DNA REPLICATION
WHAT IS DNA REPLICATION?

DNA replication is the process by which DNA makes a copy of itself. This happens during cell division, making sure that each new cell receives the same genetic information as the parent cell.

➤ STRUCTURAL OVERVIEW OF DNA REPLICATION

▪ The double helix is composed of two DNA strands, and the individual building blocks of each strand are nucleotides. The nucleotides contain one of four bases: adenine, thymine, guanine, or cytosine.

▪ The AT/GC rule or Chargaff's rule, is the basis for the complementarity of the base sequences in double-stranded DNA. The strands within a double helix have an anti-parallel alignment.

➤ Existing DNA Strands Act as Templates for the Synthesis of New Strands

▪ During the replication process, the two complementary strands of DNA come apart and serve as template strands for the synthesis of two new strands of DNA.

▪ The two newly made strands are referred to as the daughter strands, while the original strands are called the template strands or parental strands.

<u>CONSERVATIVE MODEL</u> – Both strands of parental DNA remain together following DNA replication. In this model, the original arrangement of parental strands is completely conserved, while the two newly made daughter strands are also together following replication.

<u>SEMICONSERVATIVE MODEL</u> – The double-stranded DNA is half conserved following the replication process. In other words, the double-stranded DNA contains one parental strand and one daughter strand.

<u>DISPERSIVE MODEL</u> – Proposes that segments of parental DNA and newly made DNA are interspersed in both strands following the replication process.

BACTERIAL DNA REPLICATION

➤ Bacterial Chromosomes Contain a Single Origin of Replication

The site on the bacterial chromosome where DNA synthesis begins is known as the origin of replication. The synthesis of new daughter strands is initiated within the origin and proceeds bidirectionally (in both directions) around the bacterial chromosome

➤ Replication Is Initiated by the Binding of DnaA Protein to the Origin of Replication

▪ The origin is named oriC (for origin of Chromosomal Replication).

▪ There are three types of DNA sequences within oriC that are functionally important:

- **an AT-rich region**,
- **DnaA box sequences**, and
- **GATC methylation sites.**

▪ DnaA proteins bind to the four DnaA boxes in oriC to initiate DNA replication.

▪ With the aid of other DNA-binding proteins such as HU and IHF, this causes the DNA to bend around the complex of DnaA proteins and results in the separation of the AT-rich region.

▪ The DnaA proteins, with the help of the DnaC protein, recruit DNA helicase enzymes (also known as DnaB helicase, or simply as helicase) to bind to this site.

▪ The action of DNA helicases promotes the movement of two forks outward from oriC in opposite directions.

▪ This initiates the replication of the bacterial chromosome in both directions, an event termed bidirectional replication.

➤ DNA Strand Separation and the Synthesis RNA Primers Are Necessary Before Daughter Strands of DNA Can Be Made

▪ An enzyme, known as DNA gyrase (a topoisomerase type II enzyme) travels ahead of the helicase enzyme and alleviates this supercoiling.

▪ The single-strand binding protein is to bind to both of single strands of parental DNA and prevent them from re-forming a double helix.

▪ The next event involves the synthesis of a short strand of RNA (rather than DNA) called an RNA primer.

▪ This strand of RNA is synthesized by the linkage of ribonucleotides via an enzyme known as DNA primase or simply primase.

▪ This enzyme synthesizes short strands of RNA, typically 10 to 12 nucleotides in length.

➤ DNA Polymerases Link Nucleotides to Synthesize the Daughter Strands

▪ The enzymes known as DNA polymerases are responsible for covalently attaching nucleotides together to make new daughter strands.

• In E. coli, there are five distinct proteins that function as DNA polymerases, designated pol I, II, III, IV, and V.

• Poll and pol III are involved in normal DNA replication

• Pol II, pol IV and pol V play a role in DNA repair and the replication of damaged DNA.

▪ The DNA polymerase cannot begin DNA synthesis by linking together two individual nucleotides. Rather, this enzyme can only elongate a strand starting with an RNA primer or existing DNA strand.

▪ A second unusual feature is the directionality of strand synthesis. DNA polymerases can attach nucleotides only in the 5' to 3' direction, not in the 3' to 5' direction.

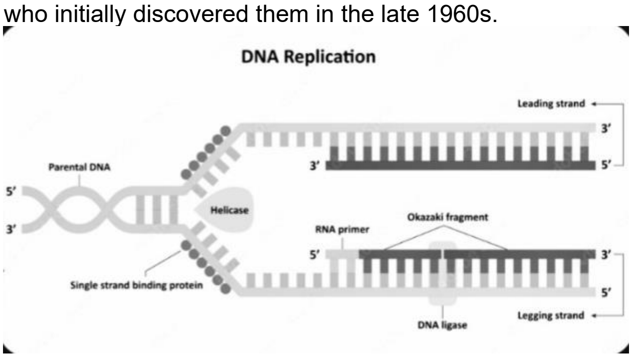
• The two new DNA strands (namely, the leading and lagging strands) are synthesized in different ways.

• In Leading Strand, synthesis of the daughter strand is continuous.

• In Lagging Strand, synthesis of DNA occurs in short Okazaki Fragments.

• An enzyme known as DNA ligase catalyzes a covalent bond between these adjacent DNA fragments to complete the process in the lagging strand.

• Okazaki Fragments named after Reiji and Tuneko Okazaki, who initially discovered them in the late 1960s.



➤ Replication Is Terminated When the Replication Forks Meet at the Terminus Sequences

• On the opposite side of the E. coli chromosome from oric is a pair of termination sequences. Sequences called ter

• A protein known as the termination utilization substance (Tus) binds to the ter sequences and stops the movement of the replication forks.

• DNA replication ends when oppositely advancing forks meet, usually at T1 or at T2.

• Interlocked circular molecules are known as catenanes. Topoisomerases introduce a temporary break into the DNA strands and then rejoin them after the strands have become unlocked. This allows the catenanes to be separated individual circular molecules.

➤ Certain Enzymes of DNA Replication Bind to Each Other to Form a Complex

• The replication of double-stranded involves an assembly of proteins called the replisome.

• DNA helicase and primase are physically bound to each other to form a complex known as a primosome.

➤ The Fidelity of DNA Replication is Ensured by Proofreading Mechanisms

• The DNA replication exhibits a high degree of fidelity.
• The hydrogen bonding between G and Cor A and T is much mores stable than between mismatched pairs.
• Proofreading function the ability to remove mismatched bases.

➤ Bacterial DNA Replication is Coordinated with Cell Division

▪ E. coli can divide every 20 to 30 minutes.
▪ DNA replication take place only when a cell is about to divide.
▪ The DnaA protein binds to DnaA boxes within the origin of replication and opens the DNA helix at the AT-rich region within the origin.
▪ Immediately following DNA replication, there are twice as many DnaA boxes, and so there is insufficient DnaA protein to initiate a second round of replication.
▪ Since it takes time to accumulate newly made DnaA protein, DNA replication cannot occur until the daughter cells have had time to grow.
▪ Another way to regulate DNA replication involves GATC sites within oriC. These sites can be methylated by enzyme known as Dam methylase (DNA adenine methyltransferase).
▪ The Dam methylase recognize 5'-GATC-3' sequence, binds there, and attaches a methyl onto the adenine base (fig.11.160).
▪ Prior to DNA replication these sites are methylated in both strands. This full methylation of the 5'- GATC-3' sites facilitates the initiation of DNA replication at the origin. Following DNA replication, the newly made strands are not methylated, since adenine rather than methyladenine is found in the daughter strands (fig.11.16b).

EUKARYOTIC DNA REPLICATION

➤ Initiation Occurs at Multiple Origin of Replication on Linear Eukaryotic Chromosomes

• Eukaryotes have a long linear chromosomes, they require multiple origins of replication so that DNA can be replicated in a reasonable length of time.
• DNA replication proceeds bidirectionally from many origins of replication. The multiple replication forks eventually make a contact with each other to complete the replication process.
• As DNA replication radiates bidirectionally from each origin, regions are formed that contain two double helices. These regions are punctuated by other regions that have not yet replicated and consist of double helix.
• In Saccharomyces cerevisiae, several replication origins have been identified and sequenced.
• They have been named ARS elements (Autonomously Replicating Sequence).
• ARS elements, which are 100 to 150 bp in length, are necessary to initiate chromosome replication in vivo.
• They have a higher percentage of A and T bases than the rest of the chromosomal DNA.
• Three or four copies of the sequence (A or T) TTTAT (A or G) TTT (A or T) are interspersed within all ARS elements.
• The origin recognition complex (ORC) is a six-subunit protein complex that acts as the initiator of eukaryotic DNA replication.
• ORC binds directly to ARS elements.
• ORC requires ATP to bind to ARS elements.

➤ Eukaryotes Contain Several Different DNA Polymerases

• Four of these, designated a (alpha), δ (delta), & (epsilon), and y (gamma), have the primary function of replicating DNA.
• The DNA polymerase a/primase complex is to synthesize a short RNA-DNA hybrid of approximately 10 RNA nucleotides followed by 20-30 DNA nucleotides.
• This short RNA-DNA strand is then used by DNA polymerase 8 or & for the processive elongation of the leading strand and lagging strand.
• The exchange of DNA polymerase a for å or & is called a polymerase switch.

➤ Nucleosomes Containing New Histone Proteins Are Quickly Formed After DNA Replication

• The DNA within eukaryotic cells is wrapped around histone proteins to form a nucleosomes structure.
• The histones are assembled into octamer structures and associate with the newly made DNA very near the replication fork.
• Each daughter strand contains a random mixture of original histone octamers and newly assembled histone octamers.

➤ The Ends of Eukaryotic Chromosomes Are Replicated by Telomerase

• The telomere refers to the complex of telomeric sequences within DNA and the special proteins that are bound to these sequence.
• Telomeric sequences consist of a moderately repetitive tandem array and a 3' overhang region that is 12 to 26 nucleotides in length.
• Telomerase it recognizes telomeric sequences at end of eukaryotic chromosomes and synthesizes additional repeats of telomeric sequences.
• The adjacent part of the RNA is used as a template to make a short, six-nucleotides repeat of DNA.
• Telomerase move six nucleotides to the right and then synthesizes another repeat.
• The bottom strand would be made by DNA polymerase, using an RNA primer at the end of the chromosomes.