Lecture 9. Interactomics (the research of protein-protein interactions)

Learning outcomes:
1. Give the definition to the terms “interactome” and “interactomics”.
2. Characterize the experimental methods of interactomics: yeast two-hybrid system (Y2H-assays), phage display, solid phase affinity chromatography, molecular fishing on the chip of optical biosensor, mass-spectrometry and microscopic methods. Give the specific examples.
3. Analyze and compare the bioinformatical methods of interactomics: phylogenetic trees, interaction networks and etc.

In molecular biology, an **interactome** is the **whole set of molecular interactions in a particular cell**. The term specifically refers to physical interactions among molecules (such as those among proteins, also known as **protein–protein interactions, PPIs**; or between small molecules and proteins) but can also describe sets of indirect interactions among genes (genetic interactions). The interactomes based on PPIs should be associated to the proteome of the corresponding species in order to provide a global view ("omic") of all the possible molecular interactions that a protein can present. The word "interactome" was originally coined in 1999 by a group of French scientists headed by Bernard Jacq. Mathematically, interactomes are generally displayed as graphs. Though interactomes may be described as biological networks, they should not be confused with other networks such as neural networks or food webs.
The study of interactomes is called **interactomics**. The basic unit of a protein network is the **protein–protein interaction (PPI)**. While there are numerous methods to study PPIs, there are relatively few that have been used on a large scale to map whole interactomes.
The **yeast two hybrid system (Y2H)** is suited to explore the binary interactions among two proteins at a time. Affinity purification and subsequent mass spectrometry is suited to identify a protein complex. Both methods can be used in a **high-throughput (HTP)** fashion. Yeast two hybrid screens allow false positive interactions between proteins that are never expressed in the same time and place; affinity capture mass spectrometry does not have this drawback, and is the current gold standard. Yeast two-hybrid data better indicates non-specific tendencies towards sticky interactions rather while affinity capture mass spectrometry better indicates functional in vivo protein–protein interactions.
Phage display is a laboratory technique for the study of protein–protein, protein–peptide, and protein–DNA interactions that uses **bacteriophages** (viruses that infect bacteria) to connect proteins with the genetic information that encodes them. In this technique, a gene encoding a protein of interest is inserted into a phage coat protein gene, causing the phage to "display" the protein on its outside while containing the gene for the protein on its inside, resulting in a connection between genotype and phenotype. These displaying phages can then be screened against other proteins, peptides or DNA sequences, in order to detect interaction between the displayed protein and those other molecules. In this way, large libraries of proteins can be screened and amplified in a process called in vitro selection, which is analogous to natural selection. The most common bacteriophages used in phage display are **M13** and **fd filamentous phage**, though **T4**, **T7**, and λ phage have also been used.
Affinity chromatography is a method of separating a biomolecule from a mixture, based on a **highly specific macromolecular binding interaction** between the biomolecule and another substance. The specific type of binding interaction depends on the biomolecule of interest; antigen and antibody, enzyme and substrate, receptor and ligand, or protein and nucleic acid binding interactions are frequently exploited for isolation of various biomolecules. Affinity chromatography is useful for its high selectivity and resolution of separation, compared to other chromatographic methods.
Molecular fishing is a variant of affinity-based isolation of target proteins from a lysate of the biological material due to specific interaction between the immobilized ligand (a bait molecule) and its putative (one or several) functionally competent partners (pray molecules). Various

compounds have been used as the bait molecules; these include small organic molecules, proteins and nucleic acids.

Once an interactome has been created, there are numerous ways to **analyze** its properties. However, there are two important goals of such analyses. First, scientists try to elucidate the systems properties of interactomes, e.g. the topology of its interactions. Second, studies may focus on individual proteins and their role in the network. Such analyses are mainly carried out using bioinformatics methods and include the **validation, predicting PPIs, text mining of PPIs and protein function prediction.**

Interaction networks can be analyzed using the tools of **graph theory**. Network properties include the degree distribution, clustering coefficients, betweenness centrality, and many others. The distribution of properties among the proteins of an interactome has revealed that the interactome networks often have **scale-free topology** where functional modules within a network indicate specialized **subnetworks**. Such modules can be functional, as in a signaling pathway, or structural, as in a protein complex. In fact, it is a formidable task to identify protein complexes in an interactome, given that a network on its own does not directly reveal the presence of a stable complex.

The questions for self - control:

1. What are the “interactome” and “interactomics”?
2. Experimental methods of interactomics.
3. Bioinformatical methods of interactomics.

Recommended readings:

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6. Mukherjee, K; Slawson; Christmann; Griffith (June 2014). "Neuron-specific protein interactions of Drosophila CASK-ß are revealed by mass spectrometry". Front. Mol. Neurosci. 7: 58. doi:10.3389/fnmol.2014.00058. PMC 4075472. PMID 25071438.
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9. Kehoe JW, Kay BK (November 2005). "Filamentous phage display in the new millennium". Chem. Rev. 105 (11): 4056–72. doi:10.1021/cr000261r. PMID 16277371.
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13. ""Introduction to Affinity Chromatography"". bio-rad.com. Bio-Rad. 2020-09-14. Retrieved 2020-09-14.
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