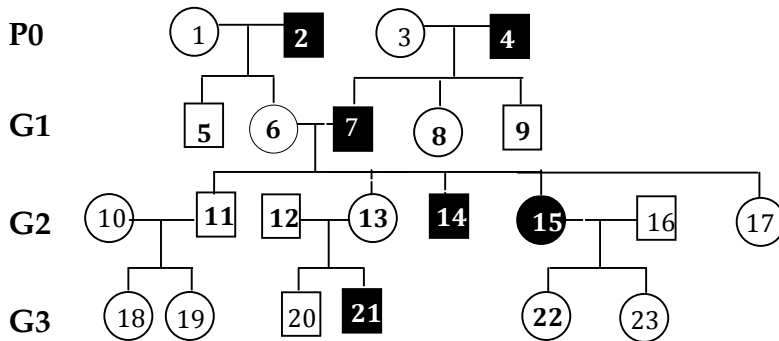


# 7.013 Problem Set 3- 2013

## Question 1

You are analyzing the following human pedigree for a disease. **Please note:** The filled squares or circles represent the abnormal phenotype. The disease related allele is rare in the population and individuals marrying into the family for generations G1- G3 do not have the disease allele. Assume that this disease is caused by the mutation of Gene A and no other mutation arises within the pedigree. Also assume complete penetrance.



**Note:** Use the symbol  $X^A$ ,  $X^a$ ,  $A$  or  $a$  where appropriate. In each case, use the letter "A" to represent the allele associated with the dominant phenotype and "a" to represent the allele associated with the recessive phenotype.

a) Give the most likely **mode of inheritance of disease** shown in this pedigree.

b) List **all** the possible genotypes of the following individuals in the above pedigree.

**Individual 1:**

**Individual 6:**

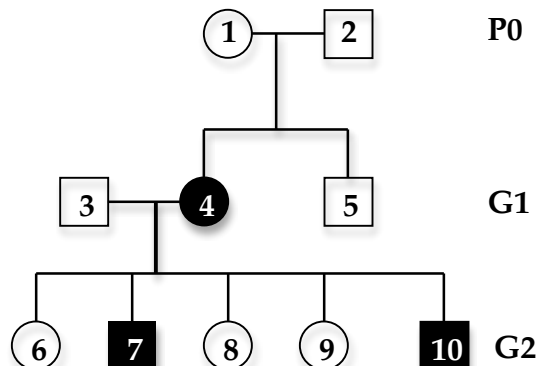
**Individual 20:**

**Individual 22:**

c) **Individual #8** from this pedigree mates with a person who has wild-type allele(s) of Gene A associated with this disease. Together they have a son and a daughter.

- i. What is the probability of their **son** being **affected**?
- ii. What is the probability of their **daughter** being **affected**?
- iii. What is the probability of their **daughter** being a carrier?

d) Your friend shows you the following pedigree and says that the disease shown in this pedigree has the same mode of inheritance as the pedigree above. Would you agree or disagree with your friend? Provide an **explanation** for your choice.



**Question 2**

a) Complete the table below **for a eukaryotic cell**, by choosing from the following keywords. If there is more than one good choice, **include all**. Choose from *lysosomes, rough endoplasmic reticulum (RER), golgi body, nucleus, mitochondria, smooth endoplasmic reticulum (SER) cytoskeleton, extracellular matrix (ECM), ribosomes, nucleolus and cell membrane*.

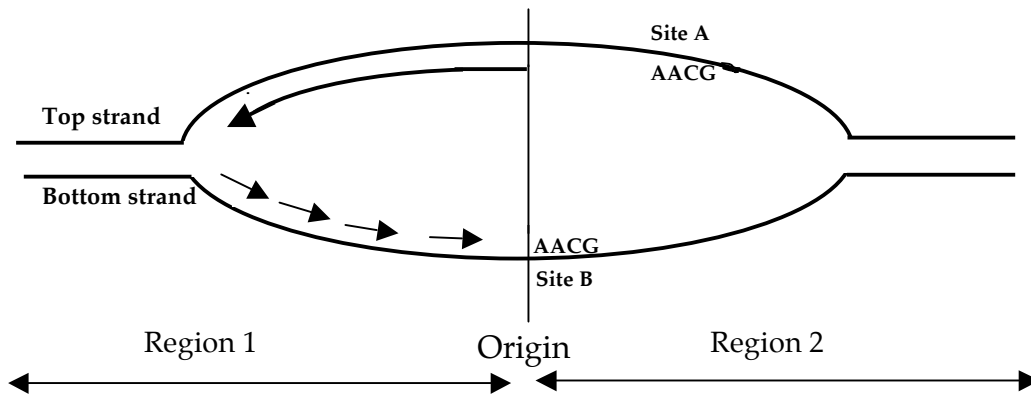
Description	Location or organelle in a eukaryotic cell
The location of <b>circular deoxyribonucleic acid (DNA)</b>	
The location of enzymes, in a eukaryotic cell, that are involved in digesting cell's own toxic material. The abnormal functioning of these enzymes may result in Tay Sachs disease.	
Membrane bound tubules that are involved in lipid metabolism and cholesterol synthesis and are NOT studded with ribosomes	
The component of cells where the ribosomes are synthesized	
The final location of proteins that glue the eukaryotic cells together and may participate in cell-cell communication	
Membrane bound tubules that are involved in protein synthesis and are studded with ribosomes	
Stacked membrane bound tubules, in a eukaryotic cell, where a newly synthesized protein goes through post-translational modification i.e. glycosylation and phosphorylation.	
Non-membrane bound structures that are involved in protein synthesis and comprised of RNA and proteins	
The final location of proteins that are a part of a dynamic network of fibers that maintain the shape and motility of a cell	

b) You label the DNA of a eukaryotic cells using BrdU (a thymidine analog). You then remove the nucleus of this cell (enucleated cell). You are surprised to see that the enucleated cell still has some DNA that is BrdU labeled. How can you **explain** this?

c) Ebola is a hemorrhagic virus, which disrupts the blood vessels. A recent publication demonstrated that this virus gains entry into the cells with the aid of the lysosomal protein NPC, found in the target cells. Briefly describe an experiment that supports the involvement of NPC proteins in Ebola infection.

**Question 3**

Shown below is a schematic of a replicating DNA in a bacterial cell.



- a) On the diagram, label the 5' and the 3' ends of the parental DNA strands.
- b) Which parental DNA strand (*choose from top or bottom*) serves as a template for the synthesis of the **leading strand in Region 2**?
- c) To which site (*choose from A, B, or both*) can the primer **5' UUGC 3'** bind?
- d) You perform **one round** of DNA replication in a test tube (in vitro) **using a single-stranded linear DNA as the template and the appropriate DNA primer**. Complete the following table, for **one round** of DNA replication.

Protein / Enzyme	Function	Required for <u>one round</u> of replication (Yes/ No)?
Primase		
DNA polymerase		
Ribonuclease		
DNA Ligase		
DNA Helicase		
Topoisomerase		

**Question 3 continued**

e) While studying replication in a bacterial cell type you find a mutant that shows a 100 fold decrease in the fidelity of replication.

- i. Name the protein(s)/ enzyme(s) that is mutated in this bacterial mutant and accounts for decreased replication fidelity.
- ii. What particular activity does this protein(s)/ enzyme(s) lack that regulates the fidelity of replication?

f) The DNA polymerase enzyme binds to the "ori site" and starts elongating the 3'OH end of the RNA primer by adding the nucleotide bases that are complementary to the sequence of the template DNA strand. Each replication cycle results in the shortening of chromosomal ends.

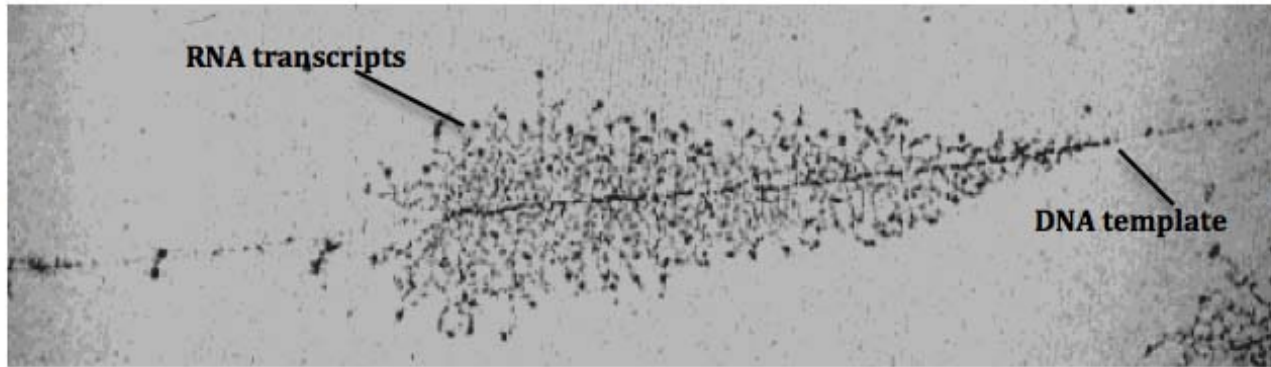
- i. **Explain** why the **Ori site** in general is rich in adenine (A) and thymine (T) bases.
- ii. The rate of mitotic cell division in an embryonic cell is much higher compared to a normal somatic cell in an adult. Briefly **explain** how the telomerase activity may regulate the number of mitotic divisions a cell can have during its life- time.

g) DNA, unlike RNA, is stable and therefore serves as the hereditary material for most organisms.

- i. What feature of RNA makes it less stable than DNA?
- ii. The fidelity of transcription is always less than the fidelity of replication. **Explain** why is this so.
- iii. A cell much better tolerates errors during transcription than errors during DNA replication. **Explain** why is this so.

**Question 4**

a) Below is an electron micrograph of a gene that is being transcribed. The DNA strand runs horizontally with RNA transcripts extending vertically outward.

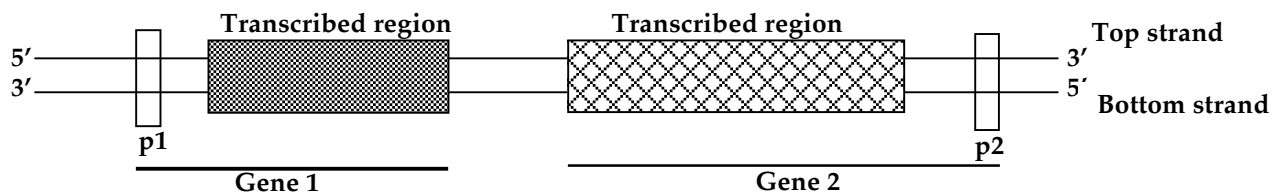


- Draw an arrow to indicate the direction of movement of RNA polymerases along the length of DNA strand. Why did you choose this direction?
- Below is a partial sequence of the gene that is being transcribed in the schematic above. **Its orientation is the same as that shown in the schematic.** Which strand is the template strand, the top or the bottom strand? **Explain** your choice.

5' ACTCGATGCTAG3'  
3' TGAGCTACGATC5'

- Give the sequence of the mRNA that would be transcribed from the above sequence and label its 5' and 3' ends.

b) The following schematic shows the chromosomal location of Gene 1 and Gene 2. The corresponding promoters for Gene 1 (p1) and Gene 2 (p2) are shown.



- Would the direction of transcription of Gene 1 be the same as that for Gene 2 (*Yes/ No*)? **Explain** your choice with reference to the promoters.
- On the schematic above, show the direction of transcription of Gene 1 and Gene 2 by drawing the arrows.
- Circle** the region(s) on each gene where the transcription factors would **most likely** bind and regulate transcription.

**Question 4 continued**

c) You realize that Gene 1 is expressed in intestinal cells of mice and mutations in this gene are associated with Crohn's disease. Further analysis reveals...

- *That the coding sequence of Gene 1 is the same in affected and wild-type mice.*
- *There is much less of mature mRNA transcribed from Gene 1 in the affected mice relative to that in the wild-type mice.*
- *The intestinal cells of the affected mice show no detectable protein unlike the wild-type mice.*

Give two possible modifications of RNA, whose absence may explain the differences that you have observed between the affected and wild-type mice.

d) Gene 2 is expressed in mouse heart muscle cells and its over-expression (too much of it) is associated with heart disease. You try to reduce the expression of Gene 2 using a particular micro RNA (miRNA-32) that you think may regulate Gene 2.

- i. **Circle** the sequence (*choose from promoter, nascent (primary) RNA, mature mRNA, 5' UTR and 3' UTR*) to which the miRNA is most likely to bind?
- ii. Briefly describe how the miRNA may inhibit expression of Gene 2.

e) From your 7.013 lectures, you have learnt that although almost all cells in an organism contain an identical genome, gene expression differs in different cell types and is highly regulated by chromatin structure. Given the following treatments, explain whether chromatin structure **compacts** or **becomes less compact** and whether gene expression is **enhanced** or **reduced**.

- i. Cells are treated with DNA methylase, an enzyme that adds methyl groups to cytosine bases in DNA.
- ii. Cells are treated with histone acetylase, an enzyme that adds acetyl groups to the histone proteins thus making them less basic.

f) As we mentioned in lecture, Prader-Willi (PW) syndrome is a rare disorder that results in intellectual disability, a constant sense of hunger and obesity. This syndrome is caused by deletion or inactivation of seven genes that lie on Chromosome 15. Normally, these genes are INACTIVE on the maternally-contributed chromosome, but ACTIVE on the paternally-contributed chromosome. PW syndrome occurs because the paternal genes that should be expressed are not for one of the following reasons:

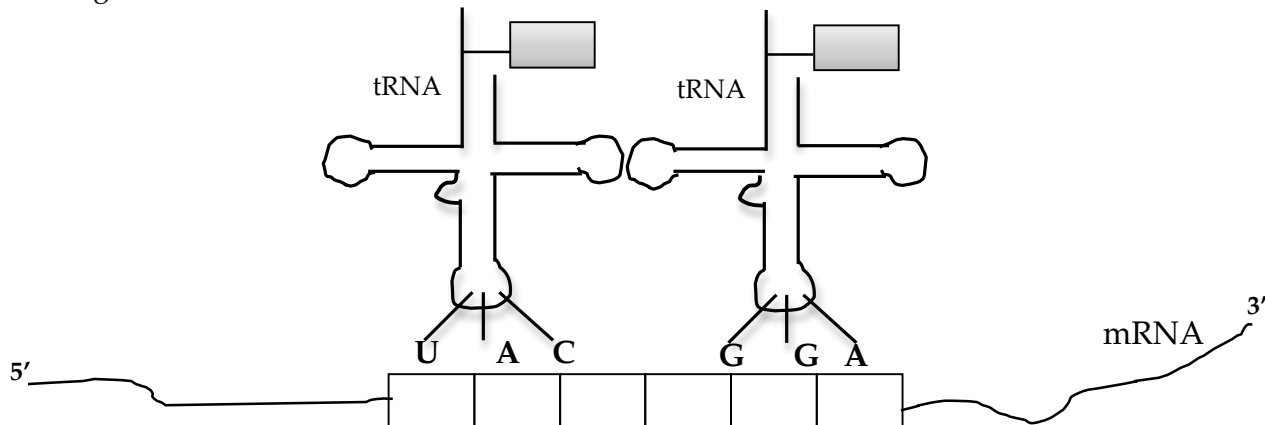
- *Paternal genes on chromosome 15 are missing or defective.*
  - *The patient has inherited two copies of chromosome 15 from the mother and no chromosome 15 from the father.*
- i. Of what is the PW syndrome an example? **Explain** the reasoning behind this answer.

**Question 4 continued**

- ii. You isolate cells from a PW patient and try to activate the Chromosome 15 genes. Circle the option from below that you would try. **Explain** your reasoning.
- Treat cells with 5-azacytidine that inhibits DNA methylase
  - Treat cells with 2-chloroethyl-N-nitrosourea (CNU) that activates DNA methylase.

**Question 5**

a) Below is a diagram of two tRNAs and an mRNA in the active site of the ribosome during translation of the mRNA into protein. Three nucleotides from the sequence of each tRNA are shown for you. In the diagram...



- Label the 5' and 3' ends of each tRNA.
- Fill in the **empty boxes** in the mRNA with the 6 nucleotides that would be present there.
- Fill in the **shaded box** attached to one end of each tRNA with the name of the amino acid that would be attached there. **Note:** A codon chart is provided on the last page of this problem set.
- Which tRNA is about to transfer its attached amino acid over to the other tRNA to elongate the peptide chain (choose from the tRNA on the left or the tRNA on the right)?

**Question 5 continued**

b) The following schematic represents the 3D- conformation of a tRNA molecule. One end of the tRNA has an anti-codon that base pairs with the appropriate codon on the mRNA and the other end has an amino acid arm that binds to a specific amino acid. Below are three anti-codon sequences for three tRNAs. Fill in the corresponding amino acid on the blanks. *Note: A codon chart is provided on the last page of this problem set.*



anticodon found on tRNA	amino acid attached to tRNA
5' AGU 3'	
5' AUG 3'	
5' CUG 3'	

- Give the anticodon used in the tRNA encoding tryptophan (trp) and label its 5' and 3'.
- Would a substitution within a codon for trp **always change** the resulting protein sequence? **Explain** your answer.
- Would a substitution within a codon for threonine (thr) always change the resulting protein sequence? **Explain** your answer.

**Question 6**

a) The following is the partial DNA sequence of Gene 1. *Note: The underlined sequence (from position 20-54) represents the promoter for Gene 1 and the underlined and italicized sequence (from position 71-90) represents the ribosome binding (RBS) site of Gene 1. Transcription begins at and includes the bold T/A base pair at position 60.*

```

1         10        20        30        40        50        60        70
I-----I-----I-----I-----I-----I-----I-----I
5' ATCGGTCTCGGCTACTACATAAAACGCGCGCATATCGATATCTAGCTAGCTATCGGTCTAGGCTACTAC
3' TAGCCAGAGCCGATGATGTATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGATCCGATGATG
                                     Promoter

          80        90        100       110       120       130       140
          I-----I-----I-----I-----I-----I-----I
5' CAGGTATCGGTCTGATCTAGCTAGCTTCTCTTCTCTCTCCCCGCGGGGCTGACTATCATGCGTGC
3' GTCCATAGCCAGACTAGATCGATCGAAGAGAAGAGAGAGGGGGCGCCCCGACATGATAGTACGCAGC
          RBS

          150       160       170       180       190       200       210
          I-----I-----I-----I-----I-----I-----I
5' TCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTACGTAAG
3' AGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGAGCCGATGATGCATTT
    
```

- Which strand (*Top/ bottom*) is the template strand for transcription of Gene 1?



**Question 6 continued**

- ii. What are the first 6 nucleotides of the mRNA transcribed from Gene 1?
- iii. What are the first 4 amino acids encoded by Gene 1? *Note: A codon chart is provided on the last page of this problem set.*

b) You have found mutant alleles of Gene 1 that have insertion of the following three base pairs immediately **after** the C/G base pair at positions, 100 and 150. Complete the table below for each of the following mutations.

5' CGA 3'  
3' GCT 5'

	Positions of insertions of the above sequence	
	After 100 <sup>th</sup> base pair	After 150 <sup>th</sup> base pair
Effect on the length of mRNA ( <i>longer than/ shorter than/ same as the mRNA produced by the wild-type allele of Gene 1</i> ). <b>Explain.</b>		
Effect on the length of protein ( <i>longer than/ shorter than/ same as the protein produced by the wild-type allele of Gene 1</i> ). <b>Explain.</b>		
If the protein is produced, will it be functional? <b>Explain.</b>		

**Question 7**

Following are three genes that encode small peptides. The splice site donor **always** has the composition 5'-GUG-3' (i.e. for the 5' end of the splice site) and the splice site acceptor always has the sequence 5'-UUG-3' (i.e. for the 3' end of the splice site), meaning that these sequences and everything between them will be removed from the mature mRNA. Only the **non-template/ mRNA like strands of DNA** for each gene are shown. *Note: A vertical line is drawn after every 10<sup>th</sup> base in each sequence. The bold and underlined ATG represents the correct reading frame.*

a) Box the **intron** in each sequence.

**Gene 1**

5' 5' -GACTAAG**ATG**ATTTGGGCAAACACAGTGCACTTTCCAGT|TTTCCTTTCT|TGGCGAACGC|CTGA-3'

**Gene 2**

5' -ACA**ATG**AATACGGGAATGGTGAACGATATCACGCTAGAGATTAATTGTGAGATCGTAGAGGACGCCAACGC|ATGA-3'

**Gene 3**

5' 5' -GACT**ATG**AATAACTATCGTGACAGACCCGGAAGCCAGAAAACCACTGGATTGTCTGGCAGAGAGGCCA|CATGATC-3'

**Question 7 continued**

b) Which of the above genes (*choose from Genes 1, 2 and 3*) encodes the smallest peptide? Give the amino acid sequence of this peptide in an N → C direction. Note: A codon chart is provided on the last page of this problem set.

c) You create the following mutants that have changes in one of the three gene sequences above. State the effect of each of these mutations on the peptides produced.

- i. The 27<sup>th</sup> base in the **non-template strand** of **Gene 1** was changed from **T** to **A**.
- ii. The 49<sup>th</sup> base in the **non-template strand** of **Gene 2** was changed from **G** to **T**.
- iii. The 4<sup>th</sup> base in the **non-template strand** of **Gene 2** was changed from **A** to **T**.
- iv. The 47<sup>th</sup> base in the **non-template strand** of **Gene 3** was changed from **C** to **T**.

	<b>U</b>	<b>C</b>	<b>A</b>	<b>G</b>
<b>U</b>	UUU Phe (F)	UCU Ser (S)	UAU Tyr (Y)	UGU Cys (C)
	UUC "	UCC "	UAC "	UGC "
	UUA Leu (L)	UCA "	UAA <i>Stop</i>	UGA <i>Stop</i>
	UUG "	UCG "	UAG <i>Stop</i>	UGG Trp (W)
<b>C</b>	CUU Leu (L)	CCU Pro (P)	CAU His (H)	CGU Arg (R)
	CUC "	CCC "	CAC "	CGC "
	CUA "	CCA "	CAA Gln (Q)	CGA "
	CUG "	CCG "	CAG "	CGG "
<b>A</b>	AUU Ile (I)	ACU Thr (T)	AAU Asn (N)	AGU Ser (S)
	AUC "	ACC "	AAC "	AGC "
	AUA "	ACA "	AAA Lys (K)	AGA Arg (R)
	<b>AUG</b> Met (M)	ACG "	AAG "	AGG "
<b>G</b>	GUU Val (V)	GCU Ala (A)	GAU Asp (D)	GGU Gly (G)
	GUC "	GCC "	GAC "	GGC "
	GUA "	GCA "	GAA Glu (E)	GGA "
	GUG "	GCG "	GAG "	GGG "

MIT OpenCourseWare  
<http://ocw.mit.edu>

7.013 Introductory Biology  
Spring 2013

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.