Overview

- Introduction
- Semiconservative replication of double-stranded DNA
- Modes of replication
- Unwinding, stabilization and stress relief
- Initiation by a primosome complex
- Chain elongation and proofreading
- Discontinuous replication of the lagging strand
- Terminator sequencing of DNA
- Molecular mechanisms of recombination

Problems of initiation, elongation, incorporation errors

- Initiation:
  - Replicons & origins of replication
  - RNA primers
- Elongation:
  - 5’ to 3’ only
  - Leading versus lagging strand
- Errors or incorporation
  - Proofreading

“It didn’t escape our attention that ...”
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**Meselson-Stahl experiment**

- Newly synthesized strands contain 15N only (heavy); these strands are denser and move to the lower band.
- Newly synthesized strands contain 14N only (light); these strands move to the upper band.

**Equilibrium density gradient centrifugation**

- Lower Cs\(^+\) concentration: more dense
- Higher Cs\(^+\) concentration: less dense

**Semi-conservative replication of DNA in chromosomes**
Semi-conservative replication of DNA in chromosomes

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Theta replication of circular chromosomes

- Actual length 1.6 mm (4.6 x 10^6 base pairs)
- Unreplated parental duplex
- Direction of movement of replication fork
- Replicated daughter strand
Theta replication of circular chromosomes

Rolling circle replication

Multi-ori
gins and bidirectional pg replication in eukaryotes

Rolling circle replication
Multiple origins and bidirectional replication in eukaryotes

Replication speed and duration in pro- and eucaryotes

- Eukaryotes:
  - 10-100 nt/sec => days / chr. (10^7)
  - => multiple origins of replication
  - => 5-10 hrs
- Prokaryotes:
  - 1,500 nt / sec => 30 min / genome
  - ↔ 20 min. generation interval!

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Unwinding, stabilization and stress relief
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Initiation by a primosome complex
- RNA primer
  - Procaryotes: primase (dnaG), 2-5 RNA residues
  - Eucaryotes: primosome (polymerase alpha + 15-20 proteins), 12 RNA residues + > 20 DNA residues
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Chain elongation and proofreading

- Elongation
- Procaryotes: polymerase III holoenzyme (2 x DNA polymerase III + > 7 proteins)
- Eucaryotes: polymerase delta
- Replication errors: rate and cause
- Proofreading ⇔ exonuclease 3’ to 5’ activity

5’ to 3’ chain elongation

Proofreading
Proofreading precludes 3’ to 5’ elongation

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Discontinuous replication of the lagging strand
- Leading vs lagging strand, Okazaki fragments
- Joining of the precursor fragments
  - Procaryotes:
    - Pol I (5’ to 3’ exonuclease activity)
    - + ligase
  - Eucaryotes
    - Replication Protein A
    - Pol delta
    - Ligase

Leading vs lagging strand, Okazaki fragments
**Leading vs lagging strand, Okazaki fragments**

- Replicated daughter strand
- Single-stranded region in lagging strand
- Unreplicated parental strand

**DNA joining of precursor fragments - procaryotes**

- 5’ to 3’ exonuclease activity of pol I
  - Removes RNA primer
  - Generates 5’ P-end (vs 5’ PPP-end of primer)
- + ligase

**DNA joining of precursor fragments - eucaryotes**

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**Sequencing of DNA**

**Dideoxyterminators**

3′-OH in normal DNA allows elongation.

A DNA strand terminating in a dideoxynucleotide cannot be elongated because a 3′-OH is necessary for polymerization.

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**Sanger sequencing**

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**Massive Parallel Sequencing**

**Reversible terminators**

Genomic DNA is sheared and adaptors for PCR primers attached. The fragments are separated into single strands, and each is attached to a random position on a surface of about 6 cm² (2.5 cm²) that is densely packed with immobilized primers. Sequencing reactions are carried out with all four nucleotides, each in the form of a reversible terminator with a unique fluorescence emission.

In each round of PCR, the template strand binds with a nearby immobilized primer, and multiple rounds of PCR eventually create a cluster of about 1000 copies of each original template strand.
Massive Parallel Sequencing Pyrosequencing

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Recombination: double-strand break and repair model
Recombination: Mismatch repair and gene conversion