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Final Version of Research Proposal

Identifying Cytoglobin’s Protein-Protein Interactions using yeast-two hybrid screening

Introduction:

 Proteins are essential macromolecules that provide structural support and defense against germs. There are many different types of proteins like hormonal proteins, structural proteins, transport proteins, contractile proteins, enzymes, globular proteins and storage proteins. Vertebrates contain different types of respiratory globin or protein that varies in terms of structure, function and tissue distribution. Globin is found in bacteria, plants, fungi, animals and it plays an important role in respiratory system. Hemoglobin, myoglobin, cytoglobin and neuroglobin are different types of globin. Hemoglobin facilitates the transport of oxygen in blood, myoglobin serves as oxygen transport and oxygen storage in muscle cells, neuroglobin plays an important role in nerve cells.Cytoglobin is an important member of recently discovered globin family. It is a small globular metalloprotein predominantly expressed in fibroblast, stomach, bladder, small intestine and many other tissues of our body. Since cytoglobin is a heme-binding globin, it has a higher affinity for oxygenbut it is not higher than that of hemoglobin. Cytoglobin expression increases in response to various stress conditions including hypoxia, which leads to brain injury due to lack of oxygen in the brain, oxidative stress (causes damage to lipids, proteins and DNA) and fibrotic stimulation. In addition, cytoglobin acts as nitrate reductase in mammals***1***. It plays crucial role under hypoxic conditions2. It is essential to identify other proteins that potentially interact with cytoglobin in order to study its functions and to study its crucial role during brain injury. This major task can be achieved by using a technique called yeast two-hybrid screening.

 Yeast two-hybrid screening is a molecular tool that aids for the study of protein-protein interactions. Two or more proteins interact once a reporter gene is transcriptionally activated. Gal4 protein is involved in this process and it contains DNA binding and activating domains6. This process is achieved by splitting the yeast Gal4 gene is divided into two parts: the activating domain and the binding domain7. The two parts of the protein should be in physical contact in order to make this chimeric protein, where each of the two proteins are fused together. This chimeric protein is made in yeast as “translational gene fusions” and a plasmid is formed: it fuses with binding domain to form activating domain7.



 Fig1. This picture shows the reporter gene interacting with another gene to form protein-protein interactions9.
Gal 4 is a molecule used in the yeast-two hybrid process. There is one activating domain and one binding domain. When both of them are fused together, then a protein-protein interaction is recognized.

Experiment:

 The purpose of this experiment is to identify proteins that potentially interact with cytoglobin. The yeast two-hybrid screening process involves using Gal4 yeast strain. For this particular experiement, mouse DNA can be used along with Polymerase Chain Reaction. Yeast two-hybrid system will be used in this experiment to find cytoglobin’s interaction with another protein. Yeast two-hybrid screening is a process used in vivo to detect the protein- protein interactions using a complimentary DNA. Gal 4 yeast strain is used and then a binding and an activating domain of cDNA will be used to predict the unknown protein that interacts using cytoglobin.

1. Yeast two-hybrid screening process is used in this experiment. A genetically engineered strain of yeast is used in which certain nutrients like amino acids are missing. When the yeast strain fails to survive a mutant strain is formed using plasmids. The binding domain and the activation domain are introduced to mutant strain of the yeast. The plasmids are engineered to interact with the binding domain and the activation domain. A bait protein is known as the protein that binds to the binding domain and used by a researcher to identify new binding partners using the yeast two-hybrid screening. The activation domain, also known as the prey protein is an unknown protein. Thus, in this experiment, cytoglobin (a protein) is the bait protein or binding domain and the activation domain is the protein that we need to find using this process. If the binding domain(cytoglobin) interacts with the activation domain(unknown protein) then the transcription factors also interact and the reporter gene’s transcription occurs. However, if those two do not interact then the transcription of the reporter gene does not occur. A change in cell phenotype represents a successful protein-protein interaction. This is a process used in my experiment.

Immuno-precipitation: Another process used by an experiment conducted by the School of Pharmaceutical Sciences, Wenzhou Medical College, Wenzhou,Zhejiang, China and it is known as immune-precipitation. It is a process of that uses antibodies specific to a protein to remove those proteins from a solution. In an experiment conducted by Neuroprotection Research Laboratory, Departments of Neurology and Radiology, Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Charlestown, MA, USA.10, immuno-precipitation was conducted using polyclonal antibodies to identify proteins that interact with neuroglobin. Polyclonal antibodies were used against mouse Ngb and the precipitates were centrifuged. The antibodies are important because they will help bind neuroglobin and form a chain of molecules. This can help identify other protein-protein interactions. As a result of that experiment, 36 different proteins were found to be interacting with Neuroglobin.

Discussion:

 Through the use of yeast two-hybrid screening, different proteins interacting with cytoglobin, an important member of globulin family, can be found. By the use of immuno-precipitation, an antibody is used to attach each cytoglobin molecule and then form a network. After that, that network of molecules is centrifuged to find out different proteins. The results can provide us with information of potential Cytoglobin-interacting multiple cellular proteins. This is necessary to study the function of this globin as well as its major role in preventing any kind of brain injury. This experiment should be conducted to test cytoglobin’s interaction with other important organelles of our body (like mitochondria). The findings of this experiment will show the proteins that will bind specifically with cytoglobin. However, the interaction network and functions of cytoglobin is not solely dependent upon this experiment. Cytoglobin’s multiple interaction with multiple proteins can help us determine number of metabolic functions, cellular pathway for cellular functions. Therefore, this experiment should be conducted to elucidate the physiological functions of cytoglobin with the help of other proteins and then apply that information to prevent hypoxia or extreme cases of brain injuries and damages to other tissues of the body.

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