



# DP IB Biology: HL



## 7.1 DNA Structure & Replication

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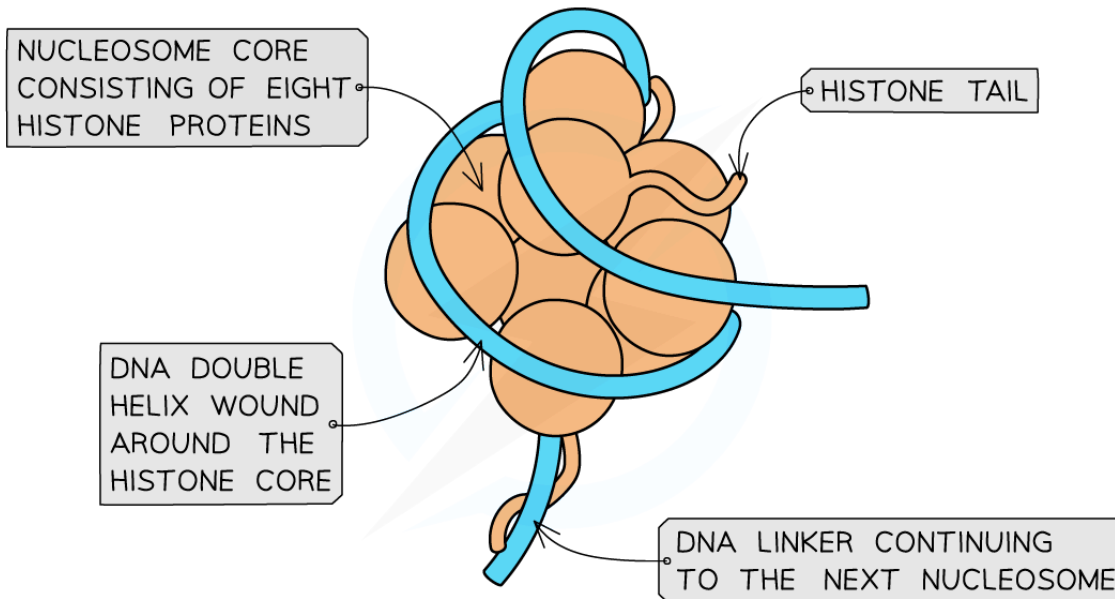


Your notes

## 7.1.1 DNA Structure

### Nucleosomes

- Unlike most prokaryotic DNA which is referred to as 'naked', eukaryotic nuclear DNA is associated with proteins called **histones** (to form **chromatin**)
- Histones package the DNA into structures called nucleosomes
  - The **nucleosome** consists of a strand of DNA coiled around a core of eight histone proteins (octamer) to form a bead-like structure
  - DNA takes **two turns** around the histone core and is held in place by an additional histone protein
  - The DNA molecule continues to be wound around a series of nucleosomes to form what looks like a '**string of beads**'
- Nucleosomes help to **supercoil the DNA**, resulting in a compact structure which saves space within the nucleus
  - Nucleosomes also help to **protect DNA** and **facilitate movement of chromosomes** during cell division
  - An analogy for supercoiling is **twisting an elastic band** repeatedly until it forms additional coils
- Nucleosomes can be **tagged with proteins** to promote or suppress transcription

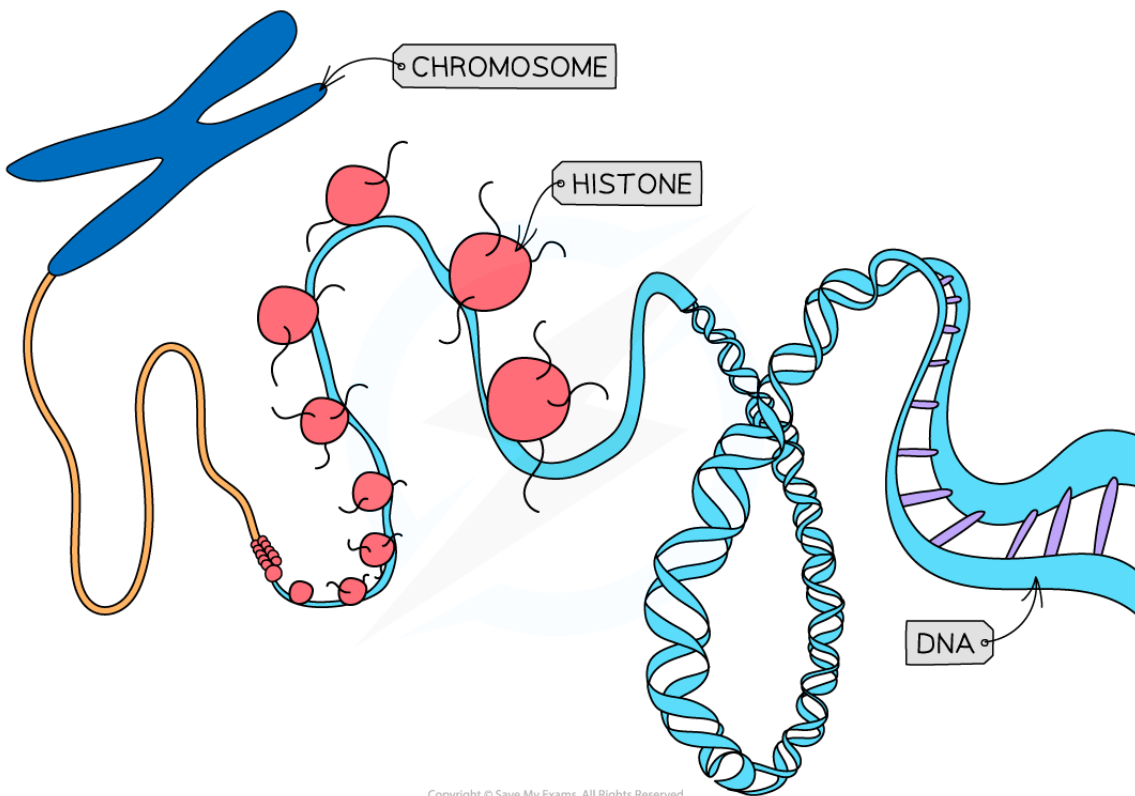


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**Structure of a nucleosome**



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***DNA is wrapped around a series of nucleosomes.  
Nucleosomes coil tightly around each other to form the chromosome structure.***

## Franklin's Investigations

**NOS Making careful observations—Rosalind Franklin's X-ray diffraction provided crucial evidence that DNA is a double helix.**

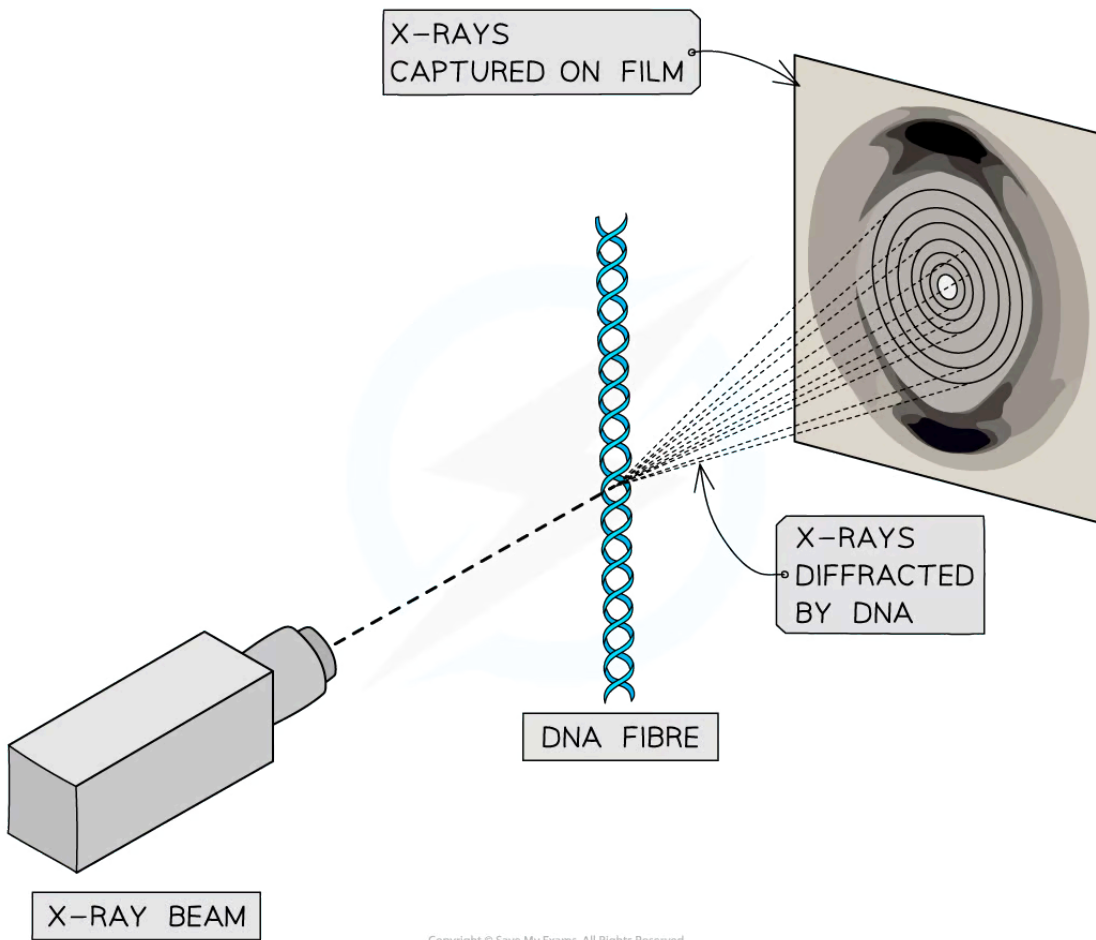


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- In the 1950s **Rosalind Franklin** and **Maurice Wilkins** used a technique called **X-ray diffraction** to study the structure of DNA
  - Franklin's work was **instrumental to Crick and Watson's model** as the diffraction patterns indicated that DNA had a **double-helical** structure
- X-ray diffraction involves directing a **beam of X-rays** onto the molecule being studied
  - X-rays have a **shorter wavelength** and **higher energy** than visible light
- The short wavelength allows X-rays to pass **through the molecule**, interacting with any **electrons** within the atoms
  - The interaction **causes X-rays to scatter** (diffraction) at angles that indicate the arrangement of atoms
  - The scattering pattern can be **recorded on a film** (similar to having an X-ray of a bone), with dark marks appearing where the X-rays strike the film
  - Rotating the sample allows for the **three-dimensional molecular structure** to be studied
- Franklin was able to **refine her methods** and produce a clear diffraction pattern of DNA
- Using mathematical techniques and available knowledge about DNA, Franklin deduced that
  - DNA strands were **helices** - as represented by the X-shape
  - The **pitch** of the helix - as represented by the angle of the X-shape
  - The **distance** between nucleotides
  - Phosphates are located on the **outside** of the molecule
  - DNA was **double stranded**



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**Summary of Rosalind Franklin's X-ray diffraction investigation, the diffraction pattern represents the position of atoms in the sample of DNA**

## DNA Structure Suggests Semi-conservative Replication

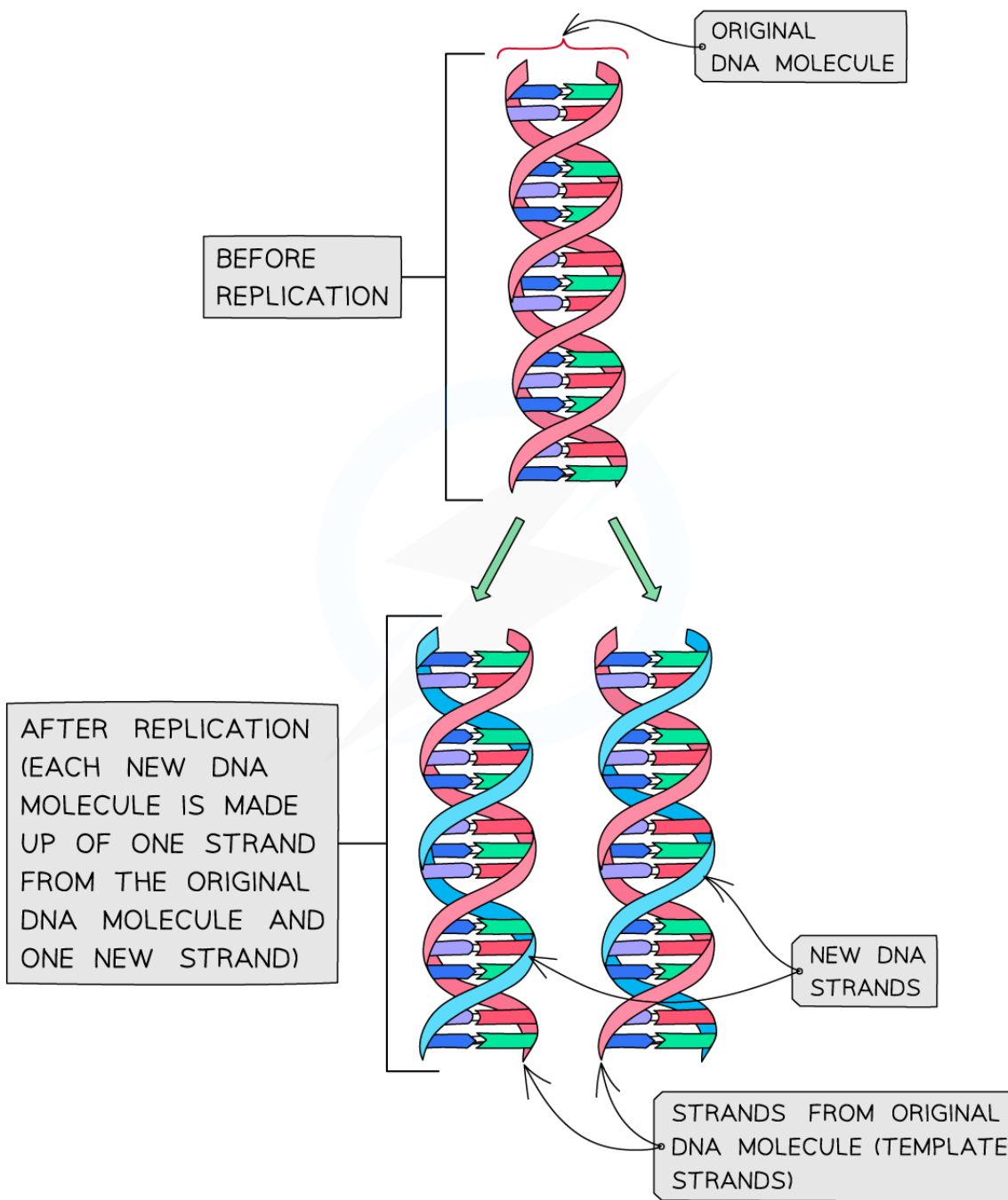
- The discovery of the structure of DNA was due to **experimental evidence** and inputs from a **range of independent researchers**
  - Franklin's X-ray diffraction patterns identified a **compact double helix**
  - Erwin Chargaff showed that DNA was composed of an equal number of **purine and pyrimidine** bases which suggested base pairing
  - Crick and Watson used this evidence to build various **physical models of DNA**
  - One model had the bases facing outwards but Franklin argued they should **face inwards** due to their hydrophobic nature
  - It was determined that if adenine paired with thymine and cytosine paired with guanine in an **antiparallel** orientation a **highly compact structure** would result
- When Crick and Watson proposed their model for the structure of DNA, they realised that the double stranded structure suggested **a mechanism for its replication** during the cell cycle
  - This was a **key question** that any model would have to address
- Crick and Watson stated that as one chain of the double helix was the complement of the other, **either chain could act as a template** during replication
  - They postulated that **hydrogen bonds break**, allowing separation of the chains
  - Each separate chain then **acts as a template** for the formation of a new complementary chain on itself
- This theory was called **semi-conservative DNA replication** as half of the original DNA molecule is kept (conserved) in each of the two new DNA molecules
- An **opposing theory** suggested DNA replicated 'conservatively'
  - The entire original DNA double helix would stay together and serve as a template for a new DNA molecule
- Crick and Watsons' theory of semi-conservative DNA replication was later **proven by Meselson and Stahl**



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**Semi-conservative replication of DNA**

 **Examiner Tip**

You don't need to memorise the nature of purine and pyrimidine bases in DNA; C and T are pyrimidines; A and G are purines. A purine always bonds to a pyrimidine in the A-T and C-G rules of base-pairing.



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## 7.1.2 Mechanism of DNA Replication



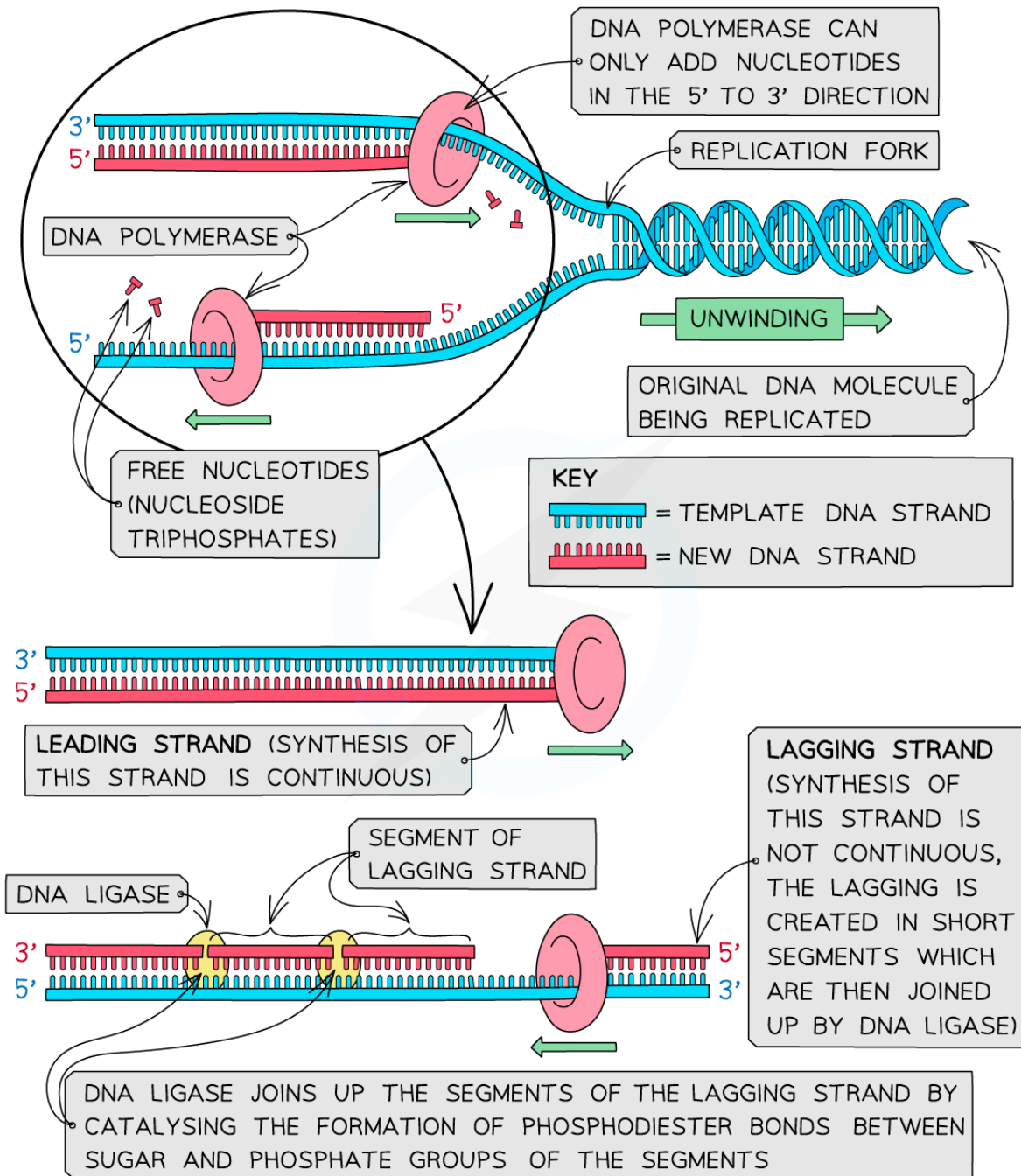
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### Leading Strand & Lagging Strand

- Double-stranded DNA consists of **two antiparallel strands** (oriented in opposite directions)
- During DNA replication, the two strands are 'unzipped' and DNA polymerase moves along each template strand linking nucleotides together to form a new strand
  - Crucially, **DNA polymerase can only add new nucleotides in a 5' to 3' direction**
  - As the template strands are antiparallel, replication needs to proceed in opposite directions
- As the replication fork opens up in one direction only, each new strand is synthesised differently
  - The **leading strand** is made **continuously**, following the fork as it opens
  - The **lagging strand** is made **discontinuously**, in short fragments, away from the fork
- As more template strand is exposed, new fragments (called Okazaki fragments) are created
  - Okazaki fragments are later joined together by DNA ligase to form a continuous complementary DNA strand



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**During DNA replication, synthesis of the leading strand is continuous but synthesis of the the lagging strand is discontinuous in small fragments (not all the enzymes involved are shown)**

## Enzymes Involved in DNA Replication

- DNA replication is carried out by a **complex system of enzymes** working as a team
- **Helicase** unwinds the DNA double helix at the replication fork by flattening out its helical structure
  - Analogy - think about untwisting a rope ladder
- Helicase then causes the hydrogen bonds between the two strands to break
  - Analogy - unzipping a zipper
- **DNA gyrase** releases the strain within the supercoiled areas to allow helicase access to the helix
- **Single-stranded binding proteins** keep the separated strands apart whilst the template strand is copied
- **DNA primase** generates a short RNA primer on the template strands
  - Providing an initiation point for DNA polymerase III to add new nucleotides
- A **number of polymerases** are involved in DNA replication, each with different functions
  - Two of these polymerases are
    - **DNA polymerase III**, which starts replication next to the RNA primer linking nucleotides in a 5' to 3' direction to form a new strand
    - **DNA polymerase I**, which removes the RNA primers on the leading and lagging strands and replaces it with DNA
- **DNA ligase** joins up the Okazaki fragments by catalysing the formation of sugar-phosphate bonds



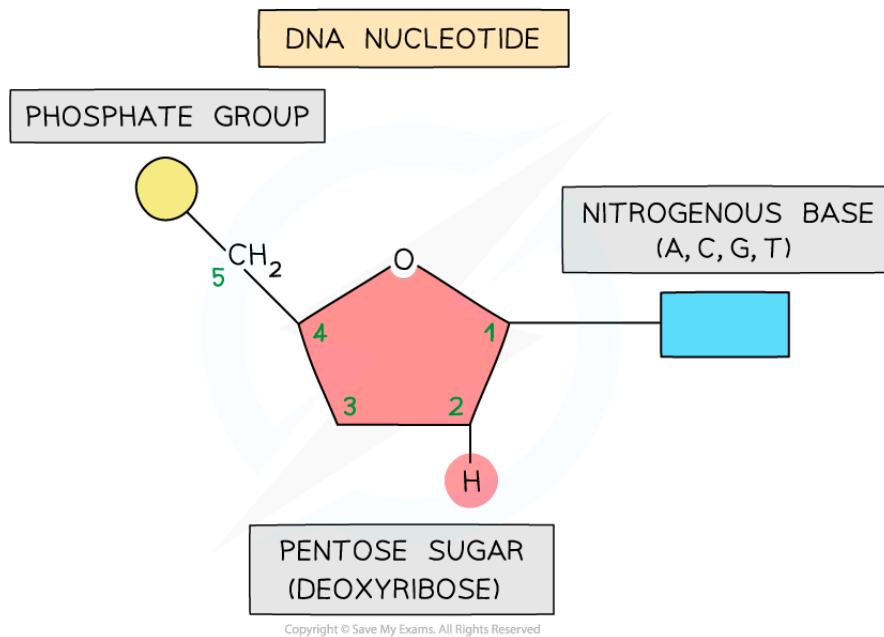
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## Direction of Replication

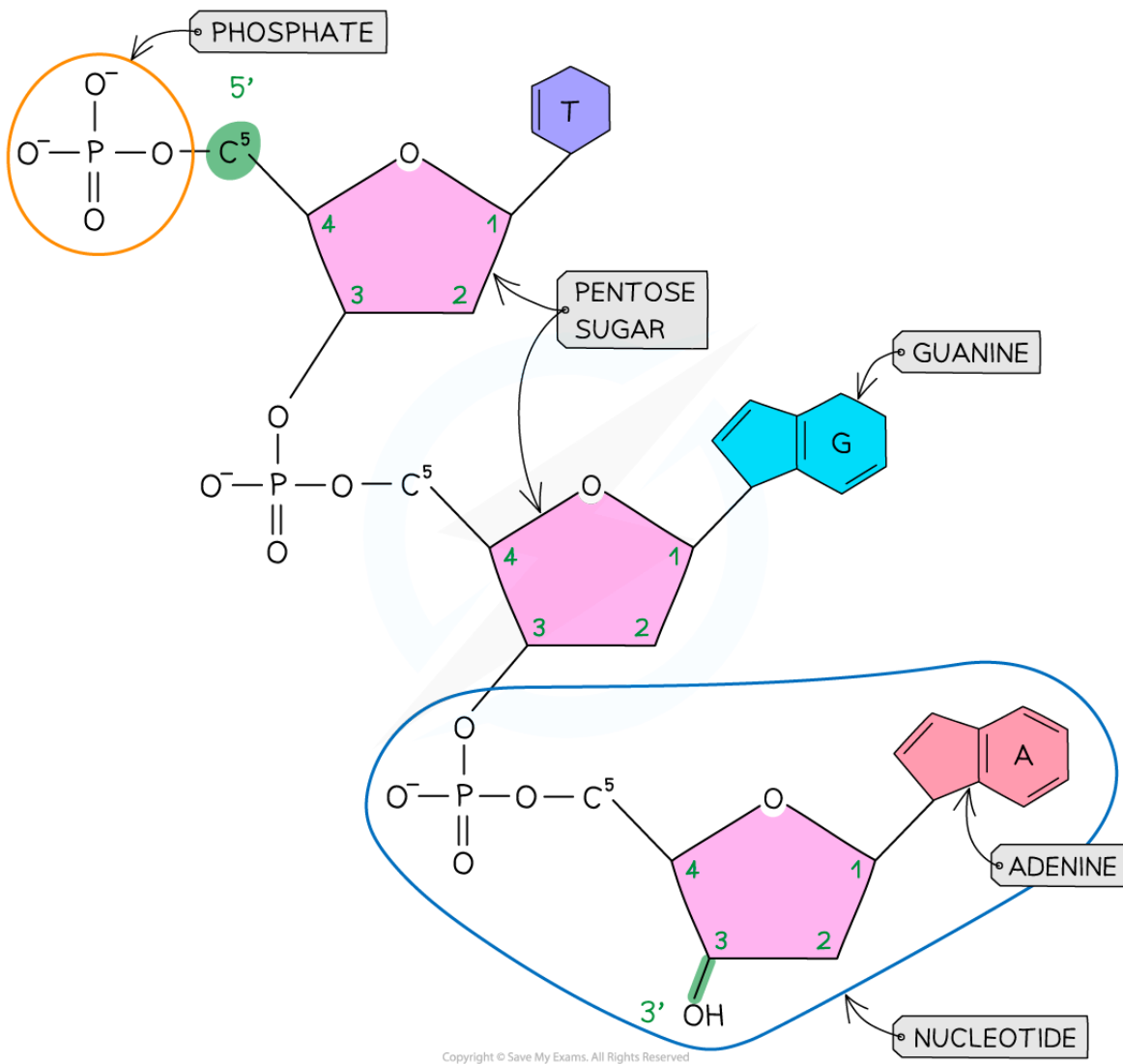
- Similar to transcription and translation, DNA replication must occur in the **5' to 3' direction**
- DNA polymerase only works in a 5' to 3' direction, **adding nucleotides to the 3' end**
  - DNA polymerases can only add nucleotides to the 3' end of a primer
- DNA nucleotides have a **phosphate** bonded to the **5' carbon** of the deoxyribose sugar
- When DNA polymerase adds a new nucleotide to extend the DNA strand, the 5' phosphate group of the **incoming DNA nucleotide** bonds to the free 3' -OH group on the **growing strand**



**DNA nucleotides have a phosphate bonded to the 5' carbon of the pentose sugar**



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**When DNA polymerase adds a new nucleotide, the 5' phosphate group of the incoming nucleotide bonds to the free 3' -OH group on the growing strand**



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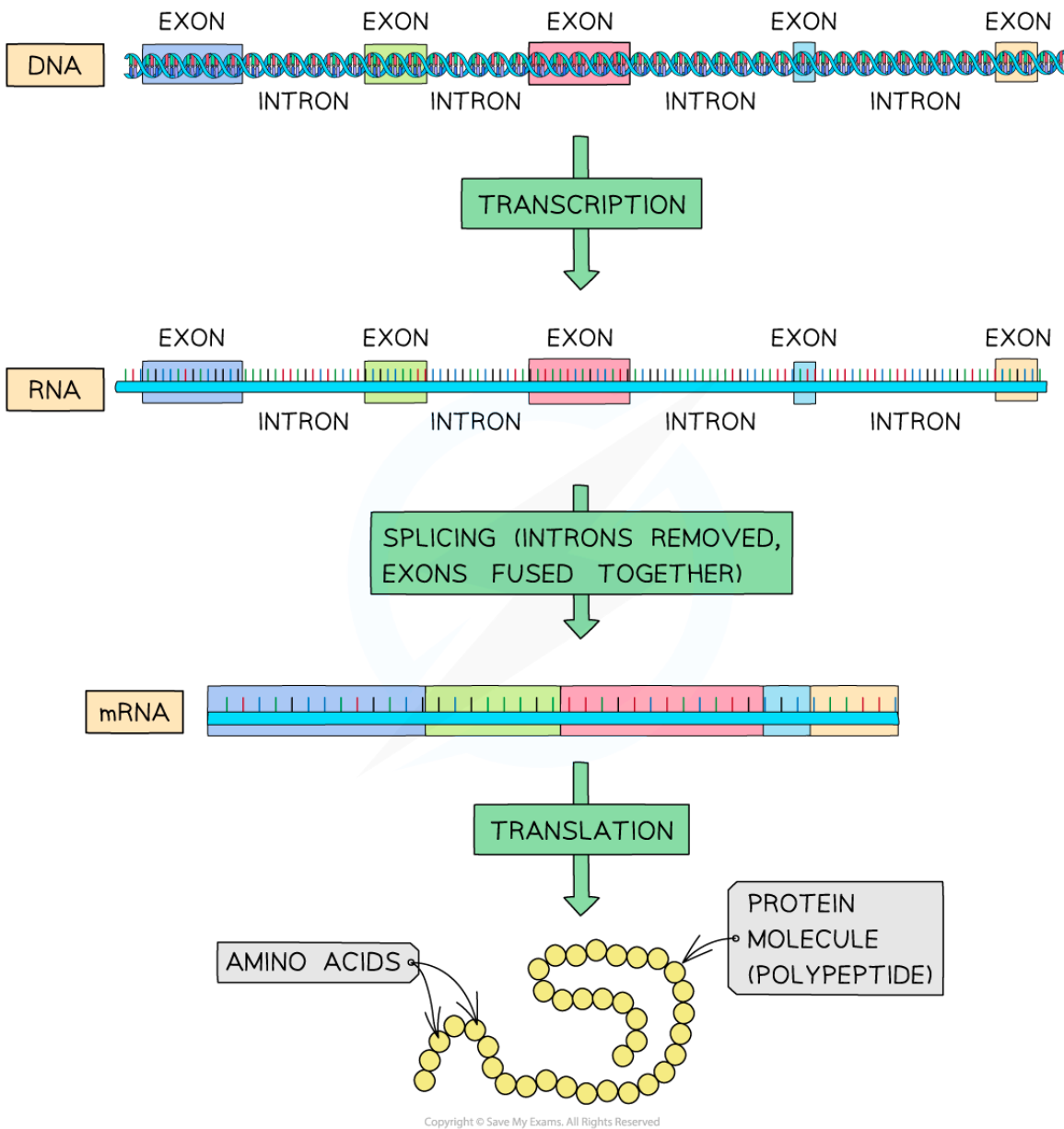
## 7.1.3 Non-coding DNA

### Non-coding Regions of DNA

- DNA molecules are very long but **only certain regions code** for the production of polypeptides
  - These are called **coding sequences**
- In humans only **1.5% of the genome** contains coding sequences
- The majority of a eukaryotic genome contains non-coding regions of DNA that do not code for polypeptides but **have other important functions**
- Non-coding gene **regulatory sequences** are involved in the control of gene expression by enhancing or suppressing transcription
- Non-coding sequences can produce functional RNA molecules like transfer RNA (tRNA)
- **Introns** are non-coding sequences of DNA found within genes of eukaryotic organisms
  - Different proteins can be produced from a gene depending on how introns are removed
- **Telomeres** are regions of **repeated nucleotide sequences** at the end of chromosomes that provide protection during cell division
  - The repeated sequence **facilitates binding of an RNA primer** at the end of the chromosome leading to synthesis of an Okazaki fragment
  - Without telomeres, DNA replication could not continue to the end of the DNA molecule and **chromosomes would become shorter** after every cell division
  - Nonetheless, telomeres shorten with age due to oxidative damage within cells
    - **Loss of telomeres** during ageing can be accelerated by smoking, exposure to pollution, obesity, stress and poor diet
    - **Antioxidants** in the diet are claimed to reduce the rate of telomere shortening



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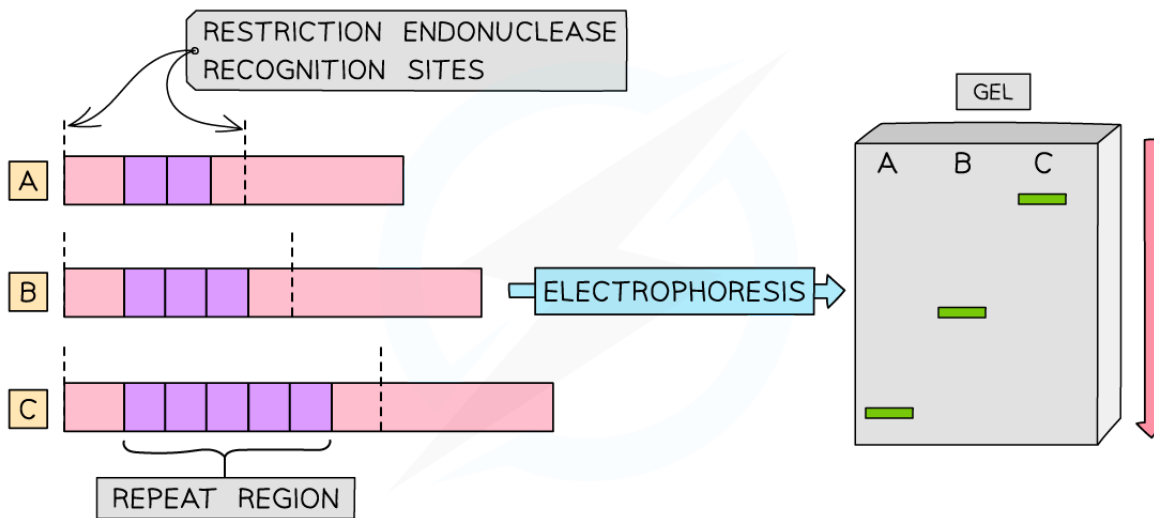
**The RNA molecule produced from the transcription of a gene contains introns that must be removed before translation can occur.**



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## DNA Profiling

- **DNA profiling** (sometimes called genetic fingerprinting) enables individuals to be identified based on their DNA profiles
  - It can be used in **forensic investigations** or **paternity testing**
- Short, non-coding regions of DNA called **variable number tandem repeats (VNTRs)** are analysed
  - The frequency that VNTRs are repeated is **unique between different individuals**
  - VNTRs are inherited and are **similar in close relatives** but **different in unrelated people**
  - Monozygotic (identical) twins **inherit identical VNTRs** so can't be differentiated through profiling
- To compare the respective DNA profiles of individuals, different regions of DNA containing the VNTRs can be **excised with restriction enzymes** or **amplified by PCR** (Polymerase Chain Reaction)
- The VNTR region for individuals will be **a different size** as they have **different numbers of repeats**
  - The resulting restriction fragment or amplified DNA will also be a different size
  - Different sized fragments will generate **a unique DNA profile** in **gel electrophoresis**



**DNA profile of specific VNTRs from three individuals. Different VNTRs could be analysed simultaneously which would result in more bands in each column**





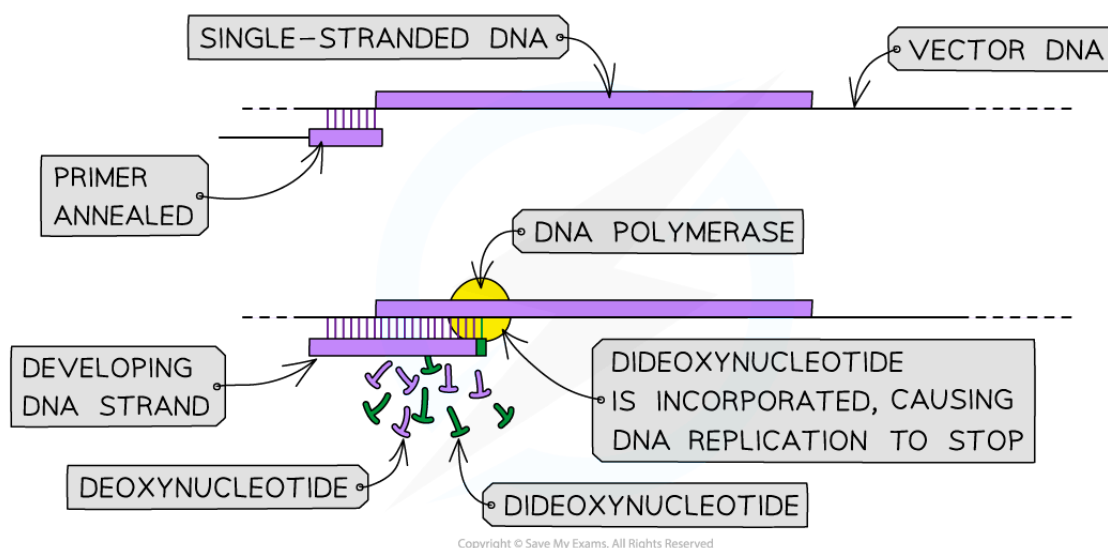
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## 7.1.4 DNA Sequencing

### DNA Sequencing

**Application: Use of nucleotides containing dideoxyribonucleic acid to stop DNA replication in preparation of samples for base sequencing**

- DNA sequencing allows for the nucleotide base sequence of an organism's genetic material to be determined
- Most methods for sequencing DNA involve the use of chain-terminating dideoxynucleotides
  - The dideoxy **chain-termination method** was developed by Frederick Sanger in 1977
- The chain-termination method uses **modified nucleotides** called **dideoxynucleotides**
  - Dideoxynucleotides have a slightly different structure to standard nucleotides
  - They lack the 3'-hydroxyl group so **cannot form a covalent bond** with the next nucleotide to be incorporated by DNA polymerase
  - Dideoxynucleotides **prevent elongation of the nucleotide chain**, which therefore terminates
- Advances in technology have enabled the development of **rapid high-throughput sequencing methods** which allow scientists to sequence the genomes of organisms rapidly



**Once the dideoxynucleotide is added to the developing strand DNA polymerase stops the replication of the developing DNA strand to produce a shortened DNA chain**

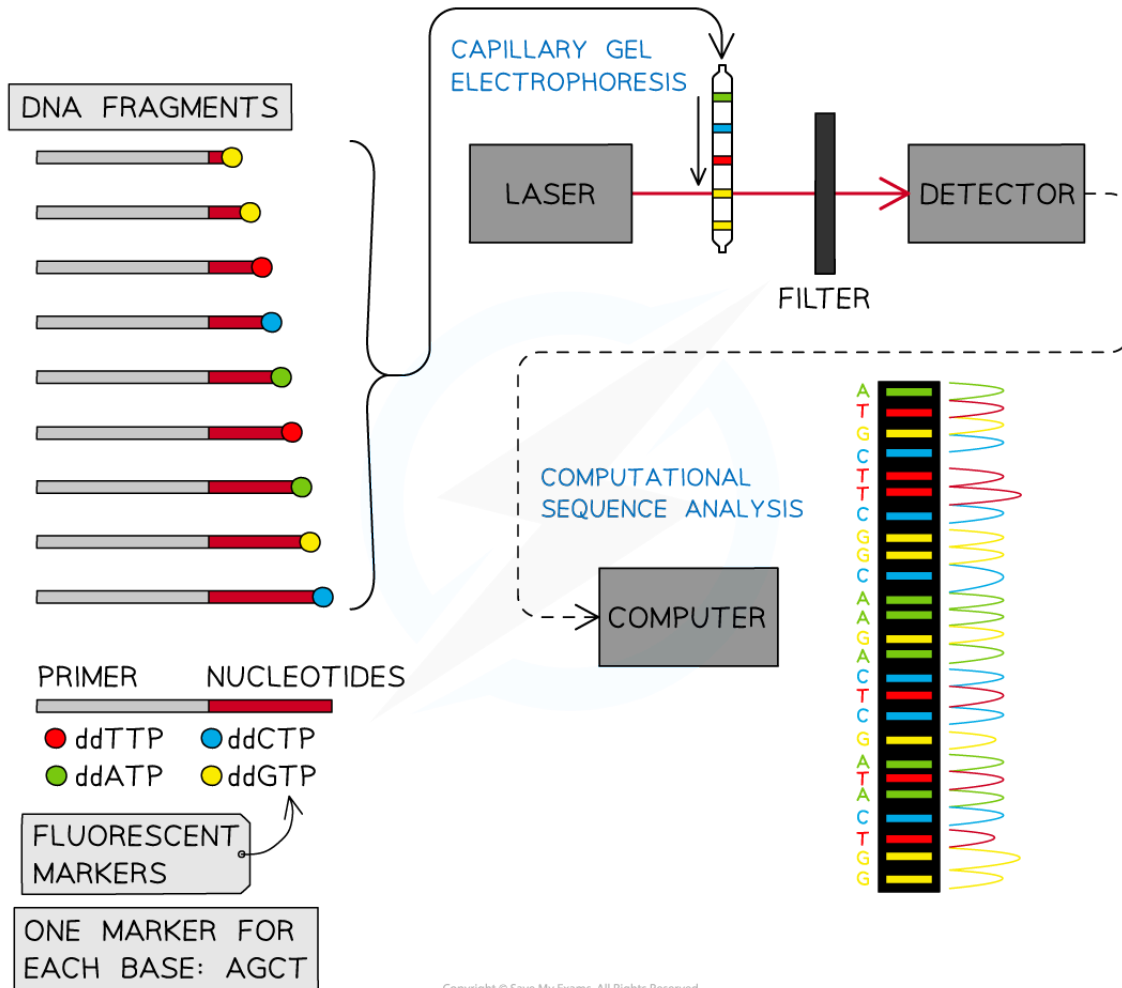
#### The chain termination method in action

- DNA sample of interest is used as a template in chain-termination PCR
- Deoxynucleotides and fluorescently-labelled **dideoxynucleotides** are used
- In the extension step of PCR, DNA polymerase will incorporate deoxynucleotides



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- If a dideoxynucleotide is **randomly incorporated**, extension stops
- Because of the nature of PCR, **billions of copies of the DNA sequence** of interest will be produced that will be terminated (by a dideoxynucleotides) at random lengths
- The fragments can **separated by size** in gel electrophoresis
- The fluorescent marker corresponds to a particular 'terminator' nucleotide and can be visualised
  - This allows the base sequence to be built up one base at a time



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**High-throughput method of carrying out the chain termination method**



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## 7.1.5 Skills: The Hershey & Chase Experiment

### Skills: The Hershey & Chase Experiment

#### Which Biomolecule is the Heritable Material?

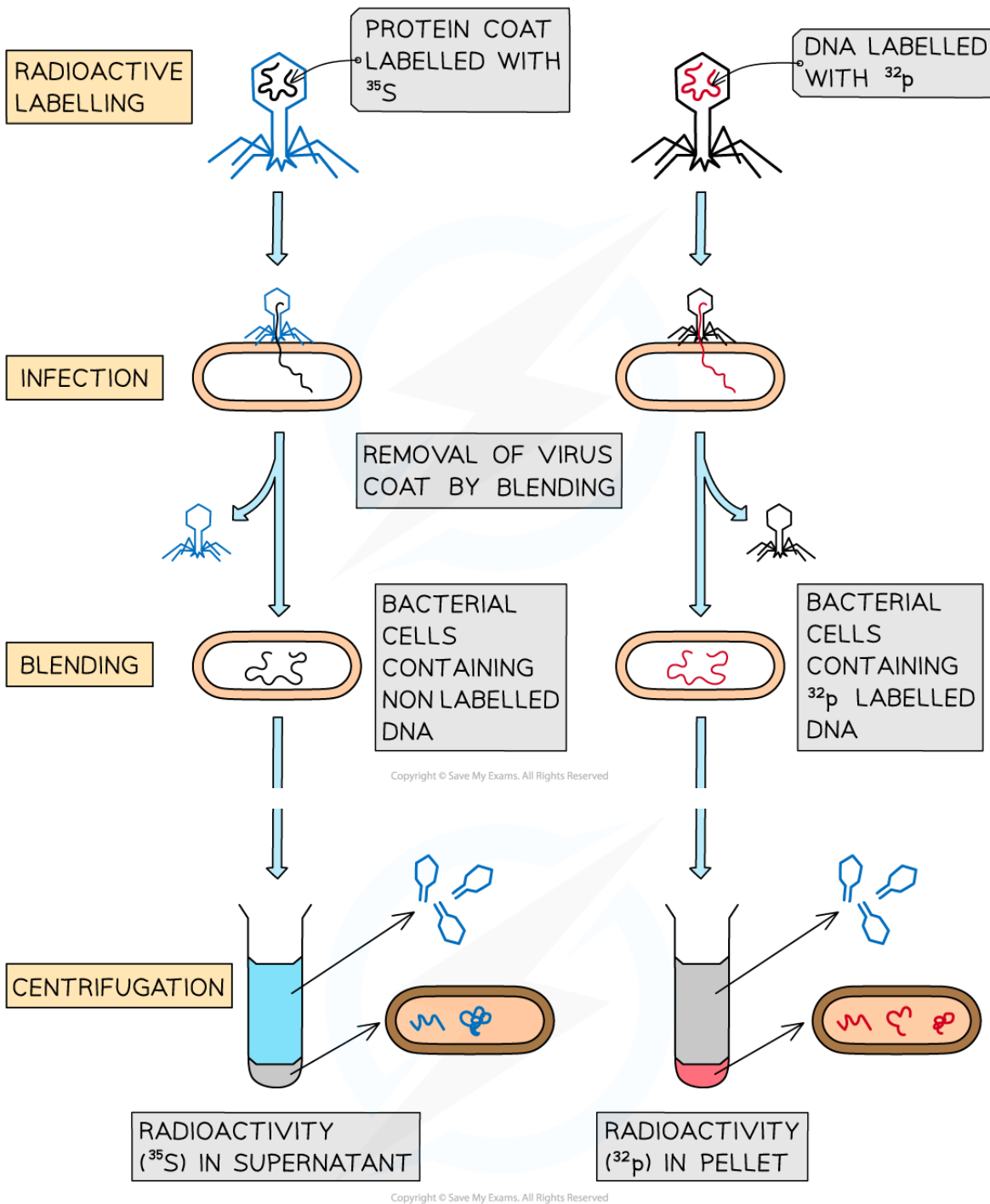
- DNA was identified in 1869 but many scientists assumed that protein was the heritable material
  - owing to the fact that there are 20 amino acids and only 4 nucleotide bases
- In the 1950s, Alfred Hershey and Martha Chase showed that DNA, not protein, is a factor of heredity responsible for carrying genetic information from one generation to another
- Viruses that infect bacteria were used in their experiment as they only consist of DNA encapsulated by a protein coat
- This would allow the biomolecule of heredity (ie. the one that caused bacterial cells to be used to produce viral progeny) to be easily determined

#### Analysis of results of the Hershey and Chase experiment provided evidence that DNA is the genetic material.

- Hershey and Chase took advantage of the **chemical differences** between DNA and proteins
  - **DNA** contains **phosphorus** but **no sulfur**
  - **Amino acids** (that make up proteins) contain **sulfur** but **no phosphorus**
- Bacteria grown in separate media containing either radioactive sulfur ( $^{35}\text{S}$ ) or radioactive phosphorus ( $^{32}\text{P}$ ) were infected with viruses
- The progeny viruses contained either  $^{35}\text{S}$  labelled proteins or  $^{32}\text{P}$  labelled DNA
- Unlabelled bacteria were then infected separately with either type of virus
  - Bacteria would be expected to contain the heritable material following infection
- A blender was used to remove attached viruses from the bacterial cells and centrifugation was used to isolate the bacteria
  - Viruses are small so remained in the supernatant in the centrifuge tube
  - Bacteria are larger so formed a pellet
- Only the bacteria infected by  $^{32}\text{P}$  **labelled viruses (DNA)** were shown to be radioactive
- This suggested that **DNA** (and not protein) was transferred to bacteria and **is the hereditary (genetic) material**



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**Hershey and Chase's experiment provided unequivocal proof that DNA is the heritable material**

## 7.1.6 Skills: Nucleosomes & Molecular Visualisation Software



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### Skills: Nucleosomes & Molecular Visualisation Software

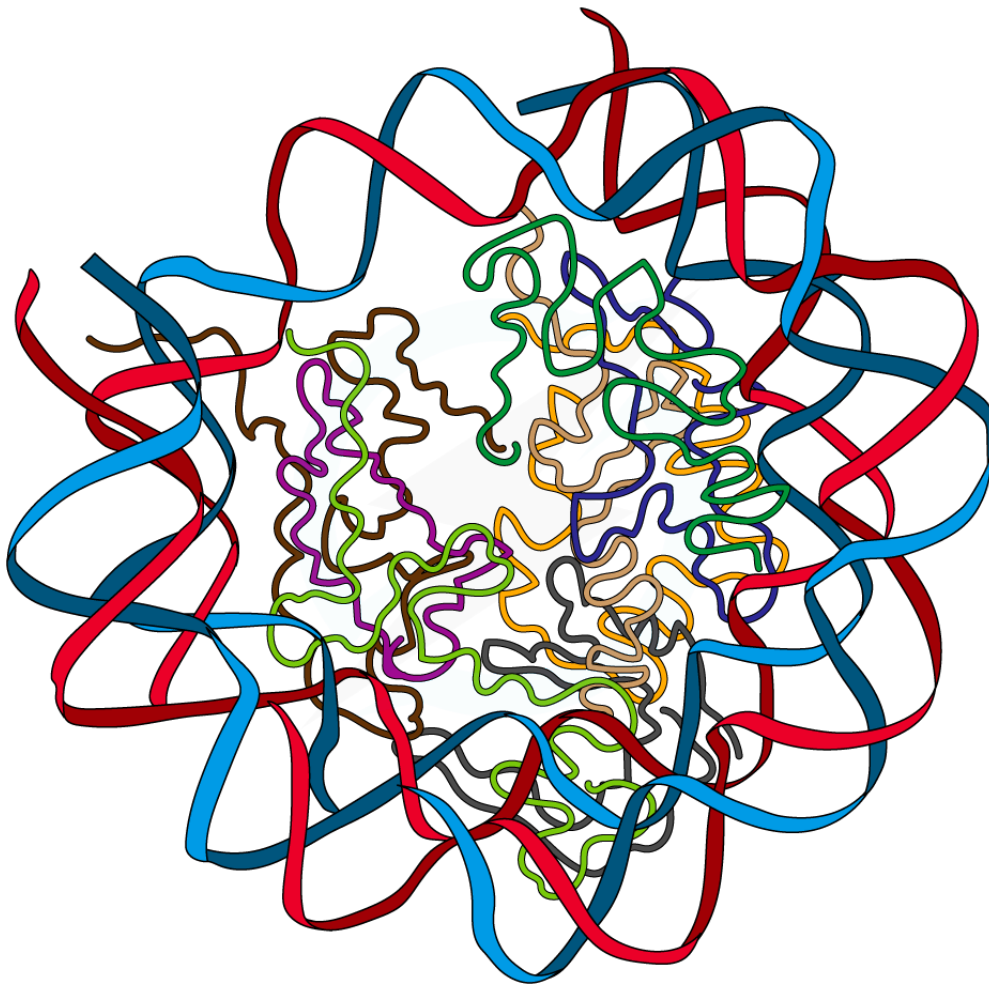
- **Molecular visualisation software** can be used to help understand molecular structures
  - Macromolecules like **protein, DNA, RNA** and **complex carbohydrates** can be **visualised as 3-D structures**
- This allows researchers to analyse macromolecules and/or study interactions between them
  - **Primary sequence** information can be related to structure and function
  - This helps to relate how **structure** might relate to **chemical** or **biological behaviour**
- Macromolecules can be represented in many different ways including **ball and stick atom models** or **simplified ribbon** representations that show the protein backbone
- Most molecular visualisation software is **freely available on the Internet** or can be accessed through many bioinformatics repositories such as the Protein Data Bank (PDB)

### Analysing the association between protein and DNA within a nucleosome

- Visit the **Protein Data Bank PDB site** and search for: **6T79 structure of human nucleosome** (do not put the search term in quotes)
- Select the “**3D view**” to view the protein structure in **mol\***
  - The 3-D structure of the nucleosome can be viewed
  - The **DNA double helix** can be clearly seen surrounding the **histone proteins**
  - **Rotate** or **zoom** into the image to visualise the different components
  - The DNA can be seen to make **two loops around the histone** octamer core
- Look carefully - the tails of each histone protein can be seen projected from the nucleosome core
  - These can be **chemically modified** to help **regulate gene expression**
- Try **changing different settings** in the viewer or select a different viewer such as JSmol



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**Structure of human nucleosome yeast tRNA showing the association between DNA (in 2 loops around the edge) and histones (central region)**