The NASA GeneLab Project

Sylvain V. Costes, PhD
GeneLab Project Manager

Special Seminar
Genetics, Genomics, & Systems Biology
University of Chicago
September 25, 2018
“…genomics, transcriptomics, proteomics, and metabolomics offer an immense opportunity to understand the effects of spaceflight on biological systems…”

“…Such techniques generate considerable amounts of data that can be mined and analyzed for information by multiple researchers…”
This is truly an exciting time for cellular and molecular biology, omics and biomedicine research on ISS with these amazing additions to the suite of ISS Laboratory capabilities.

**Omics Acquisition in Space is Now a Reality**

**Sample Preparation Module**

**Oxford Nanopore MinION Gene Sequencer**

**Cepheid Smart Cycler qRT-PCR**

**Reaction tube containing lyophilized chemical assay bead (proprietary)**

**Mini-PCR**
GeneLab Data Democratization

Community

- Citizen scientists
- Biologists
- Data scientists

Data type

- Disease states
- Pathway changes
- Differential expression
- Mapped reads
- Raw data

Missions/Experiments

GeneLab Sample Processing

Open Data

Visualization Portal

New Knowledge

Analysis Working Groups

Web Portal

Analysis Toolshed

User Data Workspace

GeneLab

Data Federation

ANL MG-RAST

EBI PRIDE

NCBI GEO
Phased Implementation

Phase 1
Searchable Data
FY2014 – 2015

Phase 2
Data Exchange
FY2016-2017

Phase 3
Tool Integration
FY2018– 2019

Phase 4
Maintenance
FY2020 – 2021

**Data System**
- Public Website
- Searchable Data Repository
- Top Level Requirements
- New Data and Legacy Data

**Data System**
- Link to Public Databases via Data Federation
- Integrated Search (e.g., data mashup)

**Data System**
- Integrated Platform across model organisms
- Build Community via AWG
- Provide access to biocomputational tools for omics analysis
- Provide collaboration framework and tools

**Open Source Maintenance**
- User community becomes primary provider of new tools/knowledge
- Maintain integrity of data, and data system

Oct 1, 2017
GLDS 2.0 Release

April 23rd, 2018
AWG workshop

Oct , 2018
GLDS 3.0 + ASGSR

June 2018
Summer interns

Nov 2018
Visualization Portal

Oct 1, 2019
GLDS 4.0 Release

March 2019
Visualization Portal

Visualization
Portal

Summer interns

Sequencing cost in 2017

- Labor, administration, management, utilities, reagents, and consumables
- Sequencing instruments and other large equipment (amortized over three years)
- Informatics activities directly related to sequence production (e.g., laboratory information management systems and initial data processing)
- Submission of data to a public database
- Indirect Costs (http://oamp.od.nih.gov/dfas/faq/indirect-costs#difference) as they relate to the above items
The real cost of OMICS

GLDS 2.0 – Oct 1st 2017
GLDS 3.0 – Oct 1st 2018
GLDS 4.0 – Oct 1st 2019
GLDS Systems Architecture
Phase III

GLDS

Extramural Computing
Data Consumers
Curators, Archivists
Intramural Data Scientists

Repository
Human Data (Raw)

Data Curation Tools
Internal Data Processing Tools
Data Processing Platform

Workspace
Visualizations
Data Submitters
Data Scientists

Open-Access
Controlled-Access
Data Analysis
Data Sharing
Data Curation/Archiving
Guiding principles to look at GeneLab data

- Increasing Genetic Diversity (more samples/payload)
- Increasing Human Relevance
- Identify Shared Processes/ Molecular Signatures
  - Hypoxic Response
  - Oxidative Stress
  - Common Tissue (e.g. muscle, liver, heart, eyes, brain,…)

ANIMAL AWG

Human (future GL database?)

MICROBE AWG

PLANT AWG

MULTI-OMICS AWG
Overview: Database content

**STUDY TYPE**
- Spaceflight: 51%
- Ground: 48%
- Parabolic: 1%

**ASSAY TYPE**
- Transcription profiling: 72%
- DNA methylation profiling: 8%
- Protein expression profiling: 9%
- Genome sequencing: 4%
- Metabolite profiling: 2%
- Environmental gene survey: 2%
- Deletion pool profiling: 1%

**ORGANISM**
- Rodents: 34%
- Microbes: 19%
- Plants: 10%
- Human: 22%
- Invertebrates: 7%
- Fruit flies: 5%
- Fish: 3%

**TRANSCRIPTION PROFILING**
- RNA-seq: 25%
- Microarray: 75%

Total # of studies: 172
The Radiation Factor

**2½ Years, 2,600 X-Rays**

Americans on average absorb the radiation equivalent of at least 7 chest X-rays each year.

Space missions, outside of Earth’s protective atmosphere and magnetic field, expose astronauts to many times more.

Source: Brookhaven National Laboratory, U.S. Department of Energy
69 Ground Data Sets: Radiation and simulated microgravity

A) Radiation only: 23 (33.33%), Microgravity Only: 2 (2.9%), Radiation + Microgravity: 26 (37.68%), Other: 18 (26.09%)

B) Homo Sapiens: 14 (50%), Mus Musculus: 1 (3.57%), Rattus norvegicus: 1 (3.57%), Microbes: 1 (3.57%), Plant: 11 (39.29%)

C) Irradiation Ground Datasets On GeneLab

- Gamma: 20
- Proton: 5
- $^{16}$O: 5
- $^{12}$C: 5
- $^{28}$Si: 5
- $^{56}$Fe: 5
- Neutron: 5

Beheshti et al., Radiation Research 2018
Radiation Dosimetry for STS samples (ISS to follow)

Beheshti et al., Radiation Research 2018
GeneLab Analysis Working Groups: Letting the scientific community take the lead

- Monthly meetings + “Homework”
- Deliverables:
  - Consensus pipelines for primary analysis of data (Microarray, RNASeq, Bisulfite sequencing, Proteomics, 16S metagenomics, Whole genome metagenomics)
  - Recommendations for visualization of data

Total AWG Members: 114

AWG Members Per Group:
- Animal: 47
- Multi-Omics/System Biology: 33
- Plants: 24
- Microbes: 21

*Some members are in multiple groups

Map created with https://www.zeemaps.com/

Annual Workshop (April 2018)

2018 Summer Internship: Generate all higher order data
<table>
<thead>
<tr>
<th>Year</th>
<th>Title</th>
<th>Journal</th>
<th>Authors</th>
<th>Status</th>
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<tbody>
<tr>
<td>2017</td>
<td>Validation of Methods to Assess the Immunoglobulin Gene Repertoire in Tissues Obtained from Mice on the International Space Station.</td>
<td>Gravit Space Res.</td>
<td>Rettig TA, Ward C, Pecaut MJ, Chapes SK</td>
<td>Published</td>
</tr>
<tr>
<td>2018</td>
<td>A microRNA signature and TGF-β1 response were identified as the key master regulators for spaceflight response</td>
<td>PLoS One</td>
<td>Beheshti A, Ray S, Fogle H, Berrios D, Costes SV</td>
<td>Published</td>
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<tr>
<td>2018</td>
<td>Global transcriptomic analysis suggests carbon dioxide as an environmental stressor in spaceflight: A GeneLab case study</td>
<td>Scientific Reports</td>
<td>Beheshti A, Cekanaviciute E, Smith DJ, Costes SV</td>
<td>Published</td>
</tr>
<tr>
<td>2018</td>
<td>Exploring the Effects of Spaceflight on Mouse Physiology using the Open Access NASA GeneLab Platform</td>
<td>JoVE</td>
<td>A Beheshti, Y Shirazi-Fard, S Choi, D Berrios, SG Gebre, JM Galazka, SV Costes</td>
<td>Undergoing revision</td>
</tr>
<tr>
<td>2018</td>
<td>GeneLab: Omics database for spaceflight experiments</td>
<td>Bioinformatics</td>
<td>S Ray, S Gebre, H Fogle, D Berrios, PB Tran, JM Galazka, S V Costes</td>
<td>Submitted</td>
</tr>
</tbody>
</table>
Cage Effects with rodent experiments: Carbon Dioxide as an Environmental Stressor in Spaceflight

Carbon Dioxide as an Environmental Stressor in Spaceflight

A) Cage Types

Animal Enclosure Module (AEM)

Sample vivarium cage

B) GeneLab Study Mission Species \( \text{CO}_2 \) (ppm) Duration (days) Tissue Type

<table>
<thead>
<tr>
<th>GeneLab Study</th>
<th>Mission</th>
<th>Species</th>
<th>( \text{CO}_2 ) (ppm)</th>
<th>Duration (days)</th>
<th>Tissue Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLDS-21</td>
<td>STS-108</td>
<td>mouse</td>
<td>(~3000)</td>
<td>11.8</td>
<td>skeletal muscle (gastrocnemius)</td>
</tr>
<tr>
<td>GLDS-111</td>
<td>BF</td>
<td>mouse</td>
<td>(~600)</td>
<td>30</td>
<td>soleus muscle</td>
</tr>
<tr>
<td>GLDS-111</td>
<td>BF</td>
<td>mouse</td>
<td>(~600)</td>
<td>30</td>
<td>extensor digitorum</td>
</tr>
<tr>
<td>GLDS-25</td>
<td>STS-135</td>
<td>mouse</td>
<td>(~3000)</td>
<td>13</td>
<td>liver</td>
</tr>
<tr>
<td>GLDS-63</td>
<td>STS-70</td>
<td>rat</td>
<td>(~3000) (est)</td>
<td>9</td>
<td>mammary gland</td>
</tr>
</tbody>
</table>

Beheshti, et al., Scientific Reports, 2018
PCA Plots Suggest Strong Cage Effect

AEM = Animal Enclosure Modules (now referred to as Rodent Habitats)
Vivarium = normal ground based rodent cages

Beheshti, et al., Scientific Reports, 2018
An increase in aldosterone is associated with metabolic syndrome, which is characterized by chronic inflammation; aldosterone secretion can be triggered by hypoxia.

Beheshti, et al., Scientific Reports, 2018
Systems Biology analysis reveals biological spaceflight master regulators

Beheshti, et al., PLOS One, 2018
GeneLab Data Used to Generate Results

Process after mice are sacrificed
Sample Processing
Data Sharing
Data Collection & Curation
Next Generation Research
Modeling and Validation

International Space Station (ISS) Missions

Mice Sacrificed on ISS

Mice flown on STS and Sacrificed after Re-entry

Liver
Skeletal Muscle
Mammary Gland
Thymus

Time in Space for Mice (days)
0 10 20 30 40 50 60 70 80 90 100

Space Shuttle (STS) Missions

Extensor Digitorum Longus Muscle
Soleus Muscle
Gastrocnemius Muscle
Quadriceps
Tibialis Anterior Muscle
Adrenal Glands
Kidney
Liver
Skin
Number of Significant Genes from Each Dataset

- Fold-Change ≥ ±1.2
- Pathway/Functional Predictions:
  - Ingenuity Pathway Analysis (IPA)
  - Gene Set Enrichment Analysis (GSEA)

Beheshti, et al., PLOS One, 2018
• p53 found in all tissues
• p53 is a transcription factor and in response to genotoxic stress, DNA damage, oncogene activation, and hypoxia, it is recruited to sites in chromatin, thus promoting transcription of apoptosis related genes

Beheshti, et al., PLOS One, 2018
Key Genes and the Connections

**TGFβ1 found to be central regulator of key genes**

- TGFβ is known to play a context specific role in sustaining tissue homeostasis predominantly via transcriptional regulation of genes involved in differentiation, cell motility, proliferation, cell survival along with regulating immune responses during homeostasis and infection.
- Previous Studies found reduction in gravitational force to diminish TGF-β expression and apoptosis with higher carcinoembryonic antigen expression in 3D human colorectal carcinoma cells, as compared to 3D cultures in unit gravity.
- In another study, differential regulation of blood vessel growth using basic fibroblast growth factor was identified in modeled microgravity with induction early and late apoptosis, extracellular matrix proteins, endothelin-1 and TGFb1 expression

Beheshti, et al., PLOS One, 2018
A recent report showed that inactivation of p53 altered TGF-β signaling, which ironically displayed both tumor-suppressive and pro-oncogenic functions. p53 functions to integrate crosstalk between Ras/MAPK and TGF-β signaling via binding to Smad3, dislocating the Smad3/Smad4 complex formation and differentially regulating subsets of TGF-β target genes.
Analysis Working Group (AWG) Member related work determines novel systemic biological factors causing damage due to spaceflight

Work in progress
• **Primary Goal:** To determine the best pipelines, workflow, and tools to analyze relationships between multiple omics platforms (including: transcriptomic, proteomic, genomics, epigenetic, methylation, miRneme, and metabolomics).
  – Develop pipelines/workflows
  – Develop the optimal visualization tools to implement on GeneLab

• **Secondary Goal:** To provide the most biological significant data impacting space biology based on the existing GeneLab data from multiple platforms and species. This will be done through an unbiased Systems Biology approach with the optimal tools decided from the Primary Goal to determine the “master switch or regulator” that is affected due to space flight.
Omics Available on GeneLab and Challenges to Overcome

Use Public databases with multiple omics datasets to determine overall behavior of the system

Challenges to multi-omics datasets

- Different Omics Platforms
  Integration of Data
- Computing Power
  Lack of Computer Clusters
  Time to Preprocess Data
- Datasets
  Poor Controls
  Bad biological replicates
  Bad experimental setup
- Analysis Methods
  Normalization
  Biased analysis
  No uniform data processing

Possible Solutions

- Develop Software Tools to integrate different platforms
- Supercomputer clusters made available on the cloud to process data
- From analysis done on public data demonstrate how to setup proper future experiments
- Create universal pipeline for unbiased analysis of all omics data
1) Determine, and get consensus for best tools to use for each type of omics and species.
   a) Specific R and python packages
   b) Other platforms (i.e. GenePattern, Galaxy, homemade, etc)

2) Determine best approach to correlate cross-platform/omics comparisons.
   a) Use tools available
   b) Create own possible tools between collaborations from the AWG members labs and people

3) Use newly created pipeline and tools to analyze existing GeneLab data and develop optimal visualization tools
   a) Create an unbiased Systems Biology analysis pipeline with the determined tools to determine overall master regulator being affected by space flight for each organism on GeneLab (rodents, plants, microbes, invertebrates, and humans)
      i. First determine individual master regulators for each omic (i.e. Transcriptome, proteins, miRNAs, metabolites, methylated factors, and DNA mutations).
      ii. Then determine through the tools created through cross platform/omics the overall master regulators.
   b) Create an unbiased cross-species analysis to determine overall master regulators that commonly impact all organisms.

4) The final tools that were used for the analysis in 3 will be compiled in one group and assessed by the rest of GeneLab
   a) These tools will include both existing and homemade analysis tools from the people included in the AWG
   b) The most optimal Visualization tools (both existing and homemade from the AWG members) will be included in our final assessment
   c) Best Systems Biology approach will be assessed and packaged into an easy to use tool for the community

5) Each step will produce at least one to two papers for publication headed by various members of the AWG
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AWG Members Involved

Chris Mason  Cem Meydan  Jonathan Foox  Flavia Rius

Susana Zanello  Scott Smith  Sara Zwart

Afshin Beheshti  Sylvain Costes
Health Risks On Astronauts in Space

**EFFECTS OF SPACE ON THE HUMAN BODY**

**SENSORIMOTOR**
- Sensorimotor disturbances can impair a person's movement control.

**CARDIOVASCULAR**
- Decreases in vascular function may reduce oxygen intake, which could lead to poor performance of physically demanding tasks.

**SPINE**
- A body gets a little taller in space due to the expansion of the vertebrae. Could cause back pain on return to Earth.

**MUSCLE**
- Lack of gravity causes muscle fibers to shrink, leaving a person weaker.

**BONES**
- Prolonged exposure to space can cause loss of bone mass and bone minerals.

**RADIATION**
- The body is at risk for radiation sickness and cancer.

**SLEEP**
- Loss of sleep can lead to fatigue and psychological problems.

SOURCE: NASA
Janet Loehrke, USA TODAY

**USA TODAY**
Specific Datasets and Tissues
AWG Members Analyzed

- Additional Datasets that are being analyzed:
  - Human datasets
    - GLDS-54, GLDS-174, GLDS-86, GLDS-118, GLDS-53, GLDS-54, GLDS-13, GLDS-52, or GLDS-114 (Tyson McDonald and Yared Kidane)
Lipid Accumulation in the Liver

Level 7: The Body
Level 6: Organs

Triglycerides and lipids Levels

Oil Red O Positivity (%)

CASIS       RR1       RR3
C57BL/6     BALB/c

Flight
Ground

*
Lipid Accumulation in the Liver
And Ion Diffusion

Level 5: Tissues
Triglycerides and lipids Levels

Level 4:
The Cell and its Organelles

Lipid metabolism

A: HEPATOCYTE
- HBDL metabolism-cholesterol homeostasis

B: ADIPOCYTE
- TAG intracellular accumulation

Inflammation

Mitochondria – All Cells

Diabetes Type 2

Monomeric Units

Mitochondrial Stress

Level 1:
Monomeric Units


Gary
Hardiman

Willian
da Silveira
Liver at the Center of Metabolic Disorders

Level 7: The Body  Level 6: Organs

Diabetes Complications

Non-Alcoholic Fatty Liver Disease Complications

Level 4:
The Cell and its Organelles

Ballestri et al. World J Gastroenterol. 2014.

Schmidt & Goodwin, Metabolomics (2013).

Willian da Silveira
Gary Hardiman

Schmidt & Goodwin, Metabolomics (2013).
Ballestri et al. World J Gastroenterol. 2014.
RR1: C57BL/6
mice strain
(female)

RR3: BALB/c
mice strain
(female)

Diabetes Type 2 induced by High Fat Diet Model:

• Th1 Immune Response (more inflammatory),
• More susceptible to adiposity, liver inflammation, and liver fibrosis

• Th2 Immune Response (more tolerogenic),
• More susceptible to liver steatosis
• Radiosensitive
RR1 and RR3 Experimental Detail

**ISS**

- **RR1** - C57BL/6
- **RR3** - BALB/c

**Exposition time**: ≈ 40 days

**RR1**
- Space Flight  n = 6
- Ground Control  n = 6

**RR3**
- Space Flight  n = 6
- Basal Control*  n = 6
- Ground Control  n = 6

**Level 5: Tissues**

- Liver
- Kidney
- Lymph node
- Ovary

**Earth**
RR1 Liver (C57BL/6)
181 DE genes adj.P < 0.05

RR3 Liver (BALB/c)
0 DE genes adj.P < 0.05

Willian da Silveira
Gary Hardiman
Brin Rosenthal
Kathleen Fisch
**GeneLab**

**RR3 (BALB/c)**

**System Level Analysis**

**GSEA - G.O Biological Process:**

<table>
<thead>
<tr>
<th>NAME</th>
<th>NOM p-val</th>
<th>FDR q-val</th>
</tr>
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<tbody>
<tr>
<td>GO_POLYOL_CATABOLIC_PROCESS</td>
<td>0.002</td>
<td>0.21</td>
</tr>
<tr>
<td>GO_FOLIC_ACID_CONTAINING_COMPOUND_METABOLIC_PROCESS</td>
<td>0.002</td>
<td>0.20</td>
</tr>
<tr>
<td>GO_PTERIDINE_CONTAINING_COMPOUND_METABOLIC_PROCESS</td>
<td>&lt;0.001</td>
<td>0.21</td>
</tr>
<tr>
<td>GO_RESPONSE_TO_LEAD_ION</td>
<td>&lt;0.002</td>
<td>0.23</td>
</tr>
<tr>
<td>GO_REGULATION_OF_CHROMATIN_SILENCING</td>
<td>&lt;0.003</td>
<td>0.22</td>
</tr>
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Related to eye damage and cataract Risk in **Diabetes Type II**

- William da Silveira
- Gary Hardiman
Vision Changes after Spaceflight Are Related to Alterations in Folate- and Vitamin B-12-Dependent One-Carbon Metabolism$^{1,2}$

Sara R. Zwart,$^3$ C. Robert Gibson,$^4$ Thomas H. Mader,$^5$ Karen Ericson,$^6$ Robert Plourz-Snyder,$^5$ Martina Heer,$^7$ and Scott M. Smith$^8$

$^3$Division of Space Life Sciences, Universities Space Research Association, Houston, TX; $^4$Wyle Science, Technology and Engineering Group, Houston, TX, and Coastal Eye Associates, Webster, TX; $^5$Alaska Native Medical Center, Anchorage, AK; $^6$Department of Chemistry, Indiana University-Purdue University Fort Wayne, Fort Wayne, IN; $^7$University of Bonn, Bonn, Germany; and $^8$Human Adaptation and Countermeasures Division, Space Life Sciences Directorate, National Aeronautics and Space Administration Johnson Space Center, Houston, TX

GSEA - G.O Biological Process:

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Related to eye damage and cataract Risk in Diabetes Type II

Willian da Silveira

Gary Hardiman

44
RR1 Differentially Expressed Genes: Space Flight vs Ground Control

RR1 Kidney
Space Flight - Ground Control

RR1 Adrenal
Space Flight - Ground Control

RR1 Liver
Space Flight - Ground Control

Color Key

RR1 Kidney
Space Flight - Ground Control

RR1 Adrenal
Space Flight - Ground Control

RR1 Liver
Space Flight - Ground Control

Kathleen Fisch
Brin Rosenthal
Kathleen Fisch

45
RR1 Shared DE Genes

GSEA
Zinc Transporters (Reactome)
Apoptosis by TGF31 via MAPK1...
Alpha Linolenic Acid Metabolism (KEGG)
PARK2_ Hepatocyte_Proliferation...
LG4_Targets_Up
Amplification hotspot...
EGFR ligand binding... (Reactome)
Response to TGFB1...
Breast Cancer...
Apoptosis by CDKN1A not via TP53...
EGFR signaling...
Systemic lupus erythematosus...

Liver
167
12

Kidney
83
2

17
Adrenal_adjP<.5

Cbx7 Arntl Cdkn1a Gale Per3 Nfil3 Npas2
Pnkd Loxl4 Bh1he41 Dbp Leo1

Toppgene
Circadian Rhythm
TF activity
DNA binding
Stress induced premature senescence
Response to redox state

GSEA
Core Duplication Genes
Signal Attenuation (Reactome)
Post-Chaperonin Tubulin (Reactome)

Rbm3 -- downregulated
RBM3 is a cold-induced RNA binding protein and is involved in mRNA biogenesis exerts anti-
apoptotic effects. Expression of this gene is induced by cold shock and low oxygen tension. RBM3 is a proto-
unignogene that is associated with tumor progression and metastasis and is a potential cancer biomarker. Based on patient survival data, high levels of R3M3 protein in tumor cells is a favourable prognostic biomarker in colorectal cancer.

Downregulated in all three conditions
Tef
Txnip

Brin Rosenthal
Kathleen Fisch
RR1 (C57BL/6)
All Tissues Network Analysis

Cluster 0
Metabolic pathways

Cluster 1
Cell cycle

Cluster 2
Chemical carcinogenesis

Cluster 3
Immune system

Cluster 4
Adaptive immune system

Cluster 5
Metabolic pathways/Lipid metabolism

Cluster 6
Circadian rhythm

Cluster 7
Cytokine interactions/Chemokine signaling

Cluster 8
Muscle contraction

DE in liver

DE in kidney

DE in all muscle
downregulated
upregulated

Kathleen Fisch
Brin Rosenthal
Kathleen Fisch
RR1 (C57BL/6): Systems Analysis (GSEA Analysis)

**RR1 – ALL Tissues**

Enrichment plot:
GO_INSULIN_LIKE_GROWTH_FACTOR_RECEPTOR_BINDING

Enrichment plot:
KEGG_MATURITY_ONSET_DIABETES_OF_THE_YOUNG

**RR1 – Kidney**

Enrichment plot: REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT

Enrichment plot: REACTOME_GLUCOSE_METABOLISM

**RR1 – Adrenal Gland**

- William da Silveira
- Gary Hardiman
- Brin Rosenthal
- Kathleen Fisch
Cite this: Photochem. Photobiol. Sci., 2013, 12, 536

Dietary vitamin D alters the response of the skin to UVB-irradiation depending on the genetic background of the mice.

R. C. Melley, H. K. Muller, M. Norval and G. M. Woods

<table>
<thead>
<tr>
<th>Strain</th>
<th>BALB/c</th>
<th>C57BL/6</th>
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<tbody>
<tr>
<td></td>
<td>D+</td>
<td>D−</td>
</tr>
<tr>
<td>Male</td>
<td>112 ± 14</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>(n = 17)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>110 ± 24</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 23)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D+</td>
<td>D−</td>
</tr>
<tr>
<td>Male</td>
<td>137 ± 32</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>(n = 24)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>129 ± 34</td>
<td>15 ± 5</td>
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Mean ± SD, nmol L−1

Strains of mice differ in their ability to be suppressed by UVB irradiation: BALB/c mice are considered “sensitive” and C57BL/6 “resistant”. The results indicate that dietary vitamin D3 can reduce UVB-induced suppression of the contact hypersensitivity response depending on the genetic background of the mice.

(Endocrinology 150: 1051–1060, 2009)

Vitamin D Deficiency Modulates Graves’ Hyperthyroidism Induced in BALB/c Mice by Thyrotropin Receptor Immunization

Alexander Misharin, Martin Hevison, Chun-Rong Chen, Venu Lagishetty, Holly A. Aliesky, Yumiko Mizutori, Basil Rapoport, and Sandra M. Mclachlan

In conclusion, we found a strain-specific difference in handling vitamin D, with BALB/c mice having reduced ability (compared with C57BL/6 mice) to generate the active metabolite.
Despite vitamin D supplement use during flight, serum 25-hydroxycholecalciferol was decreased after flight (P < 0.01).

Vitamin D acts to prevent diabetes by maintaining low levels of Ca^{2+} and ROS. Berridge, 2017

Vitamin D Receptor has been defined a gatekeeper of mitochondrial respiratory chain activity.
Skin, Reactive Oxygen Species, and Circadian Clocks

Mary A. Ndiaye, Minakshi Nihal, Gary S. Wood, and Nihal Ahmad

Abstract

Significance: Skin, a complex organ and the body's first line of defense against environmental insults, plays a critical role in maintaining homeostasis in an organism. This balance is maintained through a complex network of cellular machinery and signaling events, including those regulating oxidative stress and circadian rhythms. These regulatory mechanisms have developed integral systems to protect skin cells and to signal to the rest of the body in the event of internal and environmental stresses. Recent Advances: Interestingly, several signaling pathways and many bioactive molecules have been found to be involved and even important in the regulation of oxidative stress and circadian rhythms, especially in the skin. It is becoming increasingly evident that these two regulatory systems may, in fact, be interconnected in the regulation of homeostasis. Important examples of molecules that connect the two systems include serotonin, melatonin, vitamin D, and vitamin A. Critical Issues: Excessive reactive oxygen species and/or dysregulation of antioxidant system and circadian rhythms can cause critical errors in maintaining proper barrier function and skin health, as well as overall homeostasis. Unfortunately, the modern lifestyle seems to contribute to increasing alterations in redox balance and circadian rhythms, thereby posing a critical problem for normal functioning of the living system. Future Directions: Since the oxidative stress and circadian rhythm systems seem to have areas of overlap, future research needs to be focused on defining the interactions between these two important systems. This may be especially important in the skin where both systems play critical roles in protecting the whole body.
Most up-regulated Gene:
- **Gene**: Defb7 (ENSMUSG00000037790.3)
- **Role**: Beta-defensin 7 has bactericidal activity and is involved in defense response to bacteria
- **Log₂ fold-change**: 22
- **Adjusted p-value**: $2.94 \times 10^{-7}$

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### GSEA Analysis

#### Enrichment plots:

- **Enrichment plot: GO_DETECTION_OF_LIGHT_STIMULUS**
  - Enrichment score (ES) vs Rank in Ordered Dataset
  - Positive correlation
  - Zero cross at 0.05

- **Enrichment plot: REACTOME_ION_CHANNEL_TRANSPORT**
  - Enrichment score (ES) vs Rank in Ordered Dataset
  - Zero cross at 0.05

- **Enrichment plot: REACTOME_INSULIN_SYNTHESIS_AND_PROCESSING**
  - Enrichment score (ES) vs Rank in Ordered Dataset
  - Zero cross at 0.05

- **Enrichment plot: KEGG_FATTY_ACID_METABOLISM**
  - Enrichment score (ES) vs Rank in Ordered Dataset
  - Zero cross at 0.05

#### Table: Enriched Pathways

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Analysis Relating Liver to the Eye

https://www.eyedoctorophthalmologystnyc.com

Cone Cells - Eye

1st Amplification  2nd Amplification  3rd Amplification
Data processing: PCA plot of normalized & ComBat treated data

Normalized to the proteins of the baseline samples (B)

G1 and F3 seem to be outliers. I removed these two samples for the further analysis.

Deanne Taylor  Hossein Fazelinia
T-test analysis with %5 FDR

Heat map of significantly changed proteins

Deanne Taylor
Hossein Fazelinia
## Data processing: GO term analysis for significant proteins

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Protein Pathway Analysis
RR3 – Liver: Proteomics

Data processing: GO term analysis for significant proteins

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Related mitochondria functions are revealed from the initial proteomic analysis!!
Protein Pathway Analysis
RR3 – Liver: Proteomics

Data processing: GO term analysis for significant proteins

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Related mitochondria functions are revealed from the initial proteomic analysis!!
Deviation from mean separate for each experiment
• Spaceflight changes the physical properties of the cell components impacting from the molecular to the whole body level.

• The Mitochondria are the principal cellular component affect.

• The Liver is the principal organ affected in issues related to the metabolism.

• Overall circadian rhythm pathways are being disrupted

• Possible disease that can be associated with liver damage and pathways is: **Oculocerebrorenal Syndrome of Lowe**

  • “Extensive research has demonstrated that OCRL-1 is involved in multiple intracellular processes involving endocytic trafficking and actin skeleton dynamics. This explains the multi-organ manifestations of the disease.”
  • “The classic form of the oculocerebrorenal syndrome of Lowe (OMIM #309000), first described by Lowe et al. in 1952 [1], is characterized by the triad of congenital cataracts, severe intellectual impairment, and renal tubular dysfunction with slowly progressive renal failure”
  • Patients with this disease manifest Cataract, Glaucoma and Muscle hypotonia.

Assumptions:
- Gravity is a physical property.
- Alteration in Gravity must have a primary impact in physical properties of a cell, like ion diffusion.
- Electrostatic properties of proteins influence protein-protein interaction and/or protein folding, the same can happen with RNA structures.
- Ions are critical for Electrostatics properties of the proteins, RNAs and etc.

Hypotheses:
- By altering Ion Diffusion, microgravity can influence cellular events by altering Protein-Protein Interaction and “Binding”, Protein folding and RNA structures what would influence RNA splicing.
- Is expected that other Physiological properties dependent of Ion diffusion will be impacted too.

http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch05s04.html
Hypothesis Developed and Being Worked On (Ions and Mitochondria)

Mitochondria – All Cells

1st Amplification  2nd Amplification  3rd Amplification

Cone Cells - Eye

• Mitochondria can be disrupted by one single particle traversal in one single mitochondria and create a cascade of event, including oxidative stress lasting several days in the exposed cell.
Systems Biology Analysis Used to Understand Spaceflight Impact on Health Risk

Level 1: Monomeric Units
Level 2: Macro molecules
Level 3: Supra molecular Complexes
Level 4: The Cell and its Organelles
Level 5: Tissues
Level 6: Organs
Level 7: The Body

Inflammation, Lipid Accumulation, alteration of cell migration and etc.

Mitochondrial Stress And Cytoskeleton alteration

Protein Complexes and The Electron Chain Reaction

Electrostatic Properties and Folding

Ion Diffusion

Level 1: Monomeric Units
Level 2: Macro molecules
Level 3: Supra molecular Complexes
Level 4: The Cell and its Organelles
Level 5: Tissues
Level 6: Organs
Level 7: The Body
Visualization Tools to Implement on GeneLab Platform

**Top Picks from AWG**
- GSVA/GSEA
- Webgestalt
- G-Tex
- cBioPortal

**Other Open Source Tools**
- REVIGO
- Toppfun
- GOrilla
- Cytoscape
- VisJS2Jupyter
- MetaSub

**Proprietary Software**
- IPA
- iPPathwayGuide
- Blast2Go
- TOAST
Members of G-Tex have offered to help with development of G-Tex for our GeneLab platform. Multi-Omics AWG member helped make the connection with G-Tex.
Engage broadest community of researchers, industry, and citizen scientists to advance innovations

https://genelab.nasa.gov

- Weekly social media posts:
  - @NASAAmes Facebook
  - Twitter #GeneLab 🔗
  - ResearchGate: [https://www.researchgate.net/project/Omics-for-Space-Biology-The-GeneLab-project](https://www.researchgate.net/project/Omics-for-Space-Biology-The-GeneLab-project)

- GeneLab database listed in science journals:
  - Scientific Data, Oxford e-Research

- GeneLab issues Digital Object Identifiers (DOI) via DataCite

- Customer Support: Respond and resolve all inquiries from science community, academia, public
- **Conferences, Workshops, & Public Outreach Events**
  - ASGSR: Presentations, Galaxy platform training sessions
  - AMIA: Learning Showcase – Spotlight on GeneLab
  - AAAS: Session - NASA’s GeneLab Project and the Systems Biology of Spaceflight
  - COSPAR, ISSR&D: Presentations
  - Experimental Biology, Plant Biology, Cell Biology, ASM Microbe: Exhibit booths
  - BioData West Open Targets: Workshop & Data-thon
  - California Academy of Sciences, San Francisco Exploratorium

- **Social Media, Website, Newsletter, Podcasts, Videos**
  - Facebook, Twitter, ResearchGate, LinkedIn (weekly posts)
  - New Drupal Website (rollout Sept. 28); links to NASA portal, Space Biology
  - Quarterly newsletter (700 subscribers)
  - NASA Silicon Valley Podcasts
  - New GeneLab” video in collaboration with ISS Program Science Office
  - GeneLab User Training videos (Oct. 31)
- **Seminars, Webinars**
  - Broad Institute, JPL, JSC, UC San Diego, Stanford, Osaka City Univ - Japan, CSA
  - LabRoots, Genes in Space

- **SciComm Publication**
  - NASA’s Researcher’s Guide to GeneLab

- **Internship**
  - GeneLab for High Schools
    - Space biology training initiative for high school students on teaching the importance of omics studies and space biology. Students analyze GeneLab datasets and develop research proposals.