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ASBMB TODAY

THE MEMBER MAGAZINE OF THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

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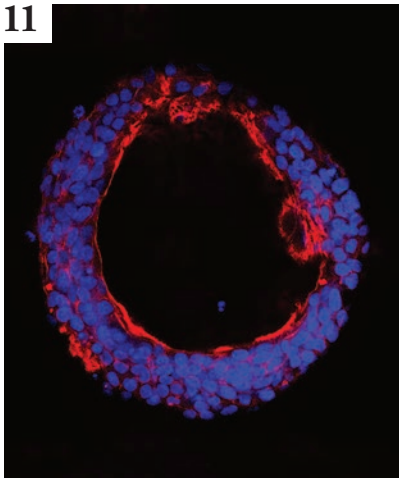

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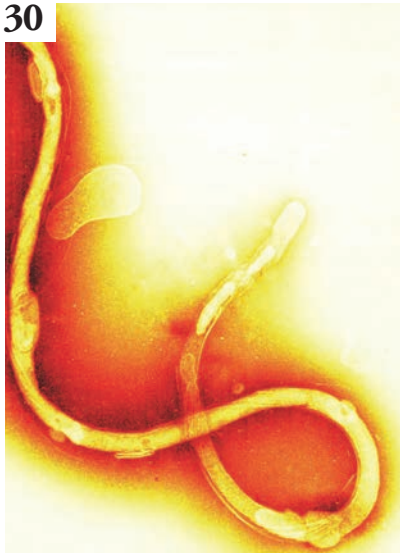

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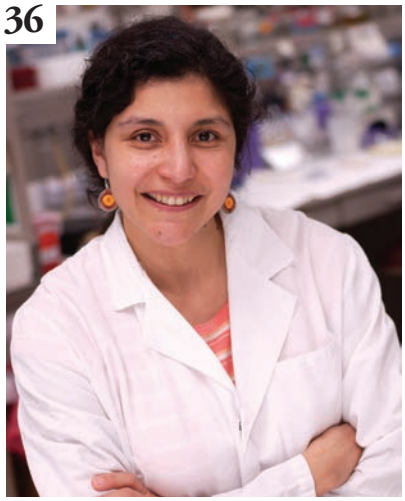

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Not an end, but a beginning

By Natalie Ahn

I write my final president's message with some thoughts about the state of the American Society for Biochemistry and Molecular Biology and goals for our future.

First, congratulations again to our next president, Gerald Hart. Throughout his career, Jerry has been a dedicated contributor to the ASBMB with his service in many groups, including Council, and current work as associate editor for both the Journal of Biological Chemistry and Molecular & Cellular Proteomics. Jerry is an inspirational leader, and with his wisdom and devotion, our society is in good hands.

The ASBMB is in excellent financial shape. And we see other upward trends:

Our three journals continue to publish high-quality molecular life science research. Under the stewardship of Lila Gierasch, the Journal of Biological Chemistry has implemented many changes to enhance transparency, rigor and the speed of reviews. The publishing experience for authors has improved, and this has led to an upturn in manuscript submissions this year. The best practices learned now are being adopted by Molecular & Cellular Proteomics and the Journal of Lipid Research; both remain the highest impact journals in their fields.

Attendance at the annual meeting has grown steadily by an average of 8 to 9 percent each year, thanks to the work of the Meetings Committee. We have heard tremendous enthusiasm for their revamp of the meeting into a more vibrant format, with symposia topics on the latest discoveries and more opportunities for participants to present their work. Discussions are ongoing with other Experimental Biology societies to coordinate parts of our programs to offer attendees a more integrated meeting experience. We welcome your feedback as we work to build fresh, interdisciplinary perspectives through collaborations among our societies.

The Educational and Professional Development and Student Chapters committees have created resources to empower the next generation and support the careers of scientists at every stage. They are developing new workshops and webinars on how to prepare for, enter, advance and succeed in diverse science careers. The ASBMB biochemistry accreditation program is expanding nationally to provide students with degree certification and the means for departments to adopt innovative concepts-based teaching methods and assess student mastery of core concepts in the molecular life sciences.

The Minority Affairs Committee works to ensure a diverse science workforce at all levels. At the annual meeting, MAC members organize the Issues in Depth Symposia, covering recent scientific advances and their

CONTINUED ON PAGE 6

The appropriations outlook

By Benjamin Corb

As the weather heats up in Washington, D.C., so too does discussion around funding the government for fiscal year 2019, which begins Oct. 1. The annual appropriations process has begun in earnest, with legislators developing their funding plans and advocates making the case for special interests.

After months of continuing resolutions and two government shutdowns, fiscal 2018 was a boom year for science funding. The National Science Foundation and Department of Energy saw modest budget increases, and the National Institutes of Health saw an unexpected $3 billion increase.

Will 2019 be another year of support for the life sciences? There are three reasons to be hopeful that 2019 will continue the trend and three reasons to be concerned.

Three reasons to be hopeful:

It is an election year. The Republican majority in Congress is looking to prove it can govern and avoid lurching from one legislative crisis to the next as has become the norm on Capitol Hill. The party could demonstrate leadership by completing appropriations work on time without the threat of continuing resolutions or government shutdowns.

Appropriators already are saying good things. During an Act for NIH celebration event this spring, Senate Appropriations Committee Chairman Roy Blunt., R-Mo.; House Labor, Health and Human Services Appropriations Committee Chairman Tom Cole, R-Okla.; and ranking member of the House Appropriations Committee Nita Lowey, D-N.Y., told attendees that another increase of $3 billion to the NIH was more than in the air for fiscal 2019.

Budget caps already are negotiated. In recent years, the appropriations process has been delayed by the need to renegotiate spending caps. Once the caps were raised, lawmakers had to renegotiate spending levels at federal agencies. Those increased spending caps are in effect again for fiscal 2019, so we already have a clear sense of how much money Congress can spend.

Three reasons to be concerned:

It is an election year. The legislative calendar is more condensed than it is in nonelection years. Congress has an annual recess from August through Labor Day. This year, in addition to the summer recess, lawmakers will be home to campaign from mid-October through early November. This will make it harder to do all the legislative work required before the end of fiscal 2018. In three of the past four election years, appropriations work was delayed to the next calendar year.

The election will change things. After the election, we'll have a lame-duck Congress, with a potential change of majority when the new Congress is seated in January. Partisans could try to delay or speed up the appropriations process in concert with changing political priorities. This uncertainty could complicate negotiations.

The NIH has been doing very well. The $3 billion increase for fiscal 2018 followed a $2 billion increase for fiscal 2017 and a $2 billion increase for fiscal 2016. This much-needed growth will not continue into perpetuity. We are beginning to hear grumbling on Capitol Hill about NIH funding fatigue, with some lawmakers saying Congress has done enough to support the NIH.

This summer, the American Society for Biochemistry and Molecular Biology public affairs office will continue our advocacy to keep funding of basic life-science research a priority for lawmakers. With a trend of funding increases and with medical research largely viewed as important, we remain hopeful that the positive trends will continue. For updates on the process and how you can help, follow our blog at policy.asbmb.org.

Benjamin Corb (bcorb@asbmb.org) is director of public affairs at the ASBMB. Follow him on Twitter @bwcorb.

Member update

By Erik Chaulk

Four elected to academy

Four American Society for Biochemistry and Molecular Biology members are among the newly elected members of the American Academy of Arts and Sciences.


GRONENBORN


AHN


BERMAN


LANDICK

Angela M. Gronenborn is the University of Pittsburgh Medical Center Rosalind Franklin professor and chair in the department of structural biology and a distinguished professor of structural biology at the University of Pittsburgh School of Medicine. In her lab, she seeks to understand the structural basis of cellular interactions.

Natalie Ahn is a professor of distinction at the University of Colorado Boulder and outgoing president of the ASBMB; she previously served on the ASBMB Council. Her research focuses on understanding the mechanisms of cell signaling.

Helen Berman is the board of governors distinguished professor emerita of chemistry and chemical biology at Rutgers University. A co-founder of the Protein Data Bank, Berman specializes in the study of nucleic acids, protein–nucleic acid interactions and collagen.

Robert Landick is a professor in the department of biochemistry at the University of Wisconsin–Madison. His research explores the structure and function of RNA polymerase.

Founded in 1780, the American Academy of Arts and Sciences is one of the oldest academic societies in the United States; it admits leaders from a variety of backgrounds, including the humanities, arts and education as well as science, engineering and technology.

Farnham named interim director

Peggy Farnham has been named interim director of the University of Southern California Norris Comprehensive Cancer Center.


FARNHAM

Effective June 1, Farnham took over direction of the center, a leading institution in the research, treatment and prevention of cancer. She is co-leader of the epigenetics and regulation program and interim associate director for basic science at the center.

She serves as the William M. Keck professor of biochemistry as well as chair and professor of biochemistry and molecular medicine in the Keck School of Medicine at USC. Her research focuses on the study of chromatin regulation and its control of transcription factor binding and function.

Elgin receives faculty award

Sarah C.R. Elgin, a professor at Washington University in St. Louis, has received the university's Holly Compton Faculty Achievement Award.


ELGIN

The award is one of several recognizing the outstanding achievements of members of the university's faculty.

Elgin is the Viktor Hamburger professor of arts and sciences and is a professor in the department of biology. A member of the Washington University faculty since 1981, her research explores the role of chromatin structure in gene regulation.

Elgin has been involved in several scientific outreach programs, including founding the forerunner to the Institute for School Partnership, an initiative focusing on improving K–12 education.

She also founded the Genomics Education Partnership, a program providing undergraduate students with opportunities to participate in genomics research.

Elgin and other faculty award recipients will be honored at a ceremony in October.

Fliesler named distinguished professor

Steven J. Fliesler has been appointed as a distinguished member of the faculty by the State University of New York.

Fliesler is among 15 faculty members recognized as distinguished professors for significant contributions

toward research and scholarship.

He is a professor in the departments of ophthalmology and biochemistry and holds the Meyer H. Riwchun endowed chair in ophthalmology at the University of Buffalo Jacobs School of Medicine and Biomedical Sciences.


FLIESLER

A leading figure in the field of ophthalmology, Fliesler's research explores the retina and pathologies that affect vision.

He is a former president of the International Society for Eye Research and recently was elected president of the Association for Research in Vision and Ophthalmology.

Kelly named Gustavus provost/dean

Brenda Kelly is the new provost and dean of Gustavus Adolphus College in Minnesota.


KELLY

In this role, Kelly will serve as the chief academic officer for the university, leading the institution's academic initiatives and strategic planning.

Kelly has been a faculty member at Gustavus Adolphus College since 2002. She served as chair of the department of chemistry from 2011 to 2015 and director of the biochemistry and molecular biology program from 2013 to 2014.

She has served as interim provost since 2016 and assumed her new responsibilities in May.

Five named Goldwater scholars

Five members of American Society for Biochemistry and Molecular Biology Student Chapters are among the 211 students recognized as Barry Goldwater Scholars.

The Goldwater scholarship program recognizes outstanding college sophomores and juniors in the natural sciences, engineering and mathematics. Recipients are awarded up to $7,500 to help cover tuition and fees for their education.


PANNULLO


ZUBAIR


DALAPATI


GEORGE


SHORT

Rochester Institute of Technology National Technical Institute for the Deaf student Nicole Pannullo is the first deaf RIT student to win this award. Pannullo hopes to earn a Ph.D. in regenerative medicine and develop therapies for genetic disorders.

Humza Zubair, a student at Arizona State University, hopes to earn a Ph.D. in biochemistry and biological sciences and then teach and do research.

Trisha Dalapati, a student at the University of Georgia, hopes to earn an M.D./Ph.D. in infectious diseases and then conduct research on pathogeneses of infectious diseases.

Stephan George, a student at the University of Georgia, hopes to earn a Ph.D. in biochemistry and then teach and do research on mechanisms of disease progression underlying hereditary neural disorders.

Audrey Short, a student at Miami University in Ohio, hopes to earn a Ph.D. in biophysics and then teach and conduct research.

In memoriam: Yves Louis Marcel

Yves Louis Marcel, professor emeritus at the University of Ottawa, passed away in February. He was 81.


MARCEL

Born in Bordeaux, France, on Feb. 11, 1937, Marcel obtained his bachelor's degree from the University of Rouen and his Ph.D. from the University of Toulouse.

Marcel worked as a research scientist at the Montreal Clinical Research Institute before joining the faculty at the University of Ottawa Heart Institute, where he was a professor in the department of pathology and laboratory medicine as well as in the department of biochemistry, microbiology and immunology.

In 1992, Marcel formed the Atherosclerosis Research Group at the University of Ottawa Heart Institute, serving as chief scientific officer. He also served as director of the High-Density Lipoprotein Biology Laboratory until retiring in 2013.

Marcel spent much of his career studying high-density lipoproteins. He contributed significant research on apolipoprotein A1 structure and reverse cholesterol transport.

Marcel was honored with the Royal Society of Canada's McLaughlin Medal in 1997 and with an Ottawa Life Sciences Council award in 2001.

He is survived by his wife, Ruth McPherson; their daughter, Gabrielle; and his children, Valerie and Christophe.


Erik Chaulk (echaulk@asbmb.org) is a peer-review coordinator and digital publications web specialist at the ASBMB.

CONTINUED FROM PAGE 2

societal implications, especially for minority populations. Members also organize instructive, hands-on mentoring for young scientists, including the yearly Interactive Mentoring Activities for Grantsmanship Enhancement, or IMAGE, workshop, one of the best grant writing workshops around. Open to early-career investigators, the IMAGE mentorship lifts participants' confidence in grant writing, resulting in a phenomenal success rate; 80 percent of attendees receive funding within two years.

These days, every scientist must be an effective communicator. The Public Outreach Committee creates effective tools to teach scientists how to tell the public what we do and why it's important. You can learn these skills by enrolling in the Art of Science Communication online course and workshops at the annual meeting. The upcoming ASBMB special symposium on science outreach in October will foster peer connections and education in the growing field of science outreach. Not only will outreach training help you promote science in society, it teaches you skills for more effectively conveying ideas in grant applications and job interviews.

Members of the Public Affairs Advisory Committee are on the front lines, advocating for the needs of the ASBMB membership. They keep us up to date on science policy and disseminate information to legislative leaders and federal agencies on issues that impact us all. At the annual ASBMB Capitol Hill Day, the PAAC provides an opportunity for scientists to meet their congressional representatives, one of the most effective actions that we as individuals can take in advocating for sustainable, predictable investments in science; enhancing STEM education; and strengthening America's scientific workforce through immigration reform.

Looking forward, the most important challenge for the ASBMB is to grow our membership, especially among young scientists entering our field. The newly formed Membership Committee has taken on this important task and soon will reach out to members like you for assistance. Now more than ever, we need your help to keep our society strong.

I hope I've conveyed the many ways in which the ASBMB directly benefits you, the scientists and educators in our field of molecular life sciences. Our society is a resource for you and stands up for you at every stage of your career. In return, please stand with the ASBMB. If you're not an ASBMB member, it's time to join. If you're already a member, please invite a colleague or student to join. Help us amplify our message about the value of your work and ensure a sustainable future for science discovery and education.

Natalie Ahn (natalie.ahn@colorado.edu), a professor of chemistry and biochemistry at the University of Colorado, Boulder, is president of the ASBMB.

2018 honor society inductees announced at annual meeting

The American Society for Biochemistry and Molecular Biology Honor Society (Chi Omega Lambda) recognizes exceptional undergraduate juniors and seniors pursuing degrees in the molecular life sciences at colleges or universities with ASBMB Student Chapters. Students are recognized for their scholarly achievement, research accomplishments and outreach activities. Information on nominating a student for the honor society is at asbmb.org/education.

ASBMB PHOTO

American Society for Biochemistry and Molecular Biology Honor Society inductees pose for a group photo in April during the 2018 ASBMB Annual Meeting in San Diego.

The 2018 inductees are:

Benjamin Anderson, Purdue University
Will Barr, Wesleyan University
Shannyn Bird, University of Nebraska–Lincoln
Emily Bliss, Otterbein University
Kyle Boulanger, Grandview University
Kelly Budge, Goucher College
Michael Chen, University of Massachusetts Amherst
Stephanie Choi, University of Massachusetts Amherst
Jacob Crosser, Purdue University
Jocelyn Daubendiek, University of Nebraska–Lincoln
Lauren DeLong, Salisbury University
Amanda Duplan, Grandview University
Grace Ferri, Boston University
Corey Gallen, Saint Leo University
Nina Marie Garcia, University of San Diego
Kate Harris, Purdue University
Victoria Henderson, Trinity University
Megan Horita, Saint Louis University
Evan Huggins, Otterbein University
Thomas Hynes, Rochester Institute of Technology
Aria Jordan, University of Massachusetts Amherst
Thomas Kania, University of Massachusetts Amherst
Emily Kessler, Wesleyan University
Sophia Kisling, University of Nebraska–Lincoln
Auston Larratta, Saint Leo University
Cindy Le, University of Massachusetts Amherst
Christine Little, Wesleyan University
Sonoor Majid, University of Nebraska–Lincoln
Victoria Mak, Saint Louis University
Antoinette Martinez, University of St. Thomas
Julie McDonald, Wesleyan University
Rachel Nguyen, Otterbein University
Rubye Peyser, Wesleyan University

Sphingolipid metabolism: a potential therapeutic target in sickle red blood cells

By Nathan Chiappa, Hannah Song & Edward Botchwey

About 300,000 babies around the world are born each year with sickle cell disease, a genetic disorder that affects about 100,000 Americans. The disease causes pain and inflammation due to blood flow blocked by sickle cells, resulting in serious complications such as strokes in childhood, acute chest syndrome, organ damage and premature death, with an average life expectancy of 45 years.

Sickle cell disease is caused by a point mutation in the gene for the beta subunit of hemoglobin. When red blood cells enter low-oxygen environments, the mutated hemoglobin polymerizes, leading to alteration of the cells' shape. Sickle hemoglobin also has a high affinity for membranes, leading to accumulation of denatured sickle hemoglobin on the inner plasma membrane surface. This contributes to a wide range of dysfunctions of sickle red blood cells, including increased oxidative stress, increased surface phosphatidylserine exposure, increased adhesion to endothelium and hyper-production of extracellular vesicles.

While alterations of membrane shape and glycerophospholipid composition in sickle red blood cells have been analyzed extensively, the involvement of bioactive sphingolipids is less well studied. Sphingolipids such as sphingomyelin and ceramide are important structural components of the plasma membrane. They regulate the lateral domain structure of membranes, including formation of lipid rafts, which is critical for protein sorting and membrane signaling. Beyond structural functions, generation of ceramide by hydrolysis of sphingomyelin through the action of sphingomyelinase causes increased surface phosphatidylserine exposure on the cell surface, increased microparticle formation and increased adhesion of red blood cells to endothelium.

"Most comprehensive studies of the red blood cell metabolome have ignored sphingolipids, and no comprehensive sphingolipid profile of red blood cells exists in the literature."

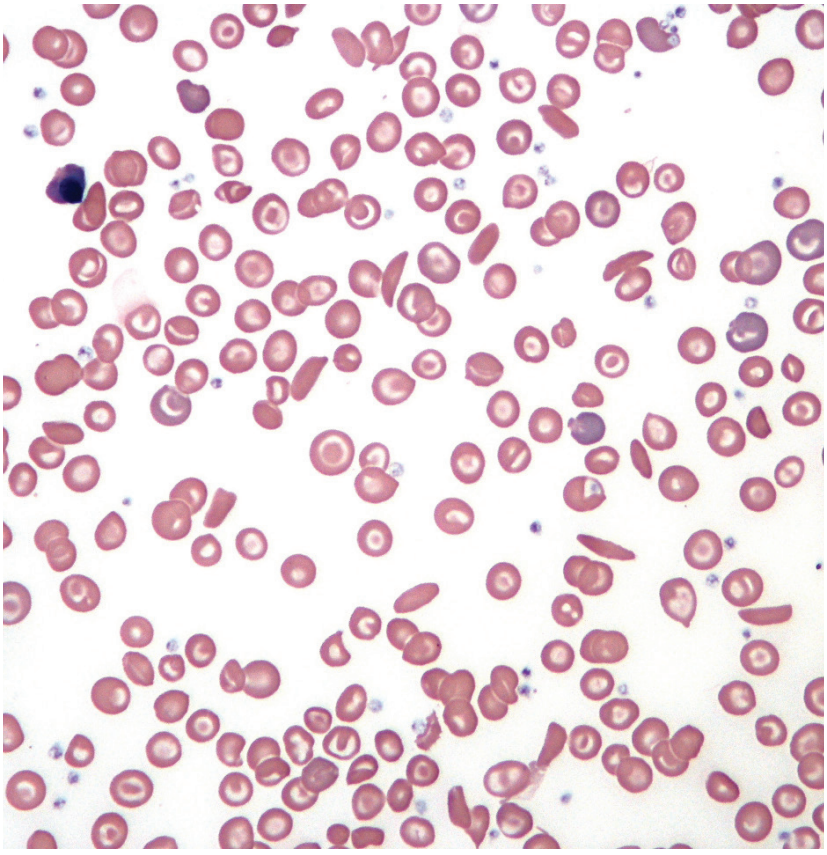
The bioactive sphingolipid that has received the most attention in the context of sickle cell disease is sphingosine 1-phosphate, or So1P. Red blood cells store So1P at a concentration of about 1 nanomole per milliliter of cells, which is higher than most cell types in the body (1). Red blood cell-derived So1P also can be released into the plasma, where it binds primarily to albumin and high-density lipoprotein. Once in the plasma, So1P regulates a range of physiological systems, most notably endothelial barrier integrity and the trafficking of lymphocytes and hematopoietic stem cells (2). New evidence suggests that So1P plays a role inside red blood cells as well. So1P may bind directly to hemoglobin, altering its affinity for oxygen and its affinity for the plasma membrane. Importantly, studies from our lab and others have shown that So1P is elevated in sickle red blood cells (1, 3). This suggests new therapeutic opportunities for treatment of sickle cell-associated pathologies by manipulation of So1P production and metabolism.

Increased So1P in red blood cells could stem from changes in different metabolic pathways. Evidence from our laboratory and others suggests that sphingosine kinase activity is significantly elevated in sickle cell disease red blood cells (1, 3). However, our studies and others show that sphingosine concentration also is elevated in these cells (3). Since the rate of So1P production is dependent on both the activity of sphingosine kinase and the availability of substrate sphingosine, the relative contributions of enzyme activity changes and substrate concentration to the increased red blood cell So1P are not clear. Further, it is unclear how red blood cell sphingosine is increased. One possibility is

through increased ceramidase flux. Although we do not know whether the activity of ceramidase is affected in sickle red blood cells, our previous studies have demonstrated an increase in sphingomyelinase activity (3). This could increase the concentration of ceramide, which is the substrate for ceramidase. An additional possibility is that there is increased influx of sphingosine from the plasma, since our studies have shown increased plasma sphingosine concentration in sickle cell disease (3).

Most comprehensive studies of the red blood cell metabolome have ignored sphingolipids, and no comprehensive sphingolipid profile of red blood cells exists in the literature. In comprehensive proteomic studies of red blood cells, few sphingolipid-metabolizing enzymes have been reported. It is unclear whether this is because these enzymes are absent or because they are scarce. Additionally, the available literature on red blood cell sphingolipid metabolism is not entirely consistent. Some studies have identified activity of certain enzymes, while other studies have found none. The only enzyme consistently observed across multiple studies of red blood cell sphingolipid metabolism is sphingosine kinase. Given the inherent complexities of sphingolipid metabolism, an incomplete knowledge of the sphingolipid metabolism network in red blood cells may confound research efforts to exploit the role of So1P in sickle cell disease pathology.

The picture of sphingolipid metabolism in red blood cells that emerges includes multiple enzymes, fluxes and sphingolipid concentrations that likely are affected in sickle cell disease. The relative importance of these changes and the optimal therapeutic strategy for addressing them as a whole is difficult to determine.



COURTESY OF KEITH CHAMBERS/SCOOTER PROJECT

A blood smear stained with Giemsa shows the distinctive sickle shape cells and cells with concentric target-like rings of red stain that are found in sickle cell disease.

Recent technological and methodological advancements may enable researchers to better understand the metabolism of bioactive lipids in sickle cell disease. First, lipidomics approaches using liquid chromatography–tandem mass spectrometry allow researchers to measure several lipids under steady-state and dynamic conditions. Second, computational systems biology allows researchers to integrate those measurements into a mathematical modeling framework, which allows for enhanced insight into system behavior and prediction of optimal therapeutic interventions.

Ultimately, we believe that the integration of lipidomics technology and systems biology can allow researchers to create a more coherent picture of the lipid dysregulation that underlies the many manifestations of sickle cell disease pathology.

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A phospholipid pathway from plants to parasites

By Sasha Mushegian

Findings by researchers at Washington University in St. Louis may aid in the development of therapies to treat parasitic infections, including malaria, and may help plant scientists one day produce hardier crops. The research team's work was published in the **Journal of Biological Chemistry**.

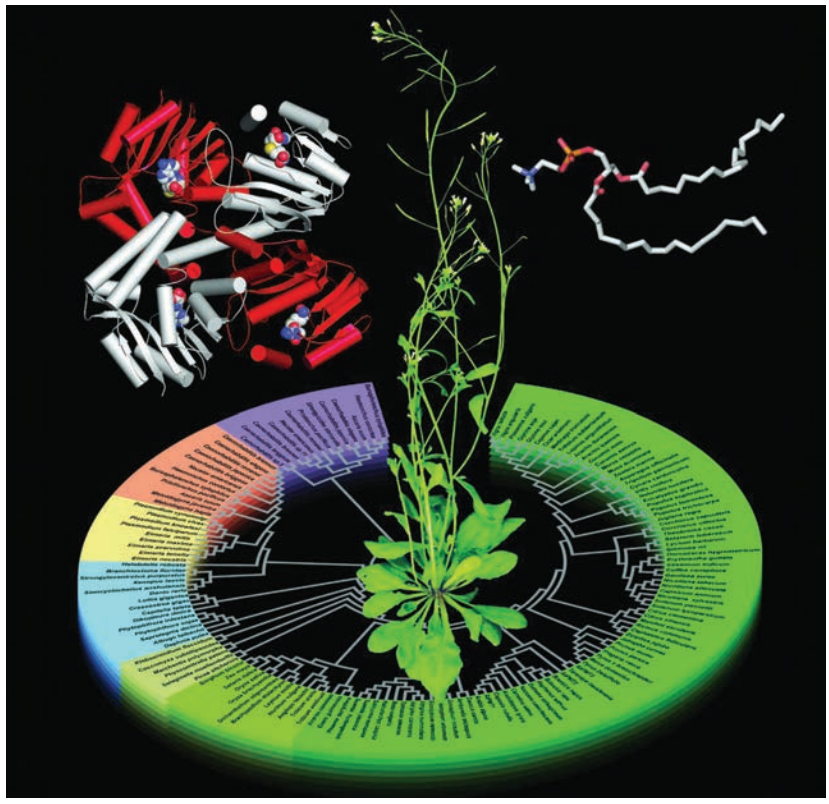
Choline is an essential nutrient that we get from certain foods, including eggs, meat, leafy greens and nuts. The human body converts choline into phosphocholine, or pCho, which it in turn converts into (among other essential building blocks) phosphatidylcholine, or PtdCho, a component of cell membranes. Plants can't acquire the nutrient from the environment and so must synthesize pCho from scratch. The biochemical pathway plants use to synthesize pCho also is found in nematodes and the malaria parasite Plasmodium.

In plants, the enzymatic reaction that produces pCho is essential for normal function and for responding to stresses. Plant pCho is converted into PtdCho, which builds membranes that can adjust their rigidity in response to temperature changes. pCho also gets converted into molecules that help plants survive high salt. The enzymes that produce plant pCho are called phosphoethanolamine methyltransferases, or PMTs.

Soon Goo Lee, a postdoctoral research fellow at Washington University in the lab of Joseph Jez (a JBC associate editor), has been fascinated by PMTs in both plants and parasites for many years.

"Understanding the PMT enzyme is key to engineer plants with improved stress tolerance and enhanced nutrients," Lee said.

Since the PMT-catalyzed pathway is found in parasites but not humans, Lee and Jez's team is looking for inhibitors of this enzyme to treat diseases caused by these parasites.



COURTESY OF SOON GOO LEE AND JOSEPH JEZ

A study explains how structures of Arabidopsis phosphoethanolamine methyltransferase, or PMT, (left) are evolutionarily related to PMT sequences from different organisms. Phosphatidylcholine (right) is PMT's product.

The new study explains that PMTs of the model plant Arabidopsis thaliana share core features of PMTs from parasites, with almost identical structure at the active site. But the plant PMTs are roughly twice as large as the parasite ones, with large sections that can rearrange themselves to carry out multiple chemical reactions.

The three PMT types in the plant — which were thought to carry out the same function — actually appear to play different roles depending on where they are found in the plant. Plant growth experiments showed that one type of PMT was essential for root development and salt tolerance, whereas the other two had no effect on roots and instead seemed to be found primarily in leaves.

In the long run, this big-picture view of PMTs in different organisms offers routes to engineer enzymes with different functions.

"I love these kinds of stories," Lee said, "where I can look from the atomic (structure) to the physiological level to explain why these enzymes have different forms and how they work."

DOI: 10.1074/jbc.RA117.000106

Sasha Mushegian (amushegian@asbmb.org) is scientific communicator for the Journal of Biological Chemistry.

A sugar-attaching enzyme defines colon cancer

By Sasha Mushegian

Researchers have identified an enzyme that is absent in healthy colon tissue but abundant in colon cancer cells, according to a paper published in the **Journal of Biological Chemistry**.

The enzyme appears to drive the conversion of normal colon tissue into cancer by attaching sugar molecules, or glycans, to certain proteins in the cell. Understanding the role that sugar-modified proteins play in healthy and cancerous cells is an emerging area of cancer biology that may lead to new therapies.

Hans Wandall's team at the University of Copenhagen studied 20 enzymes that initiate the first step in a particular kind of glycan modification, called GalNAc-type O-glycosylation, found on diverse proteins. These enzymes, called GalNAc transferases, or GalNAc-Ts, are found in different amounts in different tissues, but their functions are poorly understood.

Wandall's team, led by then-graduate student Kirstine Lavrsen, found that one of the GalNAc-Ts, called GalNAc-T6, was absent in healthy colon tissue but abundant in colon cancer cells. The team used CRISPR/Cas engineering of a colon cancer cell line with and without GalNAc-T6 to understand to which proteins the enzyme helped attach sugars and what effect this had on the cells.

"When we look at the 3D growth of a cancer cell line that has GalNAc-T6, it can form tubular structures with formation of something that looks like colon cancer tissue," Wandall said. "When we take out GalNAc-T6, then suddenly the tissue formation changes to look more like the crypt structures that you would find in a healthy colon."

Using mass spectrometry, the team categorized the proteins that GalNAc-T6 acted on in these cells.

"Our data suggest (that) this specific enzyme seems to affect a subset of proteins that could be involved in cell-cell adhesion," Wandall said. In other words, the glycan modifications changed the patterns in which cells stuck together, leading the cells to develop as something that looked more like a tumor than a healthy tissue.

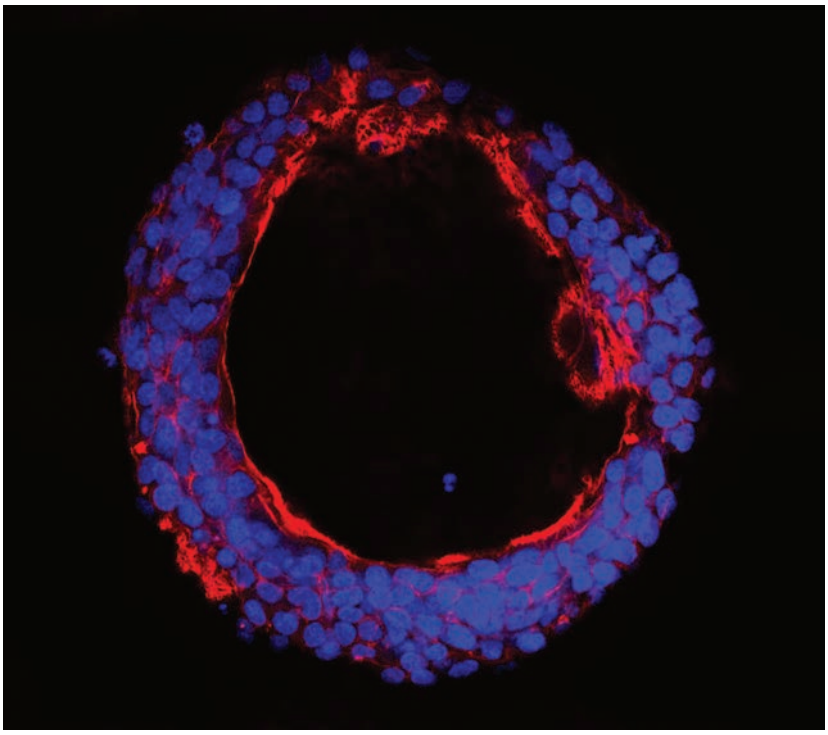
The next step is to understand precisely why adding sugars to the specific protein sites modified by GalNAc-T6 leads colon cells to develop abnormally. Glycan modifications can affect protein function in myriad ways. For example, they can make proteins that usually are cleaved into two unable to be cleaved, or prevent two proteins from binding to each other.

Wandall hopes that understanding glycosylation in cancer cells will lead to better early diagnostic tools, drugs or immunotherapies.

"Glycans add an additional context layer that could help us create more specific interventions," he said.

"Glycans look so different in cancer compared to normal tissue, and it's a really understudied field," Lavrsen said. "There are a lot of things to be discovered."

DOI:10.1074/jbc.M117.812826



COURTESY OF KIRSTINE LAVRSEN/UNIVERSITY OF COPENHAGEN

The enzyme GalNAc-T6 is upregulated selectively in colon adenocarcinomas, and its expression is associated with a cancerlike growth pattern.

Sasha Mushegian (amushegian@asbmb.org) is scientific communicator for the Journal of Biological Chemistry.

Real-time proteomics may one day speed up cancer surgery

By Laurel Oldach

Isabelle Fournier and her team are out to change surgical oncology.

"Better surgery is associated with better prognosis and higher survival," said Fournier, a professor at the University of Lille and co-director of a proteomics center of INSERM, the French national institute of health. Her laboratory has worked for several years on a device they call the SpiderMass that will enable surgeons to look for markers of cancer in a living patient's tissue, during an operation. In an article in **Molecular & Cellular Proteomics**, the team reports on an important step toward finding protein biomarkers during surgery.

COURTESY OF ISABELLE FOURNIER

In vivo analysis of the skin using the SpiderMass. The device leaves a white trail of dehydrated skin, but the traces disappear within a few minutes.

Surgery to remove a primary tumor involves a wait. After the tumor and some healthy surrounding tissue are removed, the surgical team pauses while a pathologist checks the tissue margins under a microscope. Although this process is important for preventing recurrence of the cancer, it can add up to 45 risky minutes under anesthesia.

With the new device, Fournier said, "We think that it is possible to open the way to in vivo real-time proteomics," which could help surgeons find stray cancer cells faster, perhaps even as they make incisions.

Fournier's device uses mass spectrometry, which measures the mass of molecules from complex mixtures. But turning an in vivo tissue sample into gas phase ions for measurements can be a challenge. Until now, no one knew how to extract ions from living tissues without doing harm.

So Fournier's team got creative. Riffing on MALDI, an ionization strategy that uses a carrier molecule mixed with the analyte of interest, they decided to use the water that makes up a majority of human tissue as a carrier to produce a water-assisted laser desorption/ionization, or WALDI. If they could excite the water in a tiny area, it should vaporize, taking ionized organic molecules with it.

"It was an idea at the beginning, and many people thought that it would not work," Fourier said. "Finally, we have it working beautifully."

The team built a pulsed laser excitation device tuned to heat water precisely by causing vibration in the oxygen-hydrogen bond. In a 2016 paper, they described using this laser to ionize the outermost layer of tissue, penetrating less than one-twentieth of a millimeter. The human volunteers reported a slight tingling sensation. But the ions that appeared were mostly small molecules and lipids, which are more apt than proteins to adopt a negative charge. The team hoped to measure proteins as well.

In this new paper, Fournier and colleagues report that they have cracked the protein puzzle. By using a more sensitive mass spectrometer and looking for positively instead of negatively charged ions, they found peaks representing purified proteins they had introduced into a cow liver sample. Now that they know the proteins are detectable, the next step will be finding ways to amplify the protein signal over more abundant lipids and metabolites.

In the meantime, the device is already in use for four-legged patients. Fournier's lab has worked with the veterinary biotech company Oncovet Clinical Research to run a pilot trial, comparing biopsies from pet dogs with sarcoma to healthy tissues. The team developed a lipidomics- and metabolomics-based classification system to robustly identify healthy, necrotic and cancerous tissues. Soon, they will introduce a prototype into a veterinary operating room. If it is successful there, Fournier said, she hopes to reach human clinics, improving tumor removal surgery to give patients better health outcomes.

DOI: 10.1074/mcp.TIR117.000582

Laurel Oldach (loldach@asbmb.org) is a communications intern at the ASBMB.

What can a tasty milkshake teach us about the genetics of heart disease?

Study uncovers new gene variants that quietly affect cholesterol

By Laurel Oldach

From 2002 to 2005, about 800 people in Minnesota and Utah drank more than 1,500 milkshakes, and they got to say they were doing it for science.

These self-sacrificing study subjects are participants in the Genetics of Lipid-Lowering Drugs and Diet Network, or GOLDN, and this wasn't their first rodeo. Some have been put under the microscope for about 25 years now to help researchers better understand cardiovascular risks.

This time they were helping scientists determine how genes influence cholesterol levels after high-fat meals and how genes affect the response to a medication for lowering triglyceride and bad cholesterol.

COURTESY OF WIKIMEDIA COMMONS

After study participants drank a strawberry- or chocolate-flavored milkshake, researchers drew their blood to measure changes in HDL, LDL and triglycerides.

Ultimately, the team of medical geneticists at the University of Texas Health Science Center at Houston, University of Alabama at Birmingham, the University of Kentucky and seven other institutions discovered new uncommon gene variants that affect patients' response to the cholesterol drug. They reported their findings in the **Journal of Lipid Research**.

Chronically high cholesterol results in the gradual deposition of lipids on artery walls, increasing the risk of heart attacks and stroke. This clogging process is known as atherosclerosis. A diet high in fat is just one of several risk factors. Family history strongly affects a person's risk of developing it.

"Triglyceride and cholesterol levels are influenced by what people eat and whether they take drugs to lower fat and cholesterol," said the study's lead author, Xin Geng, a postdoctoral fellow at the University of Texas Health Science Center at Houston. "We know, however, that not everyone's triglyceride and cholesterol levels respond the same to diet and drugs. Previous research suggests that these differences in response to diet and drugs may be caused by genetic factors inherited from parents."

While researchers have identified genetic variants that affect lipid responses, the medical geneticists who worked on the milkshake study set out to identify additional variants that have so far escaped detection.

Drinking milkshakes, giving blood

Each GOLDN participant came to the lab with an empty stomach and drank a milkshake concocted from heavy whipping cream, chocolate or strawberry syrup, and crushed ice. More than four-fifths of the calories in the meal came from fat.

The scientists drew their blood before and after the meal and measured high-density lipoprotein (known as "good" cholesterol), low-density lipoprotein (known as "bad" cholesterol) and triglycerides (basically, fat).

They sent the patients home to take fenofibrate, a medication that can lower cholesterol and triglyceride levels, for three weeks. After that treatment, patients returned to the lab for another milkshake and another set of blood draws.

The scientists also sequenced all of the genes and examined the gene expression level in the participants' blood cells.

Novel rare gene variants

"Until recently," said Degui Zhi, senior author from UTHSC at Houston, "it was thought that common genetic variants were the ones that caused variability in people's triglyceride and cholesterol levels."

However, common genetic variants explained less than a quarter of inherited variability in triglyceride levels. "Our hypothesis was that at

least some of the 48 percent of unexplained variability could be driven by rare genetic variants, those that occur in less than 5 percent of the population," Zhi said.

Indeed, the researchers found variants of at least three genes that were not widespread in the study population but did correlate with lipid changes in the people who had them.

"We found that variants in one gene, called SIPA1L2, predicted triglyceride level changes after consuming the milkshake. Variants in another gene, ITGA7, predicted LDL-cholesterol level changes after taking the drug. And variants in a third gene, CEP72, predicted triglyceride response when comparing the pre-fenofibrate milkshake experiment to the post-fenofibrate milkshake experiment," said Ryan Irvin, another collaborator from UAB.

The researchers say their findings should spur other scientists to look for additional rare gene variants, including non-protein-coding DNA that fell outside the scope of this study.

They also emphasize that their study population was not diverse. Most of the GOLDN participants are white. Looking for rare variants in other populations may turn up additional important genes.

"Future studies can begin where we left off by trying to uncover exactly how the metabolic pathways and mechanisms these genes are part of interact with dietary fat and fenofibrate to change triglyceride and cholesterol levels," said Donna Arnett, dean of the UK College of Public Health and the GOLDN principal investigator. "Armed with this knowledge, we will be one step closer to finding new ways to prevent and/or treat unhealthy triglyceride and cholesterol levels."

DOI: 10.1194/jlr.P080333

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Upcoming ASBMB events and deadlines

JULY

10: Frontiers in RAS Pathobiology and Drug Discovery oral abstract submission deadline
10: Frontiers in RAS Pathobiology and Drug Discovery discounted symposium registration deadline
19: Transcriptional Regulation by Chromatin and RNA Polymerase II oral abstract deadline
20: Frontiers in RAS Pathobiology and Drug Discovery poster deadline
27: Frontiers in RAS Pathobiology and Drug Discovery poster programming abstract submission deadline
28: World Hepatitis Day

AUG

August is for Advocacy Month
9: Frontiers in RAS Pathobiology and Drug Discovery registration deadline
13–17: Fungal Disease Awareness Week
14: Frontiers in RAS Pathobiology and Drug Discovery registration cancellation deadline
14: Transcriptional Regulation by Chromatin and RNA Polymerase II early registration deadline
30: Transcriptional Regulation by Chromatin and RNA Polymerase II poster deadline

SEPT

Childhood Cancer Awareness Month
Ovarian Cancer Awareness Month
3: The Art of Science Communication online course application site open
4: Transcriptional Regulation by Chromatin and RNA Polymerase II registration deadline
12: The Many Faces of Kinases and Pseudokinases oral abstract deadline
13–16: Frontiers in RAS Pathobiology and Drug Discovery, Stratton, Vt.
25: The Many Faces of Kinases and Pseudokinases early registration deadline

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American Society for Biochemistry and Molecular Biology

From the journals

By Sasha Mushegian & Laurel Oldach

We offer a selection of recent papers on a variety of topics from the **Journal of Biological Chemistry**, the **Journal of Lipid Research** and **Molecular & Cellular Proteomics**.

Diet regulates a metabolite (but not in the brain)

Ketogenic diets, which reduce carbohydrate intake and prompt the body to rely on fat-derived ketone bodies instead, are a popular treatment for epilepsy and thought to have neuroprotective effects on some other diseases. Mild caloric restriction also is believed to protect neurons. Researchers aren't sure of the exact molecular mechanism of these diets, but Svenja Heischmann and colleagues at the University of Colorado in Denver have taken a step toward characterizing their effects on the brain.

In a study reported in the **Journal of Lipid Research**, researchers conducted a metabolomics analysis of both the plasma and brain tissue of mice eating normal or ketogenic chow. They subdivided each diet group into mice eating their fill or eating a restricted amount of chow. The researchers found that, in the bloodstream, kynurenine metabolism changed dramatically. Kynurenine, made from the amino acid tryptophan, can be converted into vitamin B3 or several other metabolites with effects on neurons. However, in the brain, the level of kynurenine changed relatively little.

The research suggests that, while tryptophan degradation is a target of the ketogenic diet, changes in plasma metabolism may not always cross the blood-brain barrier. The researchers intend to explore other metabolic changes in future publications.

DOI: 10.1194/jlr.M079251

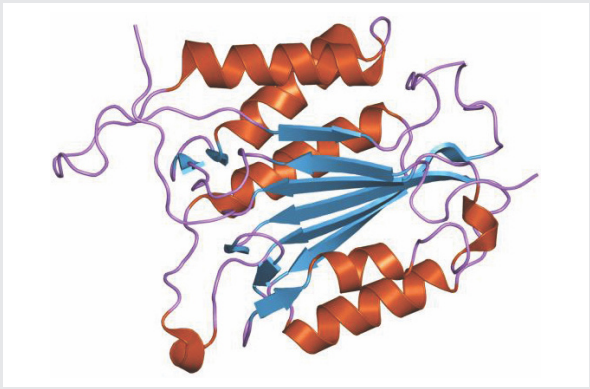
Caspase switch shows how to kill a killer

The mammalian protein caspase-3 is an executioner with several faces. The protease initiates various processes involved in breaking down cells during apoptosis; for this reason, its regulation is important for controlling cancers. But low levels of caspase-3 activity are required for normal development.

Researchers from North Carolina State University and the University of Texas at Arlington have gained new insights into how caspase-3 is regulated, potentially providing opportunities for fine-tuning its activity. Postdoctoral research associate Melvin Thomas III led the study, which was published in the **Journal of Biological Chemistry**.

"Caspase-3 has been well-characterized in apoptosis; (it starts) the process of programmed cell death," Thomas said. "But over the past decade, there's been a lot of evidence that shows that caspases in general, but caspase-3 specifically, is involved in other non-apoptotic functions, such as erythroid differentiation, macrophage differentiation, neuron pruning and a variety of other developmental phenotypes."

Thomas, working in Clay Clark's laboratory at North Carolina State, investigated how caspase-3 was regulated allosterically by phosphorylation. He saw that phosphorylation of different sites in the protein had different effects on its activity. In particular, phosphorylation of a site conserved across all apoptotic caspases reduced caspase-3 activity, whereas phosphorylation of a site specific to mammalian caspase-3 completely abolished its activity. Thomas hypothesizes that these two different types of switches allow the enzyme to be regulated to the right level of activity for different functions.



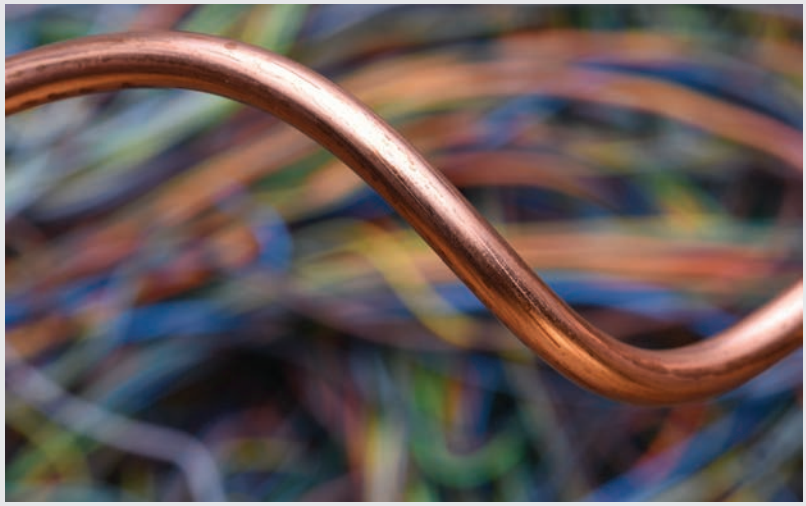
COURTESY OF JAWAHAR SWAMINATHAN/EUROPEAN BIOINFORMATICS INSTITUTE

This ribbon diagram represents a structure of caspase-3 (Protein Data Bank number 1qx3).

"You can use these posttranslational modifications to either decrease activity of the enzyme or, in the case of the kill switch, decrease it to zero," Thomas said. "(The cell can) bring the total level of caspase activity below a threshold to allow for these developmental phenotypes without committing the cell to death."

DOI: 10.1074/jbc.RA117.000728

The structure of bacterial sensitivity to copper



Copper is a ubiquitous antibiotic. Some door handles in hospitals are made of copper alloys to impede bacterial growth, and within the human body, the innate immune system mobilizes copper to sites of infection to help kill bacteria. For some pathogenic bacteria, therefore, the ability to tolerate copper is key to their ability to infect humans.

Researchers at the University of Queensland and Griffith University in Australia investigated the structural and redox biology of a bacterial protein involved in copper tolerance. Their findings, published in the **Journal of Biological Chemistry**, could spur the development of new tools to combat antibiotic-resistant infections.

Jennifer Martin of Griffith University, who oversaw the research, studies the suppressor of copper sensitivity, or Scs, family of proteins, which are critical for copper resistance in pathogens such as Proteus mirabilis, which causes urinary tract infections and hospital-acquired infections. Scs proteins are diverse; although they are related to the well-characterized disulfide bond-forming proteins found in Escherichia coli, the mechanisms by which they allow bacteria to tolerate copper are poorly understood.

The new study, led by University of Queensland graduate student Emily Furlong, focused on ScsB-alpha and ScsC in Proteus mirabilis. The biochemical data showed that the two proteins formed a redox relay in the bacterial cells. The structural data showed that ScsB-alpha interacts with ScsC via two immunoglobulinlike folds, a structure also found in an E. coli disulfide bond-forming protein.

"What was quite surprising was the structure of ScsB-alpha," Furlong said. "Even though there's a very low sequence identity (between ScsB-alpha and the E. coli protein), only 12 percent, the structural similarity is quite high."

Understanding these structure-function relationships in Scs proteins will allow them to be targeted therapeutically. Disarming bacteria by making them newly sensitive to copper represents a new approach to treating infections. But questions remain as to how this approach might work in clinical settings.

"We're still at the stage of trying to find small molecules to inhibit the (disulfide bond-forming) proteins, and we haven't got to that stage with the Scs proteins, because it's still fairly early in the piece," Martin said. "There's a lot of unknowns. What happens if you have an established infection and then you take a drug that disarms bacteria? Will that have an impact on the course of infection? Will it reduce the propensity of bacteria to develop resistance? Can it be used in combination with antibiotics that are currently unusable because of resistance? These are all questions that we don't yet know the answers to."

DOI: 10.1074/jbc.RA118.001860

Sugars can slow diabetes drugs

Liraglutide is an analogue of glucagonlike peptide-1, or GLP-1, that is used in the treatment of Type 2 diabetes and cardiovascular diseases. Liraglutide binds to human serum albumin, which helps it traffic to the GLP-1 receptor. Angélique Gajahi Soudahome and colleagues at Université de La Réunion showed that liraglutide binding to albumin decreased with increasing albumin glycation. As albumin glycation increases with diabetes progression, this factor might affect the therapeutic efficacy of the drug. The study was published in the **Journal of Biological Chemistry**.

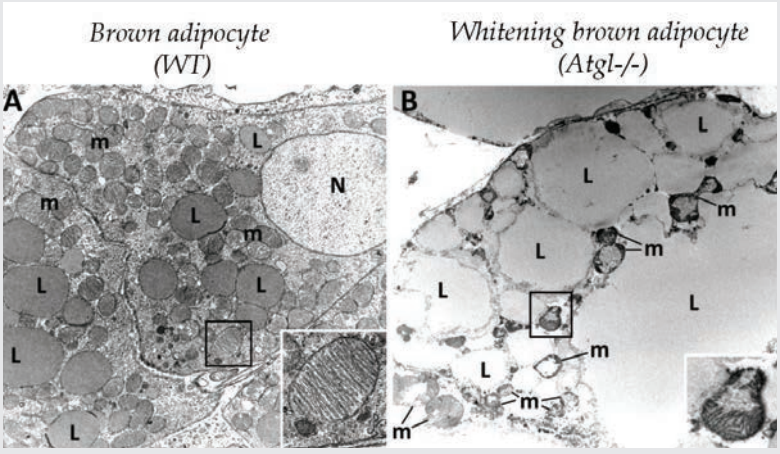
DOI: 10.1074/jbc.M117.815274

Engineering a better matriptase inhibitor

Matriptases are membrane-anchored serine proteases. When dysregulated, matriptase activates growth factors involved in cancer proliferation and metastasis. In healthy tissue, matriptase is inhibited by hepatocyte

Brown tissue whitening causes cell death, inflammation

There's more than one type of fat cell. Besides the white fat that stores triglycerides in lipid droplets in preparation for lean times later, mammals also have heat-generating brown fat, which acts more like a radiator than a storage closet. Brown fat cells are smaller, with more abundant mitochondria than white fat cells, and they hold a lot fewer lipids. In many models of obesity, brown adipose tissue converts to white tissue, with changes in the morphology and function of the cells.



COURTESY OF KOTZBECK ET AL/ UNIVERSITY OF GRAZ

A brown adipocyte (left) has numerous small lipid droplets and healthy mitochondria (inset). In a whitening brown adipocyte (right), lipid droplets are much larger, and mitochondrial structure is disrupted.

In a recent paper in the **Journal of Lipid Research**, Petra Kotzbeck, Antonio Giordano and colleagues investigated what happens to brown fat cells after whitening. The researchers, based at the University of Graz, Austria, and the University of Ancona, Italy, found that whitened brown adipocytes enlarged by addition of lipids were more likely to die than white adipocytes of a comparable size. Whitened adipose tissue also had more macrophages, presumably there to clean up the dead cells, and more inflammation under way. The vulnerability of whitened brown adipocytes may explain why gaining fat in the abdomen, where most whitened brown fat is located, is worse for your health than gaining subcutaneous fat.

DOI: 10.1194/jlr.M079665

growth factor activator inhibitor type-1, or HAI-1. Aaron C. Mitchell and colleagues at Stanford University engineered a matriptase inhibitor based on HAI-1 in which an inactive domain was replaced with a second matriptase-binding domain, resulting in greater binding activity and inhibition of growth factor activation in lung, breast and prostate cancer cells. The study was published in the **Journal of Biological Chemistry**.

DOI: 10.1074/jbc.M117.815142

Monitoring proteomics in real time

Nothing is more frustrating than being forced to throw out data. After taking the time to prepare samples, take measurements and analyze the data, a researcher may realize that a problem with the samples or an instrument compromised the results. With luck, the realization happens early — but work still must be repeated. In long-running clinical proteomics studies, sometimes the realization doesn't occur until all of the data are pooled, and the cost can be high.

In a recent article in **Molecular & Cellular Proteomics**, researchers at the Pacific Northwest National Laboratory working on a longitudinal study of the development of Type 1 diabetes announced that they have developed a tool that can tell in real time whether the quality of proteomics data has dropped. Bryan Stanfill and colleagues developed an algorithm that considers statistical features of mass spectrometry data to make a constantly adjusted model for comparison to a baseline of known high-quality data. By intentionally manipulating the instrument — for example, by changing an ion lens setting — they showed that the algorithm could identify times when the instrument needed to be recalibrated. The software also helped to flag points when unscheduled cleaning and maintenance were required. The software, called QC-ART, is freely available on the software sharing site GitHub.

DOI: 10.1074/mcp.RA118.00064

HDAC's role in endosomal pH

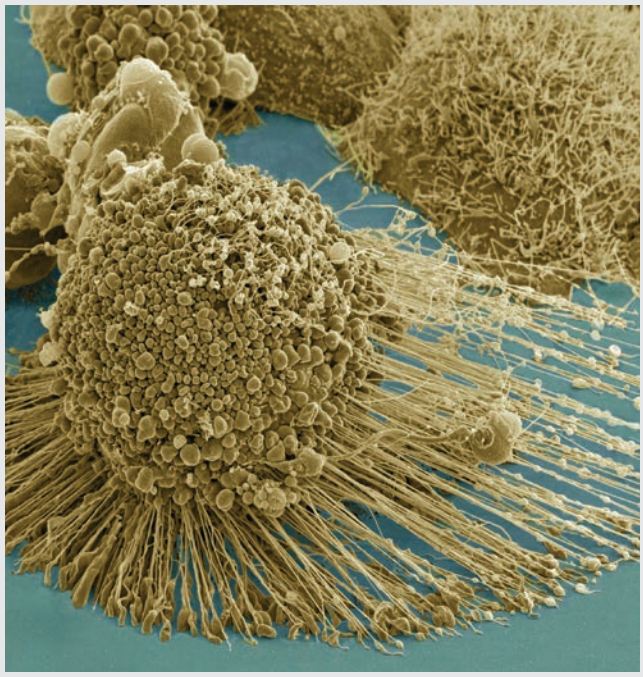
The endolysosomal system is characterized by a precisely controlled pH gradient along the pathway, ending with the acidic lysosome. Defective pH regulation in this pathway is associated with numerous disorders including neurodegenerative diseases. Hari Prasad and Rajini Rao from Johns Hopkins University performed a meta-analysis of factors affecting the regulation of an endosomal ion exchanger. They found, conserved across yeast, flies and mammals,

Looking at cells, living and dead

Researchers at the Karolinska Institute were using a proteomic screen to determine the mechanism by which cancer drug candidates killed cells when they realized a potential flaw in the standard experimental design. Amir Ata Saei and colleagues usually treated adherent cancer cells with a compound and then rinsed away any cells that detached and measured how the proteome had changed in cells that survived. But, the researchers reasoned, the dying cells were responding the most strongly to the experimental drug. Shouldn't their responses be examined?

In a study published in **Molecular & Cellular Proteomics**, the researchers compared proteomes of cultured cancer cells that had survived with those that had not after treatment with various drug candidates. Using drugs with known targets, they found that including dying cells improved the accuracy of target identification. To their surprise, the researchers also found that some proteins were upregulated in all detached and dying cells, regardless of the drug they used. They propose that these proteins, which previously had not been linked to cell death, may be cellular decision makers and promising chemotherapeutic targets.

DOI: 10.1074/mcp.RA118.000610



COURTESY OF THE NATIONAL INSTITUTES OF HEALTH

Amir Ata Saei and colleagues at the Karolinska Institute assessed the proteome of adherent and dying cultured cancer cells after treatment. In this scanning electron micrograph, a HeLa cell in the midst of apoptosis begins to detach from its substrate.

that these exchangers (and therefore endosomal pH) were regulated transcriptionally by inhibition of a histone deacetylase in response to nutrient limitation. Pharmacologically increasing expression of the histone deacetylase corrected endosomal pH and improved clearance of amyloid proteins in a cell model of Alzheimer's disease. The research was published in the **Journal of Biological Chemistry**.

DOI: 10.1074/jbc.RA118.002025

An oncogene ally against multiple myeloma

Transmembrane prostate androgen-induced protein, or TMEPAI, is a poorly understood oncoprotein that is overexpressed in solid cancers but not in liquid cancers such as leukemia and lymphoma. Yanyun Du and colleagues at Suzhou Municipal Hospital and Soochow University in China showed that TMEPAI actually may protect against multiple myeloma, a plasma cell cancer. The authors found that TMEPAI overexpression induces multiple myeloma cell apoptosis through TMEPAI's effect on a myeloma-promoting transcription factor. The study was published in the **Journal of Biological Chemistry**.

DOI: 10.1074/jbc.RA117.000972

Diabetes lessons from diverse mice

Type 2 diabetes risk is influenced significantly by genetics, but most previous diabetes research in mice has focused on only a few mouse genotypes, limiting mechanistic insight into the genetic determinants of diabetes. In a study published in the **Journal of Biological Chemistry**, Kelly Mitok and colleagues at the University of Wisconsin–Madison examined diabetes-related metabolic traits and pancreatic islet proteomics in the genetically diverse Collaborative Cross panel of mice. They uncovered mouse strain-specific differences in diabetic phenotypes and pancreatic islet proteomic profiles. They also showed that in only one strain, pancreatic synthesis of dopamine inhibited insulin secretion, demonstrating the importance of a genetically diverse reference panel.

DOI: 10.1074/jbc.RA117.001102

Syntaxin 17 promotes lipid droplet formation

Cells store energy in lipid droplets, and many such droplets are made in the liver, which plays an important role in coordinating fat metabolism. As new lipid droplets form within

the endoplasmic reticulum, acyl coA synthetase 3, or ACSL3, is indispensable for helping them mature. ACSL3 turns free fatty acids into the neutral lipids that fill the lipid droplet.

In a recent article in the **Journal of Lipid Research**, Hana Kimura and colleagues studying droplet synthesis at Tokyo University of Pharmacy and Life Sciences in Japan report that the binding and scaffolding protein Stx17 is required to move ACSL3 to the nascent lipid droplet at mitochondria-associated membranes within the ER. This new role may explain why Stx17 is expressed abundantly in the liver and adipocytes.

DOI: 10.1194/jlr.M081679

How soy resists salt

How will crops cope with rising sea levels and increasing salt in the water table as the climate changes? In a recent study in **Molecular & Cellular Proteomics**, researchers at Hangzhou Normal University studied soybeans' response to salt stress. Salt disrupts mitochondria and increases the reactive oxygen species in the plant cells. Therefore, increasing production of antioxidant molecules like flavonoids may protect the plant. By combining phosphoproteomics and metabolomics, Erxu Pi and colleagues described a salt-stress signaling pathway in soybean roots that increases flavonoid synthesis and improves salt tolerance.

DOI: 10.1074/mcp.RA117.000417

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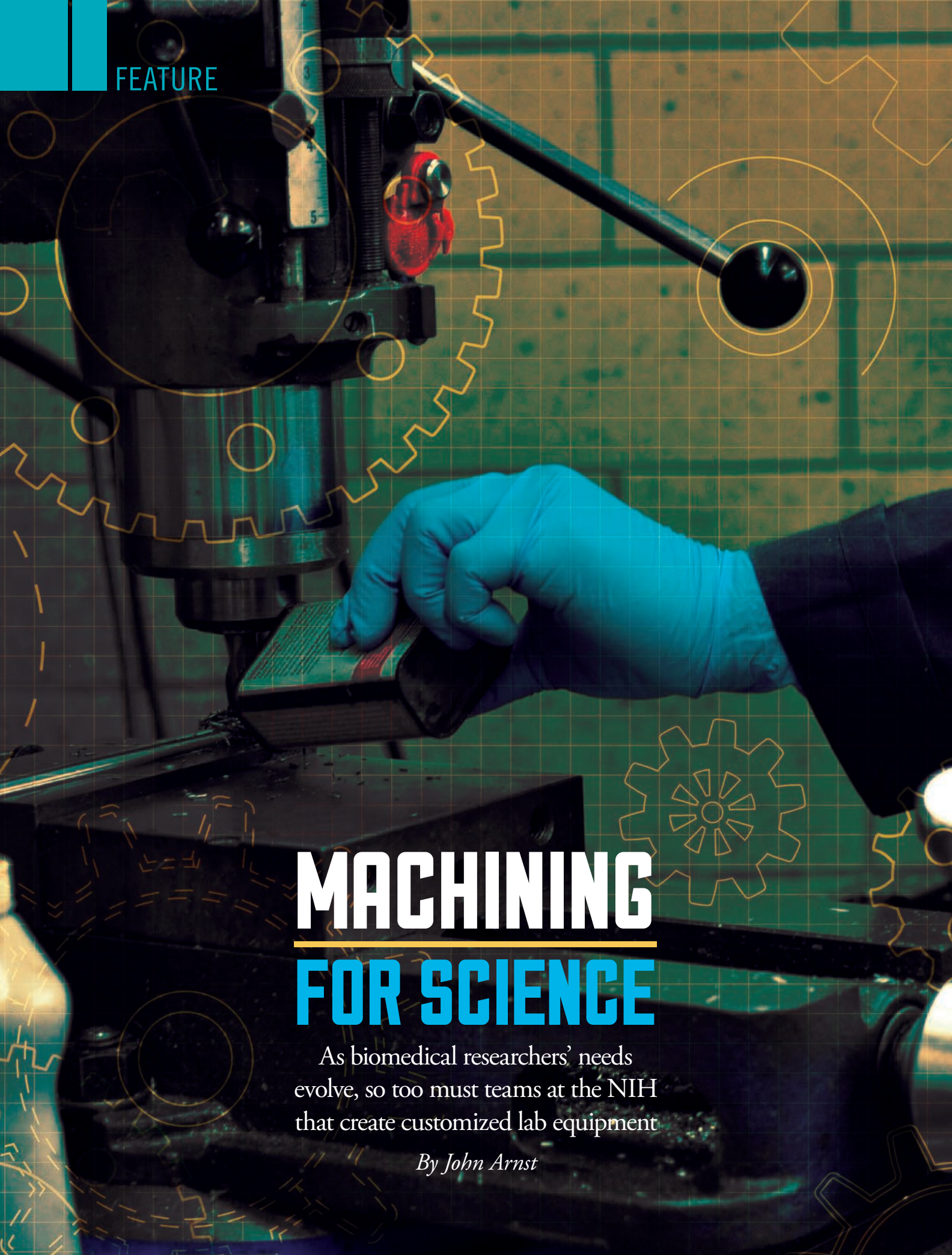


MACHINING FOR SCIENCE

As biomedical researchers' needs evolve, so too must teams at the NIH that create customized lab equipment

By John Arnst

In the basement of Building 13 at the National Institutes of Health's Office of Research Facilities, George Dold and his crew of engineers and machinists in the Section on Instrumentation strive daily to meet the needs of investigators with unconventional demands.

Their group is one of two on the NIH campus that build devices commercial lab manufacturers don't, products ranging from metal-free pneumatic flavor droppers to eight-armed radial mouse mazes.

The crew is fond of a clip from the 1999 animated adaptation of the comic strip "Dilbert" in which the underappreciated office drone recalls a childhood visit to a doctor.

"It's worse than I'd feared. I'm afraid your son has the knack," the doctor tells Dilbert's mother. "It's a rare condition characterized by an extreme intuition about all things mechanical and electrical, and utter social ineptitude."

The latter half of the characterization may be self-deprecation, but the former rings true — at age 7, Dold couldn't keep his hands off tools in his father's lab, which was at the National Institutes of Health.

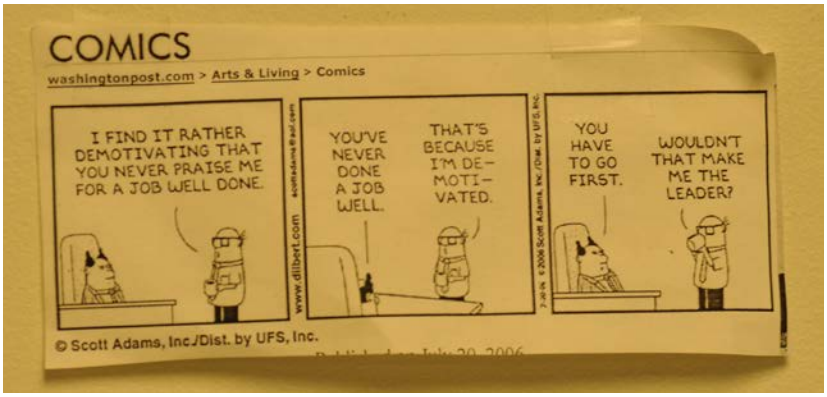
"I've always been a tinkerer," Dold said. "I also know how to fabricate quite a bit. Don't ask the machinists about that, though, because they'll say otherwise."

The Section on Instrumentation has its roots in a technical support group founded in the 1950s by the intramural divisions of the National Institute of Mental Health, National Institute of Neurological Disorders and Stroke, and National Institute of Child Health and Human Development to share developments among the institutes. As computer use increased in the following decades, the group shifted to provide support for NIH labs through engineering, machine shop work and information technology services. When the IT work was spun off to another group, the engineering and machine work subgroups became the Section on Instrumentation.

When Tom Talbot, a mechanical engineer in the section, began working at the NIH in 1975, one of the primary tasks of the more than two dozen machinists with a different group in Building 13 was automating the process of inserting cotton into freshly washed test tubes.

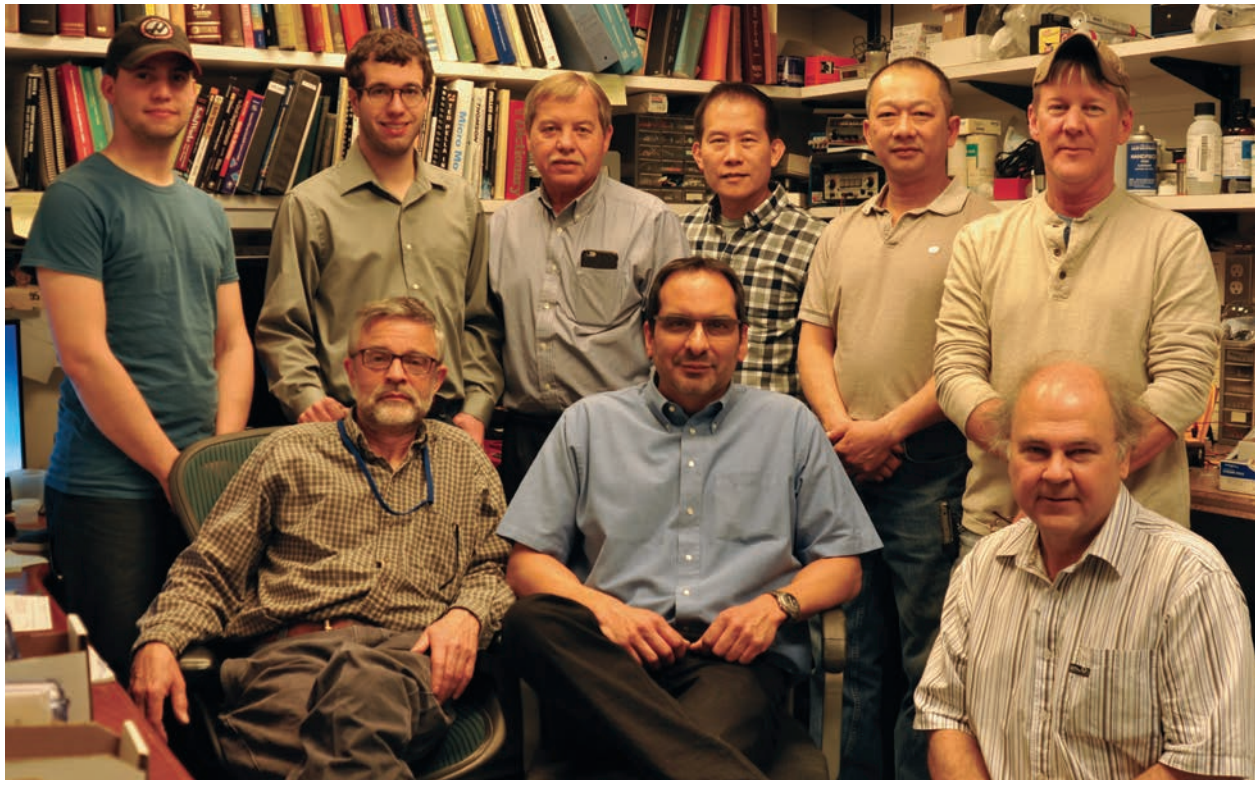
"All of that stuff went away with disposable glassware and what have you," Talbot said, "so those skills had to evolve or else go away, and to a large degree they went away."

The change in skill sets corresponded to a shrinking staff. Soon after Talbot arrived, the machinists were cut from two full-time shifts to one, which numbered about 35 mechanical, electrical and chemical engineers and 15 machinists, by



DANIEL PHAM/ASBMB

George Dold's group of engineers and machinists in the NIH's Section on Instrumentation is fond of the comic strip Dilbert. This panel is posted on the wall of their lab.



DANIEL PHAM/ASBMB

The engineers and machinists that make up the Section on Instrumentation help investigators across the NIH turn out hundreds of projects each year. Pictured in back are, from left, Daniel Yochelson, William Bennett, Tom Talbot, Danny Trang, Phuoc Pham and Daryl Bandy; in front are David Ide, George Dole and Bruce Pritchard.

Talbot's count.

Today, Dold's group includes four engineers and four machinists, or technicians, each with skills uniquely suited to the projects they handle.

Taste tests and mouse mazes

David Ide, a biomedical engineering technician, specializes in electrophysiology devices, such as microdrives that help move electrodes into the skulls of rodents and primates. He worked as a machinist at a small business in Maryland for 15 years before coming to the Section on Instrumentation 22 years ago.

"The specifics of the job change enormously; it continues to change," Ide said. "We used to make electrophoresis equipment, but no more. It's all commercially available.

"The guy I replaced said when he came to NIH they were sticking electrodes in frog legs. By the time he left, they were sticking electrodes in brain cells."

Another machinist, Daryl Bandy, and William Bennett, an engineer, operate the section's four 3-D printers. The printers are essential for constructing plastic devices, ranging from custom multiangle electrode holders to tables with trapdoors to conceal and reveal objects, that go into MRI machines for behavioral studies.

Talbot is an NIH lifer. After completing his master's degree in mechanical engineering, he took a position studying hemodynamics at the National Heart, Lung and Blood Institute; he later moved to that institute's clinical center to help develop surgical tools for open-heart research. He followed almost two decades there with a 10-year stint at the institute's Electron Microscopy Core before joining Dold's group, where he tackles electromechanical work.

In 2010, Talbot helped construct an MRI-friendly contraption of valves and tubes, a gustometer for a joint

study with researchers at the National Institute of Mental Health and National Institute of Diabetes and Digestive and Kidney Diseases.

"(The researchers) wanted a way to drop different tastes onto a patient's tongue while they're in the MRI," Talbot said. "Then they would look at different areas of the brain that would light up when the patients tasted salt, bitter, sweet, sour, et cetera."

The design for the gustometer, Talbot said, was copied and picked up in research hospitals across the country for studies on obesity. More than flattery, the section workers seem to take this imitation as a point of pride.

A more recent addition to the group, Bruce Pritchard, worked as an electrical engineer at Hewlett Packard in Oregon for nearly 30 years before a terminal cancer diagnosis brought him to Bethesda for a clinical trial at the NIH in 2011. During one of his many hours-long visits to the campus, Pritchard found his way to the Section on Instrumentation, where he struck up a friendship with his fellow tinkerers.

With his cancer in remission, Pritchard returned to the West Coast but stayed in touch with Dold. When a position in the section opened up, he applied, and he has been with the group for four years. At HP, Pritchard had designed equipment to pick up biophysical signals, and he hoped to continue that work at the Section on Instrumentation.

"I was looking forward to getting involved in amplifier design, and, lo and behold, I get here and there's very little need for it, but it's because the industry has stepped up to the plate," Pritchard said. "Fortunately, there's plenty of other fascinating stuff, and it's more (cutting edge) than what I came here thinking I was going to be able to contribute to."

Every few years, the group is tasked with an enormous project that draws on their collective skills and human power. A recent years-long project involved the construction of a laboratory that met the latest guidelines for good manufacturing practices, a facility with lead-lined radioactive ovens, or hot cells, in which robots forge the radionuclides essential for the countless PET scans done at the institute.

"They had the hot cells, (but) we basically had to integrate everything inside of them," Dold said.

On a smaller scale, Dold works with mouse mazes run by the systems engineering software LabVIEW. The legions of scientists involved in behavioral research at the National Institute of Mental Health regularly request the mazes, and Dold recently built one with eight branched pathways, each with automated doors and mouse tracking systems.

"The maze was 4, 5 feet in diameter," Dold said. "But we made it where you could take it apart pretty easily and robustly, and put it together. That was a fun project."

Sides of a coin

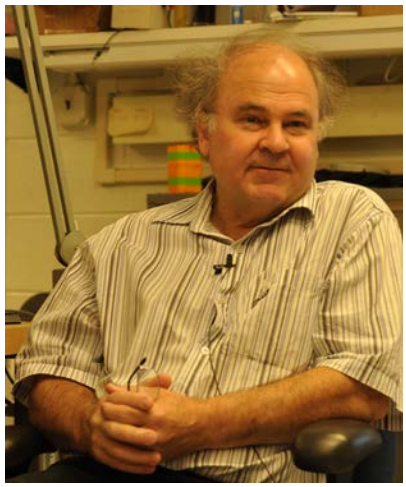
Tom Pohida's engineering group, the Signal Processing and Instrumentation Section, or SPIS, at the NIH fulfills a similar, complementary role to Dold's team, whose electromotive machinations are primarily used by researchers at the National Institute of Mental Health, National Institute of Neurological Disorders and Strokes, and National Institute of Child Health and Human Development. The Pohida group supports the whole NIH intramural research program when the needs of researchers grow beyond the reach of commercially available lab equipment and software or established laboratory and clinical methodologies.

POHIDA

Pohida's group of seven full-time engineers, with 10 to 15 interns throughout the year, was part of the NIH team that helped invent laser capture microdissection in the mid-'90s. The technique allows scientists to isolate specific cell types from tissue samples, an important purifi-

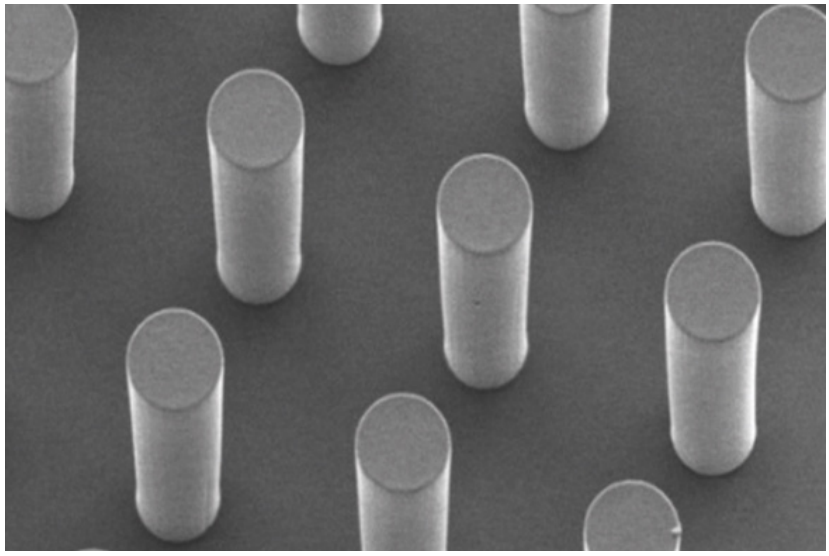
DANIEL PHAM/ASBMB

One of the many MRI-friendly components Daryl Bandy has helped build is a waveguide to help send signals to and from the control room.



DANIEL PHAM/ASBMB

One of Bruce Pritchard's current projects is helping a neuroscientist develop a hyper-localized electrode interface for neurostimulation in patients with epilepsy and depression.



COURTESY OF NICOLE MORGAN

The bioreactors that Nicole Morgan and Tom Pohida's engineering group in the Office of Intramural Research at NIH's Center for Information Technology designed for Michael Gottesman are small enough for cancer cells to grow around, measuring approximately 240 microns tall and 82 microns wide.

cation step that precedes molecular analyses.

"That was fully prototyped in-house, validated, patented and licensed. It's a gold standard in the tissue microdissection field," Pohida said. "But then, more recently, we developed a microdissection new method."

The first method is microscope-based and involves a user selectively firing a laser to melt and bond a dyed light-absorbing film to the cells of interest. This allows the targeted cell populations to be removed from the slide by pulling off the film when it's cooled. The new method, targeted activated microdissection, uses cell- or organelle-specific staining to absorb diffuse light to melt the clear film only at the desired targets. This multiyear, multi-institute technology development evolution is typical of the group's interdisciplinary projects, which are collaborative efforts that span 18 NIH institutes and centers.

"Our projects at NIH are rarely a one-off, a one-time effort," Pohida said. "We'll place an instrument in (a researcher's) lab, and then their ideas change, methods evolve, instruments need to be modified, and then there's a second- or a third-generation system to design and build."

In one of Pohida's recent collaborations, his group and Nicole Morgan, the acting chief of the Trans-NIH Shared Resource on Biomedical Engineering and Physical Systems, developed a novel micron-sized scaffolding device that acts as a bioreactor for the NIH researcher Michael Gottesman, who studies mechanisms of multidrug resistance in cancer.

"This is a problem we've been working on since the early 1980s, and it persists," Gottesman said. "We get better and better at developing drugs, and almost all of them fail because of resistance."

One obstacle to studying cancer cells is the surface the cells are grown on; many cancer cells have a strikingly different pattern of gene expression in a two-dimensional monolayer in a petri dish than they would exhibit in a human body. To better replicate that environment — for which mice make a poor proxy — and the blood vessels the cells use for oxygen delivery, Gottesman and his colleagues turned to Pohida and Morgan for a microfabricated polymeric vessel mimetic.

The device, which consists of

micron-size pillars the cells can grow around, is essentially an artificial capillary system that delivers oxygen directly to the tumor cells rather than relying on diffusion through a medium.

Gottesman and his team are still at the early stages of characterizing the device.


GOTTESMAN

"The recent data suggest that the growth of the cells and the gene expression of the cells is very different when they're grown in this new bioreactor," Gottesman said, "so we're hoping that that reflects changes in how they respond to drugs and mechanisms of resistance."

Fabricators, forensics and charters of freedom

The work done by Dold and Pohida's groups is not uncommon outside the NIH. Machine shops at public and private research universities fabricate equipment to fit researchers' needs, with some supporting single departments and others serving entire schools within a university.

The Section on Instrumentation and SPIS also aren't the only resources for National Institutes of Health scientists who require the help of mechanically skilled hands. When researchers need a piece of lab equipment modified, they often call upon Howard Metger and Robert Clary in the Office of Research Facilities.

Metger and Clary improve existing lab equipment, including centrifuges, microscopes and incubators, and design devices that can locate eyeballs, separate blood and support knees.

After a four-year apprenticeship at Landis Machine Company in the 1980s and local work with a trucking company, Metger began his federal government career as a machinist at the Naval Ordnance Laboratory in White Oak, Maryland, in 1987.

Mentoring over the long term

Like many groups within the NIH, the engineers in the SPIS are strongly committed to mentoring college and high school students. Through the NIH's Pathways co-op program, Tom Pohida and his co-workers bring on about a half-dozen engineering students from local colleges for full-time work each summer, which they often continue part-time during the fall and spring semesters.

"Our engineers are outnumbered by interns in the summer," Pohida said. "We're able to give the students a meaningful exercise for their career development and get them to a point where they're productive and working on research projects at NIH, developing instruments and software algorithms."

Pohida's group also participates in the 10-week National Institute of Biomedical Imaging and Bioengineering Summer Internship Program, which selects 16 interns between their junior and senior years of college to work on collaborative projects on the NIH campus.

The section also participates with mentor programs at local high schools that allow students to learn engineering skills during the week as a part of their curriculum.

A number of the Pathways interns stick around through their college years, Pohida said, and some are hired as federal employees after they graduate. At the moment, three of the six engineers working in the SPIS are former interns.

Sarah Anderson, a second-year medical student at Virginia Tech, worked in the SPIS through the co-op program for three years when she was an undergraduate at the University of Maryland. An electrical engineering major, Anderson began working at the SPIS the summer after her freshman year and continued through her graduation in 2017.

"I really liked how different all of the projects were and how much equipment we had," she said. "It's very unique in that the projects changed and you could learn very different skills that we don't learn in our curriculum."

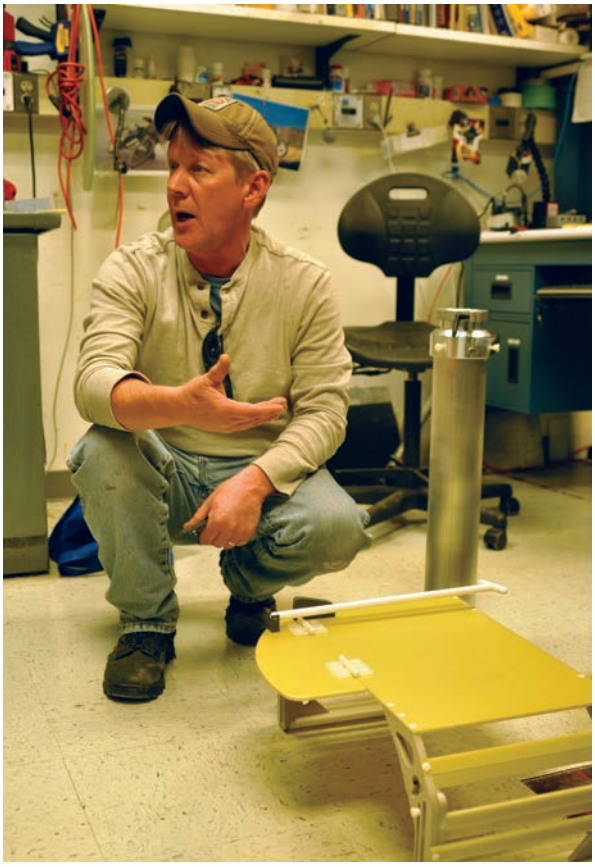
A relatively new medical school, the Carilion School of Medicine and Research Institute at Virginia Tech incorporates longitudinal research projects into students' curricula. Anderson believes her time at the SPIS made her a stronger candidate for both the application process and for choosing which lab she would work in.

"I really wanted to continue doing the same kind of work I was doing at NIH, so I talked to a lot of different people and I was able to have the practical skills where I could sell myself," she said.

Anderson now balances her coursework with work in the lab of Alan Asbeck, who designs and builds motion-assisted exoskeletons for lifting heavy objects.

Anderson's project in the lab involves designing low-cost circuit boards that filter and process the electrical signals produced by skeletal muscles, allowing a user or patient to control the exoskeleton. The project has additional applications for stroke patients who have suffered loss of motion and have difficult extending, flexing or bending an arm.

"The idea would be to have these sensors placed on the forearm," Anderson said. "If you could only partially do the motion, the muscle impulses would tell the exoskeleton to actively assist you."

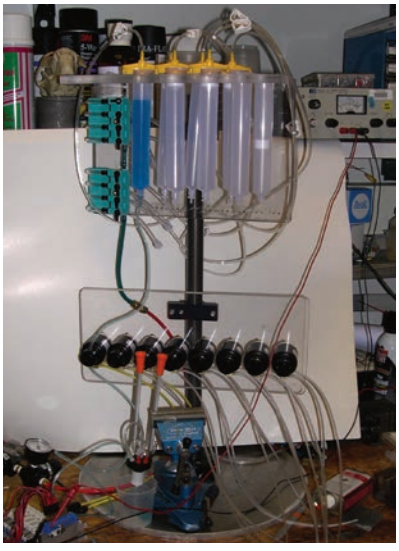


DANIEL PHAM/ASBMB

One of the MRI-friendly devices Daryl Bandy has helped construct with 3-D printing is a plastic table with a door that allows a user to reveal and conceal objects. The effect this has on areas of the brain can then be observed by researchers monitoring the MRI output.

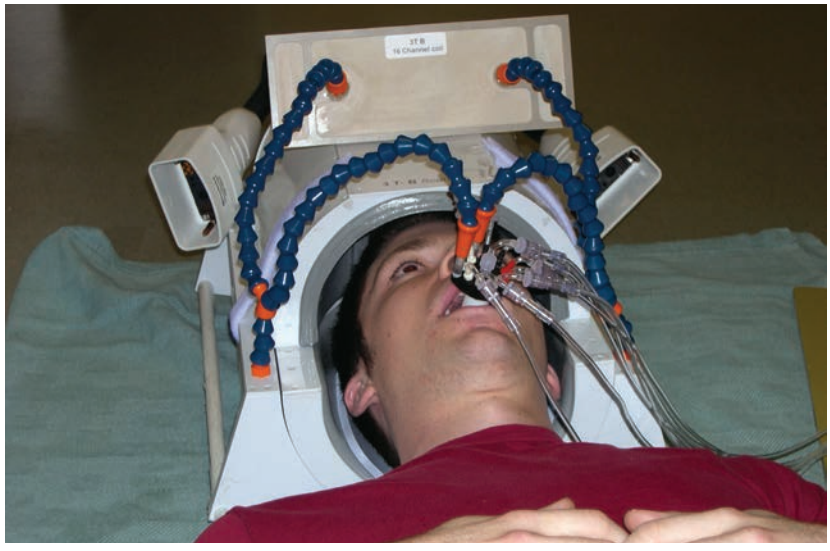
DANIEL PHAM/ASBMB

George Dold, who oversees the Section on Engineering, is a Maryland native with a bachelor's degree in electrical engineering and a master's degree in mechanical engineering.



COURTESY OF GEORGE DOLD

The Section on Engineering began developing their own prototype gustometers, which are used for a variety of taste test research, in 2009.



COURTESY OF GEORGE DOLD

The gustometers are designed to fit in an MRI machine, here demonstrated by Mark Reddish, a former postbaccalaureate worker in the lab. Tom Talbot recently updated the fluid regulating manifold and mouthpiece to a 3-D printed design.

COURTESY OF NATIONAL ARCHIVES AND RECORDS ADMINISTRATION

A visitor to the National Archives views the Declaration of Independence in its updated encasement, which Howard Metger and Robert Clary helped construct while working at the National Institute of Standards and Technology.

In 2004, Metger began working at the Office of Research Services' Mechanical Instrumentation Design and Fabrication Branch, which was transferred to the Office of Research Facilities last fall due to a new funding model. Clary, who began working at the National Institute of Standards and Technology in a co-op position in 1990 after graduating from Carroll County Vocational Technical School in Westminster, Maryland, moved to the ORS in 2006.

During his time at the NIST, Clary helped develop a standard calibration tool for the ballistics reference databases used by all 50 states and the FBI, known as the Integrated Ballistic Identification System.

As a bullet is fired from a gun, the gun's chamber leaves a serial number-like series of grooves on the bullet. When a bullet is found at a crime scene, those grooves can be run through a ballistics reference database, which will assign a score for how likely a match the grooves are to a gun in the system.

"When you take those (scores) to a court of law and you have a defense attorney ask how good it is, you need a reference material to compare individual systems," Clary said. "The only way to do that was to take one bullet and send it around to all 50 states and to the FBI and have them measure it, and hopefully all of their scores come up the same. But a better way to do it would be if we had 50 copies of the exact same bullet."

To cut those identical reference grooves into fifty-plus bullets, Clary and his colleagues at the NIST used a lathe equipped with a diamond crystal sharpened to a near-atomic point. They then sent the bullets to each ballistics center to get their tools — what Clary calls "a computer with

DANIEL PHAM/ASBMB

One of many prototypes built by the machinist Daryl Bandy in the Section on Instrumentation is a device that allows a researcher to simultaneously run dyes on multiple slides while using minimal reagents.

a microscope" — on the same page.

"It didn't solve any problems, but what it did do was it allows the court systems to lean a little heavier on the IBIS system," Clary said. "We can't just use the score to say a crime was committed, but it opens up avenues that are much more reliable."

In 1999, Metger and Clary, then working together at the NIST, were contracted to help build new versions of the bronze and glass cases in the National Archives Building that preserve and display the United States Declaration of Independence, Constitution and Bill of Rights, otherwise known as the Charters of Freedom.

"I built all of the cases and document platforms and lids for them, and Bob worked on a lot of the electronics that went on the inside of the cases," Metger said.

The documents, first encased and displayed at the National Archives in 1952, now are kept in sealed cases of glass, titanium and aluminum.

At the ORF, Clary is working on a similar project to preserve and display the charts and notes of Marshall W. Nirenberg, a researcher at the National Heart, Lung and Blood Institute who shared the 1986 Nobel Prize in physiology or medicine for discovering the molecular mechanisms responsible for translating DNA into proteins.

"The charts that he used when he was doing his research back in the day are now these Rosetta stone-type things, and the National Library of Medicine has decided that they want to encase these in some anaerobic boxes," Clary said. "We're not having luck with the commercially available boxes as of yet, so that's an ongoing project."

Some assembly required

Outside the public sector, working on equipment in molecular biology labs largely comes down to calibrating the instruments and replacing worn-out parts. Matt Helmrick is a laboratory technician at Dynex Technologies, a company mostly known for its multiplate enzyme-linked immunosorbent assay processing machines. Helmrick services the machines designed to detect the presence ofsubstances in liquid or wet samples.

"Some of the ones you don't want to deal with (can be) in a very high-throughput lab, where the technicians come in on shifts and the machines just don't stop," Helmrick said. That nonstop use means the machines' valves and tubes need to be replaced much sooner than expected. "And if (the machines) are in forensic toxicology or something, they're covered in things you don't want to touch."

On the production side, Dynex's group of fabricators oversees the assembly of each machine, testing circuit boards and tubing before sending them out to labs across the country.

For many of the fabricators, "this is the first job they got as an intern out of high school, and they ended up working here for 20 years," Helmrick said. "I know one guy was really good at pinball machines — he joined quite a while back, and now he's overseeing one of the departments."

Helmrick began as a research scientist in San Francisco, bouncing around biotech companies and eventually winding up at Bristol–Meyers Squibb, where he worked in research and development related to genomic technology.

"Higher and higher genomic and PCR work requires a lot of back-end work, so it helps if you have some coding experience to write your own searches, format files correctly, and look for genes of interest," he said.

The less sophisticated lab work, though — pipetting, plating and every manner of PCR-prepping — is already in the realm of automation and likely to be completely overtaken by it, Helmrick believes.

"I think lab technicians are going to get replaced by machines long before truck drivers ever have to worry about self-driving trucks," he said.

Creativity, however, is currently beyond the purview of machines and plays a large part in the work Clary and Metger perform for researchers.

"I think, internally, I've changed my attitude of being a machinist to being more of a researcher's assistant. When I first started in the trade back in the day, I was a machinist, and I still am at heart very much a machinist," Clary said. "I like to cut precision things, I like to make things that don't exist, but at the same time … I spend a tremendous amount of time gathering details and making suggestions."

Despite the pace of development in biomedical technology, Dold is optimistic about the future of the Section on Instrumentation.

"I think that we're going to have to continue to try to figure out ways in which to build equipment quicker and adapt more technology quicker," Dold said. "I think that the 3-D–printing revolution that was supposed to keep on exponentially growing (has) tapered off, but I still think it's going to have continued impact with us."

Every year, Dold's group averages around 500 projects, a number of which are lower tech and do not require tools like the 3-D printers.

"There's still a lot of people that need simple things done, and they need it done quickly," he said. "The jobs range from relatively low tech to very complex engineered systems with many features. We have to be able to do both ends of the spectrum."

John Arnst (jarnst@asbmb.org) is ASBMB Today's science writer. Follow him on Twitter at twitter.com/arnstjohn.

Video

Engineers and machinists of the Section on Instrumentation at the National Institutes of Health describe their work in a video at **asbmb.org/asbmbtoday**.

How two grad students at a poster session cooked up a study and cracked a puzzle

By Laurel Oldach

It was January 2008.

Lyme disease was — and remains — the most common vector-borne disease in the Northern Hemisphere. Doctors across the United States were beginning to prepare for a summer when nearly 29,000 new cases would be diagnosed.

Celebrex was a blockbuster anti-inflammatory drug, with $2.5 billion in sales that year for treatment of arthritis and acute pain — including the arthritis that can be a complication of untreated Lyme infection.

And in Big Sky, Montana, two graduate students at a Keystone Lipids Conference were cooking up research that would explain why, at least in mice, treating Lyme with Celebrex is a bad idea.

For Victoria Blaho and Matt Buczynski, the conference was the launching pad for an independent career in science. Both came to the meeting as graduate students with well-established projects, looking to expand on a certain angle. Both came away with an exciting new project and, eventually, the answer to a puzzle neither could have cracked alone.

"It's a great example of why it's important for graduate students and postdocs to go to meetings," Blaho said, "especially these small meetings."

A mouse model of Lyme disease

Lyme disease in humans is caused by infection with the bacteria Borrelia burgdorferi, a spirochete that is transmitted by tick bite. Antibiotics given early can clear the infection. But if left untreated, it can spread to the joints and other tissues, causing arthritis in about 60 percent of cases. In some patients, the arthristis persists even after a course of antibiotics.

At the time of the conference, Victoria Blaho was a graduate student studying a mouse model of Lyme infection in Charles Brown's newly opened lab at the University of Missouri. The group knew that after being infected with B. burgdorferi in the hind paw, their mice developed arthritis in the nearest ankle joint, mediated by the innate immune system. The arthritis resolved as the immune system cleared the bacteria from the joints. While studying this model, Blaho had found that treating mice with Celebrex could prevent their arthritis from healing.

BROWN

"It didn't make the disease worse," she said about the drug. "But it didn't decrease the inflammation, and it prevented the ability of the arthritis to resolve."

Celebrex inhibits a specific cyclooxygenase enzyme, Cox-2, which converts arachidonic acid released from the cell membranes by the phospholipase A2 enzyme, or PLA2, into lipid mediators of inflammation. So it was surprising that inhibiting the Cox-2 enzyme would turn a short inflammatory response to infection into a chronic inflammatory disease.

"It's exactly the opposite of what you'd observe in other models," Blaho's graduate mentor, Charles Brown, said.

In other mouse models of arthritis, which are driven by an antibody response to the mouse's own tissue, inhibiting Cox-2 prevents joint pain. But unlike most mouse models, the Lyme disease model involved a whole pathogen, rather than a single molecular component, and a systemic immune response to it. The lab knew that the drug did not affect development of an antibody response compared to untreated animals, or clearance of Lyme bacteria from the joint where Blaho had introduced it.

To understand how the drug changed the course of infection, Blaho and Brown reasoned that they needed to understand how Cox-2 products change during infection. But the compounds are difficult to extract and study; at the time, no commercial services offered lipidomic assays, and they didn't know anyone in the lipid field.

A novel technique and a felicitous meeting

Matt Buczynski, a student of lipid biochemist Edward Dennis at the University of California, San Diego, was at the forefront of the new wave of lipidomics. He had been second author on a recent paper out of the Dennis lab that introduced a new technique for simultaneously identifying and quantifying up to 60 lipid species in the eicosanoid family. Eicosanoids are water-soluble mediators of inflammation that all are derived from arachidonic acid released from the cell membrane by PLA2 and then modified by various enzymes, including Cox-2.

DENNIS

The eicosanoids have various biological effects, but because they are so similar, each representing a different minor modification to arachidonic acid, it was difficult to tell them apart. Being able to measure them separately was an important advance. The lab had piloted the development of the lipidomics field by exploiting a cell model of inflammation.

"We were working with macrophages, and doing these really cool lipidomics approaches," Buczynski said. "B ut of course if you're working with one cell type, you're only seeing part of the picture."

When she first encountered his work, Blaho appeared to feel that way. Buczynski recalls sitting in the audience at the start of the 2008 Keystone Conference on Eicosanoids and Other Mediators of Chronic Inflammation as Dennis gave the plenary lecture. Buczynski introduced himself to the graduate student sitting next to him, Blaho, who didn't know his connection to the speaker.

"She was like, 'This guy's only looking at one cell type; what does he think he's going to find? This is really cool stuff, but he's got to look at better models,'" Buczynski said.

With a chuckle, he recalls the look on her face when Dennis concluded the talk with an acknowledgments slide and Buczynski's picture.

"She just looked over at me like, 'Ohhhhh, man.'"

Fortunately, Buczynski wasn't offended.

At the poster session after the plenary lecture, where posters were arranged alphabetically, the two found themselves next to one another once again.

"It gave us a great opportunity to talk about the really cool mass spec approaches that we were using and

COURTESY OF SANFORD BURNHAM PREBYS MEDICAL DISCOVERY INSTITUTE

Victoria Blaho is a research assistant professor at Sanford Burnham Prebys in California.

COURTESY OF VIRGINIA TECH

Matt Buczynski is an assistant professor at the School of Neuroscience at Virginia Tech.

the really amazing model that she had developed," Buczynski said.

Between them, they realized that the two projects were perfectly complementary.

Blaho had seen a change in lipid-mediated inflammation but had no way to measure the change in lipids. Buczynski had a technique for measuring changes to many lipids at once but was in search of an interesting question to attack with it.

"It was a very propitious time," Blaho said of the meeting. "It just so happened that Matt really wanted to get some sort of in vivo relevance and I really needed someone who had the biochemical expertise for measuring these (lipids)."

Cooking up a collaboration

Over the rest of the conference, the pair fleshed out a plan to work together.

"It was scientific, but it was also building a rapport," Buczynski said of their ongoing conversation. "That's one of the things that makes conferences so valuable. You can read about someone's work online, but until you meet them, it's hard to tell if this is someone that you could really work with for the next one to three years."

Back in the lab, it took time to work out how to extract lipids from the hard tissue of the mouse ankle and ship them from Missouri to California unharmed. Methodology is often a hurdle for lipid biologists, Blaho said.

"Despite the fact that we've known about these things for decades and decades, there's still so much we don't know," she said, "and so much we have to do on our own, for experimental protocols."

While Brown had approved the pilot experiments, the Dennis lab was already busy with quite a few collaborations. This one, with its untested tissue extraction protocols, was risky. So Buczynski decided to run a few covert pilot experiments to see if the idea could be fruitful.

"We had just started running a lot of samples from lots of different collaborators, and so sneaking in one or two pilot samples wasn't too challenging," he said. "It was important, because you don't want to invest a lot of money doing this elaborate time course if there's literally nothing in the sample."

With the technical difficulties sorted out, the pair presented their preliminary data to both PIs. They made a strong case that they could track eicosanoids from arthritic joints over time and that the collaboration was worth investing in. In April, just four months after their first meeting, they presented a preliminary lipid time course in B. burgdorferi-infected wildtype mice at the 2008 American Society for Biochemistry and Molecular Biology Annual Meeting in San Diego. They published their work in the ASBMB's Journal of Biological Chemistry in August 2009.

A curiosity-driven project

Dennis called the mouse model "a beautiful model of an infection and its resolution." After being infected, the mice have a dramatic, localized inflammatory response as B. burgdorferi invades the ankle. The joint swells up, becoming infiltrated with innate immune cells, and the mice start to show symptoms of arthritis. As the adaptive immune system comes to the rescue, generating antibodies against B. burgdorferi, the bacterium is cleared, after which the arthritis generally resolves.

Because Blaho had found that inhibiting Cox-2 prevented resolution of arthritis, the grad students compared lipid profiles in two groups of animals: one with and one without the Cox-2 gene.

"We measured as many different (lipid metabolites) as possible, regardless of whether we had any idea whether they were involved," Blaho said. "It was kind of our version of shotgun lipidomics."

The two graduate students spent

hours on the phone, each looking at the same spreadsheets, searching for differences between the Cox-2-deficient mice and the untreated controls.

"We could see, corresponding with the peak of infection, the production of all of these bad prostaglandins that cause inflammation and swelling," Dennis said of the wild-type controls. "Then after a small time delay, we could see the production of protectin."

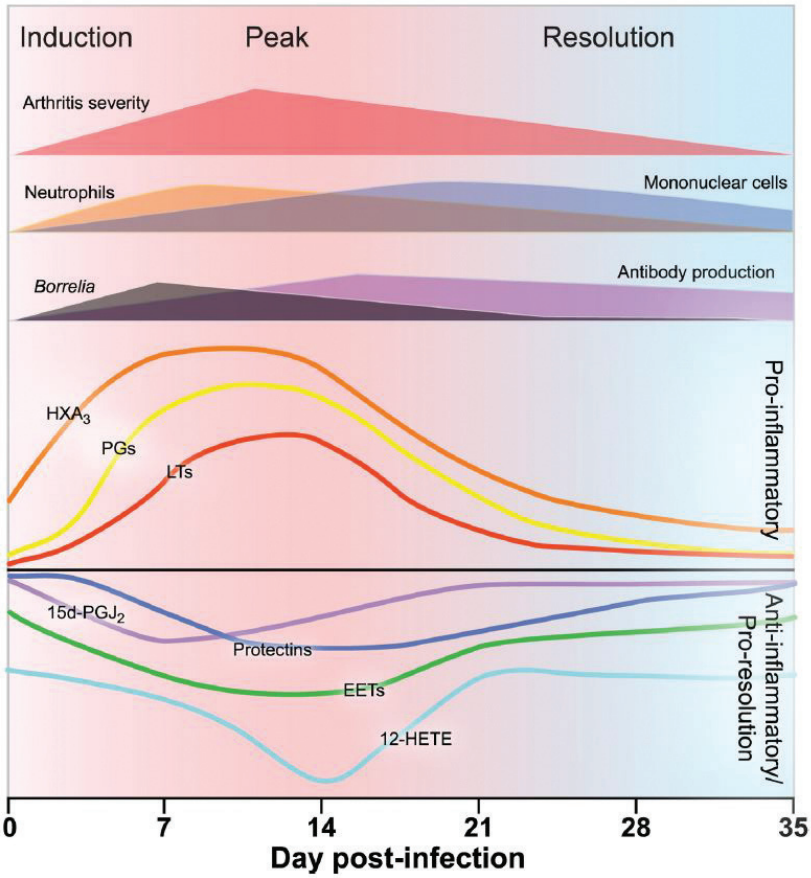
Prostaglandins are eicosanoid products of Cox-2; protectin, which signals for resolution of inflammation, is another lipid made by the enzyme 5-Lox. Strangely, in the mice with a knockout of Cox-2, there were not only lower levels of the eicosanoid products of Cox-2 products but also lower 5-Lox products.

Inhibiting Cox-2, and thereby reducing the circulating prostaglandin eicosanoids, would have been expected to stop inflammation in its tracks. But Blaho and Buczynski's findings reflect a role for Cox-2 in the resolution of inflammation. Some of its products are potent anti-inflammatory molecules, and others are precursors to production of pro-resolution molecules, such as protectin, by other enzymes. When mice were treated with Celebrex to inhibit Cox-2, or never had Cox-2 to begin with, the protectins never were made; therefore, the inflammatory response continued indefinitely.

The work contributed to a growing sense in the field that resolution of inflammation is an active process rather than a vague fizzling out of ongoing pro-inflammation signals.

Two careers launched

When they left graduate school, Buczynski and Blaho moved on from the mouse Lyme problem to different questions about the biological roles of lipids, but it isn't hard to see interests that they described in this project reflected in their current research programs. Both have become assistant professors in the last two years.



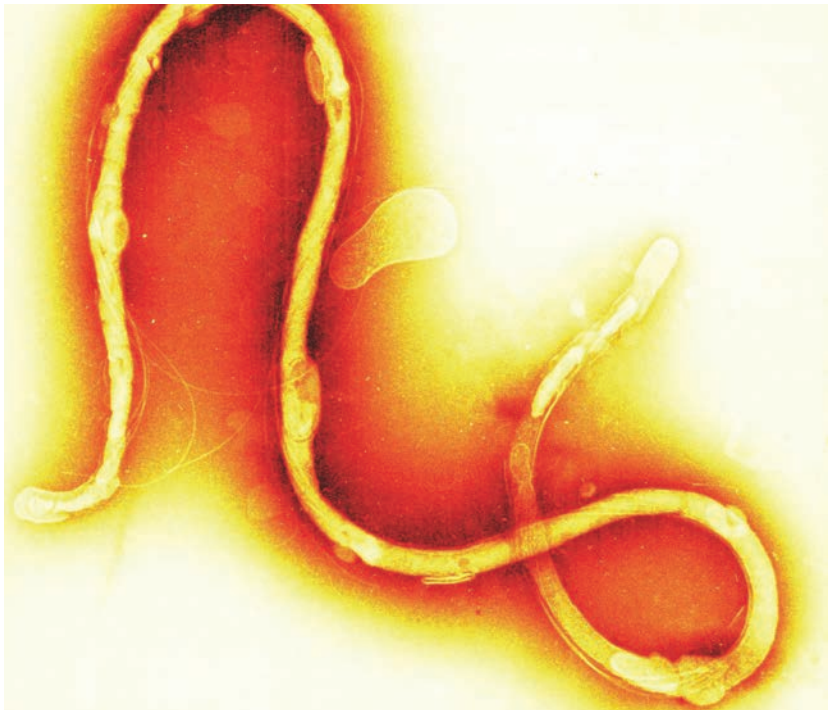
COURTESY OF VICTORIA BLAHO AND MATT BUCZYNSKI

A figure from Victoria Blaho and Matt Buczynski's 2009 paper in JBC summarizes the production of pro-inflammatory and anti-inflammatory signaling lipids over the course of infection in wild-type mice infected with Borrelia burgdorferi.

Following an interest in neuroscience derived from another graduate school collaboration, Buczynski joined the lab of the late Larry Parsons at The Scripps Research Institute as a postdoc. He studied behavioral and lipidomic changes that happen during nicotine addiction, work he has continued since starting his own lab as an assistant professor at Virginia Tech in 2016.

Blaho, who started graduate school as an immunologist, has continued to surf the intersection of lipid biochemistry and inflammation. She went to Weill Cornell Medical College to study sphingosine-1 phosphate, or S1P, as a postdoc with Timothy Hla and now works on S1P signaling in the immune system as a research assistant professor at Sanford Burnham Prebys Research Institute in La Jolla, California.

Dennis and Brown have continued to collaborate on eicosanoids in Lyme disease, co-authoring four subsequent



COURTESY OF TINA CARVALHO, UNIVERSITY OF HAWAII AT MANOA

Borrelia burgdorferi, the bacterium behind Lyme disease in humans, does not usually cause symptoms in infected mice. Blaho and Buczynski worked in the C3H mouse line, which is unusually susceptible to borreliosis and arthritis.

research articles and a review last year on the model system.

"We're still trying to understand how the innate immune system recognizes Borrelia and how that leads to development of disease," Brown said. "When does the immune system decide that it's time to resolve? What are those signals for resolution?"

Meanwhile, Dennis has expanded his lab's focus to understand the role lipid signaling plays in other infections, lately adding an influenza angle.

"In patients, we identified numerous eicosanoids that increased with influenza, including both pro-inflammatory prostaglandins and anti-inflammatory (pro-resolution) eicosanoids," he said. "So lipidomics has led to identifying new therapeutic approaches to infectious disease."

Medical guidelines for Lyme disease do not suggest non-steroidal anti-inflammatory drugs, also known as NSAIDs. But they also don't contraindicate them. "That paper would suggest that for people diagnosed with Lyme disease, it would be prudent not to take NSAIDs," Brown said. "I'm not sure that the clinicians are paying attention to that part."

Physician-scientist Linda Bockenstedt of Yale University, a clinician whose lab studies possible mechanisms for lasting Lyme-related arthritis, sees it differently.

"We don't have any evidence in humans that taking anti-inflammatories — steroids, or NSAIDs, or selective Cox inhibitors — is detrimental after antibiotics have been started," Bockenstedt said. "Obviously, with an infection, we would like to eliminate the cause of the inflammation first, before giving drugs to suppress the inflammation."

BOCKENSTEDT

She adds that anecdotes from scattered patients link steroid injections, administered to joints to alleviate arthritis before the start of antibiotics, with antibiotic-refractory arthritis. However, starting antibiotics is the standard of care, and there never has been a controlled study of steroids to see if they cause the complications.

Though their research interests have taken them in different directions, Blaho and Buczynski keep in touch. They both have fond memories of the the collaboration and the sense of community it gave them.

"Having the ability to talk to people who you know are experts and will be interested makes such a difference," Blaho said, "versus a shot in the dark and people who don't know what's important or exciting or worth funding."

Doing the study together "made me realize how much more fun it is to collaborate," Buczynski said. "I don't feel like there's anything I've accomplished in science that didn't involve other people's help, and it's more fun when they feel like they got kudos for that."

Laurel Oldach (loldach@asbmb.org) is a communications intern at the ASBMB.

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Mentors guided a career that now aims at a cure

Marina Ramirez-Alvarado, a professor of biochemistry and molecular biology at the Mayo Clinic in Rochester, Minnesota, studies the molecular determinants that drive protein misfolding and amyloid formation in light chain amyloidosis, with the goal of ameliorating the organ damage caused by this disease.

Ramirez-Alvarado earned a B.S. in biochemistry and an M.S. in biotechnology at the Universidad Nacional Autonoma de Mexico. She earned her Ph.D. in biology at the European Molecular Biology Lab, Ruprecht-Karl-Universitat Heidelberg.

Here, she talks about how she became interested in protein research and describes how numerous mentors guided her on her career path and supported her research.

What key experiences and decisions enabled you to reach your current position?

When I decided to study abroad, I came to the realization that scientists in developed countries were not better than my colleagues in Mexico. This was key for me, because I was always intimidated by the big shots in the field. It helped me to learn more from their work and to believe that I could make a difference in the field of biochemistry.

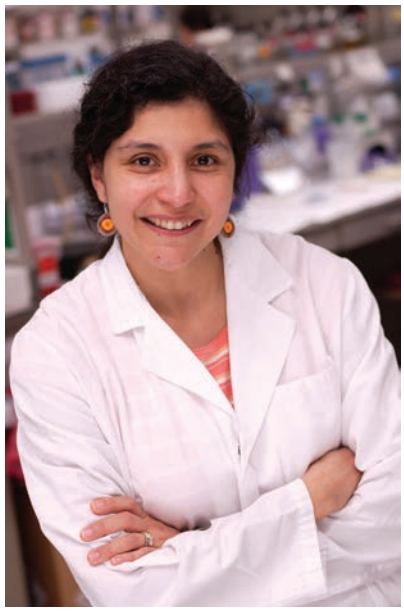
How did you first become interested in science?

When I was 15, I wanted to become a travel agent because I loved traveling and seeing new places. A friend of mine in middle school, Margarita Castrejón Balderas, pointed out that my talent for science would be wasted at a travel agency. She helped me develop my love for chemistry. When I went to college, I minored in food chemistry because I wanted to develop nutritious food to solve world hunger. In my second year, I learned about proteins from my biochemistry professor, Homero Hernandez, and I fell in love with their complexity and their beauty. Soon after that, I started doing protein research.

Were there times when you failed at something critical to your path? How did you regroup and get back on track?

I was very naive about authorship as a student, and I gave away my position in a joint first authorship as a sign of friendship. This happened at a time when journals didn't give much credit to the second name in the list. After that experience, I discuss authorship even before we start writing the first draft of a paper.

When I was a junior assistant professor, my grants and papers constantly were rejected. I felt very inadequate and questioned my ability to run an independent research program. My mentor, Grazia Isaya, told me that, in general, female scientists take failure as a sign of inadequacy. Hearing these words from an established investigator motivated me to keep trying. Another colleague, Tom Spelsberg, used to tell me, "Persistence is what we need in academe — keep trying, kiddo!"



COURTESY OF THE MAYO CLINIC

Marina Ramirez-Alvarado studies light chain amyloidosis, a rare disease caused by the build-up of an abnormal protein.

What are your hobbies?

I am a martial artist. I started taking taekwondo classes with my son, and now we are both black belts. I sing and play guitar in a band. I am a new but enthusiastic gardener for native species and pollinator-friendly plants.

What was the last book you read?

"The Hot Zone" by Richard Preston (thanks, Dr. Sara Holditch, for the recommendation). It's a terrifying story about filoviruses written

in 1995 and very relevant today. I followed it up with "Love is the Cure: On Life, Loss and the End of AIDS" by Elton John — a human, inspiring story about the AIDS epidemic in the 1980s.

Do you have any heroes, heroines, mentors or role models?

I have many, starting with Homero Hernández, who taught me cell biology and biochemistry as an undergraduate. My first research mentor, Agustin Lopez-Munguía, taught food enzymology. Agustin became my undergraduate thesis research mentor and is now a dear friend. Eduardo Bárzana taught industrial fermentations and helped me decide my path for graduate school.

During my Ph.D., Anne Ephrussi was my first female scientist role model. She was a scientist and a mother who inspired me to seek work-life satisfaction and integration.

During my postdoctoral training, Andrew Miranker was a role model. He was our next-door lab neighbor. I was the only one in my lab working on amyloid, and Andrew had just started his laboratory working on amyloid studies.

As an assistant professor, my first mentor was Frank Rusnak, who helped me so much in a very short time and died tragically six months after I started to work at Mayo. Grazia Isaya became my mentor at that point and offered me her wisdom and calm during the hard days of rejections.

Outside Mayo, I received support from Jeff Kelly, who has always supported my research. When I was invited to be an editor in chief for a protein misfolding book, Jeff agreed to edit it with me and recruited Chris Dobson, who was a pleasure to work with and whose work I have admired since my years in graduate school.

Joel Buxbaum has been a fantastic role model since we met in Tours, France, during the Amyloidosis Symposium. Joel has been a source of wisdom and good humor in my life as a professor. Writing a book chapter with him was an incredible experience.

An unusual role model and hero is Tim Stepanek, the first amyloidosis patient I met. Tim was involved in ways I never thought patients would get involved. He was the first person to believe with all his heart in what I was doing. He advised me in ways he probably never realized. He faced a devastating disease with grace and dignity. When he died 11 years ago, Tim trusted me with the task of trying to understand his disease so people like him would not have to die anymore.

What keeps you working hard every day?

I have the honor to conduct biochemical studies on a rare, devastating, incurable disease. What keeps me working hard every day is the notion that one day, possibly before I retire, our research will contribute to finding a cure for light chain amyloidosis.

Interacting with young scientists in training and seeing them learn and grow is inspiring and rewarding for me. I love learning new things every day.

About the Research Spotlight

The American Society for Biochemistry and Molecular Biology's Research Spotlight highlights distinguished biomolecular and biomedical scientists from diverse backgrounds as a way to inspire up-and-coming scientists to pursue careers in the molecular life sciences. Eligible candidates include Ph.D. students, postdoctoral fellows, and new or established faculty and researchers. To nominate a colleague for this feature, contact education@asbmb.org.

Carbon dioxide 400

or how a biochemist became a climate activist

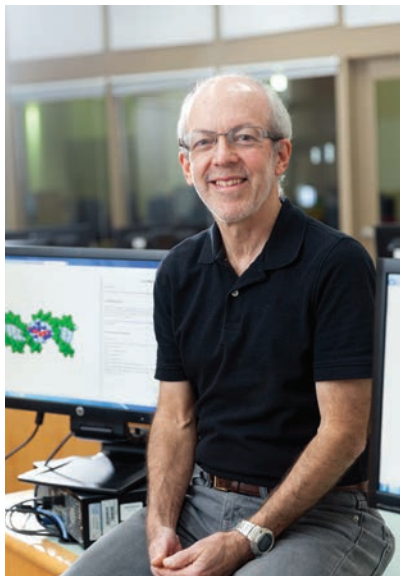
By Henry Jakubowski

When the concentration of atmospheric carbon dioxide passed 400 parts per million in June 2016, I felt a sense of urgency. The last time CO_2 was 400 ppm was 3.5 million years ago in the Pliocene Epoch, when the poles were 10 F warmer and sea levels were 16 to 131 feet higher.

As I listened to my children speak of both global and local economic and social upheavals arising from climate-related migrations and environmental changes, I started to envision a dismal future for them. I heard young people questioning whether they should have children in an increasingly unstable and insecure world. I felt a growing sense of guilt. As a member of the generation that bequeathed them this future, and as a scientist/educator, have I failed in communicating the power of science to address societal concerns? Am I partly to blame for the growing disbelief in the process of science, especially as related to climate change?

When the U.S. pulled out of the Paris climate accord and began removing environmental regulations, my sense of urgency increased. After decades of teaching, I asked myself, What can I do? More importantly, what should I do?

I have taught aspects of climate change, but mostly to nonscience majors in courses designed to address social issues. In those classes, I could focus on the process of science. For example, by reading sections of Thomas Kuhn's "Structure of Scientific Revolutions," my students began to grasp how scientific consensus develops, matures and changes; these ideas are critical to nonscientists as they evaluate information. In my science majors' courses, however, I follow a prescribed syllabus, and we have little time to discuss the science related to societal issues.



COURTESY OF COLLEGE OF ST. BENEDICT/ST. JOHN'S UNIVERSITY

Henry Jakubowski, a professor of chemistry at the College of St. Benedict/St. John's University, talks about the effects of climate change in all his classes and organized "Our Climate Futures," a panel discussion with faculty from diverse disciplines.

After January 2017, I felt ethically compelled to discuss climate change in all my classes. Since then, I have found ways to relate background infrared spectra (which students take each lab) arising from atmospheric CO_2 to climate warming arising from absorption of infrared light by greenhouse gases.

In our separations/chromatography lab, when I discuss doing 60-liter blood preps to isolate clotting proteins from cows, I take the opportunity to talk about the climatic implications of raising 41 million tons of plant protein to produce an estimated 7 million tons of animal protein for human consumption, and I note that the U.S. could feed 800 million people with the grain that livestock eat. Add greenhouse methane emissions from those cows and the carbon-hydrogen infrared stretch they see each day in the lab, and climate change can become real.

When covering influenza virus hemagglutinin binding to cell surface receptors, I discuss future pandemics arising from emerging viruses and their links to climate change. The role of bicarbonate transporters and carbonic anhydrase could be studied in coral formation and the global carbon cycle.

I often can't gauge students' immediate responses, but one teaching assistant, after taking my lab, told me how much my discussion of animal and plant protein generation affected her understanding of the interrelatedness between unquestioned societal practices and climate change, leading her to decrease her meat consumption.

Addressing climate change requires a multidisciplinary approach, so in November 2017, I organized a panel discussion titled "Our Climate Futures" with faculty from chemistry, biology, nutrition, environmental studies, economics, political science and theology. After the discussion, one of my students wrote: "I hope to counteract climate change in my own small way now, and you help

COURTESY OF DAVID MATTHEW, ST. CLOUD ROTARY CLUB

Henry Jakubowski talks to a local Rotary Club in Minnesota about climate change and a carbon fee-and-dividend economic approach to reduce CO_2 emissions.

give us concrete examples of how to do so. The climate panel really made me look at the situation differently and not simply from a scientific facts point of view."

I wanted to reach out beyond the classroom and university communities, but initially I was uncertain how to proceed. The Citizen's Climate Lobby, a bipartisan national advocacy group, opened doors for my activism. With their support, I've given talks to Kiwanis and Rotary Clubs, the League of Women Voters, a senior citizens' center and a church on climate change and a carbon fee-and-dividend economic approach to reduce CO_2 emissions. I've written op-ed pieces and letters for our local paper on science and policy and political barriers to climate change action. I have appeared on a local radio show. The opportunities to speak out are more numerous than I imagined.

As scientists and educators, we have a unique ability and opportunity to educate the public about climate change and make them aware of potential solutions, such as those offered by carbon pricing models. I encourage you to use your talents to address climate change. Once you begin, opportunities to make a difference will present themselves. Take those opportunities for the sake of our children and grandchildren, and our collective futures.

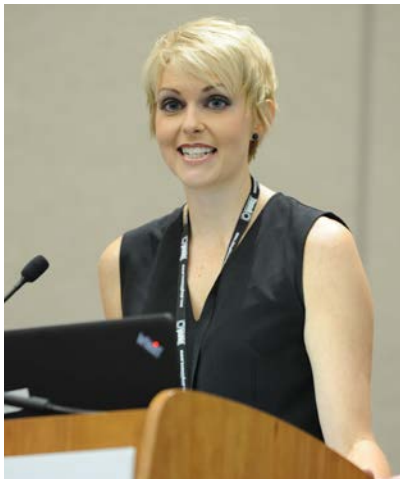
Share your story

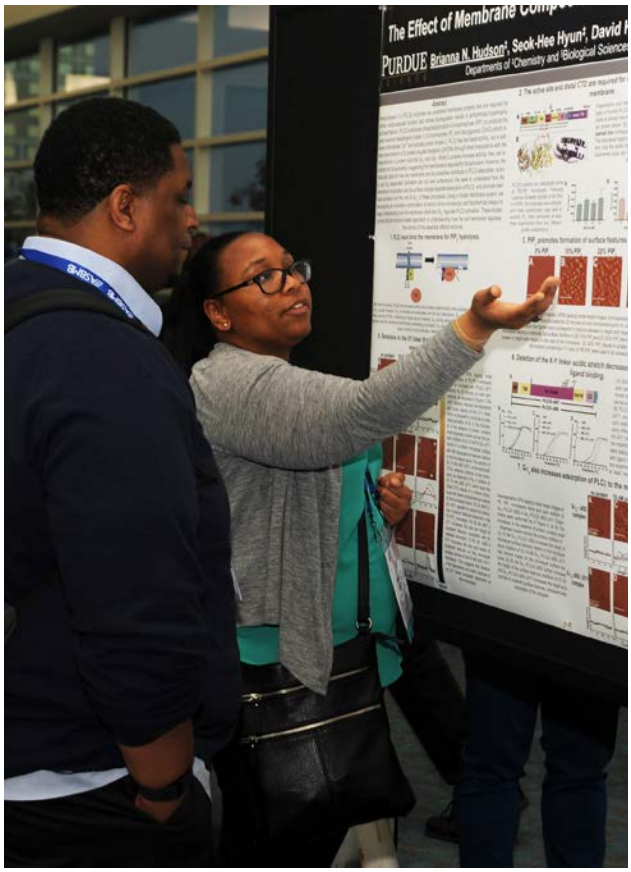
Are you incorporating climate change into your biochemistry and molecular biology classes and labs? If so, contact Henry Jakubowski at hjakubowski@csbsju.edu. As a member of the editorial board of Biochemistry and Molecular Biology Education, he is interested in expanding coverage of the science of climate change into traditional biochemistry and molecular biology curriculums.

Henry Jakubowski (hjakubowski@csbsju.edu) is a professor of chemistry at the College of St. Benedict/St. John's University.

Scenes from the annual meeting

In San Diego, members present their work and make connections.

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The GRE is a waste

I was thrilled to read the two articles (*The GRE hurts graduate schools — and science* by Alexander Shames and *GRExit or retain* by Rajini Rao, ASBMB Today, April 2018) discussing the value of the Graduate Record Exam. Since I took the GRE in 1971, I have questioned its relevance. However, my time as director of graduate studies of the Division of Biological Sciences at the University of Missouri made it crystal clear that the GRE is a waste of time, energy and money. In fact, the graduate school at the University of Missouri has not required the GRE for several years.

As director of graduate studies, I evaluated hundreds of applicants, and I can say confidently that the GRE has essentially no predictive value. We have had many students with poor GRE scores do extremely well in their graduate studies and go on to have very good careers. Conversely, we have seen students with excellent GRE scores who did not have what it takes to launch a career in science. Grades, research experience, personal statements (life experiences), letters of recommendation and interviews are all better predictors.

Most importantly, in order to realize their full potential, students need a committed and caring Ph.D. mentor. Shortcomings can be overcome in the proper training environment, and incoming students should be encouraged to do their homework carefully when choosing a lab for their studies. I applaud ASBMB Today for bringing this issue out into the open.

Stephen Alexander
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Professor emeritus
Division of Biological Sciences
University of Missouri

In defense of the GREs

Theoretically, one major motivation for using a standardized test is to allow people of all genders, skin colors, countries of origin, etc., to compete openly against one another in a platform independent of school ranking or exclusive club admittance.

An Ivy League student takes the same GRE as a community college student, and their scores are compared directly against one another. The GRE allows a high-scoring community college student who wasn't granted the privilege of attending the Ivy League school to demonstrate ability in a fair and open competition. That higher GRE score may be the only competitive advantage for a student who hasn't had the opportunity to obtain the extracurricular record of a more privileged applicant. A high GRE score may make the difference for a skilled but low-opportunity student to get into a graduate program with only a 10 percent admission rate and a 90 percent rate of denial.

Is the GRE a good predictor of graduate school success? Yes, the subject section does seem to be. A paper published by Orion D. Weiner in Molecular Biology of the Cell, Vol. 25, No. 4, Feb. 15, 2014, 427-548, said that, "Only the number of years of research experience and subject graduate record exams (GREs) were strong discriminators between the highest- and lowest-ranked students, whereas many other commonly used admissions metrics (analytical, verbal, and quantitative GREs, grade point average, and ranking of undergraduate institution) showed no correlation with graduate performance."

Anonymous graduate student

What the GRE can't measure

Graduate schools definitely should get rid of the GRE. As a biochemistry major, I am excited to learn everything about chemistry. While taking organic chemistry, I realized chemistry came very easily to me. My life would be very satisfying if I could do aldol reactions forever. However, I am not a great test taker. During my junior year of high school, I took the ACT twice. I never made higher than a 20. Personally, I was satisfied. The ACT suggested that I major in medical technology instead of biochemistry. I was both hurt and offended but decided to follow my dreams.

While taking college chemistry, I thrived in class. I often got the highest test score and plenty of commendation from my professors for my lab reports. I knew I made the right decision to major in biochemistry, and I wanted to be a research scientist. Currently, I am researching graduate schools. Should I pursue a master's or a doctorate? When should I take the GRE? Should I focus on schools that do not require the GRE? These are questions I ask daily. Standardized tests cannot measure my enthusiasm for biochemistry or how much I love being in lab. Why can't grad schools focus more on my grades in biology, chemistry, physics, etc.? Why aren't my three letters of recommendation enough?

I believe graduate schools should enforce the policy of maintaining a B or better average to stay in their respective programs. That is the best way to determine if a student is fit for the program. I'm pretty sure that when we are finally in the chemistry industry, no one will judge a biochemist based on his or her standardized test results.

Sherika Wright
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Post-publication review: 'catch & kill' at the NCBI?

Reference checking when reviewing a paper or grant application is easily accomplished by pasting the title in Google or in the PubMed page of the National Center for Biological Information. The latter page displays an abstract, and, with a further click, one can often access the cited paper. In 2013, the utility of PubMed abstracts was greatly increased when, with some fanfare, the NCBI announced the addition of a commenting facility for credited, nonanonymous contributors. It would be called PubMed Commons. In addition to the well-established pre-publication peer-review process, there was now an opportunity for post-publication peer review. When inspecting an abstract, a reader, perhaps engaged in pre-publication review, would see that there had been comments, which were a mere click away. Thus, post-publication review had the potential to assist pre-publication review, a process greatly influencing career progress, research funding and much more.

In the fields where I have some expertise, I soon noted contributions from people with reputations in those fields, and I found that both their opinions and those of many others were generally cogent and insightful. So I joined in, taking as much care as when normally reviewing papers or grant applications and adding appropriate references that the authors of the papers might have missed. The software made it easy, on each occasion, to notify the authors that a comment had been made. Sometimes an author whose paper had been targeted posted a reply or emailed me directly. More often, there was silence. This did not disconcert me, since I knew that the fields were active and they were busy people. Given that the operation was sponsored by the well-established NCBI and given the generally high quality of the comments, the possibility that PubMed Commons might not continue long into the foreseeable future did not enter my mind.

Then, in February 2018, the NCBI declared that PubMed Commons had been merely "an experiment." Moreover, it was deemed to have been an experiment that had failed. This determination had been based not on the quality of the contributions but on the quantity (about 7,000). There were protests but to no avail. In March, the NCBI declined to accept further contributions for PubMed Commons. Had this been all, the matter might then have been closed. After all, although not offering the same advantages as PubMed Commons, other outlets for post-publication peer review were growing (e.g., PubPeer).

However, not only did the NCBI decline further commenting, but it also cut all existing links from PubMed abstracts to the 7,000 comments made over the five-year period during which PubMed Commons had operated. The comments were siloed away in relatively inaccessible Excel files. Fortunately, a private agency, Hypothesis, was able to rescue the comments in accessible form (at https://hypothes.is/search, enter tag PubMedCommonsArchive). Nevertheless, the links to PubMed abstracts remained broken.

Thus, when accessing the PubMed abstract to check a citation in a paper or grant application, a potential reviewer no longer can determine whether comments exist for that paper or grant application. Five years' worth of comments have been squirreled away. The esteemed NCBI appears to have engaged in what is now popularly known in the tabloid industry as "catch and kill." We await a scientific Michael Avenatti.

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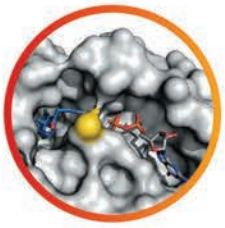

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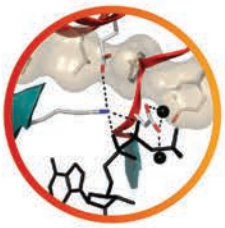

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