DNA polymerase Summary

1. DNA replication is semi-conservative.
2. DNA polymerase enzymes are specialized for different functions.
3. DNA pol I has 3 activities: polymerase, 3’→5’ exonuclease & 5’→3’ exonuclease.
4. DNA polymerase structures are conserved.
5. But: Pol can’t start and only synthesizes DNA 5’→3’!
6. Editing (proofreading) by 3’→5’ exo reduces errors.
7. High fidelity is due to the race between addition and editing.
8. Mismatches disfavor addition by DNA pol I at 5 successive positions. The error rate is ~1/10⁹.

DNA replication is semi-conservative

Meselson–Stahl experiment
1. Grow E. coli on ¹⁵N (“heavy”) ammonia
2. Switch to ¹⁴N (normal, “light”) ammonia
3. Harvest aliquots as a function of time
4. Isolate DNA
5. Separate on the basis of DNA density using density gradient centrifugation
   A. Pour CsCl₂ gradient into a tube
   B. Layer DNA on top
   C. Centrifuge until DNA stops moving (DNA floats when the density matches that of the salt solution)
Predictions of Meselson-Stahl experiment

<table>
<thead>
<tr>
<th>Conservative</th>
<th>Semi-conservative</th>
<th>Distributive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental strands stay together -- HH maintained</td>
<td>Parental strands separate every generation -- no HH after 1 generation</td>
<td>Parental strands broken -- no LL in generation 2</td>
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Results of Meselson-Stahl experiment

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Graph Picture
DNA replication is semi-conservative

**Conservative**
0
Parental strands stay together -- HH maintained

1
Parental strands separate every generation -- no HH after 1 generation

2
Parental strands broken -- no LL in generation 2

**Semi-conservative**

**Distributive**

**Results**

Graph  Picture

Arthur Kornberg discovered DNA dependent DNA polymerase

*Used an “in vitro” system: the classic biochemical approach*

1. Grow *E. coli*
2. Lyse cells
3. Prepare extract
4. Fractionate extract
5. Search for DNA polymerase activity using an ASSAY
   (Incorporate radioactive building blocks, Precipitate DNA chains (nucleotides soluble), Quantify radioactivity.)
Arthur Kornberg discovered DNA dependent DNA polymerase

Used an “in vitro” system: the classic biochemical approach

1. Grow *E. coli*
2. Lyse cells
3. Prepare extract
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Requirements for DNA polymerase activity

<table>
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<tr>
<th>Requirement</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Template</td>
<td>[Basis for heredity]</td>
</tr>
<tr>
<td>dNTPs (not ATP, not NDPs, not NMPs)</td>
<td>[Building blocks]</td>
</tr>
<tr>
<td>Mg^{2+}</td>
<td>[Promotes reaction]</td>
</tr>
<tr>
<td>Primer - (complementary bases at 3’ end, removed by fractionation and added back)</td>
<td>[DNA pol can’t start!]</td>
</tr>
</tbody>
</table>

DNA polymerase mechanism

Each dNTP provides the nucleophile (3’-OH) for the next round

PPI hydrolyzed to 2 PO_{4}^{=}
### Nobel Prize for DNA polymerase I

#### Comparison of DNA Polymerases of *E. coli*

<table>
<thead>
<tr>
<th>DNA polymerase</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural gene*</td>
<td>( \beta )</td>
<td>( \beta' )</td>
</tr>
<tr>
<td>Subunits (number of different types)</td>
<td>1</td>
<td>≥4</td>
</tr>
<tr>
<td>( M )</td>
<td>103,000</td>
<td>88,000*</td>
</tr>
<tr>
<td>3'→5' Exonuclease (proofreading)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5'→3' Exonuclease</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Polymerization rate (nucleotides/sec)</td>
<td>16–20</td>
<td>40</td>
</tr>
<tr>
<td>Processivity (nucleotides added before polymerase dissociates)</td>
<td>3–200</td>
<td>1,500</td>
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Mutant viable? Yes! Yes!
### Nobel Prize for DNA polymerase I

**Comparison of DNA Polymerases of *E. coli***

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<th>DNA polymerase</th>
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<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural gene*</td>
<td>polA</td>
<td>polB</td>
<td>polC (dnaE)</td>
</tr>
<tr>
<td>Subunits (number of different types)</td>
<td>1</td>
<td>≥4</td>
<td>≥10</td>
</tr>
<tr>
<td>M₆</td>
<td>103,000</td>
<td>88,000¹</td>
<td>830,000</td>
</tr>
<tr>
<td>3'→5' Exonuclease (proofreading)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
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<td>Yes</td>
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<td>No</td>
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<tr>
<td>Polymerization rate (nucleotides/sec)</td>
<td>16–20</td>
<td>40</td>
<td>250–1,000</td>
</tr>
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<td>Processivity (nucleotides added before polymerase dissociates)</td>
<td>3–200</td>
<td>1,500</td>
<td>≥500,000</td>
</tr>
</tbody>
</table>

Mutant viable? | Yes! | Yes! | No
Function | repair | replication

+ DNA pol IV: mutagenesis
+ DNA pol V: error-prone repair
Examples of eukaryotic DNA polymerases

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>HUGO name</th>
<th>Class</th>
<th>Other name</th>
<th>Proposed role/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>α (alpha)</td>
<td>POLA</td>
<td>B</td>
<td>POLI</td>
<td>DNA replication</td>
</tr>
<tr>
<td>β (beta)</td>
<td>POLB</td>
<td>A</td>
<td>MPR1</td>
<td>Mitochondrial DNA repair</td>
</tr>
<tr>
<td>γ (gamma)</td>
<td>POLG</td>
<td>B</td>
<td>POLG</td>
<td>DNA replication</td>
</tr>
<tr>
<td>δ (delta)</td>
<td>POLH</td>
<td>Y</td>
<td>RAD50, YPD</td>
<td>Bypass synthesis</td>
</tr>
<tr>
<td>ε (epsilon)</td>
<td>POLQ</td>
<td>Y</td>
<td>EXP50, DMC1</td>
<td>Bypass synthesis</td>
</tr>
<tr>
<td>γ (gamma) (mitochondrial)</td>
<td>POLG (mitochondrial)</td>
<td>Y</td>
<td>RAD50, YPD</td>
<td>Bypass synthesis</td>
</tr>
</tbody>
</table>

Mass
- α: 300,000
- β: 40,000
- δ: 170-230,000
- ε: 250,000
- γ (mitochondrial): 180-300,000

**DNA polymerase activities -- 5’-->3’ nucleotide addition**

- Primer has a free 3’-OH
- Incoming dNTP has a 5’ triphosphate
- Pyrophosphate (PP) is lost when dNMP adds to the chain
DNA polymerase reactions -- editing

3’--→5’ exonuclease

Opposite reaction compared to polymerase (But no PPi used or dNTP made)

DNA polymerase reactions -- nick translation

5’--→3’ exonuclease

Creates single-stranded template in front for repair
DNA pol I Klenow fragment lacks 5'-->3' exonuclease

Structure of the DNA complex of the Klenow fragment of DNA pol I
Functional sites in RB69 DNA polymerase
+ primer-template + dTTP

Fold conserved in DNA polymerases

"Fingers"  "Palm"  "Thumb"
Two different active sites for nucleotide addition and 3'--5' exonuclease

Polymerization is a race against the 3'--5' exonuclease. Relative rates of addition and exonuclease control the net reaction.

Fidelity is due to the race between the polymerase and exonuclease.

Model: Rate of nucleotide addition dominates the net reaction.

DNA pol I reads shape (and polarity) of the incoming dNTP

Nonpolar A & T analogs add and direct synthesis efficiently!
How does the polymerase “sense” mismatches?

**Mismatches distort the polymerase active site**

**Experiment**
1. Crystallize Klenow fragment plus primer-template
2. Add one Mg-dNTP
3. Wait for nucleotide addition in the crystal
4. In different crystals, create all 12 possible mismatches
5. Determine all 12 crystal structures
6. For G-T mismatch, add the next dNTP to move the mismatch
7. Solve structure
8. Repeat 6 & 7 four times to move mismatch away from the entry site.
9. Compare structures with correctly paired primer.

Results: Mismatches at n-1 to n-5 distort pol active site -- FIVE CHANCES TO CORRECT THE MISTAKE!


Mismatched base pairs in the “entry site” distort the Pol I active site.

**Left: Mismatch H-bonding pattern.**

**Right: Molecular surface of the mismatch (red) compared to a cognate G-C pair (green) highlights the change in the structure of the primer terminus.**

Four categories of distortions by insertion-site mismatches

2. Disruption of primer strand arrangement and catalytic site (T-T, C-T).

Active site distortions continue for five succeeding additions!

H-bonds to mismatched G-T pair (dashes) at each position after incorporation. Spheres are water molecules. N-3 and n-4 require base ionization or tautomerization of a base.

Mismatch slows polymerase for 5 successive additions, favoring the exonuclease reaction.
**Bacterial DNA polymerase III: a distinct polymerase fold**

Pol III structure \(\rightarrow\) Model for DNA complex

- Revealed conserved features of the DNA polymerase that copies bacterial genomes.
- Established a new model of the elongation complex including binding sites for DNA and interacting proteins.


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