Vol. 16 / No. 1 / January 2017

ASBMB TODAY

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

A CHAIN OF EVENTS

LINEAR UBIQUITIN CHAINS AND THEIR ROLE IN A DISEASE

CONTENTS

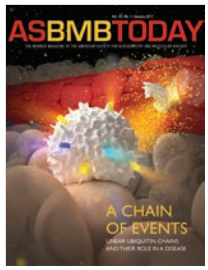
NEWS

13

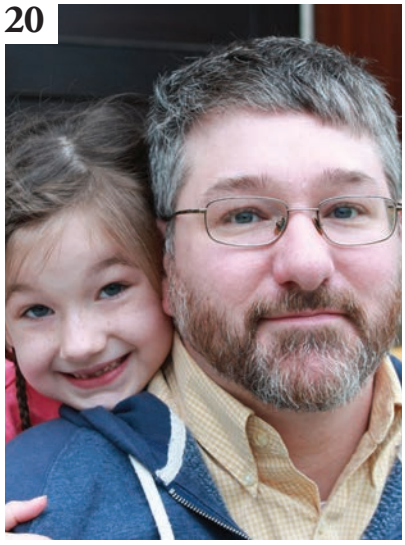
FEATURES

14

Linear ubiquitin chains, whose very existence once was debated, now are known to play a critical role in an inflammatory disease, thanks to the discovery of an enzyme.



20



PERSPECTIVES

34

31

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

ASBMB
American Society for Biochemistry and Molecular Biology
www.asbmb.org/asbmbtoday
PRINT ISSN 2372-0409

Introducing 'The do-over' series

By Rajendrani Mukhopadhyay

I wish I hadn't skipped freshman year of college.

I know I come across as a driven, ambitious woman now, but many don't realize that I have mellowed a bit over the years. At age 19, I was on fire. I had a plan, and I was going to execute it, come hell or high water (high water in the forms of ice and snow because I was in Canada for college).

As a good number of firstborns will appreciate, I carried a weight on my shoulders to make my parents proud of me. As a woman originally from India, the weight was even heavier, because I felt I had to prove that girls could be as successful as boys. My grand life plan involved getting an education that would allow me to become a financially independent, self-sufficient woman who would achieve great things. (What those great things were going to be was to be determined, but whatever they were, they were going to be dazzling.) Nothing was going slow me down, and that included freshman year of college.

Growing up in the Middle East and India, I attended schools that followed the British system of education. With my four Advanced Levels in physics, chemistry, biology and French, I had enough credits to leapfrog over the requirements for freshman year at McGill University in Montreal, declare a major and get on with earning the remaining 90 credits for a bachelor's degree.

The adviser for new students who reviewed my school transcripts and exam records said I was eligible to skip freshman year. But as he started to say that doing freshman year still remained an option, I cut him off. I heard what I was waiting to hear. I told him I already had decided to major in biochemistry, so where did I have to go and whom did I have to meet to make that happen?

The man looked at me through his black-rimmed glasses with a mix of amusement and exasperation and gave me directions to the McIntyre building, where the biochemistry department was housed at McGill. As soon as he finished giving me directions, I grabbed my folder of papers and shot out the door. It was the middle of a Friday afternoon. I wanted everything settled by 5 p.m. so that I'd be ready for classes on Monday. I barged into a couple of offices in the McIntyre building until I found the right person who could get me enrolled into the program. By 5 p.m. that Friday, I was a biochemistry major.

Now, I'll say here that I'm not one to wallow in the past. I can't change it, so why bother overthinking it? I live more for the future and deal with the present.

But if I do spare a thought for the one time in my life that I could have done differently, freshman year was it. I was not ready to skip that year. In my zeal to reach for the stars, I had not accounted for how unprepared I was for my new life. I knew in my head that things were going to be different, but I wasn't prepared for the never-ending vortex of confusion.

I was fluent in English and French, which was useful in the bilingual city of Montreal, and, thanks to music, books, TV shows and movies, my cul-

tural references were mostly Western. But coming from the more conservative societies of Kuwait and India, where I watched censored versions of "The Sound of Music" and Disney's "The Lion King," I was painfully innocent. I remember being very puzzled at a professor's reaction when he said he hadn't gotten around to writing the midterm exam. I responded with, "Oh, you naughty boy! Hope you don't get punished for that." The professor immediately left the room. When I recounted the incident in total bewilderment to a fellow student, she laughed so hard that she got the hiccups.

I never had been out and about on my own. I never had been to movie theaters. I never had to do laundry or shop for groceries. I never had seen snow and ice, let alone learned how to walk on them. For the first time, I was far away from my parents, aunts, uncles, cousins and parents' friends and felt unmoored by the lack of eyes of scrutinizing my every move.

In the midst of all these life-changing experiences, I found myself caught in a challenging major. My reason to major in biochemistry was nothing more sophisticated than that I wanted to be a researcher and I had excelled in school in chemistry and biology. Never mind the fact that I hadn't done a day's work in a laboratory and had no clue what I was working toward. Even the most fundamental aspect of education, the learning part, threw me for a loop. Instead of being in a classroom of 15 high-school students where I kept correcting the teacher's spelling of "Escherichia coli," I found myself anonymous and lost in the back of a lecture hall of 600 students, all jockeying to be ahead of the grading curve for Molecular Biology 101.

I later qualified to do honors in biochemistry. That meant 87 out of the 90 credits I needed for the bachelor's degree went toward fulfilling the major's requirements. My days were filled with an endless stream of molecules, numbers and equations in courses such as Organic Chemistry III, Calculus III and Methods in Biophysics. With my last remaining three credits, I splurged on a class about Alfred Hitchcock films offered by the School of Arts. It was the only course I took outside of science. I was so crunched by the demands of my science classes, I didn't even have time to think what it meant that I loved that Hitchcock class, with the writing I had to do for it, more than all my other classes.

If I had done freshman year, I would have been forced to slow down and take stock instead of being caught in a vortex of cultural and academic chaos from the get-go. I would have had the time to weather more evenly the shocks of being in a new place. I would have had more time to grasp the overwhelming sense of novelty of going out on my own to grab a meal at Nickel's on St. Catherine Street. I could have focused on learning the life skills I was lacking, such as knowing how to write a check. If I had done freshman year, I would have had time to recognize that I was at a large educational institution with more options than my school back home offered. If I had done freshman year, maybe I would have realized much sooner that I was better suited to be a writer than a researcher. How much heartache would I have saved myself if I had realized that sooner? I will never know.

The personal essay series we're running this year in ASBMB Today is called "The do-over." We have invited members of our community to share their reflections on what they would have done differently in their lives. In this issue, Stefan Lukianov describes in his essay how he wrecked an important relationship in the pursuit of science. We have essays coming up in forthcoming issues in which writers wonder how their lives would have turned out if they had picked a different graduate school, part of the world to live in or a career. Each essay, in going into reflection, carries senses of hope, growth and humor. We hope you'll enjoy them and feel inspired to share an essay of your own (we've extended the deadline for the series).

Rajendrani Mukhopadhyay (rmukhopadhyay@asbmb.org) is ASBMB Today's managing editor. Follow her on Twitter at twitter.com/rajmukhop.

THE DO-OVER

If you could erase a part of your life and do it over again, which part of your life would that be? What would you do differently?

For an essay series in 2017, ASBMB Today is asking its readers to send in essays about do-overs. Maybe you regretted your choice of college. Maybe you trusted someone who let you down. Perhaps you wonder what would have happened if you had picked that other research project. Whatever it is, be honest and true.

Essays must be unpublished and between 500 to 1,000 words. Submissions can be sent to **http://asbmbtoday.submittable.com/**. Submit under "The Do-Over." **Deadline extended: Aug. 31.** Please include in your essay a title, complete contact information and an author bio of no more than 50 words.

Hope and concern

By Benjamin Corb

Happy New Year! We enter 2017 with a sense of hope and optimism, tinted with a deep sense of concern and realism about the challenging environment we have ahead of us. The 115th Congress will be seated this month, and Donald Trump will be sworn in as the 45th president of the United States. The new Congress and administration will come with a new political agenda that will influence the direction of the American scientific enterprise.

There is political uncertainty in the weeks and months ahead, but the commitment of the American Society for Biochemistry and Molecular Biology Public Affairs Advisory Committee to represent the needs of our community to policymakers remains unchanged. We enter 2017 with a clear message: The nation benefits from investments in the biomedical research enterprise. The work that our members do plays a critical role in ensuring that America is the global leader in biomedical innovation.

We are excited about the plans we have for the upcoming year. We will start 2017 by preparing a report to share with the new Congress and president that outlines the core pillars of our policy agenda. In our report, we will explain how investing in biomedical research strengthens the economy, creates jobs and helps to reduce the burden of disease through improved treatments and cures for millions of Americans. We will be advocating for increases in funding at federal agencies that support our mission and promoting policies that ensure a fertile environment for biomedical research. To do so effectively, we will start the year with an initiative to educate the newly elected members of government on what fundamental biomedical researchers do and how they, as our elected officials, can help us.

Beyond legislative actions, we remain committed to developing a sustainable biomedical research enterprise, an effort we have been leading for more than two years. Next month, we'll have a discussion about the accomplishments we've made in working with stakeholders and following up on the recommendations that came out of the workshop we hosted last February on building a sustainable research enterprise. In 2016, we planted the seeds for workforce analysis, new tools to help us advocate for sustained funding, and a platform to meet the needs of postdoctoral scholars; in 2017, we are ready to reap what we have sowed.

Additionally, we'll be calling on you. In addition to our annual Hill Day event of visiting the U.S. Congress and the August advocacy push, we'll have more opportunities for you to be involved in advocating for your science. We will be launching a new "letter to the editor" campaign this spring and are working with our colleagues in the Public Outreach Committee to develop an online course to teach you how to communicate with policymakers effectively.

This year's political environment will not be without challenges. But with your support and involvement, we can rise to the occasion and work toward a bright future for science.

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

Interested in science policy?

Follow our blog for news, analysis and commentary on policy issues affecting scientists, research funding and society. Visit **policy.asbmb.org**.

ASBMB members elected to National Academy of Medicine

Bonnie Bassler, Stanley L. Hazen, Aziz Sancar and Michelle A. Williams are among the 79 new members elected to the National Academy of Medicine.


BASSLER

Bassler is the Squibb professor and chair of the department of molecular biology at Princeton University and an investigator at the Howard Hughes Medical Institute. She has received numerous awards for her recent work on quorum sensing.


HAZEN

Hazen holds the Jan Bleeksma chair in vascular cell biology and atherosclerosis and the Leonard Krieger chair in preventative cardiology at the Cleveland Clinic. He has made multiple discoveries that link gut microbial metabolites to the pathogenesis of cardiovascular and metabolic diseases.


SANCAR

Sancar is the Sarah Graham Kenan professor of biochemistry and biophysics at the University of North Carolina at Chapel Hill. His research focuses on the circadian clock as well as DNA repair. Sancar won the Nobel Prize in chemistry in 2015 and the ASBMB's Bert and Natalie Vallee Award in Biomedical Science in 2016 for his work on DNA repair.


WILLIAMS

Williams is the dean of the faculty at the Harvard T.H. Chan School of Public Health. She studies reproductive and perinatal epidemiology. Among the research and teaching awards she has received are the American Public Health Association's Abraham Lilienfeld Award and the Presidential Award for Excellence in Science, Mathematics, and Engineering Mentoring.

Established in 1970 as the Institute of Medicine, the National Academy of Medicine is a nonprofit that addresses policy issues related to health, medicine and science.

Gordon receives Beering Award


GORDON

Jeffrey I. Gordon, the Dr. Robert J. Glaser distinguished university professor and director of the Center for Genome Sciences at Washington University in St. Louis, received the Stephen C. Beering Award from the Indiana University School of Medicine for his research on the human microbiome.

Gordon and his students study the genomic and metabolic bases of our relationship with beneficial gut microbes, with a focus on how gut microbial communities form and how they affect nutritional status, notably obesity and childhood malnutrition

Established in 1983, the Beering Award honors the legacy of Steven C. Beering, who served as dean of the medical school from 1974 to 1983 and later as president of Purdue University.

The annual award recognizes an individual whose research has helped to advance biomedical or clinical science. The award carries a $25,000 prize.

Lippard earns Welch award


LIPPARD

Stephen J. Lippard, the Arthur Amos Noyes professor of chemistry at the Massachusetts Institute of Technology, is a recipient of the Robert A. Welch Award in Chemistry.

Presented by the Welch Foundation, the award seeks to encourage basic chemical research, recognizing individuals who contribute outstanding chemical research to the benefit of humankind.

Lippard is considered a leading figure in the field of bioinorganic chemistry, a discipline that covers both biological and inorganic chemistry. He has contributed significant research on the mechanism of the anti-cancer drug, cisplatin.

Lippard will share the award, which carries a $500,000 purse, with Richard H. Holm of Harvard University.

Bruchas and Huganir win BRAIN Initiative grants

Michael Bruchas and Richard Huganir are recipients of the National Institutes of Health's recent grants to support the Brain Research through Advancing Innovative Neurotechnologies, or BRAIN Initiative. In 2013, President Barack Obama introduced the BRAIN Initiative as a means of supporting research to better understand and treat the wide variety of neurological diseases.


BRUCHAS

Bruchas and Huganir have been recognized as a part of the Tools for Cells and Circuits grant, which supports research designed to develop novel techniques for rapidly identifying cells and genes that control certain brain circuits.

Bruchas is an associate professor in the departments of anesthesiology and neuroscience at the University of Washington in St. Louis. He is developing and validating a broader array of next-gen, optically controlled G-protein–coupled receptors.

Huganir is professor and director

CONTINUED ON PAGE 6

CONTINUED FROM PAGE 5


HUGANIR

of the department of neuroscience at Johns Hopkins School of Medicine. He is developing new genetically encoded fluorescent biosensors to help visualize cell-specific and circuit-specific signaling pathways.

— By Erik Chaulk

Smith wins lectureship award


SMITH

Janet L. Smith, the Margaret J. Hunter collegiate professor in the life sciences and professor of biological chemistry at the University of Michigan, has been honored with the university's Distinguished Faculty Lectureship Award in Biomedical Research. Established in 1979 by the Biomedical Research Council, the award is the highest honor bestowed by the medical school upon a faculty member for excellence in biomedical research, teaching and service to the university and the scientific community at large.

Smith's numerous accomplishments in structural biology include the development and implementation of methodologies that help us better understand macromolecules. She is a key developer of the multi-/single-wavelength anomalous diffraction technique, most widely used for de novo macromolecular phase determination, and is the scientific director of the GM/CA @ APS beamlines for biological crystallography at the Argonne National Laboratory. Smith became a fellow of the American Association for the Advancement of Science in 2007 and was the recipient of the National Institutes of Health MERIT award from 1998–2008.

— By Vivian Tang

Zhu wins Tabor award for novel proteomic approach

By Lee D. Gibbs

Haojie Zhu, assistant professor in the department of clinical pharmacy at the University of Michigan, was named the recipient of a Journal of Biological Chemistry/Herbert Tabor Young Investigator Award for his work on a novel approach for precise quantification of drug-metabolizing enzymes and transporters. This pharmacoproteomics assay has been used to identify and characterize the variants that regulate gene expression at a protein level. Zhu's work has the potential to provide essential information for effectively guiding personalized medicine. The JBC's deputy editor, Fred Guengerich of Vanderbilt University, presented the Tabor award to Zhu at the 21st International Symposium on Microsomes and Drug Oxidation in early October in Davis, California.

"My laboratory has developed a novel pharmacoproteomics approach combining pharmacogenomics and proteomics for identification and characterization of genetic and non-genetic biomarkers that are associated with individual variability in drug response," says Zhu. He adds that these biomarkers eventually can be used in clinical practice to improve the efficacy and safety of pharmacotherapy. A better understanding of individual variability in the function of drug-metabolizing enzymes and transporters will help clinicians develop more personalized therapeutic regimens to improve outcomes for their patients.

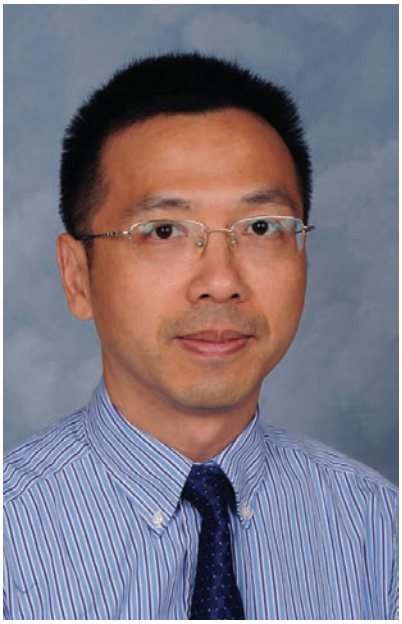


PHOTO PROVIDED BY HAOJIE ZHU

Haojie Zhu

Zhu was born and raised in Yangzhou, a city in east China. He received a pharmacy diploma from the China Pharmaceutical University and returned to the same university several years later for graduate school. After completing his Ph.D. in pharmacology, he joined the laboratory of C. Lindsay DeVane and John Markowitz at the Medical University of South Carolina, where he conducted his postdoctoral research in pharmacogenomics and neuropharmacology. After his postdoctoral training, he continued his research in translational pharmacogenomics as a research assistant professor, first at MUSC and then at the Center for Pharmacogenomics at the University of Florida College of Pharmacy. Zhu joined the University of Michigan in 2013.

Lee D. Gibbs (Lee.Gibbs@live.unthsc.edu) is a Ph.D. candidate at the University of North Texas Health Science.

LAG3 helps to transmit fibrils in Parkinson's disease

By Dawn Hayward

"There's gotta be a way it's getting into cells," says Ted Dawson of the Johns Hopkins University School of Medicine. He's referring to fibrillar alpha-synuclein, the culprit of Parkinson's disease, which is the second-most common neurological disorder.

More than 60,000 people get diagnosed with Parkinson's disease in the U.S. each year. The disease is one of several brain disorders where the root cause is the transmission of a protein in the form of aggregates through neurons. The aggregated protein in Parkinson's disease is alpha-synuclein. Although usually monomeric with a function that's not known, alpha-synuclein can misfold and form clumps that cause neuronal cell death.

In a study published Sept. 30 in the journal Science, members of the Dawson laboratory identified LAG3 as a receptor for the pathological alpha-synuclein. LAG3 preferentially bound the alpha-synuclein fibrils, suggesting that LAG3 acted as a doorway into cells for alpha-synuclein aggregates and permitted their transmission.

For their experiments, the investigators used preformed fibrils, or PFFs. PFFs are an experimental tool that mimics alpha-synuclein fibrils. They were developed by Virginia Lee's laboratory at the University of Pennsylvania. It was the initial study using PFFs by the Lee group, published in 2011 in the journal Neuron, that motivated the Dawson group.

"It was just a beautiful experiment," says Dawson. The Lee group added PFFs to wild-type neurons and got the "disease in the dish," says Dawson. In cultured neurons in which alpha-synuclein was knocked out, no hallmarks of Parkinson's disease were observed. This suggested that fibrils somehow bound and entered cells to cause toxicity. So how exactly did these fibrils gain access to cells?

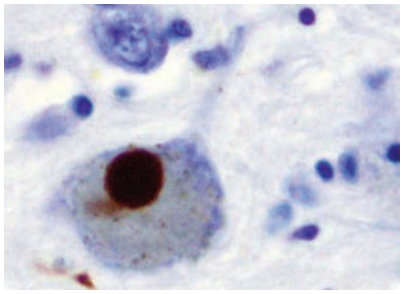


IMAGE COURTESY OF WIKIMEDIA

Fibrillar alpha-synuclein (brown) shows up in Parkinson's disease.

The Dawson group screened a library of 352 proteins for binding partners of PFFs. The library, which had been used by Stephen Strittmatter's group at Yale University, consisted of transmembrane proteins. The Dawson group found three proteins that bound fibrillar alpha-synuclein. Lymphocyte activation gene 3, or LAG3, had the highest affinity for the fibrils.

When the Dawson group investigated LAG3, it preferred to bind fibrils rather than monomers. It was expressed on neurons but not on astrocytes or microglia. The number of internalized PFFs was significantly lower in LAG3-knockout neurons.

The investigators next used a microfluidic device. In this device, three adjacent chambers of cultured neurons were connected by grooves. Initially, wild-type neurons were cultured in all three chambers and PFFs were added to the first chamber. PFFs were observed to be transmitted to the third chamber. However, when LAG3-knockout neurons were cultured in the second chamber, fewer PFFs were transmitted to the third chamber. When LAG3 was added back to the second chamber, transmission of PFFs was restored. These experiments "showed that LAG3 was really responsible for the cell-to-cell communication," says Dawson.

New questions now arise. First, why is LAG3, an immune-system protein, expressed on neurons at all? Second, LAG3 contains an intracellular domain that may signal when bound to fibrillar alpha-synuclein. "It's got to be doing something," says Dawson. But, he adds, no one knows what LAG3's intracellular domain does. Third, while LAG3 may be important for taking in alpha-synuclein, other pathways of entry are possible. Dawson underscores that knocking out LAG3 does not completely halt fibrillar transmission.

Preliminary studies in LAG3-knockout mice support the use of antibodies against LAG3 as therapeutics; a more in-depth study is underway. Dawson is quick to note that it's still unclear if these antibodies can cross the blood–brain barrier.

LAG3 is a new piece of the Parkinson's disease puzzle. It requires more attention to understand its role as the partner in crime for alpha-synuclein.

Dawn Hayward (dhaywar5@jhmi.edu) is a graduate student at the Johns Hopkins University School of Medicine.

Proteins and lipids — a complicated relationship?

By Eva Sevcsik & Gerhard J. Schütz

Researchers have been discussing for many years the role of the lipid matrix in regulating the activity and the organization of membrane proteins. A variety of effects have been singled out and studied qualitatively and quantitatively in model systems. However, the applicability of those results to living cells is — in many cases — unsatisfactory. Here, we would like to make the point that the complexity of the lipid–protein matrix in cells alters the physico-chemical mechanisms of protein–lipid interactions to an unknown extent when compared to model systems.

We shall discriminate between global and local mechanisms. Global mechanisms are mediated by lipid-bilayer properties; local mechanisms denote a direct molecular interaction between a protein and a lipid molecule. Examples of global effects include curvature, hydrophobic mismatch and preferential partitioning in phase-separated membranes ("rafts") (1–3); examples of local mechanisms are the direct binding of cholesterol to CRAC domains (4) or of phosphatidylinositol 4,5-bisphosphate to protein subunits (5).

Formally, we may characterize a protein via its chemical potential μ, with the values for two different functional or structural states, μ_1 and μ_2. If $|\Delta\mu| = |\mu_1 - \mu_2| \gg RT$ (R is the gas constant, T the temperature), a pronounced preference for one of the two states will occur. In contrast, for $|\Delta\mu| \ll RT$, we expect no preferred state.

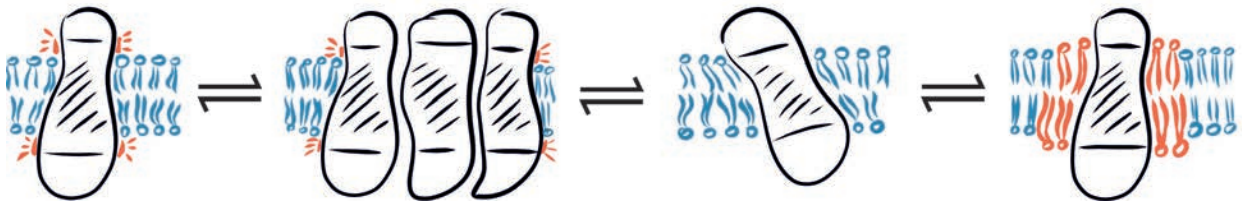
We would like to make the point that the complexity of the lipid-protein matrix in cells alters the physico-chemical mechanisms of protein-lipid interactions to an unknown extent when compared to model systems.

Most of the data for global mechanisms come from studies on simple, well-defined model systems, which allow for specifically addressing individual parameters. To emphasize the effects, such model systems usually are selected to achieve substantial contrast between μ_1 and μ_2. Examples would be the partitioning of proteins into ordered versus disordered phases in phase-separated lipid bilayers (6) or the recruitment of proteins to lipid vesicles with different curvature (7). Also, for local mechanisms, chemical potentials are the appropriate means of quantitating a protein's state: μ_1 denotes the lipid-bound state, μ_2 the unbound state, and $K_D = \exp\left[-\frac{\Delta\mu}{RT}\right]$ the equilibrium binding constant.

In cell membranes, however, a plethora of lipid species with varying properties, such as different head groups, acyl chain lengths and degrees of saturation, increases the complexity of the situation. The consequence for global mechanisms will be myriad chemical potentials describing the possible states of the protein, which can be approximated by a continuous energy landscape: Proteins essentially fluctuate between the different states. In some cases, cells may amplify the difference in chemical potential by de novo assembly of membrane structures, such as clathrin-coated pits, so that the partitioning or activity contrast will become more pronounced.

Also, in the case of local mechanisms, a variety of lipids may be able to interact with the protein of interest, potentially with only slightly different affinities. This leads to the recruitment

CONTINUED ON PAGE 10



SCHEMATIC PROVIDED BY EVA SEVCSIK AND GERHARD J . SCHÜTZ

In a complex environment, hydrophobic mismatch may cause a protein to fluctuate between different states: (from left to right) hydrophobic mismatch, protein aggregation, protein tilting and recruitment of long-chain lipids.

CONTINUED FROM PAGE 9

of specific lipid species to the vicinity of the protein and an essentially continuous distribution of chemical potentials. Again, in some cases, preferred interactions of the protein with one type of lipid occur, yielding additional discrete values of μ.

What would be the consequences of a continuous distribution of chemical potentials? There would be no clear-cut states of a protein. For example, hydrophobic mismatch, on a stochastic basis, may lead transiently to demixing of the protein, the recruitment of a shell of long-chain lipids and membrane curvature. The system would fluctuate between these scenarios. Only in cases where the energy continuum splits up, or where distinct extra-states exist, can we expect distinct states of a protein. In conclusion, studies of well-defined model systems certainly help our understanding of fundamental physico-chemical properties, but the complexity of the live cell environment provides many more options to minimize the global energy of the system.

REFERENCES

1. Milovanovic, D. *et al. Nat. Commun.* **6**, 5984 (2015).
2. McMahon, H.T. & Boucrot, E. J. *Cell. Sci.* **128**, 1065 – 1070 (2015).
3. Van Meer, G. *et al. Nat. Rev. Mol. Cell Biol.* **9**, 112 – 124 (2008).
4. Fantini, J. & Barrantes, F.J., *Front. Physiol.* **4**, 31 (2013).
5. Hamilton, P.J. *et al. Nat. Chem. Biol.* **10**, 582 – 589 (2014).
6. Dietrich, C. *et al. Proc. Natl. Acad. Sci. USA* **98**, 10642 – 10647 (2001).
7. Hatzakis, N.S. *et al. Nat. Chem. Biol.* **5**, 835 – 841 (2009).

Eva Sevcsik (sevcsik@iap.tuwien.ac.at) is a researcher at the Institute of Applied Physics at TU Wien in Austria. Gerhard J. Schütz (schuetz@iap.tuwien.ac.at) leads the biophysics group at the Institute of Applied Physics at TU Wien.

The old-dog catalase has a new trick

By Amber Lucas

One way the human body protects itself against invading bacteria is by generating reactive oxygen species that harm the bacteria. Helicobacter pylori, a pathogen present in the stomachs of almost half of the world's human population, has developed mechanisms to resist the damage induced by ROS. For more than 100 years, scientists have known that catalase helps protect the H. pylori against ROS by breaking down harmful hydrogen peroxide. But in a recent Paper of the Week in the **Journal of Biological Chemistry**, Stéphane Benoit and Robert Maier at the University of Georgia showed that catalase is no one-trick pony: In addition to its enzymatic activity, catalase has another mechanism for protection against harmful ROS that is independent from what was originally thought to be catalase's only enterprise.

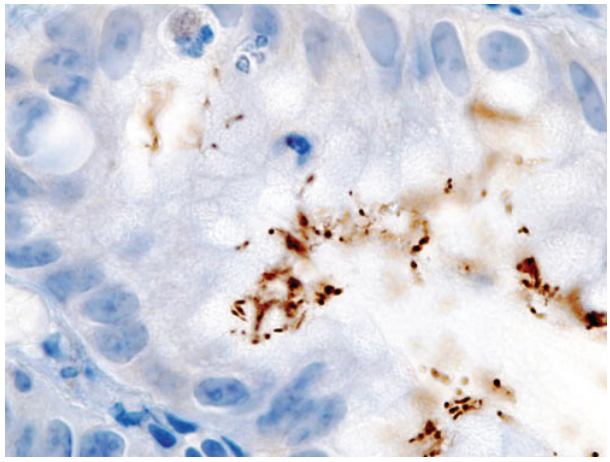


IMAGE COURTESY OF WIKIMEDIA

Immunohistochemical staining of H. pylori (brown) from a gastric biopsy.

Part two of catalase's story began 20 years ago with the observation that methionine residues in proteins undergo oxidation in the presence of ROS to form methionine sulfoxide. Scientists hypothesized that these methionine residues were acting as antioxidants to save other sites, such as DNA bases. However, evidence to support this hypothesis was lacking.

A few years ago, researchers from the Maier lab showed that methionine residues in catalase undergo oxidation in the presence of hypochlorous acid, an ROS produced by many white blood cells in response to an infection. This oxidation is reversible. Methionine sulfoxide reductase physically interacts with catalase to reduce methionine sulfoxide back to methionine.

Taken together, this led Benoit and Maier to hypothesize that the methionine residues in catalase serve as recyclable antioxidants to protect the bacteria from the host-generated ROS, something that never had been observed in an organism before. In order to show that catalase's methionine antioxidant role is independent from its enzymatic function, Benoit and Maier engineered enzymatically inactive catalase, known as apo-catalase. They treated media containing hypochlorous acid, an oxidant that is normally lethal to H. pylori, with apo-catalase before exposing H. pylori to the media. The survival of H. pylori indicated that the apo-catalase protected the bacterium from oxidative stress in vitro. The investigators were able to show that this was also true in vivo by growing H. pylori expressing the apo-catalase on agar media containing hypochlorous acid. The H. pylori expressing the apo-catalase grew just as well as H. pylori expressing enzymatically active catalase, while H. pylori lacking catalase were more sensitive to hypochlorous acid.

Benoit and Maier showed that this process is dependent on methionine oxidation by knocking out the Msr enzyme that recycles methionine sulfoxide back to methionine after oxidation. The apo-catalase/Msr knockout showed pronounced sensitivity to hypochlorous acid when compared to the apo-catalase alone, signifying that the methionines must be recycled for apo-catalase to act effectively as an antioxidant.

Next, Benoit and Maier explored whether H. pylori expressing apo-catalase could colonize the stomachs of laboratory mice. The authors showed that H. pylori expressing apo-catalase was able to colonize the stomachs as efficiently as H. pylori expressing wild-type catalase and was several orders of magnitude better than H. pylori catalase knockouts. This revealed that catalase is conferring a fitness advantage for H. pylori that is completely independent of its enzymatic activity by protecting the bacterium from host-generated ROS in the mice.

The results of this study revealed that catalase is multifaceted, protecting invading H. pylori from host-generated ROS through its ability to break down hydrogen peroxide enzymatically as well as quenching ROS via methionine oxidation. H. pylori infections have been linked to higher rates of stomach ulcers and cancers, and, with the serious threat that antibiotic resistance poses to human health, understanding these protective mechanisms can lead to the design of new drugs.

Amber Lucas (allucas@andrew.cmu.edu) is a Ph.D. candidate at Carnegie Mellon University.

Fold DAT right, sleepless flies!

By Kamalika Saha

In a recent Paper of the Week in the **Journal of Biological Chemistry**, investigators reported using small molecules to correct the misfolded receptor for the neurotransmitter dopamine and restoring its function in mutant fruit flies that have trouble falling asleep.

Dopamine is a neurotransmitter that plays critical roles in many processes, including movement, memory, cognition and behavior. The dopamine transporter, known as DAT, controls the availability of dopamine in the brain, because it is involved in the movement of dopamine from the synapse into the neuron.

Point mutations in DAT are associated with a rare form of Parkinson's disease in which the dopamine transporter is deficient. The rare disease primarily affects children of consanguineous couples. These mutations lead to a misfolded DAT. This condition also is known as infantile parkinsonism-dystonia, since the patients typically have problems with movement. Dystonia is characterized by involuntary sustained muscle contractions.

To understand better how a misfolded DAT causes diseases, researchers had developed mutant fruit flies. These flies have a misfolded DAT that can't reach the brain's sleep regulation center. As a result, the mutant flies are less sleepy than normal flies.

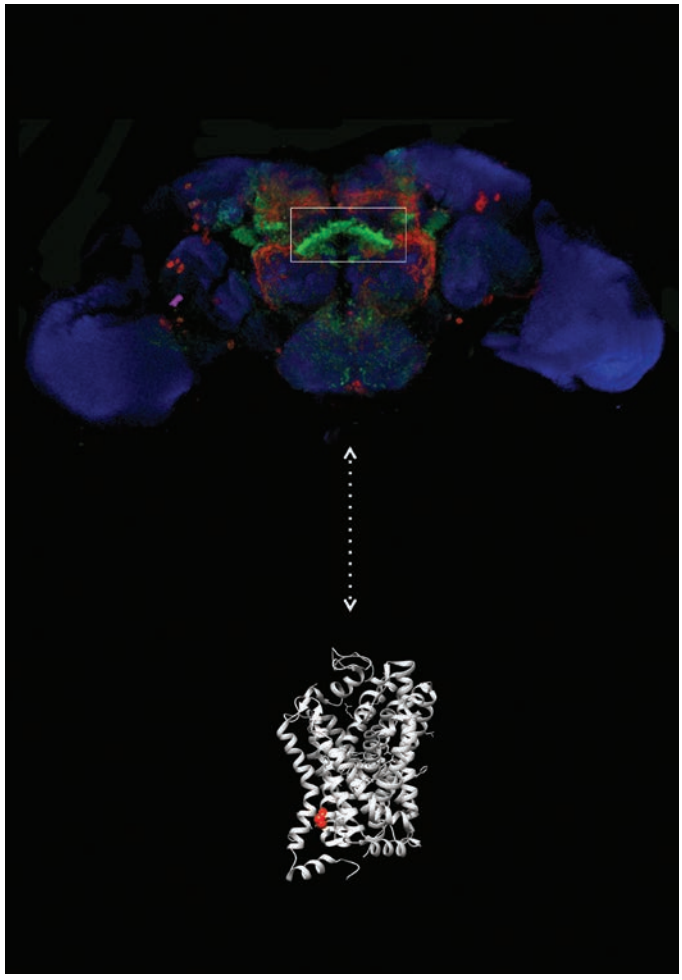


IMAGE PROVIDED BY MICHAEL FREISSMUTH

A Drosophila brain (blue) with the sleep-regulation center (green). The white ribbon diagram is the structure of the dopamine transporter with the mutation site (red).

Endogenous chaperones regulate the efficient folding of proteins. Proper folding also can be brought about by introducing small molecules, a process known as pharmacochaperoning. In this JBC paper, Michael Freissmuth at the Medical University of Vienna in Austria and colleagues set out to see if they could carry out pharmacochaperoning in the mutant sleepless fruit flies and correct the misfolding of DAT. "The ability to restore the function of a misfolded dopamine transporter by a drug is of general interest," says Freissmuth.

Using a host of molecular dynamic simulations and molecular biology techniques, the investigators established that the sleepless phenotype in flies harboring the mutant DAT indeed was caused by a folding defect in the mutant protein. Treatment of cells in culture with two small molecules, noribogaine and pifithrin-μ, rescued the misfolded protein and resulted in its efficient localization to the membrane surface and dopamine transport. When the investigators treated the mutant flies with one of the two small molecules, the flies slept the same length of time as normal flies because the mutant DAT was able to get to the brain's sleep-regulation center.

The findings of this study have the potential to have beneficial implications in patients suffering from the rare disease of dopamine transporter deficiency. Freissmuth says, "Dopamine transporter deficiency is a devastating disease. The affected children suffer from a syndrome of dystonia and parkinsonism with rigid limbs. The majority have poor prognosis and die young." Freissmuth adds that their work has the potential to be translated into a treatment soon.

Kamalika Saha (kamalika.saha@gmail.com) is a postdoctoral fellow in medical communications at MedImmune.

How now, chilled cow?

By John Arnst

Many strains of E. coli would like nothing more than to have a nice home in your lower intestine, protecting the intestinal microbial community from harmful pathogens while giving back with vitamin K. However, one strain, E. coli 0157:H7, would like to take a wrecking ball to that community. It often finds its way in through contaminated produce or beef products. In a recent paper published in the journal of **Molecular & Cellular Proteomics**, researchers reported how chilling and desiccation of beef during meat processing affect the expression of proteins critical to the survival of E. coli 0157:H7. Their findings may help reduce cases of food poisoning through beef products.

Bovine-carcass processing is a messy, complicated business that currently lacks a foolproof method for preventing E. coli contamination. "We wanted to explore whether there were some vulnerabilities in the response of E. coli that we could exploit to improve intervention methods for killing off E. coli on the carcass," says Thea King, the lead author on the MCP paper. King is a research scientist in the Food Safety & Stability group at the Commonwealth Scientific and Industrial Research Organisation, the federal agency for scientific research in Australia.

Each year, E. coli 0157:H7, which produces Shiga toxin, is responsible for about 36 percent of the estimated 265,000 cases of food poisoning in the U.S. While the symptoms of the infection typically include diarrhea, fever and abdominal pain, severe cases can involve dehydration, gastrointestinal bleeding and kidney failure.

Australia is the largest exporter of beef to the U. S. In 2015, the U.S. consumed about 12.4 million tons of beef, or enough to make 124 billion Big Macs. Of that amount, roughly 418,000 tons, or 4.2 billion Big Macs, came from Australia.

The MCP paper built on a 2014 article published in the International Journal of Food Microbiology by one of King's co-authors at the University of Tasmania, Tom Ross. In that paper, Ross and his team examined the growth responses of E. coli under separate and combined conditions of chilling stress and low-osmotic stress.

When subjected to the combined chilling and desiccation process by King and colleagues, as outlined in the MCP paper, the E. coli exhibited a sharp adaptive growth arrest, in which the bacteria made several changes in protein regulation, followed by a regrowth phase. This was similar to what had been hypothesized based on the previous paper by Ross' team.

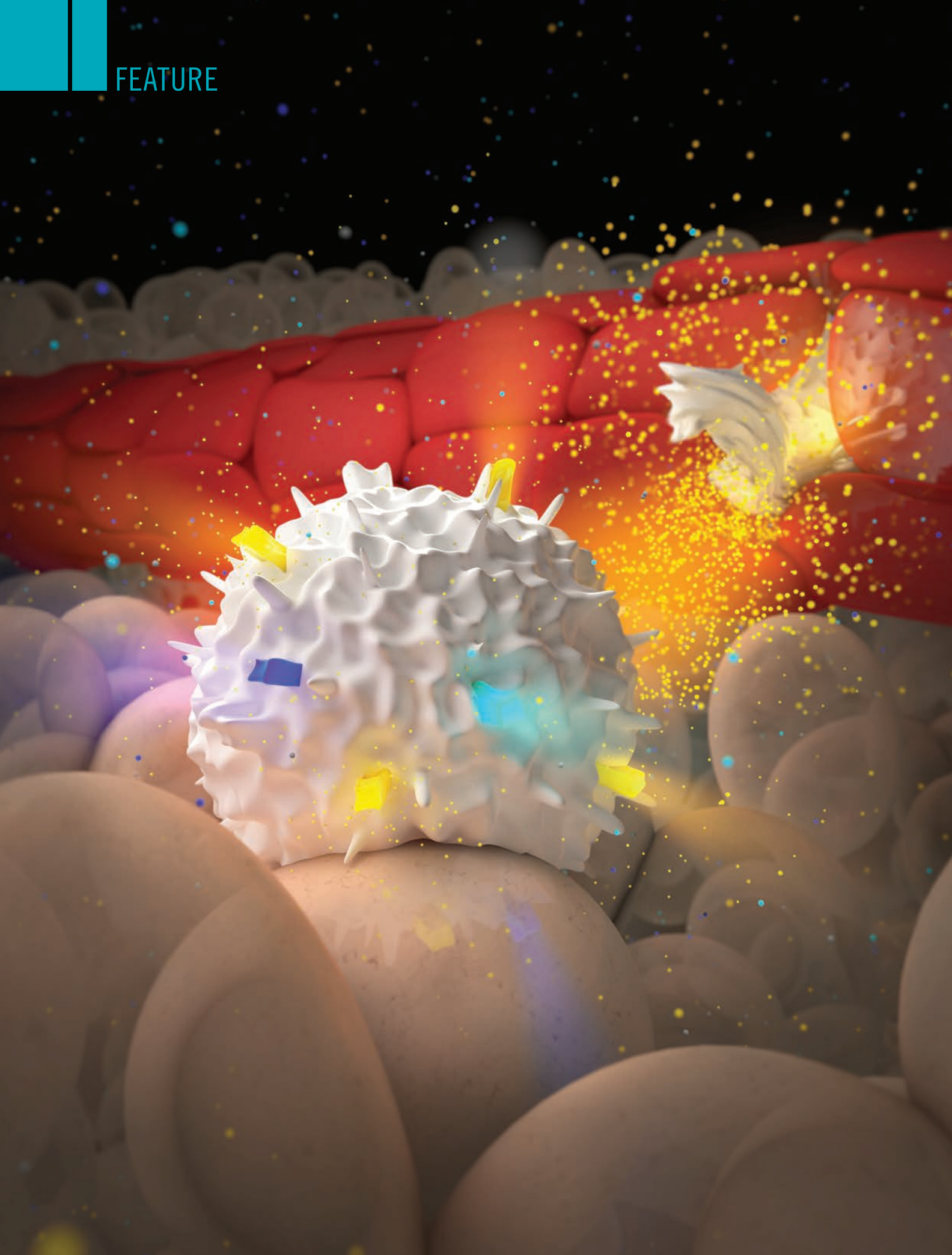
During the growth arrest, King and her colleagues noticed a window of cell susceptibility: The bacteria were undergoing DNA damage, reductions in the consumption of carbon sources and a downregulation of molecular chaperones and proteins associated with the response to oxidative damage. This was noticed at the transcriptomic and proteomic levels. The data indicated that there was a disruption of crucial energy-generating processes.

At the same time, the analysis indicated that the E. coli were busy upregulating a number of stress-related proteins by way of regulons, groups of genes that activate together in response to an external stimulus. In addition to a highly expressed cold-shock protein, "one of the first changes that we saw is that E. coli activates the RpoE regulon, which might be an emergency response for cells to repair protein misfolding in the cell envelope," says King.

King goes on to explain, "The most interesting thing that came out of this was that we found that under conditions of low water activity, E. coli seemed to lose its culturability." If the bacteria can't replicate, they can't contaminate the cow carcass. However, "when E. coli were just exposed to chilled temperatures, we didn't see this loss of culturability," adds King.

King and colleagues plan to expand the study to examine the effects of oxidative damage in additional strains of E. coli as well as Salmonella. "We really want to explore this loss of culturability further," King says, adding that the aim is to try to "exploit the physiology of E. coli under these conditions and try to benefit meat safety."

John Arnst (jarnst@asbmb.org) is ASBMB Today's science writer. Follow him on Twitter at twitter.com/arnstjohn.



A chain of events

Linear ubiquitin chains, whose very existence once was debated, now are known to play a critical role in an inflammatory disease, thanks to the discovery of an enzyme

By Rajendrani Mukhopadhyay

A few years ago, David Komander was tipped off to a mysterious gene. Komander, a biochemist at the Medical Research Council Laboratory of Molecular Biology in the U.K., is an expert in the enzymes of the ubiquitin system. A colleague told him that he had found a gene that made a protein of unknown function called FAM105B. "If any protein is called FAM," says Komander, "it usually means nobody knows anything about it."

The gene for FAM105B bore some of the hallmarks of those that make deubiquitinases. Deubiquitinases are enzymes that remove chains of ubiquitin. After doing their analyses, Komander and his colleagues concluded in 2013 that FAM105B was a deubiquitinase.

But it wasn't just any deubiquitinase. It was specific to a special kind of ubiquitin chain. After finding this particular deubiquitinase, several groups of researchers, including Komander's, discovered that the enzyme was important for keeping immune cells on an exquisitely delicate balance. If something went wrong with the enzyme, the immune cells went off the rails and caused a rare inflammatory disease.

OTULIN outed

Ubiquitin is a protein of merely 76 amino acids. When tacked onto proteins, ubiquitin affects them in a number of ways. For one, it can set off the degradation of the tagged protein, a discovery that got Irwin Rose at the University of California, Irvine, as well as Aaron Ciechanover and Avram Hershko at the Technion–Israel Institute of Technology the Nobel Prize in chemistry in 2004. Ubiquitination also can change a protein's location, activity or ability to interact with other proteins.

A triad of enzymes known as E1s, E2s and E3s attach ubiquitin to proteins. Ubiquitin can be attached either as a single molecule or as a chain. Chains of ubiquitin have a variety of forms, because the chains can be linked in eight different ways, either through one of the seven lysines or a specific methionine called Met1 on the ubiquitin molecule. Given the different ways for attaching ubiquitin — single or one of the eight types of chains — the repertoire of ubiquitin modifications is extensive. Different types of modifications have their own effects and sets of enzymes.

However, the existence of one of the ubiquitin chains, the linear chains made through Met1, once was contested. About five years ago, it was "hotly debated whether these linear ubiquitin chains are very important cellular signals," says Komander. Researchers were unable to detect Met1-linked chains readily and they didn't know of any enzyme responsible for removing the Met1-linked chains. Scientists had found the linear ubiquitin assembly complex, known

CONTINUED ON PAGE 16

The image on the opposite page and on the cover shows what happens in the absence of functional OTULIN. The defective immune cells can't control signaling pathways regulated by linear ubiquitin chains. They send out signals that lead to a systemic inflammatory response. The hyper-active immune cells damage healthy tissues. The image was created by Lesley McKeane at the MRC-LMB Visual Aids Department.

CONTINUED FROM PAGE 15

as LUBAC, which built up the linear ubiquitin chains, but no one had found the enzymes that specifically removed them. "This was really the key missing activity," says Komander. If a signal is important, there must be enzymes in place to put the signal on and take it off for the sake of regulation.

With the deubiquitinase for Met1-linked linear ubiquitin chains missing, some researchers were doubtful that Met1-linked linear ubiquitin chains were critical in cell physiology. But after analyzing FAM105B, Komander and others had their hands on the missing enzyme. It was the proof that linear chains are important in cell signaling.

In a 2013 Cell paper, Komander and his team described how their deubiquitinase specifically cleaved linear ubiquitin chains. Because the enzyme belonged to the OTU family of deubiquitinases and was targeted solely for linear ubiquitin chains, the investigators put OTU and "lin" together to give "OTULIN." The same protein also was found by Frank Sicheri and Sabine Cordes at Samuel Lunenfeld Research Institute in Canada; they called the protein Gumby in their 2013 Nature paper.

Molecular biologists now had the deubiquitinase that was specific for breaking down linear ubiquitin chains.

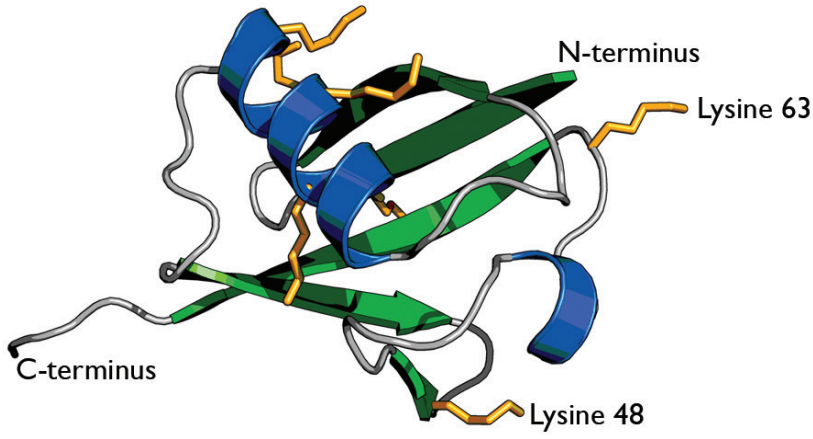


IMAGE COURTESY OF WIKIMEDIA

Cartoon of ubiquitin highlights its secondary structure.

But clinicians soon became intrigued by the enzyme.

The gene hunt

Ivona Aksentijevich at the National Human Genome Research Institute has devoted her medical career to studying patients who have disorders that involve systemic inflammation, the causes for which are unknown. "I've been studying patients with rare inflammatory diseases for more than 25 years," notes Aksentijevich. "I have participated in gene discoveries for nine diseases."

So it wasn't out of the ordinary that she and her colleague, Daniel Kastner, also at the NHGRI, were asked to examine a British Pakistani family with a child who displayed severe systemic inflammation from birth. The child was kept alive by heavy doses of anti-inflammatory medications and steroids. The child had two unaffected siblings but had first cousins who had symptoms of systemic inflammation at birth and died early in childhood.

The important clue clinicians had was that the family had intermarried for several generations. The disease was unknown outside of the family and not described in literature. Whatever was causing the disease had to be a genetic mistake that was carried in a single gene in both the parents' DNA.

Aksentijevich, Kastner and the rest of their team collected blood samples from the family members and carried out whole-exome sequencing. They noted that the child with the rare disorder "had a homozygous mutation in this protein which is known as OTULIN." Aksentijevich adds that the researchers first came across the name FAM105B, "which misled us for some time because we thought it was some poorly characterized gene." But once they realized they were dealing with OTULIN, the clinicians knew what they were working with.

The investigators knew about patients who had a deficiency in LUBAC and suffered from an unusual

phenotype that involved the immune system. OTULIN was known to interact with the LUBAC complex, which suggested that mutations in OTULIN also could cause systemic inflammation.

But, Aksentijevich says, "in studies where we define a new disease-causing gene, it makes for much stronger evidence to have additional patients with mutations in the same gene." So she went to the database that she and her team had built up over the years of patients with mystery disorders and searched for those who had similar features to the British Pakistani patient they had analyzed. She found another patient from Turkey who carried a different homozygous mutation in OTULIN. And like that, Aksentijevich identified the second family with an OTULIN mutation.

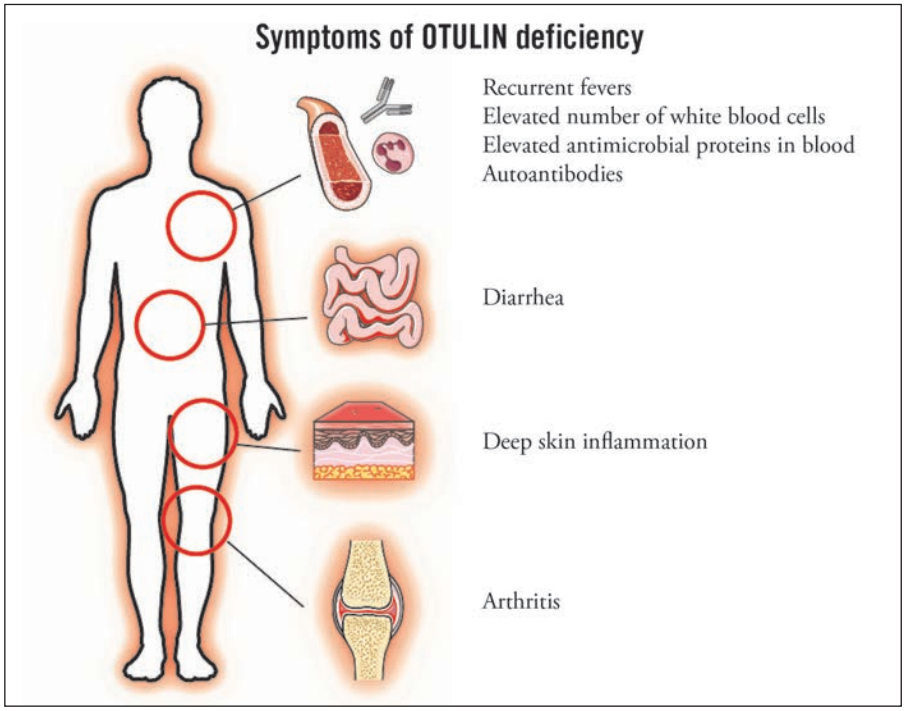


IMAGE PROVIDED BY RUNE DAMGAARD & DAVID KOMANDER

Mutations in the gene for OTULIN cause a rare systemic inflammatory disease.

Aksentijevich also reached out to collaborators in Turkey, a country where intermarriage is common, and asked if they knew of any other families that had the clinical symptoms of systemic inflammation and mutations in the OTULIN gene. Together, they identified a third family with a child who carried yet another type of mutation in OTULIN. This now meant "we had three different homozygous mutations in this gene," says Aksentijevich. "That was a solid genetic evidence that OTULIN is a disease-causing gene." Aksentijevich, Kastner and colleagues did experiments to show that these mutations cause the deficiency of OTULIN activity in the patients' cells.

Later, during a conference in Europe, Aksentijevich heard about a Japanese girl who had similar clinical features but different mutations in OTULIN. So far, four patients have been identified with the OTULIN deficiency.

Across the Atlantic Ocean, Komander and his team also were concluding that homozygous recessive mutations in OTULIN caused inflammatory symptoms. Excited by the finding that OTULIN was the missing deubiquitinase for linear ubiquitin chains, Rune Damgaard, a postdoctoral fellow in Komander's laboratory, began to develop knockout mice based on OTULIN in close collaboration with the group of Andrew McKenzie at the MRC Laboratory of Molecular Biology.

Researchers already knew that complete removal of OTULIN killed mice at the embryonic stage. When Damgaard selectively knocked out the gene in different cell types of the immune system, the mice developed signs of systemic inflammation. They had high levels of inflammatory mediators in their blood. Their white-blood cell count was high, as if the body were trying to fend off an imaginary infection. The overactive immune system was damaging the liver and other healthy organs.

Around this time, a clinician named Eamonn Maher got in touch

CONTINUED ON PAGE 18

CONTINUED FROM PAGE 17

with Komander because he had heard of the work going on in his laboratory. Maher had been treating the British Pakistani child in Birmingham, U.K., since the late 1990s with heavy doses of anti-inflammatory medications to keep the child alive. Maher had investigated the genes of this patient and the patient's family and found mutations in FAM105B. But he had no idea what FAM105B did and couldn't make sense of his finding. Then he learned about the Komander group's work in identifying FAM105B as OTULIN.

Maher met with Komander and Damgaard over coffee and described his patient. Damgaard found himself nodding along as Maher listed the child's symptoms. He was seeing similar symptoms in the OTULIN-knockout mice he was creating. Both the child and mice had overactive immune systems. The only difference was that the child had additional complications of large, patchy skin rashes and persistent diarrhea. Aksentijevich, Kastner and colleagues noted the same symptoms with their Turkish patients and independently had examined the genome of the British Pakistani family as had Maher.

When Komander and his colleagues published their paper in Cell in August, they called the disease ORAS for OTULIN-related autoinflammatory syndrome. Aksentijevich, Kastner and colleagues, in their paper, which appeared two weeks later in the Proceedings of the National Academy of Sciences, called the disease otulipenia. The two groups of investigators were aware of each other's work and were excited to learn that their results, taken from cell lines, mice and patients (both groups had analyzed cells from the British Pakistani family), corroborated one another.

"We expect more patients to be diagnosed now that we know what to look for," says Aksentijevich. She points out that identifying the disease and its cause helps physicians, because they will be able immediately to prescribe an anti-inflammatory treatment instead of first trying aggressive steroid treatments, which can cause major health complications.

A fine balance

As investigators gather molecular and clinical information about OTULIN, they are marveling at the potency of linear ubiquitin chains. One of the critical pathways regulated by these chains is the NF-κB signaling pathway, which plays a key role in regulating the immune response to infection.

Under normal conditions, the linear ubiquitin chains mediate a time-dependent activation of NF-κB. The OTULIN mutations that have been found in patients all cause a severe reduction in the activity or the amount of the enzyme in cells compared with what normally is found in people. With lower levels of the enzyme, the linear ubiquitin chains don't get chopped off, and this keeps the NF-κB signaling pathway firing without restrictions. At the physiological level, the immune system keeps chasing after an imaginary infection.

Scientists find it interesting that mutations in LUBAC also cause the immune system to go into overdrive and cause inflammatory disorders. It appears that the linear ubiquitin chain type is so highly regulated that, if the system goes awry in either way, the result is an overactive immune system.

"It just shows how exquisitely balanced this signal is in the cell," says Komander. "For me, that is the most striking aspect. We're talking here about a chain type that many people contested even to exist."

Rajendrani Mukhopadhyay (rmukhopadhyay@asbmb.org) is the managing editor of ASBMB Today. You can follow her on Twitter at twitter.com/rajmukhop. John Arnst, ASBMB Today's science writer, contributed to this story.

The Art of Science Communication

Winter session begins Feb. 6

The Art of Science Communication online course provides participants with fundamental communications training, focusing on how to present science to a nonexpert audience in a formal setting.

Registration deadline is Feb. 3

Take part in the ASBMB class from wherever you are for only **three to four hours** a week.

During the course, you will learn to:

- construct and deliver an effective presentation
- get your message across
- generate interest in your topic
- engage your audience
- effectively use nonverbal communication

The course is eight weeks long and costs $25 for ASBMB members.

www.asbmb.org/Outreach/Training/ASC/

ASBMB
American Society for Biochemistry and Molecular Biology

Burning fat and balancing liver lipids

An interview with Michael Wolfgang of the Johns Hopkins University

By John Arnst

Michael Wolfgang of the Johns Hopkins University, a member of the American Society for Biochemistry and Molecular Biology, wants to figure out on a biochemical level how your body burns fat. One of the projects his laboratory members are working on is looking at the liver's role in oxidizing fatty acids in mice. Wolfgang's research also focuses on metabolic regulation and genetics in the liver and neurons as well as their effects on diabetes, obesity and brown-fat accumulation.

However, Wolfgang, who joined the Hopkins faculty in 2008 after completing his postdoctoral training at Johns Hopkins University School of Medicine, didn't always see himself as a metabolic researcher. A childhood surrounded by sheep, chickens and pigs on a small farm in Illinois initially sent him on a path to becoming a pig farmer. This led him to an undergraduate degree in animal sciences from the University of Illinois–Urbana Champaign followed by a Ph.D. in endocrinology and reproductive physiology from the University of Wisconsin–Madison.

PHOTO PROVIDED BY MICHAEL WOLFGANG

Michael Wolfgang with his daughter Alexandria.

John Arnst, ASBMB Today's science writer, spoke with Wolfgang about his life and research and how a watershed moment witnessing in vitro fertilization led to a career ferreting out the interplay of metabolic regulation and genetics in both the liver and the brain. The interview has been edited for length and clarity.

What are some projects you're working on?

In the brain, we're trying to understand how several unique enzymes regulate lipid flux within the central nervous system and what impact that has on the behavior and the life and death of neurons and astrocytes. On the other end, a couple of the things we're working on are how mitochondrial fatty acid oxidation affects hepatic — and really all kinds of cells — function in vivo. We have an ongoing project on adipose-tissue fatty-acid oxidation and hepatic fatty-acid oxidation and what roles they have.

What are you starting to find regarding fatty-acid oxidation?

Well, the liver is quite interesting. I never thought of myself as a liver biologist, although we study metabolism, because I thought we knew everything there was to know about fatty (-acid) oxidation in the liver. When we knock out hepatic fatty-acid oxidation in livers, we would have expected the mice not to survive, and we certainly would not have expected them to

survive a 24-hour fast. They actually survive both of those things. The mouse makes adaptations to survive that actually become rather normal from a whole-body standpoint and are fascinating. We're trying to understand how this happens and how the liver is communicating this dysfunction to the other tissues.

How long did the mice survive with hepatic fatty-acid oxidation knocked out?

They can survive forever.

Wow.

Right? So this is kind of a basic biochemical textbook thing. I was so surprised by this that I actually didn't believe it for the longest time until I actually got in and did a couple of experiments with the graduate student myself. Fatty-acid oxidation is essential to gluconeogenesis, essential to ketone production. It's the major fuel that the liver uses during fasting, and the amount of plasticity and organismal flexibility is just incredible to me.

What led you to exploring these two avenues?

When I was a postdoc, we were studying a very atypical enzyme that's very neuron-specific, carnitine palmitoyltransferase (CPT) 1C. We know a lot about CPT 1A and CPT 1B and what those functions are in vivo as a rate-setting step in fatty acid oxidation. Neurons express this bizarre enzyme that's encoded by another gene. It looks very similar to both of those enzymes but in a very unusual place. It's fairly neuron-specific under normal circumstances. We knocked it out. Mice have an interesting phenotype, but trying to understand what those phenotypes were in that context was difficult, because I didn't think we knew enough about how the brain utilizes lipids at a very basic level.

I also wanted to ask what the role of fatty-acid oxidation was in the nervous system. That's sort of how all of these projects got started — by just re-examining, well, do we really understand how these basic processes work in a physiological context?

Who would you say are some of your mentors?

Probably the biggest mentor for me was Dan Lane. (Author's note: M. Daniel Lane was a biochemist at Hopkins who studied biotin-dependent carboxylation, adipogenesis and insulin signaling. He passed away in 2014.) Just how he approached science, how he mentored students and postdocs, has probably been the biggest influence on me scientifically. He was one of the first adopters of the 3T3-L1 system for adipocyte differentiation, so he made a lot of progress in that. (Author's note: The 3T3-L1 system is a cell line derived from mouse cells that is essential for research with adipose tissues.) When I joined his lab, he was interested in understanding how some of these processes work in the nervous system.

What do you think are some of the major issues or trends that might affect scientists' livelihoods?

The reproducibility issues, I think, are kind of coming to the fore, and certainly this type of thinking is impacting how we think about moving forward. Of course, funding is always an issue — not just short-term funding but longer-term funding — because I'm not old. I'm just a young buck now. (Author's note: Wolfgang is 40 years old.) So that's a little bit daunting, thinking about how this will be carried on for the next 20 years.

CONTINUED ON PAGE 22

CONTINUED FROM PAGE 21

Did you always know that you wanted to be a scientist?

Oh no, no, no — I didn't go to school to become a scientist. I went to school to become a pig farmer.

How did that transition happen?

I took a course at the University of Illinois. I was an animal-sciences major, and there are two tracks in animal science. There's a management track where you manage industrial hog farms, which is what I thought I was going to do, and then the other track is science-focused. I took a course in reproductive biology at U of I, and it was just so fascinating to me. They have a really interesting undergraduate program where there's a lot of hands-on science. In one of the labs that we took, we were doing in vitro fertilization. To watch that process in front of me was just one of the most amazing things I've ever seen. It hooked me, and I wanted to learn more about science.

What was it that made you want to be a pig farmer?

We had a small farm, 35 acres. We had sheep and chickens and a couple of pigs. When I was 16, I got a job on a large family farm harvesting and planting horseradish. They also raised a lot of hogs. After the horseradish season was over, I started working in the farrowing houses and really loved it. So much so that I thought it would make an interesting career.

Is there any advice you'd give to graduate students?

Well, you have to do something that you love, because there are few rewards other than discovering something. That's probably the best reward there is — when you have data that you've discovered. If that doesn't get you up in the morning, there's no reason to do this. You really have to be able to have the love of it to keep going.

We've talked about your life in the lab. How about your life outside of it?

I have three children. I basically do two things: I work and I spend time with my family. And that's about it. A lot of our free time is devoted to them. There are a lot of things to do within two hours of Baltimore. We go to the beach, go to a park. We're always doing something.

In regards to your work and your life, you have an overarching motto?

No. You know who David Sedaris is? He says life is like four burners. Do you know this? (Author's note: See "Four burners.")

I think he mentioned if you want to be successful, you have to turn off one, and if you want to be really successful, you have to turn off two.

Right, right. My wife keeps telling me I need to exercise, but I was like, "Well, David Sedaris said I can't do that."

Four burners

In a 2009 article in The New Yorker, humorist David Sedaris mentioned a friend's concept of life management as a stove with four burners: family, friends, health and work. The idea put forth is that, a person has to turn off one of the burners to manage a career successfully; to be truly successful professionally, a person must turn off two burners.

John Arnst (jarnst@asbmb.org) is ASBMB Today's science writer. Follow him on Twitter at twitter.com/arnstjohn.

Upcoming ASBMB events and deadlines

JAN

Jan. 20: Student Chapters annual meeting travel awards deadline
Jan. 31: DEUEL Conference on Lipids abstract and registration deadline

FEB

Feb. 6: Online communication course begins
Feb. 8: ASBMB annual meeting late-breaking abstract deadline
Feb. 17: ASBMB annual meeting Outstanding Student Chapter Award deadline
Feb. 16: PROLAB application deadline
Feb. 23: ASBMB annual meeting early registration deadline

MAR

March 15: Accreditation application deadline

APR

Apr. 22-26: ASBMB annual meeting, Chicago

ASBMB
American Society for Biochemistry and Molecular Biology

Promoting Research Opportunities for Latin American Biochemists

The American Society for Biochemistry and Molecular Biology, the International Union of Biochemistry and Molecular Biology and the Panamerican Association for Biochemistry and Molecular Biology have instituted a program (PROLAB) and committed funds to foster interactions among biochemists in Latin America, Portugal and Spain with those in the United States.

This program is open to postdoctoral fellows, graduate students and tenure-track faculty members (within five years of their training).

The application deadline is Feb. 16.

Learn more at *www.asbmb.org/pabmb*.

ASBMB
American Society for Biochemistry and Molecular Biology

IUBMB

PABMB

A figure worth 1,000 words

By Kaoru Sakabe

As the data integrity manager for the American Society for Biochemistry and Molecular Biology, I wear two hats. I investigate manuscripts submitted to and published in ASBMB journals for violations of ASBMB policies on publication ethics. (The ASBMB publishes the Journal of Biological Chemistry, the Journal of Lipid Research and Molecular & Cellular Proteomics.) I also educate authors regarding ethical issues in publishing and how best to handle them. The first role, albeit necessary, can be a roller coaster. I've

heard a lot of different excuses from authors I've investigated for violations, such as erasing blemishes and bands, reusing data from different publications and cutting and pasting bands to create data that never existed. These excuses run the gamut from somewhat credible to incredible — although I haven't yet heard that someone's dog ate it.

Educating authors about ethics is vitally important. I realize that not everybody has the exposure I had as a Ph.D. student. My mentor instilled zero tolerance for misconduct in all of his trainees. There was also a great culture in the lab of sharing best practices for data presentation.

As a publisher, the ASBMB can

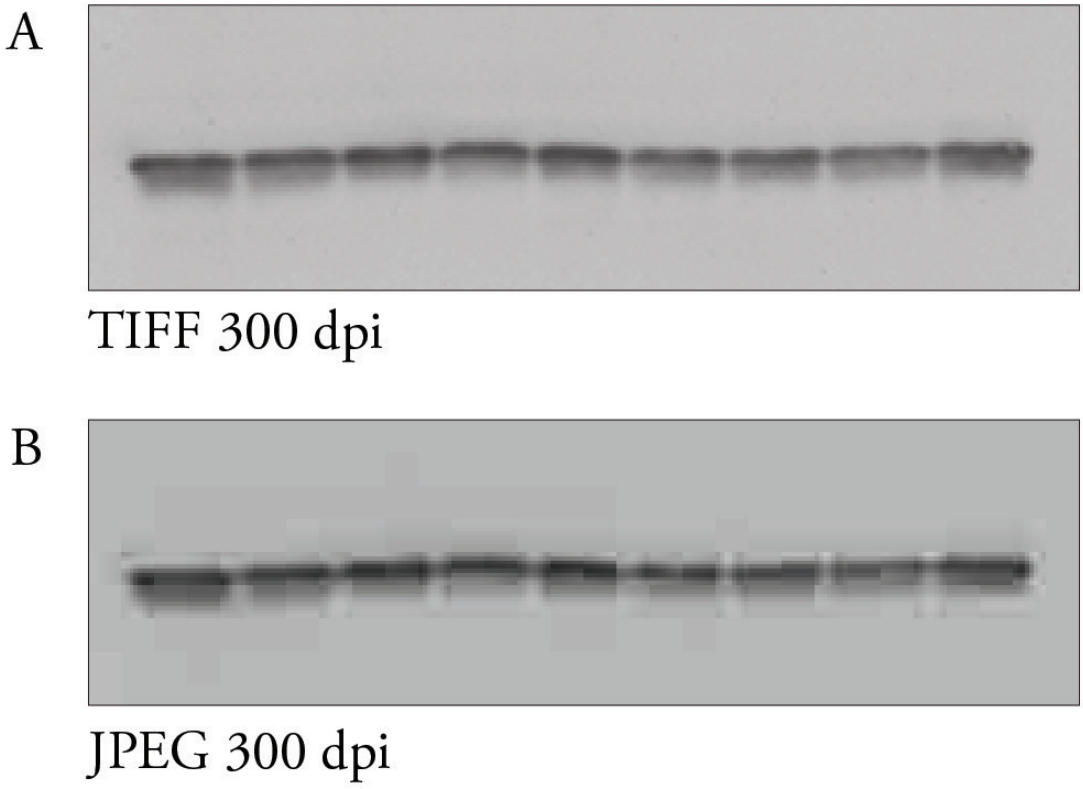


Figure 1: Images should be saved in TIFF format. The same image was saved at the same resolution of 300 dpi, but A was saved in TIFF format, while B was saved as a JPEG. Note the pixelation in B.

help fill that gap for authors unfamiliar with these practices in figure presentation, since everyone may not have had this kind of exposure as a student or postdoctoral researcher. Learning best data-presentation practices doesn't end with your formal training, though. I'm still learning, especially as publishing standards continue to evolve.

Over the next few months, I will be writing a series for ASBMB Today in which I will tackle different topics regarding images and figures and delve into ethical issues.

For now, I'll start with the basics — how to best prepare manuscript figures for submission. A manuscript is like a picture book that tells a narrative (your research) with the aid of some pictures (your figures). In telling your story, you need to present the pictures in a clear manner so that reviewers and, eventually, readers will be able to understand and interpret your data. Here are a few pointers:

Read the instructions for authors

This may seem like a no-brainer, but you always should read the instructions to authors for the journal to which you plan to submit. The instructions contain valuable information about what the journal expects. This way, you avoid the frustration of having your manuscript sent back for formatting issues or because a reviewer can't make out a blot.

Figure preparation begins at data acquisition

Preparing publication-quality figures begins during data acquisition, long before you have a story, much less know where to submit your work. Whether it's scanning a film or taking a picture, overexposing or underexposing an image leads to loss of the fine details in the data. How can you tell your image is over- or underexposed? Take a look at the histogram. The histogram graphically displays the tonal distribution of an image by showing the number of pixels that are black, white and all the different shades of grey in between. Ideally, the pixels should be distributed throughout the range and not clustered at either end of the spectrum. While it is tempting to acquire a clean-looking image with no background or speckles, reviewers know what real data look like. Additionally, the images should be acquired at a minimum resolution of 300 dots-per-inch.

Save images using loss-less compression

Scientific images should be saved in the TIFF format, because it uses a loss-less compression algorithm to save your data. Avoid the JPEG format because it uses an algorithm that results in loss of data (lossy compression). Lossy-compression algorithms approximate the original data, which can result in parts of your data being discarded. Although saving an image as a JPEG may save you computer disk space, the problems that this compression method may introduce, by essentially throwing out information, are not worth the benefit of more disk space or faster upload time (Figure 1).

CONTINUED ON PAGE 26

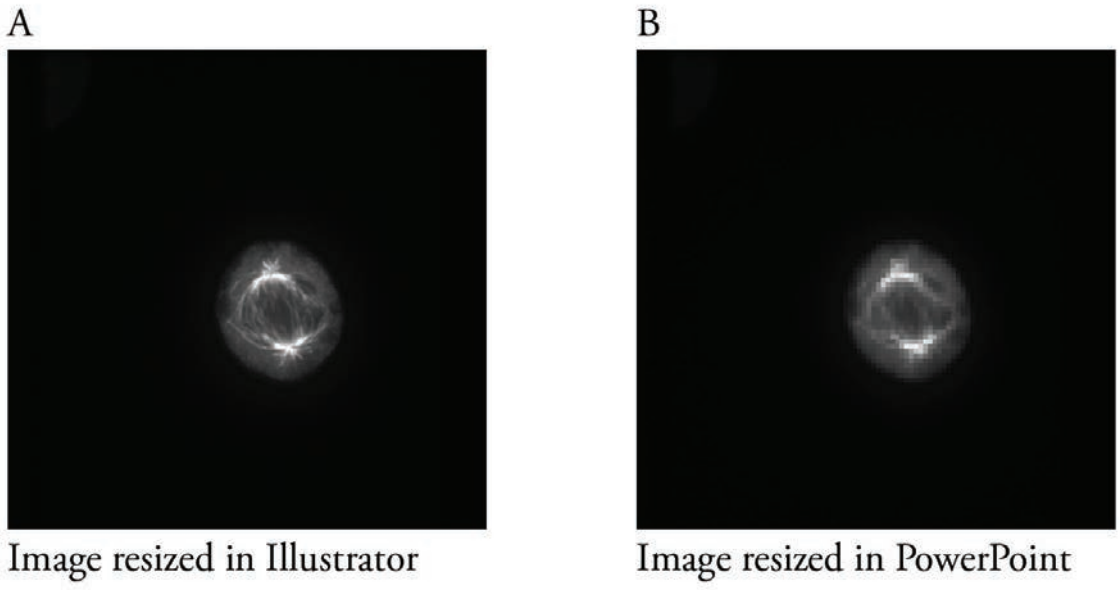


Figure 2: Figures should be created using appropriate software. The same image was resized, but A was resized in Adobe Illustrator, while B was resized in PowerPoint. Note the pixelation of the image in B. A free alternative to Adobe Illustrator is Inkscape.

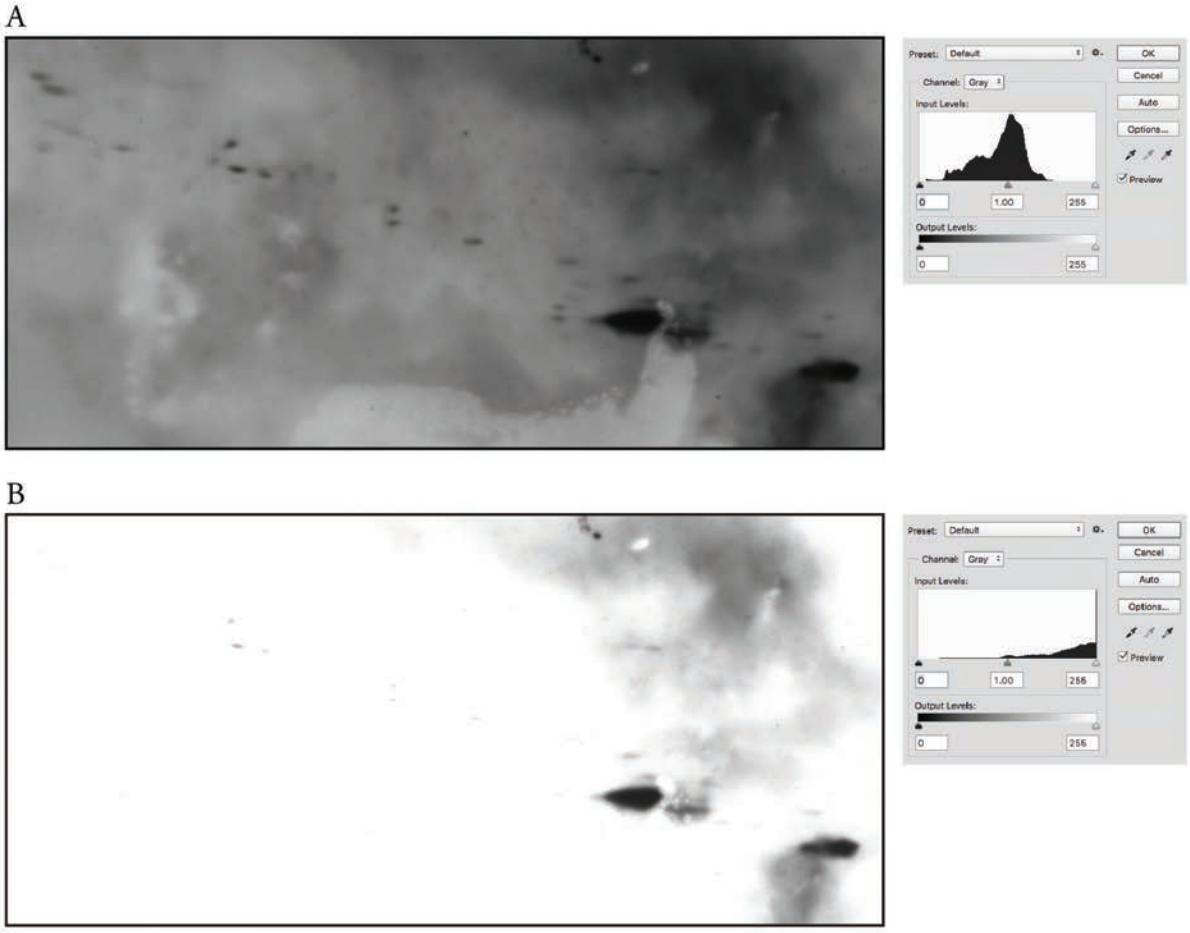


Figure 3: Avoid excessive manipulation. The original unmanipulated scan is shown in A along with the accompanying histogram. In B, the brightness and contrast were adjusted excessively. Note the absence of background, the disappearance of some spots and the shift in the accompanying histogram.

CONTINUED FROM PAGE 25

Prepare figures using appropriate software

PowerPoint is an attractive option for generating your figures, but avoid PowerPoint. The reason is that PowerPoint is designed for an onscreen resolution of 72 dpi and not print, which requires at least 300 dpi. Resizing images using PowerPoint can lead to loss of data, since it applies a lossy compression (Figure 2). Adobe Illustrator and Inkscape are good options for preparing figures.

Avoid excessive manipulation

This topic will be covered in more depth in future articles, but, in brief, you should manipulate your image as little as possible when preparing the figures for publication. Your final image should be a true representation of the film or image when you captured the original. Aggressively contrasting your image or adjusting the levels to reduce the background may draw questions from reviewers and readers. Again, take a look at the histogram to make sure you are staying within acceptable limits. That pesky band or spot that you find troubling actually may be very informative for readers. It could indicate the performance of a certain antibody, or it could be a differentially modified form of your protein of interest (Figure 3). Importantly, those bands or spots are the actual data! Hiding or omitting them misrepresents your experimental results to the reader.

Check your figures by printing them

It's a good idea to print out your figures before submitting them. If you have a hard time viewing your images, chances are so will the reviewers.

Submit!

And try to relax until the reviews come in.

Kaoru Sakabe (ksakabe@asbmb.org) is the data integrity manager at the ASBMB.

American Society for Biochemistry and Molecular Biology

ACCREDITATION & ASSESSMENT *for* B.S./B.A. PROGRAMS IN BIOCHEMISTRY & MOLECULAR BIOLOGY

The ASBMB has launched a national accreditation program for departments and programs offering baccalaureate degrees in biochemistry, molecular biology and other related degrees. Accredited programs gain access to an independently developed and scored examination for assessing student performance that leads to the conferral of an ASBMB-certified degree.

Programs seeking ASBMB accreditation will be evaluated on criteria such as:

- Faculty credentials
- Support for undergraduate research
- Faculty access to professional development programs
- Commitment to diversity
- Student advising programs
- Well-rounded curriculum that includes a robust experiential learning component

Next application deadline: March 15

For more information, visit www.asbmb.org/accreditation.

ASBMB accredited programs include:

- University of Montana
- University of Arizona
- Oklahoma State University
- University of Virginia
- Hendrix College
- Hope College
- Wellsley College
- Boston University

ASBMB
American Society for Biochemistry and Molecular Biology

Intellectual property and its place in science curricula

By Ben Caldwell

How many of you can identify with one of these situations? You are working with a commercial kit in the lab and having issues with the kit performing as expected. Your thoughts come around to the reagents in the kit, but the documentation has limited information about the kit's components. Or, in order to stretch your lab's budget, you attempt to replicate solutions or buffers in a kit from your own store of reagents, but your mix doesn't produce the same type of results. In both cases, a call to the company's technical service help gets you the reply that they cannot tell you all of the specific reagents in the kit because the composition is proprietary.

Consider another situation new employees face when they enter the workforce. Before they can enter the lab, they must sign a nondisclosure agreement. What is this and what does it have to do with the job?

These examples all have a corporate component in common. To some people, proprietary information and nondisclosure agreements may appear to have elements of secrecy and conspiracy to suppress information. What is it about commercial entities that requires such secrecy? It can be summed up in two words: intellectual property.

Intellectual property, or IP, is what gives a company a competitive edge in the marketplace. As educators, we often talk about the value of openly sharing research through publication and how the peer-review system helps to keep us honest. Publishing is the norm for academic research, but publishing in the industry arena is less common. Divulging the proprietary ingredients that help a kit to perform so well or disclosing information on company projects gives away a company's intellectual property.

One of the biggest gaps academic faculty have in preparing students for careers in industry is that most academics have had little or no experience working in a commercial setting. We do our best to make sure students are properly prepared to work in a laboratory setting. But intellectual property is a topic that rarely is discussed formally within the undergraduate or graduate curriculum, partly because many faculty do not know the subject well themselves.

What is IP? Simply put, IP can be anything that is a creation of the mind. While scientific discoveries and innovations certainly can fall into this category, intellectual property also can be literary or artistic works, industrial designs or trademarks, to name a few examples. Often ideas are developed into tangible substances or devices that serve a particular role or purpose (inventions). But IP also can be a process used for manufacturing or production or even a business practice.

Ways to protect IP

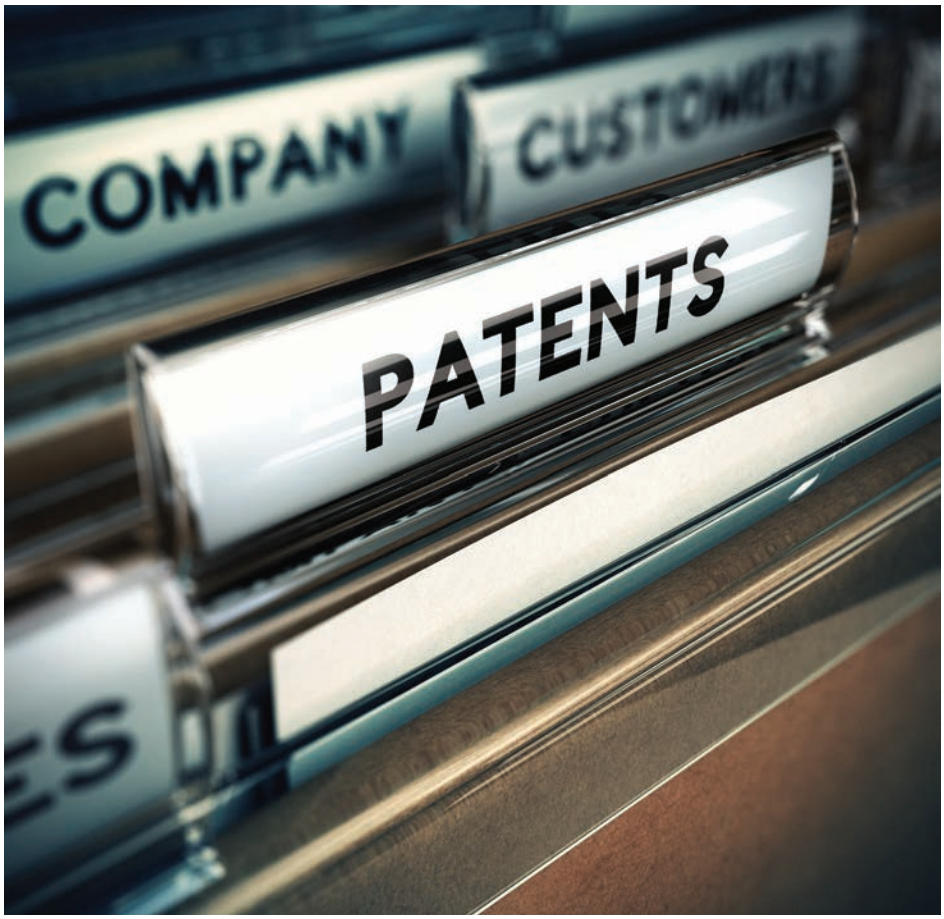
If an inventor or the IP owner wishes to protect his or her ideas or inventions, a number of strategies can be used. Keeping the IP from competitors can be as simple as holding the information privately as a trade secret. Most people are familiar with famous trade secrets, such as the formula for Coca-Cola or Colonel Sander's 11 herbs and spices for finger-lickin'-good fried chicken. Generally, the only real protection for a trade secret is that as long as the IP is held internally in a company, competitors cannot take advantage of it. If the trade secret somehow is released to competitors or the public, the proverbial cat then is out of the bag. Anyone can take advantage of that information. The originator of that trade secret may no longer have a competitive advantage and may lose out in the marketplace if competitors begin using that IP.

An individual or a group of inventors can file an application for a patent, which, if approved, will give the patent holder exclusive rights and protections. To be approved, a patent application must describe the subject matter and demonstrate that the invention is novel, has utility and is not obvious to a person skilled in the art (someone who would regularly use the item).

Unlike with trade secrets, once a patent is granted, the information within the patent becomes freely available to the public. However, although the information is now public, a patent prevents anyone, except the owner, from using the subject matter for a period of up to 20 years. Essentially, no one but the patent assignee can produce, use or sell the patented subject matter. Anyone wishing to use, make or sell the item or invention must request permission from the patent owner. The assignee can grant permission to others by issuing a contract outlining the terms of usage, which usually includes fees to be paid to the

patent owner for licensing or royalties. After the patent expires, the patent holder loses the exclusive ownership rights. At that point, anyone may make use of the information in the patent without compensating the patent holder.

As patents begin to approach their expiration date and limit of protection, a patent holder may seek to extend the life of patented inventions by making improvements to the original invention and pursuing additional new patents that protect the improvement of the original invention. There are a few caveats to this though: Any improvements must be significant and nonobvious. Making a simple change is not enough to warrant issuing a new patent, and patent examiners at the U.S. Patent and Trademark Office have the final say.



IP course for scientists

The concern here is that discussions about IP are hardly ever introduced in the undergraduate and graduate curricula, and most students, especially at smaller universities or colleges, never even have heard of IP. Undergraduate science majors are drilled continually about the importance of properly documenting experimental procedures and data in their lab notebooks. But students rarely truly understand the importance of fully recording their work until they are employed in a professional environment where documentation is an essential part of the job. Recent graduates often are surprised by the amount of time they spend documenting their efforts compared with the time actually spent working at the bench.

So how do we better prepare these scientists? While IP is not a common topic in most science curricula, the typically heavy course requirements for science majors often prevent undergraduates from taking business courses. Therefore, most science students have little appreciation or understanding of business terminology, corporate structure, basic accounting practices or marketing strategies when they graduate. Advising students to take a business class or two as electives is one way to help them become more aware. Another option that my department took was to create a business concentration for one of our undergraduate degrees. This requires students to take several business courses; the flip side is that some science content is sacrificed.

When my university began exploring possibilities for new graduate offerings to support the chemical and life science industry in our region, it became obvious to us that IP was a topic area these employers felt was important. We developed an IP course specifically for scientists that has been well received by both students and employers. Students in the masters of business administration program at our institution will have the option of taking this course in the future. I expect that having a blend of scientists and business professionals in the course will lead to some thought-provoking discussions.

We only have scratched the surface, but I believe this is something we as educators should consider emphasizing more in our science curricula.

Ben Caldwell (caldwell@missouriwestern.edu) teaches "Intellectual Property in the Scientific Setting" at Missouri Western State University, where he is a professor of chemistry and biochemistry and dean of graduate studies.

New Year's resolutions and summer research applications!

By David G. Oppenheimer

As an undergraduate student, the new year might include making self-improvement goals such as getting better organized, more sleep (and less Netflix), and attending office hours to make meaningful connections with professors. But if you also include exploring your summer research options before the semester is in full swing, you won't lose out on an incredible opportunity simply because you miss an application deadline.

The spring term is time to explore summer-program and fellowship opportunities, even if your institution doesn't have the specific experience you want or if you're interested in an off-campus adventure. Many programs have application deadlines months before the start date. Some require a combination of personal essays and recommendation letters. It's easy to underestimate the time needed to identify the right experience and to put together a professional application packet. An early start will be the insurance policy you need.

Before you outright dismiss the idea of a potentially expensive summer away from your home campus, know that many positions include a stipend, room and board, or both. You won't get rich by participating, but, if the stipend is substantial enough, your summer away from home might be quite affordable.

Fellowship and internship opportunities are available at colleges, research centers, government laboratories and industry. Basically, there is no one-size-fits-all summer research experience. You'll want to consider the requirements and advantages of each program during your selection process.

To start, check with your campus office of undergraduate research and ask about known programs. Next, do an online search using terms such as "paid summer undergraduate research programs," "undergraduate research national lab" or "undergraduate research internship." Of course, you also could include your field of interest in your search, such as "undergraduate summer research chemistry."

For many programs, it won't take long to determine if you should pursue the possibility or move on to the next search result. First, read the program overview. If you're genuinely interested in the science, go straight to the eligibility requirements. If you meet them, put the application deadline on your calendar. Then make a list of what you need to complete the application so you can check off each item as you complete it.

Some programs, however, will take longer to consider, because you may need to apply to work on a specific project or in a specific laboratory. This will require reading several project descriptions to determine which one you connect with the most. You should not underestimate the importance of this task. Thoroughly reading the project descriptions will help you choose the right position and write a compelling personal statement about why you want to devote your summer to the program. Mentors want to work with students who believe in the science and the project. Plus, you'll be much happier if you choose a project that is meaningful to you.

After you've selected the programs you want to apply to, put a target date for submitting your application on your calendar three weeks before the due date. Don't wait for that target date to start the application process, but instead consider it your final warning to complete it. Be sure to follow up with recommenders who have not submitted their letters.

A summer research experience can be one of the most challenging and rewarding adventures you have as an undergraduate. It would be a shame to miss out on this adventure because the application deadline passed before you even considered applying.

For a listing of summer-research openings in the U.S., go to www.asbmb.org/summerresearch

David G. Oppenheimer (oppenhe@ufl.edu) is an associate professor in the department of biology at the University of Florida and co-author of the book "Getting In: The Insider's Guide to Finding the Perfect Undergraduate Research Experience."

Love and biochemistry

By Stefan Lukianov

I met my first love when I was 18 years old in the spring of 2002. We both attended Framingham High School in Massachusetts and met through mutual friends. Our interaction developed into something more significant over that summer. I was preparing to leave for college at the University of Maine in Orono; she had finished her junior year in high school. We decided to attempt a long-distance relationship after dating through the summer. The first year apart passed by without difficulty. Our feelings for each other continued to grow despite the geographical gap. She finished her senior year and decided to take a break before college. She eventually joined me at the University of Maine after another year or so. I figured we were completely committed and, therefore, set for life.

Divergent paths

My first love and I had different majors in college. Mine was biochemistry and hers was visual art. Our different curricula entailed distinct assignments and separate schedules. Biochemistry majors were consumed with regimented classes, endless testing and abundant laboratory work. I remember spending nearly all my time studying and experimenting. My agenda was maintaining a perfect 4.0 grade point average, soaking up research experiences and getting into a premier Ph.D. program. I did not have time for extracurricular activities, clubs or social events because of my workload. Little time was left for my first love. The dangerous distance this created began to seal us off from each other, and we gradually forgot the feelings we once had. The relationship became an entrenched formality.

Crumbled world

My first love and I dated all through my college years, even getting engaged in January 2006. I was accepted into the biological and biomedical sciences program at Harvard Medical School in my last semester of senior year. She transferred to the Massachusetts College of Art and Design so that we could be together in Boston after marriage. There was, however, no genuine kindness or affection between us at that point. She met another young man in one of her classes. As we grew apart, they grew closer. He gave her the attention I had forgotten how to provide. I was taking her and our relationship for granted. She left me for him at the close of the school year, after four years of struggling to keep the relationship alive.

I mentally collapsed into hysteria. Our engagement, my life, was destroyed, and I was unsure whom to blame. It felt like the world had flipped upside down, and my emotions rapidly shifted between anger, despair and resignation. I had nothing but questions running frantically through my mind. Was it my fault? How could she do this to me? Should I try to get her back, find someone else or stay single? Each day slipped into the next, and I kept in constant motion by running a lot and visiting people. I was trying all the while to avoid thinking about what they could be doing together at that moment. A part of me died in the sudden rupture between my first love and me. Thankfully, I had no professional obligations that summer, and my strong connections to family, friends and faith buffered the agony. I survived one grueling day at a time and entered Harvard in September 2006.

The lesson

I have had ample time to reflect in the 10 years since my first love left. I learned that work–life balance is crucial to success in the personal and professional spheres. Life-science researchers often work long hours to obtain desired results, write grants or papers or prepare presentations. I can attest that it is easy to forget the world outside the laboratory when you are in a groove or a bind. I've discovered that this forgetfulness, however, hampers my objectivity and disconnects me from my relationships. I've found that it is important periodically to step back and take a break. Preserving my sanity is necessary for me to do good science. The people around me are the anchor I need. My present objective is to maintain balance in my personal and professional life so that I can enjoy fully both love and science.

Stefan Lukianov (stefanlukianov@gmail.com) is a Ph.D. candidate at Harvard Medical School.

A journey to sobriety

By Dr. 24hours

I spent a long time in graduate school. I got a master's degree and a doctorate, and it took me 10 years. I did everything slowly on purpose, from stringing out my classes to taking summers off to travel and changing research topics again and again. My adviser eventually pulled my stipend to force me to finish. Even then, it took me another year. In August 2005, I sent my adviser an email at 2 a.m. to inform him I was going to abandon my dissertation. He wrote back ordering me to his home the next morning. We spent from then until November working side by side, page by page, to get me finished. I remember writing, "If you say I must defend the indefensible, I will try."

So I was not a model graduate student. My dissertation was barely adequate. But I defended it and was granted leave by my institution to call myself "doctor." "All the rights and privileges that entails," I believe says the letter that bestows the degree. I didn't graduate feeling like I'd earned any rights or privileges. I was lost and bewildered and had little idea what to do next or how. It was all supposed to be so much easier. My mother had done this. Her uncle had. How was I failing so painfully?

I was failing because I am an alcoholic. I began drinking either early or late, as alcoholics go. When I was 5 years old, I stole a bottle of liquor from my parents — crème de menthe — and got drunk, threw up and hid the bottle so I could do it again. I ate toothpaste to conceal the smell. Already I knew that drunkenness was shameful. Already I knew to lie. Two or three sporadic binges aside, I didn't really drink again until my senior year in college. That's when I began in earnest. Graduate school took me so long because, almost from the outset, I drank to intoxication nearly every single day.

I would awake at 10 or 11 a.m., go to class, work for perhaps an hour or surf the Internet in the lab, leave work at 4 p.m., and drink. A glass of wine with homework. Whiskey while studying. Then until I stumbled to bed or passed out on the sofa. I did this practically every day. I regularly drove drunk to get more liquor and cigarettes. I became obese and sedentary. The greatest efforts I invested were in the lies I told to protect and maintain my access to alcohol.

Alcoholism often is entwined deeply with a sense of entitlement. I was wildly defensive about my academic talent, my value and the scope of my contribution. I insisted on believing I was doing something worthwhile, because the truth was so manifestly the opposite: I was wasting a great deal of everyone's time and money. No one had any patience for me, and I had too much for myself. I slowly lost the thing I should have cared most about: the respect of the people invested in my success. I betrayed a great deal of support and confidence so that I could drink myself to oblivion daily.

Alcoholism isolates us first emotionally; we become internalized. We can't share what we do, because we know others don't approve of the way we drink. My internal process became a sea of resentment. I wanted to be able to conduct my drinking unmolested. But that conflicted with other things I wanted, like relationships and a degree. I did have relationships. Like many relationships among younger people, they began based in part on my apparent potential. They ended when that eventually was revealed as corrupt. My drinking was always more important to me than any other person was. Nothing mattered if it interfered with my ability to drink.

Eventually, drinking isolated me so thoroughly that I was literally locking myself in my bathroom, sitting in the bathtub, drinking vodka and cutting myself. I would watch little smoke-like curls of blood dissipate into the bathwater, imagining some black bile exiting my body to be replaced with clear, pure water. By this time, I had a fiancée and soon-to-be stepson. And by this time, I recognized my problem. I would stand in the bathroom with the bottle of vodka I kept hidden in the access panel to the plumbing where it wouldn't be stumbled upon, pour myself four ounces in a plastic cup, look in the mirror at myself and go, "You are ruining three lives with this drink."

I would say it out loud but quietly enough that I'd be the only one to hear it. And then I would drink. And then I would sit in the bath, and if I'd remembered to bring my knife, I'd cut myself again. This was my routine, over and over. This is what my alcoholism wants from me. From this haze of entitlement, self-hatred, selfishness and pity, my adviser dragged a dissertation out of me, a testament not to my fortitude but to his.

Days before my graduation ceremony, I was arrested for drunk driving. My blood alcohol content was 0.19. But because my arrest was across a state line from where I lived, I was able to conceal it from most of the people to whom it would have been a topic of serious concern. My fiancée was distraught and enraged,

but I always had been successful in lying to her. Our relationship counselor had growing suspicions about my ability to function. I convinced them both my BAC was only 0.11 and that I'd been pulled over for a routine stop, not dangerous swerving.

All throughout this time, I was seeing a therapist of my own as well. For years. I was "getting help" that some people knew I needed. But I almost never spoke to her about my drinking. I used it as a balm for the other childhood pains I imagined were my real problem. The pains I used as an excuse for my drinking. The things I drank at furiously.

I graduated and stagnated. No work. No purpose. And yet I married. Seven months into our marriage, our counselor told us, "There is no hope for this marriage, unless he addresses his drinking." I finally reached out for help. I went to an inpatient rehab starting in early 2008. I began attending meetings of Alcoholics Anonymous while there.

Among alcoholics, I am fortunate in that I have not yet relapsed. I have worked the 12 steps of the program in AA despite the fact that I am not particularly spiritual. I was offered a postdoctoral-like position in a hospital about six months into my sobriety. Sober, I was able to devote the talents I was born with to the tasks I was given with more industry. Within 18 months, I was promoted to a principal-investigator position.

Today, I have changed positions again and am now a program manager at a large academic hospital on the East Coast. As I write this, I have been continuously sober for 3,169 days. I haven't cut myself deliberately since day 4. My depression and entitlement have flared occasionally. My self-sabotage is a constant companion that I work regularly to minimize. Sobriety, like everything else in life, changes over time. But my alcoholism has not abated. There is no safe amount of alcohol I can drink.

But I don't miss alcohol. I'm not ashamed of being an alcoholic, even though I've done many shameful things. My marriage didn't survive, but I wish my ex-wife well, wherever she is. I'm sorry for what I put her and her son through. I did my best to make amends, but some things are unamendable. I continue to attend AA meetings. There, I have discovered many things I used to search for at the bottom of glasses but never found. I guard my sobriety first, and everything else is second, because if I am not sober, nothing else will persist.

In sobriety, I have become healthy and useful. I quit smoking seven years ago. I've published a score of peer-reviewed papers, won grants for my institution and made differences in the lives of the patients who seek care. I have a new life partner. Together, we have begun running marathons. My partner, a biochemist by training, is "normal," as we say. But she respects the work I have to do for my sobriety. We have a dozen pictures of ourselves, hand-in-hand, crossing finish lines of races. I've risen in my profession. I have, as the program promises, become intuitively able to handle situations that used to baffle me.

Graduate school is a crucible by design. I, like many of us, fanned the flames hotter with mental illness and resistance to aid. But I have emerged. I am a researcher. I am a marathoner and a triathlete. I am a partner. I am a mentor. I am still a student in and of my life. And I am a sober member of AA. My graduate education taught me to build tools and investigate the world. But my sobriety, and AA, taught me to live in it.

Dr. 24hours (infactorium@gmail.com) is a pseudonymous health-systems engineer and researcher at an academic hospital on the East Coast. Follow him on Twitter at twitter.com/Dr24hours.

Connecting with Legos

By Lego Grad Student

Legos were my favorite toy as a kid. I had a 5-gallon tub of pieces that I slowly accumulated over birthdays and other holidays. When I went to college, I reluctantly had to hand off my tub to my younger cousins. I figured that was the end of Legos for me.

I hit some rough patches in graduate school when I felt burnt out. It seemed like I'd forgotten how to take a break to recharge myself. I would waste time on my computer watching videos, and, by end the day, I would feel neither productive nor refreshed.

At those times, when I was looking for a pure distraction, I found myself thinking about Legos repeatedly. So once in 2013 and once last year, I went out to a Lego store and bought two large sets, larger than anything I'd had as a kid. Each time, I felt like I was living some sort of childhood dream — my mind would have exploded at age 12 if I'd had sets as large and expensive as these. They were a lot of fun to build, and I realized that Legos were still one thing that I really could enjoy without feeling guilty about work.

Once I finished building the second set, I remembered that my favorite part of playing with Legos was breaking everything apart and creating my own things. I now had more than 5,000 pieces from the two sets, so there was a lot that I could do. The first original thing I built was a small bathroom. I draped a small minifigure over the toilet to make it look like the figure was retching, just because I thought it made for an amusing image.

I wanted to create more things, but I wasn't sure what to do next. I looked at my small bathroom creation and thought it might be interesting to come up with a story of why and how this minifigure ended up clinging to a toilet. Perhaps because I was in my own head, I decided I would create a short series of pictures where the figure was a graduate student who had a terrible meeting with his adviser. I worked backward to create the four images that ended up coming before it. Once I looped back to the bathroom scene, I was having too much fun. I realized that graduate school offered a lot of ideas for posts, so I decided to keep going.

When I started making these Lego scenes, I put them up on my personal Facebook account for friends. After I posted a few, some friends suggested that I also put them up on other social media platforms. I had no idea that they would take off after a couple months of posting. I truly didn't expect any large number of people to see these posts, much less react so positively to them. (Editor's note: Lego Grad Student now has more than 9,000 followers on Twitter.)

I first made these as a dark joke to myself (and I fully admit I have a dark sense of humor), but it was remarkable to hear people say that the posts really resonated with them. That was never my intent, but I am glad that these posts can help people feel like they're not alone.

Lego Grad Student is a pseudonymous social-sciences graduate student. Follow Lego Grad Student (@legogradstudent) on Twitter, Instagram, Facebook and Tumblr.

Suffering from writer's block, the grad student stares at a screen as empty as his hopes and dreams.

Updating an old friend in the city about his life, the grad student hears himself say the word “still” a disconcerting number of times.

Perusing the latest journal issue, the grad student comes across an article that is uncomfortably similar to his dissertation.

CONTINUED ON PAGE 36

CONTINUED FROM PAGE 35

Listening to a professor's remarks, the grad student learns that "three small points" means "three missiles designed to obliterate your work and self-worth."

Feeling no less confined after coming to a spacious coffee shop, the grad student confronts the reality that his work is his prison.

2017 ASBMB
Special Symposia Series

Reminder: ASBMB members save on meeting registration.

Evolution and Core Processes in Gene Expression

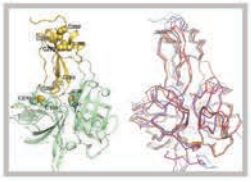
Organizers: Julia Zeitlinger, Stowers Inst., David Arnosti, Michigan State Univ. & Justin Fay, Washington Univ. in St. Louis

July 13 – 16, 2017, Kansas City, Mo.

Transforming Undergraduate Education in the Molecular Life Sciences

Organizers: Michael Carastro, Univ. of Tampa, J. Ellis Bell, Univ. of San Diego, Jim Lawrence, Univ. of Wisconsin–Stevens Point & Regina Stevens-Truss, Kalamazoo College

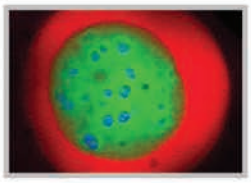
July 20 – 23, 2017, Tampa, Fl.



Membrane-Anchored Serine Proteases

Organizers: Qingyu Wu, Cleveland Clinic & Karin List, Wayne State Univ, Sch. of Med.

Sept. 14 – 17, 2017, Potomac, Md.



Emerging Roles for the Nucleolus

Organizers: Jennifer Gerton, Stowers Inst. & Thoru Pederson, Univ. of Massachusetts Med. Sch.

Oct. 26 – 29, 2017, Kansas City, Mo.

ASBMB

www.asbmb.org/specialsymposia/2017

WHERE **DISCOVERERS** MEET

Scientific research and professional connections converge at the 2017 ASBMB Annual Meeting — and you should be a part of it. As the year's most important event for the biochemistry and molecular biology community, the ASBMB's annual meeting is the ideal forum at which to share your research, exchange ideas and explore potential collaborations with leading investigators. Join the conversation on this year's hot topics, including antibiotic resistance, organelle trafficking and signaling, genetic analysis and more.

EARLY BIRD REGISTRATION DEADLINE: FEB. 23.

Register early and save up to 48%!

NEW

Meet the speakers!
Join world-renowned biochemists and molecular biologists for informal networking and scientific discussion during midday poster sessions.

ASBMB '17
ANNUAL MEETING
CHICAGO | APRIL 22 – 26

SUBMIT YOUR ABSTRACT TODAY! | ASBMB.org/meeting2017

LATE-BREAKING ABSTRACT SUBMISSION DEADLINE: FEB. 8.

Take this opportunity to advance your work and discuss the molecular mechanisms of life. **Submit your poster session abstract today.**