# **C17 Protein Synthesis**

Proteins are links between genotype and phenotype.

**Protein synthesis: (DNA🡪 RNA 🡪 protein)**

1. **Transcription** – DNA to RNA

## **Translation** – RNA 🡪 aa 🡪 protein.

Prokaryotes vs eukaryotes in protein synthesis:

## Prokaroytes have no nucleus. Transcription and translation are coupled.

## In eukaryotes, nuclear envelope separates transcription and translation. Transcription occurs in a 2 step process: pre-mRNA (**primary transcript**) then mRNA **RNA processing**.

**Triplet code** – each 3 nucleotides codes for 1 aa. (43 or 64 possible).

**Template strand** – either of the 2 DNA strands that becomes the form for the RNA transcript. mRNA is complementary, not identical.

**Codon** – the mRNA base triplets.

First codon deciphered in 1961 by **Nirenberg** of the NIH. It was UUU = phenylalanine. All 64 were deciphered by mid-1960’s.

Genetic code must have **evolved very early** as it is shared by organisms from the simplest bacteria to the most complex plants and animals.

**Details of transcription:**

**RNA polymerase** – enzyme pries the 2 strands of DNA apart and hooks together the RNA nucleotides as they base-pair along the DNA template in the **5’ 🡪 3’** direction.

**Transcription unit** – stretch of DNA transcribed into an RNA molecule.

Prokaryotes vs. eukaryotes regarding RNA polymerase:

* Bacteria have only **1 type** of RNA polymerase that synthesizes mRNA and other types of RNA.
* In bacteria, the RNA polymerase recognizes and binds to the promoter, itself.
* Eukaryotes have **3 types** of RNA polymerase, numbered I, II, and III. For mRNA synthesis, RNA polymerase II.
* In eukaryotes, a collection of proteins, called **transcription factors**, mediate the binding of RNA polymerase and the initiation of transcription.

**Three stages of transcription:**

**Initiation:**

**Promoter** – region of DNA where RNA polymerase attaches and initiates transcription. Also determines which strand is used.

**Transcription initiation complex** – the completed assembly of transcription factors and RNA polymerase bound to the promoter.

**TATA box** – a crucial promoter DNA sequence located in DNA template strand.

**Elongation:**

The RNA polymerase moves along the DNA template exposing about 10-20 DNA bases at a time pairing with RNA nucleotides.

**Termination:**

**Terminator** – the transcribed RNA sequence that signals the end of transcription.

**RNA processing:**

The pre-mRNA ends are both modified.

The 5’ end, (made 1st) is capped off with a modified form of guanine.

**5’ cap functions**:

* Helps protect the mRNA from degradation by hydrolytic enzymes
* After the mRNA reaches the cytoplasm, the 5’ cap functions as part of an “attach here” sign for ribosomes.
* Becomes the “leader” on the final mRNA

3’ end gets a **poly(A) tail** consisting of 30-200 adenine nucleotides

**Poly(A) tail functions (3):**

* Inhibits degradation of the RNA
* Helps the ribosome attach to it
* Facilitates the export of mRNA from the nucleus

**Introns** – noncoding segments that lie between coding regions, also called intervening sequences.

**Exons** – expressed regions (made into amino acid sequences).

**RNA splicing** – cut and paste function that removes introns.

**Small RNAs (formally called snRNPs** (pronounced snurps) –recognize splice sites, i.e. short nucleotide sequences at the ends of introns, small nuclear ribonucleoproteins.

**Spliceosome** – made of several different snRNA’s (small nuclear RNA) as well as additional proteins to form a large assembly, almost as big as a ribosome. Functions to cut the introns out and splice the exons together.

**Ribozymes** – RNA molecules that function as enzymes.

Functional and evolutionary **importance of introns**:

* Introns may play regulatory roles in cells; some control gene activity.
* “Split genes” often code for different **domains** of a protein, having introns may lead to more diversity of proteins.
* Introns increase the opportunity for crossing over between two alleles, increasing variability in organisms.

**Domains** – the modular protein architecture made of discrete structural and functional polypeptide units.

**Details of translation:
Transfer RNA (tRNA)** – the translator, functions to transfer aa’s from the cytoplasm’s aa pool to a new polypeptide.

**3 parts:**

* **RNA strand** of ~ 80 nucleotides folded in middle.
* **Anti-codon** – nucleotide triplet which binds to a complementary codon on mRNA.
* **Amino acid** – attachment site has a 3’ end; unique for the anti-codon.

**Wobble** – relaxation of base pairing rules occurs during translation. The 3rd position can sometimes bind flexibly. Ex. the U in the 3rd position of the tRNA, can bind with either an A or G of the mRNA codon. The most versatile bases have an (inosine) I in the third position and can bind to U, C, or A.

**Aminoacyl-tRNA synthetase** – enzyme that joins each aa to the correct tRNA (energized by ATP).

**Ribosomes** are made of two subunits, large and small. Made of proteins and **ribosomal RNA** (rRNA). Made in the nucleolus. rRNA is the most abundant type of RNA.

Eukaryotic ribosomes are slightly larger and differ in molecular composition from bacterial ribosomes. Thus, antibiotics, like tetracycline and streptomycin target bacteria without harming eukaryotes.

Ribosomes have 3 identified binding sites for tRNA.

* **E site** – exit site.
* **P site** – peptidyl-tRNA site, holds the tRNA carrying the growing polypeptide chain.
* **A site** – aminoacyl-tRNA site, holds the tRNA carrying the next aa to be added to the chain.

**3 stages of translation**:

**Initiation stage** – brings together mRNA, the 1st tRNA, and the 2 subunits of a ribosome.

* 1. Small subunit binds to both mRNA at 5’ end and a special initiator tRNA.
	2. The initiation codon, AUG, signals the start of translation.
	3. Initiator tRNA, carrying methionine, attaches to the initiation codon.
	4. Attachment of large subunit aided by initiation factors (proteins).
	5. Initiator tRNA sits in P site and vacant A site is ready for next tRNA.
	6. GTP (guanosine triphosphate) molecule helps provide energy for translation.

**Elongation** – aa’s are added to the 1st aa… Energy from GTP.

1. **Codon recognition** – H bonds form between A site tRNA and mRNA.
2. **Peptide bond formation** – rRNA molecule, functioning as a ribozyme, catalyzes the formation of a peptide bond, then new aa added.
3. **Translocation** – tRNA in the A site (holding the polypeptide) translocated to the P site. Then P to E.

**Termination** – A stop codon in the A site ends elongation. A protein called release factor binds to stop codon in the A site which adds a water molecule instead of an aa to the chain, freeing the chain.

**Polyribosomes** – several ribosomes work on the same strand of mRNA.

Proteins destined for the endomembrane system or secretion:

**Signal peptide** – sequence of ~20 aa’s near the leading end of the polypeptide gets recognized by the **signal-recognition particle (SRP).** The SRP then takes the ribosome to the ER for completion of translation.

**Mutation** – change in the genetic material of a cell (or virus).

**Point mutation** – change in 1 or a few base pairs in a single gene.

**Base-pair substitution** – (type of point mutation) replacement of 1 nucleotide and its partner in the complementary DNA strand with another pair of nucleotides. Some are silent.

**Missense mutation** – still codes for an aa, but the wrong one.

**Nonsense mutation** – aa codon changed to a **stop** codon leading to nonfunctional proteins.

**Frameshift mutations** - caused by insertions or deletions, usually results in extensive missense ending sooner or later in nonsense.

**Mutagens** – physical or chemical agents that interact with DNA to cause mutations. Ex, X-rays, UV light, chemicals, excess heat.

**Gene** – a region of DNA whose final product is either a protein or RNA.