D1.1 DNA replication

Continuity and change—Molecules

Standard level and higher level: 2 hours

Additional higher level: 2 hours

Guiding questions

• How is new DNA produced?

• How has knowledge of DNA replication enabled applications in biotechnology?

Recommended prior learning: A1.2 Nucleic acids

SL and HL

D1.1.1—DNA replication as production of exact copies of DNA with identical base sequences
Students should appreciate that DNA replication is required for reproduction and for growth and tissue replacement in multicellular organisms.
DNA replication is the process of producing exact copies of DNA with identical base sequences. This is required for reproduction in order to pass genes to offspring, in addition to growth and tissue repair in multicellular organisms. Although the complexity of the process differs between prokaryotes and eukaryotes, the general mechanism is similar.

D1.1.2—Semi-conservative nature of DNA replication and role of complementary base pairing
Students should understand how these processes allow a high degree of accuracy in copying base sequences.
Semi-conservative replication entails the separation of the two parental strands and using each one as a template for the synthesis of the new complementary strand. This results in two DNA molecules, each with one original strand and one new strand (the parent DNA molecule is thus only semi/half conserved). The reason why many organisms have evolved such a mechanism of replication remains unclear. In order to reduce the number of errors during replication, the chemical structure of the 4 DNA nucleotides allows bonds to occur only between pairs of A + T and C + G (**complementary base pairing**). So, if an A were to be added to a C molecule during replication, it would be rejected as they are not chemically compatible/complementary, thus errors are reduced, and a high degree of accuracy is achieved.

D1.1.3—Role of helicase and DNA polymerase in DNA replication
Limit to the role of helicase in unwinding and breaking hydrogen bonds between DNA strands and the general role of DNA polymerase.
DNA replication is heavily dependent on enzymes, for instance: • The enzyme **helicase** (donut-shaped) unwinds (separates) the two DNA strands at **origins of replication** (regions of DNA where replication is initiated) by breaking the hydrogen bonds between complementary base pairs • The enzyme **DNA polymerase** adds nucleotides one-by-one to the growing template strand through complementary base pairing by utilizing energy from ATP

D1.1.4—Polymerase chain reaction and gel electrophoresis as tools for amplifying and separating DNA

Students should understand the use of primers, temperature changes and Taq polymerase in the polymerase chain reaction (PCR) and the basis of separation of DNA fragments in gel electrophoresis.

When collecting DNA samples, often the number of molecules is too small for analysis, so it needs to be amplified. This is done through the **Polymerase chain reaction (PCR)**, which involves the following steps:

1. **Denaturation**: in order to separate the two strands without enzymes (to reduce costs), the DNA sample is exposed to high temperatures in order to unwind the strands
2. **Annealing**: the sample is cooled in order to anneal (add) the RNA primers which are needed for initiating DNA polymerase activity
3. **Synthesis**: the sample is warmed again and **Taq polymerase** (a special type of DNA polymerase derived from the bacterium *Thermus aquaticus* which can withstand high temperatures, thus function faster than its human counterpart) is added along with nucleotides in order to carry out DNA replication. Each cycle (replication) exponentially increases the amount of DNA, so around 10-13 cycles are done per sample to ensure sufficient quantity

Gel electrophoresis is a biotechnological tool used to separate DNA fragments (or other biomolecules like proteins and RNA) based on size and charge. However, since all DNA molecules have the same charge per mass, electrophoresis separates DNA fragments by size only. This is useful in order to isolate chromosomes and specific genes from the entire genome for analysis. Electrophoresis is usually done after a PCR in order to collect enough quantities DNA.

The apparatus for gel electrophoresis involves a tray (box) containing a gel with a **cathode** (negative terminal/pole/electrode) on one side and an **anode** (positive terminal) on another side. The DNA samples are collected using a pipette and inserted in slots (**wells**) on the cathode (negative) side with a fluorescent marker to better visualize them. Once power is supplied to the electrodes on both sides, an electrical field is applied to the DNA molecules (which are negatively charged) and causes them to move towards the anode (positive terminal). Shorter fragments move further due to their smaller size. A well-defined and clear line/strip of DNA on the gel appears after a while and is called a **band**. Each band contains a group of same-sized DNA fragments as individual ones are too small to be seen (hence why a PCR is needed beforehand).

The type of gel and strength of electrical field influences the distance the DNA fragments travel. Usually, one well is reserved for a **DNA ladder**, which is a standard reference that contains known lengths of DNA fragments in order to compare the sample to it. Figure 1(B) does not specify which direction the DNA fragments are travelling to, but the 'hooks' towards the end of each band point upwards, indicating that the wells are up and so the direction of DNA travel is downwards towards the anode.

Common units of measuring DNA fragment length are **bp** (base pair) and **kbp/kb** (kilo base pairs).

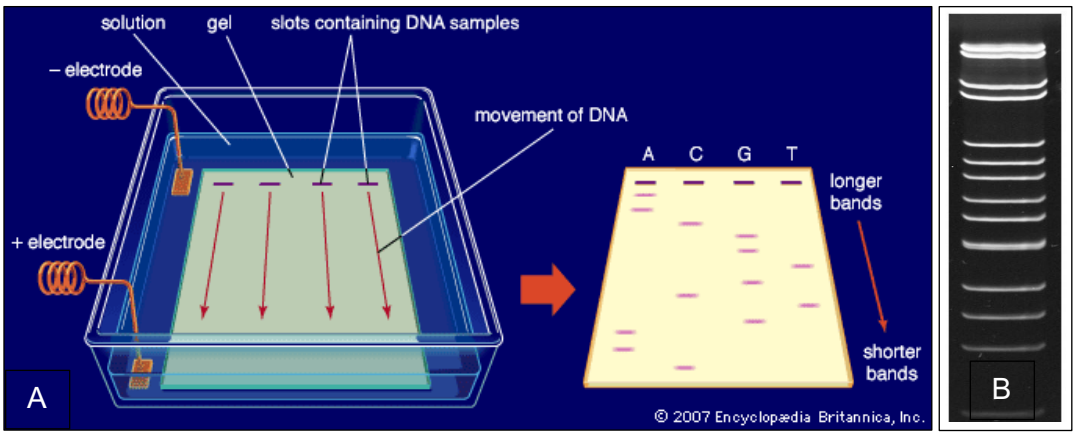

Figure 1: (a) gel electrophoresis (Rogers), (b) DNA ladder (Tan Laboratory, Penn State University).

D1.1.5—Applications of polymerase chain reaction and gel electrophoresis

Students should appreciate the broad range of applications, including DNA profiling for paternity and forensic investigations.

PCR tests are commonly used to detect viruses (like COVID-19) and other diseases. They are also useful when conducted before gel electrophoresis, like paternity tests in which the biological parents of a child are identified, and in DNA profiling for forensic investigations, both of which depend on STRs.

Short Tandem Repeats (STRs) (also known as microsatellites) are DNA base sequences between 1-6 base pairs in length that are repeated consecutively and form series spanning a maximum of 100 nucleotides. They are found widely in both prokaryotes and eukaryotes, and are scattered almost evenly across the human genome to make up around 3% of the entire genome. Residing in mostly noncoding regions, STRs have a high mutation rate and thus are highly variable between individuals, so it is very unlikely that two people have the same STR lengths.

For paternity tests, some STRs are passed to the child from both parents. Multiple STR loci from the parents and child undergo PCR and then are separated by electrophoresis before being compared. The person with the greatest number of similar bands to the child is the biological parent. For DNA profiling during forensic investigations, the suspect with the greatest number of similar bands to the DNA sample collected at the crime scene is the criminal.

NOS: Reliability is enhanced by increasing the number of measurements in an experiment or test. In DNA profiling, increasing the number of markers used reduces the probability of a false match.

Increasing the number of STR loci used for DNA profiling or paternity tests reduces the probability of a false match, as the likelihood of having all chosen STR loci be similar will be reduced.

Additional higher level

D1.1.6—Directionality of DNA polymerases

Students should understand the difference between the 5' and 3' terminals of strands of nucleotides and that DNA polymerases add the 5' of a DNA nucleotide to the 3' end of a strand of nucleotides.

DNA polymerases are a family of enzymes involved in DNA replication. **DNA polymerase III** is the main enzyme that adds nucleotides to synthesize new DNA, and it can only do this in a 5’ to 3’ direction by connecting the 5’ OH group of a free/new nucleotide with the 3’ OH pentose group of a nucleotide on the parental strand. This is evolutionarily advantageous because the energy from the phosphate group on a free/new nucleotide is used to join it to the growing nascent DNA strand. If a mismatched nucleotide was added, it can be removed and the energy from the correct free/new nucleotide can then be used to join it to the strand. If polymerase instead synthesized in the 3’ to 5’ direction, then the energy would be derived from the nucleotide on the existing strand, thus if an error occurred, a new correct nucleotide cannot provide energy for its addition, causing inefficiency.

D1.1.7—Differences between replication on the leading strand and the lagging strand

Include the terms “continuous”, “discontinuous” and “Okazaki fragments”. Students should know that replication has to be initiated with RNA primer only once on the leading strand but repeatedly on the lagging strand.

Due to the antiparallel nature of DNA and directionality of DNA polymerase III, one strand will be synthesized continuously towards the replication fork, and the other complementary parent strand will be synthesized in fragments (called **Okazaki fragments**) away from the fork. This means that replication has to be initiated with RNA primer only once on the leading strand but repeatedly on the lagging strand.

D1.1.8—Functions of DNA primase, DNA polymerase I, DNA polymerase III and DNA ligase in replication
Limit to the prokaryotic system.
The prokaryotic system for DNA replication involves several enzymes: • **DNA primase**: adds **RNA primers** (segments of RNA ~5-10 nucleotides in length) complementary to the parent strand to provide a 3' OH group that allows DNA polymerase III to start synthesizing the new strand. • **DNA polymerase III**: adds nucleotides to the 3' end of primers. • **DNA polymerase I**: removes RNA primers and replaces them with DNA bases> • **DNA ligase**: seals gaps between Okazaki fragments by forming phosphodiester bonds between adjacent nucleotides to form one continuous DNA strand.

D1.1.9—DNA proofreading
Limit to the action of DNA polymerase III in removing any nucleotide from the 3' terminal with a mismatched base, followed by replacement with a correctly matched nucleotide.
Errors in DNA replication are mitigated through **DNA proofreading**, which is a mechanism by which DNA polymerase III immediately reads every new nucleotide after adding it to the growing DNA strand and checks whether it is correct or not. The enzyme removes any nucleotide from the 3' terminal with a mismatched base and replaces it with a correctly matched nucleotide.

Linking questions

• How is genetic continuity ensured between generations?

• What biological mechanisms rely on directionality?

Review questions

SL and HL

- Suggest how the discovery of DNA's helical structure immediately provided an idea as to how DNA is passed to daughter cells. [1]
- State the role of the origin of replication. [1]
- The semi-conservative process of DNA replication is widespread across the domains of life. Suggest how this feature of heredity contributes to genetic stability. [1]
- A research team observes that a particular strain of bacteria exhibits a high frequency of mutations after UV exposure, specifically in regions where DNA is normally synthesized discontinuously. Suggest which enzyme involved in DNA replication might be defective. [2]
- Evaluate how a mutation that increases the error rate of DNA polymerase could affect the evolution of a rapidly reproducing virus. [2]
- Explain the role of short tandem repeats in biotechnology. [3]
- Explain the role of enzymes in DNA replication. [4]
- Discuss how knowledge of DNA replication enabled applications in biotechnology. [7]
- Discuss how a DNA sample is amplified and then separated in a paternity test. [8]

Additional higher level

- A cell strain is isolated and it is discovered that the joining of Okazaki fragments is impaired due to a mutation to an enzyme found at the replication fork. Suggest the enzyme most likely to be mutated. [1]
- Outline how genetic errors are mitigated by the cell. [2]
- Explain the directionality of DNA polymerases. [3]
- Explain how genetic material is passed onto daughter cells in prokaryotic organisms. [7]

References

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