Chapter 4.2
(textbook: “Molecular Cell Biology” 6 ed, Lodish section: 4.5-4.6)

DNA Replication, Repair, and Recombination
Cell division - mitosis

Cell growth

DNA replication

Spindle pole body duplication

Chromosome condensation

Spindle formation

Chromosome segregation

Nuclear division

Cytokinesis

G1

S

G2

M
S-phase is tightly regulated by kinases

1. DNA prereplication complexes assemble at origins
2. G_{1} cyclin-CDK inactivates Cdh1
3. G_{1} cyclin-CDK activates expression of S-phase cyclin-CDK components
4. G_{1} cyclin-CDK phosphorylates S-phase inhibitor
5. SCF/proteasome degrades phosphorylated S-phase cyclin-CDK inhibitor
6. S-phase cyclin-CDK activates prereplication complexes
7. Mitotic cyclin-CDK
8. APC/C-Cdc20/proteasome degrades securin
9. CdcA phosphatase activates Cdh1 and APC/C-Cdh1/proteasome degrades mitotic cyclins
Mitosis can be divided into six phases:

- **Interphase**
  - Chromosome duplication and cohesion
  - Centrosome duplication

- **Prophase**
  - Breakdown of interphase microtubule display and its replacement by mitotic asters
  - Mitotic aster separation
  - Chromosome condensation

- **Prometaphase**
  - Nuclear envelope breakdown
  - Chromosomes captured, bi-oriented and brought to the spindle equator

- **Metaphase**
  - Chromosomes aligned at the metaphase plate
Mitosis can be divided into six phases

- **Anaphase**: APC/C activated and cohesins degraded
  - Anaphase A: Chromosome movement to poles
  - Anaphase B: Spindle pole separation

- **Telophase**: Nuclear envelope reassembly
  - Assembly of contractile ring

- **Cytokinesis**: Reformation of interphase microtubule array
  - Contractile ring forms cleavage furrow
Meselson-Stahl experiment
Proved that DNA replication is semi-conservative
There are various “problems” that must be overcome for DNA polymerase to copy DNA

- DNA polymerases are unable to melt duplex DNA in order to separate the two strands that are to be copied.

- All known DNA polymerases can only elongate a preexisting DNA or RNA strand (the primer) and are unable to initiate chains.

- The two strands in the DNA duplex are opposite in chemical polarity, but all DNA polymerases catalyze nucleotide addition at the 3’-hydroxyl end of a growing chain, so strands can grow only in the 5’ to 3’ direction. Hence, the 3’ – 5’ strand (lagging strand) synthesis cannot be straightforward.

- For linear DNA: The ends (telomere regions) of the 3’-5’ strands cannot be primed. Hence, the replicate strand would be shorter than the parent strand.
Replication fork contains a leading-strand and lagging-strand
Enzymes involved in replication

1: **Helicase** unwinds parental DNA strands

2: Single strand regions are bound and stabilized by multiple copies of the protein RPA (stabilizes a DNA conformation optimal for processing by DNA pol δ)

3: Leading strand synthesis via an enzymatic complex: DNA Pol δ, PCNA, and Rfc

4: Primers for lagging strand synthesis (RNA, DNA) are synthesized by a complex of DNA pol α and Primase resulting in a mix RNA-DNA primer

5: The 3' end of each RNA-primer is then bound by a PCNA-Rfc-Pol δ complex, which extends the primer and synthesizes most of each Okazaki fragment (incl proofreading!)

6: Finally the Okazaki fragments are then joined into a complete lagging strand by the enzyme “Ligase”
Synthesis of the lagging strand

1. Primase attaches RNA oligonucleotides (primers) to the old strand copied from DNA.
2. DNA polymerase III elongates RNA primers with new DNA to form Okazaki fragments.
3. DNA polymerase I removes 5' RNA at the end of neighboring fragment and fills gap.
4. DNA ligase joins adjacent fragments, completing the lagging strand.

- Primase
- DNA pol α
- DNA pol δ
- Ligase
Replication is bidirectional.
DNA replication begins at specific chromosomal sites called replication origins

Consensus sequence of the minimal bacterial replication origin

Replication origins, regardless of organism, are
(1) unique DNA segments with multiple short repeats,
(2) recognized by multimeric origin-binding proteins,
(3) usually contain an A-T rich stretch
Type I topoisomerase relax DNA by nicking and then closing one strand of duplex DNA

The role of topoisomerase in DNA replication

- DNA molecules can coil and bend in space, leading to changes in topology such as formation of supercoils.
- Topoisomerase are enzymes that control DNA topology and perform essential functions at several different steps in replication.
Replicated circular DNA molecules are separated by type II topoisomerases.

Linear daughter chromatids also are separated by type II topoisomerases.
Telomerase prevents shortening of lagging strands during eukaryotic DNA replication

Mammalian telomere repeat: sequence

(TTAGGG)*1000*X
DNA damage and repair and their role in carcinogenesis

- A DNA sequence can be changed by copying errors introduced by DNA polymerase during replication and by environmental agents such as chemical mutagens or radiation.

- If uncorrected, such changes may interfere with the ability of the cell to function.

- Hence, several mechanisms to repair DNA damage have evolved.

- All carcinogens cause changes in the DNA sequence --> DNA damage and/or failing repair can lead to cancer development.

- Prokaryotic and eukaryotic DNA-repair systems are analogous.
General types of DNA damage and causes

<table>
<thead>
<tr>
<th>DNA Lesion</th>
<th>Example/Cause</th>
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<tbody>
<tr>
<td>Missing base</td>
<td>Removal of purines by acid and heat (under physiological conditions ≈ 10⁴ purines/day/cell in a mammalian genome); removal of altered bases (e.g., uracil) by DNA glycosylases</td>
</tr>
<tr>
<td>Altered base</td>
<td>Ionizing radiation; alkylation agents (e.g., ethylmethane sulfonate)</td>
</tr>
<tr>
<td>Incorrect base</td>
<td>Mutations affecting 3’ → 5’ exonuclease proofreading of incorrectly incorporated bases</td>
</tr>
<tr>
<td>Bulge due to deletion or insertion of a nucleotide</td>
<td>Intercalating agents (e.g., acridines) that cause addition or loss of a nucleotide during recombination or replication</td>
</tr>
<tr>
<td>Linked pyrimidines</td>
<td>Cyclotubyl dimers (usually thymine dimers) resulting from UV irradiation</td>
</tr>
<tr>
<td>Single- or double-strand breaks</td>
<td>Breakage of phosphodiester bonds by ionizing radiation or chemical agents (e.g., bleomycin)</td>
</tr>
<tr>
<td>Cross-linked strands</td>
<td>Covalent linkage of two strands by bifunctional alkylation agents (e.g., mitomycin C)</td>
</tr>
<tr>
<td>3’-deoxyribose fragments</td>
<td>Disruption of deoxyribose structure by free radicals leading to strand breaks</td>
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</tbody>
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Proofreading by DNA polymerase corrects copying errors
Schematic model of the proofreading function of DNA polymerase
Chemical carcinogens react with DNA directly or after activation, and the carcinogenic effect of a chemical correlates with its mutagenicity.

**DIRECT-ACTING CARCINOGENS**

- β-Propiolactone
- Ethylmethane sulfonate (EMS)
- Dimethyl sulfate (DMS)
- Nitrogen mustard
- Methyl nitrosourea (MNU)

**INDIRECT-ACTING CARCINOGENS**

- Benzo(a)pyrene (3,4-benzpyrene)
- 2-Acetylaminofluorene
- Dibenz(a,h)anthracene
- 2-Naphthylamine
- Dimethylnitrosamine
- Vinyl chloride
- 2-Aflatoxin B₁ (Aspergillus flavus)
- Safrole (sassafras)
Deamination leads to point mutations

2-Deoxyribose

5-Methylcytosine

Thymine

Wild-type DNA

Mutant DNA

Wild-type DNA

Base-excision repair

Deamination

Replication

1

2
Base excision repair of T*G mismatch

1. DNA glycosylase
2. APEI endonuclease
3. AP lyase (part of DNA Pol β)
4. DNA Pol β

Repaired wild-type DNA
Mismatch excision repair in human cells

1. Template strand
   - MSH2
   - MSH6

2. Newly synthesized daughter strand
   - MLH1 endonuclease, PMS2
   - DNA helicase
   - DNA exonuclease

3. Gap repair by DNA polymerase and ligase
Formation of thymine-thymine dimers

Two thymine residues

UV irradiation

Sunlight
Space radiation

Thymine-thymine dimer residue
Nucleotide excision repair in human cells

1. Initial damage recognition
2. Opening of DNA double helix
3. XP-F and XP-G endonucleases
4. DNA polymerase DNA ligase

NB:
Transcription-coupled repair
Nonhomologous end-joining
Double-strand break repair

1. DNA-PK
   KU80/KU70 heterodimer

2. Other proteins

3. Ligase
Double-strand breaks frequently lead to genetic aberration ... And cancer

<table>
<thead>
<tr>
<th>Proto-oncogenes</th>
<th>Normal Function of Genes</th>
<th>Examples of Gene Products</th>
<th>Effect of Mutation</th>
<th>Genetic Properties of Mutant Gene</th>
<th>Origin of Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Promote cell survival or proliferation</td>
<td>Anti-apoptotic proteins, components of signaling and signal transduction pathways that result in proliferation, transcription factors</td>
<td>Gain-of-function mutations allow unregulated cell proliferation and survival</td>
<td>Mutations are genetically dominant</td>
<td>Arise by point mutation, chromosomal translocation, amplification</td>
</tr>
</tbody>
</table>

| Tumor-suppressor genes | Inhibit cell survival or proliferation | Apoptosis-promoting proteins, inhibitors of cell-cycle progression, checkpoint-control proteins that assess DNA/chromosomal damage, components of signal pathways that restrain cell proliferation | Loss-of-function mutations allow unregulated cell proliferation and survival         | Mutations are genetically recessive | Arise by deletion, point mutation, methylation |

| Caretaker genes     | Repair or prevent DNA damage       | DNA-repair enzymes                                                                   | Loss-of-function mutations allow mutations to accumulate                         | Mutations are genetically recessive | Arise by deletion, point mutation, methylation |
Recombinatorial repair of collapsed Replication fork

Double-strand break repair

1. Replication fork collapse
2. 5’-exonuclease acts on broken end. Other daughter strand (pink) ligated to repaired parental strand (light blue) in unbroken chromosome.
3. RecA- or Rad51-mediated strand invasion
4. Branch migration
5. Cut strands at crossover (arrows)
6. Ligate ends
7. Rebuild replication fork and continue replication
Homologous recombination (CrossOver)

Double-strand break repair

- Recombination provides a means by which a genome can change to generate new combinations of genes (pro-evolution)

- Homologous recombination allows for the exchange of blocks of genes between homologous chromosomes and thereby is a mechanism for generating genetic diversity

- Recombination occurs randomly between two homologous sequences and the frequency of recombination between two sites is proportional to the distance between the sites