Mitochondria Minisymposium 2008

Mitochondria and their Proteomics

Poster Listing

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

56 ***Reconstitution of promoter-specific mitochondrial transcription using proteins produced in E coli***
Maria F. Lodeiro

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

57 ***The Role of Ceramide Channels in Mitochondria-Mediated Apoptosis***
Marco Colombini

58 ***Fate of Double Strand Breaks in Mammalian Mitochondrial DNA***
Senyene Hunter

59 ***Role of C-terminal tails of tubulin in its interaction with mitochondrial channel VDAC***
Kely Sheldon

60 ***Function of Phosphodiesterase 3B in regulatory circuits controlling white versus brown adipocyte differentiation***
Youn Wook Chung

61 ***Stress Induced Mitochondrial Remodeling in Neurons***
Patrick Kaifosh and Linda R Mills

62 ***The Potential Role of Mitochondria in Death Resistance and Survival Signaling***
Kristen Wright

Sponsors

National Institutes of Health
OFFICE OF DIETARY SUPPLEMENTS

National Institutes of Health
OFFICE OF Rare Diseases

NATIONAL INSTITUTES OF HEALTH

NATIONAL CANCER INSTITUTE

National Eye Institute
U.S. National Institutes of Health Research Today...Vision Tomorrow

National Heart Lung and Blood Institute
People Science Health

NIAAA
National Institute on Alcohol Abuse and Alcoholism

NIDA
NATIONAL INSTITUTES ON DRUG ABUSE

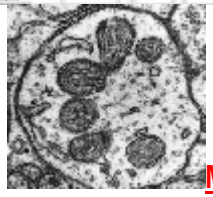
NIDDK

NIEHS
National Institute of Environmental Health Sciences

NIMH
National Institute of Mental Health

National Institute of Neurological Disorders and Stroke

National Institute of Nursing Research



MIG

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 Copelanda.** **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.