1 Introduction

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1.1 Proteomics: an overview

1.1.1 Proteome

The suffixes -*ome* and -*omics* find great popularity in today's biology research. The New York Times pointed that out in 2012 in an article from which I quote this excerpt *"The age of "omes" is here. It began with the genome, continued with the proteome…The list goes on. In fact, it goes on for about 18 pages if you go to the Omics.org site and print out its list of omes and omics."*[1]

The Oxford English Dictionary defines -*ome* as "all of the specified constituents of a cell, considered collectively or in total" and thus -*omics* refers to the study of a certain field in biology.

Genomics is the field that implies the study of the genetic material (Genome) of different species. The sequence of the human genome is now fully known.[2, 3] The advances in DNA sequencing methodology together with the decreasing costs [4] allowed, as of 2011, the complete sequencing of 2907 genomes with another 8562 in progress as indexed in the Genomes OnLine Database (GOLD).[5]

However, genomes are not everything and understanding genomes solely is not enough to understand the complexity and diversity of biological processes. Genomes are known to be the building blocks of life, but it is the proteins that are responsible for the variation in cellular structure and functions.[6]

The term *Proteome* was introduced back in 1995 and means "The entire PROTEin complement expressed by a genOME, or by a cell or tissue type".[7]

All human cells, except mature red blood cells, contain the same complete genome, but the expression, regulation and post-translational modifications (PTMs) of proteins are responsible for the vast diversity in structure and function of human cells and organs (Figure 1).[8, 9]

The study of this dynamics allows for a better understanding of cellular events in normal and disease states such as dynamics of genome-encoded events (e.g., protein translation) and non-genome-encoded events (e.g., PTMs of proteins and interactions between proteins, nucleic acids, lipids, carbohydrates, etc.) and how this knowledge could help in developing new drugs and treatment strategies.[10]

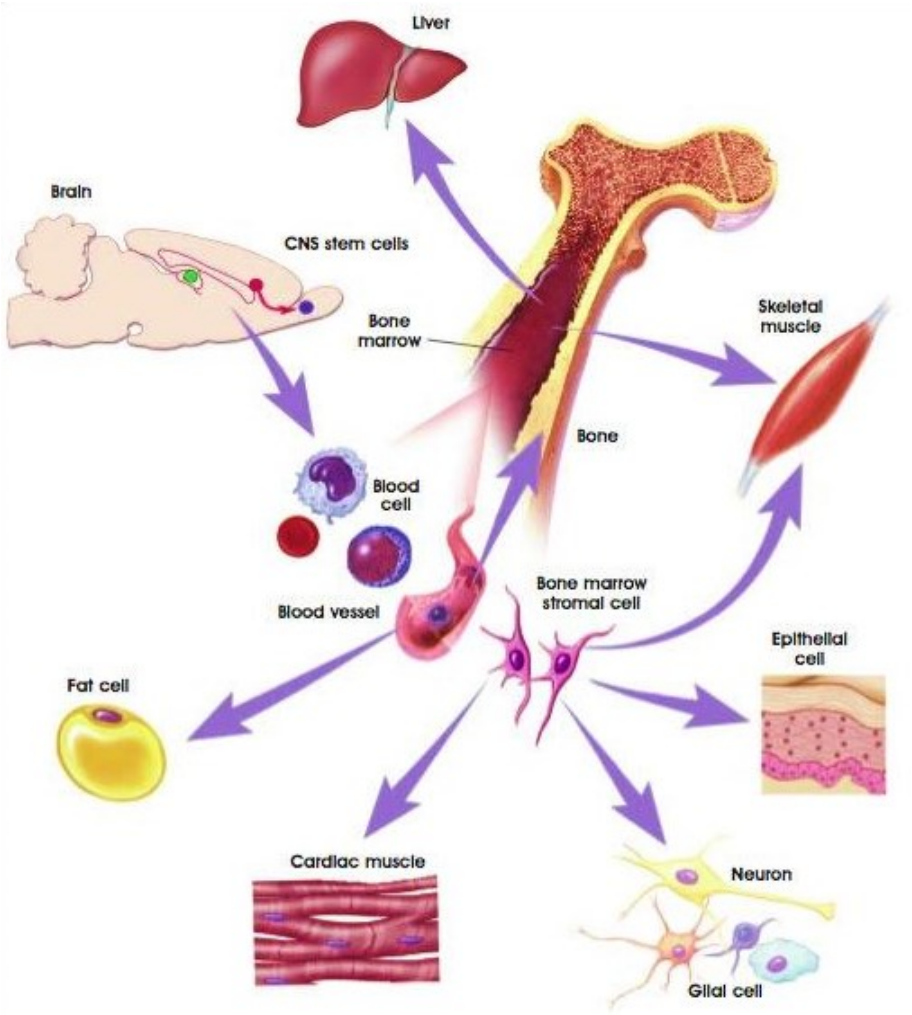

Figure 1. Stem cell differentiation. Stem cells can be differentiated into a variety of cells that share the same genetic material but vary considerably in arrangement, structure and function. Red blood cells do not contain genetic material as they contain no nuclei. (Adapted from National Institute of Health (NIH) *http://stemcells.nih.gov*).

A striking example of how the structure of protein can be involved in disease states was the outbreak of the fatal transmissible spongiform encephalopathies in mammals (known in cattle as bovine spongiform encephalopathy or simply mad cow disease, in sheep as Scrapie and in humans as Creutzfeldt-Jakob Disease). The infectious agent for this disease is called *Prion,* which is a unique form of disease-causing agents. Prions, unlike bacteria, fungi, viruses and all other infectious agents do not have a genetic material; they are merely naturally occurring proteins in a misfolded form. The disease is caused when prions enter a healthy organism; it triggers the transformation of an existing protein to the misfolded form (Figure 2). The accumulation of this misfolded form is responsible for the disruption of tissue structure and thus the fatal symptoms of the disease.[11-13] For his discovery of prions and its new biological principle of infection, the Nobel Prize in Physiology or Medicine in 1997 was awarded to Stanley B. Prusiner.

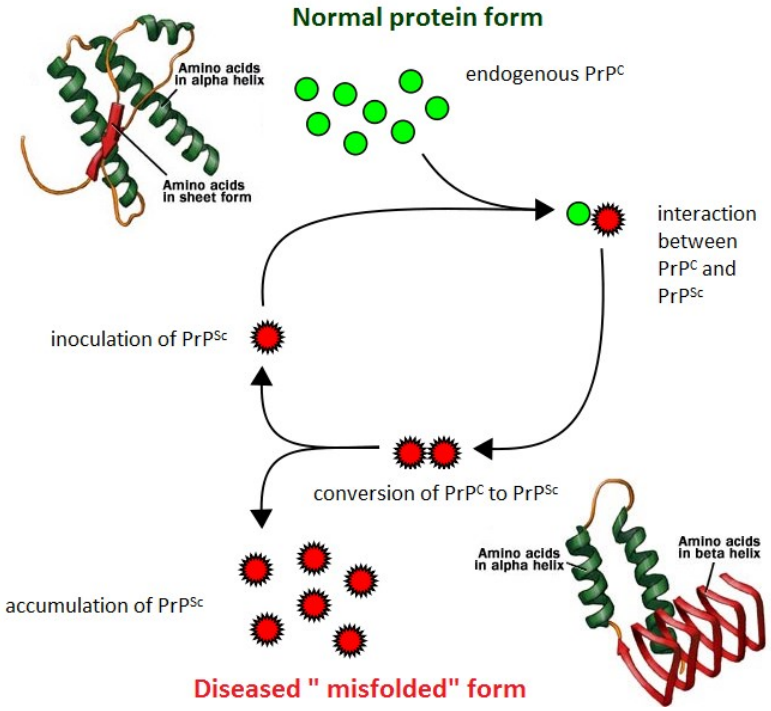

Figure 2. The Prion cycle. Upon ingestion of the misfolded prion protein (PrP^{Sc}) it triggers the misfolding of the harmless naturally-occurring prion protein (PrP^{C}) leading to accumulation of PrP^{Sc} and eventually the loss of function of the affected tissues and death of the animal/human. (Modified from Mayo Foundation for Medical Education and Research).

1.1.2 Significance of post-translational modification (PTM)

Although the human genome is estimated to include between 20,000 and 25,000 genes [14], the total number of proteins in the human proteome is estimated to be over 1 million.[15] These numbers emphasise the belief that a single gene can encode multiple proteins. This tremendous increase in complexity from the level of genome to the proteome is largely attributed to protein PTMs.

PTM is a mechanism by which the amino acid residues in peptides and proteins undergo biochemical modifications which alter the properties of these peptides and proteins in response to developmental or physiological conditions throughout the life of the cell. These modifications comprise either proteolytic cleavage of regulatory subunits or covalent addition of a modifying group to one or more amino acid residue in the peptides and proteins. Multisite PTM leads to an enormous number of potential molecular states (Figure 3) creating the basis for sophisticated forms of cellular structure and function.[16, 17]

PTMs play a key role in functional proteomics, because they are involved in the regulation of the activity, localization and interaction with other biological molecules including proteins,

nucleic acids, lipids, and cofactors. Therefore, identifying, understanding and quantifying PTMs represent a major challenge in the study of cell biology and the potential disease treatment and prevention.[17]

The list of PTMs is long and includes phosphorylation, glycosylation, disulphide bond formation, cysteine oxidation, proteolysis, nitrosylation, methylation and acetylation. The detection of PTMs is usually hindered by many factors including the stability of PTMs, the lack of suitable methodologies, sample complexity, the small size and low abundance of PTMs.[18]

Cysteine oxidation and sulfenic acid formation, detection and quantification were tackled in this work and will therefore be discussed in more detail as model to the assessment of PTMs.

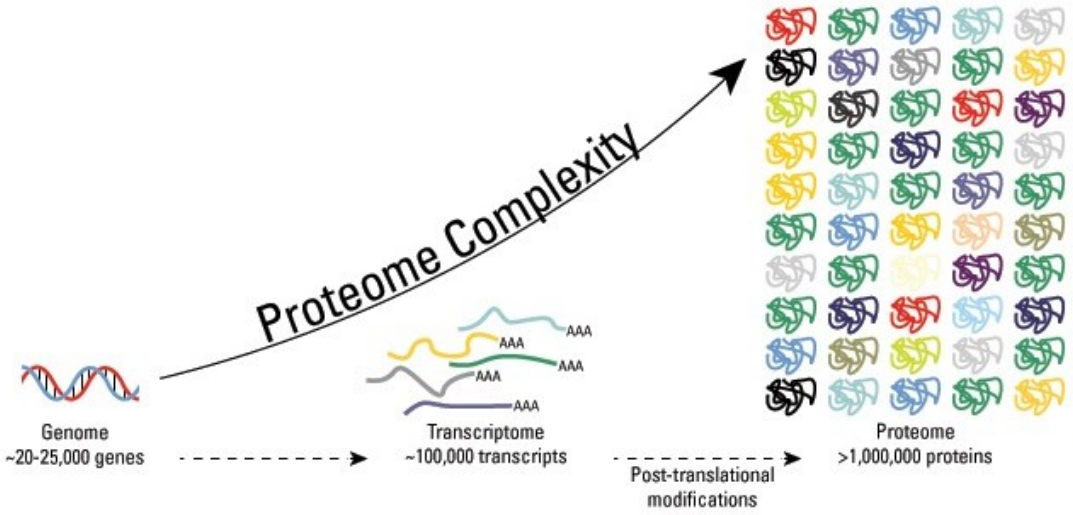

Figure 3. The enormous complexity in proteome owing to post-translational modifications. (Modified from Piercenet).

Sulfenation as PTM

The most oxidation-sensitive targets in proteins are the sulfur-containing cysteine and methionine residues.[19] Cysteine thiol groups undergo intracellular oxidation through reactive oxygen and nitrogen species (ROS/RNS) such as hydrogen peroxide (H_2O_2), nitric oxide (NO) or peroxynitrite ($ONOO^-$) produced by xanthine oxidase, lipoxygenase, cytochrome P-450 systems or by the respiratory chain in mitochondria. The oxidation of cysteines leads to a series of products namely sulfenic acid (R-SOH), sulfinic acid ($R\text{-}SO_2H$) and sulfonic acid ($R\text{-}SO_3H$) (Figure 4). The presence of such oxidative modification has long been considered as unwanted damage but nowadays there are evidences indicating that oxidation is also involved in redox-based homeostasis and defense mechanisms.[20, 21]

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