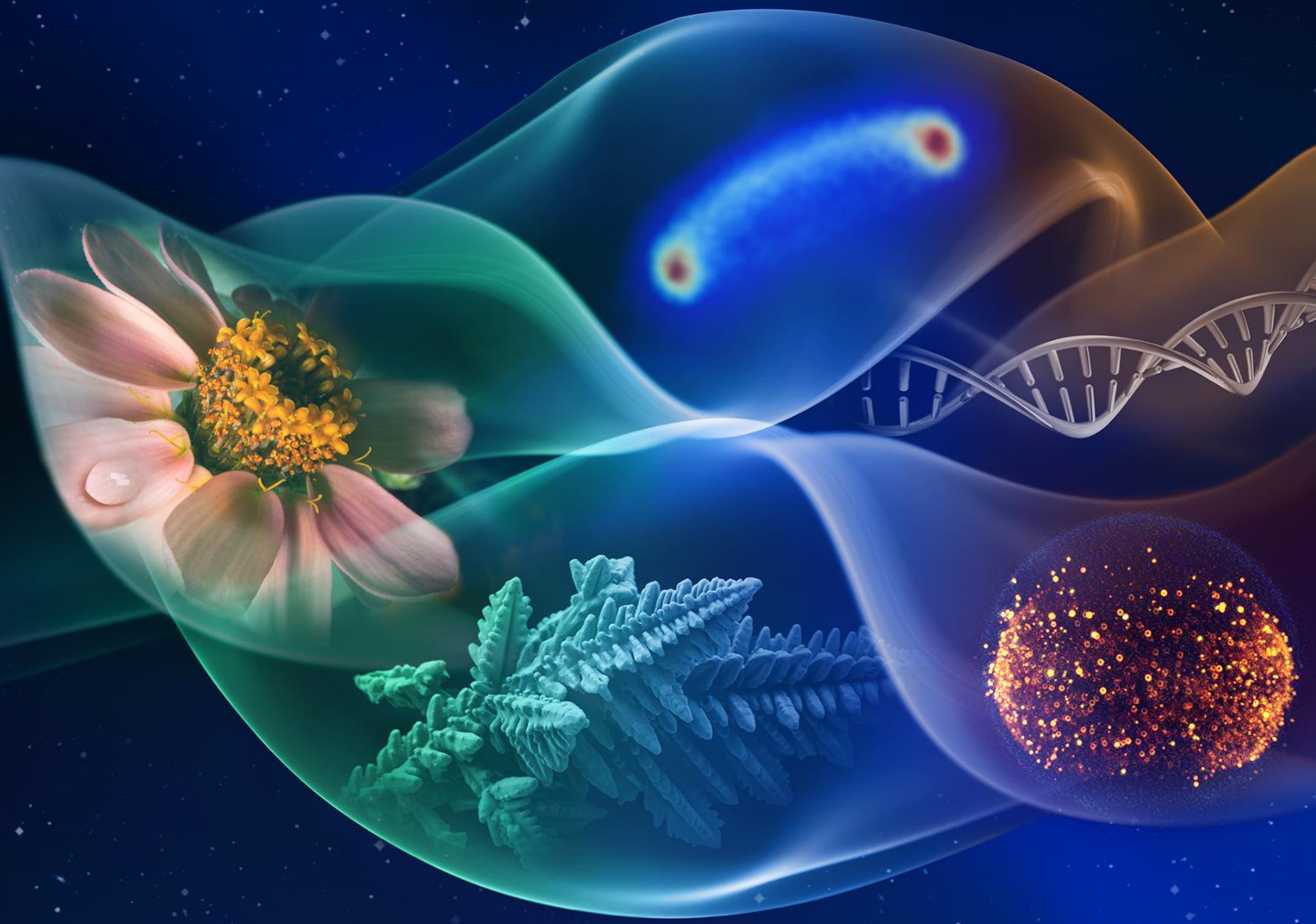
National Aeronautics and
Space Administration

Biological and Physical Sciences



SPACE BIOLOGY PROGRAM

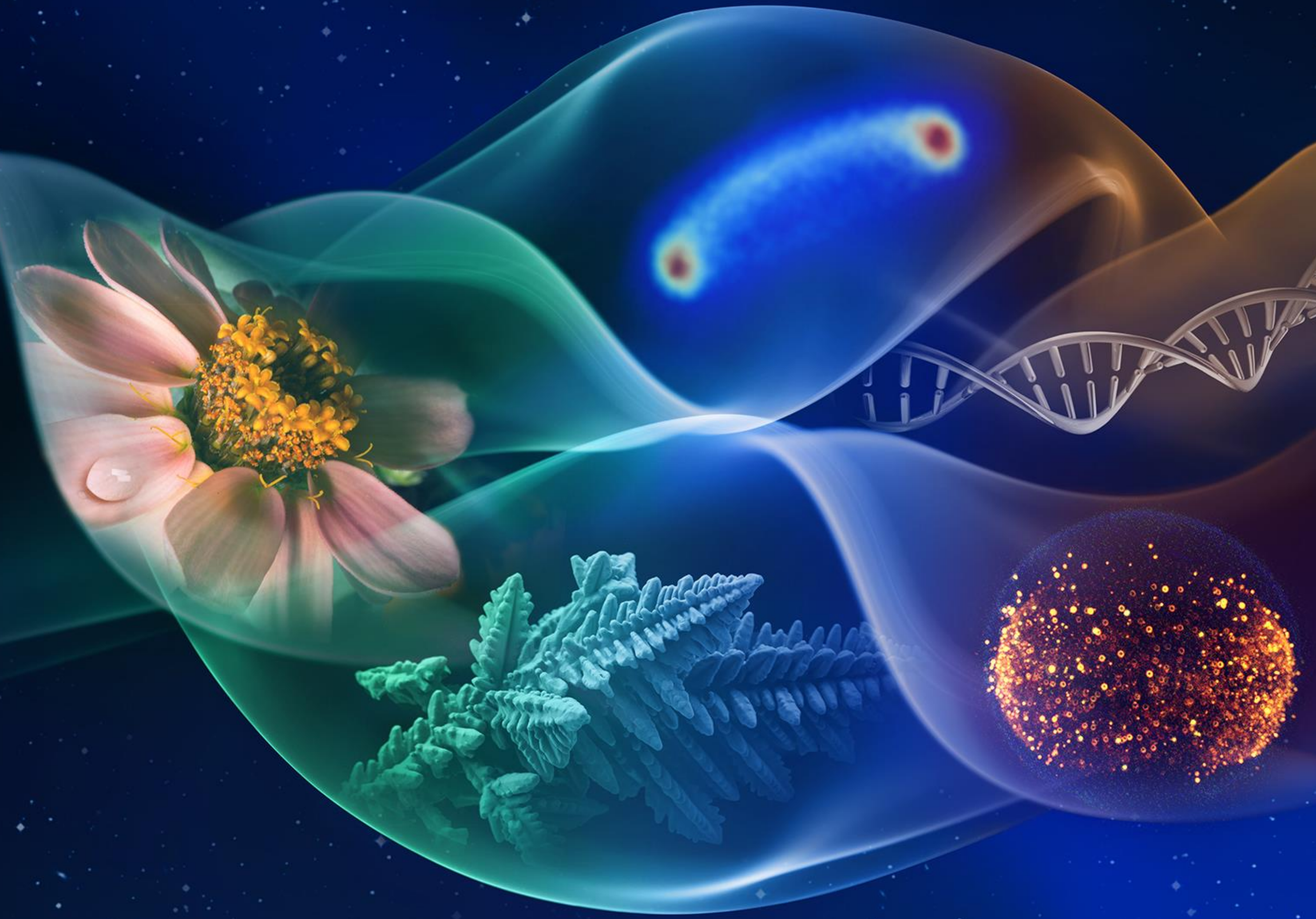
Sharmila Bhattacharya
Space Biology Senior Program Scientist
Anthony Hickey
Space Biology Senior Support Scientist

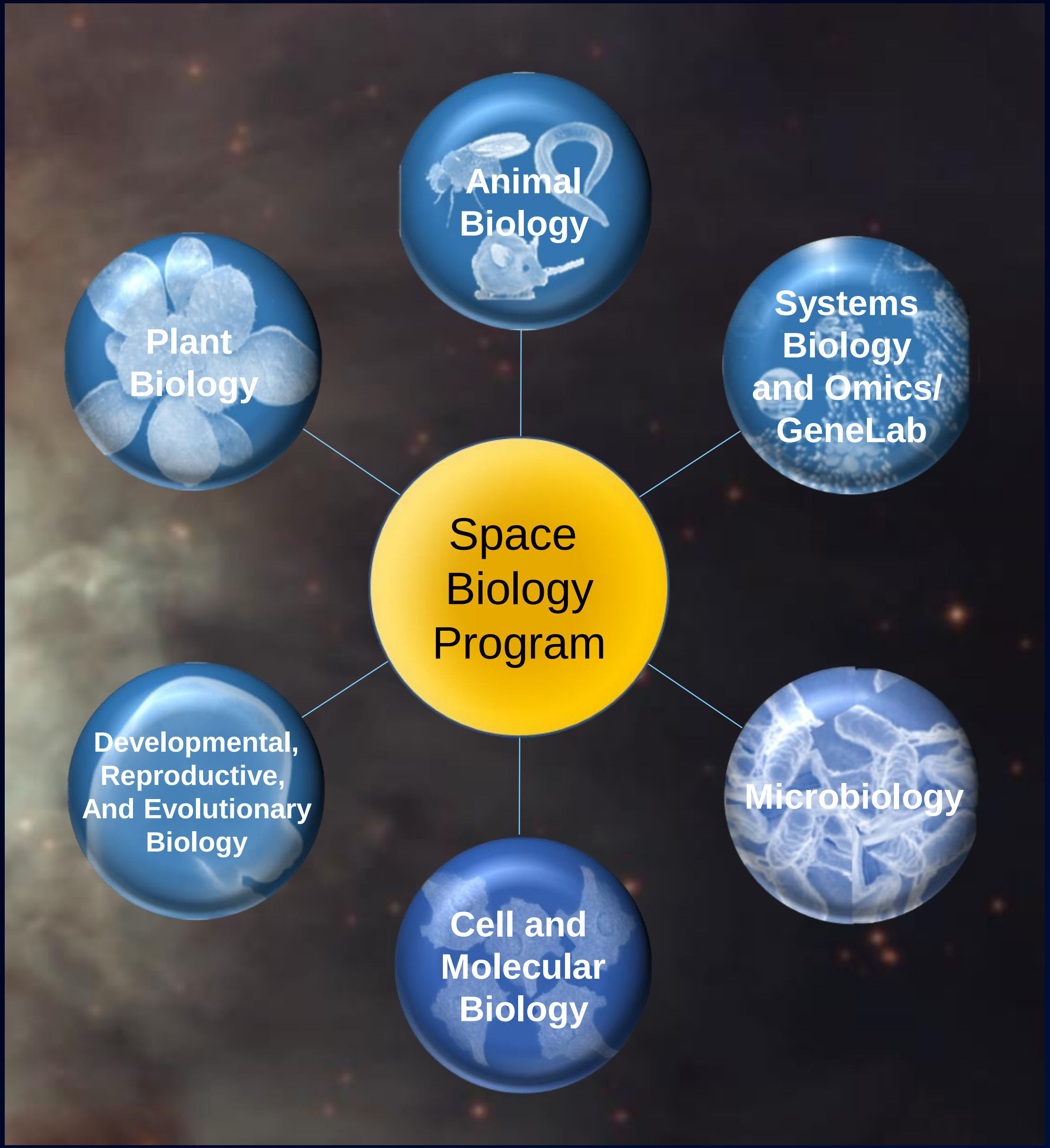
Presentation to the National Academy’s Committee on Biological & Physical Sciences in Space
Oct 20, 2022

Agenda

- **Overview of the Space Biology program**
- **Research Focus - Thriving in Deep Space**
- **Artemis I Space Biology Payloads**
- **Space Biology Lunar Payload (LEIA and PRISM)**
- **Accomplishments in Open Science**
- **Recent and Current Space Biology Opportunities**
  - Plant Biology
  - Animal Biology
  - Beyond Low Earth Orbit (Artemis II) Deep Dive
- **Recent Science Highlight**

Overview of the Space Biology Program

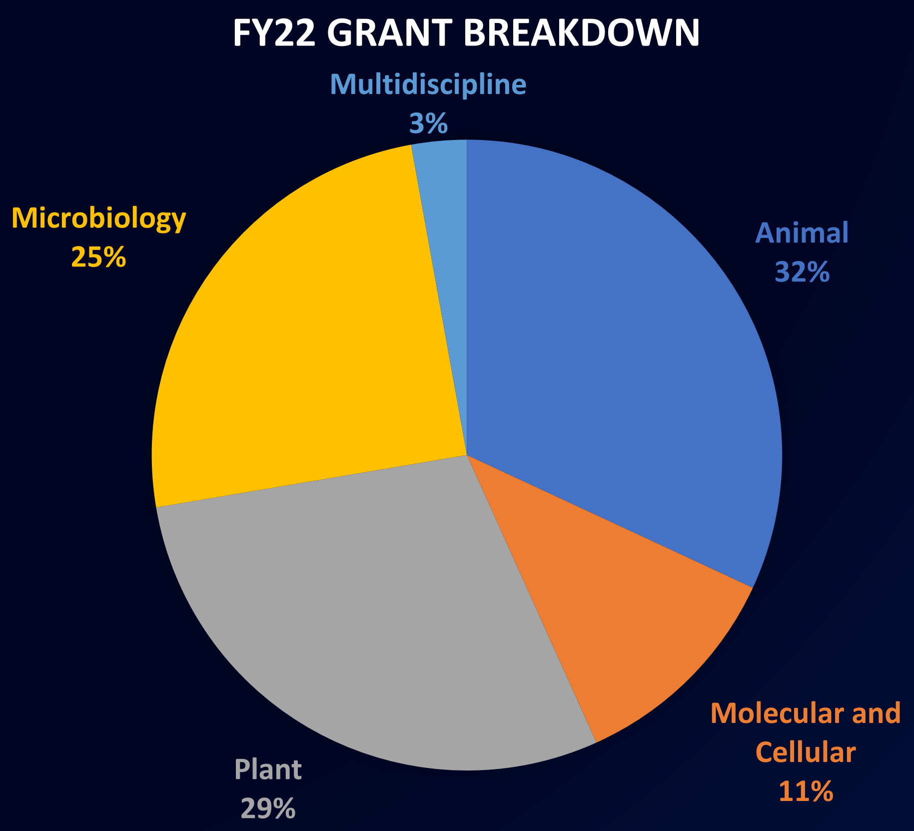




Objectives

- 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



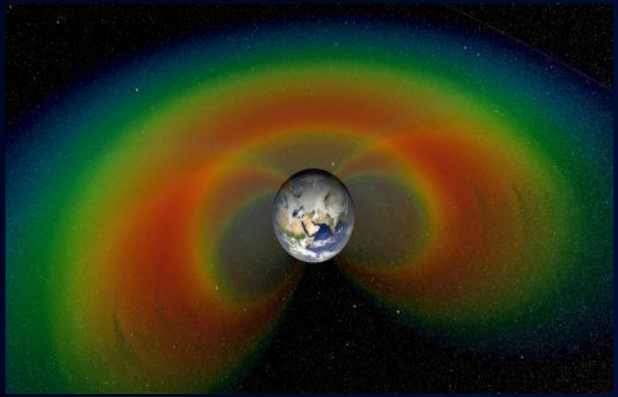
Total SB Grants	141
Flight	62
Ground	79
Flight and Ground	7

Number Directed vs Competed	
Directed	9
Competed	132

Biologically Relevant Environmental Factors Encountered in Spaceflight

Microgravity/Reduced Gravity

Ionizing Radiation

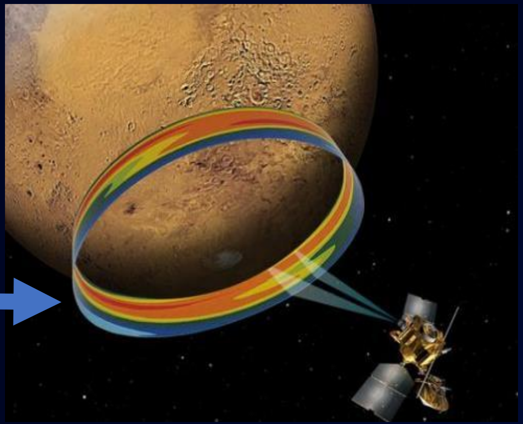


Credits: NASA/Goddard Space Flight Center/Scientific Visualization Studio

Altered Day/Night Cycles: Circadian Rhythm Changes

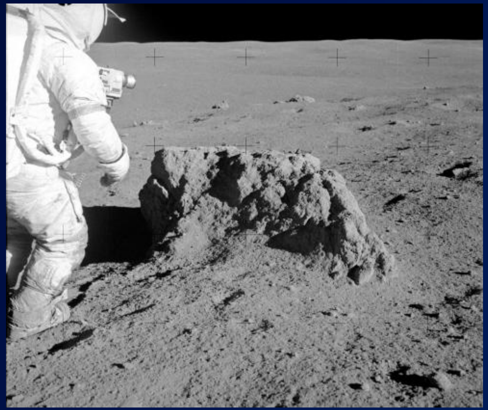
Altered Temperature and Atmosphere

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



Isolation

Regolith



COMBINATION OF MULTIPLE STRESSORS

Thriving in Deep Space (TIDES)

- Ground studies
- Space studies
- Ground & space studies

Understand Fundamental Mechanisms

Use model organisms to determine how animals/humans respond to deep space environments

+

Build the Blocks to Support Human Life

Understand how model plants, crops, & seeds respond to and can thrive in deep space environments

=

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

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

MODEL ORGANISM PROGRESSION

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

Unicellular (e.g., yeast, fungi) → Simple model plants (e.g., *Arabidopsis*) → Edible & others (for micronutrients, etc.) → Humans

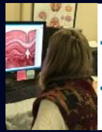
Newly Selected Animal Grants: ***HRP Partner; Radiation emphasis*** (2022)

ROSES 2021-E.11: Space Biology: Animal Studies

Insights into the impacts of continuous, low dose-rate neutron radiation exposure on maternal and fetal skeletal physiology
Heather Allaway, Ph.D. Louisiana State University and A&M College

Integrated CNS assessment in rodent models of altered gravity and irradiation
Joshua Alwood, Ph.D. NASA Ames Research Center

Unraveling the role of mitochondrial dysfunction and senescence on inhibition of tissue regeneration during spaceflight and amelioration by a novel countermeasure, PQQ
Elizabeth Blaber, Ph.D. Rensselaer Polytechnic Institute



Feasibility study: Use of neural networks to predict adaptability and multiday performance saving in dual motor-cognitive tasks after exposure to space flight stressors
Ashley Blackwell, Ph.D. Eastern Virginia Medical School

Characterization of Female Reproductive Health Risks for Long-Duration Spaceflight using Federated Machine Learning
Sylvain Costes, NASA Ames Research Center

Sex-specific physiological and transcriptomic CNS responses to combined effects of spaceflight stressors in *Drosophila melanogaster*
Janani Iyer, Ph.D. NASA Ames Research Center

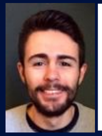
Sustained effects of spaceflight on Anemia and severity of effects dependency on age.
Cassandra Juran, Ph.D. NASA Ames Research Center

Stressors to Spaceflight: Identification of Transposon-Driven Changes to Gene Networks in GeneLab Data
Caralina Marin de Evsikova, Ph.D. Bay Pines Foundation, Inc

Circadian rhythm disruption and gravitational disturbance in a Lunar mission analog: consequences for muscle function during and after the mission
Marie Mortreux, Ph.D. Beth Israel Deaconess Medical Center, Inc.

Partial Gravity and Sex-Difference Effects on the Venous Circulation
Anand Narayanan, Ph.D. Florida State University

Acute and long-term effects of combined radiation and partial unloading on neurological and musculoskeletal systems in male and female rats
Seward Rutkove, Ph.D. Beth Israel Deaconess Medical Center, Inc.



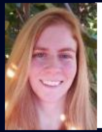
The effect of different genetic mutations and pharmacologic interventions on transcriptional responses to spaceflight *in C. elegans*
Craig Willis, Ph.D. Ohio University

Newly Selected Plant Grants: ***HRP Partner; Radiation emphasis*** (2022)

ROSES 2021-E.9: Space Biology: Plant Studies

Determining the impact of space radiation and simulated microgravity on plant root microbial community composition and function.

John Baker, Ph.D. Medical College Of Wisconsin, Inc.



How do carbon fixing strategies affect nutritional content under high CO_2? A comparison of C3 vs. C4 microgreens?

Colleen Doherty, Ph.D. North Carolina State University

Growing Food on Mars: Determining the impact of radiation, atmospheric composition, and rock substrate on plant growth in a Space Rock Garden Experiment

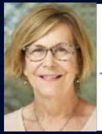
Rebecca Lybrand, Ph.D. University Of California, Davis

Plant Trek: Investigating Strategies for Regolith Pre-Conditioning to Support the Establishment of Plant-Microbe Systems in Martian Habitats

Kennda Lynch, Ph.D. Universities Space Research Association, Columbia

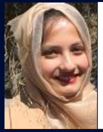
Temporal Lighting Optimization to Improve Lettuce Productivity and Nutritional Quality Under Superelevated CO_2 Stress

Qingwu Meng, Ph.D. University Of Delaware



Telomere dynamics and oxidative stress in Arabidopsis in the space radiation environment

Dorothy Shippen, Ph.D. Texas A&M AgriLife Research



Leaf Sensor Network for In Situ and Multiparametric Analysis of Crop Stressors

Shawana Tabassum, Ph.D. University of Texas, Tyler.

Space Biology Awardees from ROSES in 2021/2022

ROSES-2020 E.12: GeneLab Research Proposals

Alternative Splicing and Transcriptome Modulation under Space Flight Response in Plants
Pankaj Jaiswal, Ph.D., Oregon State University, Corvallis, Oregon

In silico Modeling of Gene Networks and Mechanisms Associated with Plant Gravitropism
Sushma Naithani, Ph.D., Oregon State University, Corvallis, Oregon

Comparative Analysis of Multi-Gravity Studies on Earth and ISS
Sarah Wyatt, Ph.D., Ohio University, Athens, Ohio

ROSES-2020 E.12: Early Career Investigation Proposals

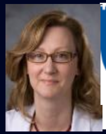
Responses of Microbes and Microbial Communities to Prolonged Exposure to Space Radiation
Jonathan Galazka, Ph.D., NASA Ames Research Center

Responses of Microbes and Microbial Communities to Prolonged Exposure to Space Radiation
Jessica Lee, Ph.D., NASA Ames Research Center

These awards are supported in part by the Science Mission Directorate Research Catalyst Fund

Featured Animal Biology Ground-based Projects

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

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

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

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

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

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

Featured Animal Biology Flight-based Projects

Megakaryocytes Orbiting in Outer Space and Near Earth: The MOON Study

Hansjorg Schwertz, Ph.D. University of Utah, Salt Lake City, UT

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

Role of Mesenchymal Stem Cells in Microgravity Induced Bone Loss

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

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

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

UMAMI: Impact of Spaceflight on Beneficial Animal-Microbe Interactions

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

Featured Plant Biology Spaceflight Projects

APEX-08: Can Polyamines Mitigate Plant Stress Response under Microgravity Conditions?
Patrick Masson, Ph.D., University of Wisconsin, Madison, Wisconsin

APEX-09: C4 Photosynthesis in Space (C4Space)
Pubudu Handakumbura, Ph.D., Pacific Northwest National Laboratory, Richland, Washington

APEX-11: 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, Florida

BRIC-24: Membrane Contacts in Plant Gravity Perception
Marcella Rojas-Pierce, Ph.D., North Carolina State University, Raleigh, North Carolina

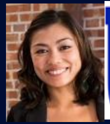
BRIC-27: From Antarctica to Space: Molecular Response and Physiological Adaptation of Moss to Simulated Deep Space Cosmic Ionizing Radiation and Spaceflight Microgravity.
Agata Zupanska, Ph.D. SETI Institute, Mountain View, California

BRIC-LED-002: BRIC: Exploring Spaceflight-Linked Changes in Plant Defense Capabilities
Simon Gilroy, Ph.D., University of Wisconsin, Madison, Wisconsin

MVP-Plant-01: RNA Plant Regulation Redux (in MVP)
Imara Perera, Ph.D., North Carolina State University, Raleigh, North Carolina

PH-03: Epigenetic Adaptation to the Spaceflight Environment - Accumulated Genomic Change Induced by Generations in Space
Anna-Lisa Paul, Ph.D., University of Florida, Gainesville, Florida

PH-08: Evaluation of Small Plants for Agriculture in Confined Environments (SPACE) Tomatoes for Space Flight Applications
Robert Jinkerson, Ph.D., University of California, Riverside, California



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

Featured Plant Biology Ground-based Projects

Suborbital: The Role of Ca2+ Signaling During the Early Events of Plant Adaptation to Spaceflight
Rob Ferl, Ph.D., University of Florida, Gainesville, Florida

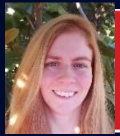
Antarctic EDEN ISS: Spectral Imaging within the EDEN ISS Project – An Antarctic Analog for Enhancing Exploration Life Support
Rob Ferl, Ph.D., University of Florida, Gainesville, Florida

Leveraging Spaceflight Genomic Data to Uncover Developmental and Cell Type Specific Gene Regulatory Networks in Plants Responding to Gravity
Simon Gilroy, Ph.D., University of Wisconsin, Madison, Wisconsin

RNA-Seq Guided Mutant Analysis to Discover New Components of Gravity Signaling in Plants
Scot Wolverton, Ph.D., Ohio Wesleyan University, Delaware, Ohio

Modeling Leafy Greens Physiological and Biochemical Responses to Light Intensity and Successive Harvest
Kellie Walters, Ph.D., University of Tennessee, Knoxville, Tennessee

Using *Brachypodium distachyon* to Study the Molecular Mechanisms that Underlie Directional Root-Growth Responses to Low-Speed 2-Dimensional Clinorotation
Patrick Masson, Ph.D., University Of Wisconsin, Madison, Wisconsin



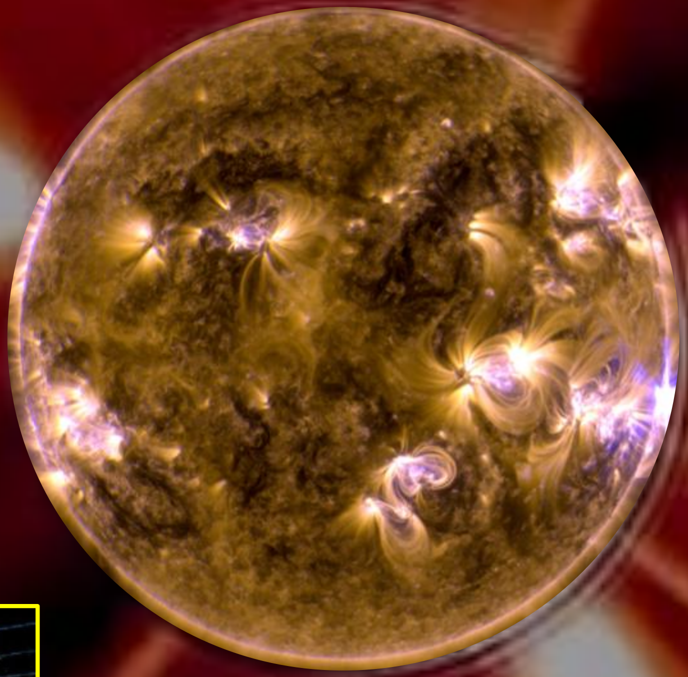
Time and Space, Determining the Interactions Between the Circadian Clock and Microgravity
Colleen Doherty, Ph.D., North Carolina State University, Raleigh, North Carolina

The Use of Microgravity Simulators for a Mechanistic Understanding of Cytoskeletal-mediated Regulation of Root Growth
Simon Gilroy, Ph.D., University of Wisconsin-Madison, Wisconsin


EXPLORE
MOON to MARS

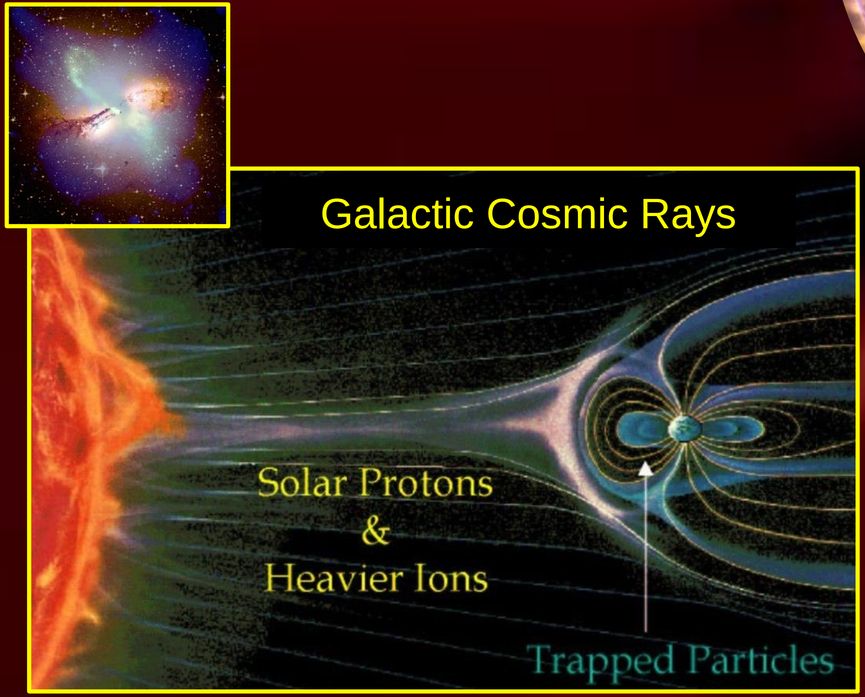
Interplanetary space radiation

What are we going to encounter beyond Low Earth Orbit (LEO)?



Ionizing radiation that will affect biology:

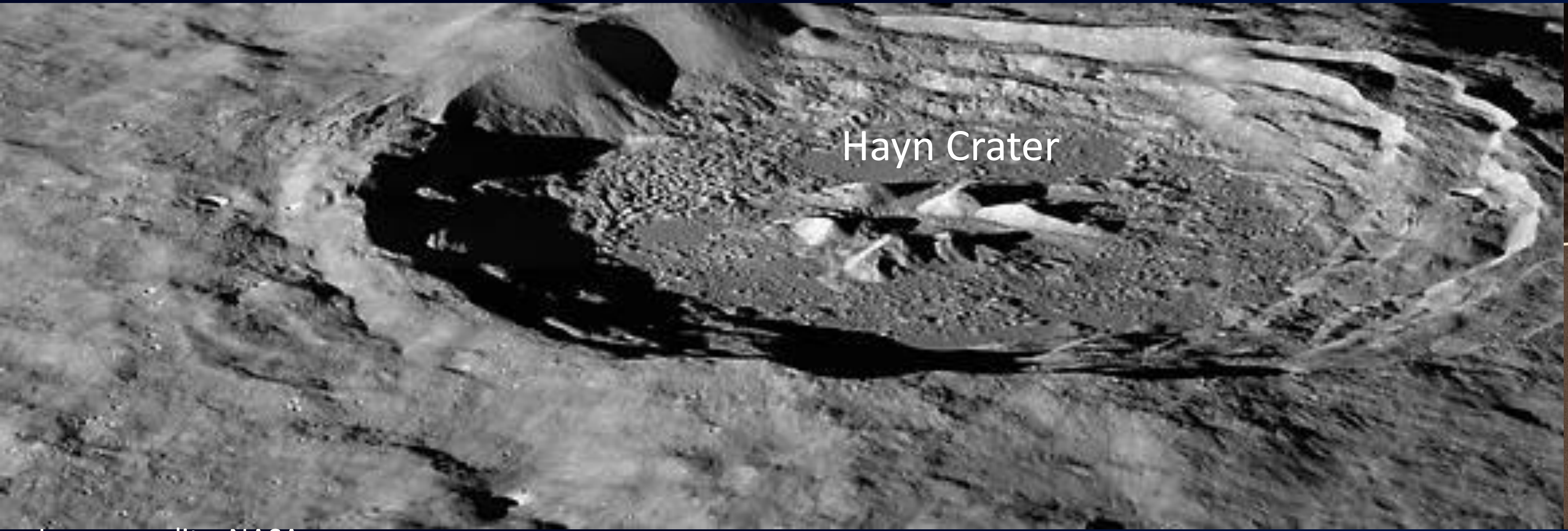
- ❖ Galactic Cosmic Radiation (GCR)
- ❖ Solar Particle Events (SPEs)



Limits of life in space, as studied to date:

- ✧ 12.5 days on a lunar round trip
- ✧ 1.5 years in low Earth orbit on ISS

Radiation Environment of the Moon:
Lunar surface radiation is a distinct environment from deep space



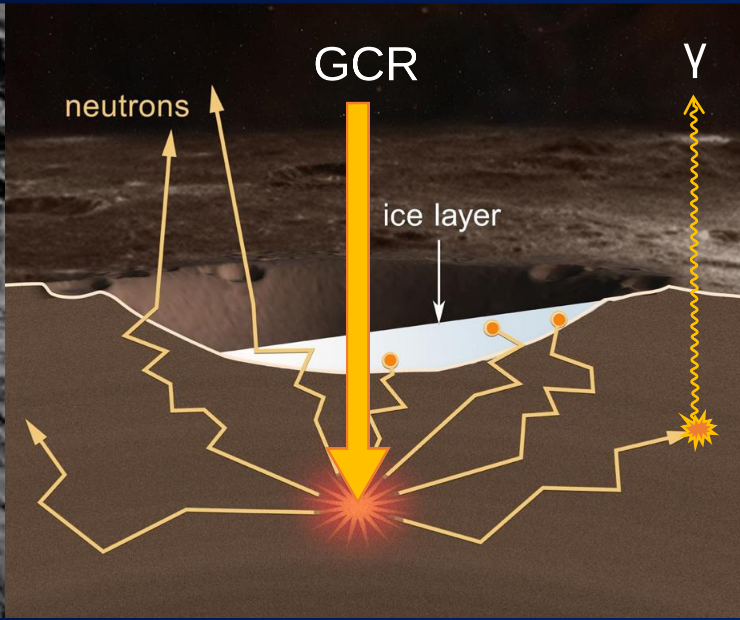


Image credits: NASA

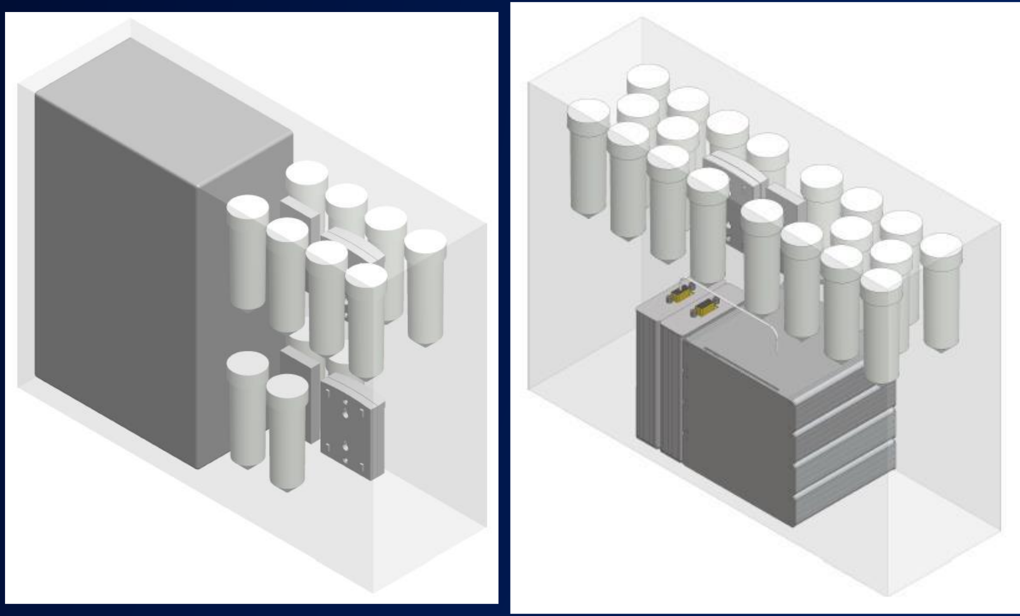
Lunar regolith absorbs galactic cosmic radiation (GCR) to produce secondary albedo neutrons

- High-energy, fast neutrons cause direct cellular damage
- Fast neutrons also produce ionizing radiation
- Estimates of surface neutron doses vary widely

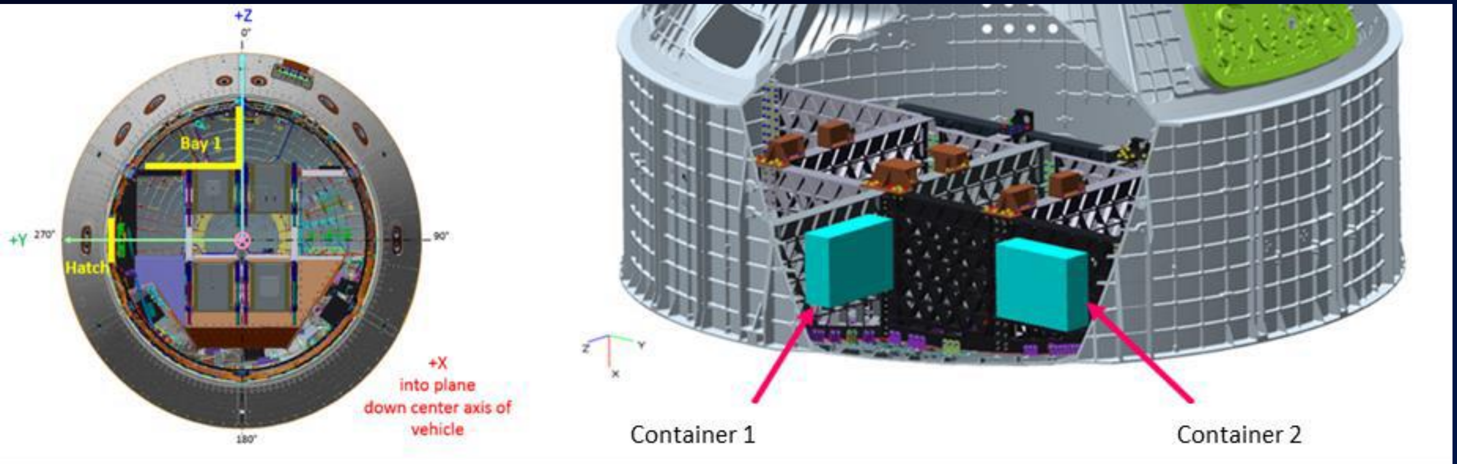
BioExpt-1: Flying on Artemis-1

Anticipated Launch: November 2022

- Plant seeds *Arabidopsis thaliana*: *Life Beyond Earth: Effect of Space Flight on Seeds with Improved Nutritional Value*; PI Federica Brandizzi, Michigan State University
- Algae *Chlamydomonas reinhardtii*: *Fuel to Mars*; PI Timothy Hammond, Institute for Medical Research; *Chlamydomonas reinhardtii*
- Fungi *Aspergillus niger*: *Investigating the Roles of Melanin and DNA Repair on Adaptation and Survivability of Fungi in Deep Space*; PI Zheng Wang, Naval Research Laboratory
- Yeast *Saccharomyces cerevisiae*: *Multi-Generational Genome-Wide Yeast Fitness Profiling Beyond and Below Earth's Van Allen Belts*; PI Luis Zea, University of Colorado-Boulder



SBP Payload Container Assembly configuration for Drs. Zea & Brandizzi (left) and Drs. Wang & Hammond (right).

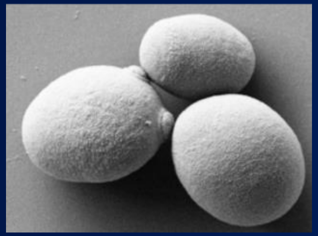


The SBP Payload Container Assemblies are installed onto backbone panels 1 and 2 in Bay 1 of the Orion capsule.

3D printed ½ scale mockup of SBP Payload Container Assembly with blue Science Bag contained within.

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



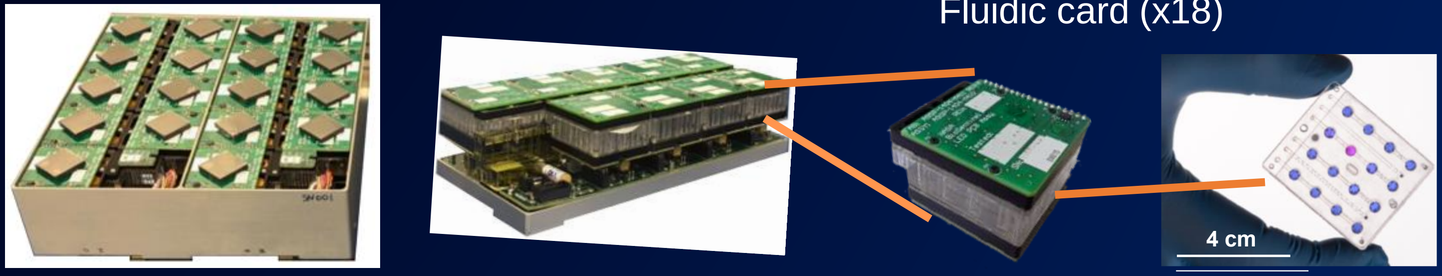
Accommodates yeast and related cellular systems

4U BioSensor Payload

(2U Pseudo Bus not shown in diagrams below)

LET spectrometer attached at one end of payload

Fluidic card (x18)



Biosensor payload with fluidic cards and optical sensors

LEIA: Lunar Explorer Instrument for space biology Applications

A Funding opportunity released as Program Element E.10 in ROSES 2021 on NSPIRES

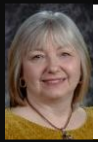
(Selections Announced: September 3, 2021)

We solicited proposals for an autonomous biological research experiment using yeast as a model organism as part of the project-formulation phase in the work of the Lunar Explorer Instrument for space biology Applications (LEIA) call

Image credit: NASA

- Solicited for experiments to be integrated into the Biosentinel hardware and be tested on the ground to establish a functional experimental payload that could be flight-ready in future.
- The overarching goal of the proposed research studies themselves were to investigate the effects of lunar environmental conditions on yeast biology, however, proposals are to be written with the understanding that the LEIA project is still in its ground-based testing

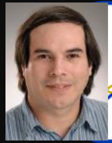
Three investigations were selected:



LSU Health SHREVEPORT

Investigating Lunar Stress and Parkinson's Disease using an Alpha Synuclein Yeast Model

Lynn Harrison, Ph.D. Louisiana State University System, Shreveport, LA



Ames Research Center

ORGANA: Oxidation-Reduction potential and Genetic Assessments for New mission Applications

Sergio Santa Maria, Ph.D. NASA Ames Research Center, Mountain View, CA

Ames Research Center

Feasibility of synthetic biology countermeasures for human exploration beyond low Earth orbit

Andrew Settles, Ph.D. NASA Ames Research Center, Mountain View, CA

PRISM: Payloads and Research Investigations on the Surface of the Moon

This Funding opportunity was released by NASA **ESSIO (Exploration Science Strategy & Integration Office)**
(Selected July 2, 2022)

Investigations that include development and flight of science-driven suites of instruments payloads that will be delivered to the lunar surface by the Commercial Lunar Payload Services (CLPS)

- Two of the selected LEIA projects were combined and submitted to the 2021 PRISM call as part of an investigation to further develop the BioSentinel hardware or delivery to lunar surface by the Commercial Lunar Payload Services (CLPS)

Image credit: NASA

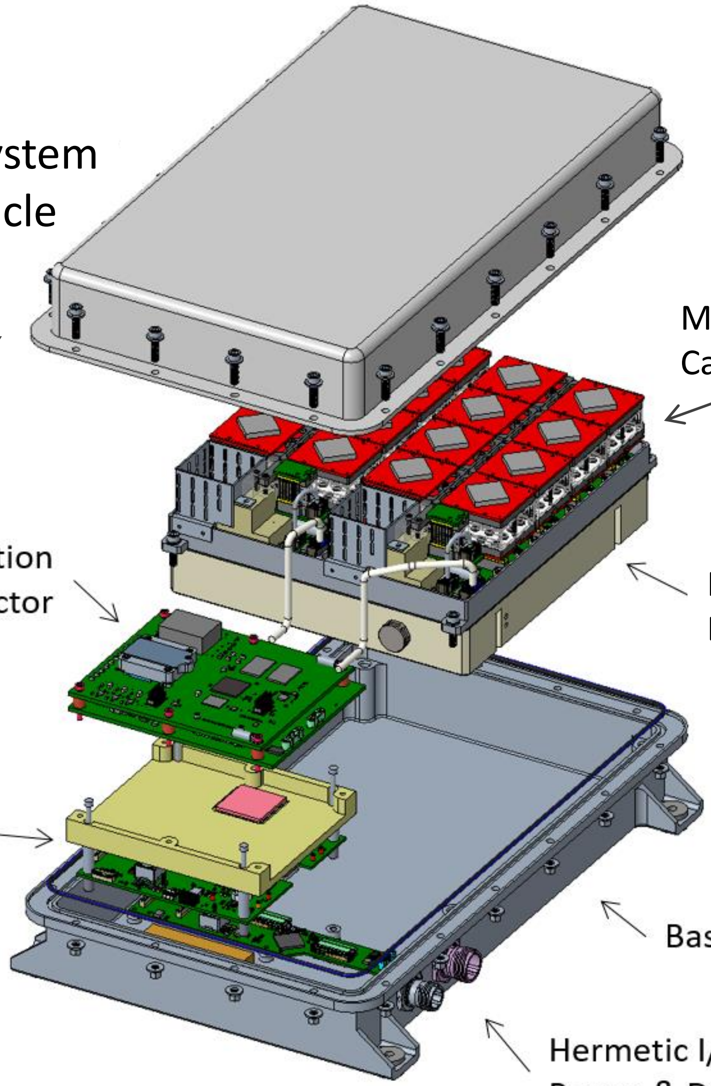
ORGANA: Oxidation-Reduction potential and Genetic Assessments for New mission Applications
Sergio Santa Maria, Ph.D. NASA Ames Research Center, Mountain View, CA

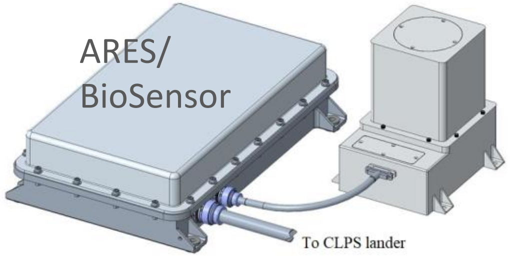
Feasibility of synthetic biology countermeasures for human exploration beyond low Earth orbit
Andrew Settles, Ph.D. NASA Ames Research Center, Mountain View, CA

- Both investigators and the Biosentinel hardware were selected for a CLPS flight as part of a future Artemis Mission

The LEIA-PRISM Experiment

Fluidic culturing system with charged-particle radiation detector

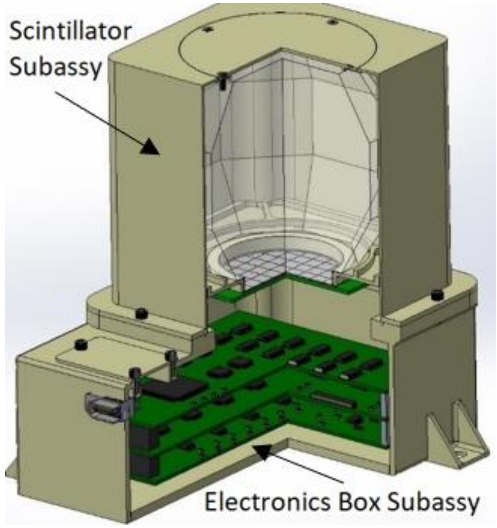




*MLI not shown

Mini-FND

fast neutron detector



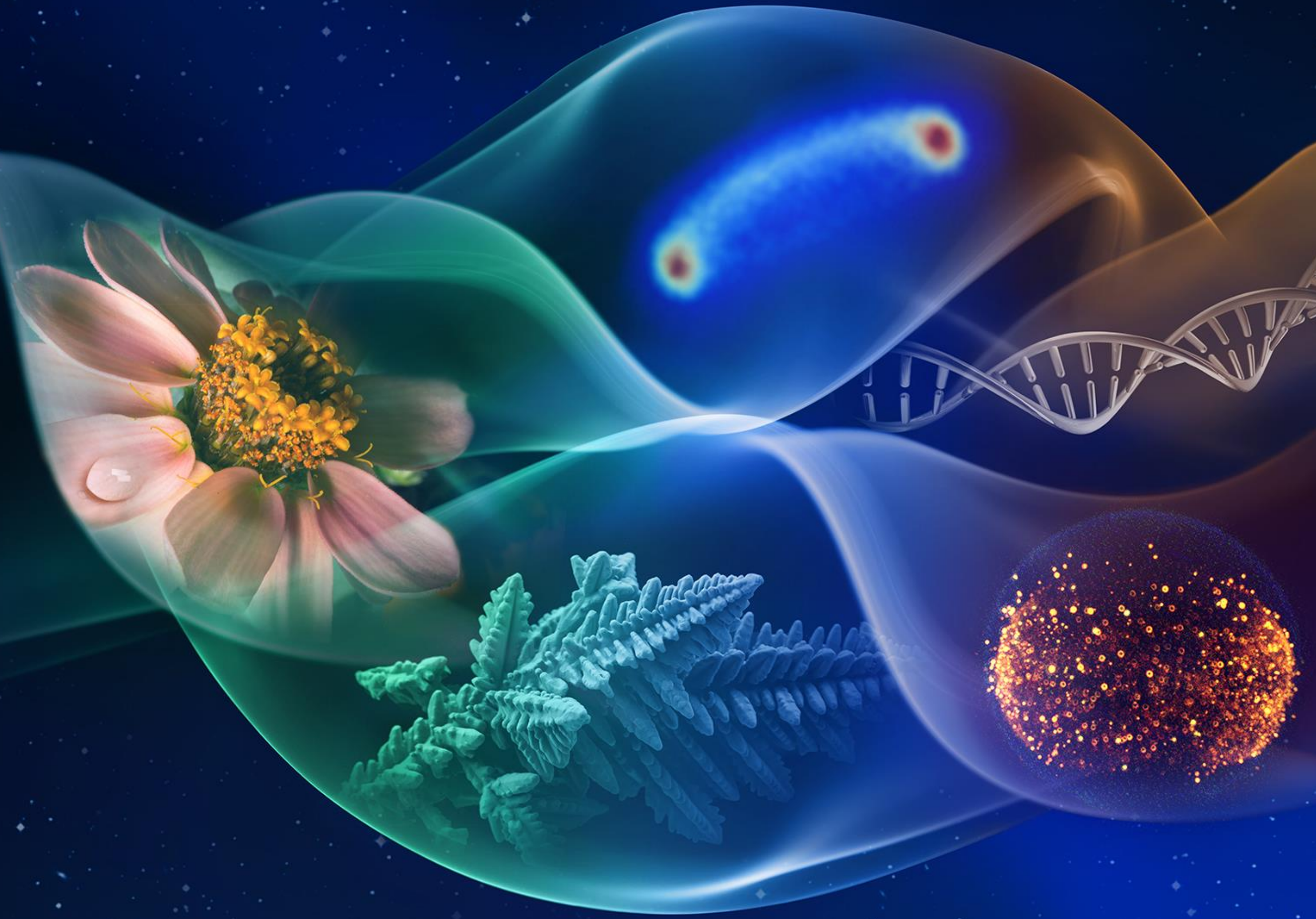
Approach:

- Engineer yeast strains to test growth and metabolism for sensitivity to radiation.
- Measure biologically relevant radiation in transit and on the lunar surface.

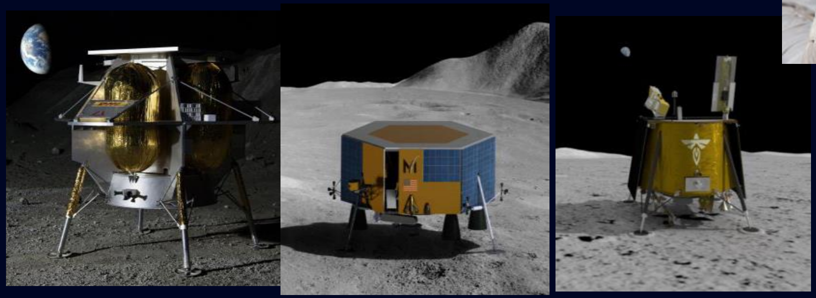
Expected Outcomes/Impact:

- Determine cellular sensitivity to the lunar environment.
- Evaluate feasibility of bioproduction on the Moon.
- Test genetic strategies to enhance cellular tolerance to the lunar environment.
- Determine radiation risks for crew.

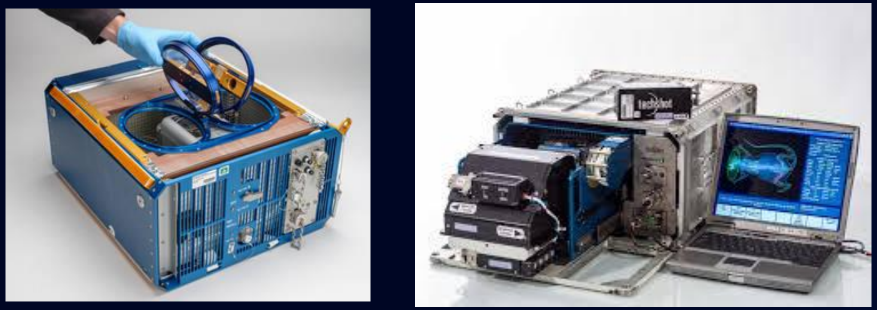
Future Needs for Science Beyond LEO



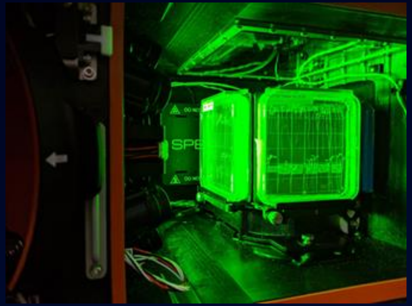
Capabilities for Animal & Model Organism Studies on the Moon



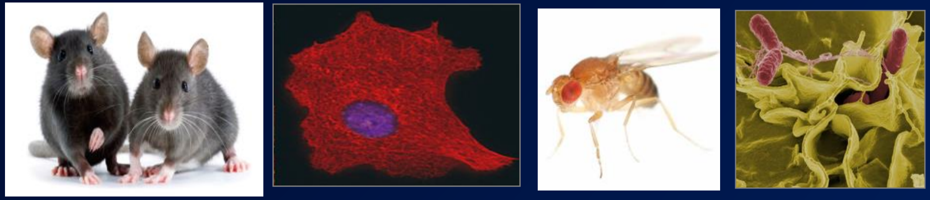
Taking science to the lunar surface

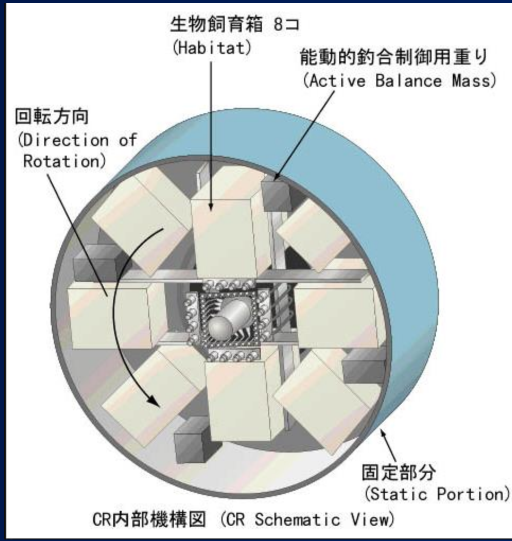


Habitats & analytical tools for research



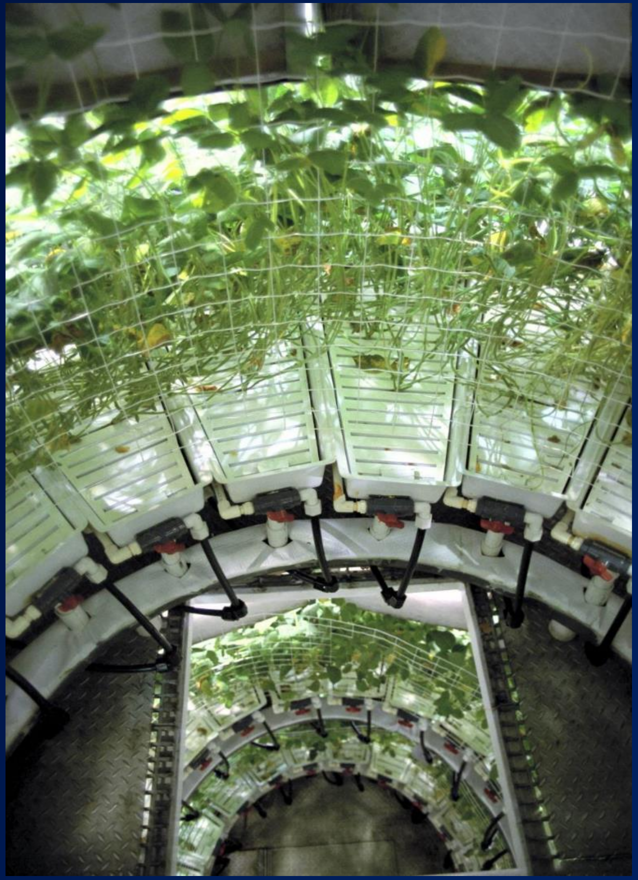
- Use well-characterized animal model organisms & cellular systems to understand the complex biological consequences of exposure to deep space's unique environment.
- Facilities/equipment/habitats required for lunar surface experimentation with animals and related systems, need to be developed for Space Biology.
- In-flight entrifuges that can simulate 1g or fractional g loads, can be used to differentiate between the biological effects of altered gravity vs other stressors like radiation.



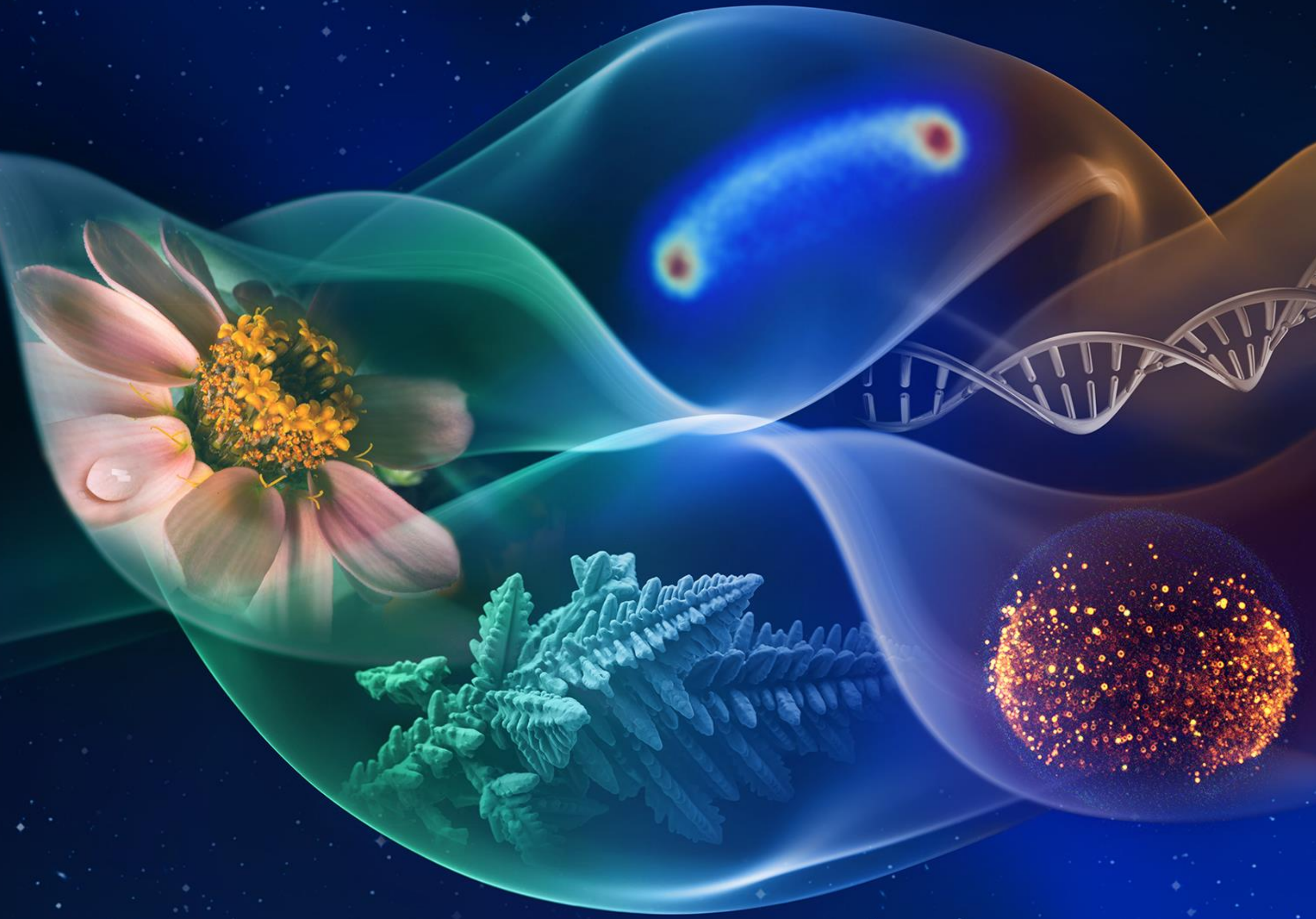


Growing Plants on the Moon

Develop a plant growth facility on the lunar surface to understand the complex biological consequences of exposure to deep space's unique environment and validate the ability to produce supplemental nutrients for future space explorers



Accomplishments in Open Science

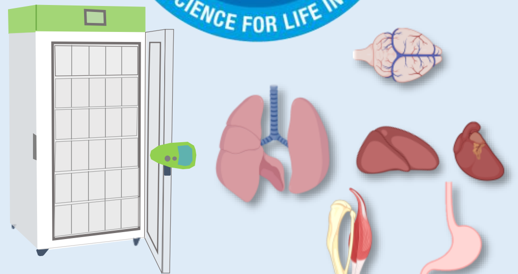


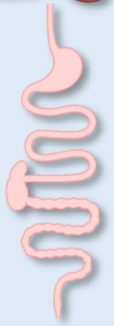
Open Science Projects

Biospecimen Sharing Program (BSP)

- *Dissects and preserves* rodent tissues from Flight and Ground investigations
- *Coordinates* internal tissue sharing

NASA Biological Institutional Scientific Collection (NBISC)

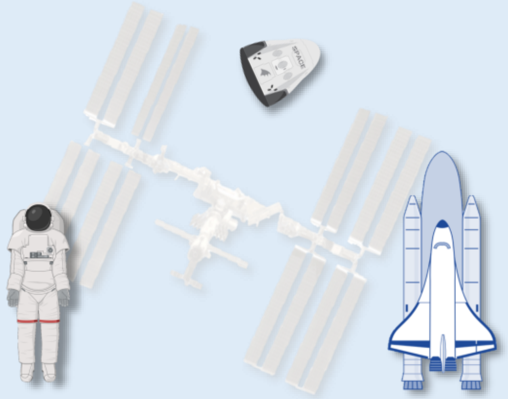




- Collection of biospecimens from non-human subjects exposed to spaceflight and ground studies
- Coordinates and fulfills biospecimen requests and awards

Ames Life Sciences Data Archive (ALSDA)

- *Collects* and *curates* physiological, mission, and imaging data

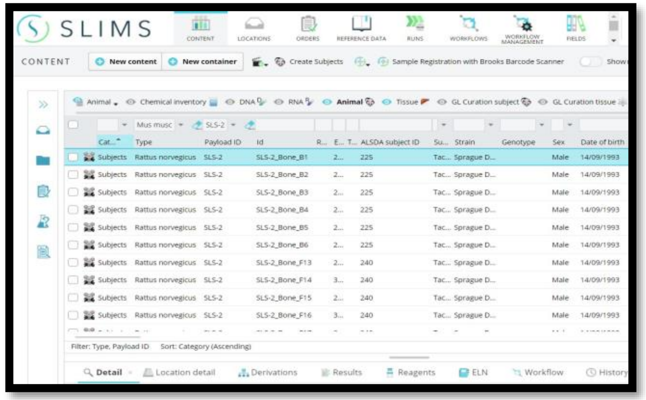


GeneLab (GL)

- *Collects* and *curates* omics data from space-relevant samples

BSP FY22 Accomplishments

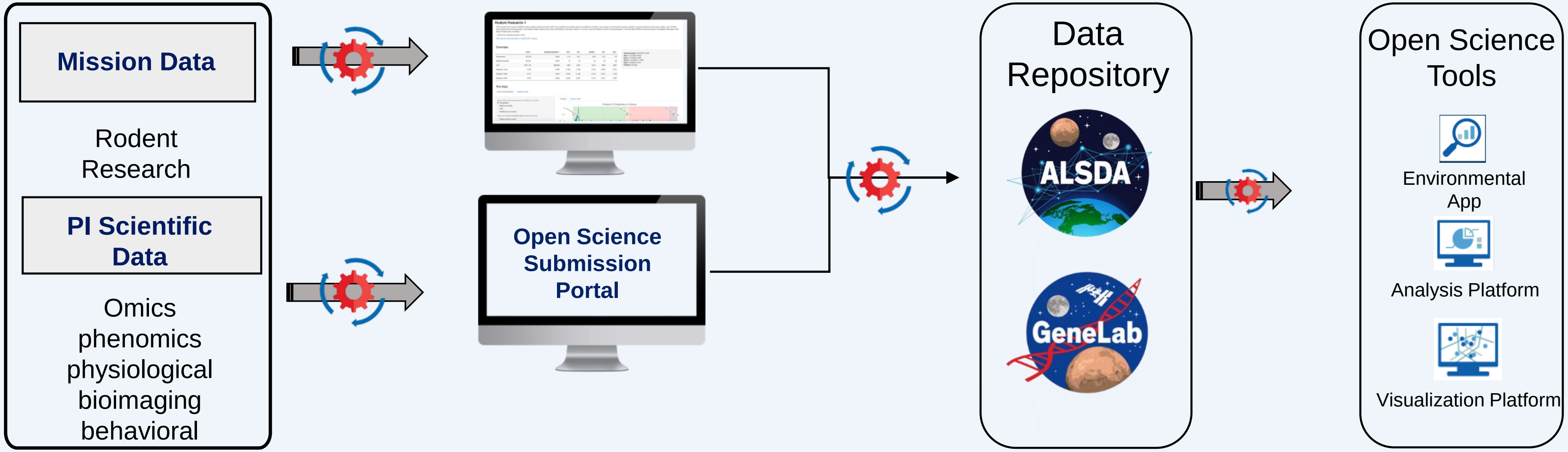
- **Rodent Research-12 (RR-12) BSP**
  - Dissections successfully completed at Loma Linda University (California) October 11-26, 2021, after postponement from March 2020 due to COVID-19.
  - ~3,500 biospecimens were collected and transferred to NBISC.
- **Rodent Research-18 (RR-18) BSP**
  - Live Animal Return dissections successfully completed at The Roskamp Institute (Florida) in January 19 – February 7, 2022, after SpX-24 return.
  - Readaptation group dissections successfully completed at Loma Linda University April 16 – May 9, 2022.
  - ~12 tissues types are available in NBISC for the greater research community to request.
- **Ground investigation: *Bone Remodeling Under Differential Gravitational Environments* (Komatsu) BSP**
  - Remote rat dissections (3 of 8) successfully completed at Stony Brook University (New York) on May 5, May 31, and August 15.
  - 5 tissues types will be available in NBISC for the greater research community to request.
- **Space Radiation Element/Space Biology Collaboration**
  - Pilot study initiated between Space Radiation Element, BSP, and NBISC to ingest biospecimens and data from SR PI Dr. Honglu Wu into NBISC.
  - Over 7 tissue types will be available in NBISC for the greater research community to request. Data entry into the Laboratory Information Management System completed in August 2022.
  - Joint BSP/NBISC/ALSDA/GL presentation completed at the inaugural Space Radiation Biospecimen & Tissue Sharing Summit on September 13, 2022, where over 140 participants engaged in discussions about open science and specimen and tissue sharing.
- **BSP and NBISC Biospecimen Data Migration to Cloud**
  - ~32,000 biospecimen records transferred into the shared, cloud-based Laboratory Information Management System to automate workflows and better manage sample metadata. Completed in September 2022.



Biological Data Repository

ALSDA and GeneLab Data Systems Integration (2022)

The Open Science Data Repositories will introduce a multi-project web-based submission portal to support self-service metadata curation and data submission within FAIR Guidelines.



AI/ML 4 Life in Space *(SMD Funded)*

- **Ensure data is AI/ML ready**
- **Generate benchmark datasets to train new AI/ML algorithms**
- **Utilize current AI/ML tools to analyze space biology data**

Analysis Working Group (AWG)

- **400+ members from multiple space agencies, international institutions, and industry**
- **Scientists meet monthly with each group to provide feedback, standards and analyze data**
- **Five Groups: Animal, Microbes, Plants, Multi-Omics, ALSDA**
- **Published 11 collaborative peer-reviewed papers to date**

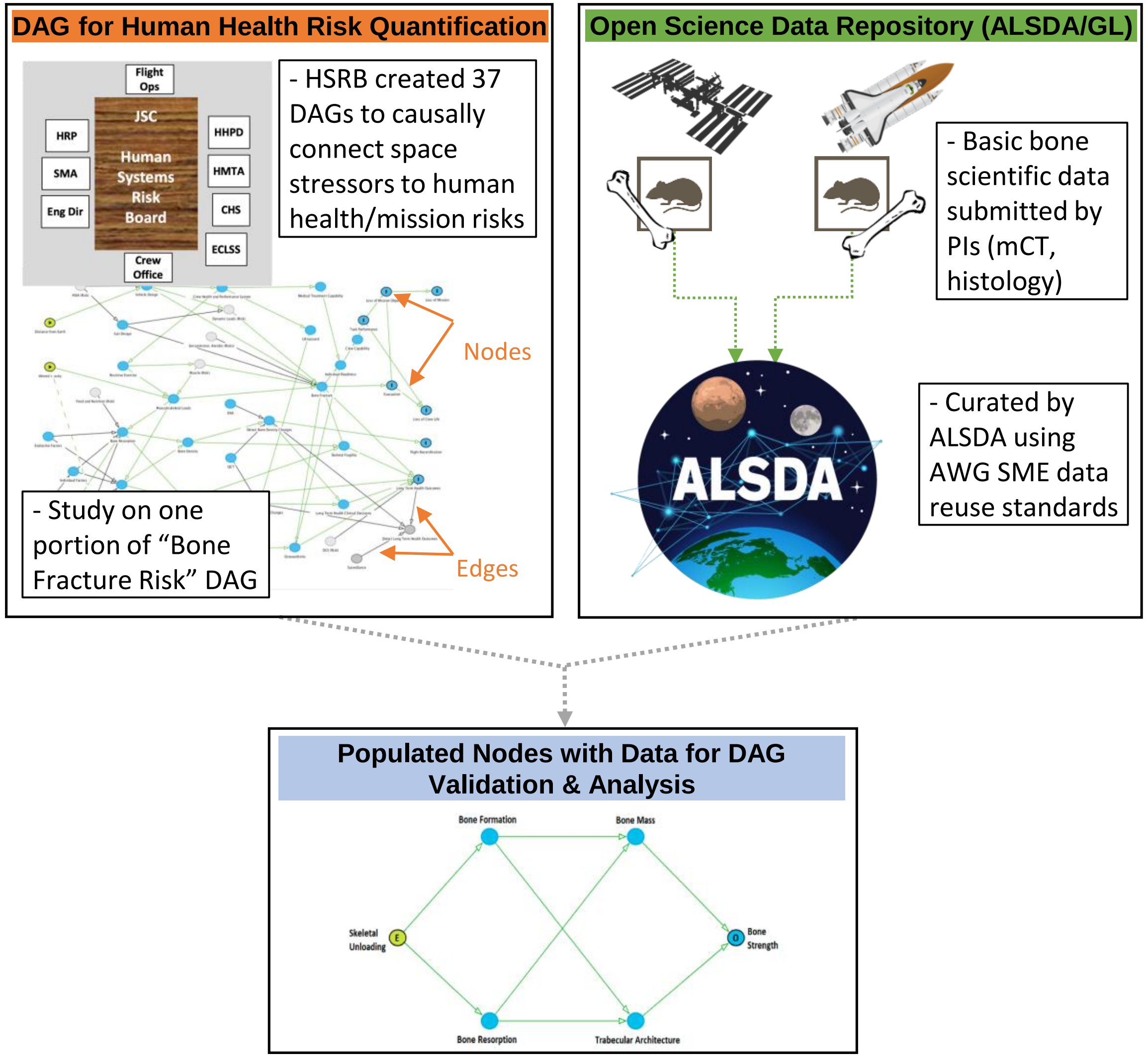
Open Science Collaboration with JSC to Improve Astronaut Health Risk Management via ALSDA Data Reuse

A study was published last month Reynolds et al., 2022 ***(Validating Causal Diagrams of Human Health Risks for Spaceflight: An Example Using Bone Data from Rodents),*** which began the effort to quantify human health risks for deep space missions using empirical data. This publication came out of a collaboration between JSC, ARC, and ALSDA Analysis Working Group SMEs.

Directed acyclic graphs (DAGs) were used to model causal diagrams for 37 human astronaut health risks, designed by 200 experts organized by the NASA Human Systems Risk Board (HSRB). The Reynolds et al. study aimed to support or disprove causal relationships within the HSRB “Bone Fracture” DAG using empirical data from the Ames Life Sciences Data Archive (ALSDA).

Using statistical computing software, 4 NASA ALSDA space-flown and ground datasets from rodent bones largely validated the causal DAG structure. Further work will 1) combine a DAG network with a Bayesian network to enable risk probabilities, 2) compare model organism to human mechanisms, 3) evaluate appropriateness of variable parameters, 4) fill in other DAGs (cardiovascular, space associated neuro-ocular syndrome, radiation carcinogenesis, etc.).

With small sample sizes and the inability to conduct assays that destroy tissue, deep space astronaut health is reliant on model organism evidence. Further research for these types of translational science projects (i.e., basic to applied to operational) is critical for improving astronaut health. This study provides evidence for HSRB (and SMD-BPS) to embrace open science and citizen science and justifies data sharing and curation for machine-readability. This work verifies the need for strong PI data submission policy, and efficacy of the ALSDA/GeneLab open science database structures to ensure reusable quality data.



Workflow of collaboration and analysis between HSRB, ALSDA, and SMEs for ‘DAG’ validation. Study involves one portion of Bone Fracture Risk DAG, which ‘reused’ rodent bone data from the ALSDA/GL Open Science Data Repository.

GeneLab Metrics

In FY22, GeneLab has added **56 studies** publicly for access worldwide.

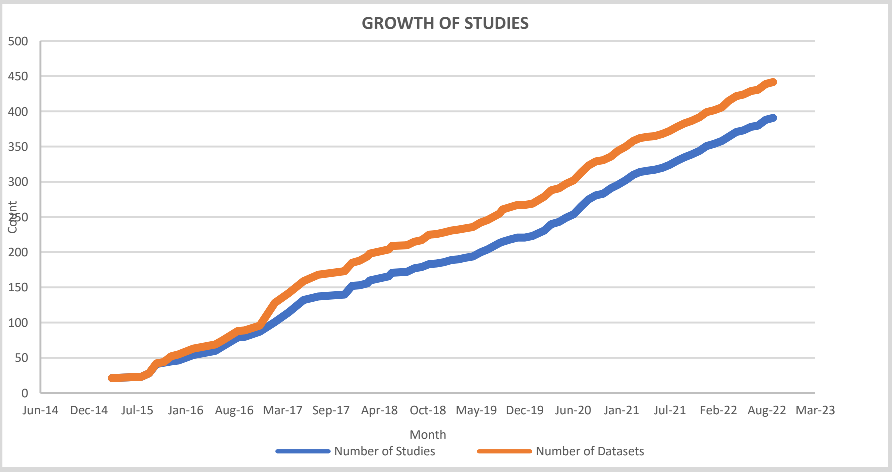
391 Studies

442 Datasets

45 Species

>10 Assays

>20K GB Data



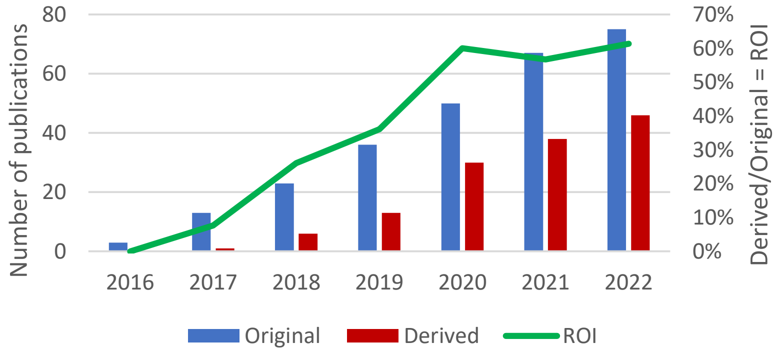
75 Original Publication linked to GeneLab

46 Derived Publication linked to GeneLab

70+ Presentations linked to GeneLab

100+ Datasets used in derived publications

ROI grows faster than publications linked to original



Original – Publications with original dataset submitted to GeneLab

Derived – Publications using data submitted to GeneLab repository

New features on the Data Systems

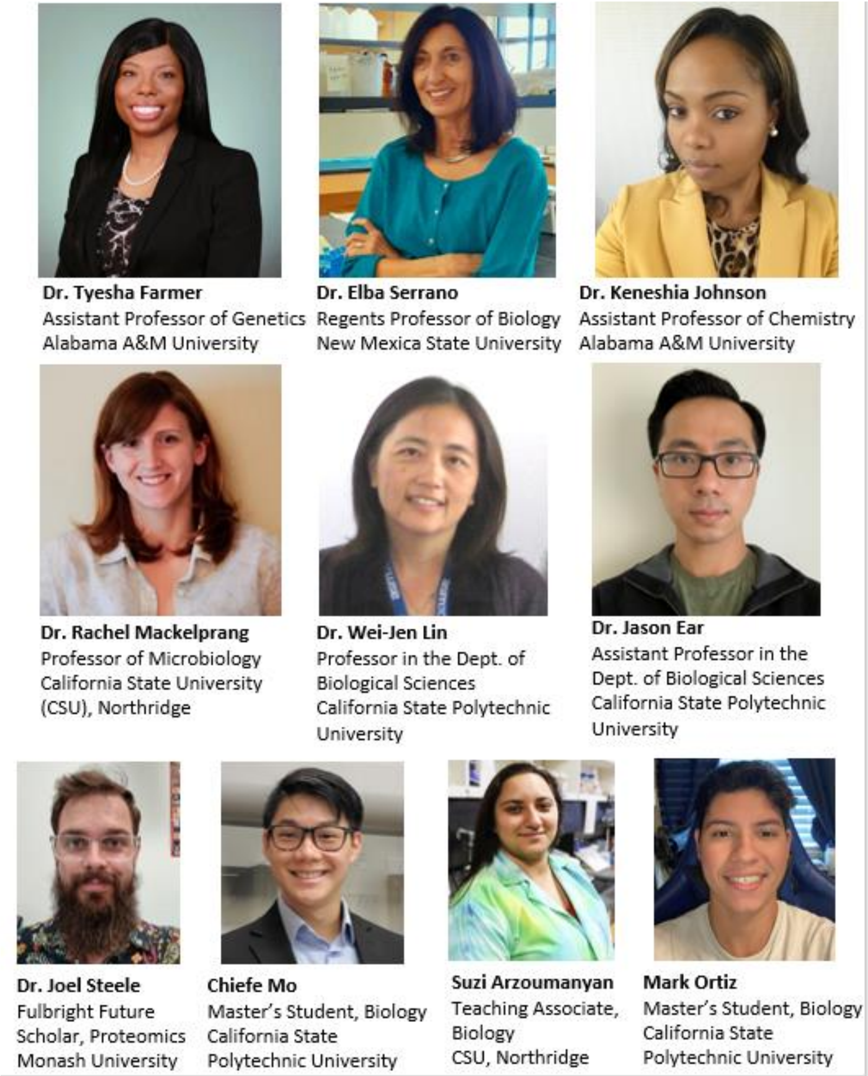
- The visualization portal has now expanded to include Gene Set Enrichment Analysis (GSEA) plots. GSEA is an analysis method that determines whether a defined set of genes shows statistically significant and concordant differences between two biological states.
- GeneLab Repository gets a new user interface with a new design and additional search capabilities to enable discovery.

Science Communication & Outreach

- On June 30th, GeneLab hosted the 2022 AWG Symposium from 10 am – 2:30 pm PT. This virtual event allowed the pubic an opportunity to view some exciting omics science stemming from the AWG community and was attended by 350 unique participants. During the event, attendees had an opportunity to sign-up for the AWGs which led to 45 new members, including early career scientists and university professors, resulting in over 400 AWG members to date.

GeneLab Expands Bioinformatics Training to HBCUs and MSIs

- **GeneLab for Colleges and Universities (GL4U) is partnering with Jet Propulsion Laboratory's (JPL) Planetary Protection Center of Excellence to provide space biology-relevant training in bioinformatics to educators at historically black colleges and universities (HBCUs) and minority serving institutions (MSIs).**
- GL4U: RNAseq Educator Bootcamp was held virtually from May 31st - June 10th, 2022, for 6 educators and 4 graduate students.
    - One student, Chiefe Mo, was so inspired that he composed and performed a GeneLab anthem on the last day - https://www.youtube.com/watch?v=szC3y2HWFcQ
- During the bootcamp, educators received training via lectures and hands-on instruction using Jupyter Notebooks (JNs) on how to 1) analyze GL RNAseq data and 2) run the RNAseq bootcamp for students at their home institutions, thereby extending the reach of this initiative to more students.
- To ensure educators from underserved colleges and universities are able to successfully implement the RNAseq training at their home institutions, the required computer resources will also be made available to them through the NASA Science Managed Cloud Environment (SMCE) funded by SMD AWS Space Act Agreement.



Dr. Tyesha Farmer
Assistant Professor of Genetics
Alabama A&M University

Dr. Elba Serrano
Regents Professor of Biology
New Mexica State University

Dr. Keneshia Johnson
Assistant Professor of Chemistry
Alabama A&M University

Dr. Rachel Mackelprang
Professor of Microbiology
California State University (CSU), Northridge

Dr. Wei-Jen Lin
Professor in the Dept. of Biological Sciences
California State Polytechnic University

Dr. Jason Ear
Assistant Professor in the Dept. of Biological Sciences
California State Polytechnic University

Dr. Joel Steele
Fulbright Future Scholar, Proteomics
Monash University

Chiefe Mo
Master's Student, Biology
California State Polytechnic University

Suzi Arzoumanyan
Teaching Associate, Biology
CSU, Northridge

Mark Ortiz
Master's Student, Biology
California State Polytechnic University

Recent and Current Space Biology Funding Opportunities

2022's Space Biology Annual Ground/Flight Funding Opportunities

Space Biology Plant Studies Released as Program Element E.9 in ROSES 2021:
Released: December 16, 2021; Final Proposals due: April 28, 2022
Selections Announced: Late October 2022

- This program element was released the NASA Human Research Program (HRP)
  - Space Biology will make and fund awards to investigators through E.9, while SRE will provide funded investigators proposing relevant studies access to the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory for ground radiation studies
- Science Emphases:
  - Proposed studies must address fundamental questions that will advance the understanding of how plants accommodate to the spaceflight environment (e.g., alterations in gravity, radiation, elevated carbon dioxide levels, etc.).
  - Proposals must be for hypothesis-driven research projects that study spaceflight environmental effects on plant physiology, biochemistry, cellular, and molecular biology during development, growth, and/or propagation.
  - Space Biology is interested in understanding biological processes that will enable NASA's long-term goal of sustained agriculture
  - HRP is interested in the psychological and nutritional benefits for astronauts of growing plants to support long duration human exploration to deep space destinations such as Mars.

Awards were made to seven investigators from seven institutions in six states. Total value of awards = ~$1.7M

2022's Space Biology Annual Ground/Flight Funding Opportunities (Cont)

Space Biology Animal Studies Released as <u>Program Element E.11 in ROSES 2021</u>:
Released: December 16, 2021; Final Proposals due: April 21, 2022
Selections Announced: Late October 2022

- This program element was released the NASA Human Research Program (HRP)
  - Space Biology will make and fund awards to investigators through E.9, while SRE will provide funded investigators proposing relevant studies access to the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory for ground radiation studies
- Science Emphases:
  - Proposed studies must address fundamental questions that will advance the understanding of how animal systems accommodate to the spaceflight environment for important stressors such as ionizing radiation and/or changes in gravity.
  - Proposals must be for hypothesis-driven research projects that study spaceflight environmental effects on animal physiology, biochemistry, cellular, and molecular biology responses.
  - Emphasis on physiological systems that are known to respond to the spaceflight and radiation environments (e.g., neurobehavioral, cardiovascular, immune, musculoskeletal, etc.) are encouraged especially those with relevance or translation to the human system.
  - The use of omics/systems biology type approaches that examine the effects of radiation, altered gravity, or other stressors encountered in the spaceflight environment are encouraged.
  - Space Biology and HRP are interested in understanding biological processes that will enable NASA's goal of long-duration human exploration to deep space destinations such as Mars.

Awards were made to twelve investigators from eight institutions in seven states. Total value of awards = ~$3.5M

Research Pathfinder for Beyond Low Earth Orbit Space Biology Investigation (Artemis II) Funding Opportunity

Released as Program Element E.11 in ROSES 2022:
Released: August 2022; Final Proposals due October 13 (+2 weeks extension due to hurricane), 2022
Anticipated Selection Announcement: Early 2023

- This opportunity solicited proposals for biological research experiments with invertebrates to be conducted on the Artemis II mission. Proposed projects must use one of the two following model organisms:
  - *Drosophila melanogaster* (fruit flies)
  - *Caenorhabditis elegans* (nematodes
- The goal of these projects will be to study early changes in physiological systems due to exposure to the deep space environment.
- Selection of projects for this opportunity will be contingent on whether the Artemis II mission is able to accommodate the program’s biological payloads on the Orion spacecraft

NASA anticipates selecting two projects for award, each with a maximum budget of $750K

Space Biology's Upcoming ROSES Solicitations:

FY2022 Annual Ground and Flight Research Announcement

To be Released under ROSES-2022
Release: ~Late Fall / Winter 2022, Proposals Due: ~Spring 2021
Selections Announced: ~Late Summer/Early Fall 2023

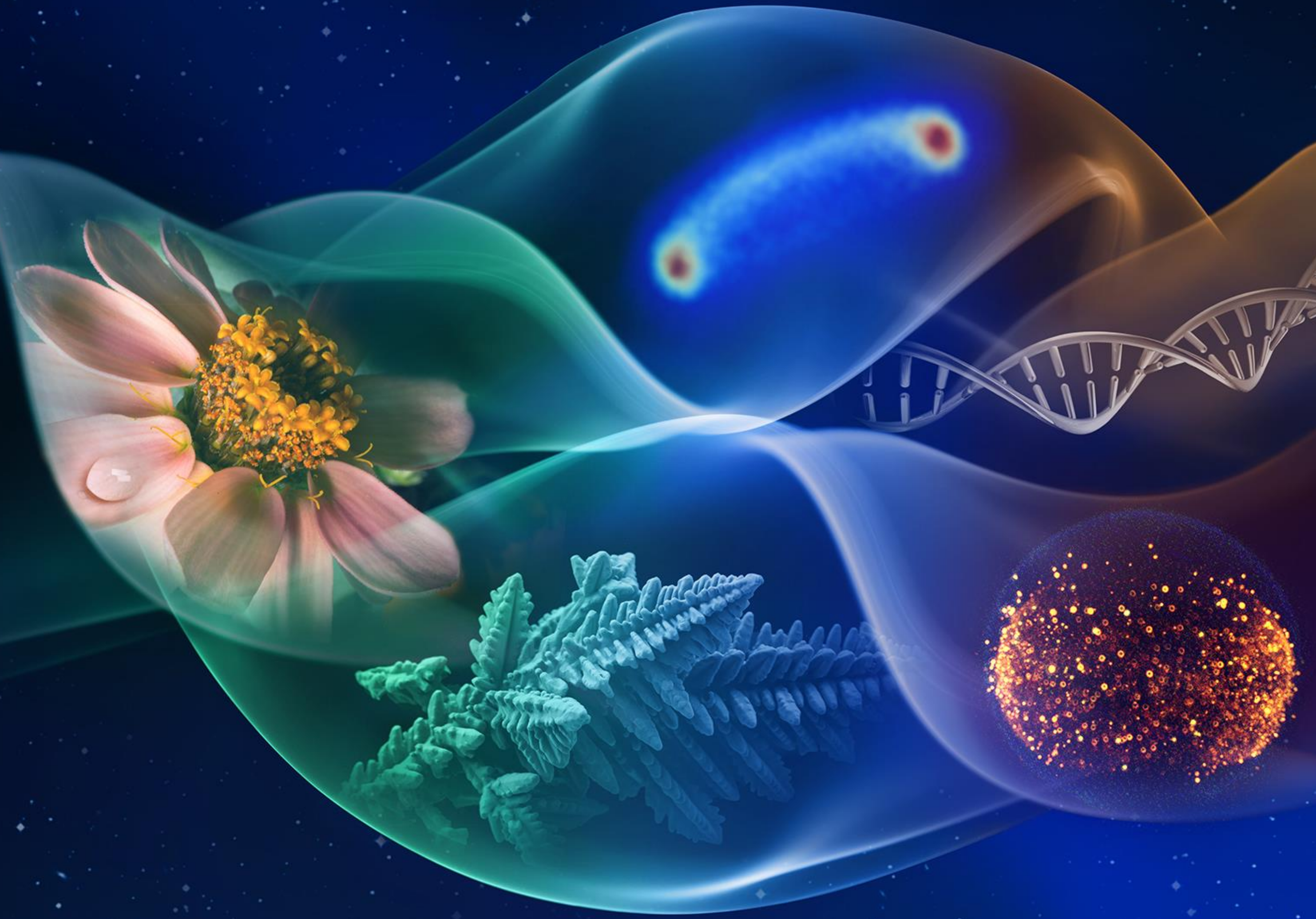
Solicited Topics: TBD

Challenges

Thoughts/Concerns:

1. Total available $ to award Space Bio grants have reduced in the past 2 years
2. 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.
3. Mission Integration & Operations costs for flight experiments were born by the ISS office. This ends in FY2025. Not clear about funding for the path forward.
4. 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. Therefore, the requisite facilities need to be maintained.
5. It will be important to develop the capabilities to conduct long duration experiments in the lunar environment in preparation for Mars missions: need to do comparative science between different spaceflight platforms and between different organisms.
6. We need to grow as a program to be able to start taking full advantage of deep-space exploration opportunities.
7. There is no decadal wedge funding set aside for us to respond to the upcoming recommendations.

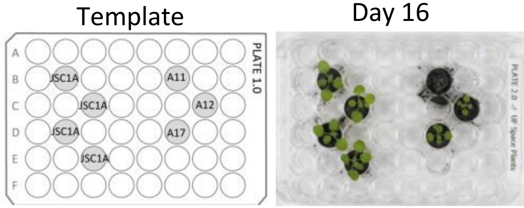
Recent Science Highlight



Plants grown in Apollo lunar regolith present stress-associated transcriptomes that inform prospects for lunar exploration.

Paul AL, Elardo SM, **Ferl R**.

Commun Biol. 2022 May 12;5(1):382. DOI: [10.1038/s42003-022-03334-8](https://doi.org/10.1038/s42003-022-03334-8)



Anna-Lisa Paul
Robert Ferl

Univ. of Florida, Gainesville

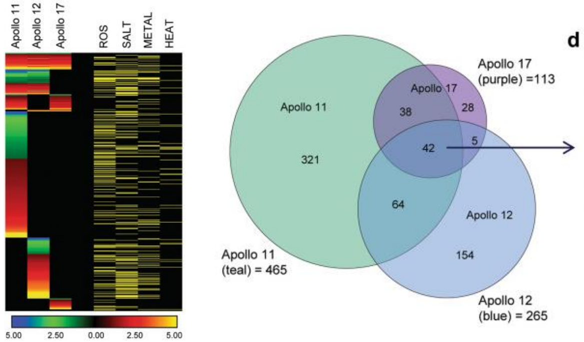
Arabidopsis thaliana germinates and grows in regolith

- Regolith samples from Apollo 11, 12, and 17 were used.
- JSC-1A = simulant control.

- Germination rates were close to 100% in all sources of Apollo regolith.

- Regolith-grown seedlings, however, did not thrive compared to JSC-1A controls.

Plant transcriptomes differ by Apollo samples (site)



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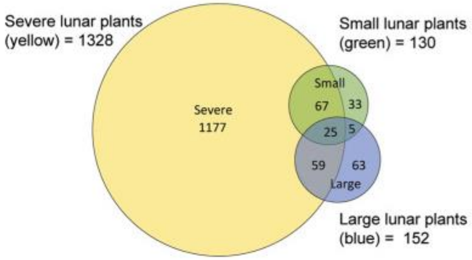
AtG	Short description	A11	A12	A17
AT5G54190	protochlorophyllide oxidoreductase A	-3.77	-2.85	-3.44
AT2G34655	associated with sulfer deficiency	-2.35	-1.85	-1.49
AT1G12570	Glucose-methanol-choline oxidoreductase	-1.63	-1.25	-1.33
AT2G23010	serine carboxypeptidase-like 9	2.44	2.00	2.43
AT3G54990	Integrase-type DNA-binding superfamily	2.47	2.32	2.40
AT1G52040	myrosinase-binding protein 1	2.49	2.97	2.15
AT2G05540	Glycine-rich protein family	2.58	2.30	2.43
AT1G24070	cellulose synthase-like A10	2.64	2.92	2.00
AT4G37150	methyl esterase 9	2.71	2.38	2.44
AT1G52400	beta glucosidase 18	2.85	2.47	2.54
AT5G24380	YELLOW STRIPE like 2	2.89	2.11	2.20
AT3G09260	Glycosyl hydrolase superfamily protein	2.93	1.72	2.66
AT1G76790	O-methyltransferase family protein	3.27	3.56	3.63
AT3G28220	TRAF-like family protein	3.33	3.15	3.30
AT5G24770	vegetative storage protein 2 / metal	3.33	4.34	3.37
AT2G30766	associated with iron defeciency	3.55	3.26	4.10
AT1G52100	Mannose-binding lectin superfamily protein	3.98	3.07	3.42
AT5G10625	metabolite signaling	4.09	2.59	3.42
AT5G26270	salt stress	4.13	3.35	3.99
AT1G13609	Defensin-like (DEFL) family protein	5.02	5.59	7.08
AT3G28290	associated with aluminium stress (DUF677)	5.44	5.34	4.21
AT3G28300	associated with drought (DUF677)	5.44	5.34	4.21
AT3G55970	jasmonate-regulated gene 21 / salt	5.53	4.39	4.60
AT3G44300	nitrilase 2 / metal	6.26	4.97	5.80

Expression data were parsed based on lunar sample site replicates compared to controls

- All regolith samples, irrespective of Apollo site, significantly evoked differentially expressed genes (DEGs) indicative of a strong stress response.
- 71% of the DEGs typically were associated with salt, metal, and reactive oxygen species stress.

Plant transcriptomes differ by morphology

- Plants with a "severe" phenotype (tiny, abnormal morphology and reddish-black pigmentation) had 1000+ DEGs, demonstrating severe reactions to the regolith.



Relevance: Terrestrial plants can grow in lunar regolith, however, further characterization and optimization is required before regolith can be considered a routine in situ resource, as it is not a benign growth substrate.

Media Resources: Twitter Spaces: https://twitter.com/NASA/status/1524788473521217538
Gravity Assist Podcast: https://www.nasa.gov/mediacast/gravity-assist-how-to-grow-food-on-the-moon

Conclusion

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

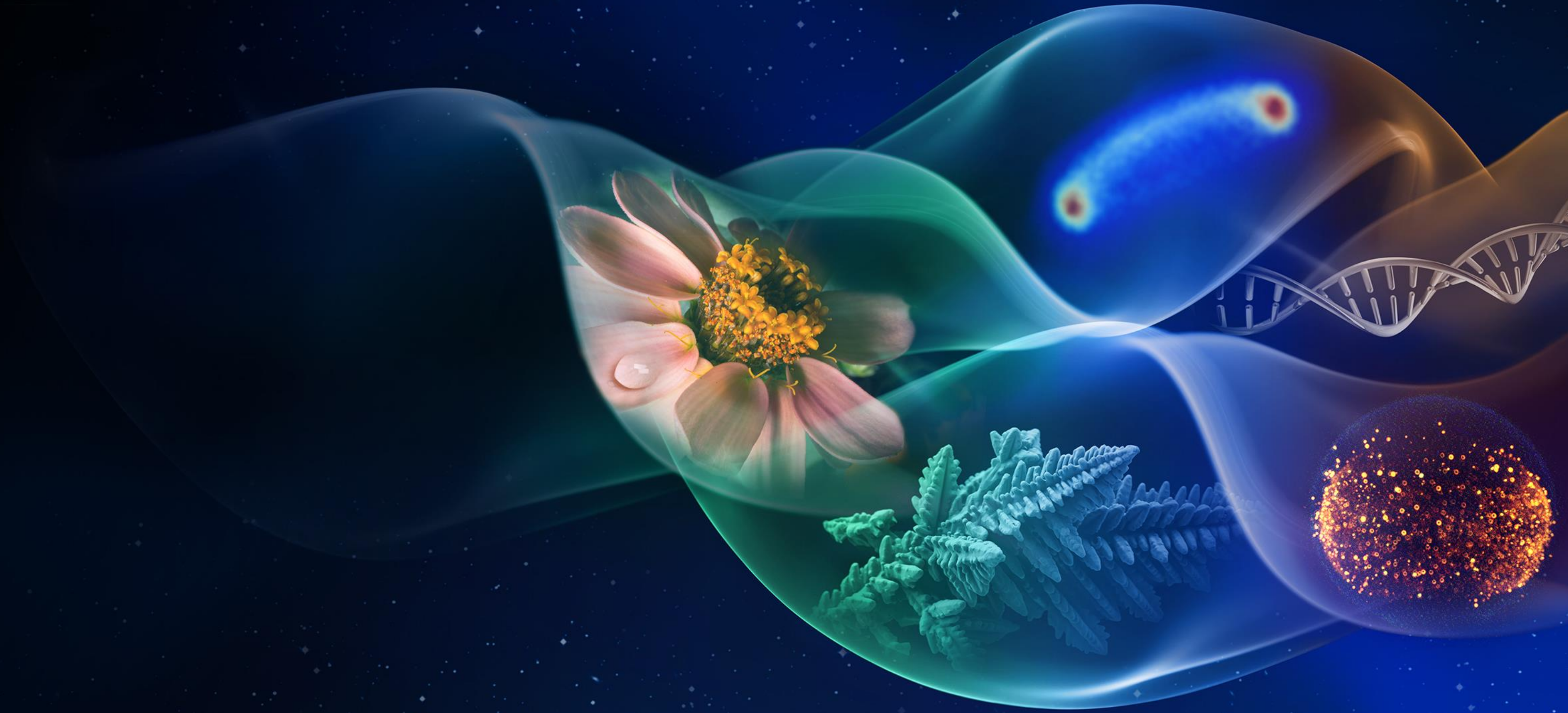
- **Space Biology intends to…**
  - utilize multiple biological model systems and
  - spaceflight platforms

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

➢ **To enable exploration,**

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

Thank you!



More Science Highlights

Plants Grown in Apollo Lunar Regolith Present Stress-Associated Transcriptomes that Inform Prospects for Lunar Exploration

Paul A-L, Elardo SM, Ferl R. *Commun Biol* **5**, 382 (2022). https://www.nature.com/articles/s42003-022-03334-8

Background/Objectives/Methodology

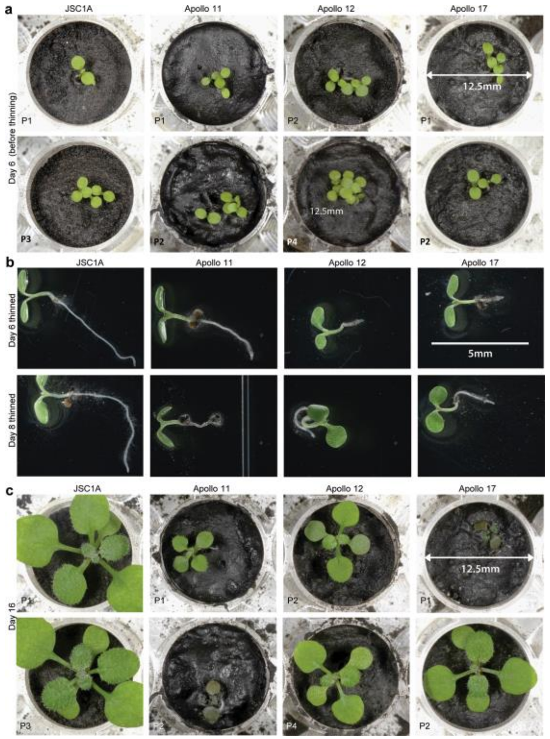
- The extent to which plants can enhance human life support on other worlds depends on the ability of plants to thrive in extraterrestrial environments using *in-situ* resources.
- To test plant growth in lunar regolith, *Arabidopsis thaliana* seeds were planted on regolith collected from lunar sites during three Apollo missions (Apollo 11, 12, and 17) and regolith simulant (JSC-1A) as a control.
- Plants grew in 900 mg of material; four replicates form each site, alongside 16 replicates of JSC-1A simulant.
- The replicates were arranged in four, Nunc 48-well cell culture plates such that each plate held a replicate of each Apollo site regolith and four replicates of JSC-1A. Each well is 12.5 mm in diameter and 15 mm deep.
- The growth plates were moistened with nutrient solution daily by placing the plates in trays of solution until the regolith was wetted from below and then allowed to drain.
- The plants were grown for 20 days then the leaves and hypocotyls were harvested, placed in microcentrifuge tubes, and snap-frozen in liquid nitrogen. Samples were stored at −80°C until RNA extraction.

Key Results

- The plants germinated and grew in the diverse lunar regoliths, but growth was challenging.
- The lunar regolith plants were slow to develop and many showed severe stress morphologies.
- All plants grown in lunar soils differentially expressed genes indicating ionic stresses, similar to plant reactions to salt, metal and reactive oxygen species.
- Although *in-situ* lunar regoliths can be useful for plant production in lunar habitats, they are not benign substrates.
- The interaction between plants and lunar regolith will need to be further elucidated, and likely mitigated, to best enable efficient use of lunar regolith for life support within lunar stations.

Relevance/Impact

- Plants have long been envisioned as part of lunar habitats and exploration environments. However, until the current study, the interactions between lunar materials and terrestrial biology were unaddressed in the era of modern molecular biology, and there had yet to be any experiments where plants were actually grown in the true lunar regolith.
- Understanding the impact of sustained exposure of terrestrial biology to lunar regolith, and determining the efficacy of lunar regolith as a viable *in-situ* resource, is important to the concept of returning to the Moon for long durations.
- This study used plant growth and gene expression to both tests the fundamental impact of lunar regolith on terrestrial biology, and provide an initial evaluation of regolith as a matrix for plant growth systems in lunar exploration habitats.



a Germination rates were close to 100% in all sources of Apollo lunar regolith and indistinguishable from rates in JSC-1A simulant. Two representative wells for JSC-1A and each Apollo site are shown. **b** The seedlings thinned from each well on day 6 or 8 indicated that root growth in lunar regolith is not as robust as in JSC-1A. **c** While germination was uniform among controls and lunar sites, the lunar regolith-grown seedlings did not thrive as compared to the JSC-1A controls. The diameter of the culture plate wells is 12.5 mm (scale bar provided in **c**). All microscope images in **b** are shown to the same scale (scale bar shown in the Apollo 17 image).

Artificial Gravity Partially Protects Space-induced Neurological Deficits in Drosophila melanogaster

Mhatre SD*, Iyer J*, Petereit J, Dolling-Boreham RM, Tyryshkina A, Paul AM, Gilbert R, Jensen M, Woolsey RJ, Anand S, Sowa MB, Quilici DR, Costes SV, Girirajan **S, Bhattacharya** (2022). *Cell Reports*, *40*(10), 111279.

Background/Objectives/Methodology

Evaluated the effects of spaceflight on CNS following a 34-day mission to the International Space Station (ISS) in response to microgravity and artificially simulated Earth-gravity (SF1g) *via* inflight centrifugation as a countermeasure using *Drosophila melanogaster*

Key Results

- Artificial gravity (SF1g) partially protects the neurological deficits observed in flies reared in spaceflight microgravity (SFμg)
- Acclimation to Earth conditions (R+25) showed progressive neuro-morphological deficits in both SFμg and SF1g conditions, with pronounced phenotypes noted in SFμg.

Relevance/Impact

This study shows that oxidative stress during spaceflight leads to differential regulation of metabolic pathways, oxidative phosphorylation, and synaptic transmission resulting in neuronal deficits, glial changes, increased apoptosis, and behavioral impairments in *Drosophila*. Further, AG provides partial protection toward spaceflight-related neurological deficits, suggesting that μg is an important but not an exclusive environmental factor contributing to the neurobehavioral outcomes during long-term deep space missions