14.1 - Introduction

Replisome is the multiprotein structure that assembles at the bacterial replicating fork to undertake synthesis of DNA. It contains DNA polymerase and other enzymes.

A quick-stop mutant is a type of DNA replication temperature-sensitive mutant (dna) in *E. coli* that immediately stops DNA replication when the temperature is increased to 42°C.

A slow-stop mutant is a type of DNA replication temperature-sensitive mutant in *E. coli* that can finish a round of replication at the unpermissive temperature, but cannot start another.

In vitro complementation is a functional assay used to identify components of a process. The reaction is reconstructed using extracts from a mutant cell. Fractions from wild-type cells are then tested for restoration of activity.
14.2 - DNA polymerases are the enzymes that make DNA

The parental duplex is replaced by two identical daughter duplexes, each of which has one parental strand and one newly synthesized strand. It is called **semiconservative** because the conserved units are the single strands of the parental duplexes.
Repair of damaged DNA can take place by repair synthesis, when a strand that has been damaged is excised and replaced by the synthesis of a new stretch. It can also take place by recombination reactions, when the duplex region containing the damaged is replaced by an undamaged region from another copy of the genome.
A DNA polymerase is an enzyme that synthesizes a daughter strand(s) of DNA (under direction from a DNA template).

Any particular enzyme may be involved in repair or replication (or both).

A DNA replicase is a DNA-synthesizing enzyme required specifically for replication.
**E. coli** has 5 DNA polymerases

Only one DNA polymerase is the replicase

The others participate in repair of damaged DNA

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td><em>polA</em></td>
<td>major repair enzyme</td>
</tr>
<tr>
<td>II</td>
<td><em>polB</em></td>
<td>minor repair enzyme</td>
</tr>
<tr>
<td>III</td>
<td><em>polC</em></td>
<td>replicase</td>
</tr>
<tr>
<td>IV</td>
<td><em>dinB</em></td>
<td>SOS repair</td>
</tr>
<tr>
<td>V</td>
<td><em>umuD’2C</em></td>
<td>SOS repair</td>
</tr>
</tbody>
</table>

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14.3 - DNA polymerases have various nuclease activities

*E. coli* DNA polymerase I has a unique 5’-3’ exonuclease activity that can be combined with DNA synthesis to perform nick translation.
14.4 - DNA polymerases control the fidelity of replication

**Processivity** describes the ability of an enzyme to perform multiple catalytic cycles with a single template instead of dissociating after each cycle.

**Proofreading** refers to any mechanism for correcting errors in protein or nucleic acid synthesis that involves scrutiny of individual units after they have been added to the chain.

DNA polymerases often have a 3′-5′ exonuclease activity that is used to excise incorrectly paired bases.

The fidelity of replication is improved by proofreading by a factor of \(~100\).
14.5-DNA polymerases have a common structure

Many DNA polymerases have a large cleft composed of three domains that resemble a hand.

DNA lies across the "palm" in a groove created by the "fingers" and "thumb".

A palm that contains the catalytic site, fingers that position the template, a thumb that binds DNA and is important in processivity, an exonuclease domain with its own active site, and an N-terminal domain.
Crystal structure of phage T7 DNA polymerase: the template strand takes a sharp turn in order to be exposed to the incoming nucleotide (Charles Richardson and Tom Ellenberger)
14.6 - DNA synthesis is semidiscontinuous

The leading strand of DNA is synthesized continuously in the 5’-3’ direction.

The lagging strand of DNA must grow overall in the 3’-5’ direction and is synthesized discontinuously in the form of short fragments (5’-3’) that are later connected covalently.

![Diagram showing two new DNA strands with different features. The leading strand is synthesized continuously, while the lagging strand is synthesized discontinuously. The lagging strand fragments are later connected covalently.](image-url)
Okazaki fragments are the short stretches of 1000-2000 bases produced during discontinuous replication; they are later joined into a covalently intact strand.

Semidiscontinuous replication is the mode in which one new strand is synthesized continuously while the other is synthesized discontinuously.

The DNA replicase advances continuously when it synthesizes the leading strand (5′-3′), but synthesizes the lagging strand by making short fragments that are subsequently joined together.
14.7 - The $\phi$X model system shows how single-stranded DNA is generated for replication

Replication requires a helicase to separate the strands of DNA using energy provided by hydrolysis of ATP.

The single-strand binding protein (SSB) attaches to single-stranded DNA, thereby preventing the DNA from forming a duplex.
The combination of helicase (Rep, unwinding), SSB, and A protein (nicking) separates a φX174 duplex into a single-stranded circle and a single-stranded linear strand.
14.8 - Priming is required to start DNA synthesis

The **priming** end can be provided by an RNA primer, a nick in DNA, or a priming protein.

For DNA replication, a special RNA polymerase called a **primase** synthesizes a short RNA chain that provides the priming end.
*E. coli* has two types of priming reaction, which occur at the bacterial origin (oriC) (involves dnaG) and the φX174 origin (primosome)

Priming of replication on double-stranded DNA always requires a replicase, SSB, and primase

DnaB is the helicase that unwinds DNA for replication in *E. coli*
14.9 - Coordinating synthesis of the lagging and leading strands

Different enzyme units are required to synthesize the leading and lagging strands.

In *E. coli* both these units contain the same catalytic subunit (DnaE).

In other organisms, different catalytic subunits may be required for each strand.
The upper model for the action of lagging strand polymerase is that when an enzyme unit completes one Okazaki fragment, it moves to a new position to synthesize the next fragment.

The lower model is that the lagging strand polymerase dissociates when it completes an Okazaki fragment, and a new enzyme unit associates with DNA to synthesize the next Okazaki fragment.
14.10 - DNA polymerase holoenzyme has 3 subcomplexes

The *E. coli* replicase DNA polymerase III is a 900 kD complex with a dimeric structure.

Each monomeric unit has a **catalytic core** (α subunit, DNA polymerase; ε subunit, 3′-5′ proofreading exonuclease; θ subunit, stimulate exonuclease), a **dimerization** subunit (τ), and a **processivity** (β subunit) component.

A clamp loader (γ, 5) places the processivity subunits on DNA, and they form a circular clamp around the nucleic acid.

One catalytic core is associated with each template strand.
A β dimer plus a γ complex recognizes the primer-template to form a preinitiation complex.

The γ complex uses hydrolysis of ATP to drive the binding of β to DNA and thus enables core polymerase to bind.

A τ dimer binds to the core polymerase and provides a dimerization function.

The γ complex is responsible for adding a pair of β dimers to each parental strand of DNA.
14.11 - The clamp controls association of core enzyme with DNA

The β subunit of DNA pol III holoenzyme consists of a head to tail dimer (in red and orange) that forms a ring completely surrounding a DNA duplex (in the center) (John Kuriyan)
The core on the leading strand is processive because its clamp (a circle of subunits) keeps it on the DNA.

The $\gamma$ clamp loader binds an open form of $\beta$ ring preparatory to loading it on to DNA.

The clamp associated with the core on the lagging strand dissociates at the end of each Okazaki fragment and reassembles for the next fragment.

The helicase DnaB is responsible for interacting with the primase DnaG to initiate each Okazaki fragment.
The helicase creating the replication fork is connected to β dimer, each is held on to DNA by a sliding clamp (γ clamp loader).

The pol that synthesizes the leading strand moves continuously. The pol that synthesizes the lagging strand dissociates at the end of an Okazaki fragment and then reassociates with a primer in the single-stranded template loop to synthesize the next fragment.
Each catalytic core of Pol III synthesizes a daughter strand. DnaB is responsible for forward movement at the replication fork. DnaB contacts the $\tau$ subunits of the $\gamma$ complex; increase the movement of DNA pol 10$\times$ and increase processivity.
Core polymerase and the $\beta$ clamp dissociate at completion of Okazaki fragment synthesis and reassociate at the beginning.

Recognition of the sites for initiating Okazaki fragments: in oriC replicons, the connection is DnaB.
14.12-Okazaki fragments are linked by ligase

DNA ligase makes the bond that connects the 3’ end of one Okazaki fragment to the 5’ beginning of the next fragment.

Each Okazaki fragment starts with a primer and stops before the next fragment.

DNA polymerase I removes the primer and replaces it with DNA in an action that resembles nick translation.
DNA ligase seals nicks between adjacent nucleotides

The *E. coli* DNA ligase uses NAD cofactor, while T4 DNA ligase uses ATP
14.13-Separate eukaryotic DNA polymerases undertake initiation and elongation

A replication fork has 1 complex of DNA polymerase $\alpha$/ primase and 2 complexes of DNA polymerase $\delta$ and/or $\epsilon$

The DNA polymerase $\alpha$/primase complex initiates the synthesis of both DNA strands

DNA polymerase $\delta$ elongates the leading strand and a second DNA polymerase $\delta$ or DNA polymerase $\epsilon$ elongates the lagging strand
<table>
<thead>
<tr>
<th>DNA polymerase</th>
<th>Function</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>High fidelity replicases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α</td>
<td>Nuclear replication</td>
<td>350 kD tetramer</td>
</tr>
<tr>
<td>δ</td>
<td>&quot;</td>
<td>250 kD tetramer</td>
</tr>
<tr>
<td>ε</td>
<td>&quot;</td>
<td>350 kD tetramer</td>
</tr>
<tr>
<td>γ</td>
<td>Mitochondrial replication</td>
<td>200 kD dimer</td>
</tr>
<tr>
<td>High fidelity repair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>Base excision repair</td>
<td>39 kD monomer</td>
</tr>
<tr>
<td>Low fidelity repair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ζ</td>
<td>Thymine dimer bypass</td>
<td>heteromer</td>
</tr>
<tr>
<td>η</td>
<td>Base damage repair</td>
<td>monomer</td>
</tr>
<tr>
<td>π</td>
<td>Required in meiosis</td>
<td>monomer</td>
</tr>
<tr>
<td>κ</td>
<td>Deletion and base substitution</td>
<td>monomer</td>
</tr>
</tbody>
</table>
The high processivity of DNA polymerase δ results from its interaction with RF-C and PCNA. The RF-C and PCNA are analogous to the *E. coli* γ clamp loader and β processivity unit. The RF-C is a clamp loader that catalyzes the loading of PCNA to DNA. It binds to the 3′ end of iDNA and uses ATP-hydrolysis to open the ring of PCNA. The processivity of polymerase δ is maintained by PCNA, which tethers the polymerase δ to the template.
14.14 - Phage T4 provides its own replication apparatus

Phage T4 provides its own replication apparatus, which consists of DNA polymerase, the gene 32 SSB, a helicase, a primase, and accessory proteins that increase speed and processivity.

<table>
<thead>
<tr>
<th>Function</th>
<th>E.coli</th>
<th>HeLa/SV40</th>
<th>Phage T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helicase</td>
<td>DnaB</td>
<td>T antigen</td>
<td>41</td>
</tr>
<tr>
<td>Loading helicase/primase</td>
<td>DnaC</td>
<td>T antigen</td>
<td>59</td>
</tr>
<tr>
<td>Single strand maintenance</td>
<td>SSB</td>
<td>RPA</td>
<td>32</td>
</tr>
<tr>
<td>Priming</td>
<td>DnaG</td>
<td>Polα/primase</td>
<td>61</td>
</tr>
<tr>
<td>Sliding clamp</td>
<td>β</td>
<td>PCNA</td>
<td>45</td>
</tr>
<tr>
<td>Clamp loading (ATPase)</td>
<td>γδ complex</td>
<td>RFC</td>
<td>44/62</td>
</tr>
<tr>
<td>Catalysis</td>
<td>Pol III core</td>
<td>Polδ</td>
<td>43</td>
</tr>
<tr>
<td>Holoenzyme dimerization</td>
<td>τ</td>
<td>?</td>
<td>43</td>
</tr>
<tr>
<td>RNA removal</td>
<td>Pol I</td>
<td>MF1</td>
<td>43</td>
</tr>
<tr>
<td>Ligation</td>
<td>Ligase</td>
<td>Ligase 1</td>
<td>T4 ligase</td>
</tr>
</tbody>
</table>
14.15 - Creating the replication forks at an origin: Initiation at oriC
DnaA binds to short repeated sequences and forms an oligomeric complex that melts DNA

6 DnaC monomers bind each hexamer of DnaB and this complex binds to the origin

A hexamer of DnaB forms the replication fork. Gyrase and SSB are also required
14.16 - Common events in priming replication at the origin

The general principle of bacterial initiation is that the origin is initially recognized by a protein that forms a large complex with DNA.

A short region of A•T-rich DNA is melted.

DnaB is bound to the complex and creates the replication fork.

Transcription initiating at $P_R$ is required to activate the origin of lambda DNA.
The lambda origin for replication comprises two regions. Early events are catalyzed by O protein, which binds to a series of 4 sites, then DNA is melted in the adjacent A-T-rich region.
14.17 - The primosome is needed to restart replication

The primosome describes the complex of proteins involved in the priming action that initiates replication on φX-type origins. It is also involved in restarting stalled replication forks.

Initiation of φX replication requires the primosome complex to displace SSB from the origin.

A replication fork stalls when it arrives at damaged DNA.

After the damage has been repaired, the primosome is required to reinitiate replication.

The Tus protein binds to ter sites and stops DnaB from unwinding DNA, which causes replication to terminate.
When replication halts at damaged DNA, the damaged sequence is excised, and the complementary (newly synthesized) strand of the other daughter duplex crosses over to repair the gap. Replication can now resume, and the gaps are filled in.
The **primosome** is required to restart a stalled replication fork after the DNA has been repaired.
Tus binds to ter asymmetrically and blocks replication in only one direction.
14.18 - Does methylation at the origin regulate initiation?

Hemimethylated DNA is methylated on one strand of a target sequence that has a cytosine on each strand.

oriC contains **11 GATC** repeats that are methylated on adenine on both strands.

Replication generates hemimethylated DNA, which cannot initiate replication.

There is a **13 min delay** before the GATC repeats are remethylated.
Only fully methylated origins can initiate replication; hemimethylated daughter origins cannot be used again until they have been restored to the fully methylated state.

While it is hemimethylated, the dnaA promoter is repressed.
14.19 - Origins may be sequestered after replication

*SeqA* binds to hemimethylated DNA and is required for delaying rereplication. SeqA may interact with DnaA. While the origins are hemimethylated, they bind to the cell membrane, and may be unavailable to methylases.
14.20 - Licensing factor controls eukaryotic rereplication

**Licensing factor** is necessary for initiation of replication at each origin.

It is present in the nucleus prior to replication, but is inactivated or destroyed by replication.

Initiation of another replication cycle becomes possible only after licensing factor reenters the nucleus after mitosis.
Licensing factor in the nucleus is inactivated after replication.

A new supply of licensing factor can enter only when the nuclear membrane breaks down at mitosis.
14.21 - Licensing factor consists of MCM proteins

The prereplication complex is a protein-DNA complex at the origin in *S. cerevisiae* that is required for DNA replication. The complex contains the ORC complex, Cdc6, and the MCM proteins.

The postreplication complex is a protein-DNA complex in *S. cerevisiae* that consists of the ORC complex bound to the origin.
The ORC is a protein complex that is associated with yeast origins throughout the cell cycle.

Cdc6 protein is an unstable protein that is synthesized only in G1.

Cdc6 binds to ORC and allows MCM proteins to bind.

When replication is initiated, Cdc6 and MCM proteins are displaced. The degradation of Cdc6 prevents reinitiation.

Some MCM proteins are in the nucleus throughout the cycle, but others may enter only after mitosis.