



2004
Meeting *of*
International
Research Scholars

Program *and* Abstracts

Tallinn, Estonia ■ June 23–26, 2004

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Office of Grants and Special Programs

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HOWARD HUGHES MEDICAL INSTITUTE PROGRAMS

The Howard Hughes Medical Institute was founded in 1953 by aviator-industrialist Howard R. Hughes. Its charter, in part, reads: *The primary purpose and objective of the Howard Hughes Medical Institute shall be the promotion of human knowledge within the field of the basic sciences (principally the field of medical research and medical education) and the effective application thereof for the benefit of mankind.*

Biomedical Research Program

HHMI is a nonprofit medical research organization dedicated to basic biomedical research and education. Its principal objectives are the advancement of fundamental knowledge in biomedical science and the application of new scientific knowledge to alleviate disease and promote health. Through its program of direct conduct of medical research, it employs about 300 independent investigators based at laboratories throughout the country. To find out about their research, go to www.hhmi.org.

Grants and Special Programs

To complement the research activities of its investigators, HHMI has a grants program committed to strengthening education in the biological and related sciences and supporting research by non-U.S. scientists. Other important objectives of HHMI's grants program are to advance public understanding and appreciation of science and to broaden access to science for all persons, including women and members of underrepresented minority groups. HHMI grants, which are administered by the Office of Grants and Special Programs, provide funding for pre-K–12 and undergraduate science education, graduate science education and research training, and international research and education. For information on HHMI's grants programs, go to www.hhmi.org/grants.

International Research Scholars Program

Since the inception of the International Research Scholars Program in 1991, HHMI has awarded more than 300 grants totaling approximately \$100 million to support the work of outstanding scientists in 32 countries outside the United States. Grants are awarded to promising biomedical scientists who have made significant contributions to fundamental research—that is, to the understanding of basic biological processes or disease mechanisms.

In 1991, Canada and Mexico (as immediate neighbors of the United States) were selected as

eligible countries for the first round of grants, which totaled \$10.8 million in support of 24 scientists. In 1992, \$13.3 million was awarded to 29 scientists in Australia, New Zealand, and the United Kingdom. In 1995, grants totaling more than \$13 million were made to 90 scientists in 10 countries of the Baltics, Central Europe, and the former Soviet Union and 58 collaborating scientists around the world. The 10 countries were Belarus, the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Russia, the Slovak Republic, and Ukraine. In 1997, grants were made to scientists in Canada and five countries of Latin America: Argentina, Brazil, Chile, Mexico, and Venezuela. HHMI awarded 47 five-year awards, ranging from \$255,000 to \$400,000 and totaling \$15 million.

Recognizing that microbial and parasitic diseases are worldwide problems with a significant impact on world health, HHMI awarded 45 grants totaling \$15 million in spring 2000 to scientists around the world in its Infectious Diseases and Parasitology initiative. This was the Institute's first competition under the International Research Scholars Program that was topic-specific and not restricted to a particular geographic region. In fall 2000, 46 new grants, totaling \$15 million, were made to scientists in countries of the Baltics, Central and Eastern Europe, and the former Soviet Union.

HHMI's 2001 international initiative was the second for Argentina, Brazil, Chile, and Venezuela and the third for Canada and Mexico. With this initiative, HHMI recognized the contributions of biomedical researchers in these countries and the need for support to sustain their research. Forty-three awards were made, totaling more than \$16 million.

New Initiatives

In April of this year, HHMI announced competitions for two new initiatives in the International Research Scholars Program. Both competitions will award five-year grants of between \$50,000 and \$100,000 per year. Applications for the 2005 Infectious Diseases and Parasitology competition must be submitted by September 15, 2004, and the awards will be announced in May 2005. Applications for the 2005 Baltics, Central and Eastern Europe, Russia, and Ukraine competition must be submitted by November 17, 2004, and the awards will be announced in December 2005. More details, including eligibility requirements and the Web-based competition system, are available at www.hhmi.org/grants/funding.

INTRODUCTION

Welcome to the 2004 Meeting of HHMI International Research Scholars in Tallinn, Estonia, at which scientists from 29 countries are gathered. Joining their fellow scholars from Estonia are scientists from Argentina, Australia, Bangladesh, Brazil, Bulgaria, Canada, Chile, the Czech Republic, France, Germany, Greece, Guinea, Hungary, India, Israel, Lithuania, Poland, Mexico, Russia, the Slovak Republic, South Africa, Switzerland, Taiwan, Uganda, Ukraine, United Kingdom, Uruguay, and Venezuela.

In its 13-year history, the International Program has expanded nearly eightfold with respect to the number of scholars supported at any one time and has spread its geographic reach from the two nearest neighbors of the United States to now 29 countries around the globe, reflecting an increasing recognition of science's international scope. In addition to our three current International Research Scholar initiatives, we entered into a partnership with EMBO to foster the early careers of the next generation of scientists in countries where HHMI and EMBO funding currently overlap. Newly independent scientists from Hungary, Poland, the Czech Republic, and, as of this year, Estonia are eligible to apply for the three-year research grants; awardees of this joint program are in attendance for the first time.

Building on experience gained from a 12-year collaboration with the National Academy of Sciences to fund scientific workshops and laboratory courses, the International Program also sponsors advanced laboratory training courses that capitalize on the expertise of our scholars and aim to build a regional network of scientists with common interests and skills. The first such course was organized by international research scholar Rashidul Haque and held last September at the Centre for Health and Population Research, International Centre for Diarrhoeal Disease Research, Bangladesh. To give instruction on the topic of infectious disease research, Dr. Haque assembled a distinguished faculty from Bangladesh, India, Thailand, Canada, and the United States. The course attracted 18 students from the following nine countries: Bangladesh, Nepal, India, Thailand, Vietnam, Turkey, Peru, Guatemala, and Malawi. For 2004, two courses are planned. Scholar Rafael Radi will host a course in Montevideo, Uruguay, that will offer advanced training in host-cell interactions with trypanosomes. The other will be held in Rosario, Argentina. It is being organized by our three scholars there, Diego de Mendoza, Fernando Soncini, and Alejandro Vila, and will focus on the molecular basis of the bacterial stress response.

HHMI's Janelia Farm Research Campus, a 281-acre site in Virginia that is about 30 miles from HHMI headquarters in Chevy Chase, Maryland, is now under development. The campus will bring together biologists, computer scientists, engineers, physicists, and chemists; free them from many of the

constraints that dominate more traditional research environments; and encourage them to create the new tools of biology, technologies that may be out of the reach of many academic institutions (see *Janelia Farm Research Campus: Report on Program Development* on the HHMI website at www.hhmi.org). Janelia Farm will provide research opportunities for scientists from the United States and abroad. Gerry Rubin, HHMI vice president and director of the Janelia Farm Research Campus, is joining us in Tallinn not just to describe the creation of the new campus but to solicit your ideas for "1000-person-year projects": biomedical research goals worthy of investing the energy of 100 scientists for a 10-year period.

We continue to be impressed by your research achievements, to which the abstracts contained in this book attest, and are mindful that such high standards are frequently attained under circumstances that most researchers would find extremely challenging. We thus anticipate that your oral presentations and posters will generate lively and stimulating discussion. To accommodate the wish that many of you voiced to go into more depth in your presentations and to have fewer parallel sessions, we decided this year to lengthen the talks to 20 minutes, with 5 minutes for discussion. To make this possible, half of you will be presenting posters, an arrangement that will be reversed at the next international meeting. The results of the 14 collaborations funded by last year's minigrant awards, which were created from funds originally allocated for a 2003 meeting, will be presented in separate sessions, and we are eagerly awaiting the outcomes of this project. Although we are not planning to fund new minigrants at this time, we hope that the meeting will provide a further opportunity for you to form productive collaborative alliances.

The book also contains summaries of the lectures of our keynote speakers, who are both HHMI investigators. William Dietrich, associate professor of genetics at Harvard Medical School, will speak about his research on host resistance to infectious disease. Celeste Simon, associate professor of cell and developmental biology and associate investigator at the Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, has been probing the connections between hypoxia, angiogenesis, and tumor progression.

We hope that you enjoy your visit to Estonia.

Thomas R. Cech, Ph.D., *President*

Peter J. Bruns, Ph.D., *Vice President
Grants and Special Programs*

Jill G. Conley, Ph.D., *Director
International, Precollege Science Education
and Research Resources Programs*

PROGRAM SCHEDULE

2004 MEETING OF INTERNATIONAL RESEARCH SCHOLARS TALLIN, ESTONIA

Wednesday, June 23, 2004

- 2:00–5:00 p.m. Welcome/Registration
- 5:00–6:00 p.m. **Welcome Reception**
- 6:00 p.m. Proceed to Hansa Room
- 6:15 p.m. **Opening Ceremonies and Keynote Address**
*Structure of Human Telomeres: Molecular Basis for Chromosome
End-Protection and Telomerase Inhibition*
Thomas R. Cech, Ph.D., President, Howard Hughes Medical Institute
- 7:30 p.m. Dinner
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Thursday, June 24, 2004

- 7:00 a.m. Breakfast
- 8:00–9:40 a.m. **Scholars' Presentations**
Innate and Enhanced Immunity, *Hansa Room*
Cellular Architecture and Transport, *Bremerhaven Room*
- 9:40–10:10 a.m. Break
- 10:10–11:50 a.m. **Scholars' Presentations**
Protein Structures: Primary Through Quaternary, *Hansa Room*
DNA Modification and Repair, *Bremerhaven Room*
- Noon Lunch
- 1:15–2:15 p.m. **Plans for the Janelia Farm Campus, Hansa Room**
Gerald M. Rubin, Ph.D., Vice President and Director of Planning for Janelia Farm,
Howard Hughes Medical Institute
- 2:15–3:30 p.m. **Poster Session, Hansa Room**
- 3:30–5:10 p.m. **Scholars' Presentations**
Functional and Comparative Genomics, *Hansa Room*
Development and Expression, *Bremerhaven Room*
- 5:10–5:30 p.m. Break
- 5:30–6:30 p.m. **Keynote Address, Hansa Room**
Genetic Analysis of Host Resistance to Infectious Disease
William Dietrich, Ph.D., Assistant Investigator, Howard Hughes Medical Institute, and
Associate Professor of Genetics, Harvard Medical School
- 7:00 p.m. Dinner

Friday, June 25, 2004

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| 7:00 a.m. | Breakfast |
| 8:00–9:40 a.m. | Scholars' Presentations Parasitic and Viral Invasion, <i>Hansa Room</i> RNA Binding, Transcription, and Replication, <i>Bremerhaven Room</i> |
| 9:40–10:10 a.m. | Break |
| 10:10–11:50 a.m. | Scholars' Presentations Pathogen Ecology, Transmission, and Host Clinical Symptoms, <i>Hansa Room</i> Biosensors and Biosynthetic Pathways, <i>Bremerhaven Room</i> |
| Noon | Lunch |
| 1:15–3:20 p.m. | Scholars' Presentations Pathogenesis and Resistance, <i>Hansa Room</i> Gene Function and Cell Cycle Progression, <i>Bremerhaven Room</i> |
| 3:20–4:30 p.m. | Poster Session , <i>Hansa Room</i> |
| 4:30–5:30 p.m. | Minigrant Presentations Session 1, <i>Hansa Room</i> Session 2, <i>Bremerhaven Room</i> |
| 5:30–6:30 p.m. | Keynote Address , <i>Hansa Room</i> <i>Hypoxia, Angiogenesis, and Tumor Progression</i> M. Celeste Simon, Ph.D., Associate Investigator, Howard Hughes Medical Institute, and Associate Professor, Cell and Developmental Biology and Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine |
| 7:00 p.m. | Dinner |

Saturday, June 26, 2004

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| 7:00 a.m. | Breakfast |
| 8:00–9:40 a.m. | Scholars' Presentations Markers of Infection and Protection, <i>Hansa Room</i> Signaling and Circuits, <i>Bremerhaven Room</i> |
| 9:40–10:10 a.m. | Break |
| 10:10–11:25 a.m. | Scholars' Presentations Muscle Force, Dynamics, and Signaling, <i>Hansa Room</i> Signaling and Energy Transfer Pathways, <i>Bremerhaven Room</i> |
| 11:30–12:50 p.m. | Minigrant Presentations Session 3, <i>Hansa Room</i> Session 4, <i>Bremerhaven Room</i> |
| 1:00 p.m. | Lunch |
| 2:15–5:00 p.m. | City tour and free time |
| 6:30 p.m. | Depart for final evening program |

KEYNOTE SPEAKERS

Genetic Analysis of Host Resistance to Infectious Disease

WILLIAM DIETRICH, PH.D.

Assistant Investigator, Howard Hughes Medical Institute, and Associate Professor of Genetics at Harvard Medical School, Boston, Massachusetts

■ Genetics provides a powerful means of determining the molecular basis of biological function. In the absence of any specific a priori hypotheses about the molecular mechanisms of a heritable trait, we can define and isolate genes important for virtually any biological process. Our current interest is in using genetics to study a host's innate defenses against infection. We are working on several models of infectious disease for which common laboratory mouse strains exhibit heritable resistance differences; two of the models are described here.

Chlamydial Infections

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that causes a variety of human pathologies, including trachoma, pneumonia, a host of urogenital syndromes (including infertility), and invasive lymphadenopathic disease. The pathogen is a major cause of blindness in endemic regions and may be the most common source of bacterial sexually transmitted disease in the world. Although effective treatment is available, controlling the incidence of infection remains a major public health effort.

Different inbred strains of mice exhibit distinct courses of chlamydial infection. For example, C3H/HeJ (C3H) mice allow greater chlamydial replication and/or survival during the early stages of a systemic infection than do C57BL/6J (B6) mice. We have analyzed splenic chlamydial replication in cross-progeny of the C3H and B6 strains and found three quantitative trait loci (QTL) on chromosomes 2, 3, and 11 that affect this trait. In the future, we hope to understand the molecular nature of the gene differences that are responsible for these QTL effects and to study genital tract infection models to compare and contrast the resistance genes for each type of infection.

Legionnaire's Disease

Legionnaire's disease (LD) is a surprisingly common form of pneumonia, accounting for about 5–10 percent of community-acquired pneumonia cases. Unfortunately, even though effective treatments for LD exist, the overall mortality rate approaches 15 percent. LD is caused by infection with a gram-negative facultative intracellular bacterium called *Legionella pneumophila*. An important aspect of the pathogenesis of *L. pneumophila* infection is its ability to grow inside the macrophages of the lung.

Purified populations of macrophages from different inbred mouse strains exhibit differences in susceptibility to the intracellular replication of *L. pneumophila*. These differences can be dramatic: inbred mouse strain A/J macrophages allow virtually unchecked replication of the parasite, whereas inbred mouse strain C57BL/6J macrophages allow virtually none. The progeny of crosses between these two strains exhibit a susceptible or resistant phenotype, depending on the parental origin of a single gene on mouse chromosome 13 that we call *Lgn1*. We and others have shown that mutations in a gene called *Birc1e* (also called *Naip5*) are ultimately responsible for the *Lgn1* phenotype. *Birc1e* is a relative of innate immune proteins of plants that are responsible for detecting intracellular pathogens. We are beginning to investigate the molecular mechanism whereby *Birc1e* affects *Legionella* susceptibility.

Dr. Dietrich received his bachelor's degree in biological sciences from Carnegie Mellon University and his Ph.D. in biology from the Massachusetts Institute of Technology, where he studied genetic disease models in mice in the laboratory of Dr. Eric Lander. For his post-doctoral work, he extended his genomic mapping work as a research scientist at the Whitehead Institute/MIT Center for Genome Research. Dr. Dietrich is currently an HHMI assistant investigator and associate professor of genetics at Harvard Medical School.

Hypoxia, Angiogenesis, and Tumor Progression

M. CELESTE SIMON, PH.D.

Associate Investigator, Howard Hughes Medical Institute, Associate Professor of Cell and Developmental Biology and Associate Investigator at the Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

■ Proper development and function of the cardiovascular system require complex signaling between hematopoietic cells, vascular endothelial cells, and various support structures (pericytes). The specification of hematopoietic and endothelial cell fates from common precursors and establishment of a differentiated vascular network are driven by a genetically controlled pathway, as indicated by loss-of-function mutations in numerous genes encoding specific growth factors (e.g., vascular endothelial growth factor [VEGF], angiopoietin-1, and angiopoietin-2) and their receptors. However, this genetic program appears to be regulated, in turn, by metabolic cues. Specifically, we demonstrated that an inability to modulate gene expression in response to different physiological O₂ levels has profound effects on development of all components of the embryonic cardiovascular system (blood, vessels, heart, and placenta). These results suggest that O₂ levels and O₂ gradients function as critical developmental signals in many aspects of cardiovascular differentiation.

Activation of the hypoxia-inducible factor (HIF) transcriptional complex represents the primary molecular mechanism by which O₂ regulates gene expression. HIF complex accumulation is inversely related to O₂ tension: the complex becomes detectable in cells grown at 8–10 percent O₂, and levels increase almost linearly as O₂ concentration drops. Interaction of the HIF complex with its con-

sensus DNA binding site is required for hypoxia-induced target gene expression. Approximately 100 HIF target genes have been identified that encode a wide array of proteins associated with cellular metabolism and survival, including glycolytic enzymes, glucose transporters, and paracrine growth factors (VEGF and erythropoietin). By creating targeted mutations in murine HIF subunit genes, we have begun to assess the developmental consequences of impaired O₂ signaling. Our results demonstrate an absolute requirement for HIF function in hematopoietic, vascular, cardiac, and placental development. These findings underscore the importance of O₂ signaling in mammalian ontogeny and offer potential targets for hematopoietic and angiogenic therapies.

Dr. Simon obtained her bachelor's degree from Miami University (Oxford, Ohio), her M.S. in microbiology from Ohio State University, and her Ph.D. in biochemistry from the Rockefeller University. She conducted postdoctoral research in the laboratories of Dr. Joseph Nevins (Rockefeller University) and Dr. Stuart Orkin (Harvard Medical School). As an HHMI associate in Dr. Orkin's laboratory, she began her work on hematopoietic development using mouse embryonic stem cells as a model for differentiation.

Dr. Simon was appointed to her first faculty position in 1992 at the University of Chicago, Department of Medicine, where she continued her studies on hematopoiesis. She became an HHMI assistant investigator in 1994, focusing on the role of the PU.1 transcription factor in specifying hematopoietic cell fate. At this time, she developed an interest in the relationship between angiogenesis and hematopoiesis and in the role of O₂ availability in regulating hematopoiesis and angiogenesis. In 1999, she joined the new Abramson Family Cancer Research Institute at the University of Pennsylvania School of Medicine, where she is an associate professor. She was promoted to HHMI associate investigator in 2000.

SCHEDULE OF SCHOLARS' PRESENTATIONS

Innate and Enhanced Immunity

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- 8:00 a.m.** Enhancing innate immunity to treat infectious diseases
B. Brett Finlay, University of British Columbia, Vancouver, Canada
- 8:25 a.m.** The role of cross-presentation in self tolerance and viral immunity
William Ross Heath, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
- 8:50 a.m.** Prime-boost immunization against malaria
Adrian V.S. Hill, Oxford University, United Kingdom
- 9:15 a.m.** The role of plasmacytoid dendritic cells and neutrophils in the generation of antiviral immune response
Gunasegaran Karupiah, Australian National University, Canberra, Australia

Protein Structures: Primary Through Quaternary

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- 10:10 a.m.** Prediction of protein-folding rates from tertiary, secondary, and primary protein structure
Alexei V. Finkelstein, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia
- 10:35 a.m.** Structure, protein engineering, and inhibitors of the *Trypanosoma cruzi* virulence factor trans-sialidase
Alberto Carlos Clemente Frasch, Institute for Research in Biotechnology, National University of General San Martin, Buenos Aires, Argentina
- 11:00 a.m.** Structural studies on regulatory proteins and their complexes with RNA
Maria B. Garber, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia
- 11:25 a.m.** High-order quaternary arrangement confers increased structural stability to *Brucella spp.* lumazine synthase
Fernando Goldbaum, Leloir Institute Foundation, Buenos Aires, Argentina

Poster Session, 2:15–3:30 p.m.

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- Poster 1** Hematopoietic cell differentiation
Petr Bartůněk, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
- Poster 3** Expression and functional characterization of human ABCG1
László Homolya, Research Group for Membrane Biology and Immunopathology, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary
- Poster 5** Intestinal mucosa barrier repair: effects of glutamine and alanyl-glutamine on in vitro and in vivo models of mucositis induced by enterotoxin A from *Clostridium difficile*, indomethacin, and 5-fluorouracil
Aldo A.M. Lima, Federal University of Ceará, Fortaleza, Brazil
- Poster 7** Rotavirus enters the cell through a non-clathrin-, non-caveolin-dependent mechanism
Susana López, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico
- Poster 9** Endoplasmic reticulum export of glycosyltransferases depends on interaction of a cytoplasmic di-basic motif with Sar-1
Hugo J.F. Maccioni, National University of Cordoba, CONICET, Cordoba, Argentina
- Poster 11** Fine mapping and candidate gene analysis of complex immunity to infection with *Salmonella typhimurium* in mice
Danielle Malo, McGill University, Montreal, Canada

THURSDAY
HANSA ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

- Poster 13** Sequence of events leading to SNARE complex assembly during acrosomal exocytosis
Luis Segundo Mayorga, Institute of Histology and Embryology (CONICET), National University of Cuyo, Mendoza, Argentina
- Poster 15** The relict chloroplast of malaria parasites: what does it do and can we kill it?
Geoffrey McFadden, University of Melbourne, Melbourne, Australia
- Poster 17** Three-dimensional structure of the bacteriophage T4 baseplate
Vadim V. Mesyanzhinov, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
- Poster 19** The isolation and characterization of novel *Drosophila* tissue polarity genes
József Mihály, Biological Research Center, Institute of Genetics, Szeged, Hungary
- Poster 21** Chronic CD40 signaling resembles the action of the Epstein-Barr virus oncoprotein LMP1 in germinal center suppression but differs in its ability to induce inflammatory and autoimmune reactions
George Mosialos, Institute of Immunology, Biomedical Sciences Research Center "Al. Fleming," Vari, Greece
- Poster 23** Nuclear receptors regulate lineage specification and the function of antigen-presenting cells
László Nagy, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary
- Poster 25** Linear genomes in yeast mitochondria: implications from an analysis of the complete DNA sequence
Jozef Nosek, Comenius University, Bratislava, Slovak Republic
- Poster 27** A novel pathway for heme detoxification in the midgut of the tick *Boophilus microplus*: aggregation inside the hemosome, a specialized organelle
Pedro L. Oliveira, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
- Poster 29** Helical order in myosin filaments requires the closed conformation of the myosin head
Raúl A. Padrón, Venezuelan Institute for Scientific Research (IVIC), Caracas, Venezuela.
- Poster 31** De novo synthesis of bacterial glycogen: *Agrobacterium tumefaciens* glycogen synthase is involved in glucan initiation and elongation
Armando J. Parodi, Leloir Institute Foundation, Buenos Aires, Argentina
- Poster 33** T cell epitope mapping in endemic populations: are we seeing the whole picture?
Magdalena Plebanski, Austin Research Institute, Melbourne, Australia
- Poster 35** Transgenic spontaneously hypertensive rats with targeted expression of Cd36 in muscle exhibit dyslipidemia and insulin resistance
Michal Pravenec, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic
- Poster 37** Pex30p, Pex31p, and Pex32p constitute a family of peroxisomal integral membrane proteins regulating peroxisome size and number in *Saccharomyces cerevisiae*
Richard A. Rachubinski, University of Alberta, Edmonton, Alberta, Canada
- Poster 39** Amino acid specificity on the ribosome and the genetic code
Janus Remme, Estonian Biocentre and Tartu University, Tartu, Estonia
- Poster 41** The modular architecture of the proopiomelanocortin gene promoter controls neuronal-specific expression in the brain
Marcelo Rubinstein, Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina

THURSDAY
HANSA ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

Poster Session, 2:15–3:30 p.m. (continued)

- Poster 43** Function of CaCrz1p: a calcineurin-dependent transcription factor in *Candida albicans* and analysis of its target genes
Dominique Sanglard, Institute of Microbiology, University Hospital Lausanne, Lausanne, Switzerland
- Poster 45** Potential application of mutant variants of the multidrug transporter ABCG2 protein in progenitor cell-based gene therapy
Balázs Sarkadi, Institute of Haematology and Immunology, National Medical Center, and Research Group for Membrane Biology and Immunopathology, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary
- Poster 47** Major susceptibility and resistance loci for severe malarial pathogenesis within the murine natural killer complex
D. Louis Schofield, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
- Poster 49** Deciphering the capacity of *Bordetella* adenylate cyclase to cross cellular membranes and to deliver vaccinal antigens for induction of cellular immune responses
Peter Sebo, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
- Poster 51** Assembly of cytochrome *c* oxidase in health and disease
Eric Alan Shoubridge, McGill University, Montreal, Canada
- Poster 53** Study of membrane-bound serine and aspartyl proteases in *Toxoplasma gondii*
Dominique Soldati, Imperial College of Science, Technology and Medicine, London, United Kingdom
- Poster 55** Translation initiation factor eIF4B is a direct substrate of S6K1/2 and functions to stimulate translation of mRNAs harboring a structured 5' UTR
Nahum Sonenberg, Department of Biochemistry and McGill Cancer Centre, McGill University, Montreal, Canada
- Poster 57** Taming the superbugs: structural and inhibitory analysis of the key determinant of broad-spectrum beta-lactam resistance in MRSA
Natalie C.J. Strynadka, University of British Columbia, Vancouver, Canada
- Poster 59** Yeast myosin light chain Mlc1p interacts with translation termination factor eRF1 and affects [*PSI*⁺] prion induction
Michael Ter-Avanesyan, Cardiology Research Center, Russian Academy of Medical Sciences, Moscow, Russia
- Poster 61** Analysis of the chromatin attachment and partitioning functions of the bovine papillomavirus type 1 E2 protein
Mart Ustav, Estonian Biocentre and Tartu University, Tartu, Estonia
- Poster 63** Eukaryotic DNA damage checkpoint clamp and clamp loading complexes: their correlated evolutionary conservation and molecular interaction
Česlovas Venclovas, Institute of Biotechnology, Vilnius, Lithuania
- Poster 65** Molecular and genetic dissection of female gametogenesis in *Arabidopsis thaliana*
Jean-Philippe Vielle-Calzada, Center for Research and Advanced Studies, IPN, Irapuato, Mexico
- Poster 67** Dopamine D5/GABA_A heteromerization: a mechanism to regulate synaptic weight
Yu Tian Wang, University of British Columbia, Vancouver, Canada
- Poster 69** High-throughput mapping of a dynamic signaling network in mammalian cells
Jeff L. Wrana, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada
- Poster 71** Investigation of the regulatory cascade of the homeobox gene *Anf*
Andrey Zarskiy, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

THURSDAY
HANSA ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

Functional and Comparative Genomics

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- 3:30 p.m.** Global mapping of the yeast genetic interaction network: discovering gene and drug function
Charles Boone, University of Toronto, Toronto, Canada
- 3:55 p.m.** Functional studies of mycobacterial genes using genomic and functional genomic approaches
Ross Leon Coppel, Monash University, Melbourne, Australia
- 4:20 p.m.** Genetic analysis of hybrid sterility in the mouse X-chromosome substitution strain
Jiří Forejt, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
- 4:45 p.m.** Comparative genomics, metabolic reconstruction, and analysis of regulation in bacterial genomes
Mikhail S. Gelfand, Research Institute for Genetics and Selection of Industrial Microorganisms (State Scientific Center GosNIIGenetika), Moscow, Russia

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SCHEDULE OF SCHOLARS' PRESENTATIONS

Cellular Architecture and Transport

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- 8:00 a.m.** The granzyme B–serglycin complex from cytotoxic granules requires dynamin for endocytosis
R. Chris Bleackley, University of Alberta, Edmonton, Canada
- 8:25 a.m.** Regulation of the actin cytoskeleton by Vav2
László Buday, Semmelweis University Medical School, Budapest, Hungary
- 8:50 a.m.** LIMK1 regulates Golgi dynamics, traffic of Golgi-derived vesicles, and process extension in primary cultured neurons
Alfredo O. Cáceres, Mercedes and Martin Ferreyra Institute for Medical Research, CONICET, Cordoba, Argentina
- 9:15 a.m.** The role of the variable domains of the light chains of kinesin in its interaction with a cargo
Fatima K. Gyoeva, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

DNA Modification and Repair

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- 10:10 a.m.** Role of chromatin proteins in maintaining the specific patterns of DNA methylation in *Arabidopsis thaliana*
Andrzej Jerzmanowski, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, and Warsaw University, Warsaw, Poland
- 10:35 a.m.** Activation of *trans* geometry in bifunctional platinum complexes by heterocyclic ligands: mechanistic studies on antitumor action
Jana Kašpárková, Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic
- 11:00 a.m.** Double covalent modification of cytosine by DNA methyltransferases
Saulius Klimašauskas, Institute of Biotechnology, Vilnius, Lithuania
- 11:25 a.m.** Novel Rad51^{SP}-dependent pathway of recombination/repair in *Schizosaccharomyces pombe* mediated by Dds20 protein
Vladimir I. Bashkirov, Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia

Development and Expression

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- 3:30 p.m.** A telomere position effect is involved in the differential expression of subtelomeric genes in *Plasmodium falciparum*
Brendan Crabb, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
- 3:55 p.m.** Changes in cholinergic sensitivity and functional efferent innervation of cochlear inner hair cells during development
Ana Belén Elgoyhen, Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina
- 4:20 p.m.** Analysis of transcription and search for the protein product of a ubiquitous gene that changes expression during T cell development
Pawel Kisielow, Institute of Immunology and Experimental Therapy, Wrocław, Poland
- 4:45 p.m.** Endothelial cell-specific growth inhibition and block of angiogenesis by an extracellular fragment of CD44
Priit Kogerman, National Institute of Chemical Physics and Biophysics and Tallinn Technical University, Tallinn, Estonia

THURSDAY
BREMERHAVEN
ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

Parasitic and Viral Invasion

page 52

- 8:00 a.m.** Interaction of rotavirus with hsc70 and lipid membrane microdomains during cell entry
Carlos F. Arias, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico
- 8:25 a.m.** The central role of Duffy binding-like (DBL) domains in the interaction of malaria parasites with host receptors for invasion and cytoadherence
Chetan E. Chitnis, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India
- 8:50 a.m.** The role of ligands in sialic acid-dependent and -independent invasion of *Plasmodium falciparum* into human erythrocytes
Alan F. Cowman, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
- 9:15 a.m.** Chemically substituted RNA aptamers as tools for the development of novel therapeutic measures against trypanosome infections
H. Ulrich Göringer, Darmstadt University of Technology, Darmstadt, Germany

Pathogen Ecology, Transmission, and Host Clinical Symptoms

page 54

- 10:10 a.m.** *Cryptococcus neoformans* var. *gattii* in Australia: the natural ecology of a pathogenic yeast
Deidre A. Carter, University of Sydney, Sydney, Australia
- 10:35 a.m.** Transmission of tuberculosis to household contacts of pulmonary tuberculosis patients in southern Mexico
Ma. de Lourdes García García, National Institute of Public Health, Cuernavaca, Mexico
- 11:00 a.m.** Human immunity to *Entamoeba histolytica* infection: evidence from a prospective study
Rashidul Haque, International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh
- 11:25 a.m.** Analysis of the bystander adrenoceptor reactivity of anti-*Trypanosoma cruzi* antibodies leads to the characterization of a novel clinical symptom of Chagas disease
Mariano Jorge Levin, Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina

Pathogenesis and Resistance

page 56

- 1:15 p.m.** Immunopathogenesis of mucosal leishmaniasis
Edgar M. Carvalho, Federal University of Bahia, Salvador, Bahia, Brazil
- 1:40 p.m.** Fas ligand regulation of *Leishmania* infection targets macrophage interactions with neutrophils
George A. DosReis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
- 2:05 p.m.** Targeting the neurotoxic species in Alzheimer's disease: inhibitors of the formation of A-beta soluble oligomers and fibrils
Sérgio T. Ferreira, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
- 2:30 p.m.** Characterization of loci contributing to host response to *Leishmania major* in the mouse
Simon Foote, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
- 2:55 p.m.** The LPS-induced inflammatory response requires HIAP1 function in macrophages
Robert G. Korneluk, University of Ottawa and Apoptosis Research Center, Children's Hospital of Eastern Ontario, Ottawa, Canada

FRIDAY
HANSA ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

Poster Session, 3:20–4:30 p.m.

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- Poster 2** Structure–function relationships and protein engineering of dehalogenating enzymes
Jiří Damborský, Masaryk University, Brno, Czech Republic
- Poster 4** Helicase loading factors: a multifunctional plasmid-encoded replication initiation protein recruits and positions helicase at the replication origin
Igor Konieczny, University of Gdansk, Gdansk, Poland
- Poster 6** Functional heterogeneity of loci controlling the response to *Leishmania major* in mice
Marie Lipoldová, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
- Poster 8** Protein trafficking in *Giardia lamblia*
Hugo D. Lujan, National University of Córdoba, Córdoba, Argentina
- Poster 10** Enzymatic mechanism of actinomyosin
András Málnási Csizmadia, Eötvös Loránd University, Budapest, Hungary
- Poster 12** Regulation of *msx* by a BMP gradient is essential for neural crest specification
Roberto Mayor, University of Chile, Santiago, Chile
- Poster 14** Identification of new metabolic pathways in parasitic protozoa
Malcolm J. McConville, University of Melbourne, Melbourne, Australia
- Poster 16** Conditional mutagenesis using site-specific recombination in *Plasmodium berghei*
Robert Ménard, Pasteur Institute, Paris, France
- Poster 18** *Trans*-splicing and protein translocation in trypanosomes
Shulamit Michaeli, Bar-Ilan University, Ramat-Gan, Israel
- Poster 20** The individual and collective roles of Y family DNA polymerases in genetic adaptation and long-term survival of mycobacteria
Valerie Mizrahi, University of the Witwatersrand and the National Health Laboratory Service, Johannesburg, South Africa
- Poster 22** COP1 and multiple photoreceptors control degradation of the negative regulator PIF3, a transcription factor required for light signaling in *Arabidopsis*
Ferenc Nagy, Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary
- Poster 24** Distinct role of TNF produced by different cell types in infectious disease and experimental hepatitis
Sergei A. Nedospasov, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia
- Poster 26** Persistently active cannabinoid receptors mute a subpopulation of hippocampal interneurons
Zoltán Nusser, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
- Poster 28** The DCX domains of doublecortin and their interactions with microtubules and tubulin
Jacek Otlewski, Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland
- Poster 30** Ammonia signaling and long-term development of *Saccharomyces cerevisiae* colonies
Zdena Palková, Charles University, Prague, Czech Republic
- Poster 32** Molecular and behavioral analysis of circadian rhythms in sandflies and mosquitoes
Alexandre A. Peixoto, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

FRIDAY
HANSA ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

- Poster 34** Structure and dynamics in solution of the complex of human dihydrofolate reductase with trimethoprim and NADPH
Vladimir Polshakov, Center for Drug Chemistry, Moscow, Russia
- Poster 36** A LEE-encoded regulatory cascade controls virulence gene expression in attaching and effacing pathogens
José L. Puente, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico
- Poster 38** Fast-reacting thiols in two-cysteine peroxiredoxins as a mechanism of peroxynitrite detoxification in infective microorganisms
Rafael Radi, University of the Republic, Montevideo, Uruguay
- Poster 40** Neuronal correlates of a perceptual decision in the ventral premotor cortex
Ranulfo Romo, Institute of Cellular Physiology, National Autonomous University of Mexico, Mexico City, Mexico
- Poster 42** Molecular mechanisms regulating myogenic specification of adult stem cells
Michael A. Rudnicki, Ottawa Health Research Institute, Ottawa, Canada
- Poster 44** How *Shigella* overwhelms the innate immune response: a model for inflammatory bowel diseases?
Philippe J. Sansonetti, Pasteur Institute, Paris, France
- Poster 46** Molecular genetic and functional studies of progressive myoclonus epilepsy
Stephen W. Scherer, Hospital for Sick Children and University of Toronto, Toronto, Canada
- Poster 48** Toward *Taenia solium* cysticercosis control through vaccination and specific diagnostic and genetic epidemiology
Edda Sciuotto, Institute of Biomedical Research, National Autonomous University of Mexico, Mexico City, Mexico
- Poster 50** Cellular models with altered expression of genetic products of the myosin light chain kinase locus
Vladimir P. Shirinsky, Cardiology Research Center, Russian Academy of Medical Sciences, Moscow, Russia
- Poster 52** Restriction enzymes interacting with two recognition sites: are two sites better than one?
Virginijus Siksnys, Institute of Biotechnology, Vilnius, Lithuania
- Poster 54** Molecular analysis of the *Salmonella enterica* magnesium regulon
Fernando C. Soncini, Institute of Molecular and Cellular Biology of Rosario, CONICET, National University of Rosario, Rosario, Argentina
- Poster 56** Molecular dissection of the multiprotein, high molecular weight, membrane-bound presenilin complexes
Peter St George-Hyslop, University of Toronto, Toronto, Canada
- Poster 58** Mapping of neutralizing epitopes on domain I of the yellow fever virus envelope glycoprotein with recombinant human antibodies generated through phage display
Jan ter Meulen, Institute of Virology, Philipps University, Marburg, Germany, and Viral Hemorrhagic Fever Project, University of Conakry, Republic of Guinea
- Poster 60** The “roll and lock” model of actin-myosin-ATP interaction during muscle contraction
Andrey K. Tsaturyan, Institute of Mechanics, Moscow State University, Moscow, Russia
- Poster 62** Reestablishing tolerance to DNA in a murine model of lupus
Tchavdar L. Vassilev, Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

FRIDAY
HANSA ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

Poster Session, 3:20–4:30 p.m. (continued)

- Poster 64** Enzymatic mechanism and physiological role of dUTPase
Beáta G. Vértessy, Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary
- Poster 66** Shaping substrate binding and recognition in metallo-beta-lactamases by directed evolution
Alejandro J. Vila, Institute of Molecular and Cellular Biology of Rosario, CONICET, University of Rosario, Rosario, Argentina
- Poster 68** Physiological functions of glycogen synthase kinase-3 as discerned through analysis of cells lacking each or both isoforms
James Robert Woodgett, Ontario Cancer Institute, Toronto, Canada
- Poster 70** Variable potency of calcium trigger signals to induce cardiac calcium release
Alexandra Zahradníková, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic
- Poster 72** Analysis of the transcriptional complexity of the TFIID complex in *Drosophila*
Mario Zurita, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

Minigrant Presentations (Session I)

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- 4:30 p.m.** A novel dendritic cell–targeting delivery system induces humoral and cellular immunity against rotavirus
Magdalena Plebanski, Austin Research Institute, Melbourne, Australia; and Susana Lopéz and Carlos F. Arias, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico
- 4:50 p.m.** Serological and genotypic studies of EBA-175 in severe and mild malaria in Ugandan children
Thomas G. Ewang, Medical Biotechnology Laboratories, Kampala, Uganda; and Chetan E. Chitnis, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India
- 5:10 p.m.** Treatment of *Trypanosoma cruzi* infection in murine experimental models using inhibitors of endogenous sterol synthesis
Julio A. Urbina, Venezuelan Institute for Scientific Research (IVIC), Caracas, Venezuela; and Miguel A. Basombrío, National University of Salta, Salta, Argentina

FRIDAY
HANSA ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

RNA Binding, Transcription, and Replication

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- 8:00 a.m.** Hepatitis D virus transcription in vitro: characterization of cellular RNA polymerase and viral protein
Pei-Jer Chen, National Taiwan University, Taipei, Taiwan
- 8:25 a.m.** Recognition of RNA templates by Q-beta replicase
Alexander B. Chetverin, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia
- 8:50 a.m.** Mutations in ribosomal RNA that affect different stages of translation
Olga Anatolievna Dontsova, Department of Chemistry, Moscow State University, Moscow, Russia
- 9:15 a.m.** Control of alternative splicing by RNA pol II elongation
Alberto R. Kornblihtt, University of Buenos Aires, Buenos Aires, Argentina

Biosensors and Biosynthetic Pathways

page 80

- 10:10 a.m.** Molecular mechanism of low-temperature sensing in bacteria
Diego de Mendoza, Institute of Molecular and Cellular Biology of Rosario, CONICET, University of Rosario, Rosario, Argentina
- 10:35 a.m.** Biosynthesis of ornithine-containing lipids, a widespread class of bioactive lipids in eubacterial membranes
Otto Geiger, Center of Nitrogen Fixation Research, National Autonomous University of Mexico, Cuernavaca, Mexico
- 11:00 a.m.** Molecular and cellular responses to phosphorus deprivation in *Arabidopsis*
Luis Herrera Estrella, Center for Research and Advanced Studies, IPN, Irapuato, Mexico
- 11:25 a.m.** Unraveling the regulation of a central pathway involved in the synthesis of plastidic isoprenoids in plants
Patricia León Mejía, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

Gene Function and Cell Cycle Progression

page 82

- 1:15 p.m.** Identifying genes affecting locomotor behavior in *Drosophila*
María Fernanda Ceriani, Leloir Institute Foundation, Buenos Aires, Argentina
- 1:40 p.m.** The mechanisms of long-distance interactions and insulator action in *Drosophila melanogaster*
Pavel Georgiev, Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia
- 2:05 p.m.** Involvement of error-prone DNA polymerase pol IV in stationary phase mutagenesis in *Pseudomonas putida*
Maia Kivisaar, Estonian Biocentre, Tartu, Estonia
- 2:30 p.m.** Novel functions of the p53 tumor suppressor
Boris P. Kopnin, Institute of Carcinogenesis, Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia

FRIDAY
BREMERHAVEN
ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

Minigrant Presentations (Session 2)

page 84

- 4:30 p.m.** Stabilization of the doublecortin domain through rational mutagenesis
Jacek Otlewski, Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland; and Janusz Bujnicki, International Institute of Molecular and Cellular Biology (IIMCB), Warsaw, Poland
- 4:50 p.m.** Induction and development of the anterior neural fold in *Xenopus* embryos
Roberto Mayor, University of Chile, Santiago, Chile; and Andrey Zarskiy, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
- 5:10 p.m.** Bioinformatics-guided engineering of DNA methyltransferases
Saulius Klimašauskas, Institute of Biotechnology, Vilnius, Lithuania; and Janusz Bujnicki, International Institute of Molecular and Cellular Biology (IIMCB), Warsaw, Poland

FRIDAY
BREMERHAVEN
ROOM

SCHEDULE OF SCHOLARS' PRESENTATIONS

Markers of Infection and Protection

page 86

- 8:00 a.m.** Immunogenetic and gene expression analyses of *Mycobacterium tuberculosis* infection in mice
Alexander S. Apt, Central Institute for Tuberculosis, Russian Academy of Sciences, Moscow, Russia
- 8:25 a.m.** Immunogenicity of *Trypanosoma cruzi* mutants: characterization of biochemical markers and models of protection against disease
Miguel A. Basombrío, National University of Salta, Salta, Argentina
- 8:50 a.m.** Molecular and cellular basis of *Listeria monocytogenes* infection: new aspects
Pascale Cossart, Pasteur Institute, Paris, France
- 9:15 a.m.** Biomarkers associated with mild or severe malaria in Ugandan children
Thomas G. Egwang, Medical Biotechnology Laboratories, Kampala, Uganda

Muscle Force, Dynamics, and Signaling

page 88

- 10:10 a.m.** Two-step mechanism of force generation in muscle
Sergey Y. Bershtitsky, Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia
- 10:35 a.m.** TGF-beta signaling during skeletal muscle formation
Enrique Brandan, Catholic University of Chile, Santiago, Chile
- 11:00 a.m.** Molecular mechanics of skeletal muscle titin and its recombinant fragments
Miklós S.Z. Kellermayer, University of Pécs, Faculty of Medicine, Pécs, Hungary

SATURDAY
HANSA ROOM

Minigrant Presentations (Session 3)

page 89

- 11:30 a.m.** Genetic analysis of the role of neutrophils in *Leishmania major* infection
Marie Lipoldová, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic; and George A. DosReis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
- 11:50 a.m.** Role of lipid-activated nuclear receptors in lipid body formation induced by *Mycobacterium bovis* BCG
László Nagy, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary; and Patrícia T. Bozza, Oswaldo Cruz Institute, Rio de Janeiro, Brazil
- 12:10 p.m.** Protection against blood-stage malaria induced by DCtag-MSP4/5 vaccination: engagement of multiple immune effector mechanisms promotes “sterile” protection
Magdalena Plebanski, Austin Research Institute, Melbourne, Australia; and Ross L. Coppel, Monash University, Melbourne, Australia
- 12:30 p.m.** Functional relevance of trans-spliceosomal and ribosomal trypanosome proteins as assessed using two-hybrid and RNAi systems
Mariano J. Levin, Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina; and Shulamit Michaeli, Bar-Ilan University, Ramat-Gan, Israel

SCHEDULE OF SCHOLARS' PRESENTATIONS

Signaling and Circuits

page 92

- 8:00 a.m.** Endocannabinoid modulation of cortical circuits linked to anxiety
Tamás F. Freund, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary
- 8:25 a.m.** P_{2Y} receptor-mediated signaling in mouse taste cells
Stanislav S. Kolesnikov, Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia
- 8:50 a.m.** Opioids directly inhibit peripheral nociceptors: effect on P2X receptors
Oleg A. Krishtal, Bogomoletz Institute of Physiology, Ukrainian Academy of Sciences, Kiev, Ukraine
- 9:15 a.m.** Altered synaptic vesicle function in *ipp*, a *Drosophila* mutant of inositol metabolism, is mimicked by lithium and rescued by exogenous inositol
Pedro Labarca, Center for Scientific Studies, Valdivia, Chile

Signaling and Energy Transfer Pathways

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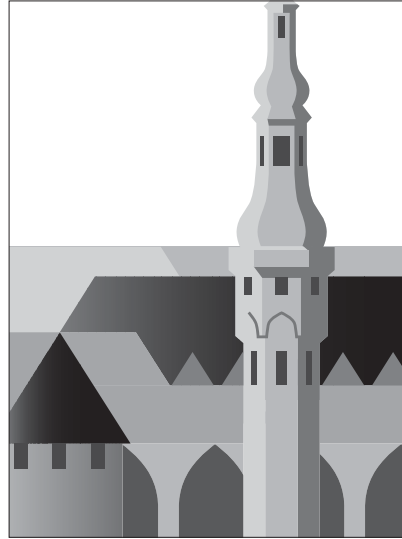
- 10:10 a.m.** Mechanisms of leukocyte lipid body formation and function in innate immunity to intracellular pathogens
Patrícia T. Bozza, Oswaldo Cruz Institute, Rio de Janeiro, Brazil
- 10:35 a.m.** Signaling assemblies formed in mast cells activated via Fc-epsilon receptor I dimers
Petr Dráber, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
- 11:00 a.m.** Electrogenic electron and proton transfer by cytochrome *c* oxidase
Alexander A. Konstantinov, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

Minigrant Presentations (Session 4)

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- 11:30 a.m.** Suppression of allograft rejection by the inhibition of apoptosis
Robert G. Korneluk, University of Ottawa, Ottawa, Canada; and R. Chris Bleackley, University of Alberta, Edmonton, Canada
- 11:50 a.m.** Development of a new antigen delivery system based on a polymeric bacterial protein carrier to improve the anticysticercosis vaccine
Edda Sciuotto, Institute of Biomedical Research, National Autonomous University of Mexico, Mexico City, Mexico; and Fernando Goldbaum, Leloir Institute Foundation, Buenos Aires, Argentina
- 12:10 p.m.** Toward engineering of restriction enzymes
Virginijus Siksnys, Institute of Biotechnology, Vilnius, Lithuania; and Jacek Otlewski, Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland
- 12:30 p.m.** Structural studies on the EST3 protein subunit of *Saccharomyces cerevisiae* telomerase
Vladimir I. Polshakov, Center for Drug Chemistry, Moscow, Russia; and Olga Anatolievna Dontsova, Department of Chemistry, Moscow State University, Moscow, Russia

SATURDAY
BREMERHAVEN
ROOM



**ABSTRACTS OF
PRESENTATIONS**

8:00 a.m.

Enhancing innate immunity to treat infectious diseases

B. BRETT FINLAY, University of British Columbia, Vancouver, Canada

■ The innate immune system is a highly conserved and effective system that immediately overcomes nearly all potential infectious agents. However, some pathogens are able to successfully circumvent the innate system, causing disease, as well as acquired immune responses. Work in our laboratory has focused on various bacterial pathogens and the host innate response to pathogens such as *Salmonella*, pathogenic *Escherichia coli*, and others. By using array technology, we found that the predominant host responses to these pathogens are standard innate responses. We also found natural compounds that activate many of the same innate responses as those triggered by the pathogens. We reasoned that if innate responses could be enhanced using derivatives of these compounds, the derivatives would serve as both preventatives and therapeutics. Results with *Salmonella* and other organisms in animal infection models suggest that such an approach to treating infectious diseases is a viable alternative to antibiotics.

THURSDAY
HANSA ROOM

8:25 a.m.

The role of cross-presentation in self tolerance and viral immunity

WILLIAM ROSS HEATH, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

W.R. Heath,¹ G.T. Belz,¹ C. Smith,¹ G. Behrens,¹ G.M. Davey,¹ R. Allan,² D. Eichner,³ K. Shortman,¹ G. Karupiah,³ F.R. Carbone² ■ ¹Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; ²University of Melbourne, Melbourne, Australia; ³Australian National University, Canberra, Australia

■ We have previously shown that mice expressing ovalbumin (OVA) in their pancreatic islet beta cells induced the deletion of CD8⁺ T cells specific for this autoantigen. This Bim-dependent deletion process involves cross-presentation of islet antigens by a CD8⁺ subset of dendritic cells, which appear to scan all tissues for self-antigens. In an attempt to understand the need for such a peripheral surveillance system, we investigated the role of dendritic cell subsets in viral immunity. This work revealed that the same subset of CD8⁺ dendritic cells was heavily involved in the generation of cytotoxic T cell immunity to three different viral infections: herpes simplex virus type 1, influenza virus, and vaccinia virus. Given that CD8⁺ dendritic cells are the major subset responsible for cross-presentation of cellular antigens, our data suggest that they form a surveillance network that captures cell-associated antigens from peripheral tissues and, in the absence of infection, induces tolerance, but during viral infection is responsible for cross-priming cytotoxic T cell immunity. Examination of the mechanism by which the CD8⁺ dendritic cells obtain viral antigen suggests that this lymph node-resident subset may require the participation of other dendritic cells for the trafficking of viral antigens from the site of infection to the draining node.

8:50 a.m.

Prime-boost immunization against malaria

ADRIAN V.S. HILL, Oxford University,
United Kingdom

A.V.S. Hill,¹ E. Prieur,¹ P. Bejon,¹ S.J. Dunachie,¹ M. Walther,¹ D.P. Webster,¹ S.J. McConkey,¹ V.S. Moorthy,¹ J. Vuola,¹ S. Keating,¹ T. Berthoud,¹ W. Reece,¹ G. Butcher,² K. Watkins,¹ K. Marsh,¹ T. Peto,¹ R. Sinden,² S.C. Gilbert¹ ■ ¹University of Oxford, United Kingdom; ²Imperial College, London, United Kingdom

■ A large variety of approaches to developing an effective malaria vaccine are under investigation worldwide. Pre-erythrocytic vaccines have recently shown particular promise. A protein-adjuvant vaccine encoding the circumsporozoite protein RTS,S/AS02 has shown substantial but short-term efficacy in a Gambian field trial. Heterologous prime-boost immunization focuses on maximizing effector T cell responses against the liver-stage parasite. In animal models of malaria, we have found prime-boost immunization using two viral vectors (fowlpox-MVA or adenovirus-MVA) to be more immunogenic and protective than DNA-MVA regimens.

In a series of Phase I and IIa clinical trials of this approach, three different candidate malaria vaccines (plasmid DNA, modified vaccinia virus Ankara [MVA], and fowlpox strain 9) have been administered in various combinations to more than 400 individuals in Oxford, The Gambia, and Kenya. The vaccines each encode either a polyepitope string fused to the whole *Plasmodium falciparum* TRAP antigen or the entire circumsporozoite antigen. When delivered with MVA as the boosting vaccine, heterologous prime-boost immunization regimens with these vaccines induced exceptionally high frequencies of interferon-gamma (IFN-gamma)-secreting antigen-specific CD4⁺ and CD8⁺ T cell responses. Responses declined over time but were re-boostable at one year with a single MVA immunization. These vaccination regimens have been safe and well tolerated without any serious adverse vaccine-related events. Vaccination induced repeatable partial and complete protection against heterologous strain sporozoite challenge in some volunteers, with the best regimens inducing on average a greater than 85 percent reduction in liver parasite burden. Further potential improvements to this vaccination approach, using a six-antigen polyprotein as an insert, and antibody-inducing and blood-stage antigen components are under development. DNA-based vaccines used in prime-boost regimens are safe and immunogenic and have repeatable quantifiable efficacy against malaria in humans.

9:15 a.m.

The role of plasmacytoid dendritic cells and neutrophils in the generation of antiviral immune response

GUNASEGARAN KARUPIAH, Australian National University, Canberra, Australia

G. Karupiah, L. O'Rance, G. Chaudhri ■ Australian National University, Canberra, Australia

■ The host response to viral infection consists of an early, innate response that profoundly influences and directs the type of adaptive response that is generated. We have investigated the contribution of neutrophils, one of the earliest leukocyte subsets to respond to infection, in the development of effective adaptive responses for the clearance of virus pathogens. We used the monoclonal antibody (mAb) RB6-8C5, which recognizes Gr-1 (Ly6G) on neutrophils. It also cross-reacts with Ly6C, which is expressed on plasmacytoid dendritic cells (PDC) and on activated CD8⁺ T lymphocytes. We utilized the mousepox model (ectromelia virus infection of mice), a disease that is similar in many ways to smallpox, in C57BL/6 mice to show that Gr-1 cells play a significant role in the resolution of virus infection. In contrast to control animals in which virus is cleared effectively without any observable symptoms, animals depleted of Gr-1⁺ cells are highly susceptible to mousepox, with mortality rates of up to 100 percent. Intriguingly, virus-specific, cytotoxic T lymphocyte (CTL) lytic activity and interferon-gamma (IFN-gamma) production by splenocytes were at least 9- to 27-fold lower in Gr-1⁺ cell-depleted mice compared with control antibody-treated animals. This suggested that Gr-1⁺ cells are critical for the generation of an optimal CTL response and cytokine production. Flow cytometric analysis indicated that activated CD8⁺ T lymphocytes are not depleted by RB6-8C5 treatment. Further, depletion of Gr-1⁺ cells did not affect the generation of CTL response to vaccinia, a poxvirus that is closely related to ectromelia virus. We are currently elucidating the Gr-1⁺ cell-dependent mechanisms that regulate the adaptive arm of the immune response, namely CTL and IFN-gamma production, which in turn results in ectromelia virus clearance. The availability of mAb specific for PDC will now allow us to dissociate the roles of PDC and neutrophils in the generation of adaptive immunity.

THURSDAY
HANSA ROOM

10:10 a.m.

Prediction of protein-folding rates from tertiary, secondary, and primary protein structure

ALEXEI V. FINKELSTEIN, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

A.V. Finkelstein, D.N. Ivankov, S.O. Garbuzinsky, O.V. Galzitskaya ■ Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

■ It is known that a basic estimate of protein-folding rate can be obtained from the length of a protein chain alone and that a more accurate estimate is obtained when, in addition, the protein-fold topology is taken into account in the form of Baker's "contact order." We show that an even better estimate can be obtained from the protein's secondary structure content and, given that this content can be computed from the primary structure, we developed a method for predicting folding rates of proteins from their amino acid sequences alone. This method achieves 82 percent correlation with experiments on a set of 64 "two-state" and "multistate" proteins and peptides studied to date; the folding rates of these proteins and peptides differ by 10 orders of magnitude.

10:35 a.m.

Structure, protein engineering, and inhibitors of the *Trypanosoma cruzi* virulence factor trans-sialidase

ALBERTO CARLOS CLEMENTE FRASCH, Institute for Research in Biotechnology, National University of General San Martin, Buenos Aires, Argentina

G. Paris,¹ L. Ratier,¹ A. Buschiazzi,² P. Alzari,² A.C.C. Frasch¹ ■ ¹Institute for Research in Biotechnology, National University of General San Martin, Buenos Aires, Argentina; ²Pasteur Institute, Paris, France

■ American (*Trypanosoma cruzi*) and African (*T. brucei*) trypanosomes express a surface trans-sialidase (TS), a unique enzyme that transfers sialic acid from host glycoconjugates to terminal galactoses in surface parasite molecules. In *T. cruzi*, TS has relevant functions in cell infection and protection of the parasite and is involved in the pathology caused by the trypanosome. The relevant functions of TS make it a good target for the development of alternative chemotherapies against the infection. We have recently obtained the crystal structure of the *T. cruzi* enzyme (Buschiazzi et al., *Mol. Cell* 10:757, 2002), which has allowed us to identify critical residues in the sialic acid and galactose binding sites. This information allowed the characterization of the catalytic nucleophile (Watts et al., *J. Am. Chem. Soc.* 125:7532–7533, 2003) and the construction of mutants with the aim of obtaining trans-sialylation activity from a sialidase scaffold (Paris et al., submitted). Five point mutations were enough to obtain a sialidase mutant with trans-sialidase activity, but a sixth mutation reduced the activity to about 10 percent of that present in the wild-type TS. The structure obtained prompted us to test compounds for their biological activity. Given that the galactose site is unique to TS, lactose derivatives able to compete with the widely used acceptor acetylactosamine were tested. The glucose open-chain derivatives lactitol and lactobionic acid were found to be good acceptors of sialic acid. In vivo, lactitol effectively inhibited both the transfer of sialic acid to acetylactosamine and the re-sialylation of parasite mucins. Lactitol also diminished *T. cruzi* infection in cultured Vero cells by 20–27 percent. These results indicate that structure-based design of lactose derivatives might allow the identification of compounds directed to the lactose-binding site of TS to prevent parasite sialylation and, thus, infection.

THURSDAY
HANSA ROOM

11:00 a.m.

Structural studies on regulatory proteins and their complexes with RNA

MARIA B. GARBER, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

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■ The primary objective of this work is to determine the three-dimensional structures of proteins and RNA-protein complexes involved in regulating gene expression. One of the major components of this process in bacteria is regulation at the level of translation, which provides fast and sensitive response to changing conditions and precise tuning of the gene expression profile. This type of regulation involves global and operon-specific regulators. Protein Hfq regulates expression of genes encoding many different proteins and is considered a global regulator of translation. A molecular model has been built for the intact *Escherichia coli* Hfq protein, including its N- and C-terminal variable tails, the structure of which had not been determined before. We found an essential difference between the structure of intact and truncated *E. coli* Hfq. Another typical example of regulation at the level of translation is coordinated synthesis of ribosomal components during ribosome biogenesis. When synthesized in excess over rRNAs, some primary rRNA-binding ribosomal proteins interact with their mRNA and inhibit translation of their own genes and the other genes in their operons. X-ray quality crystals of several bacterial and archaeal LI-mRNA complexes have been obtained, and structural studies are well under way. Determination of the structures of complexes between ribosomal proteins and specific fragments of their mRNAs would help in understanding the molecular details of translational repression mechanisms and allow comparative analysis of RNA-protein interactions in the regulatory complexes and ribosome. In addition, analysis of the determined structures should contribute to our understanding of specificity in RNA-protein recognition.

11:25 a.m.

High-order quaternary arrangement confers increased structural stability to *Brucella spp.* lumazine synthase

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■ The penultimate step in the pathway of riboflavin biosynthesis is catalyzed by the enzyme lumazine synthase (LS). One of the most remarkable characteristics of this enzyme is the divergence in quaternary structure among different species. The protein exists as pentameric and icosahedral forms, built from almost the same structural monomeric unit. The pentameric structure is formed by five 18 kDa monomers, each with extensive contacts to neighboring monomers. The icosahedral structure consists of 60 LS monomers arranged as 12 pentamers giving rise to a capsid exhibiting icosahedral 532 symmetry. In all lumazine synthases studied, the topologically equivalent active sites are located at the interfaces between adjacent subunits in the pentameric modules. However, the *Brucella spp.* lumazine synthase (BLS) sequence clearly diverges from pentameric and icosahedral enzymes, a divergence so unusual that it prompted us to further investigate its quaternary arrangement. In the present work, we demonstrate by means of solution light-scattering and X-ray structural analyses that BLS assembles as a highly stable dimer of pentamers, representing a third category of quaternary assembly for lumazine synthases. In spectroscopic studies, we also describe the thermodynamic stability of this oligomeric protein, and we postulate a mechanism for dissociation/unfolding of this macromolecular assembly. The higher molecular order of BLS increases its stability by 20°C compared with pentameric lumazine synthases. The decameric arrangement described in this work highlights the importance of quaternary interactions in the stabilization of proteins. We also postulate that the high order in quaternary arrangement contributes to the ability of BLS to act as a transporter and reservoir of lumazine for riboflavin synthesis in the demanding survival conditions found inside eukaryotic cells.

THURSDAY
HANSA ROOM

Poster 1

Hematopoietic cell differentiation

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■ Differentiation of hematopoietic stem cells and progenitors into various lineages is controlled by a complex array of extrinsic and intrinsic factors. Myeloid and erythroid cells develop from a common myeloid progenitor, which differentiates into either thrombocytes and erythrocytes or granulocytes and macrophages.

In our work, we are interested in three types of regulators of self-renewal and differentiation in hematopoietic cells, namely, nuclear hormone receptors, growth factors and their receptors, and cell cycle regulators. We are focusing on the identification and analysis of thyroid hormone-regulated genes during thyroid hormone-accelerated erythropoiesis and new ligands and nuclear hormone receptors affecting self-renewal and differentiation of myeloid and erythroid lineages. Our efforts are also concentrated on the novel avian growth factor and its receptor that we recently identified and characterized. This growth factor affects not only committed progenitors or development of thrombocytic lineage but also very early multipotent hematopoietic progenitors. Finally, we study the role of prolyl-isomerase Pin1, an important mediator of phosphorylation-dependent signaling, in differentiation and the development of leukemia.

*EMBO/HHMI Award recipient

Poster 3

Expression and functional characterization of human ABCG1

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■ The ATP-Binding Cassette (ABC) transporters are integral membrane proteins defined by the presence of certain conserved sequences: Walker A, Walker B, and the so-called ABC signature motifs. In addition to sequence homology, these proteins also share distinguishing structural characteristics. They are built from large transmembrane domains (TMDs) and cytosolic nucleotide-binding domains (NBDs), which contain the above-mentioned conserved sequences. In most cases, a functioning ABC transporter consists of at least two transmembrane and two nucleotide-binding units. The 48 human ABC transporters have been classified into seven subfamilies, ranging from ABCA to ABCG. An intriguing group is the ABCG subfamily. The particularity of these proteins is that they consist of only one NBD and one TMD, and are therefore called ABC half transporters. Some members of the ABCG subfamily have been proven to function as homodimers (ABCG2) or heterodimers (ABCG5/ABCG8). The main focus of the present study is the characterization of the ABCG1 protein, a human orthologue of the widely studied *Drosophila white* gene product. The expression of human ABCG1 is found to be ubiquitous, but its function is still unknown. In our studies, we expressed this protein in several expression systems, including baculovirus/Sf9 and retroviral/mammalian cell systems. In addition to the wild-type form of this ABC half transporter, we also produced a mutant ABCG1 containing an amino acid replacement in the Walker A region (K124M). Experience with other ABC transporters containing the corresponding mutation would lead one to predict that this variant is a nonfunctional mutant. We have made efforts to identify substrates of the ABCG1 protein using several functional studies, including ATP-binding, nucleotide-occlusion, ATPase activity, and vesicular transport and cellular uptake measurements. Our results suggest that this ABC protein functions as an active transporter, but its physiological function still remains unclear.

*EMBO/HHMI Award recipient

THURSDAY
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Poster 5

Intestinal mucosa barrier repair: effects of glutamine and alanyl-glutamine on in vitro and in vivo models of mucositis induced by enterotoxin A from *Clostridium difficile*, indomethacin, and 5-fluorouracil

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■ This study evaluates the effect of glutamine (Gln) and alanyl-glutamine (Ala-Gln) on the intestinal barrier repair in mucositis induced by enterotoxin A from *Clostridium difficile*, indomethacin (INDO), and 5-fluorouracil (5-FU). Cell proliferation and migration were measured in intestinal epithelial cells (IEC-6) plated in Matrigel-coated dishes. The cell layer was scraped and incubated with medium alone or medium containing Gln or Ala-Gln, and proliferation and migration were assessed. Apoptosis was quantified in IEC-6 cells using the Annexin V assay. Gln and Ala-Gln, at 1–10 mM, significantly enhanced cell proliferation and migration at 6 h and 24 h. Toxin A (TxA) induced a dose-dependent (1–100 mcg) reduction in cell proliferation and migration and also caused dose-dependent apoptosis in IEC-6 cells. Gln and Ala-Gln prevented the inhibition and the apoptosis induced by TxA. The mucositis induced by INDO was partially reversed by treatment with Gln or Ala-Gln. We also evaluated the effect of Gln and Ala-Gln in mucositis induced by 5-FU. Neither Gln nor Ala-Gln prevented the intestinal structural damage or apoptosis in crypt enterocytes at 24 h after 5-FU. However, Ala-Gln, but not Gln, increased the intestinal recovery in 5-FU-treated mice, predominantly by enhancing mitotic activity and crypt length. In summary, these results demonstrate that Gln and Ala-Gln enhance the intestinal epithelial repair and reduce TxA damage and apoptosis in the IEC cell line. In addition, Gln and Ala-Gln partially reverse mucositis induced by INDO whereas Ala-Gln accelerates intestinal mucosa repair in 5-FU-treated mice.

Poster 7

Rotavirus enters the cell through a non-clathrin-, non-caveolin-dependent mechanism

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■ Rotaviruses are the single most important cause of severe diarrhea in the young of many animal species. While we have recently learned much about the viral and cellular proteins involved in the initial attachment of rotaviruses to their host cells, the mechanism through which these viruses reach the cell cytoplasm is poorly understood. Here, we report our studies on the effect on rotavirus cell infection of drugs and dominant negative mutations known to impair clathrin-mediated endocytosis and caveolae-mediated endocytosis. Rotaviruses were able to enter cells in the presence of compounds that inhibit the clathrin-mediated endocytosis, as well as cells overexpressing a dominant negative form of Eps15, a protein crucial for the assembly of clathrin coats. We also found that rotavirus was able to infect cells in which uptake by caveolae was blocked; furthermore, treatment with the cholesterol-binding agents nystatin and filipin, as well as transfection of cells with dominant negative caveolin-1 and caveolin-3 mutations, or with a short interfering siRNA to caveolin-1, which silenced the expression of this protein, had no effect on rotavirus infection. Interestingly, cells treated with methyl-beta-cyclodextrin, a drug that sequesters cholesterol from membranes, and cells expressing a dominant negative mutation of the large GTPase dynamin, a protein known to function in several membrane scission events, were not infected by rotavirus, indicating that cholesterol and dynamin play a role in the entry of rotaviruses. Taken together, our results suggest that rotaviruses infect cells by a recently described non-clathrin-, non-caveolin-dependent pathway. The roles of cholesterol and dynamin during viral entry are currently under investigation.

THURSDAY
HANSA ROOM

Poster 9

Endoplasmic reticulum export of glycosyltransferases depends on interaction of a cytoplasmic di-basic motif with Sar-1

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■ Membrane proteins exit the endoplasmic reticulum (ER) in COPII transport vesicles. ER export is a selective process in which transport signals present in the cytoplasmic tail (CT) of cargo membrane proteins must be recognized by coatamer proteins for incorporation into COPII vesicles. Two classes of ER export signals have been described for type I membrane proteins, the di-acidic and the di-hydrophobic motifs. Both motifs participate in the Sar1-dependent binding of Sec23p-Sec24p complex to the CTs during early steps of cargo selection. However, information concerning the amino acids in the CTs that interact with Sar-1 is still lacking. Here, we describe a third class of ER export motif, [RK](X)[RK], at the CT of Golgi-resident glycosyltransferases, which is required for these type II membrane proteins to exit the ER. The di-basic motif is located proximal to the transmembrane border, and experiments using cross-linking in microsomal membranes and binding to immobilized peptides showed that the motif directly interacts with the COPII component Sar-1 and, in a Sar-1 GTP-dependent form, with Sec23p. Collectively, the present data suggest that the interaction of the di-basic motif with Sar-1 participates in early steps of selection of Golgi-resident glycosyltransferases for transport in COPII vesicles.

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Poster 11

Fine mapping and candidate gene analysis of complex immunity to infection with *Salmonella typhimurium* in mice

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■ The identification of the genetic basis of the complex immune response to *Salmonella* infection has progressed considerably with the use of mice as model organisms. The development of genomic technologies combined with classical genetics contributed to the identification of major *Salmonella* resistance genes, including solute carrier family 11, member 1 (*Slc11a1*), and Toll-like receptor 4 (*Tlr4*). We have developed a model to study the contribution of genetic factors to the susceptibility of wild-derived MOLF/Ei inbred mice to infection with *Salmonella typhimurium* during the early phase of infection. Two chromosomal regions designated *Ity2* (Immunity to Typhimurium locus 2) and *Ity3* were shown to influence susceptibility to infection in multiple cross experiments with *Salmonella typhimurium*. *Ity2* is a resistance locus on chromosome 11, and *Ity3* is a susceptibility locus on chromosome 1. *Ity2* and *Ity3* cover genomic regions rich in genes that could possibly play a role in the response of mice to infection with *Salmonella typhimurium*. We combined several approaches, including interval-specific congenic strains as well as multiple-cross and inbred strain haplotype mapping to refine the location of the candidate intervals. This strategy was coupled with genome-wide and chromosome-specific tissue expression arrays as well as sequence and functional analyses to prioritize quantitative trait loci (QTL) candidate genes.

Poster 13

Sequence of events leading to SNARE complex assembly during acrosomal exocytosis

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■ Acrosomal exocytosis is a calcium-dependent secretory event resulting in the release of the acrosomal contents and the loss of the membranes surrounding the acrosome. By using a permeabilized sperm model, we have shown that SNAREs (SNAP receptors) and SNARE-interacting proteins, such as NSF (N-ethylmaleimide-sensitive factor), alphaSNAP (soluble NSF attachment protein), and synaptotagmin VI, are necessary for this complex process. We have developed two strategies to study the sequence of steps governed by these proteins that leads to membrane fusion. First, we used the acetoxymethyl ester of O-nitrophenyl EGTA (EGTA-NP), a photosensitive reagent that accumulates in the acrosome in permeabilized spermatozoa and inhibits exocytosis by chelating calcium in the dark. Upon illumination, EGTA-NP is inactivated, the acrosomal calcium pool is replenished, and exocytosis can proceed. By using this inhibitor, we have evidence that NSF and alphaSNAP are required before and that SNAREs and synaptotagmin VI are required after the release of intra-acrosomal calcium. The second approach is based on the fact that neurotoxins are specific zinc-dependent proteases and SNAREs are resistant to neurotoxins when assembled in stable complexes. By adding botulinum neurotoxin E and suppressing its activity with the zinc chelator N,N,N',N'-tetrakis(2-pyridymethyl)ethylenediamine, we can assess whether SNAREs are engaged in stable complexes at different steps during the exocytotic process. The results indicate that SNAREs are inaccessible to the toxin in resting sperm but become toxin-sensitive upon stimulation with calcium or Rab3A. Combinations of both methods show that SNAREs remain toxin-sensitive until the release of intra-acrosomal calcium. These results suggest that SNAREs forming stable *cis* complexes are disassembled by NSF and alphaSNAP upon Rab3A activation. Afterwards, the SNARE proteins assemble in loose *trans* complexes until the release of intra-acrosomal calcium activates synaptotagmin VI, which in turn promotes full assembly of the complexes and triggers membrane fusion.

Poster 15

The relict chloroplast of malaria parasites: what does it do and can we kill it?

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■ The apicoplast has emerged as a promising target for new antimalarials. Apicoplasts are indispensable, but their exact function remains uncertain. To understand more about the apicoplast, we assembled a predicted organelle proteome. The apicoplast synthesizes 23 proteins but also imports numerous nuclear-encoded proteins. Targeting of these proteins to the apicoplast requires a unique N-terminal extension. After scrutinizing a large collection of these N-terminal extensions from the *Plasmodium falciparum* genome, we were able to extract a simple set of rules that could predict targeting of proteins to the apicoplast from primary sequence. Strategic mutagenesis of apicoplast-targeting peptides demonstrated that a net basic charge and a chaperone-binding site are critical to accurate targeting. These *in vivo* analyses allowed us to fine-tune our algorithms and increase prediction accuracy. As an ultimate test of these algorithms, we generated large numbers of random peptides *in silico* and screened them with our bioinformatics tools. Synthetic peptides deemed likely to mediate apicoplast targeting were coupled to a GFP reporter and transfected into parasites. Using this approach, we have identified completely artificial targeting peptides that are sufficient to target a reporter gene to the apicoplast with high precision, which further underscores the accuracy of our prediction software. Our current estimates identify more than 500 apicoplast proteins, which represents about 10 percent of the parasite genome, engaged in this compartment. These apicoplast proteins constitute complete pathways for fatty acid and isoprenoid biosynthesis plus a partial set of heme synthesis enzymes. We believe that these anabolic pathways are essential to parasite survival because end products are exported from the apicoplast for use elsewhere in the parasite cell. Various lines of biochemical and pharmacological evidence confirm the activity and indispensability of these pathways, and numerous apicoplast enzymes for these pathways as well as additional apicoplast housekeeping activities are excellent drug targets.

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Poster 17

Three-dimensional structure of the bacteriophage T4 baseplate

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■ The *Myoviridae* tailed bacteriophage T4 is one of the most structurally complex viruses to be characterized so far. In general, every T4 particle is able to infect an *Escherichia coli* cell, probably as a consequence of a complex 1,200 Å-long contractile tail that regulates the infection process. The baseplate of bacteriophage T4 is a multiprotein molecular machine that controls host cell recognition, attachment, tail sheath contraction, and viral DNA ejection. We report the three-dimensional structure of the baseplate-tail tube complex, determined to a resolution of 12 Å by cryoelectron microscopy. The baseplate has a sixfold symmetric, domelike structure of about 520 Å in diameter and 270 Å in length, assembled around a central hub. A 940 Å-long and 96 Å-diameter tail tube, coaxial with the hub, is connected to the top of the baseplate. At the center of the dome is a needle-like rigid triple beta-helical structure that was previously identified as a cell-puncturing device. We have identified the locations of six proteins with known atomic structures (gene products gp5, gp8, gp9, gp11, gp12, and gp27) and established the position and shape of other baseplate proteins. The structures of the native baseplate and "star" conformations suggest a mechanism of baseplate triggering and structural transition during the initial stages of T4 infection. Apparently, the stability of the hexagonal baseplate is maintained by interactions of the short tail fibers, a trimer of gp12, with each other and with gp11. In the absence of the short tail fibers, the baseplate could easily switch to the star-shaped conformation. The garland of six short tail fibers wound around the gp11 vertices would seem to be a metastable conformation. After interaction of the long tail fibers with lipopolysaccharide receptors, the dissociation of the gp12 garland from gp11 should initiate the structural reorganization of the baseplate.

Poster 19

The isolation and characterization of novel *Drosophila* tissue polarity genes

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■ Polarization of cells can take many forms. Epithelial cells, for example, display a ubiquitous apical-basolateral polarity but are often also polarized in the plane of the epithelium, a feature referred to as planar cell polarity (PCP). In *Diptera*, PCP is manifested in the external cuticular structures and in the compound eye. The precise polarization patterns in the fruit fly *Drosophila melanogaster* serve as the best model system in which to study planar signaling and coordination of polarized cell shape changes in general. Polarity in flies is governed by the Frizzled (Fz) receptor and a number of additional gene products. The localized accumulation of these proteins within cells plays a key role in determining planar polarity. Notwithstanding the recent progress that has been made in understanding the molecular mechanisms that lead to correct polarization, many components of the signaling pathways involved are still missing, the links between these pathways are also not entirely clear, and the tissue-specific cellular responses are poorly understood. The goal of our research is to identify and characterize a novel set of genes required to establish planar polarity in *Drosophila*. To this end, we carried out a large-scale EMS and ENU mutagenesis experiment that allowed us to identify 16 new PCP genes. Currently, we are focusing on the molecular mapping of the new genes, which will be followed by the functional characterization of the most interesting ones. In addition, we are studying a novel *Drosophila* member of the Formin family of proteins, which is involved in the regulation of the actin cytoskeleton. Strikingly, the absence of this protein leads to severe disruption of actin organization in the tracheal system. We used a combination of genetic and cell-biological techniques to study this phenotype, which allowed us to uncover a novel aspect of *Drosophila* PCP in the tracheal tubes.

*EMBO/HHMI Award recipient

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Poster 21

Chronic CD40 signaling resembles the action of the Epstein-Barr virus oncoprotein LMP1 in germinal center suppression but differs in its ability to induce inflammatory and autoimmune reactions

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■ The Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) is a critical factor for EBV-associated lymphomagenesis. Understanding the molecular mechanism of LMP1 function and its relationship to cellular signaling pathways is important for the development of therapeutic approaches against EBV-associated malignancies. In vitro experiments have identified similarities between LMP1 and CD40 function. To compare directly the signaling properties of LMP1 and CD40 in vivo, we generated transgenic mice expressing either LMP1 or a chimeric LMP1CD40 molecule, in which we replaced the cytoplasmic signaling region of LMP1 with the corresponding region of CD40. LMP1CD40 constitutively activates the CD40 pathway instead of the LMP1 pathway. LMP1 and LMP1CD40 were expressed at similar levels in a B lymphocyte-specific manner under the control of CD19 promoter. Similar to LMP1, LMP1CD40 was able to suppress germinal center formation and antibody production in response to thymus-dependent antigens. Furthermore, the avidity of the antibodies produced against thymus-dependent antigens was lower in LMP1CD40 transgenic mice compared with wild-type and LMP1 transgenic mice. Germinal center suppression was linked to the ability of LMP1CD40 and LMP1 to down-regulate mRNA and protein levels of BCL6. In contrast to LMP1, LMP1CD40 was able to up-regulate IgM, CD69, CD80, and CD86 in B cells. In addition, LMP1CD40- but not LMP1-transgenic mice exhibited elevated numbers of marginal zone B cells and increased populations of polymorphonuclear cells and/or neutrophils. Consistent with these findings, LMP1CD40- but not LMP1-transgenic mice showed evidence of spontaneous autoimmune and inflammatory responses. Taken together with previous results, our studies support the notion that LMP1 has usurped the growth-stimulatory and anti-apoptotic functions of CD40 to an extent that possibly limits the exposure of EBV to immune surveillance.

Poster 23

Nuclear receptors regulate lineage specification and the function of antigen-presenting cells

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■ Very little is known about the transcriptional events controlling the differentiation and lineage commitment of monocyte-derived dendritic cells (DCs) and their responses to external stimuli and/or internal signals. In a search for such a mechanism, we found that the ligand-activated transcription factor peroxisome proliferator activated receptor gamma (PPARgamma) is immediately up-regulated after the induction of the differentiation of monocyte-derived DCs by IL-4 and GM-CSF. Activation of the PPARgamma receptor altered the phenotype of DCs by changing the expression pattern of cell surface receptors involved in various functions and enhanced the internalizing activity and lysosomal marker expression of immature DCs. Unexpectedly, we found that a class of molecules responsible for the presentation of self- and foreign modified lipids were coordinately regulated by PPARgamma activation. CD1a antigen levels were reduced, whereas CD1d expression was induced. Enhanced expression of CD1d was coupled to the selective induction of NKT cell proliferation in the presence of DCs exposed to ligands of PPARgamma. Taken together, the results suggest that the lipid-activated transcription factor PPARgamma is involved in orchestrating a transcriptional response in differentiating DCs, leading to lineage specification and to the development of a DC subtype with increased internalizing capacity and specific lipid presentation for NKT cells.

**THURSDAY
HANSA ROOM**

Poster 25

Linear genomes in yeast mitochondria: implications from an analysis of the complete DNA sequence

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■ Analysis of the complete sequence of the linear mitochondrial DNA (mtDNA) of the yeast *Candida parapsilosis* has revealed several unique features with implications for both the evolution and replication strategy of linear mtDNA and provides a basis for identifying specific molecular targets for possible therapeutic intervention against this fungal pathogen. The length of linear mtDNA is 30,922 bp, although longer molecules with expanded terminal tandem arrays of a 738 bp unit were also observed. The genome has a highly compact genetic organization with less than 8 percent of the sequence corresponding to non-coding intergenic spacers. In silico analysis predicted genes encoding 14 protein subunits of complexes for the respiratory chain and ATP synthase, RNAs of the large and small subunits of the mitochondrial ribosome, and a set of 24 transfer RNAs. In addition, we identified six intronic open reading frames encoding homologues of RNA maturase, reverse transcriptase, and DNA endonucleases. Comparison with the circular mtDNA of *C. albicans* revealed several conserved gene clusters, indicating that mitochondrial genomes with different molecular architectures evolved from a common ancestor. In addition to linear mtDNA, the mitochondria of *C. parapsilosis* harbor a series of minicircular molecules derived solely from the telomeric sequence motif. A survey of strains belonging to the three genetically distinct groups of *C. parapsilosis* revealed variant minicircles whose sequences correspond to modified arrays at the ends of linear mitochondrial DNA. Moreover, strains with altered molecular architecture of their mitochondrial genome were identified. Mitochondria of these strains lack telomeric minicircles and contain a circular genome, formed presumably by fusion of terminal regions of the linear form. This is in line with the hypothesis that telomeric minicircles are implicated in the telomere replication pathway and that their elimination correlates with defective telomere maintenance, ultimately leading to circularization of the organellar genophore.

Poster 27

A novel pathway for heme detoxification in the midgut of the tick *Boophilus microplus*: aggregation inside the hemosome, a specialized organelle

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■ The tick *Boophilus microplus* ingests large volumes of cattle blood, as much as 100 times its own weight. Free heme is a promoter of free radical formation; therefore, digestion of blood should create an intense oxidative challenge. We followed the fate of heme in the digestive cells of the midgut of female ticks using the peroxidase activity of heme. We also studied intracellular heme traffic in digestive cells in culture using a fluorescent metal-substituted porphyrin as a heme analogue. After uptake by digestive cells, hemoglobin is directed to a specific population of vacuoles, presumably the site of digestion. Pulse-chase experiments showed that heme leaves this digestive vacuole and passes through the cytoplasm to its final destination, a membrane-delimited, specialized organelle of the digestive cell called the hemosome. Heme accounts for 90 percent of the hemosome mass and is concentrated in the core of this structure, appearing as a compact, noncrystalline aggregate of iron protoporphyrin IX without covalent modifications. The unusual FTIR spectrum of this aggregate suggests that lateral propionate chains mediate association of heme molecules with other components of the hemosome. We propose that this is a major heme detoxification mechanism in this blood-sucking arthropod. Blood-feeding organisms are found in very different taxonomic groups, and all have heme as the main end product of hemoglobin digestion, frequently described generically in the literature as hemozoin. However, structural characterization of a heme aggregate is available only for hemozoin. To our knowledge, this is the first characterization of a nonhemozoin heme aggregate showing a defined structure. This is also the first dynamic description of heme intracellular traffic in a eukaryotic cell.

THURSDAY
HANSA ROOM

Poster 29

Helical order in myosin filaments requires the closed conformation of the myosin head

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■ Myosin heads are helically ordered on the thick filament surface in relaxed muscle. In mammalian and avian filaments, this helical arrangement is dependent on temperature, and it has been suggested that helical order is related to ATP hydrolysis by the heads. To test this hypothesis, we used electron microscopy and image analysis to study the ability and temperature dependence of analogs of ATP and ADP:Pi to induce helical order in arthropod thick filaments. ATP or analogs were added to rigor myofibrils or purified thick filaments at 22°C and 4°C, and the samples were negatively stained. The ADP:Pi analogs ADP:AlF₄ and ADP:Vi and the ATP analogs ADP:BeF₃, AMPPNP, and ATP:γNH₂ all induced helical order in these thick filaments, independent of temperature. In the absence of nucleotide or in the presence of ADP or the ATP analogue ATP:γS there was no helical ordering. According to crystallographic and tryptophan fluorescence studies, all these analogues except ATP:γS and ADP induce the closed conformation of the myosin head. We suggest that helical order requires the closed state of the myosin head, but not ATP hydrolysis. It appears that helical order is required for the normal relaxed state, given that it is found in all animals at their normal physiological temperatures. This helical organization may maintain the heads close to the filament backbone, helping to maintain the OFF state. Our results demonstrate that, to obtain an atomic model for the filament, it is necessary to dock the atomic structure of the head in the *closed* conformation to the three-dimensional filament reconstruction, which we are now carrying out. Activation of thick filaments by phosphorylation of the myosin regulatory light chain (RLC) results in disordering of the helical array. Using EM and the *in vitro* motility assay, we are currently studying the functional implications of RLC mutations that occur in the midventricular chamber phenotype of hypertrophic cardiomyopathy.

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Poster 31

De novo synthesis of bacterial glycogen: *Agrobacterium tumefaciens* glycogen synthase is involved in glucan initiation and elongation

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■ Evidence was obtained indicating that, in *Agrobacterium tumefaciens* and probably in all other eubacteria, the same protein glycogen synthase (GS) is involved in both glycogen initiation and elongation. This contrasts with mammalian and yeast cells, in which two distinct proteins are required for linear alpha(1,4)-linked glucan formation: glycogenin, an autoglucosylating enzyme for initiation, and GS for further elongation of the glycogenin-linked glucan. We found that crude extracts incubated with ADP-Glc were able to form alpha(1,4)-linked glucans even though cells used for extract preparation displayed a genotype [alpha(1,4)glucan phosphorylase and phosphoglucomutase minus] that prevented synthesis of glucose-containing sugar nucleotides and thus prevented preformation of alpha(1,4)-linked glucans; the defined growth medium used had glycerol as carbon source. We therefore concluded that initiation of glycogen synthesis in *A. tumefaciens* does not require the presence of alpha(1,4)-linked glucans. Furthermore, extracts prepared from *A. tumefaciens* mutants lacking bacterial GS but expressing vector-encoded *Saccharomyces cerevisiae* GS (an enzyme known to require alpha(1,4)-linked glucan for activity) were unable to synthesize alpha(1,4)-linked glucans in the absence of primer, but expression of *A. tumefaciens* GS restored glucan synthesis capacity. Purified to homogeneity from alpha(1,4)-linked glucan-free cells, the bacterial GS was able to build its own primer by transferring glucose residues from ADP-Glc to an amino acid or amino acids in the same protein. Primed GS then became the substrate for further GS-catalyzed glucan elongation. In *A. tumefaciens*, genes involved in glycogen synthesis and degradation are part of the same operon. Our finding that GS behaves not only as elongator but also as initiator is consistent with the clustered organization of glycogen genes.

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Poster 33

T cell epitope mapping in endemic populations: are we seeing the whole picture?

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■ Malaria affects approximately 500 million people each year, causing an estimated 2 million deaths. T cells, particularly those able to secrete interferon gamma (IFN-gamma) (T1 cells) have been shown to play a central role in protective immunity against the pre-erythrocytic stages of infection. However, recent Phase I immunogenicity studies in Gambia of the recombinant RTS,S vaccine expressing the circumsporozoite (CS) protein of *Plasmodium falciparum* emphasized the unusual conclusion from our other studies that T cells induced by infection that are restimulated rapidly to secrete IFN-gamma are different from those that can do so after in vitro expansion with specific peptides. Naturally induced CS-specific T cell reactivity was low in magnitude and broad in specificity, with a range of predicted CS T cell epitopes contributing to responses. Interestingly, another protein of the pre-erythrocytic stage, thrombospondin-related adhesive protein (TRAP), showed a similar broad and low T1 reactivity pattern in large-scale population studies in Kenya. Interestingly, this reactivity was not associated with protection. Studies of T cell reactivity in naturally exposed and vaccinated humans, such as those described above, are important in providing potential epitope targets able to induce protective immunity when incorporated into vaccines. Are we missing protective epitopes in mapping studies? Epitope mapping using overlapping peptides is “assumption free” but expensive. Our results indicate that using conventional computer algorithms to predict high-affinity MHC binding peptides is likely to eliminate from mapping studies a range of potentially protective T cell epitopes. Thus, three novel families of peptide MHC-binding modalities are noted: low-affinity (but immunodominant) binding using short anchor residues, high-affinity binding using a new MHC pocket (previously not known to anchor peptides), and the use of carbohydrate molecules in glycopeptides for anchoring to MHC. We suggest that limitations in the spectrum of T cell activities analyzed and in the ability to predict MHC binding has hampered the detection of protective T cell responses.

Poster 35

Transgenic spontaneously hypertensive rats with targeted expression of Cd36 in muscle exhibit dyslipidemia and insulin resistance

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■ In spontaneously hypertensive rats (SHR), which lack a functional form of the long-chain fatty acid transporter Cd36, transgenic rescue with wild-type Cd36 expressed under the control of a universal promoter decreases serum fatty acid levels and ameliorates insulin resistance in rats fed a high-fructose diet. To search for mechanisms by which Cd36 affects insulin resistance and glucose intolerance, we investigated the metabolic effects of expressing wild-type Cd36 under the control of a muscle-specific creatine kinase (MCK) promoter in the SHR. In a fructose-fed transgenic line of SHR expressing wild-type Cd36 under the control of the MCK promoter, we observed significant increases in serum triglycerides and glucose, which were associated with impaired glucose tolerance, compared with fructose-fed, non-transgenic SHR expressing mutant Cd36. In addition, skeletal muscle isolated from transgenic animals exhibited significantly reduced basal and insulin-stimulated glycogenesis while palmitate oxidation was increased. These findings indicate that expression of Cd36 exclusively in muscle tissue is not sufficient to ameliorate the metabolic defects in the SHR and can actually promote dyslipidemia, insulin resistance, and glucose intolerance, which is possibly attributable to enhanced utilization of fatty acids at the expense of glucose in skeletal muscle and a decreased capacity of adipose tissue to buffer lipid flux.

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Poster 37

Pex30p, Pex31p, and Pex32p constitute a family of peroxisomal integral membrane proteins regulating peroxisome size and number in *Saccharomyces cerevisiae*

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■ Peroxisomes are ubiquitous organelles involved in a myriad of biochemical reactions, most notably lipid metabolism, and peroxins are proteins required for peroxisome assembly. The peroxin Pex23p of the yeast *Yarrowia lipolytica* exhibits high sequence similarity to the putative proteins Ylr324p, Ygr004p, and Ybr168p encoded by the *Saccharomyces cerevisiae* genome. Ylr324p, Ygr004p, and Ybr168p were shown to be integral to the peroxisomal membrane and to act in controlling peroxisome number and size. Synthesis of Ylr324p and Ybr168p, but not of Ygr004p, is induced during incubation of cells in oleic acid-containing medium, the metabolism of which requires intact peroxisomes. Cells deleted for *YLR324w* exhibit increased numbers of peroxisomes, whereas cells deleted for *YGR004w* or *YBR168w* exhibit enlarged peroxisomes. Ylr324p and Ybr168p cannot functionally substitute for one another or for Ygr004p, whereas Ygr004p shows partial functional redundancy with Ylr324p and Ybr168p. Ylr324p, Ygr004p, and Ybr168p interact within themselves and with Pex28p and Pex29p, both of which have been shown to also regulate peroxisome size and number. Systematic deletion of genes demonstrated that *PEX28* and *PEX29* function upstream of *YLR324w*, *YGR004w*, and *YBR168w* in the regulation of peroxisome proliferation. Our data suggest a role for Ylr324p, Ygr004p, and Ybr168p (now designated Pex30p, Pex31p and Pex32p, respectively), together with Pex28p and Pex29p, in controlling peroxisome size and proliferation in *S. cerevisiae*.

Poster 39

Amino acid specificity on the ribosome and the genetic code

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■ During the deciphering of the genetic message, amino acids are first linked to tRNA species by a specific set of ligases; aminoacyl-tRNAs (aa-tRNAs) are then selected, and amino acids are incorporated into proteins according to the mRNA codons by the ribosome. The amino acid identity is believed to be important only in the first step because it is possible to modify its side chain in the aa-tRNA without a loss in protein synthesis activity. We have studied whether or not the amino acid side chain plays a role during aa-tRNA selection on ribosomes. We have analyzed translational properties of the amino acids Ala, Phe, and Val during poly(GUU) translation on ribosomes. Triple specific tRNA^{Ala}, tRNA^{Phe}, and tRNA^{Val} were transcribed in vitro. All tRNAs had a G3-U70 base pair and anticodon CAG, which makes it possible to charge them with any one of the three amino acids and to use the resulting aa-tRNA species in poly(GUU)-directed translation. Competition experiments were carried out to compare translational efficiencies of correctly and incorrectly aminoacylated tRNAs, for example, [³H]Ala-tRNA^{Ala} versus [¹⁴C]Val-tRNA^{Ala}. At saturating ribosome concentrations, both Ala and Val were polymerized with similar efficiency. In contrast, at limiting ribosome concentration, Val was preferentially incorporated into peptides. When Phe and Val or Phe and Ala were competing on ribosomes, Phe was preferentially incorporated into peptides. This result shows that the ribosome can discriminate between aminoacyl moieties of aa-tRNAs during mRNA translation. Translational efficiencies of amino acids coincide with the known specificities of the ribosomal peptidyl transfer center.

These results suggest that the correspondence between codons and amino acids is not accidental but is based on the catalytic properties of the ribosome. Accordingly, the universal genetic code is a result of coevolution with the ribosome.

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Poster 41

The modular architecture of the proopiomelanocortin gene promoter controls neuronal-specific expression in the brain

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■ The proopiomelanocortin (POMC) gene is expressed at significant levels in neurons of the arcuate nucleus of the hypothalamus and in pituitary melanotrophs and corticotrophs. Neuronal processing of the POMC prohormone produces melanocortins and beta-endorphin to activate satiety and analgesia, respectively, whereas pituitary ACTH triggers a widespread stress response, stimulating glucocorticoid release. Pituitary-specific expression of the POMC gene is controlled by *cis*-acting regulatory elements proximal to the TATAA box, and we have demonstrated that neuronal-specific expression requires upstream distal sequences. We have identified 4 kb present in the 5' flanking region of the mouse POMC gene that targets eutopic expression of reporter genes to POMC hypothalamic neurons. Comparison of this region with the human POMC locus led us to the identification of two highly conserved sequences we named nPOMC1 and nPOMC2. By using a deletion/truncation analysis in transgenic mice, we showed that these sites were critical for POMC gene expression in the brain. We have also observed transgenic expression of EGFP in the dentate gyrus of the hippocampus controlled by cryptic sequences dispensable for pituitary or arcuate expression. The number of EGFP-positive cells in the dentate gyrus increased after prolonged free running and decreased with age, paralleling neurogenesis. EGFP-positive neurons had immature properties, including short spineless dendrites and small action potentials. Colocalization with BrdU indicated that EGFP-labeled granule cells were approximately two weeks postmitotic. EGFP-labeled cells expressed the neuronal marker PSA-nCAM, but not the glial marker GFAP nor the interneuron marker parvalbumin. Thus, hippocampal expression of EGFP driven by mouse POMC sequences is specific for immature granule cells and provides a suitable marker with which to study the integration of newly born granule cells into the adult hippocampal circuitry. Taken together, these results demonstrate that the POMC promoter contains scattered *cis*-acting regions that function as independent modules to control cell-specific expression in different cell types.

Poster 43

Function of CaCrz1p: a calcineurin-dependent transcription factor in *Candida albicans* and analysis of its target genes

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■ Calcineurin is a major player in Ca²⁺-dependent eukaryotic signal transduction pathways. Investigations in *S. cerevisiae* have shown that the transcription factor Crz1p (calcineurin-responsive zinc finger protein) is specifically activated by calcineurin. Activated calcineurin dephosphorylates Crz1p, leading to its translocation to the nucleus and activation of the expression of genes containing a calcineurin-dependent response element (CDRE) in their promoter. In *Candida albicans*, calcineurin is essential for survival in the presence of growth inhibitors or antifungal agents. However, in this yeast, the components of the calcineurin pathway have not yet been well investigated. To determine their functions and their target genes, we characterized *CRZ1* homologues in *C. albicans*. Using sequence data of the *C. albicans* genome, we found two *CRZ1*-like genes, *CaCRZ1* and *CaCRZ2*. Only a *C. albicans* mutant lacking both *CaCRZ1* alleles showed calcium hypersusceptibility. The activation of the *C. albicans* *PMCI* (encoding a vacuolar pump) that contains a CDRE-like sequence in its promoter was calcineurin- and CaCrz1p-dependent. A GFP-tagged CaCrz1p was localized in the nucleus in a calcium- and calcineurin-dependent manner. We performed microarray experiments comparing a wild-type strain induced for one hour with 200 mM Ca²⁺ with a mutant strain lacking either the calcineurin A subunit (*CNA*) or *CaCRZ1* induced under the same conditions. The up-regulation of 81 and 77 genes was dependent on *CNA* or *CaCRZ1*, respectively. These genes function in metabolism, cell wall maintenance, response to stimuli, morphogenesis, and cation homeostasis and transport. While a few genes were specifically activated by calcineurin or CaCrz1p, 68 were dependent on both calcineurin and CaCrz1p. This suggests that calcineurin and CaCrz1p belong to the same calcium-responsive signaling pathway.

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Poster 45

Potential application of mutant variants of the multidrug transporter ABCG2 protein in progenitor cell-based gene therapy

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■ The human ABCG2 (MXR/BCRP) protein is an ABC half-transporter, involved in cancer multidrug resistance. ABCG2 is active as a homodimer and catalyzes an ATP-dependent cellular extrusion of chemically different cytotoxic agents. ABCG2 is highly expressed in the placenta, liver, and intestine, as well as in haematopoietic progenitor cells. The physiological role of this protein is most probably to protect against toxic xenobiotics. In past years, we worked out functional methods to analyze the transport and ATPase activity of the ABCG2 protein using in vitro expression systems and characterized several variants of this protein. We documented that the ABCG2 variants, containing Arg (wild-type), Gly, or Thr at position 482, show different drug-transport patterns. Based on these results, we worked out the use of substrate-mutant ABCG2 as a selectable marker for gene therapy applications.

Progenitor cell-based gene therapy is often unsuccessful because of the relatively low number of corrected and repopulating cells, whose numbers decline rapidly after transplantation. To provide a selective advantage for the modified progenitor cells, we applied the R482G mutant form of the human ABCG2 protein. The cDNA of the R482G-ABCG2 gene was co-inserted with a therapeutic gene (encoding gp91^{phox}) into a recombinant retrovirus, which is used for ex vivo transduction of progenitor cells. We demonstrated that the expression of the gp91^{phox} protein in gp91^{phox}-knockout hematopoietic progenitor cells corrected the mutation responsible for human chronic granulomatous disease, while the mutant ABCG2 protein selectively protected the transduced cells against clinically applicable cytotoxic agents. Overexpression of ABCG2 did not affect hematopoietic cell maturation or the restoration of granulocyte function by gp91^{phox}. We suggest that the mutant ABCG2 protein is an ideal, tailor-made candidate for human stem cell protection and for use as a selectable marker in gene therapy.

Poster 47

Major susceptibility and resistance loci for severe malarial pathogenesis within the murine natural killer complex

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■ The natural killer complex (NKC) is a large dense cluster of immunoregulatory loci encoding several receptor families involved in the control of natural killer (NK) and NKT cell function. In common with the major histocompatibility complex (MHC), the NKC is highly polymorphic. Both NKC and MHC polymorphisms are thought to be maintained by immunity to infection. Interestingly, diverse interactions are emerging between some of the main products of the MHC and NKC clusters, especially Class I-like molecules and NK activation and inhibitory receptors. To date, NKC biology has been elucidated largely in viral and cancer models. We have made the novel and intriguing observation that NKC receptors in general and specific members of the Ly49 family in particular are critical determinants of fatal *Plasmodium berghei* murine malarial pathogenesis. In viral systems, NKC receptors are involved in the control of pathogen replication. By contrast, in malaria, NKC alleles are major pathogenicity determinants through mechanisms that are independent of pathogen replication, that is, through significant regulatory effects on fatal inflammatory processes. Our data in malaria provide the first evidence for critical NKC involvement in the response to a nonviral infectious agent. Unlike viral infections that infect MHC Class I-positive nucleated host cells, malaria blood stages exclusively infect MHC Class I-negative erythrocytes, and Class I-restricted immune responses play no role in the immunological control of blood-stage infection. Presumably, the “Missing Self” negative regulation of NK cells through Class I-reactive inhibitory receptors operates through different mechanisms where red cells are involved. Elucidating the details of NKC/malaria biology may thus prove highly informative to our rapidly expanding understanding of innate immune system biology. Moreover, our findings are doubly important because murine *Plasmodium berghei* infection has emerged as a pre-eminent model of malarial pathogenesis, with clearly established relevance to the 2 million annual human pediatric malarial fatalities.

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Poster 49

Deciphering the capacity of *Bordetella* adenylate cyclase to cross cellular membranes and to deliver vaccinal antigens for induction of cellular immune responses

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■ The *Bordetella* adenylate cyclase toxin (ACT) targets host phagocytes bearing the CD11b/CD18 integrin receptor. ACT penetrates into the cell cytosol, where it is activated by calmodulin, and intoxicates cells by uncontrolled conversion of ATP to cAMP, thereby subverting cellular signaling. Moreover, ACT inserts into cellular membranes and permeabilizes them by forming small (hemolytic) channels, allowing cation permeation across membranes. This project aims at dissecting the mechanism of ACT penetration into cells by analyzing the activity of a set of toxin mutants that are selectively affected in individual steps of ACT action. We were able to locate the receptor-binding domain of ACT within the repeat portion of the toxin. The essential transmembrane segments of ACT were identified, which are directly involved in translocation of the AC domain into cells resulting in cAMP intoxication, in calcium mobilization into cells, and in permeabilization of cellular membranes by channels. All these ACT activities appear, indeed, to act synergistically in promoting apoptotic death of phagocytes.

In parallel, the capacity of genetically detoxified ACT variants to penetrate CD11b-expressing professional antigen presenting cells (APCs) is being exploited, with special emphasis on the toxoid-mediated delivery of vaccinal passenger antigens from major pathogens and tumors (e.g., from HIV, *Mycobacterium tuberculosis*, or melanoma) for processing and presentation on MHC molecules. This ACT capacity allows induction of MHC I-restricted and antigen-specific CD8⁺ cytotoxic T cell responses, as well as efficient simultaneous delivery of CD4⁺ T cell epitopes into the MHC class II presentation pathway and induction of specific CD4⁺ T cell responses. This opens new prospects for evaluation of ACT-based vaccines for prevention and immunotherapy of certain cancers and chronic infections, where simultaneous induction of specific CD4⁺ and CD8⁺ T cell responses is required.

Poster 51

Assembly of cytochrome c oxidase in health and disease

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■ Cytochrome *c* oxidase (COX) deficiency is one of the most frequent causes of respiratory chain diseases in humans. Patients with COX deficiencies can present with a number of different clinical phenotypes, including Leigh syndrome, hypertrophic cardiomyopathy, hepatopathy, and tubulopathy. COX, the terminal enzyme of the respiratory chain, is composed of 13 subunits; the 3 mtDNA-encoded subunits form the catalytic core of the enzyme and contain the prosthetic groups (copper, heme) that channel electrons to molecular oxygen. Assembly of the holoenzyme requires a large number of accessory proteins for the synthesis and membrane insertion of the structural subunits as well as the addition of the prosthetic groups. More than 20 different COX assembly factors have been identified in yeast, about half of which have known human homologues, but the mechanism of holoenzyme assembly remains poorly understood.

Using functional complementation techniques (microcell-mediated chromosome transfer, retroviral expression of cDNAs), we identified mutations in four different COX assembly genes in patients with fatal infantile COX deficiency. Although these assembly genes are ubiquitously expressed, the biochemical deficiencies caused by the mutant proteins are generally tissue-specific, and the resulting clinical phenotypes reflect, to some degree, the extent of enzyme deficiency in different tissues. These tissue-specific effects appear to be mediated by posttranscriptional events. There are marked differences in the abundance of some of the assembly factors, most of which exist as multimeric complexes that are both independent of enzyme content and not reflected in steady-state transcript levels. These results suggest important differences in the mechanism of holoenzyme assembly. We have investigated the molecular pathogenesis of COX assembly in patient cell lines and have uncovered assembly intermediates and demonstrated functional interactions between assembly factors, providing unique insights into the normal mechanism of assembly of the COX complex.

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Poster 53

Study of membrane-bound serine and aspartyl proteases in *Toxoplasma gondii*

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■ The apicomplexan parasites have adopted an intracellular lifestyle that allows them to successfully evade the host defense mechanisms. The survival strategy of *Toxoplasma gondii* in particular is to infect virtually all nucleated cells in its many vertebrate hosts, utilizing a distinct form of actomyosin-dependent gliding motility to propel itself into host cells. Gliding motility is driven by the discharge of adhesive complexes at the apical pole of the parasite and by their redistribution toward the posterior pole of the parasite. During invasion, the transmembrane proteins are rapidly released from the parasite surface by the action of a protease called MMP1, which is affected by 3,4-dichloroisocoumarin (DCI), a serine protease inhibitor previously shown to block invasion. The intramembrane cleavage site of MMP1 is preserved in a range of apicomplexan proteins, suggesting that the protease is present and conserved across the phylum. The rhomboids are polytopic membrane proteins forming a new family of intramembrane-cleaving serine proteases, and several genes encoding rhomboid-like proteins are present in the apicomplexan genomes, constituting plausible candidates for MMP1 activity. Members of this family are currently being characterized in order to assess their substrate specificity and their biological role in *T. gondii*.

The aspartyl proteases constitute an important class of enzymes that have been recognized as appropriate and effective targets for chemotherapy against several infectious diseases, including malaria. In *Plasmodium*, these Plasmeepsins, as these proteases are called, are targeted to the digestive vacuole and involved in hemoglobin degradation. At least one highly conserved protease related to Plasmepsin VI is also found in several other apicomplexans. In *Eimeria tenella*, Eimepsin is present within the apical tips of invading sporozoites, but its function during invasion is still unknown. In *T. gondii*, two Toxomepsins are currently under investigation to determine their subcellular distribution, maturation process, and possible function.

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Poster 55

Translation initiation factor eIF4B is a direct substrate of S6K1/2 and functions to stimulate translation of mRNAs harboring a structured 5' UTR

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■ Control of translation plays a major role in regulation of cell growth. A critical signaling pathway that affects translation and cell growth is the PI3K/mTOR pathway. Two key downstream targets of PI3K/mTOR signaling are the S6 kinases S6K1 and S6K2. S6K inactivation inhibits cell cycle progression; also, deletion of the murine S6K1 orthologue yields a small mouse phenotype. How S6K signaling regulates translation is unknown. The best-studied S6K substrate involved in translation identified to date is the S6 ribosomal protein. Notably, S6 phosphorylation levels are unchanged in S6K1 knockout (KO) mice, probably due to the presence of S6K2, suggesting that lack of S6 phosphorylation is not responsible for the small mouse phenotype. Thus, S6 phosphorylation does not appear to mediate all of the effects of the S6Ks on cell growth and proliferation. Another S6K substrate, eIF4B, is a candidate for mediating S6K's effects through the regulation of translation. The eukaryotic translation initiation factor 4B (eIF4B) stimulates the helicase activity of the DEAD box protein eIF4A to unwind inhibitory secondary structure in the 5' untranslated region (UTR) of eukaryotic mRNAs. We identified a serum/mitogen-responsive eIF4B phosphorylation site, Ser422, located in an RNA-binding region required for eIF4A helicase-promoting activity. S6K1/S6K2 specifically phosphorylate Ser422 in vitro, rapamycin-resistant S6Ks confer rapamycin resistance upon Ser422 phosphorylation in vivo, and Ser422 phosphorylation is significantly decreased in S6K1/S6K2 double KO cells. Substitution of Ser422 with Ala results in a loss of activity in an in vivo translation assay, indicating that phosphorylation of this site plays an important role in eIF4B function. eIF4B may thus mediate some of the effects of the S6Ks on translation and cell growth. eIF4B is important for the translation of mRNAs harboring structured 5' UTRs, because RNA interference against eIF4B results in selective inhibition of translation of mRNAs having complex structures at their 5' UTR.

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Poster 57

Taming the superbugs: structural and inhibitory analysis of the key determinant of broad-spectrum beta-lactam resistance in MRSA

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■ The multiple antibiotic resistance of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) has become a major clinical problem worldwide in terms of both fatalities and containment costs. The key determinant of broad-spectrum beta-lactam resistance in MRSA superbug strains is the membrane-spanning penicillin-binding protein 2a (PBP2a), a transpeptidase that is required to produce peptide cross-links that give the bacterial cell wall its necessary strength and rigidity. Due to its low affinity for beta-lactams, PBP2a provides cross-linking transpeptidase activity at beta-lactam concentrations that inhibit the other beta-lactam-sensitive cell-wall transpeptidases normally produced by *S. aureus* and other pathogenic bacteria. The crystal structure of PBP2a from MRSA strain 27r has been determined to 1.8 Å resolution. Structures of the acyl-enzyme complexes of PBP2a with nitrocefin, penicillin G, and methicillin have also been determined to high resolution and show for the first time beta-lactam binding by a resistant transpeptidase. An analysis of the PBP2a active site reveals the structural basis of its broad-spectrum resistance to the approximately 50 clinically utilized beta-lactam antibiotics and identifies features in beta-lactams important for high affinity binding. This information has been used in structure-based inhibitor design strategies aiming to combat this resistance in the MRSA superbug.

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Poster 59

Yeast myosin light chain Mlc1p interacts with translation termination factor eRF1 and affects [PSI⁺] prion induction

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■ Termination of translation in eukaryotes is controlled by two interacting polypeptide chain release factors, eRF1 and eRF3, encoded in yeast by the essential genes *SUP45* and *SUP35*, respectively. Yeast eRF3 is of special interest given its ability to undergo a heritable conformational switch, similarly to mammalian prions, that gives rise to the cytoplasmically inherited [PSI⁺] nonsense suppressor determinant. Recently, it was shown that, besides the suppressor effect, [PSI⁺] may exhibit a wide variety of heritable phenotypes that are not necessarily related to the translation function of eRF3. These findings were recently supported by data suggesting nontranslational functions for both eRF1 and eRF3. However, the mechanisms underlying such functions of eRF1 and eRF3 remain unclear.

A key to understanding the nontranslational mechanisms associated with eRF1 and eRF3 may be found by identifying the proteins interacting with them. For this reason, we performed a systematic screen for genes that suppress temperature sensitivity of some *sup45* and *sup35* mutants. The search for multicopy suppressors of the *sup45^{ts}* mutant revealed the gene encoding myosin light chain. We demonstrated that, similarly to depletion of Mlc1p, mutations in the *SUP45* gene may affect cytokinesis and that eRF1 is required for intracellular localization of Mlc1p, thus uncovering, for the first time, an essential nontranslational function of the eukaryotic translation termination factor. Mlc1p was shown to be able to interact with eRF1 but not with eRF3 in a yeast cell lysate. Moreover, the data obtained suggest that Mlc1p competes with eRF3 for binding to eRF1. Mlc1p overexpression increased the frequency of [PSI⁺] de novo generation induced by overproduction of eRF3, which suggests the possible destabilization of nonprion form of eRF3 due to its release from association with eRF1 and which couples the [PSI⁺] prion phenomenon to the yeast cytoskeleton.

Poster 61

Analysis of the chromatin attachment and partitioning functions of the bovine papillomavirus type 1 E2 protein

MART USTAV, Estonian Biocentre and Tartu University, Tartu, Estonia

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■ Recent studies have suggested that successful nuclear retention and partitioning of viral genomes during extrachromosomal maintenance in dividing cells could be achieved through tethering of viral genomes to host cell chromosomes. In the case of bovine papillomavirus type 1 (BPV1), such tethering requires viral E2 protein in *trans* and its multiple binding sites located in the upstream regulatory region (URR) of the viral genome in *cis*. In the present study, we show that efficient partitioning of the URR-containing reporter in the absence of any other viral proteins is dependent on its successful E2-mediated tethering to host mitotic chromosomes, which, in turn, requires efficient chromatin attachment of the E2 protein. The interaction of the E2 N-terminal domain with its targets during the chromatin attachment and partitioning process is also likely to involve specific receptors that do not participate in the transactivation or replication initiation functions of E2, given that the activity of E2 protein in chromatin attachment and partitioning is the most sensitive of these activities to substitutions of conserved residues on the surface of the N-terminal domain. Furthermore, in contrast to EBV EBNA1 and KSHV LANA1 proteins, we found that short linear sequences were not responsible for chromosome attachment of BPV1 E2. Instead, the overall structural integrity of the N-terminal domain, as well as perhaps the intactness of some specific subregions of E2 N-terminal domain, is required. Our data suggest that the chromosome attachment of E2 is achieved through complex interactions between composite rather than linear surface elements of the N-terminal domain and specific targets on host chromatin that are most likely different from those used in the case of EBNA1 and LANA1.

Poster 63

Eukaryotic DNA damage checkpoint clamp and clamp loading complexes: their correlated evolutionary conservation and molecular interaction

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■ The eukaryotic Rad17 clamp loader complex and the heterotrimeric 9-1-1 (Rad9-Rad1-Hus1) protein clamp are involved in the early steps of the DNA damage checkpoint response. The Rad17 clamp loader complex loads a doughnut-shaped 9-1-1 complex onto sites of DNA damage, activating cell cycle checkpoint and facilitating DNA repair.

We explored the extent of conservation of this particular DNA damage response system in eukaryotes and determined how these two protein complexes interact with each other at the molecular level. Using sequence analysis of completely sequenced eukaryotic genomes, we found that although Rad17 is conserved from yeast to humans, it is not universally present in eukaryotes. In particular, two parasite species, *Encephalitozoon cuniculi* and *Plasmodium falciparum*, appear to lack the Rad17 protein. Subsequent analysis revealed that these organisms also lack the proteins that form the 9-1-1 complex, the interacting partner of the Rad17 clamp loader complex. The correlated absence of the two interacting complexes indicates that at least two organisms lack a DNA damage response system employed by most eukaryotes. In addition, using a combination of sequence analysis and comparative modeling, we have identified a Rad17 region predicted to mediate binding to the 9-1-1 complex and have proposed a detailed molecular model for this interaction. The molecular model was tested using mutational analysis. The introduced point mutations confirmed the critical importance of the predicted sites for the interaction between the 9-1-1 clamp and the Rad17 clamp loader, providing new insights into the molecular mechanism of function for these two protein complexes.

THURSDAY
HANSA ROOM

Poster 65

Molecular and genetic dissection of female gametogenesis in *Arabidopsis thaliana*

JEAN-PHILIPPE VIELLE-CALZADA, Center for Research and Advanced Studies, IPN, Irapuato, Mexico

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■ We use a transposon-based enhancer detection and gene trapping strategy to study the genetic basis and molecular mechanisms regulating sexual reproductive development and attempt the induction of modifications that lead to apomixis in flowering plants. We routinely analyze expression patterns in all enhancer detector/gene trap lines to identify individuals that show expression during stages of female gametogenesis and early seed development. Three specific lines were used to isolate specific promoters acting during female gametophyte development or early seed formation. Whereas pFM1 is a 844 bp regulatory sequence that strongly drives expression at initiation of female gametogenesis and throughout the gametophytic phase, pCNuc1 activates gene expression in specific sporophytic cells of the developing ovule. To elucidate the function of individual genes or gene families, we modified the pGSA1131 dsRNA vector (provided by R. Jorgensen, University of Arizona) by replacing the constitutive 35S promoter by pFM1. This modified vector is being used to systematically clone fragments of genes acting in the female gametophyte in a sense and antisense orientation and to posttranscriptionally silence them by RNAi at the onset of the gametophytic phase. The strategy has proven successful for silencing genes encoding chromatin remodeling elements, transcription factors, arabinogalactan proteins, and RNA binding proteins. Whereas specific arabinogalactan proteins are essential for the differentiation of the functional megaspore, new members of the SNF2/SWI gene family appear to regulate early embryo patterning and the initiation of endosperm development. We believe that, by using such reverse genetic approaches, the specification and differentiation of cells involved in early female gametogenesis can be genetically manipulated before attempting the modification of the sexual reproductive pathways that prevail in flowering plants.

THURSDAY
HANSA ROOM

Poster 67

Dopamine D5/GABA_A heteromerization: a mechanism to regulate synaptic weight

YU TIAN WANG, University of British Columbia, Vancouver, Canada

H.Y. Man,¹ F. Liu,² W. Ju,¹ H.B. Nizik,² Y.T. Wang¹ ■ ¹University of British Columbia, Vancouver, Canada; ²University of Toronto, Toronto, Canada

■ Modulation of synaptic transmission between individual neurons is central to brain functions such as learning, memory formation, and cognition and to brain dysfunctions, including schizophrenia and other mental disorders. Modulation of neurotransmitter receptor function is one of the most important mechanisms by which the efficacy of synaptic transmission is controlled in the mammalian central nervous system. Neurotransmitter receptors can be structurally and functionally classified into two large families: ligand-gated ion channels and G protein-coupled receptors. The former contain an ion channel as an integral part of the receptor and mediate fast synaptic transmission. The G protein-coupled receptor, on the other hand, is not itself an ion channel but exerts effects on fast synaptic transmission indirectly, through regulation of the function of ligand-gated receptors. G protein-coupled receptors are conventionally thought to alter ligand-gated receptor function through activation of one or more diffusible intracellular second messengers, such as adenylate cyclase. However, in our most recent research, we have made a novel observation that suggests a new model of modulation involving a heteromerization between the two receptor families. We found that the G protein-coupled dopamine D5 receptor heterodimerizes with GABA_A receptor chloride channels through a direct binding between the intracellular carboxyl tail of the D5 and the second intracellular loop of the GABA_A receptor 2 subunit. This heteromerization enables ligand-induced endocytosis of both receptors in response to agonist stimulation of either receptors and, hence, a mutual inhibition of each other's function. Moreover, GABA_A receptor-associated protein (GABARAP) is capable of blocking the interaction by competing with the D5 carboxyl tail for gamma2 binding sites, thereby preventing receptor cotrafficking and mutual inhibition between the two receptors. Given that both dopamine and GABA_A receptors have been implicated in a wide range of brain functions and dysfunctions, our results may contribute to our current understanding of brain physiology and to identifying novel targets for new therapeutics for brain disorders such as schizophrenia and drug addiction. In addition, direct binding of D5 to GABA_A receptors represents a novel and potentially common means by which G protein-coupled receptor systems regulate the function of ligand-gated receptors and, hence, the efficacy of synaptic transmission in the nervous system.

Poster 69

High-throughput mapping of a dynamic signaling network in mammalian cells

JEFF L. WRANA, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada

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■ In metazoans, signal transduction pathways interpret extracellular cues to control cellular behavior and coordinate development and homeostasis. Signaling pathways do not function in a linear manner, but rather are organized in complex networks that likely are critical for interpreting combinatorial signals. The transmission of information through signaling networks is controlled by a host of mechanisms that include protein-protein interactions, protein-lipid interactions, proteolytic activity, subcellular localization, and membrane trafficking. Therefore, to understand how these networks interpret extracellular signals, it is essential to map signaling networks in physiologically relevant systems. Here we describe a novel high-throughput technology to map protein-protein interactions in mammalian cells. Applying the technology to components of the TGF-beta pathway, we define a dynamic hierarchical signaling network. Analysis of the interactome using self-organizing maps reveals the presence of numerous clusters that display closely related patterns of interaction with the TGF-beta signaling pathway. Analysis of one of these clusters reveals a network of TGF-beta receptor-interacting proteins that includes a number of protein kinases and their regulators. One component of this network that we term TRIK is a previously undescribed kinase that lacks residues critical for catalytic activity; we demonstrate that TRIK is a potent inhibitor of Smad activation. These studies thus reveal a dynamically connected hierarchical signaling network that interprets TGF-beta signaling and define a novel catalytically inactive kinase that is a potent inhibitor of TGF-beta-dependent Smad activation.

Poster 71

Investigation of the regulatory cascade of the homeobox gene *Anf*

ANDREY ZARAIISKY, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

A. Zاراisky, G. Ermakova, V. Novoselov, N. Martynova, F. Eroshkin, A. Bayramov, M. Tereshina ■ Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

■ We have continued to investigate the genetic cascade connected with the functioning of the key regulator of the anterior neuroectoderm patterning, the homeobox gene *Xanf-1*.

First, we examined the mechanism of activation of the *Xanf-1* expression in the anterior neural plate. The transcription factors Otx-2 and SoxD were shown to be able to bind with the distinct *cis*-regulatory elements in the *Xanf-1* promoter and to activate its expression. Second, we identified and investigated the functioning of three transcription factors, FoxA4a, Xvent-2, and X-nkx-5.1, which are responsible for the spatial and temporal restriction of the *Xanf-1* expression in the anterior neural plate. Third, we investigated the role in regulation of the anterior patterning of two genetic targets of *Xanf-1*, genes encoding the small GTPase *ras-dva* and the secreted factor *Xagr*. Specifically, we demonstrated that inhibition of translation of these genes by the antisense morpholino oligos elicits reduction formation of the forebrain and the anterior placodes. The genetic basis of these abnormalities has been investigated in depth. Finally, as an extension of the project, we developed a new noncontacting technique of cell labeling in living embryos by a novel fluorescent protein activated by the green light.

THURSDAY
HANSA ROOM

3:30 p.m.

Global mapping of the yeast genetic interaction network: discovering gene and drug function

CHARLES BOONE, University of Toronto, Toronto, Canada

■ In the budding yeast *Saccharomyces cerevisiae*, about 80 percent of the approximately 6000 genes are nonessential, indicating that many biological processes are buffered from the phenotypic consequences of genetic perturbation. To examine these functional relationships, we developed a method called synthetic genetic array (SGA) analysis, which automates yeast genetics and enables a systematic and high-throughput construction of double mutants from an ordered array of about 4700 viable gene deletion mutants. In particular, double mutants showing reduced fitness (a synthetic sick phenotype) or lethality (a synthetic lethal phenotype) define functional relationships between genes and their corresponding pathways. We have undertaken a project to generate a synthetic genetic interaction network for the yeast cell with the expectation that it will represent a global map of functional relationships amongst most genes. We found that synthetic genetic interactions are more common than anticipated previously, with an average query gene displaying about 30 different interactions. Cluster analysis of a compendium of about 132 SGA screens revealed that genes displaying similar patterns of genetic interactions often encode proteins within the same pathway or complex; therefore, the yeast genetic interaction network predicts precise molecular roles of previously uncharacterized genes. Moreover, because a gene deletion mutation provides a model for the effect of a compound that inhibits its corresponding gene product, our compendium of synthetic genetic profiles provides a key for determining the cellular targets of small molecules and thus provides a tool for antifungal drug discovery. In another application of this technology, we have backcrossed the set of yeast deletion alleles to a wild-type *S. cerevisiae* strain that is capable of filamentous growth, and we are attempting to identify a comprehensive set of genes required for polarized morphogenesis. These genes are of particular interest because the transition from budding to filamentous growth is a component of *Candida albicans* pathogenesis.

3:55 p.m.

Functional studies of mycobacterial genes using genomic and functional genomic approaches

ROSS LEON COPPEL, Monash University, Melbourne, Australia

P. Crellin,¹ D. Lea-Smith,¹ A. Pelossi,¹ M. von Itzstein,² R. Coppel¹ ■ ¹Monash University, Melbourne, Australia; ²Griffith University, Southport, Australia

■ Microbial genome sequences provide an enormous impetus to the study of microbial pathogenesis. However, approximately half the predicted genes in newly sequenced microbial genomes encode putative proteins of unknown function. Genes identified as necessary for pathogen survival provide important targets for new treatment approaches. We used a battery of bioinformatics approaches to identify such genes and have uncovered a group of genes that may be important to mycobacterial cell wall synthesis and survival as well as genes likely to be important in invasion and intracellular survival of malaria parasites.

The importance of a number of these candidate genes has been tested by targeted gene disruption in the model system *Mycobacteria smegmatis*. A system based on gene rescue under the control of a temperature-sensitive plasmid enabled us to confirm essentiality. To study the effect of these gene disruptions, we performed various analyses, including cell wall studies, on these bacteria during temperature selection. To further analyze these mutants, we constructed the first *M. smegmatis* microarray. Using the as yet incomplete genome sequence, we assembled and identified all open reading frames and used these to construct an array enriched in genes involved in cell wall synthesis. This array has been validated by probing it with RNA collected from bacteria subjected to treatments known to induce particular transcriptional changes. The arrays were then probed in a number of experiments with RNA from the mutants we had created, RNA from a panel of previously existing mutants, and RNA from bacteria treated with the novel antimicrobials we reported previously. A number of genes were identified as up- and down-regulated, and they are now being targeted in gene knockout and biochemical experiments to determine whether they function in pathways related to the genes inactivated in the parent mutant bacteria.

THURSDAY
HANSA ROOM

4:20 p.m.

Genetic analysis of hybrid sterility in the mouse X-chromosome substitution strain

Jiří FOREJT, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

J. Forejt,¹ R. Storchova,¹ S. Gregorova,¹ V. Kyselova,¹ D. Buckiova,² P. Divina¹ ■ ¹Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic; ²Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic

■ C57BL/6-X^{PWD} is one of 21 inter-subspecies chromosome substitution strains that have been created as a new tool for functional genomics. The parental mouse inbred strains PWD and C57BL/6 represent a laboratory model of two closely related subspecies of the house mouse: *Mus musculus musculus* and *Mus musculus domesticus*. Male but not female F₁ hybrids are sterile, thus obeying the classical Haldane's rule of hybrid sterility. Hybrid sterility is one of the reproductive isolation mechanisms that operate during the establishment of a new species. Thus, the new chromosome substitution strains could serve as a laboratory model for the genetic dissection of mammalian speciation mechanisms. Here we show that the introgression of the X chromosome of *M. m. musculus* origin (PWD) into the genetic background of the C57BL/6J inbred strain causes hybrid male sterility. The X-linked hybrid sterility is associated with reduced testes weight, lower sperm count, and morphological abnormalities of sperm heads. The quantitative trait locus (QTL) analysis revealed an oligogenic nature of the sterility. Mapping and cloning the genes responsible for the sterility of B6-X^{PWD}/Y^{B6} males and understanding their epistatic interaction could clarify the role of the X-linked hybrid sterility in the process of speciation. Our progress in constructing a set of 20 chromosome substitution strains and 1 conplastic strain will be summarized in the second part of the talk.

4:45 p.m.

Comparative genomics, metabolic reconstruction, and analysis of regulation in bacterial genomes

MIKHAIL S. GELFAND, Research Institute for Genetics and Selection of Industrial Microorganisms (State Scientific Center GosNIIGenetika), Moscow, Russia

M.S. Gelfand,^{1,2} A.V. Gerasimova,¹ A.E. Kazakov,² E.A. Kotelnikova,¹ O.N. Laikova,¹ A.A. Mironov,¹ E.A. Permina,¹ D.A. Ravcheev,¹ D.A. Rodionov,¹ A.G. Vitreschak² ■ ¹Research Institute for Genetics and Selection of Industrial Microorganisms (State Scientific Center GosNIIGenetika), Moscow, Russia; ²Institute for Information Transmission Problems, Moscow, Russia

■ Comparative genomics techniques were applied to the analysis of several metabolic pathways and functional systems in bacteria (e.g., vitamins: cobalamin, thiamine; amino acids: lysine, methionine, histidine, threonine, branched-chain, aromatic; sugars and polysaccharides; stress response: heat shock, general stress, heavy metal resistance). This work resulted in metabolic reconstructions of these pathways, identification of numerous new enzymes and transporters, and prediction of new regulatory systems.

In particular, we continued to study riboswitches, a new class of regulatory RNA elements. We identified numerous new instances of riboswitches, described in detail their mode of action (repression or activation, attenuation of transcription or translation), and studied their evolution and interaction with other regulatory systems. In gram-positive bacteria, the riboswitch appears to be the primary regulator of the methionine pathway, whereas in lactococci this role is assumed by T-boxes and in streptococci by the MtaR transcriptional repressor. A new mechanism of riboswitch regulation in actinobacteria involves direct sequestering of the ribosome-binding site. Lysine-dependent riboswitches may activate genes for the lysine catabolism pathway in several genomes.

Analysis of zinc regulons enabled us to identify a family of proteins likely involved in pathogenesis caused by streptococci. Repression by zinc was predicted for genes encoding paralogues of ribosomal proteins containing the zinc-ribbon motif. We identified new regulatory signals for the zinc repressor AdcR, the methionine repressor MtaR, two regulators of aromatic amino acid biosynthetic operons, and numerous transcription factors from the LacI family.

New enzymes were identified in the pathways of cobalamin, thiamine (ThiN), lysine (branch of acetylated intermediates in *Bacillus subtilis*), and methionine (recycling and reverse synthesis of cysteine from methionine branches).

Several of our predictions were confirmed through experiments conducted by collaborators and in independent studies.

THURSDAY
HANSA ROOM

8:00 a.m.

The granzyme B-serglycin complex from cytotoxic granules requires dynamin for endocytosis

R. CHRIS BLEACKLEY, University of Alberta, Edmonton, Canada

K. Veugelers, B. Motyka, C. Frantz, I. Shostak, T. Sawchuk, R.C. Bleackley ■ University of Alberta, Edmonton, Canada

■ Cytotoxic T lymphocytes and natural killer cells destroy targets via the directed exocytosis of lytic effector molecules such as perforin and granzymes. The mechanism by which these proteins enter targets is uncertain. There is ongoing debate over whether the most important endocytic mechanism is nonspecific or requires the cation-dependent mannose 6-phosphate receptor. This study tested whether granzyme B endocytosis is facilitated by dynamin, a key factor in many endocytic pathways. Uptake of and killing by the purified granzyme B molecule occurred by both dynamin-dependent and -independent mechanisms. However, granzyme B is not exocytosed in a monomeric form. Rather it is part of a macromolecular complex (>100,000 MW) that contains proteoglycan, granzymes, and perforin. Most importantly, serglycin-bound granzyme B in high molecular weight degranulate material from cytotoxic T lymphocytes predominantly followed a dynamin-dependent pathway to kill target cells. Similarly, killing by live cytotoxic T lymphocytes was attenuated by a defect in the dynamin endocytic pathway; in particular, the pathways characteristically activated by granzyme B were affected. We therefore propose a model whereby degranulated serglycin-bound granzymes require dynamin for uptake.

**THURSDAY
BREMERHAVEN
ROOM**

8:25 a.m.

Regulation of the actin cytoskeleton by Vav2

LÁSZLÓ BUDAY, Semmelweis University Medical School, Budapest, Hungary

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■ Rho-like GTPases are key regulators in signaling pathways that link extracellular growth signals to the assembly and organization of the actin cytoskeleton. Like other members of the Ras superfamily, Rho proteins cycle between a biologically inactive GDP-bound state and an active GTP-bound state. The rate of cycling between the two states is regulated by GTPase-activating proteins (GAPs) and by guanine nucleotide exchange factors (GEFs). Recently, we investigated the regulation of Vav2 in the EGF signaling pathway and found that activation of Vav2 requires PI 3-kinase activity but not tyrosine phosphorylation. In addition, we and others noticed that Vav2 overexpression in COS7 cells induced membrane ruffling. To elucidate the mechanism by which Vav2 overexpression results in membrane ruffling, we have studied some of the candidate molecules that may participate in actin polymerization downstream of Vav2. We showed that overexpression of Vav2 induced translocation of cortactin to the plasma membrane. Cortactin is an Src tyrosine kinase substrate that has been recently implicated in the regulation of cortical actin polymerization. In addition, Vav2 colocalizes with cortactin at the sites of cortical actin polymerization. Using GFP-cortactin fusion proteins, we were able to demonstrate that the N-terminal half of cortactin, which contains actin-binding motifs, is sufficient for the translocation. Vav2-dependent membrane ruffling was effectively inhibited by expression of a dominant negative Pak1 mutant. Neither EGF stimulation nor Vav2 expression induced tyrosine phosphorylation of cortactin; however, increased Pak1 activity was detected in the immunocomplexes of Pak1 prepared from cell lysates expressing GFP-Vav2. Finally, EGF-dependent cortical actin polymerization and translocation of cortactin were partially blocked by a dominant negative mutant of Vav2. These results suggest that EGF regulates cortactin translocation and subsequent actin polymerization, at least in part, through Vav2, a guanine nucleotide exchange factor for Rho GTPases.

8:50 a.m.

LIMK1 regulates Golgi dynamics, traffic of Golgi-derived vesicles, and process extension in primary cultured neurons

ALFREDO O. CÁCERES, Mercedes and Martin Ferreyra Institute for Medical Research, CONICET, Cordoba, Argentina

S. Rosso,¹ F. Bollati,¹ M. Bisbal,¹ S. Quiroga,² A. Cáceres¹ ■ ¹Mercedes and Martin Ferreyra Institute for Medical Research, CONICET, Córdoba, Argentina; ²National University of Córdoba, Córdoba, Argentina

■ In this study we examined the subcellular distribution and functions of LIMK1 in developing neurons. Confocal microscopy, subcellular fractionation techniques, and expression of several epitope-tagged LIMK1 constructs revealed that LIMK1 is highly enriched in the Golgi apparatus and growth cones, with localization in the Golgi requiring LIMK1's LIM domain and that in the neuritic tips requiring its PDZ domain. Transfection of wild-type LIMK1 enhances the accumulation of Par3/Par6 and IGF1 receptors at axonal growth cones, and it accelerates axon formation; these effects are dependent on the Golgi localization of LIMK1. In addition, overexpression of wild-type LIMK1 suppresses the formation of trans-Golgi-derived tubules and inhibits the Golgi-derived export of synaptophysin-containing vesicles, while stimulating Golgi-derived export of Par3- and NCAM-containing vesicles. These effects are paralleled by an increase in cofilin phosphorylation and rhodamine phalloidin staining in the region of the Golgi apparatus and are prevented by coexpression of constitutively active cofilin. The long-term overexpression of LIMK1 produces growth cone collapse and axon retraction, an effect that is dependent on growth cone localization of LIMK1. Taken together, our results suggest an important role for LIMK1 in axon formation that appears to be directly related to the factor's ability to regulate membrane traffic, growth cone formation, and actin cytoskeleton organization.

9:15 a.m.

The role of the variable domains of the light chains of kinesin in its interaction with a cargo

FATIMA K. GYOEVA, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

F.K. Gyoeva,¹ A.I. Akmulin,¹ A.A. Minin,¹ A.L. Khodjakov² ■ ¹Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia; ²Wadsworth Center, Albany, New York, USA

■ The motor protein kinesin is implicated in the transport of membrane-bound organelles and multicomponent complexes along microtubules. In a kinesin molecule, two motor heavy chains combine with two accessory light chains, which are represented by alternatively spliced variants. It has been suggested that each light chain targets kinesin to a specific cargo. The data we obtained support this view. Different light-chain isoforms were found to localize to different organelles, whereas a single kinesin molecule proved to contain two identical light-chain variants.

Recent findings, however, suggest that there is no general mechanism for kinesin binding to cargoes. In some cases, this interaction involves the conservative portions of the light chains; in others, it depends on the heavy chains and does not require the light chains at all. Thus, determining the significance of the light-chain variability remains a challenge.

We are currently using various experimental approaches to determine the role of variable domains of the light chains: 1) inhibition of the kinesin molecules containing an individual light-chain isoform and overexpression of this isoform together with the mutant kinesin heavy chain; 2) overexpression of the variable portions of the light chains together with the protein BicD2 known to mediate binding to organelles of dynein, the motor that transports cargoes along microtubules in the direction that is opposite to that of kinesin (the DNA constructs for this work were prepared by Dr. Anna Akhmanova, Erasmus University, The Netherlands); and 3) microinjection of the antibodies against the variable portions of the light chains. In each case, positioning and movement of particular kinesin cargoes are estimated as a result of one or another intervention.

THURSDAY
BREMERHAVEN
ROOM

10:10 a.m.

Role of chromatin proteins in maintaining the specific patterns of DNA methylation in *Arabidopsis thaliana*

ANDRZEJ JERZMANOWSKI, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, and Warsaw University, Warsaw, Poland

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■ In mammals and flowering plants, DNA methylation is a key molecular signal used to distinguish between active and inactive genes. Deficient in DNA methylation 1 (DDM1) protein is required to maintain DNA methylation status in *Arabidopsis thaliana*. We used a purified recombinant DDM1 protein to investigate whether it can remodel chromatin in vitro. We show that DDM1 is an ATPase stimulated by both naked and nucleosomal DNA. It binds to chromatin and induces nucleosome repositioning and disruption of histone DNA contacts. The enzymatic activities of DDM1 are not affected by DNA methylation. To investigate the involvement of chromatin structural proteins in DNA methylation, we used double-stranded RNA (dsRNA) silencing to suppress all the *H1* genes of *A. thaliana*. Plants with a greater than 90 percent reduction in *H1* expression exhibited a spectrum of phenotypic defects resembling those observed in DNA hypomethylation mutants. The molecular background of these phenotypes has been analyzed.

**THURSDAY
BREMERHAVEN
ROOM**

10:35 a.m.

Activation of *trans* geometry in bifunctional platinum complexes by heterocyclic ligands: mechanistic studies on antitumor action

JANA KAŠPÁRKOVÁ, Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic

J. Kašpárková, V. Marini, V. Brabec ■ Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic

■ A paradigm for the structure–pharmacological activity relationship of bifunctional platinum antitumor drugs is that the *trans* isomer of antitumor *cis*-diamminedichloroplatinum(II) (transplatin) is clinically ineffective. However, several new complexes of the *trans* structure have been identified that exhibit cytotoxicity in tumor cells that is even better than that of the analogous *cis* isomers. We have shown that replacement of one ammine ligand by heterocyclic ligands such as piperidine, piperazine, or 4-picoline in the molecule of transplatin resulted in a radical enhancement of its cytotoxicity. Sufficient evidence has accumulated to identify DNA as the relevant pharmacological target of antitumor platinum compounds. In order to shed light on the mechanism underlying activity of these transplatin analogues, we examined, by biochemical and biophysical methods, oligodeoxyribonucleotide duplexes bearing a site-specific cross-link of the transplatin analogue containing the piperidine ligand. Our results indicate that, in contrast to transplatin, *trans*-[PtCl₂(NH₃)(piperidine)] forms stable 1,3-intrastrand cross-links in double-helical DNA that distort DNA and are not readily removed from DNA by nucleotide excision repair. Hence, the intrastrand cross-links of *trans*-[PtCl₂(NH₃)(piperidine)] could persist for a sufficiently long time, potentiating its toxicity toward tumor cells. Similar to transplatin, *trans*-[PtCl₂(NH₃)(piperidine)] also forms minor interstrand cross-links in DNA; these adducts thus appear to be less likely candidates for the genotoxic lesion responsible for antitumor effects of *trans*-[PtCl₂(NH₃)(piperidine)]. Hence, structurally unique intrastrand cross-links may play a predominant role in the antitumor effects of transplatin analogues in which one ammine group is replaced by a heterocyclic ligand.

11:00 a.m.

Double covalent modification of cytosine by DNA methyltransferases**SAULIUS KLIMAŠAUSKAS**, Institute of Biotechnology, Vilnius, LithuaniaR. Gerasimaitė¹ G. Vilkaitis,¹ S. Kulakauskas,² S. Klimašauskas¹ ■ ¹Institute of Biotechnology, Vilnius, Lithuania; ²Centre de Recherche de Jouy-en-Josas, INRA, Jouy-en-Josas, France

■ Enzymatic modification of DNA by two methyltransferases (MTases) recognizing identical or overlapping sequences and normally targeting either the 5- or N4-position of the same cytosine residue can lead to the formation of N4,5-dimethylcytosine (diMC), a doubly methylated base. For example, such double methylation of cytosine (underlined) at the CCWGG sites in vitro is achieved when the C5-MTase Ecodcm acts first and is followed by the N4-specific MvaI enzyme. The formation of diMC in vivo and its consequences for the viability of *Escherichia coli* were studied in *dcm*⁺ strains carrying the MvaI MTase gene on a plasmid. We found that the transformation of the plasmids was dramatically restricted in the *ung1*, *recA*, *recD*, *red*, *mutH*, and *uvrB* mutants, which are deficient in DNA repair. Biochemical analysis of cytosine modification in DNA at different expression levels of the MvaI MTase showed that both “mono-methylated” cytosines were present in various ratios; diMC was barely detectable by conventional chromatographic analysis of nucleosides but was identified using a coupled mass spectrometric detector. Taken together, these observations indicate that diMC residues do form in vivo but are effectively removed from DNA. This hypothesis was further supported by our observation that transformation with plasmids carrying the cloned MvaI MTase leads to the induction of the *E. coli* SOS system. In line with the observed low tolerance of diMC residues in DNA, our computer modeling revealed that diMC might introduce local perturbations in the double-helical DNA. Our further experiments demonstrate that the action of certain DNA polymerases in vitro is significantly interrupted at sites of the doubly methylated cytosines. These findings suggest that enzymatically generated diMCs can be used as site-specific structural probes for studying the mechanisms of DNA damage recognition and repair in vivo.

11:25 a.m.

Novel Rad51^{Sp}-dependent pathway of recombination/repair in *Schizosaccharomyces pombe* mediated by Dds20 protein**VLADIMIR I. BASHKIROV**, Institute of Gene Biology, Russian Academy of Sciences, Moscow, RussiaE.K. Khasanov,¹ A.F. Salakhova,¹ G.V. Savchenko,¹ E. Hartsuiker,² V.G. Korolev,³ A.M. Carr,² V.I. Bashkirov¹ ■ ¹Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia; ²University of Sussex, Brighton, United Kingdom; ³St. Petersburg Nuclear Physics Institute, Russian Academy of Sciences, Gatchina, Russia

■ DNA double-strand break (DSB) repair by homologous recombination is an evolutionary conserved, Rad51 protein-mediated mechanism. The Rad51 paralogs Rad55 and Rad57 of the yeast *Saccharomyces cerevisiae*, and Rhp55, Rhp57, and Rlp1 of *Schizosaccharomyces pombe* are thought to assist in Rad51 nucleoprotein assembly. Here we show that in fission yeast, the novel Rad51-dependent pathway of DSB repair operates in parallel with that mediated by Rad51 paralogs. Dds20, a novel protein that interacts with Rad51 to promote DNA damage repair and meiotic recombination, defines this pathway. Overexpression of Dds20 partially rescues the DNA repair and replication defects of the *rhp55* mutant but not meiotic recombination defects. The *dds20*Δ cells are hypersensitive to ionizing radiation and the alkylating drug MMS and are impaired in mitotic and meiotic recombination.

Dds20 is also involved in the cellular response to UV damage through the checkpoint-independent UV damage tolerance mechanism. Dds20 is a nuclear protein that exhibits a foci-like immunostaining pattern in mitotic cells. As occurs in the *rhp55* mutant, the formation of ionizing radiation-induced Rhp51 foci is reduced in *dds20*Δ cells, suggesting a role of Dds20 in Rad51 nucleoprotein assembly. Genetic interactions of *dds20*⁺ with other DSB repair genes are discussed, and a model of recombinational DNA repair is presented.

THURSDAY
BREMERHAVEN
ROOM

3:30 p.m.

A telomere position effect is involved in the differential expression of subtelomeric genes in *Plasmodium falciparum*

BRENDAN CRABB, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

B.S. Crabb,¹ M.T. Duraisingh,¹ T.S. Voss,¹ A. Marty,¹ J. Thompson,¹ R.T. Good,¹ M. Duffy,² A.F. Cowman¹ ■ ¹Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; ²University of Melbourne, Melbourne, Australia

■ Antigenic variation and cytoadherence of infected erythrocytes play a major role in the development of severe disease and chronic infection in *Plasmodium falciparum* malaria. Both processes are mediated by the virulence factor *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on the surface of infected red blood cells. PfEMP1 is encoded by the *var* gene family, comprising approximately 60 highly diverse members per parasite genome. Most *var* genes are located at chromosome ends, which are themselves attached to the nuclear periphery. In *P. falciparum*, mutually exclusive transcription of the *var* gene family ensures that only one copy is activated in a single parasite, whereas transcription of all other *var* members remains silenced. To investigate mechanisms mediating transcriptional silencing in *P. falciparum* subtelomeric regions, we generated a transgenic parasite line. Parasite growth under drug selection artificially activates transcription of the human dihydrofolate reductase (*dhfr*) resistance gene introduced into the rep20 repeat region at one end of chromosome 3. In the absence of the drug, however, this locus is silenced, demonstrating a telomere position effect (TPE). We showed that the active and silenced transcriptional states of the *dhfr* gene are associated with an altered chromatin structure in the upstream promoter. Moreover, fluorescent in situ hybridization (FISH) analysis of these parasites transfected with a second selectable marker present on episomes and targeted to the nuclear periphery suggests the presence of a physical expression site at the nuclear periphery that is permissive for transcription.

THURSDAY
BREMERHAVEN
ROOM

3:55 p.m.

Changes in cholinergic sensitivity and functional efferent innervation of cochlear inner hair cells during development

ANA BELÉN ELGOYHEN, Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina

E. Katz,¹ E. Glowatzki,² D.E Vetter,³ M.E Gómez Casati,¹ P. Fuchs,² A.B Elgoyhen¹ ■ ¹Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina; ²Center for Hearing Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; ³Tufts University, School of Medicine, Boston, Massachusetts, USA

■ In the mature cochlea, inner hair cells (IHCs) transduce acoustic signals into receptor potentials, communicating to the brain by synaptic contacts with afferent fibers. Before the onset of hearing, occurring about postnatal day 11 in rats, a transient efferent innervation is found on IHCs. The activity of efferent neurons could modulate the firing frequency of IHCs, thus playing a role in establishing adult innervation. This synapse is inhibitory and mediated by a nicotinic cholinergic receptor probably formed by the alpha9 and alpha10 subunits. Expression of alpha9 mRNA persists in adult rat IHCs, whereas that of alpha10 does not. We studied this developmental transition using whole cell recordings from IHCs in acutely excised apical turns of the rat organ of Corti. From P3 to P14, acetylcholine (ACh) elicited inward currents at -90 mV in 88–100 percent of the cells, whereas from P16 to P22, only 1 cell out of 11 responded to ACh. The mean amplitude of the evoked current varied with age (in pA/pF, a measure of amplitude normalized to the capacitance of the cell): 4.2 ± 1.2 at P3, 47 ± 7.7 at P7, 26.3 ± 4.2 at P10, and 2.3 ± 0.4 by P13. K⁺ depolarization of efferent terminals caused inhibitory postsynaptic currents (IPSCs) in 67 percent of IHCs at P3, in 93–100 percent of cells from P7 to P12, and in only 40 percent of cells by P13. From P16 to P22, none of the cells tested had IPSCs. In situ hybridization showed that alpha10 is expressed in IHCs at P3. The alpha10 signal decreased at P13, and by P21, it was undetectable. Direct efferent synapses to IHCs, detected by immunostainings from P3 to P7, disappeared by P21. The correlation of cholinergic sensitivity with subunit expression and innervation pattern strengthens the hypothesis that the functional IHC cholinergic receptor is composed of both the alpha9 and alpha10 subunits and that the expression of the *Acr10* gene is under the control of synaptic activity.

4:20 p.m.

Analysis of transcription and search for the protein product of a ubiquitous gene that changes expression during T cell development

PAWEŁ KISIELOW, Institute of Immunology and Experimental Therapy, Wrocław, Poland

M. Cebzat, A. Rapak, A. Miążek, B. Gidzińska, L. Strządala, P. Kisielow ■ Institute of Immunology and Experimental Therapy, Wrocław, Poland

■ To better understand processes essential for the ability of the adaptive immune responses to discriminate self from non-self, we have been hunting for new genes that change their expression during intrathymic T cell development and selection. We have focused on the hypothetical gene, nicknamed *Nwc*, which, as we found, is transcriptionally active in every tissue but shows an interesting pattern of expression during T cell development. It is highly expressed in thymocytes undergoing rearrangements of the V, D, and J segments of the T cell receptor (TCR) genes and is strongly down-regulated in response to TCR-mediated signals during intrathymic selection. We also found that regulation of transcription of this gene in thymocytes differs from that in mature lymphocytes and in non-lymphoid tissues. In thymocytes, transcription of *Nwc* remains under the control of a promoter of a gene involved in V(D)J recombination, and a hybrid transcript spanning both genes is spliced to produce *Nwc* transcripts of various sizes. To learn about the possible function of the *Nwc* gene, we are attempting to use RNAi technology to inhibit its expression in various cell types.

To identify the possible natural protein product of the hypothetical *Nwc* gene, a rabbit antiserum was raised against the recombinant, histidine (His)-tagged protein, produced by bacteria transformed with His/*Nwc* cDNA. Preliminary results indicate that the antiserum identifies two proteins: one is present in the majority of tissues, and the second is present in immature thymocytes but not in T cells. Preparations for the sequencing of the recombinant protein produced in bacteria as well as the two proteins identified in thymocytes are in progress.

4:45 p.m.

Endothelial cell-specific growth inhibition and block of angiogenesis by an extracellular fragment of CD44

PRIIT KOGERMAN, National Institute of Chemical Physics and Biophysics and Tallinn Technical University, Tallinn, Estonia

T. Päll,^{1,2} A. Gad,³ L. Kasak,¹ M. Drews,¹ S. Strömbblad,³ P. Kogerman,^{1,2} ■ ¹National Institute of Chemical Physics and Biophysics, Tallinn, Estonia; ²Tallinn Technical University, Tallinn, Estonia; ³Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden

■ CD44 is the main cellular receptor for hyaluronic acid. We previously found that overexpression of CD44 inhibited tumor growth of mouse fibrosarcoma cells in mice. Here we show that tumor growth inhibition by CD44 is caused by block of angiogenesis. We found that soluble recombinant CD44 hyaluronic acid-binding domain blocked angiogenesis in vivo in chick and mouse, thereby inhibiting tumor growth of various origins. In addition, recombinant CD44 hyaluronic acid-binding domain inhibited cell proliferation in an endothelial cell-specific manner. Given that mutants deficient in hyaluronic acid binding still maintain their anti-angiogenic and anti-proliferative properties, the anti-angiogenic effect of CD44 is independent of its hyaluronic acid-binding capacity. While dissecting the active domain of CD44, we isolated a shorter peptide that can inhibit angiogenesis and migration of endothelial cells toward VEGF. The isolated CD44 peptide represents a novel class of angiogenesis inhibitors based on a cell-surface receptor and may be useful in the clinic for treatment of cancer and other angiogenesis-dependent diseases.

THURSDAY
BREMERHAVEN
ROOM

8:00 a.m.

Interaction of rotavirus with hsc70 and lipid membrane microdomains during cell entry

CARLOS F. ARIAS, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

C.F. Arias, J. Pérez-Vargas, P. Isa, P. Romero, S. López ■ Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

■ Rotavirus infection is a multistep process in which the viruses interact with several cell surface molecules to enter the cell. The virus spike protein VP4, which is cleaved by trypsin into the two subunits VP5 and VP8, is involved in some of these interactions. We have previously shown that the rotavirus strain RRV initially attaches to a sialic acid-containing cell molecule through the VP8 subunit of VP4, and then subsequently interacts with integrin $\alpha 2\beta 1$ through VP5. After these initial contacts, the virus interacts with at least three additional proteins located at the cell surface, integrins $\alpha v\beta 3$ and $\alpha x\beta 2$ as well as the heat shock cognate protein hsc70. In this work, we have shown that rotavirus RRV interacts with hsc70 through a VP5 domain located between amino acids 642 and 659 of the protein and also that hsc70 interacts with this viral domain through its ligand-binding site, located in the carboxy terminal half of the molecule, given that hsc70 ligands block the VP5-hsc70 interaction and also inhibit virus infectivity. We have also shown that the integrins, implicated as rotavirus receptors, as well as hsc70 associate with cholesterol-enriched detergent-resistant cell membrane domains (rafts) during the early interactions of the virus with the cell. It is of interest that infectious viral particles also associate with rafts upon binding to the cell surface and become enriched in these membrane lipid domains in conditions that allow viruses to penetrate the cells' interior.

FRIDAY
HANSA ROOM

8:25 a.m.

The central role of Duffy binding-like (DBL) domains in the interaction of malaria parasites with host receptors for invasion and cytoadherence

CHETAN E. CHITNIS, International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India

■ Erythrocyte invasion by *Plasmodium sp.* merozoites and cytoadherence of *P. falciparum*-infected erythrocytes to host capillaries are two important pathogenic mechanisms in malaria. A family of erythrocyte-binding proteins (EBPs), which includes *P. vivax* and *P. knowlesi* Duffy-binding proteins (PvDBP and PkDBP, respectively), mediates interaction with erythrocyte receptors during invasion. Variant antigens (also known as PfEMP-1) expressed on the surface of *P. falciparum*-infected erythrocytes bind to host endothelial receptors to mediate cytoadherence.

We have used transfection technology to disrupt the gene encoding PkDBP to study the protein's role in invasion. We demonstrate that the interaction of PkDBP with Duffy antigen mediates junction formation, a critical step during erythrocyte invasion. The receptor-binding domains of EBPs and PfEMP-1 map to conserved cysteine-rich domains that are referred to as Duffy binding-like (DBL) domains, after the first receptor-binding domains identified from PvDBP and PkDBP. DBL domains contain about 350 amino acids with 12–16 conserved cysteines. Using chimeric constructs and mild proteolysis in conjunction with functional binding assays, we provide evidence for a multidomain architecture for DBL domains from EBPs and PfEMP-1. The N-terminal region containing cysteines 1–4 of PvDBP and PkDBP forms a distinct subdomain that is nonfunctional. The region containing cysteines 5–12 of PvDBP and PkDBP forms another subdomain that is capable of receptor binding. Expression of various deletion constructs on the surface of COS cells followed by functional binding assays demonstrates that receptor-binding sites of DBL domains derived from EBPs and PfEMP-1 usually lie in the central region spanning the equivalent of cysteines 5–8 of PvDBP and PkDBP. Site-directed mutagenesis is being used to identify the receptor-binding residues within this region. Understanding the structure-function relation of the interaction of DBL domains with host receptors is key to the development of receptor-blocking strategies that inhibit invasion or reverse cytoadherence.

8:50 a.m.**The role of ligands in sialic acid–dependent and –independent invasion of *Plasmodium falciparum* into human erythrocytes****ALAN F. COWMAN**, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

T. Triglia, J. Stubbs, M.T. Duraisingh, A. Maier, J.K. Thompson, R.T. Good, J. Baum, J. Healer, A.F. Cowman ■ Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

■ The members of the phylum Apicomplexa parasitize a wide range of eukaryotic host cells. In contrast, *Plasmodium falciparum* invades human erythrocytes using several specific and high-affinity ligand–receptor interactions that define invasion pathways. This includes members of the Duffy-binding–like and the reticulocyte-binding–like (RBL or Rh) family of proteins. PfRh1, PfRh2a, PfRh2b, and PfRh4, members of the PfRh protein family, are variably expressed in different lines. In contrast, the erythrocyte-binding antigen-175 (EBA-175), a ligand that binds to glycophorin A in a sialic acid–dependent manner, is expressed in all parasite lines examined so far. Different parasite strains appear to be more reliant on specific ligands such that some are dependent on sialic acid for invasion of erythrocytes whereas others are sialic acid–independent. To understand the function of ligands in the pattern of receptor utilization in merozoite invasion of human erythrocytes, we have used a combination of targeted gene disruption and functional analysis. This has provided important insights into the functional relationships between the different ligands and their receptors.

9:15 a.m.**Chemically substituted RNA aptamers as tools for the development of novel therapeutic measures against trypanosome infections****H. ULRICH GÖRINGER**, Darmstadt University of Technology, Darmstadt, Germany

H.U. Göringer, M. Homann, A. Adler ■ Darmstadt University of Technology, Darmstadt, Germany

■ *Trypanosoma brucei* is an extracellular blood parasite, which causes African sleeping sickness in humans and Nagana in cattle. Sleeping sickness is fatal if untreated. However, the available drugs are highly toxic and difficult to administer. As a consequence, new experimental strategies for developing novel therapeutics are needed. Here we report the use of the SELEX (systematic evolution of ligands by exponential enrichment) technology to isolate so-called RNA aptamers that bind with high affinity to the surface of the parasite. One of the identified aptamers has been found to bind to a specific sub-structure of the parasite surface known as the flagellar pocket. The flagellar pocket represents the main endo- and exocytosis site of the organism. After binding, the RNAs become rapidly internalized, enter early and late endosomal compartments, and end up within the lysosome of the parasite. Binding of the RNA molecules is specific for the infective life cycle stage of the parasite because the aptamers do not recognize insect-stage trypanosomes. The RNAs fold into irregular stem/loop secondary structures with specific sequence motifs within single-stranded regions. They bind at a fast rate and with nanomolar affinity. The endocytotic uptake is sequence-specific and does not occur with randomized RNA sequences or significantly shortened aptamer fragments. Colocalization experiments with transferrin suggest a receptor-mediated uptake. The identified internalization and transport pathway was used to target aptamer-coupled compounds to the lysosome. We have now synthesized nontoxic components with membrane-disrupting properties as potential reagents, which become active only within the acidic environment of the lysosomal organelle. Responsive hydrophobically associating polymers and pH-responsive pseudo-peptides are two classes of molecules that possess the required characteristics. Representatives of the two groups are currently being tested for their trypanocidal activity when delivered as aptamer-coupled compounds.

**FRIDAY
HANSA ROOM**

10:10 a.m.

***Cryptococcus neoformans* var. *gattii*
in Australia: the natural ecology
of a pathogenic yeast**

DEIDRE A. CARTER, University of Sydney, Sydney, Australia

D.A. Carter, L.T. Campbell ■ University of Sydney, Sydney, Australia

■ Rising levels of immunosuppression worldwide have made fungal diseases increasingly prevalent. A particularly important fungal pathogen to emerge recently is *Cryptococcus neoformans*, a single cell yeast that can cause rapidly fatal meningitis. This species is split into two distinct varieties: *C. neoformans* var. *neoformans* (Cnn), which is cosmopolitan and very common in immunosuppressed patients, and *C. neoformans* var. *gattii* (Cng), which is more geographically restricted and predominantly affects immunocompetent hosts. In Australia, the incidence of Cng is relatively high due to an apparent association with certain native eucalyptus tree species. In addition, Cng can be cultivated readily from the environment, providing a unique opportunity to study the natural ecology of a fungal pathogen. Initially, it was thought that the eucalypt was the primary host of the yeast, with infective spores disseminating from the tree into the environment. An analysis of the distribution of molecular markers in two environmental populations found these, however, to be overwhelmingly clonal. This indicated that the yeast life cycle does not focus on the eucalypt, which may instead simply provide a favorable substrate on which it propagates asexually. An alternative environmental reservoir for Cng, in which it completes its life cycle, must therefore exist. The analysis was extended to clinical populations: two from humans located in regions where the normal host eucalypt species do not occur, and one from domestic animals whose geographic range was limited to the Sydney region. It was hypothesized that these infections resulted when infectious basidiospores, produced following sexual pairing on the natural environmental host, were inhaled. All three populations were again clonal, however. Furthermore, there did not appear to be any genetic differentiation between them, despite considerable geographic separation. Thus, it appears that Cng is an asexual fungus in the Australian environment and may have expanded its range rapidly in recent times.

**FRIDAY
HANSA ROOM**

10:35 a.m.

**Transmission of tuberculosis to household
contacts of pulmonary tuberculosis
patients in southern Mexico**

MA. DE LOURDES GARCÍA GARCÍA, National Institute of Public Health, Cuernavaca, Mexico

M.L. García García,¹ A. Ponce de León,² M.E. Jiménez Corona,¹ M.C. García Sancho,³ L. Ferreira Reyes,¹ M. Bobadilla del Valle,² P. Small,⁴ J. Sifuentes Osornio,² K. DeRiemer⁴ ■ ¹National Institute of Public Health, Cuernavaca, Mexico; ²Salvador Zubiran Institute of Nutrition and Institute of Biomedical Research, Mexico City, Mexico; ³National Institute of Respiratory Diseases, Mexico City, Mexico; ⁴Stanford University, Palo Alto, California, USA

■ The objective of our work is to determine factors associated with initial tuberculin reactivity and tuberculin conversion among household contacts of pulmonary tuberculosis patients. To this end, we identified, by sputum smear light microscopy and culture, patients in the Orizaba Health Jurisdiction in southeastern Mexico and enrolled them in a prospective molecular epidemiological study. Patients underwent clinical study (interview, physical exam, chest X-ray, anti-HIV antibodies) and mycobacteriologic evaluation (identification, drug-susceptibility testing, and IS6110-based genotyping). Contacts were evaluated for epidemiological and clinical characteristics and tuberculin reactivity. Every three months for up to two years, contacts with negative reactivity were monitored for conversion. Participants were referred for appropriate medical treatment to the national program of tuberculosis.

Between March 2001 and August 2003, we followed 1254 contacts of 353 patients. Tuberculin reactivity was 34 percent (426/1254); conversion was 12 percent (74/615). Paucibacillary tuberculosis (smear negative, culture positive), resistance to isoniazid and rifampin, sharing the bed with the patient, and patient noncompliance to treatment were significantly associated with tuberculin reactivity, after controlling for socioeconomic and household characteristics, age and sex of contact, and age of patient. The proportion of other contacts with tuberculin reactivity in the same household, more than one tuberculosis episode, severity of symptoms, and crowding were significantly associated with conversion. We found that the presence of drug-resistant *Mycobacterium tuberculosis* and smear positivity were associated with initial tuberculin reactivity but not with tuberculin conversion during follow-up. Inclusion of sociodemographic, clinical, and therapeutic variables did not modify the models.

This project received additional funding from NIH grants A135969 and K01-TW00001, the Wellcome Trust, and CONACyT grants G26264M and 30987-M.

11:00 a.m.

Human immunity to *Entamoeba histolytica* infection: evidence from a prospective study

RASHIDUL HAQUE, International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh

R. Haque,¹ W.A. Petri, Jr.² ■ ¹International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh; ²University of Virginia, Charlottesville, Virginia, USA

■ It was not known whether acquired immunity to *Entamoeba histolytica* infection exists. Many prior studies are impossible to interpret because *E. histolytica*-specific tests were not used. We are conducting a prospective cohort study in children in Mirpur, Dhaka, Bangladesh. In this study, we found that acquired immunity to *E. histolytica* infection was linked to intestinal IgA against the CRD of the Gal/GalNAc lectin of *E. histolytica*, and resistance to infection was seen despite the fact that children were infected with genetically diverse strains of *E. histolytica*. We are continuing to follow the children of this cohort to better understand the immunity to *E. histolytica* infection and disease. Here we report on the duration of immunity as documented by the stool anti-CRD IgA response and natural *E. histolytica* infection and on the role of MHC class II alleles in protection against *E. histolytica* infection.

During the four-year period follow-up, 80 percent (162/202) of the children had a total of 384 episodes of new asymptomatic *E. histolytica* infection, 20 percent (40/202) experienced *E. histolytica*-associated diarrhea, and 12 percent (24/202) had *E. histolytica*-associated dysentery. The duration of protection conferred by a positive stool anti-CRD and natural *E. histolytica* infection was measured using the Kaplan-Meier survival analysis. The median duration of protection after a positive stool anti-CRD IgA response was 744 days (95 percent CI, 586–902 days). The median duration of protection after the first *E. histolytica* infection was 616 days (95 percent CI, 490–741 days). We have also identified a potential protective association with the class II allele DQB1*601 and the DQB1*601-DRB1*1501 haplotype with *E. histolytica* infection among this cohort of children. Our results suggest that, along with mucosal anti-CRD IgA antibodies, human genes underlie susceptibility to intestinal infection with *E. histolytica*. The study has several implications for vaccine development to prevent amebic disease and infection.

11:25 a.m.

Analysis of the bystander adrenoceptor reactivity of anti-*Trypanosoma cruzi* antibodies leads to the characterization of a novel clinical symptom of Chagas disease

MARIANO JORGE LEVIN, Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina

V. Labovsky,¹ C. Schmulski,¹ V. Grippo,¹ G. Levy,¹ K. Gomez,¹ S. Matsumoto,² M.C. Guida,¹ M.C. Paveto,¹ M.J. Levin¹ ■ ¹Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina; ²Division of Ophthalmology, Alvarez Hospital, Buenos Aires, Argentina

■ Adrenergic and muscarinic cholinergic receptors function in the regulation of the cardiovascular system. They are bystander targets of anti-*Trypanosoma cruzi* antibodies in Chagas disease. In this regard, the best-studied of these antibodies are those reacting with the parasite's ribosomal P proteins. To date, bystander reactions have been characterized only at the pharmacological level. CHO and COS 7 cells stably transfected with human beta1- and beta2-adrenergic receptors provide a most valuable tool with which to demonstrate this immunological reaction. Moreover, crystallographic studies of complexes formed by parasite P antigens with the Fab of an anti-P Mab, and mutagenesis of Mab-derived and human recombinant anti-P antibodies, accompanied by the determination of their affinities for different targets, allowed us to model their binding to the beta1-adrenoceptor. Additional evidence of the binding of these antibodies to G protein-coupled receptors comes from studies of their binding to rhodopsin. The IgG fraction of patients with Chagas disease as well as poly- and monospecific antibodies against the P proteins of the parasite react with rhodopsin in Western blots of bovine rod outer segment (bROS) membranes. Furthermore, incubation of preparations of bROS membranes with the above-mentioned antibodies inhibited the light-induced conformational change of rhodopsin, causing a marked decrease in the membranes' cGMP-phosphodiesterase activity. Interestingly, complete electro-retinographic (ERG) and retinal fluorescein angiography studies in patients with Chagas disease showed a dissociated cone-rod ERG response, with reduction of the ERG b-wave amplitude or delayed latency under dark adaptation, and mild to moderate defects in retinal epithelium pigmentation, conditions compatible with a selective rod dysfunction in these individuals. This is the first observation of clinical retinal involvement in Chagas disease.

**FRIDAY
HANSA ROOM**

1:15 p.m.

Immunopathogenesis of mucosal leishmaniasis

EDGAR M. CARVALHO, Federal University of Bahia, Salvador, Bahia, Brazil

A.R. de Jesus, L.P. Carvalho, A. Schriefer, P.R.L. Machado, E.M. Carvalho ■ Federal University of Bahia, Salvador, Bahia, Brazil

■ While type 1 immune response is protective against leishmania infection, a Th2 response is associated with progression from infection to disease. IFN-gamma is the major cytokine that activates macrophages to kill leishmania, and an inability to produce IFN-gamma is associated with parasite dissemination. However, patients with cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML) have a strong type 1 immune response, but it does not prevent the development of disease. Our hypothesis is that in CL and, predominantly, ML there is an exaggerated inflammatory reaction with tissue damage. In the present study, the T cell responses in 30 CL and 30 ML patients were characterized in peripheral blood mononuclear cells (PBMC) and in tissue. ML patients produce higher amounts of IFN-gamma and TNF-alpha than CL patients as well as lower amounts of IL-10. This biased immune response is influenced by both parasite and host factors. *Leishmania braziliensis* isolates from ML differ genotypically from *L. braziliensis* isolates from CL, and antigen from isolates of ML patients induces more IFN-gamma and less IL-10 than isolates from CL patients. We have also observed an inability of IL-10 and the monoclonal antibodies anti IL-2 and anti IL-15 to down-regulate IFN-gamma production in cultures of PBMC of ML patients stimulated with leishmania antigen. Moreover, patients with ML produce more cells expressing CD69 and CD62L than do CL patients. These data indicate that in ML patients the frequency of activated T cells increases and that parasite antigens found in isolates of *L. braziliensis* that cause mucosal disease may induce the exaggerated T cell response. Found in high frequency in ML, these effector T cells are not down-modulated by IL-10, anti IL-2, or anti IL-15 but are responsible for the maintenance of the inflammatory response in these patients.

1:40 p.m.

Fas ligand regulation of *Leishmania* infection targets macrophage interactions with neutrophils

GEORGE A. DOSREIS, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

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■ Neutrophils (PMN) provide the first line of defense against infection but play a deleterious role in infection of susceptible hosts with *Leishmania major*. Given that FasL-deficient *gld* mice are more resistant to infection than wild-type (wt) BALB mice, Fas ligand (FasL) exacerbates *L. major* infection. *L. major* promastigotes induce FasL-dependent apoptosis in resident macrophages in vitro and in vivo, leading to FasL-dependent chemokine secretion and neutrophil (PMN) extravasation. Dead exudate PMN from both wt and *gld* mice, as well as live PMN from wt mice, exacerbate *L. major* replication in syngeneic macrophages. However, live PMN from *gld* mice failed to exacerbate and even killed intracellular *Leishmania* in macrophages. Given that anti-FasL delays apoptosis of wt PMN and blocks their exacerbating effect on parasite growth, protective effects of *gld* PMN are due to delayed apoptosis. Local adoptive transfer of PMN exacerbates infection in wt, but not in *gld* mice. Systemic depletion of PMN abolishes the increased susceptibility of wt mice, compared with *gld* controls. These results indicate that FasL exacerbates *L. major* infection by targeting PMN at two steps. First, FasL attracts PMN by promoting early macrophage apoptosis. Second, FasL also promotes rapid PMN apoptosis, thereby ensuring anti-inflammatory clearance by macrophages and increased parasite replication. FasL-mediated resident macrophage apoptosis could represent a general mechanism of "danger signals" that alert the immune system to the presence of invading pathogens.

FRIDAY
 HANSA ROOM

2:05 p.m.

Targeting the neurotoxic species in Alzheimer's disease: inhibitors of the formation of A-beta soluble oligomers and fibrils

SÉRGIO T. FERREIRA, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

EG. De Felice,¹ M.N.N. Vieira,¹ L.M. Saraiva,¹ R. Goecking,¹ A.P. Wasilewska-Sampaio,¹ J. Garcia-Abreu,¹ J.D. Figueroa-Villar,² R. Liu,³ L. Chang,³ W.L. Klein,³ S.T. Ferreira¹ ■ ¹Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; ²Military Institute of Engineering, Rio de Janeiro, Brazil; ³Cognitive Neurology and Alzheimer's Disease Center, Northwestern University Institute for Neuroscience, Evanston, Illinois, USA

■ In the past two decades, a large body of evidence has established a causative role for the beta-amyloid peptide (A-beta) in Alzheimer's disease (AD). However, the recent debate has focused on whether amyloid fibrils or soluble oligomers of A-beta are the main neurotoxic species that contribute to neurodegeneration and dementia. While this crucial aspect of the pathogenesis of AD remains controversial, effective therapeutic strategies should ideally target both oligomeric and fibrillar species of A-beta. Here we describe the anti-amyloidogenic and neuroprotective actions of a number of di- and trisubstituted aromatic compounds. Thioflavin T fluorescence measurements and transmission electron microscopy were initially used to screen for inhibitors of amyloid fibril formation in vitro. Inhibition of the formation of soluble A-beta oligomers was also monitored using a specific antibody-based assay that discriminates between A-beta oligomers and monomers. Taken together, these results led to the identification of compounds that effectively block the formation of both A-beta fibrils and soluble oligomers. Significantly, such compounds blocked the neurotoxicity of A-beta to rat hippocampal neurons. Of considerable interest is the observation that some of the compounds identified as A-beta blockers also exhibit neurite outgrowth-promoting actions in cortical and hippocampal neurons and induce differentiation in neuronal cell lines. These findings provide a basis for the development of novel small molecule A-beta inhibitors with potential applications in AD.

2:30 p.m.

Characterization of loci contributing to host response to *Leishmania major* in the mouse

SIMON FOOTE, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

S. Foote, E. Handman, A. Sakthianandeswaren, C. Elso ■ Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

■ We have mapped three loci contributing to the host response to infection by *Leishmania major* in the mouse. These loci (*lmm1*, -2, and -3) are located on chromosomes 17, 9, and X, respectively, and control the rate of skin lesion development. We have generated animals reciprocally congenic for these loci both individually and collectively. These animals show altered rates of progression of *L. major*-induced lesions. There is no immunological correlate between the phenotype displayed by these animals and any of the classical cytokines involved in the T helper T cell response. Microarray analysis of macrophages (the target cell of the parasite) showed a difference in the expression of genes controlling wound healing. We have therefore looked more closely at the lesions and found major differences in the histological structure of the healing lesion in animals infected with *L. major* or in animals given a sterile lesion. In animals in which the lesion healed rapidly, collagen bundles are well ordered and constitute the major component of the healing wound. In susceptible animals, there is less collagen deposition and the pattern of deposition is much less ordered, almost random. We therefore interpret these findings as evidence that, in this murine model of *L. major* susceptibility, the major determinant of outcome is the ability of the host to heal the lesion.

FRIDAY
HANSA ROOM

2:55 p.m.

The LPS-induced inflammatory response requires HIAP1 function in macrophages**ROBERT G. KORNEŁUK**, University of Ottawa and Apoptosis Research Center, Children's Hospital of Eastern Ontario, Ottawa, Canada

■ Activation of the nuclear factor (NF)-kappaB rapidly induces the up-regulation of inflammatory and anti-apoptotic (survival) genes. One such key survival factor is the Human Inhibitor of Apoptosis (HIAP)1 protein (also called cIAP2). We first identified HIAP1 as a member of the evolutionarily conserved IAP family of proteins that have emerged as critical cellular repressors of programmed cell death. HIAP1 has been shown to be an important component of the TNF receptor 1 and 2 (TNF-R1, TNF-R2) complex and, therefore, a constituent of TNF-alpha signaling pathways. Significantly, agents that activate NF-kappaB, such as TNF-alpha, interleukin (IL)-1-beta and bacterial lipopolysaccharide (LPS) are known to potently elicit the transcriptional up-regulation of the HIAP1 gene via NF-kappaB-responsive sites in its promoter. Moreover, NF-kappaB activation imparts increased apoptosis resistance to the target cell.

To examine the function of HIAP1, we disrupted the homologous murine gene (MIAP1) by directed recombination in embryonic stem cells. MIAP1-deficient mice showed no overt phenotype, were fertile, followed Mendelian frequency of inheritance, and appeared healthy throughout their life span. Whole mouse and organ weights, as well as primary and secondary lymphoid compartment cell numbers for MIAP1-deficient and control littermates, displayed no significant differences. However, we found that the MIAP1 null mice exhibit profound resistance to LPS-induced sepsis, specifically via an attenuated inflammatory response. We demonstrated that the main reason for this unexpected phenotype is that MIAP1 null macrophages have an enhanced susceptibility to LPS toxicity. Because of the absence of MIAP1, the macrophages are unable to survive an LPS-induced pro-inflammatory environment and hence do not maintain a normal, sustained inflammatory response. These results suggest that an approach to the treatment of septic shock in patients would be to antagonize HIAP1 expression and/or function.

Poster 2**Structure-function relationships and protein engineering of dehalogenating enzymes****Jiří Damborský**,* Masaryk University, Brno, Czech Republic

J. Damborský,¹ A. Jesenská,¹ Z. Prokop,¹ J. Kmuniček,¹ M. Boháč,¹ R. Chaloupková,¹ M. Pavlová,¹ M. Monincová,¹ I. Těšínská,¹ M. Klvaňa,¹ P. Jeřábek,¹ M. Otyepka,² P. Banáš,² Y. Nagata,³ Y. Sato,³ A. Oakley⁴ ■ ¹Masaryk University, Brno, Czech Republic; ²Palacký University, Olomouc, Czech Republic; ³Tohoku University, Sendai, Japan; ⁴Australian National University, Canberra, Australia

■ The focus of protein engineering is the analysis and design of novel proteins for fundamental, medically related, and biotechnology research. We attempt to engineer the enzymes known as haloalkane dehalogenases. Haloalkane dehalogenases are bacterial enzymes that, by a hydrolytic mechanism, cleave the carbon-halogen bond of halogenated aliphatic compounds. The enzymes have become an important model system for investigating fundamental principles of enzymatic catalysis, but also have potential use in detoxification of subsurface pollutants, recovery of industrial side products, and biosensing. Modification of the substrate specificity and activity of the enzymes is required for optimization of their catalytic properties. Our project focuses on molecular mechanisms of enzymatic catalysis, structure-function relationships, and engineering of haloalkane the mutants structurally and functionally. The project will lead to the development of new theoretical approaches for computer-assisted protein design and construction of biocatalysts with modified catalytic properties.

*EMBO/HHMI Award recipient

FRIDAY
HANSA ROOM

Poster 4

Helicase loading factors: a multifunctional plasmid-encoded replication initiation protein recruits and positions helicase at the replication origin

IGOR KONIECZNY,* University of Gdansk, Gdansk, Poland

M. Pacek,¹ L. Kowalczyk,¹ K. Herman,¹ D.R. Helinski,² Y. Jiang,² A. Toukdarian,² I. Konieczny¹ ■ ¹University of Gdansk, Gdansk, Poland; ²University of California, San Diego, La Jolla, California, USA

■ The DnaA replication initiation protein has been shown to be essential for DNA strand opening at the AT-rich region of the replication origin of the *Escherichia coli* chromosome as well as serve to recruit and position the DnaB replicative helicase at this open region. In addition to DnaA, the helicase accessory ATPase protein DnaC is also required for helicase loading at *E. coli oriC*. Homologues of the *dnaA* gene of *E. coli* have been found in most bacterial species, and the DnaA protein has been shown to be required for the initiation of replication of both chromosomal and plasmid DNA. For several plasmid elements, it has been found that a plasmid-encoded initiation protein is required along with the DnaA to bring about opening of the AT-rich region at the replication origin. The broad host-range plasmid RK2 encodes two forms of its replication initiation protein (TrfA-33 and TrfA-44) that differ by an additional 98 amino acids at the N-terminus of the larger (TrfA-44) form. Both forms initiate replication of RK2 in *E. coli* in vitro by a DnaA-dependent mechanism. However, TrfA-44 specifically interacts with the DnaB replicative helicase of *Pseudomonas putida* and *Pseudomonas aeruginosa* and initiates the formation of a pre-priming open complex in the absence of DnaA and DnaC proteins. Thus, the TrfA-44 initiation protein has the multifunctional properties of recruiting and positioning an active form of the DnaB helicase at the RK2 replication origin by a DnaA- and DnaC-independent process. This property, which is unique for a replication initiation protein, undoubtedly plays an important role in extending the host range of the RK2 antibiotic resistance plasmid.

*EMBO/HHMI Award recipient

Poster 6

Functional heterogeneity of loci controlling the response to *Leishmania major* in mice

MARIE LIPOLDOVÁ, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

H. Havelková,¹ J. Badalová,¹ M. Svobodová,² J. Vojtíšková,¹ V. Vladimirov,^{1,5} P. Demant,^{3,4} I. Kurey,^{1,2} M. Lipoldová¹ ■ ¹Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic; ²Charles University, Prague, Czech Republic; ³The Netherlands Cancer Institute, Amsterdam, The Netherlands; ⁴Roswell Park Cancer Institute, Buffalo, New York, USA; ⁵Medical College of Virginia, Richmond, Virginia, USA

■ *Leishmania major* is a protozoan parasite that infects mononuclear phagocytes of vertebrate hosts. Symptoms of disease range from none or only transient pathological changes to extensive visceral involvement with spleno- and hepatomegaly, anemia, hyperglobulinemia, and skin lesions. Progression or resolution of the disease has been assumed to be associated with CD4⁺ lymphocyte Th2 or Th1 response, respectively. Genetic analysis of susceptibility in most cases has been restricted to genes controlling development of skin lesions, under the assumption that *L. major*-induced skin and visceral pathologies are correlated, both with each other and with the Th2 response, and that they are all controlled by the same genes.

In addition to skin lesions, we investigated hepatomegaly, splenomegaly, levels of IgE, IL-4, IL-12, TNFalpha, and IFNgamma in serum, and spontaneous proliferation of lymphocytes from infected mice. Using recombinant congenic strains CcS-5, -16, and -20, derived from the susceptible strain BALB/c and the resistant strain STS, we scanned approximately 35 percent of the mouse genome and mapped 16 novel *Leishmania major* response genes, *Lmr 3–18*. We found that functionally these genes are highly heterogeneous. Each is associated with a different combination of pathological symptoms and immunological reactions, the latter being frequently influenced by gene interactions. Seven *Lmr* loci control skin lesions and/or visceral pathology. *Lmr13* and *Lmr4* determine skin lesions only; *Lmr3*, *Lmr5*, and *Lmr10* control skin lesions and splenomegaly; *Lmr3* and *Lmr14* control splenomegaly and hepatomegaly (but not skin lesions); and *Lmr15* determines hepatomegaly only. Only two immunological parameters correlated with pathology: six out of seven “pathology” loci also controlled the IgE levels in serum, and all loci controlling hepatomegaly also influenced the IFNgamma level in serum. Our findings suggest a novel paradigm for the genetics of resistance to infections, involving numerous loci, exhibiting different spectra of organ-specific and systemic (immunological) effects.

FRIDAY
HANSA ROOM

Poster 8

Protein trafficking in *Giardia lamblia*

HUGO D. LUJAN, National University of Córdoba, Córdoba, Argentina

N. Gottig, M.C. Touz, H.D. Lujan ■ National University of Córdoba, Córdoba, Argentina

■ *Giardia* is a protozoan parasite of humans and a common cause of diarrheal disease worldwide. Besides its medical importance, *Giardia* is an excellent model for the study of the evolution of several cellular processes, given that it belongs to the earliest branches of the eukaryotic line of descent. To survive outside the host's intestine, *Giardia* undergoes adaptation by differentiating into an infective cyst; cysts are released with the feces and are responsible for the transmission of the disease among susceptible hosts. The encystation process includes the synthesis of cyst wall components and the biogenesis of secretory organelles not present in non-encysting cells, such as the Golgi apparatus and encystation-specific secretory vesicles (ESVs), which are required for the transport of cyst wall constituents to the cell surface for later release and assembly in the cyst wall. Among the components of the wall are two closely related leucine-rich proteins (CWP1 and CWP2), which we previously identified and characterized. Using biochemical, immunological, and molecular biological approaches, we recently found that the alkaline C-terminal extension of CWP2 is involved in ESV formation, and we identified two proteolytic enzymes necessary for both sensing the stimulus for encystation (a membrane-associated dipeptidyl peptidase IV) and processing the basic tail of CWP2 before incorporation into the cyst wall (encystation-specific cysteine protease). Moreover, a granule-specific calcium-binding protein (GSP) was isolated and characterized. GSP expression knockdown demonstrated that this protein is essential for exocytosis of ESVs during cyst wall formation. We also developed a series of monoclonal antibodies against different regions of *Giardia* CWPs. Currently, these antibodies are being used to study how cyst wall components are assembled into the extracellular structure that protects the parasite outside the intestine of its host.

FRIDAY
HANSA ROOM

Poster 10

Enzymatic mechanism of actinomyosin

ANDRÁS MÁLNÁSI CSIZMADIA,* Eötvös Loránd University, Budapest, Hungary

■ When myosin converts chemical energy to mechanical work, it undergoes a large conformational change during the ATPase cycle that results in stepwise movement along the actin filament. The main question is how the functional regions communicate with each other. Another problem concerns the development and relaxation of mechanical strains during the catalytic cycle and their perturbation by external force. Using various single tryptophan and cysteine mutants, we have extensively characterized the energetic coupling between nucleotide binding, the so-called open/closed transition, and actin binding. By holding the conformation in a certain state, we bias the coupling of the conformational changes of the functional regions, thus allowing an assessment of the energetics of the whole cycle. We use active and passive molecular springs created between different subdomains by cross-linking the introduced double cysteine. We use vertical atomic force microscopy to characterize applied forces in the following experimental design: 1) holding the lever arm in a fixed conformation by so-called molecular springs; 2) incorporating a double cysteine in the actin binding cleft and lever arm region by mutagenesis and modifying with cross-linkers of different length; and 3) applying laser flash-directed fast strain perturbation to myosin using photoactivable azobenzene cross-linkers. Relaxation of this perturbation gives important information about thermodynamics of the cycle and may reveal the existence and function of the distribution of the mechanical strains.

*EMBO/HHMI Award recipient

Poster 12

Regulation of *msx* by a BMP gradient is essential for neural crest specification

ROBERTO MAYOR, University of Chile, Santiago, Chile

C. Tríbulo, M. Aybar, A. Glavic, C. Araya, J. De Calisto, R. Mayor ■ University of Chile, Santiago, Chile

■ The neural crest originates at the border between the neural plate and the future epidermis. It gives rise to numerous and diverse cell types, including the peripheral nervous system, the cranofacial skeleton, and pigment cells. Although considerable progress has been made recently in the molecular characterization of neural crest-inducing factors, relatively little is known about the genetic cascade of transcription factors that determine the specification of the neural crest at the neural plate border.

There is evidence in *Xenopus* and zebrafish embryos that the neural crest is specified at the border of the neural plate by a precise threshold concentration of a BMP gradient. To understand the molecular mechanism by which a gradient of BMP is able to specify the neural crest, we analyzed how the expression of the BMP target, *msx*, is regulated and the role that *msx* plays on neural crest specification.

In *Xenopus* and zebrafish embryos, we analyzed *msx* expression after experimental modification of the level of BMP activity by three different methods. All the results show that a reduction in the level of BMP activity leads to an increase in the expression of *msx* in the neural plate border. Interestingly, we show that there is a specific concentration of BMP that induces *msx1* expression.

In addition, we have analyzed the role that *msx1* plays in neural crest specification. We show that *msx1* expression is necessary and sufficient for early neural crest specification. We propose a model whereby a gradient of BMP activity specifies the expression of *msx* in the neural folds, and that this expression is essential for the early specification of the neural crest.

Poster 14

Identification of new metabolic pathways in parasitic protozoa

MALCOLM J. MCCONVILLE, University of Melbourne, Melbourne, Australia

M.J. McConville, J.E. Ralton, T. Naderer, H. Piraino, F. Sernee ■ University of Melbourne, Melbourne, Australia

■ Parasitic protozoa are the cause of a number of important human diseases, including malaria, African sleeping sickness, Chagas disease, and leishmaniasis. To date, there are no well-defined subunit vaccines against any of these diseases, and existing drug therapies suffer from low efficacy, high toxicity, expense, and/or widespread drug resistance. To identify new drug targets, there is a need to identify parasite metabolic pathways that are important for the virulence and survival of these pathogens in the mammalian host. With the sequencing of several parasite genomes, multiparallel analysis of mRNA (DNA arrays) and proteins (proteomics) has been used to model metabolism in some parasitic protozoa. However, these analyses are limited by the finding that a major fraction of all the predicted open reading frames (ORFs) in the parasite genomes have no assigned function and the difficulty of predicting how changes in mRNA or protein are translated into changes in biological function. To identify developmentally regulated and/or novel metabolic pathways, we undertook an unbiased analysis of all primary and secondary metabolites in different developmental stages of *Leishmania* (the model organism for these studies). Metabolites were sequentially extracted in aqueous-organic solvent mixtures and then analyzed by gas chromatography (GC)/liquid chromatography (LC)/mass spectrometry. These metabolomic analyses revealed 1) that several metabolic pathways are highly up-regulated when leishmanial insect developmental stages invade mammalian host cells and 2) the presence of new or previously overlooked metabolic pathways. This approach led to the discovery of a novel family of intracellular oligosaccharides that accumulate in the intracellular amastigote stage and appear to be part of the parasite stress response. The pathway leading to the biosynthesis of these oligosaccharides has been partially characterized and may be a target for new anti-parasite drugs.

FRIDAY
HANSA ROOM

Poster 16

Conditional mutagenesis using site-specific recombination in *Plasmodium berghei*

ROBERT MÉNARD, Pasteur Institute, Paris, France

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■ Despite the development of gene-targeting technology in *Plasmodium*, the parasite that causes malaria, there are still major limitations to reverse genetics in this haploid protozoan. The only stage of the parasite that can be genetically transformed is the stage that multiplies inside red blood cells (RBC). Therefore, the function of the proteins that play a role in parasite invasion and multiplication in RBC cannot be characterized using molecular genetic tools, because corresponding loss-of-function mutants are lethal. Another limitation of the gene-targeting technology in *Plasmodium* is that constitutive inactivation of genes that perform multiple functions during the parasite life only reveals their earlier role in the cycle.

Here, we describe conditional mutagenesis in *Plasmodium berghei*, a plasmodial species that infects rodents, which can be cycled routinely through mosquitoes, and whose genome can be manipulated easily by homologous recombination. Conditional mutagenesis is triggered by site-specific recombination (SSR) using the *Flp/FRT* system of yeast. By homologous recombination, a target DNA is flanked by *FRT* sequences (flirted) in RBC stages and deleted in mosquito stages of the parasite upon expression of the Flp recombinase. Deletants are generated by parasite fertilization between two clones, having either the flirted target or the *Flp* gene under the control of a stage-specific promoter. The deletants are recognized in the cross progeny by linking the SSR event to fluorescence expression. This approach should permit one to address the function of essential genes of the *Plasmodium* parasite at any time of its life cycle.

Poster 18

Trans-splicing and protein translocation in trypanosomes

SHULAMIT MICHAELI, Bar-Ilan University, Ramat-Gan, Israel

S. Michael, X-H. Liang, M. Mandelboim, S. Barth, M. Biton, A. Hury, Q. Liu, S. Uliel, Y. Lustig, L. Shainer, A. Eitam, Y. Vagima ■ Bar-Ilan University, Ramat-Gan, Israel

■ Our research aims to understand the mechanism and machinery of *trans*-splicing and protein translocation mediated by the signal recognition particle in trypanosomes. Having elucidated many of the SL RNA–snRNA interactions during *trans*-splicing, we now focus our research on RNA-binding proteins and splicing factors and their role in *trans*- and *cis*-splicing, with the goal of identifying *trans*-splicing-specific factors. Silencing by RNAi of Sm proteins that bind to the sn and SL RNA demonstrates that these proteins are essential for both splicing reactions and for the stability of the U snRNAs. Surprisingly, SL RNA that accumulated in the cytoplasm during Sm silencing lacked the fourth unique cap nt. Our data suggest that SL RNA biogenesis involves a cytoplasmic phase and that the unique cap nts may have a role in export and import of the SL RNA during this complicated pathway. Interestingly, the SL RNA that accumulated in the Sm-silenced cells carried the unique pseudouridine at position –12. By silencing the SLA1 using snoRNAi, an RNAi-related mechanism that we recently discovered, we showed that the trypanosome-specific small RNA SLA1 guides this modification. We are currently investigating the role of this modification for the function of the SL RNA. To further understand how the SL RNP is brought to the spliceosome, we tagged *in vivo* splicing factors and silenced numerous splicing factors. Our results suggest that the SL RNP is brought to the spliceosome via its association with the hexameric snRNP complex.

In the protein translocation project, we demonstrated by purifying the SRP to homogeneity that the trypanosome SRP complex is the first eukaryotic complex that lacks the Alu-domain binding proteins. Silencing the chaperone and the SRP pathways suggests that membrane protein translocation is dependent on the SRP pathway, whereas signal peptide-containing proteins can transverse the ER membrane also by the chaperone pathway.

FRIDAY
HANSA ROOM

Poster 20

The individual and collective roles of Y family DNA polymerases in genetic adaptation and long-term survival of mycobacteria

VALERIE MIZRAHI, University of the Witwatersrand and the National Health Laboratory Service, Johannesburg, South Africa

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■ The alarming increase in multidrug resistance in *Mycobacterium tuberculosis* is attributable to the evolution and global spread of strains that carry multiple chromosomal mutations. As such, there is considerable interest in defining the mutational mechanisms that operate in mycobacteria. We recently found that induced (SOS) base substitution mutagenesis in mycobacteria is mediated by DnaE2, a novel, damage-inducible C-family DNA polymerase. DnaE2 is the first example of a growing number of DnaE-type polymerases from gram-positive bacteria that catalyze error-prone repair synthesis. The central role of DnaE2 in damage tolerance in mycobacteria is particularly surprising in light of the presence in mycobacteria of multiple *umuC*-like genes encoding members of the Y-family of low-fidelity DNA polymerases, which are responsible for SOS mutagenesis in other bacteria. We are adopting an integrated genetic, biochemical, and physiological approach to investigate the individual and collective roles of the two Y-family polymerases of *M. tuberculosis* and the three of *M. smegmatis* in genetic adaptation and long-term survival in these organisms. To this end, a large panel of mutant strains with altered expression of the Y-family polymerase-encoding genes has been constructed, which includes a mutant of *M. smegmatis* that lacks all three *umuC*-like genes. To determine the effects of altered levels of Y-family polymerases on mutagenesis in *M. smegmatis* and *M. tuberculosis*, we are measuring mutation rates by fluctuation analysis and elucidating the mutational spectra in various genetic backgrounds using endogenous or specifically engineered chromosomal mutational targets for monitoring base substitutions (*rpoB*, *hisD*), frameshift mutations (*hisD*), and mutations at GC-rich (PE-PGRS) repeat loci. This mutational approach is being supplemented by biochemical studies aimed at characterizing the DNA polymerase activity of a recombinant form of the *M. tuberculosis* DinX protein and by expression studies aimed at elucidating the transcriptional regulation of these genes.

Poster 22

COP1 and multiple photoreceptors control degradation of the negative regulator PIF3, a transcription factor required for light signaling in *Arabidopsis*

FERENC NAGY, Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

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■ In a quality- and quantity-dependent fashion, light induces nuclear import of the plant photoreceptors phytochromes; promotes interaction of phyA and phyB with transcription factors, including PIF3; and is thought to trigger a transcriptional cascade to regulate the expression of about 2500 genes in *Arabidopsis*. Here we show that controlled degradation of the transcription factor PIF3 is a major regulatory step in light signaling. On the one hand, we demonstrate that accumulation of PIF3 in the nucleus in the dark requires COP1, a negative regulator of photomorphogenesis. Furthermore, we show that red and far-red light induce rapid degradation of the PIF3 protein. This process is controlled by the concerted action of the red/far-red-absorbing phyA, phyB, and phyD photoreceptors and is not affected by COP1. In addition, we provide evidence that the *poc1* mutant, a postulated PIF3 overexpressor that displays hypersensitivity to red light, lacks detectable amounts of the PIF3 protein. Taken together, our results show that PIF3, a postulated positive regulator of photomorphogenesis, acts negatively; its most likely function is to mediate phytochrome-controlled signaling during dark-to-light transitions.

FRIDAY
HANSA ROOM

Poster 24

Distinct role of TNF produced by different cell types in infectious disease and experimental hepatitis

SERGEI A. NEDOSPASOV, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

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■ To predict the outcome of continuous TNF blockage in human patients, an analysis of the physiological consequences of partial or complete TNF ablation is needed. At the previous meetings, we reported on generation and characterization of mice with TNF ablation in distinct types of cells, such as macrophages/neutrophils (M-TNF mice), T cells (T-TNF mice), and B cells (B-TNF mice). We have now evaluated the mice in three pathophysiological models; in two of these, TNF plays a deleterious role, and in the third one, a protective role. M-TNF mice are uniquely protected against acute LPS-D-Gal toxicity, consistent with the fact that only these mice lack detectable serum TNF after LPS injection. However, in ConA-induced hepatitis, both M-TNF and T-TNF mice, but not B-TNF mice, show significant protection. In a *Listeria monocytogenes* infection model, both M-TNF and T-TNF mice are susceptible and show increased bacterial loads in organs, whereas with the same parasite doses, both B-TNF and wild-type mice can effectively control the infection. These findings suggest distinct roles for macrophage-derived and T cell-derived TNF: both can be protective or deleterious, depending on the physiological conditions that trigger TNF production.

The work in Frederick was supported in part by NCI contract NO1-CO-12400.

FRIDAY
HANSA ROOM

Poster 26

Persistently active cannabinoid receptors mute a subpopulation of hippocampal interneurons

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■ Cortical information processing requires an orchestrated interaction between a large number of pyramidal cells and albeit fewer, but highly diverse, GABAergic interneurons. The impact of these nerve cells on their postsynaptic target cells is determined by a variety of factors, including the number of synapses on the postsynaptic cell, quantal size, probability of transmitter release, and short- or long-term synaptic plasticity. Many of these factors are highly variable at hippocampal glutamatergic synapses, despite the apparent uniformity of pyramidal cells. In contrast, at GABAergic synapses formed by the extremely diverse interneurons, many of these synaptic parameters seem to be alike. For example, most hippocampal interneurons elicit postsynaptic responses that are highly reliable and of large amplitude. However, it has been suggested that variability of these synaptic properties is beneficial for stabilizing the network. Furthermore, interneurons also convey an autonomic, motivational, and emotional impact on hippocampal information processing, and this type of inhibition is thought to be easily modifiable. In the present study, we tested whether all GABAergic synaptic connections in the rat hippocampus were indeed highly reliable, with apparent lack of transmission failures, or whether the output of an interneuron could be dramatically altered, implying a switch in their contribution to network behaviors. We found that persistently active cannabinoid receptors, the site of action of endocannabinoids and the psychostimulants marijuana and hashish, switch off the output (mute) of a unique class of hippocampal interneurons. In paired recordings between cholecystokinin-immunopositive, mossy fiber-associated interneurons and their target CA3 pyramidal cells, no postsynaptic currents could be evoked with single presynaptic action potentials or with repetitive stimulations at frequencies less than 25 Hz. Cannabinoid receptor antagonists converted these “mute” synapses into high-fidelity ones. The selective muting of specific interneurons, achieved by persistent presynaptic cannabinoid receptor activation, provides a state-dependent switch in cortical networks.

Poster 28

The DCX domains of doublecortin and their interactions with microtubules and tubulin

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■ Human doublecortin is a 40 kDa protein required for normal migration of neurons and formation of the cerebral cortex. Mutations in the doublecortin gene, which is localized on chromosome X, result in double cortex in females and lissencephaly (“smooth brain”) in males, leading to epilepsy and mental retardation. Human doublecortin consists of two homologous domains connected by a flexible linker and contains a S/P rich C-terminal region. Three different fragments, the N-terminal doublecortin domain (N-DCX, 45–150), the C-terminal domain (C-DCX, 170–275), and tandem (tandem-DCX, 45–275) were overexpressed in *Escherichia coli* and analyzed by NMR spectroscopy. We were able to solve the structure of N-DCX using ¹⁵N,¹³C heteronuclear NMR experiments. It consists of a beta-beta-alpha-beta-beta motif arranged in a beta-grasp fold similar to GTPase-binding domains and to other members of the ubiquitin superfamily. However, the beta-grasp motif was not found in other microtubule-associated proteins, which suggests that doublecortin interacts with tubulin in a novel way. We also present evidence that the C-DCX domain of doublecortin is partially folded and that the destabilization is due to a conserved pattern of substitutions, preserved in other DCX tandems. A study of the C-terminal domain DCX revealed its low stability and high tendency to aggregate, precluding structural analysis. Furthermore, there are no interactions between two DCX domains in solution. In vivo, doublecortin stabilizes microtubules and causes bundling. Interactions between three different doublecortin fragments and tubulin/microtubules were probed by light scattering assays and a series of ¹H-¹⁵N HSQC spectra. Our data, together with knowledge of mutations in patients, allowed us to propose a mechanism of microtubule stabilization by DCX.

Poster 30

Ammonia signaling and long-term development of *Saccharomyces cerevisiae* colonies

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■ In laboratories, microorganisms are traditionally studied as the sum of individuals often growing in liquid media. In nature, by contrast, microorganisms predominantly exist within multicellular communities (colonies, biofilms), where they are better protected against various deleterious environmental conditions (Palkova and Vachova, *Int. Rev. Cytol.* 225:229–272, 2003). Previous studies showed that colonies of yeast periodically change the pH of their surroundings from acidic to alkali. In the acidic phase, colonies grow, whereas in the alkali phase, they produce ammonia and their growth is transiently inhibited (Palkova et al., *Nature* 390:532–536, 1997; Palkova and Forstova, *J. Cell. Sci.* 113:1923–1928, 2000). Genome-wide analyses of gene expression changes occurring within *Saccharomyces cerevisiae* colonies during their acidic-to-alkali transition allowed us to propose a model of metabolic changes occurring within yeast colonies (Palkova et al., *Mol. Biol. Cell.* 13:3901–3914, 2002). The transition is linked with changes in amino acid metabolism, a progressive decrease in mitochondrial oxidative phosphorylation, switching from the citrate cycle to the glyoxylate bypass, and peroxisome activation. A parallel decrease in general stress proteins indicates an active adaptation of “alkali” colonies to stress conditions of the acidic phase. This adaptation appears to be important for long-term colony survival. Ammonia may function as a long-distance signal alarm of forthcoming unfavorable conditions. The Ato proteins, putatively involved in ammonia production in yeast colonies, and the transcription factor Sok2p, an important regulator affecting long-term colony development, were identified. Cells of *sok2* colonies do not produce ammonia, they are more fragile than cells of *wt* colonies, and they exhibit a defect in survival. *sok2* colonies are not able to effectively switch on genes of adaptive metabolisms, and they have unbalanced expression and activity of various enzymes involved in cell protection against oxidative damage. Impaired amino acid metabolism and insufficient activation of genes for putative ammonium exporter such as Ato may be responsible for the observed defects in ammonia production.

*EMBO/HHMI Award recipient

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Poster 32

Molecular and behavioral analysis of circadian rhythms in sandflies and mosquitoes

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■ Sandflies (Diptera: Psychodidae) are vectors of leishmaniasis, a group of related parasitic diseases believed to affect around 12 million people worldwide, whereas mosquitoes (Diptera: Culicidae) are vectors of a number of diseases such as dengue fever and malaria, which kill more than a million people every year. The daily rhythms of activity and feeding of these blood-sucking insects are very important to disease transmission. The molecular basis of insect circadian rhythms has been studied in detail in the model species *Drosophila melanogaster*. A number of genes have been identified, and the four most important (*period*, *timeless*, *Clock*, and *cycle*) are involved in a negative-feedback transcriptional/translational loop that is central to pacemaker function. *Clock* and *cycle* encode PAS-bHLH transcriptional factors that activate the cyclic expression of *period* and *timeless*, which in turn encode proteins that enter the nucleus of pacemaker cells and repress their own transcription by interaction with CLOCK-CYCLE. We cloned fragments of these four genes from sand flies and mosquitoes and are currently studying their circadian regulation. We are also studying the activity rhythms of these vectors using the same methodology normally applied to *Drosophila*. We believe that this comparative molecular and behavioral analysis of circadian rhythms will provide insights into the evolution of the circadian clock in hematophagous insects.

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Poster 34

Structure and dynamics in solution of the complex of human dihydrofolate reductase with trimethoprim and NADPH

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■ Human dihydrofolate reductase is a 21.3 kDa (186 amino acid) enzyme that catalyzes the NADPH-dependent reduction of folate and 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate (H₄Fol). Since H₄Fol is an important cofactor in the biosynthesis of purines and amino acids, DHFR has proved to be an excellent target for antifolate drugs that act by inhibiting the enzyme in parasitic or malignant cells. The antibacterial effectiveness of one of such drug, trimethoprim, is due to its significant increased binding to the bacterial enzyme compared with binding to the vertebrate form. This specificity of TMP binding is mainly driven by the strong positive cooperative binding effect between inhibitor (trimethoprim) and the cofactor (NADPH) when they bind to bacterial DHFR. Only a small cooperative binding effect is observed when these ligands bind to the human enzyme. Information on the structure and dynamics of complexes of *Lactobacillus casei* DHFR with TMP and NADPH was obtained earlier, and in order to explore the origins of the specificity and cooperativity of binding of these two ligands to the bacterial enzyme, similar information must be obtained for the complexes formed with human DHFR.

Essentially complete ¹H, ¹⁵N, and ¹³C resonance assignments were obtained for the protein and ligand signals from the ternary complex of human DHFR with TMP and NADPH. The family of solution structures of the complex was calculated using distance, torsion angle, and chemical shift restraints. Information on protein backbone dynamics was obtained from relaxation experiments carried out at three magnetic fields. Differences in structure and dynamics between bacterial and vertebrate forms of DHFR complexes will be reported.

Poster 36

A LEE-encoded regulatory cascade controls virulence gene expression in attaching and effacing pathogens

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■ The locus of enterocyte effacement (LEE) encodes the proteins required for the generation of the attaching and effacing (A/E) lesion by enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *Escherichia coli* and the mouse pathogen *Citrobacter rodentium*. Several studies have demonstrated that Ler is required for the expression of genes located within or outside the LEE. Ler overcomes the silencing effect, exerted by the regulatory protein H-NS on the expression of its target promoters, by modifying a repressing nucleoprotein complex that traps and/or blocks the access of the RNA polymerase. Due to its central role in A/E pathogenesis, we have been interested in the systematic characterization of *cis*- and *trans*-acting elements involved in the regulation of *ler*. The *ler*-regulatory region contains sequence motifs involved in both negative and positive regulation. The current model of the regulatory mechanism controlling *ler* expression includes different conserved regulatory proteins such as IHF, Fis, H-NS, BipA, and QseA. However, lack of *ler* expression in *E. coli* K12 suggested that A/E pathogens contain an additional regulatory factor that is necessary for *ler*'s activation. Systematic and functional analyses of the LEE in *C. rodentium* led us to the identification of a novel positive regulatory protein, GrlA (Global regulator of LEE-activator), that is highly conserved between A/E pathogens and critical for *ler* regulation. *grlA* is the second gene of an operon that also encodes a novel putative negative modulatory protein named GrlR (Global regulator of LEE-repressor). Ler, GrlA, and GrlR appear to establish a regulatory cascade that modulates virulence gene expression through protein-DNA and protein-protein interactions. These results are allowing us to better understand the mechanisms that enable the adequate and coordinated expression of virulence factors in these enteric pathogens.

Poster 38

Fast-reacting thiols in two-cysteine peroxiredoxins as a mechanism of peroxynitrite detoxification in infective microorganisms

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■ Peroxynitrite (ONOOH/ONOO⁻) is a cytotoxic effector molecule formed by activated macrophages from the diffusion-controlled reaction of the free radicals nitric oxide (·NO) and superoxide (O₂⁻). Low molecular weight thiols react with peroxynitrite via a two-electron oxidation process, with second-order rate constants of about 10²–10⁴ M⁻¹s⁻¹ at pH 7.4 and 37°C, which inversely correlate with the pK_{SH} of the thiol group. Although intracellular non-protein thiols (e.g., glutathione, trypanothione) are depleted during peroxynitrite challenge to target cells, kinetic considerations indicate that the depletion is indirect, given that other intracellular targets react faster with peroxynitrite. In this context, we have seen that peroxiredoxins, ubiquitous thiol-containing hydroperoxide reducing enzymes, react surprisingly fast with peroxynitrite. In this work, we performed stopped-flow and steady-state kinetic studies of the reaction of peroxynitrite with peroxiredoxins from different infective microorganisms, namely *Trypanosoma brucei* and *Trypanosoma cruzi* cytosolic tryparedoxin peroxidases, *Mycobacterium tuberculosis* thioredoxin peroxidase, and *Plasmodium falciparum* thioredoxin peroxidase I. We found that all the peroxiredoxins tested react rapidly with peroxynitrite with second-order rate constants in the range of 10⁶–10⁷ M⁻¹s⁻¹ at pH 7.4 and 37°C. These reactions led to enzyme thiol oxidation and were inhibited either by site-directed mutation of the fast-reacting cysteine or thiol alkylation. Moreover, peroxynitrite-mediated peroxiredoxin oxidation could be reversed by the natural reductants thioredoxin or tryparedoxin. We propose that the conserved cysteine in the N-terminal domain reacts with peroxynitrite to yield a sulfenic acid derivative that readily reacts with the second cysteine to form a disulfide bridge. Thus, our results support a role of peroxiredoxins as catalytic detoxifiers of peroxynitrite during the cellular immune response.

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Poster 40

Neuronal correlates of a perceptual decision in the ventral premotor cortex

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■ The ventral premotor cortex (VPC) is involved in the transformation of sensory information into action, although the exact neuronal operation is not known. We addressed this problem by recording from single neurons in the VPC of trained monkeys while they make a decision based on the comparison of two mechanical vibrations applied sequentially to the fingertips. Here we report that the activity of VPC neurons reflects current and remembered sensory inputs, their comparison, and motor commands expressing the result, that is, the entire processing cascade linking the evaluation of sensory stimuli with a motor report. These findings provide a fairly complete panorama of the neural dynamics that underlie the transformation of sensory information into an action, and they emphasize the role of VPC in perceptual decisions.

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Poster 42

Molecular mechanisms regulating myogenic specification of adult stem cells

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■ Adult skeletal muscle possesses a remarkable ability to regenerate following injury. New myofibers formed during repair are thought to arise solely from the muscle satellite cell lineage. The experiments described here challenge this view and suggest that a resident population of adult stem cells plays a central role in the regenerative process. CD45 is considered a lineage-restricted hematopoietic marker that is not expressed on satellite cells or on any other nonhematopoietic cell types. CD45⁺/Sca-1⁺ cells isolated from resting muscle formed hematopoietic cells in vitro but never differentiated into myocytes. However, following cardiotoxin-induced regeneration, 7–10 percent of CD45⁺/Sca-1⁺ cells expressed Pax7, MyoD, and Myf5. Furthermore, CD45⁺/Sca-1⁺ cells derived from regenerating Pax7^{-/-} muscles failed to express MyoD, and retroviral Pax7 rescued this deficiency, suggesting that the myogenic potential of CD45⁺/Sca-1⁺ cells is Pax7-dependent. These results provide the first experimental evidence that putative CD45⁺ adult stem cells have a normal physiological role for tissue regeneration in vivo. Notably, muscle-derived adult stem cells isolated from resting muscle only undergo myogenic specification in vitro following coculture with primary myoblasts. Importantly, primary myoblasts express Wnt5a, and coculture of CD45⁺/Sca-1⁺ adult stem cells with Wnt-expressing fibroblasts was sufficient to induce their myogenic specification. Moreover, injection of the Wnt-antagonist sFRP1 into damaged muscle abrogated regeneration. Taken together, these data suggest that adult stem cells undergo myogenic specification in response to Wnt signaling during muscle regeneration and play an important role in muscle regeneration.

Poster 44

How *Shigella* overwhelms the innate immune response: a model for inflammatory bowel diseases?

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■ Interaction of *Shigella flexneri* with epithelial cells involves contact with membrane rafts through engagement of CD44 and release of Ipa proteins through an activatable, type III secretory apparatus (i.e., Mxi/Spa). A cascade of signals elicited by GTPases of the Rho family and by c-src causes rearrangements of the cytoskeleton, allowing bacterial entry by macropinocytosis. Then the bacterium initiates intracytoplasmic movement, due to assembly of actin filaments caused by a surface protein, IcsA, which recruits N-WASP and Arp2/3. This allows passage to adjacent cells via protrusions, which are engulfed by a cadherin-dependent process. A paracrine pathway involving secretion of ATP by hemiconnexions and calcium fluxes facilitates cytoskeletal rearrangements, thus boosting entry and cell-to-cell spread of bacteria.

In addition, epithelial cells are able to sense intracellular bacteria through their peptidoglycan (PGN) by proteins of the NOD/CARD family that are able to activate the NF-kappaB pathway in invaded cells, thus reprogramming them to produce pro-inflammatory cytokines. Global transcriptional analysis of infected cells reveals a typical pattern of increased transcription of genes encoding pro-inflammatory cytokines and other cytokines; transcription of the latter is dominated by the massive expression of IL-8 mRNAs and correlated production of this potent chemoattractant for polymorphonuclear cells. This is particularly the case for epithelial Nod1/CARD4, which was shown to specifically recognize PGN from gram-negative microorganisms. As an increasing number of epithelial cells are invaded by *Shigella*, the colonic epithelium becomes a major provider of IL-8, thereby inducing massive recruitment of polymorphonuclear leukocytes that account for the destructive inflammatory process that is characteristic of shigellosis.

Poster 46

Molecular genetic and functional studies of progressive myoclonus epilepsy

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■ Our group studies the genetics of neurodevelopmental disease. The epilepsies constitute one of the most common neurological disorders, with more than 1 million North Americans affected. Lafora disease (LD) is an autosomal recessive form of adolescent progressive myoclonus epilepsy, which leads to death within a decade of onset. In LD, pathogenic polyglucosan (an unusual form of glycogen) inclusions are observed in brain and other tissues. Our laboratory identified the *EPM2A* (*Nat. Genet.* 20:171–174, 1998) and *EPM2B* (*Nat. Genet.* 35:125–127, 2003) disease genes, and we have also now discovered at least one additional disease-causing locus. We determined that *EPM2A* encodes a dual-specificity phosphatase named laforin that interacts with carbohydrate substrates. Although the in vivo substrate of laforin is not yet known, we have discovered (novel) interacting proteins using yeast two-hybrid methodologies. *EPM2B*, which encodes the malin protein, is a putative E3 ubiquitin ligase with a RING finger and six NHL protein interaction domains. We have shown that laforin and malin interact in the same pathway.

To further study the pathogenesis, a dominant-negative strategy was used to generate a transgenic mouse model in which laforin carrying a mutation at the invariant cysteine residue of the phosphatase catalytic site (Cys266Ser) was overexpressed. Examination of tissues revealed an elevated number of polyglucosan inclusions in the brain and liver of transgenic compared with wild-type mice. Lafora bodies were present primarily in the germ layer of the cerebral cortex and the pyramidal and Purkinje cells of the cerebellum. Immunohistochemistry and ultrastructure analysis by electron microscopy of *myc* revealed striking coinciding patterns between the inclusions and *myc*-laforin expression of transgenic mice, further suggesting that the Lafora bodies are pathologically specific and involved in LD. The continued study of the function of laforin and malin will afford further insight into the cellular defect causing the disease, leading to better diagnosis and treatment.

FRIDAY
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Poster 48

Toward *Taenia solium* cysticercosis control through vaccination and specific diagnostic and genetic epidemiology

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■ This research program deals with cysticercosis infection in humans, pigs, and mice. In patients with neurocysticercosis (NC), the most severe form of the disease, it was found that localization determines severity, the immunological profile, and evolution of the infection. In a CT scan epidemiological study performed in almost 700 inhabitants of a rural community, we detected neither familial nor household aggregation, even though multicase families were found. Thus, the susceptibility to NC infection probably involves multiple genes and does not obey simple patterns of familial segregation seen for monogenic disorders, and could be even more complicated by the possible participation of variable exposure factors.

A synthetic vaccine against pig cysticercosis to interrupt the life cycle of the parasite was developed and tested in field trial. New vaccine delivery systems have been successfully expressed and tested (filamentous phages, transgenic plants). The vaccine has proven to be therapeutic against pig cysticercosis in experimental trials. An intervention program is being implemented in a state of Morelos, Mexico, based on the developed vaccine. The program began with the determination of the magnitude, extent, and risk factors of pig cysticercosis. Prevalence, ranging from 0 to 30 percent in 1720 pigs bred in the 93 localities of the 33 municipalities sampled, was related to social, biological, geographical, and economical factors. Based on the information collected, a regional approach to control transmission in the pigs, targeted to the high-prevalence localities, is now being undertaken.

In *Taenia crassiceps* experimental murine cysticercosis, two major observations have been made: the innate immune response involved in the genetic resistance to the infection is possibly mediated by the nonclassic MHC antigen Qa2, and in an in vitro culture of *Taenia crassiceps* cysticerci, progesterone promotes the evagination and differentiation into the tapeworm while gonadotrophin promotes cysticercal reproduction by budding. This in vitro model of hormone-dependent morphogenesis of *T. crassiceps* offers new insights into the biology of the parasite.

Poster 50

Cellular models with altered expression of genetic products of the myosin light chain kinase locus

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■ The myosin light chain kinase (MLCK) locus in the higher vertebrate genome encodes three size classes of proteins involved in the regulation of cell motility through differential expression in tissues and usage of a subset of functional features. One class is represented by 17 kDa Kinase-Related Protein (KRP), the myosin-binding protein expressed in smooth and heart muscle. 108 kDa MLCK (MLCK-108) is widespread among tissues and abundant in smooth muscle. 210 kDa MLCK (MLCK-210) is mainly present in nonmuscle cells and in various undifferentiated cells during embryogenesis. Both MLCK isoforms contain the KRP domain as the C-terminal extension, whereas MLCK-210 also possesses a unique N-terminal tail involved in cytoskeletal interactions.

The in vivo role of KRP remains unclear. Similarly, the involvement of the unique N-terminal sequence of MLCK-210 in motile events has not been evaluated due to the lack of appropriate cellular models. Here we describe tissue culture models with genetically manipulated expression of KRP and MLCK-210 that may be useful for elucidating the role of these proteins in the living cell.

We designed HeLa cells stably expressing chicken gizzard KRP that were characterized by augmented adhesion, spreading, migration, and proliferation compared with mock-transfectants. Immunoblotting revealed more myosin II associated with HeLa-KRP cytoskeletons, and it is hypophosphorylated at the Ser19 of regulatory light chains. Thus, studies of the HeLa-KRP model suggest that the in vivo target of KRP is, indeed, myosin and that KRP facilitates actomyosin-based cell motility through the stabilization of myosin filaments.

We isolated and cultured lung microvascular endothelium from MLCK-210 knockout mice using immunomagnetic bead technology. The cells exhibited common endothelial markers (CD31, vWF, DIL accumulation, monolayer-type growth) but lacked MLCK-210 and showed low, if any, MLCK-108 expression. Because MLCK-210 is considered the primary regulator of endothelial barrier function, MLCK-deficient endothelium could be a suitable model to test this assumption and discern the functional impact of the unique MLCK-210 sequence.

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Poster 52

Restriction enzymes interacting with two recognition sites: are two sites better than one?

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■ The restriction endonucleases *Bse634I*, *EcoRII*, and *BfiI* belong to different subtypes of restriction enzymes. Despite differences in their structural organization and cofactor requirements, they all, however, require two recognition sites for optimal catalytic activity. Indeed, kinetic studies of *Bse634I*, *EcoRII*, and *BfiI* using supercoiled plasmid DNA substrates containing one or two recognition sites demonstrate that DNA with two sites is cleaved much faster than single-site DNA, suggesting that the interaction with two recognition sequences located in *cis* stimulates cleavage. Strikingly, it has been shown that *Bse634I* and *EcoRII* (Mucke et al., *EMBO J.* 21:5262–5268, 2002) can be “cured” of the requirement for the two recognition sites by site-directed mutagenesis (*Bse634I*) or by truncation of the wild-type protein (*EcoRII*). Indeed, engineered variants of both *Bse634I* and *EcoRII* cleaved plasmid DNA with a single site with nearly the same efficiency as a two-site DNA, indicating that interaction with two recognition sites is not absolutely required for catalytic activity. Synapsis of the two cognate DNA sites, however, might enhance the specificity of the restriction enzyme for its recognition sequence. To test whether the interaction with two DNA sites contributes to the increased fidelity of restriction enzyme, we have analyzed cleavage of cognate and noncognate oligonucleotides by wt *Bse634I* and its mutational variant that does not require two recognition sites for DNA cleavage. For the first time, the obtained data demonstrate subtle correlations between the structure, stability, activity, and specificity of restriction enzymes requiring two recognition sites.

Poster 54

Molecular analysis of the *Salmonella enterica* magnesium regulon

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■ *Salmonella* responds to environmental magnesium deprivation by inducing the transcription of the PhoP-PhoQ regulon. Expression of this regulon is necessary for intramacrophage survival, resistance to acid pH and antimicrobial peptides, modification of antigen presentation, formation of spacious vacuoles, and modulation of macrophage cell death. Repression of the regulon is also required during the first steps of the infection, entry of *Salmonella* into intestinal epithelial cells, a process called invasion. The coordination of this expression is governed by the activity of the PhoP/PhoQ two-component signal transduction system. In this system, extracellular Mg²⁺ interacts with the periplasmic domain of the sensor protein PhoQ, inducing a specific PhoP-phosphatase activity that controls the phosphorylation state of the transcriptional regulator PhoP.

We took advantage of microarray technology to characterize the PhoP-PhoQ regulon in the *Salmonella enterica* serovar Typhimurium. We analyzed the transcriptional profile of the wild-type strain grown at inducing or repressing concentrations of Mg²⁺ and compared the profile with that of the *delta-phoPQ* strain grown under the same conditions. In this way, we identified new putative PhoP-regulated genes. We created chromosomal *lacZ* transcriptional fusions with these genes and confirmed that the genes under study were under PhoP/PhoQ control. Among the genes uncovered with this strategy, we discovered that a novel two-component system and a locus encoded within the *Salmonella* pathogenicity island 1 (SPI1), necessary for invasion of epithelial cells, are activated by PhoP. Interestingly, a deletion in the latter locus increased the expression of *hilA*, which encodes the major transcription regulator of the SPI1, indicating that this *pag* locus encodes negative regulators of the invasome expression. Our analysis allowed us to uncover novel aspects of the actions taken by *Salmonella* to control gene expression in order to prosper within the infected host.

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Poster 56

Molecular dissection of the multiprotein, high molecular weight, membrane-bound presenilin complexes

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■ The presenilin proteins (PS1 and PS2) are homologous, polytopic, transmembrane proteins that are necessary for the endoproteolytic cleavage of several Type 1 transmembrane proteins, including the amyloid precursor protein (APP). The cleavage of APP generates A-beta and a series of C-terminal stubs (epsilon-stubs). Although PS1 and PS2 form independent, high molecular weight complexes, these complexes do contain several common components. The other components of the PS1 and PS2 complexes include nicastrin (a Type 1 transmembrane glycoprotein), APH-1 (a polytopic transmembrane protein with no significant homology to other proteins), and PEN-2 (a unique peptide with two transmembrane domains). Glycosylation and trafficking of nicastrin to the cell surface is necessary for the biological activity of the presenilin complexes, and nicastrin preferentially binds to the mature presenilin components (e.g., PS1 N- and C-terminal fragments). Conversely, the absence of PS1 or PS2 destabilizes nicastrin. APH-1 appears to be a stable component predominantly located in the ER and may represent the initial scaffolding molecule. APH-1 binds to both mature and immature species of nicastrin and presenilin. The role of PEN-2, which interacts with other complex components through a conserved motif at the C-terminus, is less clear. PEN-2 may be involved in final complex activation or in modulating the relative balance of cleavage at the A-beta40 and A-beta42 sites. Analysis of the presenilin complex components may provide clues to a novel form of endoproteolytic cleavage (regulated intramembranous proteolysis) and to potential therapeutics for Alzheimer disease.

FRIDAY
HANSA ROOM

Poster 58

Mapping of neutralizing epitopes on domain I of the yellow fever virus envelope glycoprotein with recombinant human antibodies generated through phage display

JAN TER MEULEN, Institute of Virology, Philipps University, Marburg, Germany, and Viral Hemorrhagic Fever Project, University of Conakry, Republic of Guinea

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■ Yellow fever virus (YFV) is a mosquito-transmitted, enveloped positive-stranded RNA virus belonging to the genus *Flavivirus*, family *Flaviviridae*. It causes hemorrhagic fever in humans in Africa and South America. Epidemics of yellow fever (YF) have been occurring with increasing frequency in recent years, with a lethality of about 25 percent. Humoral immunity follows natural YFV infection and immunization with the live attenuated YFV vaccine strain 17D. For humans, the neutralizing epitopes on the virus have not been mapped. In the present study, we constructed two phage libraries displaying human antibody scFv fragments using lymphocytes of two donors who recovered from YF during the 2000 YF outbreak in Guinea, West Africa. To isolate scFvs with specific affinity for YFV, we panned the phage libraries with purified YF 17D virions. Six monoclonal phages with high affinity for the YF 17D virus were isolated. The six different scFv displayed by the monoclonal phages were expressed as soluble molecules and precipitated the YFV envelope glycoprotein (E protein) in a radioimmunoprecipitation assay. Three scFv neutralized YFV 17D in a Plaque Reduction Neutralization Test (PRNT). To map the neutralizing epitopes on the YFV-E protein, viral escape mutants of the 17D YFV strain were generated under selective pressure of the respective scFv. Sequencing analysis of these variants identified single amino acid substitutions on domain I of the E protein at positions aa 153 and 154. Because the epitopes were mapped with recombinant antibodies generated from wild-type YFV-infected patients, we believe that we have identified the domains on the 17D vaccine strain of YFV responsible for inducing protective humoral immunity in humans.

Poster 60

The “roll and lock” model of actin-myosin-ATP interaction during muscle contraction

ANDREY K. TSATURYAN, Institute of Mechanics, Moscow State University, Moscow, Russia

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A.K. Tsaturyan¹ ■ ¹Institute of Mechanics, Moscow State University, Moscow, Russia; ²Institute of Immunology and Physiology, Urals Branch of the Russian Academy of Sciences, Yekaterinburg, Russia; ³Imperial College of Science, Technology and Medicine, London, United Kingdom

■ The popular lever arm model suggests that muscle force is produced by a tilt of the light chain domain (or “neck”) of the myosin heads (myosin subfragment 1, or S1) with respect to its catalytic domain, which remains firmly bound to actin. However, a number of observations cannot be explained by this simple model. First, using EM tomography, myosin heads were found with their catalytic domains attached to actin at different axial and azimuthal angles. Second, the coincidence of the time courses of the rise in isometric tension induced by a temperature jump (T-jump) in contracting muscle fibers with the extent of labeling of the actin helix by stereospecifically bound S1s suggests that locking of nonstereospecifically bound heads on actin is accompanied by axial displacement (a “roll and lock” transition) and is an essential part of the force-generating process. Third, the T-jump-induced tension transients are independent of strain. These findings can be quantitatively explained by a kinetic-structural model that includes two-step ATP hydrolysis by S1, a “roll and lock” transition of the catalytic domain to a state in which the domain is stereospecifically bound to actin after hydrolysis, and subsequent tilting of the lever arm coupled with release of inorganic phosphate and ADP. The model also explains the previously observed transitional drop in muscle stiffness a few milliseconds after the T-jump, changes in the two-dimensional X-ray diffraction pattern accompanying tension rise at elevated temperature, fast two-phase movement of S1s revealed from interference fine splitting of the M3 myosin meridional X-ray reflection, the time course of its intensity found in our recent experiments, and the fact that this movement is not accompanied by tension changes on this fast time scale.

Poster 62

Reestablishing tolerance to DNA in a murine model of lupus

TCHAVDAR L. VASSILEV, Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

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T. Vassilev¹ ■ ¹Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; ²College of Medicine, National Taiwan University, Taipei, Taiwan

■ Systemic lupus erythematosus (SLE) is the prototype systemic autoimmune disease, characterized by the generation of autoantibodies against native double-stranded (ds) DNA. The pathological DNA-specific B cells are a logical target for a selected therapeutic intervention. We hypothesize that it may be possible to reestablish tolerance to native DNA in animals with spontaneous lupus-like diseases by administering to them a chimeric molecule containing a monoclonal antibody against an inhibitory B cell receptor coupled to a peptide that antigenically mimics DNA. This engineered molecule is expected to be bound selectively by the immunoglobulin receptors of B cells with antinative DNA specificity and to deliver selectively to them a strong suppressive signal. We constructed a hybrid antibody by coupling a rat monoclonal antibody specific to mouse Fc-gammaRIIb receptor (CD32) to the DNA-mimicking DWEYSVWLSN peptide (Putterman et al., *J. Immunol.* 164:2542–2549, 2000) and tested the activity of the chimera in MRL/lpr mice that are known to develop a lupus-like disease at the age of eight weeks.

The administration of the chimeric antibody to seven-week-old animals prevented the appearance of IgG anti-DNA antibodies and lupus symptoms in the following month. In a separate group of experiments, treatment was started at an age of 18 weeks when the mice already had the full-blown disease. Intravenous infusions with the DNA-peptide chimera resulted in maintenance of a flat level of IgG anti-DNA antibody levels and prevention of the aggravation of lupus glomerulonephritis. The PBS-injected control mice had a sharp rise in disease-associated antibody levels and proteinuria, and 80 percent died before reaching the age of 24 weeks. The use of chimeric immunomodulatory antibodies represents a new approach for the selective suppression of the activity of autoreactive disease-associated B cells in SLE and other autoimmune diseases.

FRIDAY
HANSA ROOM

Poster 64

Enzymatic mechanism and physiological role of dUTPase

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■ The enzyme dUTPase prevents uracil incorporation into DNA. Lack of the enzyme initiates thymine-less cell death by transformation of base excision repair into a hyperactive futile cycle. A detailed understanding of mechanism and the cellular role of the enzyme would offer innovative new means of modulating the process of thymine-less apoptosis. The laboratory addresses both structural/mechanistic questions and physiological aspects of the enzyme's function.

Experimental observation of the high-energy pentacovalent intermediate in dUTPase and a series of structural snapshots along the reaction coordinate in high-resolution crystallographic studies enabled us to provide a highly detailed description of the catalytic mechanism. This result also offers direct structural evidence for the long-held central dogma of enzymology stating that the catalytic power of enzymes is due to stabilization of high-energy transition states. dUTP hydrolysis is of significant associative character. It is initiated via in-line nucleophilic attack of a water molecule oriented by an activating aspartate residue that leads to formation of an additional covalent bond on the alpha-phosphorous. Stabilization of the hyperbonded pentacovalent intermediate is achieved by 1) modulation in the interaction pattern with catalysis-assisting Mg²⁺; 2) a concerted motion of residues from three conserved enzyme motifs; and 3) a consequent remodeling of water hydrogen-bonding network.

To understand the role of dUTPase and thymine-less apoptosis in development and to address the possible therapeutic significance of inducing thymine-less apoptosis in tumor cells, we carried out developmental and cellular biology investigations. In *Drosophila*, we found multilevel regulatory patterns governing dUTPase presence and activity throughout development. The lack of the enzyme in larval stages may facilitate developmental apoptosis required in the pupal stage. To investigate this hypothesis, we created a transgenic fly strain for constitutive overexpression of dUTPase. In human tumor cells, we observed that dUTPase knockout by RNA interference induces apoptosis. This cell death pathway is currently being investigated.

Poster 66

Shaping substrate binding and recognition in metallo-beta-lactamases by directed evolution

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■ Beta-lactamases represent the main resistance mechanism developed by bacteria to escape the action of beta-lactam antibiotics. Metallo-beta-lactamases (MBLs) represent the latest generation of these enzymes. In contrast to the well-described serine-beta-lactamases, MBLs display a broad substrate spectrum, being able to hydrolyze penicillins, cephalosporins, and carbapenems. This feature has thwarted the design of a clinically useful inhibitor for these enzymes.

We have studied the binding and hydrolysis of different beta-lactam substrates to *Bacillus cereus* MBL (BcII hereafter) by pre-steady-state kinetic measurements. In all cases, the substrates are unmodified in the most populated reaction intermediate, and no chemically modified substrate species accumulates in detectable amounts. The apoenzyme was unable to bind to any of the substrates, and the binding features were restored by Zn(II) uptake, revealing that the metal ions are the main determinants of substrate binding. This evidence is in line with the lack of an optimized substrate recognition patch in metallo-beta-lactamases, which provides a broad substrate spectrum.

In an attempt to further expand the substrate spectrum, we generated different mutants of BcII by directed evolution toward cephalixin, a poorly hydrolyzed substrate. The minimum inhibitory concentration toward cephalixin of *Escherichia coli* cells exporting BcII to the periplasmic space was raised 64-fold. Selected clones were sequenced, and the resulting enzymes exhibited enhanced hydrolytic capabilities toward cephalosporins with small-size substituents in the 3-position, substituents that fit in an open groove in the enzyme active site. The catalytic efficiency toward substrates with bulkier substituents in this position was not affected. We attribute this result to the effect of changes in the hydrogen bond network of the metal ligands.

FRIDAY
HANSA ROOM

Poster 68

Physiological functions of glycogen synthase kinase-3 as discerned through analysis of cells lacking each or both isoforms

JAMES ROBERT WOODGETT, Ontario Cancer Institute, Toronto, Canada

B. Doble, S. Patel, J.R. Woodgett ■ Ontario Cancer Institute, Toronto, Canada

■ Glycogen synthase kinase-3 (GSK-3) is a ubiquitously expressed, highly conserved serine/threonine kinase that is implicated in several human diseases, including diabetes, cancer, Alzheimer's disease, and bipolar disorder. The kinase is unusual in that it displays high activity in resting cells. Stimulation of cells usually results in inactivation of the enzyme by phosphorylation. Given that many of its substrates are functionally inhibited when phosphorylated by GSK-3, inhibition of this one kinase activates its multiple targets. Substrates for GSK-3 include transcription factors (cJun, NF-AT, cMyc, C/EBP, beta-catenin), structural proteins (Tau), and regulatory proteins (glycogen synthase, cyclin D, Axin).

Mammals harbor two distinct genes for GSK-3, encoding two related protein kinases with similar catalytic properties, GSK-3alpha and -3beta. We disrupted GSK-3beta in the germline of mice and found a surprising phenotype that was similar to knockout of components of the NF-kappaB pathway. These findings revealed a critical role for GSK-3beta in regulating this transcription factor and also demonstrated that the two forms of GSK-3 were not functionally identical. We have followed up these studies by disrupting both alleles of GSK-3alpha and GSK-3beta in mouse embryonic stem cells and have also generated cells lacking both alleles of both isoforms. The cells have been used to examine the role of the enzyme in beta-catenin signaling via the Wnt pathway and in growth factor-mediated signaling. By knocking specific mutants of GSK-3 back into the GSK-3 locus, we are examining the mechanisms by which cells discriminate regulation of GSK-3. The double null embryonic stem cells are defective in differentiation and retain pluripotentiality in the absence of de-differentiating factors. The null cells are thus an important tool for dissecting the molecular mechanisms of cell fate determination.

Given the recent development of therapeutic inhibitors of GSK-3, understanding the physiological functions of these kinases should explain the effects of these drugs. In addition, the GSK-3alpha and -3beta null cells will provide information regarding the specificity of "selective" pharmacological inhibitors.

Poster 70

Variable potency of calcium trigger signals to induce cardiac calcium release

ALEXANDRA ZAHRADNÍKOVÁ, Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic

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■ Calcium release from the sarcoplasmic reticulum of mammalian cardiomyocytes is thought to be triggered by openings of single L-type calcium channels (DHPRs) that activate juxtaposed ryanodine receptor (RyR) calcium release channels. To clarify how the fidelity of coupling between these two channel types is regulated, we tested the relative potency of DHPR openings to activate calcium release. Calcium currents and local calcium transients in response to short voltage prepulses followed by a test pulse were measured in rat ventricular myocytes using whole-cell patch clamp and confocal microscopy with fluo-4 as the calcium indicator. We assumed that the extent of release is dose-dependently proportional to the effective calcium influx sensed by the RyRs. The extent of calcium release-dependent inactivation of calcium currents and the latency of local calcium release activation during the prepulse and the test pulse were used as measures of DHPR potency to activate calcium release. The relative potency of DHPR openings was larger during tail calcium currents following the prepulse than for the currents during the prepulse, and it was larger for the tail-current reopenings than for the tail-current first openings. When calcium stimuli that mimicked single DHPR openings were applied repeatedly under conditions that were close to physiological, we also demonstrated an increase in the probability of RyR activation in planar lipid bilayers. Taken together, our data suggest that isolated DHPR openings have low potency to activate RyRs and trigger calcium release. The success rate of the subsequent DHPR openings is dramatically increased due to the potentiating effect of the pre-openings, which increase the basal calcium level and/or prolong the duration of Ca²⁺ signals at the RyR sensing sites. In short, the fidelity of DHPR-RyR coupling depends on the recent history of DHPR activity.

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FRIDAY
HANSA ROOM

POSTER SESSION

Poster 72

Analysis of the transcriptional complexity of the TFIID complex in *Drosophila*

MARIO ZURITA, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

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■ The basal transcription/DNA repair factor TFIID is composed of nine polypeptides that are part of two subcomplexes. The “core” complex contains six subunits (XPB, XPD, p62, p52, p44, and p34) whereas the CAK complex contains three (Cdk7, CycH, and MAT1). During transcription, the helicase activity of TFIID is involved in the formation of the open complex. The kinase activity of TFIID phosphorylates the CTD domain of the polymerase, allowing transcriptional elongation. We have found that mutations in *hay* (XPB) and in the 140 kDa subunit of the RNA polymerase II act as dominant modifiers of the derepression phenotypes of the *Scr* and *Ubx* genes. Interestingly, all the derepression homeotic phenotypes are also sensitive to the total reduction of transcription levels by actinomycin D. In addition, different promoter control regions exhibit distinct sensitivities to different *hay* alleles. Although TFIID is a basal transcription factor, these results indicate that mutations in *hay* have a locus-specific effect.

We are also analyzing the dynamics of the TFIID complex during early *Drosophila* development. We found that the TFIID “core” is initially located in the cytoplasm of syncytial blastoderm embryos; however, during the formation of the cellular blastoderm, the “core” moves from the cytoplasm to the nucleus. Interestingly, the CAK complex exhibits a different behavior in these early embryonic stages. The CAK is mostly located in the cytoplasm during cellularization and even during gastrulation. These results indicate that CAK does not interact with the TFIID “core” in early *Drosophila* development and does not participate in transcription in these stages. Later in development, the CAK complex is mostly nuclear and colocalizes in most of the chromosomal regions with the core of TFIID, but not in all sites, suggesting that the CAK complex could be acting in transcription at some loci without the TFIID “core.”

MINIGRANT PRESENTATIONS (SESSION I)

4:30 p.m.

A novel dendritic cell-targeting delivery system induces humoral and cellular immunity against rotavirus

MAGDALENA PLEBANSKI, Austin Research Institute, Melbourne, Australia; and **SUSANA LÓPEZ AND CARLOS F. ARIAS**, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

M. Plebanski,¹ S. Gloster,¹ C. David,¹ D. Leong,¹ S. López,² C.F. Arias² ■ ¹Austin Research Institute, Melbourne, Australia; ²Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

■ Rotavirus is the leading cause of childhood viral gastroenteritis. There is currently no effective vaccine available, and treatment is largely restricted to rehydration therapy. An effective vaccine would need to deliver appropriate rotavirus target proteins to the immune system to induce high levels of protective antibodies and T cells. CD4 T cells help antibody production and sustain memory and effector cytotoxic CD8 T cells and thus play a key role in promoting and sustaining effective rotaviral immunity. The VP8 protein is generated by trypsin cleavage from the VP4 outer capsid rotavirus protein. Cleavage confers enhanced infectivity to the virus. Antibodies against VP8 are able to neutralize virus infectivity in vitro. The VP6 protein is an interior capsid protein and unavailable to the action of neutralizing antibodies; however, it is shown to play a role in protective immunity, mainly due to the induction of CD4 T cells. A dendritic cell-targeting, novel vaccine delivery system (called DCtag) was developed that is able to induce both T cells (CD4 and CD8) and antibodies and protect against a range of infectious diseases and cancer. This delivery system was tested for its ability to induce cellular immunity targeted to the previously identified protective VP6 T cell epitope and to induce antibodies to VP8 that effectively interact with whole infectious rotavirus. Dendritic cell-targeting strategies offer a powerful and feasible approach to the development of rotavirus vaccines able to promote a broad spectrum of immunity.

FRIDAY
HANSA ROOM

4:50 p.m.

Serological and genotypic studies of EBA-175 in severe and mild malaria in Ugandan children

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■ *Plasmodium falciparum* EBA-175 is a promising malaria vaccine antigen derived from the asexual blood stage of the parasite. Region II of EBA-175 binds specifically to glycophorin A on the erythrocyte membrane, and specific antibodies to region II inhibit erythrocyte invasion. Ugandan children of a single ethnic group resident in the same region manifest different clinical outcomes during malaria. Our hypothesis was that high levels of antibodies against region II acquired during natural *P. falciparum* infections result in a blockade of invasion and that inter-individual variability in the levels of these antibodies results in different clinical manifestations of malaria in infants. A case-control study was carried out to identify 6- to 59-month-old children with severe malaria (SM) and age-matched controls with mild or asymptomatic malaria (CM). Informed consent was obtained from mothers, and blood samples were collected for serological and biochemical analyses. Recombinant Pff2 was used as the antigen, and total IgG antibodies were detected by enzyme-linked immunosorbent assays. Mean IgG anti-PF2 titers in SM children were significantly lower than those in CM children. In SM but not CM children, there was a significant positive correlation between titer of IgG anti-PF2 and hemoglobin, packed cell volume, and age, whereas there was a significant inverse correlation between these titers and temperature. These data support the hypothesis that IgG anti-PF2 antibodies are associated with protection against severe malaria in Ugandan children. Genotypic analyses revealed that the prevalence of the FCR-3, CAMP, and mixed FCR-3/CAMP alleles was 35.8, 30.7, and 33.5 percent, respectively. We will present preliminary evidence that the FCR-3 allele may be associated with severe malaria. In addition, data on sequence diversity in Pff2 derived from *P. falciparum* field isolates and recognition of Pff2 variants with antibodies raised against recombinant Pff2 will be presented. These data have important implications for vaccine development based on EBA-175.

5:10 p.m.

Treatment of *Trypanosoma cruzi* infection in murine experimental models using inhibitors of endogenous sterol synthesis

JULIO A. URBINA, Venezuelan Institute for Scientific Research (IVIC), Caracas, Venezuela; and **MIGUEL A. BASOMBRIÓ**, National University of Salta, Salta, Argentina

M.R. Corrales,¹ C. Davies,¹ R. Cardozo,¹ M.A. Segura,¹ A. Barrio,¹ M. Celia Mora,¹ M. Angel Basombrió,¹ J.A. Urbina² ■ ¹National University of Salta, Salta, Argentina; ²Venezuelan Institute for Scientific Research (IVIC), Caracas, Venezuela

■ *Trypanosoma cruzi* infects an estimated 16 million persons in the Americas, causing severe heart disease and mortality in a third of them. *T. cruzi* also infects a variety of mammals, allowing the development of experimental models of the disease. In mice, both acute and chronic infection can be reproduced, replicating the histopathological alterations, antibody response, and cardiopathy observed in humans. The survival of *T. cruzi* depends on the endogenous synthesis of specific sterols. Several drugs have recently been developed that are capable of selectively blocking the pathway giving rise to these vital metabolites; one of them, the triazole TAK187, exerts a potent and selective inhibition on the C14 α demethylase of *T. cruzi*. Swiss mice were infected, and an acute, nonlethal infection was obtained, followed by a long chronic phase, which allowed us to study the effects of treatment on the long-term evolution of infection, antibodies, and pathology. Treatment consisted of 30 doses (20 mg/Kg/day) of TAK187, given every other day. A parallel group was treated with benznidazole (BZL, 200 mg/Kg/day), a currently available, toxic treatment. Four days after starting treatment, a complete and lasting clearance of blood parasites was demonstrated by microscopy and hemoculture. On day 124 posttreatment, antibody levels were determined and all animals were sacrificed and subjected to histopathological and PCR studies. A significant reduction in specific antibodies was demonstrated, both in the TAK187 and BZL groups, suggesting that infection was eradicated. The histopathological studies showed that most infected, nontreated animals displayed moderate to severe inflammatory infiltrates in several organs and the presence of few amastigote nests. These alterations, and PCR signals of infection, were eliminated in most treated animals, the effects of TAK187 being equal or better than those of BZL. Because BZL is toxic, the results obtained with TAK187, at one-tenth of the dose of BZL, are highly promising for clinical studies.

FRIDAY
HANSA ROOM

8:00 a.m.

Hepatitis D virus transcription in vitro: characterization of cellular RNA polymerase and viral protein

PEI-JER CHEN, National Taiwan University, Taipei, Taiwan

P.-J. Chen, W.-H. Huang, Y.-S. Chen ■ National Taiwan University, Taipei, Taiwan

■ Hepatitis delta virus (HDV) is a single-stranded, negative polarity RNA virus whose genome is small (1700 bases). Like other negative RNA viruses, the HDV genome begins transcription after entering host cells, translates viral protein essential for replication, and then replicates via complementary RNA by a rolling cycle mechanism. One unique feature of HDV is that, in contrast to other RNA viruses, it does not encode viral RNA-dependent RNA polymerase but has to rely on cellular RNA polymerase for its transcription and replication. Even though this RNA-dependent RNA polymerase (RdRP) in host (human or animal) cells has been sought for about a decade, it remains elusive. There is indirect evidence suggesting that cellular RNA pol II is responsible for viral mRNA transcription and for the replication of viral antigenomic RNA. We proposed that appropriately prepared cellular extracts might correctly transcribe HDV mRNA in vitro. We provided preliminary results to show the feasibility of this approach.

The other important question concerning HDV is the role viral protein plays in HDV mRNA transcription. HDV encodes only one protein, viral delta antigen, which is essential for viral genome replication. However, its role in transcription is less clear. We presented data that one mutant (delta antigen losing one of its acetyl group) augments the viral mRNA level up to 10-fold compared with that of wild type, suggesting that the delta antigen plays a role modulating viral transcription, perhaps by a different posttranslational modification. Thus, we plan to purify viral delta antigen from different cell compartments (with or without HDV replication) and characterize its posttranslational modifications. The results will then be combined with those obtained in the in vitro transcription systems to study the relevance of the delta antigen.

8:25 a.m.

Recognition of RNA templates by Q-beta replicase

ALEXANDER B. CHETVERIN, Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

V.I. Ugarov, A.A. Demidenko, A.B. Chetverin ■ Institute of Protein Research, Russian Academy of Sciences, Pushchino, Russia

■ Usually accompanying the Q-beta bacteriophage infection, Q-beta replicase (RNA-directed RNA polymerase of bacteriophage Q-beta) exponentially amplifies the genomic RNA of Q-beta and small replicable RNAs (RQ RNAs) but does not amplify other viral or cellular RNAs. In only 10 minutes, Q-beta replicase can produce as many as 10^{10} copies of a cognate RNA molecule, greatly exceeding the performance of all other amplification systems, including PCR. After 40 years of effort, however, the mechanism of template recognition by Q-beta replicase remains a mystery. The enzyme does not utilize primers, nor does it use promoters; hence, template recognition must be based on a strategy different from those used by common DNA or RNA polymerases. Here, we report on template activities of the 5' and 3' fragments obtained by cleaving one of the RQ RNAs at an internal site. Unexpectedly, we found that Q-beta replicase can copy the 5' fragment and a number of its variants, even though they lack the initiator region of RQ RNA residing at the 3' fragment. This copying can occur as a 3'-terminal elongation or through de novo initiation. In contrast to RQ RNA and its 3' fragment, initiation on these templates occurs without regard to oligo(C) clusters, is GTP-independent, and does not result in a stable replicative complex. The results suggest that, although Q-beta replicase can initiate and elongate on a variety of RNAs, only some of them are recognized as legitimate templates. GTP-dependent initiation on a legitimate template drives the enzyme to a "closed" conformation that may be important for keeping the template and the complementary nascent strand unannealed, without which the exponential replication is impossible. Triggering the GTP-dependent conformational transition at the initiation step could serve as a discriminative feature of legitimate templates providing for the high template specificity of Q-beta replicase.

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FRIDAY
BREMERHAVEN
ROOM

8:50 a.m.

Mutations in ribosomal RNA that affect different stages of translation

OLGA ANATOLIEVNA DONTSOVA, Department of Chemistry, Moscow State University, Moscow, Russia

P.V. Sergiev,¹ A.A. Leonov,¹ S. Kiparisov,¹ A.A. Bogdanov,¹ R. Brimacombe,² O.A. Dontsova¹ ■ ¹Moscow State University, Moscow, Russia; ²Max-Planck Institute for Molecular Genetics, Berlin, Germany

■ Ribosomes with the lethal mutation G2655C in *Escherichia coli* 23 S rRNA have been isolated using a system developed in our laboratory. The system is based on affinity isolation of the mutant ribosomes carrying a streptavidine aptamer, introduced into the unconserved helical element on the surface of the subunit. In vitro functional tests with the mutated ribosomes showed that mutation G2655C affects neither tRNA binding to the P- or A-sites nor the rate of ribosome-dependent GTP hydrolysis, but it does significantly decrease the level of translocation.

Substitution of C2394 in 23S rRNA for G or U was thought to be important for E-site tRNA binding. The mutations do not affect cell growth significantly even when only mutated rRNA is present in the cell. In vitro functional tests with the isolated mutated ribosomes showed that only two tRNA molecules can bind to one mutant ribosome whereas three bind to the wild type. Deacylated tRNA is released from the mutant ribosome just after translocation but not after the subsequent aminoacyl-tRNA binding, as in a wild-type case.

Mutation A1191G in 16S rRNA disturbs the subunit association and affects A-site tRNA binding as well the defined stage of translocation, as was shown by fast kinetic studies.

9:15 a.m.

Control of alternative splicing by RNA pol II elongation

ALBERTO R. KORNBLIHTT, University of Buenos Aires, Buenos Aires, Argentina

M. de la Mata, G. Nogués, J.P. Fededa, M. Muñoz, E. Petrillo, M. Alló, A.R. Kornblihtt ■ University of Buenos Aires, Buenos Aires, Argentina

■ Alternative splicing (AS) is a major contributor to proteome diversity in metazoans. AS of the fibronectin extra domain I (EDI) exon is controlled by RNA polymerase II (pol II) elongation and by the binding of the splicing factor SF2/ASF to an exonic splicing enhancer (ESE) present in EDI. Faster elongation rates elicit higher exon skipping according to a model where skipping occurs because the 3' splice site (ss) of the upstream intron is suboptimal compared with the 3'ss of the downstream intron. We tested the model in *cis*, by strengthening the suboptimal 3'ss, and in *trans*, by looking at AS when transcription is performed by a "slow" pol II mutant. We found that the better the exon is recognized by the splicing machinery, the less its splicing is affected by pol II elongation (Nogués et al., *J. Biol. Chem.* 278:52166–52171, 2003) and that transcription by a "slow" polymerase not only inhibits EDI skipping but also affects alternative splicing of other genes such as adenovirus E1a and *Drosophila's Ultrabithorax* (de la Mata et al., *Mol. Cell* 12:525–532, 2003).

We prepared minigenes with two EDI regions arranged in tandem under a single promoter. We found that the proximal AS event influences the distal one: disruption of the ESE at the proximal EDI not only prevents its own inclusion but favors skipping of a wild-type distal EDI exon. The effect is polar in nature because disruption of the distal EDI ESE does not affect skipping of the proximal one, which suggests a strong link with transcription.

The human transcriptional coactivator CA150 was reported to decrease transcriptional elongation by interacting with pol II carboxy-terminal domain. Consistent with our model, overexpression of CA150 provokes a fourfold increase in EDI inclusion. Paradoxically, reduction in endogenous levels of CA150 by RNA interference also increases EDI inclusion, indicating dual roles for this coactivator.

**FRIDAY
BREMERHAVEN
ROOM**

10:10 a.m.

Molecular mechanism of low-temperature sensing in bacteria

DIEGO DE MENDOZA, Institute of Molecular and Cellular Biology of Rosario, CONICET, University of Rosario, Rosario, Argentina

L.E. Cybulski, D. Albanesi, M.C. Mansilla, D. de Mendoza ■ Institute of Molecular and Cellular Biology of Rosario, CONICET, University of Rosario, Rosario, Argentina

■ Both prokaryotes and eukaryotes respond to a decrease in temperature with the expression of a specific subset of proteins. Although a large body of information concerning cold shock-induced genes has been gathered, studies on temperature regulation have not yet clearly identified the key regulatory factor(s) responsible for thermosensing and signal transduction at low temperatures.

Bacillus subtilis is one of nature's best-studied organisms and, in recent years, has become the principal paradigm for studies of the cold-shock response in Gram-positive bacteria. We are investigating how *B. subtilis* cells sense and transduce low-temperature signals to adjust gene expression. One important step has been accomplished in the dissection of a novel pathway for the adjustment of unsaturated fatty acid synthesis in *B. subtilis*. This pathway, termed the Des pathway, responds to a decrease in growth temperature by enhancing the expression of the *des* gene, which encodes an acyl-lipid desaturase. The Des pathway is uniquely and stringently regulated by a two-component system composed of a membrane-associated kinase, DesK, and a soluble transcriptional activator, DesR. Previously, we demonstrated in vivo that the temperature-sensing ability of the DesK protein is regulated by the extent of disorder within the membrane lipid bilayer. In this report, we present the mechanism by which the sensor protein DesK controls the signal decay of its cognate partner DesR and how this response regulator activates transcription of its target promoter. The results of these analyses are presented and discussed in the context of transcriptional regulation of membrane fluidity homeostasis.

10:35 a.m.

Biosynthesis of ornithine-containing lipids, a widespread class of bioactive lipids in eubacterial membranes

OTTO GEIGER, Center of Nitrogen Fixation Research, National Autonomous University of Mexico, Cuernavaca, Mexico

J. Gao, I.M. López-Lara, O. Geiger ■ Center of Nitrogen Fixation Research, National Autonomous University of Mexico, Cuernavaca, Mexico

■ Under phosphate-limiting conditions of growth, some eubacteria replace their membrane phospholipids with lipids that do not contain any phosphorus. In *Sinorhizobium meliloti*, the phosphorus-free lipids are ornithine-containing lipids (OL), sulfoquinovosyl diacylglycerol, and diacylglycerol trimethylhomoserine (Geiger et al., *Mol. Microbiol.* 32:63–73, 1999). Although OL are not present in eukaryotes and archaeobacteria, they are widespread among eubacteria and stimulate the mammalian immune system. Until now, neither the biosynthesis of OL was understood nor were any of the genes or enzymes involved known.

We isolated mutants of *S. meliloti* deficient in OL biosynthesis; complementation of such mutants lead to the identification of two genes (*olsA* and *olsB*) required for OL biosynthesis. OL-deficient mutants of *S. meliloti* disrupted either in *olsA* or *olsB* show wild-type-like growth behavior and are capable of inducing nitrogen-fixing nodules on alfalfa, the sinorhizobial host plant. *OlsA* shows homology to *O*-acyltransferases while *OlsB* matches the superfamily fold of acyl-CoA *N*-acyltransferases. Amplification of the genes and their expression in the corresponding OL-deficient mutant demonstrated that both are required for OL biosynthesis. Expression of the gene *olsB* is essential for the specific incorporation of radiolabeled ornithine into OL, and overexpression of *OlsB* in an *olsA*-deficient mutant of *S. meliloti* leads to the transient accumulation of the presumed biosynthetic intermediate lyso-ornithine lipid. Our results suggest that *OlsB* is required for the first step of OL biosynthesis, in which ornithine is *N*-acylated with a 3-hydroxy fatty acyl residue in order to obtain lyso-ornithine lipid. In a second step, lyso-ornithine lipid is converted to OL by an *olsA*-dependent *O*-acyltransferase activity that requires acyl-AcpP as the acyl donor (Weissenmayer et al., *Mol. Microbiol.* 45:721–733, 2002).

FRIDAY
BREMERHAVEN
ROOM

11:00 a.m.

Molecular and cellular responses to phosphorus deprivation in *Arabidopsis*

LUIS HERRERA ESTRELLA, Center for Research and Advanced Studies, IPN, Irapuato, Mexico

J. López Bucio, F. Nieto Jacobo, L. Sánchez Ramírez, A. Pérez, A. Zurita, A. Chacón, A. Cruz Ramírez, L. Herrera Estrella ■ Center for Research and Advanced Studies, IPN, Irapuato, Mexico

■ Phosphorus (P) is limiting for crop yield in more than 30 percent of the world's arable land, and, by some estimates, world resources of inexpensive P may be depleted by 2050. Improvement of P acquisition and use efficiency by plants is critical for economic and social reasons. Plants have evolved a diverse array of adaptive strategies to obtain adequate P under conditions of low P availability. These include alterations in root system architecture, excretion of low molecular weight compounds and enzymes, alterations in carbon metabolism, and the enhanced expression of numerous genes involved in the low P response. Our group has been using *Arabidopsis* and maize to study the adaptive processes to P deprivation. In *Arabidopsis*, we have found that root system architecture changes with the production of an increased density of lateral roots and longer root hairs and an alteration in root meristematic activity. Two mechanisms of P sensing have been identified in *Arabidopsis*: one is involved in the detection of the external P concentration, which regulates the formation of lateral roots and the expression of genes involved in P scavenging; the second is involved in sensing the internal P concentration, which regulates primary root elongation and genes involved in P uptake. We also investigated how carbon flux from photosynthetic tissues is redirected in the root system to promote lateral root formation, which enhances the capacity of the plant to explore new soil horizons in the search for nutrients. To identify genes involved in the P response, we have isolated mutants affecting root system architecture in response to low P conditions. Two main classes of mutants have been isolated, one affecting the internal P-sensing system and the other in the external P-sensing system.

11:25 a.m.

Unraveling the regulation of a central pathway involved in the synthesis of plastidic isoprenoids in plants

PATRICIA LEÓN MEJÍA, Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

P. León Mejía, A. Guevara, C. San Román, A. Cancino, A. Arroyo, M.E. Cortéz ■ Institute of Biotechnology, National Autonomous University of Mexico, Cuernavaca, Mexico

■ Isoprenoids are essential molecules synthesized by all organisms, but they are found in greatest variety in plants. All isoprenoids are derived from two basic units, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). In plants, these universal precursors are synthesized by two pathways that operate in two different cellular compartments. The mevalonic (MEV) pathway operates in the cytoplasm, and a novel route, the MEP pathway, operates in plastids. A variety of essential isoprenoids are synthesized via the MEP pathway, including phytohormones, photosynthetic pigments, electron carriers, antioxidants, and defense molecules. In addition to these functions, many plastidic isoprenoids are of biotechnological and medical importance. Provitamin A and vitamin E are basic nutrients for human health, and taxol is used as a chemotherapeutic agent. Furthermore, inhibitors that block the MEP pathway in *Plasmodium falciparum* are also being evaluated as anti-malarial agents.

Given the central role that the MEP pathway plays in the growth and development in plants, identification of the regulatory steps is of major importance. In contrast to the MEV pathway, the molecular characterization of the MEP pathway is still limited. Isolation and characterization of mutants in the pathway corroborate the functionality of these genes. These mutants cause alterations in chloroplast development. Furthermore, mutant analysis during embryogenesis allowed us to demonstrate that these genes act non-cell autonomously, whereby partial maternal complementation of chloroplast development is observed. Alterations in the level of MEP pathway enzymes in transgenic plants allowed us to determine that the first enzyme of this route (DXS) plays a key role in the control of the isoprenoid biosynthesis. Finally, we present a comparative analysis of the expression at the transcript and protein levels of the entire route under different conditions. These analyses show that the metabolic pathway is coordinately regulated by multiple signals at the transcript and protein levels and have uncovered the complex regulatory circuits that exist in higher plants.

FRIDAY
BREMERHAVEN
ROOM

1:15 p.m.

Identifying genes affecting locomotor behavior in *Drosophila*

MARÍA FERNANDA CERIANI, Leloir Institute Foundation, Buenos Aires, Argentina

■ In *Drosophila*, a number of key processes such as emergence from the pupal case, locomotor activity, olfaction, feeding, and aspects of mating behavior are under circadian regulation. Specifically, we are interested in unraveling the molecular mechanisms underlying the circadian control of rest/activity cycles. To that end, we have explored two complementary strategies, a forward genetic screen based on the yeast GAL4/UAS system and an oligonucleotide-based high-density array that interrogates gene expression changes at the whole genome level.

The genetic screen relies on the induction of the expression of genes tagged by the insertion of a P-element that carries a GAL4-controlled promoter, oriented so that it transcribes flanking genomic sequences and therefore most likely generating gain-of-function mutations. These “target lines” are then crossed with a “pattern line” that expresses GAL4 under the control of a specific promoter. We have generated more than 600 novel insertion lines and have monitored them for defects on rhythmic behavior using an automated locomotor activity setup. So far, we have identified both short- and long-period mutants together with arrhythmic mutants. Plasmid rescue analysis allowed the identification of the target genes. Among these, we found previously known genes never associated with clock function as well as novel genes.

In parallel, we analyzed the extent of clock-controlled transcription over a two-day time course. Among the cycling genes, we identified and characterized a cyclic potassium channel protein as a key step in linking the transcriptional feedback loop to overt behavior.

1:40 p.m.

The mechanisms of long-distance interactions and insulator action in *Drosophila melanogaster*

PAVEL GEORGIEV, Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia

P. Georgiev, E. Savitskaya, A. Golovnin, R. Kostuchenko, E. Kravchenko, O. Kirchanova, N. Gruzdeva, L. Melnikova, M. Savitsky, O. Kravchuk, A. Parshikov ■ Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia

■ Enhancers exert long-distance effects, which raises the question as to how an enhancer specifically activates its target gene without affecting adjacent genes. “Insulator” is the name given to a class of DNA sequence elements that contribute to the organization of independent domains of gene function by restricting enhancer and silencer function. The best-characterized chromatin insulator in *Drosophila* is the Su(Hw) binding region contained within the *gypsy* retrotransposon. Although cellular functions have been suggested, no role has yet been found for the multitude of endogenous Su(Hw) binding sites. Two Su(Hw) binding sites were identified in the intergenic region between the *yellow* gene and the *Achaete-Scute (AS-C)* gene complex, sites that form a functional insulator. Genetic analysis demonstrated that at least two proteins, Su(Hw) and Mod(mdg4), required for the activity of this insulator are involved in the transcriptional regulation of the *AS-C*. Several additional new insulators were identified in the regulatory region of the *Abd-B* gene. The study of the insulators in transgenic lines demonstrated that the insulators are able to block enhancers by two different mechanisms. In some cases, proteins bound to an insulator directly interact with enhancer proteins that prevent enhancer-promoter communication. The independent structural loops formed by the paired insulators can also, in some cases, prevent proper communication between an enhancer and a promoter located in the separated loops. The preliminary experimental results suggest that the insulators are also involved in supporting the long-distance interaction between enhancers and promoters.

FRIDAY
BREMERHAVEN
ROOM

2:05 p.m.

Involvement of error-prone DNA polymerase pol IV in stationary phase mutagenesis in *Pseudomonas putida*

MAIA KIVISAAR, Estonian Biocentre, Tartu, Estonia

R. Tegova, A. Tove, K. Tarassova, M. Tark,
M. Kivisaar ■ Estonian Biocentre, Tartu, Estonia

■ In nature, microbes are constantly exposed to variable and stressful environments. In a growth-restricting environment (e.g., during starvation), mutants arise that are able to take over bacterial populations by a process known as stationary phase mutation. Results of our recently published study (Saumaa et al., *J. Bacteriol.* 184:6957–6965, 2002) suggest that mutation processes in cells that have been starving for a short period are not entirely compatible with those required for prolonged starvation. We have studied the role of error-prone DNA polymerase pol IV in mutagenesis in *Pseudomonas putida*. We showed that pol IV is involved in generating –1 frameshift mutations in starving cells. Occurrence of base substitutions does not require the presence of pol IV. In contrast to mutagenesis in the classical model organism *Escherichia coli*, pol IV–dependent mutagenesis in *P. putida* appears to be a RecA-independent process. Moreover, our results demonstrate that involvement of pol IV in stationary phase mutagenesis becomes essential only in long-term-starved populations of *P. putida*, which indicates that mechanisms that are distinct from the RecA-dependent SOS response could elevate the pol IV–dependent mutagenesis in cells that have suffered a long-term starvation stress. The possibility that malfunctioning of methyl-directed mismatch repair (MMR) in starving cells might be involved in pol IV–dependent mutagenesis is under consideration. The species of *Pseudomonas* examined so far, like many other non-enteric bacteria, lack chromosomally encoded error-prone DNA polymerase pol V. Although genes encoding pol V homologues are frequently found in plasmids, bacteria lacking these plasmids also accumulate stationary phase mutations. Results of our study demonstrate that only a particular type of stationary phase mutation in *P. putida* requires pol IV activity. Hence, the question arises whether these bacteria would express some other error-prone DNA polymerase activities involved in stationary phase mutagenesis. Experiments to address this question are currently in progress.

2:30 p.m.

Novel functions of the p53 tumor suppressor

BORIS P. KOPNIN, Institute of Carcinogenesis, Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia

B. Kopnin,¹ A. Alexandrova,² P. Kopnin,³ L. Agapova,¹ A. Sablina,¹ P. Chumakov^{3,4} ■ ¹Institute of Carcinogenesis, Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia; ²A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia; ³Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia; ⁴Lerner Institute, Cleveland Clinic Foundation, Cleveland, Ohio, USA

■ The p53 tumor suppressor is functionally inactivated in the majority of human cancers. It is generally accepted that normal p53 function ensures the elimination of damaged and/or potentially dangerous cells from multicellular organisms by translating a variety of intracellular stress signals into growth arrest or apoptosis. In addition, p53 is involved in DNA repair machinery. It is believed that the abrogation of these p53 safeguard activities leads to dramatic accumulation of cells harboring various genomic abnormalities, thus increasing the probability of neoplastic cell transformation and tumor development.

We have found that in addition to control of cell cycle progression, apoptosis, and DNA repair, p53 performs other functions whose alterations can also contribute to genetic instability and/or affect tumor development. These functions include 1) the control of cell motility/migration (Sablina et al., *J. Biol. Chem.* 278:27362–27371, 2003); 2) a requirement for some specific types of cell differentiation, in particular for transdifferentiation of fibroblasts into myofibroblasts induced by Notch1 or TGF-beta signaling; and 3) the detoxication of intracellular reactive oxygen species (ROS) and prevention of ROS-induced genomic alterations.

FRIDAY
BREMERHAVEN
ROOM

MINIGRANT PRESENTATIONS (SESSION 2)

4:30 p.m.

Stabilization of the doublecortin domain through rational mutagenesis

JACEK OTLEWSKI, Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland; and **JANUSZ BUJNICKI**,* International Institute of Molecular and Cellular Biology (IIMCB), Warsaw, Poland

A. Mateja,¹ L. Jucezka,¹ T. Cierpicki,¹ J. Bujnicki,² Z.S. Derewenda,³ J. Otlewski¹ ■ ¹Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland; ²International Institute of Molecular and Cellular Biology (IIMCB), Warsaw, Poland; ³University of Virginia, Charlottesville, Virginia, USA

■ Microtubule-associated proteins (MAPs) regulate the dynamics and distribution of microtubules. A gene encoding a novel MAP was identified on chromosome X in connection with a genetic syndrome, lissencephaly in males and “double cortex” in females. The gene’s product, doublecortin, is 40 kDa in size and differs from any other MAP studied. Amino acid sequence analysis identified two homologous domains within doublecortin, known as DCX domains. Homologous DCX-tandems are also found in the doublecortin-like kinase DCLK, which harbors a Ser/Thr kinase domain downstream of the tandem. Similarities between amino acid sequences in the N- and C-terminal DCX domains suggest that the two domains should exhibit a common three-dimensional fold. Surprisingly, the recombinant C-terminal DCX domains of both doublecortin and DCLK are sensitive to proteolysis and bind to 8-anilino-1-naphthalene sulfonate (ANS); their HSQC spectra show significant line-broadening, none of them shows cooperative unfolding, and their measured Stokes radius is consistent with a molten globule. Both N-terminal DCX domains show cooperative denaturation and are resistant to proteolysis. In functional assays, the N-terminal DCX domain of doublecortin binds only to assembled microtubules, whereas the C-terminal one binds to both microtubules and unpolymerized tubulin. Homology modeling of the C-terminal domains revealed that the bulky aromatic side chains present in the core of the N-terminal domains are substituted by small aliphatic or polar side chains in the C-terminal domains. These substitutions may create a destabilizing cavity in the protein core. To verify this hypothesis, we prepared single (I190F, I203Y, A210F, V216L, and I220L) and multiple (I190F/A210F, I190F/I203Y, and I190F/I203Y/A210F) mutants of C-DCLK and studied them using various physicochemical techniques, including denaturation, gel filtration, ANS binding, and limited proteolysis. None of these mutants produced the natively folded domain. Currently, we are preparing and characterizing a more extensive set of multiple mutants, including the substitutions T189R, I191F, R198F, D222R, C244S, E274P, E276W, and C277S.

*EMBO/HHMI Award recipient

4:50 p.m.

Induction and development of the anterior neural fold in *Xenopus* embryos

ROBERTO MAYOR, University of Chile, Santiago, Chile; and **ANDREY ZARAISKY**, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

■ Neural folds are generated at the border of the neural plate, and they can be divided along the anterior posterior axis: the anterior fold will give rise to the forebrain, and the lateral and posterior fold will give rise to the neural crest. Progress has been made in the characterization of the molecules involved in neural crest induction, but not much is known about the molecules that induce the anterior neural fold.

We have studied the signals that induce the anterior neural fold. By making grafts of lateral neural folds into the anterior region of the embryo, we show that this region produces signals that inhibit neural crest induction and promote anterior neural fold development. We identified the inhibitory signal as an inhibitor of the Wnt pathway. In addition, we identified the novel small G protein, called ras-dva, which is expressed during early development of the *Xenopus* embryo in a crescent-shaped zone abutting the anterior neural plate. We demonstrated that Ras-dva is the founder of a new family of small GTPases. To understand the role of ras-dva in the development of the anterior neural plate, we investigated the effect of its down-regulation by the antisense morpholino oligos and dominant negative mutants on the expression of a panel of the neural fold genetic markers. As a result, we have evidence indicating the involvement of ras-dva in the regulatory cascade controlling development of the anterior and the anterior-lateral neural folds. Specifically, down-regulation of ras-dva functioning results in inhibition of the expression of the winged helix transcription factor bf-1, a key regulator of forebrain development, and some other neural folds markers.

FRIDAY
BREMERHAVEN
ROOM

5:10 p.m.

Bioinformatics-guided engineering of DNA methyltransferases

SAULIUS KLIMAŠAUSKAS, Institute of Biotechnology, Vilnius, Lithuania; and **JANUSZ BUJNICKI**,* International Institute of Molecular and Cellular Biology (IIMCB), Warsaw, Poland

J. Bujnicki,¹ M. Kosiński,¹ R. Gerasimaitė,² Z. Staševskij,² S. Klimašauskas² ■ ¹International Institute of Molecular and Cellular Biology (IIMCB), Warsaw, Poland; ²Institute of Biotechnology, Vilnius, Lithuania

■ Phylogenetic analysis of DNA cytosine-5 methyltransferases (MTases) was performed to identify lineages of relatively closely related proteins that nevertheless differ in their substrate specificity. Protein fold recognition analysis was carried out to align the sequences to the known crystal structures of HhaI and HaeIII MTases. The alignments served as the starting point for homology modeling of M.Bsp6I, M.HaeIII, and M.HpaII. The preliminary models were evaluated using VERIFY3D, and the best-scoring fragments were recombined. For each enzyme, the final model was used to predict the residues potentially involved in the recognition of the target DNA sequence. The individual residues as well as loops harboring them were targeted for site-directed mutagenesis experiments aimed at generating MTases with new specificities.

Another goal is to develop new molecular tools for sequence-specific covalent modification of cytosine residues in DNA. One strategy is to convert the catalytic function of the HhaI MTase such that the flipped-out cytosine bases would be enzymatically excised, thereby creating a sequence-specific cytosine DNA glycosylase, a new enzymatic activity. Considering two alternative mechanisms of enzymatic cleavage of the N-glycosidic bond, we performed rational computer-guided design of a glycosylase active site upon the methyltransferase scaffold. The first mechanism is used by uracil-DNA glycosylases and involves the nucleophilic attack of an activated water molecule on the carbon atom at the glycosidic bond. In the second mechanism, operational in alkylpurine-DNA glycosylases, the carboxyl group of an aspartate is directly responsible for the nucleophilic attack. To replicate the two mechanisms, we began introducing multiple mutations at positions C81, E119, R163, R165, T250, and N304. To date, 45 single mutants of M.HhaI were constructed, purified, and largely characterized. Based on their base-flipping and catalytic properties, best candidates are being selected for further studies to produce combinations of double and triple mutations.

**EMBO/HHMI Award recipient*

FRIDAY
BREMERHAVEN
ROOM

8:00 a.m.

Immunogenetic and gene expression analyses of *Mycobacterium tuberculosis* infection in mice

ALEXANDER S. APT, Central Institute for Tuberculosis, Russian Academy of Sciences, Moscow, Russia

T.V. Radaeva,¹ M.O. Orlova,¹ E. Schurr,² E.B. Eruslanov,¹ I.V. Lyadova,¹ K.B. Majorov,¹ T.K. Kondratieva,¹ A.S. Apt¹ ■ ¹Central Institute for Tuberculosis, Russian Academy of Sciences, Moscow, Russia; ²McGill Center for the Study of Host Resistance, Montreal, Canada

■ I/St and A/Sn mice display a severe and a mild course of tuberculosis (TB), respectively. TB response in the lungs of I/St mice is characterized by 1) prominent inflammation, 2) a decrease in type 1 cytokines, 3) impaired antimycobacterial macrophage function, and 4) marked proliferation of T lymphocytes. The multiplicity of phenotypes correspond well to a polygenic pattern of TB control previously mapped to loci on chromosomes 3, 9, 17, and X, loci that regulate disease severity. Recently, we demonstrated a striking feature of TB response in the lungs of I/St mice: prolonged and severe infiltration with neutrophils. Compared with neutrophils of A/Sn, those of I/St more readily migrated and phagocytosed mycobacteria in an opsonin-dependent manner in *in vitro* transwell and *in vivo* peritoneal inflammatory models. Given that tissue neutrophils showed negligible mycobacteriostatic capacity, we speculate that in TB-susceptible animals neutrophils play the role of a “Trojan horse,” hiding mycobacteria from the host immune system and serving as an important element of pathogenesis.

Using mRNA from I/St and A/Sn lung macrophages applied to Affymetrix array U74Av2, gene expression studies indicated that infection results in *iNOS*, *hsp70*, *IL-10Ra*, *Csf-2*, and *Mmp10* up-regulation, but in *Mmp8* down-regulation. Importantly, the expression of *IL-11* and *IL-6* genes was 12-fold and 3-fold higher, respectively, in both naive and infected I/St lung macrophages, compared with their A/Sn counterparts. We confirmed these data by RT-PCR and Taqman PCR analyses. We assume that the genetically determined high level of pro-inflammatory non-T cell cytokines in the lung is another feature of TB pathogenesis.

We continued to develop congenic mouse strains that differ in those segments of Chr. 3 and 9 that are involved in TB control. In the Chr. 3 set, genotyping of the BC4–BC6 progeny allowed us to select mice in which the linkage peak marker *D3Mit215* was separated by crossing over from the left flanking marker *D3Mit29* and the right flanking marker *D3Mit199*.

8:25 a.m.

Immunogenicity of *Trypanosoma cruzi* mutants: characterization of biochemical markers and models of protection against disease

MIGUEL A. BASOMBRÍO, National University of Salta, Salta, Argentina

M. Ciaccio,¹ A. Barrio,² M. Padilla,² M.A. Basombrío² ■ ¹Istituto di Biologia dello Sviluppo, Palermo, Italy; ²National University of Salta, Salta, Argentina

■ *Trypanosoma cruzi* establishes lifelong infection. Partial immunization can be achieved by experimental vaccines, often consisting of parasite antigens plus adjuvants. However, this protection is not long lasting and has not convincingly prevented tissue lesions or alterations in heart function. Working with animal models, we were able to establish in recent years that vaccination can indeed induce solid and long-lasting resistance against infection and disease. But the ability of a vaccine to do so seems to depend on two attributes that raise safety issues: that live parasites be present in the immunogen and that they retain, at least temporarily, some degree of infectivity. Much of this evidence was obtained using the long-term, culture-attenuated TCC strain. Recently, we started using two gene-targeted mutants of *T. cruzi*, derived by homologous recombination, as tools. A *gp72*-null strain retains the ability to grow *in vitro*, in spite of a 1745-bp deletion of coding sequence from both alleles of the gene. After eight years of laboratory propagation in the absence of antibiotics, the stability of the mutation was studied. Specific primer annealing sites and length of the construct replacing the gene were analyzed. The deleted gene was still absent; it was replaced by an antibiotic-resistance construct. In mice, the mutated parasites were unable to sustain infection, but the animals were protected against virulent *T. cruzi*. However, after one year, the protection by *gp72*-null was no longer detectable, in contrast with the persistently protective TCC strain. A second *T. cruzi* mutant was analyzed, in which one allele of the *cut* gene was deleted after homologous recombination with a neomycin-resistance construct. This mutant displayed a remarkable, though incomplete, reduction in virulence and the ability to protect against virulent challenge. The possibility of knocking out one or more genes from the TCC strain while retaining its capacity to provide long-term protection is being explored in our laboratory.

SATURDAY
HANSA ROOM

8:50 a.m.

Molecular and cellular basis of *Listeria monocytogenes* infection: new aspects

PASCALE COSSART, Pasteur Institute, Paris, France

O. Dussurget, D. Cabanes, J. Johansson, S. Sousa, H. Bierne, M. Lecuit, P. Cossart ■ Pasteur Institute, Paris, France

■ *Listeria monocytogenes* is a food-borne pathogen responsible for gastroenteritis, meningitis, septicemia, and abortions with a mortality rate of 30 percent. It has the capacity to cross three barriers during infection (the intestinal barrier, the blood-brain barrier, and the fetoplacental barrier). In all infected tissues, *Listeria* is intracellular due to its capacity to survive in phagocytic cells and also to invade and survive in nonphagocytic cells. Once inside cells, bacteria escape from the internalization vacuole and spread from cell to cell by using the now well-understood phenomenon of actin-based motility. Through a combination of cell biology, genomic, and in vivo approaches, as well as epidemiological data, our knowledge of the infectious process is improving, highlighting the many bacterial, mammalian, and environmental factors that control the success of an infection. New findings concern new virulence genes such as *bsb*, a gene encoding a bile salt hydrolase, an enzyme that so far has been identified only in bacterial commensals and allows *Listeria* to persist in the intestine; new regulatory mechanisms controlling expression of virulence genes, for example, an RNA thermosensor; new aspects of the cell biology of entry process, for example, the involvement of myosin VIIA, an unconventional myosin, in the internalin-E-cadherin interaction and of Wave and VASP in the InlB-mediated cytoskeleton rearrangements; and new epidemiological and histological data highlighting how *Listeria* crosses the physiological barriers. A complete picture of the human disease is emerging.

9:15 a.m.

Biomarkers associated with mild or severe malaria in Ugandan children

THOMAS G. EGWANG, Medical Biotechnology Laboratories, Kampala, Uganda

B.O. Apio,¹ B. Kanoi,¹ T. Horii,² T.G. Egwang¹ ■ ¹Medical Biotechnology Laboratories, Kampala, Uganda; ²Osaka University, Osaka, Japan

■ Currently, no information is available about the biochemical or molecular signatures of severe and complicated malaria or mild and asymptomatic malaria. The former accounts for 1–2 million infant deaths annually in Sub-Saharan Africa. The detection of biomarkers of severe malaria, along with traditional microscopy, would result in prompt antimalarial and antidiarrheal therapy. Our working hypothesis was that distinct biomarkers were associated with the various clinical manifestations of malaria in Ugandan children. A case-control study was carried out at Apac Hospital to identify children 6–59 months of age with severe malaria (SM) and age-matched controls with mild or asymptomatic malaria (CM). Informed consent was obtained from mothers, and blood samples were collected for serological and biochemical analyses. We used two approaches. First, a commercial antibody array (with specificities against 78 human growth factors, cytokines, and chemokines) was used to probe serum pools from SM and CM children. Eighteen biomarkers were uniquely expressed in the sera of SM children, including IL-6 and IL-10, whereas four proteins, including IL-8, were uniquely expressed in the sera of CM children. The results of the array experiments were validated using quantitative enzyme-linked immunosorbent assay (ELISA). SM children had significantly elevated levels (a fourfold increase) of MCP-1, a beta-chemokine, compared with CM children, whereas CM children had significantly elevated levels (a twofold increase) of RANTES, another beta-chemokine, compared with SM children. Second, a total lysate of *Plasmodium falciparum* (crude extract) and a recombinant construct SE36 corresponding to the amino-terminus of the serine-rich antigen (SERA5) were used as antigens, and total corresponding IgG antibodies in sera were detected by ELISA. Mean IgG anti-SE36 and anti-total lysate titers in CM children were significantly higher than those in SM children. These data support the hypothesis that distinct biomarkers are associated with the mild or severe forms of malaria in Ugandan children.

SATURDAY
HANSA ROOM

10:10 a.m.

Two-step mechanism of force generation in muscle

SERGEY Y. BERSHITSKY, Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia

S.Y. Bershitsky,¹ A.K. Tsaturyan,² N.A. Koubassova,² M.A. Ferenczi³ ■ ¹Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia; ²Institute of Mechanics, Moscow State University, Moscow, Russia; ³Imperial College of Science, Technology and Medicine, London, United Kingdom

■ In the lever arm model, muscle force results solely from a tilting of the “neck” domain of the myosin head. There are, however, experimental findings that suggest an involvement of a change in the actin-myosin interface, namely, a transition from non-stereospecific to stereospecific binding. We show here that when a temperature jump (T-jump) from 5°C to 30°C triples force in contracting muscle fibers, muscle stiffness remains unchanged, whereas a simultaneous increase in labeling of the actin helix by bound myosin heads is observed in the X-ray diffraction pattern. Thus, “locking” of weakly attached myosin heads in a state of stereospecific binding to actin is an intrinsic part of the force-generating mechanism, which itself produces an essential fraction of force. The T-jump induces a two-phase change in the intensity of the myosin M3 reflection that is sensitive to axial head movement: a quick drop followed by a larger rise with a time course similar to that of tension. These observations as well as measurements of axial head displacement using X-ray interferometry suggest that the actin-myosin motor works in two steps: a “roll and lock” transition to a stereospecifically bound state followed by lever arm tilting.

SATURDAY
HANSA ROOM

10:35 a.m.

TGF-beta signaling during skeletal muscle formation

ENRIQUE BRANDAN, Catholic University of Chile, Santiago, Chile

C. Cabello-Verrugo,¹ R. Droguett,¹ F. López-Casillas,² E. Brandan¹ ■ ¹Catholic University of Chile, Santiago, Chile; ²Institute of Cellular Physiology, National Autonomous University of Mexico, Mexico City, Mexico

■ The molecular mechanisms that control skeletal muscle differentiation are only partially known. Growth factors such as transforming growth factor (TGF)-beta are strong repressors of this process in vitro, and their depletion from myoblast culture media triggers muscle formation. However, it is not known how this process is controlled.

We have observed that myoblasts require and modulate proteoglycan expression during skeletal muscle differentiation. Among the proteoglycans, decorin and betaglycan (TGF-betaRIII) are able to bind to TGF-beta, but it is not clear how they modulate the biological activity of TGF-beta. Previously, we showed that the expression of decorin is up-regulated during myogenesis. More recently, we found that the expression of betaglycan is also up-regulated during muscle formation. Transcriptional activity of a mouse betaglycan promoter construct increases during differentiation, and the expression of MyoD and retinoic acid, both muscle formation inducers, activate betaglycan transcriptional activity. In differentiated myoblasts, ligand binding of TGF-beta to betaglycan is increased, and betaglycan is found on the cell surface of myotubes and in the extracellular matrix.

Decorin-null myoblasts obtained by stable antisense cDNA transfection are less sensitive to TGF-beta1 activity due to decreased intracellular signaling, but they show a significant increase in the binding of TGF-beta2 to betaglycan. This binding can be reversed by de novo synthesis of decorin or by adding soluble decorin. These results indicate that decorin could act as an important component for TGF-beta binding and signaling as a result of its receptors competing with betaglycan.

We have also evaluated the expression of the molecules involved in the signaling pathway that is dependent on TGF-beta during skeletal muscle differentiation. We found that signaling decreases significantly during differentiation even though the expression of the transducing receptors increases. The decrease in signaling is explained, in part, by a decrease in the expression of the smad-2 proteins and an augmented binding of TGF-beta to the extracellular matrix.

This study was also supported by Fondap and MIFAB.

MINIGRANT PRESENTATIONS (SESSION 3)

11:00 a.m.

Molecular mechanics of skeletal muscle titin and its recombinant fragments

MIKLÓS S.Z. KELLERMAYER, University of Pécs, Faculty of Medicine, Pécs, Hungary

M.S.Z. Kellermayer,¹ L. Grama,¹ A. Nagy,¹ A. Málnási-Csizmadia,^{1,2} ■ ¹University of Pécs, Faculty of Medicine, Pécs, Hungary; ²Eötvös Loránd University, Budapest, Hungary

■ Titin is a 3.0- to 3.7-MDa intrasarcomeric protein that determines the elasticity of striated muscle. The molecule is a linear chain of approximately 300 globular (Ig or FN) domains and unique sequences, most notable of which is the proline-, glutamate-, valine-, and lysine-rich PEVK domain. In various muscle types, titin isoforms of different sizes are expressed via differential splicing of these domains. The structure and elasticity mechanisms of titin's various domains and the biological rationale for their differential expression are poorly understood. We used single-molecule atomic force microscopy to investigate the mechanics of purified skeletal muscle titin, a recombinant eight-domain segment (octamer) from the differentially spliced region (155-62), and three (N-, C-terminal, and middle) approximately 700-residue-long recombinant fragments of the largest, soleus PEVK isoform. To facilitate specific and high-affinity binding of the recombinant fragments, one end of the molecules contained a His-tag that was captured on a Ni-chelate-coated surface, and the other end contained two vicinal cysteine residues captured with a gold-coated cantilever tip. From the force data of purified, full-length titin, apparent persistence lengths as long as about 1.5 nm were obtained for the single unfolded molecule. Furthermore, titin molecules may globally associate into oligomers that behave mechanically as independent wormlike chains linked in parallel. In the case of the octamer, the mean domain unfolding force, at a stretch rate of 1 micrometer/s, was 108 pN (± 50 SD). Considering that larger unfolding forces have been measured for other regions of the molecule, the 155-62 segment may be a mechanically less stable part of titin. The mechanical response of the PEVK fragments could be well fitted with the wormlike chain model. The persistence lengths ranged between 0.09 and 1.74 nm. At a stretch rate of 0.8 micrometer/s, no force hysteresis was observed, suggesting that the PEVK segment is an ideal spring.

11:30 a.m.

Genetic analysis of the role of neutrophils in *Leishmania major* infection

MARIE LIPOLDOVÁ, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic; and **GEORGE A. DOSREIS**, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

E.L. Ribeiro-Gomes,¹ H. Havelková,² E. Dráberová,² G.A. DosReis,¹ M. Lipoldová² ■ ¹Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; ²Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

■ Neutrophils play an important role in the early phases of infection by clearing invading pathogens and by producing cytokines, which influence later phases of infection development. Previously, we found that clearance of dead neutrophils regulates *Leishmania* replication in macrophages. We analyzed neutrophil clearance using selected recombinant congenic strains (RCS) of the CcS/Dem series of mice. Each CcS/Dem strain contains a different, random set of 12.5 percent genes of the donor strain STS that is resistant to *L. major* infection and 87.5 percent of the susceptible background strain BALB/c. We compared survival of *Leishmania* parasites in macrophages only with the survival in macrophages cocultured with apoptotic neutrophils. There were more surviving parasites in the STS than in the BALB/c macrophages. Addition of apoptotic neutrophils led to a decrease of parasite numbers in the STS macrophages, whereas the number of parasites in BALB/c macrophages increased. Some RC strains exhibited higher levels of parasite survival than any of the parental strains, whereas others showed lower levels. Although we did not observe a simple correlation between parasite growth and susceptibility to *L. major*, CcS-16, the most susceptible RC strain, had the highest parasite load both in macrophages alone and with neutrophils. We observed four basic patterns of pathogen clearance: low load in macrophages, increase after addition of neutrophils; low load and decrease; high load and steady; and high load and decrease. The relationships between these patterns and both the internal macrophage structures and production of TGF-beta and TNF-alpha are currently being studied.

SATURDAY
HANSA ROOM

MINIGRANT PRESENTATIONS (SESSION 3)

11:50 a.m.

Role of lipid-activated nuclear receptors in lipid body formation induced by *Mycobacterium bovis* BCG

LÁSZLÓ NAGY, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary; and PATRÍCIA T. BOZZA, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

P.T. Bozza,¹ P.E. Almeida,¹ C. Maya-Monteiro,¹ D. Torocsik,² A. Szanto,² L. Nagy² ■ ¹Oswaldo Cruz Institute, Rio de Janeiro, Brazil; ²University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

■ Macrophages have important roles in both lipid metabolism and inflammation and are central to the defense against intracellular pathogens. Lipid-laden (foamy) macrophages are present in *Mycobacteria* infection, but their significance is not understood. Infection by *M. bovis* BCG induced a dose- and time-dependent increase of lipid bodies (LB) in macrophages. Newly formed LB were shown to act as structurally distinct intracellular sites for prostaglandin and leukotriene synthesis during BCG infection. In spite of advances in the understanding of the roles of leukocyte LB in arachidonate metabolism, little is known about the molecular mechanisms underlying the formation of leukocyte LB. In view of the role of lipid-activated nuclear receptors (peroxisome proliferator activated receptor [PPAR] and liver X receptor [LXR]) in lipid metabolism, macrophage differentiation, and inflammation control, we investigated the role of these nuclear receptors in LB formation induced by BCG. In vitro infection of mouse peritoneal macrophages with BCG induced an increase in PPAR γ expression as detected by Western blots, whereas PPAR γ was undetectable in nonstimulated macrophages. The increased PPAR γ protein expression parallels BCG-induced LB formation. The role of PPAR γ in LB formation was investigated with synthetic ligands. PPAR γ agonists potentiated LB formation, whereas a specific PPAR γ antagonist significantly inhibited BCG-induced LB formation.

The mRNA level of the oxysterol receptor LXR α is rapidly induced to high levels in ex vivo cultures of mouse peritoneal macrophages. Interestingly, BCG infection interferes with this induction; thus infected macrophages contain barely detectable LXR α levels compared with control macrophages. These data suggest that LXR-regulated gene expression is likely to be attenuated in infected macrophages.

Our results indicate that BCG infection of macrophages significantly alters nuclear receptor expression levels and that lipid-activated nuclear receptors might modulate lipid body formation in macrophages during mycobacterial infection.

12:10 p.m.

Protection against blood-stage malaria induced by DCtag-MSP4/5 vaccination: engagement of multiple immune effector mechanisms promotes “sterile” protection

MAGDALENA PLEBANSKI, Austin Research Institute, Melbourne, Australia; and ROSS LEON COPPEL, Monash University, Melbourne, Australia

M. Plebanski,¹ D.S. Pouniotis,¹ O. Proudfoot,¹ J. Hanley,¹ R.L. Coppel² ■ ¹Austin Research Institute, Melbourne, Australia; ²Monash University, Melbourne, Australia

■ Malaria affects approximately 500 million people each year, causing an estimated 2 million deaths. There is no effective human vaccine. A protein family expressed during blood-stage malaria and comprising the merozoite surface protein 4 (MSP4) and 5 (MSP5) of *Plasmodium falciparum* and the MSP4/5 of *P. yoelii* and *P. chabudai* was identified as targeted by protective antibodies. The MSP4 and MSP5 proteins display less variability than other vaccine candidate proteins and are likely to provide good population coverage against variant parasites in endemic areas.

To induce protective immunity, it is necessary to administer antigens with suitable adjuvants carriers. We observed that crude parasite lysate conjugated to a carrier that delivers antigen to dendritic cells (DC) in vivo (DCtag) induces powerful protective immunity against malaria, protecting fully 80–100 percent of mice against lethal *P. yoelii* 17XL and *P. chabudai* challenge and conferring long-lasting protection (four months) after a single vaccination. The present study assessed the immunogenicity (humoral and cellular) and protective efficacy against lethal malaria challenge of immunization with DCtag-MSP4/5 conjugates in mice.

Immunization with DCtag-MSP4/5 conjugates induced specific IgG titers comparable to optimal protocols using leading experimental adjuvants restricted to murine use, such as Freund's adjuvant. In contrast to Freund's, DCtag did not induce any local inflammation or systemic side effects. In addition to a strong humoral response, DCtag-MSP4/5 also induced high levels of T cells able to secrete interferon- γ (IFN- γ) and interleukin-4 (IL4) (over 100 antigen-specific cells/million spleen cells). DCtag-MSP4/5-immunized animals were challenged with a lethal dose of *P. yoelii* XL parasites. DCtag-MSP4/5 conferred levels of protection comparable to Freund's/MSP4/5 (80–100 percent protection). Moreover, most DCtag-MSP4/5-immunized animals but not Freund's/MSP4/5-immunized animals failed to develop any parasitemia (“sterile” protection), showing that DCtag can induce unusually solid protection. These results support development of DCtag-MSP4 and -5 conjugates for human vaccination.

SATURDAY
HANSA ROOM

12:30 p.m.

Functional relevance of trans-spliceosomal and ribosomal trypanosome proteins as assessed using two-hybrid and RNAi systems

MARIANO J. LEVIN, Institute for Research on Genetic Engineering and Molecular Biology, CONICET, Buenos Aires, Argentina; and **SHULAMIT MICHAELI**, Bar-Ilan University, Ramat-Gan, Israel

M.J. Levin,¹ V.M. Pablo,¹ B. Natalia,¹ Y.A. Maximiliano,¹ A. Catalina,¹ F. Caro,¹ N. Benson,¹ M. Daniel,²

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²International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya; ³Bar-Ilan University, Ramat-Gan, Israel

■ Both the trypanosomes *Trypanosoma brucei* and *T. cruzi* have evolved specific variations of common eukaryotic mechanisms, such as trans-splicing and translation. Given that 1) trypanosome versions of ubiquitous proteins harbor significant differences with respect to their mammalian counterparts and that 2) protein-protein interactions are crucial for the integrity of multicomponent enzymatic machines, we reasoned that key parasite proteins involved in essential steps of spliceosomal and/or ribosomal assembly would be appropriate targets for a functional genomic generation of antiparasitic drugs.

Initially, we identified, in trypanosome genomic data banks, *T. cruzi* and *T. brucei* orthologues of the components of the above-mentioned protein complexes using as query sequences those of human and/or yeast proteins. To date, more than 45 proteins linked to trans-splicing, polyadenylation, or RNA degradation have been identified for *T. cruzi*. More than 30 have been cloned into different vectors of the Gateway system. Similarly, more than 40 ribosomal proteins of *T. cruzi* have been identified and cloned. Already, certain protein-protein interactions have been mapped, and the construction of a partial protein-protein interaction map for these complexes is under way. The genes selected for *T. cruzi* have been used to find and clone the corresponding ones in *T. brucei*.

The functional relevance of these proteins is being tested by analyzing the loss-of-function phenotypes generated using the inducible RNAi system developed for *T. brucei*. This approach is required because the RNA-mediated interference mechanism is not present in *T. cruzi*.

SATURDAY
HANSA ROOM

8:00 a.m.

Endocannabinoid modulation of cortical circuits linked to anxiety

TAMÁS F. FREUND, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

T.F. Freund, J. Halle, N. Hájos, I. Katona ■ Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

■ Convergence of unique neurochemical and connectivity features suggests that the cholecystinin (CCK)-containing subset of GABAergic interneurons is involved in the fine-tuning of cortical network activity patterns and that malfunctioning of these interneurons may lead to mood disorders, most notably anxiety. These neurons were shown earlier to selectively express CB1 cannabinoid receptors on their axons; therefore, their role in anxiety-like behaviors can be investigated by using cannabinoid agonists and antagonists, as well as wild-type and CB1-knockout animals, in anxiety tests such as the social interaction task and the elevated plus-maze. CB1-knockout mice exhibited reduced exploration of the open arms of the plus-maze apparatus as well as their partner in the social interaction paradigm: this strain thus appeared more anxious than the wild type. We hypothesized that the conflicting data in the literature about cannabinoid actions on behavior may be explained by the simultaneous modulation of GABAergic and glutamatergic transmission via CB1 and a novel CB receptor, respectively. The hypothesis is based on our earlier evidence that glutamatergic EPSCs are reduced by cannabinoids to a similar extent. However, this effect remains unchanged in CB1-knockout mice and is thus likely mediated by a novel cannabinoid receptor. To further test the hypothesis, we administered the cannabinoid antagonist SR141716A (0, 1, and 3 mg/kg) to both CB1-knockout and wild-type mice. The cannabinoid antagonist SR141716A reduced anxiety in both wild-type and CB1-knockout mice, and the anxiety scores returned to control values in the knockouts. Given that SR141716A binds to both CB1 and the putative novel receptor, our data in the CB1-knockout mice suggest that while CB1 receptors, which selectively modulate CCK-containing interneurons in the cerebral cortex, are anxiolytic the new CB receptor located on glutamatergic terminals is anxiogenic. Selective blockade of this receptor may represent an ideal new target for the pharmacotherapy of anxiety.

**SATURDAY
BREMERHAVEN
ROOM**

8:25 a.m.

P_{2Y} receptor-mediated signaling in mouse taste cells

STANISLAV S. KOLESNIKOV, Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia

S.G. Barishnikov, O.A. Rogachevskaja, Y.E. Yatzenko, S.S. Kolesnikov ■ Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia

■ We found pharmacological evidence that mouse TRCs express P_{2Y} receptors coupled to the phosphoinositide pathway (P_{2Y} receptor/PLC/IP₃ generation/Ca²⁺ release/Ca²⁺ influx). In particular, extracellular ATP and some other P_{2Y} agonists mobilized cytosolic Ca²⁺, and ATP responses were impaired dramatically both by U-73122, a phospholipase C (PLC) inhibitor, and by the IP₃ receptor inhibitor 2-APB. Data obtained favor SOC channels rather than receptor-operated channels as a pathway for Ca²⁺ influx that accompanies Ca²⁺ release. Evidence suggests that intracellular Ca²⁺ mobilized by ATP is mainly extruded by the plasma membrane Ca²⁺-ATPase, while the relative contribution of Na⁺/Ca²⁺ exchange, reticular Ca²⁺-ATPase, and mitochondria to Ca²⁺ clearance accompanying ATP response deactivation appears to be negligible. The PKA inhibitor H-89 enhanced ATP responses, indicating that activation of the phosphoinositide cascade involved is controlled by cAMP-dependent phosphorylation. We also identified two ATP-sensitive TRC subpopulations, one expressing VG Ca²⁺ channels and the other Ca²⁺-activated Cl⁻ channels. ATP closes the Ca²⁺ channels; this regulation may underlie negative feedback that tunes neurotransmitter (i.e., ATP) release. By mobilizing intracellular Ca²⁺, ATP activates the Cl⁻ channels. ATP-responsive taste cells are abundant in circumvallate, foliate, and fungiform papillae. Taken together, our observations point to a putative role for ATP as a neurotransmitter operative in taste buds of rodents, if not all mammals.

8:50 a.m.

Opioids directly inhibit peripheral nociceptors: effect on P2X receptors

OLEG A. KRISHTAL, Bogomoletz Institute of Physiology, Ukrainian Academy of Sciences, Kiev, Ukraine

N. Mamenko, E. Yudin, I. Prudnikov, I. Chizhnikov, Z. Tamarova, O. Krishtal ■ Bogomoletz Institute of Physiology, Ukrainian Academy of Sciences, Kiev, Ukraine

■ Opioids are believed to exert their analgesic effects predominantly in the central nervous system. In the primary sensory system, opioids are known to inhibit sodium and calcium channels while facilitating some potassium channels. We have found that Leu-enkephalin (in the concentration range 10^{-9} – 10^{-6} M) and other opioids inhibit P2X (ATP-activated) receptors in the membranes of mammalian sensory neurons isolated from dorsal root and nodosal ganglia of rat. Depending on the concentration of Leu-enkephalin, the effect develops within 1–10 minutes, indicating a possible G protein-mediated action of opioid receptors. Inhibition is partial (30–80 percent), partially reversible, and is prevented in the presence of naloxone. Similar effects are elicited by morphine. Both P2X3 and P2X2/3 receptor-mediated responses are subject to the blocking action of opioids. P2X receptors serve as nociceptors at the periphery. We wished to determine whether the responses of peripheral pain-sensing (nociceptive) fibers to ATP can also be blocked by opioids. To address this question, we used a skin–n. saphenous preparation dissected in vitro from the hairy skin of the hind paw of adult rats to investigate the action of ATP and opioids on the responses of peripheral sensory nerve endings. Altogether, 72 fibers were subjected to the action of ATP, which induced excitation in 28 sensory fibers. The majority (72 percent) were nociceptive C-fibers. We have found that, in 19 out of 23 tested ATP-sensitive fibers, Leu-enkephalin strongly inhibited ATP-induced activation; naloxone partially reversed this inhibitory effect. Possible involvement of P2Y receptors was excluded by using the selective P2X agonist α , β -methylene-ATP. This is a first indication of the direct effect of opioids on the system of primary nociception. The blood-brain barrier-impermeable opioid agonists may thus function as painkillers that will free the use of opiates from the major problem of abuse.

9:15 a.m.

Altered synaptic vesicle function in *ipp*, a *Drosophila* mutant of inositol metabolism, is mimicked by lithium and rescued by exogenous inositol

PEDRO LABARCA, Center for Scientific Studies,* Valdivia, Chile

■ In addition to their roles in intracellular signaling, phosphoinositides (PI) are important regulators of membrane traffic at the synapse. We used FM1-43 imaging to investigate synaptic vesicle (SV) function at presynaptic terminals of *ipp*, a *Drosophila* mutant of the lithium-sensitive enzyme inositol polyphosphate 1-phosphatase. This enzyme participates in the recovery of inositol, is needed for PI synthesis from inositol polyphosphates, and is a putative target of lithium in managing bipolar disorder. The mutation in *ipp*, as well as lithium, would be expected to deplete inositol, thus depressing PI-dependent mechanisms. Previously, *ipp* synapses were reported to exhibit abnormal neurotransmitter release properties, which were mimicked by lithium in normal synapses. We found that synaptic vesicle trafficking is grossly impaired in *ipp*, and this deficiency was mimicked by lithium in normal synapses. Exogenous inositol restored to normal SV trafficking as well as synaptic transmission in *ipp* and prevented the effects of lithium in normal individuals. Our results provide the first evidence that inositol depletion can alter SV trafficking and neurotransmitter release at the synapse and point to PI-dependent regulation of SV function at nerve endings as a target of mood stabilizers.

*The center is a Millennium Institute.

SATURDAY
BREMERHAVEN
ROOM

10:10 a.m.

Mechanisms of leukocyte lipid body formation and function in innate immunity to intracellular pathogens

PATRICIA T. BOZZA, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

H. D'Ávila,¹ R.C.N. Melo,² H.C. Castro-Faria-Neto,¹ P.T. Bozza¹ ■ ¹Oswaldo Cruz Institute, Rio de Janeiro, Brazil; ²Federal University of Juiz de Fora, Juiz de Fora, Brazil

■ Lipid bodies are rapidly inducible, lipid-rich cytoplasmic domains and sites for eicosanoid-forming enzyme localization, which may have specific roles in enhanced inflammatory mediator production during pathological conditions. We have investigated the mechanisms involved in lipid and protein coalescence to form lipid bodies and their functions as compartmentalization sites for augmented eicosanoid generation by leukocytes infected by *Mycobacterium bovis* BCG or *Trypanosoma cruzi*. Experimental infection by *T. cruzi* or BCG induced a dose- and time-dependent increase in the number and size of lipid bodies in infected leukocytes. Pathogen-induced lipid body formation was drastically reduced in toll-like receptor (TLR)2-deficient but not in TLR4-deficient mice, demonstrating a requisite role for TLR2 receptors in signaling to induce lipid body formation. Macrophage lipid bodies observed after 24 hours of infection were often localized in close proximity with digest vacuoles, suggesting an association between these structures. The increase in lipid bodies during infection correlated with increased generation of PGE₂ and localization of COX-2. Moreover, we demonstrated by intracellular immunofluorescent localization of newly formed eicosanoid that lipid bodies were the predominant sites of PGE₂ synthesis in activated macrophages. It has been previously demonstrated that uptake of apoptotic cells exacerbates parasite replication in cocultured macrophages infected with *T. cruzi* in a PGE₂- and TGFβ-dependent manner. Interestingly, lipid body formation induced by *T. cruzi* infection in macrophages was markedly enhanced by coculture of apoptotic cells. Apoptotic cells or anti-α_v antibody alone also induced lipid body formation accompanied by enhancement in PGE₂ production as well as expression of COX-2 with colocalization within lipid bodies. In conclusion, *T. cruzi* or BCG infection and apoptotic cell uptake induced lipid body formation with increased generation of PGE₂ and localization of COX-2, suggesting that lipid bodies may have roles in the heightened eicosanoid production observed during infection with intracellular pathogen infection.

10:35 a.m.

Signaling assemblies formed in mast cells activated via Fc-epsilon receptor I dimers

PETR DRÁBER, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

L. Dráberová, P. Lebduška, I. Hálová, P. Tolar, J. Štokrová, H. Tolarová, J. Korb, P. Dráber ■ Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

■ Aggregation of Fc-epsilon receptor I (Fc-epsilonRI) on mast cells and basophils induces tyrosine phosphorylation of numerous substrates, formation of signaling assemblies, and release of preformed allergy mediators. The minimal requirement for receptor aggregation is its dimerization. In an attempt to determine biochemical and other events in cells activated via dimerized Fc-epsilonRI, we analyzed rat basophilic leukemia cells stimulated by exposure to dimerizing anti-Fc-epsilonRI monoclonal antibodies and compared them with cells triggered by extensive aggregation of the receptor-immunoglobulin E (IgE) complexes by antigen or anti-IgE antibodies. We have found that, in contrast to extensively aggregated Fc-epsilonRI, receptor dimers 1) induced less pronounced association of Fc-epsilonRI with glycosphingolipid-enriched membrane microdomains; 2) slightly delayed tyrosine phosphorylation and membrane recruitment of several signaling molecules, including growth factor receptor-bound protein 2 (Grb2) and Grb2-associated binder 2; 3) contributed to slower formation of signaling assemblies containing phosphatidylinositol 3-kinase and associated proteins; 4) triggered a slower but more sustained increase in the concentration of free cytoplasmic calcium; 5) induced degranulation that was not inhibited at higher concentrations of the cross-linking monoclonal antibodies; 6) promoted less extensive production of filamentous actin after Fc-epsilonRI cross-linking; and 7) did not produce significant changes in the topography of Fc-epsilonRI and Grb2, as detected by immunogold electron microscopy on membrane sheets. Thus, despite striking differences in the topography of Fc-epsilonRI dimers and multimers, biochemical differences were less pronounced. The combined data suggest that activated mast cells propagate signals from signaling assemblies formed around dimerized/ oligomerized Fc-epsilonRI. Although these domains are very small, they harbor all components necessary for signal propagation. The formation of large Fc-epsilonRI aggregates in osmiophilic membranes promotes both strong receptor triggering and rapid termination of the signaling responses by receptor internalization. Slow internalization of Fc-epsilonRI dimers could explain a more sustained response.

SATURDAY
BREMERHAVEN
ROOM

MINIGRANT PRESENTATIONS (SESSION 4)

11:00 a.m.

Electrogenic electron and proton transfer by cytochrome *c* oxidase

ALEXANDER A. KONSTANTINOV, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

S.A. Siletsky,¹ N.A. Azarkina,¹ R.B. Gennis,² A.A. Konstantinov¹ ■ ¹A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia; ²University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

■ The mechanism of vectorial electrogenic electron and proton transfer at different steps of the cytochrome *c* oxidase (COX) catalytic cycle has been investigated with *aa*₃-type oxidase from bovine mitochondria and from the bacterium *Rhodobacter sphaeroides*. A time-resolved electrometric technique of monitoring membrane potential generation has been combined with laser flash-induced injection of single electrons into COX pre-equilibrated at different initial redox states. Transfer of the fourth electron to oxygen (the ferryl-oxo to oxidized transition) specifically requires operation of the so-called D-channel and is highly sensitive to replacements of conserved amino acid residues in this channel. The data are consistent with a sequence of proton transfer steps: water to D132 to N139 . . . to E286 to binuclear oxygen-reducing site. Deprotonation of E286 by the binuclear site is likely to be the rate-limiting step of the overall catalytic cycle, followed by rapid reprotonation of E286 by D132, probably via N139. In contrast, the “K-channel” is operative at the early steps of the catalytic cycle (transfer of the first two electrons) and is shown to be required, in particular, for the proton-dependent splitting of bound peroxide in the oxygen-reducing site. Zn²⁺ has been found to inhibit proton pumping by COX, probably by blocking the output protonic channel. Characteristics of the oxygen-reducing sites in the *bd*-type and *ba*₃-type oxidases and the mechanisms of membrane energization by the oxidases may differ significantly from those in the *aa*₃-type oxidases.

11:30 a.m.

Suppression of allograft rejection by the inhibition of apoptosis

ROBERT G. KORNELUK, University of Ottawa, Ottawa, Canada; and **R. CHRIS BLEACKLEY**, University of Alberta, Edmonton, Canada

P. Liston,¹ C. Bleackley,² R.G. Korneluk^{1,3} ■ ¹Apoptosis Research Center, Children's Hospital of Eastern Ontario, Ottawa, Canada; ²University of Alberta, Edmonton, Canada; ³University of Ottawa, Ottawa, Canada

■ Our laboratories have overlapping interests in the control of apoptosis and immunology. We are exploring the consequences of apoptosis suppression on T cell function and tolerance. Cellular transplants (islets) are gaining currency in treating diabetes, while stem cell therapy is being proposed in a variety of neurodegenerative conditions, including Parkinson's disease, multiple sclerosis, and Alzheimer's disease. In most cases, chronic immunosuppression is currently required for long-term survival of transplanted cells or organs in humans. We have discovered, in collaboration with Dr. Bruce Verchere at the University of British Columbia, that non-strain-matched islet grafts are tolerated in the kidney capsule if apoptosis is suppressed. We are extending these results in order to test the hypothesis that failure to eliminate grafted cells results in tolerance. To explore this possibility, we are examining the fate of allogeneic fibroblast cells in a subcutaneous graft model. Fibroblasts were transduced with an adenoviral vector expressing secreted alkaline phosphatase (SEAP) alone or in combination with the X-linked inhibitor of apoptosis (XIAP), the most potent of the IAP family of caspase inhibitors. Graft survival was monitored by serum levels of SEAP, and the activation state of the host's splenic lymphocyte population assessed in cytotoxic T-lymphocyte (CTL) assays. The results of these studies will be presented.

SATURDAY
BREMERHAVEN
ROOM

MINIGRANT PRESENTATIONS (SESSION 4)

11:50 a.m.

Development of a new antigen delivery system based on a polymeric bacterial protein carrier to improve the anticysticercosis vaccine

EDDA SCIUTTO, Institute of Biomedical Research, National Autonomous University of Mexico, Mexico City, Mexico; and **FERNANDO GOLDBAUM**, Leloir Institute Foundation, Buenos Aires, Argentina

E. Sciotto,¹ G. Fragoso,¹ G. Rosas,² C. Cruz,¹ A. Toledo,¹ F. Goldbaum³ ■ ¹National Autonomous University of Mexico, Mexico City, Mexico; ²Autonomous University of the State of Morelos, Cuernavaca, Mexico; ³Leloir Institute Foundation, Buenos Aires, Argentina

■ New generation subunit vaccines frequently exhibit low immunogenicity. In this study, a novel delivery system based on the use of polymeric enzyme carrier (lumazine synthase from *Brucella spp.* [BLS]) was evaluated. KETc1, one of the well-characterized peptides that constitute the vaccine against *Taenia solium* pig cysticercosis was used for this purpose. A chimeric *Brucella* enzyme carrying KETc1 (APM-STPSATSVR) was prepared. The linear KETc1 peptide was also synthesized by stepwise solid-phase synthesis. The immunogenicity and protective capacity of BLS alone, the chimeric enzyme (LSKETc1), and the linear peptide KETc1 are being studied in mice. BALB/cAnN mice were immunized with each one of the three immunogens. KETc1-immunized mice produced antibodies that specifically recognized KETc1 only when expressed in LSKETc1, indicating that, when expressed as a chimera with BLS, the peptide's antigenicity increases. LSKETc1 is also immunogenic because it elicited high levels of specific antipeptide antibodies that remain active after BLS inhibition. A specific proliferative response was detected by in vitro priming with LSKETc1 of splenocytes derived from KETc1 or LSKETc1-immunized BALB/cAnN mice. To further evaluate the role of MHC in splenocyte stimulation, we used congenic BALB/c, BALB/B, and BALB/K as well as C57BL6J and the KOIC57BI6J (knockouts in B2 microglobulin) mice. We found that the presentation of KETc1 occurs in the context of the different H2 haplotypes and is mediated by class II molecules.

In addition, sc immunization using the linear and chimeric form of KETc1 induced a high level of protection against murine cysticercosis. Oral administration of LSKETc1 also proved to induce a high level of protection, even higher than the response induced using cholera toxin as adjuvant.

These results point to BLS as a potent new delivery system for the improvement of different subunit vaccines; BLS thus represents a promising adjuvant to improve immunogenic capacity when given orally.

12:10 p.m.

Toward engineering of restriction enzymes

VIRGINIJUS SIKSNYS, Institute of Biotechnology, Vilnius, Lithuania; and **JACEK OTLEWSKI**, Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland

V. Stonyte,¹ D. Drulis,² J. Otlewski,² V. Siksnys¹ ■ ¹Institute of Biotechnology, Vilnius, Lithuania; ²Institute of Biochemistry and Molecular Biology, University of Wrocław, Wrocław, Poland

■ Restriction endonucleases recognize short nucleotide sequences usually 4 to 8 bp in length and cleave phosphodiester bonds within or close to their recognition sites. Due to their unique specificity, restriction enzymes have gained widespread application as indispensable tools for the in vitro manipulation and cloning of DNA. Engineering of new specificities of restriction enzymes therefore becomes a challenging goal. The phage display method has been successfully applied for engineering of DNA-binding proteins. Application of the phage display technology for the redesign of specificity of restriction enzymes, however, has one principal limitation. Orthodox Type II endonucleases are generally homodimeric proteins that interact with symmetrical recognition sites. The DNA recognition interface of Type II enzymes usually comprises amino acid residues from both subunits. Moreover, dimerization and DNA recognition interfaces are often closely intertwined. This creates difficulties in the phage display and selection strategies for orthodox restriction enzymes. The Type II enzymes, however, do not all look alike. Enzymes belonging to the Type IIS subset recognize non-palindromic sequences and cleave both strands at fixed locations downstream of the recognition site. The latter enzymes often comprise the separate DNA-binding and cleavage domains. Such modular organization of the Type IIS enzymes makes them a challenging target for protein design using phage display technology. To explore the usefulness of the phage display for the redesign of restriction enzymes, we cloned the DNA-binding domain of the modular Type IIS restriction endonuclease, expressed it on the surface of the filamentous phage, and selected it by affinity binding using an appropriate oligonucleotide immobilized on a solid support. This finding opens the perspective for generating protein variants on the phage particles and selecting mutants with altered specificity.

SATURDAY
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12:30 p.m.

Structural studies on the EST3 protein subunit of *Saccharomyces cerevisiae* telomerase

VLADIMIR I. POLSHAKOV, Center for Drug Chemistry, Moscow, Russia; and **OLGA ANATOLIEVNA DONTSOVA**, Department of Chemistry, Moscow State University, Moscow, Russia

Y.S. Sharanov,¹ A.V. Shirokaya,^{1,2} M.E. Zvereva,¹ O.A. Dontsova,¹ V.I. Polshakov² ■ ¹Department of Chemistry, Moscow State University, Moscow, Russia; ²Center for Drug Chemistry, Moscow, Russia

■ Telomerase is an important eukaryotic ribonucleoprotein enzyme that is required to complete replication of chromosome termini known as telomeres. Despite the great importance of the enzyme in the regulation of cell division, very little is known about the telomerase reaction cycle at the molecular level. The subject of this study is the telomerase from yeast *Saccharomyces cerevisiae*. This enzyme complex contains the three protein subunits EST1, EST2, and EST3 and the tightly associated RNA subunit TLC1. The roles of first two protein subunits and RNA have been investigated; however, very little is known about the possible function of EST3, the third protein component of telomerase complex. Deletion of this protein subunit causes progressive telomere shortening. EST3 protein exists in the cell in two different forms: long (20 kDa) and short (11 kDa). The long form appears as a result of the

frameshift at the stop codon in the short form. Only the long form was shown to be essential for maintaining telomere length. The functional role of the short form of EST3 in regulation of cell division remains unclear.

The short form of EST3 containing an additional six-His tail at the N-terminus was expressed in *Escherichia coli* and isolated under native conditions. Three different samples of protein were prepared for NMR studies: unlabeled and ¹⁵N- and ¹⁵N/¹³C-labeled.

NMR spectra show that protein is well structured and contains both beta-strand and alpha-helical elements in the secondary structure. Assignment of ¹H, ¹⁵N, and ¹³C signals is now in progress. Further analysis of NOESY spectra will allow us to obtain the distance restraints necessary for subsequent solution structure determination. The obtained structure should help us understand the functional role of the EST3 domain in maintaining telomere length.

SATURDAY
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