5.E: DNA replication I: Enzymes and Mechanism (Exercises)

Question 5.8

Imagine you are investigating the replication of a bacterial species called *B. mulligan*. The bacteria is grown for several generations in medium containing a heavy density label, $[^{15}\text{N}]\text{NH}_4\text{Cl}$. The bacteria are then shifted to medium containing normal density $[^{14}\text{N}]\text{NH}_4\text{Cl}$. DNA is extracted after each generation and analyzed on a CsCl gradient. From the results shown below, what is the mode of replication in *B. mulligan*? Explain your conclusion.

![Density distribution diagram](https://bio.libretexts.org/Bookshelves/Genetics/Book%3A_Working_with_Molecular_Genetics_(Hardison)/Unit_II%3A_Replica…)

Question 5.9

How many turns must be unwound during replication of the *E. coli* chromosome? The chromosome contains $4.64 \times 10^6$ base pairs.

Question 5.10
Which of the following comments about Okazaki fragments are true or false? Okazaki fragments:

a. are short segments of newly synthesized DNA.
b. are formed by synthesis on the leading strand of DNA.
c. have a short stretch of RNA, or a mixture of ribonucleotides and deoxyribonucleotides, at their 5' end.
d. account for overall synthesis of one DNA strand in a 3' to 5' direction.

Question 5.11.

The following experimental results are from A. Sugino and R. Okazaki (1972) "Mechanisms of DNA Chain Growth VII. Direction and rate of growth of T4 nascent short DNA chains" J. Mol. Biol. 64: 61-85.

a. *E. coli* cells were infected with bacteriophage T4 and then chilled to 4°C to slow the rate of replication. Replicating DNA in the infected cells was pulse-labeled with [3H]-thymidine (a) or [3H]-thymine (b) for 5 sec (black-filled circles), 30 sec (open circles with vertical line), 60 sec (open circles with dot) or 300 sec (open circles). The pulse labeling was stopped with potassium cyanide and ice, and the DNA was extracted, denatured in NaOH, and separated on an alkaline sucrose gradient. Fractions from the gradient were collected and assayed for the amount of ³H in the DNA (as material that bound to a filter after washing in (a) and as acid-insoluble material in (b)). The sedimentation value in Svedbergs (S) is given along the x-axis; faster sedimenting material is toward the right. What do these data tell you about the sizes of nascent (newly synthesized) DNA at the various pulse labeling times?

(b) Sugino and Okazaki used a method to break the isolated short nascent chains (completed Okazaki fragments) randomly and recover only the oligonucleotides from the 5Å ends. They found that at very short labeling times (e.g. 5 sec) the [³H] thymidine was not at the 5' ends of the DNA (hence it was internal and at the 3' ends). After longer labeling times, the [³H] thymidine was found in the oligonucleotides at the 5' end. What do you conclude is the direction of chain growth of the nascent chains? Explain your logic.
Question 5.12

We have covered two experiments from the Okazaki lab using pulse labeling for increasing times to follow the synthesis of new DNA. How would you design a pulse-chase experiment to monitor not only the initial production of Okazaki fragments, but also their incorporation into larger DNA molecules?

Question 5.13

Which enzymes, substrates, and cofactors are used in common and which ones are distinctive for synthesis of leading strands and lagging strands of DNA at the replication fork of \textit{E. coli}?

Question 5.14

Which subunit or complex within \textit{E. coli} DNA polymerase III holoenzyme has each the following functions?

a. Catalyzes 5’to 3’ polymerization of new DNA.
b. Has the proofreading function (3’ to 5’ exonuclease).
c. Dimerizes the two catalytic cores.
d. Forms the clamp that is thought to account for its high processivity.
e. Loads and unloads the sliding clamp.

Question 5.15

What are the components of the multiprotein complex known as the primosome in \textit{E. coli}? What does it do? In what direction does it travel?

Question 5.16

Which eukaryotic nuclear DNA polymerase(s) is (are) thought to account for leading strand and lagging strand synthesis?

Contributors and Attributions

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