Cell Biology: Transcription and Translation

AI-Generated Study Guide

(Based on lectures delivered by Dr. Ty C.M. Hoffman)

I. Study Guide

A. Introduction to Gene Expression

- **What is Gene Expression?**
- The process by which information from a gene is used in the synthesis of a functional gene product, typically a protein.
- Involves two main processes: transcription and translation.
- **Historical Context: The One Gene-One Enzyme Hypothesis**
- **Beadle and Tatum's Experiment:** Used bread mold (Neurospora) to induce mutations with UV light and observed their effect on metabolic pathways.
- **Minimal Medium:** A basic growth medium containing only essential nutrients.
- **Biochemical Pathways:** A series of enzyme-catalyzed reactions, where the product of one reaction is the substrate for the next. Each step requires a specific enzyme.
- **Initial Hypothesis:** One gene codes for one enzyme.
- **Evolution of the Hypothesis:One Gene-One Protein Hypothesis:** Recognized that not all genes code for enzymes; some code for other types of proteins.
- **One Gene-One Polypeptide Hypothesis:** Further refined, acknowledging that many proteins (multimeric proteins) are composed of multiple polypeptide chains, each of which may be coded by a different gene.
- **Current Understanding:** Even the one gene-one polypeptide hypothesis is not entirely accurate.
- Some genes do not code for polypeptides (non-protein-coding genes).
- In eukaryotes, a single gene can code for multiple different polypeptides due to alternative splicing.
- **Role of DNA and RNA in Gene Expression**
- **DNA's Direct Role:** Transcription (producing RNA).
- **DNA's Indirect Role:** Traits are ultimately expressed through proteins, which DNA codes for indirectly via RNA.
- **Transcription:** The process of synthesizing RNA from a DNA template.

- **Translation:** The process of synthesizing a polypeptide from an mRNA template.
- **Types of Genes:Protein-coding genes:** Genes that are transcribed into mRNA and then translated into polypeptides.
- **Non-protein-coding genes:** Genes that are transcribed into various types of RNA (e.g., tRNA, rRNA, snRNA) that are not translated into proteins but have other functions.

B. Transcription (DNA to RNA)

- **Definition:** The synthesis of an RNA molecule from a DNA template.
- **Key Enzyme:** RNA polymerase.
- Recognizes the promoter region of a gene.
- Separates DNA strands.
- Synthesizes a complementary RNA strand (template strand is read).
- Adds nucleotides to the 3' end of the growing RNA strand (RNA grows 5' to 3').
- **Transcriptional Unit:** The segment of DNA that is transcribed into an RNA molecule (essentially, the gene).
- **Promoter:** A specific DNA sequence located upstream of a gene that acts as a binding site for RNA polymerase (and transcription factors in eukaryotes) to initiate transcription.
- **Template Strand vs. Non-template (Coding) Strand:** Only one DNA strand serves as the template for transcription for a given gene, though this can vary for different genes.
- **Stages of Transcription:Initiation:**RNA polymerase (and transcription factors in eukaryotes) binds to the promoter.
- DNA strands unwind, forming a transcription bubble.
- **Elongation:**RNA polymerase moves along the DNA template strand, synthesizing RNA by adding complementary nucleotides.
- The growing RNA transcript peels away from the DNA.
- **Termination:**RNA polymerase reaches a terminator sequence (not explicitly detailed, but implied as the end of the gene).
- The RNA transcript is released, and RNA polymerase detaches from the DNA.
- DNA strands re-form a double helix.
- **Prokaryotic vs. Eukaryotic Transcription:Prokaryotes:**Transcription and translation occur simultaneously in the cytoplasm (no nucleus).
- mRNA is immediately ready for translation after transcription.
- **Eukaryotes:**Transcription occurs in the nucleus.
- Translation occurs in the cytoplasm.
- **Pre-mRNA:** The initial RNA transcript directly produced from transcription, which is not yet functional.
- **Post-transcriptional Modification:** Pre-mRNA undergoes processing before it can be translated.
- **5' Cap:** A modified guanine nucleotide added to the 5' end.
- **Poly-A Tail:** A long chain of adenine nucleotides added to the 3' end.
- **Functions of 5' Cap and Poly-A Tail:** Aid in mRNA export from the nucleus, protect mRNA from degradation, and facilitate ribosome binding.

- **RNA Splicing:** Removal of non-coding regions (introns) and joining of coding regions (exons).
- **Introns:** Non-coding, intervening sequences.
- **Exons:** Expressed sequences, which are translated into protein.
- **Spliceosome:** A complex of small nuclear ribonucleoproteins (snRNPs) and other proteins that carries out splicing.
- **snRNPs (snurps):** Small nuclear RNAs (snRNAs) complexed with proteins, recognizing splice sites in introns.
- **Alternative mRNA Splicing:** A single gene can produce multiple different mRNA transcripts (and thus multiple different polypeptides) by selectively including or excluding certain exons. This increases protein diversity from a limited number of genes.

C. The Genetic Code

- **Nature of the Code:Codon:** A sequence of three consecutive nucleotides in mRNA that specifies a particular amino acid or a termination signal.
- **Universal:** Nearly all organisms use the same genetic code, providing strong evidence for a common ancestor.
- **Redundant (Degenerate):** More than one codon can specify the same amino acid (64 codons for 20 amino acids).
- **Not Ambiguous:** Each codon specifies only one amino acid.
- **Key Codons:Start Codon (AUG):** Codes for methionine (Met) and signals the beginning of translation. All polypeptides initially start with methionine.
- **Stop Codons (UAA, UAG, UGA):** Signal the termination of translation; they do not code for any amino acid.

D. Translation (RNA to Protein)

- **Definition:** The synthesis of a polypeptide using the genetic information carried by mRNA.
- **Location:** Ribosomes in the cytoplasm.
- **Key Players:Messenger RNA (mRNA):** Carries the genetic code from DNA to the ribosome in the form of codons.
- **Transfer RNA (tRNA):Structure:** Single-stranded RNA molecule that folds into a specific 3D shape (cloverleaf in 2D).
- **Anticodon:** A three-nucleotide sequence on tRNA that is complementary to an mRNA codon.
- **Amino Acid Attachment Site:** The 3' end of the tRNA where a specific amino acid is covalently attached.
- **Function:** Delivers the correct amino acid to the ribosome based on codon-anticodon pairing.
- **Charged tRNA (aminoacyl tRNA):** A tRNA with its specific amino acid attached.
- **Peptidyl tRNA:** A charged tRNA carrying a growing polypeptide chain.

- **Uncharged tRNA:** A tRNA that has released its amino acid and is ready to be recharged.
- **Aminoacyl-tRNA Synthetase:** Enzymes that catalyze the attachment of the correct amino acid to its corresponding tRNA. This process requires ATP. There are multiple types, one for each amino acid/tRNA pair.
- **Ribosomal RNA (rRNA):** Along with proteins, forms the structural and catalytic components of ribosomes. Ribosomal RNA itself can act as a catalyst (ribozyme) in peptide bond formation.
- **Ribosomes:**Composed of a large and small ribosomal subunit.
- Come together only when translating mRNA.
- Have three binding sites for tRNA:
- **A site (Aminoacyl-tRNA binding site):** Where incoming aminoacyl tRNAs bind.
- **P site (Peptidyl-tRNA binding site):** Holds the tRNA carrying the growing polypeptide chain.
- **E site (Exit site):** Where uncharged tRNAs leave the ribosome.
- **Stages of Translation:Initiation:**Small ribosomal subunit binds to the mRNA and the initiator tRNA (carrying methionine, with UAC anticodon).
- The initiator tRNA binds to the start codon (AUG) in the P site.
- Large ribosomal subunit joins the complex. This assembly requires GTP.
- **Elongation:** (Cyclic process, requires GTP for each step)
- **Codon Recognition:** The appropriate aminoacyl tRNA binds to the A site, complementary to the mRNA codon.
- **Peptide Bond Formation:** A peptide bond forms between the amino acid in the A site and the growing polypeptide in the P site. The polypeptide is transferred from the P site tRNA to the A site tRNA. This is catalyzed by rRNA (a ribozyme).
- **Translocation:** The ribosome moves down the mRNA by one codon. The tRNA in the A site (now carrying the polypeptide) moves to the P site. The uncharged tRNA in the P site moves to the E site and exits.
- **Termination:**A stop codon (UAA, UAG, or UGA) arrives in the A site.
- A **release factor** (a protein, not a tRNA) binds to the stop codon in the A site.
- The release factor causes the addition of a water molecule instead of an amino acid, hydrolyzing the bond between the polypeptide and the tRNA in the P site.
- The completed polypeptide is released.
- The ribosomal subunits, mRNA, and release factor dissociate. This also requires GTP.
- **Polyribosome (Polysome):** Multiple ribosomes simultaneously translating a single mRNA molecule, leading to efficient production of many copies of the same polypeptide.
- **Coupled Transcription-Translation (Prokaryotes only):** In prokaryotes, translation can begin on an mRNA molecule even before its transcription is complete, due to the lack of a nucleus and post-transcriptional modification.

E. Post-Translational Modification and Protein Targeting

- **Initial Stages of Translation:** Always begin in the cytosol on a **free ribosome**.

- **Fate of Polypeptides:Cytosolic Proteins:** Proteins that function in the cytosol are completed on free ribosomes and released into the cytosol. They fold into their functional shapes spontaneously or with the help of chaperones.
- **Proteins Targeted to the ER (Endoplasmic Reticulum):** Proteins destined for the endomembrane system (ER, Golgi, lysosomes, plasma membrane, secreted proteins) or secretion.
- **Signal Peptide:** A short sequence of amino acids (usually at the N-terminus) on the newly synthesized polypeptide that acts as a signal for targeting to the ER.
- **Signal Recognition Particle (SRP):** A protein-RNA complex that recognizes and binds to the signal peptide, temporarily halting translation.
- **SRP Receptor:** A protein complex on the ER membrane that binds the SRP-ribosome complex, docking it onto the ER.
- **Translocon (Protein Pore):** A channel in the ER membrane through which the growing polypeptide chain enters the ER lumen (or is inserted into the ER membrane).
- **Modification in the ER:** Once in the ER lumen, proteins can undergo various modifications, such as folding, glycosylation (addition of carbohydrates), or cleavage of the signal peptide.
- **Further Processing:** Proteins may then be transported to the Golgi apparatus for further modification, sorting, and packaging into vesicles for delivery to their final destination.
- **Examples of Post-Translational Modification:**Cleavage of the N-terminal methionine.
- Folding into specific 3D structures (secondary, tertiary, quaternary).
- Addition of chemical groups (e.g., glycosylation, phosphorylation).
- Cleavage of polypeptide chains.
- Assembly of multiple polypeptide chains (for multimeric proteins like hemoglobin).

F. Mutations

- **Definition:** Any change in the DNA sequence.
- **Point Mutations:** Changes affecting a single nucleotide pair.
- **Base-pair Substitutions:** One nucleotide is replaced by another.
- **Silent Mutation:** A substitution that does not change the amino acid sequence due to the redundancy of the genetic code. (e.g., CCA to CCG still codes for Proline).
- **Missense Mutation:** A substitution that changes one amino acid to another. (e.g., Sickle cell anemia, where a single amino acid change in hemoglobin profoundly alters its function).
- **Nonsense Mutation:** A substitution that changes an amino acid codon into a stop codon, leading to a prematurely terminated and usually non-functional protein.
- **Insertions and Deletions (Indels):** Additions or removals of nucleotide pairs.
- **Frameshift Mutation:** Occurs when the number of inserted or deleted nucleotides is not a multiple of three. This shifts the reading frame of the mRNA, leading to a completely different amino acid sequence downstream of the mutation, and usually results in a non-functional protein. These are generally more severe than substitutions.
- **Large-scale Mutations:** Chromosomal rearrangements (e.g., translocations, inversions) that involve large segments of DNA.

II. Quiz

Instructions: Answer each question in 2-3 sentences.

1. Explain why the "one gene-one enzyme" hypothesis was later modified to the "one gene-one polypeptide" hypothesis.
2. Describe the primary role of RNA polymerase in transcription and where it initiates this process.
3. In what ways does eukaryotic mRNA undergo post-transcriptional modification before translation?
4. Define "redundancy" and "non-ambiguity" as they apply to the genetic code.
5. What is the specific function of tRNA during translation, and how does its anticodon relate to this function?
6. Distinguish between an aminoacyl tRNA and a peptidyl tRNA, explaining their roles in the translation process.
7. Briefly describe the three stages of translation (initiation, elongation, termination).
8. What is a polyribosome, and why is its formation beneficial for a cell?
9. Explain the concept of a "signal peptide" and its significance in protein targeting within eukaryotic cells.
10. Differentiate between a silent mutation and a missense mutation in terms of their effect on the resulting protein.

III. Quiz Answer Key

1. The "one gene-one enzyme" hypothesis was modified because not all genes code for enzymes; some code for other types of proteins. Furthermore, many proteins are composed of multiple polypeptide chains, each potentially coded by a separate gene, leading to the "one gene-one polypeptide" concept.
2. RNA polymerase is the enzyme responsible for synthesizing RNA from a DNA template. It initiates this process by binding to a specific DNA sequence called the promoter, located upstream of the gene to be transcribed.
3. Eukaryotic mRNA undergoes post-transcriptional modification by adding a 5' cap and a poly-A tail, which help with nuclear export, stability, and ribosome binding. It also undergoes splicing, where non-coding introns are removed, and coding exons are joined together.
4. The genetic code is "redundant" because more than one codon can specify the same amino acid, offering a protective buffer against some mutations. It is "non-ambiguous" because each specific codon always codes for only one particular amino acid, ensuring consistent protein synthesis.
5. The specific function of tRNA during translation is to transfer the correct amino acid to the ribosome. Its anticodon, a three-nucleotide sequence, ensures this by base-pairing complementarily with the corresponding codon on the mRNA.

6. An aminoacyl tRNA is a charged tRNA carrying exactly one amino acid, ready to enter the A-site of the ribosome. A peptidyl tRNA is a charged tRNA carrying a growing polypeptide chain, typically found in the P-site of the ribosome during elongation.
7. Translation begins with **initiation**, where mRNA, an initiator tRNA, and ribosomal subunits assemble at the start codon. **Elongation** follows, involving the sequential addition of amino acids to the growing polypeptide chain via codon recognition, peptide bond formation, and translocation. Finally, **termination** occurs when a stop codon is reached, leading to the release of the completed polypeptide and dissociation of the ribosomal complex.
8. A polyribosome (or polysome) is a single mRNA molecule that is being simultaneously translated by multiple ribosomes. This formation is beneficial as it allows a cell to rapidly and efficiently produce many copies of the same polypeptide from a single mRNA transcript.
9. A signal peptide is a short sequence of amino acids, often at the beginning of a polypeptide, that acts as a tag for proteins destined for the ER or secretion. Its recognition by a signal recognition particle (SRP) directs the ribosome-mRNA complex to the ER membrane, ensuring the polypeptide is processed correctly.
10. A silent mutation is a base-pair substitution that changes a nucleotide, but due to the redundancy of the genetic code, it does not alter the amino acid sequence of the protein. A missense mutation is a base-pair substitution that results in a change in the amino acid sequence, potentially altering the protein's function.

IV. Essay Questions

1. Compare and contrast gene expression in prokaryotic and eukaryotic cells, highlighting the key differences in transcription, post-transcriptional modification, and the coupling of transcription and translation.
2. Discuss the significance of the universality, redundancy, and non-ambiguity of the genetic code for the processes of gene expression and genetic engineering. Provide examples where appropriate.
3. Describe the intricate process of protein synthesis on a ribosome, detailing the roles of mRNA, tRNA (including aminoacyl and peptidyl forms), and rRNA. Explain how energy (ATP/GTP) is utilized at various stages.
4. Explain how alternative mRNA splicing contributes to the diversity of proteins in eukaryotes. Discuss the molecular machinery involved in splicing and why introns, despite being non-coding, are important for this process.
5. Analyze the different types of point mutations (silent, missense, nonsense, frameshift) and their potential impact on protein structure and function. Provide a specific example of how a point mutation can lead to a disease.

V. Glossary of Key Terms

- **Aminoacyl-tRNA:** A tRNA molecule that has been "charged" with its specific amino acid.
- **Aminoacyl-tRNA Synthetase:** An enzyme responsible for attaching the correct amino acid to its corresponding tRNA.
- **Anticodon:** A three-nucleotide sequence on a tRNA molecule that is complementary to an mRNA codon.
- **A Site (Aminoacyl-tRNA binding site):** The site on the ribosome where incoming aminoacyl tRNAs bind during translation.
- **Alternative mRNA Splicing:** A process in eukaryotes where a single gene can produce multiple different mRNA transcripts (and thus different proteins) by selectively including or excluding various exons.
- **Ambiguous (Genetic Code):** A hypothetical scenario where a single codon could specify more than one amino acid; the actual genetic code is *not* ambiguous.
- **Biochemical Pathway:** A series of enzyme-catalyzed reactions in which the product of one reaction becomes the substrate for the next.
- **Codon:** A sequence of three consecutive nucleotides in mRNA that specifies a particular amino acid or a termination signal during protein synthesis.
- **Coupled Transcription-Translation:** The phenomenon in prokaryotes where translation of an mRNA molecule can begin before its transcription is complete.
- **Deletion:** A mutation involving the loss of one or more nucleotide pairs from a DNA sequence.
- **Domain (Protein):** A discrete structural and functional unit within a protein, often coded for by a specific exon or set of exons.
- **Elongation:** The stage of transcription or translation where the RNA transcript or polypeptide chain is extended by the sequential addition of nucleotides or amino acids, respectively.
- **E Site (Exit site):** The site on the ribosome from which uncharged tRNAs exit after delivering their amino acid.
- **Exons:** The coding regions of a eukaryotic gene that are eventually expressed (translated into protein). They are joined together after introns are removed during splicing.
- **Frameshift Mutation:** An insertion or deletion mutation that is not a multiple of three nucleotides, leading to a shift in the reading frame and a change in all downstream codons and amino acids.
- **Free Ribosome:** A ribosome that is suspended in the cytosol and not attached to the endoplasmic reticulum. It typically synthesizes proteins that function within the cytosol.
- **Gene Expression:** The overall process by which genetic information from a gene is used to synthesize a functional gene product, usually a protein.
- **Genetic Code:** The set of rules by which information encoded in genetic material (DNA or mRNA sequences) is translated into proteins (amino acid sequences). It is universal, redundant, and non-ambiguous.
- **Initiation:** The first stage of transcription or translation, where the molecular machinery assembles at the start of a gene or mRNA sequence.

- **Initiation Complex (Translation):** The assembled mRNA, initiator tRNA, and small and large ribosomal subunits at the start codon, ready to begin translation.
- **Insertion:** A mutation involving the addition of one or more nucleotide pairs into a DNA sequence.
- **Introns:** Non-coding, intervening sequences within a eukaryotic gene that are transcribed but then removed from the pre-mRNA during splicing.
- **Messenger RNA (mRNA):** An RNA molecule that carries the genetic code from DNA to the ribosomes, where it serves as a template for protein synthesis.
- **Minimal Medium:** A growth medium containing only the bare minimum nutrients required for an organism to survive and grow.
- **Missense Mutation:** A point mutation where a base-pair substitution results in a codon that codes for a different amino acid.
- **Multimeric Protein:** A protein composed of multiple polypeptide chains (subunits), which may be identical or different.
- **Mutation:** Any change in the DNA sequence.
- **Non-protein-coding Genes:** Genes that are transcribed into RNA molecules (e.g., tRNA, rRNA, snRNA) that perform cellular functions but are not translated into proteins.
- **Nonsense Mutation:** A point mutation where a base-pair substitution changes an amino acid codon into a stop codon, leading to premature termination of translation.
- **One Gene-One Enzyme Hypothesis:** The initial hypothesis proposed by Beadle and Tatum, suggesting that each gene is responsible for coding for a single enzyme.
- **One Gene-One Polypeptide Hypothesis:** A refined hypothesis stating that a gene codes for a single polypeptide chain, recognizing that proteins can have multiple polypeptide subunits.
- **Peptidyl-tRNA:** A charged tRNA carrying the growing polypeptide chain at the P-site of the ribosome.
- **Point Mutation:** A mutation that involves a change in a single nucleotide pair in the DNA sequence.
- **Poly-A Tail:** A long chain of adenine nucleotides added to the 3' end of eukaryotic mRNA during post-transcriptional modification, contributing to stability and translation initiation.
- **Polyribosome (Polysome):** A complex formed by multiple ribosomes simultaneously translating the same mRNA molecule, allowing for efficient protein synthesis.
- **Post-transcriptional Modification:** Changes made to eukaryotic pre-mRNA after transcription, including splicing, 5' capping, and poly-A tail addition.
- **Post-translational Modification:** Chemical changes that occur to a polypeptide after it has been synthesized during translation (e.g., folding, cleavage, addition of chemical groups, assembly of subunits).
- **Pre-mRNA:** The primary, unprocessed RNA transcript produced directly from transcription in eukaryotes, which contains both introns and exons.
- **Promoter:** A specific DNA sequence upstream of a gene that signals the start of transcription and serves as a binding site for RNA polymerase.
- **Protein-coding Genes:** Genes that contain the instructions for building proteins; they are transcribed into mRNA and then translated.

- **P Site (Peptidyl-tRNA binding site):** The site on the ribosome where the tRNA carrying the growing polypeptide chain is located.
- **Reading Frame:** The specific grouping of nucleotides into codons during translation, which is set by the start codon.
- **Redundant (Genetic Code):** The characteristic of the genetic code where more than one codon can specify the same amino acid. Also known as degenerate.
- **Release Factor:** A protein that binds to a stop codon in the A site of the ribosome, signaling the termination of translation and release of the polypeptide.
- **Ribosomal RNA (rRNA):** A type of RNA that, along with proteins, makes up the structure of ribosomes and catalyzes peptide bond formation (acting as a ribozyme).
- **Ribosome:** A cellular organelle, composed of rRNA and proteins, that is the site of protein synthesis (translation).
- **Ribozyme:** An RNA molecule that possesses catalytic activity, similar to an enzyme (e.g., rRNA in ribosomes).
- **Rough Endoplasmic Reticulum (RER):** An organelle in eukaryotic cells studded with ribosomes, involved in the synthesis, folding, modification, and transport of proteins destined for secretion or insertion into membranes.
- **Signal Peptide:** A short amino acid sequence (typically at the N-terminus) of a polypeptide that targets it to the endoplasmic reticulum for synthesis and processing.
- **Signal Recognition Particle (SRP):** A ribonucleoprotein that recognizes and binds to signal peptides on nascent polypeptides, targeting the ribosome-mRNA complex to the ER membrane.
- **Silent Mutation:** A point mutation where a base-pair substitution changes a nucleotide but, due to the redundancy of the genetic code, does not result in a change in the amino acid sequence of the protein.
- **Small Nuclear Ribonucleoprotein (snRNP/snurp):** A complex of small nuclear RNA (snRNA) and proteins that are components of the spliceosome and recognize splice sites during RNA splicing.
- **Spliceosome:** A large molecular complex composed of snRNPs and other proteins that catalyzes the removal of introns and joining of exons in pre-mRNA during splicing.
- **Start Codon (AUG):** The mRNA codon that signals the beginning of translation and codes for methionine.
- **Stop Codons (UAA, UAG, UGA):** mRNA codons that do not code for any amino acid but signal the termination of translation.
- **Substitution:** A type of point mutation where one nucleotide is replaced by another.
- **Template Strand:** The DNA strand that serves as the template for RNA synthesis during transcription.
- **Termination:** The final stage of transcription or translation, where the RNA transcript or polypeptide is released, and the molecular machinery disassembles.
- **Transcription:** The process of synthesizing an RNA molecule from a DNA template.
- **Transcription Factors:** Proteins that bind to DNA (often at the promoter or enhancers) and regulate the initiation and rate of transcription in eukaryotes.
- **Transcriptional Unit:** The segment of DNA that is transcribed into an RNA molecule.

- **Transfer RNA (tRNA):** A type of RNA molecule that carries specific amino acids to the ribosome during translation, matching them to mRNA codons.
- **Transgenesis:** The process of introducing an exogenous gene into an organism's genome.
- **Translation:** The process of synthesizing a polypeptide (protein) from an mRNA template.
- **Translocation (Translation):** The movement of the ribosome along the mRNA molecule by one codon, shifting the tRNAs from the A and P sites to the P and E sites, respectively.
- **Untranslated Region (UTR):** Regions of mRNA at the 5' and 3' ends that are not translated into protein but play roles in ribosome binding and mRNA stability.
- **Wild Type:** The most prevalent or "normal" form of a gene or organism in a natural population, serving as a reference point for mutants.