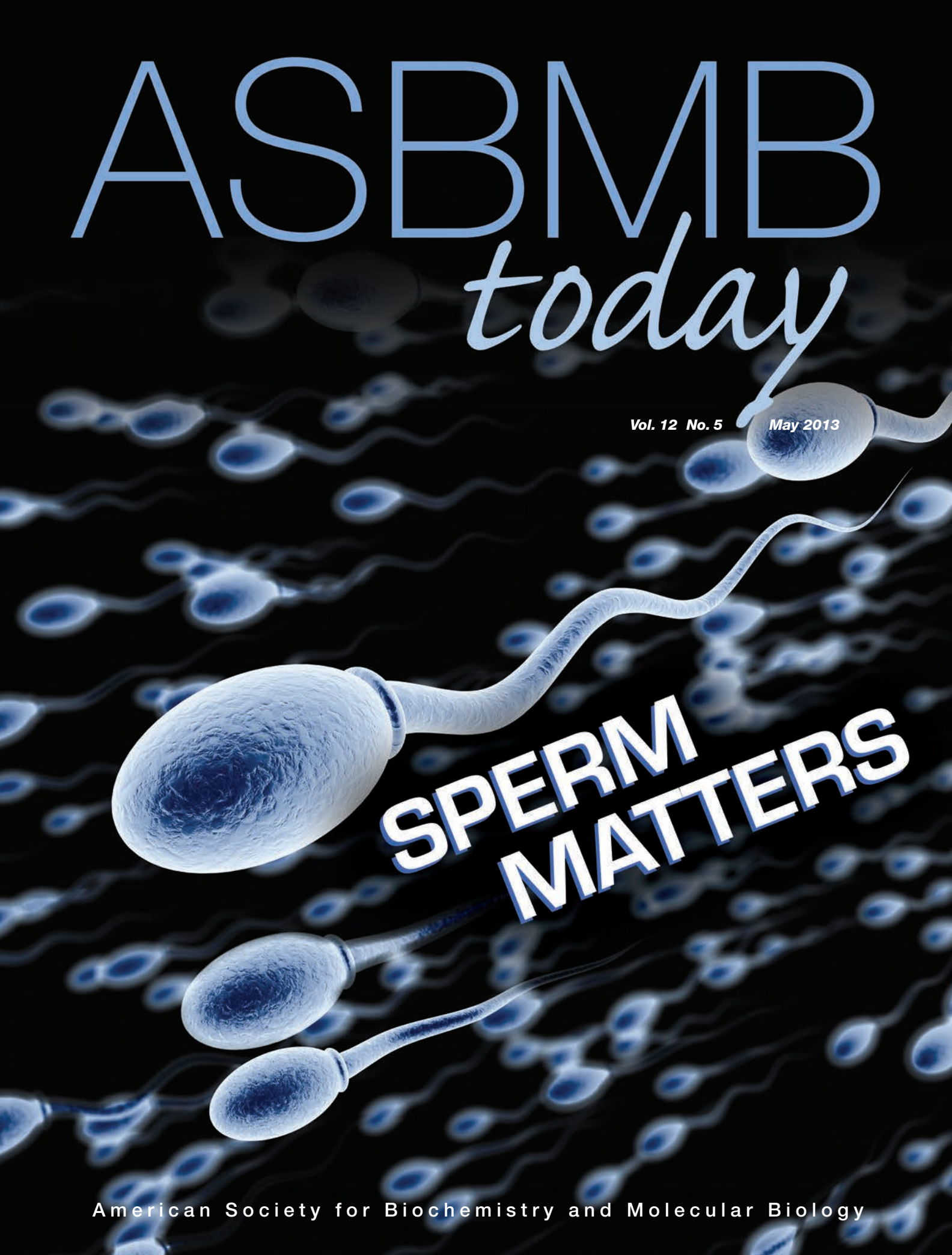
ASBMB today

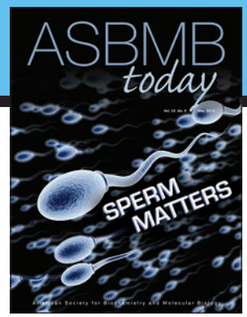
Vol. 12 No. 5 May 2013



SPERM MATTERS

American Society for Biochemistry and Molecular Biology

contents



MAY 2013

On the cover:
In this issue, we explore what we know — and what we need to know — about sperm and how it relates to male infertility. 12

news

3 **President's Message**
Schrödinger's patient

5 **News from the Hill**
Why Hill Day alone won't work

6 **Member Update**

7 **ASBMB career symposium**

8 **Retrospective**
Wm Wallace Cleland (1930 – 2013)

essays

10 **'I'd have to build the rails first'**

11 **With a lot of help from my friends**

features

12 **Sperm matters**

18 **Let's get digital**

departments

22 **Journal News**
22 JBC: Bone and skin disorders caused by disruptions in GAG synthesis
22 JBC: Story of a donut and a death machine
23 JBC: Deconstructing collagen
23 JLR: Thematic series on microRNAs
24 MCP: Spotlight on glycoscience

25 **Education and Training**
How to help students learn – and thrill your department chair

26 **Minority affairs**
In need of a new narrative

28 **Lipid News**
Letter from the new Lipid Research Division director

30 **Outreach**
ScienceOnline: Science in the Internet 2.0 era

32 **Career Insights**
Meet Rajendrani Mukhopadhyay, science writer for ASBMB Today

36 **Open Channels**

Derailed but Undeterred

The fourth and fifth pieces in our essay series are by Christine Guthrie and Jonathan Gitlin. 10-11

Retrospective
Colleagues remember Wm Wallace Cleland (1930 – 2013). 8

Vytas A. Bankaitis, the new director of the Lipid Research Division, introduces himself. 28

ASBMB today

A monthly publication of
The American Society for
Biochemistry and Molecular Biology

ASBMB Today

Angela Hopp *Editor*
ahopp@asbmb.org

Rajendrani Mukhopadhyay
Sr. Science Writer/Editor
rmukhopadhyay@asbmb.org

Marnay Harris *Designer*
mharris@asbmb.org

Andrew Harmon *Science and Technology Publishing Manager*, aharmon@asbmb.org

Nancy J. Rodnan *Director of Publications*
nrodnan@asbmb.org

Barbara Gordon *Executive Director*
bgordon@asbmb.org

For information on advertising,
contact Fox Associates Inc. at 800-440-0231
or adinfo.bmb@foxrep.com.

ASBMB

www.asbmb.org/asbmbtoday

online*exclusives*

www.asbmb.org/asbmbtoday

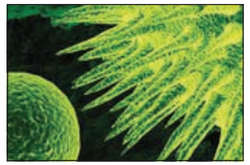
It's a wrap!
ANNUAL MEETING COVERAGE

The ASBMB crew was blogging, tweeting and Facebooking furiously during the annual meeting in Boston last month. On the ASBMB Today website, you'll find the following:

Hashtags galore

ASBMB's science policy fellow, Chris Pickett, collected tweets and photos from the scientific sessions and the special policy session.

And the winner is?



ASBMB's public outreach coordinator, Geoff Hunt, offers a report on the results of the "What is a Germ?" Challenge.

A little (fake) ink

ASBMB's marketing director, Jessica Homa, captured on camera the buzz on the exhibit floor, where staffers applied temporary tattoos for attendees.

EB2013 Blogging

ASBMB had three official meeting bloggers in Boston. Two of them, Mark Stewart and Shannadora Hollis, blogged on the ASBMB meeting blog, The Interactome, and Biochem Belle blogged at her personal blog. Check them out at www.theinteractome.wordpress.com and www.biochembelle.wordpress.com.

president's*message*

Schrödinger's patient

BY JEREMY BERG

One of the most profound revolutions in the history of science involved the discovery of the principles of quantum mechanics and the subsequent philosophical struggles to provide an interpretation of the strange probabilistic world that these discoveries revealed. The fundamental characteristic of a particle in quantum mechanics is described by its wave function. Rather than describing the position of a particle, the wave function corresponds to the probability that the particle is at any given position. According to the so-called Copenhagen interpretation of quantum mechanics (named in reference to Niels Bohr and his colleagues in Denmark), only when the position of the particle is observed does the wave function collapse to a more precisely defined location.

The peculiarity of this worldview was exemplified by a thought experiment proposed by Erwin Schrödinger in 1935. He described an experiment in which a cat is enclosed in an opaque box with a device that would release poison gas in response to a random event, such as the radioactive decay of a sample inside the box. When enough time has passed that there is a 50 percent chance that the radioactive decay has occurred, is the cat alive or dead? According to the Copenhagen interpretation, the cat exists as a superposition of a living cat and a dead cat until the box is opened and the state of the animal is observed. This interpretation led to criticism by a number of physicists, most notably Albert Einstein. However, the results of many experiments performed since Schrödinger's proposal have confirmed the predictions of this formulation of quantum mechanics. Our world seems to be much less deterministic and more probabilistic than we intuitively imagine.

There is a potential revolution underway in medicine today. This often is referred to as personalized or precision medicine. In the spirit of full disclosure, I am now the director of the Institute of Personalized Medicine at the University of Pittsburgh. Personalized medicine is driven in large part by the sequencing of the human genome. With reference genome sequences available, new technologies have driven tremendous advances in the sequencing of individual people's genomes. It is important to note that these next-generation sequencing methods depend on understanding the biochemistry of DNA replication. For example, pyrosequencing relies on the release of pyrophosphate associated with nucleotide incorporation into a DNA double helix as it is being synthesized. This pyrophosphate release is measured by coupling it to readily measurable phenomena such as luminescence.

With these technologies for DNA sequencing available, researchers have explored the genomic bases for many common and rare diseases. Some diseases, such as sickle cell disease, are caused by single DNA variations that are highly penetrant. For example, if an individual carries two copies of the variant hemoglobin β chain gene associated with sickle cell disease, that person almost certainly will display clinical symptoms under appropriate circumstances. However, for many other diseases, genomic variations are not highly penetrant, but instead only increase the risk of developing the disease by a relatively modest amount. Furthermore, variations in many genes can contribute to the predisposition to a given disease.

For a number of diseases, researchers are developing models that allow the calculation of an individual's risk for a particular disease based on these genomic variations in combination with other clinical and environmental factors. The parameters for these models generally are based on the results of studies on populations of hundreds to thousands of people. Based on the models, the risks to the individual for a range of diseases and conditions can be estimated. However, at a given time, each individual either will or will not be afflicted by a particular disease. This is analogous to Schrödinger's cat, where the genomic and other factors are used to estimate the probabilities of an individual having a disease, but the individual either does or does not have any particular disease at a given time. Only measurements, either through the development of symptoms or through appropriate tests, result in the collapsing of the probabilistic world into a relatively definite diagnosis.

In addition to the contrast between the continuous probabilistic world characteristic of the results of population studies (that is, for example, each individual

may have a 3 percent chance of having a particular disease) and the quantized world experienced by individual patients (either you do or you don't), the revolution in personalized medicine presently is limited by tremendous gaps in our knowledge of the relationships between genotype and phenotype and between genotype and environmental factors. This is true at the diagnostic level and is even truer at the level of treatment.

This can be illustrated by comparison to another type of mechanics, namely auto mechanics. Suppose that your car won't start when you go out to set off for work in the morning. Let's call this condition car-won't-start-itis. You call a mechanic, who comes out to try to start your car. If the only thing that the mechanic knows about is the role of the battery to start the car, he would perhaps check your battery or try to jump-start your car by hooking it up to his battery. If your car starts, your car-won't-start-itis is cured, at least temporarily (although a more permanent solution may require a battery transplant). However, if this approach is not successful, he may tow your car to a repair shop for further diagnosis and treatment. This could involve testing the electrical system, the fuel pump and a range of other components of your car that could lead to the source of the car-won't-start-itis.

This process, of course, depends on the experts' knowledge of all of the automobile systems. Unfortunately, our knowledge of human biology and all the mechanisms by which our systems can malfunction is much more rudimentary at present. Genomic studies can reveal some of the genes whose variants can increase the likelihood of a particular disease, but the products of these genes are almost always components of one or more systems and networks. While considerable progress has been made elucidating these systems and networks, usually through undirected fundamental studies of a range of model organisms as well as studies in humans, much more remains to be clarified and discovered. This incomplete knowledge presents great opportunities for biochemistry and molecular biology as well as younger fields such as computational and systems biology.

In 1929, Paul Dirac, one of the primary developers of quantum mechanics, noted that "the fundamental laws necessary for the mathematical treatment of a large part of physics and all of chemistry are known … and the difficulty lies only in the fact that the application of these laws leads to equations that are too complex to be solved." Of course, many scientists have worked diligently in physics and chemistry over the past 80 years developing methods to solve these equations approximately and to generate empirical data to be analyzed to reveal principles that are not generated directly from theory alone.

I expect that personalized medicine will follow an analogous path, with some insights available directly from analysis of the genome sequence but with most substantial progress dependent on the challenging integration of empirical and more mechanistic and fundamental information. In addition to the tremendous opportunities for biochemistry and molecular biology and ancillary fields noted above, we, both individually and as a society at large, will have to come to terms with the world of probabilities that is emerging before us. We all will have to become much more conversant with distinctions between relative risk and absolute risk. A certain genetic variant may be associated with an increase of 50 percent in your relative risk for developing a particular disease. This can sound quite worrisome. But if the average absolute risk of developing the disease is only 1 percent, then your absolute risk increases to only 1.5 percent. Such factors represent components of our own personal wave functions, and we will all have learn to interpret these wave functions and to deal effectively with the results when these wave functions collapse into measurable reality.

Jeremy Berg (jberg@pitt.edu) is the associate senior vice-chancellor for science strategy and planning in the health sciences and a professor in the computational and systems biology department at the University of Pittsburgh.

Why Hill Day alone won't work

BY BENJAMIN CORB

Last month, the American Society for Biochemistry and Molecular Biology's Public Affairs Advisory Committee made its biannual pilgrimage to Capitol Hill with students from across the country calling on Congress to support basic biomedical research and research funding at the National Institutes of Health and National Science Foundation. More than three dozen members took to the Capitol, armed with folders full of compelling arguments, facts on local investments in biomedical research and anecdotes underscoring the importance of such federal investment. In fact, the ASBMB wasn't the only group on Capitol Hill that day making that very argument. Our partner the Federation of American Societies for Experimental Biology was on the Hill as well. The next day, the Society for Neuroscience had its Hill day, followed a few weeks later by the Coalition for Life Sciences. Hundreds of scientists were visiting Washington, calling in chorus for federal support for biomedical research.

But Hill days are not enough.

The fact is that advocacy has gotten very difficult in this city. In this day of growing national debt and mandatory spending cuts, even an issue that traditionally has benefited from bipartisan support is getting drowned in a sea of partisanship. During our Hill Day last month, we heard a relatively common theme from officials and staffers, Democrats and Republicans alike: Congress has universal respect and support for the excellent work being done at America's research institutions to help improve Americans' quality of life; but Congress also has a universal unwillingness to do much to increase funding. For Republicans, it seems to be an issue of federal spending; for Democrats, it seems to be an issue of congressional dysfunction.

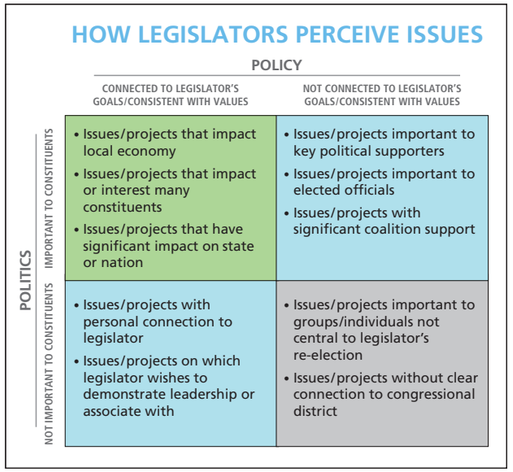
In the figure, I've shown generally how an elected official makes a decision on supporting or opposing legislation or spending. The top left box is the target zone, where issues that are important to constituents match perfectly with the representative's legislative goals or values. At the bottom right is where advocacy efforts are rejected easily, because this box represents issues of little importance to policymakers and their constituents. The remaining two boxes are the middle area, and sadly, this is where we find biomedical research funding.

Poll after poll shows Americans' broad support for biomedical research, with more than half of Americans saying they're willing to pay more in taxes to support biomedical research funding. What we are failing at is moving our advocacy from the box in the upper right — supported by constituents but not connected to a lawmaker's goals — to the sweet spot of the upper left.

Visiting Washington is simply not enough. We need more ASBMB members and more colleagues, friends and family members to get involved in our efforts locally. We need those overcoming illnesses to call their representatives and tell them how medical research saved their lives. We need supporters to attend town-hall meetings, write letters, make phone calls and contact the press. We need the message delivered twice a year in Washington during ASBMB Hill Days to be echoed throughout the year at the local level. It is only then that we will see the full potential of our advocacy efforts.

Don't know how to get involved? Visit ASBMB's Advocacy Toolkit (http://bit.ly/113fwtq) for ideas.

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



Freeze will be FASEB's next VP of science policy


FREEZE

Hudson Freeze, a professor at the Sanford Children's Health Research Center and director of the Sanford–Burnham Medical Research Institute's genetic disease program, has been elected the next vice president of science policy for the Federation of American Societies for Experimental Biology. Freeze studies inherited glycosylation disorders, which can result in inability to appropriately secrete and target proteins in the cell. He was previously the president of the Society for Glycobiology and the society's representative on the FASEB board of directors. He will begin his new FASEB term July 1.

Weisburger honored by the Toxicology Forum


WEISBURGER

Elizabeth K. Weisburger received the 2013 Philippe Shubik Distinguished Scientist Award at the Toxicology Forum in Washington, D.C., at its winter meeting. This award is given to individuals who have made significant contributions to the field of toxicology. Weisburger worked at the National Institute of Cancer at the National Institutes of Health from 1949 until her retirement.

Jones honored by Texas House

Lovell Jones, a professor at both the University of Texas MD Anderson Cancer Center and the University of Houston as well as director of the joint Center for Health Equity & Evaluation Research, was recognized on the floor of the Texas House of Representatives on April 4 for his years of service in addressing the issue of health disparities.

March of Dimes recognizes UT-Southwestern's Olson


OLSON

Eric Olson, chairman of the molecular biology department at the University of Texas Southwestern Medical Center at Dallas, has received the 2013 March of Dimes Prize in Developmental Biology for his work on identifying genetic pathways involved in heart and other muscle formation. The prize is awarded to investigators whose research helps elucidate the underlying cause of birth defects. According to the March of Dimes, one out of every 125 infants is born with a congenital heart defect each year. A number of drugs for heart disease and dysfunction based on Olson's work are currently under investigation. Olson, who will receive a $250,000 cash award and a silver medal, will be recognized on May 6 at a dinner in Washington, D.C.

Inaugural European Avanti award issued to Goni


GONI

Felix Goni, director of the Biophysics Unit at the joint Spanish National Research Council–Universidad del País Vasco/ Euskal Herriko Unibertsitatea, has received the inaugural European Avanti award for his research on lipids that induce apoptosis. Goni's research interests include membrane biophysics, sphingolipids and sphingomyelinases, membrane fusion and lipid–protein interactions. The award is intended to recognize important contributions of Europeans to the understanding of lipids and was granted for the first time by the European Biophysical Societies Association. An awards ceremony will be held during the Ninth European Congress of Biophysics, which will take place in Lisbon in July.

Dikic receives Ernst Jung Prize for medicine and Leibniz Prize


DIKIC

Ivan Dikic was named the winner of both the Ernst Jung Prize and the Gottfried Wilhelm Leibniz Prize for his groundbreaking work in understanding the role of ubiquitin in cellular signal regulation. The prize from The Jung Foundation for Science and Research, which will be presented this month in Hamburg, is 150,000 euros. The Gottfried Wilhelm Leibniz Prize, funded and presented by the German Research Foundation, is Germany's most prestigious scientific award and is accompanied by a grant of 2.5 million euros.

– Compiled by Kyeorda Kemp

ASBMB career symposium at Stony Brook

About 200 graduate students and postdoctoral scholars attended a career symposium sponsored by the American Society for Biochemistry and Molecular Biology and hosted by Stony Brook University in March.

Organized by graduate students Krithika Venkataraman and Peter Chahales, postdoctoral associate Nadine Dalrymple and professor of chemistry Peter Tonge, the event was part of an ongoing ASBMB series of symposia showcasing the many career options for young scientists.

The keynote speaker was P. Roy Vagelos, former chief executive officer of Merck and the current chairman of the board for Regeneron Pharmaceuticals.

The event's other sponsors included Pall Life Sciences, Regeneron Pharmaceuticals, Hoffman & Baron, the Stony Brook Graduate School and the departments of biochemistry and cell biology, molecular genetics and microbiology, chemistry and pharmacological sciences.

Photo credits: John Griffin/Stony Brook University

Peter Chahales and Krithika Venkataraman introduce keynote speaker P. Roy Vagelos, who participated in a conversation about his life and career. Vagelos has had a long and successful career spanning government, academia and industry.

P. Roy Vagelos speaks to graduate students and postdoctoral scholars on March 19 at Stony Brook University. He told the audience, in part, that although times are rough right now science is not going to go away and the future has great promise for the development of new drugs and therapies to treat disease.

Samuel L. Stanley Jr., president of Stony Brook University, delivers introductory remarks at the career symposium March 19. He said in part, that "a measure of a university's success is seen in the jobs that its students take after graduation." He described Stony Brook as an institution that prides itself on being a center of innovation and discovery, particularly in the life sciences, and he emphasized the importance of collaborations with the other institutions on Long Island, such as Cold Spring Harbor Laboratory, The Feinstein Institute for Medical Research and Brookhaven National Laboratory. "This region is ripe with talent who make a significant contribution to the greater bioscience community," Stanley said.

Retrospective

Wm Wallace Cleland (1930 – 2013)

BY PERRY A. FREY, GEORGE H. REED AND DEXTER B. NORTHROP

Wm Wallace Cleland, professor of biochemistry at the University of Wisconsin-Madison, passed away on March 6 of injuries sustained in an accident. He spent his last days and hours surrounded by his family.

Professor Cleland preferred to be addressed as "Mo." He was born in Baltimore on Jan. 6, 1930, to Elizabeth and Ralph Cleland. The family moved to Bloomington, Ind., where Mo's father became chairman of the botany department and dean of the graduate school at Indiana University.

Mo graduated from Oberlin College with a bachelor's degree in 1950 and from the University of Wisconsin with a master's degree in1953 and a Ph.D. in 1955. He carried out postdoctoral research under Eugene P. Kennedy at the University of Chicago and returned to the University of Wisconsin-Madison as an assistant professor in 1959. Mo advanced to professor of biochemistry in 1966. He was the Marvin J. Johnson professor of biochemistry from 1978 and the Steenbock professor of chemical sciences between 1982 and 2003.

Mo became a fellow of the American Academy of Arts and Sciences in 1977 and was elected to the National Academy of Sciences in 1985. He received the Merck Award from the American Society for Biochemistry and Molecular Biology, the Alfred Bader Award in Bioinorganic or Bioorganic Chemistry from the American Chemical Society, the Repligen Award for the Chemistry of Biological Processes from the Division of Biological Chemistry of the ACS, the Stein and Moore Award from the Protein Society and the Hilldale Award in the Physical Sciences from the University of Wisconsin-Madison. In 1978, Mo was listed among the 300 most-cited scientists.

Mo made influential contributions to enzymology throughout his career. His most widely cited work brought order into the field of multisubstrate steady-state enzyme kinetics. He published three papers on this topic in Biochimica et Biophysica Acta: "Nomenclature and rate equations," "Inhibition: nomenclature and theory" and "Prediction of initial velocity and inhibition patterns by inspection." In this work, Mo derived the basis for what are now known as Cleland's rules, which allow one to write the rate equation for a multisubstrate enzyme by inspection of kinetic patterns. Mo coined the term "ping-pong kinetics" for a kinetic pattern implicating a covalently modified enzyme-substrate intermediate.

Wm Wallace "Mo" Cleland

Early biochemists purified oxygen-sensitive proteins in the presence of mercaptoethanol. Problems with mercaptoethanol were its odor and that two molecules were required to reduce a disulfide. Mo studied the reducing properties of dithiol compounds analogous to dihydrolipoamide and found that dithiothreitol, also known as DTT or Cleland's reagent, eliminated those problems. It was highly water soluble, was nearly odor-free and displayed a low reduction potential. Cleland's reagent now can be found in most biochemical laboratories.

The enzyme-substrate binding, product-release and conformational effects intervening among the chemical steps in the action of an enzyme create a fundamental problem in mechanistic analysis. Chemists measure heavy-atom kinetic isotope effects, or KIEs, to distinguish alternative mechanisms. But binding and conformational effects in enzymes, which can limit rates, often defeat this method. In early collaborations with Wisconsin colleagues Marion O'Leary and Dexter B. Northrop, Mo set out to overcome the problems and apply KIEs to analyze chemical mechanisms in enzymatic catalysis. In the process, Mo invented the equilibrium perturbation method for measuring KIEs, especially deuterium KIEs. This method was brilliantly conceived and enabled KIEs to be measured at chemical equilibrium in a single experiment.

Mo continued with this work and became a master of enzymatic kinetic isotope effects. He neutralized the masking of chemical steps by noncovalent processes through the use of alternative substrates to increase ligand dissociation rates – through the exploitation of pH effects to find conditions at which chemical steps limit rates and through site-directed mutagenesis to make chemical steps rate-limiting. When these methods worked, KIEs on k_{cat} (turnover rate constant) could be measured.

Mo chose the internal competition method to determine KIEs on k_{cat}/K_m. In this method, the heavy atom was a trace label at natural abundance. This method gave KIEs on k_{cat}/K_m, the second-order rate constant for reaction of an enzyme with a substrate. This was the only method available for ^{14}C or ^{3}H effects, because the radioactive species were always trace labels. Mo did not generally rely on radioactivity measurements. He preferred stable heavy atoms like ^{13}C, ^{15}N and ^{18}O. Trace labeling with these isotopes often required chemical synthesis and always required chemical degradation of products and isotope ratio mass spectrometry. Mo never was deterred by the required chemistry. Moreover, he obtained exceedingly accurate values of the small KIEs for these isotopes. Mo was a master at dissecting complex physicochemical pathways and determining mechanisms by observing isotope-sensitive steps. He carried out multiple KIEs to refine structures of transition states and even to distinguish stepwise from concerted mechanisms.

Mo lived a full life that included being a patron of the arts. In his younger days, he enjoyed sailing and ice boating on Lake Mendota in Madison. Mo served as the commodore of the Mendota Yacht Club in 1966. He was an annual supporter of the Madison Symphony Orchestra. He was an opera lover and knew all the operas in the standard repertoire. He supported the Madison Opera and opera companies from coast to coast.

Mo was a world-class philatelist. He held many leadership positions in the United States Stamp Society, including its presidency in 1992. He published more than 300 articles in the Canal Zone Philatelist and the United States Specialist. Mo received the Hopkinson Memorial Literature Award in 1986, 2002 and 2006. He received the Smithsonian Institution's Philatelic Achievement Award in 2008, and in 2009 the Stamp Society inducted him into the United States Stamp Society Hall of Fame.

Mo was a devoted parent, with his former wife Joan Cleland, to Elsa Cleland and Erica Shepard, and a devoted grandparent to Max, Finn and Griffin. He was exceptionally generous to colleagues worldwide, who consulted him on enzyme kinetics. He responded mercurially to requests for assistance from students, young professionals and seasoned researchers. Mo's friends regarded him as a kind and generous adviser as well as a dominant force in enzymology.

'I'D HAVE TO BUILD THE RAILS FIRST'

BY JONATHAN GITLIN

The title of this essay series, "Derailed but Undeterred," gave me a few problems at first when it came time to write. When I think about a derailed career, it suggests a carefully thought-out plan gone awry. The trouble is, when I think back to those first few years out of grad school, a carefully thought-out plan isn't one of the things I remember!

I had accepted a postdoc at The Scripps Research Institute and moved from London to La Jolla but without any clear idea of where that would take me in the long term, and once I was there it started to become clear that an academic research job wasn't that appealing.

Getting involved with Scripps' postdoc association kindled an existing interest in science policy, but how was a postdoc from the U.K. supposed to make that leap in the U.S.? Perhaps, rather than being derailed, my career was more like a train sitting in a field: In order to get anywhere, I'd have to build the rails first.

For U.S. citizens, science policy fellowships sponsored by scientific societies such as the American Association for the Advancement of Science or the American Society of Human Genetics offered the clearest path from the lab to Washington, D.C. These weren't really an option if, like me, you were a foreigner.

Armed with useful advice from people like Peter Fiske, who specializes in helping Ph.D.s realize their career options, I understood that over the next few years I needed to gain the same sort of experience on my own. Now at the University of Kentucky, I was fortunate to work for a supportive principal investigator. In the same way that other postdocs worked on independent research that would open the door to faculty appointments, I set about building the resume I'd need to open doors into policy.

Writing for the technology publication Ars Technica taught me how to write about science for different audiences. Teaching science policy at the Patterson School on campus was invaluable for thinking about how to communicate science and science policy. Working with the National Postdoctoral Association, including a two-year stint on the board of directors, gave me actual policy experience and developed bureaucratic and organizational skills that I couldn't get from the bench.

A little luck and a good professional network later, I found myself starting work in 2009 as a policy analyst at the National Human Genome Research Institute, one of the National Institutes of Health's 27 institutes and centers and the organization behind the Human Genome Project. The past few years have involved working with some very smart and dedicated people in a very exciting area spanning the worlds of research and medicine. I may not have been able to predict this trajectory a decade ago, but it's been a great journey.

Jonathan Gitlin (gitlinjm@mail.nih.gov) is a science policy analyst within the Policy and Program Analysis Branch of the Office of the Director at the National Human Genome Research Institute. He received his bachelor of science in pharmacology from King's College London and his Ph.D. in pharmacology from Imperial College London, following which he conducted research into cardiovascular disease at The Scripps Research Institute and the University of Kentucky. Gitlin is a contributing writer for the online publication Ars Technica and taught international science and technology policy at the University of Kentucky's Patterson School of Diplomacy and International Commerce.

WITH A LOT OF HELP FROM MY FRIENDS

BY CHRISTINE GUTHRIE

I originally had no intention of ever becoming a principal investigator. I just wanted to do my science and be left alone. Besides, I had no idea how one could as a woman; certainly there were few role models. But my boyfriend had different ideas: The way he saw it, I should get a job and support us, preferably somewhere on the West Coast.

As things turned out, I received an offer to become an assistant professor in the biochemistry and biophysics department at the University of California at San Francisco. At the time, UCSF was a little-known school, commonly referred to as the Medical Center. (Indeed, until her death, my mother maintained that I was a professor at UC-Berkeley.)

I arrived in San Francisco in the late summer of 1973 a nervous wreck. The department consisted of six other faculty, all male. They were very friendly and supportive (if a bit bemused to have a female in their midst). It was the postdocs who scared me: The women were desperate to have a Role Model and made clear their high expectations of me to give them advice, yet all I could serve up was my own insecurity.

It was slow-going setting up my lab, I received a negative midcareer review, and tragedy struck when my trusted mentor, Gordon Tomkins, died prematurely. I fell into a deep, clinical depression and was hospitalized for six long weeks. Remarkably, when my colleagues came to visit me, they each said, "I know exactly how you feel; this is a really hard job." This was the first I had heard — or ever imagined — that anyone else was also feeling challenged, and the validation had an enormous impact.

Through a lucky series of connections, I became involved with a group therapy program whose belief was that emotional support and problem-solving skills were key ingredients to survival in a competitive environment. With the encouragement of the professionals leading this program, a group of friends and colleagues from various walks of academia initiated a leaderless group, in which we met to exchange experiences and offer advice in dealing with our usually shared problems. Thanks to this group, I ultimately was able to be granted tenure and to build a strong and nurturing lab environment. Now, some 35 years later, we still meet regularly every other Thursday (as Ellen Daniell suggests in her book of that name) (1), and I am happy to take this opportunity to spread the word about this empowering strategy and encourage you to consider it to enrich your own lives.

Christine Guthrie is professor of biochemistry at the University of California at San Francisco and an American Cancer Society research professor of molecular genetics. Born in Brooklyn, N.Y., she was educated at the University of Michigan (B.S. in zoology) and the University of Wisconsin (Ph.D. in genetics). The hallmark of her research is the use of genetics to understand molecular mechanisms regulating gene expression. In recognition of her pioneering use of Saccharomyces cerevisiae to understand the spliceosome, Guthrie received the 2011 ASBMB-Merck Award. She is a member of the National Academy of Sciences and the American Academy of Arts and Sciences. She has long believed that the best science happens in a nurturing environment and received the Women in Cell Biology Senior Career Recognition Award of the American Society for Cell Biology in honor of this practice.

REFERENCE

1. Daniell, Ellen. *Every Other Thursday: Stories and Strategies from Successful Women Scientists* (Yale University Press).

While an assisted-reproduction technique has helped create and change lives, concern is growing that its success has stymied fundamental research into the causes of male infertility.

BY RAJENDRANI MUKHOPADHYAY

There's no delicate way to put this: Things can go wrong with the family jewels. Experts estimate that up to 50 percent of couples' infertility cases stem from the men. But the problem, say the experts, is that the reasons for male infertility are largely unknown.

Although some genetic and lifestyle factors have been shown to affect sperm cells, "we have a very poor understanding of the basic mechanisms that regulate sperm production by the testes, the maturation and transit of the sperm through the male and female genital tracts, and events required for fertilization and early embryonic development," says Dolores Lamb at the Baylor College of Medicine, the immediate past president of the American Society for Reproductive Medicine. "Because we don't understand these molecular processes, we can't diagnose" the causes of male infertility.

One would think that not understanding male infertility would drum up support for more research. After all, the future of the human race depends on it. But researchers say that is not the case. Male infertility research is largely in the hands of a small cohort of academic investigators backed by programs such as those at the U.S.'s Eunice Kennedy Shriver National Institute of Child Health and Human Development and REPROTRAIN, a program funded by the European Commission to prepare researchers to study male reproductive biology. In recent years, the pharmaceutical players in contraceptive and fertility treatments, such as Schering Plough, Organon and Wyeth, have withdrawn from reproductive biology research and development.

Experts say that much of the pharmaceutical industry's lack of interest stems from the fact that in the past two decades assisted-reproduction clinics have been using a method that bypasses the need for functional sperm. Called intracytoplasmic sperm injection, or ICSI (pronounced ick-see), it now constitutes 68 percent of all assisted reproductive cycles done worldwide, according to ASRM. Because ICSI is effective in overcoming most forms of male infertility, Sheena Lewis at Queen's University Belfast says, "There has been no impetus over the last 20 years to do any research on the basic molecular structures and functions of spermatozoa."

SPERM CELLS UP CLOSE

So what do we know about sperm cells? There are certainly a lot of them. A fertile male usually churns out between 5 million to 250 million sperm cells per milliliter of ejaculate (the average human ejaculate is between 0.75 mL and 2.5 mL). Out of the millions, fewer than a hundred sperm cells actually arrive at the oocyte. And out of those, only one can fertilize the oocyte. It's the sperm version of the TV show "Survivor."

Human sperm are produced in several steps. Testicular stem cells undergo mitotic and then meiotic cell division over the course of about 70 days. The result is spermatids, which then proceed onto terminal differentiation. These round haploid cells undergo a series of dramatic morphological changes into the long, polarized sperm cells known also as spermatozoa.

In an ideal human sperm, there is a smooth, oval-shaped 5-micrometer head free of indentations, bulges or tapers. In it sits a sacklike organelle, called the acrosome, with various lytic enzymes that help to break down the glycoprotein shell around the oocyte. The paternal genetic material of 23 chromosomes is crammed in the nucleus next to the acrosome. About 90 percent of the genetic material is condensed by small, arginine-rich proteins called protamines; the remaining 10 percent is wrapped around histones. Next to the head is the midpiece, which houses mitochondria. And then there's the tail, which propels the sperm to the egg. The work of the tail is done by a complex microtubule structure known as the flagellum or axoneme. At the axoneme's base are the centrioles. A structure called the fibrous sheath is wrapped around the axoneme.

Sperm cells are carried out of the man in the semen, which is produced in the seminal vesicles, prostate gland and urethral glands. Once the sperm are inside the female genital tract, they undergo a subsequent maturation process called capacitation that gears them up for fertilization. (See sidebar.)

MOLECULAR MYSTERIES

From biochemical and molecular biology standpoints, sperm cells are puzzling. As spermatids go on to become spermatozoa, "they shed a lot of cytoplasm and the normal molecular biology machinery," says Joseph Tash at the Kansas University Medical Center. This is in stark contrast with what happens to somatic cells, which are filled with the cytoplasm, a fluid that provides signaling molecules a medium through which to move. Indeed, several experts refer to the sperm interior as being a solid state. Charles Muller, director of the Male Fertility Laboratory at the University of Washington, explains, "In sperm, the membranes of one organelle are pretty much placed up against the membranes of another."

"Do those second messengers function in the same way as a soluble second messenger in a sperm cell that has virtually no cytoplasm? Or do they function as second messengers more in a solid-state environment? Those are really interesting questions to ask."

– Gregory S. Kopf
Kansas University Medical Center

The sperm cell uses second messengers, such as cyclic AMP and GMP, which are soluble messengers in the somatic-cell book. "Do those second messengers function in the same way as a soluble second messenger in a sperm cell that has virtually no cytoplasm? Or do they function as second messengers more in a solid-state environment? Those are really interesting questions to ask," says Gregory S. Kopf, also at KUMC and the former director of the preclinical male contraceptive research and development program at Wyeth, which is now part of Pfizer.

That conventional signaling molecules may work differently in sperm cells is indicated during capacitation, when some proteins undergo tyrosine phosphorylation. If these tyrosine phosphorylation events do not occur, a sperm cell cannot complete capacitation and fertilize an egg. "The conundrum is that cAMP is important for these changes, but cAMP in every other cell type does not directly trigger tyrosine phosphorylation. cAMP triggers threonine and serine amino acid residues to be phosphorylated," says Tash. "The question of how cAMP triggers tyrosine phosphorylations in sperm is still unanswered."

The highly segmented character of the sperm cells raises other questions. How does biochemical information get from one compartment to another? "Many people have argued over the years that there's actually no clear path for cytoplasmic molecules to move from the head into the midpiece or tail. There do seem to be constrictions and boundaries there that might form a structural barrier to any interchange," says Muller. A case in point is ATP.

To do all that swimming and fertilizing, sperm cells need a lot of energy. How exactly does a sperm cell meet its energy requirements? The mitochondria in the sperm midpiece churn out ATP by oxidative phosphorylation. "How does that ATP get down to the dynein ATPases that are along the axoneme in the middle of the tail? That's a long distance," says Muller.

It turns out that the ATP in the tail comes from glycolysis, a topic in which Deborah O'Brien at the University of North Carolina in Chapel Hill is an expert. "We learn about glycolysis in college as this kind of boring, immutable metabolic pathway, right?" she says. "Well, sperm cells have modified nearly every step of that pathway."

Several glycolytic enzymes are distinct in sperm and may illustrate how sperm cells have adapted a metabolic pathway to occur essentially in the solid state. Multiple glycolytic enzymes are pinned to the fibrous sheath so firmly that O'Brien says attempts to strip them off the sheath, even with 6 M urea, potassium thiocyanate and detergents, fail. If any of these three glycolytic genes are knocked out in mice, the males are infertile. Thus, this sperm-specific glycolytic pathway is "a pathway that's essential for sperm function," sums up O'Brien.

Besides oxidative phosphorylation and glycolysis, which derive ATP from sugars, sperm also can get their ATP from fatty acids metabolized in mitochondrial and peroxisomal pathways. In a paper published in Molecular & Cellular Proteomics earlier this year, a team led by Rafael Oliva, REPROTRAIN's project coordinator, and Alexandra Amaral at the University of Barcelona discovered a number of peroxisomal proteins in the tails of healthy human sperm. This came as a surprise, because the conventional wisdom was that sperm didn't have peroxisomes. Some peroxisomal proteins are involved in the oxidation of very long-chain fatty acids. The implications, Amaral says, are that "sperm might be able to use fatty acids as fuel, and lipidic beta oxidation may contribute to sperm motility."

Even if the sperm make it to the oocyte, the state of the sperm DNA can greatly influence male fertility. Experts say the tightly wrapped DNA in the sperm head gives the impression that the genetic material is protected. But that's not the case. Because much of the molecular machinery is taken out of the cell toward the end of making terminally differentiated sperm, the final cells don't have the tools for repairing damaged DNA. With the mitochondria in the midpiece, the DNA sits right next to organelles that spew out reactive oxygen species that damage DNA. With the loss of transcriptional machinery during differentiation, sperm have lost the primary surveillance system for identifying ROS-induced damage. ROS aren't the only things to damage DNA. Environmental toxins, lifestyle choices such as smoking, and stresses all damage DNA.

Even with damaged DNA, sperm can fertilize eggs. The oocyte's DNA repair machinery can fix most single-strand breaks in the paternal DNA. However, researchers have observed that there are a notable number of double-strand breaks in the sperm DNA. "There is no way those can be put back in the right places," says Muller. "We don't know of a mechanism of DNA repair that can handle that. There is no template." This has implications for older men, because age has been suggested to be

SINGLE-CELL LIFE

Because it is the only cell in the human body designed to leave the confines of the body, sperm genes echo those of unicellular life forms. An example is the sperm adenylate cyclase that produces cAMP. The sperm adenylate cyclase gene in homology studies bears the most resemblance to the adenylate cyclase found in cyanobacteria. "One way to think about the human genome is that it contains the memory of unicellular life. When the gametes are made, that genetic memory becomes expressed, and genes that are actually most similar to genes found in other unicellular organisms begin to be expressed in the testes," says John Herr at the University of Virginia. "We call this the ancestral gene program that is activated" during sperm production. (Herr has developed two commercial home tests for male infertility, Sperm Check Fertility and Sperm Check Vasectomy, using biomarkers that are unique to the final stage of sperm development in the testes.)

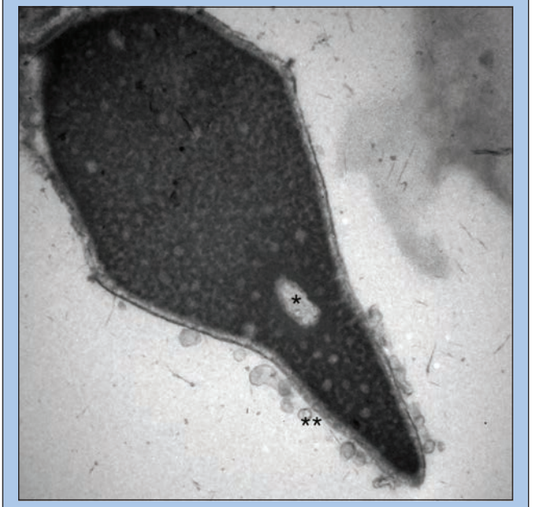
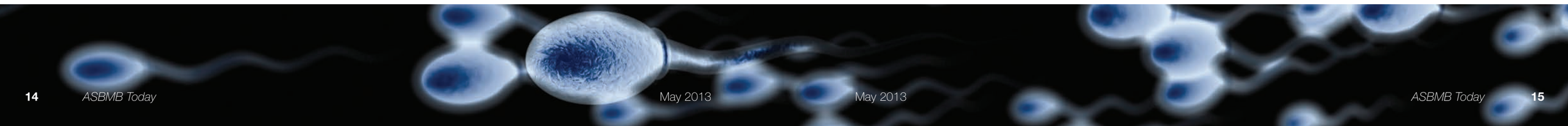


IMAGE COURTESY OF CHARLES H. MULLER AT THE UNIVERSITY OF WASHINGTON

Electron micrograph of a human sperm head at 30,000x. The (*) is a vacuole in the middle of the nucleus, which is an abnormality and may be related to DNA fragmentation. The (**) are vesicles formed from a premature acrosome reaction.



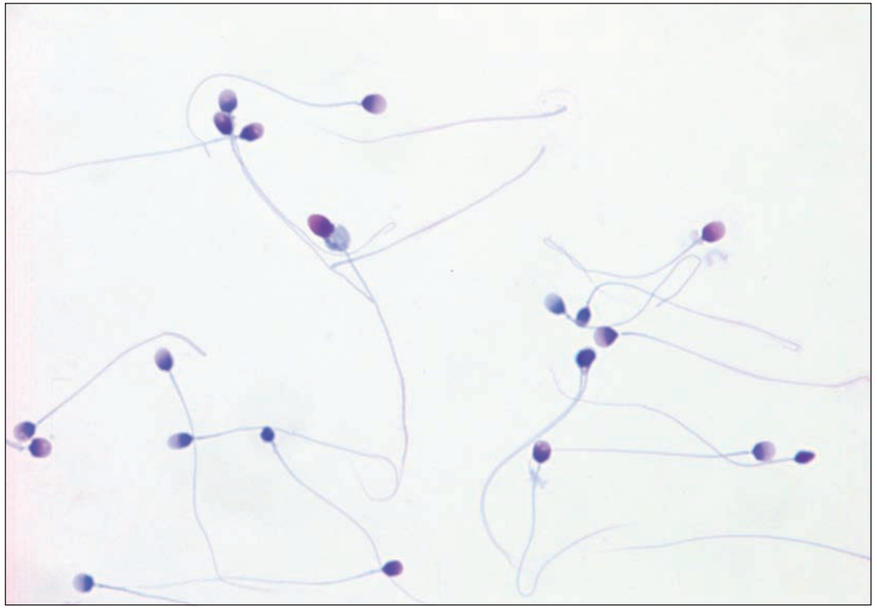


IMAGE COURTESY OF CHARLES H. MULLER AT THE UNIVERSITY OF WASHINGTON

Human sperm exhibiting many different, and almost all abnormal, shapes (morphologies). In the human, it is typical for as few as 4 percent of the sperm to be of normal shape, having a smooth oval head and normal midpiece and tail, and other characteristics. Original magnification 1000x.

a factor in creating more paternal DNA damage. For older men attempting to conceive babies, the highly damaged paternal DNA could stop fertilization from happening or, even if fertilization does happen, cause problems with fetal development.

For a long while, conventional wisdom held that sperm cells didn't have any RNA because the DNA was transcriptionally silent. In recent years, work by David Miller at the University of Leeds in the U.K. and others demonstrated that there are messenger and noncoding RNAs in sperm. But their role is unclear. Miller says that there are hints that the sperm RNA is responsible for embryonic gene activation. He also speculates that the RNA acts as a compatibility signal to the egg, telling it that the invading sperm cell is not a pathogen.

ICSI

The first demonstration of assisted reproduction was in vitro fertilization and the birth of Louise Brown in 1978. Robert Edwards, the emeritus professor at the University of Cambridge who died last month, won the Nobel Prize in 2010 for developing IVF, a process in which sperm cells and eggs are incubated in cell culture dishes for fertilization to take place.

But IVF can't tackle all fertility problems. If a man's sperm cells are few in number, have poor motility or carry morphological defects, they are not going to fertilize an oocyte even in a cell-culture dish. Until ICSI came along in the1990s, "there used to be no hope for these men," says Muller.

The technique, which bypassed the need for culture-dish fertilization, was developed by Gianpiero Palermo, currently at the Weill Cornell Medical College, while on sabbatical in Belgium. The first ICSI baby was born in January 1992 in Belgium. Approximately 4 million have been born by IVF in the past 35 years; 2 million babies have been born worldwide by ICSI in the past two decades.

For ICSI, a technician scans a semen sample under an optical microscope and picks out a single sperm cell that appears to be normal. Then the technician moves that sperm cell with micromanipulators and directly injects it into the cytoplasm of a waiting egg.

ICSI works not only with terminally differentiated sperm cells but also with immature sperm cells plucked from a man's testes. Lewis says because ICSI allows technicians to use sperm that normally wouldn't be able to fertilize oocytes on their own, it provides a way "to bypass all the laws of nature."

Experts say the method's success has killed the pharmaceutical industry's interest in understanding the fundamental biology of sperm and developing male infertility treatments. "They think ICSI is the great panacea," says Miller. "There are two arguments which factor against those of us who are working in the male reproductive field. Because of the advent of ICSI, they think that it has solved the problem of male infertility. The second thing is that even if we do discover what causes male infertility, they think there is nothing we can do about it. There is no translational benefit … They would turn around and say, 'There's nothing you can do about it. Just do ICSI and be done with it.'"

But Miller and others say that those arguments are fallacious because there may be other factors that lead to infertility that ICSI can't help. ICSI is based on sperm that seem morphologically fine under a microscope. "People have always made the assumption that the really nice looking sperm must be the best," says Muller. "That's a completely unsupported assumption."

Muller describes a couple he encountered who had visited a clinic that "believed ICSI was the answer to everything," he says. "They got zero fertilization. They spent $10,000 and got nothing out of it."

After the failed attempt, the couple went to Muller, who recounts seeing under the microscope that the man's sperm cells were devoid of acrosomes. One component in or near the acrosomes is phospholipase Cζ, which is postulated to be an activating factor that helps the fertilized oocyte initiate the first cell division. The enzyme triggers a calcium signaling cascade, so Muller's group attempted to trigger the calcium cascade with an ionophore. "Lo and behold, they got five out of six embryos developing, and the couple is currently pregnant!" Muller says.

This example and others like it point to the fact that sperm cells are not mere donors of paternal genes. Besides phospholipase Cζ, sperm also must donate their centrioles to the fertilized egg, a discovery made by Palermo and colleagues in 1994 using ICSI. Miller's work on RNA points to the possibility that sperm cells that don't contain the right RNA molecules may be ineffective for fertilization. There is probably a host of other molecules that sperm contributes to the fertilization process that haven't yet been identified.

SUPPORTING REPRODUCTIVE BIOLOGY

Given the heartache of infertility and the cost of treatments, experts argue that sperm biology needs to be investigated more thoroughly. One in five couples requires reproductive assistance, and a single round of IVF can cost up to $17,000. Data from the European Human Society of Reproduction and Embryology show that IVF and other assisted-reproduction technologies have had the same success rate over the past 30 years, somewhere between 25 and 30 percent. "We haven't really made a lot of progress," says Lewis. "A lot of things have been done to tweak superovulation regimens and things like that. But little has been done about the sperm."

Then there is the health aspect. Men who resort to ICSI usually don't get an actual diagnosis to understand why they are not producing healthy sperm in the first place, say Lamb and Oliva. Often, the lack of sperm can point to a more general health problem. For example, men with DNA-repair problems don't produce functional sperm, and there are associations between poor sperm counts and cancer in later life. Oliva also points out that, because the men are not diagnosed, if there are any genetic issues with their sperm, ICSI just passes those issues to the next generation.

So the current situation with male infertility is worrying, say experts who are working hard to understand the molecular and biochemical basis for sperm malfunction. "We cannot be treating infertility the way we are treating it now, by just taking one single sperm cell and injecting into an oocyte," says Oliva. "It seems to solve the problem, but it's temporary. The next generation is going to have the same problems as we have now."

Rajendrani Mukhopadhyay (rmukhopadhyay@asbmb.org) is the senior science writer for ASBMB Today and the technical editor for the Journal of Biological Chemistry. Follow her on Twitter at www.twitter.com/rajmukhop.

Let's get digital

As PCR becomes faster, more multipurpose and more precise, a handful of researchers are using a digital approach to find a needle in a haystack.

BY DIEDRE RIBBENS

Thirty years ago, Kary Mullis and his team at Cetus Corp. were the first to amplify short sequences of DNA in vitro. Dubbed the polymerase chain reaction, this technology involves taking a template piece of DNA, adding excess amounts of free nucleotides along with short sequences to prime the ends of the DNA molecules and specify the region of amplification, and letting a DNA polymerase copy the template during multiple amplification cycles. Their idea revolutionized modern science and now is used across the fields of forensic science, astrobiology, cancer biology and many others.

"It's amazing that one simple thing spread," says Mullis. "There are not a lot of areas of biochemistry that haven't benefitted" from PCR. The Nobel selection committee realized the impact of PCR, and Mullis was recognized for its invention with a Nobel Prize in chemistry in 1993.

"PCR has pretty much single-handedly catapulted molecular biological methodology into being a key part of almost every aspect of biological and clinical research," says Jim Huggett of the National Measurement Institute for Molecular Biology and Biochemistry in the United Kingdom. "From vaccine development to metegenomics, the simplicity and versatility of PCR has been crucial in developing many fields."

The technology of PCR has undergone revolutionary changes since its introduction in 1983. PCR is now faster, more multipurpose and more precise. The latest offshoot uses PCR to count single copies of a particular DNA sequence present in a sample. This technique, called digital PCR, can precisely quantify the amount of a given sequence of DNA among a complex mixture of sequences and express that quantity as a numerical value.

Digital PCR is related to the technique known as quantitative PCR, but it takes the quantification of DNA one step further. Quantitative PCR can compare the amount of a particular sequence to a reference sequence, one that has a known or standard amount across all samples. Because of this, quantitative PCR is actually an analog technology; the measurement uses an analog between the sample of interest and the reference sequence to make the quantification. Digital PCR does not require a reference or standard, making the quantification of DNA more precise than what quantitative PCR can achieve.

"I think digital PCR has a lot of possibility, and the digital aspect is very cool," says Rob Phillips of the California Institute of Technology. "There are many, many ways you can imagine using it."

QUANTIFYING EXACT NUMBER OF COPIES

Digital PCR involves diluting a DNA sample and placing the template DNA into micro-wells before performing hundreds or thousands of reactions with the same source material. The sample is diluted to the point at which either zero copies are contained or one copy of the template is contained in each reaction. Therefore, the reaction readout will be either positive (containing the template) or negative.

By comparing the ratio of positive to negative reactions and taking into account the dilution factor, digital PCR quantifies exactly how many molecules of the template were present in the original sample. This method is low-throughput by nature because one must run so many reactions on each individual sample, but overall it is considered more sensitive and more precise than quantitative PCR.

Several platforms exist for performing digital PCR, all involving minute reaction volumes. Emulsion in tiny droplets, microfluidics-based chips and hydrophobic/hydrophilic chambers all have been used for digital PCR. Companies specializing in these technologies, BioRad, Fluidigm and Life Technologies, have tried to optimize their systems to include features such as a large reaction number or the ability to perform quantitative PCR while the reaction cycles and then using the endpoint of the reaction to generate the digital PCR results.

Real-time PCR provides a curve or graph that measures the amplification of DNA in a reaction at the end of each PCR cycle. The real-time data for a single well of a digital PCR reaction can help determine if a detected target sequence is real as opposed to a false positive or contaminant. The readout for digital PCR requires only the endpoint value, answering the question, "Did the reaction amplify DNA or not?"

> **"Volume for volume, digital PCR may be more accurate than quantitative PCR, but if you can only get 1 µl into a digital PCR reaction, as opposed to 20 µl into a qPCR reaction, then this will reduce the physical sensitivity."**
> – Jim Huggett

For some digital PCR platforms, real-time data is not collected as the machine cycles. This means that the endpoint value is still useful for obtaining digital PCR results, but there is no way to know whether a positive reaction is a false positive. The advantage of such a system is that amplification is more rapid and can be formatted to run a higher number of reactions at once. The more reactions that can be done, the more sensitive the quantification becomes. Sensitivity depends on the volume screened, so performing more reactions equals screening a larger volume. Also, running more reactions improves precision, because more replicates can be run to determine the consistency of data between reactions.

VERSATILITY OF DIGITAL PCR

The uptake of the digital PCR technology by various research groups has been on the rise.

A search of the term "digital PCR" on PubMed shows that 14 publications recorded the use of the technique between 1999 and 2009. In 2010 alone, 15 publications did. This number stays fairly con-

KARY MULLIS RECALLS THE MOMENT HE CONCEPTUALIZED PCR

Kary Mullis originally conceived the idea for polymerase chain reaction while was working through the problem of how to diagnose sickle cell disease in a shorter amount of time. The existing method took three months from testing to diagnosis, so Mullis tried to apply his expertise in working with DNA oligonucleotides to develop a faster assay that could be done using clinical samples.

Sickle cell disease is caused by a single base-pair change in the DNA, so Mullis' strategy was to do a dideoxy sequencing reaction at that specific base pair in the genome, directed to the location by oligos designed to the surrounding sequence. Performing the reaction on both strands of the DNA would ensure accuracy.

The main obstacle, Mullis thought, was the presence of endogenous deoxynucleotides (dNTPs) in the clinical samples. These dNTPs would interfere with his sequencing reaction, because a polymerase would use them preferentially over dideoxynucleotides (ddNTPs), the basis of the sequencing technique he was using. His solution for rapidly depleting the dNTPs from the sample? Add a polymerase. The polymerase would use all the dNTPs, leaving the sample ready to be prepared for sequencing.

Mullis recounted, "I was driving to my cabin while I was thinking about this, and it was about one mile into the drive when I realized: This is going to copy both sides [of the genomic sequence], and I'll end up with two times the signal than before!" He realized the polymerase would use the dNTPs to make an exact copy of the target region, and the amount of target would be doubled. He thought, "I could do this as many times as needed!"

"Once I had the picture of that in my head," Mullis said, "I slammed on my brakes. I was in the middle of a busy highway, and I thought, 'I better get off the road before I get killed!' I pulled over, and I was just stunned. I thought, '210 was 1,024. 220 was over 1 million. I could make a million copies of the target.' Easy."

Back in the lab, Mullis industriously worked on his new technology, which he would later call polymerase chain reaction, after the file he had created on his computer (chain_reaction.pol). Although he could only stand to manually perform 10 reaction cycles, he successfully amplified plasmid DNA to an amount visible on an ethidium bromide-stained agarose gel. His idea, as he simply put, "worked, beautifully."

stant until 2013: Already, 13 publications are listed, which puts digital PCR-related publications on track to hit 52 by the end of the year, a three-fold increase over 2012.

Digital PCR is being used by regulatory agencies in the United Kingdom as the new standard in quantifying DNA. In a study published last year in the journal Nucleic Acids Research by Alexandra Whale and colleagues at LGC, the UK's designated National Measurement Institute for chemical and bioanalytical measurement, microfluidic digital PCR was compared with conventional quantitative PCR in the measurement of copy number variation, or CNV, associated with tumors.

By measuring the CNV of the HER2 gene, the group showed that digital PCR could measure reliably a smaller CNV than quantitative PCR could. The group chose to use digital PCR, because it could measure the exact number of CNV sequences and because it did not require the use of a calibration curve.

Moving from quantitative PCR to digital PCR presents some difficulties. Jim Huggett, an author on the HER2 study, had a few concerns. "At the moment, cost has to be mentioned. But technically, the most challenging is the low dynamic range of the instrument," he said. "Another big challenge is total reaction volume – and particularly the amount of sample you can get into a reaction. Volume for volume, digital PCR may be more accurate than quantitative PCR, but if you can only get 1 µl into a digital PCR reaction, as opposed to 20 µl into a qPCR reaction, then this will reduce the physical sensitivity."

Huggett said he hopes his team's publication showcases the method and the way it improves the quantitative measure of DNA. He says he sees digital PCR as a technique with the potential to considerably reduce variability between experiments done in different labs, improving the reproducibility of experiments.

"Most measurement techniques used with biological measurement are relative (such as qPCR or ELISA) and require a calibration curve to assign a value. Digital PCR does not need a calibration curve, because the results are digitized and surprisingly reproducible," says Huggett.

Digital PCR also is providing a platform to address broader questions in microbiology. In their paper "Probing individual environmental bacteria for viruses by using microfluidic digital PCR," Arbel Tadmor and colleagues at the California Institute of Technology demonstrated a creative use of digital PCR. Published last year in the journal Science, the study looked at viruses infecting bacterial cells in the termite hindgut. Although bacteriophage and other viruses are commonly found, specific virus-host interactions remain unknown due to problems with obtaining cultures from the environment.

Culturing the bacterial hosts before isolating and identifying the viruses is tedious work and can be ineffective at times. More than 99 percent of microbes cannot be cultured in a lab.

Tadmor and colleagues decided to use digital PCR to identify the viruses present in an individual bacterium from an environmental sample.

Having a lab space next to Jared Ledbetter, a colleague of Steve Quake (founder of the Fluidigm digital PCR platform), made using digital PCR a natural choice for Tadmor. "The Fluidigm product that Jared was using was this digital PCR chip," he says. After a member of Ledbetter's lab had success using digital PCR to identify functional genes present in microbes, Tadmor's adviser, Rob Phillips, wanted to try something similar.

"Rob, whose lab, among other things, works on bacterial viruses, thought, hmm, this would be cool to try to pair viral genes with the identity of the host. The reason that's an interesting problem is that most of the hosts in the microbial world cannot be cultured, and if you can't culture either the host or the virus, then you can't really know who is infecting whom in the microbial world," Tadmor explains.

By diluting their samples so that analysis could be carried out on the single-cell level, they were able to identify which viruses infected which bacteria. They multiplexed two templates in each digital PCR reaction – one to recognize a viral marker and one to recognize the bacterial 16S rRNA gene.

Tadmor, who performed the digital PCR in the study during his time as a graduate student, says he encountered unique challenges while working with viral genomes. He emphasized the difficulty of using PCR to detect ever-mutating viral genomes as well as extracting the reaction components from the physical microfluidic chips for further analysis. "It's a general problem with the method to get the (PCR) chemistry to work. (It's) really hard to get a multiplex qPCR reaction to work on the chip," he says.

Barring these troubles, however, Tadmor said he hopes that his paper will inspire others studying virus-host interactions to use this technology to ask similar questions.

Tadmor, who performed the digital PCR in the study during his time as a graduate student, says he encountered unique challenges while working with viral genomes.

"That's something which in the past has been difficult to tackle," he explains.

Overall, digital PCR has garnered excitement, but the size of its impression on the scientific community is yet to be determined. One indisputable fact, however, is the legacy of traditional PCR.

"PCR has had the same sort of impact on the world of biology that the telescope had on astronomy: It's huge," says Phillips of Caltech. "It's really hard to find any domain of biology that was not touched by PCR. In 30 years, it's amazing that we've come as far as we have."

Diedre Ribbens (diedre.johnson@gmail.com) is a graduate student at the Johns Hopkins School of Medicine and a budding science writer. Connect with her on LinkedIn.

jbc THE JOURNAL OF BIOLOGICAL CHEMISTRY

Bone and skin disorders caused by disruptions in GAG synthesis

BY DANIELLE GUTIERREZ

A number of bone and skin disorders that cause disfiguration, pain and sometimes premature death are rooted in genetic mutations that disrupt the synthesis of sulfated glycosaminoglycans, also known as GAGs.

For example, Larsen-like syndrome results in joint displacement and heart defects and is caused by mutation of β1,3-glucuronosyltransferase-I, reducing GAG production. Another disease, Omani-type spondyloepiphyseal dysplasia, results from mutation in chondroitin 6-O-sulfotransferase-1 and is characterized by shortness, joint disturbances, abnormal spine curvature, mild digit shortness, fusion of the carpal bones, altered limb length, heart defects and deafness.

In a recent minireview in the Journal of Biological Chemistry, Kazuyuki Sugahara at Hokkaido University in Japan and colleagues focus on two types of GAGs, chondroitin and dermatan sulfate, and what we know about their roles in bone and skin disorders.

To start, the authors cover the biosynthesis of chondroitin sulfate and dermatan sulfate side chains. They describe the steps of GAG synthesis: 1) the addition of a tetrasaccharide linkage region that connects the various GAGs to a serine in the core protein; 2) the addition of N-acetyl-D-galactosamine, which signals the construction of chondroitin sulfate or dermatan sulfate, or the addition of N-acetyl-D-glucosamine, which initiates the assembly of heparan sulfate; 3) elongation via the addition of repeating disaccharide units; and 4) the sulfation of these chains, which differs among various cell and tissue types, developmental states and diseases. A host of enzymes is involved with the synthesis of these diverse side chains, and their mutations can lead to a variety of diseases.

Ehlers-Danlos syndrome-progeroid type is caused by a β1, 4-galactosyltransferase-I deficiency and results in skin and bone disturbances, including shortness, osteopenia and an older physical appearance. The disease characteristics are mainly caused by disruptions in dermatan sulfate chains.

The authors also comment on disruptions to the β1, 4-N-acetylgalactosaminyltransferase-I and II enzyme activities as seen in Bell's palsy and some hereditary motor and sensory neuropathies. These conditions involve partial facial paralysis (Bell's palsy) and the ongoing loss of peripheral sensory nerve function leading to collapses and muscle weakness (hereditary motor and sensory neuropathies).

Another disease resulting from GAG synthesis enzyme mutations is Temtamy pre-axial brachydactyly syndrome. The enzyme affected is chondroitin synthase 1, and its mutation results in digit and facial deformities, hearing impairments, developmental delay and shortness.

The authors also mention adducted thumb-clubfoot syndrome, which results from mutations to a gene that encodes dermatan 4-O-sulfotransferase-1, causing increased production of chondroitin sulfate over dermatan sulfate.

While a lot has been learned about GAG function and synthesis in the past 15 years thanks to the cloning of cDNAs for the genes encoding GAG synthesis enzymes and thanks to collaborations among clinicians, geneticists and glycobiologists, the authors write, "further understanding of the molecular pathogenesis involving (chondroitin sulfate and dermatan sulfate) chains is essential to facilitate the development of therapeutics for these diseases."

Danielle Gutierrez (daniellebgutierrez@gmail.com) is a freelance science writer based in Corpus Christi, Texas.

Story of a donut and a death machine

BY PREETHI CHANDER

Not all RNAs code for proteins; instead, some of them play crucial roles in regulating other RNAs that do encode for proteins. One such class of noncoding RNA found in bacteria are those that are 50 to 300 nucleotides long, called small RNAs. Under stress, these sRNAs are induced and base pair with target messenger RNAs, leading to a positive effect (stabilization/activation) or a negative effect (repression/degradation) on mRNA translation.

In 1975, A.J. Wahba and co-workers first identified a host factor for the RNA phage Qβ, named Hfq, a protein that more recently has been shown to function as an RNA chaperone in bacteria. Hfq is a donut-shaped protein that binds sRNA on one face and mRNA on the other. This Hfq complex is essential for sRNA-based gene regulation in bacteria. This complex is an RNA death machine. At its heart is an endonuclease, RNAse E; interacting with its four arms are auxiliary functions to improve and regulate the degradation of RNA. The Hfq complex interacts with this RNaseE complex.

In a recent review in the Journal of Biological Chemistry, Nicholas De Lay, Daniel J. Schu and Susan Gottesman at the National Cancer Institute explain how Hfq, RNase E and other proteins act in collusion with sRNAs to affect negatively mRNA translation and stability. The authors describe multiple pathways by which mRNA decay occurs, thus affecting subsequent translation regulation. In eukaryotes, RNA-induced silencing complex and Argonaute proteins play roles similiar to those of bacterial Hfq and RNaseE complex. Though the machinery in bacteria and eukaryotes varies, the authors conclude that the use of sRNAs to control mRNA translation is an underlying common theme that provides "well regulated control of translation, using machinery that is sensitive to the state of the mRNA, its ability to be translated, and that can be tuned in multiple ways to fit the physiological requirements."

Preethi Chander (chander.preethi@gmail.com) is a health science analyst at the National Institutes of Health.

Deconstructing collagen

BY ADITI S. IYENGAR

Collagen metabolism is crucial to the maintenance of the structural integrity of mammalian connective tissue. Collagen triple helices form immensely tensile fibrils that link with each other to create interstitial matrices and basement membranes. The timely breakdown of collagen is an important housekeeping mechanism that helps maintain healthy tissues and organs; however, defects in normal collagenolysis have been linked to debilitating diseases such as arthritis, atherosclerosis and cancer. Therefore, there is now a burgeoning interest in gaining a deeper insight into molecular aspects that facilitate collagen degradation.

In a recent minireview in the Journal of Biological Chemistry, Gregg B. Fields of the Torrey Pines Institute for Molecular Studies in Port St. Lucie, Fla., gives an extensive account of the myriad collagenolytic enzymes, the majority of which are matrix metalloproteinases, or MMPs, implicated in mammalian collagen metabolism.

While outlining the distinct methods employed by MMPs in collagen proteolysis, the author also highlights the unique features of the cleavage sites that make different collagen fibers susceptible to the action of specific MMPs. Furthermore, this minireview delves into the molecular mechanisms that underlie the categorical breakdown of the collagen triple helix and elucidates the current models and hypotheses that have determined how MMPs gain access to cleavage sites, the enzyme conformations that are critical for hydrolysis and the diverse modes of proteolysis based on the structural complexities of collagen fibers. The minireview also describes the role of binding proteins such as integrins and processes such as MMP dimerization in providing additional strain on collagen fibers, thus facilitating the collagenolytic program.

This minireview, titled "Interstitial collagen catabolism," highlights the significance of current research in this field and the potential exploitation of differences in MMP-mediated catabolism for the development of target-specific inhibitors. "As further information on interstitial collagenolytic processes is obtained, inhibition can be fine-tuned to be disease- or pathogen-specific," writes the author.

Aditi S. Iyengar (aiyeng@lsuhsc.edu) is a graduate student in the genetics department at Louisiana State University Health Sciences Center in New Orleans.

JLR THE JOURNAL OF LIPID RESEARCH

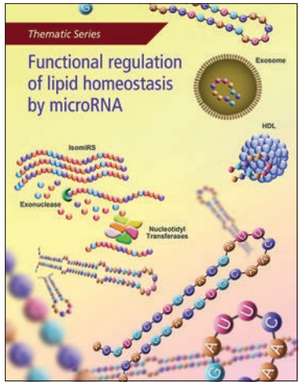
Thematic series on microRNAs

BY MARY L. CHANG

The May issue of the Journal of Lipid Research contains a new thematic series, "Functional regulation of lipid homeostasis by microRNA," coordinated by JLR editorial board member Kathryn Moore of the New York University Medical Center. Moore's introductory editorial, titled "MicroRNAs: small regulators with a big impact on lipid metabolism," and four reviews from experts in the field make up the series.

While they were first discovered only in the early 1990s, microRNAs have since been found in all standard laboratory eukaryotic systems, mammals, plants and fungi. MicroRNAs are important sections of genetic material that play a major role in gene expression. These short RNA sequences are able to bind to messenger RNA, the genetic messages that code for proteins, and affect the expression of the message, usually acting on it by silencing it.

Mireille Ouimet and Moore's contribution to the series, "A big role for small RNAs in HDL homeostasis," provides an overview of the biology of microRNAs, discusses how



the first microRNA miR-122 was discovered and covers the evolutionarily conserved miR-33 that is found in two forms in humans. With the recent discovery that high-density lipoprotein itself assists in the transport of microRNAs, Ouimet and Moore contend that there likely are many more functions mediated by these related microRNAs.

In his review, "Needles in the genetic haystack of lipid disorders: single nucleotide polymorphisms in the miRNA regulome," Praveen Sethupathy of the University of North Carolina at Chapel Hill takes a closer look at microRNA-related genetic variation and how growing evidence suggests it may be the key to the development of lipid disorders.

In "Extracellular communication via microRNA: lipid particles have a new message," Katey Rayner and Elizabeth Hennessy of the University of Ottawa Heart Institute and the New York University School of Medicine, respectively, explore the potential for microRNAs to act not just as mediators for cell-to-cell communication but also as possible detectable biomarkers for certain lipid-related diseases.

In the final review, Kasey Vickers of the Vanderbilt University School of Medicine and colleagues discuss how one particular gene location might have the potential to give rise to multiple, different microRNA isoforms, how these isoforms may affect lipid metabolism in the body and how this unexpected discovery in microRNA diversity has complicated the way researchers view microRNAs.

Mary L. Chang (mchang@asbmb.org) is publications manager for the Journal of Lipid Research and Molecular & Cellular Proteomics.

MCP **MOLECULAR & CELLULAR PROTEOMICS**

Spotlight on glycoscience

BY RAJENDRANI MUKHOPADHYAY

The April issue of Molecular & Cellular Proteomics is devoted to the area of glycoscience. The issue, co-edited by MCP Associate Editor Gerald Hart of Johns Hopkins University and MCP editorial board member Lance Wells at the University of Georgia, explores the fundamental biology of molecules modified with sugars as well as their impact in different diseases. The issue also has articles that delve into the technologies used to analyze glycosylated molecules.

Glycoscience has been growing in sophistication and importance in the past five years. In August, the National Academy of Sciences released a roadmap for glycoscience that described how the field has repercussions in a wide range of areas, including fuels, drug development and materials science. The growth of glycoscience has been driven largely by the development of techniques that can tackle properly these complex and structurally diverse molecules.

But in their editorial introducing the special issue, Hart and Wells point out that, unlike the template-driven synthesis of nucleic acids and proteins, glycosylated structures are made by complex, nontemplate processes. On top of that, carbohydrates can have a variety of linkages that produce branched structures, in contrast with DNA, RNA and proteins, which are made in a more linear fashion. "These two facts generate a considerable challenge to the analytical and bioinformatics community," wrote Hart and Wells.

To confront the challenges of studying glycosylated molecules, MCP's leadership spearheaded the development of guidelines for the publication of mass-spectrometry-based glycomics data. The guidelines, with an accompanying checklist, were the result of a meeting that was held in conjunction with the Warren Workshop for Glyconjugate Analysis in the late summer of 2012. The journal adopted the guidelines and the checklist after extensive input from the glycoscience community. The special issue of the journal contains research papers and reviews written by leading figures in the field of glycoscience, many of whom attended the meeting and contributed to the guidelines.

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

MCP-SPONSORED LECTURESHIPS

The Human Proteome Organization's 12th World Congress
Sept. 14–18: Intercontinental Grand Conference Center, Yokohama, Japan.
Associate Editor: Ralph Bradshaw

2013 Annual Meeting for the Society for Glycobiology
Nov. 17–20: Renaissance Vinoy Resort & Golf Club, St. Petersburg, Fla.
Associate Editor: Gerald Hart
Lecturer: MCP Co-Editor Al Burlingame

Visit www.mcponline.org for more information.

How to help students learn – and thrill your department chair

BY MARILEE BENORE

Choosing a career in academics? While most scientists are reasonably well-prepared to embark upon research careers, many have far less experience in teaching. The pleasure of observing students learn is as rewarding as research, although it might seem the results are less tangible. But with a tiny bit of effort, you can demonstrate evidence!

Like research, excellent teaching requires knowledge of what works. There are many ways to engage students in the classroom and abundant resources to help those wishing to find new ways to teach: Symposiums, workshops, networking events and teaching clubs on your campus are just a handful. (See the American Society for Biochemistry and Molecular Biology website!)

Once you have embarked upon a course plan, how do you know if you are dong it well? Typically, faculty members summarize student evaluations, provide examples of syllabi and exams, and solicit feedback from colleagues. These data are important. However, student learning is the real proof, and assessment of student learning is no more mysterious or challenging than research.

Planning experiments and acquiring results to demonstrate a hypothesis are hardwired into a well-trained scientist, yet a similar approach for teaching is often eschewed by those who find assessment boring or a waste of time. It is not.

Every course should have clearly defined and articulated learning outcomes. These goals should allow you to ask questions or plan exercises to evaluate the output from students against the goal. You don't need to test everything. For example, an exam answer might be graded using a rubric, which determines at what level of competencies students understood a specific concept. Answers to pre- and post-activity questions also can be measured for learning. If you can collaborate with colleagues, a similar question asked of both freshman and seniors will provide a measure of student learning as they progress through the program. Disappointing results should not be taken personally but rather as an opportunity to reflect upon the problem: What should be changed or could be different?

Just as you would work in partnership with a research colleague, you should try to collaborate with colleagues on your campus or at other institutions to expand your pedagogical training. Networking by faculty at primarily undergraduate institutions has proved to be especially useful and effective at forming learning faculty communities that are both useful and fun. This coordination of faculty to review and reflect on program goals and student learning is surprisingly informative. From these conversations emerge recognition of clear success or the need for reform and continuing discussions about the students and programs.

Reflecting upon your assessment will provide real information that you will find helpful in crafting and adapting your courses. Coupling your assessment with others' within your program or major provides a clear picture of how student learning has progressed. Aligning the learning in your classroom with that of the other courses that comprise your program will lead to the thoughtful evaluation, change and implementation needed for your program and students to flourish.

So, how to thrill your chairperson? The mere act of providing to your chair and other administrators a clear outline of what you want students to learn and how you have achieved the goals will be met with cheers of delight. Accreditation of institutions of higher learning has changed, with a more holistic emphasis on programs rather than individual courses. Program goals and outcomes need to be defined, and evidence of learning must be aligned to the goals. Furthermore, community, state and federal regulators want proof that you are using funds wisely, especially during these times of diminishing state and federal resources. With planning and nearly painless effort, you can easily document how your efforts have resulted in success.

Sharing your goals and evidence in your annual reports and program reviews will confirm your work, delight your chair and maybe even please your dean.

In need of a new narrative

BY NATASHA C. BROOKS

For better or worse, mass media play a role in the depiction of minorities but also in the way minorities define themselves. Perception can be a powerful influence, cultivating the way we think and act toward the world around us. Consider, for instance, how the dismal 2011 Science report revealing the startling disparity in RO1 funding rates between black researchers and their white counterparts and how stories in the media and minority communities rooted in distrust of science and medicine have shaped the context in which minorities view science and minorities in science. Unfortunately, narratives intended to perpetuate fear for past transgressions, those highlighting health disparities and those regarding minority scientists as exceptional and rare have become the norm. The existing narratives perpetuate the notion that science is 1) perpetrated against them and 2) not for them. This perception has not gone unnoticed among minority scientists. Danielle N. Lee, a scientist involved in outreach and a blogger for Scientific American, classifies these narratives in four groups: disempowerment, disparity, prodigy and overcoming it all.

As a minority and newly minted Ph.D., I question the validity of these narratives and realize that they have detrimental effects on the way we as minorities view and receive science. Therefore, I am committed to changing these perceptions.

The ways in which minority experiences in and with science are defined currently are antiquated and unnecessary. Current news on (human) research geared toward minorities should not be presented in the context of past unethical and discriminatory practices. Focusing on health disparities without addressing strategies to alleviate them does a disservice to minority communities. Choosing to focus primarily on exceptional minority scientists downplays the accomplishments and visibility of the majority of minority researchers. Overall, these narratives instill a negative and limited perception of science within our communities. This results in limited participation by minorities in scientific pursuits and the failure to engage minorities in meaningful dialogue about science that has an impact on their day-to-day lives.

It is time to define new narratives about and to minorities in science. These narratives should shift the consciousness and reporting on science news as it relates to minorities, implement new initiatives to tackle medical issues facing minority communities, and include the perspectives of and bring greater visibility to all minority researchers.

Disempowerment

Disempowerment narratives serve as constant reminders that science is yet another means by which minorities have been disenfranchised and exploited.

Notable stories within this narrative include the Tuskegee syphilis study, the U.S. and Guatemalan government-sponsored research involving intentional infection of Guatemalans with sexually transmitted infections, and the North Carolina eugenics program that sterilized people who were deemed unfit to raise children.

The myths surrounding these studies, in particular the Tuskegee experiments, and disregard for the historical and socioeconomic context in which these studies were performed render an unbalanced view to minorities. Further, these stories perpetuate fear and distrust of medical research.

Yes, these studies were conducted. Yes, they were terrible. But in response to these atrocities, the scientific community has enacted policies to protect human subjects, including ethical guidelines on the treatment of subjects, informed consent and oversight by institutional review boards. Therefore, there is no longer a need to present current medical research in the context of these past studies. It is imperative that we restore the trust of minorities, as clinical trials are essential to finding cures for diseases that are predominant in minority communities.

Disparity

Disparity narratives focus on how individuals from minority groups suffer disproportionately from diseases, access to medical care and so forth. Reports regarding the dismal state of health and healthcare suggest a lack of awareness and regard for health matters within minority communities. This is not a complete or accurate picture of minority health, as it fails to address 1) how intertwined socioeconomic status is with health and 2) what solutions are being put forth to close the gap in health disparities.

Grassroots campaigns in minority communities, such as Black Girls Run, are addressing the obesity epidemic. Black Girls Run is establishing running groups across the country and provides workshops aimed at developing and maintaining healthy lifestyles through exercise and proper diet. These efforts do not get as much press as they should. When I spoke with minorities in my local community, they were unaware that this program exists.

Each year the Minority Affairs Committee of the American Society for Biochemistry and Molecular Biology hosts a health symposium at the Experimental Biology conference. This year, the biology, treatment and challenges of triple-negative breast cancer within minority populations was discussed.

While it is important that scientists understand the progress and limitations of health research, it is more important that those who will benefit from this research understand it as well. I would like to see scientists going into minority communities and hosting these types of symposia.

Prodigy

A prodigy narrative focuses on the success of a young person who accomplishes an unprecedented feat. When he was just 15 years old, Neil deGrasse Tyson was asked to attend Cornell University by renowned astronomer Carl Sagan. However, Tyson chose to attend Harvard University and later earned his doctorate in astrophysics from Columbia University. As the director of the Hayden Planetarium, he made the controversial decision to rename Pluto as a dwarf planet, thus removing its place as the ninth planet in our solar system. When we focus on the accomplishments of scientists such as Tyson, it reinforces that minority scientists are anomalies. This results in science being seen as something that is not for us. Further, it supports the notion that those of us who are not prodigies have become scientists by serendipitous circumstance. In reality, most of us have taken an interest in science from an early age. We have cultivated a pathway to research-based careers by participating in science competitions at the junior level, undertaking science-intensive curricula in high school and college, and participating in undergraduate research. This all culminated in the decision to pursue science at the doctoral level. The Research Spotlight section of the ASBMB website showcases the talents and career accomplishments of minority scientists who, like me, state that the decision to become a scientist was deliberate, conscious and orchestrated.

Overcoming it all

Overcoming-it-all narratives focus on how we as minorities have overcome insurmountable odds in life to become scientists. This narrative is so pervasive that in 2010 it prompted the U.S. House to pass a bill recognizing the extraordinary number of African-Americans who have overcome significant obstacles to enhance innovation and competitiveness in the field of science in the United States. These stories suggest that we as minorities all struggle. They lend credence to the belief that to be successful one has to struggle. While these stories make for excellent reads, they do not pertain to all minority researchers.

I acknowledge that, in the past, it was difficult for minority scientists to accomplish what they did, and in some cases this still persists today. However, the road to becoming a scientist today is not blocked by as many obstacles as in the past. I am wary of overcoming-it-all narratives, as they keep us rooted in the past and, in a sense, stagnate our growth.

Consciously or unconsciously, internal and external expectations are set low for someone who is perceived to struggle. Therefore, any progress beyond menial expectations is perceived as extraordinary, further feeding into the thought that minority scientists are exceptional and rare.

The need for a new narrative

The existing narratives present a monolithic view of minorities in science and have detrimental effects on the way minorities view and receive science. The current narratives are short-sighted, lack complexity and fail to engage minorities in meaningful dialogue about science. Therefore, it is time to define new narratives about and to minorities in science.

Media consumers in the U.S. are exposed to more ideas, viewpoints and perspectives than ever before. We as scientists can use this to our advantage. We can keep minority communities abreast of research aimed at decreasing health disparities through blogs and social-

Continued on page 36

Letter from the new director of the Lipid Research Division

BY VYTAS A. BANKAITIS

In preparing to take on the directorship of the Lipid Research Division, I reflect on the genesis and accomplishments of the division and on the new challenges that lie ahead. The Lipid Research Division was born from a grassroots discussion of broad concerns shared by all lipid research scientists. These included such issues as increased national and international visibility for lipid research, representation within the American Society for Biochemistry and Molecular Biology, and increased funding for lipid research. These foundational goals were outlined in previous ASBMB Today articles, and these set a clear and appropriate course. But in moving forward, it is also of paramount importance that the lipid community actively build upon the progress already made. Realization of these foundational goals will rely on the enthusiastic support of the greater community of lipid researchers. A primary challenge faced by any director of the Lipid Research Division is to foster that enthusiasm and to help translate it into action.

But challenges lie ahead. Some can be foreseen; others cannot be so easily identified at this time. The success of the Lipid Research Division ultimately rests on gainful management of what I perceive to be a fundamental (but unofficial) concern in the lipid community. That, ironically, is one of identity.

The lipid community is composed of divergent disciplines involving chemists, biochemists, physical chemists and physiologists who study both prokaryotic and eukaryotic systems. Imprinted upon these broad disciplines are not only different areas of concentration that focus on understanding the impact of eicosanoids, sphingolipids, glycerolipids and cholesterol metabolism on basic biological processes but also a diverse array of unique technical approaches. Moreover, interest in lipids is obviously increasing in many disciplines, such as neuroscience, developmental biology, cell biology, physiology, clinical medicine and pathogenesis. The large advances made in lipidomics are and will continue to be significant drivers in these areas. With so much scientific breadth and excitement in the lipid arena, how can there be identity issues?

While the scientific diversity and expanding horizons of lipid research are undoubtedly strengths, our ability to meet our future goals and challenges demands that we embrace our primary identity as a community of investigators with a unifying interest in lipid research. It is here where diversity often becomes a double-edged sword. The expanding horizons of lipid research present growing pains as well. In my opinion, these issues play out in the perception by many in the lipid community that recent high-impact lipid research has come from directions removed from the historical core of lipid research.

On one hand, productive evolution of any vibrant discipline absolutely depends on such cross-fertilization, and the conceptual and technical advances that accompany such progress must be welcomed. On the other hand, this evolution does bring with it a rather unique potential for confusing the conceptual frontier of lipid research. This is particularly the case when "high-impact" studies are insufficiently anchored on the essential foundations so carefully laid down by lipid enzymologists and biophysicists. These foundations, and the science behind them, are often alien to researchers who come to lipid science from other disciplines. (I speak with some authority on the issues that accompany unexpected journeys into the world of organic phases and membrane surfaces.) Popularization of simplistic and occasionally naïve "high-impact" cartoon models (for which "high-impact" journals and the scientific community at large have a raging thirst) often exerts an unhealthy influence on editorial decisions at said journals. The ripple effects then flow down to funding decisions that are themselves wrapped in the subjective criterion of impact. It is this cycle that many see as a threat to the very research ultimately required to understand mechanisms (e.g., "boring" enzymological and biophysical work). Those concerns are not without merit. As we move forward, a principle responsibility for the community is to find ways to productively embrace newly developing areas in lipid research and foster their integration with its established foundations. The Lipid Research Division provides an outstanding forum for shaping this essential interface.

In closing, I am compelled to mention how important altruistic citizenship is to the health and vitality of any professional community. I take the opportunity to extend to Dan Raben, Barbara Gordon and the ASBMB the deep gratitude of the community of lipid researchers for their vision in organizing the Lipid Research Division and for their efforts in shepherding the organization through its critical birth phase. As we move forward, Dan and Barbara's experience and advice will be most valuable, and I am particularly grateful to Dan for his willingness to remain actively involved in continued development of the division. Moreover, many of our colleagues, both senior and junior, generously donated their time and talents to making the division a reality. Their contributions were central to getting the project off the ground. While I cannot name them all here, it is essential that their efforts be acknowledged, for they are deeply appreciated. To me, it is the spirit of this extended group that forecasts a bright future for the Lipid Research Division.

The Lipid Research Division set out worthwhile goals at its inception and successfully initiated organization of an infrastructure to support attainment of those goals. Now we come to the stage where execution is the name of the game. I look forward to working with the Lipid Research Division steering committee in formulating pragmatic strategies for reaching the goals already set and in charting the future activities of the organization. This committee is an outstanding and experienced group that is loaded with ideas and opinions, and I am confident that we will navigate those strategic waters effectively. Members of the steering committee and I are excited to have the privilege to serve the lipid community in these most interesting of times. We welcome input of all kinds, so please feel free to contact us directly with your ideas and concerns.

Vytas A. Bankaitis (vytas@tamhsc.edu) is the E. L. Wehner-Welch Foundation Chair in Chemistry at the Texas A&M Health Sciences Center.

ScienceOnline

Science in the Internet 2.0 era

BY GEOFF HUNT

In the seven years since ScienceOnline founders Anton Zuiker and Bora Zikovic wondered if it would be possible (or even advisable) to host a meeting for science bloggers, the conference has exploded beyond its humble North Carolina roots and birthed a new worldwide community of science communicators who are harnessing the power of the Internet to change the way science is done.

ScienceOnline represents a new paradigm for how science will be conducted in the 21st century, one that brings together a (rapidly growing) segment of the scientific community that works at the interface of research and communication. As ScienceOnline Executive Director Karyn Traphagen explains, ScienceOnline "enables connections between researchers in diverse fields of science by bringing them out of their specialized conferences and tapping into their shared experience of using the Internet to do and communicate science online."

Using the vogue un-conference style, ScienceOnline attendees propose, develop and engage in a series of sessions focused on improving the way science is communicated to the public, such as using narrative in science writing, dealing with science deniers and reaching underserved audiences. Yet for conference participants, many of whom are researchers, the best part of ScienceOnline happens outside of the session rooms, where, as neuroscience blogger Scicurious points out, "new conversations get started."

Scientists and communicators rub elbows with Internet celebrities: journalist-bloggers such as Ed Yong and Carl Zimmer, freelance writers such as David Dobbs, and online personalities such as the Huffington Post's Cara Santa Maria and Canadian rap artist Baba Brinkman. Such informal interactions cement personal and professional relationships.

"Being able to meet, and get to know, many of the biggest people in science blogs was huge [for my career]," says Scicurious.

Traphagen agrees, recalling her delight in "hearing stories of joint projects, job opportunities and other connections that have happened because people met each other at ScienceOnline."

Like the neural circuitry in the brain, the online community of science communicators grows and strengthens with each connection made, and those who participate are able to channel their enthusiasm and pass it on to their colleagues. "I've been twice," says science writer Cristy Gelling, "and both times I've come away really energized and inspired."

Thanks to the supportive atmosphere and accessible format, ScienceOnline has inspired the proliferation of spin-offs. Monthly meetings under the ScienceOnline banner have begun in Boston, Seattle, San Francisco, Denver and San Diego, along with a Washington, D.C., version co-organized by the American Society for Biochemistry and Molecular Biology. Satellite events are even taking place in Vancouver, Australia and the Netherlands, taking the movement international.

Some ScienceOnline devotees, including marine biologist David Shiffman, are going even further. Shiffman is heading the development of ScienceOnlineOceans, a three-day conference focused on all things ocean that will make its debut in October. "Marine sciences are a big topic," says Shiffman, "and there's more than enough online science to fill a focused meeting."

Likewise, a group of young scientists organized ScienceOnlineTeen, a teen-centric version of ScienceOnline that took place in April. "ScienceOnlineTeen helps to show students that there's a whole community of people just like them who want to explore and share scientific knowledge to satisfy their own curiosity," points out high-school student and conference co-organizer Hanna Ramsden.

As these satellite meetings and conferences demon-

Continued on page 36

Meet Rajendrani Mukhopadhyay, science writer for ASBMB Today

How did you get involved in science and eventually decide to become a scientist?

I grew up mostly in the Middle East and went through the British education system. In that system, at the age of 16, you pick three or four subjects to focus on ... These three or four subjects are supposed to reflect what you're going to do later in life professionally. I was torn. I excelled in chemistry, biology, English and French and loved history. I wanted to be a writer, but with my grades in school, science also was a viable option. I knew I wanted to be able to support myself financially as an adult. Through movies and books, it appeared to me that writers lived in garrets with only candlelight for warmth, and scientists appeared to be a better-fed lot. So, to the utter dismay of my English teacher, I picked chemistry, biology, physics and French (I really wanted to read Voltaire's "Candide" in its original language) … and focused on becoming a scientist. I did well … and got admission into McGill University in Montreal, Canada, with a full scholarship. I opted for the biochemistry program because it involved both chemistry and biology.

McGill's undergraduate biochemistry program is rigorous and tough. Lectures, labs and homework ate up my time. But … it mostly involved learning numbers, rules and concepts. I had the ability to absorb large bodies of material and churn them out at exam time. Even the lab courses expected me to follow protocols and come up with predetermined answers. So I did well and passed through McGill's program with honors. But I had very little hands-on experience in an actual laboratory setting working on an actual scientific problem.

Did you always know that you wanted to get a Ph.D.?

The Ph.D. became obvious to me toward the end of my second year at McGill. It seemed to me there were three options after getting a bachelor's degree in biochemistry: apply for medical school, be a laboratory technician or get a Ph.D. I never harbored any ambitions for medicine, so that was not an avenue for me. I hesitated to be a technician, because I knew within a year or two I would back at my present situation of wondering, "What next?" At the time that I was trying to figure out what to do, Canada was going through a recession. There wasn't much funding for graduate school. You had to see if an individual principal investigator could support you for the duration of your Ph.D. Also, the programs were designed as three-year programs where you started right away working on a thesis project. I didn't have any confidence that I knew enough about the different areas of biomedical research to decide on what I wanted to focus for a graduate thesis project.

I looked to the south and found the U.S. was in better financial shape and actually supporting students in their quest for a Ph.D. I got into the biochemistry, cellular and molecular biology program at Johns Hopkins University based at the university's medical campus in Baltimore. The most important aspect of the program was that it was five years long, with the first year dedicated to courses in different aspects of molecular biology, biochemistry, biophysics and genetics, and three lab rotations to explore different kinds of research. It was exactly what I was looking for.

What experiences did you have that made you realize that you didn't want to do bench science anymore?

My lab rotations helped me chose to do atomic force microscopy in the laboratory of Jan Hoh for my graduate thesis work. I loved the images taken by AFM, and I enjoyed working with the microscope's parts to get those images. Jan's lab was also different from most of the labs in the Hopkins medical campus in that he pulled together a scientifically diverse group that consisted of physicists, chemical engineers, computational biologists and folks like me with a molecular biology/biochemistry background. Lab

meetings were early training for me as a science writer to learn to talk about science without devolving into jargon.

But my Ph.D. training was the first time I was in the laboratory on my own without an undergraduate teaching assistant hovering in the background and a tested protocol in front of me. Much to my alarm, I discovered that experimental design wasn't intuitive for me. I lacked the instinct and the manual dexterity for experiments. I also lacked the patience needed for research. It was painful to learn there was no such thing as instant gratification in science.

Even more alarming, I was surrounded by peers who seemed to be more at ease in the laboratory than I was. I realized that there was no way I could compete against them when it came to academic or industry research positions after graduation.

All together, I grew miserable and scared. I realized that after all this time, I didn't have what it took to excel in research. All my dreams of following in the footsteps of Marie Curie, James Watson and Francis Crick became obviously naïve. As a 16-year-old, I had set my sights on being a scientist. Now, seven years later, it was horrifying to realize that I may have set off on the wrong path. I needed a plan B, but I didn't have one.

Was it difficult to commit to the decision to leave bench science?

My misery and fright steadily increased through the second and third year of graduate school, and I knew I had to find a way out of academic science. But in my time, the other careers in science were not publicized much, and, indeed, a number of faculty members in my program openly discouraged them. Jan was not one of them and told me on more than one occasion that there were other careers outside of academia that were just as good. But I didn't know where to find information about these other options, and I grew increasingly paralyzed with fear and a sense of failure.

After you decided you didn't want to follow the more traditional path, what path did you take?

The lucky break came from my then-boyfriend, now spouse, who, seeing my utter misery, told me to do something that would give me a break from science. I had heard of the Odyssey Program at Hopkins, which does adult-education programs. Recalling my love for English and French, I enrolled in a creative writing class that was held on Tuesday evenings. On the first day of class, the instructor told us to write about our first names. As I set pen to paper and started to describe how my father gave me the name, which means "the queen of queens" in Sanskrit, I felt a huge weight lift off my shoulders. This was what I wanted to do for the rest of my life. I wanted to write.

The realization rejuvenated me. But I needed to be practical. Even though I wanted to make a living by writing, I knew that my fiction writing wasn't good enough to bring in paychecks. I did have this extensive training in science. It wasn't that I hated science. I loved learning about science in a big-picture way. I just couldn't be bothered to know what was the buffer pH and at which temperatures the measurements were taken! So I started to ask faculty members if there was a career that combined science and writing. The chair of the department, William Agnew, immediately told me that if I knew how to communicate the excitement of science to people who were not scientists, there was a career for me.

The luscious chocolate hazelnut cake from Baltimore's Patisserie Poupon for Raj's Ph.D. graduation was a sweet way to end the chapter of her life spent hunched over the atomic force microscope.

Bill went on to be an important mentor who helped me get my first clips in Hopkins Medical News magazine. The editor of the magazine at the time, Edith Nichols, and the senior science writer, Marjorie Centofanti, quickly taught me the ropes of writing for a nontechnical audience.

Once I knew I wanted to pursue science writing, I asked every faculty member I met if they knew a science writer. That's how I got introduced to Joanna Downer, who was then at the Hopkins medical school's media office and is now at Duke University. Joanna and Marjorie helped me get into the National Association of Science Writers so I could see what science writers talked about every day and also get job alerts to see what the requirements were to break into the field.

When I was in my fourth year, Hopkins launched its Professional Development Office at the medical campus, which became an important resource for me, as well as the Science Careers website. I began to realize that there were many people with extensive scientific training who opted for careers in areas such as science policy, law, communications and management consulting. It was reassuring to know that I wasn't a failure for not continuing on in academic research and that I could do something worthwhile with my scientific training.

With the support and help of people like Bill, Marjorie, Joanna and Jan, by the time I was in my fifth year of graduate school, I was set on the path of a career in science writing. I had already devoted the evenings after I had finished up in the laboratory to building up my portfolio of clips. By the time I graduated with my Ph.D., I had written several columns for Hopkins Medical News magazine, an article for the Science Careers website, a couple of columns for the Hopkins Graduate Student Newsletter, and a creative nonfiction piece in a magazine. With that portfolio and my résumé, I landed my first job as a science writer and reporter at the American Chemical Society right after graduation. I've never looked back.

Incidentally, I met up with my high-school English teacher, Shane Heslin, shortly after starting the ACS job. When he heard that I was writing for a living, he said with a huge grin, "I told you so!"

Could you give our readers an idea of what your current job involves?

Deadlines drive my life as a science writer. I write posts for the blog Wild Types as well as stories for ASBMB Today. Every post and story has a deadline by which it has to written and be ready for publication. By the time of the deadline for each story, I need to research a topic, interview the appropriate scientists working in that field, transcribe the interviews, write a draft of the story, which will be revised several times, and fact-check it. I also work on the Journal of Biological Chemistry in writing up the Paper of the Week summaries and editing titles to eliminate jargon. All this means I have to be very organized with my time and make sure nothing falls behind. So every day I set time aside for different activities: making phone calls, transcribing interviews, revising pieces I have in development, searching the scientific literature and social media outlets like Twitter and Reddit for new story ideas, and helping with the layout of stories heading out the door for publication. As you can tell, if I do get a quiet moment, I wonder, "What am I forgetting?"

Does any of the training or education that you received help you in your current career path?

There are stellar science writers out there who don't have Ph.D.s and some who don't even have science degrees. A good science writer is someone who is curious and loves to tell stories. A science writer is also not afraid to go after topics about which he or she initially has very little knowledge. I have written about the 1976 Viking mission to Mars, art analysis, performance-enhancing drugs taken by cheating athletes and the biofuels industry. These are all topics that I didn't know much about going in. So a strong sense of curiosity, the ability to ask the right questions, and being able to frame all the information into a story that takes readers on a journey are the only requisites for science writing.

For me, though, I do feel the Ph.D. training helped me be the writer I am today. As a graduate student, I had to learn not to be intimidated by the unknown. Being a good student in high school and college gave me the unfortunate mentality that it was a sign of weakness not to know something. Being in an academic research environment for five years showed me how scientists think – shades of gray, not black and white! That has been invaluable for me when framing my interview questions for stories. I also learned that the single eureka moment is rare in science and that a single publication represents years of a student or postdoctoral fellow's work. I respect that and always keep it at the back of my mind.

But you don't have to go through a Ph.D. training program to learn these things. Just chatting long enough with scientists will teach you these same principles. I will never advocate going through a Ph.D. program if you know early on that you want to become a science writer. There are other avenues, such as science writing master's programs and internships. But if you are like me and you discover in the middle of a Ph.D. program that it's not a good fit, you can turn around that experiment gone awry in your favor.

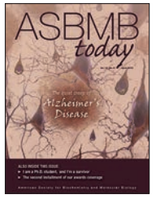
What advice would you give to undergraduates who may know that they like science and may want a career in science but don't yet know exactly what they want to do once they graduate?

Talk to graduate students, postdocs, faculty members, family members, friends, neighbors – anybody willing to hear you out. You never know who knows what and may turn you in a direction you had never imagined existed. And stop occasionally to think critically about what you are doing and how you see it steering your future. I wish I had done that earlier in my education and saved myself a lot of heartache.

Editor's note
This Q&A originally was published in Enzymatic, the newsletter for the American Society for Biochemistry and Molecular Biology's Undergraduate Affiliate Network. Here, it has been edited for length, clarity and style.

open channels

EDITOR'S NOTE



Re: The quiet creep of Alzheimer's, April 2013: Since our article was published, Jodi Bottoni placed her mother, Jacquie Berg, in an assisted-living home. Their friend KC Jones reports that Berg is very happy there. She has made friends, and she sings and dances. Bottoni now is trying to catch up on the parts of life that she missed out on while taking care of Jacquie full time.

ON THE WILD TYPES BLOG

Science and the law: Want to see how the U.S. Supreme Court hearing on gene patents went down on Twitter? Visit the Wild Types blog for a collection.

READER COMMENTS

Re: President's Message "New meets old," March 2013: I very much enjoyed your "Kluyver" article. Most beginning biochemistry students take the "Unity of Biochemistry" pretty much for granted, although they would never recognize that their, or their professor's, choice of an organism for study depends on incidental properties that have nothing to do with this Unity. You might be most interested in a long paper of mine: "From 'Butyribacterium' to 'E. coli', An Essay on Unity in Biochemistry," Perspectives in Biology and Medicine, 47, 47-66 (2004). Some people did not like it, since it did not praise Monod to the heavens, but many agreed.

– HERBERT FRIEDMANN

minority affairs continued

Continued from page 27

networking media. We can host science cafés and collaborate with civic organizations to reach out to minority communities. In particular, we as minority scientists should make a greater effort to engage our communities in scientific dialogue. In this way, we will begin to shed the negative narratives that keep us away from science. I realize that what I am suggesting will take time as well as a change in mindset. I am hopeful that, if we all do our part, we will begin to see positive narratives regarding minorities and science.

Natasha C. Brooks (ncbrooks@gmail.com) earned her degree in biochemistry and molecular biology from the University of Texas Medical Branch. She currently serves as the postdoctoral representative of the ASBMB Minority Affairs Committee.

outreach continued

Continued from page 30

strate, the demand for events such as ScienceOnline is skyrocketing. The well-documented need for scientists to become better communicators is part of the new world order for science. Being able to communicate science effectively to the public is now a required skill, a point hammered home in a recent article by Dominique Brossard and Dietram A. Scheufele in the journal Science, which stated that "a world in which one in seven people actively use Facebook and more than 340 million tweets are being posted every day is not the future of science communication any more. It is today's reality."

With science reporting by the mainstream media fading, science communicators are becoming the sole sources of reliable, accurate scientific information. Luckily, meetings like ScienceOnline are providing the avenues for novices to become experts, individuals to become part of a community, and the community as a whole to grow and improve.

Ultimately, what makes this trend so successful is the passion for science shared by those who are taking part and the unified goal of making science more accessible to the public. Now it is up to rest of the scientific community to come along for the ride. There is plenty of room on board.

To learn more about ScienceOnline, visit scienceonline.com or connect with the community on Twitter by searching for #scio13 and #scio14.

Geoff Hunt (ghunt@asbmb.org) is ASBMB's outreach coordinator. Follow him on Twitter at www.twitter.com/goodbyeshoe.

The 2014
ASBMB ANNUAL MEETING

SAN DIEGO

APRIL 26-30, 2014