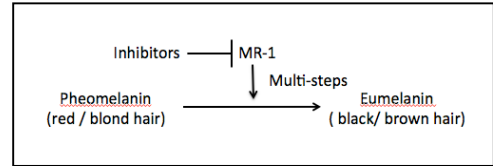


Solution key- 7.013 Problem Set 3- 2018

Question 1

Our hair color is determined by the relative amounts of two types of melanin pigment in hair follicles: **pheomelanin** (which promotes red or blond hair color) and **eumelanin** (which promotes black or brown hair color). In humans, the type of melanin produced depends on the activation of the melanocortin 1 receptor (MR-1), which triggers a series of chemical reactions within a cell that stimulates it to make eumelanin. However, if the MR-1 receptor is blocked or inactive, the cells can only make pheomelanin.

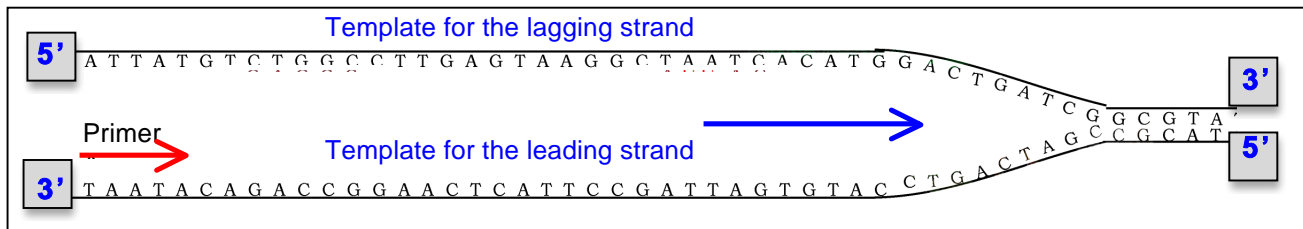
a) Represent the above information as a flowchart using -> for activation and -| for inhibition.



b) The *MR1* gene, on chromosome 16 codes the MR-1 receptor. Beside DNA, which other class of macromolecules contributes to chromosome structure: **proteins/ lipids/ carbohydrates**? Identify the most likely **non-covalent interaction** between the sugar-phosphate backbone of DNA and the class of macromolecule that you identified.

- i. Class of macromolecules: Proteins, namely histones
- ii. Likely non-covalent interaction: Ionic interaction

The diagram below depicts a replication fork at one origin of replication site (*ori*) on Chromosome 16 in a cell undergoing DNA replication. **Note:** The primer is shown by an arrow i.e. 5->3'.



c) On the DNA diagram above, label the 5' and 3' ends of the template DNA strands by filling in the shaded boxes and show the direction of movement of the replication fork by an arrow.

d) Label the parental strands as the templates for **leading (continuous)** or **lagging (discontinuous)** strand synthesis.

e) Give the sequence of the five base long primer that is shown as an arrow in the diagram above:
5' AUUAU3'

f) Give the sequence of the **next four bases** that would be added to the growing end of the primer:
5'GTCTG3'

g) Explain why **Sequence 2** shown below, is more likely the “*ori*” site as opposed to **Sequence 1**.

5' CGGGGACGCCGCGT3'
3' GCCCCTGCGGCGCA5'
Sequence 1

5' ATATATCGTTATAA3'
3' TATATAGCAATATT5'
Sequence 2

The origin is the start site of replication from which the replication fork starts to move bi-directionally. This site is often rich in A/T base pairs and therefore has a fewer hydrogen bonds compared to a G/C rich sequence. The fewer hydrogen bonds allow the DNA sequence at the Ori site to unwind rapidly.

Both Sequence 1 and Sequence 2 are of the same length i.e. they have same number of base pairs. However, Sequence 2 is A/T rich compared to Sequence 1 and therefore has a fewer hydrogen bonds/. So Sequence 2 is most likely to serve as an ori site.

Question 1 continued

h) You grow the normal, healthy melanin producing cells on plates that contain the in the presence of nutrients and the following compounds. **Note:** For this question you may assume that these compounds are easily able to get into the cells.

- **Plate 1** contains TA-65, a compound that prevents the shortening of chromosomes.
- **Plate 2** contains doxorubicin, a compound that promotes DNA supercoiling.
- **Plate 3** contains an RNase inhibitor.
- **Plate 4** contains papilloma virus E1 protein that mimics the effects of DNA helicase.

For each plate, **explain** if the cells will be able to replicate their DNA.

In Plate 1, TA-65 will likely activate the telomerase enzyme so that it is able to maintain the telomeres at the ends of the chromosomes thereby preventing their shortening. So there will be no loss of genetic information following each round of replication. Hence the cells will continue replicating their DNA and hence continue to divide.

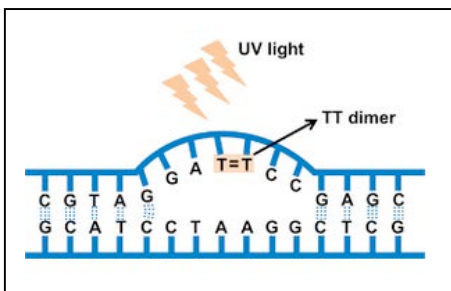
In Plate 2, doxorubicin will likely inhibit the topoisomerase enzyme, which removes the supercoils thereby relieving stress on the DNA duplex. If the DNA remains supercoiled it will NOT be able to complete replication.

In Plate 3, the cells will be able to replicate their DNA. But in the presence of the RNase inhibitor, the RNA primers will not be degraded. As a result the replication is abnormal and likely incomplete i.e. the RNA primers will not be removed and replaced by DNA sequence.

In Plate 4, the HPV will unwind the DNA thereby making the ori site available for the DNA polymerase and other proteins to bind and form an ORC complex to trigger the process of replication.

Question 2

UV radiation from the sun or tanning salons can result in the formation of thymidine dimers (T-T) in the DNA of skin and hair follicle cells. These T-T dimers, if left unrepaired can result in rapid aging of skin, freckles and even melanoma (a form of skin cancer).



a) Which process will repair the T-T dimers: **DNA Proofreading/ mismatch repair/ Nucleotide excision repair**? **Explain** why you selected this process over the others.

The T-T dimerization can happen at any time following exposure to radiation. DNA proofreading happens only during replication. A mismatch can be repaired by mismatch repair mechanism, which happens immediately AFTER replication on the hypo-methylated strand. But there is no mismatched base pair here.

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b) Does the formation of T-T dimers impact the process of ...

i. **Replication?** If so, how? **Note:** Your explanations may vary.

The T-T dimer can generate kinks in the DNA strand changing its shape. So the DNA polymerase may get stalled or read the bases incorrectly thus impairing replication. Multiple explanations.

ii. **Transcription?** If so, how? **Note:** Your explanation may vary.

Many explanations. It generates kinks in the DNA strand. This may change shape, impair normal base pairing, prevent RNA polymerase from reading the template strand to transcribe RNA.

Question 2 continued

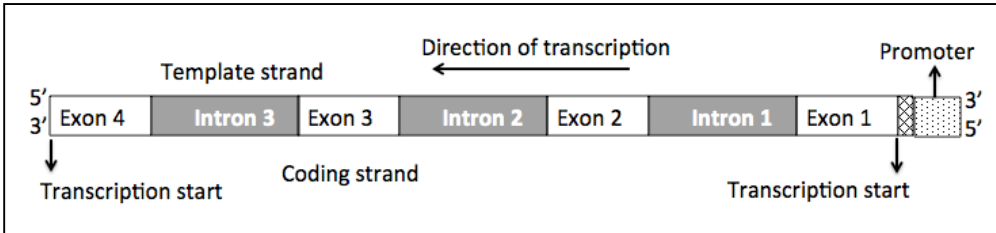
c) Once the T-T dimer has been removed...

- i. Name the enzymes/ proteins that will elongate the DNA strand and seal the gap.
 - Enzyme elongating the DNA: DNA polymerase
 - Enzyme that seals the gap: DNA ligase
- ii. DNA ligase is required in all the processes listed below except one. Circle this process: **Replication/ Proofreading/ Mismatch repair/ Nucleotide excision repair.**

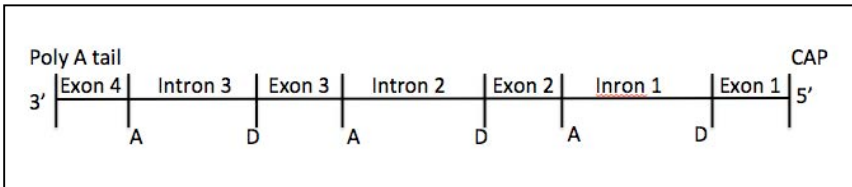
Question 3

The *MR-1* gene is comprised of four exons (Exon 1, 2, 3, and 4) that are each 1.5 kilo base (kb) long and three introns (Introns 1, 2, and 3) that are each 3kb long.

a) Based on the information provided, draw the *MR-1* gene in the box below. Label its 5' and 3' ends, draw and label the promoter, the transcription start and stop site, exons 1-4 (as blank boxes) and introns 1-3 (as shaded boxes). **Note:** The arrow in the schematic shows the direction of transcription.



b) Draw the nascent (pre-spliced/ newly synthesized) mRNA transcribed from the *MR-1* gene. Label its 5' and 3' ends, exons 1-4 and introns 1-3. Label each splice donor site with a "D" and each splice acceptor site with an "A".



c) Assuming all introns are spliced out, give all the possible mature (spliced) mRNA transcripts resulting from the *MR-1* gene.

- 3'AAAA....AA- Exon 4-Exon 3-Exon2-Exon 1-5'-CAP -> 6kb
- 3'AAAA....AA- Exon 4-Exon 3-Exon 1-5'-CAP -> 4.5kb
- 3'AAAA....AA- Exon 4-Exon 2-Exon 1-5'-CAP -> 4.5kb
- 3'AAAA....AA- Exon 4-Exon 1-5'-CAP -> 3kb

- I. Label the 5' and 3' ends of each mature transcript.
- II. Specify the exons that make the transcripts and give the size (in terms of kb) for each mature transcript.

- III. List any modification(s) to the 5' and the 3' ends of the mature mRNA transcripts and **briefly describe** their significance.
The addition of 7methyl-guanosine CAP at the 5' end and Poly A tail at the 3' end provides stability to the mature mRNA and allows its export from the nucleus into the cytoplasm where it is translated.

Question 3 continued

d) Complete the table below. **Note:** For Column 3, choose from: *chromatin structure, transcription, splicing, mRNA stability, and translation*. Please take a look at the information on eumelanin, pheomelanin and MR-1 provided thus far in the problem set.

Alteration	Hair color (Red/ black)?	Process/ feature that is the affected FIRST
Histone proteins bound to the MR-1 gene are phosphorylated	<i>Black hair</i>	<i>Chromatin structure (and transcription)</i>
Activating transcription factors (TF) that bind to the promoter region of MR-1 gene are absent	<i>Red hair</i>	<i>Transcription</i>
Promoter region of MR-1 gene is NOT methylated	<i>Black hair</i>	<i>Chromatin structure (and transcription)</i>

Question 4

The following is the DNA sequence for the transcription initiation region of the MR-1 gene. **Note:** Part of the **promoter region** is boxed and the dashes represent bases that are not shown. Transcription begins at and includes the bold and underlined T/A base pair.

5' --TGGACTGCTATGATAGCAGTTCTGCTGAGACGATGGCCATACGGCCATGG**TTC**CATAAAAGT----3' TOP
 3' --ACCTGACGATACTATCGTCAAGACGACTCTGCTACCGGTATGCCGGTACC**AAG**TATTTC-----5' BOTTOM

- a) Identify the template strand for transcription: **Top / Bottom?** *Top strand*
- b) Write the **first 6 nucleotides** of the newly transcribed MR-1 mRNA: 5' ***ACCAUG***3'
- c) Using the codon chart on the last page of this problem set, write the **first 3 amino acids** of the newly synthesized MR-1 receptor: ***N-Met-Ala-Val-C***
- d) The last (C-terminal) **5 amino acids (296-300)** of the **wild-type** form of the **MR-1 receptor** are: ***N-Val²⁹⁶-Ser²⁹⁷-Asn²⁹⁸-Ser²⁹⁹-Met³⁰⁰-C***

The DNA sequence encoding the C- terminus of the wild- type and mutant forms of the MR-1 receptor is included within the sequence below. The **point mutations** in mutants 1 and 2 are bold & shaded and the stop codon is underlined. **Note:** The codon table is provided on the last page of the problem set.

Wild- type:	5' -TCGTATCGAATTCCATGTAGC-3' 3' -AGCATAGCTTAAGGTACATCG-5'
Mutant 1:	5' -TCGTAT <u>A</u> GAATTCCATGTAGC-3' 3' -AGCATAT <u>T</u> CTTAAGGTACATCG-5'
Mutant 2:	5' -TCGTATCGA <u>AAC</u> TCCATGTAGC-3' 3' -AGCATAGCTT <u>G</u> AGGTACATCG-5'

Compared to the wild- type, which mutant allele will most likely promote red hair color and **why?**
The mutation in Mutant 2 is a silent, so the MR-1 receptor will still have the same primary sequence and will function to make eumelanin resulting in black hair. But Mutant 1 has a nonsense mutation that will result in a truncated, nonfunctional MR-1 that cannot convert pheomelanin to eumelanin. This will result in person having red hair color.

Question 4 continued

e) Approximately 5% of *MR-1* mRNA transcripts have errors relative to the *MR-1* gene sequence. However, a cell can still produce a normal concentration of functional *MR-1* receptor. Why can a cell better tolerate mutations in mRNA compared to the mutations in DNA?

Multiple correct explanations:

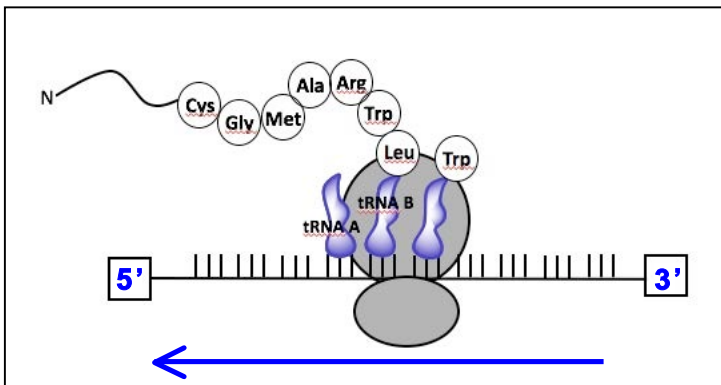
- Multiple copies of the mRNA transcripts are made during the transcription of a gene. If 95% or more have no errors we get normal amount of functional proteins.
- RNA gets degraded, so the 5% of transcripts with error will not have a deleterious effect in a cell.
- The cells do not inherit RNA errors since RNA is not the genetic material.

f) Your friend mentions that because you know the amino acid sequence of the *MR-1* receptor, you can determine the length (in terms of base pairs) and the sequence of the *MR-1* gene. Do you agree with your friend? **Why or why not?**

No, you cannot determine the mRNA sequence for the reasons outlined below:

- There are multiple codons for the same amino acid
- The mRNA has regulatory regions such as the 5-UTRs and 3-UTRs, which flank the open reading frame. The amino acid sequence of the *MR-1* receptor does not have any information on UTRs.
- The pre mRNA may have multiple introns that may be alternatively spliced to give multiple mature transcripts.

g) Below is a drawing of a ribosome actively translating *MR-1* mRNA. The horizontal line represents the mRNA, each vertical line represents a base (A, U, G, C) and two tRNA molecules (tRNA A and tRNA B) are labeled and drawn in purple on the posted version of the problem set.

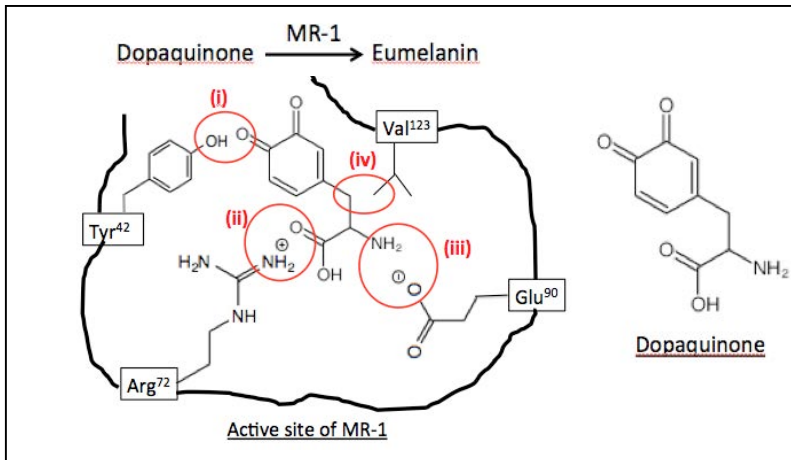


- Draw an arrow to indicate the movement of mRNA relative to the ribosome during translation.
- Label the 5' and the 3' ends of the mRNA.
- Name the amino acid that was originally bound to tRNA A: Trp
- Give the sequence of the anti-codon loop of tRNA-Trp: 5'CCA_3'

Question 5

The MR-1 receptor catalyzes the conversion of dopaquinone to eumelanin as shown in the schematic below. **Note:** Each circled interaction is critical for dopaquinone-MR-1 binding.

a) For each position (i)–(iv), name the **non-covalent interactions** between MR-1 receptor and dopaquinone by choosing from **ionic/ hydrogen/ hydrophobic interactions**.



(i): Hydrogen bond

(ii): Ionic/ Hydrogen bond, either OK

(iii): Ionic/ Hydrogen bond, either OK

(iv): Hydrophobic interaction

You analyze both alleles of the *MR-1* gene in four individuals (1-4). Each of these individuals has a specific mutation in one or both alleles of the *MR-1* gene as specified in the table below. **Note:** You should assume that the inheritance of the *MR-1* gene shows an autosomal recessive mode. You can refer to the information at the beginning of Question 1 of this problem set.

Individuals	Allele 1	Allele 2
1	5'UAU3' (Tyr) -> 5'UAG3' at (i)	Same as the wild-type
2	5'CGU3' (Arg) -> 5'CGG3' at (ii)	5'GAA3' (Glu) -> 5'GAG3' at (iii)
3	5'GAG3' (Glu) -> 5'AAG3' at (iii)	5'GUU3' (Val) -> 5'GCU3' at (iv)
4	Promoter sequence is highly methylated	5'UAU3' (Tyr) -> 5'UAG3' at (i)

b) Which of the above individuals are most likely to have black hair color and **why**?

Individual 1 will have black hair: Although Allele 1 has a nonsense mutation, its effect will be masked by allele 2 that will encode a normal functional copy of MR-1 receptor that can catalyze the conversion of pheomelanin -> eumelanin. **Individual 2** will have black hair since the mutations in both allele 1 and 2 are silent mutation resulting in a normal functional MR-1 receptor that catalyzes the conversion of pheomelanin -> eumelanin. **Individual 3** will likely have black hair: The mutation in allele 1 causes the Glu (negatively charged side-chain) -> Lys (positively charged side-chain perhaps resulting in non-functional MR-1 receptor. The mutation in allele-2 would convert Val -> Ala both of which have hydrophobic side-chains of comparable size, perhaps producing normal MR-1 receptor. **Individual 4** will have red hair: Allele 1 will not be transcribed since the promoter sequence is methylated and allele 2 has a nonsense mutation that will result in a truncated, non-functional MR-1 receptor protein.

c) Which of the above individuals are likely to be most sensitive to UV radiation and **why**?

Individual 4 will be most sensitive to UV radiation since this person has nonfunctional MR-1 receptor protein, which converts pheomelanin -> eumelanin that provides UV protection.

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