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# ***Microbiology Final- Old Material***

## ***Test 1 Material***

I. External structures of a bacteria cell
- A. Cytoplasmic membrane
  - 1. composition
    - a. phospholipids
      - 1. polar portion- hydrophilic- outside
      - 2. non-polar portion- hydrophobic- inside (fatty acids)
    - b. carbohydrates
    - c. proteins
      - 1. integral proteins- through cell mem, touching medium and cytop
      - 2. peripheral proteins- on one side of cell mem, s/t associate w/ inte
  - 2. roles
    - a. defines cell (encloses it)
    - b. transport
      - 1. semipermeable- allows necessary molecules from out to in
      - 2. transport proteins act as pumps maintaining conc grad actively
    - c. contains enzymes (proteins)
      - 1. electron transport
      - 2. oxidative phosphorylation
      - 3. cell wall synthesis
      - 4. complex lipid synthesis
    - d. DNA replication
      - 1. part of cell division where 1 copy of DNA goes to 1 cell and 1 to the other
    - e. chemotaxis
      - 1. process whereby a motile cell moves toward an attractant and away from a repellant
      - 2. depends upon motility- flagella are anchored in cell mem
  - 3. mesosomes
    - a. convoluted invaginations of the cell mem
    - b. 2 types
      - 1. septal- found near septum- uniform wall that divides 2 cells during cell division
      - 2. lateral- found anywhere except near septum
- B. Outer membrane
  - 1. found only in G- bacteria
  - 2. periplasmic space- between outer membrane and cell membrane
    - a. contains 2 things:
      - 1. proteins that would be harmful to cell if inside
        - A. ex: RNA polymerase I
      - 2. binding proteins- involved in transport
- C. Cell Wall/ Murein/ Sacculus/ Peptidoglycan
  - 1. netlike arrangement made up of covalently bonded molecules that surrounds cell in all 3 dimensions
  - 2. 3 parts:
    - a. backbone- long chain of 2 sugars:
      - 1. N-Acetyl glucosamine (NAG)
      - 2. N-Acetyl muramic acid (NAM)

b. tetrapeptide
1. S. aureus
A. L-Alanine
B. D-Glutamine
C. L-Lysine
D. D-Alanine
2. E. coli
A. L-Alanine
B. D-Glutamic acid
C. D, L (meso)- Diaminopimelic acid
D. D-Alanine
3. tetrapeptide is attached to NAM
4. important points:
A. most amino acids are in L form, in cell wall uniquely D
B. alternating L and D amino acids, ending in D
C. glutamine is modified version of glutamic acid

c. bridging group
1.connects amine end of 3rd amino acid of one tetrapeptide to carboxyl end of 4th amino acid of another tetrapeptide
2. connection= cross-linkage
A. makes cell wall into a net
B. strength and rigidity of cell wall depends on extent of c-l
1. better for bacteria to have stronger cell wall
2. antibiotics lyse the wall which inhibits cell wall synthesis
C. causes diff b/t G+ and G- cell wall
1. G- has thin cell wall, has 1 layer= 1 net surrounding bac cell
2. G+ has thick cell wall, up to 40 layers, has cross-links laterally and vertically
3. in S. aureus, bridging group is pentaglycine- chain of 5 glycines
a. attaches amine group of L-Lysine to carboxyl group of D-Alanine on another tetrapeptide

3. roles of cell wall
a. structural rigidity
1. cell mem is like soap bubble, but wall keeps shape- functional integrity
b. protection- 2 ways:
1. physical- provides barrier to keep cell intact b/c cell mem can rip
2. from lysis by internal osmotic pressure
A. cell is a bag of molecules dissolved in cytoplasm.
B. water moves toward high conc of molecules
C. cell will expand- gain osm pres- until it explodes-lysis
c. barrier- 2 ways:
1. keeps out big particles
2. keeps proteins in periplasmic space

D. Acids- only in G+ cells
1. ribitol teichoic acid
a. about 30 units of repetition of ribitol and phosphate
b. attached to cell wall, extends outward into surrounding medium
2. glycerol teichoic acid/ lipoteichoic acid/ membrane teichoic acid
a. repetition of glycerol and phosphate
b. attached to cell mem, extends through 40 layers of cell wall to medium

E. Lipopolysaccharide (LPS)- only in G- cells
1. attached to outer leaflet of outer mem of G- cell
2. 3 parts:
a. O-side chain (outer)
1. 40 repeats of 3-5 sugars
A. variations in which sugars, sequence, # of sugars in base unit (4 or 5)
2. antigenic (b/c of variability), O-antigen
A. antigen- s/t that stimulates body to produce antibodies (proteins)
1. as soon as s/t foreign enters body- usually carb or protein of bac cell, body launches immune response
B. 2 impt ideas associated with antibodies
1. specificity- antibodies for specific antigens
2. response is adaptive- antibodies only produced when antigen there, usually not there
C. typing sera- important in diagnostic microbio b/c can have antibodies for known O-antigens and can take unknown antigen and see which antibody it reacts w/ to know which bac it is
1. blood is composed of cells and fluid
2. serum- liquid part of blood
a. contains antibodies
b. core
1. 5 hexoses (6 carbon sugar)
2. 2 heptoses (7 C)
3. 3 KDO- ketodeoxyoctanoic acid (8 C)
c. Lipid A (inner)
1. composition
A. 2 glucosamines
B. phosphate
C. hydroxymyristic acid
D. fatty acids- attach it to outer mem b/c mem is lipid
2. endotoxin
A. usually toxins are exotoxins- proteins produced and excreted outside by bac cell into bloodstream where it reaches target tissue and does damage
B. endotoxin- molecule attached to bac cell, not excreted.
C. has impt bio effects:
1. less severe- fever, hyperglycemia
2. more severe- intravascular coagulation, hemorrhagic shock

F. Lipoprotein- only in G- cell
1. composition
a. lipid portion- attaches to inner leaflet of outer membrane
b. peptide (amino acid) portion- attaches to cell wall
2. role- anchors outer mem to cell wall, so it's firmly attached

G. Capsule
1. Not every bac cell has a capsule
a. those that do form mucoid colonies
b. ex: klebsiella and pneumococcus
2. composed of 2-3 sugars in long repeat
a. variations in which sugars, sequence, and number of sugars
3. antigenic- K-antigen (can use typing sera to diagnose org)
4. 2 ways to tell if org has capsule:
a. negative staining-stain body of cell with crystal-violet. Add India ink which doesn't penetrate capsule. Prepare slide. Will see dark cells with India ink around them. If have capsules, they'll be colorless.
b. quellung reaction/ capsular swelling
1. technique where treat capsule w/ antibodies that change properties of capsule, so capsule becomes visible under the microscope
5. roles of capsule
a. protection from:
1. drying out
2. bac phage
3. phagocytosis
A. virulence factor- resistant to phagocytosis by WBC
b. adhesion
1. ex: S. mutans- org that causes tooth decay can stick to teeth b/c of capsule and produces acid that destroys teeth

H. Flagella- long projection coming from body of cell
1. role- motility, rotates like propellor of ship, moving bac cell through medium
2. diagnostic microbio- 2 ways to see if cell is motile:
a. hanging drop technique
1. Use well slide- slide with depression in middle, put drop of culture on coverslip and invert cover slip so drop hangs down. Then put slip on slide. If cells are non-motile, they will wiggle randomly. If motile, they will swim in a more direct motion across the field.
b. semi-solid agar
1. Add agar to liquid to make it gel. Stab needle with bac into semi-solid agar (more liquid and less cross-strands of polysaccharides). Incubate overnight. If org is non-motile, bac growth will only be along stabline. If org is motile, growth will be along entire tube.
3. size:
a. 3-12 microns long (micron=1 millionth of a meter)
1. bac cell is only around 1 micron long
b. narrow- 12-25 nm (1 thousandth of a micron), so can't really see them
4. 2 techniques to see flagella under microscope:
a. dark field microscopy- special microscope
b. stain slide with tanning acid which clumps onto flagella and makes it visible under light microscope
5. types of flagella:
a. polar- flagella only on poles
b. peritrichous- many flagella coming out from cell body in all directions
1. proteus- type of peri. bac cell with swarming growth that forms concentric rings of growth in solid agar instead of staying put

6. composition- 3 parts:
    a. filament
        1. protein, long extending part of flagella coming off cell that extends through hook and basal body
        2. rotates in rings (cell is motor mechanism-converts chemical energy into mechanical motion)
        3. antigenic- H-antigen (can use typing sera)
    b. hook
        1. gives flagella 90 degree turn
    c. basal body
        1. made up of 4 rings:
            a. L- outer membrane
            b. P- cell wall
            c. S- periplasmic space
            d. M- cell membrane
        2. exert pressure on filament while staying fixed, so flagella moves

I. Pili- thinner, shorter, and straighter than flagella
    1. 2 types:
        a. conjugal pili (sex pili)- few in #, 1-4 per bac cell
        b. common pili- 100-200 per cell
    2. role- adhesion
        a. conjugal pili- role in conjugation
            1. conjugation- transfer of DNA from male to female cells (E. coli)
            2. male cell contains F- factor= a plasmid
                A. extrachromosomal DNA- exists in cyto, separate and distinct from chromosome of DNA of cell
                B. it's circular
                C. self-replicating- separate from chromosome replication
                D. has ability to transfer by conjugation- goes from male to female cell which requires cell-to-cell contact aided by pili
                E. some have genes for one or multiple antibiotic resistance
                    1. r-factors= resistance factors
                    2. ex: methocylin resistant streptococcus aureus
                    3. can transfer this resistance to other cells by conjug
        b. common pili-adhesion to glycoproteins on cell surface of patient.
            1. necessary to cause infection- 2 ex:
                A. gonococcus- causes gonorrhea if has pili
                B. enteropathic E. coli- only can colonize in dig tract and cause infection if has pili, otherwise it's normal flora

II. Internal structures of a bacterial cell
    A. Ribosomes
        1. responsible for protein synthesis
        2. polyribosomes
            a. occurs when a few ribosomes bind to mRNA as it's still being transcribed from DNA by RNA polymerase
            b. potential for large amount of protein synthesis b/c don't have to wait for mRNA to be made and then for 1 ribo to make all the amino acids for it
        3. varies according to growth conditions:
            a. rich medium-many nutrients, rapid growth, lots of protein syn, many ribo
            b. poor medium/ minimal medium- slow growth, few ribo

B. Mitochondria-like particles
1. found in cell mem
2. carry out electron transport and oxidative phosphorylation

C. Nucleoid= DNA
1. single, circular chromosome
2. double helix, backbone of sugar phosphates
a. phosphates have negative charge
b. to squeeze DNA into cell mem w/o the phosp repelling each other requires polyamines
1. they're small basic molecules w/ pos charge and low molecular weight that bind to phosph and neutralize charge
2. they allow DNA to collapse and compact, aid in folding of DNA
A. Klanchidt technique- break cell, release DNA, see it's twisted
3. ex: putrescine, spermidine

D. Storage granules
1. role- serve as food reserves
2. come in 3 forms:
a. as polysaccharides (carbohydrates)
1. ex: glycogen- found in enterics (org w/ intestines)
b. as lipids
1. ex: poly-B-hydroxybutyrate- found in bacillus and pseudomonas
(a chain of salt form of butyric acid with hydroxyl group in beta position)
c. as polyphosphates/ volutin granules
1. ex: found in:
A. corynebacterium diptheriae
B. mycobacterium tuberculosis
2. has metachromatic staining- stain has 1 color in bottle on shelf and a different color when stain bac cells- helps identify organism

E. Small molecules dissolved in cytoplasm
1. salts, sugars, amino acids, vitamins, metabolic intermediates, cofactors

## ***Test 2 Material***

I. Viable Counts
A. have flask with bac culture and want to know cells/mL
B. set up series of dilution tubes, each with 9 mL of broth
C. put 1 mL from flask into 1 of the tubes so have $10^{-1}$ dilution
D. put 1 mL of that into next tube, so have $10^{-2}$ dilution
E. keep doing this=serial dilution
f. if have $10^{-6}$ dilution in last tube and after incubation of 1 mL on petri dish, it has 100 colonies, then...
1. originally it had $10^{2} \times 10^{6} = 10^{8}$ cells/mL
G. if plate same dilution w/ .1 mL and get 100 colonies, then ...
2. originally it had $10^{9}$ cells/mL b/c have to multiply by 10 b/c it's .1mL
H. this is the only way that gives a viable measurement

II. Bacterial Growth Curve
- A. description of graph
  1. plot the log of the number of viable cells vs. time
  2. take a small sample of cells that grew up overnight and put them into fresh medium- time 0
  3. first no change in number of cells=lag phase
  4. then period of doubling/logarithmic growth=log phase
  5. number of cells stays the same/levels off=stationary phase
  6. number of cells starts to decrease=death phase
- B. limits to growth of a cell
  1. nutrient depletion
     a. run out of C sources, amino acids...
  2. buildup of toxic products, usually acids
     a. as acids increase, pH decreases- becomes too acidic
     b. could be other toxic molecules produced that could accumulate and inhibit growth
- C. steps to growth
  1. lag phase
     a. cell has to...
        1. adapt to new medium=readjust its internal pH from acidic to around 7
        2. take up nutrients from medium before it can grow
        3. synthesize new enzymes
           A. was process of protein degradation b/f, so need new copies of protein
        4. induce enzyme synthesis- go through regulatory processes to decide whether to make protein
           A. s/t need to synthesize regulatory factors to turn on genes to synthesize enzymes
     b. increase in cell size w/o an increase in cell number
     c. length of lag phase depends on a number of factors
        1. length of time of previous stationary phase from when incubated culture
           A. long stat phase (1-3 days)
              1.culture is depleted, missing nutrients and proteins, very acidic
              2.needs long lag phase
           B. short stat phase (2-3 hours)
              1. culture has proteins and nutrients, not too acidic
              2. needs short lag phase
        2. composition of medium
           A. rich medium
              1. has amino acids, vitamins, and nutrients, so culture can get them easily
              2. needs short lag phase
           B. minimal medium
              1. only has glucose, so needs to make vitamins and amino acids
              2. needs long lag phase
        3. nature of organism- vary in efficiency and mechanism
           A. some org do things rapidly
              1. need short lag phase
           B. some org do things slowly
              1. need long lag phase

2. transition phase
    a. there's a smooth curve b/t lag and log, not sharp break b/c...
        1. not every cell is ready at the same time for log phase
        2. not every reaction in the cell is ready to proceed at its log phase rate
3. log phase
    a. growth is logarithmic- a curve
        1. therefore, plot log number of cells vs time, not number of cells vs time
        2. this gives a straight line, so can calculate generation time
        3. slope of line shows rate of growth
    b. this is a period of...
        1. rapid growth
        2. rapid metabolism
        3. balanced growth
            A. every 20 mins, cell wall, rib, and chro are doubling- e/t coordinated
4. transition phase
    a. smooth curve b/c
        1. not every cell gets depleted at the same time
        2. not every cell slows down at the same time
5. stationary phase
    a. 2 points of view about what's happening:
        1. growth stops, culture runs out of nutrients, becomes too toxic so levels off
        2. cell growth is exactly equal to cell death
    b. this a period where...
        1. storage molecules are being made
        2. synthesis of capsule begins
        3. some cells make antibiotics- for 2 reasons:
            A. limited food supply, so kill competition to gain more nutrition
            B. to lyse the other bac cell to get nutrients from inside it
        4. sigma factors appear
            A. stationary phase is a new state of existence, so need new factors
            B. they turn off log genes and turn on stationary genes
6. death phase
    a. this is a period of depletion of energy reserves
    b. therefore, have loss of selective permeability
        1. b/c can't do active transport
    c. lysis=cell death b/c can't regulate ions into and out of the cell
    d. death is exponential like log phase
        1.lose a certain fraction of population per unit time
            A. under light microscope these cells look different
            B. cells are elongated, swollen, and distorted
    e. rate of death depends on:
        1. environment
            A. how starved, toxic, acidic it is
        2. nature of organism
            A. some fragile- start dying when not enough nutrients
            B. some can withstand harsh conditions

E. limits to growth of a colony

1. nutrient depletion
    a. oxygen has to diffuse through all cells to get to the bottom, stops after a while
2. toxic product buildup

F. kinds of info we get from growth curve
1. info about organism itself
a. some grow faster, others slower
2. info about medium
a. some support rapid rate of growth, others slower
3. add/vary C-source
a. see if it supports rapid rate of growth, slow growth, or no growth
4. add/vary molecule such as antibiotic
a. see if it will inhibit growth of these bac cells

## ***Test 3 Material***

I. Oxygen Requirements
A. classification by oxygen requirements
1. aerobes
a. require oxygen in high concentration: 10-20%
2. microaerophiles
a. require oxygen in low concentration: 2-10%
3. anaerobes
a. obligate anaerobes
1. can only grow w/o oxygen
b. facultative anaerobes
1. can grow in presence or absence of oxygen
B. why some species are obligate anaerobes
1. during electron transport, process of flavoprotein oxidation generates superoxide radical
a. superoxide radical is oxygen w/ an extra electron, so has net negative charge
b. it's highly reactive and damages DNA, proteins, and membranes- lethal to cell
2. superoxide dismutase is needed to degrade superoxide radical
a. carries out reaction: $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$
3. catalase is needed to degrade hydrogen peroxide which is also lethal to the cell
a. carries out reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$
4. which types of bac have these enzymes?
a. aerobes have both enzymes, so can grow in a lot of oxygen
b. microaerophiles have dismutase, but not catalase
1. but they have peroxidase which breaks down peroxide, too, but more slowly
2. therefore, they can only survive in limited amounts of oxygen
c. obligate anaerobes do not have these enzymes, so die if grown in any oxygen
C. types of obligate anaerobes
1. spore-forming
a. Clostridium tetani
1. causes tetanus
2. produces neurotoxin which causes spastic paralysis
A. all muscles of body contract and can't relax
B. problem b/c diaphragm needs to move in order to breath
b. Clostridium botulinum
1. causes botulism
2. produces neurotoxin which causes flaccid paralysis
A. all muscles of body relax and can't contract
B. problem b/c diaphragm can't move

c. Clostridium perfringens
1. causes gas gangrene
A. org produce gas in muscles, causing muscle fibers to explode
2. comes from shrapnel, bullet wounds- used in military context
2. non-spore forming
a. bacteroides fragilis
1. most common org in gut/intestinal tract
b. fusiform bacteria
1. found in mouth- little pockets b/t teeth and under gums
2. cause infection after oral surgery when enter bloodstream

D. how to handle obligate anaerobes
1. how to transport sample from patient to lab
a. use anaerobic culturette
1. soft plastic tube w/ screw cap w/ swab attached to cap
2. after swabbing patient, cover tube tightly
3. break ampule filled with chemicals inside tube
4. this causes chemical reactions to remove oxygen from the tube
2. how to grow up org in the lab
a. use thioglycollate broth- thioglycollate removes oxygen from the broth
b. use gas pak if grow on solid media
1. put gas pak in w/ petri dishes in large chamber that seals tightly
2. add water to gas pak which starts reaction to use up oxygen in chamber

## ***Test 4 Material***

I. Basic Nutritional Requirements
A. carbon
1. forms backbone for synthesis of small molecules in the cell
2. energy source
B. nitrogen
1. found in nitrogen-containing compounds, such as proteins and coenzymes
C. phosphorus
1. found in nucleic acids, ATP, and a large number of phosphorylated compounds
D. sulfur
1. found in cysteine and methionine (amino acids) and coenzyme A
E. potassium
1. needed for enzymatic reactions, such as protein synthesis
F. magnesium
1. found in DNA-metabolizing enzymes
2. stabilizes DNA and ribosomes
a. 2 positive charges of Mg bind to the negative charges of the phosphates in DNA
G. iron
1. found in cytochromes- part of electron transport system
H. cobalt
1. found in the vitamin B-12
I. calcium
1. involved in enzymatic reactions and sporulation
J. zinc, copper...
1. trace minerals found in metalloenzymes- enzymes that have metals as part of their structure

II. Glycolysis=Embden-Meyerhoff Pathway

A. glucose (6 C)

↓ ATP ↓ ADP

glucose-6-phosphate (6 C)

↓

fructose-6-phosphate (6 C)

↓ ATP ↓ ADP

fructose 1,6-diphosphate (6 C)

↓ ↓

dihydroxy acetone phosphate (DHAP) (3 C) ⟶ glyceraldehyde-3-phosphate (3 C)

↓

1,3-diphosphoglyceric acid (3 C)

↓ ADP ↓ ATP

3 phosphoglyceric acid (3 C)

↓

2 phosphoglyceric acid (3 C)

↓

phosphoenol pyruvic acid (PEP) (3 C)

↓ ADP ↓ ATP

pyruvic acid (3 C)

↓ → $CO_2$

acetyl coA (2 C)

III. Kreb's Cycle=Tricarboxylic Acid Cycle=Citric Acid Cycle

A. acetyl coA (2 C)

↓

oxaloacetic acid (4 C) ⟶ ⟶ ⟶ citric acid (6 C)

↑ ↓

malic acid (4 C) ↓

↑ cis-aconitic acid (6 C)

fumaric acid (4 C) ↓

↑ isocitric acid (6 C)

succinic acid (4 C) ↓

↑ ↓ → $CO_2$

succinyl coA (4 C) ⟵ ⟵ $\alpha$ - ketoglutaric acid (5 C)

↓

$CO_2$

B. glycolysis + Kreb's=breakdown of glucose into 6 carbon dioxides

IV. Pentose Cycle=Hexose Monophosphate Shunt

A. glucose (6 C)

**starts here**: ↓ ↓ ATP ↓ ADP

glucose-6-phosphate (6 C) → 6-phosphogluconic acid (6 C) → ribulose-5-phosphate (5 C) → xyolose-5-phosphate (5 C)

↓ ↓

fructose-6-phosphate (6 C) ← ← ← ← ribose-5-phosphate (5 C)

V. What can bac grow on as carbon source?

A. any molecule which can directly or indirectly fit into one of the intermediates of the central pathways (glycolysis, Kreb's, and pentose)

1. lactose- can be converted into galactose and glucose (glycolysis)
2. galactose- can be converted to glucose (glycolysis)
3. glucose (glycolysis)
4. fructose- can be converted to fructose-6-phosphate (glycolysis)
5. glycerol- can be converted to glycerol-phosphate and then to DHAP (glycolysis)
6. pyruvate- can be converted to pyruvic acid (glycolysis)
7. acetate- can be converted to acetyl coA (glycolysis)
8. citrate- can be converted to citric acid (Kreb's)
9. succinate- can be converted to succinic acid (Kreb's)
10. ribose- can be converted to ribose-5-phosphate (pentose)
    a. in the cell, these molecules exist in acid form (ex: citric acid, pyruvic acid), but we add salt form to medium (ex: citrate, pyruvate)

VI. Internal Sources of Nutrition

A. protein breakdown

1. bac break their own proteins into amino acids
2. amino acids are exposed to the enzyme deaminase which removes the amine groups
3. then they're exposed to decarboxylase which removes the carboxyl groups
4. the molecules that are left are just chains of carbons that can go through glycolysis and/or Kreb's cycle by serving as carbon source directly or indirectly

B. lipid breakdown

1. bac break their own phospholipids into fatty acids and glycerol via phospholipases
    a. glycerol can be converted into DHAP (glycolysis)
    b. fatty acids can be converted into acetyl coA (glycolysis)

C. carbohydrate breakdown

1. bac break their glycogen into glucose (glycolysis)

## ***Test 5 Material***

I. Regulation

A. control at the level of transcription- determining whether or not transcription will take place

1. background info
    a. regulatory gene causes the formation of a protein called a repressor
    b. operon
        1. it's a seq of DNA that consists of structural genes and regulatory sites and all genes are turned on or off by the same control (b/c mRNA synthesis is turned on or off for all of them by the same regulatory control)
        2. reg sites=binding sites for proteins; seq of DNA bases that don't code for mRNA
            A. promoter- binding site for RNA polymerase (transcription)
            B. operator- binding site for repressor

3. ex: of operon is lac operon
    A. it has a few parts:
        1. promoter
        2. operator
        3. z gene- codes for B-galactosidase
            A. enzyme that splits lactose into glucose and galactose
        4. y gene- codes for lactose permease
            A. enzyme that decides whether lactose will enter cell or not
        5. a gene- codes for transacetylase
            A. enzyme that we don't know what it does
    B. all these genes are turned on or off together = operon
    C. i gene makes repressor which can bind and turn off transcription

2. negative control
    a. if protein binds, it turns off transcription
    b. 2 types:
        1. induction
            A. the story of a degradative pathway- C-source utilization
                1. if you don't have substrate in medium, don't make enzyme needed to metabolize that C-source
                2. so if no lactose, turn off transcription of gene for B-galactosidase
            B. How does this happen?
                1. if no lactose in medium
                    a. repressor binds to operator site
                    b. RNA polymerase binds to promoter, but is blocked from continuing down operon and transcribing z, y, and a genes
                2. if lactose added to medium
                    a. lactose (inducer) binds to repressor
                    b. this causes conformational change in the repressor
                    c. this causes repressor to come off operator
                    d. transcription can now proceed
        2. repression
            A. the story of a biosynthetic pathway
                1. when you have a large amount of end product, you don't transcribe the genes to make the enzyme for the synthesis of that product
            B. How does this happen?
                1. if no end product
                    a. repressor doesn't bind to operator site
                    b. transcription proceeds and makes all enzymes needed for the pathway to make that product
                2. if end product is added
                    a. end product binds to repressor (made by R gene)
                    b. this causes conformational change in repressor protein
                    c. this causes repressor to bind to operator site
                    d. this blocks transcription

3. positive control
    a. if protein binds, it turns on transcription
        1. ex: catabolite repression
            A. in the presence of glucose, you don't transcribe the genes to make the enzyme needed to metabolize a poorer C-source (ex: lactose)
            B. so if have lactose and glucose in medium, cell will only use glucose and won't make B-galactosidase to metabolize lactose
            C. How does this happen?
                1. background info
                    a. CAP protein = catabolite activator protein
                    b. CAP binds to promoter site- necessary for transcription
                    c. CAP binding requires cAMP (which needs to bind to CAP)
                    d. glucose inhibits cAMP synthesis
                2. no glucose, yes lactose—>induction (explained b/f)
                3. yes glucose, yes lactose
                    a. causes cAMP to decrease so doesn't bind to CAP
                    b. this cause CAP protein to be unable to bind to promoter
                    c. transcription can't take place

II. Gene Transfer
A. transformation
1. uptake by a recipient cell of naked DNA released by a donor cell
    a. naked DNA = only DNA
    b. donor cell was lysed and released DNA that can be taken up directly by recipient cell
2. species that can undergo transformation:
    a. pneumococcus
    b. Bacillus
    c. E. coli- normally can't, only can one of 2 ways:
        1. when raise calcium level
        2. when use electroporation- expose it to elec current- makes cell permeable to DNA
3. org can only undergo transformation when they are competent
    a. protein has to build up to nec level in order for cell to be able to undergo transformation
    b. this state only happens during late log- early stationary phase of growth curve

B. conjugation
1. gene transfer by sexually differentiated bacteria
    a. requires cell-to-cll contact
2. 3 types:
    a. F+
        1. male and female cells of E. coli with male cells containing F factor
            A. F factor is a plasmid which is...
                1. extrachromosomal DNA (in cytoplasm)
                2. circular
                3. self-replicating
                4. able to be transferred by conjugation from male to female cells
                    a. requires conjugal pili
            B. only one strand of F factor transfers
                1. then single strands in both donor and recipient replicate
        2. occasionally F integrates into donor DNA
            A. F has a particular insertion site on chromosome- not random
            B. F DNA goes into DNA chromosome without messing it up
            C. when F transfers from donor to recipient, it carries genes from bac chr w/ it
            D. this is gene transfer, but gene is still not functional

3. need recombination to make gene functional
A. recipient gene and donor gene (from fragment) that code for the same thing line up by homology (all bases of gene are the same except 1 mutation)
B. reciprocal exchange of DNA (recombination)
1. gene from donor fragment and recipient gene switch
2. female cell is haploid for that gene- has only one copy
3. still female cell b/c only get a fragment of F

b. Hfr- high frequency recombination
1. same thing as F+, just F is stably integrated into the donor cell chromosome
2. F factor transfers easily and frequently, carrying genes from donor cell
3. there's reciprocal exchange of DNA like w/ F+, resulting in haploid female
4. can take place in E. coli cells

c. F prime factors
1. F is integrated into donor cell
2. know F insertion site is next to a specific gene, ex: lactose
3. 2 possibilities:
A. n/t happens: F leaves chr properly, so have bac chr and F factor separately
B. by error, loop of F factor is too big, and it takes the lac gene with it (can only take 1 gene b/c only 1 gene can become integrated into F factor)
1. F prime lac (F factor + lac) becomes integrated into female recipient
a. insertion site has base sequence that separates
b. F factor has base sequence that separates
c. each part of base sequence of F factor matches each part of base seq of insertion site on recipient chromosome
d. since F factor contains lac, it becomes integrated, too
2. therefore, recipient cell is diploid- has 2 copies of lac gene

C. transduction
1. transfer of genetic material from donor to recipient via a virus vector
a. bac virus = phage
1. it's DNA or RNA inside a protein coat
2. 2 types:
A. lytic phage
1. T4- ex: of lytic phage
2. parts of phage: head (w/ DNA inside), tail, base plate, and tail fibers
3. steps to lytic cycle:
a. phage binds to outer surface of bac cell (w/ high specificity)
b. phage inserts its DNA into bac cell- like hypodermic syringe
1. proteins remain outside the cell
c. ecclipse period- bac cell makes many copies of phage DNA
1. DNA is expressed to make dif parts of phage
d. latent period- time of assembly- dif parts of phage are put tog
1. make about 100 phage particles
e. lysis of bac cell
f. reinfection- 100 phage particles reinfect 100 bac cells to make 10, 000 phages which reinfect 100 more= 1,000,000

B. lysogenic phage
1. undergoes lysogeny
2. ex: phage lambda- can undergo a lytic cycle or lysogeny
3. phage DNA is injected into bac cell and incorporated into bac DNA
1. site-specific lambda insertion- does not mess up bac DNA
2. lamba inserted into bac cell = prophage
4. lambda codes for CI repressor which shuts off all lambda genes, so no phage parts are made inside bac cell (phage hides out and is quiet)

2. 2 types of transduction (transfer of genetic material from donor to recipient):
a. generalized transduction
1. the story of lytic phage
2. s/t by error, some bac DNA gets packaged inside phage head instead of phage DNA
3. there's lysis and reinfection- phage acts as transducing phage- carries fragment of donor DNA and injects DNA into recipient cell
4. then have homology and recombination to integrate donor DNA into recipient cell
A. this is gene transfer from donor to recipient via virus vector
B. process can occur anywhere along recipient chromosome- w/ any gene
5. product is a haploid cell
b. specialized transduction
1. the story of lambda (and other lysogenic phages)
2. there's a lambda insertion site b/t the gal and bio genes
A. gal used for galactose utilization and bio used for biotin- a vitamin
3. 2 possibilities:
A. lambda is be induced to come out and goes through regular lytic cycle
B. lambda is induced to come out, but loop is too big, so it carries another gene w/ it- gal (or bio)
1. goes through lytic cycle and 100 copies of lambda w/ gal are made
2. then there's lysis and reinfection
a. lambda w/ gal enters recipient cell and goes through lysogenic stage where it gets incorporated into recipient chr
3. this results in a diploid cell- 2 copies of gal gene
4. this is specialized transduction b/c can only take genes right next to it