Chapter 1 : Levels of Gene Control

1.1- THE PROTEIN CONTENT OF DIFFERENT CELL TYPES IS DIFFERENT

- The protein composition of various organs, tissues and cells, even among clones, can vary and are the result of gene expression.
- **Central Dogma:** DNA -> RNA ->Protein
- The action of the protein then produces the phenotype (e.g. functional globin in hemoglobin and enzymes responsible for eye color)

Specific methods can be used to study the expression of individual proteins in tissues and cells

- Investigating the expression of individual known proteins in specific tissues using a specific antibody

Methods to investigate Protein expression

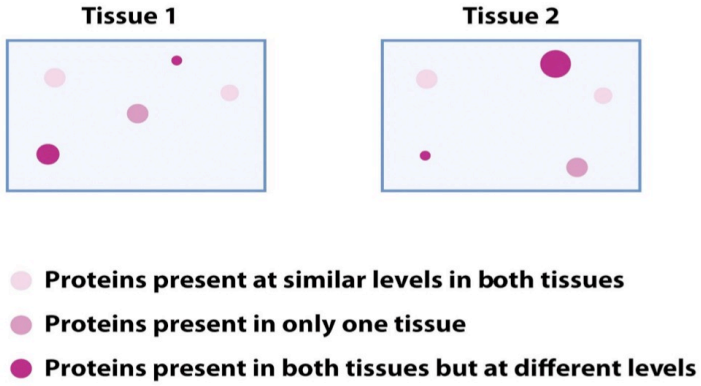
1) Western Blotting

- Analyze for presence/absence of this protein, and its abundance (relative intensity of the band)
- **Method:**
  - **STEP 1: SDS- PAGE separates Proteins of tissues A and B.** Sodium dodecyl denatures the proteins and the electrophoresis in a polyacrylamide gel separates them according to their size.
  - **STEP 2: Transfer proteins to a membrane.** The proteins are transferred to a nitrocellulose filter, which is incubated with the antibody.
  - **STEP 3: Probe with a protein-specific antibody for detection.** The antibody recognizes and binds specifically to the protein of interest.
  - The protein will be present at a particular position on the filter dependent on how far it moved in the electrophoresis step.
  - The binding of the antibody is visualized by the enzymatic or fluorescent detection procedure.
  - If the tissue contains the protein of interest, a band will be observed and the intensity will provide a measure of the amount of protein present.
- Identifies presence and absence of a protein in a particular tissue
- SDS-PAGE has non-specific coomassie staining, cannot identify particular protein with confidence

General methods can be used for studying the overall protein composition of tissues and cells

2) Two-dimensional polyacrylamide electrophoresis (2D-PAGE)

- Investigation of protein composition of different tissues
- **Method:**
  - **STEP 1: Proteins are separated by Pl value.** Separated according to their charge in a process known as isoelectric focusing.
  - **STEP 2: Soaking the gel in SDS solution and fitting it on an SDS PA gel.**
  - **STEP 3: Separating the proteins by molecular mass with SDS PAGE.** The protein moves to a position determined by both its size and its charge.
  - Allows a number of differences in the protein composition of particular tissues to be identified.



  - Housekeeping proteins are expressed in similar levels in virtually all tissues- involved in basic metabolic processes common to all cell types.

- **Tandem Mass Spectrometry**
  - Proteomics: The power of 2D gels to separate a wide range of proteins combined with the ability to study specific proteins individually.
  - Individual spots of interest are excised from the 2D gel and digested into their constituent peptides using the proteolytic enzyme –Trypsin
  - Can involve matrix-assisted laser desorption / ionization (MALDI) which allows the molecular weight of the peptide to be determined, and nanoelectrospray mass spectrometry allows the amino acid sequence of a peptide to be obtained.
  - **STEP 1: Excised proteins are digested to peptides then ionized by MALDI or nanoelectrospray.**
  - **STEP 2: Mass spectrometry determines peptide mass (MALDI) or fragment product mass (Electrospray)**