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Forewords
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JAYPEE

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27 CHAPTER

DNA Replication, Repair, Mutation and Cell Cycle

BC10.4	Describe in brief the major steps involved in DNA replication.
BC10.5	Describe the types of DNA repair, gene mutations and associated disorders.

ESSAY QUESTIONS

Q1. Discuss the steps involved in prokaryotic DNA replication. Add a note on eukaryotic DNA polymerase. (7+3)

Ans:

- DNA replication is a technique in which the ***DNA makes a copy of itself*** during the S phase of cell division.
- Two daughter DNAs are formed, and the process is semiconservative.
- Each daughter DNA has one parent or old strand and a complementary new strand.
- It maintains genetic stability, passing genetic information from parents to the offspring.

Steps involved in prokaryotic DNA replication

Initiation

A. **Two complementary DNA strands separation and unwinding of DNA**
- Replication starts at a **single** site or origin of replication (oriC in *E.coli*).
- This site is rich in **AT** sequences (**D**NA **U**nwinding **E**lement).
- **DnaA protein binds at the origin and locally opens the DNA.**
- Processive unwinding of DNA is carried by ***ATP-dependent DNA helicase*** (DnaB helicase + DnaC protein).
- ***ssDNA-binding proteins*** (SSBs) prevent premature reannealing of ssDNA to dsDNA.

B. **Replication fork formation (Fig. 27.1)**
- Unwinding and separation of two strands make a ***"V" shaped replication fork*** where active DNA synthesis occurs.
- Two replication forks run in opposite directions ***(bidirectional)*** from the origin to form ***a replication bubble***.
- Replication fork components are: ***(1) DNA helicase (2) SSBs (3) Primase and (4) DNA polymerase.***
- DNA strand separation creates a strain due to supercoiling.
- ***Positive supercoil:*** Formed due to overwinding of the DNA ahead of the replication fork.

- ***Negative supercoil***: Forms due to the underwinding of DNA behind the fork.
- The strain is solved by ***topoisomerases*** (type I and II). They cause temporary nicks and reseal the DNA strand.

C. **RNA primer**
- A **short length of RNA** (10–60 nucleotides long), synthesized by ***primase (DnaG)***.

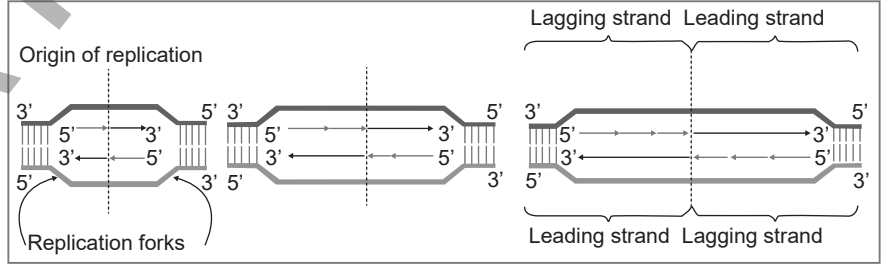

Fig. 27.1: Replication fork.

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- DNA polymerases cannot start the synthesis of a new strand complementary to the template strand.
- They ***require a free 3′-OH group*** to add the next nucleotide.
- Primer forms a complementary base pair to the template strand and provides the **free 3'-OH** group at its end.
- ***Only one primer is found at the origin of the leading strand, and many primers are required for the lagging strand (one for each Okazaki fragment).***
- The mobile complex between helicase and primase has been called a ***primosome***.

D. **The direction of DNA replication (Fig. 27.2)**

- **DNA polymerase III** can copy the parent strand in the 3'→5' direction and synthesize ***new DNA strands in the 5′→3′ direction***.
- DNA is continuously synthesized on the leading/forward strand toward the replication fork.
- On the ***lagging/discontinuous*** strand, DNA is synthesized in short (1,000–2,000 NTs long) or **Okazaki fragments**, away from the replication fork.
- Up to 1,000 fragments are synthesized and joined or ligated to become a single continuous strand.

Elongation

- Both the strands are synthesized in a **5'→3'** direction by highly processive **Pol III** enzyme.
- The replication fork moves at the rate of 1,000 nucleotides/second.
- Pol III adds 1st deoxyribonucleotide triphosphate (dNTP) to the 3'-OH of RNA primer with the release of PPi.
- The DNA strand is elongated by adding dNTP to the 3' end of the new strand and paired by the complementary base sequence of the template strand.
- **β clamp or sliding clamp binds to the Pol III enzyme and tethers it to the DNA template.**
- It enhances the ***PoI III processivity*** (dNTPs added before dissociation of polymerase from template ≥ 500,000) and the rate of chain elongation (200–1000 NTs/sec).
- PoI III elongates the primer in the lagging strand until another primer is encountered.
- Any mismatched base pairing can be corrected ***(proofreading) by the 3′→5′ exonuclease*** activity of DNA polymerases.
- ***RNA primer*** of the lagging strand is removed by the ***5′→3′ exonuclease*** activity of ***DNA PoI I***, and the gap is filled by its 5'→3' polymerase activity.
- **DNA ligase** (ATP dependent) seals the single-strand nick by forming a phosphodiester bond between the 3' OH and 5'-phosphate group.
- **Replisome** (a complex of helicase, primase, SSB, topoisomerase, Pol III, Pol I, and DNA ligase) is responsible for systematic DNA synthesis at a replication fork.

Termination

- Two replication forks meet at a terminus region containing multiple copies of **Ter (terminus)** sequences.
- The Ter sequences bind a Tus (terminus utilization substance) protein.
- The ***Tus-Ter complex can arrest a replication fork***.

– **Eukaryotic DNA polymerase**

Types	Function
1. DNA Pol α	**Primase** activity (DNA-dependent RNA polymerase) and synthesizes ***RNA primer***
2. DNA Pol β	Gap-filling and ***DNA repair***
3. DNA Pol γ	Replicate and repair the ***mitochondrial DNA***
4. DNA Pol δ	• Highly processive and synthesizes the ***lagging strand***. Proofreading exonuclease activity • Fills the gaps between Okazaki fragments following primer removal by **FEN1** (Flap endonuclease 1)
5. DNA Pol ε	Highly processive, synthesizes ***leading strand,*** and has proofreading (3′→5′ exonuclease) activity

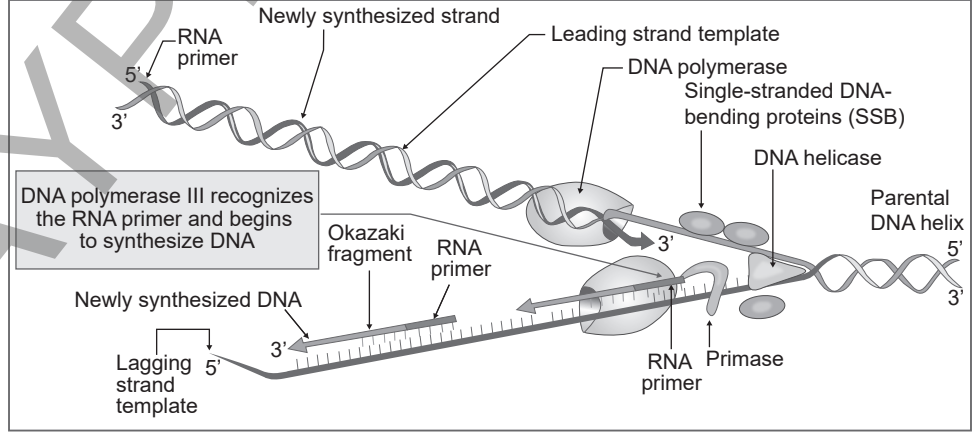

Fig. 27.2: DNA replication.

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- DNA Pol ε and δ are stimulated by **PCNA** (Proliferating cell nuclear antigen), which is a ***DNA clamp-like β subunit of PoI III that acts as a processivity factor***.

Q2. Describe the DNA replication in eukaryotes. How is it different from prokaryotic replication? (6+4)

Ans:

DNA replication in eukaryotes

The overall DNA replication features are similar in both eukaryotes and bacteria but more complex.

Steps involved in DNA replication in the eukaryotes:

A. Identification of the origin of replication
- ***Multiple ori*** (**ARS** or autonomously replicating sequences) present.
- About 80-bp **AT-rich** sequence (DNA Unwinding Element) found adjacent to ori facilitating DNA unwinding.

B. Unwinding/denaturation of DNA
- dsDNA is opened by the binding of **dnaA protein to Ori**, which facilitates further unwinding by the ***MCM (minichromosome maintenance)*** protein complex.
- ***RPA (Replication protein A)*** prevents the re-annealing of separated strands.
- Topological strain in the DNA can be relieved by topoisomerase.

C. Formation of the replication fork
- Unwinding and separating two strands forms a **"V"** shaped replication fork.
- Two replication forks move in opposite directions to form a ***replication bubble***.

D. Synthesis of RNA primer
- Primer is a short length (about 10 to 200 NT) of RNA required to initiate DNA synthesis.
- **DNA Pol α** has the primase activity.

E. Initiation and elongation of DNA synthesis
- ***The leading strand*** is continuously synthesized in the 5'→3' using the template strand in the 3'→5' direction.
- The enzyme involved in the leading strand synthesis is **DNA Pol ε**.
- Leading strand synthesis continues until it reaches the end of the chromosome or another replication fork progressing in the opposite direction.
- DNA is synthesized in short stretches called Okazaki fragments (100–250 NTs) on the lagging strand, each initiated by a different primer.

F. Removal of primers and gap filling of the lagging strand
- Primer is removed by **RNAse H**, and DNA PoI δ fills the gap.

G. Sealing the nick after gap filling: Mediated by **DNA ligase**.
- When the replication fork reaches the end of the linear chromosome, the primer for the DNA fragment to be copied at the end of the chromosome cannot be synthesized.
- These chromosomal ends thus remain unpaired and get shorter at the end of each cell division.
- ***Eukaryotic DNA replication is almost similar to prokaryotes but with some differences***

Property	Prokaryotic DNA replication	Eukaryotic DNA replication
Duration	About 30 minutes	About 9 hrs in human due to nucleoprotein
Origin of replication	**Only one**. oriC in *E.coli* ~245 bp and is **AT**-rich sequence	**Thousands. ARS** (Autonomously replicating sequence). **~** 80-bp **A+T**-rich sequence
Unwinding of DNA	**Helicase**	**MCM** (minichromosome maintenance) protein complex
Prevent re-annealing	**SSB protein**	**RPA** (Replication protein A)
Fork movement rate	1,000–2,000 nucleotides/s	~50 nucleotides/s
RNA primer	~50 nucleotides. **DNA G** is the primase	~10–200 nucleotides. **DNA Pol α** is the primase
Strand synthesis	**Pol III** synthesizes both the leading and lagging strand	**Pol ε** synthesizes the leading strand, and **Pol δ** synthesizes the lagging strand
Okazaki fragment	1000–2000 nucleotides	100–250 nucleotides
Primer removal and gap-filling	Pol I remove the primer. Pol I fill the gap	RNase H, FEN-1 remove primer, and ***Pol δ fills the gap in the lagging strand***
Termination	Mediated by **Tus-Ter** complex	Involves the synthesis of ***telomeres***
Reconstitution of chromatin structure	Absent	Present

Q3. Write down the steps of the initiation and elongation phase of the replication process in the *E. coli*. Differentiate among DNA polymerase I, DNA polymerase II, and DNA polymerase III. (6 +4)

Ans: Steps of the replication process in *E.coli* are already explained.

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Difference between DNA Pol I, DNA Pol II and DNA Pol III

	DNA polymerase I	DNA polymerase II	DNA polymerase III
3′→5′ exonuclease *(proofreading)* activity	Present	Present	Present
5′→3′ exonuclease *(degrade/excise primer)*	**Present**	Absent	Absent
Polymerization (5′→3′) rate (dNTPs/s)	10–20	40	250–1,000 **(Highest)**
Repair	**Present**	**Present**	Absent
Processivity	3–200	1,500	≥ 500,000 **(Highest)**
Major function	***Primer removal and gap filling on the lagging strand***	DNA proofreading and **repair**	Synthesis of leading strand and Okazaki fragments

Q4. Discuss DNA damage and repair mechanisms. Cite the examples of diseases occurring due to defective repair of DNA. (5+6+4 = 15)

Ans:

DNA damage

- Alteration in the chemical structure of DNA that occurs due to chemical, physical or environmental factors.
- Can be detected in the ***G1 or G2 phase*** of the cell cycle.
- If not repaired, it may lead to mutation and cancer or subject to apoptosis.
- DNA damage can be measured by **Comet Assay** or single cell gel electrophoresis (SCGE) assay.

Types of DNA damage and causative agents

Types of DNA damage	Causative agents (Mutagens)
A. Single-base alternation	
Loss of DNA base (AP)	Reactive oxygen species (ROS) and hydrolytic cleavage
Deamination of base (Ex. C→ U, A→ Hypoxanthine or G → Xanthine)	Deaminating agent (**nitrosamine**, **nitrate, and nitrite**) and hydrolysis
Alkylation of base	Alkylating agent (Nitrogen mustard, Dimethyl sulfate)
Base-analog incorporation	Bromouracil (BU) an analog of thymine
Oxidation of base	Reactive oxygen species
B. Two-base alternation:	
Adjacent **pyrimidine dimers**, usually (**T-T**) on the same strand	***Ultraviolet (UV) radiation***
Crosslink	Alkylating agent
C. Strand break	Ionizing radiation, X-ray, Oxygen radicals
D. Cross-linking of DNA (intrastrand or interstrand)	Oxidative stress, Malondialdehyde (MDA), Nitrogen mustard, Cisplatin

Repair mechanisms

- Recognition of DNA on the damaged site.
- Remove the damaged site, fill the gap left by excision using the sister strand as a template, and ligation.
- **DNA PoI I** (major enzyme) and **PoI II** are involved in prokaryotic DNA repair.

The mechanisms of DNA repair include:

A. Methyl-directed mismatch repair (MMR) (Fig. 27.3)

Recognize and repair insertion, deletion, and mis-incorporation of bases.

– **Identification of the mismatched strand:**
  - The correct or template strand is ***methylated*** on adenine at the GATC sequence.
  - Mut S and Mut L protein complexes can identify the mismatched strand.

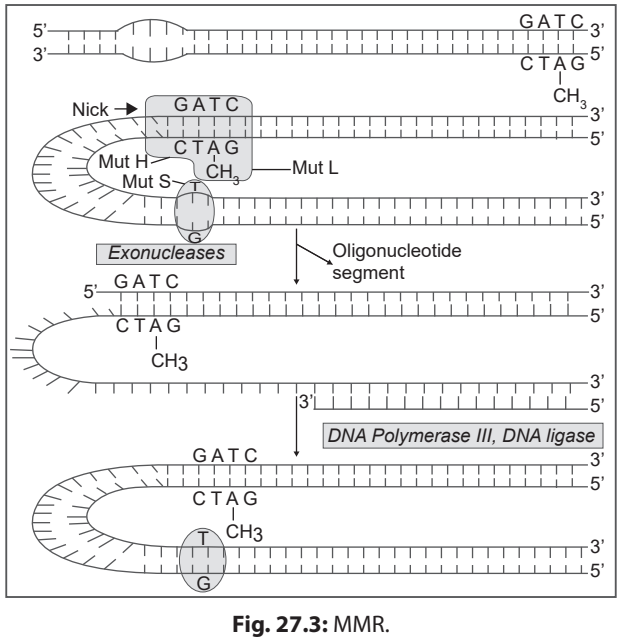

Fig. 27.3: MMR.

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- The complex binds to the mismatched base pair and activates **Mut H**, which is attached to the GATC sequence. A loop is created around the mismatched base.

– **Repair of damaged DNA**
- ***Mut H endonuclease*** cuts the unmethylated strand on the G of GATC, followed by removal of the strand towards the mismatch by a group of **exonucleases** (*Example:* UvrD helicase or DNA helicase II).
- The gap resulting is filled by a **DNA Pol III** and the nick is sealed by **DNA ligase**.

B. Base excision repair (BER)

Repair apurinic/apyrimidinic (AP) site, ssDNA break, oxidation, alkylation or deamination of the base.

1. **Removal of abnormal bases**
   Specific glycosylases can recognize and remove the abnormal bases from the sugar-phosphate backbone to form AP sites.
2. **AP site recognition and repair:**
   » Specific ***AP-endonucleases*** cleave the phosphodiester backbone near the AP site.
   » A deoxyribose phosphate lyase removes the single, base-free, sugar-phosphate residue.
   » ***DNA pol I*** removes a segment by 5'→3' exonuclease activity and later is replaced.
   » **DNA ligase** completes the repair process.

C. Nucleotide excision repair (NER) (Fig. 27.4)

– Repair of ***UV-induced pyrimidine dimer***, bulky adducts like benzo [a] pyrene-guanine or some alkylated bases.
– ***Dimer is recognized by UV-specific endonuclease (UvrABC excinuclease).***
– It cuts the damaged strand on both sides of the dimer.
– 12 to 13 dNTp fragment is removed by **UvrD helicase.**
– This gap is filled by **DNA Pol I**, and the nick is sealed by **DNA ligase.**

D. HR and NHEJ (Nonhomologous end-joining) repair

– DNA ***double-strand or single-strand breaks*** are repaired.
– Strand breaks are due to **ionizing radiation, X-ray, anti-tumor drugs (Ex. Doxorubicin) and oxidative stress**.

1. **Homologous recombination (HR) repair**
   » Active in the ***S, G2, and M*** phases of the cell cycle.
   » DNA loss is replaced by utilizing the ***homologous DNA*** as a template.
   » Enzymes involved in genetic recombination between homologous chromosomes during meiosis are used.
   » This system is much less error-prone.

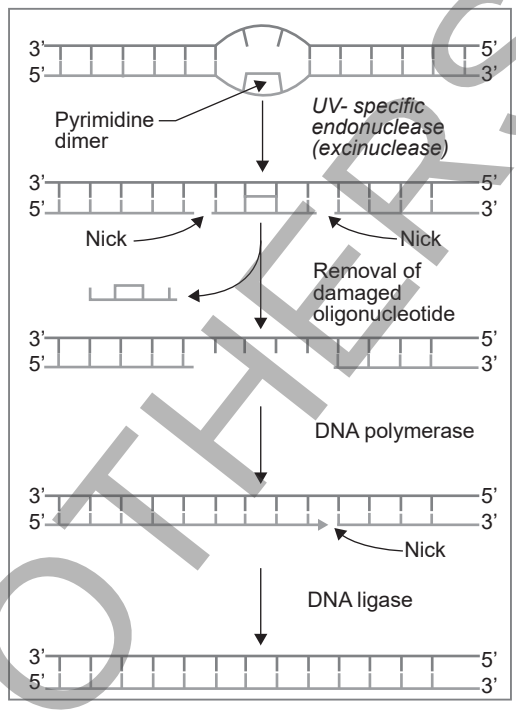

Fig. 27.4: NER.

2. **NHEJ repair**
   » Operated in **G0/G1** phases of the cell cycle.
   » Break ends are directly ligated using short homologous DNA sequences to guide the repair.
   » Some DNA is lost, and the repair is ***error-prone*** and mutagenic.

E. Direct repair

– Photoreactive **photolyase enzyme** can repair **thymine dimers** induced by UV light.
– It splits the dimer and restores the DNA to its original condition.
– The methyl group of O^6-methylguanine is removed by enzyme methyltransferase.

F. SOS repair

– Found in prokaryotes.
– It only constructs the chemical structure of DNA with the loss of hereditary information.

Diseases occurring due to defective repair of DNA

Defective DNA repair mechanism	Disease/s
NHEJ repair	***Severe combined immunodeficiency disease (SCID)***
HR repair	**AT-like disorder**, Bloom syndrome, Werner syndrome, **Breast cancer** susceptibility 1 and 2 (*BRCA1*, *BRCA 2* gene)

Contd...

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Contd...

Defective DNA repair mechanism	Disease/s
NER	***Xeroderma pigmentosum*** (XP), Cockayne syndrome (CS), Trichothiodystrophy (TTD)
Base excision repair	MUYTH–associated polyposis (MAP)
Mismatch repair: Human (h) *MLH1* and *hMSH2* genes	***Hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome*** ↑Risk of colorectal and other types of cancers like stomach, breast, pancreas, uterine and intestinal

CLINICAL CASE



Clinical Case

Q5. A 7-year-old boy presented with a photosensitive blistering skin lesion. He was diagnosed with Xeroderma pigmentosa. (10)

A. What is the molecular basis of this disease?
B. Name two agents causing DNA damage.
C. Enumerate different types of DNA repair mechanisms.
D. Name the prokaryotic DNA polymerase involved in DNA repair.
E. Write the roles of eukaryotic DNA polymerase.

Ans:

A. Xeroderma pigmentosa occurs due to mutation of genes that code for nucleotide excision repair (NER) proteins. ***Defective NER fails to repair the UV-induced pyrimidine dimer***. The patient is at high risk of developing skin cancer.

B, C, D, and E already explained.

Q6. Describe the modes of gene mutation. (7)

Ans:

- Mutation is any ***permanent change*** in the primary nucleotide sequence of DNA.
- They may be a somatic type or germline mutations.
- Most of the mutations are harmless, but they may lead to cell death or tumor formation.
- Causes are errors in DNA replication, recombination or repair, chemical mutagens, radiation, etc.
- The **Ames test** can assess the mutagenic potential of a chemical.

Modes of gene mutation

- **Mutations occur by base substitution**: ***Single-base change or point mutation***
  - May be **transition** (purine to purine or pyrimidine to pyrimidine) or **transversion** (purine to pyrimidine or vice versa).
  - Transition is more common.

Point mutation on the mRNA can lead to the following effects:

A. Silent mutation
- If a base is substituted in the ***third position*** of the codon, there may be ***no detectable effect*** of gene product.
- It is due to the ***degeneracy of the code*** (the same amino acid will be coded).
  Example: UCA→ UCU. Both code for serine.

B. Missense mutation
- A new codon with changed base codes a ***different amino acid*** forms a different polypeptide sequence.
- Depending on the location of the amino acid, it may be:
  - **Acceptable**: The new protein molecule functions as the normal one. Ex. **Hb Hikari**
  - **Partially acceptable:** The resulting protein has a ***partial but abnormal function***.
    Eample: Codon ***GAG→ GTG*** leads to the formation of ***HbS (β⁶ Glutamate →Valine)***.
    HbS binds O_2 but precipitates in a deoxygenated state, causing RBC to sickle.
  - **Unacceptable:** Resulted protein will not be normal. Ex. ***HbM Boston*** does not bind to O_2.

C. Nonsense mutation:
- Base substitution results in a ***stop codon.***
- It leads to translation arrest and mostly results in a nonfunctional protein.
  Example: ***Thalassemia*** and Duchenne muscular dystrophy (DMD)

- **Frameshift mutations**
  - ***Insertion or deletion of one or two*** nucleotides to the coding area of mRNA alters the reading frame.
  - Since the **code is non-punctuating**, the new protein either contains a thoroughly changed amino acid sequence or is truncated due to the appearance of a stop codon.
  - Addition/deletion of three nucleotides maintains the reading frame of code but can lead to severe pathology.
  - In ***cystic fibrosis (CF)***, deletion of three nucleotides results in **loss of phenylalanine at the 508th position** and forms abnormal CFTR protein.
- **Trinucleotide repeat (TNR) expansion:**
  - Occurs within the ***coding region*** of a gene, and the protein contains ***many extra copies*** of the same amino acid.
  - **CAG** codons (specify glutamine) repeat leads to ***Huntington's disease***.

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- **Splice site mutation:**
  - It leads to faulty splicing of pre-mRNA, and abnormal proteins are formed.
  - Gene silencing in ***myotonic dystrophy*** is due to altered splicing.
- **Promoter site mutation:** Leads to altered gene mutation.
- **tRNA suppressor mutation:** ***mutant tRNA*** (mutant anticodon) can suppress the mutation effect on mRNA.

SHORT ANSWER QUESTIONS

Q1. Replication fork in prokaryotes. (4)

Ans:

Replication fork in prokaryotes

- The replication fork is a key area of DNA replication.
- Following the unwinding and separation of two strands, a ***"V" shaped replication fork*** is formed.
- **The two replication forks form a replication bubble.** The movement speed is around 1000 NT/sec.
- ***Replication fork consists of four components:***
  1. **DNA helicase:** Opens DNA helix.
  2. **SSBs:** Prevent premature re-annealing of ssDNA to dsDNA.
  3. **Primase**: Forms a short length of RNA called RNA primer.
  4. **DNA polymerase:** DNA pol III initiates the leading/continuous strand in the same direction as the growing replication fork and the lagging/discontinuous strand in the opposite direction.

Q2. Okazaki fragments. (5)

Ans: Already explained.

Okazaki fragments (Fig. 27.5)

- DNA is synthesized **discontinuously** on the lagging (retrograde) strand.

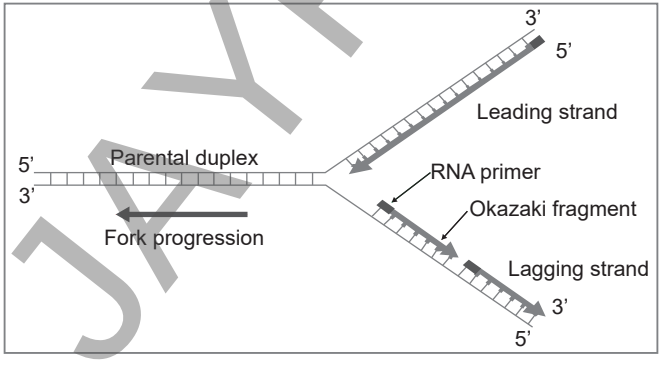

Fig. 27.5: Okazaki fragments.

- The lagging strand is synthesized away from the replication fork in a **5'→3' direction** utilizing one of the parent DNA strands as a template.
- Small fragments of DNA are added in short spurts up to thousands of times for each replication fork.
- Each fragment needs a ***separate primer*** and is synthesized by DNA PoI III in prokaryotes and DNA pol δ in eukaryotes.
- Finally, the primer is removed and the gap is filled by DNA PoI I in prokaryotes and DNA PoI δ in eukaryotes.
- They are finally joined by DNA ligase to become a single, continuous strand.

Q3. DNA polymerase I. (3)

Ans:

DNA polymerase I (Pol I)

- Discovered by ***Arthur Kornberg*** and also known as the ***Kornberg enzyme***.
- During the prokaryotic DNA replication, it connects the Okazaki fragments by deleting RNA primers and replacing the strand with DNA.
- Plays a vital role in ***DNA repair and recombination***.

Pol I

Enzymatic activities	*Function*
5′→3′ polymerase	DNA synthesis. The nucleotide addition rate is slow (10–20/second) with low processivity (3–200)
3′→5′ exonuclease	**Proofreading**
5′→3′ exonuclease	**DNA repair**. It is the chief DNA repair enzyme

Klenow fragment

- ***DNA PoI I lost its 5'→3' exonuclease (repair) domain.***
- However, it retains the polymerization and proofreading activity.

Q4. Name two inhibitors of DNA replication. (2)

- **Mitomycin** (antitumor agent) cross-links A and G on DNA strands and prevents unwinding.
- **6-mercaptopurine (6-MP)** inhibits purine synthesis.

Q5. Topoisomerase inhibitors work as antibacterial and anticancer agents. (4)

Ans:

Topoisomerases (nicking-resealing enzymes): They relieve torsional strain due to supercoiling during DNA replication and transcription.

- **Type I topoisomerase** transiently ***cuts single-stranded DNA*** in a negatively supercoiled DNA to relax, and then the cut strand is rejoined. **No ATP is required**.

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- **Type II topoisomerase *cuts both strands of double-helix DNA***, can insert and remove supercoils, and then rejoins the cut strands. They utilize ATP hydrolysis.

Antibacterial role: Fluoroquinolones (e.g., ciprofloxacin) and **nalidixic acid** are inhibitors of ***Topoisomerase II/DNA gyrase***. They prevent bacterial DNA replication and cell division.

Anticancer role: They inhibit the cell cycle in the S phase and cause DNA damage and apoptosis of cancer cells.

- **Human type I topoisomerase inhibitors: T**opotecan, Irinotecan, and Camptothecin
- **Human type II topoisomerase inhibitors: E**toposide, Doxorubicin, and Mitoxantrone

Clinical Case

Q6. An 8-year-old girl was presented to the pediatrics OPD with the signs of premature aging, i.e., coarseness of skin, alopecia, with normal intelligence. The provisional diagnosis was progeria.

A. What is a telomere? (3)
B. Explain its role in causing progeria. (2)

Ans:

i. Telomere
- It is a specialized region of **repetitive nucleotide sequences (5′-TTAGGG-3′)** at each end of a chromosome.
- These sequences interact with telomerase binding protein (TBP) and attain a looped conformation.
- It protects ends of chromosomes and prevents their fusion. It allows the proper replication of chromosomes.
- ***Telomeres shorten with each round of cell division (end replication problem) because DNA polymerase cannot synthesize the extreme 5′ end of the lagging strand. Telomere length is maintained by* telomerase.**
- When the telomere becomes too short, the chromosome cannot be replicated, which leads to cell death.
- Telomere shortening or aging is accelerated by stress, obesity, smoking, and pollution.
- Exercise, intake of fiber and antioxidants reduce the telomere shortening.

ii. Role of telomere in Progeria
- Abnormal protein **(progerin)** is formed due to a **mutation in the lamin A gene**.
- Their accumulation leads to destabilisation of the nucleus and shortening of telomeres.
- It leads to premature cell death and premature aging.

Q7. Some mutations are silent and even some are beneficial too. Explain with an example of each. (3)

A silent mutation results in the same amino acid sequence in a protein due to the degeneracy property of the genetic code. The protein performs the same biological function.

Example: UCA → UCU. Both code for serine.

Beneficial mutation: A genetic change that can provide advantages to the affected individuals.

Example: The sickle-cell trait (HbAS, one β-globin gene is normal (HbA), and other β-globin gene is HbS)

The individuals are usually asymptomatic, but the mutation confers **protection against severe malaria** caused by *P. falciparum*.

Q8. Frameshift mutation (3)

Ans:

Frameshift mutation

- Insertions or deletions (indels) of nucleotides in a DNA sequence that is not multiple of three.
- Thus, the reading frame is changed.
- The causative agent may be a chemical ***acridine or proflavin*** that intercalates (inserts) between the adjacent stacked DNA bases or the ***insertion of transposons***.
- This results in a completely different protein or premature translation.
- **Tay-Sachs disease** is caused by a frameshift mutation.

Q9. Point mutation with examples. (2)

Ans: Already explained.

Q10. Which enzyme catalyzes the synthesis of DNA from RNA? (1)

Ans: Reverse transcriptase

Reverse transcriptase (RT) or RNA-directed DNA polymerase

- Forms a complementary DNA (cDNA) from an RNA template by reverse transcription mechanism.
- RT is used by ***telomerase*** to extend the telomeres.
- Used by **retroviruses** (Ex. (HIV) and reverse transcriptase PCR **(RT-PCR)**.
- RTase activity is also seen with ***transposons***. Retrotransposons or retroposons are the transposons that involve an RNA intermediate.
- **Zidovudine (AZT)** is a thymidine analog and inhibits HIV reverse transcriptase.

Q11. Which type of DNA polymerase is required for leading strand synthesis in eukaryotes? (1)

Ans: DNA polymerase ε

Q12. Which type of DNA polymerase is Klenow fragment related to? (1)

Ans: DNA polymerase I

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Q13. Which DNA polymerase is involved in mitochondrial DNA synthesis?

Ans: DNA polymerase γ

Q14. _____ is known as Kornberg enzyme? (1)

Ans: DNA polymerase I

Q15. The moving complex formed by helicase and primase is known as _____. (1)

Ans: Primosome

Replisome: Contains primosome, DNA Pol and SSBP that creates a replication fork.

Q16. Okazaki fragments are formed during _____ synthesis. (1)

Ans: Lagging strand

Q17. Xeroderma pigmentosum is associated with DNA _____. (1)

Ans: Nucleotide excision repair defect

Xeroderma Pigmentosum (Autosomal Recessive Disorder)

- Patients are highly sensitive to **sunlight** and rapidly develop skin cancer.
- On the sun-exposed skin area, freckles and ulcerative lesions are found.
- Few manifest abnormal development, corneal ulcer, and progressive neurological degeneration.

Q18. Hereditary nonpolyposis colorectal cancer is due to defective _____. (1)

Ans: Mismatch repair

Q19. The shortest phase in the cell cycle is _____. (1)

Ans: M phase (1 Hour)

Cell cycle (Fig. 27.6)

The eukaryotic cell cycle consists of (a) interphase (G1, S, and G2 phases), followed by (b) mitotic phase (mitosis and cytokinesis), and (c) G_0 (quiescent) phase. The entire process takes about 24 hours.

- **Interphase:** The cell grows, and the DNA is duplicated.
  - **A. G1 (gap 1) phase**
    - ***Most variable phase*** and has a duration of **6–12 h**. Cell prepares for DNA synthesis in *G*1.
    - RNA, proteins, and the number of organelles are synthesized.
  - **B. S (synthetic) phase**
    - ***DNA replication*** doubles the number of DNA.
    - The centrosome is also duplicated, and the cell is prepared to divide by a mitotic process.

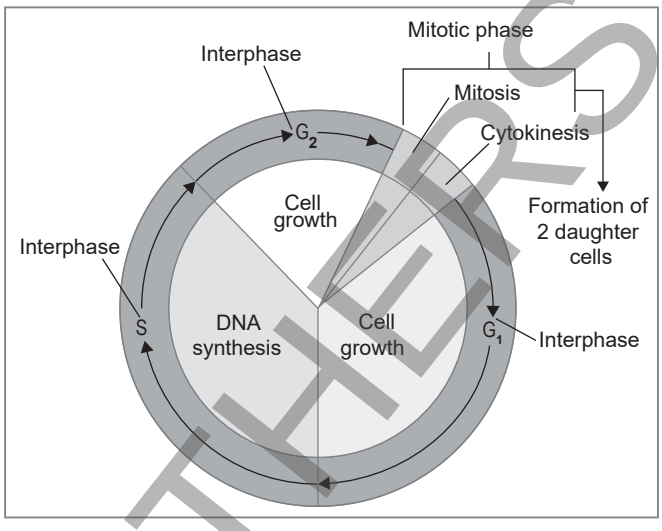

Fig. 27.6: Cell cycle.

    - RNA and proteins are synthesized slowly except histones. The duration is **6–8 h**.
  - **C. G2 (gap 2) phase**
    - Rapid cell growth and protein synthesis take place. Cell prepares for the mitosis.
    - The duration is **3–4 h**.
- **M phase**
  - It involves mitosis and cytokinesis.
  - The cell divides its copied DNA and cytoplasm into two daughter cells. The duration is **1 h**.
- **G_0 (Gap 0) or resting phase**
  - In higher eukaryotes, the **non-dividing cells** usually follow the quiescent G_0 from G_1 and may remain dormant for long periods, possibly lifelong (neurons).
  - When appropriate signals or conditions occur, cells re-enter G1 and progress normally.

Eukaryotic cell cycle regulation (cyclin and restriction/checkpoint) (Fig. 27.7)

- ***Cyclins and cyclin-dependent kinases*** (CDKs) can decide a cell's progress through the cell cycle.
- **Cyclin D** with ***CDK4 and CDK6*** complex rise in late ***G1*** Phase.
- Cyclin D-CDK complex phosphorylates the retinoblastoma (Rb) protein.
- It releases the transcription factor **(E2F)**, resulting in progression from the ***G1 to S phase (first restriction point)***.
- Cyclin E-CDK2 complex found in late *G*1.
- **Cyclin A-CDK2** is required to synthesize DNA in the ***S phase.***
- **Cyclin B-CDK1** complex is necessary for the ***G2/M transition (second restriction point)***.
- DNA damage can be detected at the G1 and G2 phases.

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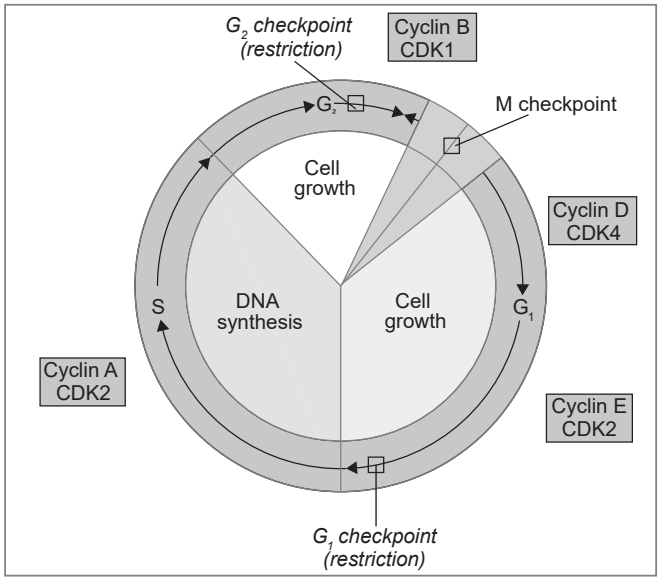

Fig. 27.7: Checkpoints.

Inhibitors affecting the cell cycle

A. Rb is a tumor suppressor protein. It is inactivated by oncoproteins produced by several DNA viruses.

B. Cyclin-CDK complex inhibitors

- p21, p27, and p57 proteins arrest cell cycle in the ***G1 phase***.
- They can bind and inactivate the cyclin-CDK complexes.

Q20. **DNA replication occurs in _____ phase of the cell cycle. (1)**

Ans: S (Synthetic)

Q21. **DNA repair occurs in _____ phase of the cell cycle. (1)**

Ans: **G1**. DNA repair occurs in both G1 and G2 phases. **G1/S and G2/M are checkpoints of DNA damage**.

Q22. **The proteins that regulate the cell cycle are _____. (1)**

Ans: Cyclins and cyclin-dependent kinases (Cdks)

Q23. **Fluoroquinolone inhibits initiation in transcription in prokaryotes. (T/F) (1)**

Ans: (F). Fluoroquinolone (Ciprofloxacin) inhibits the bacterial DNA gyrase.

Q24. **Sickle cell disease is an example of a point mutation. (T/F) (1)**

Ans: (T)

Q25. **Mutagenicity of chemicals can be detected by Ames assay. (T/F) (1)**

Ans: (T)

MULTIPLE CHOICE QUESTIONS (MCQs)

Q26. **An accurate DNA replication requires all the following, *except:* (1)**

a. DNA ligase
b. A free 5′ OH
c. The 3′→5′ exonuclease activity
d. Topoisomerase activity

A free 3′ OH end is required for polymerase activity.

Q27. **Fidelity of DNA replication is assured by: (1)**

a. Complementary base pairing
b. Phosphodiester bond formation
c. Specific nucleotide recognition
d. Specificity of DNA polymerase

DNA replication fidelity is determined by DNA polymerase activity (nucleotide selectivity and proofreading).

Q28. **A mutation that converts an amino acid codon to a stop codon is a:**

a. Nonsense mutation
b. Silence mutation
c. Frameshift mutation
d. Missense mutation

Q29. **Sickle cell hemoglobin is due to:**

a. Nonsense mutation
b. Silent mutation
c. Missense mutation
d. Frameshift mutation

Q30. **Base excision repair is primarily involved in repairing the DNA alterations caused by: (1)**

a. Spontaneous deamination of bases
b. Chemical agents and oxidative stress
c. Replication errors during DNA strand break
d. Ultraviolet radiation

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Q31. **All are due to defective DNA repair, *except:* (1)**
- **a. Xeroderma pigmentosum**
- **b. Fanconi's anemia**
- **c. Ataxia telangiectasia**
- **d. Cystic fibrosis**

Q32. **When GAC is replaced by GAA, the result is (1)**
- **a. Frameshift mutation**
- **b. Deletion mutation**
- **c. Silent mutation**
- **d. Conservative mutation**

This mutation incorporates glutamate instead of aspartate. Both are acidic amino acids.

Answers:

26. b	27. d	28. a	29. c	30. b
31. d	32. d			

SUGGESTED READING

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Extra Points

♦ **Features of DNA replication**
- Each separated strand is a template for synthesizing a complementary daughter (new) strand.
- Replication is semiconservative.
- New strands are always synthesized in a **5' to 3' direction** and require **RNA primers.**
- One stand is a continuous or leading strand, and another is discontinuous or lagging.

♦ **Topoisomerases (nicking resealing enzymes)**
- Relieve the ***torsional strain*** due to supercoiling during DNA replication and transcription process.
- They bind to DNA and cut the phosphate backbone of the DNA strands by their endonuclease activity.
- This intermediate break allows the DNA to be unwound, and the DNA backbone is resealed (nick resealing) again by ligase activity.
- **Type I** topoisomerase transiently cuts ***single-strand DNA*** in a negatively supercoiled DNA to relax and then the cut strand is rejoined. ***No ATP is required.***
- **Type II** topoisomerase cuts ***both strands*** of double-helix DNA, can insert and remove supercoils and then rejoins the cut strands. ***They utilize ATP hydrolysis.***

Important topoisomerases

Type	*Family*	*Domain(s)*	*Function*
IA	Topoisomerase **I**	Bacteria, eukaryotes	Relaxes negatively supercoiled DNA (-)
	Topoisomerase III	Bacteria, eukaryotes	Relaxes (-)
IIA	Topoisomerase **II (DNA gyrase)**	**Bacteria**	***Introduces negative supercoils (ATPase)***
	Topoisomerase **II**	**Eukaryotes**	**Relaxes (+ or -)**
	Topoisomerase IV	Bacteria	Decatenase

Topoisomerase inhibitors:
- Bacterial topoisomerase inhibitors: **Fluoroquinolones** and nalidixic acid.
- Human type **I** topoisomerase inhibitors: **Topotecan**, Irinotecan, and Camptothecin.
- Human type **II** topoisomerase inhibitors: **Etoposide**, Doxorubicin, and Mitoxantrone.

♦ **Inhibitors of DNA replication are:**

A. ***Directly interacting with DNA:***
- **Acridine**, ethidium, and doxorubicin are ***inserted (intercalated)*** between the stacked bases of the DNA duplex and prevent the DNA from acting as a template.
- **Mitomycin** (antitumor agent) ***cross-links*** A and G on DNA strands and prevents unwinding.

B. ***Inhibitors of deoxyribonucleotide synthesis or antimetabolites:***
- **6-mercaptopurine (6-MP)** inhibits purine synthesis, and **5-fluorouracil (5-FU)** inhibits thymidylate synthesis.
- **Cytarabine** is a nucleoside analog that inhibits DNA replication.
- **Sulfonamides** inhibit the synthesis of both purine and pyrimidine nucleotides.

C. ***Topoisomerase inhibitors: Topotecan, Camptothecin, and Doxorubicin***
- **Ames test**
  - » It can assess the mutagenic/carcinogenic potential of chemical compounds.
  - » It utilizes ***Salmonella typhimurium***, has a mutated histidine gene, is incapable of histidine synthesis (His -), and needs histidine in growth media.
  - » Culturing His - strain in media containing **mutagen** causes a mutation in the histidine encoding gene, making it **His +**. They can grow in a histidine-free medium.
  - » This is called ***a reverse mutation***.
- ***Other methods to detect mutation are:***
  - » **Cytogenetic analysis and FISH:** For structural abnormalities of chromosomes.
  - » **Southern blot:** Can detect large insertions, deletions, or rearrangement of nucleotides.
  - » **PCR:** Can detect the expansion of triplet repeats, gene rearrangements, and translocations.
  - » **RT-PCR**: Analyzes cDNA sequence.
  - » **RFLP and Microarray:** Point mutation, small insertions, and deletions.

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