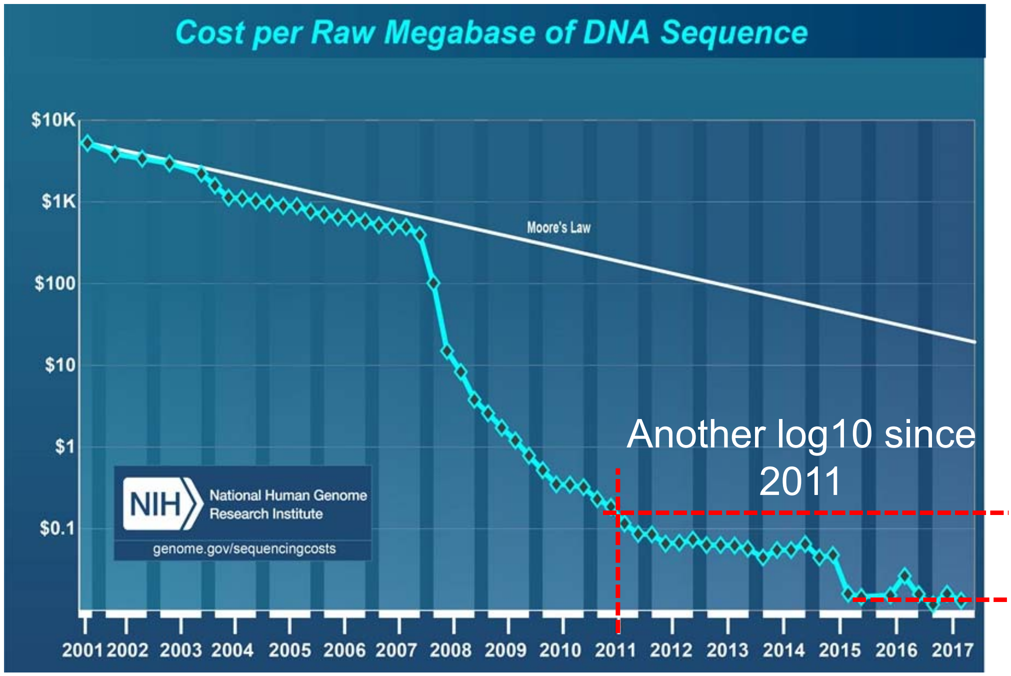
GeneLab: “Omics” Data Systems for Spaceflight and Simulated Spaceflight Environment

National Aeronautics and Space Administration

Sylvain V. Costes, PhD
GeneLab Project Manager

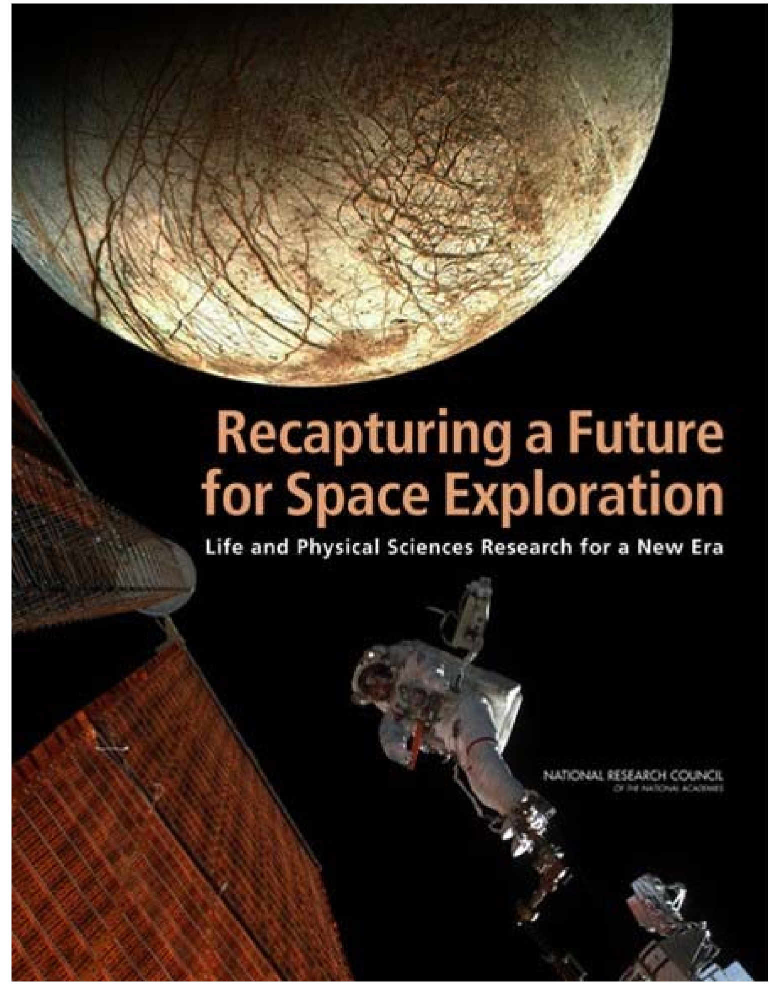
ASGSR-2018
November 2nd 2018

2011 NRC Decadal Survey and the Sequencing Paradigm Shift



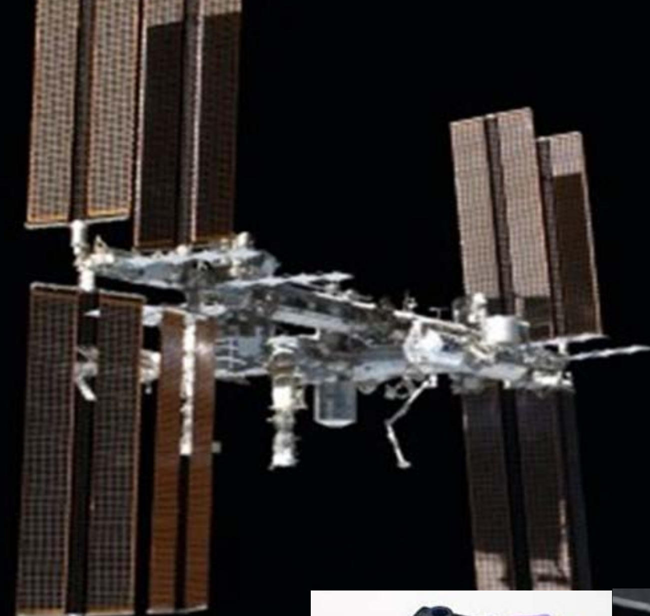
*"…**genomics, transcriptomics, proteomics, and metabolomics** offer an immense opportunity to understand the effects of spaceflight on biological systems…"*

*"…Such techniques generate considerable amounts of **data that can be mined and analyzed** for information by multiple researchers…"*



Omics Acquisition in Space is Now a Reality

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.



Sample Preparation Module

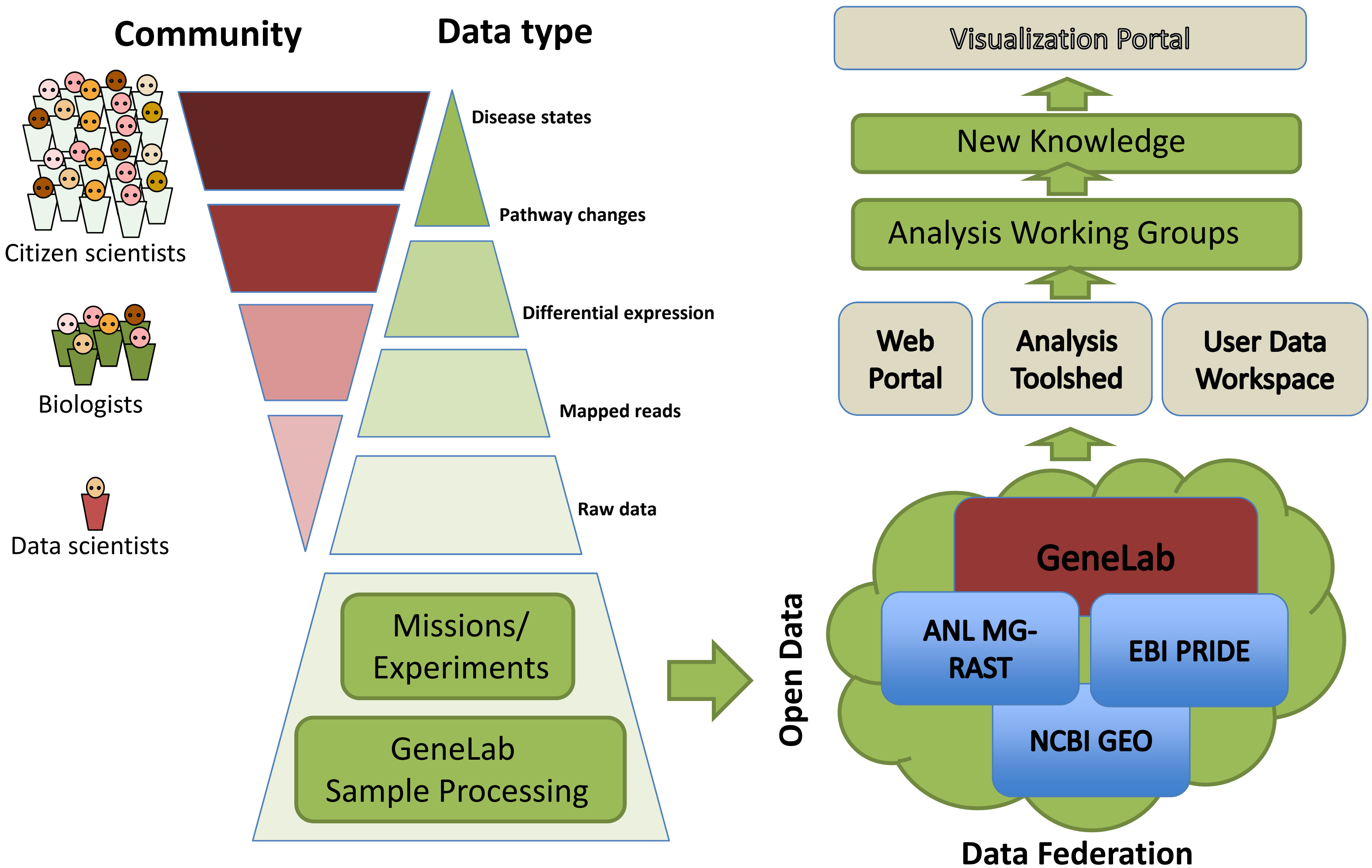
Oxford Nanopore MinION Gene Sequencer

Mini-PCR

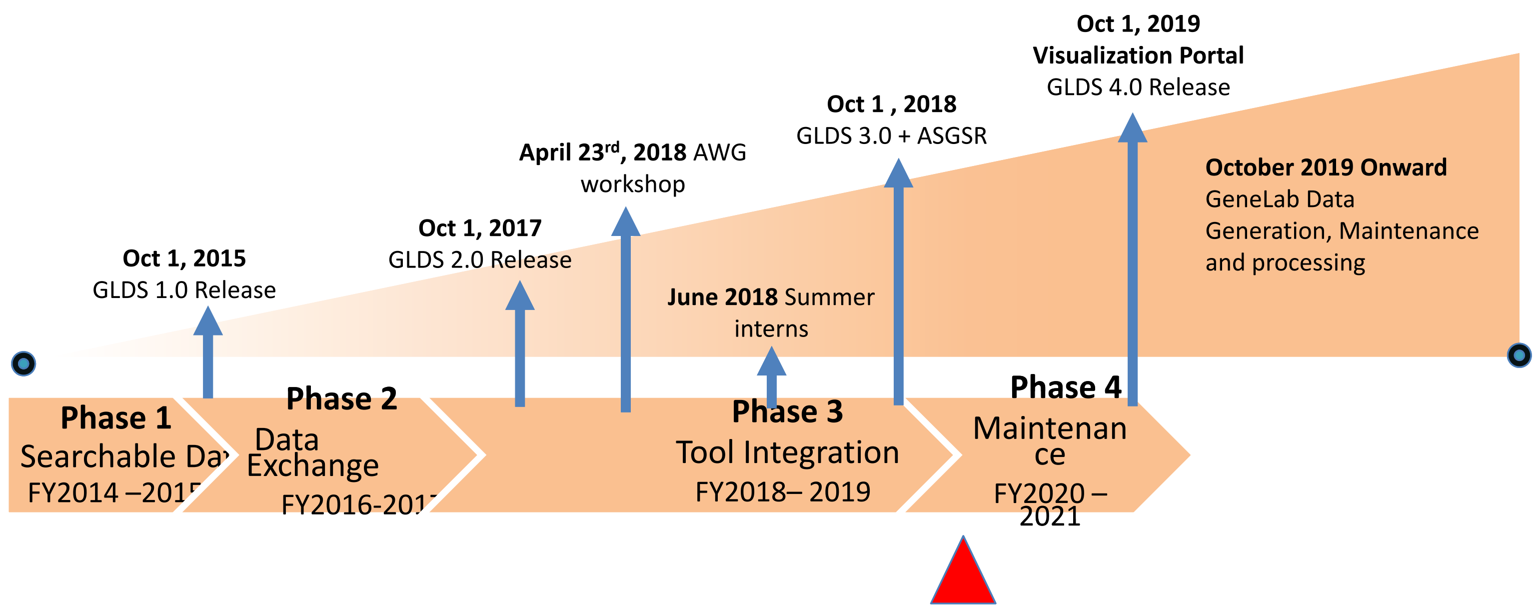
Cepheid Smart Cycler qRT-PCR

Reaction tube containing lyophilized chemical assay bead (proprietary)

GeneLab Data Democratization



Phased Implementation



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

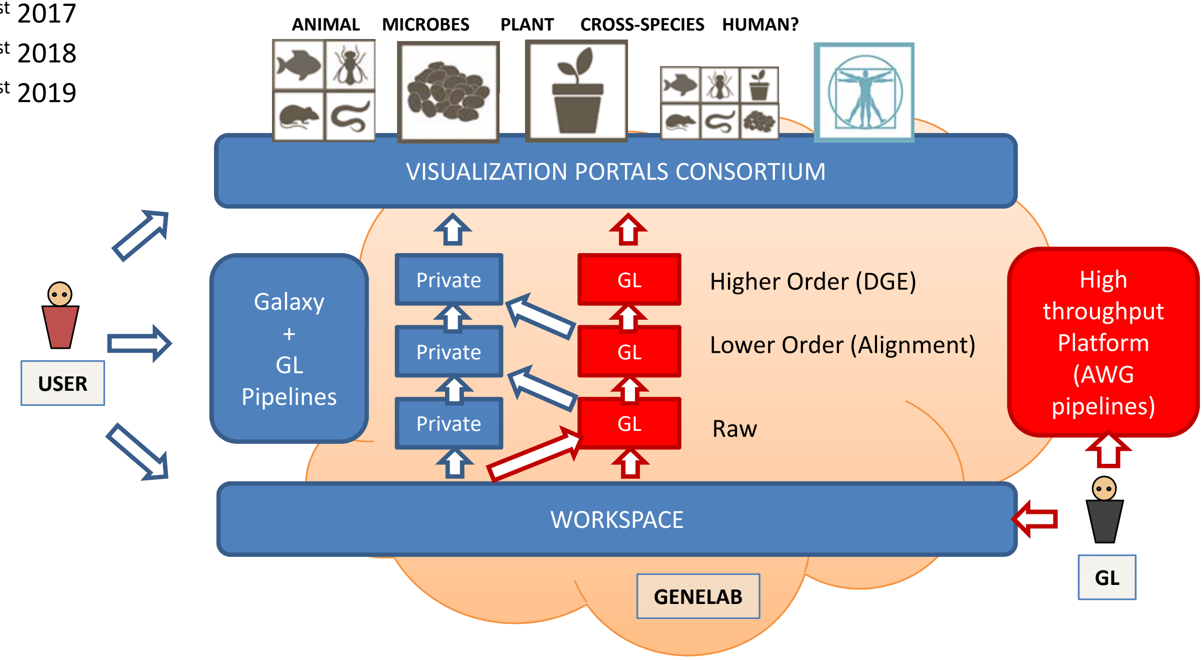
Data System
- ✓ Link to Public Databases via Data Federation
- ✓ Integrated Search (e.g., data mashup)

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

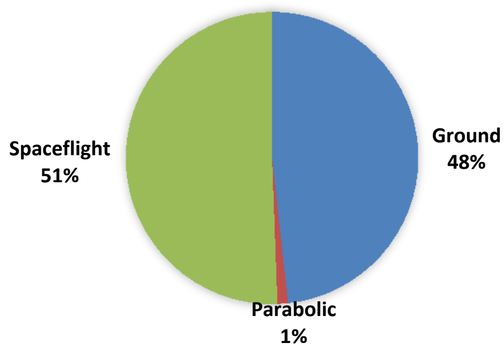
From GLDS 2.0 to GLDS 4.0

GLDS 2.0 – Oct 1st 2017
GLDS 3.0 – Oct 1st 2018
GLDS 4.0 – Oct 1st 2019

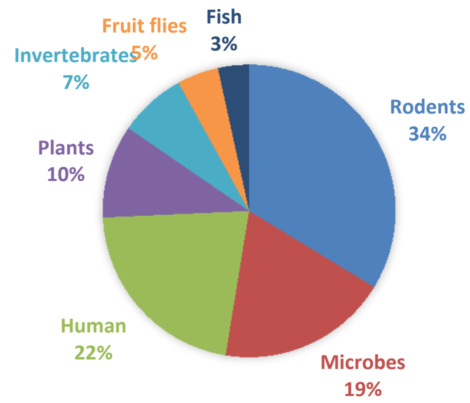


Overview: Database content

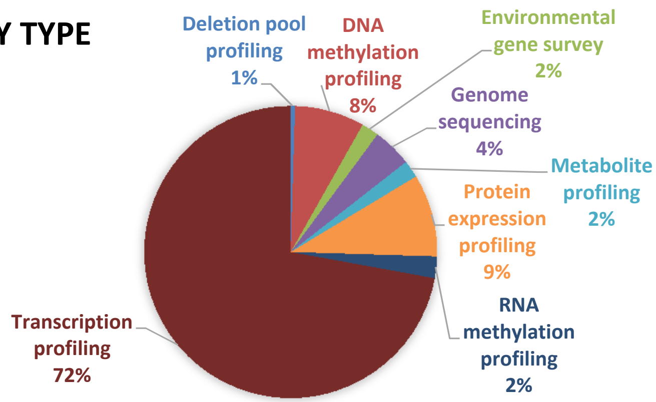
STUDY TYPE



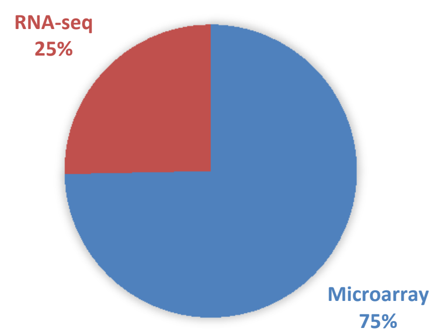
ORGANISM



ASSAY TYPE

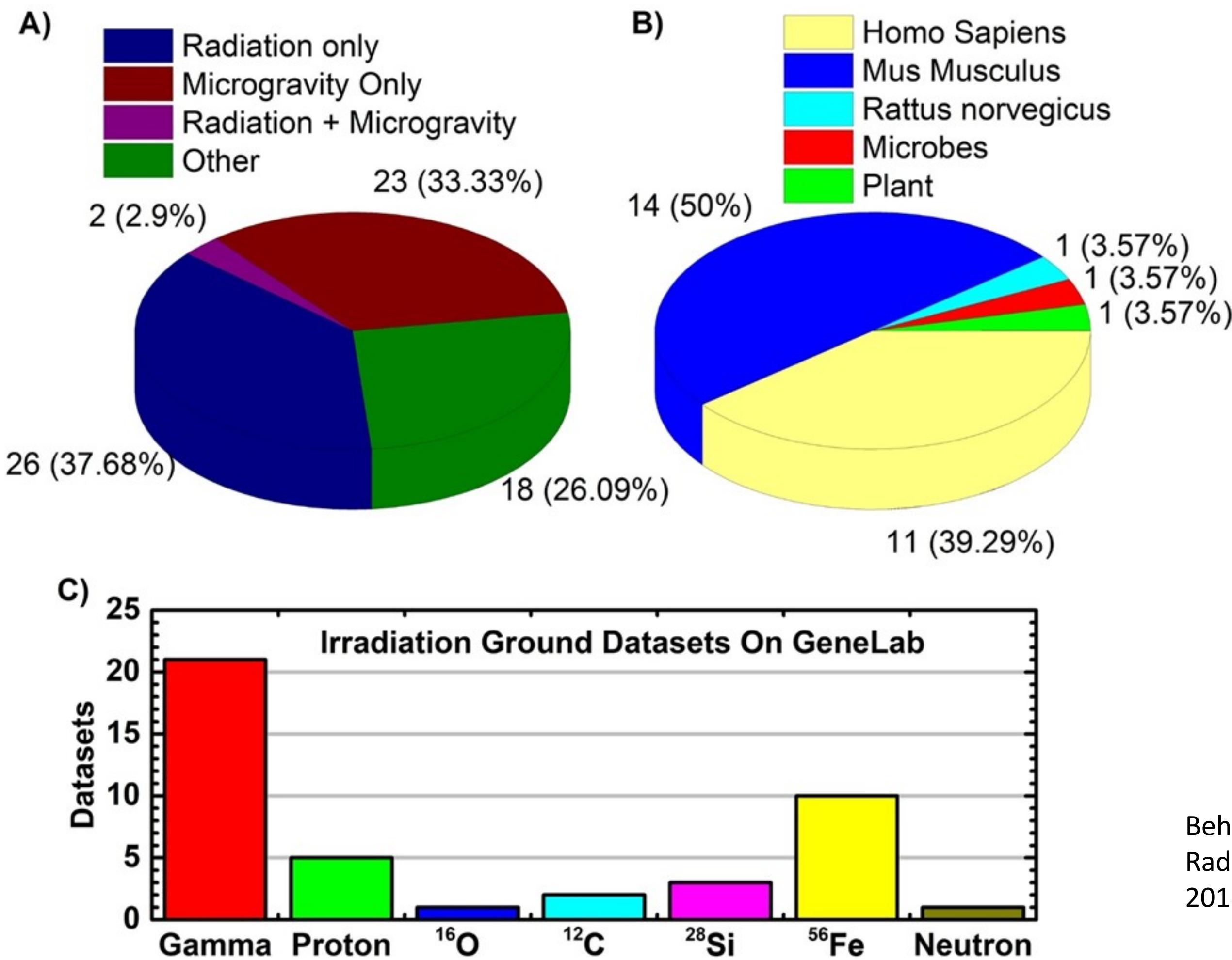


TRANSCRIPTION PROFILING



Total # of studies: 172

69 Ground Data Sets: Radiation and simulated microgravity



Beheshti et al., Radiation Research 2018

GeneLab Analysis Working Groups: Letting the scientific community take the lead

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

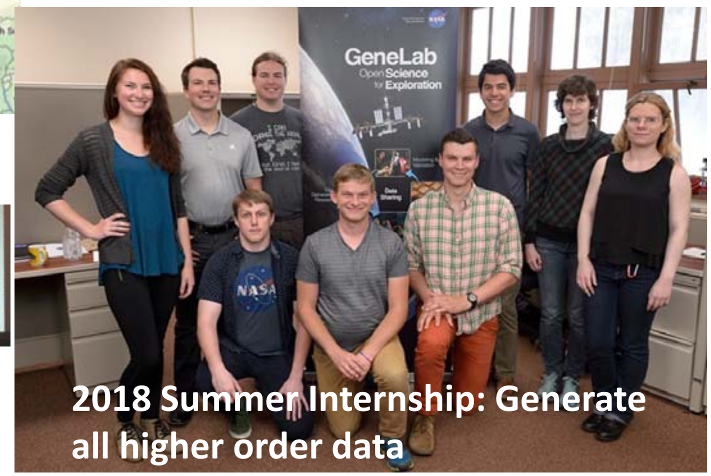
Total AWG Members: 114

AWG Members Per Group:

Animal	47
Multi-Omics/System Biology	33
Plants	24
Microbes	21

**Some members are in multiple groups*


Annual Workshop (April 2018)



2018 Summer Internship: Generate all higher order data

- **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

Publications using GeneLab

Year	Title	Journal	Authors	Status
2017	Validation of Methods to Assess the Immunoglobulin Gene Repertoire in Tissues Obtained from Mice on the International Space Station.	Gravit Space Res.	Rettig TA, Ward C, Pecaut MJ, Chapes SK	Published
2018	A microRNA signature and TGF-β1 response were identified as the key master regulators for spaceflight response	PLoS One	Beheshti A, Ray S, Fogle H, Berrios D, Costes SV	Published
2018	NASA GeneLab Project: Bridging Space Radiation Omics with Ground Studies Project: Bridging Space Radiation Omics with Ground Studies	Radiation Research	Beheshti A, Miller J, Kidane Y, Berrios D, Gebre SG, Costes SV	Published
2018	Global transcriptomic analysis suggests carbon dioxide as an environmental stressor in spaceflight: A GeneLab case study	Scientific Reports	Beheshti A, Cekanaviciute E, Smith DJ, Costes SV	Published
			Nicholson	
2018	GeneLab: Omics database for spaceflight experiments	Bioinformatics	S Ray, S Gebre, H Fogle, D Berrios, PB Tran , JM Galazka, S V Costes	Published
2018	Exploring the Effects of Spaceflight on Mouse Physiology using the Open Access NASA GeneLab Platform	JoVE	A Beheshti, Y Shirazi-Fard, S Choi, D Berrios, SG Gebre, JM Galazka, SV Costes	In Press

Cage Effects with rodent experiments: Carbon Dioxide as an Environmental Stressor in Spaceflight

Beheshti A, Cekanaviciute E, Smith DJ, Costes SV. Global transcriptomic analysis suggests carbon dioxide as an environmental stressor in spaceflight: A systems biology GeneLab case study. Sci Rep. 2018;8(1):4191. doi: 10.1038/s41598-018-22613-1. PubMed PMID: 29520055; PMCID: PMC5843582.

Carbon Dioxide as an Environmental Stressor in Spaceflight

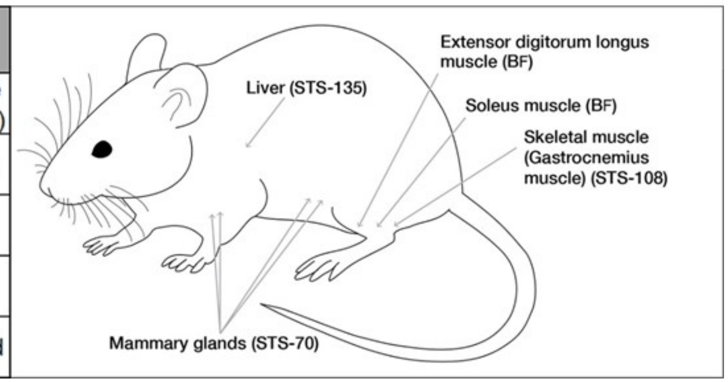
A) Cage Types

Animal Enclosure Module (AEM)

Sample vivarium cage

B)

GeneLab Study	Mission	Species	CO_2 (ppm)	Duration (days)	Tissue Type
GLDS-21	STS-108	mouse	~3000	11.8	skeletal muscle (gastrocnemius)
GLDS-111	BF	mouse	~600	30	soleus muscle
GLDS-111	BF	mouse	~600	30	extensor digitorum
GLDS-25	STS-135	mouse	~3000	13	liver
GLDS-63	STS-70	rat	~3000 (est)	9	mammary gland

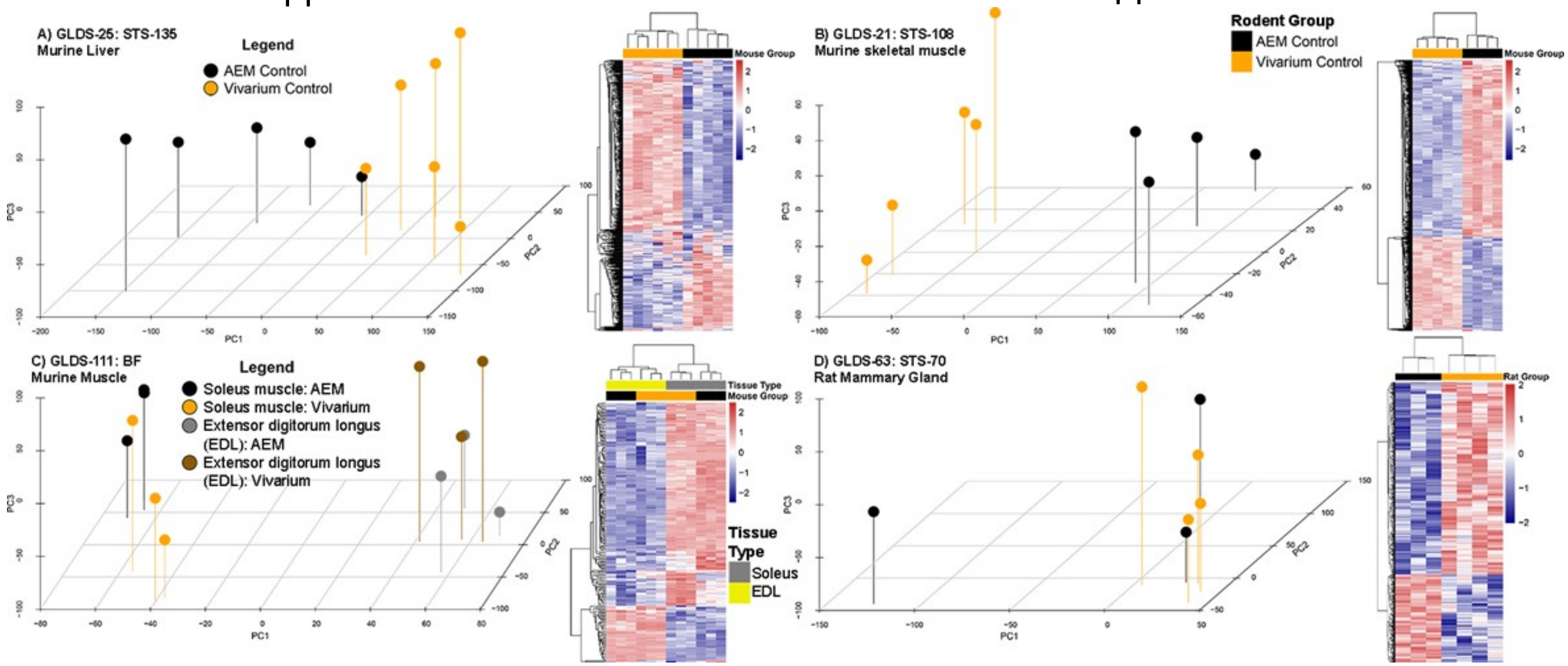


Beheshti, et al., Scientific Reports, 2018

PCA Plots Suggest Strong Cage Effect

3000 ppm

3000 ppm



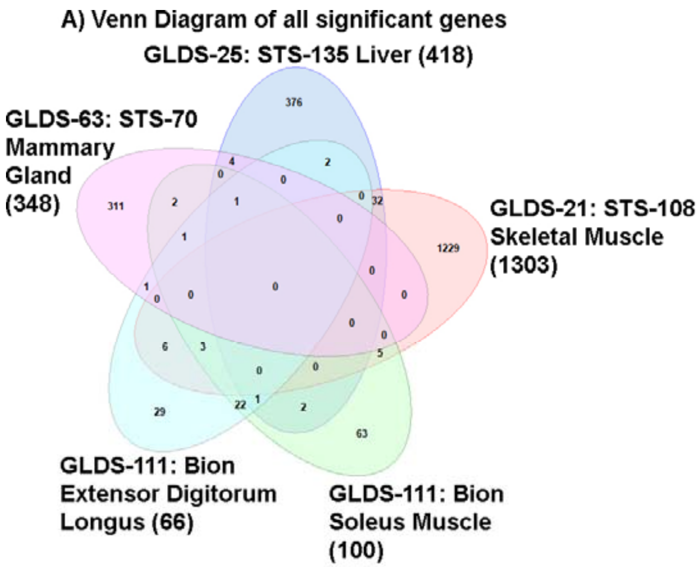
600 ppm

3000 ppm

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

Beheshti, et al., Scientific Reports, 2018

Differential Gene Expression: Cage or CO2 Effect?



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

AWG Members Involved

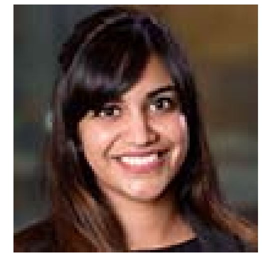
Kathleen Fisch

Brin Rosenthal

UNIVERSITY *of* CALIFORNIA, SAN DIEGO
SCHOOL OF MEDICINE

Deanne Taylor

Hossein Fazelinia



Komal Rathi

Children's Hospital of Philadelphia

Perelman School of Medicine
UNIVERSITY *of* PENNSYLVANIA

Helio Costa

Kathryn Grabek

STANFORD UNIVERSITY

J. Tyson McDonald

HAMPTON UNIVERSITY
THE STANDARD OF EXCELLENCE

Gary Hardiman

Willian da Silveira

MUSC Health
MEDICAL UNIVERSITY *of* SOUTH CAROLINA

AWG Members Involved


Chris Mason


Cem Meydan


Jonathan Foox


Flavia Rius

Cornell University


Yared Kidane

NASA
Texas Scottish Rite Hospital for Children


Susana Zanello


Scott Smith


Sara Zwart

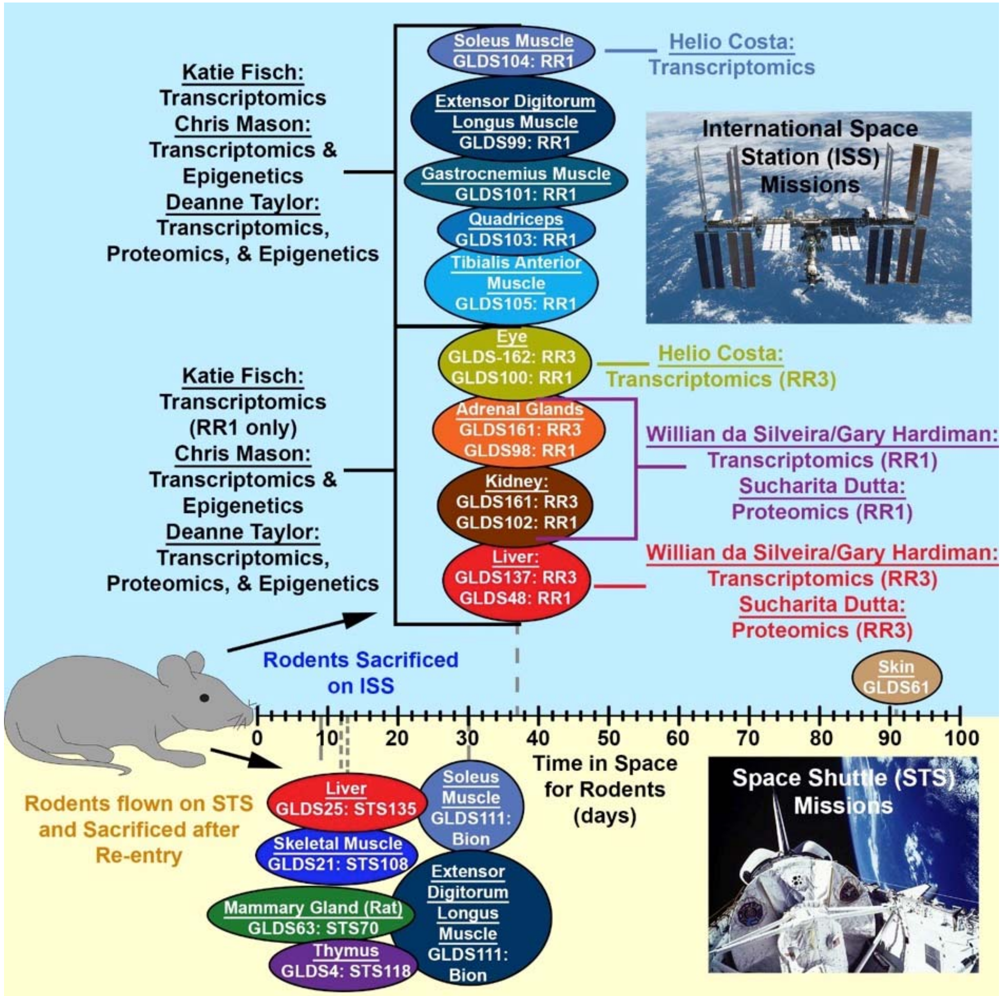
NASA
Space Center Houston
Manned Space Flight Education Foundation


Afshin Beheshti


Sylvain Costes

NASA
Ames Research Center

Specific Datasets and Tissues AWG Members Analyzed

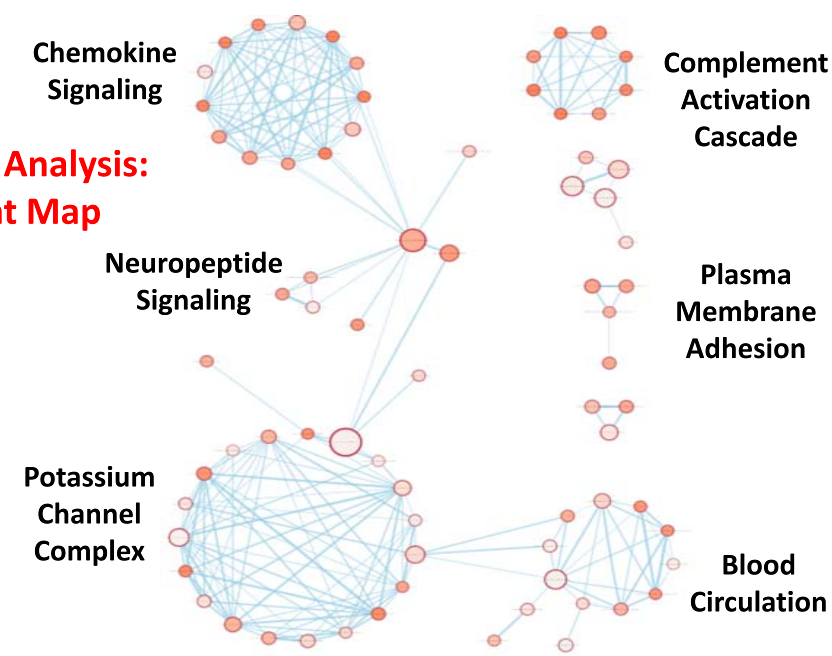


- **<u>Additional Datasets that are being analyzed:</u>**
  - Human datasets
    - GLDS-54, GLDS-174, GLDS-86, GLDS-118, GLDS-53, GLDS-54, GLDS-13. GLDS-52, or GLDS-114 (Tyson McDonald and Yared Kidane)

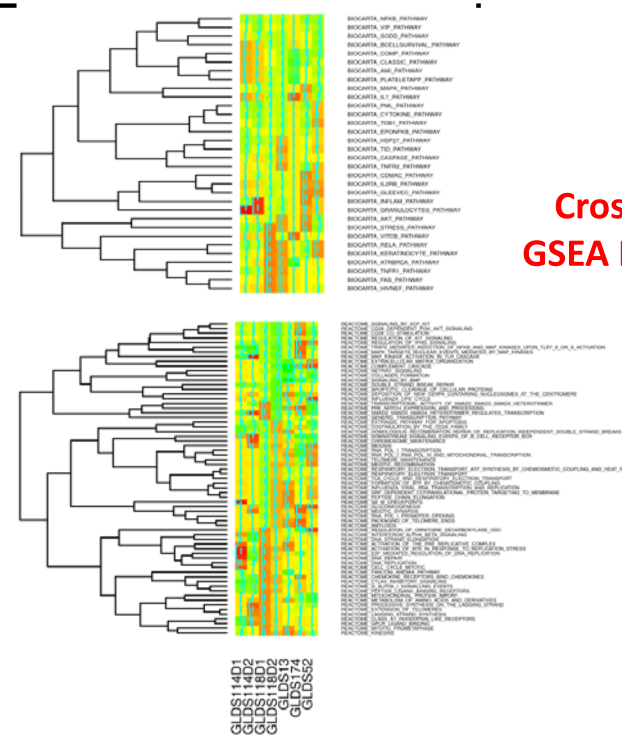
Human Dataset Analyses

GLDS	Cell/Tissue type	N	Platform	Probes	PMID
13	Primary T Cells	3	Affymetrix Microarray	54675	22750545
52	HUVEC Cells	3	Agilent Microarray	33297	23913861
54	HMVEC-dBL Cells	2	Affymetrix Microarray	45015	25102863
174	Human Hair Follicles	10	Agilent Microarray	44495	27029003
114	AG1522 Fibroblasts	4	Agilent Microarray	43457	28248986
118	Fibroblasts	4	Illumina Microarray	31426	26917741

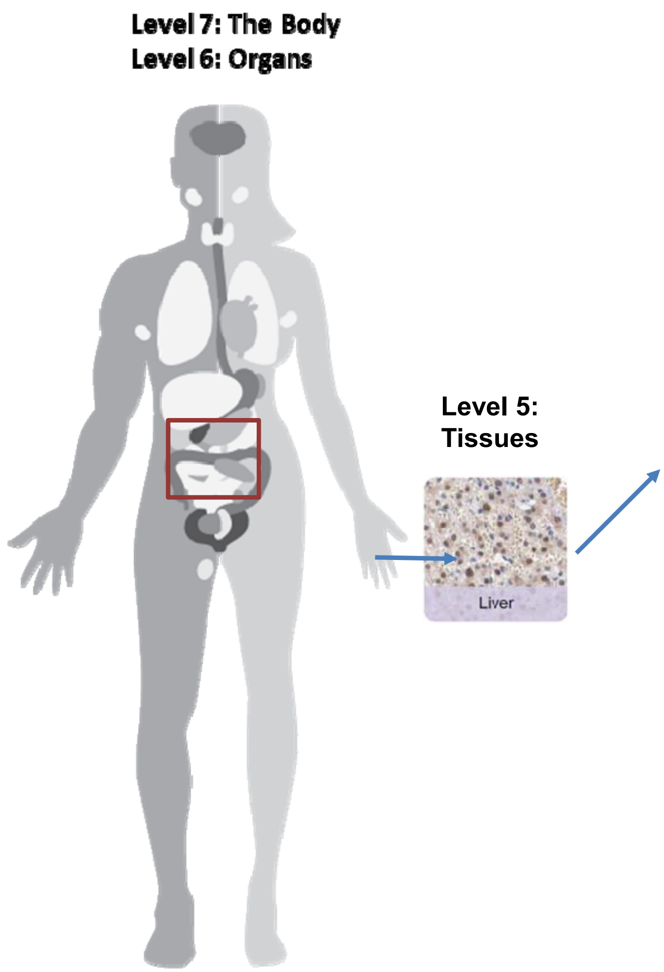
Pan-GLDS Pathway Analysis: GSEA Enrichment Map

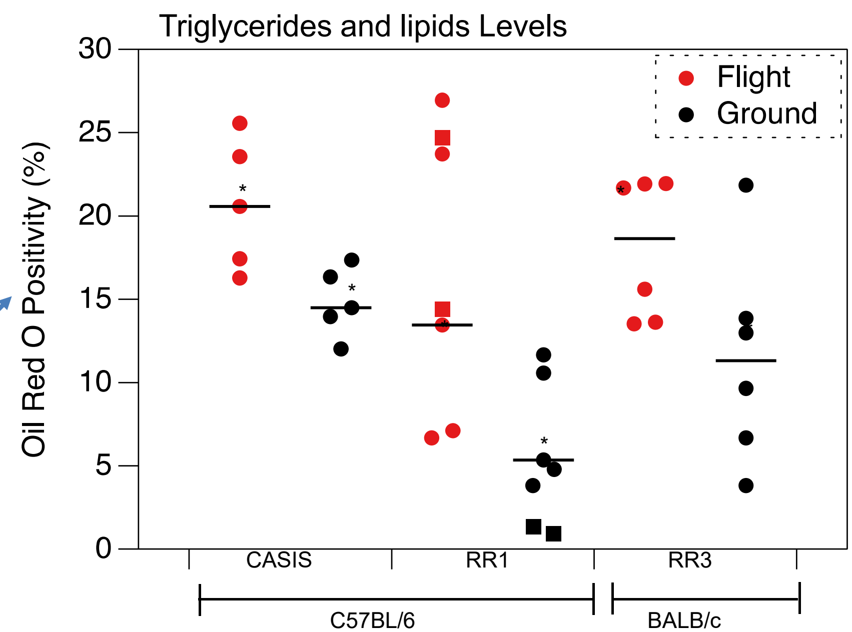


Cross-Comparison: GSEA Pathway Analysis



Lipid Accumulation in the Liver





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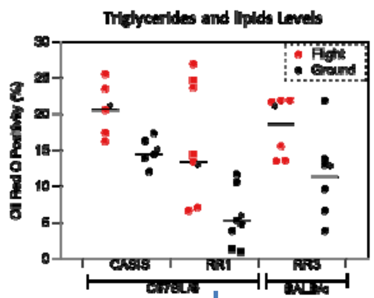
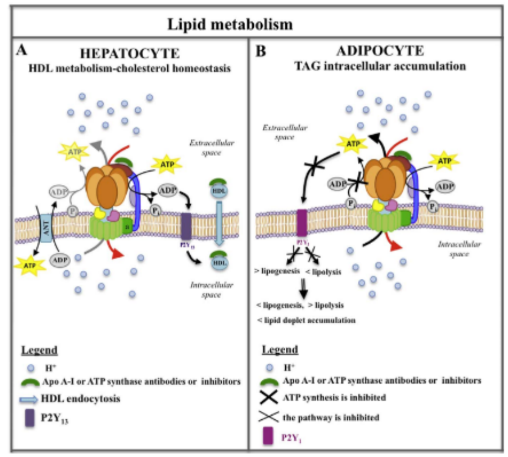
Lipid Accumulation in the Liver And Ion Diffusion

Level 5: Tissues

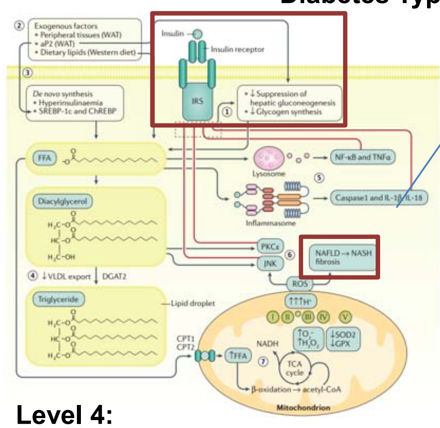


Level 1: Monomeric Units



Diabetes Type 2

Inflammation

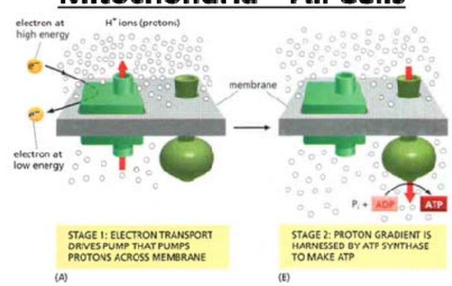


Level 4:
The Cell and its Organelles

Taurino & Gnoni. Exp Mol Pathol. 2018.
Tilg et al. Nat Rev Gastroenterol Hepatol. 2017.

Level 1:
Monomeric Units

Mitochondria – All Cells



Mitochondrial Stress

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Gary Hardiman

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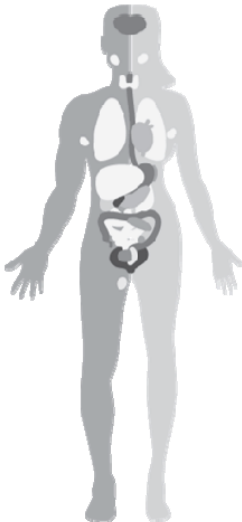
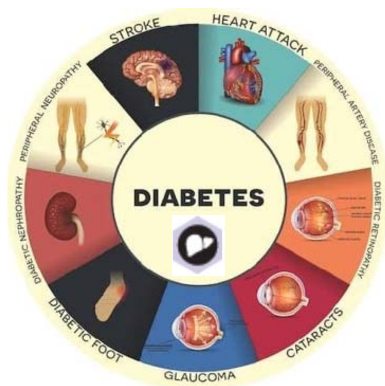
Liver at the Center of Metabolic Disorders

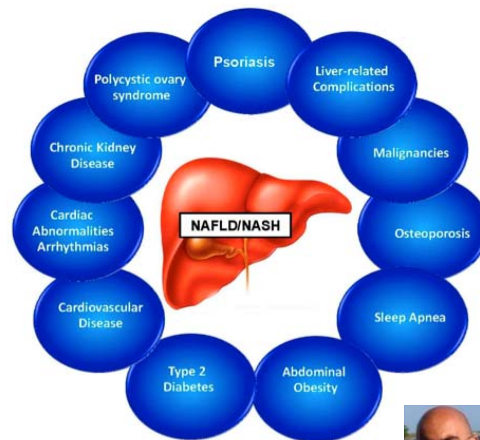
Level 7: The Body
Level 6: Organs



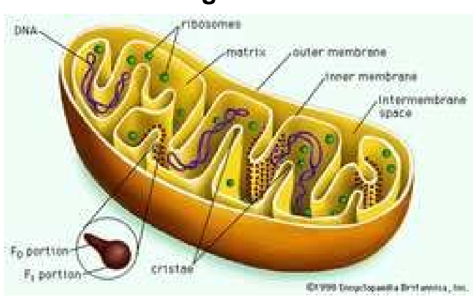
Diabetes Complications

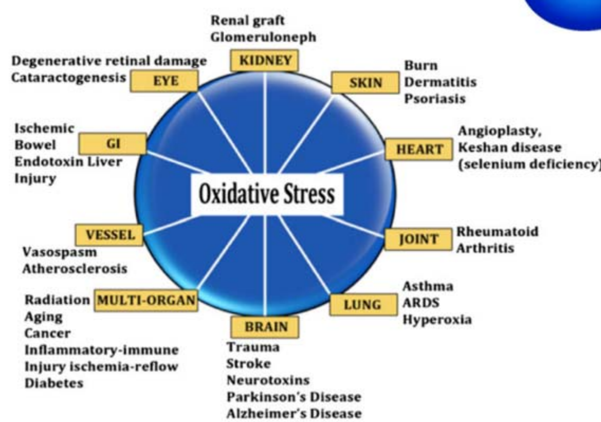


Non-Alcoholic Fatty Liver Disease Complications



Level 4: The Cell and its Organelles





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Schmidt & Goodwin, Metabolomics (2013).
Ballestri et al. World J Gastroenterol. 2014.

RR1 and RR3 Mice

RR1: C57BL/6
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

RR3: BALB/c
mice strain
(female)

- Th2 Immune Response (more tolerogenic),
- More susceptible to liver steatosis
- Radiosensitive

RR1 and RR3 Experimental Detail

RR1 - C57BL/6

RR3 - BALB/c

ISS

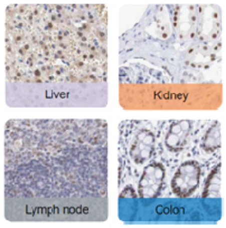
<u>Exposition time</u>: ≈ 40 days

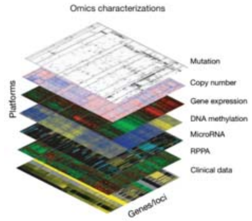
RR1
Space Flight n = 6
Ground Control n = 6

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

Earth

Level 5: Tissues





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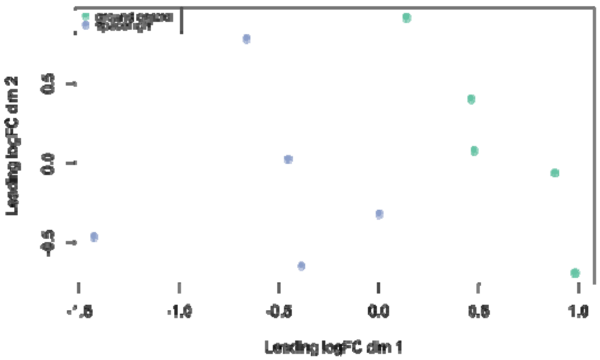
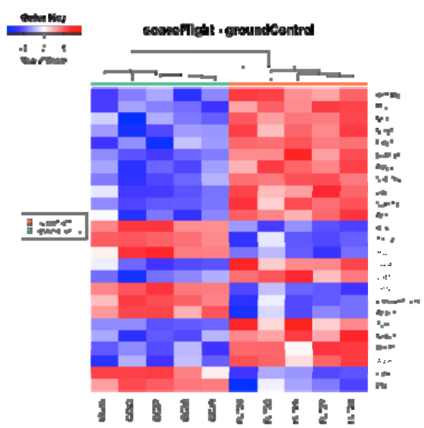
RR1 and RR3: Liver Transcriptomics

RR1 Liver (C57BL/6)

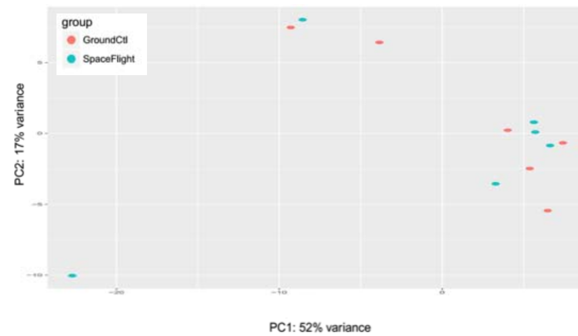
181 DE genes adj.P < 0.05

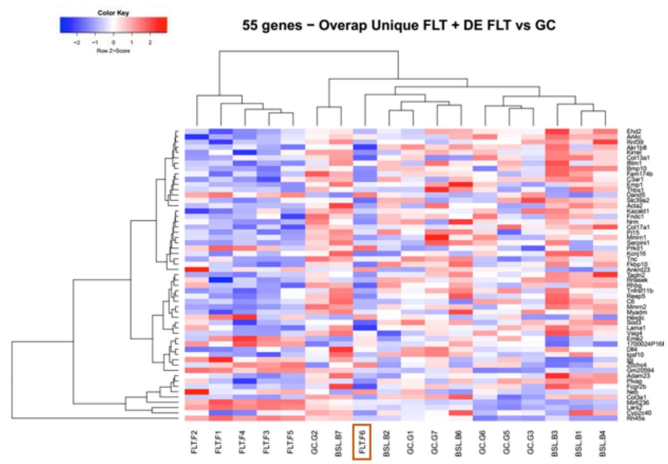




RR3 Liver (BALB/c)

0 DE genes adj.P < 0.05





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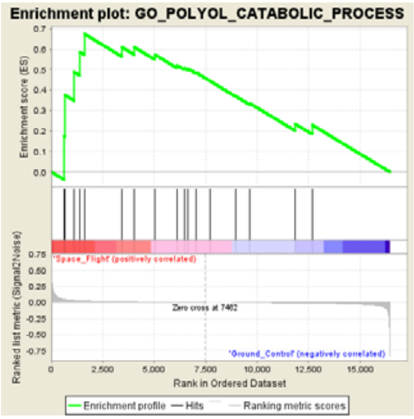
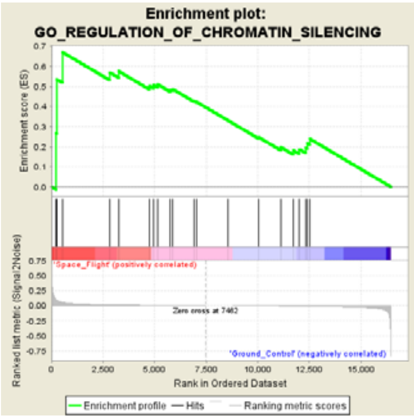
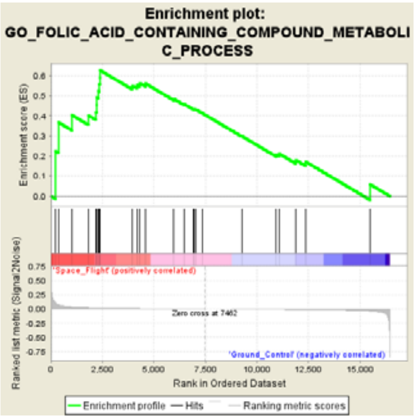
Brin Rosenthal

Kathleen Fisch

RR3 (BALB/c)
System Level Analysis







GSEA - G.O Biological Process:

NAME	NOM p-val	FDR q-val
GO_POLYOL_CATABOLIC_PROCESS	0.002	0.21
GO_FOLIC_ACID_CONTAINING_COMPOUND_METABOLIC_PROCESS	0.002	0.20
GO_PTERIDINE_CONTAINING_COMPOUND_METABOLIC_PROCESS	<0.001	0.21
GO_RESPONSE_TO_LEAD_ION	<0.002	0.23
GO_REGULATION_OF_CHROMATIN_SILENCING	<0.003	0.22

Related to eye damage and cataract Risk in **Diabetes Type II**

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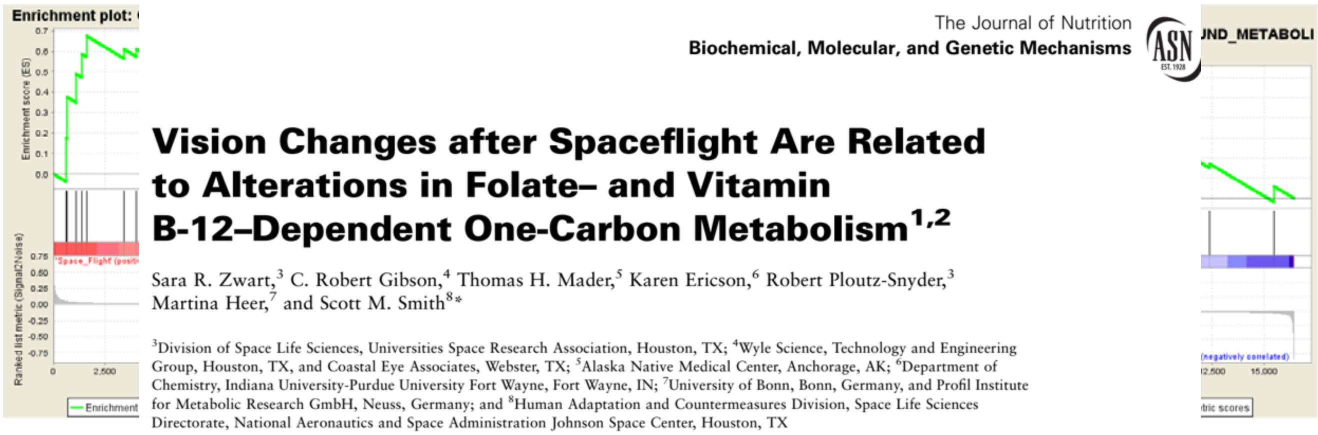
RR3 (BALB/c)
System Level Analysis


The Journal of Nutrition
Biochemical, Molecular, and Genetic Mechanisms

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 Ploutz-Snyder,[3] 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 Profil Institute for Metabolic Research GmbH, Neuss, 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:

NAME	NOM p-val	FDR q-val
GO_POLYOL_CATABOLIC_PROCESS	0.002	0.21
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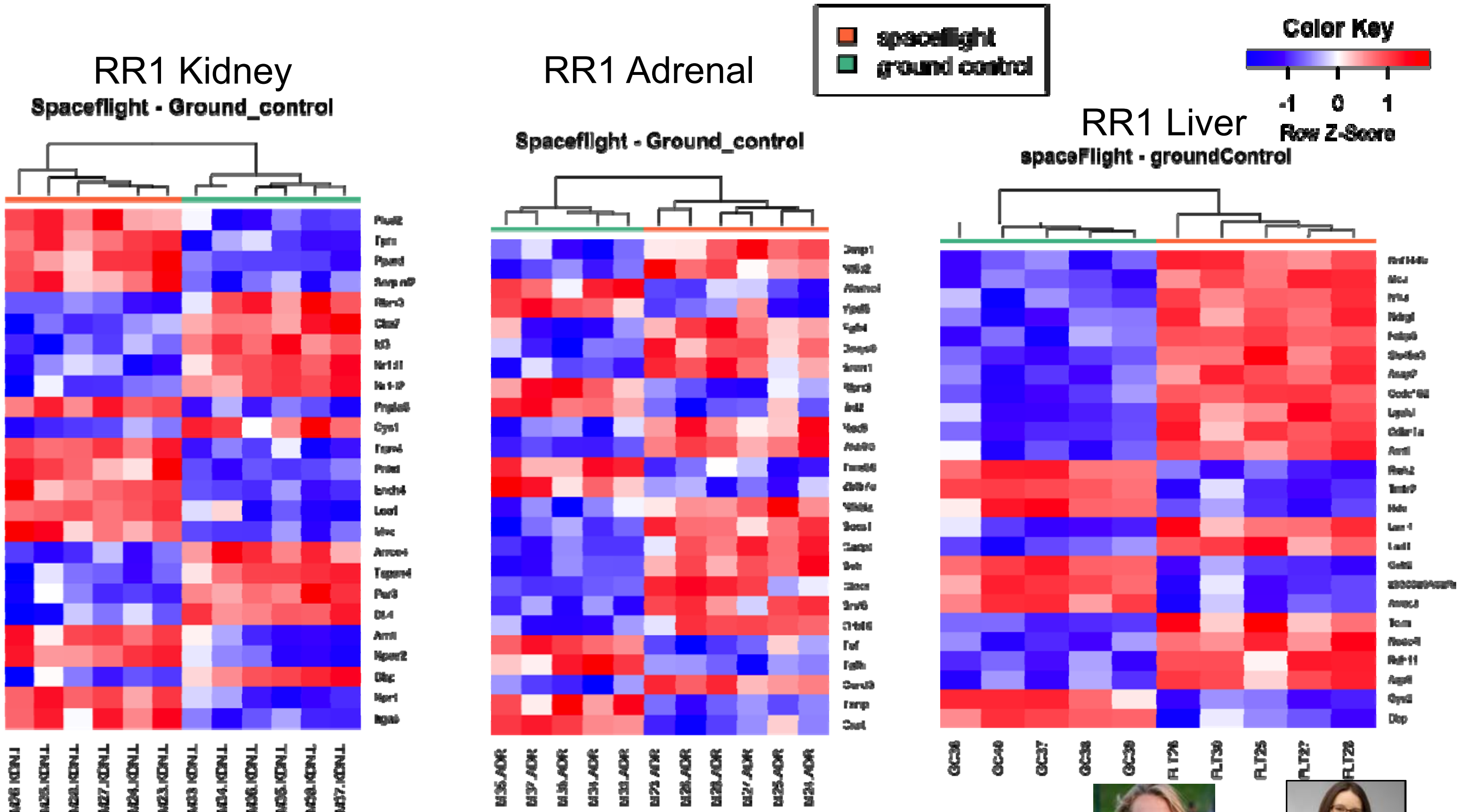
GO_RESPONSE_TO_LEAD_ION	<0.002	0.23
GO_REGULATION_OF_CHROMATIN_SILENCING	<0.003	0.22

Related to eye damage and cataract Risk in **Diabetes Type II**


Willian da Silveira


Gary Hardiman

RR1 Differentially Expressed Genes : Space Flight vs Ground Control



Brin Rosenthal

Kathleen Fisch

RR1 Shared DE Genes

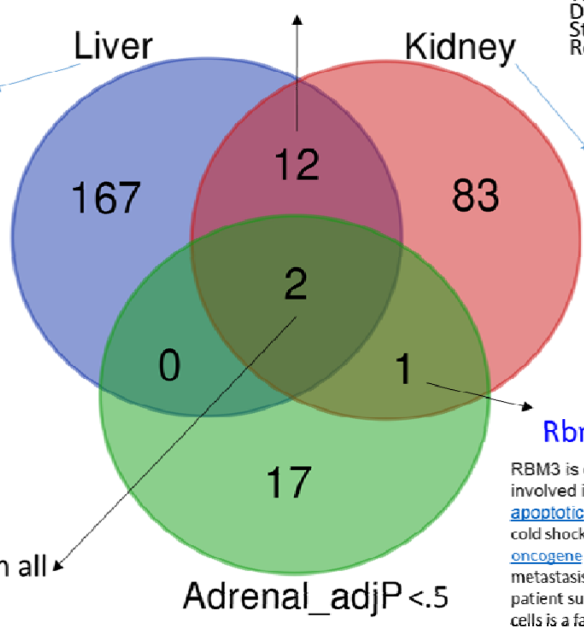
Cbx7 Arntl Cdkn1a Gale Per3 Nfil3 Npas2
Pnkd Loxl4 Bhlhe41 Dbp Leo1

Toppgene

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

GSEA

Zinc Transporters (Reactome)
Apoptosis by TGFB1 via MAPK1...
Alpha Linolenic Acid Metabolism (KEGG)
PARK2_Hepatocyte_Proliferation...
LGI1_Targets_Up
Amplification hot spot...
FGFR ligand binding... (Reactome)
Response to TGFB1...
Breast Cancer...
Apoptosis by CDKN1A not via TP53...
FGFR signaling...
Systemic lupus erythematosus...



GSEA

Core Duplicon Genes
Signal Attenuation (Reactome)
Post Chaperonin Tubulin (Reactome)

Downregulated in all three conditions
Tef
Txnip

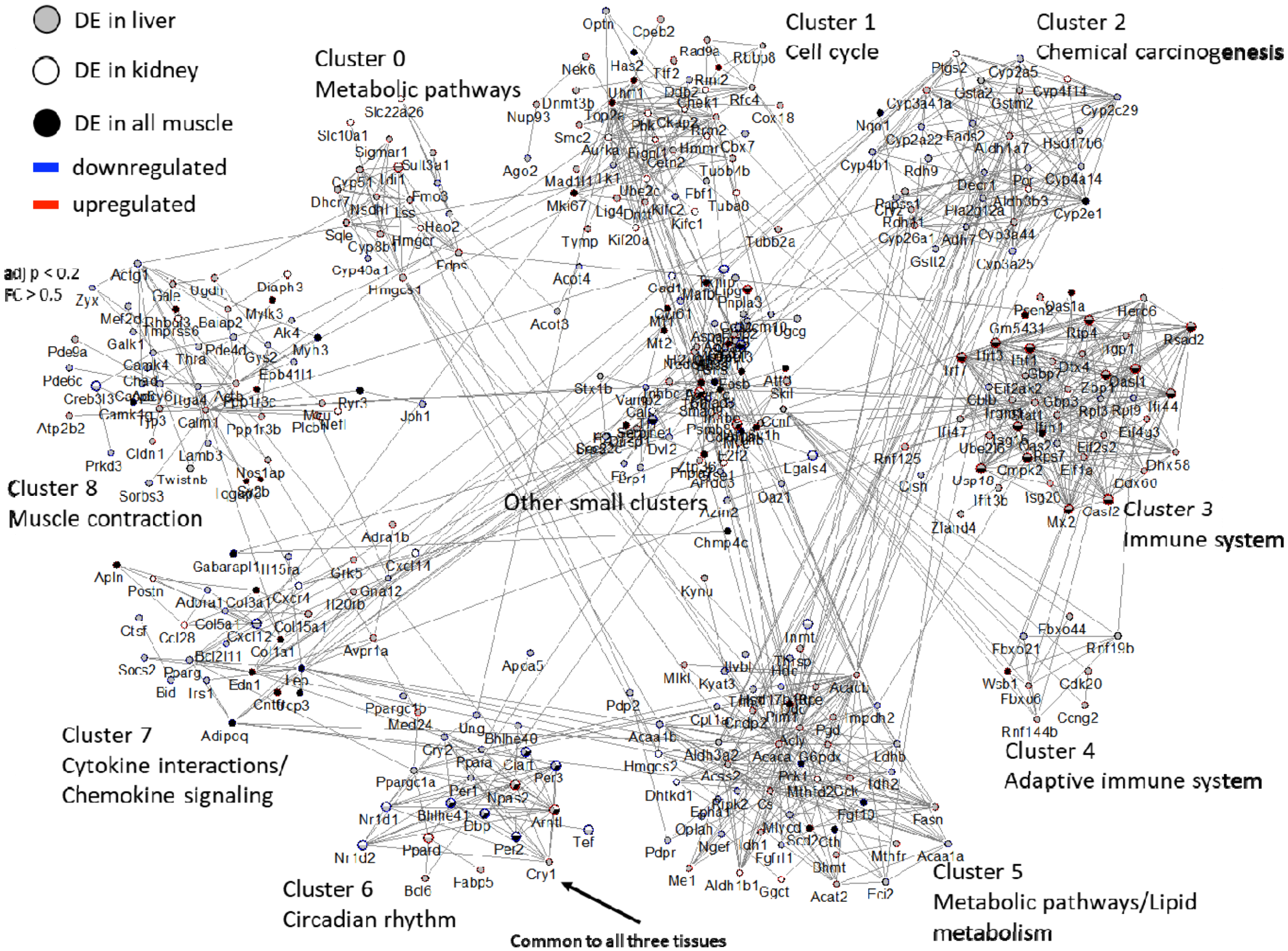
Rbm3 -- downregulated

RBM3 is cold-induced RNA binding protein and is involved in mRNA biogenesis exerts anti-apoptotic effects.[8] Expression of this gene is induced by cold shock and low oxygen tension. RBM3 is a proto-oncogene that is associated with tumor progression and metastasis and is a potential cancer biomarker.[9] Based on patient survival data, high levels of RBM3 protein in tumor cells is a favourable prognostic biomarker in colorectal cancer.[9]

Brin Rosenthal

Kathleen Fisch

RR1 (C57BL/6)
All Tissues Network Analysis



Brin Rosenthal

Kathleen Fisch

RR3 - EYE (BALB/c) :
Up-regulated: Functional Annotation Clustering

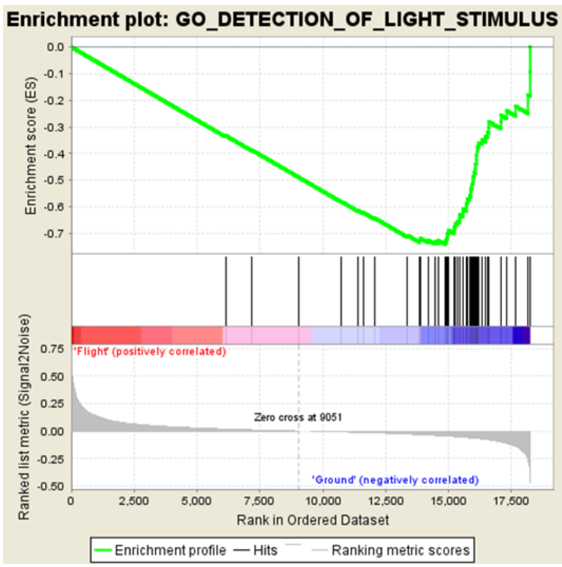
- **Most up-regulated Gene:**
  - **Gene**: Defb7 (ENSMUSG00000037790.3)
  - **Role**: Beta-defensin 7 has bactericidal activity and is involved in defense response to bacteria
  - **$\mathbf{Log_2}$ fold-change**: 22
  - **Adjusted p-value**: 2.94e-7

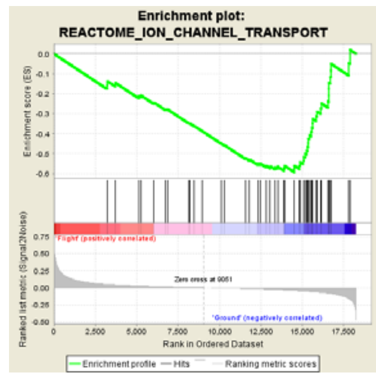
otation Cluster 1	Enrichment Score: 8.69		Count	P_Value	Benjamini
RM_BP_FAT	fatty acid metabolic process	RT	17	1.9E-10	2.1E-7
R_KEYWORDS	lipid metabolism	RT	15	2.0E-10	1.7E-8
R_KEYWORDS	fatty acid metabolism	RT	10	1.6E-8	7.9E-7
_PATHWAY	PPAR signaling pathway	RT	12	2.9E-8	2.5E-6
otation Cluster 2	**Enrichment Score: 7.69**		**Count**	**P_Value**	**Benjamini**
RM_CC_FAT	peroxisome	RT	17	6.4E-14	8.9E-12
RM_CC_FAT	microbody	RT	17	6.4E-14	8.9E-12
R_KEYWORDS	peroxisome	RT	15	1.6E-12	4.2E-10
RM_CC_FAT	microbody part	RT	8	5.6E-8	3.9E-6
RM_CC_FAT	peroxisomal part	RT	8	5.6E-8	3.9E-6
RM_CC_FAT	peroxisomal membrane	RT	6	1.1E-5	2.3E-4
RM_CC_FAT	microbody membrane	RT	6	1.1E-5	2.3E-4
Q_FEATURE	short sequence motif:Microbody targeting signal	RT	6	1.5E-4	3.8E-2
RM_BP_FAT	peroxisome organization	RT	4	1.5E-3	6.0E-2
otation Cluster 3	**Enrichment Score: 4.64**		**Count**	**P_Value**	**Benjamini**
RM_MF_FAT	carboxylic acid binding	RT	11	5.5E-8	1.0E-5
RM_MF_FAT	vitamin binding	RT	10	1.2E-5	1.5E-3
RM_MF_FAT	amino acid binding	RT	5	5.7E-4	2.1E-2
RM_MF_FAT	amine binding	RT	6	7.3E-4	2.2E-2

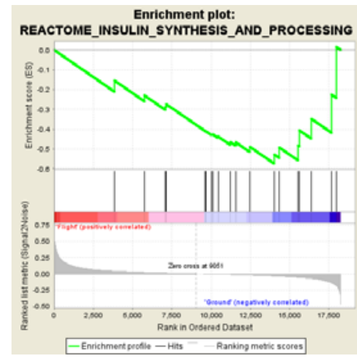
Kathryn Grabek

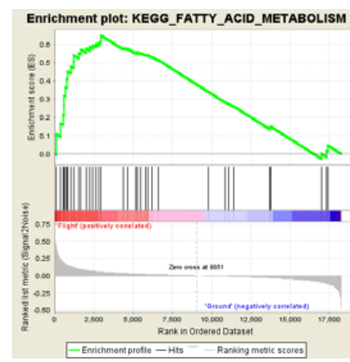
Helio Costa

RR3 - EYE (BALB/c) :
GSEA Analysis









NAME	NOM p-val	FDR q-val
GO_FOLIC_ACID_CONTAINING_COMPOUND_METABOLIC_PROCESS	0.003	0.01
GO_POLYOL_METABOLIC_PROCESS	0.014	0.17
GO_DETECTION_OF_LIGHT_STIMULUS	<0.001	<0.001
REACTOME_ION_CHANNEL_TRANSPORT	<0.001	0.01
REACTOME_INSULIN_SYNTHESIS_AND_PROCESSING	0.044	0.18
KEGG_FATTY_ACID_METABOLISM	<0.001	0.003

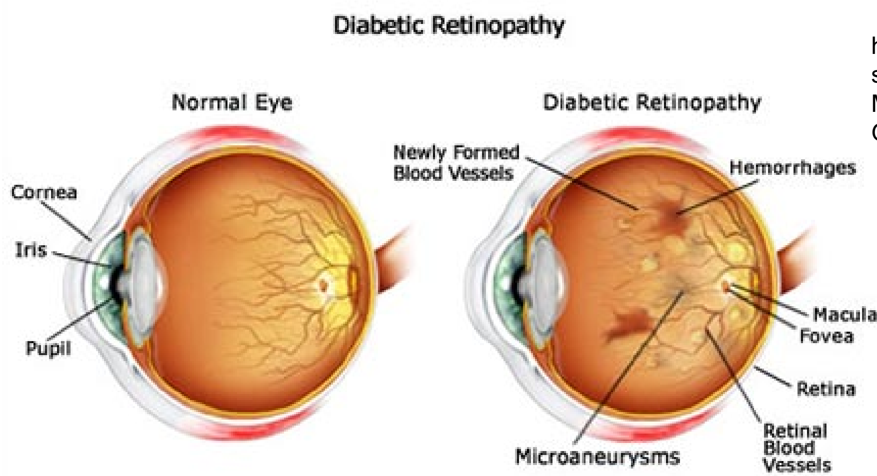
Willian da Silveira

Gary Hardiman

Kathryn Grabek

Helio Costa

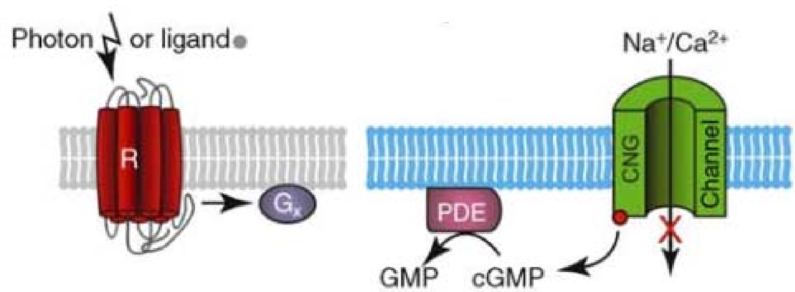
Analysis Relating Liver to the Eye



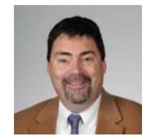
https://www.eyedoctorophthalmologistnyc.com
Molecular Biology of the Cell, Garland Science; 5th edition. 2007.

Cone Cells - Eye

1st Amplification 2nd Amplification 3rd Amplification



Willian da Silveira



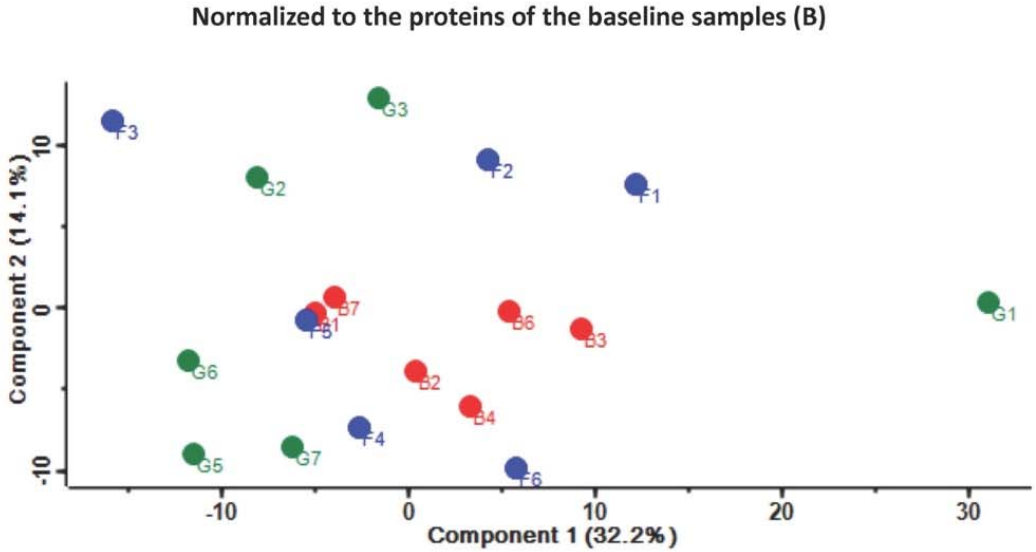
Gary Hardiman

Kathryn Grabek

Helio Costa

Overcoming Batch Effects: RR3 – Liver: Proteomics

Data processing: PCA plot of normalized & ComBat treated data



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

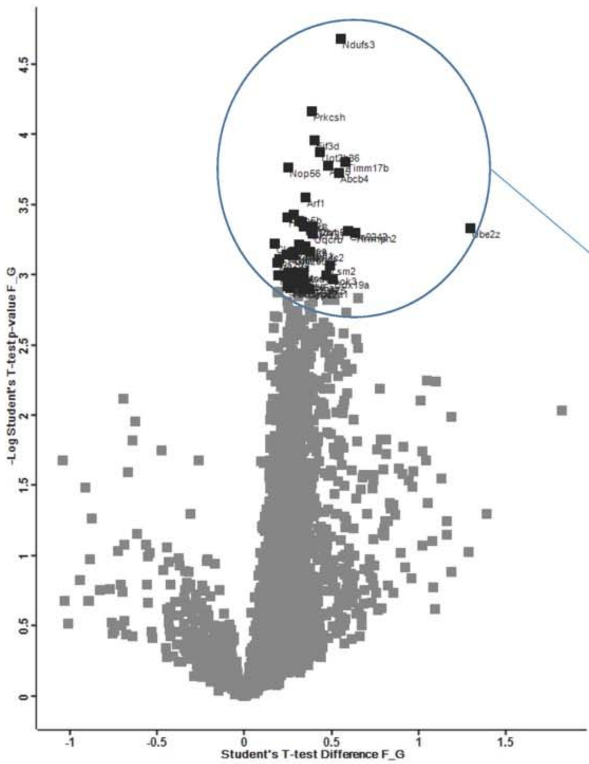
● Flight ● Ground ● Basel

Deanne Taylor

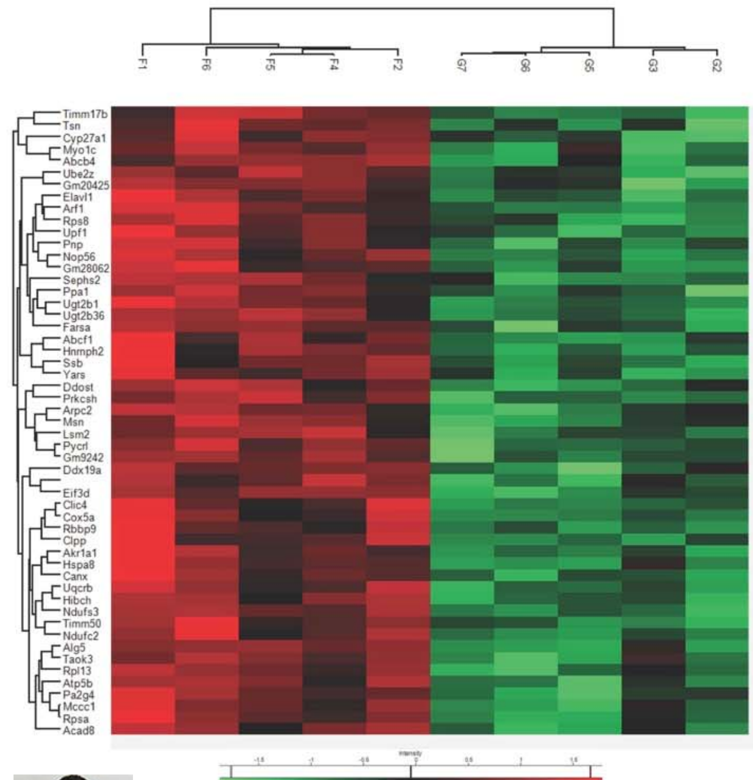
Hossein Fazelinia

Significantly Expressed Protein
RR3 – Liver: Proteomics

T-test analysis with %5 FDR



Heat map of significantly changed proteins



Deanne Taylor

Hossein Fazelinia

Protein Pathway Analysis
RR3 – Liver: Proteomics

Data processing: GO term analysis for significant proteins

#	Molecular functions	Min FDR	In Data
1	RNA binding	1.471E-09	25
2	catalytic activity	8.716E-08	42
3	heterocyclic compound binding	1.472E-05	38
4	ATP binding	1.472E-05	18
5	small molecule binding	1.472E-05	24
6	organic cyclic compound binding	1.478E-05	38
7	adenyl ribonucleotide binding	1.478E-05	18
8	adenyl nucleotide binding	1.478E-05	18
9	purine ribonucleoside triphosphate binding	3.401E-05	19
10	anion binding	3.742E-05	24
11	purine ribonucleotide binding	3.742E-05	19
12	ATPase activity	3.742E-05	10
13	purine nucleotide binding	3.742E-05	19
14	ribonucleotide binding	3.742E-05	19
15	ATPase activity, coupled	4.245E-05	9
16	carbohydrate derivative binding	9.412E-05	20
17	nucleotide binding	9.412E-05	20
18	nucleoside phosphate binding	9.412E-05	20
19	monocarboxylic acid binding	9.412E-05	5
20	ceramide-translocating ATPase activity	1.267E-04	2

#	Processes	Min FDR	In Data
1	small molecule metabolic process	1.496E-06	25
2	aromatic compound catabolic process	1.496E-06	13
3	peptide metabolic process	8.235E-06	14
4	uronic acid metabolic process	8.568E-06	5
5	glucuronate metabolic process	8.568E-06	5
6	cellular amide metabolic process	1.024E-05	15
7	protein targeting	1.075E-05	10
8	organic acid metabolic process	1.075E-05	17
9	carboxylic acid metabolic process	1.075E-05	16
10	organic cyclic compound catabolic process	1.075E-05	12
11	cellular amino acid metabolic process	1.681E-05	10
12	cellular catabolic process	1.752E-05	21
13	translation	2.470E-05	11
14	lysosomal membrane organization	2.709E-05	3
15	purine ribonucleoside triphosphate metabolic process	2.709E-05	9
16	oxoacid metabolic process	2.709E-05	16
17	metabolic process	2.713E-05	53
18	ribonucleoside triphosphate metabolic process	2.787E-05	9
19	purine nucleoside triphosphate metabolic process	2.787E-05	9
20	cellular metabolic process	2.904E-05	50

#	Localizations	Min FDR	In Data
1	cytoplasm	1.491E-10	63
2	cytoplasmic part	1.799E-08	52
3	macromolecular complex	1.349E-07	37
4	inner mitochondrial membrane protein complex	2.003E-07	8
5	intracellular organelle part	2.489E-07	48
6	organelle part	5.429E-07	48
7	mitochondrial protein complex	5.429E-07	8
8	ribonucleoprotein complex	5.429E-07	15
9	intracellular ribonucleoprotein complex	3.692E-06	14
10	mitochondrial part	4.006E-06	15
11	intracellular part	4.006E-06	63
12	mitochondrial membrane part	4.006E-06	8
13	mitochondrial inner membrane	4.038E-06	11
14	organelle inner membrane	1.103E-05	11
15	intracellular	1.315E-05	63
16	mitochondrial membrane	1.725E-05	12
17	organelle envelope	2.340E-05	15
18	envelope	2.340E-05	15
19	mitochondrial envelope	2.492E-05	12
20	myelin sheath	2.492E-05	7

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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3	heterocyclic compound binding	1.472E-05	38
4	ATP binding	1.472E-05	18
5	small molecule binding	[illegible]	24
6	organic cyclic compound binding	1.478E-05	[illegible]
7	adenyl ribonucleotide binding	1.478E-05	18
8	adenyl nucleotide binding	1.478E-05	18
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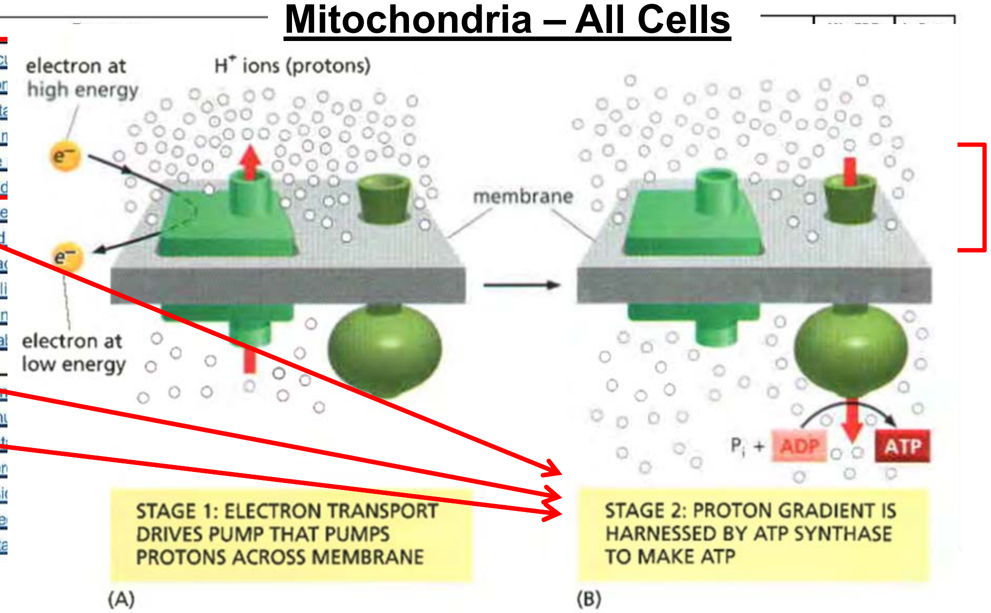
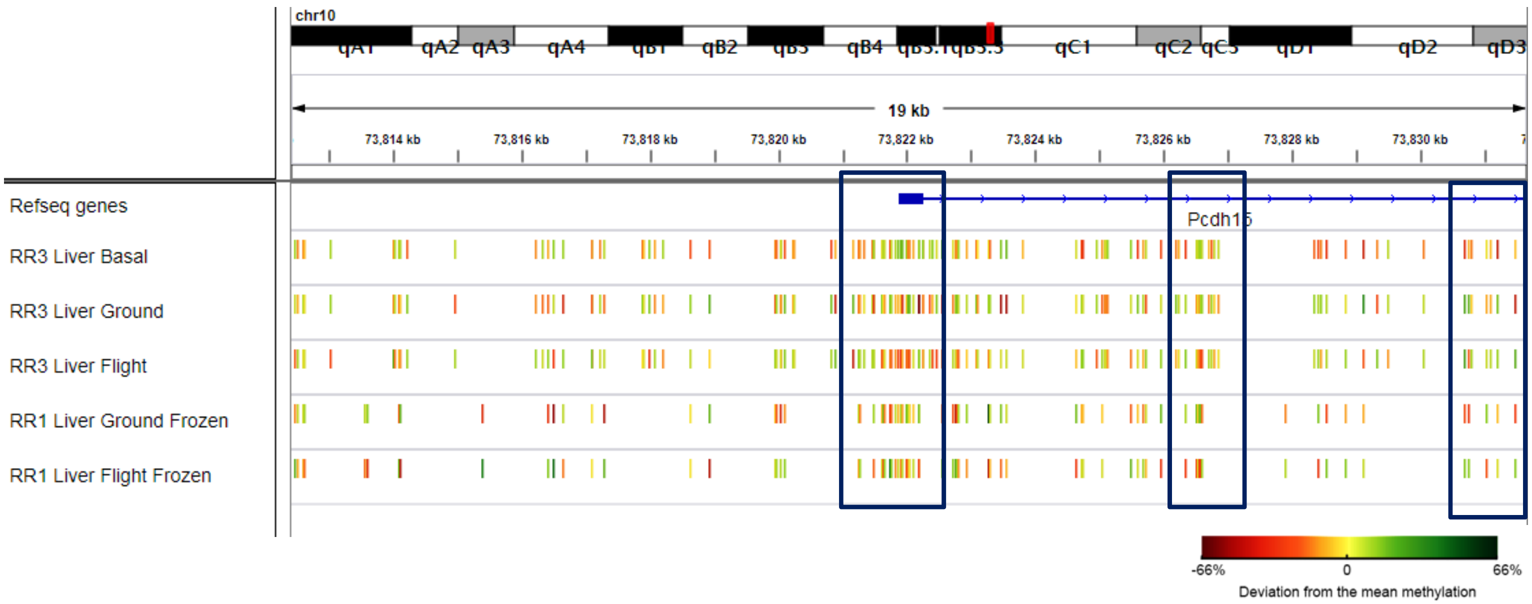
#	
1	small molecu
2	aromatic com
3	peptide meta
4	uronic acid m
5	glucuronate
6	cellular amid
7	protein targe
8	organic acid
9	carboxylic ac
10	organic cycli
11	cellular amin
12	cellular catal
13	translation
14	lysosomal [illegible]
15	purine ribonu
16	oxoacid [illegible]
17	metabolic pr
18	ribonucleosi
19	purine nucle
20	cellular meta

Mitochondria – All Cells

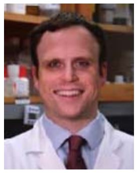


Related mitochondria functions are revealed from the initial proteomic analysis!!

Preliminary Methylation Data for RR1 and RR3 datasets



Deviation from mean
separate for each experiment

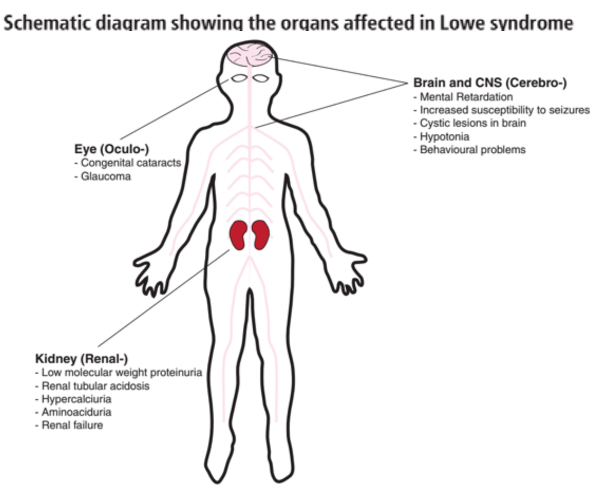

Chris Mason


Cem Meydan


Jonathan Foox

Hypothesis Developed and Being Worked On

- **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.



Mehta, Zenobia B et al. "The Cellular and Physiological Functions of the Lowe Syndrome Protein OCRL1." *Traffic* (2014).

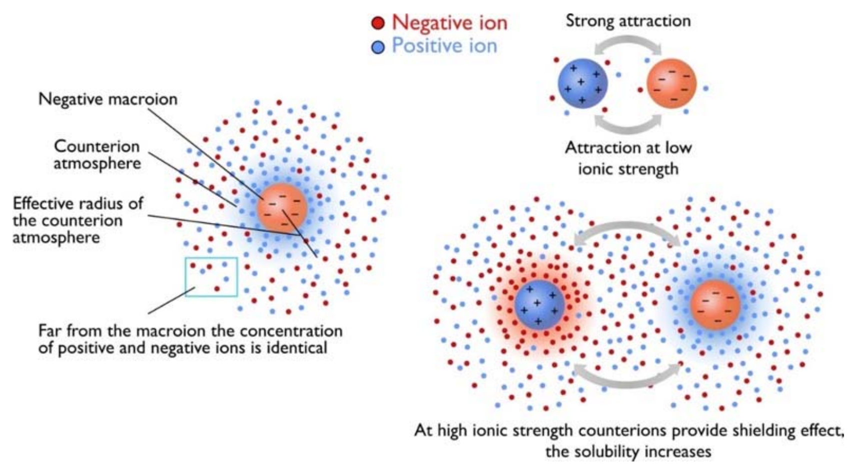
Hypothesis Developed and Being Worked On (Ion Diffusion)

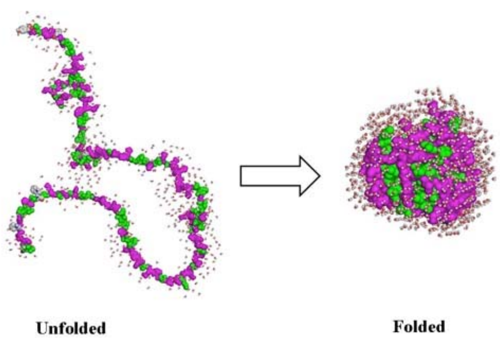
Assumptions:

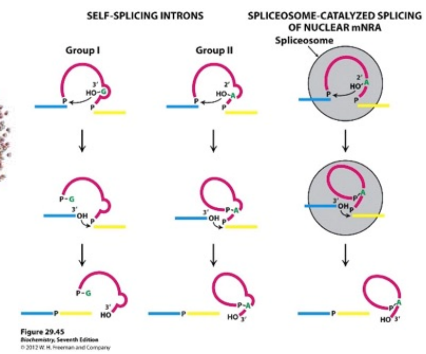
- Gravity is a physical property.
- Alteration in Gravity must have a primary impact in physical properties of a cell, like ion diffusion.
- Electrostatics properties of proteins influence protein-protein interaction and/or protein folding, the same can happen with RNA strutures.
- Ions are critical for Eletrostatics 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
https://step1.medbullets.com/biochemistry/102094/protein-folding-and-degradation

Hypothesis Developed and Being Worked On (Ions and Mitochondria)

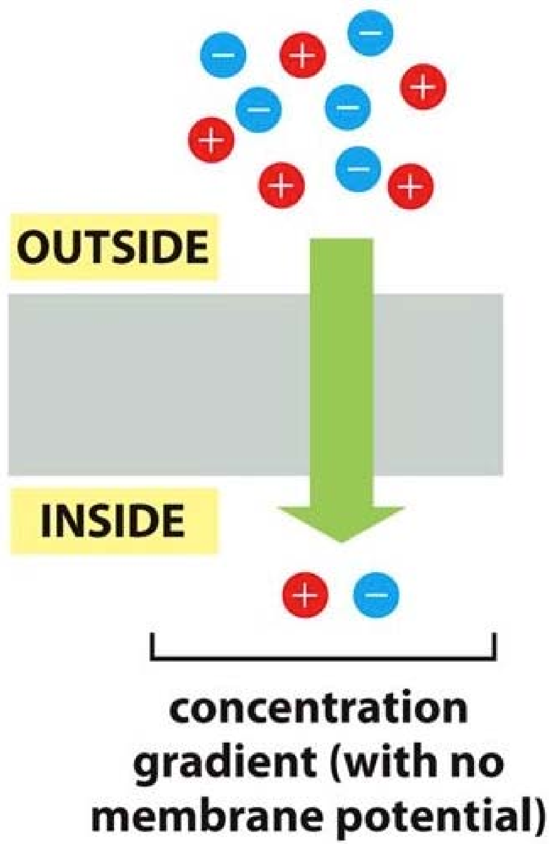
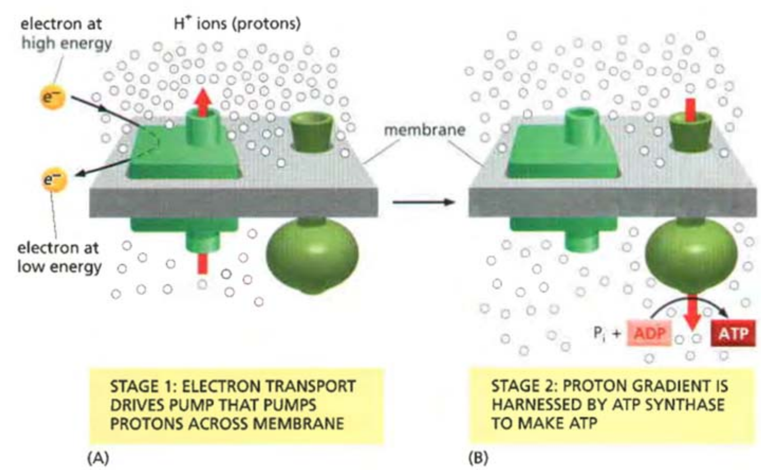


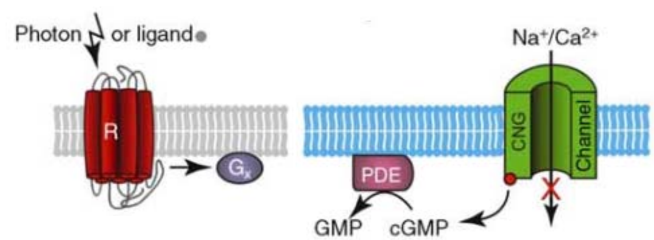
Figure 11-4b Molecular Biology of the Cell 6e (© Garland Science 2015)

Mitochondria – All Cells



Cone Cells - Eye

1st Amplification 2nd Amplification 3rd Amplification

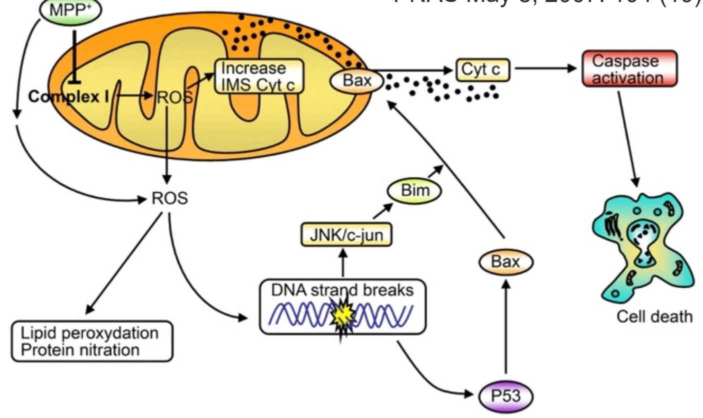


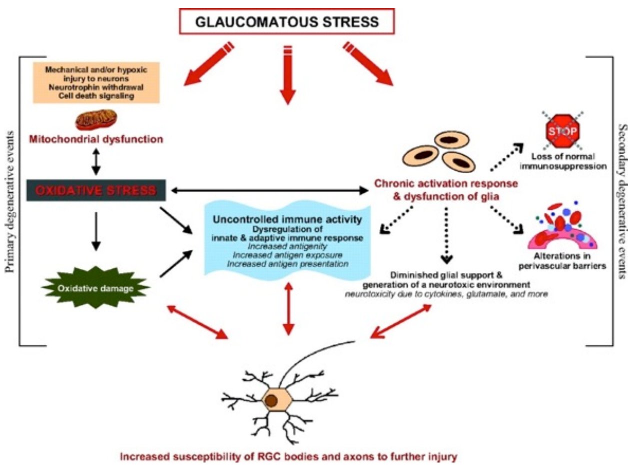
Molecular Biology of the Cell, Garland Science; 5th edition. 2007.

Alternative Hypothesis being Considered (Ions and Mitochondria)

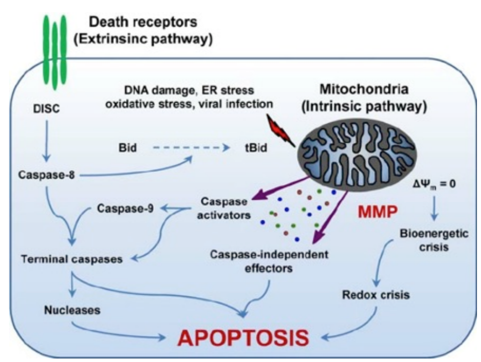
- **Mitochondria can be disrupted by one single particle traversal in one single mitochondria and create a cascade of event, including oxidative stress lasting several days in the exposed cell**

PNAS May 8, 2007. 104 (19) 8161-8166



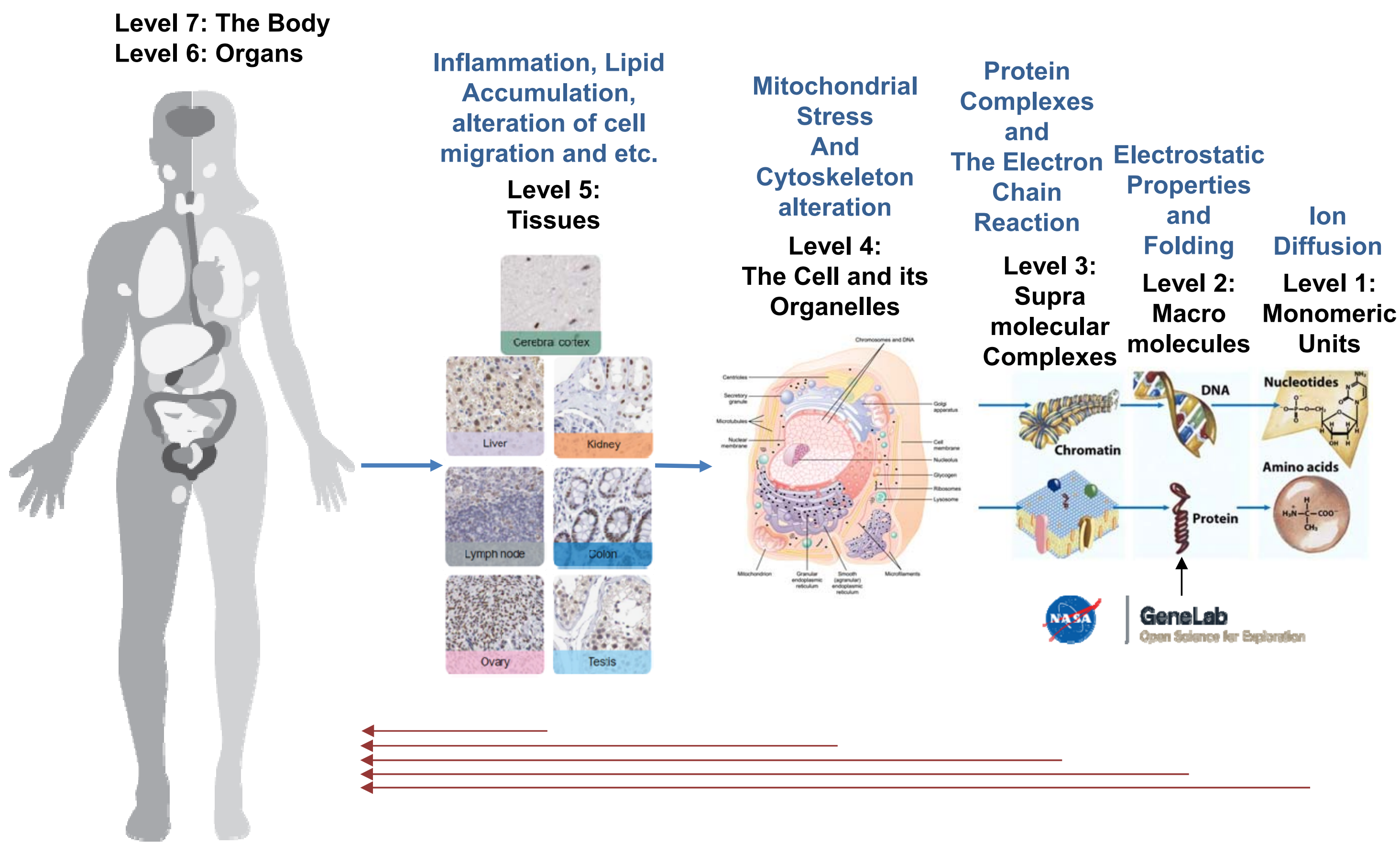


Investigative Ophthalmology & Visual Science March 2009, Vol.50, 1001-1012. doi:10.1167/iovs.08-2717



Galluzzi L, Brenner C, Morselli E, Touat Z, Kroemer G (2008) Viral Control of Mitochondrial Apoptosis. PLoS Pathog 4(5): e1000018. https://doi.org/10.1371/journal.ppat.1000018

Systems Biology Analysis Used to Understand Spaceflight Impact on Health Risk



Visualization Tools to Implement on GeneLab Platform