Name: _____

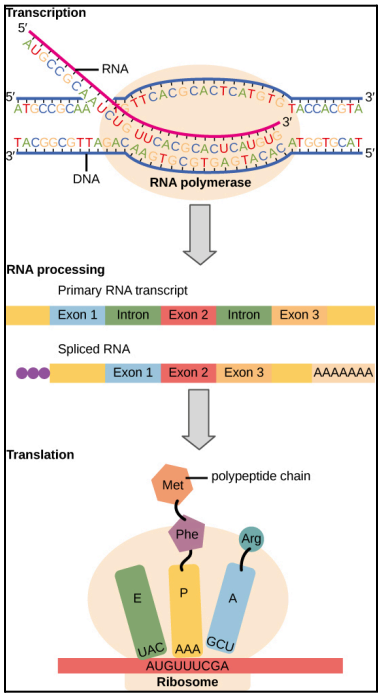
Chapter 10: Genes & Proteins

Objectives

1. Know DNA and RNA pairing rules
2. Explain the differences between transcription and replication
3. Explain the differences between DNA and RNA
4. Name the three types of RNA (full name and abbreviated)
5. Define the terms RNA polymerase, promoter, terminator, exon, intron, 5' cap, 3' poly(A) tail, start codon, stop codon, amino acid, ribosome, tRNA, peptide bond, chaperone protein, degenerate
6. Define transcription, translation, expression
7. Explain the process of transcription, including all parts and locations.
8. Explain how mRNA gets altered after transcription
9. Explain the process of translation, including all parts and locations.
10. Know what ribosomes are made of and the attachment sites on a tRNA
11. Describe the different kinds of mutations
12. Be able to convert DNA RNA using the proper 5' and 3' orientation
13. Be able to convert RNA protein (using a given genetic code table)

The Genetic Code

- 10.1: Introduction
  - Transcription - process that generates messenger RNA, also known as *mRNA*; converts nucleotide-based genetic information into a protein
  - Translation - the genetic code contained within an mRNA molecule is decoded to produce the sequence of amino acids in a polypeptide chain
  - mRNA - a mobile molecular copy of one or more genes with an alphabet of adenine (A), uracil (U), guanine (G), and cytosine (C)
  - The protein alphabet consists of 20 letters
    - Each amino acid is defined by a three-nucleotide sequence called the triplet codon
      - Different amino acids have different chemistries and different structural constraints, passing these traits on to the proteins they create



- 10.1a: The Central Dogma: DNA Encodes RNA; RNA Encodes Protein
  - Central Dogma - states that genes specify the sequence of mRNAs, which in turn specify the sequence of proteins
  - DNA is *transcribed* into RNA
  - RNA is *translated* into proteins
    - Describes the flow of genetic information in cells from DNA to mRNA to protein
    - Decoding a molecule to another is done by specific proteins and RNAs whose "recipe" is written in the DNA
    - The cell makes copies of pieces of DNA to keep the DNA intact and protected
    - Copying DNA to RNA is done by adding 1 nucleotide to the mRNA strand for every nucleotide read in the DNA strand
    - "Reading" mRNA to make a protein is more complex
      - 3 mRNA nucleotides correspond to one amino acid in the polypeptide sequence (*codons*)
  - The Genetic Code is Degenerate and Universal
    - Scientists theorized that codons, correspond to a single amino acid and that the code was *degenerate*, confirmed by Francis Crick and Sydney Brenner
      - Degenerate - when a given amino acid can be encoded by more than one nucleotide triplet
        - Believed to be a mechanism to reduce negative effects of mutations

- Codons that specify the same amino acid typically only differ by 1 nucleotide
- Amino acids with chemically similar side chains are encoded by similar codons
- This ensures that a single-nucleotide substitution mutation might either specify the same amino acid but have no effect or specify a similar amino acid, preventing the protein from being nonfunctional

- Reading Frame - sequence of triplet codons in mRNA that specify a particular protein; a ribosome shift of one or two nucleotides in either direction completely abolishes synthesis of that protein
- Genetic code was solved by translating synthetic mRNAs in vitro and sequencing the proteins they specified

- 3 of the 64 codons terminate protein synthesis and release the polypeptide from translation machinery, called nonsense/stop codons
- The AUG Sequence
  - Specifies amino acid, methionine
  - Serves as the start codon to initiate translation
  - The reading frame for translation is set by the AUG start codon near the 5' end of the mRNA
- Nearly all species use the same genetic code for protein synthesis
  - Ex.: a purified mRNA encoding the globin protein in horses could be transferred to a tulip cell, and the tulip would synthesize horse globin
  - Contributes to the idea that all life on Earth originates from the same source
- There are about 10^{84} possible combinations of the 20 amino acids and 64 triplet codons

Prokaryotic Transcription

- 10.2: Introduction
  - Prokaryotes - includes bacteria and archaea, mostly single-celled, lack membrane-bound nuclei and other organelles
  - Bacterial Chromosomes - covalently closed circle that, unlike eukaryotic chromosomes, is not organized around histone proteins but a nucleoid instead
  - Plasmid - shorter circular DNA molecules that may only contain one or a few genes
    - Can be transferred independently of the bacterial chromosome during cell division
    - May carry traits such as antibiotic resistance
  - How Transcription Differs from Replication
    - Only 1 strand is used as opposed to 2
    - Only replicating 1 gene as opposed to the entire genome
    - Both old strands reconnect at the end (reannealed)
    - The new RNA strand separates, gets modified (10.3a), and leaves
  - Transcription in prokaryotes and eukaryotes requires DNA to unwind to copy the region as mRNA
    - Transcription Bubble - region of locally unwound DNA that allows for transcription of mRNA
    - Template Strand - strand of DNA that specifies the complementary mRNA molecule
    - Sense Strand - template strand
    - Nontemplate/Nonsense Strand - strand of DNA that is not used to transcribe mRNA; this strand is identical to the mRNA except that T nucleotides in the DNA are replaced by U nucleotides in the mRNA
    - Initiation Site - nucleotide from which mRNA synthesis proceeds in the 5' to 3' direction; denoted with a "+1"
    - Upstream - nucleotides preceding the initiation site; in general, sequences toward the 5' end relative to a site on the mRNA
    - Downstream - nucleotides following the initiation site in the direction of mRNA transcription; in general, sequences that are toward the 3' end relative to a site on the mRNA

- ➢ The only difference between the original DNA and the mRNA copy is that uracil (U) replaces thymine (T)

❖ 10.a: Initiation of Transcription in Prokaryotes

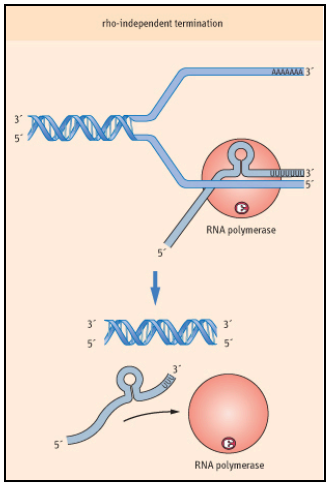
- ➢ Because prokaryotes lack membrane-enclosed nuclei, transcription, translation, and mRNA degradation occurs simultaneously
- ➢ Promoter - a DNA sequence onto which the transcription machinery binds and initiates transcription; often exist upstream of the genes they regulate; segment of DNA before the coding region
  - ■ Specific sequence is very important because it determines how often the corresponding gene is transcribed (constantly, occasionally, infrequently)
- ➢ Introns - segments that interrupt coding region; get removed
- ➢ Exons - segments of coding region

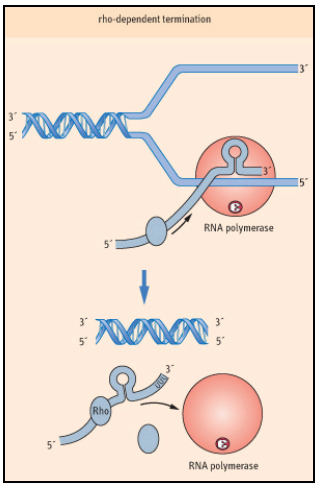
❖ 10.2b: Elongation and Termination in Prokaryotes

- ➢ Transcription elongation begins with *RNA polymerase* synthesizes mRNA (5' to 3' direction)
  - ■ RNA Polymerase - enzyme used to build an RNA molecule from a complementary strand of DNA
- ➢ DNA is continuously unwound ahead of the core enzyme and rewound behind it
- ➢ Base pairings between DNA and RNA is not stable enough to maintain mRNA synthesis, so RNA polymerase acts as a stable linker between the DNA template and the new RNA strand
  - ■ Ensures that elongation is not interrupted prematurely

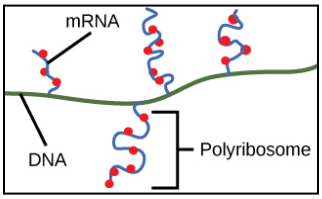
❖ 10.2c: Prokaryotic Termination Signals

- ➢ Once the gene is transcribed, the prokaryotic polymerase must dissociate from the DNA and liberate the mRNA
  - ■ 2 kinds of termination signals: protein-based and RNA-based





- Rho-**Dep**endent Termination
  - ◆ Near the end of the gene, the polymerase encounters a run of G nucleotides on the DNA template stalls
  - ◆ The rho protein collides with the polymerase
  - ◆ The interaction with rho releases the mRNA from the transcription bubble
- Rho-**Ind**ependent Termination
  - ◆ Near the end of the gene, the polymerase encounters a run of C and G nucleotides
  - ◆ mRNA forms a hairpin, the C–G nucleotides binding together
    - ➢ Hairpin - structure of RNA when it folds back on itself and forms intramolecular hydrogen bonds between complementary nucleotides
  - ◆ The complementary U–A region of the mRNA transcript forms only a weak interaction with the template DNA
  - ◆ This plus the stalled polymerase produces enough instability for the core enzyme to break away and liberate the mRNA



- ➢ Transcription is complete after termination
- ➢ By the time termination occurs, the prokaryotic transcript would already have been used to begin synthesis for the protein because these processes can occur concurrently
  - ■ The unification of transcription, translation, and mRNA degradation is possible because all of these processes occur in the same 5' to 3' direction and there is no membranous compartmentalization in the prokaryotic cell
- ➢ In contrast, the presence of a nucleus in eukaryotic cells prevents simultaneous transcription and translation

RNA Processing in Eukaryotes

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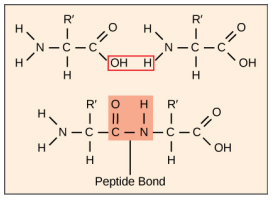
- 10.3: Introduction
  - After transcription, eukaryotic pre-mRNAs must undergo several processing steps before they can be translated
  - Eukaryotic and prokaryotic tRNAs and rRNAs undergo processing before they can function as components in the protein synthesis machinery
  - Types of RNA
    - mRNA - messenger RNA; copy of DNA
    - tRNA - transfer RNA; delivers the corresponding amino acid to the ribosome during translation
    - rRNA - RNA component of the ribosome; used to build ribosomes
- 10.3a: mRNA Processing/Post-Transcriptional Modification
  - Eukaryotic pre-mRNA goes through many processes before being translated
    - Pre-mRNAs are first coated in RNA stabilizing proteins
      - Protects the pre-MRNA from degradation during porcessing and exportation from the nucleus
    - 3 most important steps are the addition of stabilizing and signaling factors at the 5' and 3' ends of the molecule, and the removal of intervening sequences that do not specify the appropriate amino acids
  - Step 1: 5' Capping
    - While pre-mRNA is being synthesized, a *7-methylguanosine cap* is added to the 5' growing end of the transcript by a phosphate linkage
      - 7-methylguanosine cap - modification added to the 5' end of pre-mRNAs to protect mRNA from degradation and assist translation
        - This moiety (functional group) protects the new mRNA from degradation
        - Factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes
  - Step 2: 3' Poly-A Tail
    - Poly-A polymerase adds a string of A residues to the 3' end called a poly-A tail
      - Protects from degradation and signals exportation of the cellular factors that the transcript needs to the cytoplasm
  - Step 3: Pre-mRNA Splicing
    - Eukaryotic genes are made of exons and introns
      - Exons - sequence present in protein-coding mRNA after completion of pre-mRNA splicing
      - Introns - non-protein-coding intervening sequences spliced from mRNA during processing
        - Genes of higher eukaryotes often have one or more introns
        - These regions may correspond to regulatory sequences, but the biological significance of introns is unclear
        - All introns must be removed before protein synthesis
          - If the process errs by even a single nucleotide, the reading frame of the rejoined exons would shift, and the resulting protein would be dysfunctional
    - The process of removing introns and reconnecting exons is called splicing
      - Introns are removed and degraded while the pre-mRNA is still in the nucleus
      - Splicing occurs by a sequence-specific mechanism that ensures introns will be removed and exons rejoined with the accuracy and precision
      - The splicing of pre-mRNAs is conducted by complexes of proteins and RNA molecules called spliceosomes
  - More than 70 individual introns can be present, and each has to undergo the process of splicing, 5' capping and the addition of a poly-A tail just to generate a single, translatable mRNA molecule

Ribosomes and Protein Synthesis

- 10.4: Introduction



- ➢ Protein synthesis uses more of the cell's energy than any other process
  - ■ Translation, or protein synthesis, involves decoding an mNA message into a polypeptide product
  - ■ Amino acids are covalently strung together by interlinking peptide bonds
  - ■ Each individual amino acid has an amino group (NH_2) and a carboxyl (COOH) group
  - ■ Polypeptides are formed when the amino group of one amino acid forms an amide (i.e., peptide) bond with the carboxyl group of another amino acid
  - ■ Occur inside ribosomes
- ➢ Proteins account for more mass than any other component of living organisms (with the exception of water) and perform almost every function of the cell

❖ 10.4a: The Protein Synthesis Machinery

- ➢ Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors
- ➢ Ribosomes - a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides
  - ■ Pre-mRNA, the cell must use energy to make ribosomes
    - In eukaryotes, the nucleolus is completely specialized for the synthesis and assembly of rRNAs
  - ■ Exist in the cytoplasm of prokaryotes and rough endoplasmic reticulum (RER) of eukaryotes
  - ■ Ribosomes dissociate into large and small subunits when they are not synthesizing proteins and reassociate during the initiation of translation
    - Small subunit is responsible for binding the mRNA template
    - Large subunit sequentially binds tRNAs
  - ■ Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction: reading the mRNA from 5' to 3' and synthesizing the polypeptide from the N terminus (free amino end) to the C terminus (free carboxyl end)
- ➢ tRNAS - structural RNA molecules that were transcribed from genes by RNA polymerase III
  - ■ Serving as adaptors, specific tRNAs bind to sequences on the mRNA template and add the corresponding amino acid to the polypeptide chain
    - tRNAs are the molecules that actually "translate" the language of RNA into the language of proteins
    - Can do this due to an anticodon
      - ◆ Anticodon - three-nucleotide sequence in a tRNA molecule that corresponds to an mRNA codon; contains bases complementary to a three-nucleotide codon on the mRNA
  - ■ Of the 64 possible mRNA codons, 3 specify the termination of protein synthesis and 61 specify the addition of amino acids to the polypeptide chain
    - 1 codon also encodes the initiation of translation (AUG)
    - Each tRNA anticodon can base pair with one of the mRNA codons and add an amino acid or terminate translation, according to the genetic code

❖ 10.4b: The Mechanisms of Protein Synthesis

- ➢ The ribosomal subunit consists of three compartments:
  - ■ A (aminoacyl) Site - binds incoming charged aminoacyl tRNAs
  - ■ P (peptidyl) Site - site binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA
  - ■ E (exit) Site - releases dissociated tRNAs so that they can be recharged with free amino acids
- ➢ As with mRNA synthesis, protein synthesis can be divided into three phases: *initiation*, *elongation*, and *termination*

❖ Phases of Protein Synthesis

- ➢ Phase 0: Initiation of Translation

- Eukaryotic initiation uses and initiation factor (IF) that recognizes the 7-methylguanosine cap at the 5' end of the mRNA
- A cap-binding protein (CBP) and several other IFs assist the movement of the ribosome to the 5' cap
- Once at the cap, the initiation complex tracks along the mRNA in the 5' to 3' direction, searching for the AUG start codon
  - Start Codon - AUG (or rarely, GUG) on an mRNA from which translation begins; always specifies methionine
- Once the appropriate AUG is identified, the other proteins and CBP dissociate, and the large subunit binds to the complex of tRNA, mRNA, and small subunit
  - This step completes the initiation of translation in eukaryotes

➢ Phase 1: Translation
- mRNA template gives specificity
- As the ribosome moves along the mRNA, each codon cmes into register and ensures that specific binding with the corresponding tRNA anticodon

➢ Phase 2: Elongation
- Proceeds with charged tRNAs entering the A site and then shifting to the P site followed by the E site with each single-codon "step" of the ribosome
  - Ribosomal steps are induced by conformational changes that advance the ribosome by three bases in the 3' direction
- Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA
  - Amino acid bound to the P-site tRNA is linked to the growing peptide chain
- As the ribosome steps across the mRNA, the former P-site tRNA enters the E site, detaches from the amino acid, and is expelled

➢ Phase 3: Termination
- Occurs when a nonsense codon (UAA, UAG, or UGA) is encountered
  - Upon aligning with the A site, these nonsense codons are recognized by release factors in prokaryotes
  - Eukaryotes instruct peptidyl transferase to add a water molecule to the carboxyl end of the P-site amino acid
- The reaction forces the P-site amino acid to detach from its tRNA, and the newly made protein is released

➢ Aftermath
- The small and large ribosomal subunits dissociate from the mRNA and from each other and are recruited almost immediately into another translation initiation complex
- After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction

Mutations

❖ Mutations - uncorrected error in DNA replication, like DNA polymerase (DNA pol) inserting a wrong base; variation in the nucleotide sequence of a genome

❖ Most mistakes are caught by the proofreading DNA pol

➢ Proofreading - function of DNA pol in which it reads the newly added base before adding the next 1
- If it is the right base, the next nucleotide is added
- If an incorrect base has been added, the enzyme makes a cut at the phosphodiester bond and releases the wrong nucleotide

➢ This is performed by the exonuclease action of DNA pol III. Once the incorrect nucleotide has been removed, a new one will be added again

❖ Types of Mutations

➢ Induced - mutation that results from exposure to chemicals or environmental agents

➢ Spontaneous - mutation that takes place in the cells as a result of chemical reactions taking place naturally without exposure to any external agent

➢ Silent - mutation that is not expressed

➢ Point - mutation that affects a single base

- Nonsense - where a codon is changed to a stop codon; premature stop or loss of start site
- Substitution - in which one base is replaced by another; most common nucleotide mutations
  - Transition - when a purine is replaced with a purine or a pyrimidine is replaced with another pyrimidine
  - Transversion - when a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine
  - Missense - substituted nucleotide changes codon in the first or last position that changes the amino acid
- Frameshift - mutation that results in the insertion or deletion of a codon is often less deleterious than a mutation that results in the insertion of one nucleotide