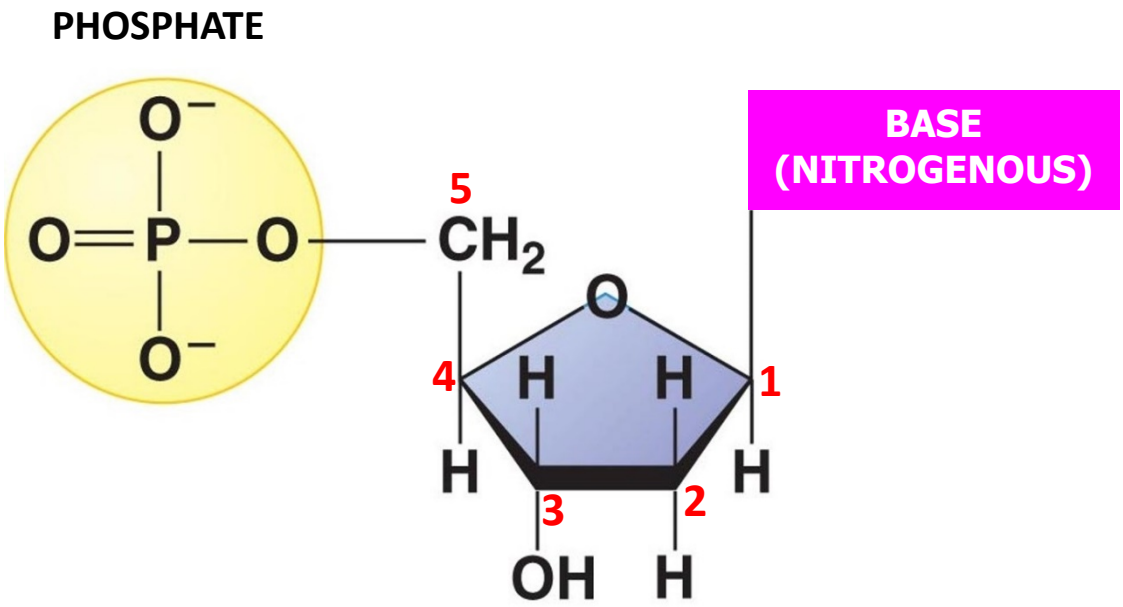
A. DNA STRUCTURE

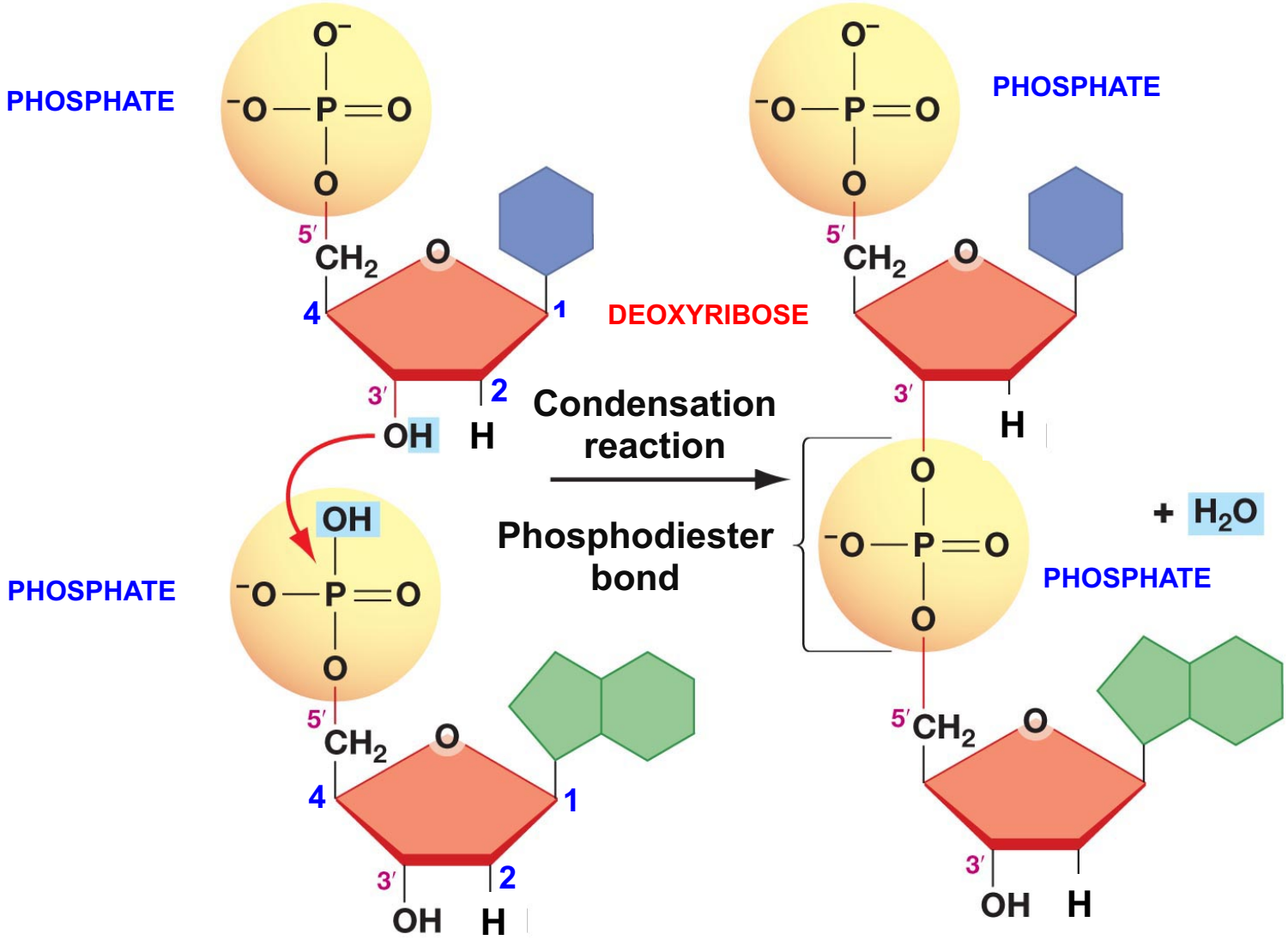
A nucleotide



DEOXYRIBOSE
has 5 carbon atoms and
the 5th is outside the ring

- The important **carbons** to remember here are **3**' and **5'**.

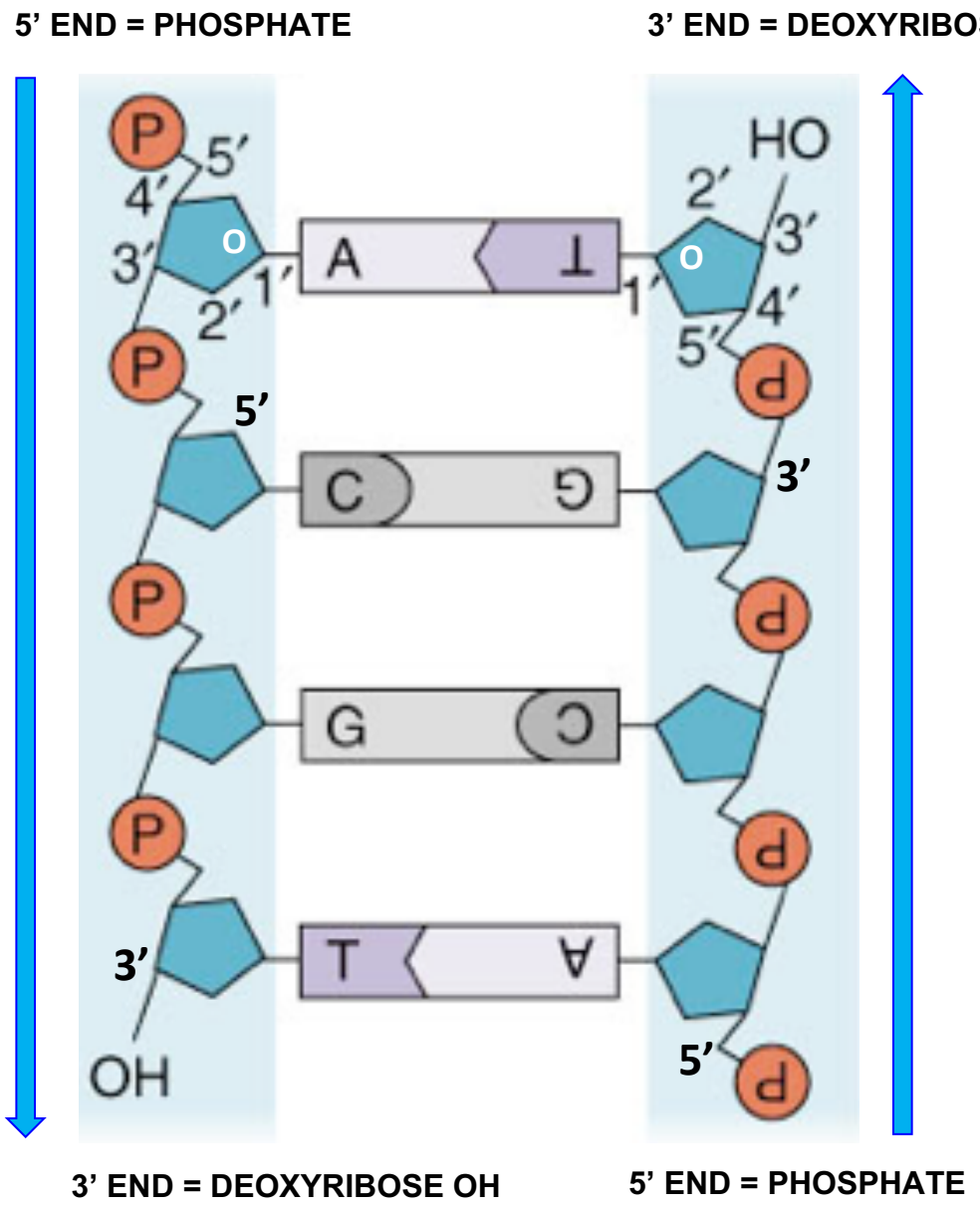
How nucleotides join together



- **New free nucleotides** are added to the **3'OH group** of the **previous deoxyribose** sugar.
- **Phosphates** are therefore **between** the **3'** and **5' carbons** of **two deoxyribose** sugars.
- The **3'OH** on **deoxyribose** reacts with the **OH** group on the **phosphate** in a **condensation reaction** to form a **phosphodiester bond**.

How the strands are labelled

- The two strands are **antiparallel** – they **run in opposite directions.**



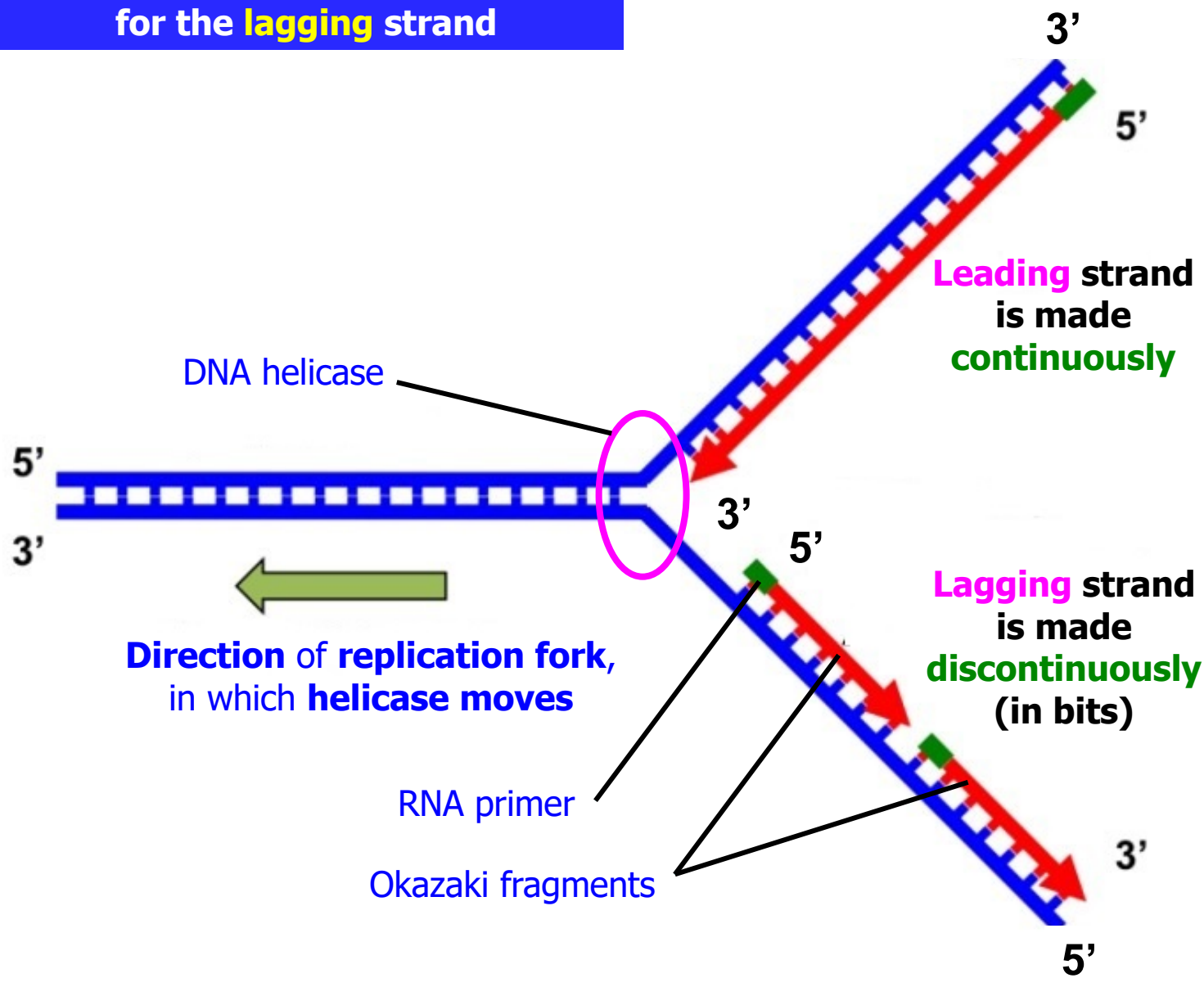
- **DNA strands** can only be **made** in a **5' → 3' direction**.
- Remember that **new nucleotides** react with the **3'OH group** on the **previous deoxyribose** sugar.

B. DNA REPLICATION

The leading and lagging strands

DNA polymerase can only add nucleotides in a 5′ → 3′ direction

This is in the opposite direction to helicase and the replication fork for the lagging strand



- **Okazaki fragments** are the **short fragments** of **DNA** made on the **lagging strand** that are eventually **joined** together.

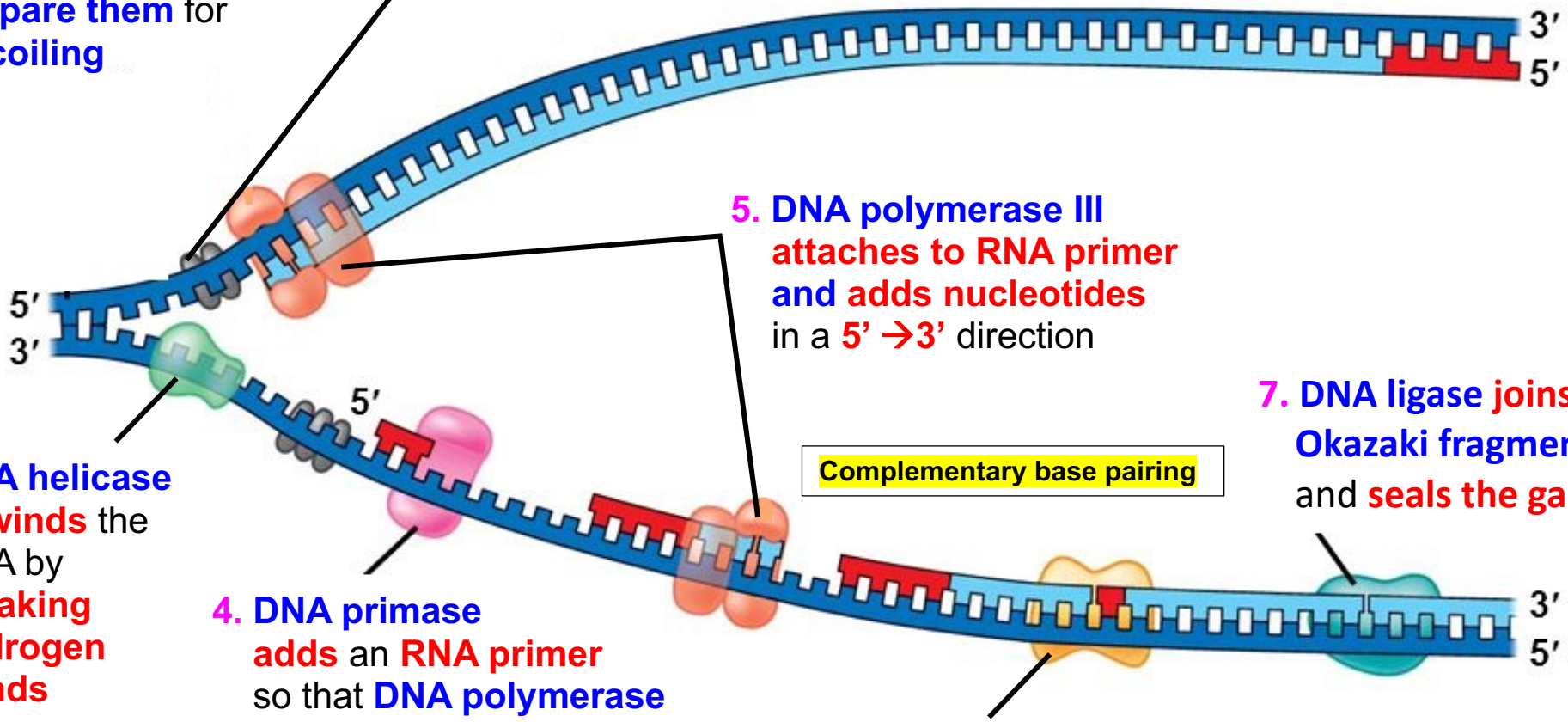
- **RNA primers** are **short fragments** of **RNA**, which are needed to allow **DNA polymerase III to attach** and **start replication.**

Enzymes involved in DNA replication

The **leading strand** is built up **continuously** and the **lagging strand** is built up in **short pieces** called **Okazaki fragments**.

1. **DNA gyrase relieves tension** in the strands **ahead** of **helicase** to **prepare them** for **uncoiling**

3. **Single-stranded binding proteins (SSBs) prevent** the DNA strands from **reattaching**



5. **DNA polymerase III attaches to RNA primer and adds nucleotides** in a **5' →3'** direction

Complementary base pairing

7. **DNA ligase joins** the **Okazaki fragments** and **seals the gaps**

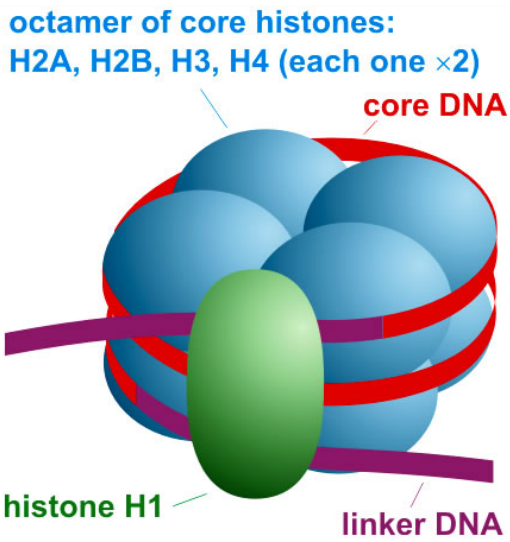
2. **DNA helicase unwinds** the DNA by **breaking hydrogen bonds**

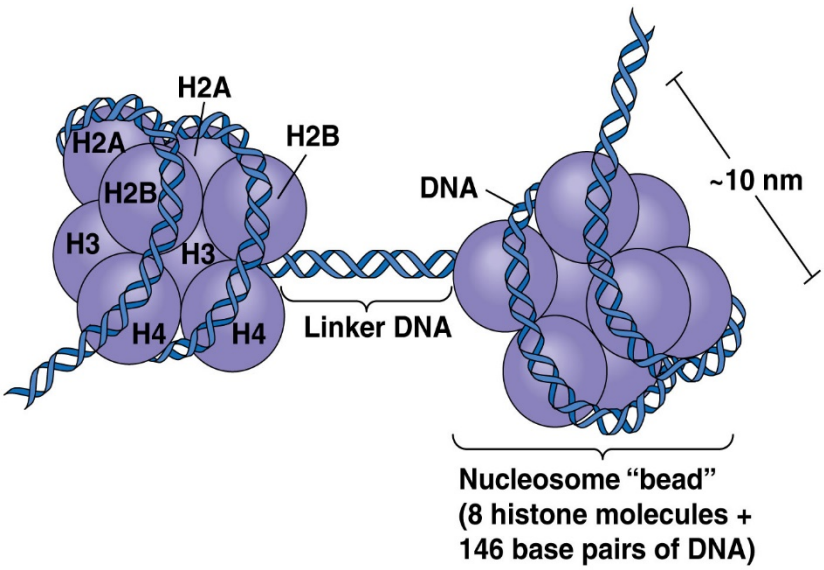
4. **DNA primase adds** an **RNA primer** so that **DNA polymerase** can **attach** and **start** to **add nucleotides**

6. **DNA polymerase I removes the RNA primers** and **replaces** them with **DNA**

C. NUCLEOSOMES

- In **eukaryotes**, DNA is **packaged** with **histone** proteins to create a **compact** structure called a **nucleosome**.





- found in **eukaryotes**
- made up of **DNA wrapped around histones (proteins)**
- **histones** are in an **octamer/group of eight**
- are **held together** by **another histone/H1**
- in **linker region**
- (function is to) help to **supercoil chromosomes**/ help in **DNA packing/supercoiling**
- (function is to) **control transcription/gene expression**

D. FUNCTIONS OF DNA BASE SEQUENCES THAT DO NOT CODE FOR PROTEINS

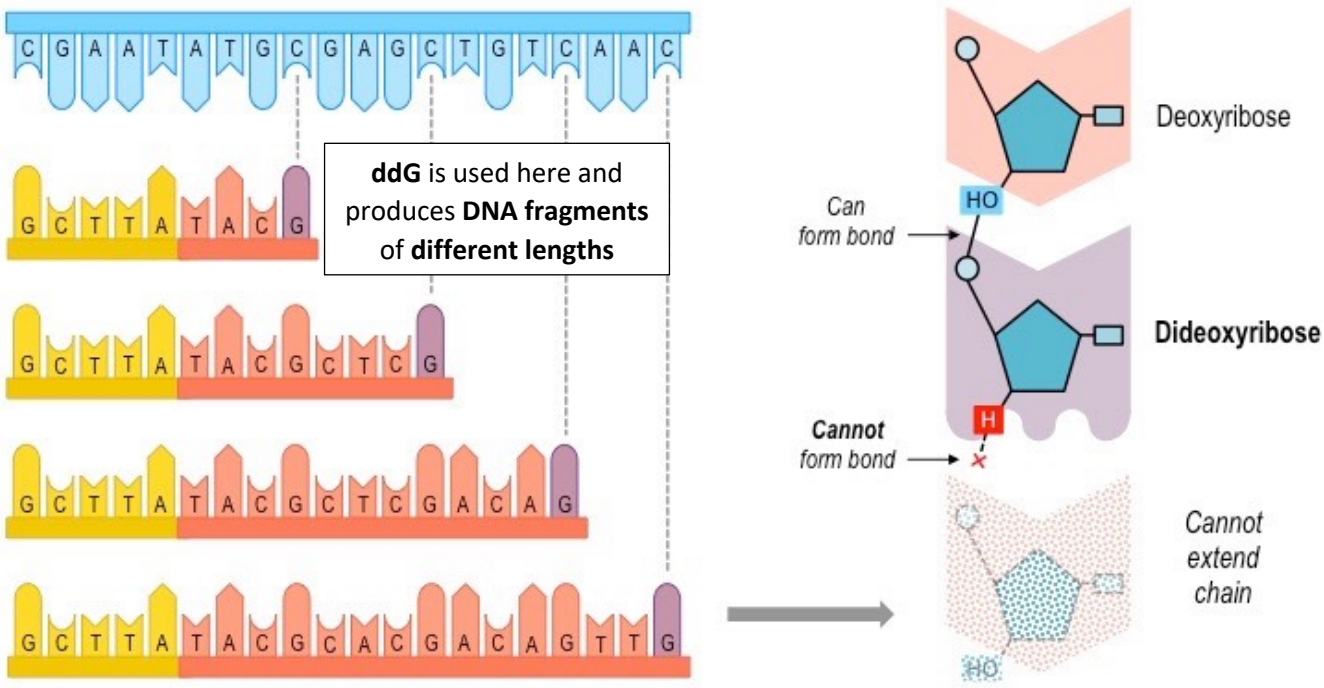
- **Non-coding** DNA base sequences can have **four** main roles:

1. **Introns** – involved in **processing mRNA**
2. **Coding for tRNA and rRNA** – these are involved in **translation**
3. **Controlling transcription/gene expression** – **binding sites** for **proteins** that can **allow** or **prevent transcription**.
4. **Telomeres** – **repetitive base sequences** at the **ends** of **chromosomes**, which **prevent parts of genes here from being lost** each time the **DNA is replicated**.

E. DNA SEQUENCING BY THE SANGER METHOD

Chain Terminators

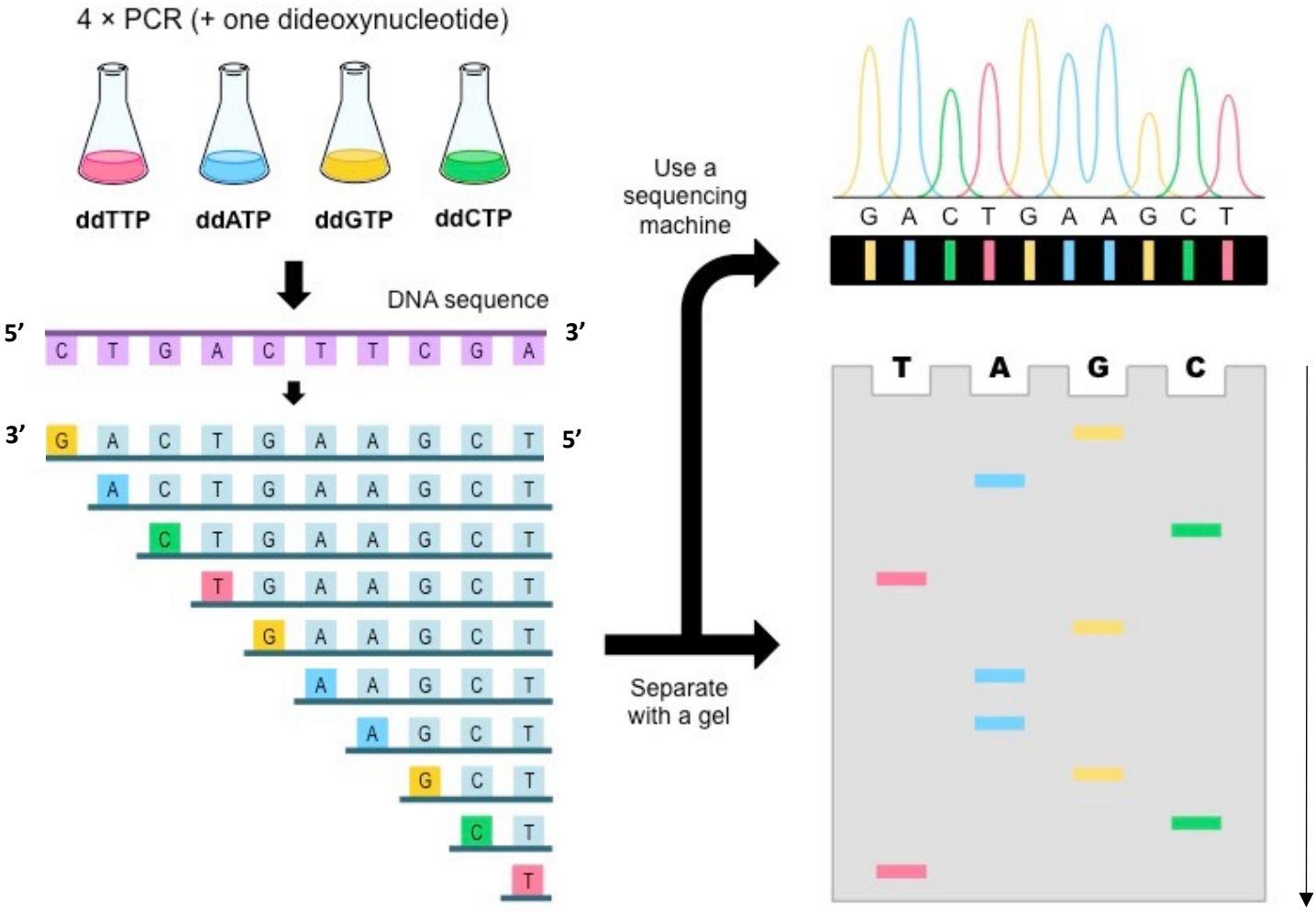
- The Sanger method is used to determine the **DNA base sequence** of a gene.
- Special DNA nucleotides called **dideoxynucleotides** (**ddNTPs**) are used that act as **chain terminators**.
- **ddNTPs** contain the sugar **dideoxyribose**, instead of the usual sugar of deoxyribose.
- There are **four** types of **ddNTP**: dd**A**, dd**T**, dd**C** or dd**G**.
- When **ddNTPs** attach to the growing DNA chain, they **stop it from growing**.



Mixing

- Four separate mixes are set up, each containing **normal nucleotides** plus **one** type of **dideoxynucleotide** (dd**A**, dd**T**, dd**C** or dd**G**).
- The DNA fragments produced will **vary in length**, depending on **how far replication got to** before a **dideoxynucleotide** was **added** to the end of a chain.
- A typical PCR will generate over 1 billion DNA molecules, so each PCR mix should generate **all the possible terminating fragments** for that **specific base**.

Separating the DNA fragments by gel electrophoresis



The newly made DNA strand is made from 5' → 3'

But this is complementary to the original DNA strand

The first base of the new complementary strand must be the smallest DNA fragment

- The gel is read from **bottom** to **top**.
- (So) base sequence of new complementary DNA strand is **5' T C G A A G T C A G 3'**
- The two DNA strands are **antiparallel**.
- (So) base sequence of original strand must be **5' C T G A C T T C G A 3'**

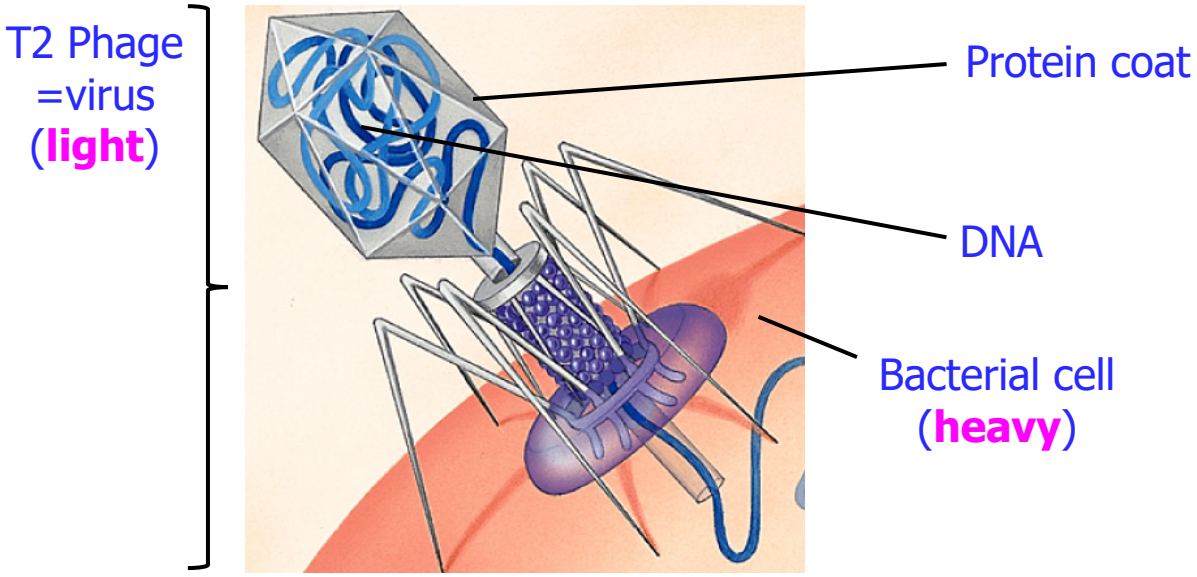
F. HERSHEY & CHASE EXPERIMENT

Background

- Showed that **DNA**, rather than protein, is the **genetic material**.

- Used a **virus** (T2 phage) that infects bacteria.

- This virus contains **DNA** inside a **protein coat**.



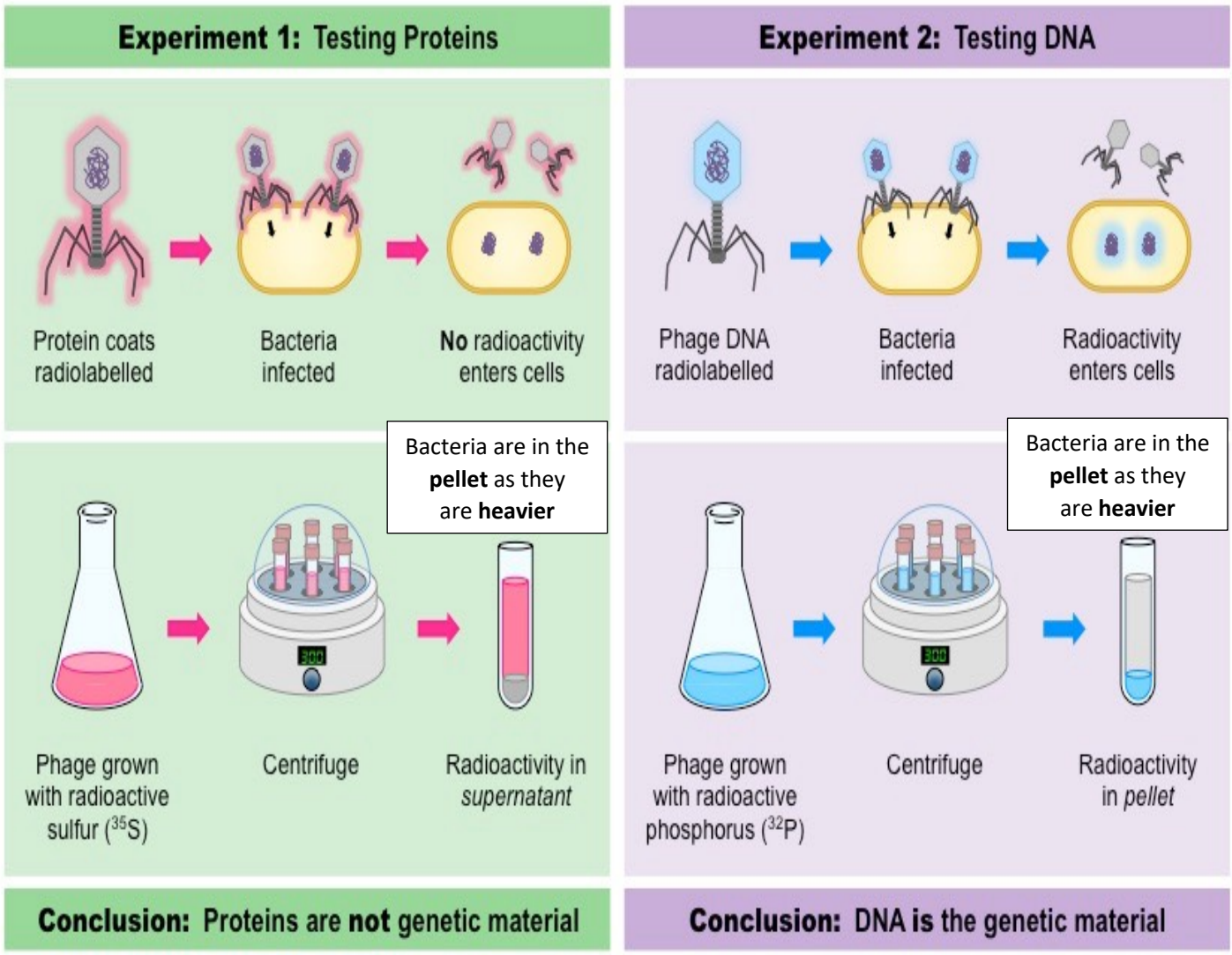
- This virus **injects** its **genetic material** into bacteria and this is used to make **more copies of the virus**.

- They used two **radioactive** isotopes, which can be **detected**:

  - ^{35}**S** (found in **protein** but not in DNA)
  - ^{32}**P** (found in **DNA** but not in protein).

- **Bacteria** are **heavier** than viruses.

What They Did & Found Out



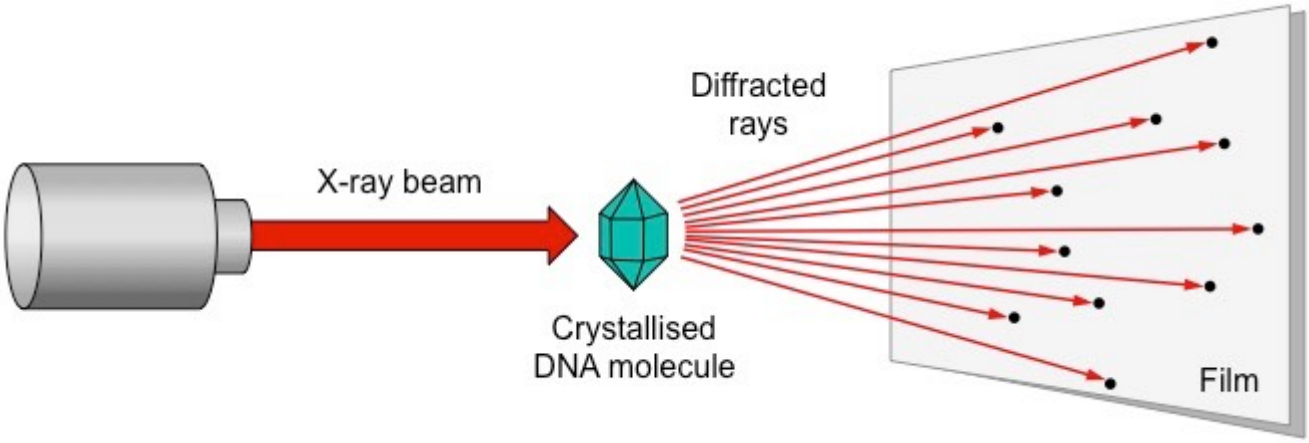
- Virus **protein coats** were labelled with **radioactive ^{35}S**
- **Infected** the bacteria with virus to allow **genetic material** to be **transferred**
- Centrifugation then used to **break attachment, separating** the virus and bacteria
- **Radioactivity detected** in the **virus** (supernatant = fluid above = lighter)
- (So) **protein** had **not** been **transferred** to the bacteria
- (So) **protein** is **not** the **genetic material**

- Virus **DNA** was labelled with **radioactive ^{32}P**
- **Infected** the bacteria with virus to allow **genetic material** to be **transferred**
- Centrifugation then used to **break attachment, separating** the virus and bacteria
- **Radioactivity detected** in the **bacteria** (pellet = pellet at bottom = heavier)
- (So) **DNA had** been **transferred** to the bacteria
- (So) **DNA is** the **genetic material**

G. X-RAY DIFFRACTION

- Rosalind Franklin and Maurice Wilkins used a method of **X-ray diffraction** to investigate the structure of DNA.

The technique



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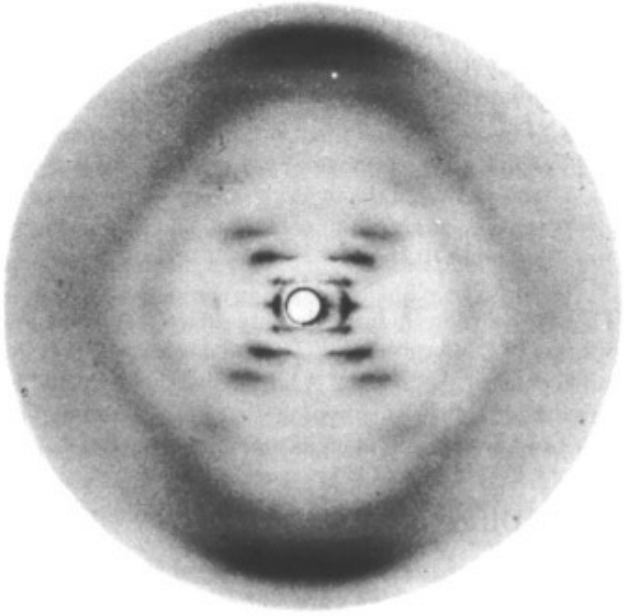
- From the **scattering pattern** produced by a **DNA molecule**, certain inferences could be made about its structure



1. It is a **double stranded** molecule
2. **Bases** are packed closely together on the **inside** and **phosphates** form an **outer** backbone
3. The molecule **twists** at **regular intervals** (every 34 Angstroms) to form a **helix**
4. There are **10** bases **per twist**