Vol. 14 / No. 1 / January 2015

ASBMB TODAY

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

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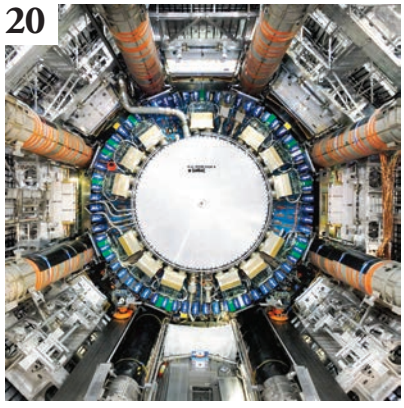

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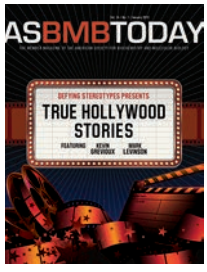
Our "Defying stereotypes" series continues with profiles of two Hollywood figures who were trained in science.



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

www.asbmb.org/asbmbtoday
PRINT ISSN 2372-0409

Anatomy of a discovery

By Steven McKnight

For hundreds of millions of years, plants and animals have been engaged in constant battle against microbial and viral pathogens. For higher metazoan organisms, the adaptive and innate arms of the immune system represent our dual lines of defense against an extensive and rapidly evolving bastion of enemies. Gerald Edelman and Susumu Tonegawa won Nobel prizes in successive generations decades ago for their discoveries showing the nature of antibodies and the elaborate means by which they are put together to facilitate adaptive immunity. More recently, Bruce Beutler and Jules Hoffmann won the Nobel prize for their groundbreaking work on Toll-like receptors as the operational nuts and bolts of the innate immune system.

Despite being blessed with these gorgeous pinnacles of scientific achievement, plenty of mysteries remain. Over the past several years, Zhijian "James" Chen and his colleagues here at the University of Texas Southwestern Medical Center have cracked open a new set of discoveries further clarifying our understanding of how innate immunity works.

Certain pathogens expose their genetic blueprint, sometimes in the form of duplex DNA, to the cytoplasmic compartment of host cells. Our own DNA belongs within either the nucleus or the mitochondrion – not in the cytoplasm. Indeed, DNA was found as an immune-response stimulant even before it was found to carry the genetic blueprint (1).

How did Chen and his team track down the mechanism by which cytoplasmic DNA stimulates the innate immune system? They, of course, started by standing on the shoulders of others who already had discovered that cytoplasmic DNA triggers activation of an endoplasmic reticulum protein designated as STING, MITA, MPYS or ERIS. The STING protein, when magically activated by cytoplasmic DNA, recruits the IKK and TBK1 kinase enzymes, which respectively activate the NF-κB and IRF3 transcription factors. Activation of NF-κB and IRF3 already was understood to trigger cells to activate the expression of genes vital for stimulation of the immune response.

To find out what might happen upstream of STING activation, Chen's team crafted a cell line missing the STING protein. They could then expose the cells to cytoplasmic DNA and prepare extracts in search of molecules responsible for propagating the pro-immune stimulatory signal. Vital to this game plan, Chen's team used permeabilized macrophage cells blessed with an intact STING pathway. By applying extracts from STING-deficient cells exposed to cytoplasmic DNA to the permeabilized macrophage cells, they could monitor IRF3 activation. This was their assay. No matter how arduous, biochemists always need a reliable assay.

The substance produced by STING-deficient cells that activated STING in the recipient macrophage cells turned out to be heat-stable and small – a metabolite, not a protein.

By use of an arduous combination of purification, analytical chemistry and synthetic chemistry, Chen and his team discovered the activating substance to be cyclic GMP-AMP, abbreviated as cGAMP (2). The precise nature of the phosphodiester linkages subsequently was found to be mixed – one was between the 2'-OH of GMP and the 5'-phosphate of AMP, and the other was between the 3'-OH of AMP and the 5'-phosphate of GMP (3, 4). That cGAMP is the STING activator has since been nailed unequivocally, including resolution of the X-ray crystal structure of metabolite-bound STING (4).

Having discovered the cGAMP metabolite as the signaling message, the Chen team turned to its mode of synthesis. They incubated cytoplasmic material from cultured cells with ATP and GTP in the presence of DNA. By applying these reactions products to permeabilized macrophages (after DNAase digestion and heat treatment), Chen's team could track fractions capable of producing the cGAMP stimulant of STING activity. Again, by use of a sophisticated array of chromatographic steps, the team was able to isolate, purify and identify the cGAMP synthase enzyme (now designated cGAS).

These breakthrough studies revealed the precise identity of the cGAS enzyme and its selective activation by DNA (5). Subsequent X-ray crystallographic experiments showed mechanistically how DNA binds and activates cGAS and offered a compelling understanding of how the enzyme converts ATP and GTP into its cGAMP product (3, 6). Those studies positioned the Chen team to generate mice selectively missing a functional gene encoding the cGAS enzyme. These cGAS-deficient mice are considerably more susceptible to lethal infection by herpes simplex virus than wild-type littermates, and studies of immune adjuvant effects of cGAMP have yielded vivid evidence of the importance of the cGAS pathway for proper deployment of the immune system (7). For-profit biotechnology companies, large and small, now are pursuing cGAS inhibitors as potential treatments for autoimmune diseases and cGAMP-related molecules for immunotherapies and vaccines.

I recount this story as a celebration of our discipline – the discipline of biochemistry. Once Chen's team threaded the needle in the discovery of cGAMP and the enzyme that makes it, other scientists could contribute rapidly. Crystallization of the cGAS enzyme was accomplished within months of the Chen team's discoveries, and nearly any molecular biologist could have made knockout mice lacking the enzyme for studies of its role in innate immunity.

What could not have been achieved without hardcore biochemistry were the rate-limiting discoveries of the cGAMP metabolite as a second message produced in response to cytoplasmic DNA as well as the enzyme that synthesizes cGAMP in response to exposure to cytoplasmic DNA. As members of the American Society for Biochemistry and Molecular Biology, we should take pride in the dominance of our scientific discipline: Textbooks are filled with the fruits of our field. Future textbooks covering disciplines ranging from immunology to cell biology to biochemistry undoubtedly will feature the contributions of Chen and his team briefly summarized in this essay. Boy, should we be proud of our field, including its illustrious past, vibrant present and promising future. Biochemistry rules!

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Wanted: lab pranks

Have you ever pranked a colleague in the lab? Have you been the victim of a (hopefully harmless) prank? Send us your story for a special feature in April. Email asbmbtoday@asbmb.org.

New year. New focus. New opportunities!

By Benjamin Corb

As we enter 2015, the American Society for Biochemistry and Molecular Biology public affairs staff is looking at a changed Washington landscape: a Republican-controlled Congress, a president in the last years of his final term and many in Washington already looking forward to the 2016 elections. Yes, 2015 is going to be a challenging year for the research community - but not an impossible year. Here are some initiatives we're looking forward to introducing in the coming months.

First, many of you certainly have read about the ASBMB Public Affairs Advisory Committee's work to build a sustainable biomedical research enterprise. In 2015, we are transitioning from the first phase of identifying systemic problems within the research enterprise (funding, training, stakeholder interactions) to the next phase of implementing the necessary changes. The ASBMB has had preliminary meetings with officials at the White House to discuss the most important needs of the biomedical research community and has submitted legislative language to a bipartisan group of members in the U.S. House that will be introducing the 21st Century Cures Act. We are excited about taking these next steps and putting our ideas into action.

As a service to you, we will begin sending monthly policy updates tailored to each geographical region. Those updates will communicate the national policies that will affect researchers across the country and explain the roles your local representatives are playing in ongoing debates. Former U.S. Speaker of the House Tip O'Neil once said that "All politics are local," and we will work to provide you a more localized perspective on science policy in 2015.

Also in 2015, we will continue to expand and improve our blog, the ASBMB Policy Blotter, which you can read at policy.asbmb.org. We will continue our timely science policy news and analysis for the reader, including our newest feature, the Policy Roundup, by Erica Siebrasse, our science policy fellow. Siebrasse's roundup highlights top news stories of the week that are most pertinent to our members, making it a terrific weekly touchstone.

Finally, we will offer more opportunities for members to engage in advocacy. With letter-to-the-editor campaigns, district meetings with elected representatives and a few exciting online advocacy opportunities, you will have a variety of new ways to get involved in the society's advocacy efforts. Opportunities will range in intensity, so those who have little free time but want to be involved will have their chance, and those who want to dedicate more time to being a leading voice for their field will have opportunities.

As always, the public affairs office at the ASBMB headquarters is looking forward to dutifully serving you in 2015, representing you proudly to policymakers in Washington and tirelessly working to ensure that this is a year of action and progress - a much needed change from the previous decade of frustration and stagnation.

Benjamin Corb (bcorb@asbmb.org) is director of public affairs at ASBMB.

Interested in science policy?

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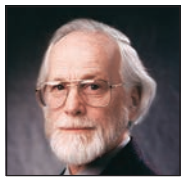
Bishop wins memorial award



BISHOP

Nobel laureate J. Michael Bishop won the Van Andel Research Institute's Daniel Nathans Memorial Award. He gave his public award lecture, titled "Uncovering the Deepest Secret of the Cancer Cell," in December. Bishop, along with Harold Varmus, won in 1989 the Nobel for medicine or physiology for the discovery of proto-oncogenes, which give rise to cancer. Bishop and Varmus also shared the Lasker Basic Medical Research Award and the National Medal of Science. "Dr. Bishop is an exemplary scientist whose contributions to cancer research cannot be overstated," said Peter Jones, VARI's research director, in a statement. "His work has had a significant impact on biomedical research and on human health, and aligns perfectly with the spirit of the Daniel Nathans Memorial Award." The institute established the award in 2000 in honor of Daniel Nathans, whose work on simian virus 40 resulted in the development of recombinant DNA and who served as a founding member of the institute's board of scientific advisors.

IN MEMORIAM: John H. Weisburger

WEISBURGER

John H. Weisburger, a research professor of pathology at the New York Medical College, in Valhalla, N.Y., died in February at the age of 92. A cancer specialist, Weisburger made special contributions to our understanding of the mechanism of 2-acetylaminofleorene. Weisburger entered the science world in the mid-1940s, earning his Ph.D. at the University of Cincinnati. He joined the National Cancer Institute in 1950 as a member of the U.S. Public Health Service. There he conducted studies of how environmental insults change the structure and function of DNA and eventually took the reins of the Carcinogen Screening Section and, later, the Bioassay Carcinogenesis Programs. He then joined the American Health Foundation and directed his attention to nutrition and cancer. Weisburger served on several editorial boards and won numerous awards over the years. In 2013, the American Association for Cancer Researched named him as a member of its inaugural class of fellows. He had been a member of the ASBMB since 1959.

IN MEMORIAM: Max Schlamowitz

Max Schlamowitz, a biochemist, teacher and lifelong music lover, died in January at the age of 94. Schlamowitz dedicated most of his career to studying the molecular bases for the transmission of antibodies across membranes. A New Yorker by birth and raising, Schlamowitz earned his bachelor's degree at the City College of New York and his master's and Ph.D. at the University of Michigan. Then, at the University of Rochester, he was in the toxicology unit of the Manhattan Project. He next joined the then-Sloan-Kettering Institute and the Roswell Park Memorial Institute. Later, he joined the University of Texas MD Anderson Cancer Center in Houston, where he conducted research and taught until retirement. Schlamowitz was a supporter of the musical arts and known to enjoy the opera, symphony, chamber music and, in particular, the violin. He had been a member of the American Society for Biochemistry and Molecular Biology since 1953.

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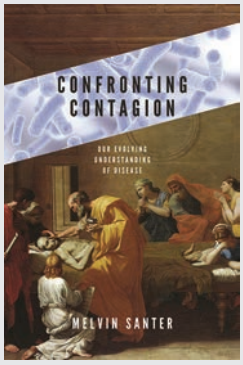
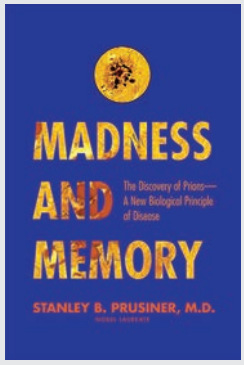
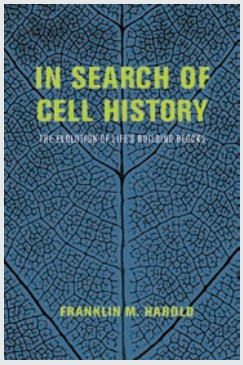
New books by members







Melvin Santer has published a new book, "Confronting Contagion: Our Evolving Understanding of Disease," with Oxford University Press. Nobel laureate Stanley B. Prusiner's book about prions, "Madness and Memory," was published by Yale University Press. Franklin M. Harold's book about the origin of cells, "In Search of Cell History," was published by the University of Chicago Press. *If you have published a book, contact us at asbmbtoday@asbmb.org*

Robert Bittman, 1942 – 2014

Robert Bittman, an organic lipid chemist and distinguished professor at the City University of New York and Queens College, died Oct. 1 of pancreatic cancer. He was 72.

Bittman won the Avanti Award from the American Society for Biochemistry and Molecular Biology in 2003 and served on the editorial board of ASBMB's Journal of Lipid Research. One of the first recipients of the National Institutes of Health MERIT awards in 1986, Bittman "set new standards in the synthesis of bioactive lipids and their analogs with ingenuity and elegance," said his longtime friend and collaborator Gabor Tigyi of University of Tennessee Health Science Center.

"His synthetic schemes benefited basic researchers and industrial chemists alike. Those of us who collaborated with him were beneficiaries of unique reagents, which provided new tools that led to many discoveries," Tigyi said.

Bittman synthesized analogs of lysophosphatidic acid; phosphatidylinositol; sphingosine 1-phosphate; phosphatidylcholine; sphingomyelin; antitumor ether lipids; immunostimulatory and immunosuppressive derivatives of galactosylceramide and FTY720; photoactivatable cholesterol and phospholipids; cell-trafficking lipid tools; and chemopreventive sphingadienes. In 1992, Bittman was the first to prepare cell-permeable ceramide analogs. He also developed many methods for the enantioselective synthesis of sphingosine, ceramide and phospholipids.

"With these new reagents, he probed the roles of sphingolipids in the fusion of alpha viruses with cholesterol-containing viruses; sterol side-chain structure and sphingomyelin structure in lipid raft formation; and the effects of ceramide structure on ceramide's dipole potential in monolayers," Tigyi explained.

A meteoric rise, a prolific researcher

Bittman was born March 19, 1942, in New York City. A gifted student, he graduated from Jamaica High School in 1958 at age 16. He earned his undergraduate degree in chemistry in 1962 at age 20 from Queens College. He then earned his Ph.D. in chemistry in 1965 at age 23 at the University of California at Berkeley, where he worked under Andrew Streitwieser. That same year, he joined the lab of Manfred Eigen (who would win the Nobel prize in chemistry shortly thereafter) at the Max Planck Institute for Physical Chemistry in Germany. Bittman was recruited to the faculty at Queens in 1966 at age 25.

Over the next almost five decades, Bittman synthesized hundreds of molecules. His work was funded continuously by a National Institutes of Health grant that ran from 1973 until earlier this year.

By all measures, Bittman was prolific: He penned more than 300 scientific papers and 64 book chapters. Nineteen U.S. patents bearing his name were granted or are pending.

Tigyi, who met Bittman at a meeting in 1995 and went on to co-author 17 papers with him, said, "He was as much a sculptor of organic molecules as he was of sentences – who would not tolerate the smallest ambiguity in his manuscripts."

Nigel Pyne of the University of Strathclyde in Glasgow, Scotland, agreed: "Although I have to say we always sent manuscripts to him with some apprehension, knowing they would come back with large numbers of mistakes corrected, he was really a line editor's dream – but probably making them largely redundant."

The "No. 1 go-to guy" for sphingolipids

Bittman's collaborators all emphasized that he never met a stranger and never came across a problem not worth trying to solve.

Julie Saba, who met Bittman at an ASBMB meeting in 2005, said she was "struck with an immediate affinity for the man, who, within minutes of our introduction, began postulating structures and chemical hypotheses while leaning casually over the banister in the fifth-floor hallway and looking out into the empty space of the gallery as though he could see the chemical structures floating there." Saba continued: "As we chatted during that first encounter, Bob's scientific brilliance, curiosity, determination, and utter unpretentiousness impressed me deeply."

Richard Kolesnick of Memorial Sloan Kettering Cancer Center called Bittman "the No. 1 go-to guy when you wanted a sphingolipid synthe-

sized, bar none."

Kolesnick quipped that a quick call to Bittman was never quick at all. "After Bob would let you explain your needs for a few minutes, he would have 10 additional ideas as to how to generate new analogs that you never could have conceived of and that would further advance your idea. After his 45-minute tutorial on the structure of sphingolipids and their organic synthesis … you realized that you really didn't know as much about your field as you thought, and you were thankful."

Gilbert Arthur of the University of Manitoba echoed that sense of gratitude. Arthur published 23 papers and a book chapter with Bittman, and they shared three patents. Their relationship began in 1992. "Bob had synthesized a number of compounds that I was sure would be invaluable to the research I wanted to pursue, and after quickly realizing the futility of attempting to synthesize them myself, I decided to write to Bob and request some of his compounds," Arthur said.

"The letter was written with the expectation that there would be no response," Arthur said. "To my surprise and delight, Bob responded almost immediately." Bittman sent the requested compounds, and a fruitful collaboration was born.

Elina Ikonen's relationship with Bittman started in a similar way. "How many times have you sent an email to a stranger and hoped for a reply – in vain?" she asked. "I sent an email to a complete stranger almost 10 years ago and was not expecting any response. This time I was completely wrong. I had hit a gold mine! This gold mine was Bob Bittman."

Ikonen continued: "My question was whether he might be willing to share one of the fluorescent lipid derivatives he had recently synthesized, BODIPY-cholesterol, with us. Not only did he reply promptly but more positively than I ever expected. This was the beginning of an active collaboration that, among other things, defined this compound as being suitable for assessing the behavior of cholesterol in cell membranes."

Antonio Gomez-Munoz also came to know Bittman in the early 1990s, back when Gomez-Munoz was a postdoc at the University of Alberta in Canada. "I became interested in ceramide 1-phosphate, a phosphosphingolipid that had been observed by Rich Kolesnick in human leukemia cells a couple of years before. This molecule was not commercially available at the time, so I needed someone who could be capable of synthesizing it for me."

He continued: "We contacted Bob, and he became even more enthusiastic than I on the project. Upon speaking with him, I could feel his energy and enthusiasm through my skin. Not only did he provide us with the compound, but he taught me excellent organic chemistry and gave me suggestions that turned out to be crucial for our studies." Gomez-Munoz went on to publish six studies with Bittman on caged ceramide 1-phosphate and has three more in the pipeline.

A problem-solver to the end

Pyne, who met Bittman in 2009, underscored that Bittman was "able to discard the periphery and to go straight to the central question." He said: "Bob was rather good at spotting these central questions, primarily because his mind was one that was unburdened and constantly searching for biology problems to solve with his compounds. His publication record is a testament to how good he was at that."

Throughout his career, Bittman dedicated himself to service to the scientific community. He served in NIH study sections, lectured across the globe, trained 20 postdoctoral researchers and 23 graduate students, served as a member and later as chairman of the Biophysics Section of the New York Academy of Sciences, won election as vice-president of the Queens College chapter of Phi Beta Kappa, and sat on the editorial boards of the JLR, Subcellular Biochemistry, Chemistry and Physics of Lipids, and the Journal of Liposome Research. In addition, he served as secretary and co-secretary of Organic Reactions from 1968 to the end of his life. He was inducted as a fellow of the American Association for the Advancement of Science in 2004.

Walter Shaw, founder of Avanti Lipids, the sponsor of ASBMB's Avanti Award that Bittman won a decade ago, met Bittman in the early 1970s. "I have seen him work his synthetic sorcery with organic molecules to produce numerous compounds that were thought to be beyond our field's current synthetic capabilities," Shaw recalled. "Our field has lost a great chemist, but more importantly, the world has lost a truly great man."

Bittman's friends say that he was reluctant to tell many people about his illness. But, Tigyi said, when Bittman did break the news to him, "he was calm and told me about his plan how to fight the disease against all odds." Tigyi continued: "He survived the diagnosis by many, many more months than projected. In a way, it was his triumph over an unbeatable disease."

To read more about Bittman's life and contributions to science, visit www.asbmb.org/asbmbtoday. There you will find a full-length Retrospective article contributed by Gabor Tigyi, Julie Saba, Nigel J. Pyne, Richard N. Kolesnick, Gilbert Arthur, Elina Ikonen, Antonio Gomez-Munoz and Walter Shaw. This report is a composite of their tributes compiled by ASBMB Today's editor, Angela Hopp.

New Tabor Young Investigator Award winner

By Alok Upadhyay

Said Izreig, a graduate student at McGill University in Canada, received the **Journal of Biological Chemistry**/Herb Tabor Young Investigator Award for his work on how cancer cells reprogram metabolic steps to their advantage. He received a plaque and a $1,500 prize at a meeting this fall in Barga, Italy.

Metabolic reprogramming is one of the hallmarks in cancer-cell proliferation. Said, who works in Russell Jones' lab at McGill, studies metabolic changes promoted by an oncogene, c-Myc, implicated in human cancer. It is known that c-Myc manipulates the metabolic program that increases nutrient consumption and enhances biosynthetic activity of cancer cells, but the molecular mechanism underlying reprograming is unclear.

Said Izreig received his Tabor award at the AMPK: Biological Action and Therapeutic Perspective conference organized by the Federation of American Societies for Experimental Biology in late September in Italy. John Kyriakis, a JBC associate editor, issued the award.

Said discovered that c-Myc regulates a family of micro-RNAs that is required for c-Myc's metabolic effects in B-lymphoma cells. These miRNAs repress the tumor suppressor and master metabolic regulator gene LKB1, leading to a hyperactive metabolism, which results in aggressive tumorigenesis.

The Tabor award is conferred at symposia and meetings throughout the year to students, postdoctoral researchers and faculty members who've not yet received tenure.

Alok Upadhyay (alok7930@gmail.com) is a postdoctoral associate at Fox Chase Cancer Center. His major research area is Notch signaling regulation during cell fate decisions and neural crest stem cell development. Follow him on Twitter at www.twitter.com/alok7667.

Why the bad taste?

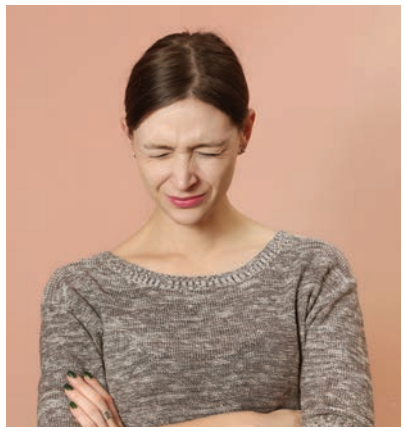
A novel mechanism that explains the bitter pill

By Sarah C.B. Guthrie

Numerous medications can affect a patient's sense of taste and smell adversely. These effects even can persist long after drug cessation. Drug-induced taste disorders can impact significantly patients' quality of life, dietary choices and emotional states. More importantly, they can provoke noncompliance problems, especially in children and elderly patients.

In a recent issue of the **Journal of Biological Chemistry**, a research team at the University of Washington described a novel mechanism for active drug accumulation and secretion in salivary gland epithelial cells that leads to the lingering bad taste of metformin, a frontline prescription drug used in the treatment of type 2 diabetes. Joanne Wang, a professor at the School of Pharmacy, led the team.

"Dr. Wang's lab has identified a transporter protein in the salivary glands that takes up drug compounds from the circulating blood and transfers them to the saliva they produce, giving us new insight into how certain medications change how foods taste," said Richard Okita of the National Institutes of Health's National Institute of General Medical Sciences, which partially funded the research. "This discovery could potentially be used in drug development to prevent excessive accumulation of a drug in saliva glands and reduce related conditions such as drug-



induced dry mouth."

While several factors might contribute to drug-induced taste disturbance, continuous secretion of drug molecules into the saliva appears to be one of them.

Very little is known regarding how the salivary gland secretory cells secrete drugs or other xenobiotics into the saliva. Most drugs have been assumed to enter saliva by passive diffusion, a process characterized by downhill, nonmediated diffusion of drug molecules across the membranes of salivary gland epithelial cells. However, passive diffusion cannot explain salivary secretion of hydrophilic drugs or account for prolonged drug presence in saliva long after systemic drug elimination.

Wang's team found that salivary glands selectively and highly express a polyspecific drug transporter called organic cation transporter 3, or OCT 3 for short. Present on both basolateral (blood-facing) and apical (saliva-facing) membranes of the salivary gland acinar cells, OCT3 is responsible for the concentration of metformin in secretory epithelial cells and its subsequent slow release into saliva.

The researchers showed that metformin was transported actively into salivary glands of wild-type mice at levels as high as those seen in the kidney and liver. Deletion of the gene for OCT3 in mice abolished active drug uptake and accumulation in salivary glands.

This is the first time that a carrier-mediated mechanism has been demonstrated for drug accumulation and secretion in salivary glands, Wang said.

The primary function of salivary glands is to secrete saliva, which plays an important role in oral health, nutrient digestion and immunity to microbial infection. Healthy adult salivary glands secrete about three-quarters of a liter to 1.5 liters of salivary fluid each day, and dysfunction of the salivary glands can lead to xerostomia, more commonly known as dry mouth.

While xerostomia may have many origins, Wang said, excessive accumulation of a drug within the salivary glands may lead to tissue toxicity and gland dysfunction. She added that, in this study, OCT3-mediated active uptake led to very high levels of drug accumulation in salivary glands, which may intensify drug toxicity to the secretory epithelial cells.

"Designing drugs that are not OCT3 substrates might prevent drug-induced taste disorders and salivary gland toxicity mediated by OCT3," Wang said.

Sarah C.B. Guthrie (gu3@uw.edu) is director of communications at the University of Washington School of Pharmacy.

Myron Goodman reflects on SOS error-prone DNA repair

By Nicole Parker

For most people, nothing goes the way they plan it, and the most unexpected things are liable to happen at any time. One person who can attest to that is Myron F. Goodman, a professor at the University of Southern California. In his recent "Reflections" article for the **Journal of Biological Chemistry**, Goodman recounts the education and career path that led him to the discovery of error-prone DNA polymerase V and its unique regulation by RecA and ATP.

"My career pathway has taken a circuitous route beginning with a Ph.D. in electrical engineering from Johns Hopkins University, followed by five postdoctoral years in biology

GOODMAN

at Hopkins and culminating in a faculty position in biological sciences at the University of Southern California," Goodman explains.

Having trained primarily in physics and electrical engineering, Goodman was not exposed to biology until his Ph.D. thesis led him to study ATP hydrolysis. His project was titled "Selective hydrolysis of adenosine triphosphate resulting from the absorption of laser light in a stretching mode of the terminal phosphate group," and he quips in his JBC article that he, at the very least, needed to learn how to measure ATP hydrolysis.

Goodman was provided a lab bench to learn how to do this assay, and from there his interest in biology expanded to a point at which he could not look back. Upon receiving his Ph.D., Goodman chose a postdoc focusing on biochemical enzymology instead of taking a job in his original field.

In 1973, Goodman joined USC as a faculty member, and his research pathway was also circuitous. It began with an attempt to identify the mutagenic DNA polymerase responsible for copying damaged DNA as

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part of the well-known SOS regulon, which led to the discovery of E. coli DNA polymerase V. E. coli DNA polymerase V is a part of the Y family of enzymes that are referred to as translesion synthesis polymerases.

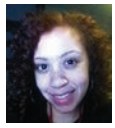
Goodman writes: "The path to the discovery of pol V is closely linked to more than 60 years of experiments and concepts in the area referred to as 'SOS error-prone DNA repair.'"

Goodman's "Reflections" highlights the discoveries that impacted the field the most. The SOS regulatory proteins of the system LexA and RecA were identified in the mid-1970s, and the discovery of the SOS mutagenesis genes umuC and umuD soon followed.

Goodman goes on to explain the various experiments his group conducted to understand SOS error-prone DNA repair from the late 1970s up till now. In 1998, Goodman's lab conducted experiments that led to the discovery that the UmuD'2C mutagenic complex could be a new type of low-fidelity DNA polymerase. By 1999, this was unequivocally shown to be a DNA polymerase with the polymerase activity in the UmuC subunit. Next, the new Y polymerase family was identified, and as of now, 13 family members have been identified in various species.

Most recently, Goodman discovered that an intrinsic DNA-dependent ATPase regulates the polymerase V function. Prior to this, no such ATPase activity or autoregulatory mechanism had been found on a DNA polymerase.

Although Goodman notes that his research pathway has been circuitous, it has truly come full circle. His interest in biology began with a study of ATP hydrolysis, and his most recent contribution to the field was the identification of the first DNA polymerase to be regulated by ATPase activity. To learn more about Goodman's work, read the full JBC "Reflections" article at www.jbc.org.



Nicole Parker (nparke11@jhu.edu) joined the University of Maryland, Baltimore County, in the Meyeroff Scholarship Program in 2007. She earned a B.S. in biochemistry and molecular biology at UMBC and is completing her Ph.D. in BMB at the Johns Hopkins School of Public Health, where she studies the biological activity of the protein GDNF and its effect on the spermatogonial stem and progenitor cells.

Exploring muscular disease in humans and cattle

Insights into treatment of Brody disease through inhibition of the ubiquitin-proteasome system

By Sapeck Agrawal

A recent study in the **Journal of Biological Chemistry** about a muscular disease in cattle may offer clues about how to treat a similar disease found in humans.

Both the Chianina cattle muscular disease pseudomyotonia and the human Brody disease are characterized by an inability of skeletal muscles to relax after strenuous physical exercise, leading to temporary muscle stiffness. The cause is a mutation in the ATP2A1 gene encoding a protein called SERCA1, which is crucial for pumping calcium from the cytosol back to the lumen of sarcoplasmic reticulum, thus enabling muscle relaxation. Because of such phenotypic and genetic overlap, Chianina pseudomyotonia is studied as a model for Brody disease.

A Chianina cow and calf in a field in Tuscany.

IMAGES COURTESY OF STEVEN WALLING, A WIKIMEDIA COMMONS USER

Interestingly, the mutated SERCA1 protein retains its basic calcium-dependent ATPase activity like the normal protein, suggesting that the mutation does not affect its function. What is affected, however, is the amount of the mutant protein in skeletal muscles, which is much lower in comparison to normal protein levels.

This is despite normal mRNA levels of the ATP2A1 gene. These key observations prompted Roberta Sacchetto and Dorianna Sandona at the University of Padova in Italy to join forces and investigate the potential roles of the ubiquitin-proteasome system in degrading the mutant proteins.

The team blocked the ubiquitin-proteasome system with different chemical inhibitors and measured the effect on the levels of mutant SERCA1 in a cellular model. Disrupting the pathway dramatically rescued the expression levels and membrane localization of SERCA1 as determined by Western blot analysis and immunofluorescence analyses.

To corroborate further the role of the ubiquitin-proteasome system in degradation of the mutant SERCA1, the team measured polyubiquitination of the mutant against that of the normal protein. Their rationale was that since the chemical inhibitor used to disrupt the ubiquitin-proteasome system pathway works downstream of the ubiquitination step, it would not affect the accumulation of polyubiquitaned forms of mutant SERCA1 protein. Results from immunoprecipitation assays confirmed the scientists' hypothesis, and, indeed, there was an increase in the polyubiquitinated mutant SERCA1.

The researchers demonstrated that the pharmacologically rescued SERCA1 was able to restore cytoplasmic calcium homeostasis in a cellular model and was also fully active in muscle fibers isolated from a PMT-affected cow.

The significance of the study is that it demonstrates for the first time the role of the ubiquitin-proteasome system in degrading the mutant SERCA1 protein, explaining the symptoms associated with Chianina cattle pseudomyotonia and some forms of Brody disease. The findings also suggest specific inhibition of the ubiquitin-proteasome system could be a sound therapeutic strategy against the two diseases.

Sapeck Agrawal is a freelance science writer with a Ph.D. in molecular biology from the Johns Hopkins University. For more stories, visit her blog at sapeckagrawal.wordpress.com.

From country girl to author on most-cited paper: Nira Rosebrough Roberts

By Rajendrani Mukhopadhyay

"I was a little country girl who really didn't know much of anything. But I was very good at what I did, and we made a good team." That is Nira Rosebrough Roberts, the technician who worked with Oliver Lowry and two others to develop the Lowry method, a famous way of measuring the amount of protein in a solution.

The **Journal of Biological Chemistry** paper that describes the method is the most-cited paper in publishing history. By late December, the paper had been cited 305,782 times. Roberts' maiden name, Nira Rosebrough, is second on the paper.

Roberts, now a vivacious 87-year-old widow living a life packed with games of bridge and other fun at an independent seniors' home in Lexington, Ky., landed in Lowry's laboratory at the Washington University School of Medicine in St. Louis by "pure happenstance," she says.

ROBERTS

Roberts grew up in the small town of Bolivar, Mo., where she excelled in school. "I got very good grades, so I was the valedictorian of a very small class," she says. "There were 44 students." Her parents didn't have money for college, so when she graduated from high school, Roberts first went to a junior college called Southwest Baptist University in Bolivar for two years. She was the first one in her family to head to college. But Roberts wanted more, so she next decided to enroll at Drury University in Springfield, Mo.

When she got to the university, Roberts weighed her options. She loved math. But in those days, the only avenue open to a woman with a math degree was teaching. "I didn't want to be a teacher," says Roberts.

So, because she had enjoyed a chemistry course in high school, she opted to pursue a bachelor's degree in chemistry with a minor in math. The degree was a four-year program, which Roberts completed in two, graduating magna cum laude in 1948.

Notably, "I'm probably the only B.S. in chemistry who got through school without taking physical chemistry!" she says with a laugh. "My chemistry professor allowed me to take a brand-new course called atomic physics instead of physical chemistry, and they gave me a bachelor's degree."

Roberts can't recall how she found out about the technician job at Washington University, but she and three young men headed to the university

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after they graduated from Drury University. The men entered the medical school, and Roberts began to work for Lowry.

Lowry had been at the institution for a year as the head of the pharmacology department. "We'd meet in the mornings and go over whatever results we had in the afternoons," recalls Roberts. "I had no idea about biochemistry or anything else when I got there, but I was a good technician."

She and Lowry had a daily routine. Lowry outlined the experiments needed to be done for the day in the mornings. Roberts made the solutions and did the experiments. Lowry came by to look at the results and discuss them in the afternoons.

Roberts describes Lowry as always brimming with ideas and being an excellent teacher: "I had no idea about biochemistry. But he explained everything to where I could halfway understand it."

Plus, she says, with a delightful chuckle, "He was very handsome!"

The protein measurement method described in the JBC paper relies on a solution called the Folin phenol reagent. The reagent, which consists of phosphomolybdic-phosphotungstic acid, binds proteins treated with copper. The reagent gets reduced, causing a quantitative color change from yellow to blue. The amount of color change is used to calculate protein concentration.

The two other people who pitched in with the protein measurement method were an M.D. named Lewis Farr and another technician, Rose Randall. Farr left research to practice medicine, and Randall was in the Lowry laboratory for only a few months. Roberts lost touch with them when they left the laboratory. Our staff's attempts to find Farr and Randall have failed so far. Lowry passed away in 1996.

In 1951, Roberts left the Lowry laboratory. She and her soon-to-be husband, DeWayne Roberts, whom she had met at Washington University, had been eking out life on technician salaries that were less than $2,000 a year. To make more money, they headed to Cactus, Texas, to work at an ammonium nitrate plant. Roberts' husband worked in the plant, and because women weren't employed there, Roberts became the administrative assistant to the plant's personnel director.

In 1953, the couple was back at Washington University, with Roberts resuming her technician position in Lowry's laboratory. She focused on micromeasurement methods while her husband pursued his Ph.D. in pharmacology.

Lowry already had written up and published the protein measurement paper in the JBC by the time Roberts returned to his laboratory. He gave her a reprint of the paper in an olive-green envelope, which Roberts still has somewhere among her possessions. She and her husband left the university in 1957 after he got his Ph.D.

Roberts says she had lost track of the JBC paper in the 1960s and 1970s while she was busy raising three children. She had become a homemaker. Her husband's work was her only connection to science.

But after Citation Classics mentioned the JBC paper in1977, a coworker of her husband's noticed it and told them about the paper's citation record. The paper's fame clued her family in that Roberts had played an important role in science.

"They were very impressed, but they didn't understand it," she says. "My family didn't realize what I was doing. They didn't know what chemistry was or anything. I was just fortunate enough that it was easy for me and I could make good enough grades."

But even if they don't understand what exactly Roberts accomplished with Farr, Randall and Lowry, "they are very proud, and so am I."

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Super microbes for biofuel production

By Maggie Kuo

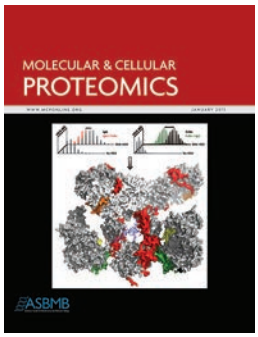
Considering the high amounts of CO_2 produced globally from burning fossil fuels, producing fuel from CO_2 would be like turning lead into gold. Bacteria and yeast can carry out this process, but the more ethanol they produce, the more their ethanol-producing capabilities are limited. Researchers at Tianjin University and the Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences in China recently reported in the journal **Molecular & Cellular Proteomics** a new target for genetic engineering that could increase microbial tolerance to ethanol.

Most bioethanol is produced by bacteria and yeast that derive their energy from sugars obtained from crops like corn, sparking the concern that crops are going toward biofuel

production instead of food production. Cyanobacteria are attractive alternatives to the sugar-using bacteria because they obtain their energy through photosynthesis and use CO_2 to produce ethanol. However, cyanobacteria are very sensitive to ethanol: A marginal amount of ethanol can slow their growth dramatically.

Ethanol, in general, is toxic to cells, but genetic engineering of sugar-using bacteria and yeast has created strains that have higher tolerance to ethanol. Several studies have suggested that these microbes employ several lines of defense to handle the excess biofuel products they produced. Manipulating the genes that regulate transcription could control this response and confer tolerance more readily than targeting individual metabolic genes. Targeting genes that control transcription has increased significantly ethanol tolerance and the efficiency of ethanol production in sugar-using microorganisms. The research collaboration led by Weiwen Zhang sought to determine if this strategy could be used for cyanobacteria.

Using the model cyanobacterium strain, the group previously had identified by proteomics and transcriptomic analyses three proteins involved in regulating transcription, Sll0792, Sll0794 and Sll1423, whose expression is influenced by ethanol exposure. In this new study, the investigators created mutant strains that were missing those genes and grew the mutant bacteria under normal conditions or in 1.5 percent ethanol. The sll0794- and sll1423-deficient cells did not grow more slowly in ethanol, but the sll0794-deficient cells did, suggesting that disrupting the sll0794 gene altered the abundance of proteins that were important for tolerating ethanol.



The researchers then used proteomics analysis to identify which proteins were expressed differently in sll0794-deficient cells when those cells were exposed to ethanol. The investigators selected the genes that changed the most and determined if sll0749 directly controlled the transcription of those genes. They found that sll0794 bound to the promoter regions of three of the genes: sll1514, which corresponds to a 16.6 kDa small heat shock protein; slr1838, which corresponds to a carbon dioxide concentrating mechanism protein CcmK; and slr1512, which corresponds to a putative sodium-dependent bicarbonate transporter.

This is the first study to identify a specific transcriptional regulatory gene, sll0794, as a target for genetically engineering cyanobacterium for ethanol tolerance. Manipulating this gene could result in strains that can better withstand and, as a result, more efficiently produce ethanol. By overcoming the ethanol sensitivity, scientists will be closer to realizing the use of cyanobacteria to recycle CO_2 to form new fuel.

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Isoform-specific function of Akt in atherosclerosis

By David B. Iaea

In normal physiological conditions, cholesterol levels are tightly regulated by a variety of homeostatic machinery and mechanisms. One such mechanism is the clearance of excess cholesterol by macrophages. However, excess dietary fat and cholesterol in the Western diet push the homeostatic machinery beyond its physiological range. Under these conditions, macrophages become overwhelmed with cholesterol and undergo cell death, forming a plaque. Progression of this process leads to the growth of an atherosclerotic plaque that impedes blood flow and can result in stroke or heart attack.

A recent study in the **Journal of Lipid Research** investigated the role of macrophage Akt isoforms in atherosclerosis. Akt is a serine/threonine kinase that is an important regulator of a variety of cellular processes including signaling, metabolism and cell survival. Macrophages express three constitutively active isoforms of Akt (Akt1, Akt2 and Akt3). However, it is unclear whether Akt isoforms have specific or redundant function in macrophages and, more importantly, what the role of these specific isoforms are in the development of atherosclerosis.

In this study, the researchers sought to elucidate the functional relevance of Akt1 and Akt2 in atherosclerosis. They utilized a genetic loss of function approach in order to selectively and specifically study the loss of these Akt isoforms in macrophages in a mouse model that lacks the low-density lipoprotein receptor.

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National Academies' report on postdocs

Committee urges higher salaries and better training

By Maggie Kuo

The National Academies released a report in December that advocates for improvements in training and salary for postdoctoral fellows in academia. The report urges action in six areas: compensation, term length, position title and role definition, career development, mentoring and data collection.

Key points from the report:

1. Postdoctoral salaries should be increased to at least $50,000 and adjusted annually for inflation. The starting salary at most institutions for many disciplines is $42,000. Furthermore, federal agencies should require institutions to provide documentation in grant proposals about the salaries the postdoctoral researchers will receive.

2. Postdoctoral appointments should be for a maximum of five years. Funding agencies should assign each postdoctoral fellow an identifier to track them better.

3. The title "postdoctoral researcher" should be used by institutions only for positions in which the individual receives significant advanced training in research. "Postdoctoral researcher" should not be used for people in positions that are more suitable for permanent staff scientists, such as lab managers, technicians, research assistant professors. The report also urges funding agencies to use "postdoctoral researcher" consistently and "require evidence that advanced research training is part of the postdoctoral experience."

4. Postdoctoral training should be viewed by graduate students and principal investigators as only a stage in which to gain advanced research training. It should not be considered the default step after Ph.D. training. Institutions should make first-year graduate students aware about careers outside of academia.

5. Training postdoctoral fellows entails more than just supervision. Mentoring should be emphasized. Postdoctoral fellows should be encouraged to seek guidance from multiple advisers besides their principal investigators, and they should seek out mentoring and resources from professional societies.

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JOURNAL NEWS CONTINUED

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Loss of Akt2, and not Akt1, in macrophages reduced both early and advanced atherosclerosis. This was demonstrated by a reduction in lesion area. Interestingly, when the plaques were stained for macrophages, loss of Akt2 resulted in a reduction in the number of macrophages that had invaded the plaque. In addition, the authors went on to show that the reduced number of macrophages in the plaque resulted from a reduction in expression of macrophage chemokine C-C motif receptor 2, known as CCR2 for short, which plays a critical role in their ability to migrate into the plaque. This indicates that deficiency of Akt2 in macrophages may have a protective role in atherosclerosis, potentially by reducing plaque invasion by macrophages.

To further determine the mechanism of this reduction in atherosclerosis, the authors investigated whether loss of either Akt1 or Akt2 altered macrophage identity. Indeed, macrophages lacking Akt2 demonstrated an immunosuppressive M2 phenotype, whereas those lacking Akt1 demonstrated a pro-inflammatory M1 phenotype. "Modulation of macrophage phenotype and CCR2 expression may be a promising strategy to treat atherosclerotic vascular disease," says MacRae Linton, the senior author of the JLR article.

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Thyroid awareness month

By Indumathi Sridharan

The pink ribbon is a well-known symbol for breast cancer awareness. But how many know about the blue paisley ribbon?

The American Association of Clinical Endocrinologists launched in 2012 the blue paisley ribbon as an icon for thyroid awareness to highlight the silent epidemic of thyroid disease. Thyroid disease is more common than diabetes, heart disease and cancer. It afflicts an estimated 30 million Americans yet half of them remain undiagnosed and untreated (1). Timely diagnosis of thyroid issues can improve general well-being and prevent incidence of fatal conditions like stroke and heart failure.

What is the thyroid gland and why is it important?

The thyroid gland is a small, butterfly-shaped gland situated in the neck below the Adam's apple. Under the feedback control of the hypothalamus and the pituitary gland, the thyroid gland metabolizes iodine to produce two key hormones, thyroxine and triiodothyronine. These hormones influence overall health since they control metabolic rate, oxygen consumption, body temperature, heart rate, cognitive function, muscle control and bone maintenance.

What happens when the gland malfunctions?

An underactive thyroid gland (hypothyroidism) produces insufficient

levels of hormones causing fatigue, depression, memory loss and weight gain. In women, it causes reproductive health complications. When the thyroid gland overproduces hormones (hyperthyroidism), it leads to muscle weakness, weight loss, sleep disorders and vision problems.

What are the molecular bases for some thyroid diseases?

Iodine deficiency and exposure to toxic halogens cause hypothyroidism by interrupting iodine uptake and metabolism. Disorders such as Hashimoto's thyroiditis and Graves' disease affect thyroid function by cell- and antibody-mediated immune processes. Thyroid nodules and cancer also disrupt thyroid function by interfering with the feedback communication between the thyroid and the pituitary gland.

What are researchers investigating now?

Terry Davies and colleagues of the Mount Sinai School of Medicine induced human embryonic stem cells to obtain thyroid cells by overexpressing two transcription factors, PAX8 and NKX2-1, and exposing the cells to activin A and thyroid-stimulating hormone. This study, which lays the ground for growing healthy thyroid tissue to replace damaged thyroid gland, was presented at the 84th annual conference of the American Thyroid Association held in October.

Other key presentations at the meeting covered the role of sex hormones in thyroid cancer and the link between the drug methimazole, which is used to treat Graves' disease, and birth defects (2).

Those who were following the meeting on Twitter noted recent advances in thyroid cancer diagnosis, including the use of next-generation sequencing to detect cancer in thyroid nodules by a group led by Yuri Nikiforov at the University of Pittsburgh Medical Center and a new microRNA-based assay developed by Rosetta Genomics that could prevent unneeded diagnostic surgeries.

This article is the first in a series of explanatory reports about diseases and conditions for which there are national and international observances. To learn more about U.S. and World Health Organization observances, visit healthfinder.gov/hov and www.who.int/campaigns/en/, respectively.

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Enrichment of acyl chains in the lipids of the phosphatidylinositol cycle

By Richard M. Epand

Phosphorylated forms of phosphatidylinositol play important roles in signaling and cell function. It has been known for many years that these lipids are synthesized in the phosphatidylinositol cycle and are highly enriched with 1-stearoyl-2-arachidonoyl species (1). Several mechanisms contribute to this enrichment, including the remodeling of synthesized phospholipids through the action of acyl transferases (the Lands pathway) (2, 3) as well as through the specificity of the enzymes of the phosphatidylinositol cycle (see figure).

In the phosphatidylinositol cycle, a diacylglycerol kinase phosphorylates diacylglycerol. Although there are 10 different diacylglycerol kinase isoforms in mammals, in addition to gene-splice variants, one isoform called DGKε shows specificity for diacylglycerol species that reflect the acyl chains found in phosphatidylinositol (4). The phosphatidic acid product of DGKε catalysis is then converted to CDP-diacylglycerol (CDP-DAG), catalyzed by the enzyme CDP-DAG synthase, known as CDS.

CDS2, one of the two isoforms of mammalian CDS, also exhibits acyl-chain specificity for its substrate, phosphatidic acid, favoring 1-stearoyl-2-arachidonoyl species, like DGKε (5).

It is important that the phosphatidylinositol cycle describes a cyclical metabolic pathway so that the intermediates of the cycle are regenerated each time the cycle goes around. As a consequence, the preferential incorporation of particular acyl chains at one step in the cycle would be magnified because of progressive enrichment in each cycle. The lipid intermediates of the cycle have to be segregated from other forms in the cell, because most species of these intermediates would not be enriched with 1-stearoyl-2-arachidonoyl species. This segregation is difficult to achieve, because different steps of the cycle occur in two different membranes: the plasma membrane and the endoplasmic reticulum. This is a current area of research.

Once phosphatidylinositol is formed, the acyl-chain enrichment with 1-stearoyl-2-arachidonoyl species does not change much in forming PI4P and PIP2 (see figure). In addition, we find that conversion

Variety is not a spice for some lipids!

We all learn that phospholipids are composed of glycerol, to which fatty acids and a defining head group are attached. We also know that many phospholipids have a characteristic set of attached fatty acids. Why such specificity exists and which physiological roles they play has eluded us. In this month's Lipid News article, Richard Epand of McMaster University describes his recent work that uncovered how this enrichment is achieved for the important signaling lipids called phosphoinositides.

Fatty-acid composition in phospholipids sometimes happens after their synthesis, at which point the attached fatty acids are exchanged for other fatty acids in a process known as remodeling. For the phosphoinositides, the selectivity occurs not only via remodeling after the synthesis of the phospholipid but also during their synthesis. These phospholipids are enriched heavily with a select set of fatty acids called stearic acid and arachidonic acid. Epand's lab showed that two important enzymes involved in their synthesis, diacylglycerol kinase-epsilon and cytidine diphosphate synthease-2, prefer precursor substrates with the selected fatty acids. Epand's article describes new findings regarding how fatty-acid selectivity is achieved for other lipids as well.

These studies offer us a glimpse of the mechanisms of fatty-acid specificity within lipid classes. Why is this important? What role do select fatty acids play in physiological processes? We now may be in a better position to start answering these questions.

– Daniel Raben

of CDP-DAG to phosphatidylinositol, catalyzed by phosphatidylinositol synthase, or PIS for short, does not result in acyl-chain enrichment. Therefore, only two enzymes – DGKε and CDS2 – are principally responsible for acyl-chain enrichment. We suggest that the extent of acyl-chain enrichment with 1-stearoyl-2-arachidonoyl species depends to a large extent on the level of expression of these isoforms in different organs.

Interestingly, the enzyme PIS, which does not exhibit acyl-chain preference, is expressed in only one form. In contrast, there are multiple isoforms for DGK and CDS, only one of which shows acyl-chain specificity. Hence, when the availability of arachidonic acid, an essential fatty acid, is low or the expression of DGKε or CDS2 is low, phosphatidylinositol synthesis still can continue with the substitution of other isoforms of DGK or CDS.

Other organisms have phosphatidylinositol species that are different from the 1-stearoyl-2-arachidonoyl forms found in mammals. Plants have mostly linoleic acid at the sn-2 position (6). The two major phosphatidylinositol species in yeast are 34:1 and 32:1, making up 56 percent of the phosphatidylinositol (7). In the case of the Amoebozoa, Dictyostelium discoideum, the major phosphatidylinositol species, is an ether-linked lipid 1-hexadecyl-2-(11Z-octadecanoyl)-sn-glycero-3-phospho-(1'-myo-inositol) (8). It appears that the phosphatidylinositol of Dictyostelium discoideum is not formed from diacylglycerol through the phosphatidylinositol cycle but rather from phosphatidic acid (8).

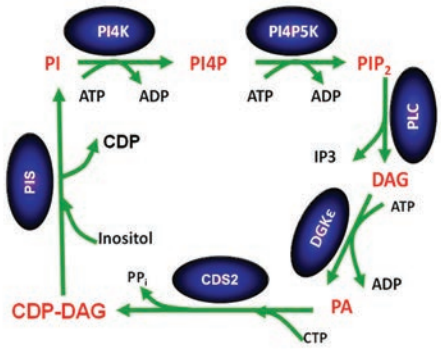

Figure: Phosphatidylinositol Cycle

All eukaryotes have only one or a few species of phosphatidylinositol with particular hydrocarbon chains. This observation suggests that the hydrocarbon chains of phosphatidylinositol and its phosphorylated species are important to their functions (8, 9). Specific acyl chains on other lipids also may be required for specific functions (10, 11, 12). We are just beginning to unravel the mechanisms by which hydrocarbon chain specificity determines physiological function.

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Upcoming ASBMB events and deadlines

JANUARY

Jan. 21: 2015 ASBMB annual meeting late-breaking abstract deadline

FEBRUARY

Feb. 2: 2015 ASBMB annual meeting discounted registration deadline

Feb. 6: Accreditation webinar

Feb. 23: 2015 ASBMB annual meeting housing deadline

MARCH

Mar. 3 – 5: ASBMB is a sponsor at the Deuel Conference

Mar. 15: Accreditation Deadline

Mar. 28 – Apr. 1: ASBMB Annual Meeting

ASBMB
American Society for Biochemistry and Molecular Biology

DEFYING STEREOTYPES:

The Hollywood experiment

Kevin Grevioux, star of the "Underworld" movie series, moved from a laboratory to an actor's trailer

By Rajendrani Mukhopadhyay and Geoffrey Hunt

Every actor has that breakthrough moment when he or she knows that acting is life's calling. For some, it's the magical lure of stardom; others are inspired after witnessing a seminal performance. For Kevin Grevioux, creator and star of the "Underworld" film series, it was an overheard conversation in a laboratory at the National Cancer Institute that changed the course of his life.

While working on his master's degree at Howard University, Grevioux was serving as a research assistant at the NCI. One day, he heard a freshly minted Ph.D. talking to someone else in the lab. "She mentioned that at 30 years old, she wasn't even making $30,000," recalls Grevioux. "I was thinking, 'Here I am, 24 or 25 years old. How long is it going to take me to finish the master's program and then get my Ph.D.? What kind of money am I realistically looking at making?'"

The depressing mental calculations sealed Grevioux's decision to abandon the lab for the bright lights of Hollywood, where he has become a major player in the world of science fiction entertainment. Yet he never fully abandoned science. "Science is my passion. I love it," he says emphatically. "All the things I do (in the film industry) are based on science."

Grevioux's interest in science dates back to a childhood fascination with the natural world, when he would spend hours pouring over encyclopedia entries about snakes, spiders and other critters and catching salamanders and locusts in the woods of Minnesota. But it was a more mythical creature that instilled his deeper fascination with science fiction: Dinosaurs, remembers Grevioux, were the "bridge at which real science and science fiction came together."

Figuring that science fiction was not a legitimate career option, Grevioux instead decided to enter a pre-med program at Howard University, from which he graduated in 1987 with a degree in microbiology. Grevioux then began working on his master's degree in genetic engineering, which led to his stint as a research assistant at the NCI. "I was the low man on the totem pole," he remembers of his time at the NCI, one whose responsibilities included "cell feeding, cell harvesting, gel electrophoresis, DNA isolation, making the solutions."

However, Grevioux always felt that he was, in his words, "atypical for science. I've fought more stereotypes when I was in science than I did when I was outside of science," he states. Colleagues would inquire why someone with his tall, broad and muscular build wasn't on the college football team. As he plodded through life as a masters student, Grevioux began

Kevin Grevioux. IMAGES COURTESY OF KEVIN GREVIOUX

taking acting and writing courses as a creative outlet.

Then came that fateful moment when he overheard his colleague's sobering conversation, which inspired Grevioux to make his move from Washington, D.C., to Los Angeles. His parents were horrified. But Grevioux reasoned that it was a now-or-never kind of moment for him to enter the entertainment industry. "School will always be there, so you might as well go for it. In a way, (the film industry) does represent the last bastion of hope you have to fulfill a dream," he says with a chuckle.

Once in Hollywood, Grevioux spent years bouncing from audition to audition, surviving on a steady diet of ramen noodles and taking up a stream of odd jobs to pay rent. Now a struggling actor rather than a struggling scientist, Grevioux still was faced with questions of whether he was in the right career. "However hard you work, you can always work harder, and someone has always worked harder than you," he points out.

Even tougher, Grevioux once again found himself being held back by factors that were completely out of his control. He remembers being rejected from auditions simply because he didn't fit the artistic vision. "You could be a guy who is 6 foot 4 inches, 220 pounds, square-jawed, have a wife and four children and [be] in very good shape. But you know what? You're told 'You look too good to be a father.'"

Grevioux finally found his footing after he landed a job doing stunt work, which he calls "the best free film school that you will ever attend." Immersed in what he calls the "crucible" of the film industry, Grevioux was able to hone his writing and acting skills, helping to set him on the path to success. "All you have to do is listen, learn and keep your mouth shut," he advises.

Such dedication eventually helped Grevioux land appearances in blockbuster movies including "The Mask" and Tim Burton's "Planet of the Apes" as well as his most recognizable role as the character Raze in the 2003 film "Underworld," a screenplay that he also wrote. He credits his deep, booming voice and physical appearance, along with his aptitude for writing, for getting the attention of directors and producers. "I think those things differentiated me from the rest of the pack," he says.

Now an established presence in the entertainment industry, Grevioux lets science pervade his work. For example, when writing the screenplay for "Underworld," Grevioux made sure that the agents responsible for turning people into vampires or werewolves were based on viruses that caused mutations rather than some mysterious fictional element. "It was comic-book science," he admits. "But it was informed by what I already knew." He also has developed comic-book characters for DC Comics and Marvel and is currently building up a company called Channel 56 Films that deals with film, television and animation projects.

Grevioux's tale of being a misfit who overcame the odds to succeed is a Hollywood cliché. While few are likely to follow in his footsteps in their quest for stardom, as Grevioux puts it, "You won't know until you try."



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DEFYING STEREOTYPES:

A quantum leap

With a Ph.D. in theoretical physics, film director Mark Levinson's résumé stands out from his Hollywood peers'

By Geoffrey Hunt and Rajendrani Mukhopadhyay

Director Mark Levinson. IMAGE COURTESY OF PF PRODUCTIONS

IMAGE COURTESY OF MK12 AND BOND/360

Film director Mark Levinson often gets asked for advice from people looking to break into the movie-making business. "I tell them, 'The good news is I didn't go to film school. The bad news is I got a Ph.D. in physics,'" he says with a laugh.

While Levinson's atypical career path is probably not the ideal template for aspiring filmmakers, it is illustrative of his perspective about the unexpected overlap between science and cinema. The "creative impulse is very similar," he points out. "I think (that similarity) underlines my whole transition."

Levinson the scientist started out in an M.D./Ph.D. program at Brown University before a professor turned him onto theoretical physics. Even though his passion for science led him to pursue a graduate physics degree at the University of California, Berkeley, Levinson admits that his interests tended to go beyond academia. "I was fascinated by art and artists and how they interacted with society and what their role was," he says.

A fan of classic movies like "2001: A Space Odyssey" and "They Shoot Horses, Don't They?," Levinson was nonetheless unaware that he could work in the film industry until he was introduced to the films of European directors Lina Wertmüller and Rainer Werner Fassbinder. Observing their distinctive styles helped Levinson become "very conscious of directors."

Becoming more and more enamored with the idea of working in the film industry, Levinson did what seemingly every Hollywood hopeful does: "I started writing a script," he says. But even then, Levinson didn't feel like his career had really made much of a change. "I went from being a graduate student in theoretical physics, where I was sitting in a room, by myself, scratching on pencil and paper, not making money," he remembers. "And then I was writing a script, and I was sitting in a room, by myself, not making much money and scratching on pencil and paper." Yet the bigger challenge for Levinson was trying to live with the feeling that he had disappointed his graduate adviser. "It was probably the hardest part," he admits.

When Levinson first entered the film industry, he scuttled through a series of jobs in various departments until he finally ended up in the editing room. While working there in 1987, he got to meet the acclaimed film editor Walter Murch, who already had won an Academy Award for his work on "Apocalypse Now." Surprisingly, Levinson's scientific training was the catalyst for his meeting with Murch.

"I was an apprentice editor in the same building where he was editing 'The Unbearable Lightness of Being,'" recalls Levinson. "He heard that there was somebody in the building with a Ph.D. in physics, so he sent his assistant down to ask me if I'd go to lunch

with him and talk to him about string theory." Levinson went on to work with Murch on "The English Patient" (which fetched Murch his second Oscar), "The Talented Mr. Ripley" and "Cold Mountain."

Despite becoming fully immersed in the glamorous world of Hollywood, Levinson always was tempted to use the opportunities afforded by the movie industry to showcase science. "I had never really seen a film that I thought accurately depicted how science really works," he says. Levinson's worlds finally collided with the 2013 release of "Particle Fever," a documentary he directed that follows researchers at the European Council for Nuclear Research in their quest to detect the Higgs boson using the Large Hadron Collider.

Rather than follow a standard documentary formula of telling a story through a single pair of eyes, the movie instead relies on six scientists to show the scientific process at work, thereby bringing a human side to research. "We needed to choose people that could bring out the various elements" of the experience, explains Levinson, pointing out that the film's protagonists range from a fresh-faced postdoctoral fellow to a senior administrator at CERN. "The focus for me always was 'How are we going to tell a good dramatic story?'" says Levinson.

"Particle Fever" has been met with overwhelmingly positive reviews and numerous accolades including the Audience Award at the 2013 Sheffield International Documentary Festival and both the Grand Jury and Brainstorm Prizes at the 2013 360° Contemporary Science Film Festival. It also enabled Levinson to resolve any lingering guilt about his decision to abandon scientific research all those years ago: One of his most gratifying moments with "Particle Fever" was the positive reception it got from his graduate school adviser. "I feel like maybe I wasn't a complete disappointment to him in the end," Levinson says. "'Particle Fever' is my gift back to the physics community."

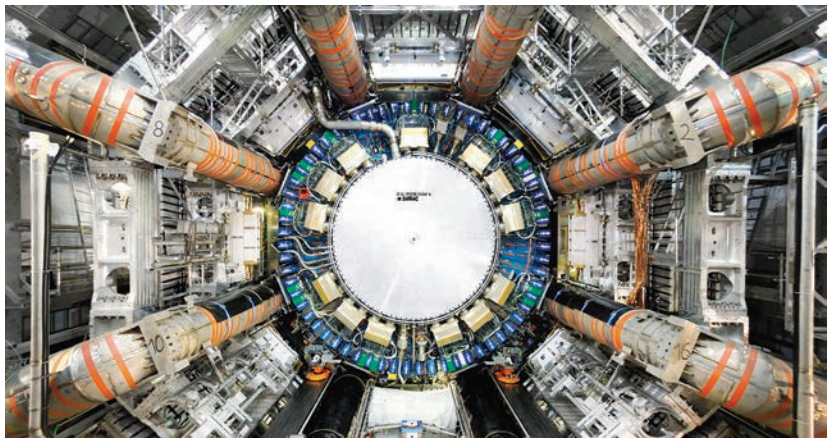


IMAGE COURTESY OF CERN

IMAGE COURTESY OF PF PRODUCTIONS

From top: Full view of the ATLAS detector at the Large Hadron Collider. Public outreach is conducted at the Globe of Science and Innovation.

The success of "Particle Fever" motivated Levinson to continue exploring the overlap between his two passions of art and science. He is working on a film adaptation of Richard Powers' novel "The Gold Bug Variations." Titled in reference to Johann Sebastian Bach's famous work called the "Goldberg Variations" and the Edgar Allan Poe short story "The Gold Bug," the novel tells of a young molecular biologist working on the genetic code in the 1950s who derails his scientific career in pursuit of love and music.

From his vantage point, Levinson sees science and cinema as two endeavors where "man is trying to find a representation of the world around him to understand it in some deeper way." Indeed, in making "Particle Fever," Levinson says, his interest in the overlap between science and art has reenergized his own belief in the importance of both realms: "These are the things that make us human," he says.

Geoffrey Hunt (ghunt@asbmb.org) is the ASBMB's public outreach coordinator. Follow him on Twitter at www.twitter.com/thegeoffhunt. Rajendrani Mukhopadhyay (rmukhopadhyay@asbmb.org) is the senior science writer and blogger for ASBMB. Follow her on Twitter at www.twitter.com/rajmukhop.

Meet Dennis Voelker

A new associate editor of the Journal of Biological Chemistry

Dennis Voelker at National Jewish Health in Denver recently joined the ranks of the associate editors at the Journal of Biological Chemistry. His laboratory focuses on phospholipid biology. The interview has been edited for length and clarity.

Would you briefly explain what your research group is studying?

My lab is focused on multiple aspects of the cell and molecular biology of phospholipids.

One problem we have investigated for many years addresses the genetics and biochemistry of intracellular phospholipid transport required for membrane biogenesis in eukaryotes. Our interest in phospholipids also has brought us to interesting problems directed at understanding the metabolism and transport of these molecules in parasites, especially apicomplexan parasites, such as Toxoplasma gondii and Plasmodium falciparum.

Another area of interest is pulmonary surfactant phospholipids. Extraordinarily high levels of phospholipids are present in a secreted lipid-and-protein complex that lines the alveolar compartment of the lung, where gas exchange occurs. We recently found that some of the minor surfactant phospholipids function as very potent regulators of innate immunity, especially the Toll-like receptors.

Tell us about your academic background and research training. Did anything occur, in a milestone sort of way, that made you choose science as a career?

My undergraduate studies were at Indiana University. I was enrolled in the multidisciplinary program in biological sciences, which, in addition to didactic teaching, placed heavy emphasis upon experimental approaches to understanding science and testing ideas. This exceptional program was developed by C.H. Werner Hirs and Anthony G. San Pietro. It was commonplace in this curriculum in both class discussions and exams to be presented with statements such as "Here is one emerging model of a biological process. Design an experiment to critically test this model."

This exposure profoundly changed my perception of science: I went from thinking of it as a passive discipline of assimilating factual material to an active process of identifying the limits of our understanding and executing experiments to develop new knowledge. This experience as an undergraduate had the greatest influence upon my choosing a career in science. All of my teachers within this program made a strong impression upon me, and I feel a lifelong debt of gratitude to them.

My graduate studies were at Oak Ridge National Laboratory. My thesis mentor was Fred Snyder, who conducted groundbreaking work in defining the mechanisms of ether-linked glycerolipid synthesis. At the time I joined the lab, Fred was developing an interest in the lipids of pulmonary surfactant and factors controlling their synthesis. This latter project caught my interest and became the focus of my thesis research. I believe I have the dubious distinction of being the only trainee in the Snyder lab not to work on ether-linked glycerolipids.

My postodoctoral studies were performed at Harvard Medical School with Eugene P. Kennedy, who is arguably the most important scientist in elucidating the biochemistry of glycerolipid synthesis. Like many who worked in this laboratory, I continually was impressed by the breadth of his knowledge of biochemistry and his ability to formulate incisive questions. He was an enthusiastic proponent of the gedankenexperiment as a means to get students and postdocs to think actively about their lines of experimentation. He also challenged trainees to think about where the broader boundaries of a field lie and to have the intellectual courage to try to address them. I always have considered it a privilege to have known

Gene personally and to have benefited from his exemplary scholarship and intellectual prowess.

When did you join the ranks of the JBC associate editors? What does it mean to you, on a personal level, to be an associate editor for JBC? What was your reaction when you were asked to be an associate editor?

I was invited to be an associate editor in November 2013 and assumed the duties in March. I must admit that my first reaction to the invitation to be an associate editor was one of trepidation, especially since I had concerns about the workload and my other commitments. Prior to assuming this position, I had served on the editorial board for three five-year cycles. I certainly consider it an honor and a privilege but also a serious responsibility to serve the JBC as an associate editor. For me, personally, the position is an important affirmation of the trust my colleagues have in me to apply high scientific standards, balanced judgment and fairness to the review process.

Do you have any advice for balancing life inside and outside of the lab?

I would like to tell you that, with all my years of experience, I have developed a clear way to balance the two. But I have not. I still spend long days at the lab, and grant-writing periods demand even more time. I think, for most in this profession, it is best to accept that life will be hectic and that adjustments to work and family demands just need to be made continually, sometimes on a daily basis. My wife is also a scientist, and we continue to wrestle with these issues. Many years ago, both of us decided on one element of our lives that would be invariant, and that is family vacations in the summer and winter. Our family never has regretted that decision, and it is one piece of advice I always offer to my junior colleagues.

What do you do outside of the lab? Hobbies?

My wife and I live in Colorado, and we spend much of our free time hiking and backcountry skiing. We are also both avid birdwatchers. I enjoy cycling and have done the 500-mile Ride the Rockies Tour twice in recent years.

For scientists in training, do you have any words of wisdom or a favorite motto?

I like to remind students and postdoctoral fellows that good science is always a battle between successes and failures. One would do well to experience and learn from both and persist in the effort. One of my favorite quotes about the broad perspective of scientists and science was written by Eugene P. Kennedy and appeared at the end of his prefatory article in the Annual Review of Biochemistry in 1992: "The anonymity that is the fate of nearly every scientist as the work of one generation blends almost without a trace into that of the next is a small price to pay for its unending progress, the great long march of human reason … To feel that one has contributed to this splendid enterprise, on however small a scale, is reward enough for labor at the end of the day."

Commit

By Chris Pickett

Finding a job away from the bench can be a frightening prospect. Are you sure you want to leave the bench? Does your training even give you the necessary skills to make this jump? What if you get a job and don't like it? What are your options then? Will you be able to go back to academia?

When I left academic science, I had all of these fears and more. I had worked at the bench for 15 years before looking for a nonacademic position. And when I started my search, I had a new family and significant student-loan and credit-card debt. Making the wrong career move could have been disastrous for all of us. On top of that, my postdoc appointment had an expiration date, making unemployment a real possibility. Not only did I have to find a new career path that provided stability, but I also had to find it before my postdoc clock ran out.

However, these pressures did not drive me to act at first. They drove me straight into a brick wall. "What are you passionate about, Chris?" was the question that stymied my early job search and caused my fears to build up into an unfocused anxiety. If I couldn't answer that question, how could I make the right career choice for me and my family?

The only way I knew how to answer these questions was to jump into everything that caught my interest. I considered a career in teaching, so I sought out local biology departments that needed an adjunct professor for a semester. I gave science writing a shot, but I found that I couldn't muster the motivation to be as productive as I needed to be successful. Finally, I found my passion in science policy, and when I did, I committed to it wholeheartedly.

You're almost certainly competing with a bunch of other newly minted Ph.D.s for the position. How will you distinguish yourself?

The time I spent working on policy issues caused friction with my postdoc adviser, and I couldn't spend as much time with my family as I would have liked. But I recognized that this was my ticket away from bench work, and I wasn't going to let it slip away.

It has been two years since I left the bench, and the biggest lesson I learned during my job search was that you have to commit. Commit to finding out what you are passionate about, and do it by getting your hands dirty. You can read about jobs and careers all day long, but you never will find out if a path is the right one for you unless you give it a try. Once you commit to a path, your confidence in your professional future will grow, and your fears about the transition will diminish.

Commit to get the job

Unless you're applying for a postdoc or staff-scientist position, your next job will require a significantly different skill set than the one you use at the bench. Thus, you will need to pick up the skills required for this new position, and you'll need to do so quickly. But your prospective employers will never question your ability to learn and apply new skills. That's the benefit of that "Ph.D." after your name.

What your potential employers will question, however, is your motivation. Given your intense involvement at the bench over the past several years, are you committed to this new career path? Is this a fleeting interest that you will ditch after three months? Or are you going to stick with it? Do you truly understand what this new job entails? To convince an organization to devote significant resources to training you, you must demonstrate your commitment.

What set me apart was my commitment to the career path. I made the time to make my voice heard consistently on policy issues. I visited with my elected representatives to advocate for science research, I wrote about policy changes at the National Institutes of Health and I talked about science policy to anyone willing to listen, all while trying to remain productive in lab.

And don't forget: You're almost certainly competing with a bunch of other newly minted Ph.D.s for the position. How will you distinguish yourself?

You have to carve out time to get involved. Whatever you are interested in, do something – anything – to gain experience and show that you are excited and committed to your new career path. This commitment will not only help you determine if this new field is right for you but also will make you stand out from your competitors.

Commit for you

I don't know you, and I don't know what your motivation was to get into science or to leave academia. But my guess is that, like me, you've put in long days, weeks and years in pursuit of your degree. You've given many presentations, read countless papers and jumped through many hoops. And, like I did, you probably also have a fair amount of fear at the prospect of leaving the bench.

We probably have one more thing in common – no matter what satisfaction you derive from your accomplishments at the bench, it probably isn't enough to overcome the negative feelings you have about that work. And this is the critical issue that you must consider. What are the personal and professional rewards you need from a job? What job gives you the best chance to excel?

Yes, bench work can be very demanding of your time. And when you're not at the bench, you're in meetings, writing papers or making time for your friends, family and yourself. But investing time and energy into your job search is essential. In my search, I slowed the pace of my experiments and spent less time than I would have liked with my family in order to write, research and participate in as many policy events as I could until I landed a position. Those sacrifices have paid off in a job that I thoroughly enjoy. And I now have more time to spend with my family than I would if I were at the bench.

So why wouldn't you make time to find what you're passionate about? Why wouldn't you make sure you are making the best possible career decision?

Do it for the job, and do it for you: Commit!

Chris Pickett (cpickett@asbmb.org) is a policy analyst at ASBMB.

Sharpen your science-communication skills at the annual meeting

Official meeting bloggers

We are accepting applications for official ASBMB annual meeting bloggers. Participants will receive complimentary press registration, entry to the press room and access to all scientific sessions of the six sponsoring societies. Bloggers with existing platforms may use them; those without will blog on The Interactome, ASBMB's meetings blog. The application deadline is Feb. 15. Contact Angela Hopp at ahopp@asbmb.org.

Official sessions tweeters

We are accepting applications for official ASBMB annual meeting tweeters. Participants will receive a special collection of ASBMB swag ¾ and plenty of retweets! The application deadline is March 15. If you would like to live tweet ASBMB sessions and events, please contact Angela Hopp at ahopp@asbmb.org.

Promote your science

We encourage you to work with your institution's media relations office on a press release about the research you'll be sharing at the meeting. If you're not sure how to get the ball rolling, visit the ASBMB Today archive at http://bit.ly/zQghRK for a handy guide. There is so much incredible science at the annual meeting that it's impossible for reporters to cover the event adequately without the help of press releases. So, do yourself, your institution and ASBMB a favor, and help get the word out about your great work. If you send a copy of your press release to media@faseb.org by March 1, our media relations team will include it in the ASBMB materials that reporters who visit the on-site press room receive.

ASBMB hashtags

#cancer	Cancer: The War at 44, Warburg at 90
#DNA	DNA Replication and Repair
#ECM	Extracellular Matrices in Health and Disease
#lipids	Lipids ¾ In Vivo Dynamics, Protein Partners and Signaling
#PTMs	Mechanistic Impacts of Post-translation Modifications
#microbiome	Microbiome Dynamics and Health Disparities
#microbiome	The Human Microbiome
#immunology	Molecular Mechanisms of Infection and Immunity
#enzyme	New Directions in Enzymology
#plants	Plant Metabolism
#protein	Protein Nonfolding as a Regulatory Phenomenon
#RNA	RNA Expression and Post-transcriptional Regulatory Events
#protein	What's New in Membrane Transport Proteins
#training	Training the Mind of an Interdisciplinary Scientist
#scicomm	Public Policy and Science Outreach

Best analogies for stem cells for public outreach

By Paul Knoepfler

I'm often asked, "How can I help people get stem cells with an analogy?" What are the best stem cell analogies? Here are the ones I've thought up that resonated most powerfully in my public outreach, including with kids.

Stem cells as blank tiles in Scrabble

IMAGE COURTESY OF ©ISTOCK.COM/RENA-MARIE

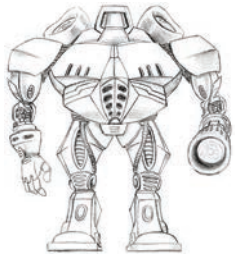
They can literally be what you want them to be if you use them properly.

They are extremely powerful.

They can make things happen.

They can change things up.

Stem cells as Transformers



They are cool.

They can transform into all different kinds of things.

They are exciting.

They are mighty.

They can do positive or negative.

Stem cells as a tree or tree trunk

They can grow.

They can branch out.

They can bear fruit.

They are a symbol of hope.

In Portuguese, "stem cells" is "células tronco," which literally translated means "trunk cells."

Paul Knoepfler (knoepfler@ucdavis.edu) is an associate professor at the University of California, Davis, School of Medicine. He received his bachelor's degree from Reed College and his Ph.D. in molecular pathology from the University of California, San Diego. He did his postdoctoral work at the Fred Hutchinson Cancer Research Center in Seattle as a Jane Coffin Childs fellow. He received the Howard Temin Award from the National Cancer Institute shortly before starting his own lab at UC Davis. The Knoepfler lab research focuses on stem and cancer cell epigenomics with a particular interest in Myc and histone variants. This article first appeared on Knoepfler's blog (http://www.ipscell.com/).

YOU'RE THE EXPERT

You're the Expert is a live show, podcast and new public radio program on WBUR that uses comedy to make academic research more accessible and exciting. This live show will be recorded in Worcester and will feature Nobel Prize winner Craig Mello alongside comedians Myq Kaplan (from Comedy Central and Netflix's "Small, Dork, and Handsome"), Jo Firestone (from Comedy Central's "Broad City") and Anna Drezen (author of "How May We Hate You?" and MTVu). Space is limited! **Get your tickets at the EcoTarium.**

You're the Expert is supported by the American Society for Biochemistry and Molecular Biology's Public Outreach Committee Seed Grant Program.

Confessions, in verse

By Aditi Dubey

A hobby,
you say?
Why, yes!
Coffee.

Coffee? Coffee. Coffee!

A juxtaposition of letters so beautiful, so perfect.
Like a Greek key.
It had to be Coffee, obviously.
What else was I supposed to fall in love with?

In times such as these,
when we are all trying to be superhuman,
perfect and accomplished,
or to maybe just graduate,
Coffee is a lifeline.

It is the blood that runs in our veins.
A propagandist tool, a cultural icon
and a grad student's best friend.
Coffee is always there when no one else is –
when all the lights, including yours, have gone out.

It is a cruel mistress,
an impudent servant who knows it is much needed.
A disease and a cure,
a color and a culture, the identity and pride of entire nations.
Savior of the Earth.

For all of Tea's royal patronage,
Coffee's disarming charm is what has all us masses rooting for it.
The reticent, subtle activist, making you oh-so-benevolent as you
drink that fair trade brew
from your local coffee shop,
as your struggle with that damned writer's block.

Coffee is the illusionist emperor of a kingdom of overachievers,
a pope of a religion of caffeine addiction,
and I am here to spread its gospel.
I believe in Coffee.

It is my addiction and my lifestyle,
my midnight-oil burner, my stress reliever
and my social tool – coffee really is why I still have friends.
Coffee is to me as a muse is to an artist,
as a cofactor is to an enzyme –
a part of my identity.
It completes me.

If I sell my life away for something, it will be Coffee.
If I invest in anything before the world economy crashes, it will be Coffee.
If I ever write this thesis, it will be dedicated to Coffee.

So, my fellow scientists,
if you ever find me lost,
with failed experiments and ill-fitting data,
wandering and helpless on the streets,
crying for the wisdom of those long gone,
just hand me a delightful cappuccino, with a helix hidden in the swirl.

Aditi Dubey (dubeyad@scarletmail.rutgers.edu) is a graduate student studying the mechanism of selenocysteine incorporation at Rutgers University Robert Wood Johnson Medical School.

Generating the NIH 3T3 cell line, the oncogene hypothesis and horses

By Rajendrani Mukhopadhyay

The NIH 3T3 cell line is one of the mainstays of cell biology, gracing more than 26,000 publications in PubMed. But the only reason the embryonic mouse cell line ever got going, muses George Todaro, was that he was a young student who was willing to head into the laboratory every three days to transfer the cells whether they needed the transfer or not. "One would have to be little crazy – or a graduate student," says Todaro with a laugh.

The cell line began in the early 1960s as part of a study to understand what properties governed the growth of cells in Petri dishes. Howard Green at New York University had just returned from a yearlong sabbatical at the Pasteur Institute in Paris, where he had learned about cell culture. Green decided to undertake a systematic study of culturing cells to understand better their growth properties. He took on Todaro, who was taking a year off from medical school to work in a research lab, to work with him on the project.

The problem with the existing cell lines at that time was that they "had very dubious history or no history," recalls Todaro. For a fresh start, Todaro and Green isolated fibroblast cells from a mouse embryo of the Swiss-Webster strain. The two researchers separated the cells into a series of Petri dishes and began to play around with conditions to see how they influenced cell growth.

TODARO

To name the series of plated cells, they went with "3T3," "3T6," "3T12" and so on. The "3" denoted the number of days the cells were allowed to grow on the plates. The "T" stood for "transfer." The number after "T" referred to the hundreds of thousands of cells transferred to a new plate for each passage, as in 300,000 cells, 600,000 cells and 1.2 million cells.

The researchers noted that the cells in 3T6 and 3T12 plates grew over each other and got crowded to the point that they started to resemble tumors. But the 3T3 cells never reached confluence. "They were strikingly different in that they were a single monolayer, a nice clean sheet of cells," says Todaro.

Out of all the permutations of cell density and culture conditions that Todaro and Green tried, "3T3 came through as an established cell line," says Todaro. "I think the secret was we transferred them every three days whether they needed it or not. As a consequence, they never got confluent." He says that while most people wait for their cells to reach confluency to transfer them into fresh plates, he was willing to head into the lab every three days, whether it was a weekday, weekend or holiday, and do the transfer.

After medical school, Todaro landed a position as a principal investigator at the National Cancer Institute in Bethesda, Maryland. With his students, Todaro says, "I basically made them repeat the 3T3 strategy with NIH Swiss cells," which were cells taken from a different mouse strain.

The new cell line from the NIH Swiss mouse embryo had the same property of being able to grow in flat monolayers, and "it had the further advantage of being highly transfectable by DNA, which the original 3T3 didn't have," says Todaro. He adds there was a 3T3 cell line from a BALB/c mouse embryo but "the NIH 3T3 became the one of choice, because you could do a lot more genetic manipulations with it."

Along with establishing an important cell line, Todaro made other

CONTINUED ON PAGE 30

The making of a man

By Andrew D. Hollenbach

I've worked hard all of my life. In high school, I worked in a library to earn money for college while studying hard to earn the grades that would get me into college. In college, I studied even harder, sometimes going weeks on end with only a few hours of sleep each night. It goes without saying that I worked hard in graduate school to earn good grades, while fighting a nearly debilitating lack of self-confidence, to get my Ph.D. I still work hard as I fight the daily frustrations of academic science, attempting to get funding, pushing to get my work published and still doing all that is expected of me as a professor.

Throughout my life, I have learned to rely on myself to achieve my goals. However, no matter how far I rise through the ranks of academic science and no matter where my path takes me in my job and in life, I never forget where I came from and who molded me and passed on to me the ideals and principles that shape my life.

I grew up in a very small town in Pennsylvania. Whenever I'm asked where I grew up I always say "just north of Philadelphia." If pushed, then I'll tell them … Perkasie. Even people familiar with the Upper Bucks County area of Pennsylvania have not heard of it. I reply: "Sellersville? Quakertown? Half way in between."

Growing up in a small town gave me strong roots, roots that have stretched all the way to New Orleans and continue to feed me and strengthen me to this day.

I can take you back to my hometown and show you the house where I was born and where my parents still live after nearly 50 years. Then I can walk you up the street two blocks and show you where my grandfather and his 11 brothers and sisters were born; two houses up from there is where my great-grandfather was born, and four blocks later, where my father was born. On the way back, we can stop by Cemetery Hill, and I can show you where many of them rest. Then I can take you out to the farm country 15 minutes out of town and show you the church that my grandfather built, the church where my parents were married and where my sister and I were baptized and grew up, a church that used to be surrounded by fields (still is, mostly) and where on a warm spring day, with the windows wide open, the breeze would bring in that "fresh country air," as we so fondly called the aroma wafting off of the fields freshly spread with manure.

Growing up in a small town is not the only thing that helped to form my life-long ideals and principles. My family, in many, respects could be said to have lived the American Dream. One grandfather drove a truck; his wife worked in the school cafeteria. My other grandfather was a minister and his wife a minister's wife. They worked hard, they saved their money, and they instilled strong family values and a solid work ethic in my parents and their brothers and sisters. Thanks to my grandparents' hard work and support, my father and mother were able to go to college, earn their degrees as schoolteachers and provide a solid, stable home for my sister and me.

More importantly, my parents were always there for us: They pushed us when we needed to be pushed; they supported us when we needed to be supported, and they provided for us on a daily basis. They never imposed their wishes for our lives on us but instead allowed us to make our own decisions, and then encouraged us to work hard to achieve our goals. Doing less than our best was never an option; however, they knew that we worked hard, and when we didn't quite live up to our own standards they knew that we had done the best we could possibly do. Because my grandparents (several of whom never finished high school) worked hard and instilled strong values in their children, my parents, who then passed these values onto my sister and myself, we were able to go on to become a professor at a university and a successful certified public accountant. Like I said, my family can be said to have fulfilled the American Dream.

No matter how far I travel from home, I never will forget where it is that I come from. I take great pride in my Pennsylvania Dutch roots, the small area of Southeast Pennsylvania that was home to the Pennsylvania Germans ("Pennsylvania Deutsch" in German, a language my grandparents were raised speaking) and the strong family values and solid work ethic imbued by the German mentality. It always brings a smile to my face when I go home and see good German names like Lichtfuss, Fenstermacher, Kramer or Binsberger.

The town has grown since I left in 1989 to attend graduate school, but it still is, at heart, the town where I grew up. I was raised by a loving

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family in this town (including my aunts and uncles, great aunts and great uncles, and cousins) and I hold dear everything that they were and are as people. This family and that small Pennsylvania Dutch town are an integral part of my life that I carry with me no matter where I go, no matter what I do or who I become, and, because they were integral in shaping me as a person, I can never, nor would I want to ever, forget my roots and what made me the man I am today.

Andrew D. Hollenbach (aholle@lsuhsc.edu), author of the book "A Practical Guide to Writing a Ruth L. Kirschstein NRSA Grant," is an associate professor in the genetics department at Louisiana State University Health Sciences Center in New Orleans.

GENERATIONS CONTINUED

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significant contributions to science. With Robert Huebner, he articulated the oncogene hypothesis in 1969. The hypothesis postulated that the human genome contained the information (oncogenes) that could be usurped by certain viruses to turn normal cells into tumorigenic ones.

For his work with oncogenes, Todarao was selected in 1970 as one of the "Ten Most Promising Men" (Mario Capecchi, who won the Nobel Prize in physiology or medicine in 2007, and Elvis Presley were among the others). This annual award was given by the U.S. Junior Chamber of Commerce to recognize 10 notable young Americans. (The program, better known as the "Jaycees," later became the "Ten Outstanding Young Americans" to include women).

It's about this point in his career that Todaro expresses regret in hindsight. "This whole thing about the oncogene hypothesis – it would have been quite easy for us to have done the experiment that really proved it. We never did that." J. Michael Bishop and Harold Varmus did do the experiment and won the Nobel Prize for it. "We should have done it," Todaro says. "I thought it was obvious that it was going to work. I do really regret that. We had all the tools."

In the 1980s, Todaro left the NCI for a job at a biotechnology firm called Oncogene and has remained in industry since then. In the 1990s, he and colleagues developed an aerosolized version of an antibiotic called tobramycin. So far, it is the only approved inhaled antibiotic in the United States for treating lung infections – it changed the way cystic fibrosis patients were treated. "When I look back on what I've done, I probably had more impact on cystic fibrosis treatment than anything else," notes Todaro. These days, Todaro works at a start-up biotechnology company called Targeted Growth to develop transgenic crops with better yields than current ones.

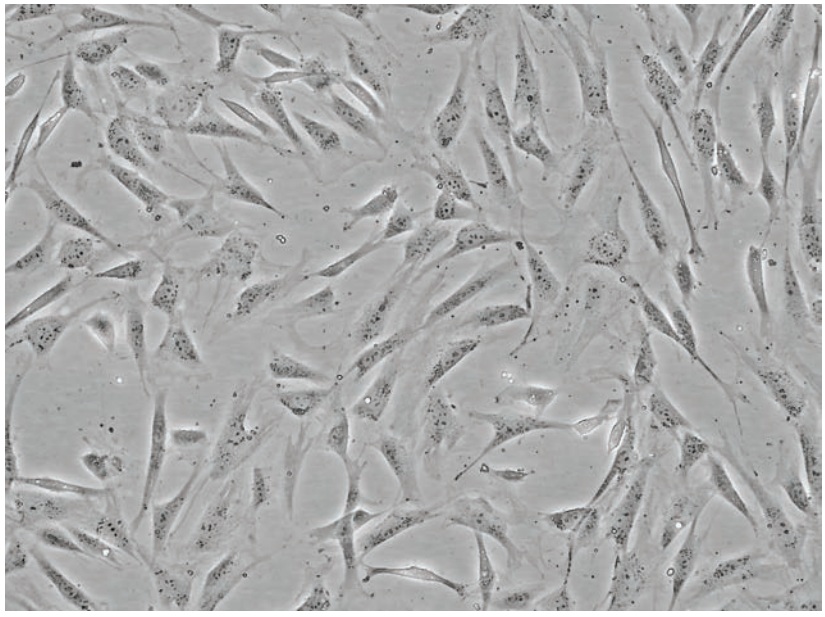

IMAGE COURTESY OF ATCC

George Todaro's group at the National Cancer Institute established the NIH 3T3 cell line in 1969.

Besides science, Todaro's other passion is racehorses. He caught the bug while living in Maryland and now is involved in breeding and horse racing. He says he sees similarities between his two passions. "You have to make a decision based on incomplete evidence," he says. "But the difference with horse racing is you get a result in a minute and 10 seconds. With science, you go for years and years before you know if you're following a useful path or if it's another one of those dead ends."

Indeed, Todaro intertwines his twin passions closely: He named one of his mares Cell Line Forever.

Rajendrani Mukhopadhyay (rmukhopadhyay@asbmb.org) is the senior science writer and blogger for ASBMB. Follow her on Twitter at www.twitter.com/rajmukhop.

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Editor's note: We know we're using an imperfect metric (pageviews), as articles published later in the year haven't had as much time to accrue hits, but we think it's an interesting glimpse at what readers are seeking nonetheless.

Correction

The necklace pictured in the profile of Raven Hanna in the December issue was incorrectly identified. The necklace depicts a part of the chlorophyll molecule.

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