Honors Research (Bio 4950H and 4952H) SAMPLE Application

Your Name: John Smith

Mentor's Name: Jane Doe

Mentor's Department: Biological Sciences

Title of Project: Cell Death within an *in vitro* model of a neural stem cell niche

Background Information on Project: Stem cell therapies have the potential to treat neurodegenerative diseases, such as Parkinson's disease and Batten disease. Batten Disease, a rare inherited disease in children, causes severe neurodegeneration, which results in blindness, seizures, and premature death. In Batten disease, the transplantation of stem cells into a patient may replace lost cells or prevent cell loss due to the disease. In one form of Batten disease, transplantation of stem cells into the retinas of mutant model mice have shown signs of neuroprotection, including enhanced survival of photoreceptors (Meyer et al. 2006). One possible method to increase the efficiency of this treatment is the transplantation of a functional unit capable of producing its own neural precursors "on demand." Such a structure, known as a neural stem cell (NSC) niche, can be found in two small areas in the brain of mammals, and is the center for adult neurogenesis throughout the lives of these animals.

Experimental Approaches: Our lab has developed a way to produce an NSC niche-like structure *in vitro* from neuralized mouse embryonic stem cells. To test how this structure is formed and maintained, I am investigating cell death within this *in vitro* NSC niche-like structure. I will perform two different tests for apoptosis or programmed cell death, Trypan Blue exclusion and TUNEL. Trypan Blue shows membrane permeability; if cells turn blue (Trypan Blue is not excluded) it is indicative of a late stage in the apoptotic process. In the TUNEL assay, nicked ends of DNA are labeled, an indication of early stage apoptosis. I also will test the effects of induced cell death on the advancement of *in vitro* niche formation.

Predicted Outcomes: Many cells in the cultures may differentiate and not survive the minimal culture media used to generate the NSC niche-like structures. If this prediction is true, differentiated cells with complicated morphologies will exhibit Trypan Blue and TUNEL labeling. We predict that the labeled cells will also label for mature neural markers, such as neurofilament and NeuN, indicating the sensitivity of highly differentiated cells to the culture conditions.

Overall Significance: These experiments will test whether programmed cell death contributes to formation of NSC niche-like structures *in vitro*. Future studies will focus on signaling events that occur in the cultures that are required for niche formation. We hope that this information will lead to a better understanding of how the *in vitro* niche forms and will provide new methods for stem cell transplantation and enhanced clinical applications of stem cell therapies.

Cited Literature:

1. Meyer, JS, Katz, ML, Maruniak JA, and Kirk MD. 2006. Embryonic stem cell-derived neural progenitors incorporate into degenerating retina and enhance survival of host photoreceptors. *Stem Cells* 24, 274-283.

Honors Research (Bio 4950H and 4952H) SAMPLE APPLICATION

Your Name: John Doe

Mentor's Name: Jane Doe

Mentor's Department: Biological Sciences

Title of Project: Structural flexibility and DNA-binding in the zinc finger protein TFIIIA

Background Information on Project: Transcription Factor IIIA is the archetypal zinc finger protein, the founding member of a family of proteins that make use of the same structural fold to recognize specific DNA sequences (1). In fact, the zinc finger structural motif is by far the most commonly used DNA-binding domain found in eukaryotic cells. Zinc finger proteins generally contain several sequential zinc finger domains that act individually to recognize small subsites within the aggregate DNA sequence recognized by the intact protein. Previous studies with TFIIIA have suggested that its nine zinc finger domains in some way interfere with each other during binding to a 52-base pair DNA sequence in the 5S rRNA gene (2). This interference is hypothesized to result from a misalignment between the zinc fingers of the protein and their respective subsites in the 5S rRNA gene, thereby necessitating an energetically unfavorable distortion in the DNA, the protein, or both that is necessary to accommodate simultaneous binding by all nine zinc fingers. I propose to test this idea by engineering variant forms of TFIIIA in which flexible protein linkers are placed between adjacent zinc fingers. These mutant proteins with flexible linkers will then be analyzed with respect to DNA-binding to determine if the affinity of the interaction is increased as a result of relieving the putative energetically unfavorable distortion.

Experimental Approaches: I will use methods of oligonucleotide-directed mutagenesis to introduce flexible linker sequences into specific sites within the DNA sequence encoding *Xenopus* TFIIIA. These variant proteins will be expressed in and purified from *E. coli*. A purified protein's ability to bind to the 5S rRNA gene in vitro will be tested using methods that quantitatively assess the affinity of the protein-DNA interaction. All of the relevant methods are well established in my mentor's lab (3-6).

Predicted Outcomes: I expect that some, but not all, of the variant proteins containing flexible linkers between adjacent zinc fingers will exhibit higher affinity binding to the 5S rRNA gene compared to what is observed with the wild-type protein. Those that do increase affinity will reveal sites of energetically unfavorable distortion within the DNA-protein complex.

Overall Significance: This project will help reveal structural features of zinc finger proteins that are important in achieving high affinity, specific DNA binding. This will be important in understanding fundamental mechanisms of genetic regulation in eukaryotic cells. Novel engineered zinc finger proteins have been designed to recognize specific DNA sequences and have numerous potential biotechnical applications. The proposed work may reveal additional ways of engineering artificial zinc finger proteins to achieve specific, high-affinity binding.

Cited Literature:

1. Miller, J., McLachlan, A.D., and Klug, A. 1985. Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. *EMBO J.* 4:1609-1614.

2. Kehres, D.G., Subramanyan, G.S., Hung, V.S., Rogers, G.W.Jr., and Setzer, D.R. 1997. Energetically unfavorable interactions among the zinc fingers of transcription factor IIIA when bound to the 5 S rRNA gene. *J. Biol. Chem.* 272:20152-20161.

3. Setzer, D.R., Hmiel, R.M. and Liao, S. 1990. A simple vector modification to facilitate oligonucleotide-directed mutagenesis. *Nucleic Acids Res.* 18, 4175-4178.

4. Del Rio, S. and Setzer, D.R. 1991. High yield purification of active Transcription Factor IIIA expressed in *E. coli*. *Nucleic Acids Res.* 19, 6197-6203.

5. Del Rio, S., Menezes, S., and Setzer, D.R. 1993. The function of individual zinc fingers in sequence-specific DNA recognition by Transcription Factor IIIA. *J. Mol. Biol.* 233, 567-579.

6. Setzer, D.R. 1999. Measuring equilibrium and kinetic constants using gel retardation assays. *Methods in Molecular Biology* 118, 115-128.