

Writing Assignment #1:

PNAS Paper: "The thioredoxin binding domain of bacteriophage T7 DNA polymerase confers processivity on *Escherichia coli* DNA polymerase I"

Authors: Ella Bedford, Stanley Tabor, and Charles C. Richardson

Online at [PNAS](#) site

The paper by Bedford and colleagues describes a detailed analysis of a modified form of the Klenow fragment of *E. coli* DNA Polymerase I (this protein is a portion of DNA Pol I that includes the DNA polymerase domain and the proofreading [3'→5'] exonuclease but lacks the 5'→3' exonuclease). A key issue in the paper is to assess the effect of adding thioredoxin on the processivity of the modified DNA polymerase. To this end, the authors use a different processivity assay than that described in class. Compare and contrast the two assays. What are the strengths and weaknesses of each approach? Although the authors demonstrate that the addition of the "thioredoxin binding domain" to the Klenow fragment makes the modified enzyme dependent on the addition of thioredoxin, the level DNA synthesis stimulation for the modified Klenow never reaches the levels of stimulation observed for T7 DNA Polymerase. Based on the data in the paper, describe the major reason for the higher levels of thioredoxin stimulation observed for the T7 DNA polymerase compared to the TBD-modified Klenow DNA polymerase.

Given that there is only one site in the T7 genome where the T7 DNA polymerase can be loaded (the T7 origin of replication), why would a strong dependence on thioredoxin be useful for the phage? (hint: What would be the consequence of loading a moderately processive polymerase at an origin?)

Instructions:

The answers to the questions should be in the form of a **2 page essay, double spaced, using #12 font size with one inch margins on top, bottom, left, and right. All papers should be left justified. No excuses!**

The essay should synopsise the important points of the paper that pertain to the question (no more than two paragraphs) and propose an answer to the questions posed. The quality of the answer will depend on the **quality of the supporting arguments** as well as the **quality of the presentation**.

Criteria for evaluation:

1. The student introduced the paper's topic effectively through a concise and clear summary of the key conclusions that can be made based on the experiments presented in the reading assignment.
2. The paper demonstrated a clear understanding of the experiments presented in the reading assignment.
3. The paper presented an insightful perspective to the study question(s). Answers were well supported with logical arguments based on the data in the paper.
4. The study question(s) were answered in the space allowed.
5. The paper:
 - a. was well organized with informative topic sentences, effective transitions, and clear expression of ideas;
 - b. had a logical flow; and
 - c. demonstrated correct grammar and mechanics.