ZO502 CELL AND MOLECULAR BIOLOGY

BLOCK–II & UNIT 10: DNA REPLICATION

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DNA REPLICATION

- **In molecular biology, DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA replication occurs in all living organisms acting as the most essential part for biological inheritance. The cell possesses the distinctive property of division, which makes replication of DNA essential.**

- **DNA is made up of a double helix of two complementary strands. During replication, these strands are separated. Each strand of the original DNA molecule then serves as a template for the production of its counterpart, a process referred to as semi conservative replication. As a result of semi-conservative replication, the new helix will be composed of an original DNA strand as well as a newly synthesized strand. Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication.**

- **In a cell, DNA replication begins at specific locations, or origins of replication, in the genome. Unwinding of DNA at the origin and synthesis of new strands, accommodated by an enzyme known as helicase, results in replication forks growing bi-directionally from the origin. A number of proteins are associated with the replication fork to help in the initiation and continuation of DNA synthesis. Most prominently, DNA polymerase synthesizes the new strands by adding nucleotides that complement each (template) strand. DNA replication occurs during the S-stage of interphone.**
- **DNA replication (DNA amplification) can also be performed *in vitro* (artificially, outside a cell). DNA polymerases isolated from cells and artificial DNA primers can be used to start DNA synthesis at known sequences in a template DNA molecule. Polymerase chain reaction (PCR), ligase chain reaction (LCR), and transcription-mediated amplification (TMA) are examples.**

STRUCTURE OF DNA

DNA exists as a double-stranded structure, with both strands coiled together to form the characteristic double-helix. Each single strand of DNA is a chain of four types of nucleotides. Nucleotides in DNA contain a deoxyribose sugar, a phosphate, and a nucleobase. The four types of nucleotide correspond to the four nucleobases adenine, cytosine, guanine, and thymine, commonly abbreviated as A, C, G and T. Adenine and guanine are purine bases, while cytosine and thymine are pyrimidines. These nucleotides form phosphodiester bonds, creating the phosphate-deoxyribose backbone of the DNA double helix with the nucleobases pointing inward (i.e., toward the opposing strand). Nucleobases are matched between strands through hydrogen bonds to form base pairs. Adenine pairs with thymine (two hydrogen bonds), and guanine pairs with cytosine (three hydrogen bonds).

- **DNA strands have a directionality, and the different ends of a single strand are called the "3′ (three-prime) end" and the "5′ (five-prime) end". By convention, if the base sequence of a single strand of DNA is given, the left end of the sequence is the 5′ end, while the right end of the sequence is the 3′ end. The strands of the double helix are anti-parallel with one being 5′ to 3′, and the opposite strand 3′ to 5′. These terms refer to the carbon atom in deoxyribose to which the next phosphate in the chain attaches.**
- **The pairing of complementary bases in DNA (through hydrogen bonding) means that the information contained within each strand is redundant. Phosphodiester (intra-strand) bonds are stronger than hydrogen (inter-strand) bonds. This allows the strands to be separated from one another. The nucleotides on a single strand can therefore be used to reconstruct nucleotides on a newly synthesized partner strand.**

DNA POLYMERASE

- **DNA polymerases are a family of enzymes that carry out all forms of DNA replication. DNA polymerases in general cannot initiate synthesis of new strands, but can only extend an existing DNA or RNA strand paired with a template strand. To begin synthesis, a short fragment of RNA, called a primer, must be created and paired with the template DNA strand.**

- **DNA polymerase adds a new strand of DNA by extending the 3′ end of an existing nucleotide chain, adding new nucleotides matched to the template strand one at a time via the creation of phosphodiester bonds. The energy for this process of DNA polymerization comes from hydrolysis of the high-energy phosphate (phosphoanhydride) bonds between the three phosphates attached to each unincorporated base. Free bases with their attached phosphate groups are called nucleotides; in particular, bases with three attached phosphate groups are called nucleoside triphosphates.**

DNA Replication

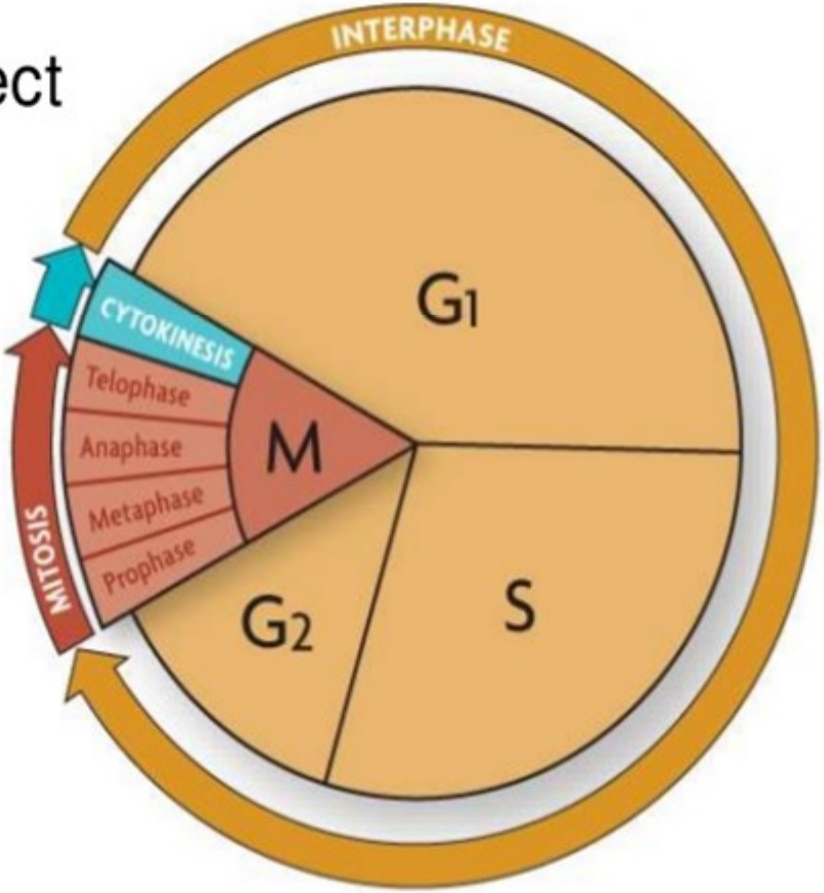
- **Types of DNA replication**
  - Semi-conservative model of DNA replication
  - Prokaryotic DNA replication
  - Eukaryotic DNA replication
  - Inhibitors of DNA replication
    (Analogues, Intercalation, Polymerase Inhibitors)

- **DNA damage**
  - Types and agents of mutations
  - Spontaneous, Radiation, Chemicals.

- **Repair mechanisms**
  - Base Excision, Nucleotide Excision, Mismatch Repair.
- **DNA-recombination**
  - In meiosis
  - Transposition

Replication copies the genetic information.

- A single strand of DNA serves as a template for a new strand.
- The rules of base pairing direct replication.
- DNA is replicated during the S (synthesis) stage of the cell cycle.
- Each body cell gets a complete set of identical DNA.



REPLICATION PROCESS

Replication fork:

- Where the DNA strands split off
- Moves with the path of the replication bubble

Primer:

Short sequence of RNA that binds to the lagging parental strand in order for DNA polymerase to copy the lagging strnad

Primase:

A protein that binds to the parental lagging strand to make a primer

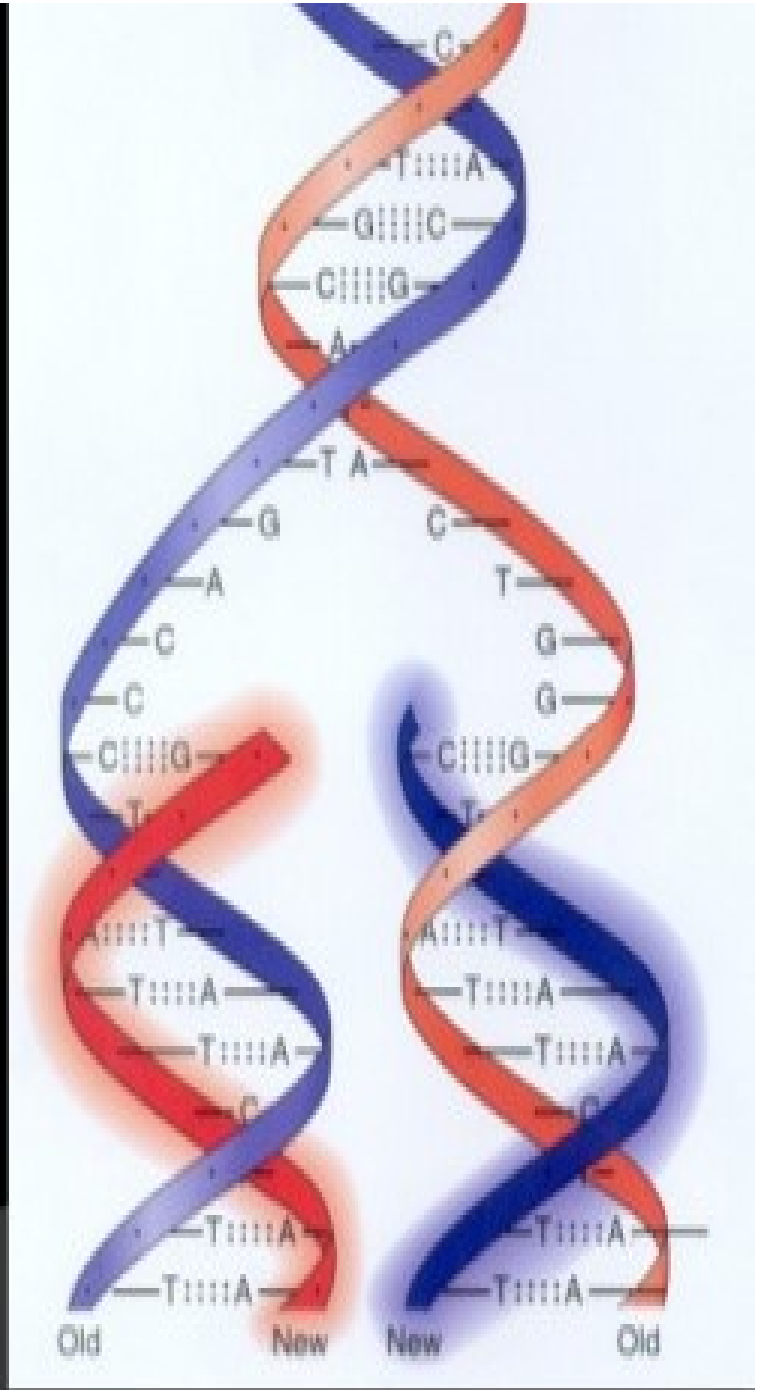
Helicase:

A protein that unwinds DNA

Proofreading and Correction

- DNA-pol I has the function to correct the mismatched nucleotides.
- It identifies the mismatched nucleotide, removes it using the 3′ - 5′ exonuclease activity, add a correct base, and continues the replication.

- Parental strands are not degraded
  - Base pairing allows each strand to serve as a template for a new strand
  - New duplex is 1/2 parent template & 1/2 new DNA



Three Stages of replication

1). Initiation

- ✓ occurs at the ***origin of replication***
- ✓ separates dsDNA, primer synthesis

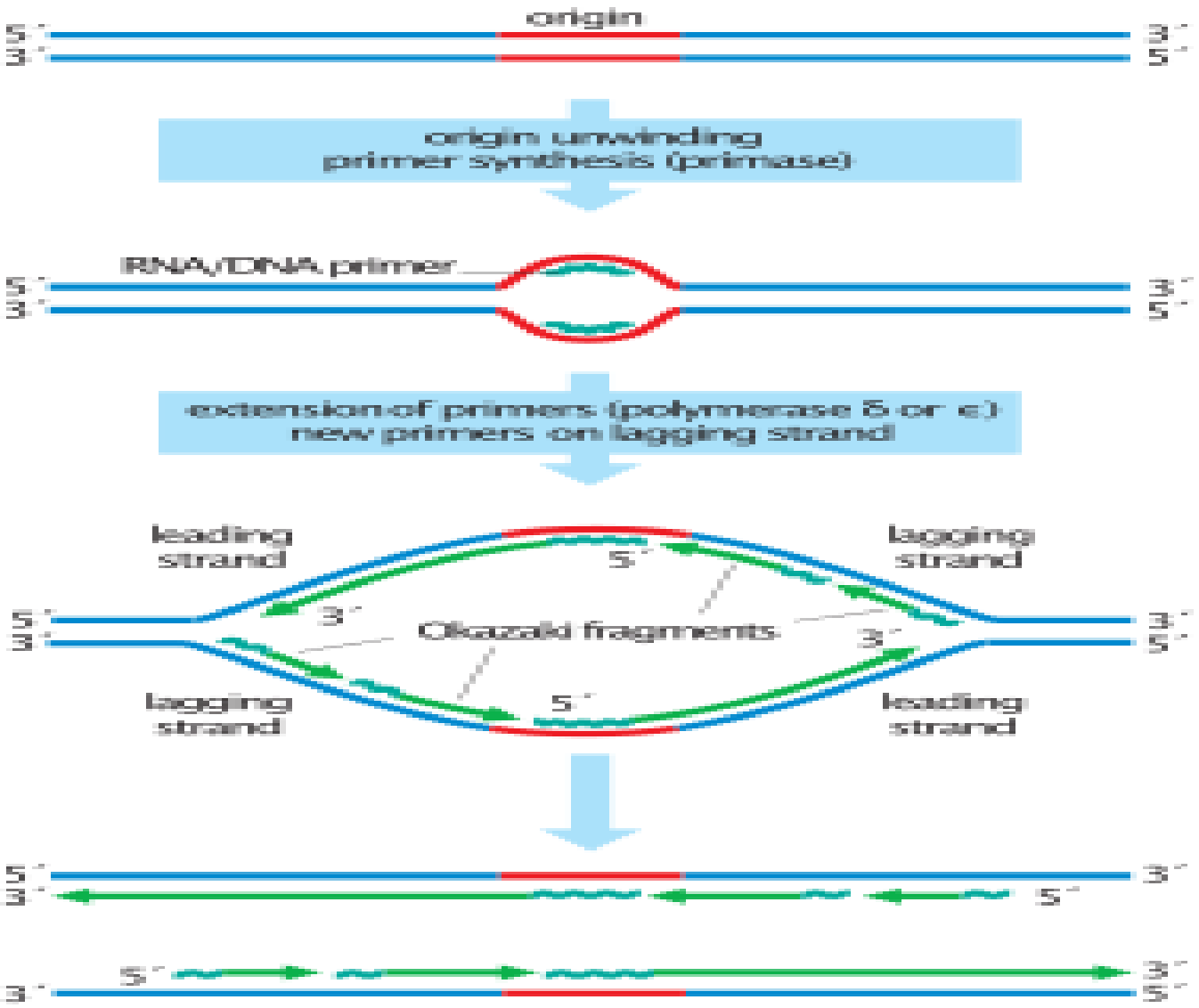
2). Elongation

- ✓ involves the ***addition of new nucleotides*** (dNTPs) based on complementarity of the template strand
- ✓ forms phosphoester bonds, correct the mismatch bases, extending the DNA strand, …

3). Termination

- ✓ stops the DNA Replication occurs at a specific ***termination site***

Overview of the steps in DNA replication

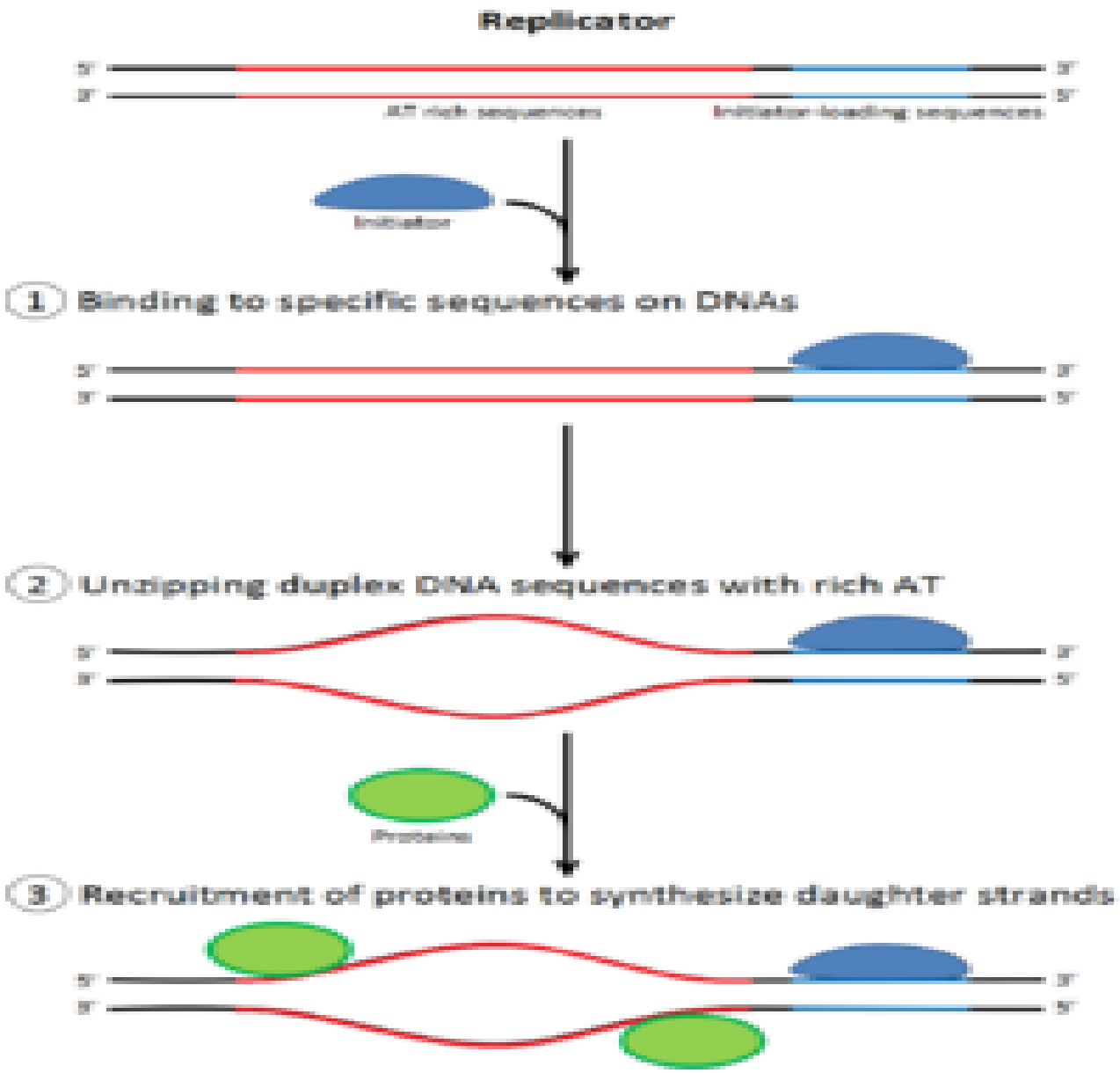


INITIATION

For a cell to divide, it must first replicate its DNA.DNA replication is an all-or-none process; once replication begins, it proceeds to completion. Once replication is complete, it does not occur again in the same cell cycle. This is made possible by the division of initiation of the pre-replication complex.

Pre-replication complex: In late mitosis and early G1 phase, a large complex of initiator proteins assembles into the pre-replication complex at particular points in the DNA, known as "origins". In *E. coli* the primary initiator protein is DnaA; in yeast, this is the origin recognition complex. Sequences used by initiator proteins tend to be "AT-rich" (rich in adenine and thymine bases), because A-T base pairs have two hydrogen bonds (rather than the three formed in a C-G pair) and thus are easier to strand-separate. In eukaryotes, the origin recognition complex catalyzes the assembly of initiator proteins into the pre-replication complex.

Role of initiators for initiation of DNA replication



Elongation Process

DNA polymerase has 5′–3′ activity. All known DNA replication systems require a free 3′ hydroxyl group before synthesis can be initiated (note: the DNA template is read in 3′ to 5′ direction whereas a new strand is synthesized in the 5′ to 3′ direction—this is often confused). Four distinct mechanisms for DNA synthesis are recognized:

All cellular life forms and many DNA viruses, phages and plasmids use a primase to synthesize a short RNA primer with a free 3′ OH group which is subsequently elongated by a DNA polymerase.

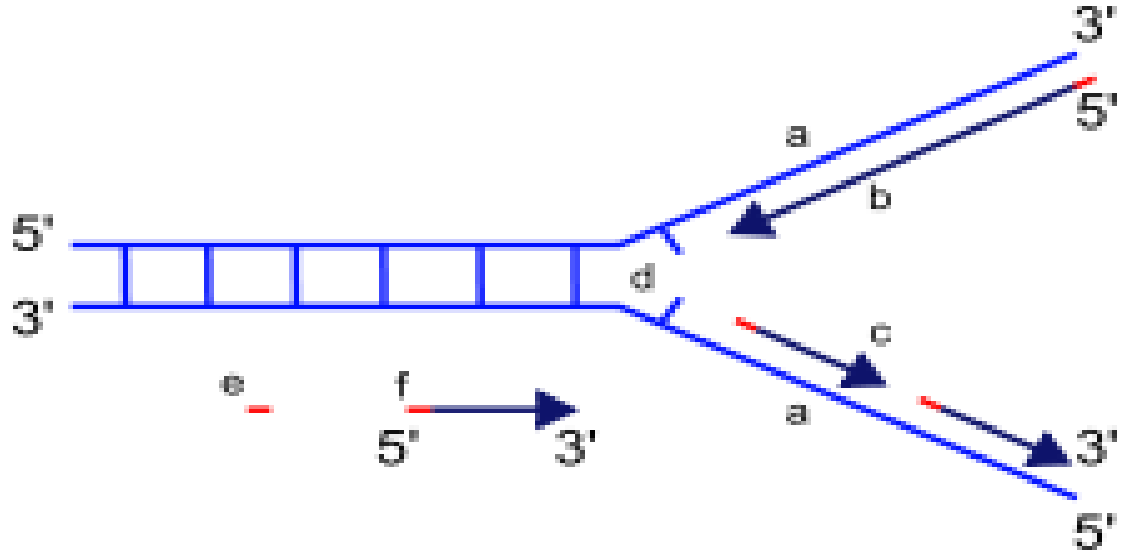
The retroelements (including retroviruses) employ a transfer RNA that primes DNA replication by providing a free 3′ OH that is used for elongation by the reverse transcriptase.

In the adenoviruses and the φ29 family of bacteriophages, the 3′ OH group is provided by the side chain of an amino acid of the genome attached protein (the terminal protein) to which nucleotides are added by the DNA polymerase to form a new strand.

In the single stranded DNA viruses—a group that includes the circoviruses, the geminiviruses, the parvoviruses and others—and also the many phages and plasmids that use the rolling circle replication (RCR) mechanism, the RCR endonuclease creates a nick in the genome strand (single stranded viruses) or one of the DNA strands (plasmids). The 5′ end of the nicked strand is transferred to a tyrosine residue on the nuclease and the free 3′ OH group is then used by the DNA polymerase to synthesize the new strand.

REPLICATION FORK

The replication fork is a structure that forms within the long helical DNA during DNA replication. It is created by helicases, which break the hydrogen bonds holding the two DNA strands together in the helix. The resulting structure has two branching "prongs", each one made up of a single strand of DNA. These two strands serve as the template for the leading and lagging strands, which will be created as DNA polymerase matches complementary nucleotides to the templates; the templates may be properly referred to as the leading strand template and the lagging strand template.



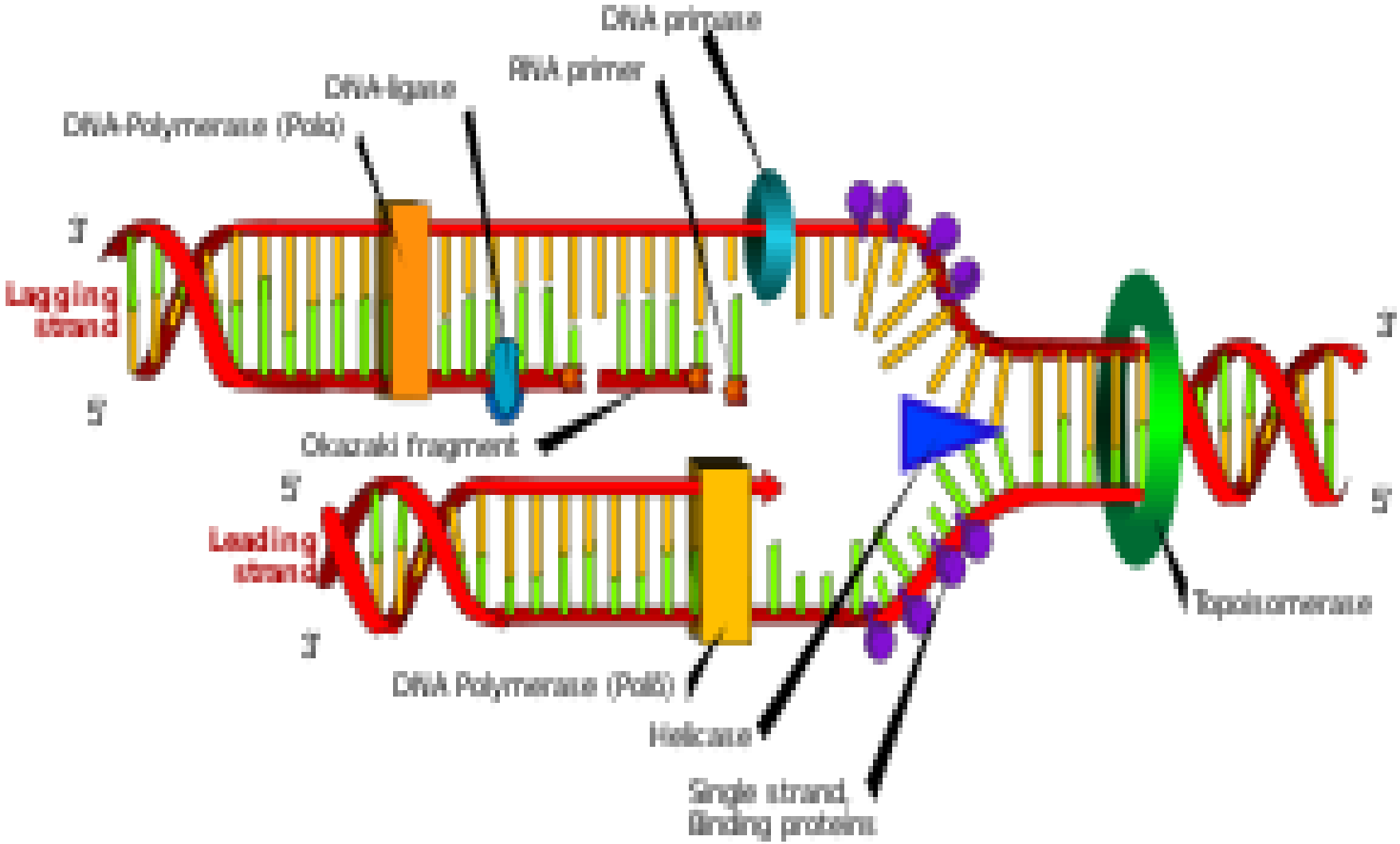
Leading strand

The leading strand is the strand of nascent DNA which is synthesized in the same direction as the growing replication fork. This sort of DNA replication is continuous.

Lagging strand

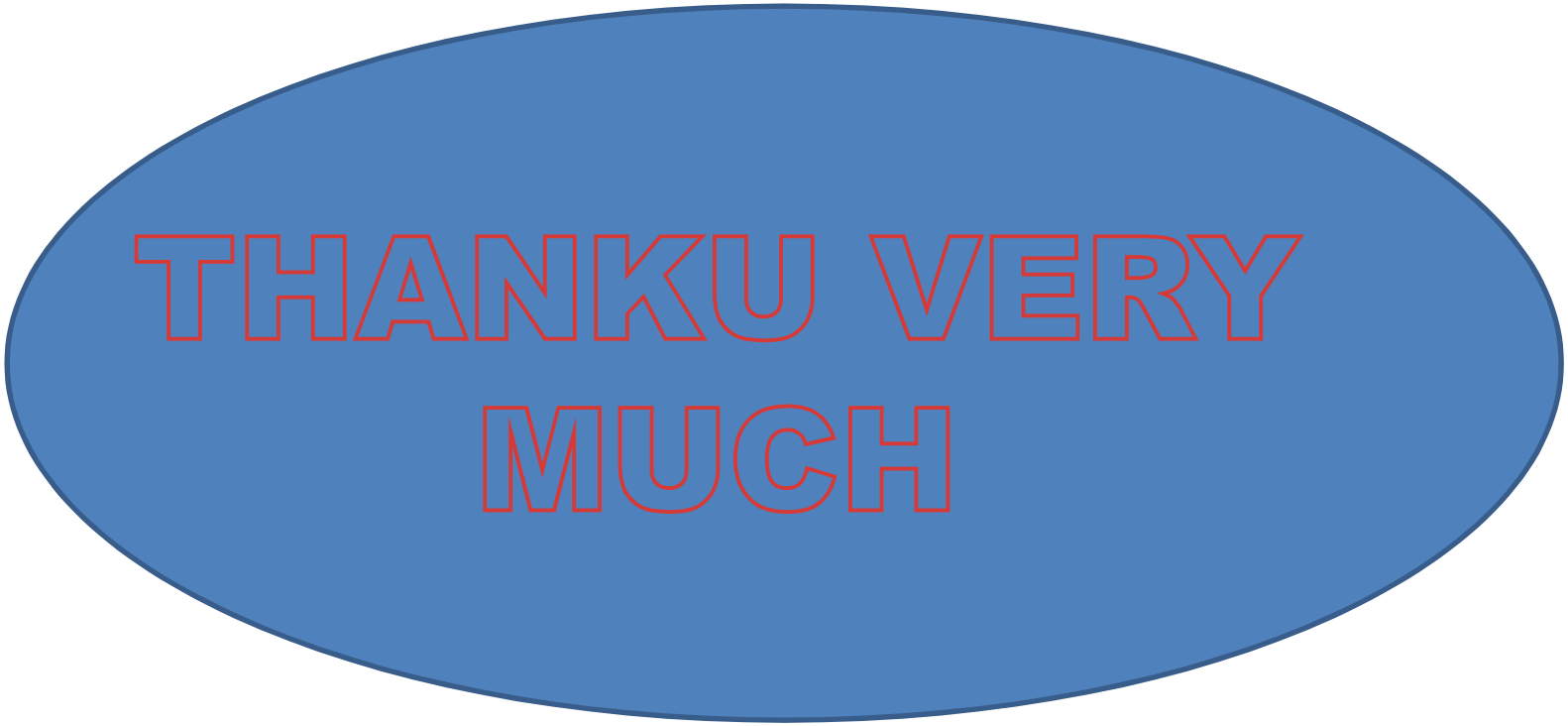
The lagging strand is the strand of nascent DNA whose direction of synthesis is opposite to the direction of the growing replication fork. Because of its orientation, replication of the lagging strand is more complicated as compared to that of the leading strand. As a consequence, the DNA polymerase on this strand is seen to "lag behind" the other strand.
The lagging strand is synthesized in short, separated segments. On the lagging strand *template*, a primase "reads" the template DNA and initiates synthesis of a short complementary RNA primer. A DNA polymerase extends the primed segments, forming Okazaki fragments. The RNA primers are then removed and replaced with DNA, and the fragments of DNA are joined together by DNA ligase.

Many enzymes are involved in the DNA replication fork



TERMINATION

- Eukaryotes initiate DNA replication at multiple points in the chromosome, so replication forks meet and terminate at many points in the chromosome. Because eukaryotes have linear chromosomes, DNA replication is unable to reach the very end of the chromosomes. Due to this problem, DNA is lost in each replication cycle from the end of the chromosome. Telomeres are regions of repetitive DNA close to the ends and help prevent loss of genes due to this shortening. Shortening of the telomeres is a normal process in somatic cells.
- This shortens the telomeres of the daughter DNA chromosome. As a result, cells can only divide a certain number of times before the DNA loss prevents further division. (This is known as the Hay flick limit.) Within the germ cell line, which passes DNA to the next generation, telomerase extends the repetitive sequences of the telomere region to prevent degradation. Telomerase can become mistakenly active in somatic cells, sometimes leading to cancer formation. Increased telomerase activity is one of the hallmarks of cancer.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