**Gregg Morin (Proteomics)**

A gene: functional unit in DNA, codes for RNA. mRNA serves a template used by ribosome to make a protein. Proteins do the work in a cell, for the most part. *Central dogma!*

Proteins can be enzymatic, structural, motor etc. Serve as scaffolds, act as enzymes, act as transporters. *Proteins interact with other proteins*. Other proteins: RNA catalyzed reaction, ribosomal RNA (highly conserved because of the functional specificity of their folding to form stem loops), tRNA, miRNA, CRISPR, guide RNA, linkRNA, pRNA.

RNA vs protein correlation – modest. So to understand protein changes, need to look at the protein. Capping, miRNAs, and other RNA specific processes guide RNA stability. Stability of proteins is not directly dependent on translation rate – its own processes regulate it. So if a protein is in a complex, it might have a different stability than if it was by itself.

***Best MS can resolve ~1000 peptides*** (assuming 10,000 proteins with 3 splice isoforms, 20 aa tryptic fragments, gives us 25 peptides per protein, 25+6 peptides for all isoforms, >310,000 peptides total).

MS measures ions in the gas phase. Was not originally designed to look at proteins.

MALDI-TOF: Theoretically all ions can be detected, and has a greater mass accuracy and detection range (no upper threshold on size!).

**Hydrophobicity based separation!** Microcapillary HPLC (flow rates <300 nl/min). Peptides are predominantly protonated, i.e. positively charged.

***Electrospray ionization***: sprayed fine droplets containing positively charged peptides. The solvent forming the droplets evaporates, leaving the peptides in the chamber (NOBEL PRIZE in Chemistry, 2002) – acetonitrile (negative pH, neutral charge) solvent. The solvent is seen as noise.

AA deconvolution from MS-MS data: experimental spectrum compared against a theoretical spectrum (derived from the protein sequence database). As long as the peptides are resolved, this works. 40% of the peptides are not matched (bad spectra, protein modifications that are not in the database) – cannot include all the modifications in the database because otherwise you’ll overmatch and overpredict.

MRM/SRM (Multiple/Selective Reaction Monitoring): highest resolution methods. Used in most present day drug testing (Olympics!).

***Phospho-peptides*** have to be specifically purified. They are low occupancy and chemically unstable.

FFPE proteomics 🡨 sounds interesting. FFPE crosslinking can add mutations to DNA and RNA. There are going to be modified peptides too, but those modifications are not present in the database being compared against, so those don’t get ‘amplified’ as they would with the PCR approach in DNA/RNA. Extraction of DNA/RNA and proteome from the same sample 🡪 technique being developed at the Morin lab currently.

Antibodies are much more sensitive for particular epitopes, than MS (or MRM) approaches, but the protocol is much more complicated and difficult. MRM is the method of the year because you can do these specific tests relatively easily. MRM based experiments used to be validated with western blots in the past!