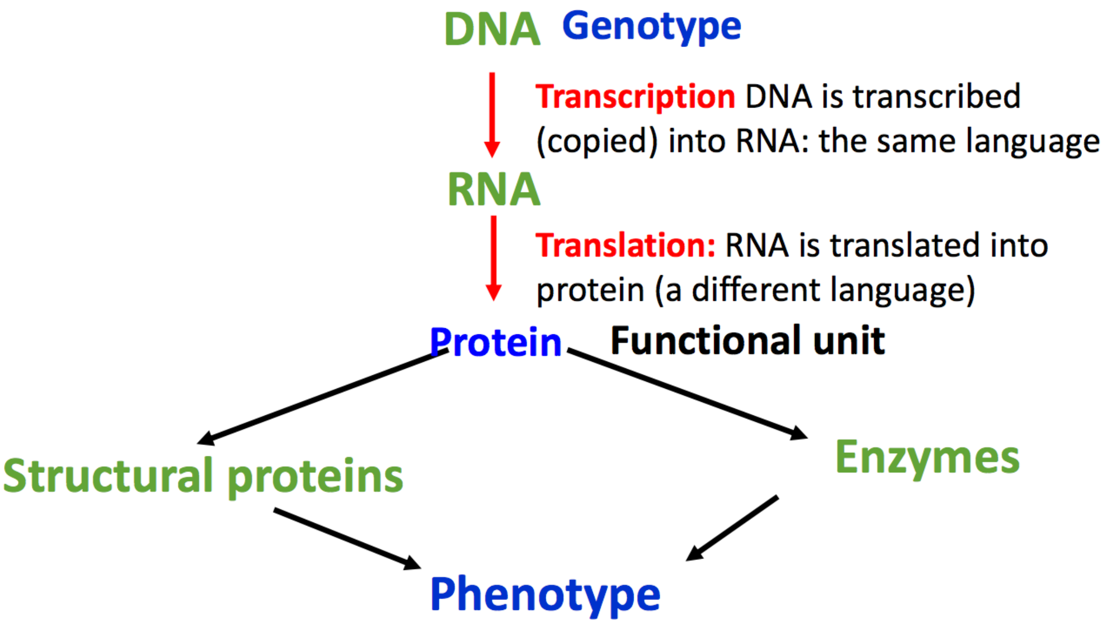
Week 9 (a) – Gene Expression:

Flow of genetic information:



- Note: as prokaryotes do *not* have a nucleus, transcription & translation in these cells both occurs in the cytoplasm.
- In eukaryotes: transcription takes place in the nucleus, translation takes place in the cytoplasm.
- The genetic code is a **triplet code**. During translation, RNA is read as a sequence of nucleotide triplets called **codons**. Each codon specifies an amino acid to be added to the growing polypeptide chain. The RNA is read in the 5’ to 3’ direction.
- AUG is the **start codon**.
- UAA, UAG, UGA are the **stop codons**.

Transcription:

- **RNA polymerases** carry out transcription (copying of DNA into RNA).
- Unlike DNA polymerase, RNA polymerase does *not* require a primer to commence protein synthesis.
- In prokaryotes, a single RNA polymerase makes all types of RNA.
- In eukaryotes, there are many types of RNA polymerases. RNA polymerase II produces mRNA.
- Transcription has 3 stages:
  - **Initiation:** where RNA polymerase recognizes (via proteins known as transcription factors) and binds to promoter DNA (the regulatory region of a gene). This causes the DNA strand to unwind, and the polymerase initiates RNA synthesis at the start point on the template strand.
  - **Elongation:** where the RNA polymerase moves downstream, unwinding the DNA and elongating the RNA transcript (5’ to 3’).
  - **Termination:** where the RNA transcript is released, and the polymerase detaches from the DNA. In prokaryotes (bacteria), termination occurs via a

terminator sequence DNA template. In eukaryotes, termination occurs via a polyadenylation sequence (AAUAAA) which causes proteins to bind and eventually cut mRNA free from the polymerase.

- Eukaryotic genes include non-coding regions called **introns** that interrupt the coding sequence. Introns are *removed* by a **spliceosome** during maturation of the final RNA product. Alternation/mutation of an intron has *no* effect on proteins produced.
- **Exons** are the regions that form *mature* RNA.
- RNA processing in the nucleus involves splicing (removing non-coding DNA such as introns), capping (adding caps (e.g. a guanine nucleotide) to the ends of RNA) & polyadenylation.
- The **5' cap** prevents mRNA from degradation and assists in ribosome binding during translation. The **3' poly-A tail** also prevents mRNA from degradation, and aids in transcription termination.

Translation:

- **Transfer RNA** (tRNA) acts as an interpreter, reading the DNA codon and interpreting it into amino acids (polypeptides).
- The **3' ACC codon** is the site of attachment for amino acids.
- The tRNA recognizes the mRNA codon via its anti-codon section.
- tRNA adds amino acids to the growing polypeptide chain when the anticodon hydrogen bonds to the complimentary codon on the mRNA.
- Translation occurs in the cytoplasm at the ribosome.
- Translation has 3 stages:
  - **Initiation:** where initiator tRNA binds to the small subunit. This small ribosomal subunit binds to mRNA at the 5' cap, and scans along until a start codon is found.
  - **Elongation:** where the ribosome reads codons, bringing in the proper aminoacyl tRNA's to translate the message into amino acids. The incoming aminoacyl tRNA is brought into the ribosome's A site, where it is matched with the codon being presented.
  - **Termination:** when a ribosome reaches a stop codon on mRNA, the A site of the ribosome accepts a 'release factor'. The release factor frees the last amino acid of the polypeptide from the ribosome.

****Note:** if a DNA strand is a *non-template* strand, it will have the *same* mRNA sequence as its DNA sequence (except thymine is replaced by uracil).
If a DNA strand is a *template* strand, the mRNA sequence will have the opposite (complement) sequence of base pairs to the template. The mRNA/complementary strand will run in the opposite direction to the direction of the template strand.

Week 9 (b) – Mutations & Errors in Protein Synthesis:

Mutations:

- **Mutations** are changes to the nucleotide sequence of DNA.
- Germline (sex-cell mutations) *are* transmitted through generations, whereas somatic cell mutations are *not* inherited.

- **Large-scale mutations** involve partial rearrangement of chromosomes, or even non-disjunction of entire chromosomes (e.g. deletion, duplication, inversion or translocation).
- **Small-scale mutations** involve one or only a few base pairs (e.g. substitution, insertion or deletion). Small-scale mutations *sometimes* have *no* effect on the amino acid sequence (known as <u>silent mutations</u>). Silent mutations normally involve substitution mutation at the *third* position of an exonic codon.
- **Missense mutations** are where a change in a *single* nucleotide results in a codon that codes for a different amino acid.
- **Nonsense mutations** result in translation being stopped prematurely (creation of a stop codon before there should actually be one).
- **Deletion & insertion mutations** can have a disastrous effect on an encoded protein as they can change the reading frame (the frame of 3 nucleotides). Mutations that change the reading frame are known as **frameshift mutations.**
- **Mutagens** (e.g. physical agents such as UV, chemical agents such as cigarette smoke) are agents that *increase* the mutation rate.
- **Somatic** (non-sex cell) **mutations** are involved in cancer. **Oncogenes** are genes that have the potential to cause cancer. **Proto-oncogenes** are normal genes which, when altered by mutation, become oncogenes that can contribute to cancer.
- **Germ-line** (sex-cell) **mutations** cause human genetic disorders.
- **Tumour-suppressor genes** *inhibit* cell division.
- **Thalassemia** describes a number of disorders caused by various types of mutations in haemoglobin genes.
    - **α–thalassemia's** involve deletion of one or more α-globin genes on chromosome 16, or a stop codon on an α-globin gene.
    - **β–thalassemia's** involve deletion of a β-globin gene on chromosome 11, or a mutation in the promoter region of the gene.

Week 9 (c) – Regulation of Gene Expression (in eukaryotes):

Factors influencing gene expression:

- **The level of chromatin condensation** (how densely DNA is packed within a chromosome): If DNA is more loosely packed (i.e. in euchromatin), it is more accessible for RNA transcription factors and therefore will be expressed more compared to DNA that is closely packed (i.e. in heterochromatin).
- **Chromatin modifications:** if acetyl groups bind to histone tails, this prevents DNA from becoming closely packed/allows for a loose chromatin structure. This allows for more transcription/gene expression. (ACETYLATION OF HISTONES = MORE GENE EXRPRESSION).
- **DNA methylation:** addition of a methyl group usually inactivates genes. For example, genes that are needed in early embryos are methylated when there is no longer a need for them.
- **Cell type-specific transcription:** different activator proteins are available in different cell types so that different genes are transcribed.
- **The rate of mRNA degradation:** some mRNAs are degraded quickly and are only translated a few times, whilst others last for weeks and are translated many times

(e.g. globin mRNAs). The shortening of the poly(A) tail will determine how quickly mRNA will break down.

- **microRNA’s (miRNA’s):** the binding of a miRNA to an mRNA will either block translation or lead to degradation of mRNA.
- **Blocking of initiation of translation:** not all mRNA’s will be translated, because the initiation of translation can be blocked.
- **Signalling cells:** can release molecules that signal nearby cells to change their gene expression.