Proteomic Analysis of Cellular Signaling

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Much of cellular signaling occurs through reversible protein phosphorylation. This enzymatically controlled process mediates cellular activity through activation or inhibition of protein function. For instance, growth factor binding to receptor triggers the kinase activity of the receptor, resulting in auto-phosphorylation and stimulating a signal transduction cascade that ultimately results in cellular proliferation and prevention of apoptosis. Mapping the cellular protein phosphorylation events and understanding their function in regulating the activity of the cell is a daunting but important task. Increased knowledge of the signaling events in pathogenic systems will provide for better drug targets, better markers of efficacy, and ultimately better drugs.

It is estimated that 30% of mammalian proteins may be phosphorylated at any given time. Correspondingly, the phosphoproteome (defined as the sum total of all phosphorylated proteins) is extremely complex, comprised of thousands of proteins. The challenge of quantifying those changes in phosphorylation that regulate intracellular signaling is similar to searching for a needle in a haystack — a given signaling pathway may alter phosphorylation stoichiometry of only a small number of proteins. Ideally, we would like to monitor the entire phosphoproteome and quantify changes in phosphorylation stoichiometry under a variety of cellular conditions. This information should be directly applicable to developing improved models of intracellular signaling pathways, leading to better understanding of the decision points which determine the fate of the cell.

Traditionally, protein phosphorylation analysis has been performed on a single protein from a single cell state at a time. Unfortunately, this lack of throughput inhibits the application of this technology to large scale systems analysis problems. However, in the past several years, new approaches to analysis of the phosphoproteome have been proposed. Although different chemistry is applied in each approach, all of these mass spectrometry-based methods include an affinity chromatography step to enrich for phosphorylated proteins or peptides. An additional reversed-phase chromatography step just prior to analysis provides for temporal separation based on hydrophobicity of the components in the analyte. Overall, mass spectrometry-based phosphoproteomic analysis will allow us to identify specific protein phosphorylation sites on hundreds to thousands of proteins in a single analysis. Relative quantification information is generated by comparison of different cell states and should allow us to track changes in the phosphoproteome through the cell cycle or on treatment with a drug. With the proper interpretation, this information will facilitate decoding of the signaling pathways regulating cellular transformation and may lead to a new class of biological markers for both disease and drug efficacy.

Keywords:
Phosphoproteome: The entire set of phosphorylated proteins from a given cell.

LC/MS/MS: Analytical technique in which a sample is chromatographically resolved on a liquid chromatography (LC) column (typically C18) prior to tandem mass analysis (in which the mass-to-charge value of an ion of interest is measured (MS), the ion is isolated, fragmented, and the mass-to-charge values of the fragment ions are measured (MS/MS)).

IMAC (immobilized metal-affinity chromatography): Method for purifying a given fraction of a sample by passing the sample over a column containing a particular metal ion. For purification of phosphorylated species, Iron(III) is chelated to the column.