Flow of Genetic Information_ Genetic Code, Mutation & Translation (Learning Objectives)

- Demonstrate understanding of the genetic code, codon, start and stop codons, redundancy of the genetic code, and reading frame by figuring out the primary sequence of the polypeptide when given the sequence of the template (anti-sense) or coding (sense) DNA strands.
- Compare and contrast the difference between point mutations: missense, none-sense, silent mutations, and insertion or deletion.
- Compare and contrast the causes of spontaneous mutations and induced mutations by chemical and/or physical mutagens.
- Explain the structure of t-RNA and its role in the process of translation.
- Describe the events of translation including the molecules and structure involved in the events of initiation, elongation, and termination.
- Explain the term polyribosome and protein folding
Flow of Genetic Information - Genetic Code, Mutation & Translation

1. Basic principles of the genetic code and determination of the primary sequence of amino acids in polypeptides.
2. Mutations and their impact on primary sequence of a polypeptide
3. RNA structure and function
4. Protein synthesis machinery and steps of translation
5. Poly-ribosomes and coupling of transcription and translation in prokaryotes
6. Review of RNA structures and different functions.
Genetic Code, Mutation & Protein Synthesis

Translation is the RNA–directed synthesis of a polypeptide

Translation from the language of nucleic acids into the language of proteins (amino acids)
mRNA Language

Alphabets: A U G C

Words: 3-letter words- codons 5’-NNN-3’

How many words are there?
Genetic code/ In-class activity

Use the genetic code table to answer the following questions:

1. How many codons are there for Leu (leucine)?
   ________________________________.

2. How many codons are there for Met (Methionine)?
   ________________________________.

3. How many codons are there for Phe (phenylalanine)?
   ________________________________.

Draw a conclusion about the number of codons for amino acids.

4. How many “stop” codons are there?
   ________________________________.

Answer the following questions using this genetic code: 5'-AUGACCCCUUUUGUUAUAC-3'

5. How long is this message in nucleotides?
   ________________________________

6. Starting with the first codon, write down the sequence of amino acids coded for by the above stretch of nucleotides.
   ________________________________.

7. How long is this polypeptide? _____.
Genetic Code

• One codon ----- one amino acid
• 61 of 64 triplets code for amino acids.
• 3 termination codons- end of translation.
• AUG codes for the amino acid methionine and indicates the start of translation.
• Redundancy of the Genetic Code
Genetic Mutation

• A change in nucleotide sequence of DNA
• Can be induced by chemical and physical agents
Types of Mutations

1. **Base substitution** (point mutation)
   a. **missense** - replaces one amino acid with another.
   
   b. **nonsense** changes codon into a stop codon (results in truncated or shorter protein)
   
   c. **silent** changes replaces one redundant codon with another (mutation at DNA level with no effect on the amino acid sequence of the protein)

2. **Insertions and deletions**

   Result in frame-shifts
1. For amino acids with redundant codons, which nucleotide position(s) are always the same, i.e. conserved? (marked next to the genetic code table)

2. Which amino acid does UUA code for?

3. Does a mutation that changes the codon UUA into a UUG change the amino acid sequence at the protein level?

4. Does a DNA mutation changing the codon UUA into a UCA change the amino acid sequence at the protein level?

5. What impact would a change of the codon UUA into a UAA have at the translational level?

In Question 3, the mutation is a ____________.
In Question 4, the mutation is a ____________.
In Question 5, the mutation is a ____________.
Reading Frames

mRNA sequence: AUG GCA AUG GCC UUU AU

Reading frame #1: AUG GCA AUG GCC UUU AU
  Methionine, Alanine, Leucine, Proline, Tyrosine

Reading frame #2: AUG GCA AUG GCC UUU AU
  Tryptophan, Histidine, Cysteine, Leucine

Reading frame #3: AUG GCA AUG GCC UUU AU
  Glycine, Isoleucine, Alanine, Leucine
Wild type

mRNA Protein
5' AUG AAG GUA UUG GCU UAA 3'
Met Lys Phe Gly Stop

Base-pair insertion or deletion

Frameshift causing extensive missense

Frameshift causing immediate nonsense
Extra U

Insertion or deletion of 3 nucleotides:
no frameshift; extra or missing amino acid
Why do mutations occur?

1. Spontaneous mutation result from mis-pairing of naturally occurring bases, DNA polymerase.

2. Induced change in the chemistry of the bases by chemical mutagens.
Mutagens interact with DNA to cause mutations

- Physical agents
  - high-energy radiation (X-rays)
  - ultraviolet light

- Chemical mutagens
  - base analogues- 5 BromoUracil (5BU)/Thymine analog pairs with G
  - Intercalating agents- Ethidium Bromide
  - Modify bases affecting base-pairing properties, deaminating agents
Deamination of Cytosine by bisulfite
Repair of DNA mutations

Proof-reading by DNA polymerase

1. Mis-match repair systems
   http://www.hhmi.org/biointeractive/media/mismatch_repair-lg.mov

2. Excision repair systems

http://www.youtube.com/watch?v=CcTayxEblio&feature=related
DNA Repair

- Excision repair systems

G1 or G2 checkpoints until all DNA is repaired
The language interpreter-transfer RNA (tRNA)

- Transfer RNA genes (DNA)
- Not translated, function as RNAs (each about 80 bases)
- Leave nucleus to cytoplasm
- Pick up individual amino acids determined by their anti-codon sequence.
- Deliver amino acid to ribosomes
- Resume picking another amino acid molecule and repeat...
t-RNA
(interpreter)

http://www.dnatube.com/groups_home.php?viewkey=f64d32a5527244d104ce&urlkey=trna&search_id=tRNA
A tRNA molecule about 80 nucleotides that folds back on itself to form a three-dimensional structure.

Has a loop containing the anticodon and an attachment site at the 3’ end for an amino acid.
Charging of t-RNA - joining the correct amino acid with its specific tRNA, **aminoacyl-tRNA synthetase**

Specific **charging** of the t-RNA

[Link to animation](http://highered.mcgraw-hill.com/sites/dl/free/0072835125/126997(animation23.html)
Protein synthesis machinery

- **Ribosomes**: two subunits each consisting of many proteins and one or two rRNA molecules
  - Eukaryotes- 40S and 60S
  - Prokaryotes- 30S and 50S

- t-RNA molecules
- Many Protein Factors
- GTP as an energy source
- mRNA
Ribosomal RNA (rRNA)

- Encoded by ribosomal RNA genes (DNA)
- Not translated
- Structural and functional part of ribosome
- Most abundant RNA inside the cell
Virtual Cell Animations

http://vcell.ndsu.edu/animations/translation/movie-flash.htm

- Translation stages:
  - Initiation
  - Elongation
  - Termination

- All three phases require protein “factors”
- Both initiation and chain elongation require GTP.
• polyribosomes trail along the same mRNA.
• A single mRNA is used to make many copies of a polypeptide simultaneously.


(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.

(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).
• Prokaryotes transcribe and translate the same gene simultaneously.
RNA has diverse functions:

- Structural
- Informational
- Catalytic

Table 17.1 Types of RNA in a Eukaryotic Cell

<table>
<thead>
<tr>
<th>Type of RNA</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Messenger RNA (mRNA)</td>
<td>Carries information specifying amino acid sequences of proteins from DNA to ribosomes.</td>
</tr>
<tr>
<td>Transfer RNA (tRNA)</td>
<td>Serves as adapter molecule in protein synthesis; translates mRNA codons into amino acids.</td>
</tr>
<tr>
<td>Ribosomal RNA (rRNA)</td>
<td>Plays catalytic (ribozyme) roles and structural roles in ribosomes.</td>
</tr>
<tr>
<td>Primary transcript</td>
<td>Serves as a precursor to mRNA, rRNA, or tRNA, before being processed by splicing or cleavage. Some intron RNA acts as a ribozyme, catalyzing its own splicing.</td>
</tr>
<tr>
<td>Small nuclear RNA (snRNA)</td>
<td>Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA.</td>
</tr>
<tr>
<td>SRP RNA</td>
<td>Is a component of the signal-recognition particle (SRP), the protein-RNA complex that recognizes the signal peptides of polypeptides targeted to the ER.</td>
</tr>
<tr>
<td>Small nucleolar RNA (snoRNA)</td>
<td>Aids in processing of pre-rRNA transcripts for ribosome subunit formation in the nucleolus.</td>
</tr>
<tr>
<td>Small interfering RNA (siRNA) and microRNA (miRNA)</td>
<td>Are involved in regulation of gene expression.</td>
</tr>
</tbody>
</table>
Transcription, RNA processing, and translation are the processes that link DNA sequences to the synthesis of a specific polypeptide chain.
A gene is a region of DNA whose final product is either a polypeptide or a functional RNA molecule.