DNA Replication, Repair & Recombination

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**Structure**

**Deoxyribonucleotides**
(Base - adenine, guanine, cytosine, or thymidine)

**Ribonucleotides**
(Base - adenine, guanine, cytosine, or uracil)
Structure

**Purines**
- Deoxyadenosine Triphosphate (dATP)
- Deoxyguanosine Triphosphate (dGTP)

**Pyrimidines**
- Deoxycytidine Triphosphate (dCTP)
- (Deoxy)thymidine Triphosphate (dTTP)
Structure

One Strand of DNA

5’ End

Phosphodiester Bonds

3’ End

Deoxyribose Numbering
Structure

- Introduction
Structure

- Introduction

Bases Paired in Middle

Phosphates and Sugars on Outside

Major Groove

Minor Groove

10.5 Base Pairs Per Turn of the Helix
DNA Structure

Strands of Opposite Polarity
DNA Forms

A-Form

Right-Handed Helix Orientation

B-Form

Left-Handed Helix Orientation

Z-Form
Semi-Conservative Replication

Discontinuous Replication on Lagging Strand (Okazaki Fragments)

Continuous Replication on Leading Strand
Leading and Lagging Strand DNA Replication

Leading Strand

Lagging Strand
DNA Replication

- 5’ to 3’ Polymerase
- Read Base in Opposite Strand
- Select Proper Bases to Put on New Strand
- Join New Base to New Strand with Phosphodiester Bond
DNA Replication

- 5’ to 3’ Polymerase
  - Read Base in Opposite Strand
  - Select Proper Bases to Put on New Strand
  - Join New Base to New Strand with Phosphodiester Bond
- 3’ to 5’ Exonuclease (Proofreading)
  - “Reads” Base Pair Just Created for Correctness.
  - Removes if Mismatched
DNA Replication

- DNA Replication Begins at a Specific Sequence in the DNA Called an Origin
- Proteins Binding at the Origin Help to “Open” the Double Helix to Allow Access of the Replication Proteins
- Replication Requires a “Reader” of Template and Catalytic Function to Make Phosphodiester Bonds
DNA Polymerase I Structure

- Thumb
- Palm
- Fingers
DNA Replication Proteins

- DNA Polymerase
- Primase
- SSB
- Helicases
- Topoisomerase
- Primer Remover
- DNA Ligase
Replication Fork
Chemical Mechanism of Chain Elongation
Leading / Lagging Strand Replication

- **Leading Strand**
- **Lagging Strand**
- **Okazaki Fragments**
- **Replication Fork**

**Action of DNA Ligase**

**Removal of RNA Primers**

**Gaps between Okazaki fragments filled by repair DNA Polymerase**

**DNA fragments sealed by DNA Ligase**
DNA Topoisomers

- Linear
- Relaxed Circle
- Supercoiled
DNA Topology
- Super-Helicity

Relaxed Circular DNA
10.5 Base Pairs Per Turn

Alteration in Number of Base Pairs Per Turn Creates Tension

Enzymes called Topoisomerasers Can Create or Relieve Tension

Super-Helix Formation Relieves Tension
Topoisomers/Topoisomerase

L = T+W

L = Linking Number

T = Twists

W = Writhes
Polymerase Chain Reaction

1. Using Knowledge of the Sequence, Design and Chemically Synthesize DNA Primers Flanking the Sequence to be Amplified
2. Mix Target DNA, Primers, dNTPs, and a Thermostable DNA Polymerase in a Tube
3. Add to a Thermocycling System
4. Begin a 3 Step Process
Polymerase Chain Reaction

Begin Process by Heating Mixture to Near Boiling

Step 1: Denature

Strands Separate
Polymerase Chain Reaction

Cool Mixture to Temperature Near $T_m$ of Primer-DNA Hybrid

Step 2: Anneal Primers

Primers Form Duplexes With Complementary Sequences in DNA
Polymerase Chain Reaction

Warm Mixture to Allow Replication to Occur

Step 3: Replicate

Primers Define Replication Locations in DNA
Polymerase Chain Reaction

Repeat Process for 30-40 Cycles

Step 1: Denature

Step 2: Anneal Primers

Step 3: Replicate

Repeat Steps 1-3
DNA Proof-reading
Prokaryotic vs Eukaryotic DNA Replication

- Introduction

<table>
<thead>
<tr>
<th>Prokaryotic DNA Replication</th>
<th>Eukaryotic DNA replication</th>
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<tbody>
<tr>
<td>Occurs inside the cytoplasm</td>
<td>Occurs inside the nucleus</td>
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<tr>
<td>Only one origin of replication per molecule of DNA</td>
<td>Have many origins of replication in each chromosome</td>
</tr>
<tr>
<td>Origin of replication is about 100-200 or more nucleotides in length</td>
<td>Each origin of replication is formed of about 150 nucleotides</td>
</tr>
<tr>
<td>Replication occurs at one point in each chromosome</td>
<td>Replication occurs at several points simultaneously in each chromosome</td>
</tr>
<tr>
<td>Only have one origin of replication</td>
<td>Has multiple origins of replication</td>
</tr>
<tr>
<td>Initiation is carried out by protein DnaA and DnaB</td>
<td>Initiation is carried out by the Origin Recognition Complex</td>
</tr>
<tr>
<td>Topoisomerase is needed</td>
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</tr>
<tr>
<td>Replication is very rapid</td>
<td>Replication is slow</td>
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Chromosomes With Telomeres
Linear DNA Replication

When RNA primers are removed, these sequences can’t be replaced.
Telomerase Action
Telomerase Action

Telomerase binds to 3’ flanking end of telomere that is complementary to telomerase RNA.

Bases are added using RNA as template.

Telomerase relocates.

Second step is repeated.

DNA polymerase complements the lagging strand.
Nucleotide Excision Repair

Bulky DNA Adduct
Nucleotide Excision Repair
Nucleotide Excision Repair
Nucleotide Excision Repair

- Thymine Dimers
Nucleotide Excision Repair

Thymine Dimer Repair

Partial Strand Removal
Base Excision Repair

Cytosine Deamination

Cytosine $\xrightarrow{\text{+ H}_2\text{O} - \text{NH}_3}$ Uracil
Base Excision Repair

Flipping out of base from helix
Base Excision Repair
Uracil-N-Glycosylase Catalysis

Excision of Base
DNA Mismatch Repair

Mismatch
DNA Mismatch Repair

Unmethylated, recognizable

Methylated, unrecognizable
Measuring Mutation

Ames Test

- Rat liver extract
- Possible mutagen
- Salmonella strain (requires histidine)
- Media with minimal histidine
- Control plate (natural revertants)

A high number of revertants (his- to his+) suggests the mutagen causes mutations.
DNA Recombination

homologous chromosomes

sister chromatids

exchanged by recombination

sister chromatids
DNA Recombination

- Strand Invasion
Structure

• Introduction
DNA Recombination

• Holliday Junction
Recombination/Repair
RecA Recombination Repair in *E. coli*