

7.014 Problem Set 3 Solutions

Question 1

After acing the 7.014 Quiz 1, you take a well-deserved break and go “looking for Baker House.” Somewhere in the tunnels you stumble on a device you have never seen before, and start playing with its dials. It turns out to be a time- and reality-transporting device. It lands you in the office of the editor of the journal *Nature* in February of 1953. This is a reality much like our own, except that in this reality no one has yet seen Rosalind Franklin’s data.

The editor is happy to see you, since he believes the knowledge you acquired in 7.014 will help him with the decision he must make. In front of him are four papers proposing various models for the structure of DNA.

For each model below, indicate whether the editor should accept or reject the paper (i.e. whether the model is plausible or not) and explain your advice. If your advice is based on data the editor has not yet seen, explain what the data is and how it will affect the plausibility of the model. If your advice is based on data the editor is familiar with, explain how that data is either consistent or inconsistent with the model.

- i. Model A: DNA is a double-stranded helix with sugar-phosphate backbones in the center, and bases sticking out into solution. In this model, the strands are running anti-parallel to each other.

This model should be rejected. DNA backbone is an alternating sugar-phosphate polymer. Phosphates are negatively charged. This model (originally favored by James Watson) would put these negative charges in close proximity. Putting negative charges that close together would be very energetically unfavorable.

- ii. Model B: DNA is a four-stranded helix with bases looking inwards. The model claims to be based on the current crystallographic data.

This model should be rejected. While this model might be consistent with all data available to the editor, it is inconsistent with Rosalind Franklin’s data. In particular, she was able to figure out that DNA adapts two forms in solution and to isolate and crystallize molecules of a unique form. It is that data that indicated to Crick that DNA was a double helix with matched bases on the inside of the molecule.

- iii. Model C: DNA is a double-stranded helix with sugar-phosphate backbones on the outside, and bases in the middle, where purines (A and G) pair with purines and pyrimidines (T and C) pair with pyrimidines. The strands are running anti-parallel to each other.

This model should be rejected. This design does not assure compliance with Chargaff rules. In addition, Rosalind Franklin’s data shows that DNA has a very regular shape on the outside of the molecule. Purine bases have two nitrogenous rings each, while pyrimidines have one. Pairing purines with purines would create a very wide section, while pairing pyrimidines with pyrimidines would give a narrow section. Thus, it would be clear from the outside of the molecule which base pair is on the inside. This would contradict Franklin’s crystallographic data that shows regular overall shape despite sequence variability.

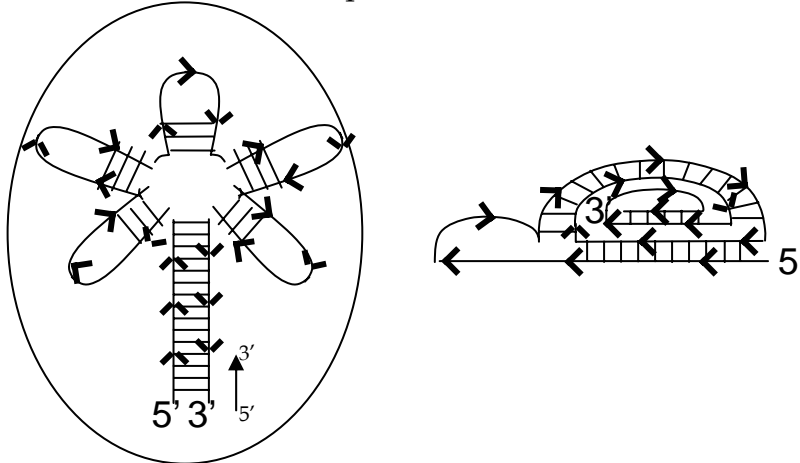
- iv. Model D: DNA is a double-stranded helix with sugar-phosphate backbones on the outside, and bases in the middle. In this model, the strands are running parallel to each other.

This model should be rejected. Attempting to base pair the bases that satisfy Chargaff’s rules (A-T and C-G) off of the parallel backbones would lead to pairs that do not have similar overall shape and angles. In that case there would be no way to assure the very regular shape on the outside of the DNA molecule that is demonstrated by Franklin’s data.

Question 2

After a 7.014 nucleic acids lecture, a budding young artist named Moe Nay wanted to explore the shapes a single-stranded DNA molecule can take. He sketched the two shapes below. His TA was impressed with Moe's imagination and artistic ability, but she informed Moe that only one of his sketches was feasible. In the sketches, the lines indicate complementary base pairing.

a) Which of the sketches below is possible? Circle the correct one.



b) What is wrong with the other drawing?

Base pairing occurs between parallel strands of nucleic acid.

c) What is the minimal number of primers that would be needed to create a complementary DNA strand to the DNA strand in the correct drawing? Why?

Only one primer would be needed because the template is a single strand. By placing a primer on the 3' end of the template, we can copy the entire strand and create a double stranded molecule.

d) On the drawing above, indicate the position(s) of the primer(s) needed to create an entire complementary strand. Label 5' and 3' of the primer(s).

e) Would the new double stranded molecule assume the shape similar to one in the drawing? Why or why not?

No, the new double stranded molecule will assume the shape of the regular double helix. Once the bases make H-bonds with the complementary bases on the other strand, it is no longer favorable for them to make the intra-molecular H-bonds that hold the molecule in the drawing together.

Question 3

a) In the Meselson and Stahl experiment, what part of the DNA gets labeled with ^{15}N ?

In this experiment, nitrogenous bases of the DNA get labeled with ^{15}N .

b) Would any other macromolecule get labeled in that experiment? If yes, what is it?

RNA also has nitrogenous bases, and protein has N in its back bone and some side chains, so both types of macromolecules would also get labeled in the experiment.

c) In the Meselson and Stahl experiment, where on the CsCl gradient would the following DNA be found (low, middle, high):

i. Double stranded DNA where both strands are labeled *low*

ii. Double stranded DNA where one strand is labeled *middle*

iii. Double stranded DNA where neither strand is labeled *high*

Semi-conservative replication was only one of the models of DNA replication proposed after the discovery of DNA structure. One of the other models was called conservative replication. In that model, new copies of both DNA strands would be made, but after replication was complete, the two “old” strands would stay together in a double helix, and the two “new” strands would form another double helix.

In Meselson and Stahl experiment, a culture is grown on media with ^{15}N , and switched to light N at time=0.

- d) If the mechanism is semi-conservative, what would you expect to see on the CsCl gradient after allowing the specified number of rounds of replication:

# rounds replication after switching to light N	Number of Bands	Location of bands (low, middle, high)
None	1	low
One	1	middle
Two	2	middle, high
Three	2	middle, high

- e) If the mechanism is conservative, what would you expect to see on the CsCl gradient after allowing the specified number of rounds of replication:

# rounds replication after switching to light N	Number of Bands	Location of bands (low, middle, high)
None	1	low
One	2	low, high
Two	2	low, high
Three	2	low, high

- f) Treating the DNA samples with heat can break the hydrogen bonds that hold paired strands together. When the DNA is cooled, strands will re-pair but not necessarily with their original partner. If replication is conservative and you briefly treated the DNA samples with heat, how would that change your results from a Meselson-Stahl experiment?

If the samples are briefly treated with heat any time after the first round of replication, we should expect to see the appearance of the middle species of DNA. That species would have one heavy and one light chain of DNA. Once that species appears, it will be maintained through any future round of replication, since in the conservative model, parental DNA strands end the round of replication re-paired with each other.

If the samples are treated before replication begins, no change in the results will occur.

- g) If you discovered a new species of bacteria, would you repeat the Meselson and Stahl experiment on it, or assume the mechanism of replication is semi-conservative? Why?

We would assume that the replication is semi-conservative. An organism employing a different mechanism would have to employ a whole host of different enzymes to make it work. Like with glycolysis, this pathway is so critical to life that it is likely to be preserved by evolution because any mutation to the original system would be likely be less fit.

Alternatively, if this bacteria is a very old system that evolved to use a different replication system, we would expect it to show up in some other organisms that evolved from this bacteria. But since only semiconservative mechanism has ever been found, this interpretation is unlikely.

Question 4

a) What (if any) editing function ($5' \rightarrow 3'$ exo; $3' \rightarrow 5'$ exo; or mismatch repair) could repair the following mistakes made by a DNA polymerase?

- i. adds an extra nucleotide
mismatch repair and possibly $3' \rightarrow 5'$ exo.
- ii. puts in a wrong nucleotide
 $3' \rightarrow 5'$ exo or mismatch repair.
- iii. slides backwards 3 nucleotides on the template strand, creating a repeat
mismatch repair

b) In the table below a number of mutant DNA polymerases are listed. For each polymerase, indicate which property of replication (rate of binding, frequency of mutation, or rate of replication) will definitely be affected, and how (decrease or increase).

Mutant DNA Pol	Rate of DNA Binding	Frequency of Mutation	Rate of Replication
Does not distinguish between DNA and RNA strands	<i>decrease</i>		
Frequently falls off of the DNA			<i>decrease</i>
Frequently misreads template DNA		<i>increase</i>	
Low specificity for correct nucleotides		<i>increase</i>	
Does not distinguish between dNTPs and dNDPs			<i>decrease</i>
Poor catalyst of the nucleotide addition reaction			<i>decrease</i>
Missing binding site for processivity factor			<i>decrease</i>

c) If a DNA polymerase contains a mutation that allows it to strongly bind RNA strands, can the polymerase now function as an RNA polymerase? Why or why not?

The polymerase would still not be able to function as an RNA polymerase because the domain that checks whether the new nucleotide coming in is complementary to the base currently on the strand is still excluding RNA bases. What the enzyme would become is in fact a reverse transcriptase – an enzyme that creates a DNA strand based on the RNA template.