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Investigation of Protein-Protein Interactions by Blue Native-PAGE & Mass Spectrometry

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Protein-protein Interactions (PPIs)

PPIs are the most fundamental structures that allow to an organelle, cell, tissue, organ or organism to survive, adapt and propagate [1-4]. PPIs are 1) static when proteins form stable protein complexes such as mitochondrial electron transport chain complexes and 2) dynamic or transient, when the interaction between proteins is short-lived and temporary (usually responding to a stimulus and having an end effect). While stable PPIs are easier to investigate, sufficient methods to study transient PPIs still do not exist. One example of transient PPIs is well-demonstrated by signal transduction pathways that are activated by receptor tyrosine kinases (e.g. insulin or Eph receptor pathways) [5,6]. Interaction of receptor tyrosine kinases with their ligands leads to the formation of receptor-ligand complexes and autophosphorylation of the receptor's kinase domain from the cytoplasmic side, which becomes the docking site for many signaling proteins. In turn, these proteins activate signal transduction pathways, which ultimately trigger a cellular event such as glucose uptake or cytoskeletal remodeling [7-9].

Methods to Study PPIs

Analysis of signal transduction pathways generally starts with the identification of proteins that interact with activated receptors. Once these proteins are initially identified, additional interacting partners may be identified and the function of these proteins may be further studied. A very common method for studying PPIs is co-immunoprecipitation. Unfortunately, this method may be employed only if preliminary data already exist. For example, if the interaction between two proteins is already measured by a different method, such as two-hybrid screen, co-immunoprecipitation may be confirmative. Therefore, analysis of PPIs has been limited to co-immunopurifications or two hybrid screens [10-12] that lead to the construction of interaction networks [13,14]. Other biochemical approaches for identification of PPIs are governed by the separating principle: affinity/immunoaffinity, electrophoresis, chromatography or mass spectrometry.

Blue Native PAGE (BN-PAGE) is an electrophoresis-based method that separates protein complexes based on external charge induced by Coomassie dye and according to their molecular mass. BN-PAGE experiments may provide information about the size, number, subunit composition, stoichiometry and relative abundance of these protein complexes [15-19]. Blue Native PAGE (BN-PAGE) has long been used to analyze PPIs and protein complexes from organelles [15-19]. This method separates protein complexes based on external charge induced by Coomassie dye and according to their molecular mass. BN-PAGE experiments may provide information about the size, number, subunit composition, stoichiometry and relative abundance of these protein complexes. Compared to previous methods used to study PPIs, there are several advantages of BN-PAGE for studying protein-protein interactions: 1) separation of protein complexes takes place under native conditions, so even transient interactions between proteins may be identified, 2) the method may simultaneously analyze association into- or dissociation from protein complexes of particular proteins as a result of ligand stimulation (e.g. time course analysis of a particular protein complex), 3) By combining BN-PAGE with liquid-chromatography-tandem mass spectrometry (LC-MS/MS), both structural and functional information may be obtained [17,20-22].

Recent advances in MS allowed scientists to identify not only one but many potential interacting partners of a protein involved in signal transduction pathways by analyzing immunoprecipitate via SDS-PAGE and LC-MS/MS [23-25]. This approach is referred to as proteomics [26-29]. Other methods, such as isotope-coded affinity tag (ICAT) [30], and stable isotope labeling by amino acids in cell culture (SILAC) [31-33], allowed detection and quantification of proteins and their phosphorylation levels involved in signal transduction from unstimulated and stimulated cells [34,35]. Therefore, combination of BN-PAGE with LC-MS/MS would allow one to simultaneously identify subunit composition and multimerization state of a protein complex.

Challenges

Although the above-mentioned approaches have advanced studies of signal transduction pathways, many questions still remain unanswered due to the technical limitations of the methodology. For example, in mass spectrometry-based experiments using unstimulated and stimulated cells, when a particular protein is identified as a participant in a signal transduction pathways, no functional assignment is made to it unless its post-translational modifications (such as phosphorylation) are dramatically increased or decreased, as determined by SILAC technology. Furthermore, even when the interaction partners of proteins involved in signaling are identified and characterized, the functional significance of the association of these proteins into multi-subunit protein complexes via ligand stimulation is still missed. Therefore, analysis of protein complexes from signal transduction pathways in terms of size, composition, post-translation modifications, multimerization level, and abundance would strongly benefit from the introduction of new approach that allows simultaneous analysis of multiple transient protein-protein interactions.

Additional theoretical and experiments challenges should also be considered. When using BN-PAGE, some special considerations and potential problems need to be expected and addressed. Since its discovery [18,19], BN-PAGE has been characterized as a robust technique that separates protein complexes based on the external charge induced by Coomassie dye and according to their molecular weight [36,37]. A potential problem for BN-PAGE is that protein complexes that have identical molecular mass may co-migrate and subunits from

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two different protein complexes may be falsely identified as being part of the same protein complex. This pitfall may be solved by adding for those particular protein complexes a pre-purification step to BN-PAGE, known as Colorless Native-PAGE (CN-PAGE), which is similar to BN-PAGE, except that the cathode buffer contains no dye. Therefore, protein complexes would be separated based on their internal charge, but not according to their molecular mass. Separation of protein complexes that result from CN-PAGE in a second dimension BN-PAGE will successfully separate the protein complexes with identical/similar molecular mass. The most difficult problem that is encountered when using BN-PAGE has to do with data analysis. The results obtained by BN-PAGE and LC-MS/MS, can be very rich in information, but do not hold too much value without included understanding of the biological significance of a particular PPI.

Perspectives

Although useful for many applications, BN-PAGE is not the method of choice for automation and large scale identification of PPIs, not due to the capability of separating/fractionating PPIs, but rather due to lack of the software that simultaneously investigates the biological significance of the PPIs identified by BN-PAGE and LC-MS/MS, as well as our capability of understanding the nature and reason of interactions such as post-translational modification-induced formation of PPIs (e.g. phosphorylation-induced PPIs). Therefore, building new PPIs-based bioinformatics software would strongly advance our current understanding of the responses of organelles, cells, tissues, organs and organisms to the internal and external stimuli through large-scale investigation of PPIs.

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