Protein-Protein Interactions

The first question is why would we want to know about protein-protein interactions? This kind of information would allow us to determine if a protein is in a complex, a metabolic of signaling pathway, or involved with other structural proteins. Sometimes a protein is identified by one function and then is found to be part of a complex with a structural or unknown role. Many of the complexes are stable such as the electron transport complexes, protein translocation complexes, nuclear pores etc., and some are transient such as the binding of a docking protein to ribosomes translating a secreted protein to take is to the ER or a G-protein involved in signaling.

There are three methods "commonly" used to look for protein-protein interactions, Yeast two hybrid, pull down or co-purification and fluorescence resonance energy transfer. We will discuss each of these.

Yeast Two Hybrid

The yeast two hybrid system is based on the presence of two domains in a transcription activator, an activation domain and a binding domain. The process is we are going to clone the protein of interest with one of the domains attached to it. This would be called the bait protein. We then take the genome of the organism and clone the other domain to all of them. This represents the prey proteins. These are then cloned into separate yeast. The yeast are allowed to mate and transfer plasmids. Selection can be made by using auxotrophs and providing genes that make amino acids. This allows the selection of those cells that contain both plasmids. Once we have the cells with both plasmids the cells that have interactions between the proteins will express the reporter enzyme and they can be detected. One example used here is the Lac Z gene. This is used in blue white screening of the cells.

There are also systems for cytosolic and membrane proteins. In the membrane system a split ubiquitin process is used. In this case ubiquitin can be split into two halves with the c-terminal ubiquitin (Cub) and the N terminal ubituitin (NubG). In this case the bait consists of the Cub protion linked to the protein of interest and followed by a transcription factor. The prey proteins will have the NubG sections attached to them. It is assumed that both of these are either membrane bound proteins or at least the bait is a membrane bound proteins. Once these two proteins are expressed and interact through the ubiquitin system the transcription factor is released by proteolysis by ubiquitin binding proteins. This allows the transcription factor to enter the nucleus and the reported protein to be expressed. A similar approach can be used for cytosolic proteins as well. It would seem to me that as long as the transcription factor can be released this would also work for some portions of organelles.

Doing this on a genomic scale takes a little more effort. The genome is basically cloned with both the bait and prey molecules and put into mating strains of yeast. When they are mixed together the strains mate and mix the plasmids. The diploid cells are then

selected for those that survive some screening process. The plasmids in the survivors can then be sequenced to determine which proteins are interacting.

Problems

There are several problems with this system. The first is that many false positives and false negatives are found. Another problem deals with the proteins that are being screened. They may not express well in the yeast system or they may not interact properly when linked to the two domains. There is also the possibility that the interactions are too transient to be measured. And lastly there are difficulties with multiple subunit complexes since two of the subunits may not be close enough for the two domains to come together.

Co-purification and Immuno-trapping.

This method is based on the concept that if the proteins are interacting in the cell they may stay together long enough to be purified together. The assumption is the the interaction will be long enough and strong enough to identify. The process is to tag one protein so that it can be pulled down with beads or columns containing antibodies to the tag. The protein is expressed in the cell, the cells lysed, and the bait protein purified by magnetic beads or on a column. Once the other proteins are washed off the protein of interest and its friends are removed by denaturation and analysis by LC/MS/MS. This slide is just to remind you of the many tags that are available for this process.

Fluorescence Resonance Energy Transfer (FRET)

In this method we are going to use the ability of one molecule to transfer its energy to another by emission of light and the uptake of that light by the other molecule. The emission of light absorbed is called fluorescence. When a second molecule whose absorption spectrum overlaps with the emission of the first molecule there can be a transfer of the light energy to the second molecule. If the second molecule is fluorescent then the light will be emitted by the second fluorescent molecule. The other possibility is that the second molecule only absorbs the energy and does not emit it resulting in quenching.

This process is highly distance dependent following a r^{-6} distance dependence. The efficiency of transfer can be determined from the quantum yield of fluorescence with and without the acceptor. This can be related to the distance by the formulas on the slides. In this system the proteins of interest are tagged with one or the other molecule. They are introduced into the system and the cells are illuminated with the excitation wavelength of the first molecule and fluorescence is look for from the second molecule. If it is observed then they are close together. The problem is how do we label them with fluorescent molecules? If there is a way to look at the interactions in vivo we can chemically label the proteins and observe the interaction. A more interesting process is to use various

forms of the green fluorescent protein and clone the proteins with one or the other attached. We then look for the energy transfer in vivo through a microscope.

Problems

As usual there are problems. We have the usual problems of protein expression. If an antibody based method is used for pull downs we have the general problems with antibodies. Lastly fusion proteins may not work due to stearic hindrance or improper folding.