DNA – semiconservative replication

New

Old

Old

New
Models for DNA replication

a) The semi-conservative model

b) The conservative model

c) The dispersive model
Meselson-Stahl experiment

- E. coli grown in $^{15}$N-labeled medium
- E. coli DNA becomes uniformly labeled with $^{15}$N in nitrogenous bases

Generation 0
- $^{15}$N-labeled E. coli added to $^{14}$N medium
- Gravitational force

Generation I
- Cells replicate once in $^{14}$N
- DNA extracted and centrifuged in gradient

Generation II
- Cells replicate a second time in $^{14}$N

Generation III
- Cells replicate a third time in $^{14}$N

$^{15}$N/$^{15}$N → $^{15}$N/$^{14}$N → $^{14}$N/$^{14}$N → $^{14}$N/$^{14}$N
Taylor experiment

(a) Replication I

$^3$H-thymidine

Unlabeled chromosome

Both sister chromatids labeled

Replication II

No sister chromatid exchange

Unlabeled thymidine

Only one chromatid labeled

Sister chromatid exchange

Reciprocal regions of both chromatids labeled

Anaphase

Chromatids migrate into separate cells

Metaphase II

Metaphase II
Bidirectional replication of the *E. coli* chromosome
In vitro requirements for DNA replication

Deoxyribose nucleoside triphosphate (A, T, C, or G)

DNA template \((d\text{NMP})_x\) and a portion of its complement \((d\text{NMP})_n\)

Complement to template strand is extended by one nucleotide \((n + 1)\)

Inorganic pyrophosphate

\[ d\text{NTP} + (d\text{NMP})_n \xrightarrow{\text{DNA polymerase I, Mg}^{++}} (d\text{NMP})_{n+1} + P-P \]
DNA polymerase at work
b) Shorthand notation

Template strand

<table>
<thead>
<tr>
<th>A</th>
<th>C</th>
<th>A</th>
<th>T</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>G</td>
<td>T</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chain growth

DNA polymerase

5' P P P P P P

3' OH OH

3' OH

5'
Molecular model of DNA replication initiation – unwinding and stabilization of open structure
Initiation of DNA synthesis requires an RNA primer.
DNA replication - Details

a) Initiation; RNA primer made by DNA primase starts replication of lagging strand (synthesis of 1st Okazaki fragment)

b) Further untwisting and elongation of new DNA strands; 2nd Okazaki fragment elongated

Polymerase III dissociates

Discontinuous synthesis on this strand

RNA primer for 3rd Okazaki fragment

1st Okazaki fragment

3' 5'

2nd Okazaki fragment elongation

5' 3'

Continued untwisting and fork movement

Polymerase III dissociates

3rd Okazaki fragment

5' 3'

5' 5'

5' 5'

5' 5'

Process continues; 2nd Okazaki fragment finished, 3rd being synthesized; DNA primase beginning 4th fragment

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DNA replication – Details cont.

d) Primer removed by DNA polymerase I

DNA polymerase I replaces RNA primer with DNA

Single-strand nick

4th Okazaki fragment

5th Okazaki fragment

RNA primer being replaced with DNA by polymerase I

Nick sealed by DNA ligase

e) Joining of adjacent DNA fragments by DNA ligase

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Molecular model of DNA replication
Overview of DNA Replication

- DNA replication is semiconservative and bidirectional.

DNA polymerases can only add deoxynucleotides to 3’ ends. As a result strands are synthesized only in the 5’ to 3’ direction.

- Both strands at the replication forks are synthesized at the same time.

- Thus, at the replication fork the leading strand is synthesized continuously, but the lagging strand is made discontinuously as Okasaki fragments.
Overview of DNA Replication
<table>
<thead>
<tr>
<th>Gene Product or Function</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA polymerase I</td>
<td><em>polA</em></td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td><em>dnaE, dnaQ, dnaX, dnaN, dnaD</em></td>
</tr>
<tr>
<td>Initiator protein; binds to oriC</td>
<td><em>dnaA</em></td>
</tr>
<tr>
<td>IHF protein (DNA binding protein); binds to oriC</td>
<td><em>himA</em></td>
</tr>
<tr>
<td>FIS protein (DNA binding protein); binds to oriC</td>
<td><em>fis</em></td>
</tr>
<tr>
<td>Helicase and activator of primase</td>
<td><em>dnaB</em></td>
</tr>
<tr>
<td>Complexes with dnaB protein and delivers it to DNA</td>
<td><em>dnaC</em></td>
</tr>
<tr>
<td>Primase; makes RNA primer for extension by DNA polymerase III</td>
<td><em>dnaG</em></td>
</tr>
<tr>
<td>Single-stranded binding (SSB) proteins; bind to unwound single-stranded arms of replication forks</td>
<td><em>ssb</em></td>
</tr>
<tr>
<td>DNA ligase; seals single-stranded gaps</td>
<td><em>lig</em></td>
</tr>
<tr>
<td>Gyrase (type II topoisomerase); replication swivel to avoid tangling of DNA as replication fork advances</td>
<td><em>gyrA, gyrB</em></td>
</tr>
<tr>
<td>Origin of chromosomal replication</td>
<td><em>oriC</em></td>
</tr>
<tr>
<td>Terminus of chromosomal replication</td>
<td><em>ter</em></td>
</tr>
<tr>
<td>TBP (ter binding protein); stalls replication forks</td>
<td><em>tus</em></td>
</tr>
</tbody>
</table>
DNA replication in eukaryotes
Replication units in eukaryotic chromosomes

Time

Origins of replication units

Template DNA (blue)

New DNA (red)

Template DNA (blue)

New DNA (red)
Linear chromosomes – problems at the ends

(Lagging strand template)

5' ──────── 3'
3' ──────── 5'

(Leading strand template)

5' ──────── 3'
3' ──────── 5'

RNA primer

Discontinuous and continuous synthesis

5' ──────── 3'
3' ──────── 5'

RNA primers removed

Gaps (a) and (b) created

5' ──────── 3'
3' ──────── 5'

(a) ──────── (b)

5' ──────── 3'
3' ──────── 5'

Gap (a) filled

Gap (b) unfilled

5' ──────── 3'
3' ──────── 5'

5' ──────── 3'
3' ──────── 5'
Synthesis of telomeric DNA by telomerase

(a) Telomerase binds to 3′ G-rich tail

(b) Telomeric DNA is synthesized on G-rich tail

(c) Telomerase is translocated and steps (a) and (b) are repeated

(d) Telomerase released; primase and DNA polymerase fill gap

(e) Primer removed; gap sealed by DNA ligase