Space Biology
February 7, 2017 3:15 – 4:15 PM

Space Life and Physical Sciences Division
Human Exploration and Operations Mission Directorate
David Tomko, Ph.D., Program Scientist
(With A Hickey, E Keller, H Levine, K Sato, N Rayl, E Taylor)

Research for Human Exploration
Space Life Sciences Research Continuum

Human Health Emphasis

Human Exploration Emphasis

Exploration Subsystems
Humans

Space Biology Science Plan
What is Translational Research by Design?

• 2011 NRC Decadal Survey Recommended Horizontal Integration and Vertical Translation within the NASA Life Sciences Portfolio

  • **Horizontal Translation**: increase integration of efforts within each level of research, toward conduct of cross-disciplinary, focused translational research and development with applications to human space risks and related areas supporting human space exploration.

  • **Vertical Translation**: Research teams to create “meaningful interactions among basic, preclinical, and clinical scientists to translate fundamental discoveries into improvements in health and well-being of crew members in space and their re-adaptation to gravity.”

• **For Space Biology and HRP management** - Identify a knowledge gap that basic research can potentially fill, and solicit research to fill that gap.
  
  • This can either be “pull”, where the program with the gap asks for the research or “push”, where the basic science program sees a potential solution to an applied problem and solicits research to fill it.
  
  • Either way, the solicitation of the fundamental research is “designed” to fill a particular gap, or answer a particular question.
### Space Biology (SB)

- Microbiology
- Cell & Molecular Biology
- Plant and Animal Biology
- Developmental, Reproductive & Evolutionary Biology
- Systems Biology & Omics (GeneLab)

### Synergies

- SB and HRP coordinate to define research priorities and identify opportunities to facilitate countermeasure development
  - Cell, Tissue & Animal Studies
  - Immunology
  - Wound healing & fracture repair
  - Radiation/Microgravity interactions
  - Oxidative Stress and Damage
  - Microbe-Host Interactions
  - Visual Impairment Syndrome
  - Artificial Gravity/Gravity as a Continuum

### Human Research Program (HRP)

- Identify, characterize, and mitigate human health & performance risks in spaceflight
  - Exercise Countermeasures
  - Physiological Countermeasures
  - Space Radiation Biology
  - Behavioral Health and Performance
  - Space Human Factors and Habitability
  - Exploration Medical Capability

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**Science exploring the unknown**

**Science addressing known risks**
Portfolio Guided by Decadal Survey

Research that enables space exploration: life and physical sciences research needed to develop advanced exploration technologies and processes, particularly those profoundly affected by operation in a space environment.

SLPS’ purpose is two-fold:
Enable exploration and Pioneer scientific discovery

Research enabled by access to space: scientific research in life and physical sciences that takes advantage of unique aspects of the space environment to significantly advance fundamental scientific understanding.
Space Life Sciences Recommendations for 2010-2020: Using the 2011 NRC Decadal Study

- Plant and Microbial Biology
  - ☐ Multigenerational studies
  - ☐ Responses to spaceflight
  - ☯ Plants and microbes in closed-loop life support
- Animal and Human Physiology
  - ☐ Bone and muscle studies
  - ☠ Drug/countermeasure evaluation
  - ☐ Vascular and interstitial pressure changes during spaceflight
  - ☠ Orthostatic intolerance
  - ☠ Deposition of aerosols in lung
  - ☐ T-cell and immune system studies
  - ☐ Multi-generation and early development
- Cross-Cutting Issues for Humans in Space
  - ☯ Artificial-G as a countermeasure
  - ☯ Animal studies to assess radiation risks
  - ☠ Cellular studies to define biomarkers for radiation toxicity
  - ☯ Understanding sex differences in adaption to spaceflight

[Link](http://www.nap.edu/catalog.php?record_id=12944)
Vision and Goals

- Create new knowledge of how different gravity levels affect biological systems important to human space exploration
- Build Links between Space Biology and Human Research
- Perform translational research by design from DNA and RNA to clinical medicine
- Leverage & amplify Space Biology findings using state-of-the-art omics, molecular/systems biology tools, & open access GeneLab data base
- Train and inspire a new generation of Space Biologists

Implementation Strategies

• Scientific Investigations
  – PI-led Individual Projects
  – Team-led
    • Solicited Science Integration Teams
    • Co-Principal Investigator Teams

• Disseminate results
  – Publication in peer reviewed journals
  – Timely data deposition
    • With space flight metadata
    • With unique analysis tools
  – Supported by GeneLab, Physical Science Informatics, MaterialsLab, etc.

• Partner to ensure value and increase capability
  – Exploration customers (AES, HRP, STMD)
  – Potential adopters (CASIS, NIH, NIST, NSF, OGAs; international partners; industry)
Topics to be covered today

• Status
• Planning
• Research findings/Accomplishments
• Genelab Update
Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Genelab Update
## Current Ground Grant Status:

- **16 Ground NRA Space Biology grants**

<table>
<thead>
<tr>
<th>PI Last Name</th>
<th>Project Title</th>
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<tbody>
<tr>
<td>Bloomfield</td>
<td>Iron Overload and Oxidative Damage: Regulators of Bone Homeostasis in the Space Environment</td>
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<td>Delp</td>
<td>Disuse Osteopenia: A Potential Vascular Coupling Mechanism--NNX14AQ57G</td>
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<tr>
<td>Foster</td>
<td>Impact of Microgravity on the Cell-Cell Interactions between a Mutualistic Bacterium and Its Animal Host</td>
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<tr>
<td>Haswell</td>
<td>Mechanosensitive Ion Channels in Plants: Genetic, Computational and Systems-Level Approaches to Understanding Microgravity-Induced Plasticity in Mammalian Utricular Hair Cells: Intrinsic or Multisensory?</td>
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<td>Hogan</td>
<td>Pre-Treatment Approaches for Improving the Response of Bone to Disuse and Recovery</td>
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<tr>
<td>Judex</td>
<td>Genes that Predict the Loss of Bone during Weightlessness</td>
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<tr>
<td>Lawler</td>
<td>Redox Regulation of nNOS Translocation and Muscle Atrophy During Mechanical Unloading</td>
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<tr>
<td>Mao</td>
<td>Role of Oxidative Stress in Mediating the Effects of Combined Exposure to Simulated Microgravity and Radiation</td>
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<tr>
<td>Mehta</td>
<td>Determination of Roles of Microgravity and Ionizing Radiation on the Reactivation of Epstein-Barr Virus</td>
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<tr>
<td>Monshausen</td>
<td>Defining the Role of the Receptor Like Kinase FERONIA in Plant Architecture Development Under Mechanical Loading</td>
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<tr>
<td>Rojas-Pierce</td>
<td>The Role of Vacuole Membrane Fusion in Plant Gravity Perception</td>
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<tr>
<td>Sabanayagam</td>
<td>Epigenetic and Protein Expression Pattern Profiling of Caenorhabditis elegans Exposed to Time-Varying Gravitation</td>
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<tr>
<td>Sams</td>
<td>Integration of Mechanotransduction and T-cell Activation Thresholds: Understanding of the Effects of Mechanobiology</td>
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<tr>
<td>Ferli</td>
<td>Hypobaric Plant Biology - Molecular Responses of Arabidopsis to the Low Atmospheric Pressures of Spaceflight</td>
</tr>
<tr>
<td>Roux</td>
<td>Rapid Signaling Changes Induced by Gravity in Cells of the Fern Ceratopteris richardii</td>
</tr>
</tbody>
</table>

## 2 Space Biology/HRP Artificial Gravity grants

- **15-15 NASA AG-0013 “Partial-Gravity Dose Response: Roles of vestibular input & sex in response to AG” - Charles Fuller (University Of California, Davis) Team Alwood, Hoban-Higgins, Ronca**

<table>
<thead>
<tr>
<th>PI Last Name</th>
<th>Organization</th>
<th>Title</th>
<th>Experimental or Tool Development</th>
<th>Model System</th>
<th>NEW SB PI or Early Career Investigator?</th>
<th>Center Mgmt</th>
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</thead>
<tbody>
<tr>
<td>Allen</td>
<td>University Of Florida, Gainsville</td>
<td>Microgravity effects on co-cultured vascular cells types</td>
<td>Experimental</td>
<td>Cocultures of endothelial and circulating stem cells</td>
<td>Yes (Young Investigator)</td>
<td>ARC</td>
</tr>
<tr>
<td>Gilroy</td>
<td>University Of Wisconsin, Madison</td>
<td>Genelab: Revealing Spaceflight- and Gravity-Response Networks in Plants</td>
<td>Experimental and Tool Development</td>
<td>Arabidopsis</td>
<td>No</td>
<td>KSC</td>
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<tr>
<td>Keller</td>
<td>University Of Wisconsin, Madison</td>
<td>Omics data mining of the ISS Aspergillus fumigatus strains in elucidating virulence characteristics</td>
<td>Experimental</td>
<td>Aspergillus</td>
<td>Yes (New)</td>
<td>ARC</td>
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<tr>
<td>Meyerowitz</td>
<td>Cal Tech</td>
<td>Using GeneLab Data to identify novel gravity sensory components in Arabidopsis</td>
<td>Experimental</td>
<td>Arabidopsis</td>
<td>Yes</td>
<td>KSC</td>
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<tr>
<td>Nicholson</td>
<td>University Of Florida, Gainsville</td>
<td>Comparative evaluation of microbial transcriptomic responses to spaceflight stress: elucidating underlying molecular mechanisms</td>
<td>Experimental and Tool Development</td>
<td>B. subtilis and other bacterial strains</td>
<td>No</td>
<td>KSC</td>
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<tr>
<td>Porada</td>
<td>Wake Forest University</td>
<td>Effects of microgravity on the risks of space radiation-induced leukemogenesis</td>
<td>Experimental</td>
<td>Human Hematopoetic Stem Cells</td>
<td>Yes (New)</td>
<td>ARC</td>
</tr>
</tbody>
</table>
Characterization of Biofilm Formation, Growth, & Gene Expression on Different Materials & Environmental Conditions in Microgravity – PI Luis Zea (Univ Colorado)

Characterize impact of long duration spaceflight on Biofilm Mass, Thickness, Morphology, & microbial gene expression of Uropathogenic Pseudomonas aeruginosa and Penicillium chrysogenum to form and maintain biofilms on different surfaces commonly used in spacecraft, cellulose, aluminum 6061, titanium alloy, polycarbonates, silicon, stainless steel and carbon fibers. Biofilm formation and risk of causing equipment malfunction and human illnesses is a problem that needs to be addressed to enable safe long-term human space exploration. Experiments will elucidate biomechanical & transcriptomic mechanisms involved in formation of “column-and canopy” biofilm architecture observed in space & assess gene expression associated with conferring resistance to oxidative stress, acidity, & antimicrobials on microorganisms in biofilms.

Polymicrobial biofilm growth & control during spaceflight - Robert McLean (Texas State - San Marcos)  Microorganisms grow as surface-adherent biofilm communities in spaceflight. Organisms in biofilms are resistant to traditional antimicrobial chemicals & can foul water treatment filters. Biofilms on ISS include many species, but studies to date have only tested single-species biofilms. Experiments will characterize how two bacterial species (E. coli and P. aeruginosa) interact, and how interactions impact biofilm’s ability to adhere to surface, antimicrobial resistance, & ability to degrade a metallic surface. Characterize biological mechanisms by which these microbes interact with each other, such as changes in quorum-sensing and other secreted proteins. Transcriptomic analyses & changes in microbial gene expression will provide insights into effects of spaceflight on microbial physiology.
Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms & Impact on Multi-System Physiology. Lead - Fred Turek, (Northwestern) Team members - Green, S (Univ III), Keshavarzian, A (Rush Univ Med Ctr), Forsyth, C (Rush Univ Med Ctr), Vitaterna, M (Northwestern)

Examine potential role(s) of disruption of microbial communities in gut (dysbiosis) in mammalian adjustment to space environment. Challenges to mammalian physiological homeostasis & immune, inflammatory, & metabolic changes in spaceflight may be attributable in part to dysbiosis. A series of experiments in mice will define mechanisms by which time in space, diet, & host genotype interact to impact the gut microbiota, & how dysbiosis relates to gene expression (by RNA-seq) & physiology in serum, colon, ileum, spleen, liver, & fat as well as the sleep/wake cycle & feeding behavior.

ISS Microbial Observatory of Pathogenic Virus, Bacteria, & Fungi (ISS-MOP) project. Lead - Crystal Jaing, (Lawrence Livermore) Team members - Mehta, S (Wyle), Pierson, D (NASA JSC), Smith, D (NASA ARC), Venkateswaran, K (NASA JPL)

In this ISS-MOP project a cultivation-based assay (Aim 1) provides a valid estimation of microbial presence, but offers a limited assessment of the phylogenetic/pathogenic diversity & physiological breadth of the microbial population present. So, the team will use novel & comprehensive LLMDA microarray specifically designed to elucidate the pathogenic viral & microbial diversity profiles of complex samples to at least the species level (Aim 2). In addition, they will characterize virulence & antibiotic resistance mechanisms associated with these ISS pathogens by developing a new Virulence & Antibiotic Resistance gene array & analyze the samples on this array. The final deliverable of the proposed project to NASA will be the ISS-MOP dataset, a compilation of all of the genomic sequences & genetic information of viruses & microbes encountered on & within the ISS habitat (Aim 3).
Integrated Omics Guided Approach to Lignification & Gravitational Responses: The Final Frontier
Lead - Norman Lewis (Washington State) Team members - Davin, L (Washington State), Hanson, D (Univ NM), Lipton, M ( Battelle Memorial Inst), Sayre, R (NM Consortium), Starkenburg, S (Los Alamos Natl Lab) Arabidopsis multi-omics study (metabolomics, transcriptomics, proteomics, phenomics) melded with integrated computational biology (ICB) approach. Plant lines include mutants with different lignin amounts & lines enhanced in C assimilation capacity. Will investigate gene/metabolic network relationships & adaptations resulting from varying lignin & C assimilation levels on photosynthesis, C allocation, water use efficiency (WUE), plant growth/development, vasculature performance, auxin transport & gravitational adaptations. integrated omics analyses of factors controlling lignin will provide new insights into global effects on plant biological processes at both 1g & in micro-g.

High dimensional biology to understand the functional response of Salmonella to long-term multigenerational growth in the chronic stress of microgravity Lead - Cheryl Nickerson, Arizona State Team members - Abu-Ali, G (Harvard) Barrila, J (Arizona State), Huttenhower, C (Harvard), Ott, M (NASA JSC), Travisano, M (U Minnesota) Investigators propose that long-term growth in spaceflight culture will induce heritable genomic & epigenetic changes in micro-organisms with adverse phenotypic outcomes that could have a profound impact on risk to crew health & vehicle system performance. They hypothesize that long-term multigenerational culture of S. Typhimurium in flight will result in stress-induced mutations & genomic instability as reflected in genomic, epigenetic, transcriptomic & select phenotypic characteristics changes (including virulence) that reflect a central role for Hfq as a global regulator of these heritable responses. They will characterize the impact of long term spaceflight culture (~300 generations) of an S. Typhimurium wild type & hfq mutant on genomic, epigenetic, transcriptomic, virulence & pathogenesis-related characteristics.
Space Biology Upcoming Payloads Status

Flown

- **SpX-8**
  - **WetLab-2** Validation
  - **Micro-9** Hammond
  - **MT-1C** Venkateswaran

- **SpX-9**
  - **PRR** Perera
  - **BRIC-23** Process Val
  - **Micro-10** Wang

- **Falcon 9**
  - **EcAMSat** Matin
  - **RR-3** BSP

- **SpX-10**
  - **APEX-4** Paul
  - **MT-2** Jaing
  - **RR-4** BSP

- **SpX-11**
  - **FFL-2** Bodmer
  - **SG-3** Medina (ESA) Kiss (NASA)
  - **APEX-02-2** Hammond

- **SpX-12**
  - **JRR-1** TBD
  - **BRIC-22** Brandizzi
  - **BRIC** Process Val
  - **EMCS-2** Wolverton

34 other PIs waiting for flight assignment...
Space Biology Upcoming Payloads Status

• **Non-ISS Free Flyer: EcAMSat NET January 2017** (Formosat-5/SHERPA)

• **SpaceX-10 (January 2017)**

  APEX-04 (PI: Paul) PI/GeneLab collaborative experiment with *Arabidopsis* will be the first spaceflight experiment to directly investigate how the plant methylome contributes to space adaptation.

  **MT-2 (PI:Jaing)** Pre-position kits

• **SpaceX-11 (March 2017)**

  **Fruit Fly Lab-2 (PI: Bodmer)** PI/translational GeneLab collaborative study of cardiac genes in the fruit fly that relate to human heart health and arrhythmia

  **Seedling Growth-3 (PIs: Kiss & Medina)** an ESA-led experiment with NASA and ESA PIs using the EMCS to study gravity and light sensing and control in *Arabidopsis*

  **APEX-02-02 (PI:Hammond)** APEX-02R research investigates *S. cerevisiae* growth and physiological responses to the multiple stimuli encountered in spaceflight environments.
Space Biology Upcoming Payloads Status

• **SpaceX-12 (June 2017)**
  - **JRR-1 (US and Russian PI Team)** First ISS based collaborative US/Russian Rodent Research Payload (Space Biology team led by Delp and including Mao and Willey)
  - **BRIC-22 (PI:Brandizzi)** Expand upon results from the BRIC-18 spaceflight experiment pertaining to the regulation of stress responses in plants exposed to microgravity conditions by the AtIRE1 protein (a master regulator of transcription).
  - **Micro-12 (PI:Hogan)**: Investigating the Physiology and Fitness of an Exoelectrogenic Microorganism Under Microgravity Conditions
  - **BRIC-24 LED SDT Mission and Hardware Validation**
  - **Veggie SN0001**

• **SpaceX-13 (September 2017)**
  - **APEX-05 (PI:Gilroy)** This experiment will determine how responses seen on orbit can be explained by the development of long-term hypoxia linked to the microgravity environment as compared to plants grown on the ground
  - **Plant Gravity Perception (PI: Wolverton)** Characterize the primary statolith-based gravity sensing mechanism, including the threshold acceleration force responsible for activation and capacity for modulating growth regulation.
  - **Plant Habitat-01 (PI:Lewis)** Multi-omics team based investigation to modulate lignin content and vascular apparatus performance by manipulation of the arogenate dehydratase multi-gene family.
### Space Biology NRAs: 2011-2016

<table>
<thead>
<tr>
<th>Solicitation Number</th>
<th>Description</th>
<th>Step 1s/NOIs processed</th>
<th>Proposals received</th>
<th>Proposals Selected</th>
<th>Selection %</th>
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<tr>
<td>2017</td>
<td>Appendix F: “Space Biology Experiment aboard the BION M2 Biosatellite”</td>
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<td>2017</td>
<td>Appendix E: Microgravity Simulation Research</td>
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<td>2017</td>
<td>Appendix C: Parabolic Flight and Suborbital</td>
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<td>2017</td>
<td>Appendix D: Balloons</td>
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<td>2016</td>
<td>SLOANMOBE2016 Sloan ISS MoBE Postdoctoral Fellowship - To Study Microbiome of ISS as a Built Environment: Using ISS as a Microbiological Observatory</td>
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<td>NNNH16ZTT001N-MoBE Appendix B: Post-Doctoral Fellowships in Space Biology to Study Microbiome of ISS as a Built Environment: Using ISS as a Microbiological Observatory</td>
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<td>NNNH16ZTT001N-GL Appendix A: GeneLab Innovation Awards for Translational Systems Biology and Informatics Research Using the GeneLab Data System</td>
<td>45</td>
<td>34</td>
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<td>NNNH16ZTT001N Research Opportunities in Space Biology (ROSBio) - 2016</td>
<td>NA</td>
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Space Biology: Recent/Current Solicitations

- NRA NNH16ZTT001N Research Opportunities in Space Biology (ROSBio) (Release 3/24/16)
  - ROSBio-2016 is an Omnibus Research Announcement that covers all aspects of basic and applied research and technology supporting Space Biology
  - Specific research/funding opportunities announced through Appendices that solicit proposals for experiments to address Space Biology’s primary objectives
  - Appendices will be released until Dec. 31st, 2017

- NRA NNH16ZTT001N-GL Appendix A: “GeneLab Innovation Awards for Translational Systems Biology & Informatics Research Using GeneLab Data System” (Release 3/24/16)
  - Solicited research projects to: 1) perform ground-based experiments to test novel hypotheses derived from analysis of data in GeneLab Database, or 2) develop novel computational tools to enhance usability & value of GeneLab. Total value ~$1.4M
  - Selected 6/32 proposals: 4 New and 2 Veteran Space Biology Investigators

- NRA NNH16ZTT001N-MoBE Appendix B: “Research Opportunities for Post-Doctoral Fellowships in Space Biology to Study Microbiome of ISS as a Built Environment” (Release 9/15/16 - Close 11/30/16)
  - Solicits research from post-doctoral fellows to conduct studies to characterize microbial populations isolated from ISS
  - Released in parallel with Sloan Foundation solicitation for same purpose
  - NASA and Sloan expect to grant 2 awards each with a duration of 2 years
Topics to be covered today

• Status
• Planning
• Research findings/Accomplishments
• Genelab Update
Understand biological processes that support human health and safety in exploration beyond LEO - moon or Mars or ??

**Space Biology Research Planning**

- **Moon**: 240,000 mi
- **Mars**: 36 million mi
- **Near Earth Objects**

**Mission Duration**

- **Minutes**: 12.5 Days
- **12 Months**
- **3 Years**

**ISS & free flyers in LEO**: 180-300 mi

**GREAT UNKNOWN**

**LITTLE KNOWN**

**BECOMING KNOWN**
Space Biology Research Planning
New Hardware to Enable Research

Bioculture System
(SpaceX-13: Sept 2017)

Veggie Unit #1 on ISS,
Veggie Unit #2 arrives on
SpaceX-12 (6/1/17)

Advanced Plant Habitat
(OA-7: Feb 2017)

BRIC LED
(SpaceX-12: 6/1/17)

MultiSpectral Fluorescent Imager/Spectrum
(SpaceX-15: April 2018)

WetLab 2
(On ISS, ready for use)
The Gravity Dose Response Curve: Threshold or Continuum?

- Hypothesis: Gravity induces biological responses at the gene expression, cellular, systems and whole organism level
- The dose response curve of any of these responses is not fully characterized
- It is not known if responses are a continuum or are based on reaching thresholds
- It is not known if responses require continuous or intermittent exposures
- It is not known if the sensitivity/dose response changes during development

**Gravity as a Continuum ToolBox** - ground & flight research on a variety of organisms to define dose response curve & adaptation mechanisms from 0 to >2+g

**Ground-based Centrifuges**

**In-flight Centrifuges** (EMCS, KUBIK, JAXA mouse centrifuge, Free-flyers)

**Parabolic Flight**

**Ground-Based Fractional G Simulators** (Clinostats, RPMs, HARVs, etc)

**Partial Unloading in Animals and Humans**
Space Biology Planning: Upcoming Solicitations

**Early 2017**

- **NRA NNH16ZTT001N Appendix C: “Space Biology Research Using Parabolic and Suborbital Flight Campaigns”** Solicit research to use parabolic or suborbital flight to conduct investigate biological effects of shortn micro- or partial-gravity on bio-systems

- **NRA NNH16ZTT001N Appendix D: “Space Biology Research Using Antarctic Balloon Flight Campaigns”** Solicits proposals to use balloon campaigns to investigate biological effects of long duration space radiation exposure on living organisms

- **NRA NNH16ZTT001N Appendix E: “Space Biology Research Using Microgravity Simulation Devices”** Solicits research to use microgravity simulators to test specific hypotheses regarding effects of altered gravity on biological systems

- **NRA NNH16ZTT001N Appendix F: “Space Biology Experiments On the Bion M2 Mission”** TBD after further discussions with our Russian Collaborators

- **NRA NNH16ZTT001N Appendix: “Plant and Microbial Biology Research”** Solicits flight & ground plant & microbe research to answer Space Biology questions & maximize ISS use

**Mid to Late 2017**

- **NRA NNH16ZTT001N Appendix: “Space Biology Ames Life Science Data Archive Biospecimen Sharing”**

- **NRA NNH16ZTT001N Appendix: “Space Biology Beyond Low Earth Orbit (Orion EM-1)”**

- **NRA NNH16ZTT001N Appendix: “Gravity as a Continuum”**

**Joint between HRP and SB in preparation**

- Effects of hindlimb unloading and space radiation in rats (at NSRL)

- Flight experiments using the JAXA Mouse centrifuge
Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Genelab Update
# Space Biology Research Accomplishments 2010-2016

<table>
<thead>
<tr>
<th>Decadal Survey Recommendations</th>
<th># Grants</th>
<th># Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 ISS Microbial Observatory: long-term, multigenerational studies</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>P2: Microbial Microbe growth and physiology</td>
<td>28</td>
<td>49</td>
</tr>
<tr>
<td>P3 Plant growth and physiology</td>
<td>41</td>
<td>146</td>
</tr>
<tr>
<td>P3 Roles of microbe-plant systems in long-term advanced life support systems</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>AH2 Preservation/reversibility of bone structure and strength</td>
<td>20</td>
<td>57</td>
</tr>
<tr>
<td>AH3 Bone loss studies of genetically altered mice</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>AH4 Osteoporosis drug tests in animal models</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>AH5 Underlying mechanisms regulating skeletal muscle protein balance</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>AH7 Daily recruitment of flexor and extensor muscles of the neck, trunk, arms, and legs</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>AH8 Basic mechanisms, vascular/interstitial pressures (Starling forces)</td>
<td>8</td>
<td>14</td>
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<tr>
<td>AH9 Microgravity and partial g (3/8 or 1/6 g) enabling levels of work capacity.</td>
<td>0</td>
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<tr>
<td>AH10 Integrative mechanisms of orthostatic intolerance (both 1 g and 3/8 g)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>AH14 Mechanism(s) of changes in the immune system; multiple organ systems</td>
<td>13</td>
<td>31</td>
</tr>
<tr>
<td>AH15 Perform mouse studies of immunization and challenge on the ISS</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>AH16 Multigenerational studies; develop rodent breeding habitat for space</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>CC2 Is artificial gravity needed? Establish dose-response relationships</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>CC8 Expand animal use for radiation research</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>CC10 Gender Differences</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>
Space Biology Research Accomplishments: Microbial Tracking Experiment MT-1 Identifies New Bacterial Species Aboard the ISS and Confirms Fungal Virulence

Decadal Survey Recommendation: P1

Researchers on the Microbial Tracking-1 experiment announce the discovery of a new species of bacteria sampled from two different locations aboard the International Space Station. Enterobacter piersonii have been named after Dr. Duane Pierson (at right) at Johnson Space Center to commemorate his many years of service to NASA in the field of microbiology. The Space Biology-funded flight investigation involved researchers from NASA’s Jet Propulsion Laboratory and is led by Dr. Kasthuri Venkateswaran. These eight strains of bacteria are most closely related to E. cloacae – a known, opportunistic human pathogen. As a member of the genus Enterobacter these newly identified strains are also likely to be resistant to drugs and more work is needed to study them in a model organism host.

Unique Fungus Discovered on the International Space Station by Microbial Tracking-1 Investigators Found to be More Virulent than Known Clinical Strains in a Zebrafish Model. Analysis of samples from the first of the three flights found that a unique strain of the fungus Aspergillus fumigatus was collected from the ISS. This fungus was characterized and compared to well-established strains isolated in experiments in Earth laboratories. Assessment of the fungal growth characteristics, secondary metabolite production, and susceptibility to chemical stresses revealed no differences between the ISS and clinical strains that would suggest special adaptation to life aboard the International Space Station. Virulence of the unique fungus was assessed using a zebrafish model, which revealed that the ISS isolates were significantly more lethal compared to clinical strains.
Manned space missions conducted over the past 50 years have expanded our knowledge of the universe, and have led to the identification of a range of challenges that must be addressed as we move towards the next phase of human space exploration. A key concern is how microgravity and other aspects of the spaceflight environment affect bacterial growth, physiology, and virulence.

Surface-associated bacterial communities, known as biofilms, were abundant on the Mir station and continue to be a challenge on the ISS. The health and safety hazards linked to the development of biofilms are of particular concern due to the suppression of immune function observed during spaceflight. While planktonic cultures of microbes have indicated that spaceflight can lead to increases in growth and virulence, the effects of spaceflight on biofilm development and physiology remain unclear. To address this issue, *Pseudomonas aeruginosa* was cultured during two Space Shuttle missions: STS-132 and STS-135, and the biofilms formed during spaceflight were characterized.

Spaceflight was observed to increase the number of viable cells, biofilm biomass, and thickness relative to normal gravity controls. Moreover, the biofilms formed during spaceflight exhibited a column-and-canopy structure that has not been observed on earth. These findings represent the first evidence that spaceflight affects community-level behaviors of bacteria and highlight the importance of understanding how both harmful and beneficial human-microbe interactions may be altered during spaceflight.

First GeneLab Reference Mission Test

Kennedy Space Center developed and flew the first experiment designed to test the cradle-to-grave process for collecting spaceflight reference mission data for the NASA GeneLab Data System. The BRIC-23 experiment, titled GeneLab Process Verification Test Using Microorganisms *Bacillus subtilis* and *Staphylococcus aureus*, was designed by KSC Project Scientist Dr. Howard Levine and Science Consultant Dr. Wayne Nicholson, University of Florida. The microorganisms were allowed to dry on Petri dishes, integrated into the BRIC-PDFU hardware, and flown to the ISS on SpaceX-9. The ISS crew initiated growth of the organisms by injecting nutrient solution into the Petri dishes. After the organisms reached the desired growth stage, they were frozen at -80°C then returned to Earth on SpaceX-9 for GeneLab "omics" analyses.
Space Biology researcher Simon Gilroy of the University of Madison-Wisconsin successfully demonstrated a long-held theory about the behavior of plants: how they "sense" gravity. The “starch-statolith hypothesis” was proposed over 100 years ago, which posits that the sedimentation of high-density starch-filled cellular organelles in cells in the plant shoots and roots is important for gravisensing of each organ. Through Dr. Gilroy’s careful study of Arabidopsis thaliana (mouse ear cress) seedlings under a microscope inside of a centrifuge he has shown that starch-filled granules called amyloplasts settling along the walls of highly-specialized statoliths in the plant shoots signal the plant to change direction. Results of the investigation demonstrated the magnitude of the plant responses to changes in the gravity vector appears to be dependent on the number of settled amyloplasts.

In the present study, Gilroy’s team constructed a new centrifuge microscope in which all the optical devices and the specimen were mounted on a single motor and rotated providing for effectively continuous visualization and illumination. The centrifuge microscope allowed imaging of the thick, less-transparent inflorescence stem specimen (approximately 0.5 mm thick). This equipment was used to investigate gravitropism and amyloplast movement induced by hypergravity conditions in wild type and gravitropic mutants that have normal starch content in amyloplasts but do not respond to gravity at 1g. By statistically correlating gravitropism and amyloplast movement they helped define the amyloplast movement/state required for gravisensing and provide support for the starch-statolith hypothesis of plant gravisensing.

Space Biology researchers Rob Ferl and Anna-Lisa Paul recently published the results of a series of spaceflight experiments where Green Fluorescent Protein (GFP) tagged genes were used to identify the activity of growth hormones in plants grown on the ISS. Their findings established the plant hormones sensitive to gravity are located in the root tips, instead of in the primary root.

- The use of fluorescent reporter genes allowed investigators to visually identify the presence and distribution of plant growth hormones in the roots.

- They found the distribution of fluorescent-tagged growth hormones in the primary roots of plants grown on the ISS was identical to that of the ground controls, but found a more restricted distribution in the root tips of the space plants.

- Their findings confirm that the establishment of the auxin-gradient system, the primary guide for gravity signaling in the root, is gravity independent and that root responses to gravity are sensed in the tips.

Results of these experiments reveal important information about which cellular parts of a plant respond to gravity. Research into how terrestrial biology responds to spaceflight will both refine our understanding of what it takes to explore space and help define how conditions on Earth have shaped biology here.

As astronauts grow, harvest, and consume fresh vegetables from ISS Garden.

The Kennedy Space Center (KSC) Vegetable Production System (Veggie) “Outredgeous” lettuce experiment (Veg-01b) demonstrated the capability to grow edible vegetables in space - from seed to mature plant. The International Space Station (ISS) crew enthusiastically harvested and consumed the lettuce on orbit. Live media broadcasts captured the vegetable harvest and consumption. The remaining lettuce samples were returned to Earth on SpaceX-8 in May 2016 for analysis by KSC scientists. The Veg-01 experiments revealed acceptable seed viability for long-term seed storage (approximately 1.5 years on ISS). The Veggie flight experiments received high praise from the ISS crew and NASA top management and became a hot-topic in the media.
A recently published study found troubling signs of fatty liver disease in mice after only two weeks in space aboard the STS-135 shuttle. These findings have contributed important information about potential risks to liver function where little is known and the results could have implications for long-duration exploration missions.

- Mice flown in space lost weight, but redistributed lipids to their livers showing approximately 3.5 times more areas of fat storage (lipid droplets) than ground controls.
- Other changes observed in the mouse livers indicate a stress response, which, if left unchecked, could lead to irreversible damage from fibrosis.
- Transcriptomic and metabolomic data collected and analyzed in this study have been entered into the GeneLab data system and are publicly available for further analysis by the scientific community.

In a study funded by a NASA Space Biology grant researchers sent cultures of mouse embryonic stem cells into low earth orbit for 15 days on the STS-131 mission to follow the progress of stem cell differentiation, both while in space and then in culture back in normal gravity on earth. What they found in this series of experiments is that mechanical unloading of embryonic stem cells in microgravity inhibits their differentiation and preserves their “stemness”; the essential characteristic of a stem cell that distinguishes it from ordinary cells.

Embryonic stem cells can differentiate into all the specialized cells needed to grow a whole organism, but are also responsible for maintaining the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues. These results may provide important clues to the basis for the inhibition of tissue regeneration in space and explain why bone and muscle cell numbers are reduced after long exposures to microgravity.

Former astronaut Dr. Millie Hughes-Fulford is a scientist who conducts research on human immunity and has performed several space flight experiments to understand the mechanisms underlying the immune system changes. She has published two papers on the results of her space flight experiments that focus on studying cells that are at the forefront of bodies' defense against disease – the T-cells. Almost every aspect of the adaptive immune response is controlled, in some way, by T-cells. Activation of T-cells is a critical event during which T-cells recognize infections within the body and initiate a defensive response.

In the first set of experiments, whole blood was obtained from 4 healthy human donors. The T-cells were extracted and maintained in cell culture and sent up to space in specialized flight hardware to keep the cells alive. Dr. Hughes-Fulford was specifically interested in tracking how miRNAs were changing in space. miRNAs are a class of small noncoding RNAs that act as posttranscriptional regulators of gene expression and play fundamental roles in regulating immune response and autoimmunity. In the data gathered from that flight experiment, she showed for the first time that the gene transcripts related to immune response were changing as a result of exposure to spaceflight.

In the second set of experiments mice were flown on the STS-131 mission for 15 days. Modified T-cells, an anti-inflammatory, and a substance that enhances the immune response were injected into the mice to see how their surrogate immune systems would respond to a specific antigen challenge while in space. The results from these experiments showed that the surrogate immune cells in the mice over reacted to the antigen creating an exaggerated inflammatory response, indicating that astronauts’ immune systems may be hypersensitive to the effects of space. This experiment was the first-of-its-kind study to examine the immune system function inside a live animal host.

Taken together these spaceflight experiments are helping to unravel the mysteries of how T-cells are impaired by exposure to space. T-cells are the sentries on the forefront of protecting us from disease. Understanding how T-cells are affected in microgravity may someday lead to countermeasures to bolster immune systems for astronauts as well as for people on Earth.

Decades of past research has revealed that spaceflight not only decreases T-cell activation, but alters the gene and protein expression patterns of these cells as well. Former Astronaut, Millie Hughes-Fulford has dedicated most of her career to characterizing these changes in both human and murine immune system.

Hughes-Fulford and colleagues have recently found that changes in T-cells extend beyond protein-coding genes to those that encode micro RNAs (miRNAs), tiny RNA molecules that attach to messenger RNAs (mRNAs) and prevent these transcripts from being translated into proteins. This novel discovery was made while conducting experiments designed to examine how spaceflight induces changes in gene expression patterns within primary cultures of human T-cells. Her work revealed that the key immunity-regulating miRNA miR-21, which targets many other mRNA transcripts, was also suppressed by microgravity. This surprising finding suggested that gravity was manipulating the immune response through multiple molecular pathways, involving both gene transcription into mRNA, and mRNA translation into proteins.

Another important finding from this study was that exposure of T-cells to partial gravity (0.5G), resulted in the restoration of miR21 levels to those of cells incubated in 1G, suggesting that partial artificial gravity can act as a viable countermeasure to the effects of microgravity during spaceflight.


A principal function of bone is to withstand mechanical loads acting upon it such as the force of gravity on Earth. Mechanical loads are required for bone maintenance and growth. When humans travel into space the absence of mechanical loading during weightlessness causes the erosion of bone and a reduction in strength. There are differences between individuals in the way bones perceive and process similar environmental cues which gives rise to very different outcomes. This investigation sought to identify the specific gene locations which drive bone’s responsiveness to unloading.

In this ground-based study adult female mice were subjected to simulated microgravity through the use of hind limb unloading and then subject to a period of reambulation (allowed to move freely in normal gravity for recovery). The femur bones were then analyzed for strength and flexibility using compression tests and the tissues were analyzed to search for the genetic locations (quantitative trait loci – QTL) related to the changes in bone morphology and strength.

The results of this study indicate that candidate genes have been identified which may be responsible for the mechanical response of trabecular (spongy) bone to gravitational unloading. By revealing novel gene targets that are responsible for responses to gravity it may help to identify and reduce the risk of bone fractures as well as maximizing the recovery of bone strength and flexibility during recovery after space travel.

Source article: Ozcivici, E., Zhang, W., Donahue, L., Judex, S. Quantitative trait loci that modulate trabecular bone’s risk of failure during unloading and reloading. Bone. 03/2014; DOI:10.1016/j.bone.2014.03.042.
A study funded by NASA Space Biology examined a novel therapeutic as a means of preventing bone loss induced by exposure to microgravity. In a ground-based study Dr. Mary Bouxsein and her team investigated the use of a sclerostin antibody (SclAbII) on mice subjected to simulated microgravity for 21 days. Sclerostin is believed to be the protein responsible for the inhibition of the cellular pathway which leads to decreased bone formation during unloading conditions. In this study, hind limb unloading was used to simulate microgravity.

The experimental animals that received the sclerostin antibody showed no bone deterioration due to disuse. Moreover, it appeared that the sclerostin antibody was so effective at preventing bone loss that the bone properties of the animals receiving SclAbII were at or above the values of the controls:

- Hindlimb bone mineral density decreased 2% in unloaded control animals, whereas it increased 13.1% ± 1.0% in unloaded animals treated with the sclerostin antibody.

- Trabecular bone volume was ~2 fold higher in unloaded mice treated with the sclerostin antibody than it was in control unloaded animals.

Scientists at NASA’s Ames Research Center found a diet of dried prunes given prior to exposing mice to whole-body radiation completely prevented bone loss.

Irradiation of control mice resulted in a 32% reduction in trabecular bone volume, a 25% reduction in trabecular number, and a 13% reduction in trabecular separation. In contrast, irradiated mice fed a diet of dried plums did not exhibit these reductions.

- Radiation-induced bone loss resembles accelerated, age-related structural changes in cancellous or spongy bone and is a risk for astronauts on long-duration, deep space missions
- Cancellous bone experiences significant damage from radiation exposure some of which is irreversible
- Drug countermeasures that have been used to prevent bone loss have limited effectiveness and carry risky side effects
- This is the first study to demonstrate the protective effects of dried prunes against radiation exposure

Space Biology Research Accomplishments:
Bion-M1 experiment sheds light on the effects of fluid shifts in space

Decadal Survey Recommendation: AH8

Michael Delp

With some astronauts reporting changes in visual acuity, this has led to speculation that the fluid pressure redistribution in space and increases in intracranial pressure may be some of the factors at work. Mike Delp from the University of Florida was one of a team of space biology investigators who participated in the international collaboration that sent mice into space for 30 days on the Bion-M1 Russian satellite. His research focuses on cardiovascular fluid shifts and pressure changes as a result of exposure to microgravity.

"Without gravity pulling body fluids down toward the feet, fluid will rise toward the brain," Delp said. "When spaceflight alters the function of arteries that precisely regulate blood flow to the brain, it could severely affect many things, including vision."

Findings from this experiment demonstrated that spaceflight did impair the distribution of brain blood flow during periods of stress. Analyzing cerebral arteries may provide potentially important information about the visual impairment of astronauts induced by spaceflight. Possible solutions may be found in future experiments Mike and his team are planning.

July 28, 2016 – 47 years after humans first landed on the moon, space biology researchers have found an increased risk of mortality from cardiovascular disease (CVD) in astronauts who traveled deep into space. Apollo astronauts experienced nearly five times greater risk of death from CVD than astronauts who’ve traveled to low Earth orbit.

Researchers first compared mortality rates of Apollo astronauts with other astronauts who either never flew in orbital missions or only flew in low Earth orbit. They found a concerning trend in rates of cardiovascular disease that warranted further investigation.

To test the effects of spaceflight on the cardiovascular system directly, investigators subjected mice to simulated conditions of zero-gravity, cosmic radiation, or both combined. After half a year (the equivalent of 20 human years), they found that only the mice that had been exposed to radiation had sustained damage to their blood vessels. In particular, the researchers found damage to the lining of the blood vessels, which is typically the first indication of long-term heart disease leading to a heart attack or stroke. Based on further research in mice, they suggest that the cause of cardiovascular disease in these astronauts may have been deep space radiation.

Principal Investigator for this Space Biology-funded study, Dr. Michael Delp, acknowledges shortcomings of the mortality study of the astronauts, but cautions that their findings indicate the need for more research on radiation effects on cardiovascular function in astronauts. This study was funded in part by the National Space Biomedical Research Institute.

Space Biology Research Accomplishments: Mission Success for Wetlab 2

Decadal Survey Recommendation: “[The ISS] is expected to serve as a world-class orbiting national and international laboratory for conducting high-value scientific research and providing access to microgravity resources for major areas of science and technology development.” Page 24.

WetLab-2 is a research platform for conducting real-time quantitative gene expression analysis aboard the International Space Station. The WetLab-2 validation experiment has been successfully completed, and, in short – it worked! The team reports that full mission success criteria have been met. April 29, astronaut Jeff Williams tested the system’s Sample Prep Module for the first time in space to extract and purify RNA from cells before performing the qPCR analysis. The WetLab-2 team at Ames received data from the space station that clearly shows that Williams isolated RNA in microgravity achieved reverse transcription—conversion of RNA to DNA—and got amplification of that DNA. WetLab 2 enables detailed studies of the quantity of RNA and DNA in identified microbial and other biological samples. See: WetLab-2 validation mission webpage. The experiment was featured in the April 29th edition of the "Weekly Recap From the Expedition Lead Scientist".


4. Choi, Gilroy, et al. (2014). Salt stress-induced Ca2+ waves are associated with rapid, long-distance root-to-shoot signaling in plants. *Proc Natl Acad Sci U S A,* 111(17), 6497-6502. doi:10.1073/pnas.1319955111 - Although plants do not have a nervous system, they do possess a sensory network that uses ion fluxes moving through defined cell types to rapidly transmit information between distant sites within the plant.


8. Judex, et al. (2013). Genetic loci that control the loss and regain of trabecular bone during unloading and reambulation. *J Bone Miner Res, 28*(7), 1537-1549. doi:10.1002/jbmr.1883– Chromosomal regions containing genes that regulate the sensitivity of trabecular bone to weight bearing have been identified in the mouse, which may in turn help to identify individuals most susceptible to unloading-induced bone loss.

9. Kim, . . . Collins et al. (2013). Spaceflight promotes biofilm formation by *Pseudomonas aeruginosa.* *PLoS One, 8*(4), e62437. doi:10.1371/journal.pone.0062437 – Spaceflight was observed to increase cell numbers, biomass, and thickness of *Pseudomonas* biofilms. Moreover, the biofilms formed during spaceflight exhibited a unique architecture that has not been observed on Earth.

10. Kiss, Millar, & Edelmann. (2012). Phototropism of *Arabidopsis thaliana* in microgravity and fractional gravity on the International Space Station. *Planta, 236*(2), 635-645. doi:10.1007/s00425-012-1633-y – First to use ISS to examine the behavior of flowering plants in fractional or reduced gravity conditions. At gravity levels ranging from 0.1 to 0.3g, red-light-based phototropism was attenuated in both roots and hypocotyls of seedlings. In contrast, blue-light negative phototropism in roots, was enhanced compared with the 1g control, and showed a significant attenuation at 0.3 g.

11. Knox, Venkateswaran et al. (2016). Characterization of *Aspergillus fumigatus* isolates from Air and Surfaces of the International Space Station. *mSphere, 1*(5). doi:10.1128/mSphere.00227-16 – Two ISS isolates of *A. fumigatus* were shown to have increased virulence in a neutrophil-deficient larval zebrafish infection model.


Education and Outreach Opportunities

**NASA Postdoctoral Program (NPP)**
- Provides early-career and more senior scientists the opportunity to share in NASA's mission
- Fellows work on 1-3 year assignments at NASA centers and institutes
- Fellows contribute to our national scientific exploration, confirm NASA's leadership in fundamental space research, and complement the efforts of NASA's partners in the national science community.

**Space Life Sciences Training Program (SLSTP)**
- Trains our next generation of scientists and engineers and enable NASA to meet future research and engineering challenges in the space life sciences
- Students conduct hands-on research, attend technical lectures, develop professional & project management skills, perform a team project and submit an abstract to a professional scientific or engineering organization for presentation
Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Outreach

- New Space Biology project
- Targets high school students
- Pilot program begins Summer 2017

GeneLab for High Schools

Consists of 3 core components:

Core Learning Modules
- Introduction to GeneLab Data Platform and ‘omics datasets and analysis tools
- Introduction to model organisms and analysis of model organism datasets

Networking and teamwork component
- Guest lectures by University Professors and Industry experts
- Networking receptions and field trips (University core facilities and Industry)

Independent Learning Project
- Analysis of dataset of choice
- Development of hypothesis and proposal of research project

Elizabeth Blaber, Ph.D.
Development and Organization Lead
Space Biology: Agenda

Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Genelab Update
• Serve as a resource for researchers to convert spaceflight Life Sciences data to new knowledge to maximize use of ISS Life Science research results.

• Collect and archive spaceflight genomic, transcriptomic, proteomic, and metabolomic data for discovery use.

• Support research community exploration of molecular network responses to spaceflight by maintaining access and tools for performing Translational Research using Genelab data.

• Make all SB spaceflight and ground-based simulation data in the Genelab database accessible to a global researcher network.

• Continually improve the database and the tools by seeking feedback and input from the community.
• The GeneLab Data System (genelab.nasa.gov) currently houses 88 datasets which are meticulously curated and fully accessible to the public: 49 datasets are from Spaceflight experiments, and 39 from relevant ground experiments.
• Averaging more than 500 file downloads per month, with a data volume of roughly 3 terabytes per month, available worldwide.
• Newsletter updates are currently sent to 4,463 subscribers.
• Collaborated with recent missions (including CASIS) to obtain either tissue samples or data for subsequent omics analyses: Rodent Research-1, Rodent Research–3, Micro-9, Micro-10, Microbial Tracking-1c, BRIC-19, BRIC-20, and the BRIC-23 Process Verification Test.
• Space Biology issued an Innovation Awards Research solicitation (NRA NNH16ZTT001N-GL) in March 2016 - 6 of 34 proposals received were funded after scientific merit and technical feasibility reviews.
Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Planning

Phased Approach:

**Phase 1**
Searchable Data
FY2014 – 2015
- At least two Collaborative Missions (BRIC19 and BRIC20)
- Establish PI Data Agreements
- Data analysis validation

**Phase 2**
Data Exchange
FY2016-2017
- Standards for samples
- Standards for metadata
- NRA for Data analysis from initial studies
- Participate in multiple missions/year
- Legacy Data
  - Minimum Data Set

**Phase 3**
System Integration
FY2018– 2019
- Continue ground controls and process enhancement
- Engage with Scientists external to NASA as part of Outreach Program
- Multiple flight experiments every year

**Phase 4**
Full Operations
FY2020 – 2021
- Full science community engagement
- Analysis and modeling
- Expanding catalog of flight experiments

**Spaceflight Experiments**
- 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)
- Integrated Platform across model organisms
- Build Community via collaborative science analysis & modeling

**Full Operations**
- Provide access to biocomputational tools for omics analysis
- Provide collaboration framework and tools
Common Threads

• NASA Space Biology objectives and research projects are well aligned with 2010 Decadal Survey priority recommendations

• Every Space Biology research task is required to comply with President Obama’s Executive order “Making Open and Machine Readable the New Default for Government Information”, enabled through the NASA GeneLab Project

• Potential for more collaboration with other NASA programs, CASIS, or international partners

• In-flight centrifugation is required across all elements and species to determine gravity thresholds, responses to fractional Earth gravity such as will be experienced on Mars or moon, and to provide 1g controls in flight.

• Ability to accomplish goals & recommendations of NRC Decadal Survey is diminished by reduced access to ISS & reduced crew time availability for implementing NASA science

• Need for ground-based research and alternative spaceflight platforms.

• Systems Biology Approach at some level present in 50-70% of all Space Biology tasks.

• Need to actively solicit users of the open science GeneLab database to develop new knowledge from spaceflight omics data.

• Attempt in each element to enlarge NASA’s research community by outreach to major scientific professional societies (e.g., Experimental Biology, American Society for Microbiology, American Plant Biology Society, American Society for Cell Biology.)
Supplementary Slides for the Committee
Space Biology Research Accomplishments

Flight Experiments Completed:

- BRIC-21, VEG-01b, VEG-01c
- SpaceX-8 (4/8/16): VEG-03 Tech Demo, MT-1c, Micro-9, Micro-10, WetLab-2 Validation
- SpaceX-9 (7/18/16): BRIC-23 GeneLab Process Verification Test, Rodent Research-3 Biospecimen Sharing

2015-16 Space Biology Publications - Top 20


2015-16 Space Biology Publications - Top 20 (CONTINUED)


Pioneering Scientific Discovery
Space Biology: External Partnership and Collaboration

- International Space Life Sciences Working Group: Forum for developing life science collaborations between NASA, ESA (CSA, CNES, DLR, ASI) and JAXA: ISS experiments, Research Announcements, and GeneLab

- JAXA: OP3 Framework: Rodent Experiments, Rodent Centrifuge and Aquatic Habitat

- U.S./Russian Joint Working Group: Forum for developing life science collaborations with Russia: ISS experiments, Bion, Foton, and GeneLab

- CASIS: collaboration on ISS based experiments with translational relevance
New Platforms

NASA and Other Governmental Opportunities

- Orion: Space Biology Pathfinder Mission
- Flight Opportunities Program
- Advanced Exploration Systems: BioSentinel technology for future LBLEO missions
- Air Force: X37B

International

- JAXA future Mouse Habitat BSP and Aquatic Habitat for GeneLab
- Bion-M2 Free Flyer Mission

Commercial

- Blue Origin
- SpaceX: Falcon and Red Dragon
- CASIS Rodent Biospecimen Sharing
Parabolic Flight Campaigns for Microgravity and Partial Gravity Life Science Experiments

Objectives:

- Establish a **yearly Parabolic Flight Campaign** for Life Scientists that will provide opportunities for short duration (20-25 sec) **micro-g and partial-g** investigations.
- Work towards the expansion of this capability to **90 sec micro-g and partial-g exposures** based upon future modifications to **Commercial F104 jets**.
- A number of conventional (but modified) aircraft are capable of flying a series of parabolas that provide 20-25 seconds of micro-g (or partial-g) at the top of the parabolas.
- Typically, 40 parabolas are flown each flight, and four consecutive flight days can be provided per campaign.

Some Commercial Parabolic Flight Providers.

- A. Starfighters Aerospace F104.
- B. Starfighters Aerospace Falcon 20.
- C. Swiss Space Systems (S3) Airbus A310.
- D. T-6 Texan WWII Warbird.
- F. Parabolic Flight Profile.
**Objective:** Establish yearly Suborbital Flight Campaigns that will provide 200-300 sec micro-g and partial-g exposures for life science investigations.

### Suborbital Flight Microgravity Durations

Microgravity ends when the vehicle re-enters the atmosphere. The duration of microgravity is primarily a function of the maximum altitude which is reached during the flight. A few examples:

<table>
<thead>
<tr>
<th>Altitude (km)</th>
<th>Micro-g Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3:10 min</td>
</tr>
<tr>
<td>200</td>
<td>5:45 min</td>
</tr>
<tr>
<td>400</td>
<td>9:10 min</td>
</tr>
</tbody>
</table>

Objective: Facilitate Deep Space Radiation High Altitude 1-3 month life science investigations.

Earth's Magnetic Field Protects us from Space Radiation:

- Most dangerous particles don't hit the Earth's surface because they are forced by the Earth's magnetic field to move around the Earth.
- Particles do enter at the magnetic north and south poles where the magnetic field points directly into the ground, so in those areas particles from deep space are free to rain in.
- The result is a Space Radiation environment comparable to what the crew will experience on the way to Mars.

A. Depiction of the magnetic field lines of the Earth showing polar region areas of deep space radiation penetration.  B. View of Auroral Zone’s ring shaped region representing the area of deep space radiation penetration with a 2500 km radius around the Earth’s magnetic pole (about 2000 km away from the geographic pole).  C. Investigators will develop hardware (either Cube-based or TBD) that piggyback on the primary science payload’s gondola.
Objective:
Provide the U.S. Space Biology research community a **ground-based micro-g & partial-g simulation capability** composed of devices that negate the directional influence of the “g” vector (e.g. 2D & 3D Clinostats, Random Motion Machines, Rotating Wall Vessels).

Clinostats currently available for use.
A. KSC Slow Rotating Clinostat for large containers (up to 32 kg).
B. KSC Slow Rotating Clinostat in ISS stowage locker configuration.
C. Rotating Wall Vessel (Micro-g configured with HARV’s attached).
D. Rotating Wall Vessel holder in 1g “control” configuration.
E. Airbus RPM 2.0 configured with experimental vessel.
F. Space Bio-Laboratories, Inc. GRAVITE RPM.
Bion-M2 Free Flyer Mission: Russian/US Collaboration

**Goal:** To investigate systemic, cellular and molecular mechanisms underlying adaptation of mammals to the combined effects of microgravity, cosmic radiation and other spaceflight factors as well as their readaptation to the terrestrial environment upon return to Earth.

- Bion-M2 experiments will be performed on space-flown C57BL/6 mice as well as ground controls housed in flight habitats and exposed to a simulated flight environment (and vivarium controls).
- Biosamples will be collected to detect acute spaceflight effects 1) approximately 2-3 hours after landing; 2) 14-17 hours after landing; 3) 3 days and 4) 7 days after landing.

**Bion Characteristics**
- 2.5 m Sphere used for spaceflight experiments
- 700 kg of payload mass (4 cubic meters) within recoverable module
- 800-1000 km circular orbit for 30 days
- Internal Pressure: 660-960 mm Hg (typically 720-760 mm Hg)
- $pO_2$: 140-180 mm Hg
- $pCO_2$: <7 mm Hg (typically <1 mm Hg)
- Relative Humidity: 30-80%
- Capsule Temperature: 18-28°C (targets 25 +/- 0.3°C)
- Average Power: 350+ Watts
- Inclination Angle: 62.8° or 82.3°
- Period: Approximately 90 minutes
- Flight Duration: 21-60 days
WetLab-2 System was developed to provide an on-orbit nucleic acid analytical capability. WetLab-2 is a research platform for conducting real-time quantitative gene expression and DNA genetic analyses. The COTS Cepheid SmartCycler and its PCR Reaction Tube were adapted for use on ISS in microgravity. The full system provides the ability to process samples on-orbit, isolate DNA and RNA, reverse transcribe RNA, and perform qPCR. The isolate nucleic acid is amplified and analyzed using the Cepheid SmartCycler. The data is downlinked for analysis within 2 hours of run completion. Purified RNA and DNA may be returned to the PI.

**Specifications:**
- Cepheid SmartCycler
  - 16 qPCR in parallel
  - 4 optical channels to measure fluorescence
- Reaction Tube
  - Carries reaction solutions and PCR primers
  - Up to quadplex reactions (currently validated for triplex reactions on-orbit)
- Sample Prep Module:
  - Capable of processing microbes, cells, and tissues
  - Sample lysis and extraction and purification of RNA and DNA
- Pipette Loader
  - Provides sample de-bubbler
- Rotor
  - Used with ISS Drill
  - Draws sample down to reaction solutions and primers in the Reaction Tube
- Thermal protocols can be uplinked from ground
Advanced Plant Habitat (APH) = a large growth volume plant habitat capable of hosting multi-generational studies with environmental variables tracked and controlled in support of whole plant physiological testing (up to 135 days) and Bioregenerative Life Support System investigations.

**Specifications:**
Max. Shoot Height 45 cm; Root Zone Height 5 cm, Growth Area 1,700 cm$^2$; Growth Volume 112,500 cm$^3$; Light Intensity <1000 μmoles [Red 630-660 nm, Blue 450±10 nm, Green 525±10 nm, Far Red 730 nm, White]; Temperature 18-30 C; Relative Humidity 50-90%; Condensate Recycled; CO$_2$ Controlled (400-5000 ppm); Air Flow @ 0.3-1.5 m/s; Ethylene Scrubbed to ≤ 25 ppb; Air Sampling Ports; Water Sampling Ports; Leaf Temperature Sensor; Root Zone Moisture Level Monitored; O$_2$ Sensors in both Shoot & Root Zone.
Spectrum: Multispectral Fluorescent Imager (SpaceX-15: April 2018)

- Images *in vivo* reporter genes using fluorescent proteins incorporated into model organisms.
- Accommodates 10 cm x 10 cm Petri plates, multi-well culture plates, & other custom containers.
- Capable of capturing high-resolution images with dissection scope level magnification.
- Data collection, storage & downlink retrieval for near-real time evaluation by the investigator team.

**Internal Environmental Conditions**
- Programmable Temperature: 18-37°C
- Programmable Light Cycles
- Relative Humidity: Ambient ISS environment
- Ethylene scrubbing (< 25 ppb)
- CO₂ control (between 400 ppm and ISS-ambient levels in units of 100 ppm)
- Airflow to prevent condensation that would interfere with imaging.
- Lighting (at 10 cm beneath the light cap)
  - Broad-spectrum white light 0-100 µmoles (400-750 nm)
  - Darkness: <1 µmoles
  - Red light: 0-100 µmoles (630-660 nm)
  - Blue light: 0-50 µmoles (400-500 nm)
  - Green light 0-30 µmoles (520-530 nm)
BRIC-LED Hardware Objective: Provide discrete illumination to biological specimens contained in 60mm Petri dishes that are subjected to a microgravity environment.

**Petri Dish Fixation Units**

*Single or Dual Fluid Injections: 17mL total fluid volume for injection of Growth media and/or liquid treatments/preservatives/fixatives.*
- N=36 60mm Petri dishes available per mission
- Capable of >150 μmoles of light
- >10% intensity resolution control for each wavelength
- 4 discrete LEDs types currently ranging from 430-750nm (blue, red, white and far-red)
- Customizable wavelengths
- Programmable lighting schedule with 1 sec resolution
- >70% light uniformity when using 4 discrete wavelengths
- Light tight from external sources

**Locker & Tray**
- Resides in US Lab on ISS
- Holds 6 BRIC Canisters
- Canisters Travel up/down
- KSC Ground Station controlled
- Commanding start/stop of expt.
- Real time telemetry of Tray and Canister temperatures & LED status
- Forced air cooling to reject heat
  - 1.5C between Canisters
  - 3C from EXPRESS AAA air
- Internal Canister Pressure Logged
- Temperature Sensors on Canister Lid Boards
- One 3-Axis Accelerometer
Veggie is an easily stowed, high growth volume, low resource facility capable of producing fresh vegetables and supporting science experiments on ISS. It also provides real-time psychological benefits for the crew, and facilitates outreach activities.

**Specifications:**

**Light:**
- 100-500 μmol m⁻² s⁻¹ PPF of Red (630 nm), Blue (455 nm) and Green (530 nm)

**Cabin Air Fan Settings:**
- Low / High / Off

**Baseplate Footprint:**
- 29.2 cm x 36.8 cm

**Max. Height:**
- 47.0 cm empty
- 41.9 cm with root mat
The Bioculture System is a perfusion culture system. The primary component is the hollow fiber bioreactor, designed to efficiently deliver nutrients and remove waste via multiple, tightly packed perfusion fibers. The hollow fiber system is particularly suited for microgravity cell culture where nutrient transfer is limited to diffusion. The increased surface area and low shear perfusion ensures that cells are receiving sufficient nutrients and gas to grow unabated.
Fig. 3. Time-course studies of positive phototropic curvature in hypocotyls of Arabidopsis seedlings in response to red light during the TROPI-2 spaceflight experiment. White circles indicate micro-g, grey circles indicate 0.1 g, hatched circles indicate 0.3 g and black circles indicate 1 g. The plots represent the mean, and different letters indicate significant differences among the first-order regression plots. Note the magnitude of the positive response at micro-g and 0.1 g. The response at 0.3 g was not significantly different from the value of the 1 g control, and there was attenuation of red light phototropism at 0.3 g and 1 g.

Fig. 4. Time course studies of positive phototropic curvature in roots of Arabidopsis seedlings in response to red light during the TROPI-2 spaceflight experiment. White circles indicate micro-g, grey circles indicate 0.1 g, hatched circles indicate 0.3 g and black circles indicate 1 g. Note the large magnitude of the positive response at micro-g. The responses at 0.1 g and 0.3 g were not significantly different from the value of the 1 g control, and the former values were attenuated compared to the robust response at micro-g.
Integrated Impact of Diet on Human Immune Response, Gut Microbiota, & Nutritional Status During Adaptation to Spaceflight. Lead - Grace Douglas (NASA JSC) Team members - Crucian, B (NASA JSC), Lorenzi, H (J Craig Venter Inst), Ploutz-Snyder, R (USRA), Smith, S (NASA JSC), Zwart, S (USRA) Compare current spaceflight diet to "enhanced" diet, with complete dietary tracking, on immune function, gut microbiota composition, & nutritional status of crew in ground-control, & on ISS. Hypothesis: Increasing consumption of fruits, vegetables & bioactive compounds (omega-3 fatty acids, lycopene, flavonoids) & enhancing overall nutritional intake will improve human immunological profiles, taxonomic profile of the gut microbiota, & nutritional status biomarkers. Will determine diet effects on immune dysregulation, including leukocyte distribution, inflammatory cytokine profiles, T cell function, & other relevant immunological markers, the taxonomic & metatranscriptomic profile of the gut microbiome, & nutritional biomarkers & metabolites at selected intervals, in spaceflight & ground-control data.

Pick-&-Eat Salad-Crop Productivity, Nutritional Value, & Acceptability to Supplement ISS Food System. Lead - Gioia Massa (NASA KSC) Team members - Douglas, G (NASA JSC) Hummerick, M (Qinetiq NA) Mitchell, C (Purdue) Morrow, R (Orbital Technologies) Wheeler, R (NASA KSC) Young, M (NASA JSC) Williams, T (Wyle) Growing nutritious, palatable food for crew consumption during spaceflight can provide health promoting, bioavailable nutrients, enhance dietary experience, & reduce launch mass for longer-duration missions. The “Veggie” vegetable-production system on ISS offers an opportunity to develop a “pick-&-eat” fresh vegetable component as a step to bioregenerative food production. Growing salad plants in Veggie during spaceflight will determine effects of light quality & fertilizer formulation on crop morphology, edible biomass yield, microbial food safety, organoleptic acceptability, nutritional value & behavioral health benefits of fresh produce. Phase A flight tests use leafy greens and Phase B dwarf tomato. Deliverable will be the development of growth protocols for these crops in a spaceflight vegetable production system.
15-15 NASA AG-0013 “Partial-Gravity Dose Response: Roles of vestibular input & sex in response to AG” - Charles Fuller (University Of California, Davis) Team Alwood, Hoban-Higgins, Ronca - Study male, female & labyrinthectomized rats, who no longer have macular vestibular input. Studies will adapt rats to 2G using chronic centrifugation. Our previous research has shown that animals exposed to chronic centrifugation establish a new steady-state in physiology & behavior. We will then return the animals to 1G Earth gravity, subjecting them to a 1G step down in ambient force. All animals will be studied for 60 days post-centrifugation. Tissues will be collected for endocrine, histological & proteomic analysis. Body temperature, activity & sleep-wake parameters. Body mass, & food & water consumption will be monitored. Body composition & bone structural analysis will be measured & video recordings will be used for behavioral phenotyping.

15-15 NASA AG-0005 “Musculoskeletal response to partial-gravity analog in rats: structural, functional & molecular alterations” - Seward Rutkove (BETH ISRAEL DEACONESS) Co-I Bouxsein One countermeasure to negative consequences of space travel is artificial gravity (AG) via centrifugation. Lower levels of force may be effective for preventing muscle & bone atrophy. This study will evaluate AG for prevention of muscle & bone atrophy & weakness by studying rats using partial unloading developed previously for mice. Musculoskeletal responses of adult male rats will be studied following exposure to 0.2, 0.4, & 0.7G as well as 1 G for 1, 2 & 4 weeks. Measures will include histological analyses, force of muscle contraction, & bone mass, microarchitecture, & strength. Serological & tissue molecular analyses will be used to understand mechanisms and time course of changes. At the conclusion, we will have defined the potential impact of partial artificial gravity for prevention of musculoskeletal dysfunction & deterioration to inform future application of AG in human research & space exploration.
Space Biology Research Accomplishments

Publications by Year

- 2010: 31 publications
- 2011: 41 publications
- 2012: 43 publications
- 2013: 65 publications
- 2014: 68 publications
- 2015: 86 publications
- 2016: 72 publications
Comparative Transcriptomic Analysis of Adult Medaka Tissues Sampled after Adaptation to a Space Environment

**Organisms** | **Factors** | **Assay Types** | **Release Date** | **Description**
--- | --- | --- | --- | ---
Oryzias latipes | Spaceflight | transcription profiling | 15-Oct-2015 | To understand how humans adapt to space environments, many experiments can be conducted on astronauts while they work aboard the Space Shuttle or the International Space Station (ISS). We also need animal experiments that can apply to human...

Bacillus subtilis strains at low-pressure: 5 kPa versus 101 kPa growth

**Organisms** | **Factors** | **Assay Types** | **Release Date** | **Description**
--- | --- | --- | --- | ---
Bacillus subtilis | strain pressure | transcription profiling | 14-Mar-2014 | Comparing the transcriptional responses of Bacillus subtilis strains WNB24 and WN1100 at 5 kPa and 101 kPa. WN1100 is a 6 kPa-evolved strain with increased fitness compared to ancestor WNB24 strain at 5 kPa. This experiment proved the difficulty...
Space Biology - Pioneering Scientific Discovery
Space Flight Data to Knowledge – Genelab Access
Space Biology Research Challenges

Crew Time:
• ISS crew time is oversubscribed; little is available for Space Biology and Physical Science

Rodent Research:
• Severely limited ability to execute rodent research on orbit

Post-ISS Exploration:
• NASA will not be the “anchor tenant” for research in low Earth orbit

New Platforms:
• Continuing research activities on ISS while developing opportunities to move beyond LEO and utilize alternative platforms
Addressing Space Biology Research Challenges

Crew Time:
• Focus near-term on ISS payloads that can fly using reserve crew time
• Focus on automating ISS flight experiments to reduce/eliminate crew time
• Partner with CASIS, Roscosmos, HRP, STMD and other Programs to co-sponsor payloads of joint interest in exchange for prime crew time from those Programs
• Target alternative vehicles to increase flight opportunities and go beyond LEO

Rodent Research:
• Implement a BSP, in collaboration with CASIS, for every Rodent Research Mission to maximize utilization of tissues returned from space and obtain tissues for Space Biology PI research
• Collaborate with JAXA and Russia for BSP and Joint Rodent Research Missions to obtain tissues for Space Biology PI research
• Seek new opportunities for Rodent Research in Space (e.g. Bion)

Post-ISS Exploration and New Platforms:
• Working to develop plans to enable increased Life Beyond Low Earth Orbit Activities
• Working to fly investigations on all NASA Exploration missions and increase utilization of new space flight carriers