National Institute on Aging
Office of Intramural Research
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National Institute on Drug Abuse
Mitochondria Interest Group

MITOCHONDRIA INTEREST GROUP

4TH INTERNATIONAL MITOCHONDRIA MINISYMPOSIUM 2008

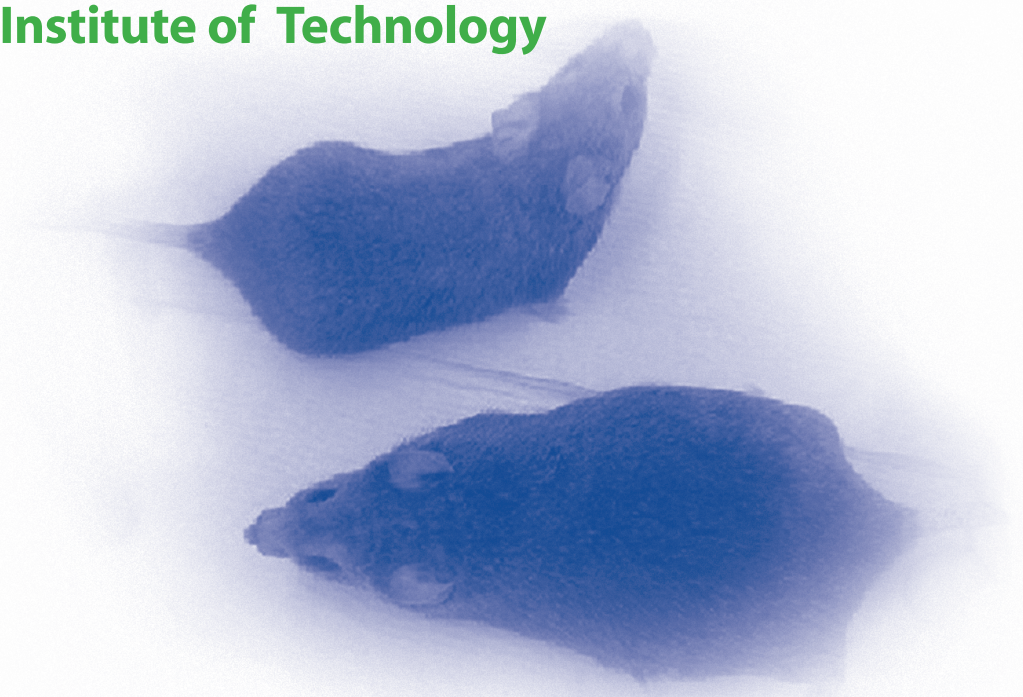
The Interaction and Independence of Sirtuins and Mitochondria: A Few NIH Perspectives

November 19, 2008 | Masur Auditorium | Clinical Center | National Institutes of Health | 8:30 a.m.

Featuring the **Wednesday Afternoon Lecture**

Sirtuins, Aging and Disease

Dr. Leonard Guarente
Massachusetts Institute of Technology
3:00 p.m.



The 4th Mitochondria Minisymposium
The Interaction and Independence of Sirtuins and Mitochondria: A few NIH Perspectives

Wednesday, November 19, 2008

Convened to accentuate the Wednesday Afternoon Lecture of Leonard Guarente (MIT).

7:45-08:30 Set Up Posters, Registration

8:30-10:00 Session I: Karen Usdin (Chair)

8:30-09:00: Karen Usdin (NIDDK) *The dark side of SIRT1*

9:00-09:30: John Hanover (NIDDK) *Sirtuins and O-GlcNAc: Interwoven threads in the fabric of the cellular stress response*

9:30-10:00: Rui-Hong Wang (NIDDK): *Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice*

10:00-10:20 Networking Break and Poster Session

10:20-11:50 Session II: Curtis Harris (Chair)

10:20-10:50: Curtis Harris (NCI): *p53-dependent cellular senescence*

10:50-11:20: Jaime Ross (NIDA-Karolinska Institutet): *Premature aging in POLG knock-in mice*

11:20-11:50: David Gius (NCI): *SIRT3 is a mitochondrial tumor suppressor gene*

11:50-12:40 Lunch and Poster Session (Guarente dines with Fellows)

12:40-2:40 Session III: Catherine Wolkow (Chair)

12:40-1:10: Catherine Wolkow (NIA): *IIS and FOXO signaling in C. elegans: Unraveling the webs of direct and indirect targets that regulate longevity and diapauses*

1:10-1:40: Mark Mattson (NIA): *Adaptive stress response pathways in neurons*

1:40-2:10: Vilhelm Bohr (NIA): *Mitochondrial DNA Repair*

2:10-2:40: Toren Finkel (NHLBI): *Sirtuin regulation of mitochondrial function*

2:40-2:55 Networking Break (Guarente prepares for WAL)

3:00-4:00 Wednesday Afternoon Lecture:
Leonard Guarente (MIT): "SIRTUINS, AGING AND DISEASE"

4:00-4:45 WAL Reception: Sponsored by NIH Scientific Directors

4:00-5:15: Poster Session

Wednesday Afternoon Lecture:

"SIRTUINS, AGING AND DISEASE"

Leonard Guarente

SIRTUINS, AGING AND DISEASE

Leonard Guarente
Department of Biology
MIT
Cambridge, MA 02139

SIR2 and related genes are NAD-dependent deacetylases that slow aging in yeast, *C. elegans*, and *Drosophila*. In yeast and flies, SIR2 genes are also involved in the longevity conferred by dietary or calorie restriction (CR). The mammalian SIRT homologs are involved in changes in stress resistance and metabolism known to be associated with CR. The CR diet not only extends life span in rodents, but also protects against many diseases of aging, including diabetes, cardiovascular disease, neurodegenerative disease, cancer and osteoporosis. In this talk, I will describe recent findings in the lab regarding SIRT1 function in specific mammalian tissues and in specific disease models. Our findings indicate a diverse array of effects exerted by SIRT1 in mammals.

Guarente, L. (2007). Sirtuins in aging and disease. *CSH Symp. Quant. Biol.* 72, 483-488.

Guarente, L. (2008). Mitochondria – a nexus for aging, calorie restriction and sirtuins?. *Cell* 132, 171-175.

SPEAKER ABSTRACTS

The Dark side of SIRT1: The case of Fragile X syndrome

Karen Usdin
National Institute of Diabetes and Digestive and Kidney Diseases
Bethesda, MD

Fragile X syndrome is the leading cause of heritable intellectual disability. The affected gene, *FMR1*, encodes a neuronally-expressed protein that regulates the translation of key brain mRNAs in response to synaptic activation. The causative mutation is an increase in the number of CGG*CCG-repeats located in the 5' end of this gene. Alleles with >200 repeats are silenced, a process which involves as yet not fully understood chromatin modifications as well as DNA methylation. Demethylation with 5-azadeoxycytidine, a DNA methyltransferase inhibitor, reactivates the gene. However, this reagent is toxic and, since it requires DNA replication for its efficacy, is not useful in neurons which no longer divide. We show here that deacetylation of lysine 16 on histone H4 is a key downstream consequence of DNA methylation. This deacetylation is carried out by SIRT1, a member of the Sirtuin family. Inhibition of SIRT1 with splitomicin allows acetylation by the histone acetyltransferase, hMOF, which relieves the gene silencing. Our data thus identify an important late step in the silencing of Fragile X alleles. Furthermore, since the efficacy of SIRT1 does not require DNA replication, our data suggest that SIRT1 inhibitors may be able to reactivate Fragile X alleles in neurons.

Sirtuins and O-GlcNAc: Interwoven threads in the fabric of the cellular stress response

John Hanover
National Institute of Diabetes and Digestive and Kidney Diseases
Bethesda, MD

The Hexosamine Signaling Pathway, terminating in the addition of O-GlcNAc to target proteins, is one of the key cellular responses to nutrient excess (See Figure 1). like the Surtuins, enzymes of O-GlcNAc addition are localized to the nucleus and mitochondia where they directly interact with the cellular acetylation and deacetylation machinery. O-GlcNAc transferase binds to histone deacetylase complexes; O-GlcNAcase has a histone acetyltransferase domain. Levels of the sugar nucleotide UDP-GlcNAc are highly responsive to concentrations of glucose, amino acids, and lipids. The large number of O-GlcNAc modified proteins includes transcription factors, nuclear pores, proteasomal subunits and signaling kinases. Enzymes of O-GlcNAc addition and removal, like the Surtuins are localized to the nucleus and mitochondia where they interact with the cellular acetylation and deacetylation machinery. To examine the function of hexosamine signaling in genetically amenable organisms, we have examined null alleles of OGT and the O-GlcNAcase (OGA) in *Caenorhabditis elegans*, *Drosophila melanogaster* and mice. Our data suggests that the enzymes of O-GlcNAc cycling "fine-tune" insulin-like signaling in response to nutrient flux. Therefore, OGT and O-GlcNAcase modulate insulin action provide a unique genetic model for examining the role of O-GlcNAc in longevity and aging, as well as insulin resistance and obesity.

Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice

Rui-Hong
National Institute of Diabetes and Digestive and Kidney Diseases
Bethesda, MD

In lower eukaryotes, Sir2 serves as a histone deacetylase and is implicated in chromatin silencing, longevity and genome stability. Here we mutated the SIRT1 gene, a homolog of yeast Sir2, in mice to study its function. We showed that a majority of SIRT1-null embryos died between E9.5-E14.5, displaying altered histone modification, impaired DNA damage response, and reduced ability to repair DNA damage. We demonstrated that SirT1+/-;p53+/- mice developed tumors in multiple tissues, whereas activation of SIRT1 by resveratrol treatment reduced tumorigenesis. Finally, we showed that many human cancers exhibited reduced level of SIRT1 than their normal controls. Thus, SIRT1 acts as a tumor suppressor through its role in DNA damage response, genome integrity, and tumor suppression.

TELOMERES "COMING UP SHORT": p53-DEPENDENT SENESCENCE

Izumi Horikawa[1], Kaori Fujita[1], Abdul Martin[1], Qin Yang[2], Borivoj Vojtesek[3], Jean-Christophe Bourdon[4], David P. Lane[4], and Curtis C. Harris[1]

[1]Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD, USA; [2]Department of Radiation Oncology, Washington University in St. Louis, St. Louis, MO, USA; [3]Department of Clinical and Experimental Pathology, Masaryk Memorial Cancer Institute, Brno, Czech Republic, [4]Department of Surgery and Molecular Oncology, University of Dundee/Inserm U858, Dundee, Scotland, United Kingdom

The p53 pathway is an intrinsic monitor and response pathway of telomeric attrition involved in cellular aging and senescence. Cellular senescence is tumor suppressive that can be activated by p53 in cancer cells. We are currently studying the molecular mechanisms of cellular senescence in normal human cells and the role of the telometric multiprotein complex, shelterin, that includes TRF2 and POT1. Our ongoing studies have revealed that p53 regulates both specific microRNAs and TRF2 expression as endogenous mechanisms of replicative senescence. In addition, POT1 isoforms are functionally diverse in both maintaining telomeric integrity and preventing p53-dependent senescence induced by telomeric shortening.

Premature aging in POLG knock-in mice

Jaime M. Ross
National Institute on Drug Abuse – Karolinska Institutet
Baltimore, MD

Mitochondrial dysfunction may underlie aging-related alterations in neuronal function and has been implicated in neurodegenerative diseases, such as Alzheimer's and Parkinson's, as well as in stroke. A homozygous knock-in mouse expressing a proof-reading deficient version of the nucleus-encoded catalytic subunit (PolgA) of mitochondrial DNA (mtDNA) polymerase has been developed to study the effects of progressive mitochondrial dysfunction, and shows many signs of premature aging (Trifunovic *et al.,* 2004). The mtDNA mutator mouse has a 3- to 5-fold increase in levels of random mtDNA point mutations as well as increased levels of random mtDNA deletions, with an approximately linear increase in mutation levels from midgestation to late adult life. The mouse has a reduced life span, reduced fertility, weight loss, reduced subcutaneous fat, enlarged heart, anemia, alopecia, kyphosis, osteoporosis, and sarcopenia. We are using Magnetic Resonance Imaging (MRI), ^{1}H Magnetic Resonance Spectroscopy (MRS), neurochemical techniques, as well as an array of microscopy-based methods to characterize the CNS in these mice. This lecture will focus on the severe metabolic alterations and striking structural disturbances observed in mtDNA mutator mice, and its relevance to the premature aging phenotype.

Loss of *SIRT3* Results in Altered Mitochondrial Integrity, Stress induced Genomic Instability, and a Tumor Permissive Phenotype

Krish Patel [1,2], Kristi Muldoon-Jacobs [1,2], Nukhet Aykin-Burns [3], Hyun-Seok Kim [4], J. Daniel Pennington [2], Riet van der Meer [5], Kheem S. Bisht [2], Phuongmai Nguyen [2], Jason Savage [2], Keshav K. Singh [6], Sarki A. Abdulkadir [5], Chu-Xia Deng [4], Douglas R. Spitz [3], and David Gius [2,6]

[2] Molecular Radiation Oncology, Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA, [3] Free Radical and Radiation Biology Program, Department of Radiation Oncology, University of Iowa, Iowa City, IA, 52242, USA, [4] Genetics of Development and Disease Branch, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA, [5] Department of Pathology, Vanderbilt University Medical Center, Nashville, TN 37232, USA, [6] Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

SUMMARY

Sirtuin family genes appear to play a role in aging-related processes and regulate factors critical to the maintenance of cellular integrity. In this regard we show that *SIRT3* knockout mouse embryonic fibroblasts (MEFs) and livers from SIRT3$^{-/-}$ mice exhibit decreased mitochondrial DNA (mtDNA) integrity, respiration, and ATP production. In addition, SIRT3$^{-/-}$ MEFs exposed to genotoxic stressors exhibit increased intracellular superoxide levels and genomic instability. These results suggest a role for *SIRT3* in the maintenance of mitochondrial integrity; however, a physiological phenotype may require a second event. Expression of a single oncogene (*Myc* or *Ras*) in SIRT3$^{-/-}$ MEFs results in *in vitro* transformation and *in vivo* tumorigenesis, and altered cellular metabolism. The addition of superoxide dismutase (SOD) prevents stress-induced genomic instability and transformation of SIRT3$^{-/-}$ MEFs by a single oncogene. These results identify *SIRT3* as a mitochondrial localized tumor suppressor gene (mlTSG) that connects mitochondrial longevity genes, oxidative metabolism, and carcinogenesis.

IIS and FOXO signaling in *C. elegans*:
Unraveling the webs of direct and indirect targets
that regulate longevity and diapause

Catherine A. Wolkow, PhD
Laboratory of Neurosciences,
National Institute on Aging
NIH
Baltimore, MD

In *C. elegans*, insulin/IGF-I-like signaling (IIS) regulates developmental diapause and adult longevity. The gene, *age-1*, encodes a PI3K p110 catalytic subunit which is the major effector for the insulin/IGF-I-like receptor (IIR) encoded by the *daf-2* gene. Loss of IIS during development leads to constitutive developmental diapause as dauer larvae, which are specialized for long-term survival in the absence of food. In adult animals, reduced *age-1* activity represses fertility and extends adult lifespan. These *age-1* phenotypes are completely suppressed by loss-of-function mutations in *daf-16*, which encodes a FOXO transcription factor. Thus, AGE-1/PI3K signaling antagonizes DAF-16/FOXO and allows larvae to bypass dauer arrest. Conditions that reduce AGE-1/PI3K signaling relieve this inhibition, enabling DAF-16/FOXO to direct the expression of dauer and prolongevity genes, such as *sod-3*.

We have previously demonstrated that IIS acts non-cell autonomously, within a variety of tissues, to rescue dauer arrest and longevity in *age-1* mutants. In contrast, DAF-16 regulates *sod-3* expression cell-autonomously. We have interpreted these findings in a model proposing that AGE-1 signaling regulates two types of outputs. Endocrine outputs (EO) of AGE-1 may coordinate dauer diapause and aging in tissues throughout the body through non-cell autonomous pathways. In contrast, cell-intrinsic outputs (CIO) of AGE-1 mediate cell-autonomous effects, such *as sod-3* expression. While the AGE-1 CIOs are likely mediated by direct regulation of DAF-16, the mediators of AGE-1 EOs are unknown. Candidates for this function are genes known to interact with, and enhance, signaling downstream of DAF-2/IIR and AGE-1/PI3K. Possible candidates include components of parallel signaling pathways, such as *sir-2.1*, one of four *C. elegans* sirtuin genes, as well as transcription factors that might act as DAF-16 co-factors, such *as hsf-1*, encoding the *C. elegans* heat-shock transcription factor.

To identify downstream pathways mediating the AGE-1/PI3K endocrine outputs, we conducted a microarray analysis of gene expression in animals with tissue-restricted IIS. These animals lacked genomic *age-1* activity, but carried integrated trangenes directing either intestine- or neuron-specific expression of a rescuing *age*-1 cDNA. This analysis identified several candidate targets of AGE-1/PI3K EOs that were rescued by either intestinal or neuronal *age-1* activity, including several HSP16 small heat shock protein genes. To confirm the microarray analysis, the expression of these candidates was also examined using transcriptional GFP reporters. In the GFP analysis, two genes, *cat-4* and *cyp-35B1,* demonstrated dauer-specific expression patterns, consistent with their regulation by AGE-1/PI3K signaling. We found that *cyp-35B1* constitutes a novel direct target of DAF-16/FOXO, whose dauer-specific expression was primarily regulated directly by IIS. In contrast, dauer-specific *cat-4* expression was indirectly regulated by IIS and DAF-16/FOXO, and was dependent on expression of the heat-shock transcription factor, *hsf-1*. Thus, HSF1 may be part of a pathway to coordinate responses to IIS in tissues at distant sites in the body.

Adaptive Stress Response Pathways in Neurons

Mark P. Mattson
Laboratory of Neurosciences, National Institute on Aging
Baltimore, MD.

Together with findings from other laboratories, our studies of neuroplasticity and animal models of neurodegenerative disorders have identified three environmental factors that protect neurons – exercise, dietary energy restriction and cognitive stimulation. These factors protect neurons by imposing a mild and transient cellular stress (ionic, energetic and oxidative) which results in the activation of adaptive stress response pathways and the induction of genes that encode proteins that promote neuronal survival, neurogenesis and synaptic plasticity. Examples of such neuroprotective signaling pathways include those involving Nrf2-ARE, cAMP-CREB and NF-□B. Gene targets of these pathways include those encoding neurotrophic factors such as BDNF, mitochondrial uncoupling proteins, protein chaperones and antioxidant enzymes. Such adaptive stress response pathways may not be activated or fail to function properly during aging, in neurodegenerative disorders and in conditions such as diabetes that place neurons at risk.

Mitochondrial DNA Repair

Vilhelm Bohr
National Institute on Aging
Baltimore, MD

DNA repair pathways in the nucleus and in the mitochondria differ significantly, and yet there is crosstalk. These pathways and recent understanding about them will be discussed. DNA repair proteins that function in the mitochondria appear to be associated with the inner membrane and there are indications that they may cooperate with proteins of the nucleotide. This will be discussed.

Sirtuin regulation of mitochondria function

Toren Finkel
National Heart, Lung, and Blood Institute
Bethesda, MD

We will discuss our recent evidence concerning a role for various sirtuin family members in the control of mitochondrial function. Our past observations have noted that Sirt1 can interact and deacetylate the transcriptional coactivator PGC-1alpha, known as a master regulator of mitochondrial biogenesis. We have also recently noted that Sirt1 can regulate autophagy in cells and that many of the proteins necessary for autophagosome formation are substrates for Sirt1. In the absence of Sirt1, electron micrographs demonstrate that abnormal mitochondria can accumulate within tissues such as the heart. This phenotype has also been seen in mice that are deficient in essential autophagy genes. Sirt1 regulation of both mitochondrial biogenesis and mitochondrial removal via autophagy suggests a potential role of sirtuins in regulating the flux of mitochondria within cells. Finally, we will discuss the role of Sirt3 in mitochondrial function and in particular the interaction of Sirt3 with Complex I of the mitochondria. Evidence will be presented regarding the role of Sirt3 in overall electron transport function.

Poster Abstracts

Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency.

G. López-Lluch,[1] N. Hunt,[2] B. Jones,[2] M. Zhu,[2] H. Jamieson,[3,4] S. Hilmer,[3,4] M. V. Cascajo,[1] J. Allard,[2] D. K. Ingram,[2] P. Navas,[1] and R. de Cabo[2]

[1]Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, 41013 Sevilla, Spain;
[2]Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, MD 21224-6825;
[3]Centre for Education and Research on Aging, University of Sydney, Concord Hospital, Concord NSW 2139, Australia; and
[4]Departments of Aged Care and Clinical Pharmacology, Royal North Shore Hospital, St. Leonards NSW 2069, Australia

Age-related accumulation of cellular damage and death has been linked to oxidative stress. Calorie restriction (CR) is the most robust, nongenetic intervention that increases lifespan and reduces the rate of aging in a variety of species. Mechanisms responsible for the anti-aging effects of CR remain uncertain, but reduction of oxidative stress within mitochondria remains a major focus of research. CR is hypothesized to decrease mitochondrial electron flow and proton leaks to attenuate damage caused by reactive oxygen species. We have focused our research on a related, but different, antiaging mechanism of CR. Specifically, using both in vivo and in vitro analyses, we report that CR reduces oxidative stress at the same time that it stimulates the proliferation of mitochondria through a peroxisome proliferation-activated receptor coactivator 1 alpha signaling pathway. Moreover, mitochondria under CR conditions show less oxygen consumption, reduce membrane potential, and generate less reactive oxygen species than controls, but remarkably they are able to maintain their critical ATP production. In effect, CR can induce a peroxisome proliferation-activated receptor coactivator 1 alpha-dependent increase in mitochondria capable of efficient and balanced bioenergetics to reduce oxidative stress and attenuate age-dependent endogenous oxidative damage.

Brain changes in the prematurely aging mtDNA mutator mouse

Jaime M. Ross[1,2], Stefan Brené[3], Johanna Öberg[4], Rouslan Sitnikov[4], Karin Pernold[2], Eva Lindqvist[2], Mügen Terzioglu[5], Takashi Yoshitake[6], Alexandra Trifunovic[5], Jan Kehr[6], Nils-Göran Larsson[5,7], Barry J. Hoffer[1] and Lars Olson[2]

(1) The National Institutes of Health, National Institute on Drug Abuse , Baltimore, MD, USA
(2) Karolinska Institutet, Department of Neuroscience., Stockholm, Sweden
(3) Karolinska Institutet, Department of Neurobiology, Health Sciences and Society, Stockholm, Sweden
(4) Karolinska Institutet, Department of Clintec, Stockholm, Sweden
(5) Karolinska Institutet, Department of Laboratory Medicine, Stockholm, Sweden
(6) Karolinska Institutet, Department of Physiology and Pharmacology, Stockholm, Sweden
(7) Max Planck Institute for Biology of Ageing, Cologne, Germany

Mitochondrial dysfunction may underlie aging-related alterations in neuronal function and has been implicated in neurodegenerative diseases, such as Alzheimer's and Parkinson's, as well as in stroke. A homozygous knock-in mouse expressing a proof-reading deficient version of the nucleus-encoded catalytic subunit (PolgA) of mitochondrial DNA (mtDNA) polymerase has been developed to study the effects of progressive mitochondrial dysfunction, and shows many signs of premature aging (Trifunovic et al, 2004). The mtDNA mutator mouse has a 3- to 5-fold increase in levels of mtDNA point mutations as well as increased levels of mtDNA deletions, with an approximately linear increase in mutation levels from midgestation to late adult life. The mouse has a reduced life span, reduced fertility, weight loss, reduced subcutaneous fat, enlarged heart, anemia, alopecia, kyphosis, osteoporosis, and sarcopenia. The aging phenotype appears not to be due to increased reactive oxygen species (Trifunovic et al, 2005). Furthermore, the prematurely aging mouse has hearing loss with apoptotic neurons in both the spiral ganglion and the cochlear nucleus (Niu et al, 2007).

We use an array of microscopy-based methods to characterize the CNS of these mice, focusing on development, cortical plasticity, neurogenesis/cell proliferation, and neurodegeneration, as well as on defined neurotransmitter systems. In situ hybridization demonstrates above-normal levels of BDNF mRNA in young mtDNA mutator mice (9 weeks), followed later in life (40 weeks) by below-normal levels. Additionally, Nogo Receptor 1 (NgR1) and Nogo mRNA levels decrease with age (after 24 weeks) in the mtDNA mutator mice. High-performance liquid chromatography (HPLC) reveals a modest increase in dopamine and DOPAC levels in striatum as the mtDNA mutator mice age. Magnetic Resonance Imaging (MRI) and histology show disturbances in cortical and hippocampal lamination, thinning of corpus callosum and cerebral cortex, and reduction in overall brain dimensions as early as 9 weeks of age. ^{1}H Magnetic Resonance Spectroscopy (MRS) is being used to monitor the in vivo metabolism of these animals. MRS demonstrates markedly increased brain lactate levels as early as 6-9 weeks of age to at least 35-38 weeks of age (oldest age group studied; average life span is 46 weeks). Additional preliminary data indicate neurogenesis differences in the dentate gyrus, altered GABA and glutamine/glutamate levels, and cell loss in the mtDNA mutator mice.

References:

Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly-Y M, Gidlöf S, Oldfors A, Wibom R, Törnell J, Jacobs HT, Larsson NG. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature. 2004 May 27;429(6990):417-23.

Trifunovic A, Hansson A, Wredenberg A, Rovio A, Dufour E, Khvorostov I, Spelbrink J, Wibom R, Jacobs H, Larsson NG. Somatic mtDNA mutations cause ageing phenotypes without affecting reactive oxygen species production. Proc Natl Acad Sci USA. 2005 Dec 13;102(50):17993-8.

Niu X, Trifunovic A, Larsson NG, Canlon B. Somatic mtDNA mutations cause progressive hearing loss in the mouse. Exp Cell Res. 2007 Nov 1;313(18):3924-34.

Physiological and gene expression changes to lipid metabolism by long-term dietary treatment with metformin in mice

Minor, Robin and de Cabo, Rafael.
Laboratory of Experimental Gerontology
NIA/NIH
Baltimore, MD..

Metformin, a biguanide agent widely used as an oral hypoglycemic agent in diabetes care, has emerged as a potential calorie restriction (CR) mimetic. The purpose of the present study was to investigate the potential for metformin in the diet to elicit beneficial effects similar to CR in mice. Groups of year-old C57BL/6 mice were fed AIN93G as the standard diet as the control and the treatment group had 1% metformin added by weight to the diet. There was also a CR group fed 30% by weight of ad libitum intake. Both CR and metformin induced weight loss in the mice and NMR analysis revealed that the metformin mice experienced a significant change in body composition that resulted in markedly increased leanness as computed by the ratio of their lean to fat mass. A gene microarray was performed on the livers of the mice. One major subset of gene changes in response to the CR and metformin diets were alterations to lipid metabolism. Some of the parallel changes included genes pertinent to fatty acid oxidation which reflected their elevated serum triglyceride levels. Several genes pertinent to sterol synthesis and transport were uniquely affected by the metformin treatment, while the regulation of lipid biosynthesis and storage was particular to the CR mice. The present findings highlight subtle differences among the effects of CR and metformin on the liver and suggest future targets for research on metformin as a mimic of CR.

Altered expression of *SSC1*, *TIM44*, *MGE1*, *SHY1* and *COX1* genes: A sign of altered mitochondrial biogenesis after low-dose ionizing radiation treatment and role in radio-adaptive response in *S. cerevisiae*.

Arya AK[*], Kumar A[*], Reena[†], Bala M[*], Sharma PK[#]
* Division of Radiation Biology, Institute of Nuclear Medicine and Allied Sciences, Brig. S.K. Mazumdar Marg, Delhi, India
# Department of Microbiology, Ch. Charan Singh University, Meerut, U.P., India.
† Department of Microbiology, Kanya Gurukula Mahavidhyalaya, Gurukula Kangri University, Haridwar, U.A., India

Reported earlier that mitochondrial mass increases after oxidative stress. Thus, strategies committed to combat with oxidative stress may prevent free radical formation and lessen the oxidative damages. Beside this, a group of researchers are working on the radio-adaptive response (RAR) induced by low-dose of ionizing radiation (LDIR). The phenomenon was confirmed in several human cell lines, tissues and PBMCs. Normally we are exposed with background radiation (cosmic fall out, medical procedures and surroundings) is very smaller than the dose-range investigated for humans as low-dose. Cuttler and Pollycove, 2003 used LDIR for cancer treatment and obtained surprisingly good results. We studied the time dependent changes in the level of transcripts of some mitochondrial genes (*COX1*, *SHY1*, *SSC1*, *TIM44* and *MGE1*) and % change in RAR after LDIR treatment.
The mid-log phase *S. cerevisiae* strain D7 was used. LDIR and lethal irradiation were given at dose rate of 0.40 rad/sec and 2.5 KGy/h respectively. After LDIR % change in RAR was measured by colony forming assay on defined synthetic medium plates. Differential gene activity was measured through RT-PCR and agarose gel assay.
After LDIR % change in RAR was found gradually increasing and significantly higher at 4.5 h and 6.0 h. After LDIR time dependent alteration in levels of transcripts of selected genes were observed.

In stress conditions mitochondria do retrograde signaling towards nucleus mediated through release of Ca^{++}, apoptosis-inducing factor, Cyt *c* and heam molecules, which in turn activate various stress responsive genetic pathways to rescue the disaster. In this direction we studied the differential activity of mitochondrial genes revealed invitation of molecular changes to compensate the cell's altered need for ATP during stress, whether it may be compensated by mitochondrial biogenesis or by importing new proteins from nucleus. In stress situations the contribution of mitochondria to rescue is appreciable and can not be ignored.

Radio-resistance in strains isolated from *Saccharomyces cerevisiae* strain D7: The role of mitochondrial *COX1, SSC1* and cytosolic *HSP82.*

Kumar A[*], Arya AK[*], Reena[†], Garg L[†], Bala M[*], Sharma PK[#]
* Division of Radiation Biology, Institute of Nuclear Medicine and Allied Sciences, Brig. S.K. Mazumdar Marg, Delhi, India
# Department of Microbiology, Ch. Charan Singh University, Meerut, U.P., India.
† Department of Microbiology, Kanya Gurukula Mahavidhyalaya, Gurukula Kangri University, Haridwar, U.A., India

Mitochondrial health may be of more concern as DNA repair and scavenging pathways for a cell endures stresses. Several stress responsive mitochondrial genes and pathways have already been reported. We have isolated strains (M1RR and M3RR) form *Saccharomyces cerevisiae* strain D7 showing resistance against ^{60}Co-γ rays. Parallel to this the expression of the *SSC1* (mtHsp70), *COX1* and *HSP82* genes were also checked. Hsp90 has stress-related and housekeeping functions as a molecular chaperone in refolding of denatured proteins, prevent protein unfolding and aggregation, docking with Tom70. MtHsp70 plays a vital role in mitochondrial protein import with TIM complex, a chaperone in matrix and a critical component of mitochondrial protein-quality control system. Whereas Cox1p is the core component of electron transport chain complex-IV.

The stationary phase *S. cerevisiae* strain D7 and isolated strains were used. Radio-resistance was checked against different doses of ionizing radiation (50-200 Gy, dose-rate 2.5 kGy/h). After irradiation % survivors were measured by colony forming units assay. Level of gene transcripts were measured through RT-PCR and agarose gel assay.
At 150 and 200 Gy the % survivors in both strains were found significantly ($p<0.01$) increased with reference to the parent strain D7. The level of transcripts of genes in isolated mutant strains were measured significantly ($p <0.01$) enhanced as compared to strain D7.
As compare to the transient radio-adaptive response, the radio-resistance is an inherent and stable property of cells endowed with. Probably, the enhanced expression of genes reflects their role in radio-resistance, because a resistant cell logically has an efficient mitochondrial system. Moreover, the functions of these genes in other stress management pathways are also appreciable which may impart radio-resistance to the cell. The study has far reaching implications in the field of radiation biology, radiation-protection strategies and space programs because *S. cerevisiae* has structural/functional homologies with humans.

Mitochondria in the Systems Biology of Aging

Furber, John. Legendary Pharmaceuticals, Gainesville, Florida.
Langley, Pat. Arizona State University, Tempe, Arizona.
Mitochondria in the Systems Biology of Aging.

The aging of the human mitochondriome plays an important, but not exclusive role in the aging of humans. The many observable signs of human senescence have been hypothesized by various researchers to result from several primary causes. Close inspection of the biochemical and physiological pathways associated with age-related changes and with the hypothesized causes reveals several parallel cascades of events that involve multiple interactions and feedback loops. We present a network diagram to aid in conceptualizing the many processes and interactions among them, including promising intervention points for therapy development. This network model includes both intracellular and extracellular processes. It ranges in scale from the molecular to the whole-body level. Important pathways include:

· Extracellular proteins become damaged by glycation, oxidation, crosslinking, and lytic enzymes, resulting in mechanical stiffness, weakness, and inflammation. Altered environmental niches for cells contribute to transdifferentiation, arrested cell division, cell death, cancer, stem cell depletion, tissue wasting, neurodegeneration, and organ malfunction. Stiffer blood vessels promote stroke and heart disease.

· Lysosomes accumulate reactive, crosslinked lipofuscin, which impairs autophagic turnover of macromolecules and organelles, and leaks into cytoplasm, triggering apoptosis of cells, which are not readily replaced.

· Mitochondrial DNA mutates. Mutations are copied, resulting in altered cell physiology.

· Lamin-A spice-variant, progerin, accumulates in nucleus, impairing cell division.

· Nuclear mutations, telomere shortening, chromosome breaks, chromatin alterations and epigenetic DNA adducts change gene expression.

· Oxidized aggregates in cytoplasm become crosslinked, resist turnover, inhibit proteasome activity, increase redox poise, and physically interfere with intracellular transport, especially in axons. Inhibited proteasomes reduce turnover of damaged molecules and of expired molecular signals. Increased redox poise alters signaling and enzyme activities, and erodes telomeres.

· Damaged molecules and sick cells promote inflammatory cascades which further damage tissues. Neuroendocrine and immune systems degrade.

This diagram is maintained on the Web as a reference for researchers and students, with the content updated as new information comes to light.

[www.LegendaryPharma.com/chartbg.html]

In addition, we are adapting the network model's contents into an interactive website with links to references and related background materials.

Loss of Mitochondrial Integrity in *SIRT3* Knockout Primary Cells Results in a Tumor Permissive Phenotype

Krish Patel [1,2], Kristi Muldoon-Jacobs [1,2], Nukhet Aykin-Burns [3], Hyun-Seok Kim [4], J. Daniel Pennington [2], Riet van der Meer [5], Kheem S. Bisht [2], Phuongmai Nguyen [2], Jason Savage [2], Kjerstin Owens [6], Keshav K. Singh [6], Sarki A. Abdulkadir [5], Chu-Xia Deng [4], Douglas R. Spitz [3], and David Gius [2,6]

[2] Molecular Radiation Oncology, Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA, [3] Free Radical and Radiation Biology Program, Department of Radiation Oncology, University of Iowa, Iowa City, IA, 52242, USA, [4] Genetics of Development and Disease Branch, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA, [5] Department of Pathology, Vanderbilt University Medical Center, Nashville, TN 37232, USA, [6] Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

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[1] The first two authors contributed equally to this manuscript.

[6] Corresponding author:
David Gius, M.D., Ph.D.
Chief, Molecular Radiation Oncology
National Cancer Institute
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892
Tel: (301) 435-9411
E-mail: giusd@mail.nih.gov

The evolutionarily conserved Sirtuin family genes appear to play a role in aging-related processes {Tissenbaum, 2001 #366}{Guarente, 2000 #222} and regulate factors critical to the maintenance of cellular integrity {Singh, 2006 #293; Guarente, 2008 #392}. In this regard we show that *SIRT3* knockout mouse embryonic fibroblasts (MEFs) and livers from $SIRT^{-/-}$ mice exhibit decreased mitochondrial DNA (mtDNA) integrity, respiration, and ATP production. In addition, $SIRT3^{-/-}$ MEFs exposed to genotoxic stressors exhibit increased intracellular superoxide levels and genomic instability. These results suggest a role for *SIRT3* in the maintenance of mitochondrial integrity; however, demonstration of a physiological phenotype may require a second genomic event. Loss of mitochondrial function is an established early step in carcinogenesis {Singh, 2006 #293}, thus we hypothesized that loss of the mitochondrial *SIRT3* gene {Onyango, 2002 #217} may result in a tumor-permissive phenotype. Expression of a single oncogene (Myc or Ras) in $SIRT3^{-/-}$ MEFs results in both *in vitro* transformation and *in vivo* tumorigenesis, and altered cellular metabolism. The addition of superoxide dismutase (SOD) prevents transformation of $SIRT3^{-/-}$ MEFs, suggesting that increased intracellular free radicals promote transformation in a $SIRT3^{-/-}$ background. These results identify *SIRT3* as a mitochondrial localized tumor suppressor gene (TSG) and suggest a connection between aging or longevity genes in mitochondrial integrity, oxidative metabolism, and carcinogenesis.

FIGURE LEGENDS

Figure 1. *SIRT3* knockout MEFs and knockout mouse livers have decreased mitochondrial integrity. (a) ***SIRT3* knockout cells have decreased mtDNA integrity. DNA was isolated from $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEFs, and mtDNA primers that amplify either the 10kb Amplicon or a 117-bp region were used for PCR. Primers to the genomic □-globin gene were used as a control. (**b) **ATP levels in *SIRT3* wild-type and knockout cells. $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEFs were lysed, and ATP levels were measured using chemiluminescence. Data are presented as arbitrary luminescent units for ATP levels.** (c) **Respiration levels in *SIRT3* wild-type and knockout cells. Oxygen consumption was measured using the YSI oxygen monitor containing a Clark-type electrode and recorded for 15 min. The data were normalized to cell number.** (d) **Livers from *SIRT3* knockout and isogenic wild type mice have increased mtDNA damage. DNA was isolated from the livers of *SIRT3* wild-type and knockout mice, and mtDNA primers were used for PCR as above.** (e) **ATP levels in *SIRT3* wild-type and knockout mice. $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEFs were lysed, and ATP levels were measured using chemiluminescence. Data are presented as arbitrary luminescent units for ATP levels.** (f) **Respiration levels in *SIRT3* wild-type and knockout mice. Oxygen consumption was measured as described above. The results off all the experiments above were done in at least three independent replicates and error bars around data points represent one standard deviation about the arithmetic mean. * P < 0.01 by t-test.**

Figure 2. *SIRT3* knockout MEFs exhibit increased superoxide levels in response to exogenous stress. (a) ***SIRT3* knockout cells grow more efficiently at low oxygen conditions. $SIRT3^{(+/+)}$ and $SIRT3^{(-/-)}$ MEF cells were plated at a 5 x 10^5 cells/100 mm dish, and cultured at or 21% Oxygen and harvested at 1, 2, and 5 days followed by quantification using a Coulter Counter.** (b) **MEFs from a transgenic mouse expressing a deacetylation null (or dominant negative, dn) *SIRT3* gene, but not $SIRT^{-/-}$ cells, have increased intracellular superoxide levels. Superoxide concentrations in $SIRT3^{+/+}$, $SIRT3^{-/-}$, and $SIRT3^{dn+}$ MEF cells were determined by DHE oxidation.** (c) **Superoxide levels were elevated in *SIRT3* knockout cells exposed to agents that induce mitochondrial damage. $SIRT3^{+/+}$ and $SIRT3^{-/-}$ MEF**

cells were exposed to either 5 Gy of IR or 5 □M of antimycin for 3 hours, and superoxide levels were monitored by DHE oxidation as compared to control, untreated cells (Cont). For all experiments the results were the normalization MFI for three independent replicates was plotted; error bars represent the standard deviation. (d) ***SIRT3*** **knockout MEFs exhibited aneuploidy following exposure to IR. SIRT3$^{+/+}$ and SIRT3$^{-/-}$ MEFs were exposed to either 2 or 5 Gy for one hour. Whole-mount chromosomes were counted in a blinded fashion. Bars show the mean chromosome number per cell from 100 separate counts. Error bars around data points represent one standard deviation about the arithmetic mean. For all the experiments in this figure * P < 0.05 by t-test.**

Figure 3. *SIRT3* knockout MEFs expressing of a single oncogene exhibit an *in vitro* transformation-permissive phenotype. (a) ***SIRT3*** **knockout MEFs spontaneously form colonies but do not immortalize. Long-term culture (28 days) of confluent *SIRT3* knockout (SIRT3$^{-/-}$) MEFs results in spontaneous colony formation. Cells were plated at 1 x 10^6/100 mm dish and fed with fresh media every 3-4 days for a total of 28 days. Colonies were evident by both phase-contrast microscopy and H&E stain. Experiments were done in triplicate and error bars represent the standard deviation.** (b) **SIRT3$^{-/-}$ MEFs infected with Myc, Ras, or both demonstrated increased *in vitro* foci formation. SIRT3$^{+/+}$, SIRT3$^{+/+}$ Myc/Ras, SIRT3$^{-/-}$ Myc, SIRT3$^{-/-}$ Ras, and SIRT3$^{-/-}$ Myc/Ras MEFs were plated at either 100 or 250 cells per plate (6-well plates) and stained with crystal violet and visualized with a Fuji Las-3000 intelligent darkbox (Fujifilm Medical Systems, Stamford, CT). All results are the mean of at least three separate experiments. Error bars around data points represent one standard deviation about the arithmetic mean. * P < 0.01 by t-test.** (c) ***SIRT3*** **knockout Myc, Ras, and Myc/Ras MEFs exhibit decreased contact inhibition. 1 × 10^6 cells were plated on a 10 cm plate and medium was replaced every 3-4 days for 28 days. Cells were subsequently stained with crystal violet and counted.** (d) **SIRT3$^{-/-}$ MEFs cells infected with either Myc, Ras, or both were examined by light microscopy for morphological alterations. Photomicrographs at 10x show (upper left) normal MEFs, (lower left) SIRT3$^{-/-}$ MEFs, (upper center) SIRT3$^{+/+}$ Myc/Ras infected MEFs, (lower center) SIRT3$^{-/-}$ Myc infected MEFs, (upper right) SIRT3$^{-/-}$ Myc/Ras infected MEFs, and (lower right) SIRT3$^{-/-}$ Ras infected MEFs.**

Figure 4. *SIRT3* is an *in vitro* and *in vivo* tumor suppressor gene. (a) ***SIRT3*** **knockout MEFs expressing Myc and/or Ras exhibit polyploidy. *SIRT3* knockout Myc and/or Ras infected MEF cells were harvested and analyzed by FACS. The percentage of cells containing greater than 4n is shown.** (b) **SIRT3$^{-/-}$ knockout Myc and/or Ras MEFs exhibit increased chromosomal aberrations. Whole-mount chromosomes were counted in a blinded fashion. Columns are the mean chromosome number per cell from 100 separate counts. Error bars indicate the standard error.** (c) ***SIRT3*** **knockout MEFs expressing Ras or Myc and Ras exhibit display anchorage independent in soft agar. SIRT3$^{+/+}$, SIRT3$^{-/-}$, SIRT3$^{+/+}$ Myc/Ras, SIRT3$^{-/-}$ Myc, SIRT3$^{-/-}$ Ras, and SIRT3$^{-/-}$ Myc/Ras cells (4000) were seeded in 2 ml of 0.4% agar in growth medium over a 3 ml base layer of agar also in growth medium. Each culture was topped with 1-2 ml of medium every 3-5 days. After 12 days, colonies were stained with methylene blue. Error bars around data points represent one standard deviation about the arithmetic mean. For all the experiments in this figure * P < 0.05 by t-test.** (d) **SIRT3$^{+/+}$, SIRT3$^{-/-}$, SIRT3$^{+/+}$ Myc/Ras, SIRT3$^{-/-}$ Myc, SIRT3$^{-/-}$ Myc/Ras, and SIRT3$^{-/-}$ Ras cells were implanted into both hind limbs of nude mice. Photograph of the hind limbs of nude mice injected with the cells are**

shown. (e) **Histological examination of SIRT3$^{-/-}$ Myc/Ras xenograft tumors stained with H and E.**

Figure 5. *SIRT3* knockout MEFs expressing Myc and/or Ras have altered biochemical properties and exhibit decreased complex I and III activity. (a) **Superoxide levels in *SIRT3* wild-type and knockout Myc and/or Ras infected MEF cells. Superoxide levels were monitored by DHE oxidation. MFI of three independent replicates was plotted.** (b) **ATP levels in SIRT3$^{+/+}$ Myc/Ras, SIRT3$^{-/-}$ Myc, SIRT3$^{-/-}$ Ras, and SIRT3$^{-/-}$ Myc/Ras. Cells were lysed and ATP levels were measured using chemiluminescence. Data are presented as arbitrary luminescent units and are a composite of three experiments.** (c) **Analysis of glucose consumption in *SIRT3* wild-type and knockout cells. Cells were counted and medium samples were obtained at 48 hours and analyzed using an YSI glucometer. Glucose consumption was determined by subtracting glucose content at the 48-hour point from the time zero sample and dividing by the number of cells.** (d) **SIRT3$^{-/-}$ Ras and SIRT3$^{-/-}$ Myc/Ras cells decreased complex I and III activity. Oxidative phosphorylation enzyme activities were measured on total cellular protein. Complex I activity was measured as the rotenone inhibitable rate of NADH oxidation. Complex II activity was measured by the succinate induced rate of reduction of DCIP. Complex III activity was measured as the rate reduction of cytochrome c (III) when stimulated with CoQ2H2. Complex activity was measured as the rate of cytochrome c (II) oxidation. All enzyme complex activities are expressed as a ratio to SIRT3$^{+/+}$ Myc/Ras. For all the experiments in this figure the error bars around data points represent one standard deviation about the arithmetic mean. * P < 0.05 by t-test.**

Figure 6. The increased growth rate of *SIRT3* knockout Ras and Myc/Ras cells is decreased by MnSOD. (a) ***SIRT3* knockout cells exhibited an increased growth rate. SIRT3$^{+/+}$, SIRT3$^{-/-}$, SIRT3$^{(-/-)}$ Myc, SIRT3$^{-/-}$ Ras, SIRT3$^{+/+}$ Myc/Ras, and SIRT3$^{-/-}$ Myc/Ras MEFs were plated at 2 × 10^4 cells per plate and harvested at 3, 4, and 5 days. The number of cells per plate was plotted as a function of days to determine growth rate and doubling times.** (b) **Infection with a MnSOD-expressing adenovirus decreases the growth rate of SIRT3 knockout cells. SIRT3$^{+/+}$Myc/Ras, SIRT3$^{-/-}$Myc/Ras, and SIRT3$^{-/-}$ Ras cells were infected with Adeno-MnSOD and cells were isolated at 72 and 90 hours to determine cell growth rates. All experiments in this figure were done in triplicate. Error bars around data points represent one standard deviation about the arithmetic mean. * P < 0.05 by t-test.**

TABLE 1. Immortalization of SIRT3$^{-/-}$ MEFs only requires a single oncogene

	Control	**Myc**	**Ras**	**Myc/Ras**
MEF SIRT3$^{+/+}$	None	None	None	Immort
MEF SIRT3$^{-/-}$ Immort	None	Immort		Immort
MEF SIRT3$^{-/-}$ + PEG-SOD	None	None	None	Immort

None = No MEF immortalization.
Immort = Immortalization.