

Genetics- Microbiology Test 5

I. General Overview

A. replication

1. info coded in the sequence of bases of DNA tells cell how to make identical copies of that DNA

B. transcription

1. info coded in the sequence of bases of DNA tells cell how to make sequence of bases of mRNA
 - a. mRNA is involved in information flow

C. translation

1. sequence of bases in mRNA tells cell how to put together sequence of amino acids (= protein)

D. mutations

1. errors in the sequence of bases of DNA
2. there are other forms of DNA damage, but there are also mechanisms for DNA repair

E. regulation

1. determines whether transcription will take place

F. gene transfer

1. the ability to transfer genetic material from one cell to another (from donor to recipient)

II. DNA Structure

A. 2 parts

1. backbone

- a. alternating sugars and phosphates in a long chain
 1. sugar is deoxyribose
- b. sugars are connected to phosphates at the 3 prime and 5 prime hydroxyl ends
- c. the DNA strand has a free 3 prime and 5 prime hydroxyl at the ends
- d. since DNA has a 3 prime and 5 prime end- it has directionality

2. bases

- a. attached to sugars
- b. 2 types:
 1. purines- adenine and guanine
 2. pyrimidines- cytosine and thymine

B. double helix

1. 2 chains, each composed of sugars and phosphates with sugars attached to bases
2. the bases of the 2 chains are attached through hydrogen bonds
3. chains are wrapped around each other (double helix) with backbones outside and bases inside
 - a. like spiral staircase with base pairs as steps
4. imp't info about double helix:
 - a. the 2 strands are antiparallel
 1. one goes in 3 prime to 5 prime direction, and the other goes 5 prime to 3 prime
 - b. helix is right-handed
 1. if you're looking down from top, it goes in clockwise direction
 - c. helix is symmetrical
 1. if dropped central line down middle, all parts would be symm around it
 - d. 10 residues per turn
 1. if you go from one base to a base one level above or below it, you pass 10 bases
 - e. base-pairing is highly specific
 1. A binds w/ T, and G binds w/ C
 2. Watson and Crick discovered structure of DNA and won Nobel Prize in 1953
 - A. they said model explains replication and transcription b/c of base-pairing
 - B. copy DNA by base-pairing to make complementary strand

C. different levels of DNA structure

1. primary structure
 - a. sequence of bases (ex: AATTGC)
2. secondary structure
 - a. helical arrangement
 - b. 3 types:
 1. B-DNA- e/t we said b/f refers to this type
 2. A-DNA
 - A. form of DNA only seen in laboratories
 - B. has 11 residues per turn
 3. Z-DNA
 - A. left-handed helix (counterclockwise)
 - B. bases zigzag (so called Z-DNA)
 - C. 12 residues per turn
 - D. bases are turned or flipped
 1. they're rotated relative to each other in all 3 planes
 2. can flip outside backbone instead of being bonded to each other
 - E. believed to have a role in regulation (DNA- protein binding)
 1. in regulation, protein has to bind to DNA to turn on transcription
 2. Z-DNA is imp't in determining if reg will take place b/c certain proteins can only bind to Z-DNA
3. tertiary structure
 - a. arrangement of genome
 - b. bac genome is...
 1. circular (double-stranded DNA is connected in circle)
 2. supercoiling
 - A. like twisting rubberband around (complete circle, but coiled)

III. Replication

- A. it starts from origin (not randomly on chromosome)
- B. it's bidirectional
 1. goes clockwise and counterclockwise from origin until the terminus (180 degrees from origin)
- C. process of replication:
 1. unwind DNA to get single strands
 2. DNA polymerase III (enzyme) copies each DNA strand by adding complementary bases
 - a. units being added are nucleotides = sugar-phosphate-base
 3. replication forks move and gets longer until have 2 double-stranded DNAs
 - a. replication fork is area where replication is taking place
 - b. have 2 forks starting from origin moving in opposite directions
 4. newly formed DNA has one old strand and one new strand = semi-conservative replication

IV. Transcription

- A. it's local- transcribes 1 gene out of the 1,000's on a chromosome
 1. dif than DNA replication which copies the entire chromosome
- B. process of transcription:
 1. localized bubble- unwinding to expose a single strand of DNA
 2. RNA polymerase attaches to DNA and starts moving across
 3. mRNA leaves and DNA closes up
- C. RNA is different from DNA in 3 ways:
 1. uracil instead of thymine
 2. ribose instead of deoxyribose
 3. single-stranded instead of double stranded

V. Genetic Code

- A. language of genetic code is in terms of mRNA- seq. of bases of mRNA is translated into amino acids
- B. unit of information is a codon
 - 1. codon- triplet of bases (so genetic code is a triplet code)
- C. code is degenerate
 - 1. this means more than one codon can code for the same amino acid
 - 2. since there are 4 bases that need to fill 3 positions in a codon, $4 \times 4 \times 4 = 64$ possible codons
 - a. 61 codons code for amino acids
 - 1. since there are only 20 amino acids, more than 1 codon codes for the same aa
 - b. 3 are termination codons- tell cell to stop making proteins
 - 1. UAA, UAG, and UGA
- D. code is colinear
 - 1. this means that 1st triplet codes for 1st amino acid, and 2nd triplet codes for 2nd amino acid...
 - 2. it's colinear w/ DNA, too- 1st triplet of DNA codes for 1st triplet of RNA which codes for 1st aa...

VI. Translation

- A. involves transfer RNA (tRNA)
 - 1. single-stranded RNA w/ a complex clover leaf structure due to intramolecular hydrogen bonds
 - 2. contains 2 essential parts:
 - a. an open end which binds an amino acid
 - b. an anticodon = sequence of 3 bases which is complementary to 1/61 mRNA codons
 - 1. ex: if mRNA codon = GGG, tRNA anticodon = CCC
 - 3. the aa that tRNA is carrying is the one that will be added when anticodon and codon match up
- B. process of translation:
 - 1. mRNA binds to small subunit of ribosome
 - a. ribosome is made up of a 30 s subunit and a 50 s subunit, but it's 70 s all together
 - 2. 1st codon is in the reading frame- window that exposes only 3 bases at a time (1 codon)
 - 3. codon-anticodon interaction = secret of translation
 - a. this is how code of bases gives seq of aa
 - b. cytoplasm is filled w/ 61 dif tRNA molecules, each with an aa
 - c. only the 1 tRNA molecule with the right seq of bases in anticodon will match seq of bases of mRNA codon
 - 4. ribosome moves over to put the next codon in the reading frame
 - 5. bring in the next tRNA carrying an aa
 - 6. have peptide bond formation b/t the 2 aa to connect them
 - 7. keep adding aa to growing peptide chain until get to termination codon
 - 8. then e/t stops and protein and mRNA leave ribosome
 - a. can make many mRNA and many proteins from one gene

VII. Mutations

- A. errors in the primary structure of DNA (base sequence)
- B. types of mutations w/ regard to change in structure
 - 1. base substitution- putting the wrong base in
 - 2. frameshift- move the entire sequence over through...
 - a. insertion- adding in an extra base or 2 bases
 - 1. changes all codons from that point on b/c sequence shifted
 - 2. this will make a full protein w/ the wrong amino acids
 - 3. if add in 3 bases, protein will have the same amino acids, just an extra one, too
 - b. deletion- removing a base or 2 bases
 - 1. changes all codons from that point on b/c sequence shifted
 - 2. this will make a full protein w/ the wrong amino acids
 - 3. if remove 3 bases, protein will have the same amino acids, just missing one aa

C. types of mutations w/ regard to effects

1. silent
 - a. change in DNA—> new codon in mRNA, but it codes for the same aa as b/f, so no effect
2. missense
 - a. change in DNA—>new codon in mRNA which codes for different aa
 - b. 2 possibilities:
 1. no effect (ex: if it's 499th aa out of 500)
 2. causes loss of activity of an enzyme b/c...
 - A. occurs at active site
 1. active site...
 - a. must have specificity for its substrate
 - b. has activity- breaks apart molecules, combines them...
 2. if an aa changed at active site, enzyme can't function
 - B. affects folding of the enzyme
 1. affects active site b/c not folded to the right shape
 2. then substrate can't fit in or active site loses its activity
 3. this causes enzyme to not be able to function
3. nonsense
 - a. change in DNA changes codon in mRNA to termination codon (so doesn't make aa)
 1. ex: UAC which codes for tyrosine becomes UAG- a termination codon
 2. this stops translation before a protein is even made

VIII. Mutagens (what cause changes in the structure of DNA)

A. 3 types:

1. physical
 - a. ultraviolet light
 1. causes thymine dimers
 - A. a covalent bond forms between 2 adjacent thymines on a DNA strand
 - B. repair mechanisms may cause errors while getting rid of the dimers
 1. can introduce new bases or change bases = mutations
 - b. x-rays/ionizing radiation
 1. cause formation of free radicals which cause single-strand breaks in DNA
 - A. breakage in the bond between sugar and phosphate (backbone) of DNA
 2. may be errors during repair causing mutations
2. biological
 - a. phage mu
 1. phage inserts its DNA anywhere in bac cell DNA, even in the middle of a gene
 2. phage can put 1,000's of phage bases in the middle of bacterial DNA = mutations
3. chemical
 - a. methyl methane sulfonate
 1. methylates bases
 2. this messes up base-pairing b/c bases can't match up properly, causing mutations
 - b. bromouracil
 1. it's a base analog of thymine b/c...
 - A. thymine is methyl uracil
 - B. bromine atom is physically and structurally similar to methyl group
 2. gets incorporated into DNA, messing up base-pairing and causing mutations
 - c. acridine orange
 1. it's a large, flat, planar molecule that works by intercalation- acts as a wedge
 2. it stretches double helix of DNA, distorting it and changing its shape
 3. therefore, when that structure is copied, the wrong bases get put in = mutations

IX. DNA Damage

A. structural changes in the DNA (not just changes in base sequence)

B. 4 ex:

1. methylated bases (from methyl methane sulfonate)
2. thymine dimers (from UV light)
3. single-strand breaks (from x-rays)
4. cross-linkage
 - a. connecting 2 different strands in DNA covalently
 - b. it's a solid strand- does not allow separation like hydrogen bonds do
 - c. thymine dimers connect thymines on same strand; this links 2 dif strands

X. Repair Mechanisms

A. direct repair

1. ex: photoreactivation

- a. requires the enzyme photolyase which requires light
- b. photolyase breaks covalent bonds of thymine dimers, so thymines are normal again

B. indirect repair

1. ex: excision repair

a. repairs damaged DNA through 4 steps, each catalyzed by a different enzyme

1. endonuclease

A. breaks the bond between sugar and phosphate to separate damaged DNA from good DNA

2. exonuclease

A. chops damaged DNA off one base at a time, starting from the free end of the DNA, leaving a gap

3. DNA polymerase I (Pol I/Pol A)

A. puts in new bases according to base-pairing rules using the other strand as a template (fills the gap)

B. Kornberg thought this was replication enzyme, but John Cairns proved him wrong. Enzyme is part of replication-like process of excision repair.

4. ligase

A. attaches new bases to old bases, closing the gap completely

XI. 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

B. control at the level of enzyme activity- will protein made by mRNA be active or not?

1. when there's a lot of end product of biosynthetic pw, it shuts off activity of 1st enzyme of pw

2. How can this happen when end product is not a substrate analog- not structurally like first reactant at all- so can't fit into active site of enzyme and mess it up?

a. enzymes are composed of 2 subunits:

1. catalytic subunit- catalyzes conversion of 1st reactant to product (A—>B)

2. regulatory subunit- binds final end product of whole pw (E)

b. when regulatory subunit binds E, it causes a conformational change in that subunit

c. that change causes a conformational change in the catalytic subunit=allosteric interaction

1. allosteric interaction- a change in the structure of 1 subunit causes a change in the structure of the 2nd subunit of the enzyme

2. allosteric interaction = feedback inhibition (FBI)

d. that inhibits the activity of the catalytic subunit

XII. 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-cell 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. eclipse 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. lambda 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 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