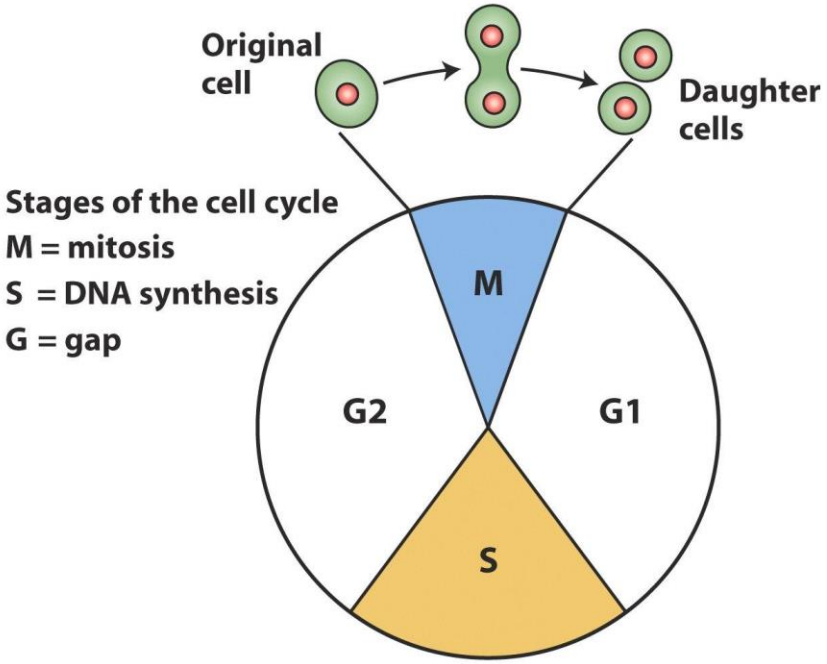
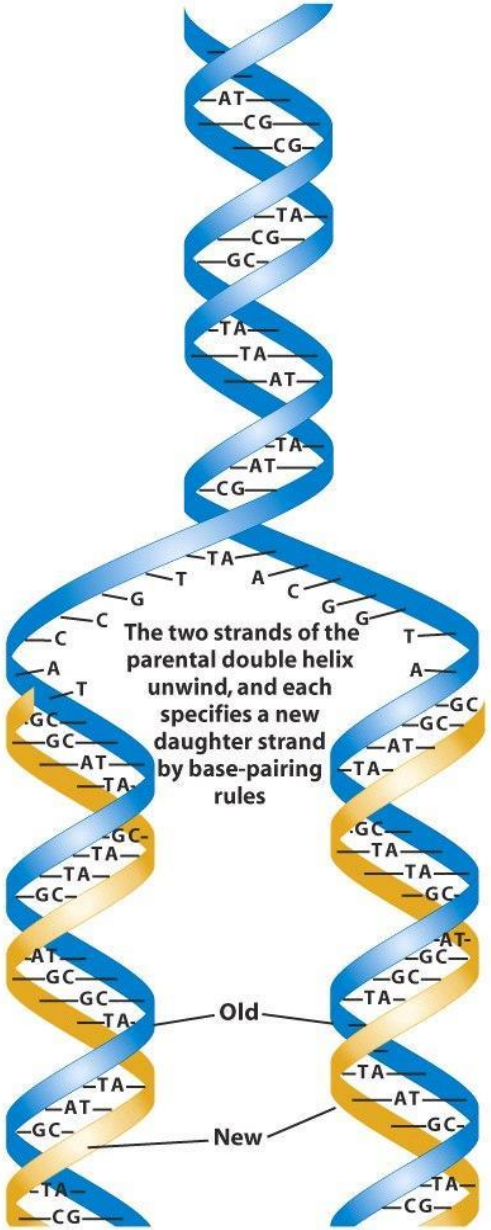
DNA Form & Function

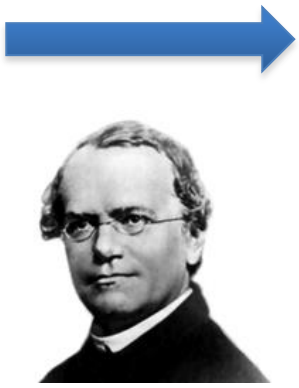




DNA: Structure & Replication

Understanding DNA replication – and the resulting transmission of genetic information from cell to cell, and generation to generation – lays the groundwork for understanding the principles of heredity

	R	R
O	RO	RO
O	RO	RO



	R	O
R	RR	RO
O	RO	OO

Understanding DNA structure and replication is a prerequisite for understanding/using the principal tools of molecular biology

DNA: Structure & Replication

Three features of DNA makes it an ideal genetic material

1. Faithful replication

2. Information content

3. Capable of change

DNA: Nucleotides

Overall structure

Nucleotides

Nucleic acids are linear, unbranched polymers of nucleotides.

Nucleotides consist of three parts:

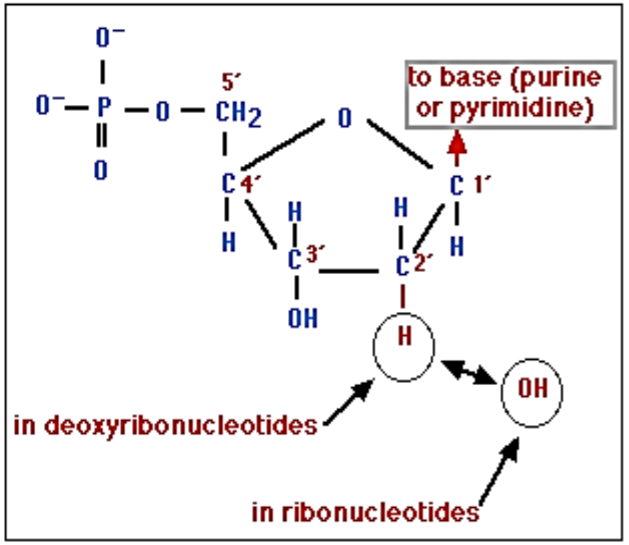
1.

A five-carbon sugar (hence a **pentose**). Two kinds are found:

- **Deoxyribose**, which has a hydrogen atom attached to its #2 carbon atom (designated 2'), and
- **Ribose**, which has a hydroxyl group there.

Deoxyribose-containing nucleotides, the **deoxyribonucleotides**, are the monomers of deoxyribonucleic acids (**DNA**).

Ribose-containing nucleotides, the **ribonucleotides**, are the monomers of ribonucleic acids (**RNA**).



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/Nucleotides.html

DNA: Nucleotides

Bases

2.

A nitrogen-containing ring structure called a **base**. The base is attached to the 1' carbon atom of the pentose. In **DNA**, four different bases are found:

1. two **purines**, called **adenine** (**A**) and **guanine** (**G**)
2. two **pyrimidines**, called **thymine** (**T**) and **cytosine** (**C**)

RNA contains:

1. The same purines, **adenine** (**A**) and **guanine** (**G**).
2. RNA also uses the pyrimidine **cytosine** (**C**), but instead of thymine, it uses the pyrimidine **uracil** (**U**).

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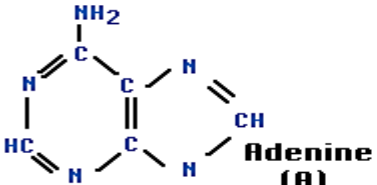
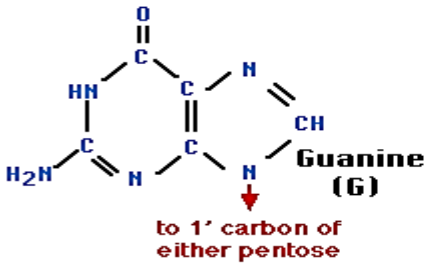
DNA: Nucleotides

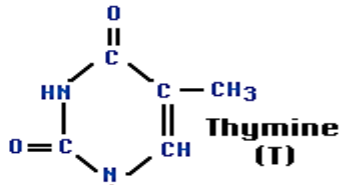
Bases

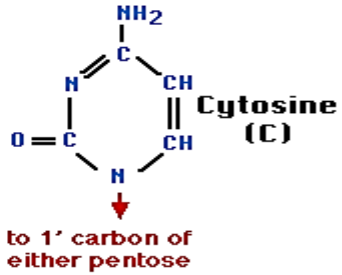
The Purines

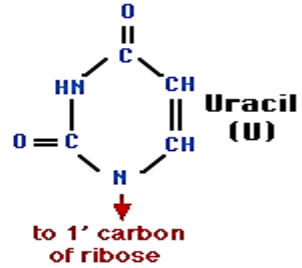




The Pyrimidines







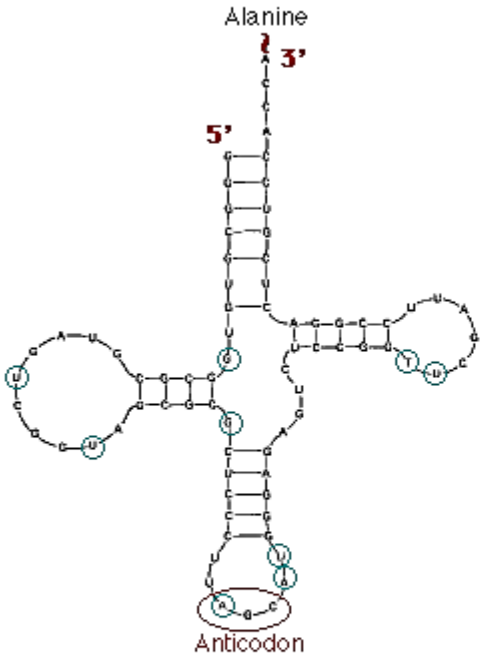
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/Nucleotides.html

The combination of a base and a pentose sugar is a **nucleoside**

DNA: Polymerization

Nucleotide + Nucleotide + Nucleotide + ……

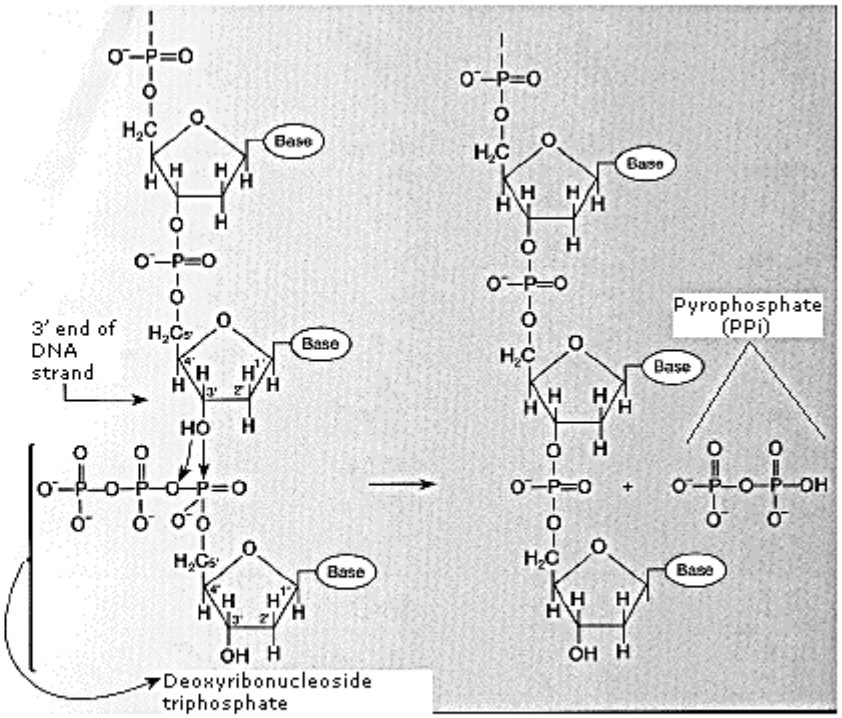
The polymerization of nucleotides.



The nucleic acids, both DNA and RNA, consist of polymers of nucleotides. The nucleotides are linked covalently between the 3' carbon atom of the pentose and the phosphate group attached to the 5' carbon of the adjacent pentose. The figure on the right shows the polymer structure of DNA.

Most intact DNA molecules are made up of **two** strands of polymer, forming a "double helix".

RNA molecules, while single-stranded, usually contain regions where two portions of the strand twist around each other to form helical regions. Alanine transfer RNA, shown on the left, is an example.



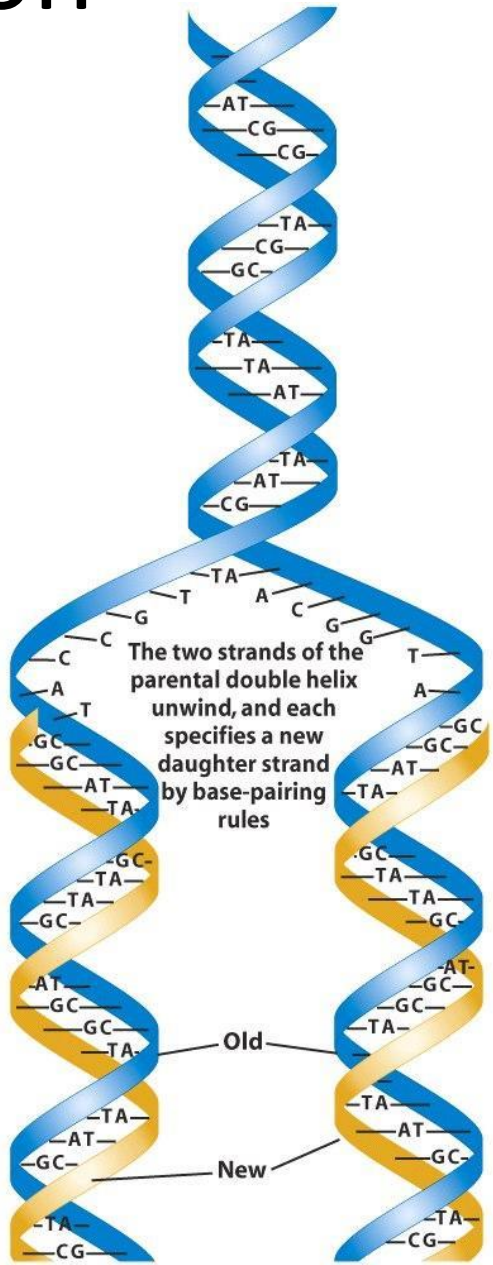
The two strands of DNA and the helical regions of RNA are held together by base pairing.

The polymerization of DNA is described more fully in DNA Replication.

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/Nucleotides.html

DNA: Replication

- Complementary base pairing and the double helix
- Replication is semi-conservative



DNA: Synthesis Essentials

1. DNTPs: dATP, dGTP, dTTP, and dCTP
2. Template DNA (a pre-existing single strand)
3. DNA polymerase

1. DNTPs

One (as shown in the first figure), two, or three **phosphate** groups. These are attached to the 5' carbon atom of the pentose. The product in each case is called a **nucleotide**.

Both DNA and RNA are assembled from **nucleoside triphosphates**.

For **DNA**, these are **dATP**, **dGTP**, **dCTP**, and **dTTP**.

For **RNA**, these are **ATP**, **GTP**, **CTP**, and **UTP**.

In both cases, as each nucleotide is attached, the second and third phosphates are removed.

The nucleosides and their mono-, di-, and triphosphates

	Base	Nucleoside	Nucleotides		
DNA	Adenine (A)	Deoxyadenosine	dAMP	dADP	dATP
	Guanine (G)	Deoxyguanosine	dGMP	dGDP	dGTP
	Cytosine (C)	Deoxycytidine	dCMP	dCDP	dCTP
	Thymine (T)	Deoxythymidine	dTMP	dTDP	dTTP
RNA	Adenine (A)	Adenosine	AMP	ADP	ATP
	Guanine (G)	Guanosine	GMP	GDP	GTP
	Cytosine (C)	Cytidine	CMP	CDP	CTP
	Uracil (U)	Uridine	UMP	UDP	UTP

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/Nucleotides.html

DNA: Synthesis Essentials

2. Template DNA (a pre-existing single strand)

ATCGGTCAACGTTAAAGTTAGCGG

DNA: Synthesis Essentials

3. DNA Polymerases

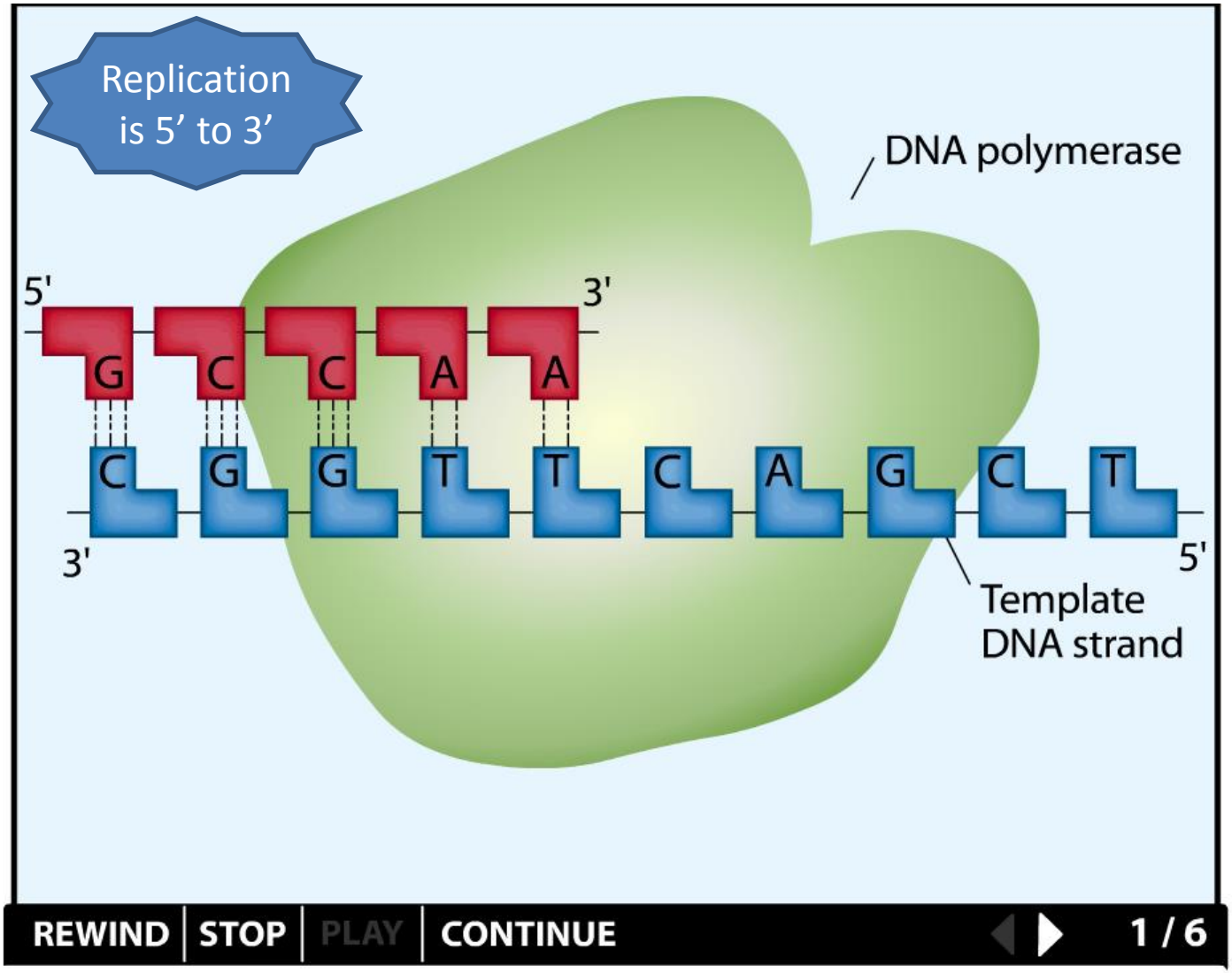
- There are multiple forms of DNA polymerase
- Different forms have different activities
  - Replicases have direct roles in replication
  - Others have secondary roles in replication and/or repair synthesis.
- DNA replication – polymerization of deoxyribonucleotides
  - Polymerases catalyze the formation of a phosphodiester bond between the 3'-OH of the deoxyribose on the last nucleotide and the 5' phosphate of the dNTP precursor
  - Process is repeated forming a synthesized DNA chain

DNA: Synthesis Essentials

3. DNA Polymerases (continued)

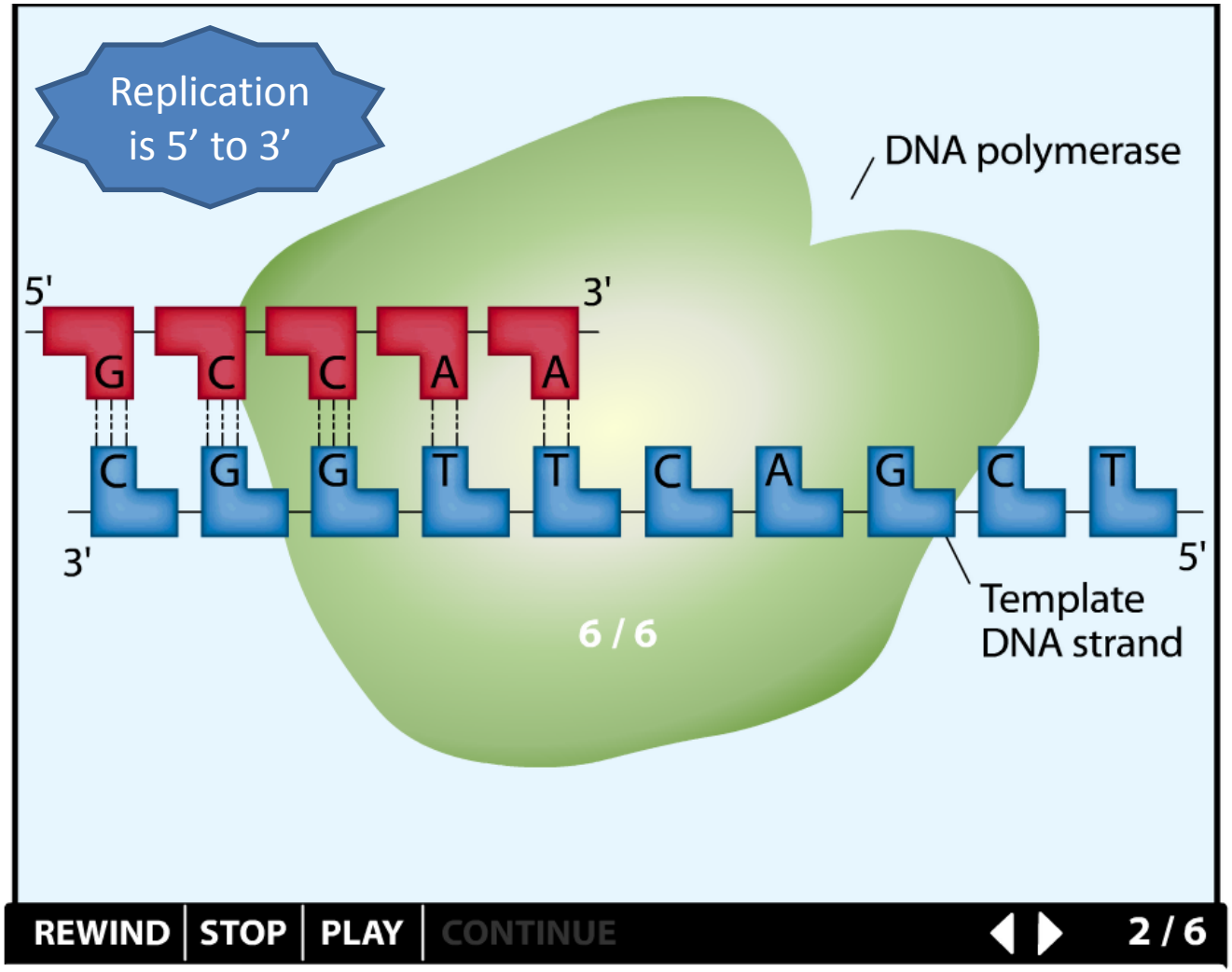
- Polymerase binds to the DNA template strand and moves along as the synthesized polynucleotide chain grows
  - At each template base, the dNTP precursor is identified that can base pair with it
  - The frequency of error is low, but errors can occur.
- Polymerases can have exonuclease activity (removal of nucleotides from the 3' end of the chain)
  - This is a proof-reading mechanism
  - An unpaired nucleotide from the 3'OH end of the growing chain triggers exonuclease activity
  - The unpaired nucleotide is cleaved from the end of the growing chain by the polymerase.

DNA: Synthesis Essentials



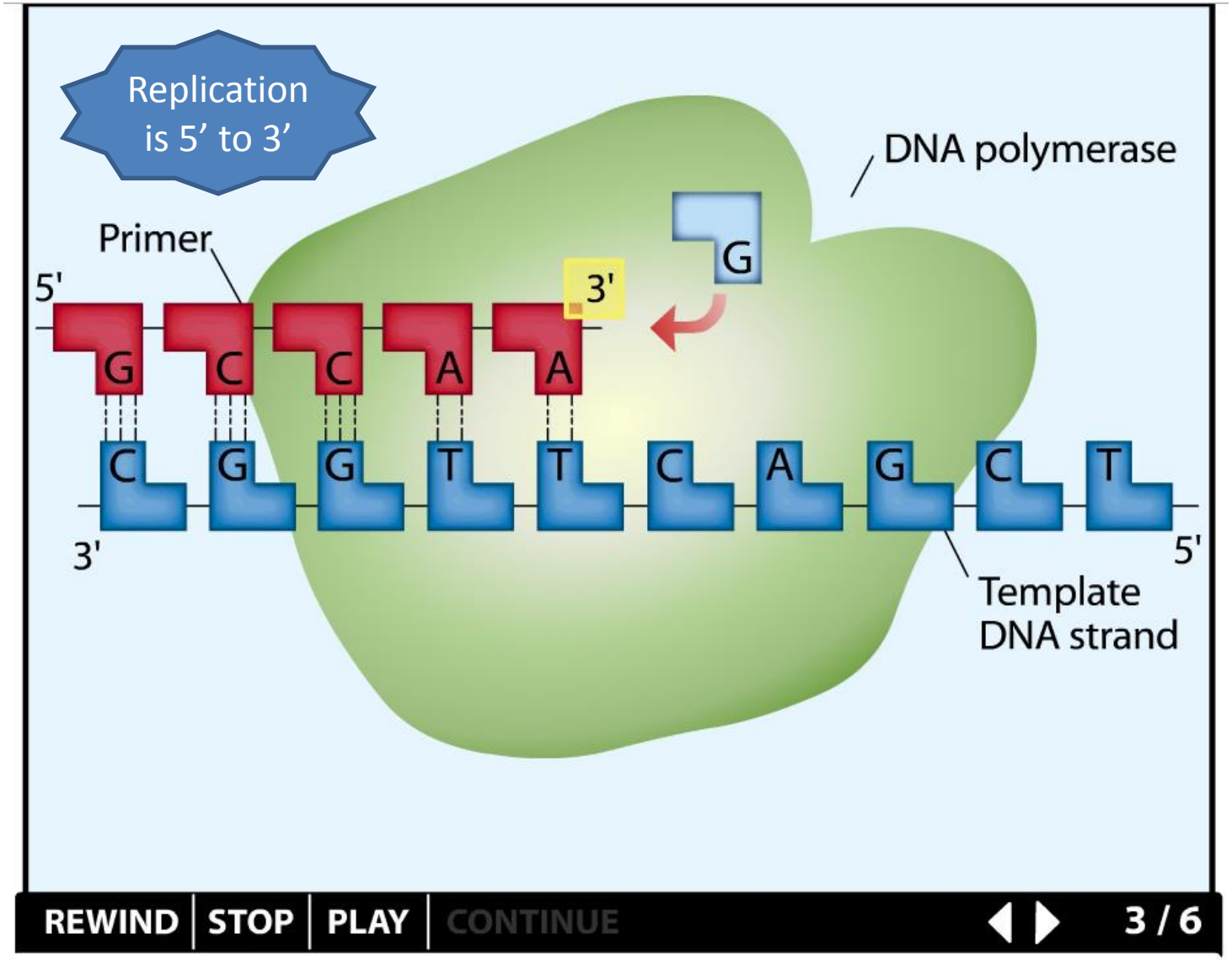
DNA polymerases catalyze the synthesis of new DNA strands from a DNA template.

DNA: Synthesis Essentials



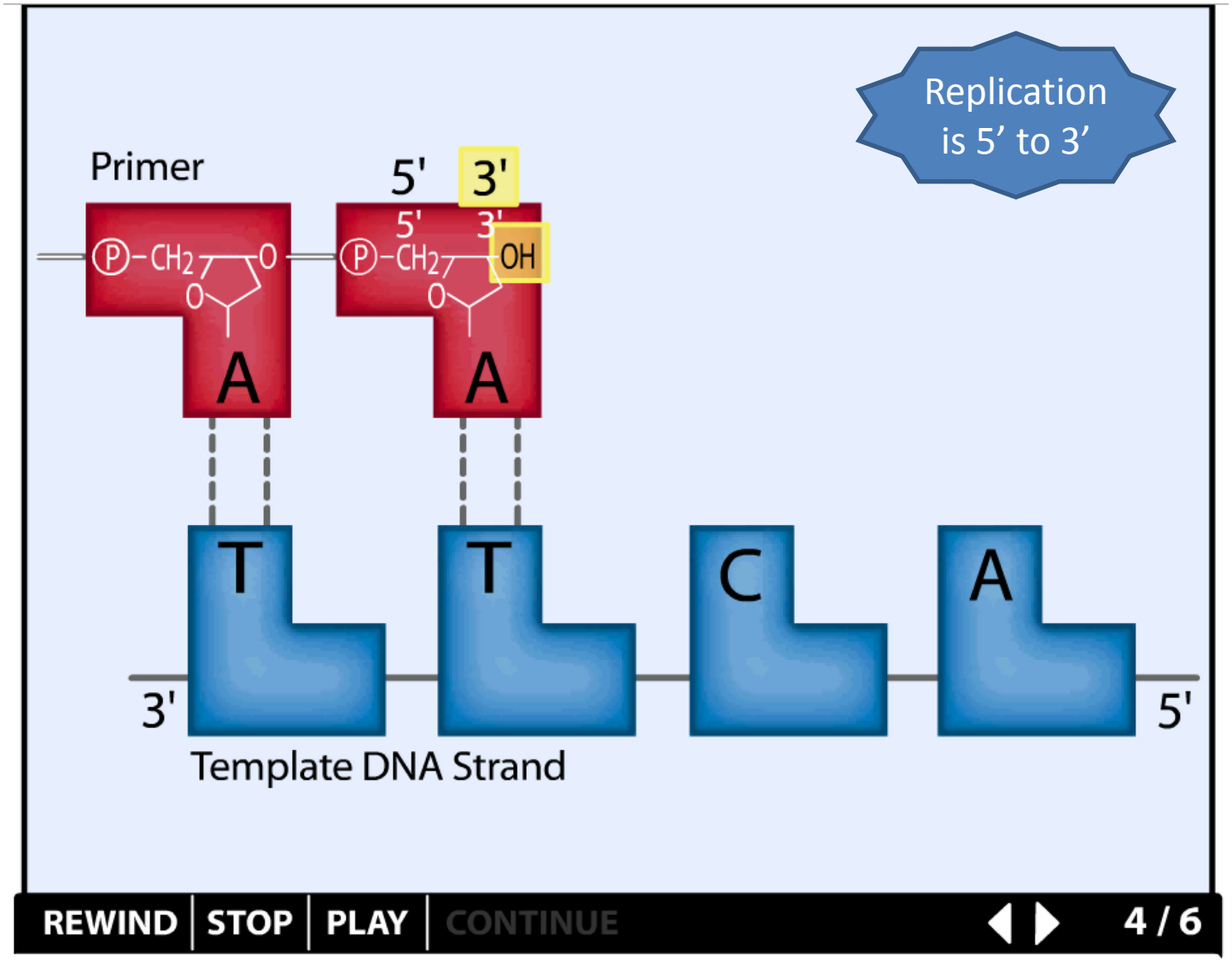
DNA polymerases require a pre-existing RNA or DNA strand, the primer, to initiate new DNA synthesis.
These polymerases add deoxyribonucleotides only to the 3' end of a growing strand.

DNA: Synthesis Essentials



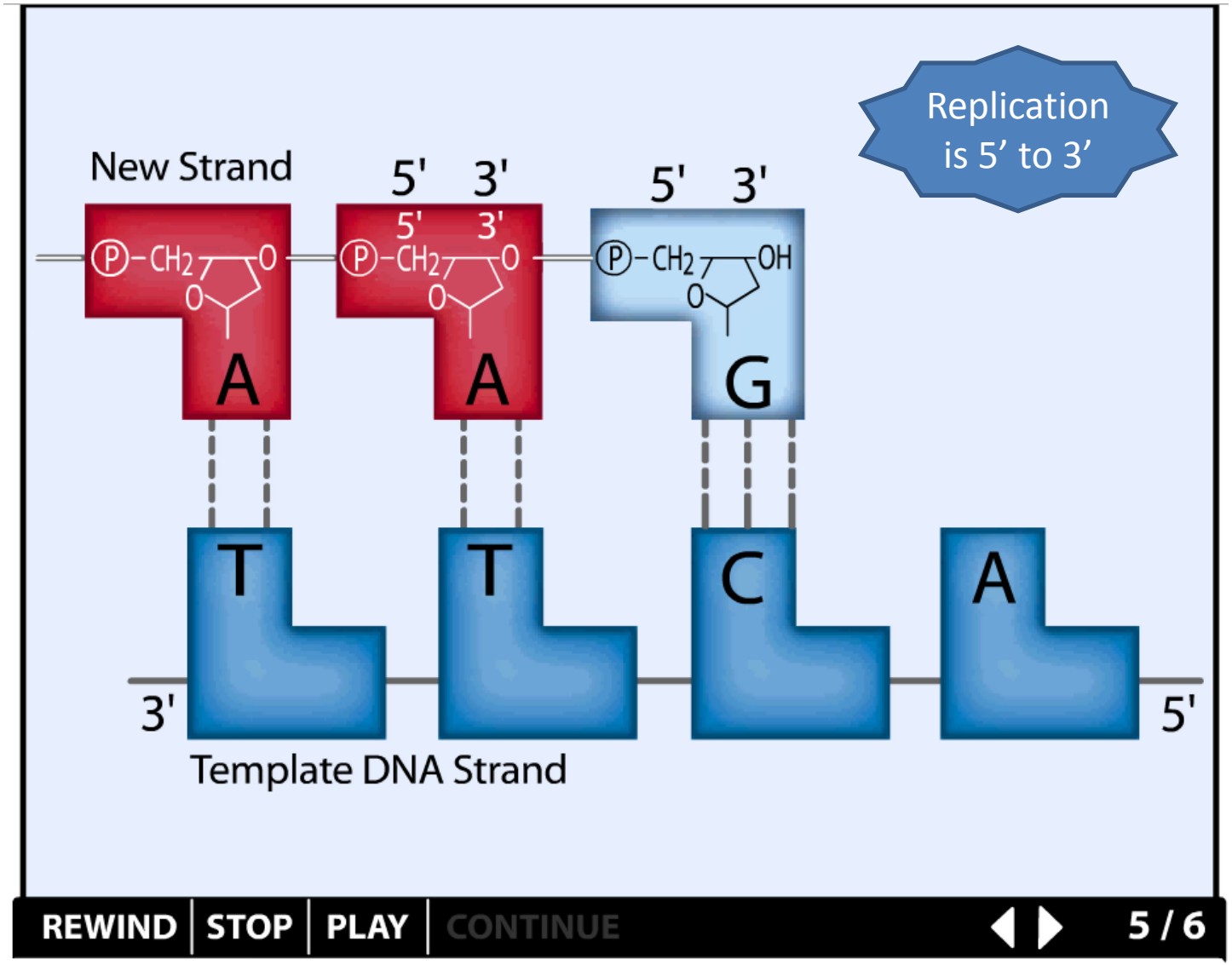
Taking a closer look at the nucleotide polymerization reaction, the 3' end of the primer contains a free 3'-hydroxyl group.

DNA: Synthesis Essentials



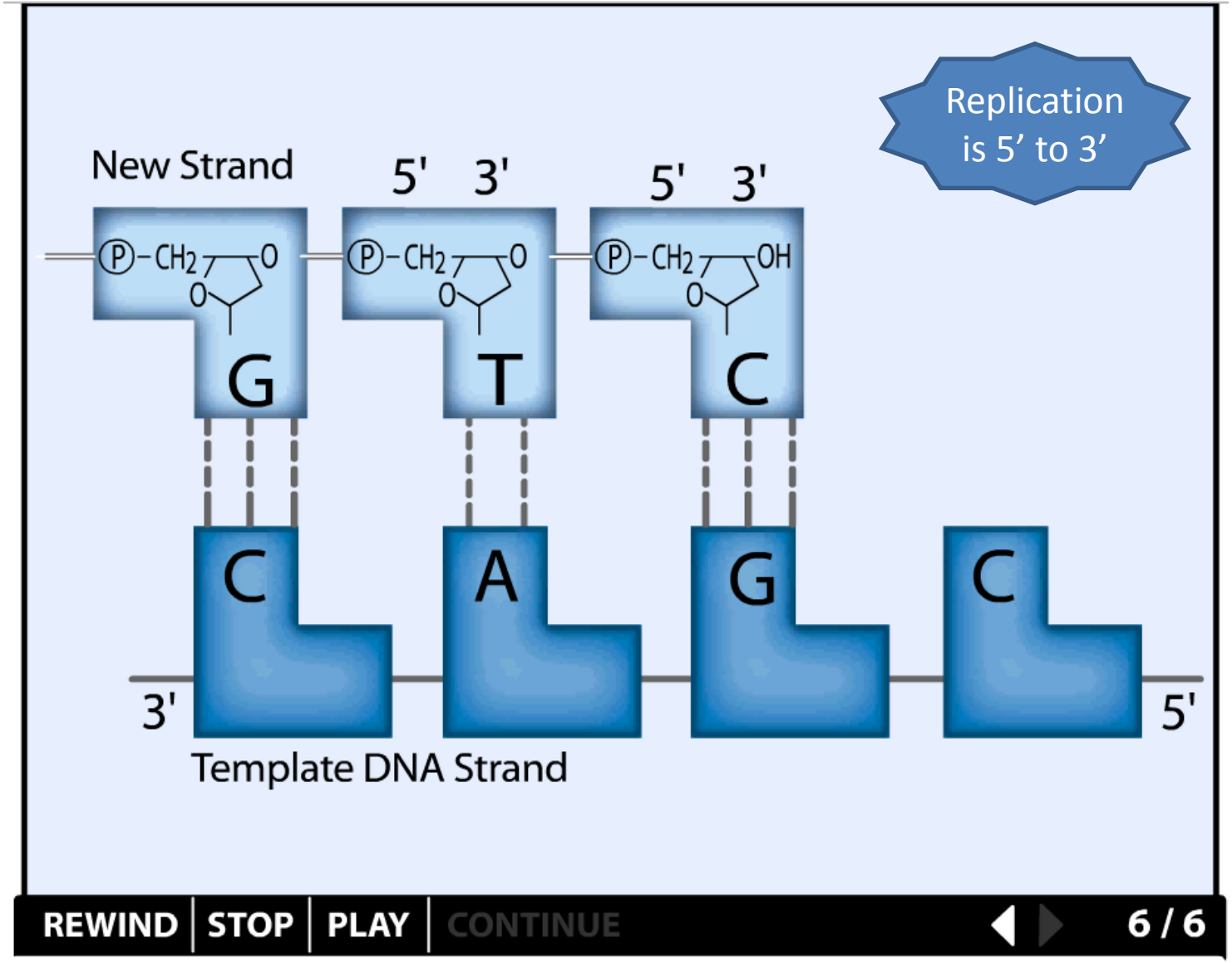
The 3' hydroxyl reacts with the 5' end of the next free nucleotide to be added.

DNA: Synthesis Essentials



Free nucleotides continue to be added to the growing DNA strand by the same type of reaction.

DNA: Synthesis Essentials



Overall, the new DNA strand grows in the 5' → 3' direction.

DNA Replication: 1-Origins

- Replication begins at a fixed point, called the origin, and proceeds bi-directionally. In a higher plant chromosome there are thousands of origins.

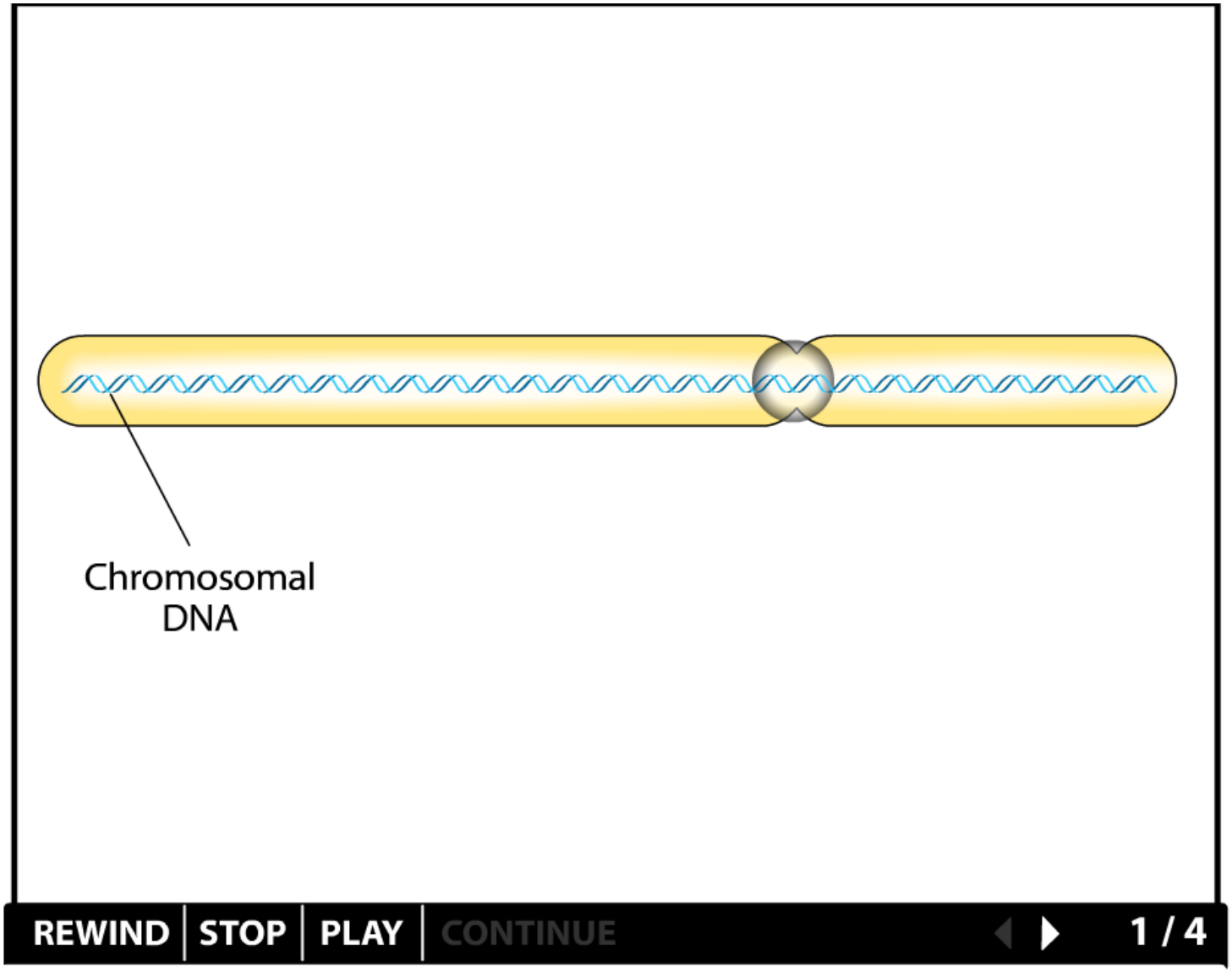
  Consider

  The size of the genome

  The rate of DNA replication

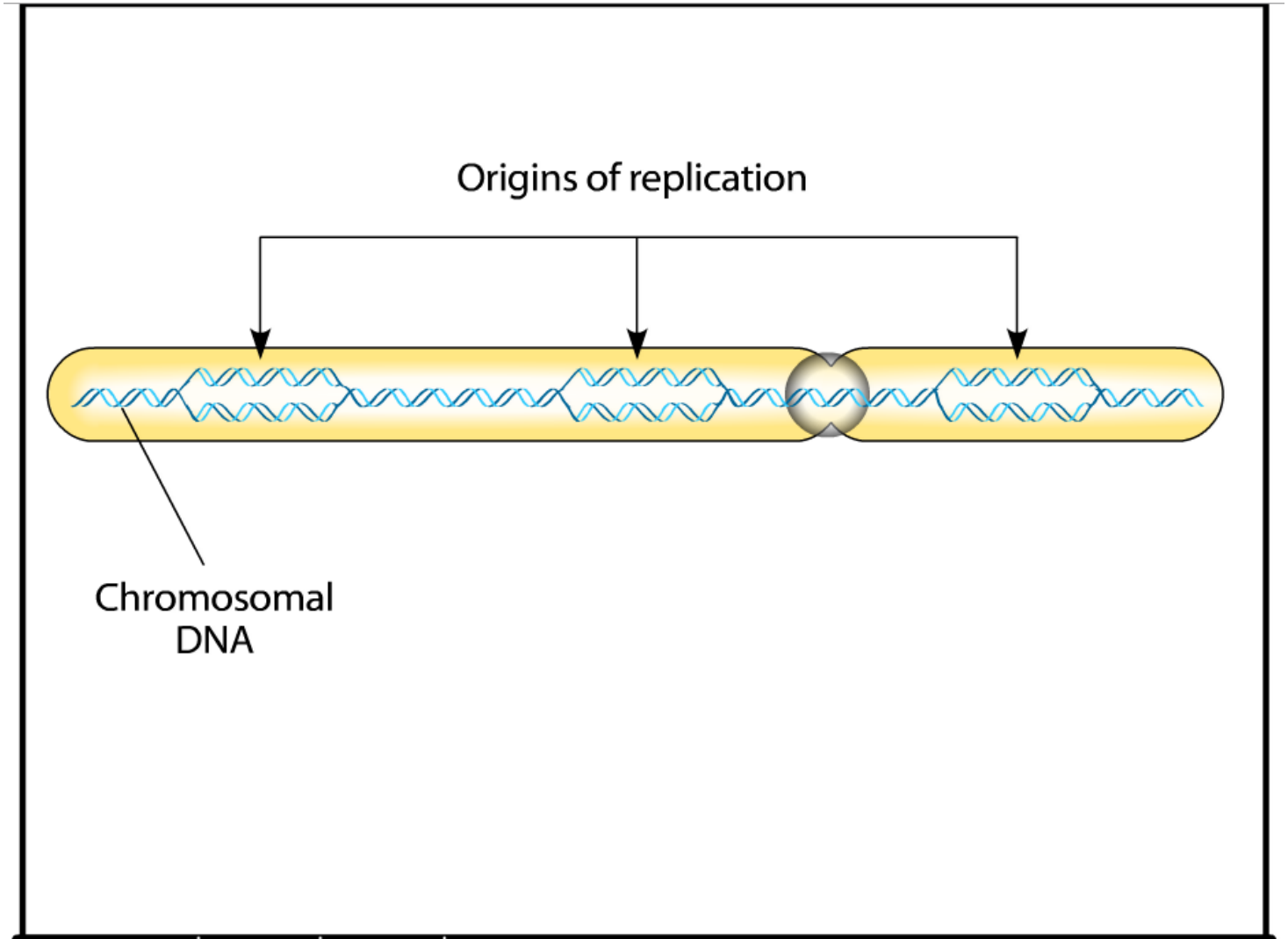
  The length of the S phase

DNA Replication: 1-Origins



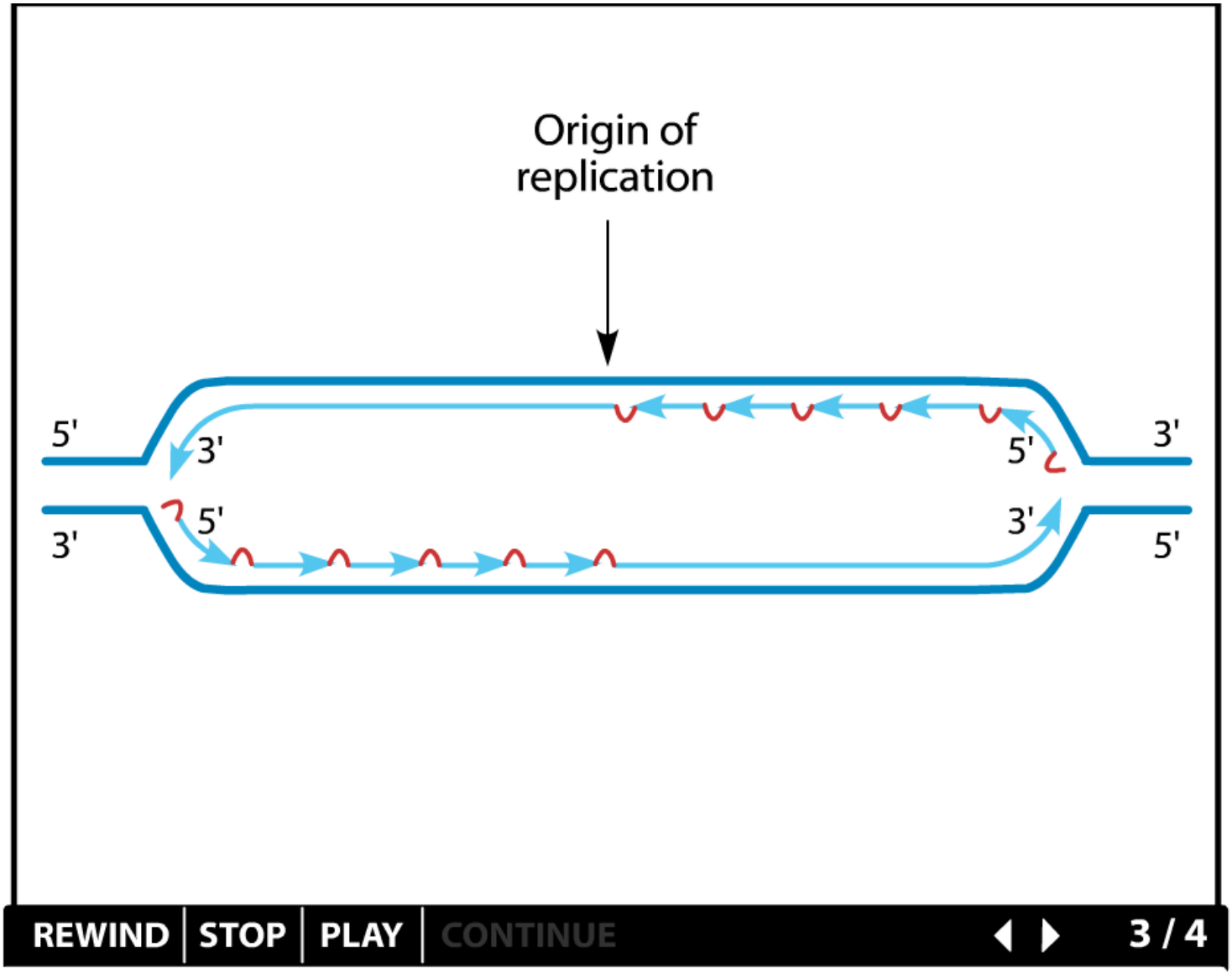
A eukaryotic chromosome contains multiple origins of replication.

DNA Replication: 1-Origins



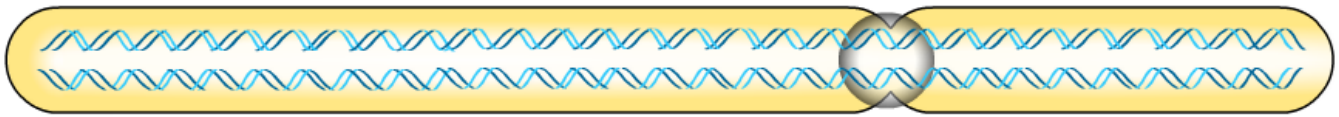
At each origin of replication, DNA synthesis proceeds bidirectionally. Two replication forks move outward in opposite directions.

DNA Replication: 1-Origins



The replicating DNA helices from each origin elongate and eventually join each other.

DNA Replication: 1-Origins

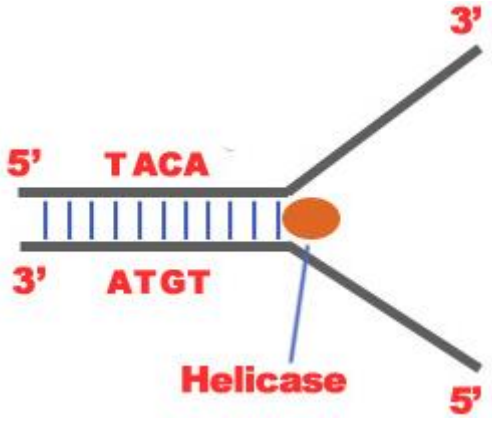


REWIND | STOP | PLAY | CONTINUE 4 / 4

The original chromosome has been replicated to form two identical daughter DNA molecules, also called sister chromatids.

Replication: 2-Unwinding, 3-Stabilization

2. *Unwinding:* The DNA helix needs to be opened up. This is accomplished by helicase enzymes, which break the hydrogen bonds holding the two strands of the helix together. Gyrase facilitates helicase action by relieving tension in coiled DNA

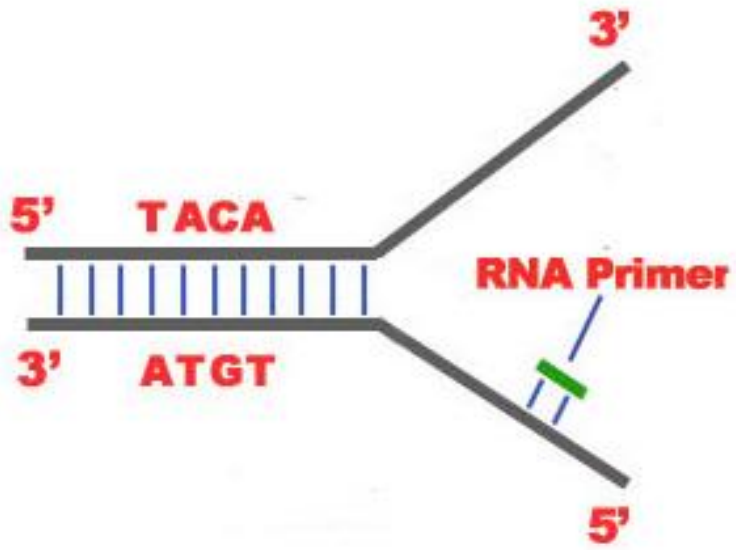


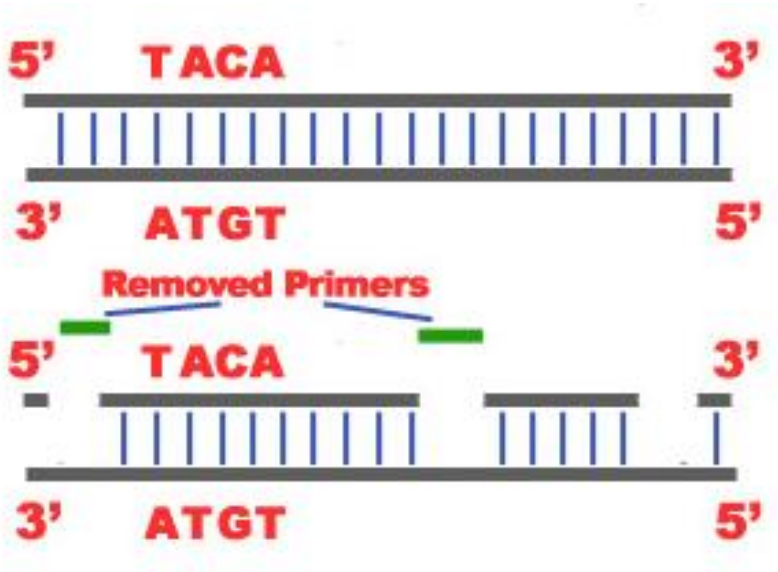
3. *Stabilization:* The unwound DNA is stabilized by a protein (single strand binding protein (SSB)), which speeds up DNA replication.

http://www.dnareplication.info/stepsofdnareplication.php

Replication: 4 - Priming

- Primases form a short RNA primer
- DNA polymerases use the primer to synthesise a new chain
  - Polymerases cannot start synthesis on their own
- RNA primers subsequently removed by exonuclease activity of a polymerase and replaced with DNA

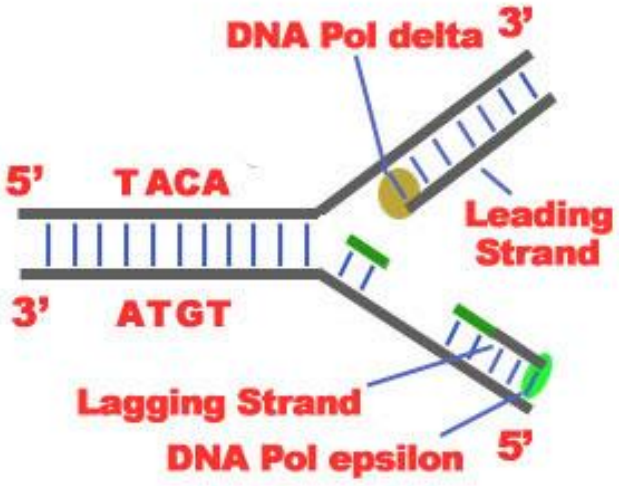


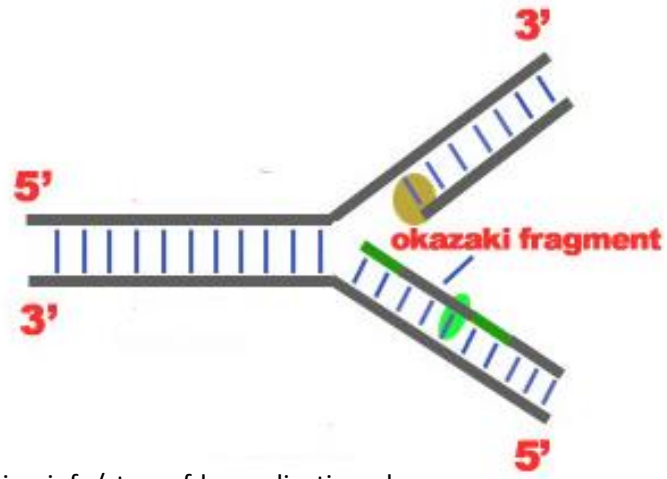


http://www.dnareplication.info/stepsofdnareplication.php

Replication: 5 – Leading & Lagging Strands

- DNA polymerases synthesize new chains only from 5' to 3‘
  - DNA molecule is antiparallel and DNA synthesis is semi-conservative.
- DNA synthesis is continuous on 5’-3’ strand - leading strand
- Synthesis discontinuous on 3’-5’ strand - lagging strand
  - Multiple priming sites on the lagging strand
  - DNA therefore formed in fragments on lagging strand - Okazaki fragments





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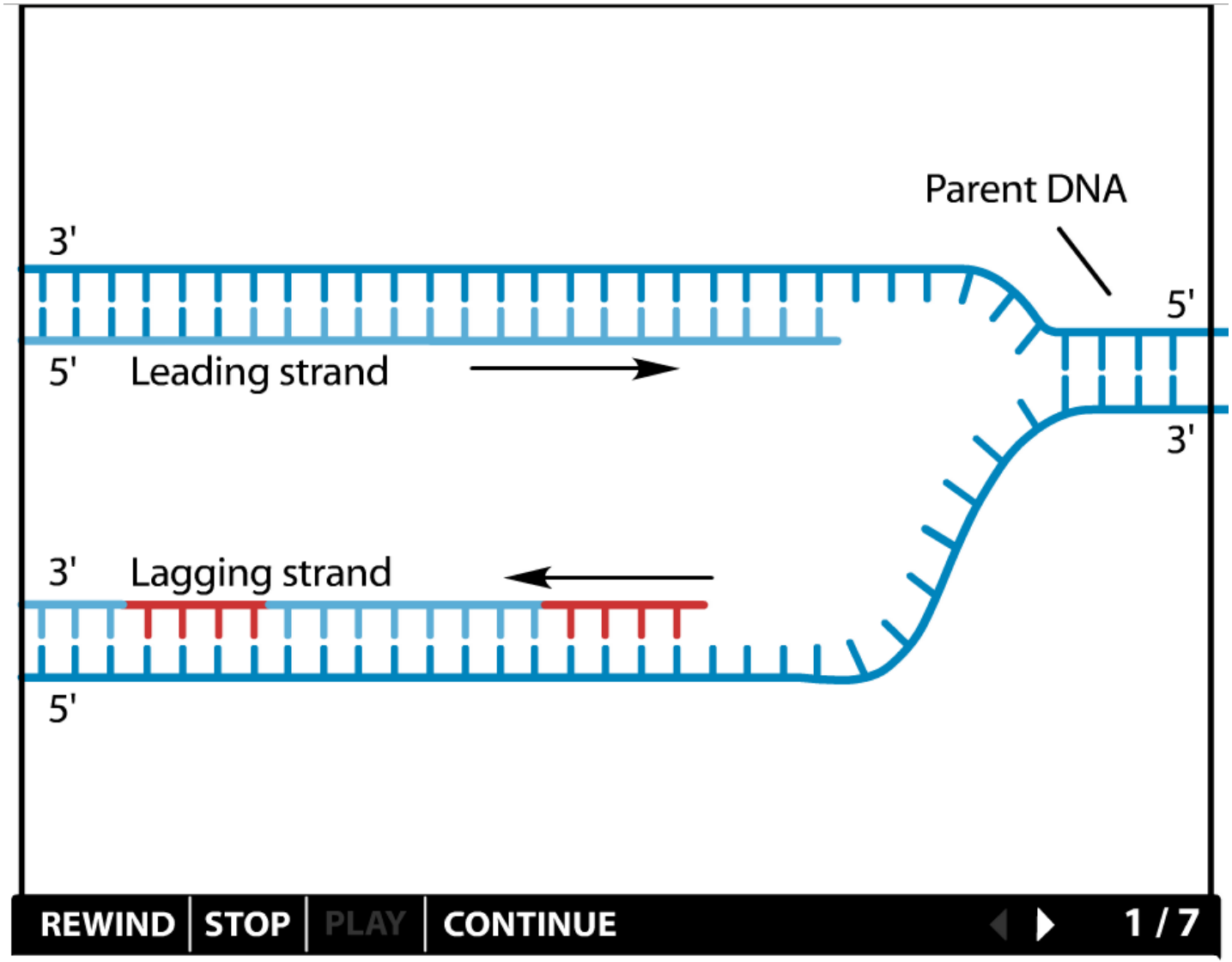
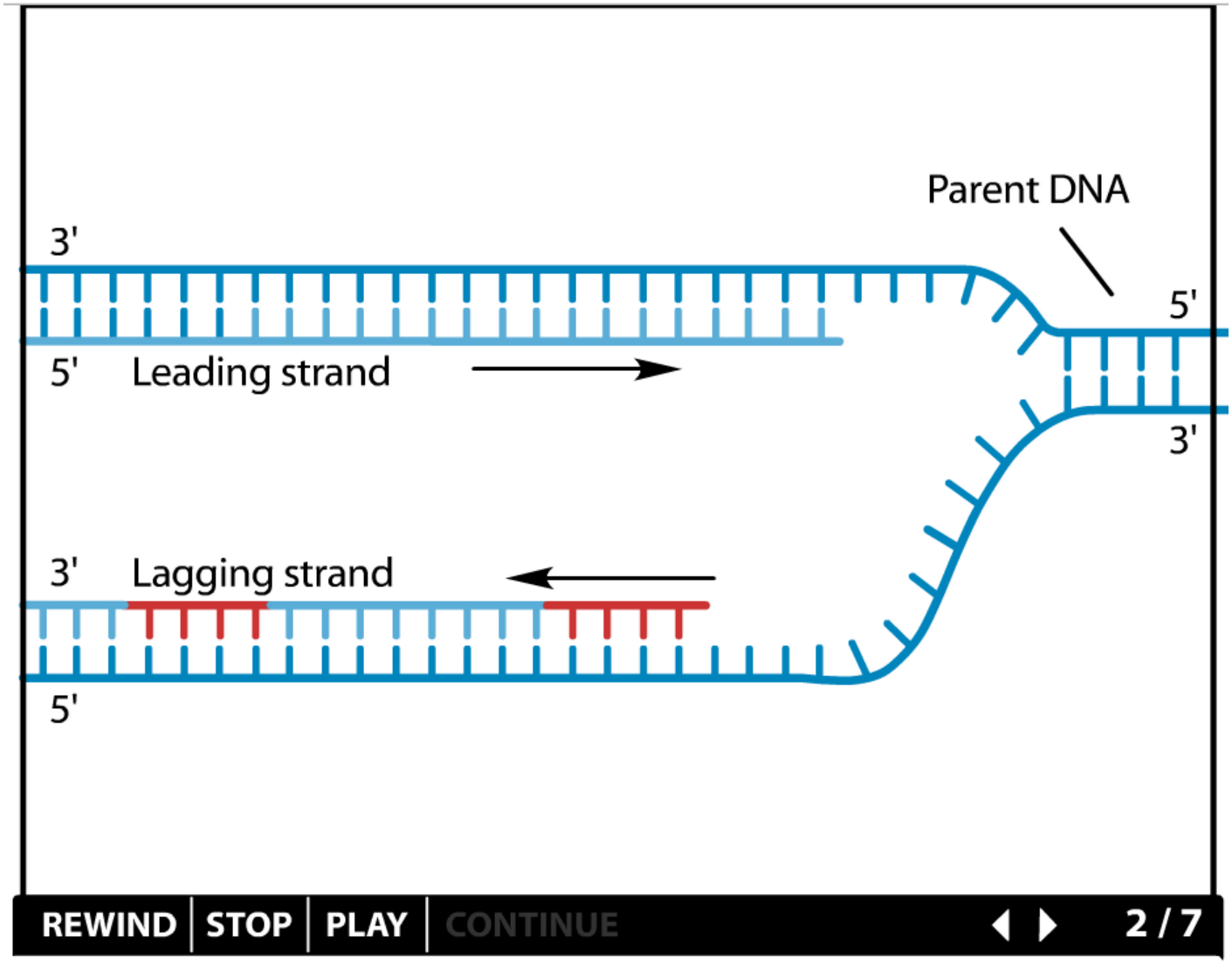
Replication: 5 – Leading & Lagging Strands



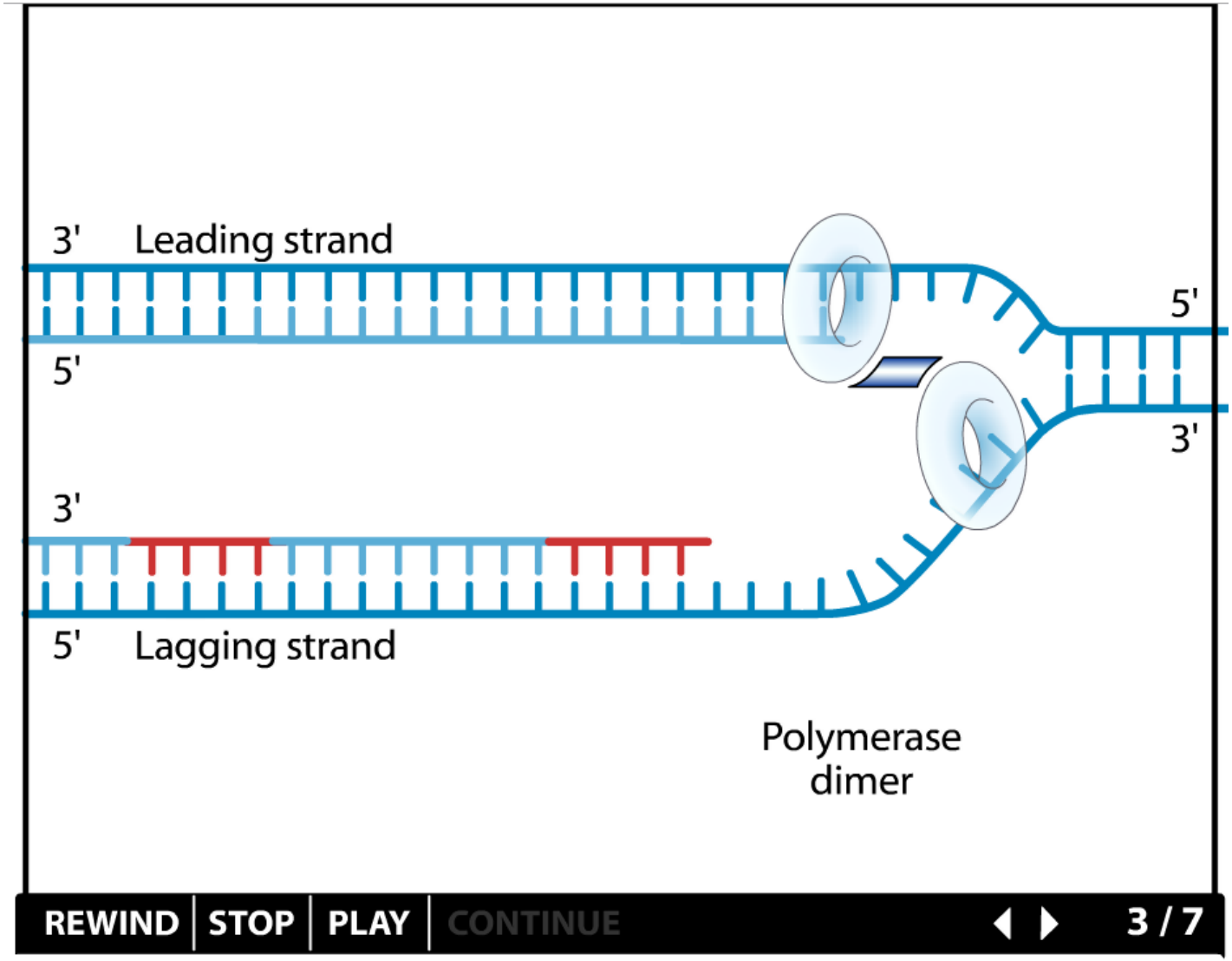
DNA is replicated by continuous synthesis of a leading strand and discontinuous synthesis of a lagging strand.

Replication: 5 – Leading & Lagging Strands



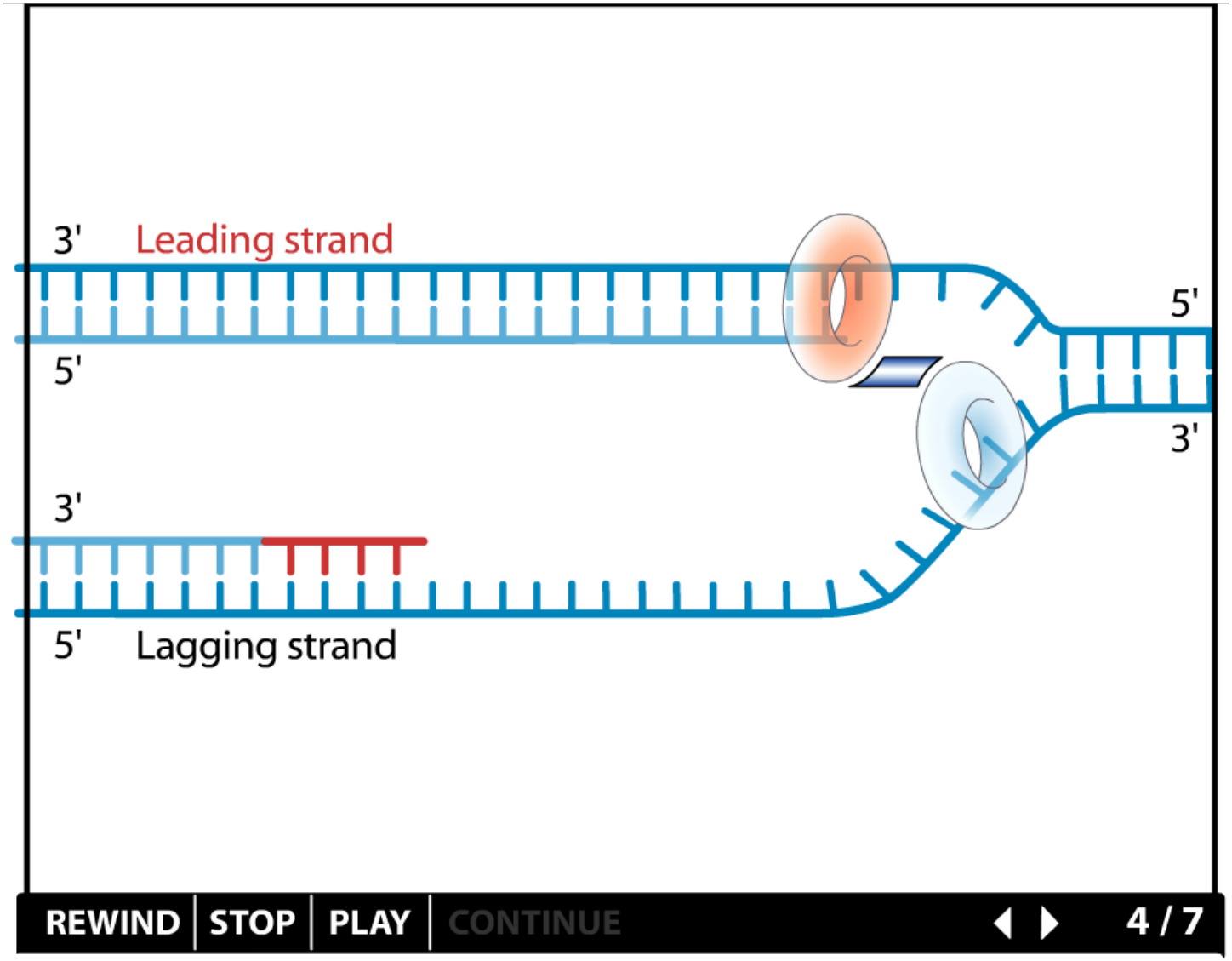
Coordination between leading and lagging strand synthesis is achieved by the dimerization of DNA polymerase molecules at the replication fork.

Replication: 5 – Leading & Lagging Strands



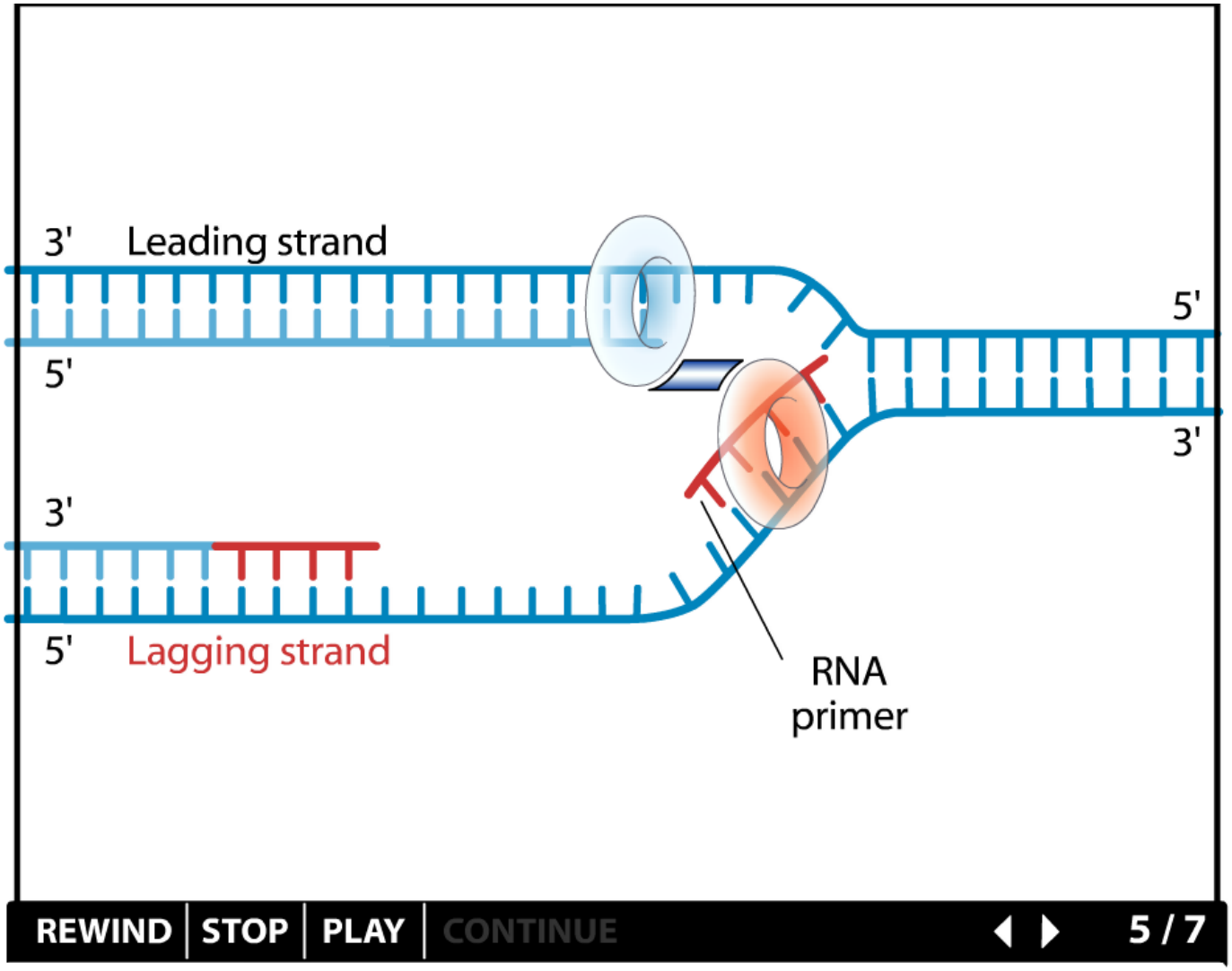
The DNA polymerase dimer moves with the replication fork. The polymerase at the leading strand template remains attached to the DNA, continuously synthesizing the leading strand.

Replication: 5 – Leading & Lagging Strands



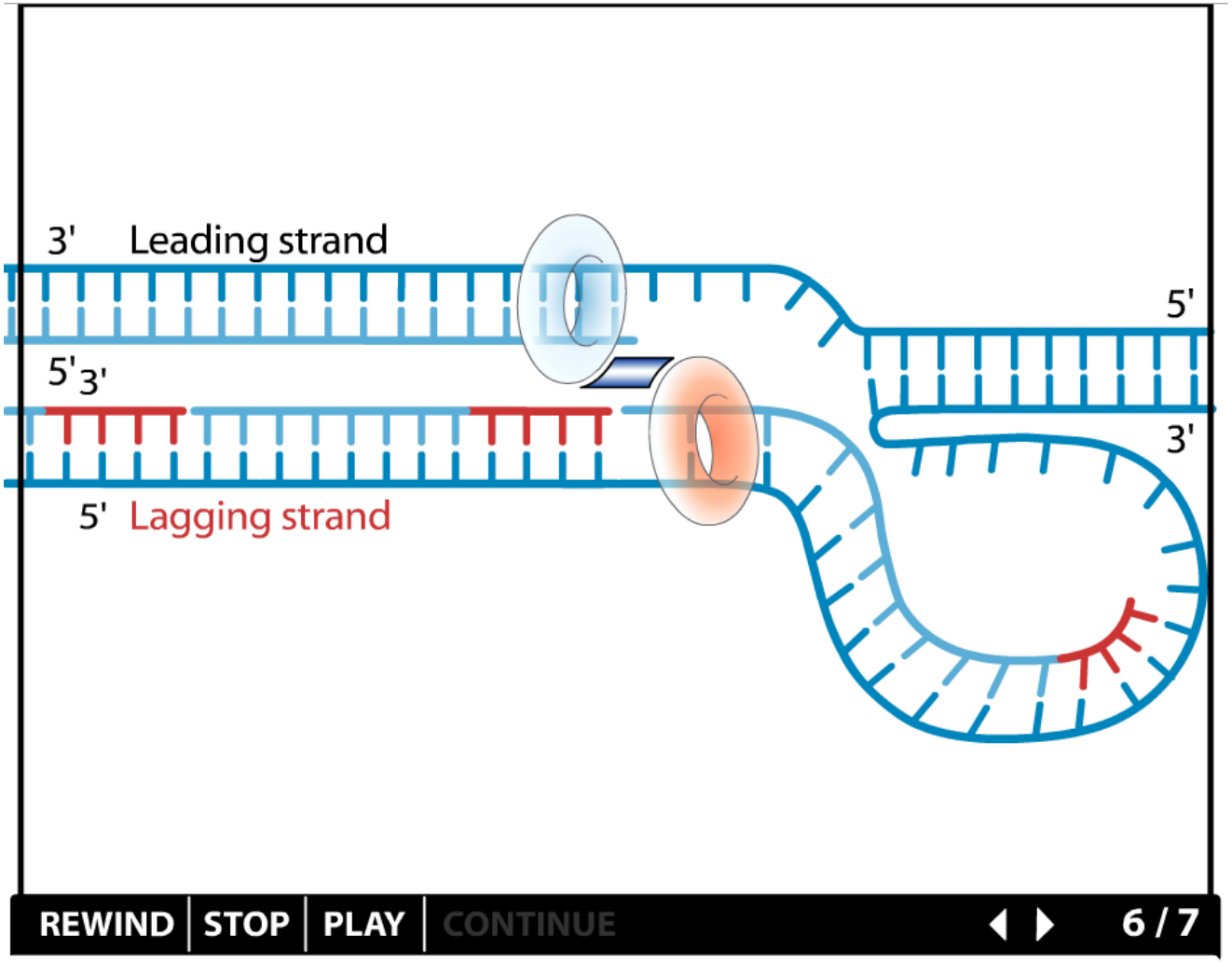
The lagging strand polymerase initiates DNA synthesis at the fork, from an RNA primer made by a primase complex.

Replication: 5 – Leading & Lagging Strands



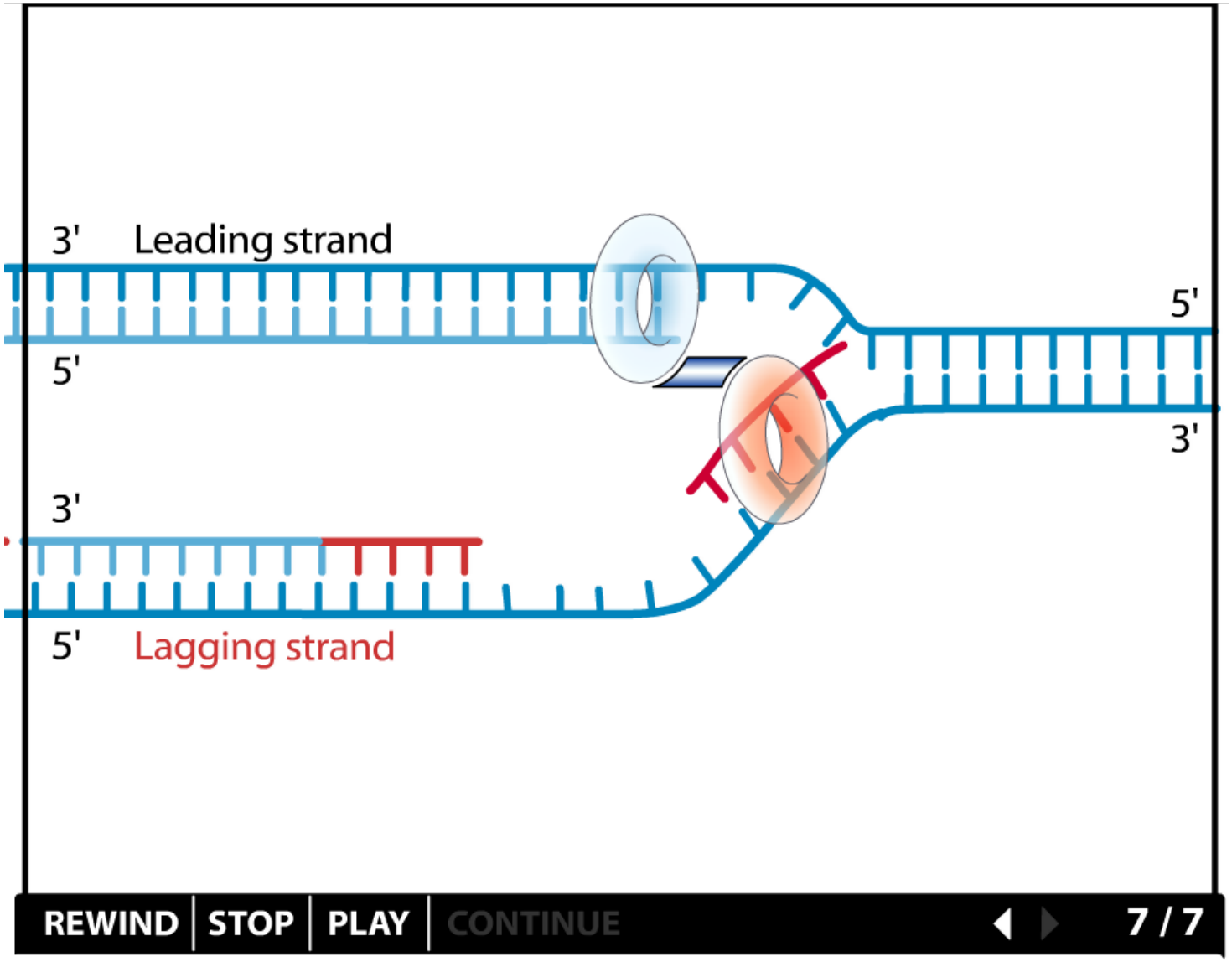
The polymerase elongates the lagging strand in a direction opposite the fork, but stays bound at the fork.
As a result, the newly synthesized lagging strand fragment loops out between the polymerase and the fork.

Replication: 5 – Leading & Lagging Strands



Once the polymerase completes an Okazaki fragment, it dissociates from the DNA template.
A new primer is produced at the fork. The polymerase reassociates with the template at this position, to continue synthesis of the lagging strand.

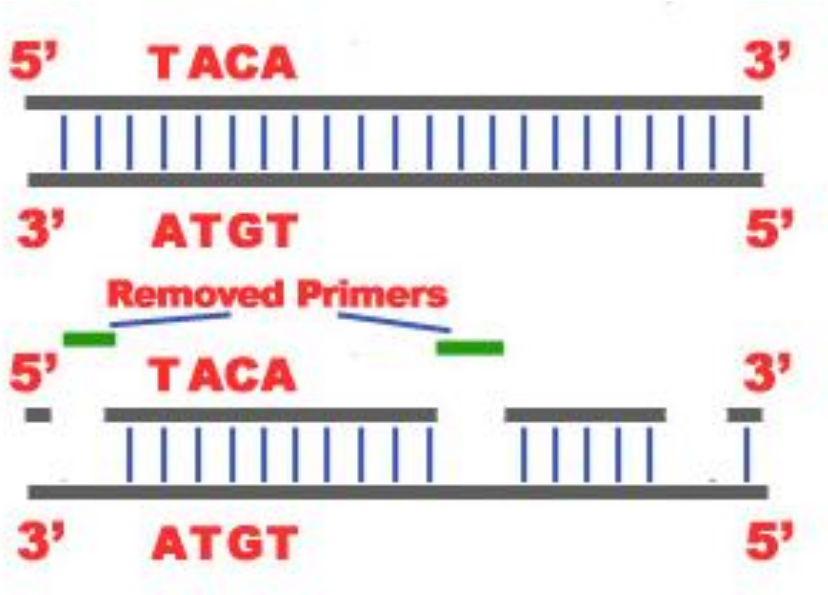
Replication: 5 – Leading & Lagging Strands



By this mechanism, the two polymerases can add nucleotides to the growing strands at the same time, and at rates up to 1000 bp/s.

Replication: 6 Termination

- **DNA Pol I exonuclease** removes RNA Primers in lagging strand
- **DNA Polymerase** adds complementary nucleotides to fill the gaps
- **DNA Ligase** adds phosphate in the remaining gaps of the phosphate - sugar backbone
- Can't backfill RNA primer site at telomere of lagging strand
  - Telomere shortening during replication
  - Role of Telomerase



The telomerase solution

https://www.youtube.com/watch?v=vtXrehpCPEE

The Plant Cell, Vol. 16, 794–803, April 2004, www.plantcell.org © 2004 American Society of Plant Biologists

HISTORICAL PERSPECTIVE ESSAY

Plant Telomere Biology

Analysis of telomeres, the nucleoprotein complexes that physically cap and protect the ends of eukaryotic chromosomes, has a long and intriguing history. The recent resurgence of plant telomere biology prompted us to recap this history to provide background and context for current investigations addressing how plants maintain a stable genome. Although many of the fundamentals of telomere biology were first uncovered in ciliates or fungi, telomere research in Arabidopsis allows us to ask basic questions in a multicellular organism with complex development and excellent genetic tools. This powerful combination of advantages is unsurpassed in other organ-

1938) and concluded that "... the terminal gene must have a special function, that of sealing the end of the chromosome, so to speak, and that for some reason a chromosome cannot persist indefinitely without having its ends thus sealed." After explaining that one difference between the terminal gene and the others is that it is unipolar, with genes on only one side of it, Muller deduced that the bipolar genes in the interior of the chromosome "... cannot be made into properly functioning unipolar ones by the simple expedient of tearing them loose from their connections on one side" (Muller, 1938). Muller coined the term telomere for this terminal gene from the

McClintock knew from her previous studies that these chromatin bridges eventually snapped, and the sister chromatids then fused to recreate a dicentric chromosome (McClintock, 1938). The question she was asking now was whether this chromosome breakage-fusion-bridge cycle would continue indefinitely. To answer the question, McClintock placed her dicentric-generating chromosome 9 in a background with a normal chromosome 9 carrying recessive alleles for plant color (*yellow green*, *yg*) and seed characteristics (*waxy endosperm*, *wx*; *colored aleurone*, *c*; and *shrunken endosperm*, *sh*) in the region between the two centromeres. Any break-

Replication: 7 Proof-Reading

- Mis-matched bases can be added in error during polymerization
- Specific polymerases with 3’-5’ exonuclease activity identify and remove any detected mis-matches on the synthesized strand
- Proofreading starts at the 3' end of the synthesized strand and proceeds 3’ -> 5’

  Two exact copies of original, each with one original and one new strand