CELL AND MOLECULAR BIOLOGY STUDENT NEWSLETTER

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IN THIS ISSUE

RESEARCH SPOTLIGHT

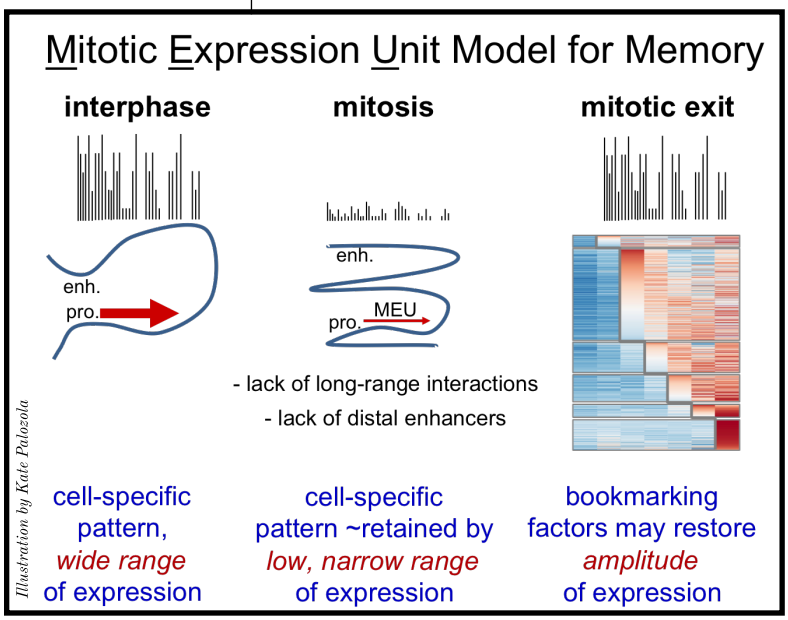
Mitotic Transcription May Help Maintain Cell Identity

Annie Chen

The human body has over 200 different cell types, and gene regulation is key to establishing and maintaining cell identity. During mitosis, chromatin condenses and long-range interactions between distal enhancers are lost.[1] As a result, scientists have long believed that transcription during mitosis is silenced, raising the question of how cells reactivate transcription to maintain cell identity. How cell identity is controlled is a fundamental biological question, and understanding this process could also provide insights for cell reprogramming and regenerative medicine.

Addressing how cells maintain identity during mitosis is not a trivial issue, given the technical challenges. Since the nuclear envelope breaks down during mitosis, one cannot isolate the nuclei to label transcripts. Previous approaches included using RNA polymerase II (RNAP2) cross-linking,[2-3] but antibody-based methods are less sensitive than direct measurements of nascent transcription. Other studies have used the thymidine analog bromodeoxyuridine (BrdU) to label nascent RNAs, but labeling live cells with BrdU, which is not cell-permeable, can be challenging.

Dr. Kenneth Zaret's lab investigates mechanisms of gene regulation involved in controlling cell identity. Dr. Kate Palozola, a recent alumna from this laboratory and a former Genetics & Epigenetics Program student, developed an assay, EU-RNA-seq, to capture nascent transcription during mitosis and mitotic exit.[4] The uridine analog 5-ethynyluridine (EU) was used to pulse-label nascent transcripts in HUH7 human hepatoma cells during nocodazole-induced mitotic arrest, mitotic exit, and in asynchronous cells. Azide-biotin was used to isolate these transcripts from total RNA, and RNA-sequencing was then performed to identify genes that are actively transcribed during mitosis and mitotic exit.



Mitotic expression unit model: A low level of transcription is maintained during mitosis. During mitotic exit, gene reactivation occurs in multiple waves, with cell type-specific genes appearing in later stages.

Surprisingly, Kate found that transcription still occurs during mitosis. Sequences from EU-RNA-seq were mostly primary transcripts, spanning introns and exons but not intergenic regions, suggesting that this method can robustly detect the nascent transcriptome. Since the mitotic population contained a small fraction of non-mitotic cells, several controls were used, including spike-in controls, to confirm the authenticity of the signal from mitotic cells. Kate identified 8074 transcripts (3689 genes) that were consistently expressed during mitosis. RNA fluorescence in situ hybridization (RNA FISH) and quantitative reverse transcription polymerase chain reaction (RT-qPCR) were used to independently assess these genes. While the mean expression level of mitotically expressed genes was approximately five-fold lower in mitotic cells compared to asynchronous cells, there were several genes with increased expression, including *KLF4* and *ATF3*, which encode transcription factors.

Kate then sought to determine whether there was a particular order in which gene expression is reactivated upon mitotic exit. Since previous work in the Zaret lab had shown that most of transcription initiates about 80 minutes after release from mitotic arrest,[2] Kate used EU-RNA-seq to monitor transcription upon mitotic exit at different intervals after release from nocodazole-induced mitotic arrest. While some transcripts appeared as early as 40 minutes, she reaffirmed that the largest burst of transcription indeed occurs at 80 minutes.[2,5] Kate found that the earliest transcripts encoded proteins involved in basic cell structure and growth. In addition, using this sensitive technique, Kate was able to identify additional waves of gene reactivation. The next wave of gene reactivation included adhesion genes (HUH7 cells are epithelial cells), followed by transcripts involved in cell cycle and DNA replication, as cells prepared for S phase. Although there were some liver-specific genes identified throughout the

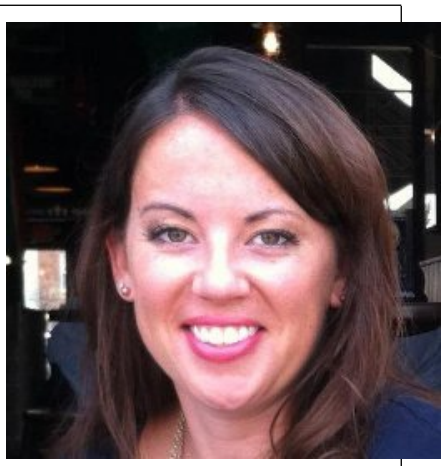
mitotic exit, most of the 149 liver-specific genes, including *APOC3* and *ASGR2*, which encode apolipoprotein C3 and asialoglycoprotein receptor 2, respectively, were reactivated much later. Kate also found that enhancer RNAs (eRNAs) were downregulated during mitosis and appeared early during mitotic exit, around the same time as their putative target genes.

Overall, these results overturned the dogma that transcription is silent during mitosis. On the contrary, RNA polymerases are still active during mitosis and may help contribute to the inheritance of a cell's transcription pattern. Based on these findings, Palozola et al. propose a model in which mitotic expression units (MEUs) maintain a low level of transcription during mitosis that could help maintain cell identity (see figure).[4] Future work may focus on how cells regulate the order in which genes are reactivated during mitotic exit, which could provide insights for reprogramming cells and tissues for regenerative therapies.

Kate is now a post-doctoral fellow in the laboratory of Jean Bennett, where she studies gene therapy for heritable forms of blindness. As a co-founder of the CAMB student newsletter, Kate is also passionate about science communication. Her favorite CAMB memories include recruitment, orientation, the annual holiday party, and the CAMB Student Newsletter.

Palozola, K.C., Donahue, G., Liu, H., Grant, G.R., Becker, J.S., Cote, A., Yu, H., Raj, A., and Zaret, K.S. Mitotic transcription and waves of gene reactivation during mitotic exit. *Science* 2017; 358(6359):119-122.

For a full list of citations, please visit our blog.



Kate Palozola, G&E

No Alternative to Proper Inner Ear Development

Iryna Shakhmantsir

Sensorineural hearing loss (SNHL) is a common sensory deficit, which affects 1 in 500 newborns, and can arise from etiologically diverse structural and functional inner ear abnormalities. The mammalian inner ear is an elegant labyrinth that contains a cochlea, the primary auditory organ, and a vestibular system that maintains body balance. Lateral cochlear duct cells, comprising Reissner's membrane and the stria vascularis, are critical for production, maintenance, and secretion of endolymph, a specialized fluid that supports hair cell function. A recent *Developmental Cell* paper by Alex Rohacek, a DSRB student from Douglas Epstein's lab, highlights a complex splicing program that is necessary for proper development of the lateral cochlear duct cells and is, therefore, essential to form a functional hearing organ in mammals.

This research story began at the Children's Hospital of Philadelphia (CHOP), during an evaluation of an 8-year-old female with congenital hearing loss. While her parents appeared to have normal hearing, her older brother was previously diagnosed with SNHL. The girl's four other siblings were asymptomatic. This finding and the absence of a family history of hearing loss suggested an autosomal recessive mode of SNHL inheritance. To identify a potential causative mutation, the researchers used whole- exome DNA sequencing, and were able to zoom in on a gene called *Epithelial Splicing Regulatory Protein 1* (*ESRP1*). It became clear that mutations in *ESRP1* were segregating with hearing loss in the family.

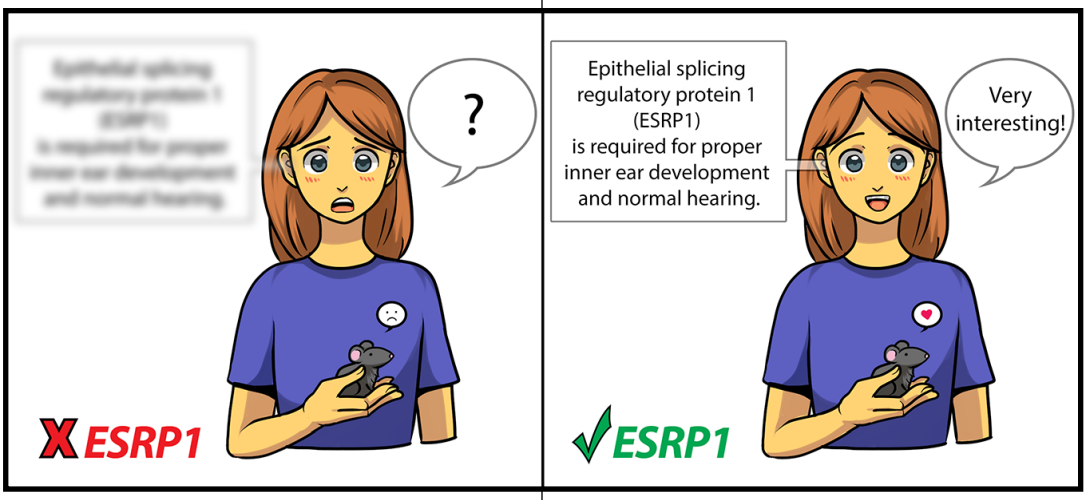


Illustration by Arwa Abbas

While these human studies performed at CHOP were essential to identify *ESRP1* as a novel hearing loss gene, the follow-up experiments done by Alex and the rest of the UPenn team were fundamental to uncovering a functional role for ESRP1 during inner ear development. In particular, the analysis of homozygous knockout *Esrp1* mouse embryos shed light onto the developmental progression of inner ear pathology and allowed the scientists to interrogate key alternative splicing events that could underlie SNHL.

As expected from the human findings, *Esrp1* null embryos had profound inner ear defects, manifesting in cochlear duct truncation and immature or absent hair cells.To probe altered gene expression events in these mutant embryos, Alex and the team performed RNA-Seq analysis to identify the top misregulated transcripts. Interestingly, many genes previously implicated in hearing loss were differentially expressed between the control and *Esrp1* null mice. Genes with the highest fold change included ion channel subunits *Bsnd* and *Kcnq1*, and their upstream regulator *Nr3b2/Esrrβ*, all of which happen to be expressed in a subset of cochlear duct cells called marginal cells.

These transcriptome data were instrumental for identifying both altered transcript levels and specific aberrant splicing events in *Esrp1* null mice. Consistent with the role of ESRP1 as a master regulator of epithelial cell-type specific programing, many epithelial-specific isoforms were substituted for their mesenchymal counterparts in *Esrp1* null embryos. Splicing of *Fibroblast Growth Factor Receptor 2* (*Fgfr2*), for example, involves a tight regulation of mutually exclusive exons IIIb (epithelial isoform) and IIIc (mesenchymal isoform). Alex and the team confirmed the switch from the normal *Fgfr2-IIIb* to mesenchymal *Fgfr2-IIIc* isoform in the cochlear epithelium of mice lacking *Esrp1*. According to Alex, making sense of alternative splicing data was by far one of the most challenging aspects of this project. "I'd never really worked with post- transcriptional processing before and very little is known about splicing in the ear. Trying to tie the switches in isoform usage we were finding to the phenotype was really difficult," he noted.

Analysis of specific nonsensory inner ear cell identities in *Esrp1* null mutants revealed a curious cell fate switch phenotype characterized by a reduction of marginal cells and a concomitant increase in Reissner's membrane cells, an imbalance that can, perhaps, explain the hearing loss phenotype in human patients. It was an in-depth look at the splicing switch in *Fgfr2* and Fgf signaling that led to surprising discoveries. It was hypothesized that the

Alex Rohacek, DSRB

observed splicing switch in *Fgfr2* would render it unable to respond to Fgf10 ligand. Since the *Fgf10* inner ear mutants had been published, Alex and the team expected to see the same phenotype, a loss of Reissner's membrane, in their *Esrp1* null mice but, instead, they observed the exact opposite - Reissner's was expanded. "After a lot of literature searching and thought experiments, we reasoned that the other isoform of *Fgfr2* was still expressed and responded to a different ligand, Fgf9, effectively giving us a gain of function phenotype. We were able to rescue the expanded Reissner's phenotype by removing an allele of *Fgf9*, validating our hypothesis. I'm honestly amazed at how complex that whole interaction is and how well that experiment worked," shared Alex.

Alex's paper is a great example of original research that has been enabled by frequent collaborations between labs at UPenn and its next-door neighbor, CHOP. A combination of patient data and cutting-edge animal research has allowed for a better mechanistic understanding of how mutations in *ESPR1* can lead to hearing loss.

Rohacek, A.M., Bebee, T.W., Tilton, R.K., Radens, C.M., McDermott-Roe, C., Peart, N., Kaur, M., Zaykaner, M., Cieply, B., Musunuru, K., Barash, Y., Germiller, J.A., Krantz, I.D., Carstens, R.P., and Epstein, D.J. *ESPR1* Mutations Cause Hearing Loss due to Defects in Alternative Splicing that Disrupt Cochlear Development. *Developmental Cell* 2017; 43(3):318-331.e5.

SPECIAL INTEREST

Getting to Know Your Friendly Neighborhood BGSA

Somdutta Mukherjee

The Biomedical Graduate Student Association (BGSA) is a student run group that represents the interests of graduate students from all seven Biomedical Graduate Studies (BGS) programs. BGSA is responsible for responding to students' suggestions and ideas, and relaying them to the BGS administration, as well as the wider Penn community.

BGSA is run by an Executive Board, which includes a Chair and Vice Chairs of Administrative Affairs, Academics, Social Affairs, Finance, and Operations. There are also four Graduate and Professional Student Assembly (GAPSA) representatives who speak on behalf of BGSA at GAPSA meetings. Furthermore, each graduate group within BGS has one program representative, with the exception of CAMB, which has three. They represent and advocate for the needs of students from their respective programs. BGS students choose members of the Executive Board, as well as their student representatives, in an election that is held annually.

The main responsibility of BGSA is to address the concerns of the BGS community. For example, many BGS students felt burdened by the costs of moving to start graduate school. BGSA brought this issue to the attention of the administration, and as a result, BGS now provides first year students with $1,000 relocation award to offset these expenses. Students can voice their concerns or make suggestions at BGSA general assembly meetings. These meetings, which are typically held once a month, are open to all BGS students. Concerns or ideas can also be submitted anonymously to BGSA through a Google form, which can be found on the BGSA website. Additionally, if students have issues pertaining to their specific graduate group, they can contact their program representative who then conveys the issue to the BGSA Executive Board. BGSA members regularly meet with the BGS administration to discuss issues raised by students, and ensure that their concerns are heard.

From left to right: BGSA Chair Bobby French, Vice Chair of Social Affairs Terra Kuhn, Vice Chair of Administrative Affairs Leah Suttner, GAPSA representative Lucas Van Gorder, Vice Chair of Finance Christin Herrmann, Vice Chair of Academics Priya Chatterji, Vice Chair of Operations Sarah Sneed, Chair of Social Media Rina Kim, and GAPSA representative Olivia Harding.

There are many ways to get involved in BGSA, and attending general assembly meetings is the easiest way to participate. BGSA Chair Bobby French says, "We always welcome people to come to General Assemnly (GA) meetings. Plus, there is free food." For those looking to get more involved, each member of the BGSA Executive Board can have a deputy. Deputies help Executive Board members with their responsibilities, and also attend the Executive Board meetings, during which the Board reviews and plans General Assembly meetings.

BGSA also interacts with other graduate groups at Penn to get ideas for improving BGS. For example, the Penn Medical Student Government conducted a survey to determine how medical students could better utilize Counseling and Psychological Services (CAPS). The BGSA Executive Board is working with GAPSA to set up a similar survey to determine how to make CAPS services more accessible for BGS students. Additionally, BGSA interacts with the wider Penn community by attending GAPSA meetings, where they represent the needs of BGS students.

In addition to listening to and addressing students' concerns, BGSA also provides funding for both individuals and student groups. Merit requests are open to individuals in BGS looking to organize an event that is open to all BGS students. French says that BGSA "has more money than usual this year, so people should submit merit requests by June." BGSA also funds events pertaining to specific BGS programs, as wells as events that involve BGS and other graduate schools at Penn. For example, BGSA provided funds to the Penn Graduate Women in Science & Engineering (PGWISE) group to help them organize events such as Pop Talks and career panels. Finally, BGSA also runs social events like the Welcome Back happy hour during orientation week and Oktoberfest.

BGSA not only gives BGS students a voice, but also brings the BGS community together. "I didn't really appreciate what BGSA could do until I got involved, and saw that I could really make a difference and advocate for BGS students directly to the administration. It's been a really great experience and I think we've been able to make an impact,"French says. The efforts of BGSA play an instrumental role in helping the BGS program continuously evolve and improve.

To learn more about BGSA and to get involved, visit their website at http://www.med.upenn.edu/bgsa/

Welcoming New-CAMB-ers:

A Faculty Profile on Shawn C. Little, Ph.D.

Ewa Stypulkowski

Shawn C. Little, Ph.D., Assistant Professor of Cell and Developmental Biology

"Why get up in the morning and fight for grant money and all this? We get to see things that no human has seen before and learn stuff that's not in any textbook. We get to work with talented students…and watch them grow into scientists!"

This is what excites Dr. Shawn C. Little, Ph.D., a newly appointed Assistant Professor in the Department of Cell and Developmental Biology at the Perelman School of Medicine.

Shawn is no stranger to Penn. He completed his doctoral thesis with Dr. Mary C. Mullins in 2008, on the role of transforming growth factor beta (TGF- β) signaling in early zebrafish development. His work elucidated how bone morphogenetic protein (BMP) gradients conferred cell fate and gene expression patterns to shape the dorsoventral axis during early embryogenesis.

Shawn built on this research as a postdoc in the lab of Dr. Eric Weischaus (in collaboration with Dr. Thomas Gregor) in the Physics department at Princeton University. While at Princeton Shawn looked deeper into how gradients and pattern information are reliably arranged within the embryo. He was additionally interested to know how subtly changing the timing and levels of transcription factors and signaling gradients can elicit specific gene expression patterns. Together with the Gregor lab, Shawn developed a novel method for imaging and quantifying single messenger RNAs (mRNA) in *Drosophila melanogaster* embryos. This fruitful collaboration resulted in many publications and unearthed new insights into the transcriptional dynamics and mechanisms governing reproducible gene expression patterns.

Now in charge of his own lab, Shawn intends to study the facets of gene expression control that generate specific patterns and programs of transcription during embryogenesis. Shawn elaborates, "the embryo needs to elicit a specific set of gene expression programs as a function of position. [In the cell] you have molecules that bump into each other at random, so how is it that at a given position, a set of cells can simultaneously decide to express a specific set of genes? At a biophysical and mechanistic level, how do enhancers and promoters at genes read input concentration levels to generate these specific responses as a function of position?"

To get at these questions, Shawn's lab uses his newly-pioneered single mRNA imaging technique as well as the live imaging of transcriptional reporters to know at any point in time how many mRNAs are being produced, how many polymerases are transcribing a gene, and how clustered these polymerases are (also known as transcriptional bursting). The lab studies how these singular bursting events are coordinated to establish a precise gene expression pattern.

Shawn is also excited to expand his studies through the many opportunities for collaboration at Penn. He feels that Penn has the best of both worlds: a vast basic sciences network and a medical school. This, in combination with the many experts here at Penn, "gives you an immediate sense that you use your questions to benefit human health," he says.

Shawn's academic and intellectual curiosity is also reflected in his journey to becoming a professor. He describes his path to academia as "fairly meandering." As an engineering undergraduate at Northwestern University, Shawn felt somewhat bored with engineering classes, so by chance he began working in the lab of Dr. Doug Engel studying cell fate during hematopoiesis. This experience ignited his interest in changes in gene expression underlying cell fate decisions. However, it was while he was a technician in the lab of Dr. Adam Driks at Loyola University that Shawn really noticed what the other labs around him were doing and began to seriously consider a career in academia.

His path led him to the graduate program at Penn for Developmental Biology. Shawn had a very successful graduate career and said that he felt lucky to have found the Mullins lab, which he described as "allowing me to apply the techniques I've acquired and… overall, a great fit." One of Shawn's favorite moments from graduate school was of an epiphany in the lunchroom. A few labmates were struggling over a set of uninterpretable results for months with no luck. Finally, after drawing out diagrams on the board, "we had a simple model that explained a complicated idea. Everything just clicked!" said Shawn.

However, his transition from graduate student to postdoc to faculty member was not easy. Shawn was challenged to develop his multi-level thinking abilities. He had to come up with broad enough questions that many people can work on, while being simultaneously specific enough to generate publications. He also felt a hurdle in the shift from a single-focus mindset to being an effective and efficient multi-tasker. This change made him realize how amazing the faculty who mentored him really have been, and is something he admits he is still working on. Another challenging experience for Shawn was the switch from being a primary data-generator to a manager. He emphasizes that learning how to respond to different needs, i.e. tailored mentoring strategies and conveying concepts across diverse audiences, is critical for success.

Despite these challenges, Shawn seems to relish the mentoring aspect of being a PI. He finds training students, especially those who have never worked in a lab outside of class, a rewarding part of being a faculty member. He finds nothing more exciting than to see how rigorous work skills are developed and how students learn to come up with a framework to interrogate hypotheses.

To students interested in pursuing academia, Shawn attributes his own achievements to taking lessons from failure, persevering, and not letting failed experiments affect his attitude since "one way or another, things work out."

Shawn strongly recommends stepping out of your comfort zone and choosing a lab where you're excited about the science and the kinds of questions asked. He believes that you should "always be willing to question what is presented to you even when the answers seem obvious. How were the facts established? Questions we think we know the answers to, we may not actually understand them at all. Look to [your] role models in academia and try

to draw things from each person, what you like about how they do an aspect of their job. Working on an idea that you think is interesting is the main driver of success, and will lead other scientists to also think your question is interesting and worthwhile pursuing."

Dr. Little is happy to speak to you about rotations! If interested, please email him at shlittle@pennmedicine.upenn.edu. For more information of Dr. Little and his lab, please visit his lab's website https://shawnlittlelab.wordpress.com/

WHERE ARE THEY NOW?

Samantha Falk

Gleb Bazilevsky

Dr. Samantha Falk, CAMB (G&E) 2016 alumnus and former student of Dr. Ben Black, loves her new job. She learns about cutting-edge science, daily. She collaborates with industry insiders and diverse professionals, constantly. She works from home, frequently.It's creative, interactive, engaging. Most likely, you've heard of it already (hint: see CAMB Student Newsletter, Volume 1, Issue 1). She is a medical writer for the Scientific Pathways medical writing agency (Hamilton, NJ), which is owned by Nucleus Global, a UK-based group of medical communications agencies.

Scientific Pathways and Nucleus Global call on their teams to act as scientific resources for clients, providing consulting and generating deliverables. "We work with pharmaceutical companies to put together communication materials for them," Samantha explains. She is a full-time medical writer, whose numerous concurrent projects include writing manuscripts for journal publication, drafting sales training plans for pharmaceutical marketing, and attending industry conferences. Samantha works alongside other Ph.D.s and M.D.s to communicate the latest research about blood and lung cancers. This group then pairs with a public services team of in-house editors, graphic designers, publishers, and others to deliver this material to the client. For instance, Samantha may be asked to summarize the pharmacological history of a drug or synthesize the most recent leukemia literature and conference presentations into an accessible synopsis. A studio team may then incorporate figures and visuals from the literature, after which the work relays across the agency's circuitry to the client for feedback and further development. Her work can vary in scope and reach. Samantha can be asked to write about the minutiae of a new drug trial or a sweeping assessment of the field as a whole. "I think about it more in a global context. How does this agent I'm working on fit into the bigger picture? Some projects have a more global scope but other projects are focused on one aspect," she relates. How, you might now ask, did Samantha come to this work for Nucleus?

Samantha chose Nucleus Global through fellow G&E alumnus Aleksandra Nall. "Networking is definitely the main reason." Nall introduced the medical writing career to Samantha, and provided a direct referral. Direct referrals greatly increase the likelihood of an applicant having their application reviewed and getting a phone interview. This proved decisive for Samantha. The idea of being a medical writer appealed to Samantha as it built on an enduring interest. "I've always been interested in communicating science to different audiences," Samantha explains. While in graduate school, she created lessons and taught genetics and epigenetics to diverse audiences at Franklin Institute events. She chose to parlay these experiences for her job search. "On the first day of my job, my boss told me one thing she really liked during my interview was the aspect of creativity, because of my involvement in the Philadelphia Science Festival. That was something she really picked up on." This demonstration of creativity helped Samantha get hired, but she emphasized that there were a number of skills that the agency looked for.

Samantha Falk, G&E

What are the skills an interested graduate student might want to cultivate for a medical writing career? "Multitasking is an important one. You have to figure out how to balance your projects, and I think having to do that in graduate school has helped a lot with my current job," Samantha states. Also, being able to find information quickly and parse out the most important points is a powerful ability to develop. Communicating clearly is another skill that has helped her succeed as a medical writer. Lastly, Samantha emphasizes the importance of learning how to structure a story by asking, "who are my audience? How does my story fit into the bigger picture?" when presenting her work to different audiences. She suggests practicing interviews and presentations with scientifically-literate people inside and outside of academia to become more comfortable communicating with people of diverse backgrounds.

Samantha enjoys her work greatly, but she has an eye on the future."I have always been interested in public-oriented scientific communication. Ultimately, I would like to focus more on communicating to patient and general public audiences." She hopes to leverage her work for Nucleus Global to reach these groups as a specialist and educator. For the full interview, please go to the CAMB Newsletter blog!

We are excited to announce our second "Pie a PI" event in this now annual tradition to raise money for the Children's Scholarship Fund Philadelphia. All members of the CAMB community can purchase a 1$ raffle ticket for a chance throw a pie in the face of Drs. Dan Kessler, Kelly Jordan-Scuitto, and Brian Keith. The 3 lucky winners will be announced on pi day (March 14th) and the pie-ing will take place on Friday March 16th at 4:30PM.