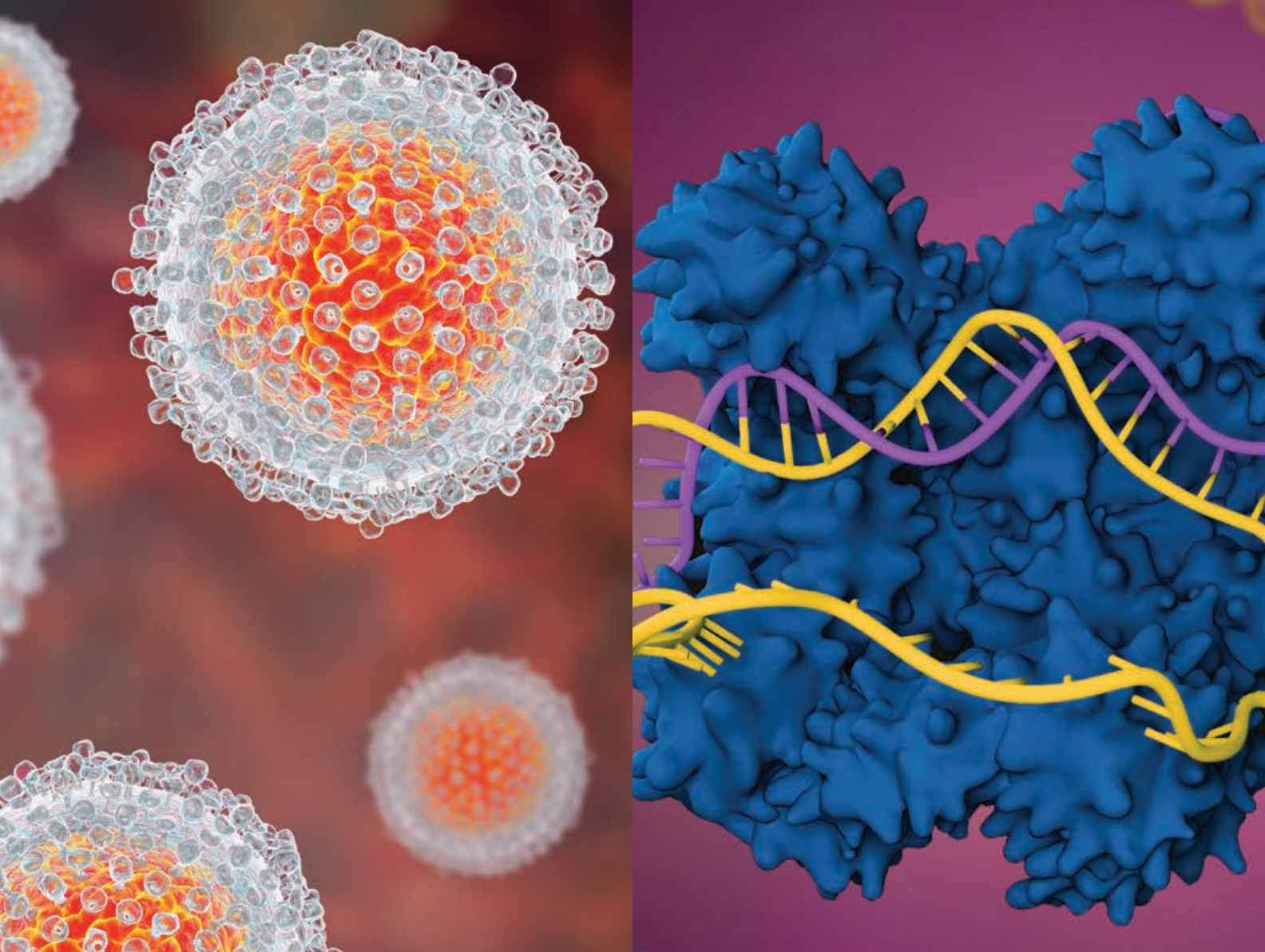


Vol. 19 / No. 11 / December 2020

ASBMB TODAY

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



A SEARCH & A SPRINT



Proteinases and their inhibitors

Feb. 24–26, 2021

This meeting covers diverse and vibrant fields of protease research, such as mechanistic studies on proteases in their molecular, cellular and organismic context.

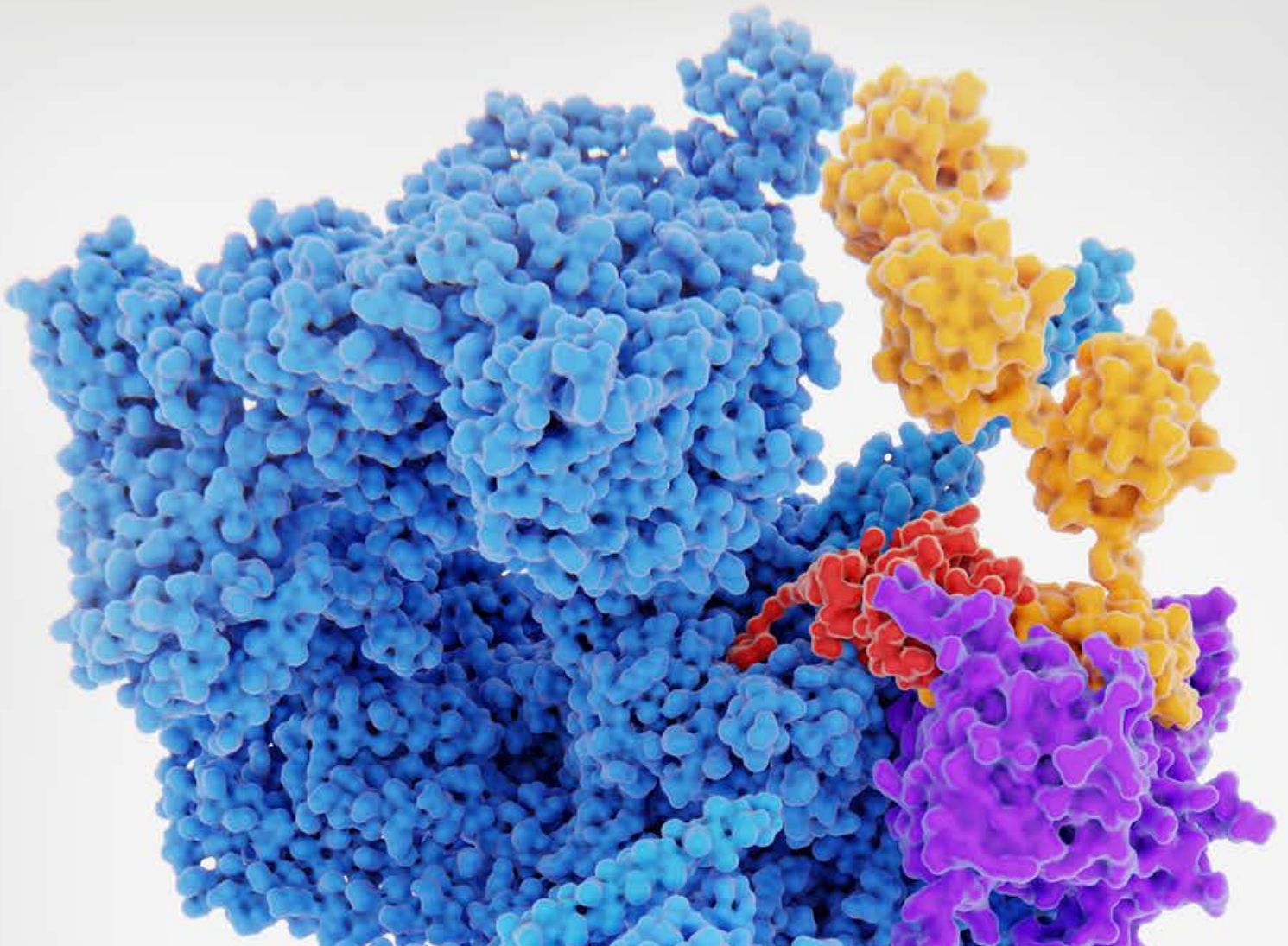
Sessions include:

- proteolysis in cancer
- proteolysis in neurosignaling and neurodegeneration
- proteolysis in blood coagulation
- discovery of protease substrates
- mechanisms and engineering of proteases, ligases, their substrates and inhibitors

Abstract submission deadline: Jan. 11, 2021

Submit your abstract and register at:

asbmb.org/meetings-events/proteinases-and-their-inhibitors



NEWS

2

PRESIDENT'S MESSAGE

Barbara Gordon, ASBMB executive director, to retire in 2021

3

NEWS FROM THE HILL

What the election results mean for science

4

MEMBER UPDATE

8

IN MEMORIAM

9

RESEARCH SPOTLIGHT

A life devoted to teaching and research

12

NEWS

12 *Targeting two-faced nuclear receptors to fight cancer*

15

JOURNAL NEWS

15 *Bubbling biochemistry*

16 *Sphingolipids show potential as biomarkers for multiple sclerosis*

18 *Georgia Tech team publishes how-to for COVID-19 test kits*

20 *From the journals*



FEATURES

26

THE CELLULAR CONUNDRUMS OF HEPATITIS C

Unraveling the genomic secrets of positive-strand RNA viruses

32

FROM BACTERIAL IMMUNITY TO SCIENTIFIC REVOLUTION

CRISPR's sprint to the gold

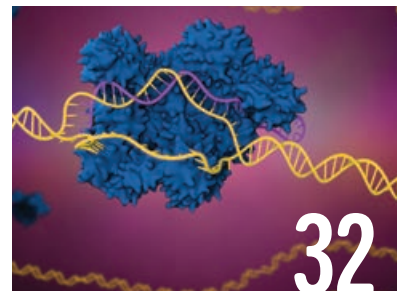
42

HOLIDAY GIFT GUIDE

44

ALEXANDRA NEWTON

A global champion of 'the big puzzle' — biochemistry



PERSPECTIVES

47

THE DEATH PROJECT

An excerpt from Nobelist Bob Lefkowitz's new memoir

52

ESSAY

Curbing the malpractice of curved grades and high-stakes exams

54

MINORITY AFFAIRS

A history-making administration — 'that believes in science'

56

FIVE QUESTIONS

Wayne Fairbrother: "You've got...to get outside of your comfort zone"



OFFICERS

Toni M. Antalis
President

Gerald Hart
Past President

Wei Yang
Secretary

Joan Conaway
Treasurer

COUNCIL MEMBERS

Suzanne Barbour

Joan Broderick

Charles Craik

Matt Gentry

Susanna Greer

Audrey Lamb

James M. Ntambi

Takita Felder Sumter

Kelly Ten-Hagen

EX-OFFICIO MEMBERS

Robert S. Haltiwanger

Carla Koehler

Co-chairs, 2020/2021 Annual Meeting Program Committee

Cheryl Bailey

Chair, Education and Professional Development Committee

Daniel Raben

Chair, Meetings Committee

Sonia Flores

Chair, Minority Affairs Committee

Nicole Waitowich

Chair, Science Outreach and Communication Committee

Terri Goss Kinzy

Chair, Public Affairs Advisory Committee

Ed Eisenstein

Chair, Membership Committee

Susan Baserga

Chair, Women in Biochemistry and Molecular Biology Committee

Sandra Weller

Chair, Publications Committee

Lila M. Gierasch

Editor-in-chief, JBC

A. L. Burlingame

Editor, MCP

Nicholas O. Davidson

Editor-in-chief, JLR

Kerry-Anne Rye

Editor-in-chief, JLR

ASBMB TODAY EDITORIAL ADVISORY BOARD

Rajini Rao

Chair

Ana Maria Barral

Natasha Brooks

Kelly Chacón

Beronda Montgomery

Bill Sullivan

Melissa Vaught

Binks Wattenberg

ASBMB TODAY

Angela Hopp

Executive Editor

ahopp@asbmb.org

Comfort Dorn

Managing Editor

cdorn@asbmb.org

Laurel Oldach

Science Writer

loldach@asbmb.org

Ed Marklin

Web Editor

emarklin@asbmb.org

Allison Frick

Multimedia and Social Media

Content Manager

africk@asbmb.org

Barbara Gordon

Executive Director

bgordon@asbmb.org

PRESIDENT'S MESSAGE

Barbara Gordon, ASBMB executive director, to retire in 2021

By Toni Antalis

It is with mixed feelings that I am writing to let you know that the American Society for Biochemistry and Molecular Biology's long-time executive director, Barbara A. Gordon, announced Nov. 16 that she will be retiring in early 2021.

Barbara has spent just about her entire career at the society, and we couldn't have asked for a more committed and caring leader. She began working for the Journal of Biological Chemistry in 1972 and went on to manage the society's meetings and its journals before being appointed executive director in 2003. During her tenure, she oversaw the journals' transition to online publishing and, most recently, their transition to open access. She also oversaw the formation of new committees and programs, such as the undergraduate degree-accreditation program and certification exam, the IMAGE grant-writing workshop, and the new MOSAIC program for diverse young investigators. Her list of accomplishments is long and impressive.

Barbara has been tremendously supportive and encouraging to all members of our ASBMB community, and we will most certainly miss her. She has freely shared her wisdom, intelligence and humor with all who have had the pleasure of working with her. On behalf of the Council and all who have benefited from working with Barbara,



Barbara A. Gordon has been executive director of the ASBMB since 2003.

I thank her for her exceptional service to the society and congratulate her on her well-deserved retirement.

The Council will soon begin the search process for a new executive director and will keep you updated on its progress. We will work closely with Barbara, the headquarters staff and the committees to ensure that there is a smooth transition.

Toni Antalis Toni Antalis (tantalis@som.umaryland.edu) is a professor of physiology at the University of Maryland School of Medicine, where she is also the associate director for training and education for the Greenebaum Cancer Center and the director of the graduate program in molecular medicine. She began her term as the ASBMB's president on July 1.



For information on advertising, contact Pharmaceutical Media Inc. at 212-904-0374 or mperlowitz@pmi.com.



www.asbmb.org/asbmbtoday

PRINT ISSN 2372-0409

Articles published in ASBMB Today reflect solely the authors' views and not the official positions of the American Society for Biochemistry and Molecular Biology or the institutions with which the authors are affiliated. Mentions of products or services are not endorsements.

What the election results mean for science

By Benjamin Corb

The election of Joseph R. Biden Jr. as the 46th president marks the end of a bitter campaign season and leaves in its wake a divided electorate struggling to survive a pandemic that has killed hundreds of thousands of Americans and has stalled the economy in ways not seen in more than a generation.

Biden sets the tone

The contrast between President-elect Biden and President Donald Trump has been stark. While Trump spent recent months downplaying the pandemic, Biden named a COVID-19 task force on his first “work day” of the transition, bringing together 12 experts on disease and public health to begin developing a pandemic response plan to be implemented on Day One of his presidency. Biden also encouraged all Americans to wear masks, a simple gesture that public health experts have begged Trump to make.

Biden has promised “disciplined, trustworthy leadership grounded in science,” which I interpret to mean more subject matter experts at the helms of agencies such as the Environmental Protection Agency and science programs throughout the federal government. I expect the next director to the White House Office of Science and Technology Policy, who acts as the science adviser to the president, will play a more prominent role.

In the Obama administration, the OSTP director was elevated to Cabinet-level importance; the Trump administration has devalued the position.

More immediately, a President Biden could reverse executive orders issued by Trump that have harmed the American scientific enterprise. Biden can act on restrictions on immigration, proposed changes to visa timing rules and restrictions on federal support for research utilizing embryonic stem cells, none of which require congressional approval.

Congress still up in the air

While the presidential election’s outcome is certain, the balance of power in the U.S. Congress remains in limbo.

Democrats maintain their majority in the U.S. House of Representatives. The Senate, however, remains uncertain. Georgia had two open Senate seats this election, and in both cases neither candidate received 50% of the vote, triggering a pair of runoff elections.

The anticipated Senate makeup favors Republicans 50–48, with the two Georgia seats unsettled. Republicans need to win only one of those Georgia seats to retain their majority, while Democrats need both to earn a 50–50 tie; Vice President-elect Kamala Harris will be able to cast tie-breaking votes.

Federal science agencies

Agencies such as the National Institutes of Health and National Science Foundation have seen their budgets increase over the past four years, a period that included a split Congress and a White House hostile to investments in science. For example, the NIH’s budget has increased 45% since fiscal year 2016 — a trend that is unlikely to change anytime soon.

Finally, the NSF director serves a six-year term in order to keep the position nonpolitical. The current director, Sethuraman Panchanathan, was appointed by Trump in late spring. I don’t expect him to be replaced, but I do anticipate that the Biden administration will seek to appoint a new head to the NIH.

Francis S. Collins, appointed by President Barack Obama in 2009, is the second-longest-serving director in the agency’s history. The incoming administration might consider looking for more diversity in the leadership. The NIH has had only one woman director (Bernadine Healy from 1991 to 1993) since its founding in the 1880s.

Benjamin Corb (bcorb@asbmb.org) is the ASBMB’s director of public affairs. Follow him on Twitter [@bwcorb](https://twitter.com/bwcorb).



Canadian society honors Cole, Fairn

Susan Cole, a professor in the department of pathology and molecular medicine at Queen's University in Kingston, Ontario, and **Greg Fairn**, an associate professor of surgery and biochemistry at the University of Toronto, are among the 2020 recipients of awards from the Canadian Society for Molecular Biosciences. Cole received the society's Jeanne Manery-Fisher Memorial Award for excellence in science, and Fairn won the New Investigator Award for outstanding accomplishments by a researcher who has been a PI for less than 10 years.

Cole, who holds the Bracken chair in genetics and molecular medicine, studied pharmacology and did postdoctoral training at the National Institutes of Health before joining



COLE

the Queen's faculty in 1994. She studies the biochemistry of chemotherapy resistance in cancer; her lab discovered a membrane protein in the ATP-binding cassette family, called multidrug resistance protein 1, or MRP1, that can render cancer cells resistant to many drugs when expressed. Subsequently, they found that beyond drug efflux, MRP1 also exports the antioxidant glutathione and numerous immune signaling molecules including leukotrienes and prostaglandins. The lab also studies other homologous proteins in the MRP family.

The award also recognizes Cole's track record of mentorship (she has trained more than 60 graduate students and postdocs) and service

to scientific societies including the American Association for Cancer Research and the American Society for Pharmacology and Experimental Therapeutics. She has been an editorial board member for the Journal of Biological Chemistry and several other journals, received multiple awards and is a fellow of the Royal



FAIRN

Society of Canada and the Canadian Academy of Health Sciences. Fairn, who joined the faculty at the University of Toronto in 2012, studies cellular membranes in a variety of physiological states. One line of research concerns contact sites between the endoplasmic reticulum and other membranes, including the cellular membrane and the phagolysosome, and how these contacts are regulated by the lipids found in each membrane. Fairn also pursues an interest in the interaction between host cells and pathogens, including the study of how macrophage sense and eliminate bacterial and fungal pathogens and the importance of protein lipidation in the process.

In 2017, Fairn won the American Society for Biochemistry and Molecular Biology Walter Shaw Young Investigator Award.

Montgomery joins MSU research and innovation office

Beronda Montgomery, a professor and plant biology researcher at Michigan State University has been named interim assistant vice president of the MSU office of research and innovation.

In this half-time position, which began in September, Montgomery will work on grants, cross-college and multi-institutional research projects and the current phase of the Global Impact Initiative to recruit new research faculty to MSU. Doug Gage, interim vice president for the office, said, "Beronda's expertise as a researcher and administrator, as well as her deep connections with federal funding agencies, will be a tremendous asset to help us pursue these important activities."

A professor in the MSU-U.S. Department of Energy plant research lab, Montgomery has appointments



MONTGOMERY

in the departments of biochemistry and molecular biology, and microbiology and molecular genetics. Her primary lab-based research is focused on the responses of photosynthetic organisms such as plants and cyanobacteria to external light cues. Her interests include biosynthesis and biochemical function of biliproteins in photosynthetic organisms, intercellular phytochrome signaling and light-dependent regulation of morphology.

Montgomery's research extends beyond biology, following a theme of understanding how individuals perceive, respond to and are impacted by their environments. She studies mentorship and faculty development to develop evidence-based strategies to foster equity and inclusion in academia. She served as assistant provost for faculty development at MSU from 2016 to 2020.

Montgomery was elected to the American Academy of Microbiology

CONTINUED ON PAGE 6

ASPET names new fellows

The American Society for Pharmacology and Experimental Therapeutics, or ASPET, recently inducted a new class of fellows. Six American Society for Biochemistry and Molecular Biology members were included in the list of 19 fellows, honored for their excellence in pharmacology research and in service to the field.

Joan Heller Brown, chair of the pharmacology department at the University of California, San Diego, studies the signaling action of G protein-coupled receptors, or GPCRs, which transduce extracellular signals into activation of intracellular enzymes. Her work focuses on cytoskeletal, growth and gene-expression changes downstream of GPCRs in vascular and heart cells. Brown also serves as principal investigator of the UCSD graduate training grant in pharmacology, one of the nation's largest, and directed the university's graduate program in biomedical sciences for years.

Marc Caron, a professor at Duke University, studies G-protein coupled receptors, with particular interest in neurotransmitter receptors. Caron developed many early techniques for GPCR purification and visualization, then moved into studying accessory proteins that modulate receptor function. Now his lab is interested in selective signaling, using allosteric modulators to activate certain signaling outcomes while blocking others. Such approaches could be used, for example, to design non-addictive pain relievers.

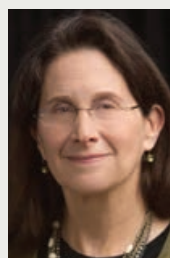
Lorraine Gudas, chair of the pharmacology department at Weill Cornell Medicine, studies retinoid compounds such as vitamin A, along with the receptors and downstream effectors through which they signal. Because retinoids can cause differentiation, she has also pursued research into carcinogenesis and potential therapeutic uses for stem cells. In addition to chairing her department,

Gudas holds leadership positions within ASPET, the American Association for Cancer Research and several other cancer research organizations.

Eric Johnson, a professor in the department of molecular medicine at the Scripps Research Institute, studies cytochrome P450 enzymes, which are key enzymes in steroid biosynthesis and drug metabolism. P450 enzymes are also involved in processing other xenobiotics, regulating blood pressure, and inflammation. His lab determined the first crystal structure of a membrane P450 enzyme and has continued to characterize the structures of mammalian P450s in complex with various substrates and inhibitors. Johnson serves on editorial boards of several journals in biochemistry and pharmacology.

John Scott, chair of the pharmacology department at the University of Washington, discovered scaffold proteins known as A-kinase anchoring proteins, or AKAPs, that bind to and organize protein kinase A and related signaling molecules. The signaling complexes that AKAPs generate have been found to regulate synaptic transmission, insulin secretion and cardiac cell contraction. Scott, a fellow of the Royal Society London, won the ASBMB's 2008 William C. Rose Award for his excellence in mentoring and has organized many conferences over the years.

David Sibley, a section chief and senior investigator at the National Institute of Neurological Disorders and Stroke, studies the pharmacology of G protein-coupled receptors, especially those that respond to dopamine. He also works on biased signaling and allosteric ligand development, leading to agonists and antagonists specific for certain dopamine receptor types. He has served as the president of ASPET and as an editor of eight scientific journals in pharmacology and biochemistry, and has edited several books.



BROWN



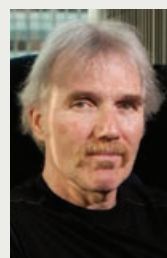
CARON



GUDAS



JOHNSON



SCOTT



SIBLEY

MEMBER UPDATE

CONTINUED FROM PAGE 4

in 2018 and recently was named one of Cell Press CrossTalk's 100 Inspiring Black Scientists. She is a member of the ASBMB Today editorial advisory board and her most recent essay for the magazine was "To support or deny: mentoring or gatekeeping?"

IUP breaks ground for Kopchick building

A groundbreaking ceremony was held in September for John J. and Char Kopchick Hall, a science research building at Indiana University of Pennsylvania. The Kopchicks, both IUP alumni, pledged \$23 million in 2018 for the new building, which will

replace structures built in the 1960s.

John Kopchick earned his bachelor's and master's degrees at IUP and his Ph.D. at the University of Texas Graduate School of Biomedical Sciences in Houston. He then did post-doctoral research at the Roche Institute of Molecular Biology and worked as a researcher at the Merck Institute of Therapeutic Research. In 1987, he accepted an endowed professorship at Ohio University where Char Kopchick is the assistant dean of students.



KOPCHICK

John Kopchick is the co-inventor

of the drug Somavert, which is used to treat acromegaly, a rare hormonal disorder. His research focuses on the molecular biology of growth hormone in relation to growth, obesity, insulin resistance, diabetes and aging. His lab also generated and characterized the world's longest-lived laboratory mouse.

Construction of Kopchick Hall is scheduled for completion in fall 2023. The four-story building will be equipped with an anatomy lab, greenhouse, imaging lab, laser lab, planetarium and vivarium. The university expected to reach the goal of raising \$75 million for the building in October. Any funds left over after construction will be used for scholarships and undergraduate research.

"Without my education here, I wouldn't be in a position to give anyone money," John Kopchick said during the dedication ceremony at IUP. "It is a way of giving back, looking forward and paying ahead. We are very proud and fortunate to be able to do this."

Tufts names research center for Levy

Tufts University has renamed its Center for Integrated Management of Antimicrobial Resistance after the late **Stuart B. Levy**, a researcher who launched the movement for antibiotic stewardship.

Levy, a molecular and microbiologist who taught and practiced medicine at Tufts for 47 years, began his research into antibiotics in the 1970s. With colleagues, he reported that antibiotics in animal feed could select for resistant bacteria, which could then be transferred to humans. Over the years he studied the molecular mechanisms of tetracycline efflux, the genetics of multidrug resistance and other related topics.

His research led Levy to conclude that rampant overuse of antibiotics, especially in agriculture, threatened both the drugs' efficacy and public health. He became an activist for antibiotic stewardship, meeting with regulators, founding a nonprofit and in 1992 publishing a book, "The Antibiotic Paradox: How miracle drugs are destroying the miracle."

Remembering Levy, his colleague John Leong, who is on the center's leadership team and led the push to rename, said, "He combined fundamental research and clinical practice and then could look down the road and see the consequences of unregulated, unthoughtful use of antimicrobial agents. He changed the way we think about how we use antibiotics."

Levy died in September 2019 at the age of 80, about a year after retiring from Tufts.



LEVY

IFCC honors Sacks for lab medicine, patient care

David Sacks, a senior investigator at the National Institutes of Health, has received the 2020 International Federation of Clinical Chemistry and Laboratory Medicine Distinguished Award for Laboratory Medicine and Patient Care.

The award recognizes Sacks' work with patients who have diabetes, notably his contribution to standardizing measurement of glycated hemoglobin. Because sugars at sufficient concentration can bind spontaneously with hemoglobin to produce a product with a month-long half-life, hemoglobin glycation is a good read-out for patients' blood sugar levels over time.

Sacks attended medical school at the University of Cape Town in South Africa and completed residencies in internal medicine and clinical pathology in the U.S. He taught and ran a lab at Harvard University and Brigham and Women's Hospital before coming to the NIH as a senior investigator and chief of



SACKS

clinical chemistry in 2011. He is a past president of the Academy of Clinical Laboratory Physicians and Scientists, an associate editor at the journal *Clinical Chemistry* and a former editorial board member for the *Journal of Biological Chemistry*. In the laboratory, his research focuses on signal transduction, with particular interest in calcium signaling.

The IFCC is a federation of professional societies in the field. This award recognizes a scientist who has made a significant contribution to laboratory medicine that improves worldwide clinical medicine and patient care.

Radford honored with OBE

For her services to molecular biology research, **Sheena Radford**, Astbury professor of biophysics at the University of Leeds, has been named



RADFORD

a civil officer of the Order of the British Empire, among the 2020 Queen's Birthday Honours announced in October. As director of the Astbury Centre for Structural Biology at Leeds, Radford leads a group of researchers who investigate the molecular basis of life. Her work focuses

Virtual seminar honors 100-year-old Eddy Fischer

Nobel laureate **Eddy (Edmond) Fischer**, an emeritus professor at the University of Washington and a member of the American Society for Biochemistry and Molecular Biology since 1955, recently celebrated his 100th birthday.

The university couldn't throw the party it had planned for Fischer in April, but Trisha Davis, chair of the UW department of biochemistry, organized a two-day virtual seminar on Oct. 29 and 30, featuring 12 speakers from throughout Fischer's scientific life. Among them was his granddaughter Elyse Fischer, a graduate student at Cambridge University, who spoke about her research on how phosphorylation affects the structure of a protein involved in the mitotic spindle checkpoint. A number of ASBMB members also spoke, including Tony Hunter, Rachel Klevit, Alexandra Newton, Nicholas Tonks, Susan Taylor and John Scott.

Eddy Fischer was born in Shanghai to European expatriates in 1920, then attended school and university in Switzerland. He came to the U.S. in 1950 to work at the California Institute of Technology. When he was offered a position at the University of Washington, Fischer and his wife found that Seattle reminded them of Switzerland, so he moved to UW and remained there for the rest of his career.



FISCHER

Fischer shared the 1992 Nobel Prize in Physiology or Medicine with colleague Edwin Krebs for their joint description of the importance of protein phosphorylation in regulating protein activity, signaling and cellular processes.

Linda Buck, a fellow Nobel laureate from the Fred Hutchinson Cancer Research Center, who has known Fischer for decades, said during the seminar, "In addition to being brilliant, Eddy is one of the most exuberant people I've ever met."

on the mechanics of protein folding and how misfolding leads to cellular dysfunction and disease. Current areas of study include protein misfolding and assembly into amyloid, and the role of chaperones and the BAM complex in folding mechanisms.

Radford earned her bachelor's degree and Ph.D. at Cambridge University. She was a reader at Leeds from 1998 to 2000 before being named a professor, and she has led the Astbury Centre since 2012. She is a fellow of the Academy of

Medical Sciences, the Royal Society and the European Molecular Biology Organization. Honors include the Biochemical Society Colworth Medal, the Royal Society of Chemistry Astra Zeneca Prize and Cornforth Award, and the Protein Society Branden Award.

The Queen's Birthday Honours for the U.K. are traditionally announced as part of Queen Elizabeth II's official birthday celebration in June but were delayed this year because of the COVID-19 pandemic.

Elie Shneour



The American Society for Biochemistry and Molecular Biology recently learned that emeritus member Elie Alexis Shneour, a neurochemist and biophysicist who joined the society 50 years ago, died March 14, 2015 in San Diego.

Born in France on Dec. 11, 1925, Shneour was raised in a Paris suburb. His father was a noted Yiddish poet and writer, Zalman Shneur. When Nazi Germany occupied France in 1940, the family escaped to Spain and then landed at Ellis Island in 1941.

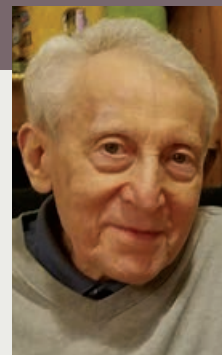
After graduating from high school in New York, Shneour joined the Army and attained the rank of captain. He earned a bachelor's degree in biology from Bard College, a master's in biochemistry from the University of California, Berkeley and a Ph.D. in biochemistry at UCLA.

Shneour began his career as a researcher and lecturer at Stanford University, then as an assistant professor at the University of Utah. He continued his research at the City of Hope National Medical Center in Los Angeles and then moved to La Jolla in 1971 to serve as director of research for Calbiochem. In 1975, he formed Biosystems Associates (later Biosystems Research Institutes), a scientific consulting company, which he ran until his retirement in 2014.

A prolific writer and a fellow of the Committee for Skeptical Inquiry, Shneour authored many articles on science and politics. His published books included "Life Beyond the Earth" and "The Malnourished Mind," and he completed a manuscript on 20th-century history, tentatively titled "Margins of Error."

Shneour was a gourmet cook and a serious photographer. He once owned a Hasselblad camera used on the moon by NASA. He was survived by his two children, Mark and Alan; and his three grandchildren, Collin, Luke and Trey.

Gregory R. Schonbaum



The American Society for Biochemistry and Molecular Biology recently learned that Gregory Richard Schonbaum, an emeritus member who joined the society in 1973, died June 26, 2019 at age 91 in Memphis, Tennessee. A biochemist for more than 40 years, his work focused on understanding enzymatic mechanisms.

Born February 21, 1928 in Lvov, Poland, Schonbaum spent the years of World War II in hiding after his parents were arrested and sent to concentration camps. After the war, he immigrated to London where he earned his Ph.D. in organic chemistry at the University of London and met his future wife, Madeleine Frydman.

The couple crossed the Atlantic, and Schonbaum held postdoctoral fellowships at the Johnson Foundation at the University of Pennsylvania in Philadelphia, with Britton Chance, and at the Illinois Institute of Technology with Myron Bender. He held positions in Pennsylvania and at the University of Alberta in Edmonton, Canada. During a sabbatical at the University of California, Berkeley, he worked with Lester Packer, a world leader in the study of antioxidants.

As a researcher at St. Jude Children's Research Hospital, Schonbaum spent almost 20 years continuing his studies on the enzymatic mechanisms of hemoproteins and seeking treatments for the side effects of chemotherapy. In the 1980s, he patented a method for alleviating kidney damage from the chemotherapy drug cisplatin with a polar dithiocarbamate compound.

In a final contribution to science, Schonbaum donated his body to the Medical Education and Research Institute. He is survived by his wife of 65 years, Madeleine Schonbaum; his children Pierre, Chris (Eunju) and Danielle; and his grandchildren, Alex and Rachel. He was predeceased by his son Michael.

A life devoted to teaching and research

Karlett Parra's scientific journey has taken her from Venezuela to New Mexico

By *Gelareh (Abulwerdi) Vinueza*

Karlett Parra remembers when she first became interested in academia. “In high school, I graded multiple-choice exams with my aunt,” she said.

Her aunt, Omaira Figueroa, an embryology and histology professor at the Universidad de Carabobo in Venezuela, gave Parra more than tests to grade: “My aunt mentored me for my first research project in high school that made me fall in love with research.”

The project involved feeding a vegetable diet to two rabbits (the control group) and a high-fat diet to another two rabbits. The experiment ended quickly when the rabbits on the high-fat diet died, but this project was Parra's first step in her scientific career. “I learned to test a hypothesis, quantify and analyze histology data, and make conclusions,” she said.

Figueroa died in 2009. In addition to starting her niece in research, her legacy includes changing the university's curriculum to add research to the didactic bioanalysis program and securing funds to build a bioanalysis education building. The undergraduate research program that she established was named *Jornadas de Investigacion en Pregrado Licenciada Omaira Figueroa* in her honor.

Born and raised in Caracas, Venezuela, Parra said the rest of her family also noticed her enthusiasm for science and cultivated it from

COURTESY OF KARLETT PARRA



Karlett Parra speaking at the Executive Leadership in Academic Medicine Program in 2017.

her teenage years. “My parents often reminded me of the value of education and the importance of pursuing a college degree,” she said.

While pursuing her bachelor's degree at Simón Bolívar University, she became involved in research. “As an undergraduate, I had my first biochemistry research experience, and it was love at the first sight,” she said, adding that the chromatography and Western blotting used to purify and visualize proteins were of great interest. “The fact that I could ‘see’ a protein fascinated me.”

In addition to her committed coursework, Parra joined a laboratory so she could conduct research and learn new methods such as purifying mitochondrial F₁-ATPase complex

“As an undergraduate, I had my first biochemistry research experience, and it was love at the first sight.”

from the parasitic nematode *Ascaris suum*. She remembers working so late at night to isolate proteins and measure the activity of enzymes that her mother had to pick her up after everyone else had left the building. “I loved every moment of working in the research lab,” she said.

Parra went on to earn her master's in biochemistry from Simón Bolívar University and decided to pursue a Ph.D. so she could continue doing research. One of her undergraduate

RESEARCH SPOTLIGHT

professors, Jose Bubis, connected her with scientists he knew in Syracuse, New York. In 1992, Parra moved to the U.S. to start her Ph.D. training in biochemistry and molecular biology at the State University of New York Upstate Medical University in Syracuse under the supervision of Patricia Kane, who became her mentor.

Parra's dissertation research focused on studying the regulation of a conserved ATP-driven proton pump called vacuolar H⁺-ATPase, or V-ATPase, in yeast. V-ATPase can pump protons across the membranes of mammalian cells; hence, it can acidify the intracellular compartment of cell organelles such as lysosomes and endosomes.

After a short postdoc at SUNY Upstate, Parra taught biology in LeMoyne College and moved to Ball State University in Indiana to take a full-time position as a tenure-track assistant professor in the chemistry department. She enjoyed teaching undergraduates and graduates, but six years later, a career opportunity presented itself at the University of New Mexico School of Medicine in

COURTESY OF KARLETT PARRA



Karlett Parra is chair of the biochemistry and molecular biology department at the University of New Mexico.

the department of biochemistry and molecular biology.

By this time, Parra was an associate professor, and the new position was for an assistant professor, but she saw it as a challenge and a way to grow in her career. In New Mexico, she would be part of a medical school where her research would have a broader biomedical impact than at BSU, a primarily undergraduate institution. Moreover, she was excited to work with a more diverse student body and research

community.

“Classrooms (at UNM) are composed of mainly underrepresented communities, with about 55% Hispanic population, 6% Native Americans, and the rest a mix of other races,” Parra said. “Seeing these students advance in their lab work and coursework is very exciting for me, and I enjoy inspiring them to pursue careers in the life sciences.”

Parra has continued her studies of V-ATPase, which plays an important role in many pH-dependent cellular processes. Its function is linked to the understanding and treatment of many disorders, including viral infections, neurodegenerative diseases, diabetes and cancer. For example, in prostate cancer, Parra's lab found that inhibition of V-ATPase affects the hypoxia-inducible factor-1-alpha function and androgen receptor expression, making V-ATPase potentially beneficial in treatment.

Continuing her interest in bioenergetics, Parra studies the communication mechanisms between glycolytic activity and V-ATPase function. “This communication is crucial,” she said, “because it allows cells to adjust V-ATPase assembly, activity and membrane distribution during periods of

COURTESY OF KARLETT PARRA



Karlett Parra (left) with her aunt, Omaira Figueroa, photographed in 1993 when Parra was a new graduate student in Syracuse, New York.

low and high energy demand.”

After five years at UNM, Parra was named chair of the biochemistry and molecular biology department — a new challenge that required her to acquire a whole new set of skills. “I am not only managing my lab and my students, but I am responsible for the success of the whole department,” she said. “I enjoy working with the junior faculty and seeing their progress to have a positive impact on science education.”

Parra admires the late Venezuelan physician–scientist Jacinto Convit, who developed a vaccine for leprosy. In 1988, he was reportedly in the running for the Nobel Prize in physiology or medicine for this work. Parra wishes she could have met Convit and asked about his research. “What were some of the limitations that he overcame?” she wondered. “How did he establish such a successful research program that impacted so many lives considering the very few resources he had at that time?”

Since 2007, Parra’s family, including her mother, Judith, and her brother, Carlos, have reunited with her in Albuquerque. Outside of work, Parra enjoys hiking, working out and cooking with her family. Her favorite dish to prepare is Spanish paella, and she has a special advantage in the kitchen: “Whenever

COURTESY OF KARLETT PARRA



Taking an outdoor break during a FASEB Science Research Conference meeting are, left to right, Summer Raines Hayek (then a postdoc, now a lecturer at UNM), Karlett Parra and Ph.D. students Chun-Yuan Chan and Yamhilette Licon Munoz.

I have cooking questions, I contact my cousin who is a chef in Florida, or I refer to her cookbook.”

Parra also enjoys visiting contemporary and traditional Southwestern art galleries, especially those on Canyon Road in Santa Fe’s historic

art district. She hopes that one day she will have visited all 300 galleries in the city.

“I love spending my weekends at the galleries,” she said, “because it makes me get a fresh perspective when I return to work.”

About the Research Spotlight

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

Gelareh (Abulwerdi) Vinueza (gelarehvinueza@gmail.com) graduated with her Ph.D. from the molecular medicine program at the University of Maryland, Baltimore. She is currently a policy fellow at the Food and Drug Administration. She has been an ASBMB volunteer writer since 2018 and is passionate about science communication and science policy. Outside of work, she enjoys photography, hiking and cooking. Follow her on Twitter [@gelareh_science](https://twitter.com/gelareh_science).



Targeting two-faced nuclear receptors to fight cancer

By Lisa Nicole Learman

For small-molecule cancer drugs, context is everything. No one is more aware of this than Stephen Safe, who designs such drugs in his molecular and cellular oncology lab at Texas A&M University. Many of the drugs his team synthesizes target nuclear receptors, which can exhibit either pro- or anti-cancer activity depending on the tissue type.

A native of Canada, Safe initially studied geology at Queens University. After a harrowing experience with a summer job in a uranium mine, he decided to switch fields. “It was easy to get killed by someone blowing you up with dynamite or rocks falling on you,” he said, “so I thought it was time to change, and I went into chemistry.”

Safe’s work as a chemist led him to a biological target that is now the focus of his lab, the aryl hydrocarbon receptor. He and his colleagues initially became interested in how organochlorine compounds such as dichlorodiphenyltrichloroethane, better known as DDT, and polychlorinated biphenyls, or PCBs, affect the environment. They synthesized derivatives of the compounds to identify how different parts of the molecular structure contribute to organochlorine toxicity. For one such chemical, dioxin, Safe said, “It turned out that the toxicity was related to binding affinity to this new receptor called the aryl hydrocarbon receptor.”

Physiologically, the aryl hydrocarbon receptor, or AhR, senses nutrient byproducts to regulate cell growth and response to inflammation. Safe and his team determined that although ligands of the AhR can be toxic, some could wipe out tumors in a rat model of breast cancer. They began to develop what Safe refers to as “selective receptor modulators,” compounds that bind the receptor and inhibit tumor growth but are relatively nontoxic.

Designing these compounds is not straightforward. Transcription factors often have different functions in different tissues, so developing safe and effective drugs can be tricky. Safe’s favorite example of this is tamoxifen,



TEXAS A&M COLLEGE OF VETERINARY MEDICINE & BIOMEDICAL SCIENCES

Stephen Safe, pictured in his lab, is the Syd Kyle chair of veterinary medicine and a professor of veterinary physiology and pharmacology, biochemistry and biophysics, and molecular and cellular medicine at Texas A&M University.

a drug that binds the estrogen receptor. In breast tumors, tamoxifen blocks estrogen receptors and significantly reduces cancer development. After using the drug for decades, researchers found that it caused a small but meaningful increase in the risk for endometrial cancer. This is because in the uterus, tamoxifen stimulates estrogen receptors, which promotes cancer. According to Safe, the potential for antagonistic functions of small-molecule drugs in different tissues is something “you don’t know offhand and you can’t predict.”

Much like the estrogen receptor, designing selective receptor modulators for the AhR can feel like trying to hit a moving target. Depending on the context, AhR receptor activity can help or hurt cancerous cells. In head and neck tumors, the receptor is pro-cancer, while in gastrointestinal tumors, it’s anti-cancer. Safe sees the dual nature of the AhR as a challenge but also an opportunity. “The beauty of it is you can design selective AhR modulators as agonists or antagonists,” he said. “So, if the AhR is a good thing, you treat with an agonist, and if the AhR is a bad thing, you treat with an antagonist.”

In a 2019 paper, Safe's lab characterized the role of the aryl hydrocarbon receptor in glioblastoma as anti-cancer, disproving a previous Nature paper. The lab didn't set out to disprove the original finding. Safe's team was helping Sharon Michelhaugh and Sandeep Mittal, glioblastoma researchers at the Karmanos Cancer Center in Detroit, who were interested in the natural AhR ligand kynurenine but were having a hard time getting their invasion assay to work. When Safe's lab looked into it for them, they could not replicate the published results demonstrating that kynurenine and its action on AhR were pro-invasion in glioblastoma. In fact, their comprehensive study yielded the opposite conclusion. Knocking out the AhR increased invasion, suggesting the receptor exhibits anti-cancer activity.

Safe and his team of scientists repeated the experiment countless times. "We just didn't believe it," he said. The Nature paper had characterized the AhR as pro-cancer in glioblastoma. Safe said his lab "had some trouble getting (our) paper published because we were disproving a Nature paper by a good group."

After characterizing the AhR knockout in many models at both functional and genomic levels, bolstered by their finding that one of their AhR agonists inhibited glioblastoma invasion, Safe was sure they were right. Neither his lab nor the authors of the original

paper have been able to reconcile the apparent contradiction in their results.

In addition to their AhR research, Safe's lab designs small-molecule cancer drugs that target other nuclear receptors, oncogenic long noncoding RNAs and the PDL-1 immune checkpoint inhibitor. They also have started to identify over-the-counter drugs with AhR receptor binding activity. Safe's group hopes that these drugs, which are already FDA-approved, could be used as cancer therapeutics.

"The one we've used the most for glioblastoma is Prilosec, the proton pump inhibitor," Safe said. In July, he and his collaborators published a study showing that Prilosec inhibits glioblastoma cell invasion and tumor growth by promoting aryl hydrocarbon receptor activity.

The lab has expanded its molecular targets, led always by their work with AhR. "Every time you go to an AhR conference, enormous new things come out," Safe said. "It's an amazing receptor."

Lisa Nicole Learman (llearman@jhmi.edu) is a Ph.D. candidate studying molecular neuroscience at Johns Hopkins University School of Medicine. She is passionate about dystopian fiction, increasing public understanding of science and the music of Charles Mingus. Follow her on Twitter @LearmanLisa.



Interest groups at the ASBMB annual meeting

The ASBMB encourages its members to hold topic-specific scientific seminars at the 2021 ASBMB Annual Meeting, held in conjunction with EB 2021.

The goal is to bring together people with similar scientific interests to facilitate communication and collaboration. During these two- or three-hour events, participants can interact, present, discuss and network within a topic-specific community.

If you are interested in organizing a scientific interest group, please submit your proposal for consideration by the Meetings Committee by Jan. 1, 2021.

Learn more at asbmb.org/meetings-events/2021-annual-meeting/networking

Don't forget to renew your ASBMB membership!

Over the past 100 years, the ASBMB has grown and changed with the times to become the supportive community of discoverers that it is today.

ASBMB members are driven to better understand what makes life work. With patience, perseverance and insatiable curiosity, and through collaboration and hard work, we seek to uncover the secrets of life.

Thank you for your membership.

If you need to renew your ASBMB membership, you can do so here:
society.asbmb.org/SignInRenew



Bubbling biochemistry

Understanding the components of sparkling wine

By Laurel Oldach

Whether or not your holiday celebrations involve imbibing, the cultural link between champagne flutes and festivities is strong. The fizz and pop of sparkling wine give breaking out the bubbly a special pizzazz.

In the first glycoproteomics study of its kind, a research team reports in the journal **Molecular & Cellular Proteomics** that glycoproteins are an important part of keeping those bubbles in solution. Cassandra Pegg and colleagues at the University of Queensland put a variety of sparkling wines under the microscope — or, more accurately, into the mass spectrometer — in hopes that the work would lead to better wine-making methods.

“Sparkling wine is really difficult to pipette,” Pegg, a postdoctoral researcher in Ben Schulz’s lab, said. “We have had some sets of samples that were gushing” — that’s a winebiz term for when bubbles won’t stay in solution — “and the tubes would pop open in the lab.”

Gushing, when champagne or cava comes foaming out of an opened bottle, entertained Pegg and her colleagues. But it’s less than desirable commercially: Winemakers want the dissolved gas to come out of solution slowly, making a beverage that continues to bubble until it’s finished.

Sparkling wine’s carbonation results from two rounds of fermentation. After producing a base wine, winemakers mix in a solution of



yeast and sugar and seal it all into a bottle to trap the carbon dioxide the yeast produces. The yeast strain, grape blend and tweaks to the production process can affect the quality of the final product — but the process is often a matter of trial and error.

To understand more about molecular attributes leading to positive prosecco properties, the researchers degassed samples of sparkling wine that had been aged for different periods or fermented with different strains of yeast. They used proteomic and glycoproteomic techniques to characterize the brews. Wine doesn’t have much protein, and most of the proteins they found were secreted by yeast or found in yeast cell walls. A surprisingly high proportion were glycosylated, or modified with com-

plex sugar molecules.

As wines age, their proteins and glycopeptide constituents change. According to Ben Schulz, the professor who led the work, changes late in the aging process, when “no biology should be happening,” probably depend on the biophysics of protein solubility. Glycosylation can make a peptide more water-soluble and less likely to clump into sediment.

Similar principles may guide a wine’s propensity to gush. Researchers previously had found that a yeast cell wall protein called seripauperin 5 can stabilize foam; this and related proteins were among the most abundant that Schulz’s team identified.

“It’s not completely understood,” Schulz said of the protein’s foam-stabilizing effect. “But from a biophysical standpoint, it makes sense: Glycans tend to be hydrophilic, and peptides hydrophobic.” By clustering at the interfaces between liquid and gas, small glycopeptides could affect a wine’s surface tension and change the rate at which dissolved gas coalesces into bubbles and escapes.

By understanding the production methods that affect seripauperin 5 and other glycoproteins, Schulz said, he hopes to work in the future with winemakers looking to optimize their products.

DOI: 10.1074/mcp.RA120.002181

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



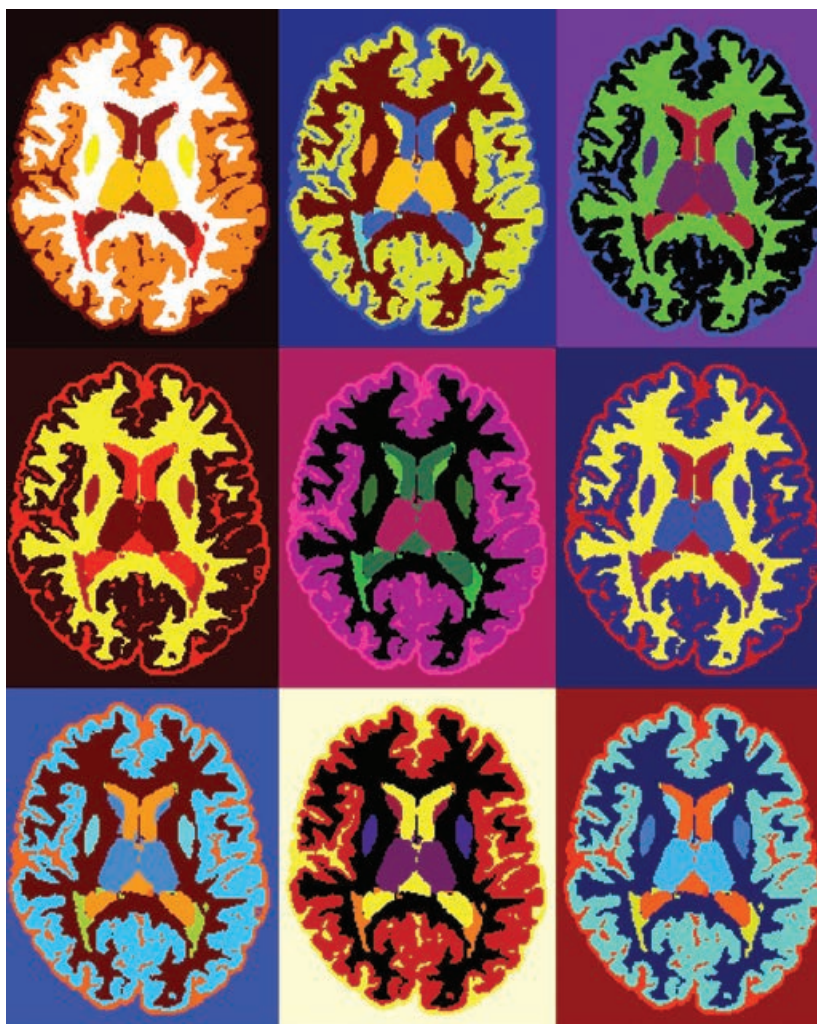
Sphingolipids show potential as biomarkers for multiple sclerosis

By Deboleena M. Guharay

Multiple sclerosis is an immune-mediated disease affecting the central nervous system. In MS, the myelin layer of the nerve cells is damaged by the immune system, creating plaques or lesions that cause problems in signal transmission between the brain and the rest of the body. According to the National Multiple Sclerosis Society, more than 1 million people in the U.S. live with MS. Symptoms include numbness of limbs, vision problems, fatigue and dizziness. There is no cure, but treatments might help to manage symptoms and disease progression. Scientists are looking for potential biomarkers to understand the stages of the disease's development.

Maria Podbielska, a researcher at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Poland, has been working to identify these biomarkers. Her interest in MS developed during her postdoctoral fellowship in Edward L. Hogan's laboratory at the Institute of Molecular Medicine and Genetics in the Medical College of Georgia, Augusta University, between 2005 and 2010. There, she worked on projects involving the pathological mechanisms of MS.

"MS is heterogeneous with respect to clinical, genetic and pathologic features," Podbielska explained. "Therefore, a set of verified and specific biomarkers for each pattern of immune-mediated brain damage needs to be developed in order to recognize them in the general non-



ILEM GEORGE AND DANIEL REICH, NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE; NATIONAL INSTITUTES OF HEALTH

Axial MRI scans of the brain of a person with multiple sclerosis have been segmented into various tissue types.

biopsied MS population."

Multiple sclerosis consists of two pathological processes: inflammation, or active phase, and neurodegeneration, or inactive or chronic phase. Both phases begin from the onset of the disease, but they develop at different rates. Sphingolipids, or SLs, are an important component of the myelin

sheath and could be biomarkers to track these phases.

In a recent paper in the **Journal of Lipid Research**, Podbielska and her colleagues wrote that they found sphingolipid species as potential biomarkers for the inflammatory and neurodegenerative processes involved in MS pathology. They did

a sphingolipidomic analysis using high-performance liquid chromatography–tandem mass spectroscopy in postmortem specimens of normal-appearing white matter from healthy central nervous systems and from patients with active and inactive stages of the disease.

Ceramide, or Cer, is an important component of SL pathways. The researchers found various Cer metabolic forms in different proportions in the active and inactive MS lesions, which clearly showed different SL pathways involved in the active and inactive phases of MS.

The tissue studies implicated sphingolipid biosynthesis in active MS lesions, Podbielska said, but

Multiple sclerosis consists of two pathological processes: inflammation, or active phase, and neurodegeneration, or inactive or chronic phase. Both phases begin from the onset of the disease, but they develop at different rates.

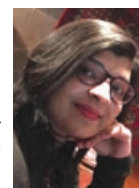
the researchers noticed a different pathological scenario for inactive MS nervous system damage, where the sphingomyelin-ceramide-hexosylceramide metabolic pathway could be responsible for damage to neurons.

“The most important observation is related to our discovery of some kind of MS diagnostic ‘red flag’ — a striking increase of the ceramide-1-phosphate levels in progressive MS lesions,” she said.

Podbielska plans to continue her work to find more potential lipid biomarkers that might help to develop therapeutic treatments for MS.

DOI: 10.1194/jlr.RA120001022

Deboleena M. Guharay
(deboleenamitra@gmail.com)
earned her Ph.D. in chemistry from Virginia Commonwealth University. She is very enthusiastic and passionate about science communication.



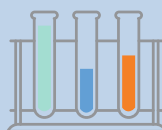
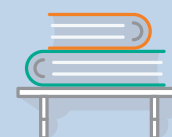
Upcoming ASBMB events and deadlines

JAN.

- JANUARY** **Cervical Health Awareness Month**
- 1 ASBMB journals become open access
 - 1 Deadline to submit an ASBMB Annual Meeting interest group proposal
 - 4 Deadline to submit ASBMB fellows nominations
 - 7 Abstract deadline for the ASBMB Annual Meeting held in conjunction with Experimental Biology
 - 7 Deadline to apply for ASBMB Annual Meeting grants and awards
 - 11 Abstract deadline for Proteinases and their inhibitors
 - 24 **International Day of Education**

FEB.

- FEBRUARY** **Black History Month**
- 1 Early registration deadline for Proteinases and their inhibitors
 - 7 **Periodic Table Day**
 - 11 **International Day of Women and Girls in Science**
 - 22 Regular registration deadline for Proteinases and their inhibitors
 - 24–26 Proteinases and their inhibitors
 - 27 **National Protein Day**
 - 28 **Rare Disease Day**



Georgia Tech team publishes how-to for COVID-19 test kits

By Lisa Nicole Learman

In the spring of 2020, the U.S. experienced severe shortages of COVID-19 tests, and a frustrated public wondered why testing kits were so hard to produce. Georgia Tech scientists took action, developing protocols that can be used by laboratories to make COVID-19 test kits in many settings and with many budgets.

Loren Williams, a professor of chemistry and biochemistry, spearheaded the effort, shifting his lab's focus from the origins of life to COVID-19 test kit production. "Like many academic scientists, we had useful expertise and resources and an intense desire to contribute," Williams said.

Seeing that reagents for RT-PCR kits were in short supply, Williams gathered a team to design a protocol for academic labs to produce kits cheaply. "Once we launched, we were nearly swamped by volunteers with all kinds of amazing skill sets," he said.

The team grew to more than 30 scientists from diverse backgrounds, including undergraduate and graduate students, postdoctoral fellows, laboratory technicians, and professors. Social distancing rules made it difficult to train recruits, but the researchers managed, sometimes using video calls to demonstrate how to use instruments or perform techniques.

The project culminated in a paper published in the **Journal of Biological Chemistry** that provides step-by-step instructions for academic

"We wanted to do something for the whole world. The U.S. didn't have enough test kits, which made me think about other, poorer countries with bad economies."

SARA FAHKRETAHA-AVAL

labs to produce COVID-19 test kits. Samantha Mascuch, a postdoc studying chemical ecology, and Sara Fahkretaha-Aval, a fifth-year grad student studying the origins of life, are co-first authors of the paper. Mascuch said working on coronavirus was not only an honor but an ethical imperative. "We're scientists," she said. "Certainly public money has gone into training us, and I think it's important for us to use what skills we have to give back."

The protocol reduces costs so that even labs with small budgets can contribute. In RT-PCR tests, pieces of the viral genome are recognized with molecular probes and amplified by an enzyme. The new protocol instructs scientists how to make the molecular probes and enzymes in-house instead of having to buy them, drastically cutting costs.

The molecular probes that bind and recognize the COVID-19 genome are short fluorescent pieces of DNA. DNA is composed of nucleotides called adenine, guanine, thymine and

cytosine, known as A, G, T and C. The researchers made a chain of these nucleotides that binds a part of the viral genome through complementary base pairing, in which A's bind to T's and G's bind to C's. Finally, they added fluorescent tags to the end of these DNA pieces so that when the viral genome is recognized and amplified by an enzyme, it can be detected by looking at the fluorescence signal.

The scientists also made the protein DNA polymerase (the enzyme that amplifies the pieces of the viral genome) by feeding bacteria the DNA instructions for making that protein. The bacteria act as little factories, turning the DNA into a functional enzyme that is purified and used in the COVID-19 test kit. The team at Georgia Tech tried many types of molecular probes and DNA polymerases before they found the most accurate and efficient combination.

Beyond being involved in exemplary scientific research, Fahkretaha-Aval is proud to have made a global resource. "We wanted to do something for the whole world," she said. "The U.S. didn't have enough test kits, which made me think about other, poorer countries with bad economies. Or countries like my own, Iran, that are under sanction or embargo."

To determine if the kits could detect SARS CoV-2, the virus that causes COVID-19, on surfaces, a subgroup of researchers took samples around the Georgia Tech campus at

COURTESY OF SARA FAKHRETAHA-AVAL



The more than 30 Georgia Tech scientists who developed a protocol to make COVID-19 test kits from scratch, top to bottom and left to right. Row 1: Samantha Mascuch, Sara Fakhretaha–Aval, Jessica Bowman, Minh Thu Ma, Gwendell Thomas, Bettina Bommaricus, Chieri Ito. Row 2: Liangjun Zhao, Gary Newnam, Kavita Matange, Hem Thapa, Brett Barlow, Rebecca Donegan, Nguyet Nguyen. Row 3: Emily Saccuzzo, Chiamaka Obianyor, Suneesh Karunakaran, Pamela Pollet, Brooke Rothschild–Mancinelli, Santi Mestre–Fos, Rebecca Guth–Metzler. Row 4: Anton Bryksin, Anton Petrov, Mallory Hazell, Carolyn Ibberson, Petar Penev, Robert Mannino, Wilbur Lam. Row 5: Andrés García, Julia Kubanek, Vinayak Agarwal, Nicholas Hud, Jennifer Glass, Loren Williams, and Raquel Lieberman.

stores, gas stations and even other labs. After the paper was published, collaborators at the University of Georgia tested the kit’s ability to detect the virus in patient samples. By every metric tested, the homemade kits work as well as, if not better than, commercially available kits.

Academic labs need an emergency use authorization, or EUA, to produce kits that can be distributed to local testing centers; the Food and Drug Administration requires data showing

the quality and efficacy of the kits. Labs can produce test kits that meet these standards more easily by following the Georgia Tech protocol.

This protocol also arms communities worldwide to respond to future shortages. Fakhretaha–Aval believes more labs need to make resources for combatting COVID-19 publicly available. “I hope they share the information like we did,” she said. “I hope that all of the scientists and companies share their information after they

produce new tests, treatments, and vaccines.”

DOI: 10.1074/jbc.RA120.015434

Lisa Nicole Learman (llearman@jhmi.edu) is a Ph.D. candidate studying molecular neuroscience at Johns Hopkins University School of Medicine. Follow her on Twitter @LearmanLisa.



From the journals

By Himanshi Bhatia, Amrita Mandal & Anand Rao

We offer summaries of recent papers in the **Journal of Biological Chemistry**, the **Journal of Lipid Research** and **Molecular & Cellular Proteomics**.

A novel cholesterol trafficking pathway in blood

Cardiovascular diseases, or CVDs, account for 31% of all global deaths. They are characterized by underlying causes such as atherosclerosis involving anomalies in cholesterol transport and deposition. Therapies such as lipid-lowering drugs have reduced the risk of CVDs but have not eliminated them, indicating the existence of additional factors that influence lipid metabolic pathways and contribute to development of CVDs.

In a multi-institutional study published recently in the **Journal of Lipid Research**, Ryunosuke Ohkawa from Tokyo Medical and Dental University and colleagues describe a unique role for red blood cells, or RBCs, in regulating cholesterol transport. This may help researchers design more effective treatments.

In this study, incubation of plasma with RBCs resulted in a time-dependent increase in serum/plasma cholesterol levels. Further lipoprotein analysis demonstrated cholesterol efflux to high-density lipoproteins and influx from low-density lipoproteins in a dose- and temperature-dependent manner with no transport to lipoprotein-depleted plasma. Using RNAi machinery and bioinformatic analysis, the researchers identified ATP-binding cassettes ABCA7 and ABCG5, lipoprotein lipase and translocator

protein as possibly involved in the transport process. These findings are expected to pave the way for better understanding of CVDs and other disorders of cholesterol metabolism. DOI: 10.1194/jlr.RA120000635

New tools to study aminoacyl-tRNA synthetases

Aminoacyl-tRNA synthetases, or aaRSs, are enzymes that polymerize amino acids to synthesize tRNA, and they have a well-known role as housekeeping proteins. However, researchers have lacked the tools to appreciate the extent of their biological functions.

In a recent paper published in the **Journal of Biological Chemistry**, Charlotta Preger of Karolinska University Hospital and collaborators created high-affinity antibodies for 13 cytoplasmic and one mitochondrial aaRS. The authors produced selected domains of these proteins in *E. coli*, which they used as antigens to yield large sets of antibodies that were validated through binding assays. Immunoprecipitation and mass spectrometry verified the antibodies' ability to capture their target proteins. These sequences of the proteins are freely available to all, and the authors believe their work will help define the noncanonical roles of aaRSs in health and disease.

DOI: 10.1074/jbc.RA120.012893

A key to inflammation in liver disease

Mallory–Denk bodies, or MDBs, are cytoplasmic aggregates of mis-

folded proteins commonly found in hepatocytes of patients with liver disease such as hepatocellular carcinoma, hepatitis B and C, and alcoholic and nonalcoholic steatohepatitis. Abnormal protein aggregation, a common hallmark of several neurodegenerative diseases, leads to the transcription factor nuclear factor- κ B, or NF- κ B, activation. This activation initiates an inflammatory cascade. However, researchers have not yet identified the mechanistic link between protein aggregation and NF- κ B activation.

In hepatocytes, the inhibitor of NF- κ B, or I κ B, family of proteins inhibits NF- κ B activation by masking its nuclear localization signal, leading to cytoplasmic retention of NF- κ B in an inactive state. In a recent article published in the journal **Molecular & Cellular Proteomics**, Yi Liu at the University of California, San Francisco, and an international team of researchers write that they've found induction of MDB production leads to sequestration of two proteins, I κ B α and I κ B β , into insoluble aggregates in the cytoplasm. As a result, NF- κ B can travel freely to the nucleus and initiate an inflammatory cascade. Using state-of-the-art proteomics analysis, the researchers also identified 10 proteins, including nucleoporins Nup153 and Nup358/RanBP2, in these aggregates, which interact with I κ B α upon MDB induction and prevent its nuclear entry and its consequent termination of NF- κ B-activation. These findings provide new therapeutic targets to study protein aggregation related to inflammation in liver disease and possibly in neurodegenerative diseases. DOI: 10.1074/mcp.RA120.002316

Vascular proteomics in citrus disease

Huanglongbing, or HLB, is a devastating citrus plant disease that causes huge economic losses to the citrus industry worldwide. The causal bacterium, *Candidatus Liberibacter asiaticus*, or CLAs, is spread by *Diaphorina citri*, a sap-sucking citrus plant bug, and ends up in the plant's vascular system. Fruits affected by the disease are greener, smaller than normal, and overall bitter in taste. Once a tree is infected with HLB, it eventually dies. Previous studies have looked at host responses in leaf, root and fruit, but scientists have lacked information regarding the proteomics of the vascular system where the bacterium resides.

To address this issue, Jessica Y. Franco and a group of researchers at the University of California, Davis, and in Texas performed proteomic analysis of plant bark samples from the sweet orange variety Washington navel inoculated with CLAs. The analysis revealed upregulation of proteins important for pathogen recognition and plant defense response. The researchers also compared and found variability in peroxidase enzymatic activity among different citrus species. Serine protease and hydrolase activity also changed dynamically during CLAs infection. These findings indicate genetics and environmental conditions influence the host defense response.

Overall, this study, published recently in the journal **Molecular & Cellular Proteomics**, showed that the Washington navel orange had a 51% change in global proteome during CLAs infection. Future investigation to assess plant host response in different genotype and environmental conditions will provide a better understanding of the disease.

DOI: 10.1074/mcp.RA120.002075



Huanglongbing disease, shown in an orange, is caused by bacteria spread by a citrus plant bug.

New work casts doubt on antibody's role in cancer studies

Scientific researchers make progress by standing on the shoulders of those who came before them. Through an often arduous process, they test and retest their own work before submitting their results to their peers to be evaluated. Cancer research is no exception to this model.

Rac1, a small GTPase involved in cytoskeleton reorganization and cell motility, is elevated in several aggressive cancers. Studies have relied on a conformation-sensitive antibody for the detection of GTP-bound Rac1 — known as Rac1-GTP — activation

in cancer cells, but Martin Baker of the University of Pennsylvania and collaborators stumbled across inconsistencies that prompted them to conduct a deeper characterization of this antibody. Using prostate and pancreatic cancer models known to have high basal Rac1-GTP levels, they demonstrated that the oft-used Rac1 antibody does not, in fact, recognize Rac1 but rather detects the filament protein vimentin. Cell lines that were devoid of Rac1 showed strong signals when using this antibody, and these signals disappeared when vimentin was knocked out.

The researchers published their findings in the **Journal of Biological Chemistry**. The work has major

implications for the study of this key GTPase in cancer, chiefly because many cancer cell lines with characteristic mesenchymal attributes show upregulation of vimentin that coincides with high basal Rac1-GTP levels when measured biochemically. This creates a misleading correlation that could steer investigators toward inaccurate conclusions and derail efforts to develop Rac1-targeted therapeutics.

DOI: 10.1074/jbc.RA120.013919

A lipid metabolite regulates neuronal firing

Lipid-signaling molecules regulate neuronal processes such as fear, anxiety, appetite, memory and pain.

Master regulator governs color production in yeast, fungi

Sterol regulatory element-binding proteins, or SREBPs, sense the intracellular lipid environment to regulate expression of key metabolism-associated genes and maintain lipid homeostasis in mammals. These functions are primarily conserved across species; in yeast and fungi, additional pathways are controlled by Sre1, the SREBP

MELISSA GÓMEZ-RIOS



This photo shows the color variations in four strains from *X. dendrorhous* seeded on an agar plate.

homolog, with roles ranging from fungal pathogenesis to growth under hypoxic conditions. Though the roles are conserved, little is known about the mechanism of Sre1 in yeast. Previous research suggests a link between production of ergosterol, the main sterol component of the fungal cell membrane, and carotenoids, the color-producing compounds in these organisms.

To gain greater understanding, authors of a recent study in the **Journal of Lipid Research** focused their efforts on the yeast *Xanthophyllomyces dendrorhous*, a natural producer of the carotenoid astaxanthin. Astaxanthin is both a coloring compound and an antioxidant, which lends commercial significance to this study. Melissa Gómez and colleagues from the Universidad de Chile performed comparative RNA-sequencing of modified *X. dendrorhous* strains to identify Sre1-dependent genes.

They also used chromatin immunoprecipitation combined with lambda exonuclease digestion, or ChIP-exo, to gain further insight into Sre1 target genes.

The researchers observed Sre1-binding motif in the promoter region of ergosterol biosynthesis-associated genes, including genes from the mevalonate, or MVA, pathway. They also identified two genes linked to carotenogenesis, *crtE* and *crtR*, as Sre1 target genes. Of these, *crtR* encodes a cytochrome P450 reductase, indicating the involvement of Sre1 in the biosynthesis of sterols and carotenoids. They found that Sre1 activates the expression of sterol-biosynthesis and MVA pathways and hence regulates carotenoid production in yeast.

These findings may benefit future studies focusing on commercially relevant compounds from yeast and fungi. DOI: 10.1194/jlr.RA120000975

Docosahexaenoyl ethanolamide, or DHEA, a type of lipid signaling molecule, is dependent on peroxisome proliferator-activated receptor, or PPAR, for its downstream neuronal functions. However, tetracosahexaenoyl ethanolamide, or THEA, has not been characterized.

In a study published in the **Journal of Lipid Research**, Lin Lin and Adam H. Methel from the University of Toronto and an

international team addressed this gap by chemically synthesizing THEA to use as a reference standard for functional analysis of naturally occurring THEA in biological samples. Euthanasia of wild-type mice and mice deficient in fatty acid amide hydrolase, or FAAH, resulted in THEA accumulation in whole-brain samples that depended on the mouse genotype (wild-type versus FAAH-deficient) and type of euthanasia

(microwave versus carbon dioxide-induced ischemia). Lower THEA levels were observed in wild-type mice and the microwave-only group. PPAR did not seem to play a role in THEA-dependent signaling. However, THEA exposure significantly increased firing from medium spiny neurons of the nucleus accumbens core. The findings from this

CONTINUED ON PAGE 24

Trimming cilia down to size

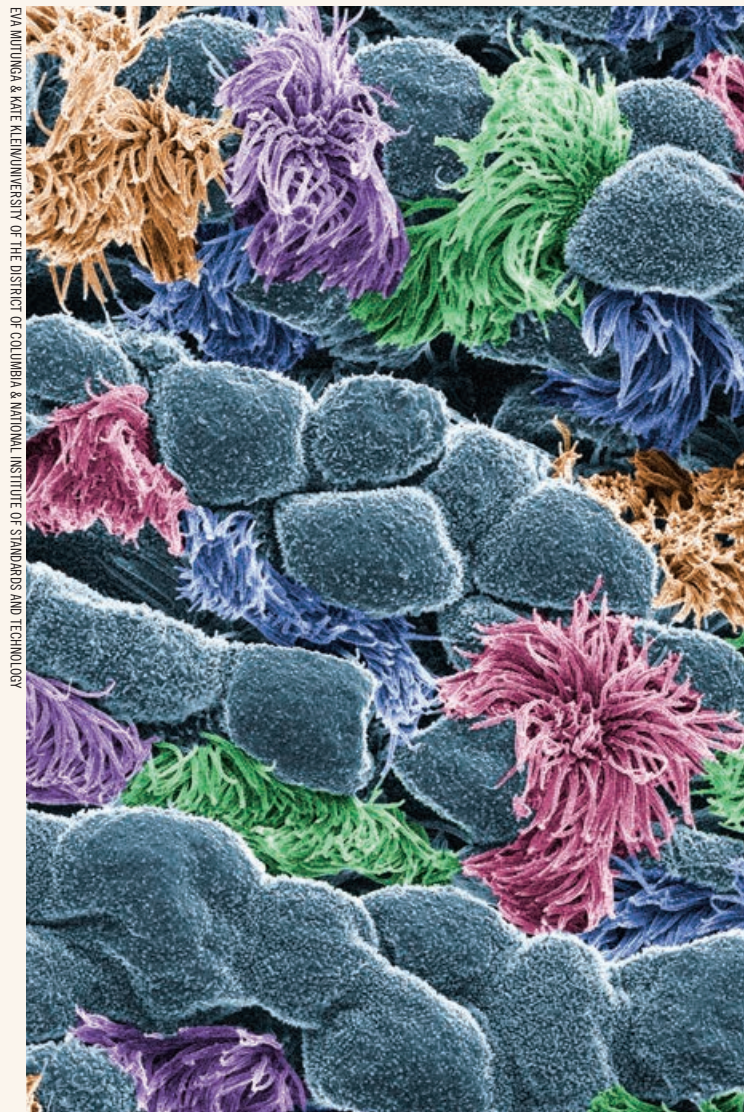
Cilia are hairlike organelles protruding from the surface of eukaryotic cells. The two types of cilia are motile and non-motile, and their structure and function can vary depending on the cell type. In the case of airway epithelial cells, tufts of motile cilia beat in concert to drive the directional flow of fluids, clearing dirt, mucus and debris from the respiratory tract. Abnormalities in the length or function of these organelles can lead to multisystem disorders known as ciliopathies, such as the hereditary respiratory disorder primary ciliary dyskinesia. While treatment options are limited for ciliopathies, uncovering the mechanisms that regulate motile cilia growth and beating could open avenues for new therapies.

Kavisha Arora and colleagues at Cincinnati Children's Hospital Medical Center discovered that autophagy, a controlled mechanism for cellular degradation, regulates motile cilia length. They did so by investigating the link between the signaling mediator adenylate cyclase 6, or AC6, and kinesin Kif19a, a dual-function molecular motor protein that moves along microtubules and depolymerizes them. Using mice genetically modified to specifically lack AC6 only in epithelial cells, the researchers showed that AC6 knockout airway epithelial cells have longer cilia than cells from mice that were not modified to lose AC6 and that this was due to decreased Kif19a protein levels in the cilia.

Finally, to learn how modulation of this novel pathway translates into changes in cilia growth and function, the authors constructed functional rescue experiments using cultured tracheal cells in combination with pharmacological inhibition or activation of AMPK activity. This approach showed that activation of AMPK caused downregulation of Kif19a and longer cilia, which compromised cilia function.

These findings, published in the **Journal of Biological Chemistry**, provide new insights into fundamental cilia biology and may lead to new treatments for ciliopathies.

DOI: 10.1074/jbc.RA120.013703



This image shows cells lining a mouse airway. The cells shown in gray secrete mucus, which traps inhaled particles. The colored cells sweep the mucus layer out of the lungs using cilia.

EVA MOUNGA & KATE KLEIN/UNIVERSITY OF THE DISTRICT OF COLUMBIA & NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY

CONTINUED FROM PAGE 22

study present a novel lipid signaling molecule that regulates neuronal excitability in the mouse brain.

DOI: 10.1194/jlr.RA120001024

Alternative splicing affects malignancy

The tumor protein p53 family is composed of three members: p53, p63 and p73. In response to DNA damage, p73 interacts with yes-associated protein 1, or YAP1, a transcriptional coactivator, and attenuates apoptosis. Researchers know that YAP1 can associate with the pro-oncogenic phosphatase known as Src homology region 2 domain-containing phosphatase-2, or SHP2. However, researchers have not identified the drivers of the pro-oncogenic effects of their interaction.

Chi Ben, Xiaojing Wu and colleagues at the University of Tokyo investigated the mechanisms underpinning the association between YAP1 and SHP2. Using experimental paradigms in cell culture and in animals, the team found that pro-oncogenic YAP1 activities are inversely regulated by alternative splicing and that certain splicing isoforms confer greater tumorigenicity than others.

The researchers recently published their results in the **Journal of Biological Chemistry**, demonstrating how abnormal splicing may exacerbate cancer malignancy. The work could lead to novel cancer therapeutics tar-

getting the tumor microenvironment.

DOI: 10.1074/jbc.RA120.013820

A proteomics study of mGluR signaling

The metabotropic glutamate receptors, or mGluRs, are G protein-coupled receptors important for synaptic transmission and neuronal excitability. Activation of mGluR signaling is implicated in behavior, learning and memory. Its disruption is associated with neurological diseases such as autism spectrum disorder and fragile X syndrome.

Group I mGluRs (mGluR1 and mGluR5) are well known for inducing long-term depression, or LTD, which involves an acute wave of new protein synthesis. These newly synthesized LTD proteins are important for endocytosis or internalization of the surface alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, or AMPARs. However, researchers have not yet identified the complete molecular signature downstream of mGluR signal activation.

In a recent study published in the journal **Molecular & Cellular Proteomics**, Charlotte AGH van Gelder, Renske Penning and a team of researchers in the Netherlands report that they have found novel regulators of the mGluR signaling pathway. The team first stimulated the cultured hippocampal neurons with the agonist (S)-3,5-dihydroxyphenylglycine to induce mGluR activation. Next, using high-resolution proteomics and bio-

informatic studies, they found several important and previously unknown modulators of mGluR signaling that provide a rich resource for future studies. Most notably, they found that AMPAR internalization was mediated by intersectin-1, an adaptor protein involved in endocytic trafficking.

DOI: 10.1074/mcp.RA120.002199

Preventing infections around prosthetics

Staphylococcus epidermidis, a Gram-positive bacterium that frequently colonizes on human skin, can cause infections around prosthetic implants. *S. epidermidis* forms amyloidlike fibrils composed of the protein Aap to create a biofilm that bolsters its resistance to host defenses and antibiotics.

In a recent paper published in the **Journal of Biological Chemistry**, Alexander Yarawsky and Andrew Herr of the University of Cincinnati College of Medicine explored how biofilms are formed. Using analytical ultracentrifugation, circular dichroism, dynamic light scattering and linkage studies, the authors showed that a pared-down biologically relevant Aap-like construct called Brpt1.5 forms a zinc ion-induced tetramer needed to produce the amyloids required for biofilm formation. Their results provide new avenues for potential therapeutic targeting of staphylococcal biofilms to reduce infections after prosthetics are implanted.

DOI: 10.1074/jbc.RA120.013936

Himanshi Bhatia (himanshi.b@gmail.com) is a postdoctoral research associate at the Washington University in St. Louis and is passionate about science communication. Follow her on Twitter @Himanshi16b.



Amrita Mandal (amritamandal09@gmail.com) is a postdoctoral fellow at the National Institutes of Health. She earned her Ph.D. in molecular biology at Cincinnati Children's Hospital in Ohio.



Anand Rao (araao@asbmb.org) is an ASBMB science communicator. Follow him on Twitter @AnandRaoPhD.





ASBMB
PUBLICATIONS



**ASBMB Journals will be
fully open access in 2021.**

**Journal of Biological Chemistry
Molecular & Cellular Proteomics
Journal of Lipid Research**

The American Society for Biochemistry
and Molecular Biology's three journals
will be open access beginning in January.

asbmb.org/journals-news/open-access

JBC

JOURNAL OF
BIOLOGICAL
CHEMISTRY

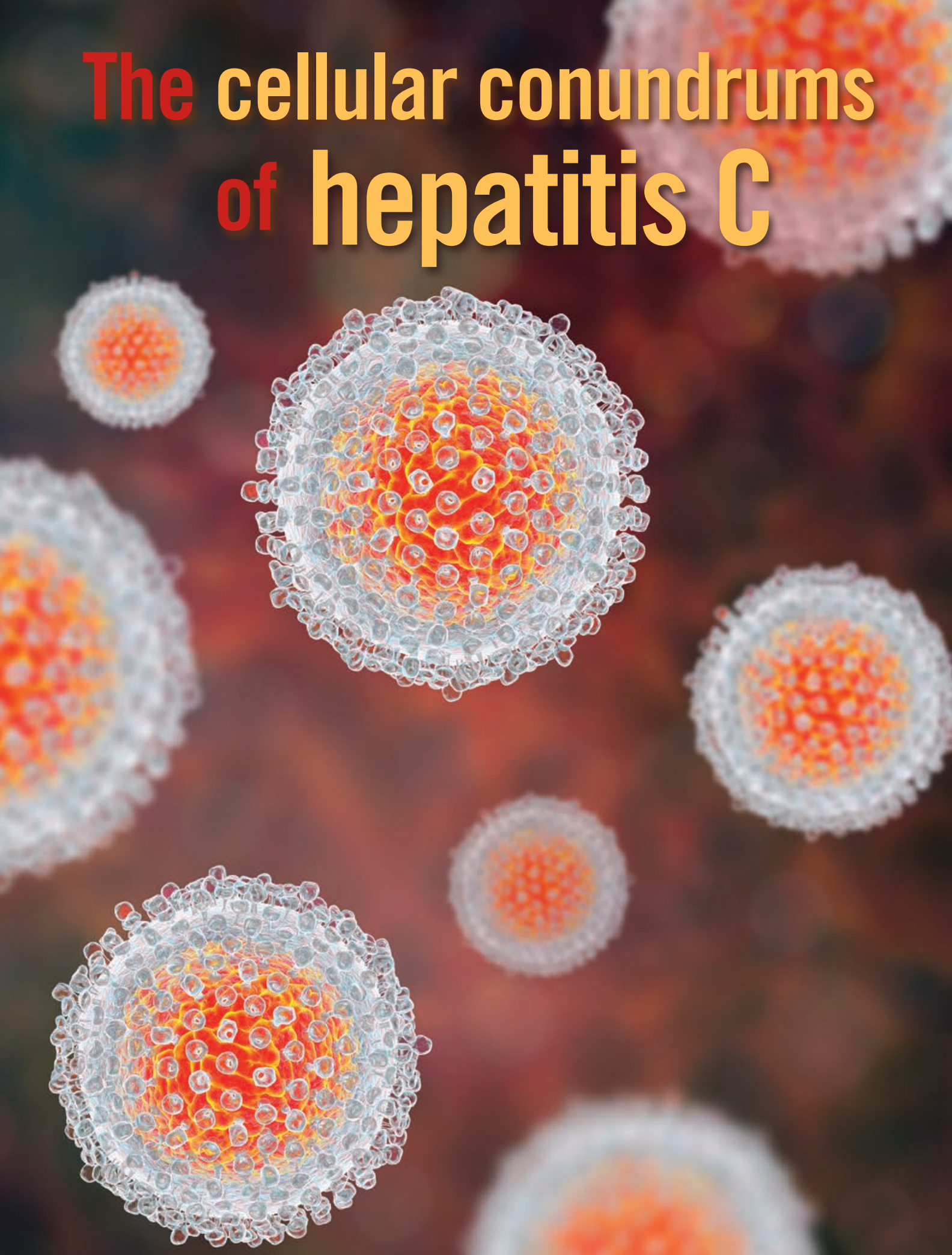
JLR

JOURNAL
OF LIPID
RESEARCH

MCP

MOLECULAR
& CELLULAR
PROTEOMICS

The cellular conundrums of hepatitis C



Charles Rice, who shared the 2020 Nobel Prize in physiology or medicine, has spent his career unraveling the genomic secrets of flaviviruses and other positive-strand RNA viruses



By John Arnst

Hepatitis C is a rarity among the viruses that infect humans on a global scale. Although an effective vaccine still is being developed for it, properly administered antivirals can eliminate the virus in 95% of the people who are infected with it.

But it hasn't been an easy nut to crack. When the virus was isolated in 1989, scientists ran into repeated dead ends as they tried to get it to replicate in cell culture. The journey to develop an effective system in which to grow and study HCV involved hundreds of scientists and took more than 15 years.

In October, Charles Rice at The Rockefeller University, along with Harvey J. Alter at the National Institutes of Health and Michael Houghton at the University of Alberta, won the Nobel Prize in physiology or medicine for their seminal discoveries leading to the identification of the hepatitis C virus.

"It was a huge surprise to me, and a shock, and a very humbling experience," said Rice. "When you think about the many people that have been involved in this success story, it's something that has spanned decades and involved thousands of dedicated scientists and clinicians to really get to where we are today."

An estimated 71 million people worldwide are chronically infected with hepatitis C virus. Without treatment, a significant number of those who are chronically infected will develop cirrhosis or liver cancer.

Epidemic jaundice

The Hippocratic physicians are credited with the first descriptions of contagious jaundice in the fifth century B.C. The condition, the hallmarks of which are yellowing of the skin and the whites of the eyes and dark urine, occurs when liver inflammation or a blocked bile duct allows bilirubin to build up in the blood.

"The connection made between the liver and jaundice was remarkable, bearing in mind that the Hippocratic physicians had not performed dissections and that their medical views were based on observation," Niki Papavramidou,

COURTESY OF CHARLES RICE



Charles Rice, who won this year's Nobel Prize in physiology or medicine with Harvey Alter and Michael Houghton, also won the Lasker Award in 2016 with Ralf Bartenschlager and Michael Sofia for developing a system to study the replication of the virus that causes hepatitis C.

The ABCDEs of hepatitis

While overconsumption of alcohol, environmental toxins and autoimmune disease play significant roles in global incidence of liver inflammation, or hepatitis, viral infections cause the majority of cases.

The Hippocratic physicians in the fifth century B.C. identified five types of jaundice based upon the color of patients' skin, urine and feces and other symptoms. By coincidence, there are five hepatitis-causing viruses.

Hepatitis A, B, C and E viruses all cause liver inflammation, but none of the viruses shares close lineage.

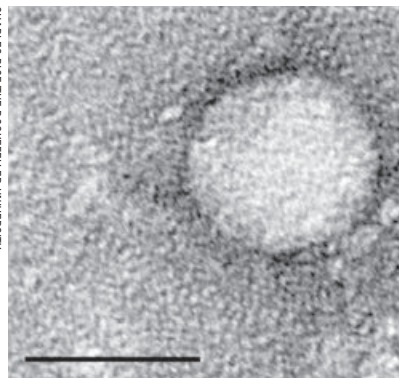
Hepatitis A and E are transmitted by contaminated food or water and cause acute disease.

Hepatitis B and C, as well as hepatitis D, which can propagate only in the presence of hepatitis B, are chronic blood-borne pathogens that spread through intravenous drug use and blood transfusions.

Together, blood-borne hepatitis viruses, which result in cirrhosis and liver cancer, cause more than 1 million deaths each year.

Hepatitis C virus, pictured here with a scale bar of 50 nanometers, currently infects approximately 71 million people worldwide.

CHARLES RICE/THE ROCKEFELLER UNIVERSITY



Elizabeth Fee and Helen Christopoulou-Aletra wrote in the *Journal of Gastrointestinal Surgery* in 2007.

Epidemic jaundice was recorded in the 17th and 18th centuries. Tens of thousands of Union soldiers during the American Civil War were sickened.

By the 1940s, scientists had begun to differentiate between hepatitis A and B by comparing their incubation times.

During World War II, hundreds of thousands of American and European troops contracted viral hepatitis. In one case, upward of 330,000 U.S. service members contracted HBV after being given a yellow fever vaccination that contained contaminated human blood serum.

Medical anthropologist Baruch “Barry” Blumberg discovered HBV serendipitously in 1967. He was interested in how inherited traits make people more or less susceptible to disease and for his studies traveled the globe collecting blood samples from remote populations.

He found a surface antigen for hepatitis B in the blood of an Indigenous Australian, which led to the identification of the virus and its role in causing acute and chronic hepatitis and liver cancer.

In 1976, Blumberg, along with D. Carleton Gajdusek, won the Nobel Prize for physiology or medicine for their work on the virus. Blumberg’s birthday, July 28, was adopted as World Hepatitis Day in 2008.

Not A or B, but C

In the early 1970s, National Institutes of Health physician Harvey J. Alter was investigating hepatitis in patients who had received blood transfusions but had tested negative for both the A and B viruses.

The new illness, then known as

“non-A, non-B” hepatitis and now known as hepatitis C, became a priority for Michael Houghton, who was working for the pharmaceutical firm Chiron at the time.

In 1989, Houghton and his colleagues Qui-Lim Choo and George Kuo isolated a near-complete genetic sequence of HCV, which allowed other scientists to develop blood-based screening tests for it.

That same year, Charles Rice, then at the Washington University School of Medicine in St. Louis, developed the first infectious clone of yellow fever virus, the prototype flavivirus in the family Flaviviridae, which also includes the mosquito-borne dengue, Zika and West Nile viruses.

Not long after, Rice received a call from Stephen Feinstone at the U.S. Food and Drug Administration, who wanted him to retool his work to tackle this new flaviviruslike agent — HCV.

Rice’s flaviviral investigations

After graduating with a bachelor’s degree in zoology from the University of California, Davis, in 1974, Rice began graduate school at the California Institute of Technology. He initially planned to continue his undergraduate research in developmental biology, but his first lab placement was with virologist James H. Strauss Jr.

“I started working in the Strauss lab, which was really the only active virology lab at Caltech,” Rice said. “And I really enjoyed that, so I just stayed there.”

Near the end of his doctoral work, completed in 1981, Rice began studying the mosquito-borne yellow fever virus, after which both the genus *Flavivirus* and family *Flaviviridae* are named. He continued his research as a postdoctoral fellow in Strauss’ lab for

the next four years.

After briefly working at the Australian National University with Lynn Dalgarno, who had co-discovered with John Shine in 1973 the Shine–Dalgarno sequence involved in mRNA translation initiation, Rice took a faculty position at WUSTL in 1986. There, he continued to work with yellow fever virus.

“At Caltech, we’d already determined the genome structure of the virus and had some ideas about what the expression strategy might be for the viral proteins,” Rice said. “But at Wash U, I was actually trying to build an infectious clone for yellow fever that would allow us to do more directed genetic manipulations on this virus to understand more about how it works.”



ARASH GRAKOU

When Rice was setting up his lab in early 1987, the first person he hired was Arash Grakoui,

who had just earned an undergraduate degree and was looking for employment as a lab tech.

“It was Charlie and me, and we had a bench, sitting across from one another. And soon there was a graduate student, and then a postdoc as well,” said Grakoui, now a professor of medicine at Emory University. “Right off the bat, he trained me as a student or postdoc in the lab and let me have a lot of freedom to be creative.”

The next few years also saw a spate of more unusual hires, and Rice’s lab began working with HCV. Brett Lindenbach, a professor of microbiology at Yale University, was a graduate student in Rice’s lab through the 1990s

and worked closely with Alexander “Sasha” Kolykhalov.

“After the Soviet Union collapsed, a lot of very excellent — like, literally, some of the top Russian



BRETT LINDENBACH

virologists — just came and worked in Charlie’s lab more or less as postdocs,” said Linden-

bach, who also did his second postdoctoral fellowship with Rice at The Rockefeller University. “Most of them came from Novosibirsk, which is where the Soviet Union did its bioweapons virus research.”

Kolykhalov and Rice began building consensus clones, full-length complementary DNAs (called cDNAs for short) of HCV that are cloned within bacterial plasmids, but the clones were never functional in cell culture or in chimpanzees, the only other animal the virus is known to infect, Lindenbach said. This led them to believe that they might be dealing with an incomplete genome.

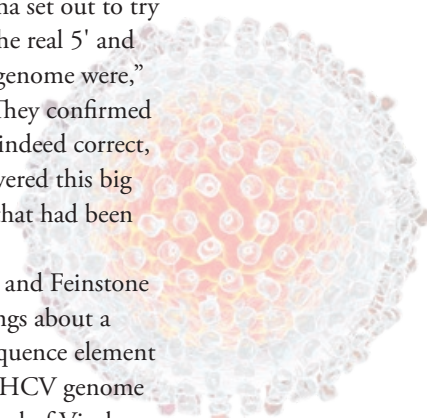
“Charlie and Sasha set out to try and discover what the real 5’ and 3’ ends of the viral genome were,” Lindenbach said. “They confirmed that the 5’ end was indeed correct, but then they discovered this big piece of the 3’ end that had been missing.”

Kolykhalov, Rice and Feinstone reported their findings about a highly conserved sequence element at the 3’ end of the HCV genome in 1996 in the *Journal of Virology*. The next year, Rice reconstructed a full-length consensus clone, a single cDNA clone representing the average of variants within the viral

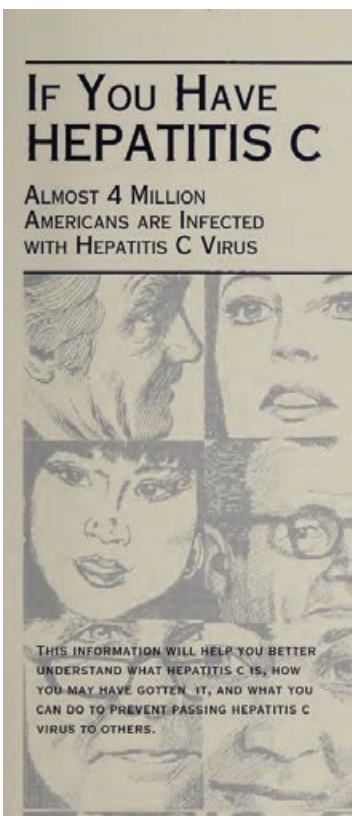
COURTESY OF ARASH GRAKOU



Arash Grakoui, left, who was the first lab tech that Charlie Rice hired in 1987 when he was starting his lab at WUSTL, has gone on several excursions to Wyoming over the years with Rice, right, whom he describes as an avid outdoorsman.



CENTERS FOR DISEASE CONTROL AND PREVENTION



In 1998, the Centers for Disease Control and Prevention put out educational pamphlets about testing and diagnoses for hepatitis C virus, with which nearly 4 million Americans were then infected.

population, but still had no system to test it in.

Despite the researchers' best efforts, the variant refused to replicate in any established cell lines. So Feinstone injected the viral variant into the livers of two chimps. (Author's note: In 2015, the National Institutes of Health announced that it would no longer support biomedical research involving chimpanzees.)

"This was a Hail Mary sort of experiment, because this is a 10,000-base-pair-long RNA," Rice said. "And if you're just injecting it into an organ in vivo and hoping that somehow it gets inside of a cell so it can be translated by that machinery, you're hoping to get lucky."

Rice, Feinstone and their co-authors did get lucky: The virus caused changes in blood and pathology resembling those seen in humans with hepatitis C, providing direct evidence that HCV was responsible for the transfusion-mediated hepatitis.

Their 1997 paper, published in the journal *Science*, noted that the scientists had fulfilled Koch's postulates on a molecular level: An infectious organism must be present during each case of a disease and must be able to be isolated and purified, and the organism must cause the disease when a new host is infected with it.

"I recall pointing out that this helps to prove that HCV is the causative agent of hepatitis C, because it fulfills sort of a molecular form of Koch's postulates," Lindenbach said. "And Charlie thought that was a great idea and wrote that into the paper."

Hepatic breakthrough

While the researchers lacked a way to study the virus in cell culture, a model arrived in 1999. Ralf Bartenschlager, a professor at the University of Mainz, and Volker

Lohmann figured out how to select for a variant of the virus to replicate in HuH7 cells originally derived from a cancerous tumor in human liver through use of a subgenomic replicon system.

"What they did was to chop out a chunk of the genome that encoded what we believed to be the structural proteins of the virus, the glycoproteins and the capsid protein, and replace that with a drug-selectable marker," Rice said.

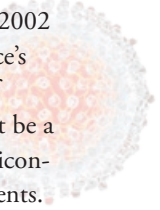
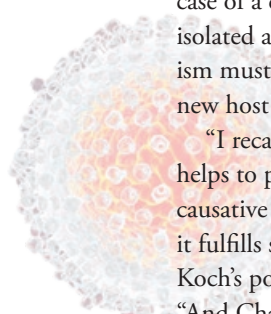
That marker was a neomycin gene that confers resistance to the drug G418, to which human cells are sensitive. By exposing the hepatic cells to G418, the cells that did not support the HCV replicon and take up the drug resistance gene were wiped out, leaving only HCV-laden cells for the researchers to pore over.

"If you're looking for a needle in a haystack, you need a big magnet. And that's what this was," Lindenbach said.

Rice and his colleagues then found that variants of HCV in the subgenomic replicon system harbored mutations that were adaptive to their environment. When those mutations then were engineered into new replicons, the researchers found that they could increase the efficiency of the system more than 10,000-fold.

Another advance came in 2002 when Keril Blight, one of Rice's postdocs, helped cure cells of replicons to see if there might be a selection for more HCV replicon-permissive cellular environments. The increased permissiveness of one of the cured cell lines allowed the researchers to detect HCV RNA and antigens early after RNA transfection, which eliminated the need for selection of replication-positive cells.

The final breakthroughs for grow-



ing hepatitis C arrived in 2005, when a team that included Japanese virologist Takaji Wakita and Bartenschlager found that a rare isolate of HCV from a patient in Japan with acute fulminant disease was able to undergo its entire life cycle in cell culture without induced mutations. At the same time, Rice's group, including Lindenbach, described a chimeric full-length HCV genome that replicated and produced virus particles that were infectious in cell culture, and a group led by Frank Chisari at Scripps Research reported a robust HCV replication system that used the JFH-1 molecular clone and HuH-7-derived cell lines.

In the years that followed, researchers were able to develop direct-acting antivirals for hepatitis C. Until that point, the viral infection had been treated with the more broadly acting antivirals pegylated interferon and ribavirin, a combination that carried significant side effects and only boasted a 50% success rate of eliminating the virus, Rice said.

In 2014, the FDA approved the direct-acting combination pill Harvoni made up of the drugs ledipasvir and sofosbuvir, which are effective in eliminating HCV in more than 95% of the people who take them.

According to Craig Cameron, a virologist at the University of North Carolina at Chapel Hill, while HCV is able to develop resistance to individual drugs, a combination of antivirals that target different viral functions kills the virus outright.

"If you hit it hard enough, long enough, you can force it to extinction," Cameron said.

Nice catch

In 2001, Rice moved his lab to Rockefeller in New York. In 2002, he convinced Grakoui, who had enrolled as a graduate student at WUSTL in

COURTESY OF ARASH GRAKOU



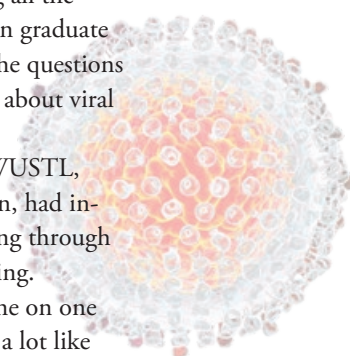
From left, John Majors, Stan Birge, Sadie (dog), Peggy MacDonald, Claire Birge, Charles Rice, Arash Grakoui and Holly Hanson visit Wyoming in 1995.

the lab of immunologist Paul Allen in 1994 after seven years in Rice's lab, to join him as a postdoctoral fellow.

"Coming back as a postdoc really enabled me to bring along all the tools that I had gathered in graduate school to tackle some of the questions that we were interested in about viral immunology," he said.

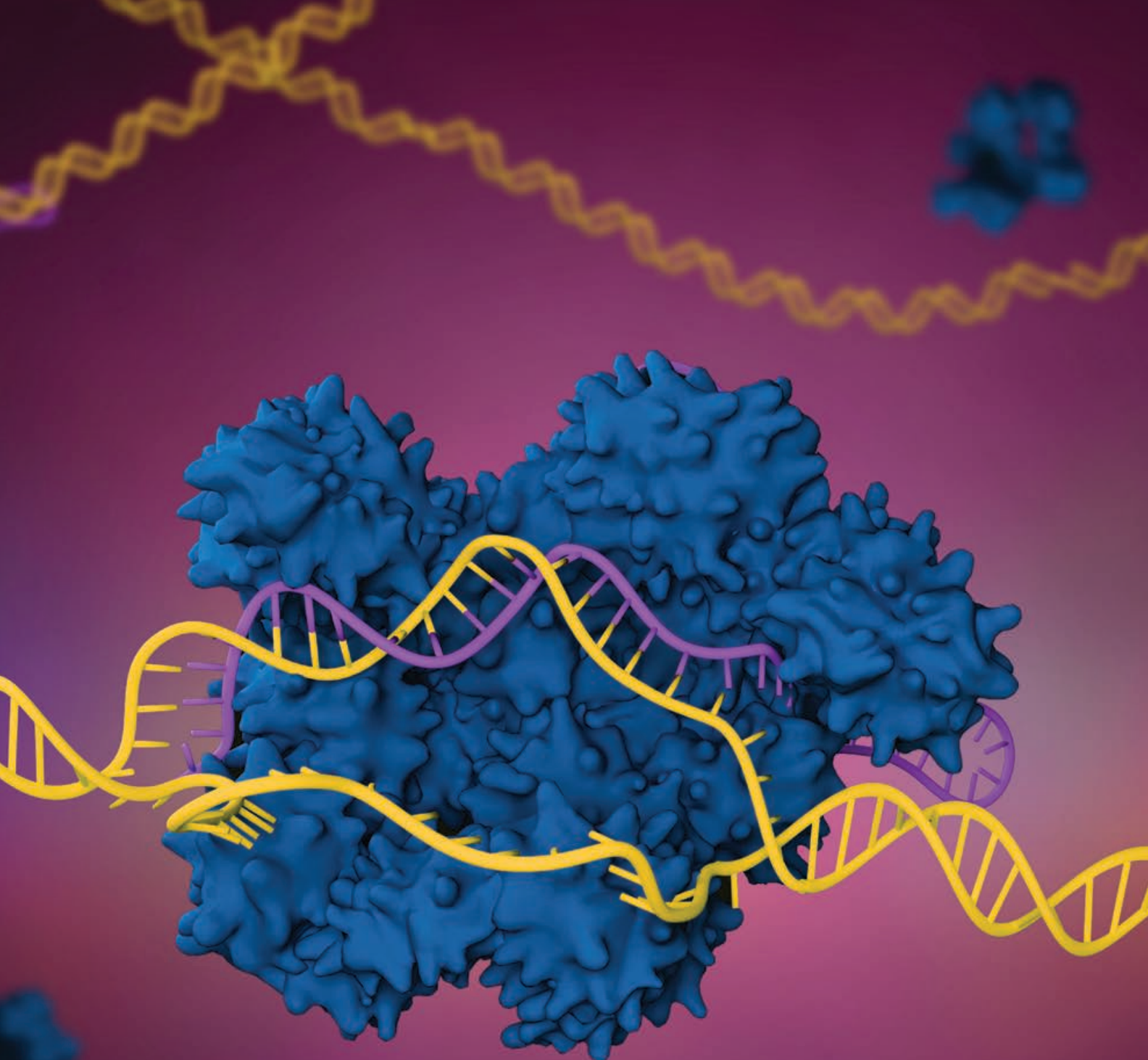
During their years at WUSTL, Rice, an avid outdoorsman, had introduced Grakoui to fishing through long excursions to Wyoming.

"Charlie described to me on one of our trips that fishing is a lot like science: You don't actually expect to catch anything, but if you do, it is incredible," Grakoui said. "You have done the best you can, and you just let nature takes its course."



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





FROM **bacterial immunity**
TO **scientific revolution**

CRISPR's sprint to the gold

By Laurel Oldach

What can I tell you about CRISPR that you haven't heard before?

The technology has been the subject of countless journal clubs and chalk talks; it has exploded in citation count and triggered a wave of creative applications that has yet to crest. It has swept through biology labs, enabling researchers to modify genomes from *Aspergillus* to zebra mussel.

No matter how groundbreaking a discovery is, by the time it wins the Nobel Prize, the dust usually has settled. The finding or technique matures, becoming another slab in the foundations of science.

CRISPR is different. Some postdocs now using the technology as a matter of course were graduate students when its potential first was grasped. The most important molecular biology discovery of the 21st century is only 8 years old.

HALBAUER & FIORETTI, BRAUNSCHWEIG, GERMANY



The most important molecular biology discovery of the 21st century is only 8 years old.

Emmanuelle Charpentier is the founding director of the Max Planck Unit for the Science of Pathogens in and a professor at the Humboldt University in Berlin.

A strange season to celebrate

On the day it was announced that this year's Nobel Prize in chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for their joint contribution to the use of CRISPR–Cas9 as a technology for genome editing, Charpentier remarked ruefully to an interviewer from the Nobel press office, “You connect to (the news), but you think it's another person, or it's surreal. ... Now it's real, and now I have to deal with it.”

A month later, Doudna told this reporter, “It's been a whirlwind, as you can imagine. But there's so much going on in the world right now that's a bit horrifying, but also motivating for scientists.”

Before the Nobel announcement on Oct. 7, Doudna was focused on pandemic response at the Innovative Genomics Institute, which she

INNOVATIVE GENOMICS INSTITUTE



Jennifer Doudna is a professor at the University of California, Berkeley, and founder of San Francisco's Innovative Genomic Institute.



COURTESY OF MEGAN HOCHSTRASSER

Megan Hochstrasser works in education and outreach at the Institute for Innovative Genomics. “One of the things that sets Jennifer apart from people is that she really sees the value in communicating things well,” Hochstrasser said.

founded in 2014 and co-leads. That work includes both a clinical testing program launched in the spring, and a more recently announced diagnostic technique for using CRISPR ribonucleoproteins and a cell phone to detect SARS-CoV-2 in patient samples.

“I wish we weren’t having to do that, obviously,” Doudna said of the IGI’s all-hands-on-deck pandemic response. Then she added, “I think this is what science needs to do. As academics, we’re not always trained to do this. But ... we need to be nimble, and we need to be able to ask ourselves: How does our expertise apply in this moment?”

Megan Hochstrasser earned her Ph.D. in the Doudna lab working on CRISPR and now does outreach and education at the IGI, which she views as Doudna’s effort “to make sure CRISPR leaves the lab and reaches the real world — and on top of that, make sure that the way it reaches the world is positive.”

Hochstrasser has witnessed the evolution of CRISPR from when she was a grad student, when she got “to see a technology develop into something wildly important, when it started out as kind of an esoteric thing.” Since moving to the IGI, she has watched it “grow from being very tiny to starting to get much bigger and more impactful.”

The IGI also brings together a team of scientists who can pool their expertise. In many cases, Doudna said, “There’s no one of us that has the whole package of expertise ... so we need to work together to do something that no one of us would be capable of doing on our own.”

Collaboration is exactly how Doudna got into the CRISPR field in the first place.

A fashionable topic

Nobel-winning duos in the biochemical sciences often are scientists who have spent a significant fraction of their careers working together. Doudna and Charpentier’s collaboration, in contrast, was brief and electrifying. They published just two research articles together — two articles with massive impact.

The idea of programmable genome engineering began long before their CRISPR work did. Restriction enzymes, which cut DNA at specific sequences, first were described in the 1960s. The discovery led researchers to reason that if they could alter a restriction enzyme’s cut site, they could modify DNA sequences with exquisite precision. For a time in the early 2000s, researchers interested in programmable DNA cutting focused on two classes of proteins called zinc finger nucleases and transcription activatorlike effector nucleases, or TALENs. Both are made up of modular DNA-binding protein domains, which can be daisy chained to recognize a given sequence. But both proved difficult to engineer.

Doudna and Charpentier demonstrated that a new programmable system for DNA cutting could be simplified to just two components: one protein and one RNA.

Microbiologists had been aware for some time of a curious repeating feature in bacterial genomes called clustered regularly interspaced short palindromic repeats, or CRISPR for short (see “Yogurt research, broad applications” on page 40). In 2005, Spanish scientists discovered that the spacer sequences in between the repeats matched up to the genes of viruses that infect bacteria. They proposed that the system might represent a kind of bacterial adaptive immunity: a way to recollect and respond to



A tool for genome editing: CRISPR-Cas9

Scientist Spotlight

CRISPR-Cas9 has been touted as one of the most revolutionary discoveries of the decade. Meet Dr. Jennifer Doudna and Dr. Emmanuelle Charpentier, the first two women to ever hold the Nobel Prize in Chemistry together for their discovery of CRISPR-Cas9. Growing up in small towns, Dr. Doudna and Dr. Charpentier (in the USA and France, respectively) never expected that this would happen to them.



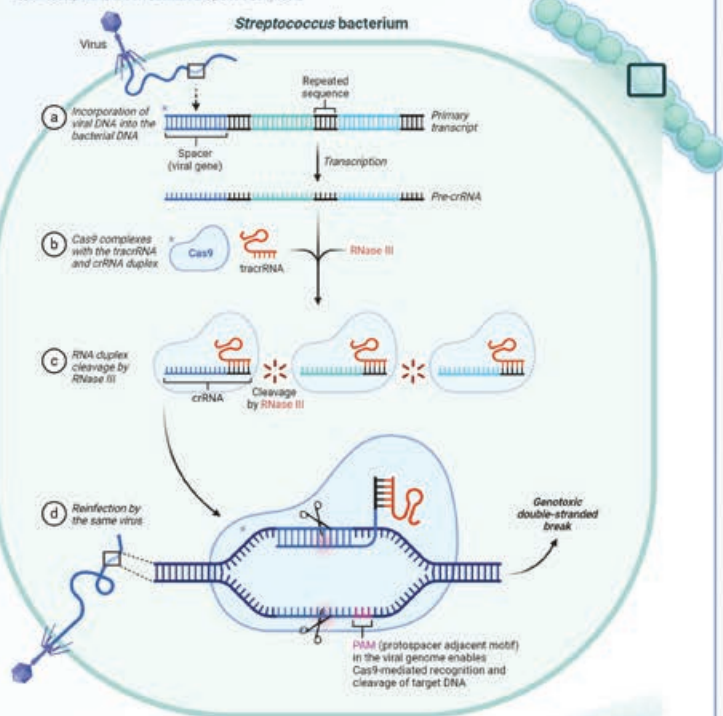
Dr. Jennifer Doudna



Dr. Emmanuelle Charpentier

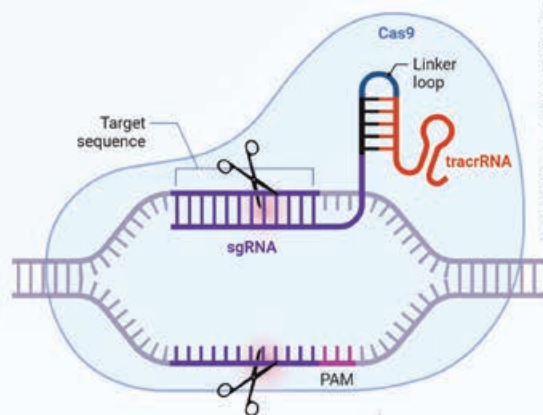
Streptococcus Adaptive Immune System against Viruses: CRISPR-Cas9

^a Schematic representations of nucleotides and proteins are symbolic.



- a** Viral DNA is inserted into the bacterial genome in between the repeated CRISPR sequences.
- b** tracrRNAs recognize CRISPR RNA sequences and target Cas9 enzymes to the CRISPR RNA.
- c** The CRISPR RNA:tracrRNA duplex is cleaved off of the longer pre-crRNA.
- d** If the bacterium is infected by the same virus, the Cas9:crRNA generated during the first infection recognizes PAM motifs in the viral genome, enabling target-DNA recognition and Cas9-mediated cleavage to prevent re-infection.

The CRISPR-Cas9 Genetic Scissors



When researchers edit a genome using the CRISPR-Cas9 genetic scissors, they artificially construct a **single guide RNA (sgRNA)**, which matches the DNA code where the cut is to be made.

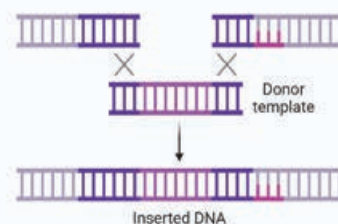
The scissor protein, **Cas9**, forms a complex with the **sgRNA**, and directs the scissors to the precise location in the genome where the cut will be made.

a Non-homologous end joining (NHEJ)



Gene disruption by small insertions or deletions

b Homology-directed repair (HDR)



Gene correction or insertion by assisted recombination

JERRY GU, JUNG-HEE LEE, VICTORIA TOKARZ & CINDY LIU / COURTESY OF BIORENDER

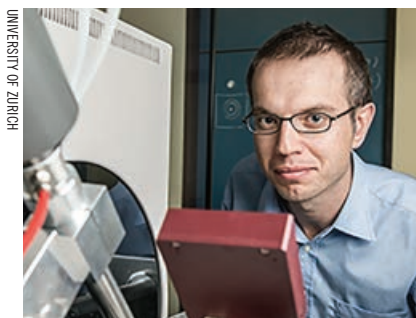
pathogens encountered before.

“CRISPR became a super fashionable topic,” said Krzysztof Chylinski, who developed an interest in CRISPR himself when he joined Charpentier’s lab. He chalked up its popularity not just to the novelty of a complex adaptive immunity system in an organism no one had expected to contain one but also to the possible applications

that occurred to many researchers.

The hypothesis of a bacterial immune system caught on, and a number of labs began to work on characterizing the CRISPR system and the CRISPR-associated, or Cas, proteins it requires.

Charpentier’s microbiology lab in the Max Perutz Laboratories at the University of Vienna was one of



UNIVERSITY OF ZÜRICH

Martin Jinek was a postdoctoral researcher in Doudna's lab and was co-first author on the 2012 paper describing the mechanism of Cas9.



COURTESY OF KRZYSZTOF CHYLINSKI

Krzysztof Chylinski was a graduate student in Emmanuelle Charpentier's lab and was co-first author on the 2012 paper describing the mechanism of Cas9.

them. The lab, which Charpentier had founded in 2002, worked on virulence factors in various pathogenic bacteria, including an RNA-regulated system in *Streptococcus pyogenes*. A bioinformatics screen they conducted in 2006 revealed that *pyogenes* too had a collection of clustered regularly interspaced repeats. They became interested in a noncoding RNA near the CRISPR locus that they thought might regulate the CRISPR system's activity.

Around the same time, Doudna, a structural biologist who had been focused on RNA interference in eukaryotic cells, learned about CRISPR through a conversation with Jillian Banfield, a colleague at the University of California, Berkeley. Intrigued by the similarities between eukaryotic RNAi and the CRISPR system, Doudna started investigating the structure and activity of Cas enzymes in *Pseudomonas aeruginosa* and, with collaborator Eva Nogales, published in 2011 a cryogenic electron microscopy structure of the *E. coli* CRISPR effector, a complex with many protein components.

Also in 2011, Chylinski was second author on a report from Charpentier's lab. They found that the noncoding RNA they had taken an interest in, dubbed the transactivating CRISPR or tracrRNA, had to be present for the *S. pyogenes* CRISPR RNAs to mature into an active form.

There are some important differences between the *E. coli* and *S. pyogenes* CRISPR systems. Whereas *E. coli* and a majority of other bacteria have systems with many proteins

guided by just one RNA, Charpentier's lab had realized that *S. pyogenes* requires two RNAs. Soon they would find that the system also involved only one effector protein, called Cas9.

The simplicity of the *S. pyogenes* system was tantalizing. Chylinski explained, "If in one system, you have like six proteins that are doing something, and in the other there is one, it must be one hell of a special protein."

The same type of scientist

Doudna and Charpentier met at a CRISPR conference in San Juan, Puerto Rico, in 2011 when the CRISPR field was still small enough to fit into a single room. They hit it off right away, both sharing a love of hard problems and careful investigation. Charpentier told a Nobel interviewer, "We are the same type of scientist, who want to see the details of the data."

The two labs came together for an often-described transcontinental and transoceanic collaboration. Though it involved just two labs, it took place in three locations: Berkeley, Vienna, and Umeå — a city in Sweden to which Charpentier was moving her lab. Soon Chylinski and Martin Jinek, a postdoc in the Doudna lab, were hard at work using purified Cas9 protein and tracr and CRISPR RNAs to understand how Cas9 cuts target DNA.



EVA NOGALES

The work went smoothly. None of the researchers recalled any extraordinary roadblocks.

"If it would be easy, everything would have been discovered already," Chylinski said. But in this project, the hurdles that arose were "just, you know, regular troubleshooting, and let's move forward."

The results, too, were clear. "Sometimes you work on systems, and it takes a long time to see what you would like to see — or the result is

not black and white, it's light gray or dark gray," Charpentier explained to a Nobel interviewer. "Here, it was really white."

The team found that base pairing between the tracr and CRISPR RNA could lead Cas9 to cut a DNA target that matched the CRISPR RNA so long as the target DNA included a protospacer-adjacent motif nearby. They also demonstrated that a single engineered hairpin RNA could perform the same role — in other words, they established a two-component system for genetic engineering.

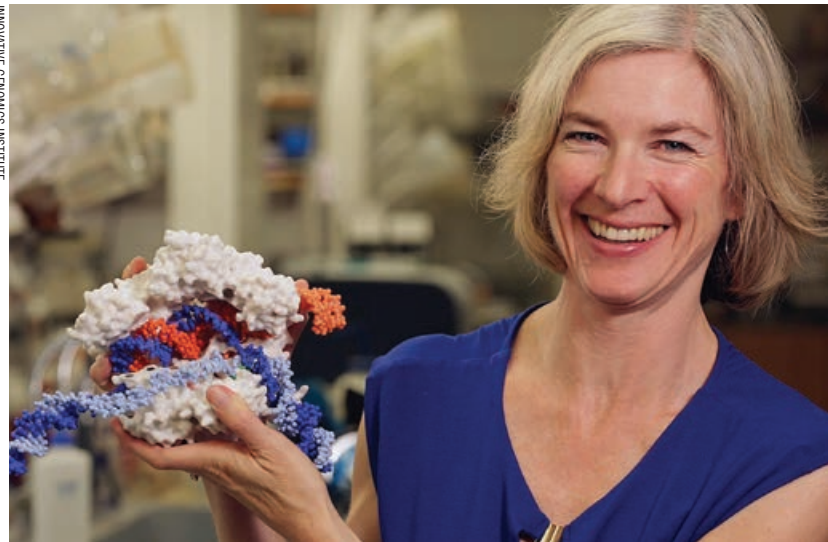
Because CRISPR had intrigued so many researchers, the team knew that they needed to publish soon to beat their competitors.

"Towards the end ... it became kind of an around-the-clock operation," Jinek said. "We took advantage of the time difference." He would wake up to an email from Chylinski reporting on the day's results and would send a similar email before he headed home.

The work was published in *Science* in 2012. A year and a half later, they followed up, again in *Science*, with a detailed structural description of two Cas9 proteins from two bacterial species and how each of them coordinated DNA and RNA.

CRISPR's big break

The CRISPR field already was moving fast. Once the 2012 paper on reprogramming Cas9 was published, it exploded. Labs around the world picked it up rapidly. Already by the time of their second paper, Doudna, Charpentier and Nogales could cite seven major articles that had used CRISPR and Cas to induce double-stranded breaks in DNA, modulate transcriptional activity and modify eukaryotic genomes — and those were just the projects that already had



INNOVATIVE GENOMICS INSTITUTE

been published.

"All of a sudden, tons and tons of labs could afford genome engineering as a very standard thing," Chylinski said. He fielded a slew of technical questions about the tool while finishing his Ph.D., and after graduation he took a job at a small nonprofit that provides technical services to help research labs implement the technology.

Doudna and Charpentier each launched biotechnology companies in 2013: Charpentier started CRISPR Therapeutics, while Doudna (who two years prior had started Caribou Biosciences based on her own lab's CRISPR work) teamed up with Feng Zhang of the Broad Institute and George Church from Harvard and MIT, along with several others, to start Editas Medicine. Later, overlapping applications for patents on Cas9-based genome editing pulled the UC system and the Broad Institute into a long-running, bitterly fought and well-publicized dispute over ownership of the rights to use CRISPR–Cas9 genome editing in eukaryotic cells.

Though it's still unclear who will win commercial rights to CRISPR–Cas9, in basic research the outcome is clear. "Everybody uses it," said RNA structural biologist Joan Steitz, who

A 3D-printed model of the structure of Cas9 (white) in complex with an RNA guide and DNA template, based on the structure that Doudna's lab solved, is one of her signature tools for science communication.

More reading on how CRISPR was discovered

A Crack in Creation

by Jennifer Doudna with Samuel Sternberg

"The biology of CRISPR–Cas: Backward and forward"

by Charpentier and colleagues in *Cell*

"A day with Jennifer Doudna"

by Lisa Jarvis for *Chemistry & Engineering News*

"The quiet revolutionary"

by Allison Abbott for *Nature*

"The promise and challenge of therapeutic genome editing"

by Jennifer Doudna in *Nature*

The many uses for CRISPR

The mechanistic understanding of bacterial immune systems that Jennifer Doudna and Emmanuelle Charpentier contributed to has engendered a huge explosion of creative uses for the technology. There are far too many to cover them all, but here are a few intriguing applications. Some are clearly translational, while others solve problems in basic research. Either way, the first thing that any CRISPR scientist will tell you about their field is that basic research can have applications no one would have predicted. Researchers have used CRISPR technology:

- To record transcription events in a living cell with reverse transcription
- As a target, in its native bacteria, for new antibiotics
- To edit DNA only in cells illuminated with blue light through photo-activated dimerization
- To knock out every gene in a cancer cell line one by one, finding drivers of drug resistance
- To alter genome architecture by bringing two DNA regions together
- To power gene drives that can propagate through mosquito populations
- To generate more realistic, genetically diverse tumors in animal models
- To manipulate RNA levels without altering DNA

knew Doudna as a postdoc at the University of Colorado and a junior professor at Yale.

Societal implications

At this year's inaugural World CRISPR Day on Oct. 20 sponsored by the CRISPR service company Synthego, Fyodor Urnov shared a story about stepping out of his office at the IGI, which he co-leads with Doudna, for an espresso a week or two before the Nobel was announced.

He happened to be wearing a CRISPR T-shirt. The barista asked, "Do you do that genome editing thing?"

Yes, Urnov said. "In fact, I work with Jennifer Doudna."

Impressed, the barista gave him the coffee on the house.

Even before the prize, Doudna and Charpentier had reached international celebrity status, racking up a series of other high-profile recognitions. The pair shared a Breakthrough Prize in 2015, a Tang Prize in 2016 with CRISPR researcher Feng Zhang, a Japan Prize in 2017, a Kavli Prize in 2018 with Lithuanian CRISPR researcher Virginijus Šikšnys and a Wolf Prize earlier this year. In the wake of their discovery, both have founded institutes that they now lead: Doudna at the IGI and Charpentier, in 2018, at the Max Planck Unit for the Science of Pathogens in Berlin.

Of the two, Doudna seems more comfortable stepping onto the global scientific stage. Both have been involved in public conversations about the ethics of CRISPR, but Doudna has been more focused on bringing that conversation to nonscientists and has become the public face of the technology and its implications.

"For many scientists, this is a foreign area: We're not trained to talk about our work more broadly," she said. "Many of us prefer to do the next experiment rather than stepping out of the lab and thinking about how to communicate our science to non-specialists — but I do think it's essential to do that."

Urnov said during his World



FYODOR URNOV

CRISPR Day talk, "I salute Jennifer, personally, for taking such a clear citizen-scientist role," adding

that he hopes more scientists will take inspiration from her sense of civic responsibility.

A gifted explainer from the start, Doudna drew inspiration from the famous Asilomar conference in the 1970s, which established scientific guardrails around recombinant DNA. When planning a conference to discuss the implications of CRISPR technology, which took place in Napa Valley in 2015, she invited two researchers who'd attended and organized the original, Paul Berg and David Baltimore.

Many of those same researchers, including Doudna and Baltimore, were at the Second International Summit on Human Genome Editing in November 2018, when Chinese researcher He Jiankui announced the astonishing news that he had edited a receptor called CCR5 out of the genome of human embryos — and that edited twins recently had been born. The work was sloppily executed, and the scientific rationale for altering CCR5 was hazy; in January of this year He was sentenced to a three-year prison term. Scientists around the

world renewed their calls for a moratorium on heritable editing, but He's announcement already had shown the limits of such appeals.

Many more CRISPR systems

While modification of human embryos sucks up a lot of the oxygen in public conversations about genome editing, Urnov may have spoken for more scientists than himself when he said at his World CRISPR Day talk, holding a hand level with his eyebrows, "I've had it up to here" with discussions of germline editing.

The technology has been applied to many societal problems that are less fraught: agriculture, drug development and nonheritable gene therapy, to name a few. Most of the scientists interviewed for this story mentioned Victoria Gray, an American whose sickle cell anemia was cured by edits to stem cells extracted from and then transplanted back into her bone marrow.

CRISPR also has been used in basic research to understand topics as esoteric as the gene controlling snail shell spiral chirality. Meanwhile, the work to understand bacterial immunity goes on — and the applications for bacterial immunity effectors are far from limited to Cas9.

"Now we don't have just plain vanilla CRISPR–Cas9 that does DNA cutting," Jinek said. "We have base editors, we have prime editors, we have transcriptional control through CRISPR ... all these applications that (are) still rooted in the basic molecular principle of having a guide RNA targeting double stranded DNA, but then apply all sorts of other molecular activities."

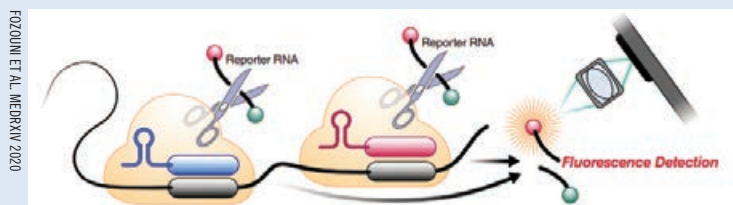
Six general types of CRISPR system are known, and researchers use them for different applications. Last year, for example, Doudna's lab pub-

CRISPR vs. COVID-19

In late September, researchers from Jennifer Doudna's lab and collaborators at the University of California, Berkeley, UC San Francisco and the Chan Zuckerberg Biohub posted a preprint introducing a method for using a CRISPR-based system to diagnose COVID-19 without amplifying its nucleic acids. Instead of the famous Cas9 protein, which cuts DNA at a site of complementarity with a guide RNA, this assay uses the protein Cas13, another effector in the arsenal of bacterial CRISPR immune systems.

The technique is called specific high-sensitivity enzymatic reporter unlocking, or SHERLOCK. Cas13 responds to a guide–target match by cutting the target RNA — and, when purified, snowballing to cut every other RNA in the vicinity. It is well-suited for detecting a small amount of nucleic acid. The assay has four parts: Cas13, its guide RNA, an RNA probe with a fluorescent moiety on one end and a quencher on the other end, and the patient sample. If base pairing happens between RNA in the patient sample and the guide RNA, then Cas13's wild reaction is triggered. Cutting the RNA probe separates the fluorophore from the quencher so that if SARS-CoV-2 RNA is present in the sample, fluorescence occurs.

In addition to adapting the chemistry from previous SHERLOCK applications, the team also came up with a detection box smaller than a shoebox with a stripped-down fluorescence excitation setup, which can be imaged using an ordinary cell phone running a custom app. Twenty-eight scientists, including biochemists, virologists, biomedical engineers and physicists, were involved.



lished cryo-EM structures of CasX, which unlike Cas9 makes a staggered cut in the DNA, producing sticky ends. Other bacterial effector proteins cut RNA instead of DNA, produce cyclic second messenger molecules or simply bind specific sequences. With input from engineers, the list of applications for these protein functions continues to grow (see "The many uses for CRISPR" on page 38).

Researchers have scrambled to find and lay claim to other effector proteins of bacterial immunity by scanning and mining bacterial genomes. Jillian Banfield, the Berkeley microbiologist who introduced Doudna to CRISPR in the first place, runs a company called Metagenomi mining



JILLIAN BANFIELD



Joan Steitz knew Jennifer Doudna as a postdoc and a junior faculty member at Yale. “She didn’t need much mentoring, but everybody can stand some advocacy,” Steitz said.

microbial genomes for novel gene-editing systems; the pharmaceutical company Bayer recently invested \$65 million in the enterprise.

The future for CRISPR scientists

What could I possibly tell you about CRISPR technology that you haven’t heard before? There have been about 48,000 research articles’ worth of new findings this year alone. The technique has spurred close to a decade of explosive creativity in the fields of biotechnology, basic biological research and clinical medicine, and it shows no signs of slowing.

“It often feels like the genome engineering applications made possible by CRISPR are limited only by our collective imagination,” Doudna wrote in her memoir, “A Crack in Creation.” She used to keep a running list of organisms in which the technology had been used; that’s no longer possible.

When asked what it was like to have been instrumental to such an important discovery, Chylinski answered with a puckish grin, “It’s nice.”

After a thoughtful pause, he added more seriously, “At the beginning it was almost intimidating how fast things were going. ... In some places in the world, people are already getting experimental treatments partially based on something I did with my Ph.D. thesis.”

Both Doudna and Charpentier have worked on a variety of topics over their careers, and as the CRISPR field expands, they’ve found new areas within it to work on.

Charpentier has had a peripatetic career, working in France, the U.S., Austria, Sweden and now Germany. “I could feel very quickly the right time to move on,” she told an interviewer from *The CRISPR Journal* in 2019. She said she wanted “not to get stuck in a position where maybe I would not evolve the way I wanted to evolve.”

Steitz observed that Doudna is “interested in all sorts of very specific experimental problems and questions within the whole big realm of RNA and all its functions in cells. She hasn’t been monolithic.”

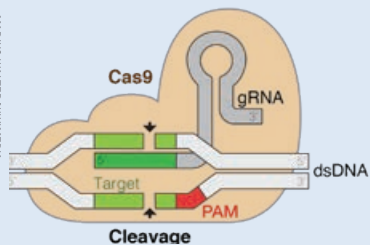
Doudna agreed with that interpretation and said her work is all about a broader exploration of numerous interesting problems. “When I think about how I choose topics, it’s more of a gut feeling,” she said. “It’s the things that I find interesting, or the things that I feel intrigued by, that tend to be the things I’m going to go after.”

Yogurt research, broad applications

After short repeating sequences interspersed with hundreds of diverse spacers were discovered in the 1990s, researchers found them in the genomes of more and more bacterial species. CRISPR-associated, or Cas, genes, always seemed to travel with them.

In 2005, CRISPR researcher Francisco Mojica raised the hypothesis that the spacers, which matched phage sequences, could help bacteria recognize pathogens. The same year, researchers reported that bacteria with more CRISPR spacers tended to be resistant to more phages. Then researchers at the yogurt company Danisco found that bacteria

that acquired resistance to a phage also picked up a spacer matching its genome. The race was on to find out how bacteria absorb snippets of phage genome into their own — and how Cas family proteins use CRISPR RNA to find and destroy phage DNA.



As it turns out, there are many answers. “It’s not like one mechanism describes everything,” said Krzysztof Chylinski, who co-discovered the mechanism of one Cas protein. “There are two classes, six types and, I don’t know, dozens of subtypes — and some uncharacterized systems.”

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





Wednesdays | 12 p.m. EST

Lipid Research Division Seminar Series

Weekly presentations from researchers highlighting their recent work in the field of lipids. Hosted by the ASBMB's Lipid Research Division.

LEARN MORE AT:

asbmb.org/meetings-events/lrd-seminar-series



ASBMB FELLOWS

Call for nominations: the first class of ASBMB fellows

DEADLINE: JAN. 4, 2021

Selection as a fellow of the American Society for Biochemistry and Molecular Biology is an honor to be bestowed upon our most distinguished members.

The ASBMB Fellows Program encourages nominations that reflect the breadth and diversity of the society's membership. Nominees must be regular, industry or emeritus members of the ASBMB.

asbmb.org/about/asbmb-fellows



1



2



3



4



5

We hope these gift recommendations help you check some of your favorite scientists off your holiday shopping list!

- 1. Women in Science 100 postcards: www.penguinrandomhouse.com
- 2. Portrait in Cells silk charmeuse scarf from Artologica: www.etsy.com
- 3. Black Lives Matter neuron T-shirt: www.bonfire.com
- 4. Black Scientist personalized notebook from By Keeks with Love: www.etsy.com
- 5. Diversity in Science pin from Science on a Postcard: www.etsy.com
- 6. "Goodnight Lab" children's book: shop.sourcebooks.com
- 7. Genotypes and A Perfect Model Organism stickers from DaniEdmunds: www.redbubble.com
- 8. ATP necklace: store.madewithmolecules.com
- 9. Women of Science tarot deck: www.mitpress.mit.edu
- 10. DNA scientific illustrations T-shirt: shop.amnh.org

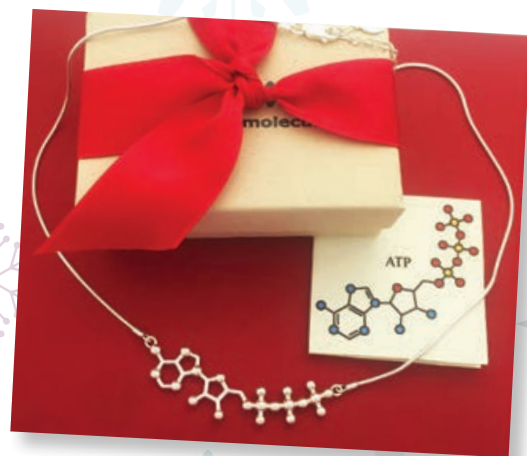
HOLIDAY GIFT GUIDE



6



7



8



9



10

A global champion of ‘the big puzzle’ — biochemistry

Alexandra Newton will be the third woman to serve as IUBMB president

By *Arti Dumbrepatil*

COURTESY OF ALEXANDRA NEWTON



Alexandra Newton, president-elect of the IUBMB, displays her love of science in the form of her favorite amino acid jewelry.

After more than three decades as a biochemist, Alexandra Newton remains on a quest to figure out how molecules interact and work with each other. Specifically, she wants to understand the mechanisms of target molecules such as enzymes in maintaining human health and manifestation of disease.

“For the development of effective therapies, in-depth understanding of the biochemical mechanisms is critical,” she said. “Biochemistry is like a big puzzle, where you are finding and piecing together different pieces. A new piece might take you into new directions. It’s like I’m the detective working toward solving a problem, considering all the clues available. There is never a dull moment.”

Now a professor of pharmacology at the University of California, San Diego, Newton has lived on three continents and completed her education in seven countries. Her father was English, and her mother is from Cyprus. Born in Cape Town, South Africa, she grew up in Canada, Greece, France and other countries. She speaks Greek, English, French and German.

These factors, along with her dedication to biochemistry, helped Newton grow as a scientist and become a mentor to students across the globe. Now they will serve her as the third woman president of the International Union of Biochemistry and Molecular Biology. The union unites biochemists and molecular biologists in 79

countries, promoting research and education throughout the world. The American Society for Biochemistry and Molecular Biology is a member of the IUBMB, and Newton will be the first woman ASBMB member to lead the IUBMB.

Becoming a scientist

As a little girl, Newton was intensely curious about science. “I began experimenting very young,” she said.

For her first experiment, instead of sending a baby molar to the tooth fairy, she measured the tooth’s diameter before and after submerging it in soft drinks to determine the sodas’ effect on dental decay. “My parents told me that soft drinks are not good for you, but I wanted to find out myself,” she said. “So, I do not drink soft drinks.”

During her undergraduate years at Simon Fraser University in Vancouver, Canada, her teachers nurtured her interest in biochemistry. When she was a grad student at Stanford University, membrane biochemistry sparked her interest. She did a postdoc in Daniel Koshland Jr.’s lab at the University of California, studying how lipids modulate the activity of protein kinase C, or PKC, an enzyme involved in metabolic regulation, immune signaling and cell proliferation. Her work has helped researchers understand the mechanism and regulation of PKC.



As a distinguished professor of pharmacology at UC San Diego and co-director of the molecular pharmacology track in the biomedical sciences graduate program, Newton is an advocate for basic biochemistry research. She is also director of Cell Signaling San Diego, a new center that brings together local experts in the field. She continues to explore the molecular mechanisms of cell signaling and their deregulation in diseases, with emphasis on her favorite molecule, PKC, and the phosphatase PHLPP.

Newton's team reversed a 30-year paradigm when they reported their finding that PKC suppresses, rather than promotes, tumors. For decades, attempts to develop drugs that inhibit PKC as a part of cancer treatment had failed, she said. "Our study showed that we needed to develop anti-cancer drugs that would boost PKC activity."

Newton uses PKC as an example of the importance of understanding the biochemistry of a target before developing therapies. Her lab has

found that PKC's activity is enhanced in neurodegenerative diseases: In Alzheimer's, a few atoms' difference in one isozyme of PKC increases its activity, which, in a mouse model, is enough to cause cognitive decline.

An international vision

Newton has been IUBMB's president-elect since July 2018; she will become president in July 2021, and past president in 2024 — a nine-year commitment. Her vision as president is to foster scientific alliances that are inclusive about geographical and gender diversity. The union already provides opportunities to scientists and students from developing countries in programs such as Promoting Research Opportunities for Latin American Biochemists, or PROLAB, a joint initiative with the ASBMB and the Pan-American Society for Biochemistry and Molecular Biology that brings Latin American scientists to work in North American labs.

The IUBMB executive commit-

Pictured at a 2019 conference in Kuala Lumpur are IUBMB Executive Committee members, left to right, Past-President Joan Guinovart, who is from Spain; Alexandra Newton; Treasurer Francesco Bonomi, from Italy; and Chair of Meetings Iлона Concha Grabinger, from Chile.



COURTESY OF ALEXANDRA NEWTON

Alexandra Newton in her lab at UC San Diego with graduate student Alex Jones and postdoc Gema Lordén.

tee has an equal number of men and women, and Newton views the organization as a model for gender diversity. “It is tricky for our virtual executive committee meetings at the moment, which span 18 time zones,” she said, “but our leadership team has members from all over the world.”

Representation of women in science has improved since Newton started as a graduate student; her Stanford class in chemistry included two women. Later, she was the only woman among 43 faculty members in the Indiana University chemistry department. She is proud that her department at UC San Diego has equal numbers of men and women researchers, but she realizes the difficulties women still face in the sciences, and she works to find solutions that will promote inclusion and diversity in the field.

“I always tell anyone who is starting a career in science, focus on the positives,” she said. “Follow your passion and love what you are doing to overcome all the negatives.”

Other challenges have been subsumed by the COVID-19 pandemic, but Newton believes now is

the time for scientists worldwide to come together. She supports communicating science across boundaries in the form of virtual conferences and webinars, seeing the new necessity as an opportunity: “This pandemic has also allowed me to open my courses and lectures for students from different countries.”

She noted that the IUBMB now offers virtual conference fellowships, allowing students from all over the world to attend meetings.

Newton is unfazed at holding a world leadership role.

“I see a silver lining for the unusual year of 2020,” she said. “This pandemic has brought together scientists around the globe. Physical boundaries did not stop scientists from trying to work together. Virtual conferences have not only helped provide access to a larger community but also bought out the innovative ways to communicate science.”

The Newton Lab at their 30-year lab reunion with Alexandra Newton at the bottom of the stairs and the grad students in chronological order, with the current ones at top of the stairs.

TIM BAFFI



Arti Dumbrepatil (artidumbre@gmail.com) is a science writer covering topics ranging from nanorobots to virology. She has a Ph.D. in biochemistry and writes for *Microbiome Digest* and *Bio Voice News*. Follow her on Twitter @rtisciwrites.

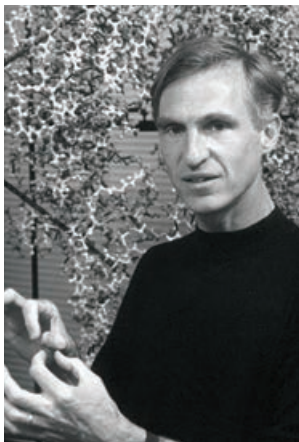


The Death Project

by Robert J. Lefkowitz with Randy Hall

This piece is excerpted from “A Funny Thing Happened on the Way to Stockholm: The Adrenaline-Fueled Adventures of an Accidental Scientist” by Robert Lefkowitz with Randy Hall (published by Pegasus Books) due to be released Feb. 2, 2021.

The phone rang in my office, and when I answered it I received shocking news: my collaborator, the legendary Harvard structural biologist Don Wiley, was missing. Don and I had been collaborating on trying to determine the three-dimensional structure of the beta-2 adrenergic receptor. In the 1970s, my lab had pioneered the purification of the receptor, and in the 1980s we had discovered the receptor’s gene sequence. Now, in the early 2000s, we were collaborating with Wiley’s group to perform X-ray crystallography on purified beta-2 receptors in order to see the receptor’s structure and understand precisely how molecules like adrenaline bound to it. My lab was adept at purifying receptors and Don Wiley was



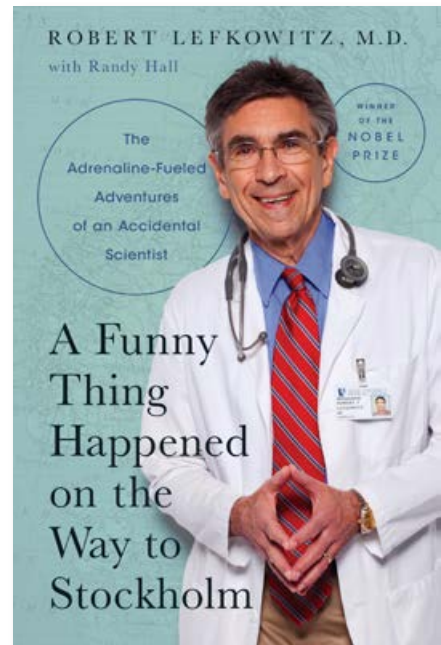
Harvard structural biologist Don Wiley was “one of the best X-ray crystallographers in the world” and an expert on infectious diseases, such as Ebola.

one of the best X-ray crystallographers in the world, so I felt confident about our chances for success. There was only one problem: Wiley had now vanished without a trace.

Don was an amazing scientist and brilliant wit who lit up every room he entered. He could give a talk about a seemingly dry topic, like the structure of a protein, and weave a spellbinding narrative that would have an audience hanging on his every word. As a storyteller myself, I recognized Don

as a kindred spirit and was thrilled to have him as a collaborator. My affection and admiration for Don made his unexplained absence all the more difficult to accept.

Wiley’s disappearance became a national news story, so between reading the news and talking with colleagues



who knew Don, I became aware of all the details of the case. Don had been at a meeting in Memphis at St. Jude Children’s Research Hospital, where he served on a research advisory board. He’d had dinner with some colleagues, and everyone at his table said Don was his usual upbeat and jovial self. Don left the dinner in a rental car to visit his father, who lived in the area, but then never showed up at his father’s house. Police found Don’s car parked on the Hernando de Soto Bridge, a huge overpass spanning the Mississippi River. One obvious possibility was that Don might have stopped on the bridge and jumped to his death. However, Don had never shown the slightest hint of depression and by all accounts was in especially good spirits that evening.

Conspiracy theories

As weeks passed without any break in the case, conspiracy theories began to pop up. Don was one of the world’s top experts on the structure of dangerous viruses, such as the Ebola virus, which led some people to speculate that he may have been kidnapped by terrorists who wanted to develop a biological weapon. This was shortly after the terrorist events of 9/11, so terrorism was very much on everyone’s mind. Others speculated that maybe Don knew too much about some sort of dark secret and was murdered. Several other virus experts had died under mysterious circumstances around the same time, which only fueled speculation about Don’s case.

About a month after Don’s disappearance, his body



TREVOR BIRCHETT

Don Wiley disappeared in November 2001 after a meeting in Memphis at St. Jude Children's Research Hospital. His car was found parked on the Hernando De Soto Bridge, which spans the Mississippi River.

was found in the Mississippi River about three hundred miles south of Memphis. FBI agents and local law enforcement officials examined his body and also performed a careful examination of his car, which had some damage on the right front end. The official theory of Don's death, put forward by the Shelby County Medical Examiner, was that his car had grazed a post on the bridge, which led him to stop his car and get out to inspect the damage. Somehow, he had then accidentally fallen off the bridge to his death. A large truck may have gone past, creating a strong surge of wind that knocked Don off the bridge. Alternatively, as Don did have an epileptic condition and was prone to the occasional seizure, he may have experienced a seizure event (perhaps related to the stress of hitting the post) and fallen over the railing.

In whatever way Don's death actually happened, it was a jolt to the scientific community to lose a man who many thought might win a Nobel Prize in the next few years, and it was especially shocking to me, as we were actively collaborating with his lab.

A cursed project?

One of the most macabre aspects of Don's death was that he was actually our third collaborator on the X-ray crystallography project to die unexpectedly. Prior to collaborating with Don on this project, we had been working with Paul Sigler at Yale. Shortly after that collaboration began, the talented postdoctoral fellow who

was leading the project, Serge Pares, died tragically (along with his pregnant wife) aboard TWA Flight 800, which exploded and crashed into the Atlantic Ocean shortly after takeoff in 1996. The project continued in the Sigler lab with contributions from several other postdocs. Then, in early 2000, Paul Sigler was walking near the Yale campus one day when he suddenly had a massive heart attack and dropped dead. Paul was a wonderful man and a hell of a scientist, so it was a devastating loss for all who knew him.

One of the postdocs in Paul's lab who was working on the project, Ben Spiller, then moved to the lab of Don Wiley at Harvard, and Don expressed an interest in continuing the collaboration with my lab. Thus, when Don's body was pulled out of the Mississippi River on December 20, 2001, he was our third collaborator on this project in six years to have met an untimely demise. In my lab, there was hushed talk about the X-ray crystallography studies being the "Death Project," and concerns were raised that anybody working on this project might be cursed.

Though he had every reason to feel shaken, Ben Spiller was undaunted by talk of a curse. After suffering through the deaths of two consecutive mentors, Ben moved to the lab of yet another famous structural biologist, Stephen Harrison at Harvard, and told me that he wanted to keep working on the project. Amazingly, Steve Harrison was also on board with the idea. Steve had actually taken in quite a few of the mentorless young scientists from Don Wiley's lab and was trying to help them continue their work. I called Steve and offered him the chance to decline this particular project, noting that the last two investigators who took on this collaboration were both now dead. In an act of defiance and bravery, Steve said he didn't believe in curses and was excited about the project, and he insisted on continuing the collaboration with Spiller as the point man.

Squirmy proteins

At the same time as these X-ray crystallography efforts were going on in my lab at Duke, we also had a number of other projects that were yielding interesting findings. For example, we were discovering novel G protein-independent signaling pathways emanating from the beta-2 receptor and other G protein-coupled receptors. While these groundbreaking studies were in full swing, we also were purifying large amounts of beta-2 adrenergic receptors on a weekly basis and sending these samples to Ben Spiller in Steve Harrison's lab at Harvard. It was a



COURTESY OF ROBERT LEFKOWITZ

During a break from a scientific meeting in California in the late 1990's, Bob Lefkowitz does his best to keep up with former trainees, Brian Kobilka and Sheila Collins, who by that time were both faculty members directing their own labs.

tough situation for Ben, having lost two mentors in tragic fashion and now doggedly trying to carry on his attempts to solve the crystal structure of the beta-2 receptor.

Technically, the project was incredibly demanding, as by definition receptors are squirmly proteins that are prone to rapidly changing conformations. If you think about it, that's what receptors do for a living: rapidly change conformations in response to ligands. Thus, it's incredibly challenging to get a receptor to hold still for a photograph, which is basically what you have to do in order to capture an X-ray crystal structure.

After a couple years of work on the project, Ben finally threw in the towel, and my lab moved on completely from the X-ray crystallography efforts because we were so focused on other interesting lines of research. Thus, when it came to the X-ray crystallography studies, aka the "Death Project," the bad news was that we were unable to solve the crystal structure of the beta-2 receptor. The good news, though, was that no further deaths were associated with the project: Spiller, Harrison, and the folks in my lab who were pursuing these studies all survived to tell the tale.

A couple of years later, in 2006, my former trainee Brian Kobilka and I shared a visiting lectureship at the University of Illinois at Urbana–Champaign. This joint lectureship provided Brian and me with some quality

time to catch up with each other. Brian had been on the Stanford faculty for about sixteen years at that point.

At a dinner together during our visit in Illinois, Brian confided to me that he had been focusing much of his lab's efforts on solving a crystal structure for the beta-2 adrenergic receptor. I told him that we also had pursued efforts in this area for a while but dropped the project several years earlier because of concerns that success might not be possible with the current state of the technology. Brian said he also had encountered many technical difficulties and was simply hoping to get a crystal structure before he retired, perhaps at some point in the next twenty years.

When Brian said this, I patted myself on the back for having the wisdom to drop the X-ray crystallography project and focus on other research directions, because it seemed crazy to labor for twenty years trying to achieve a goal that might end up being just a pipe dream.

A dazzling leap

About a year later, in the spring of 2007, I received a call from Brian with some surprising news. He said that his lab had achieved a number of technical breakthroughs and now had a crystal structure for the beta-2 adrenergic receptor! Indeed, that November, Brian, Ray Stevens

PERSPECTIVES

and their collaborators published back-to-back papers in *Science* describing the crystal structure of the beta-2 adrenergic receptor. These papers were a technical tour de force. Brian's lab innovated at every step of the process: improving the purification process to obtain much larger quantities of pure receptor than was previously possible, engineering the receptor to be much more stable than it normally was, and a dozen other technical twists that other scientists couldn't even dream up, much less actually attempt.

This breakthrough had an enormous impact on the field, as everyone now saw the path forward to solving the crystal structures for the hundreds of other G protein-coupled receptors encoded in the human genome. I was dazzled by this quantum leap in technical capability and absolutely thrilled for Brian, who had gambled a great deal in pursuing these structural studies and now saw his gamble pay off in the biggest way imaginable. What I didn't know at the time, however, was that an even more dramatic advance was on the way.

Several years later, in July 2011, Brian's group published a lead article in *Nature* reporting the crystal structure of the beta-2 adrenergic receptor in complex with a G protein. This work was done collaboratively with

several other labs, including the labs of Roger Sunahara, which had provided the purified G proteins used to achieve the docked structure, and Yiorgo Skiniotis, which had performed crucial electron microscopy work. This work was even more of a tour de force than the 2007 papers reporting the first structure of the beta-2 receptor. If you think it's hard getting one squirmy protein to sit still for a photograph, try getting two hyperkinetic proteins to hold still and pose together long enough to take a perfect photo. The technical advances in Brian's 2011 paper were staggering, and the structure was absolutely beautiful. Many new insights into how receptors work could be gleaned from this structure, and Brian went on a barnstorming tour to describe his lab's latest findings.

Nobel calling?

That summer, I was speaking at a Nobel Symposium in Stockholm. Brian was not giving a talk at the meeting, but people were talking about him. He had just shown his receptor-G protein structure at a European biophysics meeting, and Gunnar von Heijne, a member of the Nobel Committee for Chemistry, had witnessed the presentation. He then returned to Stockholm for the Nobel Symposium

COURTESY OF ROBERT LEFKOWITZ



In the lab, Bob Lefkowitz sketches out a plan of action with medical student Erin Bressler.



COURTESY OF ROGER SUMAHARA

Brian Kobilka and Bob Lefkowitz shared the 2012 Nobel Prize in Chemistry. This photo was taken just a few moments after the two finished their Nobel lectures.

where I was speaking, and when I chatted with him during a break in the meeting I could tell that he was over the moon about Brian's latest breakthrough. The sentiment seemed to be shared by several other members of the Nobel Chemistry Committee who were attending the meeting. Of course, I had long ago given up trying to read into comments made by Nobel Committee members, as I had received many enthusiastic comments over the years from various Nobel Committee members without ever being tapped for a Nobel Prize.

That October, the Nobel Prize announcements came and went, and neither Brian nor I received a call from Stockholm. As usual, numerous people asked me questions about whether I would ever win. I kept thinking about what Al Gilman said after he won his Nobel: "The best thing about winning the Nobel Prize is never again having to answer the question, 'When are you going to win the Nobel Prize?'"

Admittedly, my situation in 2011 was not as bad as it had been in 2003, when my local paper, the Durham Herald-Sun, had run a front-page, above-the-fold photograph of me with a headline that read, "Nobel Calling? Alas, Not This Year." At the time, I was thinking: who

else in the world makes headlines for NOT winning the Nobel Prize? In 2003, that headline had bothered me a little bit. By 2011, I was just enjoying my day-to-day work as a scientist and mentor, and was absolutely done with worrying about awards. Little did I know it then, but my life was about to change in dramatic fashion.

Robert J. Lefkowitz (lefko001@receptor-biol.duke.edu) is a Nobel Prize-winning scientist (Chemistry, 2012) who is best known for showing how adrenaline works via stimulation of specific receptors. He was trained at Columbia, the National Institutes of Health and Harvard before joining the faculty at Duke University and becoming an Investigator of the Howard Hughes Medical Institute. In addition to being a researcher, he is a cardiologist as well as a cardiac patient.



Randy Hall (rhall3@emory.edu) was a postdoctoral trainee of Robert Lefkowitz in the 1990s and is now a professor in the Emory University School of Medicine. He has published more than 100 scientific papers and received major awards for his research. He is also a prize-winning educator with strong interests in science writing and public outreach about science and medicine.



Curbing the malpractice of curved grades and high-stakes exams

By Melanie M. Cooper & Mike Klymkowsky

Faculties on college campuses all over the U.S. responded to recent Black Lives Matter demonstrations with calls for a renewed commitment to a more inclusive and equitable learning environment for our students. Many of us vowed to educate ourselves about systemic racism through activities such as reading, discussion groups and attending seminars.

This work is necessary and important, but if you are like us, you are ready for something more immediately actionable that is under faculty purview and has been shown to produce more equitable outcomes. If so, we suggest you look closely at your assessment and grading policies and think about their purpose. Faculty members have the opportunity to make immediate changes in these areas that can impact large numbers of students and help to increase the diversity of the science, technology, engineering and mathematics student pool.

The curve

It is well documented that some course policies not only actively discourage students but are actively discriminatory. Two major research studies, “Talking about leaving” and its more recent updated companion “Talking about leaving revisited,” both cite common approaches to determining course grades as a major factor for why students leave STEM disciplines. The practice of grading on a curve (that is, predetermining the number of As, Bs and Cs that will be given in a course) sends the clear message that the purpose of the course is to sort students and enforce competition rather than to teach.

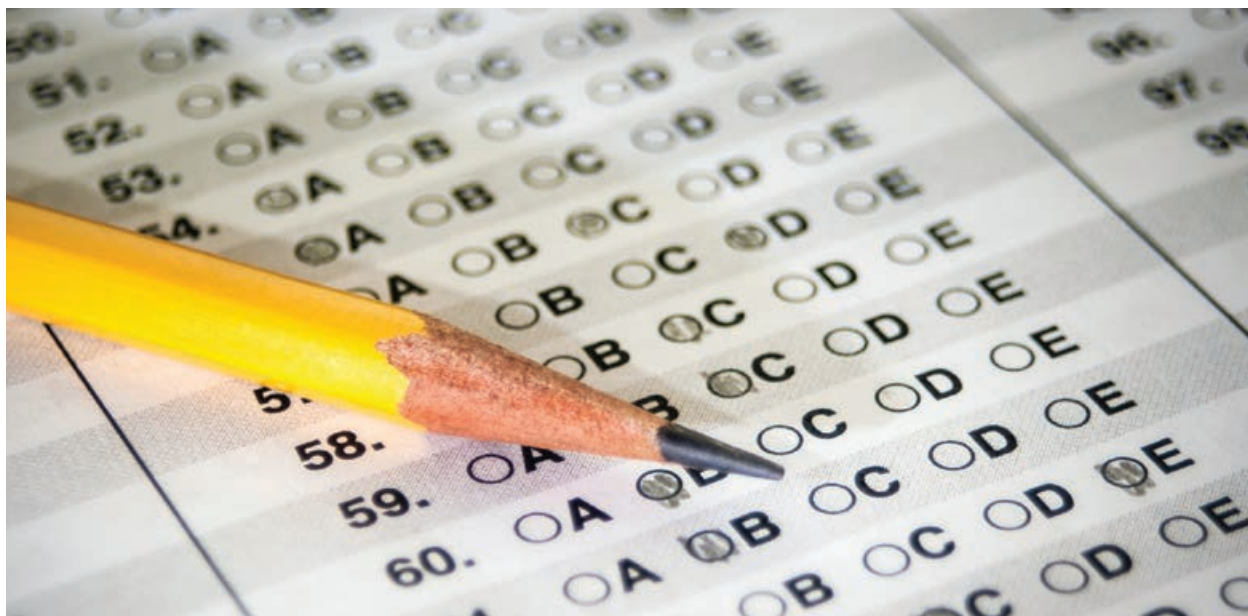
While this practice seems intrinsically misguided to many of us, there is ample evidence that it survives — particularly (but not exclusively) in large introductory STEM courses. These courses typically are characterized by what we call high DFW rates, meaning that a large percentage of students are predetermined to receive a D, an F or a withdrawal grade that does not allow them to continue on to the next course.

Research also shows that grading on a curve with predetermined outcomes has a disproportionate effect on students in underrepresented and underserved minority groups. In a recent paper looking at outcomes in an introductory general chemistry course at the University of Washington where grades are curved (around an average of a 2.6 grade points), researchers write that they found a marked difference in the fates of students who received a C in a course (and were able to continue) and those who got a C- and had to retake the course, despite the fact that there is little demonstrable difference in learning outcomes between the two groups.

Even more distressing are the different outcomes for these two groups of students. Underrepresented students who were allowed to move to the next courses were more successful in terms of completing their degree programs than their majority peers. However, those who had almost identical grades but were deemed to have failed (forced to repeat the course) were less successful than their majority peers. That is, a capricious decision to predetermine what percentage of the class must fail had a profound impact on underrepresented students. What if these students had not been graded on a curve? What if 30% of the class had not been predetermined to fail? As David Asai noted in a commentary in the journal *Cell*, persons excluded because of their ethnicity or race, known as PEERs, “leave STEM at rates much higher than non-PEERs, and the pattern of poor PEER persistence is essentially the same as it was nearly three decades ago,” an outcome that may be due in part to grading practices.

Rethinking assessments

Certainly, some faculty might defend the practice of curving as a way to prevent grade inflation, but this kind of thinking is aligned with the sorting ethos — only the most deserving should be allowed through the gates into the promised land. Multisection large-enrollment classes often curve grades to ensure that grades are similar



across sections, but wouldn't it be fairer to set standards (mastery goals) and help students meet them?

We need to rethink our approach to assessment, moving from high-stakes tests that have almost no connection to the actual things scientists do to an approach that supports the development of knowledge in use and provides understandable goals for students. Our recent transition to remote education can provide us with the impetus to move away from the high-stakes testing that is so prevalent in many college classes and so detrimental to many students.

Instead of obsessing about students cheating on high-stakes exams and going to extraordinary lengths to prevent this by employing invasive technologies or randomized multiple-choice exams that can test only recall and rote exercises, now is the time to implement mastery learning, continuous assessment and alternate approaches to grading. What if we explicitly spell out what students need to know and what they must be able to do with that knowledge? Our learning objectives should lay out these requirements explicitly.

Rather than requiring students to memorize and regurgitate the essential amino acids, what about asking them to respond to prompts such as "Construct a representative amino acid structure and explain why these substances are soluble in water at pH 7" or "How do differences between amino acids influence the folding of proteins or their catalytic activities?" Instead of having students memorize biological pathways, we could ask them to explain the role of adenosine triphosphate (or thermodynamically favorable and unfavorable reactions)

in such pathways.

Rather than a few high-stakes tests, we could provide more lower-stakes checkpoints and other activities that support desired learning outcomes. While some faculty members inevitably will resist these suggestions (after all, it is much easier to use publishers' test banks that can be randomized and graded automatically), ample evidence exists that the curving practices discussed here are exclusionary and inequitable. If we seriously are committed to more equitable outcomes, we can act to remove one known barrier for underrepresented students.

Now is the time to make these changes, before our commitment to diversity, equity and inclusion fades and we go back to business as usual. Let's seize the moment and make some real changes that will directly benefit our students.

Melanie M. Cooper (mmc@msu.edu) is a professor of chemistry at Michigan State University.



Mike Klymkowsky (michael.klymkowsky@colorado.edu) is a professor of molecular, cellular and developmental biology at the University of Colorado Boulder.



A history-making administration — ‘that believes in science’

A statement from the American Society for Biochemistry and Molecular Biology’s Minority Affairs Committee:

We congratulate President-elect Biden and Vice President-elect Harris on their election to the highest offices in the country. Their election is a win not only for the United States, because we will have an administration that believes in science, trusts the data and respects researchers, but also for the world, because Biden and Harris have vowed to rebuild America’s relationships with allies and work with them to solve global problems, starting with the COVID-19 pandemic.

In the U.S., more than 13 million people have been infected with the virus SARS-CoV-2, and more than 265,000 families have lost loved ones to the devastating disease it causes. Black, Latino and Indigenous communities have been affected disproportionately. The numbers are predicted to climb in the coming months, in part due to poor decision making at the highest levels of government. Already, though, Biden and Harris have already assembled a task force of experts to inform their COVID-19 plan, and they have promised to make decisions based upon evidence rather than politics.

In 2020 alone, more than 160 Black lives have been taken by police. Our committee wrote about this issue in June, after the killings of George Floyd, Atatiana Jefferson, Breonna Taylor and others. We vowed to channel our grief and anger in productive ways. The election results tell us that millions of Americans did so at the ballot box. We are deeply relieved that Black lives matter to the new administration, and we will continue to work to dismantle white supremacy and the systems that uphold it, including those in the academy.

Immigrants, many of whom have worked hard to contribute to American society, and indeed American science, have been banned, scapegoated and targeted by the current administration. We look forward to having leaders whose immigration policies demonstrate both compassion for humanity and appreciation for the contributions that immigrants make to the United States. We look forward to the new administration giving Dreamers the chance they deserve and ending the cruel and abusive policy of separating children from their parents at the border.

The contributions of nonimmigrant foreign students and researchers to the American scientific enterprise cannot be overstated. The ASBMB policy team has done an excellent job of tracking and responding to the current administration’s attempts to limit certain visas and discourage international collaboration. We have confidence that the new administration will maintain the United States’ standing as the world leader in science, technology, engineering and mathematics and as a beacon for the most talented and brightest minds.



LAWRENCE JACKSON/IDEN FOR PRESIDENT

Joe Biden announced U.S. Sen. Kamala Harris, D-Calif., would be his running mate on Aug. 12 in Wilmington, Delaware.

We are eager for the new administration to appoint an education secretary with expertise in education and to make decisions about STEM education based on best practices and evidence. It is also important to resume diversity, equity and inclusion training at the federal level and at federally supported colleges, universities and institutions.

Finally, the election of Harris to the office of vice president is especially momentous and meaningful. She is a Black woman of South Asian descent — the daughter of immigrants — with a blended family. Harris' election is evidence that not only women of color but women in general are brilliant, effective and well-qualified leaders. A woman in one of the two highest leadership positions in our country is empowering and long overdue.

The members of the MAC enthusiastically look forward to a presidency during which decisions are made with consideration for scientific principles and knowledge, Black lives matter, and people are not discriminated against because of their ethnicity, race, gender identity or sexuality.

As people who believe deeply in science and in the integrity of the scientific community, we recommend, as this new administration moves forward, that the policies and procedures put in place by the previous administration to politicize science, scientists and research be reversed so that our scientific community can move forward once again uninhibited to develop, design, innovate and report scientific findings in an unbiased manner and without fear of retribution.

— **Sonia C. Flores, Chair, ASBMB Minority Affairs Committee, with MAC members Ruma Banerjee, Kayunta Johnson–Winters, Deborah Neely–Fisher, Carlos Castañeda, Adela Cota–Gomez, Carlos Lopez, Vahe Bandarian, Gustavo Silva, Joseph Chaney, and Lana Saleh**

“You’ve got...to get outside of your comfort zone”

By Laurel Oldach

Wayne Fairbrother leads a department at Genentech tasked with validating disease-associated targets and determining whether they are feasible for drug development. The biophysicist, who was raised in New Zealand and has lived in California for decades, sat down virtually with ASBMB Today to talk about his career. The interview has been edited.

1 How did you come to work at Genentech?

It’s an interesting story. Genentech was looking to set up a protein nuclear magnetic resonance group back in the early 1990s. I applied for the position and, as many people do, got a polite “thank you, but no thank you” letter. Two or three months later I got a call from the director of protein engineering, who had gotten my name from one of their consultants, a senior person in protein NMR who I knew — and I got the job.

I like to tell that story to people looking for positions. You can get lost in the bureaucracy, especially nowadays when everything is computer searched, so having a network definitely helps.

2 Any other advice you often give?

I tell people going into industry the one thing I can guarantee is that in five years they’ll be working on something completely different. Things progress and come in and out of favor for various reasons, whether it’s science, strategic decisions or competition. So you need to be flexible.

That, and collaboration. You

can’t do anything without working with colleagues who have different expertise to put the pieces together and solve a problem. You’ve got to be prepared to get outside of your comfort zone.

3 How has Genentech changed in your time there?

The company has gotten bigger. In the early days we’d interact with everyone; now we’re spread over a larger campus, and it’s harder to know what’s going on everywhere at all times. You need to be more proactive in keeping your finger on the pulse. Also, the pendulum has swung a little from basic discovery to more translational research. We’re all about making therapeutics for unmet medical needs, so translation is critical.

4 Is there a project you’re especially proud to have worked on?

There’s a clear winner there: a collaboration with Abbot Labs (now AbbVie) and the Walter and Eliza Hall Institute in Australia that resulted in a treatment for chronic lymphocytic leukemia and acute myeloid leukemia. It’s a molecule that specifically targets Bcl-2, which for a long time was considered undruggable. It was an exciting project — and it’s always fun to meet patients who have benefited from something that you’ve touched. It reminds us why we do what we do.

5 Is there such a thing as an undruggable target?

I don’t think there is. It may be true to say it’s not druggable with the technology now, but technology is



Wayne Fairbrother

CURRENT POSITION

Director and senior staff scientist, Early Discovery Biochemistry, Genentech

DEGREE

D.Phil., Oxford University, 1989

POSTDOCTORAL RESEARCH

Scripps Research Institute

FIRST JOB OUTSIDE OF ACADEMIA

Scientist, Genentech

FAVORITE MOLECULE OR PROTEIN

Bcl-2

evolving. When a target is considered undruggable, it just means that we haven’t discovered the way to drug it yet. That’s the way I like to think.

Would you like to suggest an ASBMB member who works in industry for a Five Questions interview? Send an email to asmbmtoday@asbmb.com.

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



CLASSIFIEDS

Scientist, In Vivo Metabolism (Mass Spec) Calico Life Sciences

Calico is inviting applications from biologists who are excited to be part of a scientific team dedicated to unravelling the fundamentals of the aging process with a view to identifying targets and pathways suitable for therapeutic intervention. In particular, we are looking for a scientist with a background in and deep knowledge of cellular and in vivo metabolism, who is interested in applying this training to advance our understanding of the role of metabolism in aging as well as the metabolic inputs and outputs to a variety of diseases and signaling pathways. The successful candidate will be responsible for building and implementing new techniques to study the physiology of disease and aging using cell culture, in vivo systems, stable isotope tracing, and mass spectrometry.



careers.asbmb.org/job/scientist-in-vivo-metabolism-mass-spec/55230100/

(AAV) Research Associate Gene Therapy Vector Core Affinia Therapeutics

Affinia Therapeutics is rapidly growing biotech company developing transformative gene therapies for devastating diseases. We are backed by a strong syndicate of life science investors and have ambitious plans to have a dramatic impact on the lives of patients around the world.



As an early employee you will play a key role in setting our culture and values. You will be working alongside the founding management team who have a track record of success in the industry. The pace of work will be dynamic, fast and fun.

We are looking for a curious, organized and self-motivated individual to work with our Gene Therapy Vector Core team. This is not a typical Vector Core: our Vector Core is an integral part of our novel AAV discovery platform, and members work together to straddle the lines between research, process development, and support. This is an excellent opportunity to gain experience and develop in a dynamic, next-generation gene therapy environment.

careers.asbmb.org/job/aav-research-associate-gene-therapy-vector-core/54958406/

Assistant Professor of Molecular Biophysics & Biochemistry Yale University

The Department of Molecular Biophysics & Biochemistry at Yale University invites applications for tenure-track Assistant Professor positions. We seek new colleagues who will direct vibrant research programs in any area of molecular biosciences and who have an established interest in promoting equity and inclusion among diverse scientists at any level. We welcome applicants who use any approach to advance understanding of the molecular basis of life.



Successful candidates will be expected to contribute to the excellence of a lively science community at many levels: they will direct and support a research group in which diverse people can thrive, teach undergraduate and graduate students from diverse backgrounds, and be an interactive member of the department and the Yale community.

careers.asbmb.org/job/assistant-professor-of-molecular-biophysics-biochemistry/55217995/

Senior Research Associate, Analytical Development Allena Pharmaceuticals

Allena Pharmaceuticals, Inc. is a late-stage clinical biopharmaceutical company dedicated to developing and commercializing first-in-class, oral enzyme therapeutics to treat patients with rare and severe metabolic and kidney disorders. Allena's lead product candidate, reloxaliase, is a first-in-class, oral enzyme therapeutic for the treatment of hyperoxaluria, a metabolic disorder characterized by markedly elevated urinary oxalate levels and commonly associated with kidney stones, chronic kidney disease and other serious kidney disorders. Its second product candidate, ALLN-346, is also a first-in-class, oral enzyme for the treatment of hyperuricemia and gout in the setting of advanced chronic kidney disease.



The Analytical Development Senior Research Associate will be responsible for performing bioanalytical assays to support activities such as stability, characterization, and process development. Successful candidates should have laboratory training, experience working with proteins and large molecules, experience maintaining laboratory notebooks and providing data summaries.

careers.asbmb.org/job/senior-research-associate-analytical-development/55230042/

To see a full list of jobs, please visit careers.asbmb.org

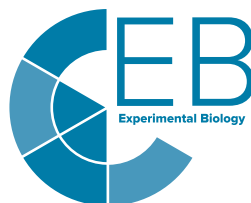


The 2021 ASBMB Annual Meeting will be virtual!

Join us April 27–30, 2021

Learn more at asbmb.org/annual-meeting

ABSTRACT DEADLINE: Jan. 7



The ASBMB annual meeting is held in conjunction with Experimental Biology.