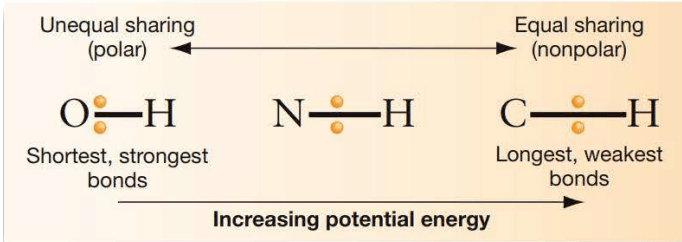
Good luck!

Energy and enzymes

Potential energy and heat ΔH



- more non-polar covalent bonds, more potential energy
- more electronegative atoms

Exothermic - a reaction with heat as a product (releases heat), $\Delta H < 0$
Endothermic - a reaction with heat as a reactant (absorbs heat), $\Delta H > 0$

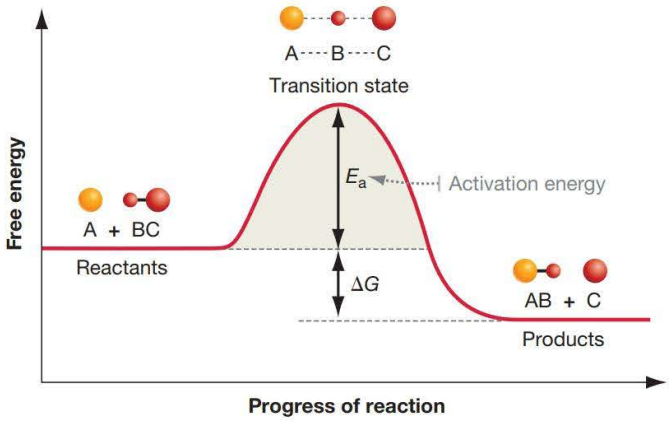
Entropy: disorder and mixing ΔS

Entropy S - the amount of disorder in a system. The universe trends to more entropy.

Gibbs free energy and spontaneous reactions $\Delta G = \Delta H - T\Delta S$

Gibbs free energy is change in enthalpy minus temperature * change in entropy
Enthalpy H - total energy in a molecule

Exergonic - $\Delta G < 0$, spontaneous reaction
Energonic - $\Delta G > 0$, non-spanteous
Equilibrium - $\Delta G = 0$



Coupling of reactions in anabolic processes

Energetic coupling allows endergonic reactions to proceed using free energy from exergonic reactions.

Anabolic pathway - any set of chemical reactions that synthesies large molecules form smaller ones. generally requires an input of energy
Catabolic pathway - breaks down large molecules into smaller ones, releases energy

Exergonic ATP hydrolysis (catabolic) is coupled with endergonic reactions (anabolic) through a shared intermediate.
Example: Sodium-potassium pump, ATP phosphoralyzes a protein pump

Enzymes

Enzymes (usually end in *-ase*) lower the activation energy of a reaction (E_a)
Specificity - an enzyme can "recognize" a specific substrate from a group of similar ones based on its structure (lock and key model)

Regulation:

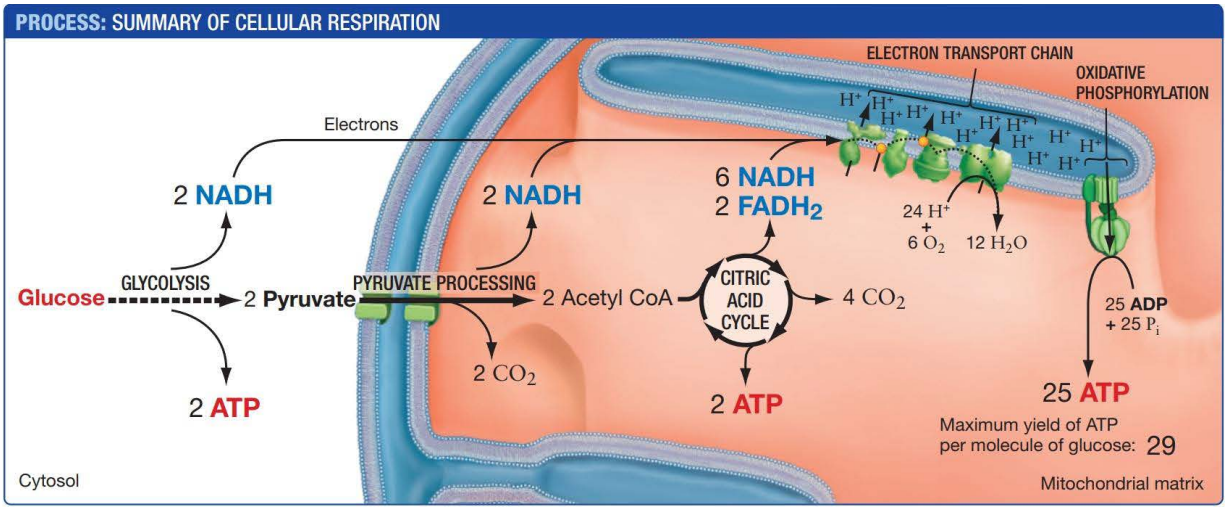
- Competitive inhibition - a regulatory molecule with similar shape to substrate sits in an enzymes active site and blocks it from binding
- Allosteric activation - a regulatory molecule attatches to the enzyme and causes a shape change, making the active site available. (putting a key in a car to start it)
- Allosteric inhibition - a regulatory molecule attatches to the enzyme and causes a shape change, making the active site UNavailable (giving a child a lollypop so they shut up)
- Phosphorylation - an added phoshpate group adds a negative charge to enzyme causing the shape change

Cellular Respiration

Purpose of celluar respiration: Using energy in glucose to make ATP

1. Glycolysis - one six-carbon molecule of glucose is broken into two molecules of the three-carbon compound pyruvate. ATP is produced from ADP and P_i, and (NAD+) is reduced to form NADH.
   Occurs in cytosol
2. Pyruvate processing - each pyruvate produced by glycolysis is processed to release one molecule of CO_2, and the remaining two carbons are used to form the compound acetyl CoA. The oxidation of pyruvate results in more NAD+ being reduced to NADH.
   Occurs in matrix of mitochondria or cytosol in prokaryotes
3. Citric acid cycle - the two carbons from each acetyl CoA produced by pyruvate processing are oxidized to two molecules of CO_2. During this sequence of reactions, more ATP and NADH are produced, and (FAD) is reduced to form $FADH_2$

4. Electron transport chain - Electron Electrons from the NADH and FADH2 produced by pyruvate processing and the citric acid cycle move through a series of electron carriers, the ETC. The energy obtained from this chain of redox reactions is used to create a proton gradient across a membrane, the ensuing flow of protons back across the membrane makes ATP



Substrate-Level Phosphorylation - creates ATP, enzyme catalyzes the transfer of a phosphate group from a phosphorylated substrate to ADP (coupled reaction)
Oxidative Phosphorylation - creates ATP, links oxidation of NADH and $FADH_2$ with phosphorylation of ADP

phosphofructokinase - regulates glycolysis, when too much ATP is produced this enzyme uses ATP to phosphorylate fructose-6-phosphate (allosteric inhibition)

Fermentation

Role of fermentation for the cell: Regenerate NAD+ - only needed to keep glycolysis going so that we can power the proton gradient to produce ATP
occurs when there isn't enough oxygen

fermentation oxidizes stockpiles of NADH to regenerate NAD+
lactic acid fermentation - regenerates NAD+ by reducing pyruvate to form lactate
fermentation is inefficient - produces only 2 ATP per glucose metabolized, while aerobic cellular respiration produces 29

Central dogma

DNA to RNA to Proteins to Phenotype
triplet genetic code - codon - 3 bases code for our 20 amino acids. we need to have 3 to code for all of them, and the extra coding is used for redundency. each coding is unique
reading frame - if our reading frame is off by even 1 base, it messes up everything and we just produce gibberish

template strand -RNA polymerase reads this strand from 3' to 5' and adds base pairs to make

mRNA
coding strand - has the same sequence of the newly synthesized mRNA

DNA to mRNA is transcription, uses RNA polymerase
mRNA to proteins is translation, uses ribosomes n shit

Transcription

synthesis of RNA from a DNA template

prokaryotes

in bacteria sigma protein must bind ot core enzyme to recognize site*s* where transcription should begin. These sites are promoters. bacterial RNA polymerase core enzyme and sigma form a holoenzyme (beads on a string)

transcription and translation can occur at same time, bacteria can translate mRNA even before its transcription is complete

eukaryotes

in eukaryotes, there are 3 major polymerases, RNA polymeras I, II, and III, which produce only certain types of RNA

eukaryotic RNA polymerase recognizes promoters using transcription factors - proteins
poly(A) signal - DNA sequence near the end of each gene, marks the end where enzyme cuts

translation and transcription are separated in time and space

exons and introns - introns are removed from growing RNA by splicing

mRNA processing

Splicing of introns and alternative splicing

5' cap and 3' poly-A tail addition - helps mRNA not desolve (protects it) and marks the end of transcription

Translation

Additional control of gene expression

mRNA stability (miRNA)

Prokaryotic regulation of gene expression

Biotechnology

Restriction enzymes - purpose and mechanism of action
Creating a recombinant DNA plasmid using restriction enzymes
Using bacteria to produce proteins

Good luck :(

Cell Signaling

Lipid soluble vs lipid insoluble signals/receptor locations

Lipid-insolible receptor proteins are on the plasma membrane

1. G protein-couple receptor
2. Receptor kinase
3. Ligand-gated ion channel

Lipid soluble signal receptors are inside the cell, since lipid soluble molecules can usually cross the plasma membrane

Transduction, amplification, and termination

- **Transduction**: Lipid-insoluble signals require transduction, convert signal from one form to another
    1. Signal reception - receptor protein recieves signal
    2. Signal transduction - Converts extracellular signal to intracellular signal
    3. Signal response - may lead to changes in cytoplasmic protein activity or changes in gene expression
- **Amplification**: Signal cascades
- **Termination**:
    - G protein turns on a down-stream enzyme, and bound GTP is hydrolized by the G protein to GDP and P_i / second-messengers
    - Ras active when bound to GTP, deactivated when it hydrolizes GTP to GDP and P_i
        * Continuously active Ras keeps on saying "divide now", cancer :(

How G Protein coupled receptors work

G proteins "peripheral membrane proteins" inside cell. Activated by a signal receptor, trigger production of a **second messenger** (transduction).

Regulated by either GTP or GDP. Activated by GTP, inactivated by GDP (removal of a phosphate group).

See figure 11.15 on textbook pg 250

Receptor tyrosine kinase signaling

Receptor tyrosine kinase (RTK) are a type of enzyme-linked receptor which trigger a phosphorylation cascade.

Activated by dimerization caused by signal*. This activates Ras protein by exchanging a GDP for a GTP. Ras activates a kinase through phosphorylation, and the active kinase starts a phosphorylation cascade.
*these signals often called mitogen-activated protein kinases, MAPKs

See figure 11.16 on textbook pg 251

Enzyme-linked receptors are similar to G-protein-coupled receptors in that both are methods of signal transduction

Development

5 essential development processes

1. Proliferation - cells generate through mitosis and cytokinesis
2. Signaling/interactions - cells talk to each other
3. Differentiation - cells begin to specialize
4. Migration - cells move to right place
5. Apoptosis - regulated cell death

Cleavage

In cleavage cells divide rapidly without going through growth phase of the cell cylce (so cells get smaller)

Development and decisions

- **totipotent** - this cell can make a full person (zygote/fertilized egg)
- **pluripotent** - cells that can form any cell in the body (embryonic stem cells)
- **multipotent** - cells that can form any cell within a group (germ layers)

cells become more specialized

3 germ layers

1. Ectoderm - nervous system, eyes, ...
2. Mesoderm - skeletal system, circulatory system, muscular system, ...
3. Endoderm - liver, pancreas, thyroid, ...

Gastrulation and Neurulation

Gastrulation is beginning of specialization. Formation of three embryonic tissue layers / germ layers. You get ectoderm on outside, mesoderm on inside, and endoderm in core with a little hole for the gut.

Neurulation: Notochord forms and signals where ectoderm should fold (where head ends up). A neural tube develops

Mitosis and Regulating the Cell Cycle

- For growth, development, and repair of cells
- **Somatic cells** (non-sperm and egg cells) divide to produce more somatic cells.
- Maintain diploid state - 2 copies of each chromasome
- 2n $\rightarrow$ 2n (equational) (n in humans is 23, we are diploid)
- Preserves genotype

Results in two daughter nuclei that have chromosomes and genes identical to parent nucleus. Cytokinesis then yields two daughter cells.

DNA replication and condensation during mitosis

1. Prophase - chromosomes condense and spindle apparatus begins to form
2. Prometaphase - nuclear envelope breaks down. microtubules contact chromosomes at kinetochors
3. Metaphase - Chromosomes complete migration to middle of cell
4. Anaphase - Sister chromatids separeate into daughter chromosomes, which are ulled to opposite poles of spindle apparatus
5. Telophase - Nuclear envelope re-forms and chromosomes de-condense

End replication problem / replication of chromosome ends

Telomere is the end of a eukaryotic chromosome. The problem is pretty much that our lagging strand becomes too short and no DNA synthesis can occur after the primer is removed. So we get this unreplicated end of the 3' coil of our lagging strand.

See figure 15.12 on page 334 in textbook

Telomeres and Telomerase

Telomerase - an enzyme that replicates telomeric DNA. It catalyzes the synthesis of DNA using an RNA template (part of the enzyme)

1. Oh no! Lagging-strand has unreplicated single-stranded "overhang" at 3' end
2. Telomerase binds to 3' end of lagging-strand, catalyzes the extension of the overhang to the end of the template region of its RNA molecule
3. Telomerase repeatedly moves down the new DNA and continuously adds more copies of the sequence it corresponds to
4. When the overhang is long enough another DNA synthesizing enzyme uses it as template strand, makes complementary strand. All is well :)

Cell cycle progression

Cyclin and CDK

Cyclin is a regulatory protein. Cyclin-dependent kinase (Cdk) catalyzes phosphorylation of other porteins to start M phase.

M phase-promoting factor (MPF) Cyclin and M phase-promoting Kinase (MPK) Cdk bind together into MPF. MPF is a regulatory molecule in cytoplasm that induces M phase.

MPF Cyclin levels... *CYCLE* throughout phaseswhile MPF CDk stays constant. When they get large enough (build during interphase) they activate Cdk and trigger M phase, then levels drop again.

Checkpoints

G1 checkpoint - if passed, cells divide or die

See pg 15 of lecture 19 (pg 46 in my pdf) for more

Social controls: mitogens, cell interactions

mitogens are extracellular signaling factors that bind to growth factor receptors, which start a signal cascade to stimulate cell division

mitogen signaling can activate Ras protein, driving cell into S-phase

Tumor suppressors and Oncogenes

p53/Rb protein regulates cell cycle

both are tumor suppressor genes and prevent inappropiate cell division

p53 is phosphorylated / activated when DNA damage is detected, which acts as a transcription factor and promotes expression of genes that inhibit cyclins to prevent progression past the G1 checkpoint

E2F - some transcription factor that triggers production of S-phase proteins, activated by cell signalling / mitogens
Rb inactivates E2F when it binds to it. if Rb couldn't bind to E2F the cell cycle would be driven forward even when it shouldn't

tumor suppressor gene / two hit

maybe you genetically get one inactive/mutated tumor suppressor gene, or maybe epigenetics causes it to become inactive. that's ok, because you have two of them, so just one should be able to do its job, but if both become inactive you'll probably get cancer

Ras regulation and role in cell cycle

Ras is activated by cell signalling / mitogens, which drives cell forward into S-phase.

Proto-oncogens (Ras) vs Tumor suppressor Genes (Rb, p53)

Proto-oncogens are our gas pedals for cell-division, and become oncogenes when they take a gain-of-function form (become overactive, cancer causing)

Tumor suppressor genes are our breaks, they prevent innappropiate cell division. if they take a loss-of-function form they stop working, can cause cancer

DNA replication

- Helicase - opens DNA double helix
- Topoisomerase - unwinds DNA strands
- RNA Primase - synthesisez RNA primer, intiates DNA snthesis (priming)
  - DNA polymerase needs a 3'-OH group to start polymerization, which is why DNA is replicated 5' to 3' end
- DNA polymerase III - extends the RNA primer
- Okazaki fragment - short lengths of DNA produced by replication of lagging strand
- Ligase - links together Okazaki fragments

DNA Mutation and Repair

Cancer

Meiosis

Germ line cells - cells that pass down genetic material to offspring (sperm/egg)

Mendelian Genetics

Good luck :)

Linkage and Recombination

Linkage is the tendency of alleles of particular genes to be inherited together. Linkage is seen when genes are on the same chhromosome

Recombinant - alleles on a chromosome are different (recombined) form the combinations present in the parent. gametes with new combinations of alleles were generated when crossing over occured during prophase of meiosis 1 in the females

Linkage and Crossig Over

Recombinant genotypes - a genotype different that both parental genotypes

recombination frequency - divide the number of offspring with recombinant chromosomes by the total number of offspring
- how often genes recombine due to crossover at meiosis

Relationship between physical distance and frequency of crossing over

Crossing over occurs frequently between genes that are far apart

Mapping genes by recombination frequency

genetic map - diagram showing the relative positions (loci) of genes along a particular chromosome

one map unit - distance between genes that produces 1 percent recombinant chromosomes

see page 311 in textbook

Extending Mendel

Some genes can have multiple alleles - i.e bloodtype has A B and O alleles in humans

codominance - a heterozygote displays the phenotype of both alleles (mix of phenotypes)
incomplete dominance - heterozygotes have an intermediate phenotype (blended phenotype)
pleiotropy -one gene may govern multiple phenotypes, even if unrelated
gene by environment interaction - the combined effect of genotype and environment (i.e if you're lactose intolerant from a gene you just don't consume lactose in your environment)
polygenic inheritance - multiple genes control expression of a phenotype (i.e height)
number of genotypes = $3^{\text{number of genes}}$ with 2 alleles per gene
quantitative phenotypes - based on polygenetic inheritance, follow a normal distribution

1. Continuous traits - vary continuously from one phenotypic extreme to the other (i.e height)
2. Meristic traits - varies with a natural number (i.e number of puppies in a litter)

3. - threshold traits - only two phenotypic classes (unaffected/affected), and each individual has some genetic risk. if your risk (genes + environment) pass some threshold you're affected (i.e schizophrenia, type II diabetes)

Genetic Disorders

Sickle cell anemia

- Inherited blood disorder affecting red blood cells (sickle shaped now)
- often results from a single amino acid change in the β-hemoglobin protein
  - Change from Glu in 6th position to Val
  - Val is nonpolar
- sickle cell diseas is autosomal recessive
- see page 6, lecture 27 for diagnosis (pg 40 in my slides)

Huntington's disease

- Inherited neurodegenerative disease, can give dementia
- Autosomal dominant
- When genes are close together along a chromosome, it is unlikely that crossing over occurs between them, so they are often inherited together
- we stick a DNA probe in a strand of DNA and get close to defective allele, then perform a souther blot anlysis?
- Huntington's disease associated Huntingtin protein has extra CAG repeats
- first disease gene mapped to a specific chromosome

Population Genetics - Hardy Weinberg and Selection

Hardy-weinberg principle

Five conditions:

1. Mating is ranodm (individuals do not choose a mate based on the gene under study)
2. No selection: All genotypes at the locus surive and reproduce equally
3. No genetic drift: Th epopulation is large enough that sampling variation does not cause random changes in allele frequency during reproduction
4. No gene flow due ot migration in or out

5. No mutaiton to generate new alleles

Equation: $(p+q)^2 = p^2 + 2pq + q^2 = 1$, where p, q are the allele frequencies

Genotype frequencies from allele frequencies: AA=p^2, Aa = $2pq$, aa=q^2
Allele frequencies from observed genotype data: sum up alleles of each type and divide by number of total alleles (remember genes usually have two alleles, extend if needed)

Random vs non-random mating - non-random mating is like another level of selection for positive traits associated with mating

Inbreeding and its effects

- Inbreeding increases homozygosity
- Inbreeding itself does not cause evolution, because although genotype frequencies change, allele frequencies do not change in the populaiton as a whole

Population Genetics and Evoluiton

Evolution represents a change in allele frequencies

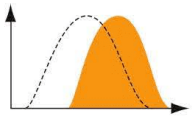
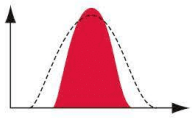
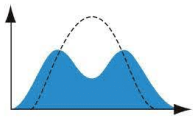
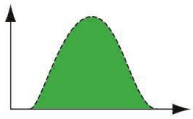
Driving forces for evolution

1. Natural selection - increases the frequency of certain alleles - the ones that contribute to reproductive success in a particular environment
2. Genetic drift - causes allele frequencies to change randomly. In some cases, drift may cause alleles that decrease fitness to increase in frequency
3. Gene flow - occurs when individuals leave one population, join another, and breed. Allele frequencies may change as a result
4. Mutation - modifies allele frequencies by continually introducing new alleles. Alleles may be beneficial, deleterious (detrimental), or neutrla in their effects on fitness

Selection

See slide 8, lecture 29 (in my slides 71)

SUMMARY **Table 23.2 Modes of Selection**

Mode of Selection		Effect on Phenotype	Example	Effect on Genetic Variation
Directional selection		Favors one extreme phenotype, causing the average phenotype in the population to change in one direction.	Average beak depth increased in ground finches during the 1977 drought.	Genetic variation is reduced.
Stabilizing selection		Favors phenotypes near the middle of the range of phenotypic variation, maintaining average phenotype.	Human babies of average size are most likely to survive.	Genetic variation is reduced.
Disruptive selection		Favors extreme phenotypes at both ends of the range of phenotypic variation.	Whitefish with low or high numbers of gill rakers are most likely to survive.	Genetic variation is increased.
Balancing selection		No single phenotype is favored in all populations of a species at all times.	Guppies with rare color patterns are favored.	Genetic variation is maintained.

Relative fitness

Relative fitness: $w =$ relative contribution of each genotype to the gene pool of the next generation
Measures the relative competitive ability between genotypes By convention the genotype with the highest combination of viability and fertilitity is assigned relative fitness $w = 1.0$

Selection coefficient: $s = 1 - w$
Measure the relative strenght of selection *against* different genotypes

Heterozygote advantage

A pattern of natural selection that favors heterozygous individuals compared with homozygotes. Tends to maintain genetic variation in a population and thus is a form of balancing selection

p_{eq} and s_{eq} are the allele frequencies for A and a at equilibrium respectiviely.
Then $\frac{s_{AA}}{s_{AA}+s_{aa}} = p_{eq}$, $\frac{s_{aa}}{s_{AA}+s_{aa}} = q_{eq}$

Genetic drift and its cosequences

- **Founder's effect** - Immigrants establish a new population, then new population is likely to have different allele frequencies than source population, by chance.
  - Change of allele frequency randomly

- **Genetic bottleneck** - High mortality strikes individuals at random, then bottlenecked population is likely to have different allele frequencies than original population, by chance
  - Like a natural disaster, similar effect

Immune System

innate vs adaptive immune system

antigen - any foreign molecule that can elicit an immune system response

- **Innate immunity** - inherent to all animals, ready to go from birth
  - First line of defense. Most effective way to prevent infections is to prevent pathogens from entering body in first place
  - skin secretes oil that creates acidic environment, mucus, earwax, saliva, eyes have antibaccterial enzyme
  - triggered when foreign invaders enter the body, too. white blood cells / leukocytes provide a *generic* response
  - Toll-like receptors (TLRS) are **pattern-recognition receptors** that detect antigens and signal innate immune system
  - inflammatory response - see page 1033 in textbook
  -
- **Adaptive immunity** - only occurs in about 1 percent of animals (vertebrates), in humans takes times to develop
  1. **specificity** components bind only to specific sites on specific antogens
  2. **diversity** - can recognize and be activated by many antigens
  3. **memory** - stronger and quicker immune response after previous exposure
  4. **self-nonself recognition** - doesn't kill itself :)

T cell B cell receptors structure/function

BCR's have these little Y's, TCR's are like ——'s. They have specific antigen-binding sites

How T cells get activated

dendritic cell - messenger cells that collect information on antigens, create MHC protein on surface carying a peptide fragment

T cells are activated by interacting with MHC-peptide complexes
TCR binds to peptide presented on MHC protein on surface of dendritic cell. Begins activation process. Activated $CD4^+$ and $CD8^+$ T cells multiply and differentiate (clonal expansion).

$CD4^+$ are helper T Cells. $CD8^+$ are Cytoxic T Cells

How cytotoxic T cells kill target cells

Cytotoxic T cells convince target cells to kill themselves (promote apoptosis)

1. Recognition - T cell recognizes and binds to infected cell
2. Directed secretion - T cell secretes a proteins that pass through cytoplasm of infected cell - Secretion of perforin and granzyme
3. Apoptosis - T cell leaves and infected cell breaks up into fragments which are consumed by Phagocytic cells.

How B cells get activated

B Cells are activated by binding to antigens and interacting with helper T Cells (see pg. 92 in my slides)

Mature B-cells (plasma cells) screte antibodies that recognize specific antigens

Viruses

Viruses:
Virus structure and function
Naked vs enveloped
Steps of the viral lytic cycle (differences between types of viruses)
How enveloped viruses enter/exit cells
What happens during lysogeny (latency) and how it differs from the lytic cycle
Antivirals function
Steps of the viral life cycle that are targets for antivirals

Good luck :)

COVID-19

Basic viral structure and life cycle

SARS-Cov2 is an enveloped virus with spike proteins on the membrane. It has an RNA genome and is an RNA virus

life cycle

- virus binds to ACE2 receptor, entry via endocytosis
- releases RNAss(+) viral genome
- genome codes for viral proteins and itself, the viral genome
- once the virus is created by the cell it uses the secretory pathway to leave
  - made in the rough ER, released to environment

ACE2 recepotry entry into cells

spike protein has receptor bbinding domain (RBD). It binds to ACE2 receptor and triggers viral entry. ACE2 receptor is often found in lungs. ACE2 receptors are less abundant and less active before puberty – perhaps this is why young children seem to get it less

Vaccine types and basics of how they work

see lecture 32, pg 12 for table (518 in my mega-slides)

Antiviral rationale

inhibit important viral lifecycle processes to prevent viral replication and spread. you would take one early on in a viral infection to give your body the edge it needs to quickly clear the virus. may target viral replication or viral entry

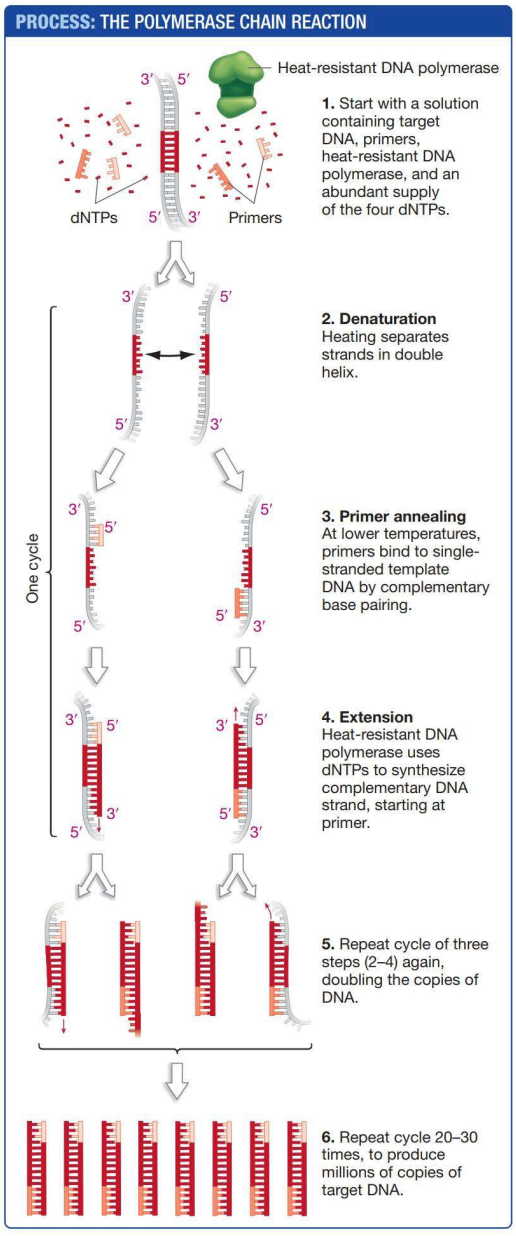
Biotechnology

PCR - purpose, components, and general steps

PCR - polymerase chain reaction - provides a quicker way to clone DNAs, amplifies a particular DNA sequence that's of interest

- requires primers to be made, short lenghts of single-stranded DNA that match sequences on either side of targeted region

- repeats 3 steps over and over again in cycles, each cycle doubles copied DNA



How gel electrophoresis can be used to separate DNA by size

- load cavities ("wells") in gel with samples of macromolecules
- hook up a power supply. negative at top, positive at bottom. molecules separate over time as some move faster than others (separate due to size and charge, larger molecules float towards the top)
- remove gel after samples have run its length. molecules that are smaller or carry more negative charge move farther than molecules that are larger or less highly charged

DNA fingerprinting method

short tandem repeats (STRs) - simple repeating units from two to about eight nucleotides long, usually occur between genes, possibly mistakes of DNA polymerase

- STRs vary widely among individuals, so we use these for fingerprinting
- use PCR to amplify an STR, then use gel electrophoresis to determine the number of repeats it contains

DNA sequencing - steps and method of Sanger sequencing

dideoxynucleotides - attatch to 3' end of a molecule, but do not have a 3'OH group. No more DNA synthesis can occur after being attatched

use PCR to synthesize DNA but use ddNTPs (labeled with color flurophore) to stop chain extension occassionally. fluroscent labels at the end of each different length fragment are imaged to get Sanger sequencing data (we can't see individual nucelotides but we can detect the labeled ddNTPs)

Genome editing

CRISPR/Cas9

bacteria already have this nifty Cas9 enzyme. It has a guide RNA sequence that base pairs with invading viral DNA, then Cas9 cuts off invading viral DNA to protect the cell. the idea is that we can change the guide RNA arbitrarily so that Cas9 cuts whatever we'd like

- sgRNA - complementary to the sequence we want to edit, guide RNA
- Cas9 - the enzyme itself
- protospace adjacent motif (PAM) requirement: limitation; this very small sequence needs to be by recognition sequence so that it can bind, but luckily it occurs on average once every 8-12 base pairs in human genome

when DNA is cut by Cas9 the cell panics, and tries to fix the DNA quickly but may make mistakes. It reconnects the DNA helix but may add insertions or deletions (INDELs). An INDEL is pretty much a gurantee of a frameshift mutation or addition of bairs, which is pretty much a gurantee of loss of gene function (gene knockout)

How genome editing can be used to treat sickle cell disease

sickle cell disease is caused by a defect in a single gene (single base pair mutation). CRISPR could reactivate the fetal hemoglobin gene by knocking out the BCL111A gene