Ch 30: DNA Structure, Replication, and Repair

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DNA Structure, Replication, and Repair

I. Overview

- Nucleic acids: Storage + expression of genetic information.
    - DNA: Repository of genetic information (genome).
    - RNA: Expression and translation of genetic information.
- Locations of DNA:
    - Eukaryotes: Chromosomes (nucleus), mitochondria, chloroplasts (plants).
    - Prokaryotes: Single chromosome + plasmids (extrachromosomal DNA).
- Genetic information flow:
    - Replication → DNA copied for daughter cells.
    - Transcription → DNA → RNA.
    - Translation → RNA → protein.

- This is the Central Dogma of molecular biology.
- Exceptions: Some viruses use RNA as genetic material.

- Gene expression:
  - Each cell expresses only the genes required for its function.
  - Fertilized egg → DNA directs development → billions of cells.
  - DNA must:
    1. Replicate precisely during division.
    2. Express selectively for functional RNA + proteins.

II. DNA Structure

- DNA = Polymer of dNMPs (deoxyribonucleoside monophosphates).

- Linkage: Covalently linked via 3′–5′ phosphodiester bonds.

- Form: Primarily double-stranded (dsDNA) helix (except some viruses with ssDNA).

- Protein association:
    - Eukaryotes → DNA + proteins = nucleoprotein (in nucleus).
    - Prokaryotes → DNA + proteins = nucleoid (non-membrane bound).
- Levels of structure:
    - Primary: Nucleotide sequence.
    - Secondary: Double helix.

A. 3′–5′ Phosphodiester Bonds

- Bond formation:
    - Joins 3′-OH of one deoxyribose to 5′-OH of next via phosphate group.
- Chain polarity:
    - 5′ end = free phosphate group.
    - 3′ end = free hydroxyl group.
    - By convention → sequence written 5′ → 3′ (e.g., TACG = thymine-adenine-cytosine-guanine).

- Cleavage:
    - Enzymatic: Nucleases.
        - DNases (DNA).
        - RNases (RNA).
    - Chemical: Hydrolytic cleavage.
    - Note: RNA (not DNA) is cleaved by alkali.

B. DNA Double Helix

- General structure:
    - Two DNA chains → coiled around common axis (helical axis).
    - Strands oriented antiparallel (5′ → 3′ paired with 3′ → 5′).
- Backbone:
    - Hydrophilic sugar-phosphate backbone → outside.
- Bases:
    - Hydrophobic bases stacked inside → ladder-like arrangement.

- Grooves:
    - Major groove (wide).
    - Minor groove (narrow).
    - Provide binding sites for regulatory proteins (sequence recognition).
- Clinical correlation:
    - Certain anticancer drugs (e.g., Dactinomycin / Actinomycin D) intercalate into the minor groove, blocking DNA + RNA synthesis → cytotoxic effect.

I. Base Pairing

- Complementary pairing:
    - A ↔ T (Adenine with Thymine).
    - G ↔ C (Guanine with Cytosine).
- Chargaff's rule:
    - In dsDNA → A = T, G = C.
    - Total purines (A + G) = pyrimidines (T + C).

- Bonding & stability:
    - 2 H-bonds between A-T.
    - 3 H-bonds between G-C → stronger.
    - Bases are stacked parallel along the axis → hydrophobic interactions + H-bonds stabilize helix.
- Implication: Knowing one strand allows determination of the complementary strand.

2. DNA Strand Separation

- Disruption of H-bonds → strand separation.
- Methods:
    - Change in pH (ionizes bases).
    - Heat (thermal denaturation).
- Phosphodiester bonds remain intact (only H-bonds disrupted).
- Melting temperature (Tm):
    - Temp. at which 50% of helix is lost (half DNA is ssDNA).

- Monitored by absorbance at 260 nm (↑ in ssDNA vs dsDNA).

- GC vs AT content:

  - High AT DNA → lower Tm.
  - High GC DNA → higher Tm (stronger bonding).

- Reannealing (renaturation):

  - Cooling or neutralizing pH → strands reform helix.
  - Occurs naturally during DNA and RNA synthesis.

3. Structural Forms of DNA

- B-DNA (biological form):

  - Right-handed helix.
  - 10 base pairs / turn.
  - Bases perpendicular to helical axis.
  - Major form in chromosomal DNA.

- A-DNA:

  - Right-handed helix.
  - 11 base pairs / turn.

- Bases tilted 20° from perpendicular.
- Found in DNA–RNA hybrids and dsRNA regions.

- Z-DNA:

  - Left-handed helix.
  - 12 base pairs / turn.
  - Backbone has zigzag pattern.
  - Found in alternating purine–pyrimidine sequences (e.g., GC repeats).
  - May play role in gene expression regulation (B ↔ Z transitions).

C. Linear and Circular DNA Molecules

- Eukaryotes:

  - Nuclear chromosomes → linear dsDNA bound with histone + nonhistone proteins → chromatin.
  - Mitochondria + chloroplasts → closed circular dsDNA.

- Prokaryotes:

  - Typically one circular dsDNA chromosome.
  - DNA compacted with nonhistone proteins →

nucleoid.

- Also contain plasmids:

    - Small circular extrachromosomal DNA.
    - Replicate independently of chromosome.
    - Often carry genes for antibiotic resistance.
    - Facilitate horizontal gene transfer between bacteria.
    - Widely used as vectors in recombinant DNA technology.

III. Steps in Prokaryotic DNA Replication

Overview

- Semiconservative replication:

    - Each daughter molecule contains one parental strand + one newly synthesized strand (antiparallel orientation).

- DNA polymerases:

    - Template-directed, Mg^{2+}-dependent.
    - High fidelity in replication.

- Origins:
    - Prokaryotes (E. coli): single origin of replication (oriC).
    - Eukaryotes: multiple origins (faster replication of large DNA molecules).
- Commitment: Once initiated, replication proceeds until entire genome is duplicated.

A. Complementary Strand Separation

- Requirement: DNA polymerases require single-stranded DNA (ssDNA) templates.
- Origin of replication (ori/oriC in E. coli):
    - Specific consensus sequence recognized by initiator proteins.
    - Contains AT-rich regions (easier to melt due to only 2 H-bonds between A-T).
- Eukaryotes: Multiple origins per chromosome.
- Outcome: Localized melting → short stretch of ssDNA exposed.

B. Replication Fork Formation

- Process:
    - Strands unwind bidirectionally from ori → two replication forks form.
    - Together they create a replication bubble.
    - Each fork resembles a Y-shaped structure.

1. Required Proteins (Prepriming Complex in *E. coli*)

- DnaA protein:
    - Binds to specific sequences (DnaA boxes) in oriC.
    - Initiates melting of AT-rich DNA unwinding element → ssDNA generated.

- DNA helicases (e.g., DnaB, requires DnaC):
    - Bind near replication fork.
    - Use ATP hydrolysis to unwind dsDNA.
    - Cause local unwinding but introduce torsional strain (supercoils).

- Single-stranded DNA-binding (SSB) proteins:

- Bind cooperatively to exposed ssDNA.
- Functions:
  - Prevent reannealing (favor ssDNA state).
  - Protect ssDNA from nucleases.
  - Keep template available for DNA polymerase.
- Note: Not enzymes — purely stabilizing proteins.

2. Solving the Problem of Supercoils

- Cause: Helicase unwinding introduces torsional strain:
  - Ahead of fork → positive supercoils.
  - Behind fork → negative supercoils.
- Effect: Positive supercoils hinder further strand separation.
- Solution: DNA topoisomerases
  - Enzymes that relieve supercoiling by transiently cleaving DNA strands (one or both), allowing rotation, then resealing.
- Analogy: Telephone cord — twisting tighter = positive supercoils; twisting opposite = negative supercoils.

III. Steps in Prokaryotic DNA Replication (continued)

a. Type I DNA Topoisomerases

- Function: Relieve supercoiling by transiently nicking one strand of DNA.

- Mechanism:

    - Cleave one DNA strand → rotate around intact strand → reseal nick.
    - Do not require ATP (energy from phosphodiester bond cleavage is reused for resealing).

- Activity:

    - *E. coli*: relax negative supercoils.
    - Many prokaryotes & eukaryotes: relax both negative and positive supercoils.

b. Type II DNA Topoisomerases

- Function: Relieve supercoiling by transiently cutting both strands of DNA.

- Mechanism:
    - Break both strands → pass another segment of dsDNA through break → reseal.
    - Requires ATP hydrolysis.
- Special enzyme: DNA gyrase (in bacteria & plants):
    - Introduces negative supercoils into circular DNA.
    - Helps neutralize positive supercoils ahead of replication fork.
    - Facilitates both DNA replication and transcription.
- Clinical relevance:
    - Anticancer drugs:
        - Camptothecins → inhibit human topoisomerase I.
        - Etoposide → inhibits human topoisomerase II.
    - Antibacterial drugs:
        - Fluoroquinolones (e.g., ciprofloxacin) → inhibit bacterial DNA gyrase.

C. Direction of DNA Replication

- Polarity:
    - DNA polymerases read template 3′→5′.
    - New strand synthesized 5′→3′ (antiparallel to template).
- Result: Both strands copied but by different mechanisms.

1. Leading strand

- Synthesized continuously.
- Oriented toward replication fork.

2. Lagging strand

- Synthesized discontinuously.
- Oriented away from replication fork.
- Produced in short DNA stretches = Okazaki fragments.
- Fragments later joined by DNA ligase into continuous strand.

D. RNA Primer Requirement

- DNA polymerases cannot initiate de novo synthesis.
- Require a short RNA primer with a free 3′-OH group to start nucleotide addition.
- Primer forms a DNA–RNA hybrid.

1. Primase (DnaG)

- A special RNA polymerase.
- Synthesizes ~10-nucleotide RNA primers complementary to template.
- Uses ribonucleoside triphosphates as substrates, releasing pyrophosphate (PPi).
- Strand differences:
    - Leading strand: only one primer at the origin.
    - Lagging strand: new primers required for each Okazaki fragment.

2. Primosome

- Formed when primase is added to prepriming complex.

- Functions:
    - Synthesizes RNA primer for leading strand.
    - Repeatedly initiates Okazaki fragments on lagging strand.
- Primer synthesis also occurs in 5′→3′ direction.

III. Steps in Prokaryotic DNA Replication (continued)

E. Chain Elongation

- General principle:
    - DNA polymerases elongate DNA by adding deoxyribonucleotides to the 3′-OH end of the growing strand.
    - Template strand dictates base sequence (complementary base-pairing).
    - Substrates: dATP, dTTP, dCTP, dGTP.
    - Reaction is 5′→3′ polymerization.

1. DNA Polymerase III

- Main enzyme of elongation in prokaryotes.

- Starts at the 3′-OH of RNA primer.

- Features:

    - Highly processive → stays bound to template.
    - Processivity due to β-subunit sliding clamp, held in place by clamp loader (ATP-dependent).
    - Hydrolysis of pyrophosphate (PPi → 2 Pi) drives reaction irreversibly.
    - Requires all 4 dNTPs; synthesis stalls if concentration drops below Km.

2. Proofreading Activity of DNA Pol III

- Has 3′→5′ exonuclease activity in addition to 5′→3′ polymerase.

- Proofreading ensures high fidelity:

    - If incorrect nucleotide is added → polymerase halts.
    - 3′→5′ exonuclease removes mispaired base.
    - Correct base is reinserted.

- Example of mutation from failed proofreading: sickle

cell anemia (A→T substitution in β-globin gene).

F. RNA Primer Excision and Replacement by DNA

- DNA pol III stops when it reaches RNA primer.
- DNA Polymerase I removes primer and replaces it with DNA.

1. DNA Pol I Activities

- 5′→3′ exonuclease: removes RNA primer nucleotides (unique to Pol I).
- 5′→3′ polymerase: fills gap with DNA.
- 3′→5′ exonuclease: proofreads new DNA.
- Functions simultaneously: primer removal, gap filling, error correction.
- Also used in DNA repair processes.

2. Comparison of Exonuclease Functions

- 5′→3′ exonuclease (Pol I): removes RNA primers (or damaged DNA) from the 5′ end.

- 3′→5′ exonuclease (Pol I & Pol III): removes mismatched bases from 3′ end of growing DNA.

- Combined activities → replication error rate ≤ 1 mistake per 10^7 nucleotides.

G. DNA Ligase

- Role: Joins DNA fragments by sealing the final phosphodiester bond.

- Links:

    - 5′-phosphate end of DNA synthesized by Pol III.
    - 3′-OH end of DNA synthesized by Pol I.

- Energy source: ATP → AMP + PPi.

H. Termination

- In *E. coli*:

    - Specific sites = Ter sites.
    - Protein = Tus (terminus utilization substance).
    - Tus binds Ter sites → halts replication fork

movement → ensures proper termination.

IV. Eukaryotic DNA Replication

General Features

- Eukaryotic DNA replication is fundamentally similar to prokaryotic replication.

- Key differences:

    - Multiple origins of replication in eukaryotes (vs. single origin in prokaryotes).
    - RNA primer removal by RNase H and flap endonuclease 1 (FEN1) (instead of DNA polymerase I).
    - Similar proteins identified: origin recognition proteins, ssDNA-binding proteins, ATP-dependent helicases.

A. Eukaryotic Cell Cycle

- Cell cycle = events of DNA replication + mitosis (cell division).

- Phases:

- G1 phase (Gap 1): Cell grows; precedes replication.
- S phase (Synthesis): DNA replication occurs.
- G2 phase (Gap 2): Prepares for mitosis.
- M phase (Mitosis): Cell division.
- G0 phase: Non-dividing/quiescent cells (e.g., mature T lymphocytes). Can reenter G1 upon stimulation.

- Regulation:

  - Checkpoints ensure each phase is complete before next begins.
  - Controlled by cyclins and cyclin-dependent kinases (Cdks).

B. Eukaryotic DNA Polymerases

- Five major high-fidelity polymerases, named with Greek letters.

1. DNA pol α (pol α/primase):

   - Multisubunit enzyme.
   - Primase subunit synthesizes short RNA primer.
   - Extended by 5′→3′ polymerase activity, producing short DNA stretches.

- Function: Initiates replication on leading strand and each Okazaki fragment.

2. DNA pol ε (epsilon):

   - Completes DNA synthesis on the leading strand.
   - Proofreads using 3′→5′ exonuclease activity.
   - Works with PCNA (proliferating cell nuclear antigen), a sliding clamp for high processivity.

3. DNA pol δ (delta):

   - Elongates Okazaki fragments on the lagging strand.
   - Has 3′→5′ exonuclease proofreading.

4. DNA pol β (beta):

   - Involved in DNA repair (gap filling).

5. DNA pol γ (gamma):

   - Replicates mitochondrial DNA.

C. Telomeres

- Definition: DNA–protein complexes (with shelterin

proteins) at chromosome ends.

- Functions:
    - Protect ends from nuclease attack.
    - Distinguish chromosome ends from dsDNA breaks.
    - Maintain chromosome integrity.
- Structure:
    - Human telomeric repeat: AGGGTT (G-rich) paired with AACCCT (C-rich).
    - G-rich strand extends beyond C-strand → leaves 3′ ssDNA overhang.
    - Overhang folds into loop structure stabilized by proteins.

I. Telomere Shortening

- Occurs in most somatic cells because gap left after RNA primer removal cannot be filled.
- Leads to progressive shortening with each cell division.
- Once critical length reached → cell becomes senescent (no further division).

- Not shortened in:
    - Germ cells
    - Stem cells
    - Cancer cells (due to telomerase activity).

2. Telomerase

- Ribonucleoprotein complex:
    - TERT (telomerase reverse transcriptase): synthesizes DNA.
    - TERC (telomerase RNA component): provides template (C-rich RNA).
- Mechanism:
    - TERC base pairs with G-rich 3′ overhang.
    - TERT extends DNA in 5′→3′ direction.
    - Process repeats, elongating telomere.
    - Pol α primase then lays primer, extends with DNA pol α, primer later removed.
- Clinical importance:

- Telomeres act as "mitotic clocks."
- Research relevance in aging, premature aging diseases (progerias), cancer biology.

D. Reverse Transcriptases

- RNA-directed DNA polymerases.
- Found in:
  - Telomerase (TERT uses RNA template for DNA synthesis).
  - Retroviruses (e.g., HIV):
    - Viral ssRNA genome → reverse transcriptase synthesizes DNA.
    - DNA integrates into host genome.
  - Transposons (retrotransposons):
    - Mobile DNA elements; transcribed to RNA → reverse transcribed → inserted into genome.

E. DNA Replication Inhibition by Nucleoside Analogs

- Modified nucleosides → converted to nucleotides in cells → incorporated into DNA → block chain

elongation.

Examples:

1. Didanosine (ddI):
    - 2′,3′-dideoxyinosine (lacks 3′-OH).
    - Prevents further elongation.
2. Cytarabine (araC):
    - Sugar replaced with arabinose.
    - Used in anticancer chemotherapy.
3. Vidarabine (araA):
    - Adenine arabinoside.
    - Antiviral drug.
4. Azidothymidine (AZT/ZDV):
    - Thymidine analog with azido group.
    - Used in HIV therapy.

V. Eukaryotic DNA Organization

General Overview

- Human diploid somatic cell: 46 chromosomes, total DNA ≈ 2 m long.
- DNA must be efficiently packaged to:
    - Fit inside the nucleus.
    - Allow replication and gene expression.
- Packaging requires DNA interaction with proteins, primarily histones.
- DNA + protein complex = chromatin.
- Basic structural unit = nucleosome ("beads on a string").

A. Histones and Nucleosome Formation

1. Histones

- Five classes: H1, H2A, H2B, H3, H4.
- Features:
    - Small, evolutionarily conserved.

- Rich in lysine and arginine → positively charged at physiologic pH.
- Bind negatively charged DNA phosphate groups via ionic interactions.
- Also stabilized by cations (e.g., Mg^{2+}).

2. Nucleosomes

- Core: Octamer of 2 × (H2A, H2B, H3, H4).
- ~146 bp DNA wraps around core nearly twice → introduces negative supercoiling.
- Histone modifications (epigenetics):
  - Acetylation, methylation, phosphorylation at N-terminal tails.
  - Regulate histone–DNA interactions → influence gene expression.
- Linker DNA (~50 bp): Connects adjacent nucleosomes.
- H1 histone:
  - Not part of core.
  - Binds linker DNA.

- Promotes tighter packing → more compact chromatin.
- Most tissue-specific and species-specific histone.

3. Higher Levels of Organization

- Nucleosomes stack → nucleofilament (30-nm fiber).
- Fiber forms loops anchored to nuclear scaffold proteins.
- Higher order folding → final chromosomal structure (visible in mitosis).

B. Nucleosome Fate During DNA Replication

- Parental nucleosomes disassembled for replication.
- After DNA synthesis → nucleosomes rapidly reassemble.
- Histones come from both new synthesis and parental histone recycling.

VI. DNA Repair

General Features

- DNA damage arises from:
    - Replication errors (mispaired bases, insertions).
    - Chemical insults (e.g., nitrous acid → base deamination).
    - Radiation:
        - UV light: induces pyrimidine dimers.
        - Ionizing radiation: causes double-strand breaks.
    - Spontaneous base loss/alteration (thousands per cell/day).

- If unrepaired → mutations → disease (e.g., cancer).

- Most repair mechanisms = excision repair systems:

    i. Recognize DNA lesion.
    ii. Excise damaged region.
    iii. Fill gap using complementary strand as template.
    iv. Seal with ligase.

- Excision repair typically removes 1–10s of nucleotides.

- DNA repair can occur outside S phase.

A. Mismatch Repair (MMR)

1. Replication Error Escape

- Sometimes proofreading fails → mismatched bases remain.
- Timing: Acts within minutes after replication.
- Error rate reduction: From 1 in 10^7 → 1 in 10^9 nucleotides.

2. Mismatch Identification

- Prokaryotes (E. coli):
    - Mediated by Mut proteins.
    - Distinguish correct (parental) vs. incorrect (daughter) strand via methylation.
    - DAM methylase methylates adenine in GATC sequences (every ~1000 bp).
    - Newly synthesized strand is unmethylated (hemimethylated DNA) → identified as daughter strand for repair.
- Eukaryotes:

- Mechanism less defined.
- Likely involves recognition of nicks in newly synthesized strand.

3. Repair Process

i. Endonuclease makes nick in daughter strand.
ii. Exonuclease removes mismatch and surrounding nucleotides.
iii. Gap filled by DNA polymerase (e.g., pol δ in humans; pol III in prokaryotes).
iv. Sealed by DNA ligase.

4. Clinical Significance

- Defective MMR in humans → Lynch syndrome (HNPCC).
  - Mutations in MSH2 and MLH1 (human homologs of Mut proteins).
  - Accounts for ~90% of Lynch syndrome cases.
- Cancer risk: ↑ risk of colorectal cancer (and others).
- Epidemiology: ~5% of all colon cancers are due to defective MMR.

B. Nucleotide Excision Repair (NER)

- Cause of damage
    - UV radiation → covalent joining of two adjacent pyrimidines (usually thymine).
    - Forms pyrimidine dimers → bulky intrastrand cross-links.
    - Block DNA polymerase → prevents replication beyond lesion.
- Recognition and excision of UV-induced dimers
    - In bacteria:
        - uvrABC excinuclease recognizes dimer.
        - Cuts on both 5′ and 3′ sides of lesion.
        - Short oligonucleotide (with dimer) excised → leaves a gap.
        - Gap filled by DNA polymerase I + sealed by DNA ligase.
    - In humans:
        - Pathway more complex → removes pyrimidine dimers + other bulky lesions (e.g., G adducts from benzo[a]pyrene in cigarette smoke).
        - NER active throughout cell cycle.

- Damage recognition mechanisms in humans
    - Global genomic repair → scans entire genome.
    - Transcription-coupled repair → fixes lesions encountered by RNA polymerase during transcription.
- Clinical correlation
    - Xeroderma pigmentosum (XP):
        - Defect in NER proteins (mutations in 7 possible XP genes).
        - UV-induced dimers not repaired → mutation accumulation.
        - Results in early and multiple skin cancers.

C. Base Excision Repair (BER)

- Cause of damage
    - Spontaneous base alterations:
        - C deamination → U.
        - A deamination → hypoxanthine.
        - G deamination → xanthine.
    - Alkylation damage: e.g., dimethyl sulfate → methylates adenine.

- Spontaneous base loss (hydrolysis) → ~10,000 purines lost per cell per day.

- Step 1: Abnormal base removal

  - Recognized by DNA glycosylases.
  - Enzyme hydrolytically cleaves altered base → leaves AP site (apyrimidinic/apurinic).

- Step 2: AP site recognition and repair

  - AP endonucleases → cut 5′ side of AP site.
  - Deoxyribose phosphate lyase → removes sugar-phosphate residue.
  - Gap filled by DNA polymerase I.
  - Sealed by DNA ligase.

D. Double-Strand Break Repair

- Causes

  - Ionizing radiation.
  - Chemotherapy drugs (e.g., doxorubicin).
  - Oxidative free radicals.
  - Physiologic: during recombination.

- Challenge
    - dsDNA breaks cannot be repaired by single-strand excision repair → both strands are broken.

- Repair systems

    1. Nonhomologous End Joining (NHEJ):

        - Proteins recognize, process, and ligate broken ends.
        - DNA loss occurs → error-prone and mutagenic.
        - Defects → predisposition to cancer + immunodeficiency syndromes.

    2. Homologous Recombination (HR):

        - Uses enzymes of meiotic recombination.
        - Requires homologous DNA as a template → accurate ("error-free").
        - Occurs in late S and G2 phases (when a sister chromatid is present).
        - NHEJ can occur at any time in cell cycle.
        - Mutations in BRCA1 and BRCA2 (HR proteins) → ↑ risk of breast & ovarian cancers.