4.6 DNA Replication (p131-137)
1. Semi-conservative
2. origins
3. priming
4. leading & lagging
5. Okazaki fragments
6. replication factors
7. bidirectional
8. regulation of replication

“It has not escaped our notice that the specific base pairing we have proposed immediately suggests a possible copying mechanism for the genetic material.” (Watson & Crick, 1953)
Conservative vs Semi-conservative replication
Do parent strands stay together or pair with daughters?

1. Grow E. coli in heavy ($^{15}N$) media
2. Transfer to light ($^{14}N$) media
3. Periodically isolate DNA from growing bacteria culture
4. Analyze DNA by equilibrium density gradient centrif.

The Meselson-Stahl Experiment

DNA Replication Requires:
1. Template
2. Primer
3. dNTP’s for 5’ to 3’ synthesis
4. Proteins

Replication Activities
Leading:
- 1 RNA primer
- 5'-3' DNA polymerization

Lagging:
- multiple RNA primers
- extended by DNA pol
- RNA degraded
- DNA pol fills gap
- ligation

1. Large T antigen (SV40 protein) - helicase
2. RPA (rep. prot. A) - binds unwound parent DNA
3. Pol δ - extends primer with dNTP's
   Rfc (rep. factor C)
   PCNA (proliferating cell nuclear ant)
   *processivity
4. Primase - syn. RNA primer
   Pol α - adds dNTPs
5. Pol δ/PCNA/Rfc complex continues extension
6. topoisomerase
   RNaseH+FEN1
   DNA ligase

Replication is Bi-Directional from the Origin(s)

Electron micrographs of linearized SV40 DNA undergoing replication

Coordination of Bi-directional DNA Replication
How is DNA Replication Regulated?

- Euk. chromosomal DNA contains multiple origins
- **ORC** binds the origins
- **MCM** helicases are activated by cellular signals to start S phase

The End Replication Problem

- **RNA primer removal**
- **DNA would progressively shorten!**

Problem solved by telomerase:
- **RNA serves as template**
- **protein carries out reverse transcription**
- shortening is prevented

Importance of telomeres:
- protection
- cellular expression of telomerase
- role in cancer
- immortality?
Proofreading (p. 961-962)

What happens if the incorrect nucleotide is incorporated during replication?

DNA Polymerase has 3'-->5' exonuclease activity

5' GCGATG 3'
3' CGCTACGTAA

Pol pauses, removes ntd.

5' GCGATG 3'
3' CGCTACGTAA

Pol resumes replication

5' GCGATG 3'
3' CGCTACGTAA

DNA Repair (p. 962-970)

<table>
<thead>
<tr>
<th>Disease</th>
<th>DNA-Repair System Affected</th>
<th>Sensitivity</th>
<th>Cancer Susceptibility</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary nonpolyposis colorectal cancer</td>
<td>DNA mismatch repair</td>
<td>UV irradiation, chemical mutagens</td>
<td>Colon, ovary</td>
<td>Early development of tumors</td>
</tr>
<tr>
<td>Xeroderma pigmentosum</td>
<td>Nucleotide excision repair</td>
<td>UV irradiation, postradiation</td>
<td>Skin carcinomas, xeroderma</td>
<td>Skin and eye photosensitivity, keratoses</td>
</tr>
</tbody>
</table>

Mutations arise when DNA repair fails

- Deamination
- 5-Methyl cytosine
- Thymine
- Base-excision repair
- DNA Polymerase
- Mutant DNA
- Wild-type DNA
DNA Repair Systems in Eukaryotes

1. **Base excision** - only incorrect ntd. is removed
   - 1. base removal
   - 2. pol adds correct ntd
   - 3. ligation
   - [Fig 23-27]

2. **Mismatch repair**
   - [Fig 23-28]

3. **Nucleotide excision**
   - 1. damaged DNA recognized
   - 2. segment containing damage removed
   - 3. pol. and ligation
   - [Fig 23-30]

**What if there is a double-stranded break?**

1. **Homologous recombination** - “error free”
   - undamaged sister chromatid serves as template
   - [Fig 23-31]

2. **End-joining** - “error prone”
   - two separated ends rejoined
   - OR
   - different broken ends joined
   - translocations

**Replicating DNA in vitro by the Polymerase Chain Reaction (PCR)**

- total DNA sample
- PCR
- amplified specific DNA sequence

**Requirements:**
1. template DNA
2. primers (DNA oligos ~20nt)
3. dNTPs
4. Polymerase*

* *Taq Polymerase*

- Cycle 1
  - Denaturation of DNA
  - 95°C
  - Denaturation of primers
  - 95°C
  - Annealing of primers
  - 50-60°C
  - Elongation of primers
  - 72°C

- [Fig 9-24]
Repeat cycle 20X --> 1-million fold amplification

- Cycle 1: Denaturation of DNA, Annealing of primers, Elongation of primers
- Cycle 2: Denaturation of DNA, Annealing of primers, Elongation of primers

Fig 23-31

Cycles 4, 5, 6, etc.