Watson Crick prediction:
Each stand of parent DNA serves as a template for synthesis of a new complementary daughter strand.
Proof of semiconservative DNA replication

Matthew Meselson-Franklin Stahl, 1958

Fig. 4.14
Visualization of bidirectional replication

John Cairns: $^3\text{H}$-thymidine labeling and visualization of 1.7mm *E. coli* chromosome (note loops $\rightarrow$ two new daughter DNAs).

Autoradiograms of *E. coli* chromosome subjected to transient pulse $^3\text{H}$-thymidine labeling implied bidirectional replication.

Lehninger Principles of Biochemistry, 4th ed., Ch 25
Termination and re-initiation occur on opposite sides of *E. coli* chromosome.

Higher specific activity [³H]-thymidine added at end of replication cycle.

Note higher grain density at points of termination and re-initiation.
Ross Inman and coworkers: replication initiates at unique origins

- Denaturing mapping studies
- Bacteriophage $\lambda \sim 48.5$ kbp chromosome partially denatured then fixed to prevent renaturation.
- Observed pattern of ssDNA bubbles via EM.
- Sequences rich in A=T base pairs served as points of origin in replicating $\lambda$.
- Confirmed concept of bidirectional replication
- $oriC$ subsequently identified in $E. coli$ chromosome
Basic chemistry of DNA chain elongation

- \[(dNMP)_n + dNTP \rightarrow (dNMP)_{n+1} + PP_i\]
- Incoming dNTP is positioned in active site by base-pairing with template nucleotide.
- Polymerase catalyzes reaction between the terminal 3′ OH on the primer strand & the 5′ α phosphorus of dNTP to form 3′– 5′ PDE bond.
- Release & hydrolysis of PP$_i$ by pyrophosphatase (~19 kJ/mol generated) favors formation of products.
- Consequently, chain growth can only occur in one direction.

Fig. 24.2
Basic features of DNA replication (constraints: direction & anti-parallel polarity)

- **Semi-conservative**
- **Ordered & sequential**
  - Starts at a fixed point
  - Synthesis in $5' \rightarrow 3'$ direction (topological problem)
- **Semi-discontinuous**
  - Leading stand (same direction as fork)
  - Lagging strand (opposite fork movement)
- **Extremely accurate**
- **Activated substrates (dNTPs)**

Okazaki fragments: In bacteria, ~1000 to 2000 nucleotides long; in eukaryotes, ~150 to 200 nucleotides long.

*Lehninger Principles of Biochemistry, 4th ed., Ch 25*
Directionality of DNA synthesis: a very simple model of a replication fork

- **DNA polymerase III**, replicates as leading and lagging strand dimer

**Leading strand**

**Lagging strand**

**Primasome**

**RNA primer**

**Fork movement**

Lagging strand synthesis requires **repeated RNA priming** due to physical constraint imposed by Pol III dimerization at the fork.

Fig. 24.1
Features of *E. coli* DNA replication coordination of enzyme activities

- **Multi-enzyme “replisome”**
  - Substrates: dNTPs (also need primer-template)
  - Enzymes: Polymerases (Pol III for rapid synthesis & Pol I for editing), DNA gyrase, helicase, primase, & ligase

- **Three main phases:**
  - Initiation: formation of specific protein:DNA complex at oriC
  - Elongation: the actual copying process (Figure)
  - Termination: separation of daughter chromosomes at Ter sites by Tus protein

SSB plays a unique, nonenzymatic role.

Lehninger Principles of Biochemistry, 4th ed., Ch 25
Enzymes and other proteins involved in DNA replication (some multi-subunit complexes)

- DNA polymerase(s)
- ssDNA-binding protein (SSB)
- Helicase (DnaB)
- Primase (DnaG)
- Topoisomerase(s) (DNA gyrase, topo IV)
- DNA Ligase
- Initiation factors (DnaA, DnaC, HU, Dam methylase)

TABLE 25-1 Comparison of DNA Polymerases of *E. coli*

<table>
<thead>
<tr>
<th>DNA polymerase</th>
<th>polA</th>
<th>polB</th>
<th>polC (dnaE)</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>7</td>
<td>≥10</td>
</tr>
<tr>
<td>$M_r$</td>
<td>103,000</td>
<td>88,000$^*$</td>
<td>791,500</td>
</tr>
<tr>
<td>3'→5' Exonuclease (proofreading)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5'→3' Exonuclease</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Polymerization rate (nucleotides/s)</td>
<td>16-20</td>
<td>40</td>
<td>250-1,000</td>
</tr>
<tr>
<td>Processivity (nucleotides added before polymerase dissociates)</td>
<td>3-200</td>
<td>1,500</td>
<td>≥500,000</td>
</tr>
</tbody>
</table>

*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dnaE* is an earlier designation for the gene now referred to as *polC*.

$^*$ Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the $\beta$, $\gamma$, $\delta$, $\delta'$, $\chi$, and $\phi$ subunits (see Table 25-2).
DNA Pol I: the first polymerase (discovered by Arthur Kornberg)

- Three activities:
  - “slow” polymerization
  - 3’ to 5’ exonucleolytic proofreading & repair
  - 5’ to 3’ exonuclease (RNA primer removal, nick translation)

- Modular structure
- Not processive enough for genome replication
- “Cleanup”/repair enzyme

DNA Pol I structure: 103 kDa peptide. Limited tryptic digestion → small N-term 35 kDa fragment and larger C-term 68 kDa fragment (Klenow, used for DNA synthesis \textit{in vitro})
DNA polymerase I: 5'→3' exo activity

Nick Translation – concerted rNMP removal from 5' end of RNA primer & dNMP addition to 3' end of Okazaki fragment

Fig. 24.5
DNA polymerase I (and Pol III): $3'\rightarrow5'$ proofreading activity

- Mechanism based on structural studies
- $3'\rightarrow5'$ exonuclease site positioned ahead of the polymerase active site
- Mismatch impedes enzyme translocation
- Enzyme stalls allowing access to exonuclease active site
- Polymerization can then resume in $5'\rightarrow3'$ direction.

Lehninger Principles of Biochemistry, 4th ed., Ch 25
Klenow family of DNA polymerases
(fingers, palm, thumb subdomains)

Bacteriophage T7 DNA Pol

Bacteriophage RB69, a cousin of phage T4, 1st one
Molecular basis of primer-template recognition and proofreading

Extensive contacts between the T7 DNA Pol enzyme and DNA minor groove explains Pol DNA-binding w/o sequence specificity, detection of base mis-incorporation.

$O^2$ of Pyr and N3 of Pur are universal H-bond acceptors of the minor groove. A-form helix has wider minor groove.

Arg429 and Gln615 are strictly conserved.
DNA polymerase active site appears capable of opening and closing


O Helix = dynamic finger subdomain
Two metal ion mechanism of catalysis by DNA polymerases (T. Steitz model)

Metal ions (Mg$^{2+}$) stabilize a multivalent transition state between phosphate oxygens and two conserved Asp residues which 1) polarizes the primer 3’ OH and 2) facilitates the leaving of pyrophosphate.