Genes: DNA to Protein

a) Introduction

A gene is a segment of DNA that encodes a protein. The nucleotides of the gene (the G's , A's, T's, and C's) are the instructions that tell the cell which amino acids must be linked together to make one particular protein. Human beings have around 25,000 genes. In contrast, bacteria have only around 2,000.

When a cell is using a gene to make a protein we say the cell is "expressing" the gene. Chapter 15 in your textbook describes all the steps of gene expression for both eukaryotic cells and prokaryotic cells. A brief review of the steps in eukaryotic cells is given below:

1) The enzyme RNA Polymerase II (RNA Pol II) binds to the gene at a region known as the promoter.

2) RNA Pol II makes an RNA molecule (called the "pre-mRNA" or the "primary transcript") that is complementary to one strand of the gene. The 5' end is the beginning of the pre-mRNA and the 3' end is the end of the pre-mRNA. This step is called transcription.

3) When the whole gene has been transcribed, the pre-mRNA detaches from the gene.

4) A 5' cap is added to the pre-mRNA.

5) A poly-adenosine tail is added to the 3' end of the pre-mRNA.

6) The introns (segments of nucleotides within the pre-mRNA that do not encode any amino acids) are removed. After this step, the RNA is called the mature mRNA.

7) The mature mRNA exits the nucleus and enters the cytoplasm.

8) A ribosome organelle binds to the 5' end of the mRNA

9) Using the codons of the mRNA as instructions, the ribosome assembles a protein from the 20 different types of amino acids. This step is called translation.

b) Sequences in the gene and the mRNA

Many of the nine steps described above rely on specific sequences of nucleotides in the gene or the mRNA. For example, the promoter has a specific sequence that allows RNA POL II to begin transcription of the gene. Transcription ends when a specific sequence near the end of the gene is transcribed into the mRNA.

The important nucleotide sequences are described below. Biologists use the term 5' to mean toward the beginning of the gene or mRNA, and 3' to mean toward the end of the gene or mRNA.

Promoter sequences: The promoters of almost all eukaryotic genes have sequences that mark where the RNA Pol II enzyme should attach to the DNA. Although there are several different sequences that allow RNA Pol II to attach to the DNA, one of the most common is the "CAT box". It has the sequence:

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5'  GGCCAATCT   3'
3'  CCGGTTAGA  5'
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The CAT box is usually located within 40 nucleotides of another promoter sequence called the "TATA box". The TATA box is important because it tells RNA Pol II where to begin transcription and what strand of the gene to transcribe. The TATA box sequence is:

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5'  TATAAAA   3'
3'   ATATTTT  5'
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The ATATTTT strand is called the **template** strand and the TATAAAA strand is called the **non-template strand**. The enzyme RNA Polymerase II does the following:

(1) It binds to the template strand then it moves along the template strand in the 5' direction of the template strand.

(2) Starting about 30 nucleotides from TATA box, it begins transcription (it begins making the pre-mRNA).

(3) It makes the pre-mRNA complementary to the template strand, and it makes the pre-mRNA starting at the pre-mRNA’s 5’ end and finishing at the pre-mRNA’s 3’ end.

The Poly-A signal: A sequence in the pre-mRNA, called the “polyadenylation signal” (or “Poly-A signal”) is NOT the 3’ end of the pre-mRNA, but it does help determine where the 3’ end will be. The Poly-A signal has the sequence:

5’ AAUAAA 3’

The RNA polymerase enzyme transcribes the poly-A signal and continues transcribing for dozens of nucleotides beyond the poly-A signal. After transcription, enzymes in the nucleus cut the pre-mRNA about 20 nucleotides 3’ from the Poly-A signal. After the pre-mRNA is cut, another nuclear enzyme will add 50 – 250 adenosine (A) nucleotides to the 3’ end of the pre-mRNA. This is called the “Poly-A tail”.

Removal of introns: The intron sequences must be removed from the pre-mRNA before it leaves the nucleus. Enzymes called snRNPs (small nuclear Ribonucleoproteins) bind to sequences at the exon/intron junctions. The spliceosome (all the snRNPs together) brings the two exons on either side of the intron together, which causes the intron to form a loop. The spliceosome ligates (joins together) the two exons and cuts off the looped intron.

The sequences that mark the beginning and end of the intron are shown below. In this diagram, the exon sequences are shown in **boxed and bold** and the intron sequences are not boxed and shown in plain text. “N” means any nucleotide and “…….” means any number of nucleotides.

5’ **NNAG**GUAAGU.........CCUCUUCUCUCUNCAG**NNNNN** 3’

There can be some variation in the above sequences. For example, note that the “…….” within the intron represents any number of nucleotides. This means introns can be of any length. After these dots, there is a region of twelve C and U nucleotides. The C’s and U’s in this region can be in any order and any mixture of C and U. The sequences at the beginning of the intron and the end of the intron can also vary somewhat, but generally they are the same as the underlined sequences shown in the figure above.

Start of translation codon: After the mRNA has left the nucleus, a ribosome will bind to its 5’ end and begin moving toward the 3’ end. The first nucleotides of the mRNA are not translated into protein. These nucleotides are called the 5’ untranslated region (5’ UTR).

How does the ribosome know where to start translation? The ribosome knows because the first AUG that the ribosome encounters is always the start of translation. This codon is for the amino acid methionine (see the genetic code table on page 7), so all proteins are initially made with methionine as their first amino acid (However, often the first few amino acids of the protein are removed after translation, so not every protein in the cell ends up with methionine as its first amino acid).

Note that the first AUG codon not only tells the ribosome where to begin translation, it also sets the “reading frame” for the ribosome. The reading frame means which nucleotides the ribosome groups into one codon. As an example, consider the following mRNA sequence:

5’ NNNNNAUGCUCUUUGAC 3’

The N’s represent the 5’ UTR. The first codon is AUG (methionine), the second is CUC (Leucine), the third is UUU (Phenylalanine), and the fourth is GAC (Aspartic acid). If, somehow, the ribosome made a mistake and skipped the first A of the AUG codon, this would change all the codons of the entire mRNA. The first codon would be UGC (Cysteine), the second would be UCU (Serine), the third would be UUG (Leucine). In both cases, the nucleotide sequence in the mRNA is the same, but the difference is the reading frame (how the ribosome groups the nucleotides).

End of translation codon: When the ribosome encounters a codon with the sequence UGA, UAG, or UAA, it stops translation. These three codons are called “stop codons”. They don’t encode any amino acid. When the ribosome encounters a stop codon it releases the mRNA.

mRNAs have nucleotides 3’ of the stop codon, but because they are 3’ of the stop codon they are never translated. This region of the mRNA is called the 3’ untranslated region (3’ UTR). The Poly-A tail is attached to the end of the 3’ UTR.

c) Instructions for today's exercise

Your lab group will be given a gene sequence (double stranded DNA). You will construct a protein from the gene by performing all 9 steps outlined on page 1 of this handout.

1) Obtain the sequence for gene #1 from pages 8-9 of this handout. Using scissors or a cutting board, cut out each part of the DNA sequence, trim away its right margins, and tape the parts together in the correct order. Note that each part is numbered to help you assemble them correctly.

2) Tape the entire gene onto your bench top.

3) Locate the promoter and transcribe the gene: Begin by scanning the ends of the gene for promoter sequences. Remember that the promoter is may be at the left or the right end of the gene.

When the promoter sequences are located, tape a blank roll of paper next to the gene and "transcribe the pre-mRNA" by writing the complementary RNA bases to the template strand. Refer to the section of this handout on promoter sequences to confirm where you should begin the transcript and which DNA strand you should use as the template.

Refer to the section on the poly-A signal to learn where you should end transcription.

When done with this step, have your instructor inspect your pre-mRNA before starting the next step.

4) After the pre-mRNA is transcribed, add a 5' cap by taping a square of paper with "GTP" written on it to the 5' end of the transcript.

5) Locate the poly-A signal and clip the pre-mRNA. Read the section of this handout on the poly-A signal to see exactly where you should cut the pre-mRNA.

6) Add a Poly-A tail. Read the section of this handout on the poly-A signal to see exactly where you should add the tail. The tail that you add on should be a piece of the roll paper with 50 A's written on it.

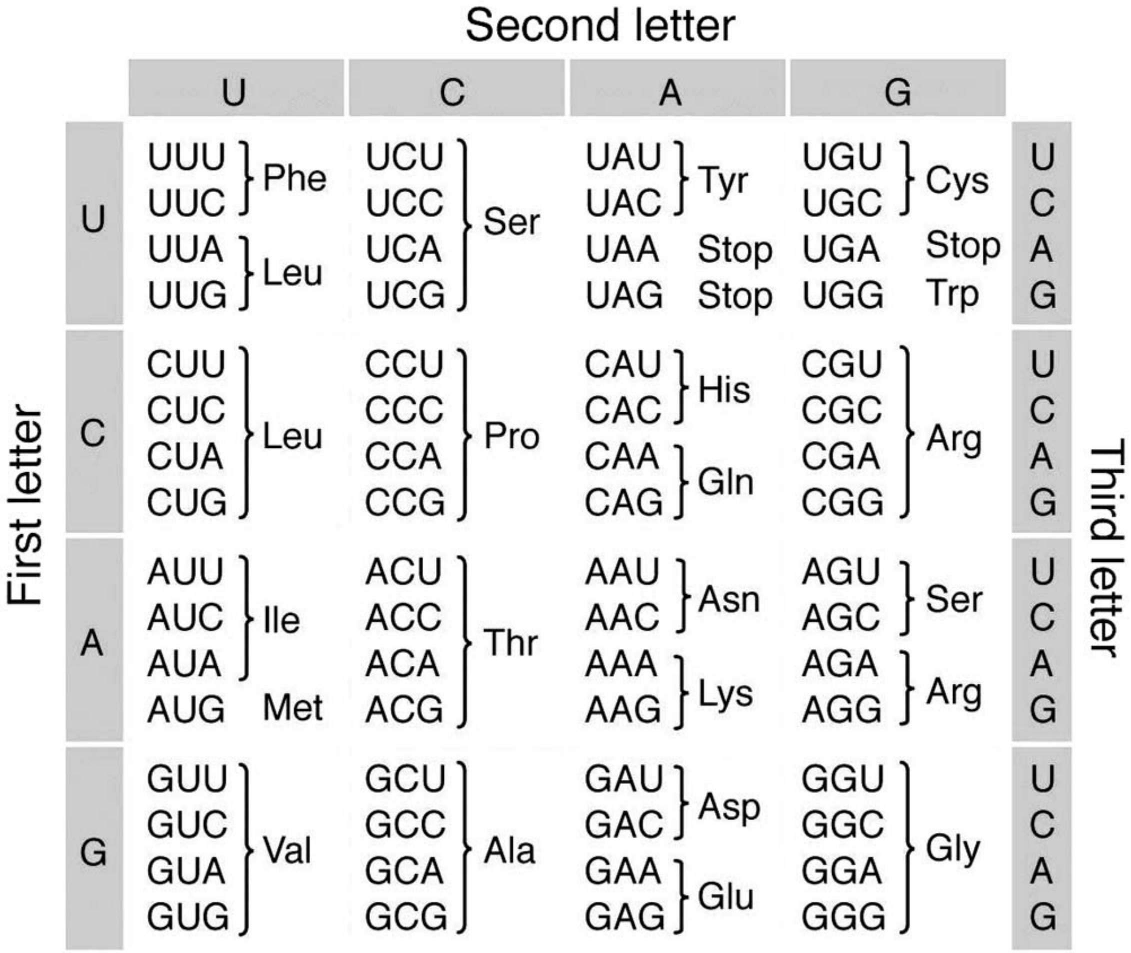
7) Identify all exon/intron boundaries. Read the section of this handout on the exon/intron boundaries help you find them.

8) Remove the introns. Cut out the introns with scissors and tape the exons together. Remember that genes usually have more than one intron.

9) After the introns are removed, the mRNA moves from the nucleus to the cytoplasm, where a ribosome will translate it. The "ribosome" student should begin by scanning the mRNA for the start codon. Once this codon is located, they should "translate" the mRNA by writing the corresponding amino acid on the roll paper under each codon. Stop translation when the "ribosome" reaches a stop codon.

Show your instructor your protein and enter the data about the protein in data table 1 on page 11.

After you have finished with gene #1, save your mRNA and protein (you will need them to answer some review questions). Repeat the procedure with gene #2. Be sure to switch roles so each student can perform any role. When your group can perform the entire process without reference to the handout, call your instructor over. The instructor will give you a new gene to demonstrate the process. Good luck!



		Second letter								
		U		C		A		G		
First letter	U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
		UUC		UCC		UAC		UGC		C
		UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
		UUG		UCG		UAG	Stop	UGG	Trp	G
	C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
		CUC		CCC		CAC		CGC		C
		CUA		CCA		CAA	Gln	CGA		A
		CUG		CCG		CAG		CGG		G
	A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
		AUC		ACC		AAC		AGC		C
		AUA		ACA		AAA	Lys	AGA	Arg	A
		AUG	Met	ACG		AAG		AGG		G
	G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
		GUC		GCC		GAC		GGC		C
		GUA		GCA		GAA	Glu	GGA		A
		GUG		GCG		GAG		GGG		G

Third letter

Gene #1

5’ GCTATCCATATTTTTGGTTTGGCACCAGTG
3’ CGATAGGTATAAAAACCAAACCGTGGTCAC

2GCCAATCTGACTTACGTGTCAGTACGTATAAT
CGGTTAGACTGAATGCACAGTCATGCATATTA

3GTGACCGCAGTAGCTATAAAAGTATGTCCCTG
CACTGGCGTCATCGATATTTTCATACAGGGAC

4TACGTAGACAGTAAGACTTTTTGTCGTCGTTG
ATGCATC TGTCATTCTGAAAAACAGCAGCAAC

5TGCAGTATCGTAGGGTATCGTGACTAGATGGC
ACGTCATAGCATCCCATAGCACTGATCTACCG

6CGATGAGGTAAGTGTCGAGAATGCTTCGATAT
GCTACTCCATTCACAGCTCTTACGAAGCTATA

7GCTTTAGTCTGATTGTAGCTAGTTGCGCGTATC
CGAAATCAGACTAACATCGATCAACGCGCATAG

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8TTCTTTTTCCTTCAGGTTGTGGCCGCACTCCC
 AAGAAAAAGGAAGTCCAACACCGGCGTGAGGG

9CCAGGTAAGTGTACGTCATGCTTCGCTCAA
 GGTCCATTCACATGCAGTACGAAGCGAGTT

10ATGATGCTAGCTGGCCTATGCTTTTTCTCCC
  TACTACGATCGACCGGATACGAAAAAGAGGG

11TCTGCAGAGAATACTTTAAAGTCGATTCGCT
  AGACGTCTCTTATGAAATTTCAGCTAAGCGA

12AGAGCAAAGTACACAGTGATTTAGCATGAC
  TCTCGTTTCATGTGTCACTAAATCGTACTG

13GTGATCCAGTAGATCGTGAAATAAACTAGTA
  CACTAGGTCATCTAGCACTTTATTTGATCAT

14GCGATAGCAACCCGTGCATGATTGGCAGTAT
  CGCTATCGTTGGGCACGTACTAACCGTCATA

15ACAGATGATTGTGAAACGATACAGTAG  3’
  TGTCTACTAACACTTTGCTATGTCATC  5’
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Gene #2

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5’    GGCACTACAGCTACTGTCACACACTAGG
3’    CCGTGATGTCGATGACAGTGTGTGATCC

2CACGTTTTATTGGTCATAAACTGGGTCACCAC
 GTGCAAAATAACCAGTATTTGACCCAGTGGTG

3     TGTGTCACCGACACACTTCAGGCTGGGGA
      ACACAGTGGCTGTGTGAAGTCCGACCCCT

4GAGGAGGGGGCTACGTCACACTGTCACTTCT
 CTCCTCCCCCGATGCAGTGTGACAGTGAAGA

5GTCAAACTACTTACCTCTTCTTTGGGCATCT
 CAGTTTGATGAATGGAGAAGAAACCCGTAGA

6TCAGTCTACTGCTACCGCTACTGGCACTCAT
 AGTCAGATGACGATGGCGATGACCGTGAGTA

7TTTATAGGTCACTAGCACTGTGTCACGCACC
 AAATATCCAGTGATCGTGACACAGTGCGTGG

8GTCTACTGCATCGTCACGTCTACTTGTGAGA
 CAGATGACGTAGCAGTGCAGATGAACACTCT
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9TTGGCCCCCACGGGGTCATCG   3’
AACCGGGGGTGCCCCAGTAGC   5’

d) Data table

Data Table:

Gene #1: Number of exons: ______ Number of introns: _______
Primary structure of protein:

Gene #2: Number of exons: ______ Number of introns: _______
Primary structure of protein:

e) Review questions (You may need to consult the textbook for some questions)

1) How many genes do human's have? ________ Bacteria? _________

2) Write the nine basic steps from gene to protein, as outlined in this handout.

3) What are the functions of the 5' cap and the poly-A tail?

4) Two promoter sequences are the CAT box and the TATA box. These two sequences have different roles in starting transcription. Contrast their two roles. Also, how would transcription change if a promoter lost its TATA box but still had its CAT box?

5) An intron always separates two exons. Review the sequences that are found at exon/intron junctions, then answer the following questions.

a) Circle any of the following that contain sequences recognized by spliceosomes:
The 5' exon   The intron   The 3' exon

b) Which amino acids can the last codon of the 5' exon encode?

6) If a gene contains 10 exons, how many introns does it have? _____

7) If a gene contains 7 introns, how many exons does it have? ______

8) Define "reading frame" and explain why it is important in translation.

9) Inspect the mRNA of gene #1 and the protein it encoded.

a) The 12th codon is AGA (Arginine). If the gene had been mutated (changed) so that an extra A had been added to the end of this codon, what would the primary structure of the entire protein become?

b) The second codon is GCC (alanine). If the gene had been mutated so that the last C of this codon had been removed, what would the primary structure of the protein become?

10) Assume that six hydrophobic amino acids in a row are sufficient to form a transmembrane domain in a protein.

a) Does gene #1 encode a membrane protein? ____ Does gene #2? ______

b) If gene #1 underwent alternative splicing, so that in some transcripts exon 1 was joined to exon 3, would it be a membrane protein? _____. Justify your answer.