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Short Communication

Protein Isolation and Beyond

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Introduction

It is the study of proteins on a large scale. Proteins being the core structure for any living being with its omnipresence. It's present right in a structural format from a muscle fibre, fragment of DNA, proteins to proteins which protect the body with the help of antibodies or send signals in the form of hormones. These proteins are present everywhere. Proteomics is helping converge the discovery science and a hypothesis driven science. Various cellular pathways and functions which were neither apparent or predictable can be made approachable. This article aims to give a brief overview of this technique and their applications. An added note on the advantages and disadvantages are also mentioned in this overview.

Terminology

The term proteome could translate to study of protein. This is equivalent to genomics of genome where the entire spectrum of the gene is studied. The word proteome was coined by a Australian, Dr Marc Wilkins at Macquarie University in 1995.

The crux of the concept

Every organisms genome is more or less constant but the proteomes of every organism may differ from cell to cell. The other important factor analysed that not every mRNA is translated into protein. The one gene and one protein does not exist, and the one genome and one protein remains the mainstay. The cellular function cannot be judged based on the genome.

- **Proteome:** The total set of proteins present at a given set of time.

The central dogma of biology

- **Genomics:** It is the complete set of DNA which also includes all the genes and the techniques used to study it.
- **Transcriptomics:** It is the sum of all RNA transcripts and the techniques used to study the same.
- **Proteomics:** The study of all the proteins at the given set of time. The dynamic protein structure and their interactions.

The two major methods include 2D gel electrophoresis which is used for separation and Mass spectroscopy which is used for identification and structure analysis. Proteomics can help us understand:

- Protein identification,
- Protein expression studies,
- Protein function,
- Protein post translational modification,
- Protein localisation and compartmentalisation,
- Protein - protein interactions.

Types of Proteomic studies

- **Protein expression comparison:** It is the simplest level of ? It consists of the quanititave study of protein expression between samples that differ by some variables less.

- **Structural Proteomics:** The three diemensional mapping of protein complexes in order to simulate the different structures of proteins.
- **Functional Proteomics:** This type of proteomics covers the various protein interaction, structure. Cellular localisation which are necessary to understand the whole set of the genome.
- **Metabolomics:** it is a measurement of all the metabolites in a biological specimen.

The process of proteome analysis

The protein mixture can be first digested and then separated or the mixture can be separated and then digested. The resultant peptides can be analysed using data reduction algorithms and subsequently identified.

- **Protein isolation:** Digestion methods
- **Protein separation:** 2 D gel electrophoresis
- **Identification:** MS analysis

Studies for interaction

- Antibody arrays: which are good for low antibody specificity.
- Array based protein interaction,
- Two hybrid analysis,

Constructing interaction maps: Grid

The mere complexity of the complete process has been a major factor which deters researchers, therefore a need for a general targeted approach to detect gene expression is needed. Further, some protein structures have more than 1000 variants.

Protein Isolation

Proteins can be obtained from a wide variety of samples extending from patient's cells or tissues. Wherever the source of protein is the extraction from them is not an easy task.

As the amount of protein available for cellular extraction can be of range of 300mg/ml. This range can be much lower in case of target proteins which can go as lower as femtomolar or picomolar concentrations. Mass spectroscopy studies can analyse samples in femtogram units. Further, there is no available way to amplify proteins unlike in nucleic acids. Protein isolation can also be tricky when cross contamination occurs by the abundantly available proteins. Proteins are unstable just like nucleic acids and can be degraded both in vivo and in vitro. Therefore the key to protein isolation is to extract as much protein in the early phase itself.

Best method of isolation of protein depends on the type of sample available. Mechanical homogenisation in case of tissues to chemical methods with a detergent solution is done. Further, density gradient ultra-centrifugation is applicable to separate out the cellular impurities. To avoid this a process called precipitation or concentration is performed which is commonly carried out by salting out or heat denaturation thus enabling the protein to be more stable.

2DE: (Two-Dimensional Electrophoresis)

This method is used to separate proteins based on molecular change and mass. These are the two dimensions. Thus parameters such as molecular weight, quantity and post translational modifications can be assessed. The disadvantages include inability to detect low hydrophobic proteins such as membrane proteins. Another variant is the 2D-DIGE or the Flourescense 2D difference gel electrophoresis. Here the proteins are labelled with Cy2, Cy3, Cy5.

Two-dimensional gel electrophoresis can separate around thousands of proteins. This method is one of leading methods of seperation of proteins and it is the first step for further analysis. This method provides a direct visual confirmation of changes proving a basis for further justification down analytical steps. 2DE applications include cell differentiation, proteome analysis, detection of bio markers and disease markers, bacterial pathogenesis and other industrial related checks. In recent studies it has been proved that 2DE has greater feasibility and robustness. This method is unsuitable for the detection of membrane associated proteins. Instead membrane solubilisation methods have been deployed the various fractions. The most commonly used methods are mass spectrometry and gel-based electrophoresis like differential in gel electrophoresis.

Databases

There are four major databases related to proteomic research. They are

- **UniProtKB:** This database contains protein sequences and information about known biological functions. The majority of information of this database are derived from the translations of genetic coding.
- **IntAct:** This database contains information about molecular protein interactions,
- **Reactome:** This database is about the proteins that play a part in biological pathways of humans,
- **PRIDE:** This database contains experimental evidence of published proteins and peptide identifications [1-4].

The subsequent step in this Proteomics is explained in the next part of this review.

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