

DNA Replication Machinery

Dr: Luu Phuc Loi, PhD.

Goals:

- Describe why the process of DNA replication is more complicated within a cell (in vivo) versus an in vitro assay.
- Demonstrate how the replication fork and its associated proteins overcome the difficulties of DNA replication within a cell.

Objectives:

After completing the materials for this unit, you should be able to:

- Label the leading strand, lagging strand, and polarity of the DNA in a replication fork.
- Describe how the structural properties of DNA helicase facilitate DNA unwinding.
- Predict helicase polarity given results from a helicase unwinding assay.
- List the properties of single-stranded binding proteins that support replication fork maturation.
- Define positively and negatively supercoiled DNA and recognize their relative positions of migration in an agarose gel.
- Compare and contrast properties of type I and type II topoisomerases.
- Diagram how the DNA polymerase III holoenzyme proceeds with replication.
- Summarize the trombone model of replication.
- Determine the best experimental assay to test a given hypothesis.
- Interpret data from DNA replication and DNA topology experiments.
- Analyze protein structures to infer functional information.

Replication in Living Cells

1. Limitations of Artificial Models

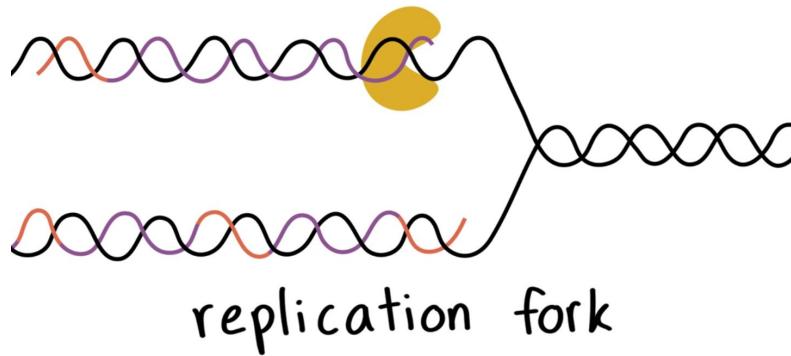
- Lab experiments often use synthetic primers and single-stranded circular DNA.
- Living cells cannot "order" primers; they must synthesize them internally.

2. Biological Challenges

- Single-stranded DNA (ssDNA) is fragile and prone to chromosomal breaks.
- The cell must minimize ssDNA exposure to preserve genomic integrity.

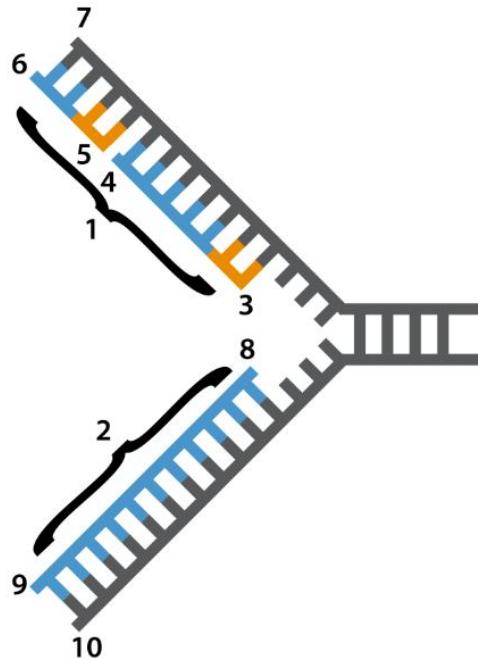
3. The Molecular Machine at the Replication Fork

- **Simultaneous Replication:** Both leading and lagging strands are synthesized at the same time.
- **Okazaki Fragments:** The solution for replicating the 3' to 5' template while polymerases only move 5' to 3'.
- **Protein Coordination:** Enzymes work together as a highly synchronized molecular machine.



Before Professor Bell discusses the replication fork, review what you remember from an Introductory Biology course like 7.00x. In this diagram, newly synthesized RNA is shown in orange, and newly synthesized DNA is shown in blue. Keep in mind that this image only shows a portion of the replication fork at a specific point in time and that the DNA strands would continue to the left and right.

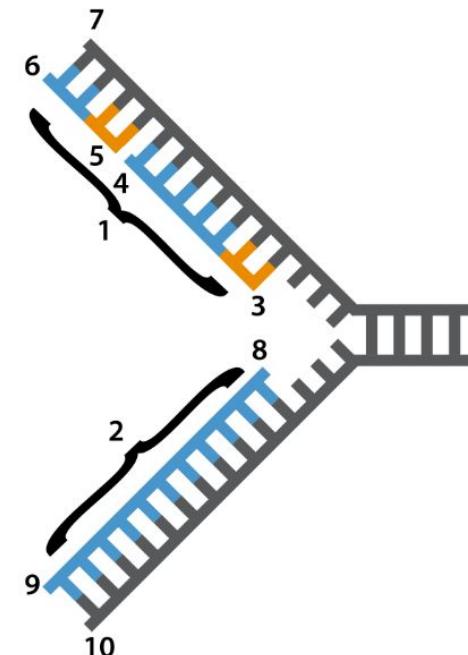
Using the dropdown options, identify which numbers in the image correspond to the leading and lagging strands and label all the 5' and 3' ends. Then describe the direction of replication.



1. Select an option strand
2. Select an option strand
3. Select an option prime end
4. Select an option prime end
5. Select an option prime end
6. Select an option prime end
7. Select an option prime end
8. Select an option prime end
9. Select an option prime end
10. Select an option prime end

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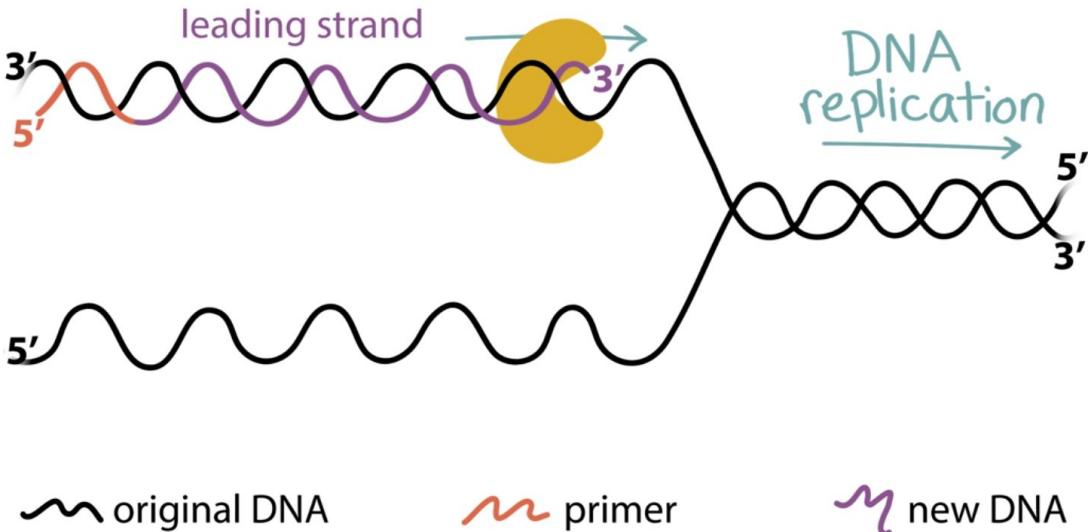


1. lagging strand
2. leading strand
3. 5' prime end
4. 3' prime end
5. 5' prime end
6. 3' prime end
7. 5' prime end
8. 3' prime end
9. 5' prime end
10. 3' prime end

The Replication Fork and Associated Proteins

Leading Strand Polymerase:

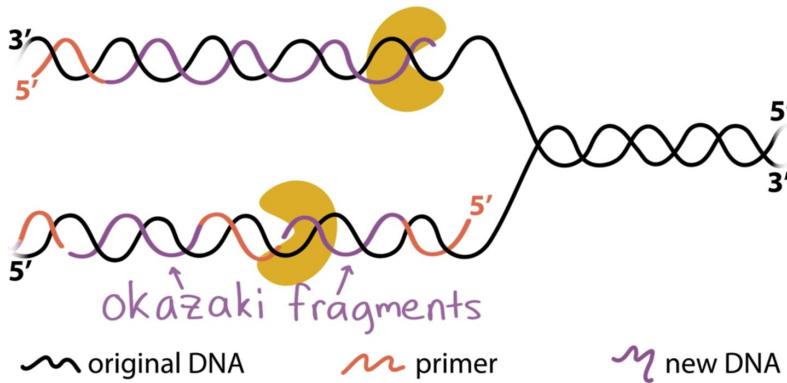
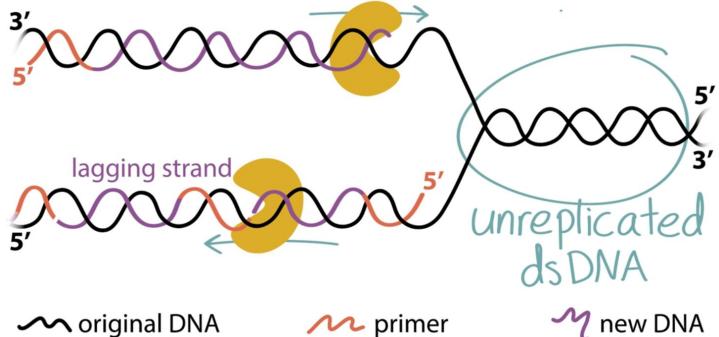
- Moves in the same direction as the overall replication fork.
- Highly efficient; typically requires only one primer at the start.



The Replication Fork and Associated Proteins

Lagging Strand Polymerase:

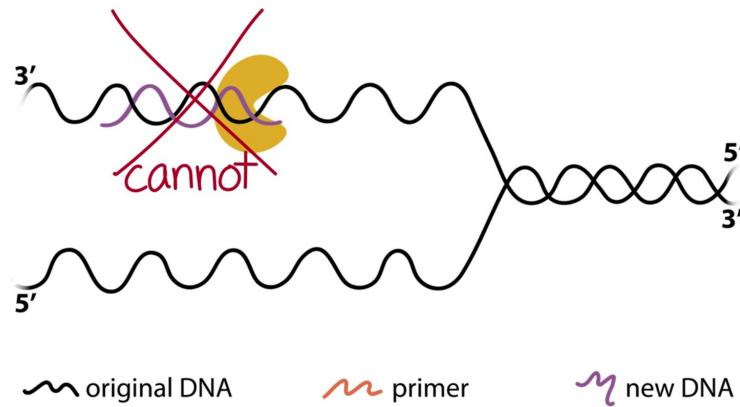
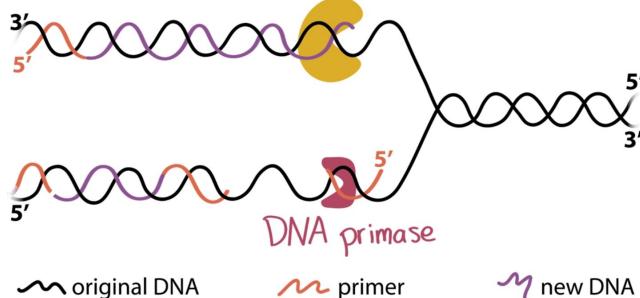
- Moves away from the replication fork direction.
- Synthesizes DNA in small chunks known as **Okazaki fragments**.



The Replication Fork and Associated Proteins

Primase (A Specialized RNA Polymerase)

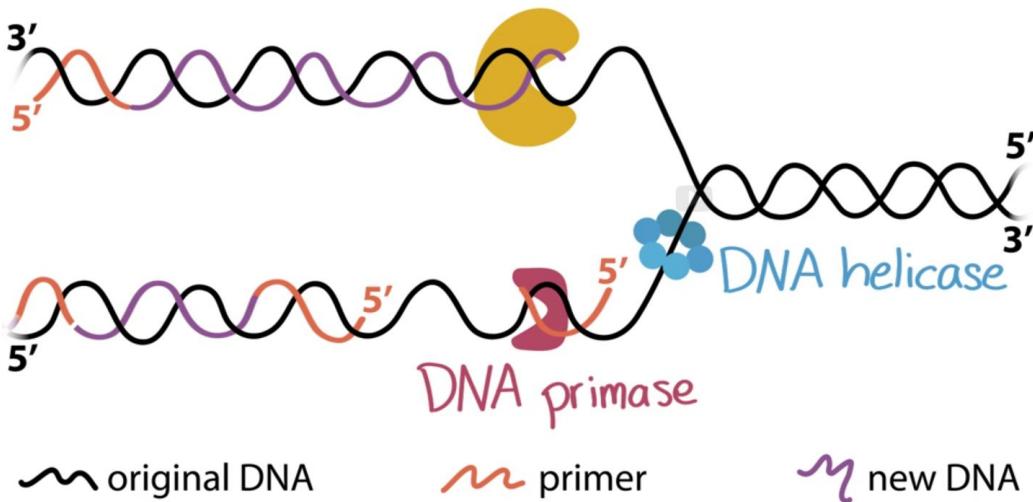
- **Function:** Synthesizes short RNA primers (6-8 base pairs long).
- **Essentiality:** DNA Polymerase **CANNOT** start a new strand from scratch; it requires a pre-existing primer:template junction.
- **Processivity:** Very low; specifically designed to fall off after making a short primer.



The Replication Fork and Associated Proteins

DNA Helicase and Enzyme Coordination

- **Helicase:** Responsible for unwinding the double-stranded DNA.
- **Activation:** Primase is activated by interacting with DNA Helicase. This ensures priming only occurs at the replication fork to prevent genomic instability.



Test Yourself: The Replication Fork and Associated Proteins

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Test Yourself: The Replication Fork and Associated Proteins

1.0/1.0 point (graded)

Which of the following are accurate statements about replication forks in living cells? Select all that apply.

- DNA polymerase extends the 5' end of the lagging strand during DNA synthesis unlike the mechanism of leading strand synthesis.
- The leading strand DNA polymerase always synthesizes DNA in the same direction as the overall direction of replication for that relevant replication fork.
- DNA polymerase can only start replication from an RNA primer:template junction.
- Only lagging strand DNA synthesis requires a primer.

Test Yourself: The Replication Fork and Associated Proteins

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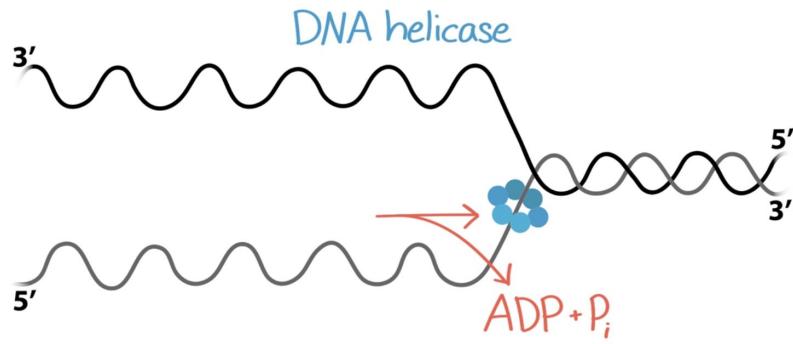
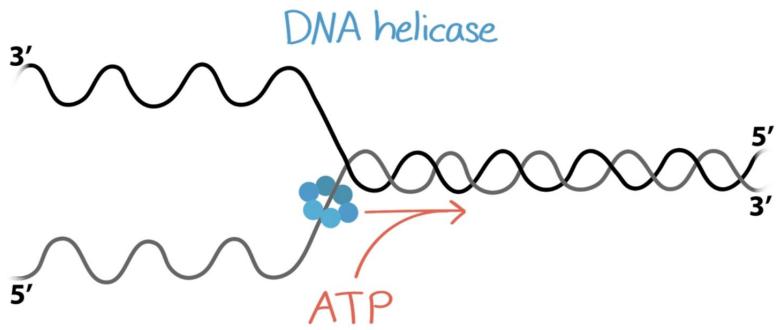
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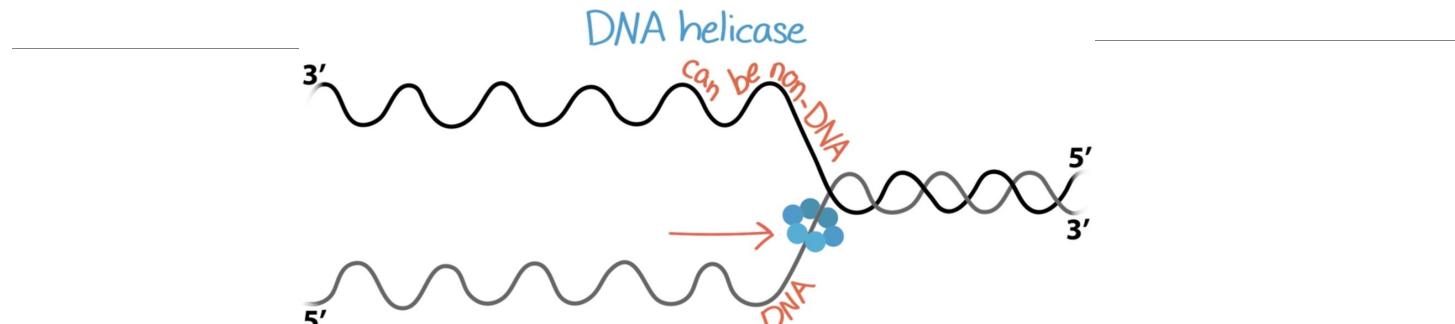
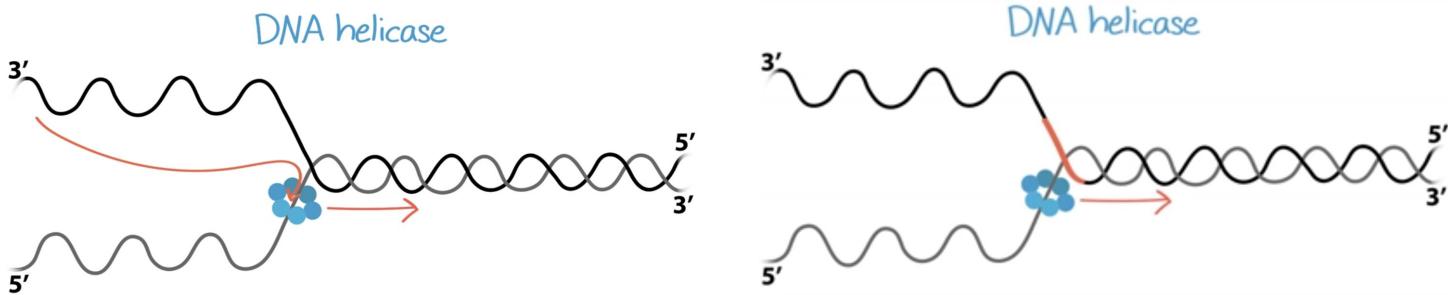
Explanation

DNA polymerase always extends DNA from the 3' end. For lagging strand synthesis, DNA synthesis occurs in the opposite direction from the overall direction of replication for that relevant replication fork. That is why DNA polymerase must replicate to a stopping point, then start again in a position closer to the replication fork. The leading strand DNA polymerase does always synthesize DNA in the same direction as the movement of the replication fork. DNA polymerase starts DNA synthesis from an RNA primer:template junction when inside a cell, however, keep in mind that DNA polymerase frequently starts replication from a DNA primer:template junction in experiments outside of the cell. DNA synthesis always requires a primer:template junction to start synthesis.

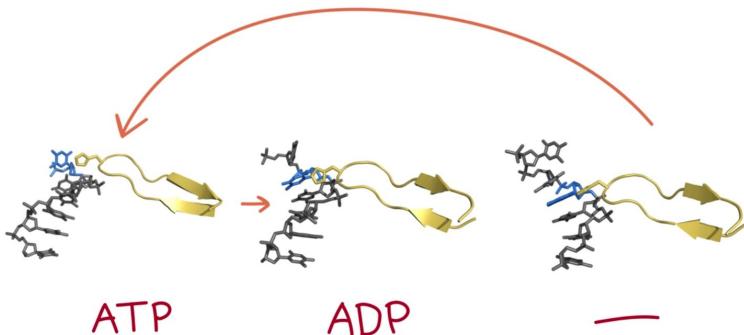
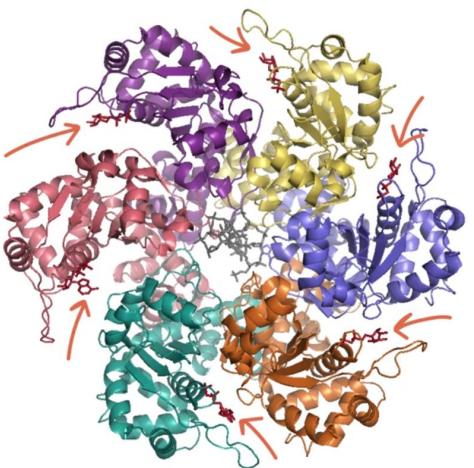
DNA Helicase: The Unwinding Engine



DNA Helicase: The Unwinding Engine



DNA Helicase: The Unwinding Engine



Test Yourself: DNA Helicase

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Select option

1. double-stranded/ single-stranded
2. different/ the same
3. binding and hydrolysis/ hydrolysis only

Test Yourself Part A: DNA Helicase

0.0/1.0 point (graded)

We can learn a lot about the function of a protein from the structure of that protein. Based on the structural properties of the replicative DNA helicase, use the dropdown options to complete the sentences describing the enzyme's activity.

1

- The replicative helicase initiates activity when bound to DNA.
- All six subunits are in state(s) of nucleotide binding throughout the process of separating the strands of DNA.
- ATP 3 is/are key to the replicative helicase translocating across the DNA.

Test Yourself: DNA Helicase

 Bookmark this page

Test Yourself Part A: DNA Helicase

1.0/1.0 point (graded)

We can learn a lot about the function of a protein from the structure of that protein. Based on the structural properties of the replicative DNA helicase, use the dropdown options to complete the sentences describing the enzyme's activity.

- The replicative helicase initiates activity when bound to ✓ Answer: single-stranded DNA.
- All six subunits are in ✓ Answer: different state(s) of nucleotide binding throughout the process of separating the strands of DNA.
- ATP ✓ Answer: binding and hydrolysis is/are key to the replicative helicase translocating across the DNA.

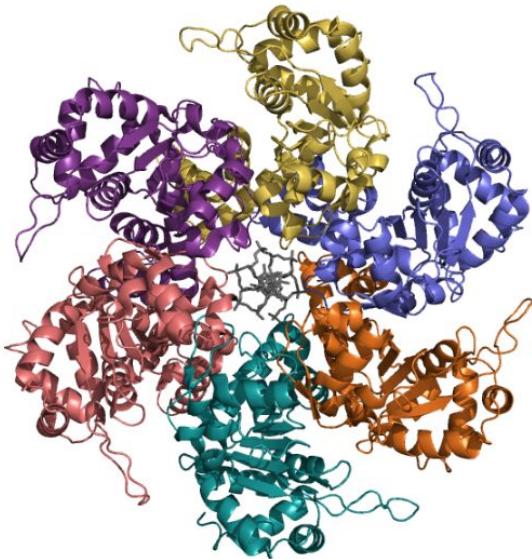
Explanation

The diameter of the central channel (or pore) of the replicative helicase is about 13 Å, while the diameter of double-stranded DNA is about 20 Å. Therefore, the structure supports the fact that the replicative helicase encircles single-stranded DNA not double-stranded DNA. Additionally, the structure supports the model of each subunit cycling through the stages of bound to ATP, bound to ADP, and unbound to a nucleotide as a mechanism for movement. The structure also indicates that the subunits move in response to ATP hydrolysis, but the subunit moves even more in response to ATP binding.

Test Yourself Part B: DNA Helicase

0.0/1.0 point (graded)

This is a representation of the structure of a replicative DNA helicase. The hexameric helicase protein complex is shown in cartoon form, and each subunit of the hexamer is shown in a different color. The single-stranded DNA in the center is shown in gray, and in stick form.



Think about what you know about the structure of a polypeptide chain at the atomic level. You may want to revisit your introductory biology notes or [7.00x Introduction to Biology](#).

Examining the cartoon representation of the helicase protein subunits in the image, what parts of the polypeptide chain are represented in the cartoon form of the structure? Select the best answer.

the backbone

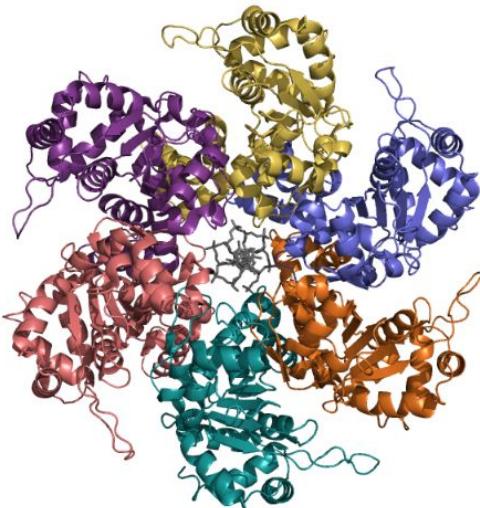
the sidechains

both backbone and sidechains

Test Yourself Part B: DNA Helicase

1.0/1.0 point (graded)

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the backbone

the sidechains

both backbone and sidechains



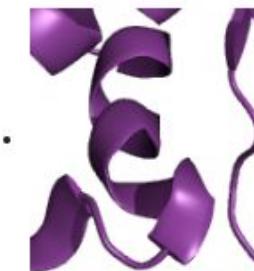
Explanation

In cartoon representations of protein structure, the backbone of the polypeptide is illustrated, and the side chains are hidden.

Test Yourself Part C: DNA Helicase

0.0/1.0 point (graded)

The replicative helicase contains alpha helices and beta sheets in its secondary structure. Use the dropdown options to identify the correct secondary structure in each of the views of the helicase shown.



Select an option ▾

1

Select option

1 -> 3: alpha helix/beta sheet



Select an option ▾

2



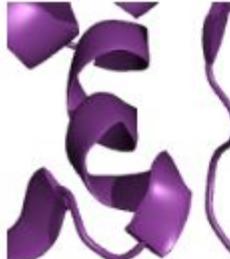
Select an option ▾

3

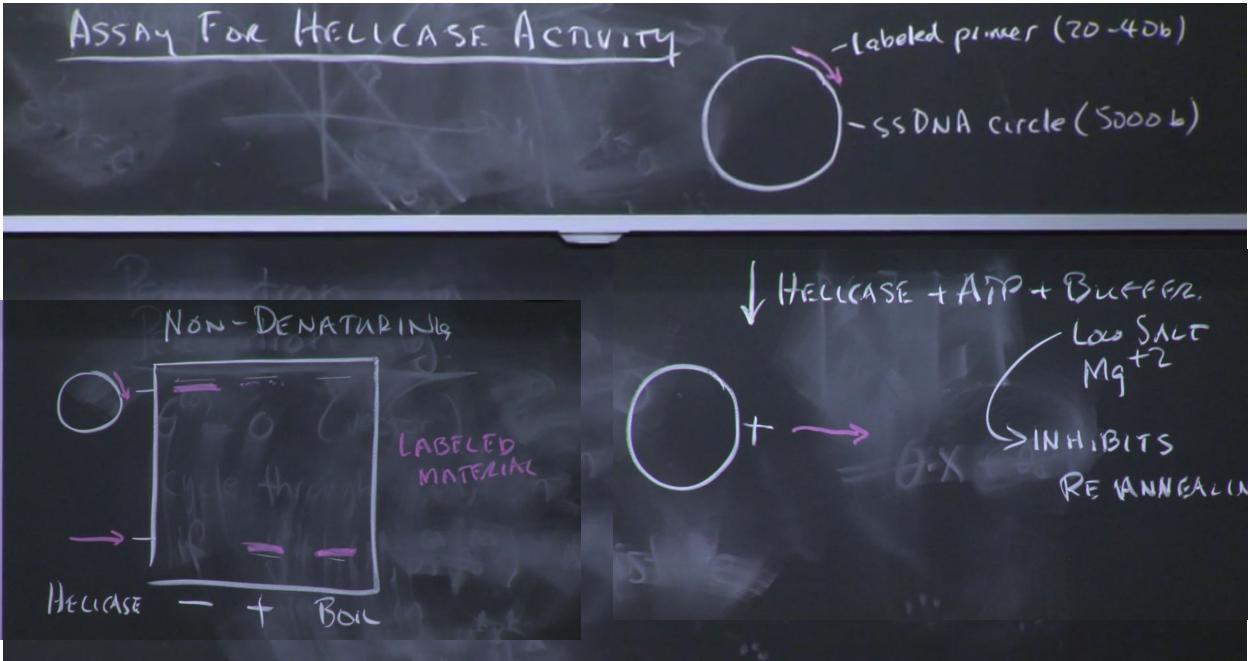
Test Yourself Part C: DNA Helicase

1.0/1.0 point (graded)

The replicative helicase contains alpha helices and beta sheets in its secondary structure. Use the dropdown options to identify the correct secondary structure in each of the views of the helicase shown.

- 
• ✓
- 
• ✓
- 
• ✓

A Basic Helicase Assay for DNA Unwinding



Test Yourself: Helicase Assay for DNA Unwinding

 Bookmark this page

Test Yourself: Helicase Assay for DNA Unwinding

0.0/1.0 point (graded)

You completed a replicative helicase assay for DNA unwinding, but your buffer had a high salt concentration.

What will you observe when you view your gel under UV? Select the best answer.

- You will observe signal for both the primer bound to the template and the primer alone with or without helicase activity.
- You will observe signal for the primer bound to template but not for the primer alone even in the presence of helicase activity.
- You will observe signal for the primer alone even in the absence of helicase activity.

Test Yourself: Helicase Assay for DNA Unwinding

 Bookmark this page

Test Yourself: Helicase Assay for DNA Unwinding

1.0/1.0 point (graded)

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You will observe signal for the primer bound to template but not for the primer alone even in the presence of helicase activity.

You will observe signal for the primer alone even in the absence of helicase activity.



Explanation

A high salt concentration minimizes the repulsive forces between the negative phosphate backbones of the DNA strands. Thus, a high salt concentration will promote re-annealing between the separated strands of DNA after helicase activity. This will result in the absence of or a significant decrease in 'primer alone' signal on your gel.

Other Helicase Properties and Assays

Definition: The unidirectional movement of Helicase along ssDNA, classified as either 5' -> 3' or 3' -> 5'.

Binding Requirement: Helicases only bind to single-stranded DNA (ssDNA) due to the narrow central channel of their ring structure.

Biological Polarity:

- **Bacteria:** Moves 5' -> 3' along the lagging strand template.
- **Eukaryotes:** Moves 3' -> 5' along the leading strand template.

Polarity Determination Assay:

- Uses a substrate with two labeled primers of unequal lengths (e.g., 20bp and 40bp) at opposite ends.
- **3' to 5' Enzyme:** Specifically displaces the longer (40bp) primer fragment.
- **5' to 3' Enzyme:** Specifically displaces the shorter (20bp) primer fragment.

Test Yourself: Helicase Polarity

 [Bookmark this page](#)

Test Yourself: Helicase Polarity

0.0/1.0 point (graded)

Which of the following changes in a helicase polarity assay protocol would cause the results to be inconclusive? Select all that apply.

- Before the restriction enzyme digest of your circular DNA with bound primer, you started out using a primer that is 5'-end labeled.
- Your reaction only includes a lagging strand DNA helicase.
- You run the reactions on a denaturing gel instead of a non-denaturing gel.
- The short, labeled DNA strands bound to the long strand are of equal length.
- You fluorescently label the DNA instead of radiolabel.
- You label the long strand of DNA rather than the short strands bound to the long strand.

Test Yourself: Helicase Polarity

1.0/1.0 point (graded)

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The short, labeled DNA strands bound to the long strand are of equal length.

You fluorescently label the DNA instead of radiolabel.

You label the long strand of DNA rather than the short strands bound to the long strand.



Explanation

Think about these scenarios and the answer that you gave. In the proper helicase polarity assay, DNA helicase only binds single-stranded DNA, you run the reactions on a non-denaturing gel, and you label both of the short strands of different lengths with either fluorescence or radiolabel.

If your initial circular DNA with bound primer includes a 5'-labeled primer, then you will not be able to visualize the other short strand of DNA in the helicase assay.

If your reaction only includes a lagging strand DNA helicase, then you will determine that the DNA helicase has 5' to 3' polarity.

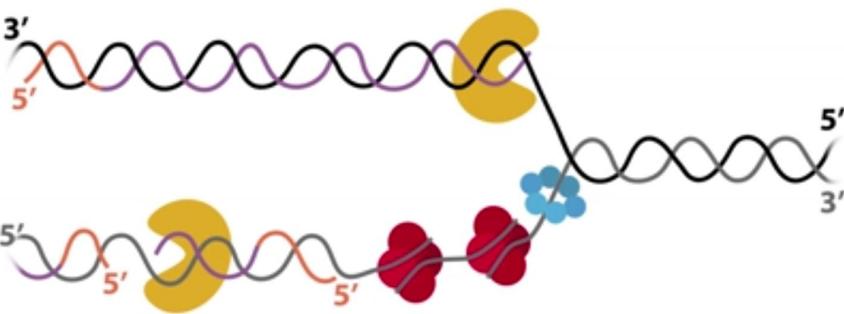
If you run the reactions on a denaturing gel instead of a non-denaturing gel, then all of the short stands of DNA will separate from the long strand of DNA. You will see both short products on the gel image and cannot determine which one separated because of helicase activity.

If the short, labeled DNA strands bound to the long strand are of equal length, then you will see the same results for a 5' to 3' helicase or a 3' to 5' helicase on the gel image.

If you label the long strand of DNA rather than the short strands bound to the long strand, then you will never see either short strand on the gel image and cannot determine the polarity of the helicase of interest.

Single-Stranded Binding Protein

- **Definition and Roles:**
 - **SSB (in E. coli) or RPA (in Eukaryotes):** A critical protein that stabilizes single-stranded DNA (ssDNA) at the replication fork.
 - **Prevents Reannealing:** Keeps separated DNA strands from immediately snapping back together or forming complex secondary structures.
- **Key Biological Properties:**
 - **High Affinity for ssDNA:** Specifically binds to single strands, showing no affinity for double-stranded DNA.
 - **Cooperative Binding:** Once one SSB molecule binds, it facilitates the rapid, adjacent binding of additional SSB molecules to cover the entire ssDNA stretch.
- **Coordination with DNA Polymerase:**
 - **Accessibility:** SSB protects the DNA while leaving the nitrogenous bases exposed for replication.
 - **Rapid Displacement:** As DNA Polymerase synthesizes the new strand, SSB is efficiently displaced.
 - **Species Specificity:** Effective displacement often requires direct interaction between the polymerase and SSB from the same species.



Test Yourself: Single-Stranded Binding Protein (SSB)

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Test Yourself Part A: Single-Stranded Binding Protein (SSB)

0.0/1.0 point (graded)

Use the dropdown options to complete the description of single-stranded binding proteins.

1

SSB proteins have sequence specificity for binding, are more prevalent on the strand template, and has the highest affinity for 3

2

Select option

1. low/ high
2. lagging/ leading
3. dsDNA/ ssDNA bound to SSB proteins/ unbound ssDNA.

Test Yourself Part A: Single-Stranded Binding Protein (SSB)

1.0/1.0 point (graded)

Use the dropdown options to complete the description of single-stranded binding proteins.

SSB proteins have



Answer: low sequence specificity for binding, are more prevalent on the

lagging



Answer: lagging strand template, and has the highest affinity for



Answer: ssDNA bound to SSB proteins.

Explanation

SSBs bind to long stretches of ssDNA and prevent them from reannealing with the other template DNA strand. DNA replication would be negatively impacted if SSBs bound specifically to certain sequences but not to others. SSBs protect ssDNA from cleavage. Lagging strand synthesis is carried out in the opposite direction by Okazaki fragment synthesis and repair and thus takes much longer so more lagging strand template is in ssDNA form for longer periods of time. ssDNA is much more fragile than dsDNA. Thus, SSBs are more prevalent on lagging strand template than leading strand template. SSB proteins preferentially bind ssDNA over dsDNA. SSBs have a much higher affinity to ssDNA already bound to SSB and will bind cooperatively. So once one binds, it is easier for a second to bind. This is likely due to enhanced protein-protein interactions between SSBs bound to ssDNA.

Test Yourself Part B: Single-Stranded Binding Protein (SSB)

0.0/1.0 point (graded)

Which roles do the SSB proteins serve at the replication fork? Select all that apply.

- impedes DNA polymerase
- activates primase
- prevents reannealing of leading and lagging strand templates
- prevents secondary structure formation within the lagging strand ssDNA

Test Yourself Part B: Single-Stranded Binding Protein (SSB)

1.0/1.0 point (graded)

Which roles do the SSB proteins serve at the replication fork? Select all that apply.

impedes DNA polymerase

activates primase

prevents reannealing of leading and lagging strand templates

prevents secondary structure formation within the lagging strand ssDNA



Explanation

There is no experimental evidence supporting an interaction with primase. SSBs do not interfere with replication and therefore do not impede DNA polymerase. SSBs do, however, prevent reannealing of the two template strands and the formation of secondary structures forming within the ssDNA of the lagging strand if there are complementary sequences.

Topoisomerase and Topological Problems of the Replication Fork

The Topological Challenge:

- Unwinding DNA strands creates excessive tension and "overwinding" ahead of the replication fork.
- Without intervention, this torsional strain stops the replication fork after only a few hundred base pairs.

DNA Linking and Structure:

- **Optimal State:** 10.4 base pairs (bp) per turn of the helix.
- **Overwound (Positive Supercoiling):** Less than 10.4 bp per turn (e.g., 8 bp/turn), leading to tightly strained DNA.
- **Underwound (Negative Supercoiling):** Greater than 10.4 bp per turn (e.g., 20 bp/turn).

Function of Topoisomerase:

- Acts as a "therapist" for DNA by relaxing suboptimal linkage.
- It unwinds overwound DNA or rewinds underwound DNA to restore the relaxed, optimal state.
- Essential for providing the energy relief needed for continuous replication.

Test Yourself: Topoisomerase and Topological Problems of the Replication Fork

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Test Yourself: Topoisomerase and Topological Problems of the Replication Fork

0.75/1.0 point (graded)

Use the dropdown options to select the best terms to describe each situation.

1

2

3

4

- Double-stranded DNA with less than 10.4 base pairs per turn of the helix is and
- Double-stranded DNA with more than 10.4 base pairs per turn of the helix is and

Select option

1. overwound/underwound
2. negatively/positively
3. overwound/underwound
4. negatively/positively

Test Yourself: Topoisomerase and Topological Problems of the Replication Fork

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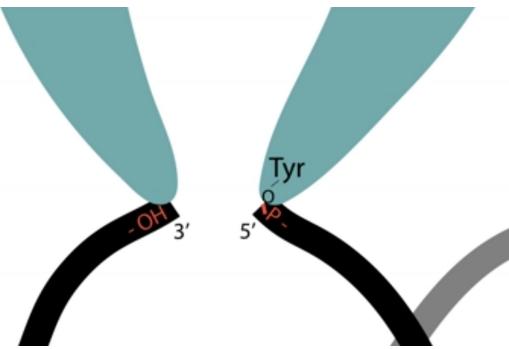
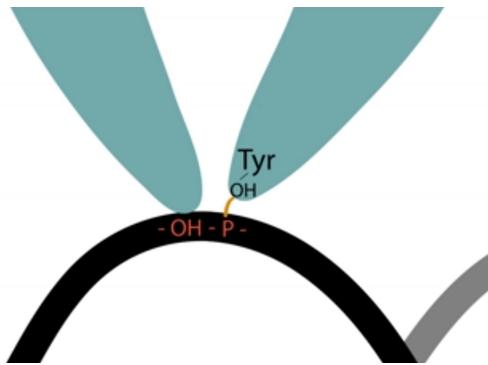
Test Yourself: Topoisomerase and Topological Problems of the Replication Fork

1.0/1.0 point (graded)

Use the dropdown options to select the best terms to describe each situation.

- Double-stranded DNA with less than 10.4 base pairs per turn of the helix is ✓ and ✓ and .
- Double-stranded DNA with more than 10.4 base pairs per turn of the helix is ✓ and ✓ and .

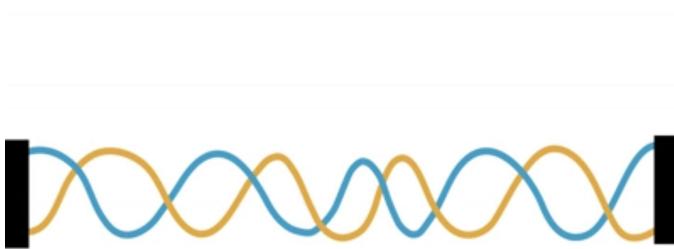
Types of Topoisomerases and Mechanisms



Types of Topoisomerases and Mechanisms

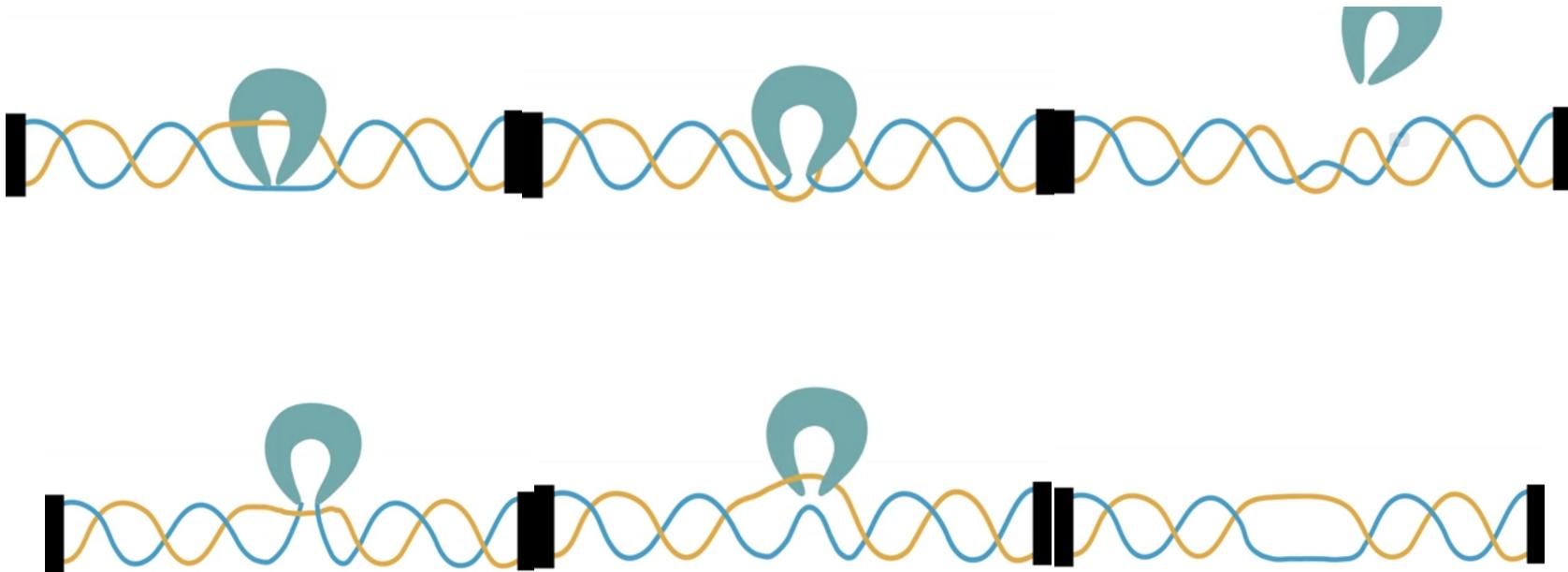


pre-topoisomerase

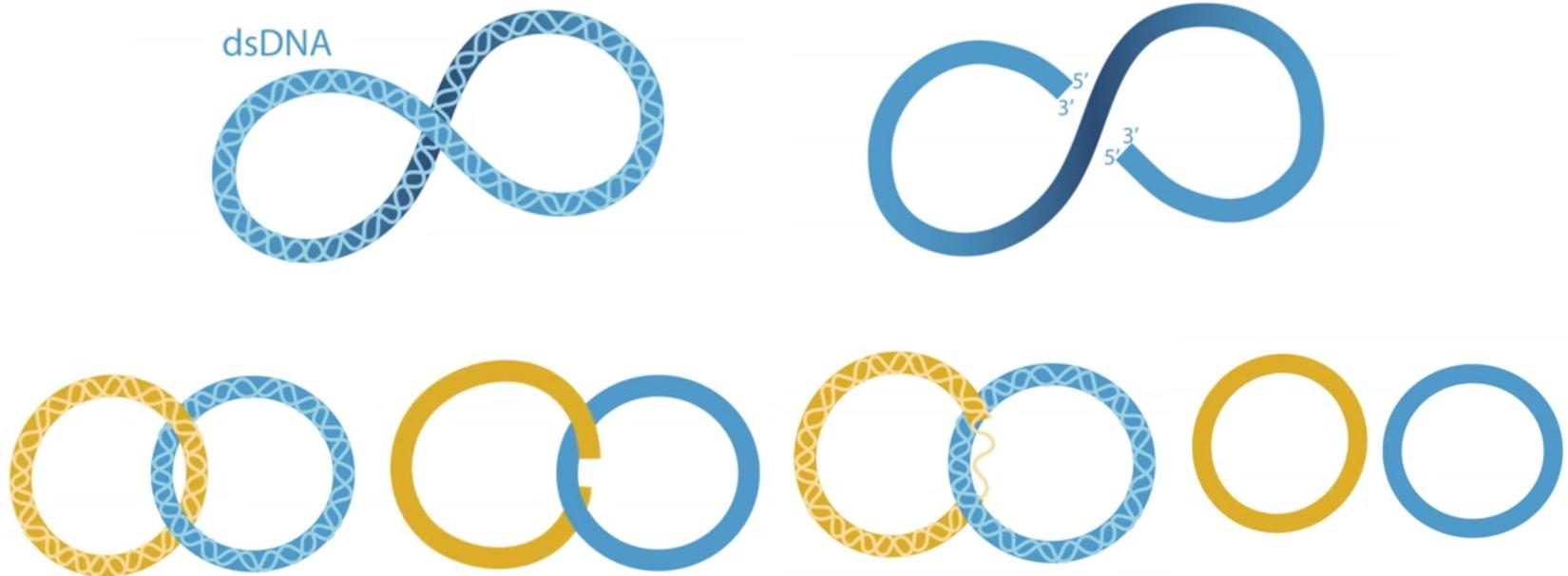


post-topoisomerase

Types of Topoisomerases and Mechanisms



Types of Topoisomerases and Mechanisms



Types of Topoisomerases and Mechanisms

Molecular Mechanism:

- Utilizes a **Tyrosine** residue to break DNA strands, forming a covalent bond with the \$5'\$ phosphate.
- **Energy Neutrality:** Type I enzymes function without ATP, driven entirely by the torsional energy stored in DNA.

Type I Topoisomerase:

- Breaks **one strand** and passes another strand through the break.
- Changes the linking number by increments of **+1**
- Features a dual-chambered structure to facilitate strand passage.

Type II Topoisomerase:

- Breaks **two strands** (double-strand break) and passes a duplex through.
- Changes the linking number by increments of **+2**
- **Unique Role:** Essential for **decatenating** interlocked DNA rings (catenanes) formed after replication.

Test Yourself: Types of Topoisomerases and Mechanisms

 Bookmark this page

Test Yourself Part A: Types of Topoisomerases and Mechanisms

0.0/1.0 point (graded)

If you incubate a covalently closed circular double-stranded piece of DNA (14.4 base pairs per turn of the helix) with a type I topoisomerase, what will happen to the DNA? Use the dropdown options to describe the outcome.

1

The ▼

2

DNA will ▼

Select option

1. overwound/ underwound/ unstrained
2. remain at the current linkage level/ rewind/ unwind.

Test Yourself Part A: Types of Topoisomerases and Mechanisms

1.0/1.0 point (graded)

If you incubate a covalently closed circular double-stranded piece of DNA (14.4 base pairs per turn of the helix) with a type I topoisomerase, what will happen to the DNA? Use the dropdown options to describe the outcome.

The

✓ Answer: underwound DNA will

✓ Answer: rewind.

Explanation

Unstrained DNA has 10.4 base pairs per turn of the helix. A piece of DNA with 14.4 base pairs per turn is underwound, and will rewind to 10.4 if a single strand cut (also called a nick) is introduced by type I topoisomerase.

Test Yourself Part B: Types of Topoisomerases and Mechanisms

0.0/1.0 point (graded)

If you treat a covalently closed circular double-stranded piece of DNA (8.6 base pairs per turn of the helix) with a type I topoisomerase, what will happen to the DNA? Use the dropdown options to describe the outcome.

The

DNA will



1

2

Select option

1. overwound/ underwound/ unstrained
2. remain at the current linkage level/ rewind/ unwind.

Test Yourself Part B: Types of Topoisomerases and Mechanisms

1.0/1.0 point (graded)

If you treat a covalently closed circular double-stranded piece of DNA (8.6 base pairs per turn of the helix) with a type I topoisomerase, what will happen to the DNA? Use the dropdown options to describe the outcome.

The

✓ Answer: overwound DNA will

✓ Answer: unwind.

Explanation

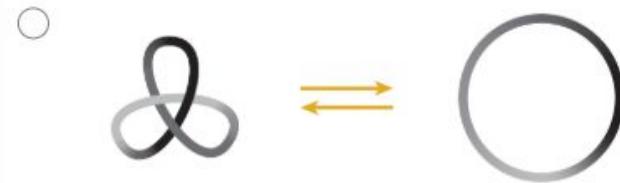
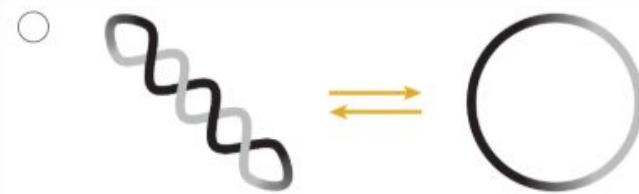
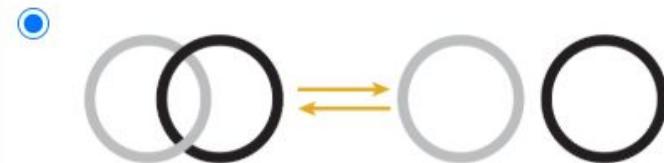
Unstrained DNA has 10.4 base pairs per turn of the helix. A piece of DNA with 8.6 base pairs per turn is overwound, and will unwind to 10.4 if a nick is made. Type I topoisomerases nick double-stranded DNA and unwind or rewind DNA in an energetically neutral fashion (with the exception of reverse gyrase, which is introduced in the next video segment). The enzyme will unwind overwound DNA and rewind underwound DNA.

Test Yourself Part C: Types of Topoisomerases and Mechanisms

1.0/1.0 point (graded)

You work in a lab studying DNA rearrangements. You are specifically interested in studying the activity of human type I topoisomerases like topoisomerase I. If you treated the following DNA products on the left with human topoisomerase I for a short time, which of the DNA rearrangements would topoisomerase I catalyze?

Note that each black or gray line represents a **double-stranded DNA double helix**.

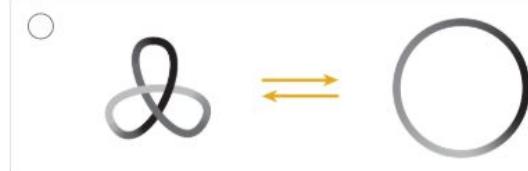
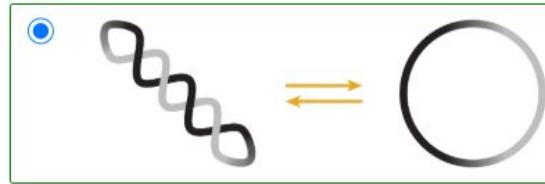
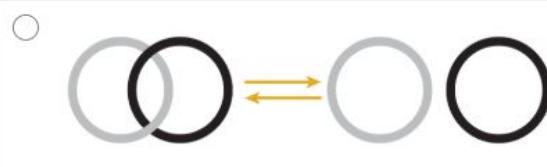


Test Yourself Part C: Types of Topoisomerases and Mechanisms

1.0/1.0 point (graded)

You work in a lab studying DNA rearrangements. You are specifically interested in studying the activity of human type I topoisomerases like topoisomerase I. If you treated the following DNA products on the left with human topoisomerase I for a short time, which of the DNA rearrangements would topoisomerase I catalyze?

Note that each black or gray line represents a **double-stranded DNA double helix**.



Explanation

Type I topoisomerases cut a single strand of double-stranded DNA and relax supercoils, so the correct answer is the image depicting a supercoiled covalently closed double helix that becomes un-supercoiled while remaining covalently closed. Type I topoisomerases do not decatenate or unknot DNA, which requires cutting both strands of double-stranded DNA.

A Type II Topoisomerase - DNA Gyrase

DNA Gyrase in Bacteria:

- A specialized Type II topoisomerase that actively consumes energy (ATP or NADH) to function.
- **Primary Function:** Introduces **negative supercoils**, causing the DNA to become underwound.

Biological Significance:

- Underwound DNA is more easily unwound by the replication machinery.
- Facilitates essential processes such as replication initiation and transcription.

Adaptation in Thermophiles:

- Utilize "anti-gyrase" (Reverse Gyrase) to add **positive supercoils** to their genome.
- This process prevents the DNA from melting (denaturing) at extreme temperatures, such as 95 degrees Celsius

Energetic Cost: Unlike Type I enzymes, these require energy to force the DNA into a non-natural, strained configuration.

Test Yourself: A Type II Topoisomerase - DNA Gyrase

 Bookmark this page

Tip

If you are unsure of the answer to this question, you may also want to watch the next segment on DNA topology, too, before answering. Then come back to try this problem.

Test Yourself: A Type II Topoisomerase - DNA Gyrase

1.0/1.0 point (graded)

Select all of the features that correctly describe the properties of type I and type II topoisomerases.

- Most Type I topoisomerases require an energy source (such as ATP or NADH).
- Most Type II topoisomerases require an energy source (such as ATP or NADH) for efficient enzyme activity.
- Type I topoisomerases change the linking number in steps of 1, while Type II topoisomerase change the linking number in steps of 2.
- Type II topoisomerases change the linking number in steps of 1, while Type I topoisomerase change the linking number in steps of 2.
- The type II DNA gyrase from *E. coli* unwinds relaxed DNA.

Test Yourself: A Type II Topoisomerase - DNA Gyrase

 Bookmark this page

Tip

If you are unsure of the answer to this question, you may also want to watch the next segment on DNA topology, too, before answering. Then come back to try this problem.

Test Yourself: A Type II Topoisomerase - DNA Gyrase

1.0/1.0 point (graded)

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Type II topoisomerases change the linking number in steps of 1, while Type I topoisomerase change the linking number in steps of 2.

The type II DNA gyrase from *E. coli* unwinds relaxed DNA.



Explanation

Some key differences between Type I and Type II topoisomerases are energy requirements and the change in linking number as a result of action. Most type I topoisomerases do not require ATP.

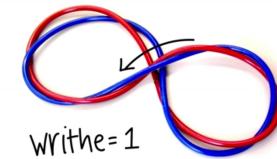
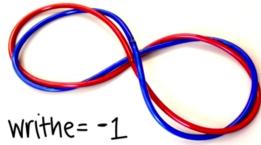
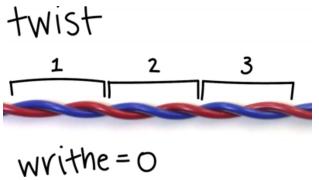
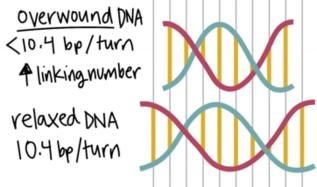
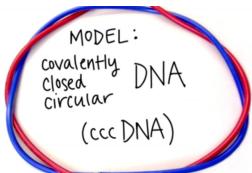
DNA Topology

DNA TOPOLOGY



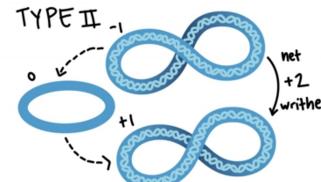
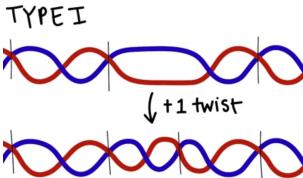
"relaxed" DNA
10.4 bp/turn

twist + writhe = linking number



TOPOISOMERASES

unwind overwound DNA
(\uparrow bp/turn) (\downarrow linking number)
rewind underwound DNA
(\downarrow bp/turn) (\uparrow linking number)



TOPOISOMERASES

TYPE I

~~ATP~~

TYPE II

+ATP/NADH

(REVERSE) GYRASE

Test Yourself: DNA Topology

 [Bookmark this page](#)

Test Yourself Part A: DNA Topology

1.0/1.0 point (graded)

How do topoisomerases help a cell operate smoothly? Select all that apply.

allow DNA to be replicated over long distances

prevent single-stranded DNA from reannealing

stabilize DNA in thermophilic organisms

Test Yourself Part A: DNA Topology

1.0/1.0 point (graded)

How do topoisomerases help a cell operate smoothly? Select all that apply.

allow DNA to be replicated over long distances

prevent single-stranded DNA from reannealing

stabilize DNA in thermophilic organisms



Explanation

By relieving overwound DNA downstream of DNA replication, topoisomerases allow these processes to proceed past several hundred nucleotides. Without topoisomerases, tension would be too high downstream of polymerases for DNA polymerase to proceed. In thermophilic organisms, the high heat of the environment relaxes DNA. This is balanced by topoisomerase (reverse gyrase), which increases winding of the DNA to prevent it from denaturing. Single-stranded binding proteins, not topoisomerases, prevent reannealing of single-stranded DNA.

Test Yourself Part B: DNA Topology

0.0/1.0 point (graded)

Use the dropdown options to complete the sentences about DNA topology and topoisomerases.

- Two replication forks approaching each other would have ¹ in between the forks.
- Type I topoisomerases break ² strand(s) of DNA, using (in most cases) ³
- Gyrases, which are ⁴ topoisomerases found in ⁵ change DNA linking number by ⁶ and ⁷ restore DNA to the relaxed state.

Select option

1. overwound/ underwound
2. one/ two
3. ATP or NADH / energy stored in the DNA
4. type I/ type II
5. Prokaryotes/ Eukaryotes
6. 0/ 1/ 2
7. do not/ do

Test Yourself Part B: DNA Topology

1.0/1.0 point (graded)

Use the dropdown options to complete the sentences about DNA topology and topoisomerases.

- Two replication forks approaching each other would have in between the forks.
- Type I topoisomerases break strand(s) of DNA, using (in most cases)
- Gyrases, which are topoisomerases found in change DNA linking number by and restore DNA to the relaxed state.

Test Yourself Part C: DNA Topology

0.0/1.0 point (graded)

It is more intuitive to understand why circular DNA becomes constrained during cellular processes. One would think linear DNA could just rotate to relieve tension. Describe the main reason you think the replication of eukaryotic DNA would still require topoisomerases. Any thoughtful answer will receive credit; click the Show Answer button to check your thoughts.

Test Yourself Part C: DNA Topology

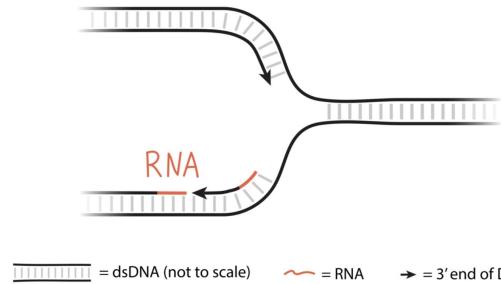
1.0/1.0 point (graded)

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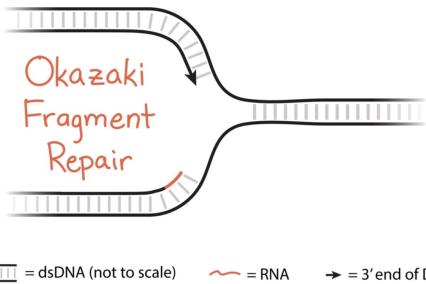
Explanation

It is true that short linear DNA can largely rotate to prevent overwinding of DNA. However, the extreme length of eukaryotic chromosomes effectively fixes the ends of the linear DNA, preventing free rotation.

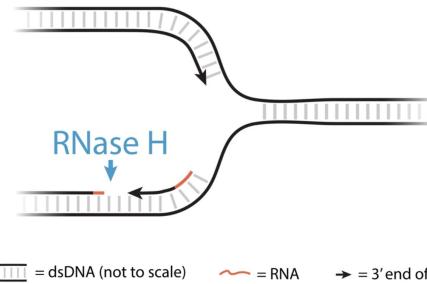
Okazaki Fragment Repair



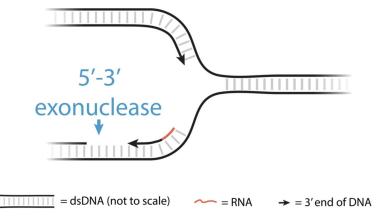
= dsDNA (not to scale) = RNA → = 3' end of DNA



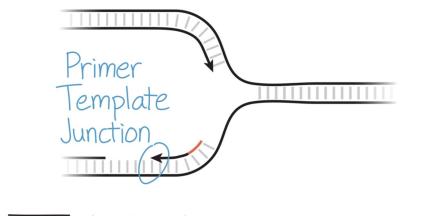
Okazaki
Fragment
Repair



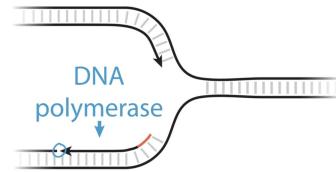
RNase H



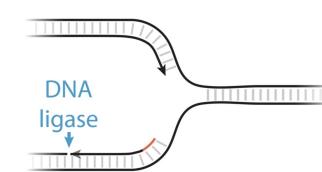
5'-3'
exonuclease



Primer
Template
Junction



DNA
polymerase



DNA
ligase

Test Yourself: Okazaki Fragment Repair

 [Bookmark this page](#)

Test Yourself: Okazaki Fragment Repair

1.0/1.0 point (graded)

Think about the role of enzymes in Okazaki fragment repair. How many different enzymes are normally required in Okazaki fragment repair? Enter your answer as a whole number.

Test Yourself: Okazaki Fragment Repair

 Bookmark this page

Test Yourself: Okazaki Fragment Repair

1.0/1.0 point (graded)

Think about the role of enzymes in Okazaki fragment repair. How many different enzymes are normally required in Okazaki fragment repair? Enter your answer as a whole number.

4

 Answer: 3 or 4

Explanation

RNAse H degrades the RNA primers that are hybridized to DNA in the Okazaki fragments. A 5' to 3' exonuclease degrades the final RNA/DNA junction at the 5' end. A DNA polymerase synthesizes DNA to fill in the gap, while DNA ligase catalyzes phosphodiester bond formation at the nicks.

Different DNA Polymerases

	exonuclease	# subunits	processivity	function
DNA Pol I	5' to 3' 3' to 5'	1	low (10-100)	Okazaki fragment repair
DNA Pol II	3' to 5' (proofreading)	1	low	DNA repair
DNA Pol IV	none	1	very low (2-4)	bypass repair
DNA Pol V	none	1	very low	bypass repair
DNA Pol III Core	3' to 5'	3	low (10-100)	see ↓
DNA Pol III Holoenzyme	3' to 5'	10	very high ($>10^5$)	chromosome replication

Test Yourself: Different DNA Polymerases

 Bookmark this page

Test Yourself Part A: Different DNA Polymerases

0.0/1.0 point (graded)

Organisms have multiple DNA polymerases, each specialized for particular roles in the cell. What would be the important characteristics for the Replicative DNA polymerase (DNA Pol III holoenzyme in *E. coli*)? Select all that apply.

highly processive

highly permissive

possessing 3' to 5' exonuclease activity

possessing 5' to 3' exonuclease activity

increased expression under stress conditions

Test Yourself Part B: Different DNA Polymerases

0.0/1.0 point (graded)

What would the important characteristics be for the Okazaki Fragment Repairing DNA Polymerase (DNA Pol I holoenzyme in *E. coli*)? Select all that apply.

highly processive

highly permissive

possessing 3' to 5' exonuclease activity

possessing 5' to 3' exonuclease activity

increased expression under stress conditions

Test Yourself: Different DNA Polymerases

 Bookmark this page

Test Yourself Part A: Different DNA Polymerases

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possessing 5' to 3' exonuclease activity

increased expression under stress conditions



Explanation

Replicative DNA polymerases synthesize the majority of the new DNA created during replication. Processivity, the number of nucleotides added before the polymerase dissociates, is important so that the DNA polymerase can replicate large tracts of the chromosome. Accuracy is also important to prevent the large-scale production of mutations, so replicative DNA polymerases contain 3' to 5' proofreading exonuclease activity.

Test Yourself Part B: Different DNA Polymerases

1.0/1.0 point (graded)

What would the important characteristics be for the Okazaki Fragment Repairing DNA Polymerase (DNA Pol I holoenzyme in *E. coli*)? Select all that apply.

highly processive

highly permissive

possessing 3' to 5' exonuclease activity

possessing 5' to 3' exonuclease activity

increased expression under stress conditions



Explanation

A DNA polymerase with a role involving maturation of Okazaki fragments would need to be accurate (thus the 3' to 5' exonuclease activity), but not processive, as this DNA polymerase is covering the short distance between Okazaki fragments. 5' to 3' exonuclease activity allows the DNA polymerase I to remove the remaining RNA nucleotide monophosphate of the Okazaki strand RNA primer from the ahead of it as this polymerase polymerizes DNA to replace the RNA primer (sometimes called nick translation).

The DNA Polymerase III Holoenzyme

Holoenzyme Components:

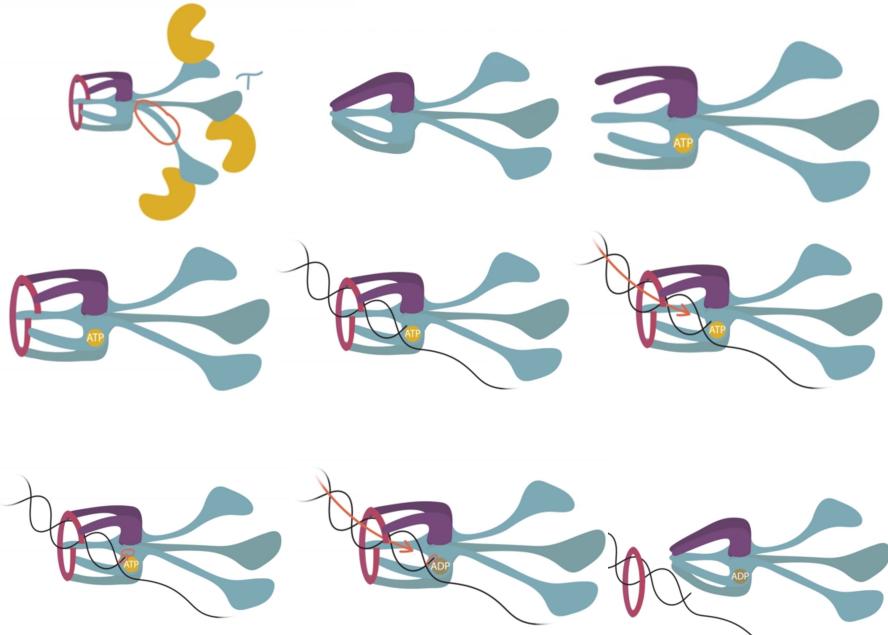
- **3 Pol III Core Enzymes:** One for the leading strand and two for the lagging strand.
- **Sliding Clamp Loader Complex:** Composed of three **tau** (τ) proteins (linking the cores), **delta** (δ), and **delta prime** (δ').

Sliding DNA Clamps:

- Ring-shaped structures (e.g., **beta** subunit in *E. coli*, **PCNA** in humans).
- **Function:** Act as processivity factors that slide along double-stranded DNA, preventing the polymerase from dissociating.

Clamp Loading Mechanism (ATP-driven):

1. **ATP Binding:** Induces a conformational change to open the sliding clamp ring.
2. **Targeting:** Specifically binds to **primer:template junctions** where dsDNA passes through the ring.
3. **ATP Hydrolysis:** Triggers the release of the loader and the closing of the clamp around the DNA.



Test Yourself: The DNA Polymerase III Holoenzyme

 Bookmark this page

Test Yourself: The DNA Polymerase III Holoenzyme

0.0/1.0 point (graded)

Which of the following statements accurately describe the DNA Polymerase III holoenzyme? Select all that apply.

- The sliding DNA clamp is a multimeric protein.
- The sliding DNA clamp enhances the processivity of DNA polymerase.
- The DNA sliding clamp encircles only single-stranded DNA.
- The sliding DNA clamp helps recruit and maintain other DNA replication enzymes to the replication fork through physical interactions with them.

Test Yourself: The DNA Polymerase III Holoenzyme

 Bookmark this page

Test Yourself: The DNA Polymerase III Holoenzyme

1.0/1.0 point (graded)

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- The sliding DNA clamp helps recruit and maintain other DNA replication enzymes to the replication fork through physical interactions with them.



Explanation

The sliding DNA clamp is a dimer in *E. coli*; or a trimer in other organisms. The sliding DNA clamp encircles double-stranded DNA at the primer:template junction. The sliding DNA clamp interacts with Okazaki repair enzymes, chromosome assembly proteins, and DNA repair enzymes.

Enzymes at the Bacterial Replication Fork

Enzymatic Cooperativity:

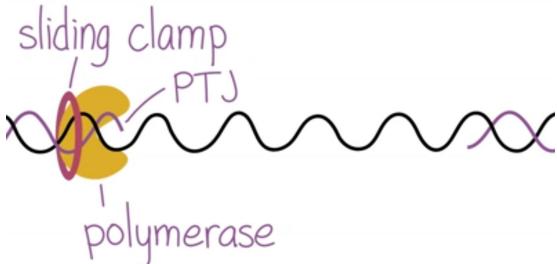
- **Tau (τ) Subunit \leftrightarrow Helicase:** Stimulates helicase activity. If Pol III stalls, the helicase slows down, ensuring the replication fork stays coordinated.
- **Helicase \leftrightarrow Primase:** Primase activity is specifically triggered by its proximity to the helicase.

Sliding Clamps as Processivity Enhancers:

- **Physical Tethering:** Prevents Pol III from diffusing away after dissociating from the primer:template junction.
- **Efficiency:** Dramatically increases processivity from <100 bp to >50,000 bp by forcing immediate re-engagement with the template.

Dynamic Affinity:

- The clamp maintains a high-affinity grip on Pol III while a single-stranded template is present.
- Polymerase is rapidly released only upon completion of the double-stranded DNA segment.



Test Yourself: Enzymes at the Bacterial Replication Fork

 Bookmark this page

Test Yourself: Enzymes at the Bacterial Replication Fork

0.0/1.0 point (graded)

The proteins at the replication fork work together in *E. coli* to facilitate DNA replication. Which of the following relationships accurately reflect the collaboration between proteins at the replication fork? Select all that apply.

- The τ (tau) subunit of DNA polymerase III holoenzyme binds to DNA helicase and stimulates DNA helicase activity.
- The τ (tau) subunit of DNA polymerase III holoenzyme binds to DNA primase and stimulates DNA primase activity.
- DNA primase stimulates the activity of DNA helicase.
- DNA helicase stimulates the activity of DNA primase.
- The strength of DNA Pol III binding to the DNA sliding clamp varies depending on the DNA substrate.

Test Yourself: Enzymes at the Bacterial Replication Fork

 Bookmark this page

Test Yourself: Enzymes at the Bacterial Replication Fork

1.0/1.0 point (graded)

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DNA primase stimulates the activity of DNA helicase.

DNA helicase stimulates the activity of DNA primase.

The strength of DNA Pol III binding to the DNA sliding clamp varies depending on the DNA substrate.



Explanation

DNA Pol III strongly binds to the clamp at a primer:template junction and weakly binds to the clamp at sites of double-stranded DNA or nicks in the DNA.

The Trombone Model of DNA Replication

The DNA Looping Mechanism:

- The lagging strand template loops back to allow the polymerase to synthesize DNA in the same physical direction as the replication fork.
- The loop grows and shrinks periodically, mimicking the slide of a trombone.

The Three-Polymerase System:

- **One core** for continuous leading strand synthesis.
- **Two cores** for the lagging strand to eliminate lag time between Okazaki fragments.

Key Steps in the Cycle:

- **Priming:** Primase interacts with helicase to create a new primer every ~1 kb.
- **Clamp Loading:** A sliding clamp is placed at the new primer:template junction while the previous fragment is still being extended.
- **Hand-off:** The "spare" polymerase engages the new clamp immediately, preventing Okazaki fragment synthesis from becoming the rate-limiting step.

Efficiency: Enables a replication rate of 1,000 bp/sec and extreme processivity (>2 megabases per binding event).

Test Yourself: The Trombone Model of DNA Replication

 [Bookmark this page](#)

Test Yourself: The Trombone Model of Replication

0.0/1.0 point (graded)

In your own words, describe how the proteins at the replication fork work together in the *E. coli* trombone model of replication. Any thoughtful answer will receive credit; click the Show Answer button to check your thoughts.

Test Yourself: The Trombone Model of DNA Replication

 Bookmark this page

Test Yourself: The Trombone Model of Replication

1.0/1.0 point (graded)

In your own words, describe how the proteins at the replication fork work together in the *E. coli* trombone model of replication. Any thoughtful answer will receive credit; click the Show Answer button to check your thoughts.

In the trombone model, the leading strand polymerase follows the helicase as it unwinds DNA. Mean



Explanation

The leading strand polymerase follows helicase, as the first lagging strand polymerase moves away from the fork. Helicase continues to generate single-strand DNA, and primase synthesizes a new primer. The clamp loader adds a clamp. Then the second lagging strand polymerase binds the new primer:template junction/clamp to start a new Okazaki fragment. Finally, the first lagging strand polymerase completes synthesis and releases, ready for the next PTJ/clamp.

Home assignment

The Interaction of DNA with Moxifloxacin and Topoisomerase

0.0/2.0 points (graded)

Your lab is studying the mechanisms of drug resistance in bacteria with a focus on quinolones. Quinolones are a class of antibiotics that kill bacteria by increasing the number of double-strand breaks caused by type II topoisomerases. Recall that most bacteria carry two type II topoisomerases, gyrase and topoisomerase IV. Topoisomerase IV is a tetramer of two ParC and two ParE proteins. Your labmate, Alice, created a fusion protein of the bacterial ParC and ParE from *Acinetobacter baumannii*, and then crystallized the fusion protein in the presence of DNA and the quinolone moxifloxacin. Examine the structure that she determined, shown in Appendix 1, which you should already have open in a separate window.

Which of the following statements accurately describe the structure? Select all that apply.

- Moxifloxacin binds at the site of a break in the DNA strands.
- The structure shows that the catalytic tyrosine of topoisomerase IV attacking a 5'-phosphate of the DNA.
- The structure shows the ParC-ParE fusion protein in complex with uncut double-stranded DNA.
- Moxifloxacin primarily interacts with the DNA.
- The secondary structure of the active site of the ParC-ParE fusion primarily consists of beta sheets.

Home assignment

Chemical Interactions with Moxifloxacin

0.0/3.0 points (graded)

What types of significant direct interactions does moxifloxacin make with different features in the crystal structure? Use the dropdown options to identify each type of interaction moxifloxacin has with the specified component.

- DNA
- Mg^{+2}
- ParC-ParE

Magnesium Coordinating Residues

0.0/1.0 point (graded)

Two amino acids of the ParC-ParE fusion protein help the protein bind the Mg^{+2} associated with moxifloxacin. Enter these amino acids in the input fields using the three-letter amino acid code followed by the number with no spaces (example format: Arg234), one amino acid per box. Enter them in **ascending** order (lowest amino acid number first).

Save

Home assignment

Analyzing Topoisomerase Activity

Bookmark this page

Bands in Your Topoisomerase Activity Gel

0.0/2.0 points (graded)

You discover that bacteria that gain resistance to quinolone antibiotics frequently have mutations at sites within *parC* that encode amino acids that interact with the Mg^{+2} ion bound to quinolone. You decide to investigate exactly how amino acid substitutions at those positions convey resistance.

You choose a well-studied, genetically tractable organism, *Escherichia coli*, for your further experiments, and create mutant *E. coli* *parC* genes that encode S80L, S80I, S80F, and E84K substitutions (serine 80 and glutamic acid 84 being the metal chelating sites in *E. coli*), respectively (amino acid chart). All four mutant *E. coli* strains show resistance to quinolones. You purify the wild-type and mutant proteins, along with their wild-type ParE partner.

You perform an initial topoisomerase activity assay using wild-type ParC and supercoiled, covalently closed, circular plasmid DNA. You incubate the reaction components together for 30 minutes, taking samples at two time points, 0 and 30 minutes, and run your samples on an agarose gel. An image of your ethidium bromide visualized gel is shown.



Which of the highlighted bands best measures DNA relaxation and DNA cleavage, respectively? Use the dropdown menus to describe how you will examine the labeled bands in your experiment.

To measure the **relaxation** of DNA over time, you should quantify the of the orange labeled band over time.

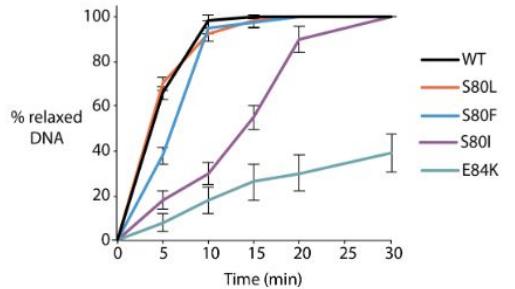
To measure the **cleavage** of DNA over time, you should quantify the of the blue labeled band over time.

Home assignment

Interpreting Topoisomerase Results

0.0/1.0 point (graded)

You want to know how your wild-type and mutant topoisomerases compare in the absence of drug. You perform your topoisomerase assay comparing the behavior of your wild-type and mutant ParC proteins in the absence of quinolone, taking samples at varying times, and quantify the gel bands from the resulting image to monitor relaxation. The data for relaxation over time are shown.



Your advisor wants you to focus on quinolone-resistant mutants that possess an otherwise **functionally wild-type** ParC protein to make your work more medically relevant. Which of the four mutants should you use? Select all that are a good choice.

 S80L S80I S80F E84K

Home assignment

Why Not this Mutant?

0.0/1.0 point (graded)

Your labmate, Rajeev, has his own mutant *E. coli* ParC protein, which has an S80R mutation. The S80R mutation confers quinolone resistance, but S80R ParC-containing topoisomerases are catalytically dead (unable to relax DNA).

Why is Rajeev's mutant a poor choice to compare to wild-type ParC if you are trying to understand topoisomerase quinolone resistance? Select all that apply.

- The pathogenic/resistant bacteria we care about medically are more likely to have a resistant **and** functional ParC, so this mutant is less medically relevant.
- The new arginine site will more tightly bind divalent metal cations, affecting other DNA replication-related processes.
- To understand how topoisomerase can continue to function in the presence of quinolone, ParC must **be** functional to begin with for comparison.
- It is never informative to compare wild-type cells with cells with null mutations (mutations that completely abolish the function of a protein).

Home assignment

Choose a Helicase Activity

0.0/1.0 point (graded)

Replication forks can collapse or fall apart in the midst of DNA synthesis, jeopardizing the viability of the cell. Sites of fork collapse are unlikely to be near origins of replication, so replication reinitiation is required independent of an origin. The primosome is the multi-subunit complex of proteins that restarts replication in this situation. Your lab is studying DNA helicase, using *E.coli* DnaB as a model protein. You decide to determine what factors are required for DnaB loading at collapsed replication forks.

You need an assay for DnaB loading. Which *in vitro* assay from the brief descriptions given could distinguish whether DnaB loads onto a given DNA substrate or not? Select the best answer.

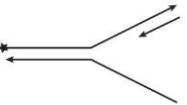
- Monitor incorporation of unlabeled dNTPs by DNA polymerase into a primer:template junction (PTJ) with a labeled primer in the presence of helicase and other factors.
- After pre-incubating DNA polymerase with labeled PTJs, start elongation by adding dNTPs, helicase, and excess unlabeled PTJ.
- Using double-stranded DNA with one labeled strand and a single-stranded region, incubate with helicase and monitor for dissociation of the labeled strand.
- Mix labeled, supercoiled, covalently, closed, circular DNA with helicase, monitoring formation of relaxed products by gel electrophoresis.

Home assignment

Predict the Outcome of Helicase Activity

0.0/1.0 point (graded)

You decide to use a helicase unwinding assay to monitor DnaB loading. You use a labeled substrate that simulates arrested replication forks (shown) and imagine how DnaB will act on the substrate. In all representations, a star indicates the radiolabeled 5' phosphate and an arrowhead represents a 3' OH.



Which diagram represents the products after DnaB acts on the substrate? Select the best answer.

-
-
-

Home assignment

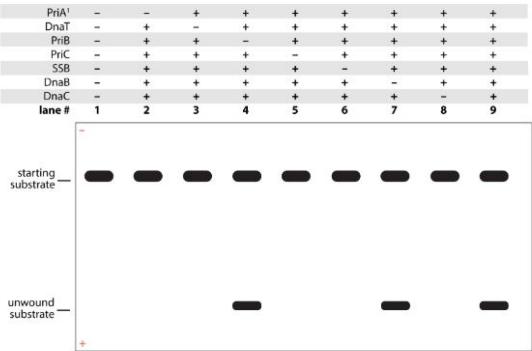
Conclusion from Your Labmate's Gel

0.0/1.0 point (graded)

In your investigation of how DnaB assembles at arrested replication forks, you research and purify proteins known to be associated with the primosome: PriA, PriB, PriC, DnaC, DnaT, SSB, and DnaB. Because the assay you intend to run depends on DnaB being the only helicase present, you use a mutant form of PriA (indicated by superscript 1) that does not have any helicase activity of its own. The steps of your assay are:

1. Incubate your labeled DNA substrate with single-stranded DNA binding protein (SSB), known to be important for arrested fork recovery) to coat ssDNA.
2. Add a mixture of primosome proteins to the substrate, setting up multiple samples with each lacking a single primosome protein.
3. Incubate the reaction components for 15 minutes at 37 °C.
4. End the reactions with addition of EDTA, SDS, and protease.
5. Separate the DNA by gel electrophoresis; detect unwound labeled products by autoradiography.

You come in to the lab the morning of your planned experiment to find that your overzealous, habitually sleep-deprived labmate, Brian, has decided to perform your assay himself overnight. Bleary-eyed, he shows you his autoradiogram, claiming that his results support that PriB is not required for helicase loading.



Besides being a bit upset that Brian did not think to ask you before using your reagents, what do you think of his data and conclusion? Select the best answer.

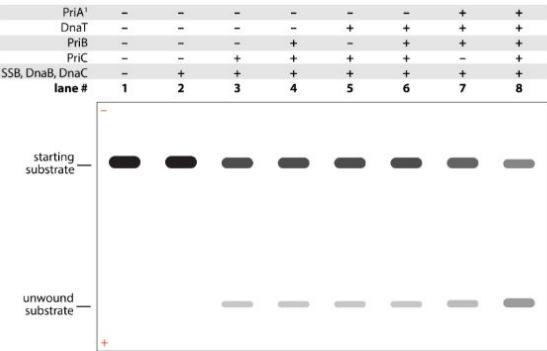
- His interpretation is correct. You got scooped!
- Brian's data is suspect because the lane for the sample lacking the helicase DnaB indicates unwinding, suggesting something is wrong.
- Brian's data is suspect because you expect to see a variety of unwinding intermediates in a helicase assay.
- Brian's conclusion is incorrect; from this data, only PriB is required for helicase loading.

Home assignment

Conclusion from Your Gel

0.0/2.0 points (graded)

Your sleepy, reagent-stealing labmate goes home to bed and you decide what to do next. You decide to slightly modify your protocol; instead of leaving out individual primosome proteins, you add them in different combinations. Your labeled autoradiogram is shown.



What conclusions can you make from your data? Select all that apply.

Note: protein names separated by slashes imply that the proteins act together for the purposes of helicase loading.

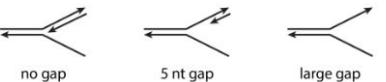
- PriC, SSB, DnaC are sufficient to load DnaB onto the substrate.
- PriC requires DnaT, SSB, and DnaC to load DnaB onto the substrate.
- PriB interferes with PriC loading DnaB onto the substrate.
- PriA, DnaT, PriB, SSB, DnaC are sufficient to load DnaB onto the substrate
- PriA/DnaT/PriB and PriC could work together to more efficiently load DnaB on the substrate than they do separately.
- PriA/DnaT/PriB and PriC could work in separate pathways to load DnaB on the substrate.

Home assignment

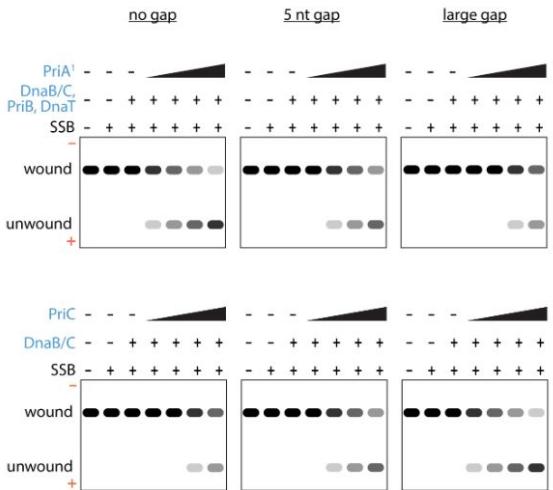
Gel Data from Helicase Experiments with Different Substrates

0.0/1.0 point (graded)

Your newest labmate, Zoe, offers to do the next experiments while you are traveling for a conference. From her reading, Zoe knows that replication forks can collapse to produce varying gap distances between the 3' end of the nascent leading strand and the replication fork. So she creates a series of DNA substrates shown that produce varying gap sizes.



Zoe repeats your helicase assay with these substrates and varying concentrations of PriA or PriC (and other associated proteins). She sends you an image of her results and asks you for your thoughts.



What substrates do PriA and PriC prefer? Use the dropdown options to describe the preference of each helicase.

PriA has a preference for substrates with no gap

PriC has a preference for substrates with large gap

gaps in the nascent leading strand.

gaps in the nascent leading strand.

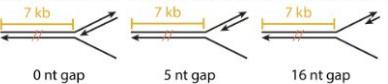
Home assignment

How to Run Your Gel

0.0/1.0 point (graded)

You and Zoe present your results of the DNA substrate preferences of PriA- and PriC-dependent helicase loading to your advisor. He wants to confirm that the effect you see in helicase loading affects the outcome of DNA replication and suggests using a gel separation-based incorporation assay. He suggests setting up reactions containing:

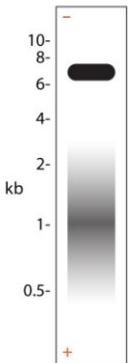
- Modified versions of Zoe's DNA substrates (~7 kb of double-stranded DNA with a fork-like end with gaps of 0 nt, 5 nt, or 16 nt)



- Enzymes required for replication: DNA Pol III, the sliding clamp (DnaN), primase (DnaG)
- Either PriA or PriC (and associated proteins as necessary)
- Start the reaction by adding radiolabeled dNTPs.
- After 30 minutes of incubation, end the reactions with EDTA and separate the reaction products on an agarose gel.

Zoe sets up a pilot reaction of the incorporation assay described with the 5 nt gap DNA substrate, both PriA and PriC, and associated proteins as required. She shows you the autoradiogram of her gel.

Hint: Think about what products you expect Zoe to have from her reaction, and think about how those products would run on a denaturing gel or on a non-denaturing gel.



What kind of gel did Zoe use? Use the dropdown options to select the best answer.

Given the DNA substrate and the included enzymes for this incorporation assay, Zoe must have run a Select an option gel.

Home assignment

Identify Gel Bands

0.0/2.0 points (graded)

What DNA products do the bands in the gel correspond to? Use the dropdown options to describe the identity of each band.

- The band at the top of the gel most likely represents the migration of
- The smear at the bottom of the gel most likely represents the migration of

Home assignment

Explain the Results

0.0/1.0 point (graded)

What is the best explanation for the lack of a smear in the lanes of reactions without primase (DnaG), **in this experiment?**
Select the best answer.

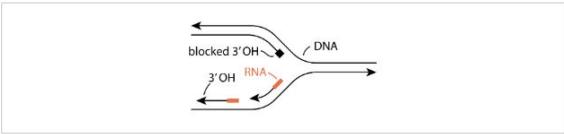
- In the absence of primase (DnaG), there is no nuclease activity that created the blur on the gel image representing labeled DNA fragments.
- Primase (DnaG) is only involved in lagging strand synthesis.
- Okazaki fragment maturation proceeds much more efficiently in the absence of primase (DnaG).
- Primase (DnaG) is more heavily involved for leading strand synthesis.

Home assignment

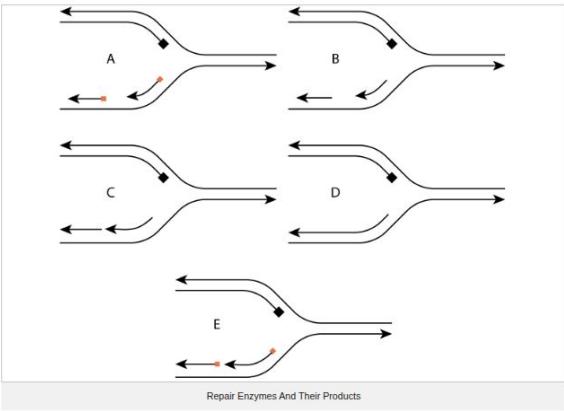
Okazaki Repair Proteins

0.0/0.0 points (graded)

You are working on an *in vitro* Okazaki fragment repair system using *E. coli* enzymes. For your experiments, you have produced the DNA substrate shown that mimics a DNA replication fork but has a leading strand that has a blocked 3' OH such that DNA polymerases will not bind to the leading strand.



The following are representations of possible products of the repair process. Which product will be produced if the substrate is incubated with each **combination of replication-associated enzymes** listed in the table? Use the dropdown options to associate the enzyme combinations with their product.



Repair Enzymes And Their Products

Repair enzyme combination	Which products produced?
RNase H, DNA ligase, 5' to 3' exonuclease	<input type="button" value="Select an option"/>
RNase H	<input type="button" value="Select an option"/>
RNase H, DNA Pol III	<input type="button" value="Select an option"/>
RNase H, DNA Pol I, DNA ligase	<input type="button" value="Select an option"/>
DNA Pol III, DNA ligase	<input type="button" value="Select an option"/>
RNase H, DNA Pol I	<input type="button" value="Select an option"/>

Save

THANKS FOR LISTENING