STUDENT RESEARCH GRANT APPLICATIONS
EXAMPLE I:

The Role of Connexin 43 in Cardiac Muscle Tissue in Hyperhomocysteinemic Mice

Project description: (no more than 1,000 words) The project description should begin by setting the proposed research question in the context of the broader field (including a brief discussion of the background and the significance of the general question of interest) and then describing the specific tasks that will be undertaken to investigate the question. Proposals should also provide: 1) a summary of results of any relevant preliminary studies already done by the student, and 2) a detailed description of the specific tasks to be undertaken by the student with this study.

Hyperhomocysteinemia (HHcy) is a condition that has been linked with cardiovascular disease, notably atherosclerosis and venous thrombosis. Often caused by a B vitamin/folate deficiency, or in rare cases, a genetic mutation in the Methyltetrahydrofolate reductase (MTHFR) gene, HHcy is the buildup of homocysteine, an intermediate product in the conversion of methionine to cysteine. While present in only 5% of the general population, HHcy emerges in 40% of individuals diagnosed with cardiovascular disease, thereby suggesting a causal or, at the least, a correlational relationship between the disease and the pathologies.

The mechanisms of HHcy are not entirely understood. HHcy imapirs vasodilation in the endothelium by the inhibition of nitric oxide and of gap junctional coupling (my current study). There has been growing evidence that HHcy alters cardiac structure and function as well. Tyagi et al. associated oxidative stress with HHcy's instigatin of the uncoupling of endothelial cells lining the cardiac chambers from cardiac myocytes. HHcy activates matrix metalloproteinases that disconnect the endothelium from myocytes, which allows the accumulation of oxidized matrix, thereby inhibiting synchronization of the layers. This remodeling results in impaired diastolic relaxation of the heart with as little as a two fold increase of plasma Homocysteine levels (3, 5). Beyond extracellular matrix remodelling, Joseph et al. delineated 3 methods by which HHcy results in diastolic dysfunction: 1. A disproportionate increase in collagen relative to myocyte hypertrophy, 2. mast cell infiltration, and 3. thickening of the coronary arterioles (1). Another study further supported the remodeling hypothesis when it demonstrated that Hcy elicits an acute negative inotropic effect on ventricular myocardium with similar levels of pathological Hcy as those seen in humans (2).

My current study focuses on HHcy's deleterious affects on the vascular endothelium and its ability to engage in gap junctional communication by an endothelial derived hyperpolarizing (EDHF) mechanism. For assessment, C57BL/6 mice have been divided into four groups: one control group and three experimental groups. Two experimental groups were subjected to a 1% methionine diet to induce HHcy. The first cohort (n=10) was placed on the diet for 4 weeks and experienced a 2 fold plasma homocysteine increase. The second cohort (n=9) remained on the diet for 8 weeks and Hcy blood levels are still to be determined. The third experimental cohort (n=10) are being subjected to an "ultra" diet with 1% methionine/ low B vitamin/folate component. The second and third experimental cohorts├ results are still pending. The control cohort (n=15) remained on a normal diet. All groups were administered water ad libidum and were exposed to 12:12-h light/dark cycles.

Thus far, both vascular function tests and connexin 37, 40, and 43 evaluations (using RNA assessment and immunohistochemistry) are showing hopeful trends. It is with this progress and with the accumulating literature suggesting the occurrence of cardiac remodeling during hyperhomocysteinemia in rats, that analyzing cardiac connexin expression in mice becomes an

enticing tangent for my project.

Because of the known abundance of connexin 43 in cardiac tissue and its critical role in electrical conduction through cardiac gap junctions, expression of this protein will be the focus of the proposed study. Cx43 mRNA in the heart will be analyzed using an already optimized method for RNA isolation and Real-time PCR quantification. The Cx43 protein will be measured using Western Blotting techniques. As a qualitative measurement, select tissues will be immunohistochemically labeled using Taqman probes. Cardiac tissues will be taken from the animals in the third experimental cohort (ultra diet group) of my current study. Control hearts from earlier groups have been frozen (-80 Celsius) and preserved in RNAlater (Qiagen) for use as well.

One of the major benefits of the proposed study is that cardiac tissues collected from animals in the current vascular function project can be utilized in the new cardiac Cx43 expression project. Also, additional exploration into the enigmatic condition of hyperhomocysteinemia will provide further insight into cardiovascular disease and perhaps even diabetes mellitus: a culprit of cardiac remodeling by a similar endothelial-myocyte uncoupling mechanism as seen with HHcy (3).

1. Joseph J, Joseph L, Shekhawat NS, Devi S, Wang J, Melchert RB Hauer-Jensen, Kennedy RH. Hyperhomocysteinemia leads to pathological ventricular hypertrophy in normotensive rats. Am J Physiol Heart Circ Physiol. 285: H679-H686, 2003.

2. Kennedy RH, Owings R, Shekhawat N, Joseph J. Acute negative inotropi ceffects of homocysteine are mediated via the endothelium. Am J Physiol Heart Circ Physiol. 287: H812-H817, 2004.

3. Tyagi SC, Roadriguez, W, Patel AM, Roberts AM, Falcone JC, Passmore JC, Fleming JT, Joshua IG. Hyperhomocysteinemic diabetic cardiomyopathy: oxidative stress, remodeling, and endothelial-myocyte uncoupling. J Cardiovasc Pharmacol Ther. 10 (1):1-10, 2005.

4. Varga, Elizabeth A., Amy C. Sturm, Caron Misita, and Stephan Moll. Homocysteine and MTHFR Mutations: Relation to Thrombosis and Coronary Artery Disease. Circulation 11: e289-e293, 2005.

5. Walke E, Black J, Parris C, Bryda EC, Cansino S, Hunt L, Chappell J, Wehner P, Studeny M, Wright GL. Effect of experimental hyperhomocysteinemia on cardiac structure and function in the rat. Ann Clin Lab Sci.34(2):175-80, 2004.

EXAMPLE II:

Title of Student_Research_Grant Project:

A Role for the Two-Component Signal Transduction System hp0166-hp0165 in the Adherence of H. pylori to Human Gastric Epithelial Cells

Project description: (no more than 1,000 words) The project description should begin by setting the proposed research question in the context of the broader field (including a brief discussion of the background and the significance of the general question of interest) and then describing the specific tasks that will be undertaken to investigate the question. Proposals should also provide: 1) a summary of results of any relevant preliminary studies already done by the student, and 2) a detailed description of the specific tasks to be undertaken by the student with this study.

BACKGROUND: Since its discovery in 1983, the Helicobacter pylori has been linked to gastric

and duodenal ulcers, gastritis and has been classified as a class I carcinogen for its putative role in the development of gastric cancer (3). H. pylori infects approximately fifty percent of people worldwide, though only a fraction of these cases ever result in symptomatic disease. Though the majority of the bacteria are free within the muscous overlying the gastric epithelial cells, a small subset of the population adheres tightly and directly to the epithelial layer. The adhesin proteins BabA and SabA have been identified as important in this interaction. Previous studies from our laboratory have demonstrated that the two-component signal transduction system, composed of histidine kinase hp0165 (jhp0151) and response regulator hp0166 (jhp0152), controls expression of sabA as transcription of this gene is derepressed in the histidine kinase mutant. (Forsyth et al., 2002) In this study, we are using bacterial adhesion assays to determine how an hp0165 mutation affects SabA levels and thus affects binding of H. pylori strain J99 to AGS cells (human gastric adenocarcinoma cells) in vitro. Data obtained from these studies will be presented at the 2005 American society for Microbiology General Meeting in Atlanta, and ultimately we hope to publish this work.

PRELIMINARY RESULTS: Strain J99-jhp0151-, in which sabA is derepressed, binds more than twice as efficiently as wild-type J99. In strain 26695, in which sabA is predicted to be out of frame, there is no significant difference between the binding of wild-type and hp0165 mutant strains. These results indicate that the two-component signal transduction pathway encoded by hp0166-hp0165 (jhp0151-jhp0152) plays a role in the regulation of adhesion in strain J99, and suggest that this could be due to an effect on SabA expression.

GOALS: 1) To determine if the increased binding of the jhp0151 null mutant is due to regulation of SabA, we will create a jhp0151/sabA double mutant in strain J99. We hypothesize that this double mutant will have a binding activity profile similar to wild-type J99, since the deregulated SabA will not be expressed at high levels. 2) In order to confirm that SabA is responsible for the observed binding differences between the histidine kinase mutant and wild-type J99, we will use neuraminidase to specifically remove sialic acid, the binding target of SabA, from glutaraldehyde fixed AGS cells. These modified cells will then be used to repeat the binding assays performed previously. We predict that the removal of sialic acid will significantly reduce binding of both the mutant and wild-type strains, and both strains will bind with a similar frequency. 3) To ensure that our preliminary results are not due to downstream effects caused by the insertion of the gene deletion construct into strain J99/jhp0151- we will complement the mutant with jhp0151. The binding activity of the complemented strain will then be compared to wild-type J99 and the jhp0151 mutant. We predict that the complemented strain will have binding levels similar to wild-type J99.

METHODS: Confluent monolayers of human AGS cells were infected at MOI 100:1 with the appropriate number of wild-type or isogenic hp0165 mutant strains of H. pylori. After three hours, monolayers were washed 3x and lysed with 1% saponin. Lysates are serially diluted and cultured for 3 days to determine CFUs. All assays are done in triplicate and repeated on multiple days. For assays involving enzyme modifications, AGS cells are first fixed in with 2% glutaraldehyde and specific fluorescently-labeled lectins are used to detect membrane structures using microscopy, and this is used to confirm the specific removal of sialic acid (St. Geme, 1994).

REFERENCES Forsyth M. H., Cao P., Garcia P. P.¢¥. Hall J. D.*, and T. L. Cover. 2002. Genome- wide transcriptional profiling in a histidine kinase mutant of Helicobacter pylori identifies members of a regulon. J. Bacteriol. 184:4630-4635.

St. Geme JW 3rd. 1994. The HMW1 adhesin of nontypable Haemophilus influenzae recognizes sialylated glycoprotein receptors on cultured human epithelial cells Infect. Immun. 62:3881-3889.

Intended Outcome of Research Project (for example, Honors Project, Publication, or

Presentation):

Publication, Presentation at the 2005 American Society for Microbiology General Meeting in Atlanta

Total Dollar Amount Requested:	400

Budget: The budget should detail the specific expenses for which you are requesting grant support. The program will not normally fund requests in excess of $400 (though joint proposals will be accepted; i.e., students from two different labs pooling resources to buy supplies or equipment totaling more than $400). For successful applications, payment will be made through reimbursement of direct expenses (details will be provided upon notification of funding).

Budget will be used for the purchase of tissue culture supplies and media, neuraminidases, fluorescent lectins for use in the sialic acid removal experiments