2. DNA function: replication – heredity

The topic of today's lecture is DNA function: replication as a molecular basis of heredity. The lecture is part of Module 1: Animal Genetics, that is a part of the ISAGREED project. This presentation was supported by Erasmus+ KA2 Cooperation Partnerships Grant "Innovation of the content and structure of study programmes in the field of management of animal genetic and food resources using digitalization".

As part of the lecture, we will first explain the concept of replication and its importance, we will talk about proposed replication models and the enzymes catalyzing this reaction. We will then explain in detail the so-called semi-conservative mechanism of replication.

DNA replication is the process of making copies of a deoxyribonucleic acid molecule, thereby transferring genetic information from one DNA molecule to another molecule of the same type. It has to occur before cell division (mitosis or meiosis) and is the basic assumption that each daughter cell receives DNA typical for the given species. During division, a cell must copy its entire genome so that both daughter cells carry the same information.

You will learn about subsequent events, which are transcription and translation, in other lectures.

You have already heard about the structure of DNA in the previous lecture, so I will only briefly mention that DNA is composed of two complementary polynucleotide strands twisted into a so-called double helix. These strands are connected by hydrogen bonds based on the complementarity between the nitrogen bases, where adenine is always connected to thymine and cytosine to guanine. The complementarity ensures that a newly synthesized strand will correspond to the original one.

We distinguish the so-called 3 prime and 5 prime end on each strand. The strands are oriented antiparallel to each other, which means that opposite the 5 prime end of one strand lies the 3 prime end of the other strand and vice versa.

DNA replication happens in a so-called semi-conservative mechanism, meaning that each newly created molecule consists of one strand of the original DNA molecule, the so-called template, and one strand newly synthesized according to base complementarity. Both strands of the original DNA molecule can serve as a template or matrix.

In the past, the so-called conservative replication model was also proposed, in which both daughter DNA strands are newly synthesized, and both template strands of the parent molecule are left in their original composition. However, this model is currently being overcome, similar to the model of so-called dispersive replication, which produces two helices in which each strand contains alternating segments of old and new DNA.

Several enzymes catalyze replication. They are primarily DNA polymerases that catalyze the synthesis of the complementary DNA strand from deoxyribonucleotides on the DNA matrix strand. This ability is called endonuclease activity. Polymerization occurs from the 5 prime end to the 3 prime end. For their activity, DNA polymerases need a short oligonucleotide, the so-called primer, from the 3 prime end of which the synthesis is started.

Some polymerases also have exonuclease activity, which means removing nucleotides from the end of the daughter strand. Exonuclease activity is necessary for removing RNA primers used

in DNA replication and is called proof-reading or control activity. It is also important if the wrong nucleotide is included as it allows a step back and correction - including the correct (complementary) base. Therefore, proof-reading activity reduces the frequency of spontaneous mutations caused by the DNA polymerase wrong activity, estimated at an average of one error per 10 to power of 7 replicated base pairs.

Three types of DNA polymerases are known in prokaryotic cells. DNA polymerase I has the function of polymerization as well as exonuclease activity. DNA polymerase II has exonuclease activity and plays a role in polymerization termination.

DNA polymerase III is a so-called holoenzyme. It has three subunits with multiple functions, which combine for better efficiency (or processivity) into a dimer of twice three subunits. Together with other proteins, it recognizes the RNA primer complex with the DNA template strand. It polymerizes at a speed of approximately thirty thousand nucleotides per minute.

Other enzymes in the replication process include DNA helicases, which catalyze the unwinding of the DNA strands of the helix by disrupting hydrogen bonds. Another enzyme is primase, which catalyzes the synthesis of an RNA primer (oligoribonucleotide) from the 3 prime end of which a short polydeoxyribunucleotide is synthesized. This complex is called the Okazaki fragment - it will be explained later. The following enzyme is DNA ligase, which catalyzes the joining of polynucleotides. It plays a role in connecting Okazaki fragments into a continuous strand.

As already mentioned, DNA replication takes place in a so-called semi-conservative mechanism, which means that both strands of the original DNA can serve as templates for synthesizing new (daughter) strands. New DNA is, therefore, always made up of one original strand and one newly synthesized strand based on the complementarity of the nucleotide bases. The DNA polymerase moves along the template chain from the 3 prime to the 5 prime end, and new strands are created in the opposite direction, that means from the 5 prime to the 3 prime end.

Here I would like to remind you again of the importance of complementary base pairing, which is necessary for the newly entering daughter's DNA to match the original parental DNA. The picture shows that cytosine always bonds with guanine with three hydrogen bonds, while adenine with thymine with two hydrogen bonds. This complementarity is an essential prerequisite for preserving and transmitting genetic information.

Self-replication begins with the denaturation of double-helix DNA when initiation proteins that bind to the DNA unfold its structure by breaking hydrogen bonds. The places where the DNA structure is first disrupted are called origins of replication and are determined by a unique nucleotide sequence.

The enzyme DNA helicase catalyzes the process of breaking the double helix structure of DNA.

Y-shaped formations called replication forks are typical for replication origins. At one origin of replication, two forks are formed that move away from each other, which is why replication is referred to as bidirectional.

Once the initiation proteins are bound to the DNA and open its double helix structure, a group of proteins binds to the origin of replication. It cooperates in synthesizing a new strand of DNA.

These are, for example, so-called single-strand binding proteins, which protect single-stranded DNA released by helicase from re-pairing.

Since DNA polymerase (the main enzyme catalyzing replication) cannot start synthesizing a new strand, there must be another enzyme that can join two free nucleotides and start synthesizing a new strand according to single-stranded DNA. This enzyme is called primase and forms short stretches of approximately ten nucleotides in length, referred to as primers. However, these are not sections of DNA, but sections of a similar molecule known as ribonucleic acid, hence the so-called RNA primers. DNA polymerase can already extend these RNA primers as a new DNA strand. Later, the RNA primers are removed thanks to the exonuclease activity of the DNA polymerase.

Since DNA can only be synthesized in the 5 prime – 3 prime end direction, a particular problem occurs at the replication fork. The replication fork is asymmetric since the strands are in opposite orientations in the original double helix. One new strand is synthesized at the replication fork according to the template in the 3 prime → 5 prime end direction. (A 5 prime → 3 prime end strand is formed). This strand that is formed continuously is called a leading strand.

The second new strand is synthesized at the replication fork according to the template in the 5 prime → 3 prime end direction. However, no DNA polymerase can extend the 5 prime end of DNA. Therefore, it grows discontinuously in this direction, meaning short sections of DNA (so-called Okazaki fragments) are synthesized in the direction 5 prime → 3 prime, which are joined into a continuous strand later.

The so-called lagging strand is then made up of many separate sections of DNA, the Okazaki fragments. The following enzymes are needed to create a continuous DNA strand from Okazaki fragments: DNA polymerase I, which removes RNA primers and replaces RNA primers with DNA, and DNA ligase, which finally joins all the sections together.

This strand that is formed discontinuously is called a lagging strand.

Replication of the prokaryotic chromosome ends at specific sequences, the so-called replication terminators (TER), to which a protein inhibiting helicase activity binds and thereby stops the formation of the replication fork.

DNA replication in eukaryotic organisms is similar to prokaryotes. However, it is more complicated. For example, replication at the ends of linear molecules - telomeres constitutes a specific problem that is solved by the RNA-containing enzyme telomerase.

This picture again shows a model of the entire replisome – the unwound DNA and protein machinery at the replication fork.

In conclusion, we will summarize the most important findings from this lecture.

DNA replication occurs by a semi-conservative mechanism, where each original DNA strand is a template for creating a new molecule.

It is a process catalyzed by several enzymes. The important thing to remember is DNA polymerases, in particular.

The polymerization of the new strand always takes place only in the 5 prime – 3 prime direction. DNA polymerase needs short stretches, RNA primers, to initiate synthesis.

Synthesis of the new strand is continuous on one template strand (this is the so-called leading strand) and discontinuous on the other with the formation of so-called Okazaki fragments (the so-called lagging strand).

In eukaryotes, replication occurs in the S-phase of cell division, starting at many locations.

Replication is essential for preserving genetic information and is the essence of heredity at the molecular level.

At this moment I would like to thank you for your attention. If you have any questions, you can use the email listed here.