GMB Quiz 3 Rubrics

1) Name different types of RNA found in cells, and what are their specific locations and functions within the cellular context?

Table 13.2 Location and functions of different classes of RNA molecules

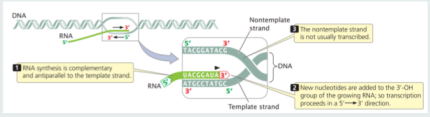
Class of RNA	Cell Type	Location of Function in Eukaryotic Cells*	Function
Ribosomal RNA (rRNA)	Bacterial and eukaryotic	Cytoplasm	Structural and functional components of the ribosome
Messenger RNA (mRNA)	Bacterial and eukaryotic	Nucleus and cytoplasm	Carries genetic code for proteins
Transfer RNA (tRNA)	Bacterial and eukaryotic	Cytoplasm	Helps incorporate amino acids into polypeptide chain
Small nuclear RNA (snRNA)	Eukaryotic	Nucleus	Processing of pre-mRNA
Small nucleolar RNA (snoRNA)	Eukaryotic	Nucleus	Processing and assembly of rRNA
Small cytoplasmic RNA (scRNA)	Eukaryotic	Cytoplasm	Variable
MicroRNA (miRNA)	Eukaryotic	Cytoplasm	Inhibits translation of mRNA
Small interfering RNA (siRNA)	Eukaryotic	Cytoplasm	Triggers degradation of other RNA molecules
Piwi-interacting RNA (piRNA)	Eukaryotic	Cytoplasm	Thought to regulate gametogenesis, but function poorly defined

2) Write the components of transcription unit. Give a brief overview of mechanism of transcription alongwith well-labelled diagram. Also mention the mechanism of action of α-amanitin on in inhibition of transcription.

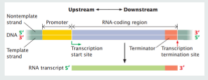
Components required for Transcription

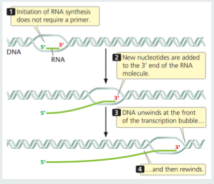
Transcription requires three major components:

1. a DNA template
2. the raw materials (substrates) needed to build a new RNA molecule
3. the transcription apparatus, consisting of the proteins necessary to catalyze the synthesis of RNA.



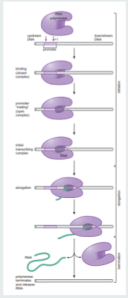
Transcription unit: promoter,an RNA-coding region, and a terminator





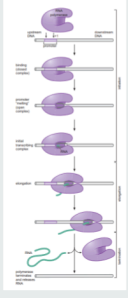
$RNA_n + rNTP \rightarrow RNA_{n+1} + PPi$

Mechanisms of Transcription



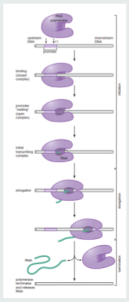
The phases of the transcription cycle: Initiation, elongation, and termination. The figure shows the general scheme for the transcription cycle. The features shown hold for both bacterial and eukaryotic cases. Other factors required for initiation, elongation, and termination are not shown here but are described in the text. The DNA nucleotide encoding the beginning of the RNA chain is called the transcription start site and is designated the "þ1" position. Sequences in the direction in which transcription proceeds are referred to as downstream from the start site. Likewise, sequences preceding the start site are referred to as upstream sequences. When referring to a specific position in the upstream sequence, this is given a negative value. Downstream sequences are allotted positive values.

Initiation



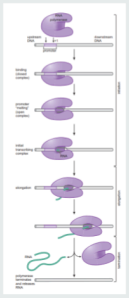
1. A promoter is the DNA sequence that initially binds the RNA polymerase (together with any initiation factors required).
2. Once formed, the promoter–polymerase complex undergoes structural changes required for initiation to proceed.
3. As in replication initiation, the DNA around the point where transcription will start unwinds. The base pairs are disrupted, producing a "transcription bubble" of single-stranded DNA.
4. Again, like DNA replication, transcription always occurs in a 50 -to-30 direction: the new ribonucleotide is added to the 30 end of the growing chain.
5. Unlike replication, however, only one of the DNA strands acts as a template on which the RNA strand is built.
6. Because RNA polymerase binds promoters in a defined orientation, the same strand is always transcribed from a given promoter.

Elongation



1. Once the RNA polymerase has synthesized a short stretch of RNA (10 bases), it shifts into the elongation phase.
2. During elongation, the enzyme performs an impressive range of tasks in addition to the catalysis of RNA synthesis.
3. It unwinds the DNA in front and reanneals it behind; it dissociates the growing RNA chain from the template as it moves along; and it performs proofreading functions.

Termination



1. Once the polymerase has transcribed the length of the gene (or genes), it must stop and release the RNA product (as well as dissociating from the DNA itself).
2. This step is called termination. In some cells, specific, well characterized sequences trigger termination.
3. In others, it is less clear what instructs the enzyme to cease transcribing and dissociate from the template.

α-Amanitin inhibits eukaryotic RNA polymerase II, which is critical for mRNA synthesis. By binding to the enzyme, α-amanitin blocks the elongation phase of transcription, leading to the inhibition of gene expression and cellular protein synthesis, potentially causing cell death.

3) A biologist is studying a gene involved in cell cycle regulation, called GeneX. To investigate the regulation of GeneX, he used CRISPR-Cas9 technology to delete the promoter region of GeneX in a cultured human cell line. After verifying that the promoter deletion was successful, they monitored the cells to observe any changes in GeneX expression levels, as well as any downstream effects on cell cycle progression. Explain why deleting the promoter region of GeneX would impact the transcription rate. Would you expect GeneX transcription to increase, decrease, or cease entirely?

Ans: Deleting the promoter region of GeneX would have a significant impact on its transcription rate because the promoter is a critical DNA sequence where RNA polymerase and transcription factors bind to initiate transcription.
Without the promoter, RNA polymerase cannot recognize or bind to the transcription start site of GeneX.
Consequently, transcription of GeneX would likely cease entirely since the necessary machinery for initiating transcription would not be able to function at that gene's location.
With no transcription occurring, GeneX mRNA levels would drop to undetectable levels, which would halt the production of the GeneX protein.

4) A hospital lab is studying a bacterial population with temperate phage infections. They notice that the OD readings for Culture C remain stable over time, but some of the bacteria begin showing antibiotic resistance even though antibiotics were not used in the culture. Upon introducing a stressor, mitomycin C, the researchers observe a rapid decline in OD.
What does the stable OD over time suggest about the initial phage infection cycle in Culture C?
Explain how the decline in OD following mitomycin C treatment relates to the phage infection cycle.
How might antibiotic resistance spread through the bacterial population due to phage infection, even without direct antibiotic exposure?

Ans : 1) Stable OD Over Time and Initial Phage Infection Cycle in Culture C:

The stable optical density (OD) over time suggests that the bacterial culture is experiencing a lysogenic cycle of phage infection. In this phase, the temperate phage DNA integrates into the bacterial genome (as a prophage) rather than causing immediate lysis. The bacteria continue to grow and divide, passing the integrated phage DNA to daughter cells without cell lysis. This stable population density results in consistent OD readings.

2) Decline in OD Following Mitomycin C Treatment:

Mitomycin C is a known DNA-damaging agent that can trigger prophages to exit the lysogenic cycle and enter the lytic cycle. This process, known as prophage induction, involves the excision of phage DNA from the bacterial genome, followed by rapid replication of the phage and production of phage particles. Eventually, the host bacterial cells lyse to release new phages. This lysis leads to a rapid decline in OD, as bacterial cells are destroyed in large numbers, reducing the culture density.

3) Spread of Antibiotic Resistance Due to Phage Infection Without Direct Antibiotic Exposure:

Phages can facilitate horizontal gene transfer, including the transfer of antibiotic resistance genes, through a process called transduction. During lysogeny, if the phage integrates into a bacterial genome containing antibiotic resistance genes, it can potentially package these genes accidentally when it transitions to the lytic cycle. When these phages infect new bacterial hosts, they can introduce resistance genes into these cells, enabling the spread of antibiotic resistance within the population.

Even without antibiotic exposure, this transduction process allows antibiotic resistance genes to spread horizontally across the bacterial population. This phenomenon can lead to antibiotic resistance emerging in the bacterial population as more cells acquire these resistance genes through phage-mediated gene transfer.

5) A geneticist isolates two bacteriophage r mutants (r13 and r2) that cause rapid lysis.he carried out the following crosses and counts the number of plaques listed below:
Calculate the recombination frequencies between (r2 and h) and between (r13 and h).

Genotype of parental phage	Progeny	Number of plaques
$h^+ r_{13}^- \times h^- r_{13}^+$	$h^+ r_{13}^+$	1
	$h^- r_{13}^+$	104
	$h^+ r_{13}^-$	110
	$h^- r_{13}^-$	2
Total		216
$h^+ r_2^- \times h^- r_2^+$	$h^+ r_2^+$	6
	$h^- r_2^+$	86
	$h^+ r_2^-$	81
	$h^- r_2^-$	7
Total		180

The recombination frequency (RF) is calculated using the following formula:
Recombination Frequency (RF) = (Number of recombinant plaques / Total number of plaques)

1.) Cross between $h^+ r_2$ and $h^- r_2^+$
Genotypes and counts:
- $h^+ r_2^+$: 6 (recombinant)
- $h^- r_2$: 7 (recombinant)
- $h^+ r_2$: 86 (parental)
- $h^- r_2^+$: 81 (parental)
Total number of plaques: 180

Recombinant plaques: 6 + 7 = 13
Recombination frequency between r_2 and h:
Recombination frequency (%) = (13 / 180) * 100 = 7.22%

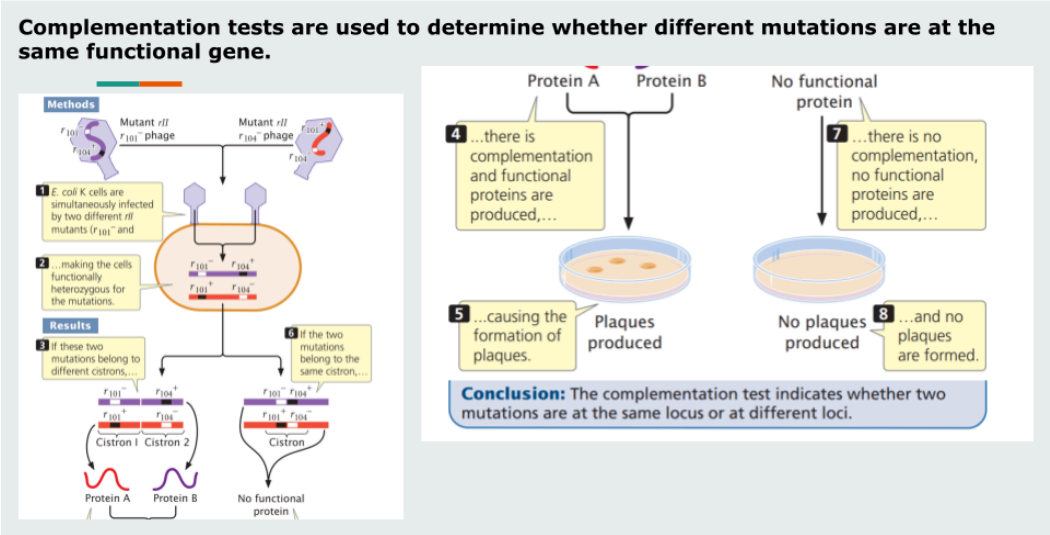
2.) Cross between $h^+ r_{13}$ and $h^- r_{13}^+$
Genotypes and counts:
- $h^+ r_{13}^+$: 1 (recombinant)
- $h^- r_{13}$: 2 (recombinant)
- $h^+ r_{13}$: 103 (parental)
- $h^- r_{13}^+$: 110 (parental)
Total number of plaques: 216

Recombinant plaques: 1 + 2 = 3
Recombination frequency between r_{13} and h:
Recombination frequency (%) = (3 / 216) * 100 = 1.39%

6) You are studying two strains of bacteriophages, Phage A and Phage B, which both exhibit a mutation that prevents them from lysing a specific bacterial host. You decide to perform a complementation test to determine whether the mutations in these phages are in the same gene or in different genes.

a) Describe the steps you would take to perform the complementation test with these bacteriophages.
b) What results would you expect if the mutations are in different genes?
c) If the mutations are found to be in the same gene, what implications would this have for the understanding of the genetic basis of the phage's ability to lyse the host?

Ans
a)



b) If the mutations in Phage A and Phage B are in different genes, complementation will occur, meaning that each phage provides the functional version of the gene that the other lacks. Together, they can produce all the necessary functions to lyse the bacterial host.
Expected Result: You would observe lysis (clear plaques) in the bacterial culture, indicating successful complementation.
c) If the mutations in Phage A and Phage B are in the same gene, no complementation will occur because neither phage can provide a functional version of the gene required for lysis. As a result, they cannot jointly overcome the defect.
Expected Result: The bacterial culture will remain intact (no lysis), indicating that both mutations are in the same gene.
Implications of Mutations in the Same Gene:
If the mutations in both phages are in the same gene, it suggests that a specific gene is critical for the bacteriophage's ability to lyse the host. This would imply that the gene is essential for producing a protein or enzyme required for the lytic cycle. Understanding this genetic basis can help identify key components of the lytic machinery and might reveal targets for disrupting phage infections or for applications in phage therapy, where controlled lysis is beneficial.