Table of Contents

The Annual Report of Caltech's Division of Biology and Biological Engineering presents major research accomplishments of Caltech's faculty, students, and staff. Through their Principle Investigators, each Caltech group, laboratory, or center submits a report on research projects that were active during the prior Caltech academic year. These reports summarize research progress and results, and list personnel, sponsors, and their publications.

2012-2013 News, Events and People
Division of Biology Highlights
Press Releases
New Biology Faculty Members
Biology Annual Retreat
Ferguson Prize
Professorial Awards and Honors
Division of Biology Seminars
Named Lectures
Mark Konishi’s 80th Birthday Symposium
Biology Graduate Students
Biology Graduates
Financial Support and Donors
Faculty & Research Staff
Administrative Staff

Developmental and Regulatory Biology Faculty
Bronner, Marianne - Albert Billings Ruddock Prof. of Biology
Davidson, Eric - Norman Chandler Prof. of Cell Biology
Elowitz, Michael - Prof. of Biology and Bioengineering
Goentoro, Lea – Asst. Prof. of Biology
Hay, Bruce - Prof. of Biology
Mazmanian, Sarkis - Prof. of Biology
Meyerowitz, Elliot - George W. Beadle Prof. of Biology
Rothenberg, Ellen - Albert Billings Ruddock Prof. of Biology
Stathopoulos, Angelike - Prof. of Biology
Paul Sternberg - Thomas Hunt Morgan Professor of Biology

Structural, Molecular and Cell Biology
Aravin, Alexei - Asst. Prof. of Biology
Baltimore, David - President Emeritus, Robert Andrews Millikan Prof. of Biology, Nobel Laureate
Bjorkman, Pamela - Max Delbrück Prof. of Biology
Campbell, Judith - Prof. of Biology and Prof. of Chemistry
Chan, David - Prof. of Biology
Deshaias, Ray - Prof. of Biology
Dunphy, William - Grace C. Steele Prof. of Biology
Fejes-Tóth, Katalin - Thomas Hunt Morgan Senior Research Fellow
Jensen, Grant - Prof. of Biology
Kennedy, Mary - Allen and Lenabelle Davis Prof. of Biology
Mayo, Stephen - Bren Prof. of Biology and Chemistry, Chair
Newman, Dianne – Prof. of Biology and Geobiology
Phillips, Rob – Fred and Nancy Morris Prof. of Biophysics and Biology
Varshavsky, Alexander - Howard and Gwen Laurie Smits Prof. of Cell Biology

Molecular, Cellular and Integrative Neuroscience
Adolphs, Ralph - Bren Prof. of Psychology and Neuroscience, Prof. of Biology
Allman, John - Frank P. Hixon Prof. of Neurobiology
Andersen, Richard - James G. Boswell Prof. of Neuroscience
Anderson, David - Seymour Benzer Prof. of Biology
Gradinaru, Viviana - Asst. Prof. of Biology
Lester, Henry - Bren Prof. of Biology
Meister, Markus - Lawrence A. Hanson, Jr. Professor of Biology
Patterson, Paul - Anne P. and Benjamin F. Biaggini Prof. of Biological Sciences
Prober, David - Asst. Prof. of Biology
Shimojo, Shinsuke - Gertrude Baltimore Prof. of Experimental Psychology
Siapas, Athanasios - Prof. of Computation and Neural Systems
Tsao, Doris - Asst. Prof. of Biology
Zinn, Kai - Prof. of Biology

Biology Facilities
Flow Cytometry Facility
Genetically Engineered Mouse Production Facility
Millard and Muriel Jacobs Genetics and Genomics Laboratory
Monoclonal Antibody Facility
Nucleic Acid and Protein Sequence Analysis Computing Facility
Protein Expression Center
Protein/Peptide Microanalytical Laboratory
The California Institute of Technology, in a move that creates an academic division unlike any other among its peer institutions, has combined the disciplines of biology and biological engineering into a new Division of Biology and Biological Engineering (BBE). The division, formally approved by the Caltech Board of Trustees in April, expands Caltech's Division of Biology, which was founded in 1928 by Nobel Prize–winning geneticist Thomas Hunt Morgan. Biological engineering focuses on using a "bottom up" approach to manipulate biological substrates, such as genes, proteins, and cells, to produce a given outcome or to encourage fundamental discovery—as opposed to the "top down" engineering of chips, medical implants, or other macroscopic devices.

"Biological engineering represents an engineering discipline that is based on the fundamental science of biology, and the formation of BBE further highlights Caltech's distinctive nature, as we tend to be extremely quantitative in our approach," says Stephen Mayo, William K. Bowes Jr. Foundation Chair of the division and Bren Professor of Biology and Chemistry. "Although other schools have biological engineering programs within their schools of engineering, none have a college or school in which biological engineering is integrated directly with biology, so they can enhance each other—allowing those people who are doing engineering to interact more closely with those who are doing fundamental work and obtaining basic knowledge. The potential synergy is powerful and important."

"The creation of BBE is a critical part of an effort at Caltech to enhance bioengineering and biological sciences and to continue Caltech's position at the forefront of these fields," says Edward M. Stolper, Caltech's provost and interim president.

As part of this change, a total of 11 professors have been added to BBE from other Caltech divisions; they represent research areas spanning genetic engineering, translational medicine, synthetic biology, molecular programming, and more. The restructured division will consist of three administrative groupings: biology, biological engineering, and neurobiology. Caltech's undergraduate program in bioengineering, previously administered by the Division of Engineering and Applied Sciences (EAS), will be managed by BBE, and the existing bioengineering graduate program also will move to BBE.

The division will manage the existing biology graduate and undergraduate options; a newly established neurobiology graduate option; the biochemistry and molecular biophysics (BMB) graduate option in collaboration with the Division of Chemistry and Chemical Engineering (CCE); and the computation and neural systems (CNS) graduate option in collaboration with EAS. Caltech's Donna and Benjamin M. Rosen Bioengineering Center, founded in 2008 through an $18 million gift from the Benjamin M. Rosen Family Foundation, will remain the campus hub for bioengineering activities and will continue to be jointly administered by BBE, EAS, and CCE.

"The formation of BBE is a reflection of the diversity and breadth of the activities in biological sciences and engineering at Caltech—from the structure and function of proteins at the atomic level to developing nanoprobe electrodes that can simultaneously measure the activity of thousands of neurons in the brain," says Mayo. "Putting these activities into one division increases the potential and the pace for providing transformative solutions to some of the biggest problems in science, medicine, and health."

The last time a division at Caltech changed its name was in 1970, when the Division of Geological Sciences became the Division of Geological and Planetary Sciences.

Written by Kathy Svitil
Director of News
Caltech Marketing and Communications
PRESS RELEASES

NEW FACULTY MEMBERS

ANNUAL BIOLOGY RETREAT

FERGUSON PRIZE

PROFESSORIAL AWARDS AND HONORS

DIVISION OF BIOLOGY SEMINARS

SPECIAL LECTURES

MARK KONISHI’S 80TH BIRTHDAY SYMPOSIUM

BIOLOGY GRADUATE STUDENTS

BIOLOGY GRADUATES

FINANCIAL SUPPORT AND DONORS

FACULTY AND RESEARCH STAFF

ADMINISTRATIVE STAFF
07/10/12
Caltech Biologist Stephen Mayo Named Inaugural Bowes Division Chair
Bowes Foundation gift will seed innovative projects in the biological sciences.
Stephen Mayo

07/12/12
Caltech Receives Gift from Sackler Foundation to Advance Biomedical Science Research
The California Institute of Technology (Caltech) and UCLA have launched highly productive collaborations in cancer research and other areas of biomedicine in recent years, frequently through the Caltech lab of Nobel Laureate and President Emeritus David Baltimore.
David Baltimore

07/17/12
Caltech Researchers Find Evidence of Link between Immune Irregularities and Autism
Scientists at the California Institute of Technology (Caltech) pioneered the study of the link between irregularities in the immune system and neurodevelopmental disorders such as autism a decade ago.
Paul Patterson and Sarkis Mazmanian

08/28/12
Modeling the Genes for Development
Caltech biologists create the first predictive computational model of gene networks that control the development of sea urchin embryos.
Eric Davidson

09/20/2012
Moving Targets
Caltech biologists gain new insight into migrating cells.
Paul Sternberg

09/25/12
Two Caltech Researchers Receive NIH Director's Awards
Two members of the California Institute of Technology (Caltech) faculty have been given National Institutes of Health (NIH) Director's Awards.
Dianne Newman and Doris Tsao

09/26/12
Ready for Your Close-Up?
Caltech study shows that the distance at which facial photos are taken influences perception.
Ralph Adolphs

10/02/12
Caltech Biologist Named MacArthur Fellow
Sarkis Mazmanian, a microbiology expert at the California Institute of Technology (Caltech) whose studies of human gut bacteria have revealed new insights into how these microbes can be beneficial, was named a MacArthur Fellow and awarded a five-year, $500,000 "no strings attached" grant.
Sarkis Mazmanian
10/08/12
**Traveling with Purpose**
Biologist spends summer vacation volunteering in India.
*Pamela Bjorkman*

10/12/12
**Wordy Worms**
Lurking in the crevices of our planet are millions and millions of microscopic worms.
*Paul Sternberg*

10/16/12
**Two Faculty Members Named Packard Fellows**
Two Caltech faculty members have been awarded Packard Fellowships for Science and Engineering. Biologist Alexei Aravin and astronomer John Johnson each were awarded $875,000, to be distributed over five years.
*Alexei Aravin*

10/31/2012
**Developmental Bait and Switch**
Caltech-led team discovers enzyme responsible for neural crest cell development.
*Marianne Bronner*

11/20/2012
**A Fresh Look at Psychiatric Drugs**
Caltech researchers propose a new approach to understanding common treatments.
*Henry Lester*

11/20/2012
**An Eye for Science: In the Lab of Markus Meister**
*Markus Meister*

12/04/2012
**Brain Control with Light**
Watson Lecture Preview
*Viviana Gradianru*

12/12/2012
**Social Synchronicity**
New Caltech-led research finds a connection between bonding and matched movements.
*Shinsuke Shimojo*

12/13/2012
**Research Update: Wordy Worms and Their Eavesdropping Predators**
For over 25 years, Paul Sternberg has been studying worms—how they develop, why they sleep, and, more recently, how they communicate.
*Paul Sternberg*
01/30/2013
**Sorting Out Stroking Sensations**
Caltech biologists find individual neurons in the skin that react to massage.

David Anderson

03/19/2013
**Mayo Appointed to National Science Board**
President Barack Obama has appointed Stephen Mayo, Caltech's William K. Bowes Jr. Foundation Chair of the Division of Biology and Bren Professor of Biology and Chemistry, to the National Science Board, the governing body of the National Science Foundation.

Steve Mayo

03/21/2013
**The First Genetic-Linkage Map**
From the Caltech Archives - One hundred years ago, in 1913, Alfred H. Sturtevant helped lay the foundations of modern biology by mapping the relative location of a series of genes on a chromosome.

03/25/2013
**Developing Our Sense of Smell**
Caltech biologists pinpoint the origin of olfactory nerve cells.

Marianne Bronner

04/19/2013
**Caltech Senior Wins Gates Cambridge Scholarship**
A senior bioengineering major and English minor at Caltech, has been selected to receive a Gates Cambridge Scholarship, which will fund her graduate studies at the University of Cambridge for the next academic year.

Catherine Bingchan Xie

04/25/2013
**Fifty Years of Clearing the Skies**
A Milestone in Environmental Science

05/21/2013
**Keeping Stem Cells Strong**
Caltech biologists show that an RNA molecule protects stem cells during inflammation.

David Baltimore
Assistant Professor, Mitchell Guttman received his B.A. in Molecular Biology and Computational Biology and his Masters of Biotechnology in Computational Biology and Bioinformatics from the University of Pennsylvania in 2006. During this period, he developed novel statistical and computational methods for studying copy-number aberrations in cancer and applied them to identify previously uncharacterized regions important for progression in breast cancer. He then joined the Biology graduate program at Massachusetts Institute of Technology (MIT) where he worked with Eric Lander at the Broad Institute of MIT and Harvard for his Ph.D. and then as a Fellow. His work was a stunning combination of genomics, statistical (i.e., bioinformatic) analyses and genetic studies that allowed him to discover thousands of large noncoding RNAs (large intergenic non-coding RNAs; lincRNAs) encoded in the human genome. He established his laboratory on the second floor of the Kerckhoff Laboratory to study the function of lincRNAs, pursuing the hypothesis that lincRNAs act as 'flexible scaffolds' that assemble complexes of chromatin-binding proteins to regulate gene expression and hence cell state decisions in embryonic stem cells.
During the fall term, the Biology Division Faculty, postdocs, and graduate students gather for an annual retreat. The Biology Retreat provides an opportunity for new faculty and new graduate students to meet, socialize and familiarize themselves with the diverse research taking place in various labs. It also assists the grad students in selecting their rotation labs. In addition, many collaborations begin as a result of the talks and discussions that take place during the three-day event.

**Session I**

**Alexei Aravin**  
Assistant Professor of Biology  
*Welcome and Orientation*

**Bruce Hay**  
Professor of Biology  
*“Engineering the composition and fate of wild populations”*

**Markus Meister**  
Professor of Biology  
*“Neural computations in the retina”*

**Katalin Fejes-Tóth**  
Thomas Hunt Morgan Senior Research Fellow of Biology  
*“Small RNA mediated transcriptional silencing in Drosophila”*

**Shin Shimojo**  
Gertrude Baltimore Professor of Experimental Psychology  
*“Internal state of the brain is critical for perceptual decision making: Psychophysics, EEG, and causality”*

**Grant Jensen**  
Professor of Biology  
*"The role of ESCRT in Sulfolobus cell division"*

**John Grotzinger**  
Fletcher Jones Professor of Geology  
*Keynote Speaker: “Mars science laboratory: initial results”*
**Session II**

**Alejandro (Alex) Balazs, Ph.D.**
Postdoctoral Scholar in Biology
David Baltimore Lab

“Engineering Immunity Against HIV”

**David Prober**
Assistant Professor of Biology

“A screen for small molecules that affect zebrafish circadian rhythms and sleep”

**Ameya Champhekar**
Postdoctoral Scholar in Biology
Ellen Rothenberg Lab

“PU.1 regulates T-lineage gene expression and progression via indirect repression during the early stages of T-cell development”

**David Chan**
Professor of Biology, Bren Scholar

“Regulation of mitochondrial fusion”

**Pamela Bjorkman**
Max Delbrück Professor of Biology

**Alexei Aravin**
Assistant Professor of Biology

“Small RNA in bacteria”

**Long Cai**
Assistant Professor of Chemistry

**Session III**

**Lili Yang**
Project Manager & Lead Scientist
Baltimore Lab

“Title”

**Eun Jung Hwang**
Postdoctoral Fellow
Andersen Lab

“Inactivation of the parietal reach region causes optic ataxia”

**Mitchell Guttman**
Assistant Professor of Biology

“Functional integration of large ncRNAs in the molecular circuitry of the cell”

**Ankur Saxena**
Postdoctoral Scholar in Biology
Bronner Lab

“Sox10-dependent neural crest origin of olfactory microvillous neurons”, and a picture is attached. Sorry for the delay”
Dr. Adler Dillman, this year’s Ferguson Prize awardee, has an infectious passion of science, indicated by his presentation of the 2012 Everhart Lecture.

Adler came to Caltech to work with Professor Sternberg on an insect killing nematode that can jump (*Steinernema carpocapsae*) and that is used in biocontrol. Working with Michael Dickinson, Adler obtained stunning high-speed videos of the worms’ takeoff. He developed an assay for odor-evoked jumping in which he puffed odors on worms (who are standing on their tails) with a syringe and scores whether they jump or not. Using this assay, Adler demonstrated that jumping is stimulated by host odors, including carbon dioxide. He isolated natural hosts for some nematodes, including mole crickets from a local golf course, and identified by gas chromatography and mass spectrometry some of the volatile organic compounds made by the host insects. As a first step in understanding the molecular biology of host response and jumping, Adler sequenced and annotated genomes of five *Steinernema* species. He also helped annotate the genome of a close outgroup, *Panagrellus redivivus*, and discovered a dramatic expansion and contraction of particular families of ubiquitin ligases. His love of comparative biology and genomics led him to initiate a number of genome projects for interesting nematodes while he was writing his PhD thesis.
David Anderson, Seymour Benzer Professor of Biology
2013 Advisory Committee to the NIH Director, Obama BRAIN Initiative
2012 Waelsch Memorial Neuroscience Grand Rounds Lecture, Columbia University, NY
2012 Distinguished Scientist Lecture, New York University, NY
2012 Givaudan Prize Lecture, Association for Chemoreception Sciences Meeting - Huntington Beach, CA
2012 Nobel Forum Lecture, Stockholm, Sweden

Alexei Aravin, Assistant Professor of Biology
2012 Packard Fellowship for Science and Engineering

David Baltimore, President Emeritus, Robert Andrews Millikan Professor of Biology, Nobel Laureate
2012 Honorary Centennial Council Founding Member, Smithsonian Institution, National Museum of Natural History
2012 University of Malaysia Nobel Fellow
2012 University of Malaysia High Impact Research Advisory Council
2012 Honorary Doctorate of the University of Buenos Aires
2013 Elected Fellow of the Academy of American Association for Cancer Research Inaugural Class

Marianne Bronner, Albert Billings Ruddock Professor of Biology
2013 Conklin Award, Society for Developmental Biology
2012 Women in Cell Biology Lifetime Achievement Award

Viviana Gradinaru, Assistant Professor of Biology
2013 NIH Director’s New Innovator Award
2013 Named a “World Economic Forum Young Scientist”
2013 Pew Scholar Award
2013 Human Frontier Science Program (HFSP) Young Investigators Grant
2012 Mallinckrodt Award

Sarkis Mazmanian, Assistant Professor of Biology
2012 MacArthur Fellow Genius Award

Elliot Meyerowitz, George W. Beadle Professor of Biology
2013 Marker Lectures, Pennsylvania State University
2013 EMBO Keynote Lecture, Presidential Symposium, Society for Developmental Biology, Cancun, Mexico
2013 Howard Hughes Medical Institute–Gordon and Betty Moore Foundation Investigator

Dianne Newman, Professor of Biology and Geobiology
2012 NIH Director’s Transformative Research Award

Rob Phillips, Fred and Nancy Morris Professor of Biophysics and Biology
2013 Society of Biology, Undergraduate Biology Book of the Year

Paul Sternberg, Thomas Hunt Morgan Professor of Biology
2012 Thomas Hunt Morgan Lecture, University of Kentucky, Lexington Kentucky
Alexander Varshavsky, Smits Professor of Cell Biology
2013 Scottish Institute for Cell Signaling (SCILLS) Lecture, Univ. of Dundee, UK
2013 Stein Memorial Lecture, Rockefeller University, New York, NY
2013 Mellon Lecture, University of Pittsburgh, Pittsburgh, PA
2012 Keynote Lecture, Jacques Monod Conference, Roscoff, France
2012 Distinguished Visitor Lecture, Max Planck Inst. for Biochem., Martinsried, Germany.

Other Awards

Elaine Hsiao, Caltech BBE and CCE Senior Postdoctoral Scholar
2013 NIH Early Independence Award
October 2012

Uri Alon, Prof., Dept. Molecular and Cellular Biology and Dept. Physics of Complex Systems, Weizmann Institute  
*Design Principles in Biology*
*Love and Fear in the Lab: guitar and discussion of the importance of the subjective and emotional sides of doing science*  
Host: Michael Elowitz  
*Davidson Lecture – Xiaodong Wang*, Director, National Institute of Biological Sciences  
*Biochemical pathways of cell death*  
Host: David Chan

November 2012

*Wiersma Lecture – Margaret Livingstone*, Prof., Dept. Neurobiology, Harvard Medical School  
*Cortical modules: how do we get them and what good are they?*  
Host: Doris Tsao  
*Maksim Plikus*, Asst. Prof., Dept. Developmental and Cell Biology, Stem Cell Research Institute, UC Irvine  
*Stochastic and self-organizing behaviors during the regeneration of hair stem cells*  
Host: Michael Elowitz  
*Nir Friedman*, Senior Scientist, Dept. Immunology, Weizmann Institute  
*Complex decisions of immune cells: parallels with engineering*  
Host: Long Cai  
*Horowitz Lecture – Catherine Dulac*, Chair & Higgins Prof., Dept. Molecular and Cellular Biology, Harvard University  
*Neurobiology of social behavior in the mouse*  
Host: David Anderson

December 2012

*Jeffery Ravetch*, Prof., Dept. Molecular Genetics and Immunology, Rockefeller University  
*Solving the antibody paradox: the diverse roles of Fc receptors in immunity*  
Host: Pamela Bjorkman

January 2013

*Thomas Clandinin*, Asso. Prof., Dept. Neurobiology, Stanford University  
*Dissecting neural computation in the fruit fly*  
Host: David Anderson  
*Joanna Wysocka*, Asso. Prof., Dept. Chemical and Systems Biology and Dept. Developmental Biology, Stanford University  
*Making faces: what epigenomics can teach us about human development and variation*  
Host: Marianne Bronner  
*Thomas Silhavy*, Prof., Dept. Molecular Biology, Princeton University  
*Outer membrane biogenesis in gram-negative bacteria*  
Host: Dianne Newman  
*Kroc Lecture – Cori Bargmann*, Prof., Laboratory of Neural Circuits and Behavior, Rockefeller University  
*Using fixed circuits to build flexible behaviors*  
Host: David Anderson  
*Charles Yokoyama*, Director, Research Administration, RIKEN Brain Science Institute  
*Conceptual advance: the hidden syntax of scientific publication*  
Host: Thanos Siapas
Oliver Hobert, Prof., Dept. Biochemistry and Molecular Biophysics, Columbia University
*Gene regulatory mechanisms that generate neuronal diversity – lessons learned from C. elegans and beyond*
Host: Katalin Fejes-Tóth

Yoav Soen, Senior Scientist, Dept. Biological Chemistry, Weizmann Institute
*Reprogramming development – mechanisms and trans-generational implications*
Host: Michael Elowitz

February 2013

Gerry Rubin, VP and Executive Director, Janelia Farm Research Campus
*A molecular geneticist's strategy for understanding the fly brain*
Host: David Anderson

Informal - Jonathan Dworkin, Asso. Prof., Dept. Microbiology and Immunology, Columbia University
*Regulation of metabolic dormancy by reversible phosphorylation of translational GTPases*
Host: Michael Elowitz

March 2013

Informal - Ichiro Taniuchi, Group Director, Laboratory of Transcriptional Regulation, Riken
*The road to become CD4+ helper T cells*
Host: Ellen Rothenberg

Alfred Goldberg, Prof., Dept., Cell Biology, Harvard Medical School
*Functioning of the Proteasome: from protein breakdown to cancer therapy*
Host: Ray Deshaies

John Lis, Prof., Dept. Molecular Biology and Genetics, Cornell University
*New views of transcription and its regulation*
Graduate Student Host: Eric Erkenbrack

Stephen Quake, Prof. & Co-Chair, Dept. Bioengineering and Applied Physics, Stanford University
*Single cell genomics*
Host: Rob Phillips

April 2013

Carl-Philipp Heisenberg, Prof., Dept. Cell Biology, Institute of Science and Technology
*Cell and tissue mechanics in zebrafish gastrulation*
Host: Angela Stathopoulos

Informal - Sebastian Munck, Dept. Molecular and Developmental Genetics, K.U. Leuven
*From the fast track to super-resolution to the spatio-statistical analysis of protein distributions on the cell membrane*
Host: Grant Jensen

Michel Nussenzweig, Prof., Laboratory of Molecular Immunology, Rockefeller University
*The HIV vaccine problem*
Host: Pamela Bjorkman

Horowitz Lecture – Eric Wieschaus, Prof., Dept. Molecular and Cellular Biology, Princeton University
*Cellular mechanics and cell shape change during drosophila gastrulation*
Host: Rob Phillips

Mark Ptashne, Program Leader, Molecular Biology Program, Center for Stem Cell Research, Sloan-Kettering Institute
*Nucleosomes and the logic of gene regulation*
Host: Eric Davidson
Victor Ambros, Prof., Dept. Molecular Medicine, UMass. Medical School
*MicroRNAs and developmental robustness in C. elegans*
Graduate Student Host: Alex Webster

Stephen Small, Prof. and Chair, Dept. Biology, New York University
*Gradient and time-dependent mechanisms that pattern the Drosophila embryo*
Host: Eric Davidson

May 2013

Erez Raz, Prof., Institute of Cell Biology, ZMBE
*Motility and directed migration of primordial germ cells in zebrafish*
Host: Angela Stathopoulos

James Engel, Prof. and Chair, Dept. Cell and Developmental Biology, Univ. of Michigan Medical School
*GATA factor regulation of HSC homeostasis and T cell development*
Host: Ellen Rothenberg

Troy Margrie, Head of Division of Neurophysiology, National Institute for Medical Research
*Intrinsic biophysical diversity and connectivity of neuronal circuits*
Host: Thanos Siapas

Phillip Sharp, Prof., Koch Institute for Integrative Cancer Research, MIT
*The synthesis and function of non-coding RNAs*
Host: Alexei Aravin

June 2013

Bonnie Bassler, Prof., Dept. Molecular Biology, Princeton University
*Manipulating quorum sensing to control bacterial pathogenicity*
Graduate Student Host: Jessica Ricci

Johan Wessberg, Prof., Dept. Physiology, University Gothenburg, Sweden
*Pleasant touch: the human unmyelinated C-tactile (CT) afferent system*
Host: David Anderson
October 2012

Xiaodong Wang
Director, National Institute Biological Sciences

"Biochemical pathways of cell death"

Host: David Chan

November 2012

Margaret Livingstone
Prof., Dept. Neurobiology, Harvard Medical School

"Cortical modules: how do we get them and what good are they?"

"What art can tell us about the brain"

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November 2012

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Chair and Higgins Prof., Dept. Molecular and Cellular Biology, Harvard University

*“Neurobiology of social behavior in the mouse”*

Host: David Anderson

April 2013

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Prof., Dept. Molecular and Cellular Biology, Princeton University

*“Cellular mechanics and cell shape change during drosophila gastrulation”*

Host: Rob Phillips

January 2013

**Cori Bargmann**
Prof., Laboratory of Neural Circuits and Behavior, Rockefeller University

*“Using fixed circuits to build flexible behaviors”*

Host: David Anderson
The Division of Biology and the
Computational and Neural Systems Option
at Caltech are delighted to invite you to

MARK KONISHI’S
80TH BIRTHDAY SYMPOSIUM

FRIDAY, FEBRUARY 22, 2013
9:00 A.M. – 5:30 P.M.

Beckman Institute Auditorium
California Institute of Technology

SPEAKERS:
ANTHONY LEONARDO, Janelia Farm
ERIC KNUDSEN, Stanford Univ.
HERMANN WAGNER, RWTH Aachen
ALLISON DOUPE, UCSF
MIKE LEWICKI, Case Western
DAN MARGOLIS, Univ. of Chicago
BRIAN FISCHER, Seattle Univ.
ROLAN EGOR, Janelia Farm
RICHARD MOONEY, Duke Univ.
GEOFF MANLEY, Univ. of Oldenburg
RALPH ADOLPHS, Caltech

Please register to attend this event
www.biology.caltech.edu/conference_information
In Celebration of Mark Konishi’s 80th Birthday

Steve Mayo  
William K. Bowes Jr. Foundation Chair, Division of Biology and Bren Professor of Biology and Chemistry  
Opening Remarks and Welcome

Ralph Adolphs  
Bren Professor of Psychology and Neuroscience, Caltech  
“What I learned from studying owls with Mark Konishi to apply to cognitive neuroscience studies of humans”

Roian Egnor  
Postdoctoral Fellow, Janelia Farm Research Campus  
“Whistling in the dark: mouse social and vocal behavior”

Anthony Lenardo  
Group Leader, Janelia Farm Research Campus  
“The art of behavior, from songbirds to dragonflies”

Mike Lewicki  
Associate Professor of Electrical Engineering and Computer Science, Case Western Reserve University  
“Computational neuroethology: What the natural environment tells us about neural representation and processing”

Geoff Manley  
Professor of Neuroscience, Oldenburg University  
“Appreciating amniote aural adaptations”

Allison Doupe  
Professor of Psychiatry, University California San Francisco  
“Neuroethological lessons from songbirds about basal ganglia circuits, social context, and plasticity”

Eric Knudsen  
Professor of Neurobiology, Stanford University  
“A Konischesque approach to the study of attention”

Dan Margoliash  
Professor of Neuroscience, University of Chicago  
“A new model of sensorimotor vocal encoding, with some implications for Mark Konishi’s template theory of birdsong learning”

Hermann Wagner  
Professor of Zoology and Animal Physiology, RWTH Aachen  
“Representation of interaural time difference: detection and remodeling”

Brian Fischer  
Asst. Professor of Mathematics, Seattle University  
“Coding for optimal performance in the owl’s brain”

Richard Mooney  
Professor of Neurobiology, Duke University  
“Where there’s fire, there’s smoke: using light to study vocal learning in birds”
Anna Abelin
Michael Abrams
Alyssia Ahmed
Michael Anaya
Stephanie Barnes 1
Labeed Ben-Ghaly
Alexandria Berry 2
Danielle Brown-Bower
Katherine Brugman 2
Anna Basalova Buchman
Kenneth Chan
Shijia Chen
Wen Chen 2
Mohsen Chitsaz 2
Cindy Chiu
Julie Cho
Ke-Huan Chow
Suk-Hen Elly Chow
Miao Cui
Marissa Morales-Del Real
Emzo de los Santos 3
Gilberto de Salvo
Adler Dillman
Gregory Donaldson
Eric Erkenbrack
Katherine Fisher
Nicholas Flytzanis
Christopher Frick 2
Zhongzheng Fu
Rachel Galimidi
Erika Garcia
Avni Ghandi
Alma Gharib
Srimoyee Ghosh
Nathaniel Glasser 2
Say-Tar Goh
Mark Goldberg
Abigail Green-Saxena
Virgil Griffith 1
Samy Hamdouche 2
Peng He
Gilberto Hernandez, Jr.
Margaret Ho
Andreas Hoenselaar 1
Xiaodi Hou
Elaine Hsiao
Na Hu
Brad Hulse

Yonil Jung 2
Joycelyn Kim
Arya Khosravi
Natalie Kolawa
Naomi Kreamer 2
Eugene Kym
Amit Lakanpal
Lauren Lebon 1
James S. Lee
Sung-Eun Lee
Toni Lee 2
Daniel Leighton
Hanqing Li
Seth Lieblisch 2
Seung-Hwan Lim
Yong-Jun Lin 1
Justin Liu
Raymond Liu
Olive Losson
Geoffrey Lovely 2
Georgi Marinov
Arnav Mehta
Timothy Miles
Ruzbeh Mosadeghi
Sandy Nandagopal 1
Ravi Nath
Janna Nawroth
Weston Nichols
Shay S. Ohayon 1
Gwen Owen 2
Jin Park 3
Soyoung Park 1
Rell Parker
Sonal Patel
Edward Perkins
Anh Pham
Yutao Qi 2
Jessica Ricci
Alicia Rogers
Rebecca Rojansky
Michael Rome
Alexander Romero 2

Akram Sadke 1
Jeremy Sandler
Catherine Schretter
Ma'ayan Schwarzkopf
Sheel Shah
Adam Shai 3
Anna Shemorry
Pei-Yin Shih
Va Si
Zakary Singer 1
Bernardo Sosa Padilla Araujo 2
Tsu-Te Su 2
Zachary Sun
Frederick Tan 2
Nicole Tetreault
Cory Tobin
Nathanic Trinandi
Vikas Trivedi 3
Benjamin Uy
Jonathan Valencia
Tri Vu 2
Brandon Wadas
Ward Walkup 5
Shuo Wang 5
Xun Wang 5
Yun Elisabeth Wang
Timothy Wanner
Alexandre Webster
Yunji Wu
John Yong
Jimmy Zhao

1 Computational & Neural Systems (CNS)
2 Biochemistry & Molecular Biophysics
3 Bioengineering
Maja Bialecka-Fornal  
Biochemistry and Molecular Biophysics  
M.S., Jagiellonian University, 2008.  
Thesis: Single-Cell Analysis of the Physiology of Mechatosensation in Bacteria.

Danielle Vera Brown Bower  
Biology  
A.B., Harvard College 2005;  
M.S., University of Cambridge 2006.  
Thesis: Chemical and Neural Regulation of Embryonic Branching Morphogenesis.

Julie YoungHee Cho  
Biology  

Marissa Morales Del Real  
Biology  
B.A., College of Notre Dame of Maryland 2006.  
Thesis: Analysis of a Transcriptional Network Involving PU.1, Notch, and Gata3 in the Lymphomyeloid Lineage Decision during Early T-cell Development.

Adler R. Dillman  
Biology  
B.S., Brigham Young University 2006.  

Gilberto Hernandez  
Biology  
B.A., University of California, Los Angeles 1996;  
M.S., California Institute of Technology 2001.  

Elaine Yih-Nien Hsiao  
Biology  
B.S., University of California, Los Angeles 2007.  

Na Hu  
Biology  
B.A., University of California, Berkeley 2008.  
Thesis: Epigenetic Regulation in Neural Crest Development: Role of DNA Methyltransferases 3A and 3B.

Natalie J. Kolawa  
Biology  
B.A., University of California, Berkeley 2007.  
Thesis: Proteomic Analysis of the Cdc48/Ubx Network Identifies a Role for Ubx2 in the Regulation of Lipid Biosynthesis.

Sung-Eun Melanie Lee  
Biology  
B.S., Harvey Mudd College 2004.  
Thesis: Mechanism of Intestinal Colonization by Symbiotic Bacteria.

Janna C. Nawroth  
Biology  
B.S., Ruprecht-Karls-Universitat Heidelberg 2004;  

Anh H. Pham  
Biology  
B.S., University of California, Berkeley 2004;  
M.D., University of Southern California 2007.  

Abigail Green Saxena  
Biology  
B.S., University of Texas 2002.  

Maayan Schwarzkopf  
Biology  
B.S., Tel Aviv University 2004;  
M.S., 2006.  
Anna Shemorry  
*Biology*  
B.S., University of California, San Diego 2007.  

Nicole Anne Tetreault  
*Biology*  
B.S., University of California, Davis 2002;  
M.S., University of California, Los Angeles 2006.  
Thesis: Microglia in the Cerebral and Cerebellar Cortices in Individuals with Autism.

Ophelia S. Venturelli  
*Biochemistry and Molecular Biophysics*  
B.S., M.S., Stanford University 2007.  
Thesis: Role of Feedback and Dynamics of a Gene Regulatory Network.

Jimmy Liu Zhao  
*Biology*  
A.A., Pasadena City College 2004;  
B.A., University of California, Berkeley 2006.  

---

**M A S T E R  O F  S C I E N C E**

Renata Christina Cummins (*Geobiology*)  

Rod Seung-Hwan Lim (*Biology*)  
B.S., University of California, San Diego 2008.

Alexander Charles Romero (*Biochemistry and Molecular Biophysics*)  

Va Cheung Si (*Biology*)  
B.S., University of California, Los Angeles 2007.

Elizabeth Trembath-Reichert (*Geobiology*)  
Biology Graduates 2013

California Institute of Technology

Bachelor of Science

Michelle Esther Bobrow* Great Neck, New York Biology
Stephanie Claire Bohaczuk* Wyckoff, New Jersey Biology
Yi Elise Cai* Albany, California Biology
Sandhya Chandrasekaran* Fremont, California Biology and Chemistry
Victor Eric Chen* San Jose, California Biology
Race Everest Rial DiLoreto University Place, Washington Biology
Marvin Ho-Yan Gee* Frederick, Maryland Biology
Yang Hu* Richland, Washington Biology
Amol Deepak Kamat* Northville, Michigan Biology
Philip L. Kong* Los Angeles, California Biology
Stephanie Ya-Wen Kwan Rancho Palos Verdes, California Biology and English (Minor)
Susan Elizabeth Liao Princeton, New Jersey Biology
Vishnu Manoranjan* Pullman, Washington Biology and Philosophy (Minor)
Mahati Mokkarala* Budd Lake, New Jersey Biology
Nina Zhixuan Ng-Quinn Alta Loma, California Biology
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*Max Delbrück Professor of Biology; Investigator, Howard Hughes Medical Institute*

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*Professor of Biology: Bren Scholar; Investigator, Howard Hughes Medical Institute*

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*Norman Chandler Professor of Cell Biology*

Raymond Deshaies, Ph.D.
*Professor of Biology; Investigator, Howard Hughes Medical Institute*

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*Professor of Biology and Bioengineering; Investigator, Howard Hughes Medical Institute*

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*Professor of Biology*

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*Professor of Biology; Investigator, Howard Hughes Medical Institute*

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*Bren Professor of Biology*

Stephen L. Mayo, Ph.D.
*Bren Professor of Biology and Chemistry; Chair, Division of Biology and Biological Engineering*

Sarkis Mazmanian, Ph.D.
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Professor of Computer Science, Computation and Neural Systems, and Bioengineering

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Bren Professor of Molecular Biology; Director, Beckman Institute

Changhuei Yang, Ph.D.
Professor of Electrical Engineering and Bioengineering

Kai Zinn, Ph.D.
Professor of Biology

ASSISTANT PROFESSORS

Alexei A. Aravin, Ph.D.
Biology

Lea Goentoro, Ph.D.
Biology

Viviana Gradinaru, Ph.D.
Biology

Mitchell Guttmann, Ph.D.
Biology

David Prober, Ph.D.
Biology

Lulu Qian, Ph.D.
Bioengineering

Doris Ying Tsao, Ph.D.
Biology
### Faculty and Research Staff 2013

**Lecturers**
- L. Elizabeth Bertani, Ph.D.
- Justin Bois, Ph.D.
- Alex Cuna, Ph.D.
- Ciro Donalek, Ph.D.
- Kelsey Homyk, B.S.
- Suzanne Kern, Ph.D.
- Carol Chace Tydell, Ph.D.

**Senior Research Associates**
- R. Andrew Cameron, Ph.D.
- Akiko Kumagai, Ph.D.
- Mary An-yuan Yui, Ph.D.

**Senior Fellows**
- Bader Al-Anzi, Ph.D.
- Lindsay Bremner, Ph.D.
- Stijn Cassenaer, Ph.D.
- Katalin Fejes Toth, M.D., Ph.D.
- Hyosang Lee, Ph.D.
- Evgeniy V. Lubenov, Ph.D.
- Hans-Michael Muller, Ph.D.
- Shuyi Nie, Ph.D.
- Isabelle S. Peter Lashgari Faghani, Ph.D.
- Kaoru Sugimoto, Ph.D.
- Allan Wong, Ph.D.
- Cheng Xiao, M.D., Ph.D.

**Senior Faculty Associate**
- Alice S. Huang, Ph.D.

**Visiting Associates**
- Elaine L. Bearer, Ph.D., M.D.
- Maria Elena deBellard, Ph.D.
- Susan Ernst, Ph.D.
- Pamela L. Eversole-Cire, Ph.D.
- Jordi Garcia-Ojvalo, Ph.D.
- Yongning He, Ph.D.
- Carmel Levitan, Ph.D.
- Tetsuya Matsuda, Ph.D.
- Carol Ann Miller, M.D.
- Eric Mjolsness, Ph.D.
- Florian Mormann, M.D., Ph.D.
- Carmie Puckett Robinson, M.D.
- Dinesh Rao, M.D., Ph.D.
- Ian Ross, Ph.D.
- Ueli Rutishauser, Ph.D.
- Russell E. Jacobs, Ph.D.
- Eugene Akutagawa, B.S.
- Janet F. Baer, D.V.
- L. Elizabeth Bertani, Ph.D.
- Bruce Cohen, Ph.D.
- Rochelle A. Diamond, B.A.
- Ali Khoshman, Ph.D.
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- Ker-hwa Ou, M.S.
- Shirley Pease, B.Sc.
- Andrew J. Ransick, Ph.D.
- Alice Schmid, Ph.D.
- Peter Siegel, Ph.D.
- Qiang Tu, Ph.D.
- Julian Michael Tyszka, Ph.D.
- Jost Vidmetter, Ph.D.
- Anthony P. West, Jr., Ph.D.
- Xiaowei Zhang, Ph.D.
- Jie Zhou, Ph.D.

**Senior Postdoctoral Scholars**
- Julius Barsi, Ph.D.

**Postdoctoral Scholars**
- Tyson Aflalo, Ph.D.
- Omar Akbari, Ph.D.
- Yaron Antebi, Ph.D.
- Kenta Asahina, Ph.D.
- Hiroki Asari, Ph.D.
- Young-Kyung Bae, Ph.D.
- Antoinette Bailey, Ph.D.
- Sreeam Balasubramanian, Ph.D.
- Alejandro Balazs, Ph.D.
- Namrata Bali, Ph.D.
- Samuel Barnes, Ph.D.
- Michael Bethune, Ph.D.
- Lacramioara Bintu, Ph.D.
- James Bonaiuto, Ph.D.
- Mark Budde, Ph.D.
- Haijiang Cai, Ph.D.
- Emily Capra, Ph.D.
- Robert Carrillo, Ph.D.
- Nickie Chan, Ph.D.
- Yi-Wei Chang, Ph.D.
- Audrey Chen, Ph.D.
- Shun Jia Chen, Ph.D.
- Yi-Wei Chen, Ph.D.
- Yung-Chia Chen, Ph.D.
- Xueqi Cheng, Ph.D.
- Vikram Chib, Ph.D.
- Vasileios Christopoulos, Ph.D.
- Hiu tung Chu, Ph.D.
- Trinity Brian Crapse, Ph.D.
- Sagar Subhash Damle, Ph.D.
- Marissa Del Real, Ph.D.
- Willem den Besten, Ph.D.
- Fangyuan Ding, Ph.D.
- Meenakshi Kshirsagar Doma, Ph.D.
- David Doughty, Ph.D.
- Brian J. Duistermars, Ph.D.
- Roberto Feuda, Ph.D.
- William Tyler Gibson, Ph.D.
- Arnulf Graf, Ph.D.
- Stephen A. Green, Ph.D.
- Piercesare Grimaldi, Ph.D., M.D.
- Cai Guo, Ph.D.
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- Markus Hauschild, Ph.D.
- Brandon Henderson, Ph.D.
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- Lisa Marie Hochren, Ph.D.
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Na Hu, Benjamin Uy

**Research and Laboratory Staff:**
Constanza Gonzalez, Martha Henderson, Matthew Jones, Joanne Tan-Cabugao

**Contributors:**
Paul Kulesa, Didier Stanier, Rob Maxson

**Lab Website:** [http://www.bronnerlab.com](http://www.bronnerlab.com)

**Financial Support:**
National Institutes of Health (NHGRI, NIDCR, NICHD)

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**CELLULAR AND MOLECULAR STUDIES OF NEURAL CREST DEVELOPMENT**

**Summary:**
This laboratory’s research centers on the early formation of the nervous system in vertebrate embryos. The peripheral nervous system forms from two cell types that are unique to vertebrates: neural crest cells and ectodermal placodes. We study the cellular and molecular events underlying the formation, cell lineage decisions and migration of these two cells types. The neural crest is comprised of multipotent stem-cell-like precursor cells that migrate extensively and give rise to an amazingly diverse set of derivatives. In addition to their specific neuronal and glial derivatives, neural crest cells can also form melanocytes, craniofacial bone and cartilage and smooth muscle. Placodes are discrete regions of thickened epithelium that give rise to portions of the cranial sensory ganglia as well as form the paired sense organs (lens, nose, ears). Placodes and neural crest cells share several properties including the ability to migrate and to undergo an epithelial to mesenchymal transition. Their progeny are also similar: sensory neurons, glia, neuroendocrine cells, and cells that can secrete special extracellular matrices.
MARIANNE BRONNER LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

Our laboratory focuses on understanding the molecular mechanisms underlying the induction, early development and evolution of the neural crest and placodes. This research addresses fundamental questions concerning cell commitment, migration and differentiation using a combination of techniques ranging from experimental embryology to genomic approaches to novel gene discovery and identification of gene regulatory regions. These studies shed important light on the mechanisms of neural crest and placode formation, migration and differentiation. In addition, the neural crest and placodes are unique to vertebrates. In studying the evolution of these traits, we hope to better understand the origin of vertebrates.

Because these cell types are involved in a variety of birth defects and cancers such as neurofibromatosis, melanoma, neuroblastoma, our results on the normal mechanisms of neural crest development provide important clues regarding the mistakes that may lead to abnormal development or loss of the differentiated state.

PUBLICATIONS

2013


Simões-Costa M, Bronner ME. (2013) Insights into neural crest development and evolution from genomic analysis. Genome Res. 23, 1069-80


Summary:
The major focus of research in our laboratory is the systems biology of the gene regulatory networks (GRNs) that control development, and the evolution of these networks. Our research is done on sea urchin embryos, which provide key experimental advantages. We pursue an integrated, "vertical" mode of experimental analysis, in that our experiments are directed at all
levels of biological organization. Our work extends from the transcription factor-DNA interactions that control spatial and temporal expression of specific genes, to the system-level analysis of large regulatory networks, to the sets of downstream effector genes they control. It has become apparent that only from the GRN system level of analysis can causal explanations of major developmental phenomena directly emerge, and this is our main focus. The sea urchin embryo is the first in which major portions of the developmental process have been encompassed in experimentally solved GRNs. Modeling demonstrates that these networks provide a predictively sufficient explanatory framework for understanding how the genomic regulatory code causes the progression of regulatory states that underlie all downstream developmental process. In current projects GRN analysis is being extended to the complex spatial regulatory domains of the oral ectoderm and the postgastrular gut, as well as to the mesoderm and the ciliated band. We are also isolating cells expressing given regulatory states by FACS, and from their transcriptomes determining the specifically expressed effector genes, so that their cell-type specific control systems can be directly related to the hierarchically upstream GRNs. A large scale transcriptome analysis is providing invaluable information on gene use in embryonic and adult tissues, on gene models, and on gene expression dynamics. One reason for the advanced knowledge of sea urchin embryo genomic control systems is their accessibility to cis-regulatory analysis, and a recent technological development in our lab has enabled high throughput cis-regulatory analysis in which expression of 10 to >100 constructs can be determined simultaneously. Both specific genes of key interest and relevant sets of genes are currently targets of cis-regulatory examination. Knowledge of the genomically encoded control processes of development opens the way to exploration of their evolution. In collaboration with the Human Genome Sequencing Center at Baylor College of Medicine, genomic sequence from other echinoderms at phylogenetically strategic distances is being obtained, which potentiates a variety of evolutionary projects. One such is exploration of the divergence of the GRNs underlying the embryonic specification of sister groups of sea urchins that diverged before the Permian/Triassic extinction. Another project is aimed at the cis-regulatory evolution of a gene expressed differently in sea stars and sea urchins. Closely related to evolutionary rewiring of developmental GRNs is experimental rewiring of GRN circuitry, which has now been greatly facilitated by the advent of the predictive model referred to above, so that the consequences can be studied a priori in silico.

The main research initiatives in our laboratories at the present time are as follows:

i. **Gene regulatory network underlying endomesoderm specification in S. purpuratus embryos**: Many of the individual projects reported below are contributing to understanding of this GRN. At present, over 60 regulatory and signaling genes have been linked into this network. The architecture of the network is emerging from an interdisciplinary approach in which high resolution spatial and temporal regulatory gene expression data are combined with perturbation data obtained by gene expression knockouts and the results of cis-regulatory analysis to provide a causal explanation of the observed embryology. A model of the GRN through time is emerging which indicates the inputs and outputs of the cis-regulatory elements at its key nodes. This model essentially provides the genomic regulatory code for specification of the endomesodermal territories of the embryo, up to gastrula stage (30 h after fertilization). This year we published the
dynamic mechanism by which the endoderm/mesoderm fate decision is made, as well as that by
which future anterior vs. posterior endoderm is specified. By this point, the pre-gastrular
skeletogenic lineage GRN and the endodermal GRNs are largely solved. The endodermal GRN
project is now focused on the specification of the development of the post-gastrular gut, which
consists of many distinct regions (foregut, midgut, hindgut, sphincters, blastopore/anus region).
The initial major effort is to achieve a comprehensive determination of the dynamic regulatory
states of these regions. Within the next year or two the pre-gastrular oral and aboral mesodermal
GRNs, which produce different mesodermal cell types, will have been brought to a similar level of
completeness as the pre-gastrular endodermal GRNs, including a complete analysis of mesodermal
Notch target genes. (endoderm: Dr. Isabelle Peter, Jonathan Valencia, Miao Cui, Jina Yun;)
mesoderm: Dr. Andrew Ransick)

ii. Dynamic Boolean model of endomesoderm gene regulatory network: We have constructed
a dynamic model representing the control system operative in life, such that the regulatory
response capabilities of each gene in the endomesoderm GRN are formalized in a vector equation
indicating the inputs and logic processing functions executed by the relevant genomic cis-
regulatory module(s). The vector equations encompass all the regulatory interrelations stated
explicitly in the GRNs, and the model as a whole provides a direct test of the overall completeness
of the experimental analysis underlying the GRN. Original strategies for incorporation of signaling
interactions, embryonic geometry, and lineage, were devised. A wholly novel computational and
graphic display apparatus was created to support model operations. Each hour the outputs of every
gene in the model (if any), are computed from the inputs available then, for each endomesodermal
spatial domain (skeletogenic, mesoderm, anterior and posterior endoderm); thus, the model
computes the dynamically changing regulatory states of the embryo. The relation between real
time and change in transcriptional status had been calculated for sea urchin embryos earlier, in a
first principles kinetic model (Bolouri and Davidson, PNAS, 2003), and these kinetics were applied
to the temporal animation of the Boolean model. The results thus far are as follows: i, The model
perfectly predicts the observed spatial domain of expression of each gene throughout the
endomesodermal domains. ii, The model recreates the temporal dynamics directly observed for
the spatial patterns in expression of almost all genes, with a few exceptions; thus the model
demonstrates by direct comparison between data and observation that the GRNs are essentially
sufficient to explain causally the progression of spatial regulatory states (the oral and aboral GRNs
only up to 18h, the remainder to 30 h). iii, The model immediately pinpoints exactly where gaps
in our knowledge remain. iv, The model can be used for in silico perturbation of the effects of
gene knockouts and experimental embryology, and thus we have shown that it almost perfectly
predicts the regulatory changes occasioned by certain gene over-expressions and gene knockouts,
and even recreates the regulatory results of a famous experiment in which transplantation of early
cleavage skeletogenic cells from the vegetal to the animal pole produces a second perfectly
organized endomesoderm. (Dr. Isabelle Peter, Eric Davidson)

iii. Oral and aboral ectoderm GRNs: In an effort to extend GRN analysis to most of the
domains of the embryo, we are working out the GRNs for oral and aboral ectoderm specification,
including about 50 more regulatory genes (the one remaining major territory, the apical neurogenic
The ectoderm is a complex mosaic of spatial regulatory states. Both the aboral and oral ectoderms produce numerous sub-regional regulatory state domains, and they are separated by another territory with its own regulatory state, the neurogenic ciliated band. A very large amount of spatial expression analysis has been required to complete the roster of regulatory genes expressed in the ectoderm, and to unravel the constituent regulatory genes of the ectodermal domains abutting the endoderm, the remaining oral and aboral epithelia, the mouth region on the oral side, and the ciliated band. Complex inter- and intra-domain signaling events must also be taken into account. Based on extensive perturbation analyses and cis-regulatory data, a GRN is emerging for the oral ectoderm that will soon approach the completeness of the endomesodermal GRNs. The aboral ectoderm GRN will require more analysis. GRNs will be encompassed in the predictive dynamic Boolean model, with the ultimate goal of extending such a model to nearly the whole embryo. (Dr. Enhu Li, Dr. Julius Barsi)

iv. Specific cis-regulatory projects using high throughput methods: Cis-regulatory systems at certain GRN nodes are of particular importance, and many of these are the subjects of particular experimental analysis. During this year cis-regulatory systems of the following genes, among others, were studied at the level of their sequence specific inputs and their functional meanings (some of these projects are now complete and have been or will soon be published): Among genes currently or recently thus characterized are alx1, foxa, brachyury, gcm, one-cut, tbx2/3, dlx, hox11/13b. (Dr. Julius Barsi, Dr. R. Andrew Cameron, Dr. Andrew Ransick, Miao Cui)

v. Embryonic transcriptome database and analysis: A large-scale S. purpuratus transcriptome sequencing and analysis project has been carried out. RNAs from 10 timed embryonic stages, from various feeding larval stages, and from all accessible adult tissues have been sequenced in depth and assembled, and quantitative per transcript databases are in construction. Three valuable kinds of data have been obtained and after computational analysis are being mounted on our public sea urchin genomics database: i, We were able to correct erroneous gene models based on priori predictions from the genome sequence in over 1/3 of cases, using the actual mapped mRNA structure(s); we also added several thousand new genes to the genome annotation; and we verified the remaining gene model predictions. The transcriptome data have vastly improved the usefulness and accuracy of the genome sequence. ii, The sets of transcripts expressed in each stage and tissue have been discovered, and classified in terms of a custom ontology of our own construction. This ontology reflects the classes of particular interest to the research community to which we belong and which we serve, such as transcripts coding for immune proteins, for cytoskeletal proteins, for transcription factors, for signaling factors, for biomineralization proteins, etc. The ontological classes were based on the expert annotations of genes in the S. purpuratus genome project. iii, We now possess global data on dynamic changes in prevalence of given transcripts during development and on absolute values. These values lock in nicely with NanoString and QPCR measurements in most cases. There is an innumerable wealth of data of biological interest in this data set. As one example, the egg transcriptome provides a comprehensive definition of maternal mRNA (which was first discovered in sea urchin eggs) both qualitatively and quantitatively, a subject now revisited for the first time in 30 years. (Dr. Qiang Tu, Dr. R. Andrew Cameron, Eric Davidson)
vi. Physical isolation of embryonic cells expressing given regulatory states: Another technological breakthrough has been the development of methods for disaggregation of sea urchin embryos to the single cell level, and efficient FACS sorting, without significant loss of cells or reduction of viability. The cells are sorted on the basis of expression of recombined BAC vectors, in which a fluorophore is expressed under control of the cis-regulatory system of a gene canonically representing a given domain-specific regulatory state. Recoveries of expressing cells are quite acceptable, and controls show that the procedure does not affect the distribution of transcripts. The availability of this technology leads in two different directions: First, it will allow us to characterize the transcriptomes of many developmental compartments at different times, including complete knowledge of differentially expressed regulatory genes. This is the primary requirement for systematic extension of GRN analysis to later and more complex developmental stages, a major near future laboratory objective. Second, we can obtain the transcriptomes of cells expressing given regulatory states. For example in skeletogenic cells isolated on the basis of expression of two different specifically expressed BACs all known biomineralization gene transcripts are enriched and other effector genes expressed specifically in these cells can now be accessed. In situ hybridization demonstrates that this procedure is extremely accurate in assigning cell type specific genes. This in turn will lead to construction of "Global GRNs" in which the control systems of all specifically expressed downstream genes (of given ontological classes) are discovered and linked into our current upstream GRNs. (Dr. Julius Barsi, Dina Malounda, Dr. Qiang Tu, Erika Vielmas, Carlos Gomez Marin)

vii. Evolutionary co-option at the cis-regulatory level: The major mechanism of evolutionary change in GRN structure is co-option of regulatory and signaling genes to expression in new spatial/temporal domains of the developing organism. This means change of cis-regulatory modules at the sequence level, so that they respond to different regulatory states; or alternately, changes in the cis-regulatory modules of genes encoding the spatial allocation of regulatory states. An excellent example is the use of Delta-Notch signaling to promote mesoderm specification in sea urchins, but to promote endoderm specification in sea stars (the sea urchin mode is the derived co-option). Sea stars and sea urchins shared a last common ancestor about 500 million years ago. To determine what happened in the lineage leading to sea urchins, we are carrying out a cis-regulatory study of sea star delta, for comparison to sea urchin delta, including cross-specific transfer of expression constructs. Current results show that though it is expressed quite differently in sea stars, a cis-regulatory module of sea star delta produces expression in sea urchin skeletogenic lineages, though no such lineage exists at all in sea stars. Thus it was aspects of the upstream regulatory state to which the delta gene responds that were co-opted in the evolution of the sea urchin skeletogenic lineage. (Dr. Feng Gao)

viii. Eucidaris tribuloides, an evolutionary window on the origins of the euechinoid endomesoderm specification GRN: The euechinoids are the modern sea urchins, of which the main research model is S. purpuratus, for the last 40 years our laboratory workhorse. The euechinoids diverged from the Paleozoic precursor echinoid lineage about 275 million years ago. Eucidaris tribuloides is a descendant of the other surviving branch of echinoids deriving from the same common ancestor stock. Its endomesodermal specification process is quite different from
that of *S. purpuratus*; for example, it lacks a precociously invaginating skeletogenic micromere lineage altogether. Current results show the endodermal specification functions of *E. tribuloides* are similar to those of *S. purpuratus*, but its mesodermal specification is different in several respects. Its micromeres apparently produce *delta* signals as do those of *S. purpuratus*, but control of their specification is differently wired, and they fail to express key skeletogenic genes in pre-gastrular development. The pleisiomorphic specification GRN of *E. tribuloides* mesoderm will reveal exactly how that of *S. purpuratus* evolved since divergence. In addition, we are attempting to reprogram the development of the skeletogenic cell lineage in *E. tribuloides*, by inserting regulatory apparatus from *S. purpuratus*. We term this Synthetic Experimental Evolution. *(Eric Erkenbrack)*

ix. **New genomics projects:** A large amount of additional echinoderm sequence is in process of being obtained. The leaders in this project are Richard Gibbs and Kim Worley at the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC) in Houston, in close collaboration with us. An initial draft sequence of the genome of *Lytechinus variegatus* has been obtained, and the genomes of the sea star referred to above, *Patiria miniata*, and of *E. tribuloides* have been sequenced. Much additional genome sequence of *S. purpuratus* has also being obtained, so as to significantly improve its quality; and earlier skim sequences of two congeners, *S. franciscanus* and *Allocentrotus* (*Strongylocentrotus*) *fragilis* have been augmented. New sequencing projects underway will provide the genomic sequences of a brittle star and a sea cucumber; thus we will have genomes of four of the five echinoderm classes. All of these data are being curated and mounted on the public genome databases that we maintain and continuously augment. *(BCM-HGSC, R. Andrew Cameron, Eric Davidson)*

x. **Additional research endeavors:**

*Principles of developmental GRN design.* We are formulating a general view of developmental GRN structure, and its implication for development and evolution. (*i*) Developmental GRNs are deeply hierarchical. (*ii*) They are composed of modular subcircuits executing discrete logic functions; (*iii*) These subcircuits evolve at different rates within the same GRN and may have diverse evolutionary origins; (*iv*) Multiple subcircuits are brought to bear on given developmental processes, including dynamic lockdowns by feedback circuitry, to ensure that they function accurately and resiliently: the "wiring" is clearly not parsimonious in design; (*v*) Different processes, e.g., embryonic spatial specification, terminal differentiation, physiological response, are controlled by differently structured GRNs, which have different depths and are composed of different types of subcircuit. (*vi*) GRN structure provides specific guides to processes by which body plans have evolved. *(Dr. Isabelle Peter, Eric Davidson)*

*Recombineered BACs.* Our BAC libraries have provided the source material for *in vitro* recombineered BACs used by the outside research community as well as ourselves. More than 100 different recombinant BACs from five echinoderm species have been constructed for use as reporter constructs, with the use of our own in house sequencing instrumentation. This includes constructs in which a fluorescent protein coding region (GFP, RFP, mCherry, Cerulean) has been inserted into the coding region of a gene of interest as well as numerous constructs in which *cis-*
regulatory modules (CRM) have been deleted or specifically mutated. (Dr. R. Andrew Cameron, Julie Hahn, Ping Dong, Miki Jun)

Additional Note: The Sea Urchin Research Resource.

Sea urchin embryos (as well as embryos of other echinoderms) have remarkable advantages as an experimental system, and now, after 40 years of molecular biological experimentation on them a significant array of resources has become available. These embryos offer an easy gene transfer technology, with high throughput technologies available, which makes the sea urchin embryo an experimental system of choice for studying the genomic regulatory code. Reliable methods have been developed for high throughput measurement and for specific perturbation of gene expression in the embryo, as well as sensitive and dramatic means of visualizing spatial gene expression. For the species we work with (Strongylocentrotus purpuratus) embryonic material is available at all seasons of the year. The embryos are optically clear, easily handled, remarkably able to withstand micromanipulations, injections and blastomere recombination and disaggregation procedures; well understood and relatively simple embryonic process is known from over a century of research; and in-house egg-to-egg culture is routine (in a special culture system we have developed, located at Caltech's Kerckhoff Marine Laboratory). Recent additions to our special research arsenal include the NanoString nCounter for simultaneous measurement of hundreds of transcript levels and a NanoString codeset targeting >200 interesting regulatory genes and some signaling ligands and receptors expressed during embryogenesis; plus >100 custom recombineered BACs, most including relevant regulatory genes and some also special vectors or regulatory mutants. We have a rich collection of arrayed BAC libraries for many other species of sea urchin, and other echinoderms, at various degrees of relatedness to S. purpuratus. The genome of S. purpuratus has been sequenced and annotated at the Human Genome Sequencing Center (Baylor College of Medicine), as has the genome of another sea urchin used as a research model. We utilize additional experimental echinoderm models for evolutionary GRN comparisons, viz. the sea star Patiria miniata also of local provenance, and the (in certain respects) pleisomorphic "pencil urchin" Eucidaris tribuloides. Their genomes are also sequenced. The embryos of both these animals prove to be as excellent subjects for gene regulation molecular biology as is that of our usual sea urchin.

The Center for Computational Regulatory Genomics

R. Andrew Cameron, Director

The Center for Computational Regulatory Biology and its subsidiary, the Genomics Technology Facility, is an integrated unit whose goal is to develop, refine and test computational approaches in genomics broadly and cis-regulatory analysis specifically. It conducts three overlapping areas of activity.

The Genomics Technology Facility is a high-throughput library arraying and printing operation that generates arrayed libraries and clones (provided on request to the community). The operation of the Facility centers on a Genetix Arraying Robot, a large flatbed robotic arm with video camera
used to produce bacterial macro-array libraries and filters. We currently maintain in -80°C freezers 27 different echinoderm libraries comprising a total of approximately three million arrayed clones.

The Research Center carries out genomically oriented wet lab research, and works collaboratively with the transcriptome and genomics efforts. A major project at present is the use of newly available sequence data to explore the mechanisms and rules of functional cis-regulatory evolution within the range of divergence times available in the various species of sea urchins for which genomic sequence and expression vectors are available.

The Computational Branch supplies software and analysis to sea urchin developmental biologists and maintains databases fundamental to the Sea Urchin Genome Project, an initiative that began in the Davidson laboratory. Its major functions are maintenance of the sea urchin genome database and solution of ongoing genomics problems. An extensive website providing access to many kinds of genomics, transcriptome and gene expression data is maintained. The main work of the Computational Branch is continuous development and improvement of sea urchin genomics resources, including genome annotations, gene models, updates of sequence assemblies, and incorporation of the stream of new genomic sequence from HGSC. This information is mounted on the Sea Urchin Genome Project web site (http://spbase.org/). (Dr. R. Andrew Cameron, Dr. Qiang Tu, Dr. Ung-jin Kim, Dr. Susan Gordon, Parul Kudtarkar, David Felt)

Images from left to right:
Professor Eric Davidson
Portion of gene regulatory network controlling specification of skeletogenic lineage of sea urchin embryos. (P. Oliveri, Q. Tu)
Sea urchin embryo, nuclei revealed by fluorogenic histone H2b. 3D confocal reconstruction. The green cells are expressing the regulatory gene foxa in endoderm and future mouth. (E. Faure, I. Peter)

Publications

2013


2012


Professor of Biology and Bioengineering:
Michael B. Elowitz

Collaborators:
Jordi Garcia-Ojalvo

Postdoctoral Scholars:
Yaron Antebi, Lacramioara Bintu, Mark Budde, Fangyaun Ding, Joseph Levine, Pulin Li, Yihan Lin, James C. Locke, Adam Rosenthal, Klara Stefflova

Graduate Students:
Amit Lakhanpal, Lauren E. LeBon, Nagarajan Nandagopal, Jin Park, Zakary S. Singer, Frederick E. Tan, Yutao Qi, John Yong, Jonathan W. Young

SURF Undergraduate Students:
Bogdan Bintu, Heidi Klumpe, Jennifer Ky, Sophia Quinodoz, Kevin Yin

Research and Laboratory Staff:
Maria Jesus Hernandez Jimenez, James Linton, Tara Orr, Leah A. Santat

Financial Support:
Bren Foundation
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KAUST Research Fellowship
Moore Foundation
National Institute of Health (NIH)
National Science Foundation (NSF)
Packard Foundation
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Natural and Synthetic Gene Circuit Dynamics in Cell and Developmental Circuits

Summary:
Cells process information, signal to one another, and control differentiation using circuits of interacting genes and proteins. A central problem in biology is to understand the principles of gene circuit design that govern the architecture and function of these circuits. Our lab tries to address this problem in three ways:

First, we construct synthetic genetic circuits and study their behavior in individual cells. These synthetic circuits are simpler counterparts to the complex circuits one finds in nature. This
Michael Elowitz Lab Annual Report | 2013

California Institute of Technology

approach – "synthetic biology" – allows one to analyze and compare alternative circuit architectures in cells, and identify minimal systems sufficient to confer key biological functions. For example, we have constructed circuits that exhibit oscillations and other dynamic phenomena, (e.g., Elowitz & Leibler, 2000). We have used synthetic circuits to analyze the dynamics and variability of gene regulation at the single-cell level, (e.g., Elowitz et al., 2002, and Rosenfeld et al., 2005). We also make use of 're-wiring' perturbations to alter the architecture of natural genetic circuits, as in our recent studies of the genetic competence and stress response systems of Bacillus subtilis (Süel et al., 2006; Süel et al., 2007; Locke et al., 2011).

Second, we analyze the dynamics of natural genetic circuits in order to understand basic principles of their operation. We have developed the ability to acquire and quantitatively analyze large time-lapse movie datasets. These movies allow tracking of circuit dynamics individual cells as they grow and develop. By incorporating several distinguishable fluorescent protein reporter genes in these organisms, we can track multiple circuit components simultaneously. The results constrain models of the corresponding circuits and provide insight into basic principles of differentiation (see Süel et al., 2006 and Süel et al., 2007), and regulation (Cai et al., 2008; Locke et al., 2011). Most recently, we have analyzed signaling through the Notch pathway in and between individual mammalian cells. This work showed that same-cell (cis) interactions between Notch and Delta lead to a situation where individual cells can 'send' or 'receive' signals, but cannot do both at the same time (Sprinzak et al., 2010).

Third, we are analyzing the generation of variability within cell populations. Genetically identical cells appear to actively generate variability, even in homogeneous environmental conditions. We focus specifically on two complementary questions: How do cells use intrinsic "noise" (stochasticity) in their own components to make effectively random cell fate decisions? And how do they suppress noise in order to operate reliably despite of variability. Current projects are examining these issues in Bacillus subtilis, a very simple prokaryote that exhibits both differentiation and development, as well as in more complicated mammalian cell culture systems. Recently, we have examined the role that noise plays in enabling an alternative mode of evolution through partially penetrant intermediate genotypes (Eldar et al., 2009). We have also studied the way in which dynamic correlations of fluctuations in gene network dynamics can help identify active regulatory interactions (Dunlop et al., 2008). We have also begun to address these issues in mouse embryonic stem cells, which exhibit extensive functionally important heterogeneity.

Projects in the lab make extensive use of mathematical models of genetic circuits. We are also developing software and tools to improve gene circuit construction and quantitative analysis of movie data.

Images from left to right:
Professor Michael Elowitz

Bacillus subtilis bacterial micro-colony responding to stress by modulating the frequency of stochastic pulses of activation of a key transcription factor. Variability in the intensity of green staining reflects heterogeneity in the pulsing.

Single-molecule RNA-FISH enables analysis of the states of individual stem cells. Each dot shown here is a single molecule of mRNA.
PUBLICATIONS

2013


2012


Assistant Professor of Biology:
Lea Goentoro

Professorial Awards and Honors:
2011 NIH Director’s New Innovator Award
2013 James S. McDonnell Scholar in Complex Systems

Graduate Students:
Michael Abrams, Christopher Frick, Xun Wang

Undergraduate Students:
Julian Kimura, Bryan Ryba, William Yuan

Research Staff:
Ty Basinger, Jae Hyoung Cho

Lab website: http://goentoro.caltech.edu/

Financial Support:
James S. McDonnell Award for Complex Systems
NIH Innovator Award

ROBUSTNESS IN MOLECULAR PATHWAYS, PLASTICITY IN ORGANISMS

Summary:

My lab this year has delightfully converged on two seemingly opposing themes: Robustness and Plasticity. The majority of my lab is focused on elucidating the mechanisms behind the robustness we discovered in the Wnt signaling pathway. We propose that integral to the robustness is the idea that cells respond to relative, rather than absolute, level of signal (which we call fold-change computation; Goentoro and Kirschner, 2009; Goentoro et al., 2009). We are using biochemistry to reconstitute the process of fold-change computation in test tubes. We are using timelapse imaging to follow the dynamic of fold-change computation in living single cells. Excitingly, using mathematical modeling, we have recently found evidence for fold-change computation in another signaling pathway, suggesting that fold-change computation may be a more general principle in cell signaling. We are now testing this using experiments in cells.

A new project started in the lab this year. We began working on the moon jellyfish Aurelia aurita. We discovered a new phenomenon of self-repair and incredible plasticity in these creatures. We are now investigating the underlying mechanisms using molecular, embryological, and microscopy approaches.
Professor of Biology:
Bruce A. Hay

Research Fellows:
Omar Akbari, Nikolai Kandul, Juan Li

Graduate Students:
Anna Buchman, Kelly J. Dusinberre

Undergraduate Students:
Wen Min Chen

Research Staff:
Haixia Huang, Katie Kennedy, Danijela Markovic

Collaborators:

1 Heinrich-Heine Universität, Düsseldorf, Germany
2 Department of Neurology, UCLA
3 Kansas State University, Kansas
4 Princeton University, New Jersey
5 National Health Research Institutes, Taiwan
6 Kyung Hee University, Seoul, Korea
7 UC Irvine
8 Virginia Tech
9 Imperial College
10 Caltech Genomics Facility
11 Joint Sciences Department, Claremont Colleges

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Foundation for NIH Research
Hicks Alzheimer’s Fund
National Institutes of Health
Sanofi

CELL DEATH, NEURODEGENERATION, microRNAs, SELFISH GENETIC ELEMENTS, POPULATION GENETICS, LONG-TERM CONTRACEPTION, AND INFECTIOUS DISEASE
Summary:

We are interested in multiple questions in basic and applied biology. For further information on Hay lab research consult our web page (http://www.its.caltech.edu/~haylab/). One goal of our work is directed towards understanding the genetic and molecular mechanisms that regulate cell death, proliferation, innate immunity, microRNA function, and spermatogenesis. We use Drosophila melanogaster as a model system to identify genes that function to regulate these processes. Important cellular regulatory pathways are evolutionarily conserved; thus, molecules identified as regulators of these processes in Drosophila are likely to have homologs in vertebrates and the pathways that link these molecules are likely to be regulated similarly.

A second goal of our work addresses three questions in population biology. 1) Can we bring about reproductive isolation (speciation) between populations of plants or animals that otherwise freely interbreed? Answers to this question have application to the growing number of situations in which plants and animals are engineered to show specific pharmaceutical or agricultural traits. In brief, we would like to be able to limit gene flow between engineered organisms and their wild counterparts. 2) Can we engineer the genetics of populations so that they drive themselves to local extinction? For example, invasive non-native plants and animals cause substantial economic losses. A number also cause substantial environmental damage, leading in many cases to extensive range reduction and/or extinction of unique, endemic species. Our goal is to develop genetic tricks that drive local extinction of invasive species and disease vectors. 3) Can we drive genes into wild populations so that all individuals express a trait of interest? With regard to this last aim, we are particularly interested in developing transgenic insects that will prevent transmission of mosquito-borne pathogens that cause malaria and dengue fever. More than 500 million people are infected with the malaria parasite each year, resulting in 1-3 million deaths, while dengue, a mosquito-borne virus, infects more than 100 million people each year, resulting in more than 25,000 deaths. Effective vaccines do not exist, and in the case of malaria, the causative agent, the parasite Plasmodium falciparum has acquired resistance to many drugs. Vector suppression through the release of sterile males, the use of insecticides, or modification of the environment provides an important tool for limiting mosquito-borne disease. However, each approach has limitations. Release of sterile males provides only transient population suppression, insecticides affect many non-target species and mosquitoes often evolve resistance to these compounds, and wholesale modification of the environment may not be feasible, or desirable in many situations based on ecological concerns. Our goals are two-fold: to develop transgenic insects that lack the ability to transmit these pathogens (primarily as collaborations with other labs); and to develop genetic tools for driving these genes into wild populations of insects, thereby blocking disease transmission.

Approaches similar to those described above can also be used to tackle diseases of agricultural interest. One disease of current interest is known as citrus greening disease (also known as Huanglongbing; HLB). HLB is caused by the bacteria Candidatus Liberibacter, which is transmitted to the citrus plant by an insect, the phloem feeding citrus psyllid, Diaphorina citri. The disease is difficult to detect and current methods of control involve either regular use of insecticides or –once the tree is infected – tree destruction. HLB threatens to effectively eliminate the citrus industry in many areas in the US. We are interested in working with the citrus industry to develop transgenic insect-based approaches to prevent HLB.

The world's human population is 7.1 billion and projected to rise to 10-11 billion by 2100. Of the roughly 208 million pregnancies each year, about 85 million are unintended, resulting in 50 million abortions, which are associated with 104,000 maternal deaths. Thus there is a large unmet need for modern contraception. There is a particular need for cheap long-term methods that can be
implemented in resource-poor settings in which access to health care is sporadic. There is also a need for non-lethal methods of population control for many free roaming animals. Examples include feral cats and dogs, as well as deer, horses, burros, elephants, and a number of invasive species. We are working to develop single-shot very long term contraceptives for a number of mammalian species.

**Drosophila models of human neuro-degenerative diseases** (Ming Guo (and the Guo lab), Haixia Huang, Bruce A. Hay, Nikolai Kandul). In collaboration with the Guo lab at UCLA we are studying Drosophila models of the two most common neurodegenerative diseases, Alzheimer's disease and Parkinson's disease (Guo, M. et al. (2003) *Hum. Mol. Genet.* 12:2669-2678; Clark, I.E. et al. (2006) *Nature* 441:1162-1166). We are particularly interested in understanding how disruption of mitochondrial function contributes to these diseases.

**Gene activation screens for cell death regulators:** MicroRNAs, small non-coding RNAs, define a new family of cell death regulator (Haixia Huang, Bruce Hay). We have carried out several screens for cell death regulators in the fly and have identified a number of new molecules. Among these are multiple microRNAs, small noncoding RNAs that function by inhibiting translation of target transcripts. We are interested in determining when and where these molecules regulate death, as well as the nature of their targets. We are also designing microRNAs that target known cell death regulators as a way of probing the function of these proteins in specific contexts.

**Cell death, caspases and IAPs** (H. Arno J. Müller, Soon Ji Yoo, Bruce A. Hay). In flies and vertebrates most, if not all, cells can undergo apoptosis in the absence of new gene expression, indicating that the components required to carry out apoptosis are present and ready for activation. The core of the cell death machine consists of members of a family of proteases known as caspases, which become activated in response to many different death signals. Active caspases then cleave a number of different cellular substrates that ultimately lead to cell death and corpse phagocytosis. Most if not all cells constitutively express caspase zymogens (inactive precursors) sufficient to bring about apoptosis. Thus, the key to cell death and survival signaling revolves around controlling the levels of active caspases in the cell. Several basic strategies are used to regulate caspase activity, and the core proteins that drive caspase-dependent death are evolutionarily conserved. In Drosophila many cells experience chronic activation of the apical cell death caspase Dronc. If unrestrained, active Dronc cleaves and activates downstream effector caspases that bring about cell death. Cells survive because they express the IAP DIAP1, which suppresses Dronc activity, as well as that of caspases activated by Dronc. One major pathway through which caspase-dependent cell death in flies is induced is through the regulated expression of pro-apoptotic proteins that disrupt DIAP1-caspase interactions through several different mechanisms, each of which has the effect of unleashing a cascade of apoptosis-inducing caspase activity. We are interested in several questions. 1) What are the signals that lead to caspase activation in cells that would normally live? 2) How do IAPs regulate caspase activity and when and where does this regulation define points of control? 3) How is IAP activity regulated? 4) And finally, as discussed further below, how do caspases, IAPs and their regulators work to regulate non-apoptotic processes? We are using both genetic screens and biochemical approaches to identify the critical molecules.

**Caspases and their regulators in a non-apoptotic process, spermatid differentiation** (Haixia Huang, Geoffrey Pittman). We have found that multiple caspases, acting through distinct pathways, acting at distinct points in time and space, are required for spermatid individualization, a process in which spermatids (which develop in a common cytoplasm) become enclosed in individual plasma membranes and shed most of their cytoplasm Huh, J. et al. (2004) *PLoS Biology*
Spermatid individualization is an evolutionarily conserved process, but little is known about how it is brought about. Several questions are of interest to us: 1) What are the upstream signals that drive caspase activation? 2) What are the nonapoptotic targets that facilitate differentiation? 3) How is cell death prevented in the face of high levels of caspase activity that would normally be associated with cell death? 4) Do caspases play similar roles in promoting spermatid differentiation in mammals? 5) Can we manipulate the biology of spermatogenesis so as to bias gamete production so that males produce gametes carrying the Y chromosome, but not the X chromosome? Elements with these characteristics, if they are located on the Y chromosome, are predicted to drive a population to extinction through the generation of male-only populations.

Cell death and the innate immune system (Bruce A. Hay). As discussed above, many IAP family proteins inhibit apoptosis. IAPs contain N-terminal BIR domains and a C-terminal RING ubiquitin ligase domain. *Drosophila* DIAP1 protects cells from apoptosis by inhibiting caspases. Apoptosis initiates when proteins such as Reaper and Hid bind a surface groove in DIAP1 BIR domains via an N-terminal IAP-binding motif (IBM). This evolutionarily conserved interaction disrupts IAP-caspase interactions, unleashing apoptosis-inducing caspase activity. DIAP2 overexpression also inhibits Rpr- and Hid-dependent apoptosis, but little is known about DIAP2's normal functions. We generated *diap2* null mutants, which are viable and show no defects in developmental or stress-induced apoptosis. Instead, DIAP2 is required for the innate immune response to Gram-negative bacterial infection (Huh, J. et al. (2007) *J. Biol. Chem.* **282**:2056-2068). DIAP2 promotes cytoplasmic cleavage and nuclear translocation of the NF-κB homolog Relish, and this requires the DIAP2 RING domain. Increasing the genetic dose of *diap2* results in an increased immune response, while expression of Rpr or Hid results in down-regulation of DIAP2 protein levels. Together these observations suggest that DIAP2 can regulate immune signaling in a dose-dependent manner, and that DIAP2 is regulated by IBM-containing proteins. Therefore, *diap2* may identify a point of convergence between apoptosis and immune signaling pathways.

Driving genes for disease refractoriness into wild pest insect populations with Medea selfish genetic elements (Haixia Huang, Omar Akbari, Wen Min Chen, Anna Buchman, Chun-Hong Chen, Bruce A. Hay). An attractive approach to suppressing mosquito-borne diseases involves replacing the wild-insect population with modified counterparts unable to transmit disease. Mosquitoes with a diminished capacity to transmit *Plasmodium* have been identified in the wild and created in the laboratory, demonstrating that endogenous or engineered mosquito immunity can be harnessed to attack *Plasmodium*. However, a critical unanswered question is how to spread these effector genes throughout the areas inhabited by disease-transmitting insects. Epidemiological and modeling studies suggest that it will be necessary to rapidly replace a large percentage of the wild mosquito population with refractory insects in order to achieve significant levels of disease control. Because insect disease vectors are spread over wide areas and can migrate significant distances, mass release of refractory insects associated with simple Mendelian transmission of effector-bearing chromosomes is unlikely to result in a high enough frequency of transgene-bearing individuals. Compounding this problem, enhancement of immune function in insects is often costly, requiring tradeoffs with other life history traits such as longevity and fecundity that decrease fitness. Therefore, it is likely that insects carrying effector transgenes will be less fit than their wild counterparts, resulting in a decrease in the fraction of individuals carrying genes for refractoriness over time. These observations argue that population replacement will require coupling of genes conferring disease refractoriness with a genetic mechanism for driving these genes through the wild population at greater than Mendelian frequencies.
Maternal-effect lethal selfish genetic elements in the flour beetle *Tribolium castaneum* have the following behavior: when present in a female, they must be inherited in the next generation in order for the offspring to survive. The molecular nature of these elements (known as *Medea* elements) is unknown, but their spiteful genetic behavior (they cause the death of those who fail to inherit them, giving a relative transmission advantage to those that do carry them) makes them attractive candidates to mediate drive because it is predicted to lead to rapid spread of the element within the population even if it carries an associated fitness cost. *Medea*'s ability to spread, and the time it takes to become present in all individuals, is a function of fitness cost and introduction frequency. The plot in Figure 1 describes the number of generations required for *Medea* to be present in 99% of individuals, for a *Medea* element with an embryonic fitness cost (resulting from the presence of a cargo transgene designed to protect from disease, for example). Homozygous *Medea*:non-*Medea* introduction ratios are indicated on the Y axis, and embryonic fitness cost on the X axis. Area between lines indicates regions of parameter space within which a specific number of generations (indicated by numbers and arrows) are required for the frequency of *Medea* individuals to reach a frequency of 99% or greater. Line color, shown in the heat map at right, provides a measure of how many generations are required. Black lines (50+) indicate that fifty or more generations are required. The border between the black-lined region and the lower unlined region defines the critical *Medea*:non-*Medea* introduction ratio, below which *Medea* will be eliminated from the population.

The molecular biology of endogenous *Medea* elements is unknown, but the genetics suggests a model in which *Medea* consists of two linked genes: The first encodes a toxin that is expressed only in the female germline, with effects that are passed to all progeny. The second encodes an antidote, expressed under the control of an early zygote-specific promoter (Figure 2). Mothers that carry a *Medea* element express a toxin (red dots) that is inherited by all oocytes (small ovals). Embryos (large ovals) that do not inherit *Medea* die because toxin activity (red background) is unimpeded (lower left square). Embryos that inherit *Medea* from the mother (upper left square), the father (lower right square) or both (upper right square), survive because expression of an antidote early during embryogenesis (green background) neutralizes toxin activity. We imagine that *Medea* is comprised of two closely linked genes (upper left).
We created synthetic Medea elements in *Drosophila* that can drive population replacement (Figure 4) and that are resistant to recombination-mediated dissociation of drive and effector functions. These elements (Figure 3) result from zygotic rescue of a maternal loss-of-function that results in embryonic arrest. During oogenesis a maternal transcript is synthesized (green dots), whose product is required for early embryogenesis. In females carrying a Medea, the first transgene (the toxin) drives maternal drives maternal germline-specific expression of microRNAs that silence expression of the gene whose product is required for early embryogenesis. This results in inheritance of a lethal condition - the loss of an essential maternally deposited product - by all oocytes/embryos. Progeny survive the embryonic arrest thereby induced if they inherit from their mother a tightly linked transgene driving early zygotic expression of the maternally silenced gene just in time to restore embryo development, but they die if they fail to inherit it.

**Sensing and killing dengue and yellow fever virus-infected cells in their insect host** (Kelly J. Dusinberre, Zachary Sun) Dengue and yellow Fever virus infect mosquitoes during a blood meal. The virus must enter and replicate inside mosquito midgut cells, disseminate throughout the body and ultimately infect the salivary gland (7-14 days later), in order to be transmitted to a new individual during a subsequent blood meal. Our goal is to develop transgenes that are phenotypically neutral when expressed in uninfected individuals, but that kill virus-infected cells and/or the mosquitoes themselves. The virus encodes several activities that are not present in uninfected host cells. These include a viral polyprotein protease, and RNA-dependent RNA polymerase. We are developing molecules that sense these activities and cause the death of cells and insects in which they occur, thereby preventing disease transmission to humans.
Engineering reproductive isolation and population replacement using a synthetic underdominance system (Anna Buchman). The Medea system detailed above is very good at spreading genes into populations distributed over large areas, provided that modest levels of migration occur. This is ideal for situations in which the goal is to carry out population replacement in large regions. However, some communities may favor an approach in which population replacement is restricted to a local environment (Let's see how it does in your back yard, before trying it in mine). This creates a challenge: how to spread genes within a local environment, but maintain a barrier to migration-driven spread and fixation in surrounding regions. To address this need we are developing the synthetic underdominance system illustrated in Figure 5. In this system homologous chromosomes carry toxin-antidote pairs in which the toxin present on chromosome A (Killer 1) is linked to an antidote (Rescue 2) that represses Killer 2. Killer 2 is located at the same position on the homologous chromosome B, linked with an antidote (Rescue 1) that represses Killer 1 (Figure x). In such a system, organisms can only survive if they carry A and B chromosomes (in A/B individuals), or only wildtype (+) chromosomes (in +/+ individuals). A/+ and B/+ individuals die. A and B chromosomes will also carry genes that confer resistance to disease transmission. Such a system has two interesting features.

First, it constitutes a simple method for engineering reproductive isolation (speciation). Matings between +/+ individuals produce viable progeny, as do matings between A/B individuals. However, mating between +/+ and A/B individuals produce only A/+ and B/+ progeny, which all die. This simple technology has a number of potential applications and provides a platform from which to explore some of the evolutionary consequences of reproductive isolation. Second, it provides a method for driving genes into a local environment in such a way that they are unlikely to spread to fixation in surrounding regions through migration. In brief, for underdominance, as with Medea elements that carry a fitness cost, a threshold frequency must be achieved in order for spread to occur at all. With single locus underdominance this threshold is quite high (66%) (Figure 6, left panel). In two-locus underdominance (Figure 6, right panel), the two toxin-antidote cassettes are located on non-homologous chromosomes. In this configuration more transgenic progeny can survive in crosses to wildtype, and thus the introduction threshold required for spread to occur is significantly lower, 33%. Once the threshold is crossed, these underdominant systems drive the wildtype chromosomes out of the population by causing their death in individuals that carry A or B, but not both. The A/B genotypes have great difficulty in spreading into surrounding regions through migration because as they migrate into areas composed largely of +/+ individuals, they are more likely to mate with +/+ individuals than with A/B individuals, resulting in the likely death of progeny that carry one but not the other. We are developing several versions of underdominance in Drosophila and are working to move these systems to mosquito species.
**UD^{MEL}, a high-threshold gene drive system.** (Omar Akbari, Kelly Matzen, John Marshall, Katie Kennedy, Bruce Hay) We have built a novel gene drive system that contains features of zygotic underdominance, described above, and Medea. In this system, known as Underdominance, Maternal Effect Lethal (UD^{MEL}). Two maternally expressed toxins, located on separate chromosomes, are each linked with a zygotic antidote able to rescue maternal-effect lethality of the other toxin. As illustrated in Figure 7, this system shows threshold-dependent population replacement in single- and two-locus configurations in *Drosophila*. Models suggest that transgene spread can often be limited to local environments. They also show that in a in a population in which single-locus UD^{MEL} has been carried out, repeated release of wild-type males can result in population suppression, a novel method of genetic population manipulation.

![Figure 7](image_url)

**Sensing and responding to normal and abnormal microRNA expression (Nikolai Kandul).** MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression by suppressing the translation or promoting the degradation of transcripts to which they hybridize. Importantly for our purposes, when miRNAs are perfectly complementary to their target transcripts, transcript cleavage and degradation results. It is clear that miRNA expression is deregulated in many disease states. In addition, many viruses encode miRNAs that promote viral replication and/or suppress host defense systems. Our goal is to develop methods for sensing the expression of a particular miRNA, and then transducing this signal into changes in gene or protein expression. This will allow us to monitor the levels of miRNA expression in living animals. It will also allow us to regulate cellular physiology in response to the levels of particular miRNAs.

**PSR, a selfish chromosome in Nasonia Vitripennis.** (Omar Akbari, Patrick Ferree) One of the most distinguishing characteristics of hymenoptera such as wasps, is haplodiploid reproduction, in which males are haploid and arise from unfertilized eggs, while females are diploid and arise from fertilized eggs. Some strains of the jewel wasp Nasonia vitripennis carry a supernumerary B chromosome known as paternal sex ratio (PSR). PSR is a small highly heterochromatic chromosome. It has the interesting feature that when present in a male it...
somehow causes the loss of all paternal chromosomes during the first mitotic division in the early embryo. This has the effect of making these diploid embryos, which should become female, into PSR-transmitting haploid males. embryos male, , chromosomes non-essential, enigmatic, highly heterochromatic mini-chromosome, that somehow encodes factors that mark the male genome during spermatogenesis, ultimately resulting in its loss in the embryo, while at the same time protecting the PSR chromosome (also present in the sperm whose genomes are being marked for loss). Thus, PSR males give rise to more PSR males

**Predicting the fate of gene drive systems and their cargos in the wild** (*John Marshall, Bruce Hay*), As we develop gene drive strategies we need to be able to predict how they are likely to behave. A number of questions arise: Under what ecological and population genetic conditions will drive chromosomes spread? What are the likely epidemiological consequences of spread in terms of disease prevention? What are the likely functional lifetimes of these elements in the wild? What are the possibilities for removal and replacement of first-generation elements with second-generation elements? We are using mathematical modeling and computer simulations to address these issues for a number of different drive strategies.

**How many possible ways are there for driving genes into populations, resulting in either population replacement or population elimination?** (*John Marshall, Bruce Hay*) We are interested in identifying all the ways in which genes, gene complexes, or entire chromosomes can promote their own spread into populations. This analysis may identify novel mechanisms by which populations have been shaped in the wild. It may also identify mechanisms that could be used to drive genes into populations, either providing them with some desirable trait, or driving the population towards an inviable genotype and extinction. We are particularly interested in identifying those mechanisms that can be thought of as consisting of combinations of genes with toxin and antidote activities as these can in principal be engineered, and may also have evolved in the wild as a consequence of epistatic interactions between genes.

**Long-term contraception.** (*Juan Li, Bruce Hay*) The world's human population is 7.1 billion and projected to rise to 10-11 billion by 2100. Of the roughly 208 million pregnancies each year, about 85 million are unintended, resulting in 50 million abortions, which are associated with 104,000 maternal deaths. Thus there is a large unmet need for modern contraception. There is a particular need for cheap long-term methods that can be implemented in resource-poor settings in which access to health care is sporadic. There is also a need for non-lethal methods of population control for many free roaming animals. Examples include feral cats and dogs, as well as deer, horses, burros, elephants, and a number of invasive species. We are working to develop single-shot very long term contraceptives for a number of mammalian species.
PUBLICATIONS

2013


2012


Evolutionary Mechanisms of Host-Bacteria Symbiosis During Health

Summary:
The Western world is experiencing a growing medical crisis. Epidemiologic and clinical reports reveal a dramatic increase in immune disorders: inflammatory bowel disease, asthma, type 1 diabetes, and multiple sclerosis. Emboldened by the 'hygiene hypothesis' proposed two decades ago, scientists have speculated that lifestyle changes (vaccination, sanitation, antibiotics) have predisposed developed societies to these disorders by reducing bacterial infections. However, the hypothesis remains without explanation as our exposure to most bacteria does not result in disease. Mammals are colonized for life with 100 trillion indigenous bacteria, creating a diverse ecosystem whose contributions to human health remain poorly understood. In recent years, there has been a revolution in biology toward understanding how (and more importantly, why) mammals harbor multitudes of symbiotic bacteria. We have recently demonstrated for the first time that intestinal bacteria direct universal development of the immune system; thus fundamental aspects of
mammalian health are inextricably dependent on microbial symbiosis. Furthermore, it is now clear that all of the diseases in question astonishingly involve a common immunologic defect found in the absence of symbiotic bacteria. As we have co-evolved with our microbial partners for eons, have strategies used against infectious agents reduced our exposure to health-promoting bacteria, ultimately leading to increased disease? We propose that the human genome does not encode all functions required for health, and we depend on crucial interactions with products of our microbiome (collective genomes of our gut bacterial species). Through genomics, microbiology, immunology, neurobiology and animal models, we wish to define the molecular processes employed by symbiotic bacteria that mediate protection from disease. Advances in the past year have now made it possible to mine this untapped reservoir for beneficial microbial molecules. Ultimately, understanding the immune mechanisms of these symbiosis factors may lead to natural therapeutics for human diseases based on entirely novel biological principles.

Images from left to Right:
Professor Sarkis Mazmanian
Bacteria Colonizing the Gut
**Publications**

**2013**


**2012**


George W. Beadle Professor of Biology, Investigator, Howard Hughes Medical Institute:
Elliot Meyerowitz

Professorial Awards and Honors:
2013 Marker Lectures, Pennsylvania State University
2013 EMBO Keynote Lecture, Society for Developmental Biology, Cancun, Mexico

Senior Research Fellows:
Vijay Chickarmane, Zachary Nimchuk, Kaoru Sugimoto

Postdoctoral Scholars:
Paul Tarr, An Yan, Yun Zhou

Graduate Students:
Labeed Ben Ghaly, Cory Tobin

Undergraduate Students:
Daniella Allevato, Stephanie Bohaczuk, Edward Pursifull, Lawrence Wang

Research and Laboratory Staff:
Arnavaz Garda, Rachel Li, Daphne Shimoda

Lab Website: http://www.its.caltech.edu/~plantlab/

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GENETICS OF PLANT DEVELOPMENT

Summary:
Our laboratory has the goal of understanding the mechanisms of plant development, using both experimental and computational methods to test hypotheses. Land plants develop in two directions, up and down – with up being the shoot and its accompanying leaves and flowers, and down the root. We concentrate on the shoot, and on the set of stem cells that continuously provides the cells for the shoot throughout the growth of the plant. This set of cells is called the
shoot apical meristem. It utilizes a number of different pattern-forming processes that are as yet poorly understood. First, the maintenance of the stem cell populations in the shoot meristem is mediated by peptide hormone communication between different regions of the meristem. The peptide CLAVATA3 signals to the cells below the pluripotent stem cells in the apical region called the central zone via transmembrane receptor serine-threonine kinases that include CLAVATA1 and additional and related members of the plant leucine-rich repeat receptor kinase family. Recent progress on this system includes the finding that CLAVATA1 experiences ligand-induced internalization, which buffers the receptor system to wide ranges of CLAVATA3 concentration, and that in the related brassinosteroid receptor system, a step in formation of the active kinase signaling complex is movement of a receptor-associated protein from plasma membrane to cytoplasm.

Secondly, there is a system of small-molecule hormone perception and feedback involving the plant hormones termed cytokinins. These have been shown to play a central role in maintenance of the fixed gene expression domains in the shoot meristem, which remain constant even as cells move through the domains to become differentiated parts of the plant (stem, leaves and flowers). One recent advance in this area has been the development of a computational model that relates cytokinin concentration to the formation and maintenance of different domains of gene expression in the shoot apical meristem. One prediction of the model, a feedback of the CLAVATA system on cytokinin synthesis, was confirmed.

Finally, there is another large feedback network in which the plant hormone auxin is actively moved through the meristem by its transporter, and initiates formation of leaves and flowers in the geometric patterns that are easily recognized in pine cones, sunflowers, and the like. A recent discovery here is that the subcellular position of the PINFORMED1 auxin transporter, which determines the direction of auxin flow, is determined in response to physical stresses in the meristem. The auxin transport system therefore responds both to chemical and physical cues, and serves as a nexus in the mediation of plant responses to mechanical stress. A recent step in this area has been the demonstration that the microtubule cytoskeleton, which reads out the direction of anisotropic stress, is under stress control in plant cells other than meristem cells as well as in meristem cells, giving some generality to the meristem observations.

Encapsulating the dynamic data and feedback between different modes of signaling in these developing tissues has led us to develop mathematical models of plant development, in which the dynamic data we gain from live imaging of growing plant tissues leads to hypotheses expressed as sets of equations, which when solved in a computer model the processes occurring in the real plant. The results from the computer are then used to predict experimental results, and new results are used to refine and alter the models. This iteration brings us closer to robust models of development, and therefore to an understanding of developmental principles. We call this approach to developmental biology Computational Morphodynamics.
Shoot apex with epidermal nuclei in green, chloroplasts in red (photo by Adrienne Roeder)

**PUBLICATIONS**

**2013**


**2012**


Gene Regulatory Mechanisms for T-Cell Development from Stem Cells

Summary:
The Rothenberg group studies the gene regulatory mechanisms that guide blood stem cells to ultimate fates as T lymphocytes. This developmental process is distinct from many of the developmental systems studied at Caltech, because hematopoietic stem cells provide a continuing source of new T cell precursors throughout life, and development of new T-cell cohorts is mobilized in fetal life, neonatal life, and on through adulthood. This system is also distinctive because it is particularly good for shedding light on the stepwise choices the cells need to make in order to complete their differentiation as T cells. Blood precursor cells need to migrate to the...
thymus and expose themselves to sustained Notch1-Delta-like 4 (DL4) interactions in order to be triggered to differentiate into T cells. All the steps from multipotent precursor to committed T-lineage cell occur in this thymic environment, where cells in each stage are relatively easy to isolate, characterize, and manipulate. Thus we have been able to learn that these cells pass through a hierarchical decision tree that involves: the choice not to become a red blood cell or a platelet, the choice not to become a B cell, the choice not to become a macrophage or granulocyte, the choice not to become an antigen-presenting dendritic cell, and finally the choice not to become a natural killer cell, which leaves only various T-cell fates as the last options. This last decision concludes the T-lineage commitment process. The goal of research in this lab is to understand not only how the cells acquire the properties they will need to work as T cells, but also why the options that remain open to the precursors still are open, and how the cells make the decisions they do at each branch point. The answers we are interested in provide explanations in terms of specific transcription factor actions in gene regulatory networks.

A convergence of cell biological and molecular biological studies has revealed that the main events in early T-cell development can be broken into two major phases, split by the conclusion of commitment. Although both phases are normally dependent on Notch1-DL4 signaling, they involve different “jobs” for the cells. The first phase seems to drive the precursors to proliferate, with only limited acquisition of T-cell characteristics. The cells then cross the boundary into the second phase, when they reduce their proliferation and activate the full T-cell differentiation program. The clean division between these two phases appears to be crucial to avoid derangement of T-cell development and progression toward lymphoma.

One of the regulators we have studied for many years, the Ets-family transcription factor PU.1, now emerges as a principal actor in the first phase. This factor can participate in gene regulatory networks pushing the cells to several different fates, but its early T-cell role is kept focused by interaction with Notch pathway signals. We have found evidence that in this context, PU.1 is a direct positive regulator of multiple genes involved in the self-renewal circuit operating in phase 1 pro-T cells, based on a convergence of data chromatin immune precipitation analyzed by deep sequencing (ChIP-seq) and on gain and loss of function perturbation experiments. PU.1 must then be repressed during commitment, and we have gained insight into new cis-elements and unexpected deployments of trans-acting factors that probably cause PU.1 to be repressed during the transition from phase 1 to phase 2.

We have also determined the identity of a factor that may be a major switch controller at the transition from phase 1 to phase 2, and this turns out to be the T-cell specific zinc finger factor Bcl11b. We have shown that if Bcl11b is deleted, phase 1 pro-T cells fail to undergo commitment, spawning non-T cells abnormally even in the presence of Notch ligands. Intriguingly, Bcl11b knockout pro-T cells uncouple proliferation from differentiation, gaining the ability to keep proliferating as long as growth factors are available without developmental progression. The cis- and trans-elements required to turn Bcl11b on can be equated with those that define T-lineage identity, and so they are a major focus of our current work. Further, the mechanism through which Bcl11b works to bring about commitment involves identifying its own direct target genes and interaction partners, and that has become another important focus. Bcl11b’s action at the last
major identity determination point for T-cell precursors may involve network interactions with competing phase 1 regulators, and the gene regulatory network aspects of its role are another important project.

The strong punctuation created by the phase 1—phase 2 transition machinery provides a new framework in which to view the roles of other essential T-lineage factors, like GATA-3, that have long appeared to have paradoxical roles. GATA-3 and TCF-1 (encoded by the Tcf7 gene) are the two factors that are initially induced by Notch signaling to distinguish the first T-cell developmental stages before commitment. GATA-3 especially has been difficult to study because its level needs to be very precisely regulated in developing T cells. The methodology we have developed to dissect stage-specific actions of PU.1 and Bcl11b has now given us more insight into the reasons why GATA-3 levels must be so tightly titrated for T cell development to proceed. Our ChIP-seq analyses of GATA-3 binding sites reveal that the phase 1—phase 2 split may not only alter the constellation of available regulatory factors in the nucleus but also alter the deployment of those factors that are present throughout the transition.

We proposed an initial gene regulatory network model to account for the T-cell development pathway three years ago, based on the effects of transcription factor perturbation on the expression of multiple developmentally regulated genes. The newest iteration of our network model has just been published. Network construction has illuminated the need for three additional kinds of information in order to complete and confirm the model. First, a more complete “parts list” for the T-cell specification process: we needed to know all the transcription factors and potential signaling systems that might be candidates for regulatory roles. Second, we needed a way to locate the candidate cis-regulatory sites at which these factors might work on their target genes. Third, we needed better tools for dissecting the roles of these factors via stage-specific loss or antagonism of function. To address the first and second needs, we have carried out a major survey of all the changes in both RNA expression and epigenetic histone marks throughout the genome as the cells progress from the earliest T-cell development stages to commitment and beyond. This enterprise, carried out through a collaboration with the Wold lab, has yielded a broad and detailed picture of the cis- and trans-regulatory changes at each stage of the T-cell specification process. Now, to verify direct functional effects of transcription factors on target genes in a stage-specific way, we have also developed a combination of inducible deletion and dominant negative strategies that resolve direct and indirect positive and negative regulation.

Another way we have sought to establish causality is by tracking the regulation of PU.1 and Bcl11b expression over time in individual cells by live imaging. This work, carried out in collaboration with the Elowitz lab, is based on following the expression of key regulatory genes under defined developmental conditions by tracking fluorescent protein transgenes inserted into the genome under the control of the PU.1 or Bcl11b cis-regulatory elements. We are able to track cells and their descendants across at least three cell cycles as they select different developmental fates in real time, and thus transcription factor gene regulation changes can be directly coupled with the changes in developmental status of living cells.
The commitment process is not only a way for T-cell precursors to renounce other hematopoietic fates; it is also closely intertwined with poorly understood events that will go on to influence the subspecialization of T-cell fate that the cells will undertake, and even to determine whether or not they will be allowed to survive in the T-cell lineage. A long-standing project in the lab has been to study the variants of this program in genetically distinct mouse strains with potentially altered T-cell generation. Genome-wide transcriptome analysis now suggests that one genetic background associated with immunological defects also causes important defects in phase 1 to phase 2 progression of thymocytes. These early defects can undermine later developmental checkpoint control and lead to a high-penetrance preleukemic phenotype. At substantial frequency, these cells can then progress to malignancy, in which the persistent phase 1 gene expression serves as a hallmark for a specific early T-cell precursor type of acute lymphoblastic lymphoma related to a virulent form of T-ALL in humans. Thus the accurate regulation of the transition from phase 1 to phase 2 in the early stages of T-cell development not only works to regulate the size of the pro-T cell pool, but also may be a matter of life and death for the organism.

Rothenberg lab projects and investigators:

Precise definition of lineage commitment and developmental branch points:
Hao Yuan Kueh, Mary Yui,
GATA-3 roles in early T-cell development:
Sagar Damle
PU.1-Notch network competitively determining lymphomyeloid fate:
Marissa Morales Del Real
Reciprocal PU.1/GATA-3 antagonism gated by Notch signaling in early T-cell development:
Marissa Morales Del Real
Bcl11b roles in early T-cell development:
Long Li
Bcl11b target genes and interaction with GATA-3:
Long Li, Jingli Zhang
Manipulation of the T-cell differentiation progression gene regulatory network:
Ameya Champhekar, Sagar Damle, George Freedman
Cell cycle kinetics as an integral component of gene regulatory network dynamics:
Hao Yuan Kueh
Computational modeling and quantitative analysis of early T cell developmental kinetics
Hao Yuan Kueh, May Yui, Erica Manesso*, Carsten Peterson*
Cis-regulatory elements of Bcl11b:
Long Li, Hao Yuan Kueh, Kenneth Ng
A high-penetrance model for variant T-ALL linked to checkpoint violation
Mary Yui

*University of Lund
Middle: imaging of hematopoietic progenitors developing in culture, green fluorescence from PU.1-GFP expression, red fluorescence from lineage tracker (courtesy: Hao Yuan Kueh)

Right: heat map of transcription factor expression patterns across five stages of early T cell development, two to three biological replicates per stage, as determined by RNA-seq. Red: highest expression, blue: lowest expression, reads per million per kilobase range >10,000 fold (courtesy: Jingli Zhang)

**PUBLICATIONS**

**2013**


Rothenberg, E. V. 2013. GATA-3 locks the door to the B-cell option. *Blood*. 121, 1673-1674. PMID: 23471221. PMCID: PMC3591792


**2012**


Zarnegar, M. A. and Rothenberg, E. V. 2012. Ikaros represses and activates PU.1 cell-type-specifically through the multifunctional Sfpi1 URE and a myeloid specific enhancer. *Oncogene*, 31, 4647-4654. PMID: 22231443

**Dynamics of Developmental Systems**

**Dorsalventral Patterning Gene Regulatory Network**

The dorsal-ventral (DV) patterning gene regulatory network (GRN) of *Drosophila* embryos is considered one of the most extensive GRNs in terms of number of characterized genes and cis-regulatory modules. Subdividing the embryo into distinct domains of gene expression is an important function of the DV GRN, which encompasses the first three hours of development: the embryonic period up to and including cellularization just preceding gastrulation. In part, this subdivision is necessary to set-up activation of signaling pathways at later stages through differential expression of receptors and ligands. Subsequently, these early patterning events support tissue differentiation and also control cell movements required for the generation of a multilayered embryo: the developmental actions that encompass gastrulation. Only recently has it come to light that the transcription factor levels in the early embryo can be dynamic. We hypothesize these dynamics support robust patterning in the face of variation in embryo size, which occurs naturally within the population.

Most studies of early zygotic gene expression consider one or two time-points spanning the first four hours of early *Drosophila* development, and yet our recent analysis suggests that gene expression patterns change on the order of minutes rather than hours. For example, recently, we uncovered dynamics for the transcription factor Dorsal, a morphogen and as such a pivotal
player in DV patterning. The levels of this factor almost double from one nuclear cycle to the next, in a matter of minutes (~10'). In addition, the activation of many signaling pathways is delayed, as signaling is not active until the embryo is cellularized about three hours following fertilization. Therefore, one major limitation of the current *Drosophila* DV GRN is that in its current form it considers all of early development as a single time-point.

We aim to expand our understanding of the DV patterning GRN: a developmental system, which uses morphogens to support patterning and undergoes rapid development. We will integrate spatiotemporal information into the DV patterning GRN with the objective of obtaining insight into the role of transcription factor and target gene dynamics. In particular, we are interested in why some target genes appear ‘plastic’, with levels changing constantly both upwards and downwards; whereas others exhibit more of a ‘ratchet’ effect in that levels continue to steadily increase. Furthermore, we have found that the size of the DV axis can change as much as 20% due to naturally occurring variation. Some patterns change accordingly, they ‘scale’, whereas other patterns remain constant. How is robust development of embryos supported in the face of such natural variability in embryo size? Why do genes exhibit different dynamics, and how does this impact developmental progression?

**Coordinate Action of CIS Regulatory Modules**

Many genes are pervasively expressed throughout development and exhibit changes of expression in a stage-specific manner. It is appreciated that different cis-regulatory modules (CRMs) act to control dynamic expression; however, not much is known about how CRM order of action is regulated. Using the *Drosophila* embryo as a model system, we have the exceptional opportunity to investigate how CRMs support spatiotemporally-regulated gene expression during the animal’s developmental course. Current experiments focus on advancing understanding of how CRM order of action is controlled. We are capitalizing on the availability of ample background information and our knowledge of dorsal-ventral (DV) patterning in the *Drosophila* embryo to help guide choice of particularly relevant cis-regulatory systems for study of CRM temporal action.

Continuous gene expression during development is supported by CRMs acting in sequence. However, it is challenging to discern the distinct roles of individual CRMs; as when one CRM stops acting and another takes over is not always clear. We are interested in studying how CRM action at the *brinker* (*brk*) gene is regulated, and contend this locus is particularly well-suited to provide insight into the regulation of temporal gene expression. Our preliminary data show that the sequence just upstream of the *brk* minimal promoter contributes to sequential, coordinate action of CRMs controlling expression in the early embryo. However, how this promoter proximal sequence as well as chromatin conformation supports timing of CRMs acting in series is not yet understood, and will be investigated.

Many molecular and genetic tools are available to support these studies of CRM temporal action and regulation in *Drosophila*. They include ease of genetic approaches in the *Drosophila* system as well as the ability to manipulate large transgenes through recombineering to facilitate functional assays of CRMs and other regulatory sequences in native context. Furthermore, we will use chromatin conformation capture assays (e.g. 3C) to provide insight into how chromatin conformation may impact the temporal action of CRMs. However, standard
chromatin conformation capture experiments that utilize homogenized embryo samples to analyze the ‘conformation’ of DNA-DNA associations in vitro may lead to mixed results; genes in the developing embryo are often expressed in distinct domains, and therefore multiple chromatin conformation states may be present. Therefore, a necessary technical advance for analysis of dynamic developmental systems is analysis of chromatin conformation on a cell by cell basis, which will support studies of when and how particular CRMs interact with the promoter with temporal and spatial resolution. To this end, we are developing various technologies to acquire this information. We are also looking broadly at the regulation of genes in time and how the action of CRMs is regulated.

**Fibroblast Growth Factor Signaling**

Fibroblast growth factor (FGF) signaling impacts a number of different cellular functions important for supporting embryonic development. FGF ligands are polypeptide growth factors that bind to cell surface fibroblast growth factor receptors (FGFRs). These receptor ligands trigger tyrosine kinase activity associated with the intracellular domains of their receptors, and thereby elicit signaling responses within cells. Both ligands and receptors exhibit diverse and dynamic patterns of expression that support directional signaling across epithelial-mesenchymal boundaries. In early embryos, FGF signaling controls mesoderm induction and patterning, cell growth, migration, and differentiation; while later functions include organ formation and maintenance, neuronal differentiation and survival, wound healing, and malignant transformation.

Differential expression of genes encoding ligands and receptors is one mechanism by which signaling pathway activation is regulated; while another is that only a subset of all possible ligand-receptor interactions are functional. For instance, we have found that only three of six possible FGF-FGFR interactions are acting in *Drosophila melanogaster*: Pyramus and Thisbe FGF ligands each support activation of the FGFR Heartless, while only the FGF ligand Branchless supports activation of the Breathless FGFR. Specificity of ligand-receptor protein interactions also limits functional combinations in vertebrates, nevertheless, the system remains quite complex with over 120 FGF-FGFR combinations possible. Moreover, up to five FGF ligands may function concurrently to activate a single receptor in an apparently redundant fashion, making dissection of individual activities linked to particular ligands quite challenging. Studies in *Drosophila* have yielded valuable insights into the functions of many signaling pathways during development, as this system is amenable to molecular and genetic techniques as well as to live in vivo imaging. Therefore, *Drosophila*, with only three FGF-FGFR combinations acting, offers a simpler system to study FGF signaling.

We contend that the *Drosophila* model system is particularly well-suited to advance understanding of the molecular mechanism by which FGF signaling coordinates cell movement, an instrumental process during embryonic development. Previous studies on FGF signaling in *Drosophila* embryos have demonstrated that mesoderm cell movements are disorganized in the absence of FGF signaling. For instance, signaling through the Heartless FGFR is important for controlling mesoderm spreading during gastrulation and also, subsequently, for migration of caudal visceral mesoderm cells in the embryo. To support these collective cell migrations, our preliminary studies have suggested a number of possible roles for FGF signaling but the exact role, understood at a molecular level, remains unknown.

In addition, using this system, we have found novel insight into signaling as we have acquired evidence that FGF ligand choice, levels, and cleavage-state can all affect FGFR-dependent
outputs. Moreover our results demonstrate that FGF ligands that act concurrently to activate the same receptor are not redundant, contrary to the generally accepted belief in the FGF field at large, and instead show that FGF ligands fulfill distinct roles in the *Drosophila* embryo. In future directions, we are interested in answering the following questions: How are FGF ligands different and how is their activity regulated? How does FGF signaling regulate cell movement? Is there a link between FGF signaling and regulation of cell adhesion?

**Collective Migration of Groups of Cells**

Cell migration is a very influential process during embryonic development as it results in rearrangement of cells from one part of the embryo to another, effectively controlling cell-cell interactions to drive cell differentiation and organogenesis. The shape of most complex organ systems arises from the directed migration of cohesive groups of cells. Thus cell migration must be regulated temporally and spatially for organisms to develop properly. The overlying goal of our research objective is to provide insight into how cells within a migrating groups sense their environment and how this contributes to their collective movement. To accomplish this goal, we must first develop methodology to facilitate study of a specific group of cells in *Drosophila* which we propose serves as an excellent model system to study cell migration *in vivo*.

Caudal visceral mesoderm (CVM) cells exhibit directed cell migration as two distinct groups on either side of the body, from the posterior-most position of the embryo toward the anterior. The cells undergo the longest migration in all of *Drosophila* embryogenesis, but little is understood about how they are directed along their course. CVM cells are so named because they originate from a cluster of cells located at the posterior-most end of the embryo, the caudal mesoderm. First, the cluster separates into two, in a symmetric fashion, such that half the cells distribute to the left and the other half to the right of the body. Subsequently, these two groups, of approximately twenty cells each, undergo coordinate and directed movement toward the anterior of the embryo. The migration ensues over six hours and throughout the entire course of the migration the two groups migrate synchronously. This migration is necessary to position CVM cells along the entire length of the developing gut. At the end of their migration, CVM cells fuse with fusion-competent myoblasts to form the longitudinal muscles which ensheath the gut.

To start, our current research plan capitalizes on our prior experience with developing and implementing an *in vivo* imaging protocol that allowed visualization of all cells within a developing embryo. Our previous work was focused on an earlier stage of development, gastrulation, but we intend to apply similar methods to study migration at later stages of embryogenesis during germband retraction, when CVM cell migration proceeds. *Live in vivo* imaging of CVM cell nuclei will provide cell tracking data, and visualization of CVM cell membranes has the potential to provide insight into how cells interact with their environment. Quantitative analysis of cell tracking data and cell protrusion number and orientation can provide important information about the cell migration process in wildtype embryos, and can be used subsequently to interpret mutant phenotype. One aim is to use develop an imaging strategy to describe the behavior of CVM cell migration. In addition, we are developing new approach for creating mutant clones and studying coordinate cell migration using light-activated molecules. There is much to learn about coordinate cell migration through study of CVM cells, which is a relatively little studied (but excellent) model system.
**Publications**

**2013**


**2012**


Thomas Hunt Morgan Professor of Biology:
Paul W. Sternberg

Senior Research Fellows:
Mihoko Kato, Hans-Michael Müller

Research Fellows:
Meenakshi Doma, Yen-Ping Hsueh, Amir Sapir, Hillel Schwartz, Ryoji Shinya

Graduate Students:
Allison Akagi, Katie Brugman, Jonathan Liu, Julie Cho, Elly Chow, Adler Dillman, Srimoyee Ghosh, Margaret Ho, James Lee, Daniel Leighton, Paul Minor, Ravi Nath, Pei-Yin Shih

WormBase Staff:

Collaborators:

Visitors:
Carmie Puckett-Robinson, Andrea Choe, Michael Kawczynski, Gary Wong, Ruby Lopez-Zenteno

Research and Laboratory Staff:
Mary Alvarez, Christopher Cronin, Shahla Gharib, Gladys Medina, Barbara Perry

Financial Support:
California Institute of Regenerative Medicine
Howard Hughes Medical Institute
Japan Society for the Promotion of Science
National Institutes of Health, USPHS
National Science Foundation

Nematode Systems Biology

Summary:
We seek to understand how a genome controls development, physiology, and behavior. We use Caenorhabditis elegans molecular genetics to understand detailed mechanisms, and functional genomics to obtain global views of development and behavior. We try to couple tightly computation and experimental data, in part to use computation to make experimental tests more
efficient. Moreover, we study other genomes, genetics, and biology of other nematodes to help us comprehend *C. elegans*, to learn how development and behavior evolve, and to learn how to control parasitic and pestilent nematodes.

Our behavioral studies focused this year on sleep, sexual attraction, and response of nematodes to fungal predators. We have continued to study the chemicals (ascarosides) that constitute mating pheromone made by hermaphrodites (morphologically females but that make sperm for internal self-fertilization) and sensed by males. We hypothesize that ascarosides are a diverse family of nematode signaling molecules. To test this hypothesis we continued our collaboration with the labs of Art Edison and Frank Schroeder to purify mating cues from other nematode species. We discovered the male and the female cues from *Panagrellus redivivus* to be ascarosides. We then looked directly for ascarosides in a variety of species and found that each species makes a distinct but partly overlapping set of ascarosides and the species tested respond to different spectrum of ascarosides. The ascomycete *Arthrobotrys oligospora* attracts and kills soil nematodes. We are analyzing the odors produced by *A. oligospora* that attract *C. elegans* and characterizing the neural response to those odors at a molecular and circuit level. We found that this nematode trapping fungus senses the presence of nematodes by detecting ascarosides, suggesting that the ascarosides provide a molecular pattern of the presence of nematodes.

We have used channel rhodopsin to faithfully activate a neuron, as evidenced by whole-cell patch electrophysiology neuronal activity in a pre-synaptic cell expressing channel rhodopsin and then in its post-synaptic partner. Now that this system is validated, we expressed channel rhodopsin and a genetically-encoded calcium sensor in a range of specific neurons to be able to examine neuronal circuit properties. One of these properties is the sleep-like state exhibited by *C. elegans* during four periods of developmental lethargus preceding each larval molt. We find that multiple levels in a sensory-motor circuit are modulated during sleep. Not only are sensory neurons dampened but oscillation of command interneurons are decorrelated during sleep.

The infective juveniles (IJs) of some parasitic nematodes such as *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* are analogous to the dauer larvae of *C. elegans*. Developing *C. elegans* larvae choose between proceeding directly to reproductive development or to arrest development as dauer larvae, depending on population density (signaled by several ascarosides) and the amount of food available. We are studying how larvae make this all-or-none decision by deep transcriptome sequencing (RNA-seq) during the decision process.

In the area of cell regulation, we have continued to study WNT and EGF signaling to define new components, how these two pathways interact, and what determines the specific outcomes of common signals. For this study we focus on the *C. elegans* vulva, a paradigm for analyzing organogenesis. In one project, we are using the polarity of the vulval secondary lineage to study how multiple types of WNT receptors act in concert or antagonistically. We discovered that fibroblast growth factor (FGF) signaling works with WNT in this process. EGF controls development via the RAS/MAPkinase pathway and behavior via phospholipase C-gamma pathway. We had previously found that the EGF-receptor acts in a single neuron, ALA, to control a sleep-like
state. We are testing other conserved signaling pathways for common roles in sleep regulation, and using calcium imaging to examine neuronal function during worm sleep. We had discovered that a network of three homeobox-containing transcriptional regulatory proteins regulate expression of the EGF-receptor and other genes in the ALA neuron, and are now defining the cis-regulatory elements that respond to these homeobox proteins. By single cell transcriptional profiling of the ALA neuron we found several neuropeptides genes that we hypothesize might mediate the systemic function of this sleep-promoting neuron.

We are trying to learn how to efficiently define cis-regulatory elements using computational analysis to predict elements, and functional assays in transgenic C. elegans to test our predictions. For example, we tested some of our methods on elements that direct expression in the DVA neuron, which we had previously shown to control the extent of body flexion during locomotion.

For a number of projects, we want to identify all the genes that are expressed in a particular cell at a particular time. The ALA neuron mentioned above is one such cell. We thus are trying different methods of obtaining a transcriptional profile from a single cell; our current method is to microdissect a GFP-labeled cell using a modified patch clamp electrophysiology preparation, and amplify the cDNA and sequence libraries of cDNA. The male linker cell described below was our first test case. We have started extending this approach to other neurons, including the ALA and several sensory neurons. ALA expressed striking number and level of neuropeptides, which we are now testing for effects on sleep induction.

We started a new project on cell migration to understand both normal organogenesis and potential migratory programs that might be accessed by metastatic tumor cells. The C. elegans male linker cell (LC) undergoes a complex migration with changes in direction, speed, and morphology. An initial functional screen for genes involved in LC migration identified the Tlx ortholog nhr-67 as being necessary for the middle parts of the migratory program, such as negative regulation of the netrin receptor unc-5 to allow a ventral turn. We have profiled the transcriptome of individual LCs by microdissection, amplification, and cDNA deep sequencing. This study identified about 800 LC-enriched genes whose functions we are now analyzing, including a number of conserved proteins of unknown function that we predict will have roles in migration in human cells. We have tested the roles of genes up-regulated in metastatic cancer cells for roles in cell migration in C. elegans as a starting place to define the molecular pathways in which they act.

Our efforts in genomics are experimental and computational. We worked with Caltech’s Millard and Muriel Jacobs Genetics and Genome Laboratory to determine the genomic sequence of several nematode species. The first was a new Caenorhabditis species (angaria) that is an outgroup for the five existing sequenced species of this genus. We used cDNA sequence data to help assemble larger than gene-size pieces of this genome. By comparing the C. angaria genome to other Caenorhabditis species, we identified thousands of short, high conserved sequences that we hypothesis are regulatory. We have sequenced, assembled and annotated the genome of Steinernema carpocapsae, an insect-killing nematode that can jump onto hosts and four other Steinernema species as well. We helped a sequence and analyze the genomes and transcriptomes of
the sheep parasite *Haemonchus contortus* and insect parasite *Heterorhabditis bacteriophora*. To understand parasites we think it is useful to understand free-living nematodes, and thus analyzed *Panagrellus redivivus*, a worm whose development and behavior we have studied for comparisons to *C. elegans*.

We continue to organize, store, and display information about *C. elegans* and to extend these efforts to other nematodes. With our international team of collaborators, we present this information in an Internet-accessible database, WormBase ([www.wormbase.org](http://www.wormbase.org)). Our major contribution is to extract information from the literature, focusing on gene, protein, and cell function; gene expression; gene-gene interactions; and functional genomics data. Annotation of gene function includes use of the Gene Ontology (GO; [www.geneontology.org](http://www.geneontology.org), and we are extending these ontologies as part of the GO Consortium. To facilitate these processes, we have developed Textpresso ([www.textpresso.org](http://www.textpresso.org)), a search engine for biological literature. In collaboration with other model organism databases, we have applied Textpresso to the literature of *C. elegans*, *Drosophila*, *Arabidopsis*, nematodes in general, mouse, and several human diseases, the latest being cancer. We use this system to automate some steps in the extraction of information from full-text papers. We are extending this system to facilitate Gene Ontology curation by the Consortium. Lastly, we are exploring ways of visualizing biological information.

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**Images from left to right:**
Professor Paul Sternberg
Jumping insect – Killing Worms respond to host odors
Sleeping worm on microfluidic pillow

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**Publications**

**2013**


Harris, Todd; Baran, Joachim; Bieri, Tamberlyn; Cabunoc, Abigail; Chan, Juancarlos; Chen, Wen; Davis, Paul; Howe, Kevin; Done, James; Grove, Christian; Kishore, Ranjana; Lee, Raymond; Li, Yuling; Müller, Hans-Michael; Nakamura, Cecilia; Oszersky, Philip; Paulini, Michael; Raciti, Daniela; Schindelman, Gary; Tuli, Mary Ann; Van Auken, Kimberly; Wang, Daniel; Wang, Xiaodong; Williams, Gary; Wong, JD; Yook, Karen; Schedl, Tim; Hodgkin, Jonathan; Berriman, Matt; Kersey, Paul; Spieh, John; Stein, Lincoln; Sternberg, Paul. (2014). **WormBase 2014: New views of curated biology.** Nucleic Acids Res. 2013 Nov 4. [Epub ahead of print] PubMed PMID: 24194605.


2012


Alexei Aravin  
Assistant Professor of Biology

David Baltimore  
President Emeritus, Nobel Laureate  
Robert Andrews Millikan Professor of Biology

Pamela Bjorkman  
Max Delbrück Professor of Biology

Judith Campbell  
Professor of Biology and of Chemistry

David Chan  
Professor of Biology

Raymond Deshaies  
Professor of Biology

William Dunphy  
Grace C. Steele Professor of Biology

Grant Jensen  
Professor of Biology
Mary Kennedy
*Allen and Lenabelle Davis Professor of Biology*

Dianne Newman
*Professor of Biology and Geobiology*

Stephen Mayo
*Bren Professor of Biology and Chemistry; Division of Biology Chair*

Rob Phillips
*Fred and Nancy Morris Professor of Biophysics and Biology*

Alexander Varshavsky
*Howard and Gwen Laurie Smits Prof. of Cell Biology*
SMALL RNAs AND EPIGENETICS

Summary:
Gene silencing via the RNA interference (RNAi) pathway is an evolutionary conserved process that is critical for the control of gene expression in organisms ranging from yeast to humans. Targets of RNAi are recognized through complementary base-pairing interactions with small RNAs that act as guides to RNAi effector complexes. Several distinct classes of endogenous small RNAs regulate gene expression states to impact diverse biological processes. Our lab focuses on understanding the nature and biological functions of small RNA pathways in animals.

We have identified and characterized an evolutionary conserved small RNA pathway that operates in germ cells and that is critical both for germline stem cell maintenance and for gametogenesis. Working in Drosophila and mice, we discovered a new class of small RNAs, Piwi-interacting (pi)RNAs. Piwi/piRNA pathway plays an important role in genome integrity by repressing selfish repetitive elements. A characterization of piRNA sequences in combination with genetic studies revealed that the biogenesis and function of piRNAs differs from that of other classes of small RNAs. While canonical small RNAs, such as microRNAs, affect gene expression post-transcriptionally, our studies suggest that piRNAs most likely serve as guides for de novo DNA methylation in mouse male
germ cells. We are interested in two general questions: biogenesis and function of small non-coding RNAs.

Finding small RNA and DNA species in bacteria
Eukaryotic Argonautes bind small RNAs and use them as guides to find complementary RNA targets and induce gene silencing. Though homologs of eukaryotic Argonautes are present in many bacteria and archaea their small RNA partners and functions were unknown. We found that the Argonaute of Rhodobacter sphaeroides (RsAgo) associates with small RNAs that correspond to the majority of transcripts. RsAgo also binds single-stranded small DNA molecules that are complementary to the small RNAs and enriched in sequences derived from exogenous plasmids as well as genome-encoded foreign nucleic acids such as transposons and phage genes. We showed that expression of RsAgo in the heterologous E. coli system leads to formation of plasmid-derived small RNA and DNA and plasmid degradation. In a R. sphaeroides mutant lacking RsAgo, expression of plasmid-encoded genes is elevated. Our results indicate that RNAi-related processes found in eukaryotes are also conserved in bacteria and target foreign nucleic acids.

Biogenesis of piRNA
Processing of piRNAs differs from that of other known classes of small RNAs. It was shown piRNA are produced independently of Dicer, the nuclease that generates siRNAs and microRNAs from double-stranded substrates; however, the proteins that are responsible for producing piRNAs are only partially understood.

Our investigations of piRNA biogenesis led us to the ping-pong model that proposes amplification of piRNAs in a cycle that depends on the nuclease activity of Piwi proteins themselves. One of the central mysteries of repeat silencing in both mammals and flies is how repeats are distinguished from genes and selectively silenced. We are investigating the nature of the determinants that make a particular sequence a target of the Piwi pathway. We are using biochemical purification of Piwi-piRNA complexes and genetic approaches to identify proteins involved in piRNA biogenesis.

Functions of the Piwi pathway and piRNA-guided de novo DNA methylation
We showed that the piRNA pathway is linked to de novo DNA methylation in the mouse germline. One of the three murine Piwi proteins is specifically found in germ cell nuclei during the critical window when de novo methylation patterns are established. We also showed that Piwi proteins at that developmental timepoint are associated with piRNAs that target several classes of transposable elements. The same transposons are de-repressed and their genomic sequences lose methylation in Piwi-deficient mice. The discovery that piRNAs may guide DNA methylation in germ cells is an important finding for several reasons. First, it provides a new paradigm for how small RNAs can affect gene expression. Second, it explains how a subset-of-sequences are tagged for de novo methylation. How methylation sites are defined remains a central mystery of epigenetics. An important goal of my lab is to define the pathway by which piRNAs guide de novo DNA methylation. We also study whether the piRNA pathway can be re-programmed to new targets and can be used to manipulate DNA methylation patterns in somatic cells.
It is clear that germ cells, somatic stem cells and probably cancer stem cells possess unique pathways for small RNA-mediated silencing. Our long-term goal is to understand how diverse RNA silencing mechanisms are integrated with other pathways in context of development and pathology. Eventually, the knowledge gained from the investigation of silencing mechanisms in stem and germ cells will help us to understand the unique biology of these cells and will impact our general understanding of gene regulation and how it is altered in disease.

**Epigenetic regulation of transposable elements in cancer**

Genomes of mammalian species, including humans, are swamped by genomic parasites, transposable elements (TE). About one half of the human genome is occupied by hundreds of thousands of TE copies. It is likely that transposable elements deeply intervene with cellular regulatory networks. It was speculated that on evolutionary timescale TEs are beneficiary for their hosts providing genomic plasticity necessary for natural selection. Analogously, it is possible that TEs help to increase genome and epigenome plasticity of cancer cells and bring them competitive advantage and adaptability. We attempt to comprehensively investigate the role that TEs play in cancer. We study changes in chromatin structure, expression and mobilization of TEs associated with cancer development using several complementary approaches.

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**Publications**

**2013**


**2012**


President Emeritus,
Robert Andrews Millikan Professor of Biology,
Nobel Laureate:
David Baltimore

Professorial Awards and Honors:
2012 Honorary Centennial Council Founding Member, Smithsonian Institution, National Museum of Natural History
2012 University of Malaysia Nobel Fellow
2012 University of Malaysia High Impact Research Advisory Council
2012 Honorary Doctorate of the University of Buenos Aires
2013 Elected Fellow of the Academy of American Association for Cancer Research, Inaugural Class

Postdoctoral Scholars:
Alex Balazs, Michael Bethune, Shuai Jiang, Alok Joglekar, Mati Mann,
Devdoot Majumdar, Alex So,

Visiting Postdoctoral Scholar:
Raj Kulkarni

Graduate Students:
Rachel Galimidi, Vanessa Jonsson, Geoffrey Lovely, Arnav Mehta, Jocelyn Kim

Undergraduates:
David Cheng, Xiaomi Du, Meghana Pagadala, Conway Xu

Lead Research Technician:
Yvette Garcia-Flores

Research Technicians:
Christin Hong, Stella Ouyang, Reeshelle Sookram

Administrative Staff:
Julie Kelly, Joanne Laurence

Financial Support:
amfAR: The Foundation for AIDS Research
Broad Foundation
Ellison Medical Foundation
Melanoma Research Alliance
National Institutes of Health
NIH Program Project
Sackler Foundation
The Ragon Institute
Basic Immunology and Engineering of the Immune System

Summary:

Our laboratory combines two different styles of work: basic studies in immunology and translational studies that draw on immunology.

The basic science revolves around various aspects of control of immune function. Over 25 years ago we discovered the inducible transcription factor NF-kB, later shown to be a master regulator of inflammatory and immune processes, and we continue to examine its properties. Most recently we have concentrated on two aspects of NF-kB, how it can produce a response that varies over more than 24 hours after its induction and how it is tuned down after induction. The timing issue has turned out to involve control by intrinsic properties of the different genes induced by NF-kB, mainly the half-life of the mRNAs and control over the timing of splicing. The tuning down involves many factors, one being feedback regulation by the NF-kB-induced microRNA miR-146a. We have shown that miR-146a downregulates TRAF-6 and IRAK-1 in macrophages and T cells so that a knockout of this microRNA leads to hyperactivation of the cells by LPS and a slower resolution of T cell responses to antigen. The consequence is hyperproliferation of the two cell types and, after a year, frank myeloid cancer. We are deconvoluting the roles of the two cell types in cancer induction. We have found that miR-146a is needed to maintain the health and longevity of hematopoietic stem cells and are trying to understand just how regulation of NF-kB is involved in this process.

We have also examined other microRNAs that are involved in immune processes like miR-155 and miR-125b. MiR-125b overexpression induces aggressive cancer in less than six months involving both myeloid and lymphoid disease. It appears to act through lin28.

In a separate program, we are investigating how lentiviruses activate dendritic cells. Surprisingly, this doesn’t involve any of the TLR-driven pathways but we are not yet sure what is the operative process.

The translational studies derive from the development of viral vectors that can mediate changes in immune function, a program we call Engineering Immunity. In one aspect, we are focusing on lentiviral vectors that encode T cell receptor genes able to program patient T cells to react with melanoma cells. Here we collaborate with colleagues at UCLA and have an active clinical program under way. In a second program, which we call Vectored ImmunoProphylaxis or VIP, we are using Adeno-Associated Virus-derived vectors to program muscle cells to make broadly reactive and potent antibodies against HIV and other pathogens. This program, presently carried out using mice that harbor a human immune system, is in transition to clinical evaluation in humans in collaboration with the Vaccine Research Center at NIH.

Images from left to right:
Professor David Baltimore
Immunofluorescence microscopy of muscle tissue following administration of AAV vector expressing ZsGreen
Structural representation of Adeno-Associated Virus 8 used to deliver anti-HIV antibody genes to muscle tissues for Vectored ImmunoProphylaxis.
**PUBLICATIONS**

**2013**


**2012**


Max Delbrück Professor of Biology: Pamela J. Bjorkman  

Member of the Professional Staff: Anthony P. West, Jr.  

Research Fellows and Associates: Jennifer Keeffe, Collin Kieffer, Blaise Ndjamen, Louise Scharf, Stuart Sievers, Beth Stadtmueller  

Visiting Associate: Yongning He  

Graduate Students: Alysia Ahmed, Rachel Galimidi, Gwen Owens, Yunji Wu  

Visiting Student: Sandra Gandre  

Undergraduate Students: Courtney Chen, Siduo (Stone) Jiang, Misha Raffiee  

High School Students: William Lange, Danielle New, Brian Williams  

Research and Laboratory Staff: Tim Feliciano, Han Gao, Priyanthi Gnanapragasam, Alexander Heide, Beth Huey-Tubman, Terri Lee, Mark Ladinsky, Lynda Llamas, Marta Murphy, Alejandra Olvera, Nick Pachon, Maria Suzuki Politzer  

Financial Support:  
American Cancer Society (fellowship to Louise Scharf)  
California HIV/AIDS Research Program (fellowship to Stuart Sievers)  
Cancer Research Institute (fellowships to Blaise Ndjamen and Beth Stadtmueller)  
Ragon Institute of MGH, MIT and Harvard (fellowship to Collin Kieffer)  
Bill and Melinda Gates Foundation  
Technology Transfer Grubstake Award  
Howard Hughes Medical Institute  
NIH HIVRAD P01, P50 and R01  
NIH Director's Pioneer Award  
CASIS (Center for the Advancement of Science in Space)  

Structural Biology of Antibody Receptors and Immune Recognition of Viruses  

Summary:  
We are interested in structural mechanisms of recognition in the immune system, specifically in the structure, function, and therapeutic uses of antibodies and their receptors, and in homologs and
viral mimics of class I major histocompatibility complex (MHC) proteins. In addition to using X-ray crystallography and biophysical techniques to analyze protein-protein interactions in solution, we use electron tomography and confocal microscopy to image interactions in cells, examining, for example, HIV infection in gut-associated lymphoid tissue and transport pathways mediated by the class I MHC-related neonatal Fc receptor (FcRn), a receptor for immunoglobulin G (IgG). We also are applying our antibody structure expertise to “engineer immunity” against HIV.

Our efforts in the area of HIV therapeutics focus upon improving the binding and neutralization properties of antibodies with the ultimate goal to design and generate antibodies or antibody-like proteins with desired properties; for example, neutralizing antibodies or designed antibodies engineered to bind more tightly to a pathogen and/or to recruit immune effector cells. The antibodies could be produced in vivo by gene therapy techniques, thus allowing long-term production. We have focused our studies on anti-HIV antibodies, in part because HIV is very successful at evading the human immune system and conventional vaccine candidates have failed to elicit an effective response. Developing potent reagents that could be delivered through gene therapy or passive immunization would therefore greatly impact the field of HIV research and treatment. Although HIV has evolved to evade most or all antibodies (hence the difficulty of finding an immunogen capable of eliciting a strong neutralizing antibody response in vaccine development efforts), an attractive feature of a gene therapy approach is that we are not limited to the traditional architecture of an antibody. Thus we can produce and express antibody-like proteins of different sizes (to facilitate access to hidden epitopes) and valencies (i.e., with different numbers of combining sites) and/or link antibodies to HIV-binding proteins such as the host receptor CD4.

In initial efforts, we developed CD4-antibody fusion proteins that cross-react to neutralize a broad range of HIV strains, and characterized a dimeric form of an anti-carbohydrate antibody, 2G12, that displays a 50- to 80-fold increased potency in the neutralization of clade B HIV strains. We also proposed a previously unappreciated general mechanism that HIV uses to evade antibodies. Our hypothesis states that an anti-HIV antibody fails to potently neutralize because it can only bind using one of its two antigen-binding sites. Simultaneous engagement of both antigen-binding sites leads to a synergistic effect called avidity, in which the antibody-antigen interaction can become nearly irreversible. With most viruses, antibodies bind with avidity because the antigenic spikes are present on the viral surfaces at high densities, a feature that is absent on HIV. The small number of antigenic spikes on the surface of HIV are mostly separated by distances that are too large to allow simultaneous engagement of both antibody-combining sites. In addition, the structure of the HIV spike trimer prohibits simultaneous binding of both combining sites to a single spike. We are currently generating libraries containing two HIV-binding proteins joined using either protein or DNA linkers and are developing high-throughput screening and selection strategies to identify bivalent reagents that enable simultaneous binding by both antigen-binding sites, either within a spike or between spikes. A potent reagent that exhibits avidity would reduce the concentration of antibody required for sterilizing immunization to realistic levels.

In addition to designing new architectures of antibodies, we are using structural biology to investigate the features that make anti-HIV antibodies broad and potent. We solved a co-crystal
structure of the CD4-induced antibody 21c in complex with CD4 and a clade C gp120. This was the first crystal structure of containing a clade C gp120, and also revealed the first visualization of an auto-reactive antibody complexed with both “non-self” (HIV gp120) and “self” (CD4) antigens, supporting hypotheses that auto-reactivity is a feature of many anti-HIV antibodies. We also determined the structure of another antibody-antigen complex (NIH45-46-gp120). We then used structure-based design to create NIH45-46G54W, a CD4-binding site (CD4bs) antibody with superior potency and/or breadth compared with other broadly neutralizing antibodies against HIV. We produced effective variants of NIH45-46G54W designed using analyses of the NIH45-46/gp120 complex structure and sequences of antibody-resistant HIV clones. One mutant, 45-46m2, neutralizes 96% of HIV strains in a cross-clade panel and viruses isolated from an HIV-infected individual that are resistant to all other known bnAbs, making it the single most broad and potent anti-HIV antibody to date. The information we gain using a combination of structural biology and bioinformatics allows us to both design more broad and potent reagents and gain a better fundamental understanding of the neutralization mechanisms of anti-HIV antibodies.

In addition to improving the therapeutic properties of IgG antibodies through enhancing their binding to antigens, IgGs can be improved by increasing their interactions with Fc receptors that mediate effector functions or regulate their serum half-life. We have a long-standing interest in structural studies of Fc receptors; for example, on-going efforts include structural studies of pIgR, a receptor for polymeric immunoglobulins, and Fc receptors involved in phagocytosis of IgG-antigen complexes. Previous crystallographic and biochemical studies involved elucidating the mechanism by which FcRn, an MHC-related Fc receptor, interacts with IgG. FcRn serves as the protection receptor for IgG in the blood, rescuing bound antibodies from a default degradative pathway, and also transfers maternal IgG to the bloodstream of fetal and newborn mammals, thereby passively immunizing the neonate against pathogens likely to be encountered prior to development of its own fully functional immune system. Transfer of IgG across epithelial barriers and rescue of IgG from degradation involves trafficking of FcRn-IgG complexes in acidic intracellular vesicles. A general question exemplified by FcRn trafficking is how cargo-containing intracellular vesicles are transported to their correct ultimate locations—for example, how does the cell know that FcRn-IgG complexes should be transported across a cell for eventual release of IgG into the blood, whereas other receptor-ligand pairs should be transferred to degradative compartments?

To study the process by which FcRn-IgG complexes are correctly trafficked across cells, we use electron tomography, a form of electron microscopy, to derive three-dimensional maps of transport vesicles in neonatal rat intestinal epithelial cells at resolutions of 4–6 nm. To facilitate these studies, we developed gold-labeling and enhancement methods to locate individual IgG fragments bound to FcRn inside intracellular vesicles. Our three-dimensional images of IgG transport revealed tangled webs of interlocking IgG-containing transport vesicles, some of which were associated with microtubule tracks to allow movement via motor proteins. Other IgG-containing vesicles included multivesicular bodies, normally associated with degradative functions but apparently functioning in IgG transport in the specialized proximal small intestinal cells of a neonate.
To complement high-resolution, but static, studies, we do fluorescence imaging in live cells, which allows tracking of labeled vesicles and quantification of the velocities and directions of FcRn-positive vesicles. We have used fluorescent imaging to characterize the intracellular trafficking pathways of two other Fc receptors: the polymeric immunoglobulin receptor (pIgR), which transports polymeric IgA antibodies into secretions, and gE-gI, a viral Fc receptor for IgG. We discovered that gE-gI exhibits a pH-dependent affinity transition for binding IgG that is opposite that of FcRn: FcRn binds tightly to IgG at acidic, but not basic, pH, so as to bind IgG inside acidic vesicles during transport and to release IgG upon encountering the slightly basic pH of blood; by contrast, gE-gI binds IgG at the pH of blood but not at the pH of intracellular vesicles. We have shown that IgG-antigen complexes bound to gE-gI and internalized by receptor-mediated endocytosis are destined for degradation after dissociating from gE-gI in acidic intracellular vesicles, which could form part of a viral mechanism to escape from antibody-mediated host immune responses.

**Images from left to right:**
- Professor Pamela Bjorkman
- 3-D reconstruction derived from electron tomography of the lateral intercellular space between two intestinal epithelial cells. Gold spheres represent antibodies transported by the neonatal Fc receptor.
- Crystal structure of a broadly neutralizing antibody bound to an HIV envelope spike protein.
- Confocal fluorescent image of polarized cells expressing Fc receptors that transport IgG and dimeric IgA.

**Publications**

**2013**


Ndjamen, B., Farley, A., Lee, T., Fraser, S.E., Bjorkman P.J. (2013) **The Herpes virus Fc receptor gE-gI mediates antibody bipolar bridging to clear viral antigens from the cell surface.** Ms. submitted.


**2012**


West AP Jr, Diskin R, Nussenzweig MC, Bjorkman PJ. (2012) **Structural basis for germ-line gene usage of a potent class of antibodies targeting the CD4-binding site of HIV-1 gp120.** *Proc Natl Acad Sci USA* **109:**E2083-90. Epub 2012 Jun 27. [PMCID: PMC3409792](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3409792)

Professor of Biology and Chemistry: 
Judith L. Campbell, Ph.D.

Postdoctoral Scholars: 
Kenny Karanja, Suhash Pokharel

Members of the Professional Staff: 
Elizabeth Bertani, Martin E. Budd, Piotr Polaczek

Graduate Student: 
Barbara Kraatz-Fortini

Research and Laboratory Staff: Stephanie Cox, Santiago Laparra

Financial Support: 
CDMRP Breast Cancer 
Ellison Foundation 
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MECHANISMS AND REGULATION OF DNA REPLICATION AND REPAIR

Summary: 
A hallmark of cancer cells, in addition to uncontrolled proliferation, is genomic instability, which appears in the form of chromosome loss or gain, gross chromosomal rearrangements, deletions, or amplifications. The mechanisms that suppress such instability are of the utmost interest in understanding the pathogenesis and treatment of cancer. Our lab studies the components of the DNA replication apparatus that promote genomic stability. We use yeast genetics and biochemistry, *Xenopus* egg extracts, and human cells.

At least seven human diseases characterized by cancer predisposition and/or premature aging are correlated with defects in genes encoding DNA helicases. The yeast genome contains 134 open reading frames with helicase motifs, only a few of which have been characterized. Martin Budd in our laboratory identified the first eukaryotic helicase essential for DNA replication, Dna2. He showed by interaction studies that it was a component of the machine that is required for accurate processing of Okazaki fragments during lagging-strand DNA replication. Enzymatic studies to elucidate the sequential action of the DNA polymerases, helicases, and nucleases required for this processing constitute an ongoing mechanistic biochemistry project in the laboratory. Okazaki fragment processing represents the heart of the replication machine, and our studies have revealed that, as in prokaryotes, the replisome is not a machine made up of dedicated parts like its namesake the ribosome. Instead, the replisome is a dynamic structure with proteins constantly exchanging...
protein and DNA partners to coordinate the rapid and high fidelity synthesis of the anti-parallel leading and lagging strands of the DNA template. Our current work focuses on the regulation, by reversible acetylation and phosphorylation, of the protein/protein and protein/DNA hand-offs that we have defined over the last decade.

One model of cellular aging suggests that accumulation of DNA damage leads to replicative senescence. Most endogenous damage occurs during S phase and leads to replication fork stress. At least three human diseases of premature aging or cancer predisposition - Werner, Bloom, and Rothmund-Thompson - are caused by defects in helicases that interact with Dna2. Martin Budd and Laura Hoopes found that dna2 mutants have a significantly reduced life span. Microarray analysis by Isabelle Lesur showed that the dna2 mutants age by the same pathway as wildtype cells; they just age faster. Interestingly, the human Bloom and Werner genes complement the replication defect of dna2 mutants, suggesting that Dna2 works in the same pathway with these genes. We have now shown that the Dna2 helicase works with the yeast BLM ortholog, Sgs1, in the major pathway of double-strand break repair in yeast and are studying the same process in both yeast and human cells. Together Dna2 and Sgs1 are involved in the initial resection of the 5' terminated strand of the DSB to produce a single-stranded 3' end. This is a crucial step because it is where the cell decides whether to pursue the relatively error-free homologous recombination pathway or the more error-prone non-homologous end-joining repair. The 3' end generated by Dna2/Sgs1 is involved in strand invasion of the homolog and thus, the initiation of strand exchange. Perhaps even more important the single-stranded DNA is a key intermediate in the activation of the cell cycle checkpoint that protects the cell from genome instability in the presence of a double-strand break arising from replication fork failure. In collaboration with Dunphy lab, we readily showed that Dna2 also participates in resection in Xenopus egg extracts. We have now reconstituted the recombination machine both from purified yeast proteins and from purified human counterparts, including Dna2 and BLM helicase. BLM helicase is defective in one of the most cancer-prone diseases yet described, Bloom syndrome. Cells from these patients show a high frequency of sister chromatid exchanges and quadriradials. The biochemical approach provides a mechanistic basis for this dynamic recombination processing machine. Especially for the human proteins, this provides insights previously unavailable due to the difficulty of performing recombination experiments in human cells.

Telomeres, i.e., the ends of linear chromosomes, are a special case of the type of ends found at DSBs. Not surprisingly, Dna2 also plays a significant role at telomeres. In fact, the bulk of Dna2 is localized to telomeres and in yeast, this localization is dynamic. During G1 and G2 phases of the cell cycle, Dna2 is at telomeres. During S phase Dna2 leaves telomeres and is present on the replicating chromatin. Dna2 is also mobilized from telomeres in response to the induction of intrachromosomal double-strand breaks with agents such as bleomycin. At the end of S phase, telomeres become single-stranded in all organisms and this occurs through 5' resection to produce single-stranded 3' overhangs. We have now shown that Dna2 is one of the major enzymes involved in resection at telomeres, as well as internal DSBs. It will be important to investigate if the same holds true in human cells with Dna2 knocked down by shRNA.
**Supplementary Figure 1: Model for DNA end resection after replication stress.** Camptothecin or cisplatin exposure blocks replication due to formation of topoisomerase-DNA adducts (red star) or interstand cross links (red link between strands), respectively. Approaching replication forks are unable to proceed past the lesions and may subsequently collapse to generate DSBs. DSBs are first processed by MRN (brown circles)/CtIP (yellow hexagon) to generate short 3’ ssDNA. BLM (blue circles), DNA2 (red pacman) or EXO1 (not shown) are necessary for long range resection to produce ssDNA that is capable of binding RPA (purple oblongs). Long range resection is also needed to effect an ATM to ATR switch. RPA bound to DNA is hyperphosphorylated thus promoting ATR phosphorylation of Chk1, induction of cell cycle checkpoint and efficient DNA damage repair. Long range resection precludes the engagement of the NHEJ pathway by preventing the hyperphosphorylation of DNA-PKcs.

**PUBLICATIONS**

**2012**

PHYSIOLOGICAL FUNCTIONS AND MECHANISMS OF MITOCHONDRIAL DYNAMICS

Summary:
The primary focus of our lab is to understand the role of mitochondrial dynamics in normal cellular function and human disease. Mitochondria are remarkably dynamic organelles that undergo continual cycles of fusion and fission. The equilibrium of these two opposing processes determines not only the overall morphology of mitochondria in cells, but also has important consequences for mitochondrial function.

Our research falls into several broad areas:

(1) What are the cellular and physiological functions of mitochondrial fusion and fission?
(2) What is the molecular mechanism of mitochondrial membrane fusion and fission?
(3) What role do mitochondrial dynamics play in human diseases?
(4) How are mitochondrial genomes packaged and maintained?
(5) What regulatory mechanisms maintain the quality of mitochondria?
To address these issues, we use a wide range of approaches, including genetics, biochemistry, cell biology, and structural biology.

**PUBLICATIONS**

**2013**

Loson, O.C., Song, Z., Chen, H., and Chan, D.C. (2013). *Fis1, Mff, MiD49 and MiD51 mediate Drp1 recruitment in mitochondrial fission*. Mol Biol Cell, 24, 659-67. PMID: 23283981


**2012**


**Summary:**
Ubiquitin serves as a molecular tag that marks proteins for degradation. Ubiquitin is attached to proteins by a cascade of enzymes comprising ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s). We investigate protein degradation via the ubiquitin-proteasome system (UPS) at multiple levels, including the regulatory circuitry that acts upstream of ubiquitin conjugation pathways that control cell division, the ubiquitin ligases that mediate attachment of ubiquitin to proteins, and the mechanism by which ubiquitin-modified proteins are brought to the proteasome and degraded. We seek to discover new components of the UPS and uncover basic knowledge about how components of the UPS work, how their activities are regulated, what their substrates
are, and how they contribute to cellular regulation. We also seek to develop drugs that target UPS enzymes for use as research tools. Given that defects in the UPS lead to cancer, neurodegenerative diseases, and autoimmune disease, we anticipate that a better understanding of the UPS will provide insight into diseases that afflict millions of people. We hope that this insight leads to novel therapies.

**SCF and the Cullin-RING Ubiquitin Ligase Family:** Our work to understand the biochemical mechanism by which yeast cells enter into the S phase of the cell division program where they duplicate their chromosomes led us to discover the SCF<sup>Cdc4</sup> ubiquitin ligase, one of whose functions is to attach ubiquitin to a protein known as Sic1. Sic1 restrains chromosome duplication by binding to and inhibiting the S-phase–promoting cyclin-dependent kinase. Immediately prior to S phase, SCF<sup>Cdc4</sup> binds Sic1 and attaches ubiquitin to it. An enzyme complex known as the 26S proteasome then degrades ubiquitin-modified Sic1. In this manner, active cyclin-dependent kinase is released from Sic1, so that it can promote chromosome duplication.

Although SCF<sup>Cdc4</sup> was discovered as a regulator of chromosome duplication in yeast, we now appreciate that it is the archetype of a large family of ubiquitin ligase enzymes that share a similar modular architecture. This family is defined by a module composed of a cullin subunit and a RING subunit, and hence these enzymes are referred to as cullin-RING ligases (CRLs). The cullin-RING module recruits both an E2 enzyme via the RING subunit and a substrate-binding module. This brings substrate and E2 enzyme into proximity to enable substrate ubiquitination.

There are nine cullin-related proteins in humans. The Cul1 subunit that underlies SCF ubiquitin ligases binds the linker protein Skp1. Skp1, in turn, binds the F-box domain of a substrate receptor protein. There are 69 F-box proteins in human cells, and thus potentially as many as 69 distinct SCF ubiquitin ligase complexes, each with a different substrate specificity. Each cullin uses a different linker protein to recruit a different family of substrate receptors. All told, there may be up to 240 CRLs in human cells. These enzymes control processes as diverse as circadian rhythms, the innate immune response, and glucose sensing. Given their key role in numerous regulatory pathways, we seek to understand how CRLs work, how they are regulated, what their substrates are, and how they are deployed in regulatory circuits to control processes such as gene transcription and cell division.

**How CRLs Work:** Despite their pivotal role in numerous regulatory pathways, we are only beginning to understand how CRLs work. Degradation of CRL substrates such as Sic1 is sustained by assembling upon the substrate a chain of ubiquitins linked together via their lysine-48 residues. We seek to understand how SCF and other CRLs work by using chemical biology approaches to develop novel substrates and tools, by devising new assays based on stop-flow and quench-flow techniques to measure real-time dynamics of enzyme-substrate complex formation and ubiquitin ligation with millisecond time resolution, and by developing structural and mathematical models to help us understand how the assembly of a ubiquitin chain proceeds.
Regulation of CRLs by the Ubiquitin-like Protein Nedd8: Because of the broad role that human SCF and other CRL enzymes play, we sought to identify proteins that control SCF activity. This led us to discover that COP9 signalosome (CSN) is a key regulator of all CRLs. CSN detaches an ubiquitin-like protein, Nedd8, from the cullin subunit of CRLs. This reaction is catalyzed by the Csn5 subunit, which together with the related Rpn11 subunit of the proteasome defined a new family of JAMM (Jab1/MPN-domain metalloenzyme) metalloproteases. CSN was previously implicated in multiple developmental processes, including photomorphogenesis in plants, neuronal differentiation, and axon guidance. Our observations suggest that the diverse activities of CSN may arise from its ability to control CRLs and other Nedd8-modified proteins.

Unexpectedly, both attachment of Nedd8 to cullins and its subsequent removal by CSN stimulate CRL activity, suggesting that active CRLs are sustained by a continuous cycle of Nedd8 attachment and removal. How this cycle operates, how it controls CRL activity, and how it is regulated remain unsolved mysteries. Interestingly, assembly of Cul1 into an intact SCF complex and the presence of substrate can both reduce the rate of deneddylation, suggesting that SCF complexes that are engaged in ubiquitination are refractory to deneddylation by CSN. We are continuing to address the mechanism and regulation of CSN by a combination of enzymological approaches, chemical biology, and quantitative proteomics.

The cycle of Nedd8 attachment to and removal from CRLs is interdigitated with a second regulatory cycle, in which a CRL from which Nedd8 has been removed binds to Cand1, leading to displacement of the substrate-binding module from the cullin scaffold. The Cand1-cullin complex is subsequently displaced by another substrate-binding module. In this manner, CRLs are thought to undergo continuous cycles of disassembly and reassembly. This would allow the cullin scaffold to remain in rapid equilibrium with the available pool of substrate-binding modules. We seek to understand this cycle of CRL assembly and disassembly and the role of Cand1 in this process through a combination of biochemical reconstitution, enzymology, and quantitative mass spectrometry-based proteomics.

How CRL Substrates Are Delivered to the Proteasome and Degraded: For many CRL substrates, ubiquitination is followed by delivery to the proteasome and subsequent degradation. Although the proteasome can degrade ubiquitinated substrates without the participation of other factors, degradation of some proteasome substrates requires the AAA ATPase p97. P97 is an ubiquitin-selective chaperone that promotes degradation of ubiquitinated substrates by mechanisms that remain poorly understood. Adding to the mystery is the fact that there are more than a dozen different p97 complexes in human cells. We believe that these complexes act at the proteasome to help unfold substrates as a prelude to their degradation. However, we don't really know how p97 is doing this, nor do we understand why so many different Ubxd-p97 complexes exist, and how their activities relate to ubiquitin receptor proteins that guide substrates to the proteasome. What is known is that p97 is important for the proper function of the ubiquitin system, and that mutations that cripple p97 activity underlie the syndrome known as Inclusion
Body Myopathy, Paget's disease of the bone, and Fronto-temporal Dementia (IBMPFD) and some inherited cases of Amyotrophic Lateral Sclerosis (ALS; Lou Gehrig's disease).

Several years ago, we discovered through proteomic studies that p97 assembles with a broad range of CRLs through the agency of the adaptor protein Ubxd7, suggesting that p97 plays an intimate role in the numerous regulatory processes governed by this family of ubiquitin ligases. Recently, we have found that Ubxd7 contains a domain that binds to the Nedd8 modification that is present on active CRLs. We now seek to understand how recruitment of Ubxd7 influences the ubiquitination and degradation of CRL substrates. We also seek to identify novel functions and substrates of p97 by using a combination of biochemical, molecular genetic, and proteomic approaches in yeast. Finally, we seek to understand how recruitment of Ubxd7 influences the ubiquitination and degradation of CRL substrates. We also seek to identify novel functions and substrates of p97 by using a combination of biochemical, molecular genetic, and proteomic approaches in yeast. We will continue to use chemical biology, proteomic, genetic, and biochemical reconstitution approaches to investigate the role of yeast and human p97 in degradation of CRL substrates.

**Proteomics:** The human ubiquitin system comprises hundreds of different enzymes, including ~570 ubiquitin ligases, ~40 E2s, ~95 deubiquitinating enzymes, and about a dozen each of ubiquitin chain receptors and Cdc48/p97 complexes. Collectively, these factors target thousands of human proteins for degradation. One of the most intractable problems in the ubiquitin field is the identification of the proteins that are targeted for degradation by any one component of the UPS. To identify the proteins whose ubiquitination is altered in cells that are lacking individual components of the UPS, we use powerful mass spectrometry-based approaches. We are also using quantitative mass spectrometry methods to catalog the network of CRLs and how it changes in response to chemical and genetic perturbations.

**Regulation of Proteasome Production:** Eukaryotic cells treated with proteasome inhibitors respond by up-regulating the synthesis of proteasome subunits. This conserved response (which we refer to as 'bounce-back') is of clinical significance because it may blunt the impact of proteasome inhibitor therapy. We seek to understand how the bounce-back response works in human cells and whether it can be modulated. We have discovered that the transcription factor Nrf1 mediates bounce-back. Nrf1 accumulates and is released from its normal location in the ER membrane upon inhibition of the proteasome. The processed form of Nrf1 that accumulates localizes to the nucleus, where it activates expression of proteasome genes. We are now investigating the mechanisms that underlie the processing of Nrf1. We are also seeking ways to block the activity of Nrf1. An Nrf1 inhibitor, used in combination, could potentially enhance the efficacy of proteasome inhibitors in cancer chemotherapy.

**Role of the UPS in ES Cell Differentiation:** Many key transcription factors (TFs) that control cell fate are unstable. Thus, UPS factors that control the stability of TFs are likely to play a broad role in regulating differentiation. To identify UPS factors with this property, we did an siRNA screen for genes that, when silenced, resulted in enhanced formation of cardiomyocytes from ES cells. This screen uncovered multiple genes, including two substrate-specificity subunits of CRL.
enzymes. Surprisingly, in-depth study of one of these proteins revealed that it does not assemble to form a CRL, but instead forms a novel complex with protein phosphatase 2A (PP-2A). Proteomic studies implicate this protein as being a negative regulator of PP-2A. We now seek to understand how this protein regulates PP-2A and how this regulation governs the differentiation of cardiomyocytes during normal development.

**Regulation of the Exit from Mitosis:** As with the entry into S phase, the exit from mitosis/cell division is triggered by ubiquitin-dependent degradation. The mitosis phase of the cell cycle program requires an enzyme known as mitotic cyclin–dependent kinase. As long as mitotic cyclin–dependent kinase is on, the cell is in mitosis. At the end of mitosis, the mitotic cyclin is degraded, which shuts off the associated kinase activity, allowing the cell to exit mitosis. The protein phosphatase Cdc14 is required to switch on mitotic cyclin destruction. For most of the cell cycle program, Cdc14 is sequestered in an inactive state within the cell nucleolus by the nucleolar protein Net1. At the end of mitosis, Cdc14 is released from Net1 and spreads throughout the nucleus and cytoplasm. The freed Cdc14 switches on anaphase-promoting complex/cyclosome (APC) ubiquitin ligase by dephosphorylating its regulator Hct1/Cdh1. Active APC attaches ubiquitin to mitotic cyclin, triggering its degradation. We are investigating the signals that govern Cdc14's release from the nucleolus and dispersal throughout the cell. Most recently, we showed that a nuclear localization sequence in Cdc14 is directly phosphorylated in late mitosis, which promotes its redistribution to the cytoplasm, where it can dephosphorylate and activate Hct1/Cdh1.
PUBLICATIONS

2013


2012

REGULATION OF THE CELL CYCLE AND MAINTENANCE OF GENOMIC INTEGRITY

Summary:
Our laboratory has been generally interested in how cells proceed through the cell cycle in an orderly manner. In order to undergo division, cells must replicate their DNA during S-phase and then distribute the duplicated copies of their genomes equally to daughter cells at M-phase or mitosis. In earlier years, we focused mainly on the enzymatic network that induces the entry of cells into mitosis. A master regulatory kinase called MPF triggers mitotic entry by phosphorylating a myriad of cellular proteins. These phosphorylations lead to the hallmark events of mitosis such as chromosome condensation, nuclear envelope disassembly, and assembly of the mitotic spindle. MPF, which stands for maturation- or mitosis-promoting factor, is a heterotrimer containing a cyclin, a cyclin-dependent kinase (Cdk), and a small ancillary protein Cks protein. The kinase subunit of MPF is Cdk1, the founding member of this family--it was historically known as Cdc2. MPF also typically contains one of the B-type cyclins.

In order for MPF to induce mitosis, it is essential that prior events in the cell cycle have occurred normally. Notably, the cell must have copied all of its genomic DNA accurately during S-phase. In addition, the DNA must also be free of damage in order for the cell to begin division. If a cell has not replicated its DNA accurately or has suffered damage in the genome, various checkpoint
mechanisms impose a blockade to mitotic entry. This delay allows time for the cell to repair DNA lesions. These checkpoint responses have additional physiological consequences. For example, these pathways can influence the transcriptional program of the cell, help to stabilize aberrantly stalled replication forks, and participate in the decision to engage in apoptosis in the event of very severe damage.

Checkpoint pathways consist of sensor proteins that detect problems with the DNA and effector proteins that, for example, regulate the function of cell cycle control proteins. Various mediator proteins manage interactions between sensor and effector proteins in order to control the specificity and efficiency of checkpoint pathways. In cells with incompletely replicated DNA, a master regulatory kinase known as ATR functions near the apex of the checkpoint pathway. The action of ATR ultimately leads to the activation of a downstream effector kinase known as Chk1. A distinct kinase called ATM becomes activated in cells with various forms of damaged DNA, such as DNA with double-stranded breaks (DSBs). Both ATR and ATM are members of the phosphoinositide kinase-related family of protein kinases (PIKKs).

Much of our work now involves a study of the molecular pathways that lead to the activation of ATR. We are also interested in the targets of this kinase and the roles of these targets in checkpoint responses. In recent years, we have found that the activation of ATR occurs through interaction with a specific activator protein called TopBP1. We have also identified a novel mediator protein called Claspin that enables activated ATR to recognize and phosphorylate Chk1. We are now pursuing a thorough characterization of this pathway in order to elucidate new players and regulatory principles. These efforts have led to the identification of a novel replication protein called Treslin that associates physically with TopBP1. Overall, these studies should eventually help us understand how cells maintain the integrity of their genomes. This issue is very relevant to human health because an overarching problem with cancer cells is that such cells have suffered a catastrophic deterioration in the mechanisms that maintain genomic stability.

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**Publications**

**2013**


**2012**

HIGH RESOLUTION CYRO-EM IMAGING OF CELLS AND VIRUSES

Summary:
If we could simply look inside a cell and see its molecular components in all their complexes and conformations, cell biology would be all but finished. While this is of course still just a dream, we are developing electron-cryomicroscopy-based technologies to do this for at least the largest structures, hoping to show both how individual proteins work together as large "machines" and how those machines are organized into "assembly lines" within living cells.

The principle technique we're developing and using is electron cryotomography (ECT). Briefly, purified proteins, viruses, or even cell cultures are spread into thin films across EM grids and plunge-frozen in liquid ethane. Quick-freezing causes the water to form vitreous ice around the proteins and other macromolecules, preserving their native structure but solidifying the sample so it can withstand the high vacuum within an electron microscope. Projection images are then recorded through the sample as the sample is tilted incrementally along one or two axes. The
microscope we use is one of only a few like it in the world: a 300 kV, helium-cooled, energy-filtered, dual-axis tilting, FEG cryo-TEM with a lens-coupled 4k x 4k CCD. Three-dimensional reconstructions, or "tomograms," are then calculated from the images. In this way we can produce 3-D structures of heterogeneous proteins, viruses, and even whole bacterial cells in near-native states to "molecular" (~4-7 nm) resolution.

The first cells we've begun imaging are small bacteria. Now that over a thousand bacterial genomes have been sequenced, a variety of "omic" technologies are being used to document which genes are transcribed and when, which macromolecules are synthesized and how many of each type are present in the cell, and how they interact in pathways to mediate metabolism and regulate gene expression. Despite this encouraging progress, our persistent ignorance about many of the fundamental physical and mechanical processes that occur in a bacterial life cycle is sobering. We still don't know, for instance, how bacteria generate and maintain their characteristic shapes, establish polarity, organize their genomes, segregate their chromosomes, divide, and in some cases move. Thus in some sense the "omics" technologies are giving us lists of parts and reactions, but bacterial cells are not merely bags of enzymes. Structural and mechanical details are also needed. This is where ECT is poised to make an important contribution.

In recent years, we have used ECT to show by direct visualization that bacteria do indeed have an elaborate cytoskeleton. We have documented structural details of different cell motility mechanisms, chemoreception apparati, flagellar motors, and metabolic microcompartments. We continue to work on all these subjects and hope also to begin shedding light on the structure and management of the nucleoid and cell wall. In addition, we are also imaging the smallest known eukaryote, Ostreococcus tauri.

We have also worked to apply the power of ECT to the structure and maturation of the human immunodeficiency virus type 1 (HIV-1). HIV-1 is an interesting structural story: following its discovery in the mid-1980's, thousands (!) of different structures of its 15 different proteins and pieces of its RNA genome have been solved. Nevertheless we still don't know just how these proteins fit together to form intact, infectious virions, or how their organization changes during assembly, maturation, and infection. The main technical obstacle is that while all HIV-1 virions have the same basic features, each virion is unique in its details. Techniques like X-ray crystallography or NMR spectroscopy, which require a large number of identical objects, have not therefore, been able to reveal "supramolecular" details. So far, we have imaged HIV-1 in its immature and mature states, and are now analyzing these at higher resolution and endeavoring to image HIV-1 structures in living host cells, as well as host factors involved in the HIV-1 life cycle.

Technologically, we are working on optimizing sample preservation, recording better images through improved instrumentation, obtaining more images through automation, and extracting as much biological insight as possible from the images through more sophisticated image processing. For more information, see http://www.jensenlab.caltech.edu.
**PUBLICATIONS**

**2013**


**2012**


Summary:
Memories are stored in the brain as connected neurons "encoding" simultaneous events and impressions. Activation of one of the connected neurons can lead to activation of all of them. Formation of new memories requires the formation of new connections among neurons. One way the brain accomplishes this is to strengthen synapses among neurons that fire together during an event.

Synapses are strengthened in response to their own activation by a process termed “synaptic plasticity.” Our brains have evolved complex mechanisms for controlling the circumstances under
which such changes will occur. For example, one of the receptors for the excitatory amino acid neurotransmitter glutamate (the NMDA-type glutamate receptor), is able to trigger a long-lasting increase in the strength of a synapse, but only when simultaneous activation of several synapses on the same neuron causes the postsynaptic neuron to fire an action potential. This "plasticity rule" is used to form memories. Synaptic plasticity occurs by a mechanism in which, in addition to depolarizing postsynaptic neurons, activation of the receptors also initiates biochemical changes in the signaling machinery of the synapse. The biochemical changes can either increase or decrease the size of the signal produced by the synapse when it fires again.

Our lab is studying the biochemical signal transduction machinery in central nervous system synapses that control synaptic plasticity. In past years, we employed a combination of microchemical and recombinant DNA methods to elucidate the molecular structure of a scaffolded network of signaling enzymes located near the postsynaptic membrane of excitatory synapses in the CNS, and called the postsynaptic density (PSD). This network controls the cellular changes that occur to strengthen or weaken synapses. For example, it regulates insertion and removal of glutamate receptors and elaboration of the postsynaptic actin cytoskeleton that underlies the shape of postsynaptic spines.

Recently, we have begun to study the postsynaptic signaling network as a system in order to learn how it regulates the delicate mechanisms of synaptic plasticity. Our work involves an interplay between spatially accurate computer simulations of biochemical reactions in the postsynapse, and experiments to test the accuracy of simulations and to help us build new models. Building of computer simulations involves a long-standing collaboration with Terry Sejnowski and Tom Bartol of the Salk Institute. Experiments involve a wide array of techniques including in vitro enzymatic assays with purified proteins, cellular pharmacology and electrophysiology with intact neurons, construction of mutant mice by homologous recombination, and mass spectrometric assays of protein phosphorylation in vitro and in vivo. In a major new initiative, we are building a plunge-freeze apparatus to harvest stimulated brain slices at defined times after a stimulus. We will construct a time course of changes in activation state of the enzymes in synaptic regulatory circuits following various stimuli. To do this, we are developing MRM (multiple reaction monitoring) mass spectrometric assays. The data will allow us to build and test kinetic models of large signal transduction pathways.
**Publications**

**2013**


*Keller, DX, *Bartol, TM, Kinney, JP, Bajaj, C, Harris, KM, †Kennedy, MB, †Sejnowski, TJ, Calcium pumps isolate calcium transients in reconstructed dendritic spines of hippocampal CA1 pyramidal neurons*, submitted for publication.

**2012**

Stephen L. Mayo

Professorial Awards and Honors:
2012 William K. Bowes Jr. Foundation Division Chair

Graduate Students:
Alexandria H. Berry, Jackson Cahn, Mohsen Chitsaz, Emmanuel L.C. de los Santos, Samy Hamdouche, Gene Kym, Toni M. Lee, Seth Lieblich, Andrew Lim, Kurt Mou, Bernardo Sosa Padilla Araujo, Timothy Wannier

Research and Laboratory Staff:
Marie L. Ary, Rhonda K. DiGiusto, Jan Kostecki, Alex Nisthal, Lilian Porter

Financial Support:
Department of Defense: National Security Science and Engineering Faculty Fellowship Program
Donald Bren Foundation
Monsanto
National Institutes of Health
National Science Foundation
Protabit Inc.

PROTEIN FOLDING AND PROTEIN DESIGN

Summary:
My research group focuses on developing quantitative approaches to protein engineering. Our work has been at the interface of theory, computation, and wet-laboratory experimentation and has been aimed at understanding the physical/chemical determinants of protein structure, stability, and function. We were the first to show that a force-field-based description of protein structure and stability could be coupled with combinatorial search algorithms capable of addressing the enormous combinatorial space available to protein sequences. In our 1997 Science article we firmly established the field of computational protein design by experimentally validating that a computationally designed protein sequence actually folded to its intended 3-dimensional structure. This and related work have been viewed as the harbinger to a complete solution to the inverse protein-folding problem (that is, the problem of predicting amino acid sequences that will fold to specific protein structures). A solution to this problem will have a profound impact on our ability to understand the evolution of protein sequences, structures, and functions, as well as on prospects for
continued development of protein-based biotechnologies. Relative to the later point, I have been engaged in significant translational activities through companies that I have co-founded: Molecular Simulations, Inc. (currently Accelrys) is focused on chemical and biological information technologies; Xencor is focused on engineered antibodies for oncology applications with several biologics in human clinical trials; and, Protabit is focused on integrating and developing next generation computational protein design software technology.

**PUBLICATIONS**

**2013**


**2012**


Dianne Newman Lab Annual Report 2013

California Institute of Technology

Professor of Biology & Geobiology:
Dianne Newman

Professorial Awards and Honors:
2012 NIH Director's Transformative Research Award
2012 Featured Speaker, Carnegie Capital Science Evenings
2012 Gavin Borden Lecturer, Cold Spring Harbor
2011 Cozzarelli Prize National Academy of Science
2011 Geochemical Society OGD Award for Best Paper
2011 Senior Visiting Research Fellowship, St. Johns College Oxford

Visiting Associates:
Ian Booth, Stuart Conway, Sean Elliot

Postdoctoral Fellows:
Megan Bergkessel, David Doughty, Ryan Hunter, Cajeetan Neubauer, Lisa Racki, Nicholas Shikuma, Chia-Hung Wu

Graduate Students:
Lina Bird, Nate Glasser, Suzanne Kern, Sebastian Kopf, Naomi Kreamer, Jessica Ricci

Undergraduate Students:
Elise Cowley (visiting HHMI EXROP) Michael Dieterle, Yang Hu, Alice Michel, Cristian Salgado (visiting HHMI EXPOR), Ben Wang

Research Staff:
Hannah Grotzinger (Summer Student/Tech), Alejandro LaRiviere (Research Technician), Lindsay Van Sambeek (visiting HHMI summer medical fellow)

Member of the Professional Staff:
Gargi Kulkarni (Staff Scientist), Shannon Park (Lab Manager), Kristy Nguyen (Administrative Assistant)

Lab website: http://dknlab.caltech.edu/Newman_Lab.html

Financial Support:
HHMI
NIH
NASA
NSF
CHLA.WEBB
Joint Center for Translational Medicine
**Physiology and Mechanisms of Metabolite Utilization by Bacteria**

**Summary:**
Electron-transfer reactions are fundamental to metabolism. Whether an organism is autotrophic or heterotrophic, free living or an obligate parasite, every cell must solve the energy-generation problem to survive. At the cellular level, most of our knowledge of electron transfer comes from mechanistic studies of oxygenic photosynthesis and aerobic respiration in prokaryotic and eukaryotic systems. While we know in exquisite detail the structure and function of various membrane-bound proteins involved in electron-transfer processes (e.g., cytochrome c oxidase in mitochondria), we know far less about the electron-transfer agents of more ancient forms of metabolism. As geobiologists interested in the origin and evolution of the biochemical functions that sustain modern life, our work has focused on probing the co-evolution of metabolism with Earth’s near-surface environments. Understanding how modern microorganisms with archaic metabolisms function is a step towards this end. Moreover, because many biological microenvironments are anaerobic, including those in most bacterial infections, this path of inquiry leads inexorably to insights about cellular electron-transfer mechanisms that potentially have profound biomedical implications.

Because rocks provide the primary record of ancient events and processes, our laboratory initially explored microbe-mineral interactions. In particular, we investigated how bacteria catalyze mineral formation, transformation, and dissolution, focusing on how these processes relate to cellular energy generation or membrane organization, and how they affect the geochemistry of their environment. For every pathway that we studied, we chose model organisms that we could genetically manipulate. Through a combination of classical genetic, biochemical, and molecular biological approaches, we identified the genes and gene products that controlled the processes of interest. For example, we discovered how bacteria use sediment-bound arsenate as a terminal electron acceptor in anaerobic respiration and convert it to arsenite, a more toxic and mobile form; how anoxygenic photosynthetic bacteria utilize ferrous iron [Fe(II)] as an electron donor in photosynthesis, thereby precipitating rust anaerobically; and how magnetotactic bacteria position the magnetosome, an organelle-like structure in which nanoparticles of magnetite are made. As our work progressed, however, it became increasingly clear that our findings transcended microbe-mineral interactions. Accordingly, our focus has shifted towards exploring more basic physiological questions that are relevant to diverse biological systems. Still, a geobiological perspective imbues our approach, compelling us to evaluate the functions of modern biomolecules in an evolutionary context.

We are currently exploring two major thematic areas:

I. The "light side": evolution of photosynthesis (focusing on how certain anoxygenic phototrophs utilize Fe(II) as an electron donor to power their metabolism, and determining the cellular function of 2-methylbacterial hopanoids— isoprenoids found in the membranes of both anoxygenic and oxygenic phototrophs, but whose molecular fossil derivatives have been used as biomarkers for the rise of oxygenic photosynthesis in the rock record).

II. The "dark side": physiological functions of redox active "secondary" metabolites (focusing on phenazine “antibiotics” produced by Pseudomonas aeruginosa PA14, an opportunistic pathogen that colonizes the lungs of individuals with the disease cystic fibrosis).
**Publications**

**2013**


**2012**


Fred and Nancy Morris Professor of Biophysics and Biology
Rob Phillips

Postdoctoral Fellows:
James Boedicker, Robert Brewster, Franz Weinert

Graduate Students:
Stephanie Barnes, Nathan Belliveau, Yi-Ju Chen, Daniel Jones, Geoffrey Lovely, Gita Mahmoudabadi, Mattias Rydenfelt

Lab website: http://www.rpgroup.caltech.edu/

Financial Support:
National Institute of Health (NIH)
National Science Foundation (NSF)
Howard Hughes Medical Institute (HHMI)
Rosen Scholarships in Bioengineering
Hertz Foundation

Physical Biology of the Cell

Summary:

Our work focuses on three primary areas which serve as case studies in the physical dissection of biological problems.

First, we have had a long standing interest in how viruses transfer their genetic material to their infected hosts. On the theoretical side, we have explored the free energy cost of DNA packing within viruses and how that stored energy can be used to power genome transfer. These efforts are complemented by single-molecule studies in which we watch individual viruses deliver their genomes in real time. These experiments reveal a rich interplay between the free energy which drives ejection and the friction that the DNA encounters as it enters the infected host.

Second, we have been fascinated by the interplay between the informational and physical characteristics of DNA which has lead to efforts on single-molecule and single-cell studies of how transcription factors interact with, deform and loop DNA. These single-molecule approaches are coupled with statistical mechanical modeling which permit the determination of the nature of the DNA-protein interactions that mediate many genomic transactions. Until recently, our efforts have primarily focused on bacterial transcription, but of late we have generalized these efforts to V(D)J recombination as a signature eukaryotic example of the interplay between information and physical processes on DNA.
Third, cells are subjected to forces of all kinds. One of the most severe mechanical perturbations that cells can suffer is osmotic shock. Our interest in these systems began with theoretical calculations of how mechanosensitive channels in bacteria work. Insights from these models have led us to undertake single-cell osmotic shock experiments in which we watch the response of cells harboring various combinations of mechanosensitive channels to osmotic shock.


**P U B L I C A T I O N S**

**2013**


Brewster, R., Jones, D., and Phillips, R. “Tuning Promoter Strength through RNA Polymerase Binding Site Design in Escherichia coli” PLOS Computational Biology 8(12), e1002811. PMID 23271961.


2012


**Smits Professor of Cell Biology:**
Alexander Varshavsky

**Professorial Awards and Honors:**
2012 Otto Warburg Medal, German Society for Biochemistry and Molecular Biology
2012 King Faisal International Prize in Science, Saudi Arabia
2012 BBVA Frontiers of Knowledge Award in Biomedicine, Spain

**Research Assistant:**
Elena Udartseva

**Research Associate:**
Xia Wu

**Staff Scientists:**
Christopher Brower, Konstantin Piatkov

**Postdoctoral Scholars:**
Yuan Liu, Jang-Hyun Oh

**Graduate Students:**
Anna Shemorry, Tri Vu, Brandon Wadas

**Undergraduate Student:**
Connor Rosen

**Financial Support:**
Howard and Gwen Laurie Smits Professorship in Cell Biology
National Institutes of Health

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**The Ubiquitin System and the N-end Rule Pathway**

**Summary:**
Our main subject is the ubiquitin-proteasome system. The field of ubiquitin and regulated protein degradation was created in the 1980s, largely through the complementary discoveries by the laboratory of A. Hershko (Technion, Israel) and by my laboratory, then at MIT.

In 1978-1985, the elegant biochemical studies by Hershko and coworkers produced the initial understanding of ubiquitin-mediated protein degradation in cell extracts, including the isolation of enzymes that mediate ubiquitin conjugation. In 1984-1990, these mechanistic (enzymological) insights with cell-free systems were complemented by our genetic and biochemical discoveries with mammalian cells and the yeast Saccharomyces cerevisiae that revealed the biology of the ubiquitin system and regulated protein degradation. These interconnected discoveries included the first
demonstration that the bulk of protein degradation in living cells requires ubiquitin conjugation and
the discovery of the first ubiquitin-conjugating (E2) enzymes with specific physiological functions,
in the cell cycle (Cdc34) and DNA repair (Rad6). These insights initiated the understanding of the
massive, multilevel involvement of the ubiquitin system in the regulation of the cell cycle and DNA
damage responses. We also discovered the critical roles of the ubiquitin system in other biological
processes, including stress resistance, protein synthesis and transcriptional regulation. In 1986, we
discovered the first primary degradation signals (degrons) in short-lived proteins. These signals
included degrons that give rise to the N-end rule, which relates the in vivo half-life of a protein to the
identity of its N-terminal residue. The discovery of the N-end rule pathway identified the first
specific pathway of the ubiquitin system. Other contributions by our laboratory in the 1980s
included the first polyubiquitin chains, their specific topology and their necessity for proteolysis; the
subunit selectivity of protein degradation (this fundamental capability of the ubiquitin system makes
possible the remodeling of oligomeric proteins); the first physiological substrate of the ubiquitin
system (MATalpha2 repressor); the first genes that encode deubiquitylating enzymes and ubiquitin
precursors (a linear polyubiquitin chain and ubiquitin fusions to specific ribosomal proteins); and the
first cloned and molecularly characterized E3 ubiquitin ligase, termed Ubr1. The 1990 discovery,
cloning and analysis of the Ubr1 ubiquitin ligase, which mediates the N-end rule pathway, opened a
particularly large field, because we now know that the mammalian genome encodes more than 1,000
distinct E3s. The targeting of many distinct degrons in cellular proteins by this immense diversity of
E3 enzymes underlies the unprecedented functional reach of the ubiquitin system. The term
"ubiquitin ligase" denotes either an E3-E2 complex or its E3 component. For accounts of the early

By the end of the 1980s, our studies had revealed the major biological functions of the ubiquitin
system as well as the basis for its specificity, i.e., the first degradation signals in short-lived proteins.
The resulting discovery of the physiological regulation by intracellular protein degradation has
transformed the understanding of biological circuits, as it became clear that control through
regulated protein degradation rivals, and often surpasses in significance the classical regulation
through transcription and translation. Just how strikingly broad and elaborate ubiquitin functions are
was understood more systematically and in great detail over the next two decades, through studies by
many laboratories that began entering this field in the 1990s, an expansion that continues to the
present day.

Our current work at Caltech focuses on the ubiquitin system and regulated protein degradation, with
an emphasis on the N-end rule pathway. The N-end rule relates the in vivo half-life of a protein to the
identity of its N-terminal residue. The N-end rule pathway polyubiquitylates proteins that contain
specific degrons, thereby targeting these proteins for degradation by the 26S proteasome. Degrons
recognized by the N-end rule pathway include a set called N-degrons. The main determinant of an
N-degron is a destabilizing N-terminal residue of a protein. Recognition components of the N-end
rule pathway, called N-recognins, are specific E3 ubiquitin ligases that can target N-degrons. The
N-end rule pathway consists of two branches, the Ac/N-end rule and the Arg/N-end rule pathways.
The Ac/N-end rule pathway recognizes proteins with N-terminally acetylated (Nt-acetylated) residues. The Arg/N-end rule pathway targets unacetylated N-terminal residues. The primary destabilizing N-terminal residues Arg, Lys, His, Leu, Phe, Tyr, Trp, and Ile are directly recognized by E3 N-recogins, whereas N-terminal Asp, Glu, Asn, Gln, and Cys function as destabilizing residues through their preliminary modifications. These modifications include the N-terminal deamidation, by the Ntan1 and Ntaq1 N-terminal amidases (Nt-amidases), and the N-terminal arginylation (Nt-arginylation), by the Ate1 arginyl-transferase (R-transferase).

Regulated degradation of specific proteins by the Arg/N-end rule pathway mediates a legion of physiological functions, including the sensing of heme, nitric oxide (NO), oxygen and short peptides; the selective elimination of misfolded proteins; the regulation of DNA repair and cohesion/segregation of chromosomes; the signaling by G proteins; the regulation of peptide import, meiosis, viral and bacterial infections, fat metabolism, cell migration, actin filaments, spermatogenesis, neurogenesis, and cardiovascular development; and the functioning of adult organs, including the brain, muscle, testis and pancreas. The recent discovery of the Ac/N-end rule pathway further expanded the already broad functional scope of the eukaryotic N-end rule pathway, and has also revealed the main physiological functions of Nt-acetylases and Met-aminopeptidases (Hwang et al., 2010). Prokaryotes, which lack a bona fide ubiquitin system, nevertheless contain the N-end rule pathway, ubiquitin-independent versions of it. Many years after the initial discovery of the N-end rule pathway, this proteolytic system continues to be a fount of biological insights (Varshavsky, 2011). Our current studies focus on the Arg/N-end rule pathway and the Ac/N-end rule pathway in the yeast S. cerevisiae and the mouse.

References cited:


For more information, see http://biology.caltech.edu/Members/Varshavsky
PUBLICATIONS

2013


2012


Molecular, Cellular and Integrative Neuroscience Biology Faculty

CALIFORNIA INSTITUTE OF TECHNOLOGY

Ralph Adolphs
Bren Professor of Psychology and Neuroscience, Professor of Biology

John Allman
Frank P. Hixon Professor of Neurobiology

Richard Andersen
James G. Boswell Professor of Neuroscience

David Anderson
Seymour Benzer Professor of Biology

Viviana Gradinaru
Assistant Professor of Biology

Henry Lester
Bren Professor of Biology

Markus Meister
Lawrence A. Hanson, Jr. Professor of Biology

Paul Patterson
Anne P. and Benjamin F. Biaggini Professor of Biological Sciences
David Prober  
*Assistant Professor of Biology*

Doris Tsao  
*Assistant Professor of Biology*

Shinsuke Shimojo  
*Gertrude Baltimore Professor of Experimental Psychology*

Kai Zinn  
*Professor of Biology*

Athanasios Siapas  
*Professor of Computation and Neural Systems*
EMOTIONAL AND SOCIAL COGNITION IN HUMANS

Summary:
Our laboratory investigates the psychological and neural bases of social cognition, using a number of different approaches. Some studies focus on the psychological level, using behavioral data from healthy people to make inferences about how emotion modulates memory, attention, or conscious awareness. A second approach uses neuroimaging and electrophysiology to investigate the neural mechanisms behind emotional and social processing. A third approach studies the performances, and the brains, of special populations. At Caltech, we have been recruiting people with agenesis of the corpus callosum to investigate the functional consequences of disruption in
long-range connectivity. Dr. Lynn Paul leads this work. In collaboration with Joe Piven at the University of North Carolina, we have also been studying people with autism. At the University of Iowa, we have ongoing collaborations that involve neurological populations with focal brain lesions, and, together with hospitals in the Los Angeles region, which involve neurosurgical patients in whom we can record intracranially.

A major focus in the past year has been on making comparisons across some of these populations and approaches. For instance, we are comparing people with autism and with amygdala lesions tested on the same tasks. Many of these comparative studies build on years of data accrual in our laboratory involving a significant amount of work by our staff, as well as the graduate students and post-docs. A second area where we are making comparisons is across methods. For instance, we are comparing responses measured in the amygdala to features of faces, and doing so using both the signal typically measured in fMRI studies (the BOLD response), as well as recording action potentials from single neurons in neurosurgical patients who have depth electrodes in the amygdala. Finally, we are continuing to collaborate with colleagues in the social sciences at Caltech who bring a model-based approach to understanding human behavior. Taken together, these studies of social cognition across a variety of populations, using multiple measures, and complemented with computational modeling, are giving us powerful insights not only into how specific structures might work (like the amygdala), but also how they might function in a network of multiple components. Extending our understanding of social cognition to the systems level, and examining the connections between different brain regions, constitutes a major thrust for future studies in our laboratory.
PUBLICATIONS

2013


2012


**Summary:**

We are continuing to explore the structure and function of the von Economo neurons in health and disease. In collaboration with Prof. Barbara Wold and Dr. Brian Williams, we are investigating gene expression in microdissected von Economo neurons using RNA-Seq. We are also using this technique to discover differences between specific cell types in neurotypical and autistic brains. We are analyzing the comparative structure of the frontal and insular cortex in primates using connectivity based on high resolution diffusion tensor magnetic resonance imaging, and cellular and fiber architecture. Finally we have initiated a study of the brains of non-demented centenarians with Prof. David Bennett and his colleagues at Rush University using these same methods as part of an endeavor to understand brain aging in extremely old individuals who had preserved cognitive functioning.
PUBLICATIONS

2012


Neural mechanisms for visual-motor integration, spatial perception and motion perception. While the concept of artificial intelligence has received a great deal of attention in the popular press, the actual determination of the neural basis of intelligence and behavior has proven to be a very difficult problem for neuroscientists. Our behaviors are dictated by our intentions, but we have only recently begun to understand how the brain forms intentions to act. The posterior parietal cortex is situated between the sensory and the movement regions of the cerebral cortex and serves as a bridge from sensation to action. We have found that an anatomical map of intentions exists within this area, with one part devoted to planning eye movements and another part to planning arm movements. The action plans in the arm movement area exist in a cognitive form, specifying the goal of the intended movement rather than particular signals to various muscle groups.

Neuroprosthetics. One project in the lab is to develop a cognitive-based neural prosthesis for paralyzed patients. This prosthetic system is designed to record the electrical activity of nerve cells in the posterior parietal cortex of paralyzed patients, interpret the patients' intentions from these
neural signals using computer algorithms, and convert the "decoded" intentions into electrical control signals to operate external devices such as a robot arm, autonomous vehicle or a computer.

Recent attempts to develop neural prosthetics by other labs have focused on decoding intended hand trajectories from motor cortical neurons. We have concentrated on higher-level signals related to the goals of movements. Using healthy monkeys with implanted arrays of electrodes we recorded neural activity related to the intended goals of the animals and used this signal to position cursors on a computer screen without the animals emitting any behaviors. Goal decoding has advantages including the coding of multiple sequences, fast decoding, and improving trajectory decoding by knowing the final goal. Interestingly we have recently found that trajectories are also represented in posterior parietal cortex that is a tremendous advantage since goals and trajectories can be decoded from a single area rather than having to implant two different areas. We are also ramping up for clinical studies in paralyzed patients using recordings from the posterior parietal cortex. One prosthetic will be a communication device operating a tablet (e.g., iPad). The second will control a state-of-the-art robotic limb that has been developed by the Applied Physics Laboratory at Johns Hopkins.

Coordinate frames. Our laboratory also examines the coordinate frames of spatial maps in cortical areas of the parietal cortex coding movement intentions. One new discovery is the finding of a novel, "relative" coordinate frame used for hand-eye coordination. Neurons in the dorsal premotor cortex and area 5d of posterior parietal cortex encode the position of the eye to the target and the position of the hand to the target. Interestingly the dorsal premotor cortex also encodes the relative position of the hand to the eye. A similar relative coding may be used for other tasks that involve the movements of multiple body parts such as bimanual movements.

Local field potentials. The cortical local field potential (LFP) is a summation signal of excitatory and inhibitory dendritic potentials that has recently become of increasing interest. We have reported that LFP signals in the saccade and reach regions provide information about the direction of planned movements, as well as the state of the animal; e.g., baseline, planning a saccade, planning a reach, executing a saccade, or executing a reach. This new evidence provides further support for a role of the parietal cortex in movement planning. It also shows that LFPs can be used for neural prosthetics applications. Since LFP recordings from implanted arrays of electrodes are more robust and do not degrade as much with time compared to single cell recordings, this application is of enormous practical importance. We have also been comparing the correlation of spikes in one area with LFPs in another to determine how cortical areas communicate with one another during different tasks.

Cortical repair. We are currently performing functional magnetic resonance imaging (fMRI) experiments in awake, behaving monkeys. This technique is important since fMRI experiments are routinely done in humans and monitors the changes in blood flow during different cognitive and motor tasks. However, a direct correlation of brain activity with blood flow cannot be achieved in humans, but can in monkeys. Thus, the correlation of cellular recording and functional MRI activation in monkeys provides us with a better understanding of the many experiments currently being performed in humans. We are also using monkey fMRI for clinical studies. We are temporarily inactivating parts of cortex to see how brain circuits adjust to compensate for inactivation. In the future we will use electrical stimulation of cortical areas determined by fMRI to be active during the compensation process. These studies are aimed at developing medical devices that can accelerate brain repair from traumatic brain injury and stroke.
PUBLICATIONS

2013


Hwang, E.J. The basal ganglia, the ideal machinery for the cost-benefit analysis of action plans. Frontiers in Neural Circuits. Vol. 7, Article 121. No PMID or PMCID available.


2012


Summary:
Research in this laboratory is aimed at understanding the neurobiology of emotion, using the laboratory mouse and the vinegar fly (*Drosophila melanogaster*) as model organisms. We seek to elucidate how fundamental properties common to emotional states or responses, such as arousal, are encoded in the circuitry and chemistry of the brain, and how sensory stimuli eliciting specific emotional behaviors, such as fear or aggression, are transformed and organized into behavior by the nervous system. Our work employs molecular genetic tools to mark, map and functionally manipulate specific circuits, to determine how identifiable populations of neurons contribute in a
causal manner to behavior. These interventional studies are complemented by electrophysiology and functional imaging to measure activity in neural circuits.

**Emotion circuits in the mouse brain**

Research using the laboratory mouse *Mus musculus* focuses on understanding the neurobiology of fear, anxiety, aggression and pain, and the interrelationships between the circuitry that processes these emotions.

Our studies of fear are currently centered on the function of circuits in the amygdala, a medial temporal lobe structure that plays an important role in Pavlovian learned fear, a form of classical conditioning. We have identified genes that mark several subpopulations of neurons that form a dynamic microcircuit within the central nucleus of the amygdala. The function of this microcircuit in fear behavior is being dissected using optogenetic tools, such as channelrhodopsin, and pharmacogenetic tools, such as the ivermectin-gated glutamate sensitive chloride channel (GluCl) (Lerchener *et al.*, 2007), together with acute slice electrophysiology and genetically-based anatomical tracing of synaptic pathways. (Haubensak *et al.*, 2010)

Similar approaches are being used to understand the role of another brain region strongly implicated in stress and anxiety, the lateral septum (LS). There are conflicting data on whether the LS promotes or inhibits anxiety, due to a lack of information about its microcircuitry. We have gained a point-of-entry into this structure by focusing on neurons expressing the type 2 corticotrophin releasing hormone receptor (Crhr2), and are using a combination of transgenic and novel viral tools to understand the functional circuitry in which these neurons participate.

In the case of aggression, we are focusing on circuits within the hypothalamus, an area that has extensive connectivity with both the LS and the amygdala, through which it receives input from the olfactory system (Choi *et al.*, 2005). We are using chronic in vivo multi-unit recording to probe the relationship between neuronal activity and aggression, as well as mating, and are also employing genetically based functional perturbations to understand how these two related social behaviors are processed by a common structure. An initial report of these studies has recently been published (Lin *et al.*, 2011).

Pain has both a sensory and an affective component. We are using genetically targeted methods to probe the functional roles of different subpopulations of primary sensory neurons identified by the expression of Mrgrps, a family of orphan G protein-coupled receptors that we previously characterized (Dong *et al.*, 2001). We are interested in the extent to which these receptors mark neurons specialized for particular types of painful stimuli, and if so how this specificity is conveyed to the brain.

**Emotion circuits in Drosophila**

The pioneering work of the late Seymour Benzer proved that the powerful genetics of *Drosophila* could be used to dissect the genetic underpinnings of many types of complex behaviors. Until recently, however, it was not clear whether this model system could also be applied to
understanding the neurobiology of emotion and affect. We are taking two complementary approaches to study the neurobiology of emotion in the fly. One approach is to dissect the neural circuitry underlying behaviors that are analogous to defensive behaviors in higher organisms, such as avoidance (Suh et al., 2004, 2007), aggression (Wang et al., 2008) or immobility (Yorozu et al., 2009). The other is to model internal states or processes that are fundamental to many types of emotional responses, such as arousal, to ask for example whether arousal is unitary, or whether there are different types of behavior-specific arousal states (Lebestky et al., 2009). In both cases, we are developing novel behavioral assays, as well as machine vision-based approaches (Dankert et al., 2009) to automate the measurement of these behaviors (in collaboration with Pietro Perona, Allen E. Puckett Professor of Electrical Engineering), and are using molecular genetic-based tools to image and perturb neuronal activity in order to identify the specific circuits that mediate these behaviors.

Images from left to right:
Professor David Anderson
Neurons in the lateral septum that are involved in anxiety (blue), receive synaptic input from other neurons expressing specific neuropeptides (red spots). These neuropeptides may modulate anxiety.
Aggression between two male Drosophila melanogaster. The fly on the right is initiating an attack towards the fly on the left.

PUBLICATIONS

2013


2012


Assistant Professor of Biology:
Viviana Gradinaru

Honors and Awards:
2013 NIH Director’s New Innovator Award
2013 Named a World Economic Forum Young Scientist
2013 Pew Scholar Award
2013 Human Frontier Science Program (HFSP) Young Investigator Grant
2012 Mallinckrodt Award

Talks:
2013 Allen Brain Institute 10th Anniversary Symposium, Seattle, USA
2013 UCLA Learning and Memory Symposium; USC Seminar
2013 TEDxCaltech: The Brain on “Brain Control with Light - Development and Application.”
2012 SfN Minisymposium on “Optogenetics for Long-Timescale Control.”
2012 Watson Lecture at Caltech on “Brain Control with Light” as the Richard C. Biedebach Memorial Lecture.

Research Fellows:
Lindsay Bremner, Jennifer Treweek, Cheng Xiao

Graduate Students:
Claire Bedbrook, Ken Chan, Nick Flytzanis

Research and Laboratory Staff:
Sripriya Ravindra Kumar, Bin Yang

Undergraduate Students:
(Summer) Christopher Finch (Amgen), Snigdha Kumar (Caltech), Victoria Servin (MURF)

Lab Website: http://glab.caltech.edu

Financial Support:
The Beckman Institute
Human Frontiers in Science Program
The Mallinckrodt Foundation
The Moore Foundation
The Pew Charitable Trusts

CONTROL OF BRAIN FUNCTION AND BEHAVIOR

Summary:

The Gradinaru Lab studies the mechanism of action for deep brain stimulation (DBS), a therapeutical option for motor and mood disorders such as Parkinson’s and depression. Our
previous work highlighted the importance of selectively controlling axons and not local cell bodies in modulating behavior, a principle that might play a generalized role across many effective deep brain stimulation paradigms. We are now particularly interested in the long-term effects of DBS on neuronal health, function, and ultimately behavior.

In addition, the lab will continue to push forward optogenetic technologies by developing tools for electrical and biochemical control and localizing them to subcellular compartments. To achieve the goals of neuronal circuits investigation and tool development for neuroscience the Gradinaru lab will use advanced Molecular and Synthetic Biology; Electrophysiology (in vitro and in vivo); Behavior; Imaging (2-photon), Optogenetics (gene delivery of photosensitive proteins to specific cell types) and CLARITY (slicing-free whole brain imaging and molecular phenotyping).

Gradinaru Lab will be a great fit for any interdisciplinary-minded person. Projects in the lab range from studying the impact of neuromodulation on neurodegeneration and behavior to engineering needed tools (molecular, cellular, hardware) for neuroscience research. If you are interested in joining our team, please email Dr. Gradinaru (viviana@caltech.edu) your CV and a brief description of your scientific interests.

Images from left to right:
Assistant Professor Viviana Gradinaru
Hippocampal Neuronal Culture with Optogenes
LED Array for Optogenetic Biochemical Control

PUBLICATIONS

2013

Sukhotinsky I; Chan AM; Ahmed OJ; Rao VR; Gradinaru, V.; Ramakrishnan C; Deisseroth K; Majewska AK; Cash SS. Optogenetic delay of status epilepticus onset in an in vivo rodent epilepsy model. PLoS One, 2013; Apr 24;8(4):e62013. [PMID: 23637949]

Bren Professor of Biology:
Henry A. Lester

Members of the Professional Staff:
Bruce N. Cohen

Associate Biologist:
Purnima Deshpande

Senior Research Fellows:
Julie Miwa, Cheng Xiao

Postdoctoral Scholars:
Brandon Henderson, Beverley Henley, Rachel Penton, Chris Richards,
Rahul Srinivasan, Ying Wang

Graduate Students:
Crystal Dilworth\(^1\), Shawna Frazier, Weston Nichols, Rell Parker

Research and Laboratory Staff:
Pamela Fong, Eloisa Imel, Atsuko Kobayashi, Elisha Mackey, Sheri McKinney, Jonathan Wang

SURF Students:
Mahati Mokkarala, Mark Starbird (CIRM), Adela Wu, Caroline Yu

Financial Support:
CIT-UCLA Joint Center for Transitional Medicine Program
Della Martin Foundation
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Gordon & Betty Moore Foundation
Michael J. Fox Foundation
National Institute of Mental Health
National Institute of Neurological Disorders and Stroke
National Institute on Aging
National Institute on Drug Abuse
University of California, Tobacco-Related Disease Research Program

\(^1\)Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA
\(^2\)Applied Physics, Division of Physics, Mathematics and Astronomy, California Institute of Technology, Pasadena, CA
"INSIDE-OUT" MECHANISMS IN NEUROPHARMACOLOGY; SYNAPTIC TRANSMISSION; ION CHANNELS; MOUSE MODELS; NICOTINE ADDICTION; PARKINSON’S DISEASE

Summary:

Neurotransmitters and drugs acutely activate or inhibit classical targets on the plasma membrane: receptors, ion channels, and transporters. Which mechanisms underlie the effects of chronic exposure to drugs, during days to weeks of exposure? In the conventional view, drugs exert their chronic or continuous effects via the classically understood pathways of second messengers, protein kinases, and downstream effectors. Our lab is testing hypotheses in a novel scientific area, “inside-out” neuropharmacology. “Inside-out” mechanisms of chronic drug action begin with binding to the classical targets, but when those targets reside in the endoplasmic reticulum and cis-Golgi. Sequelae of this binding include pharmacological chaperoning, modification of endoplasmic stress and the unfolded protein response, escorting and abduction of other proteins. These mechanisms first arose in our studies of the neural events that occur when an animal is chronically exposed to nicotine. We hypothesize that “inside-out” pharmacology underlies the pathophysiology of nicotine addiction, the world’s largest preventable cause of death.

“Inside-out” neuropharmacology also arose in our approach to an inadvertent therapeutic effect of smoking: the inverse correlation between a person’s history of smoking and his/her susceptibility to Parkinson’s disease. There will never be a medical justification for the use of smoked tobacco. However, the organism’s responses to chronic nicotine probably also underlie this apparent neuroprotection.

We are studying these complex neural processes at several appropriate levels: the genes, the receptor proteins, the effects on neurons, the organization of neurons in circuits, the resulting behavior of animals. We have produced subcellular movies depicting the first 24 hours of nicotine addiction—thought to be the most crucial-stage in the process, especially for adolescents. These images display the spread of newly chaperoned, fluorescent receptors as they travel from the endoplasmic reticulum to the cell membrane. We are now studying gene activation during the early stages of exposure to chronic nicotine.

Other lab members have generated and studied mice with genetically modified nicotinic receptors—gain of function, not knockouts. Some mice have a hypersensitive subunit; in such mice, responses to nicotine represent selective excitation of receptors containing that subunit. Other mice have a fluorescent subunit, so that we can quantify and localize upregulation of receptors containing that subunit.

The field of psychiatric drugs seems ripe for testing “inside-out” ideas, because nobody understands the events that occur during the two to three week “therapeutic lag” in the actions of antidepressant and antipsychotic drugs. We hope to define the action of the novel antidepressant ketamine.
Several of our projects lead naturally to drug discovery procedures. We have a drug discovery collaboration with Michael Marks and his group at the University of Colorado, Boulder; and with Targacept, Inc. We also collaborate with Robert Freedman and his colleagues at the University of Colorado, Denver, to generate scientific knowledge and therapies around the heavy smoking by schizophrenics.

We continue to study the biophysics of ion channels that respond to the neurotransmitters acetylcholine, serotonin, GABA, glycine, and (among invertebrates) glutamate. These are termed "Cys-loop receptors." At the most fundamental level, with Professor Dennis Dougherty's group in Caltech's Division of Chemistry and Chemical Engineering and Professor Sarah Lummis of Cambridge University, we apply new types of chemistry to understand how Cys-loop receptors transduce the binding of agonists into the opening of the channels.

We also have interests in new techniques at the intersection of biophysics, single-molecule imaging, chemistry, mouse genetics, and neuroscience. We're delighted to host visitors in our lab on the third floor of the Kerckhoff Laboratory.

**Publications**

**2013**


Limapichat W, Yu WY, Branigan E, Lester HA, and Dougherty DA (2013) Key Binding Interactions for Memantine in the NMDA Receptor. ACS chemical neuroscience 4:255-60. PMID: 23421676

Marotta CB, Dilworth CN, Lester HA, and Dougherty DA (2013) Probing the non-canonical interface for agonist interaction with an α5 containing nicotinic acetylcholine receptor. Neuropharmacology 77C:342-349. PMID: 24144909

O’Neill HC, Laverty DC, Patzlaff NE, Cohen BN, Fonck C, McKinney S, McIntosh JM, Lindstrom JM, Lester HA, Grady SR, and Marks MJ (2013) Mice expressing the ADNFLE valine 287 leucine mutation of the β2 nicotinic acetylcholine receptor subunit display increased sensitivity to acute nicotine administration and altered presynaptic nicotinic receptor function. Pharmacology, biochemistry, and behavior 103:603-21. PMID: 23123803


Van Arnam EB, Blythe EE, Lester HA, and Dougherty DA (2013) An unusual pattern of ligand-receptor interactions for the α7 nicotinic acetylcholine receptor, with implications for the binding of varenicline. Mol Pharmacol 84:201-7. PMID: 23680636

2012


Lawrence A. Hanson, Jr. Professor of Biology:
Markus Meister

Postdoctoral fellows/scholars:
Hiroki Asari
Evan Feinberg (working at Harvard)
Julijana Gjorgjieva (working at Harvard)
Max Joesch (working at Harvard)

Staff Scientist:
daniel Wagenaar (now at U Cincinnati)

Graduate Students:
Margarida Agrochao, Brenna Krieger, Melis Yilmaz

Undergraduate Student:
Alex Jose (SURF), Debbie Tsai (SURF)

Function of Neuronal Circuits

Summary:
We try to understand how large circuits of nerve cells work. In particular we study the visual system, and much of the research has been centered on the very first visual circuit: the retina.

The main challenge for our visual system is to absorb the massive onslaught of raw data from images on the retina and extract from that the few morsels that are needed to guide moment-to-moment behavior. To be concrete: The human eye receives about 1 billion bits per second of raw visual information; of this the owner of that eye uses at most 20 bits per second in deciding what to do. Of course those precious 20 bits are deeply hidden in the raw images, in ways that depend entirely on the task at hand, say playing a piano sonata, or steering a car through traffic. So the biggest mystery of vision is how our neural circuits can boil the billion bits at the input down to 20 bits at the output, and do this in real time, with less than 0.1 second delay. Solving this problem will not only provide a deep answer to how we see, but also allow us to build machine vision systems with such powerful capabilities.

For a long time, the retina of the eye was thought to be a simple camera that encodes the
image essentially in raw form and transmits that to the brain. It now emerges that the retina is considerably smarter. It already begins the process of selective filtering, and discards all but a few percent of the raw information it receives. The rest is sent to the brain along ~20 parallel pathways, each of which extracts a different visual feature at each point in the scene. We want to understand:

1) *What* information is encoded by each of these parallel channels? This involves recording the electrical signals from many of the retina’s output neurons while stimulating the input receptors with visual patterns (e.g. Zhang 2012, Leonardo 2013). Interpreting the relationship between sensory input and neural output often requires judicious use of mathematical modeling.

2) *How* are these computations performed? For this we gain access to the innards of the retina to track the signals through the various interneurons and synapses (e.g. Asari 2012). The ultimate goal here is to summarize retinal function with a neural circuit diagram that efficiently simulates the function of the real retina.

3) *Why* is the retina built this way? Much of retinal structure and function is conserved across mammals from mouse to man and probably serves a common purpose. What might this be? Perhaps to pack information efficiently into the optic nerve (e.g. Pitkow 2012); or to facilitate downstream computations of complex visual features (e.g. Guetig 2013); or perhaps to directly extract some signals that are essential for survival (e.g. Yilmaz 2013).

In my new Caltech lab, we start from this core research to explore several new directions. One is a more principled study of visual behavior in the mouse (e.g. Yilmaz 2013). Rather little is known about what these animals do with their eyes, and this needs to change if we want to forge a clear connection between the neural circuits of the visual system and the behaviors they implement. Another direction leads us further into the visual system by simply following the retinal output fibers. Most of them connect to the superior colliculus, a brain area that already integrates vision with other senses and is also intimately involved in the control of action. We are beginning to record neural signals from large circuits in this structure to understand the second stage of visual computations. Finally we are curious to see how animals use all these brain circuits under natural conditions, outside the constraints of the laboratory. For this we have developed a radio-telemetry system that can wirelessly record 64 neural signals in parallel from rodents moving freely in the wild.
PUBLICATIONS

2013


2012


Paul Patterson Lab Annual Report | 2013

California Institute of Technology

Anne P. and Benjamin F. Biaggini Professor of Biological Sciences:
Paul H. Patterson, Ph.D.

Professorial Awards and Honors:
2012 Plenary Lecture, Hereditary Disease Foundation Conference, Cambridge;
2012 Keynote speaker, Western Perinatal Research Conference, Banff

Senior Research Scientist:
Ali Khoshnan

Senior Research Fellow:
Benjamin Deverman

Research Scientists:
Rajesh Gaur, Natalia Malkova

Research Fellows:
Puja Saluja, Wei-Li Wu

Graduate Students:
Grace Chow, Elaine Hsiao

Research and Laboratory Staff:
Sophia Hsien*, Jan Ko, Marlyn Moore*, Laura Rodriguez,
John Sanchez***, Howard Yan*, Collin Yu**, Mario Zubia*

Financial Support:
Anne P. and Benjamin F. Biaggini Chair in Biological Sciences
Autism Speaks Foundation
Binational Science Foundation
California Institute of Regenerative Medicine
Caltech-City of Hope Biomedical Research Initiative
Caltech Innovation Initiative
CIRM Bridges to Stem Cell Research, Pasadena City College
CIRM Bridges to Stem Cell Research, CSLA-Pomona
Congressionally Directed Medical Research Programs, Department of Defense
Elizabeth Ross Fellowship for the Study of Mental Illness
Hereditary Disease Foundation
International Rett Syndrome Foundation
McGrath Foundation

*Undergraduate student, Caltech
**Undergraduate student, Pasadena City College
*** Undergraduate student, Cal State LA
INTERACTIONS BETWEEN THE NERVOUS AND IMMUNE SYSTEMS

Summary:
Much of the research in this laboratory involves the study of interactions between the nervous and immune systems, both in normal development and in several disease models. Using knockout mice and over-expression in vivo with viral vectors, we are exploring the role of the neuropoietic cytokine leukemia inhibitor factor (LIF) in regulating neural stem cell proliferation and fate in the adult brain. In the context of neuroimmune interactions during fetal brain development, we are investigating a mouse model of mental illness based on the known risk factor of maternal influenza infection. Huntington's disease (HD) is another focus, where we are investigating potential therapies using intracellular expression of antibodies (intrabodies) and also manipulating NFkB activity. We are also studying the regulation of MeCP2 by IKKa, because MeCP2 mutations are responsible for Rett syndrome, which frequently involves autism symptoms.

Cytokines are diffusible, intercellular messengers that were originally studied in the immune system. Our group contributed to the discovery of a family that we termed the neuropoietic cytokines, because of their action in both the nervous and hematopoietic/immune systems. We demonstrated that one of these cytokines, LIF, can coordinate the neuronal, glial and immune reactions to injury. Using both delivery of LIF in vivo and examination of the consequences of knocking out the LIF gene in mice, we find that this cytokine has a powerful regulatory effect on the inflammatory cascade. Moreover, LIF can regulate neurogenesis and gliogenesis. LIF is a critical regulator of astrocyte and microglial activation following stroke, seizure or trauma, and this cytokine also regulates inflammatory cell infiltration, neuronal and oligodendrocyte death, gene expression, as well as adult neural stem cell renewal. These results highlight LIF as an important therapeutic target. We use gene therapy to deliver LIF in a chemical model of multiple sclerosis, and find that LIF increases oligodendrocyte number and stimulates remyelination. We are currently modifying the viral vector so that LIF can be delivered in the peripheral circulation and be regulated by exogenous signals, in a cell-specific manner.

Cytokine involvement in a model for mental illness is also being investigated. This mouse model is based on findings that infection in pregnant women can significantly increase the likelihood of schizophrenia and autism in the offspring. We are using behavioral, neuropathological, molecular, and brain imaging methods to investigate the effects of activating the maternal immune system on fetal brain development and how this leads to altered behavior in young and adult offspring. The cytokine IL-6, acting on both the placenta and fetal brain, is key in mediating the effects of maternal immune activation (MIA) on the fate of the offspring. We have new evidence that MIA alters the endogenous immune cells in the placenta, as well as hematopoietic stem cell fate and lymphocyte subsets and reactivity in the adult offspring. These changes are connected with
behavior, as bone marrow transplants from naïve, control mice can correct the abnormal behaviors seen in the MIA offspring. In collaboration with the Amaral laboratory at UC Davis, we have extended the MIA model to non-human primates, and find that the offspring of immune-activated mothers display a series of autism-like behaviors. In collaboration with the Mazmanian laboratory at Caltech, we are also examining the effects of MIA on gastrointestinal tract inflammation in the offspring. We find that adult MIA offspring display deficits in gut permeability. Moreover, this leaky gut and the abnormal behaviors can be corrected by administration of probiotic bacteria, suggesting a novel therapeutic approach for these mental illnesses.

We are utilizing intracellular antibody expression to block the toxicity of mutant huntingtin (Htt), the protein that causes HD. We produced single-chain intrabodies that bind to various domains of Htt, and these can either exacerbate or alleviate Htt toxicity in cultured cells, acute brain slices, and in Drosophila HD models. Gene therapy using one of these intrabodies in five different mouse models of HD is highly effective in ameliorating the behavioral deficits and neuropathology caused by mutant Htt in these models. We have also implicated the NFkB signaling pathway in the pathogenesis of HD, and identified several steps in this signaling cascade as potential therapeutic targets. In collaboration with the Langen laboratory at USC, using electron paramagnetic resonance spectroscopy, we have obtained new structural information on the domains of mutant Htt when it forms fibrils.

Images, left to right:
Professor Paul Patterson
Remyelination 12w cuprizone
Remyelination 12+6w Ad-lacZ
Remyelination 12+6w Ad-LIF

Publications

2013


2012


**Summary:**
More than 10% of Americans suffer from chronic sleep disorders, with an estimated annual cost of $100 billion and for which therapeutic options are poor. Despite the impact of sleep disorders, the fact that we sleep for a third of our lives, and the evolutionary conservation of sleep-like behaviors, the mechanisms that regulate sleep remain poorly understood. It is therefore important to develop simple and cost-effective systems to study the genetic and neural regulation of sleep. Zebrafish are a useful system for these studies because: 1) unlike invertebrates, fish have the basic brain structures thought to regulate mammalian sleep; 2) larval zebrafish are transparent, which makes it easy to monitor and manipulate their neurons; and 3) zebrafish are amenable to high-throughput screens that can identify genes, drugs and neurons that regulate sleep. Zebrafish are therefore a useful system for unraveling the mysteries of sleep. The goal of our lab is to address two fundamental questions: What genetic and neural mechanisms regulate sleep? We are addressing these questions by performing genetic and small molecule screens, and by testing candidate genes and neurons for their roles in regulating sleep/wake behaviors.
These neural populations are comingled but Hypocretin and QRFP are never coexpressed in the same neuron.

Transgenic zebrafish larvae that express Brainbow in Hypocretin neurons. Brainbow allows each Hypocretin neuron to be labeled with a different color, which allows the projections of each neuron to be traced throughout the larva.

**PUBLICATIONS**

**2013**


**2012**

Psychophysical and Neural Studies of Perception and Decision Making in the Humans

Summary:
While we continue to examine the dynamic/adaptive nature of human visual perception – including it's crossmodal, representational, sensory-motor, developmental, emotional, and neurophysiological aspects, we continue our research on "Implicit Brain Functions" and "Interpersonal Implicit Communication" supported by JST (Japan Science and Technology Corporation) CREST (Core
Research for Evolutional Science and Technology, started in April, 2010). In these projects, we focus on implicit cognitive processes, emotional decision making, social communication, plasticity, and their neural correlates.

Vigorous collaborations have been conducted between our psychophysics laboratory here, and the CREST Japan site located at NTT Communication Science Laboratories, as well as Harvard MGH, Boston University, Gordon College London, Occidental College, and MetaModal Inc. Besides, we continue collaborative efforts on "social brain," under the Caltech-Tamagawa gCOE (grand Center Of Excellence) program (supported by MEXT, Ministry of Education, Culture, Sports, Science and Technology, Japan, which was started in September, 2008).

Using a variety of methods including eye tracking, high-density EEG, fMRI and MEG, we examine how exactly peripheral sensory stimuli, neural activity in the sensory cortex, and the mental experience of perception are related to each other in the highly plastic fashion. In particular, we aim to understand implicit, as opposed to explicit or conscious, somatic and neural processes that lead to, and thus predict, conscious emotional decision such as preference. Amongst all, most challenging on-going attempts in the laboratory include: (1) the intriguing interactions between predictive processes (prior to and thus predicting the mental event or behavior) and postdictive processes (posterior); (2) the inter-brain causal connectivity under social cooperative interactions; (3) remote tDCS modulation of subcortical reward system; and (4) sensory substitution by visual-auditory devise.

**PUBLICATIONS**

**2013**


Gomi, H., AbeKawa, N. & Shimojo, S. The hand sees visual periphery better than the eye. -Motor dependent visual motion analyses-. *J. Neurosci.*, 33(42), 16502-16509, 2013. PMID 24133255


Liao, H-I, Wu, D-A, Halelamien, N. & Shimojo, S. Cortical stimulation consolidates and reacti
vates visual experience: Neural plasticity from magnetic entrainment of visual activity. Sci. Repor
ts, 3:2228. doi:10.1038/srep02228, 2013. PMID 23863977

Liao, H-I, Shimojo, S. & Yeh, S-L. Happy faces are preferred regardless of familiarity-sad faces are
preferred only when familiar. Emotion, in press. PMID 23356560


Stiles, N. R. B. and Shimojo, S. Sensory Substitution and a Third Kind of “Qualia.” In Johan Wagemans (ed.), The Oxford Handbook of Perceptual Organization, Chap. 43, Oxford University Press, in press.

Yun, K., Watanabe, K., & Shimojo, S. Interpersonal body and neural synchronization as a marker of implicit social interaction. Sci. Reports, 2, 959, doi:10.1038/srep009592012. PMID 23233878

2012


Network Mechanisms of Learning and Memory

Summary:
Our research focuses on the study of learning and memory formation in freely behaving animals at the level of networks of neurons. Previous research has shown that the hippocampus is critical for the formation of long-term declarative memories, and that this hippocampal involvement is time-limited. The current predominant conjecture is that memories are gradually established across distributed neocortical networks through the interactions between cortical and hippocampal circuits.

However, the direct experimental investigation of these interactions has been difficult since, until recently, simultaneous chronic recordings from large numbers of well-isolated single neurons were not technically feasible. These experiments became possible with the advent of the technique of chronic multi-area tetrode recordings in freely behaving rodents. Using this technique we monitor...
the simultaneous activity of large numbers of cortical and hippocampal cells during the acquisition and performance of memory tasks, as well as during the sleep periods preceding and following experience.

Our research efforts focus on analyzing the structure of cortico-hippocampal interactions in the different brain states and on characterizing how this structure is modulated by behavior; how it evolves throughout the learning process; and what it reflects about the intrinsic organization of memory processing at the level of networks of neurons.

Our experimental work is complemented by theoretical studies of network models and the development tools for the analysis of multi-neuronal data.

Images from left to right
Professor Thanos Siapas
Assistant Professor of Biology: Doris Y. Tsao

Professorial Awards and Honors:
2012 NIH Pioneer Award
2012 McKnight Technological Innovations in Neuroscience Award (joint with Dr. William Tyler)

Postdoctoral Scholars:
Xueqi Cheng, Trinity Crapse, Piercesare Grimaldi, Sebastian Moeller

Visitor:
Jonathan Charlesworth

CNS Graduate Student:
Shay Ohayon

Research and Laboratory Staff:
Nicole Schweers

Financial Support:
DARPA Young Faculty Award
Klingenstein Foundation
Merck Foundation
NSF
NIH
Searle Foundation
Alfred Sloan Foundation

Neural Mechanisms for Visual Perception

Summary:
The goal of our lab is to understand the neural mechanisms for vision: how does the brain create a three-dimensional world of objects? We are making three major efforts towards this goal: (1) functionally dissecting the macaque face processing system; (2) functionally dissecting the macaque scene processing system; and (3) developing a new theory of topological optics to explain how visual objects first arise in the brain. We use a combination of fMRI, electrophysiology, optogenetics, and anatomy in monkeys, as well as mathematical modeling.
Face cell: Responses of a face-selective neuron recorded from the middle face patches to 16 real faces, 80 non-face objects, and 432 part intensity stimuli consisting of 12 face regions varying in brightness. The cell has strong selectivity for particular contrast relationships, and this could explain how the cell detects faces.

Face patches: An inflated left hemisphere of the macaque brain showing locations of the six temporal lobe face patches, which each respond significantly more strongly to faces than to non-face objects. A major goal of our lab is to map each of these patches to distinct steps in face processing.

Publications

2012

Professor of Biology:
Kai Zinn, Ph.D.

Postdoctoral Scholars:
Bader Al-Anzi, Namrata Bali, Robert Carrillo, Peter (Hyung-Kook) Lee, Kaushiki Menon

Graduate Student:
Michael Anaya

Staff:
Elena Armand, Patrick Arpp, Violana Nesterova

Financial Support:
Beckman Institute, Caltech
JJSI – Caltech Translational Innovation Partnership
NIH (NINDS)

Molecular Genetic Studies of Insect Nervous System Development

Summary:
Our group is interested in the general problem of how genes control the patterns and functions of synaptic connections in the brain. Our primary experimental system is the fruit fly Drosophila melanogaster. We continue to work on the fly because it has unique advantages for the study of neural development. Although the anatomy of the fly brain does not resemble that of the human brain, about two-thirds of the key genes involved in control of nervous system development in Drosophila have human orthologs or relatives. These include many genes implicated in diseases of the human nervous system. Furthermore, the nervous system is mostly hard-wired by genetics and differs little between individual flies. Many neurons can be individually identified and genetically characterized. This allows investigators to clearly evaluate the contributions of genetics to neural wiring, without the complication of variations in the animal’s experience of the world.

Most of our recent published work has focused on the larval neuromuscular system, which is a subset of the central nervous system (CNS). This is an ideal arena in which to study axonal pathfinding, synaptic targeting, and synaptic plasticity, because of its simplicity and ease of experimental access. In the larva, 32 motoneurons innervate 30 body wall muscle fibers in each abdominal segment. Each axon is targeted to a specific muscle fiber during embryonic development, and the resulting pattern of neuromuscular junction (NMJ) synapses in the larva is highly stereotyped. NMJ presynaptic terminals continue to expand and change as the larva grows, because their strengths must be matched to the sizes of the muscle fibers they drive. This growth is
a form of synaptic plasticity, and is controlled by retrograde signals from the muscle to the neuron. Studies of NMJ synapses in flies are relevant to an understanding of synaptic plasticity in the mammalian brain, because the fly NMJ is a glutamatergic synapse, organized into boutons, that uses ionotropic glutamate receptors that are homologous to vertebrate AMPA receptors.

In addition to the neuromuscular system, we also study the development of the embryonic and larval CNS, and of the larval and pupal optic lobe.

**Receptor tyrosine phosphatases (RPTPs) and their ligands**

RPTPs are neural cell surface receptors that control axon pathfinding, synapse formation, and synaptic plasticity. Mammalian RPTPs are also required for the development of many other organs. RPTP ligands and downstream signaling pathways are poorly understood. We have identified 16 cell-surface ligands for four Drosophila RPTPs. We are investigating how interactions between RPTPs and these ligands control events in neural development, and have created an ‘interactome’ defining the extracellular interaction network for these RPTPs. One ligand, Stranded at Second (Sas), is expressed on glia and interacts in trans with neuronal Ptp10D. Binding to Ptp10D downregulates glial Sas signaling. Sas controls the subcellular distribution of glial transcription factors. When Sas is overexpressed in glia, it produces tumor-like overproliferation that may represent a new fly glioblastoma model. Sas has signaling motifs in its cytoplasmic domain that resemble those in the mammalian amyloid precursor protein, APP, which is implicated in Alzheimer’s disease. We are currently investigating the mechanisms by which Sas signaling alters glial cell biology.

**Dprs, DIPs, and synaptic circuit formation**

Chris Garcia’s group at Stanford, in collaboration with us, conducted a large-scale in vitro interactome study that identified a network in which a subfamily of 21 immunoglobulin-like (Ig) domain cell-surface proteins, the Dprs, interact selectively with an 11-member Ig domain subfamily, the DIPs. Individual Dpr-DIP interactions can be observed in vivo. We are now investigating whether interactions between individual Dprs and DIPs program the formation of synaptic connections in the nervous system. Each Dpr and DIP is expressed in a small subset of neurons in the embryonic, larval, and adult CNS, and the proteins are localized to synapses. At the larval NMJ, interactions between one Dpr and its DIP partner regulate presynaptic terminal development. The data suggest that this is a transsynaptic interaction between presynaptic Dpr and postsynaptic DIP proteins. In the pupal optic lobe, each Dpr and DIP we have examined is expressed in a unique subset of neurons in the higher-order visual areas: the medulla, lobula, and lobula plate.

**Control of synaptic translation**

Local synaptic translation is a mechanism that can allow neurons to separately adjust the strengths of individual synapses and maintain synaptic properties over long time periods. We identified the translational repressor Pumilio (Pum) as a regulator of postsynaptic translation at the NMJ. Pum
negatively regulates expression of the essential translation factor eIF-4E and the glutamate receptor subunit GluRIIA, and binds selectively to the 3'UTRs of their mRNAs. Pum also represses expression of its cofactor Nanos (Nos). Although Nos is required for Pum function during early development, Pum and Nos have antagonistic functions at the NMJ. Pum represses translation of GluRIIA, while Nos represses expression of the alternative glutamate receptor subunit GluRIIB. Arthropod, nematode, and vertebrate Pum orthologs contain aggregation-prone regions, and we have found that one of these is a negative regulator of synaptic Pum function. We are currently attempting to determine whether aggregation is a mechanism used for control of Pum activity in vivo.

**Systematic generation of monoclonal antibodies (mAbs) against native cell-surface proteins**

The study of human proteins and cells, and the development of new therapeutics, would be greatly facilitated by the ability to systematically generate mAbs against large numbers of cell-surface proteins. At present, mAbs are typically made by injecting single purified proteins into mice, then conducting spleen fusions on these mice and screening large numbers of hybridomas for rare antibody lines making antibodies against the protein of interest. This is an expensive and time-consuming process. We have developed a new method in which we express large numbers of human cell-surface protein extracellular domains on the surfaces of 3T3 cells, then inject live cells into syngeneic mice and screen spleen fusions from these mice for reactivity against all of the expressed surface antigens. Using this method, we hope to be able to make high-affinity mAbs against many human proteins in a single fusion, without the necessity of purifying any of the antigens. In addition to distributing these mAbs to the community, we hope to systematically make mAbs against collections of cell-surface proteins involved in tumor metastasis and angiogenesis, and thereby identify new targets for therapy.
PUBLICATIONS

2013


2012


Flow Cytometry Facility
Rochelle Diamond

Genetically-Engineered Mouse Production Facility
Shirley Pease

Nucleic Acid and Protein Sequence Analysis Computing Facility
David Mathog

Protein Expression Center
Jost Vielmetter

Millard and Muriel Jacobs Genetics and Genomics Laboratory
Igor Antoshechkin

Monoclonal Antibody Facility
Susan Ker-hwa Ou

Protein/Peptide Microanalytical Laboratory
Jie Zhou
Summary:
The Caltech Flow Cytometry/Cell Sorting Facility is located in Kerckhoff 020 and 026. The mission of the facility is to foster scientific research by providing the expertise, state-of-the-art resources, and training necessary to solve complex biological research problems and promote cutting edge research on a fee-for-service basis. The facility strives to provide cost effective analysis and cell separation on several different platforms using a myriad of protocols to enhance the scope and quality of the investigator’s research.

The facility is equipped with two research grade flow cytometer cell sorters and two analyzers. This instrumentation can analyze and separate various types of cells and micro-organisms according to their measurable properties of light scatter and fluorescence. The BD FACSAria IIu is capable of analyzing at least nine colors utilizing three lasers (407nm, 488nm, and 633nm), and of carrying out 4-way sorting up to 10,000 cells per second with reliable efficiency and recovery, or 1-way sorting, such as for single-cell cloning, into various cell culture plate configurations. The iCyt Mission Technology Reflection 5-laser/9color (UV, 405, 488, 561, and 633nm) cell sorter with one Highly Automated Parallel Sorting (HAPS) module is contained in a Baker Sterilguard Advance Biosafety cabinet (BSL2). This instrument will be replaced in fall 2013. The Miltenyi Biotec MACSQuant VYB is a 3 laser (405nm, 488nm, 561nm), eight-color analyzer. This analyzer is equipped with automatic startup/wash/shutdown features, absolute counting from specific volume uptake, 96 well plate chilled mini-sampler and chilled tube rack, and robotic reagent handler. It was designed in collaboration with the Caltech facility to provide detection of an increased range of fluorescent proteins used as lineage tracers and gene expression reporters. This utilizes the 561nm yellow laser to accommodate the red fluorescent proteins such as mTomato, mCherry, and DsRed, as well as the standard lasers for CFP (cerulean), YFP (Venus, citrine), EGFP, and others. These reporters can be combined with commonly used fluorochromes like FITC, APC, APC-Alexa 750, Pacific Blue, PE and others depending on the fluorochrome panel. The BD FACSCalibur is a four-color analyzer, together with an offline workstation. The analyzers are available to researchers for self-service analysis provided that they demonstrate competence to use the instrument or take training provided by the facility.

The facility provides consultation services to all researchers on issues relating to flow cytometry, cell sorting, and cell separation techniques (139 consultation appointments with 30 Caltech lab groups, administrative, and JPL, and 21 external consultations last year). In addition, the facility makes Treestar's FlowJo off-line analysis program available to its clients (52) for free and non-
clients (2) for a fee through a network license. The facility has negotiated discounts with two antibody vendors and placed over 85 orders for its clients this past year.

This past two years the facility provided service to 24 laboratories from the Divisions of Biology, Chemistry and Chemical Engineering, Applied Physics, Geology and Planetary Science, and JPL, 60 users were supported. Five researchers were trained in flow cytometry and the use of the BD FACSCalibur analyzer.

Images from left to right:
Rochelle Diamond
Macsquant VYB – Flow Cytometer
Janice Grimm
Diana Perez
Patrick Koen

PUBLICATIONS

2013

Alejandro B Balazs, Jesse D Bloom, Christin M Hong, Dinesh S Rao & David Baltimore. Broad protection against influenza infection by vectored immunoprophylaxis in mice. Nature Biotechnology (2013) doi:10.1038/nbt.2618 Published online 02 June 2013


2012


Genetically Engineered Mouse Services
Director and Member of the Professional Staff:
Shirley Pease

Cryopreservation, Re-derivation and Mouse Colony Management:
Jennifer Alex

Microinjection and Embryonic Stem Cell Culture:
Shirley Pease

**GENETICALLY ENGINEERED MOUSE SERVICES**

**Summary:**
Gene addition in the mammalian system is accomplished by injecting DNA into the pronucleus of a fertilized egg (Gordon *et al.*, 1980). This is a non-targeted event. Targeted disruption of specific genes, however, requires the manipulation of pluripotent embryonic stem (ES) cells *in vitro* and their subsequent return to the embryonic environment for incorporation into the developing embryo (Zijlstra *et al.*, 1989). The resulting chimeric mouse born is useful for two purposes: 1) it is comprised of tissue from two sources, the host embryo and the manipulated stem cells. More importantly, 2) it can be mated to produce descendants that are entirely transgenic, resulting from the ES cell contribution to the germline of the chimeric mouse. (The Nobel Prize in Physiology or Medicine was awarded in 2007 to the pioneers of this technology, Mario Capecchi, Martin Evans and Oliver Smithies.) The facility, in collaboration with Anderson, Baltimore, Fraser, Kennedy, Lester, Patterson, Rothenberg, Simon, Varshavsky and Wold laboratories, has generated multiple transgenic, knockout and knockin mouse strains, amounting to nearly 180 mouse strains. The Facility together with the Baltimore lab, participated in the development of a new method for the introduction of DNA into early-stage embryos (Lois *et al.*, 2002). This method makes use of non-recombinant lentivirus as a vector for the introduction of DNA into one-cell embryos. The method has proven to be highly efficient and promises to be useful for studies in mice and rats, where large numbers of constructs need to be tested. This new methodology also makes feasible the generation of transgenic animals in species that were hitherto impractical to work with, due to the very low numbers of embryos available for use. Since the lentiviral vector method was established, 79 transient or established mouse models have been generated by this means, together with one Tg rat model. Facility staff has performed all embryo manipulation involved in the production of these new lines.

With regard to the injection of DNA into pro-nuclei of pre-implantation stage embryos, this year, GEMs staff have assisted the Fraser lab in an early embryonic developmental study of *Oct4* kinetics, for the prediction of cell lineage patterning, by the injection of DNA into single nuclei of embryos at 2 cell stage, or into the cytoplasm of 2 cell stage blastomeres. The work has been published online: "Oct4 kinetics predict cell lineage patterning in the early mammalian embryo.” Gems staff have also derived new ES cell lines from *Oct4/Nanog* mice, which have been used for quantitative live imaging by Carol Readhead in the Fraser lab.
In tissue culture and the use of murine embryonic stem (mES) cells the Facility has generated over forty new and as yet untested, embryonic stem cell lines, the majority of which are from C57BL/6 mice. This was a by-product of our wish to determine the most efficient approach to deriving such cell lines, since we anticipate that investigators may wish to use ES cells derived from their own genetically altered strains of mouse. Indeed, five such new mES cell lines were derived for the Rothenberg lab. We have multiple murine ES cell lines available for use. Several are on a 129 background, some on a C57BL/6 background and others are F1 cell lines, which are a mix between 129 and C57BL/6 strains. We are able to manipulate and obtain germline transmission from all these ES cell types. C57BL/6 ES cells provide a significant advantage in that the mutation will be established initially on this well understood genetic background, instead of undertaking a two-year breeding program to reach the same point, having initially established the mutation on a sub-optimal genetic background. Hybrid mES cells have been reported to be useful for their vigor. Unlike mES cells from an inbred background, (e.g., C57BL/6 and 129), it is possible to derive from hybrid mES cells live pups that are wholly of ES cell origin. (Nagy et al., 1993) This is made possible by first, the production of tetraploid embryos. These are made by fusion of two blastomeres at the two-cell embryo stage, resulting in the production of a single viable blastomere that has twice the normal number of chromosomes. Such embryos can develop to blastocyst stage, but thereafter, can only contribute to extraembryonic cell lineages. Thus, mES cells injected into the blastocoel cavity in this case, are sole contributors to the developing embryo. Not every mES cell line is able to support development to such a degree. However, we have seen that animals appearing to be wholly of ES cell origin can be produced by injecting mES cells into earlier stage embryos (Valenzuela et al., 2010). The facility is able to offer the use of human ES cells, - two lines from WiCell are available, H1 and H9. We also have close contact with the hES facility at USC, for advisory purposes.

For the fourth year, we organized, set up and taught a four-week course for ten “Bridges to Stem Cells” students. This was in conjunction with PCC and funded by CIRM. Students had the opportunity to derive fibroblasts and mES cell lines, plus execute a gene targeting experiment. Students also successfully derived new C57BL/6 embryonic stem cell lines, using media containing two kinase inhibitors. Some of these cell lines have karyotyped well and are currently being evaluated for use in the generation of new mouse models. These fibroblasts and ES cells will also be useful for teaching at PCC in the Biotechnology course, which is directed by Pam Eversole-Cire, (a former Caltech post-doc).

Once a new mouse model has been characterized, it may be cryopreserved by GEMs staff, or sent to the Mutant Mouse Resource Center, to be made available to the research community in general. We currently have over 100 mouse models cryopreserved. For each line, between 200 and 500 embryos at eight-cell stage have been preserved in liquid nitrogen. There are currently 34,752 embryos frozen in total. We shall continue to preserve embryos from mouse strains carrying multiple mutations. Mouse strains carrying a single mutation will be archived by sperm cryopreservation. Sperm cryopreservation is much more economic than embryo cryopreservation, although the recovery and establishment of the strain by in-vitro fertilization is more costly. The advantages of archiving mouse strains are many. Unique and valuable mouse strains that are currently not in use may be stored economically. In the event that genetic drift should affect any
strain, over time, then the option to return to the original documented genetic material is available. Lastly, in the event of a microbiological or genetic contamination occurring within the mouse facility, we have the resources to set up clean and genetically reliable mouse stocks in an alternative location. We also offer re-derivation as a service, whereby investigators can bring in novel mouse strains from other Institutions without risk of introducing pathogens to CIT stocks. This involves the washing and transfer of pre-implantation embryos from “dirty” incoming mice to “clean” CIT recipient animals.

In addition to the maintenance of nearly 100 different targeted and non-targeted strains, we also maintain colonies of inbred and outbred animals, which are used to support the development of new lines, by investigators at Caltech. We also have many mouse models on both an inbred and an outbred background, plus intercrosses between two or three different, but related, mouse models. In total, we currently maintain nearly 200 separate strains of mouse. GEMs Facility staff have been working with IMSS in the development of software that will assist technicians and investigators in the management of their mice. Amongst its features, this inter-relational system will track the breeding history of each strain and have the ability to generate family trees. The system will also report on production levels for each strain. Users will access the system to enter genotype results and work requests. An electronic signal will be sent to CLAS staff when work requests are made, helping us to manage work requests in a timely manner. The system is basic but easy to use and of value for the reports the system will be able to generate. We are currently offering investigators the use of the system. GEMs is a fee for service facility.

Shirley Pease co-edited "Advanced Protocols for Animal Transgenesis 2011" and previously, Mammalian and Avian Transgenesis, which was published in 2006.
Listed below are the names of the thirteen principal investigators and their postdoctoral fellows or graduate students who are presently using GEMs services.

David Anderson
Haijiang Cai, Celine Chiu, Li Ching Lo,
Wheizhe Hong, Hyosang Lee, Prabhat Kunwar, Ryan Remedios, Dong-Wook Kim,
Moriel Zelikowsky

Alexei Aravin
Dubravka Pezic

David Baltimore
Alex Balazs, Yvette Garcia-Flores, Galimidi,
Shuai Jiang, Jocelyn Kim, Devdoot Majumdar, Arnav Mehta, Raskatov, Alex So, Jimmy Zhao,

Ray Deshaies
Narimon Honapour

Scott Fraser
David Koos, Carol Readhead, Max Ezin

Mary Kennedy
Leslie Schenker,

Henry Lester
Purnima Deshpande, Julie Miwa, Elisha Mackay, Sheri McKinney, Rell Parker,
Andrew Steele, Tegan Wall

Linda Hsieh-Wilson
Joshua Brown, Chaubard,
Chithra Krishnamurthy, Greg Miller,
Claude Rogers, Andrew Wang

Paul Patterson
Antoinette Bailey, Grace Chow, Ben Deverman, Natalia Malkova, Ali Koshnan,
Jan Ko, Wei-Li Wu

Ellen Rothenberg
Mary Yui, Hao Yuan Kueh, Long Li,
Quiloan

David Tirrell
Alborz Mahdavi

Alexander Varshavsky
Christopher Brower, Tri Vu

Barbara Wold
Brian Williams, Ram, Brian

PUBLICATIONS

2011


Millard and Muriel Jacobs Genetics and Genomics Laboratory Director:
Igor Antoshechkin

Staff:
Vijaya Kumar

Lab Website: http://mmjjggl.caltech.edu/

Financial Support:
Millard and Muriel Jacobs Family Foundation

GENETICS AND GENOMICS LABORATORY

Summary:
The Millard and Muriel Jacobs Genetics and Genomics Laboratory provides support for genomics research to the Caltech community with an emphasis on high throughput sequencing. During the period of this report, the Laboratory has worked with groups from the Division of Biology and Biological Engineering, the Division of Chemistry and Chemical Engineering, and the Division of Geological and Planetary Sciences.

Research Support:
Division of Biology and Biological Engineering - The Laboratory performed high throughput sequencing experiments for the groups of professors Alexei Aravin, Angela Stathopoulos, Barbara Wold, Bruce Hay, David Chan, David Baltimore, Ellen Rothenberg, John Allman, Henry Lester, Marianne Bronner, Michael Elowitz, Katalin Fejes Tóth, Sarkis Mazmanian, and Paul Sternberg. The projects ranged from identification of novel transcripts expressed from the selfish PSR chromosome in Nasonia vitripennis (Bruce Hay), to studies of gene regulation by nicotine in dopaminergic neurons (Henry Lester), to de novo sequencing of the genome and developmental transcriptome of the strongylid nematode Haemonchus contortus (Paul Sternberg).

Division of Chemistry and Chemical Engineering – The Laboratory manufactured carbohydrate microarrays for the Hsieh-Wilson group. ChIP-Seq and RNA-Seq experiments were performed for laboratories of Peter Dervan, Long Cai and Jacqueline Barton. Whole genome sequencing and assembly of a bacterial strain were done for the group of Rustem Ismagilov and amplicon analyses were carried out for the group of Bil Clemons.

Division of Geological and Planetary Sciences – Structural variation and SNP identification as well as RNA-seq analyses of several bacterial strains were performed for the members of Dianne Newman laboratory.

Infrastructure and capabilities:
The Laboratory operates Illumina HiSeq2500 high throughput sequencer that features two run modes, rapid run and high output run mode, and has the ability to process one or two flow cells simultaneously. This provides a flexible and scalable platform that supports the broadest range of applications including ChIP-Seq, RNA-Seq, small RNA analysis, de novo genome sequencing, mutation discovery, etc. and is easily adaptable to different study sizes. Rapid run mode provides quick results, allows efficient processing of a limited number of samples, and offers support of longer paired-end 150 base pair reads, while the high output mode is well-suited for larger studies with more samples or when the greatest depth of coverage is required. The Laboratory has all the necessary equipment to support the HTS workflow, including analytical instruments such as Agilent 2100 Bioanalyzer, LightCycler 480 qPCR system, Qubit fluorometer and Nanodrop ND-1000 spectrophotometer that are used for the sample quality assessment and library validation.

The Laboratory has developed an extensive computational infrastructure that allows us to carry out sequence data extraction using the Illumina Sequence Analysis Pipeline and to perform such computation-intensive secondary analyses as identification of binding sites for DNA-interacting proteins, genome assembly, transcriptome analysis, etc. A local copy of UCSC Genome Browser allows us to visualize HTS data within the context of genomic annotations.

**Publications Acknowledging the Laboratory**

**2013**


Adam Z. Rosenthal, Xinning Zhang, Kaitlyn S. Lucey, Elizabeth A. Ottesen, Vikas Trivedi, Harry M. T. Choi, Niles A. Pierce, and Jared R. Leadbetter. Localization transcripts to single cells suggests an important role of uncultured deltaproteobacteria in the termite gut hydrogen economy. *PNAS October 1, 2013 vol. 110 no. 40 16163-16168*


**2012**


Monoclonal Antibody Facility

Summary:
The Monoclonal Antibody Facility provides assistance to researchers wishing to generate monoclonal antibodies (mAbs), ascites fluid and other related services. In addition, the Facility conducts research on the development of novel immunological techniques. By applying the adult tolerization or cyclophosphamide immunosuppression methods, we enhance the probability of producing mAbs against a particular target antigen in a mixture, or against a specific part of a molecule.

We also produce polyclonal ascites Abs by immunizing mice with antigens and then induce the mice with sarcoma cells to obtain high titer, polyclonal ascites fluid. This method can provide 10-18 ml polyclonal ascites fluid per mouse while using small amount of antigen.

In its service capacity, the Facility produced Abs for the following group in 2012-13. Li lab from USC obtained mAbs against Heal Shock protein 90 alpha-F5. Zipurski lab from UCLA obtained mAbs against Drosophila Frazzled protein. Transmembrane Bioscience obtained mAbs against Nora virus capsid protein. Mazmanian lab obtained mAbs against mouse cecal contents.

We are also currently working with the following groups:

Zinn lab is trying to generate Mabs that specifically recognize the native states of cell surface and secreted (CSS) proteins from humans and Drosophila. Transmembrane Bioscience is generating mAbs against Lepto LipL32 & Lepto Lipl 41 (recombinant protein from Leptospira Interrogans).


Nucleic Acid and Protein Sequence Analysis Computing Facility

Manager:
David R. Mathog, Manager

Supervisor:
Stephen L. Mayo

Summary:
The Sequence Analysis Facility (SAF) provides software, computers, and support for the analysis of nucleic acid and protein sequences. Current SAF hardware consists of a Linux web server, a Sun Netra running Solaris, a small 20 node Beowulf cluster, a file server, a 26 ppm duplexing laser printer, and a 16 ppm duplexing color laser printer. The PCs that comprise the "structure analysis facility" are also located in our facility.

Most common programs for sequence analysis are available on the primary server http://saf.bio.caltech.edu/. These include the GCG and EMBOSS Packages, PRIMER3, Phred, Phrap, Cross_Match, Phylip, and HMMER. Many of these may be accessed through the W2H or EMBOSS-Explorer web interfaces. Other programs, custom written programs, or special databases are available on request. The PCs support hardware stereo under both Linux and Windows. Under Linux the programs Coot, O, PyMol, Molscript, CCP4, and Delphi are available. Under Windows WinCoot, Swiss PDB Viewer, O, PyMol, POVray, and various drawing and animation programs may be used. The searchable documentation for these programs is available on the SAF web server. The lecture notes and homework from the introductory course "Fundamentals of Sequence Analysis" are also available on the SAF web server. A web interface allows common compute intensive jobs to run locally on the SAF Beowulf cluster. BLAST executes in a parallel mode so that searches complete faster than they do at the NCBI server. An enhanced parallel HHMER server offers the full set of HMMER programs plus the unique ability to search any of the installed BLAST databases with an HMM. Personal BLAST sequence databases up to 50Mb may be uploaded and searched. The multiple sequence alignment programs T-COFFEE, POA, Probcons, MAFFT, and Muscle are also available. Traces from any DNA sequencing facility may be uploaded and analyzed. The SAF also distributes these site licensed programs for PCs and Macs: DNASTAR, Gene Construction Kit, and, ChemSketch.
Protein Expression Center Director:
Jost G. Vielmetter

Supervisor:
David A. Tirrell

Faculty Advisors:
Pamela J. Bjorkman, Mary B. Kennedy

Staff:
Sravya R. Keremane, Alejandra I. Olvera, Inderjit K. Nangiana, Michael Schamber, Max T. Scott

Financial Support:
Beckmann Institute Fund, HIV Vaccine Research and Design (HIVRAD) Program (P01) (Pamela Bjorkman)
NIH-ENCODE III Consortium Grant (Barbara Wold)

PROTEIN EXPRESSION CENTER

Summary:
The Protein Expression Center (PEC) was established in 1996 to provide protein expression and purification for Caltech and outside researchers. The center provides heterologous expression of recombinant proteins using *E. coli*, insect cells (Baculovirus) and mammalian cells (HEK 293).

The PEC has evolved over the last four years to provide additional capabilities that include expression optimization using multiwell-plate based miniaturization and parallelization. Enhanced purification and analytical capabilities and more recently we assist in developing and applying automated plate based biochemical protein and cell based bioassays. We continue to provide support in the experimental design and execution for Surface Plasmon Resonance (SPR) based measurements of protein-protein interactions or generally of bio-molecular interaction studies. Two instruments are now available for these SPR studies a Biacore T100 (a gift from the Tirrell lab) and a T200 instrument. These instruments continue to enjoy broad interest and use and have become a valued asset in the Caltech research community.

The majority of proteins produced in the mammalian expression system are active human antiviral (influenza and HIV) antibodies and engineered antibody derivatives (Bjorkman and Mayo groups). Mainly we use protein expression based on transient DNA transfection but occasionally we also generate stable cell lines expressing anti-HIV antibodies and in one case a tRNA-synthetase mutant allowing incorporation of non-natural amino acids into expressed proteins in these cell lines.
We produced many "CHIP-able" mAbs for the ENCODE project, (Barbara Wold). "CHIP-able" mAbs are monoclonal antibodies capable of genome wide extraction and characterization of transcription factor specific DNA control sites. We have developed a production pipeline to generate antibodies in mice that are then screened for transcription factor specificity using robotic liquid handling technology. We have produced a total of over a hundred monoclonal antibodies against transcription factors BHLHB2, CSDA, FOX-M1, FOX-P2, GAPBA, HES1, MYF5, NANOG, NRSF, PER1, RBPJ. We are currently focusing on the characterization of the CHIP-ability and other properties of those mAbs.

This year’s highlights at the PEC are the development of several automated bioassays on the Tecan Evo Freedom robotic liquid handling workstation which is an instrument that was purchased by the Steven Mayo group and upgraded with grants from Pamela Bjorkman's and Barbara Wold's group. The instrument is equipped with a variable span-8 liquid handling arm, a 96-channel pipetting arm, a robotic gripper manipulator arm and the following integrated instruments: CO2 incubators (12 slots), a plate shaker, a heating/cooling plate carrier, a filter-plate vacuum manifold, several plate standard and stacking carriers, a PCR machine, a plate reader, and a plate washer. The whole instrument is enclosed in a Biosafety level II cabinet to allow sterile work and work with biohazardous material.

The fully automated ChIP assay has been successfully validated with known ChIP reagents and allows production of up to 96 ChIP samples starting with chromatin extracts and delivering enriched chromatin running in 22 hours unattended.

The second fully automated assay is a cell-based HIV pseudovirus neutralization assay originally developed by David Montefiori and routinely used by the Collaboration for AIDS Vaccine Discovery (CAVD) core neutralization facility. We have validated our automated version of this assay with known assay reagents and have successfully generated a large amount of neutralization data.

These automated assays exemplify the power of laboratory automation and demonstrate how automation can increase the productivity of experimental biology at Caltech.
PUBLICATIONS

Pamela J. Bjorkman group (mammalian cell expression, baculovirus expression and Biacore support):


Barbara Wold group (ENCODE project):


Collaborative Biacore project:


Collaborative binding assay (ALPHA screen) project:

Protein/Peptide Microanalytical Laboratory

Activity
Mass spectrometry of large biomolecules and small organic molecules
Proteomics (In-gel enzymatic protein digestion; LC/MS/MS and data base search)
Protein (Edman) chemical sequencing
Study of novel functions of Os-complexed polymer particles and application on clean-up of SDS detergent in protein and peptide samples.

Equipments
Quadrupole time-of-flight mass spectrometer (ABI QstarXL)
Triple quadrupole mass spectrometer (MDS Sciex API 365)
MALDI-TOF mass spectrometer (ABI Voyager-DE.STR)
Capillary Protein sequencer (Procise cLC, ABI 492)
HPLC nanoflow, 2D (Eksigent)
HPLC (ABI microbore 140D pump, PE UV monitor)
MASCOT server

New Developments
We have been continuing the investigation of insoluble and cross-linked [Os(II)(dmebpy)2Cl]2+-derivatized acrylamide and vinylimidazole copolymer. Sodium dodecyl sulfate (SDS) is a widely-used detergent for the solvation and denaturation of proteins. SDS interferes with the LC separation and suppresses the electrospray ionization signals in mass spectrometry. Our preliminary experiments show that Os-complexed copolymer has the function of anion exchanger, which prefers the adsorption of SDS to protein and peptide in strong acidic condition. The new function makes it possible to clean up SDS present in protein or peptide samples. We are also working on the alkylation of cysteine with NEM(N-Ethylmaleimide) to identify disulfide bonds in proteins. We had collaboration with Professor Helena Ritchie at University of Michigan School of Dentistry to identify a cleavage site of protein with Edman sequencing. The results have been published as the reference.

Services
During the first eight months of fiscal 2014 PPMAL interacted with 13 laboratories. Samples were analyzed from the Division of Biology, and Chemistry and Chemical Engineering (see list). A total of 780 samples were analyzed, including 66 proteomic samples from 7 research groups. Only 4 proteomic samples were analyzed in the same period of previous fiscal year. We got 22 protein samples from four groups for Edman chemical sequencing. In addition to our work for campus faculty and staff, work was also performed for off-campus institution.

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OFF-CAMPUS
Oelschlaeger- Mayo, S. postdoc, former
Chan, Sunney Grad Student Caltech Sopin-Off Transmembrane Biosciences

3

3