Lysine acetylation on histones is key to the epigenetic regulation of gene expression, and is mediated by the action of acetyltransferases (writers) and deacetylases (erasers). Not surprisingly, the enzymes implicated in such a critical function are often found altered in cancers; in this regard, histone deacetylase inhibitors now constitute a prime target for cancer therapies. It has been recently realized, however, that acetyltransferases and deacetylases modify not only histones, but also a wide variety of proteins throughout the cell. Like phosphorylation, which can be recognized by dedicated protein modules (or “readers”, e.g. the SH2 domain), acetylation is recognized by a specific protein domain, the bromodomain. Bromodomain-containing proteins are often mutated and/or amplified in cancers, and bromodomains, which have deep pockets to accommodate acetylated lysine residues, are also viable targets for small molecule inhibition (for example, the BRD4 inhibitor JQ1 is a potent antitumor agent). Unfortunately, our current knowledge of bromodomain specificity and function is limited, hampering the rational design of therapeutic agents.

The Gingras and Pawson laboratories have developed extensive expertise in interaction proteomics and in the identification of post-translational modifications. Within the context of this application, we have recently developed optimized methods to identify protein-protein interactions for proteins associated with chromatin and identify and accurately quantify the acetylated sites. We have already cloned and stably expressed in mammalian cell lines 55 of the 75 proteins known to participate in acetyl lysine signalling, and initiated a systematic interaction mapping effort. This work has been done in coordination with a systematic effort at the Structural Genomics Consortium (SGC; Oxford) by co-applicant Filippakopoulos to systematically determine the structures of all human bromodomains and begin to define their specificity for acetylated lysines *in vitro* (Appendix 1; Filippakopoulos et al., *Cell, in press*). Through the efforts at the SGC, molecular probes that target the interaction of the bromodomains with acetylated peptides have also been developed. In summary, in the past year we have built the technical know-how and a series of resources which put us in an excellent position to provide a new view of the acetylome.

***Here, we propose to systematically define the specificity and function of human bromodomains through identification of their binding partners and acetylated targets. This knowledge will assist in the development of new inhibitors of bromodomains, accelerating the development of new therapeutics****.*

**We propose the following specific aims:**

1) Identify the interactome for all components of the human acetylation machinery, including translocation products and mutated proteins

2) Define the specificity for each bromodomain-containing protein towards acetylated lysines

3) Reconstitute the acetylome specificity map

4) Test new bromodomain inhibitors

This project will enable us to better understand the function for each bromodomain-containing protein, as well as the mode of recognition of acetylated lysine residues by the bromodomain. With the growing interest in developing specific bromodomain inhibitors, this knowledge and the reagents generated will serve to rapidly assess, in a cellular context, the consequences of treatment with bromodomain inhibitors, and accelerate their progression to preclinical studies.