DIVISION of BIOLOGY

2011 ANNUAL REPORT

California Institute
of Technology

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06/03/10

Caltech Biologists Provide Molecular Explanation for the Evolution of Tamiflu Resistance

Biologists at the California Institute of Technology (Caltech) have pinpointed molecular changes that helped allow the global spread of resistance to the antiviral medication Tamiflu (oseltamivir) among strains of the seasonal H1N1 flu virus.

David Baltimore

06/30/10

Caltech Researchers Show How Active Immune Tolerance Makes Pregnancy Possible

Understanding of mouse immune-system response to specific fetal antigens also may provide insight into issues that arise during human pregnancies

David Baltimore

07/01/10

Caltech Biologists Discover How T Cells Make a Commitment

When does a cell decide its particular identity? According to biologists at the California Institute of Technology (Caltech), in the case of T cells—immune system cells that help destroy invading pathogens—the answer is when the cells begin expressing a particular gene called Bcl11b.

Ellen Rothenberg

07/19/10

Of Bugs & Brains: Caltech Researchers Discover that Gut Bacteria Affect Multiple Sclerosis

Biologists at the California Institute of Technology (Caltech) have demonstrated a connection between multiple sclerosis (MS)—an autoimmune disorder that affects the brain and spinal cord-and gut bacteria.

Sarkis Mazmanian

08/02/10

Gain and Loss in Optimistic Versus Pessimistic Brains

Our belief as to whether we will likely succeed or fail at a given task—and the consequences of winning or losing—directly affects the levels of neural effort put forth in movement-planning circuits in the human cortex, according to a new brain-imaging study by neuroscientists at the California Institute of Technology (Caltech).

Richard Anderson

08/04/10

Caltech Biologists Discover MicroRNAs that Control Function of Blood Stem Cells

Finding is important for diagnosis and treatment of cancer and anemia

David Baltimore

08/18/10

Two Caltech Scientists Receive 2010 NIH Director's Pioneer Awards

Michael Roukes, Pamela Bjorkman recognized for their "highly innovative approaches" to biomedical research

Pamela Bjorkman

10/04/10

Two Caltech Scientists Named Among 2010 NIH Director's New Innovator Awardees

As part of a National Institutes of Health (NIH) initiative to stimulate highly innovative research and support promising new scientific investigators, two scientists from the California Institute of Technology (Caltech) were named among the 2010 class of the NIH Director's New Innovator Award recipients.

Alexei Aravin

10/27/10

Controlling Individual Cortical Nerve Cells by Human Thought

Five years ago, neuroscientist Christof Koch of the California Institute of Technology (Caltech), neurosurgeon Itzhak Fried of UCLA, and their colleagues discovered that a single neuron in the human brain can function much like a sophisticated computer and recognize people, landmarks, and objects, suggesting that a consistent and explicit code may help transform complex visual representations into long-term and more abstract memories.

Christof Koch

11/09/10

Caltech Scientists Describe the Delicate Balance in the Brain that Controls Fear

Two different neural subtypes act like a seesaw to control the level of fear output from the brain's amygdala

David Anderson

02/01/11

Neurobiologists Find that Weak Electrical Fields in the Brain Help Neurons Fire Together

Coordinated behavior occurs whether or not neurons are actually connected via synapses

Christof Koch

02/09/11

Caltech-Led Team Pinpoints Aggression Neurons in the Brain

Finding could lead to new treatments for impulsive violence

David Anderson

04/21/11

Learning to Tolerate Our Microbial Self

The human gut is filled with 100 trillion symbiotic bacteria—ten times more microbial cells than our own cells—representing close to one thousand different species. "And yet, if you were to eat a piece of chicken with just a few Salmonella, your immune system would mount a potent inflammatory response," says Sarkis K. Mazmanian, assistant professor of biology at the California Institute of Technology (Caltech).

Sarkis Mazmanian

06/03/11

From Pre-Gut Cells to Glory

Caltech researchers discover a genomic control system that regulates gut formation in sea-urchin embryos

Eric Davidson

06/16/11

Caltech Scientist Awarded $5 Million Grant for Plant Research

Elliot Meyerowitz, a plant genetics and developmental biology expert at the California Institute of Technology (Caltech), has been awarded one of 15 five-year, $5 million grants for fundamental plant science research.

Elliot Meyerowitz

06/17/11

Caltech Students Win National and International Prizes

Students from the California Institute of Technology (Caltech) won a large number of awards this spring, including a Fulbright grant, a Gates Cambridge Scholarship, three Paul & Daisy Soros Fellowships for New Americans, two Barry M. Goldwater Scholarships, a Xerox Technical Minority Scholarship, and 36 National Science Foundation Fellowships.

06/21/11

$6 Million Gift To Spur Innovative Research Collaborations Between Caltech, City of Hope

As part of a program to foster innovative biomedical research projects, an anonymous donor has pledged $3 million each to the California Institute of Technology (Caltech) and City of Hope to strengthen scientific collaborations between the two leading research institutions.
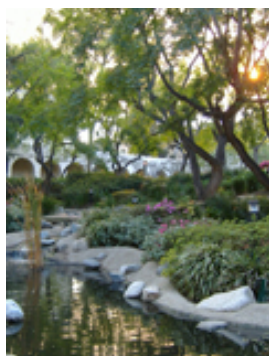
07/25/11

Sharper, Deeper, Faster: Interdisciplinary Team Develops Advanced Live-Imaging Approach

For modern biologists, the ability to capture high-quality, three-dimensional (3D) images of living tissues or organisms over time is necessary to answer problems in areas ranging from genomics to neurobiology developmental biology.

Scott Fraser

Assistant Professor Lea Goentoro joined Caltech's Biology faculty in June, 2011. Lea received a B.S. in Chemical Engineering in 2001 from the University of Wisconsin, Madison and Ph.D. in Chemical Engineering from Princeton University in 2006 under the guidance of Stanislav Shvartsman and Trudi Schüpbach. At Princeton, she combined elegant mathematical modeling and experimental developmental biology approaches to enable new insights into morphogen gradient formation in fly development. She subsequently became a Damon-Runyon Cancer Research Postdoctoral Fellow in the lab of Marc Kirschner at Harvard Medical School where she moved in 2006. There, she studied the Wnt signaling pathway, one of the most important systems for intercellular communication during development. Again she combined mathematical modeling and experiments to address a fundamental question: how the activation the intracellular regulator beta-catening depends on the level of pathway-activating ligands. Remarkably, she discovered that the system controls not the absolute level of beta-catenin, but rather the fold-change in beta-catenin in response to changes in the level of its input. This behavior resembles Weber's law in classic sensory physiology, and provokes a set of new questions about how and why fold-change detection is implemented in cells. Lea intends to pursue such questions using frog eggs and cell culture. This research has already been recognized by an NIH New Innovator award. Her new laboratory is on the second floor of the Broad building.

Professor Rob Phillips officially joined the Division of Biology in 2011, recognizing his active involvement in the Division's graduate, undergraduate and research missions. Rob received his PhD in condensed matter physics at Washington University in 1989. He built a successful career as a theoretical condensed matter physicist and solid mechanician at Brown University (1993-2000), where he was named the first chaired assistant professor. Brown University promoted him to associate professor after 4 years and to full professor after 6 years on the faculty. His research focused on the mechanics of materials and was rounded out by the publication of his book "Crystals, Defects and Microstructures" published by Cambridge University Press. Upon coming to Caltech in 2000, Rob Phillips essentially redefined his area of interest to the interface between biology and physics. His research and teaching in this area has been innovative, including an intense Physical Biology Bootcamp and a novel lab-lecture course Bi1X. His efforts have culminated in the publication of his recent book "Physical Biology of the Cell" written with Jane Kondev and Julie Theriot (published by Garland Press) and a book to be published by Cold Spring Harbor Laboratory Press entitled "Cell Biology By the Numbers", written in collaboration with Ron Milo of the Weizmann Institute. Rob's research follows three complementary threads: theoretical modeling based upon statistical mechanics, experiments using the tools of single-molecule biophysics and *in vivo* measurements at the single-cell level. Work in his laboratory is predicated upon the idea that quantitative data demands quantitative models and that such models call for systematic and precision measurements, which test their predictions stringently.

DR. LIMING WANG IS THE WINNER OF THE FERGUSON AWARD FOR THE 2010-2011 ACADEMIC YEAR. THIS AWARD GOES TO THE STUDENT WHO IS JUDGED BY THE FACULTY TO HAVE PRODUCED THE BEST PH.D. THESIS OVER THE PAST YEAR. DR. WANG PERFORMED HIS GRADUATE STUDIES IN THE LABORATORY OF PROFESSOR DAVID ANDERSON.

CHAIR, STEPHEN MAYO   LIMING WANG   PROF. DAVID ANDERSON

His thesis focused on aggression. Aggression is an evolutionarily conserved behavior across the animal kingdom, critical for the survival and reproduction of animals. Therefore, it is of particular interest to understand how this behavior is regulated. He used the fruit fly Drosophila melanogaster as a model system to understand the regulation of aggression. He identified Cyp6a20, a cytochrome P450, as a gene mediating the suppressive effect of social experience on the intensity of male-male aggression. Notably, Cyp6a20 has been previously identified by profiling Drosophila strains subjected to genetic selection for differences in aggressiveness. Therefore his findings reveal a common genetic target for environmental and heritable influences on aggressiveness. Interestingly, Cyp6a20 is expressed in a subset of non-neuronal support cells associated with pheromone-sensing olfactory sensilla, suggesting that olfactory pheromone(s) may contribute to the regulation of aggression. Consistent with this idea, he found that cis-11-vaccenyl acetate (cVA), a previously identified olfactory pheromone, promotes male-male aggression via a group of olfactory receptor neurons expressing Or67d.

Despite its robust behavioral effect, cVA is not required for baseline male-male aggression, and exogenous cVA does not induce male-female aggression, suggesting that sex specificity of male aggression is independent of cVA. Subsequent studies show that the sex specificity of male social behaviors is determined by a different class of pheromones, named male cuticular hydrocarbons. Male flies perform significantly less aggression and more courtship towards male flies lacking male CHs, both of which can be rescued by synthetic (Z)-7-tricosene (7-T), the most abundant male cuticular hydrocarbon. The opposite influences of 7-T on aggression and courtship are independent, but both require the gustatory receptor Gr32a. Surprisingly, sensitivity to 7-T is required for the aggression-promoting effect of cVA, but not vice versa. Furthermore, the increased courtship in the absence of male cuticular hydrocarbons is induced by pheromone(s) detected by an olfactory receptor Or47b. Thus, male social behaviors are controlled by gustatory pheromones that promote and suppress aggression and courtship, respectively, and whose influences are dominant to olfactory pheromones that enhance these behaviors.

Taken together, Dr. Wang and the laboratory have identified three classes of chemical cues, which are detected by distinct chemosensory modalities and work interdependently to shape the appropriate male social behaviors in Drosophila. How these chemical cues are perceived and conveyed into the central brain, and how they interact to regulate different aspects of male social behaviors, are important questions to be answered in subsequent studies.

David Anderson, Seymour Benzer Professor of Biology
2011 Seymour Benzer Lecture, Oberlin College
2011 Kuffler Lecture, Harvard Medical School
2010 Allen Distinguished Investigator Award

Alexei Aravin, Assistant Professor of Biology
2011 Searle Scholar Award
2010 NIH Director's New Innovator Award
2010 Damon Runyon-Rachleff Innovation Award

David Baltimore, Presient Emeritus, Robert Andrews Millikan Professor of Biology, Nobel Laureate
2010 Gregor Mendel Award
2010 President's Council of Advisors on Science and Technology (PCAST)
Working Group on Influenza Vaccinology

Pamela Bjorkman, Max Delbrück Professor of Biology
2010 NIH Director's Pioneer Award
2010 Honorary Doctor of Science degree; Memorial University of Newfoundland
2011 Named Among Most Powerful Moms in STEM
(Science, Technology, Engineering, and Math) in Working Mother magazine
2010 Special Lectures: Keynote Address, FASEB meeting, Snowmass, CO

Raymond Deshaies, Professor of Biology
2010 Elected Fellow of the American Academy of Arts and Sciences.

Sarkis Mazmanian, Assistant Professor of Biology
2010 Burroughs Wellcome Fund, Investigator in Pathogenesis of Infectious Diseases

Elliot Meyerowitz, George W. Beadle Professor of Biology
2011 Sibthorp Medal by Oxford University
2010 Distinguished Speaker Lecture at the European Laboratory for Molecular Biology in Heidelberg
2011 Blackman Lecture at Oxford University
2011 Named a Howard Hughes Medical Institute-Gordon and Betty Moore Foundation Investigator,
an appointment that will start at the beginning of 2013.

David Prober, Assistant Professor of Biology
2011 Rita Allen Foundation Milton E. Cassel Scholar
2011 NARSAD Young Investigator Award

Athanasios Siapas, Professor of Computation and Neural Systems
2011 NIH Director's Pioneer Award

DIVISION OF BIOLOGY SEMINARS | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

October 2010

Harmit Malik, Div. Basic Sciences, Fred Hutchinson Cancer Research Center
Genetic Conflict: The Usual Suspects and Beyond

Matthew Dalva, Asst. Prof., Dept. of Neuroscience, Univ. Pennsylvania
Deciphering Excitatory Synapse Development

Alan S. Brown, Assoc. Prof., Dept. of Psychiatry, Columbia Univ. Medical Center
Prenatal Risk Factors for Schizophrenia

Matthew Scott, HHMI - Dept. Dev. Biology, Genetics & Bioengineering, Stanford School of Medicine
Communicating with Hedgehogs: Development and Disease

Christopher Harvey, Institute Integrative Genomics, Princeton Univ.
Dissecting the Cellular and Circuit Mechanisms Underlying Navigation in Virtual Reality

November 2010

Roger Perlmutter, Exec. V.P. of Research and Development, Amgen
Bridging Science and Medicine: Molecular Genetics as a Tool for Drug Discovery

Angelika Amon, Center for Cancer Research, MIT
Consequences of Aneuploidy

Kees Murre, Prof. of Molecular Biology, UCSD
Global networks that orchestrate lymphocyte development

Carole LaBonne, Assoc. Prof. Dept. Biochemistry, Molecular Biology and Cell Biology, Northwestern U.
Modulating the Activity of Context Dependent Transcription Factors using Ubiguitin and SUMO

December 2010

John Boothroyd, Prof., Dept. Microbiology and Immunology, Stanford Univ.
Viennese waltz or Pasadena rave: how a score of injected, polymorphic "kinases" determines the character of Toxoplasma's dance with its host.

Chiara Cirelli, Assoc. Prof. Psychiatry, Medical Science Center, Univ. Wisconsin-Madison
Sleep and Synaptic Homeostasis

Sue Celniker, Genome and Computational Biology, Lawrence-Berkeley National Lab
Exploration of the Drosophila Transcriptome in Time and Space

January 2011

Owen Witte, David Geffen School of Medicine, UCLA
Targeting Tissue Stem Cells for Epithelial Cancer

Peter K. Jackson, Director and Staff Scientist, Genentech, Inc.
How the Cell Smells: How Deficiencies in Primary Cilia Cause Sensory, Neural, and Renal Defects & Obesity

Weigle Lecture - **Fred Alt**, HHMI, Dept. Genetics, Children's Hospital Boston
Two Short Stories: A Master IgH V(D)J Recombination Control Region and the B Cell Translocatome

Marcus Meister, Dept. Molecular and Cell Biology, Harvard Univ.
Neural computations in the Retina and Beyond

William Greenleaf, Research Fellow, Dept. Chemistry and Chemical Biology, Harvard Univ.
The Power of the Force: Probing Transcription and Riboswitch Folding with High-resolution Optical Tweezers

Division of Biology Seminars | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

February 2011

Kitai Kim, Children's Hospital Boston, Harvard Medical School
Nature and Nurture: Generation of Histocompatible Pluripotent Cells and Role of Epigenetic Memory

Andreas Hochwagen, Whitehead Fellow, MIT
The Rules of Meiotic Chromosome Fragmentation

Viviana Gradinaru, Research Assoc., CNC Center, Stanford Univ.
Bioengineering Tools for Mapping and Mending Dysfunctional Brain Circuits

Alexander Rich, Prof. Biology, MIT
The Role of Z-DNA and its Binding Proteins in Immunity and Infection

Bosiljka Tasic, Dept. Biology, Stanford Univ.
Expanding the Molecular Genetics Toolkit in Flies and Mice: Transgenesis and Mosaic Analysis

Alex Palazzo, Asst. Prof. Biochemistry, Univ. Toronto
Beyond the Signal Sequence Hypothesis: Nuclear Export and Endoplasmic Reticulum Targeting of mRNAs

Vanessa Ruta, Columbia Univ. Medical Center
From the Perifery through the Protocerebrum: Tracing Olfactory Circuits in the Fly Brain

March 2011

Athanasios Typas, Post doc Research, Dept. Microbiology and Immunology, UCSF
High Throughput Interaction Profiling Provides Mechanistic Insights into Key Bacterial Cellular Process

Todd Roberts, Duke Univ. for Brain Scieince
Imaging the Early Stages of Behavioral Learning

Marta Morey-Ramonell, HHMI, UCLA
Molecular and Genetic Approaches to Study Neural Circuit Assembly

Ilana Witten, Post doc scholar Bioengineering, Stanford Univ.
The neuromodulatoy control of reward: an optogenetic approach

Daniel Huber, HHMI, Janelia Farm Research Campus
Learning under the Microscope: Long-term Imaging of Neuronal Activity in the Mouse Motor Cortex

Greg Hannon, Cold Springs Harbor Lab
DNA Methylation in Developing Germ Cells

Cris Neil, Postdoc Research, Dept. Physiology, UCSF
Function and Assembly of Visual Circuits in Mouse Cortex

Zemer Gitai, Asst. Prof., Dept. Molecular Biology, Princeton Univ.
The Expanding Universe of the Bacterial Cytoskeleton

Ronald Germain, Deputy Chief, Lymphocyte Biology, NIH/NIAID
Developing an Integrated Understanding of the Immune System Using Molecular Studies, Movie-making, and Modeling

Hugo Bellan, Prof. Developmental Biology, Baylor College Medicine
Mitochondria and Neurodegeneration

DIVISION OF BIOLOGY SEMINARS | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

April 2011

Veronica van Heyningen, MRC Human Genetics Unit, Western General Hospital, Edinburgh
Making Eyes: Gene Regulation, Networks and Modulation

Ann Hochschild, Dept. Microbiology and Molecular Genetics, Harvard Medical School
Transplanting Yeast Prion Proteins into *E. Coli* Cells

Brenda Andrews, Centre for Cell and Biomolecular Research, Univ. Toronto
Mapping Biological Pathways and Networks Using Yeast Functional Genomics

David Glover, Dept. of Genetics, Univ. Cambridge
Roles of the Greatwall, Polo and Plk4 Protein Kinases in the Centrosome Duplication Cycle

John Dani, Dept. Neuroscience, Baylor College Medicine
Cellular and Synaptic Mechanisms Contributing to Nicotine Addiction

Magdalena Zernicka-Goetz, Prof., Gordon Institute, Univ. Cambridge
The switches between pluripotency and differentiation in open view and behind closed doors in the mouse embryo

Gerry Weinmaster, Prof., Biological Chemistry, David Geffen School Medicine, UCLA
Ligand Endocytosis and Mechanical Force in Activation of Notch Signaling

May 2011

Horowitz Lecture - **Ruth Lehman**, Prof., Dept. Cell Biology, Langone Medical Center, New York Univ.
Setting and Protecting Germline Fate

Neil Burgess, Inst. Cognitive Neuroscience, Univ. College London
Neural Mechanisms of Spatial Cognition

Jay Neitz, Dept. Ophthalmology, Univ. Washington
Gene Therapy for Red-Green Color Blindness

Lou Staudt, Center for Cancer Research, National Cancer Institute
Converging on the Achilles Heel of Cancer through Functional and Structural Genomic

Daniel Portnoy, Dept. Molecular and Cell Biology, UC Berkeley
Discrimination of Pathogenic and Nonpathogenic Microbes by the Innate Immune System

Weigle Lecture

Physist - Caltech Research Associate

Renowned Swiss scientist, took his Ph.D.in physics at the University of Geneva and returned as head of the Department of Physics in 1931, He came to Caltech as a research associate in Biophysics and focused on investigations of x-rays, solid state physics, and the interactions of waves. In Biology he researched the genetics of bacteriophages--viruses that attack bacteria. The Weigle fund was established to bring persons of outstanding ability to Caltech to speak, work or study in the Division of Biology. This includes scholars, researchers and students who bring new ideas and concepts, new teaching methods, and high potential to Caltech.

Jean Jacques Weigle (1902-1969)

January 2011
Fred Alt
HHMI Investigator - Department of Genetics - Children's Hospital Boston, Harvard Medical School
"Two Short Stories: A Master IgH V(D)J Recombination Control Region and the B Cell Translocatome"
Host: David Baltimore

Wiersma Visiting Professor Lecture

Caltech Professor of Biology, 1933-1976.

Wiersma was born and educated in the Netherlands. His early work on comparative physiology followed in the footsteps of Thomas H. Huxley, who wote the classic 1879 book The Crayfish, and of Willem Einthoven who invented the electrocardiogram. Thomas Hunt Morgan recruited both Wiersma and his friend Anthonie Van Harreveld to Caltech. Wiersma's major contributions to neuroscience concerned crustacean nervous systems, and his mentees in these studies included Harold Atwood, Edwin Furshpan, Raymon Glantz, and Katsuo Ikeda. He originated the practice of studying neurons that could be identified from one animal to the next, leading to his concept of "command neurons". He and his wife funded Caltech's Wiersma Visting Professor program.

Cornelis A. G. Wiersma (1905-1979)

January 2011
Marcus Meister, Ph.D.
Jeff C. Tarr Professor of Molecular and Cellular Biology, Harvard University
"Neural computations in the Retina and Beyond"
Host: David Anderson

Horowitz Lecture

Genetist - Caltech Professor

This lecture series was endowed by the renowned geneticist Norman H. Horowitz, Professor of Biology at Caltech from 1946 to 1982. Studying the "biochemical genetics" of the fungus Neurospora, his work helped to support the "one gene - one enzyme" hypothesis proposed by George Beadle and Edward Tatum. In the 1960s and 1970s, he was involved in the exploration of Mars and worked with the Jet Propulsion Laboratory on several missions. He was Chair of the Division of Biology at Caltech from 1977 to 1980.

Norman Horowitz (1902-1969)

May 2011
Ruth Lehmann, Ph.D.
Director –Skirball Institute, HHMI Investigator
Laura and Issac Perlmutter Professor of Cell Biology, NYU Langone Medical Center
"Setting and Protecting Germline Fate"
Host: Alexei Aravin

IN CELEBRATION OF ELLIOT MEYEROWITZ' 60ᵀᴴ BIRTHDAY

Steve Mayo, Chair, Division of Biology
Opening Remarks

Mark Garfinkel (1980-1988 graduate student)
University of Alabama at Birmningham
Maggot Spit and Optic Lobes: The Drosophila Days

Eric Mjolsness (2010 - present visiting professor), Moderator
UC Irvine

Adrienne Roeder (2005 - present postdoc)
Caltech
The Meyerowitz lab today: integrating plant development over space and time

Marcus Heisler (2001 - 2008 postdoc)
EMBL Hiedelberg
Cell polarity in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport

Jeff Long (1999-2003 postdoc)
Salk Institute
Making heads and tails our of Arabidopsis embryogenesis

Toshiro Ito (1997-2005 postdoc)
Temasek Life Sciences Laboratory
A competition model for timing control of floral meristems

Neil Gutterson
Mendel Biotechnology
Application of plant pathway regulation to enhance crop productivity

Doris Wagner (1995 - 2000 postdoc)
University of Pennsylvania
What can we learn by identifying the direct targets of a master transcriptional regulator?

Xuemei Chen (1995 - 1999 postdoc)
UC Riverside
Metabolism and modes of action of plant microRNAs

David Smyth (1988 visiting scientist), Moderator
Monash University

Steve Jacobsen (1993 - 1998 postdoc)
UCLA
Genetics and genomics of gene silencing in Arabidopsis

Leslie Sieburth (1990 - 1994 postdoc)
University of Utah
Long-distance signaling: Toward identifying the bps1 long-distance signal

Hajime Sakai (1990 - 1997 postdoc)
DuPont
Two big remaining questions in science

Detlef Weigel (1989 - 1993 postdoc)
Max Planck Institute in Tubingen
Harnessing the power of next-generation genetics: the plant immune system at the nexus of trade-ofs affecting fitness and gene flow

Hong Ma (1988 - 1990 postdoc)
Penn State University / Fudan University
Cell-cell signaling and transcriptional regulation required for normal anther development

Marty Yanofsky (1987 - 1990 postdoc)
UC San Diego
Flowers, fruit and other stories

John Bowman (1986 - 1991 graduate student)
Monash University
Patterning genes in land plants

Sponsors:

Division of Biology - Caltech
Beckman Institute
Pioneer Hi-Bred, a DuPont Business
Mendel Biotechnology
International Plant Molecular Biology (I-PMB)
American Society of Plant Biologists
Monsanto Company

Organizers:

Caren Chang
(1984 - 1988 graduate student; 1989 - 1994 postdoc)

Thomas Jack
(1990 - 1993 postdoc)

Jose Luis Riechmann
(1993 - 1998 postdoc)

Special thanks to:
Cynthia Carlson, Assistant to the Chair of Biology

DIVISION of BIOLOGY
2011 ANNUAL REPORT
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DIVISION OF BIOLOGY GRADUATE STUDENTS | 2011

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Marie Suver[1]

Frederick Tan[2]
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Cory Tobin
Sina Tootonian[1]
Nathanie Trisnadi
Vikas Trivedi[3]
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Jonathan Valencia
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Lawrence Wade
Ward Walkup[2]
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Shuo Wang[1]
Yun Elisabeth Wang
Timothy Wannier
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Alexandre Webster
Yunji Wu

Jennifer Yang[2]
John Yong
Jonathan W. Young
Kenneth Yu

Jingli Zhang
Jimmy Zhao

[1]*Computational & Neural Systems (CNS)*
[2]*Biochemistry & Molecular Biophysics*
[3]*Bioengineering*
[4]*Control and Dynamical Systems*

DOCTOR OF PHILOSOPHY

Nicholas R. Ballor, Ph.D.
Biochemistry and Molecular Biophysics
B.S., Michigan Technological University 2005;
 M.S., California Institute of Technology 2009.
Thesis: Hydrogenases and Hydrogen Sensors in the
Symbiotic Microbial Communities of Wood-Feeding
Termites

Matthew Edward Barnet, Ph.D.
Biochemistry and Molecular Biophysics
B.S., California Institute of Technology 1999.
Thesis: Dynamics of Sea Urchin Gastrulation Revealed
by Tracking Cells of Diverse Lineage and Regulatory
State

Charles Walter Bugg, Ph.D.
Biochemistry and Molecular Biophysics
A.A., Mississippi Gulf Coast Community College 1997;
B.S., University of Southern Mississippi 2000;
M.S., California Institute of Technology 2004.
Thesis: Domain Organization of Mutant Huntingtin
Fibrils

Janet Chow, Ph.D.
Biology
B.S.,University of California, Los Angeles 2006.
Thesis: A Pathobiont of the Mammalian Microbiota Balances
Intestinal Inflammation and Colonization.

Sagar S. Damle, Ph.D.
Biology
B.S., University of California, Los Angeles 1998.
Thesis: A Study of Information Processing in the Sea
Urchin Embryo by Rewiring Mesodermal Gene
Regulatory Networks and *cis*-Regulatory Analysis of
Skeletogenic Regulators

Barbara Karmen Kraatz Fortini, Ph.D.
Biology
B.S., California Institute of Technology 2002.
Thesis: Biochemical and Genetic Studies of Genomic
Stability

Tara Adele Gomez, Ph.D.
Biology
B.S., University of California, Los Angeles 2005.
Thesis: Mutational Analysis of Ubiquitin Shuttle
Receptor Docking Sites on the 26S Proteasome

Anne Christina Hergarden, Ph.D.
Biology
B.S., University of Texas at Austin 1997.
Thesis: The Role of Peptidergic Neurons in the
Regulation of Satiety in *Drosophila*

Steven Gregory Kuntz, Ph.D.
Biochemistry and Molecular Biophysics
B.S., University of California, San Diego 2003.
Thesis: *h/h-1* and the *C. elegans* Body Wall Muscle
Transcriptional Differentiation Network

Oren N. Schaedel, Ph.D.
Biology
B.A., Technion – Israel Institute of Technology 2005.
Thesis: Dynamic Regulation of the Dauer Decision

Arbel David Tadmor, Ph.D.
Biochemistry and Molecular Biophysics
B.S., Technion – Israel Institute of Technology 1997;
M.S., Weizmann Institute of Science 2007.
Thesis: Phage-Host Interaction in Nature

Sarah Lynn Tulin, Ph.D.
Biology
B.S., The Johns Hopkins University 2003.
Thesis: Analysis of *Drosophila* Fibroblast Growth
Factor Functional Domains

Lawrence A. Wade, Ph.D.
Molecular Biology and Biochemistry
B.A., California State University, Fullerton 1980.
Thesis: An Evanescent Perspective on Cells

Liming Wang, Ph.D.
Biology
B.S., Peking University 2005.
Thesis: Genetic and Neural Regulation of Aggressive
Behavior in *Drosophila melanogaster*

Catherine Marie Ward, Ph.D.
Biochemistry and Molecular Biophysics
B.S., North Carolina State University 2003.
Thesis: *Medea* Selfish Genetic Elements as Tools for
Altering Traits of Wild Populations: A Theoretical
Analysis

MASTER OF SCIENCE

Joy Lin *(Geobiology)*
B.S., California Institute of Technology 2011.

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B.A., B.S., University of North Carolina, Chapel Hill 2009.

Daniel Aaron Stolper *(Geobiology)*
B.A., Harvard College 2008.

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Erin Noelle Zampaglione San Jose, California *Biology*

* *Students whose names are followed by an asterisk are being graduated with honor in accordance with a vote of the faculty.*

† *Students whose names are followed by a dagger are close to completion and will receive diplomas at the end of the academic year in which all graduation requirements are met.*

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[1]*Joint appointment with Howard Hughes Medical Institute*

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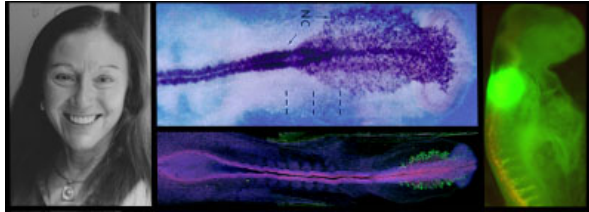# DEVELOPMENTAL AND REGULATORY BIOLOGY FACULTY | 2011

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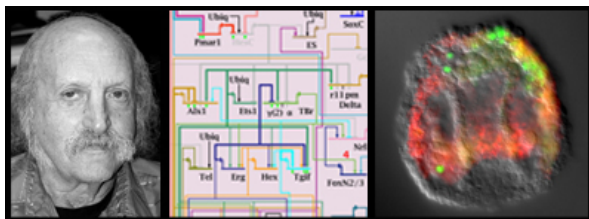# DEVELOPMENTAL AND REGULATORY BIOLOGY FACULTY | 2011
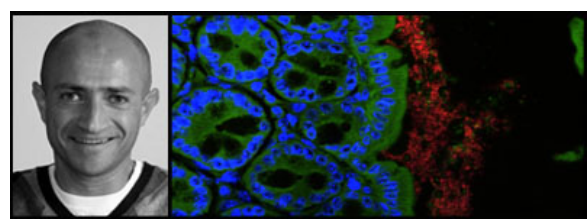
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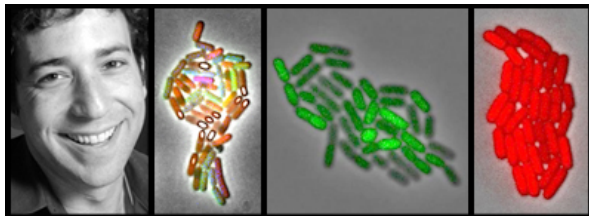*Albert Billings Ruddock Professor of Biology*

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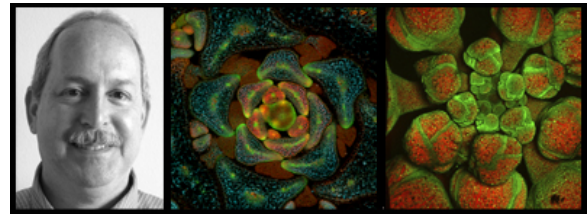*Professor of Biology*

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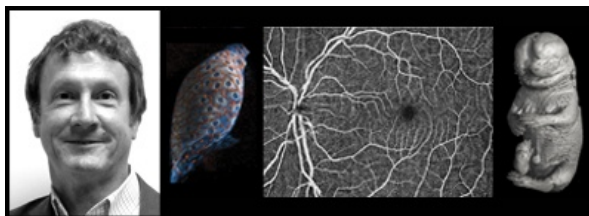*Norman Chandler Professor of Cell Biology*

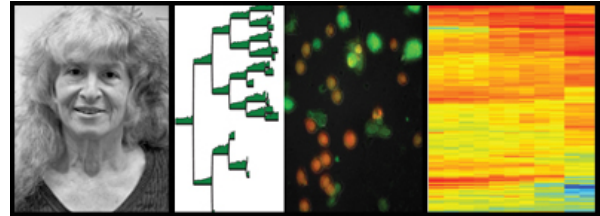Sarkis Mazmanian
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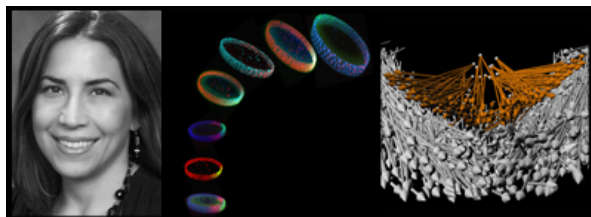*Anna L. Rosen Professor of Biology and
Professor of Bioengineering*

Ellen Rothenberg
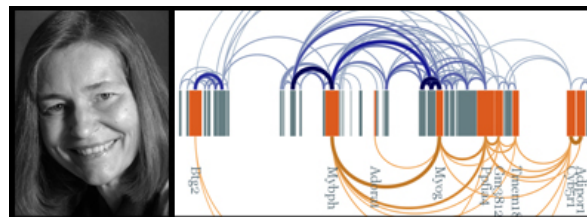*Albert Billings Ruddock Professor of Biology*

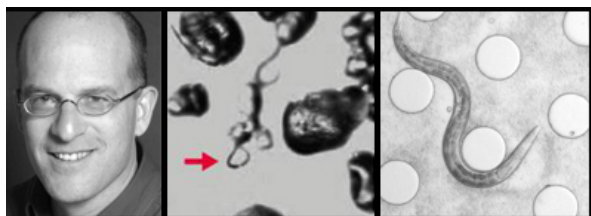# DEVELOPMENTAL AND REGULATORY BIOLOGY FACULTY | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

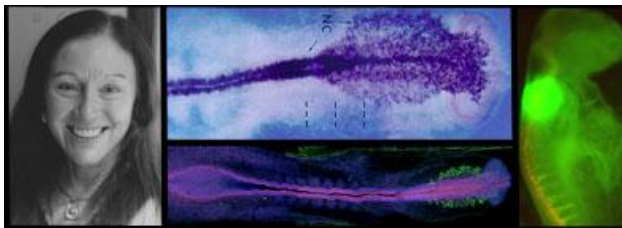Angela Stathopoulos
Assistant Professor of Biology

Barbara Wold
Bren Professor of Molecular Biology

Paul Sternberg
Thomas Hunt Morgan Professor of Biology

MARIANNE BRONNER LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

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Senior Research Fellows:
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Postdoctoral Fellows:
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Graduate Students:
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Research and Laboratory Staff:
David Arce, Mary Flowers, Constanza Gonzalez, Martha Henderson, Matthew Jones, Joanne Tan-Cabugao

Contributors:
Robert Cerny, Daniel Medeiros, Alexey Terskikh, Jose Xavier-Neto

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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.

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.

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Images, left to right:
Professor Marianne Bronner
In situ expression pattern of transcription factor Snail2
Antibody staining for HNK-1 epitope
GFP reporter expression for an enhancer encoding transcription factor Sox10.

PUBLICATIONS

2011

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Eric Davidson Lab Annual Report | 2011

California Institute of Technology
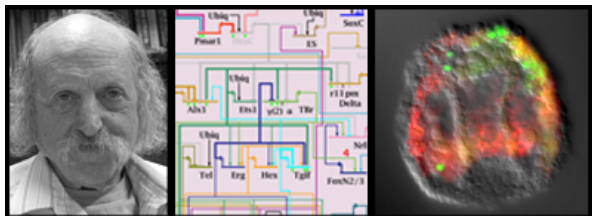
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Eric H. Davidson

**Distinguished Carnegie
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Roy J. Britten

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Senior Research Associate:
R. Andrew Cameron

Member of the Professional Staff:
Andrew Ransick

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Undergraduates 2011:
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KML Staff:
Patrick S. Leahy, Julie Gilbert

Computational Staff:
David Felt, Parul Kudtarkar, Ung-Jin Kim, Alex Tang

Administrative Staff: Jane Rigg, Deanna Thomas
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Douglas H. Erwin, National Museum of Natural History, Washington, DC
Richard Gibbs, Human Genome Sequencing Center, Baylor College of Medicine
Sorin Istrail, Brown University, Providence, RI
David McClay, Duke University, Durham, NC

An Integrated Approach to the study of Embryonic Development in Sea Urchins

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. Among these are: 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 for high throughput measurement and for specific perturbation of gene expression in the embryo; sensitive and dramatic means of visualizing spatial gene expression; availability of embryonic material at all seasons of the year; an optically clear, easily handled embryo that is remarkably able to withstand micromanipulations, injections and blastomere recombination and disaggregation procedures; a very well understood and relatively simple embryonic process, known from over a century of research; and in-house egg-to-egg culture of the species we work with, *Strongylocentrotus purpuratu*s (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 cDNA and BAC libraries for many other species of sea urchin, 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). We utilize additional experimental echinoderm models for evolutionary GRN comparisons, viz. the starfish *Patiria miniata* also of local provenance, and the (in certain respects) pleisiomorphic "pencil urchin" *Eucidaris tribuloides*. The embryos of both these animals prove to be as excellent subjects for gene regulation molecular biology as is that of the sea urchin. We pursue an integrated, "vertical" mode of experimental analysis, in that our experiments are directed at all levels of biological organization, extending 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 main research initiatives in our laboratories at the present time are as follows:

ii. 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. As of late 2011, 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 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, Natnaree Siriwon;* mesoderm: *Dr. Andrew Ransick, Dr. Stefan Materna*) **Graduated 2011

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, oral and aboral 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 of 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 complete (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, Dr. Emmanuel Faure, 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 region, is being studied in other sea urchin laboratories). The ectoderm is a complex mosaic of

spatial regulatory states. Both the aboral and oral ectoderms produce several 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, GRNs are emerging that will soon approach the completeness of the endomesodermal GRNs. These GRNs will then be used for expansion to nearly the whole embryo of the predictive dynamic Boolean model. *(Dr. Enhu Li, Dr. Smadar Ben-Tabou deLeon, Dr. Julius Barsi)*

iv. High throughput *cis*-regulatory analysis, and its impact on GRN analysis: A recent technological breakthrough is revolutionizing the processes of GRN validation and discovery, as well as vastly improving the efficiency with which *cis*-regulatory control systems can be analyzed. This is the development of multiplexed *cis*-regulatory analysis using vectors marked with "bar-coded" sequence tags, up to 130 of which can be injected together into a single batch of sea urchin eggs ("nanotags"). The individual vectors are regulated independently *in vivo* and their outputs can be de-convolved at once by NanoString technology. To this end a NanoString codeset was designed to recognize the barcode tag sequences. Expression of individual vectors can also be examined spatially since each vector also expresses GFP. The effects of perturbations of gene expression can now be determined at the same time on endogenous genes and on their *cis*-regulatory systems isolated by high throughput functional genomic scans. The uses of *cis*-regulatory nanotag technology include: *i,* scans of very large genomic regions to find *cis*-regulatory sequences in which large numbers of constructs can be assessed together; *ii,* time course measurements of quantitative output of >100 diverse *cis*-regulatory constructs at once; *iii,* effects of perturbations of regulatory state on large numbers of *cis*-regulatory constructs; *iv,* analysis of >100 mutant constructs in single experiments. *(Dr. Jongmin Nam, Ping Dong)*

v. 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, hnf6, tbx2/3, dlx, hox11/13b. (Dr. Smadar Ben-Tabou deLeon, Dr. Julius Barsi, Dr. R. Andrew Cameron, Dr. Andrew Ransick; Dr. Sagar Damle*, Miao Cui)*

vi. Embryonic transcriptome database and analysis: We have embarked on a large-scale *S. purpuratus* transcriptome sequencing and analysis effort. 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 in the genome sequence in over 1/3 of cases from the a priori predictions to the actual 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 (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)*

vii. 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 recombineered BAC vectors, in which a flourophore 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 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. 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, Dr. Qiang Tu, Erika Vielmas)*

viii. 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 in sea stars. ***(Dr. Feng Gao)***

ix. ***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 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)***

x. 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* are in process of being 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. 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)***

xi. 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)***

Biotapestry. The GRN visualization software BioTapestry, developed by our collaborator Wm. Longabaugh (Institute for Systems Biology), is now in wide use, and we are further expanding its capacities so that it will automatically generate allowed network architectures from machine readable time and space of expression data plus results of perturbation analysis. A second-generation version with much enhanced capacities has been published. ***(Wm. Longabaugh, 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. ***(Julie Hahn, Ping Dong, Miki Jun)***

Dynamic imaging of regulatory state clones. A method was perfected to allow periodic confocal imaging of immobilized embryos for many hours during development, and utilized to track clones of cells expressing given BAC constructs. A main result was the distinct cell behavior during gastrulation of veg2 cells expressing *foxa*, which execute convergent extension to build the archenteron after 30h, vs.veg1 cells expressing *evenskipped*, which much later invaginate as a coherent cone to generate the hindgut. The immobilization method is also being used to create a standardized canonical digital embryo through time, in which gene expression patterns and ultimately network circuitry can be mounted. ***(Dr. Emmanuel Faure, Dr. Isabelle Peter, Dr. Mat Barnett*)***

Re-engineering the GRN to achieve a predicted cell fate switch. We used recombineered BAC vectors to place under control of a skeletogenic regulatory system a gene encoding an upstream regulator of pigment cell specification. On introduction into the embryo this BAC causes skeletogenic cells to switch fate and become differentiated pigment cells. Some cells adopt a mixed fate but many complete or nearly complete transformation to the pigment cell state. We observed a cryptic repression function that down regulates the skeletogenic GRN in the transformation process. ***(Sagar Damle*)***

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, Parul Kudtarkar, David Felt, Autumn Yuan)*

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)
Expression of endogenous hnf6 gene (red) in ciliated band of a sea urchin embryo, visualized by double in situ hybridization, together with mosaic expression of hnf6 cis-regulatory construct (green). (J. C. Barsi)

PUBLICATIONS

2011

Damle, S. and Davidson, E.H. (2011) **Precise *cis*-regulatory control of spatial and temporal expression of the alx-1 gene in the skeletogenic lineage of *S. purpuratus*.** *Dev. Biol*. **357**:505-517. PMID: 21723273.

Davidson, E.H. (2011) **Evolutionary bioscience as regulatory systems biology.** *Dev. Biol*. **357**:35-40.

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MICHAEL ELOWITZ LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY
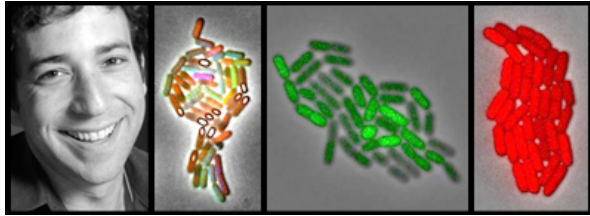
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NATURAL AND SYNTHETIC GENE CIRCUIT DYNAMICS IN CELL AND DEVELOPMENTAL CIRCUITS

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 approach – "synthetic biology" – allows one to analyze 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.

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Images from left to right:
Professor Michael Elowitz
Sporulation synopsis
Energy
Ethanol

PUBLICATIONS

2011

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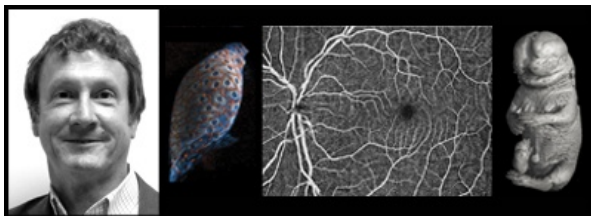
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*Graduated - or, are no longer at Caltech
**Rotation student*

I MAGING C ELL L INEAGES AND C ELL I NTERACTIONS IN D EVELOPING E MBRYOS

Summary:

Our laboratory has dedicated itself to an integrative approach to defining the cell and molecular basis of embryonic patterning, in which *in vivo* imaging tools play a central role. Our approach has been to develop imaging tools that are sufficiently robust so that the movements, lineages and gene expression in living cells can be analyzed as an adjunct to the techniques more typically employed by systems biology. Systems biology provides a means to organize the explosion of data from molecular approaches and the dramatic progress from *in vitro* culture assays, but it requires some means to test the proposed linkages. Our goal is to test these proposed mechanisms in the intact embryo, with the hope of moving forward to an understanding of which of the potential mechanisms operate in the natural biological context. There are many challenges to such tests, including the tagging of cells or molecules so that they can be followed in the intact system, the visualization of the tagged structures, and the interpretation of the time-varying events these images represent. Solutions to these challenges require the coordinated efforts of researchers spanning the life and physical sciences.

In the past year we have made significant advances in imaging fast events in the developing embryo, ranging from the movements of cilia in the developing ear and left-right organizer and the flows these cilia generate, to the workings of the beating heart as it takes shape. This has required the development of new image acquisition and processing tools that offer quantitative analyses of the key events. These tools are proving to be generalizable to other systems such as the *Drosophila* embryo, offering access to events that have previously been too difficult to address.

In parallel with the refinement of new imaging tools, we have been creating new and more efficient means for creating embryos with genetically encoded fluorescent tags. Through random insertion into the genome, this approach permits the creation of functional fusions between a host protein and a fluorescent protein, permitting cells and developmental questions to be posed in normally developing embryos. The creation of these new lines is dramatically more efficient than previous approaches, making it possible for even modest-sized facilities to embark on large-scale screens. This increased efficiency of creating marked strains requires a parallel increase in imaging technologies, and the refinement of *in toto* image acquisition and analysis tools answers this challenge.

Recently we have translated our imaging tools to clinical utility, with the hope of creating fast and accurate diagnostic tools for age-related macular degeneration and diabetic retinopathy. These tools are able to generate striking images of the retinal vasculature and determine photoreceptor health, offering rapid and non-invasive diagnostics for eye disease processes before vision loss.

There have been dramatic advances in other areas as well, ranging from the refinement of new sensor technologies with researchers in the Kavli Nanoscience Institute, to improved tools for acquiring information from MRI images and collecting 4D light microscopic images. Our

combination of modern and classical technologies continues to offer surprises and insights.

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Images from left to right:
Professor Scott Fraser
Imaging of live heart in a transgenic zebrafish using our new gene trap technology (blue: tropomyosin; red: desmin)
Imaging of the vasculature in a healthy human eye without the use of exogenous dyes.
MRI microscopy of developing mouse embryo, offering new insights into cleft palate and other birth defects.

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CELL DEATH, NEURODEGENERATION, MICRORNAS, SELFISH GENETIC ELEMENTS, POPULATION GENETICS 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.

We are also interested in the molecular mechanisms that underlie parthenogenesis – the ability of females to reproduce without males. While parthenogenesis is known to occur in many different animals, the molecular mechanisms that allow a switch from sexual to parthenogenetic reproduction are unknown for any species. To get at this question we have sequenced genomes of sexual and parthenogenetic versions of *Drosophila pallidosa* and are using classical mapping and RNA-seq approaches to identify the genes involved.

***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*

1:E15). 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-kB 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.
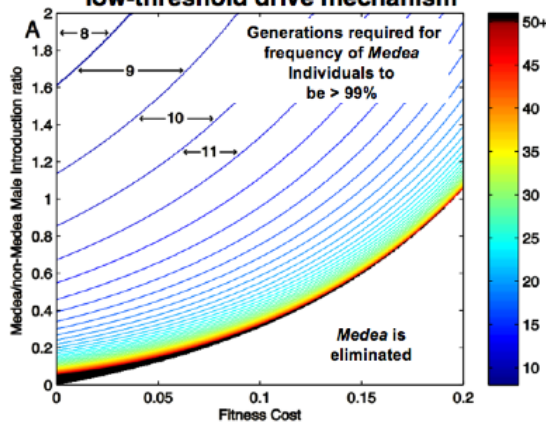
Driving genes for disease refractoriness into wild pest insect populations with Medea selfish genetic elements (*Haixia Huang, Catherine Ward, Geoff Pittman, Omar Akbari, Arun Kumar, Zachary Sun, Philippos Papathanos, Jeremy Sandler, Bruce A. Hay*). An attractive approach to suppressing mosquito-borne 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 casteneum* 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.

Figure 1

Population replacement with *Medea*, a low-threshold drive mechanism

Figure 2
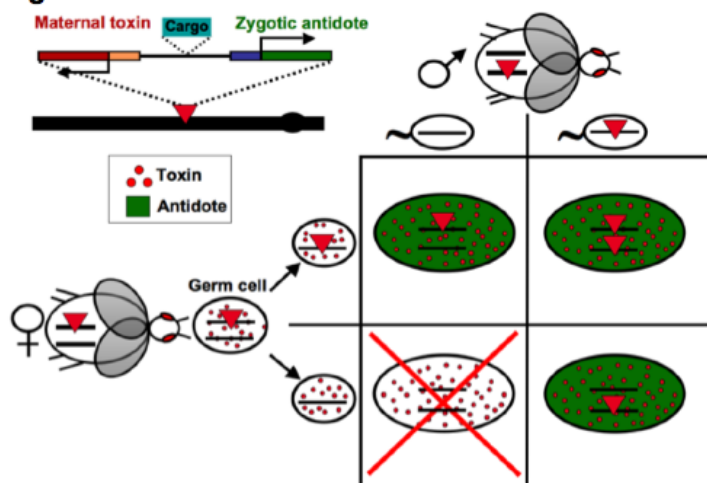
The molecular biology of endogenous *Medea* elements is unknown, but the genetics suggests a model in which *Medea* consists of two linked genes: The first encodes a toxin that is expressed only in the female germline, with effects that are passed to all progeny. The second encodes an antidote, expressed 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
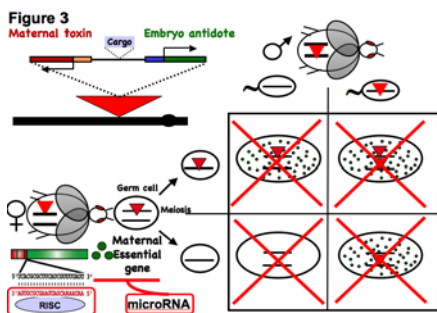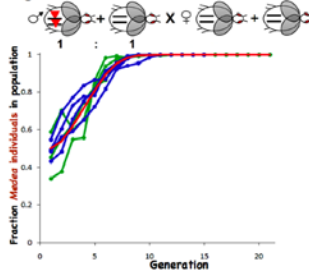

Figure 3

Figure 4

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.

Figure 5
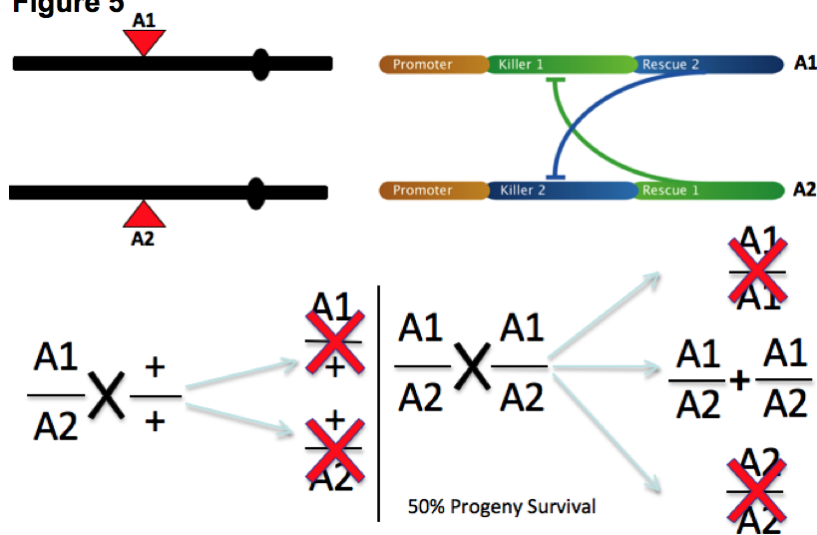
Engineering reproductive isolation and population replacement using a synthetic underdominance system (*Kelly Dusinberre, Katie Kennedy, Mario Zuba, Jennifer Hu, Anna Buchman*). The *Medea* system detailed above is very good at spreading genes into populations distributed over large areas, provided that 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 (Lets 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 spread to fixation in surrounding regions through migration. In brief, for underdominance, as with

Figure 6
singe locus underdominance

two locus underdominance

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
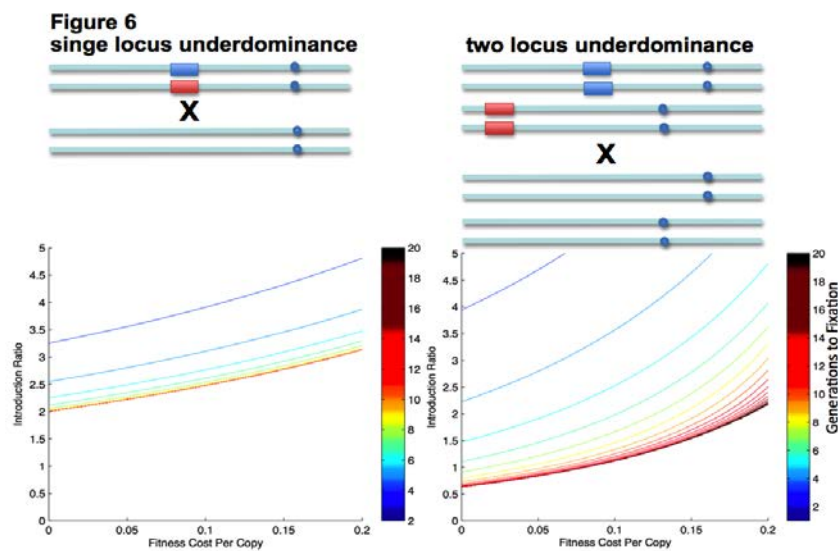
toxin-antidote cassettes are located on non-homologous chromosomes. In this configuration more transgenic progeny can survive in crosses to wildtype, and thus the introduction threshold required for spread to occur is significantly lower, 33%. Once the threshold is crossed, these underdominant systems 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.

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.

Predicting the fate of gene drive systems and their cargos in the wild (*John Marshall, Bruce Hay, Catherine Ward, Jennifer Hu*). 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.

_____

Images from left to right:
Professor Bruce Hay
Title
Title

PUBLICATIONS

2011

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SARKIS MAZMANIAN LAB ANNUAL REPORT | 2011

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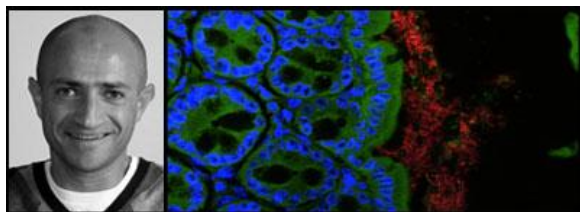
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EVOLUTIONARY MECHANISMS OF HOST-BACTERIA SYMBIOSIS DURING HEALTH

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 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.

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

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ELLIOT MEYEROWITZ LAB ANNUAL REPORT | 2011

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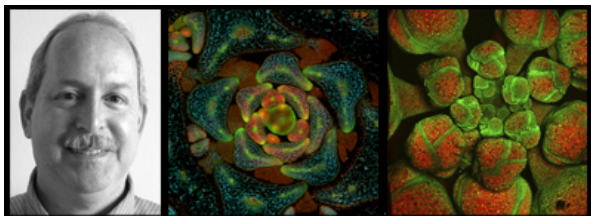
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GENETICS OF PLANT DEVELOPMENT

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 another receptor-like molecule, CORYNE. 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 CORYNE is not, as previously thought, a kinase.

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).

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.

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.

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Images from left to right:
Professor Elliot Meyerowitz
PIN1-GFP REV-VENUS
Roeder Image

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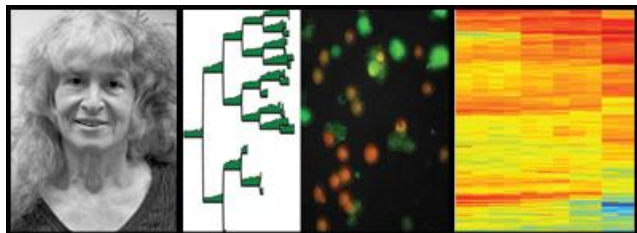
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GENE REGULATORY MECHANISMS FOR T-CELL DEVELOPMENT FROM STEM CELLS

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 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:
Mary Yui, Ni Feng

Genome-wide analysis of RNA expression and histone modification through T cell development:
Jingli Zhang

Genome-wide analysis of PU.1 and GATA-3 binding in early T cells:
Jingli Zhang

GATA-3 roles in early T-cell development:
Sagar Damle

Bcl11b roles in early T-cell development:
Long Li

Bcl11b target genes and interaction with GATA-3:
Long Li, Jingli Zhang

A PU.1-dependent timing network in early T-cell development:
Ameya Champhekar

PU.1-Notch network competitively determining lymphomyeloid fate:
Marissa Morales Del Real

Single-cell dynamics of Bcl11b and PU.1 expression during fate determination:
Hao Yuan Kueh

Cis-regulatory elements of Bcl11b:
Long Li, Hao Yuan Kueh, Kenneth Ng

Cell type specific cis-regulatory elements of PU.1:
Mark Zarnegar

A high-penetrance model for variant T-ALL linked to checkpoint violation
Mary Yui, Ni Feng, Patricia Vegh

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Images, left to right:

Professor Ellen Rothenberg

Pedigree of a clone of PU.1-GFP expressing cells tracked in culture over time (x axis), showing maintenance of PU.1 expression across multiple cell cycles; PU.1-GFP expression intensity in each cell at each time point indicated by thickness of green bar (courtesy: Hao Yuan Kueh)

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)

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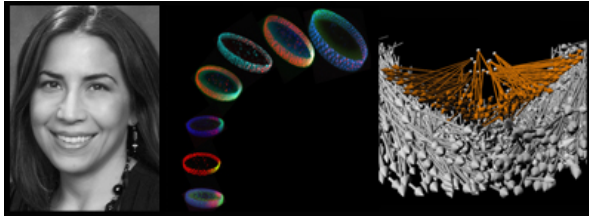
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DYNAMICS OF DEVELOPMENTAL SYSTEMS

Summary:

Inherent in the study of developmental biology is that cells within the embryo change with time. Therefore, to gain a comprehensive and dynamic understanding of embryonic development is a formidable challenge, as hundreds of genes function at any particular moment to effect developmental change. To assay progression of developmental events, we develop and employ novel technologies for making temporally relevant observations using a combined approach of imaging, computation, and molecular biology. In particular, we have focused significant efforts towards development of technology and methodology to support quantitative data acquisition, which lends to more clear comparison of wildtype and mutant phenotype.

Two goals of our research program are to better understand (i) the *cis*-regulatory mechanisms by which spatial and temporal gene expression is controlled and (ii) how signaling pathways function to control differentiation and to regulate cell movements. We use large-scale whole genome observations to reveal the genes and *cis*-regulatory modules (CRMs) that comprise these networks and defined experiments to understand how they come together to control cellular behaviors within the *Drosophila melanogaster* model system. Most of our studies have focused on early embryonic development up to and including gastrulation, as large gene expression changes and strong cell movements are required to turn the totipotent blastula into a multi-layered embryo of distinct cell types. In addition, recent efforts have extended our focus to the cis-regulatory and signaling mechanisms guiding development of specialized tissues, in the wing and female germline.

Through our studies, we strive to understand the underlying logic of *cis*-regulatory control of gene expression, how gene regulatory network structure can influence patterning/differentiation/signaling, and how cellular behaviors are organized during collective cell movements. These are inter-related problems that are relevant for the development of all animals, and as such have far reaching implications.

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Images from left to right:
Professor Angelike Stathopoulos
Cross-sections of Drosophila embryos showing Dorsal levels and gene expression along the dorsal-ventral axis
Quantitative analyses of mesoderm cell spreading during gastrulation shows movements are directed

PUBLICATIONS

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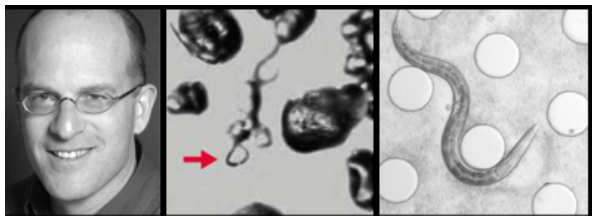
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Visitor:
Carmie Puckett-Robinson, Gary Wong

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

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MOLECULAR GENETICS OF C. ELEGANS DEVELOPMENT AND BEHAVIOR

Summary:
We seek to understand how a genome controls development and behavior. We use *C. 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 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 using only short sequencing reads. 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. In addition, we have sequenced, assembled and annotated the genome of *Steinernema carpocapsae*, an insect-killing nematode that can jump onto hosts (see below) and four other *Steinernema* species. We are also helping to sequence and analyze the genome and transcriptomes of the sheep parasite *Haemonchus contortus*.

Our behavioral studies focused this year on sexual attraction, sleep, and host finding by parasitic nematodes. 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 are continuing our collaboration with the labs of Art Edison and Frank Schroeder to purify mating cues from other nematode species. To study parasite behaviors, we are using two genera of insect killing nematodes that are used in insect biocontrol because they deliver toxic bacteria to their hosts. One key discovery this year is that *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* use the same sensory neuron as *C. elegans* to respond to carbon dioxide. *Steinernema carpocapsae* is able to jump onto insects, and we are trying to understand the genetic and cellular basis for this amazing behavior, as well as its evolutionary origin, as only members of this genus can jump.

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 are expressing channel rhodopsin and a genetically-encoded calcium sensor in a range of specific neurons to be able to examine neuronal circuit properties.

The infective juveniles (IJs) of *H. bacteriophora* and *S. 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. As worms exit the dauer stage they resume reproductive development and we have analyzed how the organization of genes into operon might facilitate a rapid transition to growth.

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. This year 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.

We are trying to learn how to efficiently define *cis*-regulatory elements using functional assays. We have established establishing pipelines for *cis*-regulatory computational analysis to define genomic elements that we test in transgenic *C. elegans*. 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.

We are developing new assays for regulatory elements. For a number of projects, we want to identify all the genes that are expressed in a particular cell at a particular time. We thus are trying different methods of obtaining a transcriptional profile from a single cell; the male linker cell is our first test case.

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 examined several molecular aspects of nematode life-cycle decisions. We used L1 larval arrest to study nutritional control of these decisions, and went on to use microarrays and NanoString technology to examine transcriptional changes. We were early adopters of chromatin immunoprecipitation analyzed by deep sequencing (ChIP-seq) and discovered that RNA polymerase accumulates at the 5' end of transcriptional units during L1 arrest. We then examined the genomic organization related to arrested states and the transition back to growth. We used this analysis to develop a model for the selective advantage of operons in metazoans, namely that operons decrease the need for transcriptional resources in the initial stages of transition to growth, either release from L1 arrest or recovery from dauer larvae. We are now examining how the entry into dauer is controlled by dauer pheromones (mixture of ascarosides) and steroid hormones (dafachronic acid). We collaborated with Adam Anteb (Max-Planck-Institute for Biology of Ageing) to analyze the role of dafachronic acid in pheromone response, in particular how worms respond to a shift form high to low pheromones when larvae are deciding to undergo reproductive or dauer development.

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). 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, and we are developing these ontologies as part of the GO Consortium. To facilitate these processes, we have developed Textpresso (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*, mouse, and several human diseases. We use this system to automate some steps in the extraction of information from full-text papers. Extension of Textpresso to neuroscience is part of the Neuroscience Information Framework. 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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2011

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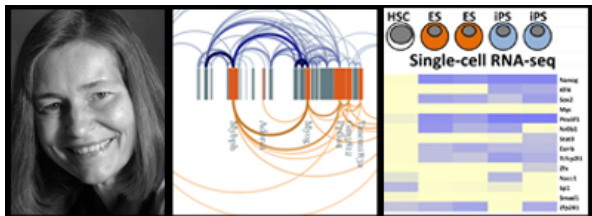
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REGULATION OF MUSCLE DIFFERENTIATION

Summary:
Our group is interested in the composition, evolution and function of gene regulatory networks. We often use muscle development, degeneration, and regeneration as a model system. This network is one among a large class whose members govern how cell fates and states are specified, executed and maintained. The approaches we take to these problems make heavy use of genome-wide and proteome-wide assays to extract connectivity relationships and evolutionary relatedness. Evolution of networks is studied in collaboration with Paul Sternberg and his group to compare mammalian and worm networks, and with John Allman and his group to compare selected brain networks of primates and rodents. A longstanding goal for us is to understand regulatory events that drive the progression of multipotential mesodermal precursor cells or adult stem cells to become determined unipotential muscle progenitors and, finally, fully differentiated cells. We currently study these cell states and transitions using functional genomics tools based on high throughput DNA sequencing methods (RNA-Seq, ChIP-Seq etc., coupled with comparative genomics. In mouse and other vertebrates, the muscle lineage arises from paraxial mesoderm to produce muscle (also bone, skin and fat, among other derivatives). Skeletal myogenesis is governed by both positive- and negative-acting regulatory factors. The MyoD family of four closely related, positive-acting transcription factors are key. Upon transfection, each can trigger nonmuscle recipient cells enter, and often to execute, the myogenic differentiation program. Our working model emphasizes that MyoD family factors are able to do this because they are highly connected and cross talk within their group and with other collaborating factors (like MEF2 factors) and other regulators that now include microRNAs. It is also clear that negative regulators

of skeletal myogenesis are probably just as important for regulating the outcome, as are the positive regulators. Multiple negative regulators of skeletal muscle are expressed in multipotential mesodermal precursors and in proliferating muscle precursors (myoblasts). It is generally believed that some of these are important for specifying and/or maintaining precursor cells in an undifferentiated state, though exactly how the system works is unknown. This focuses attention on defining the *in vivo* target repertoire for the repressors, and learning their relationship with the positive-acting regulators. To obtain the genome-wide input and output maps for multiple activating and repressing regulators, we were motivated to develop ChIPSeq and RNASeq methods (Johnson *et al.*, 2007; Mortazavi, Williams *et al.*, 2008 and below). We have been working to add to and improve these methods and the related informatics, which are increasingly concerned with integrating these diverse datasets. The basic methods produce high resolution *in vivo* protein:DNA interaction maps and quantitative RNA expression profiles. By mapping *in vivo* factor occupancy maps for key regulators and learning how the maps change from one cell state to another, members of the group are trying to address some longstanding puzzles: What distinguishes sequence motifs in the DNA that are actively bound by their cognate regulators *in vivo* from other motif instances that are identical in sequence but are not similarly bound? How does factor occupancy relate to transcriptional output of target genes? What is the target promoter repertoire for a given bound regulator? Work by Brian Williams, Ali Mortazavi, Gordon Kwan, Tony Kirilusha, Georgi Miranov, Katherine Fisher and Gilberto DeSalvo contribute to this series of studies. The MUSSA/MUSSAGL informatics tools (previous work of Tristan DeBuysscher, Diane Trout and Brandon King) have been used to find candidate conserved regulatory elements in both worm and mammalian phyla. This is part of an ongoing partnership with the Sternberg lab that involves several joint projects (see Steven Kuntz and Ali Mortazavi entries below and in the Sternberg lab section). The ultimate goal is to learn enough about the structure and function of cis-regulatory elements to be able to identify them in the genome computationally and to be able to predict their functional output with minimal new experimental data. At the network level, we seek insights into the evolution ancient gene networks that drive myogenesis across the large phylogenetic distances separating worms, mice and humans to define protein:protein complexes in the network more comprehensively, we developed a collaborative effort with Ray Deshaies and Sonya Hess in the Beckman Proteome Exploration Lab. This enabled postdoctoral fellow Libera Berghella to identify a repressor activity (encoded by the MSY3/csda gene) that acts on myogenin in mature myotubes (Berghella *et al.*, 2008). It operates through a very highly conserved *cis*-acting DNA sequence element in myogenin, which appears to have two distinct functions, one acting in early development to open the gene for transcription, and a second, repressive function that operates later on during muscle innervation and maturation.

Our group is also part of the NIH Project ENCODE, in collaboration the Rick Myers, Wing Wong and Gavin Sherlock groups at Stanford and Hudson Alpha Institute. In this project we contribute measurements of transcription factor:genome interactions and transcriptome structure in human ES cells and other cell lines and develop methods of analysis, including a study of RNA editing across the transcriptome (see entry by Ali Mortazavi, and SoCAL BSI summer interns, Wendy Lee and Alicia Rogers).

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STRUCTURAL, MOLECULAR AND CELL BIOLOGY FACULTY | 2011
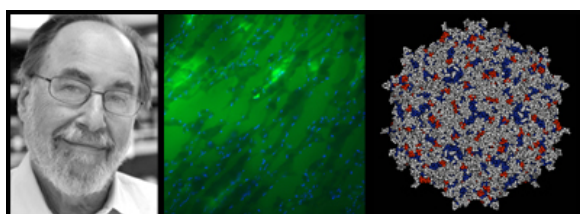
DIVISION of BIOLOGY

2011 ANNUAL REPORT
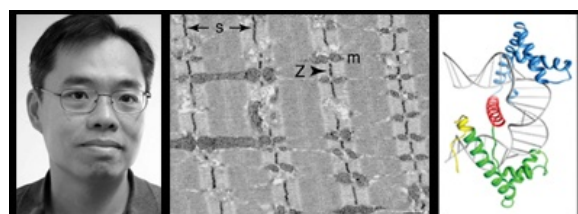
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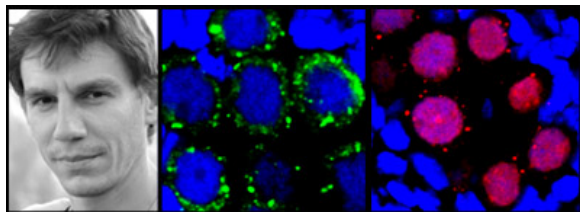
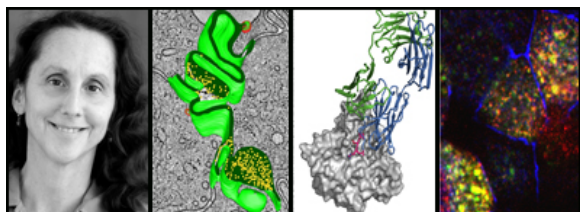**Alexei Aravin**
Assistant Professor of Biology

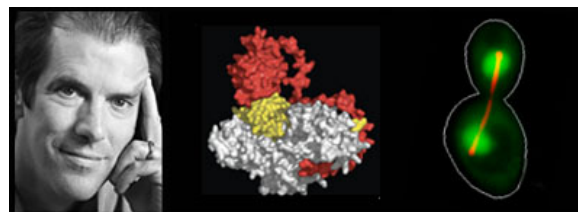
Judith Campbell
Professor of Biology and of Chemistry

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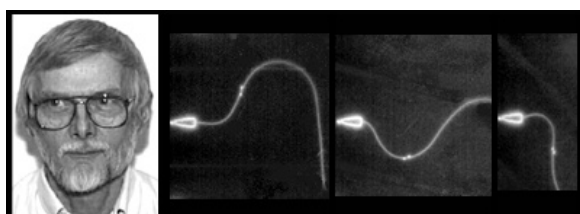*President Emeritus, Nobel Laureate*
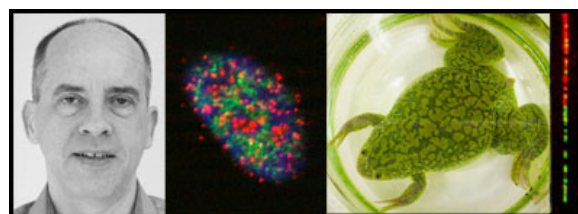*Robert Andrews Millikan Professor of Biology,*

David Chan
Professor of Biology

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Professor of Biology

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Grace C. Steele Professor of Biology

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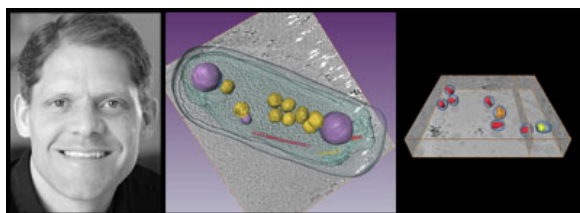
Grant Jensen
Professor of Biology
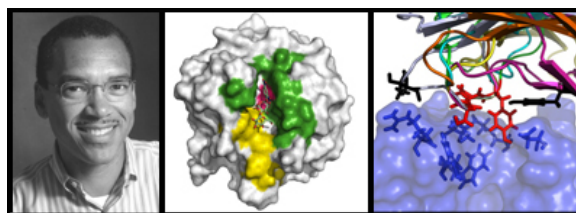
Stephen Mayo
Bren Professor of Biology and Chemistry;
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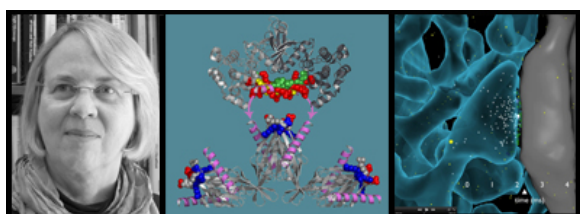
Mary Kennedy
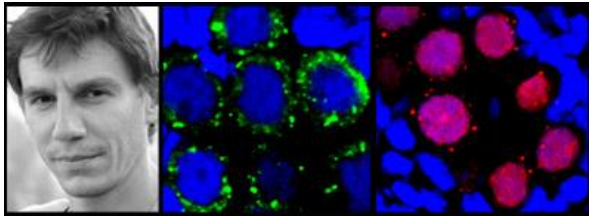*Allen and Lenabelle Davis Professor of Biology*

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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.

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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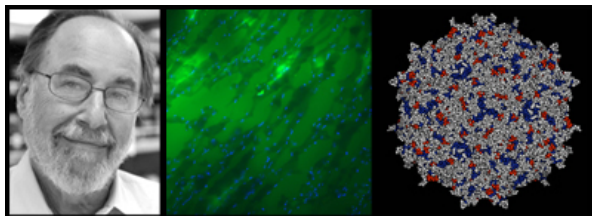
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BASIC IMMUNOLOGY AND ENGINEERING OF THE IMMUNE SYSTEM

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 cells 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 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. In a separate program, we are investigating how lentiviruses activate dendritic cells.

The translation studies derive from the development of viral vectors that can mediate changes in immune function, a program we call Engineering Immunity. In one program, 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. This program, presently carried out using mice that harbor a human immune system, is in transition to clinical evaluation in humans. Originally, we had hoped to do this with lentiviral vector programming of hematopoietic stem cells and that program remains active, focusing on IgA production, which appears more potent than IgG, especially for blocking mucosal challenge.

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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.

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PAMELA BJORKMAN LAB ANNUAL REPORT | 2011

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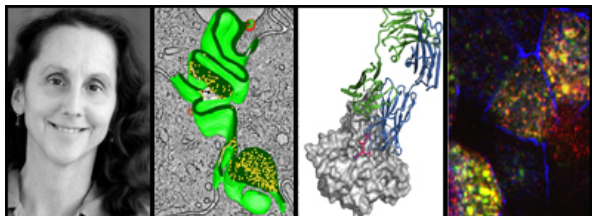
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STRUCTURAL BIOLOGY OF ANTIBODY RECEPTORS AND IMMUNE RECOGNITION OF VIRUSES

Summary:
We are interested in structural mechanisms of recognition in the immune system, specifically in homologs and viral mimics of class I major histocompatibility complex (MHC) proteins, and in the structure, function, and therapeutic uses of antibodies and their receptors. In addition to using X-ray crystallography and biophysical techniques to analyze protein-protein interactions in solution, we also use electron tomography and confocal microscopy to image interactions in cells, examining, for example, 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 a new area for us: "Engineering Immunity" against HIV.

Classical class I MHC proteins present peptide antigens derived from self- and nonself proteins to T cells during immune surveillance. The MHC structure seems ideally suited for its antigen presentation function, in that it includes a groove that is perfectly shaped to accommodate short peptide antigens. MHC homologs share similar three-dimensional structures with classical MHC

molecules but have different functions, including immune functions (antibody transport by FcRn; evasion of host immune responses by viral MHC mimics) and nonimmune functions (regulation of iron or lipid metabolism by HFE and ZAG; chaperoning pheromone receptors by M10 proteins).

Our crystal structures revealed that FcRn, HFE, ZAG, M10, and the poxvirus protein 2L do not present peptides and therefore play no role in conventional adaptive immune responses. The FcRn, HFE, and 2L grooves are collapsed, and cocrystal structures with their respective protein ligands show that each uses a protein-protein interaction mode different from MHC interactions with peptides or T cell and other receptors. Our recent structure of 2L complexed with the host inflammatory cytokine tumor necrosis factor-α (TNFα) revealed the structural basis for its picomolar affinity for TNFα and may facilitate design of anti-inflammatory protein drugs. By contrast, the grooves of ZAG, M10, and the human cytomegalovirus MHC mimic UL18 are open and theoretically capable of antigen binding, but only UL18 associates with peptides. These results raise questions about the primordial function of the MHC fold: Did it originally arise for peptide presentation/T cell interactions as part of the adaptive immune response (a relatively recent acquisition of the vertebrate immune system), or did it arise for the seemingly more ancient functions of protein transport or metabolite regulation? Perhaps surprisingly, our results suggest the former. Our studies of MHC homologs provide striking examples that structure does not always dictate function: similar structures can adopt different functions, and conversely, similar functions can be accomplished by very different structures.

We extended our characterizations of FcRn, an MHC-related receptor for IgG antibodies, to include cell biological studies of intracellular trafficking. FcRn is the receptor that 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 involves trafficking of FcRn-IgG complexes in acidic intracellular vesicles across an epithelial cell barrier in the placenta (for prenatal transfer) or the intestine (for postnatal transfer). 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 the 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 reveal tangled webs of interlocking IgG-containing transport vesicles, some of which are associated with microtubule tracks to allow movement via motor proteins. Other IgG-containing vesicles include 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 these 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 are testing the hypothesis that circulating IgG taken up by gE-gI by receptor-mediated endocytosis is 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.

In addition to studying antibody receptors, we began a new project to improve upon the binding and neutralization properties of antibodies themselves. This work is part of a collaboration with David Baltimore's laboratory (California Institute of Technology) to "Engineer Immunity" against HIV. The idea is to direct lifelong production of specified antibodies or antibody-like proteins with desired properties; for example, neutralizing antibodies or designed antibodies engineered to bind more tightly to a pathogen or to recruit immune effector cells. The antibodies would be produced in vivo by gene therapy techniques, thus allowing longterm production of anti-HIV proteins.

Our portion of the project involves designing, producing, and testing novel anti-HIV protein reagents in an effort to find proteins with increased efficacy in HIV neutralization. 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 the Engineering Immunity 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 our 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 anticarbohydrate 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 constructing hinge-extended antibodies that would enable simultaneous binding by both antigen-binding sites, either within a spike or between spikes, thereby reducing the concentration of antibody required for sterilizing immunization to realistic levels.

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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.

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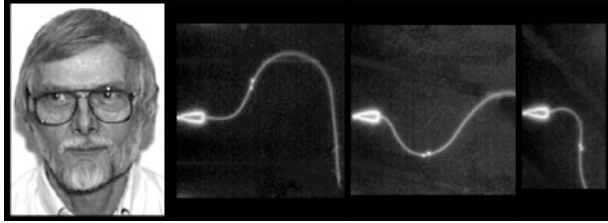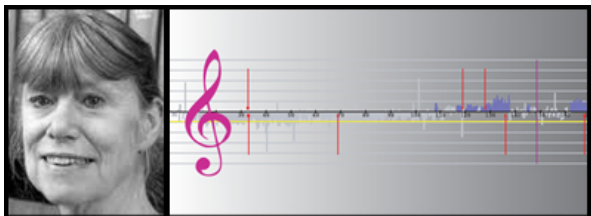
Professor Emeritus:
Charles Brokaw

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Summary:

Motor enzymes — dyneins, kinesins, and myosins — convert energy from ATP dephosphorylation into most of the movements performed by eukaryotic cells. We think that myosin and kinesin are reasonably well understood, although new experimental results from time to time surprise us. On the other hand, we have very little knowledge or understanding of the functioning of the axonemal dyneins that power the movements of flagella and cilia; these molecular complexes are a major challenge for the future. Since retiring from experimental work in the last century, I have continued to use computer simulation methods to explore ideas about motor enzyme function in situations ranging from experimental studies on individual motors to an intact flagellum containing tens of thousands of dyneins. Current work is focused on identifying aspects of dynein function that are required to fully explain experimental observations of cyclic bending movement of a single pair of axonemal doublets.

More information at: http://www.caltech.edu/~brokawc

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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.
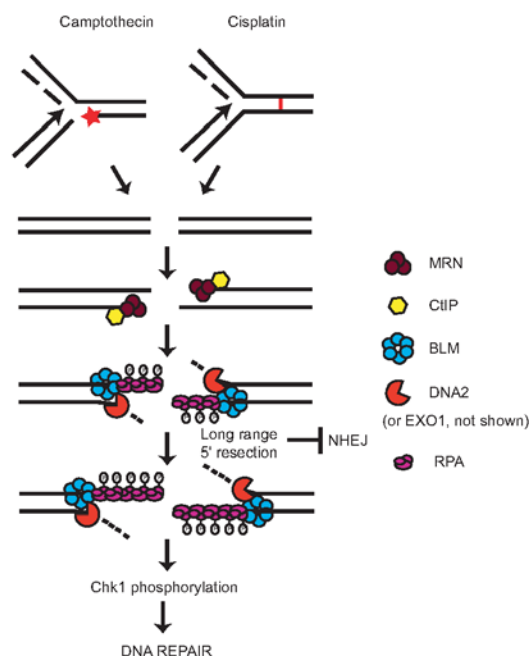
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Supplementary Figure 1: Model for DNA end resection after replication stress. *Camptothecin or cisplatin exposure blocks replication due to formation of topoisomerase-DNA adducts (red star) or interstand cross links (red link between strands), respectively. 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.*

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DAVID CHAN LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY
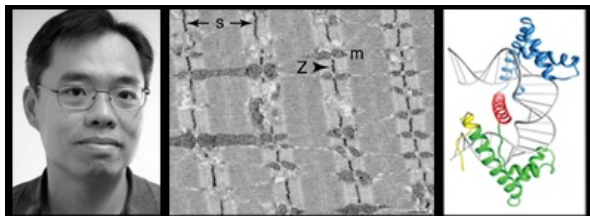
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PHYSIOLOGICAL FUNCTIONS AND MECHANISMS OF MITOCHONDRIAL DYNAMICS

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.

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Images from left to right:
Professor David Chan
Electron microscopy of mitochondria in skeletal muscle
X-ray structure of the TFAM bound to promoter DNA

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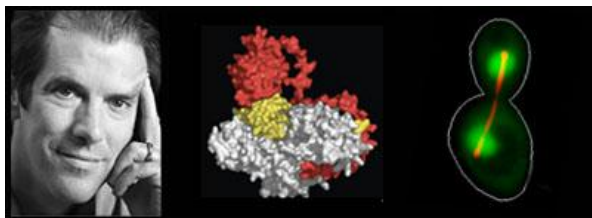
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RAYMOND DESHAIES LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

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Francesco Parlati

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MECHANISM AND REGULATION OF UBIQUITIN-DEPENDENT PROTEOLYSIS

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^{Cdc4} 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^{Cdc4} 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^{Cdc4} 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. We seek to address these questions by applying enzymological approaches, chemical biology, and quantitative proteomics to devise new assays and tools to study CSN and the cycle of cullin neddylation and deneddylation both in vitro and in cells.

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 identify selective and potent small-molecule inhibitors of p97 ATPase activity that can be used as tools to study the connection between p97 and CRLs. 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.

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Images, left to right:

Professor Raymond Deshaies
Cdc34 Dock
Dane Cell

PUBLICATIONS

2011

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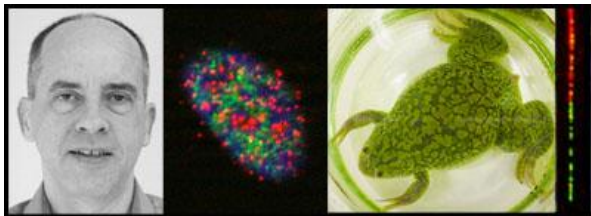
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Research and Laboratory Staff:
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REGULATION OF THE CELL CYCLE AND MAINTENANCE OF GENOMIC INTEGRITY

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 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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Images from left to right:

Professor William Dunphy
Localizations of regulators of DNA replication in human cells
Xenopus laevis Frog
Replicating DNA fibers in human cells

PUBLICATIONS

2011

Kumagai, A., Shevchenko, A., Shevchenko, A. and Dunphy, W.G. (2011) **Direct regulation of Treslin by cyclin-dependent kinase is essential for the onset of DNA replication.** *J. Cell Biol.* **193**:995-1007.
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GRANT JENSEN LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY
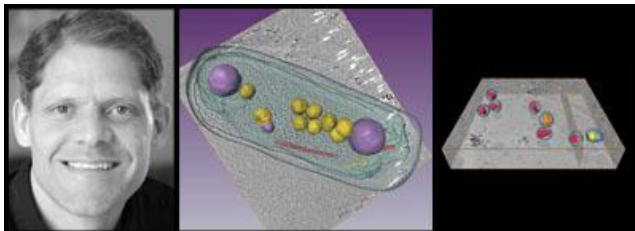
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HIGH RESOLUTION CYRO-EM IMAGING OF CELLS AND VISRUSES

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.

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Images, left to right:

Professor Grant Jensen
3-D view of a Halothiobacillus neapolitanus cell
3-D view of a field of HIV-1 virions

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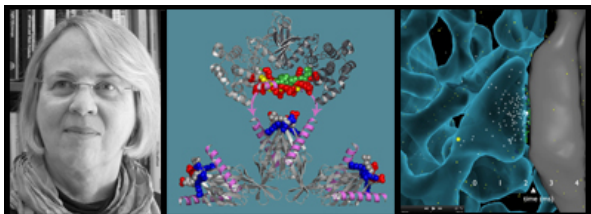
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MOLECULAR MECHANISM OF SYNAPTIC REGULATION

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.

 Neurons communicate through synapses that release chemical transmitters to activate a target neuron. Many transmitters also initiate biochemical changes in the signaling machinery of the synapse itself. The biochemical changes can either increase or decrease the size of the signal produced by the synapse when it fires again. This is called "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 glutamate (the NMDA-type glutamate receptor), triggers an increase in the strength of an active synapse only when simultaneous activation of several synapses causes the postsynaptic neuron to fire an action potential. This "plasticity rule" is used to form memories.

Our lab is studying biochemical signal transduction machinery in central nervous system synapses. 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.

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 will use MRM (multiple reaction monitoring) mass spectrometric assays. The data will allow us to build and test kinetic models of large signal transduction pathways.

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Images from left to right:
Professor Mary Kennedy
Structure of a portion of CaMKII
*Calcium flowing through spine*

PUBLICATIONS

2011

Carlisle, H.J., Luong, T.N., Medina-Marino, A., Schenker, L.T., Khorosheva, E.M., Indersmitten, T., Gunapala, K.M., Steele, A.D., O'Dell T.J., Patterson, P.H. and Kennedy, M.B. (2011) **Deletion of densin-180 results in abnormal behaviors associated with mental illness and reduces mGluR5 and DISC1 in the postsynaptic density fraction**. *J. Neurosci.* **31**:16194-16207.

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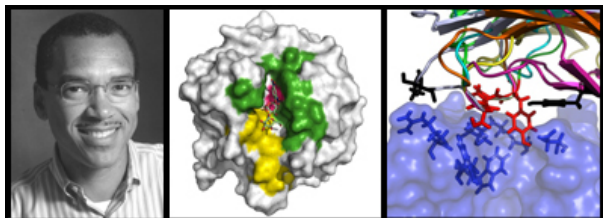
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PROTEIN FOLDING AND PROTEIN DESIGN

Summary:
The focus of the lab has been the coupling of theoretical, computational, and experimental approaches for the study of structural biology. In particular, we have placed a major emphasis on developing quantitative methods for protein design with the goal of developing a fully systematic design strategy that we call "protein design automation." Our design approach has been captured in a suite of software programs and has been applied to a variety of problems ranging from protein fold stabilization to enzyme design.

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Images from left to right:

Professor Stephen Mayo
Designing thermostable proteins for biofuel production
Designing novel protein-protein interfaces

PUBLICATIONS

2011

Hemmert, A.C., Otto, T.C., Chica, R.A., Wierdl, M., Edwards, J.S., Lewis, S.L., Edwards, C.C., Tsurkan, L., Cadieux, C.L., Kasten, S.A., Cashman, J.R., Mayo, S.L., Potter, P.M., Cerasoli, D.M. and Redinbo, M.R. (2011) **Nerve agent hydrolysis activity designed into a human drug metabolism enzyme**. *PLoS ONE* **6**:e17441. PubMed ID: 21445272.

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Shannon Park (Lab Manager), Olga Batygin (Admin)

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

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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).

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Images from left to right:
Professor Dianne Newman
Banded Iron Formations (BIF) in rock samples showing alternating layers of chert and iron oxides.
Biofilm of a phenazine knockout strain of Pseudomonas aeruginosa exhibiting a wrinkled morphology.

PUBLICATIONS

2011

P.V. Welander, D.M. Doughty, C.H. Wu, S. Mehay, R.E. Summons and D.K. Newman (2011) **Identification and characterization of *Rhodopseudomonas palustris* hopanoid biosynthesis mutants.** *Geobiology*, PMID: 22221333

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THE UBIQUITIN SYSTEM AND THE N-END RULE PATHWAY

Summary:
Our main subject is the ubiquitin 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 insights 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) advances 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, including 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. The first primary degradation signals (degrons) in short-lived proteins were discovered by us in 1986. 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 up a particularly large field, because we now know that the mammalian genome encodes at least a thousand distinct E3s. The targeting of many distinct degrons in cellular proteins by this astounding number of different 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 history of the ubiquitin field, see Hershko *et al.* (2000); Varshavsky (2006, 2008).

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 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 work at Caltech continues to focus 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-recognins, whereas N-terminal Asp, Glu, Asn, Gln, and Cys function as destabilizing residues through their preliminary modifications. These modifications include 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, this ancient 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.

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

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Images from left to right:
Professor Alexander Varshavsky
Petri Dishes
Genetic research at Laboratory

PUBLICATIONS

2011

Hwang, C.-S., Sukalo, M., Batygin, O., Addor, M.-C., Brunner, H., Aytes, A.P., Mayerle, J., Song, H.K., Varshavsky, A. and Zenker, M. (2011) **Ubiquitin ligases of the N-end rule pathway: assessment of mutations in *UBR1* that cause the Johanson-Blizzard syndrome.** *PLoS One* **6**:e24925. PMID: 21931868.

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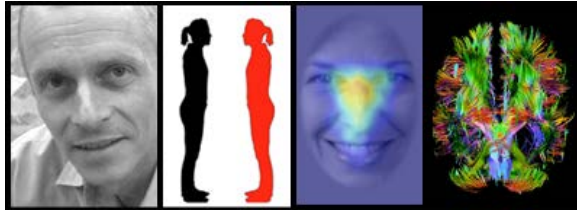
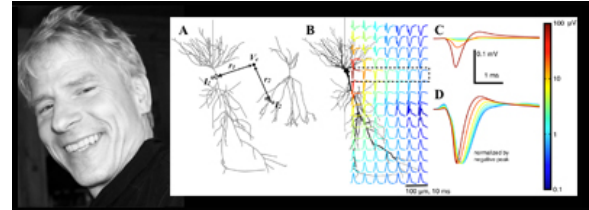# MOLECULAR, CELLULAR AND INTEGRATIVE NEUROSCIENCE BIOLOGY FACULTY

2011

DIVISION of BIOLOGY

2011 ANNUAL REPORT

California Institute of Technology

Molecular, Cellular and Integrative Neuroscience Biology Faculty | 2011
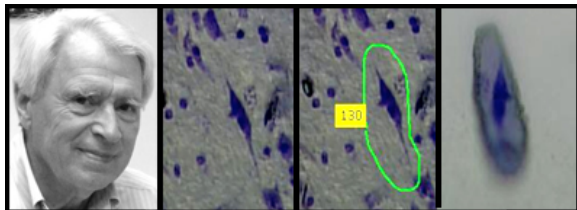
CALIFORNIA INSTITUTE OF TECHNOLOGY

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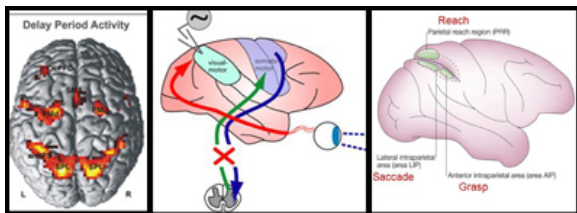*Professor of Biology*

Christof Koch
Lois and Victor Troendle Professor of Cognitive and
Behavioral Biology and
Professor of Computation and Neural Systems

John Allman
Frank P. Hixon Professor of Neurobiology

Masakazu Konishi
Bing Professor of Behavioral Biology

Richard Andersen
James G. Boswell Professor of Neuroscience
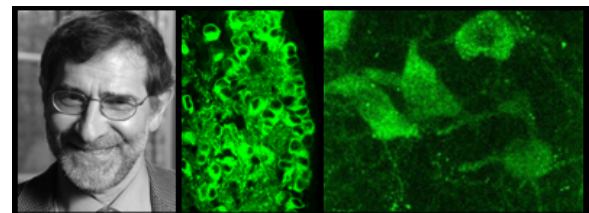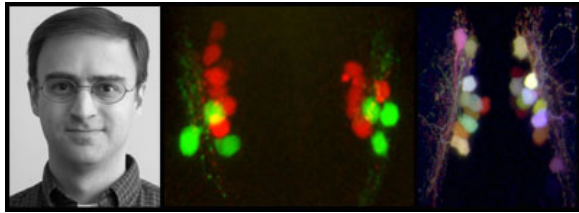

Henry Lester
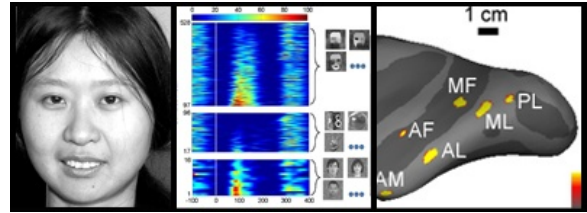*Bren Professor of Biology*
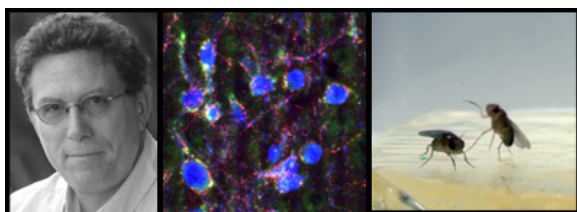
David Anderson
Seymour Benzer Professor of Biology
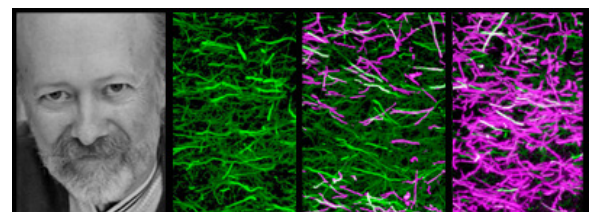
Paul Patterson
Anne P. and Benjamin F. Biaggini Professor of
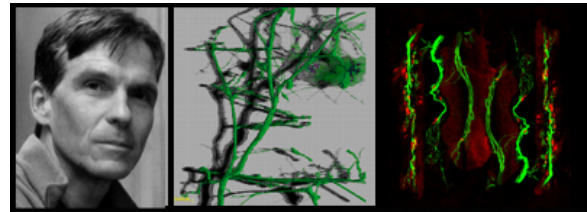*Biological Sciences*
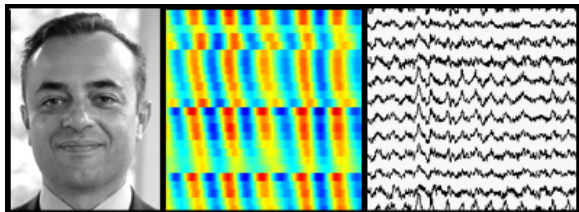
David Prober
Assistant Professor of Biology

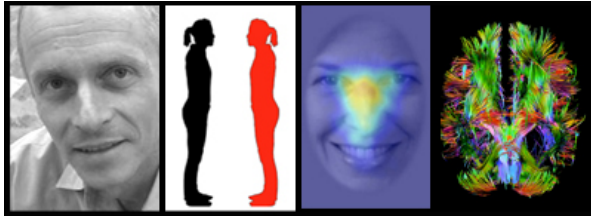Doris Tsao
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RALPH ADOLPHS LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

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Research Staff:
Tim Armstrong, Catherine Holcomb, Remya Nair

Senior Research Staff:
Lynn Paul

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EMOTION 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.

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Images from left to right:
Professor Ralph Adolphs
Measuring personal space in patients with amygdala lesions
Eye tracking to faces in people with autism
Connectivity of the brains in agenesis of the corpus callosum as visualized with MR imaging

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JOHN ALLMAN LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY
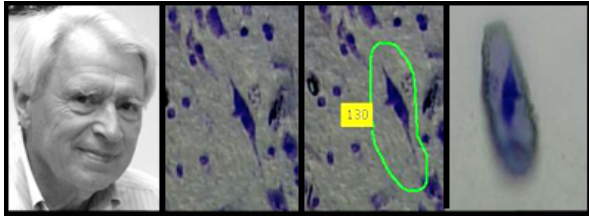
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BRAIN AND BEHAVIOR IN PRIMATES

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 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.

Images from left to right

Professor John Allman

The microdissection of a von Economo neuron (VEN), which is the large bipolar cell in the center of the field; the VEN outlined for laser microdissection; the microdissected VEN.
Microdissection by Nicole Tetreault.

PUBLICATIONS

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RICHARD ANDERSEN LAB ANNUAL REPORT | 2011

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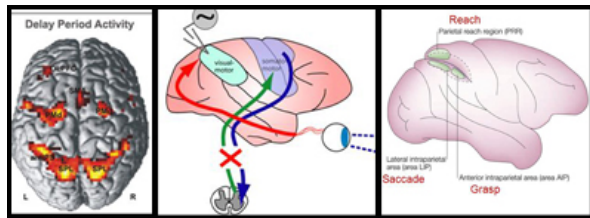
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NEURAL MECHANISMS FOR VISUAL-MOTOR INTEGRATION, SPATIAL AND MOTION PERCEPTION

Summary:

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.

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Images from left to right:
Functional magnetic resonance imaging of human during movement planning
Schematic of concept of a cognitive neural prosthetic
Area of the posterior parietal cortex involved in planning different actions

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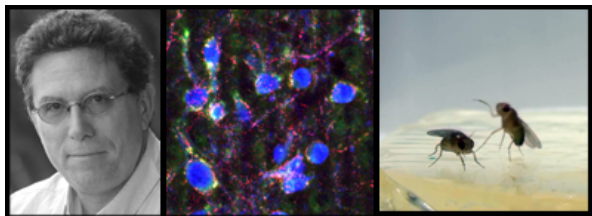
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DAVID ANDERSON LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

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Seymour Benzer Professor of Biology:
David J. Anderson

GENETIC DISSECTION OF NEURAL CIRCUITS CONTROLLING EMOTIONAL BEHAVIORS

Summary:
Research in this laboratory is aimed at understanding the neurobiology of emotion. 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 responses, 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. We use two model organisms in the laboratory: mice and the vinegar fly *Drosophila melanogaster*, with roughly equal emphasis on both.

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.

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.

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 Mrgprs, 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 determine the extent to which this is possible, and if so what we can learn from it.

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.

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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.

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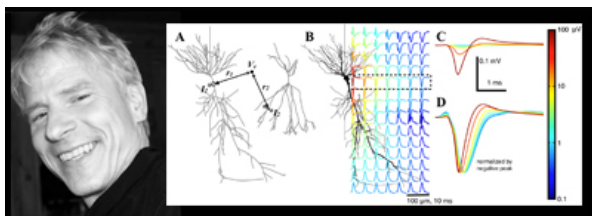
CHRISTOF KOCH LAB ANNUAL REPORT | 2011

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Philippe P. Brieu, Heather Hein, Leonard Mlodinow

PROCESSING INFORMATION WITH NEURONS

Summary:
Research in the laboratory focuses on three areas: (1) biophysics of computation in nerve cells; (2) understanding selective visual attention and visual consciousness at the neuronal, behavioral and computational levels; and (3) develop biological-motivated vision algorithm that predict where people and animals will look in natural scenes (gaze prediction). For more details and publications, see http://www.klab.caltech.edu.

Research carried out as part of a "Biophysics of Computation" focuses on how the electrophysiology, synaptic architecture, and dendritic morphology of groups of individual neurons subserve information processing. We are working on the *forward* problem of determining the local field potential (LFP) that results from the synaptic and electrical activity of 1,000 of neurons in the hippocampus. We are also considering the *inverse* problem, studying the extent to which the local field influences the timing of action potentials of neurons. Our experimental (recordings with up to 12 patch electrodes in layer 5 pyramidal neurons *in vitro*) and computational research clearly shows that gradients as low as 1 mV/mm in the extracellular field can significantly affect the synchrony of neurons. That is, not only synaptic but also ephaptic effects will influence the firing behavior of population of neurons. This work is done in collaboration with the laboratories of Gyuri Buzsaki at Rutgers/New Jersey and Henry Markram at the

EPFL/Lausanne.

Our laboratory collaborates with the neurosurgeon and neuroscientist Itzhak Fried at UCLA, recording from 128 electrodes in the medial temporal lobe of awake patients with pharmacologically intractable epilepsy who are implanted with depth electrodes to localize the focus of seizure onset. This unique setting allows us to observe invariant recognition, imagery and representation of familiar objects and famous individuals in conscious humans by listening in on the spiking activity of many individual neurons - complemented by local field analysis. This work requires sophisticated data processing skills and the careful design of the appropriate behavioral-physiological paradigms that will work in a clinical context. In on current work, we demonstrated that patients can selectively, rapidly, transiently and voluntarily control the firing behavior of selective neurons in the medial temporal lobe, enhancing the firing rate of some, while simultaneously suppressing the firing rate of others and leaving the bulk of neurons unaffected.

Understanding the action of selective, visual attention (both saliency-driven, bottom-up, as well as task-dependent, top-down forms) requires a firm grasp of how visual object recognition in natural scenes can be solved at the computational level, and how the resulting algorithms can be mapped onto the known architecture of the visual cortex and associated cortical and sub-cortical areas. We use analytical methods, coupled with computer simulations of the appropriate circuitry in the primate visual system, visual psychophysics, eye tracking and functional brain imaging at Caltech's 3.0 T Trio scanner to investigate human attentional selection (via saliency) and object recognition in the near-absence of focal attention, in visual search, in natural scene perception and as reward is modulated. Aspects of this work are done in collaboration with Antonio Rangel, Ralph Adolphs and Colin Camerer of Caltech and with Laurent Itti at USC. Together with Tomaso Poggio at MIT, we investigate neurobiologically plausible models of both, the ventral, object-recognition and the dorsal, attention visual streams. We continue to work on understanding how neurons in regions LIP and FEF - modulated by top-down information - implement a visual saliency map.

We study the neuronal correlates of consciousness, developing a neurobiological framework to understand how subjective feelings (in particular, conscious visual perception) can arise in the mammalian forebrain. Using `Continuous Flash Suppression' (CFS) and other techniques we have invented to hide images from conscious perception, we can show that visual, selective attention is a distinct process from visual consciousness. In collaboration with Chris Adami of the Keck Graduate Institute and Giulio Tononi of the University of Wisconsin at Madison, we are studying Tononi's *Integrated Information Theory* of consciousness and apply the underlying complexity measures to both abstract, as well as concrete biological circuits and motifs.

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Images from left to right:
Professor Christof Koch

A, Illustration of the extracellular potential Ve calculation in a population through the superposition of contributions from all compartments in all cells. Individual compartment contributions are primarily determined by their transmembrane currents and distances from the electrode. B, Location dependence of the extracellular action potential (EAP) for the pyramidal cell model. The peak-to-peak voltage range is indicated by the color of each trace. Subthreshold currents have been removed, and each EAP waveform is an average over 25 points at a fixed radius from the apical axis (the vertical direction here) to remove the dependence on the precise dendritic geometry. EAPs are calculated at the location of the start of each trace. C, EAPs within the cell body layer (dashed box in panel B) with voltages drawn to scale. EAP amplitude decreases rapidly with distance. The largest EAP is calculated 20 μm from the soma center, then at 50 μm intervals. D, Same traces as in C, but normalized by the negative peak.

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**Bing Professor of Behavioral Biology**:
Masakazu Konishi

Broad Senior Research Fellow in Brain Circuitry:
Guangyin Wu

Member of the Professional Staff:
Eugene Akutagawa

THE NEUROBIOLOGICAL STUDY OF NATURAL BEHAVIOR

Summary:

All birds use sounds to communicate with each other. The so-called songbird is particularly interesting to us, because young birds imitate song either from their fathers or other males in the vicinity. Song learning consists of two stages, memorizing a song during youth and reproducing it in adulthood. We would like to know where in the brain and in what form tutor song is stored. We know the chain of discrete brain areas that control song. These areas are unique to bird species that learn song and the areas are collectively referred to as the song control system. However, our own follow-up studies have revealed several new connections between areas that were thought to form a simple chain. Thus, our findings make the song control system much more complex than it was originally reported (Figure below). A shows the old figure and B shows the new figure based on our data. The red lines indicate the newly found connections.
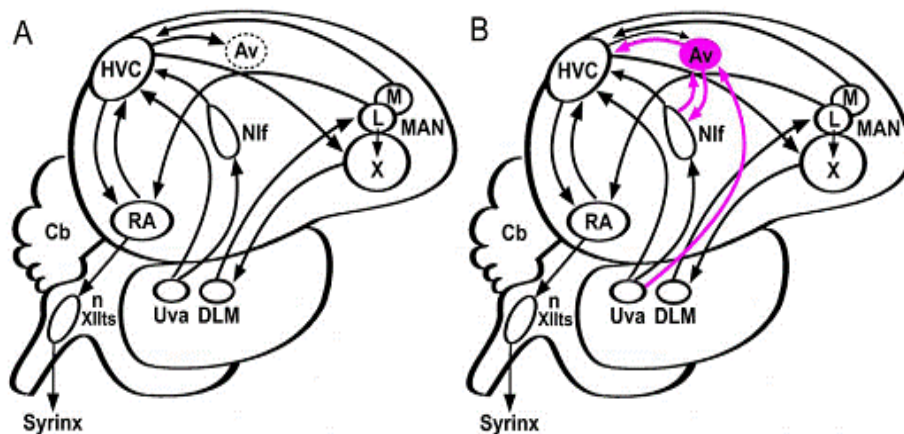
Images from left to right:
Professor Masakazu Konishi
A shows the old figure and B shows the new figure.

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2011

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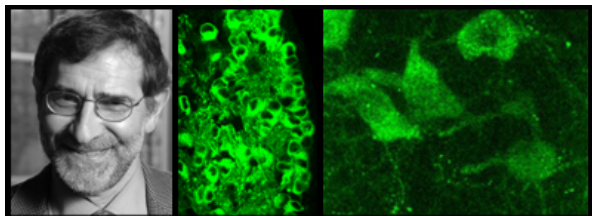
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HENRY LESTER LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

Bren Professor of Biology:
Henry A. Lester

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Associate Biologist:
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Research and Laboratory Staff:
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SYNAPTIC TRANSMISSION; ION CHANNELS, MOUSE MODELS; NICOTINE ADDICTION; PARKINSON'S DISEASE

Summary:
Our lab studies 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 are describing the neural events that occur when an animal is chronically exposed to nicotine. We hypothesize that this set of responses underlies the pathophysiology of nicotine addiction, the world's largest preventable cause of death.

There is no medical justification for the use of smoked tobacco. However, the organism's responses to chronic nicotine also underlie the benefits of two inadvertent therapeutic effects of smoking: the inverse correlation between a person's history of smoking and his/her susceptibility to Parkinson's disease, and the preventive effect of nicotine in autosomal dominant nocturnal frontal-lobe epilepsy.

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, and even neural events in humans. Several lab members are describing the molecular/biophysical aspects of SePhaChARNS, for "selective pharmacological chaperoning of acetylcholine receptor number and stoichiometry." We hypothesize that SePhaChARNS is a thermodynamically driven process leading to the classical observation that chronic exposure to nicotine causes "upregulation" of nicotinic receptors. Other proteins modify SePhaChARNS; and lab members are studying two of these: the modulatory protein lynx, and the auxiliary nicotinic subunit $\alpha5$. If the hypothesis is proven, SePhaChARNS is the molecular mechanism that shapes an animal's response to chronic exposure to nicotine. We are now producing 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.

Other lab members are generating 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.

We are also engineering Cys-loop receptors in order to manipulate neurons. Now we are optimizing suitably mutated C. elegans GluCl receptor channels to have strong responses to minuscule concentrations of the anthelmintic ivermectin and its derivatives. In collaboration with Professor David Anderson's group, we have introduced these channels into mouse neurons. When the animal is injected with ivermectin, the neurons can be selectively silenced—and eventually, we hope, selectively activated. If we can improve the system, there are important applications for both research and therapy.

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 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.

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Images from left to right:
Professor Henry Lester
Fluorescent α3 nicotinic receptor subunits in the medial habenula and fasciculus retroflexus of a knock-in mouse.
Unmixed alpha4YFP

PUBLICATIONS

2011

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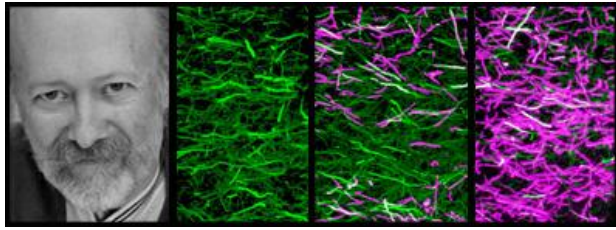
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Paul H. Patterson, Ph.D.

Visiting Professor:
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Senior Research Fellow:
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Research and Laboratory Staff:
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[*]*Undergraduate student, Caltech*
[**]*Undergraduate student, Pasadena City College*

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. 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 are currently examining the role of LIF in a chemical model of multiple sclerosis, where exogenous LIF can increase oligodendrocyte number and stimulate remyelination.

Cytokine involvement in a model for mental illness is also being investigated. This mouse model is based on findings that maternal infection 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 lymphocyte reactions to stimulation in the adult offspring. In collaboration with the Mazmanian laboratory at Caltech, we are also examining the effects of MIA on gastrointestinal tract inflammation in the offspring.

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. Recent findings indicate that viral delivery of 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.

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

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2011

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2010

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DAVID PROBER LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY
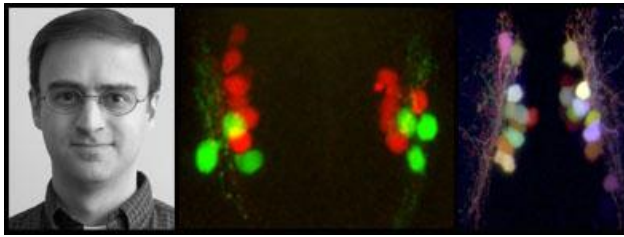
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Research Staff:
Viveca Sapin, Kenna Molinder

_____

GENETIC AND NEURAL CIRCUITS THAT REGULATE SLEEP-LIKE STATES

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.

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Image from left to right:

Professor David Prober

Transgenic zebrafish embryos that express red fluorescent protein in Hypocretin neurons and green fluorescent protein in QRFP neurons. 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

2010

Rihel, J., Prober, D.A. and Schier, A.F. (2010). **Monitoring sleep and arousal in zebrafish.** *Methods Cell Biol.* **100**: 281-94.
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Gertrude Baltimore Professor of Experimental Psychology:
Shinsuke Shimojo

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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.

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Images from left to right:
Professor Shinsuke Shimojo
Interpersonal EEG
Subcortical activity under a pressure

PUBLICATIONS

2011

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Yotsumoto, Y., Seitz, A.R., Shimojo, S., Sakagami, M., Watanabe, T. and Sasaki, Y. (2011) **Performance dip in motor response induced by task-irrelevant weaker coherent visual motion signals**. *Cereb Cortex.* PMID: 21940704.

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2010

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Saegusa, C., Shimojo, E., Park, J. and Shimojo, S. (2010) **Visual attractiveness is leaky (2): hair and face.** *J. Vis.* **10**(7):652, doi:10.1167/10.7.652.

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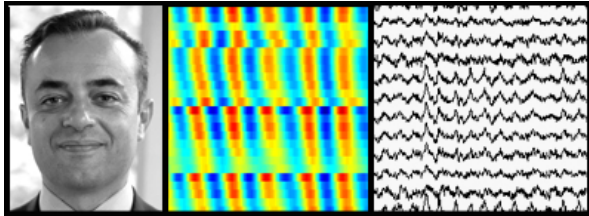
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Graduate Students:
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NETWORK MECHANISMS OF LEARNING AND MEMORY

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

DORIS TSAO LAB ANNUAL REPORT | 2011

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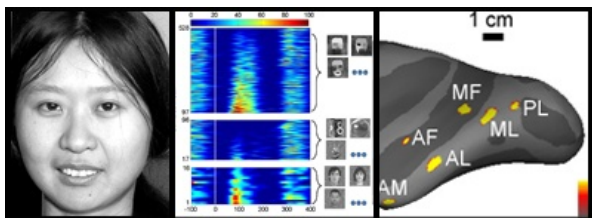
Assistant Professor of Biology:
Doris Y. Tsao

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

CNS Graduate Student:
Shay Ohayon

Research and Laboratory Staff:
Nicole Schweers

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.

Images from left to right:

Professor Doris Tsao

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

2011

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Nallasamy N., Tsao DY. (2011) **Functional connectivity in the brain: effects on anesthesia.** *Neuroscientist.* **17** (1): 94-106. PMID: 21343409

2010

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KAI ZINN LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY
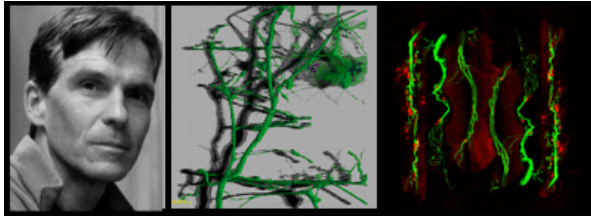
Professor of Biology:
Kai Zinn, Ph.D.

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

Staff:
Elena Armand, Violana Nesterova

MOLECULAR GENETIC STUDIES OF INSECT NERVOUS SYSTEM DEVELOPMENT

Summary:
The fruit fly *Drosophila melanogaster* is our primary experimental system. Most of our work has been centered around the study of the molecular mechanisms by which synaptic connections are formed and modified during development. In the embryo, we examine signaling systems involved in motor and central nervous system (CNS) axon guidance. We also study the control of tube geometry in the tracheal (respiratory) system; this process uses some of the same phosphotyrosine signaling pathways that regulate axon guidance. In the larva, we focus on synaptogenesis and synaptic plasticity in the neuromuscular system. In the adult, we investigate the neural and molecular mechanisms that control fat storage. Our approaches combine genetics, molecular biology, electrophysiology, biochemistry, and cell biology. We have also begun a project to develop a new strategy for systematic generation of monoclonal antibodies (mAbs) against cell-surface and secreted proteins. We will use this to generate mAbs against both *Drosophila* and human proteins. We plan to use the anti-human mAbs for investigation of vascular development and tumor angiogenesis.

Motor axon guidance and muscle targeting

The *Drosophila* motor axon network has provided one of the best systems in which to study axonal pathfinding and targeting mechanisms. The network is simple: 32 motoneurons innervate 30 body wall muscle fibers in each abdominal segment. Each motoneuron axon is targeted to a specific muscle fiber, and few projection errors are made during normal development. Thus, the motor axon network is a genetically hard-wired map, and is an ideal system in which to study how genes control the formation of specific synaptic connections. In much of our work, we have focused on the roles of tyrosine phosphorylation in regulating motor axon guidance decisions. In particular, we have studied receptor tyrosine phosphatases (RPTPs), which are cell surface receptors that

antagonize the activities of tyrosine kinases. Our current focus is on the identification and characterization of ligands recognized by the extracellular domains of the RPTPs. We have shown that one cell-surface ligand interacts with an RPTP in both *cis* and *trans*, and *that* cis and *trans* interactions have different signaling outcomes.

We have also conducted genetic screens to identify cell surface proteins that label specific muscle fibers for recognition by motor axon growth cones. These studies showed that members of one family of cell surface molecules, the leucine-rich repeat (LRR) proteins, are likely to be function as muscle target labels. We are continuing to examine the roles of several LRR proteins in controlling recognition of specific muscle fibers.

Synaptogenesis and synaptic plasticity in the larval neuromuscular system

Motor growth cones reach their muscle targets during late embryogenesis and then mature into presynaptic terminals that are functional by the time of hatching. The pattern of Type I neuromuscular junction (NMJ) synapses in the larva is simple and highly stereotyped, with boutons restricted to specific locations on each muscle fiber. These synapses 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 represents a form of synaptic plasticity, because it is controlled by feedback 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.

Our recent work on synapses has focused on control of synaptic protein translation. Local translation at synapses has been studied in *Aplysia*, mammalian, and arthropod systems. It has attracted interest because it is a mechanism that allows neurons to separately adjust the strengths of individual synapses. Our work on local translation began when we identified *pumilio* (*pum*) in a genetic screen. Pum is an RNA-binding protein that represses translation of mRNAs, and maternal Pum controls patterning during early embryonic development. However, our work showed that Pum is also an important mediator of synaptic growth and plasticity at the NMJ. Postsynaptic Pum negatively regulates expression of the essential translation factor eIF-4E and the glutamate receptor subunit GluRIIA, and it binds selectively to the 3'UTRs of the *eIF-4E* and *GluRIIA* mRNAs.

Pum also represses expression of its cofactor Nanos (Nos), and binds to the 3' UTR of *nos* mRNA. Nos is required for Pum function during early development, and Pum and Nos form a complex that represses translation of *hunchback* mRNA during early development. However, our results show that 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.

We are currently attempting to develop reporters to measure local postsynaptic translation in live transgenic animals. The current reporter is a fusion of GluRIIA to the photoconvertible fluorescent protein Eos. We hope to use these reporters to determine whether Pum and Nos actually regulate local translation of GluRIIA and other postsynaptic mRNAs. We will also attempt to test a model for control of Pum activity by aggregation-prone glutamine-rich sequences.

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Images from left to right:

Professor Kai Zinn

The pattern of motor axons and synapses in the ventral region of a third-instar larval hemisegment, visualized using the 3D rendering program Imaris. Cover image from Current Biology, March 2001. Image generated by Rachel Kraut.

An array of neuromuscular junctions on muscles 6 and 7 in the third instar larva, visualized with anti-Futsch (green) and anti-eIF-4E (red). Cover image from Journal of Neuroscience, April 2009. Image by Kaushiki Menon and Violanal Nesterova

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Lee, H-K., Cording, A., Vielmetter, J., and Zinn, K. (2011) ***cis* and *trans* interactions between a receptor tyrosine phosphatase and a cell surface ligand regulate axon guidance and glial-neuronal communication.** Manuscript submitted for publication.

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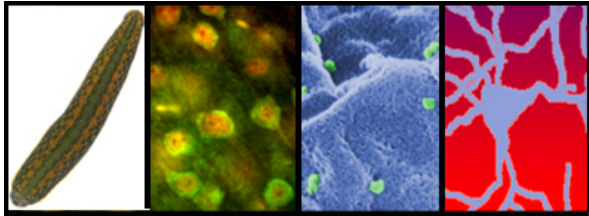
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Broad Senior Research Fellows in Brain Circuitry:
Stijn Cassenaer, Sotiris Masmanidis, Andrew Steele,
Daniel Wagenaar, Guangying Wu

Research and Laboratory Staff:

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Laurent Moreaux, Maria Papadopoulou

Masmanidis Lab:
Jiangang Du

Steele Lab:
Daniel Chang, Cynthia Hsu, Christian Gallardo, Keith Gunapala, Matthew Luby, and Scott Shuster

Wagenaar Lab:
Pieter Laurens Baljon, Cynthia Harley, Andrew Krause, John Nagarah

Wu Lab:
Yao Ding, Richard I. Kuo

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BRAIN CIRCUITRY

Summary:
Neuroscientists study each of the 100 billion neurons in the human brain. But while they understand individual neurons, they've been stumped by how neurons work together, how they encode information, and how they generate thoughts, emotions, and actions. That pioneering area of study is behind the Broad Fellows Program in Brain Circuitry, made possible through an $8.9 million grant from the Broad Foundations and philanthropist Eli Broad in 2005.

Recipients of the Broad Fellowship in Brain Circuitry are able to devote up to five years to their projects, without having to worry about finding another postdoctoral appointment in a year or two, or limiting themselves only to research that will lead to tenure. These researchers are at a level between postdoctoral fellow and assistant professor, which means that they are very independent and don't have to worry about the tenure clock. The program is designed to give researchers the freedom and flexibility to advance their work in whatever way is most productive, and may include the development of specific technologies or the invention of new instruments. Broad Fellows are given individual space to do their work in the Beckman Laboratories of Behavioral Biology. The

program strongly encourages independent cutting-edge research and provides commensurate funding, space and facilities for Fellows.

The program is funded by a generous gift from the Eli and Edythe Broad Foundation, a Los Angeles-based venture philanthropy focused on entrepreneurship for the public good in education, science, and the arts. Further funding is provided by various federal and private institutions that support the research of individual fellows. The program is overseen and directed by Prof. Christof Koch. The director is Prof. Henry Lester as of a few months ago. Currently, five Fellows are in residence.

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PUBLICATIONS

2011

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Wagenaar, D.A., Hamilton, M.S., Huang, T., Kristan, W.B. and French, K. (2010) A hormone-activated central pattern generator for courtship. *Curr. Biol.* **20**(6):487-495.

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KATALIN FEJES-TOTH LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY
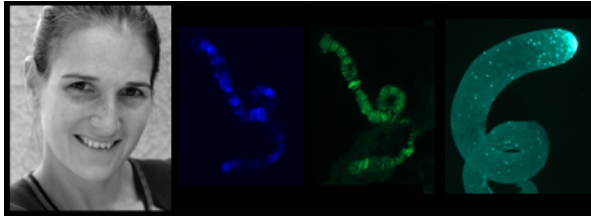
Thomas Hunt Morgan Senior Research Fellow:
Katalin Fejes Toth
Postdoctoral fellow:
Sypriya Kadam

Research Technician:
Miranda Timmons

Visiting Student:
Marcel Dammert

Undergraduate Student:
Susan Liao

Volunteer:
Mary Ha

NON-CODING RNAS IN REGULATION OF GENE EXPRESSION

Summary:

The sequencing of eukaryotic genomes and transcriptomes revealed that a remarkably small fraction of both is occupied by protein-coding sequences (<2% in human). Instead, much of what was thought to be "junk DNA" turns out to encode for so called non-coding RNAs (ncRNA) that, similarly to proteins, regulate important biological processes. We use cell culture and fruit fly as models and a combination of biochemistry, molecular biology and high-throughput sequencing techniques to address how different classes of small non-coding RNAs regulate chromatin structure and transcription. Currently we are focusing on the role of two independent classes of small RNAs: genic small RNAs and piRNAs.

We have recently described and characterized a new class of small RNAs in human cell lines, termed genic small RNAs, that map to the promoter regions (promoter associated small RNAs or PASRs) and the bodies of protein coding genes. Our studies revealed that the majority of protein-coding genes give rise to such short non-coding RNAs and expression levels of genic small RNAs, in general, correlates with the transcription of the corresponding mRNA and the presence of an active chromatin state. While the function of genic small RNAs is still unclear, several lines of evidence indicate that the RNAs or the act of their transcription *per se* regulate the expression of the protein-coding genes to which they are linked. We wish to investigate how the expression of these newly identified non-coding RNAs is controlled and how they in turn affect expression of protein-coding genes.

Establishing the correct chromatin state is crucial for maintaining the genomic integrity of the germline. Piwi proteins and their small RNA partners, the Piwi interacting RNAs or piRNAs, function in the germline to repress transposon activity thereby maintaining genomic integrity. Much is known about the cytoplasmic function of Piwi proteins where they repress expression of

transposable elements by cleavage of transposon mRNA. Most animals express at least one member of the Piwi protein family in the nucleus, but the biological significance of this localization is not known. We are testing the hypothesis that Piwi exerts its effects on transposon expression in part by altering the epigenetic state and transcription of transposable elements. We are using high-throughput sequencing methods to identify genomic loci at which Piwi is enriched and are testing the effect of Piwi depletion and tethering on histone modification patterns.

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Images from left to right:
Senior Research Fellow Katalin Fejes-Toth
D. melanogaster nurse cell polytene chromosome immunostaining
testis of D. melanogaster expressing GFP-Piwi

PUBLICATIONS

2011

ENCODE Project Consortium, Myers RM, Stamatoyannopoulos J, Snyder M, Dunham I, Hardison RC, Bernstein BE, Gingeras TR, Kent WJ, Birney E, Wold B, Crawford GE. (2011) **A user's guide to the encyclopedia of DNA elements (ENCODE).** PLoS Biol. Apr;9(4):e1001046. PMID: 21526222

Caudron-Herger, M., Müller-Ott, K., Mallm, J.-P., Marth, C., Schmidt, U., Fejes-Tóth, K. and Rippe, K. (2011). **Coding RNAs with a non-coding function: maintenance of open chromatin structure**. Nucleus. Sep 1;2(5):410-24. PMID: 21983088

Fejes Toth K, Hannon GJ (2011). **Chapter 13: Non-Coding RNAs as Regulators of Transcription and Genome Organization** in *Genome organization and function in the cell nucleus*, Rippe, K., ed., pp. 309-351, Wiley-VCH, Weinheim. Print ISBN: 9783527326983

Genetically Altered Mouse Production Facility
Shirley Pease
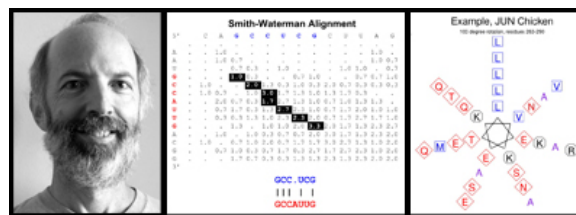
Nucleic Acid & Protein Sequence Analysis
David Mathog
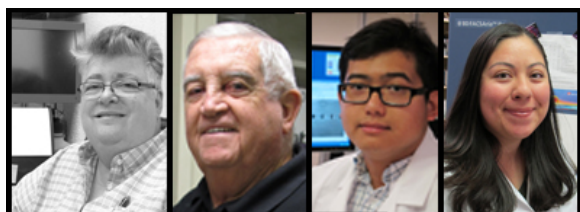
Flow Cytometry Facility
Rochelle Diamond

Protein Expression Center
Jost Vielmetter

Genetics and Genomics Laboratory
Igor Antoshechkin

Protein Peptide Microanalytical Laboratory
Jie Zhou
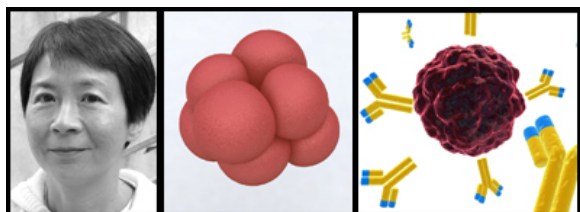
Monoclonal Antibody Facility
Susan Ker-hwa Ou
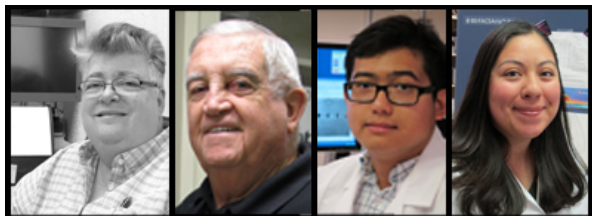
Flow Cytometry and Cell Sorting Facility

Facility Manager:
Rochelle Diamond

Faculty Supervisor:
Ellen V. Rothenberg

Sorting Operators:
Diana Perez, Joshua Verceles

Optics and Maintenance Specialist:
Patrick Koen

FLOW CYTOMETRY AND CELL SORTING FACILITY

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). 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 (over 200 consultation appointments with 32 Caltech lab groups and 17 administrative, JPL, and external consultations last year). In addition, the facility makes Treestar's FlowJo off-line analysis program available to its clients (39) for free and

non-clients (15) for a fee through a network license. The facility has negotiated discounts with two antibody vendors and placed over 125 orders for its clients this past year.

This past two years the facility provided service to 30 laboratories from the Divisions of Biology, Chemistry and Chemical Engineering, Applied Physics, Geology and Planetary Science, and JPL, 80 users were supported. Twenty two researchers were trained in flow cytometry and the use of the BD FACSCalibur analyzer.

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Images from left to right:
Rochelle Diamond
Patrick Koen
Joshua Verceles
Diana Perez

PUBLICATIONS

2011

Feng, N., Vegh, P., Rothenberg, E.V. and Yui, MA. (2011) **Lineage divergence at the first TCR-dependent checkpoint: preferential γδ and impaired αβ T cell development in nonobese diabetic mice.** *J Immunol.* **186**(2):826-837. PMID: 21148803.

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Genetically Engineered Mouse Services
Director and Member of the Professional Staff:
Shirley Pease
Cryopreservation, Re-derivation and Mouse Colony Management:
Jennifer Alex
Microinjection:
John Earle
Embryonic Stem Cell Culture:
Simon Webster

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."

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 that appear wholly of ES cell origin can be produced by injecting mES cells into earlier stage embryos (Valenzuela *et al*., 2010) In the light of recent publications, we are currently evaluating the benefit of altered mES cell culture conditions, by supplementing media with two kinase inhibitors. (Ying *et al*., 2008) Our goal is to make our gene targeting system more robust and efficient. 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 second year, we organized, set up and taught a three-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. The students successfully developed GFP expressing ES cell lines that will be useful for teaching at PCC in the Biotechnology course, which is directed by Pam Eversole-Cire, (a former Caltech post-doc). 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

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. The advantages of such a resource 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 will be basic but easy to use and most valuable for the reports the system will be able to generate. We anticipate this will be a very useful animal management tool and anticipate launching the software in the next few weeks. GEMs is a fee for service facility.

Shirley Pease co-edited "*Advanced Protocols for Animal Transgensis 2011*" and previously, *Mammalian and Avian Transgensis*, which was published in 2006.

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Images from left to right:
Director Shirley Pease
Cyropreservation
Blue stem cell cluster with pink nuclei

Listed below are the names of the seventeen principal investigators and their postdoctoral fellows or graduate students who are presently using GEMs services.

David Anderson
Ben Deneen, Li Ching Lo, Sophia Vrontu, Dayu Lin, Hyosang Lee, Prabhat Kunwar, Haijiang Cai

Alexei Aravin
Dubravka Pezic

David Baltimore
Alex Balazs, Aadel Chaudhuri, Eun Mi Hur, Daniel Kahn, Jocelyn Kim, Xin Luo, Ryan O'Connell, Param Ramakrishnan, Alex So, Xin Luo, Shengli Hao, Lili Yang, Jimmy Zhao

Mark Davis
(Chemistry and Chemical Engineering)
Jonothan Choi, Han Han, Leonard Medrano, Jonathan Zuckerman

Ray Deshaies
Narimon Honapour

Michael Elowitz
Julia Tischler

Scott Fraser
David Koos, Carol Readhead, Nicholas Plachta, Periklis Pantazis, Max Ezin

Mary Kennedy
Leslie Schenker, Holly Carlisle, Edoardo Marcora, Tinh Luong

Christof Koch
Andrew Steele

Henry Lester
Purnima Deshpande, Herwig Just, Ryan Drenan Julie Miwa Elisha Mackay, Sheri McKinney, Rachel Penton

Linda Hsieh-Wilson
Binquan Zhuang Joshua Brown, Peter Clarck, Chithra Krishnamurthy, Claude Rogers, Andrew Wang

Sarkis Mazmanian
Sarah McBride

Paul Patterson
Ben Deverman, Natalia Malkova, Limin Shi, Sohila Zadran, Elaine Hsiao, Ali Koshnan, Jan Ko

Ellen Rothenberg
Mary Yui, Hao Yuan Kueh, Long Li, Jingli Zhang

Melvin Simon
Sang-Kyou Han

Alexander Varshavsky
Christopher Brower, Brandon Wadas Konstantin Platkov

Barbara Wold
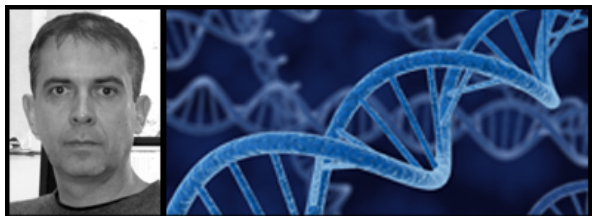Brian Williams

PUBLICATIONS

2011

Plachta, N., Bollenbach, T., Pease, S. and Fraser, S.E. (2011) **Oct4 kinetics predict cell lineage patterning in the early mammalian embryo.** *Nature Cell Biol*. PMID: 21258368.

Shipping of Mice and Embryos (2011) In: *Advanced protocols for animal transgenesis, an ISTT manual,* 1st edition, Pease, S. and Saunders, T.L. (Eds.), Springer, pps. 601-613.

Millard and Muriel Jacobs Genetics and Genomics Laboratory

Director:
Igor Antoshechkin

Staff:
Vijaya Kumar, Lorian Schaeffer

Web site:
http://mmjggl.caltech.edu/

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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 and microarray analysis. During the period of this report, the Laboratory has worked with groups from the Division of Biology, the Division of Chemistry and Chemical Engineering and the Division of Engineering and Applied Sciences.

Research Support:

Division of Biology - The Laboratory performed high throughput sequencing experiments for the groups of professors Angela Stathopoulos, Barbara Wold, Bruce Hay, David Chan, Ellen Rothenberg, Elliot Meyerowitz, Eric Davidson, Grant Jensen, John Allman, Mary B. Kennedy, Michael Elowitz, David Prober, Alexei Aravin, Katalin Fejes Toth, Sarkis Mazmanian, and Paul Sternberg. The projects ranged from genome-wide identification of transcription factor binding sites during T-cell development (Ellen Rothenberg) to the *de novo* sequencing of nematode genomes (Paul Sternberg) and transcriptome analysis of *Aedes aegypti* (Bruce Hay). Microarray experiments were carried out for the laboratories of David Baltimore and Dianne Newman.

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 the laboratories of Peter Dervan and David Tirrell.

Division of Engineering and Applied Science - The Laboratory conducted HTS experiments for the laboratory of Jared R. Leadbetter, including genome sequencing and transcriptome analysis of multiple *Treponema* strains.

Infrastructure and capabilities:
The Laboratory operates Illumina GAIIx and HiSeq2000 high throughput sequencers that allow us to perform a wide variety of experiments, including ChIP-Seq, RNA-Seq, small RNA analysis, *de novo* genome sequencing, mutation discovery, etc. 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.

Some of the equipment involved in microarray work includes Affymetrix GeneChip system, Agilent and GenePix Microarray Scanners, multiple hybridization ovens, etc. The Laboratory has the ability to manufacture custom microarrays using MicroGrid II arrayer, which have been recently used for studies of carbohydrate – protein interactions.

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. Additional microarray and HTS software tools and analysis packages, both public and commercial, are also available.

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Images from left to right:
Director, Igor Antoshechkin
DNA Strand

PUBLICATIONS ACKNOWLEDGING THE LABORATORY

2011

Budd, M.E., Antoshechkin, I.A., Reis, C., Wold, B.J. and Campbell, J.L. (2011) **Inviability of a DNA2 deletion mutant is due to the DNA damage checkpoint.** *Cell Cycle* **10**(10):1690-1698. PMID: 21508669.

Chen, S., Beeby, M., Murphy, G.E., Leadbetter, J.R., Hendrixson, D.R., Briegel, A., Li, Z., Shi, J., Tocheva, E.I., Muller A., Dobro M.J. and Jensen, G.J. (2011) **Structural diversity of bacterial flagellar motors.** *EMBO J.* **30**(14):2972-2981. PMID: 21673657.

Ozdemir, A., Fisher-Aylor, K.I., Pepke, S., Samanta, M., Dunipace, L., McCue, K., Zeng, L., Ogawa, N., Wold, B.J. and Stathopoulos, A. (2011) **High resolution mapping of twist to DNA in *Drosophila* embryos: efficient functional analysis and evolutionary conservation.** *Genome Res.* **21**(4):566-77. PMID: 21383317.

Rogers, C.J., Clark, P.M., Tully, S.E., Abrol, R., Garcia, K.C., Goddard, W.A 3rd and Hsieh-Wilson, L.C. (2011) **Elucidating glycosaminoglycan-protein-protein interactions using carbohydrate microarray and computational approaches.** *Proc. Natl. Acad. Sci. USA* **108**(24):9747-9752. PMID: 21628576.

Rosenthal, A.Z., Matson, E.G., Eldar, A. and Leadbetter, J.R. (2011) **RNA-seq reveals cooperative metabolic interactions between two termite-gut spirochete species in co-culture**. *ISME J.* **5**(7):1133-1142. PMID: 21326336.

Tai, P.W., Fisher-Aylor, K.I., Himeda, C.L., Smith, C.L., Mackenzie, A.P., Helterline, D.L., Angello, J.C., Welikson, R.E., Wold, B.J. and Hauschka, S.D. (2011) **Differentiation and fiber type-specific activity of a muscle creatine kinase intronic enhancer.** *Skelet. Muscle,* **1**:25 .PMID: 21797989.
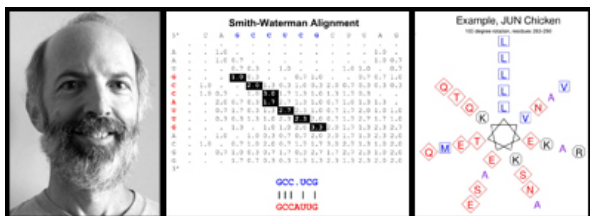
Nucleic Acid and Protein Sequence Analysis Computing Facility

Manager:
David R. Mathog, Manager

Supervisor:
Stephen L. Mayo

NUCLEIC ACID AND PROTEIN SEQUENCE ANALYSIS COMPUTING FACILITY

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.

Images from left to right:
David Mathog
Smith-Waterman Alignment
JUN Chicken

Protein Expression Center
Director:
Jost G. Vielmetter
Supervisor:
Barbara J. Wold
Faculty Advisors:
Pamela J. Bjorkman, Mary B. Kennedy
Staff:
Michael Anaya, Timothy Feliciano, Clarke Gasper, Angela Ho

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. The instrument used for these studies has been upgraded from a Biacore T100 to a T200 instrument providing higher resolution for SPR studies. The instrument continues to enjoy broad interest and use and has 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 (Engineering Immunity, Pamela Bjorkman). Mainly we use protein expression based on transient DNA transfection but recently we succeeded in generating 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 and llamas 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 two 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: CO_2 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 for 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 this our automated version of this assay with known assay reagents and have successfully generated data.

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

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Images from left to right:
Director Jost Vielmetter

Liquid handling robot in a biosafety hood. The liquid handling robot contains an 8-probe liquid handling device with fixed tips, a multi-channel pipetting device with disposable tips, and a multitude of integrated devices that can all be accessed by a robotic gripper/manipulator. All aspects of pipetting speeds, volumes, styles, and movements of labware are controlled by Tecan's Evo-specific control software (EvoWare), which runs on a PC (far right). Next to the PC is the external PCR machine control unit, and on top of it, an external CO2/air mixer to provide a stable percentage of CO2 atmosphere for the integrated CO2 incubators.

Robot arms and devices integrated into the Tecan Evo Freedom liquid handler. (a) 8-probe Liquid Handling arm (LiHa), which can move in the x, y, z directions. Probes can spread in the y-dimension to accommodate different well distances and move independently in the z-dimension to allow "cherry picking."

PUBLICATIONS

2011

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

Diskin, R., Scheid, J.F., Marcovecchio, P.M., West, A.P. Jr., Klein, F., Gao, H., Gnanapragasam, P.N.P, Abadir, A., Seaman, M.S., Nussenzweig, M.C. and Bjorkman, P.J. **Increasing the potency and breadth of an HIV antibody by using structure-based rational design.** *Science*, published online 27 October 2011.

He, Y. and Bjorkman, P.J. (2011) **Structure of FcRY, an avian immunoglobulin receptor related to mammalian mannose receptors, and its complex with IgY.** *Proc. Natl. Acad. Sci. USA* **108**:12431-12436. PMID: 21746914.

Yang, F., West, A.P., Jr., Bjorkman, P.J. (2011) **Crystal structure of a hemojuvelin-binding fragment of neogenin at 1.8 Å.** *J. Struct. Biol*. **174**:239-244. PMID: 20971194.

2010

Diskin, R., Marcovecchio, P.M. and Bjorkman, P.J. (2010) **Structure of a clade C HIV gp120 plus CD4 and a CD4-induced antibody reveals anti-CD4 polyreactivity.** *Nature Struct. Mol. Biol*. **17**:608-613. PMID: 20357769.

Klein, J.S.*, Webster, A.*, Gnanapragasam, P.N.P., Galimidi, R.P. and Bjorkman, P.J. (2010) **A dimeric form of the HIV-1 antibody 2G12 elicits potent antibody-dependent cellular cytotoxicity.** *AIDS* **24**:1633-1640. PMID: 20597163.

Luo X.M., Lei M.Y.Y., Feidi R.A., West A.P. Jr., Balazs A.B., Bjorkman, P.J., Yang, L. and Baltimore D. (2010) **Dimeric 2G12 as a Potent Protection against HIV-1.** *PLoS Pathog*. **6**(12):e1001225. PMID: 21187894.

BIOLOGY FACILITIES LAB ANNUAL REPORT | 2011

CALIFORNIA INSTITUTE OF TECHNOLOGY

Protein/Peptide Microanalytical Laboratory Director:
Jie Zhou

Associate Biologist:
Felicia Rusnak

Faculty Supervisor:
Mary Kennedy

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
Development of Os complexed polymer column for the on-column trypsin digestion of proteins

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
Our facility published one paper of title: Fast Trypsin Digestion of Proteins on a Cross-Linked $[Os(dmebpy)_2Cl]^{+/2+}$-Derivatized Copolymer of Acrylamide and Vinylimidazole Column. (Rapid Commun. Mass Spectrum; 24: 2236-2244).

We have been continuing the investigation of insoluble and cross-linked $[Os(dmebpy)_2Cl]^{+/2+}$-derivatized copolymer of acrylamide and vinylimidazole, and found some new biological applications. Second paper has been submitted for publication (Title: SDS-Tolerated Trypsin Digestion of Protein and LC/MS on a Hybrid Column of $[Os(dmebpy)_2Cl]^{+/2+}$-Derivatized Acrylamide and Vinylimidazole Copolymer/C_{18}).

Services
During the first seven months of fiscal 2011 PPMAL interacted with 19 laboratories. Samples were analyzed from the Division of Biology, and Chemistry and Chemical Engineering (see list). A total of 578 samples were analyzed. In addition to our work for campus faculty and staff, work was also performed for off-campus institutions.

PPMAL October 2010-April 2011 (7 months)

ON-CAMPUS

	#Samples	#Mass	#Proteomics	#SeqCycles
Arnold, Frances	14	12	2	
Barton	17	17		
Bjorkman	4	4		
Chan, David	7	7		
Clemons	3		3	
Dervan	1		1	
Gray	186	186		
Grubbs	1	1		
Heath	206	206		
Hsieh-Wilson	31	31		
Mayo	19	19		
Rees	74	28	46	
Roukes	1	1		
Tirrell	33	8	4	21
Varshavsky	1		1	
Vielmetter	6			6

OFF-CAMPUS

	#Samples	#Mass	#Proteomics	#SeqCycles
Glyport - Former Scott Fraser Group	3	3		
Princeton University-Prof James Link-Former David Tirrell Group	6			6
UCLA	21	1		20
TOTALS	**634**	**524**	**57**	**53**
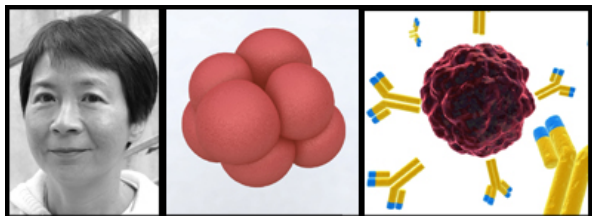
Monoclonal Antibody Facility
Director:
Susan Ker-hwa Ou

Supervisor:
Paul Patterson

Staff:
Shi-Ying Kou

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MONOCLONAL ANTIBODY FACILITY

Summary:
The Monoclonal Antibody Facility provides assistance to researchers wishing to generate monoclonal antibodies (mAbs), ascites fluid or other related services. In addition to these services, the Facility also conducts research on the development of novel immunological techniques.

By applying the adult tolerization or cyclophosphamide immunosuppression methods, we can 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 antigen and then inducing 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 amounts of antigen.

In its service capacity, the Facility produced Abs for the following groups during the past year. Transmembrane Bioscience obtained mAbs and polyclonal ascites against recombinant proteins from Bartonella, Scrub Typhus, *Orientia tsutsugamushi*, Coxiella Burnetti, Coxiella AdaA, and ESAT 6 (antigen from Mycobacterium tuberculosis). The Zinn laboratory obtained polyclonal ascites against *Drosophila* Toll 7.

As part of the NHGRI ENCODE project with Dr. Wold's group, we aim to produce more than 50 chromatin immunoprecitation (ChIP)-validated mAbs against transcription factors for which ChIP-grade reagents are not available. To increase the success rate of usable reagents, we tested antigens fixed by formaldehyde in the same manner as is used in ChIP studies. Animals immunized with fixed and unfixed NRSF N250 were fused and mAbs were obtained from both groups. Therefore, we immunize animals with fixed Ag only. After cleaving the GST from the fusion protein, there is always some GST left in the sample, and several mice generated responses against the GST instead of the antigen. The adult mouse tolerization method (Lebron *et al.*, 1999) was applied with the MYF5 antigen and mice showed significant improvement in recognizing the Ag. Therefore, we tolerize mice with GST before immunization. Using these procedures, we obtained mAbs against NRSF N250, N150, C250, FOX P2 Ag1, FOX P2 Ag2, and GABPA 200-310 inclusion body, HES1, PER1, MYF5, BHLH, and FOXM1.

We are also currently working with the following groups: The Wold laboratory is immunizing with the human transcription factors NANOG, RBPJ, RORC, STAT, CSDA, NCOR, and RORC. Transmembrane Bioscience is immunizing with recombinant proteins from Rickettsia typhi OmpB

and Crimean-Congo hemorrhagic fever (CCHF). The Chan laboratory is immunizing with human TFAM (mitochondria transcription factor A). The Jung laboratory from USC is immunizing with Rubicon.

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Images from left to right:
Director Susan Ker-hwa Ou
Solid Pink Cell Cluster
Cancer Cell - antibodies

PUBLICATIONS

2011

Gehman, L.T., Stoilov, P., Maguire, J., Damianov, A., Lin, C.H., Shiue, L., Ares, M., Mody, I. and Black, D.L. (2011) **The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain.** *Nature Genetics* **43**:706-U133. PMID: 21623373.

Jayaraman, M., Thakur1, A.K., Kar, K., Kodali, R. and Wetzel, R. (2011) **Assay for studying nucleated aggregation of polyglutamine proteins.** *Methods* **53**:246-254. PMID: 21232603.

Khoshnan, A., Southwell, A.L., Bugg, C.W., Ko, J.C. and Patterson, P.H. (2011) **Recombinant intrabodies as molecular tools and potential therapeutics for Huntington's Disease. In: Neurobiology of Huntington's disease: Applications to drug discovery**. Chapter 10, CRC Press. PMID: 21882417.

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