Lecture 20: Protein-Protein Interaction

Proteins are responsible for several functions in a cell ranging from a catalyzing reaction to several complex functions. Protein-protein interaction plays an important role within the cellular machinery for specific functions like signal transduction, translation, transcription, replication, control on Cell Cycle etc. The interaction between protein molecules leads to the formation of a larger protein complexes performing a specific function. Interaction among proteins depend upon various factors associated with the protein itself, such as its amino acid sequence, associated co-factors, and finally most important is the three-dimensional shape of protein. The different forces which help in the protein-protein interaction include various non-covalent interactions such as Van-der Waals force, hydrogen bonds formation etc.

Methods to Detect Protein-Protein Interaction

(1) Two Hybrid System

Two hybrid system is one of the most important method for protein-protein interaction studies. This method is used primarily for initial identification of interacting proteins, not for detailed characterization of the interaction. This method cannot provide thermodynamic and other parameters of protein-protein interaction. There are other methods used for thermodynamic and kinetic studies of the interactions (out of scope of the course).

The two hybrid system is a genetic method which uses transcriptional factor in order to identify protein protein interaction. Transcriptional factor contains DNA binding domain and transcriptional activation domain. The DNA binding domain refers to the promoters of specific gene and the trancriptional activation domain helps RNA polymerase enzyme to initiate the transcriptional activity of the gene, thus with the help of these two domains the expression of the gene takes place. The yeast two-hybrid system uses this unique feature of eukaryotic transcription factors. As explained earlier, these proteins consist of a DNA binding domain (DBD) which recognizes and binds to a defined promoter sequence upstream of a gene and an activation domain (AD) which interacts with the RNA polymerase II complex. When the DBD and the AD are expressed as separate polypeptides, transcription will not take place. However,

DBD still binds to promoter sequence but is unable to activate transcription. The AD can still interact with the RNA polymerase II complex, but since it is not located near the gene anymore, no transcriptional activation is taking place. The two hybrid systems for study of protein-protein interaction is based on the molecular setup of transcription factor.

The Two hybrid system uses this approach for detecting interaction among different proteins. It has been found that there is no need of covalent attachment between the two domains for the transcriptional activity. Transcription can be activated if the two domains can be brought together, which is done with the help of two interacting proteins attached with each domain. This aspect is also used in order to check whether two different proteins interact among themselves or not, if they interact then there will be the expression of reporter gene otherwise no expression.

The Construction of Two hybrid system includes creation of two different gene containing vectors i.e

1. Gene of DNA binding domain fused Bait protein (X- Bait protein)

2. Gene of Transcriptional activation domain fused Prey protein (Y-Prey protein)

These hybrid constructs are expressed in Yeast cells containing Reporter genes. If the Protein X (Bait protein) and Protein Y (Prey protein) interact, they will bring the transcription activation domain into close vicinity with the DNA binding domain, thus triggering the transcriptional process by allowing the RNA polymerase to bind and initiate transcription which is detected by the expression of the specific reporter gene (e.g. color change in case of gal1-lacZ - the beta-galactosidase gene).

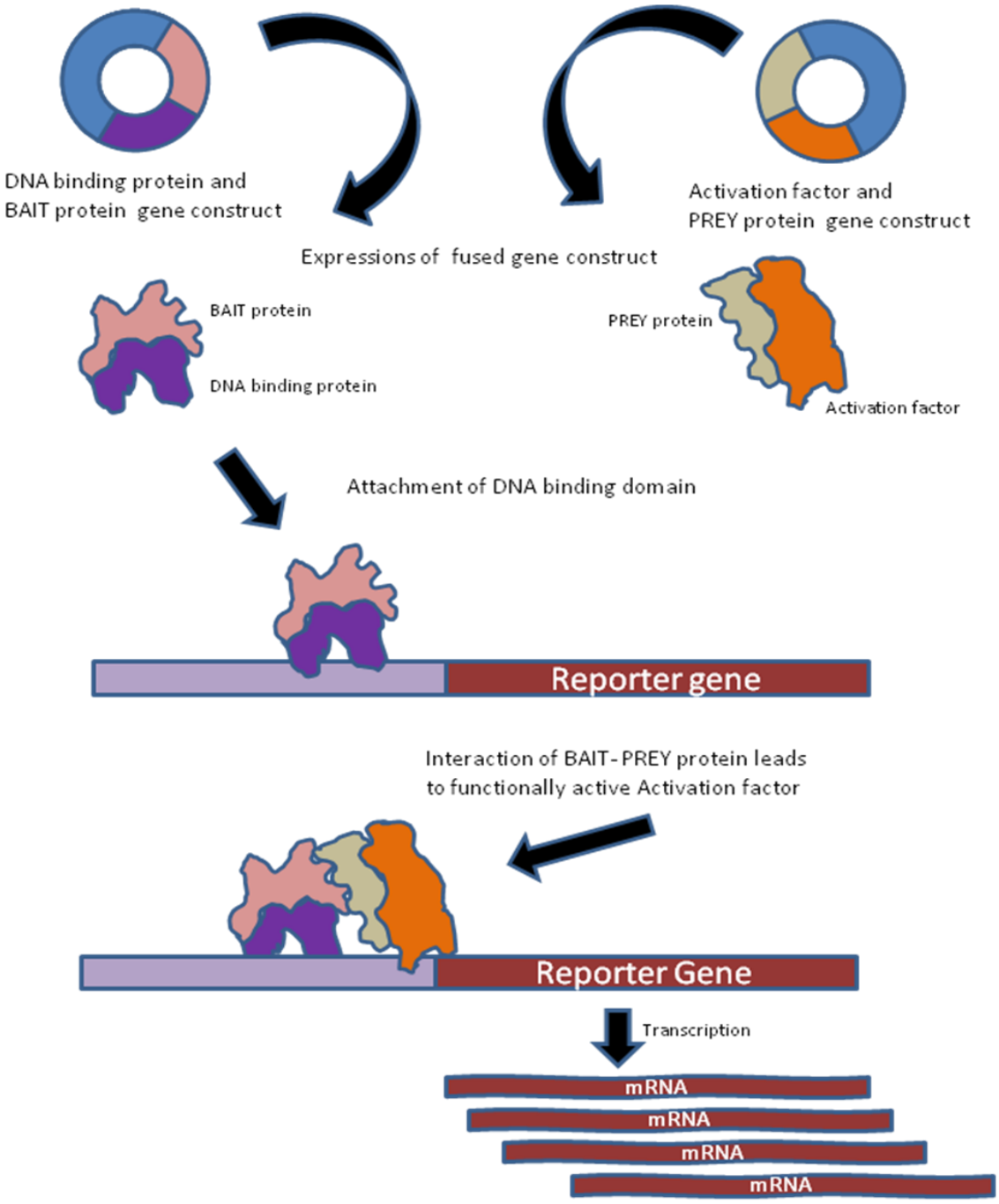
Steps for detecting Protein-protein interaction.

1. A bait vector is created consisting of selected protein to act as a bait (protein of interest against which interaction is to be checked) and DNA binding domain. The product of this vector is a Fusion protein consisting of bait protein and DNA binding domain.

2. Another Prey vector is created consisting of transcription activation domain and a specific protein whose interaction with respect to bait protein is to be checked. The product of this vector is also a fusion product of both prey protein and transcription activation domain.
3. The Fusion protein of bait vector binds to the specific promoter region of the reporter gene with the help of DNA binding domain but unable to initiate transcription as lacks activation domain.
4. The prey protein fused with transcription activation domain, if interacts compatibly with bait protein then the activation factor is able to locate itself in the vicinity of reporter gene and able to initiate the transcription by allowing RNA polymerase to act.
5. Thus, the change in color i.e. expression of the reporter gene clears that the bait protein is in interaction with prey protein and thus conclusion can be made about their functional activity further.

The Two hybrid system can be used to check the interaction of a single bait protein against thousands of prey proteins as only those cells would grow into which there is proper interaction taking place and allowing the selected gene essential for growth to be expressed.

The steps of two Hybrid System is summarized in animation


DNA binding protein and
BAIT protein gene construct
Activation factor and
PREY protein gene construct
Expressions of fused gene construct
BAIT protein
DNA binding protein
PREY protein
Activation factor
Attachment of DNA binding domain
Reporter gene
Interaction of BAIT-PREY protein leads
to functionally active Activation factor
Reporter Gene
Transcription
mRNA
mRNA
mRNA
mRNA

Affinity Chromatography

Affinity Chromatography is an essential method used to separate desired protein from complex mixtures, however it can also be used for studying Protein-Protein interaction. It is based on the affinity interaction among various groups of compatible proteins for its separation from complex mixtures. Affinity chromatography involves the retention of the desired sample within the column from the sample mixture applied. In this method one of the protein (against which interaction is to be checked) is coupled with suitable matrix in the chromatographic column.The sample mixture containing the protein of interest from suitable source is applied against it. Most of the proteins pass down through the column however, some proteins may get retained into it. The protein which comes into affinity bonding or interaction with the immobilized protein within the column gets retained and is further eluted by suitable treatment. The eluted sample is collected for further analysis (Fig. 1)

Care should be taken during the preparation of chromatographic column i.e.

- The coupling of protein with the matrix should not inhibit its functional activity otherwise it would become inactive and incapable of interacting and retaining the desired sample.
- Pure protein sample is to be used for column preparation in order to prevent unwanted protein interaction.
- The amount of sample protein applied must not be too low to be unnoticeable for detection within column and also must not be too high for many small proteins to get eluted without binding.

The advantages of the affinity chromatography is that it is highly sensitive and even detect weak interactions in proteins, also it tests all the sample proteins equally for interaction with the coupled protein in the column. However sometimes false positive results also arises in the column which is due to highly specific interaction among proteins, even through they do not get interacted in the cellular system. Thus protein interaction studies cannot be fully dependent on affinity chromatography and hence requires some other methods in order to cross check and

verify results obtained. The Affinity chromatography can also be associated with SDS-PAGE technique and Mass Spectroscopy in order to generate a high throughput data.

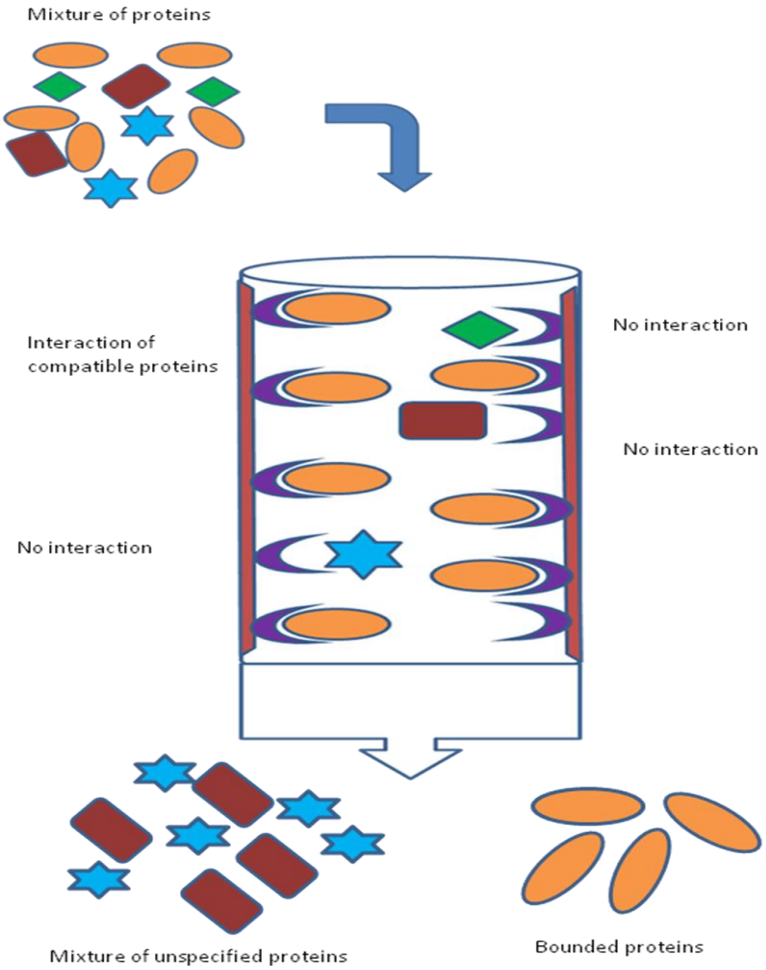


Fig 1. Diagrammatic representation of Affinity Chromatography

Figure 1: Diagrammatic representation of protein-protein interaction studies by affinity chromatography

(2) Co-Immunoprecipitation

This technique works by using an antibody which immunoprecipitate the respective antigen and with it co-immunoprecipitate any interacting protein. The protein molecules involved in the complex formation must be bound tightly to each other so that on being pulled, entire complex can be precipitated out. The method checks the interaction in the protein complex in their natural conformation. However one disadvantage is that sometimes concentration of the antigen gets lower thus unable to be detected effeciently.

Important steps involved in Co-immunoprecipitation technique (Fig. 2.)

1. Antibody- Antigen selection

It is very important to select specific antigen and antibody (for precipitating the antigen bounded with protein of interest), for easy and specific precipitation. Sometimes it happens that the antibody precipitate a non specific protein other than desired antigenic protein.

2. Cellular lysis procedure.

Care should also be taken during the cellular lysis procedure as not to disturb the proteins complex. The lysis procedure should be mild.

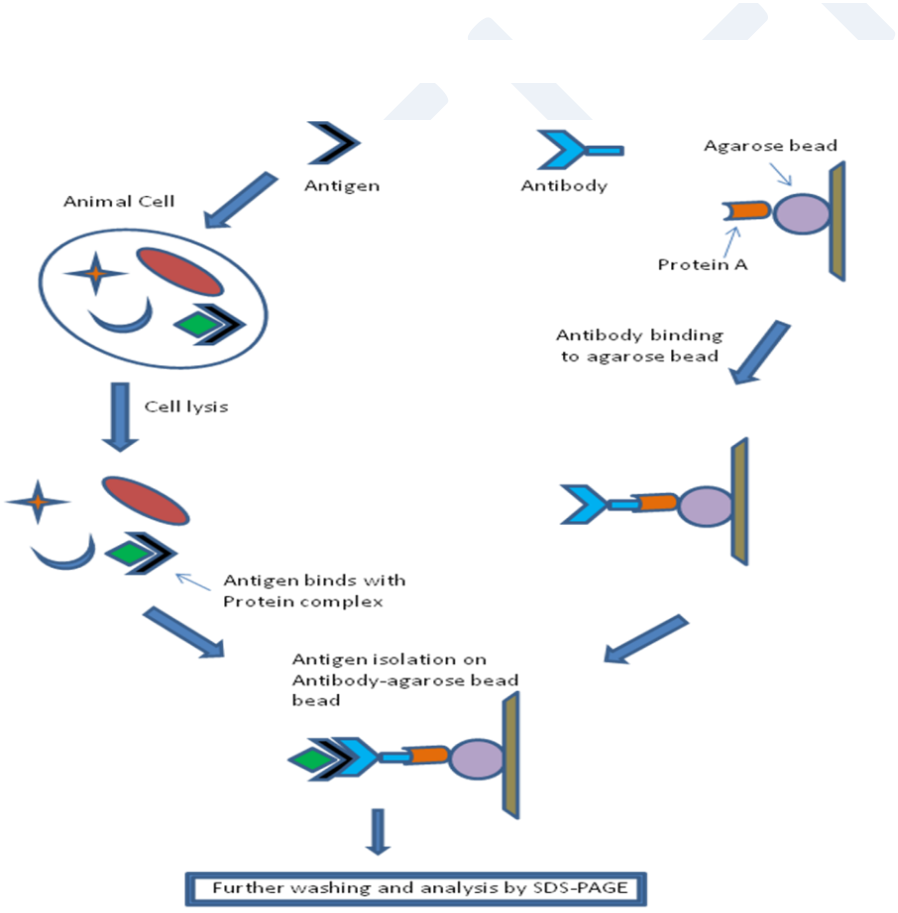


Figure 1: Diagrammatic representation of protein-protein interaction studies by co-immunoprecipitation

References

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