# Protein Synthesis

Learning Outcome B7

## Learning Outcome B7

- Demonstrate an understanding of the process of protein synthesis

## Student Achievement Indicators

*Students who have fully met the prescribed learning outcome are able to:*

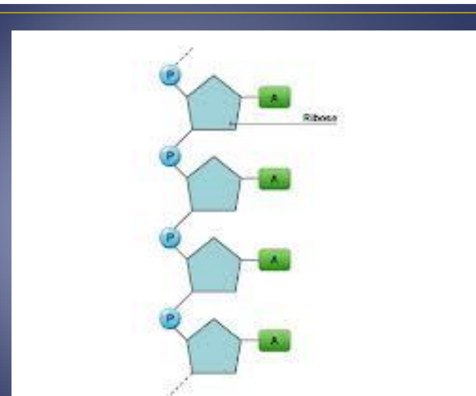
- Identify the roles of DNA, messenger RNA (mRNA), transfer RNA (tRNA), and ribosomes in the processes of transcription and translation, including initiation, elongation, and termination.
- Determine the sequence of amino acids coded for by a specific DNA sequence (genetic code), given a table of mRNA codons.
- Identify the complementary nature of the mRNA codon and the tRNA anti-codon

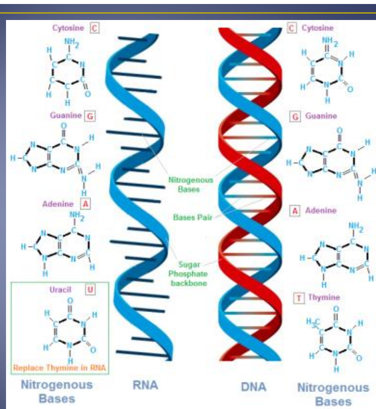
## Gene Expression

- Genetic Disorders have a missing or malfunctioning enzymatic or structural protein
- *Example* – Huntingtons's Disease
- The protein for Huntington's disease is altered and not able to carry out normal functions.
- Proteins are the link between genotypes (genetic codes) and phenotypes (appearance)
- Genes are segments of DNA that specifies the amino acid sequence of proteins.
- Differences in base sequences cause a difference in protein structure.
- A gene does NOT directly control proteins synthesis; it passes genetic information on to RNA which is involved in protein synthesis.

## RNA

- Contains a ribose sugar
- Has the nitrogen base uracil instead of thymine
- Single-stranded nucleic acid
- Three Classes of RNA:
  - ✓ Messenger RNA (mRNA) – takes messages form DNA to ribosomes
  - ✓ Ribosomal RNA (rRNA) – along with proteins make up ribosomes, ribosomes are where proteins are synthesized.
  - ✓ Transfer RNA (tRNA) – transfers amino acids to ribosomes





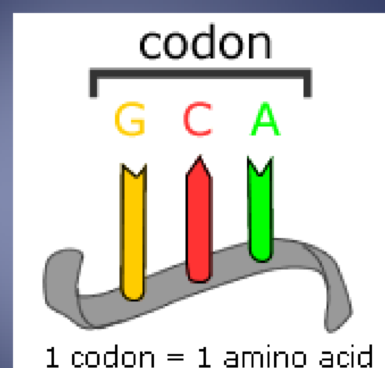

## Protein Synthesis

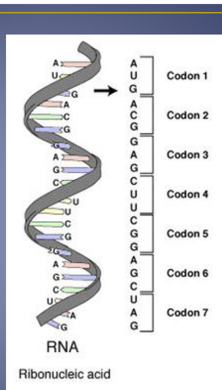
- There are 2 Steps in Protein Synthesis (AKA Gene Expression)

1. **Transcription (TXN)**
   - Information is transferred from DNA to RNA
2. **Translation (TSL)**
   - An RNA transcript directs the sequence of amino acids.
   - This means that a nucleotide sequence from DNA directs amino acids sequences.

## Genetic Code

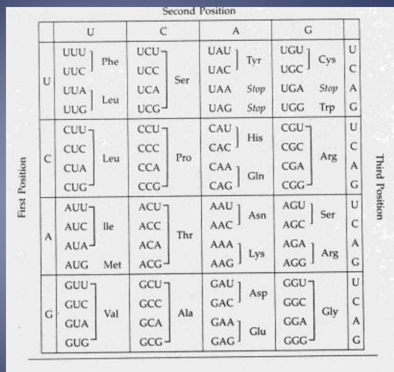
- Code is a triplet; 3 bases stand for one amino acid.
- Each three-letter unit of an mRNA molecule is called a codon.
- 64 mRNA codons have been determined
- 61 mRNA codons code for a particular amino acids
- 3 mRNA codons are known as stop codons
- Stop codon single polypeptide termination



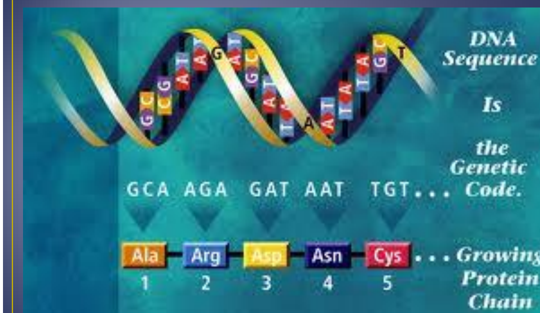

## Genetic Code

- The codon for methionine (AUG) also is an initiation codon
- Most amino acids have more than one codon. *WHY?*
- *Example* – Leucine
- Having more than one codon offers some protection against harmful mutations that change base sequences.



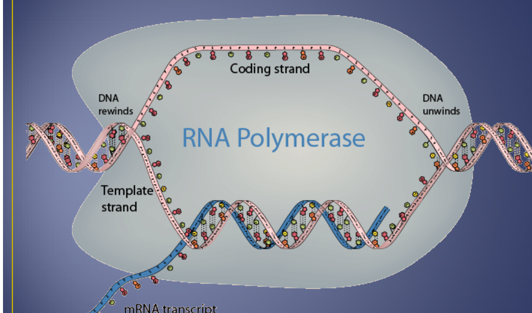
Second Position

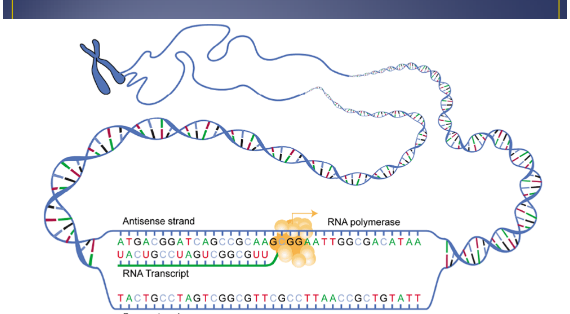
| First Position | U | C | A | G | Third Position |
|---|---|---|---|---|---|
| U | UUU Phe | UCU Ser | UAU Tyr | UGU Cys | U |
| | UUC Phe | UCC Ser | UAC Tyr | UGC Cys | C |
| | UUA Leu | UCA Ser | UAA Stop | UGA Stop | A |
| | UUG Leu | UCG Ser | UAG Stop | UGG Trp | G |
| C | CUU Leu | CCU Pro | CAU His | CGU Arg | U |
| | CUC Leu | CCC Pro | CAC His | CGC Arg | C |
| | CUA Leu | CCA Pro | CAA Gln | CGA Arg | A |
| | CUG Leu | CCG Pro | CAG Gln | CGG Arg | G |
| A | AUU Ile | ACU Thr | AAU Asn | AGU Ser | U |
| | AUC Ile | ACC Thr | AAC Asn | AGC Ser | C |
| | AUA Ile | ACA Thr | AAA Lys | AGA Arg | A |
| | AUG Met | ACG Thr | AAG Lys | AGG Arg | G |
| G | GUU Val | GCU Ala | GAU Asp | GGU Gly | U |
| | GUC Val | GCC Ala | GAC Asp | GGC Gly | C |
| | GUA Val | GCA Ala | GAA Glu | GGA Gly | A |
| | GUG Val | GCG Ala | GAG Glu | GGG Gly | G |

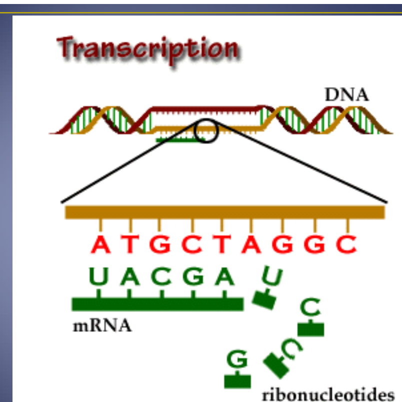



## Transcription

- A segment of the DNA helix unwinds ("unzips")
- RNA nucleotides pair complementary with the DNA nucleotides of one strand.
- The nucleus contains an RNA nucleotide pool.
- The RNA nucleotide joins to the DNA by the enzyme RNA polymerase.
- When mRNA forms it has sequences of bases complementary to DNA.
- These bases are grouped in threes and are known as codons



## Transcription

| | | | | |
|---|---|---|---|---|
| **DNA** | **A** | **G** | **C** | **and T** |
| **RNA** | **A** | **G** | **C** | **and U** |

*Example*

DNA A C G C T A A C T

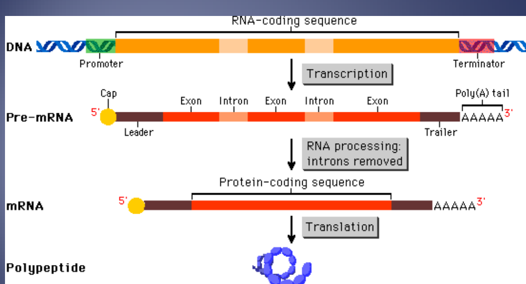
mRNA U G C G A U U G A

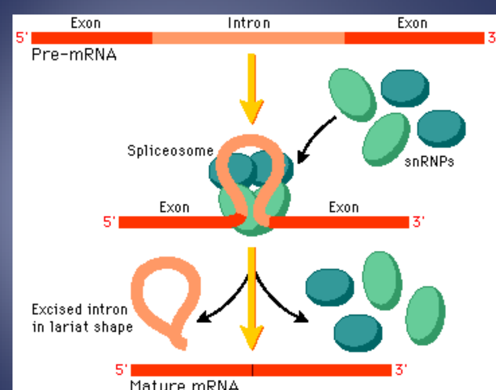
- mRNA is processed and then moves out of the nucleus into the cytoplasm where it can meet a ribosome for translation.

## Transcription

Processing of mRNA

- Introns are segments of DNA that are not part of the gene
- Exons are part of the gene that code for proteins
- When DNA is transcribed to mRNA, the mRNA contains both exons and introns.
- Before mRNA can move out of the nucleus it must be processed to get rid of the introns.
- Splicing mRNA to remove introns is done by ribozymes which are organic catalysts composed of proteins not RN
- Processed mRNA is called mature mRNA and exits the nucleus and enters the cytoplasm where it can become associated with ribosomes.




## Translation

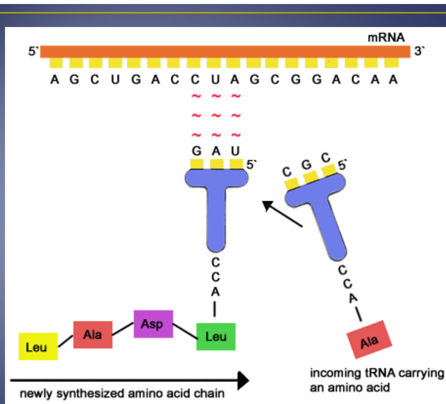
- Codons in mRNA specify the order of amino acids in a protein
- Requires several enzyme and two types of RNA

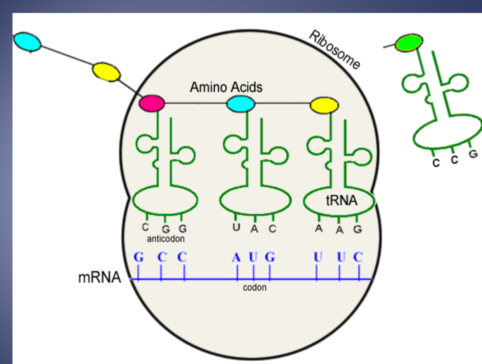
## Translation

1. Transfer RNA (tRNA)
   - ✓Brings amino acids to the ribosomes (strands of DNA)
   - ✓Single-stranded nucleic acid than doubles back on itself to create regions where complementary base pairing results in a boot-like shape.
   - ✓One tRNA molecule for each of the 20 amino acids found in proteins.
   - ✓Amino acids bind at one end (matches the mRNA strand)
   - ✓An anticodon is on one end of the tRNA molecule


## Translation

- An anticodon is a group of 3 bases that are complementary to a specific condon of mRNA.
- Amino acids and tRNA with anticodons are known as a tRNA-amino acid complex.
- When a tRNA-amino acid complex comes into contact with a ribosome, its anticodon pairs with the mRNA codon.
- *Example:* Codon – ACC Anticodon – UGG
- Codes for amino Acid – threonine
- The mRNA codon determines which tRNA-amino acid complex must come to the ribosome and therefore determine the sequence of amino acids in a protein.

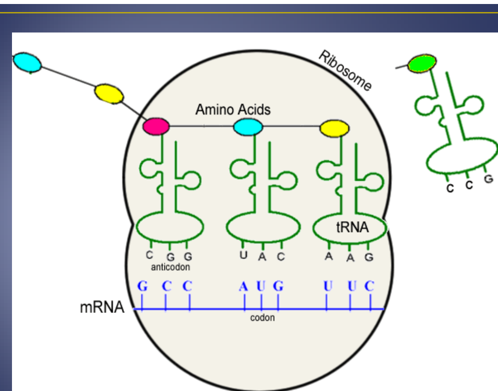



## Translation

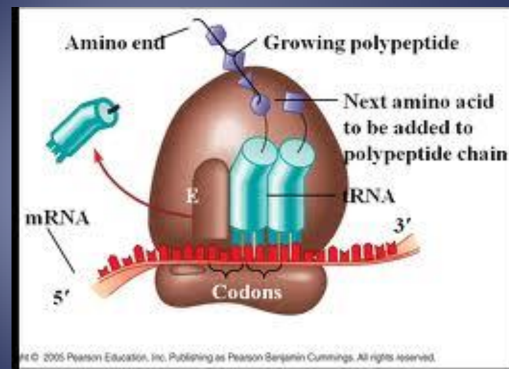
2. Ribosomal RNA (rRNA)

- Produced in nucleus
- Joins with proteins and is manufactured in the cytoplasm
- Composed of two ribosomal subunits
- There is 1 large and 1 small ribosomal subunit which contains rRNA and many different proteins.
- These subunits leave the nucleus and join together in the cytoplasm just before protein synthesis begins.
- rRNA has a binding site for mRNA and two for tRNA.



## Translation

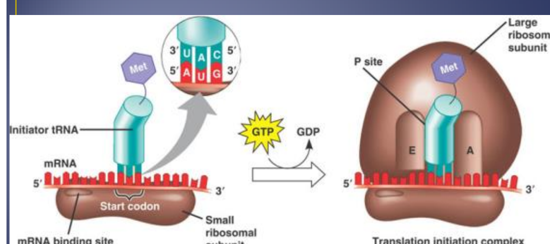
- ✓ Binding sites facilitate bonding of the tRNA anticodons and mRNA codons.
- ✓ As the ribosome moves down the mRNA molecule; new tRNA's arrive and they polypeptide grows.
- ✓ Translation terminates once the peptide is formed and the ribosome subunits dissociate and fall of the mRNA molecule.
- ✓ Several ribosomes may be attached on a single mRNA, this is known as a polyribosome.




## Steps in Translation

1. Chain Initiation

- Small ribosomal subunits, mRNA, initiator tRNA and large ribosome subunits all come together.
- Small ribosomal subunits attaches to mRNA near the start codon AUG
- The anticodon on the tRNA called the initiator codon pairs with the mRNA codon.
- The large ribosomal subunits join the small subunit; there are now two binding sites for tRNA's.




## Steps in Translation

2. Chain Elongation

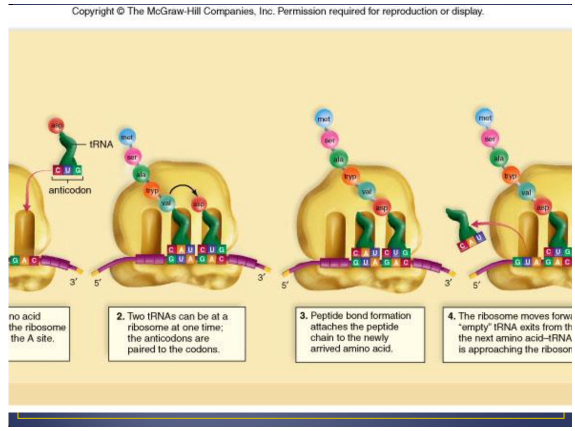
- tRNA at first binding site contains an attached peptide
- The initiator tRNA passes its amino acids to a tRNA – amino acid complex that has come to the second binding site.
- Ribosomes move forward and the tRNA in the second site is now the first site.
- This process is known as translocation.

## Steps in Translation

- Translocation occurs again and again
- Growing peptide is transferred to a newly arrived amino acid.
  - This requires energy and ribozyme
  - Ribozyme is a large part of the ribosomal subunit.
  - After translocation, outgoing tRNA will pick up another amino acid before it returns to the ribosome

## Translocation

- http://www.phschool.com/science/biology_place/biocoach/translation/elong1.html




## Steps in Translation

3. Chain Termination
- End of protein synthesis; stop codon (does not code for an amino acid)
- Polypeptide is enzymatically cleaved form the last tRNA by a release factor.
- tRNA and polypeptide leave ribosome and it dissociates.
- Newly synthesized polypeptide may function alone or become part of a protein that have more than one polypeptide.

## Control of Gene Expression

- Cells undergo mitosis (growth) in humans and are known as totipotent.
- This means that each cell has the potential to develop into a new organism because it still contains the same gene that were present in the original cell.
- Differences in gene expression account for specializations within the cell.
- Gene expression is how much of the gene is being expressed.
- Genes are turned off and on at different times, so that each gene's protein is only made when it is needed.
- *Example* - skin cells turn on genes that make keratin and red blood cell precursors turn on genes to make hemoglobin.

## Control of Gene Expression

- Many steps in transcription and translation are required for gene expression and regulation of the gene can occur at any of these steps.
- There are positive and negative controls
- Positive controls increase the final amount of protein being made.
- Negative controls decrease the final amount of the protein being made.
- Many genes code for essential metabolic enzymes.
- These are known as "housekeeping genes" and are not finely regulated because they are always needed.
- Cells also all express a set of genes necessary to maintain general cellular function such as transport and acquiring energy.

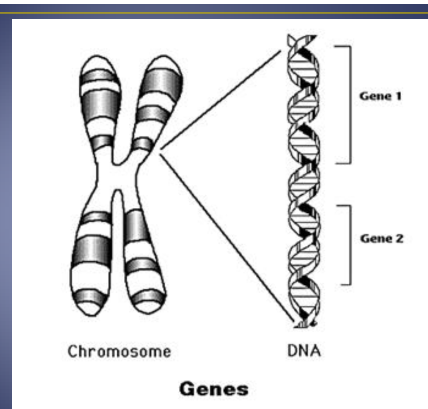
## Levels of Gene Control

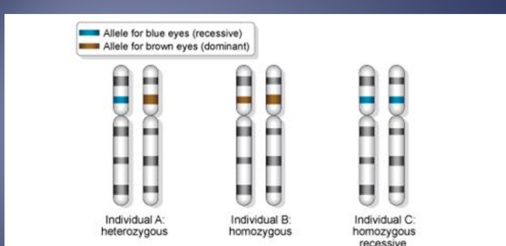
- Gene expression is controlled at 6 different levels:

1. Chromatic Structure
   - ✓ Chromatic packing is a way to keep genes turned off.
   - ✓ Genes with darkly stained areas of the chromosome on a micrograph have highly condensed chromatin and are known as heterochromatin.
   - ✓ Heterochromatin is inactive
   - ✓ This occurs on the XX chromosome in mammalian females.
   - ✓ Females are XX and males are XY

## Levels of Gene Control

- So females each have one inactive X chromosome in every cell of their body.
- Each inactivated X chromosome is known as Barr body and appears as a darkly stained mass of condensed chromatin along the inner edge of the nuclear envelope.
- During the pre-natal development, one X chromosome is shut off and it is turned back on at different times in different individuals.




## Levels of Gene Control

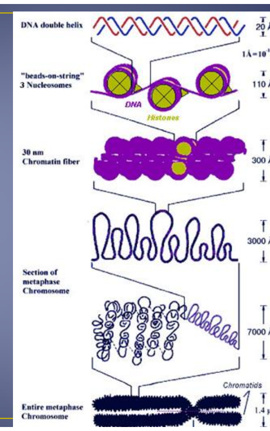
- *Example* – calico/tortoise-shell cats
- Alleles (sets of genes) for black or orange coats are carried on the X chromosome
- Random X-inactivation occurs in females and therefore in a heterozygous (two different alleles) individual some of the cells express the alleles for a black coat and some express alleles for an orange coat.
- If X-inactivation occurred early in fetal development; large patches would result. This pattern is known as a calico pattern.

## Levels of Gene Control

- If X-inactivation occurred late in fetal development, small patches would develop.
- This pattern is known as a tortoise shell pattern.

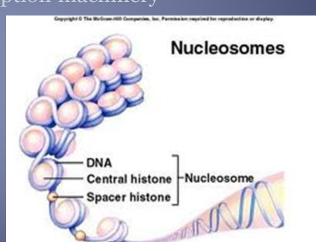
## Levels of Gene Control

- White color in calicos is due to another gene.
- All cells formed in cell division will not have the same X chromosome inactivated; therefore patches of tissue will differ in which X chromosome is being expressed.
- If a female is heterozygous for a particular X-linked gene she will be a mosaic containing patches of cells expressing different alleles.
- Active genes are associated with loosely packed chromatin known as euchromatin.
- Even euchromatin must be “unpacked” before it is transcribed.



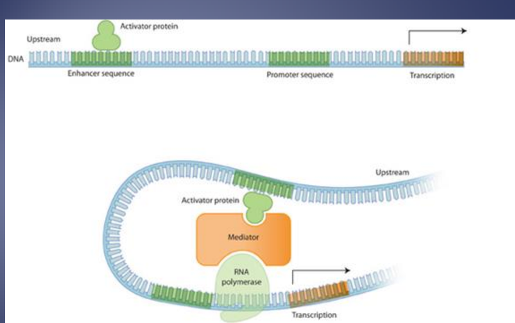

## Levels of Gene Control

- Nucleosomes limit access to DNA by the transcription machinery




## Levels of Gene Control

2. Transcriptional Control
   - Most important level of control
   - Interactions of proteins with particular DNA sequences.
   - These proteins are known as transcription factors and activators, while the DNA sequences are known as promoters and enhancers.
   - Transcription factors are proteins that help RNA polymerase bind to a promoter.
   - Several transcription factors per gene form a transcription-initiation complex that helps pull double-stranded DNA apart and even acts to release RNA polymerase so transcription can begin.
   - Some transcription factors can be used over and over again at different promoters.




## Levels of Gene Control

- If one transcription factor malfunctions it can be disastrous.
- Transcription activators are proteins that speed up transcription dramatically
- They bind to DNA regions known as enhancers; which may be quite a distance away from the promoter.
- A loop in DNA can bring the transcription activator into contact with the transcription-initiation complex.
- A group of 4 related transcription factors called the Myo-D family are responsible for the development and differentiation of muscle cells by controlling the expression of muscle specific proteins.
- The 4 transcriptions factors appear to act at different times during the development of muscle cells, allowing cells to become fully differentiated muscle cells.

## Levels of Gene Control

3. Post-Transcriptional Control

- ✓ Some proteins are not active immediately after synthesis.
- ✓ *Example* – after translation insulin is folded into a 3-D shape that is inactive.
- ✓ Then a sequence of30 amino acids is enzymatically removed from the middle of the molecule leave two polypeptide chains that are bonded together by a disulphide bond.
- ✓ The disulphide bond activates the protein.
- ✓ Many proteins only function for a short period of time before they are degraded or destroyed.

## Levels of Gene Control

4. Signalling between Cells

- ✓ Cells are constantly communicating with one another.
- ✓ Generally a cell produces a signalling molecule that binds to a receptor protein in the target cell's plasma membrane.
- ✓ A signaling molecule causes the receptor protein to initiate a series of reactions.
- ✓ These reactions will change the receiving cell's behavior.

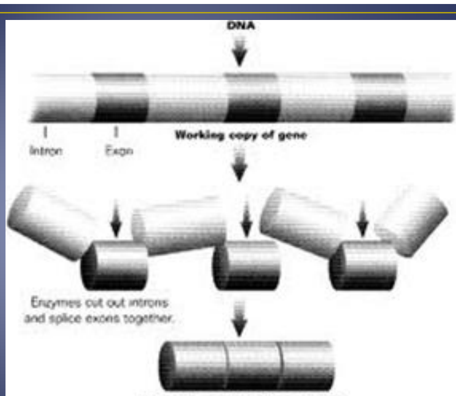
## Levels of Gene Control

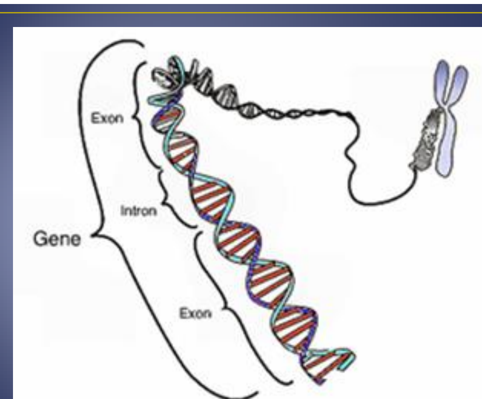
- ✓ This series of reactions is called signal transduction pathway.
- ✓ This pathway results in a stimulation of transcription activators.
- ✓ Activators will help turn a gene on and this allows a specialized cell to come on and is coordinated so the cell usually assumes a structure and function suitable to their location in the body.

## Levels of Gene Control

5. Post-Transcriptional Control

- ✓ After transcription mrRNA is processed before it leaves the nucleus to the cytoplasm.
- ✓ Primary mRNA → mature mRNA
- ✓ Mature mRNA has the addition of a poly-A tail and guanine cap.
- ✓ Introns are removed and exons are spliced back together.
- ✓ Introns are the nucleotide sequences that are removed as mRNA reaches its mature state.



## Levels of Gene Control

- mRNA can be sliced in different ways to get slightly different products (proteins) in different tissues
- *Example* – both the hypothalamus and thyroid gland produce the hormone calcitonin but the calcitonin mRNA that exits the nucleus contains different combination of exons in both the two tissues.
- Speed of mRNA movement form the nucleus to cytoplasm affects the amount of gene production following transcription (amount of protein produced).
- mRNA exits the nucleus via the nuclear pore, and mRNA varies in the amount of time it needs to exit the nucleus.

## Levels of Gene Control

6. Translational Control
- The longer the mRNA is in cytoplasm before it is broken down the more protein that can be translated.
- Differences in poly-A tail and guanine caps can determine how long a particular transcript can remain active in the cytoplasm before it is destroyed by ribonuclease.
- Ribonuclease is an enzyme associated with ribosomes.
- Hormones can cause the stabilization of certain mRNA transcripts so they are not broken down.
- *Example* – mRNA for vitelline (egg membrane protein) can persist for three weeks if exposed to estrogen as opposed to 15 hours without estrogen.