

~~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<sub>II</sub>) and heat-killed smooth bacteria of type III (S<sub>III</sub>), 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?~~

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

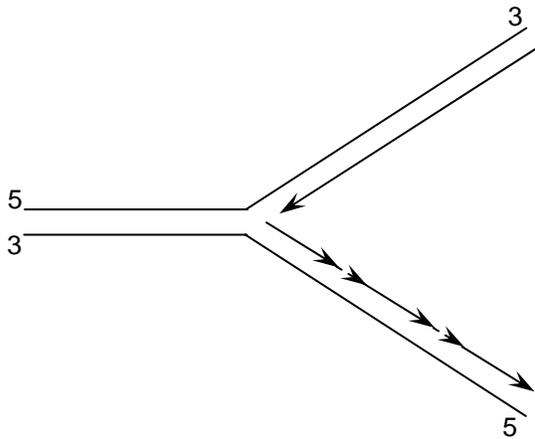
~~6. How did the transfer enable the appearance of virulent bacteria?~~

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

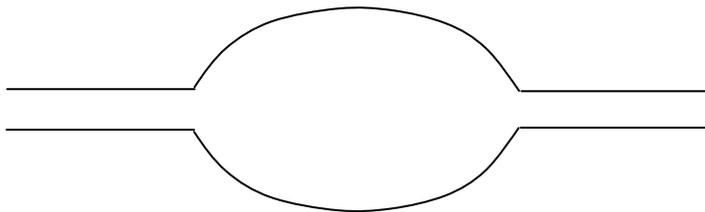
## **B. DNA Replication**

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?

2. We know that A pairs with T and C pairs with G. What is biochemically remarkable about these two pairs?
3. What enzyme is responsible for replicating DNA?
4. How does that enzyme know what base to put in next?
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?

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

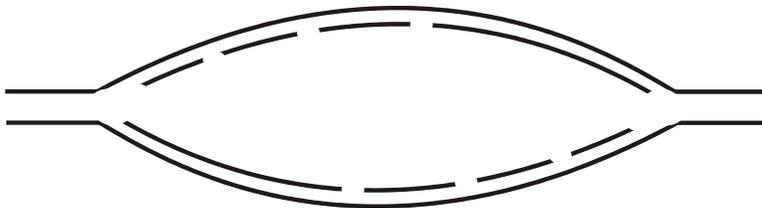
6. What are some of the types of information encoded in DNA?

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

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.)

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.

Figures removed due to copyright reasons.

Please see figure 9 and the left portion of figure 10 in

[http://hshgp.genome.washington.edu/teacher\\_resources/modules-view.htm](http://hshgp.genome.washington.edu/teacher_resources/modules-view.htm)

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

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

~~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.*

Figures removed due to copyright reasons.

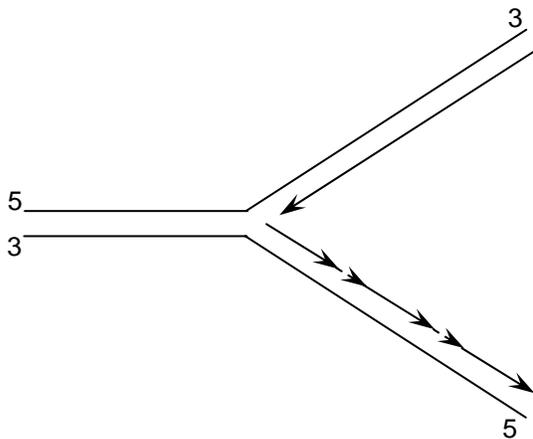
3. What enzyme is responsible for replicating DNA?

*DNA Polymerase.*

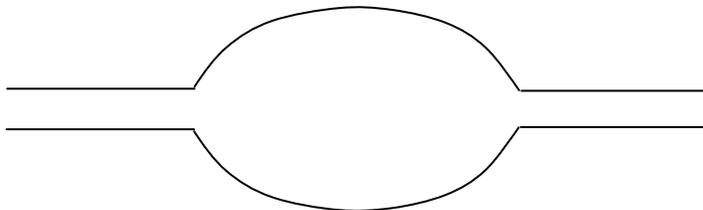
4. How does that enzyme know what base to put in next?

*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?

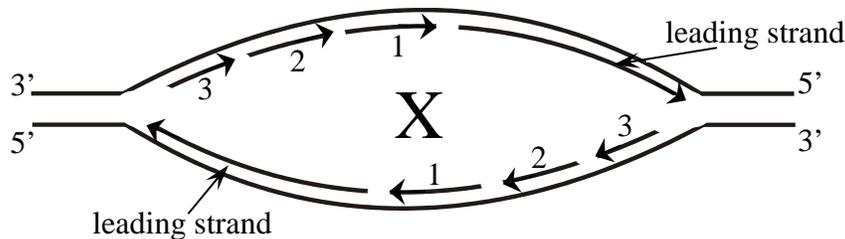
*The information is essentially written in different languages that are recognized by different machinery.*

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.



- indicate direction of DNA synthesis with arrows.
- label the ends of the original strands with 5' and 3'.
- indicate the origin of replication with an X.
- label the leading strands
- 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 a 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  $3' \rightarrow 5'$  might have been less energetically favorable for the cell overall.

*Because the energy for polymerization is coming from the phosphates, if polymerization was happening  $3' \rightarrow 5'$ , 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  $5' \rightarrow 3'$  polymerization, in a case where a base is wrong for some reason, only that base is wasted.*