

Solutions to 7.012 Problem Set 4

Question 1

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

Image removed due to copyright reasons.

a) Draw an arrow indicating the direction that the RNA polymerases are transcribing. Why did you choose this direction?



The mRNA molecules near the right side are short whereas the mRNA molecules towards the left are longer. The longer mRNA molecules will be towards the end of the gene.

b) Below is a partial sequence of the above gene. Its orientation is the same as pictured above. Which strand is the template strand, the top or the bottom strand? Explain your choice.



mRNA is only made 5' → 3', so the top strand will be the template strand in the 

c) What would be the mRNA sequence transcribed from the above sequence? Be sure to label the 5' and 3' ends.



Question 2

A tRNA molecule (shown below) is composed of an RNA chain that folds into a 3-D shape like that shown below. At one end it has an anti-codon that base pairs with the appropriate codon on the mRNA and at the other end it 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.

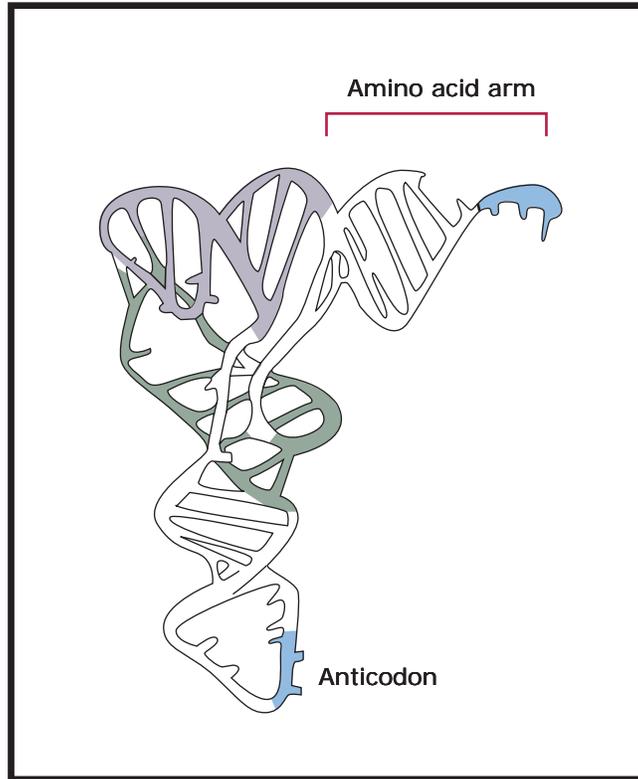


Figure by MIT OCW.

anticodon found on tRNA	amino acid attached to tRNA
5' AGU 3'	<u>Threonine</u>
5' AUG 3'	<u>Histidine</u>
5' CUG 3'	<u>Glutamine</u>

b) You are studying a certain enzyme. You have two mutant versions, one that works just as well as the wild-type and another that doesn't work at all. When you sequence the genes that code for these two mutant enzymes you find the enzyme that works has a 9 base pair deletion, whereas the enzyme that does not work has only a single base pair deletion. Explain why the sequence with the 9 base pair deletion results in an active enzyme when the sequence with a single base pair deletion produces an inactive enzyme.

A nine base-pair deletion in the coding region of the gene could result in a protein that is missing two amino acids, but otherwise has the same sequence of amino acids. A single base pair deletion in the coding region of the gene would cause a frame shift which would result in a completely different protein.

c) The first 17 nucleotides from an mRNA molecule are 5' GAAUGGCCACUUAGCAA... 3'

Write out the first 5 amino acids encoded by this sequence.

There are only 3 amino acids encoded by this sequence because the 4th codon is a stop codon.

Met- ala-thr

Question 3

a) How does the structure of the lac operon ensure that the lac genes are coordinately controlled? Why would this be beneficial?

The lac operon produces a polycistronic mRNA encoding three protein that all are important in lactose utilization. Having genes important to a single process regulated together is efficient way to ensure all need proteins are present when needed.

b) Describe the regulation of the lac operon (inducible, uninducible, or constitutive) in the case of each of the following mutants. In all cases the concentration glucose is low. Explain your choice.

i) A mutation in the lac repressor such that it can't bind DNA
constitutive

ii) A mutation that makes lac permease nonfunctional
uninducible

iii) A mutant promoter that has a high affinity for polymerase
inducible

iv) A mutant lac repressor that doesn't bind lactose
uninducible

c) You have a cell where the expression of the lac operon is constitutive due to a mutation in the operator. If you add to that cell a completely wild-type version of the lac operon would normal regulation be restored? Explain.

No, normal regulation is not restored. Operators function at the DNA level and thus can only act in cis. The Z, Y, and A genes attached to the mutant Operator region will always be constitutively expressed.

Question 4

Lambda phage is a virus that infects E. coli bacteria. After infecting the bacteria it can take one of two paths, lysis or lysogeny. In the lysis path, the phage makes many copies of itself, breaks out of the cell and infects new cells. In lysogeny, the phage incorporates its DNA into the bacterial DNA and lays dormant in the cell. The presence of a protein called lambda repressor influences which path is taken by inhibiting lysis. When lambda repressor is present it turns off or represses the genes that allow the virus to reproduce, thus no phage progeny are produced and no cell lysis occurs.

a) In studying this protein you find that lambda repressor binds DNA at a site that overlaps the promoter for the lysis genes. Propose a mechanism for repression based on this information.

The mechanism for repression would be analogous to that of the lac operon. When lambda repressor binds, it prevents transcription of the genes required to complete the lysis steps of phage replication.

b) Activators typically work by binding near a promoter site and recruiting RNA polymerase to the promoter by forming non-covalent bonds with it. This helps the polymerase attach to the promoter and increases transcription from that promoter.

Although Lambda repressor turns off the transcription of the lysis genes, it stimulates the expression of its own lambda repressor gene (acts as an activator). In studying lambda repressor you find that there are two amino acids, Asp and Glu, which are essential to the activator function. Based on this information what type of bonds do you expect Lambda repressor to make with RNA polymerase and what type of amino acids do you expect to find on polymerase participating in these bonds?

You might expect the Lambda repressor protein to form ionic bonds with the RNA polymerase. You could predict that positively charged amino acids such as lys, arg, and his would be found on the polymerase participating in these bonds.