Lecture 5 (FW)
February 4, 2009
Translation, tRNA adaptors, and the code
Reading .Chapters 8 and 9

Lecture 5. **How DNA governs protein synthesis.**
Primary goal: How does sequence of A,G,T, and C specify the sequence of amino acids in a protein?

I. The breaking of the code. Marshall Nirenberg at NIH.

A.What triplets of nucleotides actually encode which amino acid. This was discovered by Nirenberg in 1960, and Khorana at MIT. Nirenberg worked with the bacteria, E. coli, and found that he could encourage extracts of bacteria to synthesize protein if one gave it synthetic RNAs. The first synthetic RNA tried was composed of only one nucleotide, U (a relative of T). This made a protein composed only of one animo acid, phenylalanine. Eventually, all 64 combinations were tried. Examine the codon table in the text (p. 131)

B. The genetic code
The code is universal
The code is redundant. I i.e., there are synonyms.
The code has punctuation, i.e., signals for Start and Stop.

II. tRNA as the adaptor molecules

A. Now it is possible to see how Crick's adaptor hypothesis might work. Each amino acid is attached to an end of an adaptor RNA. This **adaptor** RNA is called **tRNA** (for transfer RNA).

B. The tRNA molecule possesses a nucleotide triplet in the middle of its sequence that has the exact complement of the code for the amino acid that it carries. The 3 letter code in the mRNA is called a **codon**. The complement in the tRNA is called the **anti-codon**. tRNAs are shaped so that the anti-codon is exposed and can pair (A with T; G with C) with the codon in the mRNA.

III. **Translation** (protein synthesis)

A. Translation is the step wise addition of one amino acid to another to form a protein. The first amino acid is always methionine; the methionine codon is the signal for "start". The sequence of the subsequent amino acids is strictly dictated by the sequence of codons in the mRNA.

B. The **ribosome** is the factory that uses the instructions in the mRNA. The mRNA "threads" through the ribosome (which has many enzymes associated with it) like an audio tape through a tape recorder. As the mRNA passes, the tRNA adaptors with their appropriate amino acids form "base-pairs" (i.e., G to C, U to A, etc) with codons in the mRNA. As each new adaptor comes into place, a bond is formed by enzymes between the newly incoming amino acid and the one just put in place.

C. When the ribosome hits a "stop" codon, the assembled string of amino acids falls off.

D. That's not the complete end of the story. Proteins may be modified, e.g, adding carbohydrates, phosphate, etc. They also have to be sent to the place in the cell where they belong, or secreted outside the cell.

IV. Return to Sickle Cell hemoglobin (HbS)

A. Let's look again at HbS.

1. How could Glutamic acid be replaced by Valine?
   One Glu codon is: GAA, another GAC. One Val codon is: GUA, another GUC.
2. Changing one nucleotide (called a SNP (**single nucleotide polymorphism**) can have profound consequences.

B. In-class Problem. Refer to the table on p. 131 of your text.

Let's suppose that amino acid # 37 in the β chain of Hb is specified by the codon UGC. What if a single C is changed to A?

A. Sickle cell Hb would form. B. There would be no effect, i.e., a synonymous change. C. The charge of the protein would change. D. A shortened, probably mutant form, of Hb would form.

V. The Central Dogma. Is it inviolate?

A. How do we know that the central dogma is true? Well, first of all, it seems to explain the known facts, and let's face it, it's beautiful. But we need some hard evidence.

B. Predictive value: if you make a DNA of known sequence, you can transcribe it to make an mRNA. This mRNA can be mixed with ribosomes and appropriate enzymes and it will make a protein whose sequence of amino acids is absolutely predictable.

Change any of those things, i.e., DNA sequence or the mRNA, in a living organism, and you can predict the outcome. This is what genetic engineering of crops and animals is all about.

Take home message: <u>Sequence in DNA specifies sequence of codons in mRNA which specifies sequence of amino acids in protein</u>.

Some things to think about:

1. What kinds of therapies are possible when a condition is due to a SNP? Do you treat the basic cause, or treat the symptoms?
2. What is a desirable use of genetic counseling for those afflicted with a serious condition caused by a SNP?

Word List: tRNA, genetic code, codon, anti-codon, ribosome, translation, single nucleotide polymorphism, base pair.

Reading for next lecture: pp. 37-45 in Ch 3; 51-62 in Ch. 4.

The Genetic Code

1st position (5' end) ↓	2nd position U	2nd position C	2nd position A	2nd position G	3rd position (3' end) ↓
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Amino Acids and Their Symbols

			Codons					
A	Ala	Alanine	GCA	GCC	GCG	GCU		
C	Cys	Cysteine	UGC	UGU				
D	Asp	Aspartic acid	GAC	GAU				
E	Glu	Glutamic acid	GAA	GAG				
F	Phe	Phenylalanine	UUC	UUU				
G	Gly	Glycine	GGA	GGC	GGG	GGU		
H	His	Histidine	CAC	CAU				
I	Ile	Isoleucine	AUA	AUC	AUU			
K	Lys	Lysine	AAA	AAG				
L	Leu	Leucine	UUA	UUG	CUA	CUC	CUG	CUU
M	Met	Methionine	AUG					
N	Asn	Asparagine	AAC	AAU				
P	Pro	Proline	CCA	CCC	CCG	CCU		
Q	Gln	Glutamine	CAA	CAG				
R	Arg	Arginine	AGA	AGG	CGA	CGC	CGG	CGU
S	Ser	Serine	AGC	AGU	UCA	UCC	UCG	UCU
T	Thr	Threonine	ACA	ACC	ACG	ACU		
V	Val	Valine	GUA	GUC	GUG	GUU		
W	Trp	Tryptophan	UGG					
Y	Tyr	Tyrosine	UAC	UAU				

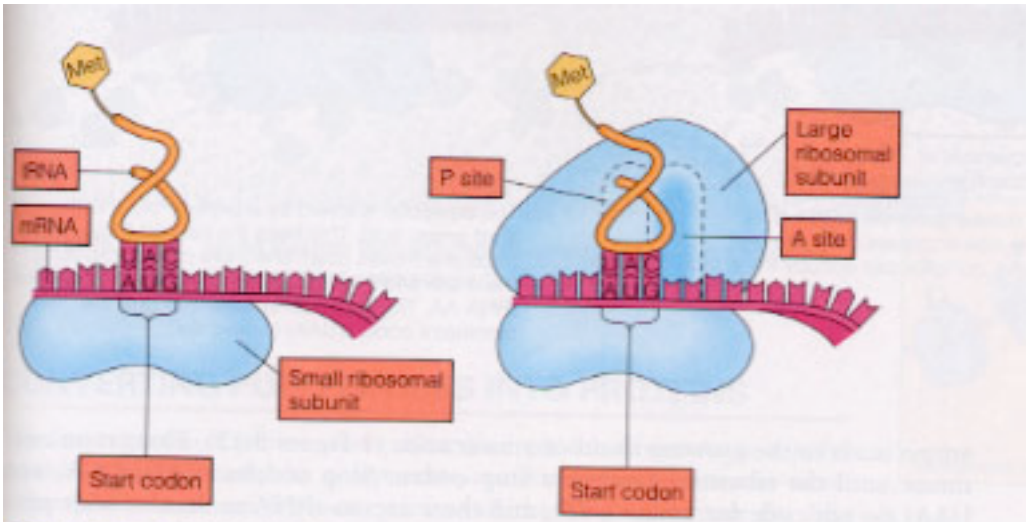
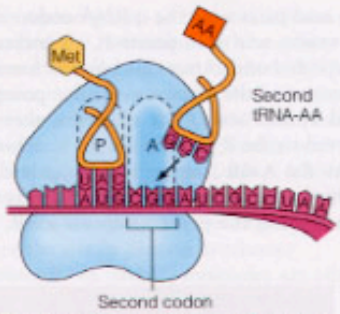
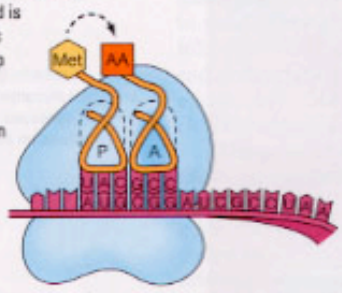


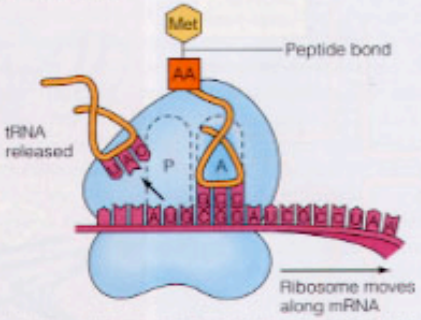
FIGURE 9.10 Initiation of translation. An mRNA molecule in the cytoplasm binds to a small ribosomal subunit. A tRNA that carries an amino acid (usually methionine) binds to the first codon (the start codon) in the mRNA. In a second step, a large ribosomal subunit binds to the small subunit, forming the initiation complex.

FIGURE 9.11 Elongation during translation. (a) After initiation, the anticodon of a second tRNA binds to the second mRNA codon, which occupies the A site of the ribosome. (b) During peptide bond formation, the two amino acids are linked together by a covalent chemical bond. When the peptide bond is formed, the tRNA in the P site is released, leaving this site empty. (c) In translocation, the ribosome shifts down the mRNA by one codon, moving the tRNA that carries the growing polypeptide chain to the P site and moving the next mRNA codon into the A site. (d) A tRNA that carries an amino acid binds to the codon in the A site, and another peptide bond is formed. This process continues until a stop codon in the mRNA (UAA) is reached. When the stop codon occupies the A site, the translation complex comes apart, and the ribosome, mRNA, and completed polypeptide are separated.

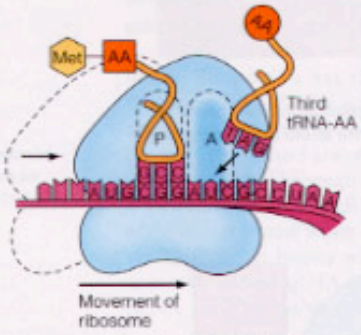


(a) As the first step in elongation, a tRNA-AA complex binds to the codon in the A site.

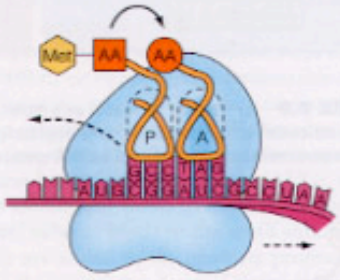




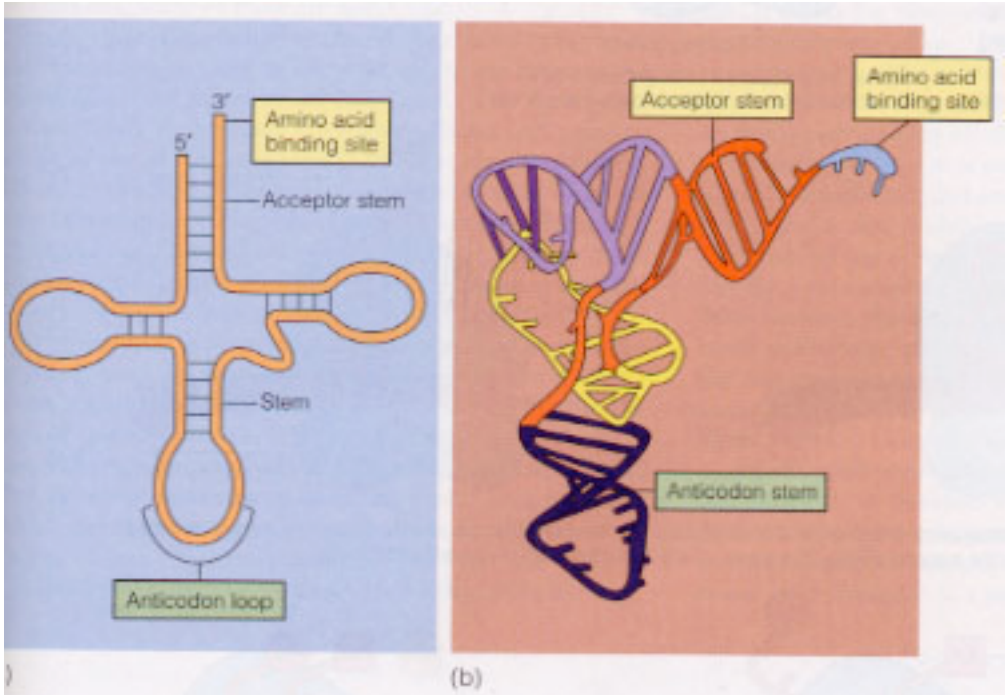
(b) An enzyme catalyzes the formation of a peptide bond between the two amino acids. The dipeptide that forms is attached to the second tRNA. This frees up the first tRNA, which vacates the P site.



(c) The ribosome moves down the mRNA. The tRNA-dipeptide now occupies the P site and another tRNA-AA complex can occupy the A site.



(d) The dipeptide is linked by a peptide bond to the third amino acid. This frees the second tRNA. The ribosome moves down one more codon, exposing the A site and freeing it up for the addition of another tRNA-AA. This process repeats itself until the terminator codon (UAA) is reached.



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(b)

FIGURE 9.9 (a) Transfer RNA acts as a molecular adapter. It recognizes mRNA codons (at