UN2005/UN2401 '18 -- Lecture 16-- Last Edited: 10/31/18

Handouts You will need 15A & 15B from last time. Also 16A & 16B.
16A -- Induction vs Repression; Repression vs Feedback Inhibition
16B -- Operons
Paper handouts are provided in class; for PDFs go to the handout list.

I. Wrap up of Protein Synthesis -- Energy & Enzymes

A. Loading of tRNA -- Here are the major issues:

1. Major Issues

a. Specificity: How do we get the right AA on the matching tRNA? We need accurate loading enzymes to get the right AA on the corresponding tRNA. (See below for multiple names for 'loading' enzymes.) For the reactions, see (a) and (b) on handout 15B.

b. Energy: Where does the energy come from to load up tRNA? It must ultimately come from hydrolysis of ATP, but how is the energy from ATP used to load tRNA? See handout 15B and topic IV-C in notes of Lecture 15 for all the individual reactions and a diagram of the energy part.

2. Solution(s)

a. Overall: Charging/loading reaction is very complex, but it serves 2 functions: accuracy/specificity and energy. Two step part (reactions a & b on handout 15B) increases accuracy/specificity; overall rxn. hooks ATP hydrolysis to protein synthesis. For a diagram, see Sadava fig. 14.11 (14.12) or Becker fig. 19-6 (22-5).

b. Names of enzymes involved -- why multiple names? Different names emphasize multiple functions of reactions involved in putting AAs on tRNAs. Enzymes are called loading enzymes, activating/charging enzymes or aminoacyl-tRNA synthetases. Different names emphasize different job(s) of enzymes, as follows:

- Loading enzyme -- ferries AA ('ferries the load') to mRNA; also 'loads' the tRNA with the cognate AA.
- Activating enzyme -- locks in energy for formation of peptide bond
- AA-tRNA synthase (or synthetase) -- does accurate matching of AA and tRNA; ensures specificity
- Charging enzyme -- both loads up AA and activates it -- locks in energy for formation of peptide bond.

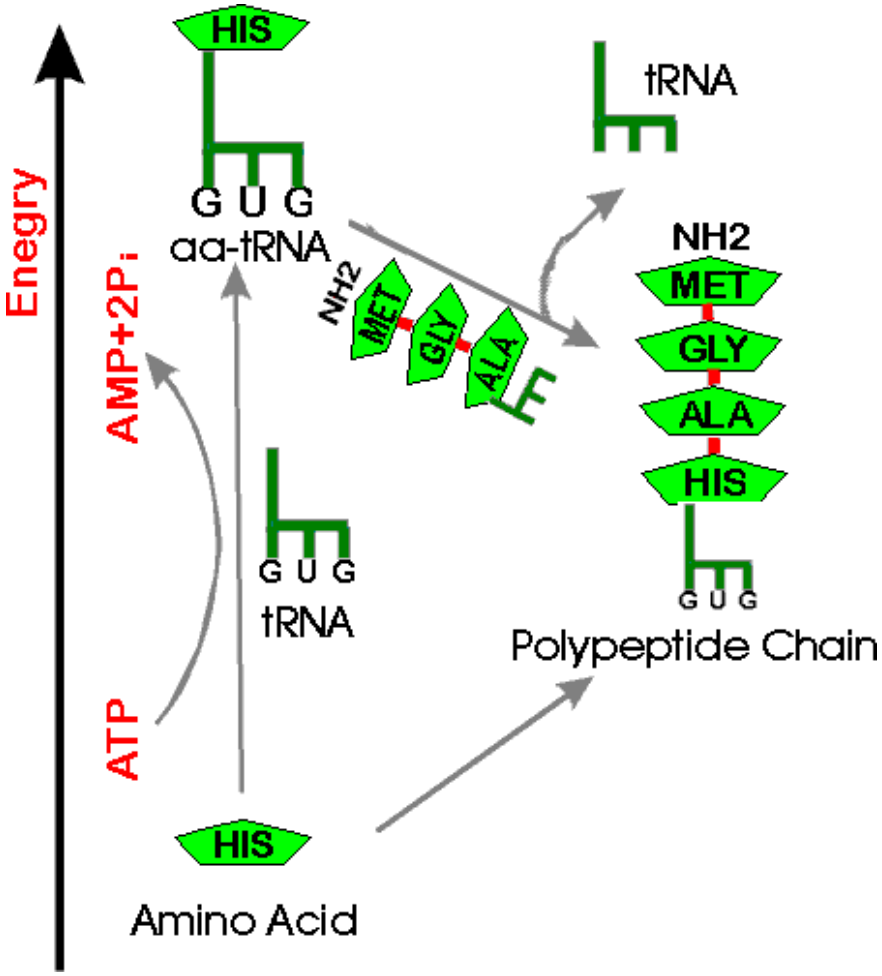
B. Loading of tRNA -- Where does the energy come from to load up tRNA? How do loading enzymes fit in?

1. Overall ATP Balance -- How does ATP fit in? There are two ways to explain how ATP fits in here:

Approach #1 -- uphill/downhill picture. See handout 15 B. (Compare to similar picture for making DNA.) Approach #2 on next page.

Basic idea: it's uphill from AA → chain, but downhill from AA-tRNA → chain. 2 P are split off ATP to get the AA onto the tRNA, and it's a downhill slide from there (AA-tRNA) to make the chain. Note that a tRNA-AA connection is broken to add an amino acid, but the connection that is broken is between the last amino acid in the peptide chain and the tRNA, not between the new AA and its tRNA. (The peptide chain adds to the AA on the next tRNA, not the reverse.)

Note: In the diagram below, the arrow for the ATP reaction (split of ATP) and the arrow for the loading of the tRNA go in the same direction. This is the usual way of writing coupled reactions, as discussed by Dr. Jovanovic. It is important to realize that the change in free energy is downhill for one reaction (splitting ATP) and uphill for the other (adding phosphates to XMPs).



Approach #2 -- sum up the reactions for charging

(rxn 1) tRNA + AA → AA~tRNA (+ water)

(rxn 2) ATP (+ water) → AMP + PP_i

(rxn 3) Pyrophosphatase: PP_i (+ water) → 2 P_i

Rxn1 is uphill, so you must couple tRNA loading (rxn 1) to hydrolysis of ATP (rxn 2).

Δ G^o for (1) + (2) is about zero, but PP 'tase = pyrophosphatase removes product (rxn. 3) and pulls the overall reaction to the right (as in nucleic acid synth.). In other words, sum of ΔG^o for (1) + (2) + (3) is negative. (Net reaction is shown on the bottom of handout 15B.)

Now the AA~tRNA can be used in protein synthesis to provide free energy for formation of peptide bonds.

2. How are reactions (1) and (2) actually combined?

a. A single enzyme actually catalyzes a 2 step rxn. for each AA & tRNA pair, such as his and $tRNA_{his}$. Having 2 steps (using same enzyme and matching same AA and same tRNA) increases accuracy as with proofreading by DNA polymerase.

b. Net result is to couple ATP hydrolysis and tRNA loading as follows (also on handout):

(a) ATP + AA → AMP~AA + PP_i

(b) AA~AMP + tRNA → AA~tRNA + AMP

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net of (a) + (b) = ATP + AA + tRNA → AMP + PP_i + AA~tRNA = same as (1) + (2) above. (Match the colors!) Note that the AMP~AA cancels out.

As explained above, ΔG^o for this is about zero, but pyrophosphatase pulls it as for DNA synthesis. If you include action of pyrophosphatase, overall net = same as (1) + (2) + (3) above.

Click here for animated protein synthesis (In Netscape >= 4, you can control the animation via the right mouse button. In Netscape 3, you can restart the animation with the Reload button and stop it at any point with the Stop button). This animation was made by a TA in this class. There are many more animations on the web. Go to Google and type in 'protein synthesis animation' for a whole collection. (Let Dr. M know if you find one that's really helpful.) Another animation is at http://highered.mcgraw-hill.com/olc/dl/120077/micro06.swf.

3. Two ways to get enough energy from splitting ATP. Addition of a monomer to a growing chain of a nucleic acid or protein requires a lot of energy. There are two different ways to get the energy from two "high energy" bonds from ATP.

Method 1: You split two molecules of ATP, removing one phosphate each time:

$$2ATP + water \rightarrow 2ADP + 2P_i$$

Method 2: You split one molecule of ATP, removing two phosphates:

$$ATP + water \rightarrow AMP + 2P_i$$. (Usually you generate PP_i and then split it to 2 P_i)

Either way the "energy cost" is the same. Synthesis of XTP's for nucleic acid synthesis uses the first method; synthesis of AA~tRNA for protein synthesis uses the second method.

C. Molecular Structure of Ribosomes This is diagramed on bottom of handout 15A.

1. Parts. See Sadava fig. 14.12 (14.13); Becker fig. 19-1 (fig. 22-1).

a. How many subunits per ribosome? Each ribosome is made of two subunits.

b. Each subunit is a ribonucleoprotein or RNP -- each subunit is made of at least one kind of rRNA and many proteins.

c. How made? Each subunit is made separately.

d. When do whole ribosomes exist? Only during translation. See below.

2. Names of Parts. See handout 15A and/or Becker table 19-1 & fig. 19-1 (table 22-1).

a. Use of S values. Whole ribosomes, subunits of the ribosome, and different ribosomal RNA's, are identified by their sedimentation constants (S values) in an ultracentrifuge.

b. Sizes in eukaryotes and prokaryotes are slightly different. There are two values (of S) given for the sizes of the RNA's and subunits -- the smaller number is for prokaryotes; the larger # for eukaryotes.

3. Self assembly -- How does ribosome structure form? The structure of each subunit is determined by the primary sequences of the rRNA's and proteins in it. Just as a protein folds up into the most stable (lowest energy) 3D conformation, so rRNA + proteins of each subunit fold into a ribonucleoprotein particle or RNP with proper 3D shape and function.

*4. rRNA vs ribosomes***.** Be careful not to confuse *ribosomes* (containing both rRNA and protein) with *ribosomal RNA.*

D. Function of Ribosomes, cont.

1. How Ribosomes attach to & move down the mRNA

a. Attachment of individual ribosomes

(1). When not in use, ribosomes come apart into subunits. The cell contains a pool of large subunits and a pool of small subunits.

(2). When translation starts, subunits come together. One small subunit and one large subunit clamp onto the mRNA to form a ribosome and begin translation.

(3). When translation ends, the two subunits come apart. The subunits fall off the mRNA, and return to the pool -- ready to be used again.

(4). Prokaryotes vs Eukaryotes. Mechanism of attachment to mRNA (and finding the right AUG start codon) is different in prokaryotes and eukaryotes, so we will ignore the details. See texts if you are curious.

b. Polysomes -- More than one ribosome can read a single message at one time.

(1) The first ribosome attaches near the 5' end of the mRNA.

(2) Then the ribosome moves relative to the mRNA (see note below). The ribosome moves down the mRNA toward the 3' end, or the ribosome stays put, and the message slides through the ribosome. Either way, the protein chain grows, and the ribosome is farther from the 5' end of the mRNA.

(3). A 2nd ribosome attaches. Once the ribosome is located far enough from the 5' end of the mRNA, a second ribosome can attach behind it (on the 5' side) and follow the first ribosome down the message.

(4). More ribosomes attach. As each ribosome moves toward the 3' end, making protein, another ribosome attaches on the 5' side until the entire mRNA is covered with ribosomes.

(5). A polysome forms. The mRNA remains covered with ribosomes. Although some ribosomes finish and fall off the 3' end, others continually attach at the 5' end. The mRNA covered with multiple ribosomes is called a polyribosome or polysome for short. Sadava fig.14.16 (14.17) or Becker fig. 19-13 (not in 8th ed).

Note: Many descriptions & diagrams indicate that the ribosomes move down the mRNA, 5' to 3'. The result is the same if you assume the ribosomes stay put while the mRNA moves through the ribosomes, 5' end first. (Which is more likely.) Once enough mRNA has slid through the first ribosome, a second ribosome can attach to the space on the 5' end and the mRNA can thread through that one next, and so on.

To review polysomes, try problem 7-16, part B.

2. Catalytic role of Ribosomes

a. Peptidyl Transferase is a Ribozyme*:* Peptidyl transferase catalyzes formation of the peptide bond, and is part of the large subunit of the ribosome.

b. A catalyst, but not a protein. The catalytic activity is a property of the rRNA in the large subunit, not a protein, so this is not really an enzyme (catalyst made of protein) but a ribozyme (catalyst **made** of RNA).

c. Other ribozymes. Peptidyl transferase is not the only ribozyme -- other catalytic RNA's are known.

d. Origin/History. It is presumed that ribozymes are relics of the "RNA world" that existed before DNA and protein took over many of the early functions of RNA (which has both catalytic and informational properties).

e. Want more Background? For more details go to http://www.sciencemag.org/cgi/content/full/289/5481/878

You can reach this site from any Columbia computer; I don't know if you can get it from a personal computer if you are not a subscriber to Science Online. Note that this site has detailed "hypernotes" which list many sites useful to molecular biologists. If you find any of these useful, please tell Dr. M. so she can tell other students. (The site maybe slow to load, but the link works.)

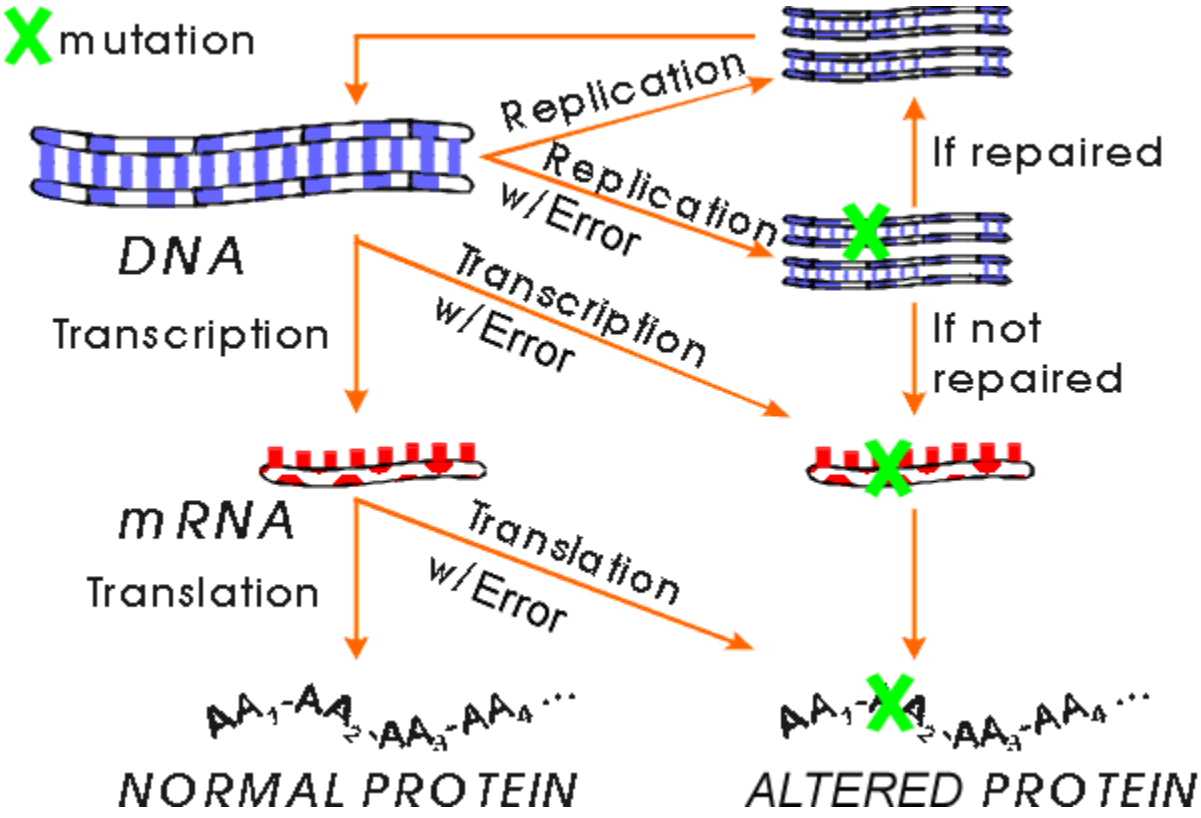
Alternatively, the article is from *Science* 11 Aug 2000: Vol. 289, Issue 5481, pp. 878-879

E. Summary of How 'RNA makes protein'. What does it take to make a protein? See part V of notes of Lecture #15.

II. Mutations

A. What are consequences of mistakes in macromolecular synthesis?

- *Protein Synthesis.* If you make a mistake when lining up the AA, so what? 1 molecule of protein bad. No big deal as long as it doesn't happen too often.
- *Synthesis of tRNA or rRNA*. If you make a mistake lining up the nucleotides in one molecule of tRNA or rRNA, results are similar.
- *Synthesis of mRNA*. If you make a mistake lining up the nucleotides in mRNA, get a few bad protein molecules. Worse, but bearable. After this molecule of mRNA gets thrown away, new mRNA and protein made will be ok.
- *Replication of DNA*. If you make a mistake lining up the nucleotides in DNA, what then? Errors can get repaired, cells have enzymes for this. If not repaired, at next replication, one descendent gets a completely changed DNA and changed sequence is passed on forever. All new RNA and protein will be altered. So this is really serious, and this is what is meant by a mutation. (Mistakes in RNA and protein synthesis -- as long as the DNA is ok -- are not called mutations. They are called mistakes.) See picture on next page and note under the picture.



Note: Picture above does not distinguish mismatched base pairs (such as G-T) which have a 'mistake' in only one strand, from entirely changed base pairs (such as A-T changed to G-C) which have a 'mistake' in both strands.

B. How do mistakes in DNA synthesis change the DNA sequence?

1. Mistakes occur: Bases can mispair (if in wrong tautomeric form or damaged) -- See Sadava fig. 15.4 (15.5). DNA polymerase can slip relative to the template and add extra bases or leave some out. Proofreading keeps mispairing mistakes low but not zero. (See below for why mutations are necessary.)

2. Repair occurs: Repair enzymes correct some mistakes. After a mistake (putting in wrong base) occurs, other strand is still okay, and can serve as template for repair. Note: in some cases, the repair enzymes are equally likely to use either strand as template. This means the 'repair' generates a matching base pair, but it is not necessarily the original one.

3. Effect of Replication: If DNA with a mistake in one strand is replicated before the mistake is corrected, one of the daughter molecules will have two changed strands. (Other daughter molecule will be okay.)

4. How mutations become 'fixed' that is, set in place: A double stranded DNA molecule with one changed strand can still be corrected (by repair enzymes) but a molecule with two changed strands cannot be corrected. Once both strands are changed, the mutation is often said to be 'fixed' meaning 'permanent.' Note that in this context 'fixed' means the *opposite* of 'corrected.'

5. Overall: Organisms keep the mutation level low, but nonzero, by extensive editing, repair, etc. of DNA. Why have mutation at all? See D below.

C. Definition/terminology of mutations See Becker fig. 19-16 (Box 22B p. 694) or Sadava fig. 15.2.

1. Mistakes vs Mutations. Mistakes in RNA or protein synthesis are called mistakes, but mistakes in DNA synthesis (that are not corrected) are called mutations. Anything that changes the base pairs in the DNA is called a mutation, and an organism with a mutation is called a mutant. The normal or starting (or standard) organism is often called "wild type." A change in the RNA or protein that does not affect the DNA is not called a mutation.

2. Types of Mutations -- Terminology See Sadava fig. 15.2 or Becker fig. 19-16 (Box 22B).

a. Substitution = change in base(s).
b. Deletion/insertion = removal or addition of base(s).
c. Frameshifts

(1). Reading Frames: There is more than one way to read a nucleic acid sequence in non-overlapping groups of three, depending on where you start. The different ways are called different reading frames. If you start with the first, 4th, or 7th.... base you get one reading frame; if you start with the 2nd, 5th, or 8th.... you get the second, and so on. There are 3 possible reading frames.

(2). A mutation that changes the reading frame is called a 'frameshift.' An insertion/deletion of 1 or 2 bases (in a coding region) causes a frameshift because the mRNA with such a mutation is misread in the wrong "reading frame" (wrong groups of three nucleotides) all the way to the end of the gene (or until ribosome reaches a stop codon). Notice the drastic difference in effects between substitutions and frameshifts.

(3). Some Questions: What sets the normal reading frame? Which is worse, deletion of 1, 2, or 3 base pairs from the DNA?

d. Nonsense vs Mis-sense. A mutation that generates a stop codon is sometimes called a "nonsense" mutation; one that changes one amino acid to another is called a "mis-sense" mutation.

3. Phenotype and genotype

The state of the DNA is known as the ***genotype***; the observable properties of the organism are known as the ***phenotype***. A mutation changes the genotype, but may or may not change the phenotype. See recitation problems #9.

D. Why are mutations important?

1. Source of evolutionary diversity -- source of all variation in phenotype for selection to act on; why there are different species (& why we're here at all). This is good overall, but not good for us when it's HIV or flu or any other infectious agent that's mutating.

2. Source of individual (& nonfunctional) diversity. Mutation leads to variations in all DNA, including noncoding DNA that does not affect the phenotype. This has little or no functional consequences, but the variations come in handy for tracing evolutionary lines of descent and making identifications. (Variations in noncoding DNA are the basis of all forensic ID's.) Variations that do not affect phenotype persist because there is no selection for or against any particular version. (Individuals that carry any particular mutation are not at any reproductive advantage or disadvantage.)

3. Cause inherited diseases like hemophilia, Tay Sachs, etc. (Can cause cancer in somatic cells.) To keep advantages of (1) and avoid disadvantages of (3), organisms keep the mutation level low but nonzero by extensive editing, repair, etc. of DNA as explained previously.

4. Mutations are a very useful tool for figuring out how things work.

- Studying effects of frameshifts allowed us to start to crack the genetic code -- see prob book, recitation problems, and texts (for details see 'frameshift mutations' in Becker, Figs 18-7 & 18-8 (21-4 & 21-5). Also see note at * below.

- Allows us to knock out one protein at a time and see what happens -- implies what function of protein was in first place. (As in figuring out pathways in prob. book.) Same affect can often be achieved with RNAi (or antisense), or with drugs.

* Note: In this course, we often tell you how it works first and then give you mutations to test your understanding. Historically, it usually works in reverse -- mutations are studied first and details of 'how it works' are figured out from analyzing the mutants. For example, the genetic code was partially 'cracked' by looking at mutations and seeing how changes in the DNA correlated to changes in the corresponding protein. (It took biochemistry to finish the job.) See texts for details.

To review mutations, see recitation problems #9 and problem 7-22. (7-23, 7-24 & 7-26 also deal with mutations.)

III. Intro to Regulation in Prokaryotes .

A. Why regulation of enzyme synthesis is reasonable and/or necessary. Consider some typical enzymes

- glycolytic enzymes

- beta-galactosidase -- needed to breakdown and metabolize lactose.
  Lactose = dimer of glucose and galactose -- See Becker fig. 20-1 (23-1)

- tryptophan synthetase (needed to synthesize trp).

When are the these enzymes needed?

1. Glycolytic enzymes -- always needed

2. Beta-galactosidase

- Catalyzes first step in catabolic (breakdown) pathway.
- Only needed if lactose ***present*** (when lactose needs to be broken down).
- Enzyme level should be low until lactose added to medium.

3. TS (trp synthetase)

- Catalyzes last step in anabolic (synthetic) pathway.
- Only needed if trp low or ***absent*** (when trp must be synthesized in order to make proteins).
- Enzyme level should be high until trp added to medium.

4. Why not make all enzymes all the time (even if not needed)? Enzyme synthesis uses a lot of energy.

B. The Phenomena -- Are enzymes (like those above) actually made only when they are needed? Graphs on handout 16A show what happens to level of appropriate enzyme if you add, or take away, the appropriate small molecule, namely lactose (lac) or tryptophan (trp). Table under graphs summarizes the terminology.

1. Example of Induction -- Lactose (small molecule) = *inducer* = signal to turn **on** synthesis of appropriate enzyme ; synthesis of beta-galactosidase (enzyme) is called *inducible*; phenomenon is known as *induction*. See Sadava fig. 16.2 & fig. 16.5.

2. Example of Repression -- tryptophan (small molecule) = *co-repressor* = signal to turn **off** synthesis of appropriate enzyme ; synthesis of trp synthetase (enzyme) is called *repressible*; phenomenon is known as *repression*.

3. Constitutive synthesis -- Synthesis of some proteins, such as enzymes of glycolysis, is called *constitutive* = synthesis of enzymes is "on" at all times.

4. Co-ordinate control – Levels of proteins of related function are controlled together -- the level of synthesis of the corresponding proteins is coordinated

C. Summary of Terminology = italicized terms in green above. See table in middle of Handout 16A.

Regulation is covered in problem set 12. To review the material in parts A-C, see Problem 12-1, A & B.

Next Time: Mechanism of Prokaryote Regulation (Handout 16B)