National Aeronautics and Space Administration

# Biological and Physical Sciences

## SPACE BIOLOGY PROGRAM

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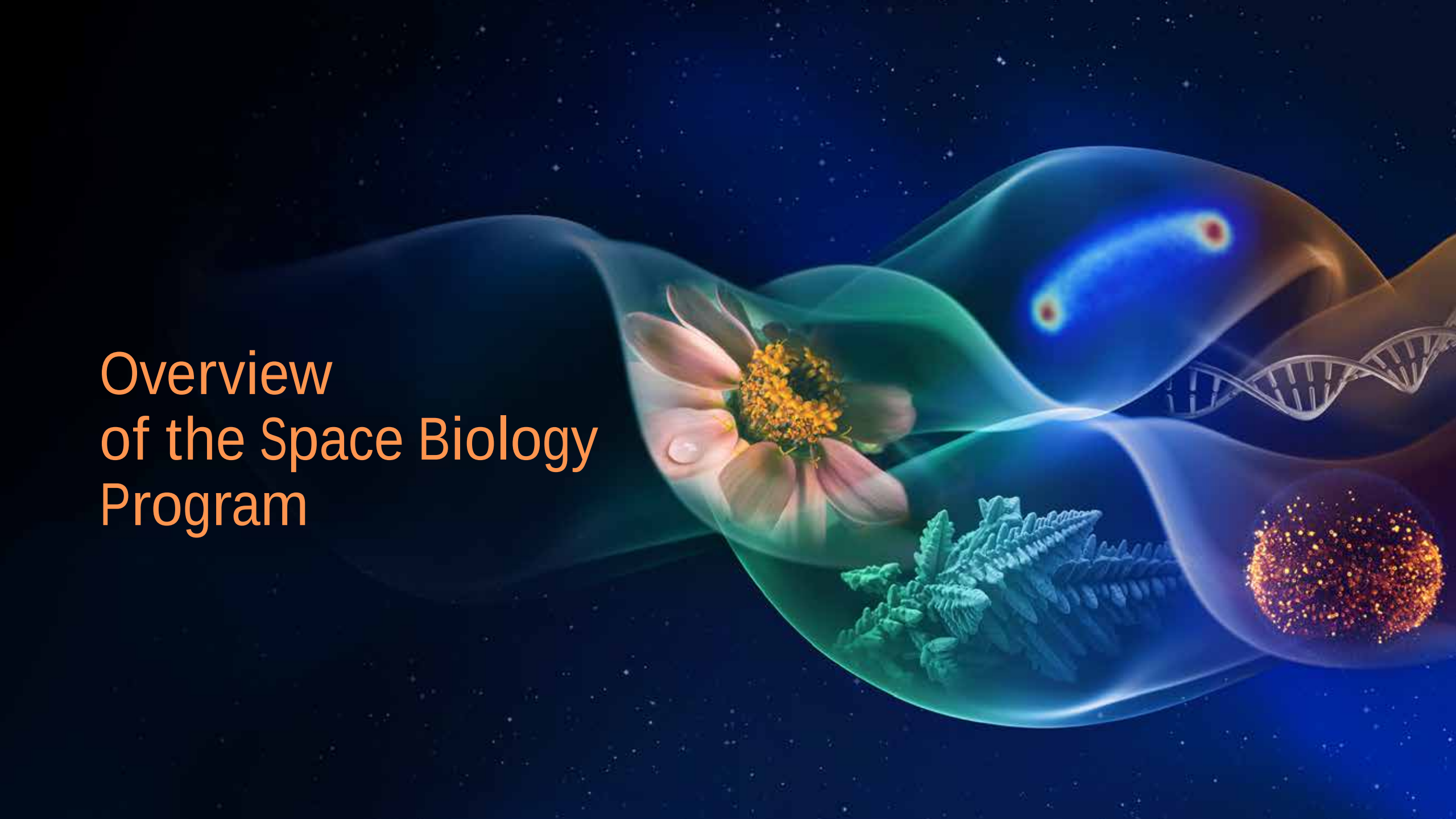
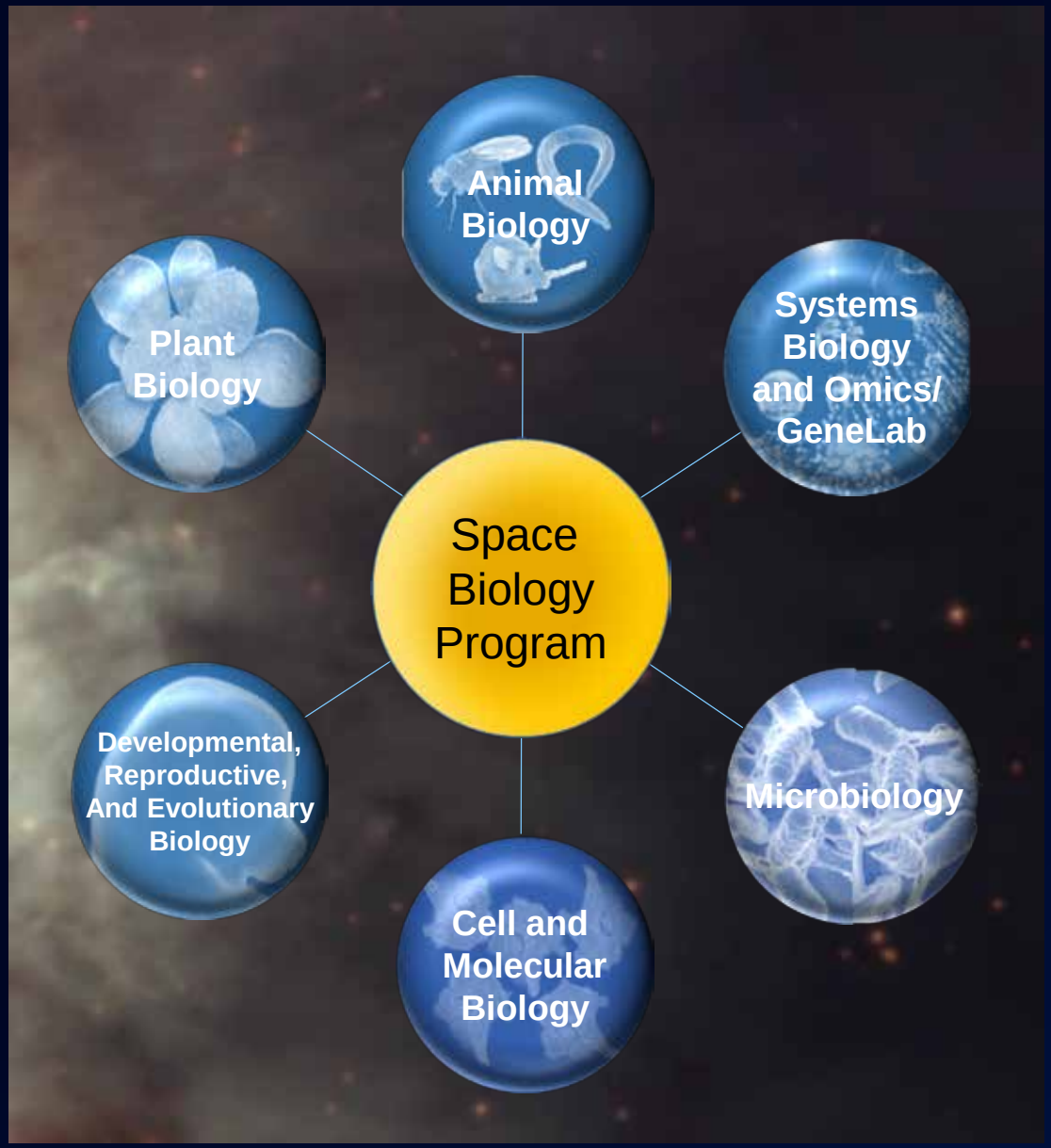
Presentation to the National Academy's Committee on Biological & Physical Sciences in Space
Oct 13-14, 2021

# Agenda

- **Overview of the Space Biology program**
- **Notable changes & transformative science areas**
  1. Omics/Systems Biology / Quantitative Genomics
  2. Genetic Engineering in Plants
  3. Effects of Regolith
  4. 3D Tissues and Organ-on-Chip models
  5. Artificial Intelligence / Machine Learning (AI/ML)
  6. Automation, Miniaturization, and Data Telemetry
- **Backup: Recent Science Highlights**

# Overview of the Space Biology Program

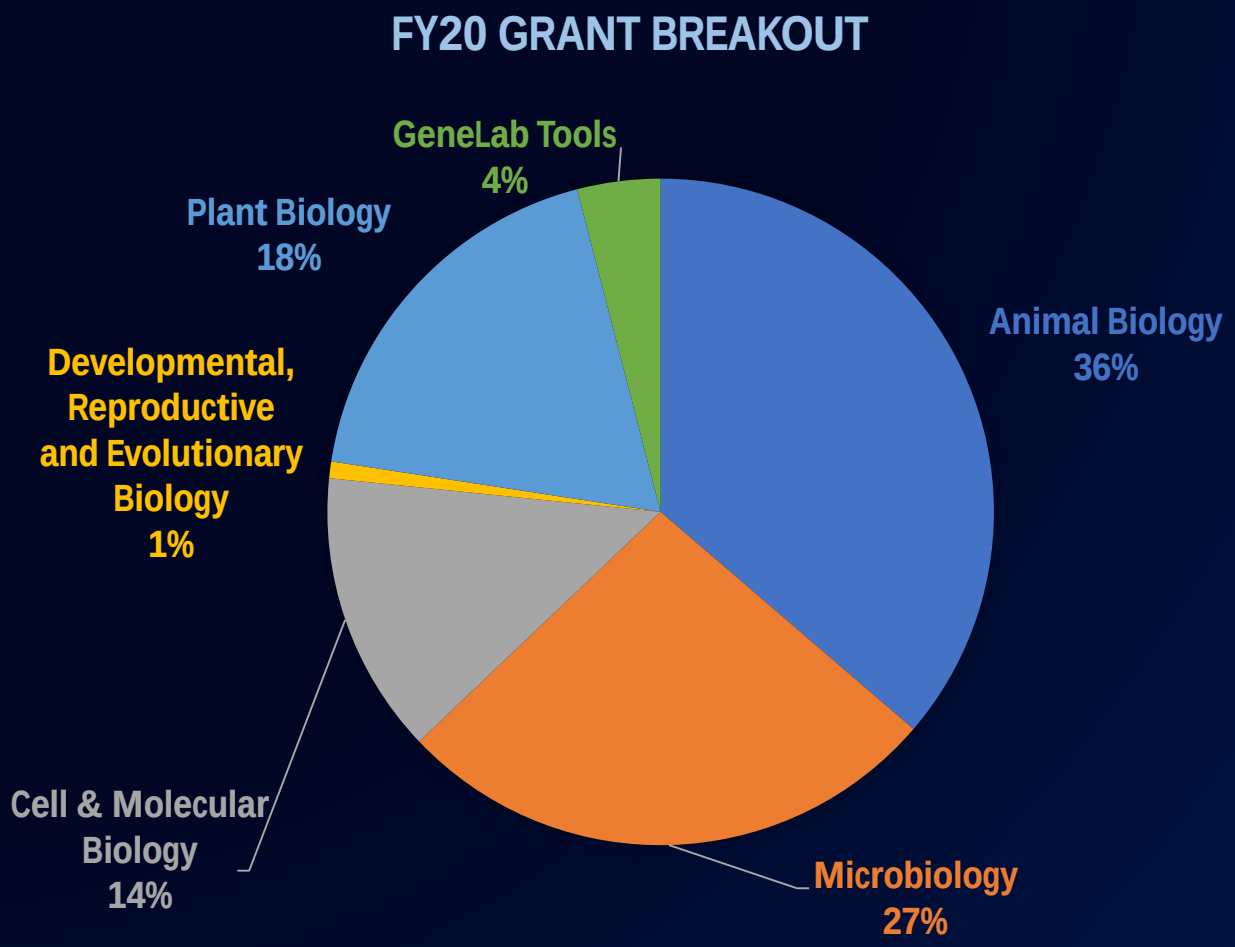





# Objectives

- **Discover how biological systems respond to the space environment**
- **Identify the underlying mechanisms and develop models for biological systems in space**
- **Provide mechanistic understanding to support human health in space**
- **Promote open science through the GeneLab Data System and Life Sciences Data Archive**
- **Develop technologies to enable spaceflight research**
- **Transfer the knowledge and technology of space-based research to benefit life on Earth**

# Space Biology Content




| Total SB FY20 Grants | 124 |
|---|---|
| Flight | 67 |
| Ground | 57 |

| Number Directed vs Competed | |
|---|---|
| Directed | 3 |
| Competed | 121 |

# Newly Selected Space Biology Projects (2021)

## ROSES 2020-E.12: Call for Flight and/or Ground Research Proposals

A Multi-omics and multi-species examination of combined environmental stressors of space exploration
Dawn Bowles, Ph.D. Duke University, Durham, NC

Developing A System for Rapid Diagnosis of Plant Diseases and Monitoring of Plant Microbiome for Spaceflight Applications
Natasha Haveman, Ph.D. University of Florida, Gainesville, FL

Spatiotemporal Mapping of the Impact of Spaceflight on the Heart and Brain
Christopher Mason, Ph.D. Weill Medical College of Cornell University, New York, NY

Integrated physiological responses of CNS and muscle in Drosophila and C. elegans along a gravity continuum
Karen Ocorr, Ph.D. Sanford Burnham Prebys. Medical Discovery Institute. La Jolla, CA

Hypobaric Plant Biology in Space Exploration - Molecular Responses of Arabidopsis to Combined effects of Low Atmospheric Pressures and Microgravity of Spaceflight Vehicles
Anna-Lisa Paul, Ph.D., University of Florida, Gainesville, FL

Microgravity Can Down-Regulate Host Resistance and thus May Up-Regulate Plant Disease Development in Space
Andrew Schuerger, Ph.D. University of Florida, Gainesville, FL

Megakaryocytes Orbiting in Outer Space and Near Earth: The MOON Study
Hansjorg Schwertz, Ph.D. University of Utah, Salt Lake City, UT

Impact of the gut microbiome on the integrative physiology of genetically diverse invertebrates
Siva Vanapalli, Ph.D. Texas Tech University, Lubbock, TX

Modeling leafy greens physiological and biochemical responses to light intensity and successive harvest
Kellie Walters, Ph.D. University of Tennessee, Knoxville, TN

From Antarctica to Space: molecular response and physiological adaptation of moss to simulated deep space cosmic ionizing radiation and spaceflight microgravity.
Agata Zupanska, Ph.D. SETI Institute, Mountain View, CA

## ROSES 2021-E.10: Lunar Explorer Instrument for Space Biology Applications (LEIA)

Investigating Lunar Stress and Parkinson's Disease using an Alpha Synuclein Yeast Model
Lynn Harrison, Ph.D. Louisiana State University System, Shreveport, LA

ORGANA: Oxidation-Reduction potential and Genetic Assessments for New mission Applications
Sergio Santa Maria, Ph.D. NASA Ames Research Center, Mountain View, CA

Feasibility of synthetic biology countermeasures for human exploration beyond low Earth orbit
Andrew Settles, Ph.D. NASA Ames Research Center, Mountain View, CA

# Other Funded Plant Biology Projects

### Evaluation of Small Plants for Agriculture in Confined Environments (SPACE) Tomatoes for Space Flight Applications

Robert Jinkerson, Ph.D., University of California, Riverside, California

### C4 Photosynthesis in Space (C4Space)

Christer Jansson, Ph.D., Pacific Northwest National Laboratory, Richland, Washington

### Assessment of Nutritional Value and Growth Parameters of Space-Grown Plants

Karl Hasenstein, Ph.D., University of Louisiana, Lafayette, Louisiana

### RNA-Seq Guided Mutant Analysis to Discover New Components of Gravity Signaling in Plants

Scot Wolverton, Ph.D., Ohio Wesleyan University, Deleware, Ohio

### Spaceflight Alters Post-Transcriptional Regulation

Sarah Wyatt, Ph.D., Ohio University, Athens, Ohio

### Membrane Contacts in Plant Gravity Perception

Marcella Rojas-Pierce, Ph.D., North Carolina State University, Raleigh, North Carolina

### Life Beyond Earth: Effect of Space Flight on Seeds with Improved Nutritional Value

Federica Brandizzi, Ph.D., Michigan State University, East Lansing, Michigan

### Microbe-Plant interactions with International Space Station microbial communities in Veggie flight crops

Christina Khodadad, Ph.D.,AECOM Management Services, Inc., LASSO, Kennedy Space Center, Merritt Island, FL

### Epigenetic Adaptation to the Spaceflight Environment - Accumulated Genomic Change Induced by Generations in Space

Anna-Lisa Paul, Ph.D., University of Florida, Gainesville, Florida

### The Role of Ca2+ Signaling During the Early Events of Plant Adaptation to Spaceflight

Rob Ferl, Ph.D., University of Florida, Gainesville, Florida

# Plant/Microbe Biology Projects

### Effect of Spaceflight and Simulated Microgravity on Plant Defense Response

Anjail Iyer-Pascuzzi, Ph.D., Purdue University, West Lafayette, Indiana

### Spaceflight Effects on Plant-Microbe Interactions

Simon Gilroy, Ph.D., University of Wisconsin, Madison, Wisconsin

# Other Funded Animal Biology Projects

Develop a Novel Single-Cell Biodosimetry for Brain Genomic Instability and Neurodegeneration to Predict Clinical Health Outcomes in Human Spaceflight Crews

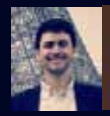
Xiaohong Lu, Ph.D, Louisiana State University, Shreveport, Louisiana

Osteocyte Plasma Membrane Disruptions in Skeletal Adaptation to Loading and Unloading

Meghan McGee-Lawrence, Ph.D., Augusta University Research Institute, Inc., Augusta, Georgia

Thrombosis in microgravity

Anand Ramasubramanian, Ph.D., San Jose State University, San Jose, California



Using Water Bears to Identify Biological Countermeasures to Stress During Multigenerational Spaceflight

Thomas Boothby, Ph.D., University of Wyoming Laramie, Wyoming

The Effects of Spaceflight and Reloading on Skeletal Muscle and Bone

Mary Bouxsein, Ph.D., Harvard Medical School, Boston, Massachusetts

Effect of Microgravity on Neuronal Cytoskeletal and Intracellular Trafficking

Kaitlin O'Connell-Rodwell, Ph.D., SCORPIO-V, HNu Photonics, Maui, Hawaii

Space Environment and Epigenetics of Endocrine Regulation of DNA Repair and Cell Cycle in Mammary Epithelial Cells

Donato Romagnolo, Ph.D., University of Arizona, Tucson, Arizona

A Technology To Measure Gait, Egress, and Locomotor Performance in Perturbed Environmental Conditions After Simulated Spaceflight

Jeffrey Willey, Ph.D., Wake Forest School of Medicine, Winston-Salem, North Carolina

Role of Mesenchymal Stem Cells in Microgravity Induced Bone Loss

Abba Zubair, Ph.D., Mayo Clinic, Jacksonville, Florida

# Animal/Microbe Biology Projects

Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms and Impact on Multi-System Physiology

Fred Turek, Ph.D., Northwestern University, Evanston, Illinois

Impact of Spaceflight on Beneficial Animal-Microbe Interactions

Jamie Foster, Ph.D., University of Florida Gainesville, Florida

# BioExpt-1: Flying on Artemis-1

Selection for NRA NNH18ZTT001N-EM1 Appendix A: "Orion Exploration Mission-1 Research Pathfinder for Beyond Low Earth Orbit Space Biology Investigations"

Federica Brandizzi, PhD, Michigan State. "*Life beyond Earth: Effect of space flight on seeds with improved nutritional value"* Characterize how spaceflight effects plants seed nutrient stores to gain new knowledge to help increase the nutritional value of plants grown in spaceflight

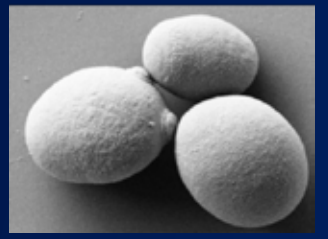
Timothy Hammond, PhD, Institute For Medical Research. "*Fuel to Mars" W*ill study photosynthetic algae, *Chlamydomonas reinhardtii*, to identify important genes that contribute to its survival in deep space

Zheng Wang, PhD, Naval Research Laboratory "*Investigating Roles of Melanin and DNA Repair on Adaptation and Survivability of Fungi in Deep Space" W*ill use fungus *Aspergillus nidulans* to study radioprotective effects of melanin & DNA damage response

Luis Zea, PhD, Univ. Colorado, Boulder "*Multi-Generational Genome-Wide Yeast Fitness Profiling Beyond and Below Earth's van Allen Belts" W*ill use yeast to identify genes that help organisms adapt to conditions of both deep spaceflight on the EM-1 mission, and of Low Earth Orbit on ISS

# Lunar Explorer Instrument for Space Biology Applications (LEIA)

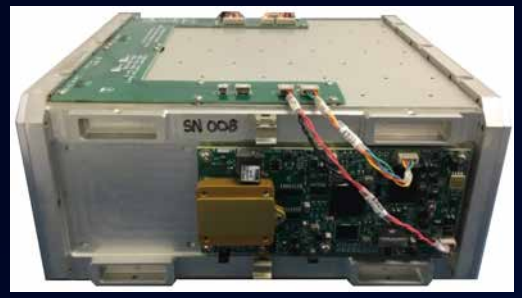
- LEIA is based on the 6U BioSentinel Small Sat
- Modified 4U Biosensor with a 2U “Pseudo Bus” to provide thermal control for the lunar surface and data conditioning



Accommodates yeast and related cellular systems

## 4U BioSensor Payload

(2U Pseudo Bus not shown in diagrams below)



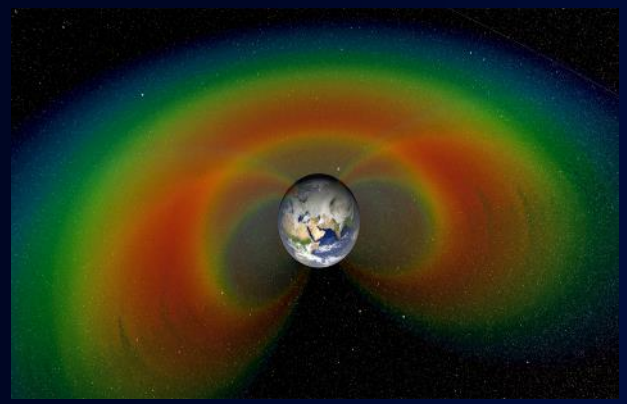

LET spectrometer attached at one end of payload


Biosensor payload with fluidic cards and optical sensors

# Biologically Relevant Environmental Factors Encountered in Spaceflight

## Microgravity/Reduced Gravity

## Ionizing Radiation

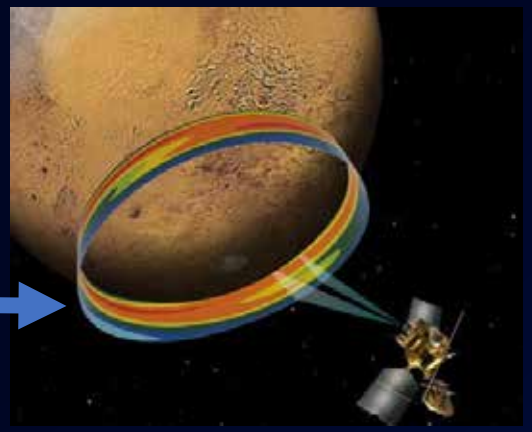


Credits: NASA/Goddard Space Flight Center/Scientific Visualization Studio

## Altered Day/Night Cycles: Circadian Rhythm Changes

## Altered Temperature and Atmosphere

- Elevated CO2
- Reduced atmospheric pressure and elevated volumetric fraction of oxygen



## Isolation

## Regolith

## COMBINATION OF MULTIPLE STRESSORS

# Thriving in Deep Space (TIDES)

Biological effects of multiple **deep-space stressors**

1. Radiation
2. Gravity
3. Temperature & Atmosphere
4. Day & Night Light Cycles
5. Isolation
6. Regolith/dust

*Cannot accurately replicate on the ground*

*Heavy ions can impact biological systems*

**Transformative** biological science and exploration applications

| | |
|---|---|
| 1. Animal Biology | Vertebrate and invertebrate models to probe analogous changes in humans |
| 2. Plant Biology | From plant models to crops to sustain life for long-term human habitation |
| Microbiology | How it influences animals and plants in space |

# Thriving in Deep Space (TIDES)

Ground studies
Space studies
Ground & space studies

**DEEP SPACE STRESSORS**

Gravity | Radiation | Temperature/Atmosphere | Day/Night/Circadian Cycle | Isolation

**PLATFORM PROGRESSION**

Ground → Sub-Orbital → LEO/ISS → Gateway → Lunar Surface → Mars Transit → Martian Surface

## Understand Fundamental Mechanisms

Use model organisms to determine the fundamental biology of the human in the deep space environment

- Utilize "omics", systems biology, physiology, genetics, etc.
- Test responses within organisms & ecosystems
- Study the combined effects of radiation, gravity, & other stressors

## Build the Blocks to Support Human Life

Study the environmental stressors in spaceflight on model plants, crops, & seeds

- Combined effects of radiation, gravity, and other stressors

## Build a Foundation for Sustained Life on Mars

Engineer habitats and ecosystems to enable astronauts' independence from Earth

- Stabilize human/animal-plant-microbial ecosystems in the context of multiple deep space stressors

**MODEL ORGANISM PROGRESSION**

Unicellular (e.g., yeast, fungi) → Invertebrates (e.g., flies, worms, tardigrades) → Vertebrates (e.g., mice & rats; fish) → Humans

Simple model plants (e.g., *Arabidopsis*) → Crops & edible plants

# Summary of results from the combination of radiation and microgravity from past spaceflight experiments

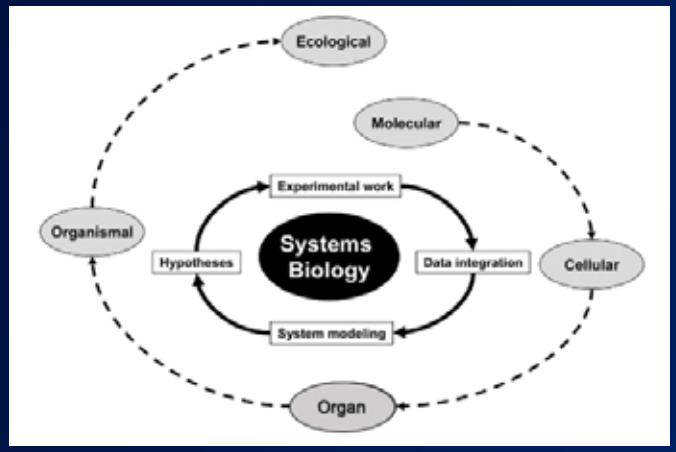
Please refer to slides # 47 to 58 from the presentation entitled “Space Biology Program” (S.Bhattacharya)
from the NAS CBPSS presentation on October 27, 2020, for a summary of the potential effects of combined stressors in deep space.

**We need to use various organisms amenable to spaceflight experimentation to obtain statistically reliable data on the combined effects of space radiation, altered gravity, and other spaceflight stressors.**

# Notable Changes & Transformative Science Areas

# 1. Biggest Changes in Past 5 Years – "Omics" and Systems Biology

- **Changes in the field as a whole**
  - Enhancement/development of methodologies:
    - "Omics"
    - Systems biology
    - Quantitative genomics




- **Changes in NASA-funded research**
  - Development of the GeneLab database (https://genelab.nasa.gov/)
    - Archive space-relevant omics data and metadata and provides collaborative space to share, analyze, visualize data
  - Update of the Ames Life Sciences Data Archive – archive of spaceflight-relevant vertebrate and invertebrate animal tissues, cells, data, etc. (https://www.nasa.gov/ames/research/space-biosciences/alsda)
    - Efforts underway to link GeneLab database closely with the NBISC (NASA Biological Institutional Scientific Collection) to facilitate efficient curation of samples along with ancillary data
      (desire to include microbiological samples in the future)

# 1a. Transformative Area: Systems Biology

- **Effects of Combined Spaceflight Stressors: e.g., deep-space radiation and altered gravity**
  - Use well-characterized biological model organisms to understand the complex biological consequences of exposure to deep space's unique environment:

***Approach:*** Conduct a sequence of Earth-based, low Earth orbit (LEO), and lunar/cis-lunar investigations, with transit to and on Mars research as the long-term goal. Populations of biological **model organisms of increasing complexity (e.g., yeast, worms, flies, mice/rodents, etc.)** will be used to gather data on changes in the underlying responses to space-unique environmental stressors, which do not occur in everyday Earth-based systems. The data from cells, organs, and the whole organism will be compared within populations and across organisms to develop a systems biology model that ultimately enables predicting physiological responses of humans to the deep space environment.

## Ground-based Studies

- NASA's Space Radiation laboratory at Brookhaven National Laboratories.
- Other radiation facilities.
- Simulated microgravity platforms.
- Hypergravity facilities.

## Flight Studies

- Compare results between ground (1g, baseline radiation) and
  - ISS/LEO (microgravity, low radiation environment)
  - Autonomous free flyers (microgravity, high radiation environment)
  - Lunar surface (1/6g, high radiation environment)
- Utilize long-duration multigenerational studies with biological model organisms
- Flight hardware should be adapted (from those used in LEO) or developed for free flyers and lunar missions.

# 1b. Transformative Area: Quantitative Genomics

- **Quantitative Genomics: Understand the genetic basis of complex traits using genotype to phenotype mapping.**
  - Use well-characterized quantitative genomics model systems (e.g., flies, mice, plants) to identify genes and pathways that affect the variation of traits relevant to the space environment. Ground-based studies may be needed preliminarily for the larger organisms (e.g., mice and plants) followed by spaceflight of selected lines. Small organisms like flies can be flown in space for genome wide association studies (GWAS) to identify genes and genetic variants that confer better survival and behavior in the space environment.

*Approach* - A *Drosophila*-based example:

Multi-generational analysis - Maintaining selected advanced intercross population (AIP) fly lines in space and Earth environment simultaneously with a generation interval, followed by freezing parent flies of each generation in their respective environment. Upon returning to Earth, the frozen flies can be used to conduct DNA, RNA, and ATAC sequencing. Additionally, the last generation larvae brought back on Earth can be grown into adults and tested for quantitative traits such as stress resistance, behavioral responses, lifespan, among others. When compared to Earth flies, the data will inform genetic, genomic, and epigenetic responses to natural selection in the space environment.

Workshop held by NASA Space Biology on May 26, 2021: See link

# 2. Biggest Changes in the Past 5 Years – Genetic Engineering in Plants

## Changes in the Field as a Whole:

- Can now evaluate all genes in combination with phenomics, proteomics, metabolomics and use as inputs to computational modeling.
- Deep insight and understanding of the gene networks and metabolic pathways allows the plant breeder to put together the best collection of 20,000+ genes using available genomic tool sets.
- This has enabled **Predictive Breeding** that shortens the time to a new crop by understanding what all the genes are doing so they can be combined into the best plant for the conditions.
- Genome editing techniques are currently being used to validate pathway models quickly, and to create predicted "quality" crops (e.g., tailored starch composition in potato or corn).
- Recent changes in plant genetic engineering
    - Sequencing technology developments
    - Genome-wide editing technology developments
    - CRISPR delivery – Nanotechnology
    - CRISPR delivery – Viral engineering
    - Multi-Omics developments
    - Breeding enhancement through AI data integration
- Accomplishments in Genetically Modified (GM) crops
    - Food security
    - Natural products
- Future Perspective: Synthetic Evolution


*Credit NASA*

*Credit NASA*

# 2. Transformative Area: Genetic Engineering of Plants

## Plant Growth and Development

- Compact size (low height and volume)
- High yield
- High edible/inedible biomass ratio (harvest index)
- Reliable germination
- Rapid growth to first yield
- Uniform growth and development between individuals
- Propagules with long shelf stability (seeds, cuttings)
- Sustained production capability over long duration
- Low debris formation (leaves, flowers, pollen, seeds, etc., remain attached)
- Custom microbiome of plant protective and growth promoting microorganisms

## Produce Nutrition

- High levels of antioxidants
- High levels of beneficial phytonutrients
- High levels of Potassium and Magnesium
- High levels of Vitamins, especially C, $B_1$, and K
- Low levels of Iron
- Low levels of antinutrients

## Produce Organoleptic Acceptability

- Intense flavors
- Good texture
- Good appearance, color, aroma

## Plant Physiology

- Stress tolerance (esp. water stress)
- Ability to grow well under conditions of:
  - Elevated CO2
  - Low relative humidity
  - Uniform temperature of 20-23°C
  - Narrow spectrum electric lights
  - Short photoperiods (to save lighting energy) or continuous photoperiods
- Tolerance of broad environment range
- Tolerance of reduced pressure
- No dormancy requirements
- Pleasing aroma
- Low release of volatile organics
- High sodium tolerance (e.g., urine recycling)
- Preference for ammonia nitrogen sources (e.g., urine recycling)

## Post Harvest

- Good produce shelf life or storage on the plant
- Reduced processing – e.g., seeds easy to remove from seed coats
- Easy composting/digestion of inedible plant material for nutrient reclamation Use of inedible waste as resources for other food system components
  - (e.g., fish, edible fungus, cellular ag feedstocks)
- Other useful materials produced from inedible waste
  - (e.g., life support, shielding, building, fuel)

# 3a. Biggest Changes– Plant Responses to Lunar Regolith

**Changes in the Field as a Whole:**

- In the 1970's, seminal work was done to show that plants could be exposed to lunar regolith without any negative effects. In fact, plants could uptake nutrients/minerals from lunar regolith and show changes in lipid and chloroplast content when grown in contact with lunar regolith.
- Given the desire to employ the *In Situ* Resource Utilization (ISRU) approach to "live off the land" when habitats are established on the moon and Mars, enabling the growth of plants in lunar regolith would be important.
- Geomicrobiology investigates the interactions of micro organisms with geological substrates and has enormous potential in the settlement of space by helping modify regolith to enable plant growth.

**NASA-Funded Research:**

- Biological and Physical Sciences (BPS) has funded ISS research efforts assessing microbial associations with plants grown on the ISS, and studies of biofilm formation on surfaces within the ISS as a segue to geomicrobiology.
- The NASA Astrobiology Program has provided prior funding to geomicrobiology.
- The bulk of research funding for growing plants in lunar regolith, however, ended after the Apollo era.

# 3a. Transformative Area: Plant Responses and Growth in Lunar Regolith

- **Limited plant studies have used lunar regolith.**
  - Limited plant growth experiments have been conducted "in contact with" small amounts of returned moon material.
  - Growing plants with archived lunar regolith and simulants and optimizing growth conditions are important for lunar habitation.
  - Projects such as farming in "Martian Gardens" are using crushed Hawaiian volcanic rock.
  - Regolith simulants are manufactured and distributed to multiple labs by NASA Johnson Space Center.

- **Microbial remediation can be used for soil/regolith conversion suitable for plants.**
  - Provides In situ Resource Utilization (ISRU) to procure bioavailable nutrients for plants.
  - Microorganisms could be used to create soil from lunar and Martian regolith.
  - Provides a foundation to support plant growth and as an element in Bioregenerative Life Support Systems (BLSS) with the ability to produce oxygen, sink carbon dioxide, and recycle organics and non organics.


- **Ecosystems studies involving multiple organisms and multi-omics approaches can be transformative.**
  - Multispecies spaceflight investigations evaluated by multi-omics techniques can provide valuable data on the response of plants to the space environment.
  - Topics for geomicrobiology extend beyond the support of plants alone and can provide bioconcretes for habitat build, production of rare minerals for electronics, magnets, and capture of lunar He-3 for safer nuclear energy production.

# 3b. Biggest Changes– Animal Responses to Lunar Dust

**Changes in the Field as a Whole:**

- In the late 1960s, a study was done that indicated that protists, invertebrates, and fish could be exposed to crushed lunar rock without any apparent negative effects. (Benschoter,  et al. Science. 1970).
  - Growth rates of protists were unaffected.
  - No mortality or morphological changes occurred in the flat worm.
  - Daily observation and histopathological findings from the cockroaches showed these insects to be in excellent condition.
  - No pathologic changes were noted during daily observation of behavioral and morphological features in the fish.
- Several studies over the next decades, however, provided additional evidence that regolith exposure had potentially negative effects on animal health at the physiological and cellular levels.

Studies with Lunar Dust (Apollo 14):

- Rat Inhalation studies with lunar dust showed concentration-dependent increases in inflammation biomarkers in bronchial alveolar lavage fluid, as measured by cellular components (total cell, neutrophil/lymphocyte counts), and cytotoxic biomarkers (enzymes [LDH, γGT and AST]). (Lam, et al., *Inhal Toxicol*., 2013).
- Rat benchmarking studies with lunar dust (intratracheal instillation) determined the safe periodic exposure estimate for humans was between 0.5-1.0 mg/m$^3$ during long stays in habitats on the lunar surface (James et al., *inhal Toxicol*., 2013).
- Lunar dust is a mild irritant to mammalian eyes (rabbit) (Meyers et al.,  *BMC Ophthalmol*., 2012).

# 3b. Biggest Changes– Animal Responses to Lunar Dust (continued)

**Changes in the Field as a Whole:**

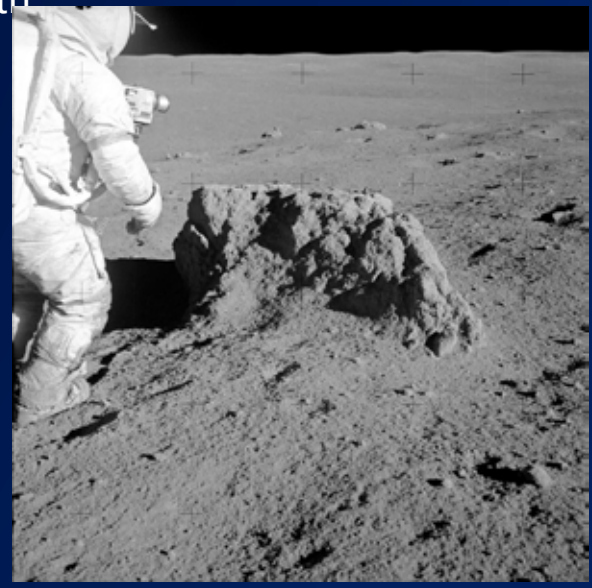
Studies with Lunar Dust/Regolith Simulant (LDS/LRS)

- LDS treatment of isolated rodent synaptosomes resulted in increased glutamate binding to the nerve terminals, indicating potential neurotoxicity (Krisanova, et al., Astrobiology, 2013).
- Treatment of macrophages with lunar regolith/soil simulant (LRS) resulted in a concentration-dependent increase of inflammatory markers with increased Induced Nitric Oxide Synthase (iNOS) (Chatterjee, et al., *J Toxicol and Environ Health A*., 2010), as well as moderate cytotoxicity, alterations of cell morphology, and phagocytosis of simulant as noted in a separate study (Li, et al., *Appl Toxicol.*, 2019).
- Lunar soil simulant treatment of neuronal and lung cell lines resulted in cytotoxicity and both genomic and mitochondrial DNA damage (as measured by qPCR – lesions block amplification) (Caston, et al., *Geohealth*, 2018).
- Rodent studies conducted indicate that the intratracheal installation of LDS in rats causes inflammatory damage that affects autonomic function, leading to inflammatory myocardial fibrosis (Sun et al., *Toxicol Res.* 2019), and the aggregation and infiltration of lymphocytes and neutrophils in the lung which may lead to inflammatory pulmonary fibrosis (Sun et al., *Environ Toxicol.* 2018).

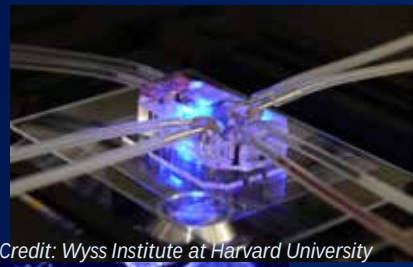
# 3b. Transformative Area: Animal Responses to Lunar Dust

- **Limited animal studies have used lunar dust.**
  - Relatively few animal studies have been conducted examining the health impacts of exposure to lunar dust. Many used lunar dust/regolith simulant (LDS/LRS).
  - While no overt negative effects were observed in animals after regolith treatment in 1970, these biological systems could not be examined on the cellular and molecular levels using the analyses tools that are available today.
  - More recent cellular studies showing that LRS can cause inflammation, cytotoxicity , DNA damage, & potential neurotoxicity, animal studies indicate that Lunar dust/LDS inhalation can have negative effects on lung, cardiac and eye tissue. Effects vary with particle size and concentration of LRS used. No knowledge of combined effects with other stressors.
  - Additional experiments are required using both simulated and genuine regolith with established animal model systems to determine the molecular, cellular, and long-term physiological consequences of exposure to regolith.
- **Future studies examining the effects of lunar dust on whole physiological models**
  - Whole physiology studies can initially be conducted using simple animal models (e.g., invertebrates) to identify physiological systems and molecular pathways that are altered from exposure to lunar dust and LDS.
    - Mutigenerational studies would indicate if exposure has long term effects on species vitality
  - Studies with vertebrate models could further define the effect that exposure has on specific systems (e.g., respiratory, cardiovascular, nervous) informed by the results of prior studies.
- **Environmental factors & additional stressors could alter physiological responses:**

  e.g., Research with humans on parabolic flights revealed differences in deposition of inhaled fine particles in the lungs between 1/6g & 1g. While the overall amount of deposition in 1/6g was lower, the deposition occurred in more peripheral regions of the lung (Darquenne and Prisk, *Eur J Appl Physiol.,* 2008).



# 4. Biggest Changes in the Past 5 Years
# 3D Tissues and Organ-on-Chip Models



*Credit: Wyss Institute at Harvard University*

*Credit: NASA*

## Background and Advances in Last 10 years

- First Human Organ-on-Chip - Lung-on-Chip Reported by Harvard in 2010
- NIH's National Center for Advancing Translational Sciences (NCATS), US Food and Drug Administration (FDA) and the Defense Advanced Research Projects Agency (DARPA) launched a $70M program in 2012 to expand capability to 10 organs-on-chip
- As of 2021: 12 Organs can be linked for comprehensive systems biology; systems available for purchase

## Changes in the field as a whole:

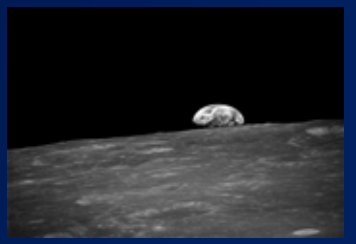
- ***Science advances***: Human induced pluripotent stem cell-derived models for personalized healthcare; Disease modeling; Cancer; Infectious Disease; Inflammation; Microbiome; Neuroscience; Drug screening and toxicity
- ***Program advances***: NCATS Tissue-Chips-in-Space Program initiated in 2017; Tissue Chip Testing Centers for validating MPS began in 2016; Clinical Trials-on-Chip Program launched in 2020; FDA Advancing New Alternative Methods awards for countermeasure development and toxicology; EPA SBIR awards using organ-on-chip for predictive toxicology
- ***Funding advances***: Government agencies, pharmaceutical companies and small businesses
- ***Commercialization advances***: global organ on chips market valued at $50.8 million in 2020 – up 60% since 2015; expected to grow to $177.8 million in 2025 and reach $350.8 million in 2030

## Changes in NASA-funded research:

- **2021 Multi-Agency Solicitation (ground-based):** Extended Longevity of 3D Tissues & Microphysiological Systems for Modeling of Acute & Chronic Exposures to Stressors (NASA Space Bio; NIH/NCATS/NIAID/NCI/NICHD; BARDA; FDA; NASA Human Rsch Prog)
- HRP and Space Biology: funded projects to assess the effects of spaceflight stressors using advanced 3D tissue models
- TRISH: awards in 2020 for organ models to study effects of space radiation (cardiac, vascular, neural, gut/microbiome, multi-organ); ISSNL & NSF partnership has resulted in awards over the past several years coupled with in-flight testing.

# 4. Transformative Areas: 3D Tissues & Organ-on-Chip Models

- **Utilize robust *in vitro* model systems that are developed and tested first in ground studies**
- **Then validate *in vitro* model system designs using ISS/LEO as a testbed**
- **Conduct long duration spaceflight missions beyond low-Earth orbit (LEO) with *in vitro* models**
  - Free-flyers: LEO or Beyond LEO – microgravity, varied radiation spectrum
  - Gateway: inside HALO will provide realistic analog of radiation exposure, similar to a Mars transit vehicle
  - Lunar Orbit: Beyond LEO – deep space radiation, microgravity
  - Lunar Surface: deep space radiation, albedo radiation effects, thermal and lunar dust challenges, 1/6 gravity
- **Determine underlying mechanisms of spaceflight response of human-derived tissue/organ systems**
  - Help assess mechanistic effects of spaceflight stressors on biology to support fundamental science and crew health
  - Increased "N" for statistics
  - Supports risk assessment
  - Combined effects of spaceflight stressors like radiation & gravity that cannot be performed on the ground or in LEO
- **Comparing results between ground, ISS, beyond LEO and Lunar Surface will uncover the contributions of multiple stressors such as altered gravity and radiation on biology**
- **Mission to send validated *in vitro* models to Mars**
  - Provide fundamental science data on the effects of spaceflight stressors during Mars transit
- **Requires technology development in automation, sensors, habitat/housing, data downlink**

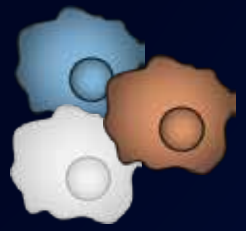


# 4. Transformative Areas:
# 3D Tissues & Organ-on-Chip Models

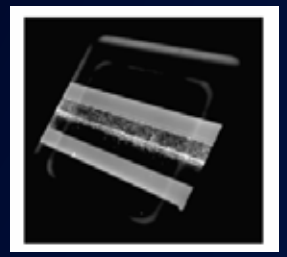
**Personalized models:**

- Genomic and epigenetic associations à mechanisms à targets for countermeasures and biomarkers
- Individual astronaut's risk

Donors



iPSC-derived cells



3D Tissue/Organ Models

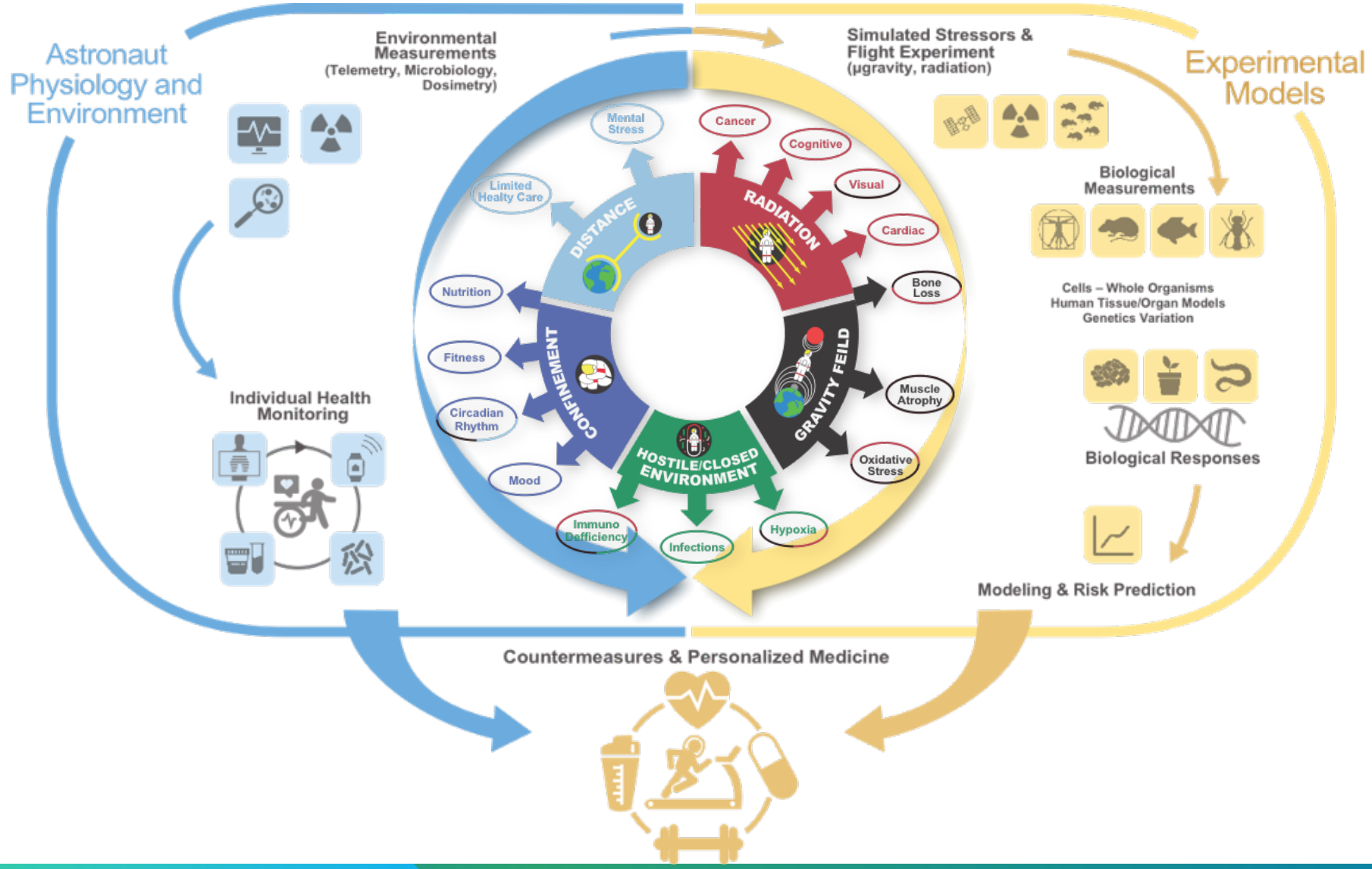

Simulated spaceflight stressors:
microgravity, ionizing radiation

LEO / BLEO / Lunar Surface payloads:

- Radiation
- Altered Gravity
- Environmental Conditions

- Fundamental space biology research
- Personalized biomarkers
- Personalized countermeasures

# Integration of Research Areas:



# 5. Biggest Changes in Past 5 Years – Artificial Intelligence/Machine Learning (AI/ML)


- **Changes in the field as a whole**
  - Protein folding prediction – game changer for drug development (e.g., AlphaFold).
  - Personalized medicine - AI/ML teams integrated into personalized medicine programs.
  - Disease prediction using omics data (e.g., 23&Me – Cancer Genome Atlas - GeneLab).
  - Wearable health devices (e.g., longitudinal data generation).
  - Diagnostics (e.g., medical imaging, breath analyzer, wearable data interpretation).
  - Image analysis, including biological images (e.g., microscopy data).
  - NLP (Natural Language Processing) which can play an important role in ingesting knowledge from publications.
  - Market growth from US$ 93B in 2021 forecast to be $ 997B by 2028 *(Grand View Research, Report ID: GVR-1-68038-955-5, June 2021)*
- **Changes in NASA-funded research:** Utilizing AI/ML to address topics like the effects of space on physiology
  - FDL : Frontier Development Lab – 2019 to 2021: Life Sciences AI/ML challenges
    - FDL2019 - *Synthetic biosensor data*
    - CRISP - Causal Relation and Inference Search Platform (CRISP)
      - *FDL2020 (Mayo Clinic) – AI Colon cancer causal inferencing model from clinical data (NASA interest in testing causal model for cancer in the context of Cancer risk)*
      - *FDL2021 (Space Biology, Human Research Program [HRP], & Intel) - Extension of CRISP in the context of radiation-induced cancer with mixed-data (human and mice) to scale risk from mice to human.*
  - Space Biology – FY21/25: AI/ML pilot CRISP + SPOKE + Modeling portal: *Radiation exposure risk prediction modeling using human & animal data (omics & phenotypic data)*
  - Human Research Program – FY22: AI/ML:
    - *Applying CRISP for risk prediction on at least two targets: bone loss, CNS, behavioral, or cancer. Using at least omics data and possibly physiological/phenotypic data (Ames Life Sciences Data Archive)*
    - *Deployment of federated learning algorithms using intel technology for analysis of private astronaut data without specific access to the data.*
  - AI/ML Workshop held by NASA's Space Biology program: June 24 – 25, 2021.

# 5. Transformative Area: Artificial Intelligence/Machine Learning (AI/ML)

- **Multi-omics data are quantitative and allow for standardization and meta-analysis**
  - AI/ML to extract knowledge from very diverse types of data (e.g., mice vs human, omics vs non-omics, sparse datasets, etc.)
- **Biological data are often qualitative (e.g., imaging, behavior, etc.) and require human scoring.**
  - Increasing role of AI/ML to interpret qualitative data and quantify them
  - AI/ML to automate data transformation, data curation, human interaction for data entry
  - AI/ML to relate back to the quantitative data
- **Biological responses are the result of very complex networks of interacting parts**
  - AI/ML methods allow the testing of such networks and identify the true cause of a given response. Causal inference methods will play a critical role in the following applications:
    - Onboard live diagnostic/prevention/monitoring for Astronauts' health status in remote situation away from Earth
    - Better risk models for mission planning
  - Microbiome is a complex system, critical to plant/animal/human health and its changes could be better interpreted by AI/ML.
- **Federated learning** is the ability to send AI tools to the data (keeping data private, but letting the tool learn from the data) – game changer to deal with private/sensitive patient data (e.g., distributed across many hospitals/institutes at once).
- **xAI (Explainable AI) – is a paradigm shift in AI/ML moving from a "black box action" to "human trustworthy recommendation" by the machine.**

# 6. Biggest Changes in the Past 5 Years – Automation & Miniaturization

- **Changes in the field as a whole**
  - Drive towards miniaturization for medical equipment, laboratory sampling tools for patients, etc.
  - Increased automation for high-throughput analyses of samples.
  - Increased use of telemedicine tools.

- **Changes in NASA-funded research**
  - Increased utilization of miniaturized, automated equipment for spaceflight.
  - Increased need for data telemetry from science experiments in deep space missions with no sample return.
  - Telemetry-based Biology Workshop was held on Aug. 18, 2021.

# 6. Transformative Area: Utilizing Automation, Miniaturization & Data Telemetry

- **Science-focused Habitat (SciHab) for Deep Space**

  - **Multi-platform habitat designed to house life science experiments for free-flyer missions, Gateway, lunar orbit, and lunar surface:**

    - Modular to accommodate multiple types of organisms
    - Autonomous readouts of biological endpoints and health of the system
    - Data telemetry for near real-time monitoring and report out
    - Conditioned environment
    - Videography / lighting
    - Radiation sensors

# Utilizing Different Platforms in Space

- **Lunar surface experiments** – partial gravity (1/6g), long-duration experiments (long-term goal of sustained lunar habitation), automated & miniaturized equipment, data telemetry, high doses of ambient radiation.
- **Cis-lunar (e.g., Gateway)** – microgravity, long-duration experiments (months to years), automated & miniaturized equipment, data telemetry, high doses of ambient radiation.
- **Free flyers/small satellites –** microgravity, very long-duration experiments (months to years), automated & miniaturized equipment, data telemetry, high doses of ambient radiation for beyond LEO free flyers.
- **The ISS and commercial LEO platforms –** microgravity, long-duration experiments (months-long), lower ambient radiation doses than in deep space, utilize available laboratory resources (e.g., freezers, microscopes, gloveboxes/biosafety cabinets, incubators, bone densitometers, PCR machines, etc.).
- **Suborbital** – short exposure to microgravity (minutes), manual or automated experiments, low ambient radiation exposure due to duration and orbital height.
- **Balloon flights** – long-duration experiments possible (weeks to months), radiation exposure can be elevated by adjusting altitude and duration of flight.

# Conclusion

- **As NASA plans to…**
  - return to the lunar surface,
  - develop sustainable lunar habitation, and
  - prepare to explore Mars

- **Space Biology intends to…**
  - utilize multiple biological model systems and
  - spaceflight platforms

Ø **To understand the mechanisms of change in biological systems in response to long duration exposure to deep space,**

Ø **To enable exploration,**

Ø **To benefit life on Earth: human health & controlled environment agriculture.**

# Deep Dive

# Thoughts/Concerns:

1. We do need to continue the capability to do animal and plant research in low Earth orbit even after the ISS is phased out since it will be a while before we can conduct complex experiments (e.g., rodent research etc.) beyond LEO.
2. Ground research will continue to be important alongside flight research as we test the effects of multiple deep-space stressors on different platforms on the ground as well as in flight.
3. It will be important to develop the capabilities to conduct long duration experiments in the lunar environment in preparation for Mars missions.
4. We need to grow as a program to be able to start taking full advantage of deep-space exploration opportunities.

# Questions:

1. **How do we continue to keep young folk/new entrants engaged in Space Biology? NASA has been successful with STEM approaches for graduate fellowships and postdoctoral fellowships, but after that, young scientists often need to leave the field because of the lack of opportunities to practice Space Biology in the larger science community.**
   a) How do we continue to sustain opportunities for these young folk and retain them in the SB field? [Grow community effectively]
   b) How do we engage universities to highlight and develop programs in Space Biology? (Develop curricula etc.)

2. **Do you have new ideas to engage citizen scientists in Space Biology goals and continue to maintain the scientific merit of the investigations?**
   - One effective way we are using citizen science is by standing up the GeneLab Analysis Working Group (GL AWG).

   *[The Public Law states citizen Science is defined as a form of open collaboration in which individuals or organizations participate voluntarily in the scientific process in various ways (P.L. No. 114-329).* Per NASA's policy document *SPD-33, SMD citizen science projects shall be held to the same rigorous standards as any SMD science program. ]*

3. **Given the high priority for IDEA, diversity, and inclusion within NASA and SMD, are there unique/new ideas to help diversify those proposing to our grants?**

# Thank you!

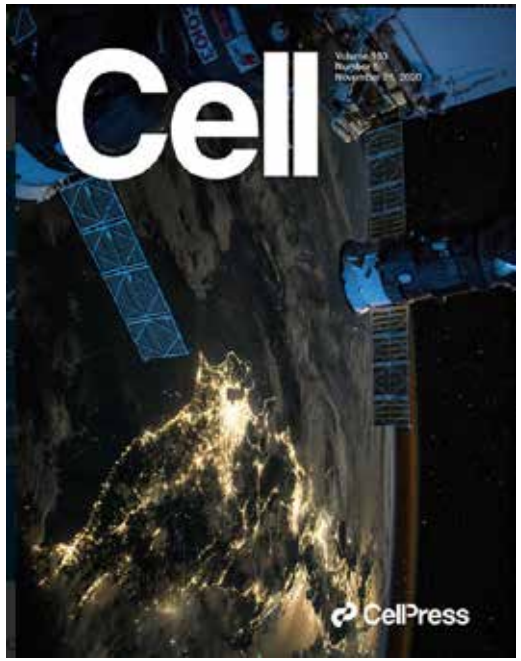
Anthony Hickey, Lisa Carnell, Mamta Nagaraja, Howard Levine, Parag Vaishampayan, Denise Freeland, Elizabeth Keller, Sylvain Costes, Lauren Sanders, Graham Lau, Siddhita Mhatre, Trudy Mackay, Gioia Massa, Ray Wheeler, Ye Zhang, Bruce Link. I am grateful to all of them for their contributions, as well as to those who are unnamed here.

# Recent Science Highlights

# Cell Press Journals Publish the BIOLOGY OF SPACEFLIGHT

*A special collection of papers on **the biology of spaceflight**, published in Cell and other Cell Press journals on November 25th, 2020, as a collaboration between NASA and other space agencies around the world.*

- **29** scientific papers, **200+ authors**
- **9 papers utilize data in GeneLab or GeneLab resources**
- *Covered by **200 News Articles worldwide***
- *https://www.cell.com/c/the-biology-of-spaceflight*



# *Comprehensive Multi-omics Analysis Reveals Mitochondrial Stress as a Central Biological Hub for Spaceflight Impact*

da Silveira WA, Fazelinia H, Rosenthal SB, Laiakis EC, Kim MS, Meydan C, Kidane Y… **Mason CE, Costes SV, Beheshti A**

*Cell 2020 Nov; 183(5):1185-1201.e20.* DOI: 10.1016/j.cell.2020.11.002

## *Background/Objectives/Methodology*

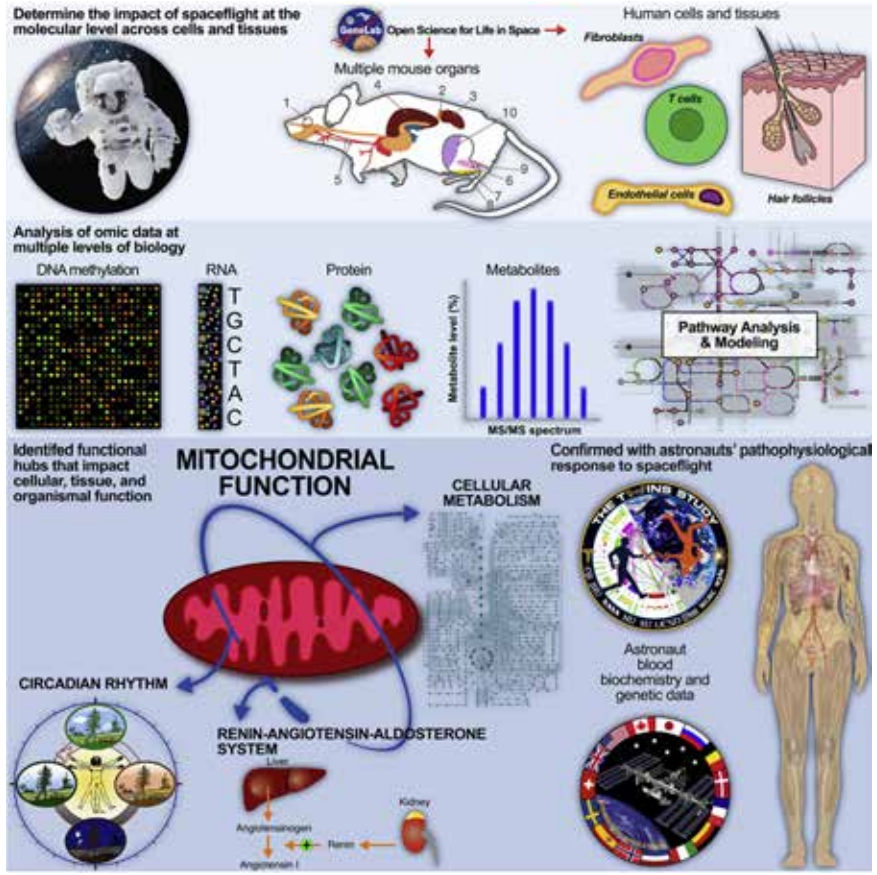
- Multi-omics analysis and techniques with NASA's GeneLab platform
- The largest cohort of astronaut data to date utilized for analysis

## *Key Results*

- Overall pathway analyses on the multi-omics datasets showed significant enrichment for mitochondrial processes, as well as innate immunity, chronic inflammation, cell cycle, circadian rhythm, and olfactory functions.
- Evidence of altered mitochondrial function and DNA damage was also found in the urine and blood metabolic data compiled from the astronaut cohort and NASA Twin Study data, indicating mitochondrial stress as a consistent phenotype of spaceflight
- NASA Twin Study data validates mitochondrial dysfunction during space missions

## *Relevance/Impact*

- Evidence of altered mitochondrial function and DNA damage was also found in the urine and blood metabolic data compiled from the astronaut cohort and NASA Twin Study data, indicating **mitochondrial stress as a consistent phenotype of spaceflight**.



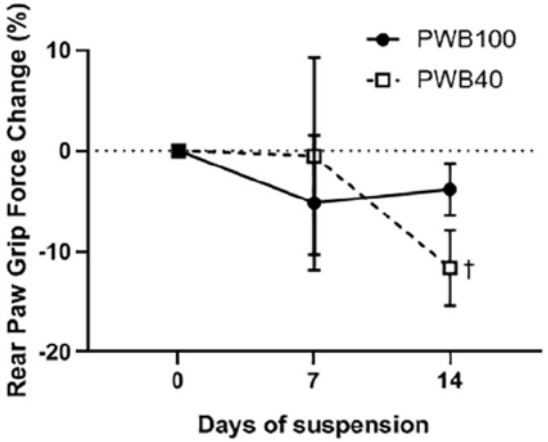

A comprehensive multi-omics analysis from 59 astronauts and hundreds of samples flown in space provides insight into fundamental biological mechanisms affected by spaceflight and highlights mitochondrial dysregulation as a central hub for space biology

# Application of a Partial Weight-Bearing model in Male and Female Rats

## *Partial Weight-Bearing in Female Rats: Proof of Concept in a Martian-Gravity Analog*

Semple C, Riveros D, Nagy JA, **Rutkove SB, Mortreux M,**

*Front. Physiol., 2020 April;* https://doi.org/10.3389/fphys.2020.00302




### *Background/Objectives/Methodology*

- The first use of quadrupedal partial weight-bearing (PWB) in female rats, which demonstrates the feasibility of partial gravity analogs in females and allows for future investigations about the impact of sex on muscle deconditioning due to reduced mechanical loading.
- Rats exposed to 2 weeks at normal loading or 40% of their normal loading (PWB40), corresponding to Martian gravity-analog

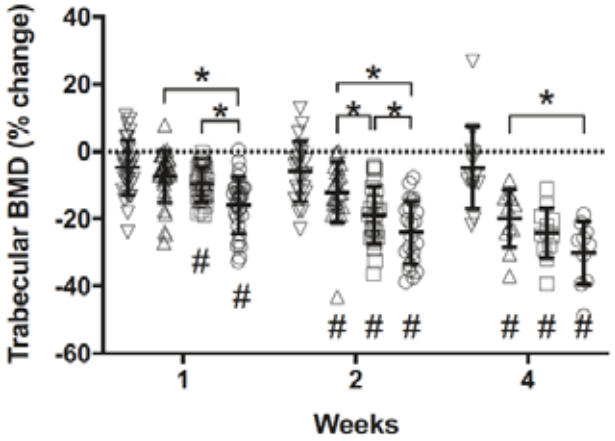
### *Key Results*

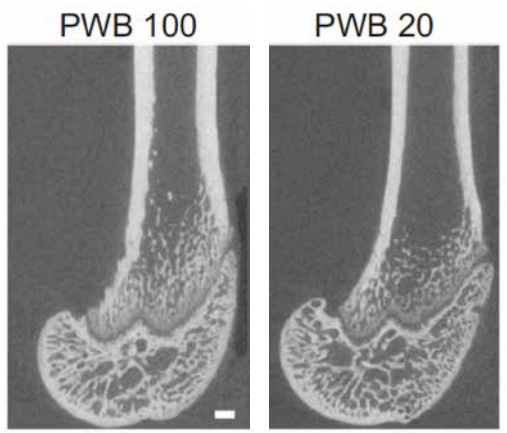
- Females exposed to PWB for 14 days showed an 11.62% decline in hindlimb grip force associated with an 11.84% decrease in soleus myofiber size.
- Rats under PWB maintained normal blood oxygenation and stress levels.

## *Dose-dependent skeletal deficits due to varied reductions in mechanical loading in rats*

*Ko FC, Mortreux M, Riveros D, Nagy JA, Rutkove SB,* ***Bouxsein ML***

*npj Microgravity (2020) May; 6(15);* https://doi.org/10.1038/s41526-020-0105-0




### *Background/Objectives/Methodology*

- Determined the structural and functional skeletal alterations in 14-week-old male Wister rats exposed to 20%, 40%, 70%, or 100% of body weight for 1, 2, or 4 weeks

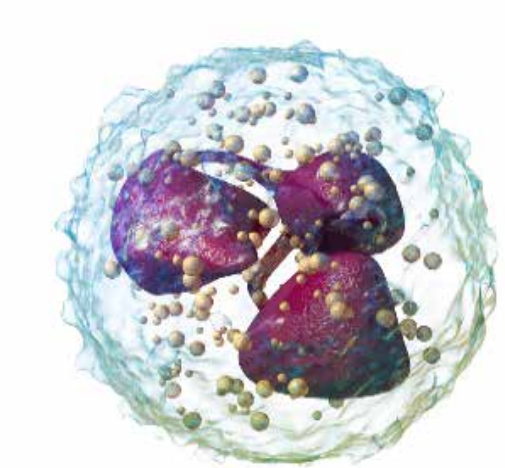
### *Key Results*

- Found trabecular bone density at the proximal tibia declined in proportion to the degree of unloading and continued progressively with time, without evidence of a plateau by 4 weeks.
- Analyses of trabecular bone in the distal femur revealed the decreased osteoblast number and mineralizing surface in unloaded rats.
- Found modest or no reductions in femoral stiffness and estimated modulus due to PWB.

# *Neutrophil to Lymphocyte Ratio: A Biomarker to Monitor the Immune Status of Astronauts*

**Paul A**, Mhatre S, Cekanaviciute E, Schreurs A, Tahimic C, Globus R, Ananad S, Crucian B, **Bhattacharya S**.

*Front. Immunol. 2020 Nov;* | https://doi.org/10.3389/fimmu.2020.564950



## *Background/Objectives/Methodology*

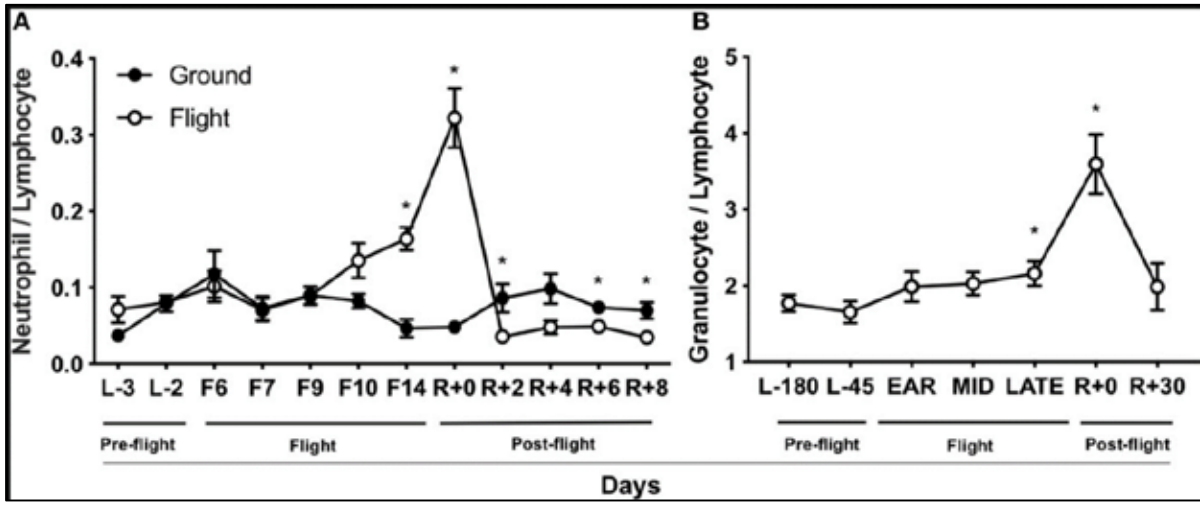
- Previous research has shown that **leukocyte differentials are altered** during spaceflight
- Simulated microgravity conditions; cultured human whole blood-leukocytes were grown in a high-aspect rotating wall vessels (HARV-RWV), plus hindlimb unloaded (HU) mice
- Both HARV-RWV simulation of leukocytes and HU-exposed mice showed **elevated NLR profiles comparable to spaceflight exposed samples**

## *Key Results*

- Results indicate an imbalance of redox processes and activation of inflammatory mechanisms, while treatment with an antioxidant treatment ***reversed*** these effects.

## *Relevance/Impact*

- These findings suggest that simulated microgravity induces oxidative stress responses that triggered inflammation and are an indicator that **an elevated neutrophil-to-lymphocyte ratio (NLR) is a potential biomarker candidate**.




Spaceflight elevates NLR and GLR. **(A)** Rodent NLR from Space Life Sciences (SLS)-2 mission (n = 5–15). **(B)** Human GLR from published data (n = 23). L, launch; F, flight; R, return on Earth denoted in days. “Early”, day 14 in-flight; “mid,” days 60–120 in-flight; and “late,” day 180.

# *Prolonged Exposure to Microgravity Reduces Cardiac Contractility and Initiates Remodeling in Drosophila*

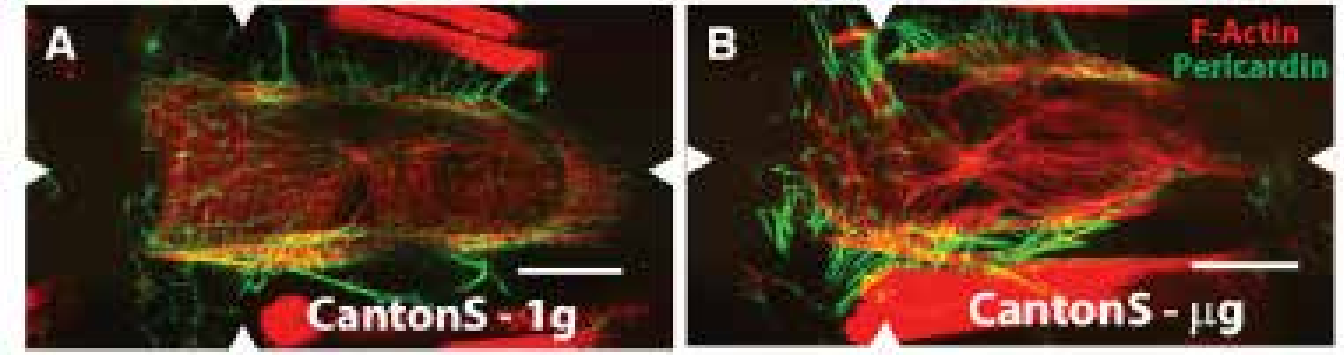
Walls S, Diop S, Birse R, Elmen L, Gan Z, Kalvakuri S, Pineda S, Reddy C, Taylor E, Trinh B, Vogler G, Zarndt R, McCulloch A, Lee P, Bhattacharya S, **Bodmer R, Ocorr K.**

*Cell Rep.2020 Dec; 33(10):108445.* doi: 10.1016/j.celrep.2020.108445

## *Background/Objectives/Methodology*

- Understanding the effects of microgravity on human organs is crucial to exploration of low-earth orbit, the moon, and beyond.
- *Drosophila* can be sent to space in large numbers to examine the effects of microgravity on heart structure and function, which is fundamentally conserved from flies to humans.

Cross section of one chamber of *Drosophila* heart




Ground control | Spaceflight

## *Key Results*

- Exposure to microgravity aboard the ISS causes heart dysfunction in fly cardiac model (2 spaceflight missions, F1 generation bred entirely in space).
- Hearts are smaller, less contractile, and exhibit changes in genes and proteins that maintain heart structure and function.
- Myofibrillar and extracellular matrix (ECM) disorganization seen from spaceflight.
- Heart defects correlate with reduced sarcomeric and extracellular matrix gene expression.
- Increase in proteasome gene expression and increase proteasome aggregates in females in response to spaceflight.

## *Relevance/Impact*

- Results indicate that cardiac remodeling and proteostatic stress may be a fundamental response of heart muscle to microgravity.

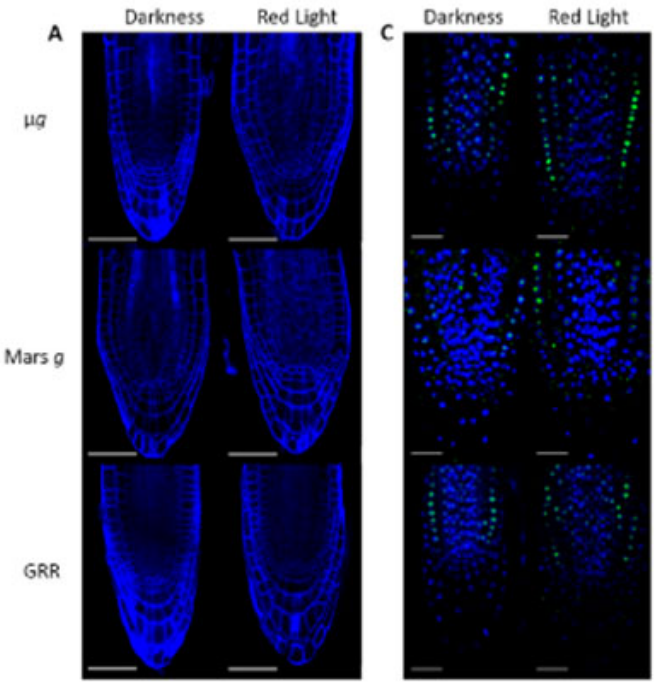
# *From Spaceflight to Mars g-Levels: Adaptive Response of A. Thaliana Seedlings in a Reduced Gravity Environment Is Enhanced by Red-Light Photostimulation*

Villacampa A, Ciska M, Manzano A, Vandenbrink JP, **Kiss JZ,** Herranz R,, Medina FJ

*Int. J. Mo.l Sci. 2021 Jan 22(2): 899.* Doi: 10.3390/ijms22020899

## *Background/Objectives/Methodology*

- The response of plants to the spaceflight environment and microgravity is still not well understood. Even less is known about plants' response to partial or reduced gravity levels.
- In the absence of the directional cues provided by the gravity vector, the plant is especially perceptive to other cues such as light.
- The response of *Arabidopsis thaliana* 6-day-old seedlings to microgravity and a Mars analogue, partial-gravity level during spaceflight, as well as red-light photostimulation, was examined.
- These experiments involve microscopic techniques together with transcriptomic studies




Confocal microscope images of cell-wall-stained root meristems of the different gravity and photostimulation conditions. Scale bar represents 50 µm.

## *Key Results*

- Plants respond differently to microgravity and partial gravity. Microgravity activates hormonal routes responsible for growth and proliferation, even when grown in darkness.
- In contrast, the Mars-analog, partial-gravity level inhibits these same routes and activates responses to stress factors to restore cell growth parameters, but only when red-light photostimulation is provided.

## *Relevance/Impact*

- Findings from this research indicate that in the absence of the directional cues provided by the gravity vector, the plant is especially perceptive to other cues such as light.
- In the long term, these discoveries can be applied to the design of bioregenerative life support systems and space farming.

# *Growth and Antifungal Resistance of the Pathogenic Yeast, Candida albicans, in the Microgravity Environment of the International Space Station: An Aggregate of Multiple Flight Experiences*

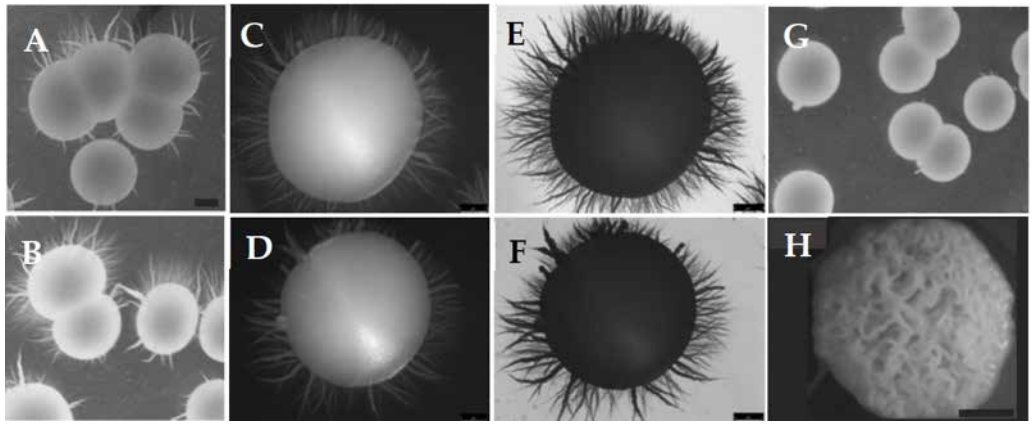
**Nielsen S**, White K, Preiss K, Peart D, Gianoulias K, Juel R, Sutton J, McKinney J, Bender J, Pinc G, Bergren K, Gans W, Kelley J, McQuaid M.
*Life* (Basel). 2021 Mar ;11(4):283. DOI: https://doi.org/10.3390/life11040283

## *Background/Objectives*

- Astronauts continue to be plagued with yeast infections from the common human pathogen *Candida albicans* that have been difficult to treat in space
- *C. albicans* samples were analyzed across Micro-6 and Micro-14 flight experiments.

## *Key Results*

- Findings from both of these studies indicate yeast cells cultivated in microgravity **demonstrated a subset of characteristics associated with virulence**
- Yeast cultures also demonstrated an **increased resistance to the antifungal agent amphotericin B** when challenged during spaceflight. Similar levels of resistance were not observed when challenged with the functionally disparate antifungal drug caspofungin.




Colony morphology and size evaluated in yeast samples returned live. Following each mission, cells were isolated immediately following hardware de-integration, counted, diluted, and spread on YPD agar plates.

## *Relevance/Impact*

- This research examines the underlying mechanisms that drive this opportunistic pathogen to become more virulent in space, as well as tests treatment methods that may be effective at protecting the astronauts from yeast infections.

# *Modeled microgravity alters lipopolysaccharide and outer membrane vesicle production of the beneficial symbiont Vibrio fischeri*

Vroom MM, Rodriguez-Ocasio Y, Lynch JB, Ruby EG, **Foster JS**

*NPJ Microgravity 2021 Mar.; 7(8) (2021).* https://doi.org/10.1038/s41526-021-00138-8

**Space Biology grant:** Effects of Modeled Microgravity on the Induction of Bacteria-Induced Apoptosis During Animal Development

## *Background/Objectives/Methodology*

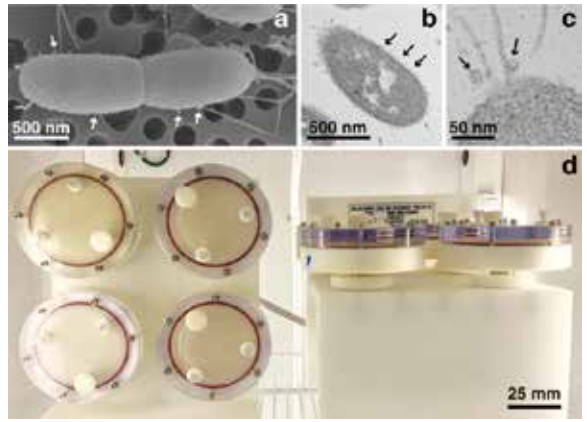
- Reduced gravity, or microgravity, can have a pronounced impact on the physiology of animals, but the effects on their associated microbiomes are not well understood.
- This study examines the impact of modeled microgravity on shedding of Gram-negative lipopolysaccharides (LPS) by the symbiotic bacterium *Vibrio fischeri* using high-aspect ratio vessels.
- LPS from *V. fischeri* induces developmental apoptosis within its symbiotic tissues, which is accelerated under modeled microgravity conditions.

## *Key Results*

- Exposure to modeled microgravity increases amount of LPS released by bacterial symbiont in vitro.
- Higher rates of shedding under modeled microgravity conditions are associated with increased production of outer-membrane vesicles (OMV), which has been previously correlated to flagellar motility.
- Mutants of *V. fischeri* defective in the production and rotation of their flagella show significant decreases in LPS shedding in all treatments, but levels of LPS are higher under modeled microgravity despite loss of motility.
- Modeled microgravity also appears to affect the outer-membrane integrity of *V. fischeri*, as cells incubated under modeled microgravity conditions are more susceptible to cell-membrane-disrupting agents.

## *Relevance/Impact*

- These results suggest that, like their animal hosts, the physiology of symbiotic microbes can be altered under microgravity-like conditions, which may have important implications for host health during spaceflight.




Overview of the beneficial microbe *Vibrio fischeri* morphology and experimental conditions. a Scanning electron micrograph of the wild-type *V. fischeri* depicting the presence of outer-membrane vesicles (OMVs) on the cell surface (arrows). b Transmission electron micrograph (TEM) of *V. fischeri* during exponential growth producing numerous OMVs during exponential-phase growth. c Higher magnification TEM visualizing OMVs associated with the bacteria flagella. d Rotary cell culture system with high-aspect ratio vessels containing *V. fischeri* cultures in the modeled microgravity (left) and gravity (right) control positions.

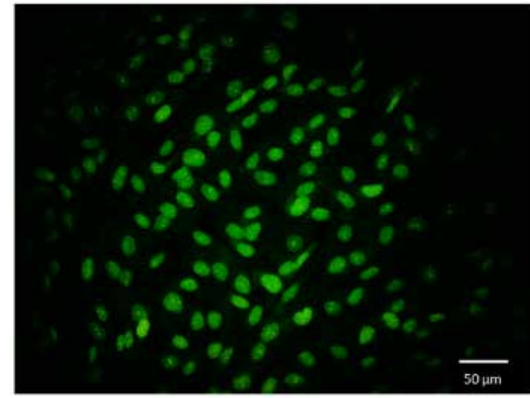
# *Module to Support Real-Time Microscopic Imaging of Living Organisms on Ground-Based Microgravity Analogs* (Postdoc)

Neelam S, Lee A, Lane MA, Udave C, Levine HG. Zhang, Y.

*Applied Sciences. 2021 April; 11(7), 3122;* https://doi.org/10.3390/app11073122

## *Background/Objectives/Methodology*

- Ground-based microgravity simulation devices (MSDs) offer accessible and economical alternatives for gravitational biology studies.
- The random positioning machine (RPM) MSD provides simulated microgravity conditions by randomizing rotating biological samples in two axes to distribute the Earth's gravity vector in all directions over time.
- To study basic cell functions (cell division, migration, proliferation) during microgravity simulation, real-time microscopy and image acquisition are required.
- These capabilities have been difficult to implement due to the constantly moving frames of the RPM as well as mechanical noise.
- The KSC Microgravity Simulation Support Facility (MSSF) team developed an image acquisition module and mounted it on an RPM to capture live images over time while the specimen is in simulated microgravity.
- The module includes a digital microscope (magnification range 20x to 700x), a high-speed data transmission adaptor for wireless streaming of time-lapse images, and a backlight illuminator to view the sample under brightfield and darkfield modes.




Fluorescent nuclei of AG01522 cells in SMG as captured using the live-imaging module.

## *Key Results*

- Using this module, the team successfully demonstrated real-time imaging of human cells cultured on an RPM in brightfield mode over an 8 hour interval and using the green fluorescent channel.
- This module was successful in monitoring cell morphology and quantifying the cell division rate, cell migration, and wound healing.

# *Fusarium oxysporum* as an Opportunistic Fungal Pathogen on *Zinnia hybrida* Plants Grown on board the International Space Station

Schuerger AC, Amaradasa BS, Dufault NS, Hummerick, ME. Richards JT, Khodadad CL, Smith, TM Massa GD.

*Astrobiology, 2021 Apr; 21(9),* https://doi.org/10.1089/ast.2020.2399

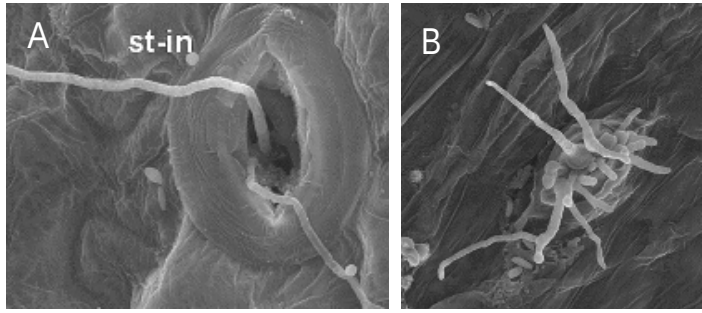
## *Background/Objectives/Methodology*

- ISS Veggie-grown *Zinnia hybrida* developed chlorosis, leaf curling, fungal growth, leaf/stem damage, and necrosis.
- Symptoms correlated to reduced airflow causing water buildup on leaves and stems in microgravity.
- Tissues were returned to Earth to determine primary causal agent of disease.
- Presumptive pathogen identified as *Fusarium oxysporum* by microconidia and conidiophore morphology.
- Germinating conidia penetrated stomata and infected host tissues.
- Ground-based pathogenicity assays exposed healthy *Z. hybrida* plants to reduced airflow and high-water stress and compared them to unstressed plants.

Under high magnification with a dissecting microscope, conidiophores and spores (arrows) observed on all infected tissues.

## *Key Results*

- Symptoms only matched ISS-flown symptomatic tissues when plants were stressed with high-water exposure.
- Unstressed plants grown under similar conditions failed to develop symptoms observed with plants on ISS.
- Pathogenicity test results imply *F. oxysporum* acted as opportunistic pathogen on severely high-water stressed plants.




SEM images of fixed *Z. hybrida* tissues returned from ISS and infected with *F. oxysporum*. (**A**) Mycelium-stomate infection points (st-in) on healthy leaf tissues exposed to the airborne microconidia of *F. oxysporum* during flight. (**B**) Fungal mycelium emerging from stomates on upper and lower surfaces of infected leaves exhibiting advanced states of decay.

# *Investigation of Spaceflight Induced Changes to Astronaut Microbiomes*

Morrison MD, Thissen JB, Karouia F, Mehta S, Urbaniak C, Venkateswaran K, Smith DS, **Jaing C.**

*Front. Microbiol. 2021 June.* https://doi.org/10.3389/fmicb.2021.659179

## *Background/Objectives/Methodology*

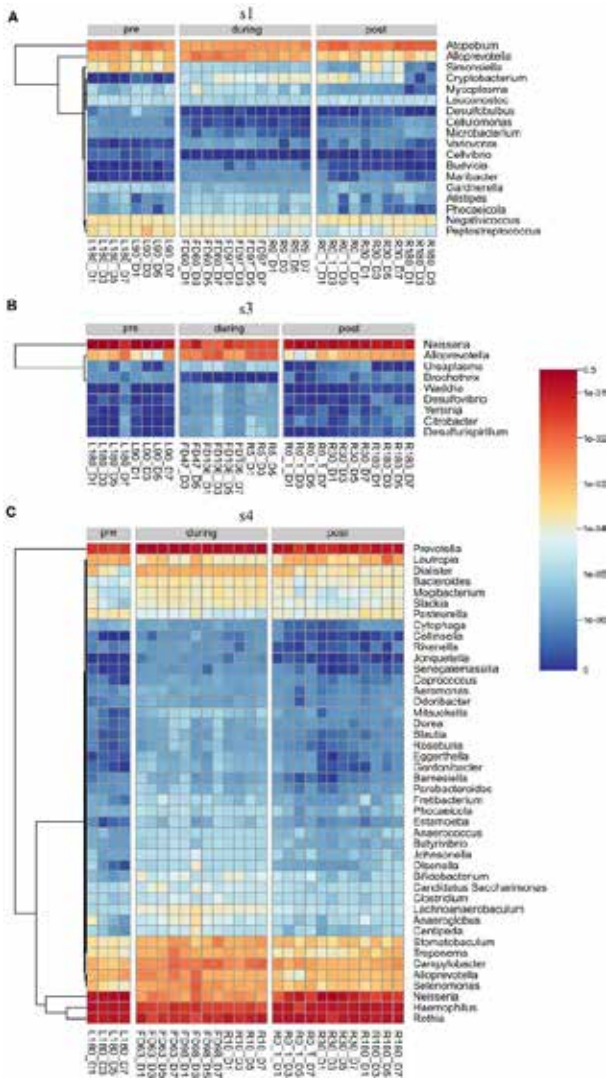
- Disruptions in the human microbiome due to exposure to space could potentially cause illness in astronauts that may be difficult to treat.
- To evaluate the effects of spaceflight on the human microbiome, body swabs and saliva samples were collected from four ISS astronauts on consecutive expeditions. Samples were analyzed to characterize the microbial biodiversity before, during, and after the astronauts' time onboard the International Space Station (ISS).

## *Key Results*

- There were some changes in each astronaut's microbiome during spaceflight, but these changes were not universal for all four astronauts.
- Two antimicrobial resistance gene markers did show a significant change in abundance in the saliva samples of all four astronauts across their collection times.

## *Relevance/Impact*

- This study was the **first** to successfully use genetic techniques (metagenome sequencing and microarrays) to identify microbes in the various sampling locations from the astronauts.
- Results from this study provide insight for future ISS microbial monitoring studies and targets for antimicrobial resistance screenings.
- The goal of the study was to characterize astronaut's microbiome changes due to spaceflight conditions and identify any changes that may pose health risks to the astronauts during their mission. Tracking the entire crew could help identify the changes that are introduced from crew interactions, which will enable better characterization of the microbiome variations due to the environmental stresses of spaceflight.

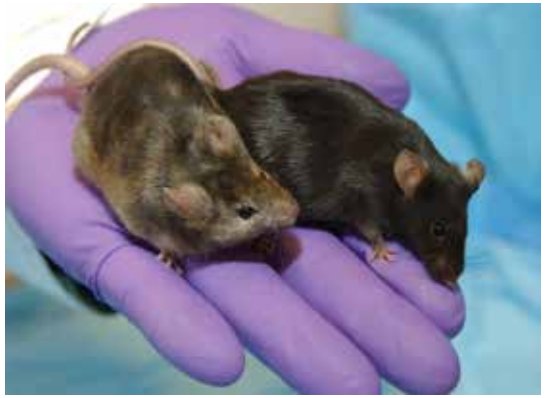



Heatmaps showing the relative abundance of genera identified as differentially abundant in Astronaut 1 (A), Astronaut 3 (B), and Astronaut 4 (C).

# *Evidence for increased thermogenesis in female C57BL/6J mice housed aboard the International Space Station*

Wong CP, Iwaniec UT, **Turner RT.**

*npj Microgravity 2021 June; 7(23).* https://doi.org/10.1038/s41526-021-00150-y



## *Background/Objectives/Methodology*

- The recommended temperature for housing mice in a laboratory setting (20–26 °C) is well below their thermoneutral zone, which depending upon strain, sex and age typically ranges from 29–31 °C
- The physiological demands required for successful adaptation to subthermoneutral housing by mice are considerable. Female mice housed at room temperature (22 °C) consumed 40% more food to achieve comparable weight gain and expressed 5-fold higher Ucp1 gene expression in brown adipose tissue (BAT).
- The activation of adaptive thermogenesis in mice housed in microgravity may introduce unrecognized and uncontrolled for confounding variables into spaceflight studies
- The effect of spaceflight on the expression of genes related to energy metabolism in BAT and white adipose tissue in female C57BL/6J mice were examined (compared to ground controls).

## *Key Results*

- Transcript abundance of 13/84 genes were significantly altered in flight animals compared to ground controls. Mice aboard ISS had 1.5x higher levels of Ucp-1 in BAT, providing direct evidence for elevated non-shivering thermogenesis.
- Authors conclude that, in spite of comparable housing temperatures, adaptive thermogenesis is increased in BAT of mice housed aboard the ISS compared to ground controls

## *Relevance/Impact*

- Increased thermogenesis may exaggerate (e.g., bone loss) or alter (e.g., response to ionizing radiation) physiological responses to spaceflight in mice. Because of species specific differences in thermoregulation, this could impact the translatability of the animal studies to astronauts.

# *Evaluating the Lettuce Metatranscriptome with MinION Sequencing for Future Spaceflight Food Production Applications* (Postdoc)

**Haveman NJ,** Khodadad CLM, Dixit AR, Louyakis AS, Massa GC, **Venkateswaran K, Foster JS**

*npj Microgravity, 2021, June, 7(22) (2021).* https://doi.org/10.1038/s41526-021-00151-x

## *Background/Objectives/Methodolgy*

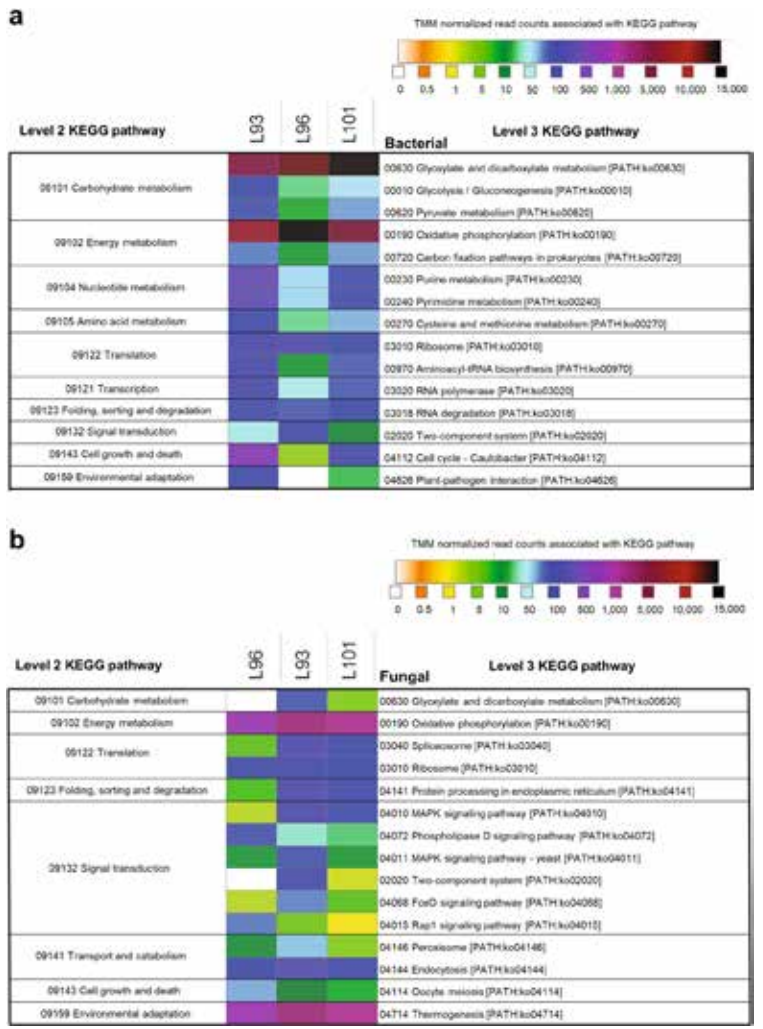
- Healthy plants are vital for successful, long-duration missions in space, as they provide the crew with life support, food production, and psychological benefits.
- Plants have complex and dynamic relationships with their microorganisms, which are influenced by various stresses and environmental factors, including spaceflight.
- The microorganisms that associate with plant tissues play a critical role in improving plant health and production.
- A methodology was developed to investigate the transcriptional activities of the microbiome of red romaine lettuce, grown under ISS-like conditions.
- Microbial transcripts enriched from host–microbe total RNA were sequenced using the Oxford Nanopore MinION sequencing platform.

## *Key Results*

- Results show that this enrichment approach was highly reproducible and could be an effective approach for the on-site detection of microbial transcriptional activity.

## *Relevance/Impact*

- Results demonstrate the feasibility of using metatranscriptomics of enriched microbial RNA as a potential method for on-site monitoring of the transcriptional activity of crop microbiomes, thereby helping to facilitate and maintain plant health for on-orbit space food production.




Heatmap showing the top 15 distribution of protein-coding reads matching KEGG pathways in the (a) bacterial and (b) fungal community. The color scale shows the TMM-normalized read counts associated with each pathway.

# *Detection of Genes in Arabidopsis thaliana L. Responding to DNA Damage from Radiation and Other Stressors in Spaceflight* (EPSCoR Rapid Research Response Selection)

**Manian V,** Orozco-Sandoval J, Diaz-Martinez V.

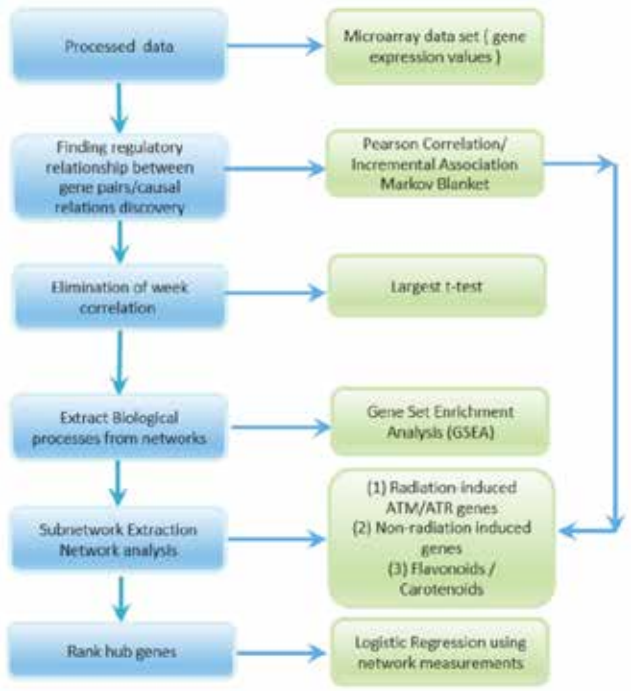
*Genes (Basel).2021June;12 (6):938.* 10.3390/genes12060938

## *Background/Objectives/Methodology*

- Plants will be a major component of advanced life support systems to enable deep space exploration that will take humans to the moon, Mars and beyond.
- In a newly-published paper, scientists analyzed plant datasets from real and simulated spaceflight studies to identify the transcription factors involved in the response to radiation and other stressors.
- GeneLab datasets were analyzed for this study.
- Advanced network analysis and machine learning approaches were used to detect key genes involved in DNA damage response (DDR) and their links with genes involved in spaceflight stress responses in the model plant *Arabidopsis thaliana.*

## *Key Results*

- Findings indicate that DDR processes are intermixed with the regulation of critical functions necessary for the plant to adapt to the spaceflight environment.
- Methods used revealed non-radiation-induced genes linking DNA damage response to root growth and plant development.
- Eighteen radiation-induced genes and sixteen non-radiation-induced gene players have been identified from the ATR/ATM protein interaction complexes involved in heat, salt, water, osmotic stress responses, and plant organogenesis




Flow diagram showing the sequence of steps followed for extraction of radiation response subnetworks from the gene expression values in the GLDS datasets and network analysis

## *Relevance/Impact*

- Organisms in space are exposed to radiation, which affects the health of humans and the anatomy and growth of plants and animals. Plants are affected by radiation resulting in damage to cellular components and damage to DNA
- Exposure to radiation causes DNA lesions in plant cells such as single-stranded breaks, Double Strand Breaks (DSB), mismatches, and modified bases. This leads to DNA Damage Response (DDR) that includes signal transduction, triggering either DNA repair, cell survival, or cell death.

# *Overexpression of catalase in mitochondria mitigates changes in hippocampal cytokine expression following simulated microgravity and isolation*

Rubinstein L, Schreurs AS, Torres SM, Steczina S, Lowe MG, Kiffer F, Allen AR, Ronca AE, Sowa MB, **Globus RK, Tahimic CGT.**

*NPJ Microgravity. 2021 Jul 6;7(1):24. doi: 10.1038/s41526-021-00152-w*

## *Background/Objectives/Methodology*

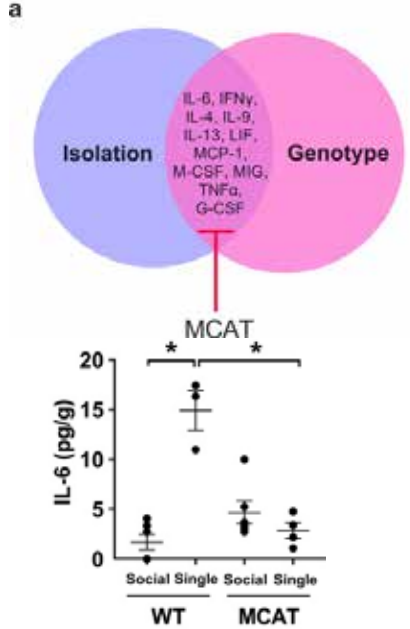
- Isolation is an important feature of the spaceflight environment. On Earth, isolation has profound effects on the central nervous system (CNS) including behavioral changes, upregulated oxidative stress pathways, neuroinflammatory responses, as well as brain cytokine alteration.
- In this study, the effects of isolation and simulated microgravity solely or combined, on the inflammatory cytokine milieu of the hippocampus were determined.
- Adult female wild-type mice underwent simulated microgravity by hindlimb unloading (HU) for 30 days in single or social (paired) housing. This study included transgenic mice that overexpressed the human mitochondrial catalase gene (MCAT) as a model for mitochondrial reactive oxygen species (ROS) quenching.
- Left-brain and hippocampal tissue was analyzed for cytokine expression and markers or DNA damage, while collected plasma was analyzed. Unbiased hierarchical clustering of cytokine protein expression was used.

## *Key Results*

- Isolation had a more pronounced impact than HU on the expression of cytokines in the hippocampus, as indicated by differential expression of 11 cytokines as a result of isolation versus 5 cytokines due to HU. In addition, HU under single and social housed conditions resulted in distinct sets of differentially expressed cytokines in the hippocampus.
- Isolation-induced cytokine responses were more extensive in the hippocampus compared to the plasma
- MCAT mice exhibited protection from the hippocampal cytokine changes caused by isolation, simulated microgravity, and in combination. In plasma, HU-induced changes in cytokine expression were mitigated in MCAT mice.

## *Relevance/Impact*

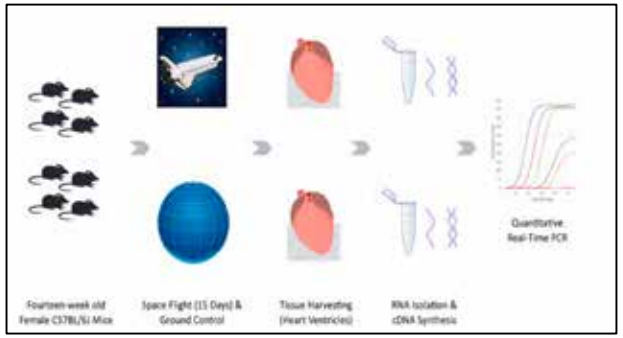
- These findings suggest a key role for mitochondrial ROS signaling in neuroinflammatory responses to spaceflight and prolonged bedrest, isolation, and confinement on Earth.
- These findings, and those of others, provide a rationale for the use of antioxidant-based approaches to address anticipated CNS changes during spaceflight and in situations of isolation and reduced mobility on Earth.




**Cytokines showing higher expression in hippocampus due to isolation and mitigation in MCAT mice**. A 11 out of 44 cytokines were upregulated in wild-type single housed vs social-housed mice and mitigated in MCAT single housed mice. B. Representative graph (IL-6) of one of the 11 cytokines elevated due to isolation and mitigated in MCAT mice. The longer horizontal line in the dot plot corresponds to the group mean while the shorter horizontal lines depict the standard error (SE). Sample sizes are WT NL social (n = 6), WT NL single (n = 3), MCAT NL social (n = 6), and MCAT NL single (n = 4). A fit model was generated to include housing state, genotype, loading state, and their pairwise interactions. Interaction effects between housing state and genotype were assessed at $p < 0.05$. *Statistically significant at $p < 0.05$ by Tukey post hoc test.

# *Spaceflight Modulates the Expression of Key Oxidative Stress and Cell Cycle Related Genes in Heart*

Kumar A, Tahimic, CGT, Almeida EAC, **Globus RK.**
*Heart. Int J Mol Sci. 2021 Aug: 22(16):9088. doi*: *10.3390/ijms22169088*




Schematic diagram of experiment design and workflow.

## *Background/Objectives/Methodology*

- Spaceflight causes cardiovascular changes due to microgravity-induced redistribution of body fluids and musculoskeletal unloading.
- Cardiac deconditioning and atrophy on Earth are associated with altered Trp53 and oxidative stress-related pathways, but the effects of spaceflight on cardiac changes at the molecular level are less understood.
- Investigators test the hypothesis that spaceflight alters the expression of key genes related to stress response pathways, which may contribute to cardiovascular deconditioning during spaceflight.
- Mice were exposed to spaceflight (FLT) for 15 days or maintained on Earth (GRD). Ventricle tissue was harvested starting ~3 h post-landing. The expression of select genes implicated in oxidative stress pathways and Trp53 signaling was measured by quantitative PCR.

## *Key Results*

- Cardiac expression levels of 37 of 168 genes tested were altered after spaceflight.
- Spaceflight downregulated transcription factor, Nfe2l2 (Nrf2), upregulated Nox1 and downregulated Ptgs2, suggesting a persistent increase in oxidative stress-related target genes.
- Spaceflight also substantially upregulated *Cdkn1a* (p21) and cell cycle/apoptosis-related gene *Myc*, and downregulated the inflammatory response gene *Tnf.*
- There were no changes in apoptosis-related genes such as Trp53.mice.

## *Relevance/Impact*

- This study revealed that spaceflight significantly altered cardiac expression of genes related to cell cycle/growth, inflammation, and oxidative stress, which may contribute to cardiac dysfunction.
- These findings provide novel data suitable for developing testable hypotheses for further study.
- Identification of specific mechanisms and molecules responsible for excess ROS generation during spaceflight is of considerable interest, and may yield new therapeutic approaches.

# Other Resources

- **Bhattacharya Presentation at the Committee on Biological and Physical Sciences in Space - 2020 Fall Virtual Meeting (Oct. 27, 2020) can be accessed here.**