Cell Bio Test 3 Study Guide

- Transcriptional Controls: gene regulatory mechanisms regulating the amount of mRNA that is transcribed from a gene(s)
    - General transcription factors are proteins that RNA polymerase requires in order to bind to the promotor region or TATA box of a gene
        - Often these proteins cause a conformational change in the DNA that allows the polymerase to bind
    - Gene regulatory sequences act as molecular switches upstream from the from an initiation site
        - Gene regulatory proteins bind to these sequences (also known as transcription factors)
            - Often interact through binding to mediators
                1. Interactions can be hydrogen bonds or hydrophobic interactions
            - Three classes of gene regulatory proteins:
                1. Homeodomain sequence: regulatory proteins with stacked alpha helices and beta sheaths
                2. Zinc-finger motif: regulatory proteins requiring zinc atoms as part of their structure
                3. Leucine zippers: regulatory proteins shaped much like zippers with hydrophobic amino acids making up the innards
            - Some gene regulatory proteins can me master regulator proteins that initiate signaling cascades while others work in tandem
            - Combinatorial Code: combinations of gene regulatory proteins that can make many different cell types
- Master Control/Regulator Genes: genes whose translated protein products can initiate or stop transcription in a host of other genes
    - Protein products can work as activators or repressors (sometimes both → ex. "ey" gene in *Drosophila* codes for a protein that acts as activators for eye formation genes but repressors for other genes)
    - Homologs: similar versions of a gene found across species
        - Sometimes a gene from one species can be inserted into another species and the second species will still develop normally (ex. "ey" gene in *Drosophila* can be inserted into mice and a mouse eye will still form thanks to similarities of genes)
        - Many master control genes are homologs
    - Can switch on organ development in any part of an organism
- Techniques in Molecular Biology:
    - DNA analysis can be used to identify various characteristics in an individual (ex. Amelogenin gene can be used to identify sex)
        - Often needs to be amplified (copied repeatedly) in order for analysis to be able to happen
    - Amplification methods:
        - Bacterial cloning: insertion of a gene of interest into a bacterial plasmid so the bacterium replicates the gene (also called genetic recombination)
            - Process:

1. Induce cut in the plasmid (often with restriction enzymes/restriction endonucleases)
2. Insert the gene of interest
3. Gene is fused with the rest of the plasmid to create a recombinant plasmid
4. Plasmid is inserted into bacteria and expression of gene begins

- Polymerase chain reaction: replication of a gene of interest using heating and enzymes
  - Process:
    1. Heat double stranded DNA in order to break the two strands apart
    2. Lower temperature and add primers for the gene(s) of interest
    3. Add polymerases to replicate the gene(s)
    4. Repeat many times to obtain potentially billions of copies of the gene(s) of interest
  - Reagents:
    1. DNA sequence
    2. Forward and reverse primers
    3. dNTPs (deoxynucleotide triphosphates)
    4. *Taq* DNA polymerase
    5. Buffer with monovalent and divalent cations
    6. Sterile H_2O

- Additional Gene Markers:
  - Short Tandem Repeats (STRs): repeated sequences of bases found in chromosomes of individuals
    - Do not code for proteins
- DNA Hybridization Techniques:
  - Genome comparison: DNA of closely related species form more hydrogen bonds and require more heat to split the helices
    - Opposite for less related species
  - Microarray/Gene Chip:
    - DNA microarray can detect if a specific gene is present
      - Works via hybridization of complementary bases from a known sequence
    - RNA microarray looks at gene expression (proteins) by analyzing mRNAs that are present
      - mRNA copied with reverse transcriptase to make cDNA
      - Amplified with PCR and analyzed
    - *In situ* hybridization is used to detect RNAs or DNAs in cells/tissues
      - Uses labeled complementary RNA/DNA (*fluorescence in situ hybridization*/FISH) to localize and label a specific sequence (often with a fluorescent component)
- Dideoxy Method:
    - 3' OH group of deoxyribose replaced with a hydrogen to make dideoxyribose
      - Stops replication of DNA

        - Allows identification of stop sites in replication for each kind of dideoxy base added
            1. Doing this for all four genes and combining the results gives the entire DNA sequence
- Transgenic animal: animals with genes of other organisms inserted into its genome (transgenes)
    - Transgenes are inserted into the male pronuclei during the embryo phase
        - Organism randomly takes up the transgene into random places in its genome (could be problematic if the transgene is inserted near a strong promotor/repressor or an important gene, etc.)
- Gene targeting: adding a specific version of a gene in an organism
    - Altered gene inserted into embryonic stem cells
        - Organisms become chimeras after the embryonic stem cells are inserted into the blastocyst
            - Knockout organisms have the old gene replaced
            - Knock-in organisms possess both genes
- CRISPR:
    - The CRISPR-Cas9 system is a DNA-protein system in prokaryotes where short repetitions of base sequences (Clustered Regularly-Interspaced Short Palindromic Repeats) bind to phage DNA, allowing the Cas9 nuclease protein to cut the sequence in question
        - Beginning to be used for gene editing (not without some issues)
- Green Fluorescent Protein (GFP): fluorescent protein found in jellyfish
    - Used to visualize where a protein is expressed in a cell
        - GFP gene *inserted into the gene itself* and is expressed
        - Protein fluoresces green when exposed to ultraviolet light (is tagged to a gene of interest first)
- Membrane Structure and Function:
    - Cellular membranes are composed of lipids and proteins
        - Lipid composition:
            - Carbon, hydrogen, and oxygen elements
            - Usually long units of polymeric molecules
            - Fats contain a large amount of energy
                1. Can be saturated fats (maximum possible number of carbons) or unsaturated fats (less than maximum possible number of carbons)
                    - ✓ Most unsaturated fatty acids are *cis*-fatty acids (*trans* fatty acids are not naturally occurring)
            - Are hydrophobic molecules
        - Lipoproteins: protein molecules used to carry around hydrophobic lipids
            - Low-density lipoproteins (LDLs) transport lipids like cholesterol into blood and tissue cells
                1. Levels elevated by saturated fats
            - High-density lipoproteins (HDLs) transport lipids like cholesterol out of tissues to the liver and eliminate them from the body
                1. Levels elevated by unsaturated fats
                    - ✓ High HDL/LDL ratio is "good"

        - Hydrogenated oils are unsaturated oils that are saturated by heating at a high temperature in the presence of hydrogen and a catalyst (usually a metal)
            - Process only partially hydrogenates the oils (full hydrogenation impossible)
            - Process also makes *trans*- fatty acids that cannot be used by the body
- Properties of Membranes:
    - Flexible thanks to lipid portion of membrane
    - Are amphipathic because they have a hydrophilic head and a hydrophobic tail
        - "Amphipathic conflict" solved by formation of the lipid bilayer hydrophobic tail innards and hydrophilic head outsides
    - Hydrophobic effect: lipid vesicles (liposomes) form when lipids are mixed in H_2O because water does not associate with the hydrophobic tail of the lipids
    - Fluid Mosaic Model: lipid bilayer acts like a 2D liquid where proteins are free to infuse with the bilayer
        - Proposed by Singer and Nicolson
- Phospholipids:
    - Structure:
        - Positively-charged choline head
        - Negatively-charged phosphate group
        - Glycerol
        - Saturated and unsaturated fatty acid chain
            - Van der Waals/dispersion attraction among tails
            - Electrostatic attraction between head and water
    - Fluidity:
        - Determined by two factors
            - Packing density (determined by how saturated or unsaturated the fatty acids are)
                1. Higher density = lower fluidity
            - Tail length: number of carbons in the fatty acid chain
                1. Shorter tail = less interaction and more fluidity (longer tail does opposite)
- Cholesterol: sterol molecule modulating fluidity of membranes in animal cells
    - Properties:
        - Nonpolar tail and polar head
        - Makes phospholipid bilayer thicker and less fluid when inserted
            - But also prevents "gelling" of phospholipids at low temperatures by straightening phospholipid tails
- Membrane proteins:
    - Carry out most membrane functions
    - Types:
        - Transmembrane: proteins spanning/crossing the lipid bilayer
        - Lipid-linked: proteins attached to lipids
        - Protein-attached: proteins attached to other proteins bound to the lipid bilayer
    - How to study them:

        - Use detergents (disruptive agents solubilizing membranes by disrupting hydrophobic associations)
- Membrane Transport:
    - Molecules have differing diffusion (movement along a concentration gradient) abilities depending on size and charge
        - Diffusion abilities ranking:
            - 1. Small hydrophobic molecules
            - 2. Small uncharged polar molecules
            - 3. Larger uncharged polar molecules
            - 4. Ions
    - Types of transport:
        - Simple diffusion: movement of molecules on along a concentration gradient
            - Cannot saturate the concentration gradient
        - Facilitated diffusion: diffusion with membrane protein "helpers"
            - Can saturate the concentration gradient (only limited by number of proteins)
            - Done with carrier proteins or channel proteins
                1. Carrier proteins move molecules that fit their binding sites (binding causes a conformational change)
                2. Channel proteins filter molecules based on their sizes and charges
        - Active transport: molecular transport against a concentration gradient while utilizing energy
            - ATP is the energy used
                1. Transport rate decreases if ATP production is blocked
- The Membrane Potential:
    - Initial conditions:
        - Cytosol:
            - High concentrations of potassium cations and assorted anions
            - Low concentrations of sodium and calcium ions
        - Extracellular medium:
            - High concentrations of sodium and calcium ions
            - Low concentrations of potassium cations and assorted anions
    - Selective, gated ion channels regulate the potential
        - Potassium ions leave cytosol via channel proteins (makes cytosol more negatively charged)
        - Electrical signal opens sodium channels and allows the cations to rush in (the potential)
    - Equilibrium potential: potential when net flow through any open channels is zero
        - Electrochemical forces are in balance
        - V = 62 mV x Log([external ion concentration]/[internal ion concentration])
- Potential Changes:
    - Depolarization: decrease in the negative membrane potential (becomes more positive) due to opening of ligand-gated Na+ channels
        - Caused by neurotransmitter binding

- Action potential: opening of voltage-gated Na+ channels in response to depolarization
- Repolarization: returning of the membrane potential to original levels caused by the closing of the inactivation gate
  - Cuts of flow of sodium ions
  - No hyperpolarization occurs unless the cell also has voltage-gated K+ channels that take longer to close
- Toxin examples affecting potentials:
  - Tetrodotoxin from puffer fish blocks the voltage-gated Na+ channels and prevents the rising phase of the action potential
  - Homobatrachotoxin acts on ligand-gated Na+ channels and inhibits repolarization

- How Concentration Gradients are Made:
  - Na-K pump:
    - ATPase that pumps three Na+ ions and two k+ ions in
      - High K+ concentration in cytosol causes negative resting membrane potential
    - Functions:
      - Helps maintain osmotic balance (cells will lyse if too much Na+ or Cl- gets in)
      - Generate concentration gradients for membrane potential
  - Uniport transport: movement of a single molecule into a cell by a molecule binding to and causing a conformational change in a channel protein
    - Form of facilitated diffusion (does not utilize *energy* from gradient)
    - Ex. Glucose transport *out* of microvilli
  - Coupled transport: movement of 2+ types of molecules
    - Done by utilizing the energy of one molecule type's concentration gradient
    - Types:
      - Symport: molecules moved in same direction across the membrane
        1. Ex. Glucose transport *into* microvilli
      - Antiport: molecules moved in different directions across the membrane
        1. Ex. Movement of Na+ into a cell and Ca2+ out of a cell for signaling and toxicity purposes
  - In the Ear:
    - Movement opens stretch-activated channels in the inner ear/cochlea
      - Channels on the stereocilia pushed opened and ions flow in (generates an action potential)
      - Action potential perceived as sound