MACROLAB TO REVEAL THE INNER WORKINGS OF PROTEINS

by Camille Mojica Rey

From reading genes to multiplying, the basic chores of a living cell rely on giant assemblies of proteins and nucleic acids working in sync—in effect, operating as molecular machines. But the limitations of available imaging and purification techniques have made studying these life-giving complexes a challenge.

Now, MCB and UC Berkeley are taking a giant step forward in this area with a new state-of-the-art laboratory funded by a $2 million grant from the W.M. Keck Foundation. The Keck MacroLab, which brings together seven MCB faculty research programs, will focus on discovering the structural basis of four essential cellular chores—DNA replication, gene regulation, protein synthesis and cell division.

“These are things that have to happen in every form of life and we don’t understand them nearly as well as we should,” says BMB professor Tom Alber, principal investigator of the MacroLab and a faculty affiliate in the California Institute for Quantitative Biomedical Research (QB3). “Our goal is to solve a major bottleneck in biochemistry.”

Keck MacroLab, which will be housed in the nearly complete Stanley

NEW PROFS

BRING NEW TALENTS

RACHEL BREM

On average, people are 99.9% genetically identical. It sounds close, but actually this translates into some 3 million genetic differences between individuals. Although most of these differences have no function, some affect us profoundly. The best understood of these are changes that alter protein structure and function, like the gene variants that affect eye color, malaria resistance, and the ability to digest alcohol. But many genetic differences exert their influence by altering the regulation of genes. This type of polymorphism has proven more difficult to study, yet remains critically important.

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Schekman takes over Proceedings,
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Biosciences and Bioengineering Facility, will combine cutting-edge biochemistry, industry-level laboratory automation, and the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory to image molecular machines. The lab will allow project researchers to make large quantities of essential proteins, crystallize them, and image them as they interact. Besides furthering basic research, the resulting experimental synergy promises to speed up the discovery of new drugs, says neurobiology professor Geoff Owen, dean of the biological sciences. “Once you know the structure of the proteins in those interactions, you can design molecules that regulate, interfere with or otherwise modulate those interactions and, ultimately, change the outcome,” Owen said.

The MacroLab will likely serve as a model for other universities interested in increasing the scale of their research capabilities through on-campus collaborative ventures. “Biochemists everywhere are limited by these problems of scale. We hope that what we do here will be replicated elsewhere,” Owen said. “This project is going to be extremely important,” he predicted.

The secret to the lab’s future success will be the integration of methods developed by an interdisciplinary group of Berkeley researchers. Among the groundbreaking discoveries that will be incorporated is a method worked out by BMB professor James Berger for expressing large amounts of protein very quickly. Berger, who will direct the MacroLab, also helped develop a new way to grow crystals from tiny amounts of protein. The lab will also take advantage of Jamie Cate’s tools for determining the structure of intact ribosomes, as well as imaging methods developed by Eva Nogales and Robert Tjian to study gene regulatory proteins. An automated data analysis system created by Alber for the ALS will further accelerate the research.

By automating both sample production and analysis, investigators will be able to tackle proteins and complexes now considered out of reach. “The scientific problems we face are increasingly large and complex, involving the most rare and complicated protein assemblies. By turning over the human work to robots, we will do more work than any one lab group could do alone,” Alber says.

Alber envisions a pipeline that begins at the Keck MacroLab and ends with scientists comparing the inner workings of different molecular machines. “The lab will provide the new front-end of an experimental process that begins with making many more samples quickly and ends with using the ALS shared beamline to discover the structures of some of the cell’s most important molecular machines,” Alber said. “It will be an intellectual center where researchers working on distinct systems can compare methods and discoveries,” he added. “We will make possible discoveries that are impossible today.”

*Assistant Professor of Genetics and Development Rachel Brem is tackling the effect of polymorphisms on gene regulatory networks through a combination of experimental and computational approaches. The subject is of great interest for both biomedical and basic research. Most traits, including susceptibility to diseases like cancer and dementia, map to multiple mutations across long stretches of genetic code, rather than to single genes. Brem hopes to discover rules or themes that distinguish important from unimportant genetic changes.

Brem joined the department in spring 2006 after completing a postdoc with Leonid Kruglyak at the Fred Hutchinson Cancer Research Center in Seattle, where she studied natural variation in gene expression. Here at Berkeley, she is beginning her study of regulatory networks with a genome-wide search for yeast genes whose protein products regulate their own expression levels through a feedback loop. In her toolkit is a collection of promoters for each of yeast’s 6200 genes hooked up to the gene for green fluorescent protein (GFP). Deleting genes for proteins that do not feed back on their own expression should have no effect on the activity of the GFP reporter construct. In contrast, the deletion of positive auto-regulators should lower GFP expression, and deletion of negative auto-regulators should raise it. It will take some time and effort to get through the yeast genome, but ultimately Brem will have in hand a complete list of genes that use feedback as a component of their regulation. The hope is that the resulting data-set can be mined for recurring themes or other clues to the design principles of gene regulatory networks, thus making it easier to distinguish important genetic variants. Synthetic biologists, who seek to build custom genetic programs for scavenging oil or manufacturing drugs, also hunger for gene regulatory data. “The better we understand how nature has engineered the regulatory circuitry of different genes, the better we can design new circuits,” Brem says. “That’s one of the driving forces in the field.”

To mine data-sets, Brem nearly always writes her own software. “I have a pathology where I don’t like to use other people’s software. There are people who build their own microscopes and other lab hardware. I’m that way with software.” It’s a common refrain from bioinformaticists. If you want to understand how the program is treating the data, you had better understand the code. But reading someone else’s code is as challenging as reading someone else’s lab notebook, Brem says. So it’s often faster, not to mention safer, to build your own.
While the emphasis on computation can be off-putting to biology students, it needn’t be, Brem says. “Students come to me and wonder if they need to be able to write code to work in my lab. My answer is no, of course not.” Brem herself knew nothing about writing computer programs until she started grad school, she says. And in any case, students will find they pick it up fast when they are exploring their own experimental results. “What’s really cool is you get to write computer code to analyze your own data,” Brem says.

Brem’s temporary lab space is in Hildebrand Hall, where she currently employs one lab manager and a rotation student. She is slated to move into the new Stanley Hall in the spring.

**MICHAEL RAPE**

The human body contains some 100 trillion cells, each of which is descended from just one fertilized egg. The countless divisions that intervene between conception and adulthood must proceed without a hitch: mistakes could lead to cancer, deformity, sterility or death. Yet each division is fraught with peril. Billions of DNA bases must be accurately copied, mitochondria must replicate, the cytosol must expand, membrane must grow, and each daughter must end up with exactly one copy of each chromosome or problems will ensue.

To ensure faithful division, the cell cycle holds up at a series of checkpoints and only proceeds when specific processes, such as DNA replication, are complete. Among the last of these before cell division is the spindle assembly checkpoint, where the cell monitors readiness to divide through the sequential degradation of regulatory proteins. Michael Rape, who joined the department as an assistant professor of cell and developmental biology this fall, would like to understand just how the cell carries out this serial destruction.

This interest naturally leads to the study of the anaphase promoting complex (APC) which coordinates progression into mitosis by tagging cell cycle regulators with a chain of ubiquitin, a biochemical earmark for destruction. The protein’s fate is sealed once the chain reaches four ubiquitins in length. As a postdoc in Marc Kirschner’s lab at Harvard University, Rape discovered how the APC ensures that its targets are destroyed in the right order. Some APC targets receive the entire four-unit chain at once and are quickly destroyed, while others receive ubiquitin in units sequentially over the course of several binding contacts with the APC. Destruction of these latter “distributive” substrates can be further delayed by the selective removal of units, or de-ubiquitination.

The upshot is a range of degradation rates that depend on the target’s processivity. For instance, the proteins Cdc20, Aurora A, Plk1 and UbcH10 are degraded in that order near the end of mitosis. This proves to correspond to their order of processivity, with Cdc20 being the most processive APC substrate. This substrate ordering depends on signal sequences within the substrate itself whose mechanistic details Rape is now trying to work out. “The end result is the same order of proteolysis through each cell cycle,” Rape says. “What’s more, the timing of degradation by APC is highly conserved,” he adds, suggesting that timing is a critical feature of the system.

Thus any substrate of APC is likely to be important in cell cycle regulation, and Rape believes that many of these remain to be discovered. But finding targets of the APC is not straightforward, since the end result of an interaction with the complex is the destruction of the protein of interest. Cell cycle proteins also tend to be present only at low concentrations and APC substrates bind only transiently.

So Rape is using an approach called in-vitro expression cloning. He makes small pools of cell products of 24 genes at a time, and mixes these with APCs in a cell-free system, either with or without an APC inhibitor. He runs the proteins out on a gel and looks for bands that appear to get chopped up by APC. So far he has screened a quarter of the library and found four new substrates.

Another area of effort for Rape is to better understand the role of de-ubiquitinating enzymes, or DUBs. APC substrates that are tagged distributively may also have ubiquitin stripped in between APC contacts by one or more DUBs, thus delaying their ultimate demise. The human genome encodes at least 80 different DUBs, and mutations in some of them have been associated with cancer and neurodegenerative diseases. Yet their role in cell cycle progression, to which they bring another layer of sensitivity and complexity, has been largely overlooked until now. “The importance of the DUBs has been totally underestimated,” Rape says.

Rape hails from Bavaria, where he did his graduate work with Stefan Jentsch at the Max Planck Institute for Biochemistry in Munich. His fiancée, also a scientist, works for a biotechnology company in South San Francisco. Rape is currently enjoying temporary space in BMB professor Stuart Linn’s laboratory. In the spring, when some faculty will move into the completed Stanley Replacement Building, he will finally settle into his new home on the fifth floor of Barker Hall.

**Orderly destruction: the cell cycle regulators Cdc20, Plk1 and UbcH10 (green) are eliminated in sequence as the cell divides.**
Among Nick Cozzarelli’s legacies was a revitalized Proceedings of the National Academy of Sciences (see Spring 2006 Transcript). Now CDB Professor Randy Schekman had taken the helm as the journal’s new Editor-in-Chief. He gave us a preview of things to come.

Do you see yourself following in Cozzarelli’s footsteps, or will you go your own way?

Nick really shook things up at PNAS. He democratized the journal, I want to do that even more. One thing Nick started is a new division of coverage called “sustainability,” which includes ocean science, biosphere research, the carbon cycle, that sort of thing—truly interdisciplinary. It takes advantage of the academy’s broad membership. Other such interdisciplinary programs are attractive to consider.

Speaking of membership, Nick ended the members-only tradition at PNAS by creating Track II, which allows non-members to publish via peer review. Yet more than half of PNAS papers are still either contributed or communicated by members. Is this a concern of yours?

My top priority will be to tighten up the review process. The perception is that PNAS benefits its members by allowing them to submit papers that would not pass anonymous peer review. For instance, in Track II, only 18% of submissions are published. That is higher than Science or Nature, but still fairly selective. On the other hand in Track III, member contributions, only six to ten papers a year are rejected for not being good enough to publish. Obviously we welcome the outstanding contributions of members, but I fear that more than just six to ten of them per year fall below acceptable standards. I will ask the Editorial Board to take a more active role in vetting the manuscripts contributed by members.

And what about Track I papers, those communicated by members on behalf of non-members?

Many members feel that communicated papers could be handled by the Track II peer review system. A member could still recommend a paper and offer to serve as a referee. But ultimately that would be at the discretion of the Editorial Board.

How quickly do you plan to make these changes?

Initially, I want to do a review of Track I. I will ask editorial board members if they feel papers are getting in that should not be published. Our standard for Track II is to publish the top 10% of papers in a given field. Surely this should apply to Track I. I also plan to poll members about applying standards for Track III. I expect a lot of agreement on this. I believe that most members want to show their best work and do not want to publish incomplete or poor work.

Nick raised the profile of the journal quite a bit. Can more be done?

Besides tightening peer-review, I want to add a small number of featured articles. These could be longer than normal for a PNAS paper, and will be prominently covered by commentary and publicity in the journal. Such featured articles would be subject to the highest standards of peer review for member and non-member submissions.

Will you move the journal further in the direction of open access?

On open access, Nick did a great job. My feeling is that some aspects of the pressure for immediate open access are not consistent with the long-term financial viability of public and private journal publishers. Already all papers are available six months after publication, and authors can pay an up-front fee for immediate release.

Will you make more use of the Internet?

We already have advance publication of papers on the Website. Mainly I’d like to use the Internet to enhance our connection to our readers. For instance, we could do video interviews with newly elected members. I’d also like to have podcasts of interviews with authors of important papers. We must also seriously reconsider how papers are published and reconcile the current disjointed publication of a fixed length hard copy and on-line supplementary material. Print versions will probably go the way of the dinosaurs.

Any other major changes?

We would like to increase the number of submissions from the physical and social sciences. Nick started on this by asking editors to find groups of papers to be published together for greater visibility in the respective communities, and I plan to enhance that effort.

You want more submissions, but don’t people often gripe about the slow turn-around at PNAS?

Right now it’s about 40 days from submission to first response. The bottleneck is often finding a member to handle the paper. To relieve this a little, Nick had instituted guest editors, non-members with expertise to handle certain papers. But we still only go to them after a list of members has been tried and exhausted. I would like staff to contact both members and guest editors right off the bat. The goal is for referees to be secured within the first week.

What’s your grand vision?

When I was a grad student, PNAS was the journal where you sent your best work. That is not true anymore. I want to re-establish its priority.

How fast can the perception of a journal be changed?

Remember what Ben Lewin did at Cell in the 1970s? In just one or two years he took it from barely on the radar screen to one of the most coveted places to publish in biology. I believe we can do that at PNAS.
The American Society for Biochemistry and Molecular Biology has designated three of Mike Chamberlin’s (BMB Emeritus) early papers as “classics.” Published in 1974 in the Journal of Biological Chemistry, the papers described for the first time how RNA polymerase initiates transcription (more at www.jbc.org/cgi/content/full/281/45/e36).

David Drubin (CDB) has received an NIH MERIT award. These research extension prizes recognize outstanding contributions and are given to no more than 5% of NIH-funded researchers.

Udi Isacoff (Neuro) is lead investigator on a $6 million NIH Roadmap grant to establish the Nanomedicine Development Center in Optical Control of Biological Function. The new effort, announced this semester, includes 13 faculty members from Berkeley, Stanford, Caltech and the Scripps Research Institute. The other MCB faculty involved are Rich Kramer, John Flannery (Neuro) and Carolyn Bertozi (BMB and Chemistry). The goal, Isacoff says, is to develop methods for remote control of protein function—and thus cellular activity—for applications in neuroscience, cell biology, and synthetic biology.

Caroline Kane (BMB) has been awarded the 2007 Excellence in Service Award of the California Alumni Association, to be conferred in March.

Jack Kirsch (BMB) was elected a fellow of the American Association for the Advancement of Science in the Chemistry Division.

Judith Klinman (BMB and Chem) received the Merck Award from the American Society for Biochemistry and Molecular Biology.

In the new year, Eva Nogales (BMB) will be a coeditor of the Macromolecular Section of Current Opinion in Structural Biology.

George Oster (CDB) was elected to the American Academy of Arts and Sciences.

Jasper Rine (G&D) has been named Director of the Center for Computational Biology, which runs MCB’s Designated Emphasis in Computational and Genomic Biology (see Spring 2006 Transcript). For more information, visit http://computationalbiology.berkeley.edu/.

Harry Rubin (CDB Emeritus) received the Alexander and Mildred Seelig Magnesium Award from the American College of Nutrition for his work establishing the central role of free intracellular magnesium in the coordinate control of cell growth and proliferation (www.amcollanutr.org).

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Jeremy Thorner (MBB) is now an associate editor for Nature’s Signaling Gateway Molecule Pages (www.signaling-gateway.org/molecule/).

Karsten Weis (CDB) received the 2006 Early Career Life Scientist Award from the American Society for Cell Biology (www.ascb.org) at the society’s annual meeting in San Diego in December.

FACULTY NEWS

NETS PIONEER AWARD

Rebecca Heald is one of 13 researchers chosen in September to receive the 2006 Pioneer Award from the National Institutes of Health. The grant provides $2.5 million over 5 years for research that might have been passed up under the usual peer-review system for being too high-risk or premature to receive support.

Heald intends to investigate how cells regulate the size of their organelles. The question is potentially important to understanding the progression of cancer, as many tumor cells carry abnormally large or misshapen organelles. In her research on cell division, Heald has found that the size of the spindle apparatus that separates the chromosomes during mitosis can vary significantly between species and that the difference depends on a cytoplasmic factor. She plans to go after the factor responsible as a first step to understanding organelle scaling.

“[The] scaling of organelles is an interesting idea that has been difficult to investigate,” Heald says. “We don’t have the first clue as to what regulates it—it’s a wide-open question.”

The NIH established the Pioneer Awards in 2003 as part of the Roadmap for Medical Research. The idea was to fund projects whose likelihood of success is uncertain, yet whose potential to advance knowledge dramatically or to influence a broad range of disciplines makes them attractive.

HIGH-RISK PROJECT

“Tug of war: spindle assembled around DNA-coated beads in a cytoplasmic extract. Spindle size varies from species to species.”
2005–2006 GRADUATES

**FALL 2005**
- Kevin D. Corbett (Berger) Structural and biochemical studies of type IIA topoisomerase from bacteria and archaea
- Brandon S. Davies (Rine) Regulation of ergosterol biosynthesis in *Saccharomyces cerevisiae* by the transcription factors Upc2p and Ecm22p
- Renee M. Deehan Kenney (Heald) A characterization of chromosomal proteins in spindle function using *Xenopus* egg extracts
- James Endres Howell (Garriga) Wnts are guidance cues in anterior-posterior neuronal migration
- John J. Engelhardt (Allison) Characterizing the function and trafficking of CTLA-4 in T cells
- Aimee Ruth Ginley (Coscoy/Raulet) MA
- Susanne J. Hoheisel (Kane) Regulation of RNA polymerase II CTD phosphatase in *S. cerevisiae*
- Timothy W. Kutzkey (Tjian) Functional dissection of long-range enhancer specificity in *Drosophila melanogaster*
- Chad D. Meliza (Dan) Spike timing and dependent plasticity of receptive fields in primary visual cortex
- Elena M. Rodriguez (Martin) Role of atypical protein kinase C in cellular transformation by Src
- Diana J. Starr (Cline) Positive selection for Sex- lethal’s germline regulators and targets in *Drosophila melanogaster*
- Lisa A. Valdin (Doudna) Master’s thesis: Characterization of alternative U2AF-like subunits in *Drosophila*
- Neil J. Webb (Welch) MA
- Claire V. Weer (Dernburg/Rubin, E.) Master’s thesis: Functional & molecular characterization of cholesteryl ester transfer protein evolution in mammals
- Scott A. Weitz (Cline/Rio) Master’s thesis: Characterization of Sex-lethal male splicing enhancers

**SPRING 2006**
- Sue Yeon Choi (Kramer) Measuring light-regulated synaptic activity in the intact vertebrate retina using optical imaging methods
- Alenka L. Copic (Schekman) Genetic and biochemical analysis of intracellular clathrin-mediated protein transport in *Saccharomyces cerevisiae*
- Shaun N. Cordes (Garriga) Genes that regulate asymmetric neuroblast divisions in *C. elegans*
- Susan E. Crown (Handel) Characterization of chemokine heterodimerization and glycosaminoglycan interactions
- Russell B. Fletcher (Harland) Signaling mechanisms regulating early neural patterning and mesoderm formation
- Dragony Fu (Collins) Molecules and mechanisms of human telomerase biogenesis and regulation
- Jamie K. Geier (Schles) Regulation of recombination at the immunoglobulin heavy chain locus
- Erin D. Goley (Welch) Dissecting the mechanism of activation of Arp2/3 complex and its role in baculovirus
- David W. Hodge (Glaser) MA
- John R. Hogg (Collins) Purification and functional analysis of ribonucleo-protein complexes containing non-coding RNAs
- Ariel R. Krakowski (Luo) Negative regulation of TGF-β signaling by SnoN and Ski: structure, localization, and roles in tumorigenesis
- Peter W. Lewis (Botchan) Identification and characterization of the *Drosophila* Myb–MuvB complex
- Eric C. Logue (Shal) Novel regulation of the co-stimulatory ligand B7
- Adam C. Martin (Drubin) The role of nucleotide in the function of the Arp2/3 complex
- John P. Mertie, Jr. (Zusman) The role of the protein FrzS in the social motility of *Myxococcus xanthus*
- Stephen E. Moyer (Botchan) CMG: A new eukaryotic DNA replication complex
- Yangling Mu (Pool) Visual experience-dependent plasticity in the developing retinotectal system
- Kathryn E. Muratore (Kirsch) Aminotransferase diversity and function
- Kristi E. Pullen (Alber) Structural and functional characterization of PstP, the PP2C-type Ser/Thr phosphatase of *Mycobacterium tuberculosis*
- Ajna S. Rivera (Weisblat) A role for Notch signaling during segmentation of the leech *Helobdella robusta*, a lophotrochozoan
- John R. ten Bosch (Cline) The TAGteam DNA motif controls the timing of *Drosophila* pre-blastoderm transcription
- David E. Wildes (Marqusee) High-energy conformations in protein folding and binding
- Jonathan J. Wong (Barnes/Drubin) Organization and function of the budding yeast spindle and Dam1 complex
- Robert P. Zinzen (Levine) Regulation of gene expression in the ventral neurogenic ectoderm of *Drosophila melanogaster*
CLASS NOTES

Kurt Ahrens [PhD 1998] is Associate Director of Systems Neuroscience at the Allen Institute for Brain Science in Seattle, where he is setting up a lab to do in vivo imaging of neural activity. Kurt says of the new job, "It's great to be back on the West Coast; and there is a Peet's Coffee shop one block from my office!"

Patrick Chen [BA 1997] received his MD from the University of Michigan in 2003 and completed his residency in family medicine at UCSF in June, 2006. He took a position as Assistant Medical Director of SOS (www.shareourselves.org), a free clinic in Costa Mesa, California. (chenpatrick@yahoo.com)

Nayiri Doudikian-Scaff [BA 1993] graduated from medical school at Hahnemann University in Philadelphia in 1997 and went on to a fellowship in plastic surgery at Wayne State University, which she finished in 2003. She now practices plastic surgery in Pasadena, California, near where she lives with her husband Daniel Scaff, a pediatrician and fellow Cal alumnus, and their two boys, ages 5 years and 17 months. (dnscalli@aol.com)

Farhad Farzaneian [BA 1998] is currently chief resident in the University of Rochester Medical Center’s Department of Radiology. He expects to finish in 2007. His wife, whom he married in 2005, is a resident in physical medicine and rehabilitation. She plans to pursue a career in sports medicine. Farhad intends on doing a fellowship in interventional radiology and hopes to move back to northern California soon. (farhadfr@yahoo.com)

James Graziano [BA 1998] has been a staff scientist at Aestherx, Inc., in Woodland Hills, California, since June 2006. He earned his PhD in the lab of Peter Schultz at The Scripps Research Institute in La Jolla. (jim.graziano@gmail.com)

Mark A. Hoffman [BA 1992] has a medical practice specializing in internal medicine and pediatrics. He lives in Pasadena, California. (hoffmanmarka@mac.com)

Jocelyn Krebs [PhD 1997] was promoted to associate professor with tenure in the Department of Biological Sciences at the University of Alaska, Anchorage, where she has been for six years. Jocelyn says she is “still studying chromatin structure and binding of charging moose.” (afje@uua.alaska.edu)

Lily Tsai [BA 2001] recently earned her MD from the University of Southern California and is now a resident in obstetrics and gynecology at the University of Rochester in New York. (mightytsail@gmail.com)

Ming M. Zhang [BA 1994] earned his MD from Duke University in 2000 and then trained in general & thoracic surgery at both Duke and West Virginia University. Since 2005, Ming has been in private practice in western Florida. He was married in September, 2006. (eurolok003@hotmail.com)

Do you have a bachelor's, master's or Ph.D. in Molecular and Cell Biology from Berkeley? Let your classmates know what you are up to by sending in a Class Note for publication in the next issue.

To send your Class Note, you can

- Clip and mail this form or
- go to mcb.berkeley.edu/alumni/survey.html or
- Send e-mail to tscript@berkeley.edu

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