Chapter 8

Transcription – the copying of information into a strand of RNA with the use of DNA as an alignment guide, or template.

Translation – When the information in RNA is converted into an amino acid chain.

Easy way to remember: C comes before L in the alphabet transcription happens before translation.

Pulse-chase experiment

An experiment in which cells are grown in radioactive medium for a brief period (the pulse) and then transferred to nonradioactive medium for a longer period (the chase). In these cases uracil is labeled to follow RNA.

- Prokaryotes: can detect the radioactive uracil after the pulse, not after the chase. Meaning that the RNA breaks down fairly quickly.

- Eukaryotes: can detect radioactive uracil in the only nucleus after the pulse, can detect the radioactive uracil mostly in the cytoplasm after the pulse.
Properties of RNA

1- single-stranded

2- ribose sugar

![ribose and deoxyribose structures]

3- Uracil instead of Thymine

![U and T molecules]

4- Can catalyze important biological reactions (function like protein enzymes).

Classes of RNA

mRNA- Messanger RNA. The RNA molecule, transcribed from the DNA of a gene, which serves as a template and encodes the amino acid sequence of a protein.

tRNA- Transfer RNA. It is responsible for bringing the correct amino acid to the mRNA during translation

rRNA- Ribosomal RNA. RNA arranged into large macromolecular machines that guide the assembly of the amino acid chain by the mRNA and tRNA

snRNA- Small nuclear RNA. They process RNA transcripts in eukaryotic cells
  - some guide the modification of rRNAs
  - some are part of spliceosomes (which remove introns)
Stages of Transcription

Prokaryotes

1- Initiation –
   -always starts at 3’ end of the DNA template strand (therefore RNA is made 5’ to 3’)
   -promoter:
      -conserved –35 and –10 regions are upstream of the gene
   -RNA polymerase holoenzyme:
      -polymerase that scans the DNA for a promoter sequence.
      -made of core enzyme (α, α, β, β’ subunits) and sigma subunit
   -sigma subunit:
      -binds to the –35, -10 region therefore positions the holoenzyme correctly
      -separates the DNA strands at the –10 region
      -once the core enzyme is bound the sigma subunit dissociates from the rest of the complex.
   -transcription starts at the +1 site, (+1 does not usually correspond to AUG, therefore there is a UTR –untranslated region- at the beginning of the mRNA)

2- Elongation
   -synthesis of an RNA strand complementary to the single-stranded region of the DNA template strand.
   -occurs in the 5’ to 3’ direction
   -energy for the addition of a nucleotide is derived from splitting the high-energy triphosphate.

3- Termination
   -transcribe beyond the end of the protein therefore get a 3’ UTR.
   -2 mechanisms
      -intrinsic
         -transcribe over a G-C rich region which forms a hairpin loop followed by a run of A’s, pause at run of A’s and fall off.
      -rho dependent
         -encounter 40-60 C nucleotides and a rut (rho utilization) site
         -rho (a hexamer) binds to rut and helps release the RNA
Eukaryotes

1- Initiation
- the promoter is a TATA region 30 bp upstream of the start site
- TATA binding protein (TBP) finds the TATA box and recruits the other components of the preinitiation complex (PIC)
- PIC is made of 6 general transcription factors (GTFs) and the RNA polymerase II core (which is made of at least a dozen protein subunits).
- Initiation ends when the carboxyl tail domain (CTD) – of a subunit of RNA polymerase II – is phosphorylated by one of the GTFs and the RNA polymerase II dissociates from the rest of the GTFs (some of the GTFs can stay at the promoter site to recruit the PIC again, others float away).

2- Elongation
- similar to elongation in prokaryotes, except that in prokaryotes translation starts during the elongation, in eukaryotes processing starts during elongation and translation happens later.

3- Termination
- transcription reaches an AAUAAA or an AUUAAA sequence and cuts off the RNA about 20 bp later

4- Processing
- 3 steps, they happen at the same time as elongation
  - addition of a cap (7-methyl guanosine linked by 3 phosphate groups) at the 5’ end
    i. protects the mRNA from degradation
    ii. required for translation
  - addition of a 3’ poly-A tail (150-200 adenine nucleotides)
    i. protects from degradation
  - splicing to eliminate introns
    i. introns: segments of unknown function that do not code for polypeptides
    ii. exons: coding sequences
    iii. alternative splicing: the fact that the pre-mRNA transcript can be alternatively spliced in different cell types.
- CTD coordinates the processing events
Splicing Mechanisms

Spliceosome
- made of several small nuclear ribonucleoprotein particles (snRNPs)
- they find a conserved sequence: ----GU------------A----AG----
- they align the intron sequence by attaching to it with hydrogen bonds
- the intron is spliced out in the shape of a lariat and the exons are joined together

Self-splicing
- looks similar to spliceosome splicing, except without the spliceosome holding the sequence in place.

- Why is this important: it was the first time that a biological molecule, other than a protein, was shown to catalyze a reaction. This provides evidence that RNA was the genetic material in the first cells.

Problem 16

A human gene was initially identified as having three exons and two introns. The exons are 456, 224, and 524 bp, while the introns are 2.3 kb and 4.6 kb.

a) Draw this gene, showing the promoter, introns, exons, and transcription start and stop sites.

b) Surprisingly, it is found that this gene encodes not one but two mRNAs that have only 224 nucleotides in common. The original mRNA is 1204 nucleotides, while the new mRNA is 2524 nucleotides. Use your drawing to show how it is possible for this one region of DNA to encode these two transcripts.
Chapter 9

Protein Structure

- a protein is made of many amino acids joined together
- the amino acids are joined by peptide bonds
- a peptide bond is made by joining the amino end of one amino acid with the carboxyl end of another amino acid

- Proteins have a complex structure that has 4 levels of organization
  - primary – the chain of amino acids
  - secondary – the R groups react to fold the chain, most commonly into an alpha helix or a beta sheet
  - tertiary – produced by folding of the secondary structure
  - quaternary – made by joining different polypeptide subunits (folded up in tertiary structures) together

Colinearity of gene and protein: the linear sequence of nucleotides in a gene determines the linear sequence of amino acids in a protein

Genetic Code

1- The genetic code is nonoverlapping
2- Three bases (codons) encode an amino acid
3- The code is read from a fixed starting point and continues to the end of the coding sequence.
   - we know this because a frame shift mutation anywhere in the coding sequence alters the codon alignment for the rest of the sequence
4- The code is degenerate in that some amino acids are specified by more than one codon.
   - With a 1 letter codon we could have 4 amino acids
   - With a 2 letter codon we could have 4x4=16 amino acids
   - With a 3 letter codon we could have 4x4x4=64 amino acids
   - With a 4 letter codon we could have 4x4x4x4=256 amino acids

How was the genetic code deciphered? By Gobind Khorana.
   - by making mRNA with 3 bp repeats (UUU, AGA, etc) and putting it in a tube with all the reagents needed to make a polypeptide.

How were stop codons found? By Brenner.
   - by comparing the ends of short mutant proteins with the wild type.
tRNA
The structure of the tRNA holds the secret to the specificity between an mRNA codon and the amino acid that it designates.
- amino acid attachment site
- aminoacyl-tRNA synthetases are enzymes which attach the amino acid to the tRNA
- once it is added the tRNA is considered charged
- anticodon loop

Wobble
The loose pairing of the third nucleotide in a codon. Therefore several codons can pair with one anticodon.

<table>
<thead>
<tr>
<th>5' Base in Anticodon</th>
<th>3' Base in Codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>U or C</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>A</td>
<td>U</td>
</tr>
<tr>
<td>U</td>
<td>A or G</td>
</tr>
<tr>
<td>I</td>
<td>A, U, or C</td>
</tr>
</tbody>
</table>

Problem 6
What anticodon do you expect for Isoleucine?

Codons from table
- AUU
- AUC
- AUA
**Translation**

1- **Initiation**
   i. ribosomal subunit finds the Shine-Dalgarno sequence in Prokaryotes and the Cap in eukaryotes with the help of initiation factors
   ii. an initiator tRNA(MET\(_i\)) binds into the P site
   iii. then the large ribosomal subunit is attached as the initiation factors are released

2- **Elongation**
   i. 2 elongation factors help the tRNAs bind in the A site and to move the complex along the mRNA strand

3- **Termination**
   i. Translation continues until one of the stop codons comes into the A site
   ii. At this point a release factor comes into the A site the polypeptide chain is released from the tRNA in the P site and the ribosomes are released.
### Practice Problems

#### The Genetic Code

<table>
<thead>
<tr>
<th></th>
<th>U</th>
<th>C</th>
<th>A</th>
<th>G</th>
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</thead>
<tbody>
<tr>
<td>U</td>
<td>Ph + ala</td>
<td>U + ser</td>
<td>U + tyr</td>
<td>U + cys</td>
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<tr>
<td>G</td>
<td>G + leu</td>
<td>C + pro</td>
<td>C + his</td>
<td>C + arg</td>
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<tr>
<td>A</td>
<td>A + trp</td>
<td>A + asp</td>
<td>A + ser</td>
<td>A + arg</td>
</tr>
<tr>
<td>C</td>
<td>C + val</td>
<td>C + ala</td>
<td>C + glu</td>
<td>C + gly</td>
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</tbody>
</table>

#### Problem 1

- **Problem:**
  - **a)** complete the table
  - **b)** label the 5’ and 3’ ends of the DNA and RNA, as well as the amino and carboxyl ends of the protein.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>t</th>
<th>g</th>
<th>A</th>
<th>DNA double helix</th>
<th>mRNA transcribed</th>
<th>Appropriate tRNA anticodon</th>
<th>Amino Acids incorporated into protein</th>
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<tr>
<td>c</td>
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- **Problem 24**

A single nucleotide addition and a single nucleotide deletion approximately 15 sites apart in the DNA cause a protein change in sequence from

Lys-Ser-Pro-Ser-Leu-Asn-Ala-Ala-Lys

To

Lys-Val-His-His-Leu-Met-Ala-Ala-Lys

- **a)** What are the old and new mRNA nucleotide sequences?
- **b)** Which nucleotide has been added and which has been deleted?