DP IB Biology: HL

Your notes

7.1 DNA Structure & Replication

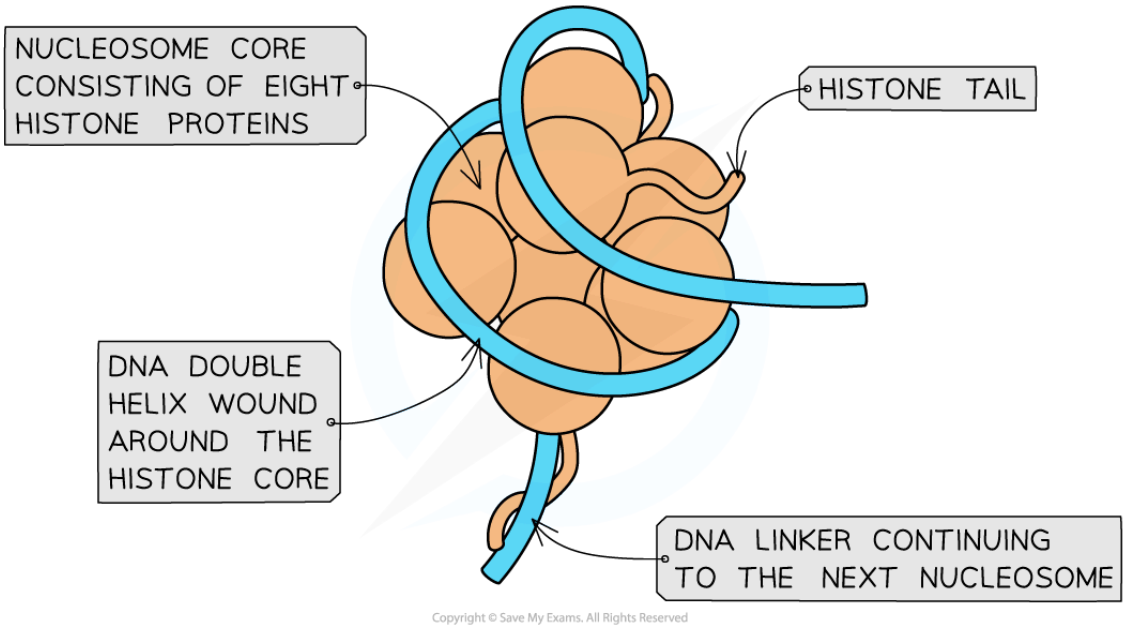
Contents

7.1.1 DNA Structure

Your notes

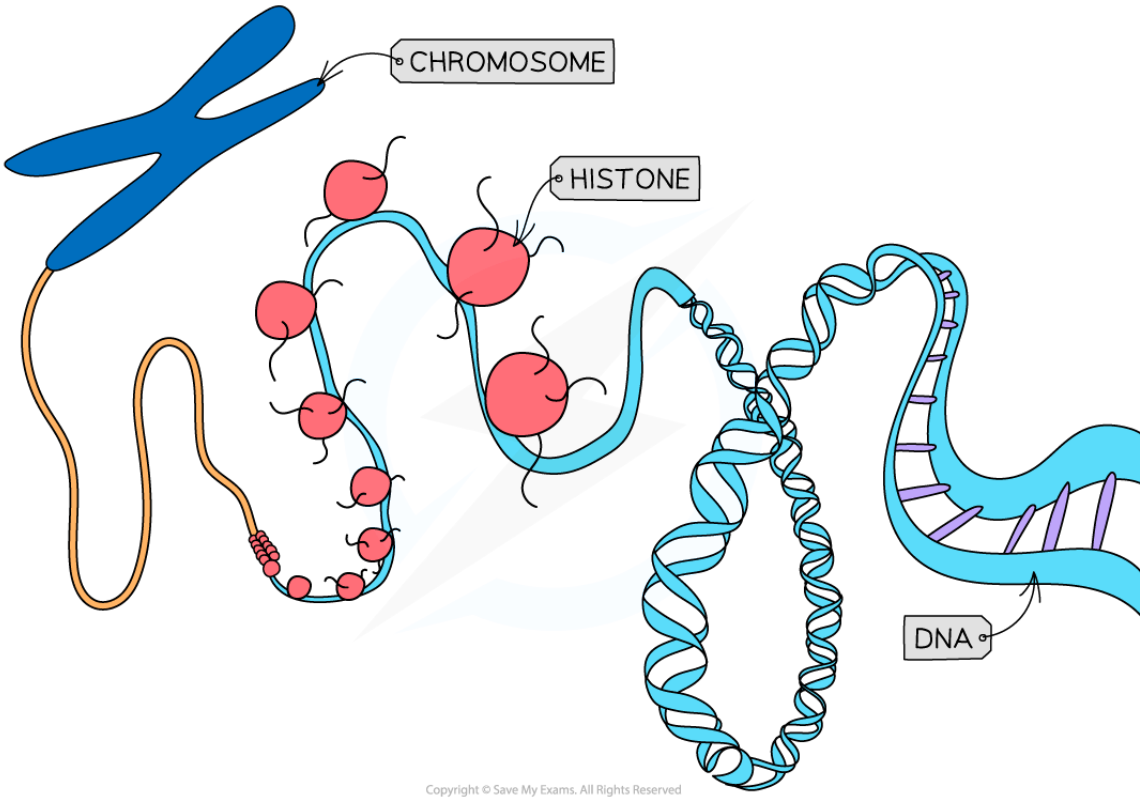
Nucleosomes

- 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



Structure of a nucleosome

Your notes



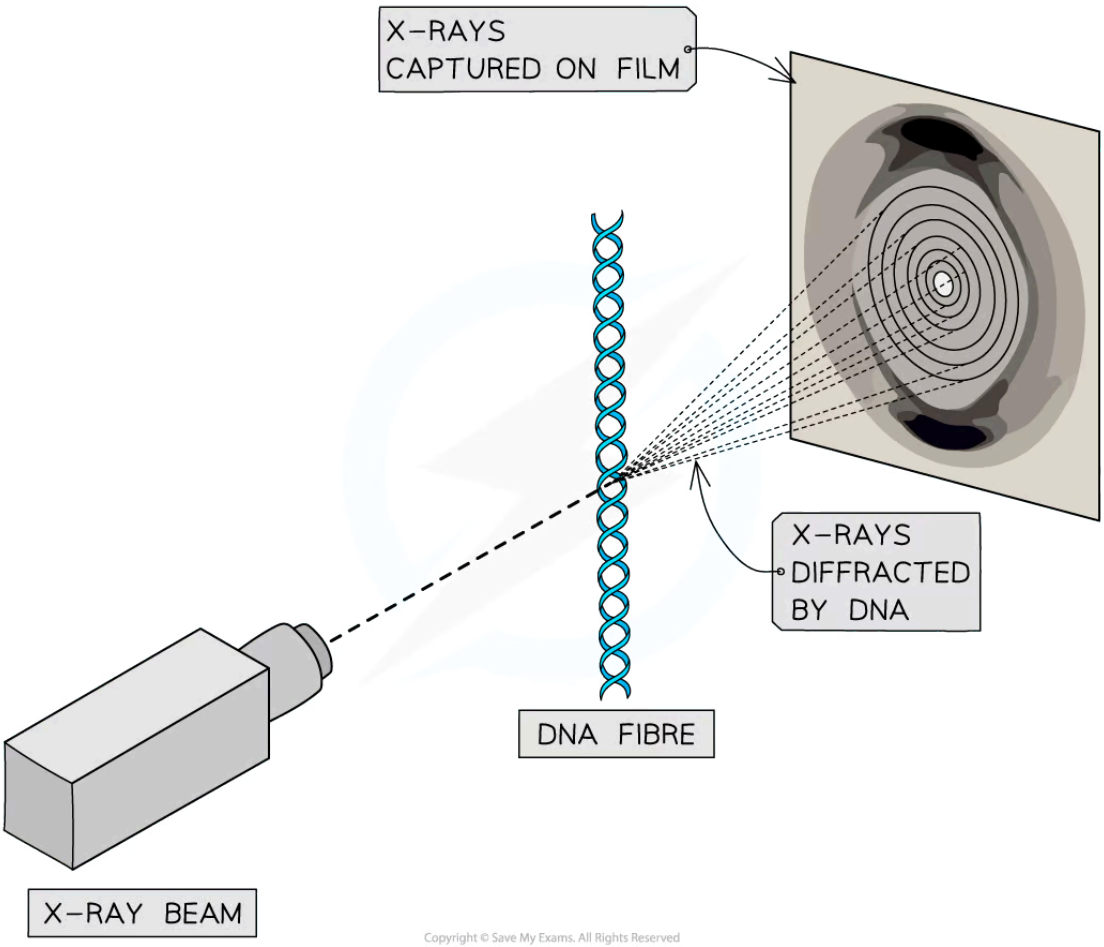
DNA is wrapped around a series of nucleosomes.
Nucleosomes coil tightly around each other to form the chromosome structure.

Franklin's Investigations

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


Your notes

- 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**



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

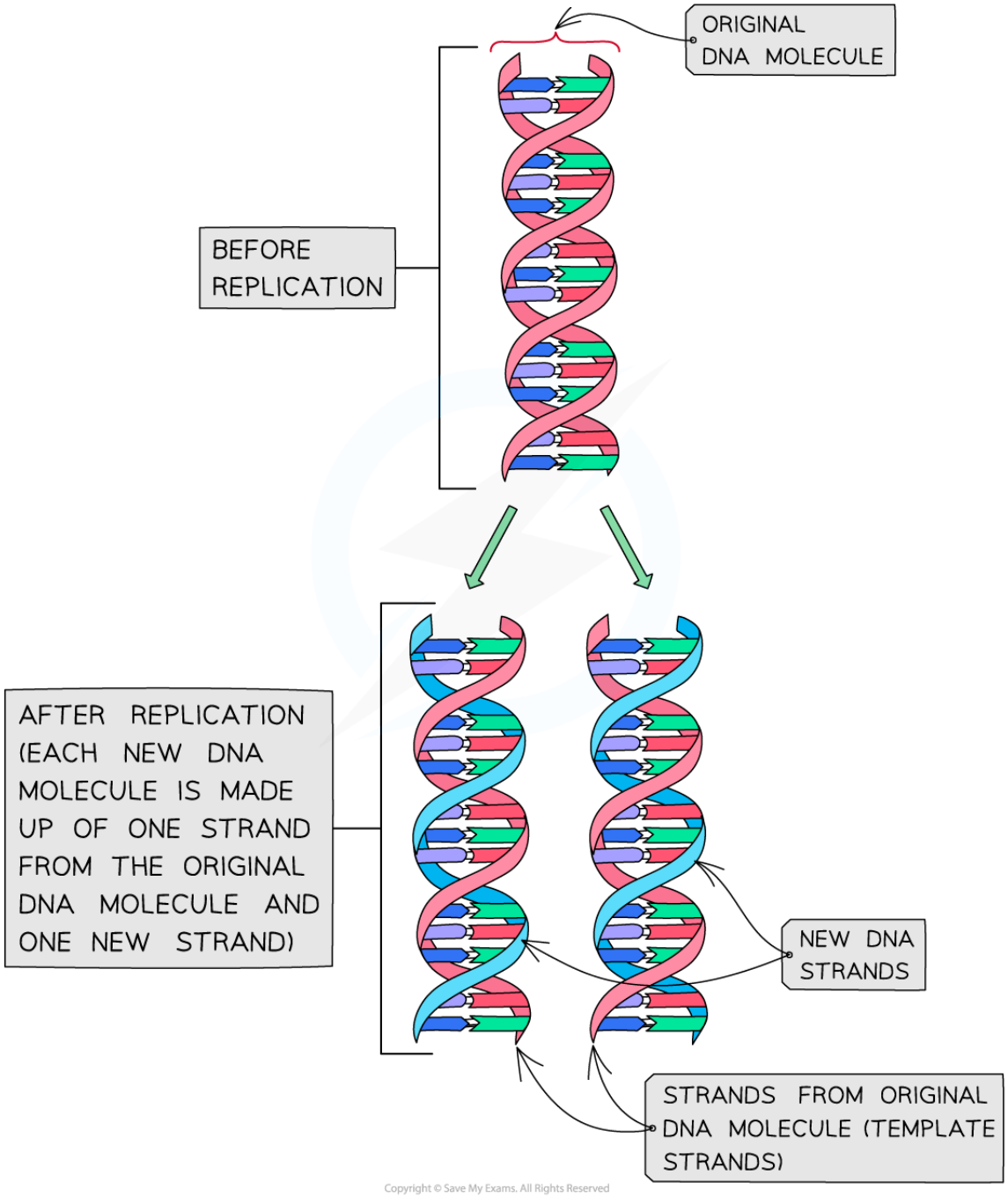
Your notes

DNA Structure Suggests Semi-conservative Replication


Your notes

- 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**

Your notes



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.

Your notes

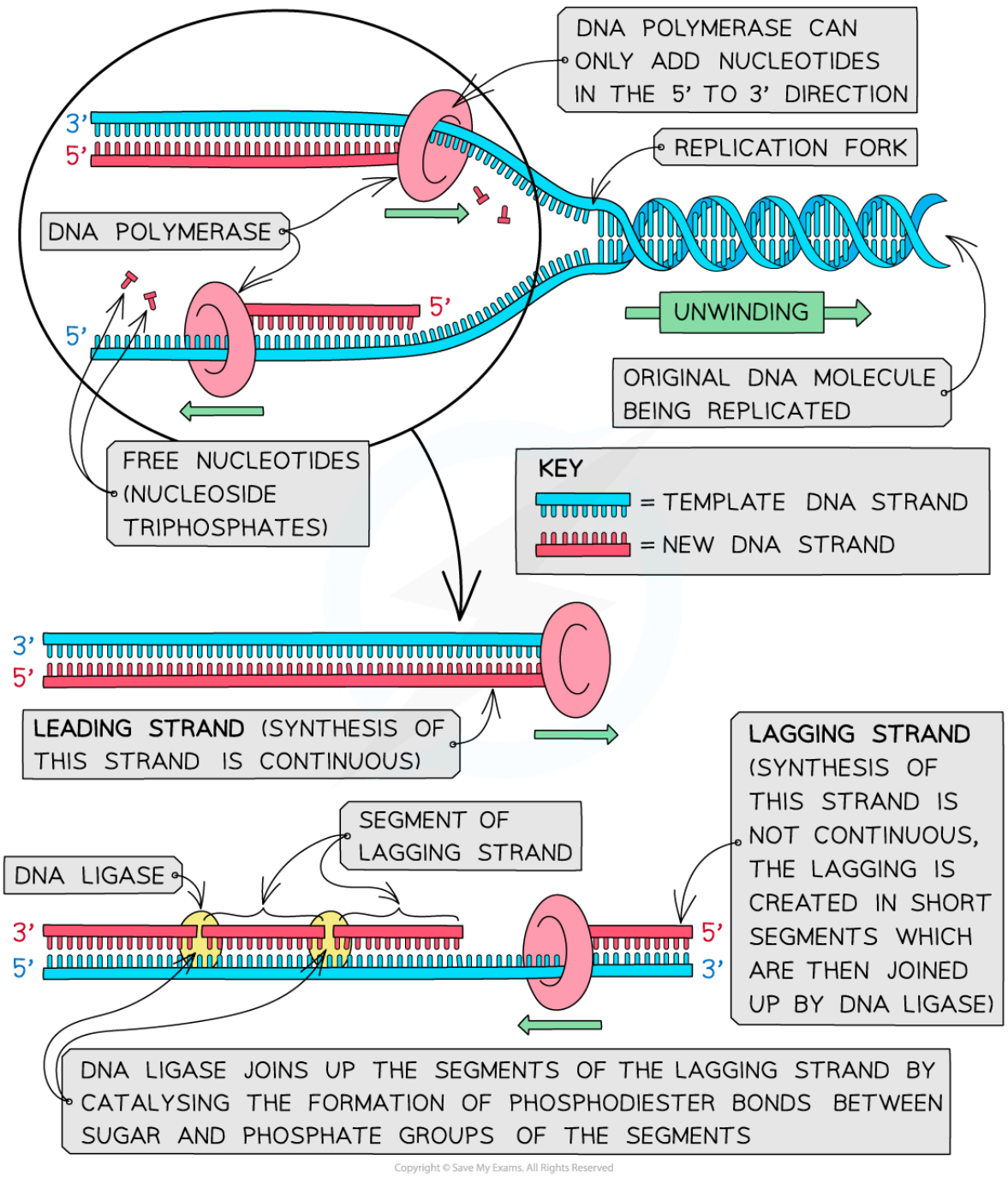
7.1.2 Mechanism of DNA Replication


Your notes

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

Your notes



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

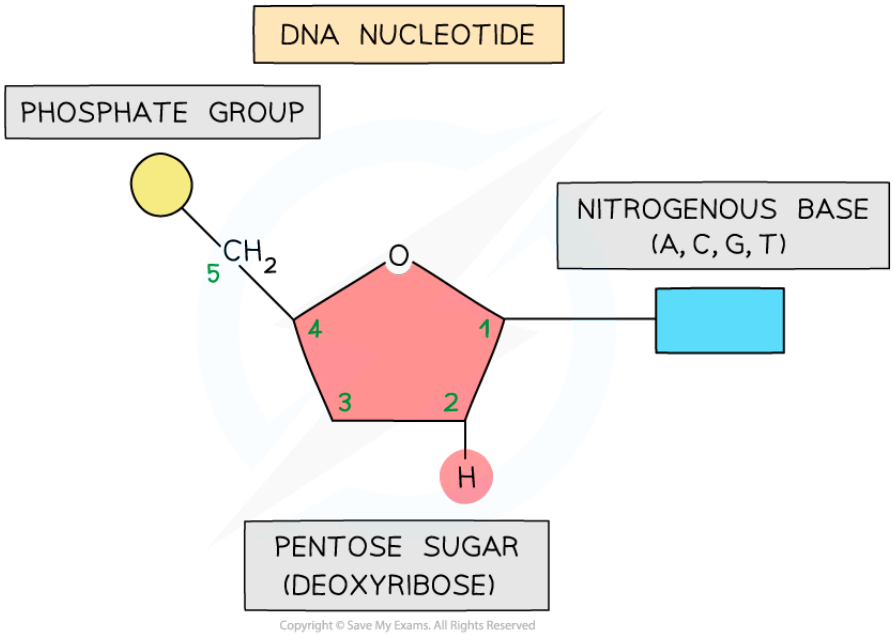

Your notes

- 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

Direction of Replication

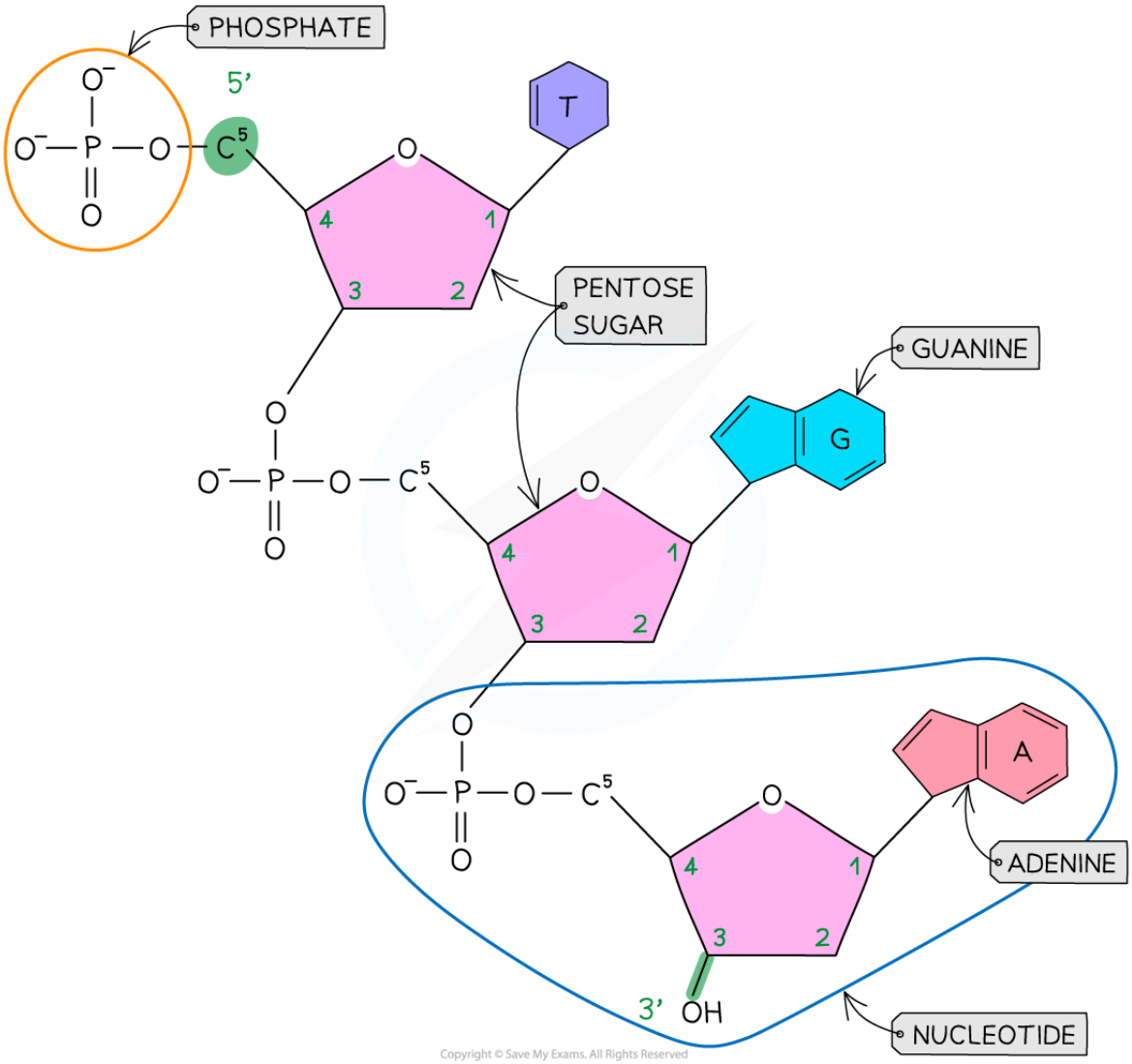
Your notes

- 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

Your notes



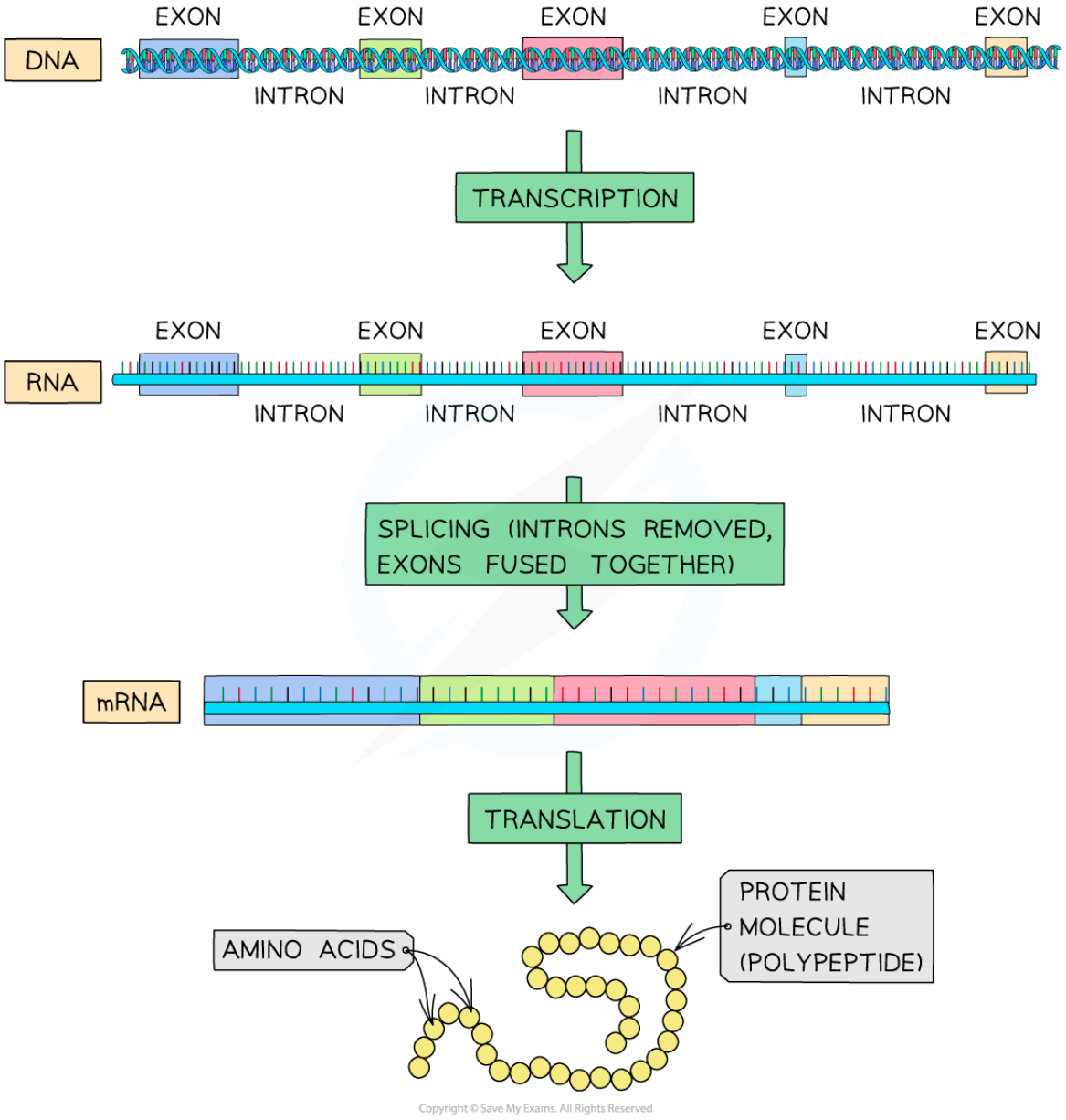
When DNA polymerase adds a new nucleotide, the 5' phosphate group of the incoming nucleotide bonds to the free 3' -OH group on the growing strand

7.1.3 Non-coding DNA


Your notes

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



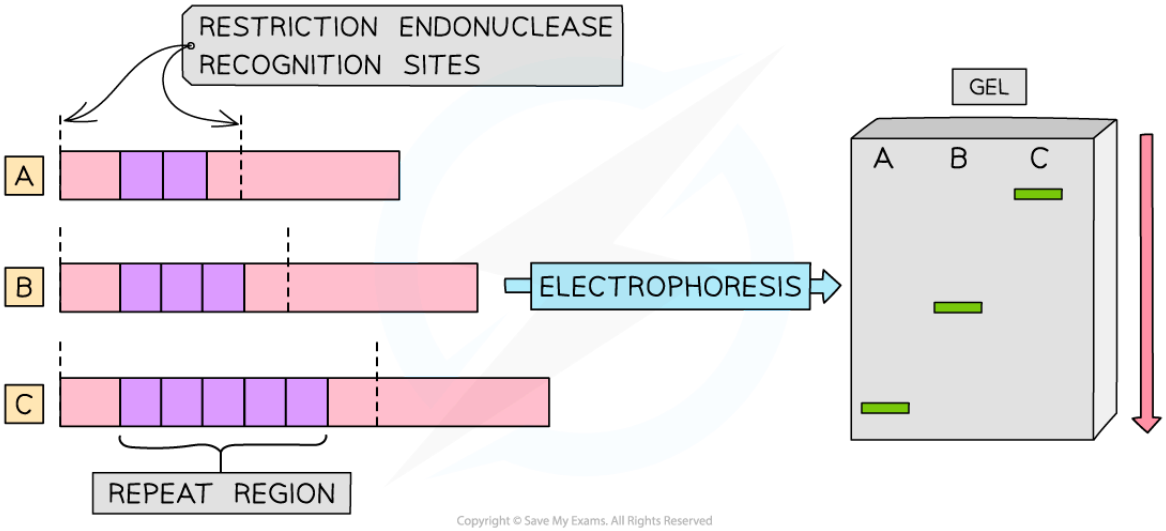
The RNA molecule produced from the transcription of a gene contains introns that must be removed before translation can occur.

Your notes

DNA Profiling

Your notes

- **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

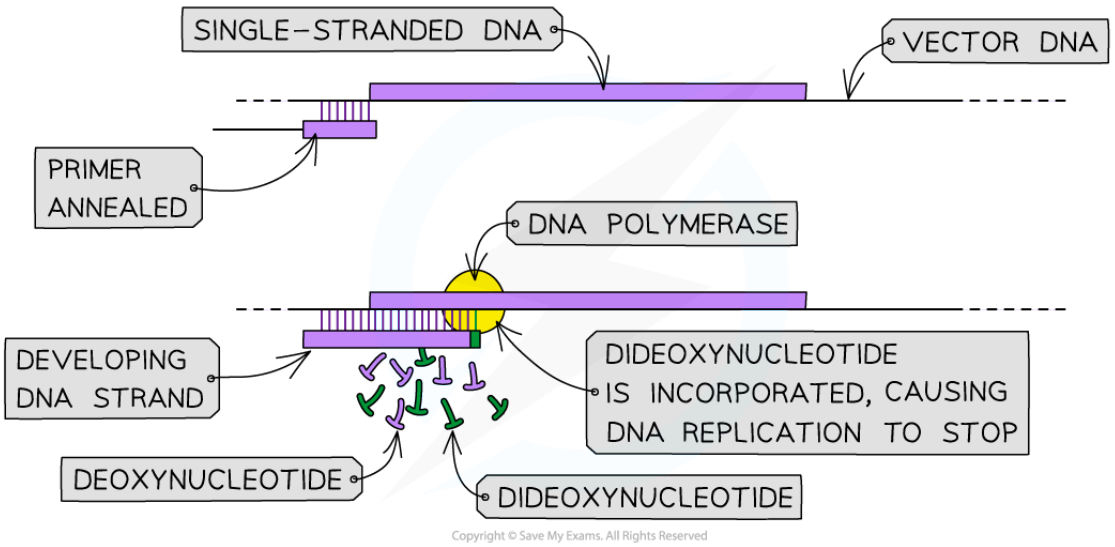
7.1.4 DNA Sequencing

Your notes

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



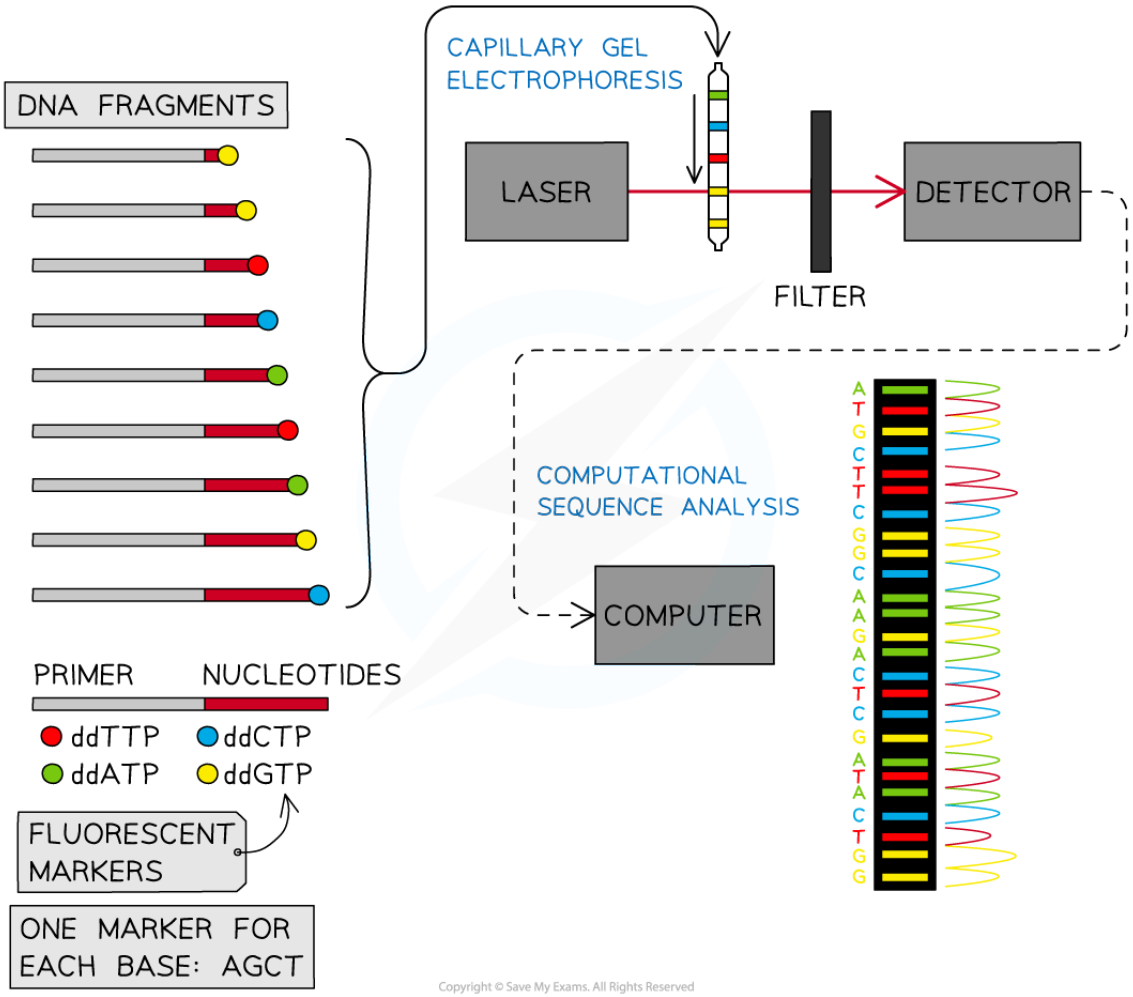
Once the dideoxynucleotide is added to the developing strand DNA polymerase stops the replication of the developing DNA strand to produce a shortened DNA chain

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
  - 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

Your notes



High-throughput method of carrying out the chain termination method

7.1.5 Skills: The Hershey & Chase Experiment

Your notes

Skills: The Hershey & Chase Experiment

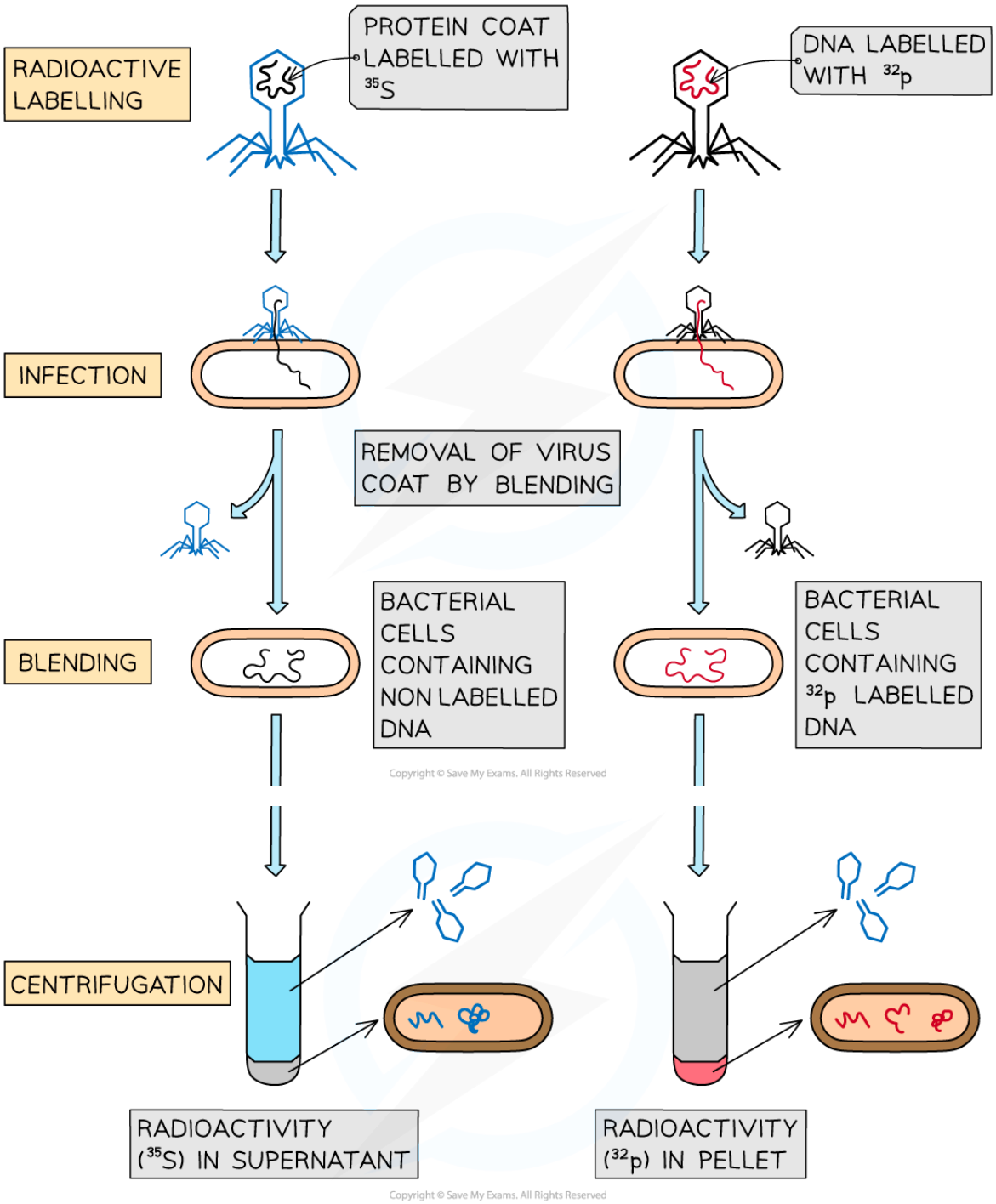
Which Biomolecule is the Heritable Material?

- 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}S) or radioactive phosphorus (^{32}P) were infected with viruses
- The progeny viruses contained either ^{35}S labelled proteins or ^{32}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}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**

Your notes



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

7.1.6 Skills: Nucleosomes & Molecular Visualisation Software


Your notes

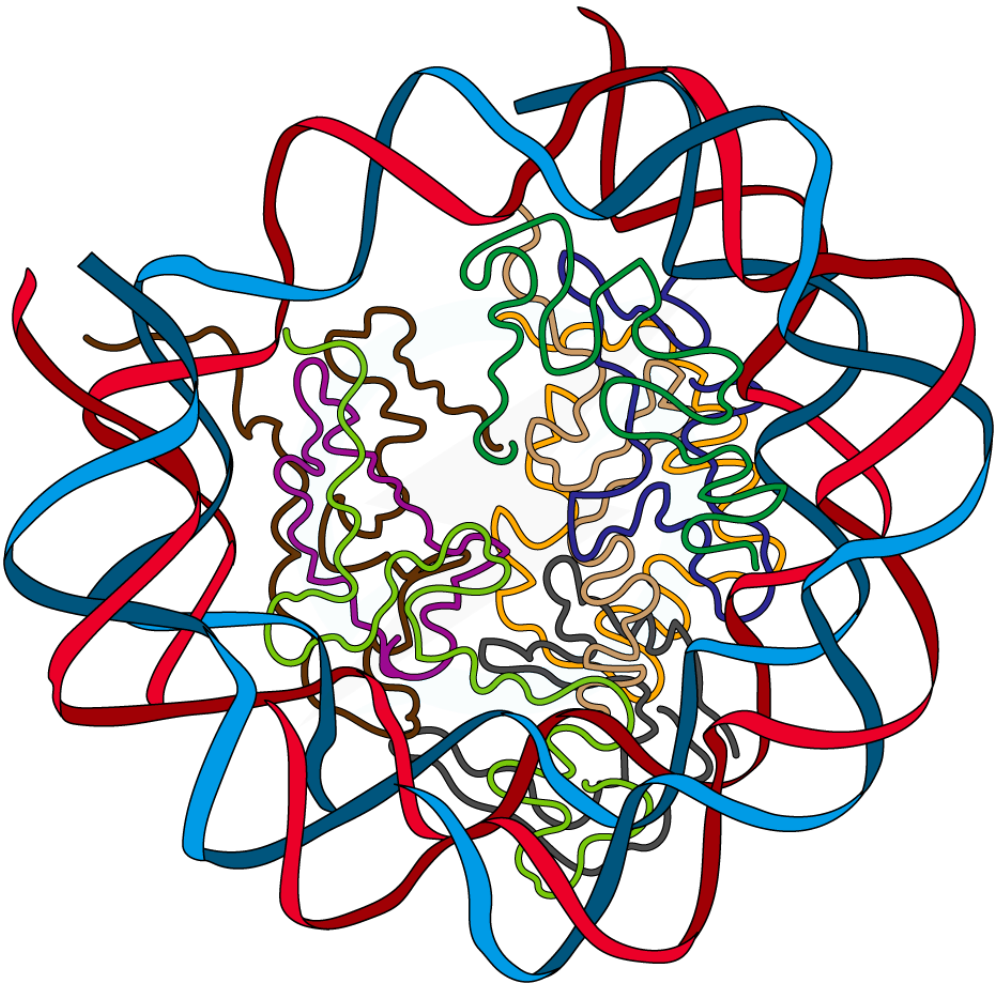
Skills: Nucleosomes & Molecular Visualisation Software

- **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

Your notes



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