

The Correlation of GluT1 Translocation to Lipid Rafts and Its Activity

Lauren Strohbehn in collaboration with Larry Louters, Ph.D.

This summer, I expanded on Professor Louters' work with a transporter protein called Glucose Transport Protein 1, or GluT1. This protein allows glucose, a sugar, to pass across the cell membrane. Glucose is the preferred fuel of the cell for maintaining function so proper glucose regulation is critical for overall health of an organism. Abnormal glucose regulation is linked to several potentially devastating diseases. Diabetes is one example, which is caused by an inefficient glucose transport system. Cancer is also related to atypical glucose regulation, although the problem is the opposite: cancer cells intake copious amounts of glucose to satisfy the high energy demands needed for rapid cell multiplication. GluT1 is more abundant than normal in many types of cancer cells. Even Alzheimer's disease is associated with improper GluT1 regulation in the brain. A deeper understanding of how this protein functions is essential for developing potential GluT1-targeted therapeutic strategies to treat such diseases.

The primary focus of my research this summer was to investigate the mechanism of GluT1 activation. Previous findings suggested that GluT1 takes in glucose faster when it is integrated into segments of the cell membrane called lipid rafts. Lipid rafts are compact parts of the cell membrane held together with cholesterol and sphingolipids. They often have a high concentration of transport and signal proteins, similar to "activity centers" on the cell surface. I hoped to enhance glucose uptake, then use a lipid raft isolation technique to compare how much relative GluT1 was in the lipid rafts in the activated cells compared to my control cells. I concluded early on that GluT1 translocation was not the primary reason for activation, and that the fibroblast cells I was working with may have unique lipid rafts. My project therefore morphed into determining the composition of these fibroblast rafts and what initially targets GluT1 to these domains.

Like the name suggests, lipid rafts are partially composed of phospholipids, which are fat derivatives. These phospholipids are less dense than the proteins that compose the membrane surrounding the lipid rafts. The surrounding membrane contains phospholipids as well, but they can be easily disrupted or removed without breaking the lipid rafts. Because of this I could isolate the rafts by creating a density "gradient" in a small tube. I loaded my cell fragments in the denser bottom portion of the tube and spun it at 200,000 times the force of gravity for 17 hours. Because lipid rafts are less dense than the proteins in the surrounding cell membrane due to phospholipid content, they float towards the top. The protein outside the lipid rafts stay in bottom, denser liquid. Then I would use an analysis called a Western blot to determine how much GluT1 was in the lipid rafts versus other portions of the cell membrane.

My findings confirmed that the mouse fibroblast cells I have been working with do not exhibit traditional lipid rafts. They are not isolated when I use a common procedure found in literature, which involves permeabilizing the cell membrane with a detergent. They also don't contain proteins usually found in rafts such as CD44 and flotillin. When I take out the cholesterol – which should dissolve the rafts – the low density structures that contain GluT1 are maintained. A paper by Kraft in 2013 suggested that these domains in fibroblasts are cohesive not due to cholesterol enrichment but sphingolipid distribution, and stable because they are tethered to the actin cytoskeleton of the cell. To test this, I inhibited the productions of sphingolipids and was excited to find a partial shift of GluT1 to high density fractions, indicating that sphingolipids are important for whatever low density microdomain GluT1 is contained in. More replicates of this data are needed. However, when I disrupted the actin cytoskeleton and microtubule formation, the lipid rafts were still present, indicating the presence of a different kind of lipid raft in my cells than the Kraft paper suggested. More inhibitions of cellular structures (or treatments with different detergents) are needed to determine the composition of these domains.

To find out what targets GluT1 for these low density domains, I inhibited the occurrence of two modifications of GluT1 that occur after the protein is synthesized in the cell. In separate experiments, I inhibited glycosylation (attachment of a carbohydrate) and palmitoylation (covalent attachment of fatty acids) to GluT1 and tried to discern if this was driving the allocation of GluT1 to lipid rafts. I found that neither of these modifications seemed to be an important factor for GluT1 location in the membrane. In the future, I would like to further explore what might be targeting GluT1 to these lipid raft domains.

Even if I do not get another chance to pursue these questions, I am extremely grateful for the time that I was given in the Louters Lab these past two summers. Coming back for a second year was especially useful because I had a significant background understanding of the project. I will most likely be pursuing a path in biomedical research and so the laboratory exposure I have gained is indispensable. It is exciting to realize how much my research skills have matured; I am much more independent and can peruse literature, analyze data, and propose next steps. I am so thankful for the people and institutions who made this experience possible.