C2005/F2401 '08 -- Lecture # 13 -- RNA & Protein Synthesis

Handouts: 13B -- Protein Synthesis; 13A -- code table & ribosome structure. (Handouts are not on web.)
Note: For this lecture, fig. and table numbers in the 6th & 7th ed. of Becker are all the same. In the 5th ed, translation is in ch. 20 instead of 22, but the fig. and table #'s are the same.

This lecture will be repeated Monday 10/27 at 7:30 in 614 Schermernhorn.

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I. Wrap up of DNA synthesis vs RNA synthesis. See handout 12-B.

A to C -- see last time.

D. Details for Starts and Stops (see picture below = bottom of handout 12B)

- Start sequences as binding sites. A start signal for transcription or replication is a sequence in the DNA recognized by the appropriate polymerase = binding site for that polymerase

- Names of start sequences
  Starts for DNA synthesis = Origins. DNA pol. recognizes (binds to) start signals for replication called origins (ori's).
  Starts for RNA synthesis = Promotors. RNA pol. recognizes (binds to) start signals for transcription called promotors (P's).

- Promotor Details:

  1. Promotors determine the direction of transcription. Promotor and enzyme are asymmetric; therefore once enzyme binds, the catalytic end of RNA pol. is "facing" in one direction, and that determines the direction of transcription (and therefore which strand will be template).

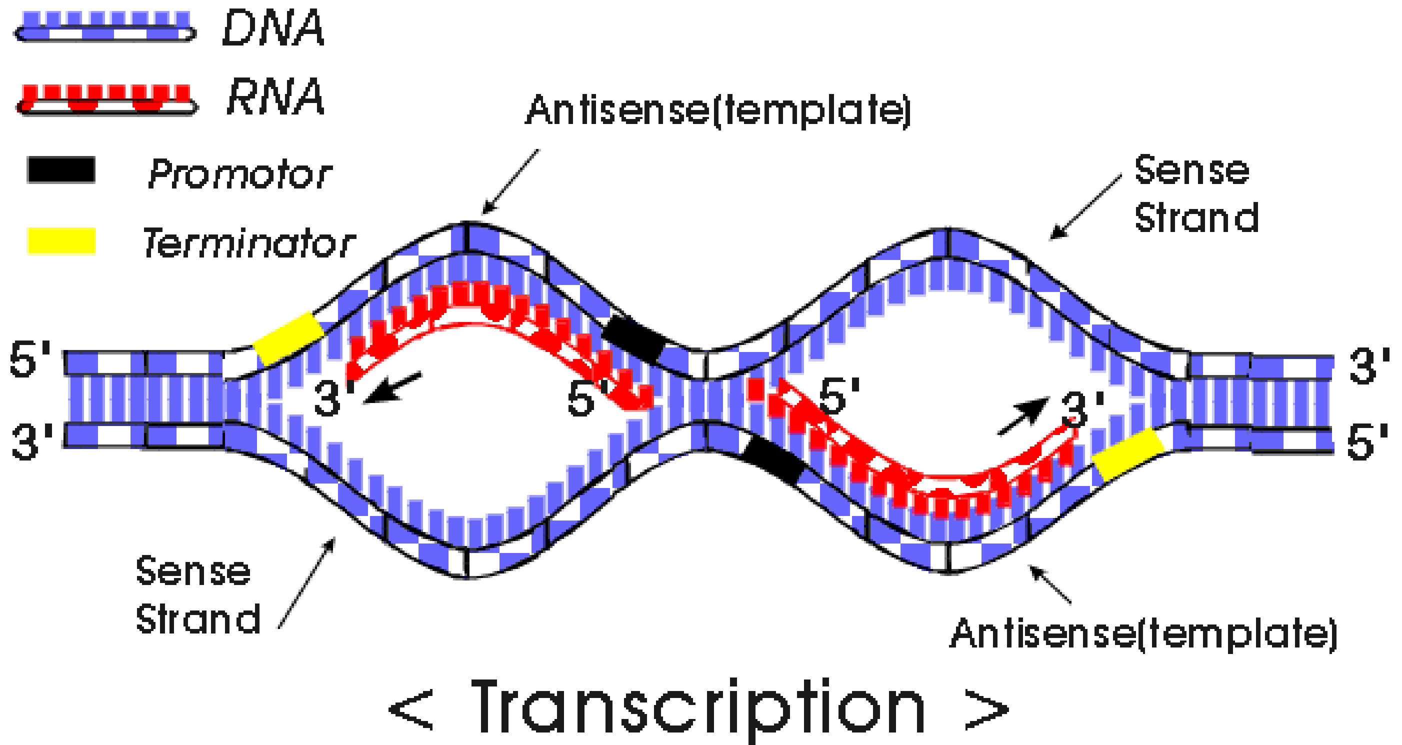
  2. The promotor will be a double stranded sequence at the end of the gene where RNA polymerase starts (= on 3' end of template strand = on 5' end of sense strand). Going along the sense strand, the way the gene is usually written (5' to 3', left to right) the promotor is "upstream" of the gene.

- How many starts? There are more P's than ori's in prokaryotic DNA. (Only need one ori per prok. DNA; need one P per mRNA made.)

- Stop (Termination) Signals. Special sequences in DNA may not be needed for DNA pol. -- enzyme may just go until it reaches the end. You do need some sort of mechanism to end synthesis of each RNA. In prokaryotes there are special sequences (often called terminators) that cause the end of transcription. The mech. for ending transcription is somewhat different in eukaryotes and prokaryotes. (We'll do euk. details next term.)

  Notes:
  (1) Stop signals for ***translation*** (stop codons) are different than the stop signals for ***transcription***

(terminators). See Sadava table (not fig.) 12.1. Translational stops are not recognized by the transcription (or replication) machinery. Each set of enzymes (for translation, transcription, or replication) recognizes only its own respective start and stop sequences.
(2) The process of starting and stopping macromolecular synthesis is often more complex than we discuss. See texts for details.



See problem 7-8.

II. Sense & Antisense

A. Why use only one strand in any one region?

1. The function argument: Messenger RNA must be single stranded to fit in a ribosome and be translated. If RNA complementary to mRNA were present, what would happen? The "sense" mRNA and the "anti-sense" complementary RNA would hybridize. The resulting double stranded **RNA** wouldn't be translated. So even though the gene was present, and transcribed, it's protein product wouldn't be made. This is what would happen if both strands were transcribed. See Sadava fig.16.14 (16.11 in 7th ed, 17.12 in 6th).

2. The evolutionary argument: If both strands are used to make mRNA, you can't optimize one without messing up the other, and vice versa. If natural selection favors the sequence of one strand so that it has optimal function or coding activity, that automatically determines the sequence of the other strand. Natural selection can't simultaneously select for the optimal sequences of both strands (if each strand has an independent function).

B. Uses of "anti-sense" mRNA

1. What good is anti-sense RNA? Gene therapy (adding DNA) should allow you to replace a defective gene that is making an ineffective product. But what do you do about a gene that is making too much product, or

making it when it shouldn't? In other words, how do you silence an over-active gene? This is an important question, because inappropriate or over expression of genes is thought to be a major factor in disease, for example, in allowing cancer cells to multiply when they shouldn't. Use of anti-sense technology should allow you to silence an over-active, or inappropriately active, gene. (Usually short double stranded RNA is added instead of single stranded antisense RNA, as explained below. See Becker Figs. 23-35 & 23-36 or Sadava fig.16.14 (16.11)).

2. How to get anti-sense RNA into cells? There are 3 ways to do it:

a. Antisense mRNA can be added to cells. Since RNA is easily degraded, modified RNA's, more resistant to hydrolysis, are used instead of ordinary RNA's.

b. Antisense mRNA can be made in the cell from a second copy of the gene. The second copy is added by genetic engineering methods; it is inverted (relative to the promotor), so that the second copy of the gene is transcribed in the opposite orientation from the original copy. Inverting a gene relative to its promotor is equivalent to moving the promotor to the opposite end of the gene (and turning it around) thereby reversing the direction of transcription. The original copy is transcribed from the usual template ("transcribed") strand to make mRNA; the second copy is transcribed from the complementary ("sense") strand to make anti-sense RNA. The two RNA's hybridize to each other and neither RNA is translated.

c. Double Stranded (ds) RNA can generate antisense RNA -- See Becker fig. 23-35 (6th or 7th ed; not in 5th).

- ds RNA can be added to cell (or cell can make some ds RNA because of genetic engineering as in b)**
- Cells have normal enzymes that cut up long ds RNA into short ds pieces, called short interfering RNA (siRNA)
- Other enzymes degrade the 'sense' strand of the short ds RNA
- The remaining short piece of antisense RNA hybridizes to mRNA and blocks translation, and/or triggers degradation of the mRNA by cell enzymes.
- This phenomenon is called RNA interference or RNAi.

** Cells can also make their own 'normal' double stranded RNA, which is then cut up by enzymes to generate a short RNA that blocks translation as above. These short antisense RNA's are called microRNAs instead of interfering RNAs. See Becker fig. 23-36 (6th or 7th ed; not in 5th).

3. Why RNAi &/or microRNA? Why do cells have enzymes to do it and labs use it?

a. RNAi is used by cells as a defense against many viruses. (The replication of many viruses generates long double stranded RNA.)

b. Regulation of translation in multicellular organisms. This is the function of microRNAs. Precursor RNAs are made that fold back on themselves to form hairpins. The double stranded hairpins are processed by the cell enzymes used in RNAi to make very short 'antisense' RNAs (here called microRNAs). The microRNAs hybridize to mRNAs and inhibit translation. This type of regulation seems to be very important during development in normal muticellular organisms.

c. RNAi is used in laboratories to block production ('knock down' expression) of specific proteins. Very short double stranded RNAs are added to cells, or the cells are genetically engineered to produce the double stranded RNAs. It is easier and more effective to block translation with RNAi

(short ds RNA) than with antisense RNA (longer, ss RNA). RNAi has been used extensively (in lab experiments) to silence specific eukaryotic genes and see what happens (in order to determine the function of the genes).

d. Therapeutic uses. Many possible uses are currently being tested, and promising results have been obtained for treatment of macular degeneration. For a review of possible therapeutic uses of RNAi click here. (You may need to use a CU computer to reach this site.) Additional info is on the Nova/PBS site.

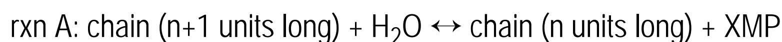
The 2006 Nobel prize in physiology and medicine was awarded to Fire & Mello for the discovery of RNA interference. For more info on RNAi, try the Nova/PBS site or the Ambion site. For a diagram of how it works, click here.

To check your understanding of antisense, see problem 7-16, part C.

III. Proofreading or Editing. This was mentioned briefly last time. Here is a review and a longer description. This will not be discussed in class, since the major points have already been made.

1. What is proof reading?

DNA pol. can back up and hydrolyze (break) phosphodiester bonds it has just made (if the wrong base was put in). This is called proof reading or editing. When it proof reads, DNA pol. catalyzes the following reaction:



rxn A: chain (n+1 units long) + H_2O ↔ chain (n units long) + XMP

2. Proofreading is not the same as catalyzing the reverse of the polymerization reaction.

Here is the normal elongation reaction catalyzed by DNA polymerase (to the right):

rxn B: Chain (n units long) + XTP ↔ Chain (n+1 units long) +PP_i

Any enzyme can catalyze its reaction in both directions, given the right concentration of substrates and products. Reversing the polymerase reaction would mean breaking the phosphodiester bond by adding pyrophosphate back and regenerating a dXTP like so:

(rxn B to the left): Chain (n+1 units long) +PP_i ↔ Chain (n units long) + XTP

However, what proof reading does is not the reverse of rxn B -- it's the hydrolysis of the phosphodiester bond (rxn A). Hydrolyzing or adding water across the newly made phosphodiester bond releases a dXMP (not a dXTP). Therefore hydrolysis is different from reversing the polymerase reaction.

Terminology:

The ability to remove nucleotides one at a time from the end of a chain is called exonuclease activity. (exo = from the exterior or end).

The enzymatic ability of DNA polymerase used in proof reading removes nucleotides one at a time from the 3' end of a chain. Therefore it is called 3' to 5' exonuclease activity.

The enzymatic activity of DNA polymerase that removes RNA primer has a different exonuclease activity -- this enzyme removes nucleotides one at a time from the 5' end of the primer (not from the 3' end). It has 5' to 3' exonuclease activity.

DNA polymerases are complex enzymes that have multiple subunits (peptide chains) and multiple enzymatic activities. The different enzymatic activities may be catalyzed by different subunits.

3. DNA polymerase can proof read, but RNA pol. probably does not

DNA polymerase has 3' to 5' exo activity but it is generally assumed that RNA pol. *does not -- once RNA polymerase catalyzes formation of a phosphodiester bond, the bond can not be hydrolyzed by RNA pol. Proof reading allows DNA polymerase to back up and remove bases (really nucleotides) that were inserted by error. If a G is added at the end of a growing chain where an A should have been (opposite a T in the template), the enzyme can back up and break off the G. Then it can try again to add the correct base (in this case an A). This allows DNA polymerase to keep the error rate low, as befits an enzyme that replicates the archival copy of the genetic information. See Sadava fig. 11.22 (a) (11.19 (a)). It is generally assumed that RNA pol. does not need to proofread, because RNA molecules are working copies that can tolerate a few errors (and can be replaced by new copies transcribed from the DNA).

*Note: There is some evidence that some RNA polymerases do have 3' to 5' exo activity and can proofread. How wide spread this is, and important it is in reducing errors (compared to DNA proofreading) is not settled. If you are interested in the experimental evidence for RNA proofreading, click here. Since RNA proofreading is not well established, we will ignore it. The important point is to understand how a proofreading polymerase works and how it differs from a polymerase that does not proofread.

4. Proof reading and the ability to start chains are linked

Table on handout 12-B says DNA polymerase edits (proofreads) and cannot start new chains; RNA polymerase does not edit (does not proofread) and can start new chains. These properties are linked. We will not go into all the details, but the structure of DNA polymerase that allows editing (proofreading) prevents it from starting new chains. (Note: the term editing is sometimes used for another process, so the term 'proofreading' is preferable and is used below.) Since DNA polymerase can add on to pre-existing chains, but cannot start them itself, it requires a primer (or primase) to get started.

The remaining two sections, 5 & 6, are FYI only. They are here in case you are interested; you will not be asked questions about these details.

5. FYI. How does proof reading work?

Every polymerase has a substrate binding site that includes the template, the last nucleotide added to the growing chain and the next dXTP to be added. With DNA polymerase, both bases, the one just added and the one about to be added, are checked each round to be sure the bases match their complements in the template. First, the last base added-template match is "rechecked" before the chain grows any longer. If the last base added turns out to have been the wrong one (perhaps it was in the wrong tautomeric form temporarily and mispaired with the template?), then the enzyme backs up and removes the last base before trying to add another. Once the enzyme checks that the last base added is ok, it checks the match between the base to be added and the template. If there is a match, the

enzyme catalyzes formation of the phosphodiester bond. So each base - template match is checked twice -- once when the base is about to be added to the growing chain and once before the next base is added to it.

RNA pol. also holds 2 nucleotides that are about to be linked by a phosphodiester bond and the template. But RNA pol. only checks the pairing between the base to be added and its complement in the template. So if the last base put in was wrong, so be it. No backing up or corrections.

6. FYI. Why does proof reading affect ability to start chains?

DNA polymerase can not start chains because the substrate binding site of DNA polymerase must hold both a nucleotide already part of a chain (the one just added) as well as the next nucleotide to be put in. There must be a phosphodiester bond that is already made, so the 3' to 5' exonuclease will have something to hydrolyze, just in case of a mismatch. At the start of a chain, there is no nucleotide already attached to the end of a chain -- there is no chain. There are only two, unattached nucleotides. So DNA pol. can't get started.

We assume that RNA pol. can start chains because its substrate binding site does not need to hold a nucleotide that is already attached to a chain. It can hold two nucleotides and hook them up.

An example of proof reading (which you should be able to do) is in problem 6-14, part B-4.

Reminder: All kinds of RNA (tRNA, mRNA & rRNA) are made in the same way from a DNA template. Product of transcription can be a tRNA, mRNA or rRNA. RNA is NOT used as template to make more RNA. So how do all three types of RNA "make protein?" That's the next question.

See problem 7-9.

IV. Details of Protein Synthesis/Translation

What are the big issues? Same as for all non repeating polymers = Order, energy and enzymes!! We'll focus on order first.

A. How is mRNA read?

1. It's read in triplets going 5' to 3' . Reading starts at a fixed point and then mRNA is read one triplet or codon at a time in the 5' to 3' direction.

2. Code table See handout 13A or texts for code table. Note that table lists codons = triplets found in the mRNA (NOT complements of codons) and corresponding amino acids. One codon specifies one amino acid. For example, CUA means leucine; UUU means phenylalanine, AUG means methionine.

3. Punctuation. Note that some codons signify "stop", not an amino acid. AUG does double duty as both "start" and "methionine." Translation usually starts at first AUG and ends at the first stop codon after the AUG. (More details on stops & starts below or next time.)

4. Leaders & Trailers. The region before the first AUG is not translated. It is called a leader, or 5'UTR (un-translated-region) or 5'UTS (un-translated sequence). Translation generally stops before the end of the mRNA (at a stop codon -- UAG, UAA or UGA). The untranslated region after the stop codon is called a trailer, or 3' UTR or 3' UTS.

To be sure you understand how to use the code table, try problem 7-12, parts A & B.

B. Structure/Function of tRNA 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/tRNA.wmv

1. Adapter Function -- how does cell know AUG is met and CUA is leu? You have the text or handout with the code table, but cell doesn't.

a. Transfer RNA (tRNA) = adaptor. Cell uses tRNA to match the codon in the mRNA (say AUG or CUA) with the corresponding amino acid (met or leu, respectively).

b. Loading Enzymes. Adaptor must carry the correct amino acid. Cell uses loading enzymes to put the correct amino acids on to their respective tRNA's. More details next time.

2. Structure of tRNA (see texts for pictures)

a. Size: About 75 bases long (relatively small). Consists of RNA chain folded back on itself.

b. Many different ones. Actual number of dif. tRNA's is more than 20 (#of dif. amino acids) and less than 64 (# of dif. codons). More exact estimate of # of different tRNA's to follow in next lecture.

c. Two headed molecule: tRNA has 2 critical parts

- one part (in middle of chain) is complementary to codon (= anticodon)
- one part (on 3' end) is acceptor end -- picks up the appropriate amino acid with the help of the appropriate enzyme.
- when tRNA is folded in 3D, acceptor end and anticodon are at opposite ends of molecule

d. General features of structure --

Secondary Structure: Each tRNA molecule is doubled back on itself to form a cloverleaf with double stranded sections. Sequences of different tRNA's differ, but all are self complementary in certain regions. Every tRNA molecule has same basic "secondary structure" = cloverleaf.

Tertiary Structure: Cloverleaf is folded into an L shaped "tertiary" structure, which has anticodon at one end and acceptor for its amino acid at the other. (See Becker fig. 22-3, or Sadava 12.8 (12.7 in 7th ed, 12.6 in 6th), for secondary and tertiary structures.) The final folded tRNA molecule is about one codon wide. That way two tRNAs can attached to neighboring codons without bumping into each other.

Important reminder: The code table lists the codons, NOT the anticodons. The anticodon in the tRNA is the ***complement*** of the triplet shown in the table.

See problem 7-18.

3. How is tRNA used to line up amino acids (AA)? 2 AA at a time are held in place by tRNAs (for forming peptide bond) -- see handout 13B. Why 2? because a ribosome can hold only 2 **loaded** tRNAs at a time that are hydrogen bonded to mRNA. (See details below.)

4. tRNA/mRNA pairing is antiparallel -- All nucleic acids pair in an antiparallel fashion. So if mRNA is written in usual way (5' → 3'), then tRNA is lined up in the opposite way, 3' → 5'. (With the amino acid or chain on its left, 3' end.) Anticodon is often written 3' → 5' to make this clear. For ex., if codon is AUG, anticodon is usually written 3' UAC 5' not CAU (or it is written upside down).

5. 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.

C. How does the new peptide chain grow? See handout 13B or Sadava fig. 12.12 (12.11) or Becker fig. 22-10.

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

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 chain is transferred as described above. This catalyst is part of the ribosome.

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.)

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. (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. 12.13 (12.12) or Becker fig. 22-11.

D. Loading of tRNA

We see what happens if you have lots of loaded tRNA. How do you get AA on tRNA in the first place and/or how do you reload the tRNA once it gives its AA away? Loading requires enzymes and energy -- we'll look at it carefully next time. For now we'll just assume each tRNA is loaded with its respective amino acid,

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

E. How do ribosomes fit in?

1. Function. You need something to hold tRNA (two loaded ones at a time) onto mRNA while amino acids are being hooked up and you need to provide necessary enzymes for making peptide bond etc. (How many weak bonds hold a tRNA and mRNA together?)

2. Overall Structure. Holding of tRNA etc. is done by a structure that contains both RNA(s) and protein(s). Anything made of both 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. So you need rRNA as well as mRNA and tRNA for translation.

For details and/or pictures of ribosome structure see Handout 13A, or Sadava 12.10 (12.9 in 7th ed, or 12.8 in 6th) and/or Becker figs. 22-1 & 22-2 & table 22-1.) See also topic F below.

3. Important Features (See Becker, fig. 22-2 or Sadava fig. 12.10 (12.9.) *See handout 13-A.*

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 bumped off the ribosome. (It's called E for exit site). This site is sometimes omitted in diagrams of elongation. (The T site shown in the 7th ed. of Purves probably does not exist and should be ignored.) The E site binds tRNA but not mRNA.

d. All ribosomes are the same. Which protein is made does not depend on the ribosome.

4. How Ribosomes Function (See Becker fig. 22-7 & 22-10 or Sadava fig. 12.12 (12.11.)

a. Attachment. When not in use, ribosomes come apart into subunits. (See handout 13A.) The cell contains a pool of subunits. When translation starts, one small subunit and one large subunit clamp onto the mRNA to form a ribosome and begin translation. When translation ends, the two subunits come apart, fall off the mRNA, and return to the pool -- ready to be used again.

b. Directions: Ribosome moves down mRNA 5' to 3' (or mRNA slides through ribosome) as peptide is made amino to carboxyl. Both peptides and nucleic acids are both made/read as written, left to right.

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).

c. A & P sites. The two binding sites for loaded tRNA are different -- 1 called A binds **a**mino acyl tRNA & 1 called P binds **p**eptidyl tRNA.

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

(1). Differences between the A & P sites allow unidirectional movement. Before peptide bond is formed, AA-tRNA is in A site and peptidyl-tRNA is in P site. 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, Since "wrong" types of tRNA are now in A & P sites, ribosome no longer fits properly and moves over one codon, shifting peptidyl-tRNA to P site, empty tRNA to E site and leaving A site empty to hold next AA-tRNA. The empty or unloaded tRNA is then released to be reloaded and used again.

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

mRNA & ribosome: Move one codon relative to each other. On handout 13B, 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.)

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

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**.** 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. So 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 will be explained next time. (For involvement of GTP in translation see Becker figs. 22-8 & 22-10.)

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

e. Polysomes.

More than one ribosome can read a single message at one time. The first ribosome attaches near the 5' end of the mRNA. Then the ribosome moves (see note below) down the mRNA toward the 3' end, making protein. 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. As each ribosome moves toward the 3' end, making protein, another ribosome attaches after it until the entire mRNA is covered with ribosomes. 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.12.14 (12.13).

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.

F. Detailed Structure & Assembly of Ribosomes: (see bottom of handout 13A). See texts for pictures.

1. Parts. Each ribosome is made of two subunits. Each subunit is a ribonucleoprotein or RNP made of at least one kind of rRNA and many proteins. Each subunit is made separately; two subunits (one large, one small) clamp onto message to form a complete ribosome for translation. The two subunits separate from each other (and the mRNA) when they reach the end of the mRNA.
See Sadava 12.10 (12.9); Becker fig. 22-1.

2. Names of Parts. Subunits of ribosome and different ribosomal RNA's are identified by their sedimentation constants (S values) in an ultracentrifuge. Two values are given on the handout for the sizes of the RNA's and subunits -- the smaller number is for prokaryotes; the larger # for eukaryotes. See handout and/or Becker table 22-1.

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* with *ribosomal RNA.*

G. Peptidyl Transferase is a Ribozyme

Peptidyl transferase is part of the ribosome. 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). It is presumed that it is a relic 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). Peptidyl transferase is not the only ribozyme -- other catalytic RNA's are known.

For more details see 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.)

Next time: Any details of the above we don't get to, plus a few more details to wrap up translation. Then (1) what happens when macromolecular synthesis makes mistakes, and (2) how is protein synthesis regulated in prokaryotes?

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