HCS The Histochemical Society Newsletter Vol. 29, No. 2

A Message from the Executive Director

Summer has arrived and I hope this newsletter finds our members enjoying the season. The first half of 2016 has been busy for the society with new programs and planning efforts for 2017.

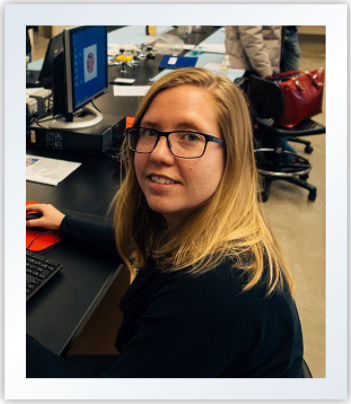
The 2016 meeting held in conjunction with Experimental Biology 2016 (EB2016) was a great success and the booth had a great deal of traffic with visitors from near and far, both old HCS friends and new. We were glad to have our community grow in San Diego with some awardees joining society committees – welcome Tirthadipa Pradhan-Sundd to the Publication Committee! Vector and Lillie awards, while a difficult decision between very highly qualified submissions, were well deserved by the recipients. The inaugural year of the HCS Capstone grants, led by the Education Committee, saw a great response and you will see more about the winners and their projects in this issue. 2017 planning continues and we already have a full program to be held in Chicago, IL at EB2017 with expanded programming to include a reproducibility workshop hosted by The American Association of Anatomists (AAA).

Efforts continue on the journal and society Facebook pages and the Communication committee has plans for the website in the coming year. Our partnership with the American Society for Investigative Pathology continues later this year at the 2016 PISA meeting (see their ad later in this newsletter). HCS continues its commitment to FASEB as a member society and discussions are underway for joint projects.

As always, I welcome our member's input – email jholland@faseb.org anytime with ideas or questions!

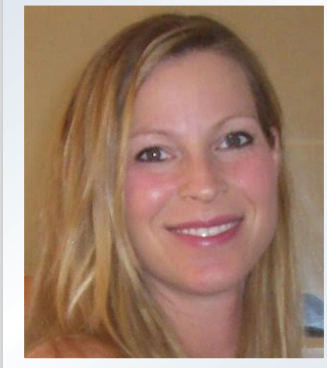
Woods Hole Travel Awardees

Evelina Sjöstedt



Evelina Sjöstedt has been a part of the Human Protein Atlas project (www.proteinatlas.org collaboration between Uppsala University and the Royal Institute of Technology in Sweden) since 2008. Initially, she started working with tissue handling and TMA construction and later focused on IHC analysis and antibody validation. After leading the antibody validation team a few years she started working as a part time PhD student but is now concentrating more on her ongoing research and projects. Her main projects focuses on antibody validation within the Human Protein Atlas, comparing IHC result with RNA-Seq data as well as investigating protein expression in the human and mouse brain. "The Immunohistochemistry and Microscopy course in Woods Hole was very useful for me. To have the opportunity to gather in a group with people from various backgrounds, but with a common interest in antibodies and IHC, was for me very encouraging and stimulating. It was nice to get in contact with people who are using similar techniques but aims to answer other types of questions within the research field. The fluorescence introduction was very valuable for me, since I normally work with chromogen staining methods, and I plan to include fluorescents based techniques in my thesis project. I got several questions answered during the course, but also questions produced which I now look forward to investigate further."

Jody Longo



My research at the Medical University of South Carolina (MUSC) focuses on cancer biology. During my time at MUSC, I have concentrated on understanding tumor evolution of Ras-driven tumors, as well as the development of targeted therapies through the use of next-generation sequencing, histology,

Jody Longo con't

mass spectrometry and mouse models. I began studying aberrant mechanisms of gene regulation in Glioblastomas (GBMs) downstream of the EGFR family in graduate school. However, studying the familial cancer syndrome Neurofibromatosis type 1 (NF1) has been the core of my more recent research efforts, but studying the biochemistry and molecular biology in both peripheral nervous system (PNS) and central nervous system (CNS) tumors is within my repertoire.

I attended the Immunohistochemistry (IHC) and Microscopy course in March of 2016 to expand my expertise in IHC. Immunohistochemical methods are critical for examining precious human samples for morphological abnormalities and protein localization patterns. IHC methods provide us with vital sub-cellular location data on our proteins of interest. Understanding the appropriate controls to perform in IHC methodology is essential for data interpretation. The IHCM course focused on teaching the foundation of appropriate controls as wells as basic IHC protocols. Significant effort was also placed on data acquisition on various instruments such as Spinning Disk Confocal, Single Point Confocal and DeltaVision microscopy systems, as well as, Brightfield image acquisition. A rooted understanding on how to choose the best instrumentation for your experimental question was stressed using both lecture based and hands-on learning. I found the design of the course highly effective in balancing out both lecture and lab time.

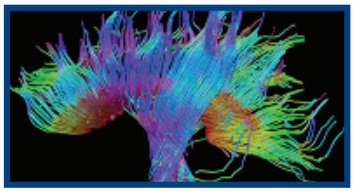
Completion of this course has given me confidence in data interpretation by teaching the appropriate critical controls to implement with each assay. The course has also expanded by research goals through education of the different microscopy systems out there. I would recommend the Immunohistochemistry and Microscopy course to anyone who utilizes microscopy and IHC techniques.

Entry Dates: July 7 — August 30, 2016

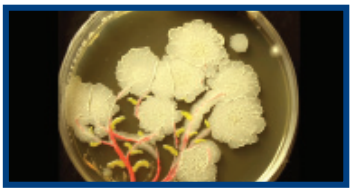
Entry Categories:

Fluorescence or Electron Microscopy Images

All Other Types of Images and Illustrations



Videos



Learn more and submit your entry at www.FASEB.org/BioArt

FASEB Report

The Federation of American Societies for Experimental Biology (FASEB) works with member societies to develop and promote policies to advance research and education in the biological and biomedical sciences. This year, the Federation developed a number of policies of interest to Histochemical Society members, including recommendations aimed to promote the reproducibility and transparency of biomedical research and efforts to recognize the importance of shared research resources.

The Federation launched the Shared Research Resources Subcommittee to lead efforts related to shared instrumentation, core facilities, and other resources that can be jointly utilized by research groups. The Histochemical Society's Immediate Past-President, **Charles Frevert, DVM, ScD**, serves on the subcommittee and on the FASEB Board of Directors.

Although the subcommittee is new to FASEB, it has been quite active. This spring, the group led efforts to aggregate information about federal agency support for apparatus acquisition, access, and development in a report called *Instrumentation: Federal Grants and Programs for the Life Sciences*. It also developed a statement affirming the importance of properly acknowledging shared resource facilities in scientific communications and issued a guide to strategies and best practices for resource acknowledgment.

Also this year, FASEB created opportunities to discuss new requirements for National Institutes of Health (NIH) grant applications to address research rigor and reproducibility. First, the Federation led a discussion about scientific rigor and reproducibility during its annual Science Policy Symposium. The conversation raised awareness of ways in which scientists can improve the reproducibility of reported research findings, particularly through cell-line authentication.

Following the success of the symposium, FASEB convened additional expert panels to discuss the challenge to rigor and reproducibility in scientific studies involving mouse models and antibodies. **Denis Baskin, PhD**, Executive Editor of *The Journal of Histochemistry & Cytochemistry*, participated in the roundtable on antibodies and helped develop recommendations to address challenges in diverse antibody-based methods, while **Charles Frevert** attended the roundtable on research using mouse models. In addition, *JHC* Editor-in-Chief **Stephen Hewitt, MD, PhD,** participated in the discussion on strategies to communicate consensus recommendations to community stakeholders.

The panel discussions culminated in a series of recommendations to help investigators navigate the new NIH guidelines, which took effect in January. The report, *Enhancing Research Reproducibility,* suggests actions for stakeholders across the research enterprise, including scientists, institutions, professional societies, journals, and federal agencies.

In other news, FASEB:

- Welcomed three new member societies. The Federation's membership to grew to 30 organizations with the addition of The Society for Experimental Biology and Medicine (SEBM), American Aging Association (AGE), and US Human Proteome Organization (US HUPO)
- Brought together nearly 50 scientists from 25 states – including **Charles Frevert** – for meetings with 100 congressional offices

FASEB report con't

for this year's Capitol Hill Day on March 3. Legislators and their staff expressed strong bipartisan support for NIH and discussed how federal funding for research benefits citizens in their states and districts

- Released up-to-date factsheets on the federal investment in research in states and districts in the U.S. The factsheets specify local funding from NIH, National Science Foundation, United States Department of Agriculture, and Department of Energy. The resources can be accessed on the FASEB website through a user-friendly map interface

*Pictured are **Tom Baldwin**, FASEB's Vice President for Science Policy, and FASEB Board of Director member **Charles Frevert** at the office of United States Senator Maria Cantwell (D-WA)*

Special meeting of the great HCS minds!

At the recent HCS 2016 meeting, a rare moment was captured as 6 of HCS's esteemed Past President's joined for a photo op.

An invaluable experience:
a junior faculty's view of the IHCM course

Francesca E. Duncan, PhD
Assistant Professor, Department of Anatomy and Cell Biology,
University of Kansas Medical Center

When I decided to attend the Immunohistochemistry and Microscopy (IHCM) course this past March at the Marine Biological Laboratory in Woods Hole, Massachusetts, several colleagues were surprised that I would elect to take five precious days away from my lab to take a course on immunohistochemistry (IHC) and microscopy. I was asked: "Don't you already know how to do those techniques?" or "Don't you have someone in your lab that can do it for you?"

I am a reproductive biologist, and although histology and IHC (primarily focused on the ovary) are central to my research and teaching endeavors, I had never received formal training in these areas. I wanted to learn about tissue processing methods and to understand how to optimize and troubleshoot protocols.

After five days of around-the-clock bootcamp led by leaders in the field, I left the IHCM course armed with just that knowledge and more. Through intimate group discussions, I learned the ins and outs of IHC and immunofluorescence – from the didactic theory to the practical details. These discussions were enhanced by hands-on laboratory experiences where, using mouse pancreas tissue sections, we performed colorimetric and fluorescence assays with glucagon and insulin-specific antibodies to detect islet alpha and beta cells, respectively. We then imaged our own slides using various types of microscopy – brightfield and fluorescence – on state-of-the-art equipment (Figure 1). Finally, we learned how to process images and generate figures according to the guidelines specified by the Journal of Histochemistry and Cytochemistry.

In the three months since attending this program, I have realized that this program has impacted my scientific career in tangible ways that I never imagined. I have highlighted some examples below that demonstrate the tremendous value of taking the time to just simply learn.

The IHCM course has made me:

- **A more effective teacher.** The IHCM course made me aware of the value of going back to the fundamentals. We must revisit the scientific method to improve the validity and reproducibilty of IHC data. As educators, we need to ensure (and lead by example) that we are training the next generation of scientists on the basic elements of experimental design – How is antibody specificity validated? What are appropriate positive and negative controls for each experiment? Although these may seem like obvious questions, they are often

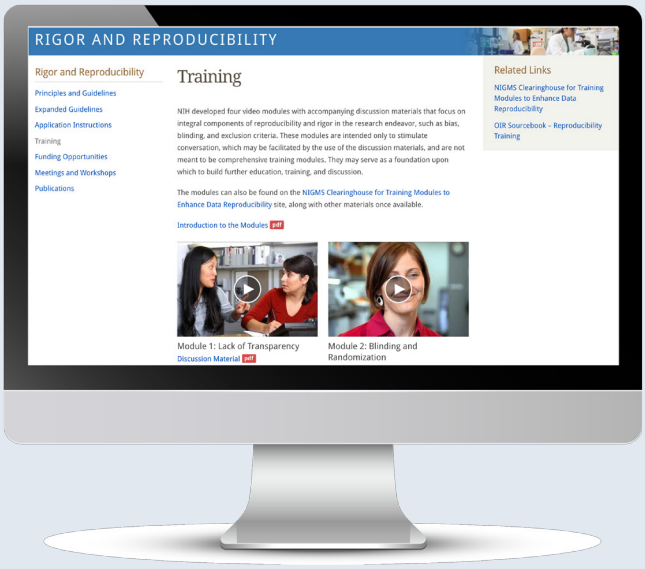
overlooked to the detriment of scientific rigor. I now consciously make sure that these fundamental concepts are integrated in each experiment performed in my own laboy and are emphasized in the graduate and medical school classes I teach.

- **A more attuned journal editor and ad hoc reviewer.** The IHCM course also introduced me to the Histochemical Society's guidelines on Controls for Immunocytochemistry (Hewitt SM et al, J Histochem Cytochem, 2014). I now use these guidelines as a foundation for critically reviewing manuscripts. I actively look to see that authors explicitly report experimental paradigm details, antibody validation methods, and use of appropriate controls in their manuscripts. These guidelines are an excellent resource, and I hope they will be adopted across reproductive science and medicine societies.

- **A scientist with new horizons. One of the most rewarding outcomes of the IHCM course was the opportunity for new collaborations.** During the IHCM course, I met Charles Frevert, DVM, ScD (course director) and Brian Johnson (instructor) who also run the University of Washington Histology and Imaging Core (UW-HIC). Since the IHCM course, I have been working with them to apply their automated IHC and image scanning services to ovarian tissue sections. We have validated proliferation, apoptosis, and vasculature markers in the ovary and obtained amazing images (Figure 2). This collaboration has greatly accelerated the pace and quality of my research and has also provided the UW-HIC with a new tissue to use as a positive control – the ovary!

Great Training Resource Videos

If you haven't seen these yet, take a look at these training videos posted by NIH. They present an excellent resource for many audiences. **CLICK HERE**



Go Figure ...

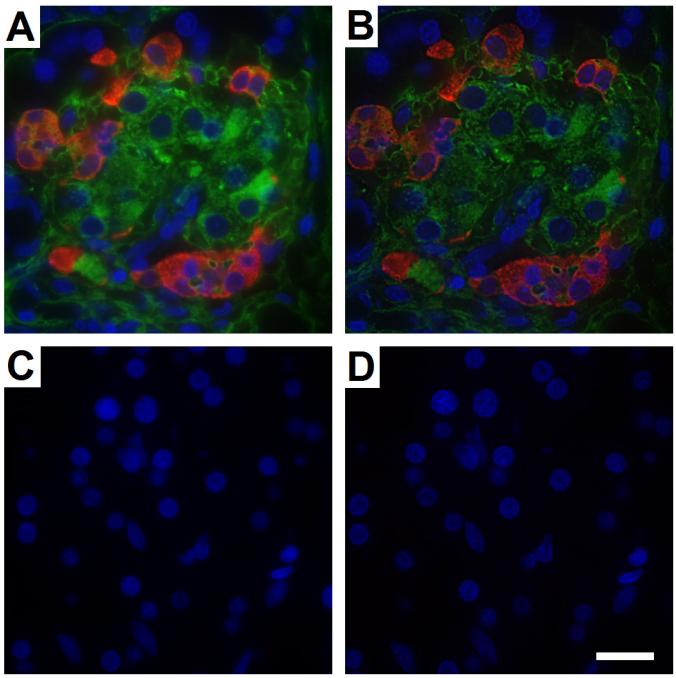


Figure 1. Immunofluorescence of insulin and glucagon in pancreatic islet cells.

(A, B) A representative image of a tissue section of a mouse pancreatic islet stained with antibodies against glucagon (α-cells) and insulin (β-cells). (C, D) are negative controls stained with non-immune IgG. Images were taken with a DeltaVision imaging system at the IHCM course. (A, C) are images taken prior to deconvolution and (B, D) are taken after deconvolution. Glucagon (red), insulin (green), DNA (blue). Scale bar = 25 μm.

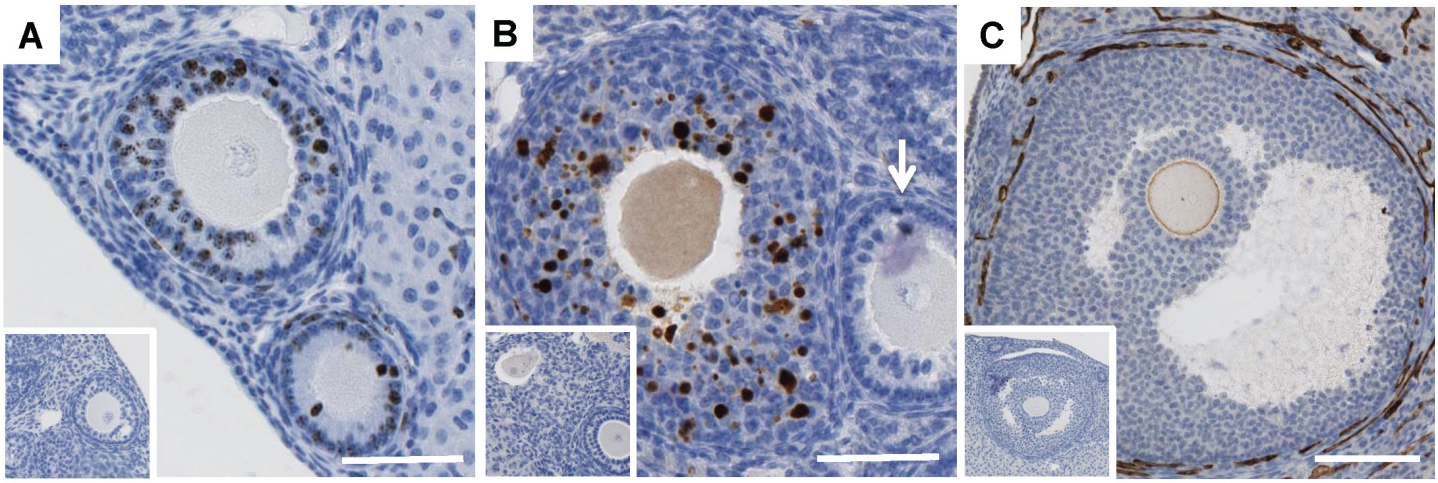


Figure 2. Immunohistochemistry of proliferation, apoptosis, and vasculature markers in the mouse ovary.

Representative images of ovarian tissue sections stained with antibodies specific to (A) Ki67 (showing proliferation in early growing follicles), (B) cleaved caspase 3 (showing apoptosis in an atretric follicle), and (C) CD31 (showing vasculature in the theca layer of an antral follicle). The arrow in (B) highlights a healthy follicle that is negative for cleaved caspase 3 staining. The insets are negative controls stained with non-immune IgG. Scale bar in (A,B) = 50 μm; (C) = 100 μm.

2016 HCS Vector Awardee:

Umesh Wankhade

University of Arkansas Medical Sciences

Abstract

Maternal Obesity Programs Offspring's Predisposition to Non-Alcoholic Fatty Liver Disease and Steatohepatitis

"I earned PhD in Human Nutrition, Food and Exercise from Virginia Tech. I investigated the role of hypothalamic transcription factor Nhlh2 in controlling energy homeostasis in mice. During the first stint of my post doc at NIDDK, NIH I explored the role of TGFbsignaling in adipose tissue biology. I was able to show that impaired TGFb signaling in adipose tissue promotes beiging/browning and improves metabolism in mice. At Arkansas Children Nutrition Center, UAMS we are interested in understanding the role of maternal obesity in shaping offspring's health and disease. I am investigating how gestational weight gain impacts offsprings predisposition to metabolic syndrome co-morbidities such as obesity and liver diseases under the mentorship of Dr. Kartik Shankar. I am also interested in understanding adipose tissue biology, especially how progenitor population in influences beiging potential of particular adipose tissue depot. I am pleased and truly honored to receive Vector Laboratories Young Investigator award from Histochemical Society. Getting recognized for the research work we do is the ultimate honor one can get in our field."

- Umesh Wankhade

2016 Lillie Awardee:

Tirthadipa Pradhan-Sundd,

University Of Pittsburgh

Abstract

Redundant role of catenins in maintaining tight junctional integrity

Lizzie's "just desserts"

HCS Travel Awardee in the news!

2016 HCS Travel Awardee, Elizabeth Stietzle was featured online recently! Join us in congratulating her!

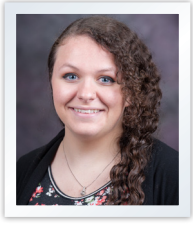
- K-State today
- CVM newsletter

Congratulations to the **2016 HCS Capstone Awardees**

Congratulations to HCS's inaugural year Capstone Grant awardees!

Vashendriya Hira, Academic Medical Center

Project Title: No place to hide: Force glioma stem-like cells out of their home, the niche, before chemo-irradiation.



Kaitlynn Bradshaw, Kansas State University

Project Title: Calcofluor White labeling of Pneumocystis fungi

MaRyka Smith, Kansas State University

Project Title: Mechanisms of Acute Kidney Injury in RVFV Infected Ruminant Tissues

Evolution of an R01 Grant: Survival of the Persistent... and Lessons Learned

By Gwen V. Childs, Ph.D.

Editor's note: Gwen Childs is Professor and Chair of the Department of Neurobiology and Developmental Sciences at the University of Arkansas for Medical Sciences. She is a Past-President of the Histochemical Society and is a member of The Journal of Histochemistry & Cytochemistry Editorial Board and reviews research grant proposals submitted to the NIH on the Reproduction, Andrology and Gynecology Study section. This document recounts her experience with the process of obtaining funding for a specific research grant from the NIH for her own research program.

In February, we learned that our NIH R01 grant proposal received a favorable review and a priority score of 14 at the second percentile. With a cut-off of 9th percentile, this grant proposal is likely to be funded. The concepts and data were originally derived from Aim 2 of an R01 that was funded in 2009. As we were celebrating we couldn't help but reflect on how we got to this point. It was clear from the beginning that the results obtained from our 2009 R01 were not going to be sufficient to completely sell the new directions emerging for this new RO1. However, they did provide ideas that were well received initially (score 27, 17%). The road to a fundable score was not smooth, as shown by scores and percentiles in Table 1.

TABLE 1

Iteration	Version	Date Submitted	Score	Percentile
1	A0	12/16/2013	27	17th
2	A1	4/16/2014	33	22nd
3	A0	8/16/2014	37	27th
4	A1	12/16/2014	26	12th
5	A0	4/16/2015	Triaged	---
6	A1	12/16/2015	14	2nd

In evaluating the data in Table 1, one needs to recognize that, whereas the same study section reviewed the grant each time, membership changed with each cycle because of ad hoc reviewers and expiration of terms of service. Therefore, it is likely that the wisdom of multiple sets of reviewers helped strengthen the proposal.

1. Exciting new ideas and concepts definitely need pilot studies backup and complementary expertise.

This is a common lesson that we teach any new grant writer. The first iteration of the proposal reported evidence that the adipokine leptin was important in the stimulation and maintenance of Gonadotropin releasing hormone receptors (GnRHR). Most unexpectedly, we had discovered that mechanisms behind leptin's actions may involve translational activation of GnRHR mRNA.1 This concept itself and supportive pilot studies merited a high impact score for Iteration 1 (Table 1). We had proposed translational regulatory mechanisms, and brought on board an expert in this area, Dr. Angus MacNicol, who became a Co-PI. Under his leadership, we proposed to test candidate microRNAs and a regulatory protein, Musashi (MSI) as regulators in leptin-mediated pathways. We also proposed to do RNA-seq to identify other candidate regulators. The study section recognized the novelty of these ideas throughout all iterations and applauded our unique complementary research team. They clearly needed to see more proof of principle, however. Thus, in the first iterations, we added EMSA to show MSI binding to GnRHR mRNA, qPCR data, which showed elevation of at least one candidate miRNA, and cytochemical evidence to show leptin regulation of MSI in gonadotropes. They didn't require that we do RNA-seq.

Evolution of an R01 Grant con't

2. Publications show positive external peer review and definitely save grant space!

Another common lesson we teach new faculty is to be sure and publish as soon as possible. Early reviews called for more experimental details, which required more space in the text of the proposal. Fortunately, our first 2014 publications[1,2] helped save space, as we could reference their Figures and Methods sections. Publishing in a well-accepted journal in your field also helped reviewers recognize that the work was being well received by others.

3. If you try to address every aspect of your animal model, your grant may become poorly focused.

In early iterations, we enthusiastically proposed studies of all phenotypic changes discovered during the previous funding period, including the metabolic deficiencies seen in the mutant males.[1] We also included exploratory studies in which we selectively deleted leptin in gonadotropes with a new floxed leptin model.[2] While these studies were considered "pioneering", they defocused the proposal. For the third iteration, reviewers stated that we were proposing the equivalent of 2 R01 grants and matched their concerns with a score that was lower than that of the previous iteration (Table 1). Our response in the A1 revision was to focus the grant on females and the infertility phenotype resulting in a score that was improved but above the 9th percentile funding cut-off for NICHD. So we forged on to Iteration 5.

4. As you prepare your significance and literature review, make certain you recognize pitfalls in published protocols.

This lesson actually underscores a new NIH requirement that we address rigor in grant proposals. It is now addressed when we discuss the "premise" on which our proposed studies are based. One of our proposed protocols involved the use of a Cre-reporter transgenic mouse to render gonadotropes fluorescent with eGFP. This would allow us to purify gonadotropes with fluorescent activated cell sorting (FACS). Reviewers of all iterations applauded this approach. It had been used by a number of laboratories (with different Cre-reporters) and therefore, we relied on their published studies to support feasibility. (We could not use our published, well-tested approaches because they depended on Gonadotropin releasing hormone receptors (GnRHR) and our mutant mice were GnRHR deficient.) So, we proposed to use the Cre-reporter transgenic mice and FACS purification of gonadotropes believing that the collective experience (by us and others) supported feasiblity. (Figure 1 is from the grant proposal).

2016 PISA

Houston Methodist Research Institute and the Marriott Houston Medical Center
Oct. 20-22, 2016 · Houston, TX

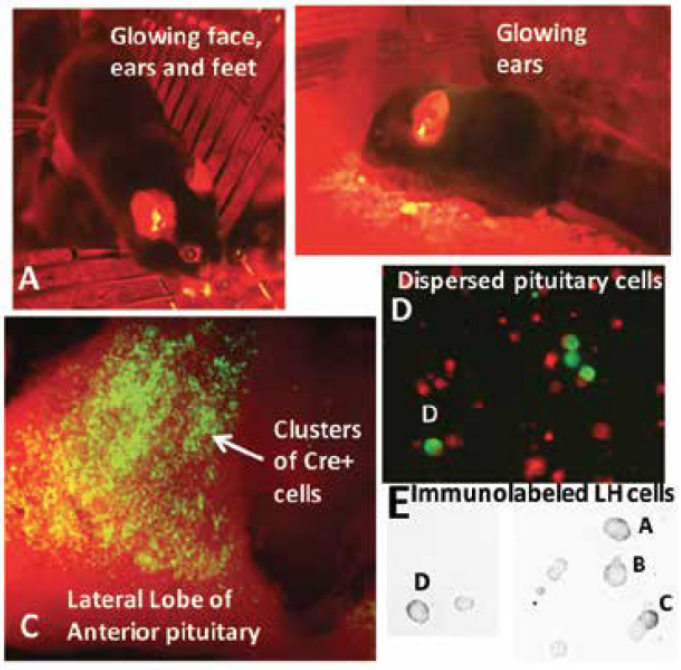
HCS Sponsoring PISA 2016 – Consider Attending!

HCS is pleased to support the 2016 PISA meeting organized by our partner's at ASIP. HCS is sponsoring the JHC Keynote Lecturer, Tanya Mayadas, PhD, Brigham and women's Hospital and Harvard Medical School, who will be speaking on Targeting Neutrophil Accumulation as a Therapy for Lupus Nephritis. **HCS members receive the discounted member registration!**

See more information about PISA 2016 HERE and mark your calendars

Evolution of an R01 Grant con't

Fig. 1. Successful development of Lhb-cre mice bearing Cre-reporter eGFP.



(A,B) Mice bearing Gt(ROSA) 26Sortm4(ACTB-tdTomato,-EGFP)Luo /J (The Jackson Laboratory) show tdTomato fluorescence (visualized by NIGHTSEA Dual Fluorescent Protein Flashlight) in the face, ears, and feet, indicating the presence of at least one allele of tdTomato-eGFP. (C) Whole pituitary from an Lhb-cre mouse showing a mass of red cells and clusters or cords of Cre-bearing cells that express eGFP (green). (D) Dispersed pituitary cells showing tdTomato-bearing red cells and Lhb-cre+ cells fluorescent for eGFP, thus marking the gonadotrope cell population. (E) Bright-field image showing immunoperoxidase labeling for LHβ in all of the eGFP+ cells (A–D) shown in (D).

What we did not realize was that no one had ever cultured and transfected FACS-sorted gonadotropes, as pointed out in the review of the triaged 5th iteration. Email from an experienced colleague confirmed that, to date, the yield of FACS-sorted gonadotropes was so low (3,000-5,000 cells/pituitary), that they were not used for more than cytochemistry or possibly qPCR assays of mRNA. Fortunately, our recent experience with high yields of FACS purified somatotropes with the same Cre-recombinase reporter mice allowed us to meet this challenge. Considering the low yields reported in the literature, we were surprised and pleased to see relatively high yields of >45,000 pure gonadotropes/mouse (as high as 66,000 gonadotropes). These yields produced protein and mRNA extracts that were sufficient for assays of all pituitary hormones and GnRHR. Thus, for the 6th iteration, we were able to report high yields and meaningful data. We also redesigned experiments so we were not as dependent on FACS-sorted cells for transfection studies. The score for the 6th iteration (Table 1) shows the study section's very positive response.

5. If you have a model for a human disease, it needs to be severe enough to raise significance levels.

In studies for Aim 2 of our original 2009 R01 grant, we had ablated the signaling domain of the leptin receptor, producing a subfertility phenotype in the females (delay in pregnancy and low pup count). Reviewers of the 5th iteration believed that this phenotype was mild. However, in all iterations, we had proposed to use a new model in which all isoforms of the leptin receptor were ablated in gonadotropes, hypothesizing that it would be a more severe phenotype. But, as the reviewers of iteration 5 correctly pointed out, we had not yet shown fertility data for this new model. Thanks to pilot studies begun in early 2015, we were able to produce and test these mice and proved our hypothesis correct. We demonstrated significant infertility in the mutant females. These data were put in the 6th iteration and received very positively (Table 1).

Chicago, IL, USA · April 22-26, 2017

HCS/EB2017 ANNUAL MEETING

COMING SOON!
SEE THE 2017 SPEAKER PROFILES

HCS PRELIMINARY MEETING PROGRAM
MONDAY, APRIL 24TH, 2017

HCS/ASIP SYMPOSIUM: Imaging signaling in vivo – from cell biology to animal models

Symposium Chairs: Douglas L. Rosene, Ph.D. and Margarida Barroso, Ph.D.

8:30 - 9:10 am — Imaging Molecular Dynamincs In Vivo, Kurt Anderson, Francis Crick Institute

9:10 - 9:50 am — Mechanisms of Membrane Remodeling in Live Animals by Intravital Microscopy, Roberto Weigert, NIH

9:50 - 10:30 am — Title TBA, Irina Larina, Baylor College of Medicine

Journal Of Histochemistry & Cytochemistry Lecture

Symposium Chair: Stephen M. Hewitt MD, Ph.D

10:30 - 11:30 am — Illuminating Biochemical Activity Architecture of the Cell, Jin Zhang, University of California, San Diego; The Johns Hopkins University School of Medicine (Adjunct)

HCS/AAA Workshop: Reproducibility in Experimental and Preclinical Research

Workshop Chairs: Charles W Frevert, DVM, ScD and Stephen M. Hewitt MD, Ph.D

Antibodies: The good, bad, and ugly, Denis Baskin, Ph.D, University of Washington

Contaminated and misidentified cell lines as a cause of irreproducible results, Speaker TBA

Animal Models for Study of Human Disease: Increasing Reproducibility and Considerations for Translatability, Charles W. Frevert, DVM, ScD

Round Table Discussion: The Role of Journals and Societies in Improving Reproducibility in Research, Stephen M. Hewitt, MD, Ph.D

6. A relatively high impact score does not guarantee success in the next submission.

This lesson shown dramatically in Table 1 was probably the hardest to explain to our young scientist colleagues. There are no guarantees that a highly scored grant will be equally well received as a revision, let alone better received. This is true even if the proposal goes back to the same study section. Different sets of reviewers may have new concerns. Also, as seen in Table 1, half of the submissions were officially new or A0 versions of the grant. The A0 version has no official "history" and you are not allowed to refer to previous A1 versions to show where you improved it, nor do reviewers see previous summary statements to learn if you were responsive.

In addition, a high impact score may carry mixed blessings as it presents fewer concerns to guide you in making revisions that ultimately may be required. In other words, if you only focus on the reviewers' few concerns for that high-scored proposal, the grant may not be revised sufficiently to satisfy a new set of reviewers in the next round. You can even be lulled into resubmitting prematurely, thinking your proposal is moving on a positive trajectory. However, for us, one advantage of high impact scores was the edge it gave for leveraging pilot studies grants from internal sources. Our 17th or 12th percentile scores impressed pilot study reviewers, producing funds that made the much-needed breeding studies possible. Without those funds, our story would have ended much differently.

7. The lowest scores and harshest reviews may provide the best information for the evolution of the proposal.

As stated previously, review of the third iteration indicated that our proposal was so poorly focused that it looked like at least 2 R01 proposals. This observation transformed the focus of our proposal and moved the score for the revision to 26 at the 12th percentile. Furthermore, the "triaged" 5th iteration of our proposal mobilized our efforts to produce FACS-sorted gonadotropes and determine that the females in the new model were infertile. Fortunately, we had the pilot funding by then to support the breeding studies that were needed.

8. Don't count a grant out because it is triaged!!!

This last lesson is for all Deans' and Chairs' offices, who are helping faculty evaluate responses to roller-coaster grant reviews. A triage needs to be evaluated in light of previous scores (if available), the comments of the reviewers, and the criterion scores. As stated above, in our response to the triaged grant, we were able to show data from FACS-sorting and our new mutant females that impressed the reviewers. The summary statement applauded our "exceptional response" to the review of the previously triaged grant.

In reviewing these lessons, we have to ask ourselves if we could have avoided this number of iterations. Could we have reached a fundable impact score sooner and, if so, what would it have taken? I think that the need for key pilot data on the mechanistic aspects (regulatory factors) was obvious and that was available early in the game. So, some of the most high-risk/high pay-off parts of our proposal (e.g., the mechanistic aim) seemed to be feasible and

acceptable. These were the obvious things we tackled immediately. However, we didn't fully appreciate the serious problems with the FACS purification of gonadotropes nor did we realize that the perceived mild subfertility phenotype might reduce the significance for at least one group of reviewers.

This experience from a well-seasoned group of grant writers shows that it may take more than 2 years to get an R01 grant. The early iteration reviews had focused on proof for our mechanistic studies and a better overall focus. So perhaps we were led to believe early on that the full development of the mouse and cellular models could be done once the proposal was funded. This unanticipated challenge clearly took extra time and money to prove infertility and produce mice bearing fluorescent gonadotropes. Considering the diversity of the opinions from the review panel, it is not obvious that any of the iterations was wasted effort that could have been avoided.

We now recognize that the real lesson might be to always do more than the reviewers expect. Overall, the most important take-home message is that we were persistent as we worked with the reviewer concerns that ultimately improved feasibility. Our "Gonadotrope R01" Research team is now excited, thrilled, and happy to actually be able to do this study.

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2016 PISA

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Registration Deadline: September 29

Thursday, October 20, 2016

- **Registration / Breakfast / Visit the Exhibits**

- **KEYNOTE LECTURE - Journal of Histochemistry & Cytochemistry Lecture: Targeting Neutrophil Accumulation as a Therapy for Lupus Nephritis**
  Tanya Mayadas, PhD, Brigham and Women's Hospital and Harvard Medical School
  Sponsored by the Histochemical Society

- **The Inflammatory Interface: The Force Awakens**
  Chairs: Cary D. Austin, MD PhD, Richard N. Mitchell, MD PhD, William A. Muller, MD PhD
  - **Age-Related Macular Degeneration: Evaluating the Role of Complement as a Therapeutic Opportunity in Geographic Atrophy**
    Lauri Diehl, DVM PhD, Genentech, Inc.
  - **Break**
  - **Leukocyte/Endothelial Cell Signaling During Transmigration**
    William A. Muller, MD PhD, Northwestern University Feinberg School of Medicine
  - **Host-Pathogen Interactions**
    David Walker, MD, University of Texas Medical Branch - Galveston
  - **Abstract-Driven Short Talks**

- **Lunch / Posters / Visit the Exhibits**

- **Microbiome and Disease**
  Chairs: James M. Musser, MD PhD & Cecelia C. Yates, PhD
  - **Host-Microbiota Interactions at the Epithelial Interface**
    Andrew Neish, MD, Emory School of Medicine
  - **Manipulating Gut Microbial Metabolism and Intestinal Inflammation**
    James Versalovic, MD PhD, Texas Children's Hospital and Baylor College of Medicine
  - **The Search for Correctible Pre-Disease States of the Microbiome**
    Jonathan Braun, MD PhD, UCLA Geffen School of Medicine
  - **Abstract-Driven Short Talks**

- **Welcome Reception / Posters / Visit the Exhibits**

Friday, October 21, 2016

- **Registration / Breakfast / Visit the Exhibits**

- **Career Development Workshop - Dancing with Journals: The Mechanics of Publishing Your Research**
  Kathryn Stockbauer, PhD, Manager, Academic Development, Houston Methodist Hospital
  Sponsored by the ASIP Committee for Career Development and Diversity

- **ASIP Young Scientist Leadership Award Lecture: Approaching Amyotrophic Lateral Sclerosis from Cellular, System and Technological Angles**
  Christi Kolarcik, PhD, University of Pittsburgh

- **Cell-Cell Communications**
  Chairs: Anirban Maitra, MBBS, Christopher A. Moskaluk, MD PhD, William Stetler-Stevenson, MD PhD
  - **Exosomes in Pancreatic Cancer Therapy and Diagnosis**
    Raghu Kalluri, MD PhD, MD Anderson Cancer Center
  - **Break**
  - **Exosomes and Extracellular RNAs in Cancer Metastases**
    Dihua Yu, MD PhD, MD Anderson Cancer Center
  - **Engineered Systems to Control Cell-Cell Communication**
    Biju Parekkadan, PhD, Harvard Medical School
  - **Abstract-Driven Short Talks**

- **Lunch /Visit the Exhibits**

- **ASIP Rous-Whipple Award Lecture: Molecular Triggers Underlying Pandemics Caused by Group A Streptococcus, the Flesh-Eating Pathogen**
  James M. Musser, MD PhD, Houston Methodist Hospital

- **Poster Discussion Breakouts**
- **ASIP Scientific Interest Group (SIG) Meetings**

Saturday, October 22, 2016

- **Registration / Breakfast**

- **Mucosal Pathobiology**
  Chairs: F. William Luscinskas, PhD, Asma Nusrat, MD
  - **Metabolic Shifts and Innate Immune Responses in the Gut**
    Sean Colgan, PhD, University of Colorado - Denver
  - **Break**
  - **Microbiome, Immune Function, and Pulmonary Disease**
    Nicholas Lukacs, PhD, University of Michigan
  - **Abstract-Driven Short Talks**

- **Awards Presentations and Business Meeting**
- **Meeting of Scientific Interest Group (SIG) Chairs**
- **Adjourn**

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