**Molecular Biology**

DNA | RNA | PROTEIN
---|---|---
transcription | translation | replication

**Proofreading (p. 961-962)**

What happens if the incorrect nucleotide is incorporated during replication?

![DNA and RNA sequences](image)

**DNA Repair (p.962-970)**

**TABLE 22-1** Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

<table>
<thead>
<tr>
<th>Disease</th>
<th>DNA-Repair System Affected</th>
<th>Sensitivity</th>
<th>Cancer Susceptibility</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention of Point Mutations, Insertion, and Deletions</td>
<td>DNA repair system for UV irradiation, chemical mutagens</td>
<td>Colon cancer, ovarian cancer</td>
<td>Early development of tumors</td>
<td></td>
</tr>
<tr>
<td>Xeroderma Pigmentosum</td>
<td>Nucleotide excision repair system</td>
<td>UV irradiation, photo-reactivity</td>
<td>Skin cancers, syndromes</td>
<td>Skin and eye photomodulability, keratoses</td>
</tr>
<tr>
<td>Repair of Double-Strand Breaks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloom’s syndrome</td>
<td>Repair of double-stranded breaks by homologous recombination</td>
<td>Mild proliferating agents</td>
<td>Carcinomas, leukemia, lymphoma</td>
<td>Photosensitivity, facial telangiectasias, chromosome alterations</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Repair of double-stranded breaks by homologous recombination</td>
<td>DNA cross-linking agents, reactive oxidant chemicals</td>
<td>Acute myeloid leukemia, sarcomas, cell malignancies</td>
<td>Developmental abnormalities including infertility and defects of the skeleton, anemia</td>
</tr>
<tr>
<td>Hereditary breast cancer, BRCA-1 and BRCA-2</td>
<td>Repair of double-stranded breaks by homologous recombination</td>
<td></td>
<td>Breast and ovarian cancer</td>
<td></td>
</tr>
</tbody>
</table>
Mutations arise when DNA repair fails

DNA Repair Systems in Eukaryotes

1. Base excision - only incorrect ntd. is removed
   - [Diagram]

2. Mismatch repair
   - [Diagram]

3. Nucleotide excision
   - [Diagram]

What if there is a double-stranded break?

1. Homologous recombination - “error free”
   - undamaged sister chromatid serves as template

2. End-joining - “error prone”
   - two separated ends rejoined
   - OR
   - different broken ends joined - translocations

Replicating DNA in vitro by the Polymerase Chain Reaction (PCR)

- [Diagram]

Requirements:
1. template DNA
2. primers (DNA oligos ~20nt)
3. dNTPs
4. Polymerase*
**Taq Polymerase**

- **Cycle 1**
  - Denaturation of DNA
  - Annealing of primers
  - 95°C
  - 50-60°C
- **Cycle 2**
  - Elongation of primers
  - 72°C

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**Features of different Eukaryotic RNAs**

<table>
<thead>
<tr>
<th>TYPE</th>
<th>POL PROCESSED</th>
<th>MATURE SIZE</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>II cap, PolyA spliced</td>
<td>variable</td>
<td>translated to protein</td>
</tr>
<tr>
<td>rRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>snRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Types of RNA**

- DNA replication
- RNA transcription
- RNA translation
- PROTEIN

- mRNA (messenger)
- rRNA (ribosomal)
- snRNA (small nuclear)
- tRNA (transfer)
- miRNA (micro)
4.2 Transcription of Protein Coding Genes & Formation of Functional mRNA (p.108-115)

1. general transcription
2. initiation
3. elongation
4. termination
5. Prok. mRNAs - operons
6. Euk. mRNAs
7. mRNA processing
8. Northern analysis

RNA Transcription Requires:
1. Template
2. NTP’s for 5’ to 3’ synthesis
3. Proteins

RNA Polymerase Catalyzes Phosphodiester bonds

Transcription Stages - Initiation

1. Polymerase binds to promoter sequence in duplex DNA. “Closed complex”
2. Polymerase makes duplex DNA near transcription start site, forming a transcription bubble. “Open complex”
3. Polymerase catalyzes phosphodiester linkage of two initial rNTPs.
4. DNA-RNA hybrid region
5. Pyrophosphate 2 Pi
**Transcription Stages - Elongation**

**ELONGATION**

4. Polymerase advances 3' → 5' down template strand, melting duplex DNA and adding rNTPs to growing RNA.

![Diagram of elongation process](image)

**Transcription Stages - Termination**

**TERMINATION**

5. At transcription stop site, polymerase releases completed RNA and dissociates from DNA.

![Diagram of termination process](image)

**Transcription starts at “+1”**

**UPSTREAM(-) +1 DOWNSTREAM(+)**

 RNA polymerase
Start site on template strand
Stop site on template strand
5' → 3' Promoter

**General Gene Structure**

**PROMOTER**

5'UTR

CODING

3'UTR

mRNA

PROTEIN
Transcription of Genes

**Prokaryotes**
- operon - 1 promoter, 1 mRNA, several genes

**Eukaryotes**
- separate genes
- introns
- pre-mRNAs capped, spliced, PolyA

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A Prokaryotic Operon

(a) Prokaryotes
E. coli genome

\[ \text{trp operon} \]

**Eukaryotic Gene Expression**

(b) Eukaryotes
Yeast chromosomes

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Gene</th>
<th>kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>TRP1</td>
<td>1580</td>
</tr>
<tr>
<td>V</td>
<td>TRP2</td>
<td>580</td>
</tr>
<tr>
<td>VII</td>
<td>TRP5</td>
<td>910</td>
</tr>
<tr>
<td>XI</td>
<td>TRP3</td>
<td>680</td>
</tr>
</tbody>
</table>

**Eukaryotic mRNA Maturation**

- \( \text{β-Globin genomic DNA} \)
- \( \text{Start site for RNA synthesis} \)
- \( \text{Poly(A) site} \)
- \( \text{Primary RNA transcript} \)
- \( \text{5' cleavage and addition of poly(A) tail} \)
- \( \text{Intron excision, exon ligation} \)
**Functions of mRNA processing/modification**

1. **5' cap:** protection, export signal, translation
2. **splicing:** yields mRNA with proper translation code
3. **polyA:** protection, export signal, translation

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**Experimental Detection of RNA**

1. isolate total RNA
2. denature and separate RNA by [ electrophoresis ]
3. transfer to blot, fix, hybridize
4. detect

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**Northern Blot of β-globin mRNA**

- What can you conclude about activation of the globin gene?
- What predictions can you make about the chromatin structure of the β-globin gene at the three different time points?