

DNA Replication

1. The enzyme **helicase** catalyzes the unwinding and the breaking of H-bonds to create a separated section of DNA. The open section is known as a *replication bubble* and in contains two *replication forks*. Many different replication bubbles are formed along the DNA structure.

2. **DNA polymerase** enzymes copy the open portions by forming the complementary strand. Nucleotide triphosphates are available in the cell. Two of the phosphate groups are removed as a new phosphodiester bond is formed, connecting the nucleotide to the chain. The hydrolysis of these phosphate groups provides the energy needed for the synthesis reaction to occur. Correct size, shape, and positioning of H-bonding groups determine which nucleotide gets incorporated at each site.

DNA polymerase is only able to travel in one direction down a strand of DNA: it can go from the 3'→5' end on the template strand. The new piece of DNA is synthesized in the 5'→3' direction. (Remember that the two strands of DNA are antiparallel – they have opposite directions; therefore, the DNA polymerases on each strand travels in opposite directions.) Moreover, DNA polymerase needs the presence of a *primer* or starting point (the primer is added by RNA primase).

On one strand (called the *leading strand*), new DNA is synthesized continuously. On the other strand (called the *lagging strand*), new DNA is made in several short segments (called *Okazaki fragments*) that are connected later by the enzyme **DNA ligase**.

To speed up the process, there are many DNA polymerase enzymes acting at the same time to copy the entire DNA at all the different replication forks in the different replication bubbles. The end result is two identical copies.

Each new DNA contains one strand that was originally part of the parent strand, and one newly-synthesized daughter strand. This is called semiconservative replication. (Each DNA is half old and half new.)

Transcription – Synthesis of RNA from a DNA template

Occurs in a region of the nucleus called the nucleolus.

RNA polymerase enzyme synthesizes a strand of RNA complementary to a section of one of the DNA strands (the template strand).

1. Initiation: A section of DNA known as the *promoter* (TATA box for eukaryotes) signals where the transcription begins. RNA polymerase recognizes and binds to this site.

2. Elongation: RNA polymerase assembles the nucleotides that complement the template stand. Just as in DNA replication, the enzyme reads the template strand in a 3'→5' direction, while building the new RNA strand in a 5'→3' direction. Again, two of the phosphate groups are removed as a new phosphodiester bond is formed, connecting the nucleotide to the chain. The hydrolysis of these phosphate groups provides the energy needed for the synthesis reaction to occur.

3. Termination: RNA polymerase encounters a *terminator*, or a sequence of nucleotides that signals the end of transcription. This completes the synthesis of the *pre-mRNA*.

Transcription is regulated by the cell according to its needs. Not all proteins are needed at all times. When a protein isn't needed, mRNA coding for it isn't formed.

Processing of pre-mRNA

Before the pre-mRNA transcript leaves the nucleus, the ends of the strand are modified to protect it from degradation in the cytoplasm (a 5' cap and a poly-A tail). The next step is the removal of non-coding segments called **introns**; the remaining **exons** (segments of coding sequences) are joined together. This mRNA strand is now ready to leave the nucleus and encounter a ribosome.

Translation – Protein Synthesis

This process occurs on ribosomes.

1. Initiation: The mRNA binds to the small subunit of the ribosome; at this point, the tRNA molecule carries the first amino acid of the polypeptide and attaches to the start codon. Then the large subunit of the ribosome attaches. This large subunit of the ribosome has 3 tRNA-binding sites, called the E, P and A sites.

Incoming tRNA binds to the A-site of the ribosome.

The P-site holds the tRNA that carries the growing polypeptide chain.

The tRNA's will exit the ribosome via the E-site.

Every three nucleotides represent a **codon**, which codes for a particular amino acid. The first codon of the mRNA (AUG – the “start” codon) goes in the “P-site” of the ribosome.

2. Elongation: A tRNA with a complementary anticodon goes to the next codon on the mRNA in the A-site of the ribosome. A peptide bond is formed between the two amino acids that are attached to the two tRNA's. Both, the tRNA's and the mRNA template, move down one site (a shift of 3 bases) to continue the process. The used tRNA is released from the E-site, and then the whole process repeats many times – a new tRNA comes to the A-site, a new peptide bond is formed – and the protein folds as it is formed.

3. Termination: When a “stop” codon is reached (UGA, UAA, or UAG), an enzyme (“releasing factor”) cleaves the polypeptide from the last tRNA (by adding a water molecule). The protein, tRNA, mRNA, and ribosomal subunits separate.

Often, the first amino acid (methionine – the “star codon”) is removed.

Several ribosomes may attach to one piece of mRNA to make several copies of the same protein at once.

Addendum: “Activated” or “charged” tRNA's are needed for translation (these are tRNA's with the appropriate amino acid attached). Aminoacyl tRNA Synthetase enzymes attach the appropriate amino acid to each tRNA. There's a different synthetase enzyme for each amino acid. It's very important for this process to be accurate! (Why?)