Exam #3 Review

Exam #3 will cover from glycolysis to complex gene regulation. This includes all glucose degrading pathways (glycolysis (Embden-Meyerhoff), Entner-Doudoroff, pentose phosphate pathway) as well as fermentation, the TCA and ETC (respiration). It also includes photosynthesis, the Central Dogma of Gene Transfer in prokaryotes (replication, transcription and translation), eukaryotic gene expression, and the regulation of gene expression (the *lac* operon).

Note: On the exam, you will be allowed to use a poster of your own making that summarizes all of the metabolic pathways and gene expression. This poster may not include large blocks / paragraphs of text. It must be a picture. It must be of your own making and it can only be one sheet (of any size). Questions will be geared more toward the understanding of these processes rather than memorization. It is not necessary to memorize most of the enzymes if you remember that the enzymes catalyzing redox reactions in which either NADH/NADPH or $FADH_2$ are formed are named: Reactant name + dehydrogenase. Reactions in which a phosphate group is added generally have a root name followed by -kinase. Enzymes catalyzing rearrangements are generally called either isomerases or mutases.

I. Metabolism (the pathways) - *Note these are for aerobic growth in chemoheterotrophs (remember that these use an organic carbon source for energy and carbon)

A. **Glycolysis** (The Embden Meyerhoff pathway) ("the splitting of something sweet") - glucose, a six-carbon molecule, is converted into two, three carbon pyruvate molecules. Energy released when the high-energy glucose bonds are broken is harvested to form ATP (substrate-level phosphorylation). The continual oxidation of glucose allows for the reduction of NAD+ to form NADH, which (when the ETC is present and functional) carries its electrons to the electron transport chain. Some of the intermediates in glycolysis can be used as precursor metabolites in anabolic pathways.

Practice: Glycolysis occurs in the _____ of eukaryotic cells.

a. nucleus
b. mitochondria
c. cytoplasm
d. vacuoles

*Important points to remember about glycolysis:

1. ATP is expended in steps 1 and 3. Thus, after step 3, two molecules of ATP have been used and no ATP has been generated.
2. Step 3: the phosphorylation of fructose 6-phosphate, is the first **committed step** of glycolysis. This step is catalyzed by **phosphofructokinase**, an enzyme that can be regulated allosterically by many molecules (two of which are ADP and phosphoenolpyruvate). **Why is this regulation important?**
3. In step 4, the 6-carbon fructose-1,6-bisphosphate is split into two 3-carbon molecules. It's important to note that there is an equilibrium between these two 3-carbon molecules so essentially both of these molecules are used in step 6. ****From here on out, every reaction occurs twice for every one molecule of glucose****.

4. Step 6 is the first step in which NAD^+ is reduced to form NADH. **Two NADH** molecules are formed for every one glucose molecule.
5. Two molecules of ATP are generated for every one molecule of glucose in step 7 (powered by hydrolysis of the high energy phosphate bond on 1,3-BPG). This is a **substrate level phosphorylation** **(Explain this by looking back at the table of phosphoryl group transfer potentials)**. At this point in glycolysis, two molecules of ATP have been used and two have been generated. Thus, there is a net gain of 0 ATP.
6. In step 10, in the production of pyruvate, two molecules of ATP are made. This leads to a **net yield of 2 ATP per glucose for glycolysis**.
    - **Practice: Which one of the following statements about glycolysis is FALSE?**
        - **a. Glycolysis occurs within the cytoplasm of both prokaryotic and eukaryotic cells.**
        - **b. During the first step of glycolysis, an ATP molecule is consumed in order to add a phosphate group to glucose. This is a reaction catalyzed by the enzyme hexokinase.**
        - **c. The fourth step of glycolysis during which the 6-carbon fructose 1,6-bisphosphate molecule is split into two 3-carbon molecules is the committed step in glycolysis. The enzyme that catalyzes this reaction is regulated allosterically by ADP.**
        - **d. ATP is created for the first time in the seventh step of glycolysis when the high-energy phosphate bond in 1,3-bisphosphoglycerate is broken.**
        - e. **Glycolysis is a amphibolic pathway that occurs in both obligate aerobes and obligate anaerobes.**

          Practice: Under what set of conditions can glycolysis occur?

            a. anaerobic conditions

            b. aerobic conditions

            c. microaerophilic conditions

            d. all of the above.
7. Don't forget that many of the intermediates in glycolysis can serve as precursor metabolites for anabolic pathways.

**Be sure that you know the net ATP and NADH yield for glycolysis!

- **Practice: If 17 molecules of glucose are oxidized by the glycolytic pathway, what is the net ATP yield for this pathway? How much reducing power is created (# of reduced NADH molecules)?**

B. **The Pentose Phosphate Pathway**

This pathway is an alternate glucose degrading pathway. It is used when biosynthesis is the primary focus of the cell! Its purpose is twofold. The purpose of the oxidative stage is to produce NADPH. The purpose of the non-oxidative stage is to produce ribose 5-phosphate (used in nucleotide synthesis). Sometimes a cell needs more NADPH than it does ribose 5-phosphate. In such a situations, excess ribose 5-phosphate will be converted to intermediates of glycolysis (fructose 6-phosphate and Glyceraldehyde 3-phosphate).

Practice: The pentose phosphate pathway

a. can proceed either in the presence or absence of O_2.
b. generates NADPH.
c. is also termed the hexose monophosphate shunt.
d. would be important in a microorganism trying to synthesize nucleic acids.
e. all of the above.

C. **The Entner-Doudoroff Pathway**

*Understand how this differs from (and yet has similarities with) glycolysis and the pentose phosphate pathway. Particularly note differences in yield.

D. **Fermentation** - a possible fate of pyruvate

- Some organisms are incapable of respiration (lack an electron transport chain) (e.g. the Lactic Acid Bacteria) and sometimes the terminal electron acceptor for respiration is not available (or in short supply). In these cases, the fate of pyruvate changes. Rather than proceeding on to the transition step and TCA, pyruvate or a derivative of pyruvate acts as an electron acceptor, thus producing commercially useful products like ethanol. This allows for the regeneration of NAD+ and thus glycolysis can continue to occur and produce ATP. **When fermentation is used, glycolysis is the only pathway by which ATP can be generated.***

Why doesn't the TCA cycle continue to run in these situations?

IMPORTANT

****The purpose of fermentation is to regenerate the NAD+. This carrier is reduced in glycolysis and must be oxidized so that it can return to glycolysis again to be reduced. This allows glycolysis to continue and thus continue to generate ATP.**

Depending upon the type of fermentation being used, different products are formed. These types of fermentation byproducts vary widely. We talked about two different types of fermentation:

1. **Alcoholic fermentation**

- pyruvic acid is converted to acetaldehyde which serves as an electron acceptor to regenerate NAD+. The byproduct is ethanol. This is the type of fermentation used by yeast when no O_2 is available to serve as a terminal electron acceptor (think about wine making).

2. **Lactic acid fermentation**

- -pyruvic acid itself, serves as the electron acceptor to regenerate NAD+. Lactic acid is the byproduct.

3. The end products of both types of fermentation are commercially useful.

- Practice: Which one of the following is NOT a potential fate of pyruvate?
  a. Pyruvate can be further oxidized to two acetyl groups in the transition step.
  b. Pyruvate can serve as a precursor metabolite for biosynthetic pathways.
  c. Pyruvate can be transported to the electron transport chain where it delivers its electrons to NADH dehydrogenase.
  d. Pyruvate can be utilized as the electron acceptor in fermentation.

D. The transition step (occurs when the fate of pyruvate is further catabolism / oxidiation) - In this step, the two, three carbon pyruvate molecules are converted to two, two carbon acetyl groups. These acetyl groups are attached to coenzyme A (a cosubstrate) to form acetyl-CoA. This is a decarboxylation reaction as two CO_2 molecules are lost (these carbons are in their final, most oxidized state). Also in this oxidation, two NADH molecules are formed.

- Know this reaction and the yield of NADH for this reaction. It is important to realize that this reaction occurs in the cytoplasm of prokaryotic cells but in the mitochondrial matrix of eukaryotic cells. In addition to yielding reducing power in the form of NADH, the acetyl group yielded in this reaction can also serve as a precursor metabolite. Why is this reaction called a decarboxylation??
- Practice: If 45 molecules of glucose are catabolized, how many NAD+ coenzymes are reduced in the transition step.
  a. 45
  b. 55
  c. 90
  d. 22.5
    - Practice: The transition step between glycolysis and the TCA cycle ...
      a. produces two NADH for every molecule of glucose.
      b. occurs in the same place in prokaryotic and eukaryotic cells.
      c. releases CO_2 as a byproduct.
      d. both a and b
      e. both a and c
    - ***Based on your experience in names of enzymes, what is the name of the enzyme that catalyzes this step?**

In what way are the transition step and step 4 of the TCA cycle the same?

a. Both are catalyzed by dehydrogenases.
b. Both occur in the mitochondrial matrix of a eukaryotic cell.
c. Both produce reducing power as well as 1 thoroughly worn out, all used up, fully oxidized carbon in the form of CO_2.
d. Both are characterized by the formation of a bond to coenzyme A (Co-A).
e. all of the above

E. **The TCA cycle** - the two carbon acetyl-CoA molecules are completely oxidized to form CO_2. A great deal of reducing power is generated during TCA in the form of both NADH and $FADH_2$. Some ATP is made via substrate level phosphorylation (step 5) and certain intermediates of the cycle serve as precursor metabolites for anabolic pathways.

*Occurs in the cytoplasm of prokaryotic cells and in the mitochondrial matrix of eukaryotic cells. In this cycle the two-carbon acetyl groups are fully oxidized to CO_2.

*Important points to remember about the TCA cycle:

1. For each glucose molecule, the cycle turns twice.

2. NADH is generated in steps 3, 4 and 8. How many NADH molecules are generated by these three steps for 1 glucose molecule?
3. $FADH_2$ is generated in step 6. Since the cycle turns twice for 1 glucose, there are a total of two $FADH_2$ generated for each glucose. Note-the enzyme that catalyzes this step is also a member of the electron transport chain. This enzyme serves as the common link between TCA and the electron transport chain.
4. ATP is produced by substrate-level phosphorylation in step 5. Two ATP are produced in this step for every glucose molecule.
    - Practice: If 23 molecules of glucose are catabolized, how many molecules of ATP are produced (via substrate level phosphorylation) by the TCA cycle? How many $FADH_2$ molecules and NADH molecules are produced by the cycle?

5. alpha-ketoglutarate produced in step 3 and oxaloacetate produced in step 8 are important precursor metabolites in the synthesis particular amino acids.

***NOTE - the TCA cycle does not directly utilize O_2 (g), however, it produces a great deal of reducing power (NADH and FADH2) that could not be regenerated without an electron transport chain. Thus, the TCA cycle is not used in organisms that lack an electron transport chain or when a terminal electron acceptor is unavailable.**

F. The electron transport chain and ox. phos.

1. Electrons from NADH and those from step 6 of the TCA (the oxidation of succinate to fumarate (FADH2)) are transferred to one of the membrane-embedded carriers of the electron transport chain. These electrons are passed from one carrier to another. Those carriers early in the chain have negative standard reduction potentials whereas those later in the chain have higher and higher positive standard reduction potentials. As electrons are transferred through this chain, energy is released.
    - Do electrons from NADH or electrons from the oxidation of succinate (FADH2) generate more energy?

    The energy released during electron transport is used to create the proton motive force (PMF)!
2. Components of the electron transport chain (ETC) in mitochondria:

*These components span the inner mitochondrial membrane.

- Practice: Which electron carrier in the ETC of mitochondria accepts electrons from NADH and ultimately transfers them to coenzyme Q.
    a. Complex I
    b. Cytochrome oxidase
    c. Complex II
    d. NADH dehydrogenase
    e. both b and c
    f. both a and d
- Practice: Which member of the mitochondrial ETC is a lipid soluble molecule that can move freely in the membrane? This

electron carrier can accept electrons from either NADH dehydrogenase or Complex II.

a. Coenzyme Q
b. Cytochrome c
c. Ctyochrome c oxidase
d. Succinate dehydrogenase

- Practice: Shuttling of electrons through which complex/es of the mitochondrial ETC results in the pumping of protons?

a. Complex II
b. NADH Dehydrogenase
c. Complex III
e. a and b
f. b and c

- Practice: If 5 molecules of NADH are completely oxidized by Complex I of the ETC, how many protons are pumped in total by Complex I, III, and IV of the chain?

3. The Chemiosmotic Theory states that the PMF can serve as an energy source for phosphorylation of ADP to form ATP!
4. ATP synthase - allows protons pumped out during production of the PMF to pass back into the cell ---> uses energy to fuel the phosphorylation of ADP to produce ATP. This is oxidative phosphorylation!
    - Practice: If 5 molecules of NADH are completely oxidized by Complex I of the ETC, how many ATP can be made by oxidative phosphorylation?
5. The components of the ETC in prokaryotes vary widely. Very few bacteria contain the carrier called cytochrome c oxidase and thus an assay that detects this enzyme (the oxidase test) can be used to help identify members of the genera *Pseudomonas* and *Neisseria*.

    The ETC of *E. coli* is commonly used as an example of a prokaryotic ETC. It has two different NADH dehydrogenase complexes and a Succinate dehydrogenase. *E. coli* also has a variety of alternative complexes that allow it to use a variety of energy sources and deliver its electrons to several possible terminal electron acceptors.
6. For eukaryotic, aerobic cells, approximately 3 ATP are produced by oxidative phosphorylation for every NADH. For every $FADH_2$, approximately 2 ATP are produced. Be certain to note that this is simply an approximation to allow for comparison. Also be aware that in prokaryotes PMF can also be used to power flagella rotation and transport --- protons diverted for this purpose do not get used to power the synthesis of ATP.
    - PRACTICE: If 24 molecules of pyruvate enter the transition step in an aerobically respiring eukaryote, how many NET molecules of ATP are yielded via both substrate level and oxidative phosphorylation (Give the total sum from glycolysis, the transition step, the TCA cycle and respiration. Assume aerobic respiration and assume that the NADH molecules generated in glycolysis generate 5 ATP via ox. phos.)?

- **This question is very important, in order to make sure you understand, here is the answer: Answer: 444 ATP molecules**

II. Anaerobic respiration

-The electron transport chain is present but an electron acceptor other than oxygen is used (e.g. in *E. coli*, nitrate may be used; anaerobes called sulfate reducers use sulfate and produce hydrogen sulfide as a byproduct). Anaerobic respiration yields less energy than aerobic respiration.

Practice: Right now, growing deep in the anaerobic depths of our Winogradsky columns is a bacterium called *Desulfovibrio*. This bacterium has the components of the ETC across its cytoplasmic membrane and utilizes sulfur or sulfate as a terminal electron acceptor in a process called _____.

a. anaerobic respiration
b. fermentation
c. photosynthesis
d. induction

III. Catabolism of compounds other than glucose

-Polysaccharides, lipids and proteins can be used as a food source by some prokaryotes. These bacteria must secrete enzymes to digest these macromolecules. The subunits can then be taken into the cell and can enter into catabolic pathways. Be familiar with the enzymes needed for digestion of these macromolecules and the point at which the subunits enter catabolism.

- Practice: A bacterium that can utilize starches as an energy source must secrete which enzyme/s?
    a. alpha-amylase
    b. oligo 1,6-glucosidase
    c. cellulases
    d. lipases and proteases
    e. a and b
    f. c and d
- Practice: Some bacteria such as *Bacillus subtilis* are capable of utilizing fats (triglycerides) as an energy source. At what point in catabolism do the carbon components of the fatty acids enter?

a. The long chain fatty acids are hydrolyzed in 3-carbon molecules that are converted to glyceraldehyde 3-phosphate. This molecule is an intermediate in glycolysis.
b. The fatty acids are degraded 2 carbons at a time to form acetyl-CoA which enters the TCA cycle.
c. The fatty acids are degraded 6 carbons at a time to form glucose.
d. Proteases degrade the peptide linkages in the fatty acid chains and each carbon enters into the TCA cycle.

Take a minute to remember back to the extra credit challenge question submitted in class.

Practice: How does the amount of energy yielded from the breakdown of the 22C long fatty acid side chains of a triglyceride compare to the energy yielded from a glucose molecule?

Practice: The bacterium *Bacillus subtilis* can utilize fatty acids as an energy source. If this bacterium breaks down the side chains of a fatty acid to produce 24 acetyl-CoA molecules, how many ATP can be generated via oxidative phosphorylation from the reducing power produced in the TCA cycle only? (*Note - assume that the ETC of this bacterium yields the same amount of ATP as a mitochondrial ETC.)

a. 216 ATP
b. 264 ATP
c. 48 ATP
d. 528 ATP

** I like this one!

** PLEASE also note how proteins can enter catabolism

IV. Chemoautotrophs thrive in extreme environments and use inorganic compounds as an energy source. These compounds are often byproducts of anaerobic respiration (e.g. hydrogen sulfide).

Practice: The hydrogen bacteria

a. are chemoheterotrophs.
b. are chemoautotrophs.
c. oxidize hydrogen for energy.
d. oxidize hydrogen sulfide for energy.
e. b and c

Practice: Some sulfur bacteria can oxidize hydrogen sulfide as their energy source and eventually form sulfuric acid as a byproduct. Because of their ability to produce this acid, these bacteria are commonly used in biomining to dissolve insoluble Cu and Au in crude ore. These bacteria are

a. photoheterotrophs
b. photoautotrophs
c. chemoautotrophs
d. none of the above

V. Phototrophs

A. Photosynthesis is the capture and conversion of light energy to chemical energy.

-It's not necessary to memorize the overall chemical reaction, however it is important to know that an inorganic carbon source (CO_2) is converted to an organic carbon source. An electron source, often water, is necessary.

1. The light-dependent reactions of photosynthesis are the reactions in which the energy of sunlight is converted into chemical energy in the form of ATP (this is the process happening in photosystems, see below). In oxygenic photosynthesis, water provides the source of electrons. In anoxygenic photosynthesis, a molecule other than water is the electron source (e.g. H_2S).
2. The light-independent reactions of photosynthesis convert CO_2 into an organic carbon source (the Calvin Cycle). These reactions don't directly require light

but they require the reducing power and ATP that are products of the light reactions.

- Practice: Although the light-independent (dark) reactions of photosynthesis do not directly require light, they do require _____ and _____ that are produced during the light reactions.
    a. oxygen, hydrogen sulfide
    b. water, carbon dioxide
    c. ATP, reducing power
    d. cyanobacteria, purple bacteria

3. Be familiar with the pigments required to absorb light energy.
    - Practice: Which one of the following type/s of bacteria is/are likely to utilize bacteriochlorophyll?
        a. the hydrogen bacteria
        b. the purple bacteria
        c. the cyanobacteria

B. Photophosphorylation (oxygenic photosynthesis)

1. The general description of a photosystem: Light of various wavelengths is absorbed by the antenna complex. These light gathering pigments funnel energy toward the reaction center chlorophyll.
    - After absorption of light by the reaction center, an electron is excited to the "top" of an electron transport chain. As always, electrons are transferred through the ETC and a proton gradient is established --> ATP is made = photophosphorylation
2. Oxygenic phototrophs have two photosystems: photosystem I and photosystem II.
    a. When a cell needs to make ATP but NOT reducing power, only photosystem I is used in a process called cyclic photophosphorylation.
    b. When a cell needs to make ATP and reducing power, both photosystem I and II are used in a process called noncyclic photophosphorylation.
    - Practice: During noncyclic photophosphorylation, electrons are syphoned from the electron transport chain of photosystem I to reduce NADP+. How are these lost electrons replenished?
    - In noncyclic photophosphorylation by oxygenic phototrophs, the electrons of photosystem I are diverted from the electron transport chain and used to make reducing power in the form of NADPH. These lost electrons are replenished by photosystem II. How does photosystem II replenish its electrons? (This response requires only a couple of words).

C. The Calvin Cycle (light-independent)

ATP and reducing power generated in the light reactions are used to power the fixation of carbon in the Calvin cycle reactions (What does it mean to fix carbon?) A total of 18 ATP and 12 NADPH are used to make one sugar. So the Calvin Cycle is the cycle during which a phototroph "makes its own food!"

Practice: When we talked about the catabolism of glucose, we looked at the overall glucose combustion reaction. How does this reaction and the ΔG value of this reaction relate to the processes occurring in the Calvin cycle?

Practice: Many reactions of the Calvin cycle

a. are anabolic.
b. are catabolic.
c. are endergonic.
d. both a and c
e. both b and c

Which one of the following is a product of the Calvin Cycle?

a. NADPH
b. ATP
c. glucose
d. water

D. Anabolism - ** PLEASE understand the basic features that differentiate anabolic and catabolic pathways.

VI. Nucleic acids and gene transfer

*Make sure that you are comfortable with the difference between replication, transcription and translation.

A. The **genome** of a bacterial cell includes both the DNA of the chromosome and that of the plasmids.

The following exercise will help you review and better understand the concepts covered in class: Draw the structure of DNA. Be sure to include the 5'-phosphate and the ribose sugar. Simply depict the bases by their letter code. Indicate the 3' and 5' ends of each strand and show the H-bonds and the antiparallel nature of the two strands.

1. When the H-bonds between two DNA strands break = **denaturation** or **melting**.
2. Because of the base-pairing rules, one strand of DNA can always be used as the template for the synthesis of another.

- Practice: In a DNA molecule
  a. there are two antiparallel strands of nucleotides; these strands are joined together by hydrogen bonds. One of the nucleotide strands runs in the 5' to 3' direction and the other runs in the 3' to 5' direction.
  b. , because of the rules of base pairing, one of the strands of DNA can always serve as the template for the synthesis of the other strand.
  c. the hydrogen bonds holding the strands of nucleotides together can be broken in a process called denaturation or melting.
  d. all of the above.

Practice: In what ways is RNA different than DNA?

B. DNA can be transcribed to give three different types of RNA. The one that we most commonly think about is **mRNA**. This type of RNA is simply a messenger and it is always translated to give a protein product. In contrast, neither **rRNA** nor **tRNA** are ever translated. Instead they have very important functions as RNA. rRNA plays a very important role in the translational function of the ribosome. tRNA binds to amino acids and delivers them to the ribosome where the polypeptide chain (that will fold into a protein) is being translated from the mRNA code.

Practice: Only mRNA, but not rRNA or tRNA

a. is composed of ribonucleotides.
b. is formed when DNA is transcribed.
c. serves a functional purpose within the cell.
d. is translated to form protein product.
e. all of the above

C. **Replication**

1. Replication of a circular chromosome or a plasmid always begins at a point on the plasmid called the origin of replication. Replication proceeds from this point in both directions (it is **bidirectional**).
   - Practice: Without using your notes, try to draw the general replication process (depict the entire plasmid). Be sure to label the replication forks and use a different color to represent newly synthesized DNA. This will help you to see that replication is semi-conservative, that is, both the final DNA molecules contain one of the original strands and one of the newly synthesized strands.
   - Practice: Replication of the *E. coli* chromosome
     a. initiates when an RNA polymerase enzyme called Tus unwinds a short stretch of DNA.
     b. originates and terminates at the same location.
     c. results in the transient formation of a region of newly synthesized DNA called the replication bubble.
2. Details of the process:
   a. Before replication begins, **helicases** unwind the helix and **topoisomerase**s relieve the tension.
   b. At the replication fork, a single complex called the **replisome** (containing **DNA polymerases**) synthesizes BOTH THE LEADING AND LAGGING STRANDS.
   c. Because it is a single complex responsible for synthesis of both strands and because DNA polymerase can only synthesize DNA in the **5' to 3' direction**, only one strand of newly synthesized DNA at the replication fork can be synthesized continuously (the **leading strand**). The other strand must be synthesized discontinuously (the **lagging strand**). The first step in the synthesis of the lagging strand is the formation of small RNA primers by the enzyme called **primase**. In the next step, DNA polymerase recognizes the free 3'OH ends of these small primers. It then builds DNA between primers forming **Okazaki fragments**. A second DNA polymerase removes the RNA primers and replaces them with DNA. Finally **DNA ligase** seals the nicks between the fragments. Be sure that you understand that DNA polymerase requires a primer in order to begin synthesizing DNA. (It sees a free 3'OH as its starting point.) Even the leading strand initially begins with a small RNA primer.
   - Practice: Based on the following illustration, which deoxynucleotide would DNA polymerase add next to the currently synthesizing strand (bottom strand)?

a. dATP
b. dGTP
c. dTTP
d. dCTP
e. dPCP

Practice: Which of the following is/are true about DNA replication?

a. It starts at the origin of replication.
b. It is bidirectional
c. It requires an RNA primer to get started.
d. It is semiconservative.
e. All of the above.

D. **Transcription (DNA to RNA)**

1. By convention, the top strand of DNA is always depicted in the 5'to 3' direction. It is called the **coding** or **plus** strand. The bottom strand is thus antiparallel to the coding strand. This strand is the strand that will actually serve as template for the synthesis or RNA. Thus the bottom strand is termed the **template** or **minus** strand. Because the lower strand serves as template for RNA synthesis, the RNA will always look like the plus (coding) strand except that it will be made of ribonucleotides instead of deoxyribonucleotides and U will replace T!

IMPORTANT PRACTICE QUESTION:

- **Observe the following region of DNA, and notice the location of the TATA box sequence within the promoter.**

-10
|
5'....TATAATGCATTCGACCTAGCAATTCGGGCCGAT....3'

3'....ATATTACGTAAGCTGGATCGTTAAGCCCGGCTA....5'

What is the sequence of the RNA transcript for the portion of the gene shown here?

a. 5'AAUGCAUUCGACCUAGCAAUUCGGGCCGAU...3'
b. 5'ACCUAGCAAUUCGGGCCGAU....3'
c. 5'ACCTAGCAATTCGGGCCGAT....3'
d. 5'AATGCATTCGACCTAGCAATTCGGGCCGAT....3'

It is important to understand that the template strand of DNA is used as the code to build the RNA. Therefore, the RNA looks like the coding strand of DNA except all Ts are replaced by Us.

2. The **promoter** is the transcription initiation site. In prokaryotes, there is often more than 1 gene under control of the same promoter. This is called an **operon**. All of the genes under control of this same promoter will be transcribed to give

a single RNA (a polygenic or **polycistronic RNA**). In eukaryotes, generally every gene has its own promoter. Promoters are consensus sequences of nucleotides that lie **upstream** of the gene: The **-35 region** and the **TATA box**.

2. INITIATION

- The **sigma factor** is the subunit of **RNA polymerase** that recognizes the promoter. Once RNA polymerase has melted and unwound a short stretch of DNA (forming the transcription bubble), and synthesized a short stretch of complementary RNA, the sigma factor dissociates from the rest of the RNA polymerase enzyme (the core enzyme) and transcription continues.
- Practice: Which of the following is/are involved with the initiation phase of transcription but not with elongation or termination?
    a. RNA polymerase
    b. the sigma factor
    c. the promoter
    d. the hairpin loop
    e. both b and c
    f. both a and d
- Practice: (T or F) RNA polymerase requires a primer in order to begin transcription.

3. ELONGATION

- RNA polymerase adds one ribonucleotide at a time as according to the DNA template strand. **Note: as the RNA strand is synthesized it dissociates from the template strand and the template strand reaneals with its original complimentary DNA strand (the plus strand).
- Practice: (T or F) A DNA template strand can be transcribed by more than one RNA polymerase enzymes at a time.
- Practice: Which terminal of the mRNA transcript is readily available while transcription is still taking place (the 5' or the 3')?

4. TERMINATION

There are two different types of termination:

a. Termination due to hairpin loop formation
b. Rho-dependent termination

VII. **Translation (RNA to Protein)**

- ***Practice drawing an amino acid. Show how a second amino acid would bond to this amino acid at the carboxyl terminal. Continue drawing, depicting how one amino acid after another would add to the carboxyl terminal to form a polypeptide chain! This will not be an exam question but it's essential to be able to visualize the process in order to understand translation.***

1. A **codon** is a 3-nucleotide sequence. There are 64 possible codons, 3 of which are **stop codons**: UAA, UAG and UGA and 61 of which encode for different amino acids. **(PLEASE do not attempt to memorize all 61 codons and the amino acids for which they encode! I will provide a copy of the table in your notes.)**
   - Since there are only 20 amino acids, it must be true that more than one codon can specify the same amino acid = **degeneracy**.
   - (Note- AUG is the start codon, however, it is only the start codon (encoding for f-Met) the first time it is encountered after the ribosome binding site (Shine-Dalgarno sequence). In all other locations, this codon simply codes for the amino acid methionine.)
   - Practice: Which statement/s regarding prokaryotic translation is/are TRUE?
     a. Only the coding region of an mRNA transcript is translated to make protein.
     b. The codons UUU, UUC and UUA all encode for the same amino acid.
     c. More than one codon specifies the amino acid Val.
     d. all of the above
     e. a and c
2. Ribosomes:
   - The factories in which translation occurs. They stabilize the mRNA and tRNAs such that the codons of the mRNA can be read 5' to 3' and the corresponding tRNAs can deliver the correct amino acids. The ribosome then catalyzes the linkage of adjacent amino acids to eventually form a polypeptide chain.
3. **tRNAs**
   - have an amino acid binding site as well as a 3-nucleotide **anticodon**. The anticodon recognizes the codon and thus allows the tRNA to deliver the correct amino acid to the ribosome where the mRNA is being read and the polypeptide chain is being built.
   - tRNAs can recognize more than one codon = **"wobble" (How does this relate to degeneracy?)**
   - Practice: A tRNA with an anticodon sequence of 3'ACG 5' would recognize which codon and carry which amino acid?
     VERY IMPORTANT CONCEPT

4. INITIATION

- *Note- Please be certain that you understand the difference between the Shine-Dalgarno sequence (ribosome binding site) and the start codon. The

ribosome binding site is generally ~7 nucleotides upstream from the start codon. Translation does not begin until the start codon. I will often represent the ribosome binding site as a black box but please understand that it is actually a particular sequence of purine-rich nucleotides.

- **Also note that in prokaryotic cells translation can begin even before transcription is complete. **Why can't this occur in eukaryotic cells??**

5. ELONGATION

- Practice: Which one of the following correctly summarizes the order of events that take place during the elongation phase of translation?

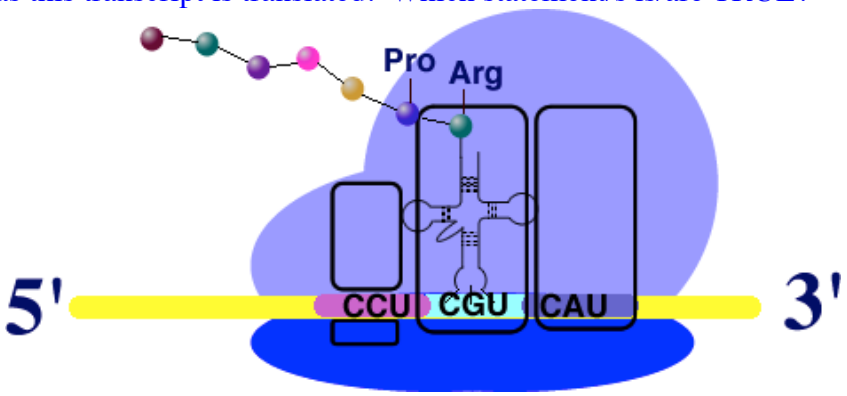
1. The initiating tRNA carrying the f-Met residue is bound in the P-site.
2. The ribosome advances the distance of one codon along the mRNA and the initiating tRNA + f-Met leave through the E-site.
3. The f-Met residue is covalently bound to the amino acid carried by the tRNA in the A-site.
4. A tRNA carrying the amino acid that corresponds to the second codon binds to the A-site.

a. 1, 2, 3, 4
b. 1, 3, 4, 2
c. 1, 4, 3, 2
d. 4, 1, 3, 2

*Note: The same RNA can be translated by several ribosomes at once = a polysome or polyribosome.

Practice: In which direction is the polypeptide chain synthesized?
a. from the carboxyl terminal to the amino terminal.
b. from the amino terminal to the carboxyl terminal.

Practice: Shown below is an prokaryotic mRNA transcript and the polypeptide that results as this transcript is translated. Which statement/s is/are TRUE?



a. arginyl-tRNAArg is bound at the P-site.
b. The amino acid that will be added next to the chain is His.
c. In this snapshot picture shown, translation is currently in the elongation phase.
d. all of the above
e. b and c

6. TERMINATION

- When the ribosome reaches a stop codon, release factors break the bond between the tRNA and the final amino acid in the polypeptide chain -> translation is terminated. The ribosome falls off the mRNA and dissociates into its subunits.

CAUTION: Often it is easy to feel comfortable with the principles and processes of replication, transcription and translation. However, I find that it is more difficult when trying to answer questions that ask one to relate the three:

Practice: Which of the following is / are involved in replication?

a. RNA polymerase
b. sigma factor
c. DNA polymerase
d. The Aminoacyl(A)-site
e. The promoter

VIII. Eukaryotic gene expression

- Practice: Which statement about eukaryotic and prokaryotic gene expression is FALSE?
  a. In eukaryotic cells only, the 5' end of the mRNA transcript is "capped" by the addition of a methylated guanine derivative.
  b. In eukaryotic cells more often then prokaryotic cells, approximately 200 adenines are added to the 3' end of the mRNA transcript.
  c. Generally only eukaryotic genes have both coding (exons) and noncoding (introns) regions.
  d. Only prokaryotic mRNAs must be transported out of the nucleus before they can be translated.

e. eukaryotic mRNAs are usually monocistronic whereas prokaryotic mRNAs can be either monocistronic or polycistronic.

IX. Regulating gene expression

A gene that is always expressed = constitutive. Some genes are usually off but can be turned on (induced) while other genes are usually on but can be turned off (repressed).

A. Mechanisms to control gene expression

Practice: What is an operon?

1. PROMOTER SEQUENCE:
   The closer a promoter sequence is to the ideal consensus sequence, the more frequently it is transcribed.
2. SPECIALIZED SIGMA FACTORS:
   Certain sigma factors are only produced under specialized situations and they recognize and bind to specialized promoters, allowing for expression of genes that are only needed under unique environmental conditions. This is one of the mechanisms used to control the expression of genes involved in endospore formation.
3. MODULATION BY DNA BINDING PROTEINS:
   A repressor blocks transcription by binding to an operator. An activator facilitates transcription by binding to an activator binding site.
   - Repressors and activators are allosteric proteins themselves. Thus, other molecules can bind to them and affect their ability to bind to the DNA:
   - Some repressors can't bind to the operator site until another molecule (called a corepressor) binds to them and allows them to bind to the operator and block transcription (e.g. the *trp* operon).
   - Some repressors bind to the operator site and block transcription until another molecule (called an inducer) binds to the repressor and keeps it from binding to the operator site (e.g. the *lac* operon).
   - Some activators cannot bind to the activator binding site until another molecule (called on inducer) binds to them (e.g. the lac operon).

B. **The *lac* operon demonstrates many types of regulation. It is under control of both an activator and a repressor:

- Practice: In lab, we inoculated a KIA tube with *E. coli*. Remember that a KIA tube contains both glucose (in short supply) and lactose (in excess). What type of growth occurs in this tube during an overnight incubation? Explain all of the changes that occur on the lac operon during this incubation.
  (*A QUESTION OF THIS VARIETY WILL DEFINITELY BE ON THE EXAM. MAKE CERTAIN THAT YOU ARE COMFORTABLE DETERMINING HOW ENVIRONMENTAL CONDITIONS TELL YOU THE STATE OF THE *lac* OPERON!)