DNA Replication II
Biochemistry 302

Bob Kelm
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Following in Dad’s footsteps…

• Original A. Kornberg *E. coli* DNA Pol I is a lousy replicative enzyme.
  – 400 molecules/cell but ~2 replication forks/cell
  – $V_{\text{max}} \sim 20$ nt/sec
  – Processivity of $\sim 3$-200 nt/encounter

• So there must be more processive DNA Pol enzymes or accessory factors….J. Cairns,1969 isolated *E. coli* ts mutant w/o Pol I polymerase and 3’ exonuclease activity but w/ 5’ exo activity.
  – Live, divide, and replicate DNA normally
  – Sensitive to UV light and alkylating agents

• R. Kornberg eventually isolated Pol II and Pol III.
# Summary of *E. coli* DNA Polymerases

## Table 24.2 DNA Polymerases of *E. coli*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Polymerase I</th>
<th>Polymerase II</th>
<th>Polymerase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural gene</td>
<td><em>polA</em></td>
<td><em>polB</em></td>
<td><em>polC</em></td>
</tr>
<tr>
<td>Molecular weight</td>
<td>103,000</td>
<td>90,000</td>
<td>130,000</td>
</tr>
<tr>
<td>Number of molecules/cell</td>
<td>400</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>$V_{\text{max}}$, nucleotides/second</td>
<td>16–20</td>
<td>2–5</td>
<td>250–1000</td>
</tr>
<tr>
<td>3$'$ exonuclease</td>
<td>Yes</td>
<td>Yes</td>
<td>No$^a$</td>
</tr>
<tr>
<td>5$'$ exonuclease</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Processivity$^b$</td>
<td>3–200</td>
<td>10,000</td>
<td>500,000</td>
</tr>
<tr>
<td>Mutant phenotype $^c$</td>
<td>UV$^\text{a}$MMS$^g$</td>
<td>None</td>
<td>$\text{dna}^f$</td>
</tr>
<tr>
<td>Biological function</td>
<td>DNA repair,</td>
<td>SOS DNA repair?</td>
<td>Replicative</td>
</tr>
<tr>
<td></td>
<td>RNA primer</td>
<td></td>
<td>chain</td>
</tr>
<tr>
<td></td>
<td>excision</td>
<td></td>
<td>elongation</td>
</tr>
</tbody>
</table>

$^a$The 3$'$ exonuclease is carried on a separate polypeptide chain, the DnaQ protein.

$^b$The number of nucleotides incorporated per encounter between polymerase and DNA (see page 894).

$^c$MMS (methylmethylene sulfonate) is a DNA-alkylating agent.

*For the holoenzyme*
Composition of DNA Pol III holoenzyme
(10 different proteins, 17 subunits)

- Subunits $\alpha$, $\varepsilon$, $\theta$ = core polymerase; $\alpha = PolC$; $\varepsilon = 3'$ exonuclease; $\theta$?
- Dimeric $\tau$ dimerizes the holoenzyme holding the lead and lag strand polymerases together at the rep fork.
- The $\beta$ subunit or sliding clamp tethers the enzyme to DNA to ↑processivity from 10 to $10^5$.
- The clamp loader ($\gamma$) complex: $\gamma$ (2), $\delta$, $\delta'$, $\chi$, and $\psi$ loads $\beta$ onto DNA.
- $\chi$ mediates switch from RNA primers to DNA and SSB interaction.
- $\psi$ mediates $\gamma$ and $\chi$ interaction.
Model for converting DNA Pol III from a distributive to a processive enzyme

1: ATP binding to γ dimer promotes conformational change (opening) of clamp loader complex.
2: Opening permits clamp loader complex binding to DNA and δ - β interaction.
3: γ forces the subunits of β apart.
4: β encircles DNA and ATP hydrolysis drives ring closure and dissociation of clamp loader complex.

Fig. 24.21

An alternative view of a replication fork asymmetric DNA polymerase III dimer

- Clamp loading need only occur once per round of DNA replication on leading strand.
- Clamp loading must occur multiple times on lagging strand because Pol III needs to rebind at the initiation of synthesis of each Okazaki fragment.

Figure from Ken Marians
Overview of *E. coli* “replisome” proteins and their functional roles

- **Topoisomerase II (DNA gyrase):** relief of topological stress (supercoiling) imposed by action of helicase
- **Helicase (DnaB protein):** DNA helix unwinding in a fixed direction 5′→3′; Rep 3′→5′
- **Primase (DnaG protein):** RNA primer synthesis – 11 mer in *E. coli*. DnaB and DnaG interact.
- **SSB:** maintain template strands in unpaired configuration
- **DNA polymerase III holoenzyme:** lead/lag strand synthesis
- **DNA polymerase I:** removal of RNA primers, gap filling
- **DNA ligase:** joins nicks in dsDNA after Pol I proofreading

Lehninger Principles of Biochemistry, 4th ed., Ch 24
Mechanism of action of type I and II topoisomerases

Type I (change L by 1)
- DNA gyrase
- Single-strand break (topo I and III)

Type II (change L by 2)
- Double-strand break (topo II and IV)
- Relaxation
- Catenation/decatenation
- Knotting/unknotting
Important biochemical features of replication fork proteins

- **Helicases (dsDNA unwinding)**
  - Helicases usually bind to ssDNA near duplex region and move in a fixed direction either 5′→3′ or 3′→5.
  - Movement requires ATP hydrolysis (some use dTTP).
  - Different mechanisms account for movement of homooligomeric dimers (Rep) vs hexameric/ring helicases (DnaB).
    - Ring helicases (DnaB) wrap around one strand displacing the other strand while rotating along the DNA.
    - Homodimeric helicases “roll” along the DNA mediated by sequential ATP binding and hydrolysis.

**Rep helicase action**

![Rep helicase action diagram](image)
Biochemical features of replication fork proteins continued:

- **Primase (DnaG):**
  - Creates new RNA primers for each round of discontinuous synthesis & first round of leading strand synthesis).
  - Requires the action of the DnaB helicase. In T4 and *E. coli*, primers are 5 and 11 nt in length, respectively.

- **SSB**
  - Binds ssDNA in a highly cooperative but non-specific manner as a tetramer.
  - Required for DNA replication, repair, and recombination.
  - Does not denature dsDNA per se but binds to ssDNA after dsDNA is partially unwound.
Biochemical features of replication fork proteins continued:

- Pol III and Pol I
  - III: replicative DNA synthesis
  - I: RNA primer removal and gap filling
- DNA ligase
  - Seals dsDNA “nicks” between Okazaki fragments.
  - Nucleotides must be adjacent and properly base-paired.
  - Activates the 5’ terminal phosphate on the DNA substrate by adenylation.

**Fig. 24.24**
Summary of fork-proximal operations occurring during elongation phase of \textit{E. coli} DNA replication

Head of the fork: Topoisomerase II relieves stress; helicase melts the duplex, SSB coats ssDNA

Leading strand: Addition of new dNTPs to 3’ end of primer - synthesis in same direction as fork movement

Lagging strand: Primase synthesizes RNA primers, Pol III extends to previous Okazaki fragment, dissociates, then reassembles at the 3’ end of a new RNA primer

Gap filling: Removal of RNA primers and filling in of gaps by Pol I, Nick stitched-up by ligase
Structural requirements for initiation of DNA replication in *E. coli*

- **Specific origin** (*oriC*, 245 bp sequence)
  - 4 repeats of 9 bp sequence (DnaA-binding)
  - 3 repeats of 13 bp sequences (A/T-rich)
  - GATC subject to methylation on $N^6$ of adenine by Dam methylase (signal for initiation?)

- **Multiprotein initiation complex**
  - DnaA (Signal for initiation may involved cycling between ATP (active) and ADP (inactive) bound states ~20-40 m.
  - HU and IHF (basic histone-like DNA-bending proteins)
Models for initiation of DNA replication in *E. coli* (only one origin, oriC)

- DnaA (~4-5 molecules/9 bp repeat) binds to the four repeats assisted by HU, IHF(?), and ATP. Strain of DNA wrapping promotes melting of the adjacent 13 bp repeats.
- Assisted by DnaC, hexameric DnaB helicase associates with opposing strands of the 13 bp loop region & continues to unwind the helix.
- Unwound DNA is prepared for use as a replication fork by loading of SSB and DnaG primase.
Fig. 24.8
Termination of DNA replication in *E. coli* occurs at specific sites

- Termination (*ter*) sites (6 related 20 bp segments, 3 each oriented in opposite directions) 180° from *oriC* function as a trap.

- Protein called Tus (*terminus utilization substance*) binds to *ter* to arrest replication fork movement....but

- Tus-DNA complex can halt a fork from only one direction (necessary to prevent over-replication by a faster fork).

- Class II topoisomerase (*topo IV*) then de-catenates daughter chromosomes.
Bacterial replication is organized into a membrane-bound replication “factory”

New origins bound to different points on plasma membrane
Features of DNA replication in eukaryotes

- Many origins > 5,000/genome which only initiate once per cell cycle
  - **Ori**: less sequence conservation

- **Origin Recognition Complex (ORC)**
  - Six polypeptides, binds to yeast ARS in ATP-dependent manner & recruits MCM helicase complex (CDC6/CDT1 plus MCM2 to MCM7)
  - Role for phosphorylation and DNA methylation (?)

- **Mitochondrial DNA**
  - Fixed origins but use two unidirectional forks (~1 h to complete replication)
Kinetic features/enzymology of eukaryotic DNA replication

• Slower fork movement but larger number of origins (~10^3 per 10^8 bp chromosome)
• Five different polymerases (distinguished by their intracellular locations, kinetic properties, and response to inhibitors):
  – Pol α - Okazaki fragment primer synthesis. (Primase activity but no 3′→5′ exonuclease activity and not very processive).
  – Pol δ - Major leading and lagging strand polymerase w/ 3′→5′ exonuclease activity. Highly processive w/ bound PCNA (analogous to *E. coli* Pol III).
  – Pol ε - DNA repair and Okazaki fragment RNA primer removal (perhaps, not definitive).
  – Pol β and Pol γ - repair and mitochondrial DNA replication.
## Properties of eukaryotic DNA polymerases

<table>
<thead>
<tr>
<th></th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>δ</th>
<th>ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell compartment</td>
<td>Nucleus</td>
<td>Nucleus</td>
<td>Mitochondrion</td>
<td>Nucleus</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Associated primase</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Biological function</td>
<td>Lagging strand replication</td>
<td>DNA repair</td>
<td>Mitochondrial DNA replication</td>
<td>Leading strand replication</td>
<td>Replication</td>
</tr>
<tr>
<td>Number of subunits</td>
<td>4</td>
<td>1</td>
<td>4 (identical)</td>
<td>2</td>
<td>?</td>
</tr>
<tr>
<td>( M_r ) of catalytic subunit, kilodaltons</td>
<td>160–185</td>
<td>40</td>
<td>125</td>
<td>125</td>
<td>210–230 or 125–140</td>
</tr>
<tr>
<td>( K_{m} ) for dNTPs, μM</td>
<td>2–5</td>
<td>10(^a)</td>
<td>0.5</td>
<td>2–4</td>
<td>?</td>
</tr>
<tr>
<td>Processivity (inherent)</td>
<td>Moderate</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Processivity (with PCNA)</td>
<td>Moderate</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>3' exonuclease</td>
<td>No(^b)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sensitivity to 2',3'-dideoxy-NTPs</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>Sensitivity to arabinosyl-CTP</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Sensitivity to aphidicolin</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

\(^a\)\( K_{m} \) values for repair synthesis in permeabilized cells are much lower.

\(^b\)A cryptic, or masked, 3' exonuclease activity is associated with polymerase α from *Drosophila.*

**PCNA ~** *E. coli* β sliding clamp of Pol III holoenzyme

**RFC ~** *E. coli* clamp loader γ complex of Pol III holoenzyme

**RFA ~** *E. coli* SSB; RNA primer removal by FEN-1/RNaseH1 or Pol ε
Nuclear genomes & linear chromosomes: nucleosome replication & gaps on 5’ gaps
Summary of DNA Replication

• Genome replication requires a large multi-protein complex ("replisome") selected for speed and processivity.
• In addition to polymerase III, *E. coli* replication forks contain primase, helicase, SSB, topoisomerases, polymerase I and DNA ligase.
• Initiation requires specific DNA sites and an ori-binding-protein, DnaA, which opens DNA for DnaB helicase and primase recruitment.
• Replication of eukaryotic DNA employs analogous factors.
Mechanisms for ensuring fidelity of DNA replication

• Metabolic: balanced levels of dNTPs
• Structural 1: complementary base pairing between dNTP and template (Error ~1 in $10^3$ to $10^4$ bp/round of replication)
• Structural 2: induced fit between Pol and DNA (Cumulative Error ~1 in $10^5$ to $10^6$)
• Enzymatic 1: proofreading by 3′ to 5′ exonuclease (Cumulative Error ~1 in $10^7$ to $10^8$)
• Enzymatic 2: mismatch and other repair systems (1 in $10^{10}$ bp/generation)
Chromosomes from different cell types exhibit different replication parameters

- **Bacteria (E. coli)**
  - Single origin in $\sim 4.6 \times 10^6$ bp chromosome
  - Speed: $\sim 850$ nt/sec/fork
  - Doubling time: $\sim 20-30$ minutes
  - Time to replicate entire genome: $\sim 40$ min
    - Daughter cell receives a chromosome well into its next round of replication.
    - Replicon firing earlier in the cell cycle implicates initiation as the key control point.

- **Eukaryotes (HeLa cell)**
  - Multiple origins on a single chromosome ($10^3-10^4$ /cell)
  - Speed: $\sim 60-90$ nt/sec/fork
  - Doubling time: $\sim 24$ hours in a typical eukaryotic cell
  - Time to replicated entire genome: $\sim 8$ hours (time to S phase of interphase)
  - Initiation is the key control point.