DNA Replication

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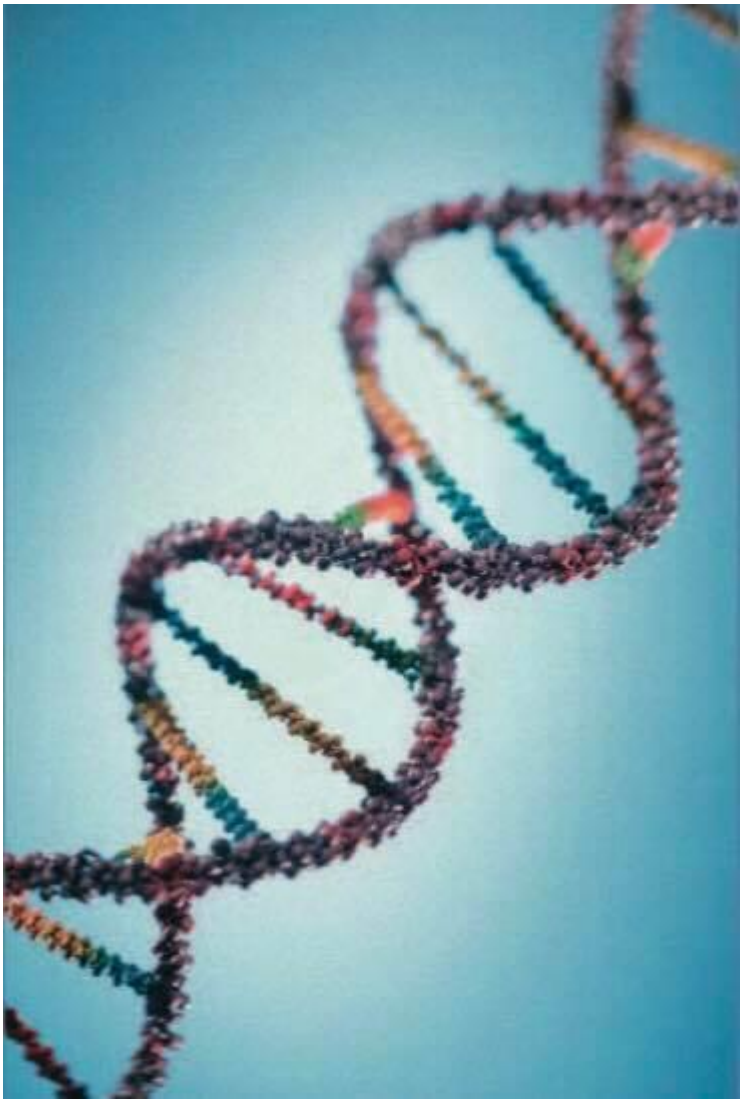
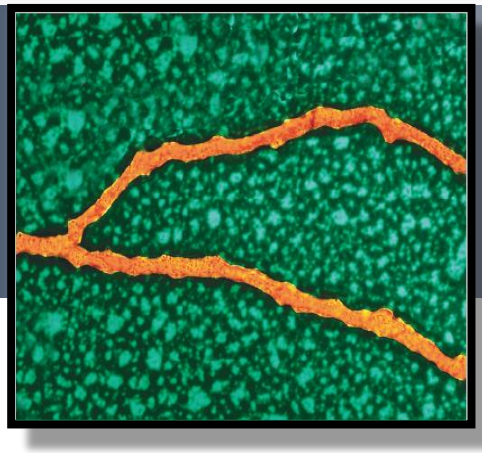


MOLECULAR BIOLOGY

SECOND CLASS

HAIDER ALNAJI

PART 1 Molecular Biology



Transmission electron micrograph of human DNA from a HeLa cell, illustrating a replication fork characteristic of active DNA replication.

LECTURE 4

DNA Replication

Lecture Contents

4.1 DNA Replication—Maintaining Genetic Information

DNA must be replicated so that the information it holds can be maintained and passed to future cell generations, even as that information is accessed to guide the manufacture of proteins.

4.2 Models of DNA Replication

- Semiconservative model: All of the atoms of one strand of the parent molecule are transferred intact and without rearrangement to one strand of the progeny IDNA molecule: the other strand is formed entirely of new atoms.

- Conservative model: Atoms of the two parental DNA strands serve as a template for two new progeny strands. The two parental strands remain intact (without rearrangement) and remain together following replication, as do the two progeny strands.

- Dispersive model: All of the atoms of each strand of the parent molecule appear in the progeny DNA, but they appear as large sections scattered throughout the length of both strands of the progeny DNA.

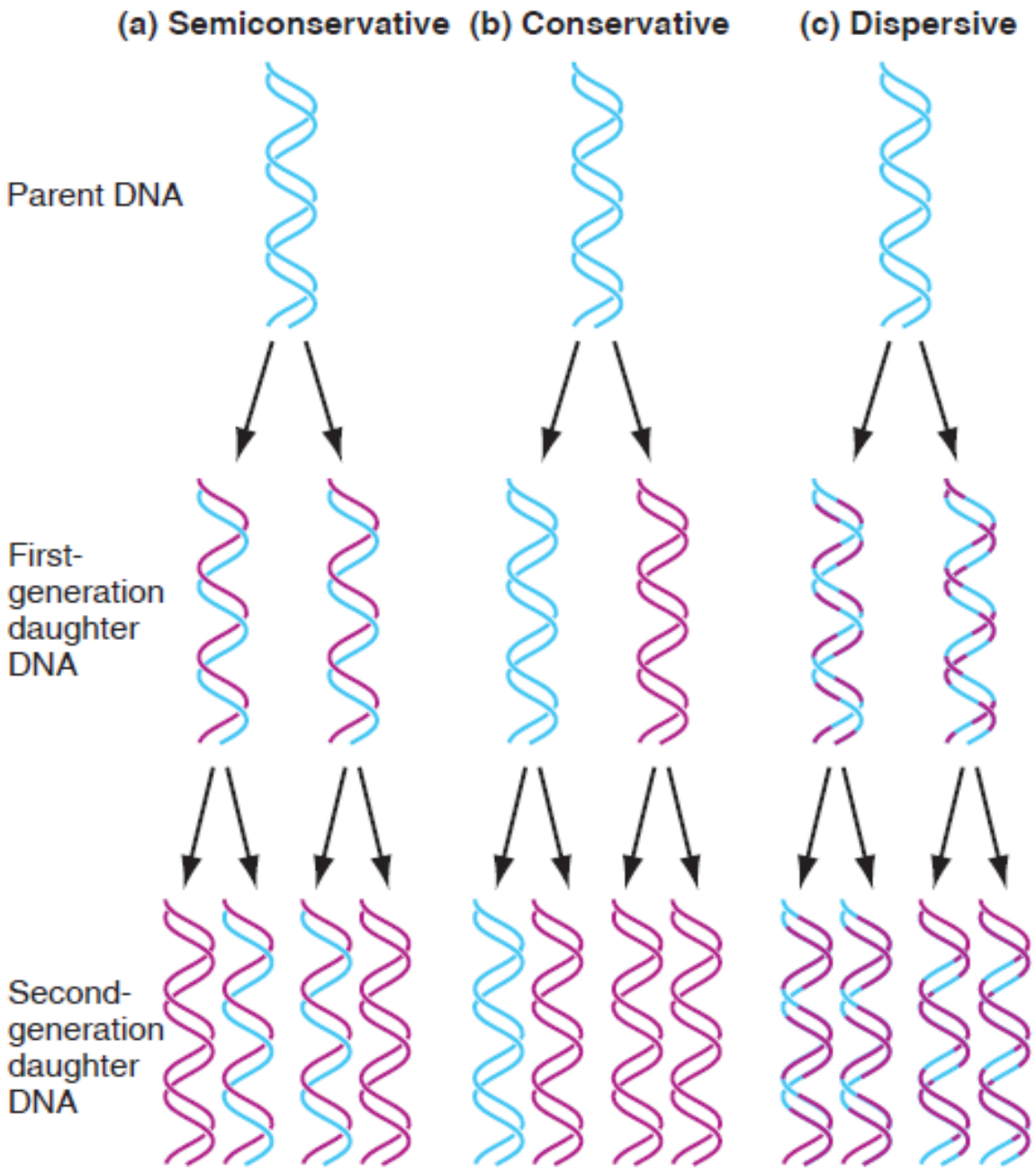


Figure 4.1 Three possible models of DNA replication. DNA from the original double helix is *blue;* newly made DNA is *magenta.* (a) Semiconservative replication (the Watson-Crick model). (b) Conservative replication: The parental double helix remains intact; both strands of one daughter double helix are newly synthesized. (c) Dispersive replication: At completion, both strands of both double helixes contain both original and newly synthesized material.

4.3 Components Required for Prokaryotic DNA Replication

components such as building blocks, enzymes, etc.-are listed

For the replication of double-stranded DNA, the following components are necessary:

1. Template DNA:

The parent sequence of nucleotides to be used as information in the synthesis of the complementary strand.

Neither DNA polymerase I nor III is capable of synthesizing DNA de novo. The enzymes will not place nucleotides in a random order.

A template is necessary, As the new DNA strand is being synthesized, each new nucleotide to be added is matching to the nucleotide on the template strand based on Chargaff base pairing. It is, then, the sequence of nucleotides of the parent DNA strand that determines the sequence of nucleotides of the new DNA strand.

2. Origin:

A specific sequence of nucleotides on the parent DNA that is recognized as the initiation site of synthesis.

Origin In prokaryotes, initiation of DNA synthesis does not begin just anywhere along the molecule. Rather, replication begins at specific locations or sites referred to as replication origins.

Prokaryotes, has a single origin site, referred to as (oriC). At this site, initiation of synthesis occurs.

Eukaryotic cells employ multiple sites on each chromosome that act as origins for the initiation of DNA replication. These sites are referred to as origins of DNA replication (ORIs).

3. **Proteins:**

DnaA, DnaB, and DnaC: Needed to recognize the origin and to separate the strands of the parent DNA.

Rep protein and single-strand binding proteins (SSB): these molecules, working together, help to uncoil the tightly packaged parent DNA (Rep protein) and unwind the double helix (SSB) in preparation for replication. These activities require ATP as an energy source.

4. **Nucleotides**:

The synthesis of DNA requires four nucleotides in the deoxyribose triphosphate form and four in the ribose triphosphate form. Needed for DNA synthesis are

1. deoxyadenine 5'-triphusphate (dATP)
2. deoxyguanosine 5'-triphosphate (dGTP)
3. deoxycytidine 5' triphosphate (dCTP)
4. deoxythymine 5'-triphosphate (dTTP)

For the synrhesis of the primer RNA, the nucleotides needed are:

1. adenosine 5'-triphosphate (ATP)
2. guanosine 5'-triphosphate (GTP)
3. cytidine 5'-triphosphate (CTP)
4. uridine 5-triphusphate (UTP)

5. **Enzymes**: Many enzymes are needed for replication, but we will deal only with the most important ones and in the order they are used:

gyrases: Uncoil the DNA in preparation for the activity of the helicase(s)

helicases: Unwind the double helix. A site where DNA is locally opened, resembling a fork, is called a **replication fork.**

RNA polymerase and primase: Needed for the synthesis of RNA primers, a requirement of DNA synthesis.

DNA synthesis is initiated by synthesis. A short piece of RNA, called primer RNA because it primes DNA synthesis, is needed before DNA chain formation can begin. The primer varies in length in different cell types, but on the order of 2-10 nucleotides. These enzymes initiate synthesis and elongate chain only in 5’ to 3’ direction.

DNA polymerase III: *The actual replicating enzyme that synthesizes DNA*

DNA polymerase III is one of the two actual replicating enzymes. As its name implies, and like the RNA polymerases, it takes its instructions from the parental- template DNA strand. Using the RNA primer as an anchor and the triphosphate deoxyribonucleotides (dATT dGTP, dCTP, and dTTT), DNA polymerase adds deoxyribonucleotides to the primer, thereby elongating the chain. As the enzyme moves along the parent strand, it "reads" each parent nucleotide and add new

nucleotide to the growing DNA chain. Synthesis is always in the 5' to 3' direction.

DNA polymerase I: *Removes the RNA primers and replaces them with DNA. Is also the "proofreading" enzyme*

DNA polymerase I is the second of the two DNA synthesizing enzymes. polymerase I is essential for both the synthesis and the repair of DNA.

DNA polymerase I has, in fact, three enzyme activities:

I. DNA polymerization

2. depolymerization in a 5' to 3' direction

3. depolymerization in a 3' to 5' direction, the proofreading activity

The last two activities are referred to as exonuclease activity, which means that the enzyme is able to remove nucleotides, one at a time, from either the 5' or the 3' end of nucleic acid chains.

DNA ligase: *Joins the Okazaki fragments by way of phosphodiester bond*

The DNA ligases are responsible for connecting DNA segments during replication, repair, and recombination. All ligases join a 5'-phosphoryl group and a 3'-phosphoryl group on adjacent fragments, thereby sealing the nick (Figure 2.6). The reaction occurs in discrete steps and requires an energy source, either ATP (eukaryotes) or nicotinamide adenine dinucleotide, NAD (prokaryotes).

4.4 Continuous and Discontinuous Synthesis

DNA synthesis process has to do with the way in which each new strand is synthesized. In this process, one strand is elongated by the continuous additions of nucleotides to the 3' end, this newly synthesized DNA is called the **leading strand**. while the other new strand is produced by repeated synthesis of primer RNAs and short lengths of DNA (Okazaki fragments), which must eventually be joined by DNA ligase. This latter method is called discontinuous synthesis of the **lagging strand** (Figure 4.2).

Because DNA synthesis is continuous on one strand and discontinuous on the other, the term **semidiscontinuous** synthesis is sometimes used to describe the overall process.

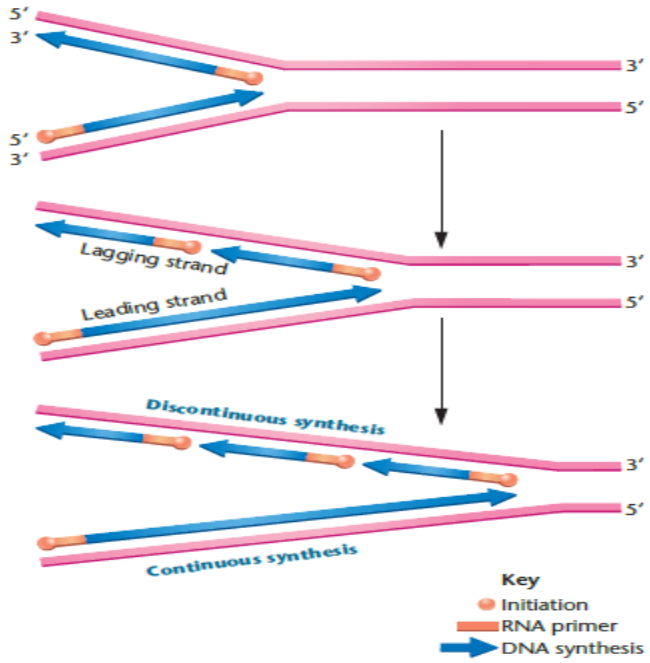


Figure 4.2 opposite polarity of synthesis along the two strands of DNA is necessary because they run antiparallel to one another, and because DNA polymerase III synthesizes in only one direction (5’ to 3’). on the lagging strand, synthesis must be discontinuous, resulting in the production of okazaki fragments. on the leading strand, synthesis is continuous. RNA primers are used to initiate synthesis on both strands.

the two strands of DNA are antiparallel; they run in opposite directions Also, keep in mind that DNA synthesis is always in the 5' to 3' direction.

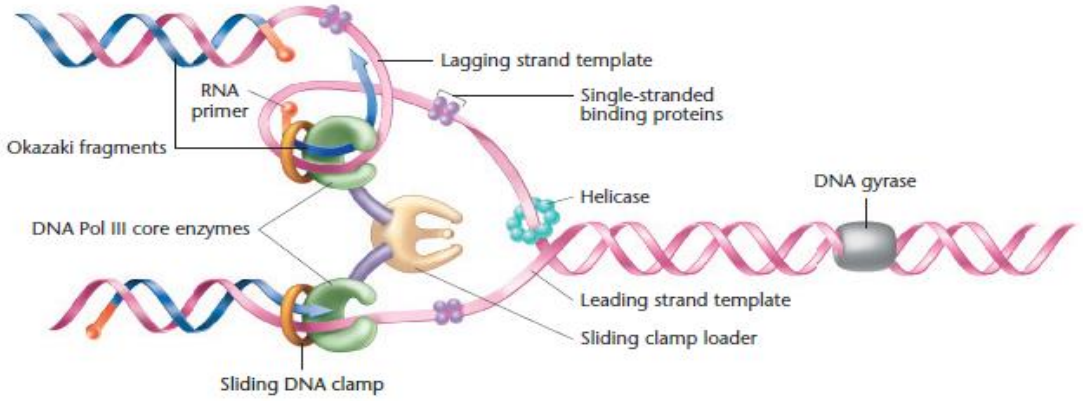


Figure 4.3 Illustration of how concurrent DNA synthesis may be achieved on both the leading and lagging strands at a single replication fork (RF). the lagging template strand is "looped" in order to invert the physical direction of synthesis, but not the biochemical direction. the enzyme functions as a dimer, with each core enzyme achieving synthesis on one or the other strand.

4.5 Summary

During replication, the DNA unwinds locally at several sites. **Replication forks** form as hydrogen bonds break between base pairs. Primase builds short RNA primers, which DNA sequences eventually replace. Next, **DNA polymerase** fills in DNA bases, and **ligase** seals the sugar-phosphate backbone.

Replication proceeds in a 5′ to 3′ direction, so the process must be discontinuous in short stretches on one strand.

4.6 Study Questions

1. Briefly explain the semiconservative model of DNA.
2. Describe the role of each of the following in prokaryotic DNA replication: triphosphate nucleotides, template DNA, primase, RNA primer, DNA polymerases I and III, and DNA ligase.
3. Describe how leading and lagging strand synthesis are similar and different.
4. What are Okazaki fragments?
5. What is the direction of DNA synthesis?