Problem Set #4
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Biochemistry

Question 1:
Name the 3 general processes of the central dogma and provide the name of the enzyme that is performing the respective function. Bonus: do the same for the 2 special processes.

Answer:
DNA replication: DNA polymerase
Transcription: RNA polymerase
Translation: Ribosome

Reverse transcription: reverse transcriptase
RNA replication: RNA-dependent RNA polymerase

Question 2:
In which direction does DNA polymerase elongate and proofread? Explain the terms processivity and synthesis rate and provide a number for these processes.

Answer:
Elongation -> 5′ to 3′
Proofreading -> 3′ to 5′
Processivity describes how much DNA is on average replicated by a single DNA polymerase before it dissociates from DNA (~50kbps).
Synthesis rate is the speed at which DNA polymerase polymerizes DNA (~750 nucleotides/s).

Question 3:
What are Okazaki fragments, where do they occur and why do they exist? How are Okazaki fragments resolved?

Answer:
Okazaki fragments are stretches of replicated DNA that occur on the lagging strand where synthesis can't occur continuously. This is because synthesis on the lagging strand occurs in the opposite direction of the replication fork movement. Resolving (fixing) the Okazaki fragments requires degrading the RNA primers (done by RNaseH) and filling the gap left by the primers with DNA nucleotides (done by DNA polymerase I) followed by ligation (done by DNA ligase) which fixes the break between individual Okazaki fragments.

Question 4:
What separates the two strands of the parental DNA to be replicated?

Answer:

DNA helicase

Question 5:
Estimate the DNA synthesis rate based on E. coli with a doubling time of 20 minutes.

Answer:
Given Information:

- E. coli genome size: ~4.6 million base pairs (Mbp)
- Replication time: ~20 minutes (one round of replication must complete in this time)
- Bidirectional replication: DNA replication starts at the origin (OriC) and proceeds in both directions with two replication forks.

Estimation:

1. Total DNA to be synthesized:
   a. Since replication is bidirectional, each replication fork synthesizes half of the genome.
   b. So, each fork replicates 4.6 Mbp / 2 = 2.3 Mbp.
2. Time available for replication:
   a. 20 minutes = 1,200 seconds
3. DNA synthesis rate per replication fork:
   a. 2,300,000 base pairs / 1,200 seconds ≈ 1,917 nucleotides per second

The estimated DNA synthesis rate in E. coli is ~1,917 nucleotides per second per replication fork.

This estimate is higher than expected because, as we have seen in Lecture 1, bacteria can divide with an overlapping cell-cycle in which E. coli can start a new round of DNA replication before the previous round finished, a process called multifork replication.

Question 6:
What are the major steps in transcription?

Answer:
Initiation, elongation, termination

Question 7:
Does RNA polymerase require a primer for transcription?

Answer:
No

Question 8:
What is the function of E. coli sigma factors?

Answer:

Sigma factors recognize specific DNA sequences and thus recruit E. coli RNA polymerase to specific promoters. This allows the expression of functionally related sets of genes.

Question 9:
What are the two types of transcriptional termination?

Answer:
Rho-dependent and Rho-independent.

Question 10:
What are the 3 sites in the ribosome that a tRNA transitions through? Name the sites and provide a brief description of the process taking place in each site.

Answer:
A-site, P-site, and E-site. Initially a tRNA binds to a vacant A-site (aminoacyl site). It then transitions to the P-site (peptidyl site) where the charged amino acid is transferred from the tRNA to the growing peptide chain. It then finally moves to the E-site (exit site) from where it is released form the ribosome.

Question 11:
What is the function of elongation factor TU (EF-TU)?

Answer:
EF-Tu delivers tRNAs to the ribosome and is thought to play a role in increasing translational fidelity.

Question 12:
Are stop codons recognized by a specific tRNA or a protein?

Answer:
Stop codons are recognized by proteins called release factors.

Question 13:
What are the two main ways in which mutations can arise?

Answer:
Point mutations and insertions/deletions.

Question 14:
Do all single nucleotide mutations result in a change in amino acid?

Answer:
No, because there is codon usage redundance in the genetic code it is possible that a DNA mutation doesn't result in a different amino acid. These are called synonymous substitutions or silent mutations. They do result in a different codon / tRNA being used, which still may result in a phenotype.

Question 15:
Translate the following DNA sequence into a peptide.

5' AGGGCATGGCTCTCAAGGTGGACTTCGGATAAGCATCA 3'

Answer:
AGGGC **ATG GCT CTC AAG GTG GAC TTC GGA TAA** GCATCA

Met - Ala - Leu - Lys - Val - Asp - Phe - Gly