DNA REPLICATION

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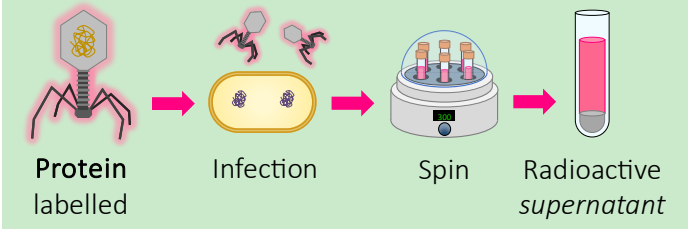
GENETIC MATERIAL

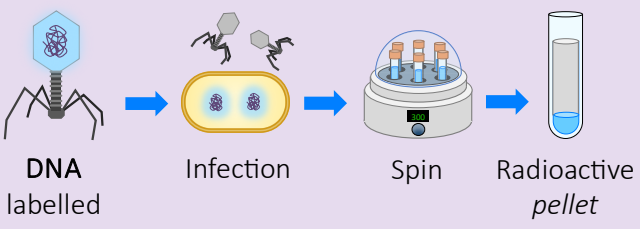
In the mid-twentieth century, scientists were still unsure whether DNA or protein was the genetic material of the cell. It was known that some viruses consisted solely of DNA and a protein coat and could transfer their genetic material into host cells. **Hershey and Chase** conducted a series of experiments to prove that DNA was the genetic material. They labelled a virus (a bacteriophage) with radioisotopes in order to track the biomolecules. Viruses grown in **radioactive sulphur** (^{35}S) had radiolabelled *proteins* (sulphur is present in proteins but not DNA), while the viruses grown in **radioactive phosphorus** (^{32}P) had radiolabelled *DNA* (phosphorus is present in DNA but not proteins). The viruses were then allowed to infect a bacterium and then the virus and bacteria were separated via centrifugation. The larger bacteria would form a solid **pellet** while the smaller viruses remained in the supernatant. Pellets were found to be radioactive when bacteria were infected by the ^{32}P–viruses (DNA) but **not** the ^{35}S–viruses (protein). This demonstrated that it was DNA, not protein, that was the genetic material (as DNA – but not protein – was transferred to bacteria).

Experiment 1: Testing Proteins with ^{35}S



Protein is **not** the genetic material

Experiment 2: Testing DNA with ^{32}P



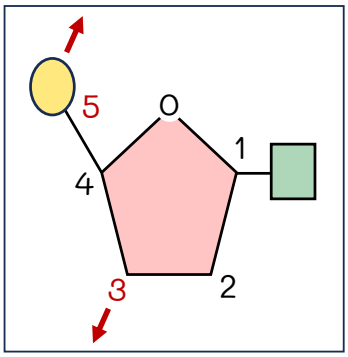
DNA **is** the genetic material

BASE SEQUENCE

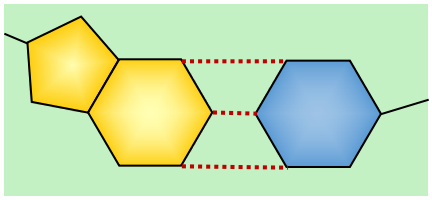
The role of the nucleotide bases in forming the coded instructions of cells was identified by **Erwin Chargaff**. Early theories proposed that the four bases existed in a repeating sequence (a *tetranucleotide* hypothesis). This would have required an equal amount of all the bases. Chargaff took DNA from a variety of organisms, then used chromatography to separate the bases and spectrophotometry to quantitate relative amounts. The frequency of the four bases were found to **not** be equal (but purines and pyrimidines *were* balanced).

DNA STRUCTURE

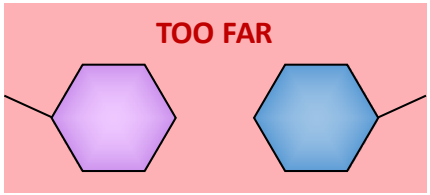
DNA is a double stranded molecule composed of two nucleotide chains. Each nucleotide consists of a pentose sugar, a phosphate group and a nitrogenous base. The carbon atoms within the pentose sugar are numbered (1 – 5). The base always connects to the 1'-end of the sugar, while phosphates attach to the 5'-end. When new nucleotides are attached to a chain, a phosphodiester bond is formed between the 5'-phosphate and the 3'-end of the sugar, with the chain said to run in a **5' → 3' direction**. Because double-stranded DNA is **antiparallel**, the two strands will run in opposite directions (5' → 3' vs 3' → 5').



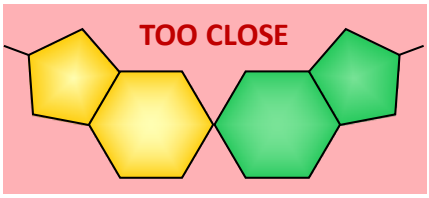
DNA consists of four nitrogenous bases – cytosine and thymine are **single-ringed pyrimidine** bases, while guanine and adenine are **double-ringed purine** bases. Double-stranded DNA is held together by bonding between complementary base pairs. A purine base is always paired with a pyrimidine – this maintains the stability of the double helix by ensuring the sugar-phosphate backbones remain separated by a constant width throughout the molecule. A pairing of two purines would result in the bases being too close together (leading to repulsion), while pairing two pyrimidines would be energetically unfavourable as bases would be too far apart to form hydrogen bonds. Adenine always pairs with thymine via two hydrogen bonds, while cytosine pairs with guanine via three hydrogen bonds. While alternative purine : pyrimidine pairings would maintain a constant diameter, the atomic configurations would not be favourable for hydrogen bonding.



Purine-Pyrimidine



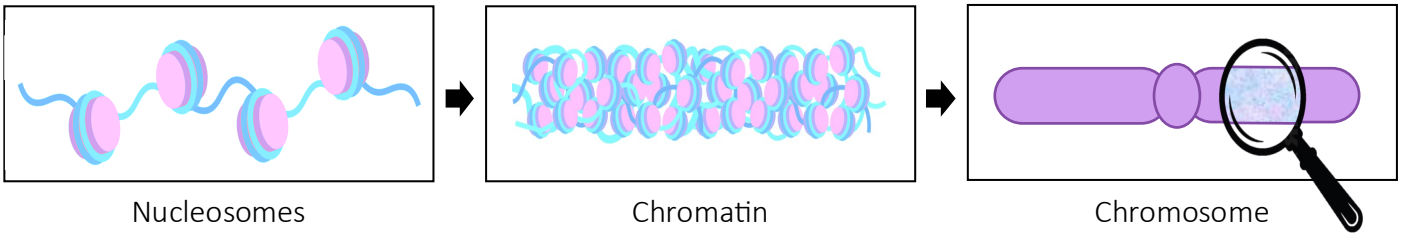
Pyrimidine-Pyrimidine



Purine-Purine

NUCLEOSOMES

In eukaryotic organisms, the DNA is packaged into a compact structure for more effective storage within a nucleus. This structure is called a nucleosome – which consists of a molecule of DNA wrapped around a core of eight **histone proteins** (an octamer). Negatively charged DNA associates with the positively charged amino acids on the surface of the histone proteins. DNA is coiled around the histone octamer in a manner that resembles thread being wrapped around a spool. Individual nucleosomes are then together linked by an additional histone protein (H1 histone) attached to linker DNA. The nucleosomes are then folded into increasingly more complicated structures, eventually forming **chromatin**. Nucleosomes help by supercoiling the DNA, which protects the DNA from damage and regulates the level of transcriptional activity. Histone proteins have N-terminal tails which extrude outwards from the nucleosome – these tails can be chemically modified (e.g. **methylation**) to determine how tightly the DNA is packaged. When the DNA is more tightly packaged, it is less accessible to the transcriptional machinery of the cell (i.e. lower gene expression levels).



Nucleosomes | Chromatin | Chromosome

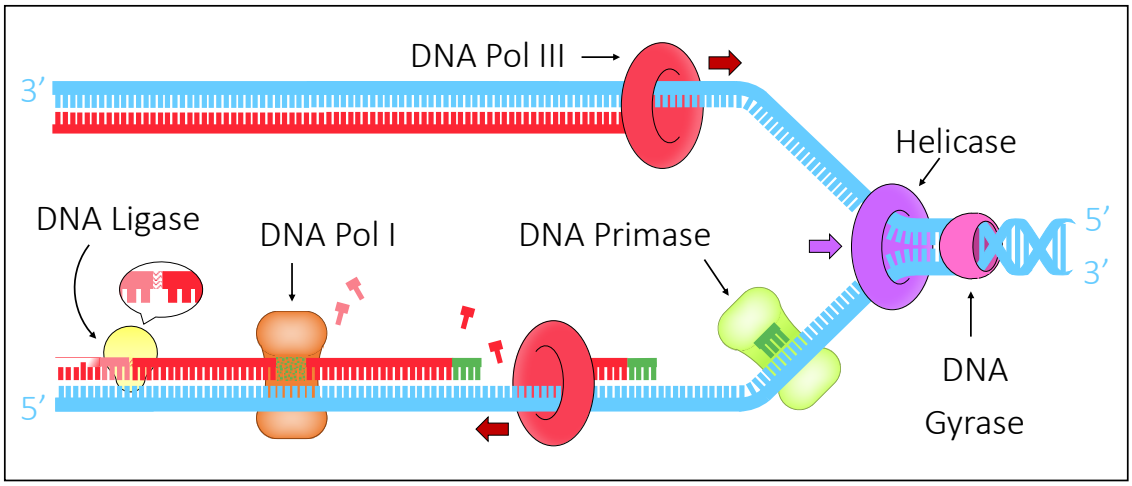
REPLICATION ENZYMES

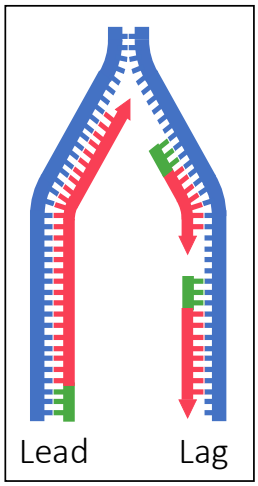
DNA replication is a semi-conservative process that is carried out by a highly complex system of enzymes:

- **Helicase** – Unwinds and separates DNA strands by breaking the hydrogen bonds between the base pairs
- **DNA Gyrase** – Reduces the torsional strain created by the unwinding of the double helix by helicase
- **DNA Primase** – Generates a short RNA primer that acts as an attachment point for DNA polymerase III
- **DNA Polymerase III** – Synthesises a new DNA strand that is complementary to the template strand
- **DNA Polymerase I** – Removes and replaces the RNA primer with complementary DNA nucleotides
- **DNA Ligase** – Joins segments of DNA (Okazaki fragments) together to form a continuous DNA molecule

DNA REPLICATION

DNA replication is initiated by *helicase* – which unwinds and separates the double-stranded DNA to create a **replication fork**. The two strands are kept apart by single stranded binding proteins (**SSBPs**), which prevent reannealing. Next, *DNA primase* generates a short **RNA primer** on each of template strand – this primer will serve as an attachment point for DNA polymerase III, which can extend a nucleotide chain but cannot start one. *DNA polymerase III* attaches to the 3'-end of the primer and covalently joins free nucleotides together in a **5' → 3' direction**. As DNA strands are antiparallel, DNA polymerase III moves in opposite directions on the two strands. On the **leading strand**, DNA polymerase III is moving towards the replication fork and can synthesise a new strand continuously. But on the **lagging strand**, the DNA polymerase III moves away from the replication fork and so must repeatedly synthesise DNA in short segments called **Okazaki fragments**. The lagging strand has multiple RNA primers along its length and these are removed by *DNA polymerase I*, which replaces the primers with DNA nucleotides. DNA ligase joins Okazaki fragments on the lagging strand together by covalently joining the sugar-phosphate backbones together to create a continuous molecule.





PROOFREADING

DNA replication is a generally accurate process, but occasionally errors can occur when copying a template strand. If these errors are not corrected, the new DNA sequence will be different to the original (resulting in **mutation**). During replication, *DNA polymerase III* can proofread the base that has just been added to the 3'-terminal before adding the next nucleotide. If a wrong base has been added, DNA polymerase III excises the nucleotide by breaking the phosphodiester bond holding it in place. In this respect, DNA polymerase III has **3' → 5' exonuclease activity** (as it cleaves the bond at the 5'-end). Once the incorrect base is removed, the correct base can be incorporated before DNA polymerase III continues to synthesis DNA in the 5' → 3' direction. Sometimes the error will not be detected until after DNA replication has been completed. In such instances, exonucleases will fully excise the entire sequence surrounding the mismatch before it is repaired.