DNA Replication
Mutation during Replication
&
It’s Repair

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Surat
The mammalian cell cycle

- **G0** phase: Quiescent cells
- **G1** phase: Rapid growth and preparation for DNA synthesis
- **S** phase: DNA synthesis and Histone synthesis
- **G2** phase: Before division, Cell Grow & preparation for cell division
- **M** phase: Mitosis

After division, Cell either go into G0 or G1

DNA synthesis and Histone synthesis
DNA replication is semi-conservative

Parental DNA strands

Each of the parental strands serves as a template for a daughter strand

Daughter DNA strands
Replication
A. Origin of replication

1. Local opening of double helix
2. Replication fork
3. Bidirectional replication continues

B. Multiple origins of replication
Initiation of DNA synthesis at the *E. coli* origin (ori)

origin DNA sequence

binding of dnaA proteins

DNA melting induced by the dnaA proteins

dnaA proteins coalesce

dnaB and dnaC proteins bind to the single-stranded DNA

dnaB further unwinds the helix
Dna A protein:
- Bind at the origin of replication
- Binds to specific nucleotide sequences
  - at AT-rich regions.
- ATP-dependent
- Strand separation
- Formation of localized ssDNA.

DNA helicases:
- Bind to ssDNA near replication fork
- Unwind double helix.
- ATP energy dependent
**Single-stranded DNA-binding (SSB) proteins:**

- Bind to the ssDNA
- Bind cooperatively
  - binding of one SSBP makes easier for another SSBP to bind tightly.
- Keep two strands of DNA separated
- Protect DNA from nucleases activity that cleave ssDNA.
DNA helicase unwinds the double helix.

Direction of movement of replication fork

Single-stranded DNA-binding proteins keep the two strands of DNA separate.
The DNA polymerases

= Copying the DNA templates
= Read parental sequences in the 3′→5′ direction
= Synthesize new DNA strands in the 5′→3′ (antiparallel)
**Leading strand:**
- Synthesized in direction of replication fork.
- Synthesized *continuously*.

**Lagging strand:**
- Strand that synthesized in the direction away from the replication fork.
- Synthesized *discontinuously*
- Synthesized in small fragments of DNA
- “*Okazaki fragments*”
- joined to become a single, continuous strand.
RNA primer

- DNA polymerases cannot initiate replication on a totally single-stranded template.
- Require an RNA primer
- Short chain of RNA base-paired.
- With free hydroxyl group on 3’-end of RNA strand.
- This hydroxyl group serves as the first acceptor of a nucleotide by action of DNA polymerase.
**Primase:**
- Synthesizes short of RNA (approx. 10 nucleotides)
- Complementary and antiparallel to DNA template.
- U in RNA pairs with A in DNA.
- On lagging strand = Multiple RNA primers
- On leading strand = Only one RNA primer require.

**Primosome:**
- The primosome makes the RNA primer.
- As with DNA synthesis, the direction of synthesis of the primer is 5’→3’ (antiparallel to the template strand).
DNA polymerase III recognizes the RNA primer and begins to synthesize DNA.
Chain Elongation

- DNA polymerases 5′→3′ direction elongate a new DNA strand.
- Add deoxyribonucleotides, one at a time, to the 3′-end.
- New strand grows in the 5′→3′ direction, antiparallel.
- DNA polymerase III is a highly "processive" enzyme:
  - Remains bound to template strand as it moves along.
  - β subunit forming a ring with template strand.
  - As a sliding DNA clamp.
- With each nucleotide add Pyrophosphate (PP<sub>i</sub>) is released.
- All four deoxyribonucleoside triphosphates (dATP, dTTP, dCTP, and dGTP) are required.
Proof-Reading of new DNA

- Misreading of template sequence make in deleterious or mutations.
- To ensure replication fidelity,
- DNA polymerase III 3′→5′ exonuclease has addition “Proofreading” activity.
- 3′→5′ exonuclease removes misplaced nucleotide.
- Than 5′→3′ polymerase then replaces it with correct nucleotide.
DNA double helix

Strand separation

Positive supercoiling
Properties of Topo-isomerase (Gyrase)

- Relieve supercoiling in downstream of DNA during replication by making break in strand & again reseal it.
- Have both action of Nuclease & Ligase
- **Type – I** = act by making break in one strand
  - Break require energy, resealing does not require energy
- **Type – II** = act by making break in both strands.
  - Breaking & Resealing both require energy.
- **Antibiotics** = Ciprofloxacin, Nalidixic acid inhibits bacterial Gyrase.
- **Anti-tumour agents** = Etoposide, Adriamycin, Doxorubicin inhibits eukaryotics topo-isomerase.
1. The left half of the circle folds over the right half.

2. The back half of the helix is cleaved.

3. The front half of the helix passes through the break, which is resealed.
**A. Polymerase Function**

An incoming nucleoside triphosphate is correctly matched to its complementary base on the DNA template and is added as the monophosphate to the growing DNA chain.

**B. Proofreading Function**

If DNA polymerase mispairs a nucleotide with the template, it uses its 3'→5' exonuclease activity to excise the mismatched nucleotide.
Excision of RNA primers and their replacement by DNA

- DNA polymerase III continues to synthesize DNA on the lagging strand until it is blocked by proximity to an RNA primer.
- DNA polymerase I excise RNA and fill the gap.

- DNA polymerase III = 5′→3′ polymerase activity that synthesizes DNA
  = 3′→5′ exonuclease activity that proofreads
- DNA polymerase I = 5′→3′ exonuclease activity, hydrolytically remove the RNA primer.
  = 5′→3′ polymerase activity.
  = 3′→5′ exonuclease activity that proofreads
**DNA polymerase I**
- locates space
- between 3’-end of New DNA & 5’-end of adjacent RNA primer.
- Hydrolytically removes RNA.
- Make $5'\rightarrow 3'$ exonuclease activity.
- Than, $5'\rightarrow 3'$ polymerase activity to fill Gap by synthesis of new DNA.
- $3'\rightarrow 5'$ exonuclease activity to make “proofreads”.
Endonuclease versus exonuclease activity

- **Exonuclease** = Remove one nucleotide at a time from the end of the DNA chain
- **Endonuclease** = Remove the chain Internally.

Dr. Payush Tailor
Differences between 5′→3′ & 3′→5′ exonucleases

- **3′→5′ exonuclease**
  - Remove nucleotides in the 3′→5′ direction
  - Remove one nucleotide at a time.
  - Important in proof reading

- **5′→3′ exonuclease**
  - Remove groups of altered nucleotides in the 5′→3′ direction
  - Removing from one to ten nucleotides at a time.
  - Important in repair of damaged DNA
Removal of RNA primer and filling of the resulting “gaps” by DNA polymerase I.
Telomere & Telomerase

1. Telomerase extends the 3'-end of the DNA.

2. RNA primer is synthesized by primase.

3. The 3'-end of the RNA serves as a primer for DNA polymerase.

4. RNA primer is removed.

Newly synthesized strand with terminal RNA primer removed.

DNA polymerase

Primase

Telomerase
Telomere

- Gap at extreme 5′-end of the lagging strand
- After removal of RNA primer
- This End is protect with proteins.
- The DNA–protein complex is termed “Telomere”.
- Consists of tandem repeats of AGGGTT
In normal somatic cells, telomeres shorten with each successive cell division.

If shortened beyond some critical length, the cell cannot survive.

In germ cells, other stem cells & in cancer cells:
- Telomeres do not shorten
- So the cells survival is longer.
Telemerase

- Enzyme = Ribonucleoprotein (Telomerase)
- Maintain length.
- Reverse transcriptase.
- Make RNA template to DNA 5′→3′
- Lengthen GT-rich strand
- Than Primase can synthesize an RNA primer.
- Than RNA primer is extended by DNA polymerase and make de novo DNA synthesis
Telomere Significant

- Mitotic clock.
- Providing information of aging and cancer.
Reverse transcriptase

- Replication of retroviruses
- Human Immunodeficiency Virus (HIV).
- Viruses carry their genome in form of ssRNA.
- Following infection of a host cell,
  - Viral enzyme uses the viral RNA as a template for the 5′→3′ synthesis of viral DNA.
  - Than Viral DNA integrated into host chromosomes.
- In eukaryotes, such elements are transcribed to RNA.
Eukaryotic DNA polymerases

- Five key eukaryotic DNA polymerases identified.

- Pol α and pol Δ:
  - **Pol α** is a multisubunit enzyme.
    - One subunit has primase activity,
  - **Pol Δ**
    - Elongation of DNA on the leading strand and elongate
    - 3′→5′ exonuclease activity to proofread the newly synthesized DNA.
    - Associates with the protein, proliferating cell nuclear antigen, which serves as a sliding DNA clamp in much the same way the β subunit of DNA polymerase III does in E. coli.

- **Pol β** and **pol ε** are involved in DNA repair.

- **Pol γ** replicates mitochondrial DNA.
Inhibition of DNA synthesis by nucleoside analogs

- Conversion of the deoxyribose to another sugar as in Arabinose, prevents further chain elongation.
- Cytosine arabinoside = Anticancer chemotherapy.
- Adenine arabinoside = Antiviral agent.
- Zidovudine (AZT) = Modifying the sugar. = termination of DNA elongation. = Use in AIDS
Drugs Structural Analogue to Nitrogen base

AZT (zidovudine)

Thymidine (naturally occurring nucleoside)

2'-3'-Dideoxyinosine, (ddl, didanosine)

Deoxyadenosine (naturally occurring nucleoside)
Can Zidovudine affect human cellular DNA replication?
DNA Damage & DNA Repair
a) 1) Guanine
   - Original base
   - Mutagen: Nitrous acid (HNO₂)
   - Modified base: Xanthine
   - Pairing partner: Cytosine
   - Predicted transition: None

2) Cytosine
   - Original base
   - Mutagen: Nitrous acid (HNO₂)
   - Modified base: Uracil
   - Pairing partner: Adenine
   - Predicted transition: CG → TA

3) Adenine
   - Original base
   - Mutagen: Nitrous acid (HNO₂)
   - Modified base: Hypoxanthine
   - Pairing partner: Cytosine
   - Predicted transition: AT → GC
<table>
<thead>
<tr>
<th>Original base</th>
<th>Mutagen</th>
<th>Modified base</th>
<th>Pairing partner</th>
<th>Predicted transition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytosine</strong></td>
<td>Hydroxylamine (NH$_2$OH)</td>
<td>Hydroxylaminocytosine</td>
<td>Adenine</td>
<td>CG → TA</td>
</tr>
<tr>
<td><strong>Guanine</strong></td>
<td>Methylmethane sulfonate (MMS) (alkylating agent)</td>
<td>O$_6$-Methylguanine</td>
<td>Thymine</td>
<td>GC → AT</td>
</tr>
</tbody>
</table>
Deamination

a) Cytosine → Uracil

b) 5-methylcytosine $\left(5^{m}C\right)$ → Thymine (T)
Thymine Dimer
DNA Damage

I. Single-base alteration
   A. Depurination
   B. Deamination of cytosine to uracil
   C. Deamination of adenine to hypoxanthine
   D. Alkylation of base
   E. Insertion or deletion of nucleotide
   F. Base-analog incorporation

II. Two-base alteration
   A. UV light–induced thymine-thymine (pyrimidine) dimer
   B. Bifunctional alkylating agent cross-linkage
DNA Damage

III. Chain breaks
   A. Ionizing radiation
   B. Oxidative free radical

IV. Cross-linkage
   A. Between bases in same or opposite strands
   B. Between DNA and protein molecules (e.g., histones)
Mechanisms of DNA Repair

1. Proofreading by the DNA polymerases
2. Mismatch (post-replication) repair
3. Base Excision repair
4. Nucleotide Excision repair
Mismatch (Post-replication) repair

- Parental DNA are methylated on certain adenine bases.
- **GATC** found approx. once every thousand nucleotides.
  - Are methylated on adenine.
Mut proteins

- Mut S
  - Scans DNA
  - Recognize mismatch base

- Mut L
  - Links Mut S & Mut H
  - Activates Mut H

- Mut H
  - Binds to hemi methylated GATC sequence
Mismatch Repair

Figure 28.36
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Repair of Mismatch DNA damage

- Mismatch is identified
- Endonuclease nicks the strand
- Exonuclease remove Mismatched nucleotide(s).
- Additional nucleotides at the 5′- and 3′-ends are also removed.
- DNA polymerase & DNA ligase fill the gap.
- E.g. = Hereditary Nonpolyposis Colorectal Cancer (HNPCC) (Lynch syndrome).
Thymine Dimer due to UV light

Pyrimidine Dimer
Mismatch repair for Thymine Dimer due to UV light

- **Dimer = Thymine dimer**
- Obstruct DNA polymerase
- Inhibit DNA replication
- **UV-specific endonuclease (uvrABC excinuclease)**
  - Recognition and excise dimer
  - Dimer containing short oligonucleotide removed.
  - Gap is filled same repair as mismatch repair.
UV radiation and cancer

- Xeroderma Pigmentosum
- Skin cancer
- Due to exposure to unfiltered sunlight.
- Defect in "UV-damage repair mechanism."
1. Pyrimidine dimer
2. UV-specific endonuclease (excinuclease)
3. Nick
4. Removal of damaged oligonucleotide
5. DNA polymerase
6. Nick
7. DNA ligase
Microsatellite instability (MSI)

(a) Increase in repeat length

(b) Decrease in repeat length
Mismatch Repair for Microsatellite instability (MSI)

- Microsatellites = repeated dinucleotide “CA”
- DNA polymerase slips out these sequences
- Forms loop
- If defects in MMR repair process
  - Increase in length of DNA
  - Decrease in length of DNA
- Corrected by MMR and NER mechanism
Base Excision Repair

- **Deamination** type of damage is repaired by Base excision repair.

- **Removal of abnormal bases only:**
  - Deamination convert Cytosine = Uracil
  - N-Glycosidic bond break first
  - Specific AP-endonucleases
  - Recognition AP site = Missing base
  - Hydrolytically cleave nitrogen base.
  - Initiate the process of excision.
  - Remove Deoxyribose phosphate
  - Than Polymerase & Ligase complete repair
Excision repair

Deamination

ATGC\textcolor{blue}{U}GCAT\textcolor{blue}{T}\textcolor{blue}{T}GA

TACGGCGTAACT

uracil DNA glycosylase

ATGC GC\textcolor{blue}{A}TT\textcolor{blue}{T}GA

TACGGCGTAACT

repair nucleases

AT GC\textcolor{blue}{A}TT\textcolor{blue}{T}GA

TACGGCGTAACT

DNA polymerase \(\beta\)

ATG\textcolor{blue}{C}CGCGAT\textcolor{blue}{T}TGAG

TACGGCGTAACTATC

DNA polymerase \(\beta\)

ATGCCGC\textcolor{blue}{A}TT\textcolor{blue}{T}\textcolor{blue}{T}GA

TACGGCGTAACTATC

DNA ligase

ATG\textcolor{blue}{C}CGCGAT\textcolor{blue}{T}TGAG

TACGGCGTAACTATC

DNA ligase

ATGCCGC\textcolor{blue}{A}TT\textcolor{blue}{T}GA

TACGGCGTAACTATC

Base excision repair

Nucleotide excision repair
Repair Of Double Strand Break

- Occur due to High-energy radiation or oxidative free radicals
- Potentially lethal
- **Non-Homologous End-joining Repair (NHER)**
  - Error prone and mutagenic.
  - Very low fidelity
  - Defects in this repair system
    - Severe immunodeficiency syndromes & Cancer
- **Homologous recombination repair (HR)**
  - Less error
  - Higher fidelity
DSB Formation

End Processing

Joint molecule formation (D-loop)

Repair DNA synthesis (Srs2)
Resolution of Intermediates (Srs2)
Ligation
Mature Recombinants

Rad50, Mre11, XRS2,
Rad51, Rad52, Rad55/Rad57, Rad54, (Srs2)
Homologous Recombination

Two originals

and

Two recombined

Defects in DNA repair or replication

**Xeroderma pigmentosum**
- Mutations in genes in *nucleotide excision repair*
- >1000-fold increase of sunlight-induced skin cancer

**Ataxia telangiectasia**
- Defect in gene that *detects DNA damage*
- Increased with exposure to X-ray
Defects in DNA repair or replication

- **Fanconi anemia**
  - caused by a gene involved in DNA repair
  - increased risk of X-ray and sensitivity to sunlight

- **Bloom syndrome**
  - caused by mutations in a DNA helicase gene
  - increased risk of X-ray
  - sensitivity to sunlight

- **Cockayne syndrome**
  - caused by a defect in transcription-linked DNA repair
  - sensitivity to sunlight

- **Werner’s syndrome**
  - caused by mutations in a DNA helicase gene
  - premature aging
DNA damage
Cell cycle abnormalities
Hypoxia

[Diagram showing:
- mdm2
- p53

Leading to:
- p53

Which results in:
- Cell cycle arrest
  - DNA repair
  - Cell cycle restart
- Apoptosis
  - Death and elimination of damaged cells

Leading to:
- Cellular and genetic stability]
p53

Function
• Role in apoptosis, genomic stability
• Anti-cancer role

Mechanism
• Activate DNA repair proteins
• Arrest growth by holding the cell cycle at G\textsubscript{1}/S
• Hold cell here for long enough
• DNA repair proteins get time to repair
• Otherwise
• Initiate apoptosis, the programmed cell death, if DNA damage proves to be irreparable.
p53

- p21 (WAF1) binds to the G1-S/CDK (CDK2)
- CDK important for the G1-S transition in the cell cycle
- $p21 + \text{G1-S/CDK (CDK2)}$ complex inhibiting their activity.
- Cell cannot continue to the next stage of cell division.
DNA Structure

- **Consists of**
  - Deoxyribose
  - Phosphate in a diester linkage
  - Bases: Adenine, Thymine, Cytosine, Guanine

- **Forms a double-stranded helical molecule**
  - Antiparallel orientation
  - Polarity: 5'-End to 3'-End

- **In the form of deoxyribonucleotides**

- **Hydrogen bonds between base pairs**
  - Adenine with Thymine
  - Cytosine with Guanine

DNA Replication

- **Replication fork**
- **Local separation of strands**
- **DNA polymerases**

- **New DNA strands**
  - 5' → 3' direction
  - Resulting in two complementary daughter strands

- **Bidirectional elongation**
  - Involving both strands
  - Templating for constructing semiconservative replication

- **Helicase**
- **Single-strand DNA-binding proteins**
- **Positive supercoils**
- **DNA topoisomerases**
- **RNA primer**
- **Primase**

- **Replication as a result of single-stranded DNA**

DNA Repair

- **DNA damage**
  - **Excision repair**
    - UV-induced thymidine dimers
    - Mismatched bases
    - Base alterations
  - **NHEJ or HR repair**
    - Double-stranded breaks

- **Corrected by**
  - Imperfect proofreading
  - Normal chemical changes
  - Environmental insults
The RNA polymerase that produces the primer necessary for DNA synthesis is called .

a. polymerase
b. helicase
c. primase
d. ligase
An enzyme that form a covalent bond between adjacent 5'-P and 3'-OH termini of separate fragments of DNA is

a. convertase
b. primase
c. ligase
d. topoisomerase
An enzymes that breaks & than seal the break of DNA strand to remove underwinding or overwinding of the DNA helix is

a. helicases
b. DNA polymerase
c. topoisomerases
d. ligases
Proof reading activity of DNA polymerase refers to

a. 5’ to 3’ exonuclease activity
b. 5’ to 3’ polymerase activity
c. 3’ to 5’ exonuclease activity
d. 3’ to 5’ polymerase activity
• What is false about DNA Polymerase I?
  a. 5’ to 3’ polymerase activity
  b. 5’ to 3’ exonuclease activity
  c. 5’ to 3’ proof reading activity
  d. None.
Arabinose (analogue of deoxyribose) is
a. Use as antiviral and anticancer drug
b. Use to inhibit replication.
c. Use as anti-diabetic agent.
d. a & b.
Which of the following is true about DNA topoisomerase

a. It unwinds DNA.
b. It always break both strand of DNA
c. It produces positive supercoiling.
d. None
The 3' end of each Okazaki fragment is joined to the 5' end of the next fragment by

a. DNA Polymerase I & DNA ligase
b. DNA Polymerase III & DNA ligase
c. DNA ligase
d. DNA Polymerase I
• Topo isomerase enzyme is inhibited by antibiotic
  a. Ciprofloxacin
  b. Adriamycin
  c. Doxorubicin
  d. Amoxycillin
- During mismatch repair, parent DNA strand is identified by its:
  a. Ribosylation
  b. Hydroxylation
  c. Methylation
  d. Phosphorylation
• Error during DNA replication can be corrected by
  a. DNA ligase
  b. Primase
  c. DNA Polymerase
  d. Topoisomerase
• All of the following is a tumor suppressor protein, EXCEPT
  a. p53
  b. mdm2
  c. BRCA
  d. UV specific endonuclease
• About “Non homologous end joining”, what is incorrect out of following?
  
  a. higher chance of gene loss.
  b. higher fidelity of fidelity
  c. higher chance of gene exchange
  d. higher chance of immunodeficiency syndrome.