

Robyn Beatty

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Mark D. Kirk

Mentor Department: Biological Sciences

Funded by: Arts & Sciences Undergraduate Research Mentorship Program

Camgaroo-2 as an indicator of function in embryonic and neuralized stem cells

Robyn Beatty, Christopher Pierret, Kathleen Spears and Mark D. Kirk

The transplantation of stem cells to replace cells that have been lost or damaged due to disease or injury is quickly becoming a conceivable treatment method. Embryonic stem (ES) cells have the capacity to become any cell in the body, so the therapeutic possibilities are vast. The ultimate goal of our research on ES cells is to induce them to differentiate into functioning neurons to replace those that are lost in patients suffering from neurodegenerative disorders. However, it is important that the differentiated cells possess the appropriate phenotype and are able to perform the correct function after transplantation. In the past, it was common to accept a differentiated cell's fate based solely on its morphology and the presence of specific membrane markers. Now, it is becoming increasingly important to determine a donor stem cell's fate based on its function, especially if the cell is to be transplanted into a subject as a means of therapy. This study used the calcium-sensitive protein Camgaroo-2 to test the function of embryonic stem cells and cells directed toward a neural lineage. Camgaroo-2 is a fusion protein that consists of calmodulin in between two halves of yellow fluorescent protein. When calcium is present, it binds to the calmodulin portion of the Camgaroo-2, inducing a conformational change that results in increased fluorescence. After mouse embryonic stem cells were transfected with Camgaroo-2, we used reagents such as potassium chloride and ionomycin, known to elevate intracellular calcium, to confirm that the ES cells were stably transfected with the plasmid, and that Camgaroo-2 was functioning correctly. Potassium chloride causes the cell to depolarize while ionomycin (a calcium ionophore) creates large pores in the cell membrane. Both reagents allow for an influx of calcium into the cell, leading to increased fluorescence. The Camgaroo-2 transfected ES cells showed the appropriate responses to KCl and ionomycin by depolarizing and showing visible increases in fluorescence. This confirms that our Camgaroo-2 construct is functioning in the ES cells. We are in the process of testing the responses of neuralized ES cells using appropriate neurotransmitters, the presence of which should induce unique fluorescent signatures in a cell specific manner. Confirming neuronal function from differentiated Camgaroo-2 ES cells is an important step toward neuron transplantation in a neurodegenerative disease model.