Lecture 2. Introduction to molecular biology. Part II.

Learning outcomes:

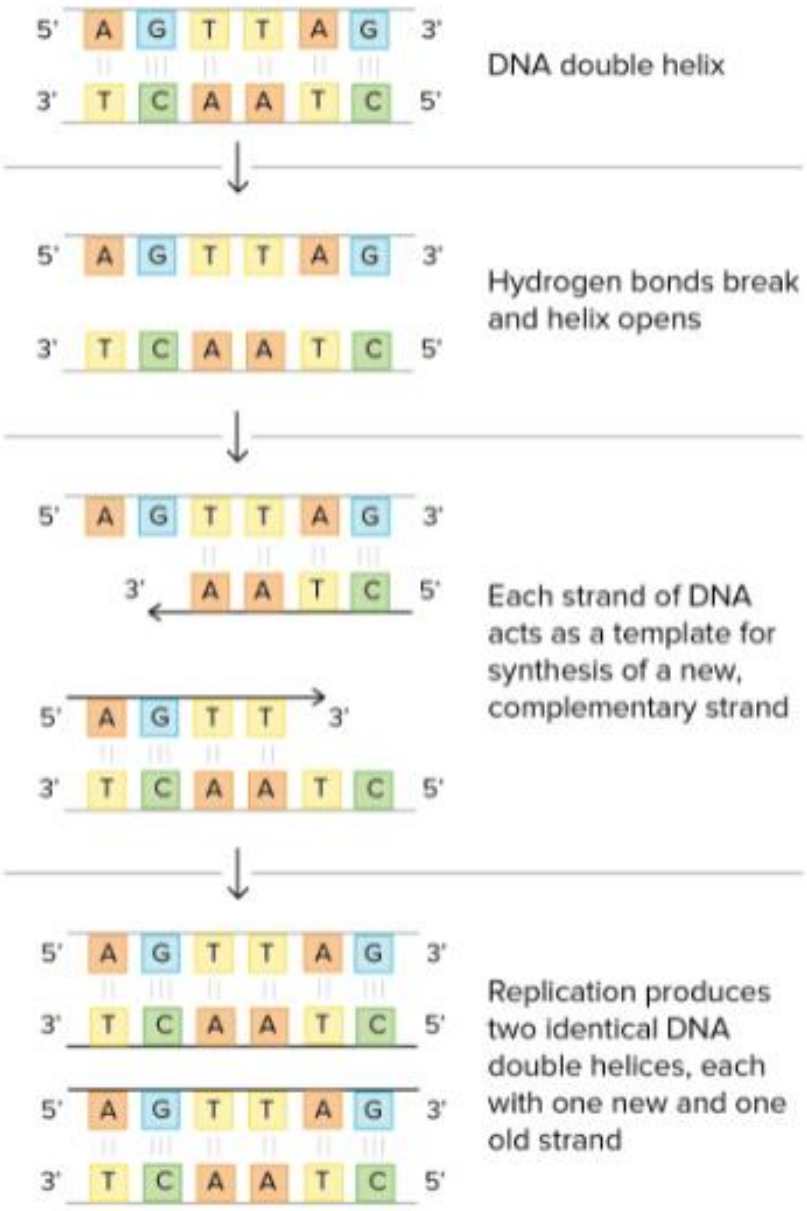
1. Describe the three hypotheses of DNA replication.
2. Describe the Meselson-Stahl experiment and explain its significance.
3. Explain the molecular mechanism of semiconservative DNA replication.
4. Explain the role of main enzymes implicated in the replication process.
5. Explain proofreading mechanisms and error correction during DNA replication.

In 1953, after the double helix structure of DNA has just been discovered. One big question concerned DNA replication. The structure of the DNA double helix provided a tantalizing hint about how copying might take place. It seemed likely that the two complementary strands of the helix might separate during replication, each serving as a template for the construction of a new, matching strand. There were three basic models for DNA replication that had been proposed by the scientific community after the discovery of DNA's structure

- **Semi-conservative replication.** In this model, the two strands of DNA unwind from each other, and each acts as a template for synthesis of a new, complementary strand. This results in two DNA molecules with one original strand and one new strand.
- **Conservative replication.** In this model, DNA replication results in one molecule that consists of both original DNA strands (identical to the original DNA molecule) and another molecule that consists of two new strands (with exactly the same sequences as the original molecule).
- **Dispersive replication.** In the dispersive model, DNA replication results in two DNA molecules that are mixtures, or "hybrids," of parental and daughter DNA. In this model, each individual strand is a patchwork of original and new DNA.

The evidence that DNA replication was semi-conservative came from an elegant experiment completed by Matthew Meselson and Franklin Stahl. They labelled the parental DNA with a heavy isotope of nitrogen (^{15}N) by growing bacteria in a growth medium that contained $^{15}NH_4Cl$. They then grew the bacteria, in a medium that contained $^{14}NH_4Cl$, in conditions such that any newly synthesised DNA would contain ^{14}N. Since DNA replication is semi-conservative, after one round of DNA replication, each cell would have a DNA molecule that contains one 'old' parental strand labelled with ^{15}N and one 'new' daughter strand labelled with ^{14}N. This was shown by analysing the density of the DNA using density-gradient centrifugation. As predicted, they observed that the new daughter DNA molecule had a density consistent with the fact that it contained both ^{15}N and ^{14}N and that this daughter DNA contained one strand with ^{15}N and another strand with ^{14}N.

DNA replication is **semiconservative**, meaning that each strand in the DNA double helix acts as a template for the synthesis of a new, complementary strand.



DNA replication can be thought of in three stages; **Initiation, Elongation, Termination**

Initiation

DNA synthesis is initiated at particular points within the DNA strand known as '**origins**', which are specific coding regions. These origins are targeted by initiator proteins, which go on to recruit more proteins that help aid the replication process, forming a replication complex around the DNA origin. There are multiple origin sites, and when replication of DNA begins, these sites are referred to as **replication forks**.

Within the replication complex is the enzyme **DNA Helicase**, which unwinds the double helix and exposes each of the two strands, so that they can be used as a template for replication. It does this by hydrolysing the ATP used to form the bonds between the nucleobases, therefore breaking the bond holding the two strands together.

DNA Primase is another enzyme that is important in DNA replication. It synthesises a small **RNA primer**, which acts as a 'kick-starter' for **DNA Polymerase.** DNA Polymerase is the enzyme that is ultimately responsible for the creation and expansion of the new strands of DNA.

Elongation

Once the DNA Polymerase has attached to the original, unzipped two strands of DNA (i.e. the **template** strands), it is able to start synthesising the new DNA to match the templates. It is essential to note that DNA polymerase is only able to extend the primer by adding free nucleotides to the **3' end.**

One of the templates is read in a 3' to 5' direction, which means that the new strand will be formed in a 5' to 3' direction. This newly formed strand is referred to as the **Leading Strand.** Along this strand, DNA Primase only needs to synthesise an **RNA primer** once, at the beginning, to initiate DNA Polymerase. This is because DNA Polymerase is able to extend the new DNA strand by reading the template 3′ to 5′, synthesising in a 5′ to 3′ direction as noted above.

However, the other template strand (the **lagging strand**) is antiparallel, and is therefore read in a **5' to 3'** direction. Continuous DNA synthesis, as in the **leading strand**, would need to be in the 3′ to 5′ direction, which is impossible as we cannot add bases to the 5′ end. Instead, as the helix unwinds, RNA primers are added to the newly exposed bases on the **lagging strand** and DNA synthesis occurs **in fragments,** but still in the 5′ to 3′ direction as before. These fragments are known as **Okazaki fragments.**

Termination

The process of expanding the new DNA strands continues until there is either no more DNA template left to replicate (i.e. at the end of the chromosome), or two replication forks meet and subsequently **terminate.** The meeting of two replication forks is not regulated and happens randomly along the course of the chromosome.

Once DNA synthesis has finished, it is important that the newly synthesised strands are bound and stabilized. With regards to the lagging strand, two enzymes are needed to achieve this; **RNAase H** removes the RNA primer that is at the beginning of each Okazaki fragment, and **DNA Ligase** joins fragments together to create one complete strand.

The questions for self - control:

1. What were the three hypotheses of DNA replication and how the experiment of Meselson and Stahl revealed the real mechanism of this process?
2. What is the semiconservative mechanism of DNA replication and how this happens?

Recommended readings:

1. Alberts B. et al. Molecular biology of the cell / 6th ed. 2015. Garland Science, pp. 239-266.
2. Cooper G.M. The Cell: A Molecular Approach. (https://www.ncbi.nlm.nih.gov/books/NBK9940/)