CELL AND MOLECULAR BIOLOGY STUDENT NEWSLETTER

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May 2018

IN THIS ISSUE

LETTER FROM THE EDITORS

Dear CAMB students, faculty, and alumni,

We would like to thank everyone who participated in our Pie a PI fundraiser this year. We had a great time while raising money for an important cause. To learn more about this year's fundraiser, see the article in this issue. Additionally, we feature research spotlights on recent work by Devin McDougald (GTV), as well as Justin Becker (CB). We also highlight the Penn Pathfinders program, and its efforts to give students exposure to a wide variety of career options. Finally, get to know the CAMB Student Newsletter team members, and enjoy a fun crossword.

For more articles, past issues, and information about the CAMB Newsletter team, visit out blog at **cambnewsletter.wix.com/blog**. We appreciate any feedback on ways we can improve future issues of the Newsletter. Additionally, new members are always welcome! For feedback or to get involved, please contact us at **camb.studentnews@gmail.com.** We hope you enjoy the May 2018 Issue!

Sincerely,

Somdutta Mukherjee and Lexy Stanley

Editors-in-chief

RESEARCH SPOTLIGHT

Focusing in on novel gene therapies for optic neuritis

Camille Syrett

The ability to target abnormal genes by introducing functional copies through gene therapy holds extreme promise for the treatment of human diseases. Since the first human therapeutic gene transfer in 1990, gene therapies have been efficacious in treating diseases like hemophilia and leukemia, and this list is continuously growing. Importantly, gene therapies reflect a shift in modern medicine from the treatment of symptoms to the permanent correction of underlying genetic causes of disease.

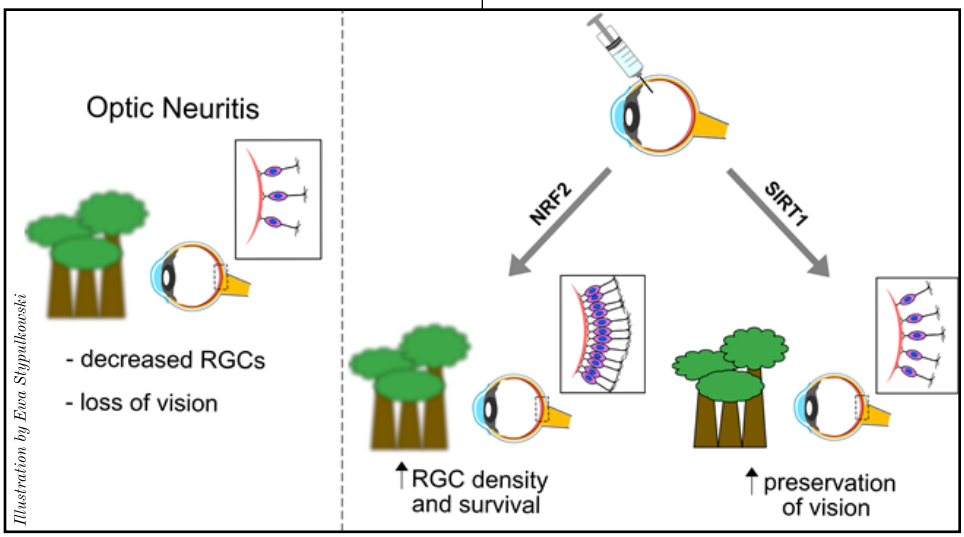
As not all diseases are created equal, gene replacement therapies are harder to implement if the genetic mutations resulting in pathologies are unclear. Recent research published by fifth year GTV student Devin McDougald aims to develop a broad-spectrum approach to gene therapy by targeting conserved pathways in disease pathogenesis. As a member of Dr. Jean Bennett's laboratory in the Department of Ophthalmology, Devin investigates canonical neurodegenerative mechanisms for novel gene therapies in ocular diseases such as optic neuritis.

Visual impairment due to optic neuritis often manifests in patients with Multiple Sclerosis (MS), which can lead to permanent visual dysfunction. In some cases, irreversible loss of vision is caused by the degeneration of retinal ganglion cells, or RGCs-neurons that are important for accurate visual processing. Working with collaborators in Dr. Ken Shindler's lab at Penn, Devin and colleagues set out to promote neuroprotection in optic neuritis using a powerful mouse model for studying MS pathogenesis. Similar to MS patients, experimental autoimmune (EAE) mice lose RGCs

Devin McDougald, GTV

and suffer from visual impairments with age. It is hypothesized that oxidative injury from the accumulation of harmful reactive oxygen species (ROS) are integral to MS pathology and optic neuritis. With this in mind, Devin hypothesized that they could promote neuroprotection by reducing oxidative injury and enhancing redox homeostasis.

Two likely candidate genes to target, NRF2 and SIRT1, both have antioxidant properties and play integral roles in promoting cell survival. Notably, Nrf2 -/- mice lose RGCs and are visually impaired, while SIRT1 overexpression promotes the survival of RGCs. To examine their neuroprotective roles in optic neuritis, Devin successfully created AAV2 expression plasmids containing human NRF2 or SIRT1 under a ubiquitous promoter. AAV2-NRF2 or AAV2-SIRT1 were transduced into cell lines to examine transgene expression and cellular localization. After confirming successful RNA and protein upregulation in transduced cell lines, AAV2-NRF2 or AAV2-SIRT1 were transduced into the retinas of 4-week postnatal C57Bl/6J mice via intravitreal delivery, with an approximately 20% RGC transduction rate for both expression vectors. Four weeks after transduction, mice were immunized with MOG to induce EAE, which begins to develop within two weeks of immunization.



Optic neuritis results in a loss of retinal ganglion cells (RGCs), resulting in irreversible vision loss. Intravitreal injection of AAV2-NRF2 or AAV2-SIRT1 demonstrates neuroprotective effects in experimental autoimmune encephalitis (EAE) mice, where NRF2 promotes RGC survival and proliferation while SIRT2 prevents further loss of vision.

To determine if NRF2 or SIRT1 gene transfer can preserve visual function in optic neuritis, transduced EAE mice were evaluated using Optokinetic Response Recordings (OKR) compared to AAV2-eGFP transduced EAE animals. This eye tracking software measures functional response of the eye, with a decline in OKR score denoting a decrease in responsiveness of the eye. Intriguingly, EAE eyes treated with AAV2-SIRT1 but not AAV2-NRF2 showed less decline in visual function, suggesting that SIRT1 can preserve functionality of the eye during EAE-mediated optic neuritis.

Because the decline in vision during optic neuritis is associated with the loss of RGCs, Devin and colleagues evaluated the ability of NRF2 or SIRT1 gene augmentation to improve RGC survival during EAE. Retinal treatment with AAV2-NRF2 showed a statistically significant increase in the number of intact RGCs, suggesting that NRF2 promotes RGC survival. While AAV2-SIRT1 also trended towards an increase in RGC density, it was not statistically significant. Finally, the effects of gene transfer on optic nerve inflammation and demyelination, two hallmarks of autoimmune-mediated lesions in MS contributing to visual decline, were determined in EAE mice transduced with NRF2, SIRT1, or eGFP. In optic nerve sections, no differences in immune cell recruitment or nerve demyelination were observed, suggesting that gene transfer does not preserve visual function through these pathways.

Taken together, Devin and colleagues successfully harnessed the neuroprotective effects of NRF2 and SIRT1 in optic neuritis using their novel gene augmentation strategy. Importantly, this study has set a framework for other experiments in the lab. Devin says, "we are actively pursuing ways to optimize the approaches outlined in the paper as well as applying them to other pre-clinical models of optic neuropathy in collaboration with other Penn investigators. We are particularly interested in the SIRT1 approach because it was able to show some degree of functional preservation in the EAE model." He also notes that "one of the major limitations in the study surround[s] the very low transduction efficiency of our AAV2 vectors, which likely played a role in observing such small effects on phenotype rescue." To enhance targeting efficiency, Devin has generated a new panel of AAVs that target RGCs. With these modifications, he hopes to increase the demonstrated neuroprotective effects of targeting oxidative injury and redox homeostasis.

McDougald DS, Dine KE, Zezulin AU, Bennett J, and Shindler KS. *Vaccine* 2016;34:5483-87. SIRT1 and NRF2 gene transfer mediate distinct neuroprotective effects upon retinal ganglion cell survival and function in experimental optic neuritis. *Invest Opthalmol Vis Sci.* 2018, 59:1212-1220.

Gradient-seq: seeking better insight into chromatin states

Clarissa Rous

Justin Becker, CB

Many of us remember being taught a simplified doctrine on histone modifications: certain marks tend to appear on transcriptionally inactive, condensed heterochromatin, while others characterize active, open euchromatin. In particular, H3K27me3 tends to mark facultative heterochromatin that may be expressed during development, whereas H3K9me3 is associated with constitutive heterochromatin. However, histone modifications do not reliably distinguish between heterochromatin and euchromatin, as numerous studies have shown, and as Justin Becker and colleagues in the Zaret lab demonstrate in their recent publication in *Molecular Cell*. They debut a new technique called gradient-seq that separates chromatin based on physical properties instead of covalent histone modifications, and use this tool to interrogate differences between heterochromatic and euchromatic DNA bearing various histone marks. The researchers began by critically assessing the standard protocol for ChIP-seq, which requires crosslinking and sonicating chromatin, precipitation using antibodies to histone marks or other proteins, and then sequencing. The sonication step is crucial, as only DNA fragments under a certain size can be sequenced. However, dense heterochromatin is resistance to shearing and may be underrepresented in the sequencing reads.

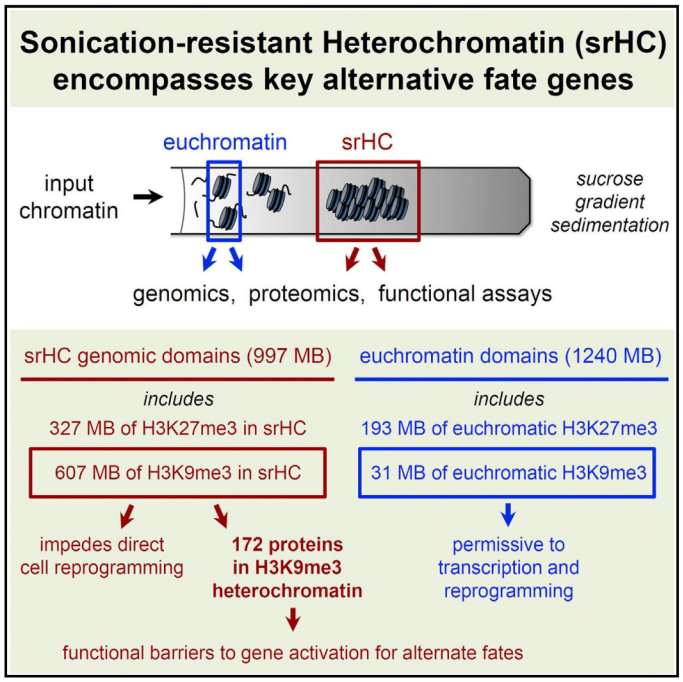
Justin developed the idea of placing crosslinked and sonicated chromatin in a sucrose gradient to separate fragments into lighter, shorter, sonication sensitive portions, and heavier, longer, sonication resistant segments. He then sequenced these purified chromatin fractions (a method termed gradient-seq) and asked whether the gradient had successfully sorted DNA into euchromatin and heterochromatin. As expected, the longest DNA segments possessed characteristics of heterochromatin: traditionally

repressive histone marks, low transcriptional activity, and CpG methylation. Sequencing revealed that genes in the heavy DNA chromatin fraction were marked with H3K9me3 and resisted activation during fibroblast reprogramming to hepatocytes, meaning that they are functionally heterochromatic. Thus, the heaviest gradient fraction earned the name "sonication resistant heterochromatin" (srHC). In contrast, the euchromatic DNA fragments were actively transcribed, unmethylated at CpG islands, enriched for traditionally permissive histone marks, and depleted for repetitive elements.

Having reliably separated heterochromatin and euchromatin, the team investigated the true landscape of histone marks on each type of DNA. The srHC fraction contained the majority of H3K9me3 and H3K27me3 marks, which generally did not overlap. Surprisingly, certain subpopulations of euchromatin also bore the "repressive" histone marks H3K9me3 and/or H3K27me3, despite being transcribed. These subpopulations also carried the H3K36me3 mark, which corresponds to transcriptional elongation. The euchromatic H3K9me3 population of DNA was highly enriched for KRAB-containing zinc finger nucleases (KRAB-ZNFs). These genes had an unusual pattern of histone marks: dual marking by H3K9me3 and the elongation mark H3K36me3, H3K9me3 in the gene body, and H3K4me3 at the promoter. Euchromatic DNA with H3K27me3 contained genes for many transcription factors, especially HOX family genes. During reprogramming of fibroblasts to hepatocytes or cholinergic neurons, genes marked by H3K9me3 and/or H3K27me3 in the euchromatin gradient portion were more prone to activation than those in the heterochromatic gradient portion. This demonstrates that the functional capacity of chromatin is more accurately represented by gradient separation than by histone modifications.

Nuclease digestion is often used to separate DNA by chromatin state, given that nucleases cut at euchromatic regions and leave denser, protein-bound heterochromatin untouched. However, when Justin compared DNA digestion by DNase and micrococcal nuclease to the fractions obtained by gradient separation, there were notable differences. The nucleases were unable to digest H3K9me3-marked DNA that is euchromatic by transcriptional activity and sonication sensitivity. Thus, gradient-seq is a more reliable indicator of chromatin state than nuclease digestion.

With a tool in hand to separate euchromatin from heterochromatin, the researchers next investigated which proteins are bound to heterochromatin, especially H3K9me3-marked heterochromatin, which is particularly resistant to transcriptional activation during cellular reprogramming. They performed mass spectrometry on srHC DNA, srHC DNA following H3K9me3 inmmunoprecipitation, and the "gradient top" fraction containing euchromatic DNA and soluble proteins. There were 217 proteins enriched in the srHC fraction, 172 enriched in H3K9me3 srHC, and 1474 unique to or enriched in the gradient top. Many of the proteins bound to H3K9me3 srHC are involved in chromatin organization, control of gene expression, and inhibition of reprogramming; such proteins include linker histones, lamin B1, HDAC2, HNRNPK, and NONO and SFPQ. Intriguingly, the srHC fraction also contained six proteins frequently mutated in Amyotrophic Lateral Sclerosis (ALS). These proteins bear domains that mediate phase separation, which has been shown to be involved in chromatin organization.



Sucrose gradient sedimentation separates sonicated chromatin into euchromatin and heterochromatin as characterized by DNA sequencing matched to mRNA sequencing, proteomics, nuclease sensitivity, and impact on cell reprogramming. The technique, called gradient-seq, is more accurate than histone modifications in identifying euchromatin and heterochromatin, and can be used to better characterize chromatin states (Graphical Abstract from Becker et al. 2017, *Molecular Cell 68*, 1023).

Mutations in these proteins could, therefore, lead to abnormal phase separation and chromatin dysregulation, which may contribute to ALS pathogenesis. The technique and the data sets that Justin created in this study certainly hold promising leads to inform research on chromatin regulation and disease states. Finally, when many of the proteins enriched in H3K9me3 srHC were knocked down using RNAi, fibroblasts became more easily converted to hepatocytes, indicating that H3K9me3 heterochromatin functions to restrict changes to cell identity.

In summary, the technique of gradient-seq, which couples DNA chromatin separation by size using a sucrose gradient with DNA sequencing, delineates heterochromatin and euchromatin based on the true functional state of chromatin rather than on histone modifications. Justin demonstrates that ChIP-seq data sets are skewed by the exclusion of large, sonication resistant heterochromatin fragments and that histone modifications alone do not reliably predict chromatin state. Gradient-seq can be paired with proteomics, histone modification mapping, or other means of characterizing DNA chromosomes in order to truly interrogate differences between euchromatin and heterochromatin.

Becker JS, McCarthy RL, Sidoli S, Donahue G, Kaeding KE, He Z, Lin S, Garcia BA, Zaret KS. Genomic and Proteomic Resolution of Heterochromatin and Its Restriction of Alternate Fate Genes. *Mol Cell*. 2017 Dec 21;68(6):1023-1037.e15.

SPECIAL INTEREST

Penn Pathfinders: Guiding Ph.D. students to achieve their career goals

Siddharth Kishore

Finding the right career can be a difficult and often times stressful decision for Ph.D. students, especially given the relative lack of resources and mentoring for those wanting to pursue careers outside of academia. The Penn Pathfinders program addresses this issue by guiding doctoral students to make informed decisions about career paths in the biomedical sciences. The program helps students thoroughly evaluate their career options, and provides a platform to explore how their passions, skills and values align with careers in academia, non-academic settings, entrepreneurship, and teaching.

The Penn Pathfinders program is a brainchild of Dr. Susan Margulies and Dr. Glen Gaulton. Susan Margulies was a professor in Bioengineering Department at Penn, and recently took over as the chair of the Department of Biomedical Engineering at Georgia Tech and Emory. Glen Gaulton is a professor in the Department of Pathology and Laboratory Medicine, as well as Vice Dean and Director of Global Health in the Perelman School of Medicine. Both, Dr. Margulies and Dr. Gaulton, have been involved in graduate education for many years, and have trained their own students who have followed diverse career paths. These experiences made them feel comfortable in providing advice to graduate students regarding non-academic career choices. Having held various leadership roles within Penn, they also realized that many mentors do not have experience in guiding students who wish to pursue careers outside academia. So they coalesced their thoughts and submitted grant applications to the National Science Foundation (NSF) to develop a structured training program that provides trainees in biomedical sciences and bioengineering with career development opportunities, and also investigate its effectiveness in comparison to non-trainee cohorts. They were awarded a $500,000 grant for three years in 2015 to pursue their efforts.

Glen Gaulton, Ph.D., Professor of Pathology and Laboratory Medicine

The program is currently in its second year of funding and has accepted two cohorts of about twenty people- a mix of biomedical and bioengineering graduate students. Each cohort meets once every month at workshops led by experts in a variety of topics. These range from learning how to effectively present your research and communicate your skills, to efficient time and project management. The workshops are frequently followed by career panels, with panelists who have successfully pursued careers outside of academia. In conjunction with these group events, students have one-on-one mentoring sessions with Drs. Gaulton and Margulies to work on individual professional development plans. The progress of individual students is tracked via comprehensive surveys before and after each monthly session. I had the opportunity to chat with Dr. Glen Gaulton about some of the program's initial findings, which are being collated to be published later this year. Gaulton says, “One of the main findings from the first two years of the program is that it isn’t enough to just have career panels, courses or workshops, but having all of these directed at the right time and sequence for students. For example, having a small workshop or informal session within the cohort about the challenges with networking followed by a career panel. There are quite a few career panels held on campus, but it’s highly beneficial to understand how to network with them and get the most out of it. Also, having people sit down and talk about their fears and reservations with their peers helps to identify and overcome them.”

My personal experience with the Pathfinders program has been extremely enlightening. I’ve had the opportunity to do an internship at Militia Hill Ventures, a life sciences venture capital (VC) firm thanks to one of the career panelists who came to speak to us. This internship has presented me with the opportunity to meet interesting people from biotech, pharma and VC firms, and as a consequence, expand my professional network. Unsurprisingly, my experience is by no means unique. In fact, about half of the students involved in the Pathfinders program are doing internships in fields that interest them. This includes a gamut of opportunities including writing, education (working at small colleges or high schools), projects at Penn Center for Innovation and Science Center, and many more. Of the two students that have graduated so far, one is a full-time teacher at Villanova, and one works at a VC firm. Both of them identified their ideal careers and explored their interest in these jobs over the two years of the program. “The key is to make students aware of all the options and learn more about the things that interest them so that they can make a more informed decision about what is right for them,” summarized Gaulton.

When asked about plans to expand the program to include more students in the future, Dr. Gaulton is hopeful and enthusiastic. He and Dr. Margulies have presented the findings from the first two years of the program to the heads of BGS and Bioengineering, and they are committed to continuing the program and mainstreaming it into the curriculum. The grant was a pilot fund and the expectation is to work out the program and share the findings with other universities that run similar programs. The goal is to launch the program on a larger scale next Fall. Gaulton envisions the limiting factor to be finding the right mentors, and he is keen on identifying and mentoring professors in the near future. He adds that, “Most of the professors are aware that not all trainees will go into academia. However, a lot of them are wrongly worried about the students’ quest to develop their professional attributes may impede the quality of their science and graduation timelines. But, there’s a way of providing professional guidance to trainees, without that negatively influencing their graduate training. The key is to develop these skills in conjunction with each other.”

Dr. Gaulton concludes with some valuable advice for grad students, “It’s never too early to start thinking about careers. Asking questions like, where do my talents lie? what kind of life do I want to lead? What are some of the doubts and fears I need to overcome? are a great step towards assessing what’s a good career fit for you. It’s always better to start thinking early in your Ph.D. life and keeping your options flexible.”

A pi(e) for a PI

Ewa Stypulkowski

In keeping with the spirit of tradition, the CAMB Student Newsletter team hosted the second annual “Pie a PI” event on Friday, March 16. This event raised money and awareness for a local charity, by having donors throw whipped cream pies at CAMB faculty. In the name of supporting a good cause, Craig Bassing (CB), Brian Keith (CB), Paul Bates (MVP), Jean Bennett (GTV), Valder Arruda (GTV), Dan Kessler (chair-CAMB), and Kelly Jordan-Sciutto (director-BGS) volunteered to have pies thrown at their faces by students and postdocs for $1 raffle tickets.

Once again, the Newsletter team dedicated the proceeds of the event to the Children's Scholarship Fund Philadelphia (CSFP http://www.csfphiladelphia.org/). Founded in 2001, CSFP is a privately funded, Pennsylvania-based partner of the national Children’s Scholarship Fund, and is committed to improving educational and social outcomes for children from low-income families. Every year, the program awards up to 2,000 need-based scholarships through a lottery drawing, helping children from low-income families attend private schools. According to their website, approximately 96% of CSFP alumni will graduate high school on time (vs. approximately 70% for Philadelphia public schools) and 80% of scholarship recipients will pursue higher-education.

CSFP currently serves about 5,200 elementary and middle school children (K-8th grade) enrolled at over 170 private and parochial schools, and supports students for up to 4 years. According to their mission statement, CSFP is proud to be the most “broad-based and diverse K-8th grade scholarship program in Pennsylvania”. The program is currently ranked in the top 7% of charities and has been awarded 5 consecutive perfect score ratings by Charity Navigator (http://www.charitynavigator.org). According

to Charity Navigator metrics, CSFP is more financially healthy, accountable, and transparent than other organizations supporting similar causes.

When asked about her feelings on getting pie-d, Jordan-Sciutto remarked, “It’s such a great event! I think the play on words pi, pie, and PI, is fun, and I’m thrilled to support a great cause!” Thanks to our donors, the Newsletter team was able to raise close to $200 for CSFP this year. The donation will go towards CSFP’s operational costs, and provide a vital and meaningful service to Philadelphia’s youth. From all of us at the CAMB Student Newsletter, we’d like to extend a huge thank you to the faculty, donors, and everyone who helped make this event a success. We hope to see you again next year!

Faculty from left to right (top photo): Valder Arruda, Craig Bassing, Kelly Jordan-Sciutto, Jean Bennett, Paul Bates, Brian Keith, Dan Kessler. Picture credit to Kathleen O’Connor-Cooley and Gleb Bazilevsky.

Getting to Know Your Fellow CAMB-ers

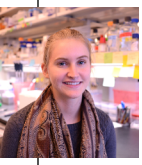
CAMB Student Newsletter Team

Somdutta Mukherjee, *Editor-in-chief and writer*

Somdutta is a 5th year student in DSRB. Her research focuses on studying liver development using human pluripotent stem cells. Outside of the lab, she enjoys doing arts and crafts projects and playing the violin.

Alexandra Stanley, *Editor-in-chief and writer*

Lexy Stanley grew up in San Diego, California. She went to the University of California, San Diego and got a B.S. in Human Biology. Lexy works on bone and muscle development and regeneration in Eileen Shore's laboratory. When not in lab, she’s playing with her dog, kickboxing, or wine tasting.



Camille Syrett, *Editor-in-chief and writer*

Camille is a 5th year DSRB student studying X-chromosome inactivation in female-biased autoimmunity as part of the Anguera lab. She's named after a hurricane, and she fosters police dogs in training and loves cooking in her free time.

Arwa Abbas, *Blog manager, graphic designer, and writer*

Arwa studies new and novel human viruses in Rick Bushman’s lab. She has farfetched plans to write her thesis as a graphic novel or as an interactive Shiny web app.

Lindsey Weed, *Design editor and writer*

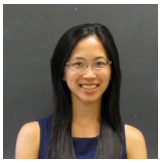
Lindsey is a 4th year GTV student developing a photoreceptor model of Leber's Congenital Amaurosis from patient-derived iPSCs in the Bennett lab. She plays in a bunch of Philadelphia sports leagues and recently completed her second marathon.

Siddharth Kishore, *Graphic designer and writer*

Sid is a 4th year student in DSRB studying pancreas development using human embryonic stem cells as a model. He spends his free time playing soccer, muay thai and attempting to paint surrealist art.

Gleb Bazilevsky, *Writer*

Gleb is a 6th year G&E student working on the biochemistry of acetyl-CoA-producing enzymes. He loves long-leaf black teas and is active on Goodreads.



Annie Chen, *Writer*

Annie is a 5th year MVP student in the Goulian lab. She is currently studying two-component systems involved in antibiotic resistance. Outside of lab, she enjoys running, reading, and playing the clarinet.

Kanika Jain, *Writer*

Kanika Jain is a first year student in the Cancer Biology program. She is passionate about pursuing a career in Tumor Immunology. She has worked as a Radio Jockey (RJ) for her college radio before and takes special interest in dancing and cooking!

Hannah Kolev, *Writer*

Hannah is a first year DSRB student studying stem cell organization and maintenance in the small intestine. She enjoys cooking, taking road trips, and exploring Philadelphia!

Sylvia Stankov, *Writer*

Sylvia is a 1st year GTV student. She enjoys cooking, kayaking and sunshine.

Clarissa Rous, *Writer*

Clarissa is a CAMB student interested in stem cell biology and dynamics of APC tumor suppressor loss during colon cancer. She enjoys exploring new places, both in person and through culinary adventures!

Iryna Shakhmantsir, *Writer*

Iryna is 6th year DSRB student who investigates how biological systems keep time. She enjoys potatoes, Soviet monumental architecture and thai kickboxing.

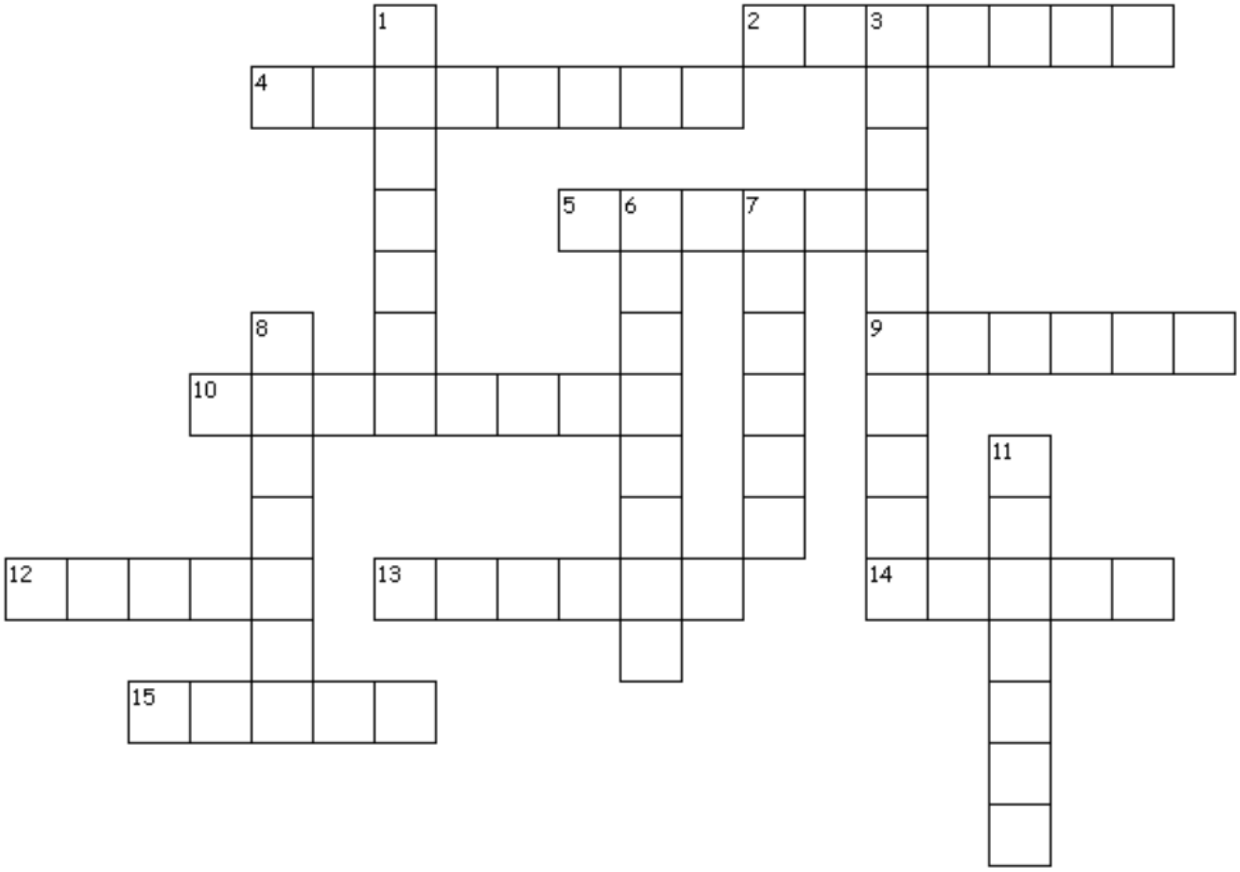
Ewa Stypulkowski, *Writer*

Ewa is a 6th year DSRB student studying the function of lipid modification on cell polarity in developmental and disease settings. She enjoys organizing, restoring furniture, and has a massive sweet tooth.

Huanjia Zhang, *Writer*

Huanjia is a Lab Technician at the PennCHOP Microbiome Program. He loves the art and craft of storytelling--stories of the science, the people, and the life behind the bench.

NAME THAT SCIENTIST!



Across
2. Inheritance of acquired characteristics academic
4. DNA X-ray crystallographer
5. Father of genetics
9. Laws of motion mathematician
10. Endosymbiotic theory proponent
12. "Cell" coiner
13. PCR method inventor
14. Citric acid cycle chronicler
15. Radioactivity Nobel laureate

Down
1. English cosmologist
3. Jumping genes pioneer
6. Theory of relativity physicist
7. On the Origin of Species author
8. Spoilage preventer
11. Penicillin knight