Recitation Section 7 Answer Key
February 28-March 1, 2005

Molecular Biology—DNA as Genetic Material and DNA Replication

A. DNA as Genetic Material

Before people used words such as "genetic material," the concept behind this term was well established. In fact, an entire industry based in large part on this concept played an enormous role in the development of our civilization.

1. What is this industry? How was the concept of "genetic material" central, if not articulated, part of this industry?

   Agricultural breeding programs have been in existence for millennia and have allowed our civilization to expand as people's capacity to produce enough food to feed the expanding population also grew. At the heart of these programs is the understanding that all living things possess heritable traits that are passed down from generation to generation. Breeding programs rely on controlled crosses and artificial selection to isolate and propagate species with particular desirable heritable traits.

After the work of Mendel was re-discovered, trying to understand what that "genetic material" was became a key part of scientific endeavor. While scientists did not discover what that substance was for some time, they fairly quickly formulated the three requirements that genetic material must satisfy.

2. What are those requirements? Why do they make sense?

   Genetic material must

   Encode genetic information
   Pass that information on to offspring (replicate)
   Change that information (mutate)

   Observations of heritable traits indicate that genetic information must be recorded somehow and also must be passed on to offspring. In addition, change over time indicates that there must also be a mechanism that allows the information to be changed.

3. Given what you now know about DNA structure, how does DNA satisfy these requirements?

   Information is encoded in the sequence of DNA bases.
   Because DNA is complementary and paired, one strand of DNA gives rise to two identical strands in DNA replication. That allows for production of two cells carrying identical copies of genetic material.
   If, as a result of environmental factors, or as a result of normal processes during replication, DNA sequence in a germ cell is changed, this mutated information is going to be passed on to the offspring, and may result in a new or changed trait.

In 1920s, Frederick Griffith experimented with smooth and rough *Streptococcus pneumoniae* bacteria. Griffith found that contrary to expectations, infecting a mouse with a mixture of live rough bacteria of type II (R_{II}) and heat-killed smooth bacteria of type III (S_{III}), killed the mouse. Griffith concluded that something was transferred in the course of the experiment from one type of bacteria to another, and as a result transformed the recipient bacteria. He named that substance "transforming principle." In fact, Griffith was able to isolate virulent bacteria from the dead mouse.

4. What was transferred in the experiment?

   DNA was transferred from one type of bacteria to another.

5. What type of bacteria was the recipient of the transforming principle, and what type was the "sender?"

   Heat-killed S_{III} was the sender of genetic information and live R_{II} was the receiver. Only a live cell can change and acquire new characteristics, so the recipient had to be R_{II}.

6. How did the transfer enable the appearance of virulent bacteria?

   After the transfer, R_{II} acquired characteristics of the virulent S_{III} strain. In fact, Griffith isolated live S_{III} from the dead mouse. We now understand it to be because some genes (segments of DNA) that encode for formation of polysaccharide coat were transferred in the experiment.

7. Were all the bacteria of the recipient type transformed? Describe the probable process from co-infection to the death of the mouse.

   It is unlikely that a significant number of R_{II} bacteria were actually transformed, or that all those transformed were transformed with the same section of DNA. It is much more likely that a single R_{II} bacterium (T) got transformed with a section of S_{III}'s genome that encodes for the polysaccharide coat. T integrated those portions into its own genome, and began producing the coat. This coat provided T with selective advantage because the immune system cells could not destroy T. Some other bacteria around T likely acquired certain other portions of the genome. However, mouse immune system acted as selection that favored T and disfavored all other variations. This is because the immune system could destroy all bacteria that did not exhibit polysaccharide coating. Thus, in effect, the immune system was clearing space for T and its descendants. Not having to compete for resources with many other bacteria, T's descendants were able to grow and divide until there were so many of them that they caused the death of the mouse.

B. DNA Replication

1. DNA replication is remarkably accurate. Do you think it would have been possible to evolve a more accurate polymerase? If so, would it have been evolutionarily advantageous? Why or why not?

   It would probably be possible to evolve a more accurate polymerase. But the cost would be slower or nonexistent rate of mutation. That would leave an organism with such a polymerase at a selective disadvantage, since the organism would lack the ability to acquire favorable mutations that would otherwise give it and its descendants a selective advantage.

2. We know that A pairs with T and C pairs with G. What is biochemically remarkable about these two pairs?

   These pairs have very similar overall width and angles. Using only these pairs, the shape of the outside of the DNA molecule remains regular.

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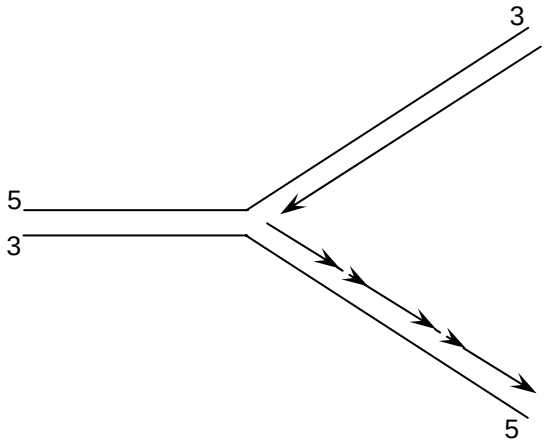
3. What enzyme is responsible for replicating DNA?

   DNA Polymerase.

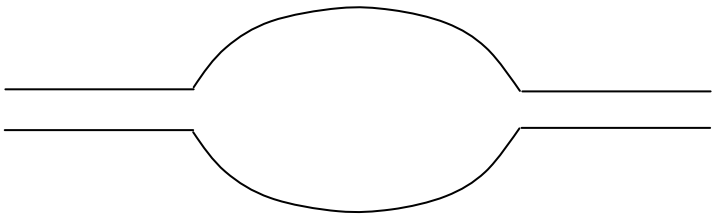
4. How does that enzyme know what base to put in next?

   The enzyme is essentially matching shapes to see whether the base it is trying will fit into the framework of the growing chain.

5. In lecture we saw the following drawing of the DNA replication fork:



How does this relate to the following diagram?



Which is a better representation of what actually happens during replication?

The bottom picture shows the replication bubble as it would open in a long piece of DNA. It is a better representation, since it takes into account more than an immediately local perspective.

DNA is a long polymer of nucleotides that only differ in their nitrogenous bases. Yet it encodes a lot of information.

6. What are some of the types of information encoded in DNA?

   Some of the examples of the various types of information encoded in DNA are: where replication begins; when a given gene would be expressed; amino acid sequence of a gene; cellular address of a protein; and information for properly segregating the chromosomes during cell division.

7. How is it possible to reliably encode these many types of information in DNA?

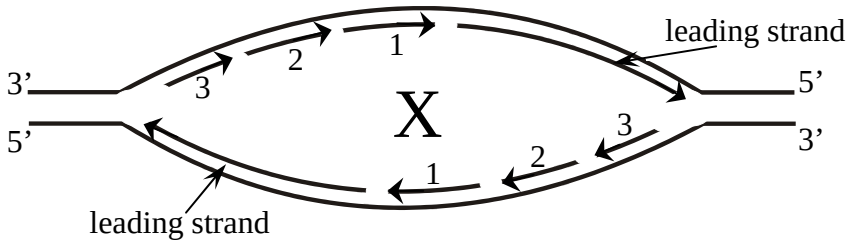
   The information is essentially written in different languages that are recognized by different machinery.

DNA is organized into chromosomes. Bacteria generally have one circular chromosome, while eukaryotes have multiple linear chromosomes.

8. How does the process of DNA replication start? Is it same or different in bacteria and eukaryotes? (Hint: think about how much DNA needs to be replicated in each case, and how much time the cell needs to replicate it.)

   For the process to be orderly and programmed, it has to start at designated places—origins of replication. In bacteria, there is one such spot per circular chromosome, ORI. In Eukaryotes, there are multiple origins of replication per linear chromosome.

9. Below is a schematic of a replication bubble that includes an origin of replication.



   i. indicate direction of DNA synthesis with arrows.
   j. label the ends of the original strands with 5' and 3'.
   k. indicate the origin of replication with an X.
   l. label the leading strands
   m. for each newly replicated strand, number the Okazaki fragments 1-3 in order of when they were created.

10. Using the schematics below, explain why base addition in DNA replication is strictly dependent on the presence of the 3' OH.

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3' OH attacks α phosphate to form the phosphodiester bond, and release the pyrophosphate. In the absence of OH, polymerization reaction is not possible.

11. What energy source is used to power nucleotide addition reaction?

Release of the phosphates powers the reaction, much like the ATP is used in other reactions.

12. Speculate as to why performing polymerization 3'→5' might have been less energetically favorable for the cell overall.

Because the energy for polymerization is coming from the phosphates, if polymerization was happening 3'→5', the energy would have to come from the phosphates at the top of a long chain of bases. If the base incorporated at the top was missing the phosphates for some reason, polymerization of the whole strand would stall. In this case, the "investment" put into building the chain so far would be wasted.
With the 5'→3' polymerization, in a case where a base is wrong for some reason, only that base is wasted.