DNA Replication

A new DNA strand is polymerized in the 5′ to 3′ direction, reading the parent strand in the 3′ to 5′ direction.

DNA Metabolizing Enzymes

- **Polymerases**: catalyze formation of the phosphodiester bond
- **Helicases**: unwind and untangle to form replication forks
- **Primase**: synthesizes a short ribonucleic acid (RNA) to prime DNA synthesis
- **Methylases**: add methyl groups to nitrogen bases
- **Deaminases**: take amino groups from nitrogen bases
- **Nucleases**: cut DNA
- **Ligases**: catalyze formation of a single phosphodiester bond
DNA Replication

- Nucleotides added based on the existing template
- Polymerases — synthesize, exonuclease, proofread
- Bases form pyrimidine to purine, T:A and C:G
- Okasaki fragments
  - Leading strand — continuous
  - Lagging strand — discontinuous
- Ligase digests primer and joins fragments of complementary strand

Despite the antiparallel nature of the two strands, DNA synthesis proceeds along both strands in the same direction.
Unwinding Template DNA

Eukaryote Genes are Interrupted

- **Exons**: sequence translated to protein
- **Introns**: transferred to hnRNA, excised
- Intron function unclear, but includes...
  - DNA and chromosome structure
  - Chromatin organization
  - Regulation of gene expression
  - Evolutionary remnants

Gene Structure

Upstream
- 5’ Exon 1
- Intron 1
- Exon 2
- Intron 2
- Exon 3
- Intron 3
- 3’ UTR (Untranslated Region)
- Promoter

Downstream
- 5’ UTR (Untranslated Region)
- Polyadenylation Signal
- 3’
RNA Polymerase Enzymes
(DNA-dependent RNA Polymerase)

- RNA Polymerase I - transcribes most rRNA genes (RNA component of ribosomes)
- RNA Polymerase II - transcribes structural genes that encode protein
- RNA Polymerase III - transcribes tRNA genes (for transfer RNAs)
- RNA Polymerase IV - is the mitochondrial RNA polymerase enzyme

DNA Transcription

RNA Structure and Classes

- Different from DNA
  - Single-stranded and Ribose not Deoxyribose
- Classes
  - Messenger (mRNA)
  - Transfer (tRNA)
  - Ribosomal (rRNA)
- Minor classes
  - Heterogeneous nuclear (hnRNA)
  - Small nuclear (snRNA)
  - Small cytoplasmic (scRNA)
Transcription - mRNA

- DNA under complex enzyme control including RNA polymerase II unwinds and rewinds
- hnRNA is assembled 5'—3' using the antisense strand as its template
- Precursor or primary transcript
- Site for removal of intervening sequences (introns)
- Moves from nucleus to cytoplasmic ribosomes
hnRNA Splicing

- Spliceosome
  - Five types of snRNA
  - 50 small nuclear riboproteins
- Splice donor site
  - Cleavage at 5' end of intron where there is a GU sequence
- Branch site within intron (A)
  - Lariat formation with GU and binding branch region
- Splice acceptor site
  - Cleavage at 3' end of intron where there is an AG sequence

RNA Processing

hnRNA Splice Junction

Primary RNA Transcript

Exon 1  Intron 1  Exon 2

...NNAG GUAAGU CAG GNNN...

5' Splice Junction 3' Splice Junction

RNA Splicing

Exon 1  Exon 2

Mature RNA Transcript

hnRNA Splicing

Splice Consensus Sequence

Donor Region  Acceptor Region

Splice Consensus Sequence

Exon 1  Exon 2

Lariat Formation

Branch Region
**CAP Region and Poly-A**

- Capping
  - Guanine added to 5’ through guanylyl transferase via 5’—5’ trisphosphate linkage
  - Not 3’—5’ phosphodiester bond
  - Confers protection
- Poly-A region added on by Poly (A) polymerase
  - Adds up to 200 adenines
  - Control, protection or stability

**Epigenetics**

**DNA Modification**

- Transcriptional gene silencing (TGS)
- Imprinting (Angelman's & Prader-Willi syndrome)
- X-inactivation
- Methylation of DNA inhibits transcription of some genes
- CpG islands are sites of methylation in human DNA

...ggaggagcgccgcggcggccagagaaaa...gccgcagcggcgcgcgcgcacccggacagccggcggaggcgggg...

**Epigenetics**

**Histone Modification**

- Euchromatin
- Heterochromatin
Translation

- Translation in the process that produces proteins
- Translation occurs in the cytoplasm of the cell
  - It requires tRNA and rRNA involvement
- tRNAs are recognized by synthetase enzymes
  - Aminoacyl tRNA synthetase attach amino acids to the
    3’ attachment site of specific tRNA molecules
- Each tRNA has a 3-base sequence (anticodon) that facilitates specific recognition and interaction with a codon in the mRNA

Translation - tRNA

- Translates mRNA code to protein by adding individual amino acids in
- ACC sequence at 3’ end provide site for amino acid esterification
- Anticodon loop binds mRNA three-base codon

Codons & Amino Acids

- The nucleic acids are combined to form a Codon (3 bp’s) coding for Amino Acids
  - 64 triplet combination of bp’s found in RNA
  - AUG at 5’ start
  - Three “stop” codons - UAA, UAG, UGA
  - So 60 codons remain, and only 20 different AA
  - Many AA are represented by more than one codon. This redundancy serves to protect the cell from the adverse effects of some genetic mutations
**Translation**

The anticodon tRNA loop 5’GAA3’ is complementary to mRNA codon 3’CUU5’. Look up 5’UUC3’ in universal code table, it codes for phenylalanine.

**RNA Universal Genetic Code**

<table>
<thead>
<tr>
<th>5’ Position</th>
<th>Second Position</th>
<th>3’ Position</th>
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<tr>
<td>U</td>
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<td>C</td>
</tr>
<tr>
<td>U</td>
<td>Phe</td>
<td>Ser</td>
</tr>
<tr>
<td>U</td>
<td>U</td>
<td>Leu</td>
</tr>
<tr>
<td>U</td>
<td>Leu</td>
<td>STOP</td>
</tr>
<tr>
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<tr>
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</tbody>
</table>

64 Possible Codons for 20 Amino Acids

5’ position

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<th>A</th>
<th>G</th>
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<td>Ala</td>
<td>Gly</td>
</tr>
</tbody>
</table>
Translation of mRNA

mRNA

5’…NNNNNNNAUG-CUC-GGG-AGC-CAU-UAA…NNNNNN…3’

Codon Usage

AUG-CUC-GGG-AGC-CAU-UAA

Translation

Met-Leu-Gly-Ser-His

Peptide Sequence

Mutations & Translation

Mutations & Translation

Mutations & Translation