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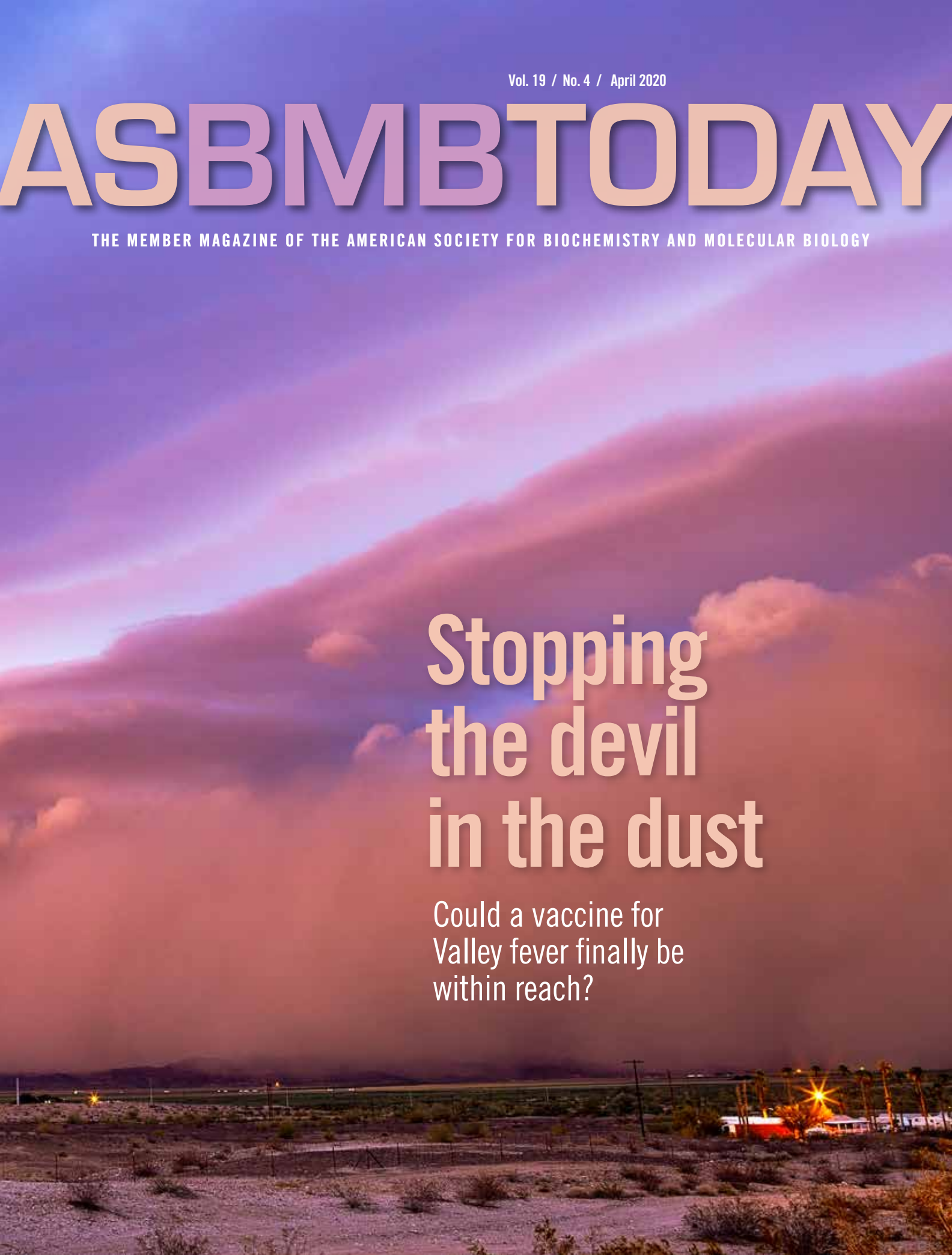
ASBMBTODAY

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



Stopping the devil in the dust

Could a vaccine for Valley fever finally be within reach?

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VIRTUAL ISSUE

Coronaviruses

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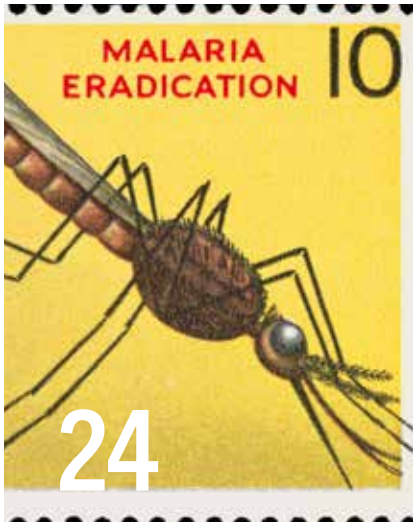

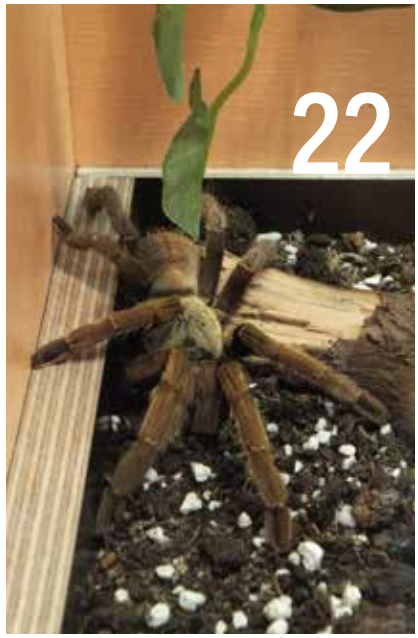

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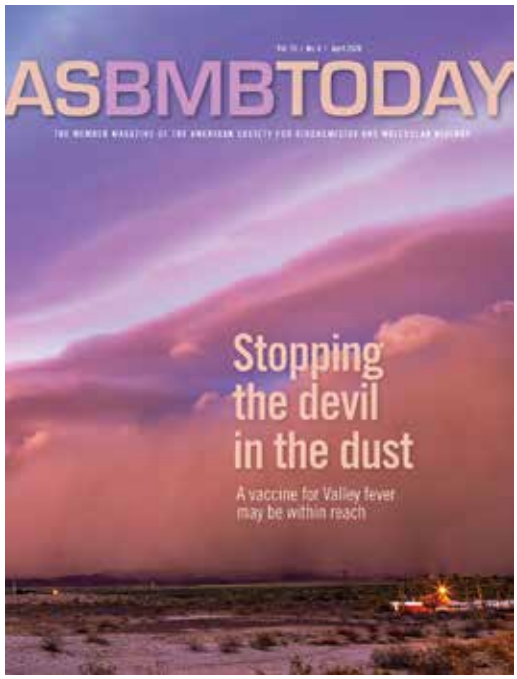
EDITOR'S NOTE

Why we're not printing the April issue

By Angela Hopp



One of the strangest things we ever did when I was in the newspaper business was print papers nobody would receive.

The first time, I was an intern at the Arkansas Democrat–Gazette in Little Rock. A big ice storm rolled in during my 4 p.m.-to-midnight shift on the news desk. On my way home, I lost control of my car twice: once on the highway (a state trooper stopped and impatiently told me to get on my way) and then on the hill leading to my apartment (I ended up leaving my car in a church parking lot at the bottom and then climbing up home on my hands and knees).

The second time was during Tropical Storm Allison. I worked at the Houston Chronicle, and the rain, of course, began during that same late shift. Long story short, I ended up walking nine miles home that night, often in waist-deep water full of sewage and floating mounds of fire ants.

Of course, these newspapers didn't print just to torture their employees. Contracts with advertisers stipulated that ads had to be printed, so it was a business decision. Printed, but not necessarily delivered. In both cases, the delivery trucks were unable to make their deliveries. Nobody read our papers. This was before the internet was, well, what it is today.

Fortunately, ASBMB Today is not beholden to advertisers.

We print the magazine as a service to ASBMB members, and most of our members receive the magazine where they work. Now, with universities and businesses closed to help stem the COVID-19 pandemic, it seems reasonable to skip printing an April issue of ASBMB Today that few will receive. We'll still post a PDF of the print issue on our archive page when it's ready, but the truth is that we're pouring our hearts and souls into our website. Our online coverage is timely, interactive — and in some cases impossible to reproduce in print.

Angela Hopp (ahopp@asbmb.org) is the ASBMB's communications director and executive editor of ASBMB Today. Follow her on Twitter @angelahopp.

Fleming receives Gordon Award


Karen Fleming and Sharona Gordon

Karen Fleming, a professor of biophysics at Johns Hopkins University and an associate editor of the Journal of Biological Chemistry, has received the first annual Sharona Gordon Award.

The award, inaugurated this year, is conferred by the Society of General Physiologists to an individual who shows "extraordinary commitment to promoting equity and inclusivity in the physiology and biophysics community." Fleming was honored for her work to support gender equity in science. On the JHU campus, that includes the Women of Hopkins project honoring alumnae, and a Women in Faculty forum that brings together faculty to address challenges unique to women faculty. Fleming has also promoted broader conversations about equity through blogging and social media and in person at workshops and forums.

Named for ASBMB member Sharona Gordon, a professor of physiology and biophysics at the University of Washington and dean for research and graduate education the UW school of medicine, the award honors Gordon's work as editor-in-chief of the Journal of General Physiology to promote equitable peer review as well as her development of mentoring networks for early-career scientists and her efforts to put an end to sexual harassment in academia.

The award was presented in a ceremony at the Biophysical Society's annual meeting in San Diego in February.

Osheroff elected to lead educators' society


Osheroff

Neil Osheroff, professor of biochemistry and medicine and John G. Coniglio chair in biochemistry at Vanderbilt University School of Medicine, has been elected president of the International Association of Medical Science Educators, a society that promotes excellence and innovation in teaching. His two-year term began in January.

Osheroff studies DNA topoisomerases, enzymes that relax supercoiling that arises during DNA replication and other cellular processes by inducing transient breaks in the double helix. Different topoisomerase classes cut either one or both DNA strands. Osheroff's lab has made foundational contributions to the understanding of topoisomerase enzymology.

Topoisomerase-targeted agents have become important cancer drugs that act by inhibiting topoisomerase-mediated ligation. As a result, the normally short-lived breaks that these enzymes introduce last longer, damaging DNA and harming cells. Some antibacterial agents, such as ciprofloxacin, act by a similar mechanism.

Osheroff is a fellow of the American Association for the Advancement of Science and a founding member of Vanderbilt's Academy for Excellence in Education, which he currently directs. He has directed courses for medical students since 1990 and helped to redesign the school's curriculum in 2013.

Khalimonchuk joins meetings committee


Khalimonchuk

Oleh Khalimonchuk is the newest member of the American Society for Biochemistry and Molecular Biology Meetings Committee.

Khalimonchuk is the Susan J. Rosowski associate professor of biochemistry and a member of the redox biology center at the University of Nebraska–Lincoln. Using yeast and mammalian cell models, his lab studies the molecular bases of mitochondrial function and dysfunction as they relate to human disease and aging.

As a member of the meetings committee, Khalimonchuk will serve a three-year term, working with other members to plan and evaluate the society's annual meetings. Members review and select abstracts for Spotlight Sessions, promote the meeting and solicit abstracts within their research communities, advise on meeting elements to meet the needs of graduate and postdoctoral attendees, and rep-

American Academy of Microbiology elects fellows

The American Academy of Microbiology has elected 68 new fellows to the Class of 2020, including three members of the American Society for Biochemistry and Molecular Biology.

Fellows of the American Academy of Microbiology, an honorific leadership group within the American Society for Microbiology, are elected annually through a selective peer-review process, based on their records of scientific achievement and original contributions that have advanced microbiology. The academy received 118 nominations this year and accepted 58% of nominees.

These ASBMB members were elected as 2020 academy fellows:

Michael Federle, professor of pharmaceutical sciences at the College of Pharmacy and director of the Center for Biomolecular Sciences, University of Illinois at Chicago. His lab studies quorum sensing, the process by which bacteria coordinate gene expression and behavior across microbial populations through chemical communication.

Henry Paulus retired in 2013, the year the Boston Biomedical Research Institute closed, after more than 50 years of service as a senior scientist at the institute and as an associate professor in the department of biological chemistry and molecular pharmacology at Harvard Medical School. His lab focused on mechanisms of protein splicing and antimycobacterial drug discovery.

Brenda Wilson, a professor of microbiology in the school of molecular and cellular biology and inaugural professor of the Carle–Illinois College of Medicine at the University of Illinois at Urbana–Champaign. Her research focuses on bacterial protein toxins and development of novel alternative toxin-based therapeutics as well as human and nonhuman primate vaginal and gut microbial ecosystems and their role in health and disease.


Federle


Paulus


Wilson

The more than 2,500 fellows in the academy represent all subspecialties of the microbial sciences and are involved in basic and applied research, teaching, public health, industry and government service. Fellows hail from all around the globe; the Class of 2020 includes fellows from Australia, Austria, Brazil, Chile, China, France, Germany, Israel, Switzerland, the United Kingdom and the United States.

resent their specific research areas by identifying trends and recommending speakers for the meeting program. The committee also evaluates proposals for smaller meetings, the ASBMB Special Symposia.

Teaching lab named for Leach


Leach

Oklahoma State University has honored the memory of the late **Franklin Leach** with an interactive teaching space for the school's department of biochemistry and molecular biology.

Leach, who retired in 1998 after teaching biochemistry at OSU for 39 years, died in 2016 at age 83. His family made a donation to have the classroom in the university's Noble Research Center renovated. The Dr. Franklin R. Leach Laboratory Classroom was opened in November.

"This whole collaboration is about coming together and thinking about that next generation," Leach's daughter Janet Weiss said. "That's really what dad was all about."

After earning a Ph.D. in chemistry from the University of Texas in 1957, Leach was a National Research Council fellow in medical science at the University of California at Berkeley until he moved in 1959 to Oklahoma State University. In addition to teaching a variety of courses, he directed multiple master's and doctoral theses, and advised pre-med students. Leach also served as the editor for the Oklahoma Academy of Science and was a founding member of the International Society for Bioluminescence and Chemiluminescence.

Patton–Vogt will lead Duquesne department


Patton-Vogt

Jana Patton–Vogt will become chair of the biological sciences department at Duquesne University in Pittsburgh in July.

Patton–Vogt, who trained at the University of Kentucky and Carnegie Mellon University, has been a professor at Duquesne since 2001. Her research focuses on phospholipid metabolism in yeast and other fungi, with a focus on glycerophosphodiester transport.

On top of maintaining a research program, Patton–Vogt also teaches three undergraduate courses, advises the university's student chapter of the ASBMB and serves on the editorial board of the Journal of Biological Chemistry.

"In this department, we want to teach well, we want to do good research, and we always want to improve," she told student journalists. "I'm looking forward to the challenge."

Her term as department chair will run through 2023. Her predecessor as department chair, Joseph McCormick, also is an ASBMB member.

Rosen wins Wiley Prize for phase condensation work


Rosen

Michael Rosen, chairman of the biophysics department at the University of Texas Southwestern Medical Center at Dallas and a Howard Hughes Medical Institute investigator, was one of three scientists recognized with the Wiley Prize in Biomedical Sciences in February. The other awardees are Cliff Brangwynne of Princeton University and Tony Hyman of the Max Planck Institute of Molecular Cell Biology and Genetics.

The award recognizes the three investigators' discovery of liquid–liquid phase-separation as a principle for organizing cellular contents into functional compartments that are not enclosed by lipid bilayers. So-called "biomolecular condensates," some of which form through phase separation, have been found throughout the cell, with roles in RNA transport and degradation, signal transduction, DNA repair and more functions. Rosen and colleagues have focused on signal transduction mediated through phase separation and on the principles that determine what molecules are included in a phase-separated compartment.

Rosen told the UT Southwestern's media team, "The award is testament to the hard work and creative insights of the students, postdocs and technicians in my lab over the past decade and the encouragement of our colleagues at UTSW and HHMI. I am grateful to them all for stepping with me into an unexplored area, and taking the risks that ultimately led to our discoveries."

The prize, which is granted by the charitable foundation of the Wiley publishing company, has a purse of $50,000. It was scheduled to be presented April 3 at The Rockefeller University, but that event was canceled due to COVID-19 closures.

Bassler receives genetics medal


Bassler

Bonnie Bassler, the Squibb professor and chair of molecular biology at Princeton University and a Howard Hughes Medical Institute investigator, has won the 2020 GSA Medal from the Genetics Society of America.

The medal, established in 1981 to recognize scientists who exemplify the ingenuity of the GSA membership through elegant and meaningful contributions to modern genetics, honors outstanding contributions to the field in the past 15 years. The society is recognizing Bassler for her groundbreaking studies of bacterial chemical communication and regulation of group behaviors.

Bassler is a leader in the field of quorum sensing, the mechanism by which bacteria communicate with chemicals, detect the number of neighboring cells present and, as collectives, change their behaviors. Such intercellular signaling among single-celled organisms, once considered outlandish, is now known to govern behaviors from bioluminescence to virulence to biofilm formation.

The society will present this award and others at The Allied Genetics Conference, which will be held online in late April.

Czech delivers lecture at Lorne Genome Meeting


Cech

Australia's annual Lorne Genome Meeting was held in February in a suburb of Melbourne. **Tom Cech**, a professor at the University of Colorado, Boulder, gave a lecture called "Curious in the nucleus: Cryo-EM of telomeres and rCHIP of chromatin binders."

Cech is best known for discovering catalytically active RNA, better known as ribozyme activity. He has received many awards for the work, including sharing the Nobel Prize in chemistry in 1989 with Sidney Altman. Since that time, Cech has enjoyed a productive career in research. He served as president of the Howard Hughes Medical Institute from 2001 to 2009 but stepped down to focus on research and teaching.

Recently, Cech's research has focused on the activity of long noncoding RNAs in the nucleus.

Chu wins educator award from ASIP


Chu

Charleen T. Chu, a professor of pathology at the University of Pittsburgh, won the Robbins Distinguished Educator Award from the American Society for Investigative Pathology. The award, named in honor of the late pathologist and textbook author Stanley L. Robbins, recognizes scientists who have made exemplary contributions to education.

Chu, a physician-researcher who studies mitochondrial biology and mechanisms regulating catabolic–anabolic responses in neurons, has contributed to education and training in numerous ways.

In 2002, she developed a course for medical and predoctoral students to demystify grant writing and reviewing. With her husband, Tim Oury, she later developed a training program to help pathology residents and fellows transition to independence. Variations of those programs and others she developed have been incorporated into meetings and other professional-development environments. In addition, she has been vice-chair for pathology faculty mentorship and development at Pittsburgh since 2016.

Chu was set to deliver an award lecture, titled "Chemical inhibition of PINK1 degradation confers neuroprotection in culture models of Parkinson's disease," at ASIP's annual meeting in April. However, the Experimental Biology conference, where ASIP, ASBMB and other societies were to hold their annual meetings, was canceled earlier this month in response to the COVID-19 outbreak.

Arne Holmgren

Arne Holmgren, a senior professor at Karolinska Institutet who was known for his groundbreaking work in the field of redox biology, died Jan. 6. He was 79.

Holmgren was born Dec. 21, 1940, on a farm in Sweden. He studied medicine at Uppsala University and, while a medical student, he joined Peter Reichard's research group. After graduating in 1964, Holmgren moved to Reichard's new lab at Karolinska Institutet. He earned his Ph.D. in 1968 with a study of E. coli thioredoxin, which had just been discovered as a reductant of ribonucleotide reductase. Holmgren spent his career at the institute, where he moved from assistant professor to university lecturer, associate professor and professor. He succeeded Reichard as professor and chairman of biochemistry in 1991 and as director of the Medical Nobel Institute for Biochemistry in 1992.

Recognized as a redox pioneer, Holmgren helped establish the study of the reduction and oxidation pathways that occur in all living cells. He discovered and named glutaredoxin, and he determined the structure and function of several members of those glutathione-dependent disulfide oxidoreductases. He was active in cancer research and in the development of antimicrobial treatments, with both of these therapeutic principles focused on the targeting of specific redox pathways.

Holmgren was a member of the Nobel Assembly at Karolinska and of the Swedish Royal Academy of Sciences. He became a senior professor in 2008 but remained an active scientist thereafter, leading a vibrant research group.

David Feingold

David Sidney Feingold, a scientist whose career ranged from chemistry to organic chemistry to microbiology, died Sept. 26 in Pittsburgh. He was 96.

Feingold was born in Massachusetts in 1922. After earning a bachelor's degree in chemistry from the Massachusetts Institute of Technology, he served in the U.S. Navy on a tank landing ship during World War II. After the war, he continued studying chemistry at the University of Zurich. He moved to Israel in 1949 and earned a Ph.D. in 1956 from the Hebrew University of Jerusalem.

On his return to the U.S., Feingold worked as a research assistant at the University of California before settling at the University of Pittsburgh School of Medicine where he was appointed a professor of microbiology and spent most of the remainder of his career.

For most of his career, Feingold studied oligo- and polysaccharides, publishing extensively on enzymes involved in bacterial saccharide synthesis and metabolism and sometimes venturing into plant and animal enzymes. His postdoctoral work involved nucleotide production in plant seedlings, a necessity for rapid growth and cell division. In 1957, along with Shlomo Hestrin and Gad Avigad, he received the Israel Prize — the Israeli equivalent of the Nobel Prize — in exact sciences.

Feingold was known for his quirky teaching style, such as throwing condoms filled with coffee beans at students to cement their knowledge of the gonorrhea bacteria's shape. He ran triathlons into old age, boasting of awards when he was the only entrant in his age category. He had a knack for quickly picking up languages and often successfully pretended to be a native speaker.

He is survived by his children, Oded Haber, Anat Feingold and Michele Feingold, as well as six grandchildren and extended family. His wife, Batia Feingold, died in 2008.

Samuel Brooks Jr.

Cancer researcher and biochemistry professor Samuel Carrol Brooks Jr., died of cancer in December. He was 91.

Born and raised in Winchester, Va., Brooks earned a bachelor's degree from Carnegie Mellon University and master's and doctoral degrees from the University of Wisconsin–Madison. He served in the United States Army and was promoted from lieutenant to captain during the Korean War.

Brooks joined the faculty of Wayne State University in Detroit in 1959. He studied estrogen signaling in cancer. Working with mentor Herbert Soule and colleague Elizabeth Locke, he described the estrogen receptor in human breast cancer cell line MCF-7, a pivotal discovery. The finding, published in the Journal of Biological Chemistry, opened the door for studies of the hormone's effect in promoting breast cancer growth. Later in his career, Brooks transitioned to studying estrogen-derived molecules as potential tumor suppressors or therapeutics.

In addition to his research, Brooks contributed to the education of many students, earning Outstanding Teacher of the Year from Wayne State's medical students. He also received the School of Medicine Distinguished Service Award in 2000 and Lifetime Achievement Award in 2005. He was the principal investigator of a training grant for the university's cancer biology program, which launched in 1985, and he remained the program director until 2005, just before he retired. He also served as deputy director of chemistry and chief of endocrinology at the Karmanos Cancer Institute at Wayne State.

Brooks lived in Steamboat Springs, Colo., at the time of his death. He is survived by Frieda Brooks, his wife of 58 years; their two children and five grandchildren, and many nieces and nephews.

Haldor Jonsson Jr.

Haldor Jonsson Jr. died in October of 2019 at age 90.

Jonsson grew up outside of Houston, Texas, occasionally arriving at school on horseback. As a young man, he served for two years in the Army during the Korean War. He managed a cattle ranch and worked for Shell Chemical before returning to Texas A&M for a master's degree in biochemistry, which he followed up with graduate and postdoctoral work.

In 1966, Jonsson joined the biochemistry department at the Medical University of South Carolina, where he spent the rest of his career there. His research, published in Science, the Journal of the American Medical Association and other notable journals, focused on prostaglandins; he investigated the abundance and role of these signaling lipids in mouse reproductive tissue, and he contributed to the discovery of novel prostaglandins in human semen. Jonsson was also a well-liked teacher, honored as Professor of the Year in 1994. He retired in 2002.

A lifelong outdoorsman, Jonsson was an avid goose and duck hunter and loved to pass the time by crabbing, fishing and shrimping with his two children and later four grandchildren. He is survived by those descendants and his wife of 55 years, Brenda Turner Jonsson.

Roger McMacken Jr.

Roger L. McMacken Jr., former chair of the biochemistry and molecular biology department at the Johns Hopkins University Bloomberg School of Public Health, died Dec. 7. He was 76.

McMacken was born in 1943 in Spokane, Washington. The son of an Air Force lieutenant colonel, he attended the University of Washington as an undergraduate, earning a degree in chemistry in 1965. During his time as a graduate student at the University of Wisconsin, he met Maria Vigo, whom he married in 1968.

After earning a doctorate in biochemistry from the University of Wisconsin in 1970, McMacken did postdoctoral work at Yale University and the University of Florida on the molecular mechanisms behind bacteriophage lambda DNA replication. He then embarked on a second postdoctoral fellowship with Nobel laureate Arthur Kornberg at Stanford University, where he explored in vitro DNA replication and reconstitution.

In 1976, McMacken joined the faculty at Hopkins, a university he would call home for 42 years. During that time, he published more than 70 peer-reviewed articles and book chapters about DNA replication. For more than 20 years, he directed the department's NIH-funded graduate training program, where he oversaw the training of more than 100 doctoral candidates and directly mentored more than 20 students and fellows. His former students and trainees established the Roger McMacken Fellowship Fund in 2018 to "recognize his deep commitment to graduate education."

McMacken is survived by his wife, Maria; daughters, Michelle, Melissa and Marisol; brothers, Steve and Paul; and four grandchildren.

Joseph Krasner

Joseph Krasner, who helped develop a widely used phototherapy treatment for jaundice in newborn babies, died Jan. 3 in his home in Amherst, N.Y. He was 93.

Born in Buffalo, N.Y., in 1926, Krasner was the son of Russian immigrants. In early childhood, he developed a leg infection that required multiple surgeries and left him with a limp. This also caused him to miss much of his early schooling. He graduated from Buffalo Technical High School in 1948, then went on to earn a bachelor's degree in biology, a master's degree in education, a master's in chemistry and a doctorate in biochemistry, all from the University of Buffalo.

Krasner went to work as a researcher at what was then the Roswell Park Memorial Institute. He then took a position at the Children's Hospital of Buffalo, where he was part of team of hospital and university researchers who introduced the use of fluorescent lights called bili-lights to get rid of excess bilirubin, a byproduct of the breakdown of red blood cells that can cause jaundice. He returned to Roswell Park, where he did gastroenterology research until he retired in 1992. He co-authored almost 30 scientific papers and gave lectures all over the world. He was a visiting scientist at the Karolinska Institute in Sweden.

He met his wife, Joan Danzig, in 1952, and they were married the next year. She died in 2005. Survivors include two daughters, Susan Krasner and Karin Axner, and two grandsons.

Ron Kaback (1936 – 2019)

By Gary Rudnick

Howard Ronald Kaback, professor of physiology at the University of California, Los Angeles, and member of the National Academy of Sciences, died Dec. 20 at the age of 83.

Ron grew up living behind his father's pharmacy in the Overbrook section of Philadelphia. In high school, he studied mechanical arts, played football and baseball, and as a senior was president of the student body. In his freshman year, Ron met Molly Schreibman, known as Teenchy, and the two started going steady. They married during Ron's third year in college and remained together for the rest of his life.

Ron said the luckiest break in his life, after meeting Teenchy, was to be admitted to Haverford College. The biology department at Haverford was staffed by a young faculty, fresh out of postdoctoral training, who taught the latest findings in molecular biology. They invited eminent scientists like David Bonner, Arthur Kornberg, Joshua Lederberg, Salvatore Luria and Linus Pauling as outside lecturers. Exposed to the exciting world of cutting-edge biochemistry, Ron began to entertain fantasies of becoming a research scientist. As a senior, he published his first paper, on intermediary metabolism of *Thiobacillus thioparus*.

The summer after graduating, Ron worked in Britton Chance's lab at the University of Pennsylvania. This was when Chance discovered respiratory control in mitochondria, which Peter Mitchell later showed was due to the creation of an electrochemical proton potential across the mitochondrial membrane — the same driving force that powers the lactose permease that Ron studied for most of his career. As a medical student at the Albert Einstein College of Medicine in New York, Ron studied bacterial resistance to D-serine in Adele Kostellow's lab. He wanted a cell-free system to examine amino acid uptake and prepared bacterial membrane vesicles by osmotic lysis. This preparation, which he jokingly called Kabackosomes, proved to be the key element of his bioenergetic characterization of bacterial transport.

After medical school, rather than being drafted to serve in Vietnam, Ron chose to perform his military service at the National Institutes of Health working in Earl Stadtman's lab. Here, Ron continued to work on his vesicles and published his second paper, characterizing proline transport by the vesicles, in Proceedings

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"Ron was an explosive and captivating bundle of knowledge, energy, humor (often a bit wild) and tennis acclaim." — Irwin Arias, professor at Einstein

> "I never missed a chance to hear him talk at meetings, fully realizing that playful ridicule would be directed at me sometime during the talk. Which I cherished."
>
> — Vincent Marchesi, Yale University

of the National Academy of Sciences. Ron also had early success with the phosphoenolpyruvate-dependent phosphotransferase system discovered by Saul Roseman. This unusual transport complex used hydrolysis of PEP to drive sugar accumulation by phosphorylating the sugar as it was transported into the cell (or vesicle). Ron interacted with many other young scientists at the NIH, including Wil Konings, Vincent Marchesi and Alan Peterkofsky, all of whom became lifelong friends. For Ron, a colleague who shared his passion for science naturally became a personal friend to be invited home and into his extended family.

While at the NIH, Ron discovered that D-lactate, oxidized by a specific dehydrogenase in the cell membrane, would drive accumulation of many substrates to high intravesicular concentrations. Ron decided to focus on lactose transport catalyzed by lac permease, coded for by the well-characterized lacY gene. Only vesicles prepared from *Escherichia coli* expressing lac permease accumulated lactose in the presence of D-lactate. He studied lac permease for the rest of his career.

In the spring of 1970, Ron moved from the NIH to the Roche Institute of Molecular Biology, a research institute set up by the Swiss drug company Hoffman–LaRoche and headed by Sidney Udenfriend. Herb Weissbach had offered Ron a position in the RIMB biochemistry department, where Ron could continue his research on transport without having to apply for external funding. He quickly found himself embroiled in a controversy over how D-lactate oxidation was coupled to lactose uptake.

Ron's initial hypothesis was that lac permease was alternatively reduced and oxidized by the respiratory chain and in doing so went through conformational changes leading to lactose transport. This flew in the face of Peter Mitchell's chemiosmotic hypothesis, which postulated that transmembrane proton potentials generated by a respiratory chain could serve as a driving force for both transport and ATP synthesis.

At first, Ron resisted, performing new experiments to test the possibility that a proton potential could be involved. Ultimately, the results swayed his opinion until he became a strong proponent of chemiosmotic coupling in transport, declaring (tongue in cheek) that Peter Mitchell must be God … or a close relative. Ron was like that, always fascinated with the latest results and how they revealed new insights into transport mechanism. It didn't matter that he had a different opinion last week; he always went with the results. In 2012, Ron became the first American awarded the Peter Mitchell Medal by the European Bioenergetics Conference.

I worked in Ron's lab during this period at RIMB, and it was a heady mixture of exciting science and childlike antics. He would test that oxygen was flowing through the tubes into the transport reactions by pointing the outlet at his lit cigar and making sure the resulting flame was sufficiently impressive. One day he called all the postdocs in the lab, one at a time, into his office. He asked us to look at some experimental results on his desk. As each hapless postdoc leaned forward to look at the data, Ron set off the CO_2 fire extinguisher hidden under the desk with its nozzle aimed at the victim. After the shock wore off, Ron told the postdoc to leave by the back door of the office

> "He could and would quickly apply new technologies and methods if they appeared promising. His broad experimental approach was always inspiring to me and I have followed the same pathway. Ron was in many ways a pioneer."
>
> — Robert Gennis, friend and collaborator in the 1980s

VLADIMIR KASHO

Ron Kaback (in hat) poses with former lab members at his 80th birthday symposium in 2016 at the National Institutes of Health. In front: Doris Hertzlinger, José Luis Vásquez–Ibar, Vladimir Kasho and Shushi Nagamori. Second row: Shimon Schuldiner, Gary Rudnick, Lan Guan, Cindy Weitzman and Molly He. Third row: Gil Privé, Kirsten Jung, Maria-Luisa Garcia, Lekha Patel, H. Ron Kaback and Etana Padan. Fourth row: Gregory Kaczorowski, Stathis Frillingos, Kazunobu Matsushita, Nancy Carrasco and Eitan Bibi. Top row: Natalia Ermolova, Heinrich Jung, Xiaoxu Jiang, M. Gregor Madej, Don Menick and Irina Smirnova.

THE GOSPEL ACCORDING TO KABACK

The most interesting and important membrane proteins are transporters, because they can transduce energy into work in the form of a concentration gradient.

In contrast, channels are boring holes, which merely allow ions and such to flow down their activity gradients. Their only interesting property is gating, because gating is similar to transport.

Receptors are obviously broken transporters that bind ligands but forgot how to transport them across the membrane.

(THIS TEXT IS FROM A SLIDE THAT RON KABACK USED TO SHOW IN ALL HIS TALKS.)

"Ron was more than a collaborator, he was a true friend."
— William Dowhan, friend

"Over these 45 years, I slowly learned to know the real Ron, mentor, colleague, counsellor and leader, but the name that fits best is Friend — with a capital F."
— Shimon Schuldiner, Kaback postdoc in the early 1970s

"I have known intense people; I have known brilliant and passionate people; I have known people who are unconditionally devoted lifelong friends; and I knew Ron Kaback, who was all that to the nth degree."
— Nancy Carrasco, Kaback postdoc in the 1980s

"Ron and I would carry on a 20-minute conversation by the candy machines at the RIMB, during which he would buy and eat four or five peanut candy bars."
— Paul Wassarman, fellow member of Roche Institute

"Although I had never worked in his lab and did not know much about transporters, he immediately treated me with kindness and respect. I learned a lot from him, as I am sure did everyone else."
— Robert Edwards, assistant professor at UCLA in the 1990s

"When Ron heard a new joke, he immediately left his office and told it to every person on the floor — his way of memorizing and improving the storytelling."
— Eitan Bibi, Kaback postdoc in the early 1990s

"I cannot recall any story about Ron that did not include hilarity in some form. Neither his passion for science nor his astonishing, roiling energy could overwhelm the sense of humor that pervaded his entire being."
— Chris Miller, friend and collaborator

"When discussing ideas with Ron, he always used to say, 'try it' and immediately give a suggestion for an experiment."
— Etana Padan, took a sabbatical with Kaback in the 1970s

"Presumably, our wrestling on a pool table at a Gordon Conference would be too revealing of our essential and cherished immaturity."
— Arthur Karlin, longtime friend

and not to tell anyone until he had run through his entire staff.

In the 1980s, Ron took advantage of the recent cloning and sequencing of lacY and the availability of site-directed mutagenesis to embark on a quest to generate mutant permeases with all endogenous cysteines removed and with each amino acid in the sequence replaced, one at a time, with cysteine. The mutants generated in this project were extremely useful in identifying residues that were absolutely required for activity (most of which later were found to directly contact bound substrate). Some of these cysteines could be oxidatively crosslinked to others, providing an early sense of the 3D structure of the protein. In later studies, Ron used these cysteine mutants to delineate the substrate permeation pathways and the conformational changes in lac permease that open and close them.

Ron was elected to the American Academy of Arts and Sciences in 1986 and to the National Academy of Sciences in 1987. The NAS honor caught Ron on a cruise ship in the Caribbean, courtesy of his brother-in-law, who owned a tire company and chartered the cruise as a perk for his buyers. Ron and Teenchy were overjoyed at the news but had no one to share it with aside from the tire buyers, who had no idea what it meant.

In 1988, Ron was recruited by the physiology department at UCLA. They made him an offer he couldn't refuse: He no longer would be a

> "Even though he was recognized and respected for his research on lacY, I'm not sure that many people realized the depth of his intuition into bioenergetics, and his understanding of how active transport occurs."
>
> — Phillip Klebba, did a sabbatical with Kaback in 2011

"An outstanding human being, who never lost interest in his former students."
— Rhonda Dunten, Kaback postdoc in the early 1990s

"We will always remember Ron as an excellent scientist, outstanding teacher, great person and wonderful friend."
— Kirsten and Heinrich Jung, Kaback postdocs in the 1990s

"Ron was like a grandfather-figure to me. I just felt so fortunate and so privileged to have known him and become his friend."
— Nieng Yan, friend and collaborator

"I liked him a lot. Besides his great science, I really appreciated his generosity and sense of humor."
— Baruch Kanner, friend

"Kaback's law: 'Darwin was wrong! Natural selection is NOT the driving force for the evolution. Rather, it is the desire of Mother Nature to screw over the investigator!'"
— Natalia Ermolova, postdoc in the 2000s

"Wondering about the hereafter, Ron said, 'I can't imagine the world without me!' Well, I can't either."
— Stephen White, friend and collaborator from the 2010s

department chair as he was at Roche, and he would become a Howard Hughes Medical Institute investigator, saving him yet again from having to apply for federal funding. The sunny southern California weather meant he could play tennis outdoors all year long.

In 2003 Ron partnered with Jeff Abramson and So Iwata at Imperial College, London, to obtain an X-ray structure of lac permease in an inward-open (cytoplasm facing) conformation. The structure confirmed all the previous biochemical results and allowed experiments addressing the conformational changes in transport, which were confirmed when he obtained a nanobody-stabilized structure of lac permease in an outward-open conformation.

The rest of Ron's career was dedicated to understanding the mechanism by which a transmembrane proton gradient was coupled to galactoside transport. To this end, Ron applied a dizzying array of techniques, including single-molecule fluorescence resonance energy transfer, double electron–electron resonance , mass spectrometry, cross-linking and Fourier-transform infrared spectroscopy. If Ron felt that any technique could provide insight into the mechanism of lac permease, he would find an expert practitioner of that technique and convince them to collaborate.

Gary Rudnick (gary.rudnick@yale.edu) is a professor of pharmacology at the Yale School of Medicine. From 1973 to 1975, he did postdoctoral research on lactose permease with Ron Kaback at the Roche Institute of Molecular Biology. Follow him on Twitter @gwrud.

Stanley Cohen (1922 – 2020)

By Laura Lowe Furge

Stanley Cohen was born Nov. 17, 1922, in Brooklyn, New York, and died Feb. 5, 2020, at the age of 97 in Nashville, Tennessee. He was co-recipient, with Rita Levi–Montalcini, of the 1986 Nobel Prize in physiology or medicine for the discovery of growth factors.

Stanley discovered epidermal growth factor, or EGF, and its receptor. Nearly every biochemistry and cell biology textbook includes at least one section or chapter devoted to the cell signal transduction pathways that subsequently were linked to EGF and receptor tyrosine kinases. His pioneering work has allowed generations of biochemists to study the elegant yet complex pathways that allow cells to respond to external events.

Stanley began his studies at Brooklyn College, where he completed majors in both chemistry and zoology in 1943. He then earned an M.A. in zoology from Oberlin College in 1945. His time dissecting earthworms at Oberlin in Robert McEwen's class helped to define his later interests. A lab exam at Oberlin asked what cells in the worm performed a function similar to mammalian liver; Stanley did not know the answer. McEwen indicated it was the cells surrounding the intestine. This idea was to influence Stanley's doctoral work greatly.

He moved to the University of Michigan to begin his Ph.D. at a time when almost everyone was working on nutrition. As he described it, the standard approach was to "find an amino acid or nucleic acid, feed it to a rat or rabbit, analyze its urine." Stanley decided that instead of nutrition, he wanted to determine whether the cells in earthworms actually did function like a liver. His adviser, Howard Lewis, gave him six months to get preliminary results.

MARLENE JANYE

Stanley Cohen hiking in Arizona in 2001.

Out on the campus green wearing a miner's headlight, Stanley collected earthworms. He kept the worms in starving conditions (no dirt or foliage) for about a month. Over that time, he would wash the worms and analyze the contents of the wash. He reasoned that the liverlike chloragogen cells should be able to make urea under those conditions and that he could measure urea in a diffusion assay with urease. Using this approach, he saw increasing amounts of urea produced over time. The longer a worm fasted, the more urea it produced. Stanley then assayed arginase activity in the worm by dissecting the cells around the intestine, adding arginine to the isolated tissue and then measuring urea production. By this method, he noted induction of arginase over

time — though at that time, induction of enzymes was not understood at all. The study was published in the Journal of Biological Chemistry in 1950. The moral of the story was, as Stanley would say, "Find something that interests you and do it; you never know what will end up being important." This was a guiding principle to him as a scientist.

After completing his Ph.D. in 1948 and then working as an instructor in the departments of biochemistry and pediatrics at the University of Colorado, he joined Martin Kamen's group at Washington University in St. Louis as a postdoctoral fellow to learn about use of radioisotopes in biomedical research. (Kamen had moved to Washington University after being fired from the University of California for talking to the Russian ambassador about using radioisotopes to cure the ambassador's daughter, who had leukemia.) After his American Cancer Society fellowship ended, Stanley moved to the lab of Viktor Hamburger and Rita Levi–Montalcini. There he began isolating a factor that caused nerve growth.

Along with his discoveries at the bench, Stanley was in a journal club that included Arthur Kornberg, Martin Kamen, Paul Berg and other faculty, which he referred to as "the best education I ever had." The journal club's daily meetings were a time for deep debate and discussion with lots of questions. Stanley presented papers almost every week to the group. He, Kornberg and Berg all went on to win Nobel Prizes (separately). They did not always reach consensus on what constituted the best science. Stanley remembered the day James Watson and Francis Crick's double helix paper was published in the journal Nature, and they discussed it in journal club. Some in the group thought it was genius; others thought it was just "paper chemistry," he said. For Stanley, these discussions demonstrated that if you thought something was important, you should follow it up — even when others may not think the results were worth pursuing.

One of Stanley's most celebrated aha! moments came during studies of snake venom induction of nerve growth. He reasoned that snake venom was made by the salivary gland, so perhaps the mammalian salivary gland had nerve growth factor as well. When the extracts were applied to mouse nerve cultures, there was nerve growth. Also, when the extracts were injected into newborn mice, there was nerve growth and, remarkably, early eye opening. Even decades after the observation, Stanley would recount the moment joyfully: "I was looking at the mice, and they were looking back at me. They weren't supposed to be doing that!" He later said it was pure luck that he isolated the salivary gland of a male mouse (as it turned out, male mice have significantly higher levels of EGF in the salivary gland than

HISTORY OF MEDICINE COLLECTIONS AT VANDERBILT UNIVERSITY

Stanley Cohen in his laboratory at Vanderbilt University in 1986.

MARLENE JANYE

Stanley Cohen hiking near Estes Park, Colorado.

female). Stanley wrote a retrospective of this work for a special Reflections article in JBC in 2008 — highly recommended reading.

When Stanley moved to Vanderbilt University in 1959 as an assistant professor, he began isolating the factor that caused this early eye opening (as well as early tooth eruption), now known as EGF. Stanley once told me, with a smile, that a colleague tried to talk him out of studying the eye-opening phenomenon — thankfully, he didn't listen. Stanley applied for support from what then was known as the Human Health and Development Division of the National Institutes of Health. His was the seventh grant funded by the division. His three-page grant application, simply titled "Epidermal growth factor," supported his work for 38 years, until his retirement in 2000.

Stanley thrived on seeing the details — the little curiosities. One curiosity he never solved, and it clearly baffled him well into retirement, was something he saw when his lab was isolating EGF from mouse submaxillary gland. They used a precipitation test in capillary tubes to track the purification at each step. He made a highly reproducible, and highly unusual, observation: Common house ants coming into his lab overnight from outdoors seemed to be very attracted to the capillary tubes containing extracts from adult male mouse salivary gland but not to the extracts made from female mouse salivary gland. During my postdoc days with him in 1998, he still had a Styrofoam tray with a row of capillaries in his lab that he had moved with him from his old building. The liquid inside had long since desiccated, but the tray and tubes represented a problem he hadn't solved.

In his later years, Stanley was fascinated by neuroscience and strongly believed that was where the next great biomedical discoveries would be made. He used to say, "You need a 21st century brain to do this work."

Stanley was always interested in talking science with other scientists; throughout his career, he regularly attended seminars, had impromptu conversations with colleagues in the halls and encouraged others in their work.

He said four things drove him as a scientist:

- Love of learning.
- Interacting with smart people.
- Not being afraid to ask questions.
- Doing what was interesting.

Stanley absolutely loved science, had a curious mind and never took an observation for granted ("Oh, isn't that interesting?" he would say). He frequently reacted to an experimental outcome with the expression, "It worked — a minor miracle." Even after earning the Nobel Prize, Stanley maintained a small research group of no more than one or two postdocs at a time and a technician because he wanted to be close to the data and results.

Stanley was not one to sit idle, either at work or at play. He learned to play the clarinet while at Washington University. In those days, there were no column fraction collectors, so on the many late nights he was isolating nerve growth factor, he practiced his clarinet between collecting fractions. He even played in quartets with other scientists (and noted that Kamen was a violin prodigy).

Stanley was an avid double tennis player — very competitive on the court despite leg mobility issues from a bout with polio as a child. He

Stanley Cohen receives his Nobel Prize medal and diploma from King Carl XVI Gustaf of Sweden on Dec. 10, 1986.

HISTORY OF MEDICINE COLLECTIONS AT VANDERBILT UNIVERSITY

Stanley Cohen discovered epidermal growth factor and its receptors, allowing generations of biochemists to study the pathways that allow cells to respond to external events.

loved to go jeeping and frequented Jeep Jamborees in his black Jeep with an "If you can read this, flip me over" sticker.

Marlene Jayne, longtime department administrator in the biochemistry department at Vanderbilt and Stanley's close personal friend, would joke that he had a problem quitting smoking. He was known for walking the halls (thinking about science, he said) with his pipe in his mouth. The dean of the medical school wrote him a letter reminding him of the smoking policy. Stan immediately returned the letter stating that he was not smoking but only used the pipe as a pacifier. The dean wrote back congratulating him and pasted a gold star on the letter; Stanley then ditched his pacifier. After Stanley won the Nobel Prize, the chancellor gave him a specially marked parking place close to his office that was his until he retired.

Stanley left a legacy of discoveries that gave us a leap forward in understanding cell signaling, cancer and disease targets. And he had many friends, to boot; his unpretentious style and reassuring smile will be greatly missed.

Stanley is survived by his wife, Jan, and three children and two grandchildren.

Special thanks to Marlene Jayne for photographs and for careful reading of this retrospective.

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The best of two worlds

Pickett's work melds cellular oxidative stress with drug discovery

By Martin J. Spiering

Many chemicals and cellular processes cause oxidative stress that can damage lipids, proteins or DNA. To sense and respond to this threat quickly, organisms have evolved enzymes that neutralize harmful oxidants such as reactive oxygen species and xenobiotics in cells.

These antioxidant enzymes include glutathione S-transferase, or GST, NADPH:quinone oxidoreductase 1, thioredoxin and hemeoxygenase-1. Many of these proteins are commonly expressed in cells exposed to oxidative stress. The antioxidant response element, or ARE, present in the promoters of many antioxidant protein genes is a major regulatory lynchpin in this cellular response.

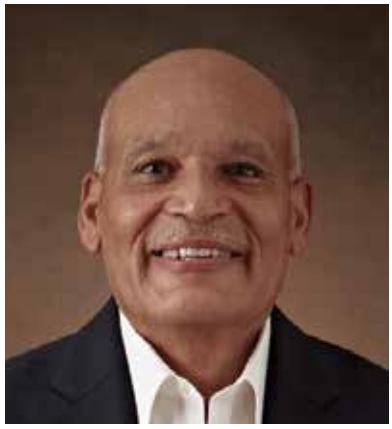
Cecil Pickett's lab at the Merck Frosst Centre for Therapeutic Research in Quebec discovered ARE as reported in the early 1990s in two **Journal of Biological Chemistry** papers now recognized as Classics.

Charting a dual career path

After completing a Ph.D. in biology and a two-year postdoc at the University of California, Los Angeles, in the mid-1970s, Pickett pursued a career in the pharmaceutical industry. He was recruited to Merck in 1978 by its then head of research and development (and later CEO), Roy Vagelos.

"I became interested in how drug-metabolizing enzymes were induced by various xenobiotics," Pickett said.

When he started at Merck, Pickett said, Vagelos encouraged the company's researchers "to really start a research program where we could build our research careers. But he also said, 'Keep in mind the long-term mission of Merck — and that is to discover novel medicines that could help people.'"



BEN WEBBER/WIKIMEDIA COMMONS

Cecil Pickett's work during his more than 40-year career has spanned both basic research and drug discovery.

Inspired by Vagelos' vision and advice, Pickett launched a vigorous research program that attracted many talented scientists.

Pickett and his team treated rats with various chemicals to induce genes involved in xenobiotic metabolism and breakdown. To isolate these genes, the team used a method for translation of the mRNAs isolated from the livers of these animals.

This approach yielded promising results. "I noticed that the profile of the translated proteins from induced animals was very different from that of noninduced animals," Pickett said. "And there tended to be a low–molecular-weight protein that was always induced."

At first, it was unclear what this small protein might be. But a colleague at Merck, Anthony Lu, suggested that it might be GST, Pickett said.

To test Lu's hunch, Pickett and his laboratory applied a technique called polysomal immunoprecipitation. The researchers used GST-specific antibodies from Barbara Hales' laboratory at McGill University in Montreal to detect and isolate mRNA–ribosome complexes called polysomes that contained GST-encoding mRNA sequences.

"That allowed us to synthesize cDNAs and isolate the structural genes for the GSTs," Pickett said.

Discovering ARE

With the GST gene sequences in hand, the researchers could home in on surrounding DNA regions that regulate the expression of the genes and identify the regulatory motifs in their promoters.

Pickett's team reported that ARE is one of the motifs required for xenobiotics-induced activation of transcription of the GST Ya gene and also defined the ARE consensus sequence.

The team's discovery of ARE caused a major shift in thinking about how xenobiotic-metabolizing genes are regulated. Their work made it clear that antioxidant genes such as GST have multiple regulatory elements in their promoters that are responsive to specific cellular stressors.

Subsequent work by Pickett's lab and others helped identify the transcription factors that recognize the

ARE and activate the transcription of GST and other oxidative stress response genes.

One of these transcriptional regulators is nuclear factor erythroid 2–related factor 2, or NRF2, identified in 1994 by another research group. Because NRF2 binds ARE, suggesting it helps regulate oxidative stress responses, Pickett and his team focused on it next and helped uncover key aspects of how NRF2 itself is regulated.

"The discovery of the ARE really laid the groundwork for much of the work that has been done in the field on NRF2," Pickett said.

A successful balance

So many fundamental discoveries came out of his lab that it might seem Pickett's main focus was on fundamental research. However, Pickett was also interested in drug discovery, and his talent in this area led him to direct major drug development efforts at Merck.

This meant working with multidisciplinary research teams. "It was a large group of people — chemists, pharmacologists, molecular biologists and biochemists," Pickett said. "I oversaw an integrated approach to how drugs need to be discovered and developed."

Pickett served on an internal committee that directed all of Merck's business in Canada; this unique vantage point further shaped his approach to drug discovery.

His scientific background in the molecular bases of inflammatory diseases was key for the discovery and development of the anti-inflammatory medicine montelukast, known by the brand name Singulair, used chiefly to prevent asthma attacks and other inflammatory lung conditions.

In 1993, Pickett joined Schering–Plough in New Jersey as executive vice president of discovery research, eventually becoming president of the institute where, he said, "I had a very good balance between the more fundamental work and also the drug discovery work."

At Schering–Plough, he drove the development of several drugs for managing metabolic disorders and diseases, including cancer, fungal and viral infections, and hypercholesterolemia.

He also continued basic research such as further deciphering the role of the ARE-binding transcriptional regulator NRF2 in oxidative stress responses. This effort helped advance drug development at Biogen Idec after Pickett joined the company as head of R&D in 2006.

"The work on NRF2 at Biogen began with some ideas that I had," he said.

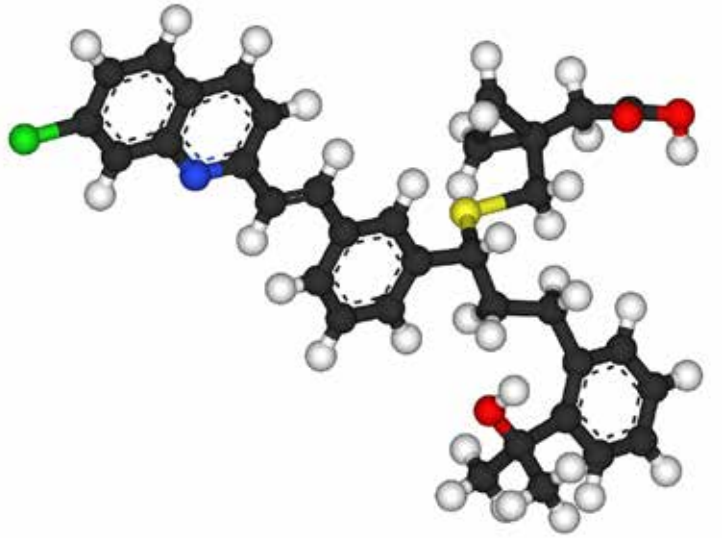
Biogen's small-molecule compound dimethyl fumarate, sold as Tecfidera, which is used to treat multiple sclerosis, activates the NRF2 pathway.

Pickett is now formally retired, but he remains involved in R&D as a member of advisory boards and committees of such organizations and companies as the American Association for Cancer Research and Zimmer Biomet.

Reflecting on the many challenges of directing both fundamental research and drug discovery efforts, Pickett emphasizes the synergy these diverse pursuits offered.

"I think having my own lab, being engaged in that research and reading the scientific literature helped me in making sure that the scientific thoughts in the (drug discovery) programs were solid."

Read more Journal of Biological Chemistry Classics at jbc.org



COURTESY OF CECIL PICKETT

Pickett not only led foundational research efforts that uncovered the molecular bases of how cells respond to oxidative stress but also directed the discovery and development of anti-inflammatory drugs such as the asthma medication montelukast (brand name Singulair), whose chemical structure is shown here.

Martin J. Spiering (mspiering@asbmb.org) is the technical editor at the Journal of Biological Chemistry. Follow him on Twitter @spieringmj.

How spider venom could help relieve pain

Modified tarantula toxins can become effective pain treatments

By Courtney Chandler

This photo was taken at the "Spiders" exhibition at the Museo di storia naturale Giacomo Doria in 2016.

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Pain is a public health problem that often is not adequately addressed by current therapies. About 100 million adults in the United States suffer from chronic pain, and those who don't will experience pain at some point in their lives, whether it be a scraped knee or surgery.

There is a need for pain treatments that are safe, effective and not addictive. This is partially due to increased awareness of the highly addictive nature of many available pain medications. A recent article in the **Journal of Biological Chemistry** aims to meet these treatment needs using an unlikely source — spider venom.

Spider venoms are complex mixtures with a wide array of biologically active compounds that have one function: to subdue the spiders' prey. Lead author Robert Neff and the Janssen Research and Development Neuroscience Drug Discovery Team focused on a peptide molecule called huwentoxin-IV found in the venom of a tarantula species called the Chinese bird spider.

This toxin is known to block a specific protein involved in pain signaling called Nav1.7. This protein is a sodium channel that amplifies pain signals to ensure they reach the brain. Neff said the researchers targeted Nav1.7 because previous research has shown that people who don't have functioning Nav1.7 can't feel pain despite being otherwise normal.

"Unfortunately, Nav1.7 is not an easy target," Neff said. "Many other groups have tried to identify small molecules that block its activity, and to date none of these efforts have resulted in a marketed drug."

As an added challenge, huwentoxin-IV is known to block other proteins in the sodium channel family that are important for normal nervous system function. Yet Neff and his team were not discouraged. They worked systematically to make small changes to huwentoxin-IV that would optimize its interaction with Nav1.7 and decrease its ability to block other sodium channel family members.

When they identified changes that had favorable effects, they combined them to make a molecule more effective at blocking pain signaling without disrupting the rest of the nervous system. However, combining single changes together did not always yield a molecule more effective than the single changes themselves, which was surprising, Neff said.

"This was a good reminder that small changes can have wide-ranging and unpredictable repercussions throughout the molecule."

Although the work is still in preliminary stages, there is reason to believe we could see spider-derived pain treatments down the line. A tarantula toxin previously identified by the group in a parallel study in 2017 has been tested in preclinical studies. While their current designer molecules are not yet in preclinical trials, Neff and his team have produced a large library of subtly different toxins and cataloged their activities in their more recent study, which should be useful for the pain research community at large.

"We hope that other interested investigators will be able to use these datasets to further their research," Neff said. "We want to help accelerate the discovery of a new, safe, and nonaddictive pain medication."

Courtney Chandler (courtneyec19@gmail.com) is a postdoctoral researcher at Johns Hopkins University and a careers columnist for the ASBMB. Follow her on Twitter @CourtneyEChan.

ANGPTL3: A promising therapeutic direction for cardiovascular disease

By Gelareh Vinueza

Despite major advances in the treatment of cardiovascular disease, or CVD, with statins, antihypertensive and antithrombotic drugs, a residual number of patients with CVD remain untreated. These patients have a genetic disorder or extremely high levels of triglycerides (hypertriglyceridemia) that render them unresponsive to current therapies. To find new therapies, researchers are targeting circulating lipids and lipoproteins.

Two main sugars in the human diet are glucose and fructose. Glucose, the major component of dietary carbohydrates, is a product of starch. Fructose is found mainly in soft drinks and other beverages, desserts and candies. Although similar in calories, glucose and fructose are metabolized differently. Glucose is absorbed rapidly by almost all cells in the body, and its levels remain balanced through insulin release. Fructose is metabolized mainly by the liver, and its levels are not regulated by insulin; increased fructose consumption increases circulating triglycerides, low-density lipoprotein cholesterol, and fat around organs and blood vessels. In the process of de novo lipogenesis, fructose in the liver metabolizes to lipids.

The liver expresses and secretes angiopoietinlike 3, or ANGPTL3, which plays a role in lipid clearance; therefore, scientists see this protein as a promising therapeutic target for developing lipid-lowering drugs that target formation of triglycerides. In a recent paper in the **Journal of Lipid Research**, Peter Havel and colleagues at the University of California wrote that consumption of dietary fructose increases circulating levels of ANGPTL3 in rhesus macaques by 30% to 40%. Increased ANGPTL3 correlated with increased levels of plasma triglycerides.

In collaboration with Arrowhead Pharmaceuticals, the authors found that inhibiting hepatic ANGPTL3 expression using RNA interference technology resulted in reduced circulating ANGPTL3 and triglycerides in rhesus macaques. Supplementing the macaques' diet with fish oil led to decreased levels of ANGPTL3.

"These are the first studies to demonstrate the effect of diet (fructose and omega-3 fatty acids in fish oil) on ANGPTL3," Havel said, "and suggest that ANGPTL3 is a promising target for management of hypertriglyceridemia."

For patients with CVD who can't benefit from current treatments, this research opens exciting possibilities for new therapies. "Suppression of ANGPTL3 production may be an important mechanism," Havel said. "Fish oil supplements, when consumed in adequate amounts, lower plasma triglycerides and reduce CVD risk."

DOI: 10.1194/jlr.RA119000423

CREDIT: COURTESY OF PETER HAVEL

Adult male rhesus macaques were fed an unrestricted diet and water along with a flavored 15% fructose solution and then treated with fish oil supplements to demonstrate the role of diet on ANGPTL3 protein levels.

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Multiomics meets antimalarials

By John Arnst

Among the species of the plasmodium parasite that cause malaria, P. falciparum is far and away the deadliest to humans, causing 405,000 deaths in 2018 alone. While public health workers have relied on three classes of drugs — quinines, artemisinins and antifolates — to treat the mosquito-borne parasitic infection for decades, the organism inevitably becomes resistant, leaving compounds such as chloroquine ineffective across swaths of sub-Saharan Africa, where 94% of cases occur.

Newer drugs, including mefloquine and dihydroartemisinin, have been deployed, and recently discovered compounds are being evaluated in drug screens and in animal models. Researchers at Darren Creek's lab at the Monash Institute of Pharmaceutical Sciences in Melbourne, Australia, recently used a multiomics approach that combined the strengths of proteomics, peptidomics and metabolomics with biochemical assays to evaluate how a promising new antimalaria drug candidate, JPC-3210, acts against the parasite. They published their results, which include a biochemical fingerprint of how JPC-3210 and other common antimalarials affect P. falciparum, in the journal **Molecular & Cellular Proteomics**.

The study was performed with collaborators from the Australian Defence Force Malaria and Infectious Disease Institute, who have been working with compounds from the Jacobus Pharmaceutical Company in Princeton, New Jersey, including JPC-3210, for over a decade. The compound was selected as a lead drug candidate in 2016.

According to Ghizal Siddiqui, a postdoctoral researcher in Creek's lab who began working with plasmodium as a graduate student, JPC-3210 kills plasmodium parasites by interfering with their ability to digest the hemoglobin in blood, which is an essential process for the organisms.

"Most compounds that we have that are antimalarials target this particular pathway, and this is because without this pathway, the parasite would effectively die," she said.

However, as with all antimalarials, even the well-studied chloroquine, researchers don't fully understand the mechanics behind this activity.

"We're still not sure exactly what JPC is doing," Siddiqui said. "We know that it's having all these effects, but we don't know exactly, still, what is the precise target."

The multiomics analyses performed by Siddiqui and colleagues showed that JPC-3210 kills the parasite in a manner unlike many antimalarials now in use, including chloroquine. However, Siddiqui said, the biochemical fingerprint for JPC-3210 is similar to that of mefloquine and dihydroartemisinin.

"It's probably not hitting the same target," she said. "But it has a very similar fingerprint profile to those two drugs … and we believe it's because both of those drugs are so fast-acting and so potent, and so is this drug."

Healthcare workers will often coadminister drugs that act against parasites at different speeds.

"Usually they like to combine drugs with different mechanisms of action," Siddiqui said. Thus JPC-3210 could be combined with a drug that has a different mechanism or even with an existing combination therapy.

Siddiqui and her colleagues in Melbourne plan to do more multiomics evaluations of JPC-3210 and other antimalarial candidates to home in on their exact mechanisms of action. Meanwhile, their colleagues at the Medicines for Malaria Venture in Geneva, who licensed the compound from Jacobus in October 2019, are continuing to develop JPC-3210 in anticipation of human trials.

DOI: 10.1074/mcp.RA119.001797

In 1962, postal administrators issued and donated tens of thousands of stamps to the World Health Organization to be sold to collectors to raise funds for the WHO's Global Malaria Eradication Programme, begun in 1955.

WELLCOME COLLECTION

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From the journals

By Deboleena M. Guharay , Anand Rao & Elizabeth Stivison

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

Activating the anti-aging enzyme

Histone deacetylase sirtuin 6, or SIRT6, an enzyme that regulates pathways related to aging, has garnered the attention of scientists aiming to tackle aging-related diseases. SIRT6's many biological functions include regulating metabolism, suppressing tumors and improving glucose tolerance. However, researchers do not have a good understanding of the mechanistic details surrounding its activation.

In a recent paper published in the **Journal of Biological Chemistry**, Mark Klein and colleagues at the Wisconsin Institute for Discovery used activity-based screening to identify compounds capable of activating SIRT6 to explore further its activation mechanisms. They discovered a novel activator of SIRT6 capable of increasing activity 18-fold to 48-fold and found that the amino acid Arg-65 is essential to this process. This work paves the way for the development of SIRT6-activating therapeutic molecules.
DOI: 10.1074/jbc.RA119.011285

GATOR aids Sestrin-mediated enzyme activation

Understanding what drives the molecular effects of exercise and nutrient starvation is of medical importance. Skeletal muscle atrophy, a major health problem, results from a number of pathological conditions including spinal cord injury and neuromuscular disorders. Obesity-associated disorders also impose a health burden on society. The processes underlying these conditions are largely unknown. Researchers studying these mechanisms and developing therapies seek to identify targetable molecules that can alter metabolic processes.

Sestrins are a family of stress-inducible proteins that coordinate metabolic processes and respond to exercise and to nutrient starvation. In work published in the **Journal of Biological Chemistry**, Allison Kowalsky and colleagues at the University of Michigan provide new mechanistic details for how this response occurs. Their findings show that Sestrin2, a homolog highly expressed in the liver, requires the protein complex GTPase-activating protein for Rag subunits A/B 2, or GATOR2, to interact with mammalian target of rapamycin 2 and activate protein kinase B, an insulin-dependent kinase. This work identifies a new signaling mechanism that may be important for metabolism, the response of skeletal muscle to exercise and the progression of obesity-associated disorders.
DOI: 10.1074/jbc.RA119.010857

Combating the flu with a fat

The 2009 H1N1 influenza outbreak, the first global pandemic in 40 years, triggered the Centers for Disease Control and Prevention to improve flu surveillance systems and highlighted the need for new approaches to treat influenza. A team of researchers led by Dennis Voelker at National Jewish Health in Denver modeled the H1N1 flu in dishes of cells as well as in mice and ferrets to demonstrate that anionic phospholipids, specifically phosphatidylinositol and palmitoyl-oleoyl-phosphatidylglycerol, or POPG, can disrupt viral infection by influenza. When the researchers challenged mice with H1N1 flu, all the mice treated with POPG survived, compared to no survivors among untreated mice. POPG also rescued ferrets from flu-induced inflammation.

This collaborative study, published in the **Journal of Biological Chemistry**, shows that lipids can disrupt flu infection and lays the foundation for development of new therapies.
DOI: 10.1074/jbc.RA119.012053

Forming stress-induced RNA–protein complexes

Under duress, cells initiate a stress response that often leads to the interruption of protein building and the formation of stress granules, RNA–protein complexes, in the cytoplasm. Though the function of stress granule, or SG, assemblies remains largely unknown, they are thought to affect cell death and modulate a cell's response to viral infection by holding mRNAs in an inactive state until cells recover. RNase L, an antiviral enzyme that cleaves RNA, stimulates the formation of SG-like complexes, but researchers have not yet identified the similarities between these complexes

Preventing chemotherapy-induced neuropathic pain

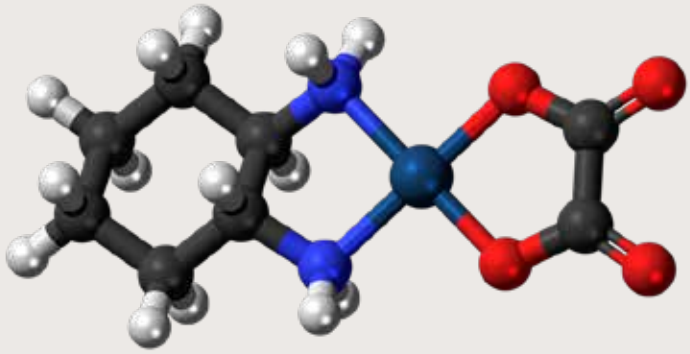
Chemotherapy, which kills rapidly dividing cancer cells to prevent their spread throughout the body, is a commonly prescribed treatment for many forms of cancer, but it can result in painful and debilitating side effects. One such side effect, chemotherapy-induced peripheral neuropathy, is caused by damage to one or more nerves; weakness, numbness and pain begin in the hands and feet and can progress to other parts of the body. Three classes of therapeutic chemicals cause peripheral neuropathy: platinum-based agents, taxanes and the proteasome inhibitor bortezomib. Discontinuing therapy with these drugs is the only treatment.

In a study published in the **Journal of Biological Chemistry**, a team of researchers led by Raymond Herr and Raghav Sundar at the National University of Singapore Yong Loo Lin School of Medicine write that oxaliplatin, a platinum-based chemotherapeutic, alters the activity of S1P, a lipid-based signaling molecule that reduces neuroinflammation. S1P interacts with five receptors, S1P1-5, which are expressed differently across various tissues and activate a range of intracellular signaling cascades.

The researchers first examined the effects of platinum-based drugs on plasma S1P levels in human cancer patients and found that oxaliplatin treatment increased the d16:1 S1P species. This increase shifted the balance of S1P interaction with S1P2, which, they argue, may provide a mechanistic explanation for the toxic side effects affiliated with oxaliplatin. To test this, the researchers used a rat model of chemotherapy-induced neuropathy to show that a selective agonist targeting this molecule reduced neuropathy symptoms, indicating the therapeutic potential of targeting S1P2 for treatment of the neurotoxic effects of platinum-based chemotherapeutic agents.

DOI: 10.15347/wjm/2014.010

— Anand Rao



JYNTO/WIKIMEDIA COMMONS

Ball-and-stick model of the oxaliplatin complex, an anti-cancer drug. Oxaliplatin forms inter- and intra-strand cross-links in DNA, preventing DNA replication and transcription, causing cell death.

and traditional SGs.

Using immunofluorescence, live-cell imaging and mass spectrometry–based analyses, James Burke and colleagues at the University of Colorado Boulder showed that the complexes formed in response to RNase L activity — known as RNase L-dependent bodies, or RLBs — are unique in their protein and RNA composition and are generated independently of SGs. They also showed that RLBs interact longer with elements in the cytoplasm, such as P-bodies, than SGs, suggesting that RLBs may differ in their ability to modulate cellular response to infections. These findings, which were published in the **Journal of Biological Chemistry**, increase understanding of how RNAse L contributes to cells' antiviral response.

DOI: 10.1074/jbc.RA119.011638

A SPEEDy way to prepare mass spec samples

To prepare samples for mass spectrometry analysis, researchers typically rely on detergents or chaotropic reagents. These methods present problems in sample digestion or extraction and require many steps, and the results can be biased. Joerg Doellinger and colleagues at the Centre for Biological Threats and Special Pathogens in Germany have reimagined how to prepare samples for mass spec, and they report on their new method in the journal **Molecular & Cellular Proteomics**.

The researchers call their method SPEED, short for sample prep by easy extraction and digestion, and it relies on acidification instead of detergents. In SPEED, pure trifluoroacetic acid is used to dissolve cells and tissues, the advantage being that the acid doesn't disrupt peptide bonds and doesn't modify amino acids. The dissolved sample then is neutralized, and the neutralized sample can be digested easily. When compared to other methods of preparation for liquid chromatogra-

New therapeutic targets for hyperactive mTOR

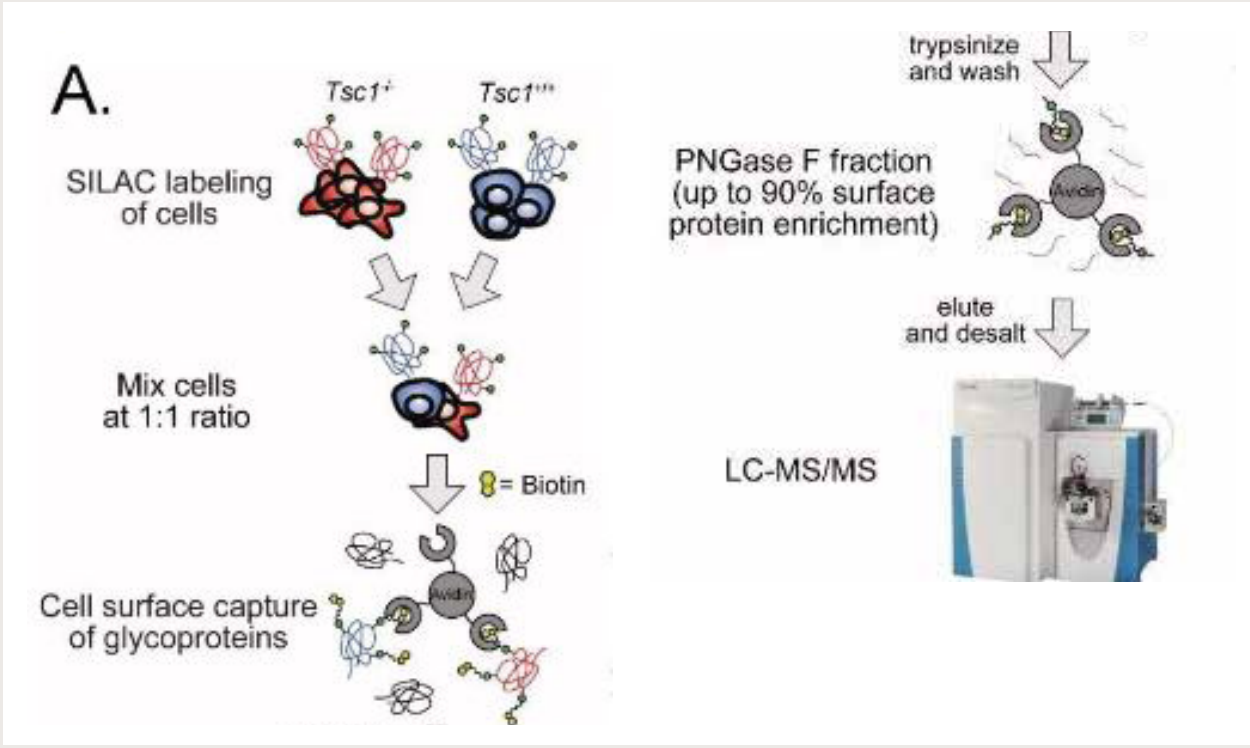
Overactive signaling of the protein kinase mammalian target of rapamycin, or mTOR, which leads to increased cell size and proliferation, is found in many cancers and other pathologies. While drugs exist that target mTOR, the authors of a new study in the journal **Molecular & Cellular Proteomics** point out that none of these drugs are effective in the long term, because cells adapt to the inhibition of mTOR.

Authors Junnian Wei, Kevin Leung and their colleagues at the University of California, San Francisco, addressed this clinical issue by finding other proteins to target in cases of upregulated mTOR activity. They used two cell lines — one with and one without tuberous sclerosis complex 1, or TSC1 — as their model, since cells without an active TSC1/2 complex have overactive mTOR signaling. The authors compared these two cell lines using stable isotope labeling by amino acids in cell culture; they labeled either the TSC1+/+ or TSC1-/- cells with heavy carbon and nitrogen, performed mass spectrometry on samples enriched for surface proteins, and looked for surface proteins whose expression changed between the strains. A number of proteins changed, and the researchers validated the top upregulated hits using flow cytometry. The metalloproteinases neprilysin, or NEP/CD10, and aminopeptidase N, or APN/CD13, were discovered to be the most highly upregulated in TSC-/- cells.

The researchers confirmed this increase in mouse cell lines and diverse human cell lines with upregulated mTOR, indicating that these proteins may be promising conserved targets. They also found that when they downregulated either of these two proteins in cancer cell lines with upregulated mTOR, the cell proliferation decreased. Similarly, the loss of TSC1 or 2 and the resulting overactive mTOR sensitized cells to the inhibition of NEP and APN. These two new targets may be useful in the future treatment of diseases with hyperactive mTOR.

DOI: 10.1074/mcp.RA119.001785

— Elizabeth Stivison



WEI, LEUNG, ET AL./JBC

This schematic shows the workflow used to find surface proteins that change in response to mTOR signaling.

HDL and Smo play a role in inhibiting beta-cell apoptosis

About 32 million Americans have Type 2 diabetes, meaning their bodies either don't produce enough insulin or resist insulin. Researchers believe that due to endoplasmic reticulum stress, these patients lose the pancreatic beta cells that synthesize insulin.

Studies in humans and in animal models have shown that high-density lipoprotein, or HDL, reduces the risk of diabetes by decreasing ER stress and beta-cell apoptosis. Researchers also have found that the hedgehog signaling pathway and cholesterol are involved in the fate of pancreatic beta cells and insulin production.

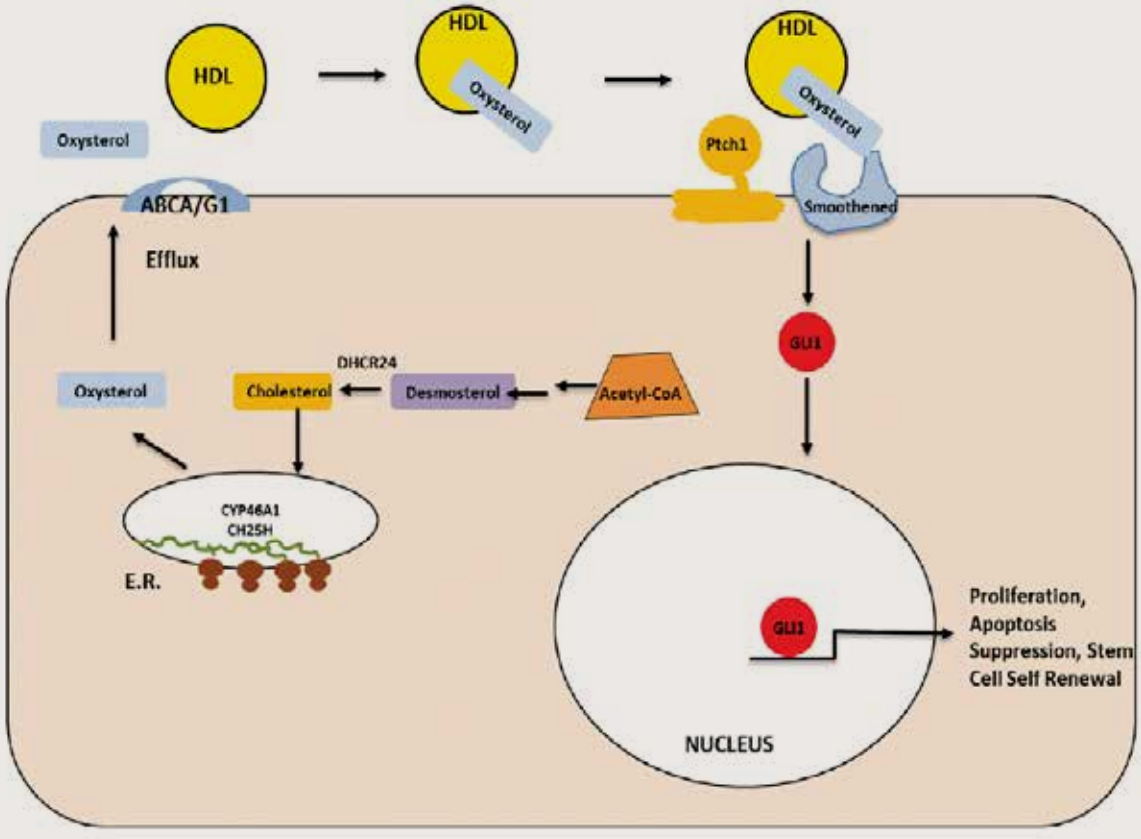
Mustafa Yalcinkaya and colleagues at the University Hospital of Zurich, Switzerland, published a paper in the **Journal of Lipid Research** on the mechanism by which HDL and the hedgehog signaling molecule Smoothened, or Smo, reduce beta-cell apoptosis induced by ER stress. They treated rat insulinoma beta cells with the ER stress inducer thapsigargin in the presence or absence of native HDL or CSL-111 (an artificially reconstituted HDL). Apoptosis studies such as free nucleosome assay and Caspase-3 activity assay showed that both HDL and CSL-111 reduced apoptosis in these cells.

Similar experiments involving the knockdown of oxysterol-producing enzyme cytochrome P450 and cholesterol transporter ABCG1 increased the thapsigargin-triggered apoptosis, indicating that oxysterols and their transport by ABCG1 increased the anti-apoptotic activity of HDL. Cholesterol and oxysterols are known to regulate the activity of Smo, which translocates the transcription factor GLI1 into the nucleus. Experiments with oxysterol showed increased nuclear GLI1, indicating Smo activation by oxysterol in the presence of HDL.

The research shows that oxysterol mobilized by HDL activates Smo and later reduces apoptosis. This work is promising; the researchers plan to conduct experiments imitating the physiological environment in primary cells for a more in-depth knowledge of the process.

DOI: 10.1194/jlr.RA119000509

— Deboleena M. Guharay



MUSTAFA YALCINKAYA ET AL./JLR

This diagram shows the mechanism of inhibition of beta-cell apoptosis by high-density lipoprotein and Hedgehog signaling molecule Smoothened.

phy–mass spectrometry, SPEED consistently had the least variation batch to batch, indicating better reproducibility. In difficult to lyse samples such as mouse lung tissue and Bacillus cereus cells, it identified up to 41% more proteins than other methods.
DOI: 10.1074/mcp.TIR119.001616

A new program to detect citrullination

Citrullination is the replacement of the imine group in the amino acid arginine with an oxygen, which converts arginine to citrulline. Recent studies have tied citrullination to autoimmune diseases such as rheumatoid arthritis and have highlighted the role that citrullination of proteins by the pathogenic bacterium Porphyromonas gingivalis may play in the development of that disorder. To understand exactly what citrullination and citrullinated proteins are doing in biology and pathology, an accurate way of identifying citrullinated proteins is required. Mass spectrometry is the most reliable method but presents problems, including misinterpretation of data.

Daniel Nyberg Larsen and colleagues at the University of Southern Denmark have developed a new computer program, Citrullia, to identify and validate citrullinated peptides. The program displays all relevant information in a single window and allows potential citrullinated residues to be evaluated on primary mass spectrum specificity, fragment ions, fragmentation pattern and retention time behavior, producing reliable results that can be validated in a straightforward manner. This study was published in the journal **Molecular & Cellular Proteomics**.
DOI: 10.1074/mcp.RA119.001700

Lipid asymmetry in a plasma membrane

The plasma membrane in eukaryotic cells is a lipid bilayer of lipids and proteins. The lipid content of this bilayer varies between the outer leaflet that faces the extracellular environment and the inner leaflet, which faces the cytoplasm. This asymmetry in the lipid distribution across the plasma membrane helps to regulate important biological processes such as apoptosis and signaling in immune cells. Scientists have tried to develop models to study it but have been unable to re-create the complex cellular environment. A more thorough methodology was needed.

Anjali Gupta and a team led by Thorsten Wohland from the National University of Singapore used two imaging systems — fluorescence lifetime imaging microscopy and imaging total internal reflection fluorescence correlation spectroscopy — to do a spatiotemporal study of the asymmetric distribution of lipids across the plasma membrane in live mammalian cells. This study was published in the **Journal of Lipid Research**. Their neat methodology demonstrated the varying lipid distribution by using fluorescent analogues of membrane lipids such as phosphatidylcholine, sphingomyelin and phosphatidylserine, and it also showed that the distribution depends on the cell line.
DOI: 10.1194/jlr.D119000364

Are apolipoproteins a key to dementia risk?

Alzheimer's disease and its related dementia are major neurological disorders, with about 44 million patients worldwide. An affordable diagnostic biomarker is needed that can identify easily the onset of these conditions in high-risk individuals. Recent studies have indicated the association of some subfractions of high-density lipoproteins, or HDL, with dementia. Researchers are looking into the relation of these subfractions, such as apolipoproteins found in HDL particles including apoA1, apoC3 and apoJ, with dementia and whether they might be potential biomarkers.

Manja Koch and colleagues at the Harvard T.H. Chan School of Public Health studied the association of HDL and its apolipoprotein subspecies in a group of 1,351 elderly men and women. Their findings recently were published in the **Journal of Lipid Research**. They found that the total concentrations of apoA1 and some of the other apolipoprotein subspecies were not related to dementia risk, so these apolipoproteins would not be effective biomarkers for risk of dementia. However, they did find that a high concentration of apoC3 related to a lower risk of dementia. The researchers plan future investigations supported with brain imaging studies in a younger population to understand better how apolipoprotein concentration relates to Alzheimer's and dementia.
DOI: 10.1194/jlr.P119000473

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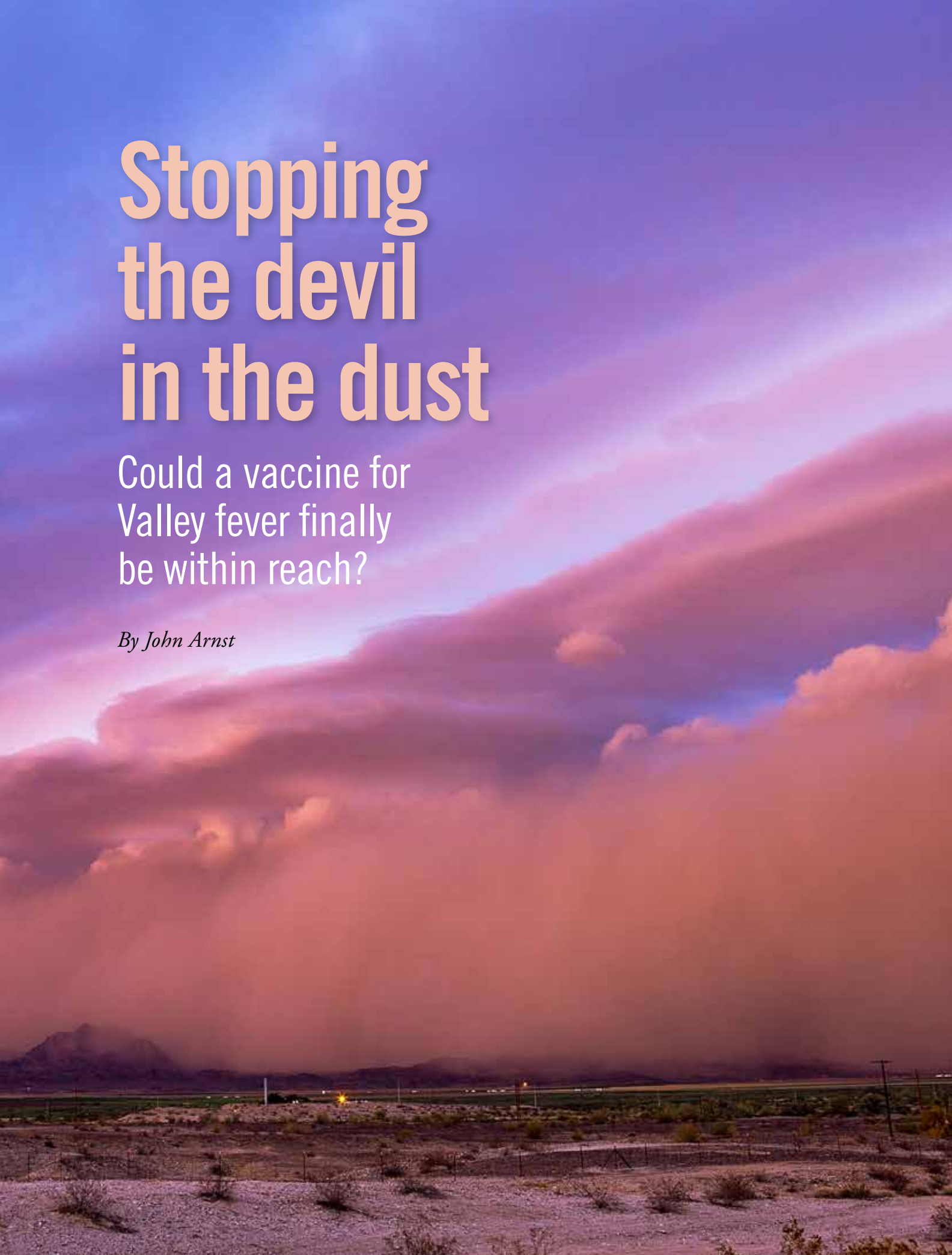


Stopping the devil in the dust

Could a vaccine for Valley fever finally be within reach?

By John Arnst

Most people who inhale the dust-borne fungal spores that cause Valley fever experience no symptoms. But in around 40% of people,the infection manifests, usually as pneumonia.

Rob Purdie knew something was amiss in early 2012 when headaches and sinus pain that were unresponsive to antibiotics began affecting his eyes. "I was having double vision," Purdie recalled.

Purdie lives in Bakersfield, California, in the southern part of the fever's namesake San Joaquin Valley. There, the fungal spores that cause Valley fever lie waiting in dense dust pockets for disruptions to carry them to a host.

Valley fever has been diagnosed as far south as Brazil, but the preponderance of cases occur in southwest Arizona — where spores can hitch a ride on increasingly frequent dust storms — and in Bakersfield, which is home to the Valley Fever Institute at Kern Medical, where Purdie sought treatment after enduring worsening headaches for five weeks.

"They got a lumbar puncture on me in the emergency department and said, 'We think we know what you have,'" Purdie said.

His formal diagnosis was disseminated coccidioidal meningitis, which occurs when spores escape the lungs and travel up the spinal cord to the brain's outer membrane. Left untreated, this stage of Valley fever is fatal. Purdie had few options. Doctors often prescribe broad antifungals, which stop the fungi from replicating but don't kill them outright.

Each year, tens of thousands of people in Arizona and California are diagnosed with Coccidioides infection. The number is likely far higher, but most go undiagnosed due to the fungi's asymptomatic or flulike presentation.

By the estimates of John Galgiani, director of the University of Arizona's Valley Fever Center for Excellence, nearly one in four cases of pneumonia in Arizona are caused by Coccidioides.

"Each year, 1% of the population of the area has an acute or long-term brush with Valley fever," he said.

Since the end of World War II, scientists have tried and failed to develop a vaccine. In 1985, the most promising candidate provided poor protection and caused swelling at the injection site.

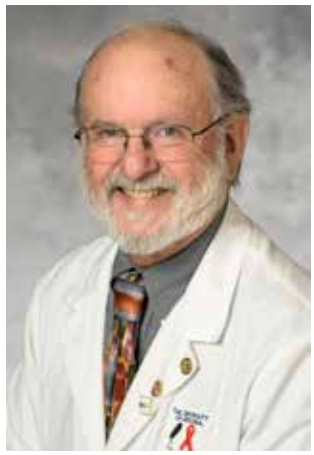
Today, two promising vaccine candidates are nearing human trials. But as the American West continues to heat up and dry out along with the rest of the world, the greatest hurdle to protecting millions of people across Valley fever's increasing range will likely be funding rather than immunology.

Valley fever in the time of COVID-19

ASBMB Today asked John Galgiani, the director of the Valley Fever Center for Excellence, what the ongoing COVID-19 pandemic might mean for people in the Southwest and California's Central Valley who are at risk of contracting both Valley fever and the new coronavirus.

"With respect to COVID-19, anyone with recently acquired Valley fever who then also gets COVID-19 infection might well have a bigger problem than someone who was only dealing with the viral infection," Galgiani said. "However, for the many people with Valley fever infections years earlier, they should be at no more risk of COVID-19 complications."

Spores and soil



COURTESY OF JOHN GALGIANI

John Galgiani, a physician who has been working with patients with coccidioidomycosis for the past four decades, is the director of the University of Arizona's Valley Fever Center for Excellence.

While the Sonoran and Mojave deserts that stretch across Mexico and the southwestern United States are the ones most known to harbor Coccidioides, the pathogen's range is expected to expand by the end of the century, according to a paper published in August in the journal GeoHealth.

Morgan Gorris, a postdoctoral fellow at Los Alamos National Laboratory, was the first author on the paper about the pathogen's range if climate change continues unabated. "In our baseline model, looking at where Valley fever is currently, we estimated that the fungus is probably in 12 states, and by the end of the 21st century that could increase up to 17 states," Gorris said. "And, as a result, the number of Valley fever cases could increase by up to 50%."

The fungus already has crept into the desert portions of Washington, and is believed to potentially also be in eastern Oregon, Gorris said. Additionally, a 240% increase in the incidence of dust storms in the Southwest between the 1990s and 2000s, possibly from climate change, was found to coincide with an 800% increase in cases of Valley fever between 2001 and 2011.

The pathogen's current and future ranges are influenced by species diversity. The two species of Coccidioides, C. immitis and C. posadasii, are morphologically

identical, but their growth rates diverge in different salt concentrations. This may be what limits C. immitis to the Central Valley but expands C. posadasii's range to northern Mexico and parts of South America.

At Northern Arizona University, biology professor Bridget Barker puzzles over what drove the cocci species to infect mammals.

"We've done a lot of soil sampling, and it looks like burrows in general for any type of animal seem to be more frequently positive than just a random soil sample," she said.

She added: "Pretty much any mammal that gets exposed is potentially at risk for disease, even like dolphins off the coast of California."

The fungi's life cycle is unique to the Coccidioides genus: It persists in soil as filamentous hyphae, then becomes infectious arthroconidia that enlarge to spherules upon inhalation, which rupture to send out endospores that grow to form spherules in adjacent tissues.

"The host has to inhale those spores from the soil. And when the spores are inhaled, they undergo a huge change, so it's basically a whole different structure inside the host," Barker said. "There's no other fungus that we know that makes that same structure; what are the evolutionary pressures that lead to that?"

A few years ago, Barker had a brush with Valley fever while taking samples in the field. Her dog and her husband also have been infected.

"My dog got it. My husband got really sick," she said. "We had been camping, my dog was digging up a rodent burrow, and dust was going everywhere."

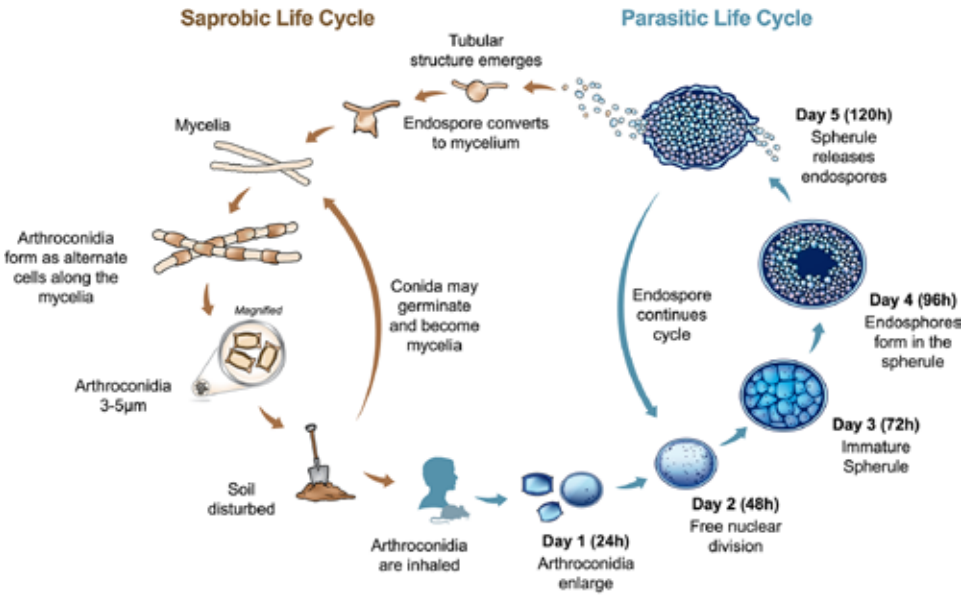
Fortunately for Barker, the disease never progressed beyond the granuloma in her lung.

"Without going in and doing a lung biopsy, they couldn't prove it … and there's always the possibility with going and getting a biopsy that you could collapse your lung," she said. "I thought it was best to just leave it alone, and that's my natural vaccination."

While Barker and her collaborators continue to examine the prevalence of cocci in soil samples in the southwestern United States, Mexico and South America, she also has her eyes trained on deserts in Africa and Asia and the novel fungal pathogens that might inhabit them.

"There are close relatives," she said, "but whether we see the same thing in other areas in Asia and in Africa, Australia … I would say the prediction is yes, but I don't know exactly what those organisms would be."

BRIDGET BARKER/PLOS PATHOGENS



Life Cycle of *Coccidioides*

Both Coccidioides species share the same asexual life cycle, switching between saprobic (on left) and parasitic (on right) life stages. The saprobic cycle is found in the environment and produces the infectious arthroconidia. The conidia may be inhaled by a susceptible host, or may remain in the environment to continue the saprobic life cycle.

In lieu of a cure

David Davis isn't sure where he was exposed to the spores that gave him coccidioidal pneumonia last summer, but he has a hunch.

"I'd been running right next to a dry riverbed and through an excavation site," said Davis, a 51-year-old Navy veteran and nurse who works in the operating room at Kern Medical in Bakersfield.

Davis, a runner and weight lifter, began to feel the effects of Valley fever while he was at work: "I was moving a patient from the operating room table back to the gurney to go to recovery, and I was shaking, sweating; my joints hurt, and I was out of breath."

After an X-ray turned up a lung mass, his primary care provider ordered a blood test for cocci.

"When I received that phone call, I was out in the dirt with my fiancée trying to exercise," he said. "I was like, 'Well, I probably shouldn't be right here in this dusty field.'"

Like many people who come down with Valley fever, Davis had a relatively straightforward recovery. He began a daily regimen of 200 milligrams of fluconazole prescribed by his primary care physician. Then Royce Johnson, the medical director of Valley Fever Institute, increased his dosage to 800 milligrams. By November, the pathogen's levels were low enough that Davis was off fluconazole, which had been causing him to lose the hair on his arms and legs.

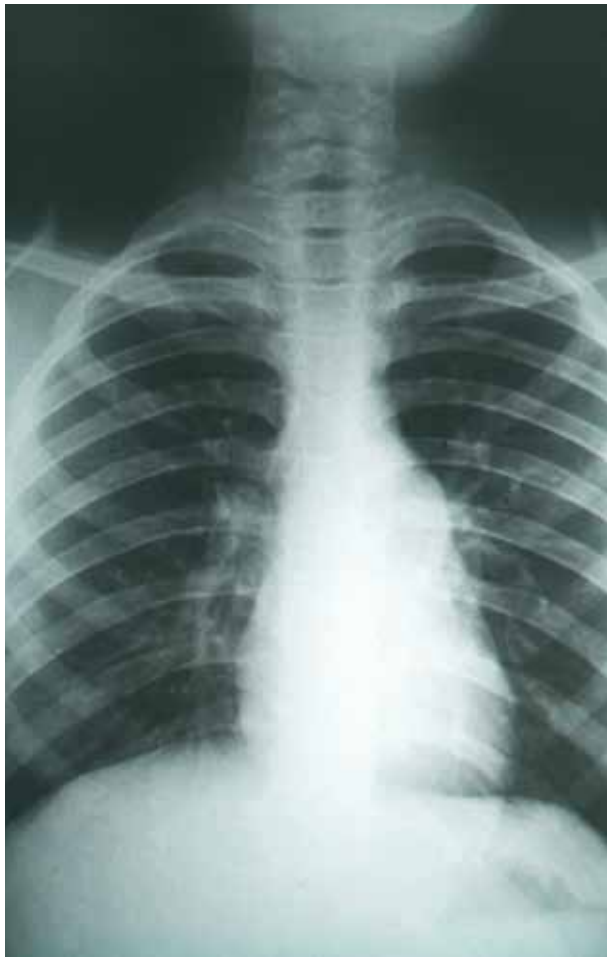
But because fluconazole doesn't kill cocci outright, Davis has lingering amounts of cocci in his body that his immune system will have to fight off.

"I was running up to 20, 30 miles a week. And now I run maybe eight, and I'm pretty tired still," he said. "So I think it's going to take a while for me to get back to the level, if ever, where I was before."

Like Davis, Judy Kingma, whose house in San Luis Obispo backs up to a field, believes she was exposed to spores in soil that had become dislodged by nearby construction.

"I've lived here for 25 years and never had a problem," she said. "And then just last year, this construction site is how I'm sure I got it."

Kingma had been traveling to the University of California, Los Angeles, to receive treatment and surgery for pheochromocytoma, a rare tumor of the adrenal glands. Not long after the tumor had been successfully removed, however, Kingma's oncologists noticed severe swelling of the lymph nodes in her chest. After months of testing failed to turn up evidence of metastasis, her physicians recommended she undergo a mediastinoscopy to open up her chest and biopsy the lymph nodes, which ultimately cost $25,000.



CDC/LUCILLE K GEORG

This chest X-ray shows pulmonary fibrosis in a patient with coccidioidomycosis. Lung scarring worsens as the fungal infection does.

"Everyone just thought I had lung cancer because, you know, you get this nodule in your lungs," she said. "That just blew my mind, like it's an $80 test blood test versus a knife."

After she was diagnosed with disseminated Valley fever, she began driving the 2.5 hours each way to Bakersfield to seek treatment at Kern Medical, where doctors increased her dose of fluconazole from 400 mg to 600 mg.

"They monitor me pretty closely," she said. "I have a lung X-ray every time I go there, about every two months."

Kingma is continuing to take fluconazole, with its unpleasant side effects, to keep the fungi's growth in check while her immune system gradually clears the cocci from her body.

"Your skin turns into like leather, your hair falls out," she said. "And it gets very expensive. All from just a little

COURTESY OF BRIDGET BARKER

Bridget Barker at Northern Arizona University swabs a dog's nose in the lab to test for the presence of coccidioides.

disease where people think, oh, you take a few pills and you're all better."

Fungal firsts

The lack of an FDA-approved vaccine against any fungi is a consequence of both the rarity of eukaryotic pathogens and the difficulty of developing vaccines for them.

The first vaccine that was developed for Coccidioides, which used formaldehyde-killed fungal spherules, stalled during clinical trials to evaluate its safety in 1985.

"There's a lot of carbohydrate in (the 1985 candidate), and it's really irritating. And it's just not acceptable," explained Lisa Shubitz, a veterinarian and research scientist at the Valley Fever Center for Excellence.

However, the immune response to Coccidioides infections still makes the pathogen an ideal vaccine target.

"Many people get infected and either have no symptoms or get symptoms which go away on their own," Galgiani said, "and then they're protected for the rest of their lives, (which) makes the idea of the vaccine very appealing."

In 1997, a funding committee affiliated with California State University called the Policy Advisory Committee for the Valley Fever Vaccine Project chose five scientists to pursue vaccine research; their five avenues of generating immunity bore out the two vaccine candidates nearing their final stages of development.

Mark Orbach, a fungal geneticist and mycologist at the University of Arizona and longtime collaborator with Galgiani, has helped develop one of those vaccines.

Orbach began this project with Coccidioides after colleagues at Cornell University performing random insertional mutagenesis knocked out a gene in a fungal maize pathogen that reduced its virulence by 60%. They then found that Coccidioides possessed an ortholog of that gene, and named it CPS1.

Orbach, who was working on a fungal rice pathogen at the University of Arizona, began working on pathogenicity in coccidioides that had the CPS1 gene knocked out, which he and colleagues named delta-CPS1. The researchers, together with scientists at the University of Kansas, Colorado State University and the pharmaceutical company Anivive Lifescience, are using those fungi to develop a vaccine.

"The vaccine itself is just spores that we harvest off of plates, clean, wash, purify separate from hyphal filaments, and then suspend in an aqueous solution," Orbach said. Developing a vaccine commercially requires a fine-tuned mixture of other ingredients, however, to ensure that the suspended spores can remain on a shelf for six to 12 months.

According to Galgiani, mice that have been bred to replicate genetic mutations that predispose people to severe symptoms of Valley fever have been protected by the delta-CPS1 vaccine. But before the vaccine can begin human trials, it must be evaluated further in animal trials, which Shubitz has helped run.

Coccidioides in canines

Shubitz treats hundreds of dogs for Valley fever every year.

"There's a high need for a Valley fever vaccine in this species," she said. "Treatment is expensive. It can fail, and Valley fever is a very common cause of relinquishment of pets."

Depending on individual cases, a diagnosis alone can cost hundreds to thousands of dollars; treatment, which can last six to 12 months, can then cost several thousand dollars.

"The medication may cost $50 to $100 a month, even if you use the least expensive medication," Shubitz said. As in humans, the most widely used antifungal is fluconazole, followed by the similar but pricier compound itraconazole.

Around 25% of dogs with Valley fever suffer severe complications, a rate more than twice that of humans. According to Shubitz, this may be a consequence of their curious noses.

Coccidiodal inequities

Researchers are attempting to unravel why certain populations, including people of Filipino and African descent, are hit harder by Coccidioides infections than others.

"Some strains cause very severe disease; some strains don't," Bridget Barker at Northern Arizona University said. "So there's clearly some genetic aspects in the fungus."

The amount of fungal spores a person is exposed to also plays a role.

"If you inhaled 10 spores, you're probably basically vaccinated. If you inhaled a million, that could end very badly for you," she said. "We think that, in some of these outbreaks that they've had, like archaeological digs or construction sites, they just happened to hit a pocket where there's a bunch of the organism and released maybe millions of spores all at once."

At the National Institute of Allergies and Infectious Diseases, Steve Holland, the director of the NIAID's division of intramural research, has been recruiting people at high risk for developing disseminated or refractory coccidioidomycosis, the more severe forms of the disease.

"We've identified now a fair number of genes that are involved in disseminated coccidioidomycosis, some of which we've reported in the past," Holland said. Those genes include those that code for the cytokines STAT1 and STAT3, which are essential for the production of the cytokines interferon gamma, interleukin-12 and interleukin-1 in response to infection with Coccidioides.

"In our current study, we've been looking at genes and mutations which are potentially a bit more common, but what makes cocci different is that you have an organism of relatively high virulence. But it's also geographically circumscribed," he said. "So if you grew up in Chicago with a high-level cocci susceptibility gene, that doesn't matter until you go to cocci territory. And trying to sort those things out is really what we're interested in."

According to Holland, the trial currently has recruited 65 participants of a targeted 200.

"I think we have genetic associations for about half of them. Which is pretty good for us," he said. "This is a disease that is being very actively studied both at the pathogenesis level and at the treatment level, and I'm absolutely confident that in the next couple of years, we're going to know a lot more about why people get sick and a lot more about how to treat them."

"Dogs may inhale larger doses (of the fungus) than humans on a routine basis, and that could contribute to the increased rate of clinical disease and complications, because their faces are in the ground all the time," Shubitz said.

While these rates are suspected to be higher than those of their closest native kin, coyotes, surveillance of wild animals, including mountain lions and other mammals, leaves the picture incomplete. "We don't know that much about coyotes, because if they die in the wild, no one knows about it," she said.

Shubitz and her colleagues recently wrapped up a clinical trial of the delta-CPS1 vaccine in dogs. While the results have yet to be released, Shubitz was optimistic about how the animals tolerated it.

"(This) should not cause a significant kind of a reaction. The mice don't care about it. The dogs had no reaction."

Better drugs

Both dogs and humans largely are limited to two categories of antifungals, prescribed off label, to treat Valley fever: the -azoles, which include fluconazole and posaconazole, and amphotericin B. They keep cocci in check, but long-term treatment can become expensive. The average lifetime cost of treatment for a person with Valley fever is estimated to be $94,000.

Since 2012, Purdie, who is now the patient/program development coordinator for the Valley Fever Institute at Kern Medical, has received injections of amphotericin B through a port called an Ommaya reservoir on the top of his skull.

"Some people get it three times a week," said Purdie, who is one of the roughly 2,400 patients who visit Kern medical for coccidioidomycosis each year. "I'm really lucky. I go in about once every eight weeks."

Like fluconazole, which was developed to treat the candida fungi that cause thrush, amphotericin B only keeps the fungi from replicating, rather than killing them. A recent clinical trial to evaluate finally the efficacy of fluconazole against coccidioidomycosis ended after falling short of the 1,000 patients it had sought to enroll.

Galgiani has been instrumental in the development of a drug, nikkomycin Z, which kills Coccidioides outright by interfering with the synthesis of chitin in their cell walls and has been evaluated in both dogs and humans. But both he and Shubitz — who collaborated with Galgiani to evaluate Nikkomycin Z in dogs — raised concerns about

the high cost of completing clinical trials needed for FDA approval.

"I don't know whether that drug will ever reach market," Shubitz said.

Outside the Valley

At the Applied Biotechnology Institute in San Luis Obispo, California — well north of Bakersfield and roughly 600 miles northwest of Tucson — John Howard is formulating another vaccine to protect against Valley fever.

Rather than a killed or live-attenuated vaccine like the 1985 candidate or CPS1, ABI is developing a subunit vaccine, a formulation that uses the smallest component of a pathogen that's able to elicit an immune response. For Coccidioides, that's Antigen 2, an immunoreactive component discovered simultaneously by mycologists at the University of Texas Health Sciences Center at San Antonio and at the Valley Fever Center for Excellence in the 1990s. By growing large quantities of the protein, referred to as Ag2/PRA, in corn and incorporating it into an edible wafer, Howard and his colleagues have created an oral vaccine.

"Oral delivery is important, because in the immune system, it gives a good mucosal response," Howard said. That immunological response includes the mucosal membranes that line the respiratory tract, which are most likely to come into contact with Coccidioides spores. "Most injections give you little or no mucosal response."

Howard, who has been working with transgenic maize for more than 40 years, was fine-tuning ABI's oral vaccine for hepatitis B when he first encountered Valley fever.

"Several years ago, my sister-in-law got sick with Valley fever, which was the first I'd heard of it," he said. "We said, 'Wait a minute, this is a fungal pathogen. There are no fungal vaccines.' So we started looking into it."

Howard reached out to Gary Ostroff, a professor at the University of Massachusetts Medical School specializing in small-molecule delivery technologies, and Chiun-Yu Hung at UTHSA, who had been continuing the institution's research into Coccidioides and Ag2. While their groups had been successful in growing and harvesting Ag2 from Escherichia coli, they had been hampered by the relatively low yield of the system.

"One of the big problems was that they can't produce very much of it in microbes," Howard said. He and his colleagues then transformed the gene for Ag2 into a maize line they already had been using and brought in a chemist from San Francisco State University, Raymond Esquerra, to assist with the process of extracting the protein from the corn. Their results, published in the Journal of Infectious Disease in July, were promising. "We got about 100 times more (Ag2) than they can get out of the microbial system."

The next steps for Howard and his colleagues are to fine-tune the processing of the Ag2 protein and to test the oral vaccines in animals.

Kamaljeet Kaur, a graduate student in Raymond Esquerra's lab at SFSU, had planned to describe improvements to the extraction process at the American Society for Biochemistry and Molecular Biology's annual meeting in San Diego before the meeting was canceled.

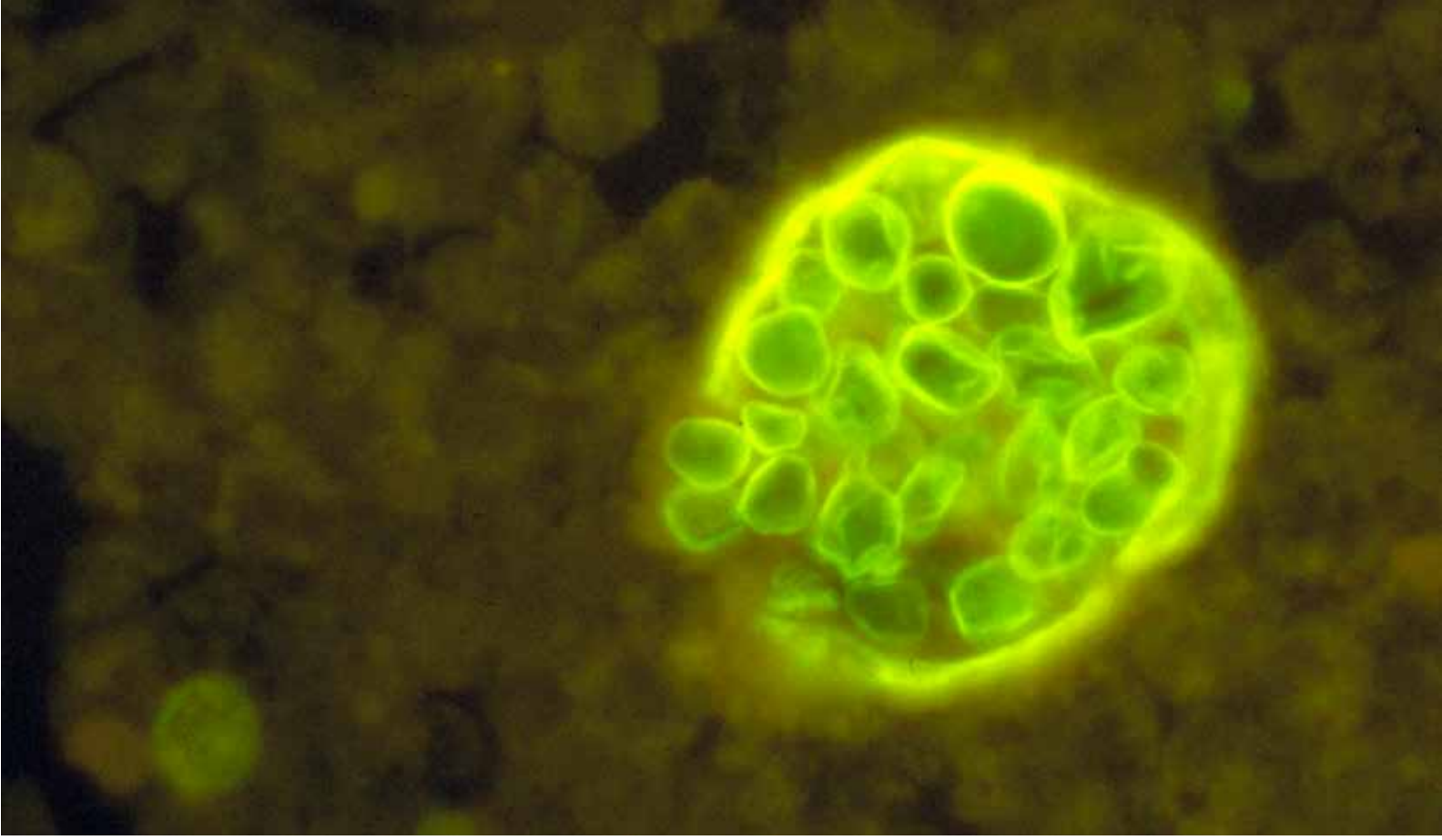
"We have tried using different detergents, and we have tried using liquid nitrogen, which is very impactful in getting the maximum extraction," Kaur said.

And in a paper recently published in the journal Vaccines, Howard, Hung and Ostroff found that mice that were simultaneously given the vaccine candidate both by injection and oral routes fared better against Coccidioides than mice that only received the vaccine candidate by one route.

LISA SHUBITZ

Lisa Shubitz, a veterinarian and research scientist at the University of Arizona, adopted a new dog, Journey, in mid-2018.

CDC/MERCY HEALTH - ST. VINCENT MEDICAL CENTER; BRIAN J. HARRINGTON



Spherules of Coccidioides immitis, here processed with calcofluor staining, rupture within hosts and release endospores that propagate in adjacent tissues, creating more spherules.

Funding the finish

While projections indicate that a vaccine for Valley fever could be profitable, they do not take into account the high costs of guiding a vaccine through clinical trials, Galgiani said.

"It's not biology. It has to do with market forces and capitalism and how things get made," he said.

One factor is the small size of the susceptible U.S. population relative to the global populations that vaccine manufacturers target to make back their production costs.

"Think about vaccines that people make for tetanus or influenza and who gets them — everyone in the world. Those are considered vaccine markets," Galgiani said. "And if you have a population that would benefit from a vaccine being in the range of, say, 25 million people, that sounds like a lot of people, but it's a small vaccine market."

A 2000 report by the National Academies titled "Vaccines for the 21st century: A tool for decision-making" estimated it would require 15 years and $360 million to develop and license a vaccine for Valley fever, money that has failed to materialize over the past two decades.

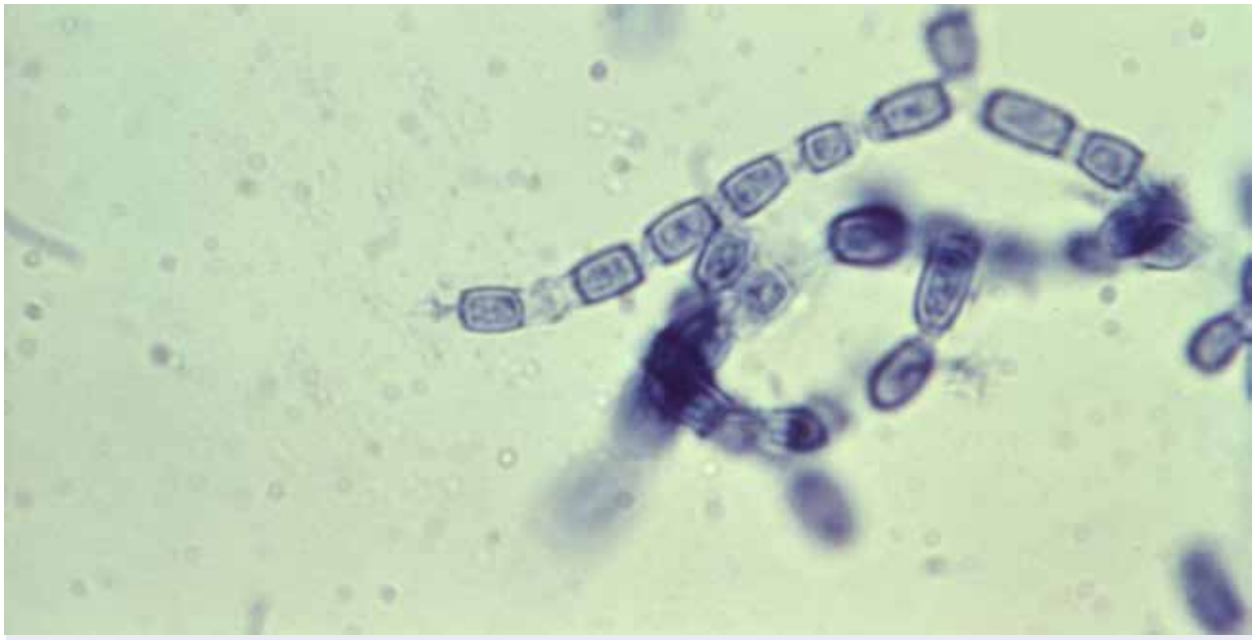
U.S. Reps. Kevin McCarthy, R-Calif., and David Schweikert, R-Ariz., who chair the Congressional Valley Fever Task Force and whose districts include Bakersfield and the northeastern suburbs of Phoenix, respectively, recently secured $2 million for Valley fever surveillance and research, but those funds, and the money they've raised in the past, fall an order of magnitude short of what's needed to bring a vaccine to fruition.

While financial barriers remain for taking a vaccine candidate through human trials, the researchers at the Valley Fever Center for Excellence have partnered with the pet-focused Anivive Lifesciences to shepherd the vaccine through the remaining animal trials. Orbach and his colleagues recently submitted paperwork to the U.S. Department of Agriculture Center for Veterinary Biologics, which regulates and approves vaccines and biologics for animals, for a provisional license to begin producing the vaccine.

"We feel that we've demonstrated safety and effectiveness. But really the USDA Center for Biologics are the people who have to decide that, and we're hoping that we'll be able to get approval by 2021," Orbach said.

"The cost is very high, so we feel that it's probably going to require government interest to help support a regional vaccine for people. And we're optimistic that we can convince them that it's a worthwhile expense."

CDC/HILLIARD F. HARDIN



Arthroconidia, the spore form of C. immitis and C. posadasii, are light-weight and easily carried by air currents.

Multiple appeals

In recent years, the federal government and the California state prison system have been sued by inmates left debilitated by Coccidioides and by families of inmates who died from exposure to it.

Ben Pavone, an attorney based in San Diego, is lead counsel for a case brought by 270 former and currently incarcerated people who contracted Valley fever while at state prisons in the Central Valley. They have been trying to sue prison officials, the former head of state prisons and former California Gov. Arnold Schwarzenegger.

In February 2019, the 9th Circuit Court of Appeals rejected the lawsuit on the grounds that the defendants reasonably could have concluded that the threat to inmates from the disease was not severe given that millions of people are exposed to Valley fever in California every year.

Pavone appealed that decision to the U.S. Supreme Court, which declined in October to hear the case.

According to Pavone, the 9th Circuit overreached by applying qualified immunity to the prison officials. The doctrine shields government officials from being sued for discretionary actions related to their line of work.

"It's a get-out-of-jail card for every time the government makes a mistake," Pavone said.

But the ruling by the 9th Circuit Court gave Pavone another pathway for redress: suing a state-run corporation appointed in 2006 to take control of prisoners' medical care after the California Department of Corrections and Rehabilitation failed to meet the standards against cruel and unusual punishment set by the Eighth Amendment of the U.S. Constitution.

Pavone's next legal move will take advantage of an earlier decision he made to split his clients into two groups of 110 and 160 each. While Pavone takes his case about the mistreatment of the initial 110 inmates against the state's receiver.

"There are two pieces of good news," Pavone said. "One, my 110 guys have another shot … and, two, we're taking a shot at qualified immunity."

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

Science Twitter: personal boundaries on a professional platform

By Kate Bradford

"Do I have to stop posting my critiques of higher education?"

"Will the leadership take me seriously if I use GIFs?"

"Am I still allowed to Tweet about cats?"

These are a few of the questions I had to navigate when I began my career as a professional development staff member at Johns Hopkins University in fall 2017. To develop my science communication skills, I had started my professional Twitter account a few months earlier when I was a postdoc at the U.S. Food and Drug Administration. I was pleased to learn that many Hopkins faculty, students, staff and leaders use professional social media accounts too. This empowered me to turn my Twitter account from a professional hobby into a critical part of my job. Twitter enabled me to grow a robust network at Hopkins, advertise my and my office's programming, and keep up with the latest research and news in my field. Among other things, I now get paid to tweet. Pretty cool, right?

Wrong. Twitter is a social networking platform. Success on Twitter has a different definition for every Twitter user. For me, success meant finding the perfect balance of personality: one that is professional, intelligent and advocates on behalf of meaningful causes but is still likable and relatable. Every day, I pressured myself to produce worthy, professional content, maintain my network with the right stakeholders and self-promote (in a non-icky way). I found myself drafting and deleting about three times as many tweets as I published, because I was so scared about my public image.

For example, one weekend there was a heated debate on Twitter about the proper way to, or not to, advertise open postdoctoral positions in labs. I wanted to respond to tweets from those who disagreed with my view on the subject. I struggled with how to express my own opinions and share the common struggles I see graduate students encounter when applying to postdocs, but I feared it wasn't my place to speak up. I spent far too long trying to write, rewrite and rewrite again my responses. Once I posted a response, I would second-guess myself. Making the whole situation even worse, the debate took place over the Thanksgiving holiday, a time I didn't want to spend thinking, and stressing, about work.

I struggled with how my own personality, sense of humor, personal stories and opinions fit in with this professional account, especially since I'm a private person and don't wish to share many details of my life online. Much of this pressure is self-inflicted; I've never felt oversight from university leadership about my account. However, in our current callout culture, Twitter users are quick to highlight and shame those who express unpopular or problematic opinions on social media. Sometimes this results in employers being notified and people losing their jobs. You can feel like you're only one controversial tweet away from facing real-world consequences, and I'm acutely aware that I do not have the security of tenure.

To use Twitter effectively for my career while simultaneously keeping myself happy, private and safe, I decided I had to develop personal boundaries and a public Twitter persona. "Twitter Kate" still has my voice, hobbies and likes, but she is a carefully curated version of me. Twitter Kate only exists 8 a.m. to 4 p.m., Monday through Friday, which are my working hours; I deleted the Twitter app from my phone to prevent myself from working in the evenings and on weekends.

Twitter Kate is a positive person who tweets about science and advocates for issues in graduate education and the Ph.D. workforce but not in a way that is controversial and might hurt my career advancement. Sometimes Twitter Kate self-promotes my work because the stakeholders (faculty, students, postdocs and leadership) are following. Outside of science and work topics, Twitter Kate makes sure to share snippets of my other interests (lately: kittens, Disney World and Star Wars), but she never talks about my friends, family or close relationships. When I go on vacation, Twitter Kate tweets about it afterward and mentions something science-related about my trip in order to connect with her mostly science-based followers. Both Twitter Kate and I love using gifs, memes and emoji to reflect my sense of humor.

These boundaries have sharpened my Twitter time management. I no

longer spend five minutes deciding whether to tweet a reply or comment on the current hot topic. I have a list of subjects I'm comfortable expressing my opinion about; all others are a quick decision of no.

Want to set your own personal boundaries on professional Twitter? Consider the following

- Is your Twitter account a part of your job or just a professional hobby? The answer will dictate how much work time vs. free time you spend on the platform.
- What parts of your personality do you want to share on Twitter? Personality often comes off in the tone, or voice, of your tweets. Tone can be hard to convey in 280 characters, so consider strategic use of GIFs, emoji and memes.
- Your ratio of professional vs. personal tweet content: What you tweet about will affect who follows you and their opinions about you, so if you want to stay connected to scientists, then you should probably tweet mostly about things they care about. Fortunately, scientists are multifaceted people. Sharing interests and experiences outside of your niche discipline can be a wonderful way to build and strengthen new connections. Some scientists find ways to blend their personal interests with their scientific expertise by tweeting on the scientific sides of art, fashion, sports, pets, pop culture, beauty, politics and the like.

- It is fine to have opinions and share them on Twitter. Accounts that lack a personal take can be seen as robotic. Use a version of the front-page test: Would you feel OK if your opinion was shared on the front page of a news site? If so, tweet it.
- Decide what personal stories you are comfortable sharing. Many scientists benefit from sharing their experiences, both good and bad, with school, mentors, careers and such. These stories allow people with shared experiences to bond and create supportive communities. Those with different experiences can learn by hearing from people outside their own communities. However, even if others share their stories, you should not feel pressured to share your own if you think there could be real-world consequences.
- There's a fine line between sharing your own experiences and sharing stories of those close to you. Consider getting permission before sharing stories and pictures of others, especially if they do not engage publicly on social media themselves.

With all these boundaries, it's important to be adaptable. Personal stories that don't feel important to share today may be easy to share in the future. Your voice will change as your career advances. Professional social media accounts are a relatively recent frontier, and new platforms like TikTok rapidly are gaining popularity. If you decide to join these, remember to educate yourself about how they are used. Then reconsider and set your personal boundaries.

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Research on a budget

By Peter Lyons

One benefit of teaching at a liberal arts institution is being in an environment that doesn't live and die on research productivity. While faculty typically are expected to do research, this may be secondary to the teaching mission of the institution and is often for the benefit of student researchers. In other words, the goal of research is to teach and inspire rather than to produce. Produce we do, but on a slower schedule and with undergrads as coauthors.

The disadvantage of this scenario is that funds and facilities for research are typically less than at research-intensive institutions. We may not have the time or resources to bring in the big bucks to pay for cutting-edge research. Often, our funds are limited to a few thousand dollars a year provided by our college's research office or from our own department's budget. What can a biochemist or molecular biologist do with this level of funds for research? We all know that molecular supplies are ridiculously expensive. Take the cost of a single antibody at $300 to $500 — there goes 10% to 20% of the yearly budget.

I started seven years ago on the faculty of Andrews University, a Michigan school with fewer than 5,000 students. We have reasonable financial resources, most equipment and facilities needed for basic molecular research, and support for writing grant applications. However, this position did not come with specific startup funds (the department was generous to get me what I needed), and I have not yet had success in winning an external research grant, even those targeted at undergraduate institutions (the competition remains tough). During this time, I have developed ways of reaching my research goals on a tight budget, and I share some of them here.

Cell culture

Cell culture media can be purchased inexpensively in powdered form. Depending on volumes needed, this can be a large savings. Unfortunately, the major cost of cell culture is in fetal bovine serum, and I have not yet found a cost-effective substitute. I once tried a product marketed as an FBS substitute. It failed to support the growth of my basic and not-very-fussy cell lines. In addition to FBS, reagents for transfection of mammalian cells are often costly; an alternative to these costly reagents is polyethylenimine, very effective for transfecting HEK293T cells as well as some other cell lines.

Western blotting and immunocytochemistry

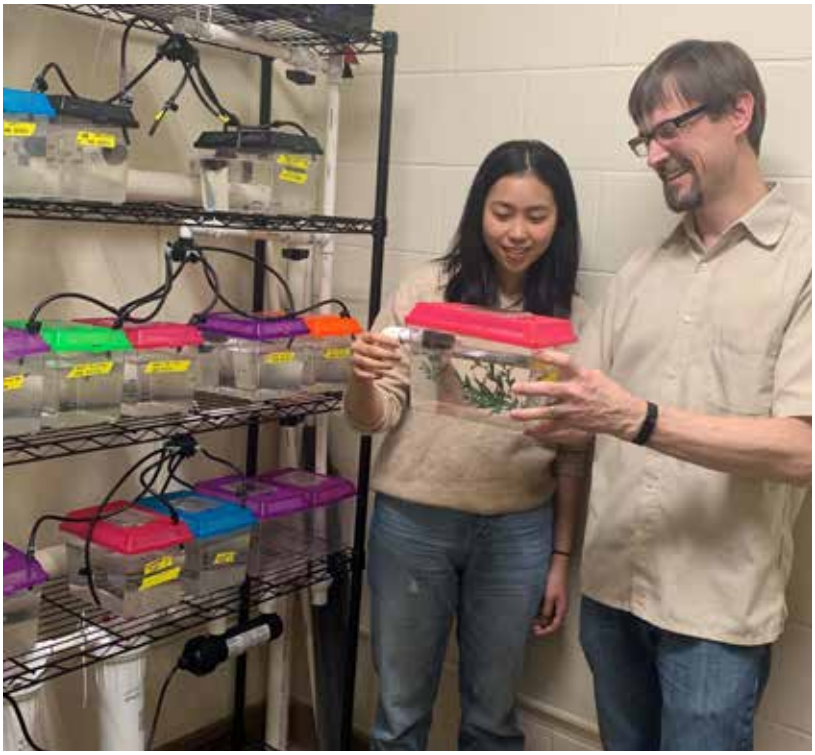
We make our own gels. This takes just over an hour; apparently, we have more time than money. Antibodies are expensive, but good antibodies can be reused multiple times with a little sodium azide as a preservative. In years past, it typically was expected that one would control for protein loading by blotting with an antibody for a housekeeping protein, such as beta-actin or alpha-tubulin. Recently, journals such as the Journal of Biological Chemistry have recommended staining membranes with Ponceau S so as to see all proteins rather than just one major protein that could be affected by experimental treatments. This has the secondary benefit that Ponceau S is cheap, much cheaper than an antibody. While I have not found a cheap source for all antibodies, the Developmental Studies Hybridoma Bank at the University of Iowa is a source of many antibodies for minimal cost. When it comes to performing immunocytochemistry, coverslips easily can be treated with polylysine and antibody volumes kept at a minimum — just 100 microliters will cover a coverslip. Mounting media can be made with simple components: buffered glycerol with an anti-fade such as p-phenylenediamine. Many recipes can be found online.

Transformation

If you only infrequently need basic subcloning efficiency competent cells, the cost of a commercially available preparation might not be too steep. However, for greater volumes, you can try making them yourself. Many protocols are available online, even one, the Inoue method, for making what the authors call "ultra-competent" cells.

Plasmid preparation and DNA markers

The cost of plasmid prep kits can add up if you do a lot of this work. However, they are unnecessary. I have prepared plasmids using the nonionic detergent method for many years now, paying pennies per prep, with results suitable for mammalian cell transfection and Sanger sequencing. The published method works well and can be scaled up for



COURTESY OF PETER LYONS

Peter Lyons and student Atalia Atmadja examine some zebrafish in front of the zebrafish system that he built from hardware store supplies for about $500.

larger quantities of plasmid. I learned recently that make-it-yourself DNA molecular weight markers, the Penn markers, are available. These consist of two plasmids that can be purified (maybe with the nonionic detergent method) and digested with the PstI and EcoRV restriction enzymes to produce 100 base pair and 1 kilobase ladders. An endless supply for just the cost of the plasmids and a couple commonly available enzymes.

RNA purification and analysis

The best method to purify RNA is one of the many commercially available column kits. However, these come at a cost. Others are cheaper but involve the use of toxic solvents, which are best avoided, especially with novice undergraduate researchers. So there is a tradeoff in these approaches. In terms of analyzing the purified RNA, a bleach gel seems a very simple and effective approach.

Challenges to working on a budget

Not everything can be done both well and cheaply. As mentioned above, sera for cell culture are just plain expensive. Protein purification can be done reasonably well with simple gravity-flow columns; however, the use of an (often pricey) chromatography system would greatly facilitate the process. I have recently constructed an 18-tank flow-through zebrafish system from hardware store supplies for about $500, significantly cheaper than the $15,000 it might cost from professionals. There have been some challenges with this system, however, that have not expedited my research, one example being when filters and pumps didn't work together as planned. Many headaches later, but with the satisfaction of having built it myself, I have it figured out.

Back when I was in graduate school, there was a technician down the hall who enjoyed tinkering in his garage in the evenings. He would construct tube rockers and similar devices for a few dollars out of basic motors and electrical clips from the hardware store. They would have cost hundreds of dollars from a scientific supply company. I think a person like this should set up shop near every major research institution.

Do you know people who are effective at making the research dollar stretch through do-it-yourself approaches? Do you have methods that work well and allow you to avoid costly purchases of kits or other supplies? Let's continue to share ideas. I wish you all the best as you do more for less.

Peter Lyons
(lyons@andrews.edu) is an associate professor of biology at Andrews University, where he and his students explore the biochemical and biological functions of metallocarboxypeptidase enzymes. Always the frugal type, he enjoys learning of new ways to save money.