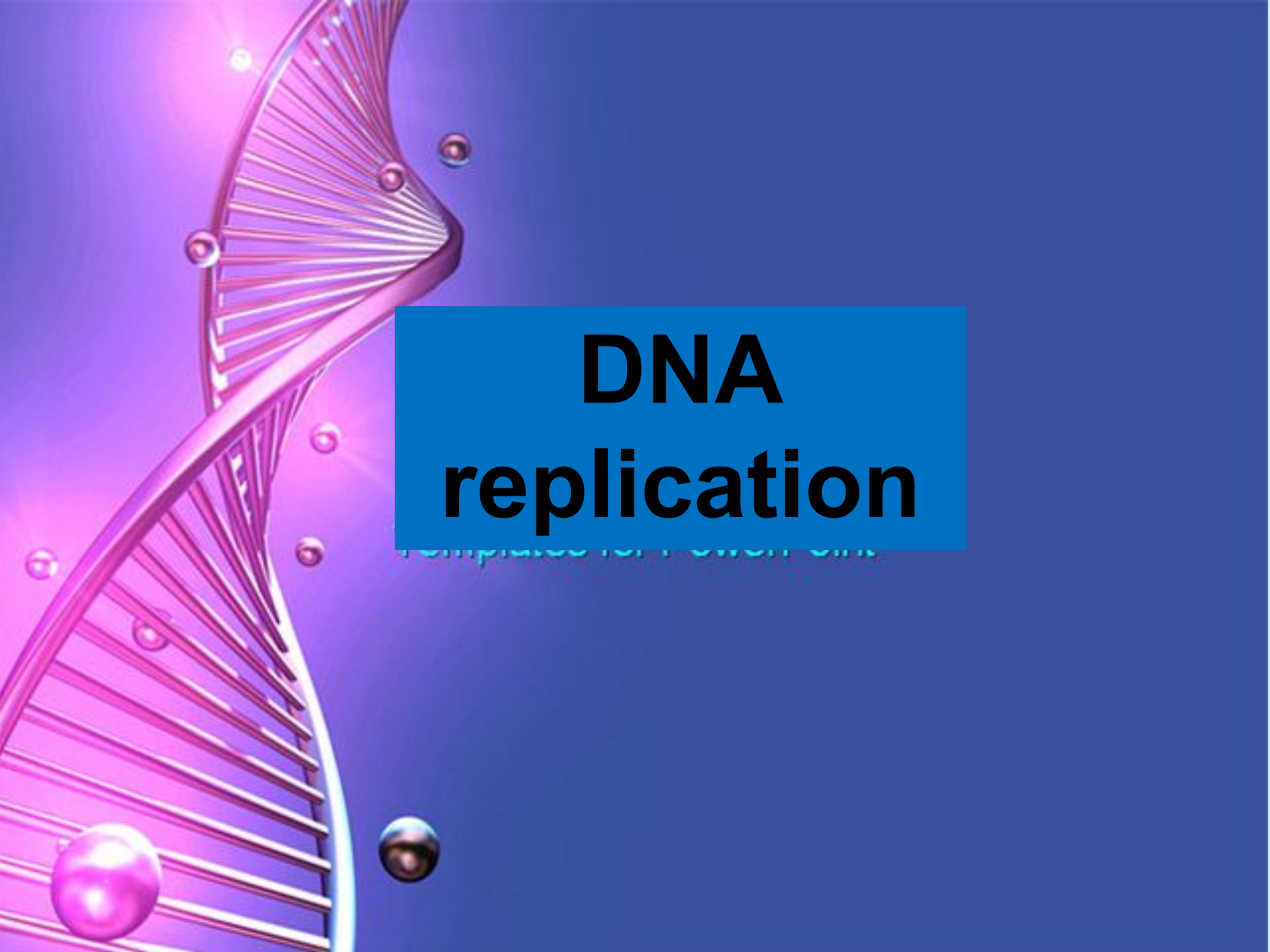
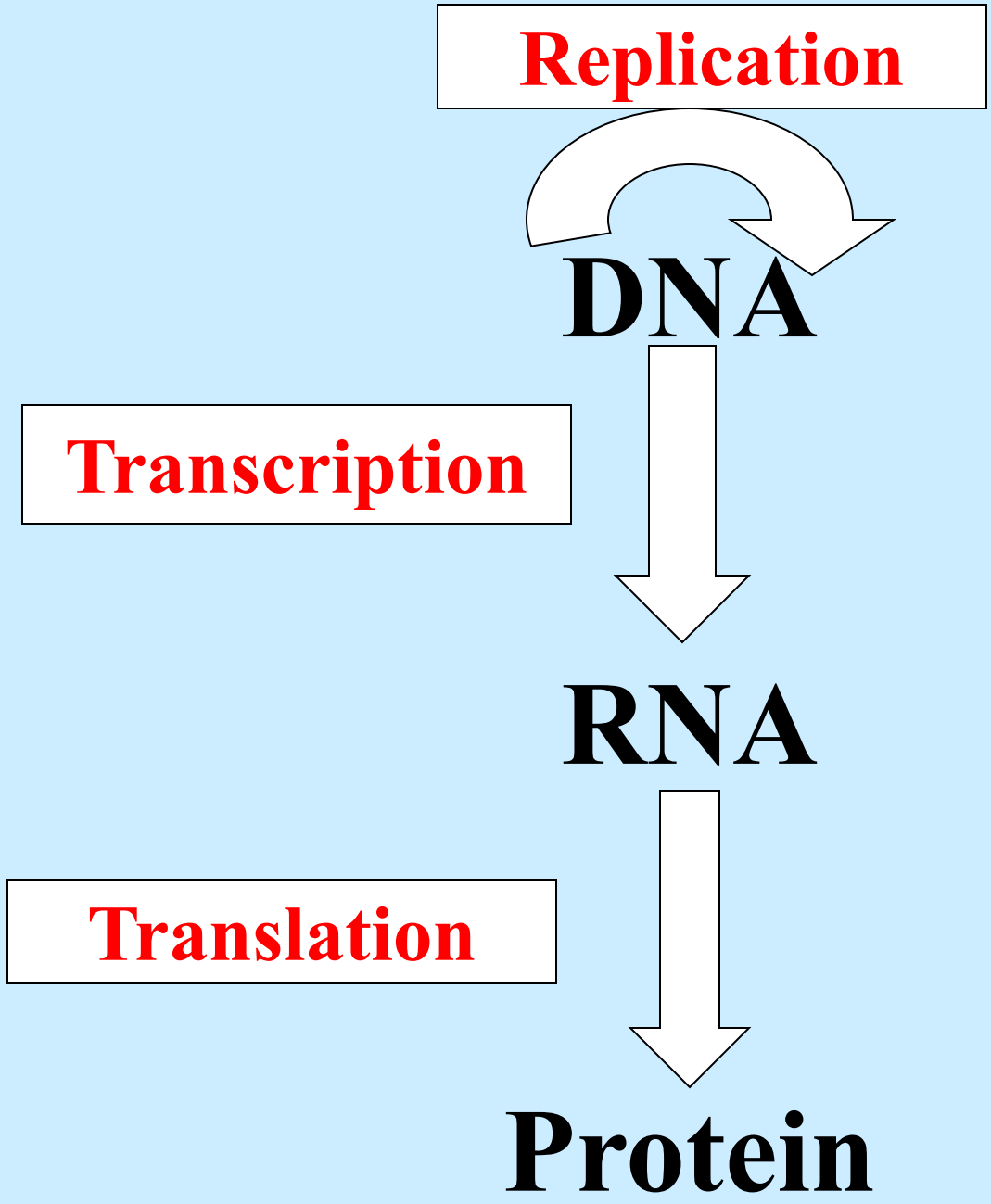
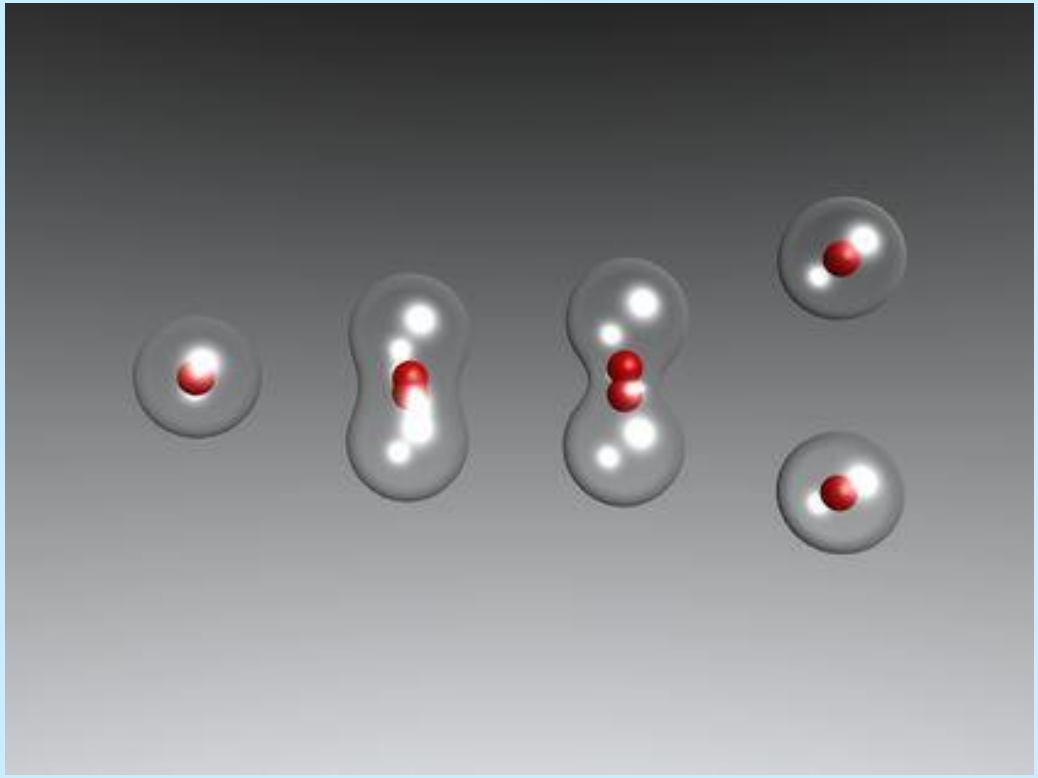

DNA
replication


Replication
DNA
Transcription
RNA
Translation
Protein

DNA replication

Occurs during cell division.



Replication: **is synthesis of daughter nucleic acid molecules identical to the parental nucleic acid.**

Replication of the DNA proceeds in stages:

- **Initiation**
- **Elongation**
- **Termination**

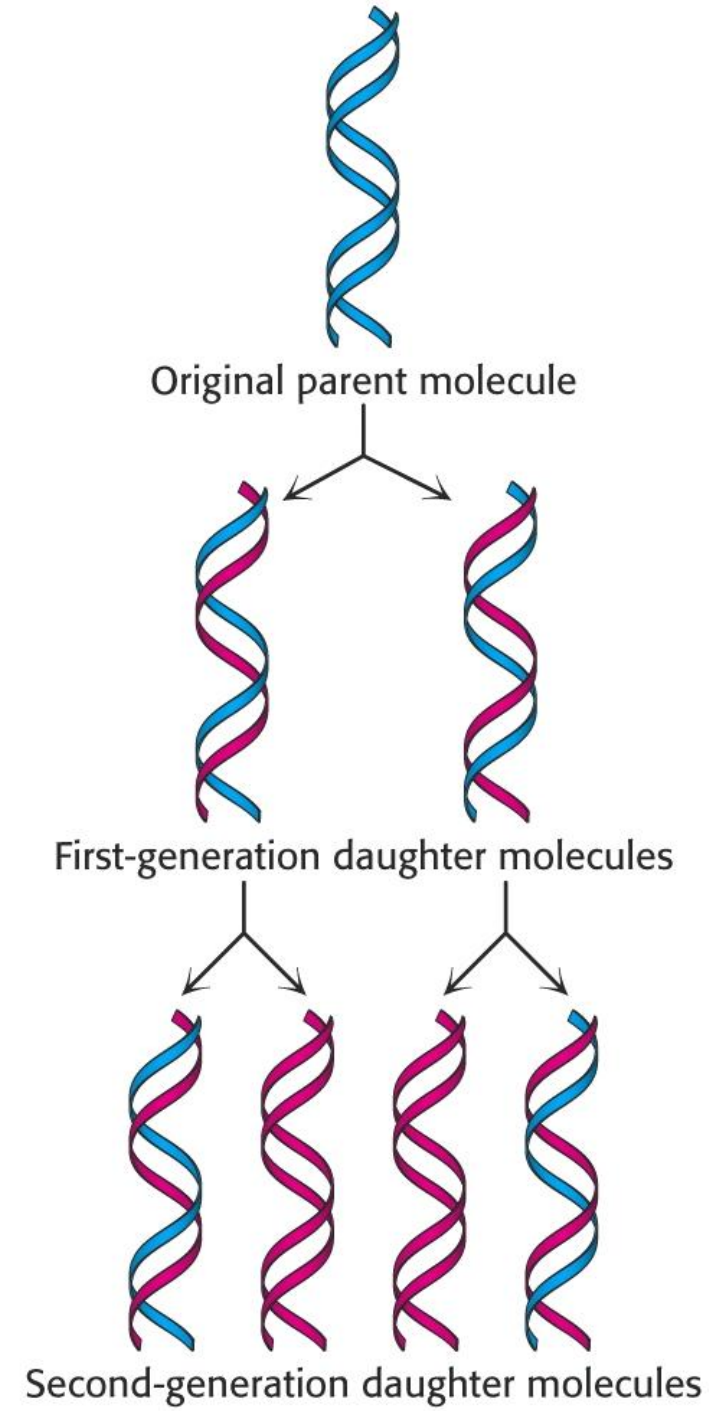
- **DNA replication requires many enzymes and protein factors.**
- **This complex has been termed**

the DNA replicase system or replisome.

The Watson-Crick Model

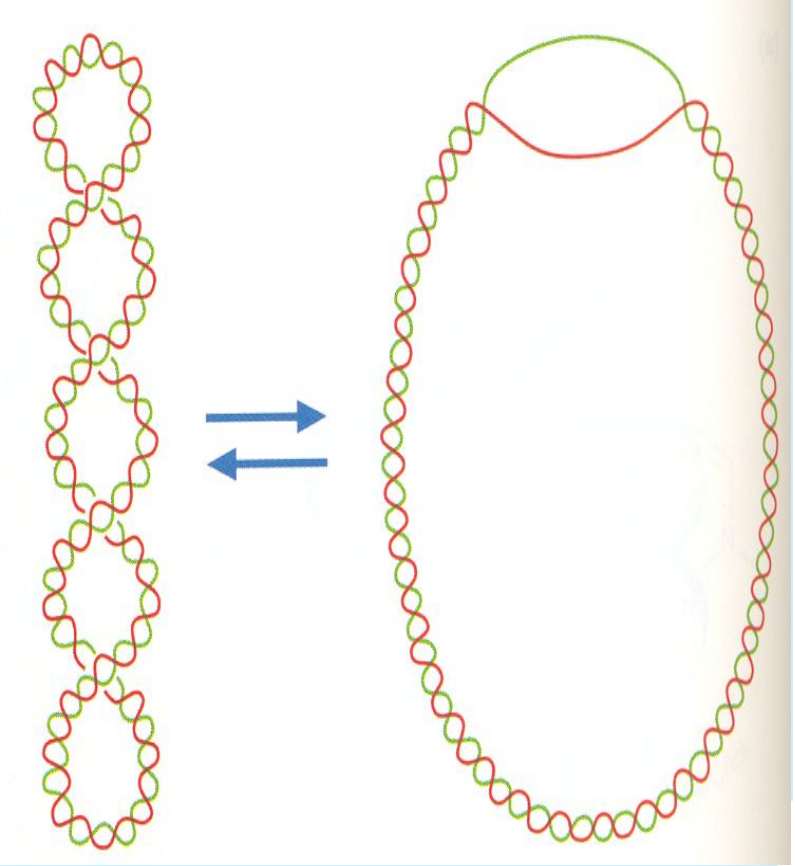
- **Semi-conservative replication of DNA**

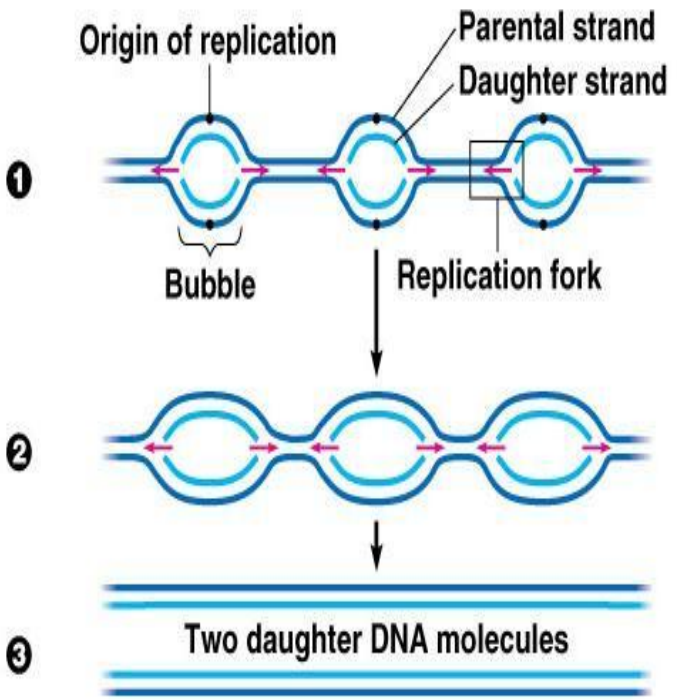
- **Replication is very accurate.**

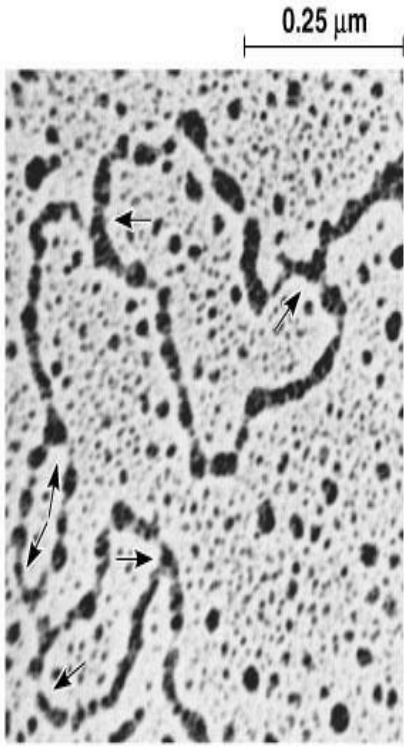


DNA Replication

1) Initiation







The initiation point where the splitting starts is called "origin of replication".

Origins of replication

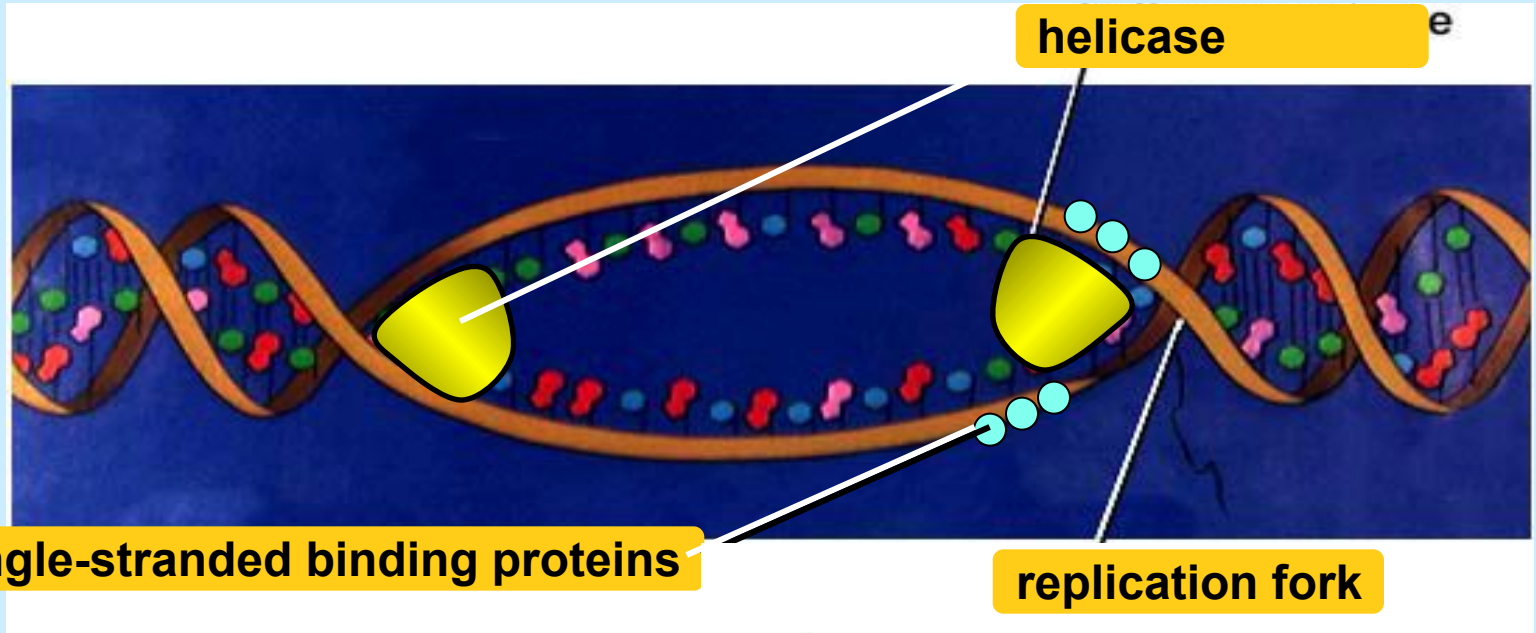
Replication Bubbles:

a. Hundreds of replicating bubbles (Eukaryotes).

b.Single replication fork (bacteria).

Initiation of replication: 1st step (Strand Separation)

1. **Helicase - unwind short segment of the parental DNA bidirectionally and create two replication forks; Helicase hydrolyzes ATP in order to break the hydrogen bonds between DNA strands**
2. **Single stranded DNA binding proteins (SSB) stabilize the separated strands and prevent renaturation of DNA**

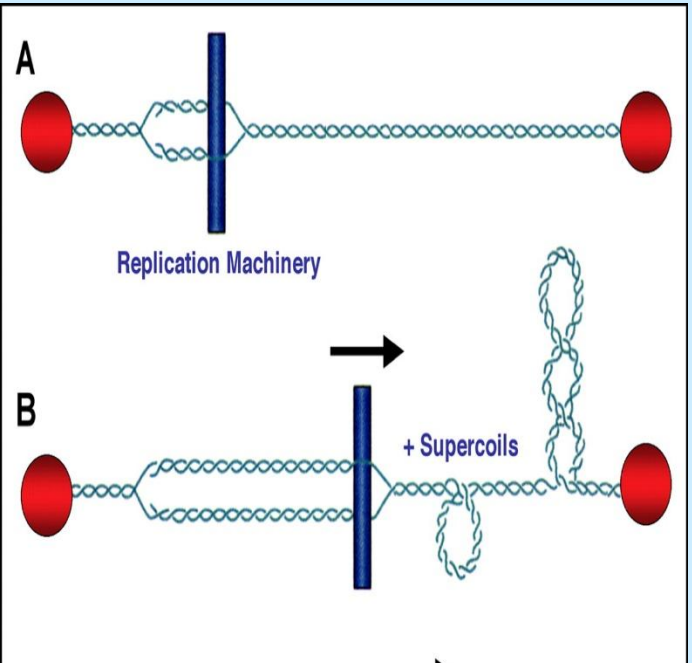


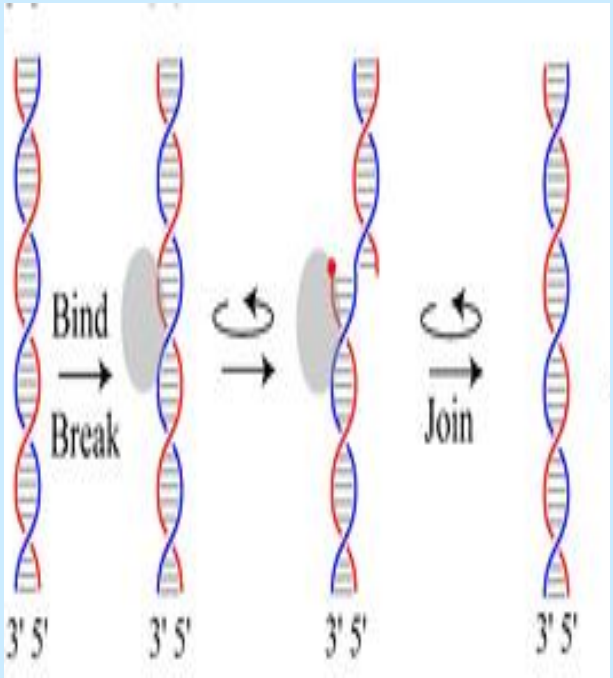
Initiation of DNA Replication

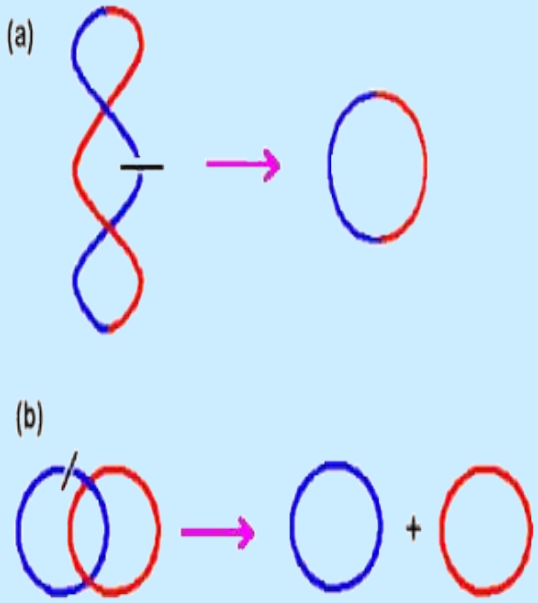
- Strand Separation:

  3. Topoisomerase: enzyme which relieves stress on the DNA molecule by allowing free rotation around a single strand.

Topoisomerase I **Topoisomerase II**



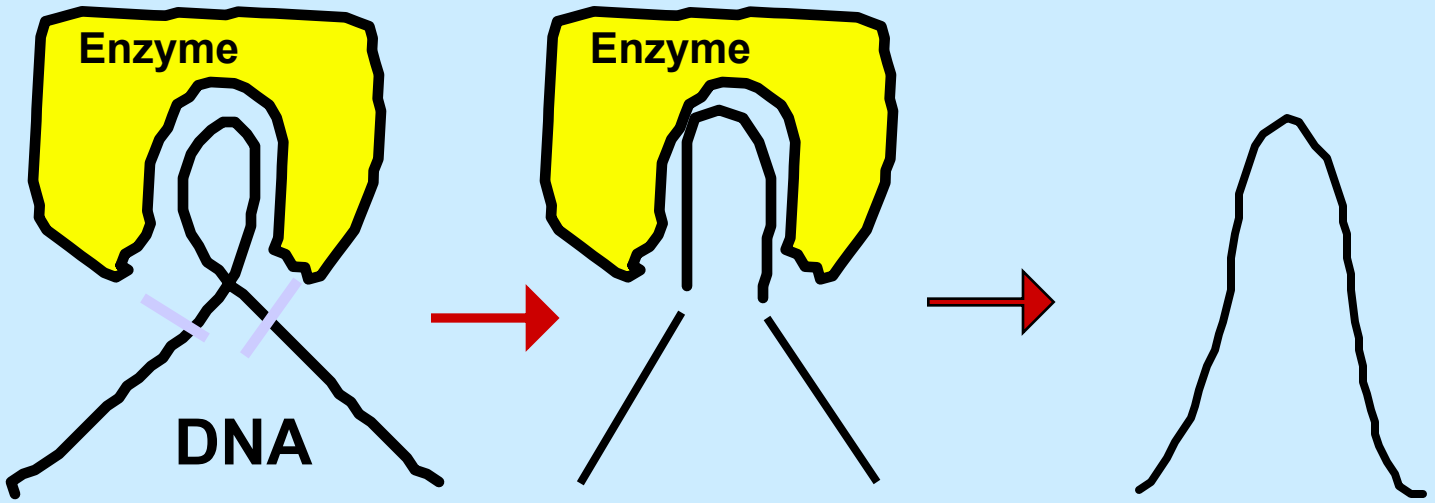




DNA Replication

- Strand Separation:

  3. Topoisomerase: enzyme which relieves stress on the DNA molecule by allowing free rotation around a single strand.



2. Elongation - Both Template strands are copied at a Replication Fork

- DNA replication is cataly by DNA polymerase which needs an RNA primer. DNA Polymerase cannot Initiate new strands, because unable to covalently link the 2 individual nucleotides together.
- RNA primase synthesizes primer on DNA strand
- DNA polymerase adds nucleotides to the 3′ end of the growing strand

DNA Replication

2. Elongation

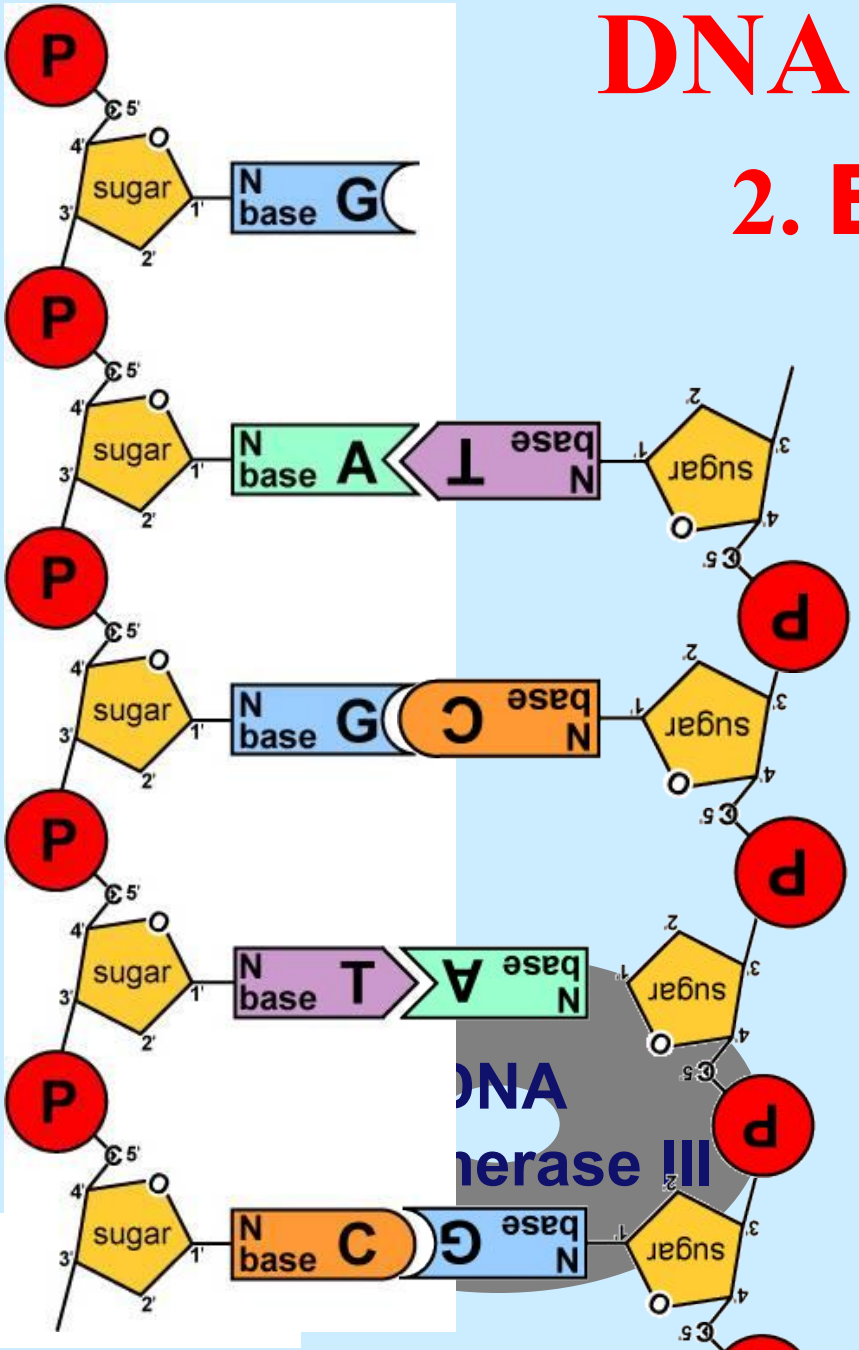
- Priming:

  1. RNA primers: before new DNA strands can form, there must be small pre-existing primers (RNA) present to start the addition of new nucleotides (DNA Polymerase).

  2. Primase: enzyme that polymerizes (synthesizes) the RNA Primer.

DNA Replication

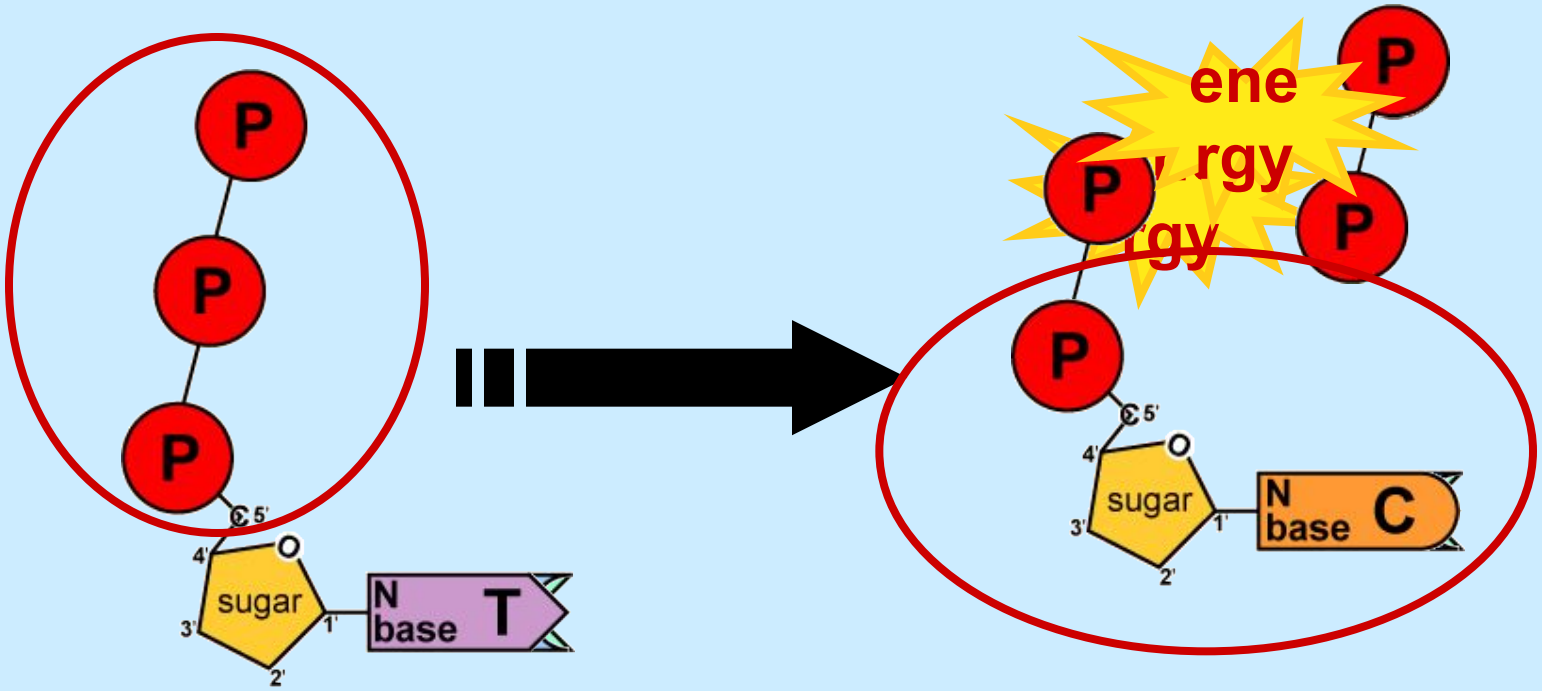
2. Elongation



- DNA polymerase III keeps pace with the replication fork.
- On the leading (forward) strand, the DNA is synthesized continuously in the direction taken by the replication fork

Energy of Replication

Where does energy for bonding *usually* come from?



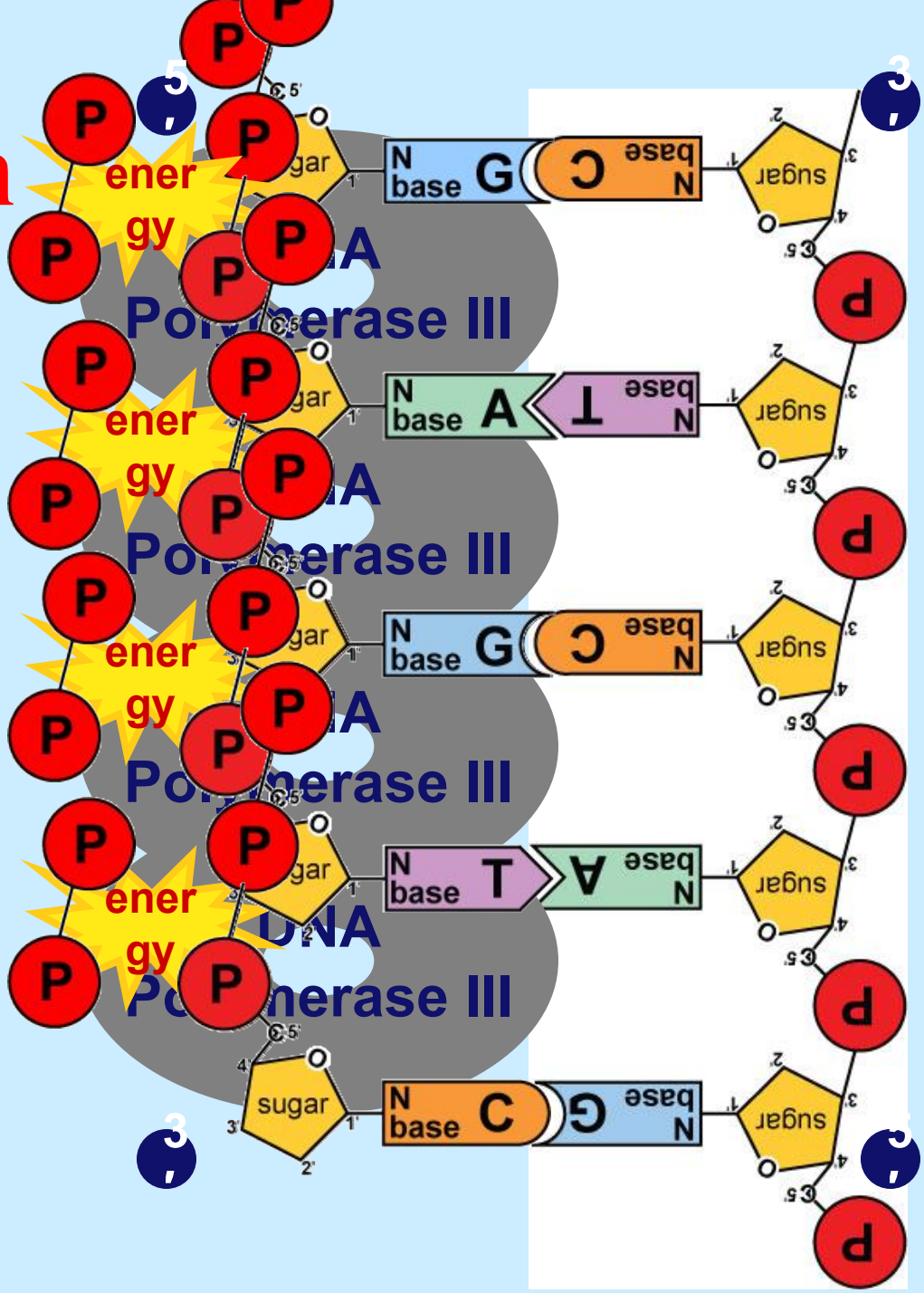
CTP

CMP

modified nucleotide

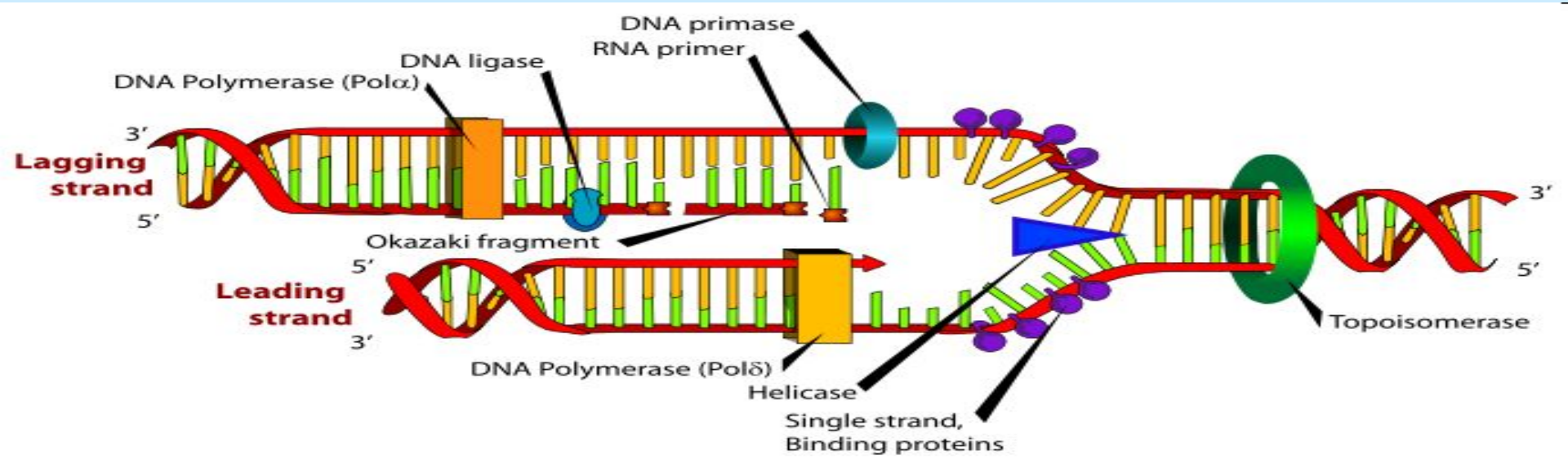
Replication

- Adding bases
  - can only add nucleotides to 3′ end of a growing DNA strand
    - need a “starter” nucleotide to bond to
  - strand only grows 5′→3′



The Mechanism of DNA Replication

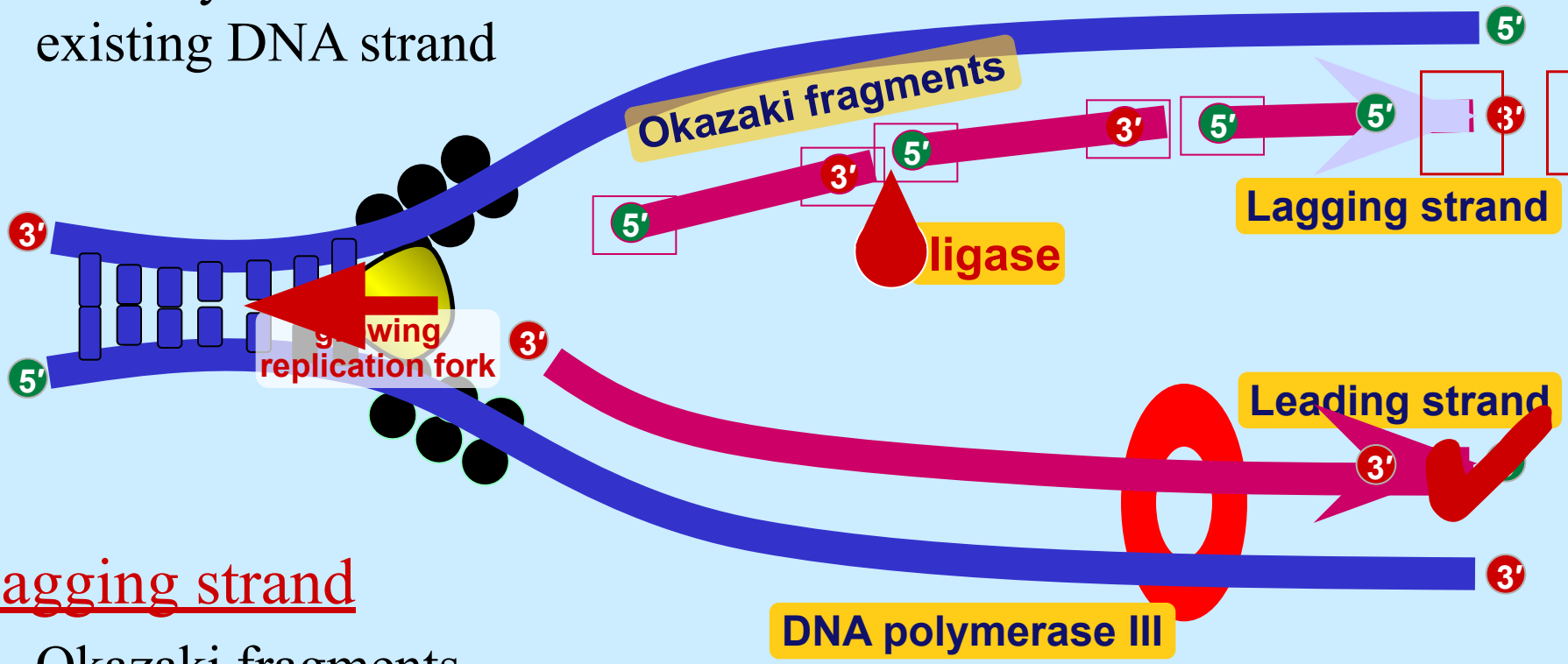
- DNA synthesis on the leading strand is continuous
- The lagging strand grows the same *general* direction as the leading strand (in the same direction as the Replication Fork). However, DNA is made in the 5′-to-3′ direction
- Therefore, DNA synthesis on the lagging strand is discontinuous
- DNA is added as short fragments (Okasaki fragments) that are subsequently ligated together



Leading & Lagging strands

Limits of DNA polymerase III

– can only build onto 3′ end of an existing DNA strand



Lagging strand

– Okazaki fragments

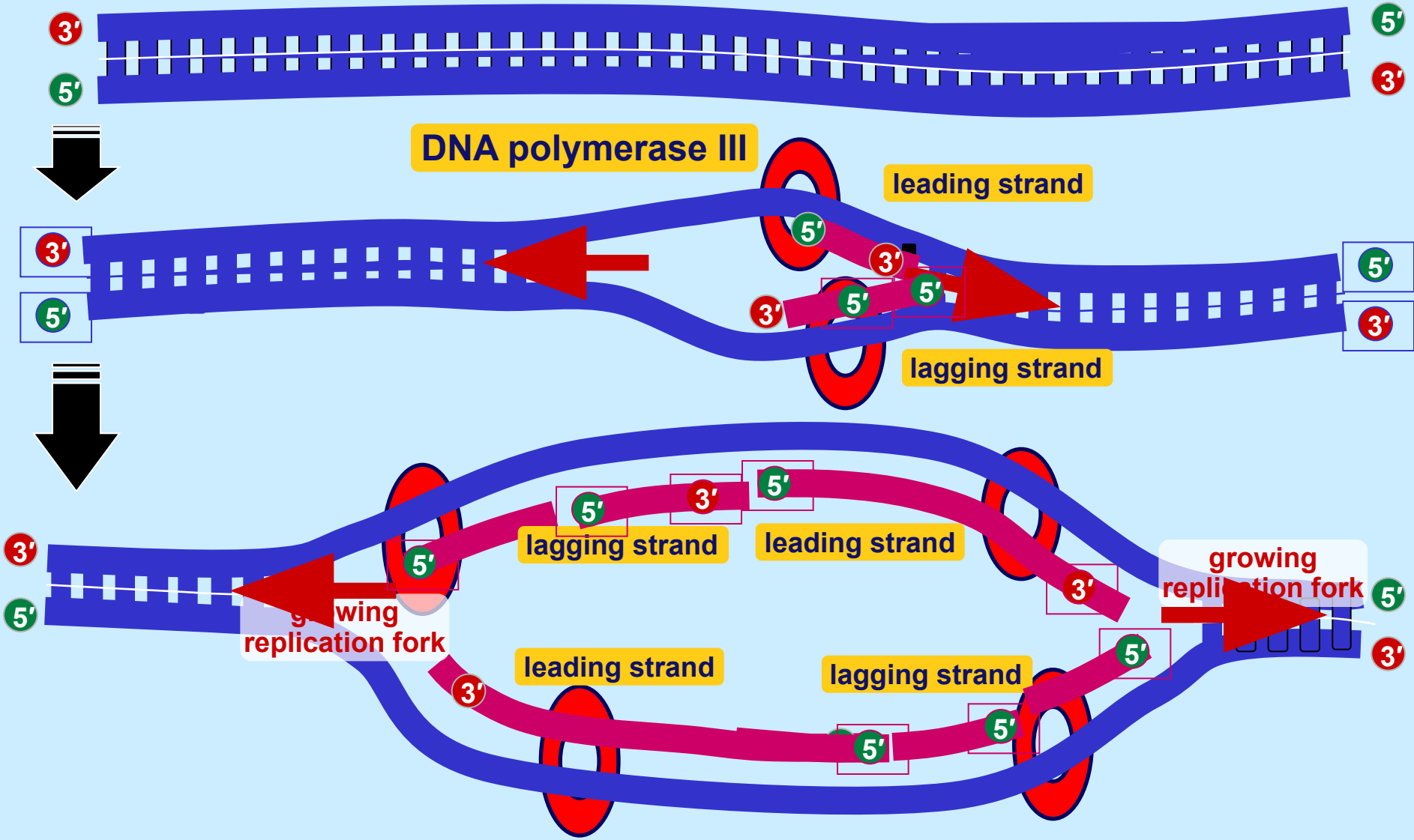
– joined by ligase

- "spot welder" enzyme

Leading strand

– continuous synthesis

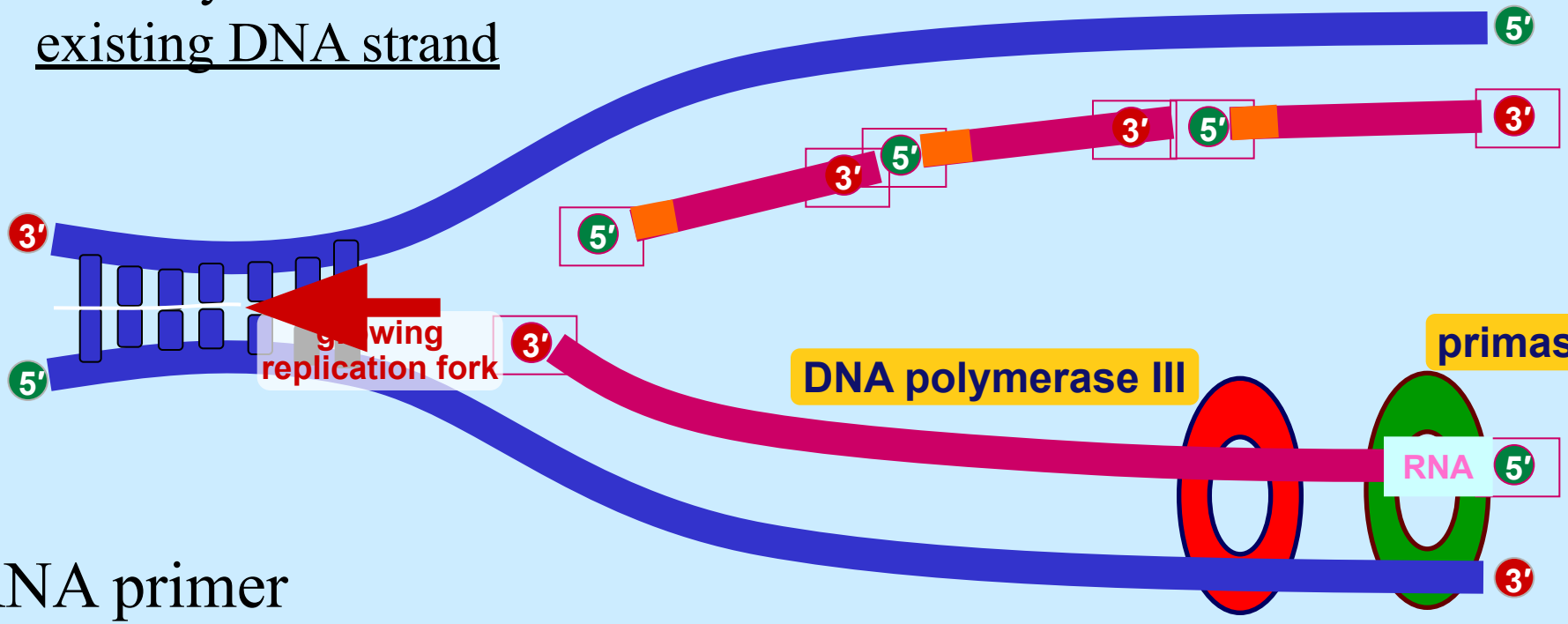
Replication fork / Replication bubble



Starting DNA synthesis: RNA primers

Limits of DNA polymerase III

– can only build onto 3′ end of an existing DNA strand

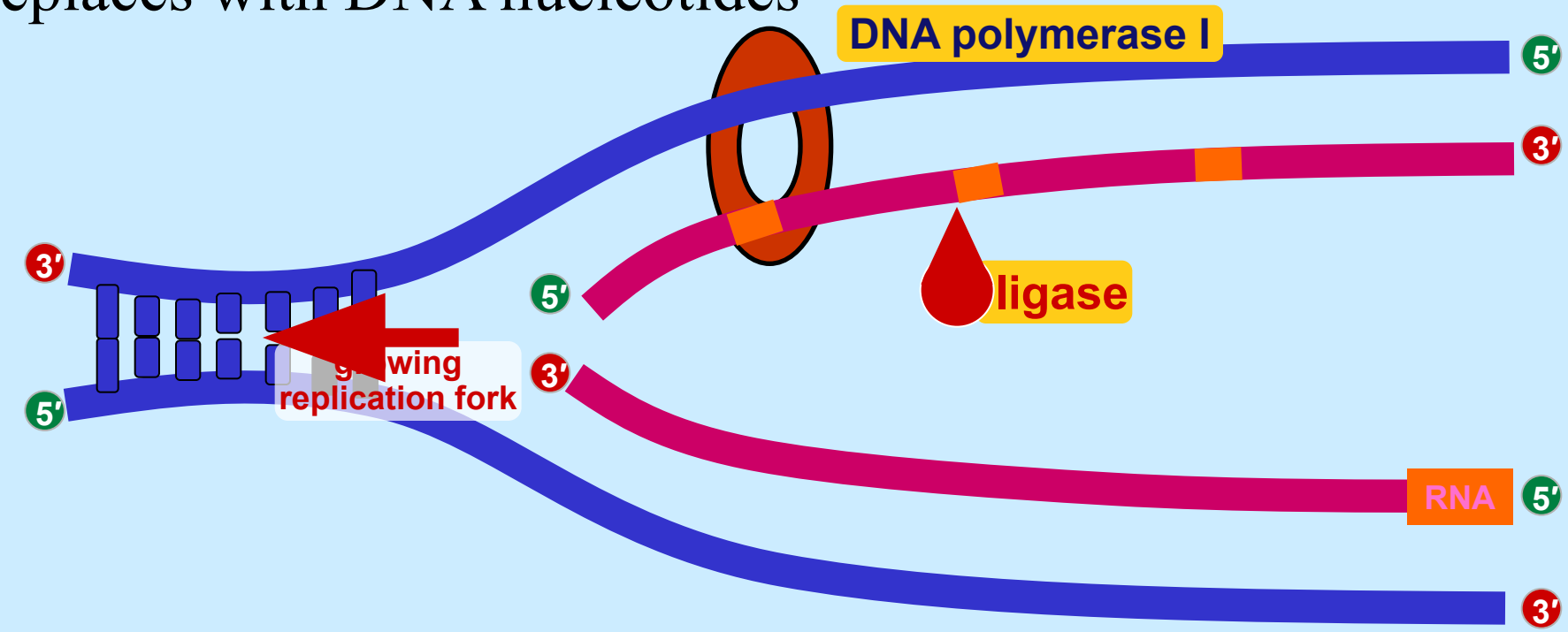


RNA primer

– built by primase
– serves as starter sequence for DNA polymerase III

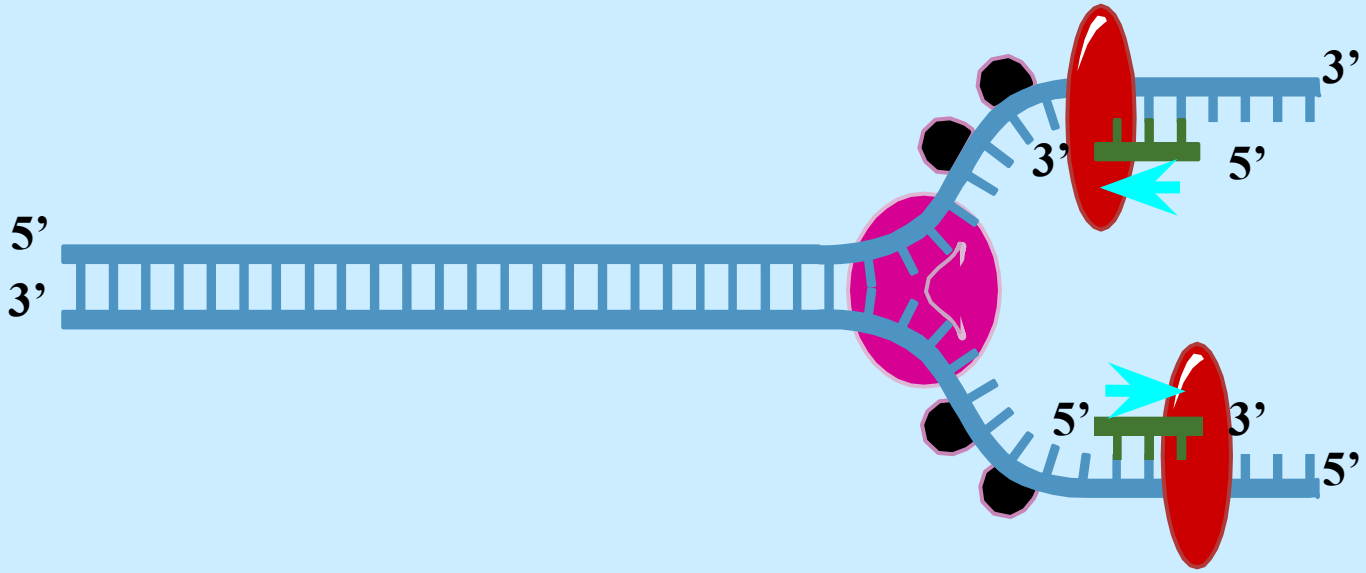
Replacing RNA primers with DNA

DNA polymerase I removes sections of RNA primer and replaces with DNA nucleotides



- Polymerase activity of DNA polymerase I fills the gaps.
- Ligase forms bonds between sugar-phosphate backbone.

Replication

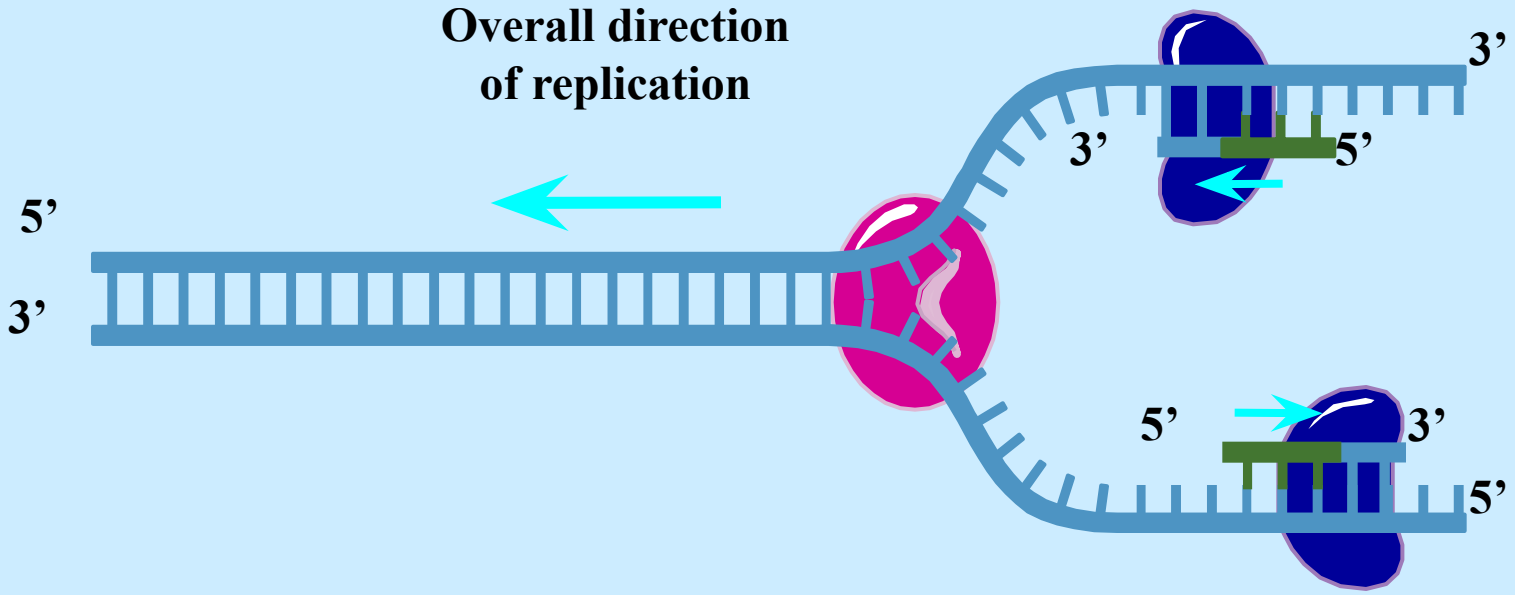


Helicase protein binds to DNA sequences called origins and unwinds DNA strands.

Binding proteins prevent single strands from rewinding.

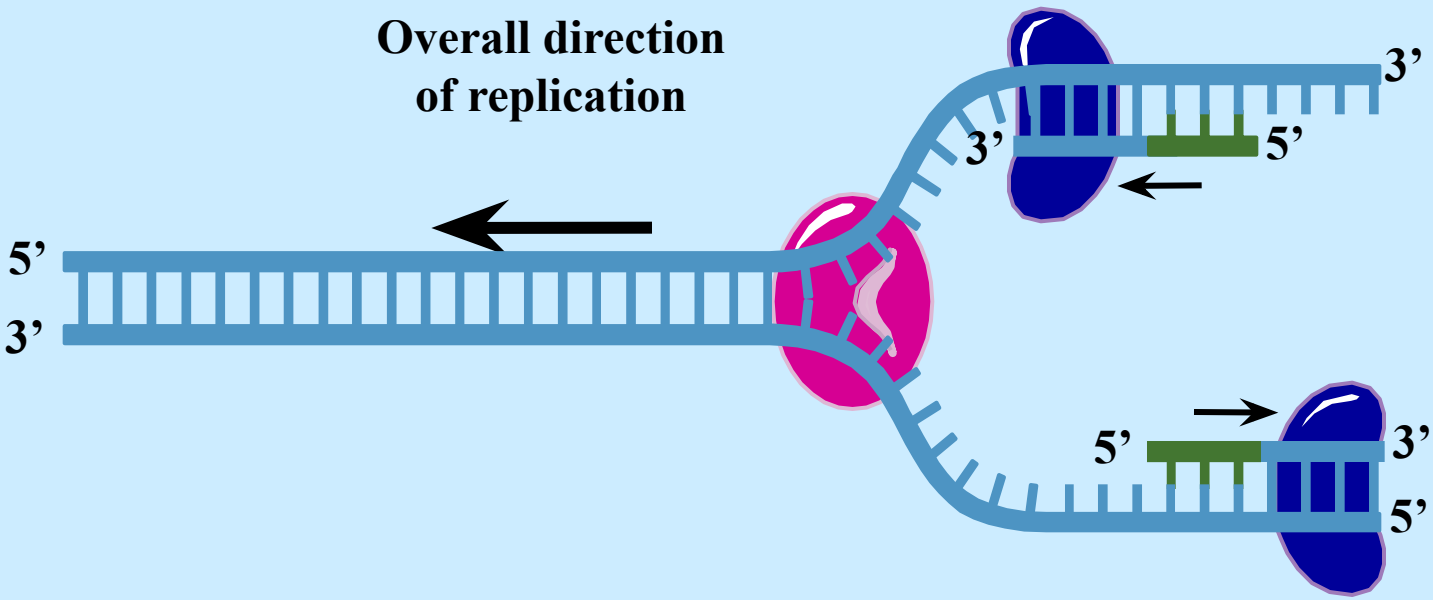
Primase protein makes a short segment of RNA complementary to the DNA, a primer.

Replication



DNA polymerase enzyme adds DNA nucleotides to the RNA primer.

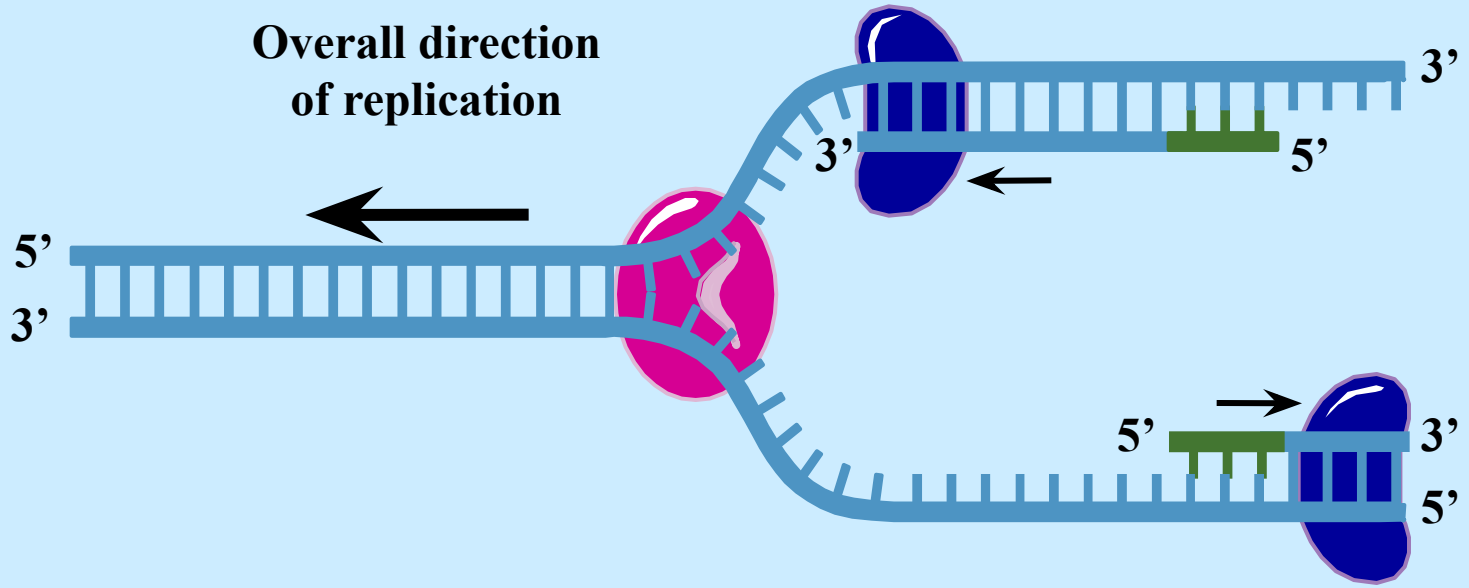
Replication



DNA polymerase enzyme adds DNA nucleotides to the RNA primer.

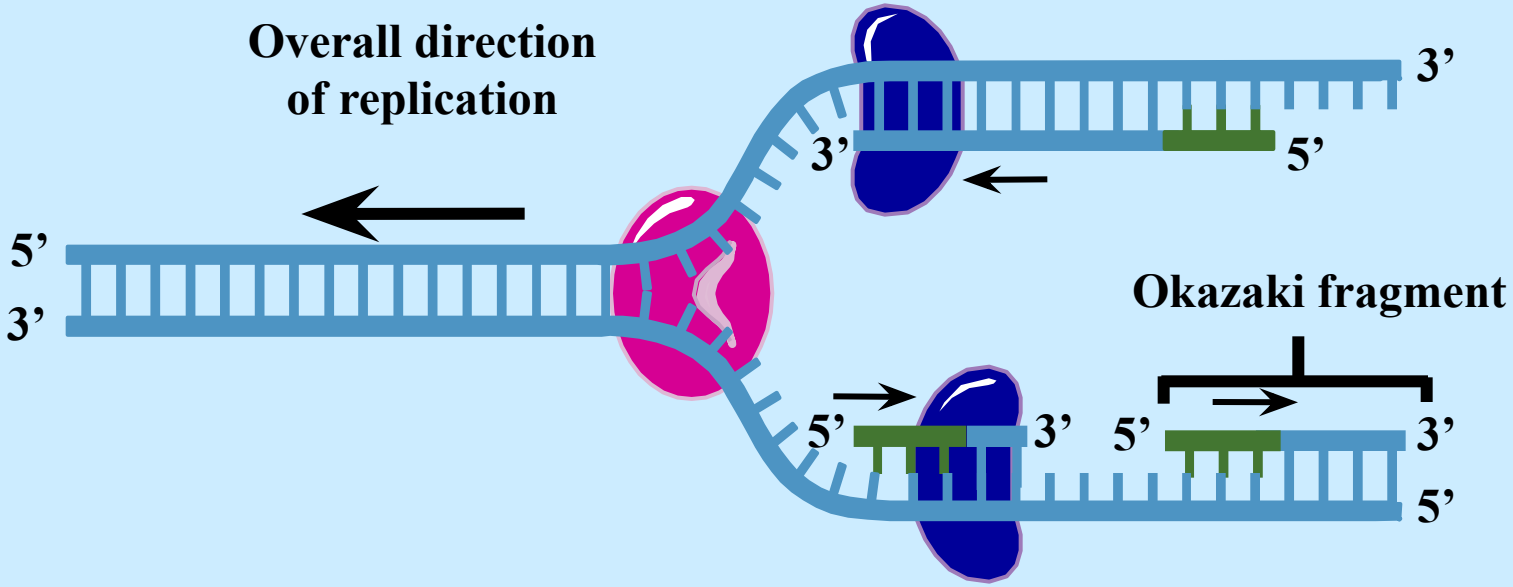
DNA polymerase proofreads bases added and replaces incorrect nucleotides.

Replication



Leading strand synthesis continues in a 5’ to 3’ direction.

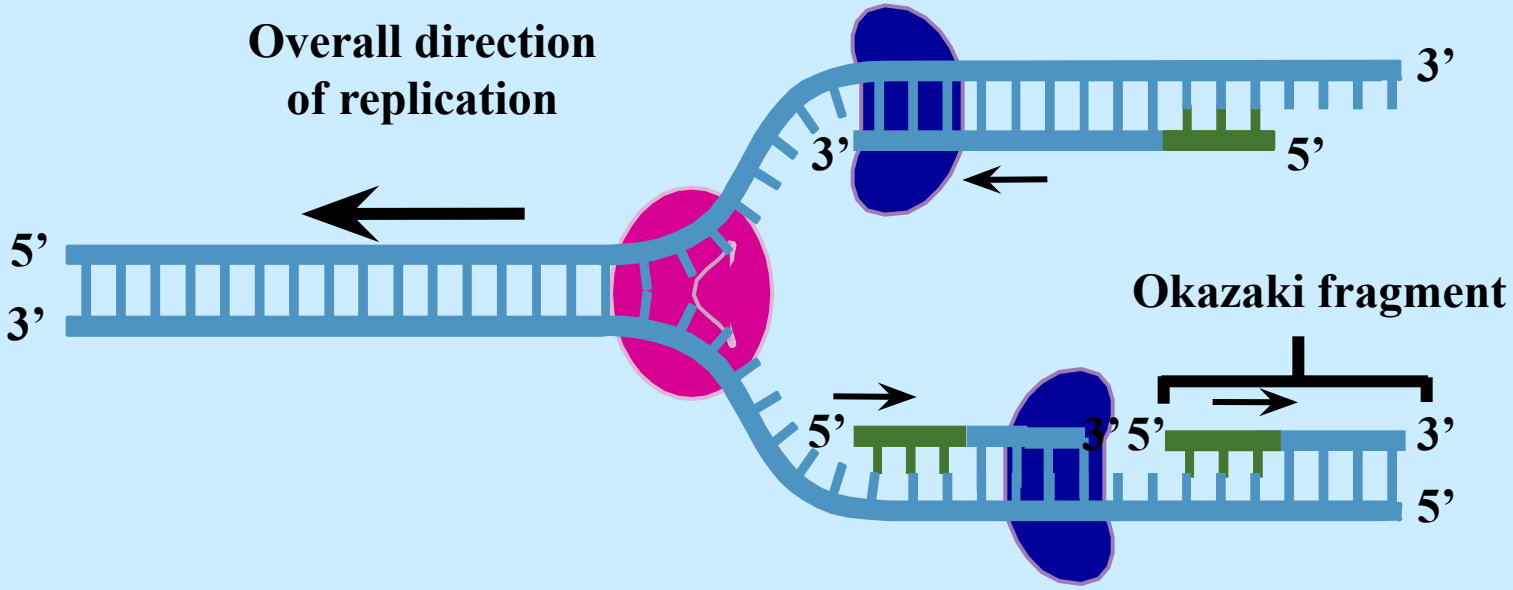
Replication



Leading strand synthesis continues in a 5’ to 3’ direction.

Discontinuous synthesis produces 5’ to 3’ DNA segments called Okazaki fragments.

Replication



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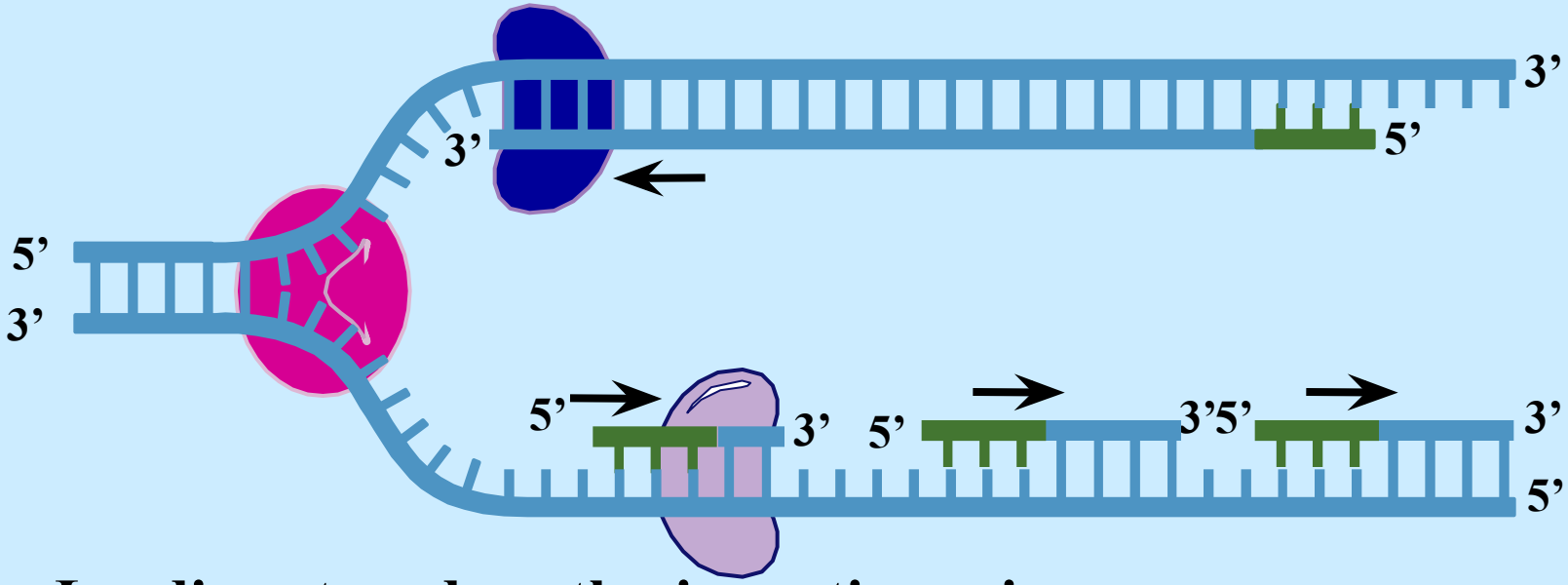

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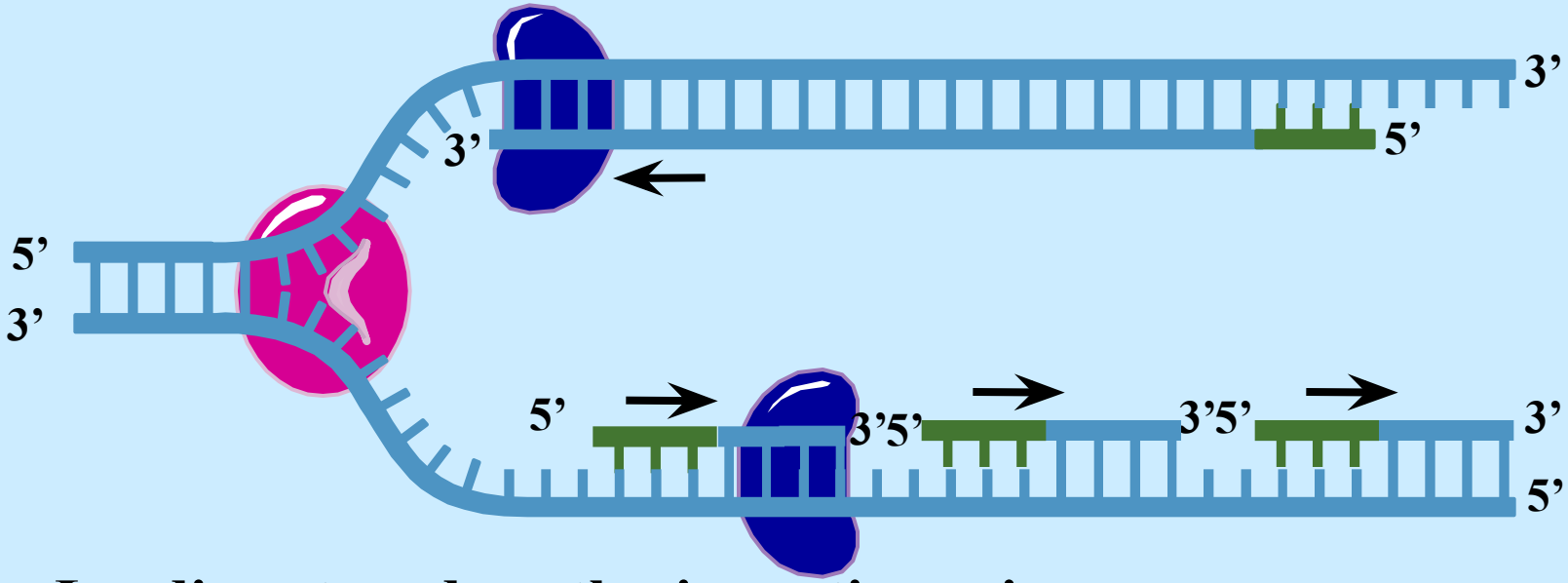

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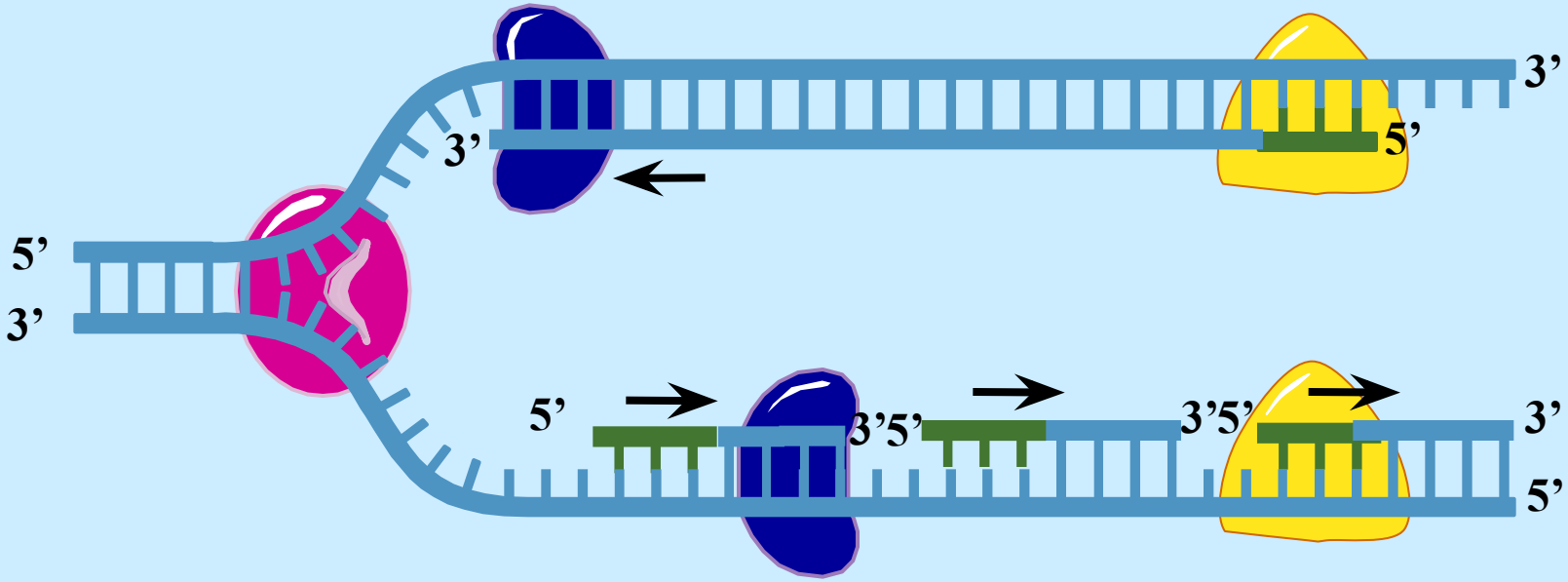
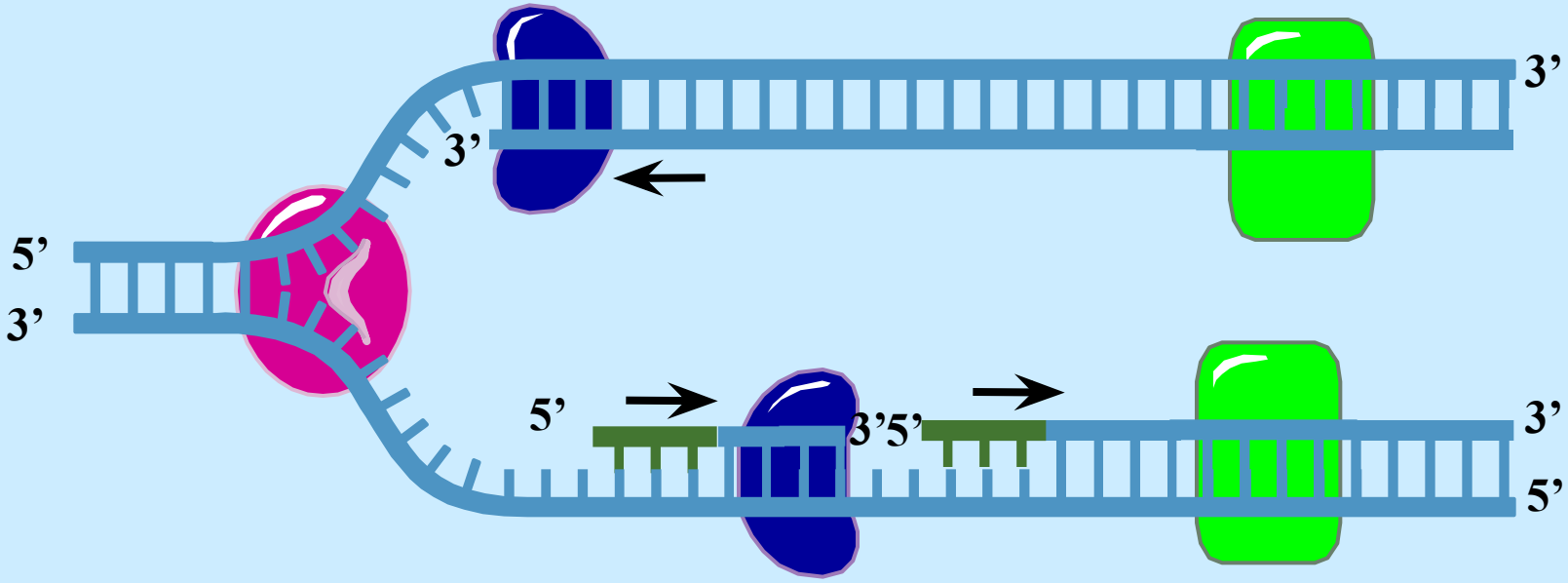


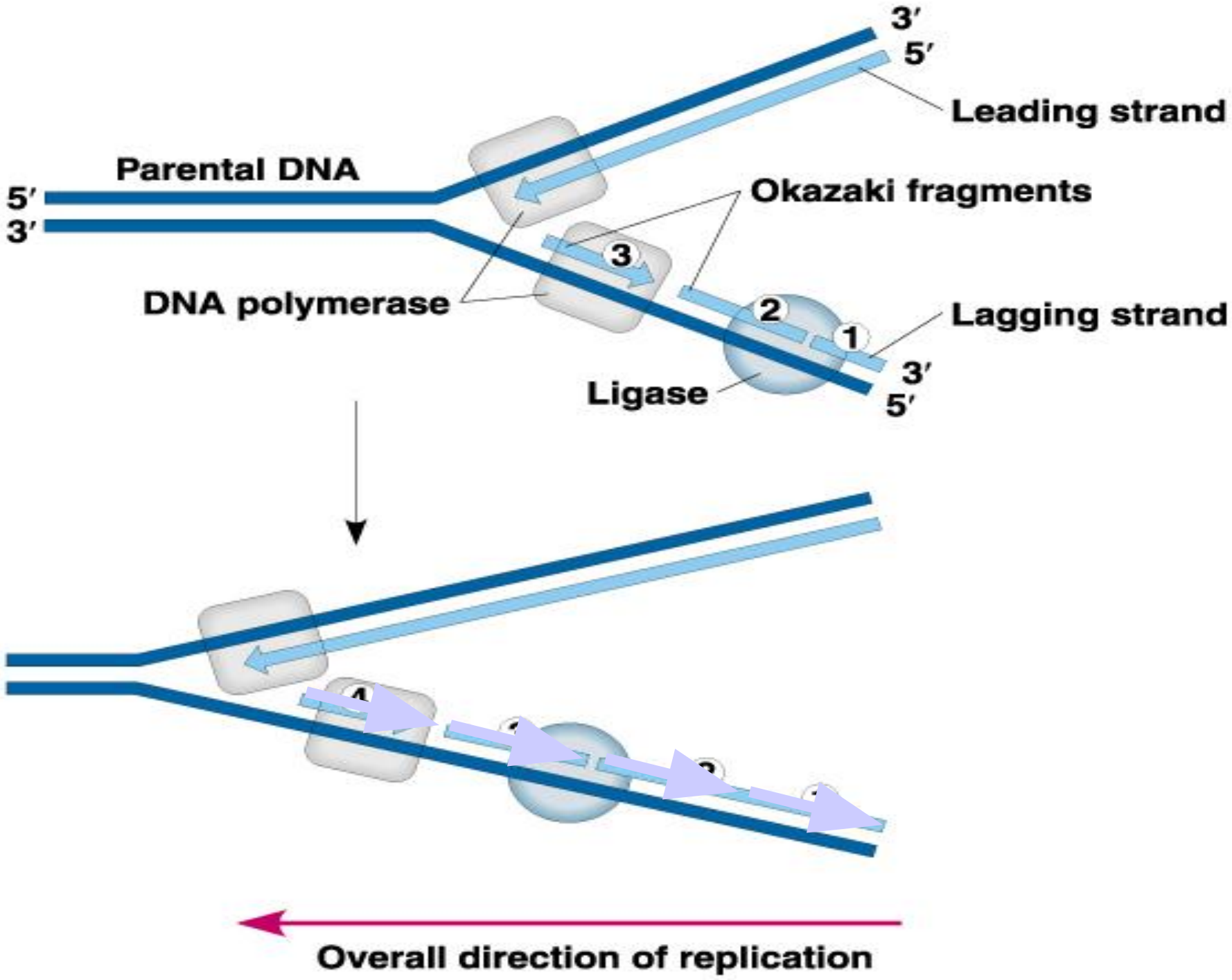
Exonuclease activity of DNA polymerase I removes RNA primers.

Replication



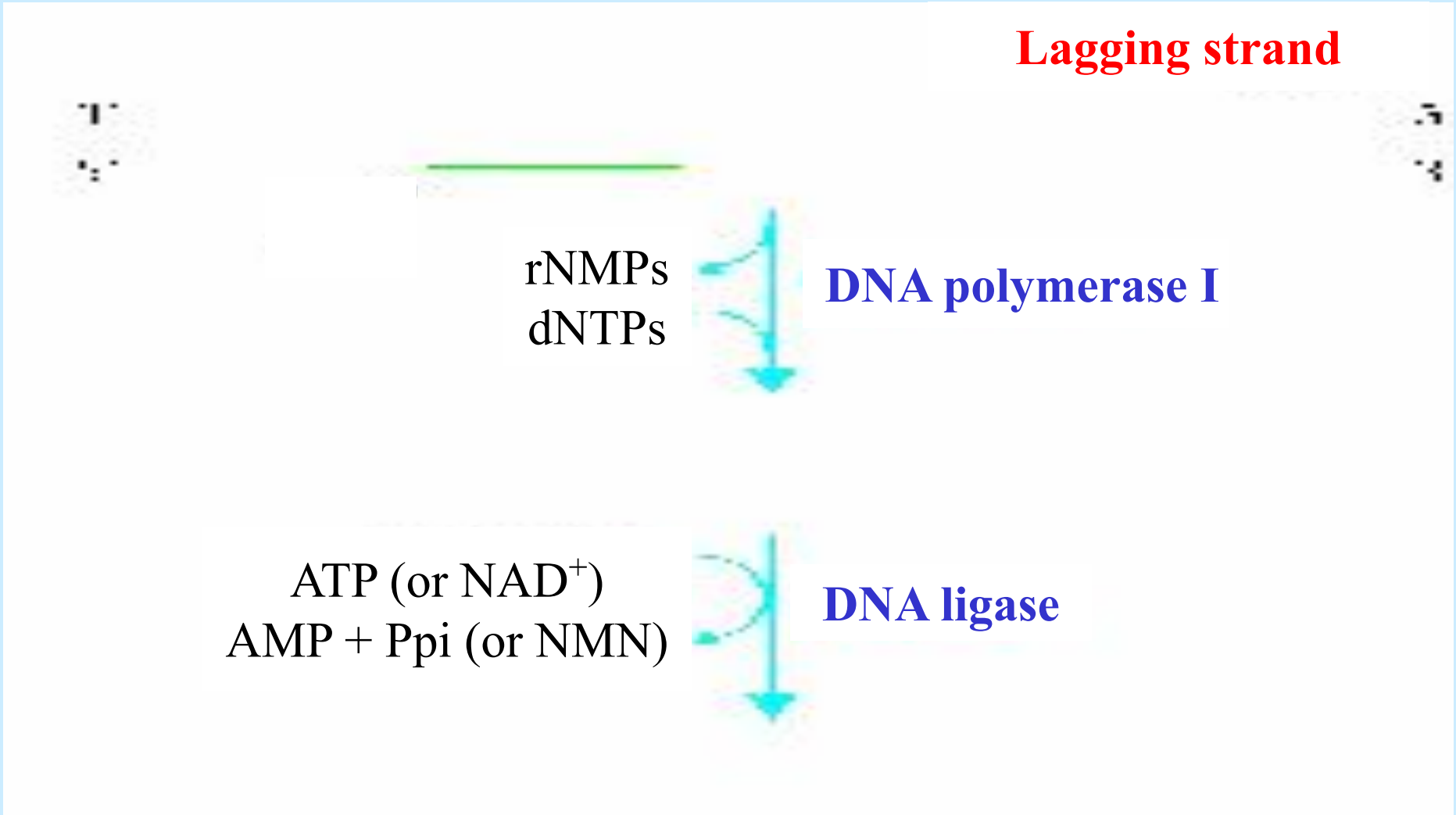
Polymerase activity of DNA polymerase I fills the gaps.

Ligase forms bonds between sugar-phosphate backbone.

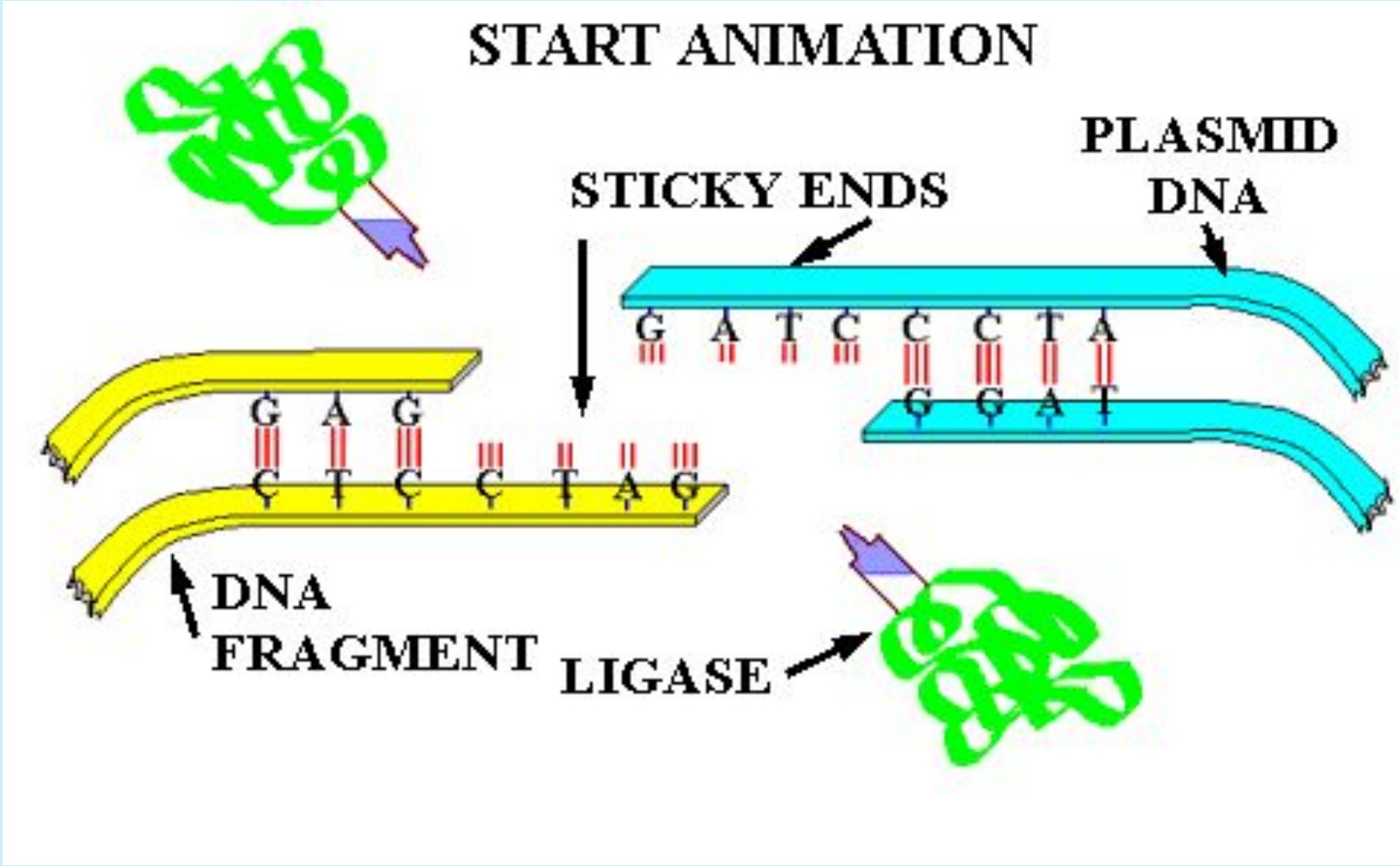

3′
5′
Leading strand
Parental DNA
5′
3′
Okazaki fragments
3
DNA polymerase
2
1
Lagging strand
3′
Ligase
5′
Overall direction of replication

Final step in the synthesis of lagging strand segments.

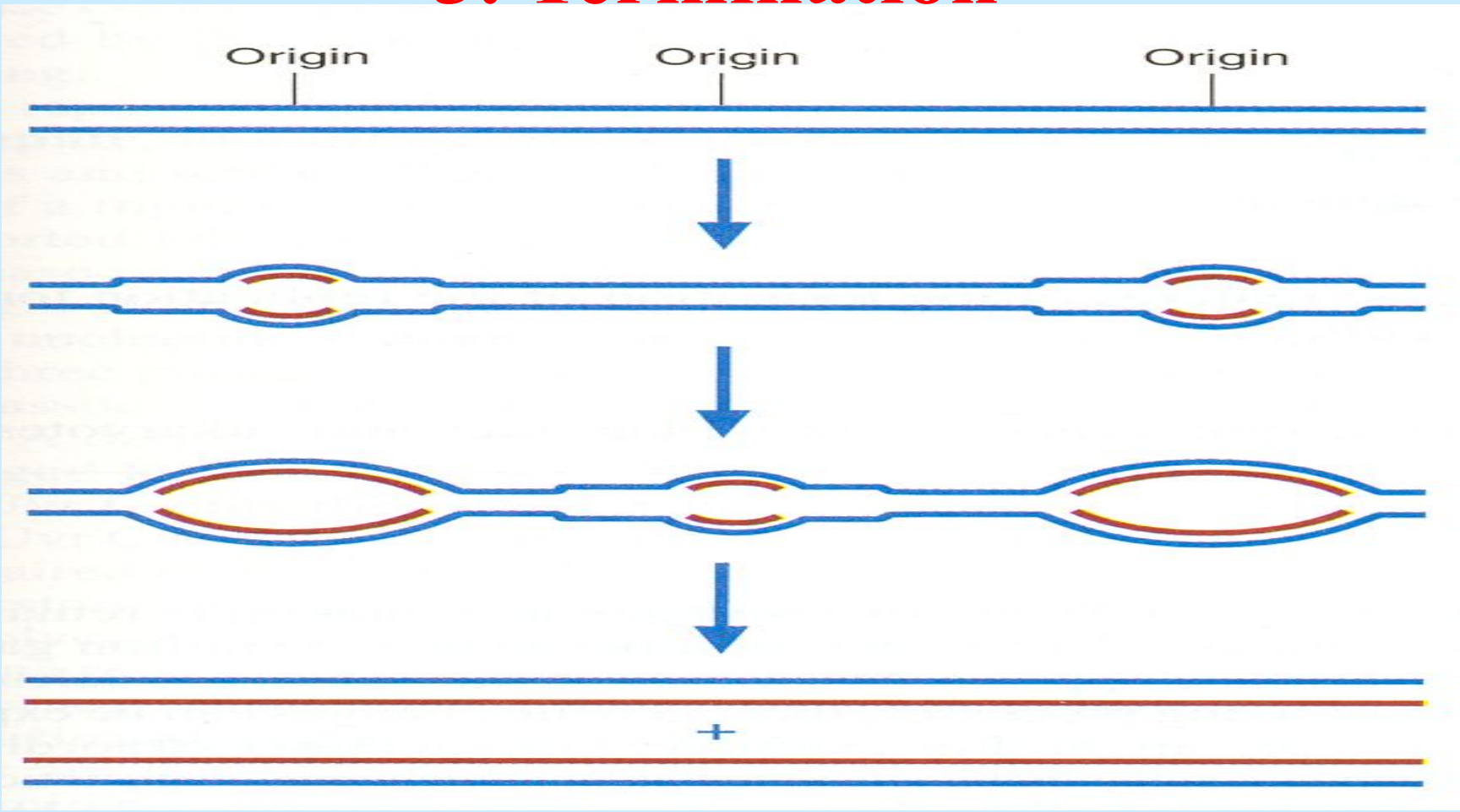
DNA ligase **catalyzes the formation of the phosphodiester bond between pieces of DNA.**



DNA ligase adds sugar phosphate back-bone between the Okazaki ragments (fill in gaps)

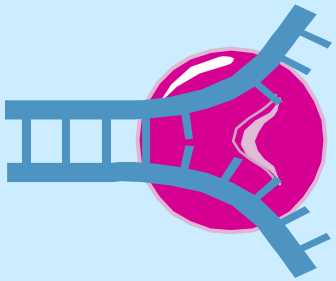


3. Termination

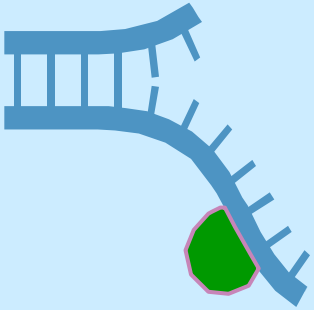


This process happens when the DNA Polymerase reaches to an end of the strands. The DNA Replication is not completed before a mechanism of repair fixes possible errors caused during the replication. Enzymes like nucleases remove the wrong nucleotides and the DNA Polymerase fills the gaps.

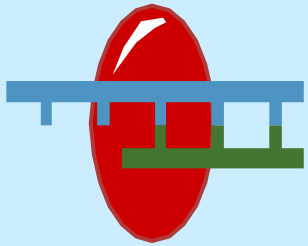
Enzymes in DNA replication



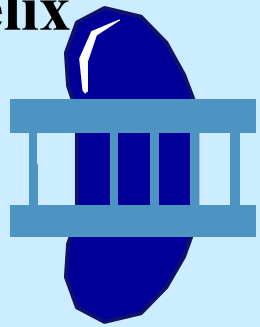
Helicase unwinds parental double helix



Binding proteins stabilize separate strands

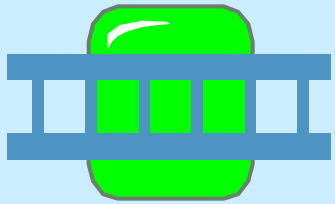


Primase adds short primer to template strand



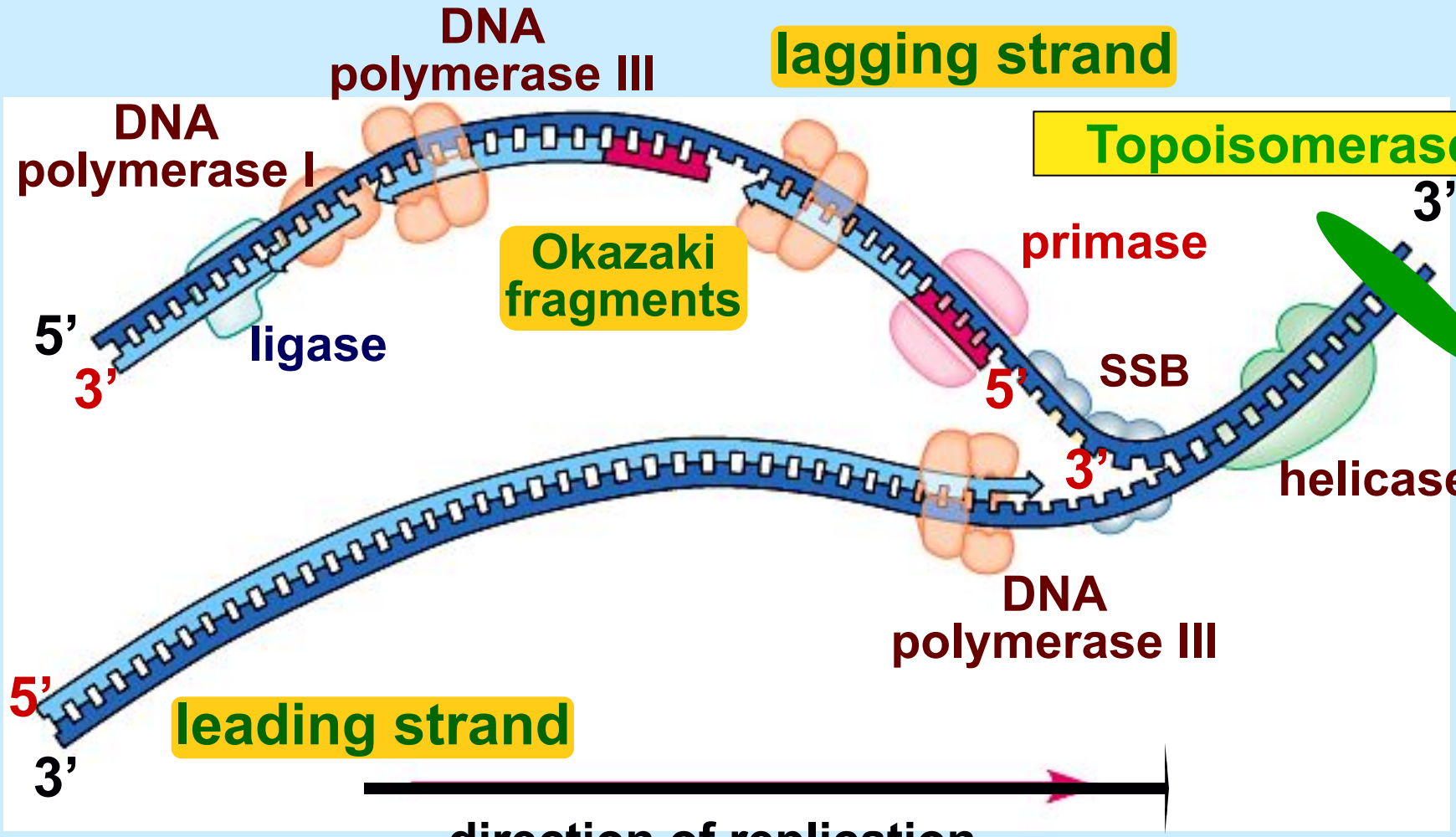
DNA polymerase III binds nucleotides to form new strands

DNA polymerase I (removes RNA primer and inserts the correct bases



Ligase joins Okazaki fragments

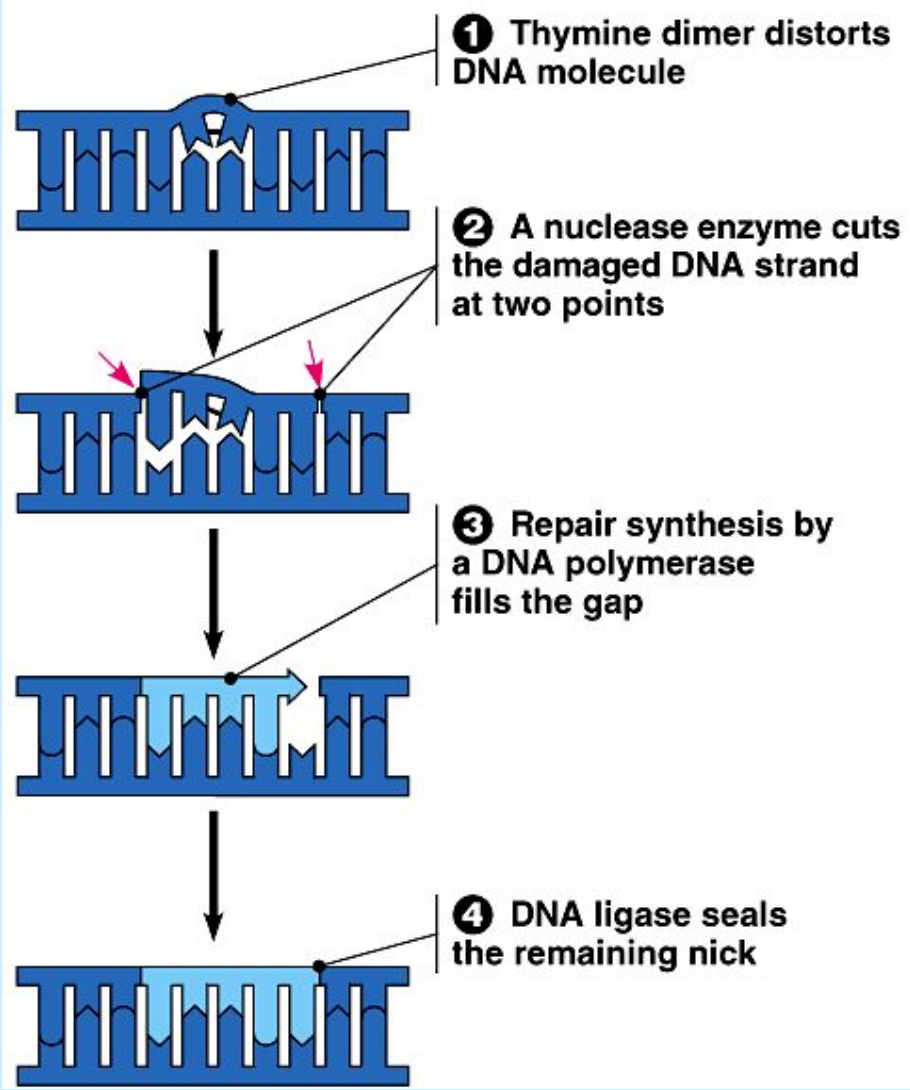
Summary of DNA replication

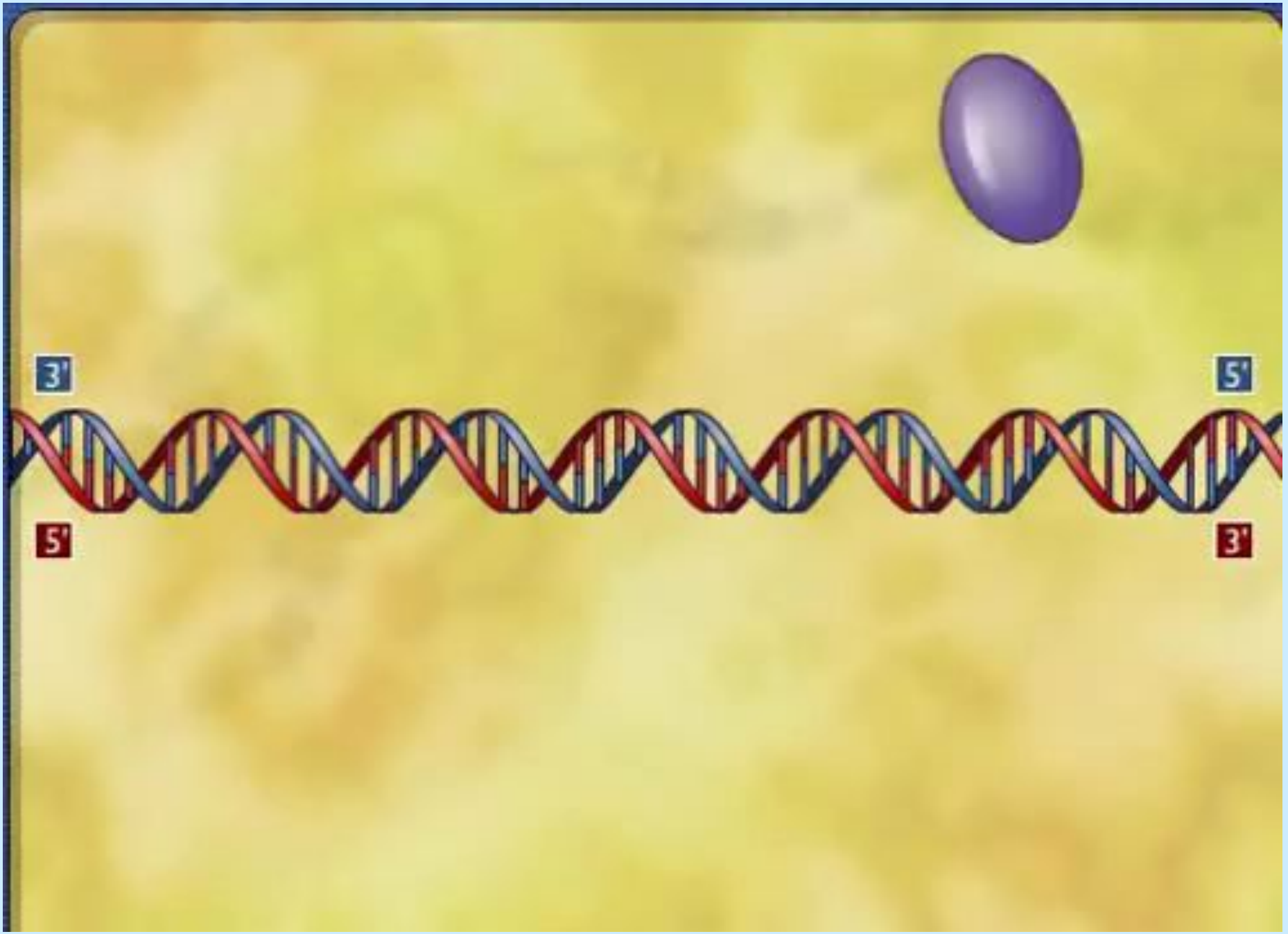


SSB = single-stranded binding proteins

Editing & proofreading DNA

- 1000 bases/second = lots of typos!
- DNA polymerase I
  - proofreads & corrects typos
  - repairs mismatched bases
  - removes abnormal bases
    - repairs damage throughout life
  - reduces error rate from 1 in 10,000 to 1 in 100 million bases




3'
5'
5'
3'

Protein sintesis

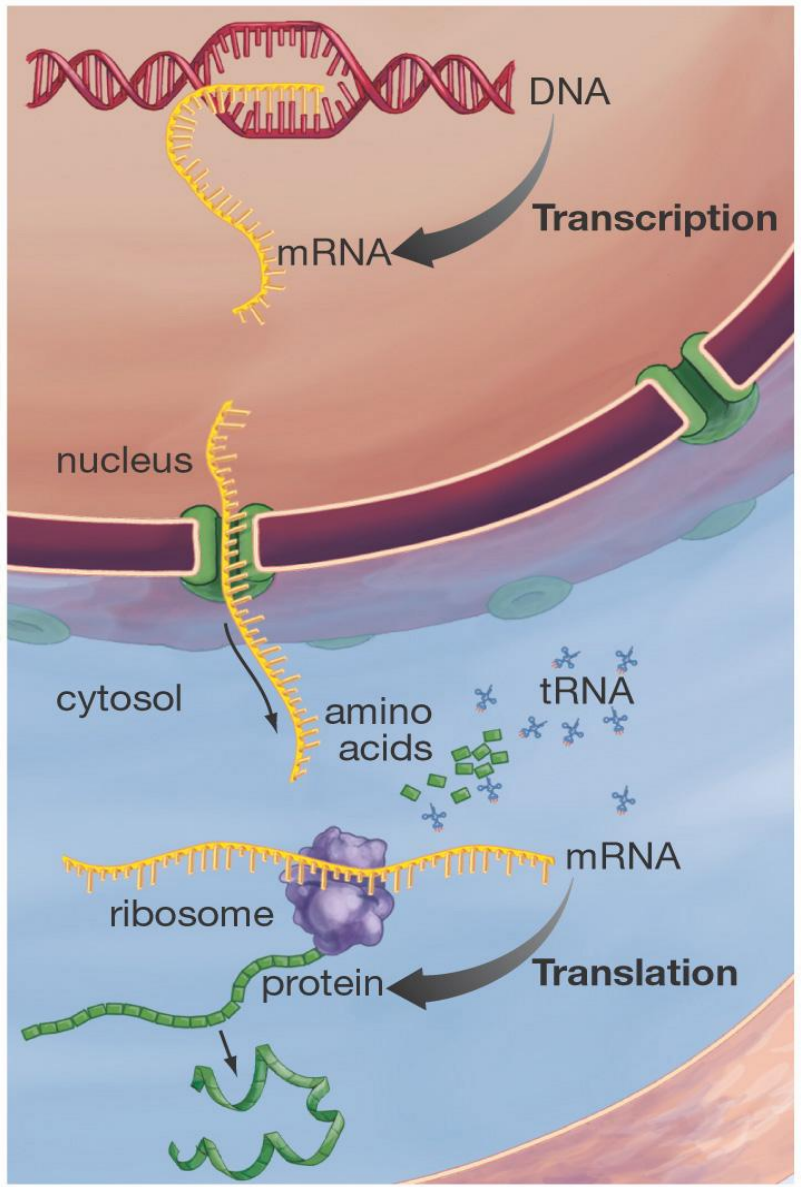
Part I

How do genotypes produce phenotypes? Protein Synthesis!

Transcription **Translation**

DNA → **RNA** → **Protein** → **Trait**

RNA processing



The chemical nature of RNA differs from that of DNA.

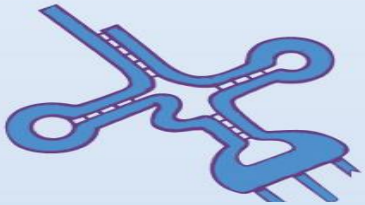
Ribonucleic acid (RNA) is a polymer of purine and pyrimidine ribonucleotides linked together by 3',5'-phosphodiester bridges.

- **1. In RNA, the sugar moiety is ribose.**
- **2. Instead of thymine, RNA contains the ribonucleotide of uracil.**
- **3. RNA exists as a single stand.**
- **4. The guanine and adenine content does not necessarily equal their cytosine and uracil content.**

The main classes of RNA molecules

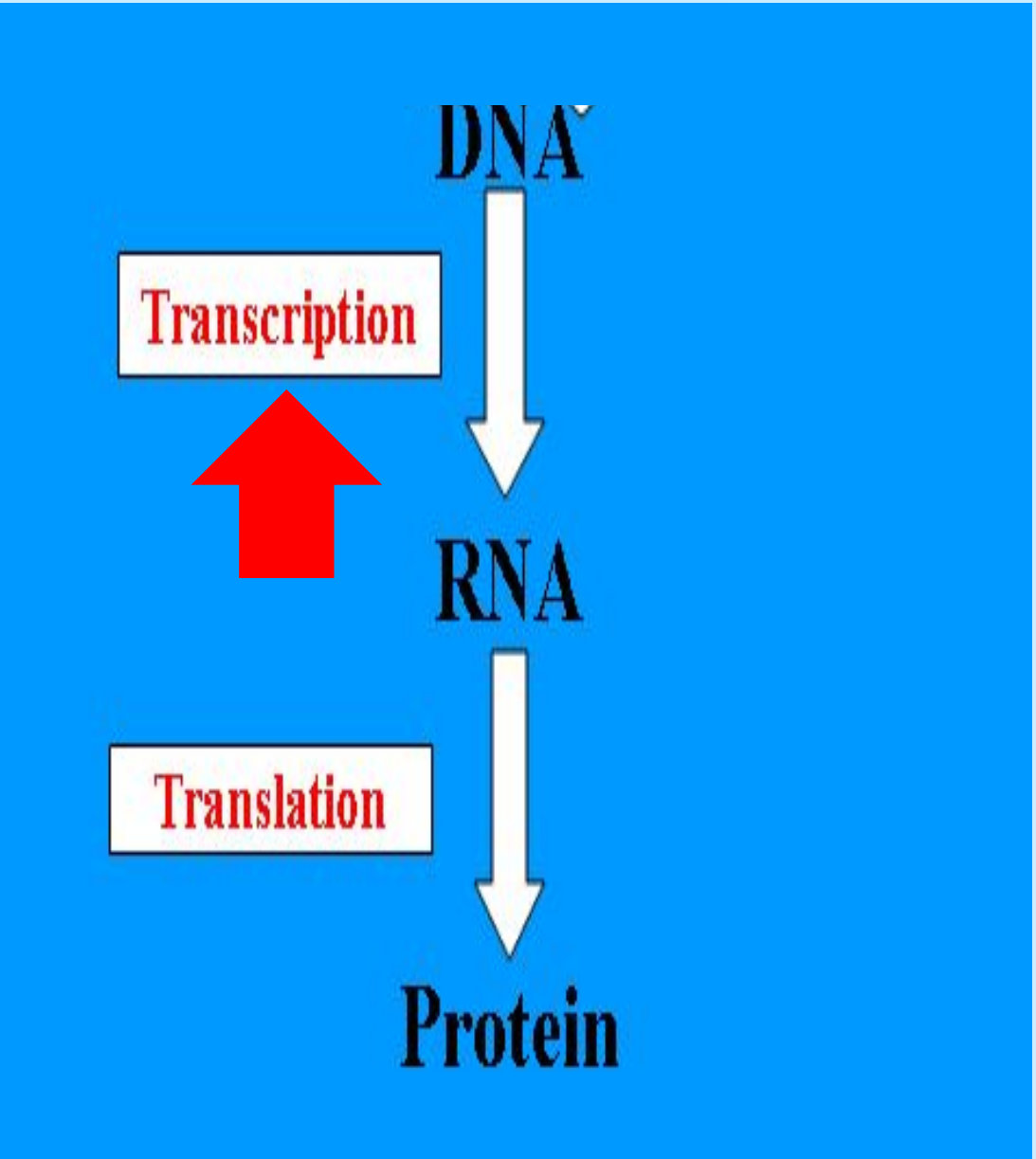
- **messenger RNA (mRNA),**
- **transfer RNA(tRNA),**
- **ribosomal RNA (rRNA)**
- **Small nuclear RNA (snRNA).**

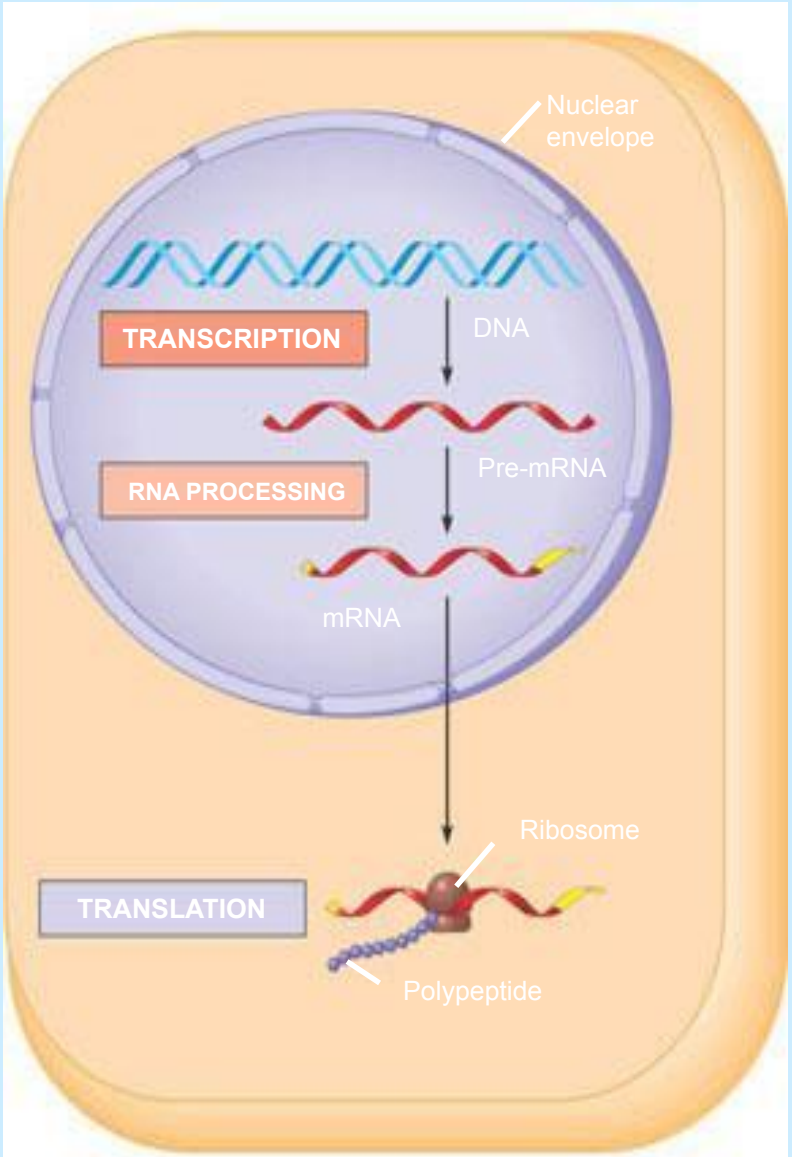
Each differs from the other by size, function, and general stability. All of them are used for protein synthesis.

Type of RNA	Functions in	Function
Messenger RNA (mRNA) 	Nucleus, migrates to ribosomes in cytoplasm	Carries DNA sequence information to ribosomes
Transfer RNA (tRNA) 	Cytoplasm	Provides linkage between mRNA and amino acids; transfers amino acids to ribosomes
Ribosomal RNA (rRNA) 	Cytoplasm	Structural component of ribosomes
Sn RNA 	nucleus	RNA splicing:

Recently, a new class of RNA, microRNA, has been shown to regulate gene expression.

Protein synthesis

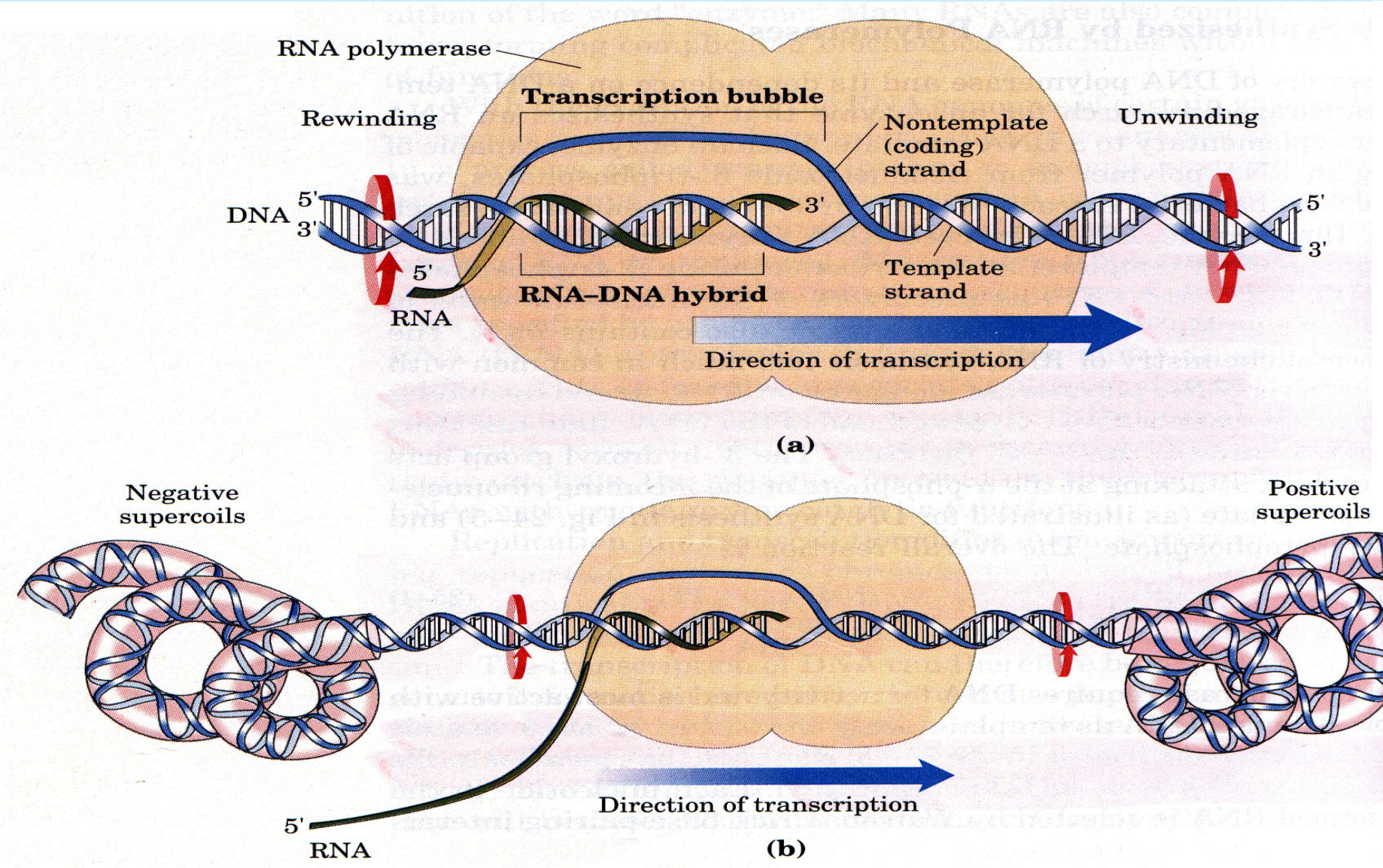




Transcription is DNA-dependent synthesis of RNA.

- **Transcription is catalyzed by RNA polymerase.**
- **RNA polymerase copies a DNA template in the 3' to 5' direction and synthesizes a single-stranded RNA molecule in a 5' to 3' direction.**
- **There are three stages of transcription:**
  1. **Initiation**
  2. **Elongation**
  3. **Termination**

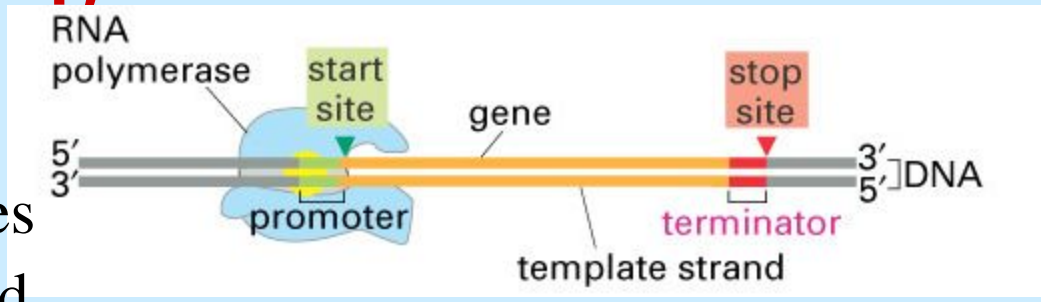
Transcription.

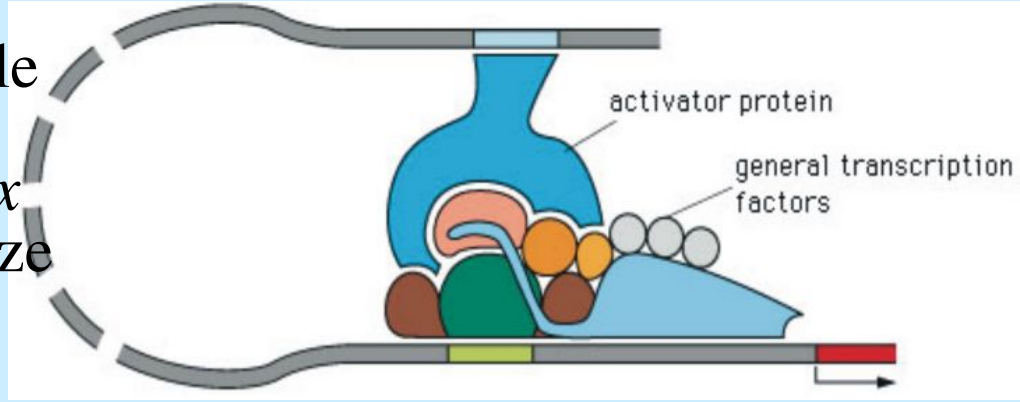


Transcription (*Initiation*)

1)

- **RNA polymerase** binds to a region on DNA known as the ***promoter***, which signals the start of a gene
- **Promoters** are specific to genes
- RNA polymerase does not need a primer
- **Transcription factors** assemble at the promoter forming a *transcription initiation complex*
  – activator proteins help stabilize the complex
- **Gene expression** can be **regulated** (turned on/off or up/down) by controlling the amount of each transcription factor

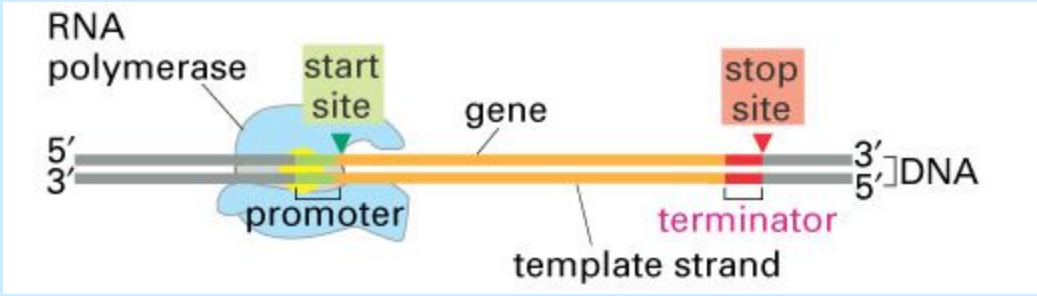


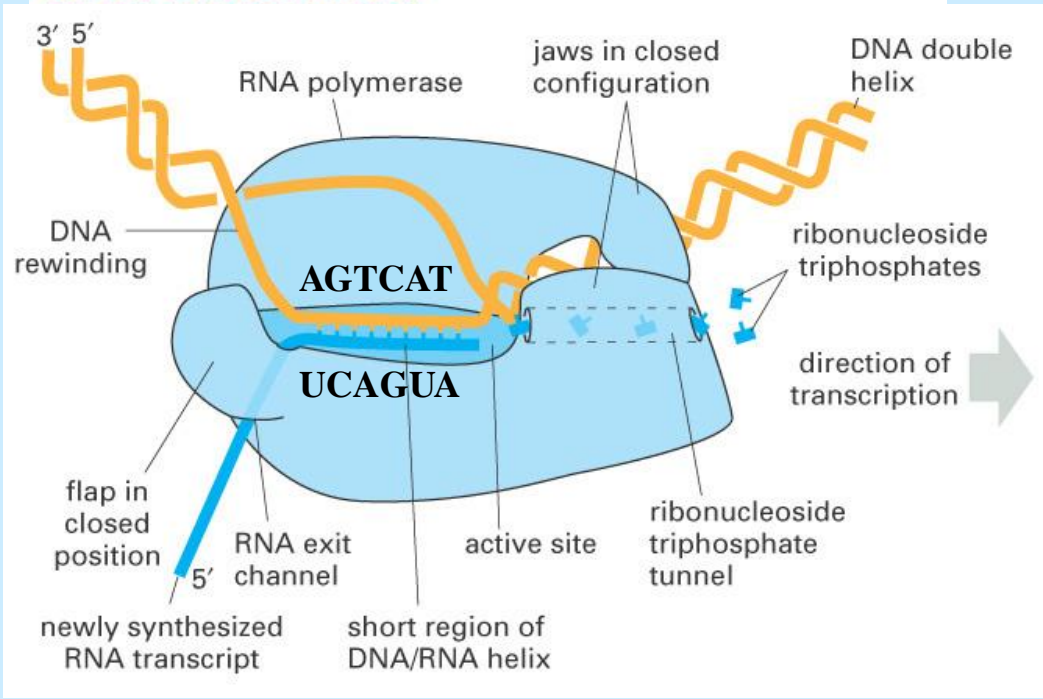


(eukaryotes)

Transcription (*Elongation*)

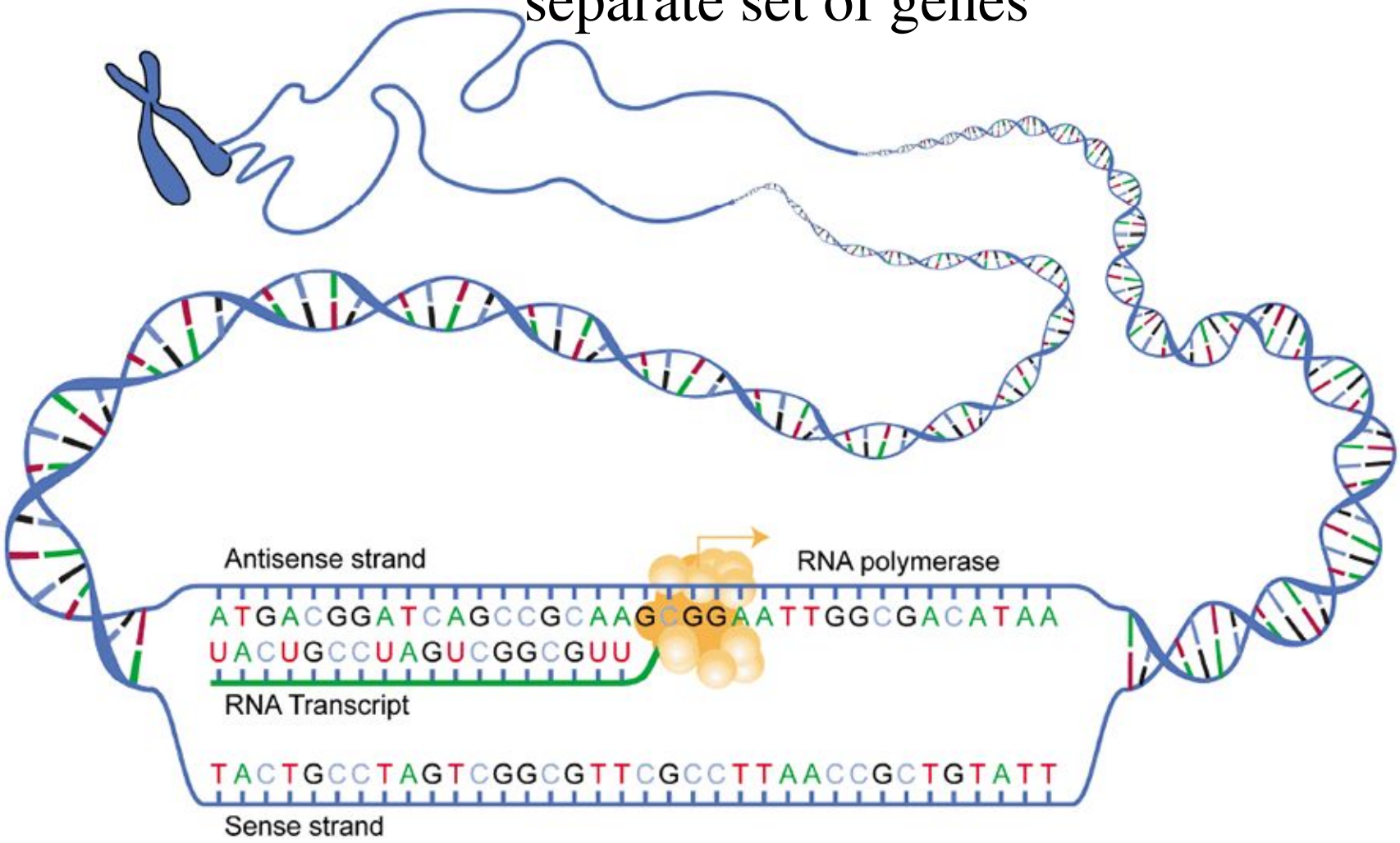
- ✔ **RNA polymerase** unwinds the DNA and breaks the H-bonds, separating them from one another
- ✔ Base pairing occurs between incoming RNA nucleotides and the DNA nucleotides
- ✔ RNA polymerase catalyzes bond to form between ribose of 3' nucleotide of mRNA and phosphate of incoming RNA nucleotide





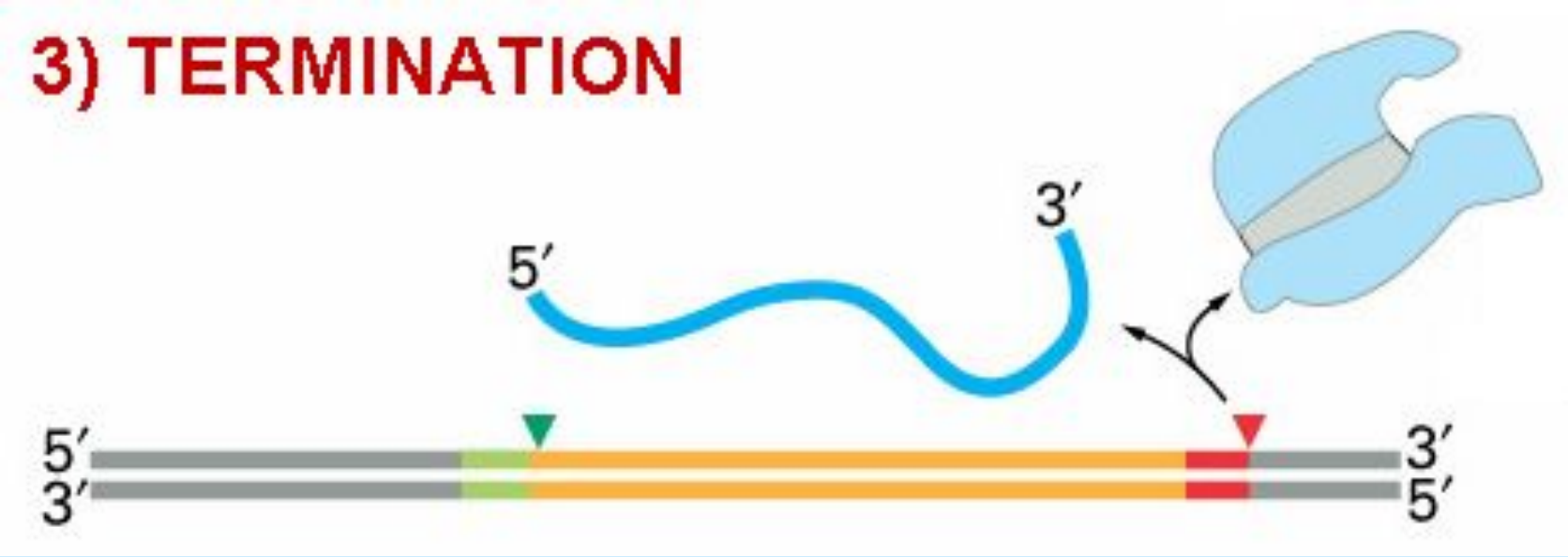
Transcription (*Elongation*)

The gene occurs on only one of the DNA strands; each strand possesses a separate set of genes

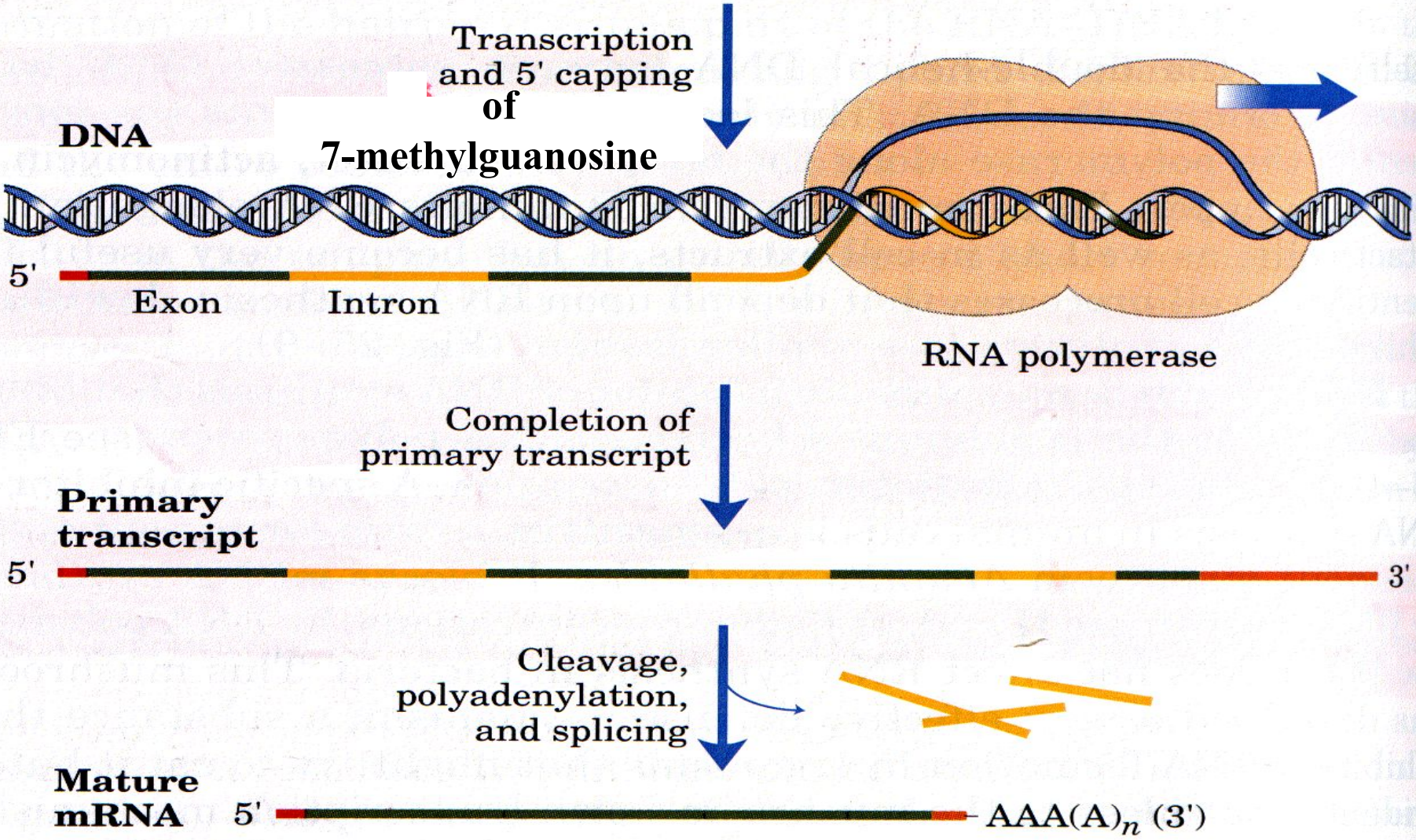


Transcription (*Termination*)

- Specific sequences in the DNA signal termination of transcription
- When one of these is encountered by the polymerase, the RNA transcript is released from the DNA and the double helix can zip up again



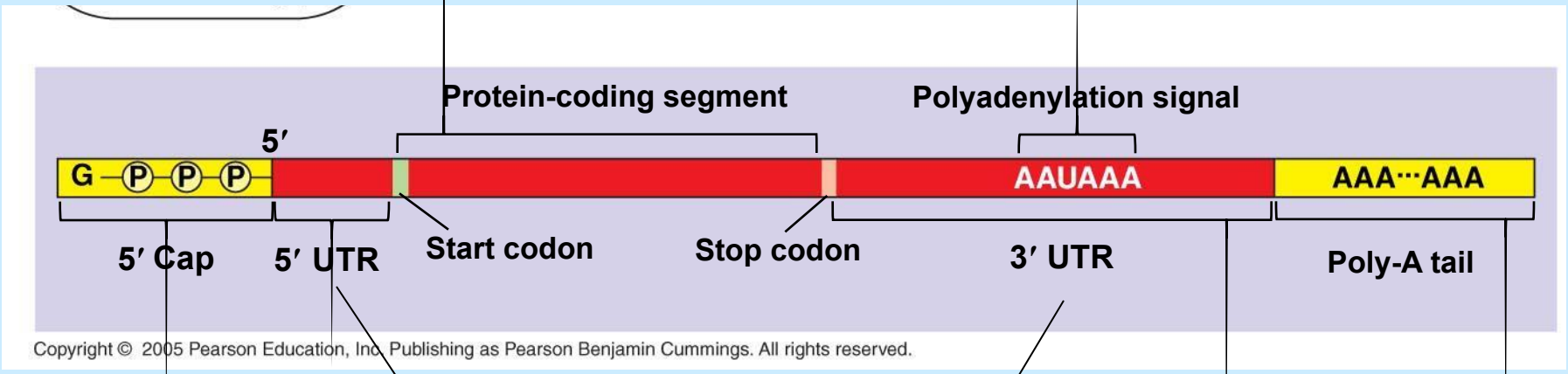
RNA processing



Each end of a pre-mRNA molecule is modified in a particular way

– The 5′ end receives a modified nucleotide cap

– The 3′ end gets a poly-A tail



UTR: untranslated regions

RNA Processing - Splicing

- The original transcript from the DNA is called pre-mRNA.
- It contains transcripts of both introns and exons.
- The introns are removed by a process called splicing to produce messenger RNA (mRNA)

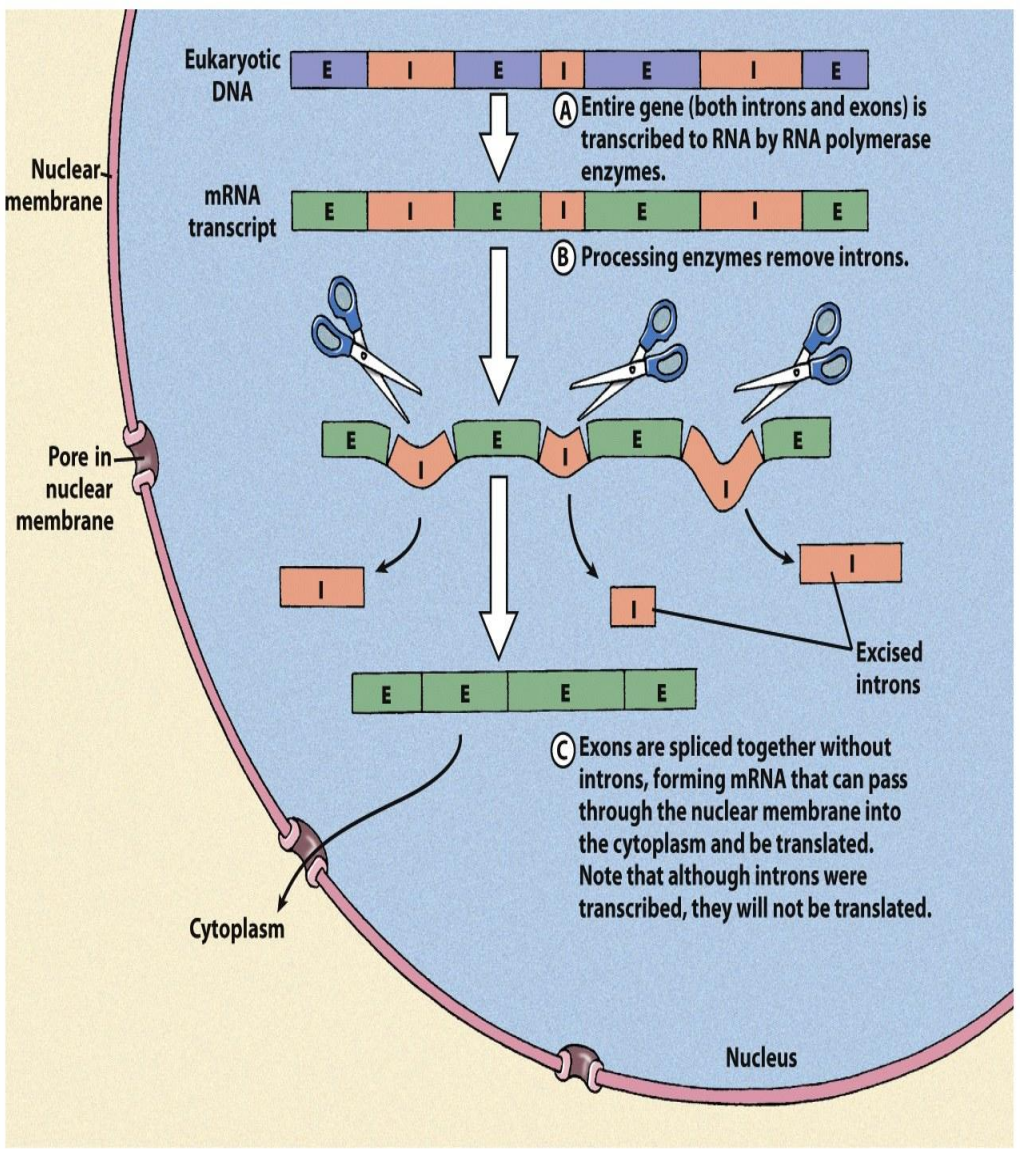


Figure 7-6 Microbiology, 7/e

RNA splicing

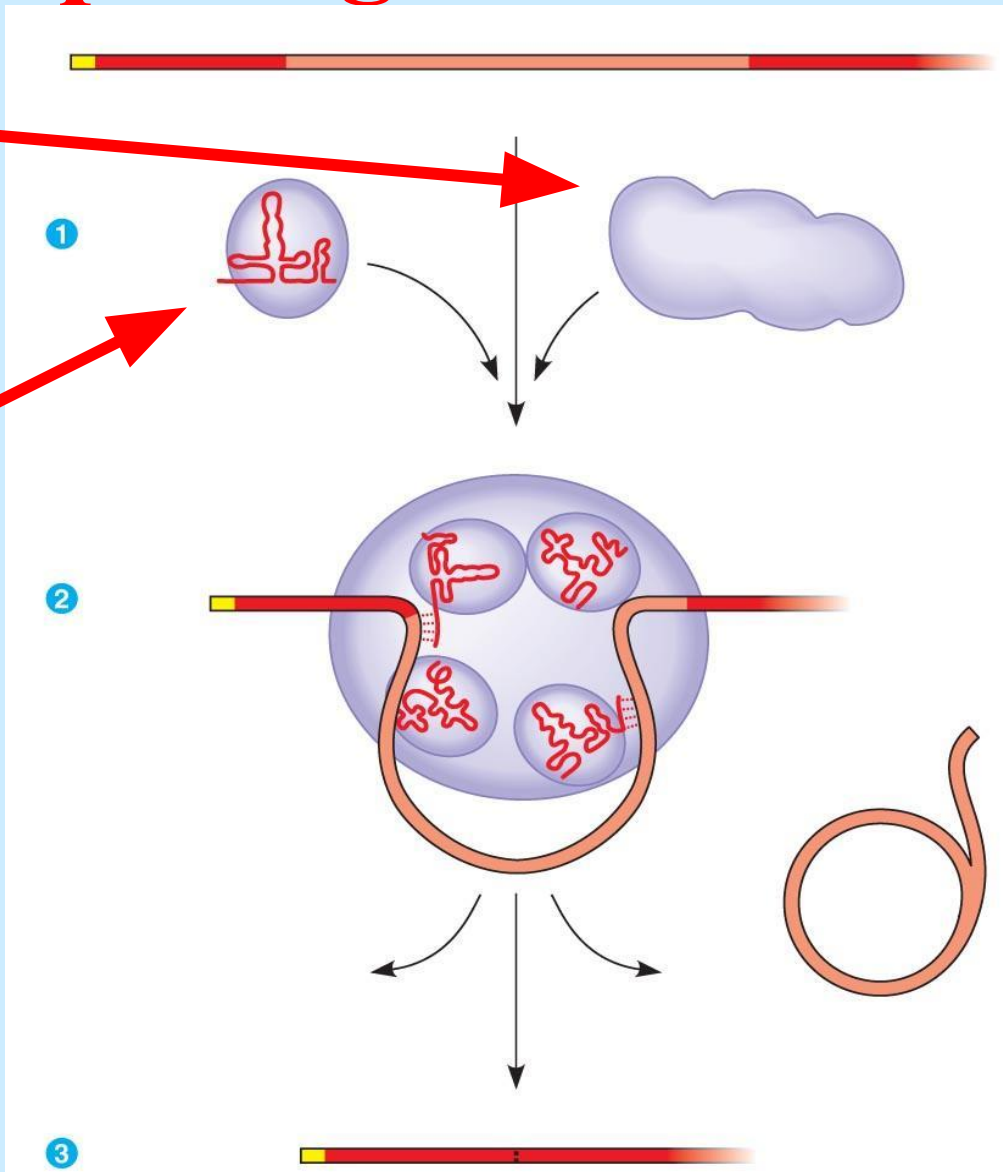
Spliceosomes -

complex of proteins

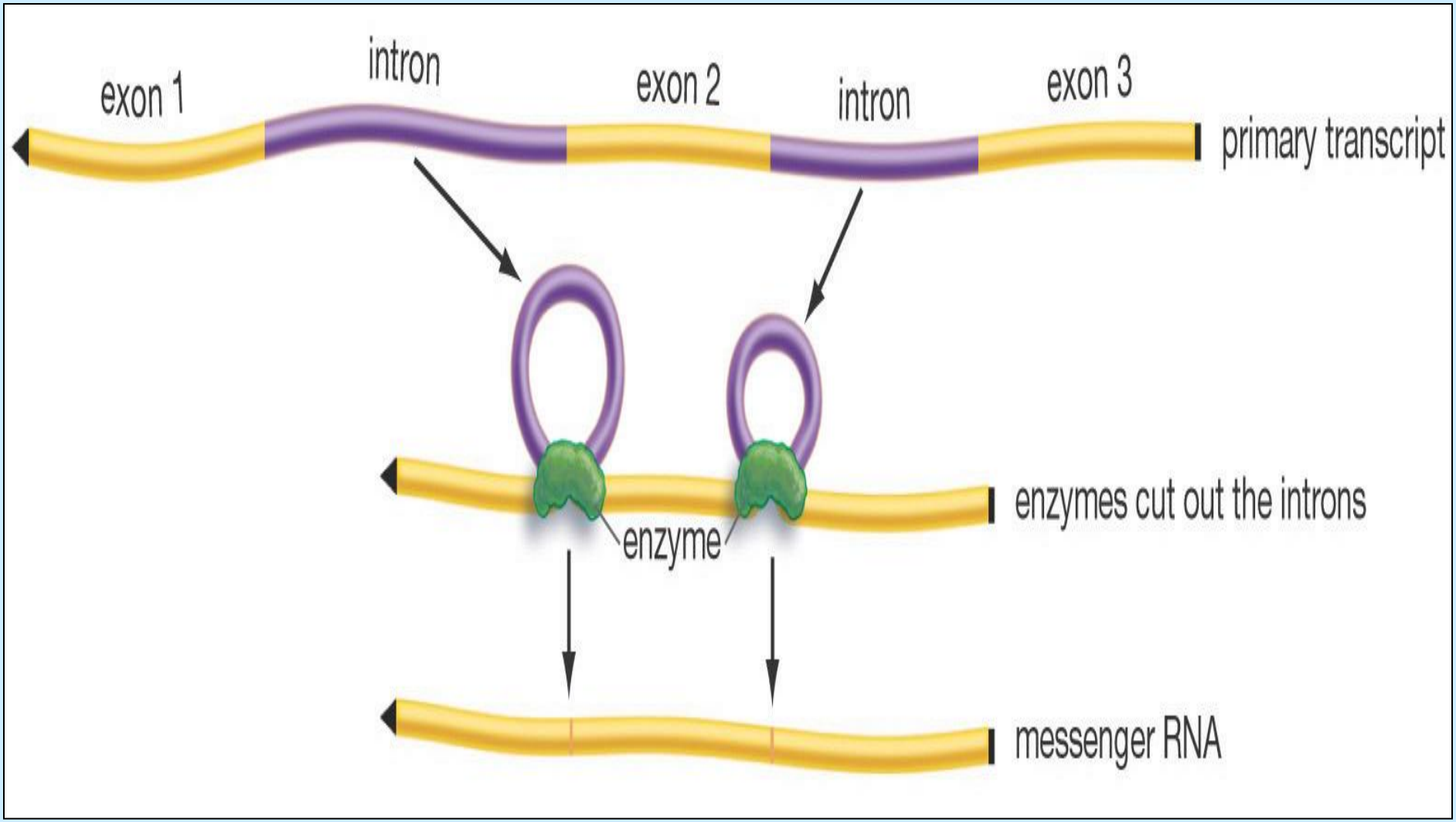
and several small nuclear ribonucleoproteins (snRNPs)

Recognize splice sites (specific RNA sequences)

cleave out introns and splice together exons (coding region)



RNA splicing



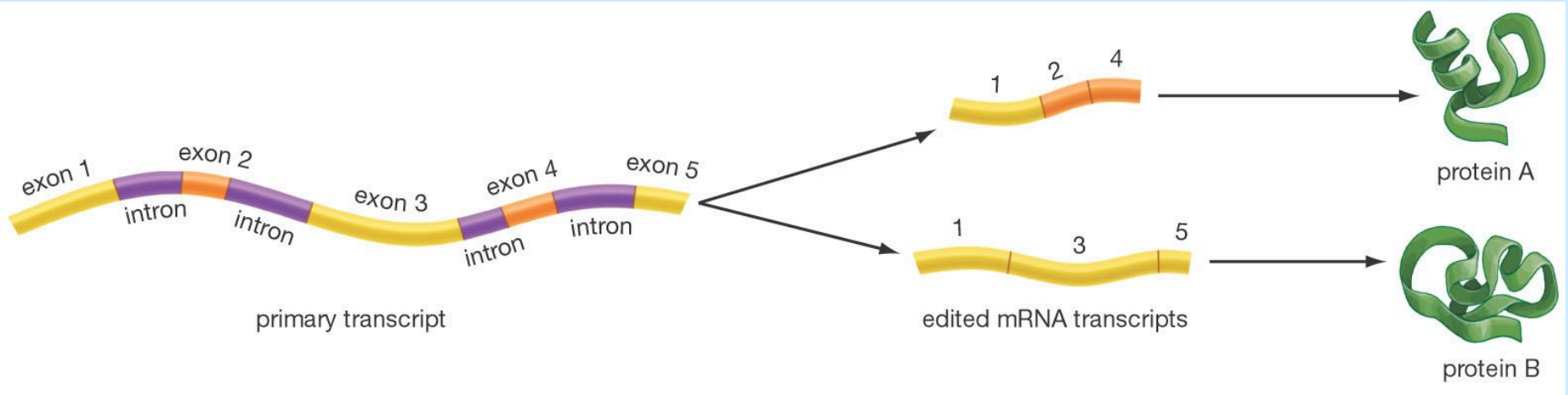
Anima

Alternative RNA splicing

- Some genes can encode more than one kind of polypeptide

  -different combinations of exons can be spliced together

- Increases the potential number of different proteins (and thus functions) in an organism




Thank
you