

VU Medical Zone

BIO 302 Current Final Term Spring 2021

Q1. What is Terminating Sequences?

2 Marks

The sequence of DNA which signals the transcription to stop is called Terminating Sequences.

Q2. Write down the importance of Phage?

2 Marks

Phage has been used extensively in the production of gene libraries, mainly because of its efficient entry into the E. coli cell and the fact that larger fragments of DNA may be stably integrated.

Q3. What is Plasmid?

3 Marks

Plasmids are autonomous self-replicating molecules of DNA (or very rarely RNA). They are not chromosomes, although they do reside inside living cells and carry genetic information. They are not regarded as part of the cell's genome for two reasons. First, a particular plasmid may be found in cells of different species and may move from one host species to another.

Q4. CTD sequences in Yeast?

5 Marks

The large subunit of Pol II has a carboxyterminal domain (CTD), which is referred to as the "tail". The CTD contains a series of repeats of the heptapeptide sequence: Tyr-Ser-Pro-Thr-SerPro-Ser. There are 27 of these repeats in the yeast Pol II CTD, 32 in the worm *Caenorhabditis elegans*, 45 in the fly *Drosophila*, and 52 in humans. The number of repeats correlate with the complexity of the genome. Each repeat contains sites for phosphorylation by specific kinases, including one that is a subunit of TFIIF. The form of Pol II recruited to the promoter initially contains a largely unphosphorylated tail, but the species found in the elongation complex bears multiple phosphoryl groups on its tail. Addition of these phosphates helps polymerase shed most of the general transcription factors used for initiation, and which the enzyme leaves behind as it

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escapes the promoter. Regulating the phosphorylation state of the CTD of Pol II controls subsequent steps—elongation and even processing of the RNA— as well.

Q5. Write a note on Operon Model?

5 Marks

Monod and Jacob proposed the operon model in 1961 to explain how the lac system is regulated. The term operon refers to two or more contiguous genes and the genetic elements that regulate their transcription in a coordinate fashion. Promoters had not yet been discovered when Monod & Jacob proposed the operon model but were readily incorporated into the operon model after their discovery.

The five major features of the model are:

1. The products of the lacZ, lacY and lacA genes are encoded in a single polycistronic lac mRNA molecule.
2. The promoter for this mRNA molecule is immediately adjacent to the lac region. Promoter mutations (p-) that are completely incapable of making β galactosidase, permease, and transacetylase have been isolated. The promoter is located between lacI and lacO.
3. The operator is a sequence of bases (in the DNA) to which the repressor protein binds.
4. When the repressor protein is bound to the operator, lac mRNA transcription can't take place.
5. Inducers stimulate lac mRNA synthesis by binding to the repressor. This binding alters the repressor's conformation so it can't bind to the operator. In the presence of an inducer, therefore, the operator is unoccupied and the promoter is available for initiation of mRNA synthesis. This state is called derepression.

Q6. CRISPRs stands for?

2 Marks

CRISPRs stands for Clustered Regularly Interspaced Short Palindromic Repeats.

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Q7. What is torpedo model? 2 Marks

Recently, however, an enzyme that degrades the second RNA as it emerges from the polymerase has been identified, and this enzyme may itself trigger termination. This is called the torpedo model of termination.

Q8. What is Alternative splicing and write its importance? 3 Marks

Alternative splicing strategy enables a gene to give rise to more than one polypeptide product. These alternative products are called isoforms.

It is estimated that 90% or more of the protein-coding genes in the human genome are spliced in alternative ways to generate more than one isoform.

Q9. Why Plasmid is not regarded as the cell's genome? 5 Marks

They are not regarded as part of the cell's genome for two reasons.

First, a particular plasmid may be found in cells of different species and may move from one host species to another.

Second, a plasmid may sometimes be present and sometimes absent from the cells of a particular host species. They are not needed for cell growth and division under normal conditions.

Q10. How Group I intron spliced DNA? 5 Marks

Group I introns splice by a different pathway.

Instead of a branchpoint A residue, they use a free G nucleotide or nucleoside. This G species is bound by the RNA, and its 3'-OH group is presented to the 5' splice site. The same type of transesterification reaction that leads to the lariat formation fuses the G to the 5' end of the intron. The second reaction now proceeds just as it does in the earlier examples: the freed 3' end

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of the exon attacks the 3' splice site. This fuses the two exons and releases the intron, although, in this case, the intron is linear rather than a lariat structure.

Q11. What is RNA processing? 2 Marks

The final RNA processing event, polyadenylation of the 3' end of the mRNA, is intimately linked with the termination of transcription, although exactly how is still not quite clear.

Q12. Name two protein used in Transcription? 2 Marks

TFIIB

TAF

Q13. Function of Lamda phage? 3 Marks

Phage λ has been used extensively in the production of gene libraries, mainly because of its efficient entry into the E. coli cell and the fact that larger fragments of DNA may be stably integrated. For the cloning of long DNA fragments, up to approximately 25 kb, much of the nonessential λ DNA that codes for the lysogenic life cycle is removed and replaced by the foreign DNA insert.

Q14. Define Sigma 70? 2 Marks

In the case of Escherichia coli, the predominant σ factor is called $\sigma 70$.

Q15. Why mRNA has short life span? 3 Marks

An important characteristic of bacterial mRNA is that its lifetime is short compared to other types of bacterial RNA molecules. The half-life of a typical bacterial mRNA is a few minutes.

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Although mRNA's short lifetime may seem wasteful, it has an important regulatory function. A cell can turn off the synthesis of a protein that is no longer needed by turning off synthesis of mRNA that encodes the protein.

Q16. Give two features of Replacement vector? 2 Marks

In a replacement vector, a central region of DNA not essential for lytic growth is removed (a stuffer fragment) by a double digestion with, for example, EcoRI and BamHI.

The central stuffer fragment is replaced by inserting foreign DNA between the arms to form a functional recombinant ϕ phage.

Q17. Define PUC plasmid? 2 Marks

The valuable features of pBR322 have been enhanced by the construction of a series of plasmids termed pUC. These plasmids are based on pBR322, from which about 40% of the DNA has been deleted.

Q18. Importance of GTF? 3 Marks

In addition, whereas bacteria require only one additional initiation factor (σ), several initiation factors are required for efficient and promoter-specific initiation in eukaryotes. These are called the general transcription factors (GTFs). In vitro, the general transcription factors are all that are required, together with Pol II, to initiate transcription on a DNA template.

Q19. What is ADAR? How many ADAR gene is present in Human? 5 Marks

Another kind of RNA editing takes place through the deamination of adenosine, which converts adenosine to inosine, which has an oxygen in place of adenine's amino group. Because inosine

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forms base pairs with cytidine in the same way as guanosine, the deamination of adenosine changes the meaning of a codon. This kind of RNA editing is directed by an enzyme called adenosine deaminase acting on RNA (ADAR).

Humans contain three ADAR genes: ADAR1, ADAR2, and ADAR3.

Q20. Write down two protein name and function of Lactose metabolism?

3 Marks

In E. coli, two proteins are necessary for lactose metabolism.

These include the enzyme **β galactosidase** and a carrier molecule, **lactose permease**, which transports lactose (and other galactosides) into the cell.

Q21. Function of A-site, P-site and E-site? 3 Marks

The A-site is the binding site for the aminoacylated-tRNA, the P-site is the binding site for the peptidyl-tRNA. The E-site is the binding site for the tRNA that is released after the growing polypeptide chain has been transferred to the aminoacyl-tRNA (E is for “exiting”).

Q22. Define Spliceosome? 2 Marks

The transesterification reactions are mediated by a huge molecular “machine” called the Spliceosome.

Q24. How many classes of spliceosome? Name them? 3 Marks

Human cells contain two types of spliceosomes the major spliceosome responsible for removing 99.5% of introns and the minor spliceosome, which removes the remaining 0.5%.

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Q25. Open complex transition?

3 Marks

Transition to the Open Complex involves structural changes in RNA Polymerase and in the Promoter DNA. The initial binding of RNA polymerase to the promoter DNA in the closed complex leaves the DNA in double-stranded form.

Q26. How nucleosome helps in change in transition machinery to bind promoter? 5 Marks

In vivo, however, the general transcription factors are not alone sufficient to bind promoter sequences and elicit significant expression. Rather, additional factors are required, including DNA-binding regulatory proteins, the so-called Mediator complex, and often chromatin-modifying enzymes. The eukaryotic core promoter refers to the minimal set of sequence elements required for accurate transcription initiation by the Pol II machinery.

Q27. Write a note on Riboswitches?

5 Marks

Riboswitches control gene expression in response to changes in the concentrations of small molecules. They do so through changes in RNA secondary structure. These regulatory elements are typically found within the 5'-untranslated regions (5'-UTRs) of the genes they control. They can regulate expression at the level of transcription or translation.

Each riboswitch is made up of two components:

- ☐ the aptamer and,
- ☐ the expression platform.

The aptamer binds the small-molecule ligand and, in response, undergoes a conformational change, which, in turn, causes a change in the secondary structure of the adjoining expression platform. These conformational changes alter expression of the associated gene by either terminating transcription or inhibiting the initiation of translation. Riboswitches are typically found upstream of genes involved in the synthesis of the metabolite ligand recognized by the

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riboswitch in question. For example, in *Bacillus subtilis*, many genes involved in the use of the amino acid methionine have a 200-nucleotide-long untranslated leader RNA that acts as a SAM (S-adenosyl methionine) – sensing riboswitch.

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