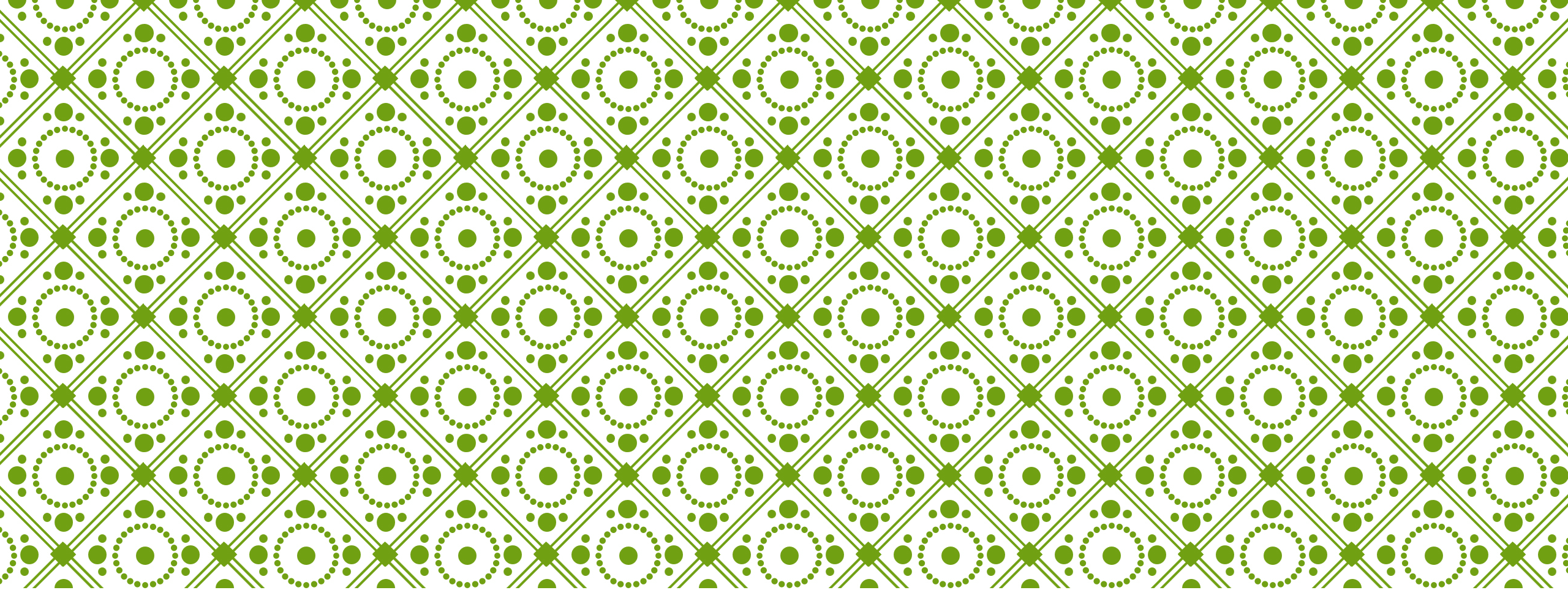


CHAPTER 2.7

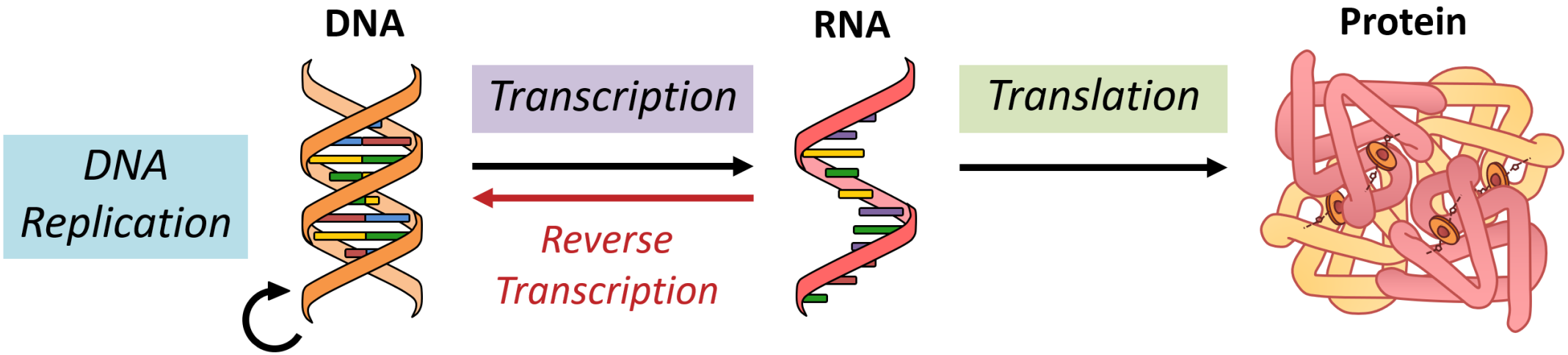
DNA Replication, Transcription and Translation

ALEXANDER PRANTNER

BASIC IDEA

The main goal of molecular biology is to describe the flow of genetic information

- DNA may be copied during the formation of new cells via ***DNA replication***
- DNA may code for RNA transcripts (mRNA) via the process of ***transcription.***
- The mRNA transcripts code for the production of proteins via ***translation.***

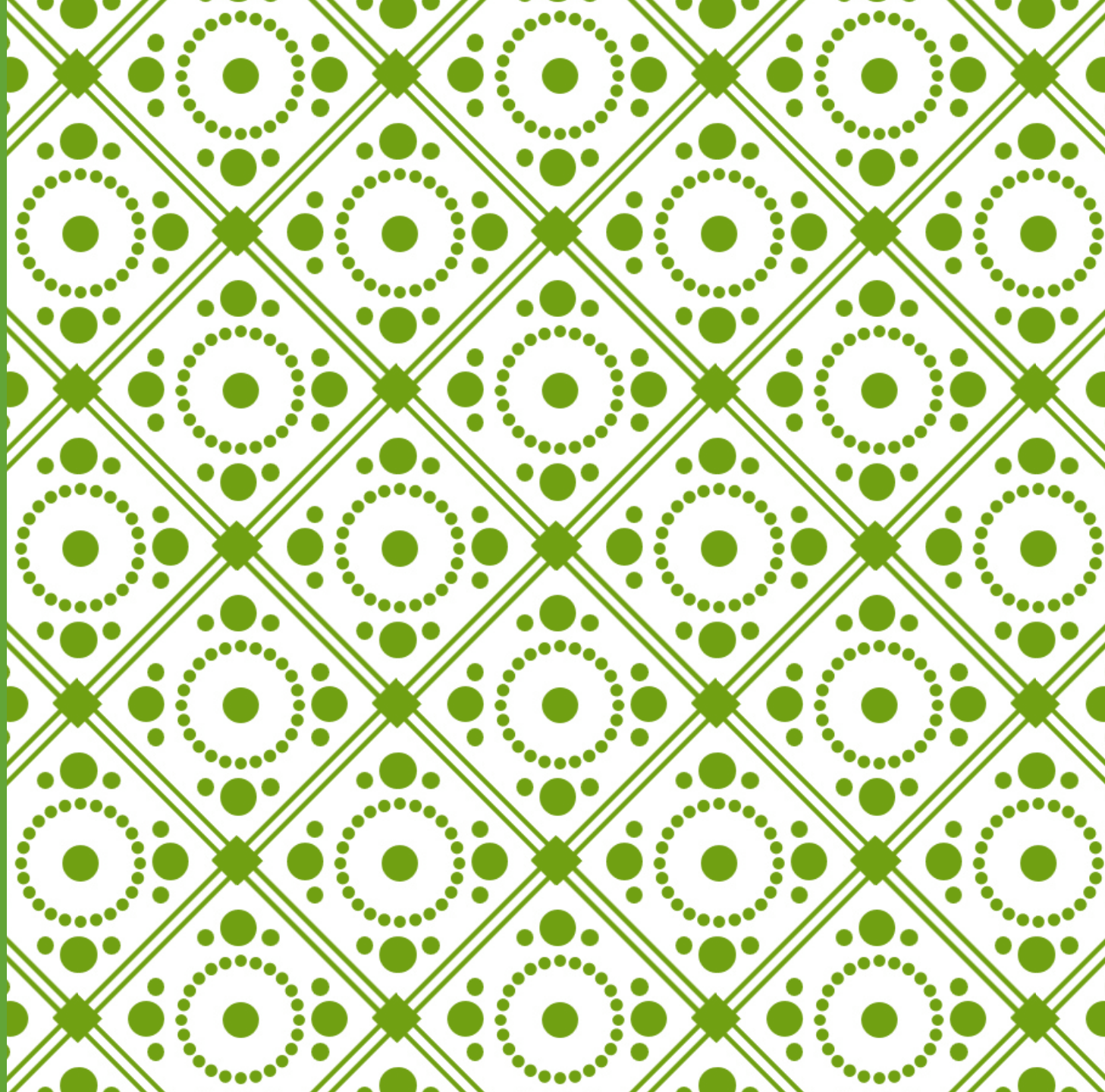


COMPARISON OF CELL PROCESSES

All three processes are vital in cells:

	DNA Replication	Transcription	Translation
When it occurs	During S phase *(cell division)*	Continuously *(all the time)*	Continuously *(after transcription)*
Location	Nucleus	Nucleus	Cytoplasm
Key components	Enzymes	Enzymes	Ribosomes
Function	Duplicate genetic information prior to cellular division	Produces RNA transcript of a DNA sequence	Makes proteins according to RNA transcript

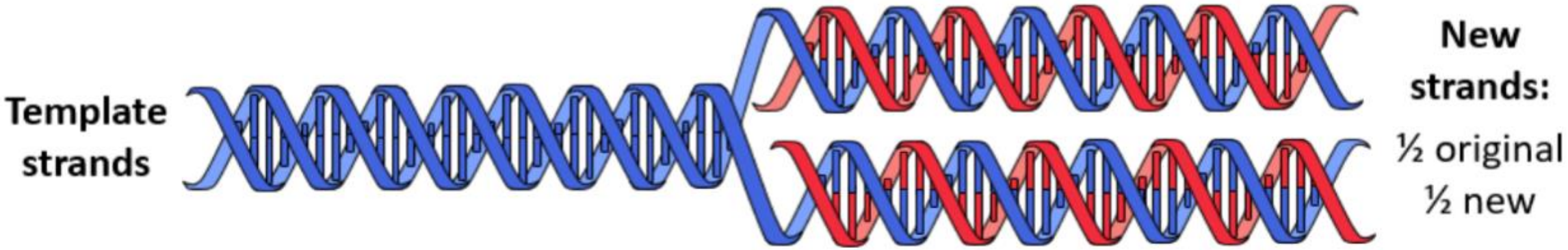
1) REPLICATION



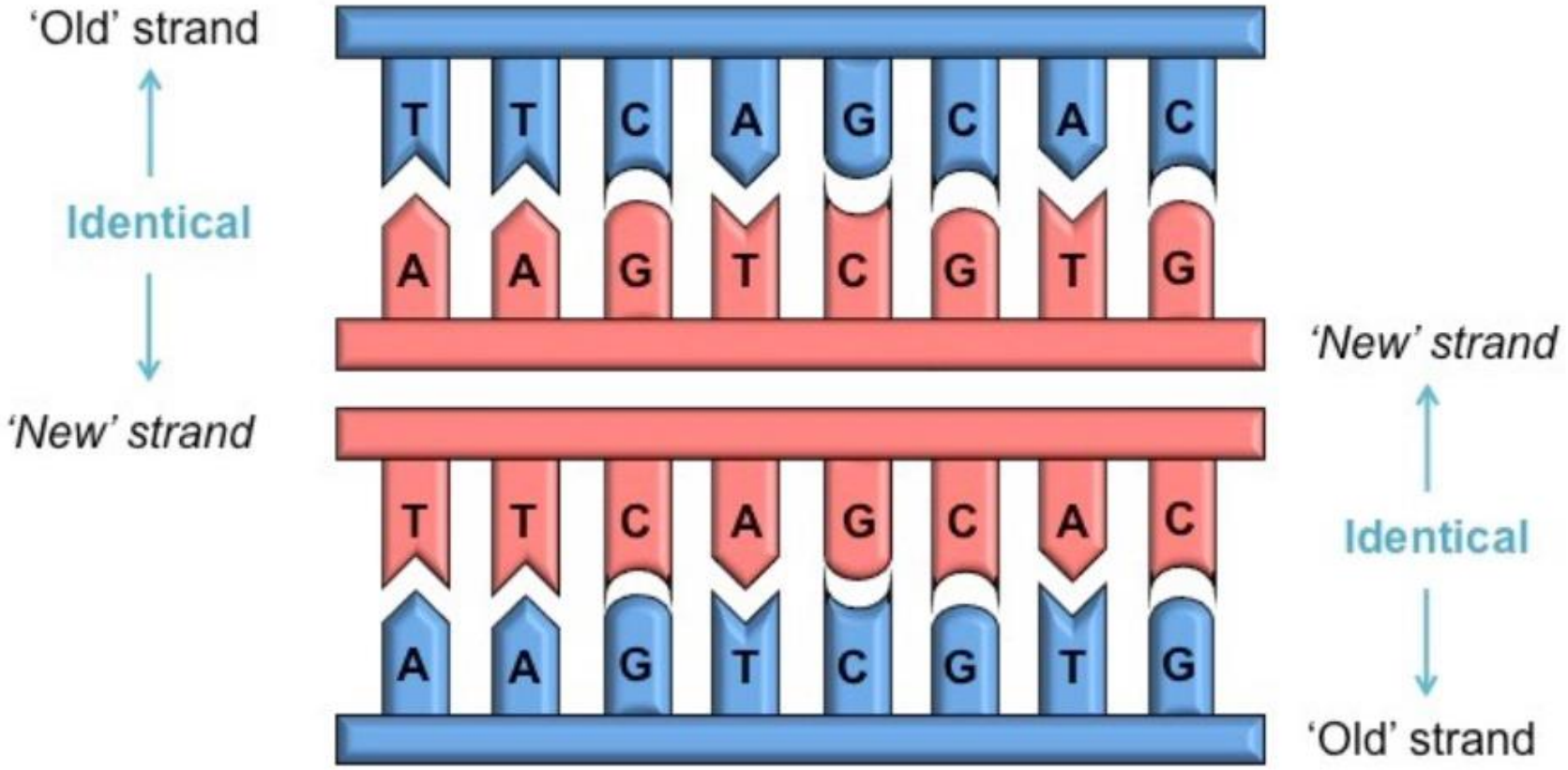
SEMI-CONSERVATIVE

DNA replication → semi-conservative, one strand from original template and one newly synthesised.

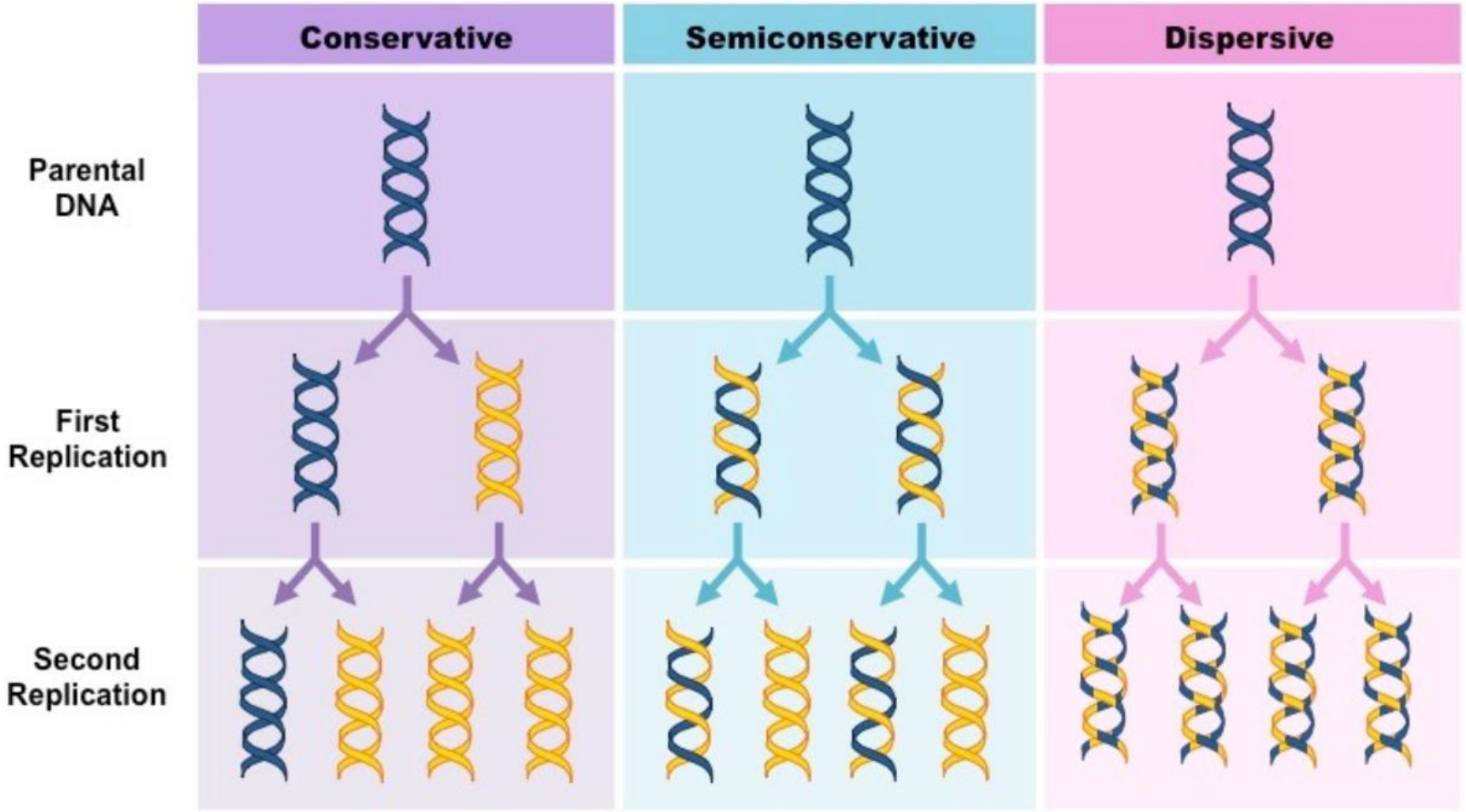
Reason: each nitrogenous base can only pair with its complementary partner (**A + T ; G + C**) → base sequence is conserved during replication



CONSERVATION OF SEQUENCE BECAUSE OF COMPLEMENTARY BASE PAIRING



PROPOSED MODELS OF REPLICATION



MESELSON AND STAHL EXPERIMENT

Data-based questions: page 113

MESELSON-STAHL EXPERIMENT

Supported semi-conservative replication theory

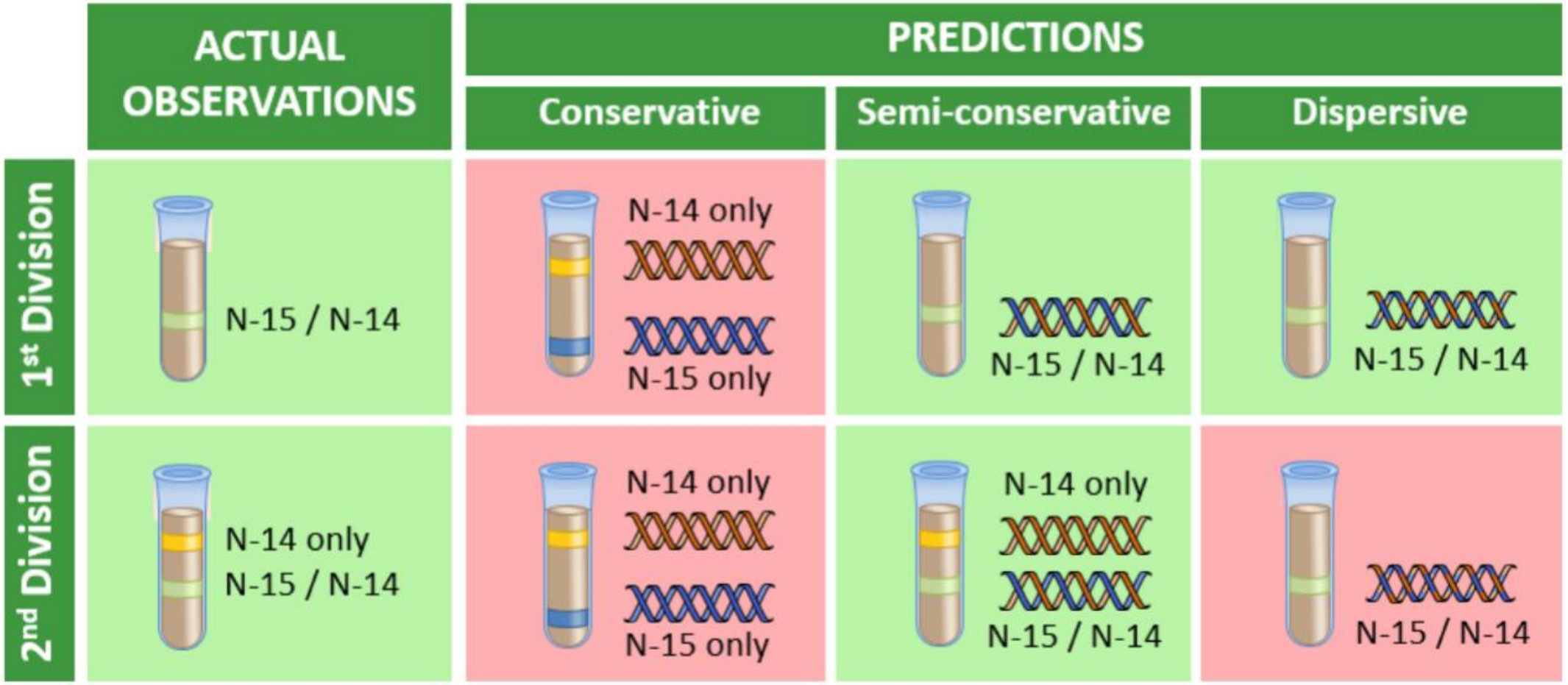
Two radioactive isotopes of nitrogen were introduced to the replication process:

- DNA molecules were first incorporated with heavier **^{15}N isotope**
- DNA was then induced to replicate in the presence of lighter **^{14}N isotope**

DNA was collected → centrifugation after division and isotope content assessed

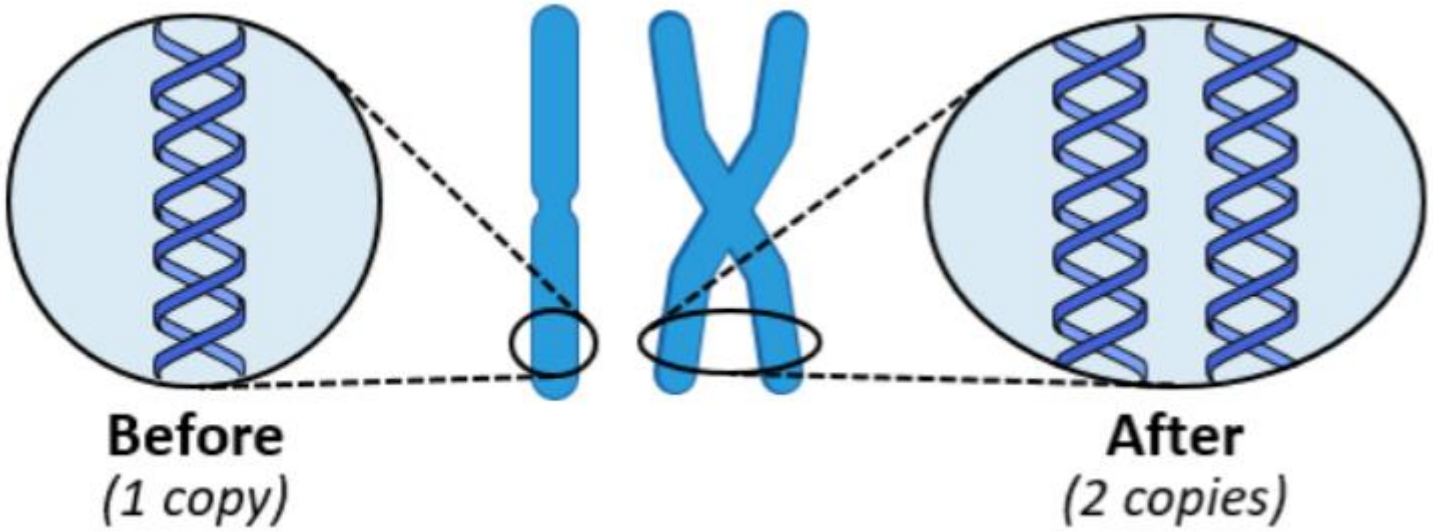
- **After 1st division:** DNA contains both **^{15}N** and **^{14}N** (*disproves conservative model*)
- **After 2nd division:** some DNA consists solely of **^{14}N** (*disproves dispersive model*)

MESELSON-STAHL EXPERIMENT

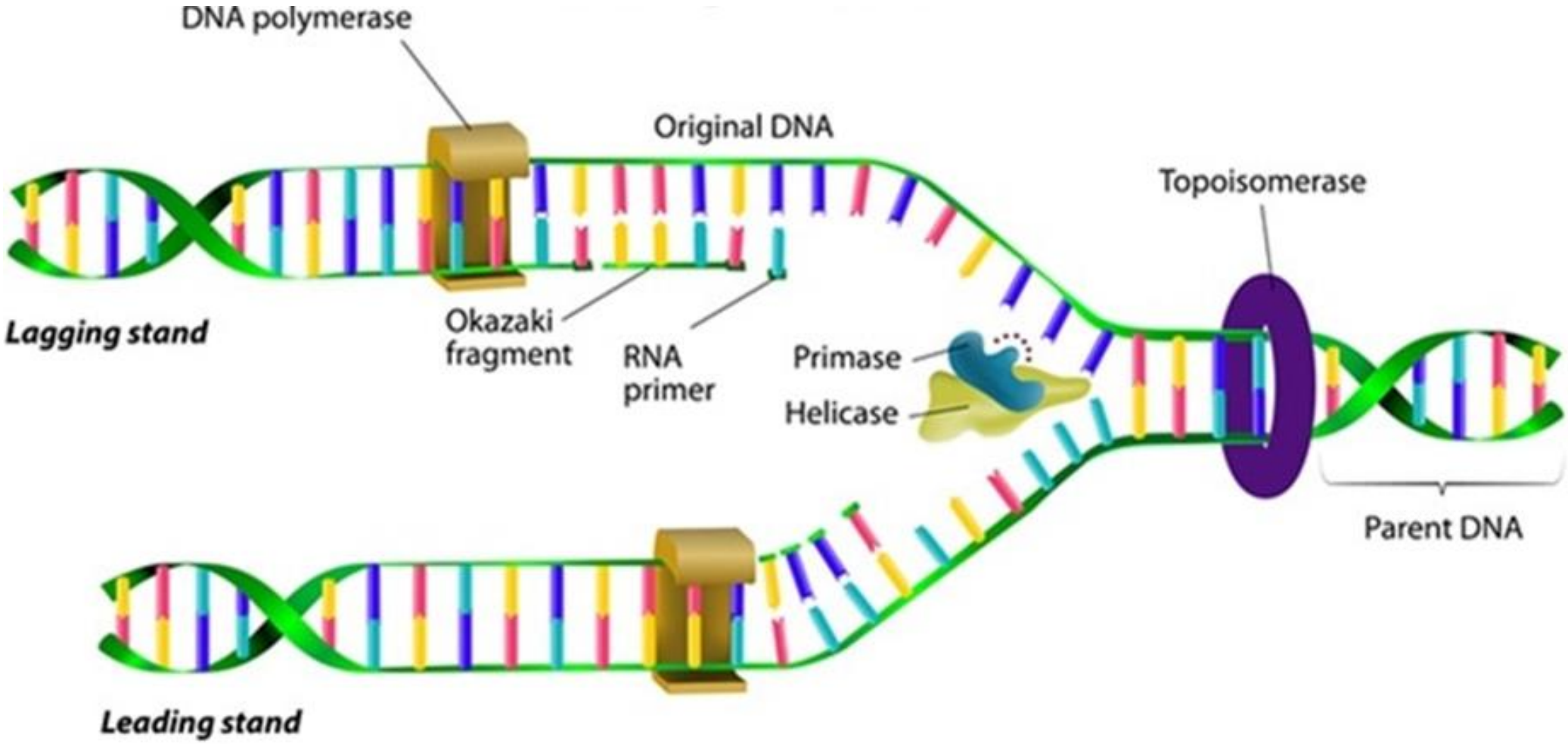


DNA REPLICATION

Purpose: Duplicate the DNA to create identical sister chromatids prior to cell division



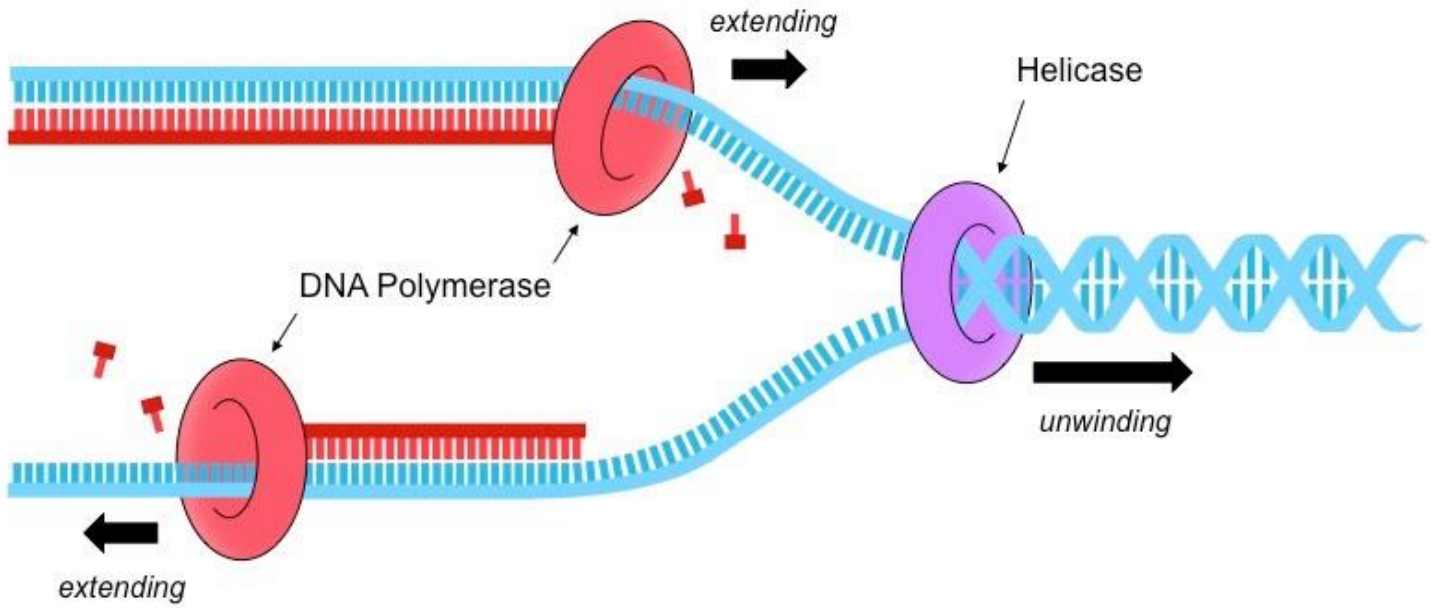
DNA REPLICATION



DNA REPLICATION

Two key enzymes:

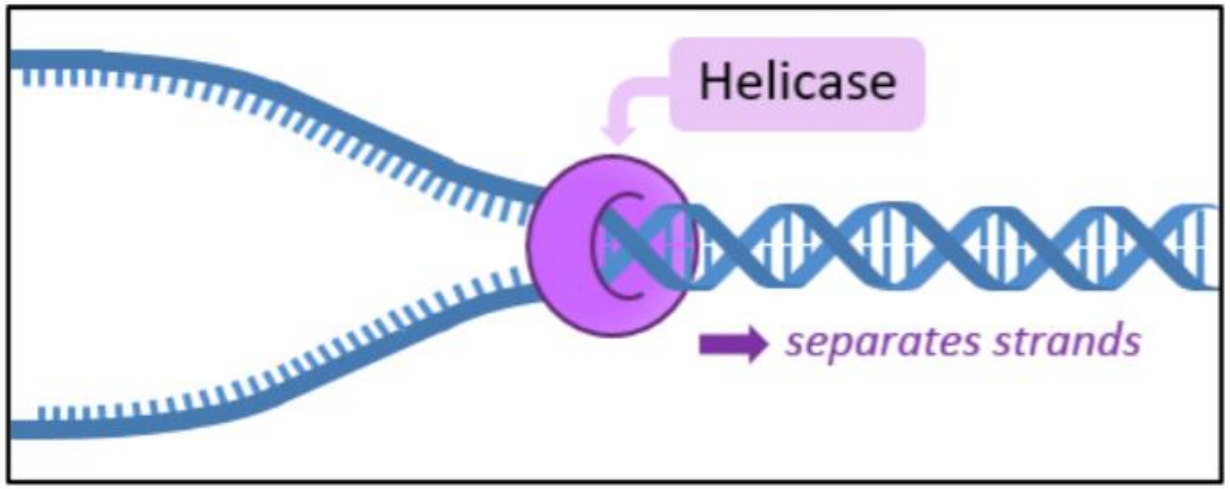
- **Helicase** separates the DNA strands
- **DNA Polymerase** copies new strands



HELICASE

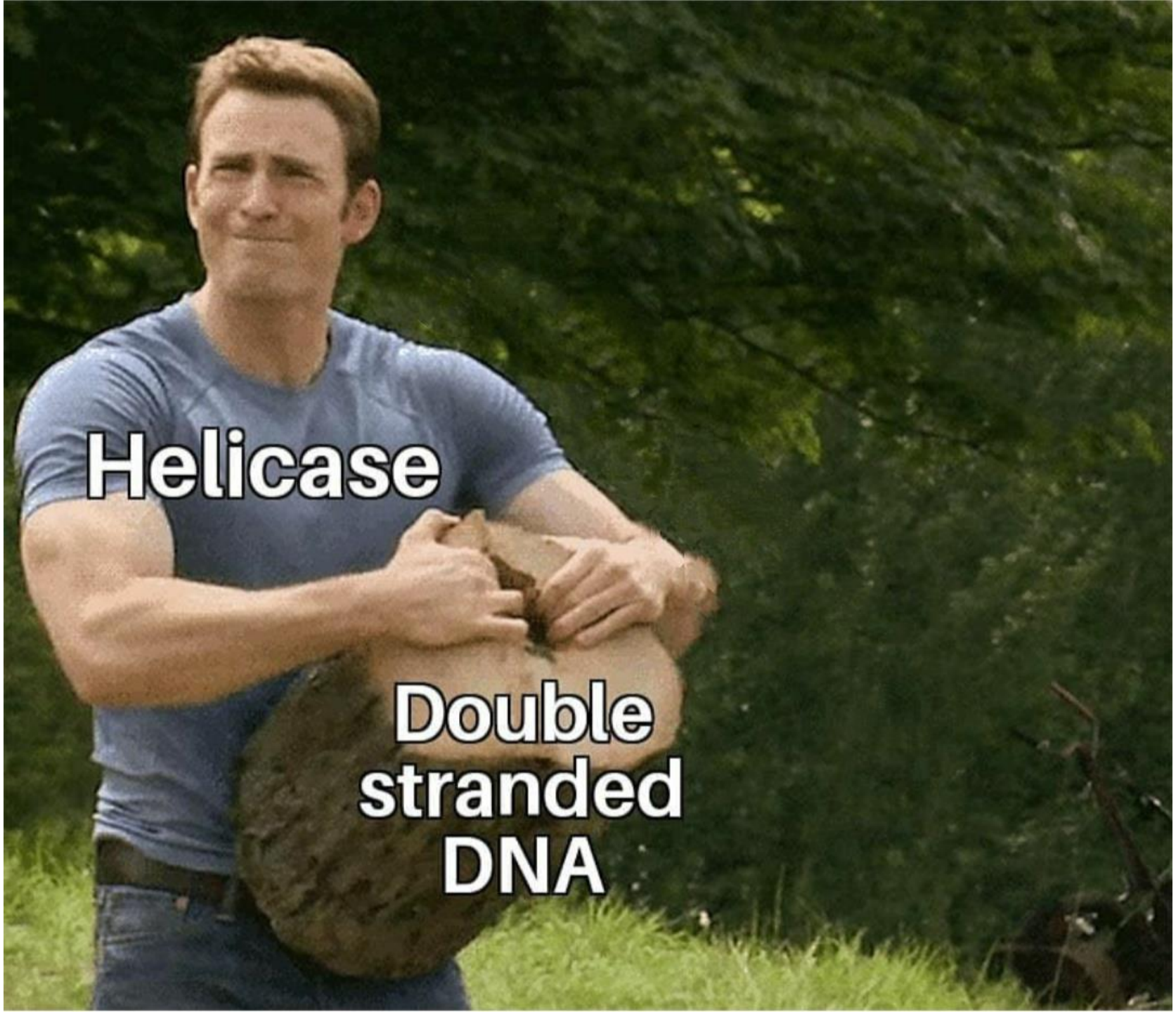
Helicase unwinds and separates the two strands → breaking hydrogen bonds between bases

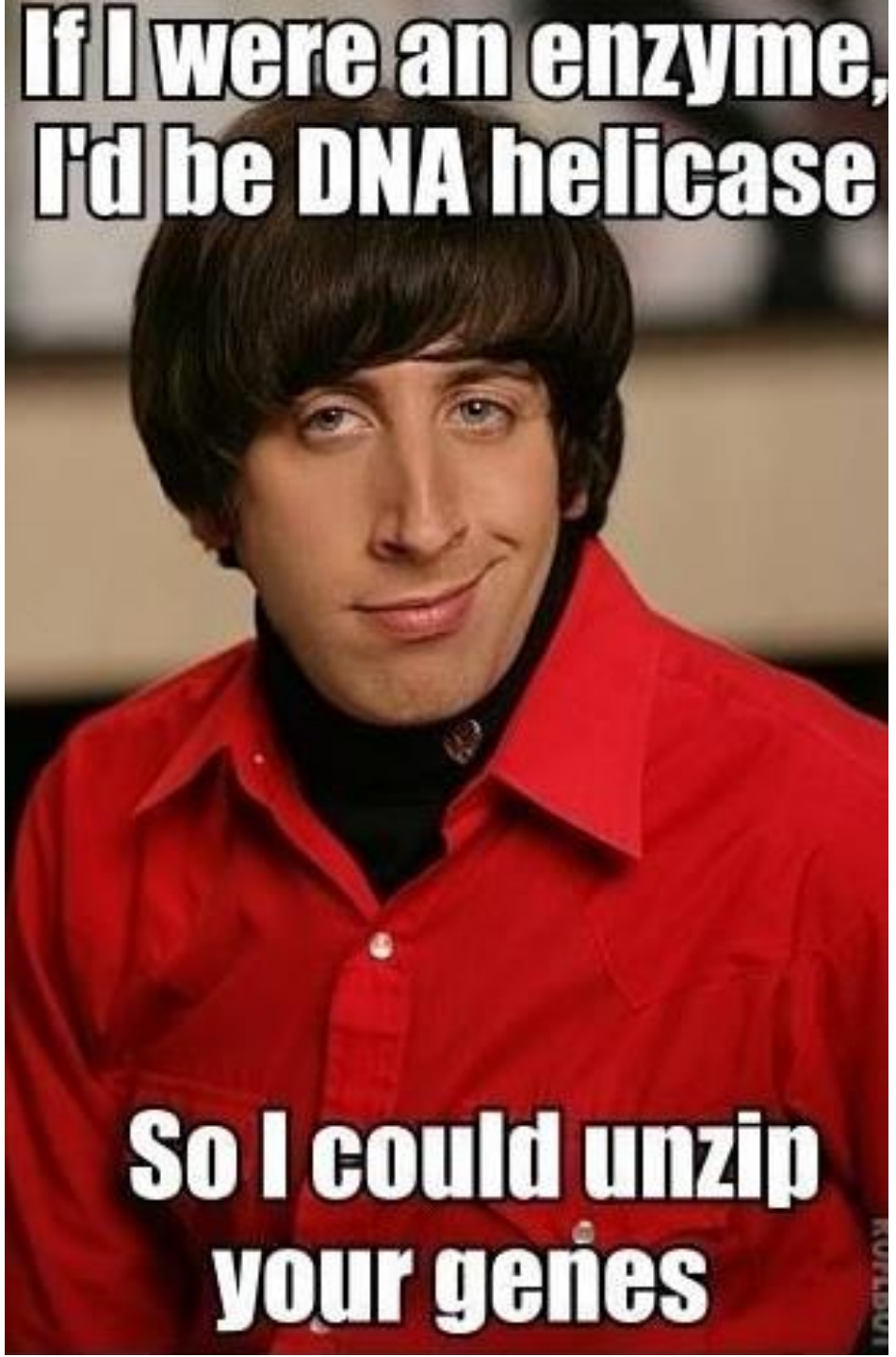
The template strands can be copied to make two sets of identical DNA molecules



Helicase:

- *Unwinds double helix*
- *Separates strands (by breaking the H bonds between base pairs*

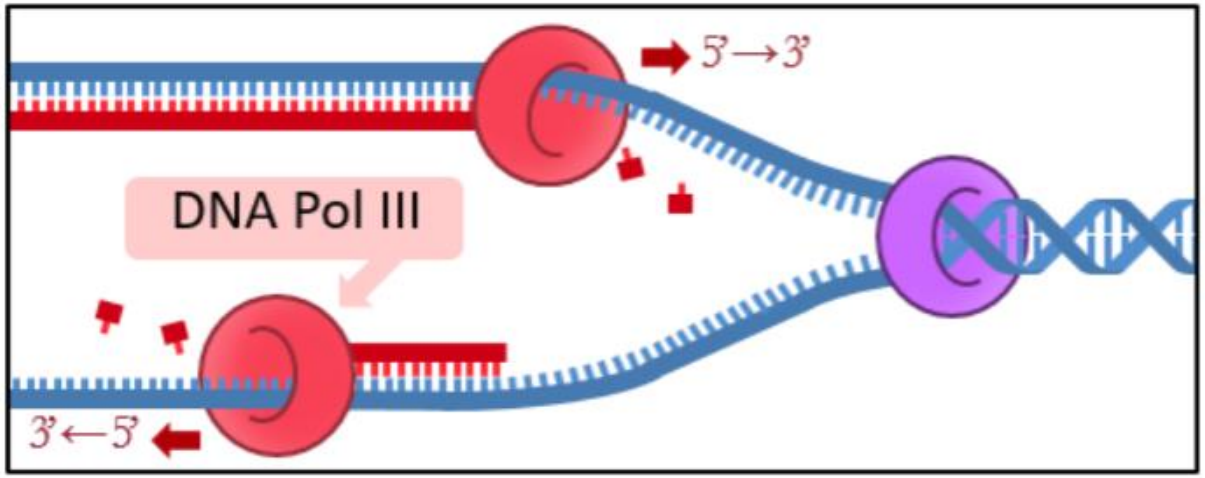

Helicase
Double stranded DNA


If I were an enzyme,
I'd be DNA helicase
So I could unzip
your genes

DNA POLYMERASE

After separation of strands by **helicase** → free nucleotides line up opposite their complementary base partnesrs

DNA polymerase III covalently joins DNA nucleotides together → new strand



DNA Polymerase III:

- *Synthesises new strand from template strand*
- *Covalently joins the nucleotides together*

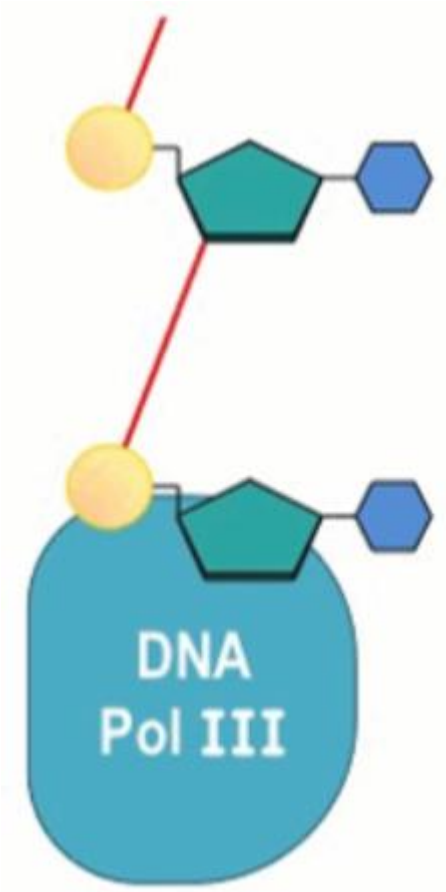
POLYMERISATION

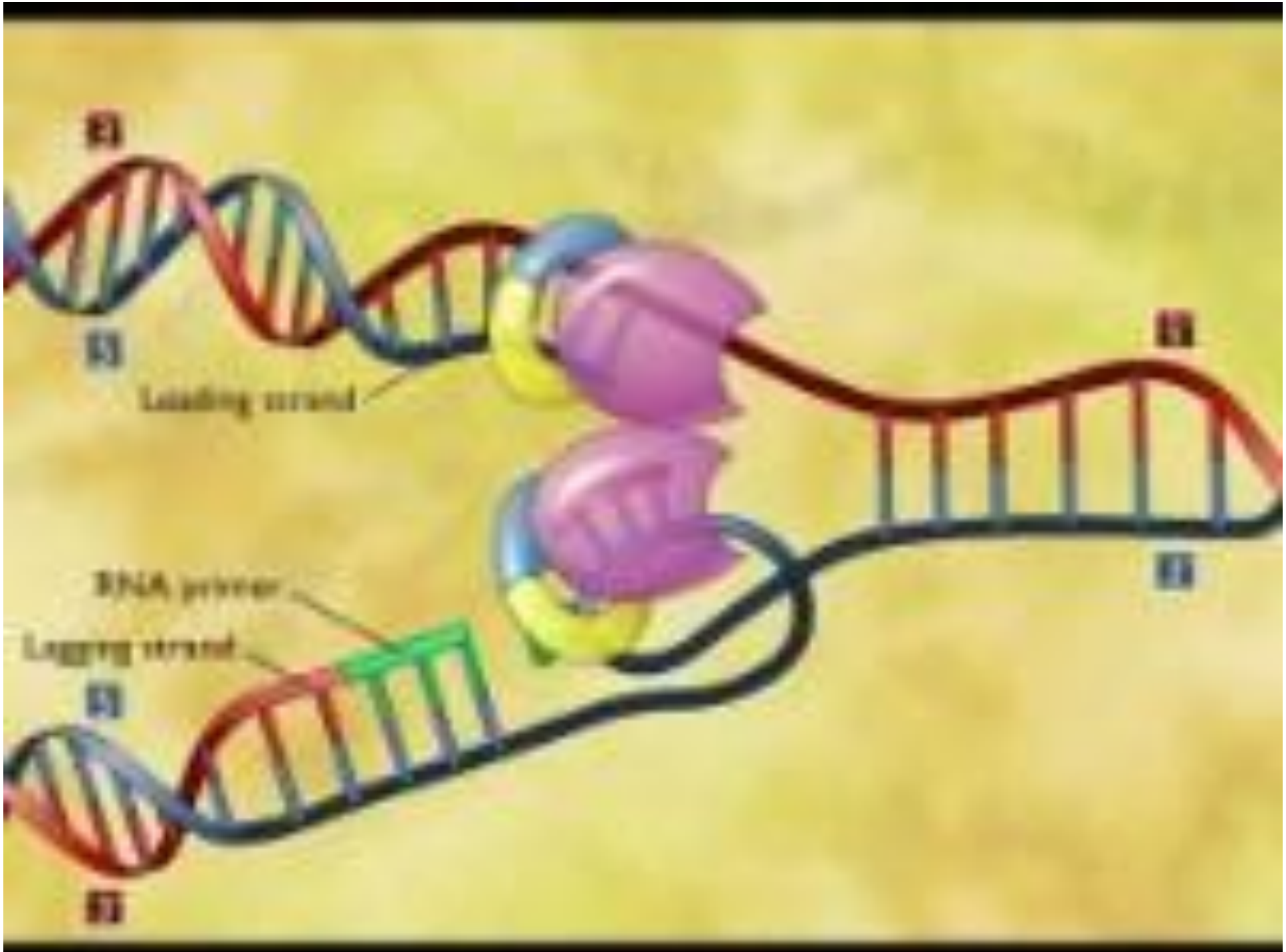
New nucleotides align opposite their complementary partner as **dNTPs** (deoxynucleoside triphosphates)

When joined to a growing chain → **DNA polymerase III** will cleave two of the phosphates, releasing energy

Energy is used by enzyme to create a **covalent phosphodiester bond** with the nucleotide chain

Synthesis occuurs in a 5‘ → 3‘ direction (*on new strand*)



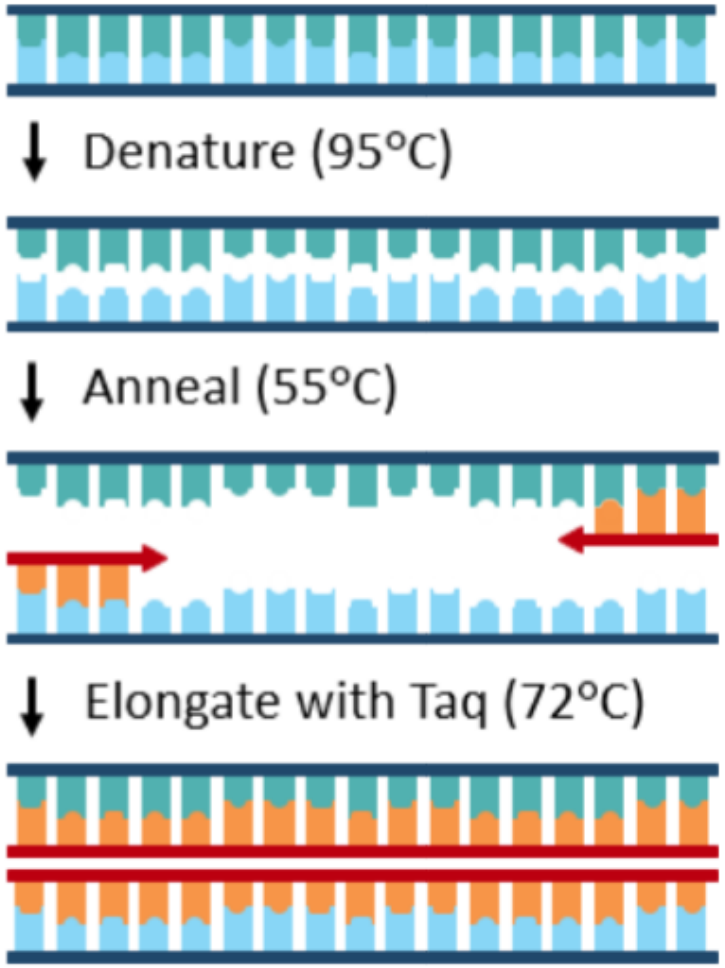

Leading strand
RNA primer
Lagging strand

PCR – POLYMERASE CHAIN REACTION

Artificial method of replication → rapidly amplifies DNA sequences

PCR involves three key steps (*which are repeated*)

- **Denaturation –** Heat separates strands (*not helicase*)
- **Annealing –** Primers designate copying sequence
- **Elongation –** Taq polymerase copies the sequence



2) TRANSCRIPTION

TRANSCRIPTION

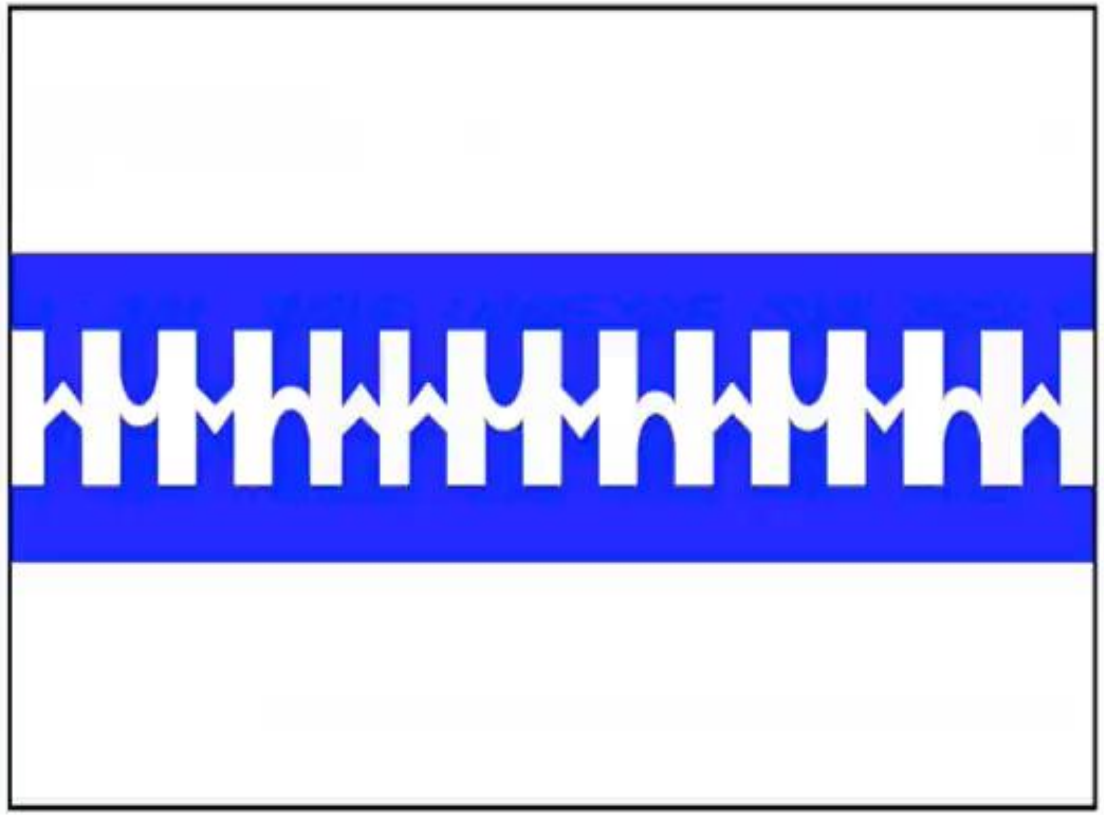
Purpose: To synthesise an RNA sequence from a DNA template (*only one strand copied*)

- The enzyme **RNA polymerase** separates the DNA strands **AND** synthesises an RNA copy (*no helicase*)

After transcription is complete → RNA sequence is released and the DNA strands reform a double helix

- RNA transcript then moves to cytoplasm → facilitate translation (*as mRNA, tRNA or rRNA*)

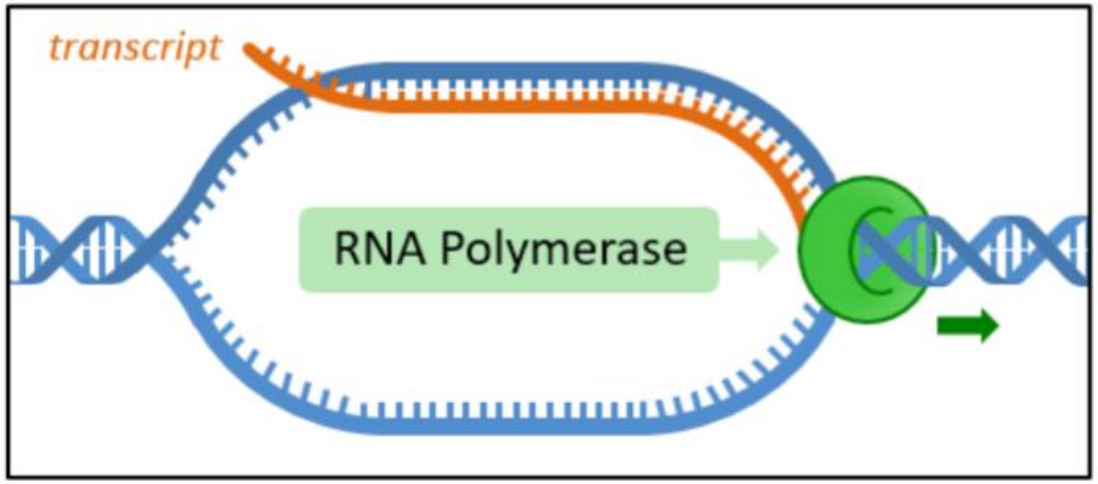
TRANSCRIPTION



TRANSCRIPTION

Is mediated by RNA polymerase which performs the following:

- binds DNA and separates the strands (*by breaking H bonds between base pairs*)
- covalently joins RNA nucleotides together to form new strand (*U instead of T*)



RNA Polymerase:

- *Separates the DNA **and** makes RNA copy*
- *RNA copy is released and DNA re-anneals*

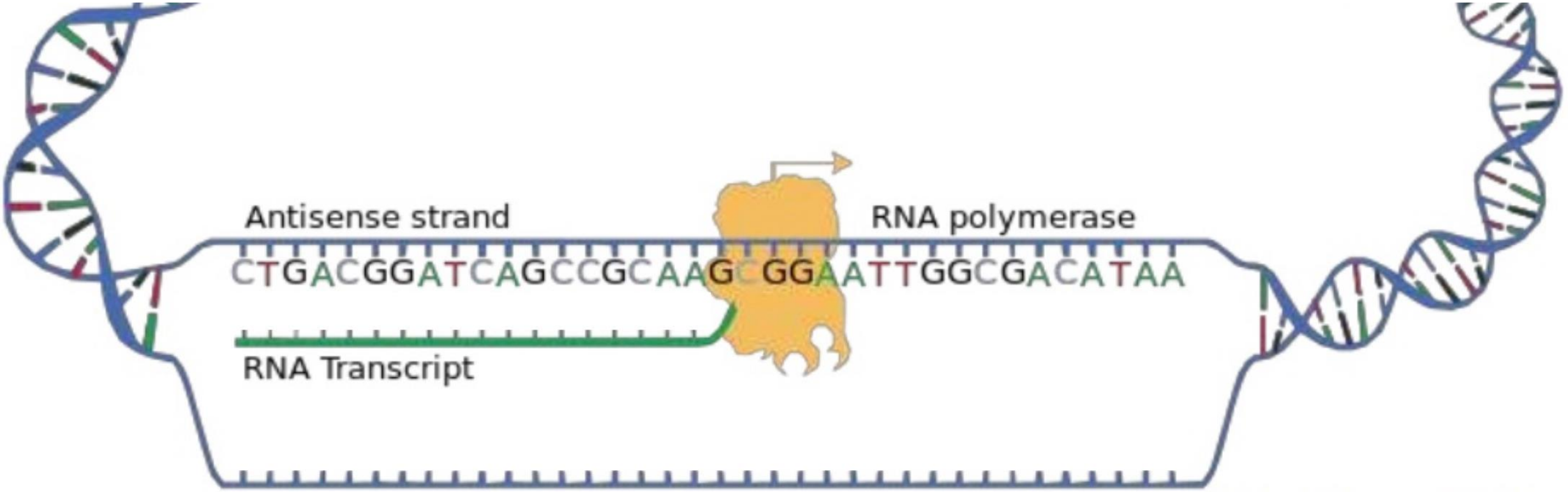
SENSE VS ANTISENSE STRAND

The sequence of DNA that is transcribed into RNA is called a gene

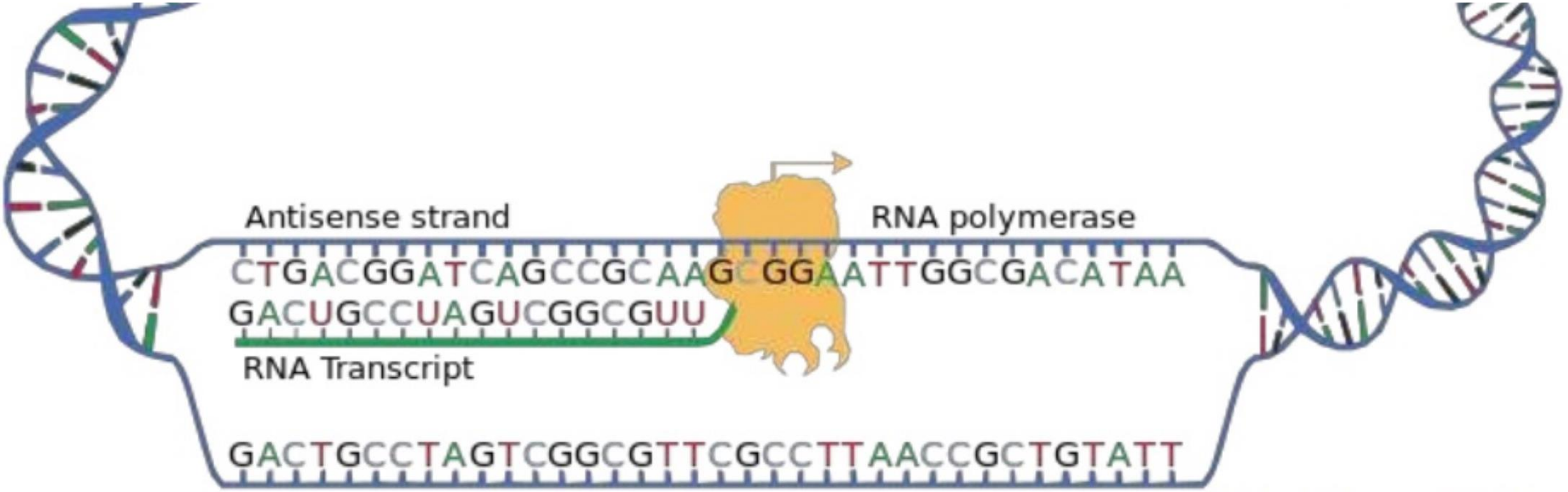
- The **antisense** strand **is** transcribed (*sequence = complementary to RNA transcript*)
- The **sense** strand is **not** transcribed (*sequence = identical to transcript – except T/U*)

Antisense:	TAC	GGT	CAC	TGA	AGT	CCC	TGC	TTA	CTG	AAT
Sense:	ATG	CCA	GTG	ACT	TCA	GGG	ACG	AAT	GAC	TTA
Transcript:	AUG	CCA	GUG	ACU	UCA	GGG	ACG	AAU	GAC	UUA

TRANSCRIPTION: EXERCISE



TRANSCRIPTION: ANSWER



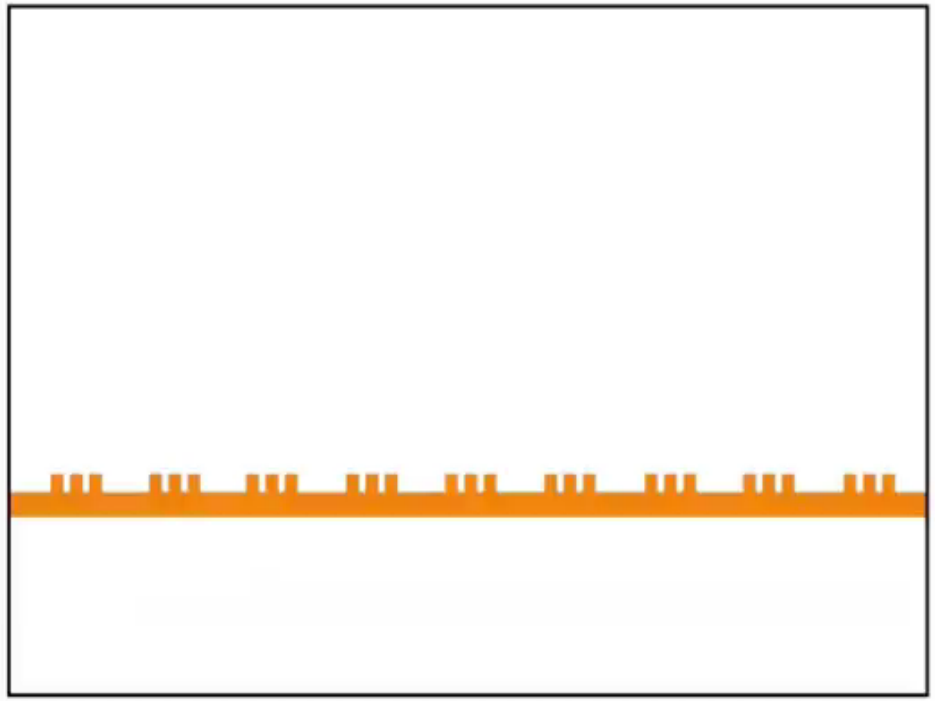
3) TRANSLATION

TRANSLATION

Involves polypeptide synthesis by ribosomes (e.g. *protein production)*

Polypeptides are encoded by genes and translated from an mRNA sequence (*which was produced via transcription)*

→ transcription and translation are linked processes in protein production

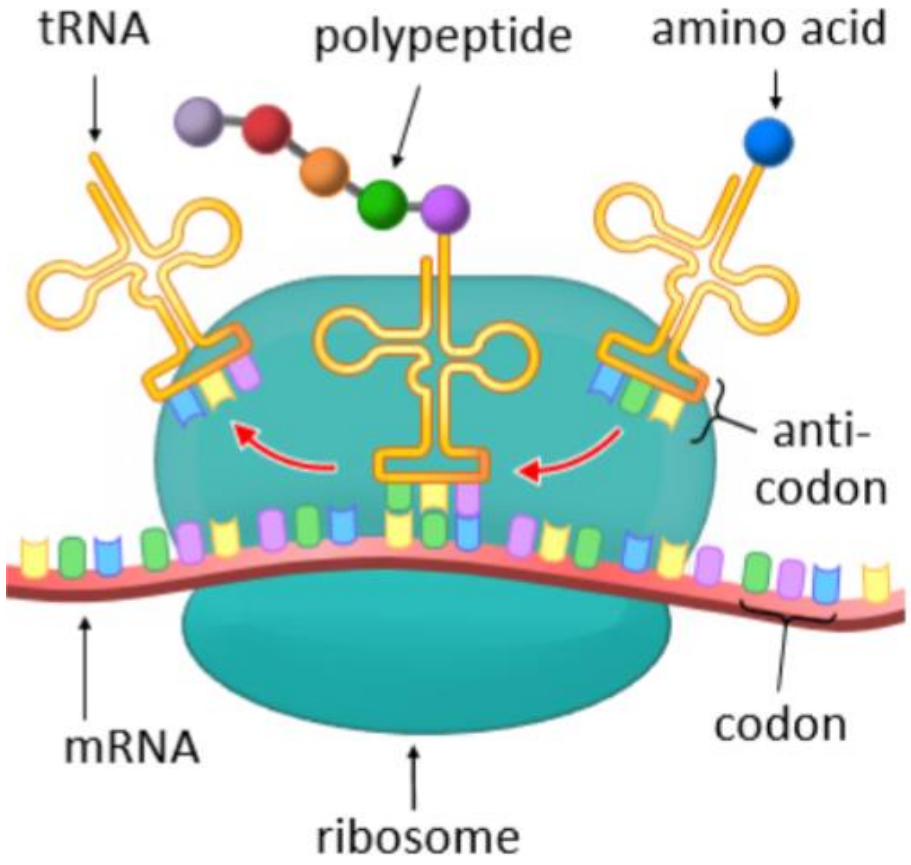


TRANSLATION: PROCESS

Messenger RNA is transported to a ribosome → reads sequence in base triplets (***codons***)

Transfer RNA molecules carry specific **amino acids** → allign opposite a specific codon (*according to complementary* ***anticodon***)

Ribosome moves along mRNA → joins amino acids together with peptide bonds



THE GENETIC CODE

Rules by which mRNA sequences are converted into protein

- codons = triplets of bases → correspond to particular amino acid
- order of codons determines amino acid sequence of polypeptide

Key features of the genetic code include:

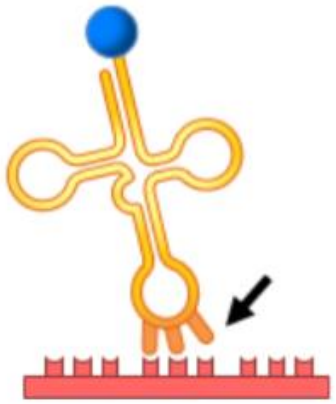
- **Universality**: all organisms use the same code (*few viral execeptions*)
- **Degeneracy**: more than one codon may code for same amino acid
    - 64 codon combinations vs 20 amino acids (*silent mutations possible*)

THE GENETIC CODE

A coding sequence of messenger RNA:

- initiates with a start codon (AUG)
- terminates with a stop codon

Degeneracy occurs in 3rd base position



'Wobble' hypothesis:
Shape of transfer RNA is not optimal for base pairing at third position

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC		UCC		UAC		UGC	
UUA	Leu	UCA		UAA	STOP	UGA	STOP
UUG		UCG		UAG		UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC		CCC		CAC		CGC	
CUA		CCA		CAA	Gln	CGA	
CUG		CCG		CAG		CGG	
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC		ACC		AAC		AGC	
AUA		ACA		AAA	Lys	AGA	Arg
AUG	Met	ACG		AAG		AGG	
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC		GCC		GAC		GGC	
GUA		GCA		GAA	Glu	GGA	
GUG		GCG		GAG		GGG	

UAG
UAA
UGA

1. Deduce the codon(s) that translate for Aspartate.

2. If mRNA contains the base sequence CUGACUAGGUCCGGA
    a. deduce the amino acid sequence of the polypeptide translated.

    b. deduce the base sequence of the DNA antisense strand from which the mRNA was transcribed.

3. If mRNA contains the base sequence ACUAAC deduce the base sequence of the DNA sense strand.

1. Deduce the codon(s) that translate for Aspartate.

   GAU, GAC

2. If mRNA contains the base sequence CUGACUAGGUCCGGA
   a. deduce the amino acid sequence of the polypeptide translated.

      Leucine + Threonine + Lysine + Arginine + Serine + Glycine

   b. deduce the base sequence of the DNA antisense strand from which the mRNA was transcribed.

      GACTGATCCAGGCCT *(the antisense strand is complementary to the mRNA, but remember that uracil is replaced by thymine)*

3. If mRNA contains the base sequence ACUAAC deduce the base sequence of the DNA sense strand.

   ACTAAC *(the sense strand is the template for the mRNA the only change is that uracil is replaced by thymine)*

READING FRAMES

mRNA transcript → organized into triplets of nucleotides = codons

- → three different reading frames for any given sequence

Open reading frame starts with **AUG** and continues until a **STOP** codon

- reading frames can be interrupted by frameshift mutations (*insertion, deletion*)

mRNA:	GUAUGCACGUGACUUUCCUCAUGAGCUGAU
Codons:	GU **AUG** CAC GUG ACU UUC CUC AUG AGC **UGA** U
Polypeptide:	**Met** His Val Thr Phe Leu Met Ser **STOP**

READING FRAME: EXERCISES

See worksheet

INTERPRETING ELECTRON MICROGRAPHS

- data based questions page 118