UN2005/UN2401 '18 -- Lecture #15 -- Last Edited: 10/27/18

Handouts: You will need 14 A & B from last time & 15A -- Wobble & Ribosome Structure
We will probably not get to15B -- tRNA Loading

I. How the Peptide Chain Grows -- Role of tRNAs & mRNAs

A. You need mRNA

1. mRNA is template -- determines order of AA.

2. How is mRNA made -- see notes of last time for details of transcription.

Reminder: all RNA molecules, mRNA, tRNA, & rRNA are made in the same way by transcription of a DNA template.

B. You need tRNA -- see notes of last time, Lecture 14, Section V, B for these topics

1. Adapter Function – what do you need tRNA for? To match codons and AA

2. Structure of tRNA

3. How does tRNA match up with the correct codon?

C. You need a full set of Loaded tRNAs.

1. Why do we need loaded tRNA (tRNA with its respective AA on it)?

a. To allow the line up of the AA in the order specified by the mRNA. tRNA acts as an 'adapter' to match AA & codons.

b. To provide the energy to drive peptide synthesis. To just hook up monomers of any polymer requires energy. Where does the energy come from to link AA? It comes from breaking the bond in a loaded tRNA between the AA and the tRNA. The energy released from breaking one AA-tRNA bond is used to make one peptide bond -- to drive addition of one AA to the growing chain. (This will be covered in detail next time – it's on 15B.)

2. How do we make and use loaded tRNA? See below.

D. How does the new peptide chain grow?

See handout 14B or Sadava fig. 14.14 (14.15) or Becker fig. 19-9 & 19-12 (22-7 & 22-10).
Note: The first figure in Becker is an overview; the second is a more detailed version of the chain elongation steps. (Note that we will be ignoring the roles of GTP, initiation factors (IFs) and elongation factors (EFs)).

For a video of the class demonstration see video (windows media file) by Peter Sloane at http://www.columbia.edu/cu/biology/courses/c2005/lectures/translation.wmv This video may take a while to load, but it does work (at least on a PC).

Dr. Price has made a more recent series of videos of most class demonstrations. These videos can be reached from the Course Menu page.

1. Chain adds to newest AA. When each peptide bond is made, the growing chain is transferred (from the tRNA that previously held it) to the next amino acid (still attached to its tRNA), not the other way around, for logistical reasons. The newest amino acid is not added to the free end of the chain. Instead, the chain is added to the newest amino acid. (The current system allows the translation machinery to slide down the mRNA reading 2 adjacent codons at a time. The other way doesn't.)

Catalyst for formation of peptide bonds is called peptidyl transferase because the growing peptide chain is transferred as described above. This catalyst is part of the ribosome. (How ribosomes fit in is discussed below.)

2. Peptide chain grows amino → carboxyl. This follows because the amino acids are held down (attached to tRNA) by their COOH ends.** So if chain must add to free end of next AA, must add to amino end of next AA.
Note for those who have had organic: From the point of view of mechanism, the electrons go the other way; the electrons of the amino attack the carboxyl.

***How are the tRNA and AA connected?* The AA is attached to the 3' end of its respective tRNA by a ester bond between the COOH end of the AA and the 2' or 3' OH on the final ribose (at the 3' end). This leaves the amino of the AA free.

3. Energy for peptide synthesis. The energy derived from splitting the tRNA~AA (really the tRNA~chain) bond drives peptide bond synthesis. In other words, the AA-tRNA connection is a high energy bond. How it is formed at the expense of ATP will be discussed next time; it is diagrammed on handout 15B. (Additional energy is required to bind the AA~tRNA and move the ribosome down the mRNA, but we will ignore the energy details of those steps, as well as the proteins needed to promote them.)

4. Stops. The peptide chain stops growing when the translation machine comes to a stop codon. There are no tRNA's for the stop codons, so there is no way that the chain can keep growing if a stop codon comes next. See Sadava fig. 14.15 (14.16) or Becker fig. 19-14 (22-11).

To review protein synthesis so far, and the role of tRNA, try problem 7-21.

II. Role of Ribosomes in Translation (We will add ribosomes to 14B.)

A. How do ribosomes fit in? Why are they needed?

1. Function

a. Accurate matching: You need something to hold tRNA (two loaded ones at a time) onto mRNA while amino acids are being hooked up. (How many weak bonds hold a tRNA and mRNA together? How many covalent bonds?)

b. Catalysis: You need to provide necessary catalysts for making peptide bond etc.

2. Structure: Ribosome contains both RNA(s) and protein(s).
Anything made of both RNA & protein is called an RNP = **r**ibo**n**ucleo**p**rotein or **r**ibo**n**ucleoprotein **p**article. This particular RNP structure = ribosome; RNA inside it is called ribosomal RNA or rRNA. Be careful not to confuse ribosomal RNA (rRNA) & ribosomes.

For pictures of ribosome structure see Sadava fig. 14.12 (14.13) and/or Becker figs. 19-1 & 19-2 & table 19-1 (figs 22-1 & 22-2 & table 22-1.) Molecular details of structure next time.

3. Important Functional Features (See Becker, fig. 19-2-a (fig. 22-2) or Sadava fig. 14.12 (14.13)

a. 1 site or groove for mRNA.

b. 2 sites for loaded tRNA (hybridized to mRNA) per ribosome -- These are called A and P; more details below. These sites bind both mRNA and (loaded) tRNA.

c. One site for unloaded tRNA This site binds binds empty, used tRNA before it is released from the ribosome. (It's called E for exit site). This site is sometimes omitted in diagrams of elongation. The E site binds tRNA but not mRNA.
(The T site shown in the 7th ed. of Purves probably does not exist and should be ignored.)

d. All ribosomes in an organism are the same. Which protein is made does not depend on the ribosome. (Q: What does it depend on??)

e. Peptidyl transferase is part of the ribosome. (Details next time.)

B. How do Ribosomes Work?

1. How Ribosomes Move (See Becker figs. 19-9 & 19-12 (figs. 22-7 & 22-10) or Sadava fig. 14.14 (14.15).

a. Directions:

(1) Ribosome movement -- Ribosome moves down mRNA 5' to 3' (or mRNA slides through ribosome, 5' end first) as peptide is made amino to carboxyl. (See below in **c. (2)** for which part actually moves.)

(2) Synthesis directions -- Both peptides and nucleic acids are both made/read as written, left to right.

(3) Transcription is separate from translation -- How mRNA is made and how it is translated happen to be in the same direction, but transcription and translation are two separate processes (which are usually coupled in prokaryotes but not eukaryotes).

b. A & P sites. The two binding sites for loaded tRNA are different

(1) A site -- A site binds **a**mino acyl tRNA

(2) P site -- P site binds **p**eptidyl tRNA.

c. Translocation -- Movement of mRNA (& tRNA's) relative to the Ribosome.

(1). Differences between the A & P sites allow unidirectional movement.

(a) Before peptide bond is formed, AA-tRNA is in A site and peptidyl-tRNA is in P site.

(b) As soon as peptide bond is formed, tRNA in A site becomes a peptidyl-tRNA, and tRNA in P site becomes unloaded or empty tRNA.

(c) After peptide bond is formed, "wrong" types of tRNA are now in A & P sites, so ribosome no longer fits properly and moves over one codon.

(d) Ribosome movement shifts peptidyl-tRNA to P site, empty tRNA to E site and leaves A site empty to hold next AA-tRNA. (FYI: This step requires energy in the form of GTP hydrolysis.)

(e) The empty or unloaded tRNA is then released to be reloaded and used again, and the next AA-tRNA arrives to repeat the cycle.

(2). Which part actually moves? Ribosome or mRNA?

(a). mRNA & ribosome: These move one codon relative to each other.

- On handout 14B, in steps 5 & 6, it looks like the ribosome moves one codon toward the 3' end of the message.
- Probably, the ribosome stays in fixed position and the mRNA advances one codon through the ribosome in the 5' direction, as shown in step 2 → 3.
- In other words, if drawn correctly, the mRNA moves to left instead of the ribosome moving to the right.

(b). Messenger RNA & tRNA: These do not move relative to each other but are pulled together.

(c). Note that the effect is the same whether the ribosome or the mRNA (& attached tRNAs) move -- the ribosome and mRNA are shifted one codon relative to each other and all the tRNA's shift down one site. Either way you look at it, the overall result is:

- The empty tRNA moves into the E site,
- The peptidyl tRNA moves into the P site, and
- The A site becomes empty, ready for the next AA-tRNA.

***(3). Protein Synthesis uses up a lot of Energy*.**

(a). Movement and binding tRNA both require energy which we are ignoring. You probably need at least 5 P's split from ATP (or GTP)* per AA added if you count all the steps involved, not just growth of peptide chain.

(b).Making proteins is a very expensive procedure, and making unnecessary proteins is very wasteful. As a result, there has been strong selection for efficient regulation of protein synthesis; how regulation works in bacteria will be explained next time.

* (If you want to know more about the details of the involvement of GTP in translation, see Becker figs. 19-10 & 19-12 (figs. 22-8 & 22-10.)

To review how the A & P sites fit in, try problem 7-12, part C.

2. How Ribosomes attach to mRNA

a. Attachment of individual ribosomes

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

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 (see note below). The ribosomes moves down the mRNA toward the 3' end, making protein.

(3). A 2nd ribosome attaches. Once the ribosome has moved far enough down, 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 after it 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: This description assumes 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.

C. Detailed Structure & Assembly of Ribosomes: This will be covered next time. (If you want a preview, See bottom of handout 15A and/or see texts for pictures.

III. Translational Starts & Stops. How do peptide chains get started? How does peptide chain growth stop?

A. Starts -- AUG is used for both 'start' (in translation) and 'methionine'

1. How does protein synthesis start? -- you need a special met-tRNA for the **P** site.

If the P site holds only tRNA's with chains, how will the first AA-tRNA fit on the ribosome? The answer is that there is a special tRNA (initiator tRNA or $tRNA_{met}i$) which is used only in starting chains. This tRNA carries met and recognizes the codon AUG, which is both the (only) codon for met and the (only) start codon. This AA-tRNA is special in that it only fits in the P site.

2. How does met get inserted in the middle of chains?-- you need a different met-tRNA for the **A** site.

If the tRNA for met fits in the P site, how will met be added to a growing chain? There is a second "ordinary" tRNA for met, one that fits in the A site. Both tRNA's for met recognize the same codon and carry the same amino acid, but one fits only in the P site and one only in the A site. The first (initiator tRNA) is used only to start chains and the other is used only in the middle of chains. Sadava fig. 14.13 (14.14) or Becker fig. 19-10 (fig. 22-8) if you are curious about all the details of initiation.

3. Processing. Why don't all protein chains start with methionine? Met is usually removed from the amino end of the protein before the peptide chain folds up. This method of synthesis (having all proteins start with met) is used for ease of manufacture, not because met is needed for protein function. It makes synthesis easier but produces a product that needs alterations before use (removal of met). Post synthetic modifications such as removal of met are common (for all macromolecules, not just proteins) and are often called processing. It is not at all unusual for a modification or processing enzyme to take off a few amino acids or nucleotides here or there, add a group or cofactor, etc. (This is how unusual bases in tRNA are formed.) These modifications are much more extensive in eukaryotes than in prokaryotes and will be discussed at length in later lectures and/or next term. (If you are curious, see Sadava fig. 14.19 (14.20)).

4. Finding the right AUG -- How do ribosomes select the right AUG to start at? The process is different in eukaryotes and prokaryotes, and we are leaving the details for more advanced courses. See texts if you are interested.

5. Start codons vs Promoters vs Origins -- all 3 are sequences that indicate 'start here' but all 3 are different, since each type of signal is used to start a different process. Start codons affect translation, promoters affect transcription, and origins affect replication. See Sadava, table 14.3 (14.2). All 3 types of sequences are encoded in the DNA, but one type of sequence (an origin) is 'read' at replication, one type (a promoter) is 'read' at transcription, and the third type (a start codon) is 'read' at translation.

B. Stops -- No tRNA is involved

1. No stop tRNA. There are no tRNA's for stop codons, so a ribosome stalls when it comes to a stop codon. A tRNA with a peptide chain is now sitting in the P site, but there is no tRNA to fit in the A site.

2. Role of protein release factor. A protein called a release factor binds to the stop codon in the A site and triggers release of the completed peptide chain. The peptide chain is released from its tRNA, folds up, and goes off to do its job.

3. Fate of last tRNA & ribosome. Once the peptide is released from its tRNA, the tRNA falls off the ribosome and then the ribosome disassociates into subunits (2 parts to be explained next time) and the subunits fall off the mRNA. The tRNA & ribosomal subunits can be used again. Sadava fig. 14.15 (14.16) or Becker fig. 19-14 (fig. 22-11).

To review starts and stops, try problems 7-12 G, 7-17, & 7-20 B.

IV. Role of Transfer RNA in Translation, revisited -- Wobble

A. Important Features of the Code – why we need 'wobble'

1. Degeneracy vs ambiguity

a. The genetic code is not ambiguous -- it is always clear what a particular word (codon) means.

b. The genetic code does contain synonyms -- multiple words for the same thing. In this case, multiple codons for the same AA. Examples:

- UUU/C (meaning UUU or UUC) is phe. See text or handout with code table for more examples.
- XYU/C always codes for the same AA; XYA/G usually does. (X, Y = any of the 4 bases.)

c. The genetic code is degenerate. Any code with synonyms -- multiple words for the same thing -- is said to be 'degenerate.'

2. Wobble -- The concept:

- What is wobble?The same tRNA could be used for two or more synonomous codons if you allow a little flexibility in pairing between the base at position 3 of the codon and its corresponding base (position 1) in the anticodon. This flexibility in pairing is known as "wobble." (See note at *.)
- Why bother? Since the code is degenerate, you could save on the number of different tRNA's required if the same tRNA could be used for two or more **synonymous** codons.
- Terminology for RNA positions: Because of wobble, position 3 of the codon and position 1 of the anticodon are known as "the wobble position" in their respective codons/anticodons.

*Wobble = Several different codons are read by one tRNA, **not** several different tRNA's are used for the same codon. (There may be multiple tRNA's for the same codon, but that's called something else, and will not be discussed in this course.)

B. How Wobble Works

1. Mechanism: By wobble, we mean that the two bases involved in pairing (the ones in the wobble position of the codon and anticodon respectively) can twist slightly relative to each other. Because of this flexibility of alignment, H bonds are possible between groups that don't normally base pair in totally double stranded nucleic acids. (Nucleotides in ds DNA or DNA/RNA linear hybrids cannot twist.) {Q&A}. Some examples are drawn in Becker fig. 19-4 (22-4) and on handout 15 A.

2. Wobble rules: The usual matches allowed at the wobble position are as shown on handout 15A, and can be summarized as follows:

Type of RNA	Position of Base	Base				
tRNA	1st position of *anticodon*	A	C	U	G	I **
mRNA	3rd position of **codon**	U	G	A or G	C or U	U, C or A

- ** tRNA contains modified bases. I = inosine (name of nucleoside) or hypoxanthine (name of base). I = A without its amino (has C=O instead of C-NH_2 at position 6.) Only the usual 4 bases are inserted during transcription, but some are modified enzymatically after the transcript is formed.

- How I pairs with U, C or A and how U pairs with G is shown on handout 15 A (or see Becker, fig. 19-4 (22-4)).

- Note that it matters which base is the tRNA and which is in the mRNA -- only certain combos are allowed. For example, an A in the tRNA (in the wobble position) can pair only with U in the mRNA. But an A in the mRNA (in the wobble position) can pair with a U or I.

- Not all possible combos allowed by these rules are compatible with the genetic code. See below.

3. Example of wobble: The same tRNA (with anticodon 3' AAG 5') can pair with both the UUU codon and the UUC codon in mRNA. (Remember pairing is antiparallel, so anticodon must be "upside down" relative to mRNA. 3'AAG 5' in tRNA pairs with 5'UUU or 5' UUC in mRNA.) Since UUU and UUC code for the same amino acid (phe), both can pair with same tRNA carrying phe, and the right amino acid (phe) gets put in every time. So only one tRNA is needed, instead of two, to read both UUU and UUC. (See handout 15 A or fig. 19-4 (22-4) of Becker.)

4. Some combinations allowed by the "wobble rules" don't occur

- *Not all possible tRNA's exist.* The combinations that do not fit the code are not allowed -- that is, the corresponding tRNA's do not exist.

- *One tRNA should read only synonomous codons.* The wobble rules (see table above & on handout) indicate that some tRNA anticodons can pair with several different mRNA codons. However, you also have to be sure that each tRNA reads only synonymous codons -- all codons that it reads should code for the same AA.

- *An example:* You can't have a tRNA with AAI in the anticodon. Why not? AAA, AAG or AAI (all written 3' to 5') could pair with the codon UUU. If you had a tRNA with AAI in the anticodon, it would pair with UUU, UUC & UUA. These 3 codons are not synomous -- they don't all code for the same amino acid. You need a tRNA that pairs with UUU and/or UUC, but not UUA. Therefore a tRNA with AAG or AAA in the anticodon is OK, but a tRNA with AAI is not.

C. How Many Different tRNAs do we need?

On the average, you need two tRNA's for each "box" in the genetic code table. Note that one tRNA can never read more than 3 codons; most tRNA's read two, and some tRNA's read only one codon. So you need about 30 different tRNA's, not close to 64. What's the exact number needed to read all the codons? Let's start with a possible max. number of 64 and consider the effects of starts, stops, wobble etc. {Q&A}

1. How starts affect the # -- (2 met tRNA's -- see above) +1

(2). How stops affect the # -- (no tRNA for stops) -3

2. How stops affect the # -- (no tRNA for stops) -3

3. If consider starts and stops, but no wobble, total would be 62.

4. How wobble affects the # -- wobble (aprox.) cuts # in half -- see details above.

5. So what's the grand total? Need around 30 different tRNA's, not 20 or 64. (If you want to guess the exact number, look at the code box by box. The usual estimate of min. # required is 32.)

To review Wobble and Degeneracy, see Problems 7-15, 7-19, & 7-25.

V. Summary of How "RNA makes Protein"

A. How many different types of RNA are needed? To Review:

1. Role of mRNA. DNA codes for mRNA, and you use the mRNA to make protein. But is mRNA all you need? Of course not.

2. Also need tRNA and ribosomes (containing rRNA) as well as mRNA.

a. Where do tRNA and rRNA come from? All RNA's are encoded by DNA just like mRNA is. So there are genes for tRNA's and rRNA's on DNA. (Genes do not just code for proteins and respective mRNA's; genes also code for other RNA's needed along with mRNA to make proteins.)

b. tRNA and rRNA are not used as templates. When tRNA and rRNA genes are transcribed, the products fold up, associate with proteins if needed (for rRNA) and do their jobs. These RNA's are not translated -- they are agents that help in the translation of other RNA's (mRNA).

3. Different types of RNA have different half lives

a. tRNA and rRNA are relatively long-lived. Individual molecules last a long time and are used over and over before they are degraded.

b. Prokaryotic mRNA is relatively short-lived. mRNA's are constantly made, used for a short time, and degraded.

c. Eukaryotic mRNAs vary -- some but not all are short-lived; some are long-lived. Examples next term.

B. Does RNA alone make protein? *No. We probably should say "Protein and RNA make protein"*

You need enzymes, initiation factors (IF's) and elongation factors (EF's), ribosomal prot. etc. too, not just mRNA or just mRNA, tRNA and rRNA. But you need protein to do everything; it's the RNA part that's unusual. Yes, this is a chicken and egg problem. It's why you need a cell -- with everything needed to make a protein -- to make (another) cell.
Note If you are interested in the details of IF's, EF's etc., see Becker fig. 19-12 (22-10) for elongation and fig. 19-10 (22-8) for initiation of translation in bacteria.

*C. How many **different** types of the various RNA's are needed?* Let's consider what it takes to make one polypeptide, and then what you have to change if you want to make a second, different peptide.

1. What does it take to make one peptide? See next page.

a. mRNA -- You need one kind to make one polypeptide.

b. rRNA -- You need several kinds (3-4) to make one ribosome; exact # depends on whether it's a eukaryotic or prokaryotic ribosome.

c. tRNA -- You need one complete set (to pick up all amino acids and read all codons but stops). This is assuming the peptide you are making contains all 20 amino acids and the mRNA uses all possible codons. We calculated above that will you need about 30 different kinds of tRNA..
Note: question here is how many *different* kinds you need, not how many of each kind. If the same codon occurs twice in a row, you will need two copies of the corresponding kind of tRNA.

2. What if you want to make a different protein?

a. mRNA -- Will you need a different mRNA to make a second (different) peptide? Yes (but see note at *) -- you need a unique sequence of nucleotides (in the mRNA template) to make each unique peptide.

**Note:* a single mRNA can sometimes carry several different sections, each coding for a different peptide. In that case you could use a different section of the same mRNA to make a different peptide.
Messenger RNAs that carry the information to make multiple peptides are called "polycistronic mRNAs" and will be discussed when we get to operons. Polycistronic mRNA's are common in prokaryotes but rare in eukaryotes.

b. rRNA -- Once you have a complete set of rRNA's (and a ribosome), do you need a new set to make a second peptide? No, the same ribosome can read any message. (A real cell has many ribosomes, but all are the same.)

c. tRNA -- Once you have a complete set of tRNA's, will you need a new set to make a different peptide? No. The same set can be used over and over to make any number of different peptides. (A real cell has many molecules of each kind of tRNA, just as it has many ribosomes.)

3. Summary: mRNA is the software which is unique to the protein being made; tRNA & rRNA (& associated proteins) are the hardware that can be used to make any protein.

To review how many different RNA's it takes to "make protein" do problems 7-14 & 7-17.

Next Time: How does tRNA get loaded? Where does the energy to drive peptide bond formation come from? (Handout 15B) What are ribosomes made of? (See handout 15A bottom) Then operons and regulation of protein synthesis in prokaryotes.