4.1 New Mass Spectrometry Technology and Applications in the Study of Cell Migration, the Histone Code, and Cancer Vaccine Development

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New technologies have been required for research involving dynamic processes such as: (a) phosphorylation on proteins involved in cell migration, (b) post-translational modifications on histones that control gene expression, gene silencing, DNA damage repair, recombination, etc, and (c) immune system regulation of cancer cells. For the direct analysis of proteins on a chromatographic time scale, we use monolithic nanocolumns, a modified linear ion trap mass spectrometer, and sequential ion/ion reactions to fragment the intact protein and convert all fragments to singly charged species. For the comparative analysis of post-translationally modified proteins, two samples are digested proteolytically and the resulting peptides from each are then converted to d0- and d3-methyl esters, respectively. The two samples are then mixed together and analyzed by nano flow HPLC interfaced to electrospray ionization on a tandem linear ion trap-Fourier transform mass spectrometer (LTQ-FTMS). This instrument operates at a resolution of 100,000, measures masses to three decimal places, and records the molecular masses of peptides in each sample at the high atomic level. For the analysis of phosphorylated proteins, immobilized metal affinity chromatography (IMAC) is employed to enrich the sample for phosphopeptides prior to analysis by nanoflow HPLC. Sequence information on these peptides is obtained by inducing fragmentation at peptide amide bonds by either collision activated dissociation (CAD) or by electron transfer dissociation (ETD).


4.2 Harnessing the Human Proteome

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One of the most compelling steps in the post-genomic era will be learning the functional roles for all proteins. The Harvard Institute of Proteomics (HIP) has initiated a project to create a sequence-verified collection of full-length cDNAs representing all human coding regions in a recombinational vector system that allows the immediate in-frame transfer of all coding regions into virtually any protein expression vector. These transfers allow the addition of peptide tags to either or both end of the proteins. This repository, called the FLEXGene Repository (for Full-Length Expression-ready), will enable the high-throughput (HT) screening of protein function for the entire set (or any customized subset) of genes using any method of in vitro or in vivo expression.

The most exciting part of this project has been the ease with which the clones from the repository can be rapidly incorporated in HT biological experimentation. Using automated gene transfer methods and protein purification it is now possible to purify thousands of proteins. Using HT retroviral methods, proteins capable of driving cell migration, altering the morphogenesis of normal epithelial structures, and affecting substrate dependent growth have been identified. A novel form of protein microarray, called nucleic acid programmable protein array (NAPPA), has been developed. This method substitutes the printing of proteins on the array with printing cDNAs encoding the proteins. Thus, the array is a DNA array that can be converted into a protein array by adding cell free protein synthesis machinery. This obviates the need to purify proteins, produces human proteins in a mammalian milieu, and avoids concerns about protein stability on the array because the proteins are made just-in-time for assay. NAPPA arrays can be used to study protein-protein interactions, protein-drug interactions and as tools to search for disease biomarkers.

4.3 Reading and Writing an Epigenetic Mark for Positive Regulation of Gene Expression

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Histone H3 lysine 4 (K4) methylation has been linked to the transcriptional activation in a variety of eukaryotic species ranging from yeast to humans. We will show that a common component of MLL1, MLL2 and hSet1 H3 K4 methyltransferase complexes, the WD40-repeat protein WDR5, directly associates with histone H3 di- and trimethylated at K4 and with H3 K4 dimethylated nucleosomes. WDR5 is required for binding of the methyltransferase complex to the K4 dimethylated H3 tail, as well as for global H3 K4 trimethylation and HOX gene activation in human cells. WDR5 is essential for vertebrate development, in that WDR5 depleted X. laevis tadpoles exhibit a variety of developmental defects and abnormal spatial Hox gene expression. Our results are the first demonstration that a WD40-repeat protein acts as a module for recognition of a specific histone modification and suggest a mechanism for reading and writing an epigenetic mark for gene activation.