

Notes on Bacterial DNA Replication

Here is what I think you should be familiar with from your reading of Chapter 1 of the text. I am assuming that you will be “re-familiarized” with the basic biochemistry of DNA replication. We’ll concentrate on things that are unique and important to bacteria, especially as they will relate to use of bacteria.

1. Table 1.1 - You should know the names and functions of the proteins/complexes, but NOT the individual components of Pol III. You should understand how these proteins work together to replicate DNA.
2. Understand the differences between DNA Pol I and Pol III, including their structures, specific functions in replication, and differences in enzyme activities. Know what the Klenow fragment of DNA Pol I is.
3. Be prepared to answer the following 2 questions in class (don’t email the answer to me)
 - a. If you were going to sell a product to replicate DNA in tube, which polymerase would you use - Pol I or Pol III? Why?
 - b. If you were going to perform a “Nick-translation” reaction in a tube to label nascently produced DNA, which enzyme would you use - Pol I (intact or Klenow?) or Pol III? Why?
 - c. How might you use an *E. coli* mutant in the *dnaQ* gene in the lab?
4. Understand how initiation and termination of chromosomal replication are regulated (in general).
5. Think about how these themes fit into plasmid biology.

DNA Polymerization

- A. Chromosome is circular
- B. Origin of replication (*oriC*)
- C. Termination region half way around
- D. Replication is regulated at initiation, not rate of synthesis (like most bacterial regulation)
- E. DnaA protein binds at *oriC*, opens DNA
- F. DnaB and DnaC enter (helicase that unwinds double strands)
- G. Single strand binding protein stabilizes the open complex
- H. Primase (DnaG) lays down RNA primer
- I. Major DNA polymerase, Pol III - a multi component complex - begins 5'-3' synthesis on leading strand
- J. Okazaki fragments are synthesized on lagging strand.
- K. Synthesis is bidirectional around chromosome - theta model
- L. DNA polymerase I (PolA) repairs Okazaki fragments, removing RNA primers and sealing joints (has 5'-3' exonuclease activity, unlike Pol III); note that the Klenow fragment of Pol I lacks 5'-3' exonuclease activity
- M. Proofreading by Pol III (3'-5' exonuclease activity) makes replication very efficient and accurate, but spontaneous mutations do occur
- N. When replication forks meet half way around, replication ceases.
- O. DNA gyrase (GyrAB) puts supercoils into DNA after it is synthesized, Topoisomerase I removes supercoils.
- P. DNA ligase seals nicks, requires 5' phosphate and 3' hydroxyl (that's why alkaline phosphatase treatment of vector DNA prevents self ligation during cloning)
- Q. It takes about 40 min. to replicate the chromosome, plus another 20 min. for the cell to divide, but *E. coli* can replicate in 20 min. There isn't time to finish the chromosome. So initiation of replication starts before termination, and there can be multiple copies of the chromosome in the cell during fast growth (this is why fast growing cells are 4X bigger than non-growing cells).