Hello everybody, exam two is likely right around the corner for all of you, so best of luck on studying! Depending on your professor, chapters 12 and/or 13 may be on your third exam. Best of luck as you prepare, and enjoy this resource!

Remember: the Tutoring Center offers free individual and group tutoring for this Genetics. Our Group Tutoring sessions will be Tuesdays from 5:15-6:15 PM at the Sid Rich basement, room 75! You can reserve a spot at https://baylor.edu/tutoring. I hope to see you there!

**Keywords:** Replication, semi-conservative, replication fork

**Key Concept in Molecular Biology:** The Central Dogma

\[
\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}
\]

The *central dogma* of molecular biology describes the flow from genetic information in a cell. DNA is the molecular storehouse of genetic code within a cell. DNA will replicate any time a cell passes through S-phase on its way to replication. Information encoded in DNA is transcribed to RNA which in turn is directly translated or assists with the translation of proteins.

*Note:* sometimes RNA will replicate, or will be reverse-transcribed (as may be seen with many viruses. Additionally, some other types of RNA may be transcribed or created that are not directly involved with translation*

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Additional images come from Campbell Biology (Pearson Education)*
**Topic of the Week: DNA Replication Models (12.2-4)**

**Origin of Replication:** the location where DNA polymerase and associated proteins bind to initiate DNA replication (ori)

**Replicon:** DNA that replicates from a single origin of replication

**Prokaryotic Models:** models of DNA replication seen on circular DNA

**Theta Replication:** DNA unwinds at a single origin of replication; replication forks on either side form with the single-stranded templates from each parent strand; replication will radiate out from either fork until two semi-conservative daughters are formed.

![Theta Replication Diagram](image)

** Rolling Circle Replication:** A single stranded break leaves a free 3’-OH group on one end of the cut and a 5’-Phosphate (P) on the other end; dNTPs are added to the 3’ end and the original strand unrolls like a spool of yarn; The original broken strand may be freed and serve as another DNA template.

![Rolling Circle Replication Diagram](image)

**Eukaryotic (Linear) Model:** models of DNA replication seen on linear chromosomes

DNA replicates from many origins of replication; Replication forks expand out linearly until two meet each other, giving long stretches of new DNA.

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Requirements for Replication:

**Template:** a template of single-stranded DNA (i.e., DNA must be unwound & separated)

**Raw Materials:** dNTPs are needed to be added to the free 3’-OH of the growing chain

**Enzymes:** needed to read, assemble, alter and join the DNA strands which are formed

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**Highlight #1: The Meselson Stahl Experiment (12.1)**

Theoretical Patterns of DNA Replication:

**Conservative Replication:** The original copy of DNA is copied each time and is completely conserved; new strands are copies and contain *none* of the ‘parental’ strand

**Dispersive Replication:** The original copy of DNA is

**Semiconservative Replication:** The original copy of DNA is split into two halves by breaking H-bonds, separating the strands. Each DNA strand acts as a *template* for DNA replication. Thus, each daughter has one *newly synthesized* and one parent strand.

**Meselson and Stahl:** a pair of scientists that showed that DNA replicated *semiconservatively*

Cells were raised in “heavy” \(^{15}\)N medium. Some cells were spun in a centrifuge and some were removed and placed into a new container with \(^{14}\)N medium. After a single replication, they were spun in a centrifuge.

<table>
<thead>
<tr>
<th>Conservative</th>
<th>Dispersive</th>
<th>***Semi-Conservative</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Conservative Diagram" /></td>
<td><img src="image" alt="Dispersive Diagram" /></td>
<td><img src="image" alt="Semi-Conservative Diagram" /></td>
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Above: these are the expected outcomes of the possible ‘pellets’ formed by the centrifugation of the DNA samples. As noted with three stars (***)}, semiconservative was the only form that Meselson and Stahl observed when they completed their experiment. Thus, they concluded that DNA replication must be semiconservative.

Highlight #2: The Replication Bubble (12.3)

Direction of Replication:
DNA polymerase adds dNTPs to the new strand (complementary to the template). dNTPs are added in the 5'→3' direction (ie we add to the 3'-OH)
The linkage from the dehydration synthesis of the 5' phosphate and the 3'-OH is a phosphodiester bond (right)

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Continuous: the section of the template exposed from the fork in the 3’ → 5’ direction which can be continually synthesized (leading strand)
Discontinuous: the section of the template exposed in the 5’ → 3’ direction. DNA-pol must ‘jump’ backwards and replicate small fragments and then repeat this process (lagging strand)
Note: the leading and lagging strands switch at the origin of replication
RNA Primer: DNA-pol needs existing DNA to bind dNTPs to. RNA primers allow replication to start by using special RNA-polymerases to create short strands complementary to the template strand. These primers are converted to DNA and DNA polymerases begin synthesis (ie. Thus, a newly synthesized strand must begin with a primer)

Comparison of Eukaryotic and Prokaryotic Replication:

Prokaryotes:

Initiation: initiator proteins bind to (ori) and begins unwinding (helicase binds)
Unwinding: helicase ‘unzips’ DNA helix by disrupting H-bonds between bases moving in the 5’→3’ direction on the lagging strand.
Single strand binding proteins (SSBs) bind DNA and prevent re-annealing. DNA Gyrase cuts, untwists and reloins DNA downstream from either replication fork to decrease torsional strain of supercoiling.
Elongation: one single primer is needed on the leading strand; each 5’ end of an Okazaki fragment needs a primer
Okazaki Fragments: segments of discontinuous DNA synthesized on the lagging strand; DNA ligase joins the disjointed Okazaki fragments into a continuous stretch of new DNA.
DNA primase binds to helicase and forms RNA primers; DNA-pol I replaces RNA with DNA nucleotides; DNA-pol III catalyzes the addition of dNTPs to the growing strands of new DNA
Termination: termination occurs when 2 ends of replication bubbles meet.
Eukaryotes:

**Initiation:** eukaryotic initiation requires “licencing” -or protein recognition- of each ori, then 2 Licensing Factors and protein ORC join to form active helicases

**Unwinding:** helicase ‘unzips’ DNA helix by disrupting H-bonds between bases

**Elongation:** one single primer is needed on the leading strand; each 5’ end of an Okazaki fragment needs a primer. Eukaryotic cells have many types of

**DNA-pol:** here are the important types:
- **DNA-pol α:** has primase activity; creates RNA primer followed by a short stretch of DNA
- **DNA-pol δ:** completes replication of the lagging strand
- **DNA-pol ε:** replicates the leading strand
- **DNA ligase:** joins the disjointed Okazaki fragments into a continuous stretch of new DNA.

**Termination:** termination occurs when 2 replication bubbles meet

**Telomeres:** the ends of linear DNA in highly proliferating cells are replicated by an enzyme called telomerase (see the linked video for more info!)

https://www.youtube.com/watch?v=2NS0jBPurWQ

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**Highlight #3: Overview of Transcription (13.2)**

**Transcription:** the synthesis of RNA from a DNA template using an RNA-polymerase

**FYI:** transcription will use a DNA-Dependent RNA-Polymerase. However, RNA replication would have an RNA-dependent RNA-polymerase, meaning it relies on an RNA template to synthesize RNA.

**Structure:** RNA is transcribed complementary and antiparallel to DNA; RNA synthesis occurs in the 5’ → 3’ direction.

**Template Strand:** only one of the two strands of DNA will be transcribed, meaning it is bound by RNA-pol and will be complementary to (coding/antisense strand)

**Non-template strand:** the DNA strand not copied by RNA-pol which bears the same sequence as the transcribed RNA, but with T’s instead of U’s (non-coding/sense strand)

**Substrates:** the nucleotide monomers joined by the RNA-pol

**Ribonucleoside triphosphate (rNTP)**
**Above:** transcription unit, or the components of the section transcribed by RNA-pol

- **Promoter:** the region of DNA to which the transcription apparatus (RNA-pol complex and associated proteins) binds
- **Transcription Start Site:** location where the first nucleotide is transcribed
- **RNA Coding Region:** the entire section of DNA transcribed by RNA-pol
- **Terminator:** the DNA sequence which causes DNA-pol to dissociate or causes cleavage of RNA transcript
- **Transcription Termination site:** the spot where transcription is completed

**Notations:**
- **Upstream:** away from terminator towards promoter
- **Downstream:** away from promoter towards terminator

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**THINGS YOU MAY STRUGGLE WITH:**

1. DNA replication will **only** happen in the 5’ → 3’ direction; that is to say, DNA replication only occurs when we add to the free 3’-OH. Thus, the 5’ end will not change but the 3’ end will **elongate**.

2. If a DNA-pol has a number, it is bacterial polymerase; if it has a greek letter, it is a eukaryotic.

3. What is the activity of DNA-pol I vs. DNA-pol III?
   a. DNA-pol III is a 5’ → 3’ polymerase and 3’ → 5’ exonuclease. This means that it can add nucleotides in the correct 5’ → 3’ direction, but can also **reverse** to correct errors.
   b. DNA-pol I is a 5’ → 3’ polymerase and 3’ → 5’ exonuclease, but **also** a 5’ → 3’ exonuclease. It has the same abilities as DNA-pol III, but the added 5’ → 3’ exonuclease property gives it the ability to remove RNA bases and replace them with DNA bases.

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**Week 7 Concept Check:**

1. What type of DNA polymerase is less processive, but has 5’→3’ exonuclease capabilities?
   a. DNA-pol I
   b. RNA-pol II
   c. RNA-pol III
   d. DNA-pol III

2. A mutation in a gene coding for DNA-pol δ causes an inactive form. What part of DNA replication would be most affected in a **Eukaryotic organism**?
   a. Leading strand primer formation
   b. Ori licencing
c. Lagging strand polymerization
d. It depends..

3. What does telomerase do? What type of cells might have telomerase? How might a mutation of telomerase affect clonal evolution (cancer development)? (see the video!)

4. Which strand am I: the template runs 3’→5’
a. What/which enzyme(s) is/are going to be more active on me

5. A certain mutation to the promoter prevents everything ________ from it, meaning that the RNA transcript will not be formed
   a. Upstream
   b. Across the river
   c. Across
   d. Downstream

You Try: Click the link to apply your knowledge!

Practice Drawing (Replication Bubble):
https://docs.google.com/drawings/d/1EOJxiH_Ftk7BTD5xXAh4apr5_4St9bgYBZEO75k-TR8/edit?usp=sharing

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CONGRATS; You made it to the end of the resource! Again, group tutoring will be every Tuesday from 5:15-6:30 PM. You can reserve a spot at https://baylor.edu/tutoring. I hope to see you there!

Key:
1. A. DNA-pol-1
2. C. Lagging strand polymerization
3. Telomerase extends the end of chromosomes
   a. Steps:
      i. G-rich 3’ overhang is extended
      ii. C-rich 5’ undehang is expanded using the RNA component of telomerase
      iii. RNA is turned into DNA
   b. Cells:
   c. Stem cells/marrow cells, germ cells, anything rapidly dividing such as cancer cells
   d. Clonal Evolution
      i. Telomerase increases the fitness of cancer cells, granting them essentially indefinite replication
4. Lagging strand
   a. DNA ligase
   b. [Prokaryotes] Helicase and primase form a complex on the lagging strand (primase is going to be very active though, be it has to keep creating new okazaki fragments)
   c. [Eukaryotes] DNA-pol-δ
5. D. downstream

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