Outline of Proposed Research

Histones are nuclear proteins that comprise the core of nucleosomes, the distinct DNA-protein complexes found scattered throughout eukaryotic genomes. Each nucleosome contains a protein core with two copies each of histones H2A, H2B, H3, and H4, to which approximately 147 base pairs of DNA is wrapped around. The primary function of nucleosomes is to package and condense DNA into the nuclei of cells. However, histone proteins within nucleosomes can also serve unique cellular functions through specific histone post-translational modifications (Suganuma and Workman, 2011).

For my Master's research project, I will be studying histone H2A.X monoubiquitylation, which is a specific type of histone post-translational modification. Histone H2A.X is a variant of the H2A histone, containing a slightly different amino-acid sequence from it; H2A.X comprises about 10 % of total H2A in cells (Krishnan et al., 2009). H2A.X monoubiquitylation in particular involves the covalent attachment of a single ubiquitin molecule to a specific lysine residue within H2A.X (Pan et al., 2011; Wu et al., 2011), ubiquitin being a 76 amino-acid polypeptide (Cole et al., 2015). Studies have shown that H2A.X monoubiquitylation is associated with the DNA damage response in cells. Particularly, H2A.X monoubiquitylation may regulate H2A.X phosphorylation, a more well-studied H2A.X post-translational modification known to occur at sites of DNA double-stranded breaks. H2A.X phosphorylation recruits proteins involved in DNA repair. If DNA double-stranded breaks do not get repaired, genomic instability will occur in cells, a feature that often leads to the development of cancers (Pan et al., 2011; Wu et al., 2011). Therefore, H2A.X monoubiquitylation is significant to study because it may provide a tumour-suppressive function by helping cells maintain genomic stability in the wake of DNA damage. This research would be significant to the field of health studies as it may provide useful information for the development of cancer therapies.

Currently, the exact mechanisms connecting H2A.X monoubiquitylation to its associated functions in the DNA damage response remains largely unknown (Pan et al. 2011; Wu et al., 2011). One approach to deducing these mechanisms is to determine proteins that specifically interact with monoubiquitylated H2A.X histones. In an effort to determine novel monoubiquitylated H2A.X-interacting proteins, I will be developing and testing a novel purification method for the isolation of monoubiquitylated H2A.X complexes. This novel purification method will be based on an original nucleosome purification method termed BICON (Biotinylation-assisted Isolation of CO-modified Nucleosomes), which was developed by Lau and Cheung. Their technique utilized the *E. coli* biotin ligase BirA, which is an enzyme that catalyzes the addition of biotin to a specific lysine residue within a unique 15 amino-acid polypeptide sequence (termed the Avi-tag) that is not present within any endogenous mammalian proteins (Lau and Cheung, 2012). The novel purification method I will be developing and testing will involve a recombinant H2A.X-BirA fusion protein and a recombinant Avi-tagged ubiquitin construct. The rationale for this purification technique is as followed: if H2A.X-BirA and Avi-tagged ubiquitin proteins are co-expressed in mammalian cells, and H2A.X-BirA becomes monoubiquitylated by Avi-tagged ubiquitin, the BirA portion could biotinylate the Avi-tagged ubiquitin conjugated to H2A.X due to their close proximity to each other. In this manner, monoubiquitylated H2A.X complexes could uniquely become tagged by biotin *in vivo*. These complexes could then be purified in pull-downs using streptavidin beads, which have very high affinity for biotin (Lau and Cheung, 2012). Proteins associated with the monoubiquitylated H2A.X complexes could be resolved on SDS-PAGE gels and silver-stained for visualization. Protein identities could then be determined using mass spectrometry analyses. If this technique is efficient in purifying monoubiquitylated H2A.X complexes and determining novel monoubiquitylated H2A.X-interacting proteins, we will be able to gain a better understanding of the mechanisms surrounding H2A.X monoubiquitylation and its roles in the DNA damage response.