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## 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<sup>1</sup>. 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 type of globin found so far<sup>2</sup>. 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<sup>3</sup>.

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<sup>4</sup>. Since cytoglobin is a heme-binding globin, it has a higher affinity for oxygen. Cytoglobin expression increases in response to various stress conditions including hypoxia( a brain injury), oxidative stress (causes damage to lipids, proteins and DNA) and fibrotic stimulation<sup>5</sup>. In addition, cytoglobin acts as nitrate reductase in mammals. It plays crucial role under hypoxic conditions. 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 domains<sup>6</sup>. This process is achieved by splitting the yeast Gal4 gene is divided into two parts: the activating domain and the binding domain<sup>7</sup>. 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 domain<sup>7</sup>.

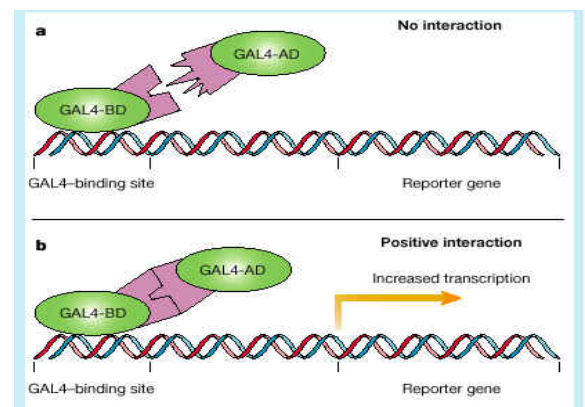


Fig1. This picture shows the reporter gene interacting with another gene to form protein-protein interactions<sup>9</sup>.

## Experiment:

The purpose of this experiment is to identify proteins that potentially interact with cytoglobin. It has already been discussed above that identification of other proteins is necessary to study the function of this important member of globin family. The yeast two-hybrid screening process involves using Gal4 yeast strain. For this particular experiment, mouse DNA can be used along with Polymerase Chain Reaction.

- a) Immuno-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<sup>10</sup>, immuno-precipitation was conducted using polyclonal antibodies. As a result of that experiment, 36 different proteins were found to be interacting with Neuroglobin.
- b) Cytoglobin's level can be examined using mouse anti-cytoglobin antibody. Cytoglobin interacting proteins were detected with its respective antibody using immune-precipitation in the experiment.
- c) Cytoglobin's interactions with other proteins can be found through this process; just like Neuroglobin's interaction with other proteins was found through the above experiment. As a result, we can compare its plasticity during brain injuries.

## 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<sup>11</sup>.

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4. Fresh blood for the vertebrate globin family

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