From Genotype to Phenotype

A. Investigators Recognize Gene Activity

1. English physician Sir Archibald Garrod introduced phrase *inborn error of metabolism*.
    a. Garrod said inherited defects could be caused by the lack of a particular enzyme.
    b. Knowing that enzymes are proteins, Garrod suggested link between genes and proteins.

B. Genes Specify Enzymes

1. George Beadle and Edward Tatum X-rayed spores of red bread mold, *Neurospora crassa*.
2. Discovered some resulting cultures lacked a particular enzyme for growth on medium.
3. They found that a single gene was mutated, which resulted in the lack of a single enzyme.
4. They stated **one gene-one enzyme hypothesis:** one gene specifies synthesis of one enzyme.

C. Genes specify a Polypeptide

1. Pauling and Itano compared hemoglobin in red blood cells of persons and normal individuals.
2. Discovered chemical properties of chain of sickle-cell hemoglobin differed from normal hemoglobin by using electrophoresis to separate molecules by weight and charge.
3. Pauling and Itano formulated the **one gene-one polypeptide hypothesis:** each gene specifies on poly peptide of a protein, a molecule that may contain one or more different polypeptide.

D. From DNA to RNA to Protein

1. Classical geneticists conceived of a gene as any of the particles of inheritance on a chromosome.
2. To molecular geneticists, a gene is a sequence of DNA nucleotide bases that codes for a product.
3. DNA is restricted to nucleus; protein synthesis occurs in cytoplasm.
4. Ribonucleic acid (RNA) was found in both regions and was likely intermediary in protein synthesis.

E. Types of RNA

1. Like DNA, RNA is a polymer of nucleotides.
2. Unlike DNA, RNA is single -stranded, contains the sugar ribose, and contains the base uracil instead of thymine.
3. There are three major classes of RNA.
    a. Messenger RNA (mRNA) takes a message from DNA in nucleus to ribosomes in cytoplasm.
    b. Ribosomal RNA (rRNA) and proteins make up ribosomes where proteins are made.

c. Transfer RNA (tRNA) transfers a particular amino acid to a ribosome.

F. The Required Steps

1. DNA undergoes transcription to mRNA, which is translated to a protein.
2. DNA is a template for RNA formation during transcription.
3. Transcription is the first step in gene expression; it is the process whereby a DNA strand serves as a template for the formation of mRNA.
4. An mRNA transcript directs the sequence of amino acids in a polypeptide.

The Genetic Code

A. Sequence of Bases in DNA

1. The central dogma of molecular biology states that the sequence of nucleotides in DNA specifies the order of amino acids in a polypeptide.
2. The genetic code is a **triplet code** comprised of 64 three-base code words (codons).
3. Codon consists of 3 nucleotide bases of DNA.

B. Finding the Genetic Code

1. In 1961, Marshall Nirenberg and J. Heinrich Matthei found an enzyme that could be used to construct synthetic RNA; discovered the codon UUU coded for phenylalanine.
2. The code is **degenerate**; there are 64 triplets to code for 20 naturally occurring amino acids and this protects against potentially harmful mutations.
3. The genetic code is **unambiguous**; each triplet codon has only one meaning.
4. The code has **start and stop signals:** there is one start codon and three stop codons.

C. The Code is Universal

1. The few exceptions to universality of the genetic code suggests code dates to very first organisms.
2. Once the code was established, changes would ve very disruptive.

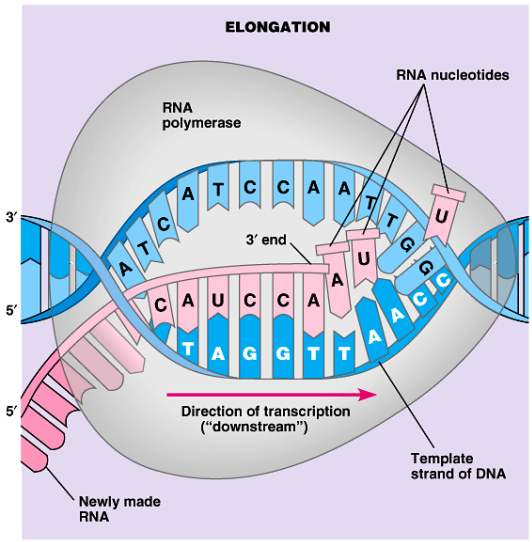
From DNA to RNA - Replication

A. Transcription

1. The first step required for gene expression; takes place in the nucleus of eukaryotic cells.
2. mRNA formation usually leads to a polypeptide gene product; however, tRNA and rRNA are also transcribed from DNA templates and are products themselves.
3. Enzymes called **RNA polymerases** are involved in transcription.

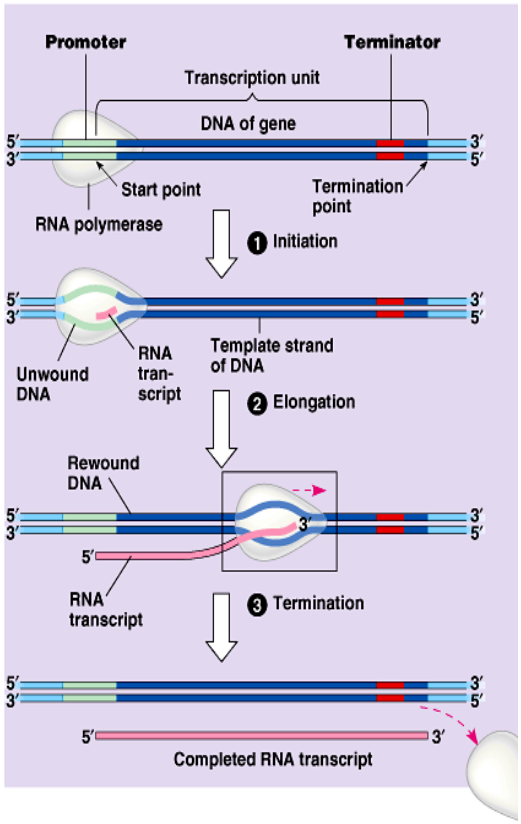
B. Messenger RNA is Formed

1. Transcription begins when RNA polymerase attaches to a promoter on DNA.
2. **RNA polymerase** is an enzyme that speeds formation of RNA from a DNA template.
3. **Promoter** is DNA region that defines start of gene, direction of transcription, and strand copied.
4. Next, a segment of the DNA helix unwinds and unzips.



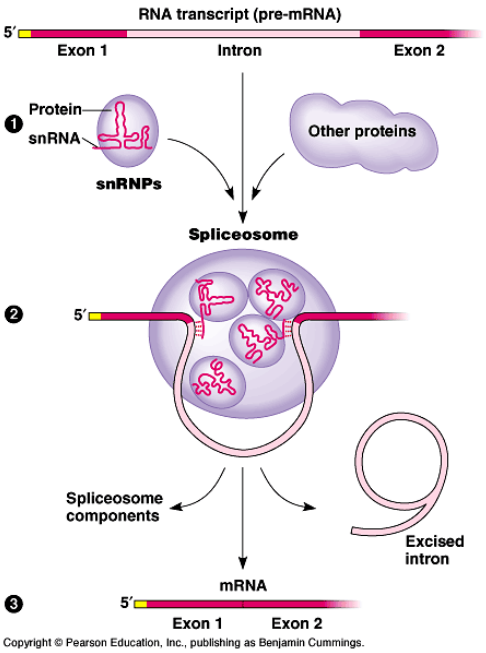
C. RNA Polymerase

1. As RNA polymerase moves along the *template* strand of the DNA, complementary RNA nucleotides pair with DNA nucleotides of the strand.
2. RNA polymerase joins the RNA nucleotides together in the 5' --->3' direction.
3. Transcription begins when RNA polymerase attaches to a region of DNA called a **promoter**; a promoter defines the start of a gene, the direction of transcription, and the strand transcribed.
4. RNA/DNA association is not as stable as DNA helix; therefore, only newest portion of RNA molecule associated with RNA polymere is bound to DNA; the rest dangles off to side.
5. Elongation of mRNA continues until RNA polymerase comes to a DNA terminator sequence.
6. Terminator sequence causes RNA polymerase to stop transcribing DNA and to release mRNA transcript.
7. RNA polymerase molecules work to produce mRNA and from same DNA molecule at same time.
8. Cells produce thousands of copies of same mRNA molecule and many copies of coded protein in a shorter period of time if a single copy of DNA were used for protein synthesis.



D. Messenger RNA is Processed

1. In eukaryotes, newly formed primary mRNA transcript is processed before leaving nucleus.
2. **Primary mRNA transcript** is immediate product of transcription; contains exons and introns.



3. Ends of the mRNA molecule are altered: a cap is put on 5-end and a poly-A tail is put on 3-end.
   a. "Cap" is a modified guanine (G) that tells a ribosome where to attach to begin translation.
   b. The "poly-A tail" consists of a 150--200 adenine (A) nucleotide chain that facilitates transport of mRNA out of the nucleus and inhibits degradation of mRNA by enzymes.
4. Portions of the primary mRNA transcript, called introns, are removed.
   a. **Exon** is portion of DNA code in primary mRNA transcript eventually expressed as a result of polypeptide synthesis.
   b. **Intron** is non-coding segment of DNA removed by spliceosomes before mRNA leaves nucleus.
5. **Spliceosomes** are a complex that contains several kinds of ribonucleoproteins; it cuts primary mRNA transcript and then rejoins adjacent exons.
6. Investigators have found that the simpler the eukaryote, the less likely that introns will be present.
7. Role of introns is being investigated: they may allow crossing over during meiosis, or introns may divide a gene into regions that can be joined in different combinations for different products; thyroid and pituitary glands process same primary mRNA transcript to produce different products.
8. **Ribozymes** are RNAs with an enzymatic function restricted to cleaving RNA at specific locations.
   a. RNA could have served as both genetic material and as first enzymes in early life forms.
   b. This hypothesis eliminates dilemma of which came first, DNA or protein; RNA came first.

The Formation of a Polypeptide - Translation

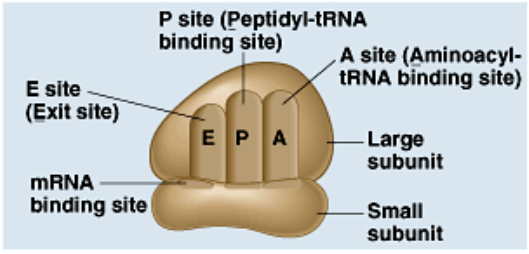
A. Translation

1. Takes place in cytoplasm of eukaryotic cells.
2. Translation is a second step by which gene expression leads to protein synthesis.
3. One language (nucleic acids) is translated into another language (protein).

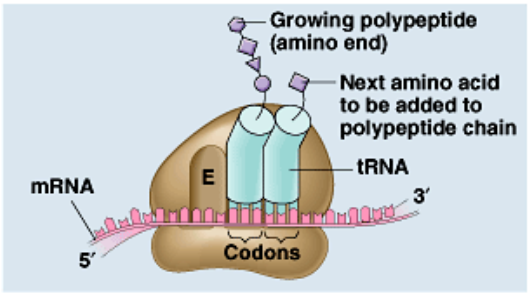
B. The Role of Transfer RNA

1. **Transfer RNA** (tRNA) molecules transfer amino acids to the ribosomes.
2. tRNA is a single-stranded ribonucleic acid that doubles back on itself to create regions where complimentary bases are hydrogen-bonded to one another.
3. At the 3' end it binds to amino acid; at other end it has an **anticodon** that binds to mRNA codon; **anticodon** is group of nucleotides on tRNA complementary to codon on mRNA.

4. There is at least one tRNA molecule for each of the 20 amino acids found in proteins; there are fewer tRNA's that codons because some tRNA's pair with more than one codon.
5. **tRNA synthetases** are amino acid-activating enzymes that recognize which amino acid should join which tRNA molecule, and then catalyze ATP-requiring reactions joining them.
6. Amino acid-tRNA complex forms, then travels in cytoplasm to ribosome for protein synthesis.



(b) Schematic model showing binding sites



(c) Schematic model with mRNA and tRNA

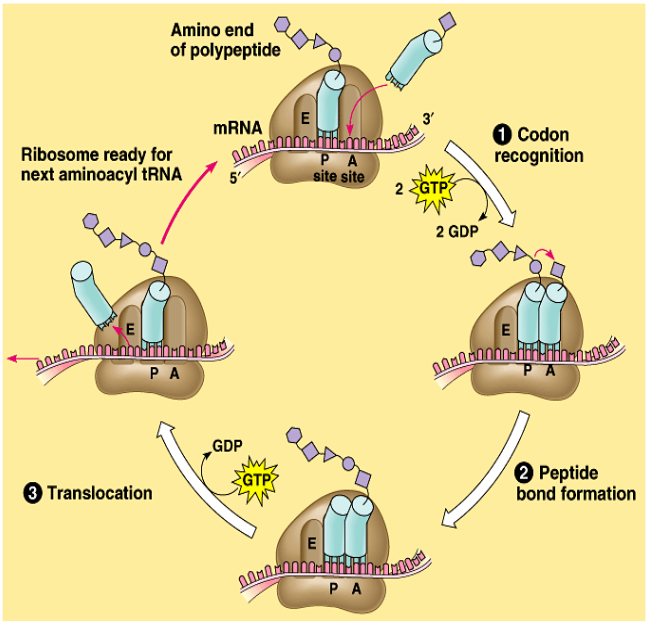
C. The Role of Ribosomal RNA

1. Ribosomal RNA (rRNA) is produced from a DNA template in the nucleolus of nucleus.
2. rRNA is packaged with a variety of proteins into ribosomal subunits, one larger than the other.
3. Subunits move separately through nuclear envelope pores into cytoplasm where they combine.
4. Ribosomes can float free or attach to endoplasmic reticulum.
5. Prokaryotic cells contain about 10,000 ribosomes; eukaryotic cells contain many times more.
6. Ribosomes have a binding site for mRNA and binding sites for two transfer RNA (tRNA) molecules.
7. They facilitate complementary base pairing between tRNA anti-codons and mRNA codons; one protein is an enzyme that joins amino acids together by means of a peptide bond.
8. Ribosome move down mRNA molecule, new tRNA's arrive; amino acids join; polypeptide forms.
9. Translation terminates once polypeptide is formed; ribosome dissociates into its two subunits. **In class we identified the three sites as Arrival, Peptide bond formation and Exit sites.**
10. Polyribosomes are clusters of several ribosomes synthesizing the same protein.
11. Ribosomes are first free in cystol; once synthesis begins, some have series of amino acids called signal sequence that enables ribosome to bind to endoplasmic reticulum.
12. Nearly all polypeptide have signal sequences that target them for final location in cell.

D. Translation Requires three steps.

1. In translation, mRNA codons base-pair with tRNA anti-codons carrying specific amino acids.
2. Codon order determines order of tRNA molecules and sequence of amino acids in polypeptide.
3. Protein synthesis involves chain initiation, chain elongation, chain termination.
4. Enzymes are required for all three steps; energy is needed for first two steps.
5. **Chain Initiation**
    a. Small ribosomal subunit attaches to mRNA in vicinity of the start codon: a base triplet (AUG).

b. First initiator tRNA pairs with this codon; then large ribosomal subunit joins to small subunit.
c. Each ribosome contains tow sites: the **P** (for peptide) **site** and the **A** (for amino acid) **site**.
d. Initiator tRNA binds to P site although it carries one amino acid; the A site is for next tRNA.
e. Initiation factor proteins are required to bring necessary translation components together.

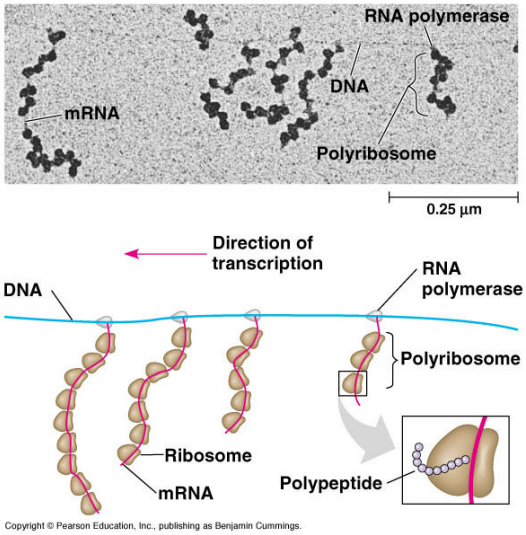


6. Chain elongation

a. tRNA with attached polypeptide is at P site; tRNA -- amino acid complex is just arriving at A site.
b. The polypeptide is transferred and attached by a **peptide bond** to the newly arrived amino acid.
c. Reaction is catalyzed by a ribozyme, which is part of the larger subunit.
d. The tRNA molecule in the P site leaves.
e. Translocation occurs with mRNA, along with peptide-bearing tRNA, moves from site A to P.
f. As ribosome has moved forward three nucleotides, there is new codon located at empty A site.
g. The complete cycle is rapidly repeated, about 15 times per second in *Escherichia coli*.

7. Chain Termination

a. Termination of polypeptide synthesis occurs at stop codon that does not code for amino acid.
b. The polypeptide is enzymatically separated from the last tRNA.
c. tRNA and polypeptide leave the ribosome, which dissociates into its two subunits.



E. Multiple Polypeptide Formation

a. The m-Rna can act as an assemble line.

b. Multiple copies of the same polypeptide can be formed at the same time.

c. Numerous ribosomes can be found on the m_RNA starand at one time.

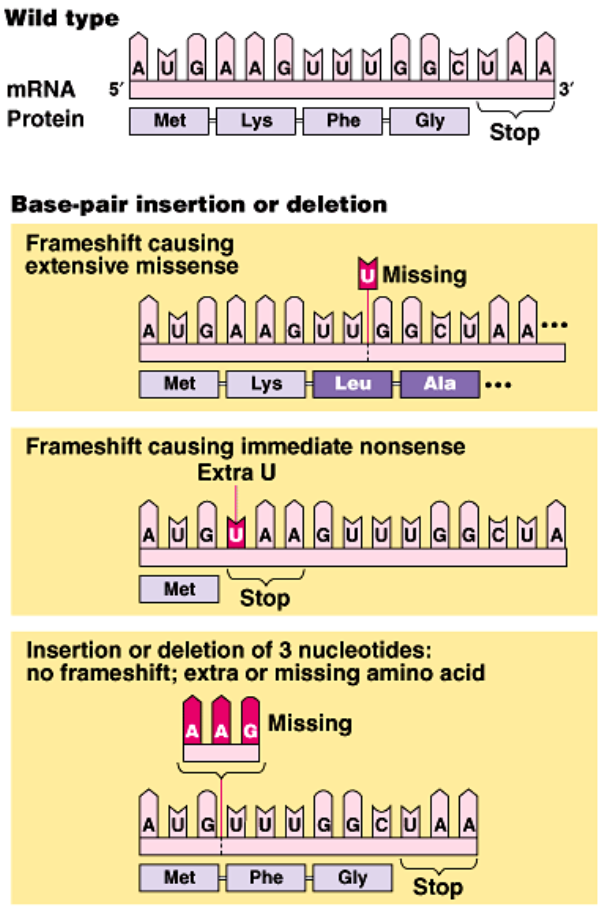
Genetic Mutations

A. A genetic mutation is a permanent change in the sequence of bases in DNA.

1. Somatic mutations only affect the organism; germ-like mutations may be passed to next generation.
2. Germ-like mutations are raw material for evolution; if phenotype is adaptive, it may have more offspring.

B. Cause of Mutations

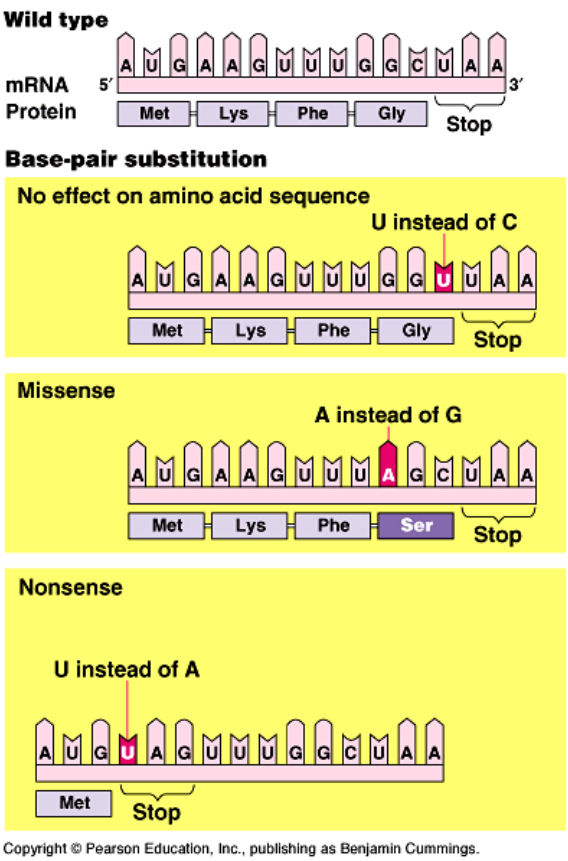
1. Some mutations are spontaneous; mutations due to replication errors are very rare.
2. DNA polymerase constantly monitors, proofreads new strand against old, and repairs any irregularities, reducing mistakes to one out of every one billion nucleotide pairs replicated.
3. **Environmental mutagens** are environmental substances that increase chances of mutations.
    a. Common mutagens are radiation and organic chemicals.
    b. If mutagens cause mutation in gametes, then offspring may be affected.
    c. Cancer is a genetic disorder caused by a failure in regulation of gene activity.
    d. Carcinogens are mutagens that increase chances of cancer.
    e. X-rays and gamma rays are ionizing radiation that creates free radicals, ionized atoms with unpaired electrons.
    f. UV radiation easily absorbed by pyrimidines in DNA.
        1. Where two thymine molecules are near each other, UV bonds them together as thymine dimers.
        2. Usually dimers are removed from damaged DNA by special enzymes called repair enzymes.
    g. Lack of repair enzymes produces xeroderma pigmentosum and higher incidence of skin cancer.
    h. Some organic chemicals act directly on DNA.
        1. 5-bromouracil pairs with thymine but rearranges to a form that pairs with cytosine at the next DNA replication: an A --T pair becomes a G--C pair.
        2. Chemicals may add hydrocarbon groups or remove amino groups from DNA bases.
        3. Tobacco smoke contains a number of chemical carcinogens.
4. Transposons
    a. Transposons are specific DNA sequences that can move within and between chromosomes.
    b. Such "jumping genes" are now recognized in bacteria, fruit flies, and other organisms.



C. Point Mutations

1. **Point mutation** changes a single nucleotide and therefore changes a single specific codon.
    a. They range in effect depending on the particular codon change.
    b. Changes to codons that have same effect have no effect; UAU to UAC both code tyrosine.
    c. Changes from UAC to UAG (a stop codon) results in a shorter protein, and a change from UAC to CAC incorporates histidine instead of tyrosine.
    d. Sickel cell disease results from a single base change in DNA where the b chain of hemoglobin contains valine instead of glutamate at one location and the resulting distorted hemoglobin causes blood cells to clog vessels.

2. **Frameshift Mutations**
    a. Reading frame depends on sequence of codons from starting point: THE CAT ATE THE RAT.
    b. If C is deleted, the reading frame is shifted: THE ATA TET HER AT.
    c. Frameshift mutations occur when one or more nucleotides are inserted or deleted from DNA.
    d. The result of a frameshift mutation is a new sequence of codons and nonfunctional protein.



3. **Nonfunctional Proteins**
   a. A single non-functioning protein can cause dramatic effects.
   b. PKU results when a person cannot convert phenylalanine and it builds up in the system.
   c. A faulty code for an enzyme in the same pathway results in an albino.
   d. The human transposon *Alu* is responsible for hemophilia when it places a premature stop codon in the gene for clotting factor IX.
   e. Cystic fibrosis is due to inheriting a faulty code for a chloride transport protein in plasma membrane.
   f. Androgen insensitivity is due to a faulty receptor for male sex hormones; body cells cannot respond to testosterone and develop as a female although body cells are XY.

Images: Campbell, Neil and Reece, Jane. *Biology* (6th ed.) San Francisco: Benjamin Cummings

Modified Notes: Mader, Sylvia. *Biology* (7th ed) New York: McGraw Hill Publishing