Chapter 30

DNA replication, repair and recombination
<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helicase</td>
<td>Begins unwinding of DNA double helix</td>
</tr>
<tr>
<td>DNA gyrase</td>
<td>Assists unwinding</td>
</tr>
<tr>
<td>SSB proteins</td>
<td>Stabilize single strands of DNA</td>
</tr>
<tr>
<td>Primase</td>
<td>Synthesis of RNA primer</td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td>Elongation of chain by DNA synthesis</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>Removal of RNA primer and filling in gap with DNA</td>
</tr>
<tr>
<td>DNA ligase</td>
<td>Closes last phosphoester gap to form phosphodiester bond</td>
</tr>
</tbody>
</table>
Leading and Lagging Strands

Leading strand

Lagging strand (Okazaki fragments)

Motion of replication fork

Parental strands
Priming of DNA synthesis by short RNA segments
E. coli DNA polymerases

<table>
<thead>
<tr>
<th></th>
<th>Pol I</th>
<th>Pol II</th>
<th>Pol III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (kD)</td>
<td>103</td>
<td>90</td>
<td>130</td>
</tr>
<tr>
<td>Molecules/cell</td>
<td>400</td>
<td>?</td>
<td>10–20</td>
</tr>
<tr>
<td>Turnover number(^a)</td>
<td>600</td>
<td>30</td>
<td>9000</td>
</tr>
<tr>
<td>Structural gene</td>
<td>polA</td>
<td>polB</td>
<td>polC</td>
</tr>
<tr>
<td>Conditionally lethal mutant</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Polymerization: 5' → 3'</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Exonuclease: 3' → 5'</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Exonuclease: 5' → 3'</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

\(^a\)Nucleotides polymerized min\(^{-1}\)·molecule\(^{-1}\) at 37°C.

E. coli DNA polymerase I (Pol I)

- Three distinct active sites
  - Polymerase activity
  - 3’ → 5’ exonuclease activity (proofreading)
  - 5’ → 3’ exonuclease activity
- Recognizing the shape of the base pair
- Repair of damaged DNA
- Nick translation
- Removal of RNA primer
Exonuclease activities of Pol I

- Mismatched bases
- 3' → 5' Exonuclease hydrolysis site
- 5' → 3' Exonuclease hydrolysis site
- Single-strand nick
Nick translation

3' 5' 3'
5' Nick

3' 5' 3'
dNTPs DNA polymerase I PP_i

3' 5' 3'
Mononucleotides
Removal of RNA primer

3’ → 5’

Fragment 1

Nick

RNA primer

dNTPs

DNA polymerase I

3’ → 5’

pp_i

Mononucleotides

5’ → 3’

DNA ligase

3’ → 5’

DNA

5’ → 3’
E. coli DNA polymerase II and III

- DNA polymerase II (Pol II)
  - Repairing DNA damage via SOS response
- DNA polymerase III (Pol III)
  - DNA replicase
  - Unable to replicate primed ssDNA or nicked dsDNA unlike Pol I
  - Many subunits
**Components of *E. coli* DNA polymerase III**

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Mass (kD)</th>
<th>Structural Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha^a$</td>
<td>130</td>
<td><em>polC</em> (<em>dnaE</em>)</td>
</tr>
<tr>
<td>$\varepsilon^a$</td>
<td>27.5</td>
<td><em>dnaQ</em></td>
</tr>
<tr>
<td>$\theta^a$</td>
<td>10</td>
<td><em>holE</em></td>
</tr>
<tr>
<td>$\tau^b$</td>
<td>71</td>
<td><em>dnaX^c</em></td>
</tr>
<tr>
<td>$\gamma^b$</td>
<td>45.5</td>
<td><em>dnaX^c</em></td>
</tr>
<tr>
<td>$\delta^b$</td>
<td>35</td>
<td><em>holA</em></td>
</tr>
<tr>
<td>$\delta'^b$</td>
<td>33</td>
<td><em>holB</em></td>
</tr>
<tr>
<td>$\chi^b$</td>
<td>15</td>
<td><em>holC</em></td>
</tr>
<tr>
<td>$\psi^b$</td>
<td>12</td>
<td><em>holD</em></td>
</tr>
<tr>
<td>$\beta$</td>
<td>40.6</td>
<td><em>dnaN</em></td>
</tr>
</tbody>
</table>

$^a$Components of the Pol III core.
$^b$Components of the $\gamma$ complex.
$^c$The $\gamma$ and $\tau$ subunits are encoded by the same gene sequence; the $\gamma$ subunit comprises the N-terminal end of the $\tau$ subunit.

Sliding clamp (β clamp) of Pol III
Unwinding DNA: Helicases and single-strand binding proteins
E. coli DNA ligase

ATP is used for T4 and eukaryotic DNA ligases instead of NAD^+.
Primase

Potential path of single-stranded template

Potential site of catalysis

RNA strand

Interaction with helicase?
The replication of *E. coli* DNA

(a) Leading strand
   - Sliding clamp
   - DNA polymerase III holoenzyme
   - RNA primer
   - Growing Okazaki fragment

(b) Lagging strand
   - Primosome
   - Primosome making new RNA primer
   - Completed Okazaki fragment
   - RNA primer to be replaced with DNA by Pol I; nick sealed by DNA ligase

(c) Newly initiated Okazaki fragment
   - Old Okazaki fragment
Fidelity of replication

One mispairing per $10^8$ to $10^{10}$ base pairs

- The proper level of dNTPs
- The binding of dNTPs leads to a conformational change from an inactive open state to an active closed state
- Proofreading (3’→5’ exonuclease activity)
- DNA repair enzymes
- RNA primers
Eukaryotic DNA replication

- Mechanism is similar to prokaryotic replication mechanism
- Much more complex than the prokaryotic system in terms of the amount of DNA to be replicated and the number of proteins required

<table>
<thead>
<tr>
<th>Properties of Some Eukaryotic DNA Polymerases</th>
<th>α</th>
<th>δ</th>
<th>ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ → 5’ Exonuclease</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Associates with primase</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Processivity</td>
<td>moderate</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Requires PCNA</td>
<td>no</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

PCNA – proliferating cell nuclear antigen (sliding clamp protein)
Each end of linear chromosomal DNA

- DNA polymerase cannot synthesize the extreme 5’ end of the lagging strand
- RNA primer cannot be replaced with DNA at the 3’ end of the DNA template
- In the absence of a mechanism for completing the lagging strand, linear chromosomes would be shortened at both ends by at least the length of an RNA primer with each round of replication
Telomeres and telomerase

- The telomeres consist of 1000 or more tandem repeats of a short G-rich sequence on the 3’-ending strand of each chromosome end.
- The 3’-single strand extension (12- to 16-nt) – Primer binding for the final Okazaki fragment of the lagging strand
- Telomerases synthesize and maintain telomeric DNAs.
- Telomerases are ribonucleoproteins whose RNA components contains a segment that is complimentary to the repeating telomeric sequence

- The gradual truncation of chromosomes in the absence of telomerase contributes to the normal aging of cells
- Enhanced telomerase activity permits the uncontrolled replication and cell growth in cancer
Mechanism for the synthesis of telomeric DNA

Telomeric DNA

Telomerase RNA

5' T T G - O H 3'

3' A A C C C C A A C

5' dGTP + dTTP

polymerize

PP_i

5' T T G G G G T T G - O H 3'

3' A A C C C C A A C

5' translocate

5' T T G G G G T T G - O H 3'

3' A A C C C C A A C
G-quartets

T-loop
DNA damage

- UV irradiation – Thymine dimers
- Chemical mutagens
  - Point mutations
    - Transitions: purine → purine; pyrimidine → pyrimidine
    - Transversions: purine → pyrimidine; pyrimidine → purine
  - Insertion/deletion mutations

- Thymine dimer
- 8-Oxoguanine (oxoG)
  (Base-pairing with either C or A, leading to a transversion)
Alkylating agents

Nitrogen mustard  Ethylnitrosourea  N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)

O^6^-Methylguanine residue

(Base-pairing with either C or T, leading to a transversion)
DNA methylation as a normal cellular process

- Bacteria’s restriction modification system
- Distinction between the methylated parental DNA and the unmethylated daughter strand
- Switching off eukaryotic gene expression
Intercalating agents cause Insertion/deletion mutations.
DNA repair

- **Direct reversal of damage**
  - DNA photolyases: Photochemical reverse reaction of pyrimidine dimer formation
  - Alkyltransferases: Removal of alkyl groups from the alkylated DNA
- **Base excision repair (BER):** the damaged bases are removed and replaced
  - DNA glycosylases: Removal of the base $\rightarrow$ apurine or apyrimidine site (AP or abasic site) $\rightarrow$ cleavage by AP endonucleases and other exonucleases $\rightarrow$ replacement of nucleotides by a DNA polymerase and DNA ligase
- **Nucleotide excision repair (NER):** In response to helix distortions, the damaged nucleotides are removed and replaced
  - *E. coli* UvrABC endonuclease – Repair of pyrimidine dimer
  - Genetic diseases caused by defective NER – Hypersensitivity to UV light and many pathological outcomes including skin cancer
- **Mismatch repair (MMR)**
- **Error-prone repair:** A process of last resort due to high mutagenicity
DNA glycosylase
Nucleotide excision repair (NER) of pyrimidine dimers
Recombination

- Homologous recombination (general recombination) – DNA segments with extensive sequence homology
- Site-specific recombination – Two short, specific DNA sequences
Holliday model of general recombination

Crossing-over

Branch migration

Two resolution pathways
RecA promotes recombination in *E. coli*
RecBCD initiates recombination

1. ATP

2. RecA

RecBCD
RuvABC mediates the branch migration

X-ray structure of a RubA-Holliday junction complex

Model of RubAB-Holliday junction complex
Recombination repairs

1. Replication fork collapse
2. Strand invasion
3. Branch migration
4. Holliday junction resolution

Repair of a replication fork with a single-strand nick

RAD51
1. Formation of two Holliday junctions
2. DNA synthesis and ligation
3. Holliday junction resolution

Repair of a double-strand break in DNA by homologous end-joining
Transposons

- Mobile genetic elements
- Common in both prokaryotes and eukaryotes
- Variation in phenotypic expression over the short term and evolutionary development over the long term
- Each transposon encodes the transposase enzymes that insert it into the recipient DNA
- No homology is required between donor and recipient DNA (Nonhomologous recombination)