Lecture 16 – Gene Transcription and Translation

“TO LIVE, TO ERR, TO FALL, TO TRIUMPH, TO RECREATE LIFE OUT OF LIFE.” – from James Joyce’s A Portrait of the Artist as a Young Man

“SEE THINGS NOT AS THEY ARE, BUT AS THEY MIGHT BE.” - a quote from the book American Prometheus which discusses J. Robert Oppenheimer and the first atomic bomb

“WHAT I CANNOT BUILD, I CANNOT UNDERSTAND.” – attributed to Richard Feynman (physicist, philosopher, badass) as the last words on his blackboard at the time of his death
In this lecture...

• Central Dogma
• A reminder: RNA and proteins
• Codons
• Transcription
  – Initiation
  – Elongation
  – Termination
• Translation
• Post-translational modification
• Mutations
Proteins link genotype and phenotype

- Albinism occurs when the protein tyrosinase is defective
- Tyrosinase directs the synthesis of melanin, a pigment found in skin and eyes
- Heterozygotes with one functional copy of tyrosinase produce enough melanin to not display the phenotype
- However, recessive homozygotes will lack all pigment
Melanin synthesis pathway and GPR413 structure
The Central Dogma

Replication

DNA → RNA → protein

Transcription

Translation
DNA sequence of tyrosinase gene

>gi|209571475:5001-122888 Homo sapiens tyrosinase (oculocutaneous albinism IA) (TYR), RefSeqGene on chromosome 11
ATCACTGTAGTAGCTGGAAGAGAAATCTGTGGA
CTCAATTGGGTTTCTGCAAGACCTTGTGAGG
ACTAGAGGAAATGTGCTCCCTGGCCTGTGGTGACTGC
CTGGCTGGAGTTTCCAGACCTGGCTGGGACTTTT
TCCCTAGAGGCCTGTGCCTCTCTCTAAAGACCTGATGG
AGAAGGAAGATCCTGCTCCACCGTGAGCGGGGAGAG
GAGTCCCTGTGAGCAGCTTTAGAGCAGAGGTCTTCT
GTGCAATAATTCCCTCTGTCAATGAGACATTTGGG
CTCTAATTTCTCTCACAGGGGTTGGAAGCCGGGA
GTCGTTGAGCTCTGGCTTTTAAAATAAGCACTGCC
AGTGGCTCTGAGAACTCTAGGGATTCAACTGTTGGA
ACTGCAGAATTTGTTTGTGGGAGGAAACACTGAC
AGAGAGAGACACTCTTGGTGAGAAGAAGAACATTTCC
ATTGAGGTGCCAGAAAAGAAGGGAACAAATTTGGG
TACCTACTTTAGCAGAAGCATACACAGCTACAGAC
TATGTCTATCCCCTAGGGGACTTTGGAAGAGC
AAAATGGAATCAACACCAGTGTAAAACAGAACTCAATA
TTTATGACTCTTTGTGTGGATGACTATTATTGT
GTCATAGGATGACTCTTGGGGTGCTGAAATCT
GGAGAGACATTGATTGGGCTCGAGCAGCAGCT
TTTCTGCCTTGGCAATAGACTCTTTTGTCGGAGG
GAACAGAAGATTCCAAGGTAGGATTGAGGAAA
ACTTCTACTTTCCATATTGGGACTGCGGAGGAGC
AAAAGGTGTGACATTGGCAAGATAGACTAGAG
AGGTGAGCACCCCCACAATCCTAATCCTACTCAGCC
CAGCATTTTTTCCTCTCGCTGCGAGGAATAT
GCTGATATACGATGCTGAGTAGGGAGGAAGAC
AAAATCACACTTCTTGAGCAAGGGATAATAATCCT
ACCTGAGAACACTTTGCAAGCCCCATCAAGGACAG
AAAGTGTCCTTGTGAAGAATGACTCTAATCTT

Primary structure of tyrosinase protein

>gi|403422|gb|AAB60319.1| tyrosinase [Homo sapiens] MLLAVLYCLLWSFQTSAGHFRACVSKNLMEKECCPP WSGDRPSGCQLSQRGSCQINLLLAPLGQFP FTGVDRESWPSVFYNTCQCSGFMGNCGKCF GFWGPNCRTERRLLVRNRNFDSAPEKDFAYLTL AKHTISSDYVIPGTYQGMNGSTPMNIDYDFVW MHYYVSMALLGGSEIWREDFAHEAPAFLPW HRLFLRWSQEIQKLTFDMFTYVWDWDAEKCDIC TDEYMGGQHPTNPLLSPASFWSWQIVCSRL TEPNSQNMHCALHYMNTMSQVQGANDPIFLL HHAIVDSIEQWLQRHPLQYEPEANAPIGH NRESYMVPFIPLRNGDFFISSKDLYDYSYLDSDPDSF QRDIKSYLEQASRIWSWLLGAAMGAVLTA LLGLVSSCRHKRKLQPEekiPQPLMEKEDYHSLOYSHL

Proteins have an N terminus and a C terminus.
One gene, one protein hypothesis
How do genes produce proteins?

**Gene expression**: the process by which genes produce proteins

Two stages:

- Transcription
- Translation
Genes can be expressed at different efficiencies

- Gene A is transcribed much more efficiently than gene B
- This allows the amount of protein A in the cell to be greater than protein B
- The lower expression of gene B is a reason behind incomplete dominance
A reminder: what is RNA?

• RNA is the bridge between genes and the proteins for which they code
<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 translator 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>Is a precursor to mRNA, rRNA, or tRNA, before being processed; 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>
</tbody>
</table>
A reminder: what are proteins made of?

• Monomers of proteins are **amino acids**

There are 20 amino acids. Each has a different property depending on its R group/side chain.
Nonpolar side chains; hydrophobic

Side chain (R group)

Glycine (Gly or G)
Alanine (Ala or A)
Valine (Val or V)
Leucine (Leu or L)
Isoleucine (Ile or I)

Methionine (Met or M)
Phenylalanine (Phe or F)
Tryptophan (Trp or W)
Proline (Pro or P)

Polar side chains; hydrophilic

Serine (Ser or S)
Threonine (Thr or T)
Cysteine (Cys or C)
Tyrosine (Tyr or Y)
Asparagine (Asn or N)
Glutamine (Gln or Q)

Electrically charged side chains; hydrophilic

Acidic (negatively charged)
Aspartic acid (Asp or D)
Glutamic acid (Glu or E)

Basic (positively charged)
Lysine (Lys or K)
Arginine (Arg or R)
Histidine (His or H)
Nitrogenous bases

Pyrimidines

Cytosine (C)

Thymine (T, in DNA)

Uracil (U, in RNA)

Purines

Adenine (A)

Guanine (G)

(c) Nucleoside components
Codons

- A **codon** is three nucleotides in a row on an RNA molecule that codes for a single amino acid.
- A specific three-nucleotide sequence encodes for each amino acid.

![Codon Table](image-url)
Template Strand

• During transcription, one of the two DNA strands, called the **template strand**, provides a template for ordering the sequence of complementary nucleotides in an RNA transcript
  – The template strand is always the same strand for a given gene
  – However, different genes may be on opposite strands
(A) DNA double helix

an RNA polymerase that moves from left to right makes RNA by using the bottom strand as a template.

(B) an RNA polymerase that moves from right to left makes RNA by using the top strand as a template.

Figure 6-13 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Figure 6-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Degeneracy of the code

- There are 64 codons, but only 20 amino acids
- One amino acid will have multiple codons
- The genetic code is said to be **degenerate** for this reason
- Each codon specifies the amino acid (one of 20) to be placed at the corresponding position along a polypeptide

Codons along an mRNA molecule are read by translation machinery in the 5’ to 3’ direction

### Figure 6-50 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Evolution of the code

• The genetic code is nearly universal, shared by the simplest bacteria to the most complex animals
  – Some species prefer certain codons (codon bias)
• Genes can be transcribed and translated after being transplanted from one species to another
Transcription

DNA → RNA

• DNA is transcribed into a special type of RNA called **messenger RNA**, or mRNA

• mRNA synthesis is catalyzed by **RNA polymerase**, which pries the DNA strands apart and hooks together the RNA nucleotides

• The RNA is complementary to the DNA template strand
  – RNA synthesis follows the same base-pairing rules as DNA, except that uracil substitutes for thymine
The Transcription Unit

- The stretch of DNA that is used in transcription
Transcription
Generic process for both prokaryotes and eukaryotes

• Initiation
  – RNA polymerase binds to the promoter of a gene and begins to unwind the DNA

• Elongation
  – RNA pol ‘reads’ the template strand in 3’ to 5’ direction and adds complementary ribonucleotides

• Termination
  – RNA pol hits a stop signal (prokaryotes) or falls of (eukaryotes)
Promoter  Transcription unit

5'  3'  DNA

Start point

RNA polymerase

5'  3'
1. Initiation

2. Elongation

3. Termination

Promoter

Transcription unit

Start point

RNA polymerase

Nontemplate strand of DNA

Template strand of DNA

Unwound DNA

RNA transcript

Rewound DNA

RNA transcript

Completed RNA transcript

Direction of transcription ("downstream")
Transcription: Initiation

- **Promoters** control when, how, and at what level a gene is transcribed
  - Composed of a distinct sequence of nucleotides

- **Transcription factors** help RNA polymerase bind to the promoter
  - Transcription factors bind to areas in and around the promoter and provide a “landing pad” for RNA pol
Regulators of gene transcription

- Promoters
- Enhancers
  - Upstream sequences in eukaryotes that help to control the expression of genes
  - Can be thousands of nucleotides away from the protein-coding region
- Silencers
  - When transcription factors bind, they prevent a gene from being transcribed
A distal enhancer and an ultraconserved exon are derived from a novel retroposon

Gill Bejerano; Craig B Lowe; Nadav Ahituv; Bryan King; Adam Siepel; Sofie R Salama; Edward M Rubin; W. James Kent; David Haussler

A retrotransposon that actively jumped around in lobed-fin fishes 410 million years ago led to the creation of enhancers and exons for a gene responsible for neurodevelopment, ISL1. ISL1 plays a hugely important role in regulating insulin gene expression, governs motor neuron generation, and helps the development of a bilateral heart in mammals.

“These add to a growing list of examples in which relics of transposable elements have acquired a function that serves their host, a process termed “exaptation,” and provide an origin for at least some of the many highly conserved vertebrate-specific genomic sequences.”
Promoters in prokaryotes

- Prokaryotes
  - Highly conserved nucleotide sequences at -10 and -35 base pairs upstream of the start of the gene
    - -10 sequence is TATA, called “TATA box”
  - **Sigma factor** is the main transcription factor
    - Binds conserved -10 and -35 sequences
Figure 6-11 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Promoters in eukaryotes

- **Eukaryotes**
  - A “basal promoter” made of a TATA box and other elements ~25bp upstream
  - A conserved region around the start of the gene called “Inr” for initiation
  - Many different transcription factors

![Diagram showing elements BRE, TATA, INR, and DPE with transcription start point and consensus sequences](image)
1. A eukaryotic promoter

2. Several transcription factors bind to DNA

3. Transcription initiation complex forms

RNA polymerase II

Transcription initiation complex

DNA

Promoter

Nontemplate strand

Start point

Template strand

TATA box

RNA transcript
Transcription: Elongation

- As RNA polymerase moves along the DNA, it untwists the double helix, 10 to 20 bases at a time
- Transcription progresses at a rate of 40 nucleotides per second in eukaryotes
  - Actually 24/s in eukaryotes and 60/s in prokaryotes
- A gene can be transcribed simultaneously by several RNA polymerases
- Nucleotides are added to the 3’ end of the growing RNA molecule
Transcription: Termination

• The mechanisms of termination are different in bacteria and eukaryotes
  – In bacteria, the polymerase stops transcription at the terminator signal and the mRNA can then be translated without further modification
Transcription: Termination

• In eukaryotes, RNA polymerase II transcribes the polyadenylation signal sequence

• The RNA transcript is released 10–35 nucleotides past this polyadenylation sequence
  – What exactly boots RNA pol off after the poly-A tail is unknown
RNA Processing

• In eukaryotes, before mRNA can be translated it has to first be processed
  – Prokaryotes don’t need processing

• Pre-mRNA is renamed to mRNA after being processed

DNA $\rightarrow$ pre-mRNA $\rightarrow$ mRNA $\rightarrow$ protein
RNA Processing

• A pre-mRNA is processed in three particular ways:
  – The 5’ end receives a modified nucleotide 5’ cap
  – The 3’ end gets a poly-A tail
  – Exons are spliced out

• These modifications share several functions
  – They seem to facilitate the export of mRNA
  – They protect mRNA from hydrolytic enzymes
  – They help ribosomes attach to the 5’ end
RNA Processing

DNA

---

Primary transcript

5' cap 5' Transcription 3' Remove 3' end 3' Add Poly-A tail 3' Splicing

mRNA 5' (A)_{100-250} 3'
mRNA splicing

• Most eukaryotic genes and their RNA transcripts have long noncoding stretches of nucleotides that lie between coding regions

• These noncoding regions are called intervening sequences, or introns

• The other regions are called exons because they are eventually expressed, usually translated into amino acid sequences

• **RNA splicing** removes introns and joins exons, creating an mRNA molecule with a continuous coding sequence
Introns cut out and exons spliced together

mRNA 5' Cap 1-146 Coding segment Poly-A tail

5' UTR 105-146

3' UTR

TRANSCRIPTION, SPLICING, AND 3' CLEAVAGE/POLYADENYLYATION

striated muscle mRNA
smooth muscle mRNA
fibroblast mRNA
fibroblast mRNA
brain mRNA

Figure 6-27 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Alternative Splicing

• The rearrangement of exons during splicing

Because of alternative splicing the number of different proteins an organism can produce is much greater than its number of genes.

Different exons can code for the different domains in a protein.

Exon shuffling may result in the evolution of new proteins.
How do we splice?

- Protein complexes called **spliceosomes** recognize and splice out introns

- **Spliceosomes** consist of a variety of proteins and several small nuclear ribonucleoproteins (snRNPs) that recognize the splice sites
Figure 6-21 Molecular Biology of the Cell 5/e (© Garland Science 2008)
The End?

• So now we have fully processed mRNA...now what?

• Prokaryotes
  – Ribosomes immediately attach and begin translation

• Eukaryotes:
  – mRNA exits the nucleus through the nuclear pores
  – Cytoplasmic or rough-ER bound ribosomes begin translating
Bacteria and eukarya differ in their RNA polymerases, termination of transcription, and ribosomes; archaea tend to resemble eukarya in these respects

- Bacteria can simultaneously transcribe and translate the same gene
- In eukarya, transcription and translation are separated by the nuclear envelope
- In archaea, transcription and translation are likely coupled
• After processing, mRNA moves through nuclear pores
Revenge of the RNA: Translation

RNA → protein

• How do we go from the language of nucleic acids to the language of amino acids?
• A cell translates an mRNA message into protein with the help of transfer RNA (tRNA)
• tRNA transfer amino acids to the growing polypeptide in a ribosome
  – An adaptor molecule composed of RNA
  – Base-pairs with itself to form a ribozyme-like molecule

Amino acids are the monomers of proteins
Translation: The players

- tRNA
- Aminoacyl-tRNA synthetase
- Ribosomes
- Amino acids
- Chaperone proteins
Structure of tRNA

(a) Two-dimensional structure

(b) Three-dimensional structure

(c) Symbol used in this book

Amino acid attachment site

Anticodon

Hydrogen bonds

Amino acid attachment site

Hydrogen bonds

Anticodon

Anticodon
More about tRNA

• Specific tRNAs will attach to specific amino acids
• How does it ‘know’ which amino acid to attach to?
  – The anticodon on the end dictates which amino acid the tRNA will attach to
  – tRNAs don’t naturally come attached to their amino acids
  – An enzyme called **aminoacyl-tRNA synthetase** catalyzes the linking of an amino acid to a tRNA
Ribosomes

• Roughly the same for both eukaryotes and prokaryotes
• Ribosomes provide a site for tRNA and mRNA to come together
• Composed of rRNA and proteins
  – Large subunit and small subunit
  – Three catalytic sites: E, P, and A
    • “Aminoacyl-tRNA binding site”
    • “Peptidyl-tRNA binding site”
    • “Exit site”
Figure 6-64 Molecular Biology of the Cell 5/e (© Garland Science 2008)
(a) Computer model of functioning ribosome

(b) Schematic model showing binding sites

(c) Schematic model with mRNA and tRNA
Ribosomes

• A ribosome has three binding sites for tRNA
  – The **P site** holds the tRNA that carries the growing polypeptide chain
  – The **A site** holds the tRNA that carries the next amino acid to be added to the chain
  – The **E site** is the exit site, where discharged tRNAs leave the ribosome
• Two populations of ribosomes in cells: free ribosomes (in the cytosol) and bound ribosomes (attached to the ER)
  – Free ribosomes mostly synthesize proteins that function in the cytosol
  – Bound ribosomes make proteins of the endomembrane system and proteins that are secreted from the cell
• Ribosomes are identical and can switch from free to bound
Translation

• Three stages:
  – Initiation
  – Elongation
  – Termination
Translation: Initiation

• mRNA binds to the small ribosomal subunit
• An special initiator tRNA binds to the mRNA+small ribosomal subunit
  – Initiator tRNA uses its anticodon to complementary base-pair with mRNA
  – Initiator tRNA will always be methionine, AUG
• Small ribosomal subunit “scans” along the mRNA until it finds the start signal
• Large ribosomal subunit binds through GTP hydrolysis and begins elongation
Translation: Elongation

- Amino acids are added one by one to the preceding amino acid at the C-terminus of the growing chain
- Each addition involves proteins called elongation factors and occurs in three steps: codon recognition, peptide bond formation, and translocation
Translocation: Elongation

• Codon recognition
  – Three ribonucleotides are exposed in the “A” site
  – A tRNA with the correct anticodon will recognize the exposed codon and enter the “A” site

• Peptide bond formation
  – A peptide bond is formed between the two amino acids of adjacent tRNAs on the “A” and “P” sites

• Translocation
  – The ribosome moves one codon forward
  – The tRNA now in the “E” site exits
  – A new codon is now exposed in the “A” site
Figure 17.19-1

Amino end of polypeptide

mRNA

5'

3'

E

P

A

site

site
Figure 17.19-2

mRNA

5’

P

A

site

site

E

3’

GTP

GDP + Pi

tRNA with correct anticodon recognizes the exposed codon in the A site and binds
Figure 17.19-3

Amino end of polypeptide

mRNA

5'  3'

E

P

A

site site

GTP

GDP + P_i

Peptide bond formation

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Ribosome translocates forward by one codon

Ribosome ready for next aminoacyl tRNA

mRNA

5’ → 3’

P site → A site

GTP → GDP + P_i

GDP + P_i → GTP
Translation: Termination

- The codons UGA, UAA, and UAG are stop signals
- Instead of a tRNA binding there, a protein called a release factor binds and causes the ribosome to release
  - The release factor causes the addition of a water molecule instead of an amino acid
  - This reaction releases the polypeptide, and the translation assembly then comes apart
Translation: Termination
Polyribosomes

- Many ribosomes can attach to one mRNA at once
- This allows for very fast translation of multiple copies of the protein
Figure 17

TRANSCRIPTION

DNA
RNA polymerase
Exon
RNA transcript
RNA transcript (pre-mRNA)
Intron
RNA transcript

NUCLEUS

RNA PROCESSING

Aminoacyl-tRNA synthetase
Amino acid
tRNA

AMINO ACID ACTIVATION

AMINO ACID ACTIVATION

CYTOPLASM

mRNA
Growing polypeptide
Ribosomal subunits
Ribosome
Anticodon
Codon
Aminoacyl (charged) tRNA

TRANSLATION

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DNA → Transcription → Splicing → Translation → Protein
Template sequence (from problem):

Non-template sequence:

mRNA sequence:

3′-TTCAGTCGT-5′

5′-AAGTCAGCA-3′

5′-AAGUCAGCA-3′
Antibiotics: Ribosome inhibitors

Figure 6-79 Molecular Biology of the Cell 5/e (© Garland Science 2008)
<table>
<thead>
<tr>
<th>INHIBITOR</th>
<th>SPECIFIC EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acting only on bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>blocks binding of aminoacyl-tRNA to A-site of ribosome</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>prevents the transition from translation initiation to chain elongation and also causes miscoding</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>binds in the exit channel of the ribosome and thereby inhibits elongation of the peptide chain</td>
</tr>
<tr>
<td>Rifamycin</td>
<td>blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)</td>
</tr>
<tr>
<td><strong>Acting on bacteria and eucaryotes</strong></td>
<td></td>
</tr>
<tr>
<td>Puromycin</td>
<td>causes the premature release of nascent polypeptide chains by its addition to the growing chain end</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)</td>
</tr>
<tr>
<td><strong>Acting on eucaryotes but not bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>blocks the translocation reaction on ribosomes (step 3 in Figure 6–66)</td>
</tr>
<tr>
<td>Anisomycin</td>
<td>blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–66)</td>
</tr>
<tr>
<td>α-Amanitin</td>
<td>blocks mRNA synthesis by binding preferentially to RNA polymerase II</td>
</tr>
</tbody>
</table>

The ribosomes of eucaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.

Table 6–4 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Post-translational modifications

• Once translated, proteins aren’t finished! They must undergo **post-translational modification**
  – Help with proper folding
  – Addition of functional groups
  – Building subunits together into a protein with quaternary structure
  – Cleaving apart a protein to activate it
Why do modification?

• Modification affects the:
  – Lifespan
  – Cellular location
  – Activity
Protein Folding

- After translation, some proteins require additional help to fold properly
- **Chaperonins** are specialized proteins that help other proteins fold correctly
  - They provide a protected, neutral chamber
Protein folding

- Newly synthesized protein
- Correctly folded without help
- Correctly folded with help of a molecular chaperone
- Incompletely folded formed digested by the proteasome

Figure 6-88 Molecular Biology of the Cell 5/e (© Garland Science 2008)
Addition of functional groups

- \(-\text{OH}, -\text{CH}_3, -\text{SH}_2\)
- Major one is \textit{glycosylation}, which attaches polysaccharides to proteins
  - Most proteins translated by ribosomes in the rough ER are glycosylated
  - Proteins are flagged to be sent to the ER by a special \textit{signal peptide}
Figure 17.22

1. Ribosome
2. SRP receptor protein
3. Translocation complex
4. Signal peptide removed
5. Protein
6. ER membrane

ER LUMEN
CYTOSOL

mRNA
Signal peptide
SRP

Quarternary Structure

- Each subunit of a protein with quaternary structure is transcribed and translated separately.
- The subunits are brought together after translation.
Cleavage

- Splitting off part of the peptide chain in order to activate the protein
  - Some cell signaling pathways signal the cleavage of other proteins
Mutations

- Large-scale, chromosome-wide
  - Translocations, inversions, deletions, insertions
- Small-scale
  - Point mutations
  - Indels
  - Frameshift
Point mutations

• Changing a single nucleotide in the template strand

• Point mutations are the cause of SNPs
Types of point mutations

- A **nucleotide-pair substitution** replaces one nucleotide and its partner with another pair of nucleotides.
- **Silent mutations** have no effect on the amino acid produced by a codon because of redundancy in the genetic code.
- **Missense mutations** still code for an amino acid, but not the correct amino acid.
- **Nonsense mutations** change an amino acid codon into a stop codon, nearly always leading to a nonfunctional protein.
Wild type

DNA template strand 3’
5’

mRNA 5’

Protein
Amino end

Stop Carboxyl end

(a) Nucleotide-pair substitution: silent

A instead of G

U instead of C

Met Lys Phe Gly

Stop

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**Wild type**

DNA template strand 3’

<table>
<thead>
<tr>
<th>T</th>
<th>A</th>
<th>C</th>
<th>T</th>
<th>T</th>
<th>C</th>
<th>A</th>
<th>A</th>
<th>A</th>
<th>A</th>
<th>C</th>
<th>C</th>
<th>G</th>
<th>A</th>
<th>T</th>
<th>T</th>
</tr>
</thead>
</table>

5’

| A | T | G | A | A | G | T | T | T | T | T | G | G | C | T | A | A |

mRNA 5’

| A | U | G | A | A | G | U | U | U | U | G | G | C | U | A | A |

Protein

- Met
- Lys
- Phe
- Gly

Stop

Carboxyl end

Amino end

(a) Nucleotide-pair substitution: missense

T instead of C

3’

<table>
<thead>
<tr>
<th>T</th>
<th>A</th>
<th>C</th>
<th>T</th>
<th>T</th>
<th>C</th>
<th>A</th>
<th>A</th>
<th>A</th>
<th>A</th>
<th>T</th>
<th>C</th>
<th>G</th>
<th>A</th>
<th>T</th>
<th>T</th>
</tr>
</thead>
</table>

5’

| A | T | G | A | A | G | T | T | T | T | T | A | G | C | T | A | A |

A instead of G

5’

| A | U | G | A | A | G | U | U | U | U | A | G | C | U | A | A |

Stop

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### Wild type

<table>
<thead>
<tr>
<th>DNA template strand 3'</th>
<th>mRNA 5'</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACCTCAAAACCGATT</td>
<td>AUGAGUUGGCUCUA</td>
<td>Met</td>
</tr>
<tr>
<td>5'</td>
<td>3'</td>
<td></td>
</tr>
<tr>
<td>ATGAAATTTTGGCTAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Amino end**

**Carboxyl end**

STOP

---

### (a) Nucleotide-pair substitution: nonsense

**A instead of T**

<table>
<thead>
<tr>
<th>DNA template strand 3'</th>
<th>mRNA 5'</th>
<th>Protein</th>
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<tbody>
<tr>
<td>TACATCAAAACCGATT</td>
<td>AUGAGUUGGCUCUA</td>
<td>Met</td>
</tr>
<tr>
<td>5'</td>
<td>3'</td>
<td></td>
</tr>
<tr>
<td>ATGATTTTGGCTAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Stop**

---

**T instead of C**

<table>
<thead>
<tr>
<th>DNA template strand 3'</th>
<th>mRNA 5'</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACATAAAACCGATT</td>
<td>AUGAGUUGGCUCUA</td>
</tr>
<tr>
<td>5'</td>
<td>3'</td>
</tr>
<tr>
<td>ATGTATTGCTAA</td>
<td></td>
</tr>
</tbody>
</table>

**Stop**

---

**U instead of A**

<table>
<thead>
<tr>
<th>DNA template strand 5'</th>
<th>mRNA 5'</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUGCUGUUGGGCUCUAA</td>
<td>AUGAGUUGGCUCUA</td>
</tr>
<tr>
<td>5'</td>
<td>3'</td>
</tr>
<tr>
<td>AUGCUGUUGGGCUCUAA</td>
<td></td>
</tr>
</tbody>
</table>

**Stop**

---

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Indels

- **Insertions** and **deletions** are additions or losses of nucleotide pairs in a gene
- These mutations have a disastrous effect on the resulting protein more often than substitutions do
- Insertion or deletion of nucleotides may alter the reading frame, producing a **frameshift mutation**
Wild type

DNA template strand 3’ T A C T T C A A A A C C G G A T T T 5’
           5’ A T G A A G T T T T G G C T A A A 3’

mRNA 5’ A U G A A G U U U U G G C U A A A 3’

Protein
Met - Lys - Phe - Gly

Amino end

Stop Carboxyl end

(b) Nucleotide-pair insertion or deletion: frameshift causing immediate nonsense

Extra A

3’ T A C A T T C A A A A C C G G A T T T 5’
           5’ A T G T A A A G T T T T G G C T A A A 3’

Extra U

5’ A U G U A A A G U U U U G G C U A A A 3’

Met

Stop

1 nucleotide-pair insertion
Wild type

DNA template strand 3’  T A C T T C A A A A C C G A T T 5’
5’  A T G A A G T T T G G C T T A A 3’
mRNA 5’  A U G A A G U U U U G G C U A A 3’
Protein Met  Lys  Phe  Gly
Amino end

(b) Nucleotide-pair insertion or deletion: frameshift causing extensive missense

3’  T A C T T C A A A C C G A T T 5’
5’  A T G A A G T T T G G C T T A A 3’

A missing

3’  T A C T T C A A A C C G A T T 5’
5’  A T G A A G T T T G G C T T A A 3’

U missing

5’  A U G A A G U U U G G C U A A 3’

Met  Lys  Leu  Ala

1 nucleotide-pair deletion
### Wild type

**DNA template strand 3’**

<table>
<thead>
<tr>
<th>5’</th>
<th>T A C T T C A A A A C C G A T T</th>
<th>3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’</td>
<td>A T G A A G T T T T G G C T A A</td>
<td>5’</td>
</tr>
</tbody>
</table>

**mRNA 5’**

<table>
<thead>
<tr>
<th>3’</th>
<th>A U G A A G U U U G G C U A A</th>
</tr>
</thead>
</table>

**Protein**

- Met
- Lys
- Phe
- Gly

**Amino end**

**Carboxyl end**

### (b) Nucleotide-pair insertion or deletion: no frameshift, but one amino acid missing

**Wild type**

<table>
<thead>
<tr>
<th>5’</th>
<th>T T C missing</th>
<th>3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’</td>
<td>T A C A A A A C C G A T T</td>
<td>3’</td>
</tr>
</tbody>
</table>

**mRNA 5’**

<table>
<thead>
<tr>
<th>3’</th>
<th>A A G missing</th>
</tr>
</thead>
</table>

**Protein**

- Met
- Phe
- Gly

**Stop**

### 3 nucleotide-pair deletion
Wild type

DNA template strand
3' TACTTCAAACCGATT5'
5' ATGAGTTTGGCTAAA3'

mRNA
5' AUGAAGUUUGGCUAA3'

Protein
Met Lys Phe Gly

Amino end
Carboxyl end

(a) Nucleotide-pair substitution

A instead of G
3' TACTTCAAAAAATT5'
5' ATGAGTTTGGCTAA3'

U instead of C
5' AUGAAGUUUGGCUAA3'

Silent (no effect on amino acid sequence)

T instead of C
3' TACTTCAACCGATTT5'
5' ATGAGTTTGGCTAA3'

A instead of G
5' AUGAAGUUUGGCUA A3'

Missense

(b) Nucleotide-pair insertion or deletion

Extra A
3' TACATTTCAAACCGATT5'
5' ATGTAGTTTGGCTAA3'

Extra U
5' AUGUAGUUUGGCUA A3'

Frameshift causing immediate nonsense (1 nucleotide-pair insertion)

A missing
3' TACATTCAACCGATT5'
5' ATGTAGTTTGGCTAA3'

U missing
5' AUGUAGUUUGGCUA A...3'

Frameshift causing extensive missense (1 nucleotide-pair deletion)

TTC missing
3' TACATTTCAACCGATT5'
5' ATGTAGTTTGGCTAA3'

AAG missing
5' AUGUAGUUUGGCUA A...3'

Nonsense

No frameshift, but one amino acid missing (3 nucleotide-pair deletion)
Mutagens

• Spontaneous mutations can occur during DNA replication, recombination, or repair

• **Mutagens** are physical or chemical agents that can cause mutations
  – **Carcinogens** are mutagens that can cause cancer
Carcinogens

- **Heterocyclic amines** are produced when meat is charred
- **Nitrosamines**, used in preserving meat, fish, and beer
- **Tobacco smoke** – 19 known carcinogens
  - Benzopyrene diol epoxide
Vocabulary

• Carcinogen
• Mutagen
• Point mutation
• Silent mutation
• Missense mutation
• Nonsense mutation
• Frameshift mutation
• Indel
• Post-translational modification
• Glycosylation
• Chaperonins
• Antibiotics
• Polyribosomes
• Stop signals
• Release factor
• Translation

• Initiation, elongation, termination
• Methionine
• tRNA
• Anticodon
• Aminoacyl-tRNA synthetase
• Ribosome
• Small subunit, large subunit
• EPA
• Spliceosomes
• Alternative splicing
• Introns, exons
• RNA splicing
• preMRNA
• 5’ cap, Poly-A tail
• Transcription
• Initiation, elongation, termination
• Promoter
• Enhancer
• Silencer
• TATA box
• -10 and -35 sequences
• Transcription factors
• Sigma factors
• RNA polymerase
• Codon bias
• Degenerate code
• Codon
• Template strand
Questions?

“Mr. Osborne, may I be excused? My brain is full.”