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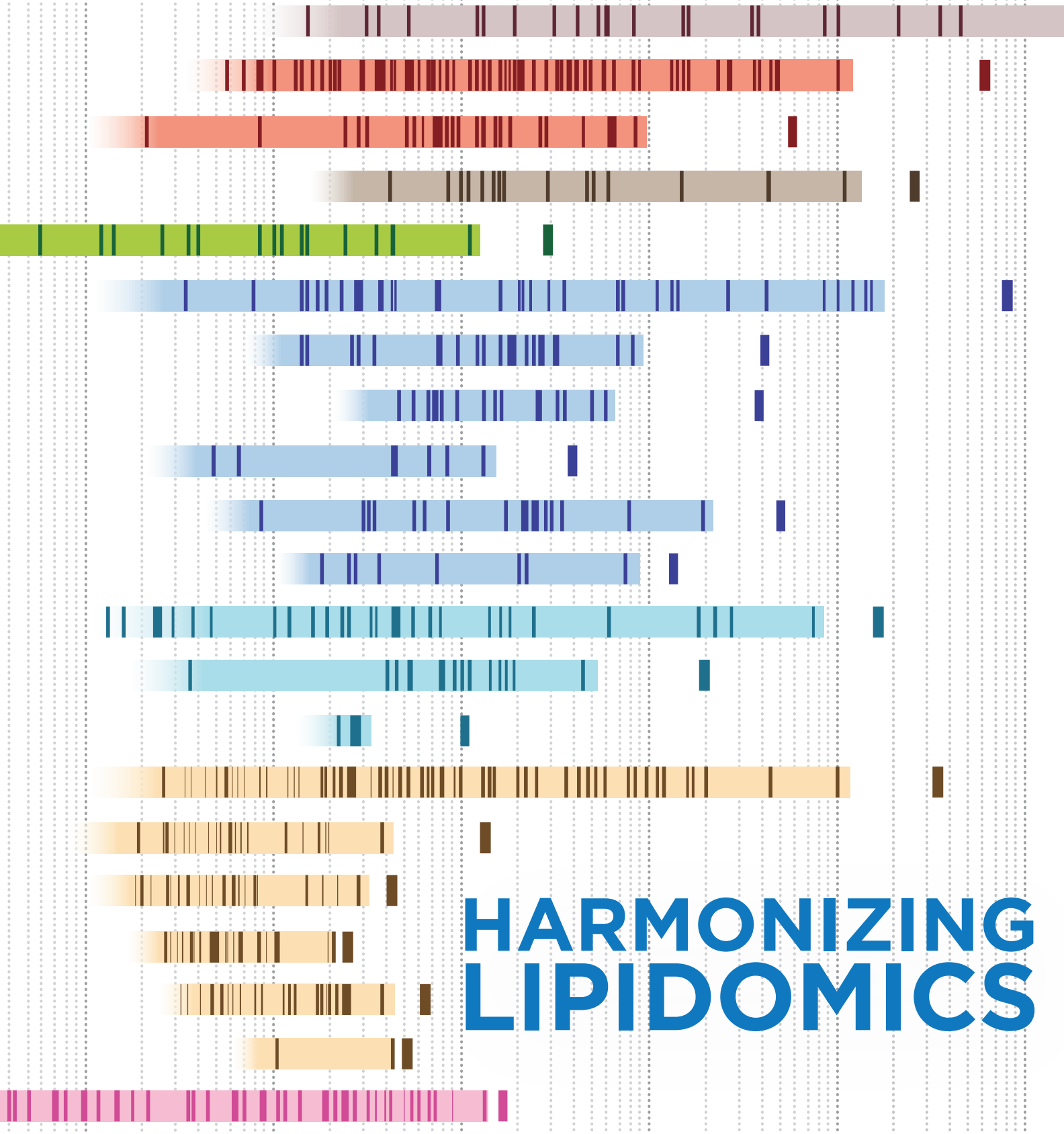
ASBMB TODAY

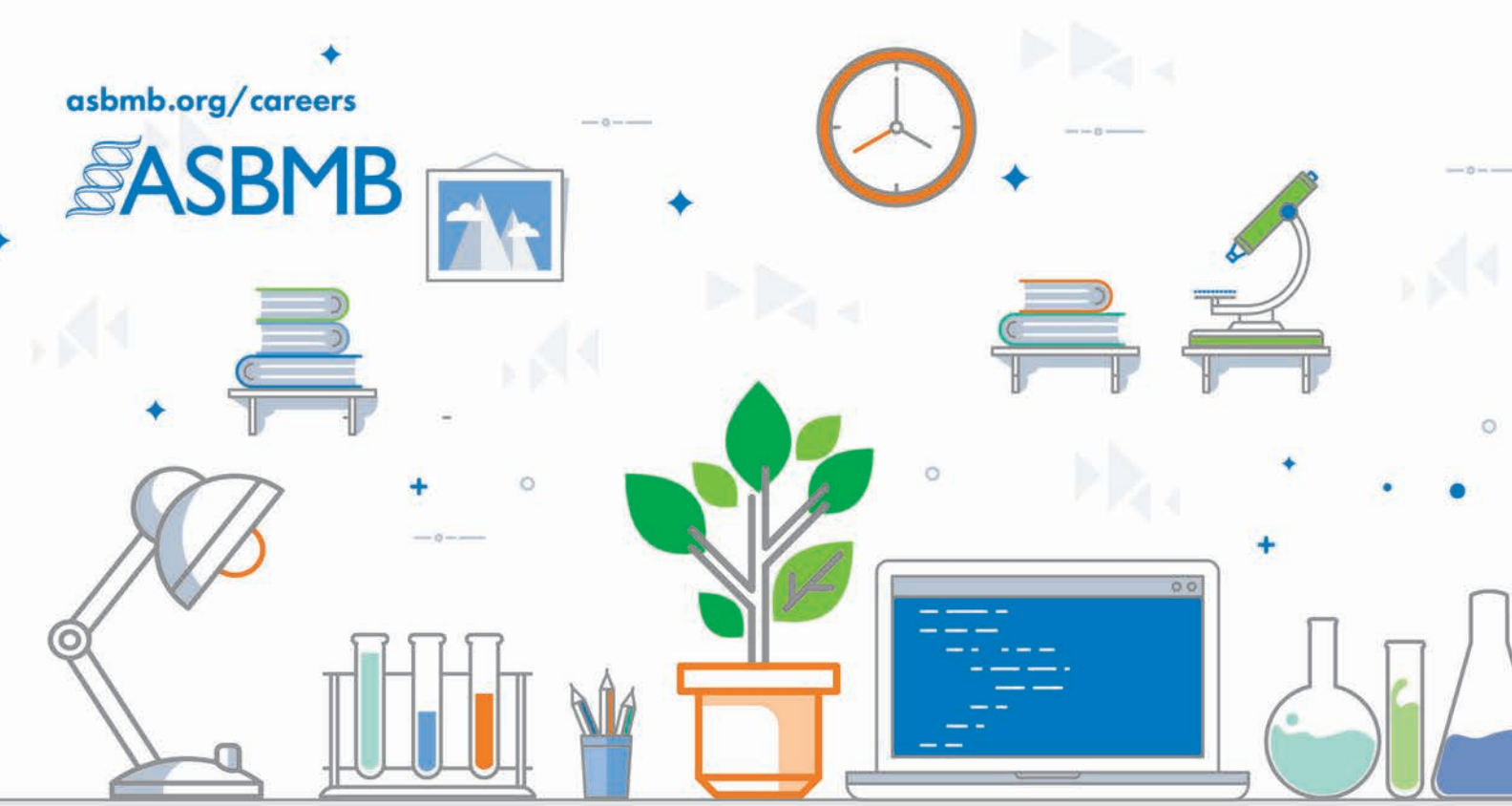
THE MEMBER MAGAZINE OF THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY



HARMONIZING LIPIDOMICS

asbmb.org/careers

ASBMB



ASBMB professional-development resources

Job board
asbmb.org/jobboard

The ASBMB job board has listings from academia, government and industry. Looking for your next hire? Members can post jobs for free.

Grant-writing training
asbmb.org/grantwriting

This Washington, D.C.-based summer workshop yields impressive results; 75% of participants end up with successful grants within two years.

Communications training
asbmb.org/commcourse

Can't travel for training? Take the ASBMB's "The Art of Science Communication" online course to gain the skills, knowledge and mindset necessary to become a great presenter.

Small meetings
asbmb.org/specialsymposia

Small meetings are offered throughout the year on a wide range of scientific topics. Interested in organizing a meeting? Members can work with the ASBMB to plan and organize a special symposium.

Careers blog
asbmb.org/careersblog

Every week, our careers blog presents insights into the current job market.

Webinars
asbmb.org/webinars

We offer live webinars and recordings of past webinars on topics including getting funding, salary negotiation, research careers in industry and more.

Video tutorials
asbmb.org/careers/tutorials

Our video series has tips on networking, dressing professionally, building a personal brand and more.

Advocacy Training Program
asbmb.org/advocacy/atp

The ASBMB ATP is a six-month externship that provides hands-on science policy and advocacy training and experience.

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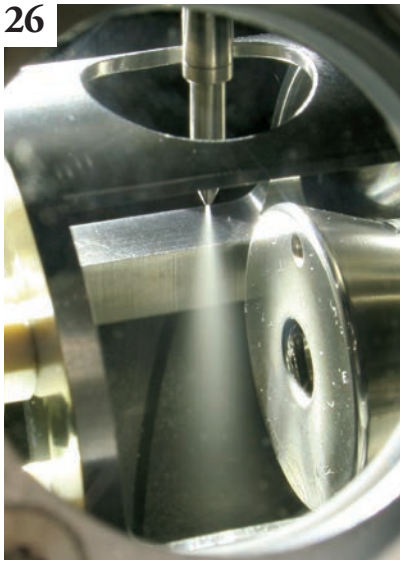

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Working knowledge

By Comfort Dorn

More than 30 years ago, I was employed as the parish administrator of a church, a huge, crumbling building in an underserved area of Washington, D.C. The parish ethos was to make maximum use of the building by opening it up to neighborhood service providers.

I was in my early 30s, a young mother with a degree in liberal arts and a vague interest in improving the world. My boss, the rector, was about 10 years older, one of the first women ordained as a priest in the Episcopal Church. The parish staff was bare bones and the budget small.

One dank winter afternoon, the heat stopped working. Complaining phone calls came from the preschool and free clinic upstairs. Our resident custodian (referred to in Anglican-speak as a sexton) was AWOL, so the rector and I made our way into the bowels of the church basement to the furnace, an ancient behemoth squatting in a dark corner. It required the regular draining of something called a McDonald & Miller valve, a bit of maintenance that had gone neglected. We wrestled the valve open and, as steaming rusty water gushed into a bucket (and onto our shoes), the rector sighed and gave me a deadpan look.

"Another thing they didn't teach me in seminary."

Which brings me to careers in biochemistry and molecular biology.

As an undergrad, grad student and (maybe) postdoc, you've learned a whole lot about science. But how much were you taught about having a career as a scientist? More to the point, how much were you not taught? How much knowledge did you have to pick up outside the lab — about choosing a career path, finding a job, starting a lab, or managing a budget and personnel?

You've probably learned a lot from your experience, which is, as they say, the best teacher. Would you be willing to share some of that hard-won knowledge?

The August issue of ASBMB Today traditionally is given over to the vast topic of careers. It's an opportunity for society members to pool their collective wisdom and help each other with the stuff they really need to know.

Every career has its McDonald & Miller valves. We'd like to hear about yours. Maybe you want to write an essay. Maybe you just have a few words of wisdom. Either way, drop me a line at cdorn@asbmb.org. Deadline for the August issue is June 3.

Comfort Dorn (cdorn@asbmb.org) is the managing editor of ASBMB Today. Follow her on Twitter @cdorn56.

Corrections

In an article about Angela Gronenborn on page 29 of the April issue the first sentence in the fifth paragraph should read, "In 1988, Gronenborn joined the National Institutes of Health where, alongside Marius Clore and Adriaan Bax, she started to work on HIV, supported by the HIV-targeted antiviral program."

In an article about JLR junior associate editors on page 15 of the April issue, Gissette Reyes–Soffer's name was misspelled and Stephen Young should have been listed as Brandon Davies' mentor.

Advocates visit Capitol Hill

By Benjamin Corb

Twenty undergraduate, graduate and postdoctoral students joined members of the Public Affairs Advisory Committee and public affairs staff to participate in the American Society for Biochemistry and Molecular Biology's annual Hill Day on March 28, visiting their elected representatives to discuss Congress' continued support for biomedical research.

Although President Donald Trump's budget request for fiscal year 2020 called for 5 percent cuts to all science funding agencies in the government, the advocates were heartened by the warm reception they received. Martha Cyert, associate chair of biology at Stanford University, said, "It was encouraging to hear staffers share their support for my science and their understanding of the importance of basic research in helping to discover treatments for diseases. They really seem to get it."

The agenda for this year's Hill Day advocates focused largely on asking for increases in funding at the National Institutes of Health, the National Science Foundation and the Department of Energy's Office of Science. But the discussions with lawmakers and their staffs extended to issues beyond funding, indicating a Congress that has a nuanced understanding of and curiosity about how science works.

Kristine Deibler, a postdoc from the University of Washington, was surprised by some of the topics. "My senator's staff was very direct and interested in hearing about my perspectives related to the issue of sexual harassment in science," Deibler said. "I was so encouraged to see Congress paying such close attention to this very serious issue."

COURTESY OF CALLAN FRYE

Callan Frye, a graduate student at the Medical University of South Carolina, shows his enthusiasm for advocacy at the ASBMB's 2019 Hill Day. For more photos, turn to page 48.

This year's advocates came from 24 states and conducted 83 meetings over the course of their day on the Hill. The student participants, selected from a pool of applicants by the ASBMB's public affairs staff, arrived in Washington, D.C., the night before Hill Day for a crash course in being an advocate. PAAC members stayed through the following day for meetings with NIH and NSF leaders.

"This Hill Day experience is among the most rewarding opportunities that ASBMB (offers) for its members," said Matt Gentry from the University of Kentucky, the outgoing PAAC chair. "ASBMB isn't the only scientific society that holds events like this, but in my experience the organization and staff put on the best show by far."

Gentry has been a member of the PAAC for five years and is a Hill Day veteran. For some, like Alex Blackburn, a Ph.D. student at the University of Idaho, this Hill Day was their first taste of advocacy.

"This was a very positive, very fun experience," Blackburn said. "I got to meet really great people on both sides of the aisle. When I get back to Idaho, I look forward to telling my colleagues that they should consider getting involved themselves. I definitely would love to do this again."

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

Member update

By Erik Chaulk

Bruns named Mr. Homecoming

Professor of chemistry Kerry Bruns has received the Southwestern University 2018 Mr. Homecoming Award.

Presented by the university's alumni association, the Mr. Homecoming Award is given to a faculty member who has garnered the respect of former students.

Bruns earned his Ph.D. from New Mexico State University in 1987 and his B.A. from Western New Mexico University in 1981. In addition to teaching general chemistry and biochemistry courses, he leads Southwestern University's premedical advisory committee, which provides professional development programming and handles the application process for students interested in attending medical school.

BRUNS

He also organizes the health care professionals' breakfast at homecoming each year, providing students the opportunity to connect with alumni.

Bruns is retiring at the end of the year. Colleagues say he has had a significant, long-lasting impact upon the Southwestern community.

Nita–Lazar promoted to senior investigator

Aleksandra Nita–Lazar was promoted in December to senior investigator with the National Institutes of Health National Institute of Allergy and Infectious Diseases.

Nita–Lazar leads the Functional Cellular Networks Section, which focuses on understanding protein modifications involved in cell signaling as well as absolute quantification of molecular representation and interaction.

NITA–LAZAR

After obtaining her Ph.D. in biochemistry at the University of Basel in 2003, Nita–Lazar completed postdoctoral training at Stony Brook University and the Massachusetts Institute of Technology.

In 2009, she joined the Program in Systems Immunology and Infectious Disease Research, now the Laboratory of Immune System Biology, at the NIAID.

Gottesman named visiting professor

Susan Gottesman is among four individuals announced in December as new Vallee visiting professors.

The visiting professor program sponsored by the Vallee Foundation promotes intellectual exchange and

In memoriam: Hugh Forrest

Former University of Texas professor Hugh Forrest died Nov. 16. He was 94.

The youngest of five sons, Forrest was born and raised in Glasgow, Scotland. He remained in Scotland for his undergraduate studies, graduating from the University of Glasgow in 1944.

He earned his first Ph.D. in 1947 studying therapeutic chemical compounds at the University of London and earned a second Ph.D. in 1951 from the University of Cambridge researching pteridine biosynthesis.

FORREST

Forrest left for the United States in 1951 after receiving a U.S. Public Health Service fellowship at the California Institute of Technology, where he continued his research into pteridines.

In 1955, Forrest joined the University of Texas at Austin as a postdoctoral fellow. He remained there for nearly five decades, becoming a professor in 1962 and a professor emeritus in 1993.

Forrest served as a mentor to numerous postgraduate students during his tenure.

The University of London awarded him a doctor of science degree in 1970, and he was elected as a fellow to the Royal Society of Edinburgh in 1979.

He is survived by his three children, Eleanor, Anne and Hugh, and his six grandchildren.

scientific partnership by offering senior scientists an opportunity to work at premier biomedical research institutes.


GOTTESMAN

A National Institutes of Health distinguished professor, Gottesman is co-chief in the laboratory of molecular biology and head of the biochemical genetics section at the National Cancer Institute.

Gottesman's research focuses on post-transcriptional mechanisms of regulation in bacterial systems.

As a Vallee visiting professor, Gottesman will spend a one-month sabbatical in another lab of her choice.

Basu, Varshney elected to World Academy

Joyoti Basu and Umesh Varshney are among 46 new fellows elected to the World Academy of Sciences.


BASU

Founded in 1983, the World Academy of Sciences is a global organization based in Trieste, Italy, that seeks to promote science in developing countries and works to support sustainable prosperity through research, education, policy and diplomacy.

Basu is a J.C. Bose national fellow in the department of chemistry, Bose Institute, Kolkata, India. She has contributed significant research in the area of Mycobacterium tuberculosis pathogenesis.

Varshney is professor in the department of microbiology and cell biology and chair of the division of biological sciences at the Indian Institute of Science, Bangalore, India. Varshney's lab uses E. coli to study mechanistic aspects of protein synthesis and DNA repair.


VARSHNEY

The election of this new class represents efforts to increase the ratio of women in the organization as well as scientists from countries that have been underrepresented or unrepresented in the academy's membership.

Corbett receives mentoring award

Emory University biology professor Anita Corbett has won the 2018 Nature Award for Mentoring in Science.

First conferred in 2005, this award focuses on a specific country or region each year, recognizing individuals for outstanding scientific mentorship. The award consists of two prizes of $10,000, one awarded for midcareer mentorship and one for lifetime achievement in mentorship.

Corbett is a biochemist, and her research primarily focuses on determining the function of evolutionarily conserved RNA binding proteins.

Mentorship has played a significant role in Corbett's career. Susan Wente, a Vanderbilt University professor and chair of the judging committee for the award, stated in a Nature press release that Corbett "reaches outside of her own laboratory and is a leader at her university and in her field, with a passion for gender equity."


CORBETT

Corbett is one of four scientists to receive the 2018 awards and shares the midcareer achievement award with Kjersti Aagaard, Baylor College of Medicine.


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

Upcoming ASBMB events and deadlines

MAY		**National Stroke Awareness Month**
	7	Serine Proteases in Pericellular Proteolysis and Signaling abstract deadline
	23	Tips for Applying for ASBMB Accreditation webinar
	28	Serine Proteases in Pericellular Proteolysis and Signaling early registration deadline
JUNE	1	Marion B. Sewer Distinguished Scholarship for Undergraduates deadline
	3–7	Art of Science Communications Course begins (first week)
	4	Transforming Education in the Molecular Life Sciences abstract deadline
	12	Transforming Education in the Molecular Life Sciences late registration deadline
	13–15	IMAGE grant-writing workshop
	27	**National HIV Testing Day**
JULY		**Eye health, in support of UV Safety Month by the American Academy of Ophthalmology**
	7	Mass Spectrometry in the Health and Life Sciences: Molecular & Cellular Proteomics final abstract deadline.
	25	Emerging Roles for the Nucleolus oral abstract submission deadline
	25–28	Transforming Education in the Molecular Life Sciences
	28	Serine Proteases in Pericellular Proteolysis and Signaling poster abstract submission deadline

WELCOME, NEW ASBMB MEMBERS

Meshal Alfheed, Qassim University

Yousef Alharbi, Qassim University

Aishah Al-Jarallah, Kuwait University

Abdullah Alrashed, Qassim University

Chenoa Arico, University of Texas at El Paso

Akanksha Bansal, University of Calgary

Ruthie Barbas, Spectrum Brands Inc.

Jessica Beckham, Middle Tennessee State University

Manas Biswal, University of South Florida

Ashis Biswas, New Mexico State University

Kaylee Brabham, St. Bonaventure University

Martin Brenneman, Ohio Northern University

Henry Brothers (no affiliation)

Alexandra Caguana, Montclair State University

Philip Cammarata, St. Bonaventure University

Mariangeles Campos, Montclair State University

Ciara Carpenter, Montclair State University

Kathy Castor, Montclair State University

Saiful Chowdhury, University of Texas at Arlington

David Cobrinik, University of Southern California

Tyler Cook, Loyola University Chicago

Brock Couch, Middle Tennessee State University

Aimee Cruikshank, University of North Florida

Anjelica DaSilva, University of North Florida

Crsitiano Dias, New Jersey Institute of Technology

Emre Dikici, University of Miami

Aiste Dobrovolskaite, University of Central Florida

Amalia Dolga, University of Groningen

Kristiann Dougherty, Palm Beach Atlantic University

Garrett Dunlap, Harvard University

Yaritza Escamilla, University of Texas Rio Grande Valley

Serena Estevez, Hillsborough Community College

Brandon Eudy, University of Florida

Baggio Evangelista, University of North Carolina at Chapel Hill

Nathan Forney, Otterbein University

Jarrod French, Stony Brook University

Callan Frye, Medical University of South Carolina

Ciara Golliher, University of North Florida

Matthew Greseth, Medical University of South Carolina

Gerald Grunwald, Thomas Jefferson University

Jeisac Guzman Rivea, University of Puerto Rico, Río Piedras Campus

Lillian Hewitt, University of North Florida

Laszlo Homolya, Hungarian Academy of Sciences

Nazar Hussein, Kent State University

Joseph Hwang, MilliporeSigma

Houssine Ikhlef, University of Central Florida

Camille Immanuel, University of Kentucky

Mahnoor Izhar, University of North Florida

Jodiene Johnson, Fisk University

Paul Johnson, University of North Georgia

Geoffrey Kapler, Texas A&M Health Science Center

Jack Kavanaugh, University of North Florida

Debra Kellogg–Yelder, BioCryst Pharmaceuticals Inc.

Saran Kone, St. Bonaventure University

Michael Larock, St. Bonaventure University

Katherine Leon Hernanzed, Montclair State University

Yunan Li, University of Minnesota

Melissa Locke, University of California, Berkeley

Broderick Lu, University of Massachusetts Boston

Phillippe Ly, California State University, Long Beach

Sonal Mahindroo, St. Bonaventure University

Ramon Martinez, University of Maryland, Baltimore

Nathaniel McClure, St. Bonaventure University

Gillian Melikian, Providence College

Michael Merchant, University of Lousiville

Carlen Merritt, Marshall University

Jyotsna Mishra, Medical College of Wisconsin

Holly Moots, University of Central Florida

Young–Jae Nam, Vanderbilt University Medical Center

Elizabeth Nolan, Massachusetts Institute of Technology

Rachel Osborn, Otterbein University

Michael Parsons, Marshall University

Kathryn Pearce, Ohio Northern University

Oksana Penezina, Tokyo Chemical Industry Co.

Otto Phanstiel, University of Central Florida

Hannah Richter, University of Pennsylvania

Neishaly Rivera, Universidad Interamericana de Puerto Rico

Antonieta Salguero, Johns Hopkins University

Fernanda Santos, Adventist University of Health Sciences

Claire Schaef, St. Bonaventure University

Samantha Schwartz, Emory University

James Scott, Kennesaw State University

Sally Seder, Montclair State University

Vandana Sekhar, University of Central Florida College of Medicine

Savita Sharma, University of Kentucky

Yujiang Shi, Harvard Medical School

Tala Shourbagitello, University of North Florida

Melanie Silva, Montclair State University

Andrew Smith, Metropolitan State University of Denver

Patrick Sochor, Arizona State University

Kennon Stewart, Tulane University

Natalie Sumser, Otterbein University

Krissie Tellez, Stanford University

Dorothea Tholl, Virginia Tech

Claire Todd, Otterbein University

Ashutosh Tripathi, Texas A&M University

Trent Tucker, Otterbein University

Takeshi Uemura, Amine Pharma Research Institute

Abraham Villa Mundo, University of Texas at El Paso

Sarah Waldron, Kennesaw State University

Adrianna White, University of North Florida

Charles White, James Madison University

Mary Jane Osborn (1927–2019)

By Sandra Weller & Lawrence Rothfield

Mary Jane Osborn, a prominent biomedical scientist, a trailblazer for women in science, and a former president of the American Society for Biochemistry and Molecular Biology, died Jan. 17 in Farmington, Connecticut. She was 91. The cause was complications following emergency surgery.

Osborn's research career began in the late 1950s, a time when women were notoriously underrepresented in all fields of science and many major university biomedical research departments had no tenured female faculty. In the decades that followed, the number of female scientists in university departments increased significantly, although female faculty were (and continue to be) significantly underrepresented at the higher academic ranks. Although she always said she personally never felt she was professionally discriminated against, Osborn was keenly aware of the systemic problems faced by women in science. In recognition of this, the University of Connecticut Medical School, where she was a faculty member for 42 years, established the annual Osborn Lectureship in 2002 to celebrate women in science.

ALL PHOTOS COURTESY OF SANDRA WELLER

Mary Jane Osborn poses with her former Ph.D. student Inger Damon, who now directs the Division of High-Consequence Pathogens and Pathology in the Centers for Disease Control and Prevention's National Center for Emerging and Zoonotic Infectious Diseases and is a captain in the U.S. Public Health Service.

After hearing of Osborn's death, Lucy Shapiro, director of the Beckman Center for Molecular and Genetic Medicine at Stanford University, said, "Mary's breadth of knowledge, scientific rigor and acute intelligence would have made her a remarkable role model of any gender, but she was a woman in a world where few women had a chance to shine. And shine she did. When she spoke, people listened. When she published, her papers became the gold standard."

> **"Dr. Osborn was a remarkable woman, a fabulously successful biochemist and microbiologist who was always the smartest person in the room. She had a profound influence on my career by serving as a role model and sounding board for ideas."**
>
> *— Sandra Weller*
> *Osborn's first female faculty recruit*

Osborn made her first important scientific contribution in 1957 as a research fellow at the University of Washington, when she discovered the mechanism of action of the drug methotrexate, one of the earliest cancer chemotherapeutic agents. The research was published in the Journal of Biological Chemistry. Methotrexate also is widely used for patients with rheumatoid arthritis and psoriatic arthritis.

As a faculty member at the New York University, Albert Einstein and University of Connecticut medical

Remembrances

These are excerpts from remembrances by scientists who worked with Mary Jane Osborn. Read the complete texts at asbmb.org/asbmbtoday.

In 1982, Mary Jane was selected to give a Harvey lecture. It's a tremendous honor to be chosen to give this lecture, and the whole lab drove to New York to hear her present "Biogenesis of the outer membrane of Salmonella." I knew MJO would give a wonderful talk, that was a given, but I honestly will never forget that night. Our group arrived and sat together. We watched as the Harvey Society members in their formal attire filed into the theaterlike hall, and then Mary Jane was introduced. From the moment she took the podium, she owned the room, expertly presenting an amazing body of work. There could be absolutely no doubt she had truly advanced the field. I returned to the lab both tremendously proud to be part of her group and inspired to continue my studies.

— Pamela A. Marino

At first, my lab was right across the hall from Mary's operation. I was a bit scientifically lonely, having moved from a large lab as a postdoctoral fellow to a new department where it was just me and a technician. Fortunately, I quickly learned that I was always welcome to join Mary at the blackboard outside her lab or in her office. She was never too busy to listen to my latest experiment or idea and then explore it with me. I always left the blackboard or her office with great advice and new ideas. My focus on DNA tumor viruses was unrelated to Mary's interests, but she generously shared her time to mentor a young colleague.

— Thomas Shenk

Her influence is such that in my current research seminar I show slides of two of her papers. Despite the fact that both of these were published in the 1970s, they remain very relevant. One of these describes the Osborn method for purifying the outer membrane. In this paper she notes that all of the components of the outer membrane are made inside the cell, and therefore mechanisms that transport these components across the cell envelope to the cell surface must exist. I have spent the better part of my career trying to understand these mechanisms.

— Thomas J. Silhavy

This photo of Mary Jane Osborn was taken for an Annual Review of Microbiology memoir she wrote recently. "It is very touching to me, as it shows frailty but also intense curiosity," Sandra Weller said of the photo.

schools, she became a leader in studies of bacterial endotoxin (also known as lipopolysaccharide). This highly toxic molecule is located on the surfaces of a large group of bacteria that include the causative agents of life-threatening diseases such as typhoid fever and meningococcal meningitis. This work prompted her to confront a puzzling paradox — many cells are capable of fabricating very large complex molecules, such as endotoxin, that lie outside the cell body despite the fact that all of the building blocks and the energy sources required for their synthesis are inside the cell. Beginning in 1972, she used a combination of bacterial genetics, biochemistry and electron microscopy to solve

ON THE WEB

For an inspiring audio interview and links to some of Mary Jane Osborn's seminal papers in the Journal of Biological Chemistry and other journals, go to asbmb.org/asbmbtoday.

Mary Jane Osborn, Lawrence Rothfield and Sandra Weller pose together at a University of Connecticut departmental retreat in 2005.

> **She was the jewel in the crown of this medical school and university.**
>
> *— Lawrence Rothfield*
> *A longtime colleague and sometime competitor*

key aspects of this problem. In her seminal 1972 JBC papers, she described the technique that made these experiments possible, now universally known as the Osborn method, which still is used in laboratories around the world.

Her research gained her international renown, leading to her election to the American Academy of Arts and Sciences in 1977 and to the National Academy of Sciences in 1978, 10 years after assuming her first faculty position. She was the president of several important biomedical societies and the recipient of many honors and awards. In 1980, President Jimmy Carter appointed her to the National Science Board, the governing body of the National Science Foundation, the country's major funder of basic scientific research.

Osborn served on the ASBMB Council from 1974 to 1975, and in 1981 she was elected ASBMB president, the second woman to hold that office.

In the 1990s, she became interested in space science research. She chaired NASA's Committee on Space Biology and Medicine, which produced a report that plotted the U.S. space biology research program in the first decade of the 21st century.

Osborn had a keen interest in poetry and the arts, and she traveled to New York City, often several times a month, to attend concerts and ballet and opera performances. She was a special fan of the Paul Taylor Dance Company, American Ballet Theater and New York City Ballet.

In 1968, she was one of the founding faculty who shaped the curriculum and character of the new medical school of the University of Connecticut in Farmington. She served as chair of the medical school's department of microbiology from1980 to 2002. She remained there as a professor of microbiology and of molecular biology and biophysics until her retirement in 2014.

Sandra Weller (weller@uchc.edu) is a Board of Trustees professor and chair of the department of molecular biology and biophysics at UConn Health.

Lawrence Rothfield (lroth@neuron.uchc.edu) is a professor emeritus in the department of molecular biology and biophysics at UConn Health.

IN REMEMBRANCE

The following ASBMB members died in 2018 and early 2019.

Alfred Alberts
1931–2018

Thomas August
1927–2019

Claude Baxter
1923–2018

Gunter Blobel
1936–2018

Subir Bose
1931–2018

Paul Boyer
1918–2018

Ray Brown
1924–2018

Peter Condliffe
1922–2018

Minor Coon
1921–2018

Richard Cowart
1949–2018

Paul Englund
1938–2019

Mark Fisher
1954–2018

Hugh Forrest
1924–2018

Julian Gomez-Cambronero
1960–2018

Dwight Hall
1940–2018

Geoffrey Hendy
1948–2018

Lowell Hokin
1924–2018

Jerard Hurwitz
1928–2019

Jack Kirsch
1934–2018

Laszlo Lorand
1923–2018

Barbara Low
1920–2019

Lewis Lukens
1927–2018

Yves Marcel
1937–2018

Julian Marsh
1926–2018

Andrei Medvedev
died 2018

Henry Metzger
1932–2018

Mary Jane Osborn
1927–2019

Woon Paik
1925–2019

Joanne Ravel
1924–2018

Peter Reichard
1925–2018

Gordon Shore
1945–2018

Donald Small
1931–2019

Thomas Steitz
1940–2018

Frank Talamantes
1943–2018

Martha Vaughan
1926–2018

John Vournakis
1939–2018

Tamio Yamakawa
1921–2018

Wu-Kuang Yeh
1942–2018

We recently learned of the passing of these members.

William Claycomb
1943–2016

Mahendra Jain
1938–2017

Kenneth Kopple
1930–2013

Robert Lester
1929–2017

Deneys Van der Westhuyzen
1945–2017

William Wells
1927–2017

For news stories, remembrances and a video tribute, go to asbmb.org/asbmbtoday.

Lipid regulation of mitochondria

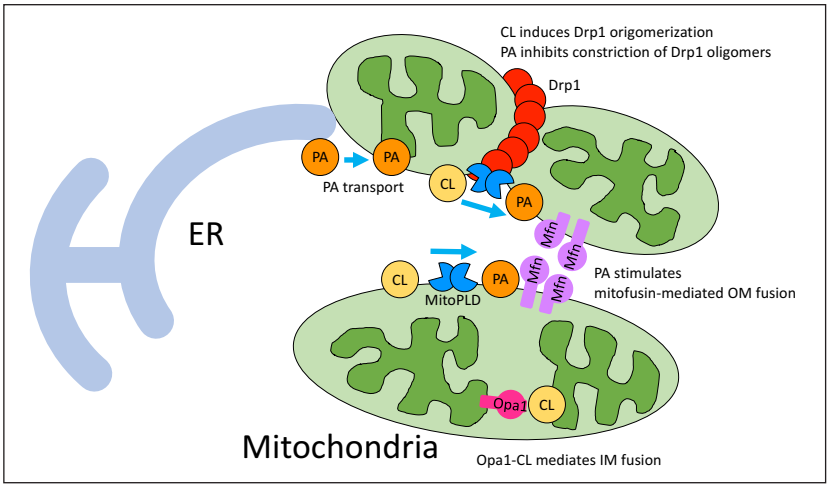
By Yoshihiro Adachi, Miho Iijima & Hiromi Sesaki

Mitochondria are dynamic organelles that grow, divide and fuse in most of the human body's cells; these dynamic membrane processes are critical for mitochondrial health.

Our laboratory studies mitochondrial division and fusion, focusing on three mechanochemical dynamin-related GTPases: Drp1, mitofusin and Opa1. Drp1 is a soluble GTPase that splits the mitochondrial membrane. Mitofusin and Opa1 are integral membrane GTPases that work together to fuse the mitochondrial membranes. Mutations in each of these enzymes lead to human diseases that mainly affect central and peripheral nervous systems.

Mitochondrial division and fusion need to be balanced to maintain functional mitochondrial size, structure and distribution within cells. This dynamic balance is controlled by several layers of mechanisms, including gene expression, post-translational modifications and protein degradation of these GTPases and their binding partners. In addition, mitochondrial phospholipids play important roles in regulation of mitochondrial dynamics.

Drp1 interacts with two phospholipids, cardiolipin, or CL, and phosphatidic acid, or PA, in the mitochondrial outer membrane, or OM. CL is synthesized in the mitochondrial inner membrane, or IM, and a fraction of CL is transported to the OM. Drp1 is recruited to the mitochondria through its receptor proteins on the surface of mitochondria; binding to CL stimulates Drp1 to assemble into high-order oligomers that function as a division machinery. The machinery is regulated further by PA. Binding to PA restrains the assembled machinery from initiating the constriction of the mitochondrial membranes, likely creating a priming step for mitochondrial division. Binding sites for CL and PA are different in Drp1; therefore, these phospholipids may create different degrees of the regulation through a combination of single or concurrent binding to Drp1.



HIROMI SESAKI ET AL.

Cardiolipin, or CL, in the mitochondrial outer membrane promotes oligomerization of Drp1 to drive mitochondrial division; CL in the inner membrane mediates fusion through heterotypic interactions with Opa1.

The production of CL and PA is a dynamic process in the OM. As described above, CL is transported to the OM from the IM. This transport may be regulated through dynamic interactions at the intramitochondrial OM-IM contact sites. In the OM, there is a phospholipase D, MitoPLD, which converts CL to PA. Since MitoPLD directly binds Drp1, conversion of stimulatory CL to inhibitory PA may happen locally in the vicinity of the division machinery. PA also is produced in the endoplasmic reticulum, or ER, and imported into the OM through ER-mitochondrial contact sites. Drp1 often divides mitochondria at these sites. Mitochondrial PA levels also may be regulated by this interorganellar interaction.

PA changes the mitochondrial membrane's biophysical properties and facilitates mitofusion-mediated membrane fusion. Opa1, similar to Drp1, binds CL, and this interaction drives membrane fusion. Therefore, lipid transport and synthesis coupled to intramitochondrial contact site dynamics and interorganellar interactions play key roles in controlling mitochondrial dynamics.

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For May, it's in your bones: calcium and phosphorus

By Quira Zeidan

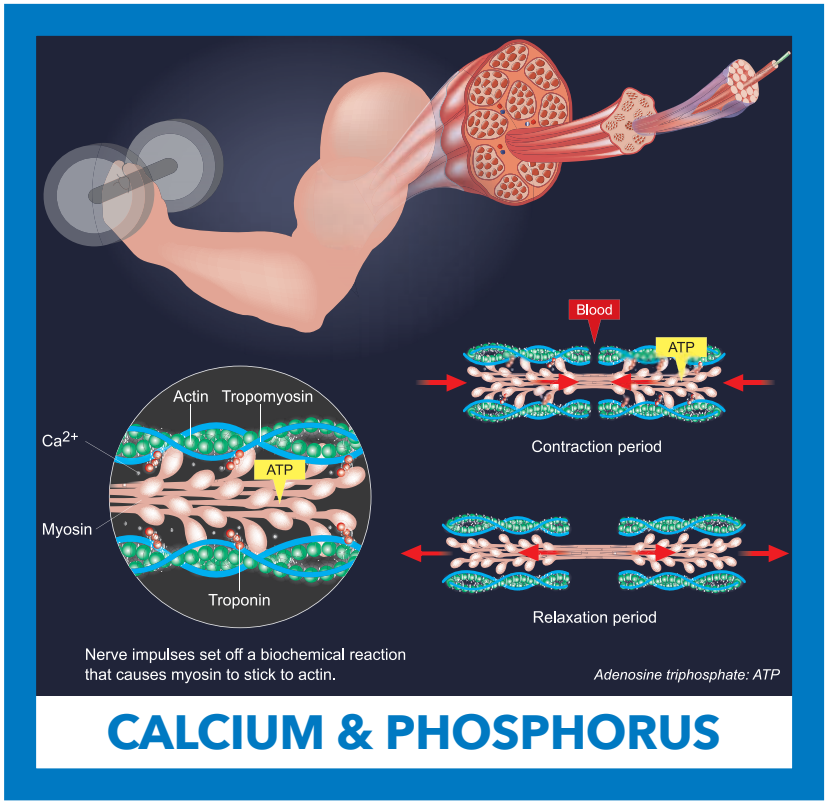
The International Year of the Periodic Table marks the 150th anniversary of Dimitri Mendeleev's periodic system. The American Society for Biochemistry and Molecular Biology is joining the celebration with a series of articles on biochemical elements. Since January, we have presented hydrogen; iron; sodium, potassium and chlorine; and copper.

May is arthritis awareness month, so we selected calcium and phosphorous, the two components of the mineral salt hydroxyapatite that makes up about 65 percent of the human adult bone mass.

With chemical symbol Ca and atomic number 20, calcium is classified in the periodic table as an alkaline earth metal. In chemical reactions, calcium easily loses the two valence electrons in its outermost orbital to form ionic compounds that contain dipositive Ca^{+2}.

At 3 percent of the Earth crust's mass, calcium is the fifth most abundant element and the third most common metal after iron and aluminum. Most of the Earth's calcium is found as a carbonate mineral in limestone — sedimentary rock that contains fossilized sea life. Calcium carbonate makes corals, sea shells and pearls when Ca^{+2} released by weathering reacts with seawater bicarbonate.

Calcium is essential in biology. Both prokaryotes and eukaryotes maintain low intracellular free Ca^{+2} via ion channels, transporters and calcium-sequestering proteins. In response to environmental changes, intracellular Ca^{+2} rapidly rises,



An electrical impulse traveling from a motor neuron results in the release of calcium from the muscle's intracellular stores. Ca^{+2} binds to the inhibitory troponin-tropomyosin complex, allowing myosin and actin filaments to slide past one another causing muscle contraction. Adenosine triphosphate is hydrolyzed in the process. Relaxation follows when cytoplasmic calcium is removed.

transmitting the outside information to the interior of the cell. In bacteria, this calcium signaling system regulates chemotaxis — or movement toward a chemical stimulus — and flagellar rotation.

In mammals, cells respond to hormones by activating the phosphoinositide 3-kinase signaling pathway that leads to high intracellular Ca^{+2} and expression of calcium-dependent genes. Excited neurons release the neurotransmitter acetylcholine, which binds to its receptor on the receiving cell, opening ion channels and allowing the influx of extracellular Ca^{+2}. At synapses, the inflow of calcium propagates the electrical signal to the receiving neuron, and at neuromuscular junctions, it triggers muscle contraction in the receiving fiber.

Phosphorus — with chemical symbol P and atomic number 15 —

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Bacterial drug synergies hide in plain sight

Metagenomics may speed discovery of future therapeutic combinations

By Laurel Oldach

While on the hunt for a molecule with therapeutic potential, Peter Mrak and his colleagues made a more sweeping discovery: Every known isolate of bacteria that produces the immunosuppressive drug rapamycin also can make a second compound that enhances rapamycin's effect.

"Put into simple words, this feels like finding an ancient treasure map in your grandfather's attic," Mrak said.

Rapamycin was isolated in the 1970s from bacteria found on Easter Island. The molecule gives those bacteria a competitive edge by suppressing the growth of fungi in the soil. At first, researchers took a cue from nature and tried using rapamycin to treat fungal infections. Then they found that it also suppresses growth and metabolism in human cells, notably those of the immune system. The drug is now used to prevent transplant rejection and stop tissues from growing into coronary stents.

Mrak and an international team at the Swiss pharmaceutical company Novartis recently sought other beneficial compounds from the same bacteria. Mining for natural products with pharmaceutical potential in bacteria from diverse environments is a standard approach with high success rates. But in this case, the researchers found more than a single molecule.

In culture media that had been used to grow a rapamycin-producing strain of Streptomyces, the scientists found a group of secondary products called actinoplanic acids. This family of molecules had been isolated from other microbes in the late 1990s, but no one had worked out how they are synthesized. Mrak and colleagues noticed that actinoplanic acids contain a chemical group called a tricarballylate that would be very difficult for a synthetic chemist to produce in the lab and wondered whether they could exploit bacterial means of synthesizing that chemical group.

HULEROYO/PIXABAY

Rapamycin is named for Rapa Nui, or Easter Island, where the bacteria that produce it were initially isolated.

The researchers used a bioinformatics tool called antiSMASH to comb through the Streptomyces genome, hunting for possible enzymes involved in actinoplanic acid production. After finding a few candidates, they showed by targeted mutagenesis that a cluster of closely spaced genes on the Steptomyces genome can function as a sort of biochemical assembly line to generate actinoplanic acids, including making the tricky tricarballylate.

When Mrak and colleagues looked for other bacteria that might carry this gene cluster, they noticed something surprising. Every one of the fully sequenced bacterial isolates known to make rapamycin also carried the genes to make actinoplanic acid. This

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is a reactive nonmetal that combines with other elements mainly by sharing electrons via covalent bonds. Free phosphorus is rare; the element normally is found in compounds in oxidation states of +3, +5 and -3.

Phosphorus is the 11th most common element on Earth. About one gram of phosphate is found for every kilogram of the Earth's crust, mostly in the form of oxidized inorganic rocks formed over millions of years.

Phosphorus is required for all life. Some bacteria derive energy for growth by oxidizing H_2PO_3– or phosphite to inorganic phosphate. Phosphate groups are major structural components of nucleotides, which are the building blocks for nucleic acids like DNA and RNA. Phospholipids — which contain a hydrophobic fatty acid "tail" and a hydrophilic phosphate "head" — form lipid bilayers that constitute cellular membranes.

Most cellular metabolic reactions are driven by chemical energy harnessed from the cleavage of adenosine triphosphate, a molecule that contains a sugar, a nitrogenous base and three phosphate groups. The addition of phosphoryl groups to proteins during phosphorylation changes protein activity and/or cellular localization, regulating a plethora of cell-signaling events.

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suggested that microbes might benefit from having both molecules.

When the researchers tested actinoplanic acid and rapamycin as fungal growth inhibitors, they found that the two molecules' combined effect was greater than you'd expect from simple addition. In other words, the molecules work synergistically. The research appears in the **Journal of Biological Chemistry.**

Doctors have found by trial and error that the effect of rapamycin in patients is amplified when the drug is combined with molecules that, like actinoplanic acid, inhibit an enzyme called farnesyltransferase. That combination of drugs is in clinical tests for cancer now. The authors say their approach could be used to find other co-occurring groups of biosynthetic genes more rapidly.

"We could potentially shorten the path toward new therapies by learning from examples which have evolved in nature over millions of years," Mrak said. "Considering that some (natural products) show their best only in combination with another molecule, there may be a number of new medicinal compounds hiding among the natural products already discovered."

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The mystery of metformin

By Martin J. Spiering

Jean Sterne discovered in 1959 that metformin, or dimethylbiguanide, lowers blood glucose levels in people with Type 2 diabetes. Researchers now are studying metformin as a therapeutic agent for managing such maladies as fatty liver disease, cardiovascular disorders and cancer. Yet while the mode of action for other drugs — including the anticancer agent Taxol, the analgesic aspirin and the antidepressant Prozac — is well understood, metformin's exact molecular mechanism remains a mystery.

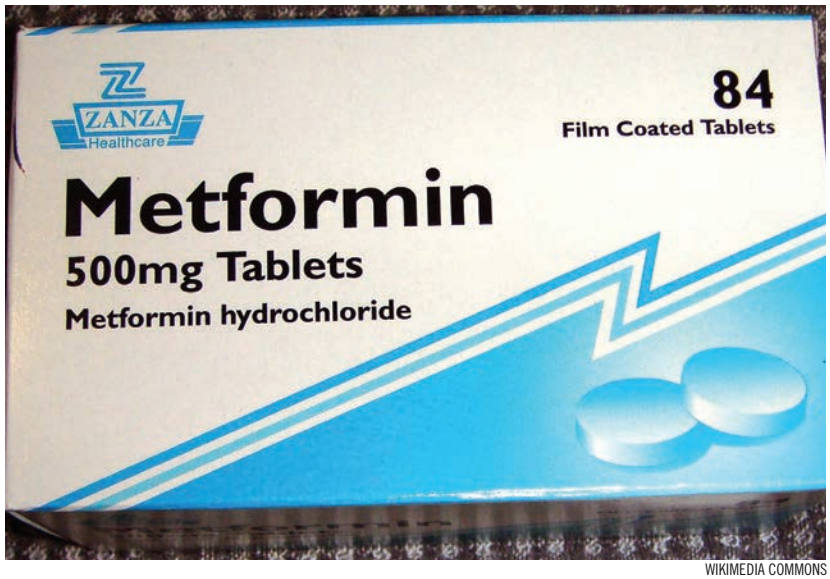
This is not for lack of trying. The journey to discover how metformin works includes two seminal studies published in the **Journal of Biological Chemistry**, now recognized as JBC Classics.

Mohamad-Yehia El-Mir and colleagues reported in 2000 that metformin inhibits respiratory complex I in the mitochondria of liver cells, or hepatocytes. This suggested that the authors had hit the bullseye of metformin's molecular target. However, there was a wrinkle.

"Metformin's effects on complex I were indirect," said Eric Fontaine, one of the researchers involved in this work and now at Université Grenoble Alpes in France. This indirect effect decreased cellular respiration and was highly specific, solely affecting mitochondrial complex I — but only in intact hepatocytes.

"If we put metformin into isolated mitochondria, we did not observe an inhibition of complex I," Fontaine said. The reason for this finding remains unclear, but metformin's indirect effect has been reproduced by the authors of the El-Mir paper and by other labs.

Later studies have reported that metformin directly inhibits complex I in isolated mitochondria, Fontaine said, but only in state 3 of complex I, when ATP is produced, a state that the El-Mir study did not examine. However, the metformin concentrations required to affect complex I activity directly are consistently higher than those yielding the indirect effect, suggesting the two effects are not linked. Confusion about metformin's effect on complex I persists in the literature and likely will be resolved only after metformin's molecular target is pinpointed.



WIKIMEDIA COMMONS

Metformin is prescribed to control Type 2 diabetes, but the drug's exact molecular mechanism remains a mystery.

Another clue to metformin's cellular target came to light in a 2001 paper reporting that metformin activates AMP-activated protein kinase, or AMPK. Gaochao Zhou and colleagues observed that metformin-induced AMPK activation suppresses glucose production in the liver through gluconeogenesis and also promotes glucose uptake into skeletal muscle. The study represented an important advance, according to David Carling, senior author of the second JBC Classics paper, which was published in 2002. However, "it wasn't clear how metformin was causing AMPK to be activated, what the molecular mechanisms were," he said.

This provided an opportunity for Carling's lab, which had a long track record of studying AMPK.

Metformin's effect on AMPK intrigued Carling because "AMPK acts as a very important, perhaps the most important energy sensor, within mammalian cells," he said.

Having previously developed a skeletal muscle cell line called H-2Kb that provided a reliable model to study AMPK in muscle cells, Carling and his group were ideally positioned to take the next steps to look at AMPK's role in mediating metformin's effects.

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"The first thing was that we got really good (AMPK) activation in this cell line, so that was brilliant," he said. "We were able to confirm and reproduce that metformin really did give a very robust activation of AMPK."

This was no small feat; metformin fails to get into cells that lack a specific transporter, organic cation transporter 1, or OCT1, a technical detail that wasn't common knowledge at the time and may still bedevil research into metformin's effects in cells. What's more, the H-2Kb cells, unlike some commonly used cell lines, express high levels of an upstream kinase — liver kinase B1, or LKB1 — that phosphorylates and activates AMPK.

"The hindsight is that (the cells) were able to take up metformin," Carling said, "and they expressed the upstream kinase that is required for activation of AMPK in response to metformin."

With all these then-unknown factors in alignment, Carling's research team set out to test whether metformin and another antidiabetic drug, rosiglitazone, activate AMPK by increasing the AMP-to-ATP ratio, a well-known AMPK inducer. The authors first demonstrated that rosiglitazone activates AMPK, a finding not previously reported, and that it does so by dramatically increasing the AMP-to-ATP ratio. However, metformin apparently did not measurably change the ratio. This finding prompted the authors to conclude that rosiglitazone and metformin activate AMPK via distinct routes.

But there was a hitch to that conclusion — with refinements in analytical methods, additional studies have shown that AMPK is sensitive even to very small changes in the AMP-to-ATP ratio. So small, in fact, that Carling and his team had been unable to see them.

"We didn't have sensitive enough methods available for detecting very small changes in AMP," Carling said.

So, although metformin is much less potent than rosiglitazone in increasing the AMP–to–ATP ratio, both appear to activate AMPK through the same general mechanism. "It's been an interesting journey for the AMPK field to realize that the levels of change in the nucleotides that you need are incredibly subtle," Carling said.

In an ironic twist, later studies have shown that AMPK is, in fact, activated via two routes: one through AMP-to-ATP ratio-stimulated phosphorylation by the upstream kinase LKB1 and another via calcium-induced phosphorylation by another enzyme, calcium/calmodulin-dependent kinase kinase 2, or CAMKK2.

"It turns out there are two distinct pathways, just not the ones that we thought," Carling said.

Both Carling's work on AMPK and that of El-Mir and colleagues on complex I show that metformin profoundly alters cellular energetics. "I think there are a lot of knock-on effects that interfere with complex I," Carling said.

This might explain why it's been so hard to pin down metformin's direct target, but metformin's elusive activity in the lab, its relatively weak effects on the AMP-to-ATP ratio and its impaired uptake into some cells might be why it continues to be an efficient and well-tolerated antidiabetic drug.

"The fact that it is a fairly poor drug turns out to be its strength — it's very unusual," Carling said. "I think metformin is a great example of why you shouldn't get too hung up with mechanism (when you start) to test whether a drug is effective."

This hasn't stopped scientists' search for metformin's direct target in hopes of unraveling how it exerts its effects and improving on those effects to more efficiently manage diabetes or cancers that have emerged as potential targets of metformin-based therapies. Recent advances such as cryo-EM have enabled detailed structural studies of larger protein complexes, including complex I.

Carling believes researchers are much closer to finding out whether or how metformin interacts with complex I or other cellular structures. "(Complex I) is an obvious one to do," he said.

Perhaps the long journey to solving the mystery of metformin's direct target will soon come to an end.

Three of the scientists who made major contributions to these findings have since died: Mohamad-Yehia El-Mir and Xavier Leverve, first and senior authors, respectively, of the El-Mir et al. paper, and Lee Fryer, first author of the Carling group study.

Fontaine worked closely with El-Mir for about two years and remembers him as a great friend and colleague. And Leverve, he said, was an inspiring mentor. "He was a fantastic man — very, very charismatic. Everybody who met him wanted to work with him."

Leverve's training and experience as a physician in an intensive care unit may have spurred his interest in bioenergetics, leading him to study complex I and the compounds acting on it. "He had a very open mind from the clinic, especially in the ICU, where there is a kind of an energy failure during a septic shock," Fontaine said.

Carling said he appreciates that his lab's work has been nominated as a JBC Classic, but he also noted, "It's a great shame that Lee is not around to see this recognition. Lee was a very productive scientist and had a big impact on my lab."

This article has been edited for ASBMB Today. Read more JBC Classics at jbc.org.

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How to catch ovarian cancer when it's curable

Researchers use proteomics to develop diagnostic tool from liquid biopsy

By Laurel Oldach

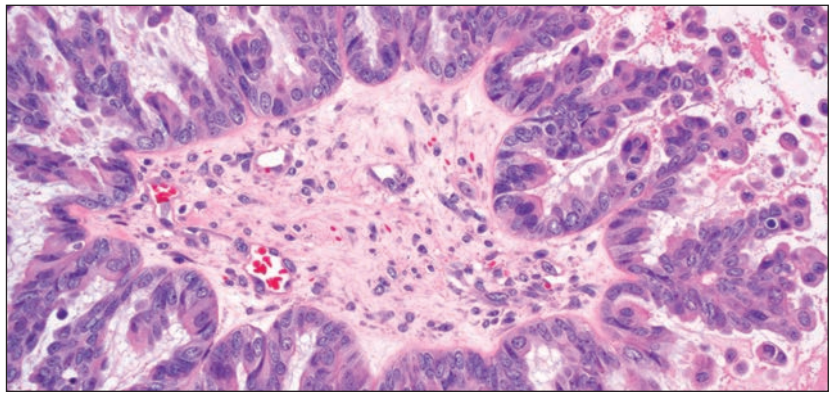
Fewer than half of ovarian cancer patients survive for five years after their diagnosis. According to the American Cancer Society, this is because only about one-fifth of ovarian cancer cases are detected early, when the chances of successful treatment and recovery are highest.

"If we could change this reality by detecting (ovarian cancer) at a curable stage, we could save many lives," said Keren Levanon, a physician-researcher at Chaim Sheba Medical Center in Israel.

In the journal **Molecular & Cellular Proteomics**, researchers led by Levanon and Tamar Geiger of Tel Aviv University report a new test for ovarian cancer that outperforms previous tests. They hope it can be used to screen women who are genetically predisposed to the disease.

The researchers searched for signatures of cancer in uterine fluid sampled by liquid biopsy during surgery. They compared samples from women with ovarian cancer who had surgical treatment and from volunteers who had gynecological surgery for reasons unrelated to cancer, such as uterine fibroids or benign ovarian cysts.

Bodily fluids contain many proteins. Strong signals from the most common proteins can mask signals from smaller amounts of cancer-linked proteins that also might be present. To overcome that difficulty, researchers isolated microvesicles from the uterine fluid. Because microvesicles are shed from cells, they contain almost none of the signal-masking blood plasma proteins. Instead, they contain protein cargo that may vary between normal and malignant tissues.



VIRGINIA COMMONWEALTH UNIVERSITY

A section of an ovary stained with hematoxylin and eosin, showing a roughly star-shaped tumor. This tumor can be identified as aggressive because of its micropapillae, or outgrowths.

Using proteomics, the researchers compared thousands of proteins in uterine microvesicles from 12 healthy volunteers and 12 cancer patients. Then they used machine-learning algorithms to search for patterns of protein abundance that differed among the samples.

"We developed a diagnostic set of nine proteins that distinguishes women with ovarian cancer from healthy women with greater sensitivity and specificity than reported before," Levanon said.

The researchers then tested the set's accuracy in 152 women, 37 of whom were known to have ovarian cancer. The test had 70 percent diagnostic sensitivity, meaning that it correctly detected cancer in 25 of the 37 study participants who truly had cancer, including all early-stage cases; it also had 76 percent specificity, meaning that it correctly identified about three out of every four healthy volunteers as healthy. It outperformed previous proteomics-based tests, which had less than 60 percent sensitivity.

Though the study used fluids collected during surgery, the proteomic test can also be run on samples collected in a minimally invasive procedure similar to intrauterine device insertion. The authors propose that it may help young women whose risk of developing ovarian cancer is known to be high in deciding whether to have their ovaries removed as a preventative measure. The method of isolating microvesicles from bodily fluids to detect faint cancer signals also may have promise for other difficult-to-detect types of cancer.

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JLR celebrates diamond jubilee with special reviews

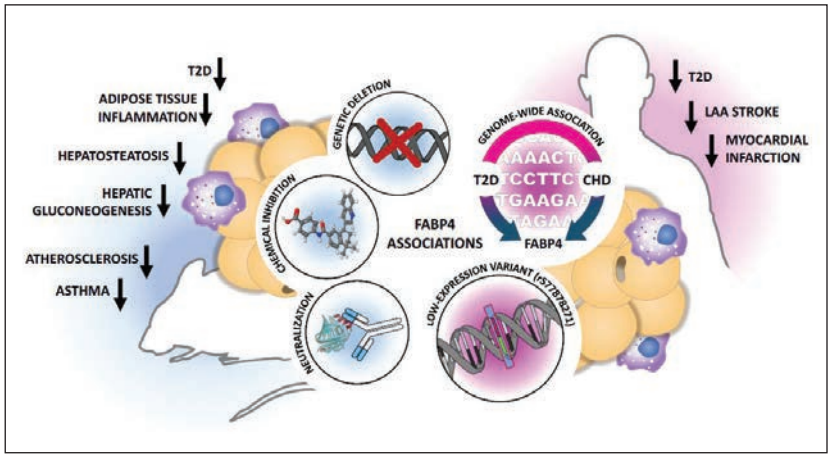
By Laurel Oldach

A diamond traditionally symbolizes a 60th anniversary, and this month, the **Journal of Lipid Research** marks 60 years with a sparkling collection of special review articles.

The JLR started as a quarterly journal in October 1959 in response to an explosion in methods and research in lipid biochemistry. It began publishing monthly in 1983, and in 2003 it joined the American Society for Biochemistry and Molecular Biology journals family.

Since its 50th anniversary, the JLR has sponsored lectures at meetings in the lipid biochemistry field. For the journal's 60th anniversary, the editors invited each of the more than 50 researchers who have been recognized with these invited lectures to write an update on his or her area of research. While not all the scientists could participate, the 60th anniversary party is off to an exciting start with the first set of reviews.

These articles touch on several broad themes in lipid science. They explain recent advances in well-known areas, such as the role of lipid storage in metabolic disorders, and also areas that may not be so familiar, such as transcellular biosynthesis of signaling molecules. Whatever your focus in lipid research, you're sure to find something interesting. Here's a quick overview of the articles published so far.



K. PRENTICE ET AL./JLR 2019

Each JLR perspective article features a graphical abstract that can be downloaded as a PowerPoint slide for teaching. This one is from a review on fatty acid binding protein 4 by Kacey Prentice and colleagues at Harvard University and Harvard School of Public Health.

Lipids in immune signaling

Many diffusible signaling molecules that affect immune activity originally were components of the cell membrane. What's more, hydrophobic molecules such as cholesterol can be recognized and promote inflammation.

• Leukotrienes are lipid signaling mediators that contribute to anaphylaxis and white blood cell traffic. The synthesis of a leukotriene from a membrane lipid sometimes requires several cells to chip in. Robert Murphy and Giancarlo Folco discuss how leukotriene synthesis is regulated in concert with other lipid metabolism pathways.

• Macrophages contribute to atherosclerosis, or blood vessel plaque formation, through inflammation. Alan Tall and Marit Westerterp review the hypotheses for how excess cholesterol can contribute to inflammatory signaling in macrophages and neutrophils.

• Sarah Spiegel and colleagues describe a transporter that moves sphingosine-1-phosphate, a lipid regulator of T and B cell activity, from within the cell where it's made to outside the cell, where it is sensed by G protein-coupled receptors.

Lipids in metabolism and metabolic disorders

Energy storage in the form of triglycerides in adipocytes in lipid droplets is a basic building block of obesity — and it also plays into insulin resistance and other metabolic problems. Several articles discuss what we've learned in recent years about coordinating triglyceride formation.

• Rosalind Coleman describes how the enzymes that build triglycerides interact with other proteins to get these energy-storing lipids made — and used — at appropriate times.

• Phosphatidic acid phosphatases clip a phosphate group from phosphatidic acid to make a simpler backbone that can be built into triglycerides.

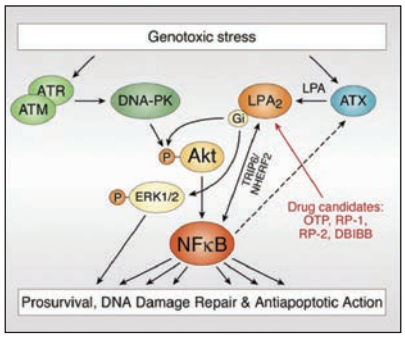
Companion reviews from Karen Reue and Huan Wang and from George Carman and Gil-Soo Han discuss the human and yeast versions of these enzymes.

• Without communication between tissues, whole-body lipid metabolism never could be coordinated. Marcus Seldin and Aldons Lusis describe their work using systems genetics to investigate variability in how adiponectin from fat tissues is received in the liver.

Lipids in the development of rare diseases

Two reviews highlight genetic disorders of sphingolipid metabolism. Sphingolipids are found in membranes, where they can act as receptors or regulate membrane protein activity; they also can work as signaling molecules after a little metabolic tweaking.

• In the past, many genetic diseases seemed unrelated, but as our understanding of sphingolipid synthesis and turnover improves, it has become clear that several are linked by disruptions to sphingolipid processing, Richard Proia and co-authors explain in their review.



G. TIGYI ET AL./JLR 2019

This graphical abstract is from a review by Gabor Tigyi and colleagues on the therapeutic potential of lysophosphatic acid type 2 receptor agonists.

• The latest genetic disorder of sphingolipids was discovered in 2017. Julie Saba describes the 50-year journey from the first description of a sphingolipid breakdown enzyme to the identification of a disease caused when it is disrupted.

Lipids in pharmaceutical science

Along with our emerging understanding of lipids' roles in physiology have come advances in how scientists can modulate lipid-related signaling. Sometimes this means a new understanding for an old and familiar drug; other times it's about targeting new signaling pathways.

• Niacin was the earliest drug for dyslipidemia. Johan Auwerx and colleagues review recent research suggesting a new mechanism of action to explain why it lowers blood triglycerides.

• Lysophosphatidic acid is a powerful antiapoptotic signal, but like many such signals, it also can promote tumor growth. Gabor Tigyi and colleagues describe their effort to develop an LPA mimic with less dramatic negative side effects.

• Finally, a review on inhibitors of the PI3 kinase pathway, a new class of drugs for cancer, highlights the importance of signaling that happens downstream of lipids. Lewis Cantley and coauthors write about how cancer cells evade PI3K inhibition and how to reduce that resistance.

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From the journals

By Gelareh Abulwerdi, Jonathan Griffin & Kerri Beth Slaughter

We offer a selection of recent papers on a variety of topics from the American Society for Biochemistry and Molecular Biology's three journals, the **Journal of Biological Chemistry,** the **Journal of Lipid Research,** and **Molecular & Cellular Proteomics**.

Peering into Alzheimer's protein aggregation

The hereditary Arctic mutation of 42-amino-acid beta-amyloid peptide, known as Abeta42, causes aggressive aggregation of the peptide in early-onset Alzheimer's disease. The mechanism connecting this mutation to the protein accumulation is unknown, however. Meng Lu and colleagues at the University of Cambridge revealed details of this process by establishing stable cell lines expressing the mutation and observing them with fluorescence-lifetime and super-resolution imaging. They found that Arctic mutant Abeta42 formed five unique types of aggregates that degraded more slowly than those in wild-type cells. The authors suggest their model lays groundwork for studying how intracellular amyloid affects cellular compartments. The study was published in the **Journal of Biological Chemistry**.
DOI: 10.1074/jbc.RA118.004511

Advancing phage proteomics

Bacteriophages, or phages, are viruses that infect bacteria, and they are ubiquitous across the planet. Phage studies have provided fundamental knowledge for the development of molecular biology and important biotechnologies such as the CRISPR-Cas system.

Researchers are working to

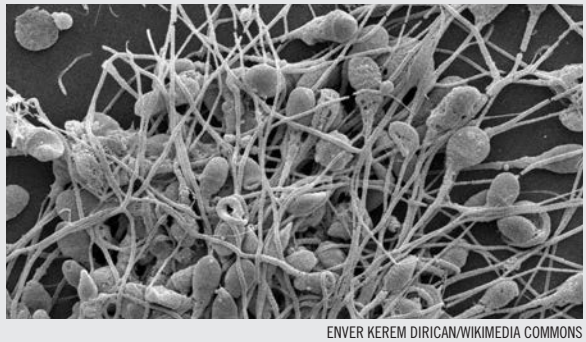
Signal transduction in sperm development

Spermatogenesis is a highly regulated cell differentiation process that gives rise to the male gamete known as the sperm cell. This process takes place within the seminiferous tubules of the testis. During the last step of the cell differentiation process, the developing gametes undergo a number of structural changes in which they elongate, lose most of their cytoplasm and gain important functional features such as the flagellum. The gametes then are released to continue their maturation process in the epididymis. Fertility deficiencies and offspring disorders can occur if any of the steps in spermatogenesis are disrupted.

Previous studies of spermatogenesis have uncovered the hormone pathways that drive the differentiation process. However, other areas of regulation, such as signal transduction during sperm development, require further exploration. For instance, protein phosphorylation is a signaling event that plays a role in cell cycle regulation, cell death, cell differentiation and other critical biological pathways. Protein phosphorylation has been identified as a crucial player in regulation of testis-specific events.

In a paper published in **Molecular & Cellular Proteomics**, Judit Castillo and colleagues from Lead Pharma and the OncoProteomics Laboratory in the Netherlands performed phosphoproteomics studies using human testicular tissue to identify important signaling events during spermatogenesis. They coupled metal oxide affinity chromatography with mass spectrometry to generate a profile of the human testis phosphoproteome that included 174 phosphorylated kinases. They used their data to identify the most active kinases in the human testis, such as cyclin-dependent kinase 12 and p21-activated kinase 4, which function in splicing regulation and cell survival, respectively. In the future, their findings may be used to validate targets for drug development for male fertility and testicular tumors.
DOI: 10.1074/mcp.RA118.001278

— Kerri Beth Slaughter



ENVER KEREM DIRICAN/WIKIMEDIA COMMONS

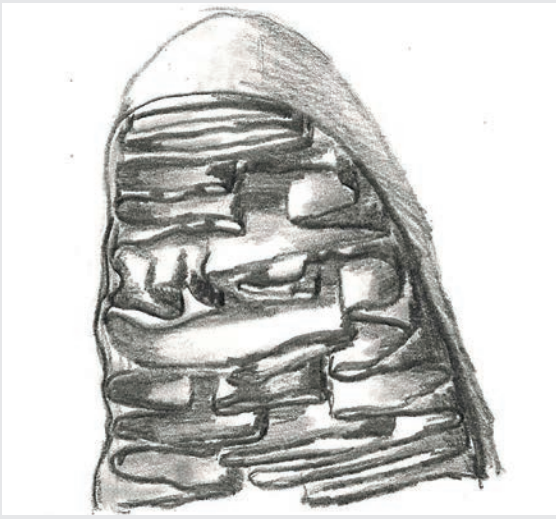
This scanning electron micrograph shows human spermatozoa at 2,500 times magnification.

Role of sphingosine in mitochondrial dysfunction and TBI

Traumatic brain injury, or TBI, is a public health concern in the developed world among young people who play contact sports and the elderly, who are prone to falling. Events after TBI can be separated into primary and secondary injuries. Primary injury occurs nearly instantly after TBI, while secondary injury begins usually within minutes or hours and includes events such as immune cell infiltration and activation, cytokine release and apoptosis. One secondary injury event is mitochondrial dysfunction. In a paper published in the **Journal of Lipid Research**, Sergei Novgorodov and colleagues at the University of South Carolina write about some mechanisms by which enzymes involved in sphingolipid metabolism lead to mitochondrial dysfunction and exacerbation of secondary injury after TBI.

Sphingolipids play important roles in cells. Some, such as sphingomyelins, are major building blocks of cellular membranes, while others, ceramides and sphingosine, are essential in cell proliferation, differentiation and programmed cell death. More than 40 enzymes in the brain metabolize sphingolipids. The researchers showed that events after TBI cause stimulation of one of these enzymes, called neutral ceramidase, or NCDase, leading to accumulation of mitochondrial sphingosine and eventually mitochondrial dysfunction exacerbating secondary injury.

To investigate the role of sphingosine in secondary brain injury, Novgorodov's team examined NCDase-deficient mice and wild-type mice that underwent controlled cortical impact injury to reproduce the histological and pathological changes associated with human TBI. The NCDase-deficient mice had 37 percent lower levels of mitochondrial sphingosine in their injured brains than the wild-type mice. The finding that sphingolipids can cause mitochondrial dysfunction, which in turn can exacerbate neuroinflammation, could open new avenues for drug discovery.



IAN MACDONALD

Mitochondria, such as the one in this drawing, are the powerhouses of the cell. In this study, researchers show that sphingosine can disrupt mitochondrial function.

DOI: 10.1194/jlr.M091132

— Gelareh Abulwerdi

unravel crucial aspects of phage biology, including the phage and bacterial proteome during infection. Transcriptomic and metabolomic studies have been conducted to analyze gene expression during phage infection, but proteomic analysis is required to understand how gene expression is regulated post-transcription.

In a paper published in **Molecular & Cellular Proteomics**, Marie-Laurence Lemay and colleagues from Laval University in Quebec City describe how they used high-throughput proteomics techniques to characterize the viral and bacterial proteomes at different stages of infection. They identified several bacterial proteins that were expressed only during infection. Their results also generated a more comprehensive view of the phage proteome that will be important for future studies of phage biology.

DOI: 10.1074/mcp.RA118.001135

Up close and personal with ribosomes

The ability to visualize macromolecular structures of single cells could aid understanding of a host of cellular activities. However, current methods are limited by low resolution and the requirement for highly purified samples. Xiunan Yi and colleagues at the University of Texas present a new technique that combines single-cell lysis with electron microscopy. In their report in the **Journal of Biological Chemistry**, they write that they used this method to image and count ribosomes of single Caenorhabditis elegans embryos throughout several stages of development. The authors conclude that this method holds significant promise for applications in developmental, evolutionary and disease-related studies.

DOI: 10.1074/jbc.RA118.006686

High fructose diet and metabolic syndrome

Have you looked at the sugar content on your favorite soft drink or juice? Soft drinks and juices are high in fructose. A high-fructose diet can lead to dyslipidemia and is a major risk factor for development of cardiovascular disease, or CVD, and insulin resistance. Andrew Butler at Saint Louis University, in collaboration with researchers at the University of California, Davis, studied the role of fructose-fed diet in developing CVD. In a paper published in the **Journal of Lipid Research**, they write that they fed rhesus monkeys beverages sweetened with fructose-containing sugar and measured plasma lipid profiles as well as lipidomic changes. They were the first researchers to show a positive correlation between high fructose consumption and an increase in the quantity of large lipoprotein particles such as very-low density lipoproteins, known as VLDLs, that are associated with CVD. Furthermore, they showed that administration of fish oil reversed the increased shift in the quantity of large lipoprotein particles. They showed a strong correlation between concentration of plasma ApoC3, a protein component of VLDLs, and elevation in the number of VLDLs. ApoC3 affects the metabolism of lipoproteins, and its expression is a risk factor for CVD. Next time, check the sugar content and think twice before you enjoy your favorite sweet drink.
DOI: 10.1194/jlr.M089508

Bacterial extracellular vesicles as virulent bombs

The bacterium Listeria monocytogenes is the cause of listeriosis, an illness that can present a major threat to pregnant women, newborns and others with weakened immune systems. This organism has been found to secrete tiny lipid-bilayered spheres called extracellular vesicles, or EVs, but their role in infection is not completely understood. Caroline Coelho at Johns Hopkins University and colleagues report in the **Journal of Biological Chemistry** that these EVs contain several virulence factors including the pore-forming toxin listeriolysin O. High-resolution fluorescence imaging captured real-time EV release from bacteria inside infected mammalian cells. The authors suggest that EVs are used for toxin release, which adds to the current understanding of L. monocytogenes' pathogenic strategy.
DOI: 10.1074/jbc.RA118.006472

Immunomic screening of the Plasmodium proteome

Malaria is caused by a single-celled eukaryotic parasite in the Plasmodium genus. This parasite is spread from mosquitoes to humans, particularly in tropical and subtropical regions of the world, leading to severe disease and sometimes death. No vaccine exists to prevent malaria, and drug resistance poses a major threat to fighting the infection.

Developing a malaria vaccine has proved challenging, and additional studies are needed to identify new parasite proteins associated with protection in humans. A limited number of malaria proteins have been investigated, and a more comprehensive screening of the malaria proteome using antibodies able to provide protection against infection could advance vaccine development.

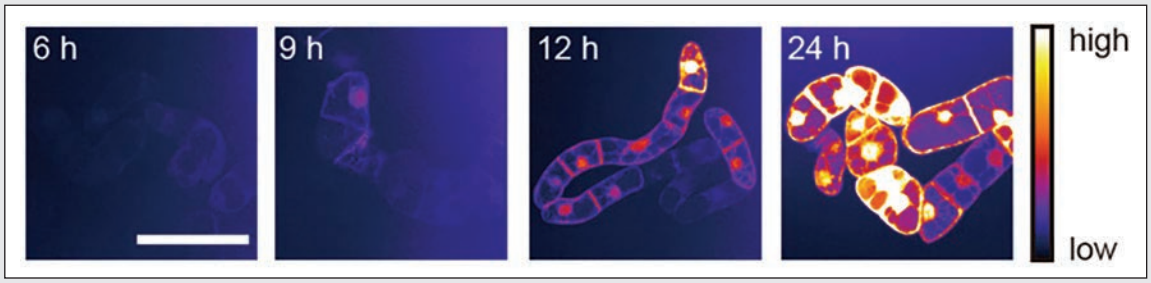
In a paper published in **Molecular & Cellular Proteomics**, Anthony Siau and colleagues from the Nanyang Technological University in Singapore screening of the malaria proteome using antibodies able to provide protection against infection. After the initial screen, the researchers used predictive criteria to calculate the likelihood of protection for each antigen. Their proteomic techniques for studying protection provided a framework for the development of a new approach to malaria vaccine development.
DOI: 10.1074/mcp.RA118.000997

Lipid profiles and chronic kidney disease

Data on the association between dyslipidemia and end-stage renal disease, the final stage of chronic kidney disease, are rare, and the results are not conclusive. Such data would be valuable for a medical practitioner putting a CKD patient on a drug regimen. Ching-Wei Tsai and colleagues at China Medical University performed a 13-year prospective study of more than 4,500 patients with CKD between the ages of 20 and 90 to find the association between lipid trajectories and risk of developing ESRD. In a paper published in the **Journal of Lipid Research**, Tsai and colleagues report that higher baseline levels of lipoproteins such as LDL cholesterol and total cholesterol correlated with higher risk of developing ESRD in patients with CKD. Overall, the incidence of ESRD in CKD patients with high baseline triglyceride was 49 percent higher.
DOI: 10.1194/jlr.P084590

A protein that propelled life toward multicellularity

The basement membrane, or BM, is an extracellular matrix component that is essential to tissue functions. BM played a key role in the evolutionary leap of unicellular organisms to multicellularity, but its exact structure and function during this ancient transition are unknown. Carl Darris and colleagues at Vanderbilt University now report that the BM protein Goodpasture antigen-binding protein, or GPBP, likely played a key role in this transition. By comparing the genomes of some metazoans and unicellular organisms, they found that GPBP-2 is the oldest isoform of GPBP and possibly functioned both



NAGASHIMA ET AL.

A reporter gene assay shows the response of tobacco BY-2 cells to the caryophyllene structural analog caryophyllene oxide.

How plants sniff out danger

Plants cannot hear or see impending threats, but they can smell them. Volatile organic compounds, or VOCs, released by herbivore-infested plants can be sensed by other plants, which then, in response to the danger nearby, upregulate defense-related genes. While animals sense odors through membrane receptors in their nervous systems, the same receptors are rarer and have different jobs in plants. It seems likely that plants have their own unique way of sensing VOCs, but molecular mechanisms that could explain these olfactory processes have evaded researchers.

In a study published in the **Journal of Biological Chemistry**, Ayumi Nagashima and a team of researchers from across Japan have made a significant step toward understanding plant olfaction by showing that gene-suppressing TOPLESS-like proteins, or TPLs, are critical for tobacco plants' sensing and responding to VOCs.

The researchers exposed tobacco cells to an array of VOCs and noticed that caryophyllene encouraged the expression of stress-responsive genes. To find which plant molecule was binding this VOC, they used a pulldown assay in which proteins extracted from tobacco leaves were incubated with caryophyllene-linked beads. After separating the beads from the extract with magnets and washing them, they found that TPLs remained bound to caryophyllene.

The role of TPLs in plant olfaction was probed further by overexpressing the protein in tobacco cells. After exposing these cells to caryophyllene, they found that the upregulation of defense-related genes was reduced, which could implicate TPLs in the response of plant gene expression to caryophyllene.

This study provides evidence for VOC sensing via a nuclear protein. Future studies could aim at illuminating the long-hidden mechanism underlying plant olfaction.

DOI: 10.1074/jbc.RA118.005843

— Jonathan Griffin

intracellularly and extracellularly in early metazoans. The GPBP-1 isoform emerged later in chordates as an extracellular component, which the authors suggest likely allowed for the development of epithelial tissues. Their results were published in the **Journal of Biological Chemistry**.
DOI: 10.1074/jbc.RA118.006225

Linking lipid transport to metabolic disease

Mutations in lipid transporting phospholipid flippases, or P4-ATPases, can lead to a variety of metabolic diseases, including insulin resistance and obesity. These mutants also have been associated with elevated levels of intracellular glucosylceramide, known as GlcCer, but how these lipids traverse cellular membranes is not well understood. In a study in the **Journal of Biological Chemistry**, Bartholomew Roland and colleagues from Vanderbilt University and Kyoto University confirmed that certain P4-ATPases play a direct role in GlcCer transport by observing uptake of fluorescently tagged GlcCer into yeast and human cells. They also found that a central glutamine of the TM4 transmembrane segment is required for GlcCer transport. The authors suggest that certain GlcCer-transporting P4-ATPases may be critical for lipid homeostasis and metabolism.
DOI: 10.1074/jbc.RA118.005876

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Circulating oxysterol levels

A new tool in detecting breast cancer

By Kian Kamgar-Parsi

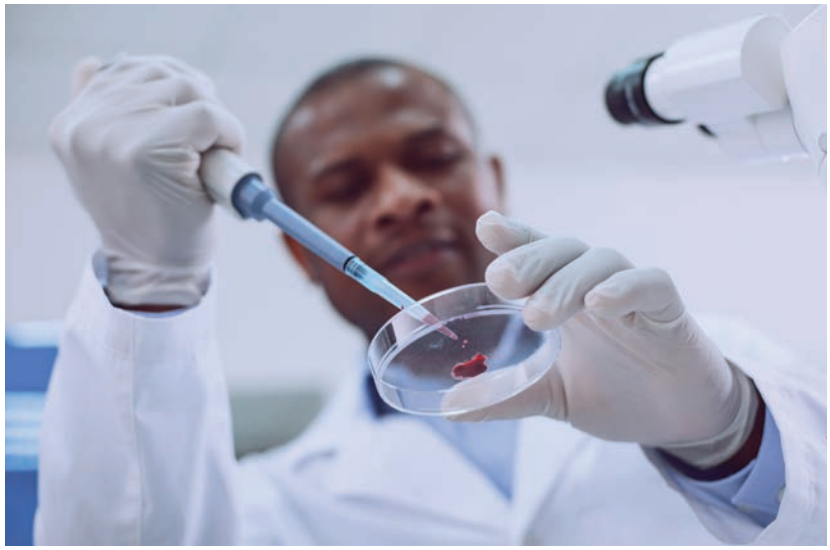
Finding cancer before it has progressed or spread is crucial when trying to fight the disease. Despite this, early cancer detection remains a challenge. Cancers often can lie undetected without symptoms until it's too late for effective treatment. Recent research, however, may present a new path to overcoming this obstacle.

In an article in the journal Clinical Chemistry and Laboratory Medicine, F. Peter Guengerich of the Vanderbilt School of Medicine and an international team showed a correlation between certain circulating molecules and the presence of cancerous cells.

Oxygenated metabolites of cholesterol, called oxysterols, are molecules formed naturally through metabolic processes and play important roles in mediating cholesterol and lipid metabolism. By measuring the blood levels of oxysterols in breast cancer patients before and after tumor removal, Guengerich and colleagues discovered that certain concentrations changed, indicating a potential role for them in cancer biology.

"In general, some people think that most cancer is caused by things in the environment, like smoking or the things they eat," Guengerich said. "Turns out there's a lot of stuff going on in our own bodies, like metabolism, that's driving cancer."

Oxysterols have been implicated in breast cancer in the past, but this new research indicates that their role may be more complex than previously thought. While researchers have known for years that there are multiple types of oxysterols, it turns out that these oxysterols can behave very differently from each other when



tumors grow and are removed.

Particularly surprising to Guengerich and his team was that one oxysterol, 7-ketocholesterol, or 7-keto, actually decreased in concentration when tumors grew and increased in concentration after tumors were removed. Previously, many scientists believed that more cancer would mean more oxysterols.

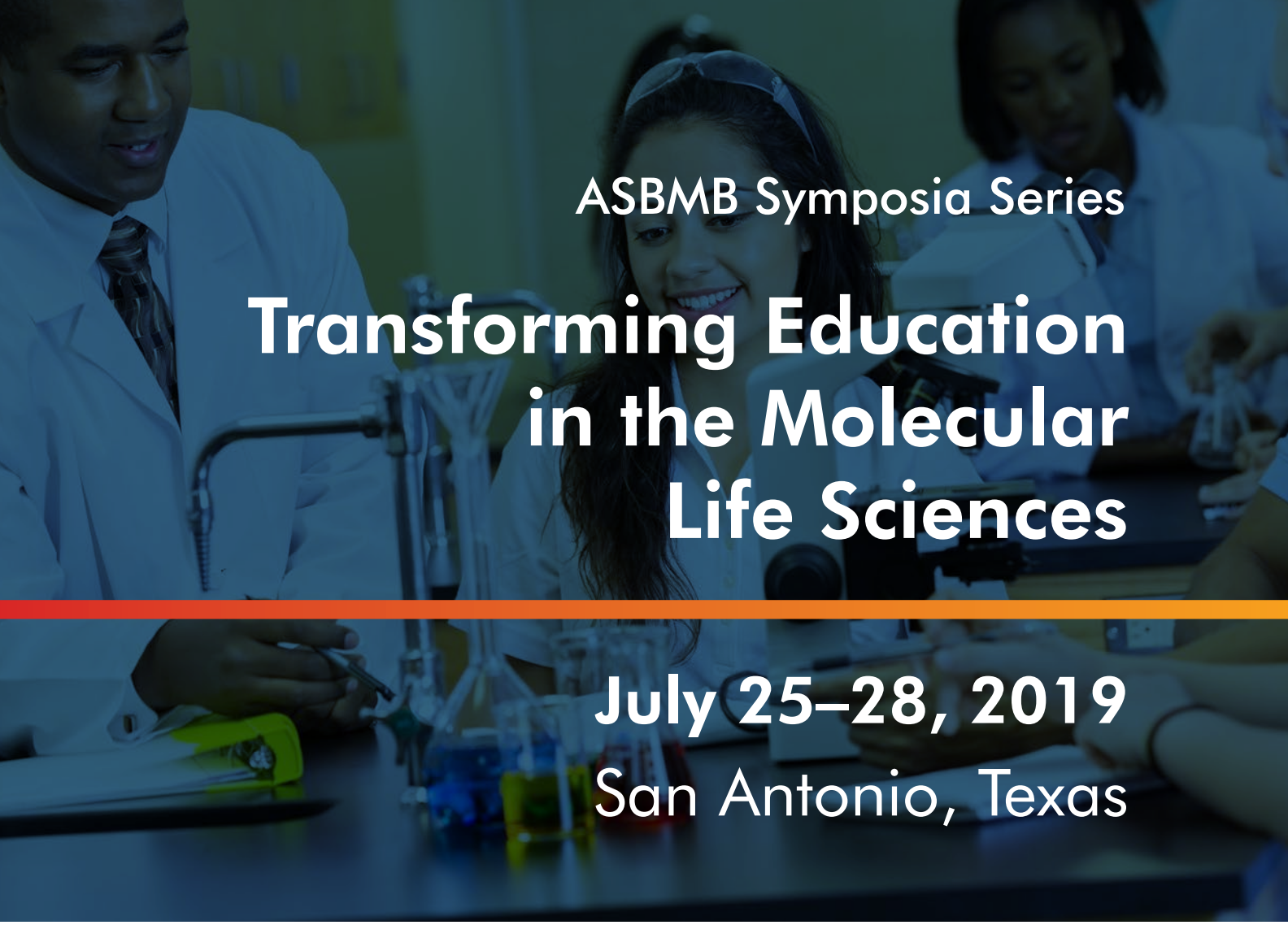
"Even in this field, people have the idea that all oxysterols are bad, but our work indicates that that may not be true," said Guengerich, who is a deputy editor of the Journal of Biological Chemistry. "Levels of individual oxysterols are going up and down in relation to a cancer, so they're not necessarily all good or all bad, but all have their own type of biology."

This richer picture of oxysterol behavior gives rise to the hope that the monitoring of oxysterols could become an early warning system for cancer, although Guengerich warns that we're not there yet. Studies are needed to determine how oxysterol levels change after tumor removal and how those changes correlate with survival rates. Researchers also want to determine if changes in oxysterols indicate cancer metastasis.

This research project was limited; only 24 patients were involved and the study lacked healthy control subjects for comparison. "Cancer is a heterogeneous type of disease, and we need more people and time to be clinically useful," Guengerich said.

Oxysterols such as 7-keto may not yet be useful biomarkers, but they present a promising avenue of research and raise new questions: What causes the change in oxysterol levels? Are these changes a cause of breast cancer or a side effect? When answers are found, they could lead to new tools in the fight against breast cancer.

Kian Kamgar-Parsi (kkamgar@umich.edu) studied biophysics at the University of Michigan and is a consultant for the pharmaceutical industry.



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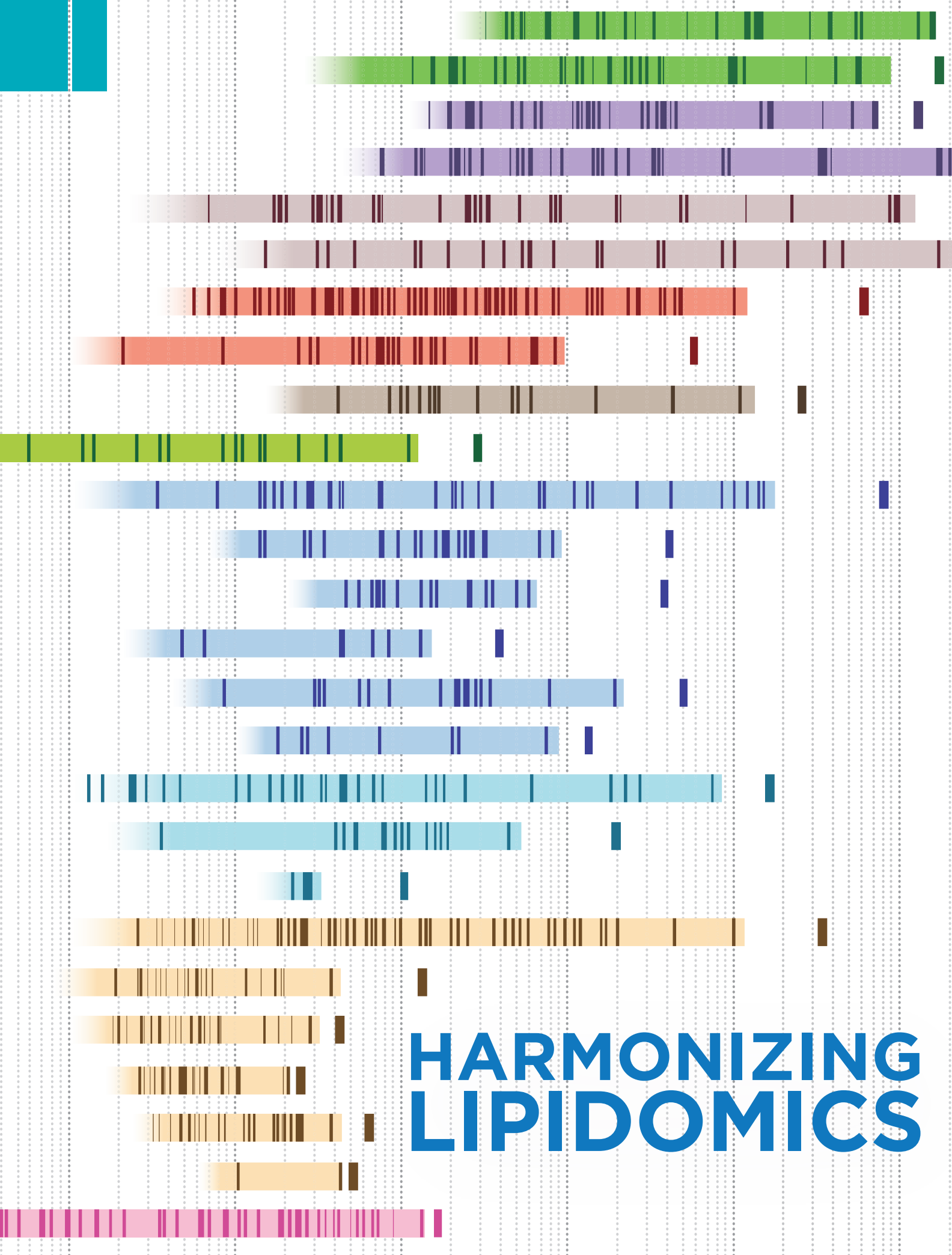

HARMONIZING
LIPIDOMICS

Harmonizing lipidomics

Researchers strive to agree on what they're measuring

By Laurel Oldach

Chatting at a conference a few years ago, lipidomics researchers Markus Wenk and Andrej Shevchenko noticed a problem.

"We were having coffee over a break during the sessions, and we were thinking, 'There are a lot of papers flying around now on plasma lipidomics,'" Wenk, of the National University of Singapore, said.

WENK

Many of those studies were looking for biomarkers, molecules that change reliably during the course of a disease and might someday be used for diagnosis. To detect potential lipid biomarkers, researchers compare the level of many lipid species in blood plasma from patients to those of healthy volunteers — often without determining an absolute molar quantity of either.

"The situation is very odd," said Shevchenko, of the Max Planck Institute of Molecular Cell Biology and Genetics. "We cannot compare data … You identify significantly changing lipids, and do maybe thousands of analyses, but I cannot compare my results with yours."

SHEVCHENKO

Not all biological studies must be strictly quantitative. Western blots, fluorescence imaging and many proteomics approaches rely on semi-quantitative measurement. Likewise, a lipidomics study might show that people with a given disease have twice as much of a certain lipid as healthy individuals. If later studies back up the finding, the molecule in question begins to look like a promising analyte to screen for the disease.

For a lipid to be useful in the clinic, however, researchers must agree on the identity of the molecule in question and on how much of it to expect in a healthy person. Lipidomics researchers are working toward such agreements; their field-wide project is mostly collegial but sometimes contentious and provides a case study of how scientists create systems of measurement.

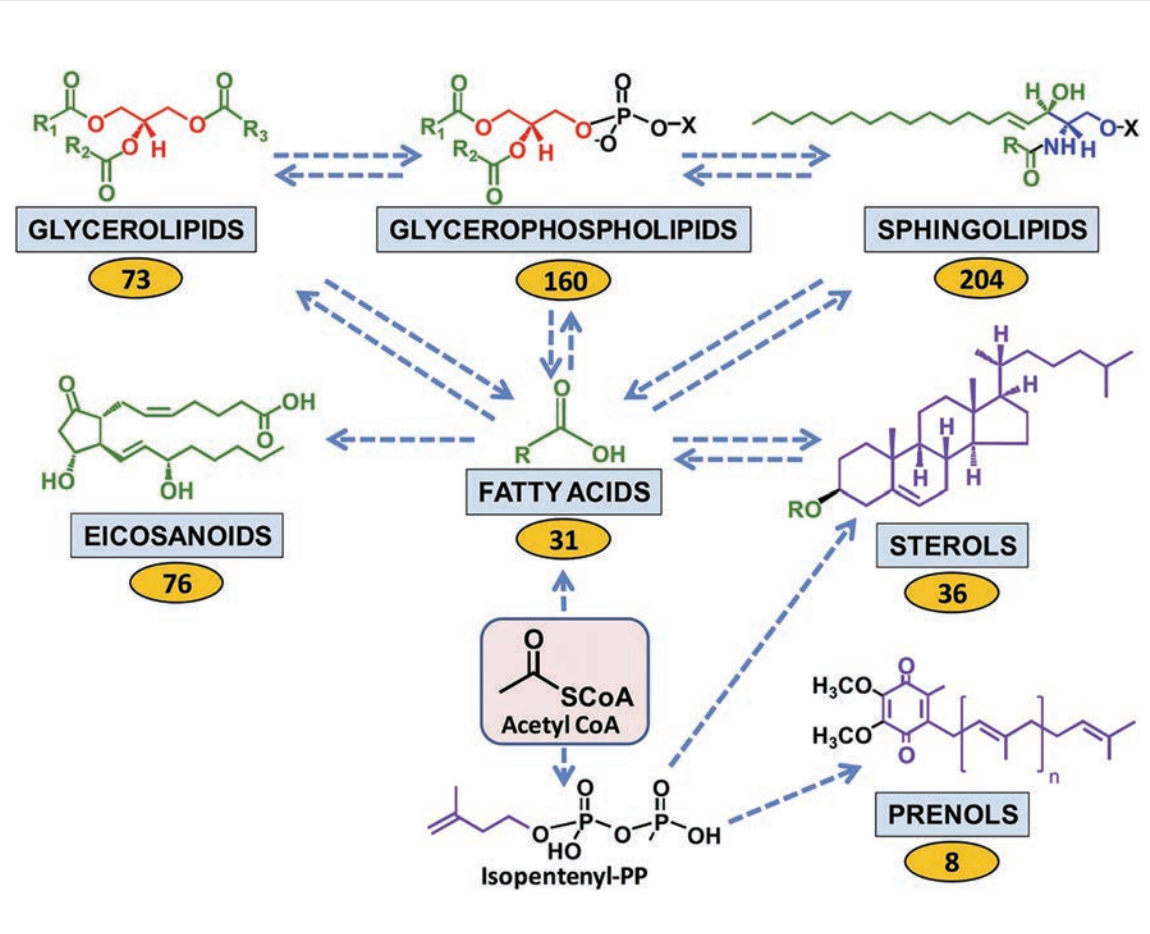
Lipidomic beginnings

The most common lipid biomarker is cholesterol. Clinical laboratories use an assay with colorimetric readout to measure cholesterol in a patient's blood plasma. A lab's confidence in its measurement comes from running control samples with each assay and from external certification.

Lipid researchers have found numerous molecules that might someday predict diseases such as Alzheimer's, cancer and metabolic disorders as effectively as cholesterol predicts the risk of heart attack and stroke. But cholesterol is by far the most abundant lipid in the blood. (See box on page 28: What is a lipid?) To measure molecules with much lower concentrations, scientists must use methods with higher sensitivity, such as mass spectrometry, or MS.

Forty years ago, using a mass spectrometer to measure lipids seemed impossible. The technique starts by ionizing molecules to measure their mass-to-charge ratio; lipids, many of

Opposite: These colorful stripes are adapted from a figure showing the wide variety of molar quantities of various lipids in human plasma. For more information, see page 37.



QUEHENBERGER ET AL. JLR 2010

The LIPID MAPS consortium established a classification system for lipid molecules. This figure, from the consortium's study of the lipidome of a pooled plasma sample, shows how the six major lipid classes (fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterols and prenols) are synthesized and interconverted as well as how they relate to other lipid metabolites.

What is a lipid?

Lipids are partly or completely hydrophobic small molecules. The simplest is a fatty acid, a carboxylic acid with a hydrocarbon tail. By adding fatty acids to other molecules via esterification, or by elaborating on a few other building-block molecules like acetyl coenzyme A, cells can make a tremendous variety of molecules that fit into the lipid class; LIPID MAPS' database includes some 40,000 molecular species. In animal cells, these lipids fall into six classes.

Lipids famously form the plasma membranes that contain cells and eukaryotic organelles, and triglycerides are stored for energy in lipid droplets. Lipids also can function as coenzymes (for example, ubiquinone in the mitochondrial electron transport chain, or vitamin K) and as diffusible signaling molecules (a well-known example is S1P).

which are nonpolar, can be difficult to ionize. (See box on page 30: How does mass spectrometry work?)

When he started measuring lipids in the 1980s, Richard Gross, a mass spectrometrist at Washington University in St. Louis, would homogenize buckets of samples to harvest enough of certain subcellular membranes to detect. Though the fundamentals of mass spectrometry are the same, instrumentation has advanced a great deal since then.

"Nothing has changed in the last 40 years," Gross joked, "except the ion sources have improved by five orders of magnitude, the ion traps have improved, and so has detector resolution. We've gone from working with garbage cans and canoe paddles to working in Eppendorfs."


GROSS

Gross' lab was among the first to measure lipids extracted from tissue by MS, publishing its first paper on the work in 1984. "From my perspective, that was the beginning of lipidomics," he said, "although lipidomics wasn't going to be a word for 20 years."

What took the technique so long to catch on? Kai Simons, former director of the Max Planck Institute of Molecular Cell Biology and Genetics, blames the molecular revolution in biology.

"It was DNA, RNA, proteins," Simons said. "There was so much to do. Many of the most creative young researchers wanted to do what they saw before them — and lipids were not included."


SIMONS

Nonetheless, analytical chemists continued to make technical progress. In 2003, the word "lipidomics" made its debut in the peer-reviewed literature. Three review articles in quick succession pointed to a trickle of papers demonstrating robust mass spectrometric measurement of lipids and argued that, like the growing field of proteomics, high-throughput lipid measurement was poised to take off.

The legacy of LIPID MAPS

Before lipidomics even entered the lexicon, Edward Dennis of the University of California, San Diego, says he made a case to the National Institutes of Health that the field would be a great investment. The NIH budget had doubled in the previous five years. In 2000, the National Institute for General Medical Sciences introduced a new funding mechanism called the glue grant intended to propel interdisciplinary science forward with up to 10 years of funding for projects with multiple principal investigators. Dennis thought he had just the project.

"Developing an integrated metabolomics system capable of characterizing the global changes in lipid metabolites is a daunting task, but one that is important to undertake," Dennis wrote in his initial application.

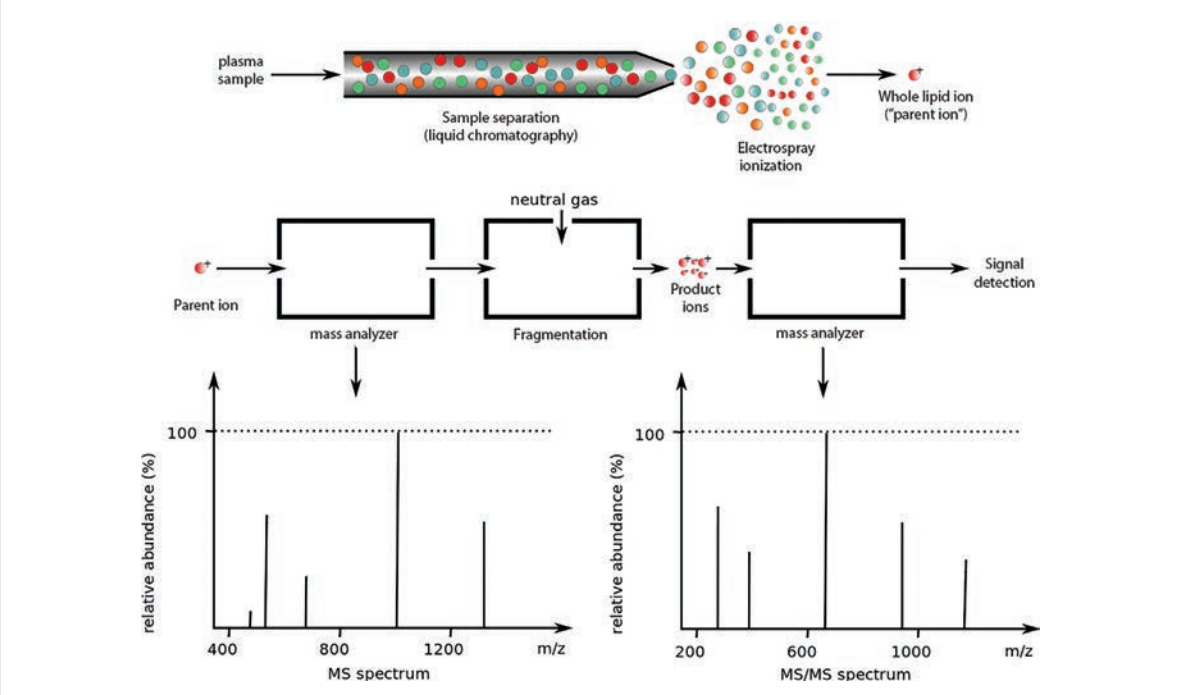

DENNIS

The Lipid Metabolites and Pathways Strategy, or LIPID MAPS for short, got the grant. Its 12 PIs set out to develop rigorous laboratory and bioinformatics techniques for lipidomics with approximately $73 million from the NIGMS over 10 years.

By current estimates, human blood contains millimoles of hydrophobic cholesterol but only tenths of a nanomole of the hydrophilic signaling lipids called eicosanoids: a 10 million-fold difference in molar abundance. With such chemical diversity and varied abundance, lipid measurement requires multiple approaches.


WIKIPEDIA/THETWEAKER

Many lipidomics studies use electrospray ionization. This photo shows the ionization source in a mass spectrometer. The sample is ionized while it's sprayed from the needle, and gaseous ions then enter the mass detector to the right.



ADAPTED FROM WORK BY PHILIPE HUPE/WIKIMEDIA

Lipidomics experiments often begin by separating a sample through liquid chromatography (top). After ionization, the sample is passed through one or more mass analyzers (middle). The spectrum from the first detector (bottom left) represents ionized whole molecules. In tandem mass spectrometry, some parent ions are broken down and further examined by a second mass analyzer, which produces the second spectrum (bottom right). For more on how product ions are formed, see page 33.

How does mass spectrometry work?

Mass spectrometry is the process of ionizing a sample, separating the ions by mass and charge, detecting the ions, and recording a spectrum.

The output can be viewed as a series of mass-to-charge ratios (also called features or m/z peaks) whose height represents their abundance. By determining the elemental composition of a peak, researchers can determine which molecule it might represent — a process called annotation.

Many experimental approaches elaborate on the core ionization-separation-detection workflow. For example, using tandem mass spectrometry, a researcher can choose a peak from the first spectrum and break it down further into constituent ions. It's also possible to separate a sample into fractions before ionizing it.

Whether to fractionate samples is a long-running debate in the field. In shotgun lipidomics, complex lipid extracts are injected straight into the mass spectrometer. By contrast, liquid chromatography separates lipids according to their size or chemical properties before ionizing them.

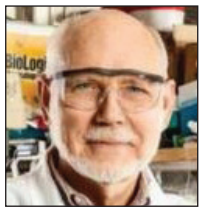
Analysis of a spectrum can be complicated by factors such as the existence of lipid isomers (compounds with the same composition but different structures) and ionization suppression, which happens when two analytes affect each other's chances of picking up a charge. Because differences in technical approaches affect ionization and other variables, instrumentation and workflow may introduce biases into different labs' estimates of lipid ratios.

To estimate the amount of a lipid in a sample accurately, a researcher must compare its abundance to a known concentration of a standard — usually a lipid synthesized either with a heavy isotope of carbon or hydrogen or with an unnatural odd-numbered acyl chain. The standard needs to have chemical properties as close as possible to the lipid of interest so it will behave roughly the same way in the mass spectrometer. According to Al Merrill of Georgia Tech, choosing standards can be difficult for researchers interested in a lot of lipids at once.

"There are so many lipids that they occupy a very large fraction of the possible masses that would be detected by mass spectrometry," Merrill said. "Once you add these new materials, there's a potential that they occupy the space of something else that you might need to analyze."

The LIPID MAPS consortium chose core labs specializing in different lipid classes to lead methods development for each class. Al Merrill of Georgia Tech led the sphingolipid core.

"From the very beginning, the hope was that the field was going to explode," Merrill said. "Our goal was to do anything we could to accelerate that process, and seeing it happen would be the biggest reward for our efforts."



MERRILL

The consortium developed a widely used system for classifying lipid types, referring to them by standard names and drawing them consistently. The American labs worked with leading Japanese and European lipid biochemists to reach consensus on the system before publishing it. According to Dennis, this collaboration helped to clarify later conversations.

"Everyone in the world has accepted our nomenclature, our classification and our structure drawing program," Dennis said. "There is no conference that argues about nomenclature … it's just accepted by the worldwide community."

LIPID MAPS labs used identical instruments to determine structures and quantities of thousands of lipid species and built a website where researchers could search for features in mass spectra and match them to known structures.

After the project's funding expired in 2013, its large-scale collaborative projects came to a halt, but most of its researchers kept working in lipidomics.

Shevchenko, who was trained in proteomics and has worked with lipids for about 20 years, believes LIPID MAPS settled on instruments and approaches a little too early in the process of global methods development.

"With all my respect to colleagues, in 2006 the field was in its infancy," he said. "Just being able to see lipids and quantify (them) … was a big step forward. People would look at this with big respect. But that doesn't mean the right thing to do was to standardize the approaches. Because there were no approaches to standardize. Right? There's no way around this — just to wait and let the field develop."

"When do you standardize? I think he makes a good point," Dennis said in reply. "(But) I don't think LIPID MAPS attempted to set the standard protocol or ever said it did. LIPID MAPS developed the best damn protocol it could, used that approach to measure plasma, and published it."

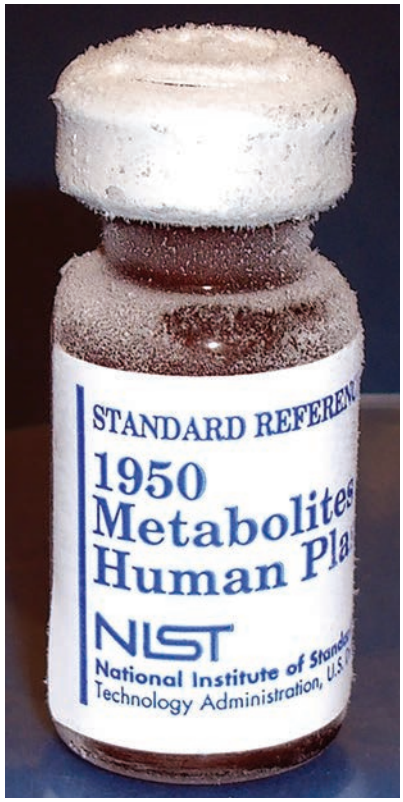
Quantifying a lipidome

A LIPID MAPS capstone project was to quantitate as many lipid species as possible in human plasma, using the technologies the consortium had developed. They chose a plasma sample supplied by the National Institute of Standards and Technology, or NIST, pooled from 100 volunteers, middle-aged men and women whose ethnicity matched the average U.S. population.

NIST develops many such reference materials for calibration of clinical assays. This particular sample, called Standard Reference Material 1950, came with certified measurements of cholesterol, triglycerides, free fatty acids, and several steroid hormones and lipid vitamins. Otherwise, the lipid content was largely unknown.

Each LIPID MAPS core laboratory used the quantitative techniques that it had optimized for its class of interest to extract lipids from a vial of SRM 1950, measure them and report back. The consortium measured just shy of 600 lipid species in six classes and published its findings in the Journal of Lipid Research.

In 2011, the year after the LIPID MAPS study was published, analytical



NATIONAL INSTITUTE FOR STANDARDS AND TECHNOLOGY

Standard Reference Material 1950, a pooled sample of plasma from 100 volunteers, has been the subject of numerous lipidomics studies.

chemist John Bowden took a job at NIST. With the field of lipidomics expanding, he saw the post as an opportunity to contribute to standardization.


BOWDEN

"I tried to spread the message at NIST that we needed to help standardize and harmonize the community in this type of measurement," said Bowden, now a professor at the University of Florida. "At the time, it was not a community-wide priority."

Bowden and his team launched a comparison exercise in 2014, using SRM 1950 to determine the variability in lipid identification and quantitation among 30 labs. Bowden told ASBMB Today in 2017, "We (asked): 'How much variability exists in the community right now, with all the different methodologies and philosophies for measuring lipids?'"


ULMER

Each lab measured lipids in triplicate according to its usual protocols, reporting concentration estimates for anywhere from 100 to 1,500 species. Candice Ulmer, a postdoc on Bowden's team, said the guidelines were intentionally vague. "We gave them free rein (in order) to see, if there's no guidance provided, how do they go about assigning concentrations?"

After anonymizing individual labs' measurements, Bowden's team calculated a consensus mean, a median of the average concentration measured at multiple labs, for each lipid species. The median of means was more robust than a weighted average to outlier values, of which there were several. Ulmer said, "Our idea with the consensus mean values was to provide concentrations that are robust and independent of the instrumentation … and the type of data processing tools you use."

The disagreement among labs was considerable. "The problem was that (NIST) wanted to be neutral," said Kai Simons, the former Max Planck Institute director who now runs Lipotype, a company that participated in the study. "They didn't want to provoke a critical discussion, which would have been quite devastating."

Reception of the study, published in the Journal of Lipid Research, was as mixed as the laboratories' estimations of lipid concentration.

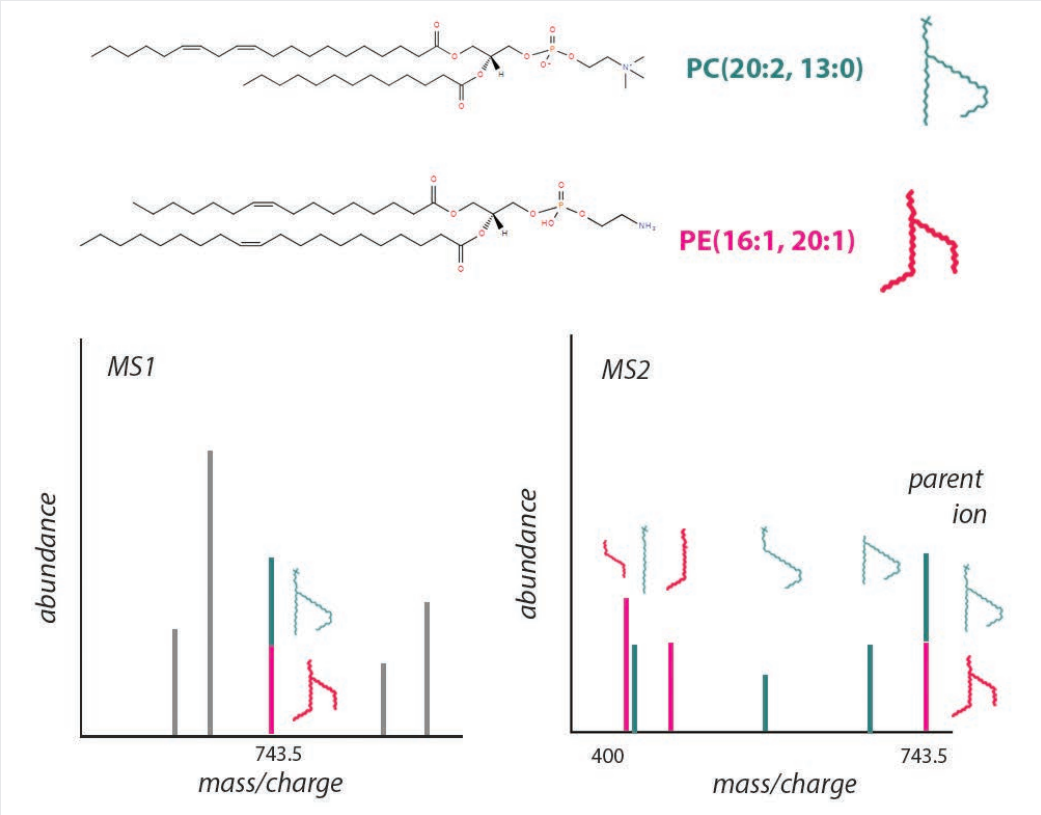
Oliver Fiehn, a metabolomics expert at the University of California, Davis, who participated, said that when measurements are technically challenging and protocols differ, expecting perfect concordance is unrealistic. "People always think that we should all get the same results … (but) every single ring trial shows that there's always a distribution of values."

Fiehn called the study a success. "Is the glass half full or half empty? If (others) say it's half empty, I'd say its half full."

Xianlin Han, who wrote one of the 2003 lipidomics review articles, called the study a failure. "It turned out the result could be a threefold difference," said Han, of the University of Texas Health Sciences Center at San Antonio. "So what do the data mean? Later on, if I want to study lipidomics in plasma, I can report any data, refer to this paper, and say, 'Hey, my data are within this range.' That's a disaster. The outcome would be very different if the organizers had spiked a few standards into the NIST plasma sample as controls."


HAN

For Bowden, establishing the lack of agreement on how to conduct and quantify lipidomics experiments was



LAUREL OLDACH

This schematic, based on information from LIPID MAPS, shows one way structural isomers can introduce challenges in mass spectrometry–based lipidomics. The phosphatidylcholine (top) and phosphatidylethanolamine (middle) molecules shown have the same chemical formula. After soft ionization, both have the same mass-to-charge ratio and appear as one peak in an MS1 spectrum (lower left), even though they may behave differently as part of a membrane. More destructive ionization enables researchers to differentiate the two by their fragmentation in MS2 (lower right).

Levels of lipid identification

According to analytical chemist John Bowden, who led a NIST lipidomics study, "Every level of identification of a lipid requires some specific piece of data to support it."

When analyzing a mass spectrometry experiment, researchers interpret a feature, usually a spectrum peak, to annotate it or label its identity. There is a limit to how much structural information a researcher can determine from a parent ion: the lipid's class or head group, the combined length of its fatty acyls, and how many carbon-carbon double bonds or rings are in the structure. It's impossible to tell how fatty acyl groups are oriented on the head group, and where in a fatty acyl chain double bonds occur, without breaking the molecule into smaller ions to be analyzed with tandem mass spectrometry.

Moreover, because distinct molecular species can have the same mass-to-charge ratio, researchers must reckon with overlapping peaks. For example, the two phospholipids pictured are structural isomers. Though they consist of different head groups with four distinct acyl chains and have distinct chemical properties, they have the same elemental makeup. To tell them apart, researchers would need to break down a parent ion into smaller parts.

LIPID MAPS and the Lipidomics Standards Initiative seek to promote annotation that specifies the degree of certainty about a lipid's exact molecular structure according to how an experiment was carried out.

precisely the point. "At the time, we really did not know what the exact issues were in lipid measurement across the community," he said.

The study helped participating laboratories spot problems in their workflows and got the community talking about measurement quality, Bowden said. "There was a thinking that everybody was doing the best we could, given the resources … but if we put enough energy into certain areas, then maybe we can actually make better lipidomics measurements."

Identifying features

What would it take to improve lipidomics measurement?

Valerie O'Donnell, a professor at Cardiff University, specializes in analysis of phospholipids and their signaling byproducts. "Up until 12 or 15 years ago, lipid research was a relatively small field," O'Donnell said. "Everyone knew each other; it's always been an extremely collaborative but relatively small field of specialists who worked on individual lipid classes."


O'DONNELL

Everything changed around 2005, O'Donnell said, when new benchtop mass spectrometers made the technology accessible to more labs. "From then on, you didn't have to be a physicist and understand how to take apart and put back together a mass spectrometer to use it to measure lipids."

Instrument resolution also improved, making it possible to distinguish lipid species more accurately. According to Bowden, "We're uncovering all kinds of new lipids, and we're able to separate out lipids you couldn't separate out before."

These factors have drawn more researchers to look into the lipidome. But that growth brings challenges. "With lots of new people coming into the field," O'Donnell said, "there are emerging issues around how you identify lipids."

The major challenge is that any one feature in an MS spectrum could represent a large number of molecular species. To be sure of a lipid's structure, a researcher needs several dimensions of information, far more than a single MS run provides. (See box, page 33: Levels of lipid identification.)

Bowden and his NIST colleagues wrote a primer on reporting one's degree of certainty in identifying a peak in a special issue of the journal Biochimica et Biophysica Acta in 2017. In the same issue, another paper explicitly called for standardization of lipidomics data. Its authors include two scientists Bowden met during the NIST study: Kim Ekroos, an independent lipidomics consultant in Finland, and Gerhard Liebisch of the University of Regensburg in Germany.


EKROOS

Liebisch and Ekroos had come across a troubling case of peak misidentification in a 2015 paper in the journal Clinical Chemistry. Using standard metabolomics, the authors reported finding six lipids that were higher in healthy volunteers' serum than in samples from people with Type 2 diabetes.


LIEBISCH

"We question the identities of the reported biomarkers and the lack of validation thereof," Liebisch, Ekroos and colleagues wrote in a reply to the editors. They pointed out that the authors had reported more information about acyl chain length and position for some lipid species than their data could reasonably provide. Moreover, some species

were at unreasonable masses, suggesting they might be wrongly identified.

The authors of the original study protested that they'd given adequate information under guidelines from the Metabolomics Society's standardization initiative. There was, they conceded, an error in one of their annotations: a lipid species with one double bond was described incorrectly as having none, accounting for the wrong mass.

Liebisch said the exchange demonstrated that "lipids should be considered a special case of metabolites, where simple matching of (spectral) features is not sufficient."

It also motivated the pair to think about problems in the field. Ekroos said, "We are starting to see quite a few errors in published data already … that we want to somehow try to clear up."

Michael Wakelam, director of the Babraham Institute associated with Cambridge University, has been doing lipidomics studies for more than 25 years and agrees with Ekroos. "We are seeing more and more of this," Wakelam said of error-prone papers. "I think it's actually a byproduct of the success of the field."

WAKELAM

LIPID MAPS returns

By 2016, it was clear to many experts that lipidomics researchers needed help with standardization and reproducibility. Today, three overlapping groups are working on the problem. One has begun work to revitalize LIPID MAPS as a resource for researchers in the field, a second has focused on technical problems in plasma lipidomics, and a third wants to reform publishing and reporting standards for all lipidomics data.

The first team, led by O'Donnell and Wakelam in the U.K., has spearheaded the continuation of LIPID MAPS. When NIH funding ended in 2013, lead bioinformatician Shankar Subramaniam, a University of California, San Diego, professor, landed a small bridge grant to keep the website online.

The LIPID MAPS website, redesigned in 2018, curates MS data on lipids and collects information about methods and tools.

"They were at the point where they were thinking that it may have to get shut down," O'Donnell said. "I felt that was really bad, because we use this (tool) all the time, and we couldn't do our work without it."

Wakelam, Dennis, Subramaniam and O'Donnell applied to the U.K.-based Wellcome Trust to revitalize the database. The project migrated to the U.K., and curation of lipid species and MS data resumed on a redesigned website.

"LIPID MAPS wants to play a big role in signposting our lipid biochemistry colleagues to what's out there for big data analysis," O'Donnell said. "Previously, it was a research project … but at the moment it's really (about) the database and having it available as a global online free open access resource."

MATEJ ORESIC

This group photo was taken at a 2017 forum on harmonizing plasma lipidomics held in Singapore. After they noticed confusion in the field, Markus Wenk (third from right) and Andrej Shevchenko (fifth from left) invited fellow lipidomics researchers to meet and hash out a path toward reference values for lipids in the blood.

The Singapore workshop

At the 2016 Lipidomics Forum, where Wenk and Shevchenko shared impressions over that cup of coffee, the two also gave the opening and closing keynotes; they discussed finding, respectively, quantitative variations within a healthy individual's plasma lipidome and further variability among individuals according to age, ethnicity, sex and prescription medications.

Both thought groundwork was needed before biomarker discovery studies went forward. "Rather than study disease or complications, one needs to first actually get an idea of healthy baselines," Wenk said.

That means finding out which lipids are stable in healthy adults and which ones vary by the hour — and determining how labs might reliably measure each of those. Wenk and Shevchenko knew that if the goal was consensus on these points, they needed other scientists to buy in.

"If you publish a small article and say, 'Hey guys, we are in a position to teach you how to do lipidomics,' the field will not respond," Shevchenko said. "We have to be united."

Wenk agreed. "I don't think you can tell scientists what they have to do. You need consensus. We've been very careful about this."

Shevchenko and Wenk reached out to established PIs working on plasma lipidomics. Dennis, Ekroos, Han, Liebisch, Wakelam and 13 others responded, and in April 2017, the two hosted a two-day meeting in Singapore. Members of the group specialized in various lipid chemistries using a variety of instruments and platforms. Their mission: figure out how to harmonize studies of the plasma lipidome.

"Of course, we all do it a little differently," Dennis said. "Nobody said, 'Oh, you do it right, Mr. X, we'll all just use your protocol,' because everybody's invested in different approaches."

The workshop hadn't been going on long when Tze Ping Loh spoke up. Loh, a consulting physician in the laboratory medicine department

at Singapore's National University Hospital, was a rare bird among the panelists, a clinician in a room full of basic scientists.

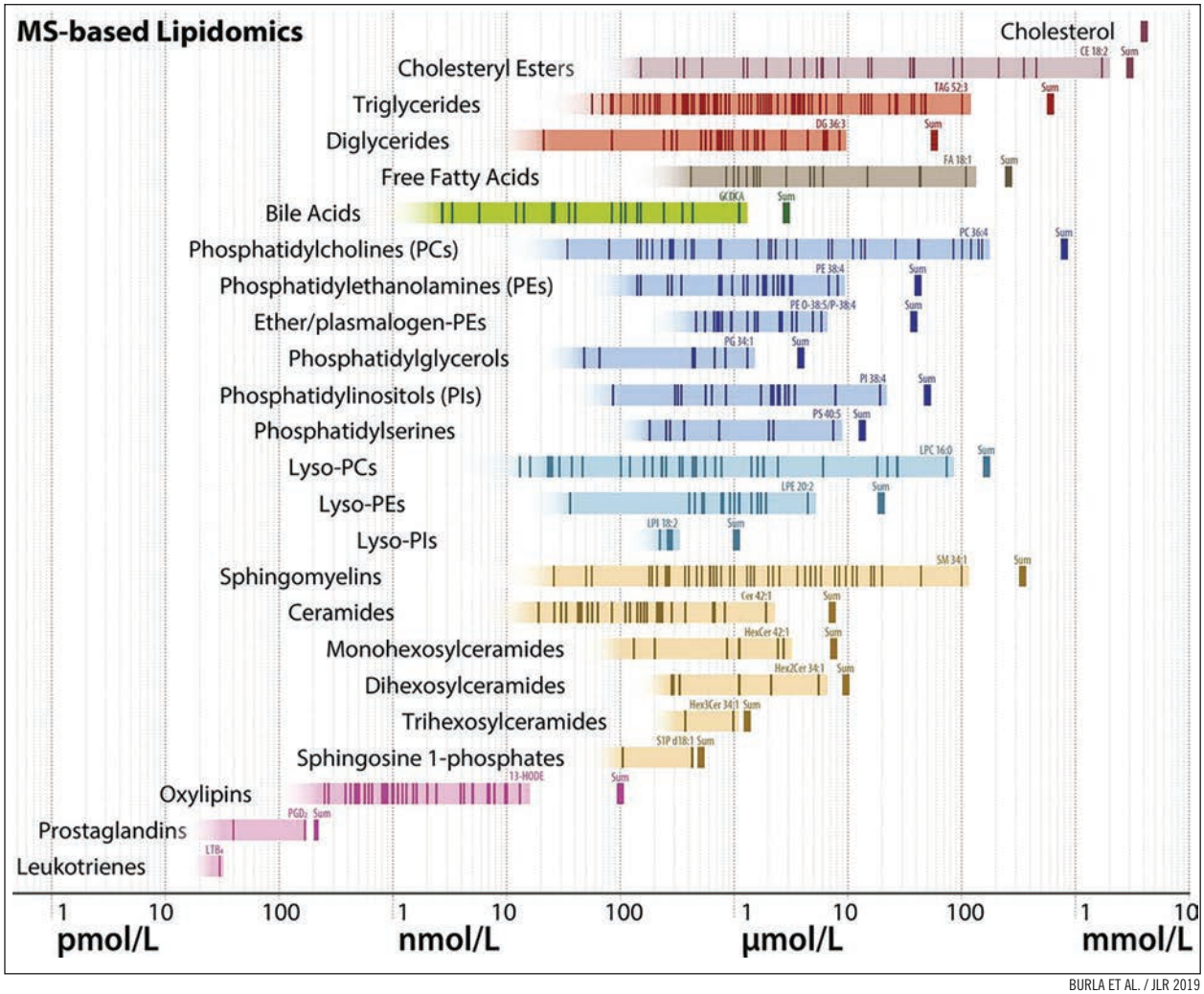
"We're all doing analytical chemistry," Shevchenko said. "To get grants, we used to call (our work) biomarker or diagnostic discovery. But we don't do diagnosis. And then Tze Ping, the guy who actually does diagnosis … explained to us what a diagnostic is actually and how far we are from (that). He really hit us in the head."

Loh explained the practice in laboratory medicine of establishing reference intervals, the range of concentrations of a molecule measured in a chosen population when all labs agree to measure in the same way.


LOH

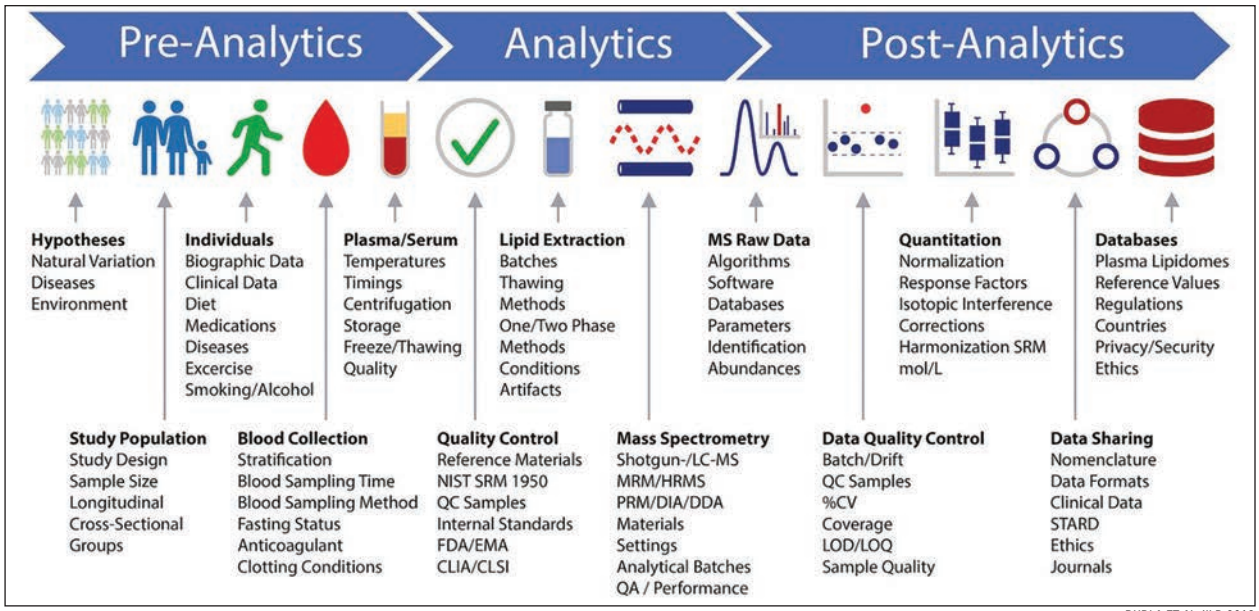
To define a reference interval is to come to a consensus on what an analyte is, how to measure it accurately and reproducibly, in which populations it should be measured, and the statistical procedures to calculate it. (See box on page 39: Talking about reference intervals.)

The cholesterol test is a good example. In its report, a clinical lab notes how the patient's total cholesterol levels compare to recommended targets. That gives a robust health



BURLA ET AL. / JLR 2019

A schematic diagram shows estimated molar quantities of various lipid classes in human plasma, measured by mass spectrometry. Each vertical line represents an individual lipid species, and horizontal bands of color represent range estimates. Lipidomics researchers are trying to narrow down these estimates and make sure they match ranges known from clinical chemistry.



BURLA ET AL./JLR 2019

A lipidomics workflow schematic developed by participants at the Singapore lipidomics forum shows many points where a researcher's decisions can introduce variability in a lipidomics experiment — even before a sample is loaded into the mass spectrometer. For example, many labs store and handle blood samples on ice to slow biochemical reactions and delay lipid oxidation. But that chilling may activate platelets, which then release bioactive lipids. Research groups interested in measuring those particular lipids reproducibly may handle samples at room temperature, perplexing colleagues from other labs.

indicator that any doctor or nurse can interpret.

Loh's interjection helped move the conversation from debate over the merits of different technical approaches to comparing measurements among these platforms, Shevchenko said. He attributes the productive conversation in part to Wenk.

"This is Markus' talent. He's a guy who has a vision to organize people without insulting them. So he got us together, with (our) different platforms, different approaches, different backgrounds," Shevchenko said.

Galvanized by that first day, the workshop's attendees started to lay out a plan for harmonization, beginning with broad-strokes guidelines for lipidomics research.

Balancing biases and adjudicating conflicts among research camps can derail attempts to standardize. The article in the Journal of Lipid Research that came out of the Singapore working group took no stance on most technical questions. Instead, the authors stressed the importance of using internal standards, determining molar concentrations of each analyte and working toward evidence-based agreements on other technicalities.

At a follow-up meeting in November, researchers were invited to help select the lipid species most amenable to clinical development. "When you come to all this granularity — they're all scientists, right?" Wenk said. "By paring it to a single task, my hope is that it will be easier to come to a general best solution to that particular problem."

The group settled on ceramides and bile acids because these two classes of molecule have well-defined roles in disease and are thought to be abundant, durable and easy to isolate. The research community can now work toward defining a method for measuring these specific lipids and determining their range in healthy individuals, Wenk said.

The standards initiative

Ekroos and Liebisch, the researchers who took biomarker hunters to task in Clinical Chemistry, participated in the Singapore meeting but found it too narrow in scope. In early

2018, they launched the Lipidomics Standards Initiative, or LSI. The two said they want to focus on getting standards for reporting methods and identifying lipids accepted by researchers in the field and demanded by journals.

"That's an initiative that is more overarching than ours, that aims at standardization in principle," Wenk said. "It's a much more heroic effort."

Modeling their project on efforts in metabolomics and proteomics, Liebisch and Ekroos started a website with guidelines for the design and analysis of quantitative lipidomics experiments, which Liebisch described as a summary of current best practices. They also provided space for discussion about the protocols.

"We can't really write these rules in stone today," Ekroos said. "They have to be pretty open. But they should at least guide people to do more what is correct."

Perceiving a common mission, the LSI quickly partnered with LIPID MAPS. The groups held a joint workshop at the European Lipidomics Meeting in October to introduce both projects.

"I haven't seen so much debate and argument, constructive discussion, in a long time at a meeting," O'Donnell said. "People got really animated about different methods and different approaches."

Participants debated "everything from the start of the acquisition process right through to the data analysis … and reporting guidelines," O'Donnell said.

A carousel of meetings

So what happens next in lipidomics?

Other fields of analytical chemistry, such as proteomics and metabolomics, have formed standing committees, and the LSI seems poised to follow that model. But Wenk believes forming a society for the harmonization of lipidomics, while helpful, could be an added burden for researchers.

"One needs to leverage what's there," he said. "One of those things is a roster, or a carousel as I call it, of small to medium-sized conferences."

Wenk envisions a decentralized approach to standardizing the field, where researchers come together at conferences, hash out clearly defined issues as they did in Singapore and then send a report on to the next conference.

Conference organizers including Keystone, Gordon and the American Society for Mass Spectrometry have added lipidomics meetings to their programming for this year and 2020. These come in addition to existing periodic meetings in Europe, Australia and Japan. The LSI and LIPID MAPS plan to host workshops at the Keystone meeting.

"I think there's a pocket of people that are spearheading this," Bowden said. "But I think the ultimate goal is that everybody gets involved at some point, and it's not just a couple of people."

However the field organizes its next steps, Shevchenko said, he sees enthusiastic support from the community. "I love the words 'critical mass,'" he said. "We've reached a critical mass of knowledge, of experience and of interest in transparent and confident lipidomics."

Scientists who contributed expertise but were not quoted in this story include Shankar Subramaniam, Eoin Fahy, Erin Baker, Bo Burla and Tze Ping Loh.

Laurel Oldach (loldach@asbmb.org) is a science writer for the ASBMB. Follow her on Twitter @LaurelOld.

Talking about reference intervals

A conversation between Andrej Shevchenko and his mother, a physician, illuminates the gap between discovery lipidomics and what might be useful in the clinic. This is Shevchenko's account of their exchange, lightly edited for clarity:

My mom is a medical doctor. She's had an M.D. for like 45 years as a practicing physician in a humble neighborhood, no science. She knows that I've been doing this plasma analysis in a clinical context, and she once said to me, "You know what, Andrej? I think you do rubbish."

I was like, "Why?"

She said, "When a clinical analysis comes to my desk — talk about cholesterol, for example, or blood pressure — I have a table. I know the norm and I know the variation … Do you have these sort of ranges for normal values in patients?"

I said, "No. We are kind of in the process."

She said, "You guys are cheating people. You're saying you are aiming at diagnostics, but how can you talk about diagnosis if you don't have normal values?"

I was stumped. I said, "Yeah, but we are finding biomarkers …"

"Forget about this, it is all crap. Before you arrive at a reference value, what the normal parameter is, it's all noise."

And she is right. That's what I think.

Meet Karin Musier–Forsyth

At Ohio State, the JBC associate editor and tRNA trailblazer uses ASBMB resources as teaching tools for grad students

By John Arnst

The pseudolife of a retrovirus is complicated.

Once it has infiltrated a host cell, the virus — here, HIV — must make use of a reverse transcriptase primer, lysine tRNA, to help transcribe its viral RNA into DNA that can be sent to a host cell's nucleus. There, the new viral DNA will be integrated into host DNA and hijack the mechanisms of DNA transcription to pump out fresh copies of viral mRNA. These will either be used as new genomes for viral cells or translated at host ribosomes to create viral proteins — the collective raw ingredients, plus some stolen shielding from the host cell's surface, for new viral particles.

In her lab at Ohio State University, Karin Musier–Forsyth probes the editing mechanisms of aminoacyl-tRNA synthetases, enzymes that attach amino acids onto transfer RNAs during synthesis of HIV proteins at ribosomes.

Originally a Floridian, Musier–Forsyth grew up in St. Petersburg, where she attended Eckerd College and received a bachelor's degree in chemistry in 1984. She did her graduate work at Cornell University, earning a Ph.D. in 1989. After an American Cancer Society postdoctoral fellowship at the Massachusetts Institute of Technology from 1989 to 1992, she started as an assistant professor at the University of Minnesota, where she was named Merck professor of chemistry in 2003 and distinguished McKnight university professor in 2006 and received the Camille-Dreyfus Teacher Scholar Award in 1996.

COURTESY OF KARIN MUSIER–FORSYTH

A proud alumna of Eckerd College, Musier–Forsyth is a member of the liberal arts school's National Advisory Council, and visits the St. Petersburg, Florida campus at least once a year.

Musier–Forsyth joined Ohio State University as an Ohio Eminent Scholar in 2007. She has been a member of the editorial board of the **Journal of Biological Chemistry** since 2012 and became an associate editor in January 2018. She spoke with John Arnst, ASBMB Today's science writer, about her work. The interview has been edited for clarity and length.

What is your group focused on?

We're interested in two broad areas, one being fidelity mechanisms in protein translation. Specifically, we're focused there on quality control (or editing) mechanisms by aminoacyl-tRNA synthetases. We're also interested in single-domain trans-editing proteins.

A long time ago, my interest in tRNA got me interested in retroviruses, because retroviruses use tRNAs to prime reverse transcription. For example, HIV-1 specifically packages a host cell tRNA group, that's lysine-specific tRNAs, into the virus particle for that purpose. We're interested in the mechanisms of how the tRNAs are selectively packaged and also how the genomic RNA is selectively packaged into the virus particle.

A big question in the field is how and why exactly two copies of the genomic RNA are packaged into every retroviral particle, when Gag assembly of an immature virus particle can happen even in the absence of genomic RNA. (Author's note: The Gag polyprotein coordinates the assembly, budding and maturation of HIV virions.) But if genomic RNA is expressed in the cell, it gets packaged, and exactly two copies get packaged. So how does that selective packaging work?

We've recently probed the conformational dynamics of the 5' untranslated region of the genome, which is a critical region for packaging of the RNA. We can see different factors, both cellular and viral factors, that shift the conformational dynamics, and we think these conformational shifts are important for the packaging process. I should mention our collaboration with James Munro at Tufts University. We're using single-molecule methods to look at the dynamics of these conformational

COURTESY OF KARIN MUSIER–FORSYTH

Musier–Forsyth's lab, currently more than 20 members strong, boasts research associates, postdoctoral fellows, lab technicians and undergraduate and graduate students from a variety of backgrounds. This group photo of the lab was taken in 2017.

changes. We've been collaborating for about two years, and we're just about to publish our first paper on this.

All these interactions and packaging processes involve protein-RNA interactions, so that's another way I sometimes describe my lab: we're interested in protein-RNA interactions.

What was your academic background and training?

When I was an undergraduate studying chemistry, I became really interested in biochemistry, so I did my Ph.D. at Cornell in biophysical chemistry, and then I did a postdoc at MIT with Paul Schimmel studying aminoacyl-tRNA synthetases and tRNA recognition. That's where I really got interested in this family of enzymes that specifically charge tRNAS, and I've been working in that area ever since.

We also have a link between aminoacyl-tRNA synthetases and HIV — they're much more than just housekeeping proteins, and their other activities are a big, emerging area. I just edited a thematic series in JBC that covers some of these noncanonical functions of aminoacyl-tRNA synthetases and their role in human disease.

Did anything occur in a milestone sort of way that made you choose science as a career?

I was interested in math and science and was deciding between medical school and graduate school — I think that's a common decision that students that are interested in those areas make. Some of my undergraduate research experiences pushed me in the direction of graduate school and a career in research. Specifically, one summer I was in a Research Experience for Undergraduates program at Georgia Tech and got a real flavor for what graduate school was like. I had two co-authored papers as a result of that, and I realized at that point that I was much more excited about research than medical school.

As a postdoc, you have to decide if you're going to go into industry or academics or some alternative career. I thought I'd give academics a shot, because I really like working with students and teaching and training students in the lab.

How have your students responded to using ASBMB resources and JBC reviews in the classroom?

For the first time, together with two colleagues at OSU, I'm teaching a writing class for graduate students. It's a small class, and I found the ASBMB and JBC resources really useful, especially Kaoru Sakabe's Due

NICHOLAS FORSYTH

Musier–Forsyth and her husband, Craig Forsyth, also a biochemist at Ohio State University, have been skiing together for 34 years. Their son took this photo.

Diligence columns. My class is a mix of first-year through fifth-year graduate students, so they have different levels of what they know and what they've experienced, but I found that these resources are very useful, especially for the early students, if they haven't published yet.

I teach my students how to write a review, because faculty often ask their students to help them write one, and also I think it's useful to have them experience that process. The JBC minireviews were perfect for that, because they're short, and the students could pick one that interests them. They've done a great job with that.

I also picked some JBC reviews; we went through parts of them and talked about how to write a good introduction. We looked at the figures, because now we have a wonderful figure consultant/artist at JBC. The reviews are perfect for second-year students, because they've had a little experience but they're just getting into the research.

KARIN MUSIER–FORSYTH

Musier–Forsyth's dog is a four-year-old Samoyed named Lola.

How is your new role with JBC so far?

I really like being an associate editor of a society-based journal that's run by scientists from the editor-in-chief down to the AEs and the editorial board members. Because it's run by scientists, I think the review process is really fair; the people that are reviewing the papers are also going to be wanting to publish someday.

I've had a great experience both as a JBC author and as a reviewer over the years, so now I'm excited to serve as an AE and help shape the future of scientific publishing at a journal that's so well-respected and has been around for so long.

What do you do outside the lab? Any advice for balancing life in the lab with life outside of it?

I think it's really important to have life outside of work. I enjoy jogging and swimming in the summer. I have a 15-year-old son; and my husband, my son and I go on vacations a few times a year. We like hiking, camping, skiing, things that get us outdoors and active. We just came back from spring break skiing in Utah. It was great — while we were there, it snowed three feet.

We also have a Siberian sled dog, a Samoyed named Lola. We didn't take her skiing, but she would have loved it. She definitely loves when it's cold outside here. I also walk/jog her every day, so that also keeps me active.

Do you have any advice for young scientists?

You should make sure that your work is something you're really passionate about. Then you'll never be bored or unhappy. It's important to have hobbies and do things with family and friends, but a significant portion of your day is going to be spent at work. So if you love and are passionate about what you do, then you're going to be happier overall.

I find the work that I do at the university and at JBC to be really satisfying. It's great to be able to teach and train graduate students and then see my graduate students go on and get postdocs and jobs and start training students of their own.

John Arnst (jarnst@asbmb.org) is an ASBMB Today science writer. Follow him on Twitter @ arnstjohn.

ASBMB webinar offers financial advice

By Jeff Pines

Roth IRA. Credit cards. Student loans. Car loans. Checking account. Personal finance can be confusing and a source of stress. Getting into good habits when you're in your 20s or 30s can reduce the likelihood of long-term problems. To help instill those habits, the American Society for Biochemistry and Molecular Biology recently offered a financial advice webinar for its members.

Eric Goodbar, managing director at BNY Mellon Wealth Management, delivered the talk. Here are some highlights.

"You can collapse the hype and jargon to a few clear concepts you can use throughout your career," Goodbar said.

The starting point is the checking account. "Your checking account is the root of the financial tree of life. It's like an organic footprint. It's essential to balance your checkbook monthly," Goodbar said. He explained that balancing your checkbook will enable you to track what you earn; what you pay out for rent, bills and other expenses; and what you keep.

"Data capture of your income and spending pattern leads to a viable budget plan," he said.

A key component of a financial plan is putting aside money for an emergency fund. Goodbar warned that anyone might be out of work for a time and it's essential to have enough money for three to six months of living expenses in a savings account. Consider how much you spend on housing, food, utilities and other monthly expenses. "Plan for the worst. Hope for the best," he said.

Once you have a budget plan and an emergency fund, consider investing for retirement. For investors in their 20s or 30s, the goal should be compounding interest, not maximizing performance, Goodbar said.

"Investing for the future is a bit like forecasting the outcome of metabolic pathways," he said.

There is a plethora of rules out there on how much to invest. Goodbar suggests the 4 percent rule. Take your salary and multiply it by 4 percent. For example, let's say you earn $50,000 a year. Multiply by 4 percent and you get $2,000 a year. Readjust the amount when you get pay raises. This percentage is for people who plan to live modestly, he said. Retirees who plan to travel extensively will need to save more.

Goodbar made a couple of reading recommendations:

- "The Infographic Guide to Personal Finance: A Visual Reference for Everything You Need to Know" by Michele Cagan and Elisabeth Lariviere
- "The Infographic Guide to Personal Finance: A Visual Reference for Everything You Need to Know" by Tycho Press

Join us on Twitter

The ASBMB will host a Twitter chat on finances for scientists in May. Follow us on Twitter @asbmb for date and time information, mark your calendar, and plan to join us with your questions and comments.

Jeff Pines (jpines5720@gmail.com) has worked as a journalist since 1994, covering corporations and investors for Bloomberg News, Bridge News and Dow Jones and writing about a variety of topics for service and housing providers for the elderly. He served in the U.S. Air Force as a public affairs specialist.

What we learned

Graduates talk about the ASBMB's Advocacy Training Program

By Daniel Pham

A goal of the American Society for Biochemistry and Molecular Biology is to provide our members with opportunities and tools to become lifetime advocates for science. Our Advocacy Training Program, implemented last June, is doing just that. More than 20 ASBMB members have gone through this six-month externship, which provides hands-on training and advocacy experience, becoming community leaders in the process.

We invited participants in the first ATP cohort to share their experience and what they have learned through the program. Read their responses in the paragraphs below.

The greatest lesson I learned from being an ATP delegate was how to refine my advocacy strategy. I started the program well aware of the numerous challenges scientists face, but the ATP gave me an opportunity to critically think about how to realistically address those challenges. I learned that one of the most important aspects of advocacy is perfecting "the ask," a specific policy request made to a legislator regarding a topic. It must be realistic, strategic and ambitious. A good "ask" also requires the advocate to possess a certain level of prudence when considering who they are advocating to. These invaluable lessons have carried over into my personal life, where I have taken initiatives to advocate for graduate students at large and evoke institutional change.

Aria Byrd
Graduate student
University of Kentucky

During the ATP I co-founded the Health Sciences Student Advocacy Association at my university (@oneWSUHS). Our advocacy group focuses on innovating ways to amplify student voices on campus and beyond. STEM culture needs to change, and the fastest way to achieve change is to train scientists to advocate for change from within and teach them how to talk with legislators about change (and funding).

Shannon Kozlovich
Graduate student
Washington State University

The ASBMB ATP made me dedicate time to connect with my congressional representatives and work on issues I find important. In the three months since the ATP program ended, I have started multiple policy projects including a pathway analysis of recycling and analyzing the scientific backgrounds of members of Congress.

Sage Arbor
Faculty member
Marian University

Participating in the ATP took me to a new level in my involvement in science communication and advocacy. Through listening to speakers such as state representatives and science advocacy experts, engaging in hands-on advocacy, and learning about the fundamentals of policy, I was able to strengthen my skills to become an effective advocate. With the support and knowledge I gained from the ATP, I founded an ASBMB Student Chapter at my institution. Currently, I'm the president of the chapter I started, a member of ASBMB's engagement working group, and vice chair of the ASBMB ATP alumni working group.

Kelly McAleer
Undergraduate
College of New Jersey

Through the ATP, I was able to learn about effective advocacy strategies for all of science. We heard from experienced advocates as well as local representatives, which allowed us to construct effective advocacy messages. We were also able to hear from other scientists and cohort members with similar goals, which gave us fresh ideas and provided a support network for questions. I was able to sit down in offices and talk face-to-face with representatives at the local and federal levels to discuss topics such as STEM education and science funding. The ATP furthered empowered me to explore this passion for science communication and advocacy, and I am so grateful to have had the opportunity.

Bailey Weatherbee
Undergraduate student
University of Delaware

Being a part of the ATP opened up the world of science policy to me, since I had very limited experience with the field during graduate school. Through working with the ATP, I've had meaningful interactions with elected officials as well as campaigns. I focused my efforts on working with my local congressional representatives to support legislation that combats sexual harassment in academic science — this is something I continue to work on. I also utilized the skills I obtained from the ATP to create a 2018 midterm science policy voter guide for my community, which is something I would have never thought to tackle before.

Christa Trexler
Postdoctoral fellow
University of California, San Diego

The ATP has granted me the tools I need to be a science advocate. Before this program, I knew little about how laws that fund scientific research actually work or how my actions could influence them. Now I understand how to set up and have effective meetings with my representatives and organize advocacy events in my own community. In the most recent government shutdown, for example, I was able to guide peers in my department in writing letters to our state representatives to make our voices heard. I would not have had the courage or knowledge to do any of this without the ATP.

Daniel Wilson
Graduate student
Carnegie Mellon University

Be an advocate

The third ATP training session begins in June. We are accepting applications through May 17. Learn more about the program and apply today at asbmb.org/advocacy/atp/.

Daniel Pham (dpham@asbmb.org) is the ASBMB's public affairs manager. Follow him on Twitter @dpham20.

Of mice and scientists

Following instructions and creativity in research training

By Daniel Bolon

When I was a 22-year-old graduate student, I was instructed to add dry ice to a cage containing a white lab mouse and to wait until the carbon dioxide released from the dry ice purged the oxygen from the cage air and the mouse died. I had been taught to follow instructions, and I did as I had been taught.

I asphyxiated two mice that day. I used an oven mitt to protect my hand from the cold of the dry ice pellets. I first weighed the pellets to ensure they would provide enough carbon dioxide to kill each mouse. Then I lifted the plastic lid on the top of the cage and poured the dry ice into a metal container with warm water in it. The warm water made the dry ice bubble rapidly and hastened the release of carbon dioxide gas from the pellets. I quickly closed the lid. Then I waited anxiously and uncomfortably. Within minutes, each mouse lay down and stopped moving. After a further minute, my mentor checked that they were dead. Later that day, I harvested organs and analyzed the levels of a few proteins, though the purpose and details of those experiments have long since departed from my memory. I do vividly remember how I felt as I sacrificed the mice. I felt a tightness in my stomach, as if I were about to vomit, and a frightening sense of power — imagine Voldemort with severe food poisoning.

COURTESY OF DAN BOLON

This photo of Daniel Bolon was taken when he was a graduate student, around the time he was first instructed to euthanize mice in the lab.

I also felt something that I did not appreciate at the time — comfort and trust in following instructions. Experienced scientists had developed these instructions, so it was okay to follow them. This trust and comfort were so strong that they overwhelmed my uncertainty about my actions. I did not consider the possibility of asking if what I was doing was okay or, even further, if it was sensible. I did not value my own concerns and ideas.

Why didn't I value my own ideas? It was a habit from my childhood, formed for good reason — it helped me to feel worthwhile. Following instructions made my parents happy, and it also made my teachers happy, which in turn gave me a sense of accomplishment and meaning and was a large factor in my decision to study science. Following instructions felt like eating ice cream on a warm day — it was satisfying and filled me with joy.

Some 20 years later, I am a professor who teaches and mentors students. I wonder about what and how

I should be teaching them. I wonder about the value of following instructions and how this can be both a critical part of science and at the same time an impediment to free thought, creativity and ethics. I am convinced that both instruction following and free thought have an important role in education. As a scientific community, we already are adept at teaching the value of following instructions.

I feel an urgent need to encourage free thinking in the early stages of science education. Devaluing personal concerns and ideas is common among graduate students in the sciences. As an educator, I seek opportunities for students to share their ideas and gain confidence and a sense of value in their perspectives.

I have tried many unsuccessful approaches. For example, in the midst of explaining a 20-step experiment, I would ask young scientists to consider why a step was important. This type of questioning drove me nuts when I was a student because it took all my energy to take in new instructions, leaving me unable to think freely or clearly. Asking such questions as a professor also drove me nuts. If a student struggled to answer, I would wonder if they felt like a failure. My attempts to foster free thinking felt inefficient or counterproductive.

In my search for more effective ways to foster confidence and creative thinking, I stumbled on ethics. During casual conversations with students and postdocs, I often would search for topics of mutual interest. What topics bring out broad interest from scientists independent of their status from student to leader? Ethical questions are the best answer I've found, and the conversations they engender can stimulate creativity and confidence. Discussions of ethical questions provide a proverbial roundtable where the perspectives of all scientists can carry equal weight: When is it appropriate to sacrifice animals in research? When is it acceptable to genetically engineer plants, animals and humans? Is it sensible for humans to design robots to kill? What human population control measures, if any, are appropriate? These discussions provide opportunities to practice free thinking and open communication while learning to value our own ideas and wisdom.

Is there a tradeoff between following instructions and creativity in research training? Maybe there is, and encouraging free thinking will come with a cost. My experience indicates that the cost is minor compared to the benefits.

So, when is it appropriate to sacrifice mice for science?

I cannot answer that question for you, but I hope my thoughts may help you to consider and value your own perspective and encourage others to do the same.

Daniel Bolon (dan.bolon@umassmed.edu) is a professor in the department of biochemistry and molecular pharmacology at the University of Massachusetts Medical School. His lab studies molecular evolution and seeks to understand how genotype contributes to phenotype. Follow him on Twitter @BolonDan.

HILL DAY!

COURTESY OF ELIZABETH HUNSAKER

Above: Elizabeth Hunsaker (left) and Callan Frye, grad students at Duke University and the Medical University of South Carolina, respectively, board the subway under the U.S. Capitol building with Michael Schaller, a professor at the University of West Virginia, during the ASBMB's Hill Day in March.

Upper right: Dorothy Shippen (left), a professor at Texas A&M University; Katherine Friedman, an associate professor and director of graduate studies at Vanderbilt University; and Daniel Bastardo Blanco, a graduate student at St. Jude Children's Research Hospital pose for a selfie in front of the U.S. Capitol dome.

Below: Jazmine Benjamin (left) and Alanna Condren, grad students at the University of Alabama at Birmingham and the University of Illinois at Chicago, respectively, ride the subway under the U.S. Capitol with Anita Corbett, a professor at Emory University.

COURTESY OF JAZMINE BENJAMIN

COURTESY OF DANIEL BASTARDO BLANCO

COURTESY OF DAN WILSON

COURTESY OF ALEXANDRA CHIRAKOS

Above, center photo: Dan Wilson (left), a graduate student at the University of Pittsburgh, meets with U.S. Rep. Michael Doyle, D-Pa., during the ASBMB's Hill Day in March. At right is Jeff Brodsky, professor of biological sciences at the University of Pittsburgh.

Above: Alexandra Chirakos, a graduate student at the University of Notre Dame, poses by the door of a congressman's office. For more on Hill Day, see page 3.

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Cryo-EM Research Specialist

Washington University in St. Louis
School of Medicine

The Washington University Center for Cellular Imaging (WUCCI) is seeking an Cryo-Electron Microscopy Research Specialist to augment current Cryo-EM staff capacity in the operation and support of the center's current Titan Krios G3 and future Talos Arctica Cryo-EM platforms. Successful applicants will work in conjunction with existing staff under the general supervision of the Center Director. They will serve as one of the point people responsible for the oversight and maintenance of the Titan Krios and Talos Arctica platforms under the supervision of the Director as well as training and assisting users. They will also have the opportunity to work on correlative microscopy technology development projects currently underway in the research arm of the center.

http://www.asbmb.org/Careers/Jobs/80002/

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The Department of Physiology & Membrane Biology at the University of California, Davis, School of Medicine, seeks to hire a full-time employee as an Assistant Project Scientist to perform research functions in development and validation of recombinant antibodies and in protein engineering.

Although the Project Scientist is expected to work independently under the general guidance of an academic member with an independent research program (i.e., Professor, Professional Researcher, Specialist in Cooperative Extension, etc.), he/she is not required to develop an independent research program or reputation. He/she will carry out research or creative programs with supervision by an individual in an academic title that carries with it automatic Principal status. The Project Scientist does not usually serve as a Principal Investigator but may do so by exception.

http://www.asbmb.org/Careers/Jobs/80000/

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We are seeking an established Principal Investigator, but will consider applicants at the Assistant Professor level, with expertise in metabolism and flux analysis as it relates to human health. Ph.D in biochemistry or related field required. Candidates for associate or full professor rank must meet criteria for appointment at rank per UW School of Medicine and Public Health guidelines for appointment and promotion on the tenure track. We seek applicants with a track record of outstanding research accomplishments and exhibiting a strong commitment to mentoring and teaching in a highly collegial and collaborative academic environment.

http://www.asbmb.org/Careers/Jobs/79941/

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COLLEGE OF THE Holy Cross

The Department of Biology and Health Professions Advising at the College of the Holy Cross invites applications for a full-time Continuing Non-Tenure-Track faculty position to begin in August 2019. We are seeking a candidate who could contribute to high demand biology courses in areas of Cell and Molecular Biology such as Microbiology, Biochemistry, Genetics, Cell Biology, or Nutrition as well as biology courses for non-majors with a preference for courses related to health and human biology. In addition, the successful candidate will join the Health Professions Advising Committee (HPAC) and also serve as an Associate Health Professions Advisor at the College.

http://www.asbmb.org/Careers/Jobs/79729/