# A.B.S.T.R.A.C.T.

Annals of Biomedical Student/Postdoc Training, Research, and Current Topics

ISSUE 2, SPRING 2008




INSIDE THIS ISSUE:



## The Top Ten by Ashley Brady and Susan Ivie

**1. Brown, Christopher** (Graduate Student, Dept. of Biological Sciences, Funk Lab): President's Prize Runner-up for Best Talk at the 2007 Entomological Society of America's Annual Meeting

**2. Buckholtz, Joshua** (Graduate Student, Dept. of Psychology , Lab): Vanderbilt Law and Neuroscience Research Prize (Inaugural Recipient), Vanderbilt University, 2008 (award for best research proposal in Law and Neuroscience Class) and Early Researcher Award; American Psychological Association, 2007 (Award for best interdisciplinary psychology research as a graduate student)

**3. Egan, Scott P.** (Graduate Student, Dept. of Biological Sciences, Funk Lab): Hickory Stick Award for Outstanding Teaching in the Dept. of Biological Sciences, Vanderbilt University.

**4. Holinstat, Michael** (Postdoctoral Fellow, Dept. of Pharmacology, Hamm Lab): Awarded K99/R00 Pathway to Independence Award from NHLBI

**5. Knutson, Sarah** (Graduate Student, dept. of Biochemistry, Hiebert Lab) Postdoctoral fellowship, Novartis Institutes for BioMedical Research, Cambridge MA.

**6. Jayagopal, Ashwath and Chinmay Soman** (Graduate Students, Biomedical Engineering, Haselton Lab (Jayagopal) and Interdisciplinary Program in Materials Science, Giorgio Lab (Chinmay): 2nd Runner Up, Nano Nexus Business Plan Competition, organized by Oak Ridge Laboratory.

**7. Levin, Scott** (Graduate Student, Dept. of Biomedical Engineering): Accepted a position as an Assistant Professor in the School of Medicine at Johns Hopkins University in the Department of Emergency Medicine.

**9. Lowe, John S.** (Graduate Student, Dept. of Pathology, Mohler Lab): Invited Oral Presentation: American Heart Association Scientific Sessions (Orlando, FL 2007) (Ion Channel Trafficking and Regulation Section) *Cardiac voltage-gated $Na_v$ channel $Na_v1.5$ requires an ankyrin-G-dependent pathway for targeting in cardiac myocytes.*

**10. Ryckman, Kelli (**Graduate Student, Center for Human Genetics Research, Williams Lab)Invited Talk: American Society of Human Genetics Meeting, San Diego, CA, Title: Racial differences in genetic association of cytokine concentrations in the presence and absence of bacterial vaginosis, October, 2007.

**11. Shirey, Jana K.** (Graduate Student, Dept. of Pharmacology, Conn Lab): Paper: Shirey, J.K., *et. al.*, An allosteric potentiator of $M_4$ mAChR modulates hippocampal synaptic transmission. *Nature Chemical Biology* 4(1):42-50, 2008.


Sponsored by: the Office of Biomedical Research, Education and Training (BRET)

# Spotlight Scientist by Erin Kristobak

*"Alcázar-Román hopes to merge his scientific career with his passion for outreach by establishing scientific ties between his native Peru and the biological research community in the United States."*

Abel Alcázar-Román, a recent Ph.D. graduate of the Cell and Developmental Biology Graduate Program, has provided significant contributions to an emerging field of research focused on the regulation of gene expression by soluble inositol polyphosphates. He defended his thesis "Inositol Hexakisphosphate and Gle1 Direct mRNA Export by Spatially Regulating the DEAD-box Protein Dbp5 at the Nuclear Pore Complex" on March 3, 2008.

Photograph by Robin Marjoram

Abel grew up in Lima, Peru, where he developed an interest in biology and decided to begin his studies at Lipscomb University in Nashville. While completing his major in Biology and participating in an NSF-Research Experience for Undergraduates (NSF-REU) program, his enthusiasm for research was solidified, and he decided to attend graduate school to pursue a research career.

Alcázar-Román began his graduate studies at Vanderbilt in 2002 in the Interdisciplinary Graduate Program. After graduating from Lipscomb and before starting at Vanderbilt, however, he had the opportunity to return home to Lima for a ten month visit. Having a special affection for his home country, Abel decided to make the trip, knowing it would be the last time he could spend a significant amount of time there before his career in science began.

In the Spring of 2003, Alcázar-Román joined the lab of Susan R. Wente, Professor and Chair of the Department of Cellular and Developmental Biology. The Wente Laboratory investigates nuclear import and export of macromolecules through the nuclear pore complex (NPC). Correct directionality of transport for large molecules such as proteins and messenger RNA (mRNA) is crucial for gene expression and cell function. Although the transport of proteins into and out of the nucleus has been extensively characterized, less is known about how mRNA is transported from the nucleus of the cell to the cytoplasm.

Prior to Alcázar-Román's studies, the process by which mRNAs get to the NPC had been investigated, but the trafficking of the mRNAs from the NPC to the cytoplasm was poorly understood. When mRNAs are transcribed, RNA binding proteins bind to the nascent chain forming messenger-ribonucleoprotein particles (mRNPs). Directionality of transport of the mRNAs is associated with the composition of the proteins in the mRNPs. Certain RNA binding proteins, which only function in the nucleus, must be removed for the mRNP to exit the nucleus. It is thought that proteins of the DEAD-box helicase family help mediate necessary changes in mRNP complexes. One of the DEAD-box helicase proteins, Dbp5, interacts with mRNPs and the NPC and was hypothesized to mediate mRNP remodeling as the mRNA leaves the nucleus though the NPC.

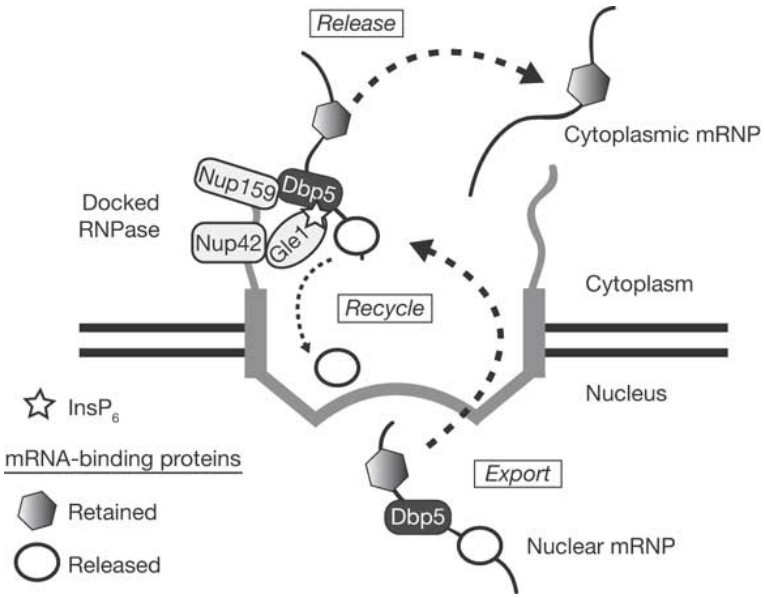
In the Wente Laboratory's investigation of mRNA export, they had discovered that deficiency of a certain inositol polyphosphate, inositol hexakisphosphate ($IP_6$), resulted in a mRNA export defect in yeast. In addition, the lab discovered another molecule necessary for mRNA export, a protein known as Gle1, which is localized to the NPC. Upon entering the lab, Alcázar-Román set out to investigate these phenomena and help decipher the mechanism of mRNA export from the NPC. Employing a genetic screen in yeast and many biochemical assays, he identified the genetic target of $IP_6$ and determined the functions of Gle1 and $IP_6$ in this crucial cellular function.

In a *Nature Cell Biology* paper published by Alcázar-Román in July 2006 (8:711-16), he described the mechanism of Dbp5/Gle1/$IP_6$-mediated mRNA export at the NPC (see Figure 1 and legend). He discovered that, like RanGTPase-mediated protein transport across the NPC, mRNA trafficking is also regulated by a nucleotide triphosphate to nucleotide diophosphate switch. The Wente Laboratory later confirmed that the ADP-bound form of Dbp5 displaces proteins from mRNA, while the ATP-bound form does not. Localized on the cytoplasmic side of the NPC, $IP_6$ and Gle1 activate Dbp5 ATPase activity allowing for the proteins to be released specifically on the cytoplasmic side of the NPC. The homologues of Gle1 and and Dbp5 are conserved in human cells and the Wente Laboratory believes that $IP_6$ may also have a similar function in human physiology. These exciting findings, along with two previous papers Alcázar-Román helped publish in the Wente lab, have provided significant insight into mRNA nuclear export.

Alcázar-Román presents a new theory in his recent review on gene regulation by inositol polyphosphates (*Chromosoma* (2008) 117:1-13.) These molecules are known to be involved in several processes: regulating chromatin remodeling, telomere length, transcription, and mRNA editing. Even though $IP_6$ deficiency resulted in an mRNA export defect, yeast continued to grow at a wild type rate. This indicates that export of all nuclear transcripts may not require $IP_6$. Instead, Alcázar-Román hyposthesizes that different populations of mRNAs require specific molecules for export, such as $IP_6$, which are not needed by all mRNAs. Therefore, regulation of gene expression may not only occur at the levels of mRNA transcription, processing, splicing, and protein translation, but at the level of mRNA export as well. Alcázar-Román's current work in the Wente lab will investigate this hypothesis.

This June, Alcázar-Román will start a post-doctoral fellowship with Pietro de Camilli, a Howard Hughes Medical Institute investigator in the Department of Cell Biology at Yale University. Focused on studying the formation and traffic of synaptic vesicles, the lab applies various approaches to investigate the cellular machinery and signaling pathways which regulate this process. Interestingly, de Camilli is currently investigating the regulation of vesicle recycling by inositol phospholipids. Alcázar-Román will join the de Camilli Laboratory's effort to understand this complex transport process by analyzing the formation of synaptic vesicles at nerve termini and applying his studies of cellular trafficking regulated by inositols,

In the future, Alcázar-Román hopes to merge his scientific career with his passion for outreach by establishing scientific ties between his native Peru and the biological research community in the United States. He knows that there are many in Peru who are very interested in scientific research, but the funding and equipment available in labs in the country can limit their opportunities. Alcázar-Román would like to foster relationships between universities in Peru and the United States to serve as a bridge for these eager Peruvian scientists to take part in the many research opportunities available in the United States.




**Schematic representation of how Gle1–$InsP_6$ activation of Dbp5 may trigger mRNP remodelling at a NPC cytoplasmic-face scaffold.**
Dbp5 associates with nascent transcripts in the nucleus and is recruited to the cytoplasmic NPC fibrils by Nup159 interaction. This juxtaposes Dbp5 and Gle1 — docked to the NPC through Nup42 — allowing maximal $InsP_6$ binding and activation of the Dbp5 ATPase activity. This activity may facilitate the release of mRNA binding proteins, or export factors, from the exporting mRNP. Triggering release and recycling of select mRNA binding proteins would impart directionality by shifting the mRNP preferentially to the cytoplasmic mRNP state during a terminal export step.

# Business News by Kevin Seale

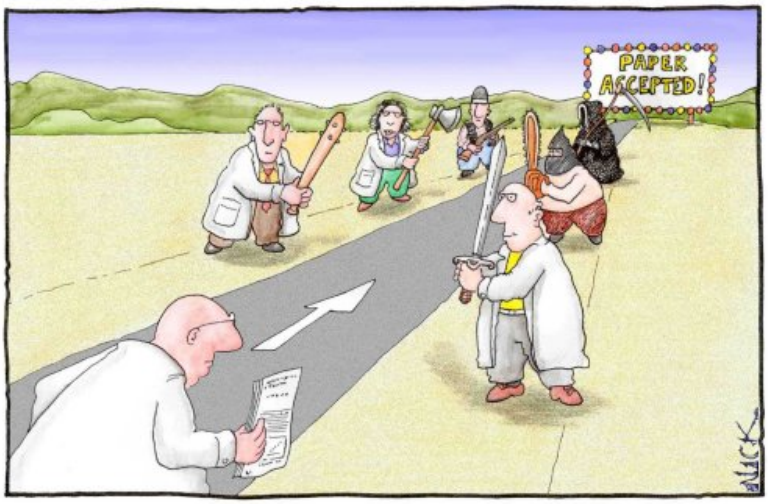
## Peer Review and the Publication of Science

Peer review – the evaluation of significance and scientific merit by your peers – is a process common to the selection of research grants for funding as well as research manuscripts for publication. Both often utilize a two-tier system of triage and careful peer review in order to relieve reviewer burnout, thereby making the workload manageable and optimizing selection. However, both systems are flawed and in major need of overhaul[1]. A peer reviewed journal with wide readership can be expensive to implement and can lead to high subscription costs that may exclude some readers. This has led some scientists to call for open access to scientific literature. Recent studies of peer review and open access have highlighted the challenges and offered suggestions for change.

On February 28 of this year, an NIH advisory committee to the director (ACD) completed a peer review self-study[2] and made recommendations for improvement that are to be implemented this spring. Seven major challenges were highlighted including the need to reduce the overall administrative burden of peer review and to optimize the funding of alternative, transformational approaches to conducting science. This study follows closely on the heels of a new policy which stipulates that NIH-funded research be made freely available in PubMed Central, a policy which has drawn criticism from Senator Arlen Specter of the US senate appropriations committee. Taken together, the NIH access policy and the peer review overhaul seem to indicate that NIH Director Elias Zerhouni would like to see a change in the way the agency's research is funded and communicated.

Peer review of research grants and peer review of scientific manuscripts may be somewhat linked, since it is plausible that research grants from well-published authors are more likely to receive positive reviews. This is a well-known problem for young researchers without a large publication track record; a problem possibly worsened by the fact that the peer review mechanism is flawed. In a peer-reviewed article about peer review, Neff and Julian[3] developed a statistical model of a two-tiered peer review system and discovered that the system inevitably results in some amount of error. That is, the rejection of suitable papers and acceptance of unsuitable papers is unavoidable and can be significant depending on the exact structure of the review process. In a recent book, former editor of the British Medical Journal Richard Smith asserts that peer review is flawed, and cites his own scientific studies of the process[4]. For good quality control, both studies recommend an editorial pre-review followed by unanimous agreement on the manuscript's suitability among multiple independent referees. Dr. Smith also suggests open web publishing following editorial pre-review as well as ranking by online readers as the ultimate measure of publication quality.

*"Peer review as a means of quality control is often overrated. Even the most prestigious journals are not immune from fraudulent science."*




Most scientists regarded the new streamlined peer-review process as 'quite an improvement.'

ACS Chemical Biology, "Experimenting with Peer Review"

Direct web publishing of some type may be the wave of the future. Grigori Perelman published his proof of the Poincaré conjecture (a 100-year old math problem) on the physics preprint server (www.arXiv.org), a repository that is not peer reviewed. He has since won the Field's Medal in mathematics for the work. When the paper was criticized for not being peer reviewed, Perelmen responded "if anybody is interested in my way of solving the problem, it's all there [on the arXiv] - let them go and read about it." Harvard Arts & Sciences faculty unanimously agreed last month to have all their completed scholarly work appear on an open access website. Although "completed" presumably means peer-reviewed, it is a step in the direction of more speedy, less expensive distribution of knowledge. Certainly, publishing accurate scientific results without peer review would remove a heavy burden from the backs of both the author and the reviewer, although there would almost certainly be a decrease in the quality of some scientific publications.

However, peer review as a means of quality control is often overrated. Even the most prestigious journals are not immune from fraudulent science[5]. Peters and Cici resubmitted fourteen recent psychology articles to the journals that originally published them and discovered that 89% of the articles were rejected on scientific grounds, with no indication from the editors or referees that the papers were recognized as having been already published[6]. In 2005, three MIT students wrote a computer program that produced a nonsensical paper which was accepted to an artificial intelligence conference after peer review[7]. Physicist Jan Hendrik Schön performed the miraculous feat of authoring or co-authoring a peer reviewed publication every eight days in 2001. Ultimately 25 of his papers were suspected to be fraudulent and many were retracted. There are many disturbing publishing practices that fall short of outright fraud that survive peer review, however. The Committee On Publication Ethics (COPE) categorizes ethical issues in scientific publications on its website, including duplicate and redundant publications, ghost and gift authorship and editorial and reviewer misconduct[8].

There are 27,015 peer-reviewed scientific journals in existence, a fraction of which are indexed by various commercial databases and search engines. With more than two new articles appearing every minute, any mechanism of effective quality control based on "expert review" is severely limiting. Considering the quantity of information being generated, the internet seems like an obvious alternative as a vehicle for the codification of knowledge. The incredible popularity of sites like Google and Wikipedia are a testament to their own usefulness, but scientists are extremely reluctant to part with anonymous peer review in favor of an open discourse on the World Wide Web. However, the world's youth may have already turned to the internet as a primary fact source – as evidenced by bans some colleges have placed on the use of Wikipedia as a sole source for research papers. Out of concern for accuracy, Wikipedia co-founder Larry Sanger has begun another website: citizendium.org - a sort of hybrid between wikipedia and peer-reviewed publications that aims at credibility rather than quantity. Age-old and creeky, anonymous peer reviewed journals face a serious challenge to be faster, more accessible and more accountable if they are to be heard above the din and hum of the internet.

1. Chubin D.E., and Hackett, E.J. (1990) *Peerless Science: Peer review and U.S. Science Policy.* Albany: SUNY press
2. http://enhancing-peer-review.nih.gov/meetings/NIHPeerReviewReportFINALDRAFT.pdf
3. Neff, B.D.; Olden, J.D. Is Peer Review a Game of Chance? BioScience, Volume 56, Number 4, April 2006, pp. 333-340(8))
4. Smith, R., (2006) *The Trouble With Medical Journals*. London: Royal Society of Medicine Press Ltd.

5. Hwang, W. S. et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. Science 303, 1669–1674 (2004). (Retracted)

6. Douglas P. Peters and Stephen J. Ceci, Peer-review Practices of Psychological Journals: The Fate of Published Articles. Submitted Again J. Behavioral and Brain Sci., 187 (1982).
7. Rooter: A Methodology for the Typical Unification of Access Points and Redundancy, Jeremy Stribling, Daniel Aguayo, and Maxwell Krohn Accepted by WMSCI 2005. -- PS | PDF | Bibtex, Also published in The Journal of Irreproducible Results, vol. 49, no. 3, pg 5-8, May 2005.
8. http://www.publicationethics.org.uk/cases

# Current News by Rachel Roberts-Galbraith

## The Plague of Plagiarism in Scientific Publication

When one reads a scientific article, a certain trust is placed in the authors–that experiments were done as described, that the data presented are accurate, and that the words used to describe the work are the authors' own. While this trust is almost always warranted, lapses of scientific integrity including, data falsification, data fabrication and plagiarism, undermine this basic trust. High profile cases have highlighted incidents of data fabrication and falsification, but plagiarism has been identified only infrequently. Now a recent work in the journal *Bioinformatics*[1] has attempted to quantify the prevalence of plagiarism in scientific publishing.

Plagiarism, defined by the Office of Research Integrity as "the appropriation of another person's ideas, processes, results, or words without giving appropriate credit,"[2] is academic theft, a form of misconduct equal in severity to data falsification and fabrication. In addition to betraying the trust of the reader, plagiarism inflates the publication and citations records of the authors, sometimes at the expense of honest research scientists. Because no accurate measurement existed on the incidence of plagiarism in scientific literature, Errami, et al[1] undertook a statistical sampling of Medline article titles and abstracts and developed search engine software to examine textual overlap. The authors found that 0.04% of abstracts shared high similarity despite having no authors in common and many of these overlapping articles may be cases of plagiarism. Extrapolating from this percentage to all Medline literature, the authors estimate that about 3500 articles could be plagiarized, with about 200 additional plagiarized articles added each year. This estimate demonstrates the abundance of plagiarism in scientific publishing, but may at best underestimate the problem, as 1) only abstracts were searched due to a lack of availability of full text articles, 2) "smart duplication" involving rewording would not be detected, 3) abstracts from non-English journals could not be searched, and 4) either old or otherwise unavailable abstracts could not be searched. However, this was the most comprehensive study of plagiarism in scientific publishing to date and identified several trends with plagiarized works, including an above-expected number of shared citations and a tendency for highly similar works to appear as one another's "most related article" in Medline. The authors are currently using these trends to hasten their search for instances of plagiarism[3].

***"Plagiarism detection software has been applied to full length papers on the physical sciences server arXiv, resulting in the discovery of almost 70 plagiarized papers."***

Remarkably, though many journals have adopted strict standards for acceptable forms of data processing and manipulation[4], few have confronted plagiarism with the same urgency. In fact, *Nature* Publishing Group is one of the few to have an easily found formal policy on plagiarism[5], which states that each accused act of plagiarism will be investigated. If plagiarism were found prior to publication, a manuscript would not be considered. If plagiarism were found after publication, the publishing group would contact the institutions that employ and fund the authors and either mark all instances of plagiarism in the online copy of the paper or consider formal retraction. Other journals do not publicize formal plagiarism policies; for example, no policy is available on the *Cell* Press website, and a representative of the journal declined to comment for this article.

One limitation of journals in confronting plagiarism is that they are unable to compare submitted papers to the bulk of work published in other journals. Whereas a single journal may only have access to its own archive, a publisher like Elsevier, the largest publisher of scientific literature (including *Cell*, the *Trends* journals, and others), would have far greater access and a greater opportunity for responsibility in confronting plagiarism. Elsevier had been previously criticized for removing plagiarized articles too quietly[6], but it has recently begun to test and employ plagiarism detection software to create a database against which a submitted manuscript could be searched[7]. Similarly, plagiarism detection software has been applied to full length papers on the physical sciences server arXiv, resulting in the discovery of almost 70 plagiarized papers[8, 9]. Significant advances in detecting (and thereby deterring) plagiarism in the sciences as a whole will likely come when larger full text databases are compiled and it becomes feasible to search submitted papers against these databases.

Finally, further discussion and education within the scientific community will be required to clarify standards for plagiarism. One of the physicists accused of plagiarism as a result of the arXiv searches defended his actions, saying he and his colleagues were "borrowing better English" as they claim many others scientists do[10]. A study of incoming graduate students at Vanderbilt and three other universities showed that 15.5% could not recognize the definition of plagiarism[11]. And ethical grey areas in plagiarism exist; for example, can you copy a sentence or two from the methods sections of one of your papers to another? Because educational backgrounds and cultural ideas about intellectual property can vary, and because even definitions of plagiarism can be unclear, it is up to institutions and journals to be explicit in what constitutes plagiarism and then relentless in pursuing and preventing cases of plagiarism.

1. Errami M., et al. *Bioinformatics* 24.2 (2008): 243-249.
2. http://ori.dhhs.gov/misconduct/definition_misconduct.shtml
3. Errami M. and Garner H. *Nature* 451 (2008): 397-399.
4. Rossner M., and Yamada K. *Journal of Cell Biology* 166.1 (2004) 11-15.
5. http://www.nature.com/authors/editorial_policies/plagiarism.html
6. Foster A. *The Chronicle of Higher Education* 10 Jan. 2003.
7. http://www.elsevier.com/wps/find/editorsinfo.editors/plagdetect
8. Sorokina, et al. arXiv:cs/0702012v1.
9. Brumfiel, G. *Nature* 449 (2007) 8.
10. Yilmaz I. *Nature* 449 (2007) 658.
11. Heitman E, et al. *Academic Medicine* 82.9 (2007) 838-845.

# International Perspective by Sabata Lund

Grad School in a foreign university is a dating experience… you begin with courtship. You need to ask professors who do not remember you at all to recommend you to a new school, saying how great you are. Some will be very supportive, but others will be slightly hurt because you are choosing to go abroad. And as with online dating, you may find surprising new meanings for words: "scenic campus" means "in the middle of nowhere" and "affordable rent" means more money than you make in a year. But once you find the best school to fit your needs (or the only one where you were accepted), you are so happy to go to the Consulate at 4:00 a.m. (I mean it! I was really happy!). You need to answer amusing questions like "are you a prostitute?" or "are you a Nazi?" or my favorite "do you plan to commit murder or genocide?" It does not matter, as long as you get your visa.

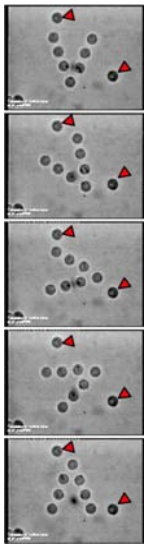
And then comes the honeymoon phase. Here, "next day delivery" means the "following day," not following month. "Your desk" means "your desk," not "yours + 15 undergrads" desk. You even get a paycheck! The classwork is only from 9-11am, three times a week so you can actually have time to be in the lab before midnight. Previously deemed exotic foods like macaroni-and-cheese, whipped cream, chocolate chip cookies, Pringles, barbecue sauce, onion rings, Buffalo wings and Big Macs are everywhere! And for simple math, lecture equals free food. Everybody thinks your accent is cute and you can even say "I pretend to be a good student and work hard in the lab" to your PI and not get fired (*pretend* means *intend* in Portuguese). Lab is so exciting: it is the first time you run an *American* gel. You are no longer splitting cells, you are splitting *American* cells. And your DMEM and the serum are no longer 'imported' reagents. The core facilities are there 24/7 and somebody else, *not you*, washes the dishes and makes the buffers. Actually, somebody else will *purchase* the buffers (before, I never heard of people buying PBS)! You feel so productive! Your enthusiasm combined with all reagents, space and equipment you need. Vanderbilt is definitely marriage-material school. This place is heaven for research.

Then comes the reality check (or the Tylenol-from-my-homecountry-is-better-than-from-here phase). It is not so good, not so bad either… it is just a stable relationship! Once you have a lab, a PI, a PhD committee and a thesis topic, American and international programs are not as different as it seems in the beginning. There is a normal distribution of hard working, lazy, happy, confused, family-oriented or Nobel prize-oriented types among both populations. Although lab productivity is more dependent on your self-motivation and discipline than where you come from, with the current funding situation, international students are still in a disadvantaged position because they cannot apply for training grants or most financial support. Joining a foreign school for your PhD offers you a great life experience, although not necessarily a better one than you would have had back home (and I will never know, because *no way* I will start a second PhD program just to make my point). The one good thing that can be said for sure, you knew it will sooner or later end up in graduation (a friendly divorce, but with more paperwork).

# Cell and Developmental Biology Department by Dominique Donato

**Matt Tyska is "tweezing" out membranes**

While optical tweezing *sounds* like a painful surgical procedure, it is actually an exciting microscopy technique being used by Assistant Professor of Cell and Developmental Biology, Matt Tyska. Optical tweezers allow him to capture membrane "tethers" from epithelial cells and measure the tension exerted as a sticky micron-sized bead attached to the tether snaps back to the cell. Using this technique, his lab has the ability to study the effect of myosins on these tethering forces. The principle, he says, is a lot like "the tractor beam idea in Star Wars." The bead is held into place by an optical trap, which is generated by a laser focused through an objective. "The bead gets sucked toward the brightest spot," Tyska says. Though the force exerted by light is too small for us to sense, the bead experiences the force in a similar way to a piece of paper being held by a hand. These forces are extremely small, measuring about $10^{-12}$ picoNewtons. Tyska's background in biophysics has helped him engineer the best system for his research, allowing him to manipulate many beads at once (see inset). These optical tweezers are currently the only ones at Vanderbilt University and are a great asset not only to Tyska's lab, but also to the greater Vanderbilt research community. Tyska looks forward to collaborating with other investigators, who will avoid having to set up their own optical tweezer system, which could easily cost them $150,000 for the most basic set up.



PictureCaption: Beads being manipulated by optical tweezers. Red arrows indicate stationary beads. Picture courtesy of Matt Tyska.

*"If we can understand the genetics and biology underlying how genes affect the way certain pharmacologica l agents modify lipid levels, it may lead to a better understanding and intuition about how to design new drugs."*

# Program in Human Genetics by Stephen Turner

As in every area of scientific research in academia, research in human genetics thrives on collaboration between investigators, students, and postdocs with different areas of expertise and resources. Dr. Dana Crawford, one of the newest faculty members in the Center for Human Genetics Research, has several ongoing and proposed projects that all involve collaborative efforts with other groups at Vanderbilt, and with groups at other academic and government research institutions.

Dr. Crawford and a 2nd year graduate student in her lab, Logan Dumitrescu, are interested in how genetic variation plays a role in common, complex human disease, such as cardiovascular disease. Crawford is a co-investigator in the Pharmacogenomics of Arrhythmia Therapy project, which is an arm of the much larger nationwide multi-center Pharmacogenetics Research Network, whose goal is to understand how genes affect the way different individuals will respond to medicines so that treatment options may eventually be tailored to each patient based on their genetic profile.

As part of this project, Crawford and Dumitrescu are studying how common single base-pair changes in the DNA sequence called single nucleotide polymorphisms (SNPs) affect cholesterol and triglyceride levels in a cohort of children. Children make for an ideal population to study the genetic influence on lipid levels because their cholesterol and triglyceride levels are presumably not affected by environmental factors as much as in adults, whose cholesterol and triglyceride levels are largely the result of decades of dietary and exercise habits, which may obscure any genetic effects. In a joint effort with the Centers for Disease Control and Prevention (CDC), a part of the US Department of Health and Human Services, Crawford and Dumitrescu plan to follow up their findings in children by replicating their findings in the CDC's National Health and Nutrition Examination Survey (NHANES). This will give Crawford and Dumitrescu access to approximately 15,000 DNA samples linked to important clinical information, including lipid measurements, in both children and adults of multiple ethnic backgrounds.

Additionally, in collaboration with St. Jude Children's Research Hospital, Crawford and Dumitrescu are studying how genes may modify how certain drugs given as part of a chemotherapy regimen can affect lipid levels. The hope is that if we can understand the genetics and biology underlying how genes affect the way certain pharmacological agents modify lipid levels, it may lead to a better understanding and intuition about how to design new drugs that may lower "bad" cholesterol or raise "good" cholesterol levels. Furthermore, understanding how genes modify the action of cholesterol-lowering drugs leads us one step closer to the goal of preventative and "personalized" medicine.

Dana Crawford, Ph.D. (crawford@chgr.mc.vanderbilt.edu) is a faculty member at Vanderbilt in the Department of Molecular Physiology & Biophysics and Program in Human Genetics.

# Microbiology and Immunology Department by Megan Johnson

For this edition of ABSTRACT, we would like to feature potential thesis projects available in the department of Microbiology & Immunology. Though we are unable to highlight the upcoming research in every laboratory, this article will give you a perspective of the breadth of research in the department that investigates bacteria, viruses, and the immune system.

Both methacillin Resistant Staphylococcus aureus (MRSA) and antibiotic resistant bacteria have recently monopolized the nightly news. In their recent Science paper, the Eric Skaar laboratory began to dissect out the dynamic relationship between *S. aureus* and the immune system. They examined the ability of neutrophils and calprotectin to defend against bacterial infection (Corbin et al. Science 2008). Future student projects will focus on the constant struggle for metal between bacterial pathogens and their hosts during infection.

In a similar fashion, the war on terror has increased public awareness of bacterial toxins, which is the focus in Borden Lacy's laboratory. Prospective projects will determine the cellular entry mechanisms for botullinum neurotoxin, large clostridial cytotoxins and H. pylori vacuolating toxin. While many of the projects center on the toxin structure, functional projects are also available. Knowledge of structure-function relationships will help to determine the mechanisms by which these toxins act.

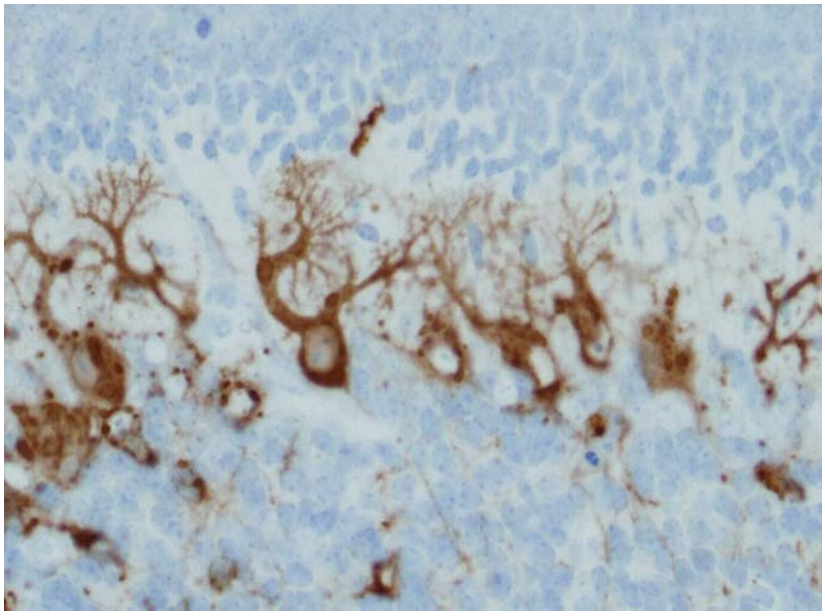
Viruses are also a pervasive health concern. Using reovirus as a model system, Terry Dermody's laboratory has revolutionized the field by developing the first plasmid-based reverse genetics system for reovirus (Kobayashi et al. Cell Host Microbe 2007). This system allows direct manipulation of the reovirus genome, enabling hypothesis-driven queries of virus-cell and virus-host interactions. Available thesis projects will explore the areas of reovirus receptor binding, cell entry, and vaccine development.

With 33.2 million people worldwide living with HIV, Chris Aiken's laboratory has focused their prospective projects on HIV-1 restriction factors ranging from elucidating the mechanism of Trim5alpha restriction, identifying cyclophilin A-dependent HIV-1 restriction factors, and identifying host factors which regulate the process of uncoating, a completely unexplored part of the HIV life cycle. The Aiken laboratory has reformed the field by developing an *in vitro* assay to directly study the process of uncoating.

For those interested in the complexities of the immune system, Dean Ballard's laboratory focuses on signaling pathways converging on the transcription factor NF-kB. Prospective projects in the lab will investigate how post-translational modifications, including phosphorylation and ubiquitination, regulate signal transduction in the immune system. Knock-in mice harboring IKK subunit mutations are available for Ph.D. thesis projects, which may help in the development of therapeutic intervention in inflammation-based diseases.

You may ask yourself what happens when our powerful immune system attacks itself. This question is the focus of the James W. Thomas laboratory, which explores how B cells malfunction in autoimmune disease. Potential projects involve investigating antigen presentation pathways, identifing novel antigens recognized by B cell receptors at the site of autoimmune attack, or investigating the role for NFAT transcription factors in autoreactive B cells. These studies will provide new insight into how autoimmune diseases are triggered and will identify new targets for therapy in rheumatoid arthritis and type I diabetes.

For more information on any of these projects you are encouraged to contact the principal investigator.



Histological section of the cerebellum of a mouse infected with reovirus. The tissue was stained with polyclonal reovirus-specific antiserum. Reovirus targets the large Purkinje cells of the cerebellum as well as neurons of the cortex, hippocampus, and thalamus. Reovirus serves as a model to better understand the contribution of viral receptors to pathogenesis.

## Biochemistry Department by Chris Barton

Normal processes subject our cells to a great deal of genomic stresses, many of which could prove to be extremely detrimental to cell survival. For example, recombination can cause the generation of "knots" in DNA that could later prevent the separation of the two DNA strands. Additionally, the unwinding of DNA during replication can result in tangled structures, known as supercoils or catenanes. Luckily, our cells employ proteins known as topoisomerases that can repair these structures by cleaving DNA, unwinding the tangled structures, and then ligating the cleaved ends back together. Because these proteins are important for DNA replication and cellular proliferation, a number of commonly used chemotherapeutics, such as etoposide, specifically poison topoisomerases to kill cancer cells by cleaving DNA and subsequently inhibiting the protein's ability to re-ligate the cleaved ends, resulting in cell death.

In work recently published in the journal *Biochemistry*, Joseph Deweese and Dr. Neil Osheroff describe a system used to study the ability of topoisomerase II to cleave DNA substrates without rejoining the cleaved ends. The separate cleavage and ligation reactions carried out by topoisomerases have previously proven tough to study independently due to the transient nature of the enzyme. "The protein-DNA complex is extremely short-lived, making it very challenging to study the separate components of the catalytic cycle," says Deweese, a 4th year graduate student in the department of Biochemistry. The system used in this study employs modified DNA substrates (3'-bridging phosphorothiolates) in which sulfur replaces a single oxygen in the backbone of DNA at the scissile bond. This modified substrate allows for DNA cleavage by the enzyme, but does not allow for the subsequent ligation reaction to occur. This is the first irreversible oligonucleotide cleavage system developed for topoisomerase II. Using this system, the authors were able to show that the topoisomerase II-targeted drugs etoposide and amsacrine had no effect on the forward rate of DNA cleavage by topoisomerase II. Consistent with previous reports, the drugs acted by inhibiting the ability of topoisomerase II to religate the cleaved DNA ends. On the other hand, abasic sites, one of the most frequent forms of DNA damage, enhanced the rate of DNA cleavage by topoisomerase II. Because it allows one to separate the cleavage and ligation steps, the authors hope that other laboratories will use this system to study the function of DNA topoisomerases in the future. Presently, Deweese and Osheroff have already taken advantage of their system to study additional aspects of DNA topoisomerases. "This system has enabled us to address questions about the role of the divalent metal ions used during catalysis, which will be addressed in a paper that is currently being written," says Deweese. Also, we are working with collaborators in an effort to use these substrates to trap the enzyme-DNA complex for X-ray crystallography."

Joseph Deweese (joseph.e.deweese@vanderbilt.edu) is a graduate student in the Department of Biochemistry.

Neil Osheroff (neil.osheroff@vanderbilt.edu) is a professor in the Department of Biochemistry and Medicine.

***"Depression is a devastating neuropsychiatric disorder, disabling and debilitating."***

## Neuroscience Program by Nellie Byun

Depression is a devastating neuropsychiatric disorder, disabling and debilitating. Despite advances in neuroscience research encompassing genetics, brain imaging, animal models, electrophysiology, and pharmacology, major depressive disorder (MDD) remains a conundrum to psychiatrists and neuroscientists. Why do some depressed patients turn to food and gain weight while others lose interest in eating altogether? Why do some individuals languor in excessive sleep while others suffer from insomnia and cannot rest? The mechanisms involved in the pathogenesis of depression remain elusive due to the polar subphenotypes that characterize MDD and the different neurotransmitter pathways involved, Maureen Hahn, PhD, along with other Vanderbilt researchers in Neuroscience, Pharmacology, and Psychiatry, embarked on a study of genomics and MDD endophenotypes to better understand "how genetic

## Neuroscience Program Continued by Nellie Byun

variation can impact subphenotypes based on an understanding of how the gene participates in sustaining basic physiological mechanisms and behavior." Specifically, since subphenotypes have significant familial associations, they hypothesized that individual symptoms could be linked to specific gene variations.

The study of the genetics of depression is not new. However, the subphenotype-genotype approach is different compared to conventional studies that have been "for the most part treating depression as a unitary disorder." With 110 well-phenotyped, solely unipolar MDD patients and genetic testing coupled with robust statistical analysis (multivariate permutation testing), they were able to investigate common polymorphic variants of genes related to central monoaminergic and cholinergic pathways. Importantly, their analyses showed significant associations of the norepinephrine transporter (NET) with depression subphenotypes, such as depression recurrence and increased appetite; the study also uncovered significant associations with serotonin receptor polymorphic variants and a choline transporter variant associated with depression severity. The cholinergic system has been receiving less attention than their monoaminergic counterparts regarding depression, but this novel finding supports recent work linking acetylcholine deficits with depression.

The genetic and phenotypic complexities underlying unipolar depression have made it a difficult disease to crack. Dr. Hahn and colleagues demonstrate a successful collaborative effort between psychiatrists, neuroscientists, and pharmacologists and the importance of a firm understanding of genetics and statistics. Of course the biology behind the variant genes is important to answer questions on how polymorphisms affect signaling. Although not brought up by the authors, it seems important to state that these polymorphisms may not be risk factors for depression *per se* and that the treatment of symptoms may not be a guarantee in treating depression, but only additional studies can resolve this. For future studies, interactions between risk genes need to be assessed, as well as the question of patients' differential responses to antidepressant medication. It is foreseeable that results from larger studies will drive a paradigm shift in treatment strategies for depression toward personalized medicine. This is the beginning for more tailored pharmacotherapies addressing variations in relevant genes for depression and should bring about change in the treatment of a broad spectrum of neuropsychiatric disorders.

Maureen Hahn (Maureen.hahn@vanderbilt.edu) is an assistant professor in the Division of Genetic Medicine and Department of Pharmacology.

Hahn MK, Blackford JU, Haman K, Mazei-Robison M, English BA, Prasad HC, Steele A, Hazelwood L, Fentress HM, Myers R, Blakely RD, Sanders-Bush E, Shelton R. Multivariate permutation analysis associates multiple polymorphisms with subphenotypes of major depression. *Genes, Brain and Behavior*. 2008; 7:487-495.

## Pathology Department by Robin Marjoram

A primary goal of graduate students is to have a manuscript published in a peer-reviewed journal. Stephen Smith, a current graduate student in Pathology, recently had a manuscript accepted in *the Journal of Biological Chemistry* [Vol. 283(11), pp.6696-6705]. After receiving his undergraduate degree in biochemistry from Murray State University, Stephen came to Vanderbilt where he entered the IGP and joined Dr. David Gailani's laboratory. Stephen's research has focused on the contribution of plasma clotting factors in normal and pathologic conditions, specifically the serine proteases, factors IX and XI (FIX and FXI, respectively).

Their recently accepted paper characterized a novel form of the activated coagulation factor XI that seems to be a component of the important protease cascade that activates thrombin to produce a fibrin blood clot. FXI exists as a homodimer in blood, and upon stimulation of coagulation, each subunit of the FXI homodimer can be activated by its physiological activators, factor XIIa (FXIIa) and thrombin. Stephen and his colleagues found was that two forms of activated FXI exist: a novel form where only one of the FXI subunits is activated (½-FXIa) and the known form where both subunits are activated (FXIa). Interestingly, ½-FXIa has properties of both FXI and FXIa, where it can bind to glycoprotein Ib found on platelets through the FX1 subunit and also serve as a fully active protease through the FXIa subunit to cleave its physiological substrate, FIX, to FIXa. The finding of ½-FXIa has new and interesting implications in the plasma clotting factor cascade as well as in platelet functions in hemostasis and thrombosis.

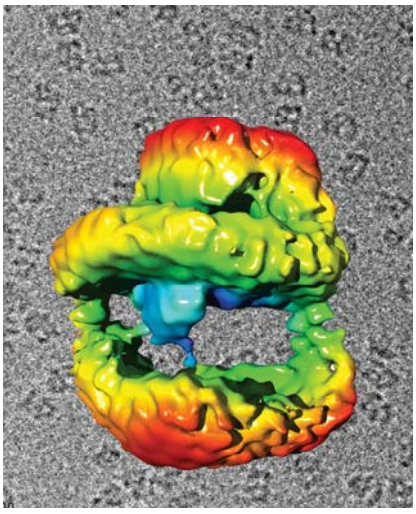
David Gailani (dave.gailani@vanderbilt.edu) is an associate professor in the Departments of Pathology and Medicine.

# Molecular Physiology and Biophysics Department by Jessica Moore

*"They may be a link between the risk factors obesity and hyperlipidemia, as well as diabetes and heart disease."*

Kim Coenen, a recent graduate of Alyssa Hasty's laboratory in the Molecular Physiology and Biophysics Department, has used a new mouse model to distinguish the contributions of obesity and hyperlipidemia, or increased levels of certain lipids and low density lipoprotein (LDL) in the blood, to multiple associated diseases such as diabetes, cardiovascular disease, and nonalcoholic fatty liver disease (NAFLD). Because lipid levels in mice normally do not correspond with the level of fat in their diet, Coenen created a double-mutant line lacking the LDL receptor ($LDLR^{-/-}$), whose lipid levels increase with increased fat intake, and ectopically expressing the *agouti* gene in all tissues ($A^y/a$), which are moderately obese due to hyperphagia and upregulation of lipogenic genes in adipose tissue. This allows the comparison of four groups: (1) $LDLR^{-/-}$; a/a fed regular chow, (2) $LDLR^{-/-}$; a/a fed a high-fat diet, (3) $LDLR^{-/-}$;$A^y/a$ fed regular chow, and (4) $LDLR^{-/-}$;$A^y/a$ fed a high-fat diet, resulting in a range of body weights, with lipid levels determined by diet. In an August 2007 paper, Coenen showed that obesity has no impact on atherosclerosis in this model, while it worsens NAFLD and insulin resistance. In her March 2007 paper in the journal *Diabetes*, she examined infiltration of macrophages into white adipose tissue (WAT), as they may be a link between the risk factors obesity and hyperlipidemia, as well as diabetes and heart disease. Increased levels of macrophages reside in the WAT of obese individuals compared with controls. Interestingly, macrophage infiltration precedes or coincides with the onset of insulin resistance in obese mice, and these cells secrete inflammatory cytokines that may contribute to a systemic pro-inflammatory state which is a risk factor for cardiovascular disease. The degree of adiposity correlated with infiltration, while lipid levels had no effect.

Dewight Williams, a former postdoctoral fellow in Phoebe Stewart's laboratory in the Molecular Physiology and Biophysics Department, recently published a seven-angstrom resolution electron cryo-microscopy (cryoEM) structure of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). This kinase regulates non-homologous end joining (NHEJ) of double stranded (ds) DNA breaks and is essential for V-D-J recombination, which generates antibody and T cell diversity. Defects in NHEJ result in severe combined immune deficiency and sensitivity to ionizing radiation. Williams has a deep interest in DNA management, including such broad, basic questions as, "How are lesions found in a vast sea of nucleotides and multiple layers of structure? How is the type of repair initiated at a DNA lesion determined?" DNA-PKcs is an excellent first candidate for study of the structure of proteins involved in DNA repair, as its large size and abundance make it suitable for cryoEM. This is one of the highest resolution structures determined by cryoEM for a single asymmetric particle, a technological breakthrough that merited the cover of *Structure* in March 2008. An extremely large data set and advanced microscopes allowed for this accomplishment, which revealed a previously unobserved structural feature, a protrusion that is likely to be the site of interaction with dsDNA.



This image is of the cryo-EM structure of DNA-PKcs that DeWight WIlliams and Phoebe Stewart just published. The color coding is for distance from the vertical center of the protein, where dark blue represents 0 angstroms and red is 75 angstroms. They model a configuration later in the paper in which dsDNA interacts with the blue central protrusion.