PS#1_2016: We will begin at 10:30 am on Thursday 1/14 with Problem 1 in BH212

Assignments
Problem 1- group 1
Problem 2- group 2
Problem 3- group 3
Problem 4- group 4

Time limit: 25 min per group.

The beginning: Designate one person in each group to give a 5 min introduction: try to be succinct and remember to describe the biological significance of the question. You don’t need to describe the whole solution in these 5 min, but you can set the stage.

The discussion: Describe the solutions and the logic behind them.

The end: Designate one person in each group to give a short summary at the end of the problem discussion.
The figure at left comes from Okazaki’s classic study of the mechanism of DNA chain growth in E. coli (Fig 2. Okazaki et al 1968 PNAS 59:598). In this experiment newly synthesized DNA of replicating cells was labeled for 5, 10, 30 or 60 seconds with radioactive thymidine before being extracted with alkali, which separates daughter strands from parental strands. This denatured DNA was then size fractionated by centrifugation in alkaline sucrose gradients, and the amount of radioactivity in each gradient fraction was measured.

Q1. Okazaki concluded from the figure above that the data “supports the predictions of those mechanisms by which two daughter strands are synthesized in a discontinuous fashion.”

(A) For the above experiment draw out what the data would look like for the following three formal possibilities:
(i) discontinuous DNA synthesis on both strands, (ii) continuous synthesis on one strand but discontinuous on the other (semi-discontinuous) and (iii) continuous synthesis on both strands.

(B) How might you account that the above results led Okazaki to favor a fully discontinuous model? How could these results still be consistent with a semi-discontinuous model?

Q2. Okazaki made another conclusion from the figure above: that the small molecular weight (MW) peak observed after the 5 sec labeling “represents an intermediary state in the formation of chromosomal DNA”. Do you agree with this conclusion? Can you think of additional experiment(s) to test this conclusion? What type of analyses of the small MW labeled fragments might help?
Bioreg 2016 Problem Set 1: Q3&4

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

5'–TTC
3'–AAGCTCA

20 µM

2 mM

200 s⁻¹

0.4 s⁻¹

0.02 s⁻¹

5.0 s⁻¹

k_{exo}^c primer

k_{exo}^l primer

k_{pol}^c primer

k_{pol}^l primer

K_D^C dNTP

K_D^C dNTP

K_D^l dNTP

K_D^l dNTP

K_D

K_D

DNA polymerase

primer-template

DNA polymerase

primer-template

bound dNTP

pseudoequilibrium constant for nucleotide binding

composite rate constant for polymerization reaction

composite rate constant for exonuclease reaction
Q3. (A) Intrinsic Fidelity: How much does the polymerase favor the correct over the incorrect nucleotide at the level of nucleotide binding in the active site? How much does the polymerase favor the correct over the incorrect nucleotide at the level of polymerase catalysis? What is the total fidelity of the polymerase in incorporating the correct nucleotide?

(B) Exonuclease Proofreading: What step shown in the diagram is the exonuclease in competition with? Why are each effectively irreversible? What is the quantitative difference in kinetic partitioning between the two competing reaction steps for a correctly incorporated nucleotide versus an incorrectly incorporated nucleotide. What is the amount by which exonuclease proofreading contributes to polymerase fidelity?

Q4. (A) You discover that phosphorylation of the polymerase at a specific residue reduces all polymerization rate constants by a factor of 10 without affecting other reaction steps. How will this phosphorylation affect the intrinsic fidelity of nucleotide incorporation or the exonuclease proofreading? What will happen to the speed and overall fidelity of the polymerase?

(B) You mutagenize the polymerase and discover a mutation that increases all exonuclease rate constants by a factor of 10 without affecting any other reaction step. How will this mutation affect the intrinsic fidelity of nucleotide incorporation or exonuclease proofreading? What will happen to the speed and overall fidelity of the polymerase? Speculate on what might be the selective disadvantage of this mutation.