Samples of Summaries for Research Progress and Research Plans

All of the summaries below were taken (with permission!) from the TAC forms of current and former GSBS students, and provide good examples of brevity and coherence.

**Summary of Research Progress Since the Last Report: Three Examples**

I have furthered previous work in the lab and validated that PAR2 blockade does confirm a protective effect to tissues under conditions of oxidative (Carbon-tetrachloride injection) or dietary (MCD) stress. In particular I have confirmed, by histological section, that PAR2 blockade reduces cellular necrosis and fibrosis in the livers of animals receiving carbon-tetrachloride injections. The data on necrosis agrees strongly with data previously generated in the lab showing that blockade reduced the level of activation of human stellate cell line (LX2), stellate cell activation is presumed to be the predominant cell type involved in fibrosis and development of the disease Nonalcoholic Steatohepatitis (NASH). These data also correspond well with work published by others showing a role for PAR2 in fibrosis and development of NASH. I have also used livers taken from mice having PAR2 blockade or deficiency and have evaluated by QPCR that the liver undergoes a dramatic alteration in its apparent expression of genes involved in lipid metabolism. I have also created a system to use for the evaluation of PZ-235 to block oxidative stress related cell death in vitro using the Vitamin K analog called Menadione.

I have constructed a number of HPK1 mutants on a kinase dead background and have done some preliminary imaging experiments with the goal of finding out what part of the HPK1 molecule is required for entrance into microclusters.

I have also designed and optimized primers for rt-qPCR for detecting Map4K family members.

- Completed experiments for the xds story: time course RIVET experiment in vitro and in vivo to determine when during infection low phosphate is sensed by V. cholerae

- Submitted the manuscript to Molecular Microbiology: "Identification of in vivo regulators of the Vibrio cholerae xds gene using a high-throughput genetic selection." Authors on the paper: EmilyKate McDonough, David Lazinski, and Andrew Camilli.

- Made G482D point mutation in pepA and tested its ability to regulate emrD using qRT-PCR, concluding that aminopeptidase activity of PepA is responsible for emrD regulation

- Began work on identification of other Vibrio cholerae phosphatases: bioinformatically and using a transposon mutant screen. One putative acid phosphatase was identified by BLAST analysis. The tn mutant screen has yielded 7 colonies that are yet to be sequenced.

- Began work on identification of the second regulator of phoX. Tested HapR as a putative regulator, but data did not support a regulatory connection. Designed a modified genetic selection to identify the regulator.
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Summary of Research Plans: Three Examples

1. To test whether integrins are involved in Gal-8-induced LEC sprouting, neutralizing Ab against different integrins, like integrin α9β1, αvβ3 and β5, will be used in the LEC sprouting assays.

2. To test whether VEGFR-3, integrins, PDNP are associated at cell surface, primary LECs were treated with VEGF-C or Gal-8 and cross-linked with DTSSP cross-linker. Immunoprecipitate VEGFR-3 or PDNP, then probe with anti-integrin Ab.

3. To test whether Gal-8-induced transdifferentiation is occurred not just in a macrophage cell line, freshly isolated bone-marrow derived macrophages will be used in the Gal-8 treatment.

4. To test whether Gal-8-induced transdifferentiation is dependent on NF-κB pathway, macrophages will treated with Gal-8 in the presence of a NF-κB inhibitor, leptomycin B and the expression of VEGFR-3 and PDNP will be assessed by flow cytometry and Western blot.

SPP
Using non-lethal doses of SPP inhibitors in parasite culture, we will test whether there is an increase in the number of gametocytes. Furthermore, we will use a SPP-GFP construct to determine the percent decrease in activity as a result of the C-terminal GFP tag.

MSP1
Using a stable cell line expressing GPA we will perform a number of binding assays to determine whether MSP1-12 is binding to GPA. I have been optimizing in vitro binding assays analogous to those for erythrocytes, while also developing a cell binding adhesion assay, and a protein-cell cross-linking experiment.

RhopH3
RhopH3 experiments will only be performed as required by the journal review process.

Pf113
We will clone, express and purify the new larger phage sequence identified for Pf113. This larger clone will then be used for invasion assays to determine whether a larger portion of Pf113 can significantly inhibit parasite invasion. Following these assays the next step will be to specifically identify its putative erythrocyte receptor.

Write Manuscript by next meeting.

- Knockdown and add-back of STING in MOLF and B6 BMDMs followed by stimulation with CDNs – to show the STING dependent effects in parental cells which have great genetic variety.
- Blot for activation of canonical and non-canonical NFkB signaling components.

- Image STING mutant localization upon signaling.
- continue analysis of microarray.
- Begin in vivo infection studies to determine dependence on STING allele for survival of Listeria infection
  - including cytokine and lymphocyte proliferation profile of mice.