3RD INTERNATIONAL MITOCHONDRIA MINISYMPOSIUM

MITOCHONDRIA AND THEIR PROTEOMICS

Natcher Conference Center (Building 45) ♦ Bethesda, Maryland

January 9–11, 2008

Sponsors

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January 9–11, 2008

Agenda

Wednesday, January 9, 2008

7:00 a.m. Poster Setup

8:15 a.m. Introductions and Meeting Kickoff
Meeting Chair: *Douglas C. Wallace, University of California, Irvine*

SESSION 1: DYNAMICS OF MITOCHONDRIAL PROTEOMES IN CARDIOVASCULAR DISEASES

8:30 a.m. **Dynamics of Mitochondrial Proteomes in Cardiovascular Diseases**
Chair: *Peipei Ping, University of California, Los Angeles*

8:45 a.m. **The Systems Biology of Mitochondria**
Robert Balaban, National Heart, Lung, and Blood Institute

9:00 a.m. **Proteomic Approaches to Organelles in the CV System**
Jan Schnitzer, Sidney Kimmel Cancer Center and University of California, San Diego

9:15 a.m. **The Phosphor Proteome of Cardiac Mitochondria in Heart Failure**
Jenny Van Eyk, Johns Hopkins University

9:30 a.m. **The MPT Pore in Cardiac Diseases**
Christopher Baines, Cincinnati Children's Hospital Medical Center

9:45 a.m. **NIH Peer Review and the Finer Points of Grant Applications**
Cathleen Cooper, NIH Center for Scientific Review

The NIH Institute/Center Locus of Review Perspective
Gerald McLaughlin, National Institute on Drug Abuse

10:15 a.m. **BREAK**

SESSION 2: MITOCHONDRIAL PROTEOMICS

10:30 a.m. **Mitochondrial Proteomics by Antibody Arraying of Functional Complexes**
Chair: *Rod Capaldi, MitoSciences*

10:45 a.m. **Cellular Bioenergetics Assays**
David Ferrick, Seahorse Bioscience

11:00 a.m. **Multi-Disease Collaboration in Mitochondrial Research**
Ronald Bartek, President, Friedreich's Ataxia Research Alliance

11:15 a.m. **Altered Gene Expression and Oxidative DNA Damage in Peripheral Blood Cells from Friedreich's Ataxia Patients**
Bennett Van Houten, National Institute of Environmental Health Sciences

11:30 a.m. **Research Opportunities at the National Cancer Institute: Using Mitochondrial Genomic and Proteomic Information**
Mukesh Verma, Director, Epidemiology and Genetics Research Program, National Cancer Institute

SELECTED ABSTRACT PLATFORM PRESENTATIONS

11:45 a.m. **Effects of Middle Domain Interactions and Posttranslational Modifications on Drp1 Function and Mitochondria Morphology**
Chunag-Rung Chang, National Institute of Neurological Disorders and Stroke

11:55 a.m. **Cytoskeleton Regulates Mitochondria Respiration Through the Tubulin-VDAC Direct Interaction**
Tatiana Rostovtseva, National Institute of Child Health and Human Development

12:05 p.m. **Biochemical Characterization of Alpers Mutants in Human DNA Polymerase g**
Rajesh Kasiviswanathan, National Institute of Environmental Health Sciences

12:15 p.m. **LUNCH AND POSTERS**

SESSION 3: NINR SESSION. BIOMARKERS IN FATIGUE AND MITOCHONDRIAL DAMAGE—BENCH TO BEDSIDE AND BACK
Chair: *Joachim Voss, University of Washington*

1:15 p.m. **Conceptual Issues of Biomarkers for Fatigue**
Judith Payne, Duke University

1:30 p.m. **Inflammatory Responses in Muscle During Chemotherapy in a Mouse Model**
Lisa Wood, Oregon Health & Science University

1:45 p.m. **Sarcopenia and Proteolysis in Muscle of a Cancer Mouse Model**
Donna McCarthy, Ohio State University

2:00 p.m. **Mitochondrial Damage and Fatigue in HIV**
Joachim Voss, University of Washington

2:15 p.m. **POSTERS AND TRAVEL TO BUILDING 10/MASUR AUDITORIUM**

3:00 p.m. **WEDNESDAY AFTERNOON LECTURE: LAURIE S. KAGUNI (MICHIGAN STATE UNIVERSITY): PROTEIN DYNAMICS AT THE MITOCHONDRIAL REPLICATION FORK (RECEPTION FOLLOWING)**

Thursday, January 10, 2008

SESSION 4: INTERGENOMIC CROSS-TALK BETWEEN THE MITOCHONDRIA AND THE NUCLEUS

8:00 a.m. **Intergenomic Cross-Talk Between Mitochondria and the Nucleus and Its Role in Tumorigenesis**
Chair: *Keshav K. Singh, Roswell Park Cancer Institute*

8:15 a.m. **Multitasking in the Mitochondrion by the Lon Protease in Protein and mtDNA Quality Control**
Carolyn Suzuki, University of Medicine and Dentistry of New Jersey

8:30 a.m. **Regulation of Mitochondrial Homeostasis and Gene Expression by the ATM and TOR Signaling Pathways**
Gerald S. Shadel, Yale University

8:45 a.m. **Mitochondrial DNA Mutations as Biomarkers for Early Cancer Detection**
Paul Wagner, National Cancer Institute

SELECTED ABSTRACT PLATFORM PRESENTATIONS

8:55 a.m. **Innate Ability of Mammalian Mitochondria to Import tRNAs by a Mechanism Distinct from Protein Import**
Juan D. Alfonzo, Ohio State University

9:05 a.m. **Mitochondrial Localization of Mammalian Ribonuclease H1**
Yutaka Suzuki, National Institute of Child Health and Human Development

9:15 a.m. **Biochemical Characterization of the Mitochondrial RNA Polymerase**
Jamie J. Arnold, Pennsylvania State University

9:25 a.m. **Pathogenic Mutations in PEO1 Cause Biochemical Defects in the Human Mitochondrial DNA Helicase**
Matthew J. Longley, National Institute of Environmental Health Sciences

9:35 a.m. **BREAK/NETWORKING**

SESSION 5: NIAAA SESSION

10:15 a.m. **Alcohol-Mediated Mitochondrial Dysfunction, Apoptosis, and Therapeutic Interventions**
Chair: *BJ Song, National Institute on Alcohol Abuse and Alcoholism*

10:30 a.m. **The Clinical Impact of Alcohol on HIV Mitochondrial Metabolism**
Mariana Gerschenson, University of Hawaii

10:45 a.m. **Oxidative/Nitrosative Stress in Various Animal Models of Heart Failure**
Pal Pacher, National Institute on Alcohol Abuse and Alcoholism

11:00 a.m. **Mitochondrial Motility and Fusion-Fission Dynamics: A Potential Target of Ethanol**
György Hajnóczky, Thomas Jefferson University

SELECTED ABSTRACT PLATFORM PRESENTATIONS

11:15 a.m. **Oxidative Stress and Mitochondrial Toxicity Relate to the Development of Alcoholic Cardiomyopathy**
Ioan Cucoranu, Emory University

11:25 a.m. **POSTERS AND LUNCH**

SESSION 6: MODEL ORGANISMS OF MITOCHONDRIAL DISEASE—RON BUTOW MEMORIAL SESSION

12:45 p.m. **Yeast as a Model for Human Mitochondrial Disease—Tribute to Ron Butow**
Chair: *Xin Jie Chen, SUNY Upstate Medical University*

1:00 p.m. **The Layered Structure of mtDNA Nucleoids**
Dan Bogenhagen, Stony Brook University

1:15 p.m. **Proteomic Technologies for Cancer Research: Strategic Plan—Translating Discoveries to Clinic**
Henry Rodriguez, Director, Clinical Proteomics Technologies Initiative, National Cancer Institute

SELECTED ABSTRACT PLATFORM PRESENTATIONS

1:30 p.m. **Oxidative Inactivation of Key Mitochondrial Proteins Leads to Mitochondrial Dysfunction and Injury in Hepatic Ischemia Reperfusion of Mice**
Kwan-Hoon Moon, National Institute on Alcohol Abuse and Alcoholism

1:40 p.m. **Seeking the Biochemical Basis of Type III 3-methylglutaconic Aciduria Through Zebrafish Models**
Wuhong Pei, National Human Genome Research Institute

1:50 p.m. **Decrease of mtDNA Replication in Xenomitochondrial Mouse Model**
Seyed Hosseini, Emory University

2:00 p.m. **Mutations in the Gene Cause Mitochondrial Mislocalization and Parkinson-Like Phenotypes in the Drosophila Ovary and Muscle**
Rachel T. Cox, Howard Hughes Medical Institute and Carnegie Institution

2:10 p.m. **Modulating Stat1 Signaling by Mitochondrial Tid1, a Human Homolog of Bacterial DnaJ and the Drosophila Tumor Suppressor Tid56**
Bin Lu, New Jersey Medical School, University of Medicine and Dentistry of New Jersey

2:20 p.m. **BREAK**

SESSION 7: ACQUIRED MITOCHONDRIAL TOXICITIES AND CLINICAL AND SOCIETAL ASPECTS OF MITOCHONDRIAL DISEASE

2:40 p.m. **Mitochondrial DNA Depletion Syndromes**
Chair: *Robert K. Naviaux, University of California, San Diego*

2:55 p.m. **Acquired and Genetic Diseases of the Mitochondrial DNA Polymerase**
Bill Copeland, National Institute of Environmental Health Sciences

3:10 p.m. **Frequent Mitochondrial DNA Mutations and Polymorphisms in Childhood Cancer Survivors Following Multi-Agent Chemotherapy**
Steve Lipshultz, University of Miami

3:25 p.m. **Frequent Mitochondrial DNA Mutations and Polymorphisms in HIV-Infected Children Receiving Highly Active Antiretroviral Therapy**
Vernon Walker, Lovelace Respiratory Research Institute

3:40 p.m. **Fetal Mitochondrial Consequences of Transplacental NRTI Exposure**
Miriam Poirier, National Cancer Institute

3:55 p.m. **Chemotherapy-Associated Mitochondrial Dysfunction**
Bruce Cohen, Cleveland Clinic

4:10 p.m. **BREAK**

4:25 p.m. **The Impact of Voluntary Health Organizations on Mitochondrial Research and Physician Outreach**
Charles Mohan, Director, United Mitochondrial Disease Foundation

4:40 p.m. **The Rare Diseases Clinical Research Network (RDCRN)**
Rashmi Gopal-Srivastava, Director, Extramural Research Program, Office of Rare Diseases, NIH

4:55 p.m. **Challenges in Dietary Supplement Research: Application to Mitochondrial Disease**
Paul M. Coates, Director, Office of Dietary Supplements, NIH

SELECTED ABSTRACT PLATFORM PRESENTATIONS

5:10 p.m. ***In Utero* Exposure of Female CD-1 Mice to Nucleoside Reverse Transcriptase Inhibitors (NRTIs) Leads to Temporal Changes in Mitochondrial Structure, Function, and Mutations**
Salina Torres, Lovelace Respiratory Research Institute

5:20 p.m. **Association Between Mitochondrial DNA Haplogroups and AIDS Progression**
Sher Hendrickson, National Cancer Institute

5:30 p.m. **Mitochondrial Oxidative Stress in HIV-1 Protease Inhibitor–Mediated Suppression of Glucose-Stimulated Insulin Release by Rat Insulinoma Cells**
Surabhi Chandra, Tulane University

5:40 p.m. **Respiratory Chain Dysfunction and Oxidative Stress Correlate with Severity of Primary CoQ10 Deficiency**
Luis Carlos Lopez Garcia, Columbia University

5:50 p.m. **Improvements in Mitochondrial Function, Morphology, and Lipid Profiles in HIV-Positive Patients Switching from Stavudine to a Tenofovir-Containing Therapy**
Courtney Kim, University of Hawaii

SESSION 8: VITAMIN AND COFACTOR THERAPY AND CLINICAL TRIALS FOR MITOCHONDRIAL DISEASE

6:00 p.m. **Introduction to CoQ10, Carnitine, Creatine, B-Vitamin, Antioxidant Cocktails, and Clinical Monitoring Guidelines for Assessing Clinical Outcomes**
Bruce Cohen, Cleveland Clinic

6:10 p.m. **Exercise and Nutriceuticals in Mitochondrial Disease and Aging**
Mark Tarnopolsky, McMaster University

6:25 p.m. **Panel Q & A**
Bruce Cohen, Mark Tarnopolsky, Paul Coates, Robert Naviaux Rashmi Gopal-Srivastava, Douglas Wallace

Friday, January 11, 2008

SESSION 9: MTDNA REPAIR SESSION

8:00 a.m. Chair: *Vilhelm Bohr, Laboratory of Molecular Gerontology, National Institute on Aging*

8:10 a.m. **Mitochondrial Topoisomerase I (Top1mt) Controls Mitochondrial DNA Replication Through D-Loop Formation**
Yves Pommier, National Cancer Institute

8:25 a.m. **Mitochondrial Respiratory Enzyme Dysfunction in Midbrain DA Neurons Elicits Parkinsonian Symptomatology: A Novel Genetic Model**
Barry Hoffer, Scientific Director, National Institute on Drug Abuse

8:40 a.m. **Is Tyrosyl-DNA Phosphodiesterase a Mitochondrial DNA Repair Enzyme?**
Lawrence F. Povirk, Virginia Commonwealth University

8:55 a.m. **Pathways for Mitochondrial DNA Repair: Relevance to Aging and Disease**
Nadja Souza-Pinto, National Institute on Aging

SELECTED ABSTRACT PLATFORM PRESENTATIONS

9:10 a.m. **Opa1 Regulates Crista Junction Sizes and Cytochrome c Accessibility**
Ryuji Yamaguchi, La Jolla Institute for Allergy & Immunology

9:20 a.m. **The Actin-Binding Protein Cofilin as the Major Promoter of Oxidant-Induced Apoptosis in Tumor Cells**
Fábio Klamt, Federal University of Rio Grande do Sul, Brazil, and Center for Drug Evaluation and Research, Food and Drug Administration

9:30 a.m. **SIRT3 as a Regulator of Intracellular Superoxide Levels**
Krish Patel, National Cancer Institute

9:40 a.m. **The DNA Polymerase GAMMA Y955C Disease Variant Associated with PEO and Parkinsonism Mediates the Incorporation and Translesion Synthesis Opposite 7,8-dihydro-8-oxo-2'-deoxyguanosine**
Rachelle J. Bienstock, National Institute of Environmental Health Sciences

9:50 a.m. **BREAK**

SESSION 10: INVITED LECTURE

10:30 a.m. **A Mitochondrial Paradigm for Metabolic and Degenerative Diseases, Cancer, and Aging**
Douglas C. Wallace, University of California, Irvine

Mitochondria Minisymposium 2008

Mitochondria and their Proteomics

Invited Speaker Abstracts

Title: **Proteomic Approaches to Organelles in the CV System**	**Schnitzer, Jan E.** **Sidney Kimmel Cancer Center, San Diego, California**	Organellar proteomic imaging in vivo: Targeting endothelial caveolae for tissue-specific penetration and drug delivery New biomarkers and targeting strategies are needed to fulfill the promise of molecular medicine. Proteogenomic technologies provide major analytical power to assess molecular expression and to generate a variety of diagnostic and therapeutic opportunities. The ability to overcome in vivo barriers to deliver imaging and therapeutic agents into specific tissues in sufficient quantities to be effective still remains an elusive goal. The endothelial cells lining these blood vessels form a key barrier restricting access inside most tissues. Yet the luminal surfaces of vascular endothelia are directly in contact with circulating blood and thereby provide an inherently accessible interface for targeting in vivo. Caveolae are abundant on many endothelial surfaces and may provide a means to cross the endothelial cell barrier. Here we use colloidal silica nanoparticles to coat luminal surfaces of endothelia in major organs & various solid tumors & to isolate this membrane and its caveolae away from the rest of the tissue. We integrate this fractionation with subtractive proteomic mapping, bioinformatic interrogation, and molecular imaging in vivo to identify and validate tissue- and tumor-induced endothelial targets that are accessible to antibodies injected intravenously. This proteomic mapping reveals distinct molecular signatures for vessels in solid tumors and major organs. Electron microscopy shows that select antibodies to tissue-specific caveolar proteins can target 5-20nm nanoparticles to caveolae for subsequent transport across the endothelial cell barrier into the tissue interstitium. Dynamic live imaging techniques using specific monoclonal antibodies demonstrated rapid tissue-specific vascular targeting in vivo including, within seconds (normal lung) and minutes to hours (solid tumors) after intravenous injection, the transendothelial transport in vivo of antibodies

		targeting caveolae. Caveolae act as active pumps transporting specific molecular cargo, even against a concentration gradient, to concentrate select antibodies in the tissue interstitium. Such pervasive access improves the efficacy of radioimmunotherapy in destroying both stromal and tumor cells and in treating a wide variety of solid tumors. The unprecedented speed in immunotargeting and caveolar transcytosis in vivo not only underscores the physiological function for caveolae in transvascular exchange but also encourages targeting caveolae as a worthwhile novel strategy to enhance molecular and functional imaging as well as drug, nanoparticle, and gene delivery in vivo. This discovery & validation strategy uncovers targets potentially useful for non-invasively detecting, characterizing, treating, and even monitoring many tumor types in the clinic.	

Title: **The MPT Pore in Cardiac Diseases**	**Baines, Christopher** **Cincinnati Children's Hospital Medical Center, Cincinnati, OH,**	Upon a cell death stimulus, cardiac mitochondria undergo a rapid increase in inner membrane permeability, the so-called mitochondrial permeability transition (MPT). This results in the inhibition of ATP synthesis, swelling, and ultimately mitochondrial rupture. The MPT pore, a non-specific channel thought to span both mitochondrial membranes, mediates these catastrophic increases in mitochondrial permeability. Based upon biochemical and pharmacological studies, the pore was proposed to consist of the voltage-dependent anion channel (VDAC) in the outer membrane, the adenine nucleotide translocase (ANT) in the inner membrane, plus CypD in the matrix. However, mice lacking ANT still exhibit a classical MPT phenomenon and respond normally to cell death stimuli. Therefore, the exact composition of the pore remains uncertain. To this end, we have studied mice lacking VDAC or CypD. Mitochondria from CypD-null mice are resistant to Ca^{2+}-induced permeability transition. Moreover, CypD-deficient cells are protected against Ca^{2+}- and oxidative stress-induced death, and CypD-null mice are less sensitive to myocardial ischemia/reperfusion injury and cardiac Ca^{2+} overload. In comparison, permeability transition and cell death progress normally in cells lacking all 3 VDAC isoforms. Therefore, only CypD remains as a defined molecular component of the MPT, where it functions in an enzymatic capacity to induce pore formation and cell death by binding and regulating unknown proteins. Consequently, we are currently employing proteomic and genetic screens aimed at identifying novel CypD-interacting proteins and characterizing their role in MPT and cell death. This work supported by: American Heart Association

Title: NIH Peer Review and the Finer Points of Grant Applications	**Cooper, Cathleen** **NIH Center for Scientific Review**	What happens to your grant application from the time it is received by NIH until you receive the score and summary statement? In this presentation, you will discover how the NIH Center for Scientific Review routes your application to both study section review and institute assignments, how the study sections and review process really work, including study section “pet peeves”, and where you can have input along the way	

Title: **NIH Institute Locus of Review: Rationale and Expectations**	**Gerald McLaughlin, Ph.D.** **Chief, Grants Review Branch** **National Institute on Drug Abuse (NIDA), NIH, HHS**	Abstract: Dr. Gerald McLaughlin's specialty is Institute-based administrative and scientific review of grants and contracts and he will describe rationales, policies, technologies and expectations for applicants and reviewers who are involved with Institute-reviewed Funding Opportunity Announcements (FOAs). In general, FOA's with Institute-based reviews emphasize emerging priorities for Institute(s) and guidelines are defined in Requests for Applications (RFA's), Program Announcements (PAR), and some Roadmap, Blueprint, Center, and emerging Eureka and Avant-Garde Announcements. Applicant and reviewer roles vary for assorted R, P, T, and K funding mechanisms and their FOA's relative to standard CSR reviews although many similarities remain. More successful and established programs that remain broad NIH priorities, tend to have their locus of review moved to CSR and several examples will be provided.

Title: **Mitochondrial Proteomics by Antibody Arraying of Functional Complexes**	**Roderick.A.Capaldi.** **MitoSciences Inc. Eugene. OR 97403.**	Many research applications related to complex diseases such as diabetes, cancer and neurodegenerative disorders, as well as efforts to screen for drug efficacy or toxicity, require a systems approach. Proteomics can provide important information on changes in protein composition, amounts and post-translational modifications. However current approaches such as 2D gel electrophoresis and MUDPIT-type mass spectrometry analysis have limitations of lack of quantification, inability to identify hydrophobic proteins and no ability to examine functional attributes. We are developing an alternative strategy in which proteins and their complexes are arrayed by immunocapture using highly specific mAbs against the targets of interest. This allows for quantification by using a detector antibody, identification and quantification of post-translational modifications, and the ability to monitor the activity of the proteins while bound to the plates. To date we have generated arrays for all of the components of OXPHOS, the fatty acid oxidation enzymes, apoptotic proteins of the mitochondrion and mitochondrial morphology proteins. The use of these arrays in screening for oxidative stress and of changes in mitochondrial functioning in response to metabolic stress will be described.

Title: **Cellular Bioenergetics Assays**	**Ferrick, David.** **Seahorse Biosciences, Billerica, Massachusetts, USA**	Prevalent age-related human diseases such as obesity, Type II diabetes, cancer, neurodegenerative and cardiovascular disease all share a common feature, dysregulation of cellular energy metabolism. Unfortunately, the ability to measure bioenergetics *in vitro* - quickly and reliably - continues to be a key challenge for researchers. This talk will focus on a novel approach for measuring bioenergetics using extracellular flux (XF). By measuring the extracellular flux or flow of nutrients, organic molecules and elements in the media one can directly determine the metabolic state and physiology of primary cells and cell lines without adding labels or touching them in anyway. Features include: 1. The cell populations being studied are not exposed to labels or manipulations that can introduce artifacts or alter the experiment 2. Multiple analytes can be measured simultaneously 3. Real-time kinetic data are generated 4. The cell populations being analyzed can be reused in other applications or chronic studies The talk will demonstrate measurement of the two major energy yielding pathways, mitochondrial respiration and glycolysis. Examples of studies investigating mitochondrial energetics and determining mechanism of action for compounds will be presented. This work supported by: Seahorse Biosciences

Title: **Altered Gene Expression and Oxidative DNA Damage in Peripheral Blood Cells from Friedreich's Ataxia Patients**	**Van Houten, Bennett** **Van Houten, Bennett[1]; Haugen, Astrid[1]; Halweg[1], Chris; Parker[2], Joel; DiProspero, Nicholas[3]; Fischbeck, Kurt[3]. [1]NIEHS, NIH; [2]Expression Analysis; [3]NINDS, NIH.** **National Institute of Environmental Health Sciences**	Frataxin, a conserved mitochondrial protein involved in iron homeostasis, is reduced in patients with Friedreich's ataxia (FRDA). Transcription profiling and DNA damage assays were performed on blood cells from 48 FRDA children. Microarrays for the analysis of gene expression were completed on a 22,000 gene Agilent array using a universal RNA standard design. These children were compared to a group of normal young adults. Significance Analysis of Microarray (SAM) revealed ~1,500 differentially expressed genes at a false discovery rate of 1%. Expression patterns identified altered immune response, signaling pathways, transcription, apoptosis, and genotoxic stress pathways. In support of this last finding, compared to a control group of young adults, FRDA patients had significantly more mitochondrial and nuclear DNA damage as measured by a gene-specific quantitative PCR assay. DNA lesions, multiplied by the years of disease duration, were highly correlated with ICAR scores. Frataxin mRNA levels correlated with age of onset, and those patients with the lowest levels of frataxin displayed a unique set of gene alterations involved in reduced protein synthesis and reduced oxidative phosphorylation. Validation of gene expression changes was performed by analysis of 14 adult French FRDA patients and analysis of 10 FRDA lymphoblastoid cell lines. This study demonstrates how analysis of blood in FRDA patients yields molecular insight into the nature and progression of the disease. This work supported by: Intramural NIH research funds and a grant from the Office of Rare Diseases to BVH and KF.

Title: **Research Opportunities at National Cancer Institute: Using Mitochondrial Genomic and Proteomic Information**	**Verma, Mukesh** **Epidemiology and Genetics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health. Rockville, MD 20852**	Mitochondria have been implicated in the carcinogenesis process because of their role in apoptosis and other aspects of tumor biology. The National Cancer Institute is interested in promoting research in utilizing mitochondrial genomic and proteomic information for detection, diagnosis, and prognosis of cancer. Along with investigator initiated projects (R01 grants), small pilot projects (R03 grants), and exploratory projects (R21 and R33) are encouraged. Investigators have now a choice of using "Multiple PI Mechanism" which has advantage of including a clinician, bioinformatics expert or a non-mitochondrial expert for large projects. Details will be discuused. To understand the utility of mitochondrial DNA in cancer epidemiology, one approach is to look for the somatic mutations in mitochondria and the other approach is to look for disease-associated haplotypes. The inheritance pattern of mitochondria in patients with cancer has been studies by haplotype analysis. Polymerase chain reaction of key polymorphic sites in the mitochondrial genome is performed in samples from cancer patients and normal individuals to determine if there is an association between mitochondrial genotype and cancer. Such analysis has been accomplished in prostate and renal cancer. One haplogroup U, with OR value of 1.95, has been identified. Inheritance of the U haplogroup is associated with high risk of developing prostate cancer and about 20 million white individuals have this haplogroup. So far 9 mitochondrial haplogroups, H, I, J, K, T, U, V, W, and X, have been identified. Thus the presence of specific haplotype group predisposes to risk of prostate cancer. Similar observations have been made, for another haplogroup, in renal cancer. By virtue of their clonal nature and high copy number, mitochondrial mutations may provide a powerful molecular marker for noninvasive detection of cancer. It has been suggested that the extent of mitochondrial DNA mutations might be useful in the prognosis of cancer outcome and/or the response to certain therapies. Research opportunities in proteomics field will also be discussed.

Title: **Conceptual Issues of Biomarkers for Fatigue**	**Payne, Judith K. PhD, RN** **Duke University School of Nursing, Durham, NC.**	Fatigue is a common and distressing problem for persons with cancer, especially for those receiving treatment. However the phenomenon is poorly understood and little is known about influencing factors or the underlying etiology of the onset, intensity, and persistence of fatigue among patients with cancer. While biomarkers have been used in the past for detecting early disease and predicting tumor behavior, ongoing research efforts have expanded the use of biomarkers to manage the symptom of fatigue. This shifting paradigm opens the door for understanding the etiology of fatigue, and may help clinicians predict and monitor responses to treatment in oncology care. However, challenges exist as we continue to investigate the scientific and clinical value of biomarkers in fatigue. Much of our research has focused on a variety of different biomarker pathways, and possible linkages to fatigue outcomes. These efforts have been confounded by lack of consistent definitions and measures, and with small sample sizes. Most importantly, there is an urgent need to examine biomarkers in fatigue within the context of a clear scientific conceptual framework. The lack of a conceptual framework has hindered our efforts to systematically build on each other's research findings, and therefore has made it difficult to compare results from one study to another. Conceptual issues need to be considered as we systematically plan our research efforts in order to evaluate our investigation of biomarkers and fatigue. To accomplish this, more collaborative, descriptive and explanatory research is needed within a concerted and perhaps multi-site research effort. This work supported by: Oncology Nursing Society; Sigma Theta Tau; NINR

Title: **Inflammatory Responses in Muscle During Chemotherapy in a Mouse Model**	**Wood, Lisa** **Collin R. Elsea[1], Dan Roberts[1], Nancy A. Perrin[1], Lillian M. Nail[1], Brian J. Druker[3,4] & Lisa J. Wood[1,2,4].** **Oregon Health & Science University [1]School of Nursing, [2]Department of Radiation Medicine, School of Medicine, [3]Cancer Institute & [4]Howard Hughes medical Institute.**	Breast cancer (BC) patients undergoing systemic antineoplastic therapy often experience changes in body composition such as a loss of lean muscle mass and bone. The inflammatory cytokine, interleukin-6 (IL-6), has been implicated in the regulation of body composition and increased serum IL-6 levels have been reported in breast cancer survivors following treatment. We have recently demonstrated that plasma levels of IL-6 rise rapidly (peak at 3-6 hours) in mice administered a single dose of Cytoxan - Adriamycin-5-Fluourouracil (CAF), a common BC treatment regimen. Accumulation of IL-6 mRNA was evident in liver, spleen, duodenum, and skeletal muscle. Mice administered 4 cycles of CAF, at 3-week intervals displayed a reduction in food intake, body weight, and physical activity during treatment. Weight loss in drug-treated mice was related to a reduction in lean body mass and bone density as determined by Dual energy X-ray absorptiometry (DEXA) but not fat mass. The purpose of this study was to determine the role of IL-6 in CAF induced changes in food intake, activity, and body composition in mice. **Methods:** Food intake, wheel running activity, and body composition, were assessed in twenty IL-6 deficient mice and twenty wildtype (WT) counterparts. Ten mice of each genotype were administered adminsitered cyclophosphamide (500mg/m^2), doxorubicin (4mg/m^2), and 5-fluorouracil (500 mg/m^2), the remaining 10 in each genotype group were sham-injected. The effects of CAF administration on daily food intake, physical activity, and body weight were determined during the course of 4 treatments administered at 3-week intervals. The effects of CAF administration on changes in body composition were assessed by measuring total lean body mass, total fat mass, and total bone mineral content by DEXA just prior to the first drug dose and 3-weeks after the last drug dose. **Results:** We found no significant difference in the pattern of food intake or physical activity between CAF treated IL-6 deficient mice and their WT counterparts. However, whereas

CAF-treated WT mice lost significant weight during treatment relative to WT control mice, CAF treated IL-6 deficient mice did not. Assessment of body composition by DEXA revealed that IL-6 deficient mice were protected from the loss of lean body mass that occurs as a result of CAF treatment. There was no significant difference in the loss of bone mineral content between WT and IL-6 deficient mice administered drug. Blood levels of IL-6 were significantly elevated in CAF treated WT mice compared to control mice 3-weeks after the last dose of drug.

Conclusion: Our findings suppport the idea that induction of BC treatment related increases in IL-6 production may be the mechanism underlying loss of lean body mass that commonly occurs in women undergoing treatment for BC with systemic antineoplastic drugs. Therefore, this work has the potential to lead to new treatment strategies aimed at decreasing the effects of drug treatment on muscle loss.

This work supported by: The American Cancer Society

Title: **Sarcopenia and Proteolysis in Muscle of a Cancer Mouse Model**	**McCarthy, Donna** **Ohio State University, Columbus OH**	Biomarkers of increased proteolysis and decreased protein synthesis in an animal model of tumor-induced skeletal muscle wasting. The loss of body weight, particularly lean body mass, is a major factor in symptoms of fatigue and reduced effort tolerance in patients with cancer cachexia. The pro-inflammatory cytokine, TNF-alpha, is thought to play a key role in the pathology of this disorder. Animal models of cancer cachexia have shown that tumor-induced skeletal muscle wasting is associated with decreased muscle expression of IGF-1 and phosphorylated p70s6 kinase, two mediators of muscle protein synthesis. It is also associated with increased muscle expression of TNF-alpha type 1 receptors, interleukin-6, and ubiquitin ligases thought to be key in proteasome-mediated degradation of myosin. However, muscle expression of TNF-alpha was not increased. Many of the proinflammatory effects of TNF-alpha are mediated by cyclooxygenase-2 (COX2) the rate limiting enzyme in synthesis of pro-inflammatory prostanoids. Inhibition of COX activity with nonsteroidal anti-inflammatory agents preserved muscle mass in mice bearing the Lewis lung carcinoma or the colon26 adenocarcinoma, but not in mice bearing the B16 melanoma. Similar results were obtained when mice were fed a diet containing 1% conjugated linoleic acid, a fatty acid that reduces the synthesis of pro-inflammatory prostanoid mediators from arachidonic acid. These data suggest that tumor-induced skeletal muscle wasting can occur by COX-dependent and independent pathways. This work supported by: NINR Intramural Research Program

Title: **Mitochondrial Damage and Fatigue in HIV**	**Voss, Joachim** **University of Washington, Seattle WA, USA.**	Fatigue is one of the most frequent and burdensome symptoms of HIV patients. Previous efforts to identify a quantifiable biomarker for HIV-related fatigue (eg. CD4, viral load) have failed to show a relationship between biological mechanisms and subjective symptom perception. Mitochondrial dysfunction due to exposure to antiretroviral treatment is a well established fact, yet we are less clear if the mitochondrial dysfunction is in causal relationship to the fatigue experiences of patients. Therefore our goal was to identify the profile of low-abundant proteins in serum of HIV-infected patients (N=10) with moderate to severe fatigue compared to healthy controls (N=10).We utilized spin column technology to deplete the seven most abundant serum proteins, digest the proteins with trypsin and analyzed them by HPLC-electrospray tandem mass spectrometry. From these acquired peptide tandem mass spectra a SEQUEST search against the human IPI database identified approximately 99 proteins that have been found to be significant in four fatigued HIV patient samples, further analysis is underway. The identification of indicator proteins representative of fatigue in HIV would provide for the first time serum makers that may be predictive of early onset of fatigue, be specific enough in clinical diagnosis of fatigue and could be a marker for effectiveness of pharmacological and behavioral intervention studies. This work supported by: NIH Clinical Center, NINR, NINDS, NIAID, and the University of Washington

Title: **Protein Dynamics at the Mitochondrial Replication Fork**	**Kaguni, Laurie S.** **Carol L. Farr, Yuichi Matsushima, Marcos T. Oliveira and Tawn D. Ziebarth** **Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824-1319**	The mitochondrial replicase (pol) comprises two subunits, a large catalytic core (pol γ-α) and a smaller accessory subunit (pol γ-β) that enhances holoenzyme activity and processivity. Mutagenesis of four conserved sequence elements located within the spacer region between the DNA polymerase and 3'-5' exonuclease active sites in pol γ-α demonstrates their functional roles in holoenzyme activity, processivity and/ or DNA binding affinity. Several mutations also affect differentially DNA polymerase and exonuclease activity, and/ or functional interactions with mitochondrial single-stranded DNA-binding protein (mtSSB). Overexpression of the catalytic core in the nervous system of *Drosophila* induces severe mtDNA depletion and reduces median life span. A parallel mutagenesis of the human accessory subunit, in combination with the determination of its crystal structure and molecular modeling, elucidates its role as a novel type of processivity factor. Loss of function alleles result in mtDNA depletion and developmental lethality in *Drosophila*. A human pol / DNA complex model was developed using the structures of the pol dimer and the T7 DNA polymerase ternary complex, which suggests multiple regions of subunit interaction between pol and the human catalytic core that allow it to encircle the newly synthesized double-stranded DNA, and thereby enhance DNA binding affinity and holoenzyme processivity. Functional complexes of pol , mtSSB and a novel mitochondrial DNA helicase reconstitute the mitochondrial DNA replication fork. The human mtDNA helicase exists as a hexamer/ heptamer and exhibits a modular architecture that is highly similar to that of bacteriophage T7 primase-helicase and *E. coli* DnaB protein. Molecular analysis of active site and selected human disease alleles of the Drosophila homolog by overexpression in Schneider cells results in a dominant-negative lethal phenotype resulting from mtDNA depletion. This work was supported by NIH grant GM45295.

Title: **Multitasking in the Mitochondrion by the Lon Protease in Protein and mtDNA Quality Control**	**Suzuki*, Carolyn K., Lu*, Bin, Santos*, Janine, Tian*, Bin, Chen§, Si-Han and Wu§, Shih-Hsiung** ***UMDNJ- New Jersey Medical School, Newark, NJ USA** **§ Academia Sinica, Taipei City, Taiwan**	Human mitochondrial Lon is an ATP-powered proteolytic machine that selectively degrades protein substrates and also specifically binds to single-stranded G-rich DNA. DNA binding by human Lon *in vitro* is inhibited by ATP and stimulated by protein substrate. DNA binding, however, has no observed effect on Lon-mediated proteolysis. Our thermodynamic analyses demonstrate that Lon selectively binds to DNA sequences that have a propensity for forming parallel G-quartets. We have shown in living cells that Lon binds to mitochondrial DNA (mtDNA) at sites distributed within one half of the genome and interacts preferentially with the control region for mtDNA replication and transcription. Bioinformatic analysis of the mtDNA regions bound by Lon *in vivo* identified a G-rich consensus sequence. The distribution of sequences showing the highest conformity to this consensus within the mitochondrial genome corresponds well to the distribution of Lon binding in cells. Although the function of Lon in mtDNA maintenance and expression remains elusive, we have recently demonstrated that cellular levels of Lon influence the vulnerability to oxidative mtDNA damage. When oxidatively stressed, cells expressing normal Lon levels exhibit an increased frequency of mtDNA lesions. By contrast, Lon-depleted cells show little if any mtDNA damage. This suggests that oxidative mtDNA damage is permitted when Lon is present and prevented when Lon levels are substantially reduced. Experiments are underway to determine the role of Lon in modulating the susceptibility to mtDNA damage and how Lon functions in linking protein and mtDNA quality control. This work supported by: This work was supported in part by grants to C.K.S. from the National Institutes of Health, the American Heart Association and the Foundation of UMDNJ.

Title: **Regulation of Mitochondrial Homeostasis and Gene Expression by the ATM and TOR Signaling Pathways**	**Shadel, Gerald S.** **Department of Pathology, Yale University School of Medicine, New Haven, Connecticut**	The role of mitochondria in human disease and aging has long been appreciated, but the precise mechanisms involved remain poorly understood. Mitochondria have multiple and tissue-specific roles and therefore can cause or exacerbate pathology by multitude of mechanism, including disruption of oxidative metabolism, production of reactive oxygen species (ROS), and deregulation of apoptosis that lead respectively to energetic imbalance/deficiency, oxidative stress, and cell death. Mitochondria are also intimately involved in cellular signal transduction pathways, providing yet another pathogenic route for mitochondrial dysfunction. Here we describe our recent studies that demonstrate mitochondria are relevant downstream targets of two conserved signaling pathways. First, evidence is provided that the Ataxia-Telangiectasia Mutated (ATM) kinase controls mitochondrial homeostasis and that mitochondrial dysfunction likely plays a role in the complex pathology of this disease. Second, I will present evidence that the nutrient-sensing, target of rapamycin (TOR) pathway regulates mitochondrial gene expression and respiration to curtail yeast life span and discuss the relevance of these findings in human cells. Altogether, our results indicate that the role of mitochondria in human disease is grossly underestimated and that disruption of cellular signaling pathways is a novel route for pathology. Furthermore, targeting these pathways may also be a therapeutic route for mitochondrial-based diseases and age-related pathology. This work supported by: NIH-NINDS, NIH-NHLBI, NIH-NIEHS, Army Research Office

Title: **Mitochondrial DNA Mutations as Biomarkers for Early Cancer Detection**	**Wagner, Paul** **Division of Cancer Prevention, National Cancer Institute**	The NCI's Early Detection Research Network (EDRN) is national consortium to discover and validate biomarkers for early cancer detection and risk assessment. Detecting tumors early, before they become invasive, can improve the patient's odds for successful treatment and survival. Several studies have suggested that mitochondrial mutations might be useful biomarkers for early cancer detection. Somatic mitochondrial DNA (mtDNA) mutations have been observed in a variety of cancers, and the frequency of mitochondrial mutations is high, with one-half to two thirds of cancers harboring at least one mutation. The EDRN in collaboration with National Institute of Standards and Technology (NIST) have undertaken two projects to examine the usefulness of mtDNA mutations for early cancer detection. The first uses an oligonucleotide-based microarray, the MitoChip, which enables resequencing of the entire mitochondrial genome. In collaboration with scientists at Johns Hopkins University and New York University, we determined the mtDNA sequence in tissues and matched bodily fluids (urine or sputum) of patients with early stage cancers. Using this high-throughput method, 83% of the tumor tissues were found to contain mtDNA mutations, but less than 50% of the body fluids from these patients contained mtDNA mutations. The second project, performed in collaboration with scientists at Genesis Genomics, examines the usefulness of 3.4kb mtgenome deletion for the detection of prostate cancer using needle biopsies. Genesis Genomics found that in comparison to histopathology for benign and malignant samples, the 3.4kb mtgenome deletion gave sensitivity and specificity of 80% and 71% respectively. NIST-EDRN in a blinded validation study determined the sensitivity and specificity were 83% and 79%. The statistical discrimination between the groups suggests that the 3.4mt deletion may be useful in distinguishing malignant from benign prostate. This work supported by: National Cancer Institute

Title: **Alcohol-Mediated Mitochondrial Dysfunction, Apoptosis, and Therapeutic Interventions**	**Song, B.J.** **National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA**	Chronic alcohol (ethanol) intake is known to activate several oxygen-producing enzymes with simultaneous inhibition of the mitochondrial complex enzymes, contributing to a markedly elevated oxidative/nitrosative stress. Despite the well-established mitochondrial dysfunction, it is poorly understood which mitochondrial proteins are oxidatively-modified under alcohol-induced oxidative/nitrosative stress. Therefore, we specifically aimed at identifying the oxidatively-modified proteins by focusing on redox modulation of Cys residues using biotin-*N*-maleimide (biotin-NM) as a specific probe and evaluating their functional modulations in alcohol-exposed animals. The biotin-NM-labeled liver mitochondrial proteins from pair-fed controls or alcohol-fed rats were purified with streptavidin-agarose and then subjected to mass spectrometric analysis for protein identification. Mitochondrial aldehyde dehydrogenase 2 (ALDH2), ATP synthase, acyl-CoA dehydrogenase, 3-ketoacyl-CoA thiolase and many other proteins, involved in chaperone activity, mitochondrial electron transfer and ion transport, were oxidatively-modified in alcohol-exposed rats. The hepatic activities of 3-ketoacyl-CoA thiolase, involved in the mitochondrial β-oxidation of fatty acids, ALDH2 and ATP synthase were significantly inhibited in alcohol-exposed rats. Inhibition of 3-ketoacyl-CoA thiolase is consistent with hepatic fat accumulation as determined by biochemical and histological analyses. Similar redox approaches were also applied to study oxidatively-modified mitochondrial proteins following hepatic ischemia-reperfusion (I/R) injury in the presence or absence of a peroxynitrite scavenger MnTMPyP. Our result showed that many proteins were oxidatively-modified under I/R injury in the absence of MnTMPyP, which selectively blocked *S*-nitrosylated proteins without affecting the oxidized proteins. Furthermore, ethanol exposure activated stress-activated protein kinases such as JNK and p38 kinase, both of which phosphorylate pro-apoptotic Bax, prior to translocation of phosphorylated Bax to mitochondria and apoptosis. Phosphorylation of Bax, its mitochondrial translocation, and apoptosis were significantly blunted by the presence of specific inhibitors of JNK or p38 kinase as well as anti-oxidants. Taken together, these results

indicate the important role of oxidative/nitrosative stress in alcohol-induced mitochondrial dysfunction, fat accumulation, and apoptosis.

This work supported by: NIAAA Intramural Program.

Title: **The Clinical Impact of Alcohol on HIV Mitochondrial Metabolism**	**Mariana Gerschenson** **University of Hawaii**	Background: Since alcohol toxicity is in part mitochondrial, we measured biomarkers of mitochondrial injury: mitochondrial isoform of aspartate aminotransferase (mAST) in sera and mitochondrial DNA (mtDNA) in peripheral blood mononuclear cells (PBMCs) to determine if there was an association with alcohol consumption. Methods: Sera from 254 VACS participants (158 HIV+, 94 HIV-) were measured for mAST and mtDNA. MAST enzyme activity was measured post-immunoprecipitation with cytoplasmic specific AST antibody. PBMC mtDNA copies/cell were quantitated by real-time PCR for mitochondrial NADH dehydrogenase 2 and the nuclear Fas genes. Pearson correlations and multivariate models were used to determine the independent association of mtDNA and mAST with drinks per week (AUDIT) and alcohol abuse or dependence (ICD-9 Diagnostic codes) adjusting for HCV (laboratory data), diabetes (patient report), obesity (BMI>29), and cumulative exposure to NRTIs (pharmacy fill data). Results: The two biomarkers did not correlated with each other (rho=-.05, p=0.5). 37% drank more than 7 drinks/week or had a diagnosis of abuse or dependence. There was no difference in alcohol consumption between the HIV +/- subjects (p=0.4). Among, HIV+ veterans, mAST correlated with a diagnosis of abuse or dependence (rho=-0.17, p=0.03) and mtDNA was correlated with more than 7 drinks/week (rho=0.24, p=0.003). In a multivariate model adjusted for obesity, hepatitis C, diabetes, and exposure to NRTIs, being HIV+ was associated with lower mAST (-10.1: 95% CI: -16.6, -3.5) as was a diagnosis of alcohol abuse or dependence (-3.0; 95% CI: -6.4, 0.5). In a similar model predicting mtDNA, the interaction between HIV status and alcohol consumption was significant (p=0.01) demonstrating that more than 7 drinks/week were associated with substantial changes in mtDNA only among those with HIV. Conclusions: Both biomarkers of mitochondrial injury were associated with indicators of alcohol consumption (>7

drinks/week and diagnosis of abuse or dependence) among HIV+ veterans.

This work supported by NIH grant U01 AA013566.

Title: **Oxidative/Nitrosative Stress in Various Animal Models of Heart Failure**	**Pal Pacher** **Partha Mukhopadhyay; Sandor Batkai, Mohanraj Rajesh** **National Institute on Alcohol Abuse and Alcoholism**	Previous experimental and clinical studies have suggested that there is an increased production of reactive oxygen species (ROS: superoxide, hydrogen peroxide, hydroxyl radical, etc.) both in animals and in patients with acute and chronic heart failure. The possible source of increased ROS in the failing myocardium include xanthine and NAD(P)H oxidoreductases, cyclooxygenase, the mitochondrial electron transport chain and activated neutrophils among many others. The excessively produced nitric oxide (NO) derived from NO synthases has also been implicated in the pathogenesis of heart failure. The diffusion limited reaction of NO with superoxide yields peroxynitrite, a reactive oxidant, which has been shown to impair cardiac function via multiple mechanisms. Here we show an example emphasizing the importance of the interplay of nitric oxide, mitochondrial superoxide and peroxynitrite in a clinically relevant animal model of heart failure induced by chemotherapeutic agent Doxorubicin, and reveal the role of these radicals in apoptotic/necrotic cell death both *in vivo* and *in vitro* in live cells. This work supported by: NIAAA

Title: **Mitochondrial Motility and Fusion-Fission Dynamics: A Potential Target of Ethanol**	**Hajnoczky, Gyorgy** **Thomas Jefferson University**	Chronic alcoholism is often accompanied by mitochondrial dysfunction and induces characteristic changes in mitochondrial morphology in a variety of tissues. Emerging research supports a close interdependence between mitochondrial morphology and function and implicates mitochondria shaping factors as major regulators of mitochondrial function and cell survival. It is likely, therefore, that the morphological changes in mitochondria evoked by chronic alcohol intake are relevant for the impaired function of mitochondria. Mitochondrial morphology is dynamically controlled, primarily by fusion, fission and motility, but these processes have been difficult to study until very recently. To elucidate the physiological and pathophysiological mechanisms and significance of mitochondrial dynamics we employ novel live cell imaging approaches including the "highlighting" of single mitochondria by photoactivated fluorescent proteins. Visualizing mitochondrial fusion in real time we identified two classes of fusion events in H9c2 cells. In addition to complete fusion, we observed transient fusion events, wherein two mitochondria came into close apposition, exchanged soluble intermembrane-space and matrix proteins, and reseparated preserving the original morphology. Both transient and complete fusion exhibited rapid kinetics of the sequential and separable mergers of the outer and inner membranes. Transient fusions usually began from oblique or lateral interactions indicating association of the mitochondria with separate microtubules whereas complete fusion resulted from longitudinal merging of organelles traveling along a single microtubule. Loss of transient fusions was caused by either the chemical disruption of the microtubules or hormone-induced suppression of mitochondrial motility, suggesting involvement of the mitochondrial motors in the rapid reseparation. These interventions also turned ATP producing mitochondria to ATP consumers. Thus, a mitochondrial quality control mechanism seems to require the transient fusion and motility of the mitochondria. We are currently using ethanol-treated H9c2 cells to define the effects of chronic alcohol exposure on the mitochondrial fusion mechanisms and motility dynamics.

Title: **Yeast as a Model for Human Mitochondrial Disease—Tribute to Ron Butow**	**Chen, Xin Jie** **Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY 13210.**	Mitochondrial function degenerates during aging and in aging-related neuromuscular degenerative diseases, which leads to the physiological decline of living organisms. We are interested in cellular factors that can potentially delay the degenerative process. Here, we show that reducing cytosolic ribosomal function is a robust cellular strategy that suppresses aging-related mitochondrial degeneration. We modelled the adult/later-onset degenerative disease, autosomal dominant Progressive External Ophthalmoplegia (adPEO), by introducing the gain-of-function A128P mutation into the yeast adenine nucleotide translocase, Aac2p. The *aac2*^{A128P} allele induces aging-dependent mitochondrial degeneration and phenotypically tractable degenerative cell death that occurs dominant-negatively and independent of nucleotide transport activity. This experimental system enabled us to demonstrate that mitochondrial degeneration is suppressed by lifespan-extending nutritional and environmental interventions, and by 8 longevity mutations, which are all known to reduce cytosolic ribosomal function. We found that the *aac2*^{A128P} allele is synthetically lethal to low mitochondrial membrane potential ($\Delta\psi_m$) conditions. Aac2^{A128P}-induced mitochondrial degeneration is accelerated by protein overloading onto the mitochondrial inner membrane. The data support the model that reduced cytosolic protein synthesis may increase the availability of critical molecular component(s). These findings could have implications for better understanding the recently emerging paradigms in which reducing cytosolic protein synthesis promotes longevity in several model organisms. This work supported by: 5R01-AG023731 (NIA/NIH), 0435047N (American Heart Association)

Title: **The Layered Structure of mtDNA Nucleoids**	**Bogenhagen, Daniel.** **Stony Brook University, Stony Brook, NY, USA.**	Mitochondrial DNA (mtDNA) occurs in cells in nucleoids containing several copies of the genome. While much of the leading work on these structures has been done in yeast, principally by Ron Butow's lab, we have studied nucleoids in Xenopus oocytes and in human cells in culture. A human mtDNA nucleoid containing 5-7 genomes contains 50-70 million Daltons of DNA and a comparable mass of protein packed in volume with a diameter of about 70 nm, so that it is likely the largest structure in a mitochondrion. We identified proteins associated with these nucleoids when they are biochemically purified by sedimentation and immunoaffinity chromatography[1]. More recently, we have used formaldehyde crosslinking and strongly denaturing conditions to determine which nucleoid proteins are in close contact with the mtDNA[2]. A set of core nucleoid proteins is found in both native and crosslinked nucleoids, including 13 proteins with known roles in mtDNA transactions. Several other metabolic proteins and chaperones identified in native nucleoids including ATAD3 were not observed to crosslink to mtDNA. Additional studies showed that an N-terminal domain of ATAD3 previously proposed to bind to the mtDNA D-loop[3] is directed away from the mitochondrial matrix, so that it is unlikely to interact with mtDNA in vivo. These results are discussed in relation to a model for a layered structure of mtDNA nucleoids as a major biosynthetic center in mitochondria in which replication and transcription occur in the central core while translation and complex assembly may occur in the peripheral region. [1] Wang, Y., and Bogenhagen, D.F. (2006). Human Mitochondrial DNA Nucleoids Are Linked to Protein Folding Machinery and Metabolic Enzymes at the Mitochondrial Inner Membrane. J Biol Chem *281*, 25791-25802. [2] Bogenhagen, D.F., Rousseau, D., and Burke, S. The Layered Structure of Human mtDNA Nucleoids. submitted [3] He, J., Mao, C.-C., Reyes, A., Sembongi, H., Di Re, M., Granycome, C., Clippingdale, A.B., Fearnley, I.M., Harbour, M., Robinson, A.J., *et al.* (2007). The AAA+ protein ATAD3 has displacement loop binding properties

and is involved in mitochondrial nucleoid organization. J Cell Biol *176*, 141-146.

This work supported by: NIH GM029681 and ES012039

Title: **Proteomic Technologies for Cancer Research: Strategic Plan—Translating**	**Rodriguez, Henry** **National Cancer Institute, Bethesda, MD**	Proteomics technologies have revolutionized cell biology and biochemistry by providing powerful new tools to characterize complex proteomes, multiprotein complexes and posttranslational modifications. Although proteomics technologies could address important problems in clinical and translational cancer research, attempts to use proteomics approaches to discover cancer biomarkers in biofluids and tissues have been largely unsuccessful. To complete the bridge from discovery to the patient, proteomic platforms, reagents and data analysis must be brought up to rigorous clinical standards. The National Cancer Institute has taken a leading role in facilitating the translation of proteomics from research to clinical application, through its Clinical Proteomic Technologies Initiative (CPTI) for Cancer (http://proteomics.cancer.gov). This program is designed to accelerate proteomics from a research tool into a reliable and robust clinical application by improving protein measurement capabilities and evaluating promising technologies for applicability in both analytical and clinical validation studies. This is to be achieved through identifying major sources of experimental variability and optimizing existing proteomic platforms to enable labs to compare data and results; developing innovative and advanced proteomic technologies capable of identifying rare cancer-related proteins circulating in body fluids such as blood; and developing a much needed clinical reagents and resources core of well-characterized biological samples, reagents, reference sets, and standards available to the scientific community. This talk will outline a strategy for the advancement of clinical proteomic technologies that perform consistently across platforms, instruments and laboratories to facilitate biomarker discovery.

Title: **Mitochondrial DNA Depletion Syndromes**	**Naviaux, Robert K.** **The Mitochondrial and Metabolic Disease Center, University of California, San Diego, 214 Dickinson St., Bldg CTF, RM C103, San Diego, CA 92103-8467. Email: Naviaux@ucsd.edu**	Defects in mitochondrial DNA replication and repair produce a broad spectrum of disorders that range in severity from catastrophic diseases like Alpers syndrome that can kill in the first year of life, to more indolent disorders with isolated progressive external ophthalmoplegia (PEO), which may not present until the 5th decade of life. Pathogenic mutations in 11 genes are known, and more genes are still being discovered. Recessive, dominant, and sporadic forms with *de novo* dominant mutations are known. Causative genes can be grouped broadly in 2 categories: replication/repair, and nucleotide salvage or import. The most commonly mutated gene known is the mitochondrial DNA polymerase gamma (*POLG*). The carrier frequency for pathogenic mutations in *POLG* may be as high as 2% of the population, and produce a disease frequency of nearly 1:10,000. Mutations in *POLG* can cause at least 7 different phenotypes, from Alpers syndrome and PEO with or without Parkinson-like features, to the most common form of inherited ataxia in northern Europe, known as MIRAS or ANS, for ataxia-neuropathy spectrum disorder. Other genes include: *POLG2, TWINKLE, DGUOK, TP, TK2, SUCLA2, ANT1, MPV17, DNC,* and *p53R2.* Both phenocopies and genocopies occur. While expert clinicians may appreciate the clinical overlap in the symptoms of many of these disorders, the vast majority of physicians will view them as discrete disorders, creating a significant diagnostic challenge. Moreover, all mtDNA disorders evolve over time, so the symptoms required for diagnosis may not be present at the onset of disease. Founder effects produce large differences in the relative frequency of these disorders, and of specific mutations in different populations. A combination of clinical, family history, neuroimaging, and laboratory findings helps to narrow the differential diagnosis. Diagnosis is confirmed by DNA sequencing of the suspected gene(s).

Title: **Acquired and Genetic Diseases of the Mitochondrial DNA Polymerase**	**Copeland, William C.,** **Mitochondrial DNA Replication Group, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, Reseaech Triangle Park, NC 27709**	DNA polymerase , pol is required for replication and repair of mitochondrial DNA. Human pol is composed of a 140-kDa catalytic subunit and a 55-kDa accessory subunit. The catalytic subunit contains DNA polymerase activity, 3'-5' exonuclease proofreading activity, and a 5'dRP lyase activity that is required for base excision repair. We are studying the role of pol in mtDNA mutagenesis and disease. Pol can produce mutation in mtDNA through three distinct mechanisms: 1.) spontaneous mutations produced in mtDNA during replication and repair; 2.) by inhibition of mtDNA replication from antiviral nucleoside analogs (ANAs) used to treat HIV infections; and 3.) from mutations in the gene for pol *POLG.* Mutations in *POLG* are linked to several mitochondrial disorders of varying intensity, including progressive external ophthalmoplegia (PEO), sensory and ataxic neuropathy, Alpers syndrome, and male infertility. To date, over 100 disease mutations and several non-synonymous polymorphisms have been identified in the *POLG* coding region (see the complete list, visit the Human DNA Polymerase Gamma Mutation Database at http://tools.niehs.nih.gov/polg/). Our group is exploring the consequences of *POLG* disease mutations by characterizing the recombinant pol enzyme with the disease substitutions and a summary of these results will be discussed. Many ANAs induce a mitochondrial toxicity characterized by loss of mitochondrial DNA, muscle myopathy, peripheral neuropathy, and lipodystrophy. This loss of mtDNA and associated symptoms are caused, in part, by the incorporation of these analogs into mtDNA by pol . We have elucidated the mechanism of this inhibition as well as amino acids in pol responsible for this incorporation and induced toxicity. This work supported by: Intramural funds from the NIEHS

Title: **Frequent Mitochondrial DNA Mutations and Polymorphisms in Childhood Cancer Survivors Following Multi-Agent Chemotherapy**	**Lipshultz MD, Steven E.** **Vernon E. Walker DVM PhD, Lovelace Respiratory Research Institute, Albuquerque, NM; Stuart R. Lipsitz, Brigham and Women's Hospital, Boston, MA; Rebecca E. Scully, University of Miami, Miami, FL; Elly Barry MD, Dana-Farber Cancer Institute, Boston, MA; Salina M. Torres MPH, Lovelace Respiratory Research Institute, Albuquerque, NM; Dale M. Walker DVM, Experimental Pathology Laboratories, Sterling, VA; Stephen E. Sallan MD, Dana-Farber Cancer Institute, Boston, MA; Tracie L. Miller MD, University of Miami, Miami, FL for the Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium**	Background: Progressive myocardial dysfunction, cardiomyopathy, congestive heart failure (CHF), and sudden cardiac death are well-recognized potential late effects of cancer treatment administered in childhood. Mounting data implicate treatment-induced mitochondrial DNA (mtDNA) mutations as one potential mechanism of cardiac toxicity. Methods: The Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia (ALL) Consortium collected peripheral blood mononuclear cell samples from 93 long-term ALL survivors who were 4 or more years post multi-agent therapy including doxorubicin, asparaginase, corticosteroids, vincristine, methotrexate, and cranial radiation. Cumulative doxorubicin dose varied among patients by treatment protocol (median=300 mg/m^2; range 45 mg/m^2 to 470 mg/m^2). Median age at treatment was 4.5 years (range=0.5 to 20.8 years) and at mtDNA screening, 14.9 years (range=6.0 to 41.1 years). The mitochondrial tRNA genes and flanking regions were screened via PCR-based denaturing gradient gel electrophoresis (DGGE). Preliminary data shows 54 confirmed polymorphisms or mutations in 44 of 93 ALL survivors screened (47%), a significantly higher rate of mutations than that found in a control group (6 out of 44 = 13%, $p<.001$). Conclusions: These data suggest that childhood ALL and its treatment may lead to mutations, over-expression of rare polymorphisms, and the induction of persistent changes that when spontaneously occurring have been associated with clinically significant cardiac effects. This work supported by: Lance Armstrong Foundation, Women's Cancer Association, Bankhead-Coley/State of Florida Bridge Grant

Title: **Frequent Mitochondrial DNA Mutations and Polymorphisms in HIV-Infected Children Receiving Highly Active Antiretroviral Therapy**	**Walker, Vernon E.** **Salina M. Torres, Consuelo L. McCash, and Dale M. Walker** **Lovelace Respiratory Research Institute, Albuquerque, NM.**	NRTIs are remarkably effective in reducing maternal-fetal transmission of HIV-1; however, perinatal exposure of infants to these drugs may impose a risk for cancer and mitochondrial disease later in life. Researchers in France have hypothesized a link between *in utero* 3′-azido-3′-deoxythymidine (AZT) and 2′-deoxy-3′-thiacytidine (3TC) exposure in HIV-uninfected children to mitochondrial abnormalities and persistent signs of mitochondrial dysfunction involving the brain, liver, and heart (Lancet 354:1084-1089, 1999). Thus, a denaturing gradient gel electrophoresis method, using unipolar psoralen-clamped PCR oligonucleotides, was adapted to screen for mtDNA mutations in the 22 tRNA genes and flanking regions of umbilical cord tissue (where endothelial cells serve as the major source of DNA) from newborn children exposed *in utero* to NRTIs versus infants born to healthy mothers. Preliminary data from healthy newborns suggested the occurrence of only a few polymorphisms in this group, including 7 sequence variants (5 distinct polymorphisms) in 6/44 (14%) patients (averaging 0.16 sequence variants/patient). In contrast, preliminary data from infants receiving prepartum AZT-based HIV prophylaxis demonstrated increased numbers and variety of changes (12 distinct novel mutations and polymorphisms/mutations at polymorphic sites) including 25 sequence variants in 13/42 (31%) patients (averaging 0.60 sequence variants/patient). At least 5/12 distinct sequence variants from NRTI-exposed infants have some pathogenic characteristics. Increased occurrence of mtDNA mutations in AZT-treated infants is consistent with the ability of this drug to induce gene and chromosome mutations in experimental systems, however, the potential mutagenic effects of 'fetal stress responses' in the face of maternal HIV infection should also be investigated. This work supported by: NIH grant number R01 HL072727 (VE Walker)	

Title: **Fetal Mitochondrial Consequences of Transplacental NRTI Exposure**	**Poirier, Miriam C.** **Einem, Tracey L., Chu, Yvonne, Divi, Rao L., LCBG, Nagashima, Kunio, Walker, Vernon E.,** **National Cancer Institute, Bethesda, MD; SAIC, FCRDC, Frederick, MD; and LRRI, Albuquerque, NM.**	Nucleoside reverse transcriptase inhibitors (NRTIs) comprise essential therapy for HIV-1 infection but cause mitochondrial compromise in adults, and in infants exposed transplacentally. A major goal of these studies was to evaluate mitochondrial integrity, in the absence of viral infection, in fetuses born to *Erythrocebus patas* monkeys given human-equivalent NRTI doses in protocols modeled on human clinical use. A second goal was to perform an inter-species comparison of mitochondrial integrity in NRTI-exposed human and monkey fetuses, all of whom appeared to function normally after birth. Pregnant patas dams were given oral NRTIs for the last 50% (10 wk) of gestation, and infants were given the same NRTIs for 6 wk after birth. Patas exposure groups included: Zidovudine (AZT); AZT plus Lamivudine (3TC); AZT plus Didanosine (ddI); and 3TC plus Stavudine (d4T). NRTI-exposed patas infants were clinically asymptomatic but had molecular evidence of heart and skeletal muscle mitochondrial compromise, at birth and 1 year of age, that included morphological damage visible by electron microscopy (EM) and abnormal mitochondrial (mt)DNA content. Monkey umbilical cords and cord blood leukocytes had evidence of mitochondrial pathology that was almost identical to that seen in HIV-1-uninfected human infants born to HIV-1-infected mothers receiving NRTI therapy during pregnancy, demonstrating that the drug alone is capable of causing mitochondrial damage in primate fetuses. In addition, in AZT-exposed human fetuses, mtDNA depletion was observed in cord blood and peripheral blood at 1 and 2 years of age. Because NRTI-exposed children and monkeys had similar mitochondrial molecular damage in umbilical cord and cord blood leukocytes, and because the monkeys also had persistent heart and skeletal muscle damage, it is possible that heart and skeletal muscle mitochondria could also be compromised in NRTI-exposed human infants. This work supported by: The CCR/NCI intramural research program.

Title: **Chemotherapy-Associated Mitochondrial Dysfunction**	**Cohen, Bruce** **Cleveland Clinic, Cleveland, OH, USA.**	Cancer is the second leading cause of death, accounting for about 23% of all deaths in the United States. It is expected that 1.4 million people will be diagnosed with cancer this year, and about 550,000 will die from the disease. Treatment consists of surgery, radiotherapy and chemotherapy. Although biologic therapies (monoclonal antibodies and hormonal therapy) are being introduced into the treatment armamentarium, cytotoxic chemotherapy remains the most common form of treatment. The cytotoxic agents can be classified as alkalating agents, antibiotics, antimetabolites, plant-derived podophyllotoxins, taxanes and alkaloids, and topoisomerase inhibitors. The frequent and nearly universal side effects of chemotherapy, such as pancytopenia, hair loss, and the multiple effects of loss of intestinal lining integrity affect rapidly dividing cells. These side effects are dose dependent, occur soon after the dose of chemotherapy and are due to DNA injury affecting the rapidly dividing cells (tumor and healthy). Many side-effects of chemotherapy are agent specific and include hearing loss, renal tubular dysfunction, cardiomyopathy, Purkinje cell loss, neuropathy, and a wasting syndrome that cannot be explained by caloric intake. Similar to what is observed in mitochondrial disorders, these later side-effects occur in tissues that are post-mitotic at birth. The mechanism of toxicity of several antitumor agents has been linked to mitochondrial injury and the discussion will focus on the basic and clinical evidence that adriamycin and cisplatin are mitotoxic, and speculate on the basis of toxicity caused by the anti-metabolites 5-fluorouricil, cytosine arabinoside and methotrexate, as well as speculate on potential therapies to possibly limit the toxicity.

Title: **The Impact of Voluntary Health Organizations on Mitochondrial Research and Physician Outreach**	**Mohan,Charles** **United Mitochondrial Disease Foundation**	Voluntary health organizations specifically the United Mitochondrial Disease Foundation (UMDF) are having a multi-faceted impact on mitochondrial research and physician outreach. UMDF provides an essential resource for funding studies of mitochondrial disease that are unlikely to garner support through conventional funding sources ultimately providing greater potential to be further funded by larger organizations, particularly the NIH. UMDF support of over $6,000,000 in “bootstrapping” grants has increased interest in basic as well as translational research. Half of the grants supported by UMDF last year were awarded to researchers outside of the United States attracting international attention. UMDF sponsored symposia has created a forum for the exchange of information and education between the scientific, medical communities, allied health professionals and affected families. UMDF has seen community awareness, interest and involvement increase as chapters spread across the country pushing mitochondrial disease into the limelight on a local level enhancing the potential to be noticed and make an impact on a national level. Members of voluntary health organizations expand the scope of necessary awareness attracting the attention of their local physician, allied health groups and political community. This enables them to provide this audience with important materials increasing their awareness and understanding of the disease. Results of increased awareness and education has help direct insurance, state, and federal attention to the plight of mitochondrial patients and their families, many of whom can no longer work, and have trouble affording the supplements

		and treatments they need. Voluntary organizations provide mitochondrial disease specialist contact information to other physicians for the purpose of consultation, as well as provide specific clinic and lab information providing greater opportunities for better diagnosis, treatments and ultimately a cure for mitochondrial disease.	

Title: **The Rare Diseases Clinical Research Network (RDCRN)**	**Gopal-Srivastava, Rashmi** **NIH Office of rare Diseases. The Rare Diseases Clinical Research Network (RDCRN)**	Since FY 2003, the Office of Rare Diseases (ORD) has collaborated with NIH Institutes and Centers (ICs) to support the Rare Diseases Clinical Research Network (http://rarediseasesnetwork.epi.usf.edu/). The RDCRN consists of 10 consortia (Rare Diseases Clinical Research Center, RDCRC) each of which focuses on a group of rare diseases that includes between three to eight rare diseases each, totaling approximately 50 rare diseases for the network. In addition, the network includes a data and technology coordinating center (DTCC) that serves all consortia. The collaborating patient advocacy groups, through a coordinating coalition, participate on the network's steering committee. The network consists of more than 70 sites and includes more than 30 patient advocacy groups. The distribution of research locations across the United States makes investigational studies and treatments more accessible to patients with rare diseases. The network through the DTCC collects clinical information to develop biomarkers and new approaches to diagnosis, treatment, and prevention of rare diseases; provides training of new clinical research investigators; and supports demonstration projects. The purpose of this cooperative research program is to facilitate clinical research in rare diseases through support for 1) collaborative clinical research in rare diseases, including longitudinal studies of individuals with rare diseases, clinical studies, phase one and two trials, and/or pilot and demonstration projects; 2) training of clinical investigators in rare diseases research; 3) a test bed for distributed clinical data management that incorporates novel approaches and technologies for data management, data mining, and data sharing across rare diseases, data types, and platforms; and 4) access to information related to rare diseases for basic and clinical researchers, academic and practicing physicians, patients, and the lay public. Each RDCRC includes a consortium of clinical investigators, institutions, and relevant organizations, including patient support organizations, for the study of a subgroup of rare diseases. This cooperative agreement program facilitates identification of biomarkers for disease risk, disease severity/activity, and measures of clinical outcome appropriate

		for applicability to clinical trials and encourages development of new approaches to diagnosis, prevention, and treatment of rare diseases. In past one and a half year more than twenty five clinical protocols have been approved by the Protocol Review Committees (PRC), Data Safety Monitoring Board (DSMB) and the NIH, and a few are under development. At this time 26 studies are included in ClinicalTrials.gov ((http://www.clinicaltrials.gov <http://www.clinicaltrials.gov/>) and 24 are actively recruiting patients. The ORD and collaborating ICs continue to discuss and decide future plans for the RDCRN and develop a timetable for the announcement and release (re-issuance) of a Request for Application (RFA).	

Title: **Challenges in Dietary Supplement Research: Application to Mitochondrial Disease**	**Coates, Paul M.** **Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA.**	The Office of Dietary Supplements (ODS) at NIH is charged with evaluating, coordinating, and conducting research, and disseminating the results of research on dietary supplements (DS) to enhance the quality of life of the US public. Overall, NIH sponsors considerable research on DS ($200-300 million per year), much of which focuses on mechanism of action and health effects. ODS contributes to these efforts and provides additional resources including: evidence-based reviews of efficacy and safety to inform research agenda development; databases to support the assessment of use in the population; and analytical tools. ODS also disseminates professional and consumer information. DS are marketed and regulated in the USA as foods, not as drugs. There is no pre-market approval for DS ingredients; manufacturers maintain documentation of their efficacy and safety. DS ingredients are intended for health promotion and chronic disease risk reduction, not for disease treatment or mitigation; however, much can be learned from the clinical evaluation of the ingredients in patients that can inform their use in healthy individuals. Dietary interventions, including the use of DS ingredients, are a mainstay in the management of many mitochondrial diseases. Nevertheless, evidence-based approaches for these dietary interventions have been infrequently employed, in large part due to the rarity of most of the individual diseases. Some of the same ingredients found in DS (coenzyme Q10, B-vitamins, antioxidants, carnitine, creatine, etc.) are also used as drugs – alone or in cocktails – in the treatment of mitochondrial diseases. Consumers use them widely for their purported energy-enhancing effect based on evidence from their use in the treatment of patients with mitochondrial diseases. The mitochondrial disease community is urged to develop sound protocols for the systematic evaluation of these agents. This will benefit not only the thousands of patients with these diseases, but also the millions in the general population who

consume these ingredients as DS.

This work supported by: National Institutes of Health.

Title: **Introduction to CoQ10, Carnitine, Creatine, B-Vitamin, Antioxidant Cocktails, and Clinical Monitoring Guidelines for Assessing Clinical Outcomes**	**Cohen, Bruce.** **Cleveland Clinic, Cleveland, OH, USA.**	Introduction to CoQ10, levocarnitine, creatine, B-vitamin, antioxidant cocktails, and clinical monitoring guidelines for assessing clinical outcomes. The symptoms of mitochondrial disease result from a relative or absolute deficiency in ATP availability. Although many disorders of metabolism may ultimately result in ATP deficiency, dysfunction of the respiratory chain and closely associated metabolic pathways are the main cause of mitochondrial disease. Respiratory chain dysfunction also results in increased superoxide production, resulting in excessive free radical concentrations within the mitochondria, and subsequent injury to the lipids and nucleic acid structure in the very organelle responsible for energy production. Many steps in intermediary metabolism are vitamin and cofactor-dependent, and several, including electron shuttling, require a specific co-factor. In an attempt to maximize energy flux in the disease state, physicians have advocated using vitamins and cofactors, as well as suggesting that supplemental anti-oxidants may help squelch excess free-radicals and thereby limit toxicity. The use of evidence-based medicine (EBM) has become part of clinical practice, but there are few randomized controlled trials that support the use of these substances. Until recently the Effectiveness of Treatment was at best, Level II-2, II-3 and III with Recommendation for Treatment at Level C for most of the vitamin and cofactors. The difficulties in conducting these studies include the vast array of different genotypic and phenotypic disorders, the unpredictable natural history of each disorder, the difficulty in developing a placebo arm, even in crossover studies, and the easy availability of the substances under evaluation. Finally, developing entry criteria and single outcome measures for an illness that may have several evaluable manifestations provides a methodological and statistical challenge. Recent clinical evidence for the use of vitamin and cofactor therapy will be presented as well as proposed methods of monitoring treatment efficacy.

Title: **Mitochondrial Topoisomerase I (Top1mt) Controls Mitochondrial DNA Replication Through D-Loop Formation**	**Pommier,Yves** **Hongliang Zhang and Yves Pommier** **National Cancer Institute, Bethesda, MD**	Somatic cells contain thousands of copies of mitochondrial DNA (mtDNA), which consist of duplex DNA circles encoding genes essential for oxidative phosphorylation and cellular metabolism. mtDNA replication must, therefore, be tightly controlled. In animal mtDNA replication, most nascent strands from the leading, heavy-strand origin (O_H) are prematurely terminated, generating a 650-base, 7S-DNA product that defines the 3' boundary of the so-called "displacement loop" (D-loop). Proper formation of the D-loop is critical to the entire replication process and therefore to the integrity of the cell, but the control elements for it have not been identified. Here we show that mitochondrial topoisomerase I (Top1mt) is responsible for that control. In intact mitochondria, Top1mt sites are confined to three sites within a 150-bp segment downstream from, and adjacent to, the site at which replication is terminated prematurely. We also find that TOP1mt knockout cells show defects in that termination process, with abnormal extension of the 7S-DNA into the coding region. Moreover, inhibition of Top1mt by camptothecin reduces formation of the 7S-DNA. Taken together, our findings demonstrate that Top1mt controls mtDNA replication by regulating the premature termination of replication.

Title: **Mitochondrial Respiratory Enzyme Dysfunction in Midbrain DA Neurons Elicits Parkinsonian Symptomatology: A Novel Genetic Mode**	**Hoffer, Barry** **National Institute on Drug Abuse, NIH, Baltimore, MD, USA.**		

Title: **Is Tyrosyl-DNA Phosphodiesterase a Mitochondrial DNA Repair Enzyme?**	**Povirk, Lawrence F.** **Konstantin Akopiants, Tong Zhou, Amy J. Hawkins, Kristoffer Valerie#, Penelope Mason*, Nadja Souza-Pinto*, Vilhelm Bohr* and Lawrence F. Povirk** **Department of Pharmacology and Toxicology and #Department of Radiation Oncology, Virginia Commonwealth University; and *Laboratory of Molecular Gerontology, National Institute on Aging.**	Mitochondrial DNA topoisomerase I is closely related to its nuclear homolog and therefore is expected to generate a low level of persistent single-strand breaks in mitochondrial DNA, with topoisomerase covalently linked to the 3′ terminus. In nuclear DNA such lesions are repaired by TDP1, polynucleotide kinase/phosphatase, XRCC1 and DNA ligase III. DNA ligase III binds directly to the N-terminus of TDP1 and is found in mitochondria. To assess the possible role of TDP1 in repair of topoisomerase-mediated lesions in mitochondrial DNA, mitochondrial extracts were screened for tyrosyl-DNA processing activity. Although TDP1 has no apparent mitochondrial targeting sequence, TDP1-like activity was detected in mitochondrial extracts from several sources, at levels very similar to those in whole-cell and nuclear extracts. Such activity was not found in mitochondrial extracts from TDP1-mutant SCAN1 (spinocerebellar ataxia with axonal neuropathy) cells, confirming that the activity was attributable to TDP1. Western blotting also confirmed the presence of TDP1 protein in mitochondrial extracts, while as reported previously XRCC1 was found in nuclear but not in mitochondrial extracts. However, screening of young and old Tdp1-/- knockout mice showed that deletions in mitochondrial DNA accumulated at rates similar to those in normal mice. The results suggest that TDP1 may be involved in mitochondrial DNA repair, but its deficiency does not lead to increased mitochondrial DNA deletions.

Title: **Pathways for Mitochondrial DNA Repair: Relevance to Aging and Disease**	**de Souza-Pinto, Nadja C. and Bohr, Vilhelm A.** **Lab. Molecular Gerontology, NIA-IRP, NIH, Baltimore, MD 21224.**	Mitochondria contain their own genome, which encodes for 13 polypeptide components of the respiratory chain and the RNA repertoire required for translation. Accumulation of DNA damage, mutations and deletions of the mtDNA has been associated with inherited or acquired diseases, as well as normal aging. While earlier results suggested that mammalian mitochondria lack DNA repair activities altogether, at least one repair pathway has been undoubtedly assigned to mitochondria. Base excision repair (BER) is the main repair pathway for small base modifications, such as oxidation and methylation. We have characterized several BER enzymes from mammalian mitochondria and shown that their activities vary significantly depending on the tissue of origin. Moreover, both aging and calorie restriction, the only intervention proven to extend both medium as well as maximum life span in mammals, modulate mitochondrial DNA repair activities, again in a tissue-specific fashion. These results indicate that mitochondrial DNA repair is likely regulated by pathways that respond to the metabolic/redox state of the tissue. Interestingly, we found that absence of mtDNA does not affect mtBER significantly. In contrast, p53, which translocates to mitochondria under stress conditions, modulates mtBER through a stimulation of the nucleotide incorporation step. However, no direct physical interaction between DNA pol gamma and p53 was observed, suggesting that another protein/pathway mediates this stimulation. P53 binds efficiently to TFAM and modifies its binding to damaged DNA (*Yoshida et al., Cancer Res, 63: 3729, 2003*). Thus, we investigated whether TFAM modulates mtBER activities. We found that TFAM binds more strongly to DNA containing 8-hydroxyguanine lesions and inhibits OGG1 activity, as well as other BER activities. Because binding of TFAM to oxidized DNA was inhibited by p53 (*Yoshida et al.*) it is possible that p53 modulates mtBER partially via an interaction with TFAM. This work supported by: NIA-IRP

Title: **A Mitochondrial Paradigm for Metabolic and Degenerative Diseases, Cancer, and Aging**	**Wallace, Douglas C.** **Center for Molecular and Mitochondrial Medicine and Genetics (MAMMAG), University of California, Irvine, Irvine, CA 92697-3940.**	Life is the interplay between structure and energy, and most of human energy is generated by the mitochondria. The mitochondria play three major roles in the cell: production of energy by the oxidative phosphorylation (OXPHOS), generation of cellular reactive oxygen species (ROS) as a by product of OXPHOS, and initiation of apoptosis by the activation of the mitochondrial permeability transition pore (mtPTP) when energy output declines and/or ROS damage becomes excessive. Mitochondrial OXPHOS is assembled from two information storage and retrieval systems: the nucleus-cytosol and the mitochondrion. Therefore, the mitochondrial genome consists of approximately 1500 genes, 37 encode by the mitochondrial DNA (mtDNA) and the remainder encoded by the nuclear DNA (nDNA). The mtDNA is present in thousands of copies per cell, can encompasses mixtures of mutant and normal mtDNAs (heteroplasmy), can drift toward more mutant or wildtype during successive mitotic cell divisions (replicative segregation), and is strictly maternally inherited. mtDNA mutations have been linked to a wide spectrum of age-related degenerative disease symptoms including diabetes, deafness and blindness, dementias, movement disorders, muscle and heart dysfunction, renal failure, etc. Moreover, ancient mtDNA polymorphisms have been identified that permitted humans to move out of tropical Africa into temperate Eurasia and arctic Siberia by shifting mitochondrial energy allocation from predominantly ATP production to more heat generation. The mtDNA has also been found to accumulate somatic mutations with age as a result of ROS damage. This results in the age-related decline in mitochondrial function thus accounting for aging. This same mitochondrial function decline can exacerbate partial nuclear defects resulting in the delayed onset and progressive course of age-related diseases. Cancer is also an age-related disease. Germline and/or somatic mitochondrial gene mutations accumulate in cancer cells which impede electron flux through OXPHOS. This increases mitochondrial ROS production which acts as both a nuclear gene mutagen (initiator) and nuclear mitogen (promotor) driving the cell into neoplastic transformation. Cancer cells must also adapt to

		new environments. This may be why they acquire some of the same functional mtDNA polymorphisms as have been found in the human populations that adapted to different climatic zones.	

Mitochondria Minisymposium 2008

Mitochondria and their Proteomics

Selected Platform Speaker Abstracts

Title: **Effects of Middle Domain Interactions and Posttranslational Modifications on Drp1 Function and Mitochondria Morphology**	**Chunag-Rung CHANG and Craig BLACKSTONE** **Cellular Neurology Unit; National Institute of Neurological Disorders and Stroke** **National Institutes of Health; Bethesda, MD 20892**	Mitochondria in cells are dynamic organelles comprising a tubulovesicular reticulum shaped by continuous fission and fusion events that are important for maintenance of mitochondria functions. The multimeric dynamin-like GTPase Drp1 is a critical protein mediating mitochondrial fission, and it harbors multiple motifs including GTP-binding, middle, and GTPase effector (GED) domains that are important for both intra- and intermolecular interactions. Reminiscent of other dynamin superfamily members, such interactions are critical for assembly of higher-order structures and cooperative increases in GTPase activity. Our lab focuses on the cellular mechanisms regulating Drp1 function, and we have recently reported that phophorylation at S637 by protein kinase A alters intramolecular interactions and decreases GTPase activity. Here we demonstrate that mutations within the Drp1 middle domain, including A395D, which has been reported to cause a lethal mitochondrial syndrome, along with G350D and G363D, alter Drp1 oligomerization, impair its GTPase activity, and interfere with intramolecular associations between GED and GTPase–middle domains. Over-expression of these mutant Drp1 proteins in HeLa cells causes tubulation of the mitochondria by inhibiting mitochondrial fission. Meanwhile, we have identified several sumoylation sites within Drp1 and are currently investigating the impact of sumoylation on Drp1 GTPase activity and distribution. With emerging evidence of multiple post-translational modifications regulating Drp1 functions, we are also examining the coordination between Drp1 phosphorylation and sumoylation. These studies will provide important insights into the dynamic regulation of Drp1 function and mitochondrial membrane dynamics.

Title: **Cytoskeleton regulates mitochondria respiration through the tubulin-VDAC direct interaction**	**Tatiana Rostovtseva** **[1]Tatiana K. Rostovtseva, [2]Dan L. Sackett, [3]Claire Monge, [3]Valdur Saks, and [1]Sergey M. Bezrukov** **[1]Laboratory of Physical and Structural Biology; [2]Laboratory of Integrative and Medical Biophysics, NICHD, NIH, Bethesda, MD 20892, USA; [3]Laboratory of Fundamental and Applied Bioenergetics, Joseph Fourier University Grenoble Cedex 9, France**	Mitochondria have long been known to localize within the tubulin-microtubule network in heart and many other cells (Appaix et al., 2003). It is also well-known that in permeabilized cardiac cells the apparent K_m for exogenous ADP in the control of mitochondrial respiration is significantly higher than in isolated mitochondria. It has been suggested that the low permeability of the mitochondria outer membrane (MOM) for ATP and ADP in cells is due to interaction of mitochondria with some cytoplasmic proteins (Saks et al., 2003). Here, for the first time, we demonstrate that tubulin is the factor which controls MOM permeability by regulating VDAC, the major channel of MOM. By direct measurements we show that nanomolar concentrations of mammalian tubulin induce highly voltage-sensitive reversible closure of VDAC channels reconstituted into planar phospholipid membranes. Analysis of VDAC single channel fluctuations in the presence of tubulin shows that channel closure occurs at very low potentials (as low as 10 mV) compared to VDAC gating in control. The tubulin-VDAC interaction requires the presence of negatively charged C-terminal tails of tubulin. Tubulin with proteolytically removed C-terminus does not induce VDAC closure. We propose a model of tubulin-VDAC interaction in which the tubulin C-terminus penetrates into the channel lumen, interacting with VDAC with high specificity and blocking channel conductance. The experiments with isolated heart mitochondria strongly confirm our findings. Apparent K_m for exogenous ADP increases 10 times after addition of 1-10 M of tubulin to isolated heart mitochondria. We conclude that tubulin strongly limits ADP entry to mitochondria across its outer membrane. Our results suggest a new general mechanism of regulation of mitochondrial outer membrane permeability under normal and apoptotic conditions.

Title: **Biochemical characterization of Alpers mutants in human DNA polymerase**	**RAJESH KASIVISWANATHAN AND WILLIAM C. COPELAND** **NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES**	Alpers' syndrome is a rare heritable autosomal recessive disorder primarily affecting young children and is characterized by refractory seizures, psychomotor regression, and hepatic failure. It generally manifests during the first few weeks to years of life and symptoms gradually develop in a stepwise manner leading eventually to death. Mutations in *POLG*, the gene encoding the catalytic subunit of human DNA polymerase (pol) causes this syndrome by depleting mitochondrial DNA. Human pol is a two-subunit complex comprising a catalytic (p140) and an accessory (p55) subunit and bears the burden of replicating and repairing the mitochondrial genome. The catalytic subunit contains an N-terminal exonuclease domain connected to a C-terminal polymerase domain by a linker region. To date, around 40 disease mutations identified in *POLG* are associated with Alpers syndrome and only 2 mutations (A467T and W748S-E1143G) have been biochemically characterized. Hence, this study attempts to dissect the biochemical defects of 6 Alpers mutations located in the polymerase domain of the catalytic subunit. DNA polymerase assays performed with purified enzymes on a poly(rA).oligo(dT) substrate revealed <1% activity for G848S, R852C and R853Q mutants and 50-80% activity for T851A, Q879H and T885S mutants compared to the wild-type enzyme. Current experiments are examining the steady-state kinetic values, DNA binding, processivity, fidelity and p55 interaction efficiency of the mutant enzymes. The results from these analyses should provide insights into the biochemical deficiencies of the mutant enzymes and may assist in understanding the severity of these mutations in patients with the syndrome.

Title: **Innate ability of mammalian mitochondria to import tRNAs by a mechanism distinct from protein import.**	Alfonzo, Juan D. Microbiology Department, The Ohio State University, Columbus, Ohio.	A majority of eukaryotes contain mitochondria where protein-mediated coupling of electron transport leads to production of the bulk of the ATP made in cells. All mitochondrial genomes encode a small subset of proteins needed for respiration. Paradoxically, in many organisms the mitochondrial genomes lack a complete set of tRNA genes needed for translation and have thus evolved mechanisms for the import of nucleus-encoded tRNAs from the cytoplasm. We show for the first time that mammalian mitochondria have the innate ability to import tRNAs, whereby two out of four nucleus-encoded $tRNA^{Gln}$ isoacceptors are localized to the mitochondria *in vivo*. These tRNAs can also be efficiently and specifically imported into mammalian mitochondria *in vitro* in the absence of added cytosolic factors. We suggest that import of $tRNA^{Gln}$ is conserved among all mitochondria-containing eukaryotes and occurs by a mechanism that is distinct from protein import. Our findings, while having direct implications for the study of mitochondrial tRNA mutations linked to diseases, also offer a cautionary view to the use of tRNA import systems as the basis for therapy.

Title: **Mitochondrial Localization of Mammalian Ribonuclease H1**	**Suzuki, Yutaka., Cerritelli, Susana M., Sakhuja, Kiran. & Crouch, Robert J.** **NIH/NICHD. U.S.A.**	Ribonucleases H (RNase H) are enzymes that cleave RNA of RNA/DNA hybrids. Using knockout mice, we have found that RNase H1 is required for amplification of mitochondrial DNA (mtDNA) during embryonic development. Transient expression of RNase H1 fused to GFP indicates that the majority of this protein localizes to the nucleus with only a minor portion targeted to mitochondria. We are interested in how this dual localization is achieved and regulated. To understand the mechanism of distribution of this protein, we constructed fusion proteins of several regions of mouse RNase H1 with GFP. Transient expression of those proteins revealed that N-terminal 26 amino acid sequence is sufficient for mitochondrial targeting signal and that S96-D285(C-terminal) region contains the nuclear targeting signal. In addition, we found that translation initiation from the second in-frame AUG codon (Met27) results in production of an RNase H1-GFP fusion protein lacking the mitochondria targeting signal. This was confirmed by introduction of M27I mutation into M1-S96-GFP fusion construct, which changed its localization from mitochondria-cytoplasm to exclusively mitochondria. The second in-frame AUG codon encoding Met27 is conserved in all of the mammalian RNase H1 known so far. We are studying the possibility that production of mitochondrial RNase H1 is regulated at the level of translation initiation.

Title: **Biochemical characterization of the mitochondrial RNA polymerase**	**Arnold, Jamie J. and Cameron, Craig E.** **Department of Biochemistry & Molecular Biology, The Pennsylvania State University, University Park, PA 16802**	We have used a synthetic scaffold to assemble a catalytically competent elongation complex containing the mitochondrial RNA polymerase that permits the kinetics and mechanism of nucleotide addition to be studied. We show that incorporation of AMP as a correct nucleotide is dependent on ATP concentration (Kd,app of 30 μM) and is fast (30/s). The frequency of GMP misincorportation was ~1 per 45,000 nucleotides incorporated. The enzyme was capable of utilizing both 2'-dATP and 3'-dATP more efficiently than an incorrect nucleotide. In addition to these nucleotide analogues, the enzyme utilized a variety of antiviral ribonucleotides, including ribavirin triphosphate and 2'-C-methyladenosine triphosphate, which are known inhibitors of RNA viruses. The cytotoxicity of the analogues tested correlates well with the mitochondrial RNA polymerase incorporation efficiency. Incorporation of certain nucleotide analogues led to rapid dissociation of the enzyme from nascent RNA. Together, these studies provide the first glimpse into the fidelity and specificity of mitochondrial RNA polymerase and suggest mechanisms for cytotoxicity of antiviral ribonucleosides.

Title: **Pathogenic Mutations in *PEO1* Cause Biochemical Defects in the Human Mitochondrial DNA Helicase**	**Matthew J. Longley, Farida S. Sharief, and William C. Copeland** **Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina, 27709**	Maintaining the integrity of the mitochondrial genome is essential for proper cellular energy metabolism, and disruption of mitochondrial DNA replication causes a range of severe mitochondrial disorders. At least 20 different missense mutations in the human *PEO1* gene, which encodes the Twinkle mitochondrial DNA helicase, co-segregate with infantile-onset spinocerebellar ataxia, recessive hepatocerebral mtDNA depletion, or dominant progressive external ophthalmoplegia associated with pronounced deletions in mitochondrial DNA. In an effort to identify the molecular mechanisms leading to mtDNA depletion and deletions, we chose a biochemical approach to characterize defects in the mutant forms of the mtDNA helicase. Wild type and mutant forms of *PEO1* were over-expressed in *E. coli*, and recombinant helicases were purified to near homogeneity by conventional chromatographic methods. The DNA helicase and ATPase activities of the purified proteins were assessed with a variety of DNA substrates. Reaction kinetics, cofactor requirements, thermal stability, and DNA-binding strength were determined for each protein. Sedimentation analyses were utilized to assess assembly into hexameric structures as well as physical interaction with other components of the mitochondrial DNA replication fork. The varied biochemical defects of each pathogenic amino acid substitution predict stalling of the mtDNA replication fork, which may promote the deletions and depletion of mtDNA observed *in vivo*.

Title: **Oxidative Stress and Mitochondrial Toxicity Relate to the Development of Alcoholic Cardiomyopathy**	**Ioan Cucoranu*1, Seyed Hosseini1, James Kohler1, David Johnson1, Elgin Green1, Amy Hoying1, Chad Haase1, Rodney Russ1, Stanley He1, Brian Day2, and William Lewis1,** **Emory University, Atlanta, GA, US1 and National Jewish Medical Center, Denver, CO, US2.**	BACKGROUND: Excessive alcohol consumption causes defects in myocardial contractility and derangement of myocyte architecture. Oxidative stress is a major source of tissue damage produced by alcohol. To study the mechanism involved in the development of alcoholic cardiomyopathy we used SOD2+/- KO mice that have limited antioxidant defense. METHODS: SOD2+/-KO and WT mice were treated with 4g/kg/d alcohol by gavages, 35 days. Left ventricle (LV) mass and left ventricle end diastolic dimension (LVEDD) were defined echocardiographically (ECHO), mitochondrial ultrastructural defects were identified by electron microscopy (EM), histological changes were identified by light microscopy, abundance of cardiac mtDNA was quantified by real-time PCR, and mitochondrial hydrogen peroxide production was measured by Amplex-Red assay. RESULTS: SOD2+/-KOs exhibited normal LV-mass and LVEDD and minor mitochondrial damage. Alcohol increased LV-mass and LVEDD in SOD2+/-KO and caused significant increase in the mitochondrial hydrogen peroxide production. There was no significant change in the LV-mass, LVEDD and mitochondrial hydrogen peroxide production in the WT treated with alcohol. Both KO and WT treated with alcohol demonstrate mitochondria that have open cristae patterns. In the SOD2+/- KO, lipid droplets appear abundant with alcohol compared to control. Histopathologically, cardiomyocites present lytic changes with increased granularity in the SOD2+/-KO mice. These changes are augmented by alcohol treatment. Also, there is increased abundance of mitochondria in SOD2+/- KO compared to WT. Determination of mtDNA abundance demonstrates a trend for increased mtDNA/nDNA ratio in SOD2+/-KO treated with ethanol.

Title: **Oxidative inactivation of key mitochondrial proteins leads to mitochondrial dysfunction and injury in hepatic ischemia reperfusion of mice**	**Kwan-Hoon Moon1, Brian L. Hood2, Partha Mukhopadhyay3, Timothy D. Veenstra2, Byoung-Joon Song1, and Pal Pacher3** **1Laboratories of Membrane Biochemistry and Biophysics and 3Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892-9410, and 2Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., Frederick, MD 21702.**	Ischemia-reperfusion (I/R) is a major mechanism of liver injury following hepatic surgery or transplantation. Despite numerous reports on the role of oxidative/nitrosative stress and mitochondrial dysfunction in hepatic I/R injury, the proteins that are oxidatively-modified during I/R damage are poorly characterized. This study was aimed at investigating the oxidatively-modified proteins underlying the mechanism for mitochondrial dysfunction associated with acute hepatic I/R injury. We also studied the effects of a superoxide dismutase mimetic/peroxynitrite decomposition catalyst metalloporphyrin MnTMPyP given before I/R insult on oxidatively-modified proteins and their functions. The oxidized and/or S-nitrosylated mitochondrial proteins from I/R-injured mouse livers with or without MnTMPyP pretreatment, were labeled with biotin-N-maleimide, purified with streptavidin-agarose and resolved using two-dimensional gel electrophoresis. Comparative analysis of 2-DE revealed markedly increased numbers of oxidized and S-nitrosylated mitochondrial proteins following hepatic I/R injury. Mass-spectral analysis identified many key mitochondrial enzymes involved in cellular defense, fat metabolism, energy supply, and chaperones as being oxidatively-modified proteins. Pretreatment with MnTMPyP attenuated the I/R-induced increased serum transaminase levels, histological damage, increased iNOS expression, and S-nitrosylation and/or nitration of various key mitochondrial proteins. MnTMPyP pretreatment also restored I/R-induced suppressed activities of mitochondrial aldehyde dehydrogenase, 3-ketoacyl-CoA thiolases, and ATP synthase. In conlusion, these results suggest that increased nitrosative stress is critically important in promoting S-nitrosylation and nitration of various mitochondrial proteins, resulting in mitochondrial dysfunction and hepatic injury.

Title: **Seeking the biochemical basis of Type III 3-methylglutaconic aciduria through zebrafish models**	**Wuhong Pei, Isa Bernardini, Christopher Wassif, Forbes Porter, Yair Anikster, Marjan Huizing and Benjamin Feldman.** **NHGRI** **Building 35, Room 1B409, 35 Convent Drive, Bethesda, MD 20892**	Type III 3-methylglutaconic aciduria (MGA-III) is a rare disorder with early-onset optic atrophy and later-onset spasticity, cerebellar ataxis, cognitive deficit and increased urinary excretion of 3-methylglutaconic acid (3MGC) and 3-methylglutaric acid (3MGA). Genetic mapping and sequencing have identified two familial mutations in the OPA3 gene associated with MGA-III. We are trying to understand OPA3's biochemical function using the zebrafish. We found that the zebrafish orthologue is expressed ubiquitously during embryogenesis and is enriched in the brain from the pharyngula stage until at least 120 hpf. Antisense-based depletion of zebrafish Opa3 causes the signature increase in 3MGC and 3MGA, but also a more severe eye defect than seen in MGA-III patients. To explore whether Opa3 acts in the leucine catabolic pathway, we delivered exogenous leucine to Opa3-deficient embryos. As a comparison, leucine delivery to an MGA-I model deficient for the leucine catabolic pathway enzyme 3-methylglutaconyl-CoA hydratase causes no morphological defects, but leads to a sharp increase in leucine, 3MGC and 3MGA. In contrast, leucine-treated Opa3-deficient embryos display severe brain dysmorphology but show no accumulation of leucine or 3MGA. To determine whether Opa3 interacts with the mevalonate pathway, we examined the effects on Opa3-deficient embryos of mevalonate-depletion via simvastatin treatment, and found that simvastatin causes additional brain defects in Opa3-deficient embryos. We have thus uncovered two classes of metabolic sensitivity that are specific to the brain of our zebrafish MGA-III model, where opa3 expression is enriched, demonstrating that zebrafish opa3 genetically interacts both with the mevalonate and leucine catabolic pathways.

Title: **Decrease of mtDNA replication in Xenomitochondrial mouse model**	**Seyed Hosseini[1], James Kohler[1], Elgin Green[1], Chad Haase[1], David Johnson[1], Victor Kapoor[1,] Tomika Ludaway[1], Rodney Russ[1], Ian Trounce[2], Carl Pinkert[3] and William Lewis[1]** **Emory University School of Medicine, Atlanta, GA, USA[1], University of Melbourne, Melbourne, Australia[2] and Auburn University, Auburn, AL, USA[3].**	Background: It has been established that treatment with pyrimidine nucleoside reverse transcriptase inhibitors (NRTI) treatment in HIV AIDS patients impacts mtDNA replication which can result in mtDNA depletion. It is also known that genetic variation between individuals plays a crucial role in drug metabolism, response, and risk of drug toxicity. Using a xenomitochondrial mouse model, we investigated the role of genetic variability of the mtDNA template in the development of NRTI mitochondrial toxicity. Methods: Genetically engineered mice (xenomice) were employed. These xenomice harbor nuclear encoded genes from *Mus musculus domesticus* (Mm) mouse species and mtDNA encoded genes from *Mus dunni* (Md). Xenomice and wild-type (WT; containing both nuclear and mtDNA template from Mm) littermates were treated with HAART (zidovudine/lamivudine/indinavir;) or vehicle for 35 days. Changes in cardiac and mitochondrial structure and function were examined by echocardiography (ECHO), mtDNA abundance was determined by real-time PCR and nucleotide sequence was determined by direct sequencing. Results: Xenomice exhibited ~ 3-log fold decrease in mtDNA abundance in all organs examined including heart, kidney, spleen, liver, brain, and skeletal muscle compared to WT. HAART treatment of the xenomice increased mtDNA abundance ($p<0.01$) in heart, liver, and kidney. An increased left ventricular (LV) mass was also found in HAART treated Xenomice. However, HAART treatment did not have a significant effect on LV mass or mtDNA abundance of WT (Mm) mice. The D-loop region sequencing of xenomice revealed a 14.2% variation from *Mus musculus domesticus* (Mm). Conclusions: Variation in mtDNA template from related murine species reduced mtDNA replication in multiple organs of the Xenomice. Moreover, the added result of NRTI combination HAART therapy causes a compensatory mtDNA increase that correlates with cardiac hypertrophy *in vivo*.

Title: **Mutations in the gene clueless cause mitochondrial mislocalization and Parkinson-like phenotypes in the Drosophila ovary and muscle.**	**Rachel T Cox and Allan C Spradling,** **Howard Hughes Medical Institute and Carnegie Institution of Washington, Dept. of Embryology, Baltimore, MD 21218.**	Mutations in mitochondrial DNA and in nuclearly encoded mitochondrial proteins are responsible for a large number of diseases, both spontaneous and inherited. A hallmark of mitochondrial and aging diseases is neuromuscular degeneration. While many neurodegenerative diseases such as Parkinson's are associated with decreased mitochondrial function, it is becoming increasingly clear that poorly functioning mitochondria may be a primary cause of the disease, and not simply a result. I found the previously unstudied fly gene clueless (clu) causes severe effects on mitochondria in both muscle and ovarian cells. Mutations in clu are semi-lethal and clu mutants are short-lived. The mutant adults that are able to eclose are very uncoordinated, suggesting neuronal problems. clu also has a striking effect on flight muscle fibers and their mitochondria, causing indistinct sarcomere banding and enlarged, empty mitochondria. The muscle phenotype, lack of coordination, and aging defects are reminiscent of fly mutants in the parkin pathway, the homolog of a gene linked to familial Parkinson's disease in humans. In the ovary, clu mutants exhibit mislocalized mitochondria clustered to one side of the cell in germline stem cell and germ cells, as well as in a subset of ovarian somatic cells. Mutant females exhibit reduced fertility and males are sterile. Clueless is a large protein that contains five tetracopeptide repeats, repeats thought to be involved in protein-protein interactions, but its molecular function is unknown. The high degree of sequence identity from humans to yeast suggests Clueless is an important conserved mediator of mitochondrial movement and function. Studying clu function offers the opportunity to elucidate the link between mitochondrial mislocalization and muscle degeneration.

Title: **Modulating Stat1 Signaling by Mitochondrial Tid1- a human homolog of bacterial DnaJ and the Drosophila tumor suppressor Tid56**	**Lu, Bin, Suzuki, Carolyn K.** **UMDNJ-New Jersey Medical School, Newark, NJ 07103, USA**	Tid1 is a human homolog of bacterial DnaJ and the Drosophila tumor suppressor Tid56 that has two alternatively spliced isoforms Tid1-Long and Tid1-Short (Tid1-L and Tid1-S), which differ only at their carboxyl-termini. Although Tid1 localizes overwhelmingly to the mitochondrial matrix, most published data demonstrate non-mitochondrial functions of Tid1. We are trying to resolve the paradox of how this mitochondrial matrix protein functions in both inside and outside of the mitochondrion. We demonstrate that Tid1 isoforms have a conserved mitochondrial DnaJ-like function that substitutes for the yeast mitochondrial DnaJ-like protein Mdj1p in the folding and degradation of proteins. Like Mdj1p, Tid1 isoforms localize to human mitochondrial nucleoids, which are large protein complexes bound to mtDNA. Thus, like Mdjj1p, Tid1 may be important for mtDNA maintenance. Unlike most DnaJs, Tid1-L and –S form hetero-complexes with one another. Our results show that Tid1-L has a longer half-life and residency time in the cytosol than Tid1-S, which may be explained by its selective interaction with Hsc70 in cytosol. Tid1-L has also been shown to interact with a number of non-mitochondrial proteins such as E7, an oncoprotein of human papilloma virus; UL9, an origin-binding protein of herpes simplex virus type 1 (HSV-1), Tax, a viral transactivator of human T-cell leukemia virus (HTLV-1), Jak2, a Janus kinase and Stat1 (Signal Transducers and Activators of Transcription). Non-mitochondrial Tid1 has been implicated in the modulation of IFN-gamma signaling mediated by Stat1. We demonstrate here that Tid1-L overexpression inhibits IFN-gamma induced gene expression whereas Tid1-L knockdown upregulates this signaling pathway. Tid1-L knockdown increases IFN-gamma induced serine phosphorylation of Stat1 and upregulates Stat1 protein levels but has no effect on Stat1 tyrosine phosphorylation. Further experiments are currently underway to explore the mechanism(s) by which Tid1 modulates of Stat1 signaling, such as the regulation of Stat1 trafficking between the cytosol and nucleus and the regulation of Stat1 degradation.

Title: **In utero exposure of female CD-1 mice to nucleoside reverse transcriptase inhibitors (NRTIs) leads to temporal changes in mitochondrial structure, function, and mutations.**	**Torres, Salina1, Divi, Rao2, McCash, Consuelo1, Einem, Tracy2, Walker, Dale3, Poirier, Miriam2, Walker, Vernon1.** **1Lovelace Respiratory Research Institute, Albuquerque, NM 2National Cancer Institute, Bethesda, MD; 3BioMosaics, Burlington, VT**	Although NRTIs are effective in inhibiting viral replication in HIV-infected patients and in reducing vertical transmission of HIV from mother to child, emerging evidence suggests that AZT-based therapies increase risk for mitochondrial dysfunction and cardiotoxicity. The relative impact of AZT, 3TC, or AZT/3TC on mitochondrial structure and function was examined in hearts of female mice exposed transplacentally during the last 7 days of gestation. Echocardiography was performed at 13 and 26 weeks of age; the female offspring were then necropsied and their hearts were sectioned for examination by light and ultrastructural microscopy. Mitochondrial functional changes were evaluated by measuring OXPHOS enzyme activities, mitochondrial DNA (mtDNA) depletion, and mutations. Analyses of echocardiographic data revealed a progressive decrease in left ventricular wall thickness that, at 26 weeks of age, was statistically less in AZT-exposed ($p=0.021$), 3TC-exposed ($p=0.006$), and AZT/3TC-exposed ($p<0.001$) mice compared with controls. The left ventricular wall measurements in the AZT/3TC-exposed group was also significantly less than those in the AZT-only ($p=0.013$) and 3TC-only ($p=0.009$) groups. Ultrastructural alterations within each treatment group were distinctive and progressive. Complex IV enzyme activities were greater than control mouse values in all exposure groups at both 13 and 26 weeks of age, with a more pronounced increase found at 26 weeks. Possible treatment-induced mtDNA depletion and mutations in the 22 mitochondrial tRNA genes and flanking regions are being assessed. These progressive changes in various biomarkers of mitochondrial toxicity suggest that this experimental system may be a useful model for investigating chemoprevention of NRTI-induced cardiomyopathy.

Title: **Association between Mitochondrial DNA Haplogroups and AIDS Progression**	**Hendrickson, Sher.** **Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD.** **Coauthors: Holli B. Hutcheson, Eduardo Ruiz-Pesini, Jason C Poole, James Lautenberger, Lawrence Kingsley, John Phair, James Goedert, Susan Buchbinder, Douglas C. Wallace, and Stephen J. O'Brien**	Increasing evidence indicates that mitochondrial function is intricately involved in both AIDS progression and highly active antiretroviral therapy (HAART) toxicity and failure, and therefore, mtDNA variation might represent one class of AIDS Restriction Genes (ARGs). The single nucleotide polymorphisms (SNP) in mtDNA have been shown to influence mitochondrial regulation of three key cellular functions of relevance to AIDS pathology: energy production by oxidative phosphorylation (OXPHOS), production of reactive oxygen species (ROS), and apoptosis. Further mtDNA SNP haplotypes show marked phylogeographic variation that has raised the prospect of genetic adaptation in distinctive climatic environments. A comprehensive gene association study of European mtDNA haplogroups among 2327 study participants in American AIDS cohort studies revealed mtDNA haplogroup J and sub-haplogroups J1, J1C, and J2 were more prone to adverse outcomes including accelerated progression to AIDS'87 and death. Haplogroup Uk proved to be highly protective against AIDS pathogenicity (CD4 count <200cells/μL and AIDS'93). A highly significant signal for protection against AIDS and death was observed in haplogroup H3. While we do not know for certain why these mtDNA lineages had varying effects of AIDS pathology, we will make the argument that it is the inter-related consequences of mitochondrial functional variation: increased ROS production in tightly coupled mitochondria which enhance innate immunity (haplogroup H), reduced ROS and energy production in partially uncoupled mitochondria which reduce disease resistance (haplogroups J and U), and in extreme cases of uncoupling (Uk), reduced ATP production which predisposes the cells to premature death following infection, thus limiting productive viral infection.

Title: **Mitochondrial Oxidative Stress in HIV-1 Protease Inhibitor Mediated Suppression of Glucose Stimulated Insulin Release by Rat Insulinoma Cells**	**Surabhi Chandra, Debasis Mondal, Krishna C Agrawal.** **New Orleans, LA-70112**	Highly active anti-retroviral therapy (HAART) with combinations of protease inhibitors (PI) and nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI and NNRTI) is the treatment regimen in HIV-1 positive patients. Long term use of HAART, however, is associated with several metabolic disorders, including cardiovascular dysfunction (CVD), lipodystrophy, and diabetes. It has been suggested that oxidative stress due to HAART may cause CVD and insulin resistance syndrome (IRS). The direct effect of NRTIs on mitochondrial DNA synthesis is implicated in manifestations of myocardial defects. Although PIs are linked to increased reactive oxygen species (ROS) production and IRS, the molecular mechanism(s) remain largely unknown. Glucose uptake by pancreatic -cells increase ATP levels which stimulate insulin release. During hyperglycemia -cell toxicity is mediated by ROS and decreased insulin production. The effect of PIs on insulin secretion and oxidative stress in the rat pancreatic-cells, INS-1 was monitored in the present study. Exposure to the PIs, nelfinavir (5-10 M) and atazanavir (8-20 M), but not saquinavir, significantly decreased insulin secretion (50%) within 24 h. Exposure to nelfinavir (10 M), but not atazanavir, increased (1.5 fold) ROS levels and decreased cytosolic superoxide dismutase (Cu/Zn SOD) levels (40%). Further, nelfinavir treatment also reduced (30%) both glutathione (GSH) and ATP levels, clearly implicating mitochondrial oxidative stress. As ROS production in mitochondria is primarily regulated at the Co-Q complex, ubiquinone treatment can suppress ROS production . We used thymoquinone (TQ), an active ingredient of black seed oil to demonstrate its possible therapeutic potential. Simulateneous treatment with PIs and TQ (2.5 M) significantly inhibited ROS production in INS-1 cells and increased glucose stimulated insulin secretion. The present findings imply that PI induced ROS production has deleterious effects on pancreatic -cell functioning, and that TQ may have a potential for ameliorating this PI induced side effect in HAART treated patients.

Title: **Respiratory chain dysfunction and oxidative stress correlate with severity of primary CoQ10 deficiency**	**Luis Carlos Lopez Garcia** **Luis C. López*,1, Catarina M. Quinzii*,1, Jakob Von-Moltke*, Ali Naini*, Sindu Krishna, Markus Schuelke , Leonardo Salviati , Plácido Navas , Salvatore DiMauro*, Michio Hirano*,2** **1These Authors contributed equally to the work.** ***Department of Neurology, Columbia University Medical Center, New York, NY, United States.** **Department of Neuropediatrics, Charité Virchow University Hospital, Berlin, Germany;** **Servizio di Genetica Clinica ed Epidemiologica, Department of Pediatrics, University of Padova, Padova, Italy.** **Centro Andaluz de Biología del Desarrollo and CIBERER, ISCIII, Universidad Pablo de Olavide-CSIC, Sevilla, Spain.**	Coenzyme Q10 (CoQ10) is essential for electron transport in the mitochondrial respiratory chain and antioxidant defense. Last year, we reported the first mutations in CoQ10 biosynthetic genes, COQ2, which encodes 4-para-hydroxybenzoate: polyprenyl transferase; and PDSS2, which encodes subunit 2 of decaprenyl diphosphate synthase. However, the pathogenic mechanisms of primary CoQ10 deficiency have not been well-characterized. In this study, we investigated the consequence of severe CoQ10 deficiency on bioenergetics, oxidative stress, and antioxidant defenses in cultured skin fibroblasts harboring COQ2 and PDSS2 mutations. For that propose, skin fibroblasts were grown in both glucose rich media and galactose media. Measurement of bionergetics (ATP synthesis, adenosine nucleotides levels and phosphofructokinase activity) and oxidative stress markers (reactive oxygen species [ROS] production, lipid peroxidation, protein oxidation and glutathione system) were performed. The results showed that defects in the first two committed steps of the CoQ10 biosynthetic pathway produce different biochemical alterations. PDSS2 mutant fibroblasts have 12% CoQ10 relative to control cells and markedly reduced ATP synthesis, but do not show increased reactive oxygen species (ROS) production, signs of oxidative stress, or increased antioxidant defense markers. In contrast, COQ2 mutant fibroblasts have 30% CoQ10 with partial defect in ATP synthesis, and significantly increased ROS production and oxidation of lipids and proteins. Our results suggest that primary CoQ10 deficiencies cause variable defects of ATP synthesis and oxidative stress, which may explain the different clinical features and may lead to more rational therapeutic strategies.

Title: **Improvements in mitochondrial function, morphology, and lipid profiles in HIV-positive patients switching from stavudine to a tenofovir-containing therapy**	**Kim, C. and Gerschenson, M.** **University of Hawaii at Manoa, Honolulu, HI**	HIV-lipoatrophy has been associated with mitochondrial dysfunction induced by nucleoside reverse transcriptase inhibitor (NRTI) therapy. Substitution by the nucleotide analogue tenofovir (TDF) may result in improvement. Ten patients receiving stavudine (d4T), lamivudine (3TC), and lopinavir/ritonavir (Kaletra®) for over 6 years switched d4T to TDF for 48 weeks. Subcutaneous fat tissue biopsies, fasting metabolic tests, HIV RNA, CD4 cell count, and whole body DEXA scans were obtained at Entry and Week 48. MtDNA copies/cell and mitochondrial morphology was assessed in adipose tissue biopsies, mtDNA 8-oxo-deoxyguanine in PBMCs, glutathione and F2-isoprostane in plasma. There was no change in limb fat by DEXA, however, trunk fat increased 14.9 % (1022 grams; $p=0.006$). Fasting total cholesterol decreased 33 mg/dL ($p=0.04$) and serum glucose decreased 4 mg/dL ($p=0.005$). MtDNA copies/cell increased from 386 to 1537 ($p<0.001$). Transmission electron microscopy showed that mitochondrial cristae were lacking or poorly defined at Entry, whereas mitochondrial inner structures were more well-defined and outer membranes were intact at 48 weeks. Oxidative damage decreased in 8/10 patients, glutathione increased, and F2-isoprostane decreased. The results from this study demonstrate that systemic and peripheral fat mitochondria improve in patients switched from d4T to TDF, while maintaining protease inhibitors.

Title: **Opa1 Regulates Crista Junction Sizes and Cytochrome *c* Accessibility**	**Ryuji Yamaguchi** **Ryuji Yamaguchi, Lydia Lartigue, Guy Perkins, Ray T. Scott, Amruta Dixit, Mark Ellisman and Donald D. Newmeyer** **La Jolla Institute for Allergy & Immunology**	Since the intercellular energy needs are different from tissue to tissue, sometimes demands made on mitochondria for more energy must be met by creating more intercristal spaces in each mitochondrion where ATP synthesis take place. Thus biogenesis of mitochondrial intercristal spaces plays important roles both in development and in aging of animals. The recent evidence suggests Opa1 is critical in regulating the size and accessibility of intercristal space by the outer-membranes. During apoptosis, Opa1 oligomers are disassembled and accessibility of cytochrome c from outer membrane increases dramatically. Several years ago, this observation led Scorrano and colleagues to suggest that crista junctions become wider during apoptosis, releasing cytochrome c from intracristal spaces. However, when we took the actual measurement of junctions using EM tomography, they became narrower during apoptosis; from average 16.5 nm in diameter to average 8 nm. 8 nm channels are still wide enough to let 100 kDa proteins through easily. We also observed the increase in the accessibility of cytochrome c by outer membrane proteins. Thus we interpreted our data as follows: the narrower 8 nm crista junctions with monomeric Opa1 are in the “open” state, letting cythchrome c and other proteins through while 16.5 nm crista junctions with oligomerized Opa1 are in the “closed” states that retain cytochrome c within intracristal speces. Furthermore mutant form of Opa1 that is recalcitrant to disassembly retains cytochrome c longer during apoptosis. Therefore it is not the narrowness of junctions, but Opa1 oligomerized at the junctions is likely to be the factor keeping cytochrome c and other soluble proteins in the intercristal spaces. Furthermore, in cells lacking Opa1 all together harbor mitochondria with almost no intercristal spaces, demonstrating the importance of opa1 protein in formation of intercristal spaces. Lastly Opa1 oligomer assembly and disassembly are regulated by GTP and during apoptosis, by Bax/Bak proteins but independent of outer-membrane pore formation.

Title: **The actin-binding protein Cofilin as the major promoter of oxidant-induced apoptosis in tumor cells**	**Klamt, Fábio [1,2], Zdanov, Stephanie[1], Baolin Zhang[1], Yaqin Zhang[1], Timothy D. Veenstra[3], Li-Rong Yu[3], and Emily Shacter[1]** **[1]Laboratory of Biochemistry, Division of Therapeutic Proteins, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Bethesda, MD 20892-4555, USA** **[2] Federal University of Rio Grande do Sul, RS, Brazil.** **[3] National Cancer Institute at Frederick, Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick Inc., Frederick, MD 21702-1201, USA**	The oxidation-reduction (redox) environment of the cell controls cell growth, differentiation, and death. Redox-sensitive proteins regulate these processes. Recent research has focused on the relationship between apoptosis and oxidative stress. However, until now, there has been no defined mechanism for how oxidants activate the apoptotic machinery. Here, using a redox-proteomics approach, we show that the actin-binding protein cofilin is a key target of oxidation that mediates induction of apoptosis by taurine chloramine, a physiological oxidant produced by activated neutrophils. In treated cells, cofilin loses its affinity with actin and translocates from the cytosol to the mitochondria. *In vitro* experiments using isolated mitochondria demonstrate that only oxidized cofilin interacts with the organelles and that it induces mitochondrial swelling and cytochrome c release. Cells become completely resistant to oxidant-induced apoptosis when Cys to Ala cofilin mutants are over-expressed. Moreover, over-expression of wild-type cofilin causes a 2-fold increase in the sensitivity of cells to oxidant-induced apoptosis. The data demonstrate that cofilin is a major intracellular redox-sensor and that, when oxidized at critical cysteine residues, activates mitochondria-dependent apoptosis (ORISE; CNPq).

Title: **SIRT3 as a regulater of intracellular superoxide levels.**	**Patel, Krish.** **Radiation Oncology Branch, National Cancer Institute, NIH, Bethesda, MD.**	Reactive oxygen species (ROS), such as superoxide, result in damage to numerous cellular components including membrane lipids, proteins, and DNA. The production and leak of these reactive oxygen species (ROS) from the mitochondria are key features of both age related diseases and carcinogenesis. Studies of the yeast Sir2 gene homologs, sirtuins, have shown these genes promote longevity in numerous organisms. There are seven sirtuins in humans, with three, SIRT3, SIRT4, and SIRT5, localizing to the mitochondria. While roles for the cytoplasmic sirtuins are starting to emerge, the exact functions of the mitochondrial sirtuins are less well known. We propose that SIRT3 plays a central role in intracellular superoxide levels and therefore, is an important anti-aging and anti-cancer entity. To evaluate this hypothesis we transfected human colon cancer cells (HCT116), which express minimal endogenous SIRT3 with vectors overexpressing either wild type (wt-SIRT3) or a dominant negative catalytically inactive SIRT3 (mt-SIRT3) to create stable cell lines. The superoxide levels in mt-SIRT3 cells are significantly higher than in wt-SIRT3 cells. These matched cell lines also show differenetial effect to known cytotoxic agents. Pretreatment of these cells with non-cytotoxic dose of H_2O_2 followed by ionizing radiation demonstrated that the wt-SIRT3 adapt to this oxidative damage and become significantly resistant to cytotoxicity. In contrast, the mt-SIRT3 cells are more sensitive to IR-induced cytotoxicity when pre-exposed to H_2O_2 suggesting the lose of an adaptive response. The increase in cell survival after a mild oxidative challenge correlated with a decrease in intracellular $O_2^{\bullet-}$ levels in wt-SIRT3 cells while a significant increase in superoxide was observed in the mt-SIRT3 cells following exposure to either H_2O_2 or H_2O_2 and IR. Additionally, $SIRT3^{(-/-)}$knockout, MEF cells show higher levels of superoxide at 21% versus 6% oxygen and grow more efficiently in 6% oxygen whereas superoxide levels and growth in wt-SIRT3 MEF cells are roughly equal at 21% and 6%. Finally, we show that SIRT3 likely provides this adaptable response to oxidative stress via regulation of MnSOD and SOC2, with increased MnSOD and SOC2 expression in wt-SIRT3 cells but decreased in mt-SIRT3 cells. Taken together these results suggest that SIRT3 regulates superoxide levels during cellular stress by activating downstream genes that remove ROS and improve electron transport.

Title: **THE DNA POLYMERASE GAMMA Y955C DISEASE VARIANT ASSOCIATED WITH PEO AND PARKINSONISM MEDIATES THE INCORPORATION AND TRANSLESION SYNTHESIS OPPOSITE 7,8-DIHYDRO-8-OXO-2'-DEOXYGUANOSINE**	**Bienstock, Rachelle J. (2), Graziewicz, Maria A. (1)$, Copeland, William C.(1)** **(1)Laboratory of Molecular Genetics and (2)Scientific Computing Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709**	DNA polymerase , encoded by the POLG gene, is responsible for the repair and replication of mitochondrial DNA . The Y955C mutation in POLG leads to autosomal dominant progressive external ophthalmoplegia (PEO) with other severe phenotypes. PEO patients with this mutation can further develop parkinsonism or premature ovarian failure. Mouse and yeast Y955C models show an increased amount of oxidative lesions and increased mtDNA damage. In DNA pol , Tyr955 plays a critical role in catalysis and high fidelity DNA synthesis. 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG) is one of the most common oxidative lesions in DNA and can promote transversion mutations. Mitochondria are thought to be a major source of endogenous reactive oxygen species that can react with dG to form 8-oxo-dG as one of the more common products. DNA polymerases can mitigate mutagenesis by 8-oxo-dG through allosteric interactions from amino acid side chains, which limit the anti-conformation of the 8-oxo-dG template base during translesion DNA synthesis. Here, we show that the Y955C pol displays relaxed discrimination when either incorporating 8-oxo-dGTP or translesion synthesis opposite 8-oxo-dG. Molecular modeling and biochemical analysis suggests that this residue, Y955, in conjunction with Phe961 helps attenuate the anti-conformation in human pol for error free bypass of 8-oxo-dG and substitution to Cys allows the mutagenic syn conformation. Collectively, these results offer a biochemical link between the observed oxidative stress in model systems and parkinsonism in patients, suggesting that patients harboring the Y955C POLG mutation may undergo enhanced oxidative stress and DNA mutagenesis.

Mitochondria Minisymposium 2008

Mitochondria and their Proteomics

Poster Abstracts

Mitochondria Minisymposium 2008: Mitochondria and their Proteomics
Table of Contents

Mitochondrial Proteomics/Friedreich's Ataxia

17 ***The small cochaperone Hsc20, a new candidate for ataxia susceptibility?***
Helge Uhrigshardt

Intergenomic Cross-Talk Between and the Mitochondria and Nucleus

18 ***Bcl-2 Mediated Enhancement Of Mitochondrial Function by Lithium And Valproate***
Yun Wang

19 ***G1- to-S Phase Cell Cycle Progression Requires a Single Electrically-Coupled Mitochondrial Network with a Continuous Lumen***
Kasturi Mitra

20 ***Cytoskeleton regulates mitochondria respiration through the tubulin-VDAC direct interaction***
Tatiana Rostovtseva

21 ***Brain-Specific Microrna-338 Regulates Oxidative Phosphorylation in the Axons of Sumpathetic Neuron***
S. Armaz Aschrafi

22 ***Parkin suppresses transcription of nuclear-encoded mitochondrial proteins through cytosolic sequestration of Estrogen-related receptors***
Jian Feng

23 ***Roles for novel mitochondrial ribosomal proteins (MRPs) in mitochondrial function oxygen consumption, cell proliferation and longevity***
Thomas W. O'Brien

24 ***Recognition and read through of human mitochondrial stop codons***
Michael P King

NIAAA Session, Alcohol and Mitochondria, Oxidative Stress and Mitochondrial Dynamics

25 ***Heat Shock Protein 90β1 is essential for polyunsaturated fatty acid-mediated mitochondrial Ca^{2+} efflux***
Bin-Xian Zhang and Hua Zhang

Model Organisms of Mitochondrial Disease

Acquired Mitochondrial Toxicities, Clinical and Societal Aspects of Mitochondrial Disease, Disease of mtDNA Replication

Vitamin and Cofactor Therapy and Clinical Trials for Mitochondrial Disease, Mutation Detection

47 ***Role of Pyruvate on Mitochondrial Oxygen and Fuel Sensing Mechanisms in Liver Following Hemorrhagic Shock and Resuscitation in Rats***
Pushpa Sharma

48 ***New Mitochondrial DNA Mutations Found in Individual Diagnosed with a Mitochondrial Disease***
Jaimie Myrkalo

49 ***Determination if Heteroplasmy Exists in Single Cells and in Single Mitochondria Through the Use of the Plexor™ qPCR System***
Michael Adam and Koren Holland Deckman

50 ***Deletion or Artifact? Screening for Deletions in the Mitochondrial DNA Genome of an Individual Diagnosed with a Mitochondrial Myopathy***
Timothy Calamaras and Koren Holland Deckman

51 ***Sensitivity of Cardiac Mitochondria Separated by Free Flow Electrophoresis into Subpopulations.***
Oliver Drews

NIA-LMG Session, DNA Repair, DNA Replication

52 ***Top1mt controls mitochondrial DNA replication through D-loop formation***
Hongliang Zhang and Yves Pommier

53 ***Bleomycin down regulates PKD expression in A549 cells and induces mitochondrial and nuclear DNA damage***
William J. Martin

54 ***Characterization of the Role of Mitochondrial Transcription Factor a in Base Excision Repair***
Anne-Cécile V. Bayne

55 ***Allotopic expression of ATP6: mtDNA mutation modeling***
David A. Dunn

NINR Session Biomarkers in Fatigue and Mitochondrial Damage-Bench to Bedside and Back

Poster No. Assigned	Title	Authors / Affiliation	Abstract
1	Title: **Antioxidant Enzymes, Hydrogen Peroxide Metabolism and Respiration in Rat Heart during Experimental Hyperammonemia**.	**Venediktova Natalia,** Elena Kosenko, and Yury Kaminsky Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences.	Excessive ammonia influx or production can cause hyperammonemia, an abnormal increase in blood ammonia level. Administration of high quantities of ammonium acetate increases the level of blood ammonia and causes animal death. Hyperammonemia is observed in epilepsy, alcoholism, cancer, radiation damage, body organs transplantation, Alzheimer's and Parkinson's diseases. Cardiac abnormalities linked with hypoxia, ischemia/reperfusion and cardiac infarction are accompanied with significant ammonia increase in blood and heart cells. Ammonia intoxication is accompanied by severe disorders of functioning of mitochondria and may intensify ROS production. Change of balance between ROS production and utilization lead to oxidative stress development. Ammonium acetate administration led to increased level ammonia in blood, brain and heart. Acute hyperammonemia decreased the rates of phosphorylating oxidation (V_3) and the respiratory rate in resting state (V_4) for pyruvate plus malate, without changing the respiratory control index (RCI) or phosphorylation efficiency (ADP/O). Ammonia intoxication leads to increased activity of antioxidant enzymes in the heart. Parallel increase in Mn-SOD activity and the rate of H_2O_2 production in heart mitochondria was observed after administration of a lethal ammonia dose. The reaction catalyzed by Mn-SOD contributed most to H_2O_2 production. Ammonium acetate injection into rats decreased antioxidant enzyme activity in the liver, brain, and erythrocytes and induced serious disturbances in the electron-transport chain of brain MX. (Kosenko et al. 1997). Ammonia concentration inhibiting antioxidant enzyme activities in the liver and brain did not suffice to such inhibition in the heart. Probably, the heart is the most adaptive organ.

2	Title**: An energetics and ion transport in sodium-loaded rat heart mitochondria.**	**Sergey Korotkov, Vladimir Nesterov, Larisa Emelyanova, Nikolay Ryabchikov, Inna Demina, Alevtina Naumkina. Sechenov** Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russian Federation.	It is known that ischemia followed reperfusion of myocardium results in sodium load of the matrix of heart mitochondria. A distribution of safranin, ion transport via the inner membrane, and a respiration of sodium-loaded rat heart mitochondria (Na^+-mitochondria) were studied in the presence of 10 mM pyruvate and 2 mM malate. State 3 and 2,4-dinitrophenol (DNP)-stimulated respiration of the Na^+-mitochondria was decreased in comparison to one of rat heart mitochondria (K^+-mitochondria) which was not subjected to a sodium load of its matrix. A fluorescent signal of safranin in experiments with the Na^+- and K^+- mitochondria was maximal across 2 min after mitochondrial energization. The Na^+-mitochondria within period from 2 till 6 min demonstrated more accelerated dissipation of the signal to be compared with one found for the K^+-mitochondria. Swelling of non-energized Na^+-mitochondria in a medium with KNO_3 or with NH_4NO_3 was markedly increased in comparison to that of the K^+-mitochondria. A contraction of energized Na^+-mitochondria in the NH_4NO_3 medium was markedly lesser than one found for the K^+-mitochondria. The energized Na^+-mitochondria in comparison to the K^+-mitochondria swelled more intensively in a medium with K acetate and sucrose. So, we can notice that the sodium load of the matrix of rat heart mitochondria caused to marked depression of mitochondrial energetics, found as the decreased respiration and as more accelerated dissipation of the safranin signal. The energy-linked potassium transport into the matrix and permeability of the inner membrane to K^+ and H^+ increased in the mitochondria as well

3	Title: **A sodium load of rat heart mitochondria stimulated opening of the mitochondrial permeability transition pore.**	**Sergey Korotkov, Vladimir Nesterov, Larisa Emelyanova, Nikolay Ryabchikov, Inna Demina, Alevtina Naumkina. Sechenov** Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russian Federation.	It was found that Ca^{2+} and Na^{+} overload of the mitochondrial matrix is resulted in ischemia with followed reperfusion of heart miocardium. A study of the mitochondrial permeability transition pore (MPTP) was made in experiments with sodium-loaded rat heart mitochondria (Na^{+}-mitochondria), or with mitochondria without the loading (K^{+}-mitochondria), in the presence of Ca^{2+}, inorganic phosphate (P_i), 10 mM pyruvate, 2 mM malate, and 4 μM oligomycine (there indicated). Incubation of energized Na_{+}-mitochondria in the presence of 100 μM Ca^{2+} and P_i stimulated their massive swelling in a medium with NH_4NO_3, or with mannitol and sucrose, as well as with K acetate and sucrose to be compared with one for the K^{+}-mitochondria. The swelling was markedly depressed by Cyclosporine A (CsA) or by ADP + Mg^{2+}. A dissipation of safranin signal after injection of 60 μM Ca^{2+} into the medium was more accelerated in experiments with Na^{+}-mitochondria than one found for the K^{+}-mitochondria. The dissipation was markedly retarded in the presence of ADP and Mg^{2+} not CsA. State 3 (-oligo) and 2,4-dinitrophenol (DNP)- stimulated respiration (+/- oligo) of the Na^{+}- and K^{+}-mitochondria was markedly decreased in a medium with KCl, sucrose, and Ca^{2+}. The respiration (-oligo) in experiments with the Na^{+}- and with K^{+}-mitochondria, and 120 μM Ca^{2+} has been markedly restored in the presence of CsA. The DNP-stimulated respiration (+oligo), decreased by 180 μM Ca^{2+}, was restored in experiments with ADP+Mg^{2+} or with CsA (only for K^{+}-mitochondria). In summary, we can conclude that the probability of the MPTP opening was additionally increased in the experiments with the Na^{+}- mitochondria after their Ca^{2+} overload in comparison to ones with the K^{+}-mitochondria.

4	Title: **Nitric Oxide Mediated Regulation of Bcl-2 Expression Dynamics in Heart.**	**Warburton, Sarah; Wang, Sujing; Khan, Aliyah; Vondriska, Thomas**. UCLA, Los Angeles, CA	Heart attacks induce irreversible damage of cardiac cells, the prevention of which is a major challenge for the treatment of ischemic heart disease. Previous investigations have demonstrated that administration of nitric oxide (NO) donors to mice induce a biphasic protective phenotype that prevents ischemic cell death, although the mechanisms temporally regulating this phenomenon are unknown. Bcl-2 is the prototypical member of a family of anti-apoptotic proteins known to antagonize mitochondrial-dependent cell death in numerous systems, including the heart. To determine the role of this protein in the temporal development of protection, the level of Bcl-2 in mouse myocardium was evaluated by immunoblotting after administration of protective doses of the NO donor DETA/NO (4 x 0.1 mg/kg). Surprisingly, Bcl-2 protein was rapidly down-regulated at 30 min after NO donor treatment. After this initial down-regulation, Bcl-2 expression began to return to baseline level at 18 hr and was completely restored at 24 hr—the same time point at which the myocardium of the mouse is resistant to ischemic cell death. This biphasic regulation of Bcl-2 expression following administration of the NO donor suggests a potentially unappreciated molecular explanation for why the cardiac protective phenotype

5	Title: **Thermodynamic Constraints in the Reversal of Adenine Nucleotide Translocase During the Reversal of F0-F1 ATP Synthase Caused by Respiratory Chain Inhibition.**	**Christos Chinopoulos, Lilla Turiak, Miklos Mandi, Katalin Takacs and Vera Adam-Vizi.** Department of Medical Biochemistry, Semmelweis University, Neurobiochemical Group, Hungarian Academy of Sciences, Szentagothai Knowledge Center, Budapest, Hungary.	Mitochondria are the main ATP producers in the cell. However, in various adverse conditions that bring the electron flow to a standstill and prevent proton pumping through the respiratory complexes, mitochondria become ATP consumers due to a reversal of the F0-F1 ATP synthase, antagonizing a collapse in membrane potential. This had led to the belief that extramitochondrial ATP producing pathways are strained to provide ATP to the mitochondrial matrix chiefly through the reversal of the ANT. Here we show that in mitochondria with a completely inhibited respiratory chain, reversal of the ATP synthase generates a sufficient membrane potential to oppose the ANT from operating in reverse mode. Furthermore, pathophysiologically relevant extra- and intramitochondrial [ATP] and [ADP] levels keep the reversal potential of the ANT above that produced by respiratory chain inhibition, thereby unfavoring ANT reversal. Under these conditions, ANT can be allowed to fully reverse only by a concomitant uncoupling, or by protracted periods of respiratory chain inhibition, leading to matrix [ATP] exhaustion. It is suggested that in disease states in which mitochondria have not suffered yet a severe loss in membrane potential such as during permeability transition or substance-induced uncoupling, these organelles cannot contribute to cytosolic ATP depletion.

6	Title: **Nitration and oxidation of tryptophan 372 in mitochondrial enzyme Succinyl-CoA:3-Ketoacid CoA Transferase (SCOT) during aging**	**Igor Rebrin, Catherine Brégère, Timothy K. Gallaher and Rajindar S. Sohal.** Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90033.	Purpose of this study was to identify targets and elucidate mechanisms of protein nitration in mitochondria during aging. Succinyl-CoA:3-ketoacid coenzyme A transferase (SCOT), the mitochondrial matrix enzyme involved in the breakdown of ketone bodies in the extrahepatic tissues, was identified in different rat tissues as a target of a novel, nitro-hydroxy, addition to tryptophan 372, located in close proximity (~10 Å) of the enzyme active site. This post-translational modification was characterized using several proteomic approaches: western blot with anti-3-nitrotyrosine monoclonal antibody, HPLC-electrochemical detection of nitrohydroxytryptophan, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) and electrospray ionization mass spectrometry (ESI-MS). Novel finding was that tryptophan, in contrast to tyrosine, was identified to be a specific target of simultaneous nitration and oxidation *in vivo*. Nitrohydroxytryptophan formation was demonstrated after *in vitro* exposure of the synthetic peptide YGDLANWMIPGK to peroxynitrite. We hypothesize that increases in tryptophan nitration of SCOT and catalytic activity in old animals constitute a plausible mechanism for the age-related metabolic shift towards enhanced ketone body consumption by mitochondria as an alternative source of energy supply in the heart.

7	Title: **Analysis of the structure and function ATP Synthase complex from site specific yeast mutants of the β Subunit mimicking known phosphorylations.**	**Lesley Kane** Kane, LA[1], Youngman, MJ[2], Jensen, RE[2], and Van Eyk, JE[1,3]. Johns Hopkins University, Departments of [1]Biological Chemistry, [2]Cell Biology and [3]Medicine.	**Introduction:** Recently, we discovered that the β subunit of the mitochondrial ATP synthase undergoes modification upon a 60 minute treatment of myocytes with adenosine and identified 5 novel phosphorylation sites on the protein. Two sites are buried within the ATP synthase complex and the others are located on the external face. The ***functional consequences*** of phosphorylation and complex assembly are assessed. **Methods:** A model system, S. cerevisiae, was chosen for high sequence homology of the β subunits and for ease of cloning protocols. Non-phosphorylatable (S/T to A) and pseudophosphorylated (T to E or S to D) analogs of 4 sites were created, T91, S246, T295 and T351. Isolation of intact F_1/F_o complex was performed using a sucrose centrifugation for all strains and equal protein amounts were used for future assays. Strains were compared to WT and a deletion strain for ATPase activity of isolated complex (measuring release of P_i from ATP), and complex assembly (whole mitochondrial Blue Native (BN)-PAGE). **Results:** On non-fermentable media all strains had WT growth, except the T295E strain, which has decrease growth. ATPase assays (n=6) on T295E strain showed a significant reduction in activity compared to WT (0.01 ± 0.004 and 0.1 ± 0.01 respectively, $p<0.0001$) and was equivalent to a deletion strain. Both internal strains T351A and T351E, had significantly decreased function (0.041 ± 0.006 and 0.035 ± 0.007, $p<0.0001$). One external site strain T91E had significantly decreased function as compared to both the T91A and WT. BN-PAGE gels revealed a complex assembly defect in the T295E, T351E mutants which lack the free F_1 component, normally found in abundance. Other strains had small changes in assembly. **Conclusions:** This data suggests that ATP synthase can be modulated by phosphorylation (both activity and assembly) and may have implications to preconditioning where the phosphorylations were first identified.

8	Title: **Electromagnetic Sensors of Mitochondrial Activity**	**John H. Miller, Jr.** Miller, John H., Jr., PhD; Fang, Jie; Mercier, George T., PhD; Vela, Luz; Widger, William R., PhD. University of Houston, Houston, Texas, USA. University of Houston Department of Physics & Texas Ctr. for Superconductivity 4800 Calhoun Rd., Ste. 617 SR1 Houston, Texas 77204-5005	We report on measurements of harmonics generated by suspensions of whole cells, mitochondria, and thylakoid membranes, in response to applied sinusoidal electric fields at kilohertz frequencies. The frequency- and amplitude-dependences of the induced (eg. 2nd and 3rd) harmonics exhibit features that appear to correlate with activity of complexes in the mitochondrial (or photosynthetic) electron transport chain. We believe that sensors based on harmonic generation spectroscopy could be developed to detect mitochondrial activity and possible dysfunction. Mitochondrial dysfunction has been implicated in obesity, type-2 diabetes, heart disease, cancer, and numerous specific mitochondrial disorders. Thylakoid membrane suspensions (from spinach chloroplasts) have also proven to be useful model organelles for preliminary studies, because the generated harmonics depend strongly on the presence or absence of light in such photosynthesizing organelles. **This work supported by** Grant R21CA122153 from the National Heart, Lung, and Blood Institute and the National Cancer Institute, NIH, and from the National Science Foundation. Additional support provided by the R. A. Welch Foundation and the Texas Center for Superconductivity.

9	Title: **Characterization of the Mitochondrial Proteome in PDK4 Wild-Type and Knock-out Mice.**	**Heather Ringham** **H.N. Ringham, P.V. Blair, N.H. Jeoung, S.M. Hong, R.A. Harris, and F.A. Witzmann. Indiana University. Indianapolis, Indiana.**	The goal of this study was to determine the effect of a PDK4 (pyruvate dehydrogenase kinase isoenzyme 4) knock-out on mitochondrial protein expression. A 2-D gel based mass spectrometry approach was used to analyze the mitochondrial proteomes of PDK4 wild-type and knock-out mice. Mitochondria were isolated from the kidneys of C57BL/6J black mice in both well-fed and starved states. Previous studies show PDK4 increases greatly in the kidney in response to starvation and diabetes suggesting its significance in glucose homeostasis. The mitochondrial fractions of the four experimental groups (wild-type fed, wild-type 48 h starved, PDK4-/- fed, and PDK4-/- 48 h starved) were separated via large- format, high resolution two-dimensional gel electrophoresis. Gels were scanned, image analyzed, and ANOVA performed followed by a pair-wise multiple comparison procedure (Holm-Sidak method) for statistical analysis. The abundance of a total of 87 unique protein spots was deemed significantly different ($p<0.01$). 22 spots were up- or down-regulated in the fed knock-out vs. fed wild-type; 26 spots in the starved knock-out vs. starved wild-type; 61 spots in the fed vs. starved wild-types; and 44 in the fed vs. starved knock-outs. Altered protein spots were excised from the gel, trypsinized, and identified via tandem mass spectrometry (LC-MS/MS). Currently, differentially expressed proteins identified with high confidence are involved in the Krebs cycle, the urea cycle, the F0F1-ATPase complex, Complexes I, II, III, and IV of the electron transport chain, fatty acid oxidation, and import into the mitochondria. The greatest differences in protein abundances were between the fed and starved wild-types. These findings suggest that starvation has a greater affect on mitochondrial protein expression than the PDK4 knock-out. Protein analysis is ongoing to identify the remaining proteins.

10	Title: **Mitochondrial sub-proteomics and cardiac resynchronization therapy: molecular insight on a metabolic therapy**.	**Agnetti, Giulio1,2. Elliott, Steven1. Kaludercic, Nina2. Sheng, Simon1. Kane, Lesley A1. Chakir, Khalid2. Samantapudi, Daya2. Guarnieri, Carlo3. Caldarera, Claudio M3. Kass, David A2. Van Eyk, Jennifer E1.** **1The Johns Hopkins Proteomics Center at Bayview, Johns Hopkins Medicine, Baltimore, MD, USA; 2 Department of Cardiology, Johns Hopkins Medicine, Baltimore, MD, USA;** **3 INRC, Dipartimento di Biochimica "G. Moruzzi", Università degli Studi di Bologna, Italia**	Cardiac resynchronization therapy (CRT), is a procedure used in the clinics to ameliorate the symptoms associated with heart failure-induced conduction disturbances and ventricular dyssynchrony. The molecular modifications underlying the beneficial effects of CRT have not been completely clarified. Mitochondria are likely to be major players in this benign transition due to their role in both energy production and apoptosis regulation. Functional data obtained on mitochondria isolated from a dog model for dyssynchrony-induced heart failure (DHF, 6 wks tachy-pacing after left bundle branch ablation) alternatively submitted to CRT (starting after 3 wks from left bundle branch ablation) show an improved ADP/O consumption ratio upon CRT. Therefore, the proteome of cardiac mitochondria from CRT and DHF hearts was investigated. Methods and results: Mitochondria-enriched fractions obtained from the left ventricular free wall of either DHF or CRT dogs were analyzed through two-dimensional gel electrophoresis (2DE, pH 4-7 and 6-11). Roughly 1200 protein spots were visualized after silver staining. Software-assisted image analysis indicated changes in the density of 40 protein spots upon CRT. These spots were identified through tandem mass spectrometry. 53% of protein changes pertained the OxPhos complexes with multiple spots identification for ATP synthase α, β and δ subunits suggesting post-translational modifications (PTM). Phosphorylation status of mitochondrial proteome was monitored by combining the differential-in-gel electrophoresis (DIGE) technology with alkaline phosphatase treatment. ATP synthase β and δ subunits showed decreased phosphorylation in CRT compared to DHF. As well, ATP synthase β and α subunits were selectively degraded in DHF compared to CRT Conclusions: The proteomic investigation of mitochondria revealed a previously unseen presence of PTM mechanisms at the mitochondria level in heart failure and CRT. Phosphorylation as well as proteolytic mechanisms in the mitochondria may play a prominent role in modulating cardiac performance, as observed for the beneficial effects of CRT.

11	Title: **Mitochondrial Protein Structural Database**	**Talapady N Bhat** **T. N. Bhat, Anh -Dao Nguyen, V. Ravichandran** **Biochemical Science Division, NIST, Gaithersburg, MD 20899 USA**	The protein Data Bank is the internationally recognized sole archival of structural data. Due to the complexity and sheer volume of the data archived by the PDB, often it is hard for the certain focused community to locate, view and compare structures of interest in the PDB. For this reason, several specialized databases (e.g. HIV Structural database - http://bioinfo.nist.gov/SemanticWeb_rt2d/chemblast.do Protein Kinase- http://www.kinasenet.org/pkr/Welcome.do dedicated to a class of proteins have been established by various institutions. Structural data plays a key role in understanding and comparing proteomics data. Here we report the availability of a database – MITODB dedicated to mitochondrial proteins with special emphasis to structural data. The goal of this Web resource is to address the needs of the proteomics community working on mitochondrial proteins and its focus is to enable visualization, comparison and analysis of structural data. Structural data obtained form the PDB were annotated for completeness, data uniformity and integrated with relevant data obtained from several other publicly available resources such as the PubMed and SWISPROT. The annotated data were incorporated into an ORACLE database and presented with a user-friendly Web interface developed using Java.Visualization tools for three-dimensional structures are also provided. We anticipate that this resource will become a structural gateway and a warehouse for proteomics community working on mitochondrial proteins. The Web site is maintained by the TRC and IT of NIST.

12	Title: **A Bioinformatics Approach to the Tissue Engineered Medical Product Chondrocytes: Human Mesenchymal Stem Cells**	**Jean Roayaei, Ph.D.** **NCI-Frederick**	We will provide a general approach to the development of the tissue engineered materials which involves the isolation and propagation of cells. This requires a bioinformatics analysis Of differentiated chondrocytes from different sources. We are particularly interested in the analysis of undifferentiated chondrogenic bone marrow derived mesenchymal stem cells. We analyze the chondrogenic Media in three different time points. We study human mesenchymal stem cells changes elucidated by their gene expression profiles. We use RMA (Robust Multi-array Averaging) to measure gene expression profiles of stem cells. This involves three preprocessing stages. The background correction, quantile normalization, and a summarization that is derived from a multi-array model fit applying the median polish algorithm. We used Bioconductor R 2.4.6 to perform our bioinformatics analyses. We have used the RMA convolution model for background correction. The summarization techniques are divided into two categories, those that are single array and the ones that are multi-array. We have used the Affymetrix Gene Chip Oligonucleotides MAS 5 for background correction where both perfect matches and the ideal mismatch correction are included. However, this approach where mismatches are subtracted from the perfect matches has been shown to over-adjust. We attempt to improve upon the Affymetrix MAS 5 by applying the RMA technique to measure gene and protein expression levels of human stem cells.

13	Title: **Comprehensive Analyses of Post-Translational Modifications on Mammalian Mitochondrial Ribosomal Proteins: Role of Phosphorylation and Acetylation in Regulation of Ribosome Function**	**Koc, Emine C.,** **Pennsylvania State University, University Park, PA**	Mitochondria produce over 90% of the energy used by mammalian cells through the process of electron transfer and oxidative phosphorylation. Thirteen of the essential protein components of the electron transfer chain and the ATP synthase are the products of genes present in the mitochondrial DNA and the synthesis of these proteins is carried out by mitochondrial ribosomes within this organelle. Although the mitochondrial translation system is unique and important for the survival of eukaryotic cells, it is one of the least understood systems. To elucidate role of post-translational modifications of ribosomal proteins, such as phosphorylation and acetylation, in regulation of mitochondrial translation, we have identified the phosphorylated and acetylated proteins of mammalian mitochondrial ribosomes using immunoblotting and mass spectrometry-based proteomics analyses. Overall, nearly half of the ribosomal proteins were determined to be phosphorylated at steady-state conditions. Interestingly, presence of ATP and several know mitochondrial kinases resulted in inhibition of ribosomal function up to 25% in in vitro translation assays. Moreover, we also discovered acetylation of several mitochondrial ribosomal proteins and demonstrated the specific deacetylation of these ribosomal proteins by a ribosome associated mitochondrial NAD+-dependent deacetylase in the presence of NAD+. Our findings constitute the first indications for regulation of protein synthesis by reversible phosphorylation and acetylation of mitochondrial ribosomal proteins as part of the metabolic regulatory network in mammalian mitochondria.

14	Title: **Glucose Deprivation Inhibits Mitochondrial Protein Import: The Role Of Tom20**	**Phan, Nam, Diec Diana, Shulyakova Natalya, and Mills LR** **11-430 TWH 399 Bathurst St Toronto ON M5T 2S8**	Most (99%), of mitochondrial proteins are nuclear-encoded and must be imported into mitochondria. The import process is complex and dependent on an array of translocases and chaperones localized to the inner and outer mitochondrial membranes. Previous studies using PC12 cells stably transfected with an inducible mitochondrially-targeted GFP (mtGFP) established that mitochondrial protein import can be inhibited by a variety of sub-lethal stressors, including glucose/glutamine deprivation-reperfusion (GD/R). Hypothesis: Overexpression of Tom20, an integral component of the protein import machinery, will ameliorate the GD/R-induced decline in import. PC12 cells were transfected with full-length human Tom20 and western blot confirmed that transfection significantly increased Tom20 expression and Tom20 levels in mitochondria. In these cells, mtGFP import also increased; mtGFP levels in mitochondria rose by 29% ± 3% and by 38% ± 4% at 24h and 48h, respectively. mtGFP expression and import in untransfected cells was unchanged immediately post-GD, but by 24hrs post GD/R mtGFP import was reduced by 27% ± 3% (assessed by flow cytometry) and 22% ± 4% (assessed by Western blot) and by 32% ± 5% at 48h Intra-mitochondrial turnover of mtGFP was unchanged. In these cells, levels of endogenous Tom20 declined significantly. Mitochondrial membrane potential and ATP levels were unchanged, but ROS levels increased by 71% ± 8% and 60% ± 14% versus controls at 24h and 48h post-GD/R. Overexpression of Tom20 prior to GD, prevented the GD-induced decline in Tom20 expression, the reduction of Tom20 in mitochondria, and restored mtGFP import to levels above controls. Our results indicate that in neurons, sublethal GD reduces Tom20 expression and Tom20 levels in mitochondria. These changes are associated with a decline in mtGFP import and the decline in Tom20 and mtGFP in mitochondria can be reversed by overexpression of Tom20. These findings argue that Tom20 is sensitive to GD and is a key loci at which protein import can be modulated.

15	Title: **Chronic Depolarization Up-regulates Mitochondrial Protein Import in Differentiated PC12 Cells**	**Fong, Jamie, Sirk D, Diec Diana, Shulyakova N and Mills LR** **11-430 TWH 399 Bathurst St Toronto ON M5T 2S8**	The majority of mitochondrial proteins (>99%) are nuclear-encoded and are imported into mitochondria. Little is known about how protein import is regulated in neurons. Hypothesis: Depolarization (50mM KCl) will up-regulate mitochondrial protein import in neurons. To assess the effects of KCl we measured the import of 3 proteins in differentiated PC12 cells; (a) mtGFP, an inducible fusion protein targeted to mitochondria, (b) mtHsp70, a mitochondrial matrix chaperone, and (c) Tom20, a key mitochondrial protein import receptor. Protein import, cytoplasmic levels of mitochondrial proteins, and protein expression were measured by autoradiography and immunoprecipitation, or by western blot, using mitochondrial fractions, cytoplasmic fractions, and whole cell lysates. In live cells mtGFP import and intramitochondrial mtGFP turnover were assessed by flow cytometry. Results: KCl (50mM, iso-osmotic) significantly increased mtGFP import; by 12hrs the mtGFP signal, which in live cells reflects only imported mtGFP (Sirk et al 2003; 2007), increased by 21%+/-1.5 (n=3, $P<0.01$) versus controls; by 24hr and 48 hrs import increased by 31%+/-7 (n=5, $P< 0.01$) and 40%+/-5 (n=5, $P<0.001$) respectively. Western blots and autoradiography confirmed that KCl increased mtGFP import; by 24hrs mtGFP levels in mitochondria were 59%+/-5 (n= 3, $P< 0.05$) higher vs controls. MtGFP expression also increased significantly but mtGFP intramitochondrial turnover was unchanged up to 48hrs. The KCl induced increase in mtGFP import was reversible and further enhanced by the L-type Ca2+ channel agonist, BayK. The effects of KCl were selective; KCl increased both expression and import of a physiological protein mtHsp70, but not of Tom20 or GAPDH. KCl also blocked an A□ mediated inhibition of protein import (Sirk et al 2007). These findings demonstrate that chronic depolarization can up-regulate mitochondrial protein import in neurons. They further suggest that the effects of KCl on import are regulated, in part, by Ca2+ influx through voltage gated Ca2+ channels.

16	Title: **Imaging Mitochondria and Mitochondrial Protein Import In Live PC12 Cells And Primary Neurons In Slice Culture.**	**Shulyakova Natalya, Phan, Nam and Mills Linda R.** **Toronto Western Research Institute, UHN Toronto Canada.**	Typically measurements of protein import to mitochondria are performed on isolated mitochondria and/or yeast systems. We have developed a technique for imaging mitochondrial protein import in real time that can be used in PC12 cells, primary neurons in dissociated cultures, and neurons in organotypic cultures. Neurons are biolistically transfected with a mitochondrially targeted GFP (mtGFPb) and individual mitochondria or clusters of mitochondria, are photobleached by a laser pulse. Under optimal conditions cell viability is not affected and in cells labeled with Rhodamine-123 immediately post-bleach the mitochondria within the bleach zone (which are now labeled with Rhodamine-123) appear normal morphologically. The capacity of the bleached mitochondria to take up Rhodamine-123 and retain it also argues that there is no sustained loss of mitochondrial membrane potential associated with the photobleaching regime. Any migration of unbleached mitochondria into the bleach zone is readily monitored by confocal microscopy since unbleached mitochondria typically have an intense mtGFP signal. In the absence of migration, recovery of mtGFP positive mitochondria within the bleach zone signal reflects the import of new mtGFP. In all PC12 cells (n=15 cells, 9 experiments) and in primary neurons in hippocampal slices (n=7 cell, 3 experiments) the mtGFP signal, which was undetectable at time zero, gradually increased to 30% of pre-bleach levels over 30-120 minutes. In PC12 cells treated with sublethal CCCP which rapidly inhibits the import of mtGFP (Sirk et al. 2003) recovery of the mtGFP signal within the bleach zone was slowed by 15-46% (n=10 cells, 5 experiments) confirming that the recovery of the mtGFP signal was due to import. This technique permits the monitoring of protein import to mitochondria in live neurons in real time at the level of individual neurons and in subpopulations of mitochondria in specific neuronal compartments i.e., axonal versus dendritic versus somal.

17	Title: **The small cochaperone Hsc20, a new candidate for ataxia susceptibility?**	**Uhrigshardt, Helge, Missirlis, Fanis and Rouault, Tracey** **Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, MD 20892, USA**	Iron sulfur clusters most likely represent the most ancient cofactor of proteins. Their in vivo assembly and insertion into the respective targets requires a complex biosynthetic system, which in eukaryotes is primarily localized in the mitochondria. Mutations in two of the proteins of this machinery, frataxin and ABCB7, cause the human neurodegenerative disorders Friedreich's ataxia (FA) and X-linked sideroblastic anemia with ataxia (XLSA/A). This raises the possibility that other components of the ISC assembly pathway are also potential disease factors. One such factor may be Hsc20, a member of the family of J-domain heat shock proteins. In yeast, reduction of Hsc20 homologue Jac1 led to decreased activity of mitochondrial ISC proteins and mitochondrial iron overload, highly reminiscent of the cellular phenotypes observed in FA or XLSA/A patients. We have therefore initiated functional analyses of Hsc20 in higher eukaryotes to determine its potential role in ISC assembly, mitochondrial iron homeostasis, and neurodegenerative disease. We found that in HeLa cells, human Hsc20 is predominantly localized to mitochondria comparable to Jac1 in yeast. Consistent with a role in ISC protein maturation, RNAi-mediated depletion of Hsc20 resulted in growth defects and severely reduced enzymatic activity of ISC-containing proteins in both the cytoplasm and mitochondria. A new twin CXXC-motif was identified in Hsc20 homologues of metazoa, which might act as a sensor of oxidative stress or might be involved in ISC binding. Its strict conservation in higher eukaryotes enabled us to detect the putative hsc20 gene of Drosophila melanogaster. P-element insertion into the fly homologue caused a homozygous lethal phenotype that could be partially rescued by the human Hsc20. These findings demonstrate that Hsc20 plays a highly conserved and apparently essential role in the assembly and/or repair of ISC-containing enzymes in higher eukaryotic organisms. Our ongoing studies are now aimed at elucidating the potential of Hsc20 as a factor for the development of neurodegenerative disease.

18	Title: **Bcl-2 Mediated Enhancement Of Mitochondrial Function By Lithium And Valproate.**	**Yun Wang** **Yun Wang, Rosilla F. Bachmann, Peixiong Yuan, Rulun Zhou, Xiaoxia Li, Salvatore Alesci, Cynthia S. Falke, Jing Du and Husseini K. Manji.** **Laboratory of Molecular Path physiology, Mood and Anxiety Disorders Program, National Institute of Mental Health, National Institute of Health, Bethesda, MD 20892, USA.**	Accumulating evidence suggests that mitochondrial dysfunction plays a critical role in the progression of a variety of neurodegenerative disorders. However, at present, treatments for these disorders are largely symptomatic. Lithium and valproate (VPA), the mood stabilizers, have recently been postulated to regulate mitochondrial function. A series of studies were undertaken to investigate their effects on mitochondrial function, and might against mitochondria-mediated neurotoxicity. In this study, chronic treatment with lithium or VPA upregulated Bcl-2 protein and enhanced cellular respiratory rate, mitochondrial membrane potential, and mitochondrial oxidation in SH-SY5Y cells. These effects were attenuated by knock-down of Bcl-2 with specific Bcl-2 siRNA. Additional in vivo study also showed that chronic lithium or VPA treatment increased Bcl-2/Bax ratio, and reversed methamphetamine (METH)-induced decrease of Bcl-2/Bax in the mitochondrial fraction of the frontal cortex, effects that were accompanied by markedly reduced METH-induced mortality. Microarray analysis demonstrated that the gene expression of several proteins related to the apoptotic pathway and mitochondrial functions were altered by METH, and these changes were attenuated by treatment with lithium and VPA. These findings indicate that lithium and VPA enhance mitochondrial function partially through Bcl-2 and protect against mitochondria-mediated toxicity. These agents have potential clinical utility in the treatment of neurodegenerative disorders associated with impaired mitochondrial function.

19	Title: **G1- to-S Phase Cell Cycle Progression Requires a Single Electrically-Coupled Mitochondrial Network with a Continuous Lumen**	**Kasturi Mitra** **Kasturi Mitra, Badri Roysam and Jennifer Lippincott-Schwartz** **Rm 101, Bldg 18T** **NICHD, NIH** **18 Library Drive** **Bethesda** **USA 20892**	Mitochondria continuously undergo fission and fusion. Their morphology, including fragmented elements and tubular networks, results from a balance between fission and fusion events. To determine if there are changes in mitochondrial dynamism at different stages of the cell cycle, we carried out live cell imaging experiments in cells stably expressing RFP targeted to the mitochondrial matrix in Normal Rat Kidney cells (NRK). We found that mitochondria exhibit distinct morphological and physiological states at different stages of the cell cycle. In mitosis, mitochondria fragmented into hundreds of small units for partitioning into daughter cells at cytokinesis. Strikingly, at G1/S, mitochondria fused together into a single huge, dynamic filamentous system, unlike at any other cell cycle stage. Photobleaching of an area across this filamentous system revealed the mitochondrial matrix was continuous. The mitochondrial network also was electrically coupled and had a higher membrane potential than mitochondria at all other stages of the cell cycle. When the filamentous network or its membrane potential was disrupted, or its dynamics perturbed, cell cycle progression from G1 into S was arrested in a p53-dependent manner. Moreover, p21-overexpression, which induces a G1/S arrest, resulted in filamentous mitochondria with reduced matrix continuity and loss of electrical coupling. The data thus revealed that mitochondria dynamism and morphology undergo critical changes during the cell cycle that are sensed by the cell at G1/S to control cell cycle progression.

20	Title: **Cytoskeleton regulates mitochondria respiration through the tubulin-VDAC direct interaction**	**Tatiana Rostovtseva** **1Tatiana K. Rostovtseva, 2Dan L. Sackett, 3Claire Monge, 3Valdur Saks, and 1Sergey M. Bezrukov** **1Laboratory of Physical and Structural Biology; 2Laboratory of Integrative and Medical Biophysics, NICHD, NIH, Bethesda, MD 20892, USA; 3Laboratory of Fundamental and Applied Bioenergetics, Joseph Fourier University Grenoble Cedex 9, France**	Mitochondria have long been known to localize within the tubulin-microtubule network in heart and many other cells (Appaix et al., 2003). It is also well-known that in permeabilized cardiac cells the apparent Km for exogenous ADP in the control of mitochondrial respiration is significantly higher than in isolated mitochondria. It has been suggested that the low permeability of the mitochondria outer membrane (MOM) for ATP and ADP in cells is due to interaction of mitochondria with some cytoplasmic proteins (Saks et al., 2003). Here, for the first time, we demonstrate that tubulin is the factor which controls MOM permeability by regulating VDAC, the major channel of MOM. By direct measurements we show that nanomolar concentrations of mammalian tubulin induce highly voltage-sensitive reversible closure of VDAC channels reconstituted into planar phospholipid membranes. Analysis of VDAC single channel fluctuations in the presence of tubulin shows that channel closure occurs at very low potentials (as low as 10 mV) compared to VDAC gating in control. The tubulin-VDAC interaction requires the presence of negatively charged C-terminal tails of tubulin. Tubulin with proteolytically removed C-terminus does not induce VDAC closure. We propose a model of tubulin-VDAC interaction in which the tubulin C-terminus penetrates into the channel lumen, interacting with VDAC with high specificity and blocking channel conductance. The experiments with isolated heart mitochondria strongly confirm our findings. Apparent Km for exogenous ADP increases 10 times after addition of 1-10 □M of tubulin to isolated heart mitochondria. We conclude that tubulin strongly limits ADP entry to mitochondria across its outer membrane. Our results suggest a new general mechanism of regulation of mitochondrial outer membrane permeability under normal and apoptotic conditions.

21	Title: **BRAIN-SPECIFIC MICRORNA-338 REGULATES OXIDATIVE PHOSPHORYLATION IN THE AXONS OF SYMPATHETIC NEURON**	**S. Armaz Aschrafi** **Aschrafi, A., Schwechter, A., Natera, O., Gioio, A., Kaplan , B.B.** **National Institute of Mental Health, Laboratory of Molecular Biology, Bethesda, USA**	MicroRNAs (miRs) are evolutionarily conserved, non-coding RNA molecules of approximately 21 nucleotides. Mature miRs regulate the expression of genes that are involved in various biological processes, such as development, proliferation, and differentiation of the cell. We investigated the highly conserved, brain specific miR-338 in primary sympathetic neurons cultured in Campenot chambers. Our results show that these neurons express significant amounts of this mircoRNA in their axons, and that the relative abundance of miR-338 increases during maturation. We also found that transfection of precursor miR-338 into the axons of sympathetic neurons decreases COXIV mRNA levels and results in a decrease in respiration as measured by Alamar Blue, an indicator of cellular oxidative phosphorylation. Conversely, the transfection of synthetic anti-miRNA oligonucleotides that inhibit miR-338 increases COXIV mRNA and protein levels resulting in a significant increase in oxidative phosphorylation in the axons. Our results point to a molecular mechanism by which this miRNA participates in the regulation of axonal respiration by modulating the levels of COXIV, a protein which plays a key role in the assembly of the mitochondrial cytochrome c oxidase complex IV.

22	Title: **Parkin suppresses transcription of nuclear-encoded mitochondrial proteins through cytosolic sequestration of Estrogen-related receptors.**	**Feng, Jian.** **Yong Ren, Houbo Jiang and Jian Feng.** **Department of Physiology and Biophysics, State University of New York at Buffalo**	Mutations of parkin, a microtubule-associated protein-ubiquitin E3 ligase, represent the most frequent cause of recessively-inherited Parkinson's disease (PD). Studies using parkin knockout mice and flies have shown that loss of parkin disrupts mitochondrial functions. Our previous studies have shown that parkin attenuates the toxicity of cytosolic dopamine by suppressing the transcription of monoamine oxidases (MAO), which are mitochondrial enzymes responsible for the oxidative deamination of dopamine. Here we show that parkin interacted with the transcription factor Estrogen-Related Receptor α (ERRα), which plays a significant role in transcriptional regulation of many mitochondrial proteins including MAO. Parkin, a cytosolic protein that strongly binds to microtubules, sequestered a portion of ERRα away from the nucleus to suppress its transcriptional activity. All three members of the ERR family greatly enhanced the promoter activities of both MAO-A and MAO-B. The effects were abolished by parkin in a manner independent of its E3 ligase activity. Our microarray studies showed that parkin altered the expression of many mitochondrial proteins encoded by the nuclear genome. Some of these genes, such as COX4i2 (isoform 2 of subunit 4 of cytochrome C oxidase) were also suppressed by parkin through its interaction with ERRs. This novel function of parkin paralleled the cytosolic sequestration of p53 by Parc, which has a similar RING-IBR-RING motif in the C-terminus; it may be linked to mitochondrial dysfunction in the absence of parkin.

23	Title: **Roles for novel mitochondrial ribosomal proteins (MRPs) in mitochondrial function, oxygen consumption, cell proliferation and longevity**	**OBrien, Thomas W., Wang, Rejean L., Sun, Luning and Singh, Amar M.** **Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610**	. Human mitochondrial ribosomes contain 80 different proteins. We have mapped the genes for many of these MRPs to chromosome locations linked to various disorders of energy metabolism such as Leigh Syndrome, Multiple Mitochondrial Dysfunction Syndrome and Deafness, implicating MRPs as candidates for mitochondrial disease (Sylvester, et al., 2004, Mitochondrial ribosomal proteins: candidate genes for mitochondrial disease. Genet Med. 6(2):73-80.). Several of the MRPs are novel, having no homologues in bacterial or extramitochondrial, cytoplasmic ribosomes. Except for MRPS29 (DAP3), the novel MRPs are of undefined function and unknown location in the ribosome (O'Brien, et al., 2005, Nuclear MRP genes and mitochondrial disease. Gene.;354:147-51). The mitochondrial small subunit contains eighteen novel proteins unrelated to other ribosomal proteins, and the large subunit contains another twenty. Surprisingly, the small subunit contains one of three very different isoforms of the ribosomal protein S18, indicating that the mitochondrial ribosomes are heterogeneous. In addition, they contain two proteins, MRPS29 and MRPS30, that have been implicated in mitochondrially-mediated apoptosis. We are using RNA interference (RNAi) with the expression of individual MRPs and proteins that interact with mitochondrial ribosomes to discern their roles in mitochondrial function, oxygen consumption, cell proliferation and longevity. RNAi studies of the novel MRPs to date indicate that the novel MRPs each play essential roles in human mitochondrial ribosomes.

24	Title: **Recognition and read through of human mitochondrial stop codons.**	**King, Michael P.** **Thomas Jefferson University, Philadelphia, PA. Stop or Go?**	We have used transmitochondrial cells to analyze a G15242A mutation in MTCYTB that changes a GGA glycine codon to an AGA stop codon. Although the homoplasmic G15242A mutation would be predicted to result in the complete loss of Complex III activity, mutant cells proliferated in culture conditions that require mitochondrial respiratory chain function. Mutant cells synthesized low levels of full-length cytochrome b and had low levels of Complex III activity. The efficiency of stop codon suppression was increased by streptomycin, but not by other aminoglycosides. In contrast, a premature AGG stop codon in MTCOX1 was not suppressed, either in the presence or absence of aminoglycosides. We hypothesize that the mtDNA-encoded tRNASer(GCU), which normally recognizes the serine codons AGC and AGU, can recognize and decode the AGA stop codon, but not the AGG stop codon, resulting in readthrough of the AGA stop codon. The genetic code used by mammalian mitochondria is distinct from that used in the nucleus. One change is the use of AGA and AGG as termination codons, in addition to the canonical UAA and UAG. The use of the non-canonical termination codons AGA and AGG requires novel class I translation release factors (RFs) that recognize and bind to these stop codons in order for translation to terminate with the release of the nascent protein. Our analyses have revealed that vertebrate mitochondria contain two Class 1 release factors, mtRF1a and mtRF1b. siRNA knockdown of mtRF1b increased readthrough of the AGA stop codon, while knockdown of mtRF1a resulted in a generalized decrease in mitochondrial translation. Our functional characterization suggests that mtRF1a recognizes UAA and UAG termination codons, while RF1b recognizes AGA and AGG termination codons.

25	Title: **Heat Shock Protein 90β1 is essential for polyunsaturated fatty acid-mediated mitochondrial Ca2+ efflux.**	**Bin-Xian Zhang, Hua Zhang** **Bin-Xian Zhang, Hua Zhang, Zhen-Hua Li.** **San Antonio, Texas.**	Defective mitochondrial function has been observed in type 2 diabetes, and proposed to be a major contributing factor in the pathogenesis and progression of the disease. While the mechanism leading to mitochondrial dysfunction in diabetes remains under intensive investigation, a critical role for nonesterified fatty acids (NFA) and/or fatty acid metabolites is emphasized by an increasing body of evidence. NFA may influence mitochondrial function by alterations in gene expression, metabolism, and/or mitochondrial Ca2+ homeostasis. We have previously reported that polyunsaturated fatty acids (PUFA) induce Ca2+ efflux from mitochondria, an action that may deplete [Ca2+]m and thus contribute to NFA-responsive mitochondrial dysfunction. Here we show that heat shock protein β1 (hsp90β1) is required for PUFA-mediated mitochondrial Ca2+ efflux (PMCE). Retinoic acid (RA) induces differentiation of human teratocarcinoma NT2 cells in association with elimination of PMCE. Proteomic analysis of mitochondrial proteins indicates that hsp90β1, among other proteins, is eliminated in RA-differentiated cells. Blockade of PMCE in NT2 cells by the hsp90 inhibitor 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG) and hsp90β1 RNAi demonstrates that hsp90β1 is essential for PMCE. We also show localization of hsp90β1 in mitochondria by western blot and immunofluorescence. Distinctive effects of inhibitors binding to the N- or C-terminus of hsp90 on PMCE in isolated mitochondria suggest that the C-terminus of hsp90β1 plays a critical role in PMCE. The novel function of hsp90β1 described here suggests that hsp90β1 is a potential target for prevention of NFA-induced mitochondrial dysfunction.

26	Title: **Dynamic Regulation of Mitochondrial Functions by Glucocorticoids and Stress**	**Du, Jing1, Wang, Yun1, Hunter, Richard2, Machado-Vieira, Rodrigo 1, Wei, Yanling1, Falke, Cynthia1, Chen, James1, Blumenthal, Rayah1, Zhou, Rulun1, Yuan, Peixiong1, McEwen, Bruce2, and Manji, K. Husseini1.** **1. Laboratory of Molecular Pathophysiology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA. 2. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY, USA**	Corticosterone plays an important role in modulating neuroplasticity and in morphological reorganization, especially during chronic stress. The mechanisms underlying corticosterone's ability to modulate neuronal functions, especially mitochondrial functions, remain unclear. In this study, we found that glucocorticoid receptors (GRs) formed a complex with Bcl-2 in response to corticosterone treatment, and translocated with Bcl-2 into mitochondria after acute treatment with low and high doses of corticosterone in primary cortical neurons. However, after three days of treatment, high corticosterone resulted in a decrease in GR and Bcl-2 levels in the mitochondria. In addition, three independent mitochondrial functional measurements—mitochondrial calcium holding capacity, mitochondrial oxidation, and membrane potential—were also regulated by long-term corticosterone treatment in an inverted "U"-shape. Acute treatment with high (1,000 nM) and low (100 nM) concentrations of corticosterone enhanced mitochondrial oxidation, membrane potential, and calcium holding capacity. However, after long-term treatment, low-dose treatment enhanced mitochondrial functions, but high-dose treatment attenuated mitochondrial functions. Similarly, after chronic stress and long-term treatment with corticosterone, GR and Bcl-2 levels in mitochondria were significantly decreased in prefrontal cortex. These findings suggest that, in response to corticosterone, GR recruit Bcl-2 into mitochondria and regulate mitochondrial functions. These findings have the potential to contribute to a more complete understanding of the mechanisms by which chronic stress and hormones regulate cellular plasticity and resilience, and to inform the future development of improved therapeutics.

27	Title: **EVIDENCE OF MITOCHONDRIAL DYSFUNCTION IN ALPHA SYNUCLEIN NEUROTOXICITY**	**Mordhwaj S. Parihar1, Rafal R. Nazarewicz1, Woineshet J. Zenebe1, Arti Parihar1, Masayo Fujita2, Makoto Hashimoto2, Pedram Ghafourifar1** **1Department of Surgery, Davis Heart and Lung Research Institute, and Institute of Mitochondrial Biology, The Ohio State University, Columbus, OH, USA; 2Laboratory for Chemistry and Metabolism, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo, Japan**	Alpha synuclein is a major protein component of Lewy bodies and Lewy neuritis that are involved in the pathology of neurodegenerative diseases. Increased aggregation of alpha synuclein into large inclusion bodies and increased accumulation of high molecular weight of alpha synuclein play a significant role in neurotoxicity particularly in dopaminergic neurons of the substantia nigra. Despite many experimentally tested models, the consequence of alpha synuclein-mitochondrial interaction and molecular mechanism by which alpha synuclein induces neuronal toxicity remains largely elusive. We investigated possible interaction of alpha synuclein with mitochondria and consequences of such interaction using SHSY cells and isolated mitochondria. We show that alpha synuclein interacts with mitochondria and causes oxidative modification of mitochondrial components. Our findings suggest a pivotal role for mitochondria in oxidative stress and apoptosis induced by alpha synuclein.

28	Title: **Effects of free radicals in mitochondria**	**Heuett, William J, and Periwal, Vipul.** **NIDDK, National Institutes of Health, Bethesda, MD, USA.**	. The tissue damaging effects of reactive oxygen species (ROS) are hypothesized to underlie many disease complications, including those associated with diabetes, Parkinson's, Alzheimer's, and atherosclerosis. We suggest, however, that it is the saturation of ROS signaling under stress that plays a more important role in disease complications. In mitochondria, where ROS are produced through a process that is very sensitive to the proton motive force, oxidative stress is reduced by scavenging enzymes and decreased membrane potential due to the activation of uncoupling proteins. Details of this regulation in mitochondria are still being established; however, based on the current published data, we present a simple model that captures the behavior of mitochondrial respiration, ATP synthesis, and ROS production in pancreatic beta-cells. Our model is consistent with experimental observations of the non-ohmic rise in the passive proton leak rate at high membrane potential as well as the dependence of the proton leak rate on increased ROS production. Furthermore, our model shows that increased nutrient levels result in a saturated response in ROS levels leading to proportionately reduced downstream signaling, rather than an accumulation of excess ROS.

29	Title: **Impaired balance of mitochondrial fission and fusion in Alzheimer disease.**	**Xinglong Wang, Bo Su, Mark A Smith, George Perry, Xiongwei Zhu** **Case Western Reserve University, Cleveland, OH 44106**	Mitochondrial dysfunction is a prominent and early feature of Alzheimer's disease (AD). Emerging evidence suggest that mitochondrial function is dependent on the dynamic balance of fission and fusion events which are regulated by a machinery involving large dynamin-related GTPases that exert opposing effects; i.e., dynamin-like protein 1 (DLP1) for fission, and Mitofusin 1 (Mfn1) for fusion. By regulating mitochondrial fission/fusion, DLP1 and fusion proteins control the morphology and distribution of mitochondria. While an impaired balance of mitochondria fission/fusion is being increasingly implicated in neurodegenerative diseases, few studies have examined this aspect in AD. To address this issue, in this study, we investigated mitochondria morphology and distribution in biopsy brains from normal subjects and those from AD patients. We found disease-related changes in mitochondrial morphology and distribution as well as changes in expression levels and distribution of mitochondrial fission and fusion proteins. To understand the underlying mechanisms of these mitochondria alterations in AD, we overexpressed or knocked down functional DLP1 and other mitochondrial proteins in M17 neuroblastoma cells or rat primary neurons. Interestingly, in situations where functional protein changes mimicking that in AD, we found similar changes in mitochondrial morphology and distribution to that observed in AD neurons. We further demonstrated that elevated oxidative stress and increased amyloid-β production are likely the potential pathogenic factors that cause impaired balance of mitochondrial fission/fusion.

30	*Title:* **Relative quantitation of mitochondrial superoxide levels *in vivo* in *C. elegans* mitochondrial mutants**	**Falk, MJ[1], Lightfoot, R[2], Dingley, S[1], Rao, M[1], Ostrovsky J[1], Polyak, E[1], Ischiropoulos H[2].** **Divisions of [1]Human Genetics and [2]Neonatology, Department of Pediatrics,** **The Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA.**	The mitochondrial respiratory chain is associated with oxidant production and altered longevity in *C. elegans*. These associations have been made largely using *in vitro* markers of oxidant damage. To better assess individual mitochondrial component involvement in oxidant species generation, we developed an *in vivo* method to quantify *C. elegans'* mitochondrial superoxide levels. **METHODS:** Synchronous young adult populations of *C. elegans* mutant for complex I (*gas-1*), II (*mev-1*), III (*isp-1*), the insulin receptor (*daf-2*), or mitochondrial manganese superoxide dismutase (*sod-3* and *sod-2*) were fed 10 uM Mitosox Red (a mitochondrial matrix superoxide indicator dye) with or without oxidant stressors (Paraquat or Antimycin A). Terminal pharyngeal bulb mean intensity in living worms was quantitated by fluorescence microscopy following 24 hour exposures. Confocal imaging was used to demonstrate overlay of mitotracker green and mitosox in the terminal pharyngeal bulb. *SOD-3* and *SOD-2* relative expression was also quantified to assess the response of the major superoxide scavenging enzyme(s) to mitochondrial dysfunction and oxidizing agents. **RESULTS:** A significant increase in steady-state superoxide levels was detected in *gas-1* (9.1%, p<0.0001) and *sod-3* (63.7%, p<0.0001) when compared with wildtype (N2). Significantly increased superoxide levels were observed in all mutants in comparison with N2 upon exposure to Paraquat (*gas-1* 55.6%, p<0.0001; *mev-1* 40.3%, p<0.0001; *isp-1* 14.5%, p<0.0001, *sod-3* 60.6%, p<0.0001), with the exception of *daf-2.* Similarly, a lethal dose of Antimycin A for *sod-3* resulted in no significant increase in superoxide levels in *daf-2*. Intrastrain comparisons with and without Paraquat demonstrated significantly increased superoxide levels only in *gas-1* (37.8%, p<0.001) and *mev-1* (34.6%, p<0.0001). RT-qPCR analysis revealed *sod-3* expression was more dramatically upregulated than *sod-2* expression in all mutants studied, but its upregulation was most pronounced (10-20 fold) both at baseline and with paraquat stress in the long-lived complex III and insulin receptor mutants compared with N2 baseline. Lifespan analyses of both *sod-2* and *sod-3* knockout mutants indicate both are significantly short-lived at 20°C to a comparable extent as the complex I mutant, *gas-1*. **CONCLUSIONS:** Terminal pharyngeal bulb fluorescence intensity quantitation of Mitosox Red-fed *C. elegans* is a sensitive and specific method to relatively quantify *in vivo* steady-state mitochondrial superoxide levels. Our results suggest the short-lived complex I, complex II, and *sod* mutants have an increased sensitivity to oxidant stress but at least for the MRC subunit mutants, a relatively limited capacity to upregulate their major

			superoxide defense enzyme(s). Among the long-lived complex III and insulin receptor mutants, superoxide levels do not increase substantially with oxidant stress; this is likely related to their dramatically increased superoxide scavenging capacity.

31	Title: **Potentiation and Defence of the Powerhouse: Positional cloning of the C57BL/6J mouse Nnt gene defect highlights its critical role in homeostasis and disease through control of mitochondrial free radical generation and defence.**	**Dr. Ayo A. Toye** **Department of Infection Immunity and Inflammation, Faculty of Medicine, University of Leicester, Leicester, UK.**	I first implicated the Nnt gene in type 2 diabetes and by extension metabolic syndrome through genetic mapping and positional cloning of the C57BL/6J mouse gene defect in diabetes QTL mapping and functional genomics study. Discovery of the C57BL/6J Nnt defect provided the first crucial clue of the role of Nnt in mammalian disease. As a result of these studies and others in model organisms including C. elegans and mice (Arkblad et al., Freeman et al., Huang et al. etc), there is now a growing appreciation of the role of Nnt in cellular / organismal function. These studies extend pioneering work over 6 decades in the discovery, and biochemical and biophysical characterisation of the Nnt molecule by Kaplan-NO, Rydstrom-J., Mitchell-P, Hatefi-Y, Jackson-BJ, and others. In this article, I describe current knowledge of Nnt and further highlight its putative vital and wide ranging roles in development, health, disease and ageing with specific emphasis on relevance to humans. Further, I highlight additional mitochondrial molecules that are critical mediators of organelle free radical status and a rationale for their further study in conjunction with Nnt.

32	Title: TH**E ROLE OF MITOCHONDRIA IN METABOLIC DEPRESSION OF LAMPREY (Lampetra fluviatilis) HEPATOCYTES.**	**Larisa Emelyanova** **Margarita Savina, Larisa Emelyanova, Sergey Korotkov, Irina Brailovskaya** **Institution: I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry of Russian Academy of Sciences**	Over winter months of pre-spawning migration, the period of starvation, the metabolic depression in lamprey hepatocytes is mediated by prolonged reversible alterations of mitochondrial functions, namely low activity of the mitochondrial respiratory chain, low oxidative phosphorylation, low content of mitochondrial adenine nucleotides, high level of reduced mitochondrial pyridine nucleotides, and leaky mitochondrial membranes owing to opening of mitochondrial permeability transition pore in its low conductance state. One can draw some analogy between molecular mechanism(s) underlying metabolic depression in lamprey liver cells in winter period of pre-spawning migration and those in cells of patients suffering from mitochondrial encephalomyopathies, neurogenerative diseases, sepsis, poisoning, and cancerogenesis. However, the cardinal difference between mitochondria of patients having the listed pathologies and those of the lamprey liver consists in the fact that mitochondria of the latter “overcome” the energetic depression and “get alive” in spring, that is likely connected with seasonal activation of lipolysis in their hepatocytes. In spring the sharp activation of oxidation and phosphorylation in the lamprey liver mitochondria followed by spawning and death of the animal is observed, i.e. the situation is under strict control.

33	Title: **Mitochondrial ABC transporter ABCme is essential for erythropoiesis in vivo.**	**Hyde, Brigham1; Elorza-Godoy, Alvaro1; Schlaeger, Thorsten2; Richey, Lauren3; Shirihai, Orian1** **Tufts University School of Medicine, Boston, MA USA1, Harvard Medical School Boston, MA USA2, Tufts University Division of Laboratory Animal Medicine Boston, MA USA3 .**	ABCme (ABCB10) is a mitochondrial ATP-binding cassette (ABC) transporter which is highly expressed in erythropoietic tissues. It is induced by GATA-1 during hematopoiesis and it's induction has been shown to enhance the erythropoietic capability of differentiating erythroid cells. It is hypothesized to play either a direct or supportive role in compartmentalization of heme biosynthesis intermediates. We investigated the role ABCme in erythropoiesis in vivo we using previously uncharacterized ABCme KO mouse. The ABCme -/- mouse was found to be embryonic lethal. Additionally, the ABCme -/- mouse was unable to hemoglobinize on days 8.5-11.5. Further analysis of the blood-island progenitors from day 10.5 PC -/- embryos found that erythroid progenitors were unable to differentiate beyond the level of CD71 + proerythroblast and exhibited a dramatically higher level of apoptosis in CD71+ progenitors. These results demonstrate for the first time that ABCme is essential for erythropoiesis.

34	Title: **Mitochondrial Dysfunction and Glutathione Depletion in a Murine Model of mut0 Methylmalonic acidemia**	**Randy Chandler1, Sara Shanske2, Patricia Zerfas3, Tina Cowan4, Gregory Enns4, Victoria Hoffman3, Salvatore DiMauro2, Charles P Venditti1** **1 Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland** **2 Department of Neurology, Columbia University College of Physicians and Surgeons, New York, New York** **3 Division of Veterinary Resources, Office of Research Services, National Institutes of Health, Bethesda, Maryland** **4 Medical Genetics Division, Stanford University School of Medicine, Stanford California**	Methylmalonic acidemia (MMA) is commonly caused by defective activity of the mitochondrial localized enzyme methylmalonyl-CoA mutase (MUT). Affected patients suffer from life-threatening intermittent metabolic decompensation, metabolic strokes, and renal failure; the etiology of these complications remains unknown. Current treatments include dietary restriction of precursors, vitamin B12 supplementation as well as liver and liver/kidney transplantation in the most severely affected patients. Paradoxically, liver transplantation does not appear to substantially reduce the plasma levels of methylmalonic acid, but does confer greatly increased metabolic stability. Mitochondrial dysfunction and oxidant stress may play a role in pathogenesis but have not studied. A methylmalonyl-CoA mutase (Mut) knock out mouse was created to recapitulate the phenotype observed in humans and to study mitochondrial morphology and function in MMA. Changes in the mitochondria ultrastructure were detected using electron microscopy in the liver, kidney and to a lesser extent in the pancreas, but not in the skeletal or cardiac muscle. These changes included the enlargement of the mitochondria, distortion of the cristae and the formation of inclusions bodies. Liver extracts showed ETC dysfunction, with a severe decrease in cytochrome c oxidase activity. Identical mitochondrial abnormalities were later observed in the liver of a patient with MMA. Glutathione pools of Mut-/- mice in the both the liver and whole blood were reduced indicating increased oxidative stress. These studies are the first to show that ETC dysfunction and glutathione depletion are inherent features of methylmalonic acidemia. Treatment strategies, in mice and patients, directed towards improving mitochondrial function, replenishing glutathione pools and protecting from oxidant stress are suggested by our results. The tissue specific mitochondrial changes caused by MMA may be the key to understanding the etiology of complications observed in this disease and the increased stability reported in liver transplantation.

35	Title: **Xenomitochondrial mice as models of mitochondrial dysfunction.**	**Cannon, Matthew V. * †; Irwin, Michael H.*; Dunn, David A.*†; Howell, Robert L.†; Trounce, Ian A. ‡; Pinkert, Carl A. *†.** ***Auburn University, Alabama; †University of Rochester, New York; ‡University of Melbourne, Australia.**	Introduction of mitochondrial DNA (mtDNA) derived from Mus terricolor fibroblasts into ρ0 Mus musculus domesticus ES cells was accomplished with the aim of engineering an animal model of mtDNA mutations (xenomitochondrial mice). Introduction of Mus terricolor mtDNA was expected to emulate a general mitochondrial impairment in mice due to sequence divergence between species. Cybrid studies supported this hypothesis, showing increased lactate production in Mus musculus domesticus cybrids harboring Mus terricolor mitochondria. However, xenomitochondrial animals failed to exhibit anticipated developmental phenotypes. Biochemical and behavioral measures were comparable in experimental and control mice. Treadmill experiments showed no difference between groups in running ability or serum lactate measurements. Post exercise histology was normal in experimental mice. Oxygen consumption was also unaltered in xenomitochondrial mice. Barnes maze data were suggestive of developmental differences; although background strain was a confounding variable. Altered gene expression of mitochondrially related genes is hypothesized to function as a compensatory mechanism leading to normal phenotypes. Based on data collected, we suggest a mild, general down regulation of genes involved in mitochondrial function and biogenesis, counter to expectations. Variations between genes and tissues are evidenced by northern and qPCR data. Understanding mechanisms leading to altered gene regulation in xenomitochondrial mice is of interest, as it will greatly supplement our understanding of nuclear-mitochondrial crosstalk in an animal model harboring extensive mtDNA polymorphisms and mutations.

36	Title: **Does Succinate Dehydrogenase Affect Centrosome Duplication in C. elegans?**	**Moore, Akilah and Golden, Andy** **NIDDK/LBG**	Various laboratories have performed genome wide RNA interference (RNAi) screens in C. elegans. The results from these screens have demonstrated that depletion of a number of mitochondrial proteins in the maternal germline by RNAi causes early embryonic lethality. We have assayed over 50 of these nuclear encoded mitochondrial genes and none of the genes studied cause a tight, stage specific arrest. However, when specific subunits of the Succinate Dehydrogenase complex are depleted by RNAi, there is a very tight one or two cell embryonic arrest. This arrest is characterized by defects in polar body extrusion, chromosome segregation, and abnormal chromosome morphology. Interestingly, in C. elegans there are very few examples of how maternal depletion of a specific protein causes a two cell embryonic arrest. Most often, an arrest at this stage in development is associated with defects in proper centrosome duplication. This project describes the unexpected arrest phenotypes that are associated with depletion of the SDH complex. We hypothesize that Succinate Dehydrogenase may be playing a role in centrosome duplication in addition to its known roles in the electron transport chain and the citric acid cycle in developing C. elegans embryos.

37	Title: **Heavy metal-induced mitochondrial dysfunction: a comparison of cadmium (II) with zinc (II) and selenite.**	**Elena A. Belyaeva** **Laboratory of Comparative Biochemistry of Inorganic Ions, Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences**	Mitochondria are found to be target organelles for such environmental pollutants as heavy metals. Recently we have shown that Cd2+ induces both necrotic and apoptotic death of hepatoma cells that is accompanied by increased formation of reactive oxygen species (ROS) at the respiratory complex III level and opening of mitochondrial permeability transition (MPT) pore. In the present work we continued to study mechanism(s) of Cd2+-induced toxicity on rat ascites hepatoma AS-30D cells cultivated in vitro. Using trypan blue and propidium iodide assays, we observed that Cd2+ disturbed the cell cycle, depressing cell growth and influencing the progression through its specific phases. Keeping in mind that Zn2+ and selenite can protect against toxic effects of Cd2+, we tested their action on the ROS-associated cell injury produced by Cd2+ and found that, at the concentrations used, they were not preventive against Cd2+-induced cytotoxicity and by themselves enhanced ROS formation and cell death. To underscore molecular mechanism(s) underlying the heavy metal-induced mitochondrial dysfunction we compared the action of the abovementioned metals on isolated rat liver mitochondria energized by glutamate and malate. Using selective electrodes, fluorescent probes and swelling technique we showed that the earliest event was the disturbance of the respiratory chain activity by the heavy metals which was partially sensitive to cyclosporine A, a strong MPT pore inhibitor, and simultaneous addition of NADH and cytochrome c. Similarities and differences in the action of the heavy metals as well as sequences of events manifested in the mitochondrial dysfunction are discussed.

38	Title: **Validation of the Mitochondrial Disease Criteria (MDC)**	**David Hsieh, MD and Andrea Gropman MD** **Children's National Medical Center, Washington DC**	Diagnosing mitochondrial disorders in children can be challenging. In the past, adult-based criteria have been published to assist with the evaluation (Bernier et al, 2002). A recent publication proposes the "Mitochondrial Disease Criteria" (MDC) as a more specific tool in children, as it emphasizes laboratory testing and imaging, areas that are given little emphasis in the adult criteria (Morava et al, 2006). Our objective was to validate the MDC in our patient population with a diagnosed mitochondrial disorder who carry a known mitochondrial or nuclear gene mutation. Methods: We applied the MDC to a known population of patients with genetically proven mitochondrial disorders. Results: 13 patients with genetically proven mitochondrial disorders were identified, whose symptoms began during childhood, and who had laboratory testing and brain MRI. 5 of these patients received a muscle biopsy. Using the MDC parameters, the mean clinical score was 3.69, with a range of 3-4. The mean pre-biopsy score was 6.76, with a range of 5-8. The mean post-biopsy score was 8.6. Conclusions: The MDC can be a useful tool for diagnosing mitochondrial disease in children. All 13 patients with genetically proven mitochondrial disease in our series had pre-biopsy scores of at least 5, correlating to "probable mitochondrial disease" by the MDC. Using the Criteria can be helpful in the consideration of laboratory testing for mitochondrial and nuclear gene mutations, or for proceeding to muscle biopsy. Imaging added to the probability of identifying a patient as having a high likelihood of having a mitochondrial disorder.

39	Title: **Strategies to Reduce NCE Attrition Due to Toxicity - Designing Novel Screening Methods**	**Yvonne Will, Lisa D Marroquin, James A. Dykens** **Pfizer Drug Safety Research and Development**	Despite regulatory vigilance, untoward toxicity and other side effects of ethical pharmaceuticals remain a major health concern. Several widely-publicized withdrawals of marketed therapeutics have revealed apparent failures in both the current models of drug development, and in the regulatory matrix designed to evaluate drug safety. Many of these drugs show toxicity to liver, cardiovascular system, skeletal muscle, nervous system, and kidneys. This toxicity is often idiosyncratic in that it is not necessarily related to dose, suggesting a genetic component, and is usually not discovered until after a large population of patients has been exposed. Recent evaluations in our laboratories and elsewhere show that many of these drugs have deleterious effects on mitochondrial function. Early identification of new chemical entities (NCE) that perturb mitochondrial function is therefore of significant importance in drug discovery if attrition due to toxicity is to be avoided. We discuss the strength and limitations of new HTS applicable screens, such as oxygen sensing probes, antibody capture methods and pH sensing and provide recommendations of where to position these assays within drug development process.

40	Title: **The effects of human ClpP in cell viability and cisplatin-induced apoptosis.**	**Yang Zhang** **Yang Zhang, Michael Maurizi** **NCI** **Bldg 37, Rm 2128** **37 Convent Dr** **Bethesda MD 20892**	The effects of human ClpP in cell viability and cisplatin-induced apoptosis. ClpXP is a bipartite chaperone/protease machine that catalyzes ATP-dependent protein unfolding and degradation in bacteria and in subcellular compartments of eukaryotes. ClpXP is present in plant chloroplasts and in photosynthetic bacteria, where it is essential for viability. ClpXP degrades specific proteins by tightly regulated mechanisms and plays essential roles in development, stress responses, and replication of phage and plasmids. In humans, hClpX and hClpP are imported into mitochondria, but the biological functions of hClpXP have not been characterized. We found that over expression of hClpP in cell culture inhibits cisplatin-induced apoptosis and delays cell death, whereas catalytically inactive hClpP did not produce the same results. Activation of caspases-3, 7, and 9 and cleavage of PARP following cisplatin treatment were significantly reduced in cells with high levels of hClpP. The release of apoptosis inducing factor (AIF), from mitochondria into the cytosol also is reduced in hClpP-over expressing cells. These results indicate that active hClpP inhibits cisplatin-induced apoptosis by interfering with the caspase-dependent and -independent pathways. Treatment of cells with hClpP siRNA leads to depletion of hClpP within 24 h and after 54 h mitochondrial membrane potential is lost and the cells undergo apoptotic cell death marked by the release of AIF from the mitochondria. Cells treated with low levels of hClpP siRNA become sensitized to cisplatin and other agents that induce apoptotic cell death. These results show that hClpP plays an important role in ensuring mitochondria integrity and modulating mitochondrial responses to stress. Future work will focus on effects of hClpP on quality control of mitochondrial proteins and its role in maintenance of mitochondrial membrane potential.

41	Title: **The Role of DNA Polymerase Gamma in Mitochondrial Disease**	**Sherine S. L. Chan and William C. Copeland.** **Mitochondrial DNA Replication Group, Laboratory of Molecular Genetics, NIEHS/NIH, RTP, NC**	Mitochondrial diseases affect 1 in 5000 children and adults in the general population. Mutations in nuclear genes encoding mitochondrial DNA (mtDNA) replication components have been linked with these diseases. In particular, POLG, the gene encoding the catalytic subunit of the mtDNA polymerase (pol gamma), is a major locus for mitochondrial disease, with more than 100 different mutations associated with the fatal early-childhood Alpers syndrome, ataxia neuropathy syndromes, progressive external ophthalmoplegia (PEO), male infertility, and susceptibility to drugs that inhibit HIV reverse transcriptase and that are commonly used to treat AIDS. Pol gamma is a two-subunit enzyme consisting of a catalytic subunit with highly faithful DNA polymerase and proofreading activities, and a smaller accessory subunit for tight DNA binding and processive DNA synthesis. As pol gamma is the only DNA polymerase within the mitochondrion, it is essential for replication and repair of mtDNA. Thus, we need to understand how and why pol gamma defects lead to such a wide spectrum of disease. We are addressing this question through a multi-faceted approach encompassing the following methods: 1. Collaborations with clinicians to identify new mitochondrial disease mutations and mechanisms of disease. 2. Structure-function and biochemical analyses to characterize mutant pol gamma proteins. 3. Mouse models of mitochondrial disease. These results provide a clearer understanding of how defects in pol gamma contribute to mitochondrial disease. Furthermore, our studies are generating crucial insights into the roles of pol gamma in mtDNA replication and repair.

42	Title: **Bioenergetic response of different transformed cells to chemotherapy provides evidence of the mitochondrial background as a determinant of tumour cell fate.**	**Stepien Georges.** **Inserm U484.** **Clermont- Ferrand, France.**	Cancer cells mainly rely on glycolysis for energetic needs and mitochondrial ATP production is almost inactive. However, cancer cells require mitochondrial functions to proliferate, such as specific metabolic pathways and maintenance of their internal membrane potential gradient (ΔΨm). The ability of tumour cells to regenerate damaged ΔΨm, an ATP consuming mechanism, is a critical factor for their survival. It thus may be predicted that ΔΨm regeneration should depend on cellular capability to produce sufficient ATP by upregulating glycolysis or recruiting oxidative phosphorylation (OXPHOS). To investigate this hypothesis, we compared the response to an anticancer agent of two transformed cell lines: HepG2 (hepatocarcinoma) with a partially differentiated phenotype and 143B (osteosarcoma) with an undifferentiated one. Treatment effects were tested on cell proliferation, O2 consumption / ATP production coupling, ΔΨm maintenance, and global metabolite profile. Our results showed an OXPHOS uncoupling and a lowered ΔΨm, leading to an increased energy request to regenerate ΔΨm in both models. However, energy request could not be met by undifferentiated cells (143B), which led them to death, while partially differentiated cells (HepG2) could activate oxidative metabolism and escape chemotherapy. We propose that mitochondrial OXPHOS background confers to tumour cells a survival advantage in response to chemotherapy.

43	Title: **Simulations of nucleoside analog drug interactions with POLG**	**Samuels, David** **Virginia Bioinformatics Institute, Virginia Tech., Blacksburg, VA, USA**	A significant fraction of the patients undergoing antiviral therapy for HIV/AIDS experience toxicity from the nucleoside analog components of the treatment. This toxicity often involves damage to the patients' mitochondria. Given the nature of these drugs and their mechanism of action (interference with the production of viral DNA), it is natural that our attention has mainly been focused on toxicity mechanisms acting though interference with the mitochondrial DNA polymerase, POLG, though other mitochondrial toxicity mechanisms are possible. The enzyme kinetics of POLG with a wide range of nucleoside analog substrates have been measured. We analyze this experimental data by carrying out a stochastic simulation of the action of POLG through the replication of the human mtDNA sequence, as a function of the concentrations of the four natural nucleoside triphosphates (dATP, dCTP, dGTP and TTP), and one or more activated nucleoside analog drugs. For each drug, we calculate the activated drug concentration necessary to give a 50% probability of interfering with the mtDNA replication process. We compare the ranking of the calculated IC50 values with the observed clinical toxicities of these drugs. This comparison indicates which drugs may reasonably be causing toxicity through this POLG mechanism, and which drugs must have other toxic mechanisms.

44	Title: **Mutations in the yeast mitochondrial DNA polymerase, MIP1, increase mitochondrial DNA mutagenesis.**	**Jeffrey D. Stumpfa, Diana Spella,b, Karen S. Andersonc, and William Copeland.** **aNational Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709, bSpellman College, Atlanta, GA 30314, cYale University, Department of Pharmacology, New Haven, CT 06520.**	Mitochondrial DNA replication is necessary for proper mitochondrial functions. Over 100 mutations in the human mitochondrial DNA polymerase, pol γ, have been linked to several mitochondrial diseases, including Alpers syndrome, progressive external opthalmoplegia, and ataxia-neuropathy (Copeland, 2007). Mitochondrial DNA from patients with pol γ related diseases is absent or contains large deletions. In the case of a few of the mutations, the amino acid change in pol γ was demonstrated in vitro to drastically reduce DNA replication (Graziewicz et al. 2004, Ponamarev et al. 2002, Chan et al. 2005, Chan et al. 2006). However, the effect of most of these mutations on disease has not been studied. To characterize DNA replication in the presence of disease mutations, we used the genetic model system Saccharomyces cerevisiae that contains the homologous mitochondrial polymerase, Mip1 (Foury, 1989). By aligning pol γ and Mip1 amino acid sequence, we found that 34 of the disease mutations in pol γ are conserved in yeast. We screened yeast strains, which contained a wildtype copy of MIP1 on the chromosome and a plasmid-encoded mutant mip1 allele, for increased mitochondrial mutagenesis and loss of mitochondrial function. Our results demonstrate that two disease mutations in the exonuclease domain and four in or near the polymerase domain significantly decrease polymerase activity or fidelity. Furthermore, we show the decrease of polymerase activity in vitro of a human mutation that corresponds to the yeast mutation that causes loss of mitochondrial function. Together, these results demonstrate the usefulness of the yeast model system to easily screen conserved disease mutations for mitochondrial DNA maintenance or mutagenesis.

45	Title: **Acute exposure of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) causes oxidative modifications of mitochondrial proteins and mitochondrial dysfunction in rat liver**	**Kwan-Hoon Moon1,4, Vijay V. Upreti2,4, Li-Rong Yu3,4, Insong J. Lee2, Xiaoying Ye3, Natalie D. Eddington2, Timothy D. Veenstra3, and Byoung-Joon Song1,** **1Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892-9410, 2Pharmacokinetics and Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, MD 21201, and 3Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., Frederick, MD, 21702-1201. 4 These authors equally contributed to the work**	MDMA (3,4-methylenedioxymethamphetamine, ecstasy) is a synthetic derivative of amphetamine and is frequently being abused. Acute exposure of MDMA alone or with another abused substance can damage many organs including liver and brain. Despite many reports about the causative role of oxidative stress in MDMA-induced hepatic toxicity, the underlying mechanism of organ damage is poorly understood. Virtually no information is available for the proteins that are oxidatively-modified during MDMA exposure. To address this problem, the oxidatively-modified mitochondrial proteins in rat livers following MDMA exposure were labeled with biotin-N-maleimide (biotin-NM) as a specific probe for oxidized Cys residues, purified with streptavidin-agarose and resolved on 2-DE. MDMA treatment significantly elevated the levels of serum transaminase activities, hydrogen peroxide, lipid peroxidation, and nitric oxide synthase activity with abnormal liver histology and decreased levels of reduced glutathione, contributing to increased oxidative stress and liver damage. Comparative 2-D gel analysis of biotin-NM labeled proteins showed marked increases in the levels of oxidatively-modified proteins following MDMA exposure compared to vehicle controls. Mass spectrometric analysis confirmed that many mitochondrial proteins involved in energy supply, fatty acid oxidation, intermediary metabolism, anti-oxidant defense, and chaperone activities were oxidatively-modified after MDMA treatment. Furthermore, the activities of mitochondrial aldehyde dehydrogenase and 3-ketoacyl-CoA thiolases, and ATP synthase were significantly inhibited following MDMA exposure. These results strongly indicate that increased oxidative/nitrosative stress and the oxidative modifications of various mitochondrial proteins contribute to mitochondrial dysfunction and liver injury in MDMA-exposed animals.

46	Title: **Mitochondria and the Undergraduate Biology Curriculum**	**Webb, Lisa S.** **Christopher Newport University, Newport News, VA**	The mitochondrion isn't just the powerhouse of the cell, it has the potential to be the workhorse of the Undergraduate Biology curriculum. It can be utilized extensively to illustrate multiple biological concepts. At Christopher Newport University, I teach both Cellular Biology and Introductory Biochemistry. I introduce the mitochondrion in my course as an organelle, but the relationship does not stop there. It is also used to illustrate the concepts of endosymbiosis and evolution. I utilize Mitochondria extensively when discussing membranes, including membrane structure and dynamics, permeability, and membrane transport. I also utilize the mitochondrial genome when discussing evolutionary processes that lead to nucleic acid sequence conservation. And don't forget meiosis and genetics, where the humble mitochondrion is an essential player. We discuss why mitochondria are maternally inherited and the processes that effect this differential pattern of inheritance. In conclusion, mitochondrial form and function can be utilized in many areas of the undergraduate biology curriculum to teach, illustrate or reinforce a variety of biological concepts.

47	Title: **Role of Pyruvate on Mitochondrial Oxygen and Fuel Sensing Mechanisms in Liver Following Hemorrhagic Shock and Resuscitation in Rats**	**Pushpa Sharma, Sean Rotolo and Paul D. Mongan** **Department of Anesthesiology, USUHS, Bethesda MD 20814**	Multiple organ failure as a secondary consequence of hemorrhagic shock is the fifth leading cause of death and disability in United States. The key events in the progression of organ failure are the inadequate supply or incomplete metabolism of substrate and oxygen consumption followed by a sharp decline in cellular ATP. The amount of cellular ATP depletion can modify the cell death mechanisms (apoptosis/necrosis). Although, mitochondria respond to the increasing need of ATP supply of damaged cells by reducing the cellular metabolic rate (similar to hibernation). We wanted to investigate the mechanism by which mitochondria sense and adapt to the decreased substrate and oxygen supply using a rat model of hemorrhagic shock (60 min) followed by resuscitation with/out sodium pyruvate (60 min). Liver was analyzed for mitochondrial oxygen consumption, HIF 1-α and pyruvate dehydrogenase complex activity (PDH). We found that in comparison to the sham animals, HS rats had significantly reduced mitochondrial oxygen consumption in the presence of complex I substrates (56%of sham) and pyruvate resuscitation of these animals significantly increased the oxygen consumption (78% of sham). Similar results were obtained with PDH. HIF1- α expression also increased partially after HS but significantly higher protein content (Western blotting) was noted after pyruvate resuscitation. In conclusion, mitochondria respond to the changing conditions of substrate and oxygen supply by decreasing the PDH activity (pyruvate sparing effect) and by elevating the HIF 1- α protein to increase vascular circulation in HS.

48	Title: **New Mitochondrial DNA Mutations Found in Individual Diagnosed with a Mitochondrial Disease.**	**Myrkalo, Jaimie and Deckman, Koren Holland.** **Gettysburg College, Gettysburg, PA. New Mitochondrial DNA Mutations Found in Individual Diagnosed with a Mitochondrial Disease.**	Respiration that occurs in mitochondria supplies most of the energy needed for cell survival. Thus, a point mutation in the mitochondrial DNA genome could interfere with the proper coding of the specific RNAs and/or protein subunits of the respiratory chain. Depending on how abundant the mutation is, an individual with this type of mutation would then be incapable of generating sufficient energy and would display symptoms of a mitochondrial disease. In this study, we sequenced approximately 99% of the mitochondrial genome of an individual who was diagnosed by the Mayo Clinic with a probable mitochondrial disorder. Two new point mutations were identified: a silent G-A point mutation located at nucleotide position (np) 12127 and an A-G point mutation located at np 13681, which codes for a T to A amino acid change within the ND5 gene. Six additional mutations, previously seen in multiple patients, contribute to amino acid changes in a total of 4 subunits (ND1, ND3, ND5 and CytB). The combination of these mutations may affect protein structure and function and could lead to a dysfunction in the protein subunits. Knowledge of the presence of mutations and polymorphisms in the mitochondrial DNA genome is important to both medical and forensic communities. Analysis of the distribution of these mutations in various tissues from this patient and maternal relatives could illuminate the relationship between the mutation and the disease.

49	Title: **Determination if Heteroplasmy Exists in Single Cells and in Single Mitochondria Through the Use of the PlexorTM qPCR System.**	**Adam, Michael and Deckman, Koren Holland.** **Gettysburg College, Gettysburg, PA.**	Single nucleotide polymorphisms (SNPs) and heteroplasmies are present in mtDNA in a wide variety of cells and can cause problems in forensic identifications. For example, a heteroplasmy found in single hair shaft from the crime scene but not in the suspect's sample could lead to ambiguity in the forensic identification. In this study, we sought to optimize a genotyping method - PlexorTM qPCR - using a C/T heteroplasmy specific to the mtDNA in the human leukocyte (HL-60) cell line. An improved genotyping method would help in successfully characterizing other ambiguous heteroplasmies. Electropherogram peak intensities in traditional amplification and sequencing of extracted total DNA shows an approximately equal ratio of C to T at nucleotide position 12071. Non-heteroplasmic control samples only contain T at 12071. The ratio of C/T was determined by three methods: 1) the ABI BigDye v.1.1 chemistry; 2) allele-specific qPCR; and 3)the PlexorTM qPCR system. The optimized method (the PlexorTM qPCR system) was then applied to single cells and single mitochondria, both isolated via the optical tweezers methodology. By this isolation method and the qPCR method, we were able to determine that this C/T heteroplasmy exists in the mitochondria of the single cell and in the mitochondrial DNA of the single mitochondrion.

50	Title: **Deletion or Artifact? Screening for Deletions in the Mitochondrial DNA Genome of an Individual Diagnosed with a Mitochondrial Myopathy.**	**Calamaras, Timothy and Deckman, Koren Holland.** **Gettysburg College, Gettysburg, PA.**	The mitochondrial genome of a Caucasian female who in her mid twenties had been diagnosed with a mitochondrial myopathy has been sequenced to identify single nucleotide polymorphisms (SNPs) and deletions within the genome. When compared to 102 reported Mitomap.org deletions, two new deletions were discovered in this individual by PCR amplification of short amplicons by pairing distant primers. The two amplicons have been sequenced and the deletion junctions have been determined. Based on nested primer studies, though, the true nature of these deletions is still under investigation. Both junction sites of the deletions rest within the binding site of one of the paired primers. This could indicate an artifact and may be related to the coiled nature of the circular mitochondrial genome. Deletions lead to heteroplasmic length polymorphisms within the mtDNA genome and can contribute to the mitochondrial myopathy symptoms exhibited by the individual. The exploration of deletions and polymorphisms within the human population has important implications for both the medical and forensic communities; this study on the variability of the mitochondrial genome may lead to a greater understanding of the causes of mitochondrial diseases and their relationship with mitochondrial mutations, SNPs, deletions and additions. The significance these amplicons generated from distant primer sites must be understood for the success of future deletions studies.

51	Title: **Sensitivity of Cardiac Mitochondria Separated by Free Flow Electrophoresis into Subpopulations.**	**Oliver Drews1, Jun Zhang1, An-Sheng Lee2, David Liem1, Peipei Ping1.** **1UCLA, School of Medicine, Los Angeles, California, USA; 2National Taiwan University, Taipei, ROC.**	High intracellular calcium levels cause mitochondrial swelling and the release of pro-apoptotic factors as a result of increased permeability and following rupture of mitochondrial membranes. In cardiomyocytes, such deleterious calcium concentrations arise from ischemic insults, leading ultimately to cell death. Previously, we have shown that cardiac mitochondria purified by zone electrophoresis in a laminar flow (ZE-FFE) separate in two major fractions (Mol Cell Proteomics. 2006; 5: S21). Mitochondria in cardiomyocytes are localized in the intermyofibrillar and subsarcolemmal space possibly explaining the two populations in our purification. Indeed, subsequent proteomics analyses showed that myosin heavy and light chain co-purified at low stoichiometric amounts with only one subpopulation, indicating the intermyofibrillar mitochondria. The lack of organelle markers, such as LAMP1 and GRP78, in the preparations confirmed the removal of common impurities in mitochondrial isolations. Both subpopulations contained the inner mitochondrial membrane protein ANT and the outer mitochondrial membrane protein VDAC, indicating the mitochondria are intact and not stripped from their outer membrane. Calcium sensitivity was assayed by Ca2+-induced swelling of the mitochondria. The magnitude of mitochondrial swelling served as an indicator for calcium sensitivity and was significantly different for the subpopulations. Since the isolation of the subpopulations is based on an electrophoretic separation in the assay buffer, the distinct sensitivity directly relates to mitochondrial differences rather than different treatment. Inhibition of mitochondrial swelling was achieved for both subpopulations by the addition of Cyclosporin A. Cyclosporin A is an inhibitor for the mitochondrial permeability transition pore. Therefore, the distinct calcium sensitivity of the mitochondrial subpopulations seemed to be related to variances in the opening of mitochondrial permeability transition pores. In conclusion, there exist mitochondrial subpopulations in the myocardium with distinct calcium sensitivity, which might contribute unequally to cardiac cell death after ischemic insults. Assessment and characterization of the subpopulations in disease phenotypes will improve the understanding of their contribution in cardiac diseases.

52	Title: **Top1mt controls mitochondrial DNA replication through D-loop formation**	**Hongliang Zhang and Yves Pommier** **LMP, CCR, NCI, NIH, Bethesda, Maryland.**	Somatic cells contain thousands of copies of mitochondrial DNA (mtDNA), which consist of duplex DNA circles encoding genes essential for oxidative phosphorylation and cellular metabolism. mtDNA replication must, therefore, be tightly controlled. In animal mtDNA replication, most nascent strands from the leading, heavy-strand origin (OH) are prematurely terminated, generating a 650-base, 7S-DNA product that defines the 3' boundary of the so-called "displacement loop" (D-loop). Proper formation of the D-loop is critical to the entire replication process and therefore to the integrity of the cell, but the control elements for it have not been identified. Here we show that mitochondrial topoisomerase I (Top1mt) is responsible for that control. In intact mitochondria, Top1mt sites are confined to three sites, adjacent to the premature replication termination site. We also find that TOP1mt knockout cells show defects in that termination process. Moreover, inhibition of Top1mt by camptothecin reduces formation of the 7S-DNA. Taken together, our findings demonstrate that Top1mt controls mtDNA replication by regulating the premature termination of replication.

54	Title: **Bleomycin down regulates PKD expression in A549 cells and induces mitochondrial and nuclear DNA damage**	**William J. Martin, M.D.,Sukhdev S. Brar1, Joel N. Meyer 2, Marcelo G. Bonini3, Bennett Van Houten4 and William J. Martin II1** **Laboratory of Respiratory Biology1, Nicholas School of the Environment and Earth Science, Duke University, Durham, NC 27708, USA2, Laboratory of Pharmacology Chemistry3, and Laboratory of Molecular Genetics4, National Institute of Environmental Health Sciences, National Institute of Health 111 TW Alexander Drive, Research Triangle Park, NC, 27709-2233, USA**	Bleomycin is a well established cancer chemotherapeutic drug but pulmonary toxicity has limited it usage. Bleomycin is known to produce reactive oxygen species (ROS) that can attack both mitochondrial and nuclear DNA and ultimately cause apoptotic cell death. Using a quantitative QPCR assay we comparatively assessed mitochondrial vs. nuclear DNA damage in A549 cells at 2, 4, 8, 12, 24, and 48 hrs after bleomycin, hyperoxia, and bleomycin+hyperoxia (combination) treatment. All three treatments caused DNA damage at some timepoints. Bleomycin and hyperoxia alone caused more mtDNA damage than nDNA (p=0.016 and 0.004, respectively). The combination caused a high level of lesions (1-1.5 lesions/10 kb at 12, 24, and48 hours); however mtDNA damage was greater than nDNA at 4 and 8hrs. Western blot analysis of A549 cells treated with bleomycin, hyperoxia, or the combination shows that the bleomycin caused some activation of Caspase-3 at 24 hrs and with the combination treatment this activation occurred at 12 hrs and continued up to 48 hrs. No Caspase-3 activation was seen with hyperoxia alone over a time period of 48 hrs. Bleomycin and combination treatment also caused translocation of Bax from the cytosol to mitochondria. Recently, the serine/threonine kinase Protein Kinase D1 (PKD1) was identified as a mitochondrial sensor for oxidative stress. PKD1 plays an important role in several cellular processes such as apoptosis, immune regulation, cell proliferation, oxidative stress signaling, and adhesion. PKC-mediated phosphorylation of PKD1 results in the translocation of the active form of PKD1to the nucleus and activates NF-kB, which results in expression of superoxide dismutase (SOD2). SOD2 is involved in elimination of free radicals in mitochondria. Bleomycin and the combination treatment resulted in down regulation of PKD1 and also reduced SOD2 level inside the mitochondria matrix. Therapies intervening these pathways of mitochondrial injury may contribute to a reduction in lung cell injury and potentially reduce its predictable outcome of pulmonary fibrosis.

55	Title: **CHARACTERIZATION OF THE ROLE OF MITOCHONDRIAL TRANSCRIPTION FACTOR A IN BASE EXCISION REPAIR**	**Anne-Cécile V. Bayne, Nadja C. de Souza-Pinto, and Vilhem A. Bohr.** **Laboratory of Molecular Gerontology, #2D-05** **National Institute on Aging-Intramural Research Program** **National Institutes of Health** **5600 Nathan Shock Drive** **Baltimore, MD 21224**	One of the main functions of mitochondria is to produce cellular ATP through oxidative phosphorylation. This process produces significant amounts of reactive oxygen species which can damage DNA. The circular double-stranded mitochondrial genome is only about 16,600 base pairs but it encodes 13 critical proteins of the respiratory chain, as well as 2 ribosomal RNAs and 22 transfer RNAs. Mechanisms to repair mitochondrial DNA (mtDNA) have been clearly identified, and there is now evidence showing that several proteins structure the mitochondrial DNA in nucleoids localized at the inner mitochondrial membrane. Mitochondrial transcription factor A (TFAM) is an essential component of the nucleoids and is sufficient by itself to organize mitochondrial chromatin. This high mobility group protein is a key regulator of mitochondrial DNA transcription and replication. However, it is at present unknown whether it is involved in mitochondrial DNA repair. The main purpose of this study was to characterize the role of TFAM in mitochondrial base excision repair (BER), the only complete biochemical pathway for oxidative mtDNA damage repair characterized so far. Recombinant human TFAM was produced in a bacterial system and binding studies showed that the presence of a single 8-oxoguanine, one of the most common oxidative damage observed in vivo, increased TFAM binding to DNA significantly, while other base excision repair intermediates did not modulate significantly TFAM affinity for DNA. Activity assays revealed that TFAM modulated negatively 7,8-dihydro-8-oxoguanine-DNA glycosylase (OGG1), uracil-DNA glycosylase (UDG), abasic endonuclease (APE1) and mitochondrial DNA polymerase gamma (POLG), all enzymes involved in mitochondrial base excision repair. Mild oxidation of TFAM led to a loss of DNA binding affinity, which resulted in the abolition of the inhibitory effect on DNA repair. Altogether, these results indicate that TFAM is a likely player in the regulation of mitochondrial base excision repair.

55	Title: **Allotopic expression of ATP6: mtDNA mutation modeling.**	**Dunn, David A.†* and Pinkert, Carl A.†*** **†Auburn University, Department of Pathobiology and *University of Rochester Medical Center, Department of Pathology and Laboratory Medicine.**	Animal modeling of mitochondrial DNA (mtDNA) mutations has trailed nuclear transgenesis due to a host of cellular and physiological distinctions. mtDNA mutation modeling is of critical importance as mutations in the mitochondrial genome give rise to many pathological conditions. The T to G mutation on nucleotide 8993 of the human mitochondrial genome results in either NARP (Neurogenic muscle weakness, Ataxia, and Retinitis Pigmentosa) or MILS (Maternally Inherited Leigh Syndrome) phenotypes. A study was undertaken to develop a mutation model where the mtDNA 8993 mutation was engineered for expression from the cell nucleus. Nuclear localization and transcription of mtDNA genes followed by cytoplasmic translation and transport into mitochondria (allotopic expression) provides an opportunity to create in vivo modeling of a targeted mutation in mitochondrial genes. A murine ATP6 gene coding for the T8993G mutation with nuclear codon substitutions and the Cox VIII N-terminal mitochondrial transport signal was synthesized de novo and stably expressed in NIH/3T3 cells. Transgenic mice that are generated using this construct are expected to recapitulate the biochemical and pathological phenotypes of NARP/MILS ATP6 mutation. A resultant transgenic mouse lineage will represent the first germline competent animal model of a specific deleterious human mtDNA mutation.

56	Title: **Reconstitution of promoter-specific mitochondrial transcription using proteins produced in E. coli.**	**Lodeiro, Maria F.; Arnold, Jamie J.; Reynolds, Shelley L. and Cameron, Craig E.** **Department of Biochemistry and Molecular Biology, The Pennsylvania State University.**	Mutations that alter mitochondrial RNA metabolism, including mitochondrial transcription, are linked to numerous diseases, for example neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, muscular dystrophies, cardiac diseases and cancer. In all cases, the molecular defects underlying this broad spectrum of pathologies have not been defined, thus precluding the development of strategies to prevent and/or treat these diseases. Although promoter-specific transcription can be reconstituted in vitro from purified components, the detailed mechanisms governing mitochondrial transcription are poorly understood. In addition to mitochondrial RNA polymerase, promoter-specific initiation/elongation requires mitochondrial transcription factors mTFA and mTFB1 or mTFB2. Moreover, the transcription machinery utilizes two different promoters, LSP and HSP. The literature suggests that only mitochondrial proteins expressed in a eukaryotic system are functional. However, our laboratory has reconstituted promoter-specific transcription by using proteins produced in E. coli. This advance greatly facilitates interrogation of mitochondrial transcription complex structure, function and mechanism. With this technology we are investigating the role of mTFB1 and mTFB2 in mitochondrial transcription initiation and/or elongation, fundamental information that is not currently available for mitochondrial transcription. Our current results for the requirement/mechanism of promoter specific initiation and elongation will be discussed.

57	Title: **The Role of Ceramide Channels in Mitochondria-Mediated Apoptosis**	**Colombini, Marco** **University of Maryland, College Park, MD**	Mitochondria-mediated apoptosis involves the release of proteins from the inter-membrane space to the cytosol leading to the execution phase of apoptosis. An excellent candidate for the pathway that is responsible for this release is a channel formed by the sphingolipid, ceramide. Early in apoptosis mitochondrial ceramide levels often rise above the mole fraction needed for hundreds of ceramide mono-mers to self-assemble, forming channels. When mitochondrial ceramide levels do not rise, inhibition of ceramide channel formation by anti-apoptotic Bcl-2-family proteins is reduced by heterodimerization with pro-apoptotic proteins, resulting in ceramide channel formation. Indeed, both the mammalian anti-apoptotic protein, Bcl-xL, and the worm version, CED-9, disassemble ceramide channels when formed in mitochondrial outer membranes or phospholipid membranes. The delta-N76 deletion of Bcl-xL is pro-apoptotic and causes the growth of ceramide channels. The pores formed by ceramide channels have been visualized by negative-stain electron microscopy and their size is approximately 10 nm in diameter. The same pore size is calculated from the size of native proteins released by ceramide treatment of rat liver mitochondria. Dihydroceramide, the inactive precursor lacking the essential 4, 5 trans double bond, does not induce apoptosis and does not form channels. Of the sphingolipids tested, ceramide is unique in forming protein-permeable channels. These channels have the ability, opportunity, and interactions necessary to be excellent candidates for the release pathway.

58	Title: **Fate of Double Strand Breaks in Mammalian Mitochondrial DNA**	**Hunter, Senyene, Collins, Leisha and Van Houten, Bennett** **National Institute of Environmental Health Sciences, RTP, NC, USA**	The process of oxidative phosphorylation in mitochondria leads to the production of highly reactive oxygen-containing molecules described as reactive oxygen species (ROS). DNA double-strand breaks (DSBs) are induced by endogenously generated ROS and exogenous agents such as ionizing radiation and certain chemotherapeutic drugs. DSB repair is essential for the maintenance of mitochondrial DNA (mtDNA) in yeast, plants and fungi. However, mammalian mtDNA repair has not been well studied. We are investigating human mitochondrial DSB repair. We have developed a highly sensitive, quantitativePCR-based DSB repair assay. Utilizing this assay, we observe the repair of restriction endonuclease-induced DSBs catalyzed by highly purified mitochondrial extracts. DNA containing cohesive ends (5' or 3' overhangs) is repaired more efficiently than blunt-ended DNA (6.6%, 4.1% and 1.5% repaired, respectively). To elucidate the mechanism of mitochondrial DSB repair, we further investigated the rejoining of PstI-generated DSBs. This DSB repair is coupled with the processing of DNA ends, resulting in the loss of approximately 50 bases surrounding the PstI site. Sequence analysis revealed several patterns of the repaired DNA, most with deletions spanning 4-7 bp direct repeats. We hypothesize that mitochondrial nucleases resect the DNA to reveal short stretches of homology thus allowing annealing and ligation of broken DNA. The nucleases responsible for DNA resection are being investigated. This type of mtDNA repair would lead to the loss of expression of critical mitochondrial encoded proteins. There are an ever increasing number of neurodegenerative diseases and mitochondrial myopathies associated with alterations in the mitochondrial genome. One clinical manifestation associated with the loss of mtDNA between direct repeats is Kearns-Sayre Syndrome. Our study of mitochondrial DSB repair might shed new light on the underlying mechanism of this and other mitochondrial associated diseases.

59	Title: **Role of C-terminal tails of tubulin in its interaction with mitochondrial channel VDAC**	**Kely Sheldon, 2Dan Sackett, 1Sergey Bezrukov, and 1Tatiana Rostovtseva1** **Laboratory of Physical and Structural Biology; 2Laboratory of Integrative and Medical Biophysics, NICHD, NIH, Bethesda, MD 20892**	Mitochondria have long been known to interact with the tubulin-microtubule system. We recently have found a direct functional interaction between bovine brain tubulin and VDAC, a channel from mitochondria outer membrane, reconstituted into planar lipid membrane. Both and subunits of the tubulin heterodimer possess anionic C-terminal tails (CTT) which regulate interaction with a number of cytosolic proteins, and which can be removed by controlled proteolysis. Here we study the role of CTT of tubulin in its interaction with VDAC. We have shown that tubulin induces VDAC channel closure with very high efficiency (equilibrium binding constant is K ~ 0.1 M-1). When CTT were proteolitically removed in tubulin-S, VDAC closure did not occur. However, we found that CTT peptides by themselves are not active. Two synthetic peptides with the sequences of mammalian and brain tubulin CTT did not induce channel closure up to micromolar concentrations. Analysis of current fluctuations through a VDAC channel in the presence of tubulin-S showed that the tailless body of the tubulin dimer does interact with VDAC, but this interaction does not induce VDAC closure. We investigated which CTT, or plays a dominant role in closing the VDAC channel. Our results suggest that when driven by an electrical field, almost the full length of CTT penetrates in to the channel lumen and reaches two binding sites from both entrances of the VDAC pore. Our findings represent a novel role for the tubulin CTT, distinct from its previously known role in mediating interactions on the microtubule surface.

60	Title: **Function of Phosphodiesterase 3B in regulatory circuits controlling white versus brown adipocyte differentiation**	**Youn Wook Chung, Yan Tang, Steven C. Hockman, Faiyaz Ahmad, Young Hun Choi, Sunhee Park, Vincent C. Manganiello** **Translational Medicine Branch, NHLBI, NIH, Bethesda, Maryland**	Cyclic nucleotide phosphodiesterase 3B (PDE3B) has been suggested to be critical in regulating energy metabolism in adipocytes, liver, and pancreatic cells. In Pde3b-KO mice epididymal white adipose tissue (EWAT) exhibits some phenotypic characteristics of brown adipose tissue (BAT), including enhanced gene expression of peroxisome proliferator activated receptor coactivator-1 alpha (PGC-1) and uncoupling protein 1 (UCP1), and increased mitochondria number and size. Mitochondria were isolated from wild-type and PDE3B knock out (KO) mice using discontinuous sucrose gradients, and were studied by electron microscopic (EM) and proteomics techniques. Sucrose gradient and EM data demonstrated two populations of mitochondria, with EWAT containing lighter and smaller mitochondria, and BAT, heavier and bigger mitochondria. EWAT from PDE3B KO contained both populations of mitochondria. Knock out of the Pde3b gene also resulted in increased adipocyte fatty acid oxidation (FAO) and oxygen consumption. Taken together, these results suggested that PDE3B might function as a molecular switch determining white versus brown adipocyte differentiation, and thereby could play an important role in regulation of energy metabolism.

61	Title: **Stress Induced Mitochondrial Remodeling in Neurons**	**Kaifosh Patrick, and Mills, Linda R,** **Toronto Western Research Institute, UHN Toronto Canada.**	Neuronal mitochondria are motile and dynamic, frequently undergoing changes in morphology. Sustained morphological changes also occur in a variety of pathological conditions, and mitochondrial swelling and fission are associated with the release of intermembrane proteins that trigger apoptosis. We used confocal microscopy to examine the effects of sublethal stress on mitochondria in differentiated PC12 cells transfected with an inducible GFP targeted to the mitochondrial matrix. In response to an osmotic challenge mitochondrial morphology rapidly changed; within 120s formerly elongated mitochondria rounded up, and in some cases swelled, as the mitochondrial network disintegrated. This remodeling was reversible upon removal of the osmotic challenge: complete recovery of pre-challenge morphology and the network occurred within 120 seconds of reintroduction of normosmotic medium. Time lapse series revealed that remodeling was repeatable: similar changes occurred during up to four cycles of osmotic challenge within the same cell. Mitochondria populations in cells undergoing sustained, but less severe challenges, showed some spontaneous, partial, recovery of normal morphology. Multiple cycles of mitochondrial remodeling did not cause significant cell death, assessed by propidium iodide (flow cytometry and confocal microscopy) or cytochrome c release (western blot). Mitochondrial membrane potential was maintained throughout cycles of remodeling, and ATP levels were not altered. Remodeling was not associated with increased reactive oxygen species, changes in mitochondrial motility, and was not prevented by respiratory inhibition, prolonged mitochondrial uncoupling, calcium loading, inhibition of the mitochondrial permeability transition, or actin depolymerization. Our results indicate that mitochondrial morphology is extremely plastic and that acute morphological changes can occur throughout mitochondrial populations without impairing mitochondrial functions or cell viability. Primary cortical neurons in vitro also displayed robust mitochondrial remodeling, and reversible remodeling also occurred in response to sublethal oxygen glucose deprivation (albeit on a slower time scale). Our results suggest that reversible mitochondrial remodeling may play a key role in recovery from events (e.g. stroke, mechanical trauma) associated with neuronal swelling.

62	Title: **The Potential Role of Mitochondria in Death Resistance and Survival Signaling.**	**Wright, Kristen, Patierno, Steven R., Susan Ceryak.** **Department of Pharmacology and Physiology, Program in Molecular Medicine, George Washington University Medical Center, Washington, DC.**	Mitochondria are critical to apoptosis and homeostatic control. The aim of this study was to elucidate the role of mitochondria in cellular death resistance and survival signaling. We used populations of BJ-hTERT (death-sensitive, DS) fibroblasts subcloned from clonogenic survivors of 24h 5μM hexavalent chromium Cr(VI) exposure that acquired resistance to genotoxin-induced death, (death-resistant, DR). Certain forms of Cr(VI) are known respiratory carcinogens and we use Cr(VI) as a model genotoxin with public health relevance. Our previous studies showed that, after genotoxin exposure, DS cells displayed increased caspase 3 cleavage, mitochondrial membrane depolarization and increased VDAC mRNA expression, in sharp contrast to the DR cells. Moreover, DR cells, exhibited genotoxin-inducible Akt activation, while Akt was downregulated in DS cells under the same conditions. Upregulated hexokinase II (HKII) protein expression has been shown in tumor cells. Akt is known to enhance HKII-mitochondrial (HKII-mito) association, correlated with decreased apoptosis, potentially through HKII binding of VDAC. Here we found low levels of HKII-mito association, in the DS cells both basally and following Cr(VI) exposure, while the DR cells had consistently higher HKII-mito association. Total HKII protein expression was also higher in the DR cells following Cr(VI) exposure. The DR cells displayed a diffuse reticular mitochondrial network as evidenced by immunofluorescence staining with Mitotracker Green, while the DS cells showed peri-nuclear mitochondrial localization. However, we found no changes in mitochondrial shape or size by electron microscopy. Additionally, flow cytometric analysis with Mitotracker-CMXRos suggested increased mitochondrial activity in the DR cells. Finally, we showed that DR cells were able to override the G2/M cell cycle checkpoint following Cr(VI) exposure. A connection between G2/M override and mitochondrial-mediated survival is currently under investigation. Our data suggest a potential role for mitochondrial regulation in survival signaling and death resistance, which may play a role in neoplastic progression.