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Post-Translational Modification (PTM) Proteomics: Challenges and Perspectives

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The genetic revolution ushered in comprehensive understanding of genes; however, proteins are the major gene products. As the effectors of biological products, proteins warrant increased study and attention and thus, a proteomic evolution has begun.

Proteins, Protein Isoforms and Protein PTMs

Proteins are the gene products and thus their effectors [1-6]. Proteins can be part of two or more different biological processes due to two main intrinsic properties: protein-protein interactions (PPIs) and PTMs of proteins [1,2,7-10]. Being part of a protein complex with inhibitory properties (towards a particular biological process, e.g. cell motility) makes that protein an inhibitor of that biological process [5,6,11-16]. However, being part of a protein complex with activatory/stimulatory properties makes that protein an activator [7,13,17-20]. Therefore, depending on its interaction partners, the same protein can have dual or multiple functions [5,7,8,11,13,15,20].

Another intrinsic property that gives proteins dual and sometimes multiple function are PTMs [5-7,10,14,16,21]. There are many protein PTMs; among the most common are phosphorylation, glycosylation, acetylation, truncation or formation of disulfide bridges [1,5,7,20-25]. There are also intracellular specific protein PTMs (e.g. phosphorylation or acetylation) and extracellular protein PTMs (e.g. glycosylation, disulfide bridges, etc) [10,14, 17,23,26-29]. These protein PTMs are involved in a large variety of processes such as protein stability, enzymatic activity, signal transduction pathways, cytoskeletal remodeling, gene regulation or cell motility. Understanding the function of these protein PTMs can help in understanding physiological and pathological processes, which in turn can help us in understanding when, where and why one should take action to modify a physiological process (e.g. to create a virus/bacteria/pest/parasite-resistant transgenic plant), to use a physiological process (e.g. to produce a recombinant antibody) or to prevent, monitor, or even treat a pathological process (e.g. diseased or disorders) [18,24,30-33]. Therefore, identification of these PPIs, protein PTMs, their location within the protein, as well as their status (e.g. covalent or non-covalent interactions, stable or transient interactions, etc) would provide valuable information about the status and function of that protein [18,24,30-33].

PTM Proteomics

Proteomics is the study of proteins, protein PTMs, PPIs of the cells, tissues, organs or organisms (or bodily fluids) at a particular stage or time-point (physiological or pathological, or during development) [1,2,7,17,34-36]. Proteomics is performed at both qualitative (protein and protein PTMs identification and characterization) and quantitative (protein and protein PTMs quantitation) [1,2,7,17,34-36].

Identification of common protein PTMs is more or less straightforward and it is usually performed at both protein level (top down or middle down proteomics) and peptide level (part of bottom up proteomics) [3,4,6,10,36]. For example, phosphoproteomics consists at identification of the phosphorylation sites at the Serine, Threonine and Tyrosine residues and their subsequent characterization and quantification [3,4,6]. The most common approaches used for identification of these phosphorylation sites are enrichment at the protein level (anti-phosphotyrosyne antibodies and less popular anti-phosphothreonine/serine antibodies) or peptide level (metal-based affinity purification such as TiO_2 affinity chromatography, immobilized metal affinity chromatography (IMAC), or a combination of both [4,7,8,15]. There are also similar approaches for other protein PTMs such as lectins for glycoproteins or glycopeptides, anti-nitrotyrosine for enrichment of nitrotyrosine residues or anti-acetyl-lysine antibodies for enrichment of the acetylated lysine residues. At the mass spectrometry level, methods like multiple reaction monitoring, information dependent data analysis (data-dependent analysis using an inclusion list) or neutral loss are common methods for identification of protein PTMs [4-8,14,15,17,22,28].

Challenges

As previously briefly described, there are many options for identification, characterization and quantification of protein PTMs [4-8,14,15,17,22,28]. The challenge is not so much in the biochemical and proteomic characterization of the stable or common protein PTMs such as phosphorylation or acetylation but rather for the transient or uncommon protein PTMs. For example, there are challenges in identification of transient phosphorylations (e.g. during signal transduction pathways) or in identification of PTMs such as nitrosylation, farnesylation, glycosylation or identification of disulfide bridges [4-8,14,15,17,22,28]. In addition, while there are established methods for identification of disulfide bridges and determination of O-and N-linked glycosylation sites and the structure of the glycan structure, it is not possible to automate these methods [4-8,14,15,17,22,28]. Furthermore, for the methods that can be automated (e.g. phosphorylation of acetylation), prior enrichment is necessary [6-8,15].

For basic research, one wonders about considering PPIs as possible PTMs. While ubiquitinylation is considered a PTM, it is in fact a covalent PPI. Therefore, functional PPIs can also be considered PTMs. The challenge in PPI PTMs is to accurately identify them and most difficult to validate them. Having these PPIs in mind, one also wonders whether conformation of one protein is also a non-covalent PTM, which can in fact lead to two different protein configurations. A very good example is the existence of identical antibodies with similar conformations, but with different configurations (different disulfide linkage) [37].

For the biotechnology and pharmaceutical companies, protein and/or antibody characterization (with or without conjugation with a cytotoxic or cytostatic drug) is the biggest challenge, since the disulfide

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bridges in these proteins may not be identical in different protein batches or the glycosylation may not happen or may even happen at different sites. An example of such a surprise was recently published by Sokolowska and colleagues [28], where they demonstrated that introduction of new glycosylation sites in a protein may in fact change its conformation and the location of the glycan group.

Another challenge lies in the significance of a PTM. What does actually a PTM in a protein mean for a 1) protein and 2) a cellular process at a particular time point. If for a protein, a PTM mostly means change of stability, conformation, function, or a combination of all of them, for a cellular process, it is more difficult to define a PTM. In the classical example of the phosphorylation at different sites of the Protein Kinase A (PKA), described in the Biochemistry textbook [38], different degrees of phosphorylation of PKA means different degrees of enzymatic activity. However, in the mass spectrometry field (especially in phosphoproteomics), many research groups report a fold-ratio in the phosphorylation level of the proteins from two different conditions. Does this reflect an accurate and perhaps direct relationship between the PTM level (in this case phosphorylation) of a protein and its function? Can this be applied to structural proteins, as well? If yes, how?

Other challenges that the researchers face when they study protein PTMs are the artificial (experimental)-induced PTMs. Methionine, cysteine and tryptophan easily oxidize, Serine, threonine, glutamate and aspartate easily loose water, arginine, lysine, asparagine and glutamime easily deamidate or cyclisize. In addition, other common experimentally-induced modifications such as alkylation can modify proteins at sites and amino acids previously unknown, and therefore, a peptide that contains such a modification will not be identified by a mass spectrometer [3-5,16,17,20,26,28].

Perspectives

The human genome has been sequenced. We have about 30,000 genes that produce, in the best case, about 100,000 protein isoforms. With the given number of genes and their protein products, we are not that complicated. However, what makes a big, a really big difference is the PTMs in proteins, whose number easily increases the number of proteins to several million isoforms, which are far from being identified and structurally and functionally characterized.

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