DNA replication
Semiconservative DNA replication
(Meselson-Stahl experiment)
Replication of DNA
New nucleotides are added to DNA only during replication in the 5’-3’ direction.
DNA synthesis takes place simultaneously but in opposite directions on the two DNA templates.
DNA synthesis is **continuous** (leading strand) on one template of DNA and **discontinuous** (lagging strand) on the other.
Priming of DNA synthesis with a RNA segment
(RNA primer)

**Primase** synthesizes short stretches of RNA nucleotides, providing a 3’-OH group to which DNA polymerase can add DNA nucleotides.
On the leading strand, where replication is continuous, a primer is required only at the 5’ end of the newly synthesized strand.

On the lagging strand, with discontinuous replication, a new primer must be generated at the beginning of each Okazaki fragment.
DNA helicase unwinds DNA by binding to the lagging-strand template at each replication fork and moving in the 5'-3' direction along the strand by breaking hydrogen bonds.

Primase forms a complex with helicase (primosome).

SSB (single-stranded binding) proteins stabilize the exposed single-stranded DNA.
How double helix unwind

- Helix unwinding
- Double-stranded break
- Gyrase
- Helix swivels

Swivel of broken strands; breaks in duplex repaired; stress relieved; more helix unwinding
DNA polymerases in *E. coli*

DNA polymerase III

DNA polymerase I
DNA polymerase III

5'-3' polymerase activity
Proofreading role of DNA polymerase III

Hydrolysis due to 3'-5' exonucleolytic activity
DNA polymerase III- a large multiprotein complex

- α- actividade polimerase 5’-3’
- ε- “ exonuclease 3’-5’
- β- aumenta processividade da enzima
- θ- necessária ao assembly de α ε τ
- τ- mantém estrutura do dímero e contacta com DnaB (helicase)

Modelo assimétrico da DNA polimerase III apoia o modelo da replicação simultânea das duas cadeias
A β subunit tethers the core of *E. coli* DNA polymerase III to DNA thereby increasing its processivity.
DNA polymerase I

5'-3' exonuclease activity

5'-3' polymerase activity

DNA ligase

DNA polymerase I also have 3'-5' exonuclease activity

1st ribonucleotide of RNA primer is trifosphatated
ACTIVITIES

(A) 5′→3′ DNA synthesis

- DNA polymerase I
- DNA polymerase III

Synthesis

(B) 3′→5′ exonuclease activity

- DNA polymerase I
- DNA polymerase III

Removes incorrect nucleotide

(C) 5′→3′ exonuclease activity

- DNA polymerase I

Displaces incorporated nucleotides
### Table 12.3 Characteristics of DNA Polymerases in *E. coli*

<table>
<thead>
<tr>
<th>DNA Polymerase</th>
<th>5’→3’ Polymerization</th>
<th>3’→5’ Exonuclease</th>
<th>5’→3’ Exonuclease</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Removes and replaces primers</td>
</tr>
<tr>
<td>II</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>DNA repair; restarts replication after damaged DNA halts synthesis</td>
</tr>
<tr>
<td>III</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Elongates DNA</td>
</tr>
<tr>
<td>IV</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>DNA repair</td>
</tr>
<tr>
<td>V</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>DNA repair; translesion DNA synthesis</td>
</tr>
</tbody>
</table>
Klenow fragment
(large DNA polymerase I fragment)

DNA polymerase I (103 kDa)

- 5'-3' polymerase activity
- 5'-3' exonuclease activity
- 3'-5' exonuclease activity

Klenow fragment (large DNA polymerase I fragment) (68 kDa)

- 5'-3' polymerase activity
- 5'-3' exonuclease activity
- 3'-5' exonuclease activity

DNA polymerase I has the unique ability to start replication \textit{in vitro} at a \textit{nick} in DNA

Small fragment (35 kDa)

- 5'-3' exonuclease activity
Topoisomerase I

Gyrase (topoisomerase II)

Helicase

DNA polymerase complex (contains Pol III)

DNA ligase

RNA primase complex (primosome)

DNA polymerase complex (contains Pol III)

Single-strand binding proteins (SSB proteins)

Unwinding of parental DNA

Twisting of DNA strands ahead of replication fork

Transient break serves as a swivel to allow free rotation of DNA strands
Replisome
Relationship between *E. coli* replication proteins at a growing fork

In this model, DNA must form a loop so that both strands can replicate simultaneously.
Model of DNA replication in *E. coli*, where two units of DNA polymerase III are connected.

The lagging strand loops around so that 5’-3’ synthesis can take place on both antiparallel strands.
Origin of replication
Model of initiation of replication at \textit{E. coli oriC} (Konberg and collab.)

- **245 bp**
- **Typical 13-mer**: GATCTATTTATTT
- **Typical 9-mer**: TTATCCACA

DNA forced to unwind in 13-mers

Ligation of DnaA (initiator proteins) occurs when DNA is negatively supercoiled

Activates DnaG (primase)

Unwinding allows helicase and other SSB proteins to attach to single-stranded DNA
<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiator protein</td>
<td>Binds to origin and separates strands of DNA to initiate replication</td>
</tr>
<tr>
<td>DNA helicase</td>
<td>Unwinds DNA at replication fork</td>
</tr>
<tr>
<td>Single-strand-binding</td>
<td>Attach to single-stranded DNA and prevent reannealing</td>
</tr>
<tr>
<td>proteins</td>
<td></td>
</tr>
<tr>
<td>DNA gyrase</td>
<td>Moves ahead of the replication fork, making and resealing breaks in the</td>
</tr>
<tr>
<td></td>
<td>double-helical DNA to release torque that builds up as a result of</td>
</tr>
<tr>
<td></td>
<td>unwinding at the replication fork</td>
</tr>
<tr>
<td>DNA primase</td>
<td>Synthesizes short RNA primers to provide a 3’-OH group for attachment</td>
</tr>
<tr>
<td></td>
<td>of DNA nucleotides</td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td>Elongates a new nucleotide strand from the 3’-OH group provided by the</td>
</tr>
<tr>
<td></td>
<td>primer</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>Removes RNA primers and replaces them with DNA</td>
</tr>
<tr>
<td>DNA ligase</td>
<td>Joins Okazaki fragments by sealing nicks in the sugar–phosphate</td>
</tr>
<tr>
<td></td>
<td>backbone of newly synthesized DNA</td>
</tr>
</tbody>
</table>
Unidirectional vs bidirectional replication

Circular DNA molecule

Linear DNA molecule
Modes of Replication

Theta
Rolling circle
Linear
Theta replication is a type of common in *E. coli* and other organisms possessing **circular DNA**

Double-stranded DNA

Double-stranded DNA unwinds at the replication of origin

Origin of replication (oriV site)

Newly synthesized DNA

Strands separate at *oriV* (vector)

The fork proceeds around the circle

Two DNA molecules are produced

The products of theta replication are two circular DNA molecules

Producing single-stranded templates for the synthesis of new DNA. A **replication bubble** forms, usually having a replication fork at each end (bidirectional replication)
Rolling-circle replication
(circular DNA)

3’-OH at the nick is the **growing point** where DNA synthesis begins. The inner strand is used as a **template**

The 3’ end grows around the circle giving rise to the name rolling-circle model

Takes place in some **virus** and in the **F factor** of *E. coli*
The cycle may be repeated

(A) One complete revolution

(B) Two complete revolutions

Two copies (or more) of same sequence of linear DNA
The linear molecule circularizes

- after serving as a template for the synthesis of a complementary strand

- or either before serving as a template
Bidirectional model of linear eukaryotic replication
<table>
<thead>
<tr>
<th>Replication Model</th>
<th>DNA Template</th>
<th>Breakage of Nucleotide Strand</th>
<th>Number of Replicons</th>
<th>Unidirectional or Bidirectional</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theta</td>
<td>Circular</td>
<td>No</td>
<td>1</td>
<td>Unidirectional or bidirectional</td>
<td>Two circular molecules</td>
</tr>
<tr>
<td>Rolling circle</td>
<td>Circular</td>
<td>Yes</td>
<td>1</td>
<td>Unidirectional</td>
<td>One circular molecule and one linear molecule that may circularize</td>
</tr>
<tr>
<td>Linear eukaryotic</td>
<td>Linear</td>
<td>No</td>
<td>Many</td>
<td>Bidirectional</td>
<td>Two linear molecules</td>
</tr>
</tbody>
</table>

- D-loop
- One or several linear molecules
Termination of DNA replication in *E. coli*: the role of terminator sequences *ter* during DNA replication in *E. coli*

Replication termini in *E. coli* are located beyond the point at which the replication forks actually meet.
Eucaryotic replication
Linear DNA replication takes place in eukaryotic chromosomes at several origins of replication.

Replication begins and is bidireccional.

Synthesis starts at all the origins of replication.

Replication bubbles fuse where they meet.
ARS- autonomously replicating sequence, that acts as an origin of replication in *S. cerevisiae*

A, B1, B2 and B3- functional sequences

**Melting of the helix** occurs within the subdomain B2, induced by the attachment of ARS binding protein (ABFI) to subdomain B3.

The proteins of the *origin of replication complex* (ORC) are permanently attached to subdomains A and B1.
Number and length of replicons

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of replication origins</th>
<th>Average length of replicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> (bacterium)</td>
<td>1</td>
<td>4 200 000</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisae</em> (yeast)</td>
<td>500</td>
<td>40 000</td>
</tr>
<tr>
<td><em>Drosophila melanogaster</em> (fruit fly)</td>
<td>3 500</td>
<td>40 000</td>
</tr>
<tr>
<td><em>Xenopus laevis</em></td>
<td>15 000</td>
<td>200 000</td>
</tr>
<tr>
<td><em>Mus musculus</em> (mouse)</td>
<td>25 000</td>
<td>150 000</td>
</tr>
</tbody>
</table>
Events in DNA replication in *E. coli* and eukaryotes

- Polimerase $\delta$ on leading and maybe lagging strand;
- Polimerase $\epsilon$ maybe on lagging strand;
- Polimerase $\alpha$ initiates both the leading and lagging strands (contains primase activity);
- Polimerase $\gamma$ in mitochondrial DNA replication.
Table 12.5 DNA polymerases in eukaryotic cells

<table>
<thead>
<tr>
<th>DNA Polymerase</th>
<th>$5' \rightarrow 3'$ Polymerase Activity</th>
<th>$3' \rightarrow 5'$ Exonuclease Activity</th>
<th>Cellular Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>α (alpha)</td>
<td>Yes</td>
<td>No</td>
<td>Initiation of nuclear DNA synthesis and DNA repair</td>
</tr>
<tr>
<td>β (beta)</td>
<td>Yes</td>
<td>No</td>
<td>DNA repair and recombination of nuclear DNA</td>
</tr>
<tr>
<td>γ (gamma)</td>
<td>Yes</td>
<td>Yes</td>
<td>Replication of mitochondrial DNA</td>
</tr>
<tr>
<td>δ (delta)</td>
<td>Yes</td>
<td>Yes</td>
<td>Leading- and lagging-strand synthesis of nuclear DNA, DNA repair, and translesion DNA synthesis</td>
</tr>
<tr>
<td>ε (epsilon)</td>
<td>Yes</td>
<td>Yes</td>
<td>Unknown; probably repair and replication of nuclear DNA</td>
</tr>
<tr>
<td>ζ (zeta)</td>
<td>Yes</td>
<td>No</td>
<td>Translesion DNA synthesis</td>
</tr>
<tr>
<td>η (eta)</td>
<td>Yes</td>
<td>No</td>
<td>Translesion DNA synthesis</td>
</tr>
<tr>
<td>θ (theta)</td>
<td>Yes</td>
<td>No</td>
<td>DNA repair</td>
</tr>
<tr>
<td>ι (iota)</td>
<td>Yes</td>
<td>No</td>
<td>Translesion DNA synthesis</td>
</tr>
<tr>
<td>κ (kappa)</td>
<td>Yes</td>
<td>No</td>
<td>Translesion DNA synthesis</td>
</tr>
<tr>
<td>λ (lambda)</td>
<td>Yes</td>
<td>No</td>
<td>DNA repair</td>
</tr>
<tr>
<td>μ (mu)</td>
<td>Yes</td>
<td>No</td>
<td>DNA repair</td>
</tr>
</tbody>
</table>
In eukaryotes the primase forms a complex with DNA polymerase α, which is shown synthesizing the RNA primer followed by the first few nucleotides of DNA.
Removal of the RNA primer from each Okazaki fragment in eukaryotes, by FEN I endonuclease

There appears to be NO DNA polymerase with 5’-3’ exonuclease activity in eukaryotes.

The “flap endonuclease” FEN I cannot initiate primer degradation because its activity is blocked by the triphosphate group present at the 5’ end of the primer.
Two models for completion of lagging strand replication in eukaryotes

(A) The flap model

- DNA polymerase δ + helicase push aside the primer
- FEN1 cuts at the branch point
- Missing phosphodiester bond
- DNA ligase links the two DNA fragments

(B) The RNase H model

- RNase H removes the primer, up to the last ribonucleotide
- FEN1 removes the last ribonucleotide, plus some of the DNA
- DNA ligase links the two DNA fragments

RNase H - degrades the RNA strand of RNA-DNA hybrids
Telomeres replication

Telomeres:
- have simple repeating sequences
- seal the chromosome ends
- are synthesized by a ribonucleoprotein enzyme
- are essential for survival
Como se resolve o problema da extremidade 3’ projectada originada durante o processo de replicação do DNA?
(c) End of a linear chromosome

- Synthesis of primer
- 3′ OH
- Elongation of DNA
- Removal of primer

Gap left by removal of primer
The mechanism of restoring the ends of a DNA molecule in a chromosome relies on an enzyme called **TELOMERASE**.

Telomerase elongates the template DNA strand at the 3’ end.

The telomerase contains an internal RNA with a sequence complementary to the telomere repeat.
Mechanism of action of telomerase

(a) $5'\text{CCCAA} 3'\text{GGGTTGGGTT}$

(b) RNA template

Telomerase

(c) $5'\text{CCCAA} 3'\text{GGGTTGGGTT}$

New DNA

(d) $5'\text{CCCAA} 3'\text{GGGTTGGGTT}$

(e) $5'\text{CCCAA} 3'\text{GGGTTGGGTT}$
Nonconventional base pairing

DNA replication
G-quartet structure formed by hydrogen bonding between four guanine bases present in a single DNA strand folded back upon itself.
Models of telomere structure in Oxytricha and Tetrahymena

(A) *Oxytricha*

(B) *Tetrahymena*
Replication slippage

A trinucleotide repeat in the act of replication

The 3’ end of the growing strand momentarily detaches from the template and reanneals to the template at a point upstream from its original location

Continued replication duplicates the region between the points of detachment and reannealing

Mismatch repair of the shorter strand creates a duplex with a trinucleotide expansion

Doenças neurodegenerativas (doenças de expansão de repetições de trinucleotídeos)
Fragile-X syndrome: CGG repeat present in the FMR1 gene

RNA transcript: the CGG repeat is in a part of the messenger RNA that is not translated.

Wildtype allele: This example represents the most common form, which has 30 repeats denoted (CGG)_{30}.

Expansion to premutation: Repeats range from (CGG)_{60} to (CGG)_{100}, in this example (CGG)_{100}. The gene is still functional but mutates in a single generation to the full mutation.

Expansion to full mutation: Repeats number more than 230, in this example (CGG)_{500}. Methylation of the repeat and other CG sites downstream shuts off transcription of the gene.

Methyl (−CH_{3}) groups added to a fraction of the cytosine nucleotides in the full mutation.

Transcription starts here

ATG "start" codon

Protein-coding sequence

Transcription eliminated
<table>
<thead>
<tr>
<th>Disease</th>
<th>Repeated Sequence</th>
<th>Normal Range</th>
<th>Disease Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinal and bulbar muscular atrophy</td>
<td>CAG</td>
<td>11–33</td>
<td>40–62</td>
</tr>
<tr>
<td>Fragile-X syndrome</td>
<td>CGG</td>
<td>6–54</td>
<td>50–1500</td>
</tr>
<tr>
<td>Jacobsen syndrome</td>
<td>CGG</td>
<td>11</td>
<td>100–1000</td>
</tr>
<tr>
<td>Spinocerebellar ataxia (several types)</td>
<td>CAG</td>
<td>4–44</td>
<td>21–130</td>
</tr>
<tr>
<td>Autosomal dominant cerebellar ataxia 37–~220</td>
<td>CAG</td>
<td>7–19</td>
<td></td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
<td>CTG</td>
<td>5–37</td>
<td>44–3000</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>CAG</td>
<td>9–37</td>
<td>37–121</td>
</tr>
<tr>
<td>Friedreich ataxia</td>
<td>GAA</td>
<td>6–29</td>
<td>200–900</td>
</tr>
<tr>
<td>Dentatorubral-pallidoluysian atrophy</td>
<td>CAG</td>
<td>7–25</td>
<td>49–75</td>
</tr>
<tr>
<td>Myoclonus epilepsy of the Unverricht-Lundborg type*</td>
<td>CCCCCGCCCCCGCG</td>
<td>2–3</td>
<td>12–13</td>
</tr>
</tbody>
</table>

*Technically not a trinucleotide repeat but does entail a multiple of three nucleotides that expands and contracts in similar fashion to trinucleotide repeats.