

7.016 Recitation 8 – Fall 2018

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Summary of Lecture 10 (9/28):

Translation: Translation begins when the ribosome orients itself at the start codon 5'-AUG-3' on an mRNA. A tRNA that recognizes and base pairs with that codon (i.e. a tRNA with the anticodon 3'-UAC-5') fits into the ribosome and donates the amino acid to which it is bound (methionine) to the new protein. From then on, three nucleotides in the mRNA are read at a time by another tRNA, which is modified with a single amino acid (an amino acyl tRNA). Each time, the next amino acyl-tRNA comes and fits into the ribosome, donates its amino acid to the new protein, waits for the next amino acyl-tRNA to come in, and then exits. This proceeds until a stop codon is reached, and the ribosome dissociates from both the mRNA and the newly made protein. Every protein has an N (amino) and a C (carboxyl) terminus, and proteins are synthesized in the N → C direction, such that the 5' end of the mRNA corresponds to the N-terminus of the protein and the 3' end of the original template DNA strand.

Mutations: Most mutations that geneticists study are single nucleotide mutations that cause phenotypic changes. The four types of single nucleotide mutations are silent, missense, nonsense, and frame shift. A silent mutation changes a codon but does not change the amino acid encoded by that codon. A missense mutation changes the identity of the amino acid at one position. A nonsense mutation causes a protein to be truncated because a codon is changed to a stop codon. A single nucleotide frame shift is a change that either inserts or deletes a single nucleotide from the coding region of a gene, leading to a change in the reading frame of that gene.

Process of Gene regulation: The production of the final, functional protein from a gene can be regulated at many steps.

- The gene may or may not begin to be transcribed, depending on whether the regulatory proteins (activator or repressor) are bound to the regulatory sites of the gene.
- If the gene is transcribed, RNA polymerase may or may not transcribe all the way through till the end of the gene.
- The introns may or may not be spliced out and the message (mRNA) may or may not be transported from the nucleus to the cytoplasm.
- The mRNA may or may not be stable.
- Translation of the message by the ribosome may or may not occur.
- If the protein is made from the message, that protein may or may not be active. (Many proteins require different kinds of covalent modifications, such as phosphorylation, in order to be active.)
- The protein may or may not be stable.
- The protein may or may not need to be transported to a specific subcellular location in order to access its substrate and perform its function.
- The transcription may not happen due to chromatin modification such as altered methylation of bases or de-acetylation of histone. Methylation of DNA inhibits transcription, and the pattern of methylation of DNA varies depending on cell type and cell age.

Questions:

1. Complete the table below.

	Translation
Subcellular organelle (s) in eukaryotic cell where translation occurs	
Monomer used to form proteins	
Rule for adding a type of incoming monomer?	
Covalent bond formed between two adjacent monomers in a growing polypeptide chain?	
Number and type of template (RNA or DNA) for protein synthesis	
In what direction is the template read?	
In what direction is the polypeptide chain synthesized?	

2. Assume that the sequence of DNA below is a short protein-encoding gene; the sequences in between the transcription start and stop sites are shown. The entire DNA sequence of the very short gene is:

5' CGCTTATAGAACCCAATCTCTCATAGGC 3'
3' GCGAATATCTTGGGTAGAGAGTATCCG 5'

a) What would the resulting mRNA be if the top strand of this DNA molecule were used as a template in transcription? Label the 5' and 3' ends of your molecule: _____

b) What is the full sequence of the protein that would be translated from this RNA? Label the N and C termini of your molecule: _____

c) What would happen to the encoded protein if the underlined nucleotide **C** were mutated to a **T**

3. Drawn below is part of a wild-type gene. The DNA sequence shown encodes the last amino acids of a protein that is normally 380 amino acids long. The **bold & underlined** codon indicates the correct reading frame of this gene. The lower strand of the gene is used as the template during the transcription of mRNA from this gene.

...**GCT**AAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3'
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5'

a) In the copy of the sequence drawn above, circle one base pair that you could change to make a mutant form of the gene that produces a protein that is now 381 amino acids long. Indicate the identity of one new base pair that could take its place

b) In the copy of the sequence drawn above, draw a slash between two base pairs where you could add one extra base pair in order to make a single mutant form of the gene that produces a protein that is 373 amino acids long. Indicate the identity of the one new base pair you are adding.

Solution key:

1. Complete the table below.

	Translation
Subcellular organelle (s) in eukaryotic cell where translation occurs	<i>Cytoplasm and/ or ER membrane (for secretory and plasma membrane proteins)</i>
Monomer used to form proteins	<i>Amino acids</i>
Rule for adding a type of incoming monomer?	<i>Adding is to the –COOH end</i>
Covalent bond formed between two adjacent monomers in a growing polypeptide chain?	<i>Peptide bond/ amide bond</i>
Number and type of template (RNA or DNA) for protein synthesis	<i>One, mRNA template</i>
In what direction is the template read?	<i>5→3'</i>
In what direction is the polypeptide chain synthesized?	<i>N→C</i>

2. Assume that the sequence of DNA below is a short protein-encoding gene; the sequences in between the transcription start and stop sites are shown. The entire DNA sequence of the very short gene is:

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5' CGCTTATAGAACCCAATCTCTCATAGGC 3'
3' GCGAATATCTTGGGTTAGAGAGTATCCG 5'

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a) What would the resulting mRNA be if the top strand of this DNA molecule were used as a template in transcription? Label the 5' and 3' ends of your molecule: *5'GCCUAUGAGAGAUUGGGUUCUAUAAGCG3'*

b) What is the full sequence of the protein that would be translated from this RNA? Label the N and C termini of your molecule: *N-Met-Arg-Asp-Trp-Val-ILeu-C*

c) What would happen to the encoded protein if the underlined nucleotide **C** were mutated to a **T**? *N-Met-Arg-Asp-C (This creates a premature stop codon resulting in a truncated protein).*

3. Drawn below is part of a wild-type gene. The DNA sequence shown encodes the last amino acids of a protein that is normally 380 amino acids long. The **bold & underlined** codon indicates the correct reading frame of this gene. The lower strand of the gene is used as the template during the transcription of mRNA from this gene.

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...GCTAAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3'
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5'

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a) In the copy of the sequence drawn above, circle one base pair that you could change to make a mutant form of the gene that produces a protein that is now 381 amino acids long. Indicate the identity of one new base pair that could take its place

You should change the stop codon immediately after the codon for 380th amino acid to get a protein that is 381 amino acids long. Please note that the codon immediately after the first stop codon is also a stop codon.

b) In the copy of the sequence drawn above, draw a slash between two base pairs where you could add one extra base pair in order to make a single mutant form of the gene that produces a protein that is 373 amino acids long. Indicate the identity of the one new base pair you are adding.

You should add a "T" before the 1st base in the 374th codon so that you get a stop codon.

		2nd base in codon				
		U	C	A	G	
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G
						3rd base in codon

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7.016 Introductory Biology
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