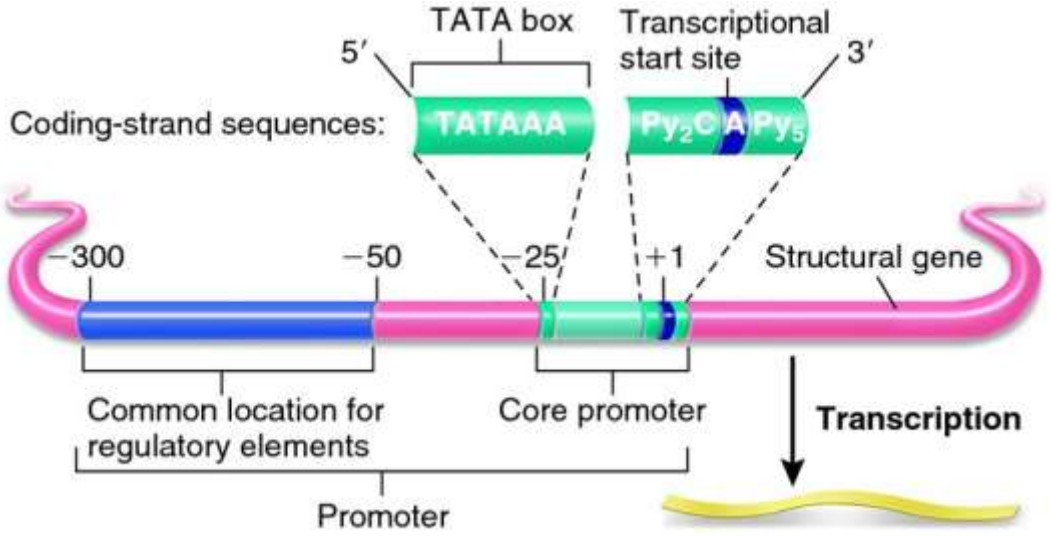
Parts of a gene

- Structural genes code for enzymes and related proteins
- Promoters are transcriptional start site
  - Often is a sequence of Py_2CAPy_5
  - Py_2 means 2 pyrimidines, C is cytosine, A is adenine, Py_5 means 5 pyrimidines



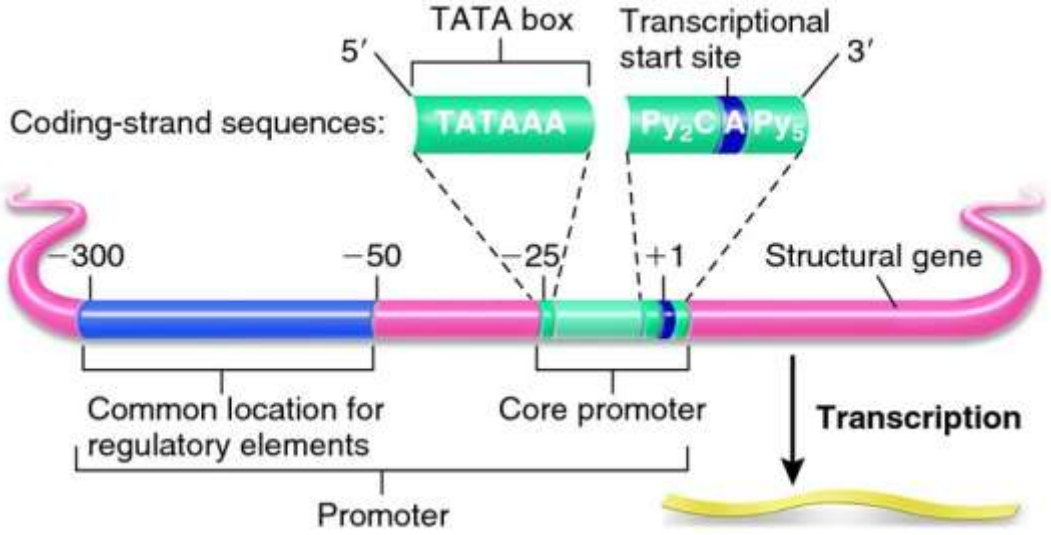
Parts of a gene

- TATA box is a site within the transcriptional start site that determines the exact point of transcription initiation.
  - Guides the RNA polymerase to the right spot
  - TATA box and transcriptional start site form the core promoter
  - The core promotor by itself results in low level basal transcription
  - About 25 base pairs upstream



Parts of a gene

- Response elements (specific DNA sequences) are sites that affect the initiation of transcription. The number of these determines the relative strength of promotion.
  - Commonly these are 50-300 base paires (b.p.) upstream (towards the 5' end) of the transcriptional start site
  - They are recognized by regulatory proteins that control the initiation of transcription
  - Also known as regulatory elements
    - Includes enhancers and silencers



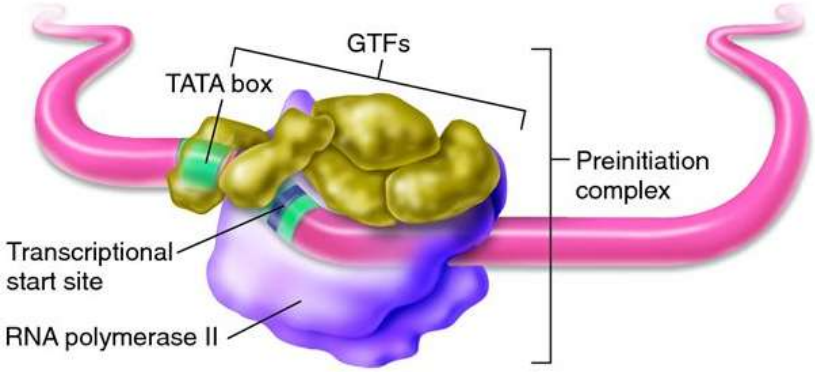
Example Question

TATA boxes function to:

a) Enhance transcription
b) Guide RNA polymerase to start site
c) All of the above
d) None of the above

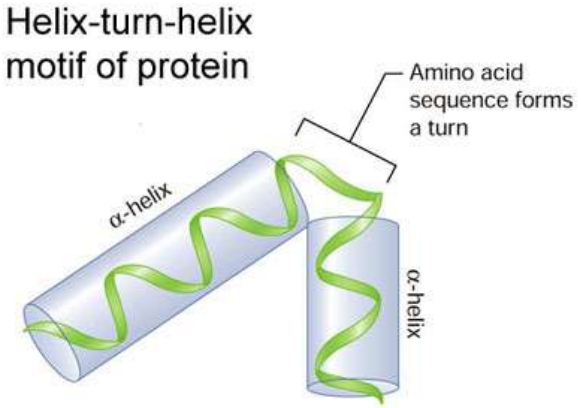
Preinitiation Complex

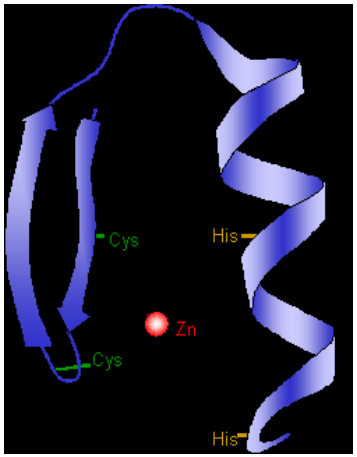
- Comprised of:
  - RNA polymerase
  - 5 different general transcription factors (GTFs)
  - GTFs and RNA polymerase come together at the core promotor
- GTFs must combine with RNA polymerase for transcription to begin
  - This occurs at the core promotor
  - GTFs and RNA polymerase together at the TATA box are called the **basal transcription apparatus**
  - Activators and repressors can bind to GTFs and mediators



Transcription Factor Motifs

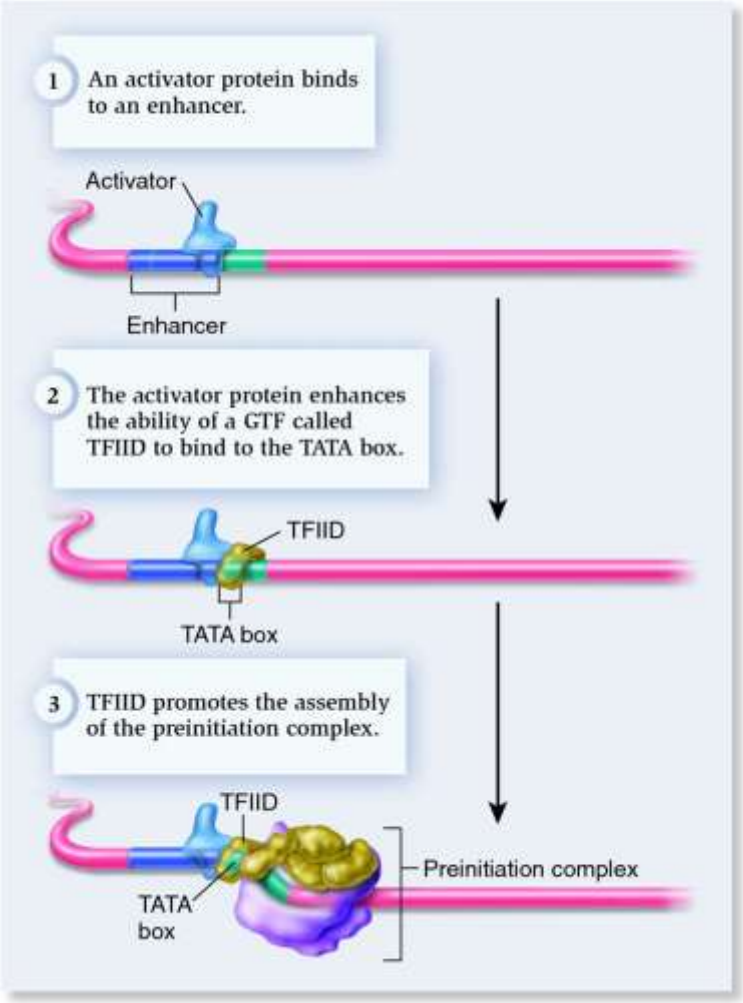
- Transcription factor proteins contain domains with specific functions
  - They can bind specific sequences of DNA
- Motifs are domains or portions of domains with similar structures in different proteins
  - Alpha helix – a double helix recognizing feature.
  - Zinc fingers – recognize DNA sequences in the major groove





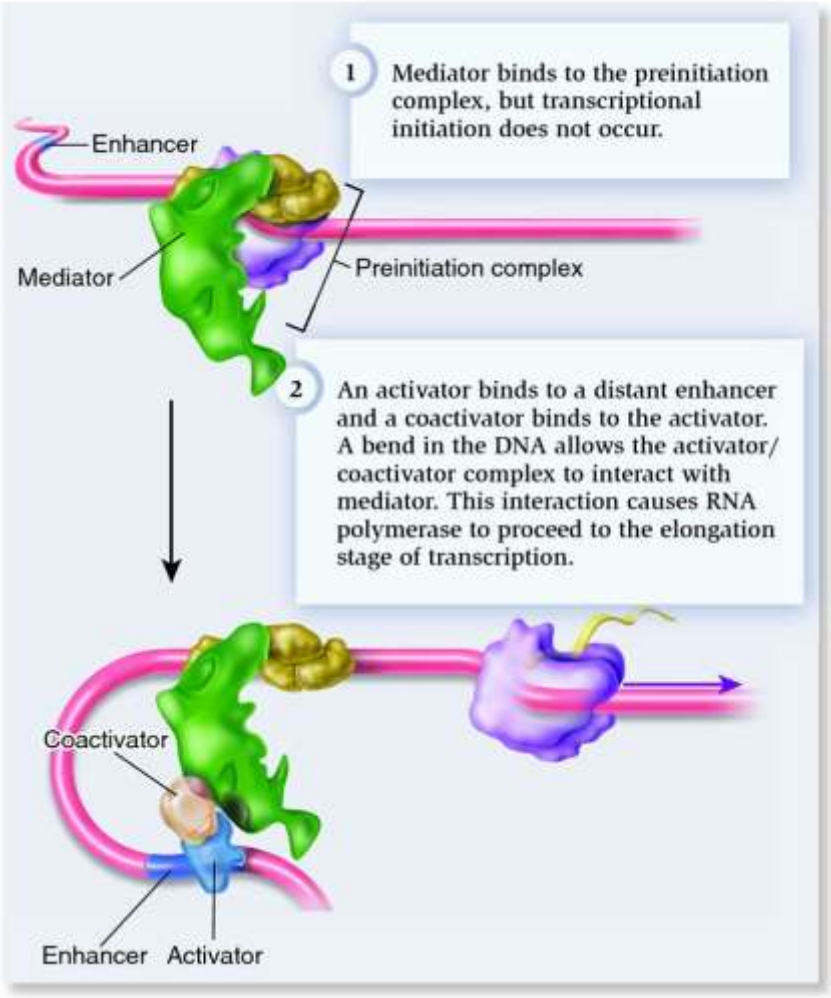
Activators/Repressors and GTFs

- Control of RNA polymerase can be accomplished via activators and/or repressors binding to GTFs
- An example of an activator:
  - Activator must come in first and is the trigger for the rest
    - Binds to regulatory elements upstream
  - TFIID helps to initiate the assembly of the preinitiation complex
  - With the activator helping TFIID bind to the TATA box, preinitiation complex formation is enhanced
    - This in turn enhances transcription of that particular gene
  - Repressors can inhibit TFIID
- The opposite of the above would be a repressor
  - Would bind to regulatory elements



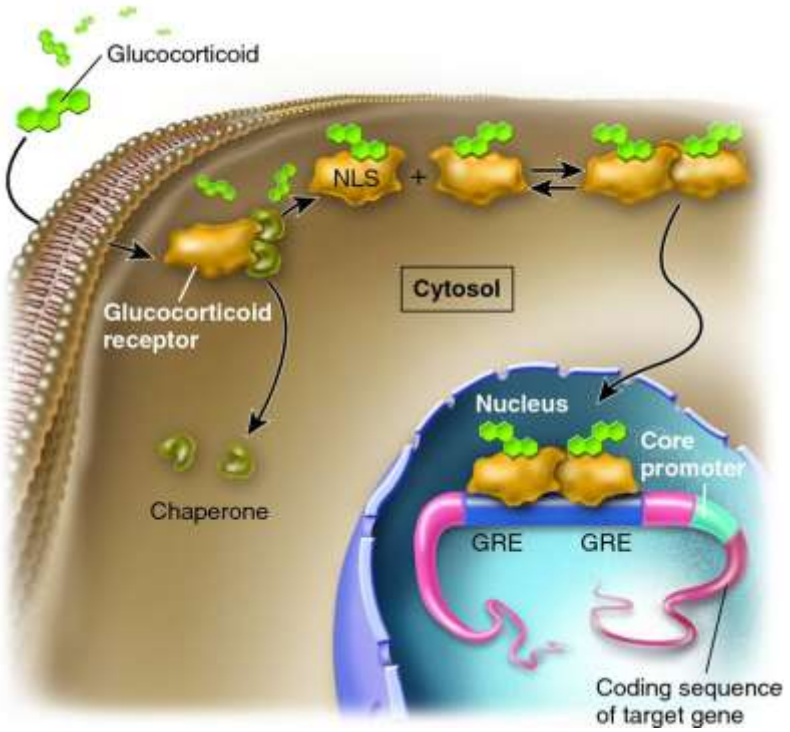
Activators/Repressors and Mediators

- Mediators (composed of multiple proteins) partially wrap around the preinitiation complex
  - Mediates interactions with activators or repressors of gene activity
  - Controls the rate at which RNA polymerase can begin transcription
  - Most do not bind directly to RNA polymerase
    - GTFs DO but these others do not



Steroid hormone example

- Some transcription factors respond to steroid hormones
  - Activators are actually steroid receptors
  - These steroid (lipid) hormones are examples of small effector molecules
  - Travel through plasma membrane and bind to receptors
    - Exposes nuclear localization signals
  - Once in the nucleus they dimerize and bind to an enhancer region to activate transcription



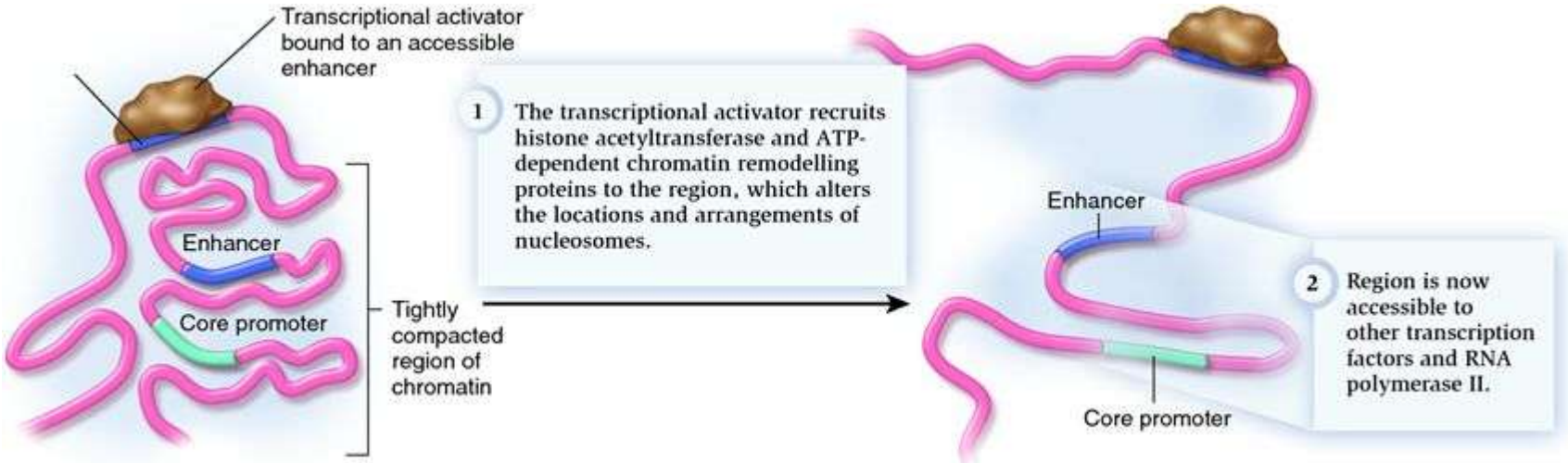
Example Question

What is not a part of the preinitiation complex?

a) GTFs
b) RNA Polymerase II
c) Mediators
d) None of the above

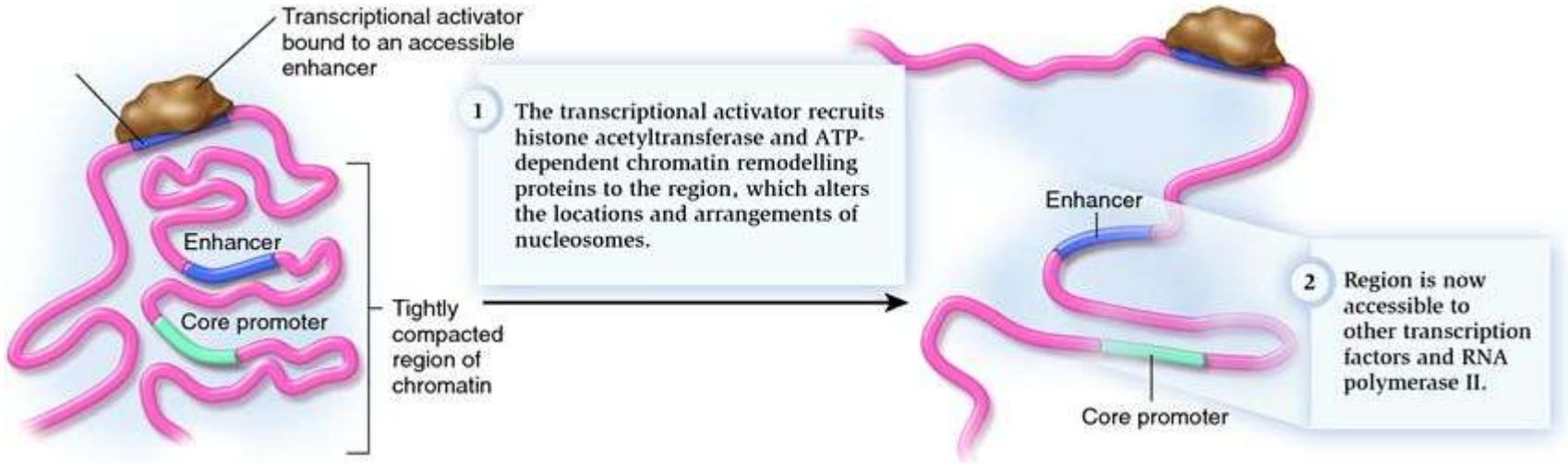
Gene Accessibility

- DNA is associated with proteins in the nucleus to form compact chromatin
  - When DNA is arranged in this fashion it is in a closed conformation
- Chromatin packing affects gene expression
  - When the DNA is tightly packed in the closed conformation (as chromatin) it is difficult or impossible for transcription to occur
  - This is because the RNA polymerase simply cannot access it
- Access to the DNA is allowed in the loosely packed open conformation



Gene Accessibility

- At any given time, certain portions of chromosomes are in the open or closed conformation
- Some activators can help decrease the amount of DNA compaction near a gene
  - Activator proteins are recruited
  - Histone acetyltransferase attaches acetyl groups to histone proteins so they don't bind DNA as tightly
    - Histones are proteins that DNA is wrapped around (and bound to) to help compact it
  - ATP dependent chromatin remodelling enzymes also help loosen DNA compaction
- Therefore the third way to control RNA polymerase is to recruit proteins that influence DNA packing



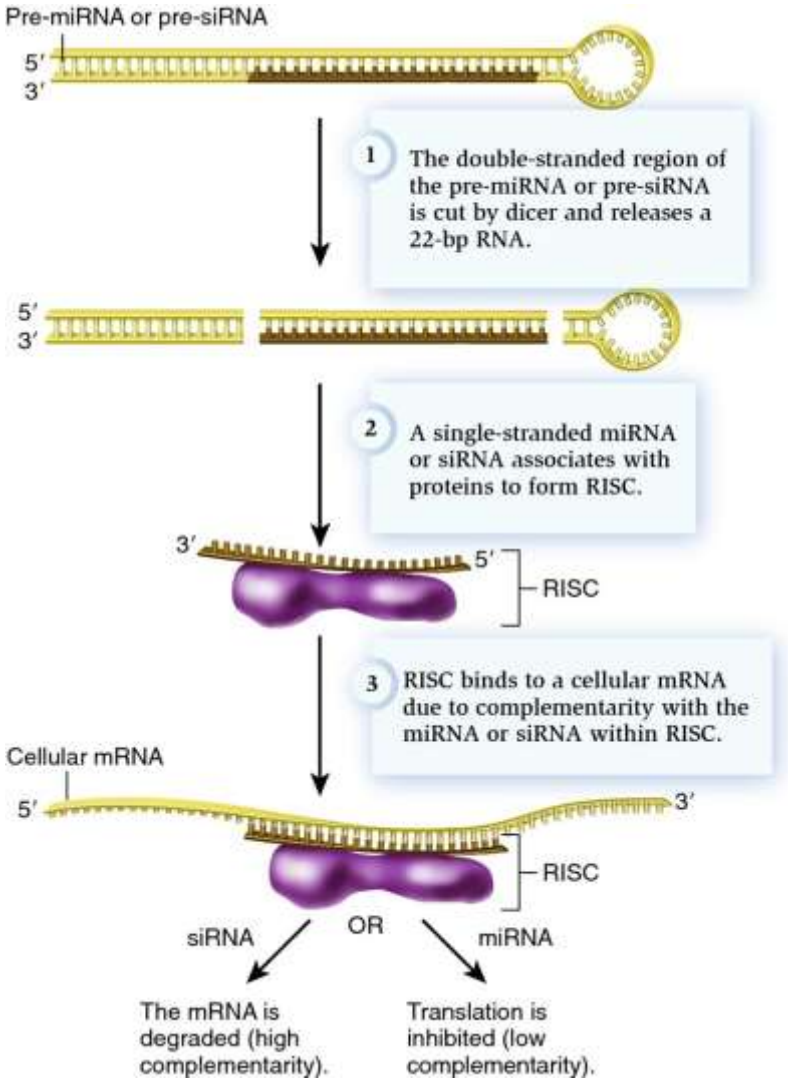
Example Question

When DNA is in a closed conformation...

a) RNA polymerase cannot access it
b) It can be accessed by histone acetyltransferase
c) It is closed off from the cell forever
d) A and B

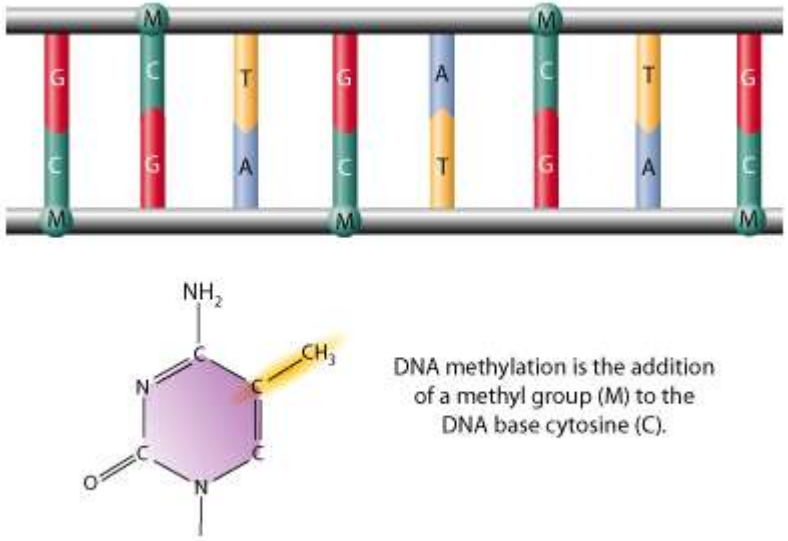
miRNA and siRNA

- Pre-miRNA (micro RNA) or pre-siRNA (silencing RNA)
- miRNA/siRNA binds in a way to expose nucleotide bases to solution
- RISC binds to a mRNA
  - Binding is due to complementarity with miRNA/siRNA within the RISC
  - If siRNA the mRNA is degraded due to high complementarity
  - If miRNA transcription is only inhibited (due to low complementarity)
- This was called RNA interference (RNAi) by Fire and Mello



DNA Methylation

- Methylation – addition of a methyl group to a carbon to prevent transcription
- Accomplished by DNA methylase which attaches methyl groups (CH_3) to cytosine
- Islands of CpG (cytosines and guanines with phosphodiester bonds) found near promotors determine whether or not the gene is activated or repressed by methylation
  - Methylated = represssed, unmethylated = active
- Methylation inhibits expression in two ways
  - Methylation prevents binding of activators to UPEs and enhancers
  - It also functions to tighten up the chromatin packing into the closed formation by recruiting proteins that condense the chromatin



DNA methylation is the addition of a methyl group (M) to the DNA base cytosine (C).

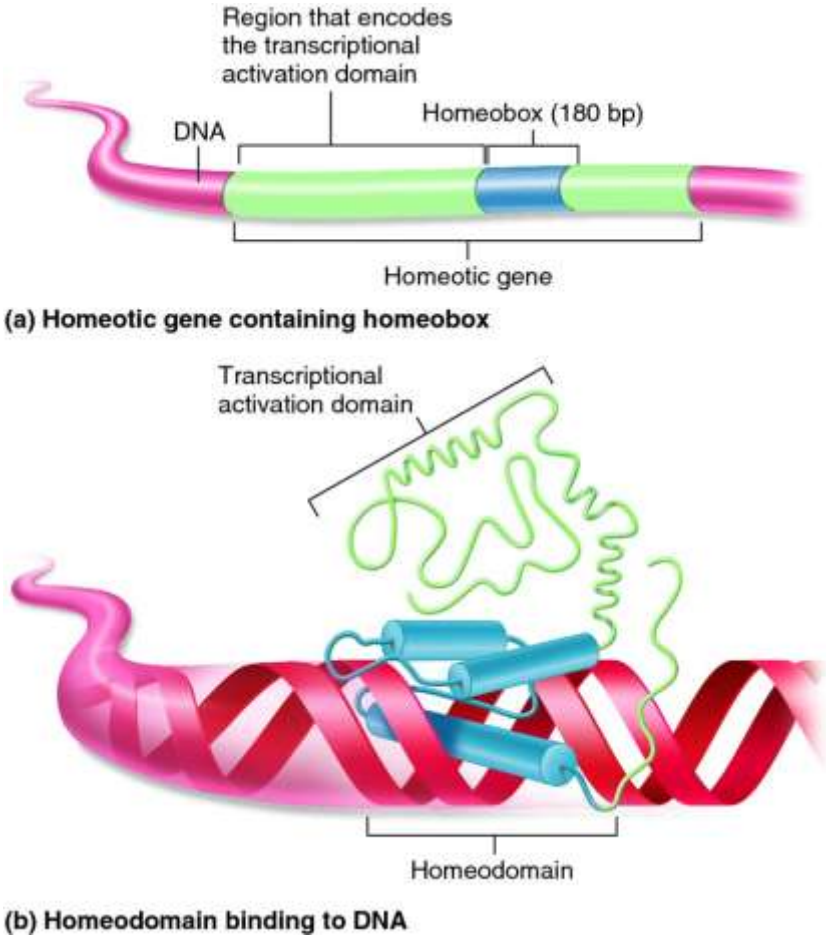
Example Question

DNA methylation inhibits transcription because...

a) It degrades DNA
b) Prevents binding of activators to upstream promotor elements
c) Packaging chromatin in a closed conformation
d) B and C

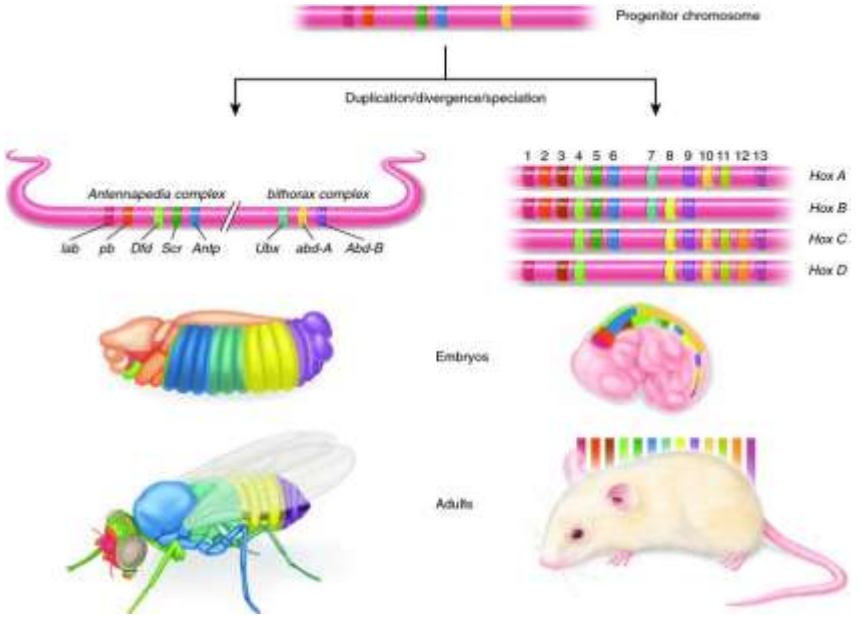
Homeotic Genes

- Developmental genes known as Homeotic Genes control formation of structures during development
- Within these genes are homeoboxes which are 180 base pair sequences
- Homeoboxes code for a protein region called a homeodomain that binds to DNA and affects transcription.
  - Homeodomain is 60 amino acids that form 4 helices



Homeotic Genes

- Homeotic genes such as Antennapedia and bithorax are often called Hox genes.
  - Hox genes are normally clustered together
- Homeotic mutations can result in one set of cells encoding for an entirely different set
  - Eg. legs expressed where antennae should be
  - So cells that would normally turn into one type of tissue are instead turned into something different
- The order of arrangement of hox genes reflects spatial organization of the animal and the order of activation during development

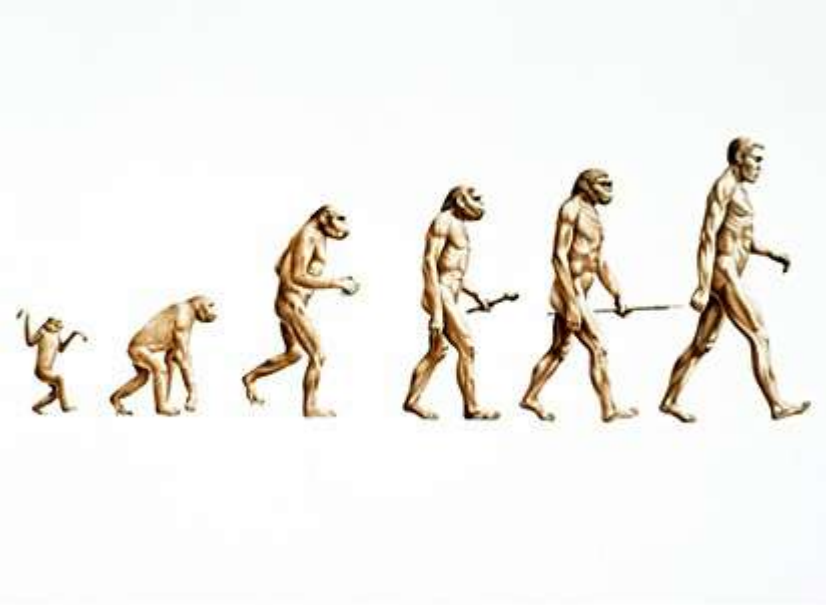


(a) Normal fly

(b) Mutant fly

Homeotic Genes

- Hox genes are seen in many different types of organisms
  - May have started off as just a single set of hox genes
  - Over evolutionary time the hox genes were duplicated and underwent mutations
    - More complex organisms have more sets of hox genes (eg. mice have more than flies) but this is not always the case
    - All hox genes share many characteristics and this is why we think they all come from some sort of progenitor species (like a sponge)
- Evolution of the regulation of genes is just as important as the sequence of the genes themselves



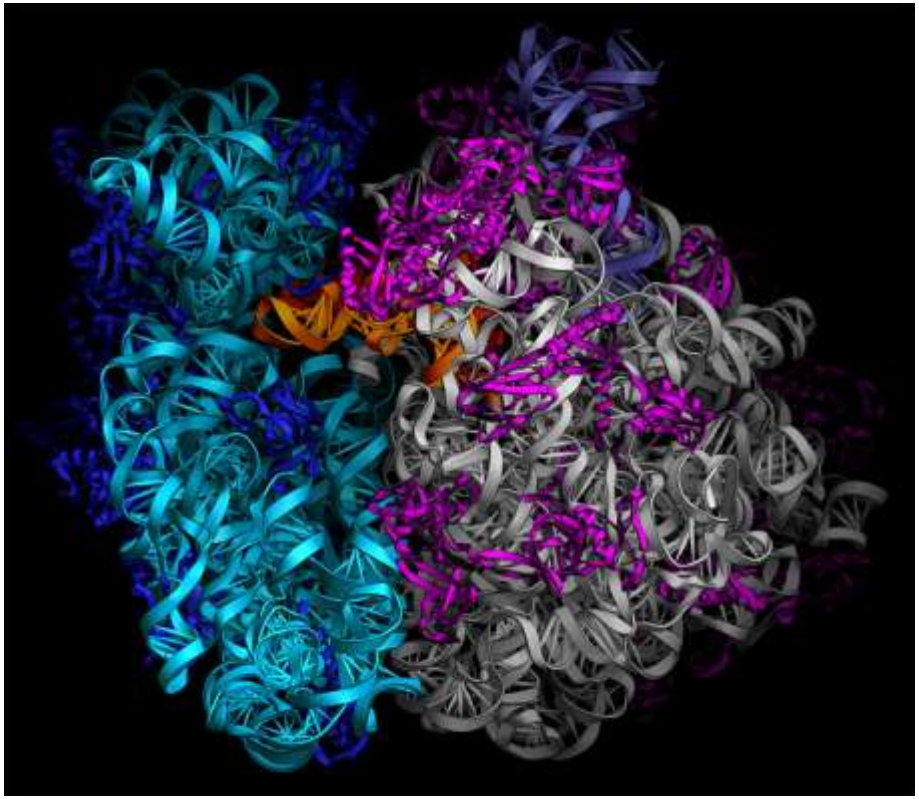
Example Question

Homeotic genes affect _____ and control _____ of the organism.

a) Transcription, development
b) Transcription, reproduction
c) Translation, development
d) Translation, reproduction

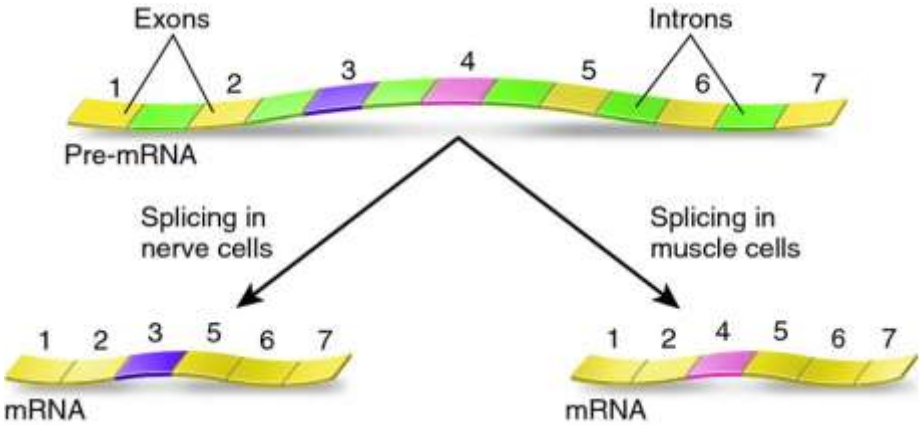
Translational Controls

- ENZYMES ARE AFFECTED DIRECTLY
- Feedback (end-product) inhibition
  - Product feedbacks to affect enzymes
- Phosphorylation
- Other enzyme modifications (eg. glycosylation)
  - Change enzyme structure
- Proteolytic processing
  - Chopping up proteins to make them into mature forms
  - Eg. proinsulin is produced from the gene (end product of transcription/translation) and its active form (insulin) is created when a portion of it is cleaved off
- Selective degradation
- Relates to turning processes on/off



Alternative splicing

- Alternative splicing of the pre-mRNA can increase the proteome size without increasing the total number of genes
  - Pre-mRNA is the RNA transcript that is created directly from transcription (contains both introns and exons)
- For organisms to become more complex (like higher plants and animals) evolution has produced more complex proteomes
  - General trend is that less complex organisms tend to have fewer genes
- Frequency of alternative splicing increases with increasing biological complexity



Example Question

Alternative splicing may explain...

a) Larger proteomes in complex organisms
b) Large proteomes with small genomes
c) All of the above
d) None of the above

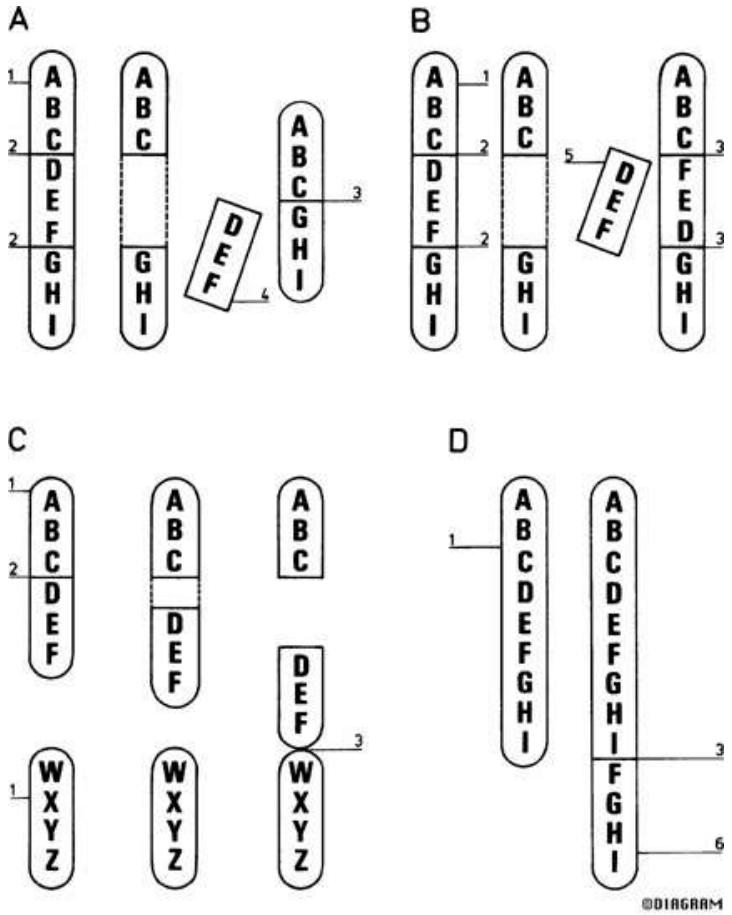
Chapter 13 – Mutation, DNA repair, and Cancer

Mutations

- Mutations are any heritable change in genetic material (DNA)
  - Can be brought about by mistakes during replication and cell division
    - Also by chemical treatments (mutagens)
- Essential to the continuity of life
  - Mutations that cause changes that can be selected for/against have the potential to drive natural selection in a species, eventually causing speciation
  - As such they are the source of variation for natural selection
  - New mutations are usually more harmful than helpful

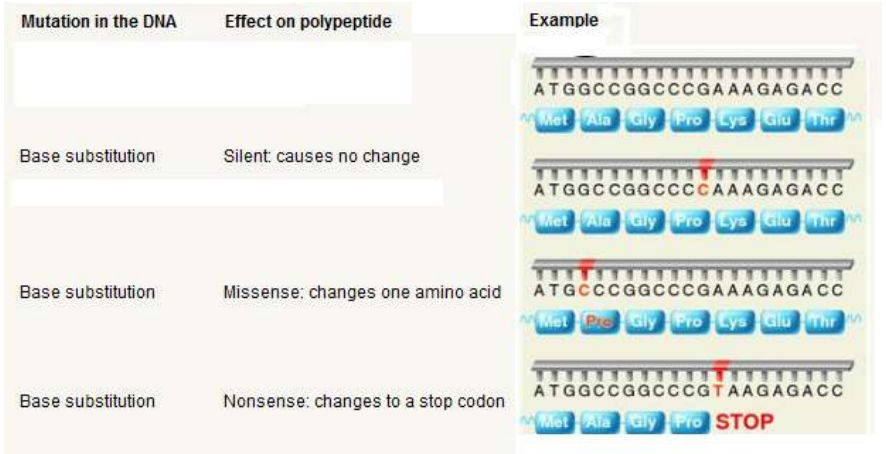
Chromosomal Mutations

- Deletion or addition of a DNA segment
  - One to multiple proteins lost
  - Often these mutations are very serious and fatal
- Fragile X syndrome
  - Mutation of addition of CGG base repeats causes methylation which organizes the X chromosome into a closed conformation
  - Large pieces of DNA have changed
- Klinefelter Syndrome
  - Two X chromosomes
  - Individual looks like a male, can be treated with testosterone patches
  - Normally these individuals are sterile and develop breasts



Point Mutations

- One nucleotide is incorrect, changes up to one amino acid within the protein
- Usually less serious
  - Silent/neutral – no effect because of redundancy in the genetic code (3rd position wobble)
  - Missence – amino acid changes
  - Nonsense – stops amino acid change by changing amino acid to a stop codon
  - Example is Sickle cell anemia
    - Missense mutation causes red blood cells to be concave rather than circular
      - Results in RBCs being unable to carry as much O_2 and makes it harder for them to fit through blood vessels
    - Gives them a heterozygous advantage against malaria
      - If they have one of each type of gene they have an advantage for battling malaria



Frameshift Mutations

- Frameshift Mutation – a shift in the reading frame away from the original alignment
  - Where the gene begins to be read (starting point) is very important because the data in genes are read in groups of three bases at a time (codons)
  - Removal or addition of 1-2 bases substantially changes the output
- Example sequence (not DNA) THEMANCANRUNNOW
  - The codons would be read THE MAN CAN RUN NOW
  - Imagine an addition of one nucleotide THE**A**MANCANRUNNOW
    - Now reads THE AMA NCA NRU NNO W
  - Imagine a deletion of one nucleotide (deleted in red) THEM**A**NCANRUNNOW
    - Now reads THE MNC ANR UNN OW

Gene Mutations Outside Coding Regions

Sequence	Effect of mutation
Promoter	May increase or decrease the rate of transcription
Transcriptional response element/operator site	May alter the regulation of transcription
Splice junctions	May alter the ability of pre-mRNA to be properly spliced
Translational response element	May alter the ability of mRNA to be translationally regulated

Example Question

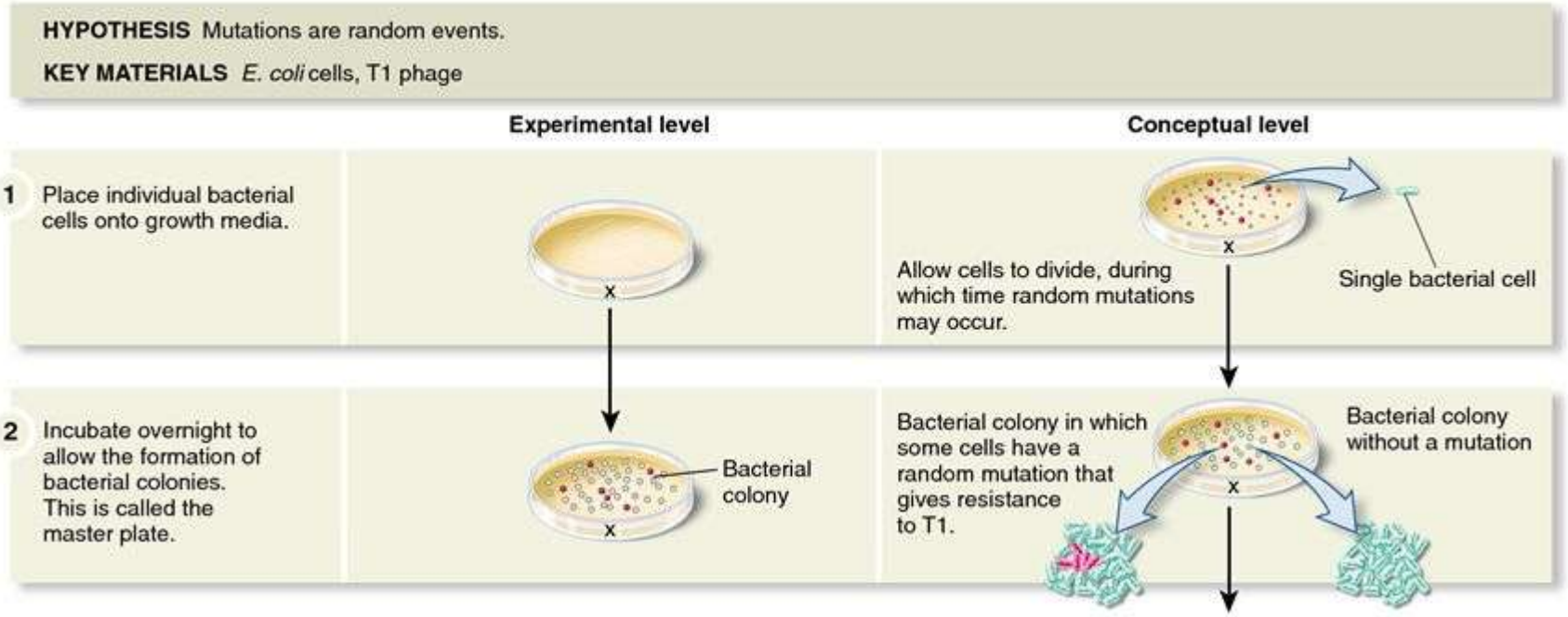
A mutation of a single nucleotide that changes a codon to a stop codon is called a:

a) Silent mutation
b) Missense mutation
c) Nonsense mutation
d) Frameshift mutation

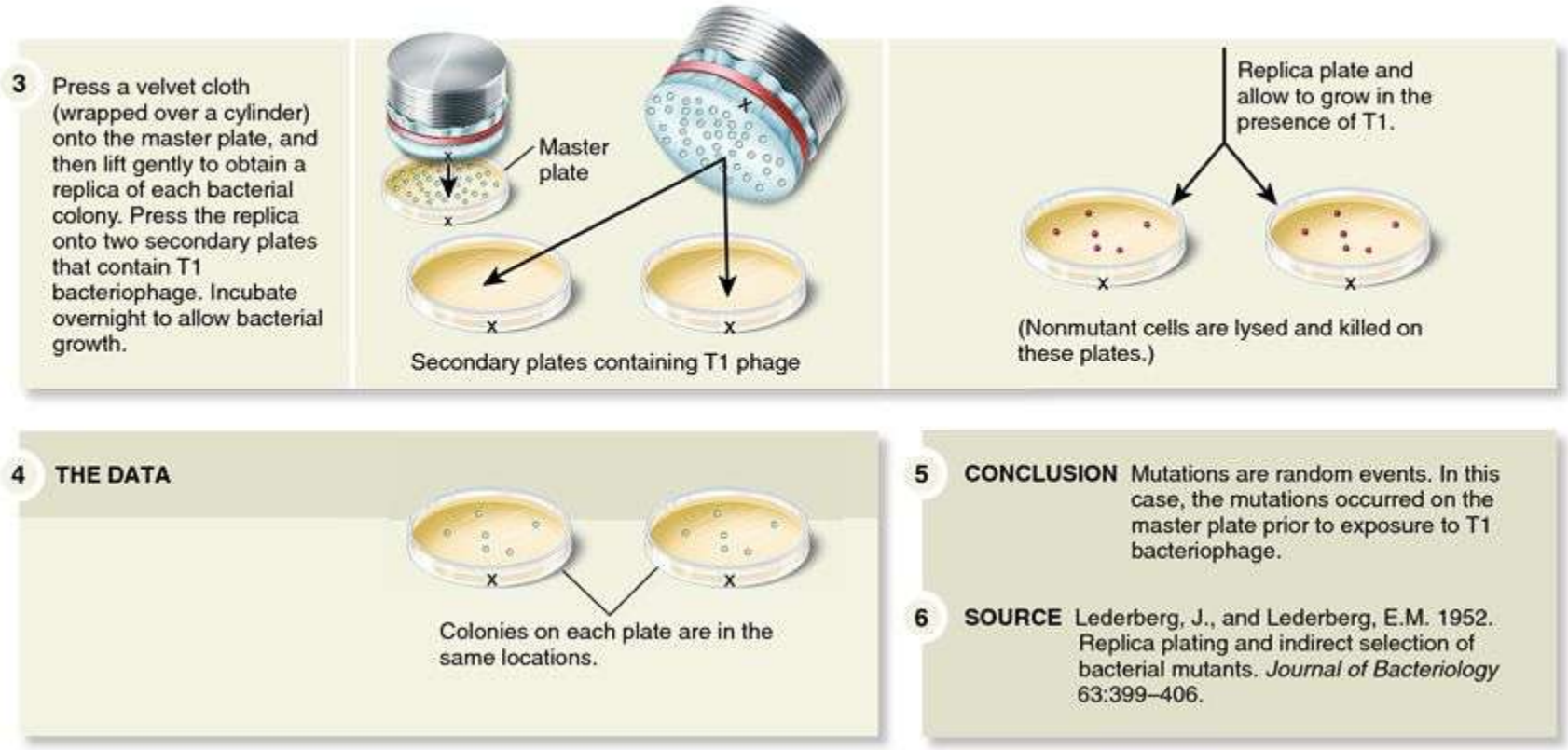
How do mutations occur?

- Pre-existing circumstances? (caused by selection pressure)
  - Eg. Does exposure to a certain set of circumstances trigger mutation?
- Unplanned random events

Ester and Joshua Lederberg



Ester and Joshua Lederberg



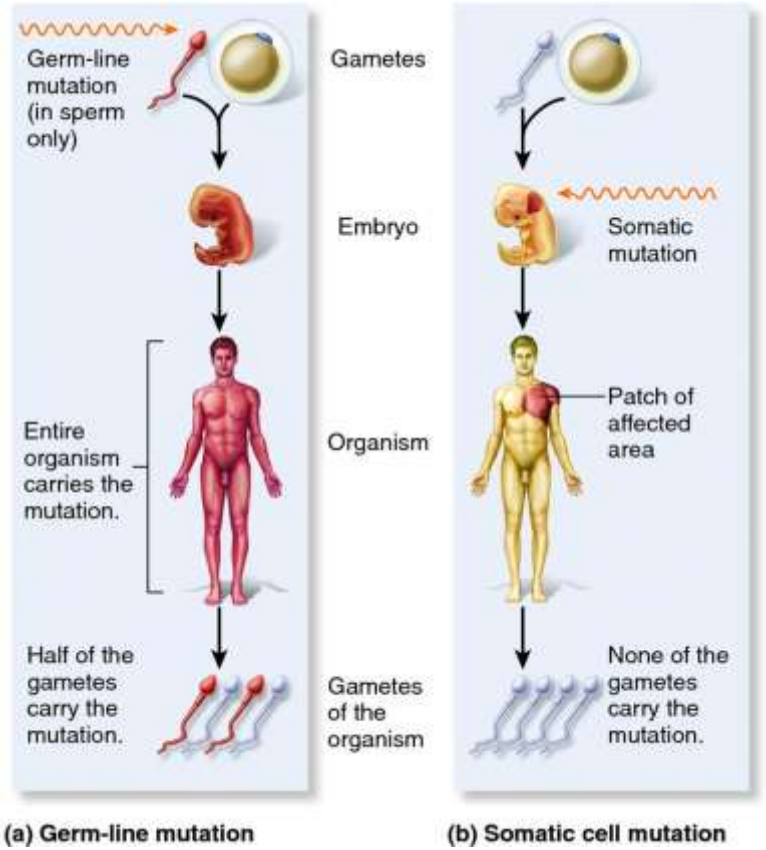
Example Question

Based on the results from the Ester and Joshua Lederberg experiments, mutations occur...

a) Under specific nutrient conditions
b) Randomly
c) Due to natural selection
d) Due to viral infection

Where mutations occur

- Timing and location of a mutation are critical to the:
  - Severity of effect
  - Ability to pass on the mutation
    - Mutations that occur in the germ line (gamete producing cells), or the gametes themselves are transmitted across generations
      - Mutation is a part of the entire zygote
    - Mutations in the somatic line are not passed on



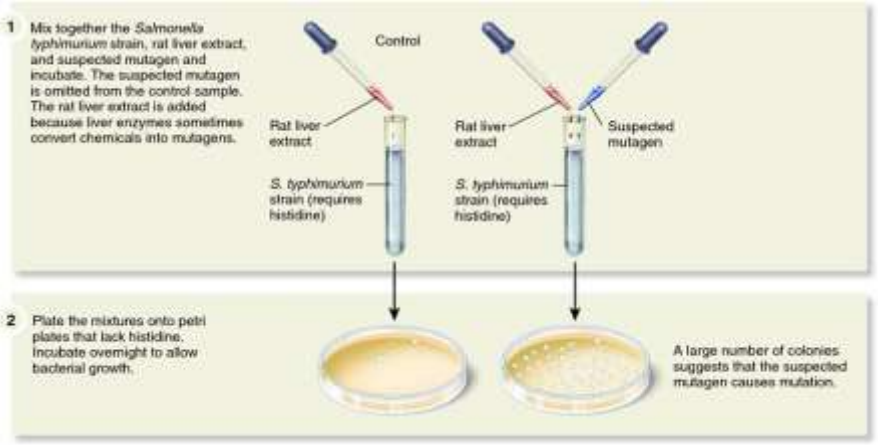
Spontaneous or Induced mutations

- Spontaneous mutations are random occurrences
  - Occur once in a million genes.
  - Rate varies between species and between genes
- Induced mutations are brought on by exposure to environmental agents
  - Rate of mutation is higher than the spontaneous mutation rate
- Environmental agents include chemicals (benzopyrene found in cigarettes) or physical agents (UV for example) increasing the rate of mutation (mutagens)

Spontaneous	
Errors in DNA replication	A mistake by DNA polymerase can cause a point mutation.
Toxic metabolic products	The products of normal metabolic processes may be reactive chemicals, such as free radicals, that can alter the structure of DNA.
Spontaneous changes in nucleotide structure	On rare occasions, the linkage between purines and deoxyribose can spontaneously break. Also, changes in base structure (isomerization) can cause mispairing during DNA replication.
Transposons	As discussed in Chapter 19, transposons are small segments of DNA that can insert at various sites in the genome. If they insert into a gene, they can inactivate the gene.
Induced	
Chemical agents	Chemical substances, such as benzo(a)-pyrene, a chemical found in cigarette smoke, can cause changes in the structure of DNA.
Physical agents	Physical agents, such as UV (ultraviolet) light and X-rays, can damage the DNA.

Ames Test

- Bruce Ames developed a test for determining if a chemical is a mutagen
- Uses *Salmonella typhimurium* strain that cannot synthesize histidine due to a point mutation
- One of two things could happen
  - Bacteria could die because they need histidine
  - Mutation occurs allowing synthesis of histidine
- The test monitors the rate at which reverting mutation occurs (back to wild type that can produce histidine)
  - Compares the growth of two plates; one that is a control and the other that contains the mutagen
  - More colonies surviving means that that substance is a mutagen



Example Question

If a mutation occurs in a germ line cell then....

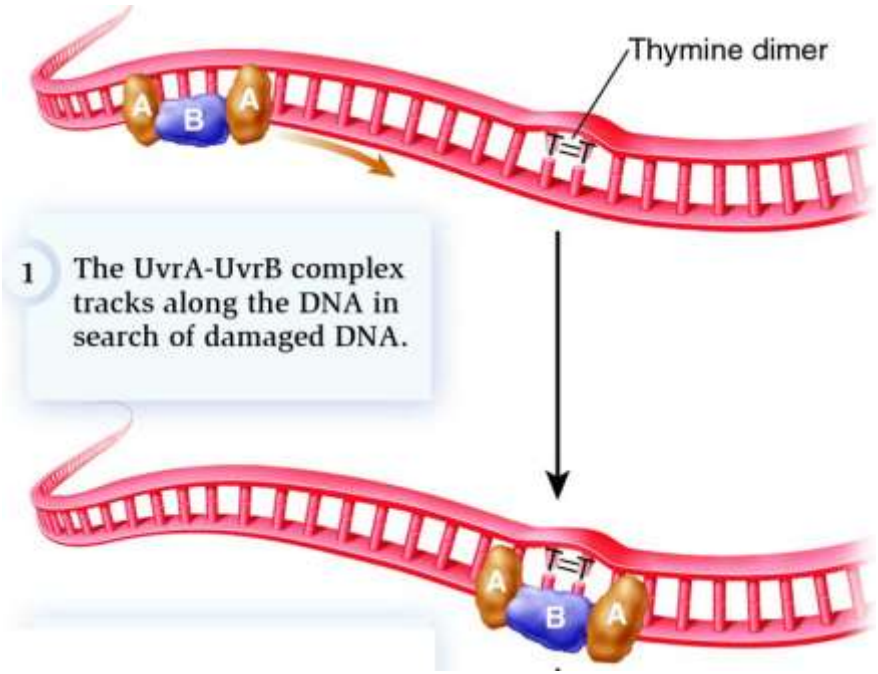
a) Only a certain area of the embryo will be affected
b) The mutation could be passed onto future generations
c) The mutation could effect the entire individual
d) B and C

DNA Repair

- Direct Repair
  - Enzyme physically removes the modification (for example an alkyl group)
- Altered DNA strand is removed and new segment is synthesized
  - More common than direct repair
  - Nucleotide Excision Repair
    - Remove the damaged DNA; use the remaining template to form the removed complimentary strand
  - Methyl-directed mismatch repair
    - Corrects non-methylated daughter strand

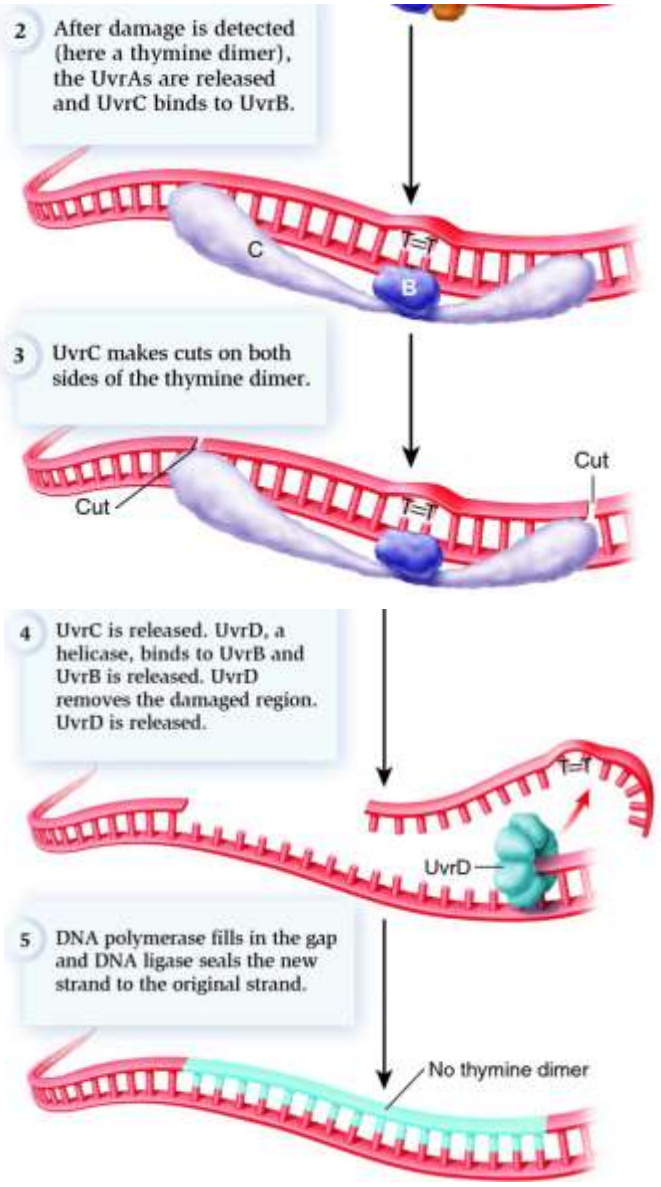
Nucleotide Excision Repair (NER)

- Most common DNA repair system
- Region encompassing several nucleotides in the damaged strand is removed from the DNA
  - Region around the mutation is removed as well



NER

- Intact undamaged strand is used as a template for resynthesis of a normal complementary strand
- Found in all eukaryotes and prokaryotes

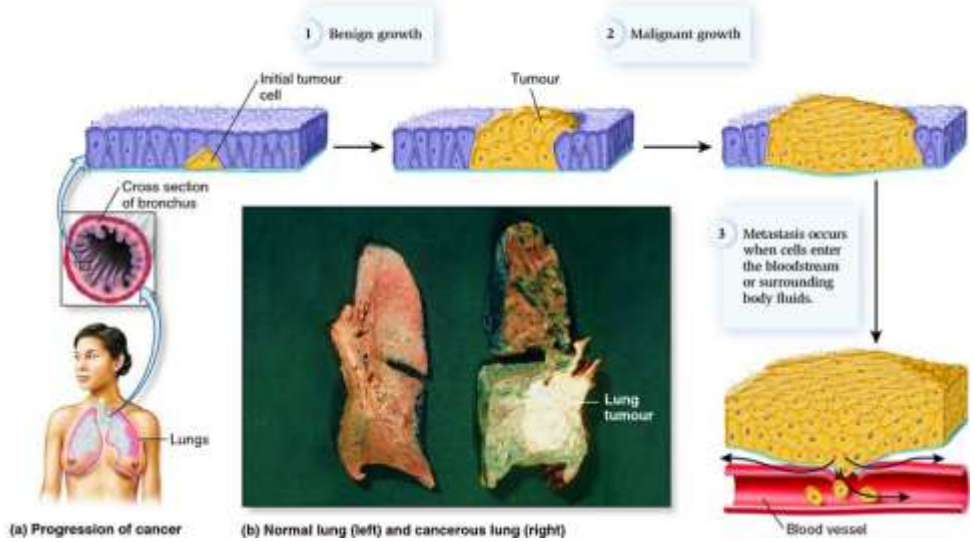


Direct repair....

a) Removes the damaged portion of DNA
b) Is more common then NER
c) Uses methylated sites
d) Physically repairs the mutation

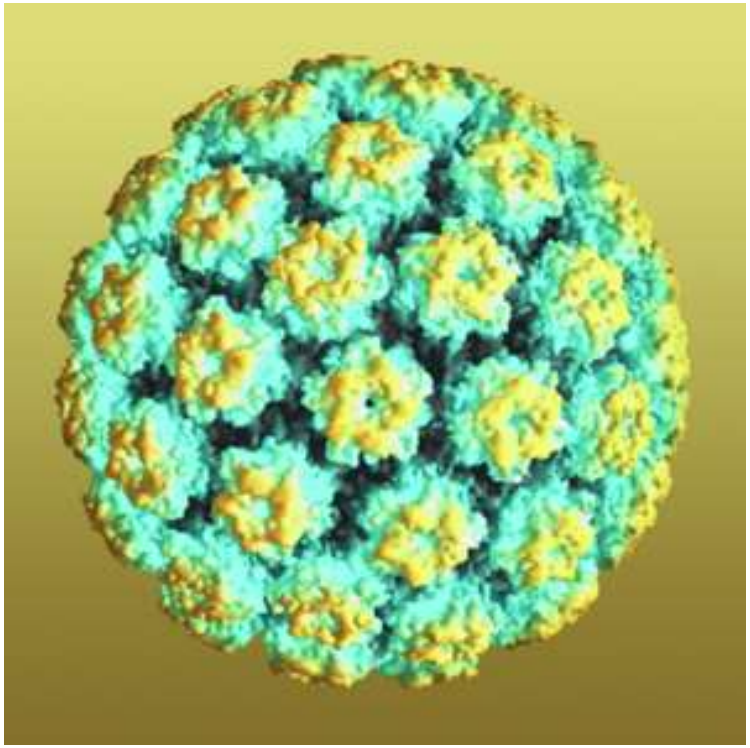
Cancer

- Uncontrolled cell division
  - Proliferation of cells or tissue where they shouldn't be proliferating
- Cancers originate from a single cell
  - That cell mutates so that the cell grows abnormally
  - Soon forms a tumour (an overgrowth of cells with no purpose)
    - Tumour may be benign or pre-cancerous
- Cancerous stages:
  - Malignant – lost normal growth regulation
  - Invasive – can invade healthy tissue
  - Metastatic – can migrate from its original location to other parts of the body
- Untreated malignant cells will cause the death of the organism
  - Get in the way of normally functioning cells



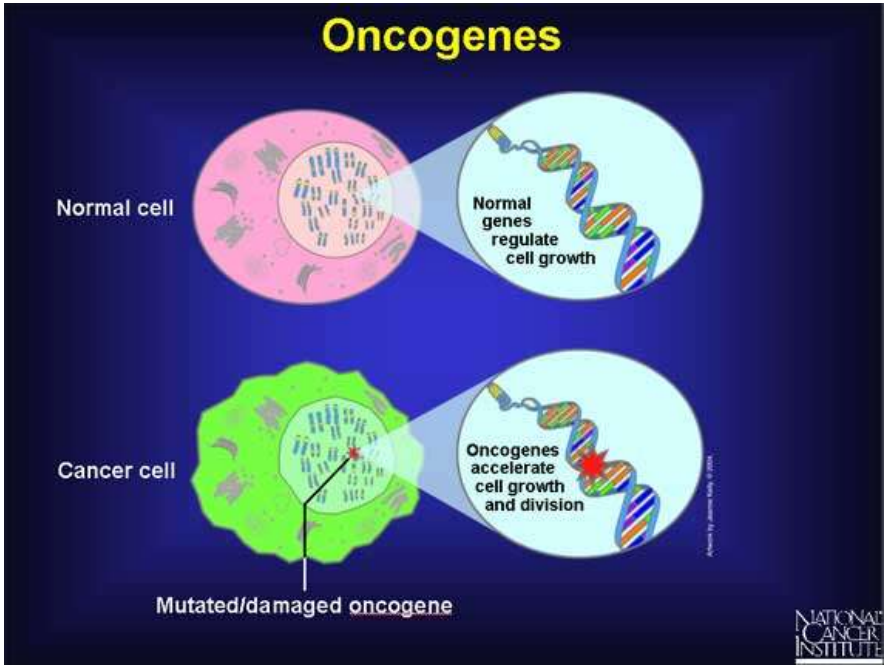
Causes of cancer

- Most often caused by mutagens
  - Either physical (eg. UV light) or chemical
- A few viruses are known to cause cancer in plants, animals, and humans
  - An example is the Human papilloma virus
  - Some viruses may cause cancer by modifying genes in the host cell
  - Others may carry oncogenes in the viral geneome



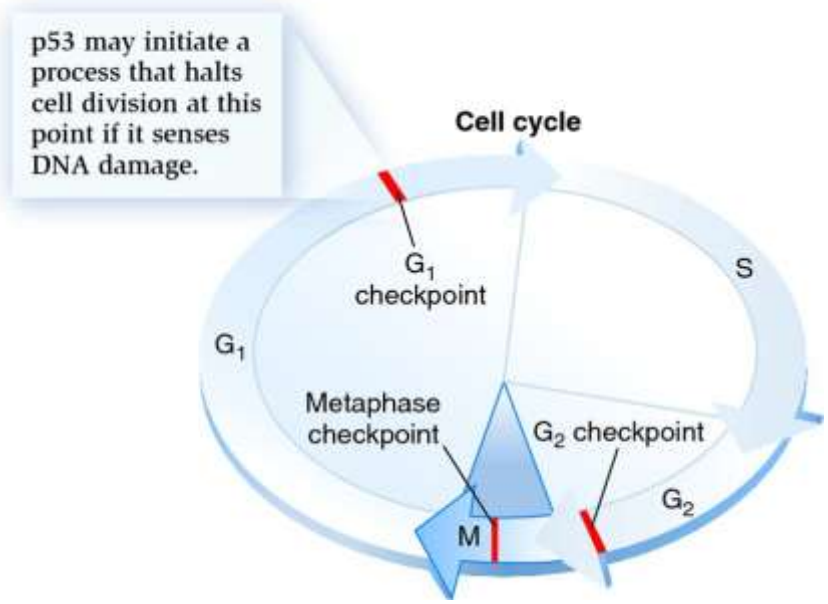
Cancer Causing Genes

- Oncogenes
  - When a mutation causes this gene to be overactive, uncontrolled cell growth occurs
- Mutations in tumour suppressing genes
  - Normally these genes encode proteins that prevent cancer
  - If a mutation eliminates this function then cancer can occur



p53

- p53 is a G1 checkpoint protein
  - *p53* gene is transcribed when DNA is damaged
- When damaged the cell cannot progress from G1 to the S (DNA synthesis/replication) phase of the cell cycle
  - If DNA is severely damaged p53 prevents the cell from entering the S phase (in a normal cell)
  - When p53 is mutated or absent then the cell is allowed to progress to mitosis despite damage to the DNA
- About 50% of all human cancers are associated with defects in *p53* gene

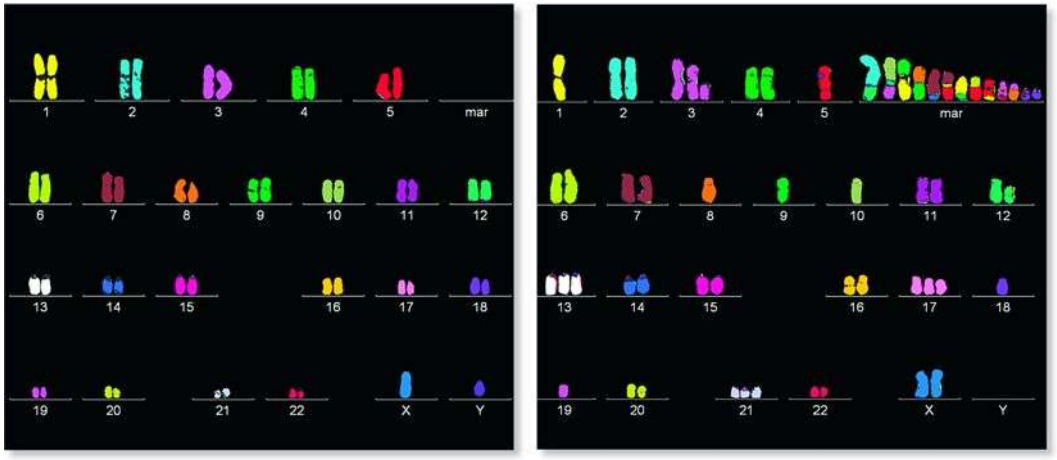


Mutations in approximately 300 Human genes may promote cancer

- Not all of these mutant genes found in cancers directly affect the growth of cells
- They can provide some type of growth advantage for the cell population from which the cancer developed
  - For example, if a gene that makes the cell is able to acquire glucose better than other cells
- Over 1% of our genes have the potential to promote cancer if their function is altered by a mutation

Abnormalities in chromosome structure and number

- If you compare cells in normal and tumour tissues you find bizarre chromosomal abnormalities including:
  - Loss of tumour-suppressing genes
    - Possibly due to DNA compaction
  - Over expression because of extra chromosomes
  - Chimeric (mixed) genes; by translocations

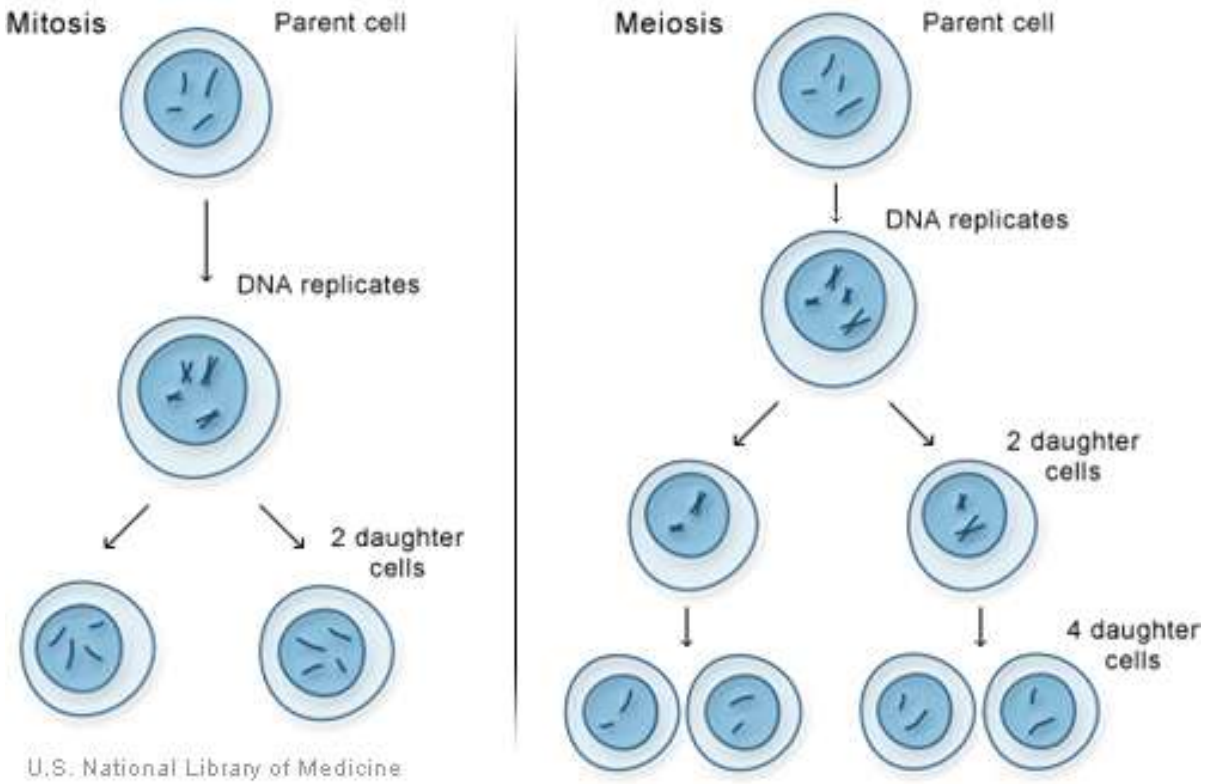


Example Question

Cancer can be caused by:

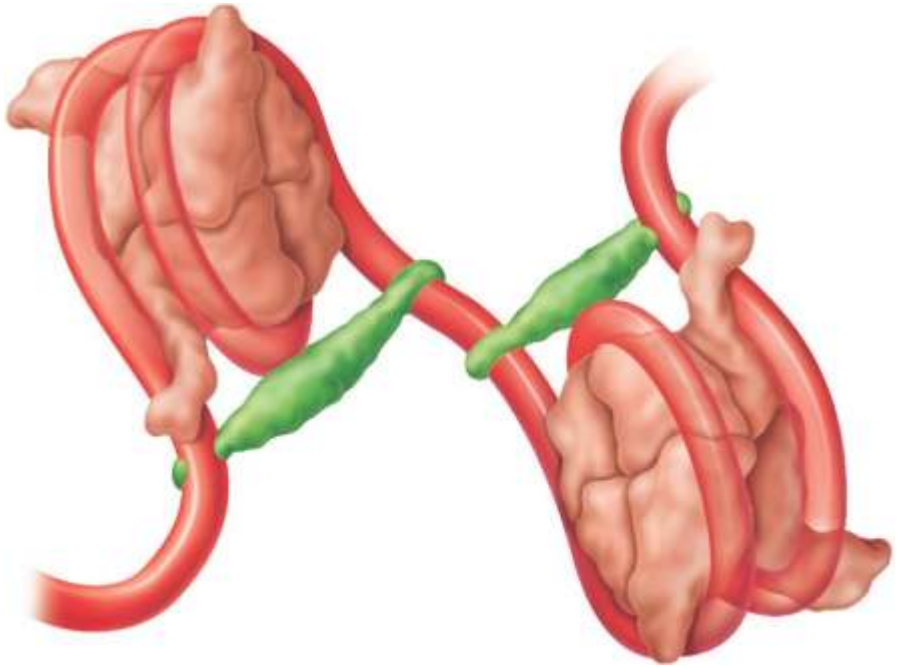
a) Viruses
b) Chemicals
c) UV light
d) All of the above

Chapter 14 – Eukaryotic Chromosomes, Mitosis, and Meiosis



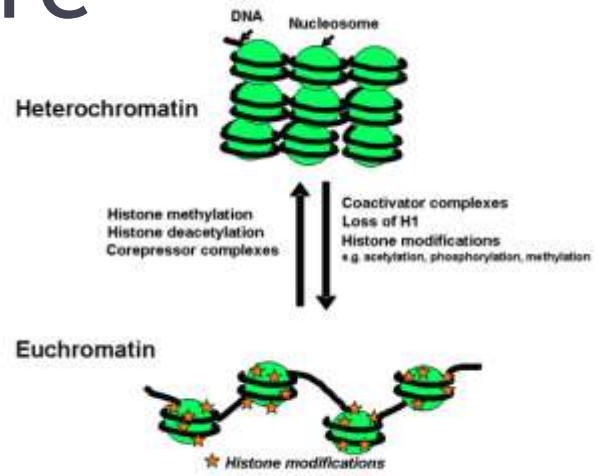
Chromosome Structure

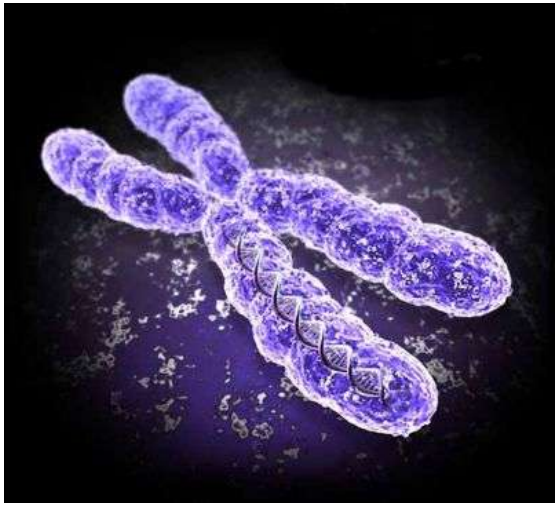
- Chromosomes come in pairs – found by Sutton in 1903.
- Cells coil around histones so to be compact
  - 8 histones with attached DNA is called a nucleosome
  - Between nucleosomes are linker regions
    - Connected by another histone called H1
  - Nucleosomes form a zig-zag patterm



Chromosome Structure

- The zig zag pattern of nucleosomes is arranged in radial loop domains
  - These are organized into heterochromatin
- The folding of heterochromatin gives chromosomes their characteristic structure





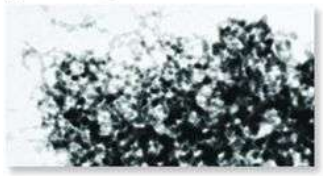
Chromosome Structure

(a) DNA double helix

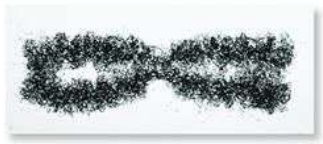
(b) Nucleosomes ("beads on a string")

(c) 30-nm fibre

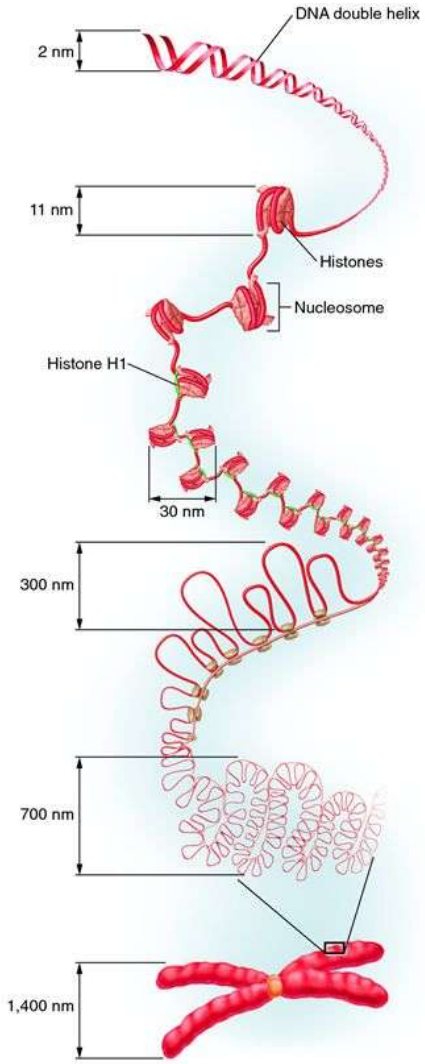
(d) Radial loop domains



(e) Heterochromatin



(f) Metaphase chromosome



1. Wrapping of DNA around histone proteins.
2. Formation of a three-dimensional zigzag structure via histone H1 and other DNA-binding proteins.
3. Anchoring of radial loop domains to the nuclear matrix.
4. Further compaction of radial loops to form heterochromatin.
5. Metaphase chromosome with two copies of the DNA.

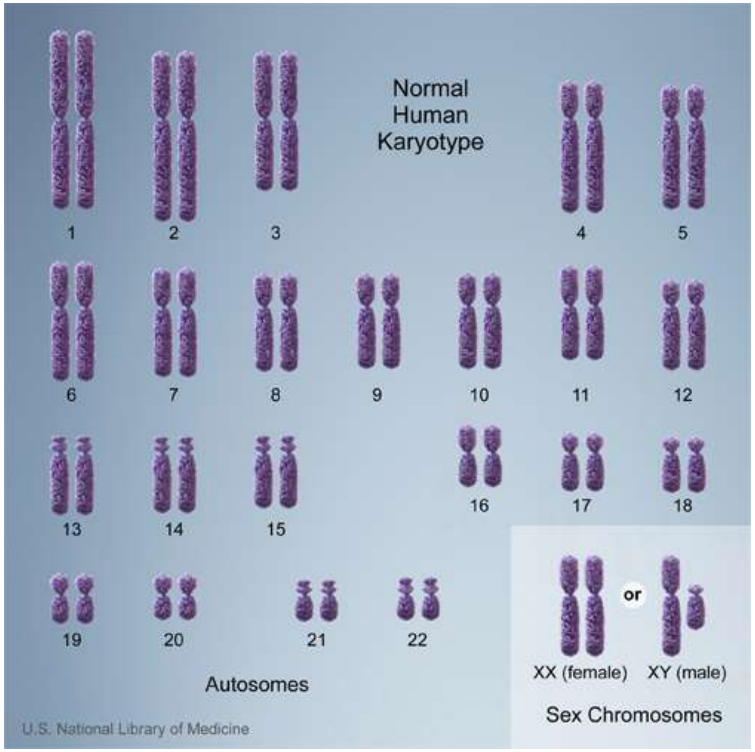
Example question

Which of the following is the most correct?

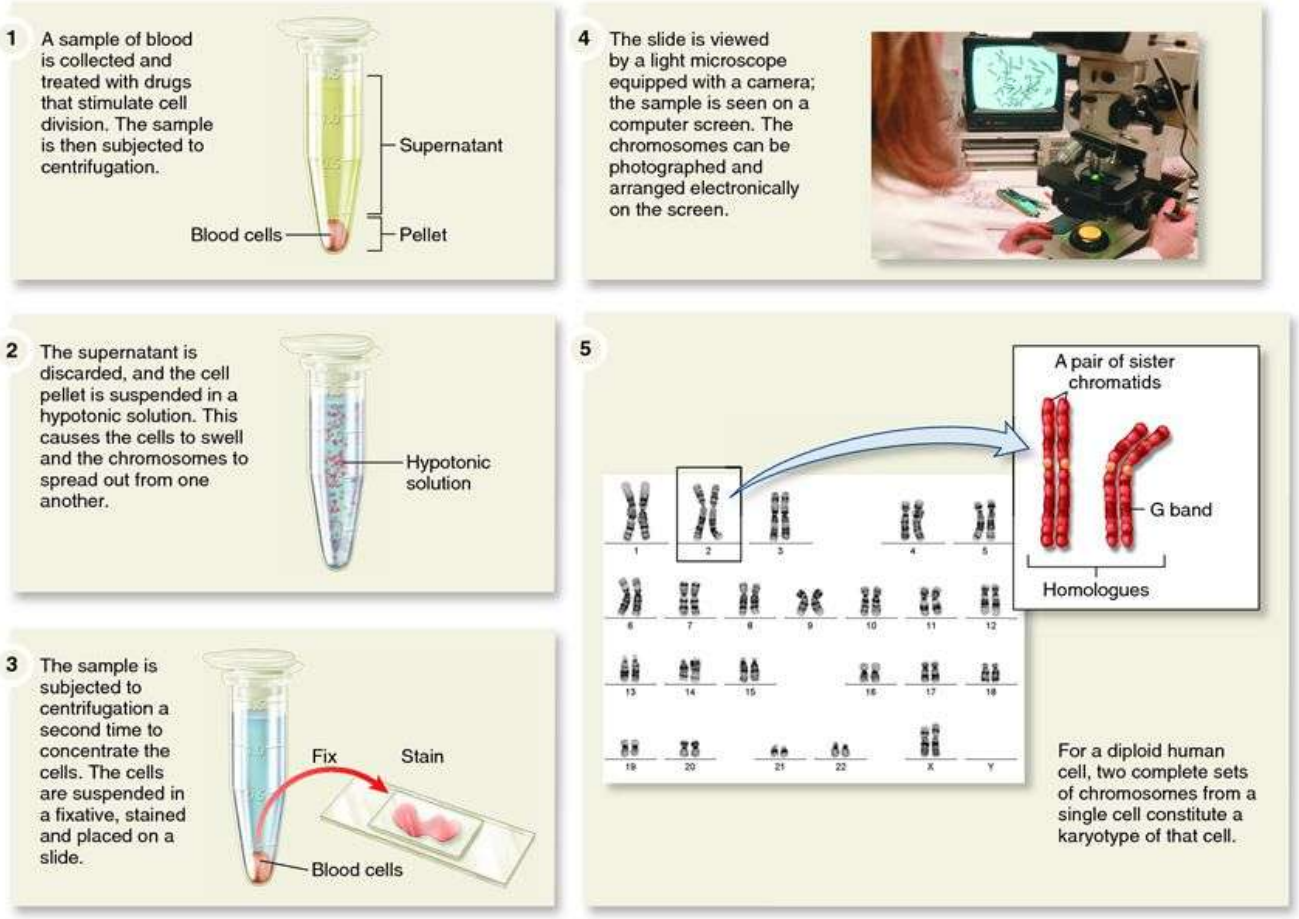
a) DNA double helixes form heterochromatin
b) Histones form heterochromatin
c) Nucleosomes form heterochromatin
d) Radial loops form heterochromatin

Human chromosomes

- Somatic (body) cells have 2 sets of chromosomes that are homologous (identical)
  - The diploid chromosome number (number of chromosomes in the 2 sets) is 46 in humans
- In the sex cells (gametes) there is only one set of chromosomes
  - If a cell possesses only one set of chromosomes it is haploid
  - Haploid chromosome number in humans is 23 (half of the diploid 46)



Karyotyping

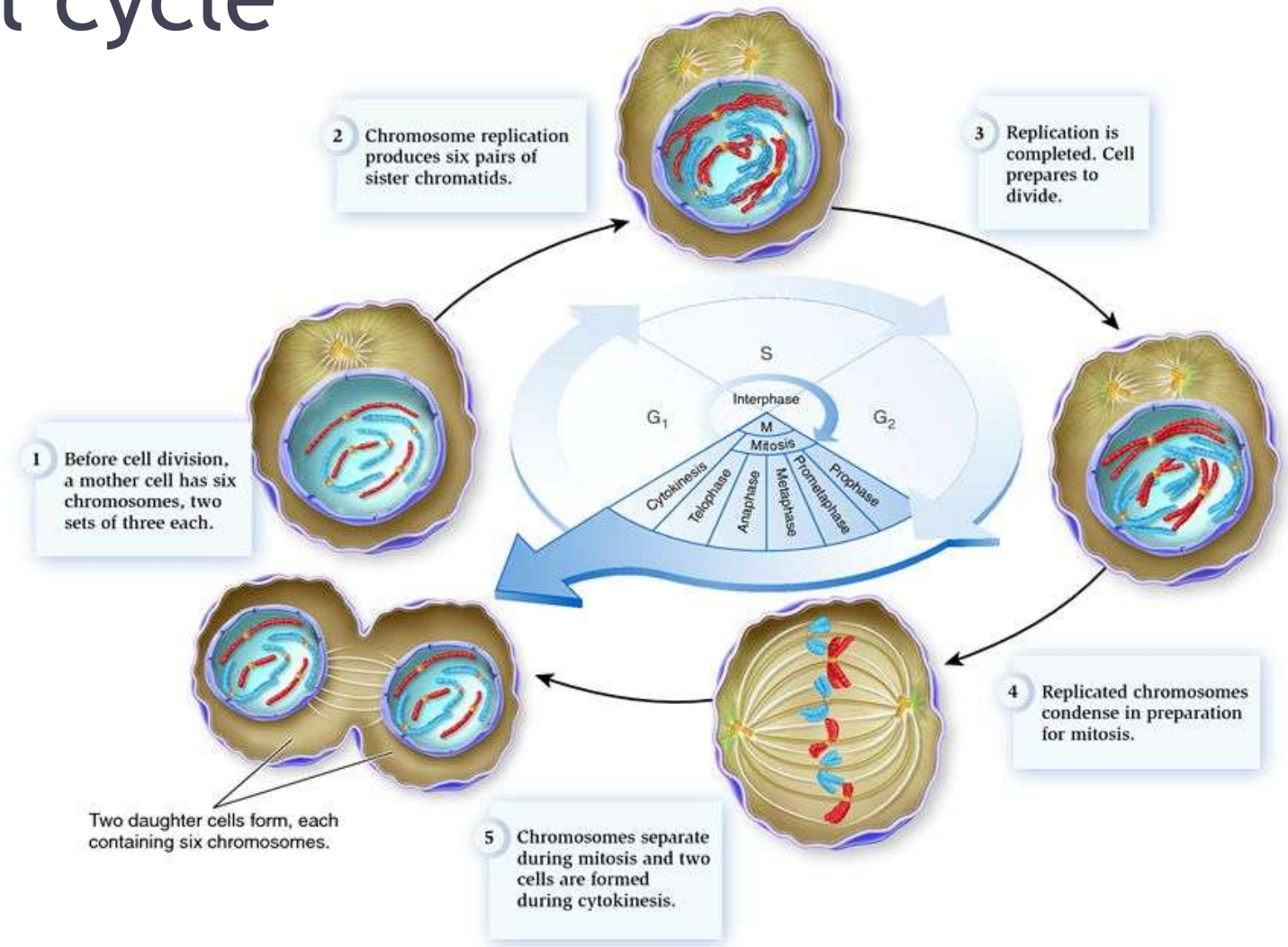


Example question

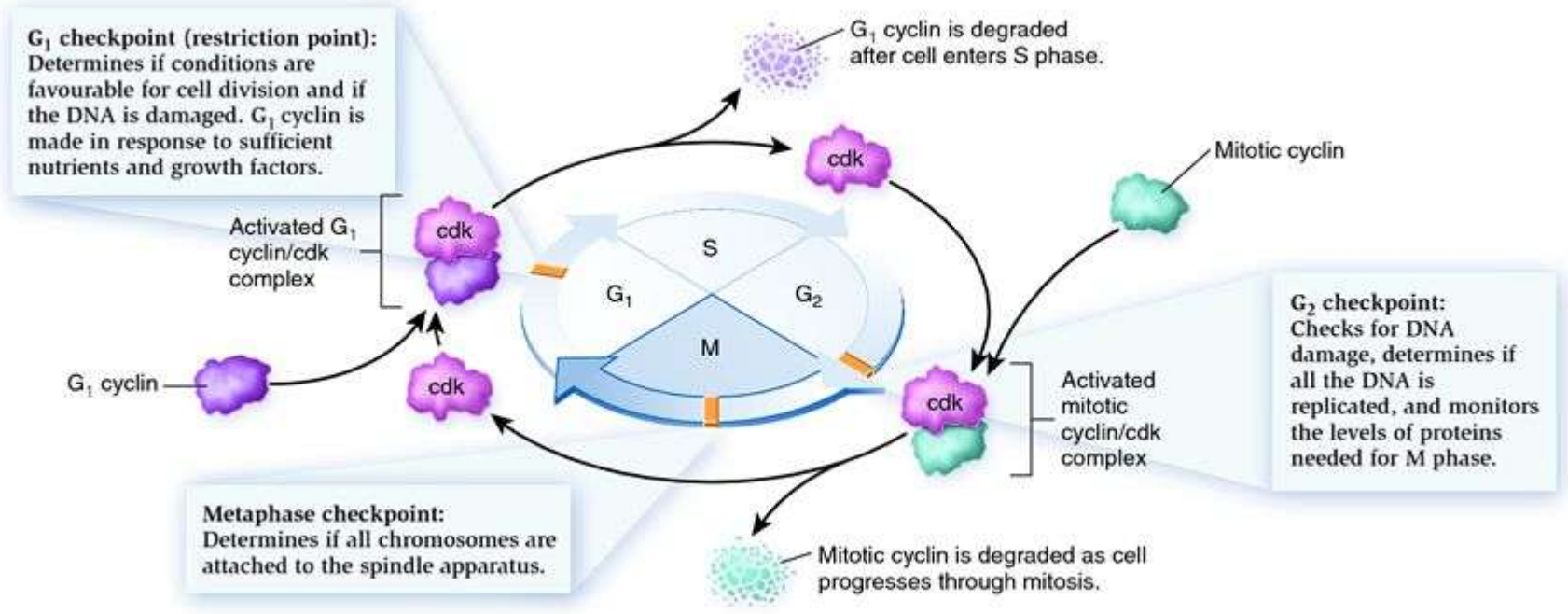
In a normal human karyotype there are ___ homologous chromosomes which means there are ___ chromosomes.

a) 46, 23
b) 44, 22
c) 23, 46
d) 22, 44

Cell cycle

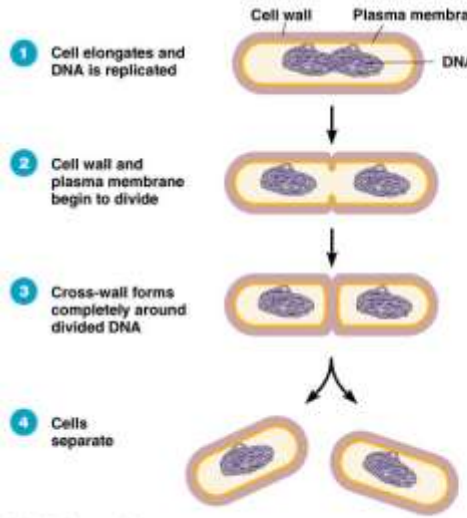

2 Chromosome replication produces six pairs of sister chromatids.
3 Replication is completed. Cell prepares to divide.
1 Before cell division, a mother cell has six chromosomes, two sets of three each.
S
Interphase
G1
G2
M
Mitosis
Cytokinesis
Telophase
Anaphase
Metaphase
Prometaphase
Prophase
4 Replicated chromosomes condense in preparation for mitosis.
Two daughter cells form, each containing six chromosomes.
5 Chromosomes separate during mitosis and two cells are formed during cytokinesis.

Regulating the Cell Cycle

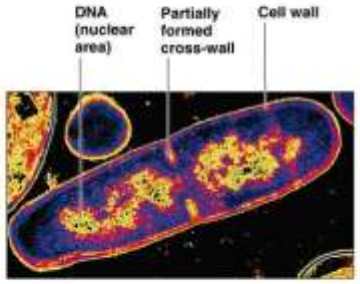


Cell Division in Prokaryotes

- In prokaryotes, there is simple fission, which creates identical daughter cells.
  - This is asexual reproduction
  - No mitotic spindle
- Circular DNA molecules separate as the cell wall forms



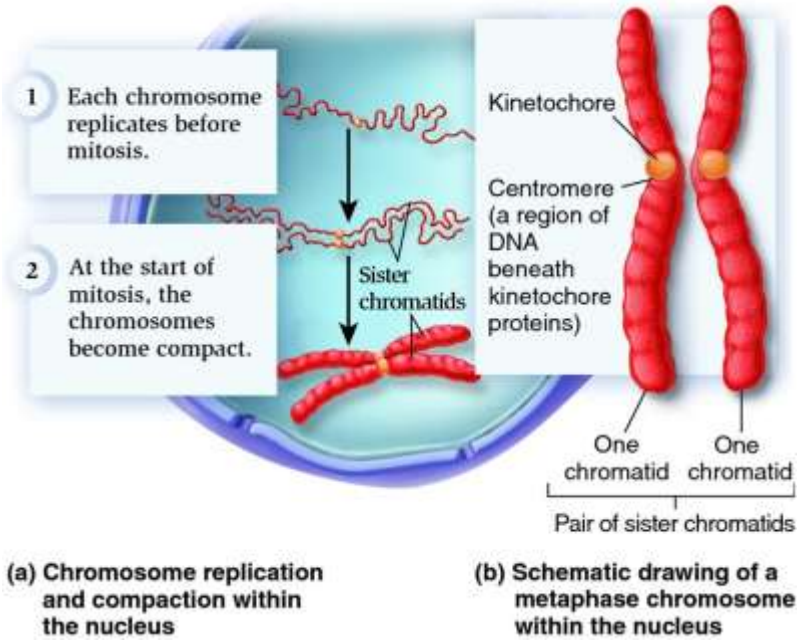
(a) A diagram of the sequence of cell division.



(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

Cell division in Eukaryotes

- Eukaryotes use mitosis to produce identical daughter cells
  - Essentially this is asexual reproduction
- Complex processes involving cytoskeletal systems
- Linear DNA strands become separated by complex motor systems

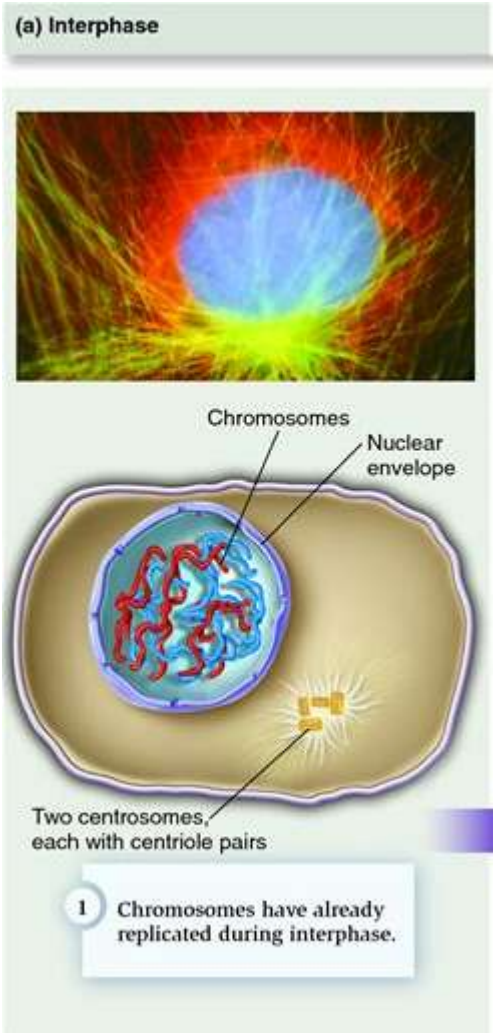


(a) Chromosome replication and compaction within the nucleus

(b) Schematic drawing of a metaphase chromosome within the nucleus

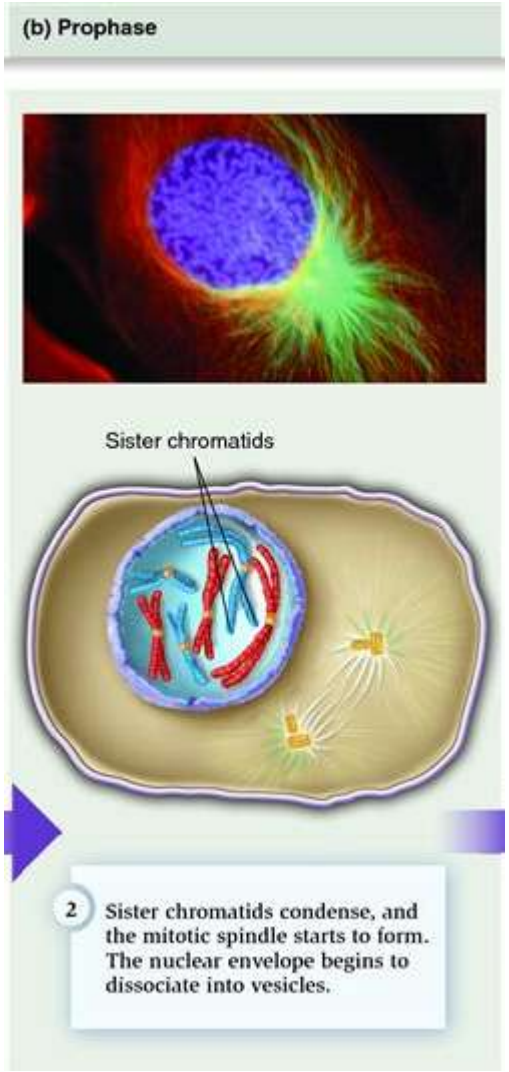
Mitosis (Interphase)

- Replication has already occurred
- Centrosomes form from centrioles pairs
- Comprises the G1, S, and G2 phases



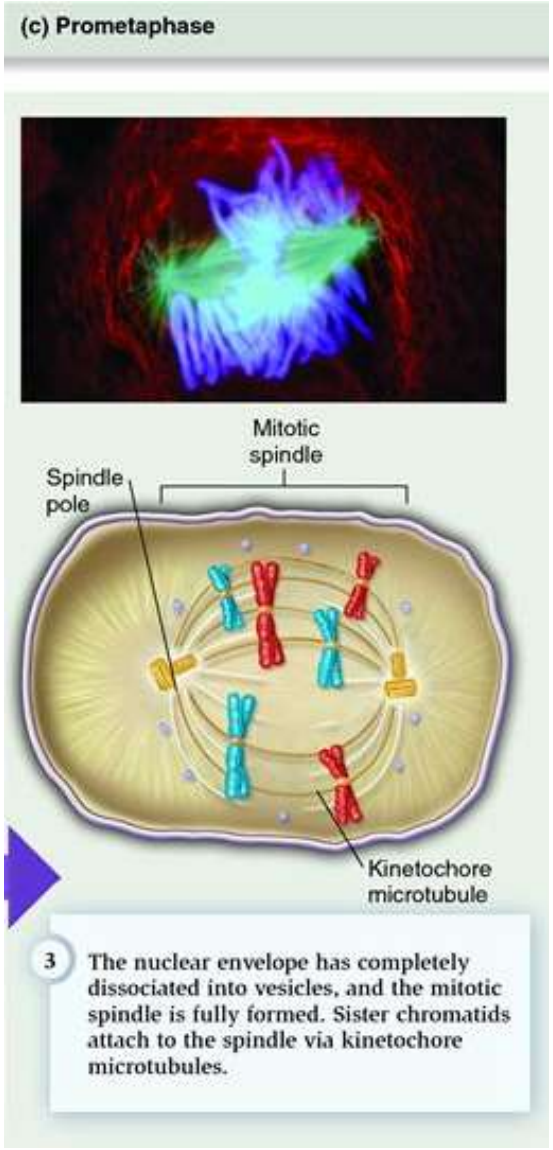
Mitosis (Prophase)

- Sister chromatids condense
- Spindle fibers start to form from the centrosomes, which are moving towards the pores
- The nuclear membrane begins to breaks apart into vesicles
  - Doesn't disintegrate, these vesicles will eventually reform the nuclear membrane in daughter cells



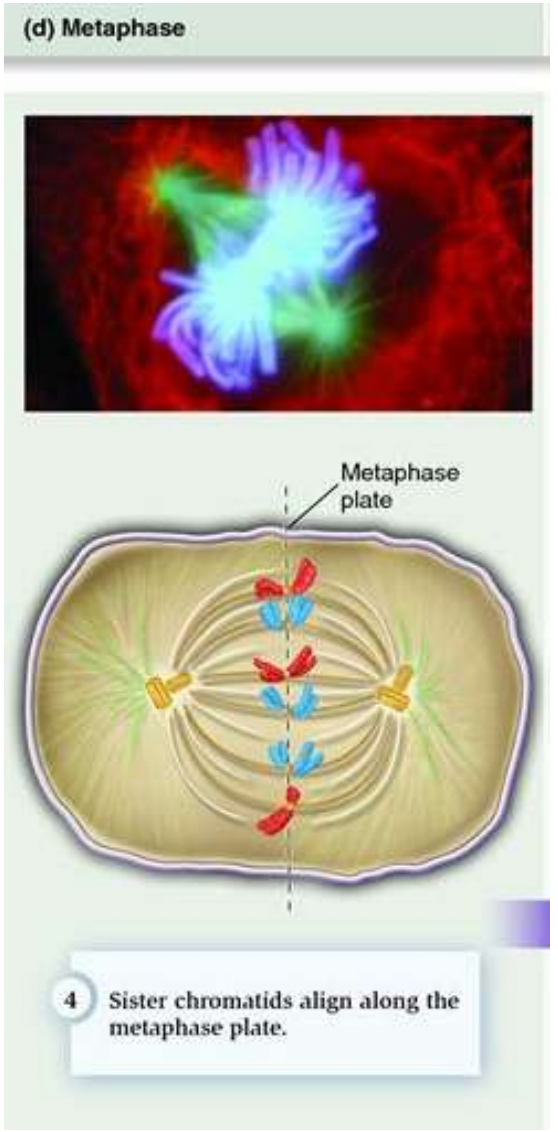
Mitosis (Prometaphase)

- Nuclear membrane is gone
- The spindle fibers are fully formed
- Sister chromatids attach to spindle via kinetochore microtubules
  - Spindle is the beginning of the mitotic spindle



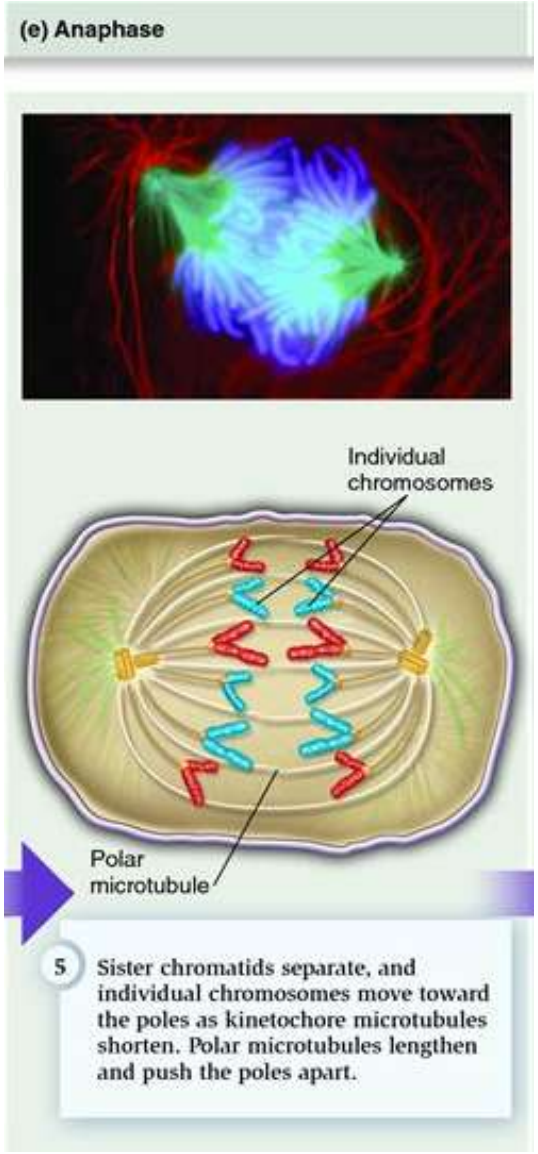
Mitosis (Metaphase)

- The chromatids align on the metaphase plate
  - Usually in the center of the cell
- The centrioles have associated microtubules
  - Also has astral structure that anchors it the centrioles to the poles of the cell
- Mitotic spindle
  - Composed of astral microtubules, kinetochore microtubules (connect to chromosomes), and polar microtubules which start at the poles but don't connect to the chromosomes
  - Also composed of centrosomes
  - Centrioles are not found in plants
    - Other proteins make the poles function for separating DNA



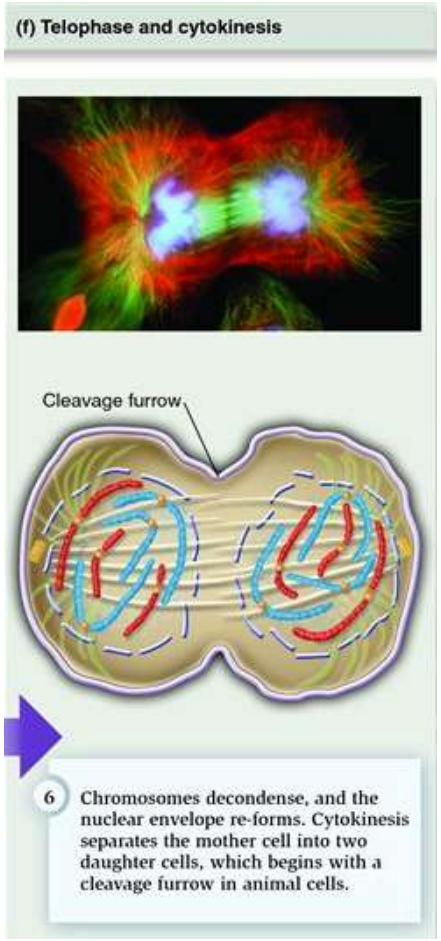
Mitosis (Anaphase)

- Sister chromatids split and move to each pole by the shortening of the kinetochore microtubules
- The lengthening of the polar microtubules pushes the two poles of the cell apart



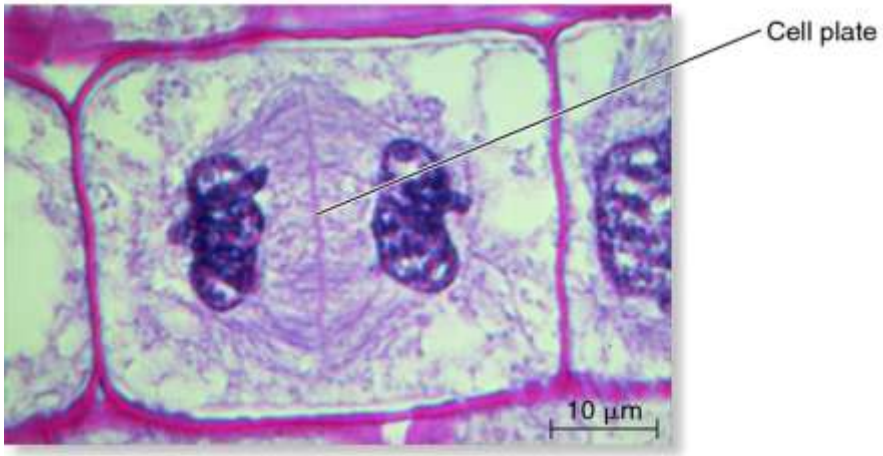
Mitosis (Telophase and Cytokinesis)

- Chromosomes decondense, and nuclear membranes reform (from vesicles)
- Cell cleavage occurs, pinching the two cells apart
- Actual separation of the cytoplasm into the two daughter cells is called cytokinesis
  - Cytokinesis is actin and myosin dependant
    - Actin and myosin are protein filaments
  - Actin/myosin fibres pull like purse strings to nip the cells apart



Mitosis in Plants

- There are no centrioles in the mitotic spindle
- Vesicles form out of the Golgi over the equatorial plate (metaphase plate)
  - They form a large vesicle and produce the new cell walls
  - Enzymes form the substances necessary for the cell walls
  - Remember that 2 cell walls form (one for each daughter cell)



(b) Formation of a cell plate in a plant cell

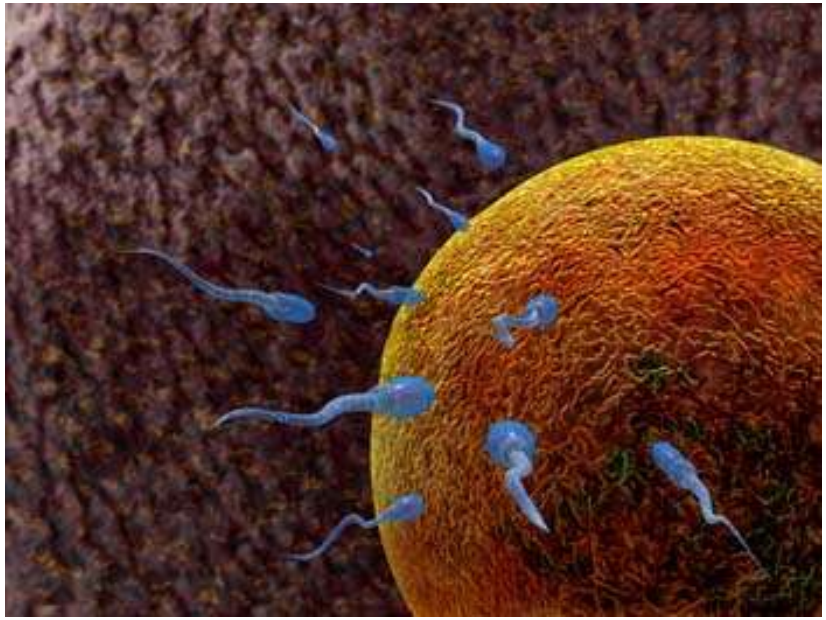
Example Question

In which phase do the sister chromatids split apart?

a) Interphase
b) Prophase
c) Metaphase
d) Anaphase
e) Telophase

Sex and Ploidy

- Progeny are made when two parental DNA come together. In order to prepare for this, the DNA must go through **meiosis**
- Gametes = Sex cells (sperm and egg)
  - They have 1 set of chromosomes and are therefore haploid (1n)
- Gametes combine to form the zygote
  - One gamete is from the mother, the other from the father
  - This fusion creates the diploid (2n) zygote
    - Homologous chromosomes refers to matching chromosomes from the mother and the father



Why Sex?

- Sex promotes diversity and variation allowing better adaptation to an environment
  - This is due to the mixing of the genes from the mother and father
  - Some of these mixes are more favourable than others
- Even though asexual reproduction is energetically cheaper, the mixing gives you a greater advantage over evolutionary time

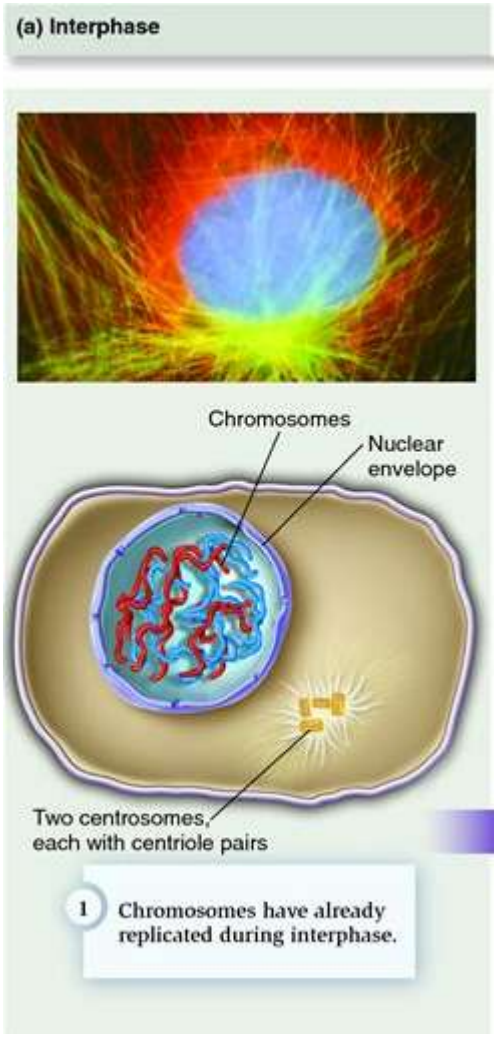
Example Question

Sex is favoured over asexual reproduction because...

a) It is energetically cheaper
b) It creates greater variation
c) It occurs faster
d) It's more fun

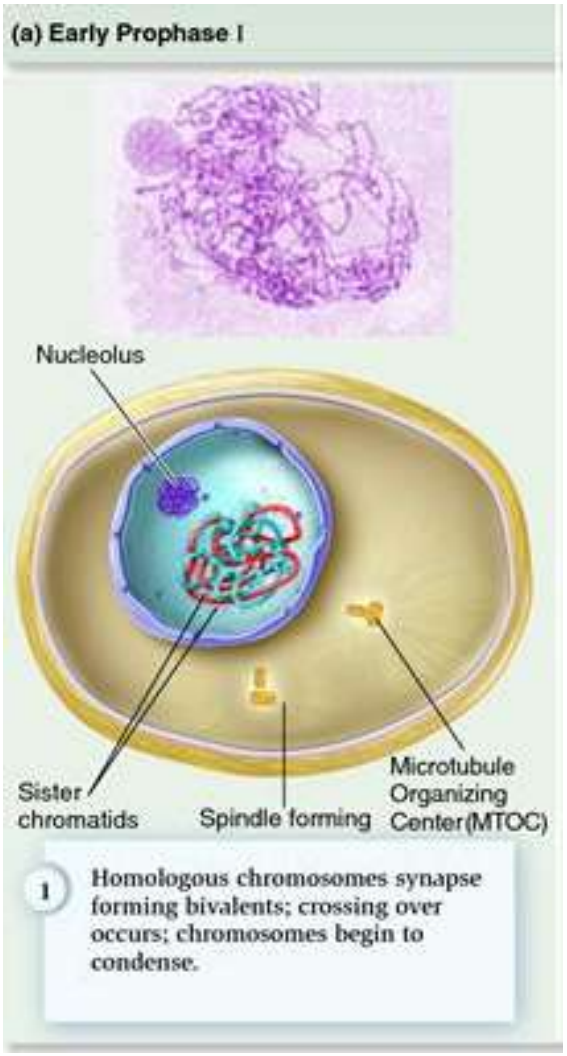
Meiosis I (Interphase)

- Same as mitotic interphase
- Comprises the G1, S, and G2 phases
- Meiosis is different because now the homologues must be separated



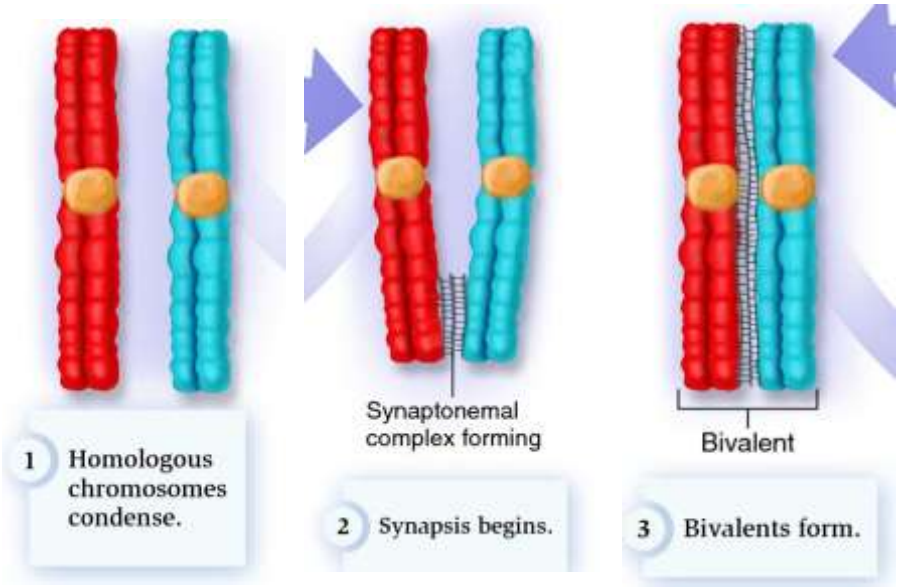
Meiosis I (Prophase I)

- Occurs after pre-meiotic interphase
- Homologous chromosomes synapse to form bivalents
  - This is when crossover occurs
- Chromosomes begin to condense



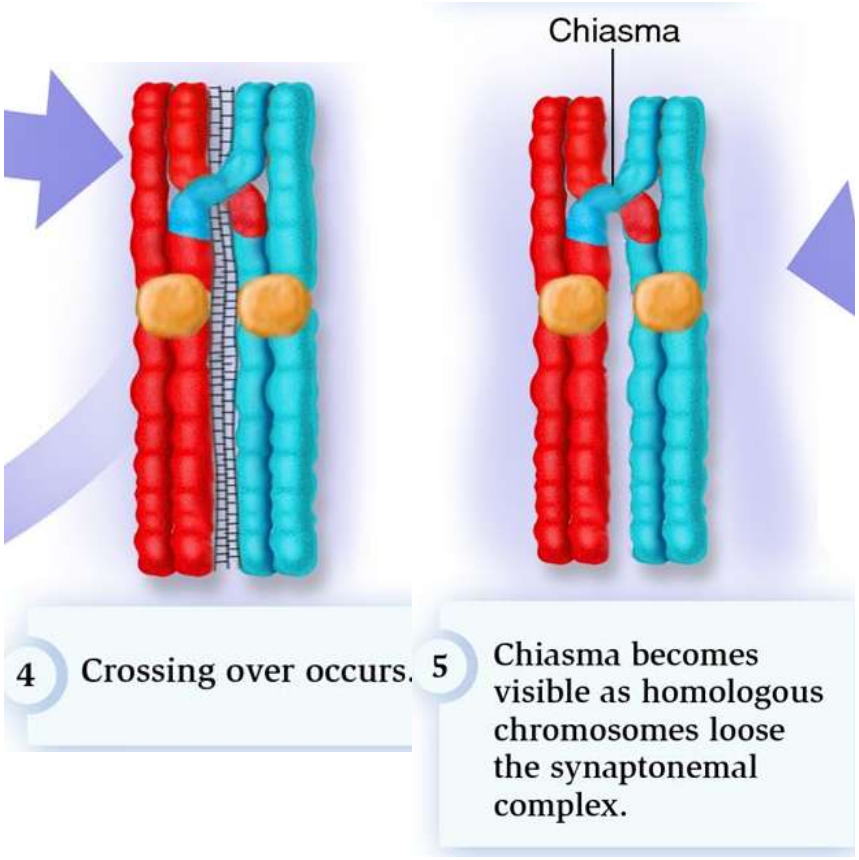
The Synaptonemal Complex

- Synaptonemal complex is two pairs of homologous chromosomes held together by various proteins
- DNA is precisely cut and recombined while part of the synaptonemal complex
- The synaptonemal complex holds the chromatids of the homologs close together until crossover is complete
  - The two homologs held together in this way are called a bivalent



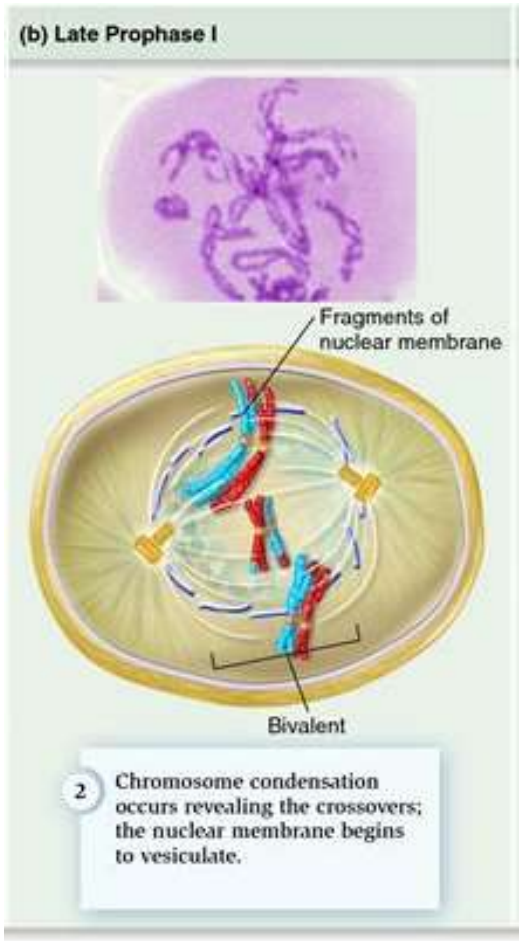
Crossover and Chiasmata

- The region of crossover forms an X shaped structure
  - DNA is cut and then crosses over with the other chromosome
  - So the maternal and paternal chromosomes become attached and mix
- This X shape is called a chiasma



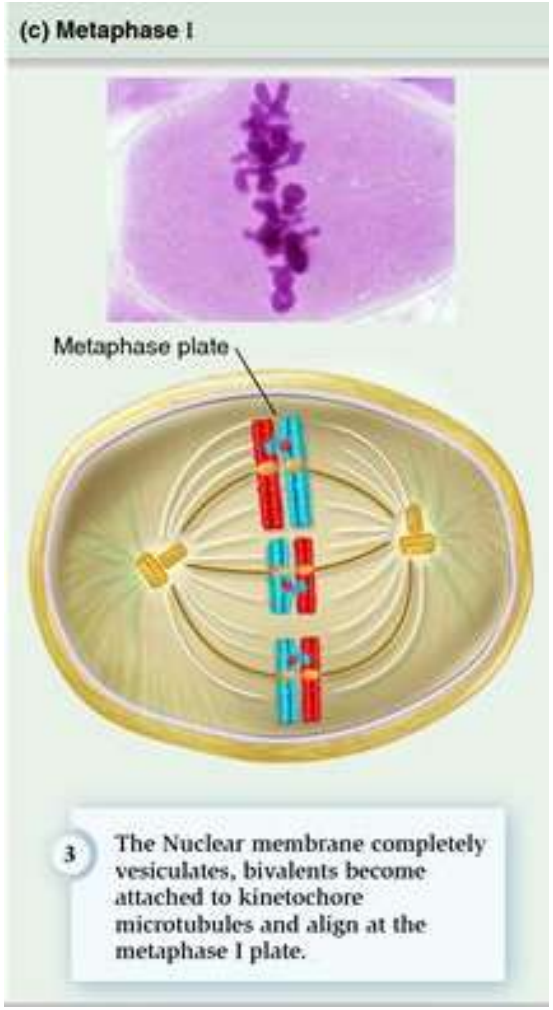
Meiosis I (Late Prophase I)

- Nuclear membrane begins to break apart as vesicles
- Bivalents become attached to kinetochore microtubules



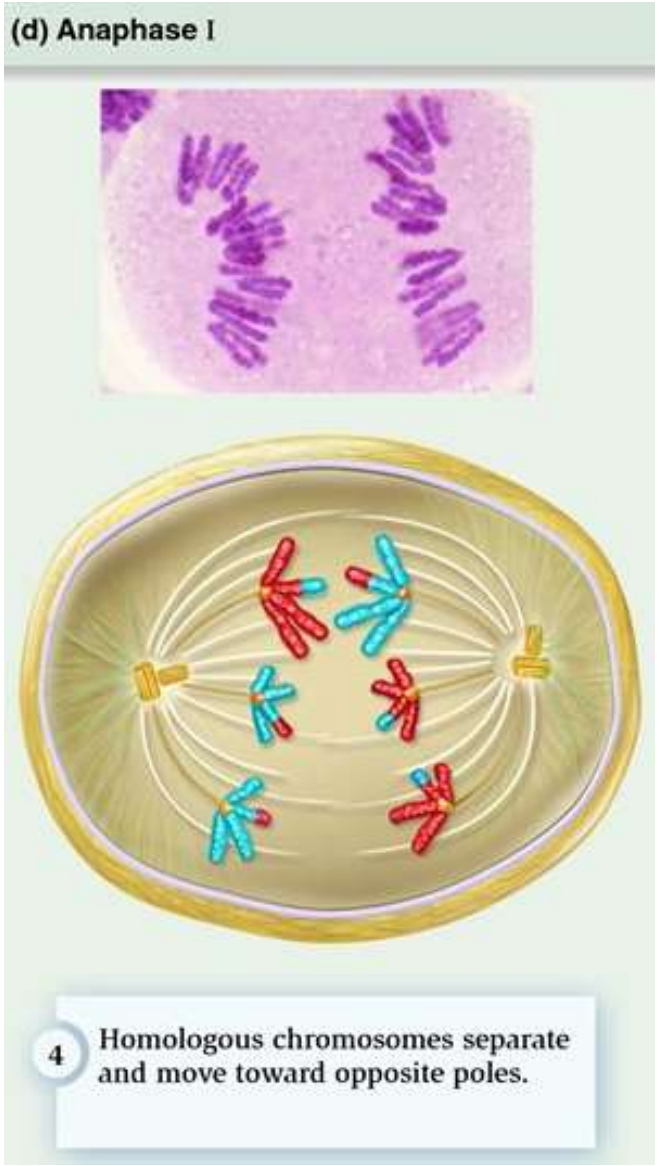
Meiosis I (Metaphase I)

- Bivalents align along the metaphase plate



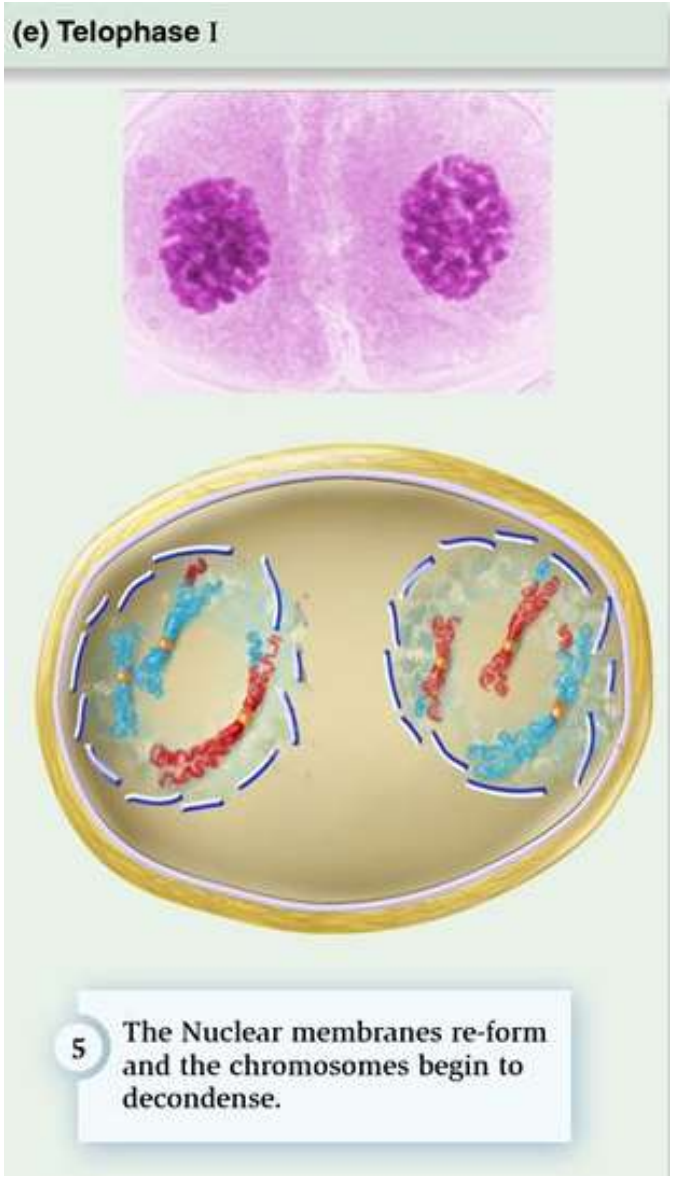
Meiosis I (Anaphase I)

- Homologous chromosomes separate and are pulled towards poles



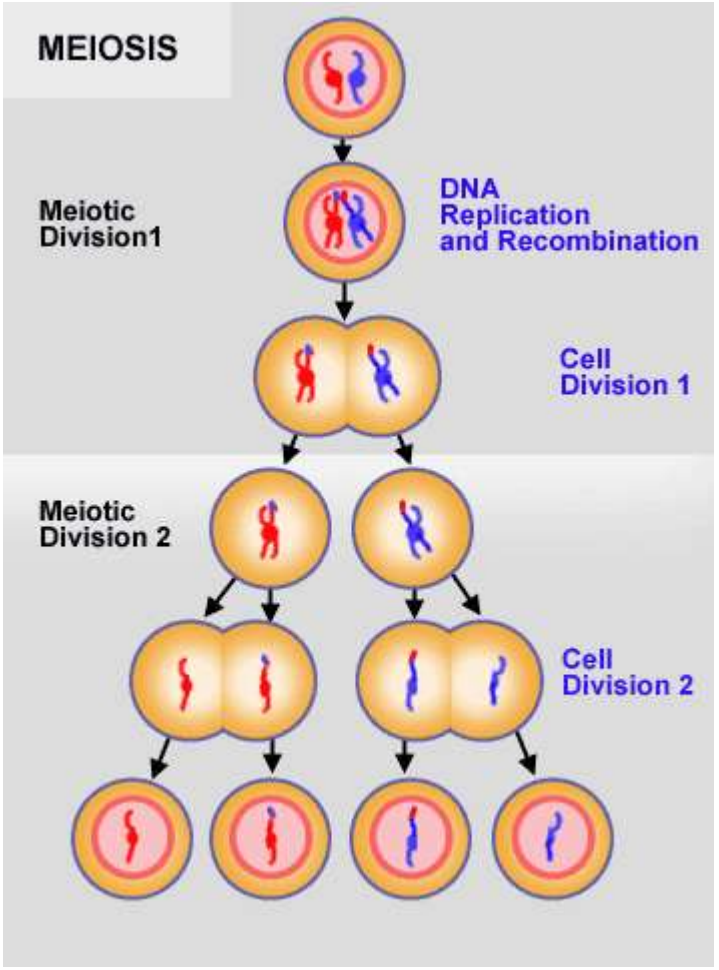
Meiosis I (Telophase I)

- Nuclear membranes reforms
- Chromosomes decondense
- Cytokinesis occurs and results in two cells



Meiosis II

- Just like mitosis but it uses the chromosomes that were created in the first meiotic division
  - No further DNA replication
- Also results in haploid (1n) sex cells called gametes
  - Mitosis results in the formation of two diploid cells



Example Question

The formation of the synaptonemal complex and crossover occurs in what phase of meiosis?

a) Interphase I
b) Prophase I
c) Metaphase I
d) Anaphase I
e) Telophase I

Chapter 15 – Inheritance and Phenotype Determination

Why Mendel?

- Gregor Mendel was the first to demonstrate the principles of inheritance.
- Systematic scheme based upon an understanding of mathematics and statistics
- He used excellent scientific method

Gregor Mendel

Blending inheritence

- Prior to Mendel people thought of inheritance like the concept of blending
  - Thought that characteristics added together like blending to form the new generation
- If this were true, after a bunch of generations, we would be identical

Mendel's Discoveries

- Mendel applied the scientific method and mathematical analysis to show:
- Unit characteristics (genes)
  - Segregation of those characteristics
  - Dominance of some characteristics
  - Independent assortment of characteristics

Mendel's Principles of Inheritence

- Segregation
  - Different genes go into the gametes
- Independent assortment
  - Genes assort independently of one another during meiosis
- Dominance
  - If a certain type of gene is present it dominates the other one

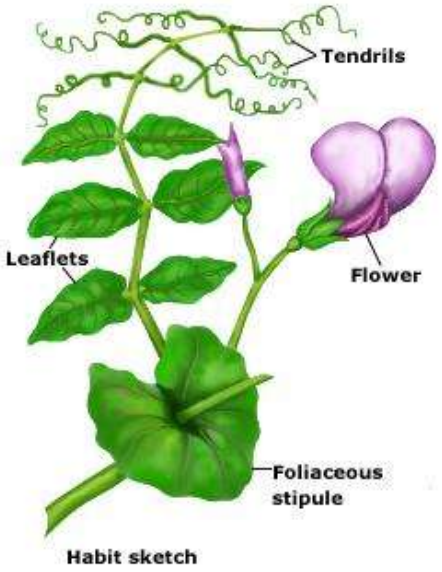
Example Question

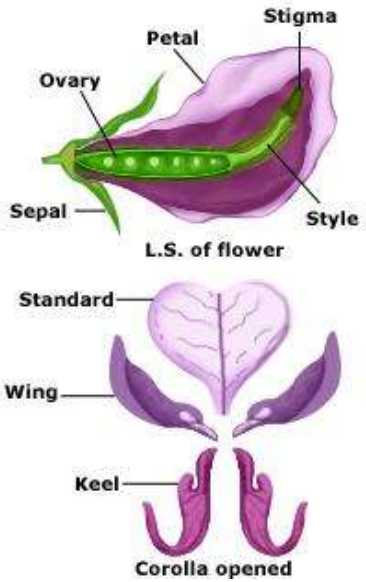
Mendel's principle of segregation states…

a) Different genes go into different gametes
b) Genes assort independently of one another during meiosis
c) Certain genes dominate others
d) Characteristics blend together in the progeny

Mendel's Pea Plants

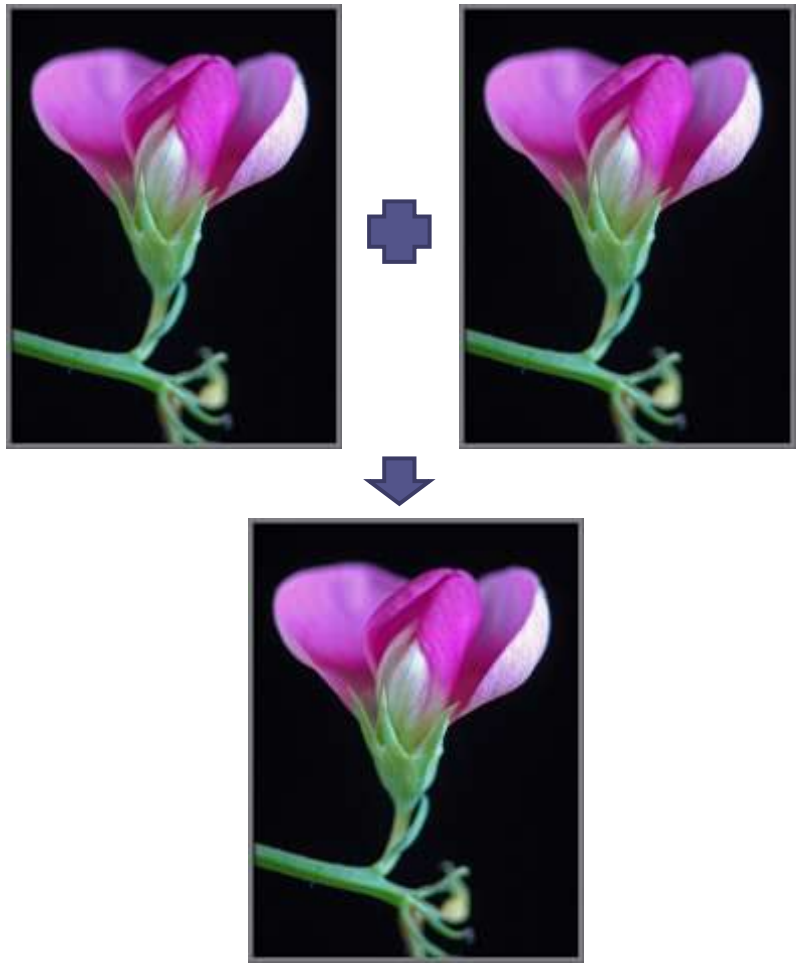
- Worked with the garden pea *Pisum sativum*
- Great model organism
  - Lots of progeny (very productive)
  - Short life cycle
  - Easily cross pollinated
    - Although typically self pollinating
    - Easy to go into the flower and snip off the anthers (male parts)
- It also has distinct phenotypic characteristics
  - Colour and shape of seeds
  - Colour of pods
  - Height





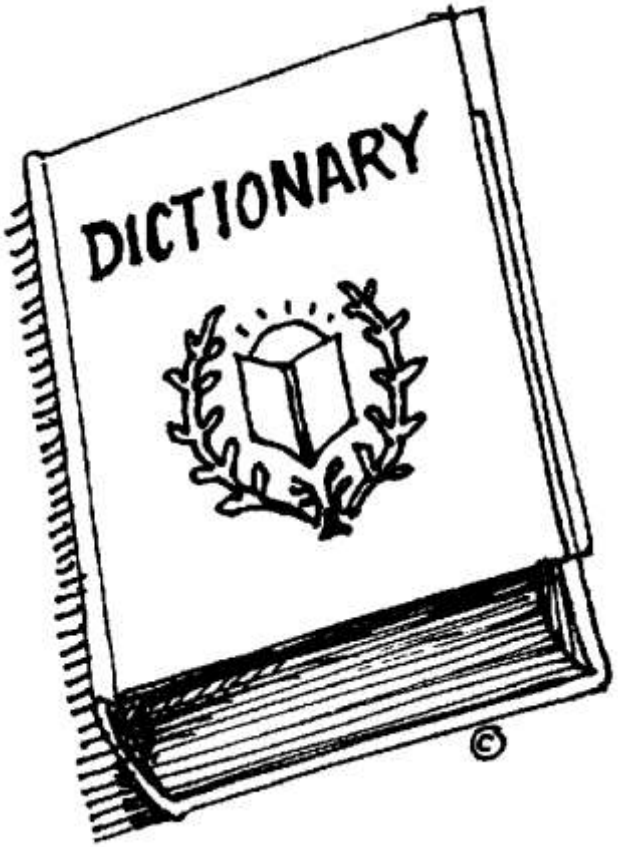
Obtaining a True Breeding Stock

- Mendel needed stocks of plants that bread in a reliable manner
  - Developed true breeding stock
    - These are plants that produce progeny that are just like the parents in regards to characteristics (so tall plants always produce more tall plants)
- True breeding stocks were developed by inbreeding for several years
  - Inbreeding is when you breed the parent to progeny or sibling to sibling
- In other words parental type crossed with parental type creates parental type



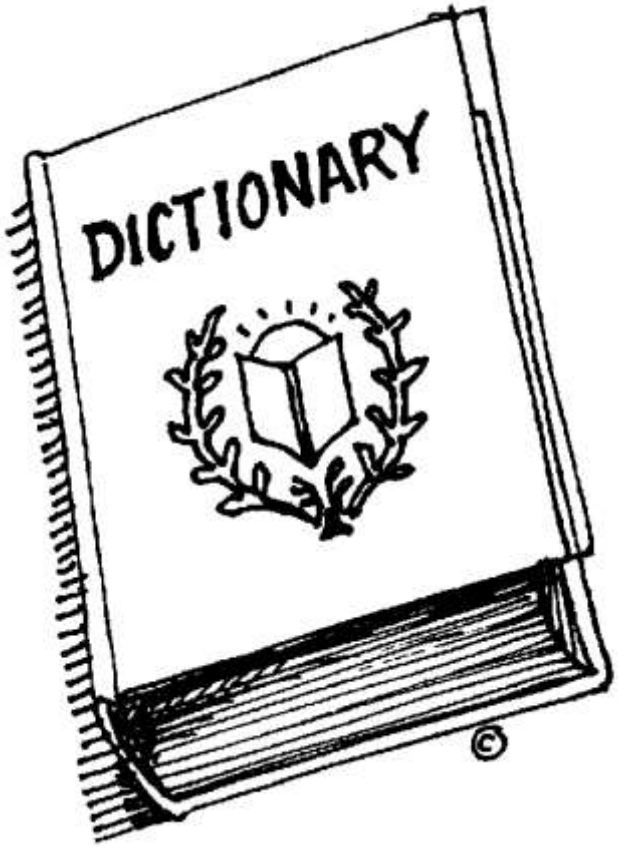
Crossing (breeding) definitions

- P is the parental generation of true breeders
- F_1 is the 1st filial generation
  - The progeny of crossing true-breeding P generation individuals that had varied in one character
- F_2 represents the second filial generation
  - Derived from crossing the F_1 generation with itself



Crossing (breeding) definitions

- Phenotype is a description of characteristics
- Genotype is the organisms genetic makeup
- Alleles are different varieties of genes
- Dominant alleles are shown with capital letters
  - T is dominant over t
- Recessive alleles are lower case letters
  - t is recessive to T
- Homozygous and heterozygous
  - Homozygous means the same alleles (eg. TT or tt)
  - Heterozygous means different alleles (eg. Tt)



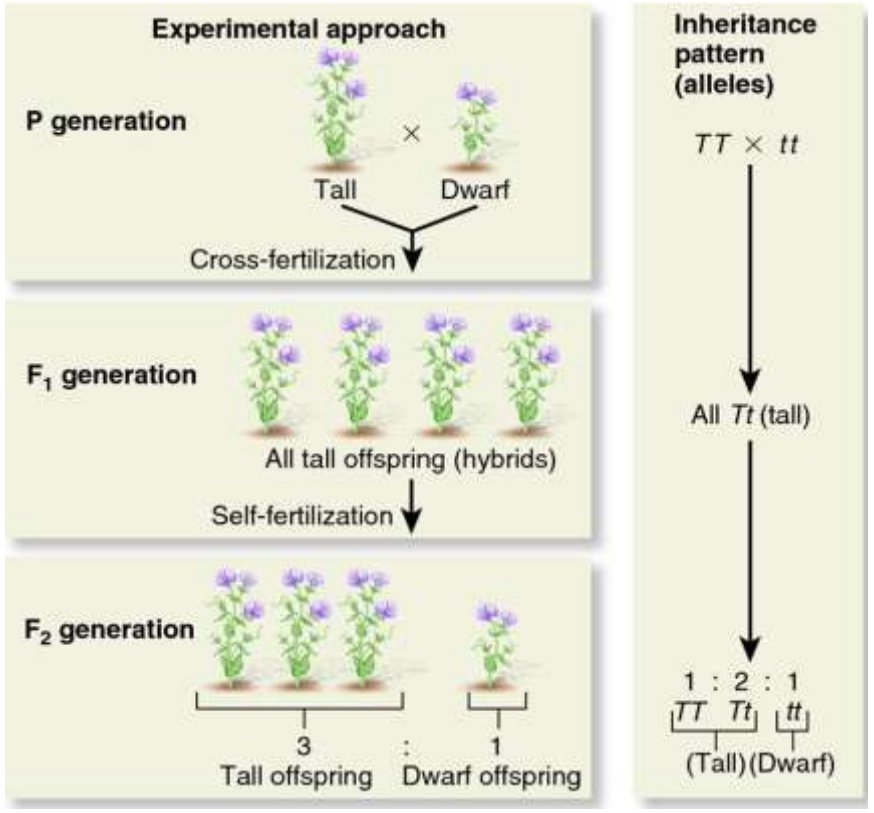
Example Question

The genotype TT would be _____ and _____.

a) Heterozygous, recessive
b) Heterozygous, dominant
c) Homozygous, recessive
d) Homozygous, dominant

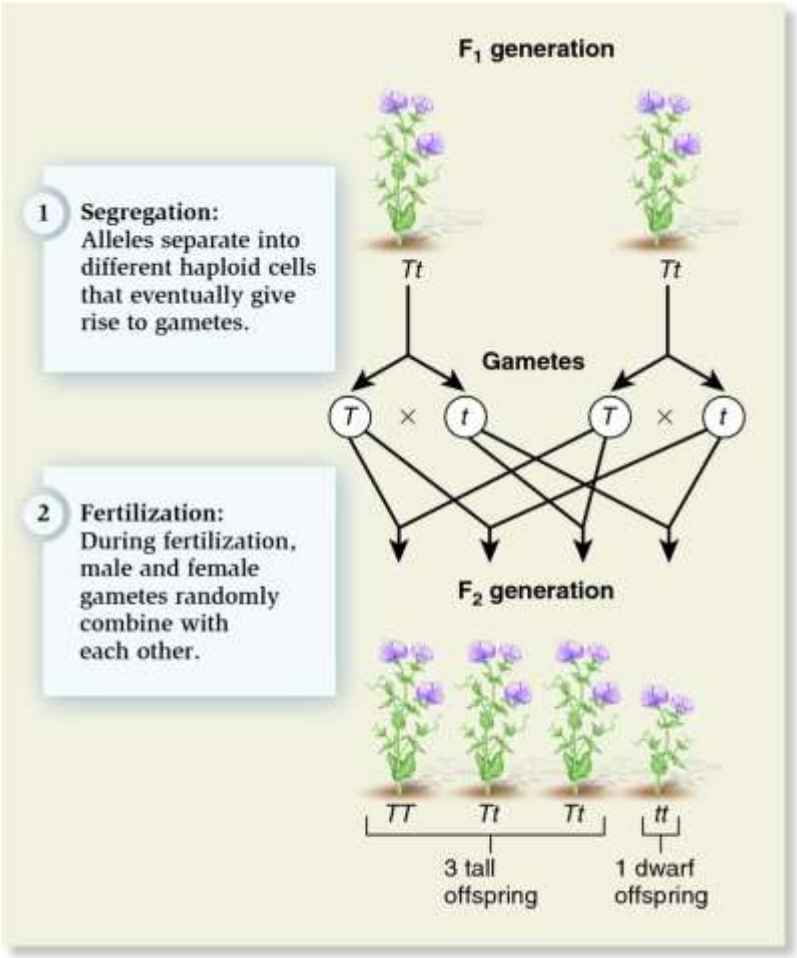
Mendel's Cross

- In the F_1 he crossed true tall (TT) with true short (tt) and obtained all tall (Tt) progeny.
  - Because they all had a tall phenotype, it can be determined that the T (tall) genotype is dominant.
  - The tall genes overrided the short therefore that gene must be dominant
  - This is evidence that blending doesn't occur
    - Would've been medium



Mendel's Cross

- Self crossing the F_1, the F_2 were 3:1 tall to short
  - This demonstrated that the F_1 did not breed true (did not create ONLY other tall plants)
- From the P generation to the F_2 the parental phenotype disappeared and then reappeared
  - This again disproves blending
- He then introduced the concept of unit characteristics
  - Genes as we now know them
- Unit characteristics separated to create the F_1
- Posited that the true breeding unit characteristics from the F_2 randomly combined to create the F_1



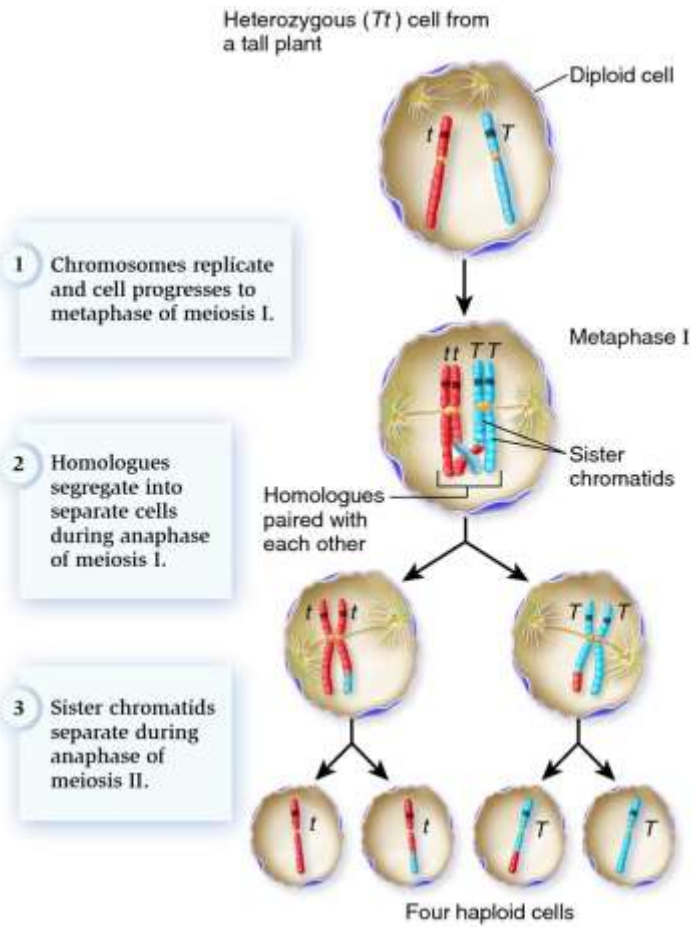
Example Question

If blending occurred in Mendel's original pea cross…

a) The F1 generation would have been the average height of the P generation
b) The F2 generation would have been half the size of the P generation
c) The F2 generation would have been all the same size
d) A and B
e) B and C
f) C and D

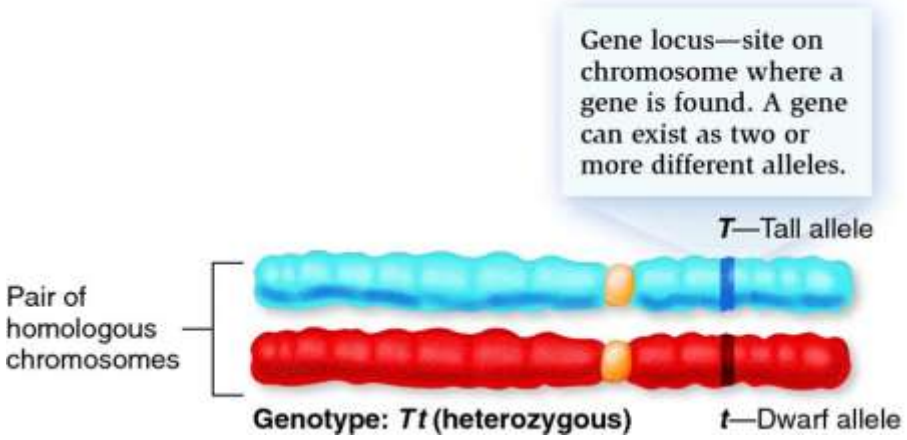
Chromosomal Basis of Allele Segregation

- Allele segregation means that characteristics must separate into the gametes and are seen in the subsequent generation
- Segregation occurs in meiosis
  - Both in meiosis I and meiosis II
    - Each sister chromatid in all cells produced is different
- Ova and sperm give different homolgous chromosomes to progeny



More on Alleles and Genomes

- The collection of genes in an organism is the genotype or genome
- Genes are positioned at different loci (singular = locus)
  - This is the physical location of a gene on a chromosome
- Alleles are varieties of genes
- In a 2n organism, there are two possible alleles for each gene locus
  - If those genes are the same the organism is homozygous at that locus
  - If different – heterozygous



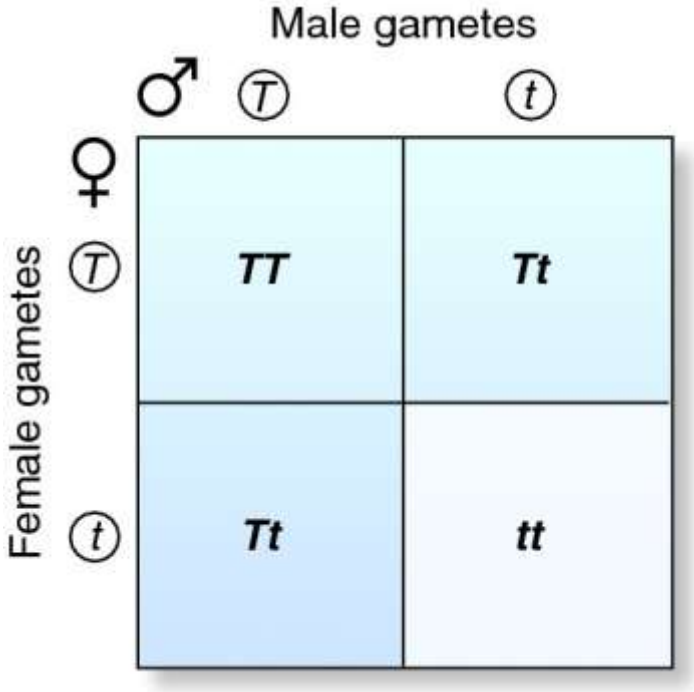
Example Question

Alleles differ at....

a) Chiasmata
b) Promotors
c) Loci
d) Operators

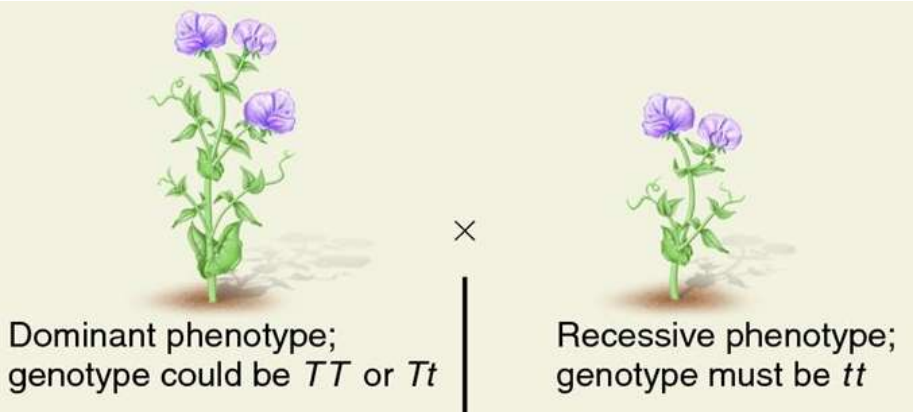
Punnett Squares

- Punnett Squares predict the genotypes and phenotypes of the next generation
- The allelic composition of the next generation (progeny) is obtained by adding the alleles of the two parents together



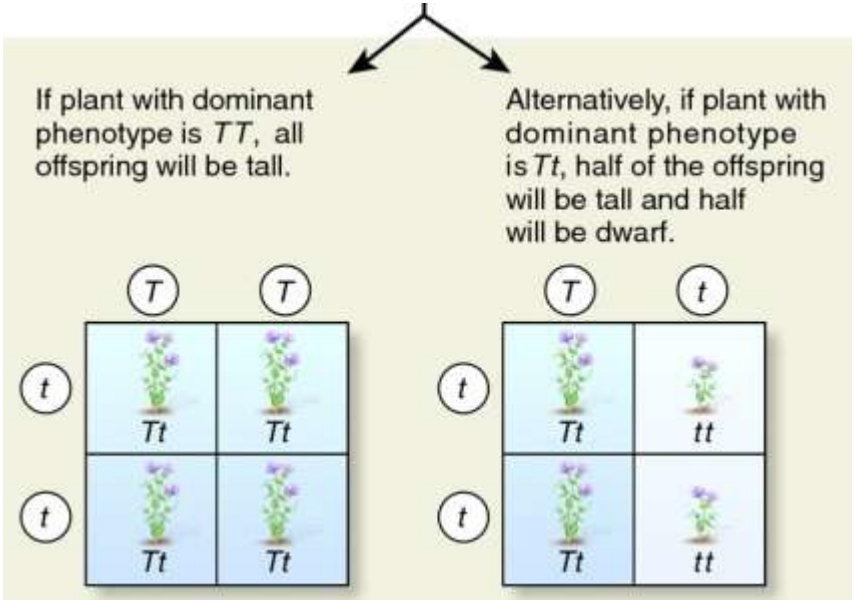
Testcross

- Tests if the dominant phenotype is homozygous dominant (TT) or heterozygous dominant (Tt)
- This is when you cross the plant with the unknown genotype (but dominant phenotype) with a plant with a recessive phenotype
  - Recessive phenotype must be homozygous recessive (tt)
- Depending on the phenotypic ratio of the progeny you can then determine what the parental genotype must have been



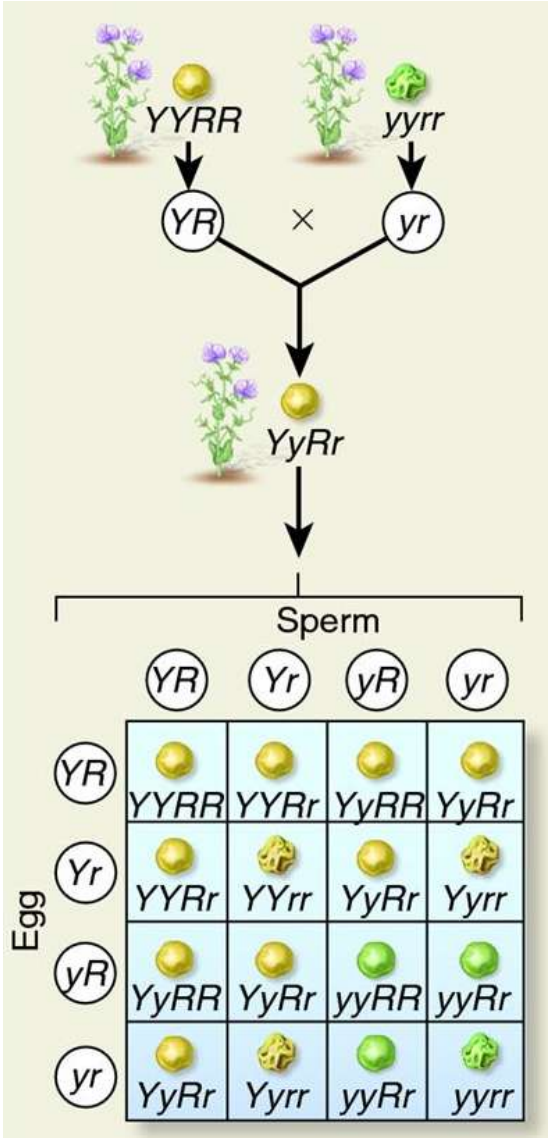
Testcross

- If all phenotypically tall then the parent must have been homozygous dominant
- If half are tall and half short than the parent must have been heterozygous dominant



Two Factor Cross

- When you are looking at more than one trait
- Must take into account independent assortment!
  - Each gene assorts into the gametes independently of each other (not physically connected to each other)
- Also called dihybrid cross



The Product Rule

- The probability that two or more independent events (phenotype of one does not affect phenotypes of others) will occur equals the product of their individual probabilities
- For example if you toss a coin twice what is the probability that you will get heads both times
  - Using the product rule you multiple the probability of each individual coin toss
    - ½ x ½ = ¼
    - Probability of each coin toss being heads is ½

$$P = \frac{\text{number of times an event occurs}}{\text{total number of possible outcomes}}$$

$$P_{\text{heads}} = \frac{1 \text{ heads}}{(1 \text{ heads} + 1 \text{ tails})} = \frac{1}{2}$$

The Sum Rule

- The probability that 1 of 2 or more mutually exclusive outcomes ("either/or" question) will occur is the sum of the probabilities of the possible outcomes
- In a cross between two heterozygous (Tt) pea plants, we may want to know the probability of a particular offspring being a homozygote
  - ¼ of the offspring will be homozygous dominant (TT) and ¼ will be homozygous recessive (Punnett Square)
  - So ¼ + ¼ = ½ , half the offspring will be homozygotes (TT or tt)

$$P = \frac{\text{number of times an event occurs}}{\text{total number of possible outcomes}}$$

$$\frac{1}{4} + \frac{1}{4} = \frac{1}{2}$$

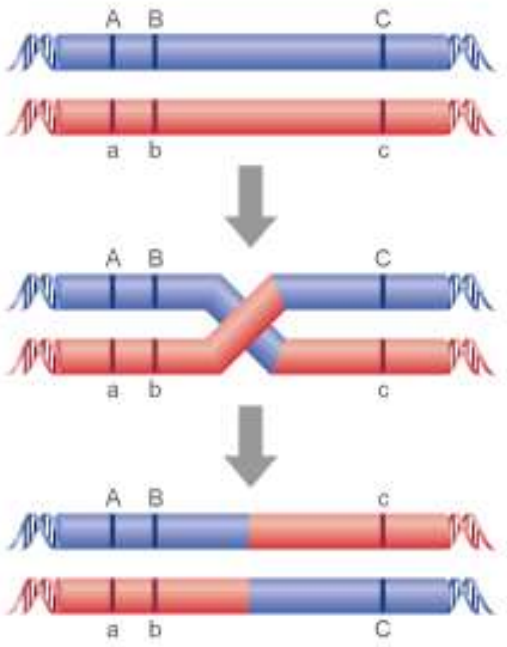
Example Question

In a cross between a heterozygous pea plant (Tt) and a homozygous recessive pea plant (tt) what is the probability of the first 3 progeny being tall?

a) 1/6
b) ½
c) 1/9
d) 1/12

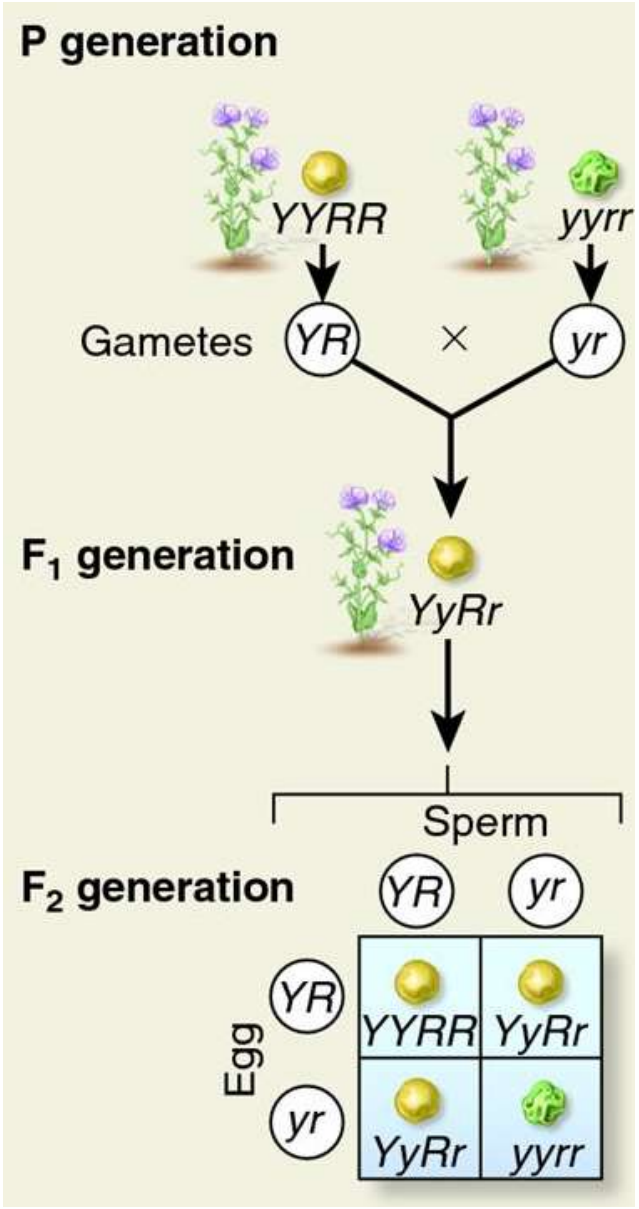
Linkage

- Genes can be linked
  - Occurs when they are physically very close to one another on the DNA strand
- When crossover occurs during meiosis, it is unlikely that the genes become separated

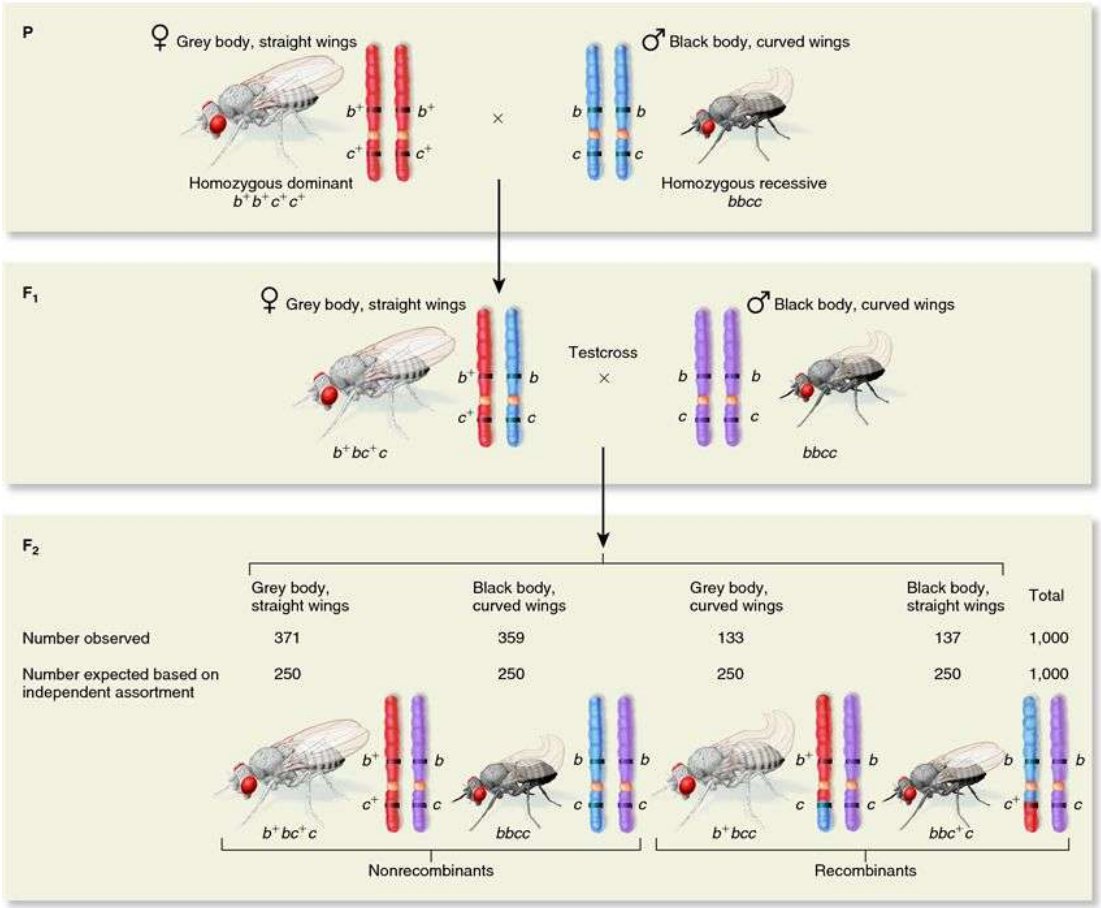


Linkage

- This would mean that they would not follow independent assortment
  - Interferes with Mendelian frequencies
  - Linked genes are therefore easily detected
    - If the frequency distribution of genes in the progeny of a dihybrid cross is not Mendelian then the genes are linked

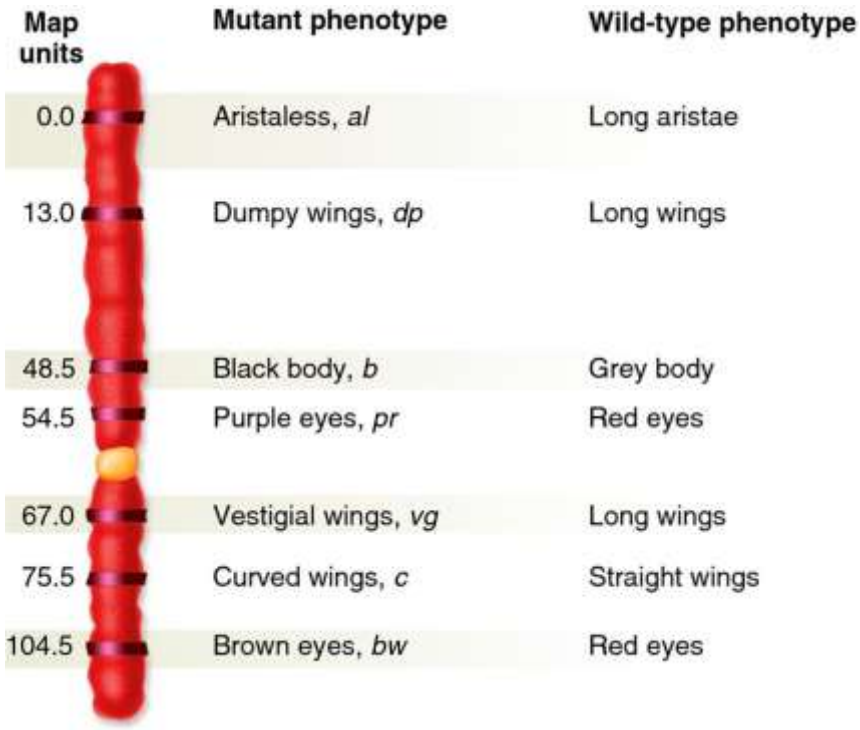


Linkage Example



Crossover determines the relative location of genetic loci

- Crossover is assumed at equal frequency for each loci along a chromosome
- The number of crossovers is proportional to the distance between 2 loci
  - The further apart two loci are, the larger number of crossovers occur between them
  - The closer they are the smaller, this can lead to linkage
- Distances between loci are related to the rate of crossover
  - One percent cross over = one map unit



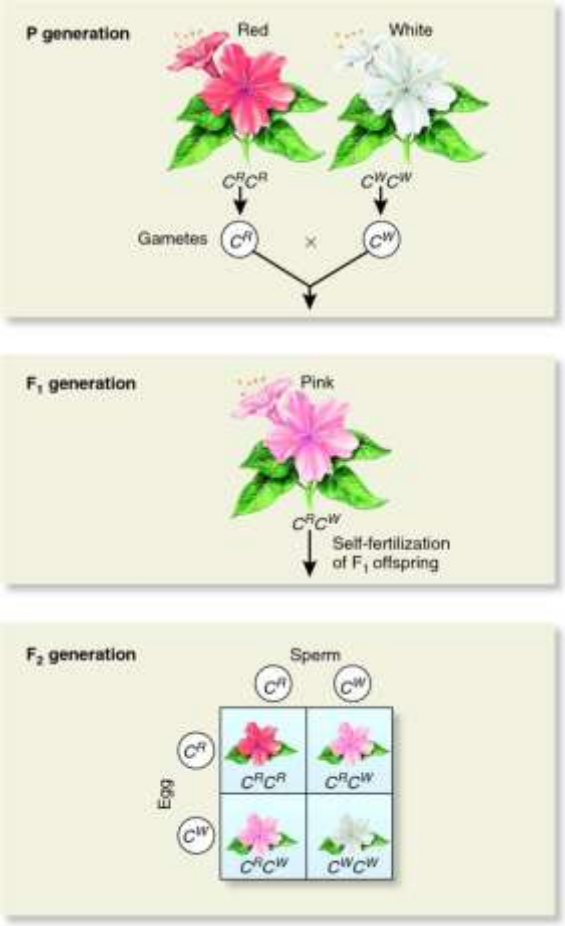
Example Question

Which of the following is false?

a) Linkage is likely when genes are 1 map unit apart
b) Linkage interferes with Mendelian genetics
c) Linkage occurs during crossover
d) Linkage follows independent assortment

Incomplete Dominance

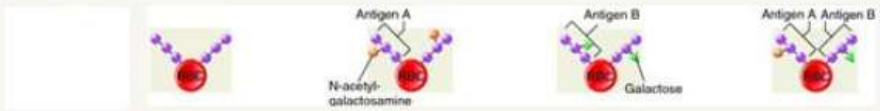
- Incomplete dominance is when the heterozygote has a 3rd, intermediate phenotype
  - So in our T/t example, Tt genotype would not produce a tall phenotype
  - Rather it would produce a medium phenotype
- Occurs when neither allele is dominant



Multiple Alleles

- 3 or more variants in a population
- Phenotype depends on which 2 alleles are inherited
- An example is ABO blood types in humans
  - Blood type determines what antigens are expressed on the outside of your blood cells
  - Type AB is codominant (both alleles are expressed equally, you express both antigens equally)

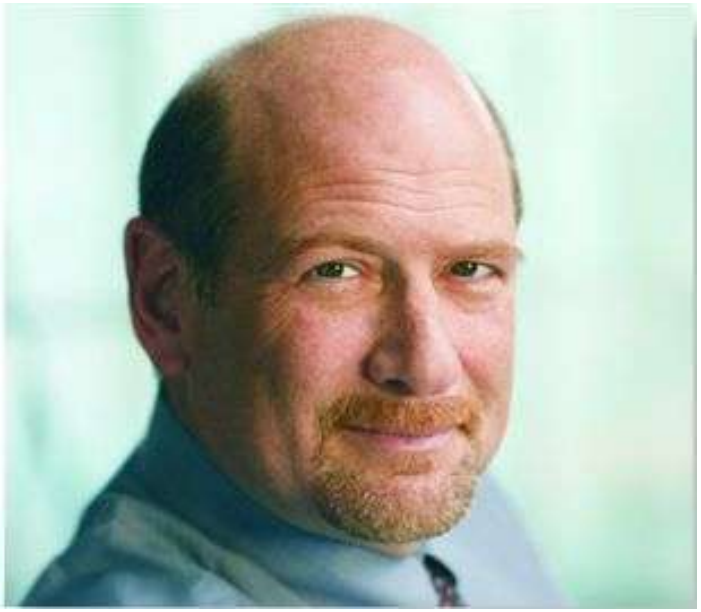
The ABO Blood Group



Blood type	O	A	B	AB
Genotype	ii	I^AI^A or I^Ai	I^BI^B or I^Bi	I^AI^B
Surface antigen	Neither A nor B	A	B	A and B
Serum antibodies	Against A and B	Against B	Against A	None

Sex influenced inheritance

- Occurs when an allele is dominant in one sex but recessive in the other
- Increase in 5 alpha-dihydrotestosterone (by 5 alpha-reductase)
- Pattern baldness
  - Baldness allele dominant in men but not in women
  - Only a woman homozygous for baldness allele would be bald (or an adrenal gland tumor causing high testosterone in females)
- NOT X LINKED
- Baldness gene is on an autosomal chromosome



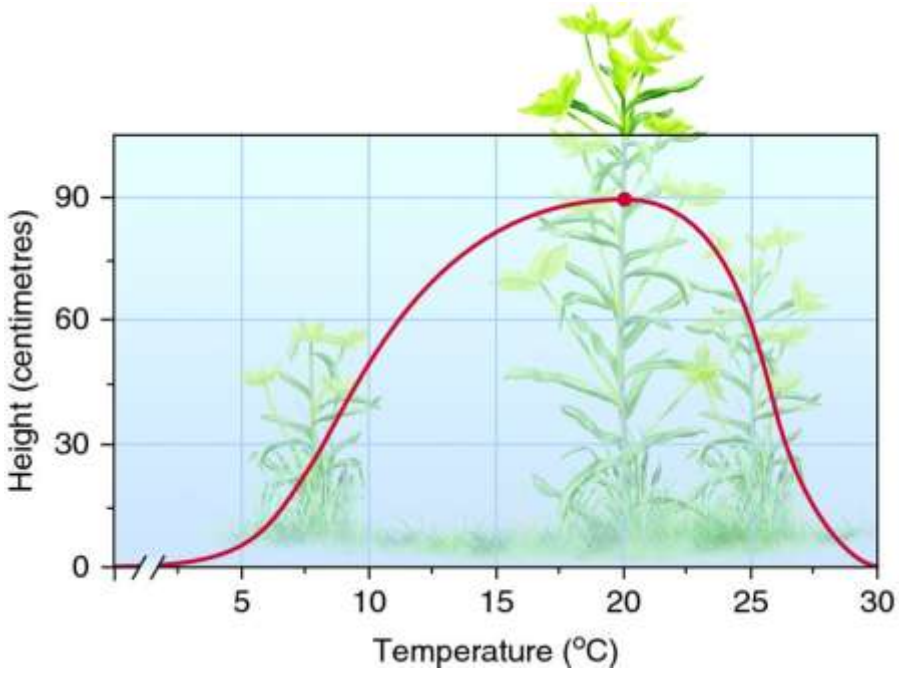
Example Question

Which of the following adheres to Mendel's Principles of Inheritance?

a) Incomplete dominance
b) Human blood type
c) Male pattern baldness
d) All of the above
e) None of the above

Role of Environment

- "Norm of reaction"
  - Effects of environmental variation on a phenotype
- An example is genetically identical plants that grow to different heights in different temperatures

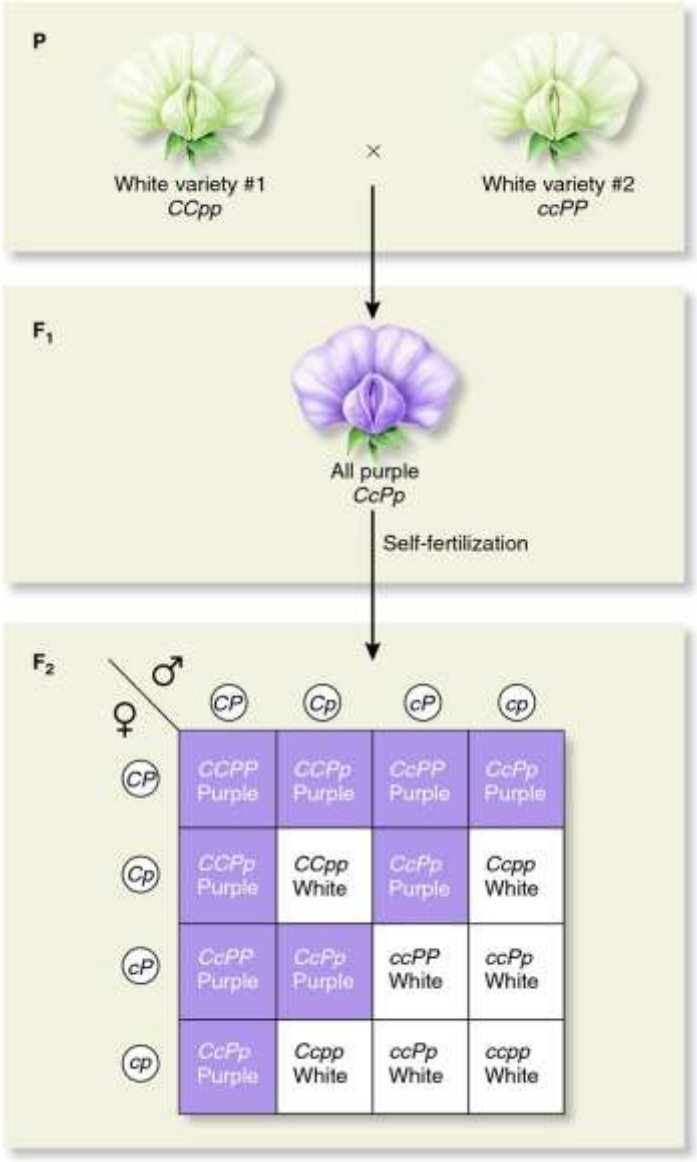


Gene interactions

- Sometimes a single trait is controlled by 2 or more genes, each of which has 2 or more alleles
  - Epistatsis
  - Polygenic inheritance

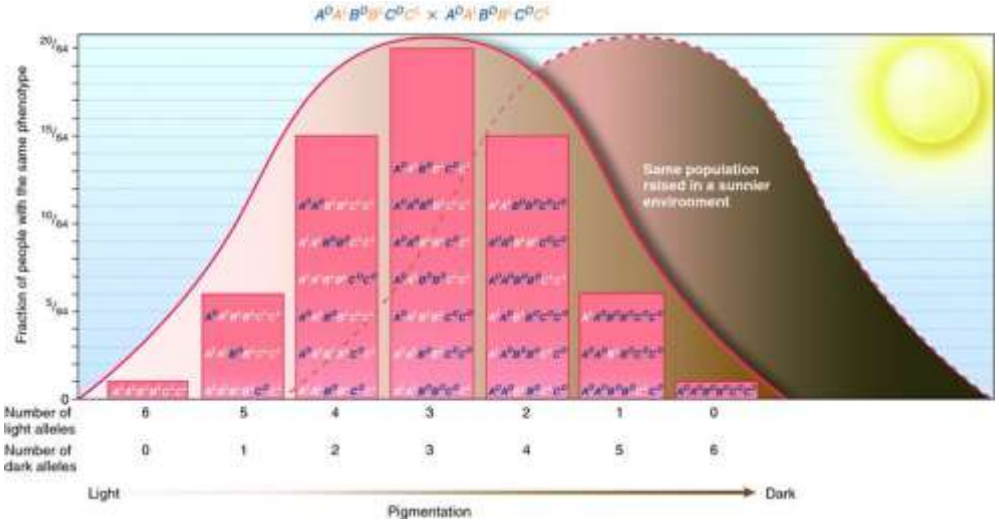
Epistasis

- Alleles of one gene mask the expression of the alleles of another
- Often arises because 2 or more different proteins are involved in a single cellular function
- In this example, the dominant form of C and P are need to create the purple flower



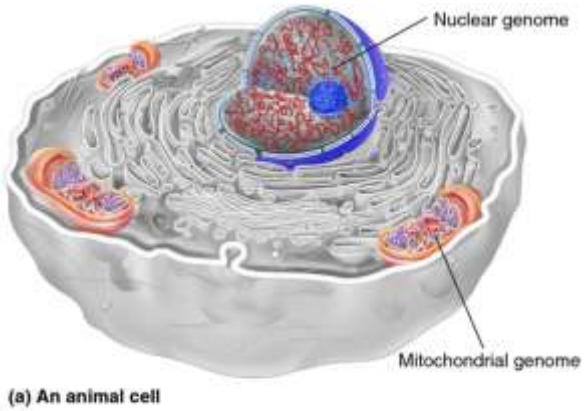
Polygenic inheritance

- In humans, skin colour is controlled by 3 unlinked loci
  - All three loci effect the phenotype
  - Different genotypes produce a wide range of phenotypes
- Follows expected frequency distribution for a Mendelian trait
- Height is also polygenic (in a normal population)

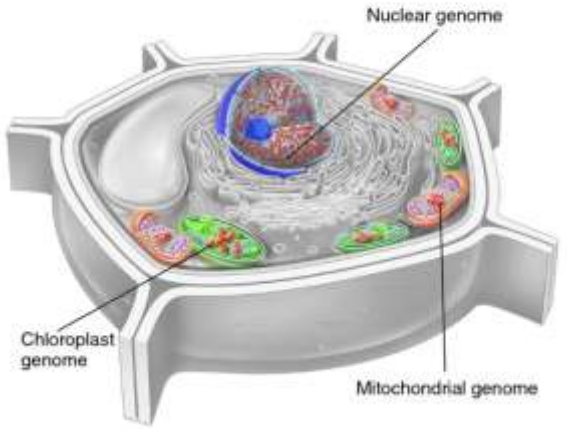


Extranuclear inheritance

- Organelles (mitochondria and chloroplasts) contain their own genomes and can impart different phenotypes
  - Often differences in these genotypes result in diseases
  - This is because they affect photosynthesis or respiration



(a) An animal cell



(b) A plant cell

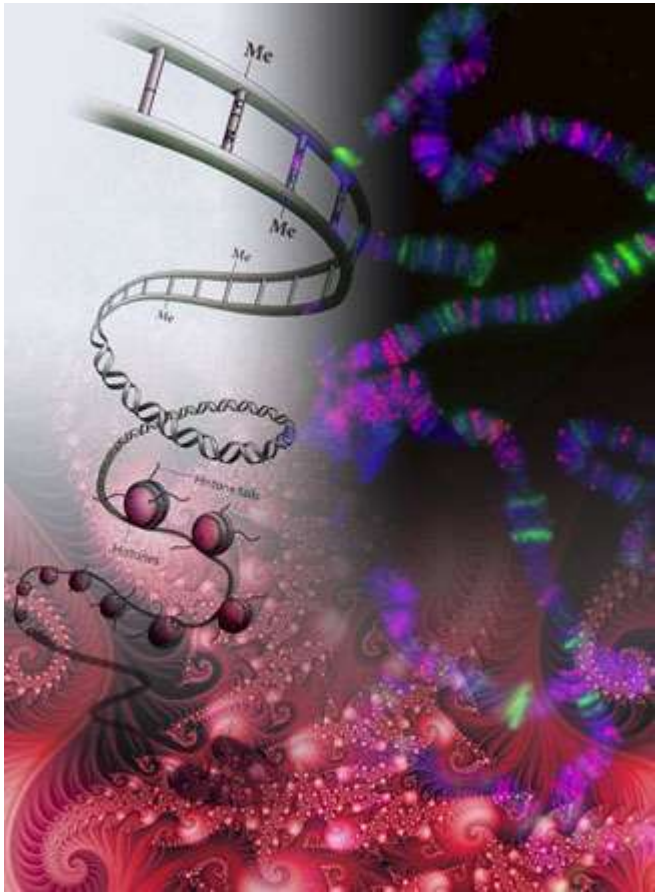
Example Question

If the alleles of one gene mask the expression of the alleles of another what is this an example of?

a) Polygenic inheritance
b) Extranuclear inheritance
c) Monogenic inheritance
d) Epistasis

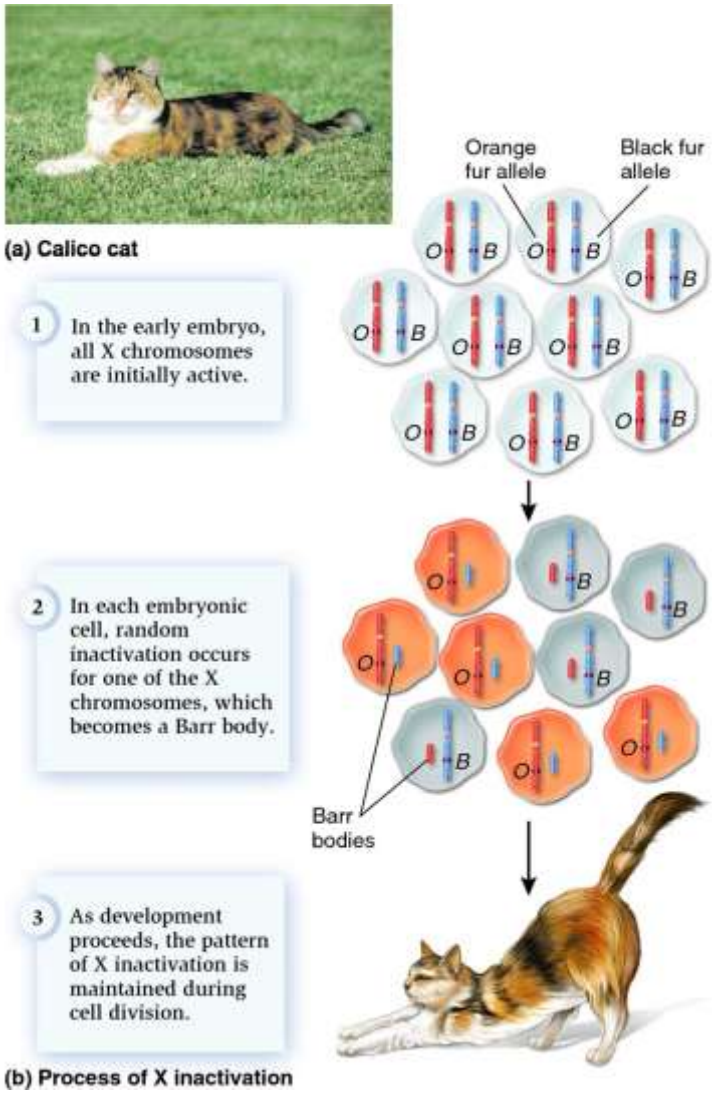
Epigenetic inheritance

- Modification of either a gene or chromosome during egg/sperm formation or the early stages of embryo growth
  - Alters gene expression in a way that is fixed during an individual's lifetime
- It permanently affects the phenotype of the individual, but the effect is not permanent over the course of many generations
  - This is because it doesn't actually change the DNA sequence
- X inactivation and Genomic imprinting



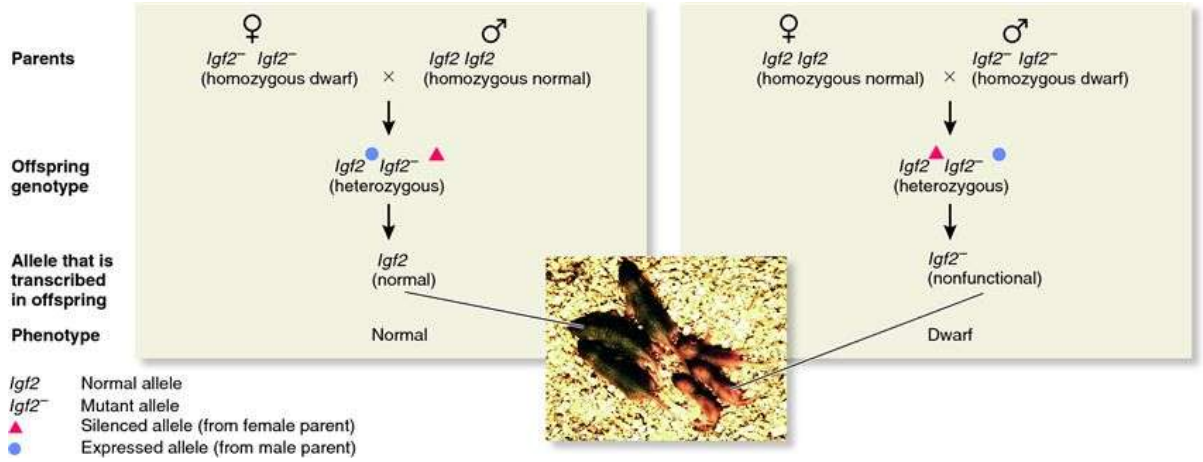
X inactivation

- One X chromosome in the somatic cells of female mammals is inactivated
  - If you have both of them functional there are too many gene products
- Lyon discovered it using 2 lines of evidence
  - Barr bodies are found in female but not male cat cells
    - Barr bodies are the closed conformation of the X chromosome
  - Calico cat coat colour pattern



Genomic Imprinting

- Segment of DNA is imprinted via methylation
- Imprinting can occur to single genes, parts of chromosomes, entire chromosomes, or all the chromosomes from one parent
  - Offspring distinguishes between maternally and paternally inherited chromosomes (or genes, parts of chromosomes, etc.)
  - Offspring express either the maternal or paternal allele, but not both



Example Question

In genomic imprinting....

a) The X chromosome is inactivate as a Barr body
b) Only maternal genes are expressed
c) Only paternal genes are expressed
d) Either paternal or maternal genes are expressed

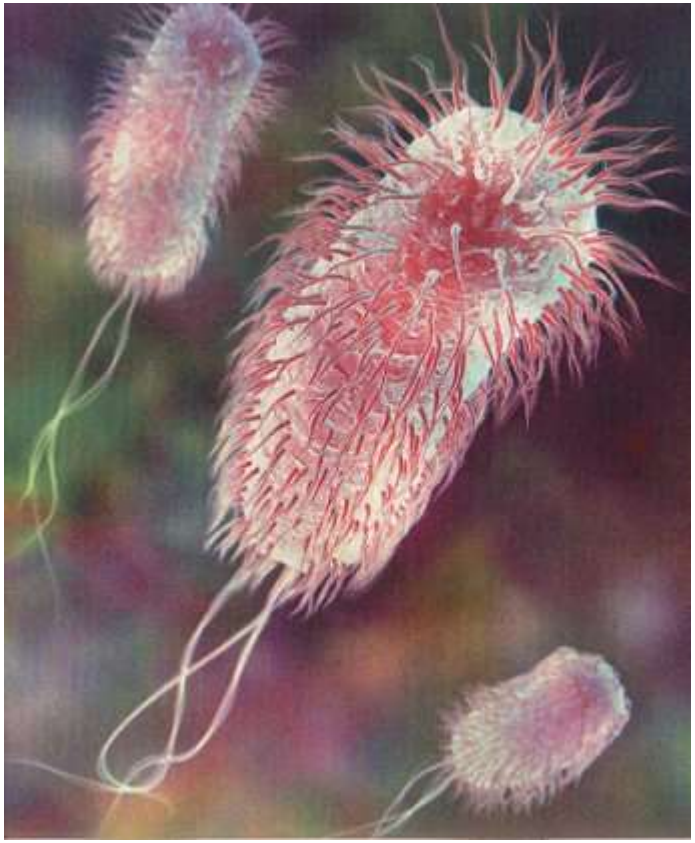
Chapter 18 – Genetic Technology

Revolution in the Biological Sciences

- Started in the mid 1970s with recombinant DNA technology
  - This is technology that redirects the genetic activity of organisms
- Techniques and approaches include
  - DNA cloning: isolating and amplifying specific DNA sequences, both *in vivo* (inside) and *in vitro* (out of) the cell
  - Genomics and Proteomics: analysis of entire genomes and proteomes of organisms
- Biotechnology: addition of new genes to create transgenic or genetically modified organisms

Restriction Enzymes

- Discovered by Hamilton Smith, Daniel Nathans, and Werner Arber
- Restriction enzymes are molecular scissors
- They are normally found in bacteria and function to destroy non-host (bacteriophage) DNA
  - A bacteriophage is a virus



Restriction Enzymes

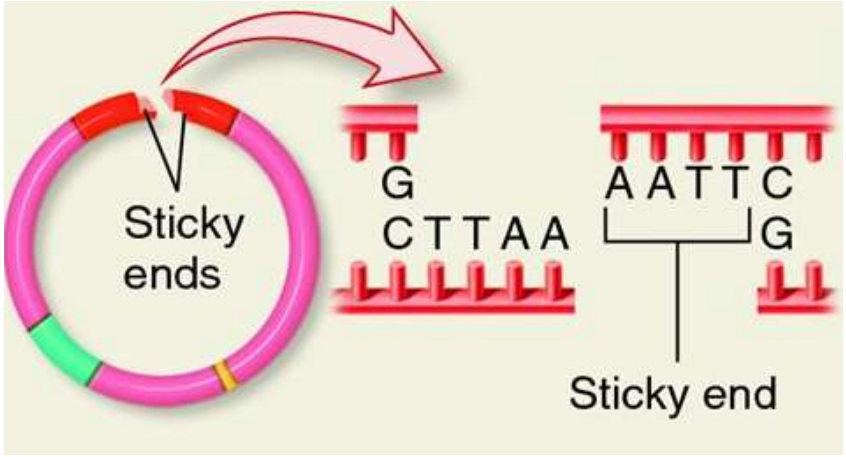
- What they do is cut DNA at specific base pair sequences
  - These sequences are usually palindromes
    - Palindromes are the same sequence forward and backwards (eg. ABBA or RACECAR)
    - So in DNA the sequence would read the same 5' to 3' as it would 3' to 5'

Examples of Restriction Enzymes Used in Gene Cloning

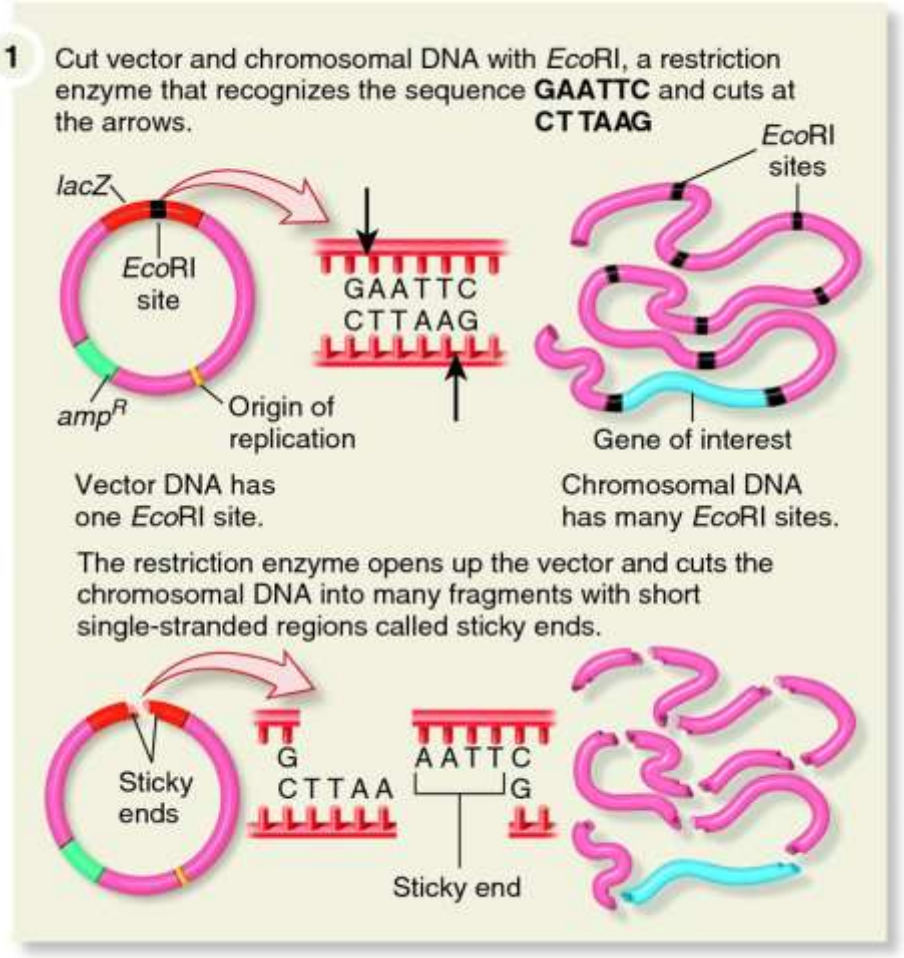
Restriction enzyme*	Bacterial source	Sequence recognized†
*Bam*HI	*Bacillus amyloliquefaciens* H	5′–GGATCC–3′ 3′–CCTAGG–5′
*Eco*RI	*Escherichia coli* RY13	5′–GAATTC–3′ 3′–CTTAAG–5′
*Sac*I	*Streptomyces achromonogenes*	5′–GAGCTC–3′ 3′–CTCGAG–5′

Restriction Enzymes

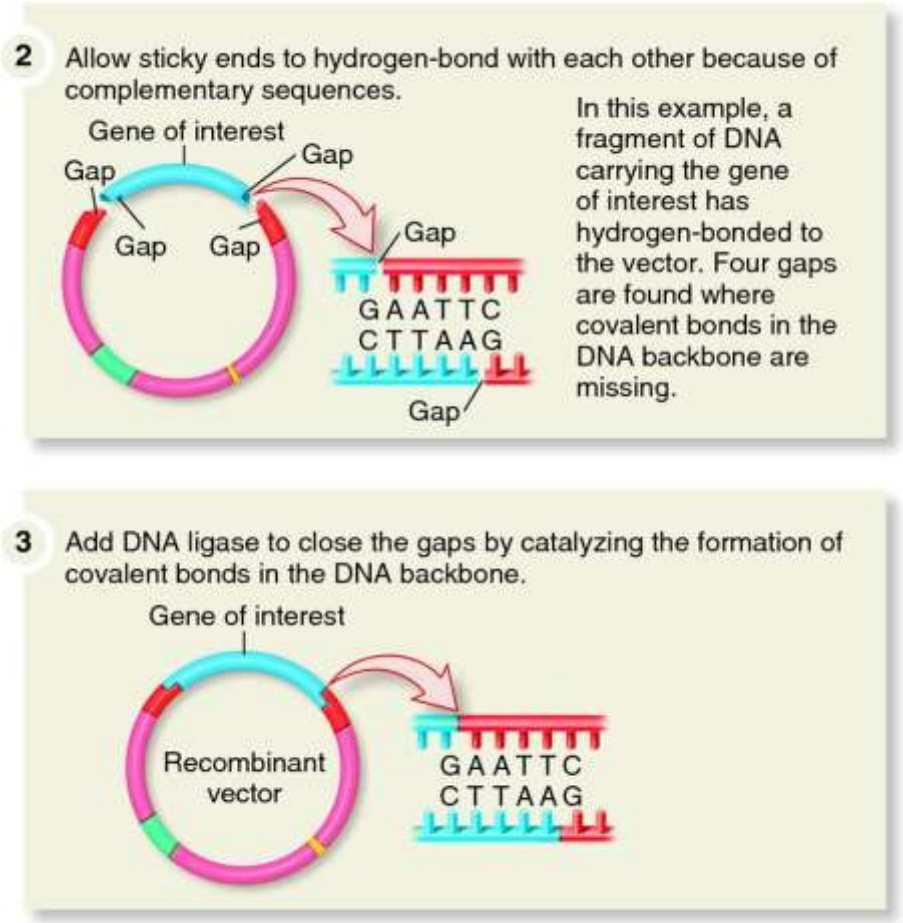
- Scientists purify them and use them to cut DNA at specific sequences so that they can then insert genes that they created into the DNA
- When they cut they leave sticky ends
  - These are the ends of the cut DNA
  - The restriction enzyme leaves little sequences of single stranded DNA when it cuts
    - Called 3' or 5' overhangs depending on what strand is single stranded



Restriction Enzymes



Restriction Enzymes



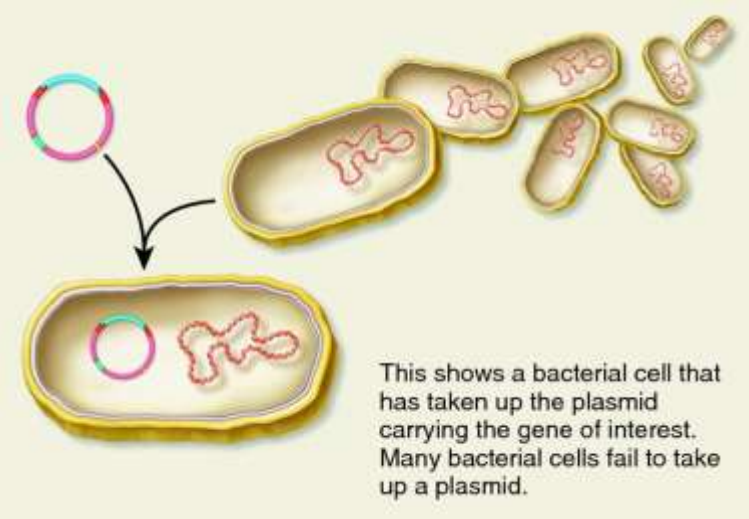
Example Question

Which of the following is false?

a) Restriction enzymes cut at palindromic sequences
b) Restriction enzymes leave sticky ends
c) Restriction enzymes were derived from viruses
d) Restriction enzymes can insert genes into vectors

Recombinant DNA technology

- Use of laboratory techniques to isolate and manipulate fragments of DNA
- Recombinant DNA contains DNA from 2 or more sources
  - Sources may both be from the same organism, just two different genes
- Once inside a host cell, recombinant molecules are replicated to produce identical copies or clones



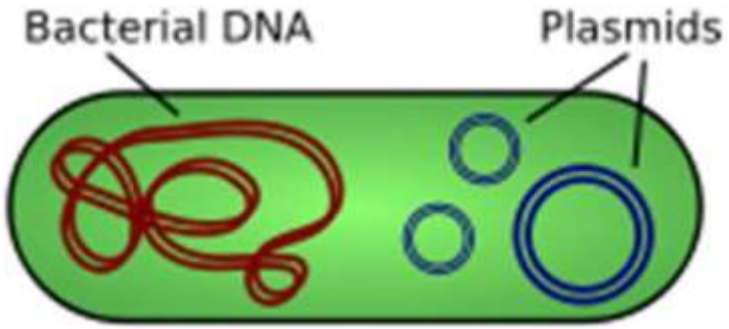
This shows a bacterial cell that has taken up the plasmid carrying the gene of interest. Many bacterial cells fail to take up a plasmid.

Why Create Gene Clones?

- Want copies of a gene for study or use
  - It creates large amounts of DNA, enough for DNA sequencing
  - It can also be used as a probe to ID the same or similar genes
- Obtain lots of gene product (mRNA or protein)
  - Creates large amounts of the protein so it can be studied
  - Can make pharmaceutical products
  - Can be introduced into plants to alter their characteristics
  - Can be used to treat diseases (gene therapy)

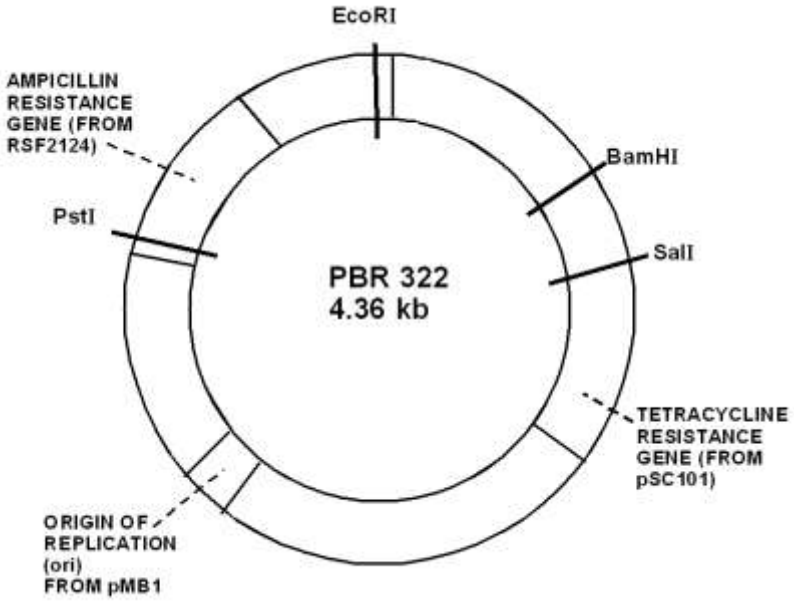
Gene Cloning

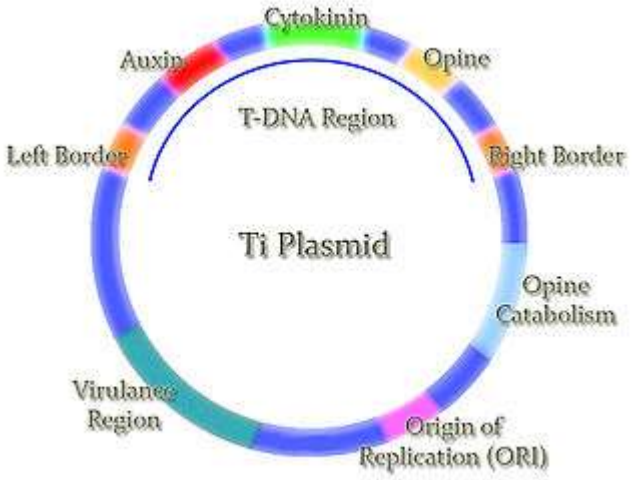
- Vector DNA is a carrier for the DNA segment to be cloned
- When a vector is introduced into a living cell, it can replicate making many copies
- Common vectors are plasmid or viral
  - Plasmids are circular DNA from bacteria
- Also need the gene of interest from chromosomal DNA digested with restriction enzymes



Plasmid Vectors

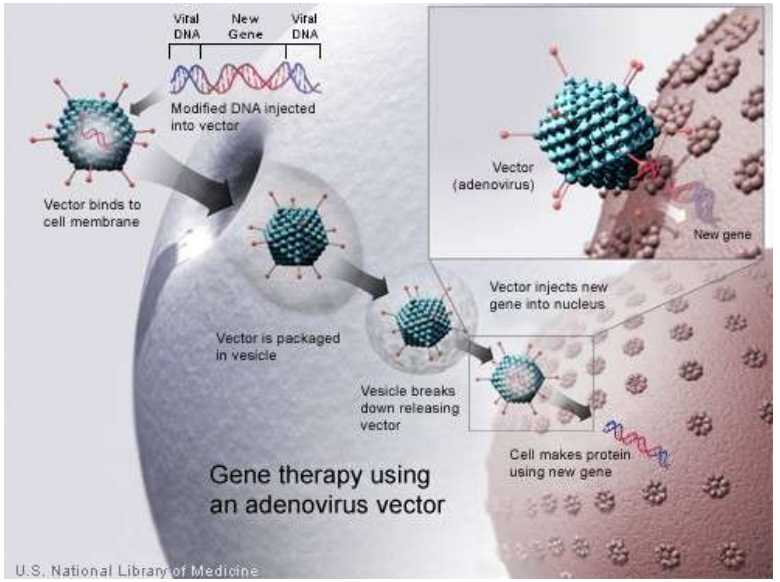
- A vector is a carrier that delivers foreign DNA into a host cell
  - Once it gets in there it becomes function (genes work)
- Bacteria and yeast
  - Plasmids are small rings of double stranded DNA
    - Since they are double stranded you can use restriction enzymes on them
- Plant cells
  - *Agrobacterium* Ti plasmid
    - Ti plasmid is very large
    - Has the ability to transfer DNA into plant genomes (only trans-kingdom gene transfer system known)
- Infection is called transformation





Viral Vectors

- Mammalian cells
  - Engineered mammalian viruses
    - Viruses deliver genes into the cells
  - When engineering a virus you take out the deleterious things and use it just as a delivery system for your gene
- Infection is called transfection

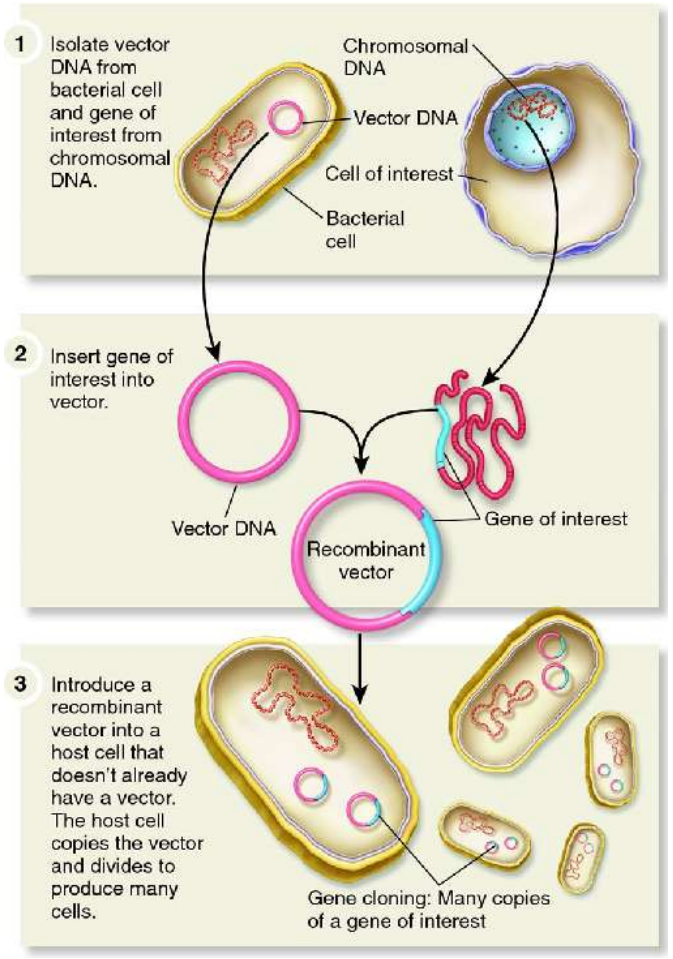


Example Question

Plasmid vectors originate from _____ and are _____ DNA.

a) Viruses, linear
b) Viruses, small rings of
c) Bacteria, linear
d) Bacteria, small rings of

Gene Cloning

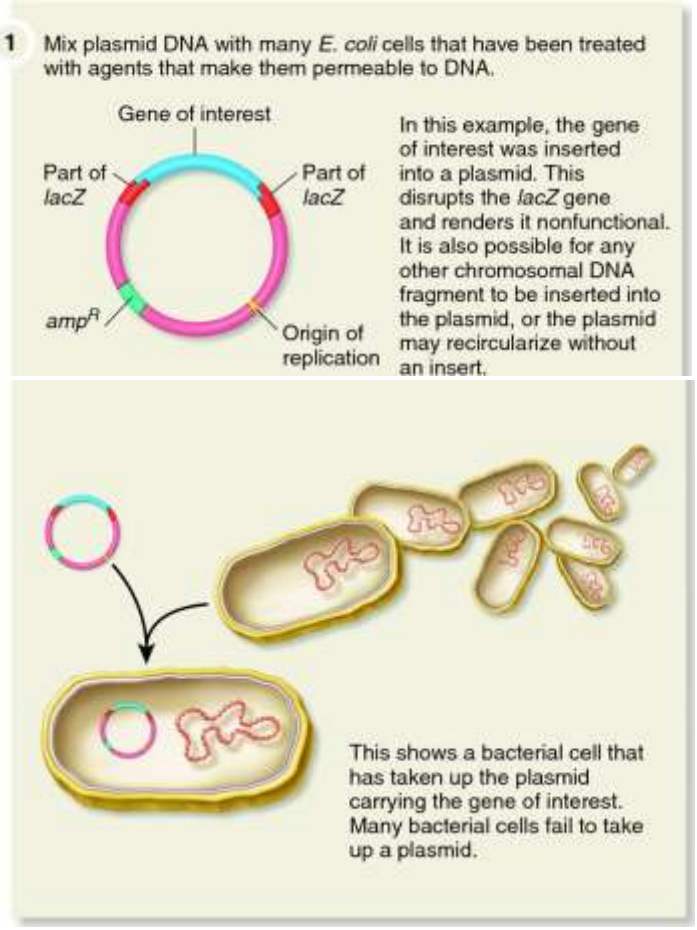


Gene Cloning

- The goal for the recombinant DNA is to be taken up by the bacteria
  - You have to treat the bacteria to make them receptive
    - Make the plasma membrane more permeable to large macromolecules like DNA
    - Electroporation is when you give the cells a short electric shock (also makes the membrane more permeable)

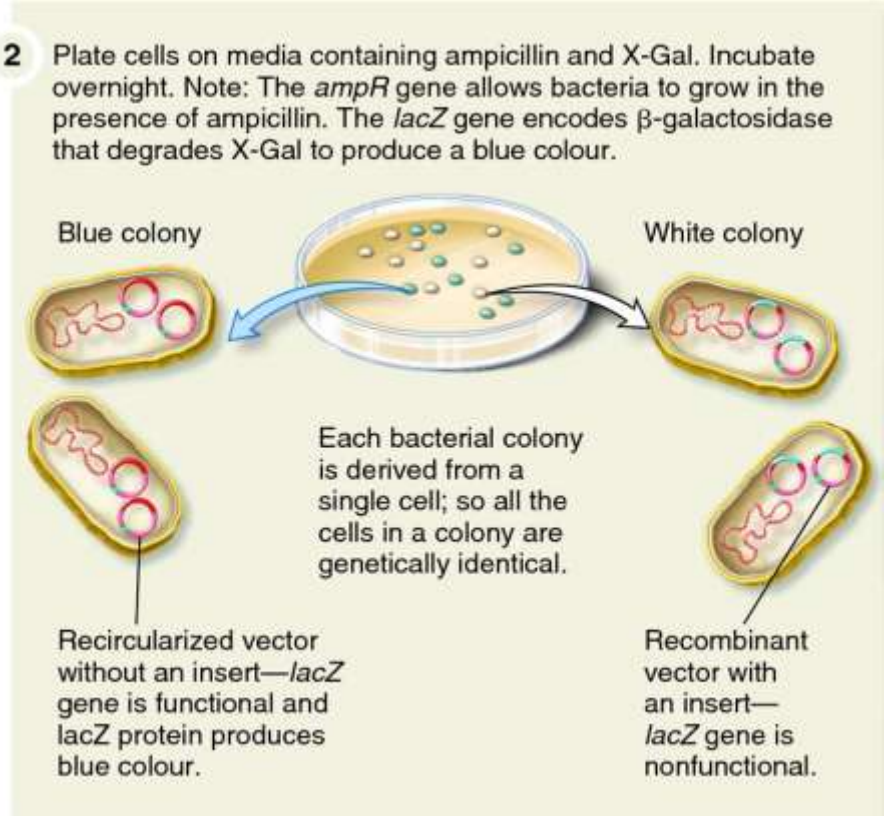
Uptake of the Recombinant DNA

- By inserting the DNA into an area of the chromosome that codes for the lacZ gene (a coding gene for beta-galactosidase) it can be seen if the DNA was taken in or not
  - If the lacZ gene is cut up, the cell can't break down galactose
- By adding X-Gal to the plate, the ones that form blue colonies are the ones coding for beta-galactosidase (showing that they didn't take up the DNA), and the white colonies broke up the lacZ gene (by adding the new DNA)



Uptake of the Recombinant DNA

- Once the DNA is in the bacteria, only the "infected" ones will grow in cultures containing ampicillin
  - This is because only the ones that have the vector with the gene for ampicillin resistance (amp^R) will be able to survive the ampicillin treatments
- The lacZ mechanism works cooperatively with the amp^R marker because sometimes plasmids will recirculate and still have ampicillin resistance but not have the recombinant DNA
  - Acts as another selectable marker



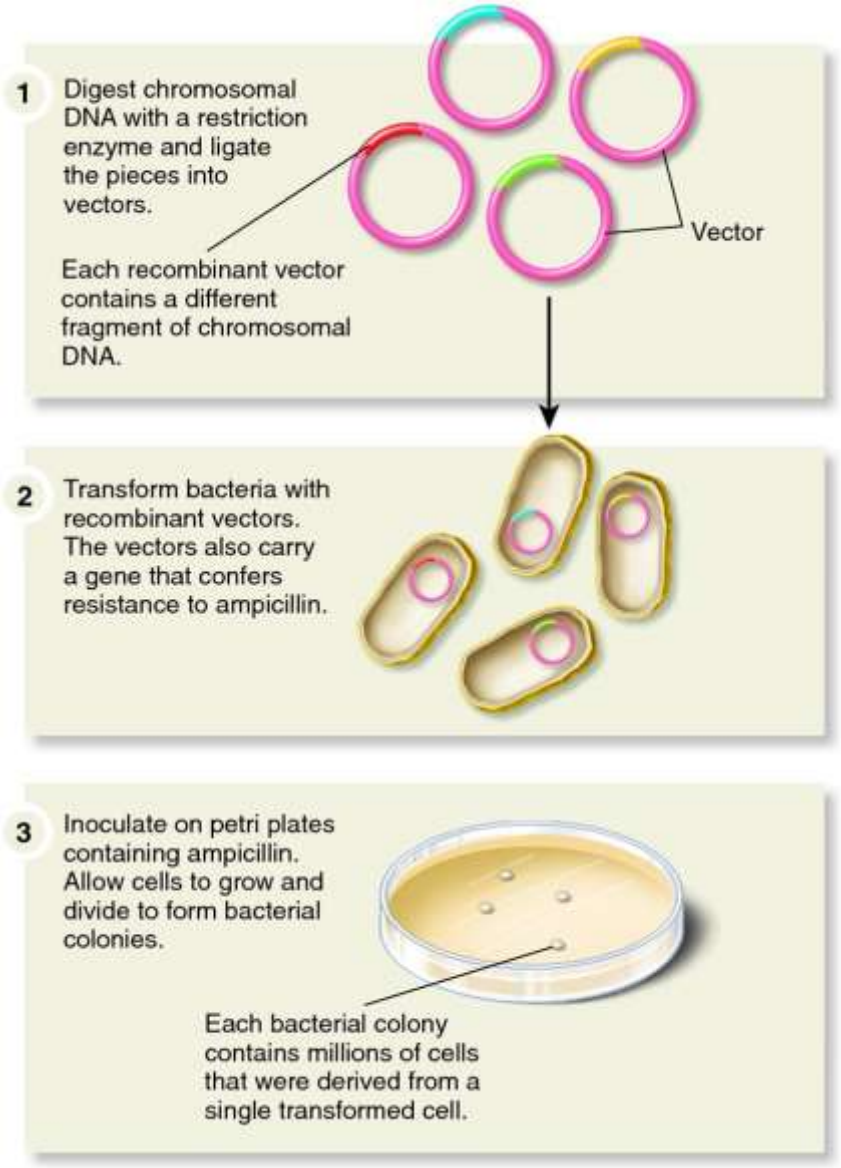
Example Question

_____ and _____ work cooperatively to identify which bacterial colonies have taken up the recombinant plasmid DNA.

a) LacI, amp^R
b) LacI, amp^Z
c) LacZ, amp^R
d) LacZ, amp^Z

DNA Libraries

- DNA libraries are collections of recombinant vectors.
  - Genomic libraries – fragments derived from chromosomal DNA
  - cDNA – obtained from mRNA using reverse transcriptase, forming the complimentary DNA.
    - Complementary DNA lacks introns so it is simpler to use

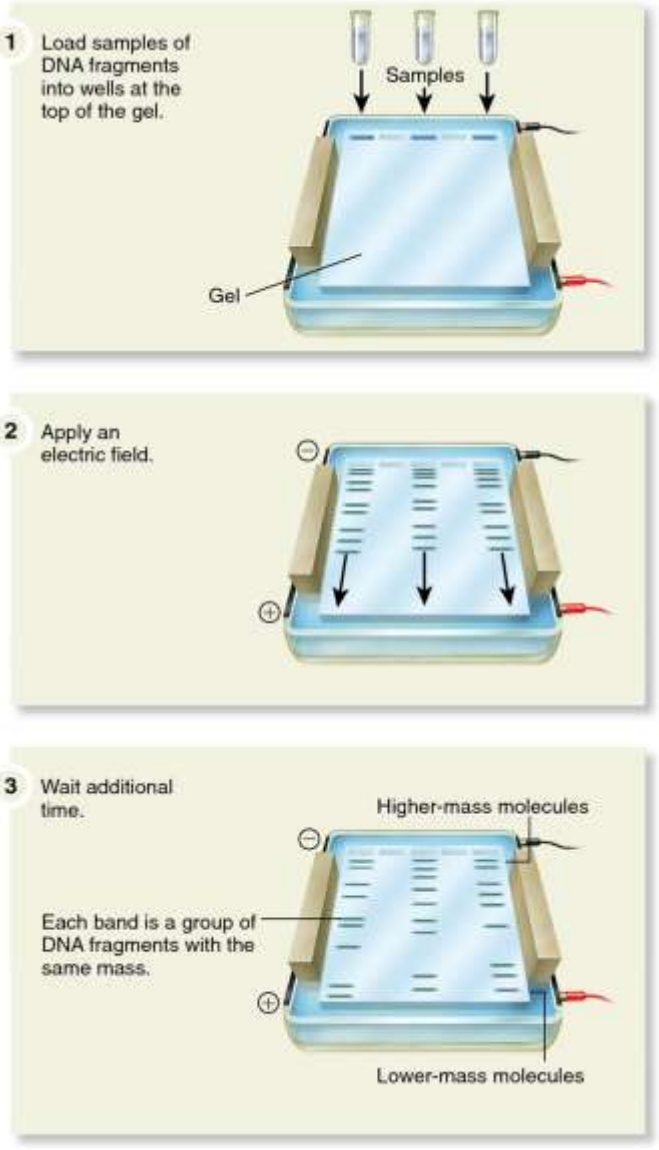


Blotting can ID gene families

- Southern blotting can detect the presence of a particular gene within a mixture of many chromosomal fragments separated on a gel
- Another common use of Southern blotting is to identify gene families
  - Two or more genes are derived from the same ancestral gene
    - The members of a gene family are homologous having similar but not identical DNA sequences
- Helps distinguish homologous members of a gene family within a single species or identify homologous genes among different species

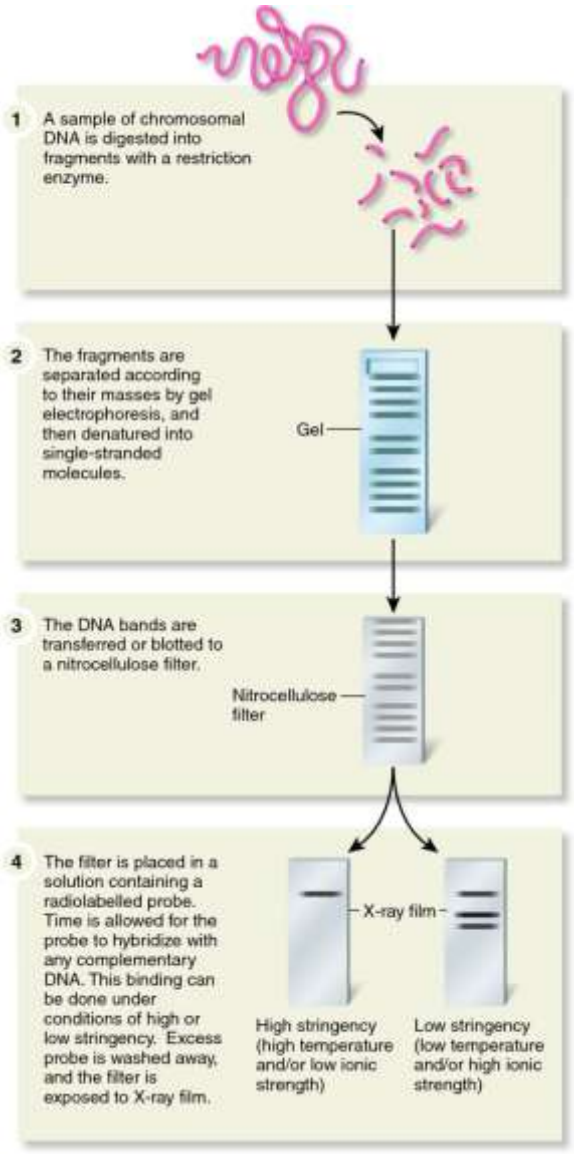
Southern Blotting

- Start by using restriction enzymes to cut DNA up into fragments then put it into an electrophoresis gel
  - The gel is made of agar or agarose
  - It is roughly rectangular and has wells (small holes) on one side
- You place you DNA into the wells
- Connect the gel to an anode (negative) at the well end and a cathode (positive) at the other end



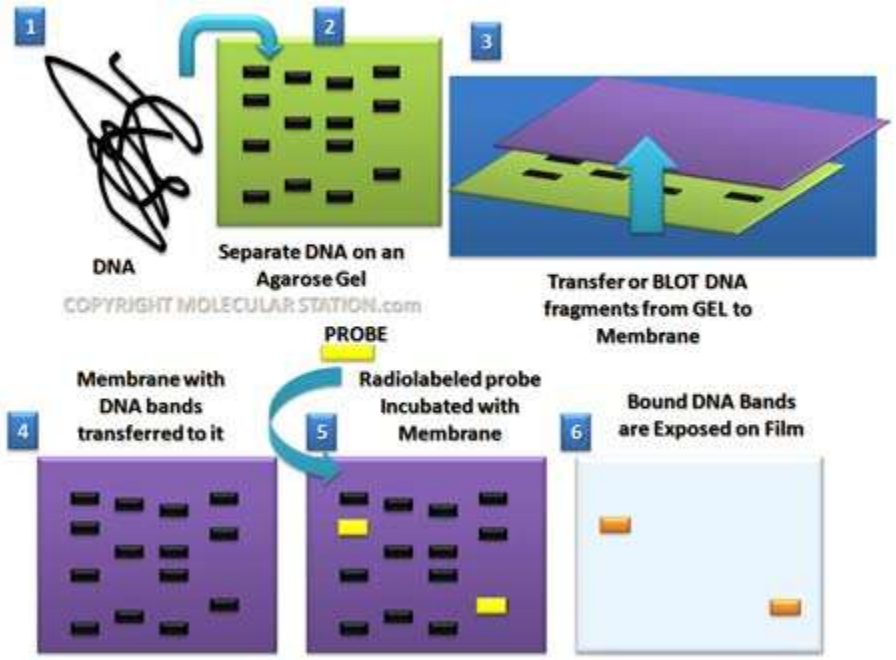
Southern Blotting

- When placed in the gel and you charge the gel it separates macromolecules based on charge and size
  - DNA is negative therefore it moves towards the positive electrode
  - Smaller fragments move faster and farther than larger fragments
- Creates a gel that has distinct bands
  - Bands can be stained or probed after being transferred to a nitrocellulose filter
- This filter acts like a negative of the gel



Southern Blotting

- High stringency Southern blotting
  - Detects close match between the probe and the chromosomal DNA
  - The sequences between the probe and what it's going to attach to will be almost identical
  - High temperature and/or low salt conditions
- Low stringency Southern blotting
  - Detects homolgous genes
  - Low temperature and/or high salt conditions



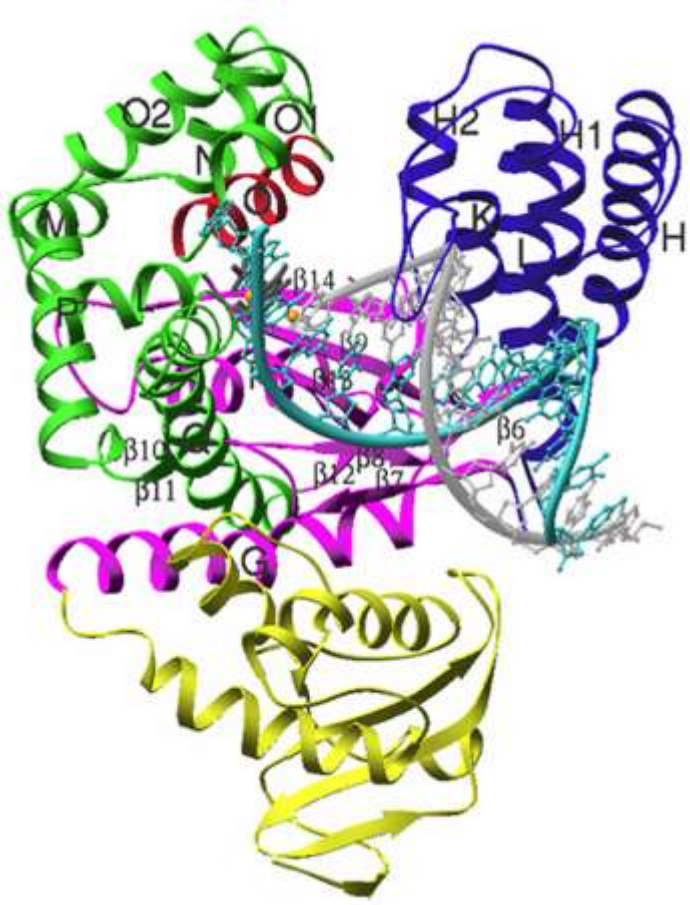
Example Question

In gel electrophoresis, large molecules of DNA will be closer to the _____.

a) Anode
b) Cathode
c) Pathode
d) Wrathode

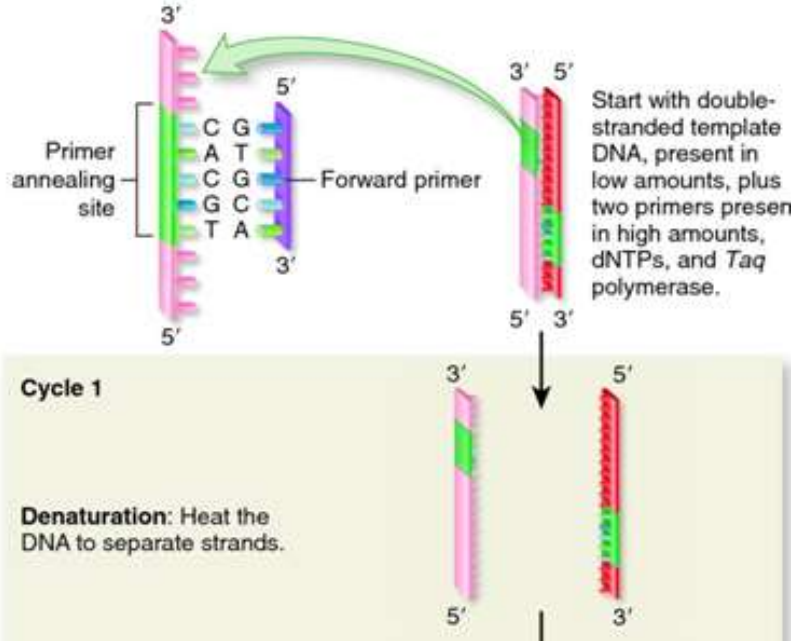
Polymerase Chain Reaction (PCR)

- Copy DNA without vectors and host cells
- Goal is to make many copies of a defined DNA region
- Uses two primers complementary to sequences at the ends of the DNA region to be amplified, deoxynucleotide triphosphates (dNTPs), and heat stable form of DNA polymerase (*Taq* polymerase)
  - dNTPs are the building blocks of DNA and the *Taq* polymerase puts it all together



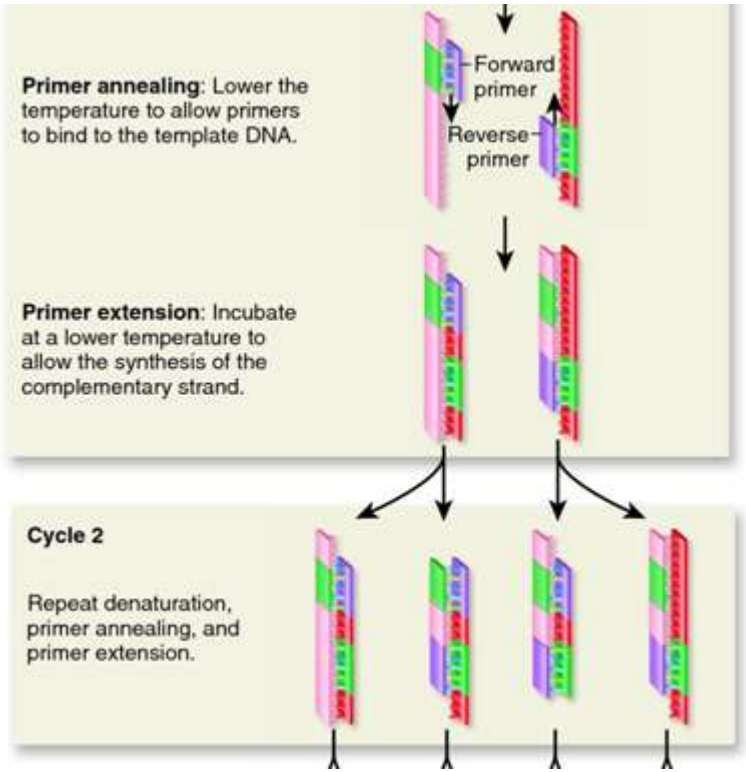
PCR

- Need to start with template DNA, the 4 nucleotides, and *Taq* polymerase
  - Also need the 2 different sequence primers that bind to the specific site that is to be amplified
- Start by adding all the ingredients and heating them up to split the DNA template into single strands



PCR

- Primer annealing
  - Lower the temperature for the primer to bind to each strand of template DNA
  - Remember that the primers are necessary because polymerases (like *Taq* polymerase) need double stranded DNA to start working
- Incubate the sample at a lower temperature to allow the synthesis of the complementary strand
- Repeat the cycle for more copies



Uses of PCR

- Examine historical figure and extinct species
- Criminal investigations (DNA fingerprints) from blood or hair
  - Uses primers that amplify several fragments simultaneously
- Each individuals DNA would prime at distinct locations giving them their own unique DNA fingerprint

Example Question

Which of the following is true

a) *taq* polymerase can survive at high temperatures
b) PCR requires vectors and host cells
c) PCR can only be done once with DNA
d) DNA needs to be heated for primers to bind