Functional genomics and proteomics

ASSIGN
BUSTER

Title: Proteomics has four branches that include sequence and structural proteomics, expression proteomics, interaction proteomics and functional proteomics. Provide one example for each branch and describe them briefly.

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Introduction

With the accomplishment of the Human Genome Project, the prominence is escalating to the protein compliment of the human organism. This has given upsurge to the science of proteomics, the study of all the proteins produced by cell and organism and their interactions in a cell (Cho, 2007). Proteomics is the protein counterpart of genomics and has seized the imagination of bio-molecular researchers globally. It comprises a wide range of technologies targeted at determining the quantity and identity of expressed proteins in cells, their three-dimensional structure as well as interaction partners (Cho, 2007). Hence, the branches of proteomics includes sequence and structural proteomics, expression proteomics, interaction proteomics and functional proteomics. As protein-protein interactions are fundamental to signal transduction to numerous regulatory processes, a systematic organisation of protein-protein interfaces is a valuable source for modelling protein complexes and for comprehending the principles of molecular recognition (Cho, 2007). An integration of functional, structural, and dynamic information is required in order to find out how one protein regulates the action of another by binding to it.

Sequence and Structural Proteomics

Structural proteomics is the large-scale establishment of the three-dimensional structures of proteins (Rastogi, Mendiratta, & Rastogi, 2008). When similar sequences give upsurge to similar structures, it is certain that protein sequence, function, and structure are often closely related (Twyman, 2004). The study of three-dimensional protein structure is strengthened by technologies such as nuclear magnetic resonance spectroscopy and X-ray crystallography. Technological advances in structural proteomics have focused on increasing the throughput of structural establishment and the instigation of systematic plans for proteome-wide structural investigation (Twyman, 2004).

In order to define the function of proteins the establishment of the three-dimensional structure of proteins is essential. The X-ray crystallography is an example of a main tool to discover the structure-function of macro-molecule. The three constituents in an X-ray crystallographic analysis are a source of X-rays, a protein crystal, and a detector (Ravi, Baunthiyal, & Saxena, 2014). X-ray crystallography is used to study molecular structures over the growth of solid crystals of the molecules they investigate. Crystallographers target high-powered X-rays at a minute crystal comprising trillions of identical molecules. The crystal scatters the X-rays on an electronic sensor. After every blast of X-rays, ranging from a few seconds to several hours, the crystal is revolved by entering its preferred orientation into the computer that handles the X-ray device (Ravi, Baunthiyal, & Saxena, 2014). This allows the researchers to capture in three-dimensions, in what manner the crystal diffracts, or scatters, X-rays. The strength of each diffracted ray is entered into the computer, which utilises a mathematical equation to analyse the

position of each atom in the crystallised molecule (Ravi, Baunthiyal, & Saxena, 2014). The outcome is a three-dimensional digital image of the molecule. Crystallographers calculate the distances among atoms in angstroms. The scattering of X-rays is also termed as " X-ray diffraction". Such scattering is the consequence of the interaction of magnetic and electric fields of the radiation with the electrons in the crystals (Ravi, Baunthiyal, & Saxena, 2014).

Expression Proteomics

Expression proteomics is dedicated to the investigation of protein abundance and encompasses the identification of individual constituents, the separation of complex protein mixtures and their systematic quantitative analysis (Twyman, 2004). The main technologies in expression proteomics are mass spectrometry for protein identification, multidimensional liquid chromatography and 2D-gel electrophoresis for protein separation, and mass spectrometry or image analysis for protein quantitation (Twyman, 2004). A developing trend in expression proteomics, and a swiftly growing business sector in the proteomics market, is the utilisation of protein chips for quantitation and analysis.

2-DE or two-dimensional gel electrophoresis technology was a crucial turning point in the field of separation and has been revealed to be a dependable and proficient technique for separation of proteins based on charge and mass. High resolution 2-D polyacrylamide gel electrophoresis can separate up to 10, 000 protein spots per gel. This method has been utilised in human tissue, serum, and plasma proteome analysis with or without preceding

fractionation. Visualization of separated proteins in the gel can be accomplished by staining techniques such as silver staining and Coomassie blue. Some of the latest improvements in silver staining products make it well-suited with mass spectrometry analysis as well. To permit direct assessment of different mixtures of proteins, differential in-gel electrophoresis (DIGE) has been created which allows simultaneous assessment of labelled proteins in dissimilar mixtures. In a typical test, two samples are labelled with dissimilar fluorescence dyes (Cy5 and Cy3) and mixed preceding to electrophoresis and run in parallel with an internal standard labelled with a third dye (Cy2) for quantitative analysis (Mesri, 2014).

Interaction Proteomics

This branch of proteomics deliberates the physical and genetic interactions between proteins as well as interactions among proteins and nucleic acids or small molecules (Twyman, 2004). The analysis of protein interactions can deliver information not only regarding the function of individual proteins but also regarding how proteins function in networks, pathways, and complexes. Theoretically the most ambitious characteristic of interaction proteomics is the construction of proteome linkage maps constructed on binary interactions among individual proteins and higher-order interactions established by the systematic analysis of protein complexes (Twyman, 2004). Main technologies in this area comprise the mass spectrometry and yeast two-hybrid system for the analysis of protein complexes (Twyman, 2004).

The most common technique for detecting protein-protein interactions is the two-hybrid method. In these analyses, a protein is bonded to a DNA binding domain and verified for interaction in yeast against a panel of constructs that contains coding sequences bonded to a transcriptional activation domain (Michaud & Snyder, 2002). A positive protein-protein interaction is logged through the activation of a reporter gene(s). This method has now been utilised to numerous organisms, comprising two large-scale research for the budding yeast S. cerevisiae. These studies have revealed more than 5100 protein-protein interactions and established a large network of interactions (Michaud & Snyder, 2002). This network has numerous clusters in which numerous proteins of similar function group together. Proteins that have not been categorised that fall into these groups are concluded to have the same function as other adherents of the group. Using this method, functional predictions could be completed for 364 proteins of unknown function (Michaud & Snyder, 2002).

Functional Proteomics

Functional proteomics is associated with the study of protein interactions and cellular, biochemical, and system functions (Twyman, Principles of Proteomics, 2014). It is a comparatively new advance where protein functions are verified directly but on a large scale. An instance is the systematic analysis of expressed proteins for dissimilar enzymatic activities. The establishment of functional protein chips, which permit high-throughput functional assays to be done in a simple fashion is also one of the key technology (Twyman, Principles of Proteomics, 2004).

Recently, it has been probable to analyse the actions of thousands of proteins via protein microarrays. Protein microarrays comprise defined set of proteins spotted and analysed at high density (Michaud & Snyder, 2002). These have massive prospective for protein profiling and analysing biochemical events on a very big scale.

Functional protein arrays comprise proteins arrayed on a solid surface and can be utilised to screen numerous proteins for function. The first proteome-wide study of biochemical events were accomplished using the proteome of S. cerevisiae (Michaud & Snyder, 2002). A library of 5800 yeast protein GST-his6-yeast open reading frame fusions were constructed. The proteins were overexpressed in yeast, then purified, and later printed at high-density on aldehyde or nickel slides. This yeast proteome microarray was exposed to a diversity of molecules. Proof-of-principle research revealed that the array could be utilised to screen protein-lipid and protein-protein interactions. By addition of biotinylated calmodulin to the chip, 39 calmodulin-interacting proteins were recognised (Michaud & Snyder, 2002). Inside the set, several estimated calmodulin-binding proteins were discovered. In addition, 33 novel calmodulin-interacting proteins were discovered, signifying that these proteins are regulated by attaching to calmodulin. A characteristic worth noting concerning protein microarrays for the high-throughput mapping of protein interactions is that, of the presently available high-throughput methods, only protein arrays can enable for validation of a binary protein interaction. If a reconstructed interaction among two purified proteins in vitro is not owing to the existence of a third protein, then the interaction is direct. The yeast proteome chip was also investigated with liposomes

encompassing different phosphoinositides. Targets of 150 were identified, displaying varying specificity reliant on the lipid head group (Michaud & Snyder, 2002).

References

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