BIOSYNTHESIS OF DNA AND RNA: REPLICATION AND TRANSCRIPTION

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Nucleic Acids are important for their roles in the storage, transfer and expression of genetic information.

HOW DO YOU TRANSFER INFORMATION TO THE NEXT GENERATION?

HOW DO YOU DECODE THE INFORMATION AND MAINTAIN THE CELL’S STRUCTURE AND FUNCTION?
Transfer and interpretation of genetic information is described in the central dogma of molecular biology.
REPLICATION OF DNA
DNA is passed on thru replication and is semi conservative.

**Figure 11-1 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons**

- (a) Semiconservative replication
- (b) Conservative replication
DNA is passed on thru replication and is semi conservative.

Step 1: DNA extracted from cells is mixed with CsCl solution and placed in centrifuge tube.

Step 2: Solution is centrifuged for a few days at very high speed.

Step 3: DNA molecules move to positions where their density equals that of CsCl solution.

CsCl forms a density gradient with the greatest density at the bottom.

Density gradient centrifugation
DNA is passed on thru replication and is semi conservative.

Direction of sedimentation

Parent $^{15}\text{N}$-DNA (both strands heavy)

Normal $^{14}\text{N}$-DNA (with two light strands)

Preliminary experiment
DNA is passed on thru replication and is semi conservative.

**Experimental results**

1. Hybrid DNA
2. After one generation on $^{14}$N-NH$_4$Cl
3. Light DNA
4. Hybrid DNA
5. After two generations on $^{14}$N-NH$_4$Cl

**Conclusions**

- First generation:
  Both DNAs contain one light and one heavy strand.

- Second generation:
  Two hybrid DNAs and two light DNAs are formed.
DNA is passed on thru replication and is semiconservative.
Replication start at discrete points on DNA and is bidirectional.
Replication start at discrete points on DNA and is bidirectional.

**Tandem array of 13-mer sequences (AT rich)**

**Binding sites for DnaA protein**

**Consensus sequence**

5’-G A T C T N T T N T T T T T-3’
3’-C T A G A N A A N A A A A A-5’
Replication start at discrete points on DNA and is bidirectional

(a) Unidirectional replication

(b) Bidirectional replication

Garrett & Grisham: Biochemistry, 2/e
Figure 30.5
Replication start at discrete points on DNA and is bidirectional
Replication is semi-discontinuous.

Leading strand is copied continuously

Motion of replication fork

Parental strands

Leading strand

Lagging strand (Okazaki fragments)

Lagging strand is copied in segments which must be joined

Figure 11-9 Concepts in Biochemistry, 3/e
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Replication is semi-discontinuous.
• If Watson and Crick were right, then there should be an enzyme that makes DNA copies from a DNA template

• In 1957, Arthur Kornberg and colleagues demonstrated the existence of a DNA polymerase - **DNA polymerase I (Pol I)**

• Pol I needs all four deoxyribonucleotides, a template and a primer - a ss-DNA (with a free 3'-OH) that pairs with the template to form a short double-stranded region
DNA elongation happens through the action of DNA Polymerases.

- template-directed enzymes
- 5 classes are identified
- all DNA polymerase have common structural features
- Klenow fragment
DNA elongation happens through the action of **DNA Polymerases**.

- specificity dictated by H-bonding and shape complementarity between bases
  - binding of correct base is favorable (more stable)
  - interaction of residues in the enzyme to the minor groove of DNA
  - close down around the incoming NTP

*Figure 23-18*  
Bachelder, Sixth Edition  
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DNA Polymerase creates the phosphodiester bond from a pre-existing 3’-OH and a dNTP creating a pyrophosphate.

Figure 4-22
Biochemistry, Sixth Edition
© 2007 W.H. Freeman and Company
The first DNA polymerases were discovered in *E. coli*.

### Table 11.1

**Comparison of *E. coli* DNA polymerases**

<table>
<thead>
<tr>
<th>DNA Polymerase Characteristic</th>
<th>I (103,000)</th>
<th>II (88,000)</th>
<th>III (900,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular mass (daltons)</td>
<td>103,000</td>
<td>88,000</td>
<td>900,000</td>
</tr>
<tr>
<td>Polypeptide subunits&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Polymerization rate (nucleotides added per second)</td>
<td>16–20</td>
<td>7</td>
<td>250–1000</td>
</tr>
<tr>
<td>3’ → 5’ Exonuclease activity</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5’ → 3’ Exonuclease activity</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>a</sup>The number of polypeptide subunits defines the quaternary structure.

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*Table 11-1 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons*
The exonuclease activity of DNA Polymerases are important for proofreading.

- proofreading mechanisms
  - Klenow fragment – removes mismatched nucleotides from the 3'' end of DNA (exonuclease activity)
  - detection of incorrect base
    - incorrect pairing with the template (weak H-bonding)
    - unable to interact with the minor groove (enzyme stalls)
The exonuclease activity of DNA Polymerases are important for proofreading.
DNA polymerase III holoenzyme replicates the *E. coli* chromosome. Several other proteins are also important:

**Table 11.2**

Proteins necessary for DNA replication in *E. coli*

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helicase</td>
<td>Begins unwinding of DNA double helix</td>
</tr>
<tr>
<td>DNA gyrase</td>
<td>Assists unwinding</td>
</tr>
<tr>
<td>SSB proteins</td>
<td>Stabilize single strands of DNA</td>
</tr>
<tr>
<td>Primase</td>
<td>Synthesis of RNA primer</td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td>Elongation of chain by DNA synthesis</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>Removal of RNA primer and filling in gap with DNA</td>
</tr>
<tr>
<td>DNA ligase</td>
<td>Closes last phosphoester gap to form phosphodiester bond</td>
</tr>
</tbody>
</table>
Unwinding of DNA helix:

- dnaB helicase – disrupts H-bonds holding the 2 strands
- DNA gyrase – introduces negative supercoils
Unwinding of DNA helix:

SSB (single-stranded DNA-binding protein)
  • binds to unwound strands to prevent annealing
  • forms prepriming complex
Primer

RNA primes the synthesis of DNA.

Primase synthesizes short RNA.
Elongation by DNA Polymerase III
DNA Polymerase I cleaves RNA primer and inserts dNTPs.
- **DNA ligase** seals breaks in the *double stranded DNA*
- DNA ligases use an energy source (ATP in eukaryotes and archaea, NAD\(^+\) in bacteria) to form a phosphodiester bond between the 3’ hydroxyl group at the end of one DNA chain and 5’-phosphate group at the end of the other.
**DNA ligase** seals breaks in the *double stranded DNA*

- DNA ligases use an energy source (ATP in eukaryotes and archaea, NAD⁺ in bacteria) to form a phosphodiester bond between the 3’ hydroxyl group at the end of one DNA chain and 5’-phosphate group at the end of the other.
DNA replica

34

Leading strand “core”
DNA polymerase III

SSB

γ

Rep protein

Leading strand

Lagging strand

Helicase II

Primosome

3rd RNA primer (most recent)

Lagging strand “core”
DNA polymerase III

2nd RNA primer

Growing Okazaki fragment

1st RNA primer

(b)

2nd RNA primer

Almost completed Okazaki fragment

1st RNA primer to be replaced with DNA by polymerase I; nick sealed by DNA ligase.

3rd RNA primer
DNA replica

sites

2nd RNA primer

Almost completed Okazaki fragment

1st RNA primer to be replaced with DNA by polymerase I; nick sealed by DNA ligase.

3rd RNA primer

4th RNA primer (most recent)

Newly initiated Okazaki fragment

2nd RNA primer

Saunders College Publishing
Linear DNA gets shorter after each replication cycle. To protect DNA, **Telomeres** are added to the ends of DNA.

DNA_telomeres
Telomeres are synthesized by Ribonuclearprotein complexes called Telomeras.
Telomerases use RNA template to synthesize DNA. (REVERSE TRANSCRIPTASE!)

- Primer required, but a strange one - a tRNA molecule that the virus captures from the host
- RT transcribes the RNA template into a complementary DNA (cDNA) to form a DNA:RNA hybrid
Reverse Transcriptase is very common in viruses

- Three enzyme activities
  - RNA-directed DNA polymerase
  - RNase H activity - degrades RNA in the DNA:RNA hybrids
  - DNA-directed DNA polymerase - which makes a DNA duplex after RNase H activity destroys the viral genome
- HIV therapy: AZT (or 3'-azido-2',3'- dideoxythymidine) specifically inhibits RT
RECAP:

1. DNA replication is semi-conservative, bidirectional and semi-discontinuous.
2. Helicases, DNA gyrase and SSB are needed to prepare a DNA for replication.
3. RNA primase creates primers to start DNA replication.
4. DNA is synthesized from a pre-existing 3’-OH in the 5’-3’ direction by DNA polymerases (with proofreading mechanism).
5. DNA ligase connects fragments.
6. DNA is shortened after replication, to prevent adverse effects, telomeres are present.
7. DNAs can be synthesized from RNA template via reverse transcriptase.
DNA DAMAGE AND REPAIR
Changes in the base sequence of DNA are called **mutations**. They may have adverse effect on the normal biological functions or **silent**. Mutations may be **spontaneous** or **induced**.

The gene defect is a known mutation of a single nucleotide (**T to A**) of the β-globin gene, which results in glutamate being substituted by valine at position 6.

Spontaneous mutations are those that occur during normal genetic and metabolic functions.

1. Substitution of base pair
   a. Transition
   b. Transversion

2. Deletion of base pair/s

3. Insertion of base pair/s
Nitrogenous bases may be modified by naturally occurring species (water) and reactive oxygen species (ROS).

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Repaired by DNA glycosidases cleave the nitrogenous bases
Environmental factors also cause mutations. These are called **mutagens**. One type of this mutagen are ionizing radiations.
Chemical Agents can modify DNA: (1) Heterocyclic base analogs, (2) Reactive chemicals, (3) Intercalating agents

5-Bromouracil (5-BU) (keto tautomer, pairs with A)      (5-BU) (enol tautomer, pairs with G)   Guanine

5-BU is an example of heterocyclic base analogs.
Chemical Agents can modify DNA: (1) Heterocyclic base analogs, (2) Reactive chemicals, (3) Intercalating agents

- Nitrous acid (HNO\(_2\))
  - Converts adenine to hypoxanthine, cytosine to uracil, and guanine to xanthine
  - Causes A-T to G-C transitions
Chemical Agents can modify DNA: (1) Heterocyclic base analogs, (2) Reactive chemicals, (3) Intercalating agents

(a) Dimethyl nitrosamine
(b) Guanine

Figure 11-17 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons
Chemical Agents can modify DNA: (1) Heterocyclic base analogs, (2) Reactive chemicals, (3) Intercalating agents

- Intercalating agents are flat aromatic compound. Causes insertion or deletion

![Chemical structures](image-url)
DNAs repair damage via nucleotide excision or base excision.

Mismatches bases

Step 1

Endonuclease, H₂O
DNAs repair damage via nucelotide excision
DNAs repair damage via nucleotide excision

Step 3

Polymerase, dATP

Figure 11-13 part 3 Concepts in Biochemistry, 3/e
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DNAs repair damage via nucleotide excision
RECAP:

1. Integrity of DNA can be jeopardized during replication.

2. Damage may also come in the form of natural reactions from the environment: via chemical or radiation.

3. DNA repairs itself via base excision or nucleotide excision processes.
TRANSCRIPTION OF DNA TO RNA
Mechanism of RNA synthesis is similar to DNA synthesis.

- RNA Polymerases
  - Template (DNA)
  - Activated precursors (NTP)
  - Divalent metal ion (Mg$^{2+}$ or Mn$^{2+}$)
Mechanism of RNA synthesis is similar to DNA synthesis.

5’-GTCCCAATTGGATGCCCTAGCTCGCTAGAATAGAGATATAGAGATAAACC- 3’

5’ DNA coding strand 3’

3’ DNA template (SENSE) strand 5’

3’-CAGGGTTAACCTACGGGATCGAGCGATCTTATCTCTATATCTCTATTTGG-5’

5’-GTCCCAATTGGATGCCCTAGCTCGCTAGAATAGAGATATAGAGATAAACC- 3’

5’ DNA coding strand 3’

5’-GUCCCAAUUGGAUGCCCCUAGCUAGCUCGUAGAAUAGAGAUAGAGUAAACC- 3’

5’-GUCCCAAUUGGAUGCCCCUAGCUAGCUCGUAGAAUAGAGAUAGAGUAAACC- 3’

5’ RNA Pol 3’

mRNA

DNA template strand

3’-CAGGGTTAACCTACGGGATCGAGCGATCTTATCTCTATATCTCTATTTGG-5’
Mechanism of RNA synthesis is similar to DNA synthesis.
Different types of RNA polymerase encode for different types of RNA. Most common of which is RNA Polymerase II

<table>
<thead>
<tr>
<th>Type of RNA Polymerase</th>
<th>Genes transcribed</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA Polymerase I</td>
<td>5.8S, 18S and 28S rRNA genes</td>
</tr>
<tr>
<td>RNA Polymerase II</td>
<td>All protein-coding genes, plus snoRNA genes and some snRNA genes</td>
</tr>
<tr>
<td>RNA Polymerase III</td>
<td>tRNA genes, 5S rRNA genes and some snRNA genes and genes for other small RNAs</td>
</tr>
</tbody>
</table>
Intrinsic markers in the DNA molecule direct transcription enzymes where to start. These are called **promoter sites**.
RNA polymerase attach to DNA and start creating RNA Primary transcripts. NOTE, no primer is needed!
In eukaryotes the alpha subunit is not a simple compound, but rather LOTS of transcription factors which have varied roles in promoting and controlling transcription.
RNA polymerase attach to DNA and start creating RNA. NOTE, no primer is needed!
RNA polymerase interacts with DNA and start creating RNA.

**NOTE:** no primer is needed!

**Step 3**

ρ factor interacts with RNA polymerase; transcription is terminated.

RNA, DNA, RNA polymerase, and ρ factor are released.

RNA polymerase is released.

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Figure 11-22 part 3 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons
A terminator factor recognizes specific DNA sequences and signals RNA Pol to stop encoding RNA from the DNA and start creating a terminal sequence.

In *E. coli* it creates a hairpin tail with many Uracils.
In eukaryotes, a poly-A tail is synthesized by poly-A polymerase and poly-A binding proteins after termination of RNA sequence.
RNA can also be synthesized from fellow RNA (via RNA replicase).
Different kinds of RNAs require different post transcription modifications

- Methylated GTP cap
- Polyadenylation
- Splicing

RNA

mRNA

Base modification
Acetylation

tRNA
Primary transcripts for tRNA production are cut up in several places by nucleases.

Nitrogenous bases are modified (mostly methylated)

Amino acyl group is added on the 3’-OH end
RNAs have a triphosphate at the 5’ end, which is capped by GMP and then methylated.

Capping ensures the fate of an mRNA as a code carrying molecule. (It allows the body to distinguish mRNA from other types of RNA.)
Primary transcripts for mRNA containing non-coding regions called introns.

- **In**trons
- **In**tervening sequences
- **Ex**ons
- **Ex**pressed sequences
Pre-mRNAs containing non-coding regions called introns.
Splicing defects can cause serious medical implications

- Figure 28.28. Splicing Defects. Mutation of a single base (G to A) in an intron of the b-globin gene leads to thalassemia. This mutation generates a new splice site (blue) akin to the normal one (yellow) but farther upstream.
Some pre-mRNA molecules can be spliced in alternative ways to yield different mRNA.

A pre-mRNA with multiple exons is sometimes spliced in different ways. Here, with two alternative exons (exons 2A and 2B) present, the mRNA can be produced with neither, either, or both exons included. More complex alternative splicing patterns also are possible.
Some pre-mRNA molecules can be spliced in alternative ways to yield different mRNA

Alternative splicing provides a powerful mechanism for expanding the versatility of genomic sequences. Alternative splicing provides an opportunity for combinatorial control.
RECAP:

1. RNA synthesis is similar to DNA except
   a. RNA polymerase is directed by transcription factors
   b. RNAs do not need a primer to start transcription
   c. There is no proof-reading mechanism
   d. Post-transcription modifications are necessary: capping, poly-A tail, splicing