Download CSIR NET UNIT 3A – DNA Replication Notes PDF

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Unit 3 – Molecular Biology: The most common choice of almost all students. If you want to start your CSIR NET preparation then this unit is perfect to start with. Questions are asked from this UNIT almost every time in the CSIR NET Exam and are to the point & direct – subject-specific questions. Here we will be discussing about UNIT 3(A) – DNA Replication.

Reference Book For UNIT 3A: DNA Replication

• Lehninger's Principles of Biochemistry by Nelson & Cox
• Biochemistry by Voet & Voet
• Molecular Biology of the Cell by Alberts
• Molecular Biology by Robert F. Weaver
• Molecular Biology of the Gene by Watson

Four requirements for DNA to be genetic material

- Must carry information – Cracking the genetic code
- Must replicate – DNA replication
- Must allow for information to change – Mutation
- Must govern the expression of the phenotype – Gene function

Mechanisms by which information is transferred in the cell are based on "Central Dogma"

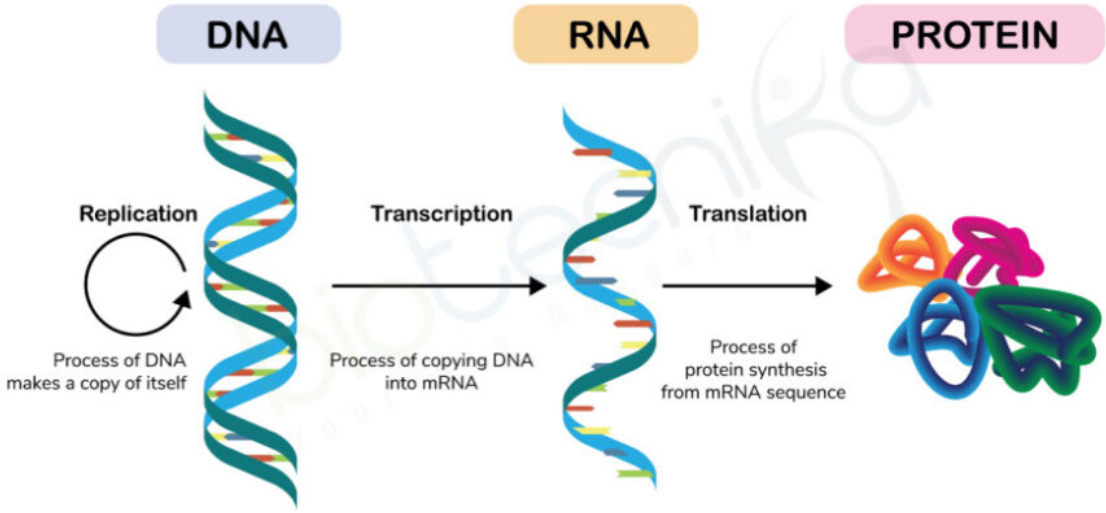


Fig: Central Dogma

- Replication: synthesis of daughter DNA from parental DNA
- Transcription: synthesis of RNA using DNA as the template
- Translation: protein synthesis using mRNA molecules as the template
- Reverse transcription: synthesis of DNA using RNA as the template

Rules of DNA Replication

- Takes place in the S phase of the cell cycle
- Occur once per cell cycle
- Starts from the origin of Replication
- DNA Replication takes place from 5'to 3'direction
- DNA Replication can be uni or bidirectional

DNA replication 3 possible models

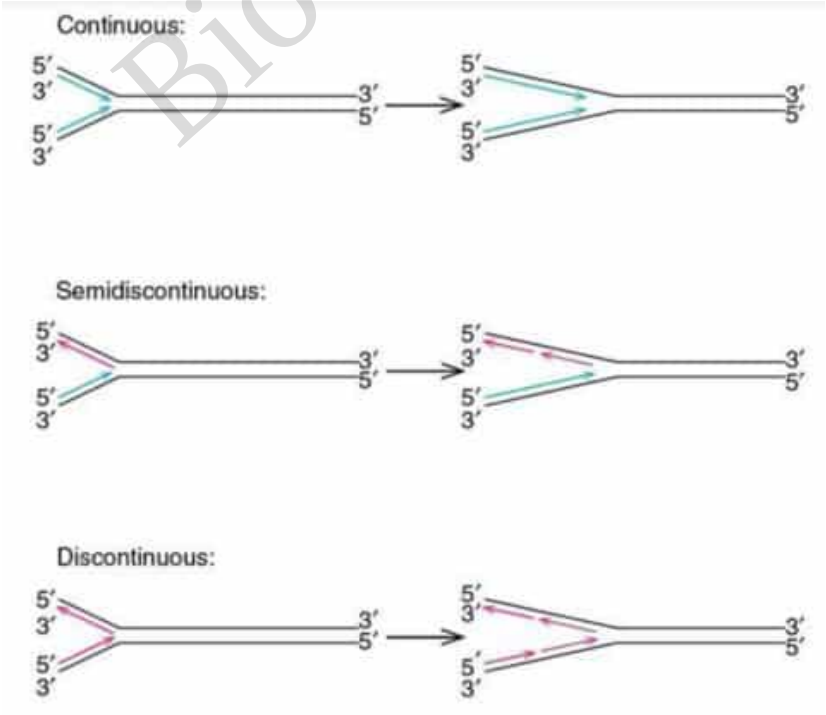


Fig: DNA Replication Models

Semi-conservative replication

One strand of duplex passed on unchanged to each of the daughter cells. This 'conserved' strand acts as a template for the synthesis of a new, complementary strand by the enzyme DNA polymerase.

How do we know that DNA replication is semiconservative?

The Meselson-Stahl Experiment

1. Meselson and Stahl (1958) grew E. coli in a heavy (not radioactive) isotope of nitrogen, 15N in the form of 15NH4Cl. Because it is heavier, DNA containing 15N is denser than DNA with normal 14N, and so can be separated by CsCl density gradient centrifugation.
2. Once the E. coli were labeled with heavy 15N, the researchers shifted the cells to a medium containing normal 14N and took samples at time points. DNA was extracted from each sample and analyzed in CsCl density gradients (Figure 3.2).

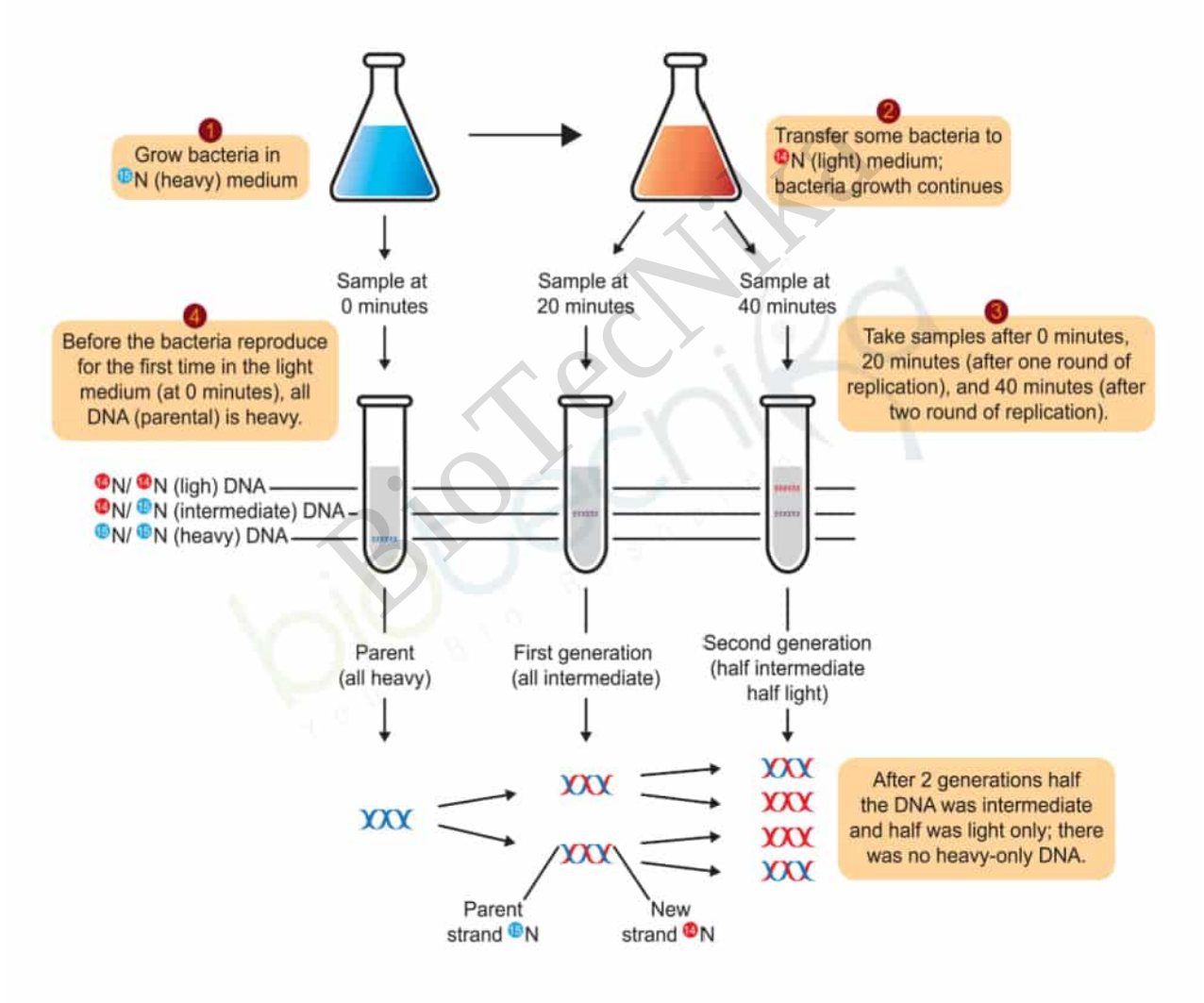
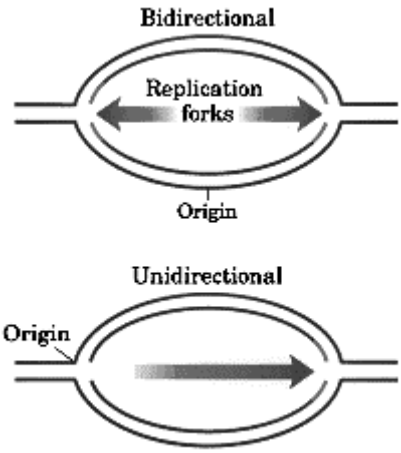


Fig: Fig: The Meselson-Stahl experiment, which showed that DNA replicates semi conservatively

Uni or bidirectional

- Replication starts from unwinding the dsDNA at a particular point (called origin), followed by the synthesis of each strand.
- The parental dsDNA and two newly formed dsDNA form a Y-shape structure called a replication fork.



Bidirectional replication

- Once the dsDNA is opened at the origin, two replication forks are formed spontaneously.
- These two replication forks move in opposite directions as the syntheses continue.

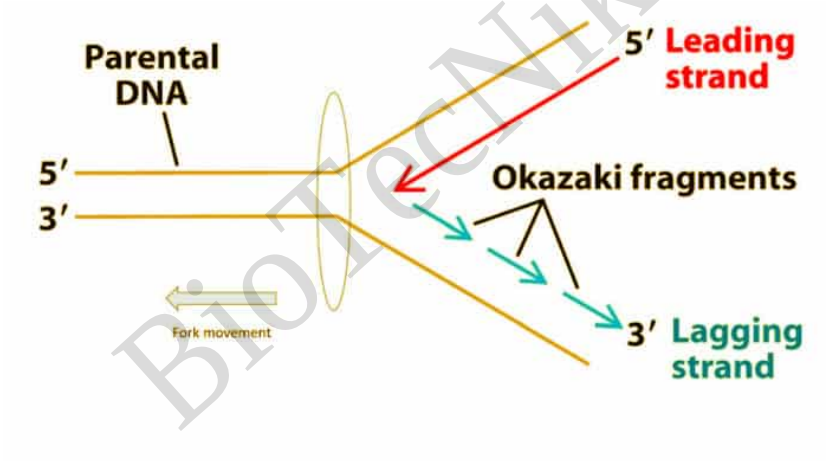
Semi-continuous Replication

The daughter strands on two template strands are synthesized differently since the replication process obeys the principle that DNA is synthesized from the 5´ end to the 3´end.

New strand synthesis is always in the 5'-3' direction.

Discovery of Okazaki fragments

Evidence for semi-discontinuous replication [3H] thymidine pulse-chase labeling



experiment

1. Grow E.coli
2. Add [3H] thymidine in the medium for a few seconds spin down and break the cell to stop labeling analyze found a large fraction of nascent DNA (1000-2000 nt) = Okazaki fragments
3. Grow the cell in a regular medium then analyze the small fragments join high molecular weight DNA= Ligation of the Okazaki fragments

Enzymes required for DNA replication

More than 20 different enzymes are involved in DNA replication. The entire complex is termed as REPLISOME

1. DNA polymerase- Catalyze the synthesis of DNA
2. Helicase- Separates the two-parent strands
3. Topoisomerases- Relieves topological stress in the helical DNA structure
4. DNA-binding proteins (SSB)- stabilizes the separated strands of DNA
5. Primase- synthesizes an RNA primer
6. DNA ligases- fills gap after RNA primer is removed
7. Nucleases- degrade nucleic acids from one end of the molecule

• Exonuclease- degrade nucleic acid from one end (5'- 3' or 3'-5'direction)
• Endonuclease- degrade at specific internal sites

Prokaryotic DNA Replication: Enzymes and Their Function

Enzyme protein	Specific Function
DNA pol I	Exonuclease activity removes RNA primer and replaces it with newly synthesized DNA
DNA pol II	Repair Function
DNA pol III	Main enzyme that adds nucleotides in the 5'-3′ direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Topoisomerase	Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA
Single-strand binding proteins (SSB)	Binds to single-stranded DNA to avoid DNA rewinding back

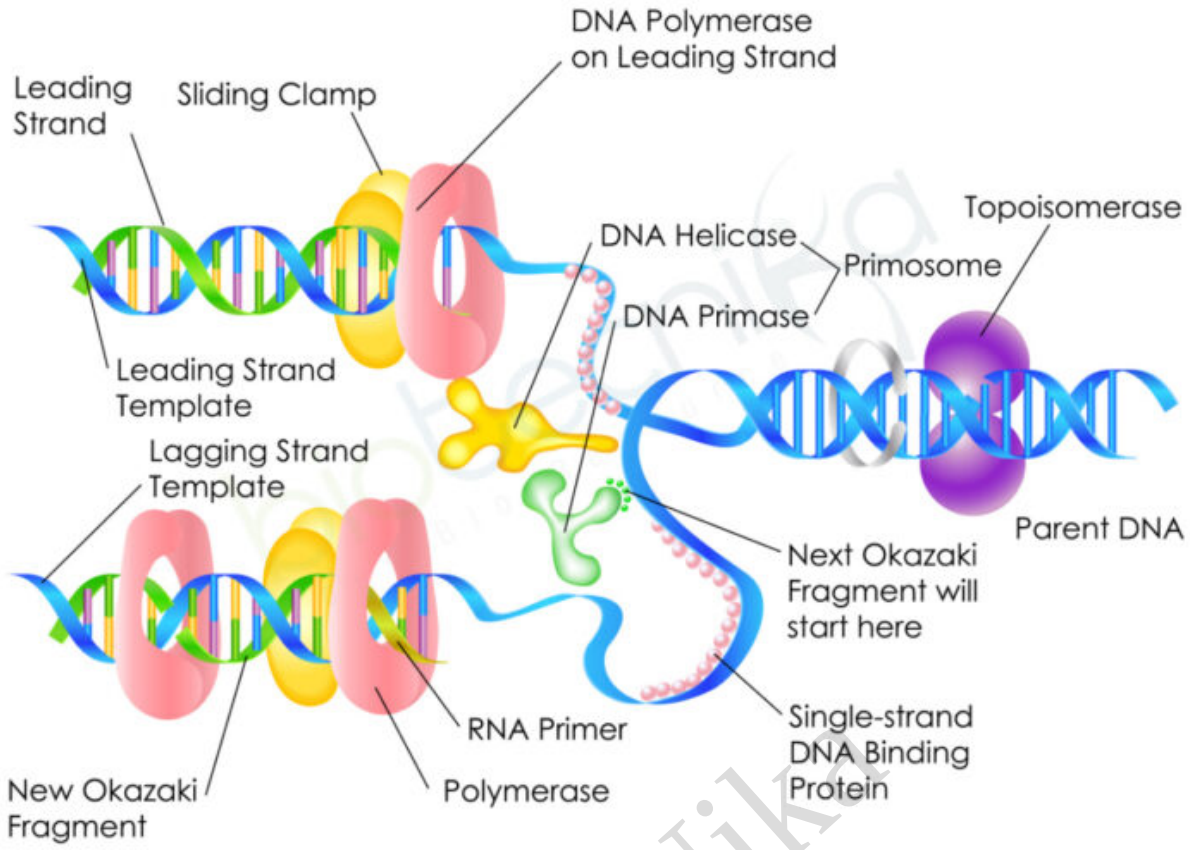


Fig: DNA Replication & Enzymes Involved

DNA Helicases

- Helicases are motor proteins that couple the hydrolysis of ATP to nucleic acid unwinding.
- The hexameric helicases have a characteristic ring-shaped structure, and all, except the eukaryotic minichromosomal maintenance (MCM) helicase, are homohexamers.

Primase

- Primase is the enzyme that copies a DNA template strand by making an RNA strand complementary to it
- Primase synthesizes a short (about 10 nucleotides) RNA primer in the 5'-3' direction
- The parental strand is used as a template for this process
- RNA primers are required because DNA polymerases are unable to initiate the synthesis of DNA, but can only extend a strand from the 3' end of a preformed "primer"
- The enzyme is active only in the presence of other proteins (including a helicase), which creates a complex called the "primosome"

DNA ligase

- The mechanism of DNA ligase is to form two covalent phosphodiester bonds between 3′
  hydroxy ends of one nucleotide, (“acceptor”) with the 5’ phosphate end of another (“donor”).
- ATP is required for the ligase reaction, which proceeds in three steps:
- Adenylation (addition of AMP) of a lysine residue in the active center of the the enzyme, pyrophosphate is released
- Transfer of the AMP to the 5′ phosphate of the donor, formation of a pyrophosphate bond;
- Formation of a phosphodiester bond between the 5′ phosphate of the donor and the 3′
  hydroxyl of the acceptor.
- DNA ligase in E. coli, as well as most prokaryotes, uses energy gained by cleaving nicotinamide adenine dinucleotide (NAD) to create the phosphodiester bond.

Topoisomerases

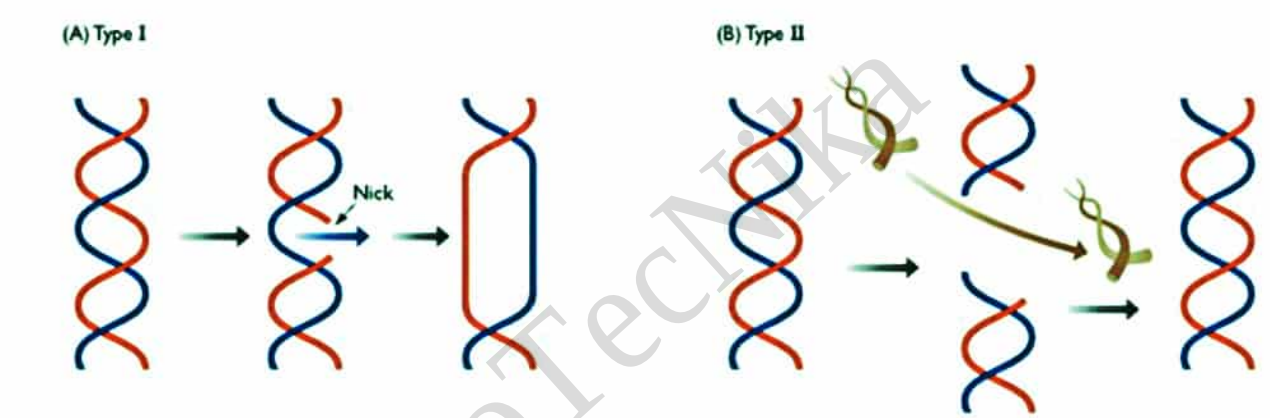


Fig: (A)- Type I topoisomerase makes a nick in one strand of a DNA molecule passes the intact strand through the nick, and reseals the gap. (B) – Type II topoisomerase makes a double-stranded break in the double helix, creating a gate through which a second segment of the helix is passed.

DNA Polymerases

- Catalyzes DNA-template-directed extension of the 3’- end of a DNA strand by one nucleotide at a time.(dNMP)n + dNTP (dNMP)n+1 + PPi
- Processivity- The average number of nucleotides added before a polymerase dissociates
- Proofreading-3’-5’ exonuclease activity

Roles of DNA Polymerases

1. All DNA polymerases link dNTPs into DNA chains Main features of the reaction:

a. An incoming nucleotide is attached by its 5’-phosphate group to the 3’-OH of the growing DNA chain. Energy comes from the dNTP releasing two phosphates. The DNA chain acts as a primer for the reaction.

b. The incoming nucleotide is selected by its ability to hydrogen bond with the complementary base in the template strand. The process is fast and accurate.
c. DNA polymerases synthesize only from 5' to 3'.

2. The enzyme Kornberg isolated was believed to be the only DNA polymerase in E. coli. However, mutations in this gene (polA1) were not lethal, indicating that other DNA polymerases must exist in E. coli.

Prokaryotic DNA polymerases

- DNA Polymerase I – In 1956, Arthur Kornberg and colleagues discovered DNA polymerase I (Pol I), in Escherichia coli. It has 5'- 3'Exonuclease activity.
- DNA Polymerase II- required in DNA repair
- DNA Polymerase III- Replication enzyme in E.coli
- DNA Polymerase IV- Involved in mutagenesis. It exhibits no 3′- 5′ exonuclease (proofreading) activity and hence is error-prone.
- DNA Polymerase V- Involved in DNA repair mechanisms

DNA Polymerase I

- Encoded by the polA gene and ubiquitous among prokaryotes.
- Pol I is the most abundant polymerase, accounting for >95% of polymerase activity in E.
  coli)
- Pol I adds ~15-20 nucleotides per second, thus showing poor processivity.
- Pol I possesses four enzymatic activities:1. A 5'→3′ (forward) DNA-Dependent DNA polymerase activity, requires a 3′ primer site and a template strand
- A 3'→5′ (reverse) exonuclease activity that mediates proofreading
- A 5'→3′ (forward) exonuclease activity mediating nick translation during DNA repair.
- A 5'→3′ (forward) RNA-Dependent DNA polymerase activity. Pol I operates on RNA templates with considerably lower efficiency (0.1–0.4%) than it
  does on DNA templates, and this activity is probably of only limited biological significance.
- The polymerase's special functions are enhanced by its 5'-3'Exonuclease activity. This activity is located in a structural domain that can be separated from the enzyme by mild protease treatment
- When the 5'-3'exonuclease domain is removed, the remaining fragment large fragment or Klenow fragment retains polymerization and proofreading activities
- Klenow fragment is extensively used for molecular biology research

DNA Polymerase II

- Pol II is encoded by the pol B gene
- Pol II has 3′-5′ exonuclease activity and participates in DNA repair, and replication restart to bypass lesions, and its synthesis in cells can jump from ~30-50 copies per cell to ~200-300 during SOS induction.
- Pol II is thought to be involved in directing polymerase activity at the replication fork and helping stalled Pol III to bypass terminal mismatches

DNA Polymerase III holoenzyme

- DNA polymerase III holoenzymes is the primary enzyme complex involved in prokaryotic DNA replication.
- The complex has high processivity and proofreading capabilities that correct replication mistakes by means of exonuclease activity working 3'→5' direction
- DNA Pol III is a component of the replisome

The replisome is composed of the following:

2 DNA Pol III enzymes, each comprising α, ε, and θ subunits.

• the α subunit (encoded by the dnaE gene) has the polymerase activity.
• the ε subunit (dnaQ) has 3'→5′ exonuclease activity.
• the θ subunit (holE) stimulates the ε subunit's proofreading.

- 2 β units (dnaN) which act as sliding DNA clamps, they keep the polymerase bound to
the DNA.
- 2 τ units (dnaX) which act to dimerize two of the core enzymes (α, ε, and θ subunits).
- 1 γ unit (also dnaX) which acts as a clamp loader for the lagging strand Okazaki fragments, helps the two β subunits to form a unit and bind to DNA. The γ unit is made up of 5 subunits which include 3 γ subunits, 1 δ subunit (holA), and 1 δ' subunit (holB). The δ is involved in copying the lagging strand.

Replication origin

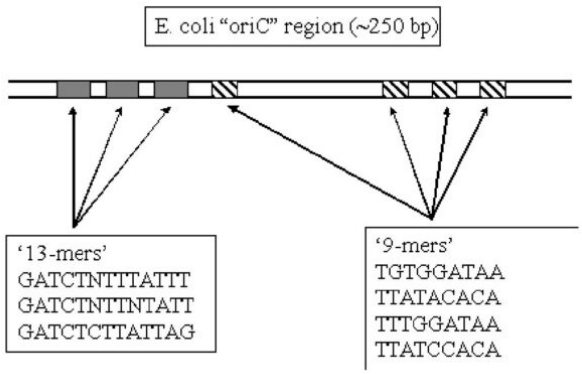
The E.coli replication origin, Ori C consists of 250bp, it bears DNA sequence elements that
are highly conserved among bacterial replication origins.

- Three repeats of a 13bp sequence (AT-rich)
- Four repeats of a 9bp sequence

In eukaryotic cells, the origin of replication is called autonomously replicating sequences (ARS) or replicators

Initiation of DNA Replication

- A complex of about 20 DnaA protein molecules, each with a bound ATP, binds to the four 9bp repeats.
- Then it specifically recognizes and denatures DNA in the region of three 13 bp repeats
- DNA C loads the helicase (DNA B). DNA B unwinds ori C and extends the single-stranded region for copying
- Single strand binding protein (SSB) binds to this single-stranded region to protect it from damage



- As the parental DNA is unwound, the resulting positive supercoiling (torsional stress) is relieved by topoisomerase II (DNA gyrase) by inducing transient single-stranded breaks
- The DNA primase then attaches to the DNA and synthesizes a short RNA primer to initiate synthesis.

Elongation

- DNA polymerase III extends the RNA primer made by primase Leading strand synthesis
  New DNA is made continuously in 5'-3'direction
- Lagging strand synthesis
- Multiple primers are synthesized at specific sites by primase and DNA pol III synthesizes short pieces of new DNA (Okazaki fragments)
- After DNA synthesis by DNA pol III, DNA polymerase I use its 5'-3' exonuclease activity to remove the RNA primer and fill the gaps with new DNA
- Finally, DNA ligase joins the ends of the DNA fragments together

Overview of DNA Synthesis

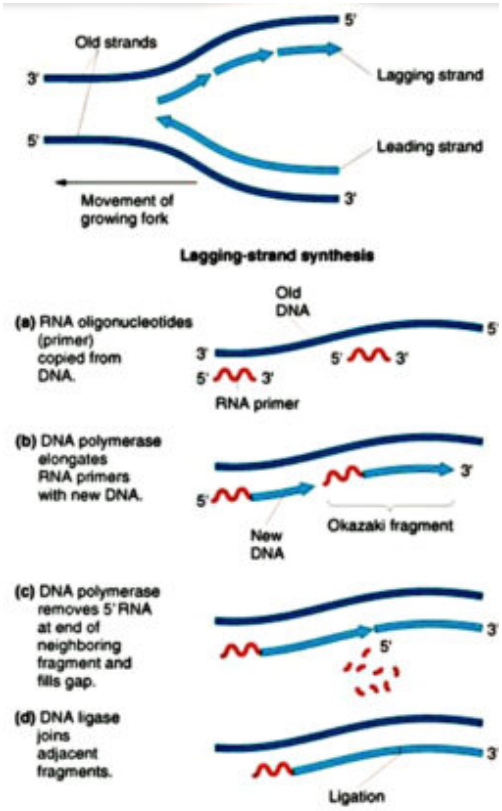
- DNA polymerases synthesize new strands in 5'-3' direction
- Primase makes RNA primer
- Lagging strand DNA consists of Okazaki fragments
- In E.coli, Pol I fills in gaps in the lagging strand and removes RNA primer
  Fragments are joined by DNA ligase

Termination

Termination requires that the progress of the DNA replication fork must stop or be blocked. Termination at a specific locus involves the interaction between two components:

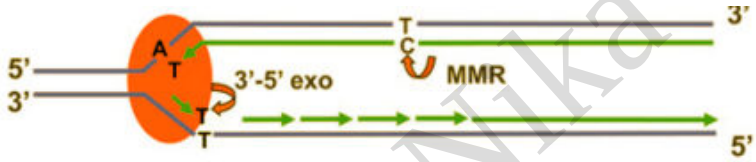
1. a termination site sequence in the DNA (Ter)
2. a protein that binds to this sequence to physically stop DNA replication

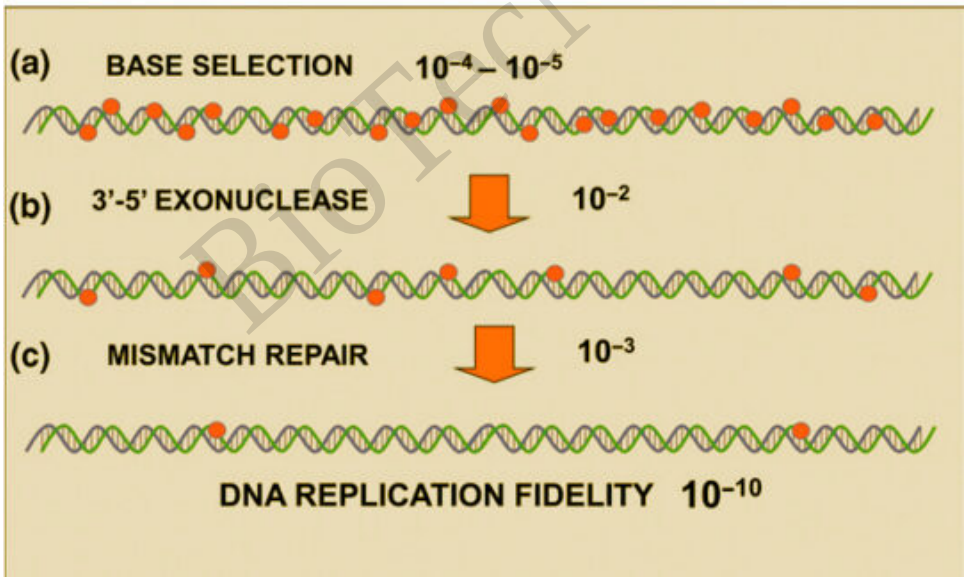
- tus (terminus utilizing substance) protein binds to Ter forming the Ter-Tus complex and
  stop replication fork completing 2 interlinked circular chromosomes (Catenated)
- DNA Topoisomerase IV separates the DNA



Replication fidelity

Important for preserving the genetic information from one generation to





another, spontaneous errors in DNA replication are very rare. e.g one error per 10^{-10} base in E.coli.

Molecular mechanisms for the replication fidelity

1. DNA polymerase: Base selection by Watson-Crick base pairing
2. 3'-5' proofreading activity
3. Mismatch repair

Detailed Notes on DNA Replication including Eukaryotic DNA Replication – Available which includes:

- Flow charts
- Koncept Tables
- Lecture Videos
- Question Discussion
- Doubt Solving

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