C2005/F2401 '10 -- Lecture # 13 -- RNA & Protein Synthesis

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Handouts: 13A -- code table & tRNA structure.
13B -- Protein Synthesis
12B -- from last lecture -- DNA synthesis vs RNA synthesis

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.

I. DNA synthesis vs RNA synthesis. The easiest way to go over RNA synthesis, given that we've discussed DNA synthesis at length, is to compare DNA and RNA synthesis. **See handout 12-B.**

A. What is the same? See Lecture 12.

B. What's Different?

1. Enzymes

- Growth of DNA chain is catalyzed by DNA polymerase (and associated enzymes)
- Growth of RNA chain is catalyzed by RNA polymerase.

2. Choice of Substrate. If you put all 8 XTP's in a test tube, what do you get, DNA or RNA? Enzyme (DNA vs RNA pol) is responsible for which nucleotides used.

- RNA pol. uses ribonucleoside triphosphates (containing U, not T).
- DNA pol uses deoxyribonucleoside triphosphates (containing T, not U).

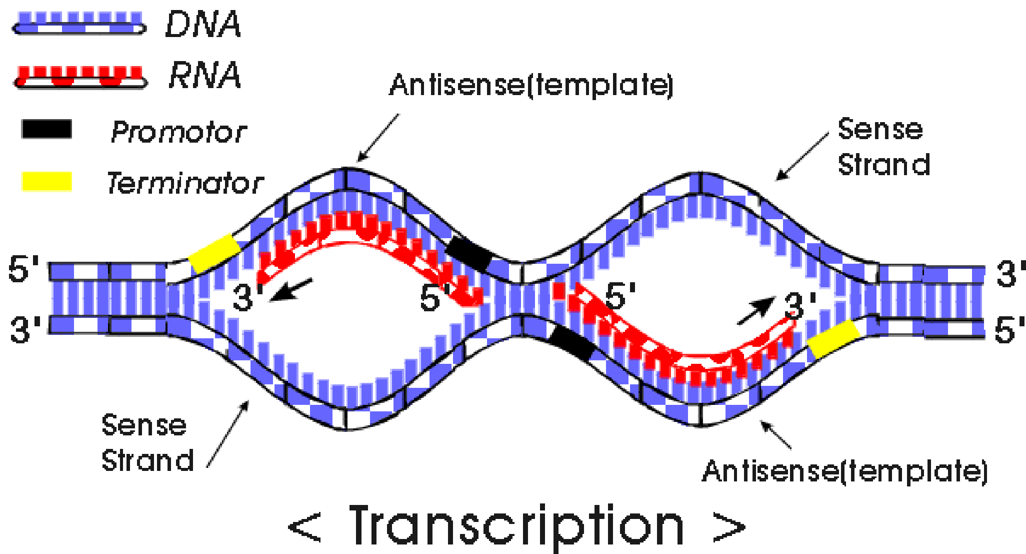
3. Products

- *DNA is long and double stranded*
- *RNA is short and single stranded*

4. Choice of which part of Template to use

- Template = short section, one strand at a time (for RNA synth.) vs all of both strands (for DNA synth.)
- Why? Because starts and stops are different. Starts & stops = sequences in DNA recognized by the enzymes = places where replication or transcription starts (or ends). These must be different for the two enzymes.
- Names of start sequences = section where polymerase binds
  Starts for DNA synthesis = Origins. DNA pol. recognizes (binds to) start signals for replication called origins (ori's).
  Starts for RNA synthesis = Promoters. RNA pol. recognizes (binds to) start signals for transcription called promoters (P's).

See problem 7-6



C. Template Details

1. One Strand is Template for RNA polymerase. For any one gene or region, RNA polymerase uses Crick or Watson, but not both, as template. RNA that is made is complementary (and antiparallel) to the template strand. Note that an entire strand is not used as template throughout. The "Watson" strand of DNA is used as template in some sections and the "Crick" strand in others.

2. Continuous vs. discontinuous synthesis.

- DNA synthesis: Replication fork moves down DNA making complements to **both** strands; one new strand is made continuously and one discontinuously. Ligase is needed for synthesis of lagging strand.

- RNA synthesis: RNA polymerase moves down DNA making complement to one strand **or** the other (in any particular region). Therefore RNA synthesis is continuous and doesn't need ligase.

3. Terminology

a. Transcribed Strand. Strand used as template is called the transcribed or template strand or the antisense strand (in that region). This strand is **complementary to** the RNA that is made.

b. Sense Strand. Strand that is **not** transcribed (in that region) is called the sense strand or coding strand. The base sequence of this strand is **identical to** the RNA that is made (except that the RNA has U and the sense strand has T).

c. An entire DNA strand (going the length of a whole molecule) is not all "sense" or "antisense." "Watson" may be sense in one section and "Crick" may be sense in the other (as in the picture on handout 12-B). The terms "sense" and "transcribed" strand are defined for each section of the DNA that is transcribed as a unit (usually a gene or small number of genes).

d. Sense RNA. The usual RNA transcribed from the DNA is said to be "sense." (Sense RNA matches the sense strand of the DNA.) The complementary RNA, if it exists, is said to be "antisense." Some practical uses of "antisense RNA" are below.

e. Why this terminology? The sense strand (not the template) actually contains the information used to line up amino acids to make proteins. (Assuming the gene codes for a peptide.) When a DNA sequence is published, it is usually the sense strand that is given. Why? If the gene codes for a protein, the amino acid sequence of the protein is much easier to figure

out using the sense strand -- you just consult the code table (details next time).

f. Additional notes FYI on terminology:

(1). Becker (and some others) call the sense strand the coding strand, meaning the "strand coding for protein." I prefer the term "sense strand" since coding strand could mean "coding for protein" or "coding for mRNA." (The term "coding strand" is almost always used the way Becker uses it, to mean "coding for protein.")

(2). The terms "template strand" or "transcribed strand" can also be interpreted in more than one way, but these terms are virtually always used to mean the strand acting as template for RNA synthesis (= the strand that is transcribed from, not the strand that is being made, during transcription). The template or transcribed strand is **not** the strand equivalent to the mRNA -- the template strand is the strand ***complementary*** to the mRNA.

4. Directions: Suppose you have a double stranded DNA template. If need to copy "Crick," RNA polymerase will go one way (say right to left -- actual direction will depend on which end of template is 5' end); if need to copy "Watson" RNA polymerase will need to go the other way (say left to right). What determines where RNA polymerases starts & which way it goes? This is discussed below.

See problems 7-3, 7-4, 7-8 & 7-9.

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 = Promoters. RNA pol. recognizes (binds to) start signals for transcription called promoters (P's).

- Promoter Details:

  1. Promoters determine the direction of transcription. Promoter 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 promoter 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 promoter 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.) 14.2 (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. (More on this when we get to operons.)
(2) The process of starting and stopping macromolecular synthesis is often more complex than we discuss. See texts for details.

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.

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.18.8 (16.14).

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 promoter), so that the second copy of the gene is transcribed in the opposite orientation from the original copy. Inverting a gene relative to its promoter is equivalent to moving the promoter 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 from its DNA either naturally -- see ** below -- or because of genetic engineering, as above)
- 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. (It is made as a single strand, but doubles back on itself to form a relatively short hairpin.) The hairpin 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.

The 2009 Horowitz prize was awarded (by Columbia U.) to two of the discoverers of microRNAs. The awardees gave lectures last November. For more info on the prize, the lectures, and the awardees research, go to http://www.cumc.columbia.edu/horwitz/

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.

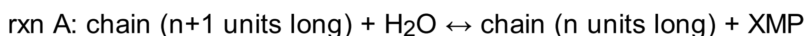
The 2006 Nobel prize in physiology and medicine was awarded to Fire & Mello for the discovery of RNA interference. For more info on RNAi, try the Nova/PBS site or the Ambion site. For a diagram of how it works, click here.

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

III. Proofreading. This was introduced last time. Here is a review and a longer description. This will not be discussed in class at length, 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. (In some older texts it is called editing, but the term 'editing' is now usually reserved for a different process.) When DNA pol. proof reads, it catalyzes the following reaction:


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

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

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. (But see ** below.) 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. 13.21 A (11.22 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 can backup and proofread (although by a somewhat different mechanism). How wide spread this is, and important it is in reducing errors (compared to DNA proofreading) is not settled. It is well known that the error rate in DNA synthesis is significantly lower than the error rate in RNA synthesis. (The difference is at least one order of magnitude, and may be much larger.) If you are interested in the experimental evidence for RNA proofreading, click here. Since RNA proofreading is not well established, we will ignore it.

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

Table on handout 12-B says DNA polymerase proofreads and cannot start new chains; RNA polymerase does not proofread and can start new chains. These properties are linked, because the structure of DNA polymerase that allows proofreading prevents it from starting new chains. 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. If you want to know more, consult an advanced text.

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 starts at an AUG, and ends when it reaches the first stop codon after the AUG. How the proper AUG is chosen is different for prokaryotes and eukaryotes. (See the texts if you are interested in the details.) More specifics on stops & starts 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.

5. 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.

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 handout 13A & 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 handout, Becker fig. 22-3, or Sadava fig. 14.12 (12.8), 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 CGG, anticodon is usually written 3' GCC 5' not CCG (or it is written upside down as on handout 13A).

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.

6. Loading of tRNA. How do you get the right AA on the corresponding 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,

C. How does the new peptide chain grow? See handout 13B or Sadava fig. 14.16 (12.12) 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 growing peptide 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. 14.17 (12.13) or Becker fig. 22-11.

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

D. 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. Ribosome contains both RNA and protein. 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. Be careful not to confuse ribosomal RNA (rRNA) & ribosomes.

For pictures of ribosome structure see Sadava fig. 14.14 (12.10) and/or Becker figs. 22-1 & 22-2 & table 22-1.) Molecular details of structure next time.

3. Important Structural Features (See Becker, fig. 22-2 or Sadava fig. 14.14 (12.10) *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 Move (See Becker fig. 22-7 & 22-10 or Sadava fig. 14.16 (12.12)

a. 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).

b. 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.

c. 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. When the next AA-tRNA arrives, 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.

5. How Ribosomes attach to mRNA

a. Attachment. When not in use, ribosomes come apart into subunits. 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. 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. 14.18 (12.14).

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.

E. 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 some more important 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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