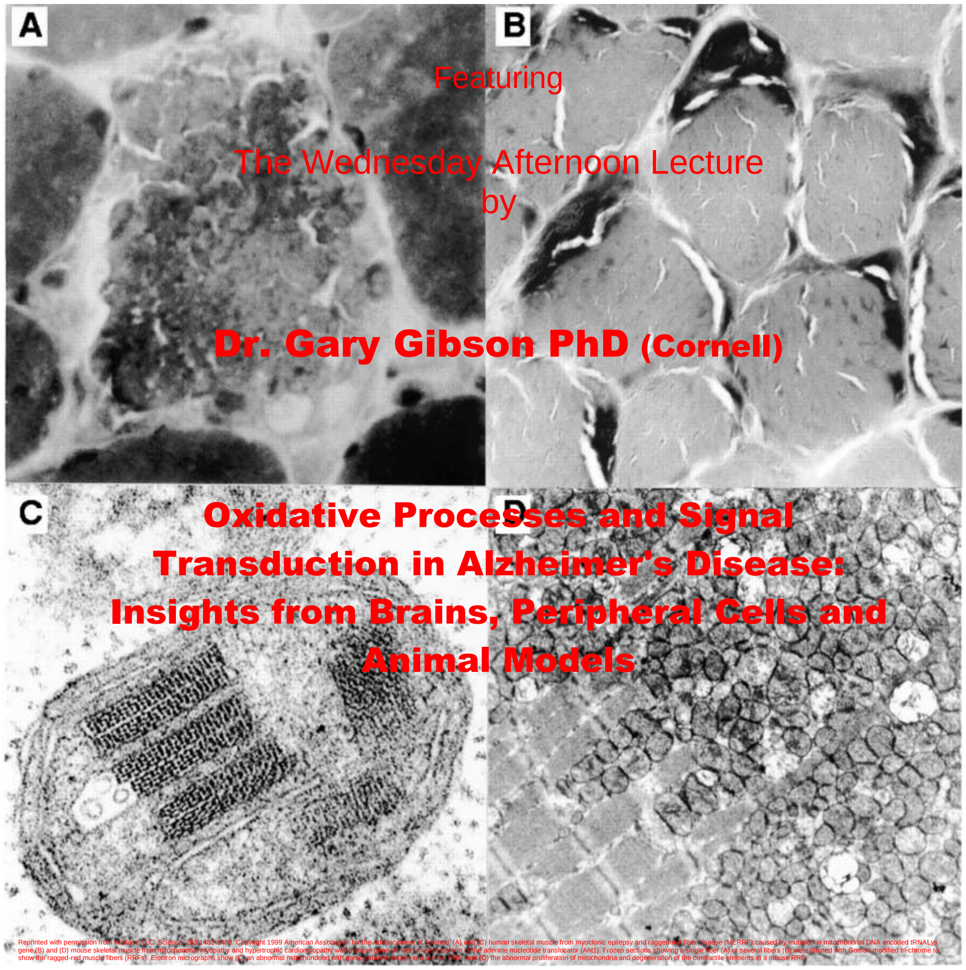
NIH Director's Wednesday Afternoon Lecture Series Event

Mitochondria Interest Group Minisymposium

Mitochondria: Interaction of Two Genomes



Reprinted with permission from Wallace, D.C. Science, 283:1482-1488. Copyright 1999 American Association for the Advancement of Science. (A) and (C) human skeletal muscle from myoclonic epilepsy and ragged-red fiber disease (MERRF),caused by mutation in mitochondrial DNA encoded tRNALys gene (B) and (D) mouse skeletal muscle from mitochondrial myopathy and hypertrophic cardiomyopathy with inactive gene of heart-muscle isoform of the adenine nucleotide translocator (Ant1). Frozen sections showing a single fiber (A) or several fibers (B) were stained with Gomori modified tri-chrome to show the ragged-red muscle fibers (RRFs). Electron micrographs show (C) an abnormal mitochondrion with paracrystalline arrays in a human RRF, and (D) the abnormal proliferation of mitochondria and degeneration of the contractile elements in a mouse RRF.

Tuesday and Wednesday 14 and 15 March 2000

Masur Auditorium, Lipsett Amphitheater, and Visitors Information Center

Clinical Center (Building 10), NIH, Bethesda, MD

Registration and Schedule Website: http://www.nih.gov/sigs/mito/March2000.html

For special accommodation needs call 301-594-5595

The NIH/FAES is accredited by the Accreditation Council for Contuinuing Medical Education to sponsor continuing medical education for physicians.
The NIH/FAES designates this Wednesday Afternoon Lecture for a maximum of 1 hour and the Minisymposium for a maximum of 15.0 hours in category 1 credit towards the AMA Physician's Recognition Award. Each Physician should claim only those hours of credit actually spent in the educational activity.

The Mitochondrial Medicine Society
MMS
www.mitosoc.org

Mitochondria Interest Group
www.nih.gov/sigs/mito/

The Mitochondria Research Society
www.mitoresearch.org

Mitochondria Interest Group

2nd NIH Mitochondria Minisymposium, convened to accentuate the Wednesday Afternoon Lecture of Dr. Gary Gibson (Cornell Medical College), meets Wednesday also

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Minisymposium Attendance/Poster Registration Form and Schedule
Mitochondria: Interaction of Two Genomes
A National Institutes of Health Director's Wednesday Afternoon Lecture Series Event
Tuesday and Wednesday March 14 and 15, 2000

(See Schedule at bottom of this page)
Masur Auditorium and Visitor Information Center
Clinical Center (Building 10), NIH Bethesda, MD

If you need special accomodations while at NIH, please contact Steve Zullo (see contact info below).

We are pleased to announce that the BETHESDA POOKS HILL MARRIOTT has been designated HOST HOTEL for the MITOCHONDRIA MINISYMPOSIUM

A Block of Rooms will be held for the nights of 13 and 14 March 2000, at reduced rates (Single or double, register at same time if you want to double). Call 1-800-228-9290 (or 301-897-9400) before 15 February 2000, and be sure to indicate "Mitochondria Minisymposium".

Thanks to NHGRI for supplying the Registration script, Jai Evans of SCRC and Wayne Rasband of RSB-NIMH for modifying and hosting it. Special Thanks to Sandy Desautels for assistance with our web page and debugging.

You can register for the Minisymposium with or without a poster. If you wish to present more than one poster, please start here to register each poster. If you are registering without a poster, enter "none" for poster title, and for abstract.

Start Registration

Maps around NIH

For maps of NIH, METRO Rail System, and area, please check the following Web Site: http://www.nih.gov/welcome/maps.html. WARNING: Parking is limited on campus, plan to use METRO! Submit via the above form or submit via e-mail before cob 29 February 2000 to: zullo@helix.nih.gov or via regular mail postmarked before 15 February 2000 to:

Steven J. Zullo, PhD
Building 10, Room 2D56
Mail Stop 1513
NIH
Bethesda, MD 20892
Phone: 301-435-3576
FAX: 301-480-9862

An NIH Director's Wednesday Afternoon Lecture Series Event

Mitochondria: Interaction of Two Genomes

2nd NIH Mitochondria Minisymposium 14 March 2000

Educational Objectives: For a working understanding of mitochondria and mitochondrial dysfunction

Lecture Venue: Masur Auditorium, Clinical Center, NIH

Poster Venue: Visitor Information Center, Clinical Center

Exhibitor Venue: Atrium, Clinical Center

- **0745 Registration, Poster Set-up, Continental Breakfast in Exhibit Area**
- **0830 Dr. Richard Boles (USC): Heteroplasmy Screening of the Entire mtDNA by TTGE**
- **0905 Dr. Bruce Cohen (Cleveland Clinic): Polarography and Spectrophotometry: Making Sense of Respiratory Chain Assays in Clinical Mitochondrial Disease**
- **0940 Dr. Edward Kaye (CHOP): Mitochondrial Diseases Presenting as Leukodystrophy**
- **1015 Poster Session/Coffee Break/Product Show**
- **1045 Dr. Richard Haas (UCSD): Results of the Open Label DCA Trial**

- **1120 Dr Craig Warden (UC-Davis): Uncoupling Proteins and the Trail to Complex Mitochondrial Phenotype**

- **1155-1255 Lunch Break/Poster Session/Product Show**

- **1200-1230 Mitochondrial Biology LunchTime Review (MBLTR): Moderators-Dr. Betsy Smith (NIAID) and Dr. Mariana Gerschenson (NCI); Clinically Induced Mitochondrial Toxicity, A Panel Discussion**

- **1255 Dr. John Shoffner (Children's Healthcare of Atlanta): Complex I Defects in Oxidative Phosphorylation Disease**

- **1330 Dr. Gino Cortopassi (UC-Davis): Cellular Models of Mitochondrial Neurological Disease**

- **1405 Poster Session/Coffee Break/Product Show**

- **1445 Dr. Robert Naviaux (UCSD): Pyrimidine Therapy of Mitochondrial Disease**

- **1520 Dr. Richard Kelley (Johns Hopkins): Autism**

- **1555 Dr. Steve Zullo (NIMH): Gene Therapy of Mitochondrial DNA Mutations**

- **1630 Poster Session/Coffee Break/Product Show**

- **1700 Mito Burger Bash**

- **1800-2000: Late-Breaking Mitochondrial News**

- **1800 Dr. Henry Weiner (Purdue): Can Mitochondrial Import be a Co-translational Event?**

- **1820 Dr. Barry Kaplan (NIMH): Synthesis of Nuclear-encoded Mitochondrial Proteins in the Presynaptic Nerve Terminal**

- **1840 Dr. Ester Fernandez-Salas (NCI): mtCLIC, a TNF-a and p53-regulated Mitochondrial Chloride Channel Protein, Induces Apoptosis through Mitochondrial Alteration**

- **1900 Dr. Mark Smith (Case Western Reserve University): Mitochondria, Metals and Alzheimer Disease**

- **1920 Dr. Nadja Souza-Pinto (NIDA): Mitochondrial DNA repair in vitro**

- **1940 Jason Arcediano (Intergen): Quantitative detection of Apoptosis markers by fluorescence RT-PCR**

- **Minisymposium Resumes Tomorrow Morning at 0830**

An NIH Director's Wednesday Afternoon Lecture Series Event

Mitochondria: Interaction of Two Genomes

2nd NIH Mitochondria Minisymposium 15 March 2000

- **0745 Registration, Poster Set-up, Continental Breakfast in Exhibit Area**

- **0830 Dr. Douglas C. Wallace (Emory): Mitochondrial Diseases of Man and Mouse**

- **0920 Dr. Martin Gonzalez (NICHD): Regulation of DNA trans-lesion synthesis in Escherichia coli by proteolysis**

- **0955 Poster Session/Coffee Break/Product Show**

- **1020 Dr. Lawrence Grossman (Wayne State): Promoter Interactions in Regulation of Cytochrome c Oxidase Gene Expression**

- **1050 Dr. Grazia Isaya (Mayo): Functional Analysis of Frataxin**

- **1120 Dr. Keshav Singh (Johns Hopkins): In vivo Evidence for Repair of Mitochondrial DNA by Nuclear Protein(s)**

- **1150 Lunch Break/Poster Session/Product Show**

- **1200-1220 MBLTR: Moderator-Dr. Andrea Gropman (NINDS and Childrens' Hospital, DC): Support Groups**

- **1240 Dr. Mariana Gerschenson (NCI): Mitochondrial Endonuclease, ENDO-G**

- **1310 Dr. Carmen Mannella (Wadsworth): New Insights into Mitochondrial Structure from EM Tomography (this title linked to images of mitochondria)**

- **1340 Dr. William C. Copeland (NIEHS): The Role of the Human DNA Polymerase Gamma in Mitochondrial DNA Mutations and Diseases**

- **1410 Dr. Stanley Rapoport (NIA): Staging Downregulation of Mitochondrial Oxidative Phosphorylation in Alzheimer Disease, using in vivo PET Imaging and Postmortem Brain Studies**

- **1440 Mitochondria Minisymposium Ends**

- **1500 Wednesday Afternoon Lecture: Dr. Gary Gibson (Cornell, Burke): Oxidative Processes and Signal Transduction in Alzheimer's Disease: Insights from Brains, Peripheral Cells and Animal Models**

- **1600 Reception/FARE Poster Session: Above VIC (Sponsored by National Institute on Drug Abuse)**

The NIH Director's Wednesday Afternoon Lecture and Minisymposium

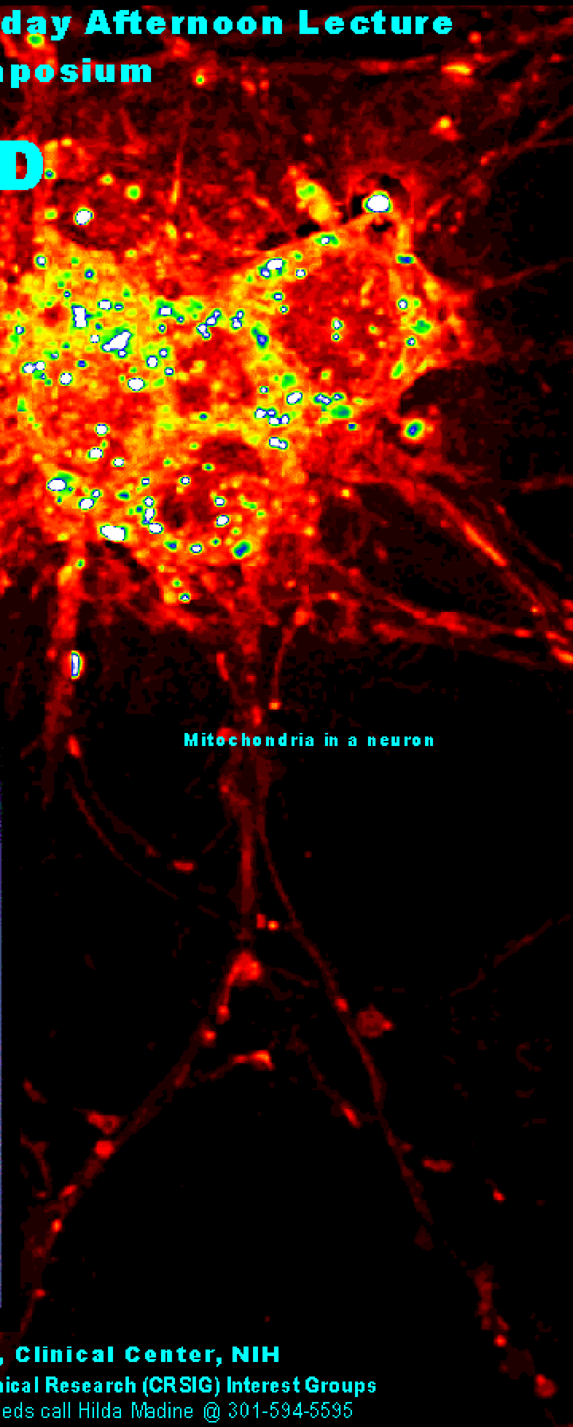
Gary Gibson, PhD

Professor, Cornell Medical College
Burke Research Institute

Oxidative Processes and Signal Transduction in Alzheimer's Disease:

Insights from Brains, Peripheral Cells and Animal Models

15 March 2000
3:00-4:00 pm



Mitochondria in a neuron

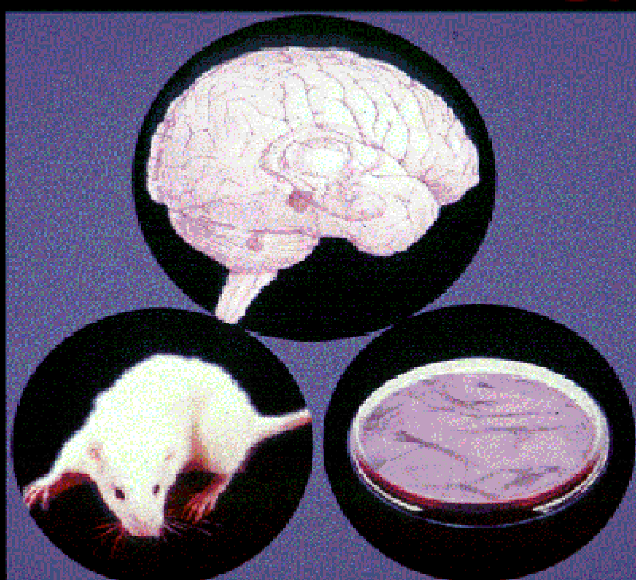


Figure and Background courtesy of Gary Gibson.

Jack Masur Auditorium, Clinical Center, NIH

Sponsors: NIH Mitochondria (MIG) and Clinical Research (CRSIG) Interest Groups

For Information/Special Accommodation Needs call Hilda Madine @ 301-594-5595

The Minisymposium

Mitochondria: Interaction of Two Genomes

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Lecture and Minisymposium for Researchers, Clinicians and the Public

1. Boles, Richard Le,Samantha T. Chaudhari,Divya Higashimoto,Tomoyasu Ito,Masamichi
Division of Medical Genetics, Childrens Hospital Los Angeles, Box 90, 4650 Sunset Blvd., Los Angeles, CA 90027 USA rboles@chla.usc.edu

Transient multisystem dysfunction or static neurodevelopmental disease in a subset of children with clinical mitochondrial dysfunction characterized by sequence heterogeneity of the mitochondrial DNA control region

Children with clinical mitochondrial dysfunction are frequently encountered in tertiary practice. Many of these children have affected matrilineal relatives in which mtDNA mutations are not found on standard analysis, suggesting the presence of novel variants. Mutation of the mitochondrial DNA control region (mtDNA-CR), an area involved in replication and transcription, is a plausible mechanism for disease, although no cases are reported. We used temporal temperature gradient gel electrophoresis (TTGE) to assay the mtDNA-CR for heteroplasmy in 85 children with clinical mitochondrial dysfunction and in 100 controls. We found heteroplasmic single nucleotide changes (known polymorphisms, if homoplasmic) in the hypervariable regions (HV1&2) of the mtDNA-CR in 11 affected children and in 0 controls. HV1 homoplasmic single nucleotide substitutions were 5 fold more frequent in the 11 cases versus haplogroup-matched controls, indicating that, in many cases, the defect was maternally transmitted over at least a few generations. Children with mtDNA-CR heteroplasmy were characterized by the presence of transient non-neurological tissue dysfunction and/or static and variable neurodevelopmental disease. Cardiomyopathy, cyclic vomiting and birth defects are common. The apparent inheritance pattern is maternal or sporadic in cases with clinical improvement, and their mothers, but not their fathers, have mtDNA-CR heteroplasmy. Two neonatal-onset fatal cases show apparent autosomal recessive inheritance, and, in one, mtDNA-CR heteroplasmy is present in both parents. Our cases likely suffer from at least 2 relatively common, novel disorders: a severe, early onset, autosomal recessive variant, and a variable maternally-inherited variant. Pathology is likely mediated by an increased mtDNA mutation rate.

2. Cohen Bruce H. Hoppel,Charles&&2
Neurology, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195 ~2~Clinical Pharmacology, Department of Veteran Affairs Medical Center, 10701 East Blvd. Cleveland, OH 44106
bhc2neuro@aol.com

Making Sense of Respiratory Chain Assays and Clinical Mitochondrial Disease

A variety of laboratory tests may be used to confirm the diagnosis of a mitochondrial cytopathy. These include pathologic findings of ragged red (or ragged blue) fibers, ultrastructural changes such as paracrystalline inclusions, or a genetic finding of a pathologic point mutation, deletion or duplication of mtDNA. Muscle, and occasionally liver, is often studied in order to better define the exact biochemical defect. Electron transport chain activity of complexes I, II, III and IV can be determined on homogenized tissue or mitochondria isolated from that tissue using spectrophotometric techniques. In addition, polarographic assay of fresh mitochondria tests the functional activity of the intact mitochondria by measuring oxygen consumption under varying respiratory states following addition of different substrates. Polarography can determine the activity of complexes I-V, the coupling of oxidation and phosphorylation, and estimate the activity of PDH,CPT and ANT. Polarography and spectrophotometry are complimentary techniques that provide different information about mitochondrial function. Using case studies, the benefits and limitations of both techniques will be discussed.

3. Kaye Edward Biochemical Genetics, Children's Hospital of Philadelphia, Philadelphia kaye@email.chop.edu

Mitochondrial Diseases Presenting as Leukoencephalopathy

Leukoencephalopathies are caused by a number of metabolic diseases such as lysosomal storage diseases, peroxisomal diseases, organic acid disorders, and myelin protein deficiencies. Many mitochondrial disorders have white matter changes although we usually consider these diseases as affecting basal ganglia and cortex. We present 4 cases in which a primary white matter disorder was initially suspected but latter proved to be a mitochondrial disease. One child presented shortly after birth and was noted to have a Complex I deficiency with hypomyelination and thalamic changes. A second child presented at age 4 months with failure to thrive, developmental delay, and an abnormal white matter on MRI consisting of diffuse hypomyelination and cystic regions in the periventricular white matter. A persistent serum lactic acidosis was also discovered due to a Complex III deficiency. The third cases presented for severe developmental delay, ethylmalonic aciduria, and MRI showed T2 high signal intensity in brainstem, cerebellum, and centrum semiovale. The MR spectroscopy demonstrated increased lactate in CSF and basal ganglia. A fourth child demonstrated severe failure to thrive and a lack of normal development at 4 months of age. MRI showed cystic changes in brainstem, spinal cord, and cerebellum in addition to diffuse hypomyelination. Lactate was noticed on MR spectroscopy (MRS). Biochemical studies revealed a defect at the level of pyruvate and a-ketoglutarate dehydrogenases. These four cases demonstrate the importance of identifying necrotic areas in white matter and of determining lactate on brain MRS to suspect a mitochondrial cause for a leukoencephalopathy.

4. Haas Richard Barshop,Bruce&&1,3 Naviaux,Robert&&1,4 Nyhan,William&&1,3 UCSD Mitochondrial and Metabolic Disease Center, 214 Dickinson Street Box #8467, San Diego, CA 92103~2~Department of Neurosciences, UCSD, La Jolla, CA~3~Department of Pediatrics, UCSD, La Jolla, CA~4~Department of Medicine, UCSD, La Jolla, CA rhaas@ucsd.edu

Results of the UCSD Open Label Dichloroacetate Trial in Congenital Lactic Acidosis

Dichloroacetate (DCA), inhibits pyruvate decarboxylase kinase, and lowers lactic acid levels. The effects of DCA were studied in a cohort of patients with mitochondrial disease and plasma or CSF lactic acid elevation (>3mM). Patients were administered 2 test doses of intravenous DCA (50 mg/kg) and 38 who showed a lactate lowering effect continued with oral treatment at a dose of 25 mg/kg twice daily. The 23 males and 15 females ranged from 7 months to 51 years. Fourteen patients had the clinical phenotype of Leigh syndrome and 13 had a MELAS phenotype with an identified A3243G mutation in 10, and T3271C in 1. Electron transport or mtDNA mutations were identified in 32 (84%) of patients. There was apparent overall improvement in 17 patients. Beneficial effects included regaining ambulation in 5 subjects. There were 9 deaths, during or soon after stopping treatment. Twenty-six continued DCA treatment for 1 year or longer. New complaints of peripheral neuropathy occurred in 6 patients (16 %), dose reduction improved symptoms. Nerve conduction studies worsened in 12 patients studied. Neuropathy occurred despite supplementation with 100 mg/day of thiamine. Side effects leading to discontinuation of treatment or dosage reduction included; neuropathy, sedation, increased ataxia, and mild elevation of liver function tests (in 1 patient, later attributed to valproate). Whilst this open label trial is continuing, most new patients are now recruited into a blinded crossover high dose / low dose study of 2 years duration. DCA treatment appears most clearly beneficial in patients with MELAS.

5. Warden Craig Dept of Pediatrics, Section on Neurobiology, Physiology and Behavior, UC Davis, Davis, CA 95616 chwarden@ucdavis.edu

Uncoupling Proteins and the Trail to Complex Mitochondrial Phenotypes

Obesity results whenever energy intake (calories consumed) exceeds energy expenditure (calories used). Although variations in food intake should be able to cause obesity, there is surprisingly little evidence that obese people overconsume calories. The relationship between energy expenditure and obesity is complex. Calories are expended to maintain the resting metabolic rate (RMR), for exercise, as the thermogenic effect of food, and during non-exercise activity thermogenesis (fidgeting). Since RMR accounts for 50 - 70% of all calorie expenditure, then low RMR could cause obesity. However, obese people have higher resting metabolic rates than lean people do. In contrast, low resting metabolic rates are correlated with weight gain in longitudinal studies. Thus, variations in RMR may contribute to weight gain. Furthermore, since RMR is itself partly genetic, then it is important to identify the specific underlying causes of individual variation in RMR. The mitochondrial uncoupling proteins are candidate obesity genes because they may decrease the yield of ATP from each calorie consumed. Uncoupling protein-1 (UCP1) is a fatty acid activated proton carrier that partially uncouples the mitochondrial proton gradient. Ablation of UCP1 by knockout in mice results in animals that cannot thermoregulate - that is, they rapidly loose body temperature when placed at 4°C. However, UCP1 knockout mice do not exhibit any obesity phenotypes, perhaps because it is only expressed in brown adipose tissue. In contrast, the mitochondrial UCP2 and 3 proteins are expressed in adipose and skeletal muscle and may have much greater quantitative influence on RMR and obesity.

6. Smith Mary E. Gerschenson,Mariana&&2 Division of AIDS, NIAID, NIH, Rockville, MD~`~Carcinogen-DNA Interactions Section, LCCTP, DBS, NCI, NIH, Bethesda, MD MESmith@niaid.nih.gov

Clinically Induced Mitochondrial Toxicity: A Panel Discussion

Clinically induced mitochondrial toxicities have been demonstrated to be due to the exposure of drugs, like nucleoside analogs and aminoglycoside antibiotics. Nucleoside analog type drugs, like fialuridine or zidovudine, have been demonstrated to cause hepatic failure and muscle myopathies in patients, respectively. These drugs are incorporated into mitochondrial DNA (mtDNA) and lead to a depletion of mtDNA resulting in a decrease in the mitochondrial polypeptides involved in ATP synthesis. Aminoglycoside antibiotics (streptomycin, gentamycin) have been demonstrated to cause irreversible, profound, high frequency sensorineural deafness in hypersensitive patients. This ototoxicity has been associated with a mtDNA mutation in the 12S ribosomal gene, thus effecting mitochondrial gene transcription. Today's panel discussion will discuss current research in this field.

7. Shoffner John Children's Healthcare of Atlanta

Complex I Defects in Oxidative Phosphorylation Disease

1. Complex I defects are one of the most commonly reported oxidative phosphorylation defects. Significant confusion exists concerning the clinical relevance of this biochemical abnormality.
2. The goals of the talk include recognition of recent diagnostic approaches that can be helpful in assessing patients who are being tested for Complex I defects.
3. Clinically relevant biochemical and genetic techniques for assessment of Complex I defects will be reviewed.
4. Physicians will obtain a better understanding of genetic mutations (nuclear DNA and mtDNA) that produce Complex I defects.
5. The issue of primary versus secondary defects in Complex I will be discussed in terms of patient diagnosis

8. Cortopassi Gino L. Cavelier1 H Collins2 MF Seldin2 ML Savontaus3 M McGrogan4, GA Cortopassi1 1Dept. Molecular Biosciences, 1311 Haring Hall, University of California, Davis, CA 95616; 2Rowe Program in Genetics, Departments of Biological Chemistry and Medicine, University of California, Davis, Davis, CA 95616; 3Department of Medical Genetics, Institute of Biomedicine, University of Turku, Finland; 4Layton Biosciences, Gilroy, CA

Novel cellular models of mitochondrial neurological disease

Mitochondrial genetic disease frequently affects neural cells. However the pathogenetic mechanism(s) of mitochondrial molecular disease have been mainly studied in non-neuronal cells, most frequently osteosarcoma cell lines. In an attempt to generate a more relevant cellular model system for the study of the neuropathogenetic mechanisms in mitochondrial genetic disease, we have demonstrated the transfer of mutant mtDNAs from patient lymphoblasts to a pre-neuronal cell line N tera2 (Nt2). Restriction digests were consistent with transfer of patient mtDNA, and homoplasmic lines were identified. A potential issue was the contamination of transmitochondrial cell lines with patient nuclear DNA, but assay of >50 variable microsatellite loci was inconsistent with nuclear contamination by donor cells. mtDNA and nuclear DNA copy number were similar in control and transmitochondrial cell lines. Nt2 cells bearing mutant mitochondria were differentiable with retinoic acid into postmitotic cells with a neuronal morphology. Such cells could represent a useful model in which to study the neuropathogenetic mechanism(s) of mtDNA mutations.

9. Naviaux Robert The Mitochondrial and Metabolic Disease Center, University of California, San Diego School of Medicine, 200 West Arbor Drive, San Diego, CA 92103-8467

Pyrimidine Therapy of Mitochondrial Disease

Background. The synthesis of pyrimidines is linked to oxidative phosphorylation through the mitochondrial inner membrane protein, dihydroorotate CoQ oxidoreductase (E.C. 1.3.99.11) (DHO-QO). This enzyme catalyzes the fourth step in the de novo synthesis pathway, the oxidation of dihydroorotate to orotate, and uses CoQ10ox as the electron acceptor. Both inborn and acquired disorders of mitochondrial metabolism may lead to secondary pyrimidine deficiency states because of shortfalls in de novo synthesis. Triacetyluridine (PN401; Pro-Neuron, Inc., Gaithersburg, MD) is a product of uridine that is rapidly converted to uridine upon oral administration. The acetylation of uridine improves its bioavailability 5-10 fold over unmodified uridine. **Patients.** Five patients with Leigh syndrome secondary to nuclear defects (complex I, pyruvate dehydrogenase, and cytochrome c oxidase), two patients with Leigh syndrome secondary to the NARP (T8993G and T8993C) mutation in mitochondrial DNA, one patient with mitochondrial encephalomyopathy and 3-hydroxyisobutyric acidemia, one patient with a MNGIE-like phenotype, and one patient with Alzheimer dementia. Four of the 10 patients also had significant renal tubular acidosis. **Treatment.** 2 g/m2 PO three times daily for one month to two years. Design. Open label pilot study of children and adults with a variety of mitochondrial disorders. Results. Four of four (100%) patients with renal tubular acidosis showed either complete correction or reduction in daily oral bicarbonate requirement. Three of five (60%) patients with Leigh syndrome resulting from nuclear defects showed significant improvements as measured by reduced frequency of infections, hospitalizations or urgent outpatient appointments, new developmental milestones, or decreased ataxia. The two patients with Leigh syndrome resulting from the NARP mutation also showed improvement in the first 3 months of therapy, then developed respiratory tract infections during the first winter and electively discontinued therapy. Quantitation of the NARP mutation before and after 3 months of therapy showed no increase in the level of heteroplasmy present in the blood. The patient with a MNGIE-like phenotype had an increase in exercise tolerance and reduction in seizures. The patient with Alzheimer dementia had a significant decrease in episodes of delirium, combative behavior, and periods of depression, and showed

improvements in activities of daily living. There was a 5 point decline in Mini Mental Status Exam score (from 24 to 19) during a 2 month drug holiday and a 4 point recovery upon reinstitution of PN401. Southern blot analysis showed no induction of mtDNA deletions in the blood of patients treated for at least 3 months. **Conclusions.** PN401 therapy was associated with symptomatic improvement in 6 of 10 (60%) patients with mitochondrial disorders enrolled in this pilot study. Prospectively randomized, double-blind, placebo, and cross-over controlled studies will be required to evaluate effects of PN401 therapy on long term prognosis of mitochondrial disease.

10. Kelley Richard Kennedy Krieger Insitute, Baltimore, MD~`~Johns Hopkins University kelle_ri

Abnormalities of Mitochondrial Metabolism in Children with Autistic Spectrum Disorders

Although developmental delay is a common characteristic of children with disorders of mitochondrial metabolism, classical autism, Asperger syndrome, and pervasive developmental disorder (PDD) have not commonly been associated with mitochondrial disease. Because our institution serves a large number of children with developmental disabilities, we have had the opportunity to diagnose many metabolic diseases among children with autistic spectrum disorders, including defects of organic acid, sterol, and mitochondrial metabolism. Among these, mitochondrial disease is the most common diagnostic category and represents a clinically significant fraction of autistic children. Although we find a variety of autistic phenotypes to have associated mitochondrial abnormalities, the most common is nonspecific PDD, typically of a form that manifests language and cognitive regression or stagnation during the second year. Most surprising among multiplex families is that the biochemical and clinical makers of mitochondrial disease often segregate in an autosomal dominant manner. Although no molecular lesion has yet been found in the autosomal dominant families, the biochemical findings are most consistent with abnormal mitochondrial complex I activity. Moreover, when identified below the age of two years, affected children often respond to therapy designed to augment complex I activity. We propose that, like the basal ganglia, areas of the brain important in language development and personal social interaction are especially vulnerable in the first two years to injury mediated by defects of mitochondrial energy metabolism, and that early and careful evaluation of autistic children for these more subtle mitochondrial disturbances may rescue them from more severe brain injury.

11. Zullo Steve Eisenstadt, JM 2 Parks, WT 3 Fenton, W 2 Merril, CR 1 Lab Biochemical Genetics, NIMH, Bethesda, MD 20892, USA; Yale U 2; NCI 3 zullo@helix.nih.gov

Gene Therapy of the Mitochondrial Genome, a Tale of Two Genomes

We have successfully transferred a vertebrate mitochondrial DNA (mtDNA) gene to the nuclear genome in order to ameliorate the effects of a mtDNA defect. This gene therapy technology, using the mtDNA-encoded ATPase6 mutant gene of a chinese hamster ovary (CHO) cell line that confers oligomycin resistance (oli-r), involved the conversion of the mitochondrial DNA code to the nuclear DNA universal code and attachment of the ornithine transcarbamylase (OTC) mitochondrial targeting sequence ("universal OTC ATPase6"-UOATP6 construct). UOATP6 is thus constructed for transformation into the nuclear genome, since there is little information on mitochondrial genome recombination, with the expressed protein being targeted to the mitochondria. The UOATP6 construct was electroporated into CHO cells sensitive to oligomycin (oli-s). Transformed CHO cell lines are capable of growing in up to 0.1 ug/mL oligomycin while untransformed sensitive CHO cells are eliminated at only 0.001 ug/mL oligomycin. In an oligomycin selected transformed cell line, we localized the transformed construct on the centromeric region of the p arm of the chinese hamster chromosome 1. We also recently transformed human cybrids containing a mutation in the human mtDNA-encoded ATPase6 gene (T8993G mutation) in 100% of the mitochondrial genomes with UOATP6, the CHO oli-r ATPase6 construct described above. The T8993 mutation results in a condition known as Leigh Syndrome, Subacute Necrotizing Encephalopathy (SNE), or

NARP (neuropathy, ataxia, retinitis pigmentosa). Cybrids transformed with UOATP6 are growing in 0.1 ug/mL oligomycin, while the untransformed cybrids are eliminated in 0.001 ug/mL oligomycin. These experimants suggest it may be possible to ameliorate both the neuropathological effects of somatic mitochondrial DNA mutations, and the effects of similar age-related mitochondrial mutations that have been observed in the brain.

12. Weiner Henry Biochemistry Department Purdue University West Lafayette IN 47907-1153 weiner@biochem.purdue.edu

Can Mitochondrial Import be a Co-translational Event?

Dogma states that mitochondrial protein import is a post translational event. That is, the entire precursor protein is translated and is free in the cytosol prior to import. The strongest data supporting this supposition is that import does occur in an in vitro assay. We tried to find evidence to support the notion that import could be a co-translational event by transforming HeLa cells with the green fluorescent protein (GFP). To this protein we fused two different signals. At the N-terminal end was attached a typical mitochondrial leader sequence that we show directs the carrier protein to mitochondria. Alternatively, at the C-terminal end was an ER-targeting signal that we found to bring to protein to ER. When both signals were on the carrier, protein was found only in mitochondria and none was detected in the ER. If import were a post translational event, protein would have been found in both organelles. We further showed that the mitochondrial leader from aldehyde dehydrogenase was destroyed rapidly by HeLa cell cytosolic extracts. Thus, it appears, at least when the leader is from aldehyde dehydrogenase, that import can be a co-translational event. Alternatively, it is possible that the entire precursor is translated, but is not free, for if it were, the leader would have been destroyed and the carrier protein folded. Either event would have prevented import from occurring. Currently we are testing the model with leaders from other proteins. The research was support in part by a grant from NIAAA.

13. Kaplan Barry Gioio,Anthony 1 Eyman,Marilenna 2 Zhang,Hengshan 1 Wen,Huajie 1 Guiditta,Antonio 2 Labratory of Molecular Biology, NIMH, NIH, Bethesda MD.~`~Department of General Physiology, University of Naples, Naples, Italy Kaplanb@intra.nimh.nih.gov

Synthesis of Nuclear Encoded Mitochondrial Proteins in the Presynaptic Nerve Terminal

Previously we demonstrated the presence of a heterogeneous population of mRNAs and biologically active polyribosomes in the giant axon and presynaptic nerve terminals of the photoreceptor neurons in squid. Recent studies employing differential mRNA display methodology and RT-PCR have established the presence of mRNAs for nuclear encoded mitochondrial proteins to include: COX17 and COQ7. The mRNA encoding HSP-70, a molecular chaperone known to be involved in mitochondrial protein import, has also been identified. These findings suggest that protein required for the maintenance of mitochondrial function is synthesized locally in the axon and presynaptic terminals. To evaluate this hypothesis, synaptosomes prepared from squid optic lobe were pulse- labeled with 35S-methione. Translational inhibition by either cycloheximide or chloramphenicol was used to discriminate between endogenous mitochondrial protein synthesis and extramitochondrial synaptosomal synthesis. Cycloheximide (which selectively inhibits eukaryotic protein synthesis) inhibited synaptosomal protein synthetic activity 70-75%, where as chloramphenical (a selective inhibitor of mitochondrial protein synthesis) reduced incorporation by 20-25%. Electrophoretic analysis confirmed that these agents inhibited the synthesis of two different sets of synaptosomal proteins. Analysis utilizing differential centrifugation to isolate a mitochondrial-enriched fraction, established that some chloramphenical-resistant radiolabeled proteins were present in the mitochondrial fraction, suggesting that they had been transported into these organelles. Taken together, these findings support the hypothesis that local synthesis of proteins involved in the maintenance and regulation of mitochondrial activity is occurring in distal regions of the neuron, a finding that focuses

attention on the intimacy of the relationship between the presynaptic nerve terminal and its energy generating system.

14. Fernandez-Salas Ester Levy,Joshua1 Pathak,Kamal1 Cheng,Christina1 Weinberg,Wendy C2 Yuspa,Stuart H.1 Lab. of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH, Bethesda, MD 20892.~`~Oral and Pharyngeal Cancer Branch, NIDCR; NIH, Bethesda, MD 20892. esterf@nih.gov

mtCLIC, a TNF-a and p53-regulated mitochondrial chloride channel protein, induces apoptosis through mitochondrial alteration

mtCLIC is a chloride channel protein that belongs to the CLIC family of intracellular chloride channels. Expression of mtCLIC mRNA and protein is upregulated by p53 and TNF-a. By cell fractionation both the fusion protein mtCLIC-GFP and the endogenous mtCLIC are localized to the cytoplasm and mitochondria. Fractionation of rat liver and subsequent purification of mitochondria by sucrose gradient confirmed this localization pattern. Overexpression of mtCLIC reduced mitochondrial membrane potential as measured by Mitotracker staining and flow cytometry analysis. This suggests that an organellular chloride channel regulated by p53 and TNF-a can alter mitochondrial function. Transfection of mtCLIC into keratinocytes induces apoptosis as shown by chromatin condensation, propidium iodide labeling of living cell nuclei, and cell surface annexin V labeling. This apoptotic pathway can be blocked by the caspase inhibitor Z-VAD, suggesting that overexpression of mtCLIC leads to caspase activation. In addition, release of cytochrome C into the cytoplasm can also be detected in cells overexpressing mtCLIC. Treatment of keratinocytes with the DNA damaging agent etoposide (VP-16) results in apoptosis that correlates with an up-regulation of mtCLIC protein. Our data indicate that mtCLIC is a p53- and TNF-a regulated gene that may be involved in mediating apoptosis by several stress-response pathways through mitochondrial alteration and caspase activation.

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MITOCHONDRIA, METALS AND ALZHEIMER DISEASE

Cell bodies of neurons at risk of death in Alzheimer disease (AD) have increased lipid peroxidation, nitration, free carbonyls, and nucleic acid oxidation. These oxidative changes are uniform among neurons and irrespective of whether the neurons display neurofibrillary tangles. Considering this localization of damage, we investigated abnormalities that may initiate and promote neuronal oxidative damage. First, we investigated whether mitochondrial abnormalities might be the source of reactive oxygen species yielding perikaryal oxidative damage. In neurons susceptible to AD, normal and 5kb-deleted mtDNA was greatly increased and was restricted to damaged mitochondria. The importance of such mitochondrial abnormalities to oxidative stress was indicated by a high correlation coefficient between mtDNA increase and RNA oxidative damage ($r = 0.93$). We suspected that abnormal mitochondria supply a key reactant that once in the cytoplasm, releases radicals. One such reactant, H_2O_2, abundant in mitochondria, can react with iron via the Fenton reaction to produce hydroxyl radicals. We found that redox-active iron is associated with vulnerable neurons. Interestingly, after removal of iron with deferoxamine, iron could be rebound to re-establish lesion-dependent catalytic redox reactivity. Characterization of the iron-binding site suggests that binding is dependent on available histidine residues and on protein conformation. Taken together with our previous studies showing abnormalities in the iron homeostatic system including heme oxygenase, iron regulatory proteins 1 and 2, ceruloplasmin and dimethylargininase, our results indicate that iron misregulation could play an important role in the pathogenesis of AD. Therefore, chelation therapy may be a useful therapeutic approach.

16. de Souza Pinto Nadja Hogue,Barbara A. Bohr,Vilhelm Lab. Molecular Genetics, GRC, NIA, NIH. 5600 Nathan Shock Dr., Baltimore, MD 21224 souzan@grc.nia.nih.gov

Changes in mammalian mitochondrial DNA repair during the aging process

Mitochondria play a critical role in the etiology of the aging processes. These organelles are exposed to oxidative stress and accumulate oxidative DNA damage with age. However, the role that DNA repair plays in this process is still unclear. Mammalian mitochondria can remove DNA lesions that are repaired by the base excision repair (BER) pathway, and many BER enzymes have been isolated from mitochondria. Here, we report the utilization of mice liver mitochondria (MLM) as a model system to study changes in mtDNA repair activities with age and in response to chronic stresses. MLM are very advantageous for such studies, since: a) a high yield of high purity material can be obtained from one single animal, b) mice can be easily subjected to stress regiments and c) a wide range of transgenic animals are now available. We measured the activities of a series of BER enzymes in MLM extracts obtained from young and old C57/Bl6 mice, MnSOD-/+ heterozygotes, and mice treated with the succinate dehydrogenase inhibitor 3-nitropropionic acid (3-NPA). We found the activity of mtODE (mitochondrial oxidative damage endonuclease) to be elevated in old compared to young animals. In contrast, mtODE activity was similar in MnSOD+/- and in wt animals, and did not change after administration of 3-NPA. In conclusion, these results suggest that DNA repair increases with the normal aging process in mice. Those changes cannot be solely explained by the exposure to oxidative stress since two different models of oxidative stress failed to induce age-associated changes.

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Quantitative detection of Apoptosis markers by fluorescent RT-PCR

Amplifluor™ Gene Systems offer a closed-tube fluorometric detection format for measuring mRNA levels using quantitative reverse transcription polymerase chain reaction (QTR-PCR). QRT-PCR has become a very popular technique as an extremely sensitive method for detecting and quantifying of mRNA expression. However, due to the exponential nature of the amplification reaction and multiple factors affecting the amplification efficiency, careful optimization of the assay is required to obtain reliable results. Furthermore, necessity of post-PCR sample processing and potential carry-over contamination of amplification products make routine applications of the methodology difficult. Amplifluor™ Gene Systems allow rapid, reproducible high throughput determination of cDNA expression levels without post-PCR manipulation. Amplifluor™ Gene Systems utilize unique, state-of-the-art technology based upon the incorporation of energy transfer-labeled primers into double stranded DNA. As Amplifluor™ primers are incorporated an amplicon during PCR, there is a corresponding increase in fluorescence. Unincorporated primers have an extremely low fluorescence signal, eliminating the need to purify the amplified product prior to detection or quantification. Amplifluor™ generated fluorescence signal can be measured during the reaction (real-time) or at its endpoint. We currently have Amplifluor™ Gene Systems for the following apoptosis-related genes: bax-á, bcl-2, bcl-X (Long) and Fas. In addition, we will soon be releasing a new line of cytokine mRNA quantification kits.

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Mitochondrial Diseases of Man and Mouse

A variety of degenerative diseases have now been shown to be caused by mutations in mitochondrial genes encoded by the mitochondrial DNA (mtDNA) or the nuclear DNA (nDNA). Mitochondria generate most of the cellular energy by oxidative phosphorylation (OXPHOS), creating most of the toxic reactive oxygen

species (ROS) as a by-product. Genetic defects that inhibit OXPHOS also cause the redirection of OXPHOS electrons into ROS production, thus increasing oxidative stress. A decline in mitochondrial energy production and an increase in oxidative stress can impinge on the mitochondrial permeability transition pore to initiate programmed cell death (apoptosis). The interactions of these three factors appear to play a major role in the pathophysiology of degenerative diseases. The importance of these factors has been demonstrated using mouse models of mitochondrial disease. A mtDNA mutation imparting chloramphenicol resistance to mitochondrial protein synthesis has been transferred into mice, resulting in growth retardation. A nDNA mutation which inactivates the heart-muscle isoform of the adenine nucleotide translocator (Ant1) results in mitochondrial myopathy and cardiomyopathy; inhibition of OXPHOS; induction of ROS production; and the compensatory up-regulation of energy, anti-oxidant, and apoptosis gene expression. A nDNA mutation that inactivates glutathione peroxidase-1 results in increased mitochondrial H2O2 production and reduced growth rate. Finally, a nDNA mutation that inactivates the mitochondrial Mn superoxide dismutase results in death at 8 days due to a dilated cardiomyopathy, which can be ameliorated by catalytic anti-oxidant treatment. Thus, degenerative diseases do result from interaction of mitochondrial energy deficiency, oxidative stress, and apoptosis.

19. Gonzalez Martín Woodgate,Roger Section on DNA Replication, Repair, and Mutagenesis(SDRRM)|SDRRM, NICHD, 9000 Rockville pike, Bethesda, MD., 20892-2725, USA| gonzalez@box-g.nih.gov

Regulation of DNA trans-lesion synthesis in Escherichia coli by proteolysis

Most DNA trans-lesion synthesis in Escherichia coli is dependent upon the UmuD and UmuC proteins. Perhaps as a consequence, the activity of these proteins is exquisitely regulated. The intracellular level of UmuD and UmuC is normally quite low but increases dramatically in lon- strains, suggesting that both proteins are substrates of the Lon protease. UmuD', the mutagenically active cleavage product of the proprotein UmuD, is relatively insensitive to Lon activity, yet in the presence of full length UmuD, UmuD' is subject to ClpXP-mediated degradation. We will show a region of UmuD that is essential for efficient degradation by the Lon protease; moreover, this same region of UmuD will be shown to be vital for ClpXP-mediated degradation of UmuD'.

20. Grossman Lawrence Center for Molecular Medicine & Genetics, Wayne State University School of Medicine, 540 E. Canfield Ave., Detroit, MI 48201
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Nuclear COX subunits: promoter regulation and evolution

Cytochrome c oxidase (COX), which catalyzes the terminal step in electron transport, is composed in mammals of three mtDNA-encoded subunits and ten nuclear-encoded subunits. We have been studying the regulation and evolution of selected nuclear subunits. The diversity of promoter elements in different COX subunits suggests integration at a higher level of circuitry. We have recently isolated the tissue-specific subunit VIIa-H gene, COX7AH. In examining its transcriptional regulation we find that muscle-specific elements within 600 bp preceeding the start of transcription are the major regulatory motifs, and are studying their interaction. We are also examining interactions among the previously identified elements in the COX7C core promoter. We find that binding YY1 at one of its two sites strongly inhibits binding at the other, consistent with an induced bending model for interaction. Because selected adaptive changes in the biochemical machinery for aerobic energy metabolism may be among important molecular changes in our evolutionary history, we investigated the evolution of COX subunits in primates and noted rapid evolution in human of one, COX IV. Subsequent phylogenetic analysis of COX4 revealed a pattern of an accelerated nonsynonymous substitution rate in the ancestors of higher primates followed later by a decelerated rate, a pattern suggestive of positive selection for adaptive amino acid replacements. Phylogenetic analyses of gene sequence data for other COX subunits, and for other proteins active in the respiratory chain, have also

provided evidence for an accelerated nonsynonymous substitution rate in our earlier anthropoid ancestors. (Supported by NIH and NSF.)

21. Isaya Grazia Pediatric Research; Mayo Clinic & Foundation; Rochester MN 55905

Functional Analysis of Frataxin

Iron is essential for many cellular functions, and yet free iron is highly toxic and extremely insoluble at physiologic pH under aerobic conditions. Hence iron-storage proteins have evolved. Ferritin, a well known iron-storage protein, can sequester up to 4500 atoms of iron in a non-toxic, bio-available form, and not surprisingly it is highly conserved in bacteria, plants, and animals. Although (-purple bacteria, probably the closest living relatives to mitochondria, possess at least two iron-storage proteins (i.e. ferritin and bacterioferritin), ferritin is only found in the cytoplasm of plant and animal cells while no storage proteins have thus far been identified in mitochondria. This is very surprising considering that substantial amounts of iron are required in mitochondria for heme and iron-sulfur cluster biosynthesis, and that uncomplexed iron together with superoxide and hydrogen peroxide can generate highly toxic hydroxyl radicals. It is probable that most iron is immediately incorporated into heme or iron-sulfur clusters as it crosses the inner mitochondrial membrane. It seems logical, however, that mitochondria should also have some mechanism to sequester, store, and "chaperone" iron. We propose that mYfh1p fulfils this function in S. cerevisiae.

22. Singh Keshav Barbara Sigala Hashmat Sikder Christine Schwimmer Grace Kim
Johns Hopkins Oncology Center, Johns Hopkins School of Medicine, Bunting-Blaustein Cancer Research Building, 1650 Orleans Street Baltimore, Maryland 12131-1000

In vivo evidence for DNA repair in mitochondria

In the past decade it has been clearly recognized that mutations in mitochondrial genome are involved in pathogenesis of a variety of diseases including ischemic heart disease,cardiomyopathies, adult-onset diabetes, degenerative diseases such as Parkinson's disease, Huntington's disease and other neurological disorders. Accumulation of somatic mutations of mitochondrila genome in heart, brain, liver and other postmitotic tissues appears to be a constant feature of normal aging in all vertebrates thus far examined. Human mitochondrial genome contains no introns and has no protective histones or nonhistone proteins. Mitochondria are the major source of endogenous reactive oxygen species (ROS) due to normal oxidative phosphorylation. According to estimates, cells produce 10 million ROS per mitochondrion per cell per day. As a result, mitochondrial genome appears to be extremely vulnerable to damage by ROS. Yeast Saccharomyces cerevisiae is an excellent eukaryotic model system. The entire genome of this organism has been sequenced and nuclear DNA repair mechanisms between the yeast and human are conserved. Therefore, we have undertaken a systematic analysis of DNA repair in mitochondria using S. cerevisiae as a model system. We report that DNA repair protein(s) that repair ROS induced damaged bases in the nucleus repair damaged bases from the mitochondrial genome. We provide in vivo evidence that inactivation of DNA repair gene(s) leads to mutations in mitochondrial genome.

23. Gropman Andrea Neurogenetics Branch, NINDS, 10 Center Drive, Building 10 Room 3B04, Bethesda, MD 20892; Neurology Department, Children's National Medical Center, Bethesda, MD 20892 301-435-9324 301-480-3365 Neurogenetics agropman@nhgri.nih.gov|

Mitochondrial Support Groups

This session, geared towards patients, families and health care providers dealing with mitochondrial disorders, will focus on the many aspects detailing the purpose, formation and maintainence of disease specific support groups. The facilitator will draw on experiences from her own patient population which is comprised of patients and families in early, mid- and late stages of diagnosis, as well as enlist the expertise of members of the UMDF national support group executive office to facilitate open discussion. Common themes to be explored include history of support groups, what support groups can achieve, how to start a group, fund raising, parental notions and expectations of the similarity and certainty of other conditions that are more highly publicized in the lay press, parental advocacy, obtaining special medical and educational services, expectations, fears about life expectancy, and aspects of grief, loss, uncertainty and isolation. Discussion wil also focus on Participant will be encouraged to share their views in an open discussion that will follow the presentations.

24. Gerschenson Mariana Carcinogen-DNA Interactions Section, Laboratory of Cellular Carcinogenesis and Tumor Promotion, DBS, NCI, NIH, Bethesda, MD gerschem@exchange.nih.gov

Mitochondrial Endonuclease G

Endonuclease G (Endo G), a 27 kDa homodimer polypeptide, is a potent Mg+2 dependent endonuclease that at low salt concentrations will extensively degrade duplex DNA, single strand DNA and RNA. At moderate ionic strength, Endo G preferentially nicks 12 guanine and complementary cytosine [conserved sequence box II (CSB-II)] residues upstream of the origin of mitochondrial DNA (mtDNA) replication. Endo G is not a nuclear contaminant. Compartmentalization studies in rat heart and calf thymus have shown guanine nicking activity located predominantly in sucrose gradient purified mitochondria, with low levels in the nuclei. The Endo G activity that has been detected in the nuclei is identical to the mitochondrial enzyme in both nucleotide sequence specificity and by amino acid sequence analysis. The function of Endo G in vivo is probably to facilitate the removal of oxidative damaged mtDNA. Endo G specific activity (per mg of mitochondrial protein or mtDNA circle) has been found to be 200-times higher in rat heart (an organ with the highest rate of oxygen consumption) than in the liver or spleen. Also, Endo G activity in the developing rat heart is elevated significantly at birth when oxygen consumption increases. These variations in the specific activity of the endonuclease, when considered along with other properties of the enzyme suggest that the endonuclease serves a role in the removal of oxidative damaged mtDNA.

25. Mannella Carmen Wadsworth Center, Albany NY, 12201-0509

NEW INSIGHTS INTO MITOCHONDRIAL STRUCTURE FROM EM TOMOGRAPHY

Tomography of conventionally fixed and embedded mitochondria indicates that the standard model for mitochondrial structure is incorrect. In mammalian mitochondria, the cristae connect through narrow tubular regions to the inner membrane (IM) surface and to each other [1-3]. This design may lead to formation of lateral gradients of important metabolites (like ADP) within the cristae. (See poster by Moraru et al.) Changes in IM morphology associated with varying osmotic or metabolic states cannot be explained by simple folding/unfolding and probably involves membrane fusion events. In yeast mitochondria, large-scale IM swelling (activation of the permeability transition), followed by osmotic shrinkage, leads to

formation of vesicular cristae with no connections to the external compartment (collaboration with D. Pfeiffer, Ohio State Univ.). This structural change may seriously impair bioenergetic function of the mitochondria. Recently, tomograms have been obtained of mitochondria that have been quick-frozen and embedded in vitreous ice, without fixatives or stain [4]. Tomograms of frozen-hydrated rat-liver mitochondria confirm the tubular nature of cristae, with larger compartments apparently formed by fused tubes. Extensive zones of contact between inner and outer membranes seen in conventionally prepared mitochondria do not occur in the frozen-hydrated specimens. Instead, 15-nm particles are observed that appear to bridge the outer and inner membranes. (Research supported by NIH grant RR01219.) 1. Mannella et al. (1994) Microscopy Res Tech 27:278 2. Mannella et al. (1997) Trends Biochem Sci 22:37 3. Perkins et al. (1997) J Struct Biol 119:37 4. Mannella et al. (1999) Proc. Microscopy & Microanalysis, 416

26. Copeland William Laboratory of Molecular Genetics, NIEHS, 111 TW Alexander Dr. Research Triangle Park, NC 27709 copelan1@niehs.nih.gov

The role of the human DNA polymerase gamma in mitochondrial DNA mutations and diseases

Animal mitochondrial DNA is replicated by the two-subunit DNA polymerase gamma. In human, DNA polymerase gamma is composed of a 140 kDa subunit containing catalytic activity and a 55 kDa accessory subunit. The catalytic subunit contains DNA polymerase activity, 3'-5' exonuclease proofreading activity, and 5'dRP lyase activity required for base excision repair. As the only DNA polymerase in animal cell mitochondria, the DNA polymerase gamma participates in DNA replication and DNA repair. We have cloned, overexpressed, purified, and characterized the human DNA polymerase gamma catalytic subunit, p140, and the p55 accessory subunit. The accessory subunit functions as a processivity factor and enhances DNA binding of the holoenzyme. Using our recombinant DNA polymerase gamma and accessory subunit we are addressing the contribution of mutations produced in the mtDNA by the DNA polymerase gamma. The fidelity of this polymerase has been determined with and without the proofreading function and the accessory subunit. The human DNA polymerase gamma has high DNA synthesis fidelity at single base pairs but rather poor fidelity in homopolymeric runs of nucleic acid. This suggests that homopolymeric runs in human mitochondrial DNA are potential hot spots for DNA replication errors. The accessory subunit lowers the fidelity of DNA synthesis in nearly all DNA substrates analyzed. The implications of the polymerase fidelity and analysis of spontaneous mutations produce in vivo will be discussed.

27. Rapoport Stanley I. Section Brain Physiology and Metabolism, National Institute on Aging, NIH, Bethesda MD 20892

Staging Downregulation of Brain Oxidative Metabolism in Alzheimer Disease (AD).

In vivo brain imaging of patients with Alzheimer disease (AD), using positron emission tomography (PET), demonstrates progressive reductions in "resting-state" brain glucose metabolism and blood flow (markers of functional synaptic activity) in relation to dementia severity, more so in association than primary cortical regions. During cognitive or psychophysical stimulation, however, blood flow and metabolism in the affected brain regions can increase to the same extent in mildly demented AD patients as in age-matched controls, despite reduced resting state values. The extent of activation declines with dementia severity and is markedly reduced in severely demented patients. Thus, there is an initial functionally responsive stage in AD, followed by progressive loss of responsiveness. Analysis of biopsy and postmortem brain from AD patients suggests that these stages reflect progressive synaptic failure and dropout. The initial "potentially reversible" stage appears associated with selective down-regulation of expression of enzymes and their subunits (whether derived from the mitochondrion or nucleus), which are involved in oxidative phosphorylation (OXPHOS). The later progressively functionally nonresponsive stage appears accompanied by accumulation of neurofibrillary tangles in more than 50% of pyramidal cell cytoplasm, and reduced transcription of both OXPHOS and non-OXPHOS mitochondrial and nuclear genes. It is followed eventually by cell death. Thus, staging mitochondrial OXPHOS dysfunction in brain tissue from AD

patients can elucidate the basis of progressive failure of metabolic and blood flow responsiveness to brain activation during life, and even suggest mechanisms to slow this failure. Refs: Chandrasekaran et al. 1997. Brain Res. Mol. Brain Res., 44, 99-104; DeKosky et al. 1996. Neurodegeneration 5, 417-421; Hatanpää et al. 1996. Ann. Neurol., 40, 411-420. Rapoport 1999. Ann. N. Y. Acad. Sci., 893, 138-153.

2000 Brain Awareness Week
March 13-19, 2000

National Brain Bee Finals 2000

State	City	Sponsor	Winner	High School
AZ	Glendale	Midwestern University	Otilia Husu (1st nationally)	Mountain Ridge High School
IL	Chicago	Northwestern University	Charles Ansell	Whitney Young High School
IA	Des Moines	Des Moines University Osteopathic Health Center	Sarah Bennett	Ankeny High School
IA	Iowa City	University of Iowa	Joe Seo	West High School
MA	Amherst	U-Mass/Amherst College	Marlee Krieger	Belchertown High School
MD	Baltimore	University of Maryland	Jeremy Friedman	Franklin High School
MN	Minneapolis	University of Minnesota	Kejia Sun	Irondale High School
NJ	New Providence	North South Foundation	Narendra Shet	Hillsborough High School
NY	Southampton	Southampton College of Long Island	Ashish Bhatt	Newfield High School
OH	Berea	Baldwin Wallace College	Don Esker	Copley High School
ON	Toronto	University of Toronto	Yvonne Chan	Toronto High School
PA	Erie	Mercyhurst College	Sophia Chung	Mcdowell High School
PA	Lancaster	Franklin and Marshall College	Sarah Edwards	Manhiem Township School District
PA	Philadelphia	David Mahoney Institute of Neuroscience	Adrian Childs	Strawberry Mansion School
PA	Pittsburgh	University of Pittsburgh	Kathleen Rubritz (3rd nationally)	North Allegheny Senior High School
PA	Scranton	Scranton University	Tom Burke	Seton Catholic High School
TN	Nashville	Vanderbilt University	Paul Justin Davis	Hillwood High School
TN	Memphis	Christian Brothers University	Mai Tran	Bartlett High School
TX	Lubbock	West Texas Neuroscience Chapter	Nikhil Rao (2nd nationally)	Lubbock High School
VA	Norfolk	Eastern Virginia Medical School	Yolandie Becker	Granby High School

Introduced immediately preceding Dr. Gibson's Wednesday Afternoon Lecture!

28. Gibson Gary E. Cornell Medical College, Burke Research Institute

Oxidative Processes and Signal Transduction in Alzheimer's Disease: Insights from Brains, Peripheral Cells and Animal Models

Abnormalities in mitochondrial function that lead to oxidative stress are central to numerous neurodegenerative disorders. The clinical symptoms of Alzheimer's Disease (AD) result from a complex interaction of aging, genetic factors, characteristic pathological structures (plaques and tangles), the loss of synaptic terminals, especially cholinergic terminals, and loss of select neuronal populations. Which of these of these features causes the others is unknown. An understanding of these interactions is critical to understanding the etiology of the disease, and will enable more effective therapeutic designs. This seminar will discuss the hypothesis that mitochondrial abnormalities that lead to oxidative stress link multiple aspects of the disease and are a critical, and essential, component in the disease process.

Mitochondrial abnormalities and oxidative stress are present in brain and peripheral tissues of AD patients. Brains of patients with both genetic and non-genetic forms of AD have evidence of abnormal mitochondrial function including oxidative stress. In a portion of patients with AD who bear a gene that predisposes them to the development of AD, reductions in the activities of a mitochondrial enzyme are better predictors of the dementia than are plaques or tangles. Abnormalities in mitochondrial function including oxidative stress persist not only in brain, but also in cells cultured from patients with AD, suggesting that they reflect inherent properties of AD cells and do not merely reflect neurodegeneration.

Changes in mitochondrial function including oxidative stress can lead to many of the features generally associated with AD. These data support the hypothesis that oxidative abnormalities are a critical component of a cascade of events that lead to neurodegeneration. This supports the suggestion that ameliorating the mitochondrial deficit will benefit the patient. Impairing mitochondrial function reduces mental function, especially memory, and the decline is associated with diminished cholinergic function. The products of the mutant genes that lead to AD cause mitochondrial abnormalities or potentiate oxidative stress. These genetic forms of AD are only a small portion of the total population suggesting that in the majority of patients other factors lead to the oxidative stress. Abnormal mitochondrial function can also lead to alterations in the processing of the proteins that form plaques and tangles.

Studies with animal models with chronically impaired cerebral oxidation support the importance of abnormalities in mitochondrial function that lead to oxidative stress in the selective loss of neuronal populations. Treatments that mimic aspects of the mitochondrial dysfunction in AD lead to loss of brain function, degeneration of neurons in select brain regions, and memory loss. As in AD, oxidative stress, vascular changes and inflammation accompany neuronal death. In these animals, changes in the protein that forms the plaques is a late change. In animal models cause/effect relations can be assessed by treatments that reduce oxidative stress and/or genetic knockout of precise steps. The compromised metabolism and oxidative "stress" occurring in brains from AD patients, and cellular and animal models suggest that these changes are a central and essential part of the cascade that leads to the disease.

POSTERS

29. Starkov Anatol Gary Fiskum ANESTHESIOLOGY, University of Maryland, 685 W Balltimore Strt, Baltimore MD 21202 USA

Mitochondrial Permeability Screening by Reduction of External Pyridine Nucleotides.

The phenomenon of mitochondrial permeability transition (MPT) is drawing increased attention since it's key role in cell apoptosis has been revealed. However, currently used methods for the detection of MPT in vitro such as measurement of the mitochondrial membrane potential or swelling are labor -intensive, indirect and require relatively high amounts of mitochondria. These methods are at best semi-quantitative

and do not generally permit a direct comparison of results obtained with different tissues or even with different MPT inducers. To overcome these limitations, we have adapted and further developed a method which allows direct measurement of the permeability of mitochondrial inner membrane (IM). The method is based on impermeability of IM to NADP+ and consist of measuring of the rate of external NADP+ reduction by mitochondrial matrix enzymes. The procedure requires minute amounts of mitochondria, it is fast, simple, and produce quantitative results which can be used directly to estimate the degree of MPT in a mitochondrial preparation. Moreover, the method allows to use high- efficient instruments such as fluorescence plate reader and pre-mixed ready-to-use solutions, which render it suitable for a high-throughput screening procedures. (Supported by NS3415)

30. Polster Brian Fiskum,Gary&&1 Kushnareva,Yulia&&1 Sokolove,Pat&&2 Kinnally ,Kathleen&&2 Department of Anesthesiology, University of Maryland School of Medicine, 685 W. Baltimore St., MSTF 5-34, Baltimore, MD 21201, U.S.A.~`~Wadsworth Center, New York State Department of Health bpolster@anesthlab.ummc.umaryland.edu

Release of Apoptogenic Cytochrome C Elicited by Mitochondrial Signal Peptides

Mitochondrial permeability transitions (PT) have been implicated in the release of cytochrome c (CytC) that frequently precedes caspase activation during apoptosis. Involvement of the classical cyclosporin A (CsA)- inhibitable PT is dependent on several factors, however, including the trigger that induces release and the tissue that is used. A unique PT can be elicited by exposing liver mitochondria to synthetic signal peptides whose sequences mimic the targeting regions of mitochondrial precursor proteins. This study tests the hypothesis that signal peptides can initiate the release of CytC from the mitochondria of liver, brain, and digitonin-permeabilized GT1-7 cells in the presence of physiologically relevant concentrations of K+, Mg2+, and ATP-4. Measurements of changes in mitochondrial volume, membrane potential, and CytC release demonstrate that a human cytochrome oxidase subunit IV signal peptide (15-100 mM) induces swelling, a decrease in membrane potential, and the release of CytC in each type of mitochondria; however, brain mitochondria and permeabilized cell mitochondria require higher levels of peptide and exhibit less swelling than liver mitochondria. Although classical PT inhibitors have no effect on these changes, dibucaine and propranolol inhibit membrane depolarization, swelling, and CytC release. Mitochondrial signal peptides can therefore evoke release of CytC under physiologically relevant conditions by a mechanism that is different from release induced by the classical PT. (Supported by NS34152 and by the Bayer Corporation)

31. Zullo S W.T. Parks(2 A. Wong(3) K.M. Sanders(4) C.R. Merril(1) (1)LBG, NIMH; (2)LCRC, NCI; (3)Molecular Biosciences, University of California, Davis; (4)SNB, NIDDK.

The "Common" Human Mitochondrial DNA 4977-bp Deletion in Cultured Lymphocytes: Decreased Oxygen Consumption and Apoptotic Characteristics

The "common" human mtDNA (mtDNA4977) deletion is associated with apoptotic changes in the cultured lymphocytes from a mother, a son with Pearson's syndrome (containing the deletion at 50% levels), and an asymptomatic daughter. The distribution of the mtDNA4977 deletions in the cells ranged from isolated regions to cells that were apparently saturated with mtDNA4977 deletions, consistent with progression of the deletion from a single locus to deletions throughout the cell. Maximal rate of oxygen consumption was measured when the electrons entered the electron transport chain (ETC) through complex I, or through complex II. The son's lymphocytes gave the lowest values and the mother's lymphocytes always gave the highest. The daughter's lymphocytes gave values comparable to the son's through complex II and intermediate between the mother's and son's (although closer to the son's) through complex I. The results clearly show that son's and daughter's lymphocytes have diminished capacities for oxygen consumption compared to the mother's, with the deficit worse in the son's. The cells from this family displayed the same susceptibility to apoptosis-inducing agents as control lymphocyte lines.

32. Zullo S Catherine Summers (2) Wendy Pogozelski (3) Carl R. Merril (1)
(1)Lab of Biochemical Genetics (LBG); NIMH Bethesda, MD 20892. Research Genetics, Huntsville, AL 35801. (3) Department of Chemistry, State University of New York at Geneseo, NY 14454.

Could the Common 4977 bp Human Mitochondrial DNA Deletion be the result of an Active Enzymatic Process?

We (LBG) have shown that conditions associated with chronic hypoxia may be important in the occurrence of the common 4977 bp human mitochondrial DNA
deletion (mtDNA4977 deletion). Analogous deletions have been observed in a
number of animal species, notably mouse, rat, and rhesus monkey. The deletions appear in the same region of the mitochondrial genome (gene order conserved in mammals) as the human mtDNA4977 deletion. In addition, anoxia has also been reported to induce mtDNA deletion-like petite mutations in yeast. The mtDNA intron-encoded protein of the fungus Podospora anserina deletes an analogous region of the mtDNA in senescing cultures (alpha-event). These observations suggest that the mtDNA4977 deletion may be a result of an active enzymatic process. Thus, we scanned a high-density membrane array of a bacterial artificial chromosome (BAC) library of the human genome with the alpha-event protein coding sequence, as a candidate gene involved in the deletion process. We realized positive BAC signals, indicating that sequences in the human and mouse genomes are hybridized by the alpha-event protein gene sequence. Identification of the hybridizing sequences contained in the BAC inserts may enable us to determine the likelihood of an alpha event-like process in human mtDNA deletions.

33. Kohn Kurt Lab. Molec. Pharmacol., DBS, NCI
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Molecular Interaction Map of Mitochondrial Function in Apoptosis

In order to comprehend the integrated function of complex networks of molecular interactions, a diagram convention has been developed and previously applied to mammalian cell cycle control (Kohn, Mol.Biol. Cell 10:2703, 1999). A map will be presented that shows the interactions of Bcl-2 family proteins in the control of cytochrome c release from the mitochondrial intermembrane space and the connections to initiator and effector caspases. Regulatory connections from cell surface receptors, PKB/Akt, and microtubules will be included. Each molecular interaction is annotated with salient facts and references.

34. SMAILI SORAYA S. HSU,YI-TE&&2 SANDERS,KAREN&&2 RUSSELL,JAMES T.&&3 YOULE,RICHARD J.&&2 INFAR,FEDERAL UNIVERSITY OF SÃO PAULO, RUA TRES DE MAIO N.100, SÃO PAULO, BRAZIL, 04044-020~`~BIOCHEMISTRY SECTION, NINDS, NIH, BETHESDA, MD 20892-1414~`~LCMN, NICHD, NIH, BETHESDA, MD 20892-4599
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Sequential events correlated with Bax insertion, mitochondrial membrane potential integrity and ATP

Several proteins from the Bcl-2 family promote cell survival or cell death. Overexpression of Bax, one member of this family, promotes cell death of a variety of cells subjected to death stimuli and upon induction of apoptosis, Bax translocates from the cytosol into organelle membranes, in particular, mitochondrial. Bax is associated with a loss of mitochondrial membrane potential (MMP), another feature associated with cell death. However, the sequential order between Bax insertion and mitochondrial processes is still unclear. In this study, using high resolution microscopy, GFP tagged Bax and a potentiometric dye that measures MMP, we investigated simultaneously the events related to Bax insertion

and MMP integrity. We have found that in Cos-7 cells expressing GFP-Bax, after 2-3 hours in the presence of staurosporine a complete loss in the MMP was observed. The disruption in the MMP occurred 8 min before the onset of Bax insertion. Disruption of MMP with the protonophore FCCP, does not induce Bax insertion. However with FCCP plus oligomycin (F0F1-inhibitor), Bax insertion was observed. FCCP plus oligomycin are able to induce more apoptotic cell death than FCCP by itself. Disruption of MMP by antimycin, an inhibitor of the eletron transport chain has also induced Bax insertion. Our results suggest that disruption of MMP by different pathways trigger Bax insertion. However, when ATP consumption is inhibited (with oligomycin) or is minimized (antimycin), Bax insertion occurs and apoptosis proceeds. In the presence of FCCP, decreased ATP levels due to excessive ATP consumption may inhibit Bax insertion.

35. Amstad Paul Johnson,Gary&&2 Lee,Brian&&2 Dhawan,Sumant&&3
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CaspaTagTM FAM-VAD-FMK: A Novel In Situ Marker For The Detection Of Activated Caspases

The central component of apoptosis is a cascade of proteolytic enzymes called caspases. These enzymes are activated in response to pro-apoptotic signals and result in cleavage of protein substrates, causing the disassembly of the cell. Activated caspases can be detected via immunoprecipitation and immuno-blotting techniques using caspase specific antibodies, or by employing pro-fluorescent substrates which become fluorescent upon cleavage by the caspase. Most of these methods require the preparation of cell extracts and therefore are not suitable for the detection of active caspases inside a living cell. Using FAM-VAD-FMK we have developed a simple and sensitive assay for the detection of caspase activity in live cells. FAM-VAD-FMK is a carboxyfluorescein (FAM) derivative of benzyloxycarbonyl-Valine-Alanine-Aspartic acid-Fluoromethyl Ketone (zVAD-FMK) which is a potent broad- spectrum inhibitor of caspases. FAM-VAD-FMK enters the cell and irreversibly binds to activated caspases. Cells containing bound FAM-VAD-FMK can be analyzed by flow cytometry, fluorescence microscopy or fluorescence spectroscopy (fluorescence plate reader). We have applied FAM-VAD-FMK to measure caspase activation in live non-adherent and adherent cells. Our results show that 20% to 30% of Jurkat cells induced with camptothecin or HeLa cells treated with staurosporine for 5 hours stain positive for active caspases using flow cytometry or fluorescence microscopy. Using a fluorescence plate reader camptothecin induced cells show a 3 to 4 fold increase in green fluorescence compared to control cells. These results show that FAM-VAD-FMK is a sensitive and reliable tool for detecting activated caspases in live adherent and non-adherent cells.

36. Melnov S S.J. Zullo(2) C.J.C. Hamel(3) P.G.S.Prasanna(3) W.K. Pogozelski(4) N. Fischel-Ghodsian(5) C.R. Merril(2) and W.F. Blakely(3) (1)Institute of Nuclear Medicine, Minsk, Belarus; (2)Laboratory of Biochemical
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CYTOLOGICAL DETECTION OF THE 4977-BP "COMMON" MITOCHONDRIAL DNA DELETION USING AN IN SITU PCR ASSAY

Cytological identification and localization of mitochondrial DNA (mtDNA) mutations can be useful in the investigation of the nature and genesis of various human diseases and in radiation epidemiologystudies. The 4977-bp "common" mtDNA (mtDNA4977) deletion has been associated with different diseases including Pearson's syndrome, Kearns-Sayre syndrome, ophtalmoplegia, cardiomyopathy, as well as exposure to ionizing radiation. Chronic exposure to free radicals created by electron-transport-chain activity, absence of sophisticated repair mechanisms, and frequent mtDNA replications can contribute to accumulation of mutations in the mitochondrial genome. We developed an in situ PCR method for

cytological identification of the mtDNA4977 deletion. We utilized established human lymphocyte cell lines, one maintains the mtDNA4977 deletion at around 50% of total mtDNA. Primer sets were selected to detect the mtDNA4977 deletion as well as total mtDNA (rRNA sequence), each with a distinctive fluorochrome, which in a multiplex application permits cytological confirmation of their co-localization. Mitochondrial localization of the signals was confirmed by the combined use of this in situ PCR assay with a mitochondrial membrane probe. We are continuing to investigate the utility of this mtDNA deletion detection method for biological dosimetry applications.

37. Zullo S S. Desautels(2) D. Maglott(3) P. Lemkin(4) C.R. Merril(1) (1)LBG, NIMH; (2)CIT, NIH; (3)NCBI, NLM; (4)LECB,NCI/FCRDC

The MitoDat Database: A Web Database of Mitochondrial Proteins

This database is dedicated to the nuclear genes specifying the enzymes, structural proteins, and other proteins, many still not identified, involved in mitochondrial biogenesis and function. MitoDat highlights predominantly human nuclear-encoded mitochondrial proteins, although proteins from other animals and those currently known only from yeast and other fungal mitochondria, as well as from plant mitochondria are included. The database is centrally maintained, with the ability of researchers world-wide to make entries that are then verified by the curators before inclusion in the database. The database consolidates information from various biological databases, eg., GenBank, SwissPro, Genome Data Base (GDB), Online Mendelian Inheritance in Man (OMIM). Because the mitochondrion has a central role in cellular metabolism, it is involved in many human diseases. This database should help us in studying these diseases. This database will also allow us to effectively identify the probe sequences needed for developing a "mitochondiral chip", to develop mitochondrial fingerprints" for different tissues under different physiological and pathological conditions.

38. Tatusova Tatiana Wolfsberg,Tyra Tatusov,Roman Ostell,James
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Organelle Resources at NCBI

The NCBI has developed a collection of eukaryotic organelle complete genome sequences and associated resources. The sequences are part of the NCBI Reference Sequence project that provides curated sequence data and related information for the community to use as a standard. At present, only the animal (metazoan) mitochondrial sequences are considered reviewed, that is, they have been manually curated by the NCBI staff. Other mitochondrial and chloroplast sequences are provisional and are presented as found in the source GenBank records. In addition to providing a list of complete mitochondrial and plastid genomes, this site also presents tools that can be used to analyze these sequences. The organisms from which the organelles derive are presented in a taxonomic hierarchy built from the NCBI Taxonomy database. The following resources are available for the reviewed reference sequences. We will integrate other reference genomes into these resources once they have been reviewed. o A manually curated reference sequence for each organelle, including standardized gene, protein, and RNA names. o The gene order (Protein List) and gene and RNA order (Gene/RNA order) by taxonomic grouping. o Collection of organelle protein sequences by taxonomic grouping. o Multiple alignments of organelle protein sequences by taxonomic grouping. o Links between the organelle protein sequences and the NCBI COG database. o Match gene, a tool to compare your protein sequence to a database of mitochondrial protein sequences, by taxonomic grouping. The NCBI Organelle website is available at
http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/organelles.html

39. Preusch Peter Pharmacology, Physiology, and Biological Chemistry Division, National Institute of General Medical Sciences, 45 Center Drive, MSC 6200, Bethesda, MD, 20892-6200, USA preuschp@nigms.nih.gov

NIH Funding of Research on Mitochondria

Data will be presented on NIH funding of research programs in mitochondrial structure/function, biosynthesis, and roles in cellular regulation. Included will be information on the distribution of funding by Institute and by study section, as well as program announcements of interest.

40. Boyer Leslie Mohan, Jr.,Charles United Mitochondrial Disease Foundation, 8085 Saltsburg Road, Suite 104, Pittsburgh, PA 15239 leslie@nb.net

Poster Presentation: United Mitochondrial Disease Foundation

The mission of the United Mitochondrial Disease Foundation (UMDF)is to promote research for cures and treatments of mitochondrial disorders and to provide support for affected families. UMDF will present, in poster format, the benefits UMDF provides both to clinicians and scientists, as well as affected families. UMDF has a well established research grant program, a quarterly newsletter, a patient registry (gathering information on patient diagnosis and symptoms), a reference library, and a family support network (linking families across the country. UMDF will also be sponsoring its third international symposium, June 1-3, 2000.

41. Biasella Susan Hillcrest Hospital~`~The Cleveland Clinic Health System~`~United Mitochondrial Disease Foundation sbiasella@meridia.org

Adult-Onset Mitochonrial Disease: Effect and Reaction of Patient and Family Members

What happens when a midlife adult is finally given the diagnosis of a Mitochonrial Disease? This poster will present the feelings and reactions of the patient and family members is three areas: 1. How does the patient feel about their diagnosis, treatment , and quality of life? 2. How does the spouse/adult child react as caregiver? 3. How does the nuclear and extended family react to this serious, chronic condition? This poster will be prepared by the adult members of the Internet Mitoldies List sponsored by Onelist, administered by Jean Sheperd and Alice Adams.

42. Lerman-Sagie Tally Lev,Dorit&&1 Yanouv,Miri&&1 Leshinski-Silver,Ester&&1 Sagie,Alik&&2 Ben-Gal,Tuvia&&2~`~Rustin,Pierre&&2~`~Munnich,Arnold&&2 Metabolic Neurogenetic Clinic, Wolfson Medical Center, POB 5, Holon,58100, Israel ~`~Cardiology Department, Rabin Medical Center, Petah Tikva, Israel~`~Department of Pediatric Genetics, Hopital de Infants Malades, 149 Rue de Sevres, Paris, 75743,France asagie@post.tau.ac.il

Dramatic Response to Idebenone in a Patient with Severe Heart Failure Due to Mitochondrial Cardiomyopathy

Idebenone is a synthetic analog of coenzyme Q10. It has been shown to improve cardiac function in patients with Friedreich's ataxia and a deficiency of respiratory chain complexes I-III. We describe a dramatic response to Idebenone in a woman with a mitochondrial cardiomyopathy. A 36 year old woman, who had been known to have a stable cardiomyopathy since the birth of her only child 5 years earlier, developed progressive combined right and left heart failure with pulmonary congestion at rest, ascites and peripheral edema (NYHA functional class IV). Echocardiography revealed mild dilatation and severe left ventricular (LV) systolic and diastolic dysfunction (EF=25%). Despite treatment with cardiac medications and diuretics her condition did not improve and she was evaluated for a cardiac transplant. Family history

was significant for a mother who died at the age of 42 from a cardiomyopathy and a son with congenital cardiomyopathy, mental retardation, and failure to thrive. His muscle biopsy showed ragged red fibers but normal respiratory chain complexes. The mtDNA did not disclose mutations in any of the nucleotides described in Maternally Inherited Myopathy and Cardiomyopathy. The patient's endomyocardial biopsy showed severely decreased activities dependent on Co Q, pointing to Co Q depletion. Adding quinones restored these activities. Idebenone 225 mg/day was started. Three months later there was a dramatic improvement in her clinical status with resolution of the ascites and improvement in her functional class (NYHA class II). On follow up echocardiography there was a significant increase in LV systolic function (EF =50%).

43. Wallace Kendall B. Department of Biochemistry & Molecular Biology, University of Minnesota, 10 University drive, Duluth, MN 55812, USA Wallace kwallace

Irreversible mitochondrial cardiomyopathy caused by adriamycin

We previously reported that adriamycin (Adr) redox cycles on Complex I of the mitochondrial electron transport chain to stimulate the oxygen free radical-mediated induction of the mitochondrial permeability transition (MPT). In vivo, this is manifested as a cyclosporin A (CsA)-sensitive decrease in calcium-loading capacity of cardiac mitochondria isolated from adriamycin-treated rats. The current study demonstrates that this shift in calcium sensitivity of the MPT is a dose-dependent and irreversible manifestation of Adr toxicity in vivo and that it is not associated with changes in coupling efficiency or cytochrome content of the isolated mitochondria. It is reversed by adding CsA but not tamoxifen or thiol reducing agents in vitro and it is associated with a decline in adenine nucleotide translocator content of cardiac mitochondria from Adr-treated rats. We conclude that interference with the calcium-dependent regulation of the MPT may be a critical event and is consistent with the dose-dependent and persistent cardiomyopathy observed clinically in patients receiving Adr cancer chemotherapy. (Supported by HL-58016).

44. Legido Agustin Miles,Daniel K Melvin,Joseph J Salganicoff,Leon Grover,Warren D Legido,Agustin Section of Neurology, Dept. of Pediatrics, St. Christopher's Hospital for Children, MCP Hahnemann University, Erie Avenue at Front Street, Philadelphia, PA 19134~`~The Barnett Center for the Study of Mitochondria Disorders, St, Christopher's Hospital for Children, Erie Avenue at Front Street, Philadelphia , PA 19134
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SEIZURE TYPES AND EEG ABNORMALITIES IN CHILDREN WITH MITOCHONDRIAL CYTOPATHIES

OBJECTIVE: To study seizure types and EEG abnormalities in children with mitochondrial cytopathies.(MC) BACKGROUND: Myoclonic epilepsy is the uniform seizure type reported in association with MC. However,in our experience, young children with MC frequently present with multiple seizure types often in association with a severe encephalopathy. DESIGN/METHODS: A retrospective analysis was made on children with the diagnosis of MC attending the Neurology Clinic. Presenting symptoms, clinical course, neurological status, neurophysiology studies where available and detailed biochemical profiles were obtained. MC was defined as defective oxidative phosphorylation detected in isolated mitochondria from skeletal muscle using polarographic techniques and/or DNA identifications of one of the commonly occurring mitochondrial DNA mutations in blood. Diagnostic investigations included histochemical and EM evaluation of muscle. RESULTS: Medical records on 32 children with MC were reviewed. Nine of them presented with motor problems or developmental delays but never had seizures. Of the remaining 23, 10 developed seizures in the first year of life: neonatal seizures (3), infantile spasms (1), myoclonic seizures (2), single generalized seizure (2), status epilepticus (1) and both partial and

generalized seizures (1). The other 13 children ranged from 1 to 17 years at age of seizure onset with all but 2 presenting in the preschool years. A total of 10 children had refractory seizures, defined as failure to respond to two or more AEDS. These children had multiple seizure types, including various combinations of myoclonic, partial, absence, tonic and tonic-clonic seizures. One child presented with epilepsia partialis continua. Children with refractory epilepsy usually had a severe accompanying encephalopathy. EEG findings in children with refractory epilepsy showed marked background abnormalities alone (1), multifocal and generalized epileptiform discharges (5), generalized slow spike wave(1), and focal discharges with or without secondary generalization (3). EEG findings in children with nonrefractory seizures ranged from normal (2) to background slowing (2), focal discharges(5), multifocal discharges (2) and generalized discharges (1). Mitochondrial abnormalities, including respiratory chain and Krebs cycle defects, and mitochondrial DNA mutations were equally distributed among children without seizures, children with refractory epilepsy and children with nonrefractory epilepsy. CONCLUSIONS: MC should be considered in the differential diagnosis of children presenting with seizures of unclear etiology in the first few years of life especially with an accompanying encephalopathy.Seizure types and EEG abnormalities are varied and usually do not correlate with specific biochemical defects.

45. Jones Jessa Song,Jason Hempen-Shively,Paula Kern,Scott Oncology, Johns Hopkins Univ, 464 CRB 1650 Orleans,Baltimore, MD, 21231,USA~`~Predoctoral Program in Human Genetics, Johns Hopkins University, Baltimore, MD

Mitochondrial Mutations in Pancreatic Cancer

Our recent sequencing efforts of the complete mitochondrial genome in several pancreatic cancer cell lines and tumor xenografts have identified several somatically acquired homoplasmic mutations. The functional significance of these mutations is unknown. We are interested in determining whether these mitochondrial mutations in tumors are due to clonal expansion of rare mitochondrial polymorphisms, or if these mutations provide a selective advantage important during tumorigenesis.

46. Fliss Makiko Usadel,Henning Caballero,Otávia Wu,Li Buta,Martin Eleff,Scott&&2, Jen,Jin, Sidransky,David Department of Otolaryngology-Head and Neck Surgery, Head and Neck Cancer Research Division Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA~`~Department of Anesthesiology & Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA fmakiko@yahoo.com

Facile detection of mitochondrial DNA mutations in tumors and bodily fluids

Examination of human bladder, head and neck, and lung primary tumors revealed a high frequency of mitochondrial DNA (mtDNA) mutations. The majority of these somatic mutations were homoplasmic in nature, indicating that the mutant mtDNA became dominant in tumor cells. The mutated mtDNA was readily detectable in paired bodily fluids from each type of cancer, and was 19 to 220 times more abundant than mutated nuclear p53 DNA. By virtue of their clonal nature and high copy number, mitochondrial mutations may provide a powerful molecular marker for noninvasive detection of cancer.

47. Tarnopolsky Mark Chorneyko,Kathy&&2 Simon,David&&3 Johns,Donald&&3,4 Neurology, McMaster University, Hamilton, CANADA~`~Pathology, McMaster University, Hamilton, CANADA~`~Neurology, Beth Israel Deaconess Medical Center, Boston, MA, USA~`~Opthalmology, Beth Israel Deaconess Medical Center, Boston, MA, USA
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Reversal of Paracrystalline Inclusions in a Patient with a G15497A Missense Mutation Following Creatine Monohydrate Supplementation.

Creatine monohydrate can enhance muscle performance in patients with mitochondrial cytopathy. A hallmark of several of the mitochondrial cytopathies is the presence of paracrystalline inclusions composed of crystals of dimeric mitochondrial creatine kinase (miCK). Creatine monohydrate has been shown to stabilize miCK in the octameric form, which could allow for reversal or prevention of paracrystalline formation. We serendipitously found many paracrystalline inclusions in a young male athlete who was involved in a study looking at intra-muscular lipid. His neurological examination was normal, yet he had a very high respiratory exchange ratio during exercise that is seen in mitochondrial disorders. His mother was diagnosed with seizures, but also had a normal neurological examination. Direct DNA sequencing of the mitochondrial genome revealed a novel missense mutation at G15497A of mtDNA that resulted in a glycine to serine conversion at a highly conserved site in the cytochrome b gene in the patient and his mother. No other potentially pathogenic mutations were identified in the mtDNA sequencing. The patient was treated with creatine monohydrate (5 g/d) for a period of 5 weeks and had a repeat muscle biopsy of the ipsilateral vastus lateralis in which no paracrystalline inclusions were found. He then discontinued the creatine supplementation for 3 months and had a repeat biopsy in the contralateral vastus lateralis which demonstrated early paracrystalline formation and electron densities in the mitochondria. These observations suggest that paracrystalline inclusions in muscle are dynamic and may be reversed by creatine monohydrate supplementation, possibly by octameric stabilization.

48. Strand Micheline Dominick,Olivia Copeland,Bill Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, 111 Alexander Dr., Research Triangle Park, NC 27709 Strand@NIEHS.NIH.GOV

The Crg1 gene of S. cerevisiae: A new copper-regulated component of the free radical detoxification system that alters mitochondrial copy number and mitochondrial mutation rates

We have isolated a new gene, Crg1, required for the protection of mitochondria against damage by free radicals. Disruption of Crg1 results in a 44-fold increase in the mitochondrial mutation rate in the mtArg8 gene. The crg1 mutant exhibits a three-fold increase in mitochondrial DNA copy number. Transcription of Crg1 is increased three-fold after ten minutes of exposure to excess copper. Disruption of Crg1 results in slow growth, increased sensitivity to copper, an increased accumulation of Cu in mitochondria, increased cytochrome c activity per cell, and an increased incidence of petite formation. We have determined that the function of this gene is to detoxify free radicals. As the primary site of oxidative damage is in the mitochondria, the effects are primarily mitochondrial. We hypthesize that mitochondrial damage by the elevated number of free radicals leads to reduced mitochondrial function, prompting the cell to increase mitochondrial replication. Sequence analysis of the Crg1 promoter predicts that this gene is regulated by Ace1, a positive transcription factor activated by copper or oxygen, and Yap1, a transcription factor regulating oxidative stress response genes. The gene is highly conserved, and a human homolog has been identified by random sequencing. The human homolog appears to be highly expressed and exhibits a significant frequency of mutations. This gene plays an essential role in the defense of mitochondria against reactive oxygen species, and mutations in the human homolog are expected to affect human health by increasing reactive oxygen species, mitochondrial mutation rates, and mitochondrial copy number.

49. Liang Min- Hui Park,John C. Wong,Lee-Jun C. Institute for Molecualr and Human Genetics, Georgetown University Medical Center, 3800 Reservoir Rd., MW; M4000; Washington, DC20007 liangm@gunet.georgetown.edu

Novel mtDNA nucleotide changes

Mitochondrial DNA (MtDNA) disorder is a maternally inherited, heterogeneous, neurological disease that is caused by mtDNA mutations. Identification of mtDNA mutation is important in confirming the diagnosis. It was suggested that the majority of pathogenic mtDNA mutations exist in a heteroplasmic state. Temporal temperature gradient gel electrophoresis (TTGE) was used to scan nucleotide changes in 11 mtDNA regions containing all 22 tRNA genes and 4 mRNA regions containing Cytb, COXI, COXII, and COXIII genes. Our results demonstrate that both heteroplasmic and homoplasmic nucleotide changes can be effectively detected by TTGE as multiple bands and band shift, respectively. Direct DNA sequence analysis revealed several novel nucleotide changes. They are as following: (1) rRNA: A723C, G769A, G1719A, C1721T (2) tRNA: T4454C, T5580C, G5821A, C5840T, C7476T, G7521A, C8359A, G12207A, C12239T, G15928A (3) mRNA: G4048A, A5496G, C5499T, T12338C, A14566G, A14587G, C14751T, A15824G, A15311G, A15830G, A15848G, C15849T (4) D-Loop: C16111A. Clinical significance of these novel mutations is currently being investigated.

50. Sekiguchi Kazumasa Sekiguchi, Kazumasa 1, 2 Kasai,Kentaro 2 Levin,Barbara C. 1 Biotechnology Division, National Institute of Standards and Technology, 100 Bureau Drive, Mail Stop 8311, Gaithersburg, MD, 20899-8311, USA~`~National Research Institute of Police Science, 6-3-1, Kashiwanoha, Kashiwa-shi, Chiba, 277-0882, Japan barbara.levin@nist.gov

INTERGENERATIONAL TRANSMISSION OF A HUMAN MITOCHONDRIAL DNA HETEROPLASMY AMONG THIRTEEN MATERNALLY-RELATED INDIVIDUALS

A cytosine:thymine (C:T) heteroplasmy at position 16291 in the HV1 region of the human mtDNA was examined in buccal cells from thirteen maternally-related family members across three generations and in additional tissues (e.g., hair, blood, and finger nails) from two members of this family. The ratio of C to T at position 16291 showed wide intra- and intergenerational variation. The variation was also observed within tissues from individuals, (e.g., one person showed different proportions of the heteroplasmy between hairs and even between the root and the shaft of single hairs). In addition to the 16291 heteroplasmy, two other polymorphisms (positions 16357 and 16188.1) were detected in two out of 24 hairs (one difference per hair) from this person. Examination of the hair, buccal cells, and blood showed the 16291 heteroplasmy in all of this individual's examined tissues, but only in one out of 24 hairs from her son. These studies indicate that the proportion of the two components of a heteroplasmy can vary widely among maternally-related family members and among tissues within a single individual. Thus, a single base pair difference should not be used as the basis of an exclusion. Such a discrepancy may be resolved by the detection of a heterplasmy in additional tissues from the individual or maternally-related family members. The observation that the transmission of a mtDNA heteroplasmy from one individual to her offspring is likely to differ between the offspring and between generations lends further credence to the bottleneck theory of inheritance of mtDNA.

51. Nyaga **Simon** Bohr,Vilhelm Laboratory for Molecular Genetics, NIA, 5600 Nathan Shock Drive, Baltimore, MD 21224 nyagas@grc.nia.nih.gov

Oxidative DNA Damage Repair in Human Lymphoblasts

Oxidative DNA Damage Repair in Human Lymphoblasts. Simon G. Nyaga and Vilhelm Bohr, Laboratory for Molecular Genetics, National Institute on Aging, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, MD 21224. Mitochondrial DNA being in close proximity with the electron transport chain, is subject to attack by reactive oxygen species associated with this transport system. These radicals target mitochondrial DNA producing lesions such as 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) and thymine glycol. Uracil in mitochondrial DNA can also result from oxidative deamination of cytosine. If unrepaired, these lesions can lead to mutations, replication block, and potentially, cell death and carcinogenesis. This laboratory has previously demonstrated that uracil is repaired by the single-nucleotide BER pathway using rat liver mitochondrial extracts. We present evidence for DNA repair activities in human mitochondria contrary to the earlier notion that mitochondria were devoid of DNA repair. Using mitochondrial extracts from human lymphoblasts, we have demostrated efficient excision repair of 8-hydroxyguanine, thymine glycol and uracil. The three lesions are repaired in a time-dependent manner, indicating that human mitochondrial DNA repair is specific for these lesions. Furthermore, our data utilizing restriction digestion analysis of the incorporated repair patch indicates that uracil may be putatively repaired by the short patch BER mechanism in humans. In addition, 8-hydroxyguanine repair in a GG sequence context is repaired more efficiently than when in a CC, TT or AA sequence context. SGN is a recipient of Kempner fellowship (1999-2000) from The University of Texas Medical Branch at Galveston, Texas.

52. Longley **Matthew** Nguyen,Dinh Kunkel,Thomas Copeland,William Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC, 27709 longley@niehs.nih.gov

Accuracy of DNA Synthesis by Human DNA Polymerase Gamma

Mutations in mitochondrial DNA cause genetic mitochondrial diseases. Mutations occur through oxidative and chemical damage to DNA, by exposure to environmental mutagens, or by errors made during DNA replication. Mitochondrial DNA is replicated by DNA polymerase gamma, the sole DNA polymerase in animal mitochondria. We measured the accuracy of human DNA polymerase gamma replicating a reporter gene in vitro. With an estimated error rate of less than one mistake for every 300,000 nucleotides synthesized, the overall fidelity of DNA synthesis is very high. A similar analysis with a 3' to 5' exonuclease deficient form of pol gamma reduced fidelity by ~20-fold, indicating pol gamma proofreads errors during DNA synthesis. The 55 kDa accessory subunit of pol gamma acts as a processivity factor, greatly enhances binding of pol gamma to DNA, and mildly reduces the fidelity of DNA synthesis. We also measured the steady state kinetics of pol gamma synthesizing a single correct or incorrect nucleotide. As expected for a faithful polymerase, pol gamma is highly biased (~10E5) against insertion of the wrong nucleotide. Extension of mispaired primer termini was also infrequent. Although the 55 kDa subunit has little effect on the kinetics of misincorporation, inclusion of this subunit increases mispair extension 2- to 7-fold, depending on the mispair examined. Preliminary evidence indicates a greatly reduced fidelity for pol gamma when synthesizing short homopolymeric runs. This effect is exacerbated by the 55 kDa processivity subunit, suggesting homopolymeric runs in human mitochondrial DNA are potential hot spots in vivo.

53. Tully Lois Schwarz,Frederick P. Levin,Barbara C.
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Development of a Heteroplasmic Mitochondrial DNA Standard Reference Material for Detection of Heteroplasmy and Low Frequency Mutations

A heteroplasmic human mitochondrial DNA (mtDNA) standard reference material (SRM) is being developed to provide quality control to forensic, medical, and toxicological scientists who wish to determine their detection limits when examining low frequency mutations or heteroplasmic sites in DNA. While the detection of a mutation present in every mtDNA molecule is routine, it is extremely difficult to detect mutations present in only a small proportion of the molecules. To address these concerns, mtDNA mixtures containing a polymorphic/wild-type site in different percentages (e.g., 1, 2.5, 5, 10, 20, 30, 40, 50) have been constructed. Using automated sequencing chemistries (ABI Dye-Terminator, dRhodamine Terminator, Big Dye Terminator), we were able to unambiguously detect the polymorphism present at the 30% level. Although visible at the 10% and 20% concentrations, it was difficult to distinguish the polymorphism from the background. With denaturant gradient gel electrophoresis (DGGE), resolution at the 5% level was achieved. With the addition of a peptide nucleic acid (PNA) complementary to the wild-type sequence, PCR of the wild-type decreased, the polymorphism selectively amplified, and detection by sequencing became possible at the 5% level. This SRM can be employed to test and perfect more sensitive mutation detection techniques.

54. Berdanier Carolyn Nutrition Laboratories
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Inheritance of OXPHOS characteristics by normal Sprague Dawley and diabetes prone BHE/Cdb rats

The OXPHOS characteristics of isolated hepatic mitochondria were studied in Sprague Dawley, BHE/Cdb and F1 crossbred rats. The rats were first screened for a mutation in the mt ATPase 6 gene. F1 progeny of BHE/Cdb and F1 progeny of female BHE/Cdb rats had the mutation whereas Sprague-Dawley and F1 progeny of Sprague Dawley females did not. Maternal inheritance of OXPHOS function was observed:Reduced OXPHOS efficiency characterized the BHE/Cdb rats and their F1 progeny whereas this was not observed in the Sprague Dawley or F1 Sprague Dawley progeny. With age impaired glucose tolerance developed and this characteristic was maternally inherited as well.

55. DasGupta Dianne Rapoport,Stanley Fiskum,Gary&&2 Chandrasekaran,Krish&&2
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The effects of high energy phosphate compounds on mitochondrial RNA synthesis in organello

The primary function of the mitochondria is to phosphorylate ADP to form ATP which is used by the cell to support its metabolic processes. Evidence suggests that mitochondrial DNA (mtDNA) exists as a functional independent unit with some degree of self-regulation. To analyze factors affecting self-regulation, we isolated mitochondria from rat liver and set up an in organello transcription system. The milieu of this system consisted of oxidizable substrates and constituents that maintained and supported transcription as seen in vivo. The rates of synthesis of mitochondrial (mt) transcripts were quantified using 32 P-UTP when we varied the external concentrations of ADP (0 mM- 6 mM). Addition of 1 mM ADP stimulated mt transcription, whereas there was a progressive decrease in transcription at higher ADP (2

mM-6 mM) concentrations. To ascertain whether mitochondrially generated ATP levels was required for mt RNA synthesis, the effect of the respiratory inhibitors rotenone and oligomycin were evaluated. Inhibition of mitochondrial respiration with rotenone completely abolished transcription. Treatment with oligomycin also resulted in an inhibition of transcription, confirming that ATP was required for transcription. The regulatory effects of ATP are currently under investigation. Our results suggest that mitochondria not only detect but also respond to changes in ATP levels by changing the amounts of RNA synthesized.

56. Brewer Gregory J. Wallimann,Theo&&2 Neurology, Med. Micro/Immuno, Southern Illinois Univ. Sch. Med., Springfield, IL 62794-9626~`~Cell Biology, Swiss Inst. Tech. (ETH), CH-8093 Zurich, Switzerland gbrewer@siumed.edu

Upregulation of Mitochondrial Creatine Kinase in Neurons Stressed by Glutamate

Mitochondrial creatine kinase (Mt-CK) converts intraluminal ATP into phosphocreatine (PCr) for use in the cytoplasm as a source of direct energy or as an energy reserve for conversion to ATP by cytoplasmic BB-CK. Neurons exposed to glutamate activate ionotropic glutamate receptors. During excitotoxic activation, toxicity results from disturbances in ionic homeostasis and generation of reactive oxygen species, which could be reduced by PCr. Recent work has shown protection from glutamate toxicity by supplementation of creatine to neurons (Brewer & Wallimann, J. Neurochem., in press). Under these conditions, the PCr/ATP ratio increased from 5 to 15, which is difficult to explain by equilibrium enzymology. Since mitochondrial respiration is rate-limited by creatine and cellular creatine content correlates with levels of CK in other tissues, we hypothesize that neurons respond to energy demands under stress from glutamate and upregulate their Mt-CK. To test this hypothesis, hippocampal neurons in serum-free culture were exposed to creatine, glutamate or creatine + glutamate, followed by immunoblot analysis of extracts for Mt-CK. A time course of treatment with creatine + glutamate showed a large increase in Mt-CK starting at 7 hr. that was maintained through 24 hr. of treatment. Another mitochondrial protein, the adenine nucleotide transporter indicated a similar increase. Creatine alone showed no change. Glutamate alone showed an increase that was smaller than glutamate + creatine. Together, these results suggest that the stress of glutamate can increase Mt-CK, if fortified with creatine, possibly in the context of an increase in total mitochondria per cell.

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Higher Respiratory Rates in Brain Mitochondria Isolated in Anti-oxidants

Methods of isolation of brain mitochondria generally follow those for liver and heart, yet brain mitochondria have higher levels of poly-unsaturated lipids. These lipids are more susceptible to degradation by reactive oxygen species. Based on the requirement of primary neurons for anti-oxidants during the radical-generating procedure of isolation (Brewer et al., 1993), we tested the effect of an anti-oxidant mixture on succinate-driven respiration in isolated rat brain mitochondria. Mitochondria were isolated in mannitol/sucrose/EGTA/BSA without or with SCAVEGR™ antioxidants (SOD, catalase, vitamin E, glutathione reduced). Respiration driven by ADP (with rotenone) was measured in the absence and presence of 20 mM creatine. For mitochondria prepared with SCAVEGR, respiratory Vmax was 2-fold higher and the respiratory response to creatine was sensitive to lower levels of ADP (Km). Other measures of yield of mitochondria and respiratory control ratio were improved with SCAVEGR, but did not reach significance for this small n of 3 preparations. Mitochondria prepared with SCAVEGR also showed an 11% decrease in thiobarbituric acid reactive material (a measure of lipid oxidation products). Altered levels of protein carbonyls were not detected, nor were differences seen by electron microscopy. However,

SCAVEGR did increase the octamer/dimer ratio in subunits of mitochondrial creatine kinase, a ratio that is exquisitely sensitive to levels of peroxynitrite. Together, these results suggest that significant improvements in brain mitochondrial function are obtained by isolation in the presence of a mixture of anti-oxidants.

58. Chandrasekaran Krish Liu,Li-Ing 2 (1) Dept. of Anesthesiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201; (2) Section on Brain Physiology and Metabolism, NIA, Bethesda, MD 20892 kchandra@anesthlab.ummc.umaryland.edu

Regulation of Mitochondrial Gene Expression in Differentiated PC12 Cells

Mitochondrial DNA (mtDNA) expression of genes of oxidative phosphorylation are thought to be regulated by energy demand (levels of ADP and ATP). We examined this hypothesis by measuring steady-state levels of mtDNA-encoded cytochrome oxidase subunit III (COX III) in a differentiated rat pheochromocytoma cell line PC12S under conditions of decreasing and increasing energy demand. In differentiated PC12S cells, inhibition of Na/K-ATPase by ouabain decreased ATP consumption and increased significantly the ATP/ADP ratio, whereas addition of a sodium ionophore, monensin, increased energy consumption and decreased significantly the ATP/ADP ratio. Measurement of steady-state levels of COX III mRNA by northern blot analysis showed an early increase particularly in monensin-treated cells, followed by a decrease in both ouabain- and monensin- treated cells. No significant decreases were seen with mtDNA-encoded 12S rRNA or with nuclear DNA-encoded beta-actin mRNA. Removal of the drugs restored the normal levels of COX III mRNA. Determination of half-lives of COX III mRNA, 12S rRNA and beta-actin mRNA revealed that the decrease in COX III mRNA was associated with a decrease in the half-life of COX III mRNA. These results suggest that mitochondrial gene expression is regulated by both transcriptional and posttranscriptional mechanisms. The transcriptional regulation represents a physiological mechanism of regulation by energy demand, while the posttranscriptional regulation may represent a mechanism that overrides the normal regulation of mitochondrial gene expression by energy demand and that operate under pathologic situations.

59. Gabrielson Kathy Hogue,Barbara 1 Bressler,Joseph 2 Souza-Pinto,Nadja 1 LMG, GRC, NIA, NIH 5600 Nathan Shock Dr., Baltimore, MD 21224 USA~`~EHS, School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA kgabriel@welchlink.welch.jhu.edu

3NPA induces mitochondrial dysfunction in cardiac toxicity in mice

3-Nitropropionic acid (3NPA), a plant and fungal toxin, produces striatal neurodegeneration in humans and in experimental animals, and has been used to produce an animal model for Huntington's disease. 3NPA toxicity is due to the irreversible inhibition of succinate dehydrogenase and reduction of cellular ATP. We tested toxicity of 3NPA and found both striatal and cardiac damaged in 4 different strains of inbred mice. The cardiac damage was sufficient to induce hypoperfusion of the brain. Since the cardiac toxicity has not been previously reported, we characterized the mechanism of toxicity. Compared to control saline treated mice, heart mitochondria from 3NPA treated mice displayed up to 95% decrease in the rate of oxygen consumption with succinate, which is a substrate of complex II, the molecular target for 3NPA. Additionally, a 30-50% decrease in the rate of oxygen consumption in mitochondria with malate and glutamate in 3NPA treated mice were observed compared to controls. We also found that heart mitochondria from 3NPA treated mice built less mitochondrial membrane potential, which collapsed much faster when compared to mitochondria from control mice. In addition, mitochondria from treated mice showed a higher sensitivity to calcium-induced membrane depolarization. These effects were completely inhibited by cyclosporin A, suggesting the involvement of the permeability transition pore in the mitochondrial damage mechanism. In conclusion, our results suggest that 3NPA-induced cardiac toxicity

may be due to accumulation of damage leading to membrane permeability. This in conjunction with a decreased ATP production would, in turn, lead to cellular dysfunction.

60. Bosetti Francesca Tendi,Elisabetta Chickale,Elsbeth Rapoport,Stanley I. SBPM, NIA, NIH Bldg 10 Rm 6C103, 9000 Rockville Pike, Bethesda, MD 20892 frances@mail.nih.gov

Aluminum exposure decreases mitochondrial cytochrome oxidase gene expression in PC12 cells

Brain and CNS are known as major targets of aluminum (Al) toxicity and high levels of accumulation of the metal in these tissues has been implicated in a number of neural diseases such as dialysis encephalopathy, Alzheimer disease and Parkinson syndrome. Although Al is apparently involved in a broad spectrum of physiological disorders, mechanisms of its toxicity remain largely unknown. Since adult neurons are difficult to maintain in culture, we used rat pheochromocytoma (PC12) cells in culture as a model for testing the effects of aluminum on neurons. Aluminum uptake into PC12 cells was evaluated at a pH of 7.4 using increasing extracellular aluminum concentrations from 1 to 200 uM. Aluminum intracellular levels were determined using atomic absorption spectrophotometry. After a 6 h incubation, internalized aluminum increased linearly in response to aluminum extracellular concentration. Northern blot analysis of total RNA from control and Al-exposed PC12 cells indicated that COX III mRNA levels were reduced between 67 and 85% in Al-treated cells as compared to control. Hence, this reduction in COX III mRNA provided experimental evidence for the hypothesis that an impairment of mitochondrial function may be involved in Al neurotoxicity. Since COX subunits I-III are required for catalytic activity of this enzyme, a decrease in expression of COX III mRNA could be associated with decreased COX activity and defective energy metabolism. A deficit in oxidative energy metabolism due to loss of COX III could interrupt neuron-to-neuron communication and result in synaptic failure.

61. Lenaz Giorgio D'Aurelio,Marilena Bernacchia,Andrea Biondi,Anna Lisa Paolucci,Ugo Bovina,Carla; Formiggini,Gabriella; Parenti Castelli,Giovanna Dept. of Biochemistry, University of Bologna, Via Irnerio 48, 40126 Bologna, Italy lenaz@biocfarm.unibo.it

BIOMARKERS OF MITOCHONDRIAL FUNCTION IN PLATELETS AND LYMPHOCYTES

The role of mitochondrial dysfunction in pathological processes and in aging has been recently emphasized. A common denominator of many such processes may be oxidative stress by reactive oxygen species produced by the mitochondrial respiratory chain and affecting mitochondrial function with a vicious circle leading to collapse of cellular bioenergetics. The search for suitable biomarkers of mitochondrial function must consider using cells containing mitochondria. The use of blood cells follows the rationale that in aging, and perhaps in most age-linked degenerative pathologies, a systemic alteration of mitochondrial function is probably present. Methods developed by now to monitor mitochondrial energetic capability in intact cells are the following: (a) decrease of antimycin A inhibition of energy-dependent platelet aggregation; (b) decrease of antimycin A-induced enhancement of lactate production (Pasteur effect) in platelets or lymphocytes; (c) increased sensitivity to uncouplers of the lymphocyte mitochondrial membrane potential, detected by flow cytometry with suitable probes; (d) overexpression of plasma membrane NADH oxidase in lymphocytes, to release excess glycolytic reducing power. We have characterized these markers in pathological conditions where the mitochondrial function is impaired and in aging. Moreover it is possible to use these markers to monitor cellular effect of molecules affecting mitochondrial function. In particular we have used Pasteur effect to assay possible cellular toxicity of Coenzyme Q homologs and analogs used as mitochondrial acceptors.

62. Kaguni Laurie Lefai,Etienne&&2 Ruiz de Mena,Inmaculada&&1,2 Fernandez-Moreno,Miguel A.&&2 Alahari,Anuradha&&1 Garesse,Rafael&&2 Department of Biochemistry, Michigan State University, East Lansing MI 48824-1319 USA~`~Instituto de Investigaciones Biomedicas

Differential Regulation of Mitochondrial DNA Replication Genes in Drosophila

The structure of the D. melanogaster genes encoding the mitochondrial single-stranded DNA-binding protein (mtSSB) and the two subunits of mitochondrial DNA polymerase (pol gamma) was determined, and their patterns of expression evaluated during Drosophila development. The mtSSB gene is transcribed at variable levels throughout development. Two Drosophila DNA replication-related elements (DRE) and a single putative E2F binding site are present within the promoter region. The DRE sites are required for efficient promoter activity in Schneider cells, and gel shift analyses show that DRE binding factor (DREF) binds to them. The pol gamma genes are located in a cluster of five genes that contains two bidirectional promoters. Northern analysis indicates that the steady-state level of pol gamma-beta mRNA increases during early embryonic development, reaching its maximum preceding the start of mitochondrial DNA (mtDNA) replication. In contrast, the steady-state level of pol gamma-alpha mRNA decreases as development proceeds and is low in stages of active mtDNA replication. The pol gamma-beta promoter contains a DRE site that is essential for promoter activity, and gel shift analyses show DREF binds to this site. In contrast, the expression of the pol gamma-alpha gene is directed by a weak promoter that lacks a DRE element. DREF regulates the expression of several key proteins involved in nuclear DNA replication. Its role in controlling the expression of the mtSSB and pol gamma-beta genes demonstrates the presence of a common regulatory mechanism linking nuclear and mitochondrial DNA replication in Drosophila.

63. LIM SUSAN Longley,Matthew J. Copeland,William C.
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The mitochondrial p55 accessory subunit of human DNA polymerase enhances DNA binding, promotes processive DNA synthesis, and confers NEM resistance

Human DNA polymerase gamma is composed of a 140 kDa catalytic subunit and a smaller accessory protein variously reported from 43 - 54 kDa. Immunoblot analysis of the purified, heterodimeric native human polymerase gamma complex identified the accessory subunit as 55 kDa. We isolated the full-length cDNA encoding a 55 kDa polypeptide, expressed the cDNA in E. coli, and purified the 55 kDa protein to homogeneity. Recombinant Hp55 forms a high affinity, salt-stable complex with Hp140 during protein affinity chromatography. Immunoprecipitation, gel filtration and sedimentation analyses revealed a 190 kDa complex indicative of a native heterodimer. Reconstitution of Hp140oHp55 raises the salt optimum of Hp140, stimulates the polymerase and exonuclease activities, and increases the processivity of the enzyme by several 100-fold. Similar to Hp140, isolated Hp55 binds DNA with moderate strength and a specificity for double stranded primer-template DNA. However, Hp140oHp55 has a surprisingly high affinity for DNA, and kinetic analyses indicate Hp55 enhances the affinity of Hp140 for primer-termini by two orders of magnitude. Thus the enhanced DNA binding caused by Hp55 is the basis for the salt tolerance and high processivity characteristic of DNA polymerase g. Observation of native DNA polymerase g both as an Hp140 monomer and as a heterodimer with Hp55 supports the notion that the two forms act in mitochondrial DNA repair and replication. Additionally, association of Hp55 with Hp140 protects the polymerase from inhibition by N-ethylmaleimide.

64. Mott J G. Denniger(1) S.J. Zullo(2) H.P. Zassenhaus(1) C.R. Merril(2)
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The intron-exon borders in the mitochondrial DNA polymerase gamma gene of mouse (Polg) and human (POLG) are located in homologous positions

Our laboratories have previously isolated the mouse mitochondrial DNA polymerase gamma (Polg) cDNA (SLU), and by utilizing the yeast pol gamma coding sequence have isolated bacterial artificial chromosomes (BAC) containing the human POLG and the mouse Polg (NIH). We localized the gene to mouse chromosome 7E and the human gene (POLG) to the homologous region of human chromosome 15q24-q26 (NIH). In this report, we have determined the intron-exon borders of the full-length mouse gene contained on one BAC. These borders of the mouse Polg coincide with the homologous positions of the human POLG intron-exon borders. We discuss the intron-exon organization with respect to the functional sites of the protein.

65. Overman R. Glenn Stokes,Jayme B. Farwell,Mary A.
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Isolation of Genomic DNA from the Human Mitochondrial Translation Initiation Factor 2 (MTIF2) Gene Using Long-Distance and Ligand-Mediated PCR

Our laboratory is interested in mitochondrial protein synthesis, in particular the genes that are required for mitochondrial translation. We have been studying the nuclear-encoded gene for human mitochondrial translation initiation factor 2, which catalyzes the binding of initiator tRNA to the small ribosomal subunit in the presence of GTP. The goal in our laboratory has been to isolate the genomic copy of MTIF2 to determine the number, position, and length of exons and introns within the gene relative to the published liver cDNA sequence. We are also interested in characterizing the promoter. Our previous approach was to screen a human genomic library in Lambda Fix™ by hybridization with the complete cDNA as a probe. Using this method, we obtained 23 Kb of genomic sequence data that contains 75% of the 3' end of the liver cDNA sequence. A second screening using the 5' half of MTIF2 liver cDNA sequence as a probe failed to isolate new clones. Using long-distance polymerase chain reaction as an alternative to further library screening, we amplified fragments of MTIF2 directly from human genomic DNA. By using Ligand-Mediated PCR (LM-PCR) in addition to LD-PCR, five genomic DNA clones were isolated that contain sequence representing the remaining 25% of the liver cDNA sequence and approximately 1.7 Kb of 5' flanking region. We are currently analyzing the flanking region to identify putative promoter elements.

66. Vernon Jamie Burr,Patrick C.&&1 Wiley,John E.&&2 Farwell,Mary A.&&1
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Genomic cloning and chromosomal localization of the human mitochondrial elongation factor Ts gene (TSFM)

We have been characterizing genes for mitochondrial protein synthesis factors, all of which are nuclear-encoded. One of these factors, mitochondrial elongation factor Ts, catalyzes guanine nucleotide exchange for a separate factor, mitochondrial elongation factor Tu. A 14.3 kb genomic clone containing part of the elongation factor Ts gene (TSFM) was isolated and sequenced with automated DNA sequencing. The clone contains exons in the 5' half of the TSFM gene and 5000 bases of sequence 5' to the AUG initiation codon. Putative promoter sequences have been identified. We used the TSFM clone in fluorescence in-situ

hybridization (FISH) analysis. Inspection of metaphase spreads show that human TSFM is located on chromosome 12. G-banding analysis refined the localization to 12q13-->q14. In addition, a somatic cell hybrid mapping panel was used to confirm the location. Two primers flanking an intron-exon junction near middle of the gene were designed to DNA sequence data. These primers were used to carry out polymerase chain reaction (PCR) with DNA from somatic cell hybrids serving as the template. Human parental DNA served as the positive control, and rodent genomic DNA served as the negative control. DNA from chromosome 12 gave a signal of the correct size on an agarose gel, confirming the localization of TSFM to human chromosome 12.

67. Wei, Baoxian Henry Weiner, Henry Biochemistry Department, Purdue University, West Lafayette IN 47907-1153 weiner@biochem.purdue.edu

Can every protein be brought into the mitochondria? An investigation of the in vivo import of a cytosolic protein into yeast mitochondria

The earliest studies on mitochondrial protein import showed that a leader could bring any carrier protein into mitochondria. We have gathered data to suggest that this might not be the case in that the N-terminal portion of some carrier proteins could affect the structure of leader and affect its ability to function. More recently we have investigated the ability of the leader of mitochondrial aldehyde dehydrogenase to bring yeast or liver cytosolic ALDH into yeast mitochondria. We found in an in vitro assay that only 10% of the liver cytosolic protein were imported compared to the real precursor construct. Experiments were performed in yeast strains that had all their ALDHs disrupted. Here we found that no detectable levels of either liver or yeast ALDH could found in the mitochondria of the transformed cells. In contrast both the actual yeast and liver precursor proteins were brought into the mitochondria during cell growth. To be imported the carrier portion of the protein must be unfolded and leader stable to non-specific proteolysis in the cytosol. It appears that in the case of cytosolic ALDHs constructs improper folding leads them to become subjected to proteolysis and not be imported. Thus if proteins are to be designed to be brought into mitochondria to complement damaged proteins it will be necessary that they interact properly with HSPs to remain unfolded and to have a leader that is not susceptible to proteolysis. This research was supported in part by a grant from NIAAA.

68. Mannella Carmen Slepchenko,Boris Mannella,Carmen&&2 Loew,Leslie Univ. Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06030-3505~`~Wadsworth Center, Albany, NY 12201-0509

ROLE OF CRISTAE MORPHOLOGY IN REGULATING MITOCHONDRIAL ADENINE NUCLEOTIDE AND H+ DYNAMICS

Mitochondrial morphology is highly dependent on the level of respiratory activity. However, it is unclear whether the transition between orthodox and condensed states plays a significant role in regulating mitochondrial function. Recent 3D images obtained by EM tomography have provided new insights into cristae morphology, suggesting that gradients in ion or metabolite concentrations may occur in the intracristal space. We used the Virtual Cell modeling system to test this hypothesis. Both 2D and 3D models showed neither steady state nor dynamic gradients in [H+] even at maximum pumping rates. However, significant gradients in [ADP] may occur. We therefore included full kinetic models of the ATP/ADP antiporter and the FoF1 ATP synthase in the simulations. Depending on the number and size of the cristae compartments, the system showed a wide range of steady state spatial distributions in [ADP], both within the matrix and the intracristal space. Static and dynamic gradients in [ADP] between cristae and cytosol were as large as 80%, with concentrations in the cristae below 10 uM. Surprisingly, the consequence of the observed distributions is that the condensed state (associated with highly phosphorylating mitochondria) appears to be less efficient, having a significant amount of inner membrane

surface working under conditions suboptimal for ATP synthesis. However, this state is more responsive to large changes in energy demands by the cytosol. (Supported by NIH grants GM35063 and RR13186)

69. CrivelloneMary Shi,Guanfang Molecular Biology Department, University of Medicine and Dentistry of New Jersey, 2 Medical Center Drive, Stratford, NJ 08084 crivelmd@umdnj.edu

Chaperone-Assisted Assembly of Complex III of the Yeast Respiratory Chain

The Cbp3 protein of Saccharomyces cerevisiae is an enzyme-specific chaperone required for the assembly of ubiquinol-cytochrome c reductase (complex III) of the mitochondrial respiratory chain. To gain an understanding of the mechanism by which Cbp3p assists in the assembly process, 40 independently isolated mutants were analyzed to identify functional regions of the protein. Three domains essential for Cbp3p activity were defined by examining the assembly of complex III and steady state amounts of Cbp3p plus the respiratory enzyme subunits in 23 point mutants. Domains #1 and #3 were required for Cbp3p function, while domain #2 was necessary for protein stability. Mutation of Glu134 in domain #1 (defined by Cys124 to Ala140) resulted in the inability of the Rieske FeS protein to assemble with the enzyme complex. Mutations targeted to domain #3 (Gly223 to Asp229) affected the assembly of the 14 kDa subunit, cytochrome c1 and the Rieske FeS protein. Domain #2 (Leu167 to Pro175) overlaps the singular, putative hydrophobic region of Cbp3p. Mutations in this area altered the association of Cbp3p with the mitochondrial membrane and resulted in a rapid turnover of the protein. Deletion mutants D12-23, D 24-54 and D 56-96 were examined to determine the role of the amino-terminus in Cbp3p functional activity. All three mutants were respiratory competent indicating that residues 12 through 96 were not essential for Cbp3p function, stability or import. Analysis of carboxy-terminal deletion mutants demonstrated that the terminal 44 residues were not necessary for Cbp3p function, however, alterations in the secondary structure of the extreme carboxy-terminal 17 residues compromised the functional activity of the assembly protein. We show that Cbp3p is an extrinsic membrane protein whose functionally significant regions are located between residues 97 and 291. Approximately 58% of the protein is essential for mitochondrial biogenesis. Our working hypothesis concerning the role of Cbp3p in complex III assembly will be presented.

70. Saada Ann Aptowitzer,Iris 1 Link,Gabriela 2 Elpeleg,Orly N 1 Metabolic Disease Unit Shaare Zedek Medical Center p.o.b.3235 Jerusalem 91031 Israel~`~Department of Human Nutrition and Metabolism, Hebrew University Hadassah Medical School, Jerusalem Israel saada@szmc.org.il

ATP Synthesis in Lipoamide Dehydrogenase Deficiency

Lipoamide dehydrogenase deficiency is an inborn error of several metabolic pathways, including pyruvate metabolism, Krebs cycle and branched-chain amino acid degradation. The clinical course is variable ranging from infantile neurodegenerative disease to recurrent episodes of liver failure or myoglobinuria starting later in life. In contrast, residual enzymatic activity in muscle tissue spans over a narrow range. Despite the recent elucidation of the underlying molecular pathology in most patients, relationships between the genotype and the biochemical and clinical phenotype remain unclear. In order to find a suitable assay for the prediciton of clinical outcome and assessment of treatment, we have evaluated enzymatic activites and energetic states in fibroblasts from lipoamide dehydrogenase deficient patients representing three different phenotypes and genotypes. Direct relationship between clinical parameters such as age of onset and disease severity and biochemical characteristics including lipoamide dehydrogenase activity, pyruvate dehydrogenase activity and ATP production ratio in fibroblasts, were identified. Clinical parameters were not reflected by lactate/pyruvate ratio. ATP production rate was in direct relationship with the severity of the neurological involvement; the patient with reduced ATP synthesis ratio to 33% of control mean had a neurodegenerative disease whereas ATP synthesis values above 45% were associated with a more favourable course. Incubation of the patients' fibroblasts with dichloroacetate and thiamine resulted in slight but significant improvenent of ATP production.

From: Merril Allen [allennco@bellatlantic.net]
Sent: Wednesday, February 02, 2000 07:36
To: zullo@helix.nih.gov
Subject: Feelings....from a mito patient..

Dr Zullo:

I am an active member of a list that is restricted to older mito patients. I am 57 years old, was first diagnosed in the 70s and have biopsy confirmed mito, OXPHOS problems, hepC (from 5x bypass 1985), a long cardiac history, and much more.

I recently was in the hospital for a very complete workup. Although I do not yet have all the results of the tests and procedures performed during the hospitalization, I certainly have the emotional scars suffered by many mito patients as they thread the maze of medical care for a very unknown (to the majority) disease.

I have tried to put those feelings into words. When I saw your reminder about the March symposium, I thought you might be quite interested in "the other side of the story."

Thank you... Merril Allen.... a long-time mito

> Steve Zullo has my permission to use my writings and also "personal" information about me at his discretion for the March Minisymposium and any events connected with it.
> Merril Allen...Forked River NJ .... 7 March 2000
>
> Thanks again, Steve, for helping me to reach out to others.
>
> PS: I was awarded the 1999 Ocean County, NJ, (Superior Court) Judicial Volunteer of the Year Award. The plaque says: "In grateful acknowledgement for the new standard you have set for promoting a better quality of justice in our courts, and a better quality of life in our community. Your selfless dedication to the youth of Lacey Township and Ocean County has left an indelible imprint which will endure well into the Twenty First Century."
>
> I went up to receive the award in an electric powered wheelchair; I thought I would still be playing softball at age 57. I have been a judicial volunteer since 1994.

****

PERSPECTIVES...

I am mourning the loss of me.
The dependable, reliable, strong, cheerful, capable, able to meet any challenge, me.
I am not handling this loss very well.

At every turn I am frustrated by the limitations of the now less dependable, less reliable, much less cheerful (often sad), much less capable, often not willing to meet a challenge, me.

There was a time when "can't" was not in my vocabulary. The jock in me said there is always a way if you try a little harder. Now I must struggle to keep that "c" word out of my mind, my words, and my actions. The jock in me says "I am still here," but the mind and the body do not seem to be able to respond without an energy output greater than I can even think about.

Is the negative response that I get from some loved ones based on their inability to accept the differences in me?? Or is it based on the fact that the "new me" is someone they would not want to be around anyhow?? If it is the first, I am not sure what I can do to help them. If it is the second, I am not even sure what I can do to help me.

I am so tired; both physically and mentally.

I am tired of managing my own care and have sought to change that, but with no real success. I am not alone. Many others that I know are in the same position.

I am tired of waiting. When you are on the receiving end of services, you have little, if any, control. It would seem that there is little understanding of what it is like to wait to hear things about yourself. "Good" or "bad", the coping and adjusting process cannot begin without the "knowing." In reality, few of us are able to take things on "blind faith."

I am tired of being told that "Lots of people are willing to help you. You must learn to depend on them. Just ask; they want to help you." I don't doubt for a moment that they WANT to help and I know the old me loved to do things for people; to be honest, the new me does, too. But, the new me struggles to try to depend on people who WANT to help, but basically have their own lives to live and must "fit me in." I do not do well with that. I am not sure that I ever will.

As my own physical resources continue to fail, one by one, and sometimes two by two, it becomes more and more difficult to maintain a cheerful outlook. Each day is a challenge that I TRY to meet. My legs work less well, the numbness in my hands is almost to the point where I am not sure I can compensate, my vision has good days/bad days, my bladder has days when I am not entirely sure who has control, and today, in addition, my ribs and diaphragm are sore for some as yet unknown reason. My liver is not well. I feel that my brain is not well, either. I keep finding new ways to compensate; how many more are there?

I yearn to go sailing, play softball or racquetball, shovel snow, even swim. I long for the days that running six errands in 55 minutes was a reality. I would like to be able to curl up on my side on the bed and have a good cry. Alas, none of this is currently available to the "new" me. My balance is bad, my limbs are weak and shake, and if I don't sleep flat on my back all night, every night, my legs become more numb than they get even sleeping on my back. Going anywhere takes a lot of planning and time.

And as I mourn the old me, I remind the new me that I am fortunate. I am. This is a world filled with technology. As I sit here at the computer I am humbled by the thought that 20 years ago I would not have had even this outlet for my frustration. I am married to a wonderful person. We are financially "comfortable." We have a lot of friends.

There are many people who have said that they really do WANT to help. Will I be able to accept that help when I am used to being the "helper", not the "helpee"? I don't know.

What will I do? Well, I guess I will locate one of those inflatable "punch-me" things. Maybe if I knock it down several times each time I feel frustrated, I will knock down some of my frustration, too. Frustration (and/or anger) is bad for the spirit and the metabolism. I will keep searching for solutions that might eliminate some of the problems. I will try to learn to depend more on other people because eventually this will not be a choice. I will continue to mourn the old me and I will do my best to accept the new me. No promises.

****

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Wednesday 1st March 2000

Dr Steve J Zullo
2nd NIH Mitochondria Minisymposium
"Mitochondria: Interaction of Two Genomes"
14th & 15th March 2000

Dear Steve,

On behalf of the Children's Mitochondrial Disease Network. Our President, Advisors & European Correspondents.

May I take this opportunity to thank you for allowing us to submit this small insert into the final abstract book. I would like to wish you, your group at NIH & all delegates a very successful, informative and fruitful symposium.

I would like to wish all your sponsors well including the UMDF, for their work in the United States, with Mitochondrial Disease. I would like to inform all the delegates about our new Mitochondrial Homepage @ www.childrensmdn.co.uk, and hope they may consider paying us a visit, the site is aimed at the professional & parent alike, offering a keyhole into a family with children affected by mitochondrial disease, additionally the work EMDN has been involved with in the past and future events. Such as the VIII International Congress of Metabolism, Cambridge UK, September. Details at the SSIEM Web Site. www.ssiem.org.uk

All our best wishes to all at the "Mitochondria Minisymposium"

Yours Sincerely

Paul Preston
Honorary Director

Providing: Information, Support & Awareness for all Mitochondrial Disorders
Patron: John Savident a.k.a. 'Fred Elliot from ITV's Coronation Street'
President: Professor Tony H V Schapira, DSc, MD, FRCP, FMedSci, Head of Department and Professor of Clinical Neuroscience,
The Royal Free Hospital School of Medicine, London, England
Hon. Director: Paul Preston (founder)
Headquarters: MAYFIELD HOUSE, 52 Winnington Lane, Northwich, Cheshire CW8 4DQ, England
Helpline & Fax: 01606 - 76112 (24hrs) Email: pprestonmitonet@netcentral.co.uk Web Site: http://www.netcentral.co.uk/emdn Est. January 1998

United Mitochondrial Disease Foundation
1998 Fact Sheet

The United Mitochondrial Disease Foundation (UMDF) is the result of a merger between a number of specific mitochondrial disease organizations to form a larger, more cohesive **UNITED** foundation representing all mitochondrial diseases and all sufferers, adult and children alike.

The three-fold purpose of the UMDF is straight-forward: To educate the public and the medical community about these complex diseases in order to help sufferers obtain an earlier diagnosis and better treatment; to support affected families; and above all, to fund research for a cure. A continued **UNITED** effort is the key to addressing these purposes.

A Brief History of UMDF
Since its Inception in 1995

- The 3rd annual UMDF "Mito-What?" Run, in Santa Barbara, was another success. In the last 3 years this "Mito-What?" run has profited more than $40,000 for mitochondrial research.

- The first ever UMDF Golf Outing in Pittsburgh, PA, was held June 25, 1998 and successfully raised over $20,000.

- Two of the UMDF founders single-handedly planned and executed the only two international mitochondrial symposia for scientists and families held in the United States. The first in Indianapolis, by UMDF Director Lee Neff in 1995; the second, in April 1997, by UMDF Director Marsha Barnett was attended by 200 families and 250 doctors and physicians from around the world.

- UMDF contributed $20,000 to underwrite the cost of parents of affected children who could not afford the travel expense to the 1997 symposium held in Philadelphia, PA.

- UMDF is investigating hosting the next symposium in the year 2000.

- UMDF awarded $30,000 in 1997, and $35,000 in 1998, to researchers supporting its mission of "promoting research for cures and treatments of mitochondrial disorders."

- UMDF plans to award a substantial grant each year and hopes to fund a half million dollar endowment by the year 2005 to ensure grant funding for the future.

- UMDF has established a web site, http://biochemgen.ucsd.edu/umdf, which provides amazing information on mitochondrial diseases and issues that affect the sufferers and their families. The web site also provides links to over 75 other informative and pertinent sites.

- The UMDF Scientific Advisory Board is composed of 17 of the top specialists and researchers in the fields of metabolism, genetics, neurology and biochemistry from around the world. These advisors represent a variety of specialties, clinics and labs.

- The UMDF maintains a database that organizes mitochondrial symptoms and diseases so that sufferers can connect with others with similar experiences and situations. This database is three years in the making and is the result of a collaboration between computer programmers, parents and physicians. What makes this registry so important is the fact that mitochondrial diseases present differently from individual to individual, so that until now affected patients had no easy way to share with others with similar symptoms.

- The UMDF maintains a library and catalogs over 1500 articles specific to mitochondrial disease.

- The UMDF publishes a newsletter that covers a variety of topics including current medical information, advocacy, insurance, chronic illness, family support and many other issues of relevance to UMDF members.

- The UMDF currently has a mailing list of over 6000 families, professionals and donors; this database includes over 1000 individuals affected with mitochondrial diseases.

- The National Leigh's Foundation (NLDF), located in Corinth, Mississippi, dissolved and conveyed all its assets and membership to UMDF in 1996.

- ABCD, located in Chicago, dissolved and conveyed all its assets to UMDF in 1998.

- The UMDF has re-located its office to the first floor of the S&T Bank Building at 7660 Saltsburg Rd., Pittsburgh, PA 15239. Mail correspondence still remains P.O. Box 1151, Monroeville, PA 15146-1151.

- The UMDF office is located in a 600 sq. ft. room with an adjoining 600 sq. ft. room available for additional storage and work space.

- The UMDF office is fully furnished with 3 state of the art computerized workstations and new phone system. Our database programmers have addressed and solved the year 2000 problem.

United Mitochondrial Disease Foundation, P.O. Box 1151, Monroeville, PA 15146-1151, Phone: (412) 793-8077, FAX: (412) 793-6477

United Mitochondrial Disease Foundation
1998 Fact Sheet

- UMDF currently has a staff of 2 part-time administrative assistants.

- UMDF has established a search committee to review résumés for a full time Executive Director.

- The UMDF is in the process of establishing a Nationwide Parent Support Network to further help families connect with one another and to provide more information about local resources.

- The UMDF office currently receives an average of 250 phone calls each month. This activity continues to emphasize the need to adhere to our mission to: "Provide support to affected families."

- UMDF has formed a Family Support Committee (FSC), chaired by UMDF Trustees certified in counseling. Meetings have taken place with the Delaware Valley and Southern California Support Groups.

Additional Facts

- There are more than 50 inherited diseases of metabolism that are known to affect mitochondria.

- Mitochondrial diseases are common affecting as many as 1 in 1,000 children with many of the cases causing severe damage to the brain, muscle and heart, and is fatal in many circumstances. Four million children are born in the US each year. This means that 1000 children could be born each year with a mitochondrial disease. By comparison, about 8000 new cases of childhood cancer are reported each year.

- Both mitochondrial disease and childhood cancers range in mortality from 10 to 50 percent per year, depending on the specific disease.

- Defects in mitochondrial function have now been linked to many of the most common diseases of aging such as Parkinson Disease, Alzheimer Dementia, Osteoporosis, and Cancer. Over 50 million people in the US suffer from these degenerative disorders.

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CARNITINE DEFICIENCY

Carnitine deficiency is a condition that results when carnitine is not present in sufficient amounts. Carnitine deficiency may be a side effect from treatments for other disorders or a result of genetic defects or dietary insufficiency.

Some treatments, including those for kidney dialysis and epilepsy, can cause carnitine deficiency. In addition, diseases such as inborn errors of metabolism also can cause carnitine deficiency.

Carnitine has two major functions: 1) transport of long-chain fatty acids into mitochondrial matrix for further oxidation; and 2) modulation of intracellular CoA homeostasis, which is reflected by the plasma A/F

SYMPTOMS[1]

Some of the clinical findings that may be associated with carnitine deficiency:

- Developmental delay
- Hypotonia
- Hyperammonemia
- Inappropriate ketosis
- Hypoglycemia
- Myopathy
- Cardiomyopathy
- Pancytopenia
- Periodic episodes of acidosis
- Reye-like syndrome
- Recurrent infection
- Seizure disorder
- Encephalopathy

PLASMA A/F RATIO (acyl/free carnitine ratio)

The plasma A/F ratio provides an excellent way of determining whether adequate free carnitine is available to clear metabolic waste products effectively.

Commonly, laboratories report only the plasma total and free carnitine levels, along with their normal values, when a carnitine assay is requested. We can learn more about the carnitine status of patients by making two simple calculations:

Plasma Total – Free Carnitine = Plasma acylcarnitines

Plasma Acylcarnitines ÷ Free Carnitine = Plasma A/F ratio

Plasma A/F ratio = 0.25 is considered normal.
Plasma A/F ratios > 0.4 indicate carnitine insufficiency. [2,3]
Plasma free carnitine < 20 µmol/L indicates carnitine deficiency.[4]

Carnitine deficiency exists when there is insufficient carnitine to buffer toxic acyl-CoA compounds.[3] This (abnormal) state is referred to as carnitine insufficiency, indicating that more carnitine is needed to handle any increased need for the production of acylcarnitines.[5] Levocarnitine is indicated in disorders with a low (plasma) free carnitine or elevated (plasma A/F) ratio.[6]
Mitochondria Minisymposium supported by Unrestricted Educational Grant from Sigma Tau Pharmaceuticals, Inc.

[1] Naviaux RK. The spectrum of mitochondrial disease. *Primary Care Physician's Guide: Mitochondrial and Metabolic Disorders* 1997; 10.
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[3] Stumpf DA, Parker WD, Angelini C. Carnitine deficiency, organic acidemias, and Reye's syndrome. *Neurology* 1985; 35: 1041-1045.
[4] De Vivo DC, Tein I. Primary and secondary disorders of carnitine metabolism. *Intl Pediatr* 1990; 5: 134-141.
[5] Carter AL, Abney TO, Lapp DF. Biosynthesis and metabolism of carnitine. *J Child Neurol* 1995; 10 (suppl 2): 2S3-2S7.
[6] Winter SC. Diagnosing metabolic disorders: one step at a time. *Contem Pediatr* 1993; 10:35-56.


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