

Solutions to 7.014 Problem Set 4

Question 1

You have discovered a new virus that contains only RNA as its genetic material. Curious as to how this virus works, you infect cells with this virus and discover that after infection, some DNA that encodes viral proteins is produced. At a later time point, lots of viral RNA molecules and various viral proteins are produced.

a) Explain what type of virus you have discovered.

A Retrovirus.

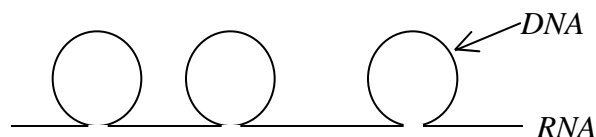
b) Outline the steps of the information flow for this virus by filling in the blanks below. Each arrow represents an activity for example, DNA polymerase. Circle the arrow that represents an activity that the host cell would not have.

RNA \longrightarrow DNA \longrightarrow RNA \longrightarrow protein

c) There is a human gene that encodes a protein identical to one of the proteins produced by the virus. When you take the mRNA for this protein and base-pair it with its complementary single-strand human DNA and viral RNA, you're surprised to find that they look different by electron microscopy.

Viral RNA + mRNA

human DNA + mRNA



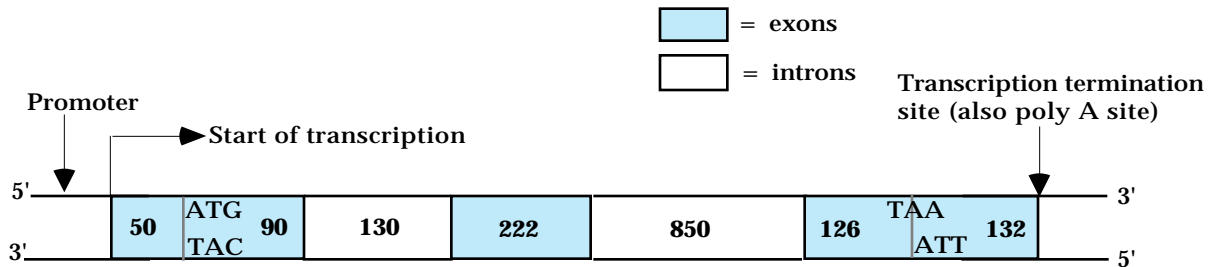
i) Label which strand is DNA and which is RNA in the human DNA + mRNA picture.

ii) Why does the human DNA + mRNA schematic have the shape it does?

The DNA contains introns that are spliced out in the final mRNA.

Question 2

Shown below is the genomic structure of the wild-type human β -globin gene. The numbers within the boxes indicate the length (in nucleotides) of each region. The DNA sequences corresponding to the start codon and the stop codon are indicated.



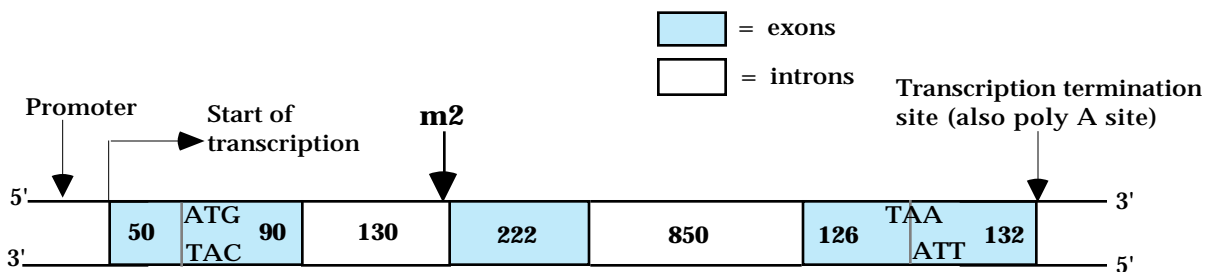
a) i) What is the length (in nucleotides) of the wild-type β -globin primary RNA transcript (pre-mRNA)? 1600

ii) How many amino acids are present in the wild-type β -globin protein? 146

b) Give the location of a mutation (m1) in the β -globin gene that would prevent RNA synthesis from the β -globin gene.

Promoter

c) A base-pair substitution mutation (m2) indicated below, destroys the 3' splice site located in the first intron of the β -globin gene.



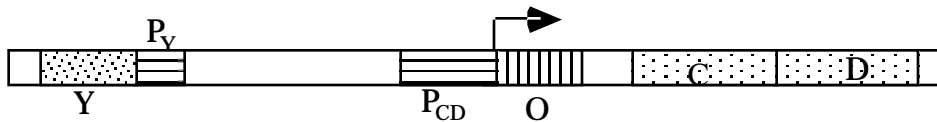
i) Would the m2 mutation **increase, decrease or not change** the length of the primary RNA transcript (pre-mRNA) made from the mutant β -globin gene? Briefly explain your reasoning.

No change, this is before the mRNA is spliced.

ii) Would the m2 mutation **increase, decrease or not change** the length of the mature RNA transcript made from the mutant β -globin gene? Briefly explain your reasoning.
Because the splice site is damaged, the intron remains and the mature mRNA is increased by 130 bases.

Question 3

A scientist has discovered a bacterium that can metabolize alcohol to pyruvate when stimulated by the presence of ethanol. Hoping to use this bacteria to eliminate the harmful effects of excessive alcohol consumption, he finds that this bacterium produces the enzymes C and D which catalyze the ethanol \rightarrow pyruvate reactions. The genes for enzymes C and D are part of an operon as diagrammed below.



a) You find two mutants, mutant 1 and mutant 2 that each carry a loss-of-function mutation in one of the components of the operon. These mutants constitutively produce enzymes C and D. Given this information, Is Y an activator or repressor? Explain.

Loss of these components, likely Y and O, allows transcription of C and D whether or not ethanol is present. Thus Y is a repressor.

You want to determine what component is missing in each of the mutants. You examine production of enzymes C and D in wild-type, mutant 1 and mutant 2 in the presence and absence of ethanol. You also create partial diploids and measure production of the enzymes.

cell type	No ethanol Production of C and D	+ ethanol Production of C and D
Wild type	low	high
Mutant 1	high	high
Mutant 2	high	high
Mutant 1 + $Y^+ P_Y^+ P_{CD}^+ O^+$	high	high
Mutant 2 + $Y^+ P_Y^+ P_{CD}^+ O^+$	low	high

b) Mutant 1 has a loss-of function mutation in which component of the operon? Explain.

Mutant 1 has lost the function of the operator, O, such that repressor cannot bind. Operators act at the level of DNA and can only control the genes to which they are physically attached. Even when a wild type copy of all components is added, the O operon will always produce C and D.

c) Mutant 2 has a loss-of function mutation in which component of the operon? Explain.

Mutant 2 has lost the function of the repressor (Y) or the promoter of the repressor (P_Y) such that the Y protein is not made, or if it is made it can not bind to the operator. When a wild type copy of all components is added, the Y repressor is made and appropriately regulates transcription of C and D.

Question 4

a) The genes needed to synthesize tryptophan are found in the *trp* operon. The presence of tryptophan controls the transcription of these genes.

i) Assume that the regulatory protein for this operon is a repressor. When do you expect the repressor to be associated with the operator? Circle one.

When tryptophan concentration is low.

When tryptophan concentration is high.

Explain your choice.

You would inhibit the production of the enzymes needed to make tryptophan if enough tryptophan is present.

ii) Assume that the regulatory protein for this operon is an activator. When do you expect the activator to be associated with the operator? Circle one.

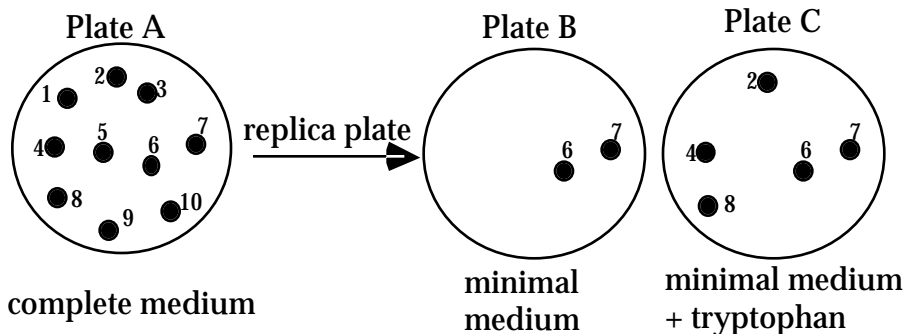
When tryptophan concentration is low.

When tryptophan concentration is high.

Explain your choice.

You would produce the enzymes needed to make tryptophan if tryptophan is low.

By using bacterial genetics you want to identify the enzymes involved in the tryptophan pathway by isolating bacteria that failed to synthesize tryptophan (these bacteria are referred to as “*trp*⁻”). You know that mutant bacteria that fail to synthesize tryptophan (and thus can not grow without addition of tryptophan) are likely to be defective in one of the enzymes involved in the tryptophan synthesis pathway. You start with a population of wild-type (“*trp*⁺”) bacteria, mutagenize it with UV light and allow the bacteria to grow into isolated colonies on plate A (see diagram). You then use the replica plating technique to transfer some bacteria from each colony onto plates B, C, and D. The contents of the growth medium are listed below each plate. Complete medium contains all nutrients; minimal medium contains nutrients sufficient to allow wild-type bacteria to grow, but bacterial cells with mutations in a gene for any nutrient synthesis pathway cannot grow unless the nutrient is added to the growth medium.



b) List the colonies that are *trp*⁻.

2, 4, 3

Question 4, continued

c) Some colonies do not grow on Plate C. Give one possible explanation for the growth behavior of these colonies.

These cells may have a mutation in a biosynthetic pathway other than the trp pathway.

d) You repeat this experiment several more times and isolate eight trp^- mutants. You then perform a complementation test on these mutants. The data is shown below. Briefly describe how a complementation test is performed.

Mutants are combined in pairwise fashion to form diploid. These diploids are then tested for their ability to grow in the absence of trp.

Complementation Test Results:

In the table below, a (+) indicates growth on minimal media, a (-) indicates lack of growth on minimal media.

	m1	m2	m3	m4	m5	m6	m7	m8	WT
m1	-	+	+	+	+	+	+	+	+
m2		-	+	+	+	-	-	+	+
m3			-	+	-	+	+	+	+
m4				-	+	+	+	+	+
m5					-	+	+	+	+
m6						-	-	+	+
m7							-	+	+
m8								-	+

e) Assign the mutants 1-8 into complementation groups.

Group 1; m1

Group 2; m2, m6, m7

Group 3; m3, m5

Group 4; m4

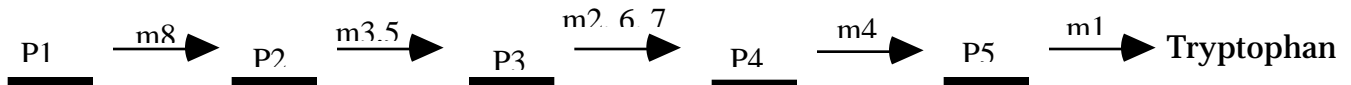
Group 5; m8

Question 4, continued

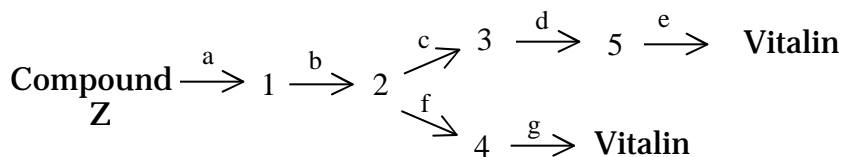
You determine that the pathway for tryptophan biosynthesis involves five precursor compounds, P1- P5. The growth requirements for each of the mutants are summarized in the table below.

Mutant	Compounds					
	P1	P2	P3	P4	P5	Trp
m1	-	-	-	-	-	+
m2	-	-	-	+	+	+
m3	-	-	+	+	+	+
m4	-	-	-	-	+	+
m5	-	-	+	+	+	+
m6	-	-	-	+	+	+
m7	-	-	-	+	+	+
m8	-	+	+	+	+	+

f) Draw the pathway for tryptophan biosynthesis. Fill in the blanks with P1-P5 as appropriate. Label the arrows with the mutants that can not complete that step.



g) You discover a different metabolic pathway that synthesizes a product necessary for survival in bacteria, where each number represents an intermediate, and each letter represents the enzyme that catalyzes that step.



i) You find that bacteria with mutations in either c or f can survive but a cell that has mutations in both c and f cannot. Explain why.

The pathway to Vitalin is branched. As long as one arm of the pathway is intact, Vitalin can be produced and the cells can survive.

ii) Which of the following mutants: c, d, or g can grow on media containing just intermediate 3? Mutants c and g can grow on media containing just intermediate 3.

iii) If a cell had a mutation in b, what intermediate would accumulate? Intermediate 1.

iv) If a cell had a mutation in c, what intermediate would accumulate?

It would not accumulate any intermediates as intermediate b will be used to produce Vitalin by the other arm of the pathway.